Charles University, Faculty of Science
Department of Zoology
PhD study program: Zoology

ALBERT DELER-HERNÁNDEZ

Taxonomy, phylogeny and phylogeography of selected groups of aquatic beetles (Coleoptera: Hydrophilidae, Hydraenidae) of the Caribbean region

Taxonomie, fylogeneze a fylogeografie vybraných skupin vodních brouků (Coleoptera: Hydrophilidae, Hydraenidae) karibské oblasti

PhD THESIS

Supervisor: Martin Fikáček

Prague, 2017

I declare that this thesis has not been submitted for the purpose of obtaining of the same or another academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed through the authorship order of the included publications and manuscripts. All literature sources I used when writing this thesis have been properly cited.

Prague, 2017 Albert Deler-Hernández

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LIST OF ATTACHED PUBLICATIONS AND MANUSCRIPTS

Part 1: Evolutionary history of selected hydrophilid beetles of Greater Antilles

1. **Deler-Hernández A,** Sýkora V, Seidel M, Cala-Riquelme F, Fikáček M. 2017. Multiple origin of the *Phaenonotum* beetles in the Greater Antilles (Coleoptera: Hydrophilidae): phylogeny, biogeography and systematics. *Zoological Journal of the Linnean Society* (in press).
2. **Deler-Hernández A**, Sýkora V, Fikáček M. 2017. A review of the genus *Crenitulus* of Greater Antilles (Coleoptera: Hydrophilidae) (manuscript draft).

Part 2: Contributions to the systematics of aquatic beetles of Greater Antilles

3. **Deler-Hernández A,** Cala-Riquelme F, Fikáček M. 2013. Description of a new species of *Phaenonotum* from eastern Cuba (Coleoptera: Hydrophilidae: Sphaeridiinae). *Acta Entomologica Musei Nationalis Pragae* 53(2): 615–622.
4. **Deler-Hernández A,** Fikáček M, Cala-Riquelme F. 2013. A review of the genus *Berosus* Leach of Cuba (Coleoptera: Hydrophilidae). *Zookeys* 273: 73–106.
5. **Deler-Hernández A,** Cala-Riquelme F, Fikáček M. 2014. A review of the genus *Oosternum* Sharp of the West Indies (Coleoptera: Hydrophilidae: Sphaeridiinae). *Deutsche Entomologische Zeitschrift* 53(2): 615–622.
6. **Deler-Hernández A,** Fikáček M. 2016. Redescriptions and lectotype designations of Central American species of *Phaenonotum* Sharp (Coleoptera, Hydrophilidae) based on the type material from the David Sharp collection. *ZooKeys* 579: 83–98.
7. **Deler-Hernández A,** Fikáček M. 2016. Larval morphology and chaetotaxy of three Caribbean *Berosus* Leach, 1817 with revised adult diagnosis of *Berosus undatus* (Fabricius, 1792) (Coleoptera: Hydrophilidae). *Aquatic Insect* 37(2): 99–113.
8. **Deler-Hernández A,** Delgado JA. 2017. The Hydraenidae of Cuba (Insecta: Coleoptera) II: Morphology of preimaginal stages of six species and notes on their biology. *Zootaxa* 4238 (4): 451–498.
9. **Deler-Hernández A,** Delgado JA. 2017. The Hydraenidae of Cuba (Insecta: Coleoptera) III. Description of two new hygropetric species *Hydraena* Kulgelann. *Zootaxa* 4250 (5): 434–446.

ACKNOWLEDGEMENTS

I would like to thank all the people (professors and colleagues) that helped me to reach my goals during my PhD studies.
My sincere gratitude to Martin Fikáček, my supervisor and friend, for giving me the opportunity first to learn and grow as a researcher, and second to see that the capitalism is not so bad.

Sincere thanks to all my family, my girlfriend Aneta Prozrová and my team of friends (Martina Hájková, Markéta Végsöová, Lukáš Slavík and Kuba Jelínek) who supported me all the time.
My greatest thanks belong to my two brothers, Franklyn Cala-Riquelme and Ondra Jelínek who supported me in many ways.

My research was financially supported by grant SVV 260 434/2017 at the Faculty of Science, Charles University, by the Rufford Foundation (15144-1) and by the Mobility Found of the Charles University. The field work in Cuba, Dominican Republic and Puerto Rico was made partly possible thanks to the funds of the Ministry of Culture of the Czech Republic to my supervisor.

ABSTRACT

This thesis is focused in the representatives of beetle families Hydrophilidae and Hydraenidae of West Indies and adjacent regions. It consists of two parts, the phylogenetic part and the systematic part.

The phylogenetic part focuses on the hydrophilid genera *Phaenonotum* and *Crenitulus* of Greater Antilles: beetles were sampled in all four main islands (Cuba, Hispaniola, Puerto Rico and Jamaica) and analyzed using the combination of molecular and morphological data. The genus *Phaenonotum* (**Chapter 1**) contains four single-island endemics, of which those from Cuba, Jamaica and Hispaniola are wingless and form a clade that diversified ca. 46 million years ago (Ma) and likely colonized the Caribbean via the GAARlandia land bridge. In contrast, the Puerto Rican endemic and the two remaining non-endemic species colonized the Greater Antilles by over-water dispersal during the Oligocene-Miocene. The analysis of the genus *Crenitulus* (**Chapter 2)** revealed that Greater Antillean species belong to two separate clades: the *Crenitulus yunque* clade endemic for Cuba and Hispaniola, and the *Crenitulus suturalis* clade containing specimens from Greater Antilles and from northern America. A detailed revision of the *Crenitulus yunque* clade using morphology and molecular-based species delimitation recognized 11 species locally endemic for particular mountain ranges in Cuba and Hispaniola.

Systematic part includes taxonomic revisions and larval studies of selected genera of the Greater Antillean Hydrophilidae and Hydraenidae. In total, taxonomic treatment is provided for 46 species, including 25 species new to science, and larvae of 9 species are described. In the family Hydrophilidae, I described a new species of the genus *Phaenonotum* from eastern Cuba (**Chapter 3)** and reviewed the type material of the Central American species of the genus (**Chapter 6)**. The Cuban fauna of the genus *Berosus* was reviewed taxonomically (**Chapter 4**) and larvae of three species were described (**Chapter 7**). Ten species were recorded in the Caribbean for *Oosternum*, of which seven are described as new (**Chapter 5**). The studies of the family Hydraenidae focused on morphological description of immature stages of Cuban species (**Chapter 8**) and description of two new hygropetric species of the genus *Hydraena* from Cuba (**Chapter 9**).

ABSTRAKT

Tato doktorská práce je zaměřena na zástupce broučích čeledí vodomilovitých (Hydrophilidae) a vodanovitých (Hydraenidae) karibské oblasti a přilehlých oblastí. Je rozdělena do dvou částí: fylogenetické a systematické.

Fylogenetická část se zaměřuje na vodomilí rody *Phaenonotum* a *Crenitulus* Velkých Antil: materiál byl nasbírán na všech čtyřech hlavních ostrovech (Kuba, Hispaniola, Jamajka, Portoriko) a analyzován na základě morfologických a molekulárních dat. Rod *Phaenonotum* (**kapitola 1**) obsahuje čtyři endemické druhy vyskytující se každý na jednom z ostrovů, z nichž ty z Kuby, Jamajky a Hispanioly jsou bezkřídlé a tvoří fylogenetickou větev, která se oddělila před ca. 46 milióny let a pravděpodobně kolonizovala karibskou oblast přes pevninský most GAARlandia. Druh endemický pro Portoriko a další dva neendemické druhy kolonizovaly karibské ostrovy přes moře během oligocénu a miocénu. Analýza rodu *Crenitulus* (**kapitola 2**) ukázala, že karibské druhy patří do dvou kládů: skupina druhů kolem *C. yunque* tvoří klád endemický pro Kubu a Hispaniolu, kdežto skupina druhů kolem *C. suturalis* je rozšířena na Velkých Antilách a v severní Americe. Podrobná revize druhové skupiny kolem *C. yunque* za pomocí morfologie a metod pro rozlišení druhů na základě molekulárních dat ukázala, že tato skupina obsahuje 11 druhů endemických pro jednotlivá pohoří Kuby a Hispanioly.

Systematická část obsahuje taxonomické revize a popisy larvální morfologie vybraných rodů čeledí Hydrophilidae a Hydraenidae Velkých Antil. Celkem jsem taxonomicky zrevidoval 46 druhů, z čehož 25 druhů jsou druhy nové pro vědu, a popsal jsem larvální morfologii 9 druhů. V čeledi Hydrophilidae jsem popsal nový druh rodu *Phaenonotum* z východní Kuby (**kapitola 3**) a revidoval typový materiál tohoto rodu ze střední Ameriky (**kapitola 6**). V rodě *Berosus* jsem zrevidoval faunu Kuby (**kapitola 4**) a popsal larvální morfologii tří kubánských druhů (**kapitola 7**). V rodu *Oosternum* jsem v celé karibské oblasti rozlišil 10 druhů, z nichž 7 jsem nově popsal (**kapitola 5**). V čeledi Hydraenidae jsem popsal nedospělá stádia kubánských druhů (**kapitola 8)** a dva nové druhy rodu *Hydraena* ze smáčených stěn z Kuby (**kapitola 9**).

1. INTRODUCTION

The West Indies (Fig. 1) (i.e. the Caribbean islands off the continental shelf; Morrissey, 1998, Peck, 2011) is a region of the Atlantic Ocean in the Caribbean, that includes Bahama Islands, the Greater Antilles (Cuba, Cayman Islands, Jamaica, Hispaniola and Puerto Rico), and the Lesser Antilles (Anguilla, Antigua and Barbuda, Barbados, British Virgin Island, Dominica, Granada, Guadeloupe, Martinique, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, United Stated Virgin Island and the northern Dutch Windward Islands) (Dávalo, 2004; Peck, 2011).

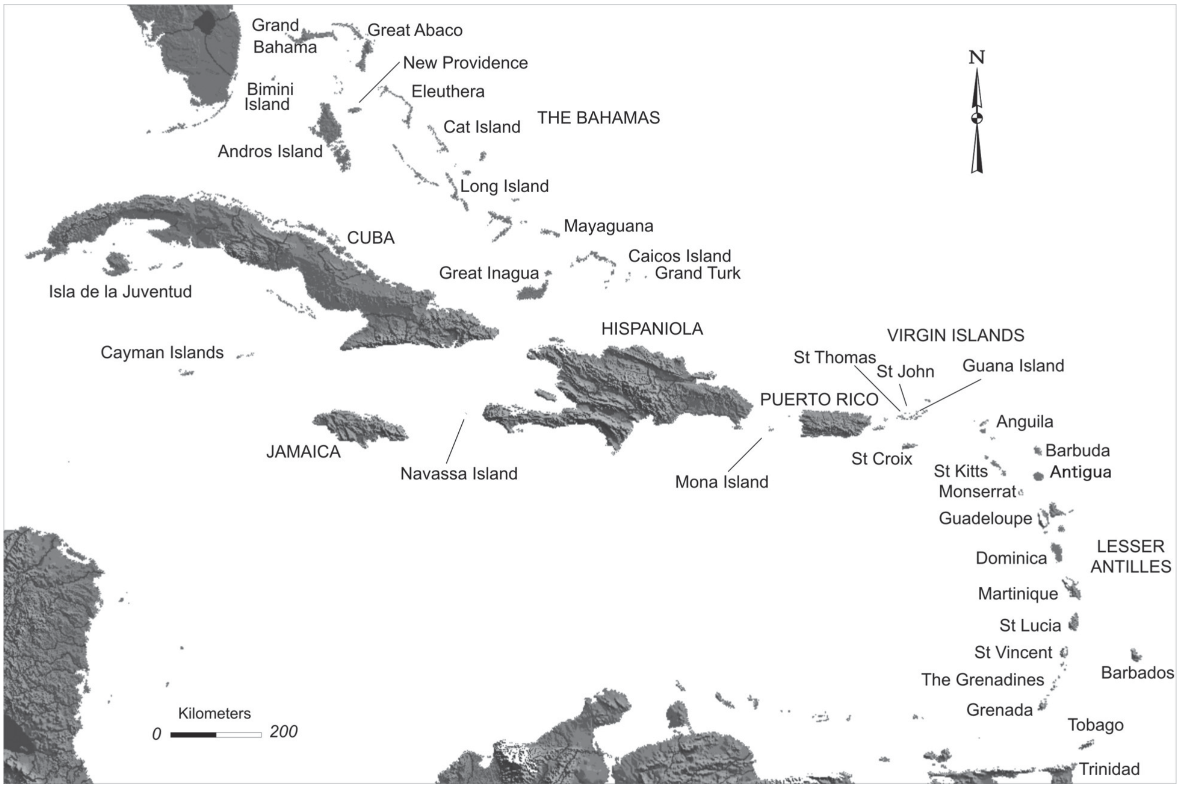

Figure 1. The islands of the central and eastern West Indies and adjacent continental land masses, showing in the east the main island arc of the Lesser Antilles (adopted from Peck, 2009, 2011).

The islands of the West Indies exhibit exceptional biodiversity and are ranked in the top five of the world's most important biodiversity hotspots, with endemism reaching over 90% in some groups (Myers *et al*., 2000; Weaver *et al*., 2016).

The flora of the West Indies has not yet undergone a comprehensive review, but there are at least 10,000 species of vascular plants, about one third of which are endemic (Adam, 1972; Gentry, 1992). Vertebrates are the best-studied organisms in the West Indies (Alonso, 2011; Hedges, 2001), of those the amphibians, reptiles and birds exhibit a high species richness, although the percentage of endemism show big differences. Only one third (35%) of the birds are endemic for the West Indies, while almost all the amphibians (99%), reptiles (96%) and freshwater fishes (96%) are endemic from the region (Alonso, 2001; Hedges, 2001).

The region is recognized as a natural laboratory for the study of biogeography and evolution (Williams, 1969; Ricklefs and Bermingham, 2008; Sato *et al*., 2016), and has hosted large radiations of lineages with limited dispersal capacity. This is the case e.g. of some *Platynus* carabid beetles (Liebherr, 1988) and *Drosophila* flies (Wilder and Hollocher, 2003), *Eleutherodactylus* frogs (Hedges *et al.,* 2008), *Anolis* lizards (Losos, 2009) and also some clades of plant families Melastomaceae (Michelangeli *et al*., 2008) and Asteraceae (Francisco-Ortega *et al*., 2008).

1.1 Aquatic beetles of the Caribbean region

Beetles (Coleoptera) is the most diverse group of insects, containing more than 380,000 described species (McKenna *et al*., 2015). Their diversity concerns not only the number of species, but also the morphology, size and ecological strategies. Although the vast majority of beetles are terrestrial, at least 12,000 species are aquatic in one or more developmental stages (Jäch and Balke, 2008) and are an important part of most freshwater ecosystems. Of the four Coleoptera suborders, three (Myxophaga, Adephaga and Polyphaga) have aquatic representatives, of which two (Adephaga, Polyphaga) are present in the Caribbean (Peck, 2005; Perez-Gilabert, 2008). The aquatic members of Adephaga, commonly named Hydradephaga, comprise eight families, four of which are present in the Caribbean: Dytiscidae (57 species), Gyrinidae (7 species), Noteridae (19 species) and Haliplidae (12 species) (Nilsson, 2001; Nilsson, 2003; Nilsson and Vondel, 2005; Peck, 2005; Megna and Deler, 2006; Deler-Hernández and Megna, 2007; Vondel and Spangler, 2008; Alarie *et al*., 2009; Peck, 2009, 2011; Megna *et al*., 2011; Megna and Epler, 2012; Megna and Sanchez-Fernández, 2014; Manuel, 2015). Polyphaga is the most diverse group of Coleoptera, containing about 90% of all known beetle species (Lawrence and Slipinski, 2014). Seven aquatic families are present in the Caribbean: Hydrophilidae (95 species), Hydrochidae (4 species), Hydraenidae (12 species), Dryopidae (1 species), Elmidae (4 species), Psephenidae (1 species) and Lutrochidae (1 species) (Perkins, 1980; Bamuel and Jäch, 2000; Peck, 2005; Shepard and Megna, 2006; Deler-Hernández and Delgado, 2010; Epler, 2010; Deler-Hernández and Delgado, 2012; Deler-Hernández *et al*., 2013a: **Chapter 3**; Deler-Hernández *et al*., 2013b: **Chapter 4**; Deler-Hernández *et al*., 2017: **Chapter 9**). However, until recently, the Caribbean fauna of aquatic beetles has been little studied, and the majority of studies are based on occasional collecting events and historical records. Recently, extensive research has been conducted at least in some groups (e.g., Short, 2004a; Short and Torres, 2006a; Spangler and Short, 2008; Megna and Epler, 2012; Megna and Sanchez-Fernández, 2014; Megna and Sondermann, 2015), and the present PhD thesis also aims in improving the knowledge about the Caribbean fauna in families Hydraenidae and Hydrophilidae.

1.1.1 Dytiscidae

The Dytiscidae (predaceous diving beetles) is at the moment divided in 11 subfamilies: Agabinae, Cybistrinae, Colymbetinae, Copelatinae, Coptotominae, Dytiscinae, Hydrodytinae, Hydroporinae, Laccophilinae, Lancetinae and Matinae (Miller and Bergsten, 2014, 2016). About, 4300 species are known, classified in 188 genera (Nilsson, 2003; Miller and Bergsten, 2016), many of them of a worldwide distribution. Members of the family Noteridae are sometimes still considered as members of Dytiscidae (as subfamily Noterinae; e.g., Fransiscolo, 1979; Pederzani, 1995; Rocchi, 2000), which is not followed here.

Six of these subfamilies are present in the Caribbean Region (Cybistrinae: 3 species; Colymbetinae: 2 species of *Rhantus*; Copelatinae: 10 species of *Copelatus*; Dytiscinae: 8 species; Hydroporinae: 25 species; and Laccophilinae: 10 species; for details see Table 1) (Perez-Gelabert, 2008; Peck, 2009, 2011; Megna *et al*., 2011). 36% of species are Caribbean endemics, and the genera *Copelatus* and *Desmopachria* have the largest number of endemic species.

Adults and all larval stages of dytiscid beetles live in water and are predaceous. They are well adapted for aquatic lifestyle. Adults are smooth and streamlined and usually compact in form. Their body shape and size are often somewhat correlated with habitat preferences, with elongate, more narrowed species often better swimmers, and short and compact species more maneuverable and often found in dense vegetation where they do less open-water swimming (Ribera *et al*., 1997). Both adults and larvae breathe atmospheric oxygen. Adults carry the air bubble under their elytra to where the spiracles are opening, and they occasionally come to the surface to replenish the oxygen in the bubble once it is depleted. Some small species of Hydroporinae, or species that live in high-oxygen environments, may exchange gases through the cuticle or through specialized pores (Madsen, 2009, 2011).

Dytiscid beetles are good indicators for conservation purposes (e.g. Ribera and Foster, 1993; Sánchez-Fernández *et al*., 2006) due to high diversity of species and the variety of habitats occupied, including desert pools, habitats with high disturbance regimes (vernal pools, desert rock pools, phytotelmata). A large number of species appear to be eurytopic, occurring in many habitat types, and these are among the most commonly encountered species in the family; other occur only in specialized habitats (e. g., *Coelambus salinarius* Wallis specimens can withstand an exceptional range of salt concentrations) (Sanchez-Fernández *et al*., 2010; Céspedez *et al*., 2013; Miller and Bergsten, 2016). Dytiscid beetles can also be found in hygropetric habitats (Miller and Spangler, 2008) and contains also at least 5 putatively terrestrial species: *Geodessus* from India and Nepal, *Typhlodessus* from New Caledonia and two *Paroster* from Australia (Brancucci and Hendrich, 2010; Brancucci and Monteith, 1996).

1.1.2 Noteridae

Noteridae (burrowing water beetles) is a group of aquatic beetles with worldwide distribution, with the highest diversity in tropical and subtropical region (Roughley, 2001; Lawrence and Slipinski, 2014). Originally, they were included within the family Dytiscidae due to their dytiscid-like appearance and recognized as representing a separate phylogenetic clade (and hence separate family) by Crowson (1955) based on morphological characters of adults and larvae. Both adults and larvae are aquatic and usually occur in shallow ponds, slow-running pools or streams (Peck, 2005). Adults are distinguished by the filiform antennae; maxillary palpi shorter than the antennae; concealed scutellum; distinctly five-segmented fore and mid tarsi; first abdominal ventrite completely divided by hind coxae; and hind tarsi with two equal claws (Epler, 2010). According to Nilsson and Vondel (2005) the family Noteridae are represented by around 258 described species in 22 genera (Nilsson, 2011). In the Caribbean, the knowledge of the family is very limited, and is based on old studies only (Chevrolat, 1863; Gundlach, 1891; Young, 1979, 1985; Spangler, 1973, 1981). In total, 19 described species have been recorded in the region, representing six genera (Megna and Deler, 2006; Manuel, 2015). 37% of species recorded in the region are endemic, with the genus *Notomicrus* containing the highest proportion of endemic species.

1.1.3 Haliplidae

Haliplidae (crawling water beetles) is a cosmopolitan family with ca. 200 described species classified in five genera (Vondel and Spangler, 2008). They occur among dense aquatic vegetation at the edges of the ponds, lakes or slow-moving streams, and in the slower parts of the rivers (Peck, 2005; Epler, 2010). Adults usually crawl on the bottom, but are able to swim using alternate leg movement. Haliplid beetles are small, and are characterized by the strongly enlarged metacoxal plates, which at least reach the fifth ventrite (Vondel and Spangler, 2008; Epler, 2010; Lawrence and Slipinski, 2014). The family has been little studied in the Caribbean, the most complete study in the region was made by Vondel and Spangler (2008) who are recording 12 species, of them six species are endemic.

1.1.4 Gyrinidae

Gyrinidae (whirligig beetles) are present in all continents with the exception of Antarctica (Miller and Bergsten, 2012). They have a unique lifestyle on the water surface of ponds and streams (Peck, 2005). There are no truly marine gyrinds, but a number of species are collected in brackish water (Ochs, 1969). Adults of all species are highly adapted to swimming on the water surface and use the surface film for support. They are characterized especially by the present of compound eyes completely divided into one 'pair' of dorsal (above the water line) and one 'pair' of ventral surface of the head (below the water line), and by meso- and metathoracic legs with broadly expanded tarsi fringed with setae for swimming. The family presents around 900 species, grouped in three subfamilies: Spanglerogyrinae (1 species), Heterogyrinae (1 species) and Gyrininae (with about 900 species) (Lawrence and Slipinski, 2014). Three genera and six species of Gyrinidae are known in Caribbean. Three species of the genera *Gyretes* and *Gyrinus* are endemics for the region, one species, *Dineutus longimanus* Olivier, 1795, is represented by four subspecies, each endemic for one of the main islands of the Greater Antilles (Gustafson and Miller, 2015). The family has been little studied in the Caribbean, but recent field work seems to indicate that the presence on new species is unlikely.

1.1.5 Hydrophilidae

Hydrophilidae (water scavenger beetles) represent one of the most ecologically diverse groups of Coleoptera, containing around 3350 described species classified in 160 genera and six subfamilies: Hydrophilinae, Chaetarthriinae, Acidocerinae, Cylominae, Enochrinae, Sphaeridiinae (Short and Fikáček, 2013; Lawrence and Slipinski, 2014; Seidel *et al*., 2016). Hydrophilid beetles occur in aquatic habitats, but many species inhabit more specialized aquatic situations, such as hygropetric (e.g. *Oocyclus* Sharp: Short and García, 2010), subterranean streams (e.g. *Troglochares* Spangler: Spangler, 1981b) and the water tanks of bromeliads (e.g. *Lachnodacnum* d'Orchymont: Clarkson *et al*., 2014). Representatives of the subfamilies

Table 1. Genus composition and endemism of the adephagan aquatic beetles fauna of West Indies.

Genus	Number of species	Single-island endemics	Wide Caribbean endemics	Continental species	Introduced species	References
Dytiscidae						
Cybister	1	-	-	1	-	Peck, 2005
Megadytes	2	-	-	2	-	Peck, 2005
Rhantus	3	-	-	3	-	Peck, 2005
Copelatus	14	7	2	6	-	Megna & Epler, 2012
Acilius	1	-	-	1	-	Peck, 2005
Eretes	1	-	-	1	-	Peck, 2005
Prodaticus	2	-	-	2	-	Peck, 2005
Thermonectus	4	-	-	4	-	Peck, 2005; Alarie *et al.*, 2009
Anodocheilus	1	-	-	1	-	Peck, 2005
Bidessonotus	4	2	2	-	-	Peck, 2005
Neoclypeodytes	1	-	1	-	-	Peck, 2005
Liodessus	1	-	-	1	-	Peck, 2005
Neobidessus	1	-	-	1	-	Peck, 2005
Hydrovatus	3	-	-	3	-	Peck, 2005
Desmopachria	6	4	1	1	-	Deler-Hernández & Megna, 2007; Megna & Sanchez-Fernández, 2012
Pachydrus	1	-	-	1	-	Peck, 2005; Alarie & Megna, 2006
Derovatellus	2	1	-	1	-	Peck, 2005
Celina	7	2	-	5	-	Peck, 2005
Laccophilus	8	1	2	5	-	Megna *et al.*, 2011
Laccodytes	1	1	-	-	-	Toledo *et al.*, 2011
Laccomimus	3	-	-	3	-	Toledo & Michat, 2015
Noteridae						
Notomicrus	4	3	-	1	-	Megna & Deler, 2006 Manuel, 2015
Pronoterus	2	1	-	1	-	Nilsson *et al.*, 2005
Mesonoterus	2	-	-	2	-	Nilsson *et al.*, 2005
Suphis	2	-	-	2	-	Nilsson *et al.*, 2005
Hydrocanthus	4	-	1	3	-	Nilsson *et al.*, 2005
Suphisellus	5	1	1	3	-	Nilsson *et al.*, 2005
Haliplidae						
Haliplus	11	2	3	7	-	Nilsson *et al.*, 2005 Vondel & Spangler, 2008
Peltodytes	1	1	-	-	-	Nilsson *et al.*, 2005 Vondel & Spangler, 2008
Gyrinidae						
Dineutus	3	(4 ssp.)	-	3	-	Peck, 2005; Perez-Gelabert, 2008; Gustafson & Miller, 2015
Gyrinus	2	1	1	-	-	Peck, 2005; Perez-Gelabert, 2008
Gyretes	2	-	1	1	-	Peck, 2005

Cylominae and Sphaeridiinae diversified in terrestrial niches: many are generalist inhabiting decaying plant remains or dung; some *Oosternum* Sharp are myrmecophilous (Spangler, 1962); some *Tormissus* Broun inhabit penguin guano (Ordish, 1974); and several genera are even found in flowers (M. Fikácek, personal observation). Adults feed mostly on plant material and decaying organic matter; larvae are predaceous (Archangelsky *et al*., 2005). Adults are distinguished by the antennal club with 3 pubescent segments beyond the cupule; long maxillary palps, almost as long or longer than antennae; and the 4-5 (6 in *Laccobius*) visible abdominal ventrites, the first not divided by metacoxae (Epler, 2010).

Until very recently, water scavenger beetles (Hydrophilidae) in the Caribbean Region were largely neglected and systematic or faunistic studies were scarce (Spangler, 1981a; Peck, 2005), but in the last years recent studies focused mainly in the Greater Antilles has allowed an increase on the knowledge of the family (Short, 2004a; Spangler and Short, 2008; Deler-Hernández and Delgado, 2010, Deler-Hernández *et al*., 2013a: **Chapter 3**; Deler-Hernández *et al*., 2013b: **Chapter 4**; Deler-Hernández *et al*., 2014; Arriaga-Valera *et al*., 2017: **Chapter 5**). In the Caribbean, the family is represented by 16 genera, eleven of which contain at least one species endemic (Hansen, 1999; Short, 2004a; Spangler and Short, 2008; Deler-Hernández *et al*., 2013a: **Chapter 3**; Deler-Hernández *et al*., 2013b: **Chapter 4**; Arriaga-Varela *et al*., 2017; **Chapter 1, 2**). 33% of species are Caribbean endemics, with a high endemism present in the genus *Crenitulus* (**Chapter 2**) (for details see Table 2).

1.1.6 Hydrochidae

Hydrochidae (elongated water scavenger beetles) has worldwide distribution except some islands (e.g., New Zealand; Hansen, 1999; Short and Fikáček, 2011). Adults are characterized by the rough, sculptured body; protruding eyes; posterior margin of pronotum distinctly narrower than bases of elytra; small scutellum; and first abdominal ventrite not divided by hind coxae (Epler, 2010). They occur in aquatic habitats, inhabiting densely vegetated standing waters, leaf debris and detritus at edges of standing and slowly running waters, and/or gravel or sandy banks of streams and rivers. Adults are slowly moving and they crawl about on vegetation or floating detritus (Archangelsky, 1997; Epler, 2010). Larvae are poorly known and are likely predaceous (Archangelsky, 1997).

The family contains ca. 180 described species, all classified in a single genus *Hydrochus* Leach (several other genera have been proposed by Makhan (1994a, 1998, 2001a, b) but are considered synonyms (Hansen 1999) or ignored). The taxonomy of the Hydrochidae is still rather poorly known in the Caribbean and the current knowledge is based primarily on occasional collecting events and historical records (Peck, 2005). Four species are recorded, of which one (*H. naraini* Makhan) is endemic to Cuba and one (*H. pallipes* Chevrolat) is a widespread Caribbean endemic.

1.1.7 Hydraenidae

Hydraenidae (minute moss beetles) is a cosmopolitan family of water beetles with around 1,500 described species classified at the moment in four subfamilies (Jäch and Balke, 2008; Jäch *et al*., 2016): Orchymontiinae (endemic for New Zealand), Prosthetopinae (eight genera with Afrotropical distribution), Hydraeninae and Ochthebiinae (both distributed worldwide). As the English name implies, most species are small, ranging from 0.8 to 2.0 mm in size and rarely reaching more than 3 mm. The highest species richness of the family is found in tropical and subtropical forests, such exceptional diversity can be related with a scarce dispersal capacity of many of its species (Jäch and Balke, 2008; Jäch *et al*., 2016). Various genera of the family are restricted to the Southern hemisphere, other genera are present only in one biogeographic region, while *Hydraena*, *Ochthebius* and *Limnebius* have a worldwide distribution (Jäch *et al*., 2016).

Hydraenid beetles inhabit all types of aquatic habitats: some genera or species groups prefer the margins of lentic environments (living among decaying leaves and aquatic vegetation), other species prefer running waters, were they are usually found in fast-flowing parts and are associated to aquatic mosses or vegetal debris; some species are found in hygropetric habitats (Deler-Hernández *et al*., 2012; Jäch and Delgado, 2014; Deler-Hernández *et al*., 2017: **Chapter 9**) and a few genera are known to have humicolous species or live associated to coastal vegetation. A Chilean genus, *Ochtheosus* Perkins, 1997 is fungicolous. Preimaginal stages are also associated with aquatic or very humid habitats. Adults are capable to pass long periods of time submersed under water, breathing with the help of a ventral microplastron, which works as an effective physical gill. In the Caribbean, two subfamilies are represented: Hydraeninae (with 12 species of the genus *Hydraena*) and Ochthebiinae (1 species of *Ochthebius*, 1 species of *Gymnochthebius*) (Bamuel and Jäch, 2000; Hansen, 1998; Spangler, 1980; Perkins, 1980; Deler-Hernández *et al*., 2012; Deler-Hernández *et al*., 2017: **Chapter 9**). The endemism of the genus *Hydraena* is high in the region, 12 species are recorded, of which 10 are endemic in the region.

1.1.8 Elmidae

Elmidae (riffle beetles) is a large family of worldwide distribution, with 1400 described species classified in 145 genera (Lawrence and Slipinski, 2014). They usually inhabit lotic habitats with rocky bottoms, clear water and higher oxygen content (Peck, 2005). Both, larvae and adults feed on decayed plant material and algae (Lawrence and Slipinski, 2014). Elmids are small to minute with hard body; filiform antennae (apical segment may be enlarged in some genera); rounded anterior coxae with concealed trochantin; 5-segmented tarsi on all legs; and first abdominal ventrite not divided by metacoxae (Epler, 2010; Lawrence and Slipinski, 2014). Elmid beetles are often good candidates for indicators of water quality (Brown, 1972) noted that "they cannot tolerate excessive pollution by such wetting agents as soaps and detergents".

Table 2. Genus composition and endemism of the polyphagan aquatic beetles fauna of West Indies. Continuation.

Genus	Number of species	Single-island endemics	Wide Caribbean endemics	Continental species	Introduced species	References
Hydrophilidae						
Berosus	10	1	-	9	-	Deler-Hernández *et al*., 2013a
Crenitulus	12	11	0	1	-	Komarek, 2005, Deler-Hernández *et al*., in prep
Derallus	2	-	-	2	-	Short & Torres, 2006a
Paracymus	8	1	1	6	-	Hansen, 1999; Peck, 2005
Laccobius	1	-	1		-	Spangler, 1968; Peck, 2005
Enochrus	9	-	2	7	-	Short, 2004a, Hansen, 1999
Helobata	1	-	-	1	-	Hansen, 1999
Helochares	3	1	-	2	-	Hansen, 1999
Hydrobiomorpha	2	-	-	2	-	Short, 2004b; Hansen, 1999
Hydrophilus	2	-	-	2	-	Hansen, 1999; Perez-Gelabert, 2008
Tropisternus	8	-	1	7	-	Spangler & Short, 2008; Perez-Gelabert, 2008
Dactylosternum	5	-	2	3	-	Peck, 2005
Phaenonotum	6	4	-	2	-	Deler-Hernández *et al*, 2013b Deler-Hernández *et al*., 2017
Cercyon	10	5	1	2	2	Arriaga-Valera *et al*., 2017
Oosternum	10	8	1	1	-	Deler-Hernández *et al*., 2014
Omicrus	6	3	1	2		Hansen, 1999
Aculomicrus	1	1	-	-		Hansen, 1999; Smetana, 1975
Hydrochidae						
Hydrochus	4	1	1	2		Peck, 2005
Hydraenidae						
Hydraena	12	8	2	2	-	Perkins, 1980; Bamuel & Jäch, 2000; Deler-Hernández & Delgado, 2012, 2017
Gymnochthebius	1	-	-	1	-	Peck, 2005
Ochthebius	1	-	-	1	-	Peck, 2005
Elmidae						
Anommatelmis	1	1	-	-	-	Perez-Gelabert, 2008
Lemalelmis	2	2	-	-	-	Perez-Gelabert, 2008
Hispaniolara	1	1	-	-	-	Perez-Gelabert, 2008
Phanocerus	3	1	-	2	-	Perez-Gelabert, 2008
Hexacylloepus	4	2	-	2	-	Shepard & Megna, 2006; Perez-Gelabert, 2008
Neoelmis	1	-	1	-	-	Shepard & Megna, 2006
Xenelmoides	1	1	-	-	-	Shepard & Megna, 2006
Phanocerus	1	-	-	1	-	Shepard & Megna, 2006
Dryopidae						
Pelonomus	1	-	-	1	-	Peck, 2005
Psephenidae					-	
Pheneps	3	3	-	-	-	Peck, 2005; Perez-Gelabert, 2008
Lutrochidae						
Lutrochus	1	-	1	-	-	Peck, 2005

The knowledge about the Caribbean fauna of Elmidae is very limited, and the most information is based on occasional collecting events and historical records (Shepard and Megna, 2006). Caribbean fauna comprises eight genera and 12 species, of which nine species are endemic for the region (Shepard and Megna, 2006; Perez-Gelabert, 2008).

1.1.9 Dryopidae

Dryopidae (long-toed water beetles) is a relatively small family with a nearly worldwide distribution, having the highest diversity in the tropics (Kodada and Jäch, 2005). Dryopid beetles are distinguished by the second antennomere which is not enlarged or heavily sclerotized, not forming a shield for remaining segments; bases of antennae are close together; eyes and antennae pubescent; and the soft body with longer, "fuzzier" pubescence. Currently, 33 genera and about 280 described species are known (Kodada and Jäch, 2005).

In the Caribbean, only the genus *Pelonomus* is recorded and referred as semi-aquatic; beetles are common inhabitants of swamps, cypress hammocks and pond margins, where they are found in plant debris, and adults come to light (Peck, 2005). Larvae have been considered terrestrial or semi-aquatic (Peck, 2005; Epler, 2010). *Pelonomus* is primarily a Neotropical genus, with a single continental species, *P. obscurus* only found in Cuba (Peck, 2005).

1.1.10 Psephenidae

Psephenidae (water pennies) is the family present in all continents with the exception of Antarctica and many islands (e.g., Hawaii, Ireland and New Zealand). Diversity is much higher in tropical than in temperate zone, with the Oriental region being the most speciose realm (Lee *et al*., 2005). Adults are not aquatic, but are commonly found resting on vegetation or rocks in riparian areas, and are often collected in streamside light traps (Peck, 2005; Epler, 2010). Psephenid larvae are strictly aquatic and the oviposition also takes place underwater. Specialized larvae are often adapted for swiftly running water by the flattened, limpet-like body shape; they are usually found attached to rocks or vegetation and cannot be confused with any other aquatic beetle larvae in the region.

The family comprises 35 genera and around 275 species grouped in four subfamilies: Eubriacinae, Psepheninae, Psephenoidinae and Eubriinae (Lee *et al*., 2005; Lawrence and Slipinski, 2014). Three single-island endemics of the genus *Pheneps* have been recorded from Cuba and Dominican Republic (Peck, 2005; Perez-Gelabert, 2008).

1.1.11 Lutrochidae

Lutrochidae (travertine beetles) is a small New World family of aquatic and semiaquatic beetles. Both, adults and larvae are aquatic, and occur on rocks in rapids and flowing areas of streams with high calcium content (Peck, 2005; Ide *et al*., 2005). The family is represented in the Caribbean only by the genus *Lutrochus* which includes ca. 15 described species in total (Ide *et al*., 2005). The only species is known from Caribbean: *Lutrochus geniculatus* Chevrolat, 1864 from Cuba and Dominican Republic (Peck, 2005).

2. Geological history and biogeography of the Caribbean Region

2.1 Theories of the origin of the Caribbean fauna

The history of the Caribbean region stretches back to almost 100 million years, when the fault that formed the Lesser Antilles began to move from west to east between North and South America (Pindell and Kennan, 2009). During a dynamic geological history of the region, islands have accreted and broken apart, and may have formed temporary connections with the mainland (Iturralde-Vinent and MacPhee, 1999; Graham, 2003; Pindell and Kennan, 2009). Four main types of islands may be found in the Caribbean: (1) islands connected with other islands or with the continent when the sea level was low (Cuba, Hispaniola and Puerto Rico); (2) continental islands separated from continental lands due to the movement of tectonic plates (Jamaica); (3) islands emerged by accumulation of marine carbonate sediments (Bahama Banks); and (4) volcanic islands (some islands form Lesser Antilles) (MacPhee and Iturralde-Vinent, 2005).

Three main models were formulated to explain the origin of the Caribbean biota, expecting different importance of the vicariance and over-water dispersal (for general discussion, see e.g. Rosen, 1985; Hedges, 1996 a, b; Crother and Guyer, 1996; Roca *et al*., 2004; Glor *et al*., 2005; Hedges, 2006; Hedges *et al*., 2008).

(1) The **vicariance model** by Rosen (1975) suggests that the present Caribbean biota represents the remnants of the ancient flora and fauna which colonized the Caribbean islands in the times when (some) islands were continuous with North and South America in the Late Cretaceous.

(2) The **overwater dispersal model** suggests that the ancestors of the Caribbean biota arrived to the islands from the continent across the ocean (over-water), e.g. on islets of floating vegetation (e.g., MacArthur and Wilson, 1963).

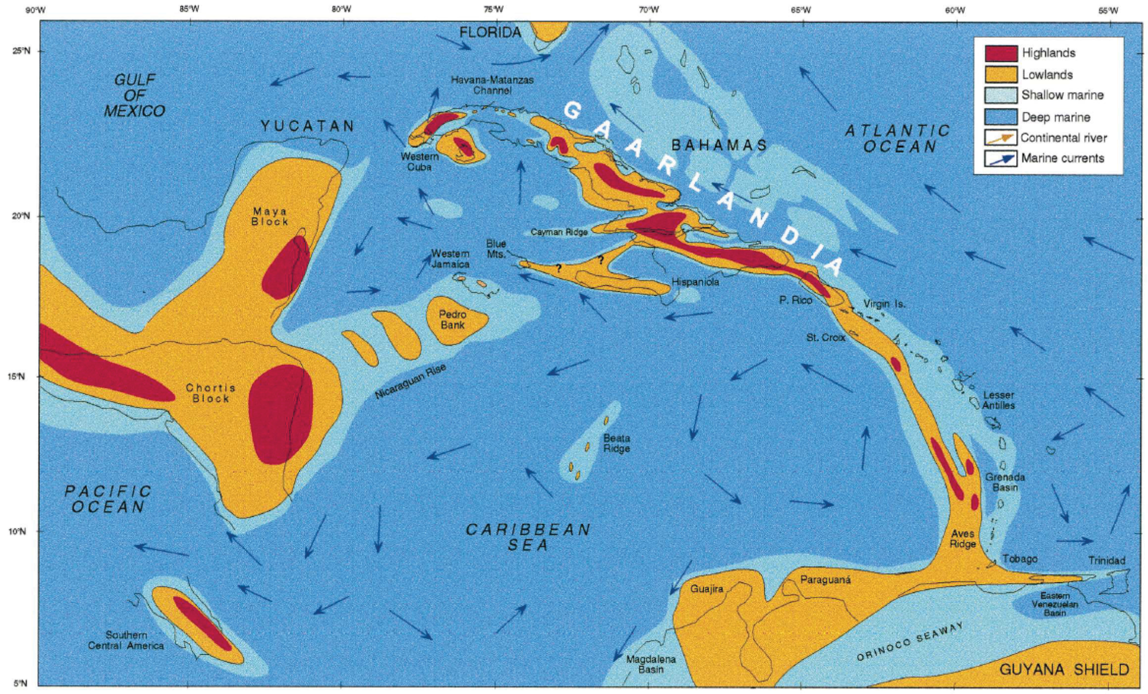

Figure 2. Map of Caribbean region as reconstructed for latest Eocene/Early Oligocene (35–33 Ma), with formation of the hypothesized GAARlandia landbridge (adopted from Iturralde-Vinent, 1999).

(3) The **GAARlandia model** was first sketched by Holcombe and Moore (1977) and complemented later by Iturralde-Vinent and MacPhee (1999) – it supposes that the ancestors of recent flora and fauna colonized the Caribbean islands via a land bridge connecting South America and the Greater Antilles during the late Eocene to early Oligocene (33-35 Ma) (Fig. 2). They called this land-bridge GAARlandia (GAAR = Greater Antilles + Aves Ridge) (Iturralde-Vinent and MacPhee, 1999; MacPhee and Iturralde-Vinent, 2000, 2005; Dávalos, 2004). The GAARlandia hypothesis comprises at least two components: (a) the emergence of the land bridge could have been used as a route for migration of the continental terrestrial biota to the Antilles, probably looking for warmer environments (Iturralde-Vinent and MacPhee 1999, Iturralde-Vinent, 2006); and (b) the effect of island-island vicariance during the subdivision of the land bridge into separate islands. Mid-Oligocene and Miocene marine transgressions and tectonics (Mann and Burke, 1984; Mann *et al.*, 1990) substantially affected the paleogeography of the Greater Antilles Ridge: several passages opened or expanded along the northern Caribbean plate boundary (Cayman Trough, Mona Canyon, Sombrero Basin/Anegada Passage), creating deep-water channels and dividing particular (paleo)islands. Under these conditions, the terrestrial biota that had colonized the land bridge stayed isolated and probably represented the first inhabitants of the Antilles (Iturralde-Vinent, 2006).

2.2 Dominican Republic amber fossils

Amber deposits are present in several countries of the Caribbean region (e.g. Haiti, Puerto Rico and Jamaica), but amber only occurs in exploitable quantities in three regions of the Dominican Republic (Iturralde-Vinent, 2001). These fossils provide an insight into the biota that inhabited the island of Hispaniola during the Miocene period, 15–20 million years ago (Perez-Gelabert, 2008) (but notice that alternative hypotheses exist about the age of the deposits: Grimaldi (1995) considers the age of 23-40 million years old). The origin of unusually large deposits of amber in the Dominican Republic can be explained by the fortunate combination of adequate conditions of relief and soil for the development of large populations of resin producing trees during a warm and humid climate optimum (Iturralde-Vinent, 2001). The fossilized resin of Dominican amber has a leguminous origin, with the *Hymenaea protera* tree being the most probable source of this resin (Poinar, 1991, Iturralde-Vinent and MacPhee, 1996).

Dominican amber contains a wide spectrum of inclusions, ranging from small invertebrates to vertebrates (e.g Anolis lizards: de Queiroz *et al*., 1998; Sherratt *et al*., 2015; *Palaeoplethodon* salamander: Poinar and Wake, 2015). An updated version of the insect in the Dominican amber includes more than 1000 species known at the moment (Perez-Gelabert, 2008); most of the described species belong to Diptera, followed by Hymenoptera and Coleoptera (Perez-Gelabert, 2008). The majority of inclusions described from Dominican amber belong to extinct species, but few insect taxa (Coleoptera: *Micromalthus debilis*, Strepsiptera: *Bohartilla kinzelbachi*, Trichoptera: *Ochrotrichia aldama*) may represent extant species (Hörnschemeyer *et al*., 2010).

Only, two aquatic beetles species have been described for amber from Dominican Republic: *Crenitulus paleodominicus* (originally described in *Anacaena*) (Hydrophilidae, Fikáček and Engel, 2011) and *Copelatus predaveterus* (Dytiscidae, Miller and Balke, 2003).

2.3 Molecular phylogenies and origin of Caribbean fauna

The relatively good knowledge of the geological history of the region, the high amount of endemic species as well as the presence of the *in situ* fossil deposits makes the Caribbean region ideal for case studies of the species assembly (vicariance *versus* long-distance dispersal), island biogeography and adaptive radiations. These studies are possible due to molecular dated phylogenies which become available for more and more Caribbean clades, and increasingly employed e.g. to test for correspondence between the ages of island endemics and/or to reconstruct past geological events (Fritsch, 2003; Hower and Hedges, 2003; Dávalos, 2004; Gifford *et al*., 2004; Roca *et al*., 2004; Glor *et al*., 2005; Hedges and Heinicke, 2007; Weiss and Hedges, 2007; Lavin and Beyra Matos, 2008; Doadrio *et al.,* 2009; Crews and Gillespie, 2010; Oneal *et al*., 2010; Rodríguez *et al*., 2010).

The ancient origin of Caribbean biota corresponding to the vicariance model by Rosen (1975, 1985) was originally proposed for solenodons and *Cricosaura* lizards (e.g., Hedges, 2006), but only the *Cricosaura* clade has been confirmed to be old enough (Noonan *et al*., 2013; Sato *et al*., 2016). Under this scenario, the ancient Antillean fauna would have had to survive the collision of the large bolide with the Earth at the Yucatan Peninsula 65 million years ago (Ma) and its consequences which exterminated most of the Cretaceous terrestrial life forms in the Caribbean region (Iturralde-Vinent, 1982; Iturralde-Vinent and MacPhee, 1999; Tada *et al*., 2003).

Long distance over-water dispersal from the surrounding landmasses during the Cenozoic (e.g. Hedges *et al*., 1992; Hedges, 2006) was documented as the way by which Caribbean islands were colonized e.g. by solenodons (Sato *et al*., 2016) and urocoptid snails (Uit de Weerd *et al*., 2016).

The colonization of Greater Antilles from northern South America via GAARlandia land bridge during the Eocene was suggested e.g. for mammals (Dávalo, 2004), *Micrathena*, *Selenops* and *Spintharus* spiders (Crews and Gillespie, 2010; McHung *et al*., 2014; Dziki *et al*., 2015), *Peltophryne* toads (Alonso *et al*., 2012), heroine cichlid fishes (Říčan *et al*., 2013), *Calisto* butterfly (Matos-Maraví *et al*., 2016), and poecilid fishes (Weaver *et al*., 2016).

Our study on the hydrophilid genus *Phaenonotum* (**Chapter 1**) illustrates that the origin of some groups may be actually the combination of the above scenarios.

The Caribbean region did not stand only as a sink of colonizations from the continent, but several recent studies revealed that it was also the source for colonizations of Central America during the Miocene to Pleistocene. Zhang *et al*. (2017) revealed the Caribbean to Central America colonizations for *Exophthalmus* weevils, Lewis *et al*. (2015) for *Heracleidos* butterflies, and several examples are also known for the vertebrates (Ricklefs and Bermingham, 2008). Central America was an archipelago for most of this time, and the colonizations of Central America from the Caribbean hence were island-to-island events, not the island-to-continent ones.

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ATTACHED PUBLICATIONS AND A MANUSCRIPTS

Part 1: Evolutionary history of selected hydrophilid beetles of Greater Antilles

CHAPTER 1

Deler-Hernández, A., Sýkora, V., Seidel, M., Cala-Riquelme F. and Fikáček, M. 2017. Multiple origin of the *Phaenonotum* beetles in the Greater Antilles (Coleoptera: Hydrophilidae): phylogeny, biogeography and systematics (in press)

Zoological Journal of the Linnean Society

Multiple origin of the Phaenonotum beetles in the Greater Antilles (Coleoptera: Hydrophilidae): phylogeny, biogeography and systematics

Journal:	*Zoological Journal of the Linnean Society*
Manuscript ID	ZOJ-02-2017-2898.R1
Manuscript Type:	Original Article
Keywords:	Caribbean < Geography, biogeography < Geography, phylogeny < Phylogenetics, endemic < Geography, new species < Taxonomy
Abstract:	The systematics and the phylogenetic position of the Caribbean representatives of Phaenonotum Sharp (Coleoptera: Hydrophilidae) are investigated in order to understand the composition of the Caribbean fauna and its origin. Phylogenetic analysis based on mitochondrial and nuclear genes has revealed the Caribbean species to be situated in three deeply nested clades, inferring multiple colonization of Caribbean islands from the continent. Time-tree analysis and BioGeoBEARS analyses of ancestral ranges estimated the oldest clade, consisting of wingless single-island endemics of Cuba (P. delgadoi), Jamaica (P. ondreji sp. nov.) and Hispaniola (P. laterale sp. nov.), to have diverged ca. 46.6 Ma from the South American ancestor and subsequently colonizing the Caribbean most likely via the GAARlandia land bridge connecting South America with Greater Antilles. The remaining three Caribbean species, including the Puerto Rican endemic, P. borinquenum sp. nov., are of more recent (Miocene to Pliocene) origin and colonized the Greater Antilles by over-water dispersal. All the Caribbean species are illustrated and diagnosed and three new species are described. The genus Phaenonotum, excluding P. caribense Archangelsky, is confirmed as a monophylum. We demonstrate that species-level taxonomy of Phaenonotum is difficult to solve by morphology alone, and ideally requires the combination of morphology and molecular markers.

Multiple origin of the *Phaenonotum* beetles in the Greater Antilles (Coleoptera: Hydrophilidae): phylogeny, biogeography and systematics

ALBERT DELER-HERNÁNDEZ[1]*, VÍT SÝKORA[1], MATTHIAS SEIDEL[1,2], FRANKLYN CALA-RIQUELME[3,4], and MARTIN FIKÁČEK [1,2]

1 Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44, Praha 2, Czech Republic.
2 Department of Entomology, National Museum, Cirkusová 1740, Praha 9 – Horní Počernice, CZ-193 00, Czech Republic.
3 Programa de postgrado, Departamento de Biología, Universidad Nacional de Colombia, Bogotá, Colombia.
4 Instituto de Ciencias Naturales, Universidad Nacional de Colombia, Bogotá, Colombia.
**Corresponding author. E-mail: adeler1982@gmail.com*

The systematics and the phylogenetic position of the Caribbean representatives of *Phaenonotum* Sharp (Coleoptera: Hydrophilidae) are investigated in order to understand the composition of the Caribbean fauna and its origin. Phylogenetic analysis based on mitochondrial and nuclear genes has revealed the Caribbean species to be situated in three deeply nested clades, inferring multiple colonization of Caribbean islands from the continent. Time-tree analysis and BioGeoBEARS analyses of ancestral ranges estimated the oldest clade, consisting of wingless single-island endemics of Cuba (*P. delgadoi*), Jamaica (*P. ondreji* **sp. nov.**) and Hispaniola (*P. laterale* **sp. nov.**), to have diverged ca. 46.6 Ma from the South American ancestor and subsequently colonizing the Caribbean most likely via the GAARlandia land bridge connecting South America with Greater Antilles. The remaining three Caribbean species, including the Puerto Rican endemic, *P. borinquenum* **sp. nov.**, are of more recent (Miocene to Pliocene) origin and colonized the Greater Antilles by over-water dispersal. All the Caribbean species are illustrated and diagnosed and three new species are described. The genus *Phaenonotum*, excluding *P. caribense* Archangelsky, is confirmed as a monophylum. We demonstrate that species-level taxonomy of *Phaenonotum* is difficult to solve by morphology alone, and ideally requires the combination of morphology and molecular markers.

KEYWORDS: Caribbean biogeography, GAARlandia, phylogeny, molecular dating, ancestral range analysis, endemics, taxon cycle, systematics, new species.

INTRODUCTION

The Greater Antilles (i.e. islands of Cuba, Hispaniola, Jamaica and Puerto Rico in the Caribbean Region) are known for their high species diversity and endemism, and are considered one of the world biodiversity hotspots (Mittermeier *et al*., 2005). The complex geological history of the region, characterized by emergence and sinking of particular islands and changes in their interconnections, has played a crucial role in generating these diverse and endemic faunas. The Antilles first emerged as the Antillean volcanic arc system in the Cretaceous in the gap between North and South America and repeatedly emerged and submerged while moving eastward into the Proto-Caribbean Sea later during the Cenozoic. The uplift of the core Greater Antilles started during the Middle Eocene and reached its maximum at the Eocene-Oligocene boundary. The area of the islands was reduced during the higher sea-level period in the Late Oligocene to Middle Miocene but the islands remained emerged, possibly with exception of parts of today's Jamaica and Hispaniola which are believed to have emerged permanently only during the Neogene (e.g. Iturralde-Vinent & MacPhee, 1999; Bartolini *et al*., 2003; Iturralde-Vinent, 2006).

The origin of the present day terrestrial and freshwater biota of the archipelago has been explained by three alternative scenarios. The first scenario assumes that ancestors of extant lineages colonized the Antillean volcanic arc system from the southern North America during the Late Cretaceous and Early Paleogene and survived there as relicts to the present (e.g. Rosen, 1975, 1985). This scenario was originally proposed for solenodons and *Cricosaura* lizards (e.g., Hedges, 2006), but only the *Cricosaura* clade has been confirmed to be old enough (Noonan *et al*., 2013; Sato *et al*., 2016). Under this scenario, the ancient Antillean fauna would have had to survive the collision of the large bolide with the Earth at the Yucatan Peninsula 65 million years ago (Ma) and its consequences which exterminated most of the Cretaceous terrestrial life forms in the Caribbean region (Iturralde-Vinent, 1982; Iturralde-Vinent & MacPhee, 1999; Tada *et al*., 2003). The second scenario suggests long distance over-water dispersal from the surrounding landmasses during the Cenozoic (e.g. Hedges *et al*., 1992; Hedges, 2006) which was recently documented e.g. for solenodons (Sato *et al*., 2016) and urocoptid snails (Uit de Weerd *et al*., 2016). In contrast to over-water dispersal, Iturralde-Vinent & MacPhee (1999) introduced the GAARlandia theory (GAAR = Greater Antilles + Aves Ridge) assuming a land bridge connection of the Greater Antilles with northern South America resulting from a tectonic uplift and sea-level fall at the Eocene-Oligocene boundary. This third scenario assumes that numerous terrestrial and freshwater organisms colonized the archipelago during a relatively short time period (cca 35–33 Ma), as was suggested e.g. for *Peltophryne* toads (Alonso *et al*., 2012), poecilid fishes (Weaver *et al.*, 2016) or heroine cichlid fishes (Říčan *et al.*, 2013). The GAARlandia hypothesis is still a subject of debate: Hedges (2006) and Ali (2012) point to the absence of geological evidence and lack of any clear signal in vertebrate phylogenetic analyses, and hypothesize GAARlandia as no more than a chain of widely spaced islands situated between northern South America and Greater Antilles.

The above scenarios have been tested by numerous phylogenetic studies, which make the Caribbean one of the model regions for understanding historical island biogeography. Paradoxically, the vast majority of these studies are focused on vertebrates (i.e. relatively species-poor clades with rather young modern crown-groups; e.g. Roelants *et al.*, 2007; Claramunt & Cracraft, 2015; Foley *et al.*, 2016) and plants (i.e. group with resistant seeds facilitating long distance dispersal; e.g., Sanmartín & Ronquist, 2004). Little is known about the origin and biogeography of Caribbean insects and other terrestrial or freshwater arthropods, i.e. species-rich old groups which may provide numerous independent examples of evolutionary histories corroborating or contradicting the above biogeographic scenarios. Dated molecular phylogenetic studies have been performed only for selected groups of Caribbean butterflies (Wahlberg, 2006; Wahlberg & Freitas, 2007; Matos-Maraví *et al.*, 2014; Lewis *et al.*, 2015), wasps (Ceccarelli & Zaldívar-Riverón, 2013; Rodriguez *et al.*, 2015) and arachnids (e.g., Crews & Gillespie, 2010; Zhang & Maddison, 2013; McHugh *et al.*, 2014; Dziki *et al.*, 2015; Esposito *et al.*, 2015; Agnarsson *et al.*, 2016). Only a single very recent study is available for Caribbean beetles (Coleoptera), i.e. the most speciose and most intensively studied insect group (Zhang *et al.*, 2017).

The beetle family Hydrophilidae comprises *ca.* 3350 species inhabiting both aquatic and terrestrial habitats, and distributed worldwide (Hansen, 1999; Short & Fikáček, 2013; Lawrence & Slipinski, 2014; Seidel *et al.*, 2016). In the Caribbean, the family is represented by 16 genera, eleven of which contain at least one species endemic to the Greater Antilles (Hansen, 1999; Short, 2004; Spangler & Short, 2008; Deler-Hernández *et al.*, 2013a, b, 2014; Arriaga-Varela et al., 2017). Among them, the genus *Phaenonotum* Sharp, 1882 contains 18 described and many undescribed species in the Neotropical and southern Nearctic Regions, and one East-African species (Hansen, 1999), all inhabiting semiaquatic or terrestrial habitats. A single widespread species, *Phaenonotum exstriatum* (Say, 1835), was originally recorded from the Caribbean, until the surprising discovery of *P. delgadoi* Deler-Hernández *et al.*, 2013 endemic to eastern Cuba (Deler-Hernández *et al.*, 2013a). Our subsequent field work revealed additional candidates for endemic species in Jamaica, Hispaniola and Puerto Rico, indicating that *Phaenonotum* possibly underwent an island radiation in the Greater Antilles. In this paper, we analyze the systematics and evolutionary history of the Caribbean *Phaenonotum*, in order to understand to what extent it corroborates the aforementioned scenarios explaining the origin of Caribbean faunas.

MATERIAL AND METHODS

DNA SEQUENCES

The molecular study is based on *Phaenonotum* specimens collected in all main islands of Greater Antilles during our field work in 2010–2016. Specimens were collected either manually from aquatic habitats, rotten plant debris and at light, or by sifting of forest leaf litter and extracting the beetles from sifting samples using Berlese and/or Winkler funnels. All specimens were collected in 96% ethanol and stored at -20°C once they got into the lab. To understand the origin of the Caribbean fauna, we included *Phaenonotum* species from Costa Rica, Guatemala, Venezuela, Suriname, Guyana, Ecuador, Peru and the USA, i.e. all continental DNA-grade material available to us. To test the monophyly of *Phaenonotum*, we also included all available DNA-grade specimens of the genera *Phaenostoma* Orchymont, 1937 and *Lachnodacnum* Orchymont, 1937 which form a strongly supported clade with *Phaenonotum* (Sýkora *et al.*, unpubl. data). Outgroup taxa consist of selected genera representing the main clades of the tribe Coelostomatini (*Cyclotypus* Sharp, 1882, *Dactylosternum* Wollaston, 1854 and *Coelostoma* Brullé, 1835) and the sphaeridiine tribes Protosternini (*Sphaerocetum* Fikáček, 2010) and Sphaeridiini (*Sphaeridium* Fabricius, 1775). The majority of the specimens used for this study was newly extracted and sequenced, but in a few cases we adopted the sequences published by Short & Fikáček (2013).

DNA was extracted from complete specimens cut into two parts between prothorax and mesothorax, using the commercial DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Voucher specimens and DNA extractions are kept in the collection of the Department of Entomology of the National Museum, Prague (NMPC).

Our gene selection corresponds with those used in previous phylogenetic studies of the family Hydrophilidae (e.g., Short & Fikáček, 2013) and contains three mitochondrial genes (3´ end cytochrome c oxidase I, cytochrome c oxidase II and 16S rRNA), and two nuclear genes (18S rRNA and 28S rRNA); sequences of 18S rRNA were amplified in two parts (3´ end and 5´ end) and subsequently combined; for primers and PCR conditions, see Supplementary File 1. 10μl of each PCR product were purified by adding 1.0μl Exonuclease 1 (Exo1 [20 U/μl]) (ThermoFisherScientific) and 2.0μl Thermosensitive Alkaline Phosphatase (FastAP [1 U/μl]) (ThermoFisherScientific) and incubating the mixture for 15 min at 37°C followed by a 15 min inactivation step at 80°C. Sanger sequencing was performed by BIOCEV (Vestec, Czech

Republic). DNA sequences were assembled and edited using Geneious 7.1.9 and submitted to GenBank under the accession numbers shown in Supplementary File 1. In total, we obtained sequences for 36 terminal taxa (25 specimens of *Phaenonotum*, 4 of *Phaenostoma*, 1 of *Lachnodacnum* and 6 outgroup taxa).

PHYLOGENETIC ANALYSES

Our molecular dataset includes sequences of three ribosomal genes which are non-trivial for alignment especially in case of distantly related taxa (e.g., Bocák *et al*., 2014), and a rather large proportion of missing data for *cox2* and *16S* genes which we failed to amplify despite repeated effort. To test the impact of these problems we compared the results of analyses of three different datasets:

(1) 35 taxa dataset: contains all *Phaenonotum*+*Phaenostoma*+*Lachnodacnum* specimens and all outgroups;

(2) 30 taxa dataset: contains all *Phaenonotum*+*Phaenostoma*+*Lachnodacnum* specimens, but is only rooted with closely related *Cyclotypus* (eliminates the problem with alignment of distantly related taxa);

(3) 22 taxa dataset: contains only *Phaenonotum*+*Phaenostoma*+*Lachnodacnum* with 3 and more genes, and is rooted by *Cyclotypus* (eliminates the problem with alignment of non-related taxa and the effect of missing *cox2* and *16S* data).

Specimens MF1736 and MF1741 from Venezuela are genetically very close (2.1 % in *cox1*), corresponding to intraspecific variation in *cox1* found in *Phaenonotum exstriatum* (0.0–3.1 %), and were hence considered as conspecific; they were combined for our analyses and included in all three datasets.

Sequences were aligned with MAFFT algorithm as implemented in Geneious 7.1.9 software, using the default settings (score matrix = 200PAM / k = 2, gap open penalty = 1.53, offset value = 0.123). The final alignment has the total length of 4827 bp, consisting of the following gene fragments: *cox1* (778 bp), *cox2* (694 bp), 16S (530 bp), 18S (1798 bp) and 28S (1027 bp). The dataset was divided into 9 partitions (by genes, plus *cox1* and *cox2* were both divided by codon positions). Each dataset was analyzed using the Bayesian inference and maximum likelihood. Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist *et al*., 2012) using four chains of 25,000,000 generations and sampling the chain every 1000 generations. We sampled across the substitution model space in the Bayesian MCMC analysis itself (Huelsenbeck *et al*., 2004; Ronquist *et al*., 2012) instead of prior testing for an appropriate model for each of nine partitions.

Results were examined in Tracer v.1.6 (Rambaut *et al.*, 2014) to check for the proper effective sample size (ESS), proper mixing of chains, and reaching the stationary phase; 25% burn-in was used for construction of the final consensus tree. Maximum likelihood analyses were performed using RAxML 8.0 (Stamatakis, 2014) with a GTR substitution model and 1,000 bootstrap replicates. Resulting trees were visualized in FigTree 1.4.3 (Rambaut, 2012).

DIVERGENCE DATING

We performed a divergence dating analysis using full dataset of 35 taxa in BEAST 2.4.5 (Bouckaert *et al*,. 2014) with fixed tree topology as revealed by the Bayesian analysis of the 35 taxa dataset; the internal topology of *P. exstriatum* was manipulated to be bifurcate in agreement with results of the ML analyses of 35 and 30 taxa. We divided the dataset into five partitions corresponding to each gene (*cox1*, *cox2*, 16S rRNA, 18S rRNA and 28S rRNA) and used PartitionFinder v1.1.1 (Lanfear *et al*,. 2012) to estimate the evolutionary model that best fitted the data for each partition separately. The following substitution models were selected using Bayesian Information Criterion (BIC) and used for particular genes in the divergence dating analysis: GTR+I+G for *cox1*, *cox2* and 28S; GTR+G for 16S; and TrNef+I+G for 18S.

Due to the absence of fossils belonging to the tribe Coelostomatini, we used the combination of a rate dating and node dating constraining the age of the most recent common ancestor (MRCA) of the Coelostomatini. Molecular clock models were linked into two partitions (mtDNA: *cox1*, *cox2*, 16S rRNA; nDNA: 18S rRNA, 28S rRNA) and substitution rates were set to 0.0133 (mtDNA) and 0.0017 (nDNA) substitutions per million years following Papadopoulou *et al*. (2010). The age of the MRCA of the Coelostomatini was constrained to 152.5 Ma (95% HPD (CI): 134–170 Ma), following the results of the time tree analysis of the whole family Hydrophilidae performed by Bloom *et al*. (2014) and based on a wide spectrum of fossil calibrations (for list of fossils used see Supplementary material in Bloom *et al*. 2014). We performed a Bayes Factor comparison to test alternative clock models (non-clock, strict clock, relaxed clock) using a stepping-stone method (Xie *et al.*, 2011) as implemented in MrBayes 3.2.6 (Ronquist *et al.*, 2012) (see Supplementary File 1).

The birth-death model was used for the tree prior as this model is commonly used to model speciations and extinctions in inferring phylogenies using Bayesian methods, thus at any point in time every lineage can undergo speciation at rate λ or go extinct at rate μ (Stadler, 2009). Due to problems with convergence of parameters, we performed two separate runs each with MCMC chain length set to 500 million generations and sampling frequency of every 25000 generations. We combined both runs using LogCombiner 2.4.0 and applied a 10% burn-in fraction after checking the convergence of parameters in TRACER 1.6 (Rambaut *et al*., 2014). The resulting tree was visualized in FigTree 1.4.3 (Rambaut, 2012).

HISTORICAL BIOGEOGRAPHY RECONSTRUCTION

For historical biogeography estimation, we used the time tree resulting from the divergence dating analysis as the input tree, from which we excluded all outgroups except *Cyclotypus*. Four specimens of *P. exstriatum* were included into the analysis, each representing a geographically distinct population. An alternative set of analyses with a single terminal for *P. exstriatum* was also performed.

The distribution ranges were divided into the following eight areas: A – North America incl. Mexico; B – Central America; C – northern South America (corresponding to Guiana Shield + northwestern Venezuela and northern Colombia, i.e. regions on the Caribbean coast and in direct contact with Central America); D – South America (remaining continent south of the former region); E – Cuba; F – Jamaica; G – Hispaniola; H – Puerto Rico. As no inter-island divergences are present in our phylogeny, we coded the Caribbean islands in their current shape, without considering their historical composition of multiple paleoislands. Islands of the Lesser Antilles were not considered as separate areas, as only the youngest and widespread *P. exstriatum* is known to occur in some of them; instead, presence/absence of the Lesser Antilles was considered when setting dispersal multipliers for the time-stratified analysis (see below). The distribution of terminal taxa is based on examined specimens in all undescribed species; distribution of *P. exstriatum* and *P. laevicolle* complex on the continent follows Smetana (1978); Hansen (1999); Oliva *et al*. (2002) and González-Rodríguez *et al.* (in press).

We carried out the historical biogeography analyses in the R package BioGeoBEARS (Matzke, 2014) in order to estimate the timing of *Phaenonotum* colonization of the Greater Antilles archipelago. This package contains three models implemented in a maximum likelihood framework: DEC model (Ree & Smith, 2008), DIVALIKE model (likelihood version of the DIVA model: Ronquist, 1998) and BAYAREALIKE model (likelihood version of BayArea model: Landis *et al*., 2013). Moreover, each model is available in its original version and with an additional parameter *j* representing the founder event, i.e. speciation following long distance dispersal; six different models are hence available in total.

We conducted non-time-stratified and time-stratified analyses to estimate ancestral area distribution on given nodes, each with all six models. Unconstrained non-stratified analyses were done using default parameter values. For time-stratified analyses, time periods were defined as follows, to reflect different paleogeography of the area in each period (Iturralde-Vinent, 2006; O'Dea *et al*., 2016): (1) 0–2.8 Ma: from present to the closing of Isthmus of Panama; (2) 2.8–9 Ma: Greater Antilles emerged, the chain of islands (Lesser Antilles) present between Greater Antilles and South America; (3) 9–32 Ma: Greater Antilles significantly reduced in area and widely separated from South America by deep sea; (4) 32–38 Ma: Greater Antilles connected to northern South America by GAARlandia land bridge; (5) 38–55 Ma: Greater Antilles started to form and were well-separated from surrounding continents; (6) 55–110 Ma: prior to the formation of the Greater Antilles. Dispersal probabilities were set to reflect the paleogeography as follows: 0.8 for adjacent continental areas; 0.5 for non-adjacent continental areas; 0.2 for adjacent islands (or island-continent) separated by less than 200 km of sea; 0.05 for connection by island chain (e.g. Lesser Antilles) or intermediate island (e.g. Hispaniola between Cuba and Puerto Rico); 0.001 for long distance dispersal (areas separated by more than 200 km of sea); 0.000001 when dispersal was not possible (i.e. when the area was submerged; we followed the BioGeoBEARS manual in setting extremely low rather than zero probabilities in such cases). Areas not present during particular time slice (e.g. Cuba in the Late Cretaceous) were not allowed for the reconstruction using the Areas Allowed matrix. Models of both non-time-stratified and time-stratified analyses were compared using likelihood values and Akaike information criterion corrected for small sample sizes (AICc) (Matzke, 2013). All input files for the biogeography analyses are available in Supplementary File 2.

MORPHOLOGY AND TAXONOMY

Examined specimens are deposited in the following collections:

BMNH	Natural History Museum, London, United Kingdom (M.V.L. Barclay);
CMN	Canadian Museum of Nature, Ottawa, Canada (R. Anderson);
DZRJ	Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Instituto de Biologia, Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil (B. Clarkson);
MCZ	Museum of Comparative Zoology, Cambridge, USA (P. Perkins);
MNHNSD	Museo Nacional de Historia Natural, Santo Domingo, Dominican Republic (C. Suriel);
NHMW	Naturhistorisches Museum, Wien, Austria (M. Jäch, A. Komarek);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček);
SBNM	Santa Barbara Museum of Natural History, California, USA (M. L. Gimmel);

SBPC	Stewart Peck Personal Collection, Ottawa, Canada;
UPRM	University of Puerto Rico, Mayagüez, Puerto Rico (A. Segarra);
ZMHB	Museum für Naturkunde der Humboldt-Universität, Berlin, Germany (J. Frisch, B. Jäger).

Habitus photographs were taken using Canon EOS 550D digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS5 and CorelDRAW Home & Student X8. Photographs of genitalia were taken using Canon EOS 1100D digital camera attached to Olympus BX41 compound microscope and subsequently combined in Helicon Focus software. SEM micrographs of the holotype of the new species were taken using a Hitachi S-3700N environmental electron microscope at the Department of Paleontology, National Museum in Prague. General morphological terminology follows Smetana (1978), Archangelsky (1989) and Hansen (1991). All Caribbean species were compared with type specimens of the Central and South American species deposited in the Natural History Museum, London (Sharp collection; see Deler-Hernández & Fikáček, 2016) and in the Institut Royal des Sciences Naturelles de Belgique, Brussels (Orchymont collection), and with unidentified material from Mexico, Costa Rica, Ecuador and Peru deposited in National Museum, Prague, in order to reveal whether or not they might be conspecific with continental species. Complete data of all examined specimens in DarwinCore-formatted Excel spreadsheet is available in Supplementary File 3 and in the dataset submitted to Zenodo.

DEPOSITORY OF PRIMARY DATA

All primary data and results of all analyses were uploaded as a .zip file into the Zenodo depository (https://zenodo.org/) under doi XXXX [will be added once accepted for publication]. Parts of the data were also uploaded to specialized archives as specified below.

RESULTS

PHYLOGENETIC ANALYSES

All analyses performed revealed a strongly supported clade consisting of Neotropical genera *Phaenostoma*, *Phaenonotum* and *Lachnodacnum*, but do not support the current status of the genera. *Phaenonotum caribense* Archangelsky, 1989 is revealed as not related to the remaining *Phaenonotum* species and is revealed as a sister-group to the rest of the Neotropical clade in most analyses (strongly supported in Bayesian analysis with 30 and 22 taxa, weakly supported in maximum likelihood analyses); in the Bayesian analysis with 35 taxa it is revealed as sister to *Phaenostoma kontax* Gustafson & Short, 2010 and *Lachnodacnum*, with moderate support). When *P. caribense* is excluded, the genus *Phaenonotum* (= core *Phaenonotum* hereafter) is revealed as strongly supported monophylum in all analyses. The genus *Phaenostoma* is revealed as sister to the core *Phaenonotum* in most analyses (moderately supported in Bayesian analysis except that with 35 taxa, weakly supported in all maximum likelihood analyses), with *Lachnodacnum* always nested within *Phaenostoma*. In the Bayesian analysis with 35 taxa, *Phaenonotum caribense* + *Phaenostoma kontax* + *Lachnodacnum* clade is revealed as sister to the *Phaenostoma posticatum* + core *Phaenonotum*.

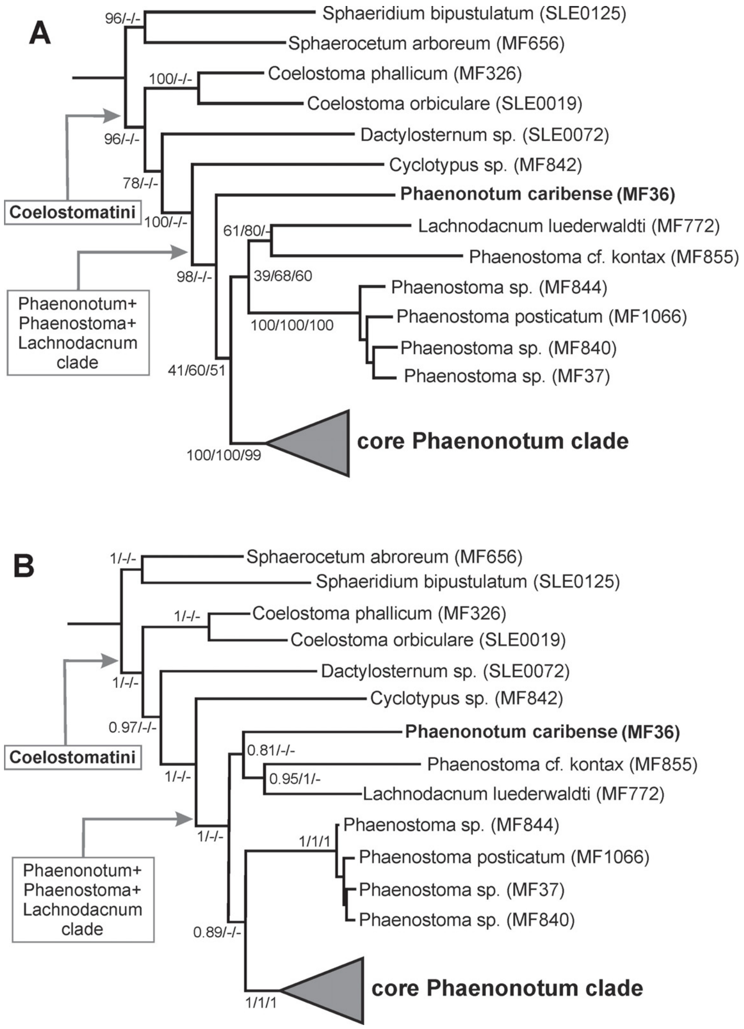

Figure 1. The phylogenetic position and polyphyly of *Phaenonotum* as it was revealed in our maximum likelihood (A) and Bayesian analyses (B). Branch support are indicated for analyses with 35, 30 and 22 terminal taxa, respectively.

Within the core *Phaenonotum* clade, all six analyses revealed the same five strongly to moderately supported clades and two undescribed species (MF1739 from Guatemala and MF861 from Peru) not closely related to other species and forming deeply divergent separate clades. The relationships between these seven principal clades are the same in all analyses performed, with exception of MF1739 which is revealed as early branching in Bayesian analyses and more deeply nested in maximum likelihood analyses. All Caribbean species are part of the strongly supported deeply nested monophylum containing three of the principal clades: the clade of the endemic Cuban, Jamaican and Hispaniolan species, the clade containing Puerto Rican endemic *P. borinquenum* sp. nov., *P. laevicolle* complex and an undescribed species from Guyana (MF1061), and the clade consisting of *P. exstriatum* and undescribed species from Venezuela and Suriname. Within the Caribbean endemic clade, the internal topology is the same in all analyses, with the Cuban *P. delgadoi* diverging first, and the Jamaican *P. ondreji* sp. nov. and Hispaniolan *P. laterale* sp. nov. as sister taxa. The internal topology of the *P.borinquenum+laevicolle* clade varies the most among analyses, with either *P. borinquenum* or the undescribed species from Guyana (MF1061) revealed as the earliest diverging taxon. The internal topology of *P. exstriatum* clade differs between Bayesian and maximum likelihood analyses, with Puerto Rican (MF1728) and Costa Rican (MF1738) revealed as sister taxa in maximum likelihood ones; the Cuban specimen (MF654) is always revealed as sister to the one from USA: Delaware (MF1063).

DIVERGENCE DATING

The relaxed clock model was identified as the best fitting clock model based on the Bayes Factor comparison (see Supplementary File 1). The core *Phaenonotum* clade was estimated to originate during the middle Cretaceous (ca. 102 Ma), with diversification of modern clades starting in Late Cretaceous (ca. 83 Ma). The clade composed of the endemic Cuban, Jamaican and Hispaniolan *Phaenonotum* diverged during the Eocene (ca. 47 Ma), with *P. delgadoi* from Cuba diverging at about Eocene-Oligocene boundary (ca. 36 Ma), and *P. ondreji* (Jamaica) from *P. laterale* (Hispaniola) during the Oligocene (ca. 26.4 Ma). The Puerto Rican endemic *P. borinquenum* diverged in the early Miocene (ca. 19 Ma). The divergence of the Cuban and Venezuelan specimens of *P. laevicolle* complex was dated to the Pliocene-Pleistocene (ca. 47 Ma), the modern populations of the widespread *P. exstriatum* diverged at about Pliocene-Pleistocene boundary (ca. 2.4 Ma). Precise ages and 95% confidence intervals are listed in Table 1 for the principal *Phaenonotum* clades, and illustrated in Supplementary File 1 for all clades.

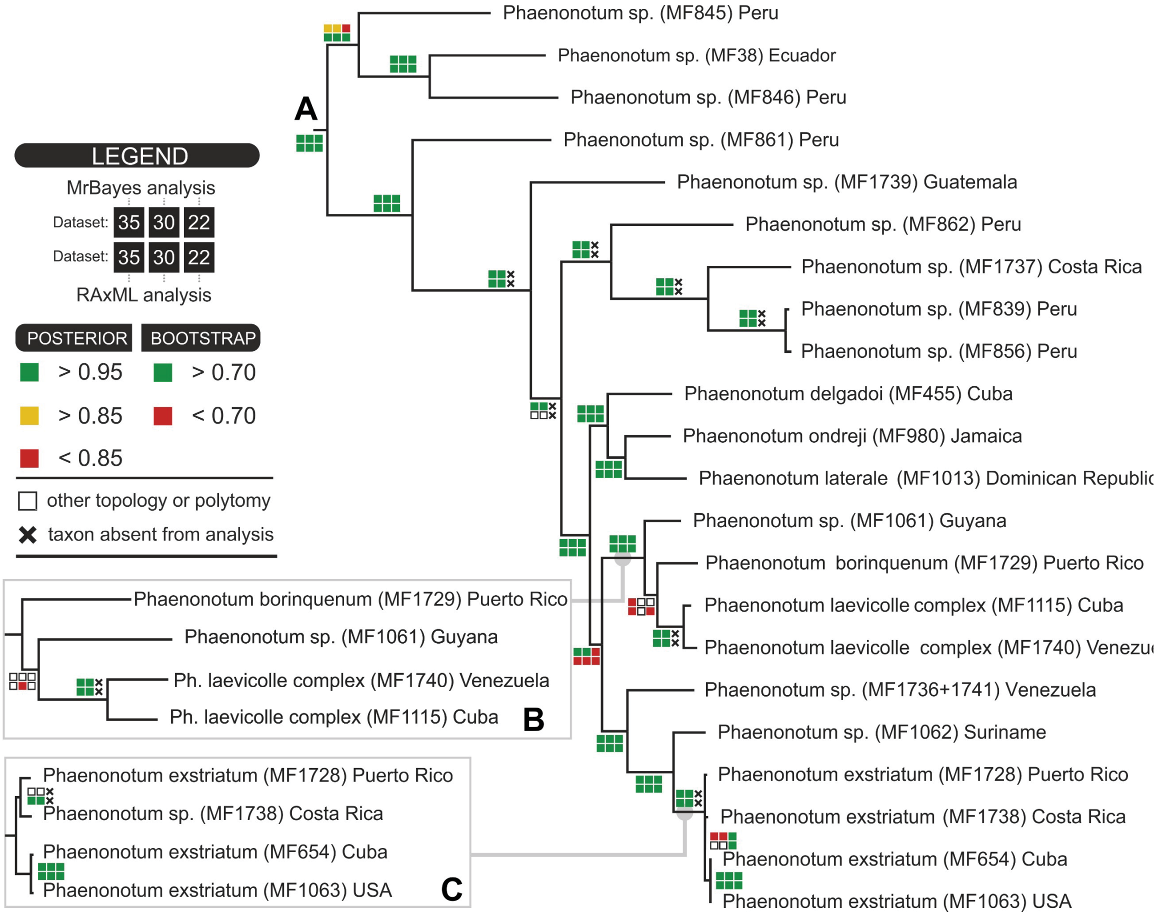

Figure 2. Summary of the internal topology of the core *Phaenonotum* clade revealed by Bayesian and maximum likelihood analyses of all three datasets (35 taxa, 30 taxa and 22 taxa, see Material and methods for details). (A) topology resulting from Bayesian inference of 35 taxa dataset, with presence and support of particular clades in all analyses mapped by color squares; (B) alternative topology of the *borinquenum–laevicolle* clade obtained from maximum likelihood analysis of the 30 taxa dataset; (C) alternative internal topology of *P. exstriatum* clade obtained from maximum likelihood analyses of 35 and 30 taxa datasets.

BIOGEOGRAPHY ANALYSES

Of the 6 models included in BioGeoBEARS, those implementing founder event (i.e. perapatric speciation, *j* parameter in the analyses) fit the data better both in time-stratified and non-time-stratified analyses than those not allowing for jump dispersal (Table 2). Analyses allowing the founder event resulted in identical ancestral area estimates in all nodes and corners in non-time-stratified analyses under all three basic models (DEC+J, DIVALIKE+J and BAYAREALIKE+J), and in identical estimates in time-stratified analyses with DEC+J and

Table 1. Divergence ages and their confidence intervals for major *Phaenonotum* clades incl. all Caribbean ones.

	Age (Ma)	**95% confidence interval (Ma)**
Phaenonotum stem	101.7	81.7–121.9
Phaenonotum crown	82.5	65.4–100.2
Caribbean *Phaenonotum* stem	46.6	35.6–58.1
P. delgadoi	35.7	25.5–47.2
P. ondreji and *P. laterale*	26.4	17.2–36.5
P. borinquenum	19.2	12.1–27.1
P. laevicolle from Cuba	4.7	2.2–7.9
P. exstriatum crown	2.4	1.3–3.7

Table 2. Comparison of the models used for non-time-stratified and time-stratified analyses of the dataset with multiple populations of *P. exstriatum*. Best performing model is marked by asterisk for each groups of analyses. LnL = log likelihood; n par = number of parameters in the analysis; d, e, j = parameres of the model (d = dispersal, e = extinction, j = founder event); AIC = Aikake information criterion; AICc = size-corrected AIC.

Non-time-constrained							
	LnL	**npar**	**d**	**e**	**j**	**AIC**	**AICc**
DEC	-91.91	2	0.0013	<0.0001	0	187.8	188.3
DEC+J*	-70.21	3	0.0004	<0.0001	0.046	146.4	147.3
DIVALIKE	-84.36	2	0.0016	<0.0001	0	172.7	173.2
DIVALIKE+J*	-69.8	3	0.0005	<0.0001	0.060	145.6	146.5
BAYAREALIKE	-125.4	2	0.0023	0.026	0	254.8	255.2
BAYAREALIKE+J	-70.56	3	0.0004	<0.0001	0.049	147.1	148
Time-constrained							
	LnL	**npar**	**d**	**e**	**j**	**AIC**	**AICc**
DEC	-104.9	2	0.016	0.0049	0	213.9	214.3
DEC+J	-98.94	3	0.011	0.0039	0.17	203.9	204.8
DIVALIKE	-100.2	2	0.017	0.0042	0	204.4	204.8
DIVALIKE+J*	-95.44	3	0.012	0.0034	0.099	196.9	197.8
BAYAREALIKE	-134.2	2	0.033	0.025	0	272.5	272.9
BAYAREALIKE+J	-101.8	3	0.0077	0.0066	0.24	209.5	210.5

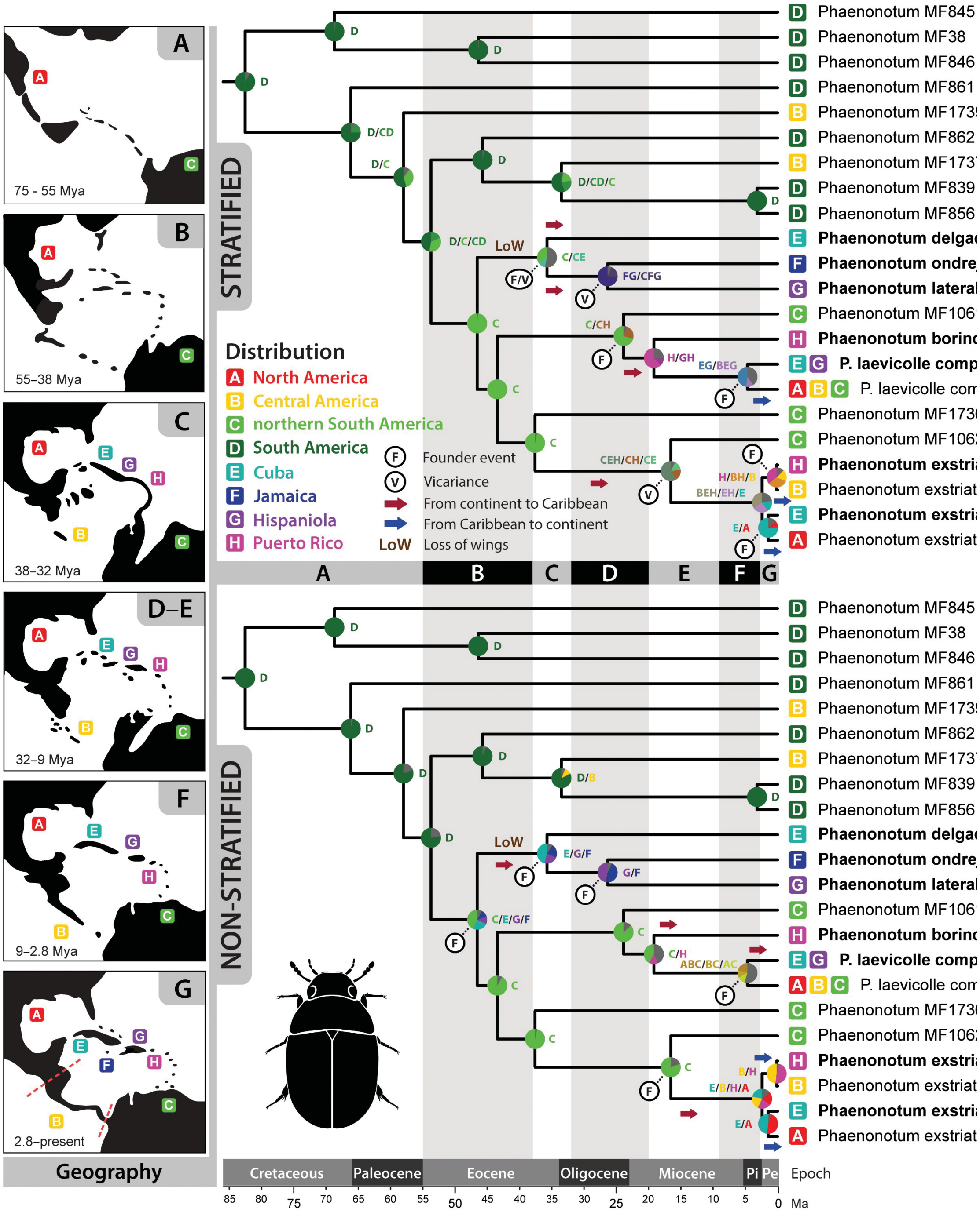

Figure 3. Results of the ancestral area estimation using time-stratified and non-time-stratified DIVALIKE+J model of BioGeoBEARS. Maps A–G show simplified continent and island positions in the respective time window used for the time-stratified analysis. Pie charts show two or three most probable ancestral areas for the respective node.

DIVALIKE+J models (time-stratified analysis with BAYAREALIKE+J model is nearly identical, only with slightly different scenario for *P. exstriatum* clade; *P. borinquenum* see Supplementary File 2 for details). Models not implementing the founder event resulted in very different ancestral area estimates for each model in non-time-stratified analyses, but in identical estimates under all three models in time-stratified ones. The number of continent-to-Caribbean and Caribbean-to-continent dispersal events (and source/sink regions on continents) for all three models in non-time-stratified and time-stratified analyses are shown in Table 3. Based on the AICc metrics, the best performing models were DIVALIKE+J for both non-time-stratified and time-stratified analyses.

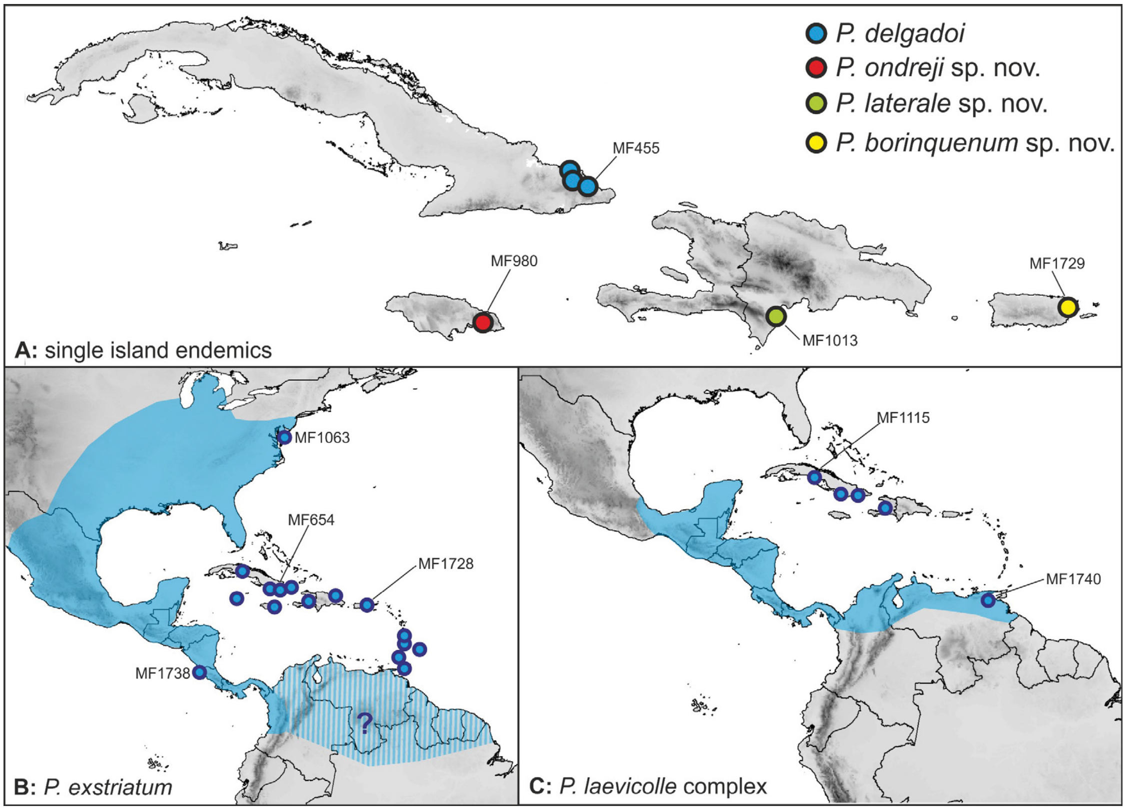

Figure 4. Current distribution of the genus *Phaenonotum* in the Caribbean. (A) single island endemics; (B) *P. exstriatum*; (C) *P. laevicolle* complex. The localities of sequenced specimens are shown and marked by the code of the respective DNA extract.

Most analyses infer South America as the ancestral range of the most recent common ancestor of *Phaenonotum*, with several independent colonizations of northern South America and Central America (through northern South America in time-constrained analyses). Most analyses (non-time-stratified allowing for founder event and all time-stratified except

BAYAREALIKE+J model) infer that ancestor(s) of *P. delgadoi*+*P. laterale*+*P. ondreji* colonized the Caribbean from northern South America: non-time-stratified models and time-stratified models not allowing for founder event infer a single colonization followed by series of founder events (non-time-stratified analyses) or combination of range expansions and vicariance events (time-stratified analyses); time-stratified analyses allowing for founder event infer two independent colonizations followed by vicariance event between Jamaica and Hispaniola. *Phaenonotum borinquenum* and Caribbean *P. laevicolle* were estimated to colonize the Caribbean independently by the non-time-stratified analysis. In contrast, time-stratified analysis favors earlier colonization of the Caribbean by the ancestor of these taxa, and back-colonization from the Caribbean to the continent in *P. laevicolle*. *Phaenonotum exstriatum* was revealed to be of South American origin in non-time-constrained analyses allowing for founder event and all time-constrained analyses; the ancestral range of its most recent common ancestor was either Caribbean or Caribbean + Central America, depending on the model implemented. The results of all analyses are available in the Supplementary File 2.

Table 3. Number of continent-to-Caribbean and Caribbean-to-continent colonization events (total number and number of colonizations from/to each continental area) inferred for the genus *Phaenonotum* in analyses including 4 populations of *P. exstriatum*. Abbreviations of source/sink areas: CA = Central America; NA = North America; nSA = northern South America. Time constraint: non-strat. = non-time-stratified analysis; time-strat. = time stratified analysis. Models best fitting the data are marked in grey.

Model	**Time constraints**	**Continent-to-Caribbean events**						**Caribbean-to-Continent events**		
		total	**NA**	**CA**	**nSA**	**CA +SA**	**NA+CA +SA**	**total**	**NA**	**CA**
DEC	non-strat	3×		1×	2×	–	–	1×	1×	–
DIVALIKE	non-strat	4×		–	4×	–	–	0×	–	–
BAYAREALIKE	non-strat	5×		2×	–	3×	–	1×	1×	–
DEC+J	non-strat	4×		–	3×	–	1×	2×	1×	1×
DIVALIKE+J	non-strat	4×		–	3×	–	1×	2×	1×	1×
BAYAREALIKE+J	non-strat	4×		–	3×	–	1×	2×	1×	1×
DEC	time-strat.	3×		–	3×	–	–	2×	1×	1×
DIVALIKE	time-strat.	3×		–	3×	–	–	2×	1×	1×
BAYAREALIKE	time-strat.	3×		–	3×	–	–	2×	1×	1×
DEC+J	time-strat.	4×		–	4×	–	–	3×	1×	2×
DIVALIKE+J	time-strat.	4×		–	4×	–	–	3×	1×	2×
BAYAREALIKE+J	time-strat.	5×	1×	–	4×	–	–	1×	–	1×

Alternative analyses with *P. exstriatum* as a single terminal resulted in estimates nearly identical to those treating the four populations of *P. exstriatum* separately, with DEC+J as the best-performing model for time-stratified and non-time-stratified analyses. Both analyses revealed northern South America as an ancestral range of the MRCA of *P. exstriatum* and its sister species (undescribed species from Suriname, voucher MF1062) Results of these analyses are available in Supplementary File 2.

SYSTEMATICS OF THE CARIBBEAN *PHAENONOTUM*

KEY TO THE CARIBBEAN *PHAENONOTUM*

1. Eyes small, interocular distance 5.0–6.0× the width of one eye in dorsal view (Figs 6A–C). Metaventrite very short, its total width 5.0–6.0× the length behind mesocoxae (Figs 7A–C). Species without hind wings (apterous). 2

– Eyes moderately large, interocular distance 3.5–4.0× the width of one eye in dorsal view (Figs 6D–F). Metaventrite moderately long, its total width 3.0–4.0× the length behind mesocoxae (Figs 7D–E). Species with fully developed hind wings (macropterous). 4

2. Metaventrite with pubescent cavities at sides of meso-metaventral process (Fig. 7A). Elytral suture strongly elevated posteriorly; elytral punctation very coarse (Fig. 5A). Pronotum with moderately coarse punctation, without microsculpture. Eastern Cuba. ***P. delgadoi* Deler-Hernández *et al.*, 2013**

– Metaventrite without pubescent cavities at sides of meso-metaventral process (Figs 7B–F). Elytral suture weakly elevated posteriorly; elytral punctation never extremely coarse. Pronotum either with distinct microsculpture, or with extremely fine (nearly invisible) punctation. 3

3. Body length 3.1–3.4 mm. Head and pronotum with mesh-like microsculpture and distinct punctation. Elytral punctation rather coarse, yellowish margins of elytra present in apical half only (Fig. 5B). Mesoventral process moderately wide, median portion of metaventrite with narrow highly elevated median keel (Fig. 7C). Aedeagus 0.6 mm long (Fig. 6H). Jamaica. ***P. ondreji* sp. nov.**

– Body length 2.5–2.7 mm. Head and pronotum without microsculpture, pronotal punctation extremely fine, almost invisible. Elytral punctation fine, whole lateral margins of elytra with yellowish stripe (Fig. 5C). Mesoventral process very narrow, metaventrite with slightly elevated median portion, without median keel. Aedeagus 0.35 mm long (Fig. 6I). Hispaniola. ***P. laterale*** **sp. nov.**

4. Median lobe of the aedeagus wide; gonopore large, situated in apical third of the median lobe. Bases of lateral struts of the median lobe weakly expanded laterally (Fig. 6L). Widespread in Greater Antilles. ***P. laevicolle*** **complex**

– Median lobe of the aedeagus narrow; gonopore small, situated subapically. Bases of lateral struts of the median lobe largely expanded laterally (Figs 6J, K). 5

5. Body length 3.3–4.0 mm. Bases of parameres meeting in single point (Fig. 6K). Widespread in Greater and Lesser Antilles. ***P. exstriatum*** **(Say, 1835)**

– Body length 3.2–3.3 mm. Bases of parameres widely joined together (Fig. 6J). Puerto Rico. ***P. borinquenum*** **sp. nov.**

PHAENONOTUM BORINQUENUM SP. NOV.

(Figs 5D; 6D, G; 7D)

Type locality: Puerto Rico, Naguabo, El Yunque National Forest, 18º16.1'N 65º48.1'W, 575 m.

Type material: Holotype: male (NMPC): ‘PUERTO RICO: Naguabo: El Yunque Nat. Forest, S part La Sabana recr area 5.25 Km N of Río Blanco above rd. PR191, 18º16.1'N 65º48.1'W, elevation 575 m, 21.vi.2016, A. Deler-Hernández lgt., PR05 / DNA isolation: MF1730, isolated by A. Deler-Hernández 2016, isolate deposited at Department of Entomology, National Museum in Prague [green printed]’ [Molecular voucher 1730]. Paratype: 1 female (NMPC; associated with the holotype by *cox1* sequence): Puerto Rico: Naguabo, El Yunque Nat. Forest, S part La Sabana recr area 5.6 Km N of Río Blanco at rd. PR191, 18º16.1'N 65º47.6'W, 510 m a.s.l., 21.vi.–2.vii.2016, Deler-Hernández, Fikáček, Seidel lgt., PR03 / DNA isolation: MF1729, isolated by A. Deler-Hernández 2016, isolate deposited at Department of Entomology, National Museum in Prague [green printed]’ [Molecular voucher 1729].

Description: Habitus as in Fig. 5D. Body size 3.2–3.3 mm (holotype: 3.3 mm). Body elongate oval, moderately convex, elytral suture not elevated. Dorsum black (dark brown in the teneral holotype), lateral margins of pronotum and elytra without distinct paler stripe; ventral surface dark brown to black; femora and tibiae black; antennae, maxillary palpi and tarsi yellowish. Head with sparse and moderately coarse punctation, without microsculpture (except posteriorly on frons); eyes moderately large, separated by 4.0× dorsal width of one eye (Fig. 6D). Pronotum

Figure 5. Habitus of the Caribbean *Phaenonotum* (dorsal and lateral views). (A–D) species endemic for Greater Antilles: (A) *P. delgadoi* Deler-Hernández *et al.,* 2013 from eastern Cuba; (B) *P. ondreji* sp. nov. from Jamaica; (C) *P. laterale* sp. nov. from southern Hispaniola; (D) *P. borinquenum* sp. nov. from Puerto Rico. (E–F) non-endemic species: (E) *P. exstriatum* (Say, 1835) from Cuba; (F) *Phaenonotum* sp. from the *P. laevicolle* complex from Cuba.

with sparse punctures slightly finer than on head, interstices without microsculpture. Elytral punctation strongly impressed, coarser than on pronotum and head; elytral interstices without microsculpture. Wings present, fully developed. Mesoventral elevation as wide as metaventral process posteriorly, narrowing anteriorly, with distinct anterior hood; metaventrite without pubescent pits on sides of metaventral process; metaventrite ca. 4.0× wider than its length posterior of mesocoxae; median moderately elevated part of metaventrite narrow throughout (Fig. 7D). Aedeagus 0.5 mm long (Fig. 6J). Median lobe rather widely triangular, ca. 1.8× longer along midline than wide; apex not reaching apices of parameres; gonopore moderately large, subapical; lateral struts projecting laterad. Parameres distinctly sinuate on lateral margin, slightly expanded subapically; widely meeting each other basally. Phallobase longer than wide.

Etymology: The species name is a Latinized adjective derived from the Spanish version of the indigenous Taíno name of the islands of Puerto Rico.

Diagnosis: *Phaenonotum borinquenum* may be distinguished from other Caribbean species by the combination of moderately large eyes, moderately long metaventrite, presence of wings and morphology of the aedeagus. It is extremely similar to *P. exstriatum* in its external morphology and genital morphology, and may be distinguished from it only by its smaller body size and widely meeting bases of parameres only. Despite the strong morphological similarity, it is not closely related to *P. exstriatum*.

Distribution: *Phaenonotum borinquenum* is only known from the eastern part of Puerto Rico (Fig. 4A).

PHAENONOTUM DELGADOI DELER-HERNÁNDEZ, CALA-RIQUELME & FIKÁČEK, 2013

(Figs 5A; 6A, G; 7A)

Type material examined: See Deler-Hernández *et al*. (2013), incl. 1 sequenced paratype from the type locality [molecular voucher MF455].

Additional material examined: **Cuba:** Holguín Prov.: 5 spec. (NMPC): La Melba, Parque Nacional Alexander von Humboldt, 20.43352°N 74.82507°W, 336 m, rainforest litter, 21.ix.2014, R. Anderson, F. Cala-Riquelme, A. Deler-Hernández lgt. (2014-002); 4 spec. (NMPC): 20.45396°N 74.82342°W, 510 m, pluviselva litter, 22.ix.2014, R. Anderson, F. Cala-

Riquelme, A. Deler-Hernández lgt. (2014-011); 19 spec. (NMPC): road out La Melba, 20.51524°N 74.81844°W, 407 m, elfin forest litter, 24.ix.2014, R. Anderson, F. Cala-Riquelme, A. Deler-Hernández lgt. (2014-015); 8 spec. (NMPC): road out La Melba, road out of La Melba, 20.59086°N 74.83627°W, 130 m, scrub forest litter, 24.ix.2014, R. Anderson, F. Cala-Riquelme, A. Deler-Hernández lgt. (2014-016).

Published records: Cuba: Guantánamo Prov.: El Yunque de Baracoa (Deler-Hernández *et al*., 2013a). Holguín Prov.: La Melba (Deler-Hernández *et al*., 2013a).

Redescription: Habitus as in Fig. 5A. Body length 2.2–2.5 mm (holotype: 2.3 mm). Body oval, strongly convex, elytral suture distinctly elevated posteriorly. Dorsum black, lateral margins of pronotum and elytra without distinct paler stripe; ventral surface brown to dark brown; femora and tibiae reddish; antennae, maxillary palpi and tarsi yellowish. Head with sparse fine punctation, without microsculpture (except posteriorly on frons); eyes small, separated by 5.2× dorsal width of one eye (Fig. 6A). Pronotum with very sparse minute punctures much smaller than on head, interstices without microsculpture. Elytral punctation very strongly impressed, much coarser than on pronotum and head; elytral interstices without microsculpture. Wings completely absent. Mesoventral elevation as wide as metaventral process throughout, not narrowing anteriorly, with distinct anterior hood; metaventrite with a deep pubescent pit on each side of metaventral process; metaventrite ca. 5.8× wider than its length posterior of mesocoxae; median moderately elevated part of metaventrite narrow anteriorly, widening posteriorly (Fig. 7A). Aedeagus 0.4 mm long (Fig. 6G). Median lobe rather narrowly triangular, ca. 1.9× longer along midline than wide; apex not reaching apices of parameres; gonopore small, subapical; lateral struts simple, not expanded. Parameres indistinctly sinuate, nearly evenly arcuate on lateral margin, not expanded subapically, broadly meeting each other basally. Phallobase slightly wider than long.

Diagnosis: *Phaenonotum delgadoi* may be distinguished from all other *Phaenonotum* known to us by the combination of extremely coarse elytral punctation, elevated elytral suture and deep pubescent pits on each side of metaventral process. See Identification Key for additional characters.

Distribution: *Phaenonotum delgadoi* is only known from eastern Cuba, all known localities are situated in the Nipe-Sagua-Baracoa mountain system (Fig. 4A).

PHAENONOTUM EXSTRIATUM (SAY, 1835)

(Figs 5E; 6E, K; 7E)

Type material: Neotype of *Hydrophilus exstriatus* Say, 1835 designated by Smetana (1978) from southeastern states of USA (deposited in Museum of Comparative Zoology, Harvard University, Boston, USA): not examined in our study. Types of *Phaenonotum dubium* Sharp, 1882 were examined by Deler-Hernández & Fikáček (2016) and confirmed as being conspecific to North American specimens treated as *P. exstriatum* (synonymy proposed by Smetana 1978).

Material examined: **Barbados:** 1 spec. (SBPC): Jack-in-Box Gully, forest, 13º11'N 59º34.3'W, 230 m, UV light, 23.vi.2007, S & J. Peck lgt. (07-25). **Cuba:** Cienfuegos prov.: 1 spec. (NMPC): Soledad, 22.12682°N 80.33289°W, 71 m, MV light, 21.v.2013, A.B.T. Smith lgt. Guantánamo prov.: 2 spec. (NMPC): Baracoa, Jobo Dulce, 20°18'18.00"N 74°27'21.60"W, 94 m, 29.x.2010, R. Correa lgt; El Yunque, 3.2 km SW of campismo, 20°19'N 74°34'W, 150m, 13.vi.2012, A. Deler-Hernández lgt. Holguín Prov.: 1 spec. (NMPC): Mayarí, Guatemala, Guarina Nuñez, 20°42'4.06"N 75°40'24.45"W, ca. 27 m, 26.iii.2013, A. Deler-Hernández lgt. Granma Prov.: 1 spec. (NMPC): Cauto Cristo, Rio Cauto, El Sitio, 20°16'22.80"N 76°29'2.40"W, 135 m, 1.v.2005, L. Chaves. Santiago de Cuba prov.: 9 spec. (NMPC): El Vivero, 1.3 km NEE of Dos Caminos, 20°10'49.36"N 75°46'41.43"W, ca. 170 m, at light, 23.v.2013, Deler-Hernández & A. Smith lgt. [1 spec: molecular voucher MF654]; 1 spec. (NMPC): Daiquirí, 1–4.vi.1985, S. Bílý lgt. **Dominica:** 8 spec. (BMNH): La Plaine, 97-67, 22.i.1889, G. A. Ramage lgt. **Dominican Republic:** 1 spec. (NMPC): Samaná, 6.4 km N of Samaná, road to El Valle, 19°15.78'N 69°20.23'W, 21 m, 5.ix.2014, Deler, Fikáček, Gimmel (DR36). **Grenada:** 1 spec. (SBPC): Grand Etang Forest Reserve, 12º04.952'N 61º42.162'W, 434 m, nursery edge forest, UV trap, 14.viii.2010, S. Peck lgt. (10-69); 25 spec. (BMNH): Grand Etang (Windward side), 1900 ft, H. H. Smith.; 1 spec. (SBPC): Par. St. Andrew, Mirabeau, Agric. Lab, UV trap, 23.ii.1990, R. E. Woodruff lgt.; 34 spec. (BMNH): Mount Gay Est. (Leeward side), H. H. Smith lgt.; 3 spec. (BMNH): Chantilly Est. (Windward side), H. H. Smith lgt.; 8 spec. (BMNH): Balthazar (Windward side), H. H. Smith lgt.; 1 spec. (BMNH): Vendome Est. (Leeward side), H. H. Smith lgt. **Cayman Islands:** 1 spec. (BMNH): Grand Cayman, by freshwater lake near George Town., UV light trap, 4.viii.1970, Joy Farradane lgt. **Haiti:** 1 spec. (BMNH): Port au Prince., 1.iii.1908, M. Cameron lgt. Jamaica: 1 spec. (BMNH): Kingston., 16.ii.1908, M. Cameron lgt.; 9 spec. (SBPC): Ewarton, St. Cath. Par., St. Clair Cave, 27.xii.1972, S & J. Peck. **Monserrat:** 1 spec. (BMNH): Salem 8.ix.1975, J. Cooter lgt. **Saint Lucia:** 1 spec. (SBPC): Mon Repos, 6.5 km W of Fox GroveInn, 13º52.5'N 60º56.4'W, 300 m, submontane forest litter, 22.vii.2007, S & J. Peck lgt. (07-76). **Saint Vincent and the Grenadines:** 1 spec. (SBPC): Saint Vincent, Brighton Bay Village, 13º07.97'N 61º10.06'W, 1 m, streamside UV trap 8.vi.2007, S & J. Peck lgt. (07-10); 15 spec. (BMNH): Saint Vincent, without additional data, H. H. Smith lgt. **Puerto Rico:** 7 spec. (NMPC): Naguabo, El Yunque Nat. Forest, S part 3.45 km N of Río Blanco at rd. PR191, 18°14.8'N 65°47.7'W, 170 m, 24.vi.2016, Deler, Fikáček & Seidel lgt. [1 spec.: molecular voucher MF1728]. **Trinidad & Tobago:** 2 spec. (BMNH): Trinidad, St. Augustine, 15.iv.1926, C. L. Withycombe lgt.; 1 spec. (BMNH): Trinidad, St. Margarita, 11.vi.1942. E. C. Humphries lgt. **USA:** Florida: 1 spec. (NMPC): Highlands Co., Venus, 4 miles W of Fish Eating Creek, 15.viii.1965, W. Suter lgt., det. A. Smetana; 1 spec. (NMPC): Alachua Co., Gainesville, black light, 19.vii.1978, F. N. Young lgt., det. A. Smetana; 1 spec. (NMPC): Kansas: Douglas Co., Bridenthal Ecological Reserve, 3.2 miles N of Baldwin City, 38.81043°N 95.18669°W, 270 m, ix.2009, Eldgedge & Fikáček lgt.; 1 spec. (NMPC): Douglas Co., Lawrence Baker Wetlands, 27.viii.2009, Gustafson, Eldgedge & Fikáček lgt.; 1 spec. (NMPC): Delaware: New Castle Co., Frenchtown Woods Natural Area, 23.v.2004, at light, A. E. Z. Short lgt. (AS-04-065) [molecular voucher MF1063]. **Costa Rica:** 1 spec. (NMPC): Guanacaste: 6.6 km from main road, roadside gravel stream running through culvert, 10°09'26.7"N 85°22'47.5"W, 50 m, 13.i.2004, Short & Lebbin lgt. (AS-04-037) [molecular voucher MF1738].

Redescription: Habitus as in Fig. 5E. Body length 3.3–4.0 mm. Body elongate oval, moderately convex, elytral suture not elevated posteriorly. Dorsum black, lateral margins of elytra with very narrow indistinct paler stripe, pronotum paler in posterolateral corners; ventral surface dark brown to black; femora and tibiae black; antennae, maxillary palpi and tarsi yellowish. Head with sparse moderately coarse punctation, without microsculpture (except posteriorly on frons); eyes moderately large, separated by 4.0× dorsal width of one eye (Fig. 6E). Pronotum with sparse fine punctures, slightly smaller than on head, interstices without microsculpture. Elytral punctation moderately impressed, coarser than on pronotum and head; elytral interstices without microsculpture. Wings present, well-developed. Mesoventral elevation as wide as metaventral process posteriorly, slightly narrowing anteriorly, with distinct anterior hood; metaventrite without pubescent pits on sides of metaventral process; metaventrite ca. 3.9× wider than its length posterior of mesocoxae; median moderately elevated part of metaventrite moderately wide throughout (Fig. 7E). Aedeagus 0.4 mm long (Fig. 6K). Median lobe narrowly triangular, ca. 2.1× longer along midline than wide; apex reaching apices of parameres; gonopore small, subapical; lateral struts expanded laterally. Parameres distinctly sinuate on lateral margin, slightly expanded subapically. Phallobase longer than wide.

Diagnosis: *Phaenonotum exstriatum* may be distinguished from Caribbean species except *P. borinquenum* by the combination of moderately large eyes, moderately long metaventrite, presence of wings and morphology of the aedeagus. It may be distinguished from *P. borinquenum* by larger body size and bases of parameres meeting in single point only. Despite the strong morphological similarity, it is not closely related to *P. borinquenum* (see Fig. 2).

Distribution: *Phaenonotum exstriatum* is widespread in the eastern USA and in Central America (Smetana, 1978; Deler-Hernández & Fikáček, 2016) as well as in Greater and Lesser Antilles (Fig. 4B). The only record from South America is from eastern Colombia (Rodríguez-González *et al*., in press), but the species is probably widespread in northern South America as it is common in southern islands of Lesser Antilles and it also occurs in Trinidad.

***PHAENONOTUM LAEVICOLLE* SHARP, 1882 COMPLEX**

(Figs 5F; 6F, L; 7F)

Type material examined: Types of *P. laevicolle* Sharp, 1882, see Deler-Hernández & Fikáček (2016).

Material examined: **Cuba:** Cienfuegos Prov.: 1 male (NMPC): Río Cabagan, Gruta Mengoa, 21.93123ºN 80.08461ºW, 651 m, 20.v.2014, A. Deler-Hernández lgt. [molecular voucher MF 1115]; 3 spec. (ZMHB): Sierra del Escambrai, 5 km N Topes de Collantes, ca. 600 m, sifting of leafs, 17.xii.2007, M. Schülke lgt. (CU7-4). Sancti Spíritus Prov.: 9 spec. (ZMHB): Sierra del Escambrai, Topes de Collantes, ca. 700 m, Streu, Totholz, 17.xii.2007, M. Schülke lgt. (CU7-3). Granma Prov.: 7 spec. (ZMHB): Sierra Maestra, La Habanita, 35 km NE Pilón, 900–1000 m, sifted hay, 20.xii.2007, M. Schülke lgt. (CU7-8). Santiago de Cuba Prov.: 1 spec. (NMPC): El Vivero, 1.6 km E of Dos Caminos, 20°10.8'N 75°46.4'W, 150m, 20–21.vi.2012, A. Deler-Hernández & Fikáček (MF18). **Haiti:** 3 spec. (BMNH): Port au Prince, 1.iii.1908, M. Cameron lgt. **Venezuela:** 1 male (NMPC): Monagas small pond between Morichal Largo & Temblador, 9°05'47.9"N 62°43'37.1"W, 29 m, 2.ii.2010, Short, García & Joly lgt. (VZ10-0202-03A) [molecular voucher MF1740].

Redescription: (refers to the Caribbean specimens examined): Habitus as in Fig. 5F. Body length 2.7–3.1 mm. Body elongate oval, moderately convex, elytral suture not elevated posteriorly. Dorsum black, lateral margins of elytra with paler stripe reaching subapically, pronotum paler in posterolateral corners; ventral brown to dark brown; femora dark brown to reddish, tibiae reddish; antennae, maxillary palpi and tarsi yellowish. Head with sparse fine punctation, without microsculpture (except posteriorly on frons); eyes moderately large, separated by 3.6× dorsal width of one eye (Fig. 6F). Pronotum with sparse fine punctures similar to that on head, interstices without microsculpture. Elytral punctation moderately impressed, coarser than on pronotum and head; elytral interstices without microsculpture. Wings present, well-developed. Mesoventral elevation slightly narrower than metaventral process posteriorly, slightly narrowing anteriorly, with distinct anterior hood; metaventrite without pubescent pits on sides of metaventral process; metaventrite ca. 3.5× wider than its length posterior of mesocoxae; median moderately elevated part of metaventrite moderately wide throughout (Fig. 7F). Aedeagus 0.5 mm long (Fig. 6L). Median lobe rather triangular, ca. 1.7× longer along midline than wide; apex reaching apices of parameres; gonopore large, wide, situated in apical third of median lobe; lateral struts very shortly expanded laterally. Parameres distinctly sinuate on lateral margin, strongly expanded subapically, broadly meeting each other basally. Phallobase longer than wide.

Comments: Both sequenced specimens (MF1115 from Cuba and MF1740 from Venezuela) form a strongly supported clade in the molecular analysis and are evidently closely related (pairwise distance of *cox1* sequences is 4.8 %). Both specimens correspond with each other in external morphology and the characteristic shape of male genitalia, and only differ in body size: the Cuban specimen is smaller (2.8 mm), the Venezuelan specimen larger (3.4 mm). In this aspect, the sequenced Cuban specimen corresponds to all additional Greater Antillean specimens examined, which are also rather small (2.7–3.1 mm). Hence, it seems probable that Venezuelan specimen is not conspecific with the Greater Antillean ones, but additional material is necessary to evaluate the intraspecific genetic and morphological variability properly to decide whether the sequenced specimens represent one or two species.

The external morphology, body size and the morphology of genitalia of the Caribbean specimens correspond to the types of *P. laevicolle* Sharp, 1882 described from Guatemala and examined by Deler-Hernández & Fikáček (2016). However, due to the absence of DNA-grade specimens of *P. laevicolle* from Central America, we are unable to determine whether the Greater Antillean specimens are conspecific. For that reason, we consider all above specimens as members of the *Phaenonotum laevicolle* species complex whose taxonomy needs to be addressed once more material will be available.

Distribution: The Caribbean specimens of the *Phaenonotum laevicolle* complex are known from central and eastern Cuba and western Hispaniola (Haiti). Outside the Caribbean, the species complex clearly occurs in southernmost Northern and Central Americas (types of *P. laevicolle*) and in northern South America (as *P. globulosum* (Mulsant, 1844) from Colombia (Hansen, 1999; not examined by us) and the Venezuelan specimen sequenced by us). The records from Argentina (types of *P. spegazziinii* Bruch, 1915 not examined by us) seem doubtful (Oliva *et al*., 2002) and are not considered here. Based on these sources, we estimate the distribution of the species complex to be as shown in Fig. 4C.

PHAENONOTUM LATERALE **SP. NOV.**

(Figs 4A; 5C; 6C, I; 7B)

Type locality: Dominican Republic, Barahona, Monumento Natural Miguel Domingo Fuerte "Cachote", 18º5.91'N 71º11.35'W, 1188 m.

Type material: Holotype: male (NMPC): ‘Dominican Republic: Barahona, MN Domingo Fuerte "Cachote", 18º5.91'N 71º11.35'W, 1188 m, 14.viii.2014, Deler, Fikáček, Gimmel DR03 / montane broad-leaf cloud forest with numerous *Cyathea*: sifting of thin layer of wet leaf litter and mosses’. Paratypes: Dominican Republic: 10 spec. (NMPC): same data as holotype [incl. molecular voucher MF1013]; 25 spec. (CMN, NMPC, MNHNSD, NHMW): MN Domingo Fuerte "Cachote", 18º5.21'N 71º11.46'W, 1205 m, sparse montane cloud forest with ferns and mosses here and there, sifting of thin layer of leaf litter, 14.viii.2014, M. Fikáček lgt. (DR03a); 49 spec. (BMNH, DZRJ, MCZ, MNHNSD, NMPC, SBMN): MN Domingo Fuerte "Cachote", 18º4.48–5.37'N 71º11.03–11.54'W, 1188 m, 14.viii.2014, secondary montane broadleaf forest with sparse ferns and moss, sifting of leaf litter, Deler-Hernández, Fikáček & Gimmel lgt. (DR04).

Description: Habitus as in Fig. 5C. Body length 2.5–2.7 mm (holotype: 2.7 mm). Body oval, moderately convex, elytral suture slightly elevated posteriorly. Dorsum brown to dark brown, lateral margins of pronotum and lateral and sutural margins of elytra with distinct yellowish stripe; ventral surface brown to reddish brown; femora and tibiae reddish; antennae, maxillary palpi and tarsi yellowish. Head with sparse fine punctation, without microsculpture (except posteriorly on frons); eyes small, separated by 5.1× dorsal width of one eye (Fig. 6C). Pronotum with very sparse and very fine punctures much smaller than on head, interstices with not very distinct mesh-like microsculpture. Elytral punctation sparse and moderately impressed, similar to that on head, much coarser than on pronotum; elytral interstices without microsculpture. Wings completely absent. Mesoventral elevation extremely narrow, as wide as metaventral process throughout, not narrowing anteriorly, without distinct anterior hood; metaventrite without deep pubescent pits at sides of metaventral process; metaventrite ca. 5.0× wider than its length posterior of mesocoxae; median weakly elevated part of metaventrite narrow throughout (Fig. 7B). Aedeagus 0.35 mm long (Fig. 6I). Median lobe rather narrowly triangular, ca. 2.1× longer along midline than wide; apex reaching apices of parameres; gonopore small, subapical; lateral struts simple, not expanded. Parameres indistinctly sinuate on lateral margin, not expanded subapically, widely meeting each other basally. Phallobase as long as wide.

Etymology: The species name refers to the conspicuous yellow stripe along lateral margins of pronotum and elytra characteristic of this species.

Diagnosis: *Phaenonotum laterale* may be distinguished from other Caribbean species, based on its brown-yellow coloration, obsolete pronotal punctation and extremely narrow meso-metaventral keel. See Identification Key for further diagnostic characters.

Distribution: *Phaenonotum laterale* sp. nov. is only known from a high altitude cloud forest region in the southwestern Dominican Republic, geologically situated on the southern paleoisland of Hispaniola (Fig. 4A).

PHAENONOTUM ONDREJI **SP. NOV.**

(Figs 4A; 5B; 6B, H; 7C)

Type locality: Jamaica, Blue Mountains National Park, 18°5'14.13"N 76°43'37.55"W, 1279 m.

Type material: Holotype: male (NMPC): 'Jamaica: Blue Mountain, 18°5'14.13"N 76°43'37.55"W, 1279 m, 14.xi.2013, F. Cala-Riquelme leg.'. Paratypes: Jamaica: 19 spec. (CMN, MCZ, NMPC, NHMW) same data as holotype [incl. molecular voucher MF980; 2 spec. (BMNH): Newcastle, 19–22.viii.1908, M. Cameron lgt.

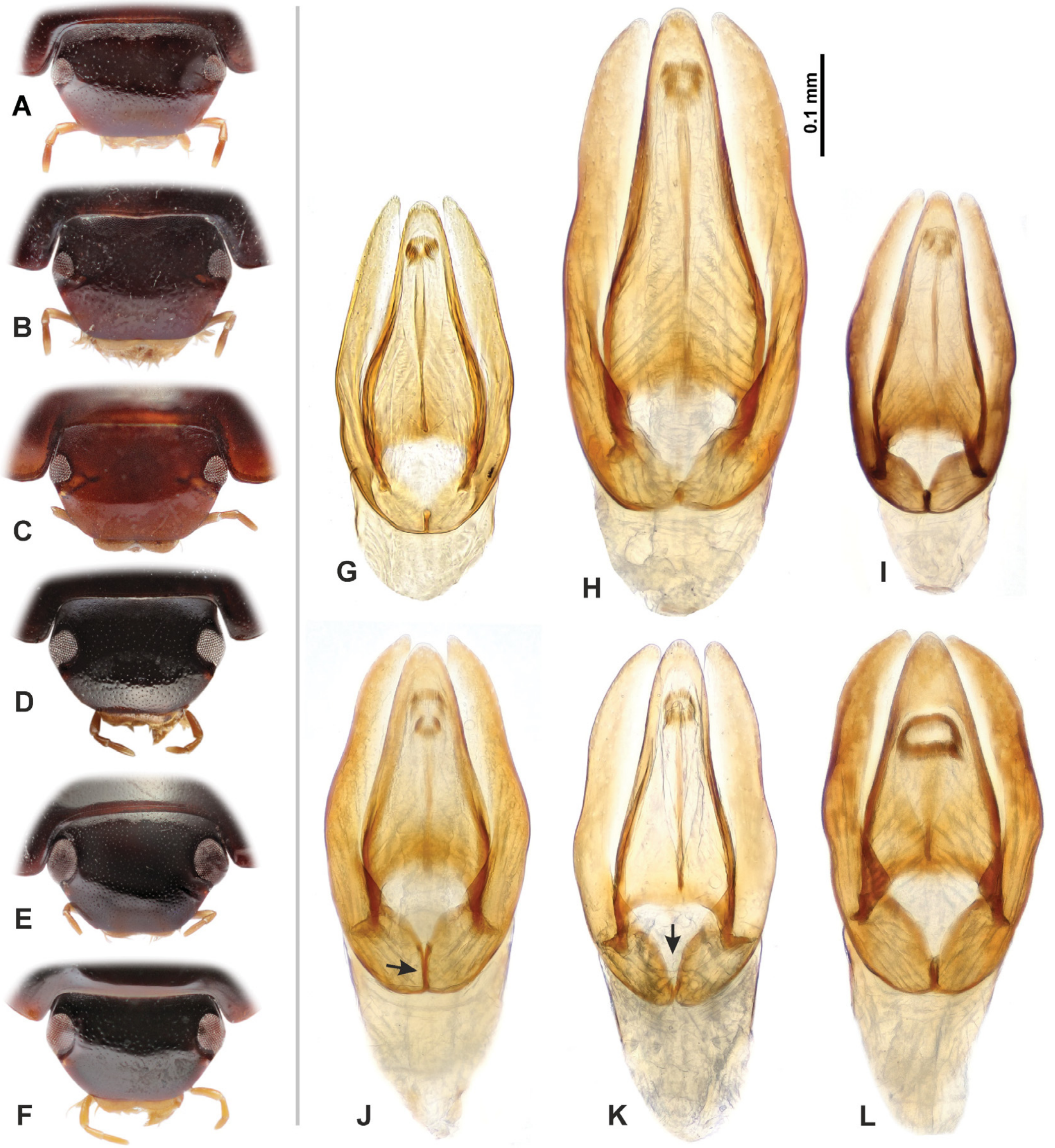

Figure 6. Head in frontal view (A–F) and aedeagus (G–L) of the Caribbean *Phaenonotum*. (A, G) *P. delgadoi* Deler-Hernández *et al.,* 2013; (B, H) *P. ondreji* sp. nov.; (C, I) *P. laterale* sp. nov.; (D, J) *P. borinquenum* sp. nov.; (E, K) *P. exstriatum* (Say, 1835); (F, L) *Phaenonotum* sp. from the *P. laevicolle* complex from Cuba. Figs A–F not to scale.

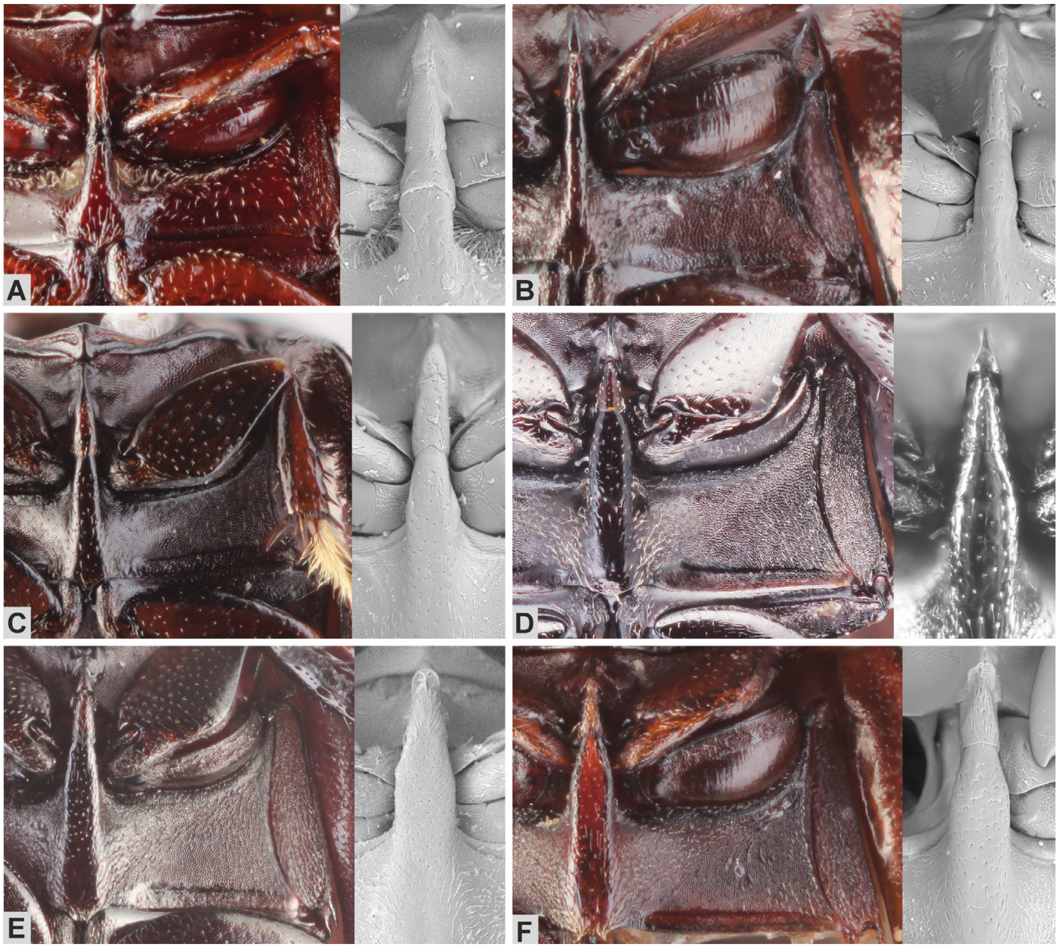

Figure 7. Meso-metaventral morphology of the Caribbean *Phaenonotum* (left: middle and right portions of complete meso-metaventrite, right: detail of mesoventral elevation and anterior metaventral process). (A) *P. delgadoi* Deler-Hernández *et al.,* 2013; (B) *P. laterale* sp. nov.; (C) *P. ondreji* sp. nov.; (D) *P. borinquenum* sp. nov.; (E) *P. exstriatum* (Say, 1835); (F) *Phaenonotum* sp. from the *P. laevicolle* complex from Cuba. Not to scale.

like microsculpture. Elytral punctation sparse, moderately impressed, coarser than on head and pronotum; elytral interstices without microsculpture. Wings completely absent. Mesoventral elevation very narrow, as wide as metaventral process throughout, not narrowing anteriorly, without distinct anterior hood; metaventrite without deep pubescent pits at sides of metaventral process; metaventrite ca. 5.1× wider than its length posterior of mesocoxae; median highly elevated portion narrow throughout (Fig. 7C). Aedeagus 0.6 mm long (Fig. 6H). Median lobe very narrowly triangular, ca. 2.3× longer along midline than wide; apex slightly overlapping apices of parameres; gonopore small, subapical; lateral struts weakly expanded. Parameres sinuate on lateral margin, weakly expanded subapically, narrowly meeting each other basally. Phallobase as long as wide.

Etymology: This species is named in honor of Ondřej Jelínek, a good friend of the senior author, in appreciation of his friendship.

Diagnosis: *Phaenonotum ondreji* may be distinguished from other Caribbean species by the combination of small eyes, head and pronotum with distinct mesh-like microsculpture, and narrow highly elevated meso-metaventral keel. See Identification Key for additional diagnostic characters.

Distribution: *Phaenonotum ondreji* sp. nov. is only known from the eastern part of the island (Blue Mountains range, Fig. 4A).

DISCUSSION

BIOGEOGRAPHY OF *PHAENONOTUM* AND ORIGIN OF CARIBBEAN SPECIES

Caribbean lineages of *Phaenonotum* diverged from their sister-taxa during the middle Eocene to early Pleistocene (ca. between 47–2.4 Ma), i.e. long after the bolide impact to the Caribbean region at the Cretaceous-Paleogene (K-Pg) boundary ca. 65.5 Ma (Schulte *et al*., 2010). This is incongruent with the vicariance hypothesis by Rosen (1975, 1988) which assumes that Caribbean lineages originated by the colonization of the proto-Antillean volcanic arc in the Late Cretaceous and survived the K-Pg boundary. The Cenozoic origin of *Phaenonotum* lineages however corresponds to other insect and spider groups studied so far, of which Caribbean clades were dated to originate between the Eocene and the Miocene (Wahlberg, 2006; Wahlberg & Freitas, 2007; Ceccarelli & Zaldívar-Riverón, 2013; Zhang & Maddison, 2013; Matos-Maraví *et al*., 2014; McHugh *et al*., 2014; Lewis *et al*., 2015; Rodriguez *et al*., 2015; Dziki *et al*., 2015; Zhang *et al*., 2017). The Caribbean *Phaenonotum* fauna originated from three to four independent colonization events from northern South America, the earliest of which correspond(s) in timing to the timespan of the GAARlandia land bridge connecting northern South America and the Greater Antilles through the emerged Aves Ridge at Eocene-Oligocene boundary (Iturralde-Vinent & MacPhee, 1999; Iturralde-Vinent, 2006). Subsequent colonizations happened during the late Oligocene to early Miocene after the Antillean archipelago lost direct connection to the continent (Iturralde-Vinent & MacPhee, 1999; Iturralde-Vinent, 2006). The evolutionary history of *Phaenonotum* may be hence best explained by the combination of colonization via a land bridge in some lineages and the over-water dispersal events (i.e. founder events) in others.

The oldest colonization of *Phaenonotum* to the Caribbean gave rise to a small clade today containing three single-island endemics: *P. delgadoi* from eastern Cuba, *P. ondreji* from eastern Jamaica, and *P. laterale* from southern Hispaniola. The divergence of this clade from modern continental clades slightly predated the timespan of the GAARlandia land bridge, which allows for two possible scenarios of colonizations: single colonization of the Greater Antilles via GAARlandia and subsequent split into multiple species in the archipelago, or parallel colonizations of Greater Antilles via GAARlandia by several species of the clade and subsequent speciation in the archipelago (our BioGeoBEARS analyses propose both scenarios as possible, depending on the model and time-stratification used and whether founder event is allowed or not). Naturally, we cannot totally exclude the over-water dispersal in case it coincided by age or slightly predated the timespan of the land bridge (Poux *et al.*, 2006; de Queiroz, 2016). However, unlike all other *Phaenonotum* examined, all three species of this clade are wingless, indicating that their most recent common ancestor likely had no metathoracic wings either. The expected limited dispersal abilities of the members of the clade together with the time overlap of their arrival to the Greater Antilles with the timespan of the GAARlandia land bridge make the scenario of the over-land colonization of Greater Antilles more probable than the over-water dispersal alternative.

The divergence of the Cuban *P. delgadoi* from the remaining two species of the clade was dated to the Eocene-Oligocene boundary (ca. 36 Ma), i.e. coinciding with the maximum landspan of the GAARlandia and pre-dating the subdivision of Greater Antilles into particular paleoislands (separation of Puerto Rico from Hispaniola+Cuba by Mona Passage ca. 30-20 Ma and separation of eastern Cuba from central Hispaniola by Windward Passage ca. 17-14 Ma; Ituralde-Vinent & MacPhee, 1999; Matos-Maraví *et al.*, 2014). Both clades may have split while colonizing separate mountain ranges of GAARlandia peninsula (Ituralde-Vinent & MacPhee, 1999; Ituralde-Vinent 2006) or already before colonizing the peninsula. In contrast, the divergence of the Hispaniolan *P. laterale* and Jamaican *P. ondreji* was dated to the Late Oligocene (ca. 26 Ma) when only the Blue Mountain range of Jamaica was emergent and widely isolated from other islands (Iturralde-Vinent & MacPhee, 1999; Iturralde-Vinent, 2006). This implies a long distance over-water founder event, improbable for wingless mountain species. Interestingly, Jamaica-Hispaniola divergences of similar age are also found in some other animal groups (ca. 22 Ma in *Osteopilus* tree frogs: Moen & Wiens, 2009; ca. 20 Ma in *Exophthalmus* weevils: Zhang *et al.*, 2017; ca. 15 Ma in *Calisto* butterflies: Matos-Maraví *et al.*, 2014). This may indicate either much closer geological connection between Hispaniola and Jamaica in Late

Oligocene to Early Miocene, or constellation largely facilitating Hispaniola to Jamaica dispersal (e.g. by strong sea currents or hurricanes; Hedges, 2006).

Puerto Rico was a part of GAARlandia land bridge but no species of the above *Phaenonotum* clade was recorded from the island, in contrast to the predictions of the over-land GAARlandia scenario. Similarly, the endemic occurrence of the Hispaniolan *P. laterale* in the southern part of the island that emerged in the Late Miocene, largely postdating the origin of the species (26.4 Ma, Late Oligocene) also disagrees with the current model of historical geography of the Greater Antilles. This indicates that range shifts and extinctions likely played a significant role in the evolutionary history of *Phaenonotum* beetles in the Caribbean. On the other hand, the absence of the members of this *Phaenonotum* clade from Lesser Antilles is congruent with the GAARlandia hypothesis: older Lesser Antilles islands are of ca. Oligocene to Miocene origin, the younger ones arose during the Pliocene only and none of them were part of the GAARlandia land bridge which was situated slightly more to the west in the place of nowadays submerged Aves Ridge (Iturralde-Vinent & MacPhee, 1999; Iturralde-Vinent, 2006; Thorpe *et al*., 2004).

The remaining *Phaenonotum* clades probably colonized the Caribbean by two or three independent events during the Late Oligocene to Pliocene by over-water dispersal. One of these events (dated to Early Miocene, ca. 19 Ma) gave rise to the Puerto Rican *P. borinquenum*, i.e. the only single-island endemic which is not a member of the Caribbean endemic lineage discussed above. Both widespread continental-Caribbean species (*P. laevicolle* and *P. exstriatum*) were estimated to originate in the Caribbean and back-colonize the continent during the Pliocene and Pleistocene in the time-stratified BioGeoBEARS analyses. Our ancestral range estimates may be, however, strongly influenced by the incomplete sampling of continental *Phaenonotum* species and limited knowledge on their distribution, and additional studies are hence necessary to corroborate our results. Nevertheless, our data suggest that the Caribbean Region may act not only as a sink of colonizations from the continent, but is also a source of taxa colonizing North and Central America. This corresponds to the situation recently revealed for the *Exophthalmus* weevils and for *Heracleidos* butterflies, both of which colonized Central America from the Caribbean during the Miocene to Pleistocene (Lewis *et al*., 2014; Zhang *et al*., 2017); multiple examples are also known for vertebrates (Bellemain & Ricklefs, 2008; Ricklefs & Bermingham, 2008). Moreover, Central America was an archipelago similar to today's Greater Antilles during the Miocene to Pliocene, and Caribbean species hence probably colonized islands with unbalanced fauna rather than a continent with diverse well-established fauna characterized by strong interspecific competition (Bellemain & Ricklefs, 2008).

All four single-island endemic *Phaenonotum* species inhabit leaf litter in lowland (*P. delgadoi*) or montane cloud forests (*P. ondreji*, *P. laterale* and *P. borinquenum*), i.e. the primary forest habitats in the island interior. In contrast, the remaining two species are more widespread (*P. exstriatum* is recorded from all Greater Antillean islands incl. Cayman Islands, and from most islands of Lesser Antilles, *P. laevicolle* is known from Cuba and Hispaniola; Fig. 4), inhabit disturbed lowland habitats (*P. exstriatum* aquatic and semiaquatic habitats and decaying plant material, *P. laevicolle* decaying plant material including hay piles and leaf litter in farmland). When biology and distribution data are correlated to the age of the respective species, they are in a good agreement with the taxon cycle hypothesis proposed originally by Wilson (1959, 1961) for Melanesian ants, and subsequently confirmed e.g. for Lesser Antillean birds (Ricklefs & Cox, 1972) (see Ricklefs & Bermingham, 1999 for review and multiple examples, and Economo & Sarnat, 2012 for detailed re-analysis of taxon cycle in Melanesian ant fauna). Following the predictions of the taxon cycle, the recently colonizing species (*P. laevicolle* and *P. exstriatum*) inhabit wider spectrum of lowland disturbed habitats and are good dispersers (range expansion phase) whereas the locally endemic species are remnants of ancient colonizations inhabiting exclusively inland primary habitats and having poor dispersal abilities (they switched from the expansion phase at the time of colonization into the range contraction phase; this may be followed by another range expansion phase or by extinction).

SPECIES-LEVEL SYSTEMATICS OF *PHAENONOTUM*

Despite the beetles being frequently collected, the systematics of *Phaenonotum* beetles was not studied properly until now, and very little is known about the continental fauna of the genus. Sixteen species are known from the continental Neotropics, and two from the Nearctic (with the widespread *P. exstriatum* inhabiting both regions). The real species diversity seems to be much higher, which is illustrated e.g. by the fact that of the ca. 13 continental species included in our analysis, we were only able to identify two of them (*P. exstriatum* and *P. laevicolle*) whereas remaining ones probably represent undescribed species. The extreme morphological similarity of the species and limited number of characters useful for species-level identification are clearly the main obstacle for species-level studies and identification of *Phaenonotum*. Moreover, extreme similarity in external morphology and male genitalia does not necessarily indicate close phylogenetic relationship of species. This is illustrated by *P. exstriatum* and *P. borinquenum* sp. nov. in our case, which are not closely related to each other, yet they are nearly identical in morphological characters. In contrast, *P. laevicolle* complex seems closely related to *P.*

borinquenum but can be very easily distinguished from it by genital morphology (compare Figs 6L and 6J). The combination of morphology and molecular data seems hence necessary for proper understanding of species limits even in distantly related species in *Phaenonotum*. The same is naturally true for closely related similar species. This is the reason we refrain from describing the Cuban and Hispaniolan species of the *P. laevicolle* complex, and do not decide whether the sequenced specimens from Cuba and Venezuela belonging to this species complex represent one or two species. Sequences of freshly collected specimens from type locality of *P. laevicolle* or its surroundings and from multiple specimens across the range of the species complex would be necessary to analyze the species limits in this case.

Neotropical Coelostomatini and the position of *Phaenonotum*

Despite a limited taxon sampling, our analyses indicate that the Neotropical endemic genera of the Coelostomatini (*Cyclotypus*, *Phaenonotum*, *Phaenostoma*, *Lachnodacnum*) form a monophyletic group. This result corresponds with topology revealed in the phylogenetic analysis containing a wider spectrum of coelostomatine genera but fewer Neotropical taxa (Sýkora, unpubl. data) and suggests that the early evolution of the Coelostomatini was strongly influenced by the paleogeography: the origin of this clade was revealed as Early-Middle Cretaceous in our analysis when South America was largely isolated from other continents. The internal topology of the Neotropical clade disagrees with current generic concepts which are based on the combination of a few easy-to-observe morphological characters (Hansen, 1991; Clarkson *et al.*, 2014): *Phaenonotum* is revealed polyphyletic when comprising the morphologically aberrant *P. caribense*, and the bromeliad inhabiting genus *Lachnodacnum* is nested within the polyphyletic *Phaenostoma*. The fact that the aberrant morphology corresponds to a separate phylogenetic position in one taxon (*Phaenonotum caribense*) but to biology rather than phylogenetic position in another one (*Lachnodacnum*) clearly indicates that the systematics of the Neotropical clade of the Coelostomatini (and in fact of the tribe as a whole) is a complex task. Additional studies combining morphological and molecular characters are necessary to understand the higher-level systematics of the group and to recognize ecology-based morphological characters from phylogeny-informative ones. Additional taxa need to be also included into the analyses – this especially concerns *Hydroglobus puncticollis* (Bruch, 1915) from Argentina which probably belongs to the Neotropical clade.

The clade of the core *Phaenonotum* (i.e. *Phaenonotum* after excluding *P. caribense*) corresponds well with the current morphology-based concept of the genus (Hansen 1991), with the only exception of the African *P. africanum* whose assignment to the genus is doubtful and needs to be tested. Based on our analyses, the core *Phaenonotum* are either sister group to *Phaenostoma*+*Lachnodacnum* (Fig. 1A) or to the *Phaenostoma posticatum* clade only (Fig. 1B). The latter topology seems to be better supported by morphology, as both *Phaenostoma posticatum* and the core *Phaenonotum* species share the unique shape of the metanepisternum (widened in posterior third, narrowing anteriorly, Fig. 7); all remaining coelostomatine species examined including *Phaenostoma kontax*, *Lachnodacnum*, *Phaenonotum caribense* and *Cyclotypus* have the metanepisternum with lateral margins parallel-sided (and hence the same width) throughout. However, the undescribed *Phaenonotum* species from Peru (MF845) bears the parallel-sided rather than anteriorly narrowing metanepisternum despite being strongly supported as member of the core *Phaenonotum* clade in our analyses. Hence, the phylogenetic significance of this character needs to be further tested.

ACKNOWLEDGEMENTS

We are grateful to the authorities and personnel of the Cuban Ministry of Science, Technology and Environment (CICA-CITMA) and the Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF) for providing access to protected areas under their control, to Carlos Suriel, Edda Martínez Caléz and Marilyn Colon Negron for their help with arranging the collecting permits in the Dominican Republic and Puerto Rico, to Robert Anderson (Canadian Museum of Nature, Ottawa), Matthew L. Gimmel (Santa Barbara Museum of Natural History), and people of the Cuba and Jamaica CarBio teams for their help during our field work, to S. Peck (Carleton University) and curators of the institutional collections (see Material and methods) for the possibility to examine their collections, and to Andrew Short (University of Kansas, Lawrence, USA), Joseph Parker (Columbia University, New York) and Bruno Clarkson (Universidade Federal do Rio de Janeiro) for providing continental specimens used in our analyses. We specially acknowledge the comments and critique of Andrew Short and two anonymous reviewers on the earlier version of this draft, and Robert Angus (BMNH) for a careful correction of our English text. The study was supported by the grant SVV 260 434/2017 at the Department of Zoology, Charles University and The Rufford Foundation (15144-1) to ADH, and by the Ministry of Culture of the Czech Republic (DKRVO 2017/14, National Museum, 00023272) to MF.

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SUPPORTING INFORMATION

Supplementary File 1: Molecular data and phylogenetic and divergence dating analyses.

Supplementary File 2: Imput data and results of the BioGeoBEARS analyses.

Supplementary File 3: Complete data of all examined specimens.

Supplementary material

to the paper

Multiple origin of the *Phaenonotum* beetles in the Greater Antilles (Coleoptera: Hydrophilidae): phylogeny, biogeography and systematics

by

ALBERT DELER-HERNÁNDEZ, VÍT SÝKORA, MATTHIAS SEIDEL,
FRANKLYN CALA-RIQUELME and MARTIN FIKÁČEK

Part 1:
Molecular data and phylogenetic and divergence dating analyses

List of specimens included in the molecular analyses [GenBank accessions will be added before accepting]
(specimens MF1736 and MF1741 are very close genetically and hence considered as representing one species, their data were combined for the final analyses).

Voucher #	**Species**	**Country**	**Included in analysis**			**Sources**	**COI**	**COII**	**16S**	**18S**	**28S**
			35	**30**	**22**						
MF656	*Sphaerocetum arboreum*		x			GenBank	KM874806	NO	NO	KM874807	KM874809
SLE0125	*Sphaeridium bipustulatum*		x			GenBank	AM287095	KC992459	KC992710	AJ810722	AJ810757
MF326	*Coelostoma phallicum*		x			GenBank	KC935244	KC992398	NO	KC935021	KC992550
SLE0019	*Coelostoma orbiculare*		x			GenBank	AM287094	AM287116	AM287072	KC935020	KC992549
SLE0072	*Dactylosternum* sp.		x			GenBank	KC935254	KC992400	KC992664	KC935031	KC992559
MF36	*Phaenonotum caribense*	Ecuador	x	x	x	GenBank	KC935315	KC992399	NO	KC935090	KC992623
MF37	*Phaenostoma* sp.	Ecuador	x	x	x	New	YES	NO	YES	YES	YES
MF38	*Phaenonotum* sp.	Ecuador	x	x	x	New	YES	YES	YES	YES	YES
MF455	*Phaenonotum delgadoi*	Cuba	x	x	x	New	YES	YES	NO	YES	YES
MF654	*Phaenonotum exstriatum*	Cuba	x	x	x	New	YES	YES	YES	YES	YES
MF772	*Lachnodacnum luederwaldti*	Brazil	x	x	x	New	YES	NO	YES	YES	YES
MF839	*Phaenonotum* sp.	Peru	x	x		New	YES	NO	NO	YES	YES
MF840	*Phaenonotum* sp.	Peru	x	x	x	New	YES	NO	YES	YES	YES
MF842	*Cyclotypus* sp.	Peru	x	x	x	New	YES	YES	YES	YES	YES
MF844	*Phaenostoma* sp.	Peru	x	x		New	YES	NO	NO	YES	YES
MF845	*Phaenonotum* sp.	Peru	x	x	x	New	YES	YES	NO	YES	YES
MF846	*Phaenonotum* sp.	Peru	x	x	x	New	YES	YES	YES	YES	YES
MF855	*Phaenostoma* cf. *kontax*	Peru	x	x		New	YES	NO	NO	YES	YES
MF856	*Phaenonotum* sp.	Peru	x	x	x	New	YES	YES	NO	YES	YES
MF861	*Phaenonotum* sp.	Peru	x	x	x	New	YES	YES	YES	YES	YES
MF862	*Phaenonotum* sp.	Peru	x	x		New	YES	NO	NO	YES	YES
MF980	*Phaenonotum ondreji*	Jamaica	x	x	x	New	YES	YES	NO	YES	YES
MF1013	*Phaenonotum laterale*	Dominican R.	x	x	x	New	YES	YES	NO	YES	YES
MF1061	*Phaenonotum* sp.	Guyana	x	x	x	New	YES	YES	YES	YES	YES
MF1062	*Phaenonotum* sp.	Suriname	x	x	x	New	YES	YES	YES	YES	YES

List of specimens included in the molecular analyses [GenBank accessions will be added before accepting]
(specimens MF1736 and MF1741 are very close genetically and hence considered as representing one species, their data were combined for the final analyses).

MF1063	*Phaenonotum exstriatum*	USA	x	x	x	New	YES	YES	YES	YES	YES
MF1066	*Phaenostoma posticatum*	Venezuela	x	x	x	New	YES	YES	NO	YES	YES
MF1115	*Phaenonotum laevicolle* complex	Cuba	x	x	x	New	YES	NO	YES	YES	YES
MF1728	*Phaenonotum exstriatum*	Puerto Rico	x	x		New	YES	NO	NO	YES	YES
MF1729	*Phaenonotum borinquenum* PT	Puerto Rico	x	x	x	New	YES	NO	NO	YES	YES
MF1730	*Phaenonotum borinquenum* HT	Puerto Rico				New	YES	NO	NO	NO	NO
MF1736	*Phaenonotum* sp.	Venezuela	x	x	x	New	YES	NO	NO	NO	YES
MF1737	*Phaenonotum* sp.	Costa Rica	x	x		New	YES	NO	NO	YES	NO
MF1738	*Phaenonotum* sp.	Costa Rica	x	x	x	New	YES	NO	YES	YES	YES
MF1739	*Phaenonotum* sp.	Guatemala	x	x		New	YES	NO	NO	YES	YES
MF1740	*Phaenonotum* sp.	Venezuela	x	x		New	YES	NO	NO	YES	NO
MF1741	*Phaenonotum* sp.	Venezuela	x	x	x	New	YES	NO	NO	YES	NO

Primers used in PCR reactions for individual genes

Gene	Primer	Direction	Sequence (5-3)
COI-3´	stev_jerryF	forward	CAACATYTATTYTGATTYTTTGG
COI-3´	stev_patR	reverse	GCACTAWTCTGCCATATTAGA
COII	TL2-J-3037	forward	TAATATGGCAGATTAGTGCA
COII	TK-N-3785	reverse	TTTAAGAGACCAGTACTT
16S	LR-N-13398	forward	CGCCTGTTTAACAAAAACAT
16S	LR-J-12887	reverse	CCGGTCTGAACTCAGATCACGT
18S 3´	18Sa1.0	reverse	GGTGAAATTCTTGGACCGTC
18S 3´	18S3'I	forward	CACCTACGGAAACCTTGTTACGAC
18S 5´	18S5'I	forward	GACAACCTGGTTGATCCTGCCAGT
18S 5´	18Sb0.5	reverse	TAACCG CAACAACTTTAAT
28S	NLF184-21	forward	ACCCGCTGAAYTTAAGCATAT
28S	LS1041R	reverse	TACGGACRTCCATCAGGGTTTCCCCTGACTTC

PCR programs used for amplification of individual genes

PCR	**COI-3´**					
Step	1	2	3	4	5	6
Temperature (°C)	94	94	50	72	72	4
Time (s)	180	30	45	60	480	forever
		Repeat 35x				

PCR	**COII**					
Step	1	2	3	4	5	6
Temperature (°C)	94	94	50	72	72	4
Time (s)	180	30	45	60	480	forever
		Repeat 35x				

PCR	**16S**					
Step	1	2	3	4	5	6
Temperature (°C)	94	94	48	72	72	4
Time (s)	120	30	30	60	600	forever
		Repeat 40x				

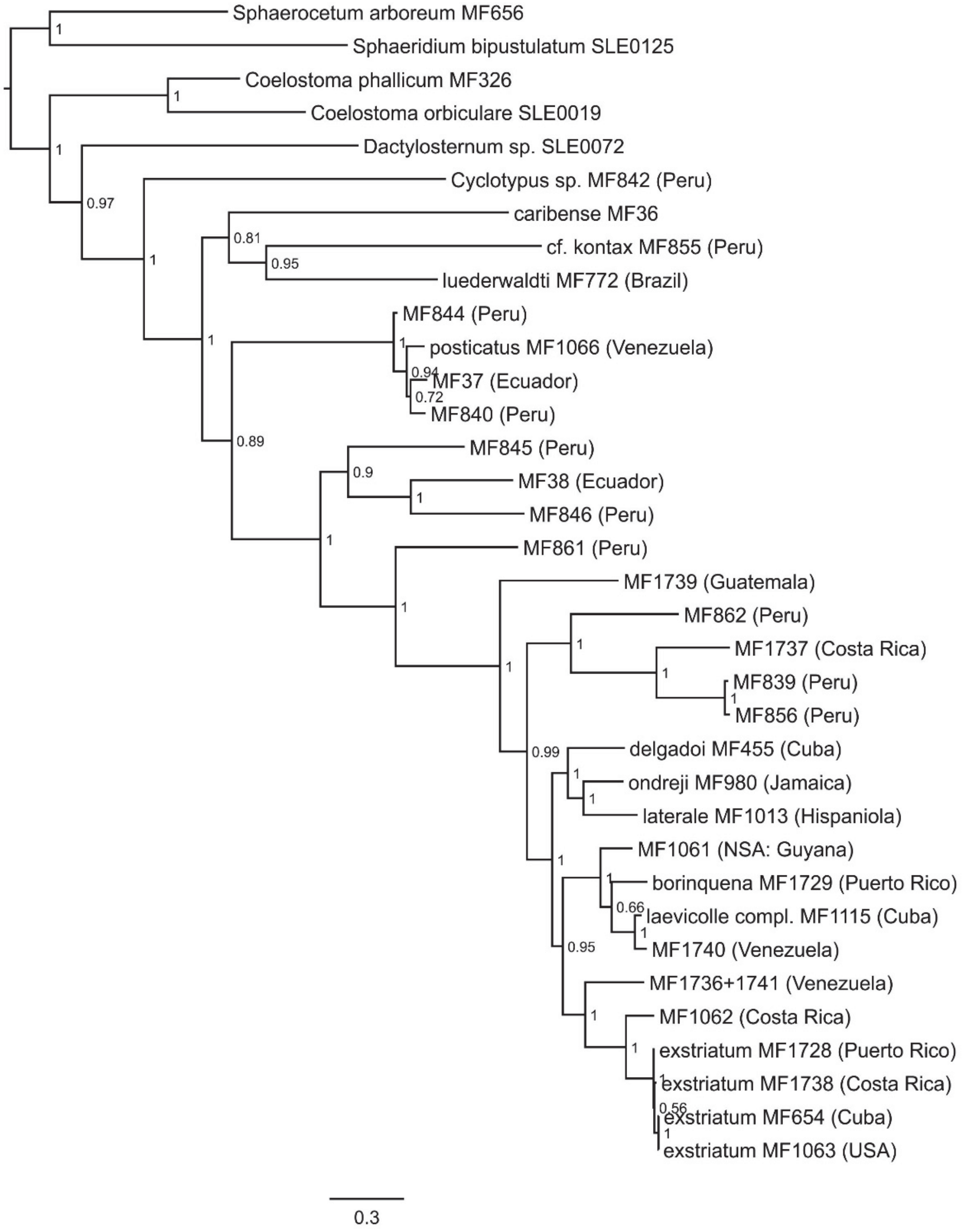
PCR	**18S 3´**					
Step	1	2	3	4	5	6
Temperature (°C)	98	98	52	72	72	12
Time (s)	30	10	30	90	480	forever
		Repeat 30x				

PCR	**18S 5´**					
Step	1	2	3	4	5	6
Temperature (°C)	98	98	52	72	72	12
Time (s)	30	10	30	60	480	forever
		Repeat 30x				

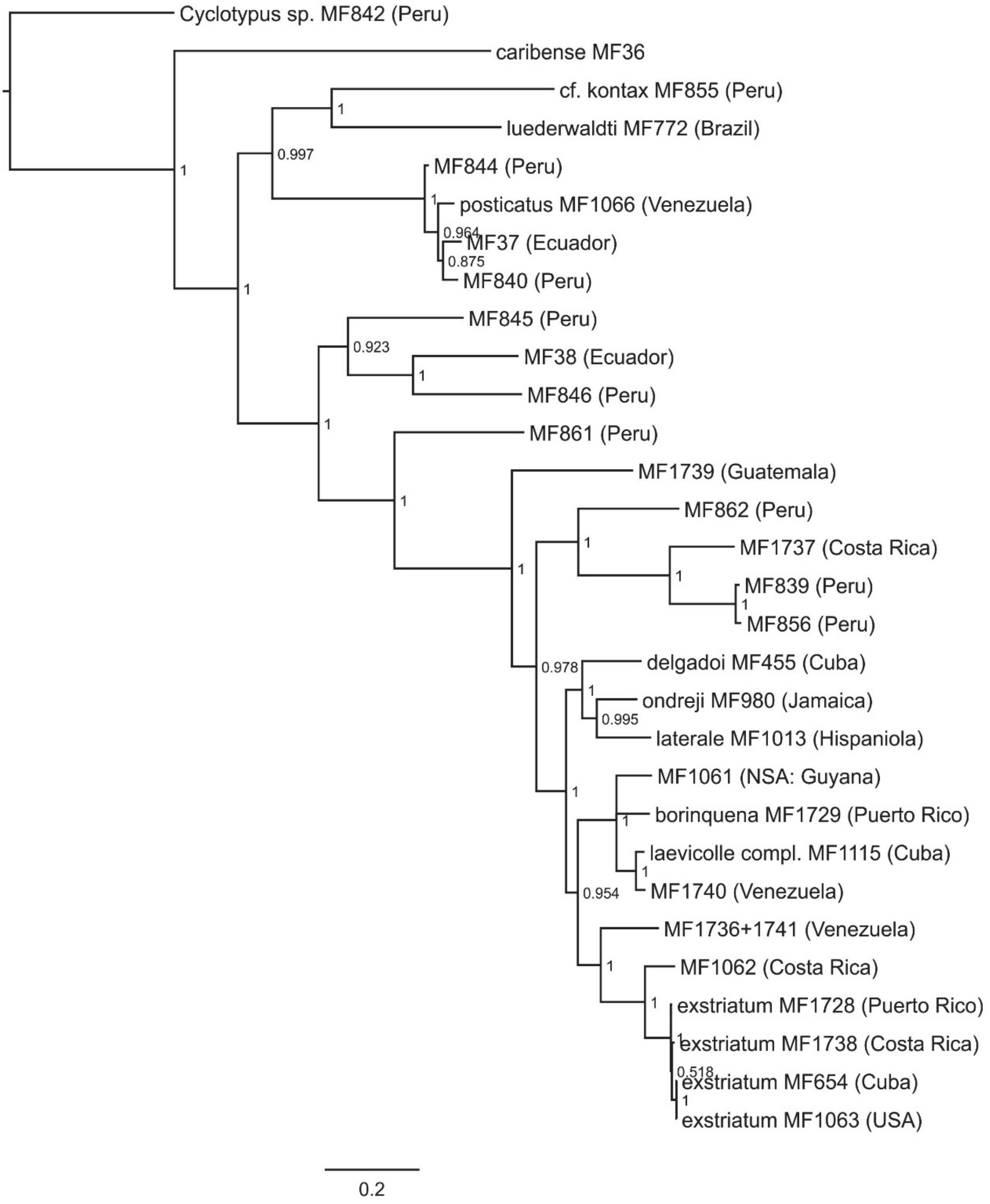
PCR	**28S**					
Step	1	2	3	4	5	6
Temperature (°C)	98	98	54	72	72	10
Time (s)	30	10	30	60	480	forever
		Repeat 30x				

RESULTS OF THE PHYLOGENETIC ANALYSES

MrBayes analysis: 35 taxa dataset
outgroup: all outgroup taxa
ingroup: all *Phaenonotum* + *Phaenostoma* + *Lachnodacnum*

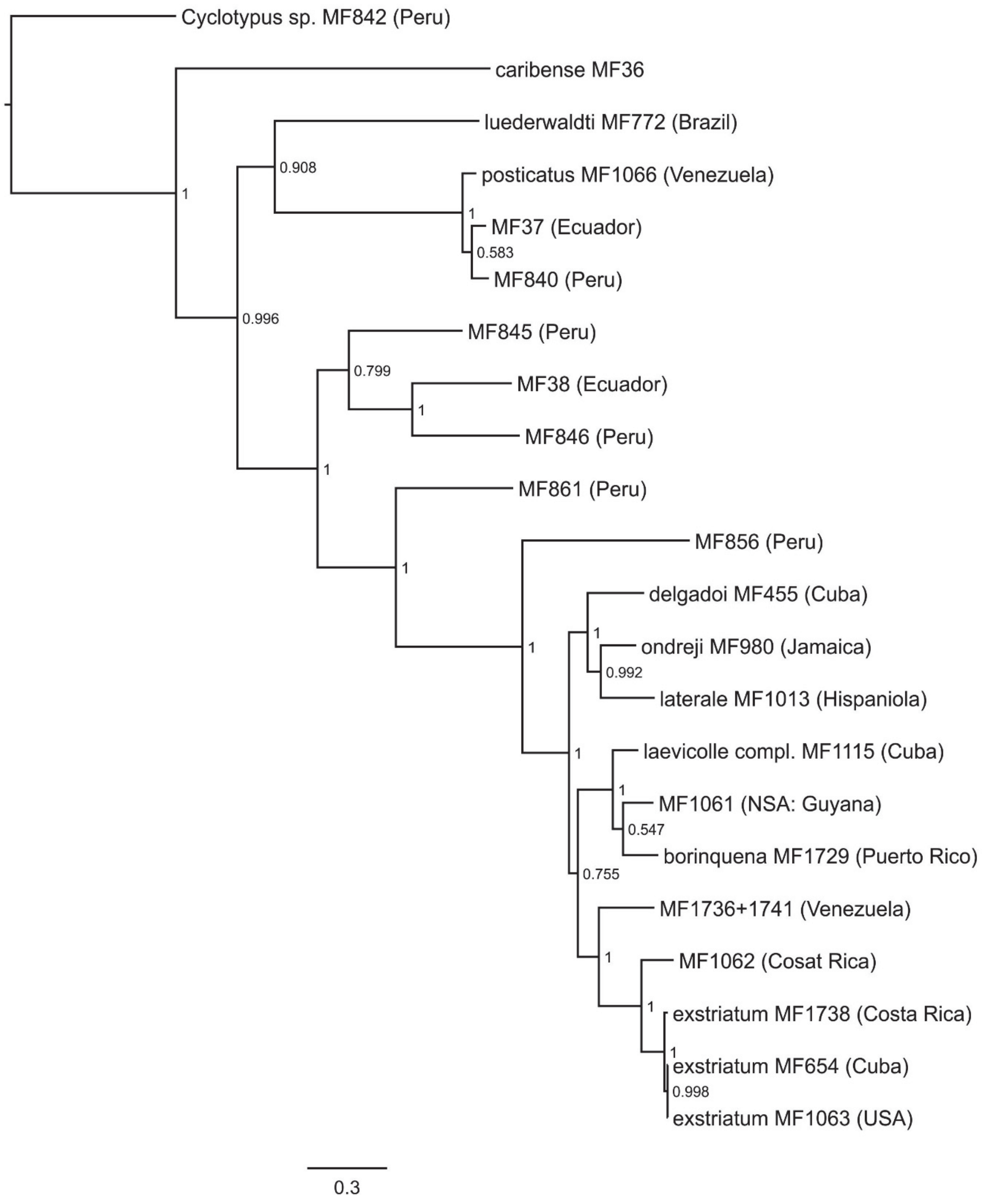


MrBayes analysis: 30 taxa dataset
outgroup: *Cyclotypus*
ingroup: all *Phaenonotum* + *Phaenostoma* + *Lachnodacnum*



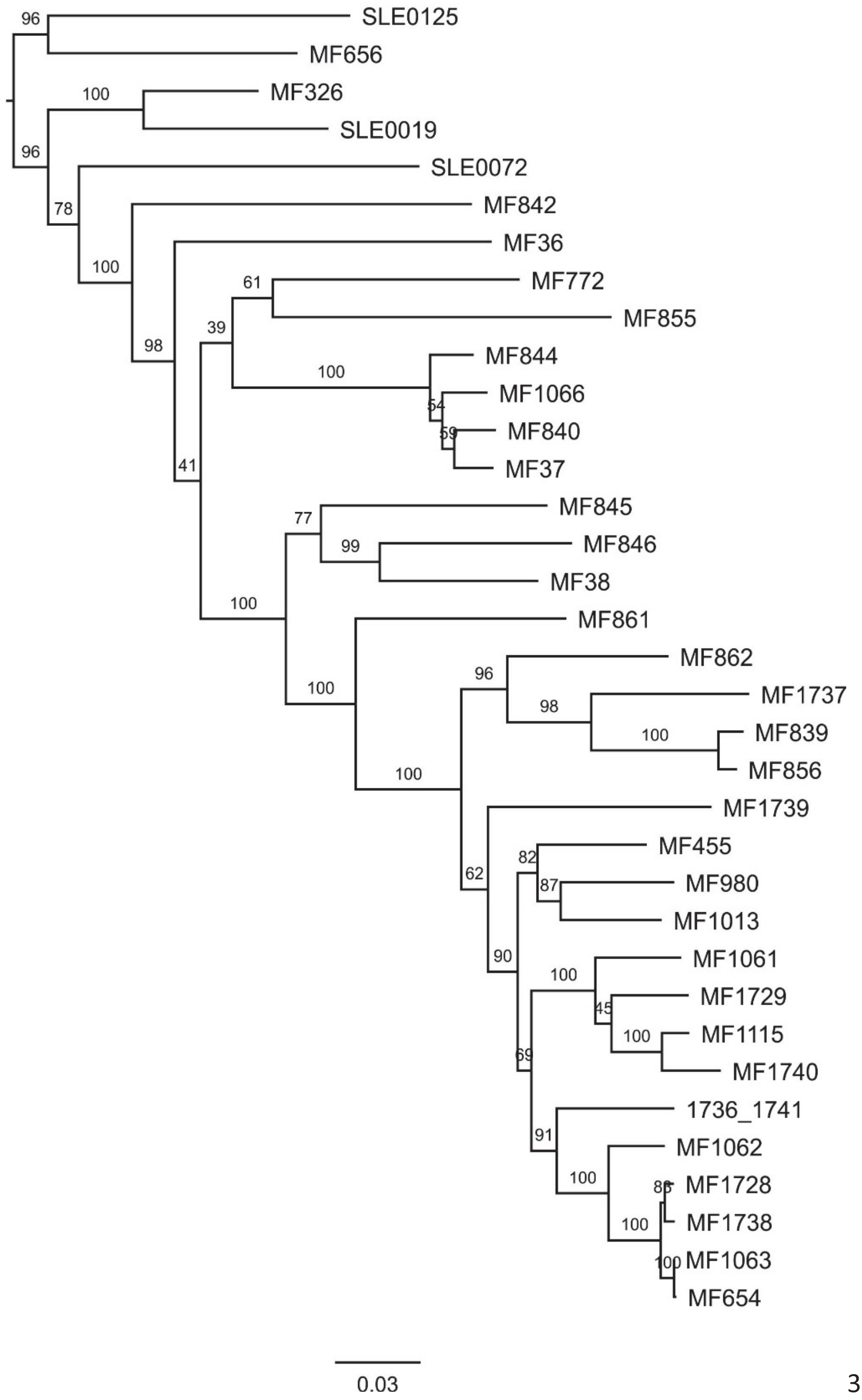
MrBayes analysis: 22 taxa dataset

outgroup: *Cyclotypus*
ingroup: *Phaenonotum* + *Phaenostoma* + *Lachnodacnum* with 3+ genes

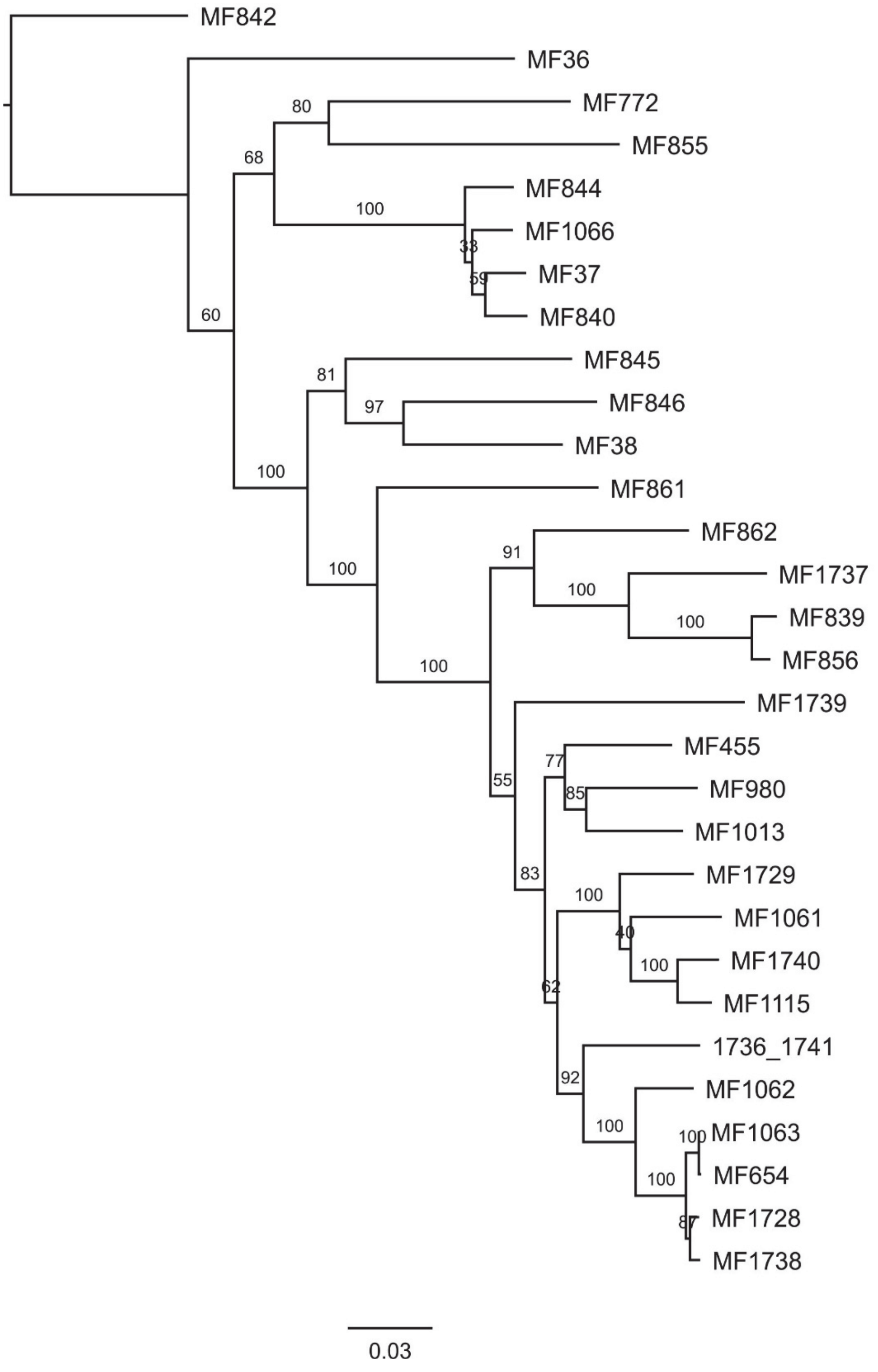


RAxML analysis: 35 taxa dataset

outgroup: all outgroup taxa
ingroup: all *Phaenonotum* + *Phaenostoma* + *Lachnodacnum*

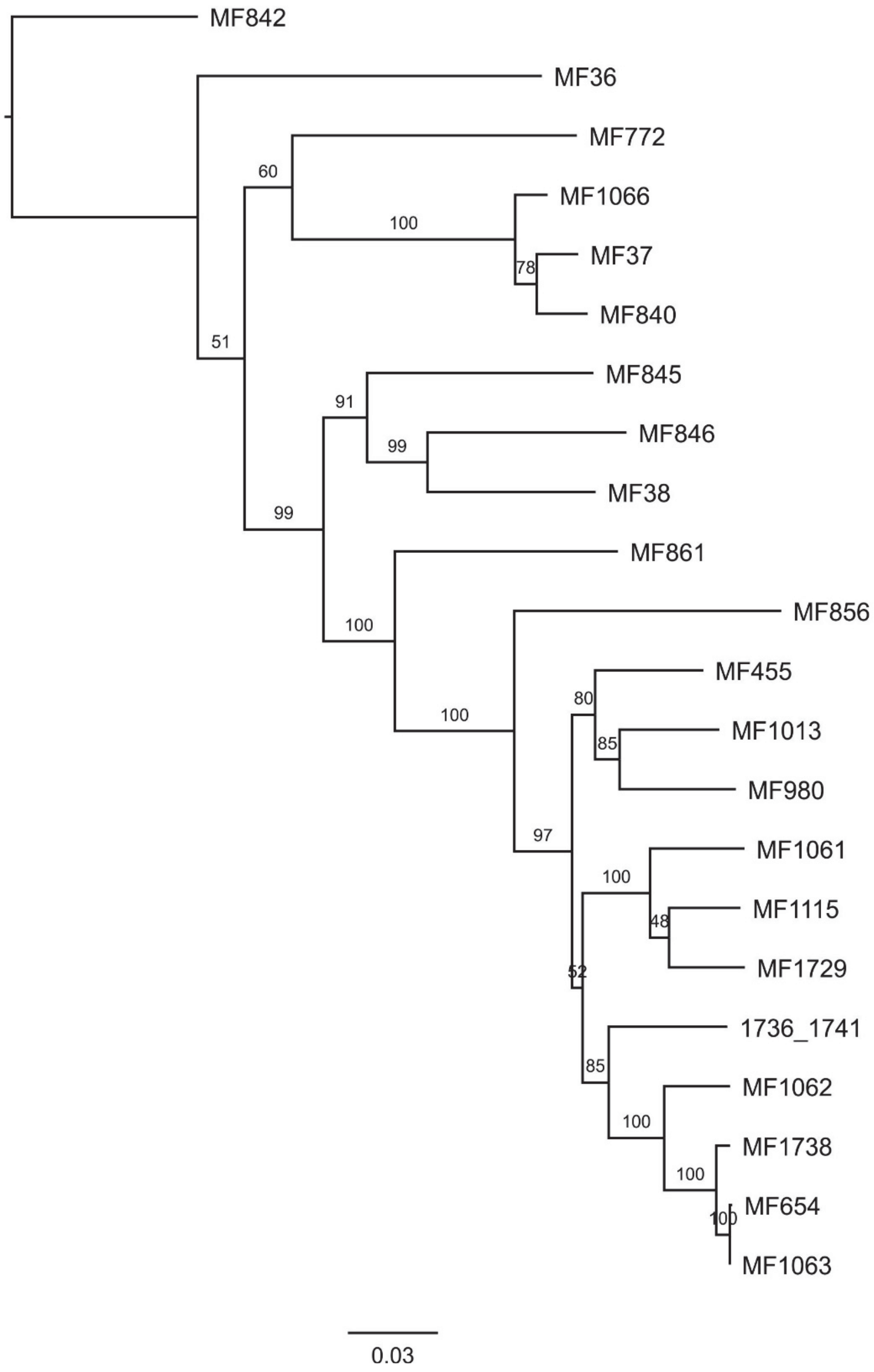


RAxML analysis: 30 taxa dataset
outgroup: *Cyclotypus*
ingroup: all *Phaenonotum* + *Phaenostoma* + *Lachnodacnum*



RAxML analysis: 22 taxa dataset

outgroup: *Cyclotypus*
ingroup: *Phaenonotum* + *Phaenostoma* + *Lachnodacnum* with 3+ genes



DIVERGENCE DATING ANALYSES

Testing molecular clock

MrBayes commands used:

```
# set up the substitution model
lset nst=6 rates=invgamma

# fix the root (preferred way, see MrBayes manual)
constraint ingroup = 1-33
prset topologypr = constraints(ingroup)

# test for non-clock
prset brlenspr=unconstrained:exp(10.0)
ss ngen=255000 diagnfreq=2500

# test for strict clock
prset brlenspr=clock:uniform
prset clockvarpr=strict
ss ngen=255000 diagnfreq=2500

# test for relaxed clock - uniform
prset brlenspr=clock:uniform
prset clockvarpr=igr
ss ngen=255000 diagnfreq=2500

# test for relaxed clock - birth-death
prset brlenspr=clock:birthdeath
prset clockvarpr=igr
ss ngen=255000 diagnfreq=2500
```

Results of the molecular clock tests:

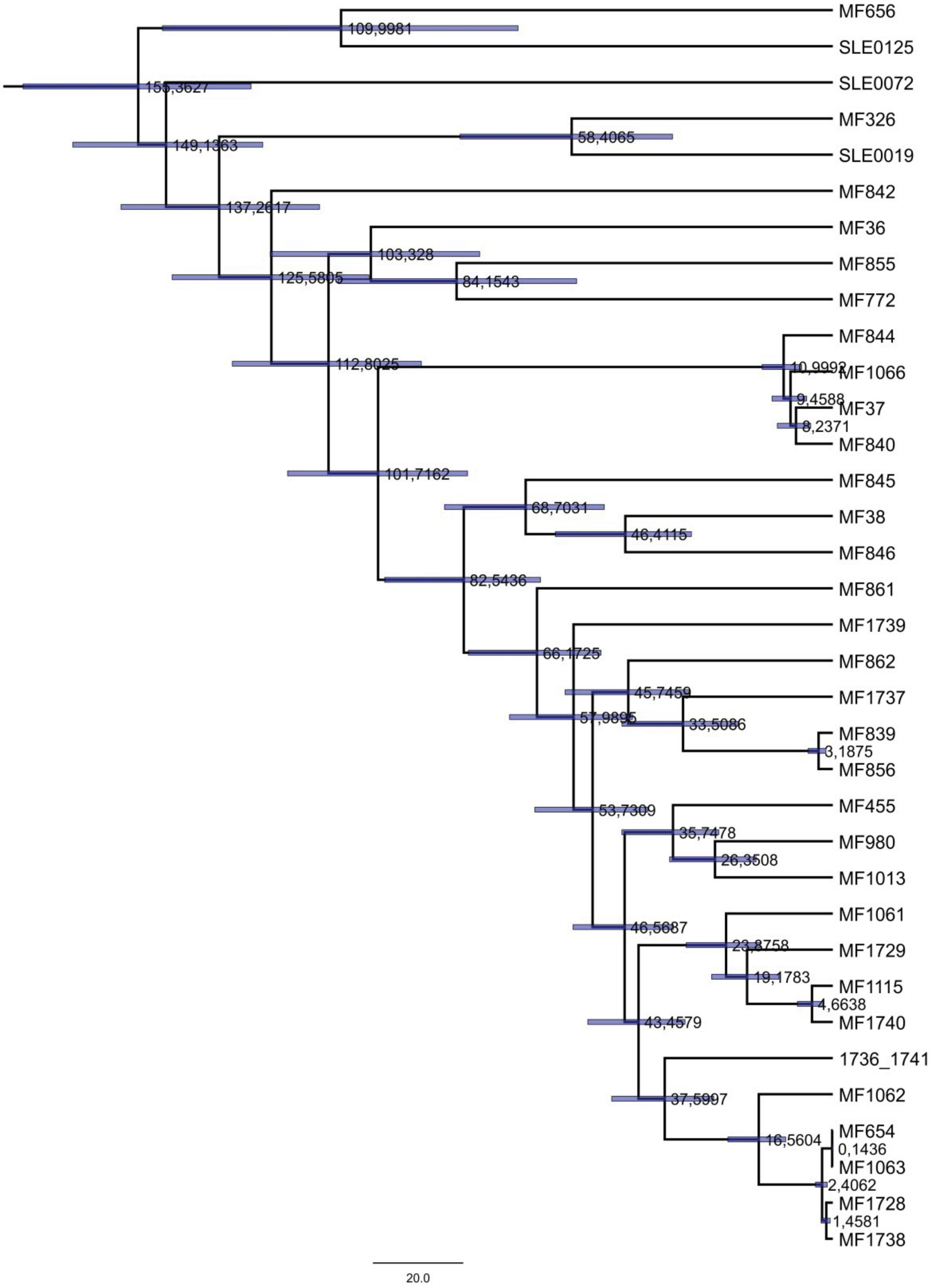
analyses of 35 taxa dataset (All_Phaenonotum_outgroup.nex)

	non-clock	strict	relaxed	relaxed_birthDeath
1	-29815.84	-29783.46	-29768.76	-29756.61
2	-29809.61	-29780.10	-29764.68	-29763.95
mean	-29810.30	-29780.76	-29765.36	**-29757.30**

BEAST timetree analysis

relaxed clock, birth-death, based on 35 taxa dataset

calibrated by:
- mitochondrial substitution rate: 0.0133 substitutions/Myr
- nuclear substitution rate: 0.0017 substitutions/Myr
- age of the MRCA of the Coelostomatini: 152.5 Ma (95% CI 134–170)



Supplementary material

to the paper

Multiple origin of the *Phaenonotum* beetles in the Greater Antilles (Coleoptera: Hydrophilidae): phylogeny, biogeography and systematics

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Part 2:
Imput data and results of the BioGeoBEARS analyses

BioGeoBEARS analysis: Dispersal multipliers used for the analyses

Time period: 0-2.8 Mya

	A	B	C	D	E	F	G	H
A	1	**0.8**	**0.5**	**0.5**	**0.2**	**0.05**	**0.05**	**0.001**
B	**0.8**	1	**0.8**	**0.5**	**0.001**	**0.001**	**0.001**	**0.001**
C	**0.5**	**0.8**	1	**0.8**	**0.001**	**0.001**	**0.001**	**0.05**
D	**0.5**	**0.5**	**0.8**	1	0.000001	0.000001	0.000001	0.000001
E	**0.2**	**0.2**	**0.001**	0.000001	1	**0.2**	**0.2**	**0.05**
F	**0.05**	**0.001**	**0.001**	0.000001	**0.2**	1	**0.2**	**0.05**
G	**0.05**	**0.001**	**0.001**	0.000001	**0.2**	**0.2**	1	**0.2**
H	**0.001**	**0.001**	**0.05**	0.000001	**0.05**	**0.05**	**0.2**	1

Time period: 2.8-9 Mya

	A	B	C	D	E	F	G	H
A	1	**0.8**	**0.05**	**0.05**	**0.05**	**0.001**	**0.001**	**0.001**
B	**0.8**	1	**0.2**	**0.05**	**0.001**	**0.001**	**0.001**	**0.001**
C	**0.05**	**0.2**	1	**0.8**	**0.001**	**0.001**	**0.001**	**0.05**
D	**0.05**	**0.05**	**0.8**	1	0.000001	0.000001	0.000001	0.000001
E	**0.05**	**0.2**	**0.001**	0.000001	1	**0.2**	**0.2**	**0.05**
F	**0.001**	**0.05**	**0.001**	0.000001	**0.2**	1	**0.05**	**0.05**
G	**0.001**	**0.05**	**0.001**	0.000001	**0.2**	**0.05**	1	**0.2**
H	**0.001**	**0.001**	**0.05**	0.000001	**0.05**	**0.05**	**0.2**	1

Time period: 9-20 Mya

	A	B	C	D	E	F	G	H
A	1	**0.2**	**0.001**	0.000001	**0.001**	**0.001**	**0.001**	**0.001**
B	**0.2**	1	**0.001**	0.000001	**0.001**	**0.001**	**0.001**	**0.001**
C	**0.001**	**0.001**	1	**0.8**	**0.001**	**0.001**	**0.001**	**0.001**
D	0.000001	0.000001	**0.8**	1	0.000001	0.000001	0.000001	0.000001
E	**0.001**	**0.001**	**0.001**	0.000001	1	**0.001**	**0.2**	**0.05**
F	**0.001**	**0.001**	**0.001**	0.000001	**0.001**	1	**0.001**	**0.001**
G	**0.001**	**0.001**	**0.001**	0.000001	**0.2**	**0.001**	1	**0.2**
H	**0.001**	**0.001**	**0.001**	0.000001	**0.05**	**0.001**	**0.2**	1

Time period: 20-32 Mya

	A	B	C	D	E	F	G	H
A	1	0.000001	**0.001**	0.000001	**0.001**	**0.001**	**0.001**	**0.001**
B	0.000001	1	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
C	**0.001**	0.000001	1	**0.8**	**0.001**	**0.001**	**0.001**	**0.05**
D	0.000001	0.000001	**0.8**	1	0.000001	0.000001	0.000001	0.000001
E	**0.001**	0.000001	**0.001**	0.000001	1	**0.001**	**0.8**	**0.05**
F	**0.001**	0.000001	**0.001**	0.000001	**0.001**	1	**0.001**	**0.001**
G	**0.001**	0.000001	**0.001**	0.000001	**0.8**	**0.001**	1	**0.2**
H	**0.001**	0.000001	**0.05**	0.000001	**0.05**	**0.001**	**0.2**	1

Time period: 32-38 Mya

	A	B	C	D	E	F	G	H
A	1	0.000001	**0.001**	0.000001	**0.05**	**0.05**	**0.001**	**0.001**
B	0.000001	1	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
C	**0.001**	0.000001	1	**0.8**	**0.5**	**0.5**	**0.5**	**0.8**
D	0.000001	0.000001	**0.8**	1	**0.5**	**0.5**	**0.5**	**0.5**
E	**0.05**	0.000001	**0.5**	**0.5**	1	**0.8**	**0.8**	**0.5**
F	**0.05**	0.000001	**0.5**	**0.5**	**0.8**	1	**0.8**	**0.5**
G	**0.001**	0.000001	**0.5**	**0.5**	**0.8**	**0.8**	1	**0.8**
H	**0.001**	0.000001	**0.5**	**0.5**	**0.5**	**0.5**	**0.8**	1

Time period: 38-55 Mya

	A	B	C	D	E	F	G	H
A	1	0.000001	**0.001**	**0.001**	0.000001	0.000001	0.000001	0.000001
B	0.000001	1	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
C	**0.001**	0.000001	1	**0.8**	0.000001	0.000001	0.000001	0.000001
D	**0.001**	0.000001	**0.8**	1	0.000001	0.000001	0.000001	0.000001
E	0.000001	0.000001	0.000001	0.000001	1	0.000001	0.000001	0.000001
F	0.000001	0.000001	0.000001	0.000001	0.000001	1	0.000001	0.000001
G	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	1	0.000001
H	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	1

Time period: 55-130 Mya

	A	B	C	D	E	F	G	H
A	1	0.000001	**0.05**	**0.001**	0.000001	0.000001	0.000001	0.000001
B	0.000001	1	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001
C	**0.05**	0.000001	1	**0.8**	0.000001	0.000001	0.000001	0.000001
D	**0.001**	0.000001	**0.8**	1	0.000001	0.000001	0.000001	0.000001
E	0.000001	0.000001	0.000001	0.000001	1	0.000001	0.000001	0.000001
F	0.000001	0.000001	0.000001	0.000001	0.000001	1	0.000001	0.000001
G	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	1	0.000001
H	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	0.000001	1

0.000001	impossible (= 0)
0.001	long distance dispersal (separated by more than 200 km of sea)
0.05	connection by island chain
0.2	adjacent islands/blocks (separated by less than 200 km of sea)
0.5	non-adjacent continental areas
0.8	adjacent continental areas

A	North America
B	Central America
C	northern South America
D	South America
E	Cuba
F	Jamaica
G	Hispaniola
H	Puerto Rico

BioGeoBEARS analysis: areas allowed matrix

Time period: 0-2.8 Mya

	A	B	C	D	E	F	G	H
A	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1

A	North America
B	Central America
C	northern South America
D	South America
E	Cuba
F	Jamaica
G	Hispaniola
H	Puerto Rico

Time period: 2.8-9 Mya

	A	B	C	D	E	F	G	H
A	1	1	1	1	1	1	1	1
B	1	1	1	1	1	1	1	1
C	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1
E	1	1	1	1	1	1	1	1
F	1	1	1	1	1	1	1	1
G	1	1	1	1	1	1	1	1
H	1	1	1	1	1	1	1	1

Time period: 9-20 Mya

	A	B	C	D	E	F	G	H
A	1	1	1	1	0	0	0	0
B	1	1	1	1	0	0	0	0
C	1	1	1	1	1	1	1	1
D	1	1	1	1	1	1	1	1
E	0	0	1	1	1	1	1	1
F	0	0	1	1	1	1	1	1
G	0	0	1	1	1	1	1	1
H	0	0	1	1	1	1	1	1

Time period: 20-32 Mya

	A	B	C	D	E	F	G	H
A	1	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0
C	0	0	1	1	1	1	1	1
D	0	0	1	1	0	0	0	0
E	0	0	1	0	1	1	1	1
F	0	0	1	0	1	1	1	1
G	0	0	1	0	1	1	1	1
H	0	0	1	0	1	1	1	1

Time period: 32-38 Mya

	A	B	C	D	E	F	G	H
A	1	0	1	1	1	1	1	1
B	0	0	0	0	0	0	0	0
C	1	0	1	1	1	1	1	1
D	1	0	1	1	1	1	1	1
E	1	0	1	1	1	1	1	1
F	1	0	1	1	1	1	1	1
G	1	0	1	1	1	1	1	1
H	1	0	1	1	1	1	1	1

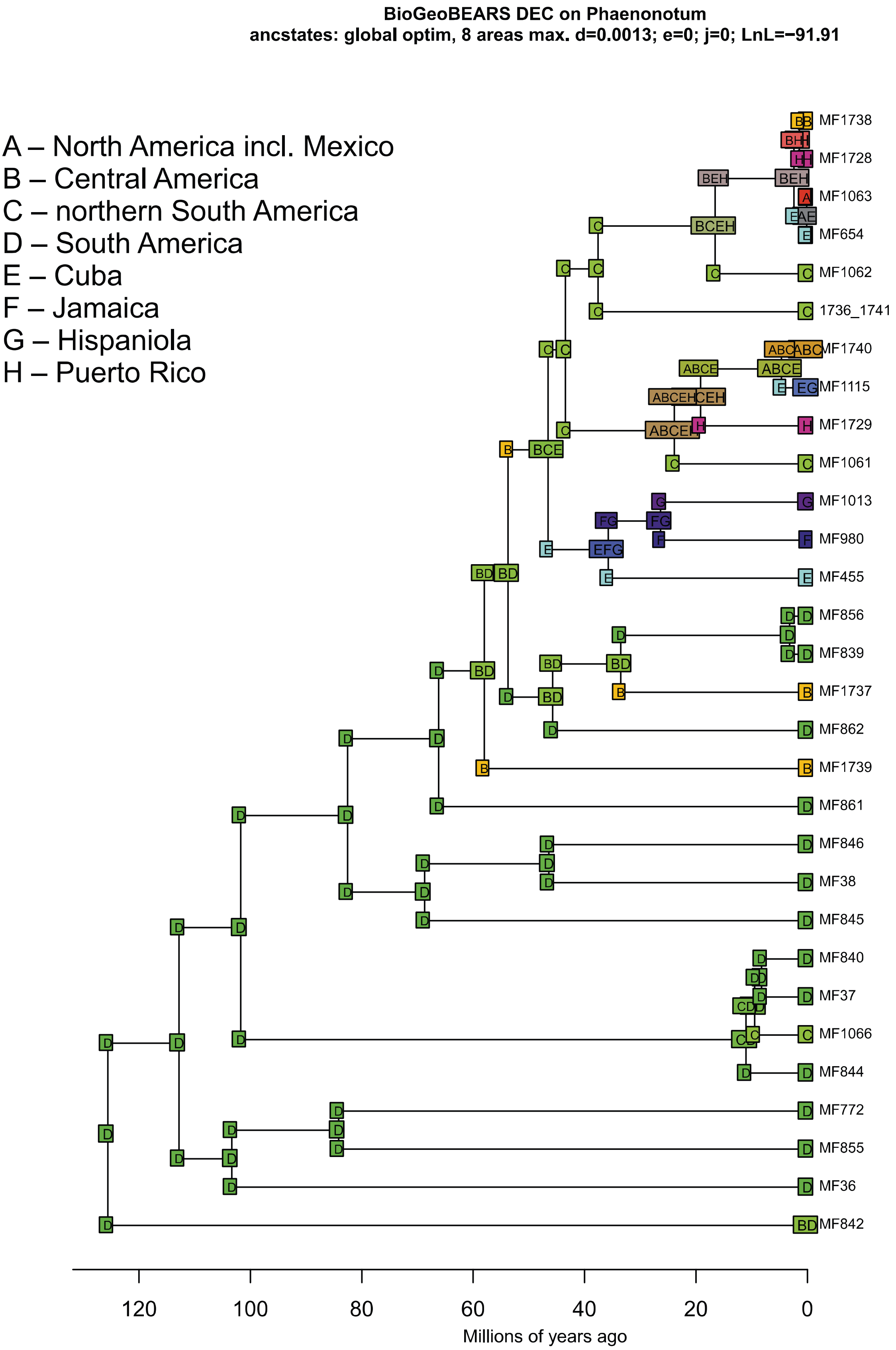
Time period: 38-55 Mya

	A	B	C	D	E	F	G	H
A	1	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0
C	0	0	1	1	0	0	0	0
D	0	0	1	1	0	0	0	0
E	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0

Time period: 55-130 Mya

	A	B	C	D	E	F	G	H
A	1	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0
C	0	0	1	1	0	0	0	0
D	0	0	1	1	0	0	0	0
E	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0
H	0	0	0	0	0	0	0	0

BioGeoBEARS analyses:
non-time-stratified analysis, DEC model



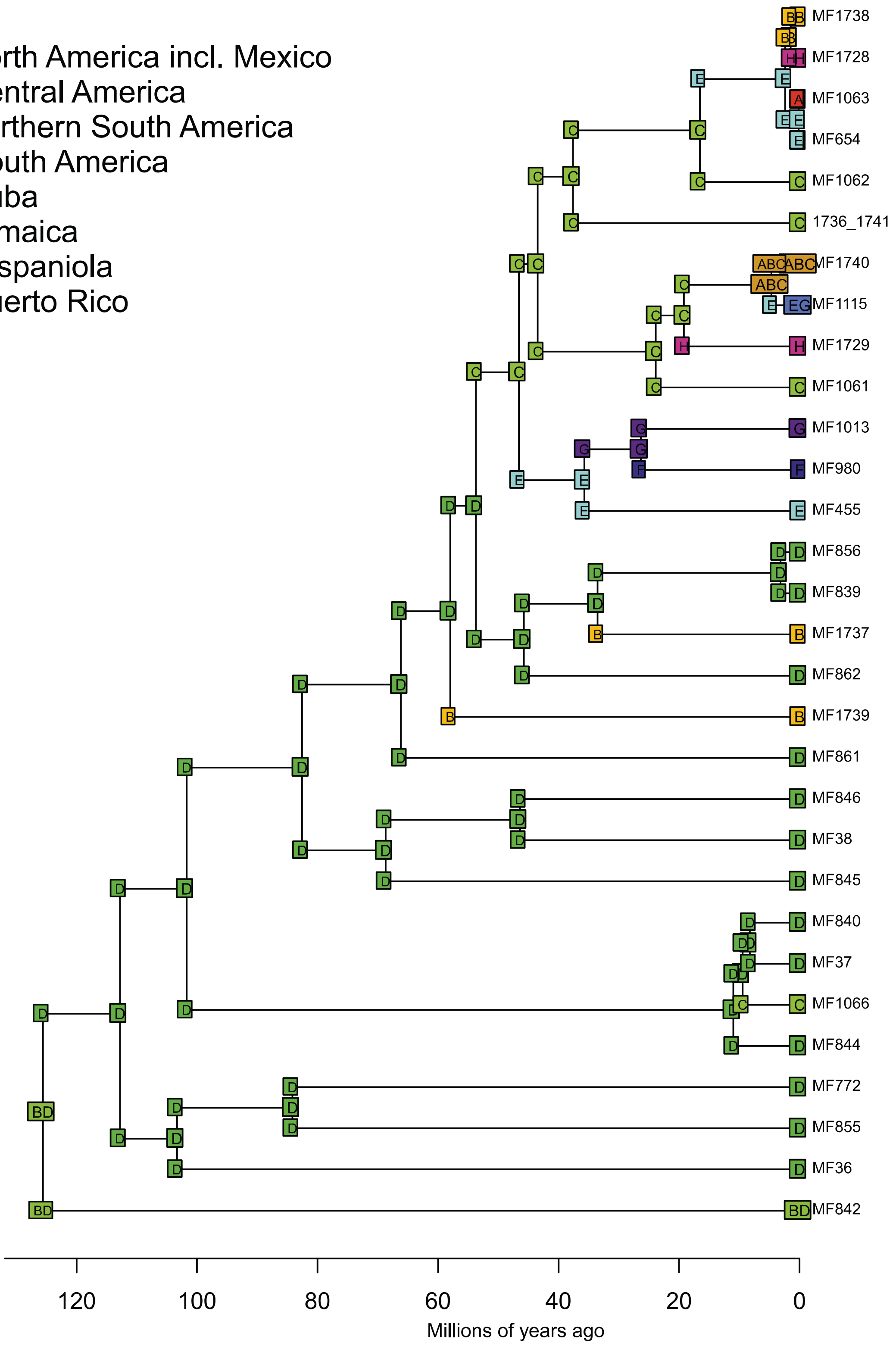
BioGeoBEARS DEC on Phaenonotum
ancstates: global optim, 8 areas max. d=0.0013; e=0; j=0; LnL=−91.91

A – North America incl. Mexico
B – Central America
C – northern South America
D – South America
E – Cuba
F – Jamaica
G – Hispaniola
H – Puerto Rico

BioGeoBEARS analyses:
non-time-stratified analysis, DEC+J model

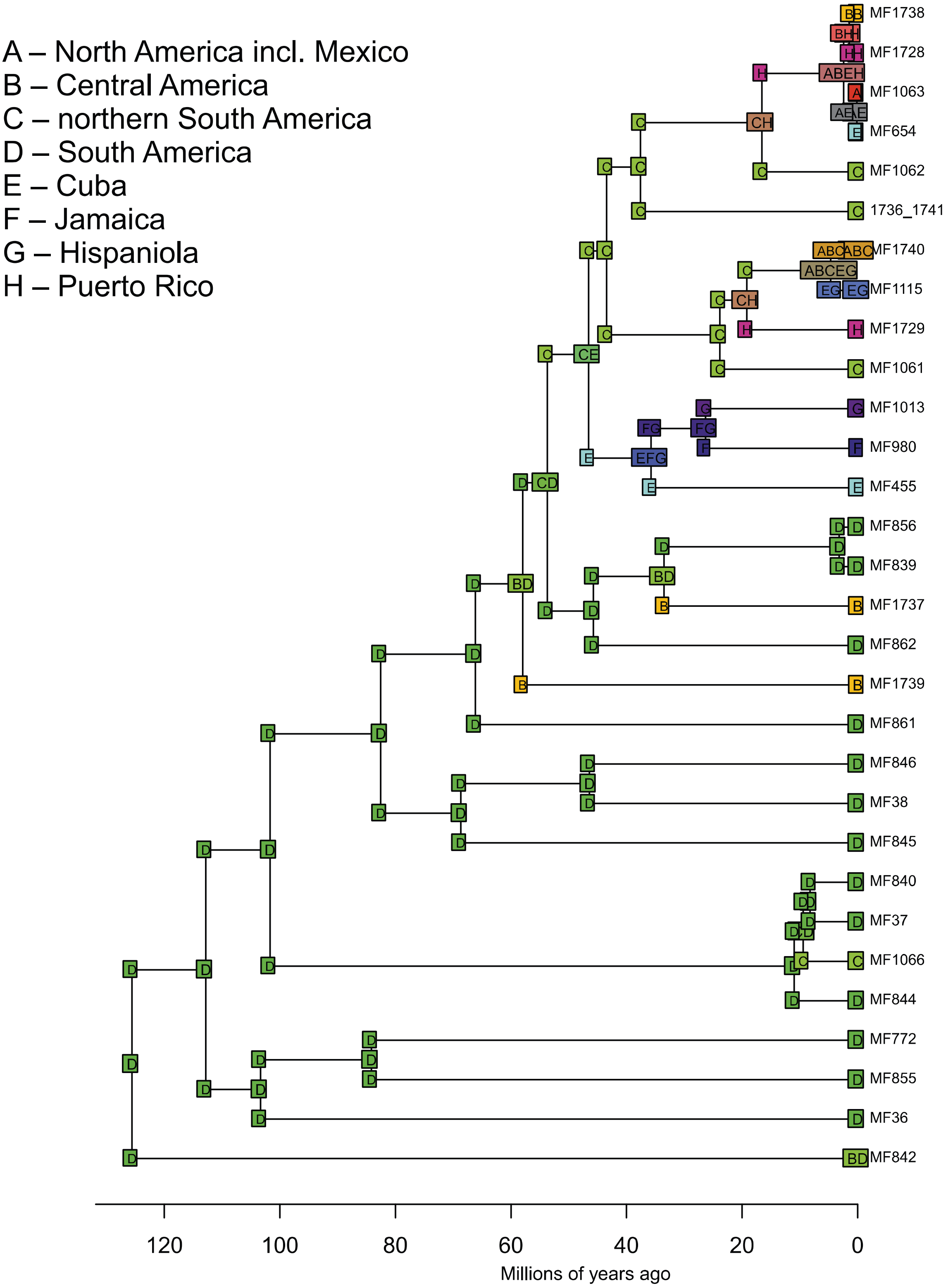
BioGeoBEARS DEC+J on Phaenonotum
ancstates: global optim, 8 areas max. d=4e−04; e=0; j=0.0464; LnL=−70.21

A – North America incl. Mexico
B – Central America
C – northern South America
D – South America
E – Cuba
F – Jamaica
G – Hispaniola
H – Puerto Rico



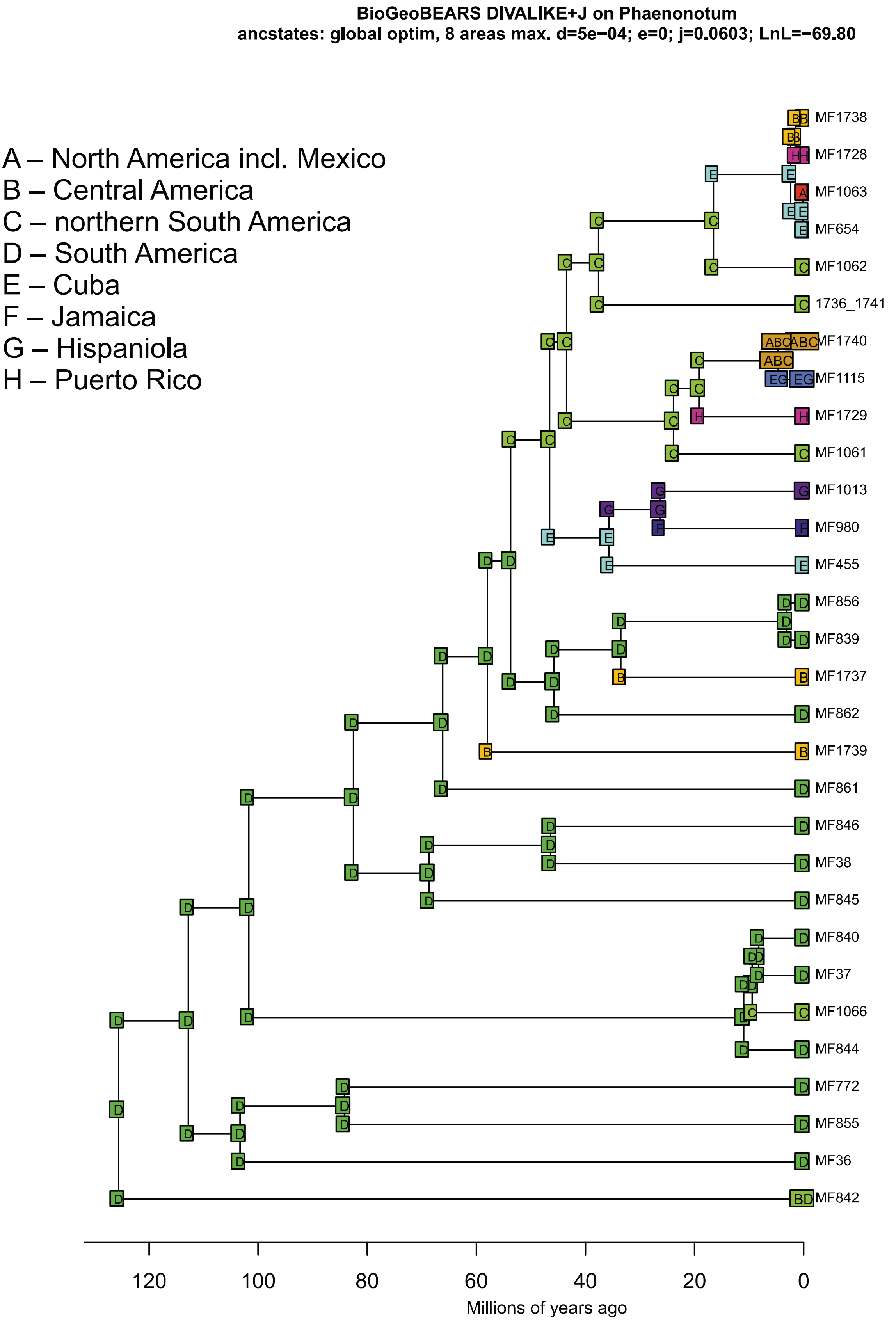
BioGeoBEARS analyses:
non-time-stratified analysis, DIVALIKE model

BioGeoBEARS DIVALIKE on Phaenonotum
ancstates: global optim, 8 areas max. d=0.0016; e=0; j=0; LnL=−84.36

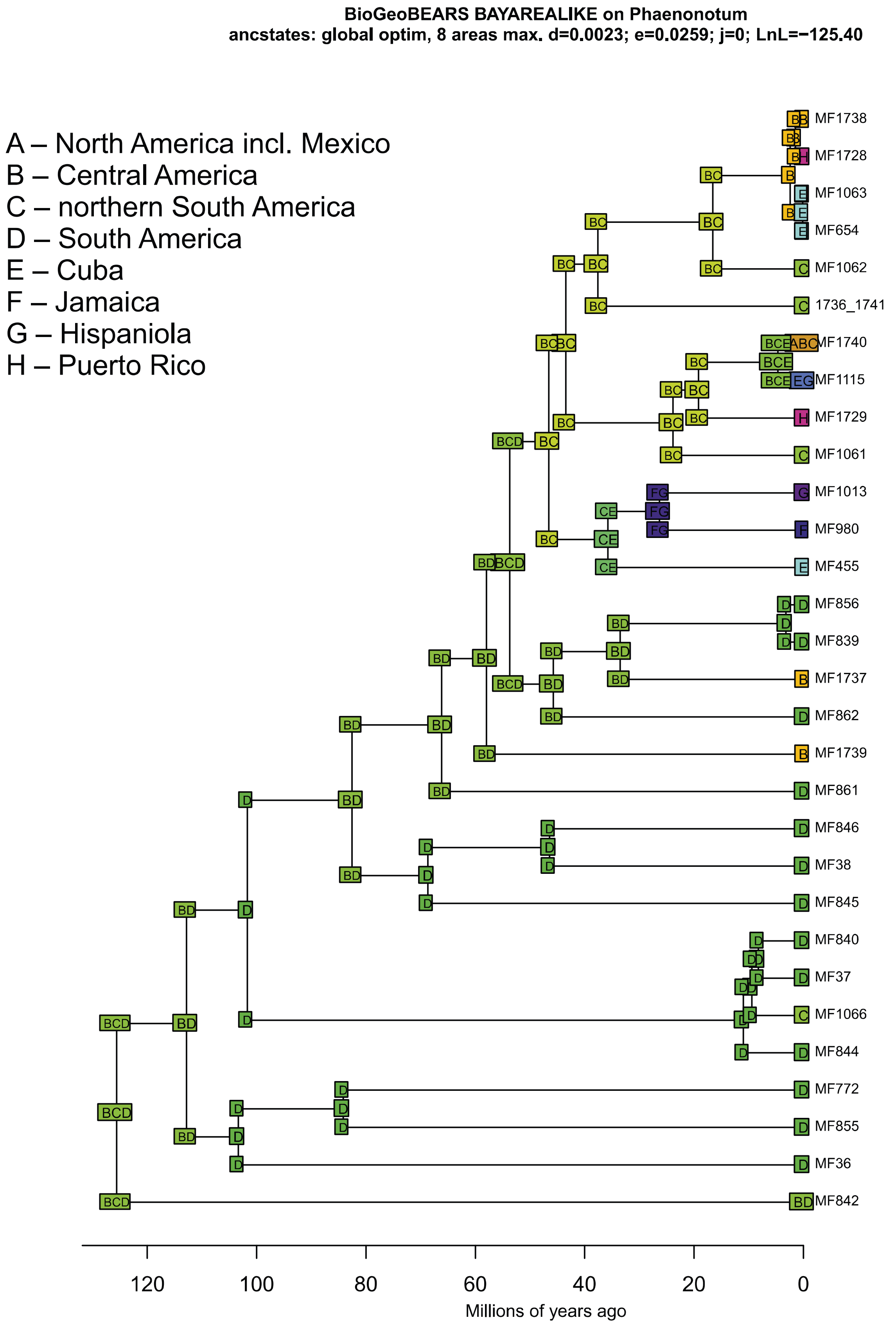


BioGeoBEARS analyses:

non-time-stratified analysis, DIVALIKE+J model



BioGeoBEARS analyses:
non-time-stratified analysis, BAYAREALIKE model

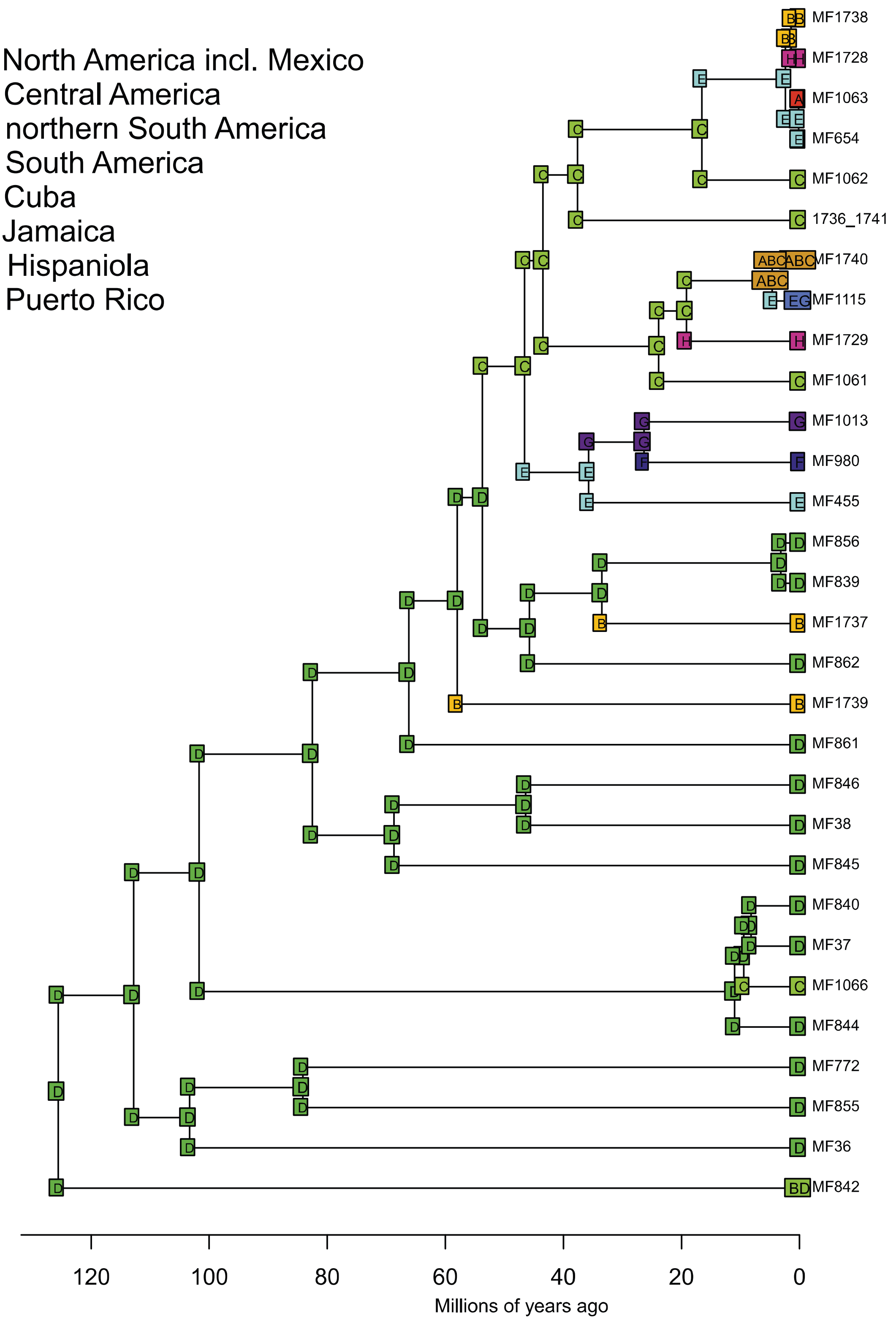


BioGeoBEARS analyses:

non-time-stratified analysis, BAYAREALIKE+J model

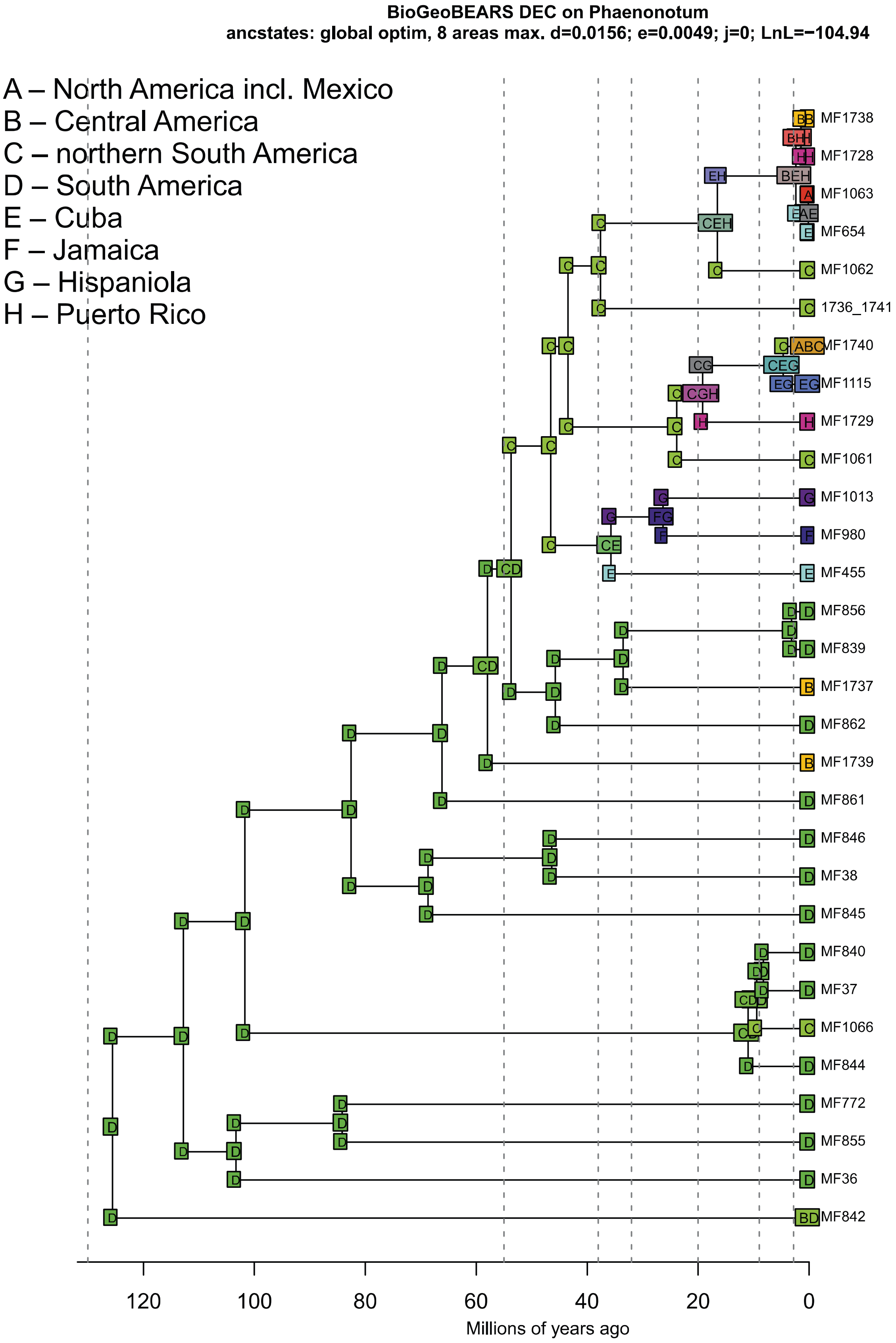
BioGeoBEARS BAYAREALIKE+J on Phaenonotum
ancstates: global optim, 8 areas max. d=4e−04; e=0; j=0.0491; LnL=−70.56

A – North America incl. Mexico
B – Central America
C – northern South America
D – South America
E – Cuba
F – Jamaica
G – Hispaniola
H – Puerto Rico

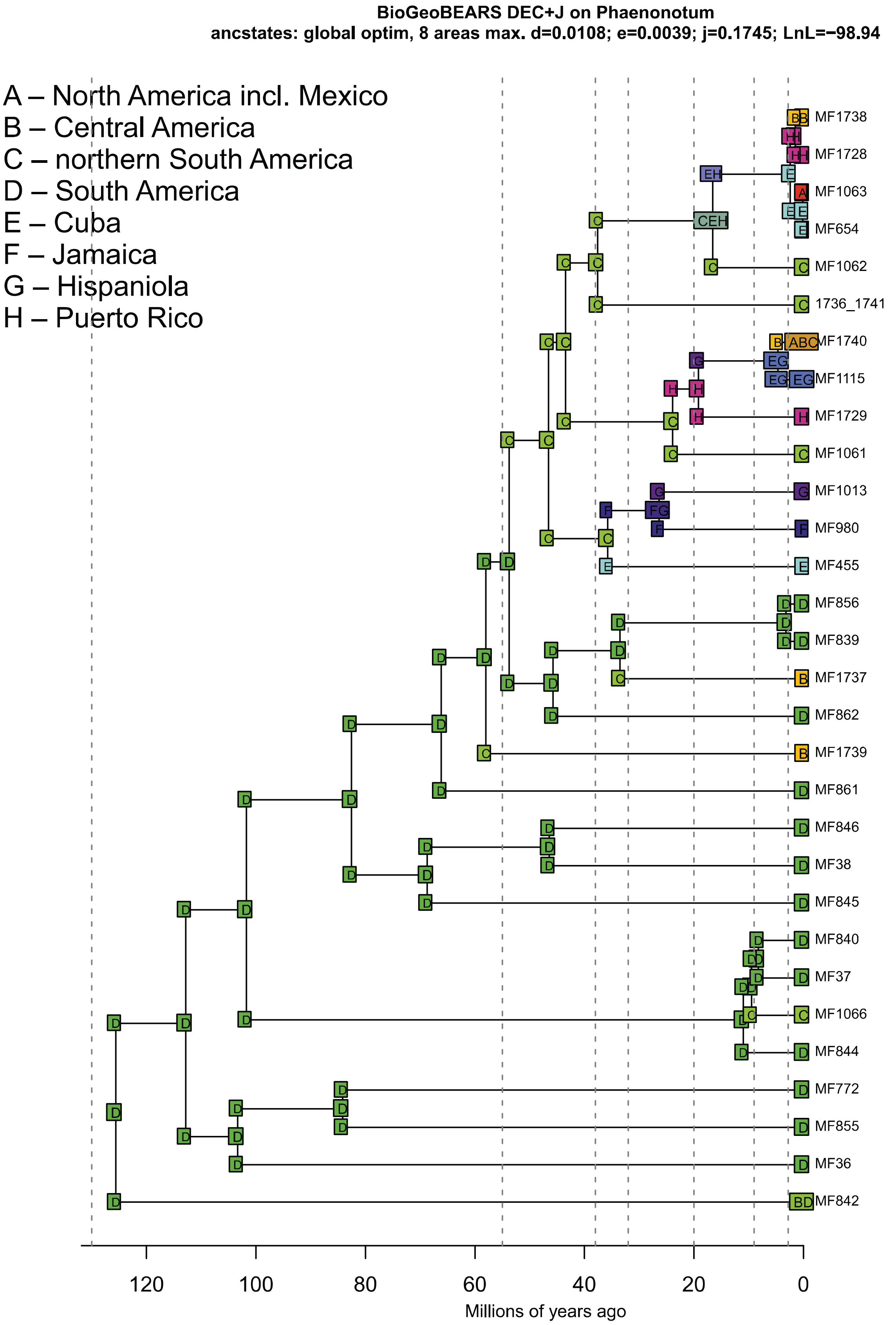


BioGeoBEARS analyses:

time-stratified analysis, DEC model

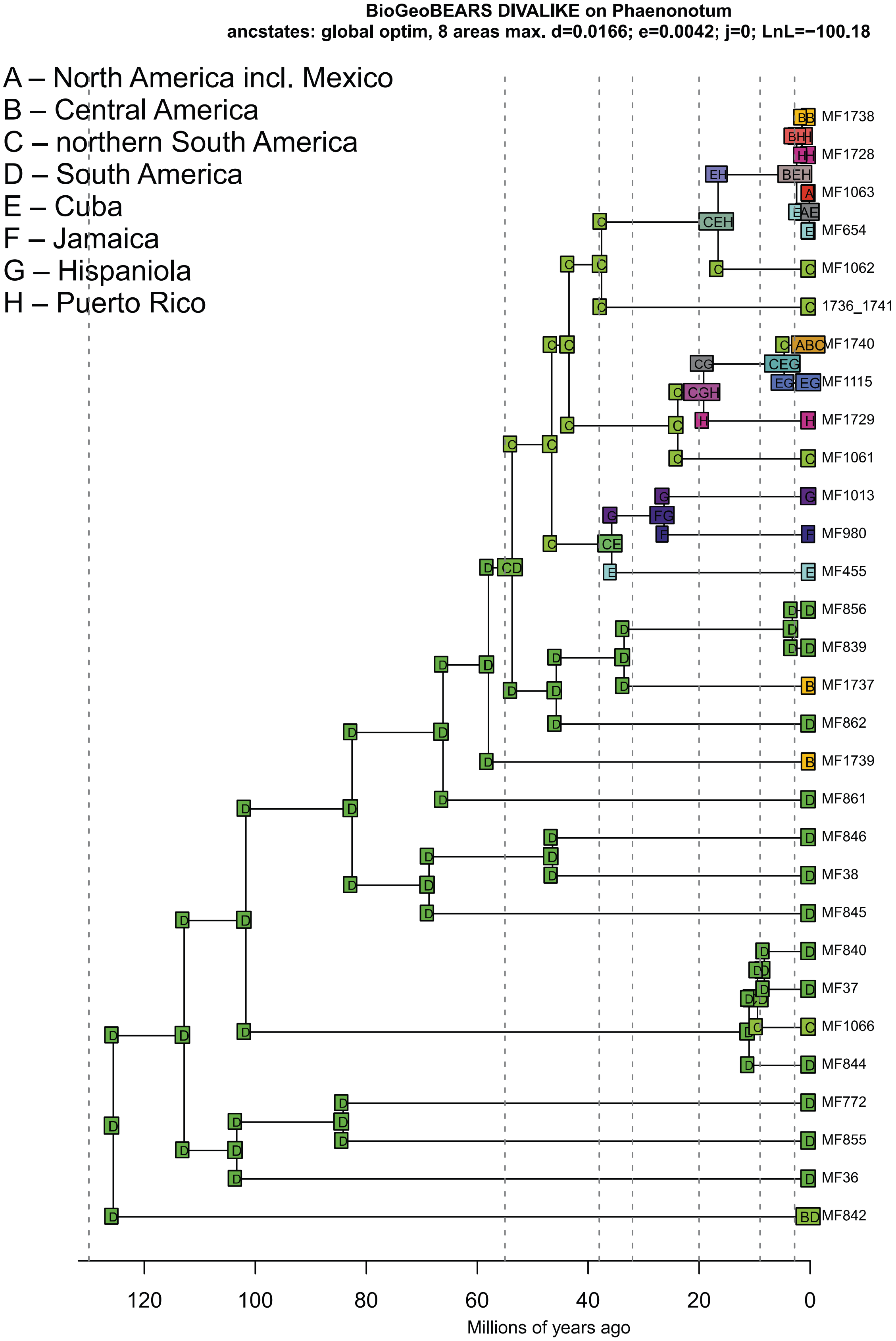


BioGeoBEARS analyses:
time-stratified analysis, DEC+J model

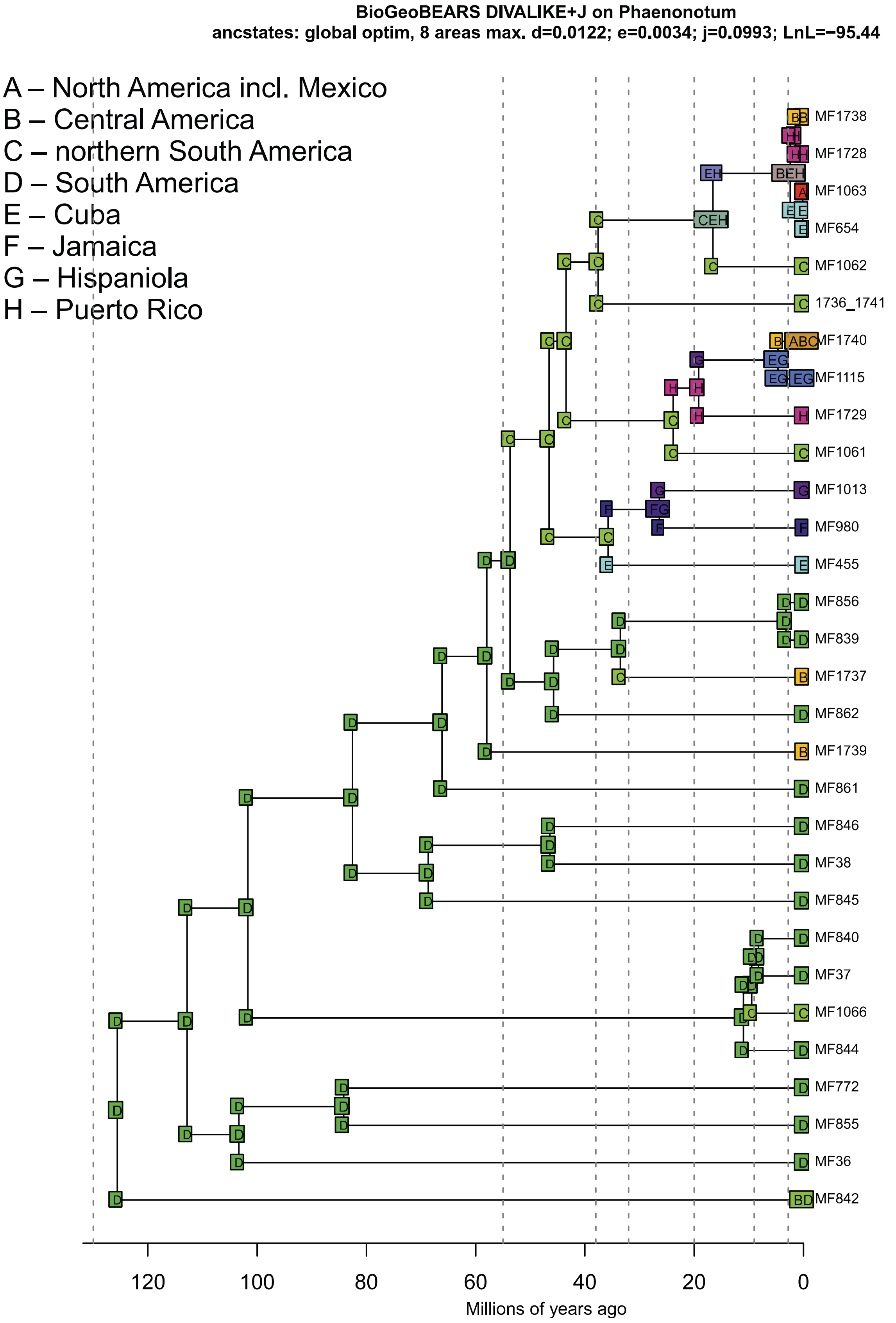


BioGeoBEARS analyses:

time-stratified analysis, DIVALIKE model

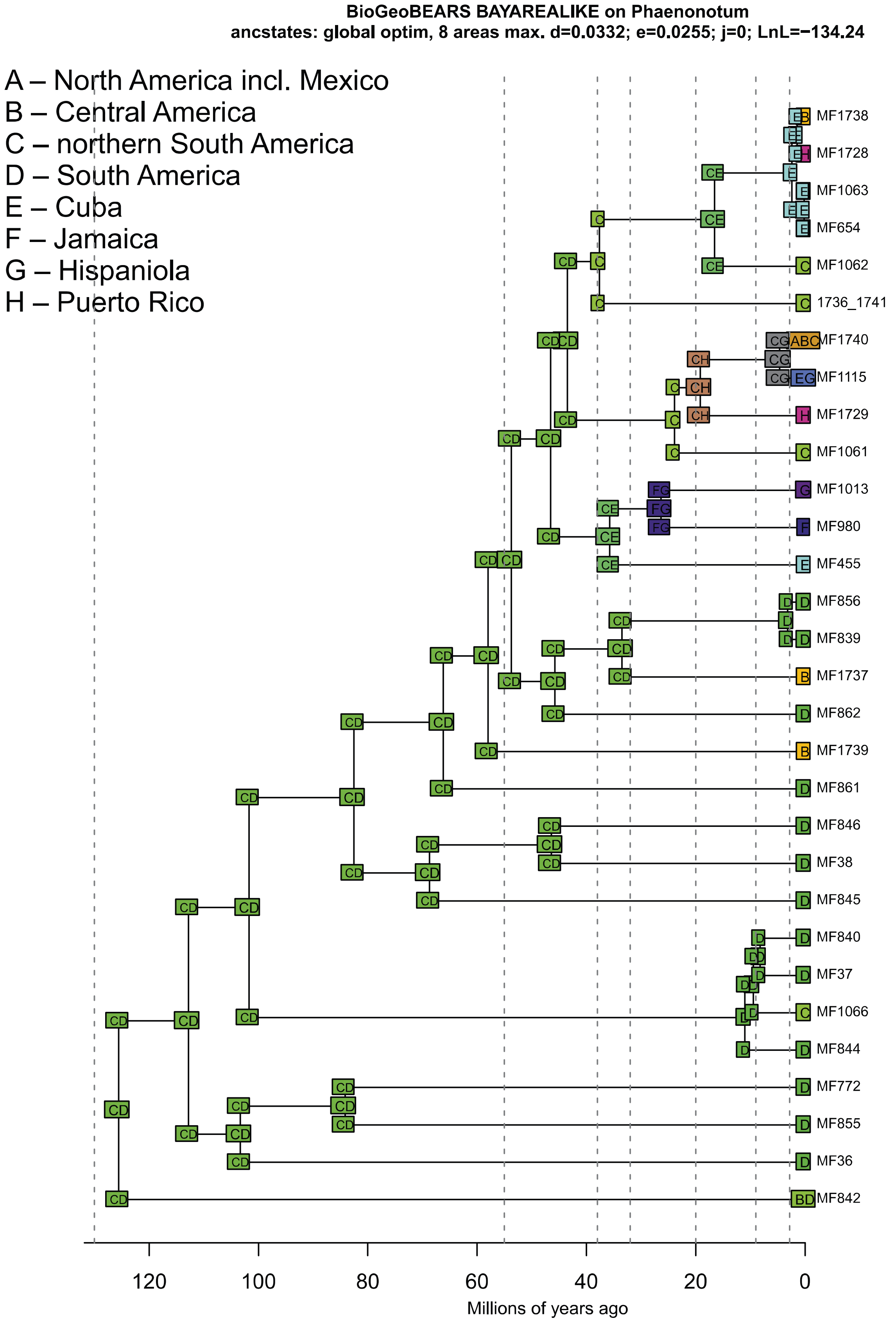


BioGeoBEARS analyses:
time-stratified analysis, DIVALIKE+J model



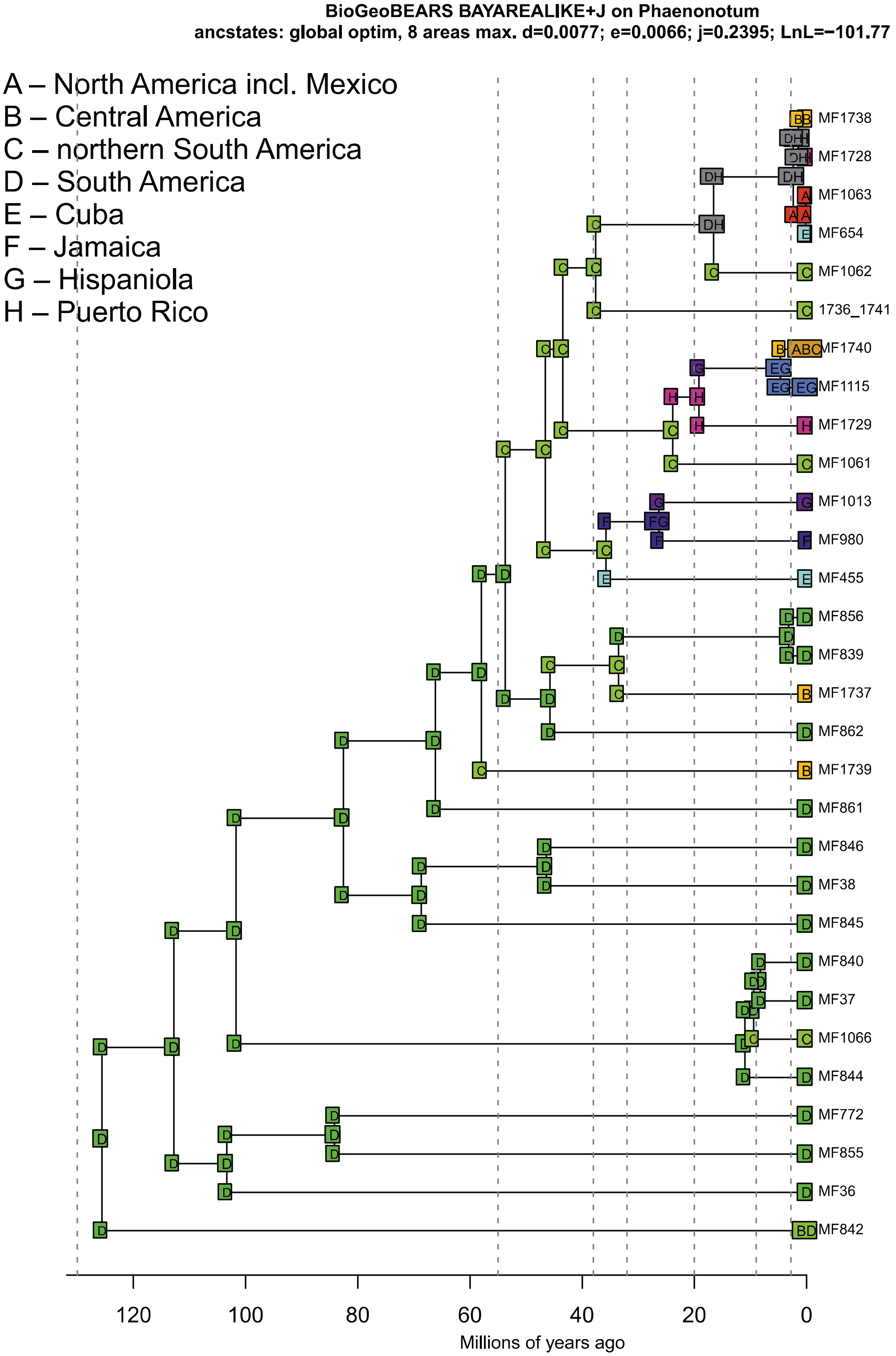
BioGeoBEARS DIVALIKE+J on Phaenonotum
ancstates: global optim, 8 areas max. d=0.0122; e=0.0034; j=0.0993; LnL=−95.44

BioGeoBEARS analyses:
time-stratified analysis, BAYAREALIKE model

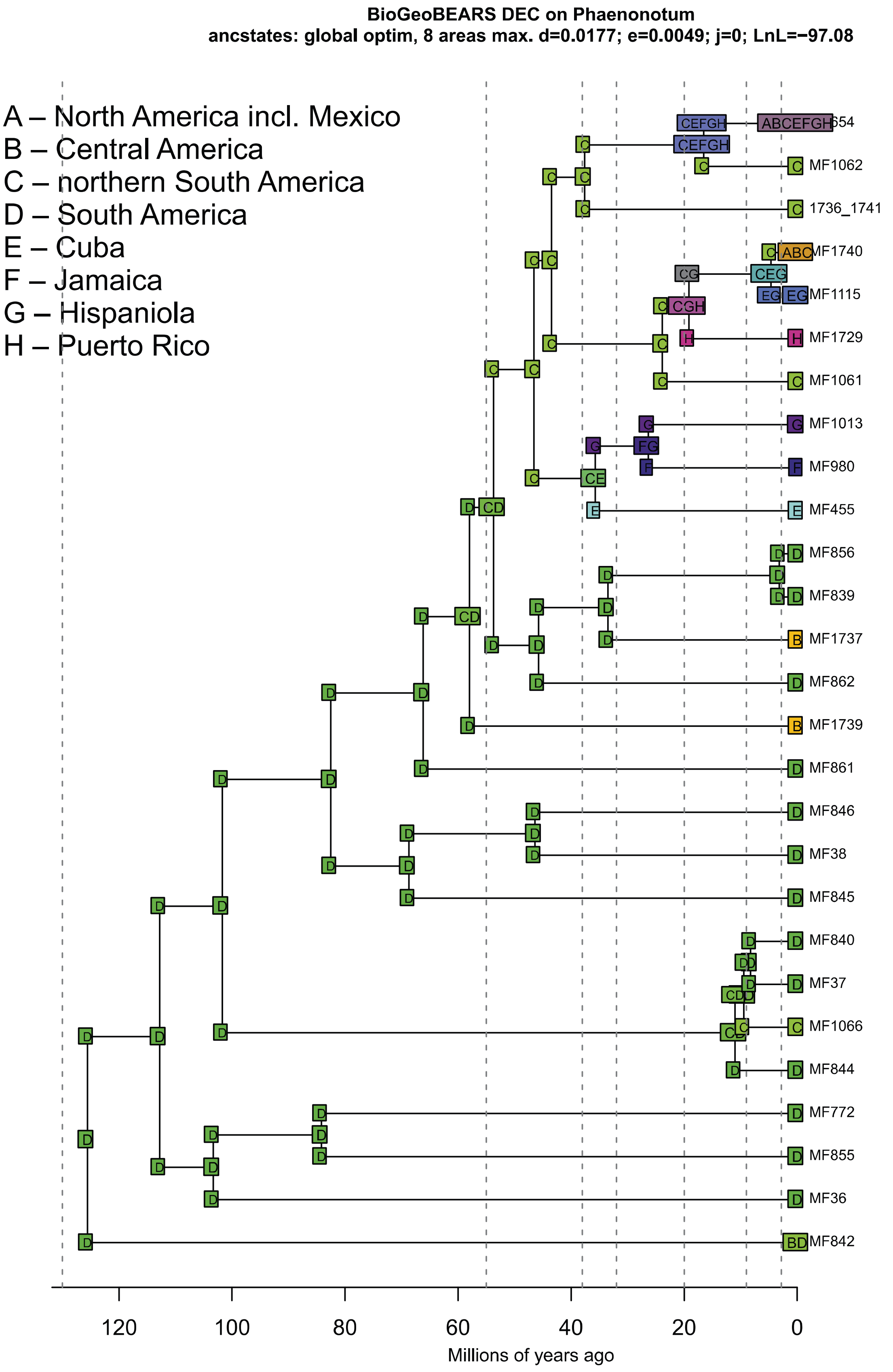


BioGeoBEARS analyses:

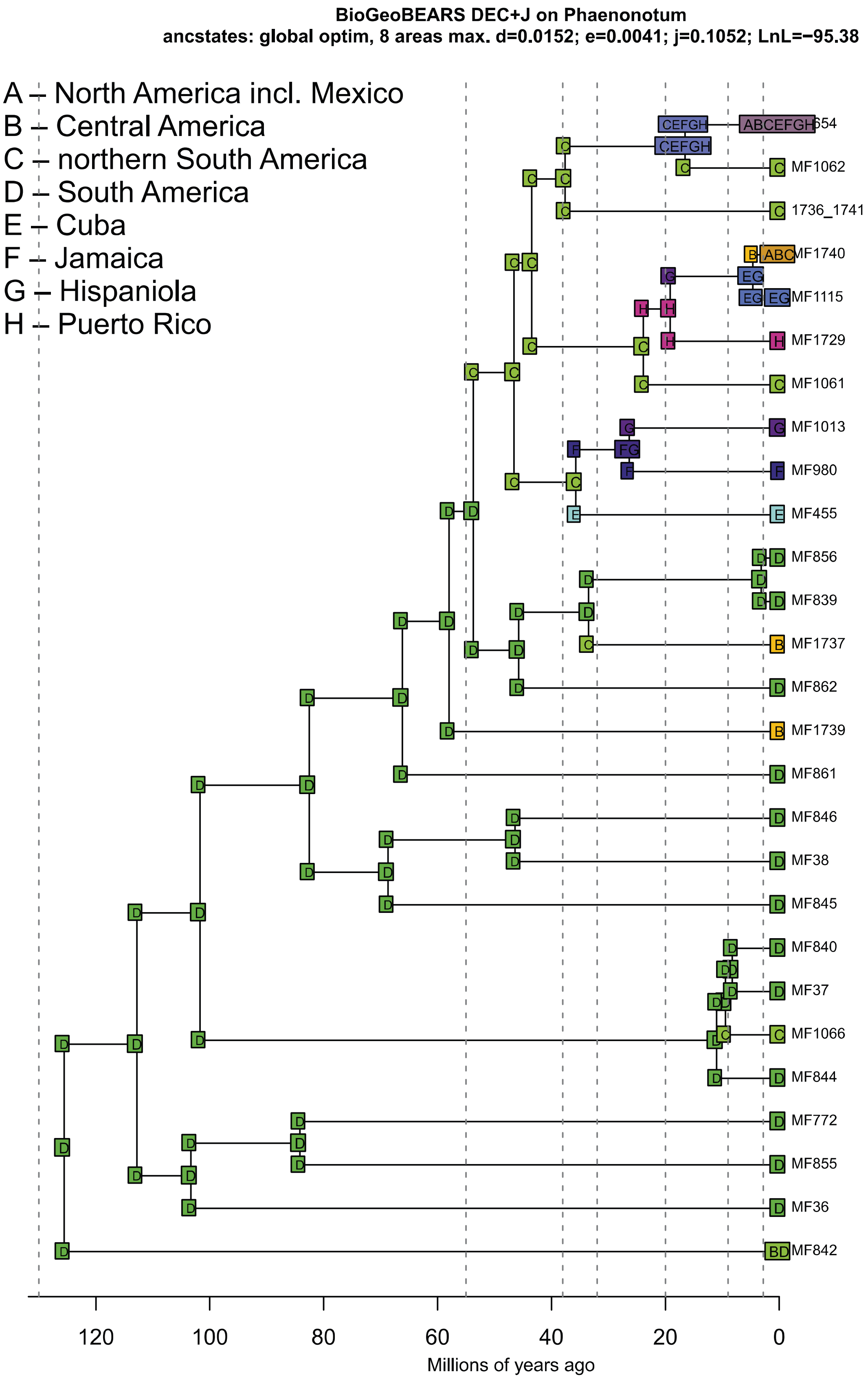
time-stratified analysis, BAYAREALIKE+J model



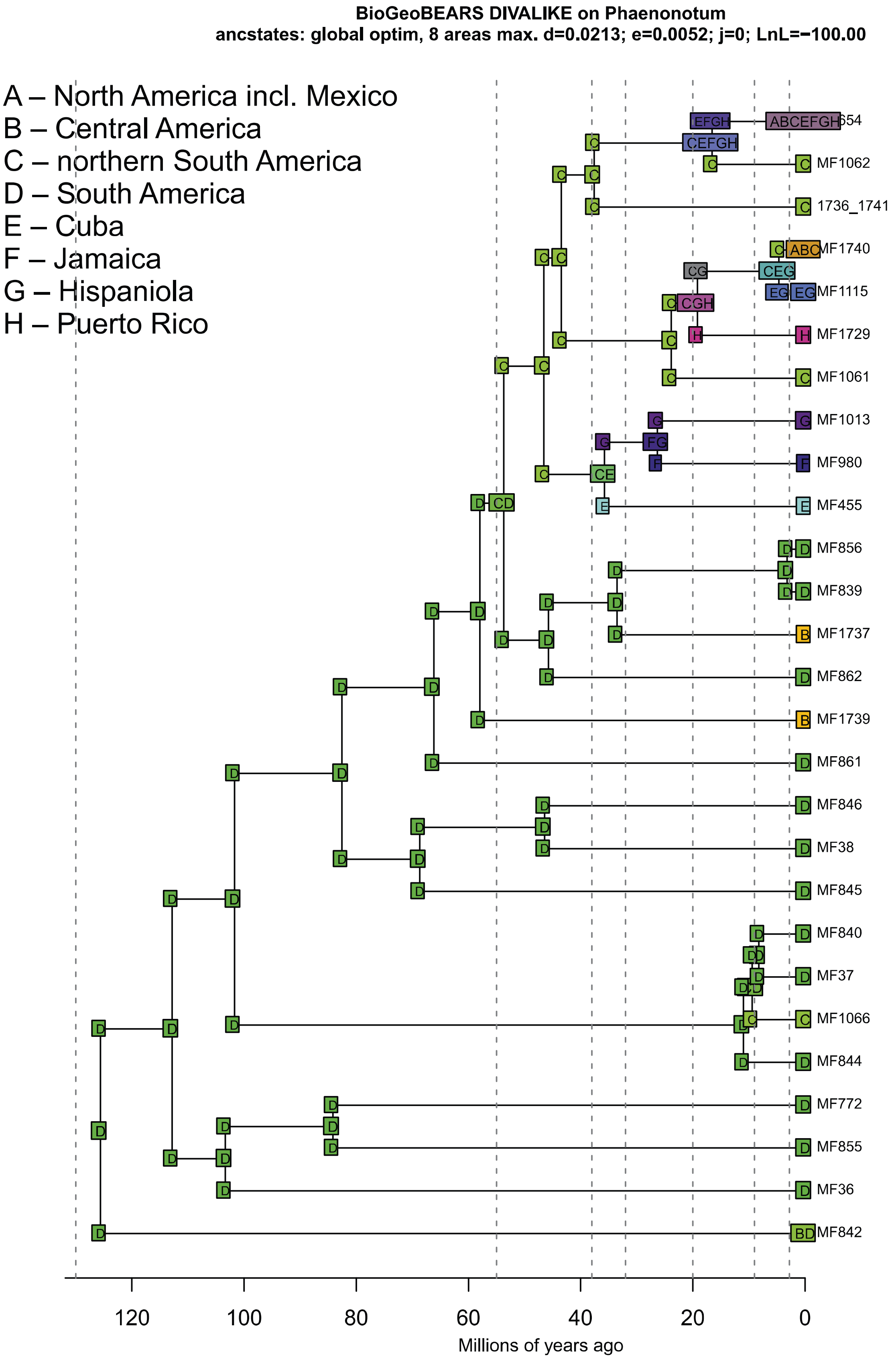
BioGeoBEARS analyses (P. exstriatum as 1 terminal): time-stratified analysis, DEC model



BioGeoBEARS analyses (P. exstriatum as 1 terminal): time-stratified analysis, DEC+J model



BioGeoBEARS analyses (P. exstriatum as 1 terminal): time-stratified analysis, DIVALIKE model



BioGeoBEARS analyses (P. exstriatum as 1 terminal)**:** time-stratified analysis, DIVALIKE+J model

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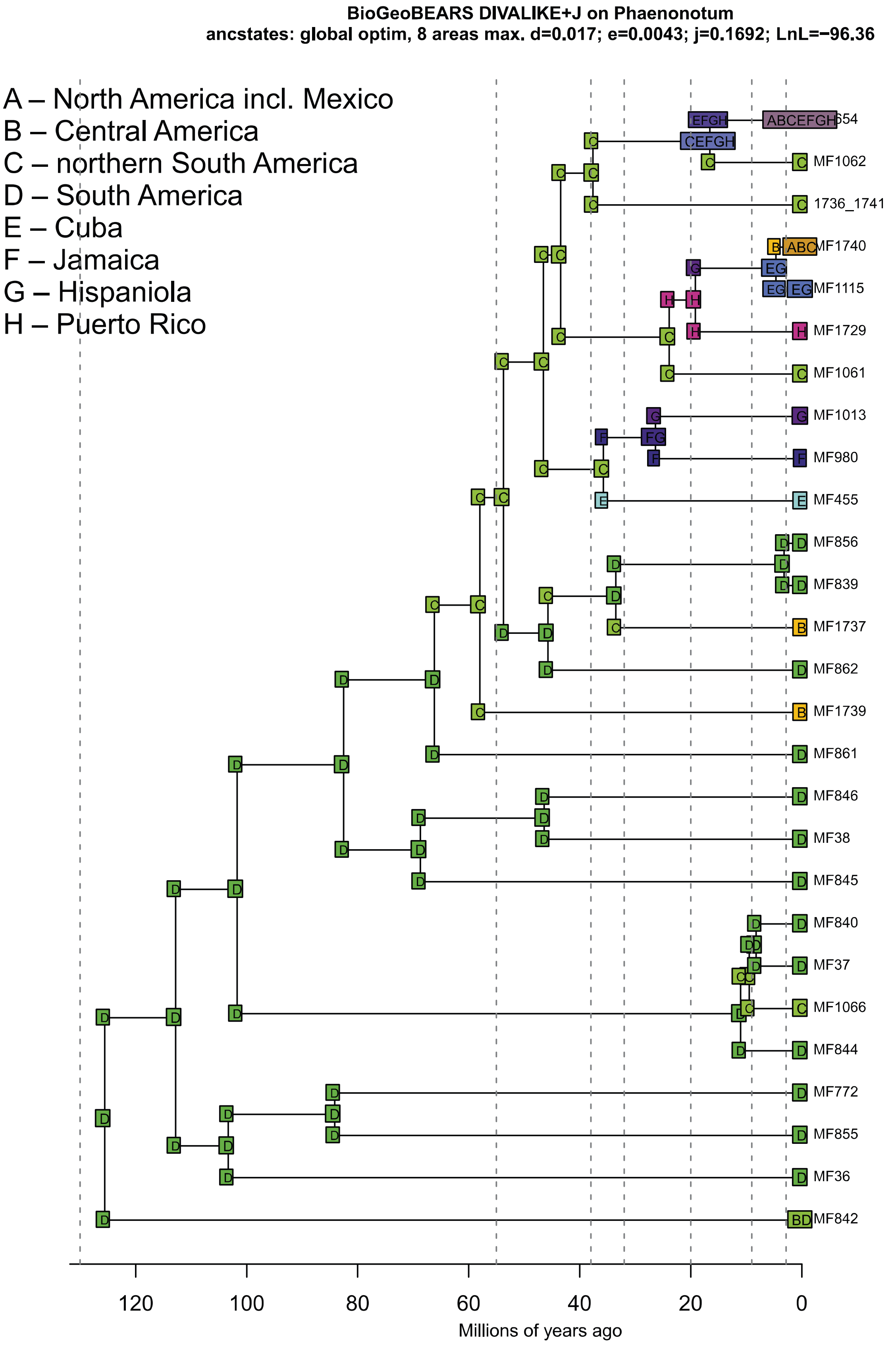

BioGeoBEARS analyses (P. exstriatum as 1 terminal): time-stratified analysis, BAYAREALIKE model

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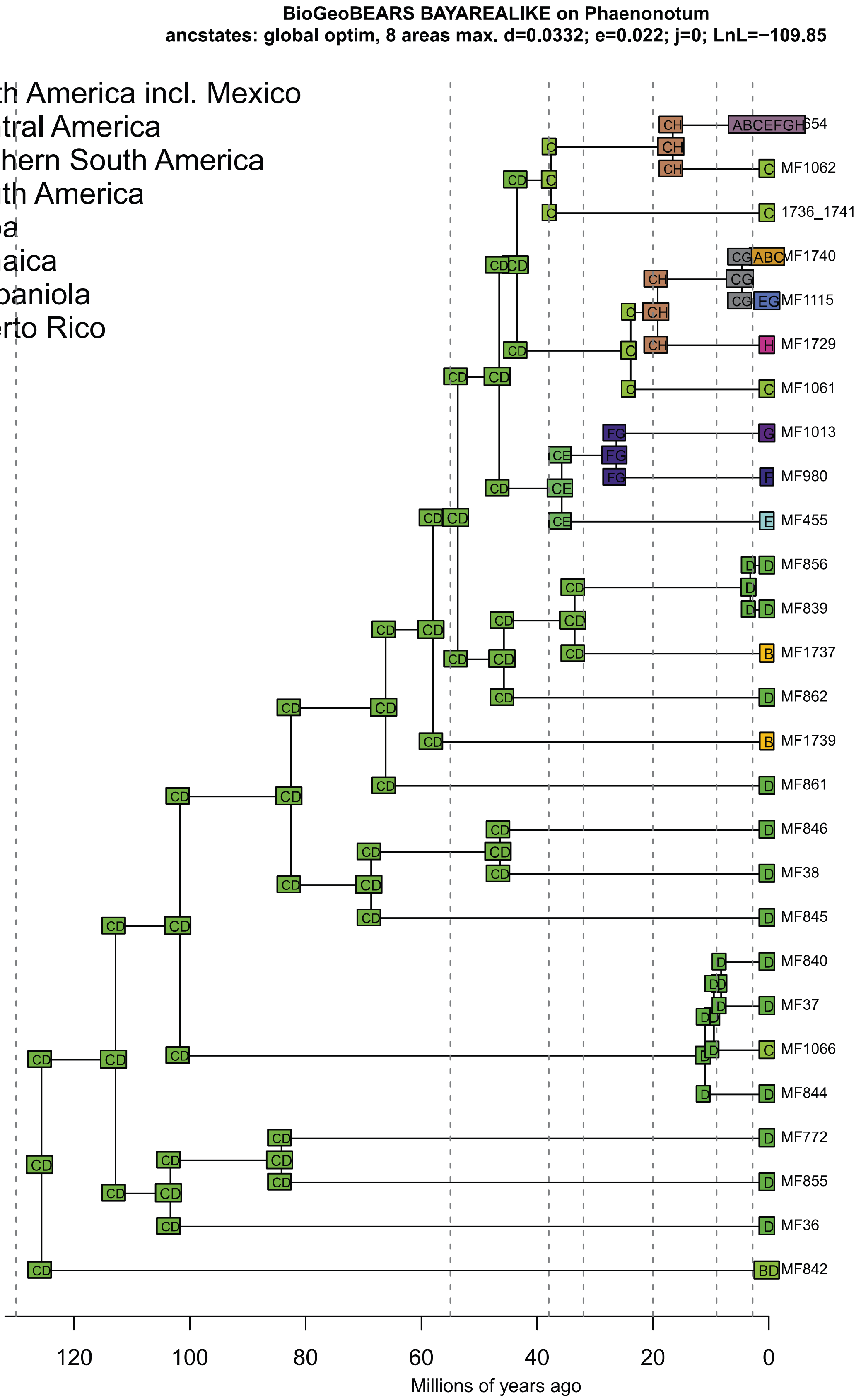
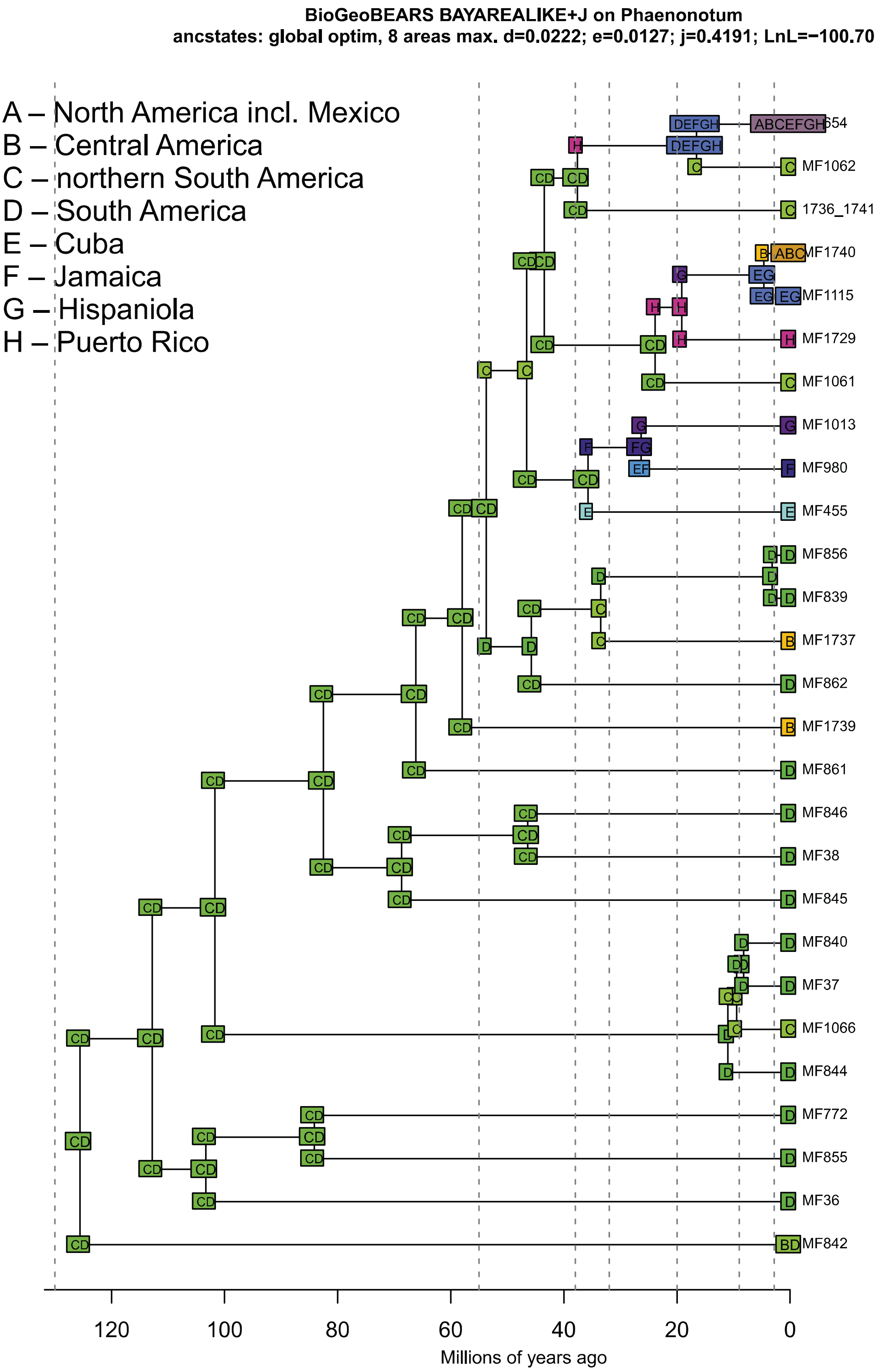
A – North America incl. Mexico
B – Central America
C – northern South America
D – South America
E – Cuba
F – Jamaica
G – Hispaniola
H – Puerto Rico



BioGeoBEARS analyses (P. exstriatum as 1 terminal)**:**
time-stratified analysis, BAYAREALIKE+J model



CHAPTER 2

Deler-Hernández A., Sýkora V, Fikáček M. A review of the genus *Crenitulus* of Greater Antilles (Coleoptera: Hydrophilidae)

(manuscript draft)

A review of the genus *Crenitulus* of Greater Antilles (Coleoptera: Hydrophilidae)

(manuscript draft)

Albert Deler-Hernández[1)], Vít Sýkora[1)] & Martin Fikáček[2, 1)]

[1)] Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 43, Czech Republic; e-mails: adeler1982@gmail.com

[2)] Department of Entomology, National Museum, Cirkusová 1740, CZ-19100, Prague 9, Czech Republic; e-mail: mfikacek@gmail.com

Abstract. The genus *Crenitulus* Winters, 1928 of the Greater Antilles is reviewed, based on the recently collected material. Phylogenetic analyses based on three mitochondrial (*cox1*, *cox2* and 16S) and two nuclear genes (18S rRNA and 28S rRNA) revealed that Greater Antillean *Crenitulus* belong to two separate clades: the *Crenitulus yunque* clade endemic for Cuba and Hispaniola, and the *Crenitulus suturalis* clade containing specimens from Greater Antilles (Cuba, Jamaica and Hispaniola) and from northern America. We performed a detailed revision of the *Crenitulus yunque* clade, using the combination of molecular data (GMYC species delimitation using three genes: *cox1*, 16S and ITS2) and morphology. Both methods revealed a highly congruent results, recognizing eleven species locally endemic for particular mountain ranges; all these species are described, diagnosed and illustrated. Two species are described from the Dominican Republic: *C. dominicensis* sp. nov. (Cordillera Central) and *C. smithi* sp. nov. (Sierra de Neiba and Cordillera Central). Nine species are described from Cuba: *C. cristal* sp. nov. (Sierra Cristal), *C. guamuhuya* sp. nov. (Guamuyaha Mts.), *C. guanyangi* sp. nov. (Cuchillas de Baracoa), *C. iviei* sp. nov. (Nipe-Sagua-Barracoa Mts.), *C. jiri* sp. nov. (Sierra Maestra Mts. and Cuchilla de Baracoa), *C. lajoncherei* sp. nov. (Sierra del Rosario), *C. naranjoi* sp. nov. (Sierra Maestra and Nipe-Sagua-Baracoa Mts.), *C. riberai* sp. nov. (Sierra Maestra Mts.) and *C. yunque* sp. nov. (Cuchillas de Baracoa). We are summarizing the known distribution of the *C. suturalis* group in Greater Antilles, but the taxonomy of the group is not examined here in detail due to insufficient material available.

Key words. Coleoptera, Hydrophilidae, water beetles, lotic habitat, Greater Antilles, Cuba, Jamaica, Hispaniola, species delimitation, new species

Resumen. El género *Crenitulus* Winters, 1928 de las Antillas Mayores es revisado basado en material colectado recientemente. El análisis filogénetico realizado, usando tres genes mitocondriales (*cox1*, *cox2* y 16S) y dos nucleares (18S rRNA y 28S rRNA) revelaron que los *Crenitulus* de las Antillas Mayores pertenecen a dos clados: el clado *Crenitulus yunque* endémico para Cuba y La Española y el clado *Crenitulus suturalis* con ejemplares presentes en las Antillas Mayores (Cuba, Jamaica y La Española) y Norteamérica. La revisión del clado *Crenitulus yunque* se realizó combinando datos moleculares (GMYC delimitación de especies usando *cox1*, 16S y ITS2) y morfológicos. Ambos métodos revelaron resultados altamente confiables, reconociendo 11 nuevas especies de ambientes montañosos; todas las especies son descritas e ilustradas. Dos especies son descritas para República Dominicana: *C. dominicensis* sp. nov. (Coordillera Central) y *C. smithi* sp. nov. (Sierra de Neiba y Cordillera Central). Nueve especies son descritas para Cuba: *C. cristal* sp. nov. (Sierra Cristal), *C. guamuhuya* sp. nov. (central Cuba), *C. guanyangi* sp. nov. (Cuchillas de Baracoa), *C. iviei* sp. nov. (Nipe-Sagua-Baracoa), *C. jiri* sp. nov. (Sierra Maestra y Cuchillas de Baracoa), *C. lajoncherei* sp. nov. (Sierra del Rosario), *C. naranjoi* sp. nov. (Sierra Maestra y Nipe-Sagua-Baracoa), *C. riberai* sp. nov. (Sierra Maestra) y *C. yunque* sp. nov. (Cuchillas de Baracoa). Un resumen de la distribución conocida de *C. suturalis* complex en las Antillas Mayores es realizado; la taxonomía del grupo no es examinada en detalles debido a falta de suficiente material.

Palabras clave. Coleoptera, Hydrophilidae, escarabajos acuáticos, ambientes lóticos, Antillas Mayores, Cuba, Jamaica, La Española, delimitación de especies, nueva especie

Introduction

Very little is known about the origin, diversity and biogeography of freshwater invertebrates of Greater Antilles, despite the fact that available data indicate that they are highly endemic (Genaro & Tejuca, 2001) and that their habitats are highly endangered (Woods & Sergile, 2001). The problem moreover does not concern only invertebrate groups: a high proportion of previously overlooked locally endemic species was recently revealed in Greater Antilles even in such a well-studied group as freshwater fishes (Weaver *et al*., 2016).

Aquatic beetles are among the most apparent groups of freshwater invertebrates in the Greater Antilles. Their fauna consists of 218 species (102 of which are endemic) representing 63 genera in 11 families. Water beetles of Greater Antilles were considered as rather well known, but recent studies revealed many new, mostly endemic species in nearly all groups examined (e.g., Short 2004a, b; Short & Torres 2006; Spangler & Short 2008; Deler-Hernández & Delgado 2010, 2012; Megna *et al*., 2011, Megna and Epler, 2012; Toledo *et al*., 2011; Deler-Hernández *et al*., 2013a, 2017; Megna and Sánchez-Fernández, 2014). However, many genera are still waiting for a detailed revision, and the general knowledge is especially fragmentary for Puerto Rico and Jamaica. Aquatic beetles, especially those inhabiting running water (streams and rivers), are considered as excellent models for studies of historical biogeography and for evaluating impacts of habitat loss and fragmentation (e.g., Ribera *et al*., 2004; Abellán *et al*., 2006; Hidalgo-Galiana *et al*., 2014; Toussaint *et al*., 2015). They are characterized by narrow habitat requirements and limited dispersal abilities, which results in deep phylogeographic structure of their populations and an increased probability of allopatric or peripatric speciation. At the same time, this makes them prone to form locally endemic species, but also puts them at a high risk of extinction due to environmental changes.

The hydrophilid genus *Crenitulus* Winters, 1926 is a typical representative of tiny-sized aquatic beetles inhabiting sides of streams and rivers in the Greater Antilles. Only a single wide-spread species, *Crenitulus suturalis* (LeConte, 1866) was recorded from the Dominican Republic and Jamaica so far (Komarek 2005). Fikáček & Engel (2011) described the second Greater Antillean species, *C. paleodominicus* (Fikáček & Engel, 2011), from the Dominican amber. Same authors concluded that the presence of the genus in Greater Antilles during the Miocene indicates that the knowledge of the modern *Crenitulus* fauna is very insufficient due to collecting bias, or that water beetle faunas went through Miocene or post-Miocene extinctions. Our field-work performed between 2010–2016 accumulated quite a rich material of the genus from Cuba and Dominican Republic which we are analyzing here. This paper is a continuation of the study of the Caribbean hydrophilid fauna led by the first author (Deler-Hernández & Delgado 2010; Deler-Hernández *et al*., 2013a, b, 2014, in press; Arriaga-Varela *et al*., 2017).

Material and methods

Material examined. The material used in this study was collected between 2010-2016 during our expeditions in Cuba, Dominican Republic and Jamaica. In Cuba, where our sampling was most intensive, we collected in various localities of the four principal mountain ranges: Cordillera de Guaniguanico (western Cuba), Guamuhaya mountains (also called Sierra del Escambray; central Cuba), and Sierra Maestra and Nipe-Sagua-Baracoa ranges in eastern Cuba. These ranges are traditionally used to define three major geographical regions in Cuba (Western, Central and Eastern), and were used in previous studies of freshwater macroinvertebrates (Naranjo *et al*., 2010). In the Dominican Republic, we sampled aquatic habitats across the main mountain systems (Cordillera Central, Sierra de Neiba, Sierra de Bahoruco, Cordillera Septentrional and Samaná). In both cases, specimens were collected using an aquatic net or small kitchen sieve, and fixed directly in 96% ethanol, and stored at -20°C once they got into the lab. From Jamaica, we only got an accidentally collected specimen from Blue Mts. region, no proper sampling was performed. In Puerto Rico, we sampled streams and rivers in various localities of Cordillera Central and on the slopes of El Yunque Mt., but we failed to find a single specimen of *Crenitulus*. The present study is hence focused mainly on Cuba and Hispaniola. In addition to the specimens collected by us, we also examined a small amount of specimens deposited in other museums.

Examined specimens are deposited in the following collections:

BMNH	Natural History Museum, London, United Kingdom (M.V.L. Barclay);
CMN	Canadian Museum of Nature, Ottawa, Canada (R. Anderson);
MNHNSD	Museo Nacional de Historia Natural, Santo Domingo, Dominican Rep. (C. Suriel);
NHMW	Naturhistorisches Museum, Wien, Austria (A. Komarek);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček).

Holotypes of the new species bear the red printed label 'HOLOTYPE, CRENITULUS [name of the species] Deler-Hernández et al. 2017'; paratypes bear the yellow printed label 'PARATYPE, CRENITULUS [name of the species] Deler-Hernández et al. 2017'

Morphological studies. Habitus photographs of dry-mounted specimens were taken using Canon EOS 550D digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS5. Male genitalia were dissected and mounted in temporary glycerine slides; photographs were taken using Canon EOS 1100D digital camera attached to Olympus BX41 compound microscope. Multiple photos in different focal levels were taken for both habitus and genitalia, were subsequently combined in Helicon Focus software. Drawings of maxillary palp are based on photographs taken using Canon EOS 1100D digital camera attached to Olympus BX41 compound microscope and subsequently combined in Helicon Focus software. General morphological terminology follows Komarek (2005).

DNA sequences. DNA was extracted from complete specimens cut into two parts between prothorax and mesothorax, using commercial DNeasy Tissue Kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. Voucher specimens and DNA extractions are kept in the collection of the Department of Entomology of the National Museum, Prague (NMPC). For species delimitation analysis, we used two mitochondrial genes (3´ end of cytochrome c oxidase I, 16S rRNA) and one nuclear gene (internal transcribed spacer 2). For species-level phylogenetic analysis we used three mitochondrial genes (3´ end cytochrome c oxidase I, cytochrome c oxidase II and 16S rRNA), and two nuclear genes (18S rRNA and 28S rRNA); sequences of 18S rRNA were amplified in two parts (3´ end and 5´ end) and subsequently combined; for primers and PCR conditions, see Supplementary File 1. 10µl of each PCR product were purified by adding 1.0µl Exonuclease 1 (Exo1 [20 U/µl]). (ThermoFisherScientific) and 2.0µl Thermosensitive Alkaline Phosphatase (FastAP [1 U/µl]) (ThermoFisherScientific) and incubating the mixture for 15 min at 37°C followed by a 15 min inactivation step at 80°C. DNA sequences were assembled and edited using Geneious 7.1.9 and submitted to GenBank under the accession numbers shown in Supplementary File 1 [GenBank submissions will be done upon the acceptance of the manuscript for publication].

Species delimitation. We used 44 specimen dataset containing all sequenced specimens of the *Crenitulus yunque* complex, and 9 outgroup specimens for *cox1* and 16S (6 representatives of *Crenitulus* belonging to other clades, *Crenitis* sp., *Horelophus walkeri* and *Anacaena globulus*) or two outgroup specimens for ITS2 (Cuban specimens of *C. suturalis* complex). We applied the Generalized Mixed Yule Coalescent (GMYC) method (Pons *et al*., 2006) in order to evaluate species limits. We used three single locus ultrametric trees (*cox1*, 16S and ITS2) with multiple individuals per species as the input. The trees were obtained with BEAST v2.4.0 (Bouckaert *et al*., 2014) with a strict clock model for each gene after prior clock model testing using stepping stone sampling in MrBayes v3.2 (Ronquist *et al*., 2012). We constrained the age most recent common ancestor of *Crenitis*+*Crenitulus* to fall between 139–90 Ma (uniform distribution) following the 95% confidence interval obtained for that node in the fossil-calibrated time tree analysis of the Hydrophilidae by Bloom et al. (2014). Moreover, substitution rate calibration was set to 0.0177 substitutions per base per million years in the *cox1* analysis, and to 0.0054 substitutions per base per million years for 16S analysis (Papadopoulou *et al*., 2010); substitution rate was not specified for ITS2 analysis. All runs were carried out with 250 million generations and sampling frequency of every 12 500 generations. The 10% burnin fraction was applied after checking convergence of parameters in Tracer v1.6 (Rambaut *et al*., 2014). The resulting tree was visualized in FigTree 1.4.3 (Rambaut, 2012).

The GMYC analysis were performed in R v3.3.3 (R Core Team, 2014) using packages ape, paran, rncl and splits. All three analyses were conducted under the single-threshold version (Fujisawa & Baraclough, 2013) and results were tested with likelihood ratio tests against the null model assuming that all individuals belong to the same species, see Supplementary File 2.

The genetic distances based on *cox1* sequences within and between species of *Crenitulus yunque* complex was calculated using the Maximum Composite Likelihood model as implemented in MEGA7.0.26 software (Kumar *et al*., 2016).

Species-level phylogeny. We used a 34 taxa dataset which contains 27 representatives of *Crenitulus* from continental America and Greater Antilles, including single representative for each species of the *C. yunque* complex and all five available specimens of *C. suturalis* complex. Outgroup taxa comprised other genera of the Anacaenini for which sequence data are available: *Anacaena* (4 species: *A. lanzhui*, *A. globulus*, *A. lindi* and *A. lineata*), *Horelophus walkeri* and *Crenitis* sp. (all adopted from Short & Fikáček, 2013), and *Pseudorygmodus versicolor* (adopted from Fikáček & Vondráček, 2014). Sequences were aligned with MAFFT algorithm as implemented in Geneious 7.1.9 software, using the default settings (score matrix = 200PAM / k = 2, gap open penalty = 1.53, offset value = 0.123). The final alignment has the total length of 4316 bp, consisting of the following gene fragments: *cox1* (742 bp), *cox2* (662 bp), 16S (519 bp), 18S (1798 bp) and 28S (993 bp). The dataset was divided into 9 partitions (by genes, plus *cox1* and *cox2* were both divided by codon positions). Each dataset was analyzed using the Bayesian inference and maximum likelihood. Bayesian analyses were performed in MrBayes 3.2.6 (Ronquist *et al*., 2012) using four chains of 25,000,000 generations and sampling the chain every 1000 generations. We sampled across the substitution model space in the Bayesian MCMC analysis itself (Huelsenbeck *et al*., 2004; Ronquist *et al*., 2012) instead of prior testing for an appropriate model for each of nine partitions. Results were examined in Tracer v.1.6 (Rambaut *et al*., 2014) to check for the proper effective sample size (ESS), proper mixing of chains, and reaching the stationary phase; 25% burn-in was used for construction of the final consensus tree. Maximum likelihood analyses were performed using RAxML 8.0 (Stamatakis, 2014) with a GTR substitution model and 1,000 bootstrap replicates. Resulting trees were visualized in FigTree 1.4.3 (Rambaut, 2012).

Results of phylogenetic analyses and species delimitation

Phylogenetic position of *Crenitulus* of Greater Antilles. All phylogenetic analyses supported the monophyly of the genus *Crenitulus* and revealed four or five strongly supported clades within the genus (Fig. 1). Both Bayesian and maximum likelihood analysis revealed two unidentified species from Peru forming Clade I as sister to remaining species analyzed. Specimens from Greater Antilles form two independent clades: all specimens of the *Crenitulus yunque* complex form a clade sister to all other *Crenitulus* except the two aforementioned Peruvian species, and all specimens of the *C. suturalis* complex form a separate clade together with the US specimen of *C. suturalis*. *Crenitulus hirsutus* from Central America forms small Clade II with one other Central American unidentified species in the Bayesian analysis (Fig. 1A), but both species form a paraphyletic assemblage at the base of *C. suturalis* clade in maximum likelihood analysis (Fig. 1B). Clade III contains the Central American representative of *C. solstitialis* and six unidentified species from Peru and Ecuador (Fig. 1).

Internal topology of Greater Antillean clades. The internal topology of the *Crenitulus yunque* clade is largely congruent in Bayesian and maximum likelihood analyses based on 5 genes: *C. guamuhaya* from Central Cuba is a sister-species to all other members of the clade which group in three clades: a clade comprising the Eastern Cuban *C. cristal*, *C. riberai*, *C. yunque* and *C. jiri*, the clade comprising both Dominican species (*C. dominicensis* and *C. smithi*) and the clade comprising the Western Cuban *C. lajoncherei* and Eastern Cuban *C. naranjoi* and *C. iviei*. Only the position of Eastern Cuban *C. guanyangi* differs between both analyses: it is weakly supported as sister to the Dominican clade in Bayesian analysis, and to the *lajoncherei-iviei-naranjoi* clade in the maximum likelihood analysis.

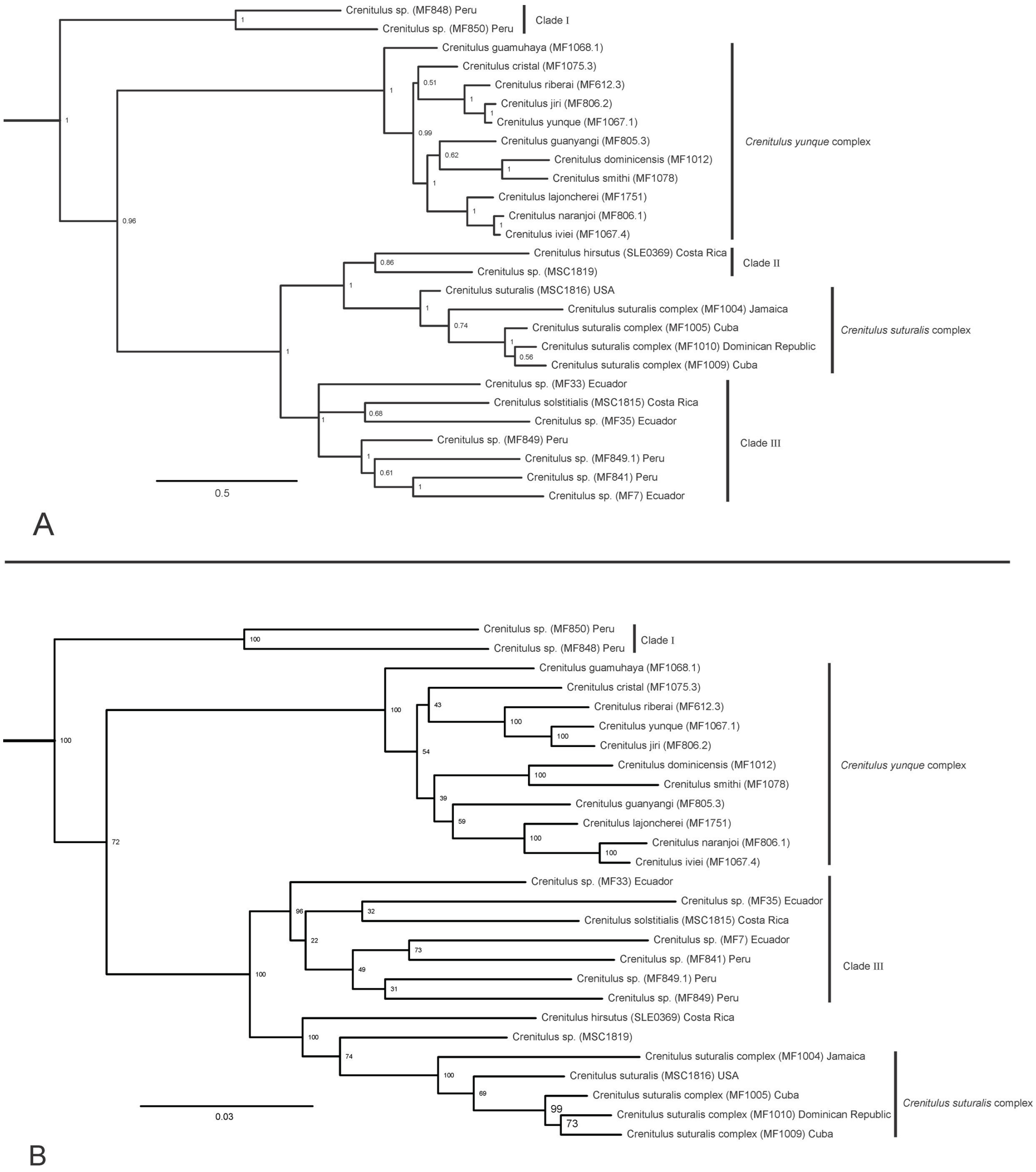


Figure 1. Internal topology of the *Crenitulus* clade, based on the concatenate of *cox1*, *cox2*, 16S, 18S and 28S sequences. A) Bayesian analysis B) RAxML analysis. The position of the *Crenitulus yunque* complex endemic to Cuba and Hispaniola and of *Crenitulus suturalis* complex which occurs in the Caribbean are indicated.

Single gene trees used for species delimitation revealed topologies only partly congruent with 5-gene analyses, with the following principal differences: (1) Dominican species form a clade which is sister to all other species (*cox1* and 16S analysis) or sister or all other species except *C. jiri* (ITS2 analysis); (2) the *cristal-yunque-jiri-riberai* clade is not monophyletic in 16S and ITS2 analyses but forms a monophylum in cox1 analysis; (3) *C. guamuhaya* is sister to *cristal-yunque-jiri-riberai* clade in cox1 analysis or to all other Cuban species in 16S and ITS2 analyses; and (4) *C. guanyangi* is sister to *iviei*-*lajoncherei*-*naranjoi* clade in cox1 and ITS2 analysis, but to *C. cristal* in 16S analysis.

The internal topology of the *C. suturalis* clade was only examined in the species-level analyses. Both Bayesian and maximum likelihood analyses reveal the Cuban and Dominican specimens forming a strongly supported clade. Bayesian analysis reveals the Jamaican unidentified species as sister to this clade, and the US *C. suturalis* as the basalmost taxon, whereas maximum likelihood analysis reveals the Jamaican specimen as the basalmost one; both topologies are however weakly supported.

DNA species delimitation of *C. yunque* species group. The GMYC species delimitation analysis recognized 9–11 species within the *C. yunque* complex (11 using *cox1* tree, 9 using 16S tree and 9 using ITS2 tree in which *C. lajoncherei* was not represented, however). Only the analyses based on *cox1* and 16S data were statistically significant; the likelihood ratio test of the ITS2 analysis resulted non-significant and does not allow reject the null hypothesis of just one species present in the dataset. Both *cox1* and 16S analyses resulted in a very similar threshold times (3.27 millions of years in *cox1* analysis, 3.34 millions of years in 16S analysis) when branching rate starts to increase, indicating within-species coalescence processes. In many species, DNA-based species delimitation based on all three genes completely agrees with genitalia morphology and distribution; this is the case of *C. guanyangi*, *C. riberai*, *C. guamuhaya*, *C. dominicensis* and *C. smithi*. In the remaining species, conflicting results were revealed and are commented in detail below:

(1) *Crenitulus iviei-naranjoi-lajoncherei* complex: four species-candidates were recognized using *cox1*, and two using both 16S and ITS2 (however no specimen of *C. lajoncherei* was included in the ITS2 analysis). *Crenitulus iviei* and *C. naranjoi*, both occurring in Eastern Cuba are recognized as genetically close in all analyses, but clearly differ in small details of morphology of male genitalia: apices of parameres are narrowly rounded in *C. iviei* but widely rounded in *C. naranjoi*, and the gonopore is apical in *C. iviei* whereas suapical in *C. naranjoi*. GMYC analysis based on ITS2 revealed the species species limits fully conforming with the genital morphology, whereas 16S reveals part of the specimens of *C. iviei* from La Melba (Fig. 3A: locality C) clustering with *C. naranjoi*, and *cox1* reveals two different clusters within *C. iviei* and clusters the species also with one specimen from La Mensura-Piloto (Fig. 3A: locality E). The latter specimen is female (and hence we lack data on genital morphology), but since genital morphology seems to match fully the ITS2 clusters, we consider this specimen as belonging to *C. naranjoi*. *Crenitulus lajoncherei* occurs in geographically distant area from the

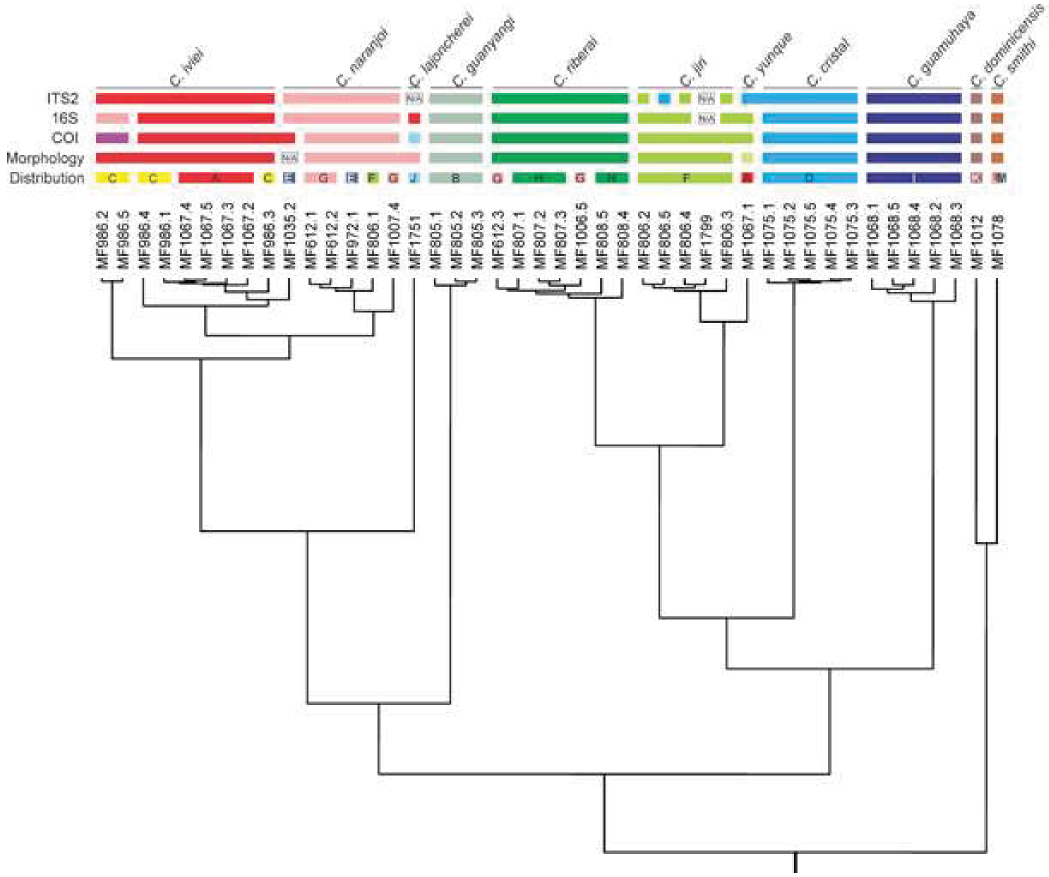


Figure 2. Results of GMYC species delimitation analysis of the *Crenitulus yunque* complex based on *cox1*, 16S and ITS2 sequences, morphology of male genitalia and distribution mapped on the Bayesian tree based on *cox1* data only. Distribution lettering refers to maps on Fig. 3.

other two species (Western Cuba) and is revealed as genetically distant in *cox1* gene tree as well as in the multigene species-level phylogeny; for that reason we consider it as a separate species despite its genitalia are identical to *C. naranjoi* and despite the GMYC analysis of 16S data clustered it with part of the specimens of *C. iviei* from Eastern Cuba.

(2) *Crenitulus jiri-yunque-cristal* complex: two species candidates were recognized in this group by all three gene trees, but not in a congruent way; both mitochondrial markers clustered the specimen from Yunque de Baracoa (Fig. 3A: locality A) with *C. jiri*, whereas ITS2 data cluster it with *C. cristal* and a specimen from Gran Piedra (Fig. 3A: locality F) otherwise assigned to *C. jiri*. In contrast to the genetic markers, genital morphology consistently indicates three morphotypes in the complex, corresponding moreover with geography: a morphotype with larger aedeagus and narrow parameres from Gran Piedra (Fig. 3A: locality F), a morphotype with smaller aedeagus, evenly convex lateral face of parameres and subapical gonopore from Pico Cristal (Fig. 3A: locality D) and a morphotype with smaller aedeagus, apically slightly concave outer face of parameres and apical gonopore from Yunque de Baracoa (Fig. 3A: locality A). The

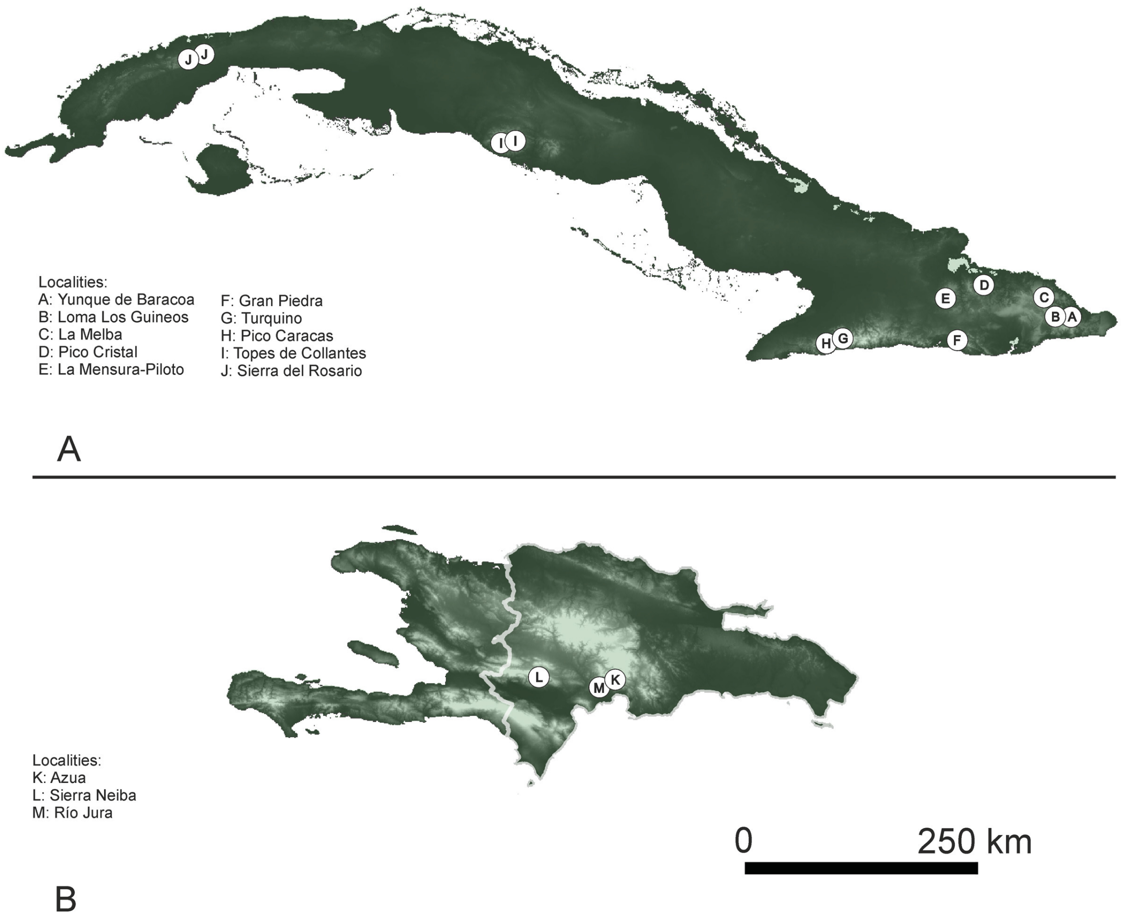


Figure 3. Map of localities in Cuba (A) and Hispaniola (B) successfully sampled for representatives of the *Crenitulus yunque* complex. Letters correspond to those used for distribution mapping of sequenced specimens in Fig. 2.

latter morphotype is moreover the one which is inconsistently clustered by nuclear versus mitochondrial markers. We hence consider all three morphotypes as separate species.

Based on the analysis above, combining the DNA-based GMYC species delimitation analysis and analysis of genital morphology and distribution, we are recognizing 11 species within *C. yunque* clade, nine occurring in Cuba and two in Hispaniola.

The mean interspecific distance in *cox1* sequences vary between 2.0 and 12.0 % in our dataset. The smallest interspecific distances were found between *C. yunque* and *C. jiri* (2.6 %) and *C. naranjoi* and *C. iviei* (2.0 %). The intraspecific mean distances vary between 0.0 and 1.5% in the species for which we were able to include more than one specimen into the analysis.

Table 1. Corrected mean intraspecific and interspecific genetic distances within the *Crenitulus yunque* species group.

	Species	**Intra-spec**	**Interspecific**									
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	*cristal*	0										
2	*dominicensis*	N/A	0.077									
3	*guamuhaya*	0.014	0.083	0.093								
4	*guanyangi*	0	0.064	0.079	0.072							
5	*iviei*	0.015	0.079	0.104	0.099	0.081						
6	*jiri*	0.005	0.088	0.091	0.094	0.082	0.111					
7	*lajoncherei*	N/A	0.089	0.082	0.089	0.055	0.058	0.104				
8	*naranjoi*	0.008	0.079	0.093	0.093	0.068	0.020	0.118	0.046			
9	*riberai*	0.005	0.079	0.090	0.095	0.088	0.120	0.039	0.105	0.122		
10	*smithi*	N/A	0.088	0.048	0.101	0.064	0.089	0.088	0.086	0.087	0.109	
11	*yunque*	N/A	0.083	0.085	0.098	0.068	0.095	0.026	0.086	0.102	0.043	0.079

Taxonomy

Checklist of the extant Greater Antillean species of *Crenitulus*

Crenitulus yunque complex

Crenitulus cristal **sp. nov.**	Cuba: Sierra Cristal
Crenitulus dominicensis **sp. nov.**	Hispaniola: Dominican Rep. (Cordillera Central)
Crenitulus guamuhaya **sp. nov.**	Cuba: Guamuyaha Mts.
Crenitulus guanyangi **sp. nov.**	Cuba: Cuchillas de Baracoa
Crenitulus iviei **sp. nov.**	Cuba: Nipe-Sagua-Baracoa Mts.
Crenitulus jiri **sp. nov.**	Cuba: Sierra Maestra and Nipe-Sagua-Baracoa Mts.
Crenitulus lajoncherei **sp. nov.**	Cuba: Sierra del Rosario
Crenitulus naranjoi **sp. nov.**	Cuba: Sierra Maestra and Nipe-Sagua-Baracoa Mts.
Crenitulus riberai **sp. nov.**	Cuba: Sierra Maestra Mts.
Crenitulus smithi **sp. nov.**	Hispaniola: Dominican Rep. (Sierra Neiba and Cordillera Central)
Crenitulus yunque **sp. nov.**	Cuba: Cuchillas de Baracoa

Crenitulus suturalis complex	
Crenitulus suturalis (LeConte, 1866) complex	Cuba, Jamaica, Dominican Rep., otherwise widespread from Canada to Argentina

Species treatment

Crenitulus yunque complex

Diagnosis. *Crenitulus yunque* complex can be distinguished from other species of *Crenitulus* by the following combination of characteristics: shape of the body elongate oval; body length 1.35–2.42 mm; coloration of clypeus, frons, pronotum and elytra variable from yellow to black; pronotum largely dark with anterolateral, lateral and posterolateral margins paler, pale with dark brown to black central patch of oval shape, or uniformly pale; nine antenomeres; maxillary palpi stout with palpomere 2 inflated and palpomere 4 elongated, darker at apex; procoxae with stout spines; ventral surface pubescent.

Differential diagnosis. The systematics of *Crenitulus* is poorly known, with potentially many undescribed species present, and it is hence impossible to provide a general differential diagnosis for the *C. yunque* group. In the Greater Antilles, the only species not belonging to this group belongs to the *C. suturalis* group and may be distinguished as follows: body very small (1.34-1.80 mm, compared to 1.35–2.45 mm in *C. yunque* complex), dorsal coloration always nearly completely dark, only with paler pronotal and elytral lateral margins (yellow to nearly completely dark in *C. yunque* complex), ventral surface always very dark (paler in representatives of *C. yunque* group, however teneral specimens of *C. suturalis* group may also look like that). The aedeagus of *C. suturalis* group is very small (0.28 mm) and has parameres ca. as long as phallobase, and is hence resembling only *C. cristal* and *C. guanyangi* of the *C. yunque* group; *C. guanyangi* can be easily distinguished from *C. suturalis* group by paler dorsal coloration (Fig. 4C); *C. cristal* is a dark species and may be distinsuished from *C. suturalis* group only by details of genital morphology (wider parameres, subapical gonopore, narrower and longer phallobase: compare Figs. 5A and 5L).

Crenitulus cristal **sp. nov.**

(Figs 4A, 5A, 6A)

Type locality. Cuba, Holguín province, Frank País, Parque Nacional Pico Cristal, 20.57017'N 75.42838'W, 565 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'CUBA: Holguín Prov., Frank País, P.N. Pico Cristal, 11.v.2013, 20.57017N 75.42838W, 565 m, Deler-Hernández, hygropetric habitat [printed]'. PARATYPES: 24 spec. (BMNH, CMN, NHMW, NMPC): same data as holotype, [incl. molecular vouchers MF1075/1, MF1075/2, MF1075/3, MF1075/4, MF1075/5].

Description. Habitus as in Fig. 4A. *Body* length 1.70−1.85 mm. *Coloration.* Clypeus and head black; pronotum dark brown with anterolateral, lateral and posterolateral margins pale; elytra dark brown with lateral margins pale; ventral surface brown. *Maxillary palpi* (Fig. 6A) stout with palpomere 4 brown, palpomere 2 club-shaped; palpomeres 3 short, palpomere 4 elongated, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5A, Table 2) 0.30 mm long; phallobase slightly longer than parameres, with basal portion narrowly subquadrate, ca. as long as long narrow manubrium; parameres widest at mid-length, with evenly rounded lateral margins and almost straight mesal margins; median lobe shorter than parameres, gonopore in subapical position, basal apophyses short and rounded, with short extensions into phallobase.

Differential diagnosis. *Crenitulus cristal* is one of the three totally black species of *Crenitulus* in Cuba (along with *C. lajoncherei* sp. nov. and *C. suturalis* complex). It can be easily distinguished from *C. lajoncherei* by the morphology of the aedeagus (compare Figs 5A and 5G). It can be distinguished from *C. suturalis* group by details of the genital morphology: gonopore is subapical (apical in *C. suturalis* group), parameres are wide, widest at midlength (narrow, widest in basal third in *C. suturalis* group), and phallobase narrow with long manubrium (wide with short manubrium in *C. suturalis* group).

Etymology. This species is named after Sierra Cristal where the type series was collected; the species name is noun in apposition.

Distribution and biology. *Crenitulus cristal* sp. nov. is only known from Sierra Cristal in the eastern part of Cuba (Fig. 3: locality D). All specimens were collected under hygropetric conditions, in a very narrow exposed streamlet with algal mats flowing over limestone rocks at the margin of pluviselva forest, together with specimens of *Hydraena blancae* (Coleoptera: Hydraenidae; Deler-Hernández *et al*., 2017).

Crenitulus dominicensis sp. nov.

(Fig. 5B)

Type locality. Dominican Republic, 13.3 km N of Azua, 18°34.61'N 70°45.24'W, 545 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'DOMINICAN REPUBLIC: Azua, 13.3 km N of Azua, E of Peralta, 18°34.61'N 70°45.24'W, 545 m, 06.ix.2014, A. Deler-Hernández, Fikáček, Gimmel, DR40 [printed]' [molecular voucher MF1012].

Description. *Body* elongate oval; body length 2.2 mm. *Coloration.* Clypeus and head black; pronotum dark brown with lateral and posterolateral margins pale; elytra black; ventral surface dark brown; legs brown. *Aedeagus* (Fig. 5B, Table 2) 0.42 mm long; phallobase slightly longer than parameres, subrectangular, ca. as long as narrow long manubrium; parameres widest at midlength, with rounded lateral margins and slightly convex mesal margins, sinuate on lateral margin subapically; median lobe slightly shorter than parameres, gonopore in subapical position, basal apophyses short and rounded, with short extension into phallobase.

Differential diagnosis. *Crenitulus dominicensis* sp. nov. can be diagnosed from remaining two *Crenitulus* species recorded from Hispaniola (*C. smithi* and *C. suturalis*) only on the basis of male genitalia: the aedeagus is relatively large (in contrast to *C. suturalis*), with median lobe ca. as long as parameres (shorter than parameres in *C. smithi*), gonopore situated subapically (in contrast to apical gonopore in *C. suturalis*). Apical portions of the parameres of *C. dominicensis* are characteristically bent out, which is not present in any other *Crenitulus* species from Greater Antilles.

Etymology. The species named refer to the Dominican Republic where the type specimens was collected; adjective.

Distribution and biology. *Crenitulus dominicensis* sp. nov. is only known from the type locality in the southern foothills of Cordillera Central, geologically situated on the northern paleoisland of Hispaniola (Fig. 3B: locality K). It was found in shallow exposed pools with algae and submerged plants at gravel banks of a small mountain river.

Crenitulus guamuhaya sp. nov.

(Figs. 4B, 5C, 6B)

Type locality. Cuba, Sancti Spíritus province, Topes de Collantes, 80º03′36″W 21º54′42″N, 891 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'CUBA: Sancti Spiritus Prov., Trinidad Municipality, Topes de Collantes, Codina, 01.vii.2010, 80º03′36″W 21º54′42″N, 891 m a.s.l.

[printed]' [molecular voucher MF1068.1]. PARATYPES: 9 spec. (NMPC, BMNH): same data as the holotype [incl. molecular vouchers MF1068/2, MF1068/3, MF1068/4, MF1068/5]; 13 spec. (NHMW, NMPC): Codina, 01.vii.2010, Y. S. Megna; 5 spec. (NMPC, CMN): same locality, 31.v.2015; 3 spec. (NMPC): Codina, 30.vi.2010; Salto del Caburní, 21º55'14.2''N 80º00'14.4''W, 381 m, A. Deler-Hernández lgt,. Caburní river.

Description. *Habitus* as in Fig. 4B. *Body* length 1.90–2.10 mm. *Coloration.* Clypeus and head dark brown; pronotum uniformly yellowish to dark brown with pale lateral and posterolateral margins; elytra yellowish to dark brown, with variably demarcated darker areas in some specimens; ventral surface dark brown, legs light brown. *Maxillary palpi* (Fig. 6B) stout with palpomere 4 reddish apically, palpomere 2 wide; palpomeres 3 short, palpomere 4 elongated, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 4C, Table 2) 0.36 mm long; phallobase slightly longer than parameres, basal portion short and narrow, manubrium only slightly narrower; parameres widest at midlength, with rounded lateral margins and almost straight mesal margins, apical region narrow; median lobe slightly shorter than parameres, gonopore in apical position, basal apophyses short and rounded, with short extension into phallobase.

Differential diagnosis. *Crenitulus guamuhaya* sp. nov. is very variable in dorsal coloration, ranging from nearly totally black to pale brown, and it may be hence easily confused with most other *Crenitulus* of Greater Antilles. By genital morphology, it can be however only confused with *C. cristal* and *C. yunque*; from *C. cristal* it may be recognized by apically situated gonopore (subapical in *C. cristal*), from *C. yunque* it cannot be distinguished at all. However, *C. guamuhaya* is larger than *C. cristal* and in contrast to the latter it only occurs in Topes de Collantes region in Central Cuba. In Central Cuba, it only co-occurs with *C. suturalis* from which it can be easily distinguished based on male genitalia (compare Figs 5C and 5L).

Etymology. This species is named after the Guamuhaya mountains where the type series was collected; the species name is noun in apposition.

Distribution and biology. *Crenitulus guamuhaya* sp. nov. is only known from the Guamuhaya mountains in Central Cuba (Fig. 3A: localities I). All specimens were collected in shaded streams and temporary pools with and muddy to sandy-stony bottom all located in highlands within well-preserved gallery forest. *Crenitulus guamuhaya* co-occurs syntopically with *Crenitulus suturalis* complex.

Crenitulus guanyangi **sp. nov.**

(Figs 4C, 5D, 6C)

Type locality. Cuba, Guantanámo province, Baracoa, Loma Los Guineos, 20º19′38.38″N 74º35′35.37″W, 530 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'CUBA: Guantanámo Prov., Baracoa municipality, Loma Los Guineos, 20º19'38.38''N 74º.35'35.37''W, 530 m, 07.iv.2012, A. Deler-Hernández, Stream (Arroyo) [printed]' [molecular voucher MF805/3]'. PARATYPES: 3 spec. (NMPC): same data as holotype [molecular vouchers MF805/1, MF805/2].

Description. Habitus as in Fig. 4C. *Body* length about 1.35−1.65 mm. *Coloration.* Clypeus and head black; pronotum yellowish brown with dark brown central patch of subrectangular shape; elytra yellowish brown, with demarcated lateral darker area at midlength; ventral surface light brown. *Maxillary palpi* (Fig. 6C) stout with palpomere 4 brown apically; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, widest in the middle, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5D, Table 2) 0.26 mm long; phallobase as long as parameres, with basal portion subquadrate, manubrium triangulate; parameres slender, widest at midlength, narrowed towards apex, with rounded lateral margins and almost straight mesal margins; median lobe slightly shorter than parameres, gonopore in apical position, basal apophyses short and sharp, with short extension into phallobase.

Differential diagnosis. *Crenitulus guanyangi* sp. nov. is a very small species of characteristic coloration (dorsal surface pale with subrectangular central pronotal spot and dark markings at sides ca. at midlength of elytra). By dorsal coloration it resembles *C. naranjoi* and some specimens of the *C. guamuhaya*, from which it can be distinguished by its small size about 1.35−1.65 mm and the demarcated lateral darker area at midlength. By genital morphology, it resembles *C. cristal*, *C. guamuhaya* and *C. yunque* from which it can be distinguished by parameres slender and phallobase subquadrate and manubrium triangulate.

Etymology. The new species is dedicated to Guanyang Zhang, a colleague of the first author and a very enthusiastic entomologist specializing on Curculionidae.

Distribution and biology. *Crenitulus guanyangi* sp. nov. is only known from Loma Los Guineos (Cuchillas de Baracoa) (Fig. 3A: locality B). All specimens were collected in shaded streams with sandy-stony bottom and without aquatic vegetation, located in highlands within well-preserved primary forest, together with specimens of *Crenitulus jiri*.

Crenitulus iviei sp. nov.

(Figs 5E, 6D)

Type locality. Cuba, Guantanámo province, Baracoa, Yunque de Baracoa, 20º313′N 74º574′W, 200 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'CUBA: Guantanámo Prov., Baracoa municipality, Yunque de Baracoa near Finca Las Delicias, 20º313′N 74º574′W, 200 m, 17.v.2015, coll.: A. Deler-Hernández, [printed]' [molecular voucher MF1067/4]'. PARATYPES: 3 spec. (NMPC, NHMW): same data as the holotype [molecular vouchers MF1067/2, MF1067/3, MF1067/5]; 6 spec. (BMNH, CMN, NMPC): Holguín, La Melba, National Park Alejandro v. Humbold, 440 m, 22.ix.2014, 20.43441 74.83916, A. Deler-Hernández [incl. molecular vouchers MF986/1, MF986/2, MF986/3, MF986/4, MF986/5]; 1 spec. (NMPC): Guantánamo prov., El Yunque, 3.2 Km SW of campismo, right tributary of Duaba river, 20º19'N 74º34'W, ca. 150 m a.s.l., 13.vi.2012, Deler-Hernández & Fikacek lgt., MF09.

Description. *Body* elongate oval; body length 1.80−2.00 mm. *Coloration.* Clypeus and head black; pronotum light brown to brown, with dark brown central patch; elytra light brown to brown, with demarcated darker area at midlength; ventral surface light brown. *Maxillary palpi* (Fig. 6D) with palpomere 4 reddish apically, palpomere 2 club-shaped; palpomeres 3 short, palpomere 4 elongated, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5E, Table 2) 0.38 mm long; phallobase longer than parameres, basal portion long and quadrate, manubrium long and only slightly narrower; parameres wide, widest in basal third, with convex lateral margins, almost straight mesal margins and apical region narrowly rounded; median lobe shorter than parameres, gonopore in apical position, basal apophyses short and rounded, with short extension into phallobase.

Differential diagnosis. *Crenitulus iviei* sp. nov. belongs, together with *C. lajoncherei* and *C. naranjoi* from Cuba and *C. smithi* in Hispaniola, to the species characterized by very long phallobase, widely rounded apices of parameres, and median lobe much shorter than parameres. It differs from all these species in rather narrow parameral apices and the apical gonopore. In Eastern Cuba, it can be only confused with *C. naranjoi* which has identical dorsal coloration, the other two species do not occur in Eastern Cuba.

Etymology. The new species is dedicated to Michael A. Ivie, an enthusiastic Coleoptera specialist from Montana University who is partly specializing on Caribbean beetles.

Distribution and biology. *Crenitulus iviei* sp. nov. is known from the eastern part of the Nipe-Sagua-Baracoa mountain range in the Eastern Cuba (Yunque de Baracoa and La Melba: Fig. 3A: localities A and C). All specimens were collected in shaded streams, muddy to sandy-stony bottom and without aquatic vegetation located in highlands within well-preserved pluviselva and rainforest, often together specimens of *Crenitulus suturalis* complex, and in Yunque de Baracoa (Fig. 3A: locality A) also together with *Crenitulus yunque*.

Crenitulus jiri sp. nov.

(Figs 4D, 5F, 6E)

Type locality. Cuba, Santiago de Cuba, Gran Piedra, 20º00′22″N 75º36′56″W, 1144 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'Cuba, Santiago de Cuba, Santiago, Gran Piedra, Stream near Museo La Isabelica, 20º00'22''N 75W36'56''W, 1144 m, 01.x.2014, coll. A. Deler-Hernández [printed]' [molecular voucher MF1799]. PARATYPES: 1 spec. (NMPC): same data as holotype, [molecular voucher MF1798]; 5 spec. (NHMW, NMPC): Santiago de Cuba, Gran Piedra, Stream near Museo La Isabelica, 20º00'22''N 75º36'56''W, 1144 m, 18.x.2012, coll. A. Deler-Hernández [molecular vouchers MF806/2, MF806/3, MF806/4; MF806/5]; 21 spec. (BMNH, CMN, NMPC): Santiago de Cuba, Gran Piedra, near Museo La Isabelica, 20º00'22''N 75º36'56''W, 1120 m, 01.x.2014, Deler-Hdez; 5 spec. (NMPC): Santiago de Cuba, Protected Area Loma del Gato, near of Loma del Gato, 26.vi.2009, 20º00'44.6''N 76º02'53''W, 800 m, A. Deler-Hernández; 1 spec. (NMPC): Guantanámo Prov., Baracoa municipality, Loma Los Guineos, 20º19′38.38″N 74º35′35.37″W, 530 m, 07.iv.2012, A. Deler-Hernández, Stream (Arroyo).

Description. Habitus as in Fig. 4D. *Body* length 2.00–2.20 mm. *Coloration.* Clypeus and head brown to black; pronotum yellowish to dark brown, with dark brown central patch in darker specimens; elytra yellow to dark brown; ventral surface light brown. *Maxillary palpi* (Fig. 6E) stout, palpomere 2 club-shaped; palpomeres 3 long, palpomere 4 elongated, with terminal infuscation. *Aedeagus* (Fig. 5F, Table 2) 0.40 mm long; phallobase slightly shorter than parameres, with basal portion narrowly quadrate, manubrium rather wide and rounded; parameres slender, widest at midlength, with rounded lateral margins and almost straight mesal margins, apical region narrow; median lobe slightly shorter than parameres, gonopore in subapical position, basal apophyses short and sharp, with short extension into phallobase.

Differential diagnosis. *Crenitulus jiri* sp. nov. belongs together with *C. riberai* to the largest species of the genus in Greater Antilles, characterized moreover by pale general coloration with or without central pronotal spot. By genital morphology, especially its large size and narrow parameres, it can be only confused with *C. riberai* from which it differs by apical gonopore (compared to subapical gonopore of *C. riberai*).

Etymology. The new species is dedicated to Jiří Hájek, a water beetle specialist from the National Museum, Prague; noun in apposition.

Distribution and biology. *Crenitulus jiri* sp. nov. is known from two of the main mountains systems of Eastern Cuba (Sierra Maestra: Cordillera de la Gran Piedra and Cordillera del Turquino, and Nipe-Sagua-Baracoa: Cuchillas de Baracoa). All specimens were collected in shaded streams and temporary pools, muddy to sandy-stony bottom and without aquatic vegetation, located in highlands in well-preserved pluviselva and rainforest. In Loma Los Guineos (Fig. 3A: locality B), *Crenitulus jiri* was collected together with specimens of *C. guanyangi*.

Crenitulus lajoncherei sp. nov.

(Fig. 5D)

Type locality. Cuba, Artemisa province, Soroa, Río Manantiales, 22º47′29.97″N 83º0′22.83″W, 180 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'CUBA: Artemisa Prov., Soroa, Río Manantiales, 22º47'29.97''N 83º0'22.83''W, 180 m a.s.l., 14.vii.2016, coll:. A. Deler-Hernández [printed]' [molecular voucher MF1751]'. PARATYPE: 1 female (NMPC): Artemisa Prov., Cañon del Santa Cruz, Río Santa Cruz, 22º45'1.29''N 83º8'58.36''W, 199 m a.s.l., 16.vii.2016, coll.: A. Deler-Hernández [molecular voucher MF1752].

Description. *Body* elongate oval, body length about 2.05−2.35 mm. *Coloration.* Clypeus and head black; pronotum brown, weakly darkened centrally, elytra reddish; ventral surface reddish. *Maxillary palpi* stout with palpomere 4 brown; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, with terminal infuscation. *Aedeagus* (Fig. 5G, Table 2) 0.38 mm long; phallobase much longer than parameres, with almost the same wide throughout; parameres wide, widest at midlength, with convex lateral margins, nearly straight mesal margin, and apical region broadly rounded; median lobe shorter than parameres, gonopore in subapical position, basal apophyses short and rounded, with short extension into phallobase.

Differential diagnosis. *Crenitulus lajoncherei* sp. nov. may be only confused with *C. naranjoi* and *C. iviei* in Cuba when genital morphology is examined: all species have the phallobase much longer than parameres, without clearly defined manubrium, short median lobe and apically rouded parameres. The species can be diagnosed from *C. iviei* by subapical gonopore (apical in *C. iviei*). Morphologically, *C. lajoncherei* seems to be extremely similar to *C. naranjoi* from Eastern Cuba with which it share the same genital morphology; both species may possibly differ only in dorsal coloration, as *C. lajoncherei* has weakly darekened center of the pronotum, whereas *C. naranjoi* seems to always have clearly defined central pronotal spot (even in teneral specimens). However, we only examined two specimens of *C. lajoncherei* and additional material is necessary to understand its color variation. *Crenitulus lajoncherei* is endemic to Western Cuba and does not co-occur with *C. naranjoi* which is confined to Eastern Cuba only.

Etymology. The new species is dedicated to Luis Lajonchere, a very enthusiastic Cuban malacologist.

Distribution and biology. *Crenitulus lajoncherei* sp. nov. is known from Sierra del Rosario in Cordillera de Guaniguanico in Western of Cuba (Fig. 3A: localities J). All specimens were collected in shaded streams, sandy-stony bottom and without aquatic vegetation, located in well-preserved gallery forest, together with specimens of *Crenitulus suturalis* complex.

***Crenitulus naranjoi* sp. nov.**

(Figs 4E, 5H, 6F)

Type locality. Cuba, Santiago de Cuba province, Gran Piedra, 20º00′22″N 75W36′56″W, 1144 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'Cuba, Santiago de Cuba, Santiago, Gran Piedra, Stream near Museo La Isabelica, 20º00'22''N 75º36'56''W, 1144 m, 18.x.2012, coll. A. Deler-Hernández [printed]' [molecular voucher MF806/1]. PARATYPES: 2 spec. (NMPC): Santiago de Cuba, Santiago, Gran Piedra, Stream near Museo La Isabelica, 20º00'22''N 75W36'56''W, 1120 m, 01.x.2014, A. Deler-Hdez; 6 spec. (CMN, NMPC): Santiago de Cuba, Gran Piedra, Arroyo [Stream], 20º00'26''N 75º37'06''W, 11.vii.2011, A. Deler-Hernández lgt.; 4 spec. (BMNH, NHMW, NMPC): Granma, PN Turquino, on the trail up ca. 0.5 Km S (by air) from of La Platica, shaded small mountain stream in secondary evergreen forest, 920 m, 24.vii.2012, 20º0.5'N 76º53.3'W, Deler-Hernández & Fikacek [molecular vouchers MF612/1, MF612/2, MF1007.4]; 1 spec. (NMPC): Holguín, Mayarí, P.N. Mensura-Piloto, 20.48310 75.78960, 647 m, 10.v.2013, A. Deler-Hernández, Stream [molecular voucher MF1035.2]; 1 spec. (NMPC): Holguín, P.N. Mensura-Piloto, Potrerillo, 20.41598 75.82008, 410 m, 12.v.2013, A. Deler-Hernández, Stream, [molecular voucher MF972.1].

Description. Habitus as in Fig. 4E. *Body* length about 1.85−2.25 mm. *Coloration.* Clypeus and head black; pronotum yellowish to dark brown, with dark brown central patch, subdivided into two lateral spots in paler specimens; elytra yellowish to dark brown; ventral surface reddish. *Maxillary palpi* (Fig. 6F) stout with palpomere 4 brown apically; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, slightly widest in the middle, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5H, Table 2) 0.42 mm long; phallobase much longer than parameres; parameres wide, widest at midlength, with convex lateral margins and straight mesal margin, apical region broadly rounded; median lobe shorter than parameres, gonopore in subapical position, basal apophyses rounded, with short extension into phallobase.

Differential diagnosis. *Crenitulus naranjoi* sp. nov. closely resembles *C. iviei* and *C. lajoncherei* in genital morphology, and seems morphologically almost identical to *C. lajoncherei*. See under *C. lajoncherei* for differential diagnosis of all three mentioned species.

Etymology. The new species is dedicated to the memory of Dr. Juan Carlos Naranjo López, Cuban entomologist, professor and mentor of many Cuban entomologist, including the senior author.

Distribution and biology. *Crenitulus naranjoi* sp. nov. is known from two of the principal mountains systems in Eastern of Cuba (Sierra Maestra and Nipe-Sagua-Baracoa Mts.) (Fig. 3A: localities E, F, G). All specimens were collected in shaded streams, sandy-stony bottom and with or without aquatic vegetation, located in well-preserved pluviselva, karst and montane forests. In Gran Piedra (Fig. 3A: localitity F), was collected together with *C. jiri*; in Turquino (Fig. 3A: locality G), together with *C. riberai* and *C. suturalis* complex.

***Crenitulus riberai* sp. nov.**

(Figs 4F, 5I, 6G)

Type locality. Cuba, Granma province, Parque Nacional Turquino, La Platica, 20º0.7′N 76º53.4′W, 880 m a.s.l.

Type material. HOLOTYPE: male (NMPC): ‘Cuba: Granma Province, PN Turquino, on the trail up to ca. 0.5 km S (by air) from La Platica, 20º0.5’N 76º53.3’W, ca. 920 m, 24.vi.2012, Deler-Hernández & Fikáček [printed]’ [molecular voucher MF612/3]. PARATYPES: 1 spec. (NMPC): same data as holotype, [molecular voucher MF1005.5]; 8 spec. (NHMW, NMPC): Granma province, PN Turquino, around La Platica, 20º0.7’N 76º53.4’W, 880 m, 25-26.vi.2012, Deler-Hernández & Fikáček lgt., MF24; 2 spec. (NMPC): Granma province, PN Turquino, on the trail up to ca. 0.5 km S (by air) from La Platica, 20º0.5’N 76º53.3’W, ca. 920 m, 23-27.vi.2012, Deler-Hernández & Fikáček lgt., MF20; 6 spec. (NMPC): Granma, Hoyo de Caracas, Protected Area Pico Caracas, 19º58'3.58''N 77º00'27.41''W, 800 m, 07.iii.2013, coll. A. Deler-Hernández [molecular vouchers MF807/1, MF807/2, MF807/3, MF808/4, MF808/5].

Description. Habitus as in Fig. 4F. *Body* length about 2.05−2.40 mm. *Coloration.* Clypeus and head dark brown; pronotum light brown to dark brown, with dark brown central patch in some specimens or lateral and posterolateral margins pale in others; elytra light brown to dark brown; ventral surface dark brown. *Maxillary palpi* (Fig. 6G) stout with palpomere 4 brown apically; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, widest in the middle, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5I, Table 2) 0.38 mm long; phallobase as long as parameres, basal portion short, subquadrate, manubrium only slightly

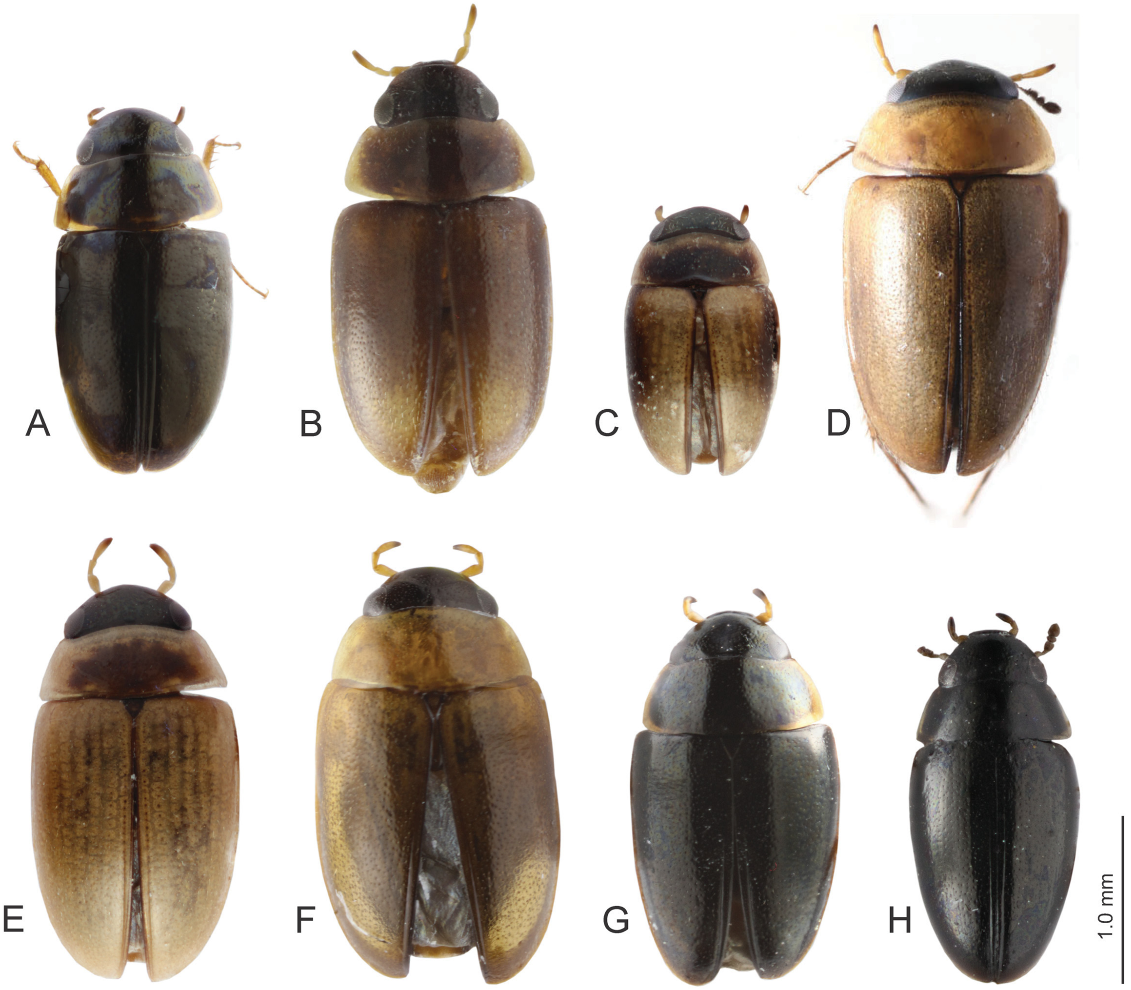


Figure 4. Habitus of selected representatives of the genus *Crenitulus* in the Greater Antilles. (A) *C. cristal* sp. nov.; (B) *C. guamuhaya* sp. nov.; (C) *C. guanyangi* sp. nov.; (D) *C. jiri* sp. nov.; (E) *C. naranjoi* sp. nov.; (F) *C. riberai* sp. nov.; (G) *C. smithi* sp. nov.; H) *C. suturalis* complex.

narrower, widely rounded; parameres elongate, with rounded lateral margins and straight mesal margins, apical region narrow; median lobe slightly shorter than parameres, gonopore in subapical position, basal apophyses short, with a very short extension into phallobase.

Etymology. This species is named in honor of Ignacio Ribera, a water beetle specialist from Barcelona, Spain.

Distribution and biology. *Crenitulus riberai* sp. nov. is known from the western part of the Sierra Maestra mountain range (Fig. 3A: localities G, H). All specimens were collected in shaded streams, sandy-stony bottom and without aquatic vegetation, located in well-preserved evergreen and montane forests. In Turquino (Fig. 3A: locality G), was collected together *C. naranjoi* and *C. suturalis* complex.

Crenitulus smithi sp. nov.

(Figs 4G, 5J, 6H)

Type locality. Dominican Republic, Barahuco province, 18°35.49'N 71°26.41'W, 720 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'Dominican Republic: Bahoruco province, 11.7 km N of Neiba [=Neyba], 18°35.49'N 71°26.41'W, 720 m, 19.viii.2014, Deler, Fikáček, Gimmel, DR14 [printed]' [molecular voucher MF1078]'. PARATYPES: 1 spec. (NMPC): same data as holotype; 44 spec. (BMNH, CMN, MNHNSD, NHMW, NMPC): Azua, 12.3 km NW of Azua, crossing of Río Jura, 18°32.54'N 70°48.91'W, 292 m, 07.ix.2014, A. Deler-Hernández, Fikáček, Gimmel, DR39.

Description. Habitus as in Fig. 4G. *Body* length about 1.80−1.95 mm. *Coloration.* Clypeus and head black; pronotum dark brown to black, with anterolateral, lateral and posterolateral margins pale; elytra dark brown to black; ventral surface dark brown. *Maxillary palpi* (Fig. 6H) stout with palpomere 4 brown; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, with terminal infuscation. *Aedeagus* (Fig. 5J, Table 2) 0.40 mm long; phallobase longer than parameres, with basal portion subrectangular, rather long, manubrium short, slightly narrower; parameres wide, widest in basal two fifths, with rounded lateral margins and straight mesal margins, apical region rounded; median lobe shorter than parameres, gonopore in subapical position, basal apophyses short, with a very short extension into phallobase.

Differential diagnosis. *Crenitulus smithi* sp. nov. can be diagnosed from the two remaining *Crenitulus* species recorded from Hispaniola (*C. dominicensis* sp. nov. and *C. suturalis* group) only on the basis of male genitalia. In comparison to the other species, it has the median lobe much shorter than parameres (as long as parameres in *C. dominicensis* and *C. suturalis*). By the general form of the aedeagus, *C. smithi* resembles the Cuban *C. iviei*, *C. lajoncherei* and *C. naranjoi*.

Etymology. This species is dedicated to Andrew Smith, a colleague of the first author and a very enthusiastic entomologist specializing in Scarabaeidae.

Distribution and biology. *Crenitulus smithi* sp. nov. is known from Sierra de Neiba and Cordillera Central in Dominican Republic (Fig. 3B: localities L, M). In both localities, it was collected in shallow exposed pools at stony banks of small rivers in mountain foothills, together specimens of *C. suturalis* complex.

***Crenitulus yunque* sp. nov.**

(Figs 5K, 6J)

Type locality. Cuba, Guantánamo province, Baracoa, El Yunque, 20.313ºN 74.574ºW, 200 m a.s.l.

Type material. HOLOTYPE: male (NMPC): 'CUBA: Guantánamo Prov., Baracoa municipality, El Yunque near Finca La Delicia, 20.313ºN 74.574ºW, 200 m, 17.v.2015, coll.: A. Deler-Hernández' [molecular voucher MF1067.1].

Description. *Body* elongate oval; body length 1.95 mm. *Coloration.* Clypeus and head dark brown; pronotum brown with anterolateral, lateral and posterolateral widely pale; elytra brown, with demarcated darker area at midlength; ventral surface brown. *Maxillary palpi* (Fig. 6J) stout with palpomere 4 brown; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5K, Table 2) 0.32 mm long; phallobase as long as parameres, with basal portion narrowly subquadrate, manubrium long and only slightly narrower; parameres wide, widest at midlength, with convex lateral margins and straight mesal margins, apical region narrow; median lobe slightly shorter than parameres, gonopore in subapical position, basal apophyses wide and rounded, with short extension into phallobase.

Differential diagnosis. In Eastern Cuba, the genital morphology of *Crenitulus yunque* resembles *C. cristal* and *C. guanyangi,* and can be diagnosed from them by apical gonopore (subapical in *C. cristal*), wide manubrium (narrow in *C. cristal* and *C. guanyangi*) and details of shape of parameres (compare Figs 5A, 5D and 5K). The genital morphology of *C. yunque* is nearly identical to that of *C. guamuhaya* from Central Cuba.

Etymology. This species is named after the El Yunque de Baracoa massif, where the type specimen was collected, the species name is noun in apposition.

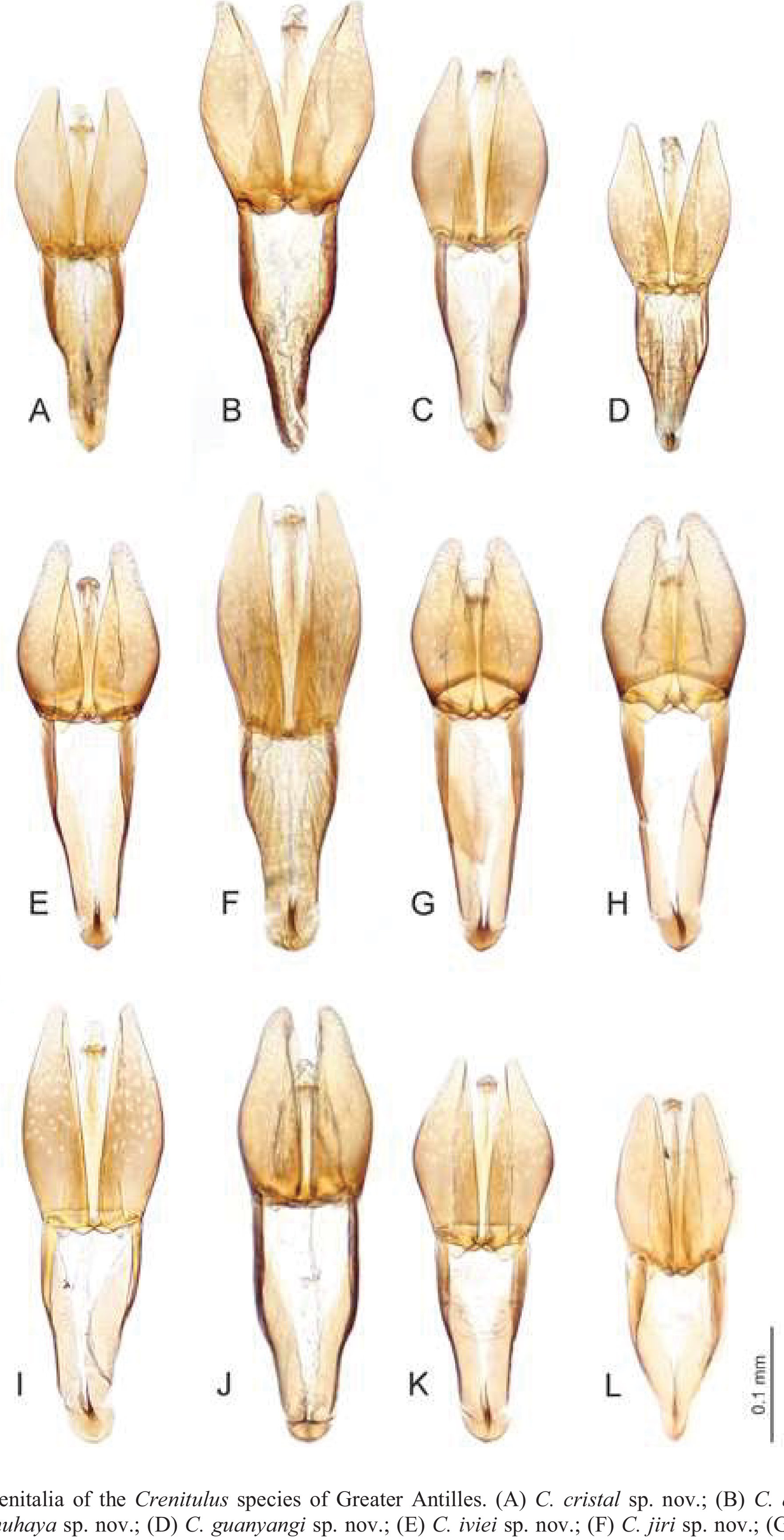


Figure 5. Male genitalia of the *Crenitulus* species of Greater Antilles. (A) *C. cristal* sp. nov.; (B) *C. dominicensis* sp. nov.; (C) *C. guamuhaya* sp. nov.; (D) *C. guanyangi* sp. nov.; (E) *C. iviei* sp. nov.; (F) *C. jiri* sp. nov.; (G) *C. lajoncherei* sp. nov.; (H) *C. naranjoi* sp. nov.; (I) *C. riberai* sp. nov.; (J) *C. smithi* sp. nov.; (K) *C. yunque* sp. nov.; (L) *C. suturalis* complex.

Distribution and biology. *Crenitulus yunque* sp. nov. is only known from the type locality at El Yunque de Baracoa in Nipe-Sagua-Baracoa mountain range (Fig. 3A: locality A). The holotype was collected in shaded streams, muddy to sandy-stony bottom and without aquatic vegetation, located in highlands within well-preserved rainforest, together with specimens of *Crenitulus iviei*.

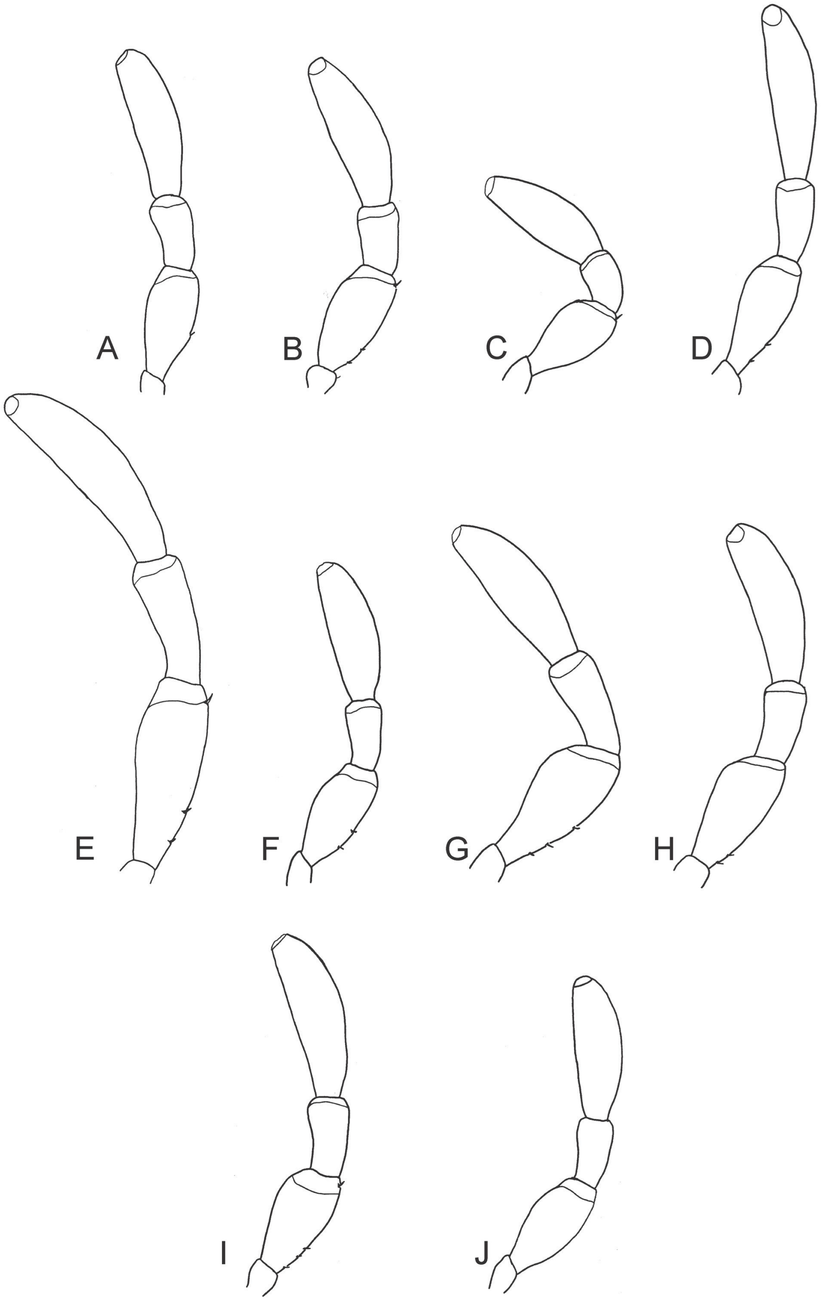


Figure 6. Maxillary palpus of *Crenitulus* of Greater Antilles. (A) *C. cristal* sp. nov.; (B) *C. guamuhaya* sp. nov.; (C) *C. guanyangi* sp. nov.; (D) *C. iviei* sp. nov.; (E) *C. jiri* sp. nov.; (F) *C. naranjoi* sp. nov.; (G) *C. riberai* sp. nov.; (H) *C. smithi* sp. nov.; (I) *C. suturalis* complex; (J) *C. yunque* sp. nov.

Table 2. Diagnostic characters of male genitalia of *Crenitulus* species of Greater Antilles.

	Length of the body (mm)	Length of aedeagus (mm)	Parameres to phallobase ratio	Parameres: shape of apex	Length of the median lobe	Position of gonopore
C. cristal	1.70−1.85	0.30	0.90	narrow	shorter than parameres	subapical
C. dominicensis	2.2	0.42	0.83	narrow	nearly as long as parameres	subapical
C. guamuhaya	1.90-2.10	0.36	0.89	narrow	nearly as long as parameres	apical
C. guanyangi	1.35-1.65	0.26	1.00	narrow	nearly as long as parameres	apical
C. iviei	1.80-2.00	0.38	0.78	rounded	shorter than parameres	apical
C. jiri	2.00-2.16	0.40	1.11	narrow	nearly as long as parameres	subapical
C. lajoncherei	2.08-2.35	0.38	0.81	broadly rounded	shorter than parameres	subapical
C. naranjoi	1.85-2.23	0.42	068	broadly rounded	shorter than parameres	subapical
C. riberai	2.04-2.42	0.38	1.00	narrow	nearly as long as parameres	subapical
C. smithi	1.81-1.93	0.40	0.74	rounded	shorter than parameres	subapical
C. yunque	1.93	0.32	0.68	narrow	nearly as long as parameres	subapical
C. suturalis complex	1.34−1.80	0.28	0.87	rounded	nearly as long as parameres	apical

Crenitulus suturalis **(LeConte, 1866) complex**

(Figs 4H, 5L, 6I, 7)

Material examined: CUBA: Artemisa: 14 spec. (NMPC): Las Terrazas, Río San Juan, 22º50′40.32″N 82º56′8.09″W, ca. 150 m, 12.vii.2016, A. Deler-Hernández lgt.; 18 spec. (NMPC): Soroa, Río Manantiales, 22º47′29.97″N 83º0′22.83″W, ca. 180 m, 14.vii.2016, A. Deler-Hernández lgt.; 6 spec. (NMPC): Cañon del Santa Cruz, Río Santa Cruz, 22º45′1.29″N 83º8′58.36″W, ca. 199 m, 16.vii.2016, A. Deler-Hernández lgt. **Sancti Spiritus**: 12 spec. (NMPC): Topes de Collantes, Salto del Caburní, Caburní river, 381 m, 16.x.2014, 20º0.5′N 76º53.3′W, A. Deler-Hernández lgt. [molecular vouchers MF1009/1, MF1009/2]. **Holguín**: 3 spec. (NMPC): La Melba, PN Alejandro v. Humbold, 440 m, 20.43441 74.83916, A. Deler-Hernández lgt. [molecular vouchers MF1005/1, MF1005/2, MF1005/3]; 4 spec. (NMPC): PN Mensura-Piloto, Potrerillo, 12.v.2013, A. Deler-Hernández lgt. [incl. molecular vouchers MF1032/1, MF1032/2, MF1036/3]. **Granma**: 4 spec. (NMPC): PN Turquino, La Platica, 920 m, 20º0.5´N 76º53.3´W, A. Deler-Hernández lgt. [molecular vouchers MF612/4, MF1017/1, MF1017/2, MF1017/3]; 15 spec. (NMPC): Protected Area Pico Caracas, Hoyo de Caracas, 800 m, 6-7.iii.2013, 19º58′3.58″N 77º0′27.41″W, A. Deler-Hernández lgt. [molecular vouchers MF1034/1, MF1034/2, MF1034/3]; 5 spec. (NMPC): Río Colorado, 20.09765 – 76.92718, 129 m, 30.ix.2014, A. Deler-Hernández lgt. **Santiago de Cuba**: 4 spec. (NMPC): Dos Caminos, El Vivero, 1.6 km E of Dos Caminos, 20°10.8'N 75°46.4'W, ca. 150 m, 20-21.vi.2012, A. Deler-Hernández & Fikáček lgt. (MF17-18); 31 spec. (NMPC): San Miguel de Parada, Río Cobre, 3 m, 20º0′56.40″N 75º52′15.14″W, 25.ix.2009, A. Deler-Hernández lgt.; 1 spec. (NMPC): Chalón, 6.iii.2005, Y.S.Megna lgt.; 43 spec. (NMPC): La Redonda, Río Sevilla, 20°00'N 75°45'W, 24.ix.2009, A. Deler-Hernández lgt. **Guantánamo**: 13 spec. (NMPC): PN Alejandro v. Humboldt, ca. 0.4 km E of administration camp, 20°30.5'N 74°40'W, 20 m, 17.vi. 2012; A. Deler-Hernández & Fikáček lgt. (MF16); 13 spec. (NMPC): El Yunque, 3.2 km SW of campismo, right tributary of Duabe river, 20°19'N 74°34'W, ca. 150 m a.s.l., 13.vi.2012; Deler-Hernández & Fikáček lgt. (MF09) [incl. molecular voucher MF634/1, MF634/2, MF634/3]; 8 spec. (NMPC): Baracoa, San Alejo, 12-14.x.2010, A. Deler-Hernández lgt. **JAMAICA:** 1 female (NMPC): Blue Mountain, 18.0238889 76.6392556, 509 m, 16.xi.2013, stream, F. Cala-Riquelme lgt. (JA-11) [molecular voucher MF1004]. **DOMINICAN REPUBLIC: Barahona**: 9 spec. (NMPC): pools on river 2.6 km SE of Polo, 18°4.82'N 71°15.53'W, 570 m, 16.viii.2014, A. Deler-Hernández, Fikáček & Gimmel lgt. (DR09) [molecular voucher MF1010]. **Puerto Plata**: 3 spec. (NMPC): MN Salto Damajagua 2.3 km SE of Imbert, 19°44.28'N 70°48.72'W, 188 m, 31.viii.2014, A. Deler-Hernández, Fikáček & Gimmel lgt. (DR26) [molecular voucher MF1011]. **Azua:** 4 spec. (NMPC): 12.3 km NW of Azua, crossing of Río Jura, 18°32.54'N 70°48.91'W, 292 m, 7.ix.2014, A. Deler-Hernández, Fikáček, Gimmel lgt. (DR39).

Redescription. Habitus as in Fig. 4H. *Body* length 1.34−1.80 mm. *Coloration.* Clypeus and head black; pronotum black with anterolateral, lateral and posterolateral margins pale; elytra black with lateral margins pale; ventral surface dark brown. *Maxillary palpi* (Fig. 6I) stout with palpomere 4 brown; palpomere 2 inflated; palpomeres 3 short; palpomere 4 elongated, slightly widest in the middle, with terminal infuscation, ca. 2x longer than palpomere 3. *Aedeagus* (Fig. 5L, Table 2) 0.28 mm long; phallobase slightly longer than parameres, basal portion long and wide, manubrium short and narrow; parameres with convex lateral margins and straight mesal margins, widest in basal third, narrowly rounded apically; median lobe nearly as long as parameres, gonopore in apical position, basal apophyses with short extension into phallobase. Detailed description provided by Komarek (2005).

Differential diagnosis. *Crenitulus suturalis* group contains small dark specimens which are externally identical with several dark species of the *C. yunque* complex (*C. dominicensis*, *C. smithi*, *C. cristal*). It can be distinguished from all these species as well as from all other species of *C. yunque* complex by the genital morphology – see the diagnosis of the *C. yunque* group for details.

Taxonomic comment. The identity of the Greater Antillean specimens of the *C. suturalis* group remains unclear. All sequenced specimens form a well-supported clade (Figs 1A, B) with the specimen of *C. suturalis* from USA (i.e. North America, from where *C. suturalis* was originally described) sequenced by Short & Fikáček (2013). Hence, they are surely closely related to true *C. suturalis*, but seem to be genetically rather distant from the sequenced US specimen to represent the same species. Moreover, the single examined Jamaican specimen of *C. suturalis* group is also genetically rather distant from Cuban and Dominican specimens (which are genetically rather close to each other), which may suggests that at least two species (one in Jamaica and another in Cuba+Hispaniola) may be present in the Greater Antilles. Genital morphology of Cuban+Hispaniolan specimens examined is rather uniform and corresponds with Fig. 5L, only in some cases show small variation in the length of the median lobe and the shape of the phallobase. We were not able to compare it with the genital morphology of the Jamaican specimens, as the only specimen in our hands is female. Genital morphology of the Cuban and Hispaniolan specimens does not correspond with the genital morphology illustrated for *C. suturalis* by Komarek (2005), without specifying the locality data for the illustrated specimen. A detailed comparison with continental specimens (especially those from North America which should represent the true *C. suturalis*) is hence necessary to reveal the identity of the Greater Antillean specimens of *C. suturalis* group. This study is not performed here and we hence assign specimens belonging to the *C. suturalis* clade to the species group only.

Distribution and biology. *Crenitulus suturalis* is considered a very widespread species occurring from Canada to Argentina by Komarek (2005) (Canadian records are considered doubtful by Smetana (1988)). In Cuba, representatives of *C. suturalis* group are the most commonly collected representatives of *Crenitulus* recorded in Cuba, in both lowland agriculture land and forested highland and mountain areas (Fig. 7). In Dominican Republic (Fig. 7), they seem to be less common based on our collecting effort in 2014, and we failed to find them in any sampled locality in Puerto Rico. Specimens were usually collected in shaded or sun-exposed pools at sandy or gravely banks of streams or small rivers (or directly at sides of streams and rivers), both without vegetation or with sparse submerged vegetation or algae.

Figure 7. Known distribution of the *Crenitulus suturalis* complex in the Greater Antilles.

Discusion

***Crenitulus* of Greater Antilles.** Our study revealed a high species-diversity of *Crenitulus* in Cuba and Hispaniola which was overlooked until now. Only a single species (*C. suturalis*) was reported from Greater Antilles (Jamaica and Dominican Republic) by Komarek (2005). In our first screening, we considered the specimens of the *C. yunque* species group as belonging to another widespread species, *C. solstitialis*. Analysis of *cox1* sequences subsequently revealed a deep genetic structure within this clade which we first considered as population structure, until the specimens were dissected and differences in genital morphology indicating the presence of multiple species were found. Moreover, after including more continental specimens and more genes into the phylogenetic analysis, it became evident that the whole clade is not standing close to the continental *C. solstitialis*. On the contrary, our analysis reveals that *C. yunque* clade is an isolated clade of *Crenitulus* endemic for Greater Antilles. In this respect, our results are in agreement with recent studies on other arthropod groups which revealed large old endemic Greater Antillean clades (e.g., *Callisto* butterflies: Matos-Maraví *et al*., 2014; *Phaenonotum* beetles: Deler-Hernández *et al*., in press; selenopid spiders: Crews & Gillespie, 2010), sometimes in groups in which the Caribbean fauna was considered species-poor and consisting of few widespread continental species (e.g., theridiid spiders: Dziki *et al*., 2015).

The majority of the species of the *Crenitulus yunque* clade occur in Eastern Cuba (7 spp.), whereas only two species were found in Hispaniola, and one species occurs each in Central and Western Cuba. Both Hispaniolan species form a clade deeply nested among the

Cuban ones, sister or close to the species from easternmost parts of Cuba (Yunque de Baracoa). All species seem to be locally endemic, occurring at most in two closely adjacent mountain ranges, but more frequently confined to one particular mountain range or its part. Mountains of Eastern Cuba are definitely the region where most of the species occur (and likely where most of the cladogenesis happened), in agreement e.g. with *Eutherodactylus* frogs which also underwent an island radiation in Cuba (Rodríguez *et al*., 2010, 2012). These studies also indicated Eastern Cuba as a source area from which Central and Western Cuba were colonized. Our results reveal that the situation was more complex in *Crenitulus*: Central Cuban *C. guamahaya* is revealed either sister to all other species (in Bayesian analyses and maximum likelihood: Fig. 1) or as early branching one (in single gene tress used for species delimitation); in contrast, the Western Cuban *C. lajoncherei* is deeply nested and sister to Eastern Cuban *C. iviei*+*C.naranjoi* (and is moreover morphologically identical to *C. naranjoi*). A more complex biogeographic scenario than "from east to the west" may be hence expected, likely also involving extinction of some species/clades. Extinction may be for example responsible for the Western Cuba–Eastern Cuba distribution pattern of the *C. lajoncherei*-*iviei*-*naranjoi* clade and its absence in Central Cuba.

In Eastern Cuba, multiple species of the *C. yunque* complex may co-occur syntopically, but the co-occurring species are never closely related: *C. naranjoi* co-occurs with *C. riberai* in Turquino and with *C. jiri* in Gran Piedra, *C. yunque* co-occurs with *C. iviei* in Yunque de Baracoa, and *C. guanyangi* with *C. jiri* in Loma Los Guineos. In contrast, closely related species are strictly allopatric. This indicates that allopatric speciation of populations isolated in mountain refugia may have been the main driving forces generating the current species diversity of *Crenitulus* in Cuba. Interestingly, the distribution ranges of most species do not correspond to two principal mountain ranges in Eastern Cuba (Sierra Meastra and Nipe-Sagua-Baracoa). Some species are either confined to small parts of the respective mountain range only (e.g., *C. jiri* in easternmost part of Sierra Maestra, *C. riberai* in westernmost part of Sierra Maestra). More widespread species (*C. naranjoi* and *C. jiri*) occur in western parts of both principal mountain ranges and are sister to species confined to easternmost part of Nipe-Sague-Baracoa range.

The Hispaniolan fauna of *C. yunque* group seems to be much poorer than in Cuba, only consisting of two species occurring in the southern foothills of Sierra de Neiba and Cordillera Central. No specimens were found in other parts of the Dominican Republic where similar habitats were sampled. Our sampling effort in the Dominican Republic was less complete than in Cuba, and we hence cannot exclude that more species will be discovered in the future. On the other hand, our phylogenetic analysis indicates that Dominican species form a deeply nested clade in the *C. yunque* clade and hence colonized Hispaniola much later than Cuba. Lower number of species in Hispaniola may be hence partly a consequence of shorter time for cladogenesis. Alternatively, extinction rate may have been higher in Hispaniola. *Crenitulus paleodominicus* from Dominican amber is not conspecific with any modern species occurring in Hispaniola (i.e. *C. dominicensis*, *C. smithi* or *C. suturalis*) based on the morphology of the male genitalia, and is definitely an extinct species.

Species-level systematics of *Crenitulus*. New World *Crenitulus* species were revised taxonomically by Komarek (2005) as a part of his *Anacaena* revision (only later the species were moved from *Anacaena* to *Crenitulus*; Fikáček & Vondráček, 2014). Six species were recognized in the Neotropics and southern Nearctics, of which two were considered extremely widespread: *C. suturalis* distributed from Canada to Argentina, and *C. solstitialis* distributed from Mexico to Brazil. Eleven species were synonymized with the above two species in the course of the revision, as their external morphology fell into the continual variation of the above species, and genitalia did not show any relevant differences which would indicate separate species (Komarek, 2005). Our study indicates that the real situation may be largely different: at least 12 species seem to be present in our random sampling of continental *Crenitulus* sequenced for the species-level phylogeny (Fig. 1). Those from Ecuador were even identified using the key provided by Komarek (2005) as *C. suturalis*, *C. solstitialis* and *C. hirsutus*, but results of the molecular analyses do not indicate them to stand close to these species. Morphology itself seems hence not to be sufficient for the identification and systematics of *Crenitulus* species, as it is rather uniform and seems to be convergent in unrelated species. Moreover, in contrast to the conclusion of Komarek (2005) who considered the genital morphology as very uniform within the genus, we found rather large differences between genitalia of the continental specimens indicated as separate species by molecular markers.

Our detailed revision of the *C. yunque* clade combined with its phylogenetic analysis complicates the situation even more. We discovered genetically distant non-related species (e.g., *C. guamuhaya* and *C. yunque*, or *C. lajoncherei* and *C. naranjoi*) which are morphologically identical and even genital morphology does not provide any diagnostic characters. In both above cases, the species are locally endemic but widely allopatric, each living in a different part of Cuba. The opposite case was found in the *Crenitulus cristal-jiri-yunque* complex in which molecular markers only indicated two species candidates, despite the fact that genital morphology allows to distinguish three morphotypes which moreover correspond with distribution pattern (all three species seem to be allopatric, but all live in same parts of Eastern Cuba in this case). Many species of *C. yunque* clade seem to be local microendemics – this can be naturally a consequence of the island environment, but it may also indicate that similar micro-endemism be also expected in the continent.

When summarized, our results seem to indicate that *Crenitulus* may be a species-rich genus in the Neotropics and that its current species diversity is largely underestimated. However, the systematic studies need to be performed as a combination of morphology-based and molecular approaches, as neither morphology nor molecules alone do not allow to understand species limits properly. Molecular barcoding (based on one or few standard markers) is recommended as a quick tool for basic screening of the species-diversity available in the material or region examined, which should be followed by an effort to find morphological diagnostic characters allowing for identification of specimens which cannot be sequenced. Descriptions of new species based on old or degraded material only should be discouraged, as they may produce huge problems in interpreting the identity of the respective species. At least some DNA-grade specimens properly deposited (i.e. in 96% alcohol and frozen) in easily accessible collections should be required even in case when the species is described only on the basis of morphology; these specimens will make the future DNA-based analysis possible which may specify the identity of the species and allow for its delimitation from related/similar ones.

Acknowledgements

We thank members of the 2013-2014 Canadian-Cuban Expedition (Robert Anderson and Andrew Smith from the Canadian Museum of Nature, Ottawa, Canada, Guanyang Zhang from Arizona State University, Tempe, U.S.A., and Franklyn Cala-Riquelme from Universidad de Bogotá, Bogotá, Colombia) for their great help during field work. We are deeply grateful to the authorities and personnel of all the Protected Area and National Parks in Cuba and Dominican Republic for providing access to both protected regions and, especially, for sharing invaluable local knowledge. This study was supported by Grant SVV 260 434/2017 and The Rufford Foundation (15144-1) to A.D.H., and by the Ministry of Culture of the Czech Republic (DKRVO 2016/14, National Museum, 00023272) to M.F.

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SUPPORTING INFORMATION

Supplementary File 1: Molecular data and phylogenetic analyses.
Supplementary File 2: Species delimitation analyses.

Supplementary material

to the paper

A review of the genus *Crenitulus* of Greater Antilles (Coleoptera: Hydrophilidae)

by

ALBERT DELER-HERNÁNDEZ, VÍT SÝKORA and MARTIN FIKÁČEK

Part 1: Molecular data and phylogenetic analyses

List of specimens included in the molecular analyses [GenBank accessions will be added before accepting]

Voucher #	**Species**	**Country**	**Sources**	**COI**	**COII**	**16S**	**18S**	**28S**
SLE0133	*Horelophus walkeri*		GenBank	KC935276	KC992431	KC992681	KC935053	KC992581
MF791	*Pseudorygmodus versicolor*		GenBank	KM262052	KM262053	NO	KM262050	KM262051
MF257	*Anacaena lanzhujii*		GenBank	KC935218	KC992455	NO	KC935002	KC992523
GB	*Anacaena globulus*		GenBank	AM287086	AM287108	AM287064	AM287125	NO
SLE0242	*Anacaena lindi*		GenBank	KC935219	KC992501	NO	KC934994	KC992524
SLE0246	*Anacaena lineata*		GenBank	KC935296	KC992500	KC992645	KC934996	KC992526
MSC1819	*Crenitis sp.*		GenBank	KC935246	KC992413	KC992660	KC935023	KC992552
MSC1816	*Crenitulus suturalis*	USA	GenBank	KC935226	NO	KC992649	KC935001	KC992531
SLE0369	*Crenitulus hirsuta*	Costa Rica	GenBank	KC935217	KC992482	KC992644	KC934993	KC992522
MSC1815	*Crenitulus solstitialis*	Costa Rica	GenBank	KC935223	NO	KC992647	KC934998	KC992528
SLE0346	*Crenitulus sp.*	Suriname	GenBank	KC935225	KC992483	KC992648	KC935000	KC992530
MF7	*Crenitulus hirsuta*	Ecuador	New	YES	YES	YES	YES	YES
MF33	*Crenitulus suturalis*	Ecuador	New	YES	NO	YES	YES	YES
MF35	*Crenitulus solstitialis?*	Ecuador	New	YES	YES	NO	YES	YES
MF612.3	*Crenitulus riberai*	Cuba	New	YES	NO	YES	YES	YES
MF805.3	*Crenitulus guanyangi*	Cuba	New	YES	NO	YES	YES	YES
MF806.1	*Crenitulus naranjoi*	Cuba	New	YES	NO	YES	YES	NO
MF806.2	*Crenitulus jiri*	Cuba	New	YES	NO	YES	YES	YES
MF806.5	*Crenitulus jiri*	Cuba	New	YES	NO	YES	YES	YES
MF807.2	*Crenitulus riberai*	Cuba	New	YES	NO	YES	NO	YES
MF808.4	*Crenitulus riberai*	Cuba	New	YES	NO	YES	YES	NO
MF841	*Crenitulus solstitialis*	Peru	New	YES	YES	NO	YES	YES
MF848	*Crenitulus hirsuta*	Peru	New	YES	YES	NO	YES	YES
MF849	*Crenitulus hirsuta*	Peru	New	YES	YES	NO	YES	YES
MF849/1	*Crenitulus solstitialis*	Peru	New	YES	YES	NO	YES	YES
MF850	*Crenitulus sp.*	Peru	New	YES	YES	NO	YES	YES

List of specimens included in the molecular analyses [GenBank accessions will be added before accepting]

Voucher #	**Species**	**Country**	**Sources**	**COI**	**COII**	**16S**	**18S**	**28S**
MF986.1	*Crenitulus sp.*	Cuba	New	YES	NO	YES	YES	YES
MF986.2	*Crenitulus sp.*	Cuba	New	YES	NO	YES	YES	NO
MF1004	*Crenitulus suturalis*	Jamaica	New	YES	NO	YES	YES	YES
MF1005	*Crenitulus suturalis*	Cuba	New	YES	NO	YES	YES	YES
MF1009	*Crenitulus suturalis*	Cuba	New	YES	NO	YES	YES	YES
MF1010	*Crenitulus suturalis*	Dominican R.	New	YES	YES	YES	YES	YES
MF1012	*Crenitulus dominicensis*	Dominican R.	New	YES	NO	YES	YES	YES
MF1035.2	*Crenitulus sp.*	Cuba	New	YES	NO	YES	YES	NO
MF1067.1	*Crenitulus yunque*	Cuba	New	YES	NO	YES	NO	NO
MF1067.4	*Crenitulus sp.*	Cuba	New	YES	YES	YES	YES	YES
MF1068.1	*Crenitulus guamuhaya*	Cuba	New	YES	YES	YES	YES	YES
MF1075.3	*Crenitulus cristal*	Cuba	New	YES	YES	YES	YES	YES
MF1078	*Crenitulus smithi*	Dominican R.	New	YES	YES	YES	YES	NO
MF1751	*Crenitulus lajoncherei*	Cuba	New	YES	NO	YES	YES	YES
MF1799	*Crenitulus jiri*	Cuba	New	YES	NO	NO	YES	YES

Primers used in PCR reactions for individual genes

Gene	Primer	Direction	Sequence (5-3)
COI-3´	stev_jerryF	forward	CAACATYTATTYTGATTYTTTGG
COI-3´	stev_patR	reverse	GCACTAWTCTGCCATATTAGA
COII	TL2-J-3037	forward	TAATATGGCAGATTAGTGCA
COII	TK-N-3785	reverse	TTTAAGAGACCAGTACTT
16S	LR-N-13398	forward	CGCCTGTTTAACAAAAACAT
16S	LR-J-12887	reverse	CCGGTCTGAACTCAGATCACGT
18S 3´	18Sa1.0	reverse	GGTGAAATTCTTGGACCGTC
18S 3´	18S3'I	forward	CACCTACGGAAACCTTGTTACGAC
18S 5´	18S5'I	forward	GACAACCTGGTTGATCCTGCCAGT
18S 5´	18Sb0.5	reverse	TAACCG CAACAACTTTAAT
28S	NLF184-21	forward	ACCCGCTGAAYTTAAGCATAT
28S	LS1041R	reverse	TACGGACRTCCATCAGGGTTTCCCCTGACTTC

PCR programs used for amplification of individual genes

PCR	**COI-3´**					
Step	1	2	3	4	5	6
Temperature (°C)	94	94	50	72	72	4
Time (s)	180	30	45	60	480	forever
		Repeat 35x				

PCR	**COII**					
Step	1	2	3	4	5	6
Temperature (°C)	94	94	50	72	72	4
Time (s)	180	30	45	60	480	forever
		Repeat 35x				

PCR	**16S**					
Step	1	2	3	4	5	6
Temperature (°C)	94	94	48	72	72	4
Time (s)	120	30	30	60	600	forever
		Repeat 40x				

PCR	**18S 3´**					
Step	1	2	3	4	5	6
Temperature (°C)	98	98	52	72	72	12
Time (s)	30	10	30	90	480	forever
		Repeat 30x				

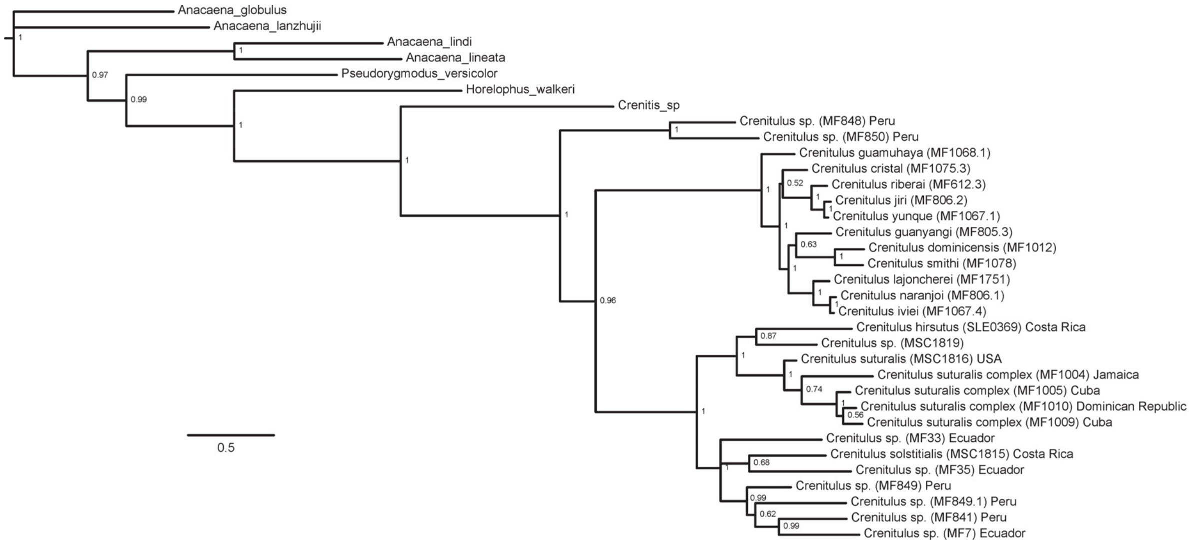
PCR	**18S 5´**					
Step	1	2	3	4	5	6
Temperature (°C)	98	98	52	72	72	12
Time (s)	30	10	30	60	480	forever
		Repeat 30x				

PCR	**28S**					
Step	1	2	3	4	5	6
Temperature (°C)	98	98	54	72	72	10
Time (s)	30	10	30	60	480	forever
		Repeat 30x				

RESULTS OF THE PHYLOGENETIC ANALYSES

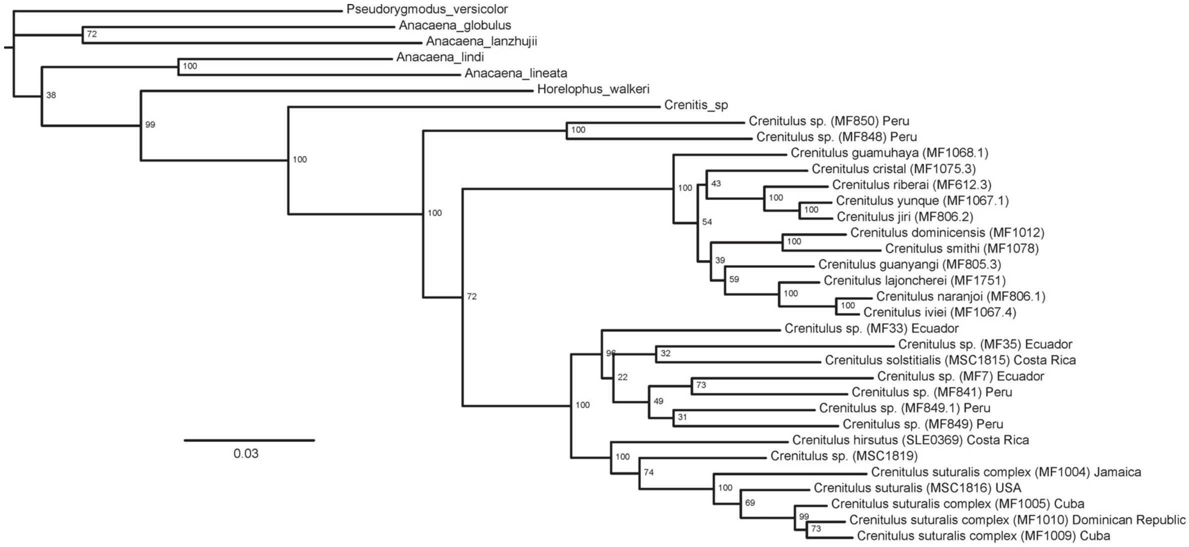
MrBayer analysis: 34 taxa dataset

outgroup: all outgroup taxa
ingroup: all *Crenitulus*



RAxML analysis: 34 taxa dataset

outgroup: all outgroup taxa
ingroup: all *Crenitulus*



Supplementary material

to the paper

A review of the genus *Crenitulus* of Greater Antilles (Coleoptera: Hydrophilidae)

by

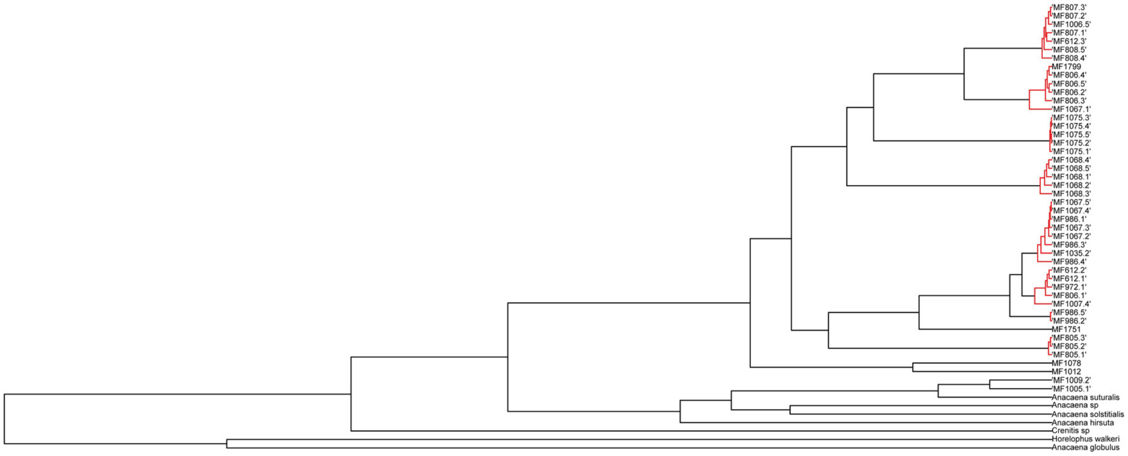
ALBERT DELER-HERNÁNDEZ, VÍT SÝKORA and MARTIN FIKÁČEK

Part 2: Species delimitation analyses

RESULTS OF THE GMYC SPECIES DELIMITATION ANALYSES

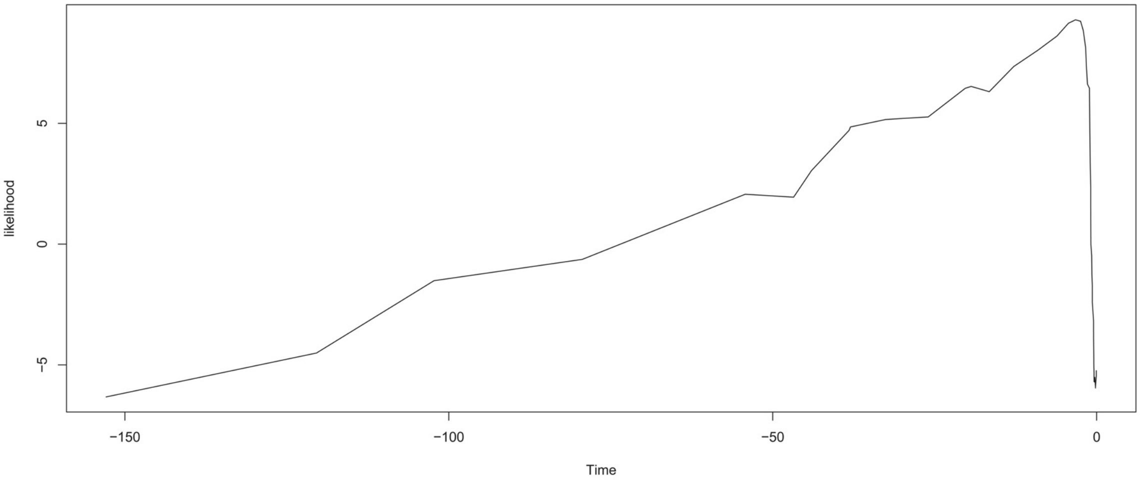
GMYC species delimitation analysis using cox1 tree: 53 taxa dataset

outgroup: *Crenitis* sp. + *Horelophus walker* + *Anacaena globulus*
ingroup: all *Crenitulus*



GMYC species delimitation analysis using cox1 genes

Maximum likelihood of GMYC model



Result of GMYC species delimitation

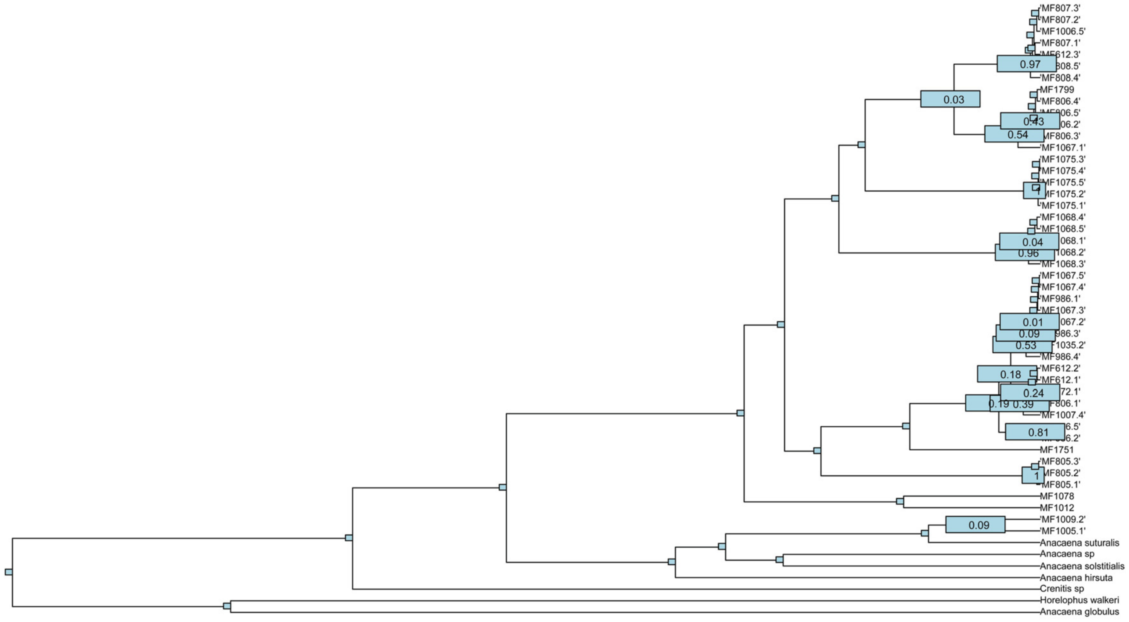
```
        method: single

        likelihood of null model:       -6.326176

        maximum likelihood of GMYC model:       9.286171

        likelihood ratio:       31.22469

        result of LR test:      1.658226e-07***


        number of ML clusters:  8

        confidence interval:    6-8


        number of ML entities:  20

        confidence interval:    16-24


        threshold time: -3.26586



  GMYC_spec          sample_name

1         1            'MF805.1'

2         1            'MF805.2'

3         1            'MF805.3'

4         2            'MF986.2'

5         2            'MF986.5'

6         3           'MF1007.4'

7         3            'MF806.1'

8         3            'MF972.1'

9         3            'MF612.1'
```

10	3	'MF612.2'
11	4	'MF986.4'
12	4	'MF1035.2'
13	4	'MF986.3'
14	4	'MF1067.2'
15	4	'MF1067.3'
16	4	'MF986.1'
17	4	'MF1067.4'
18	4	'MF1067.5'
19	5	'MF1068.3'
20	5	'MF1068.2'
21	5	'MF1068.1'
22	5	'MF1068.5'
23	5	'MF1068.4'
24	6	'MF1075.1'
25	6	'MF1075.2'
26	6	'MF1075.5'
27	6	'MF1075.4'
28	6	'MF1075.3'
29	7	'MF1067.1'
30	7	'MF806.3'
31	7	'MF806.2'
32	7	'MF806.5'
33	7	'MF806.4'
34	7	MF1799
35	8	'MF808.4'
36	8	'MF808.5'

37	8	'MF612.3'
38	8	'MF807.1'
39	8	'MF1006.5'
40	8	'MF807.2'
41	8	'MF807.3'
42	9	Anacaena_globulus
43	10	Horelophus_walkeri
44	11	Crenitis_sp
45	12	Anacaena_hirsuta
46	13	Anacaena_solstitialis
47	14	Anacaena_sp
48	15	Anacaena_suturalis
49	16	'MF1005.1'
50	17	'MF1009.2'
51	18	MF1012
52	19	MF1078
53	20	MF1751

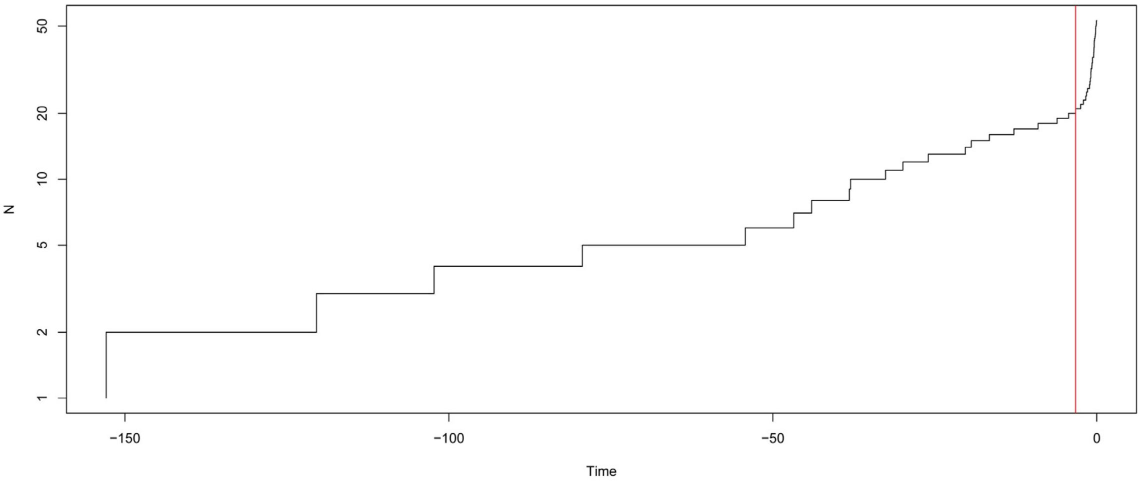
GMYC species delimitation analysis using cox1 genes

Support



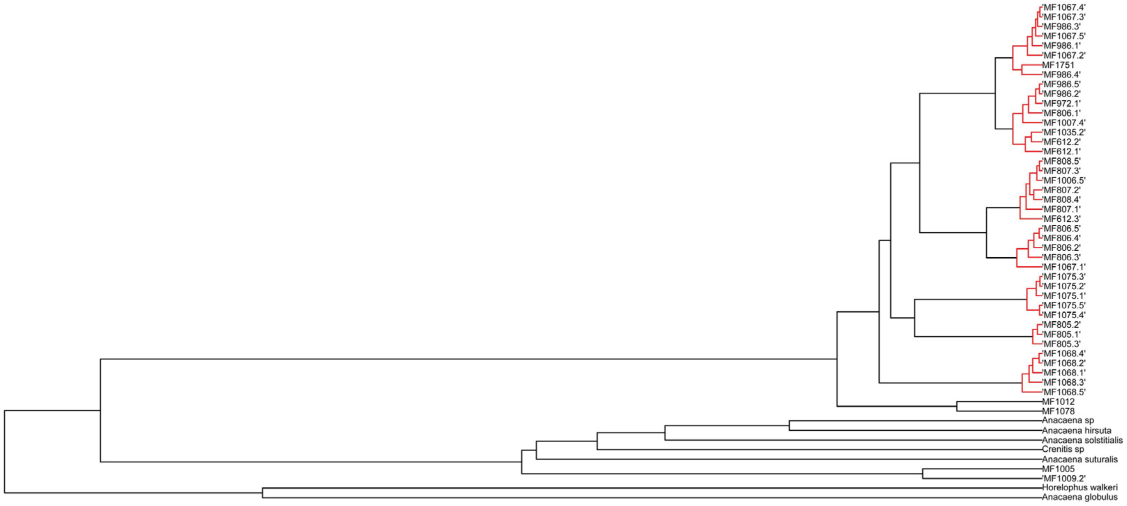
GMYC species delimitation analysis using cox1 genes

Treshold



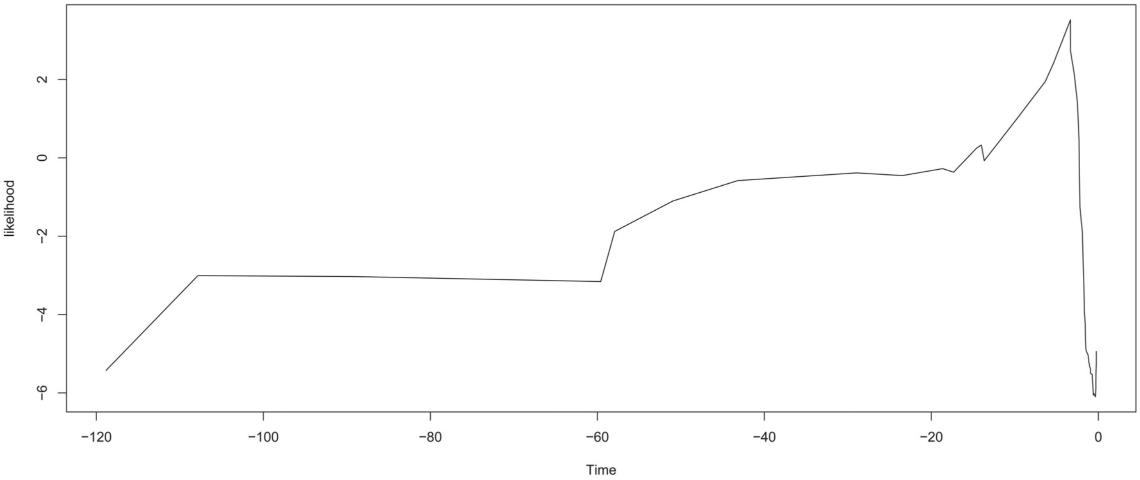
GMYC species delimitation analysis using 16S tree: 52 taxa dataset

outgroup: *Crenitis* sp. + *Horelophus walker* + *Anacaena globulus*
ingroup: all *Crenitulus*



GMYC species delimitation analysis using 16S genes

Maximum likelihood of GMYC model



Result of GMYC species delimitation

```
        method: single

        likelihood of null model:       -5.425229

        maximum likelihood of GMYC model:       3.523991

        likelihood ratio:       17.89844

        result of LR test:      0.0001298384***


        number of ML clusters:  7

        confidence interval:    5-9


        number of ML entities:  18

        confidence interval:    16-20


        threshold time: -3.339792
```

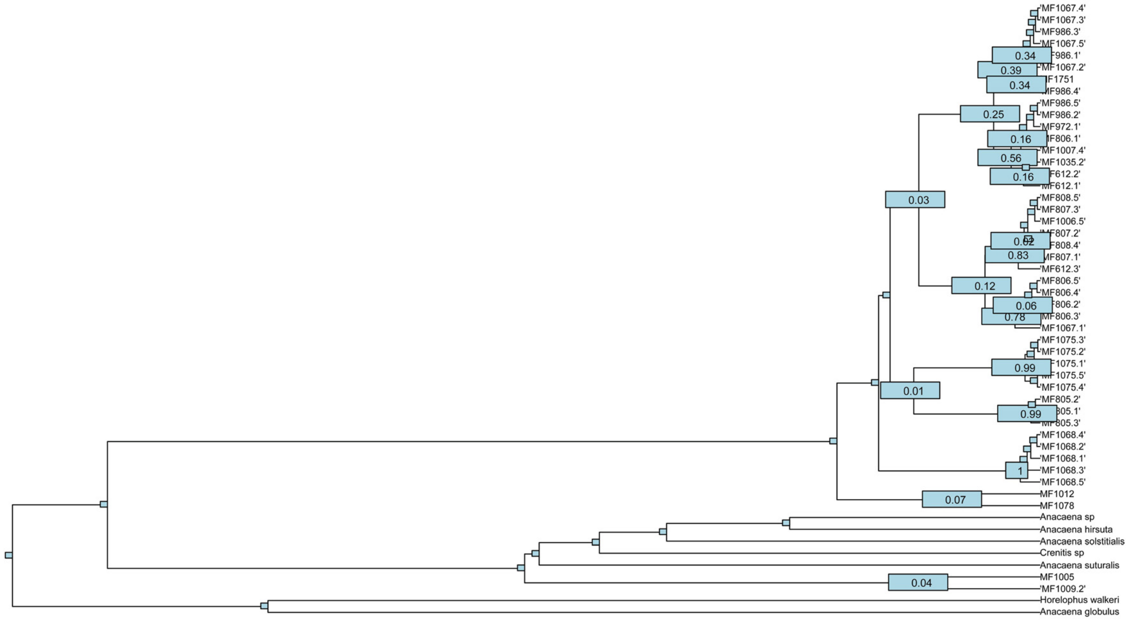
```
GMYC_spec          sample_name

1        1          'MF1068.5'

2        1          'MF1068.3'

3        1          'MF1068.1'

4        1          'MF1068.2'

5        1          'MF1068.4'

6        2           'MF805.3'

7        2           'MF805.1'

8        2           'MF805.2'

9        3          'MF1075.4'
```

10	3	'MF1075.5'
11	3	'MF1075.1'
12	3	'MF1075.2'
13	3	'MF1075.3'
14	4	'MF1067.1'
15	4	'MF806.3'
16	4	'MF806.2'
17	4	'MF806.4'
18	4	'MF806.5'
19	5	'MF612.3'
20	5	'MF807.1'
21	5	'MF808.4'
22	5	'MF807.2'
23	5	'MF1006.5'
24	5	'MF807.3'
25	5	'MF808.5'
26	6	'MF612.1'
27	6	'MF612.2'
28	6	'MF1035.2'
29	6	'MF1007.4'
30	6	'MF806.1'
31	6	'MF972.1'
32	6	'MF986.2'
33	6	'MF986.5'
34	7	'MF986.4'
35	7	MF1751
36	7	'MF1067.2'

37	7	'MF986.1'
38	7	'MF1067.5'
39	7	'MF986.3'
40	7	'MF1067.3'
41	7	'MF1067.4'
42	8	Anacaena_globulus
43	9	Horelophus_walkeri
44	10	'MF1009.2'
45	11	MF1005
46	12	Anacaena_suturalis
47	13	Crenitis_sp
48	14	Anacaena_solstitialis
49	15	Anacaena_hirsuta
50	16	Anacaena_sp
51	17	MF1078
52	18	MF1012

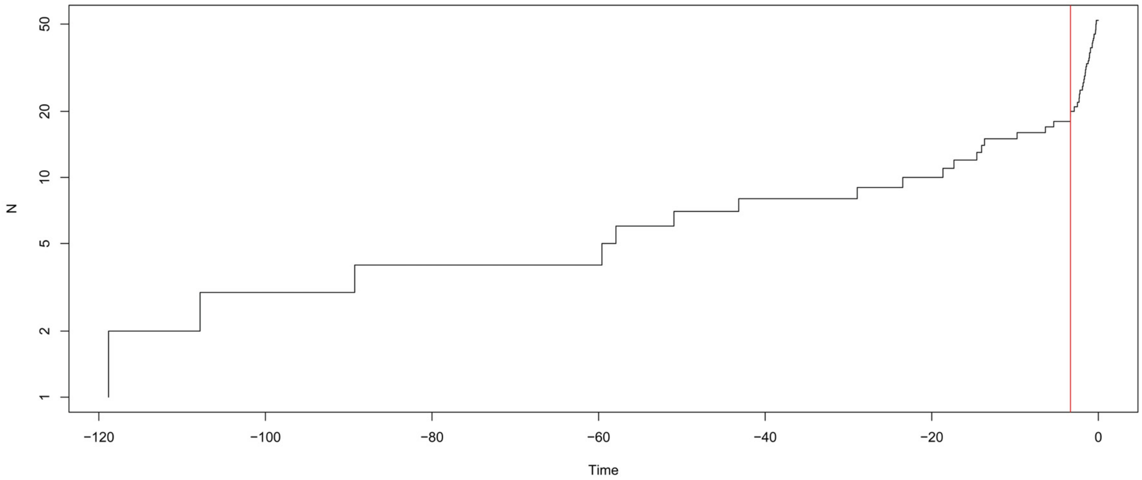
GMYC species delimitation analysis using 16S genes

Support



GMYC species delimitation analysis using 16S genes

Treshold

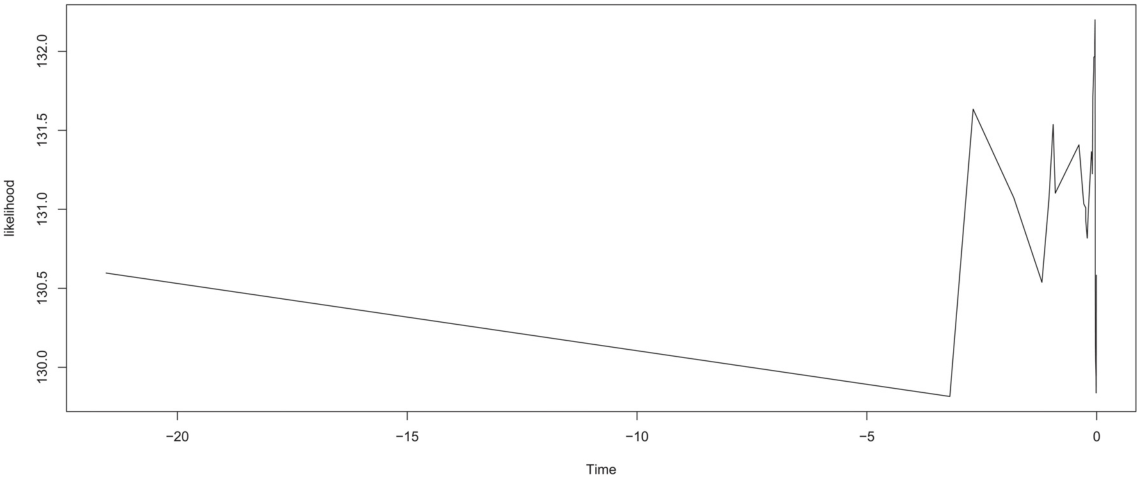


GMYC species delimitation analysis using ITS2 tree: 42 taxa dataset

ingroup: all *Crenitulus*

GMYC species delimitation analysis using ITS2 genes

Maximum likelihood of GMYC model



Result of GMYC species delimitation

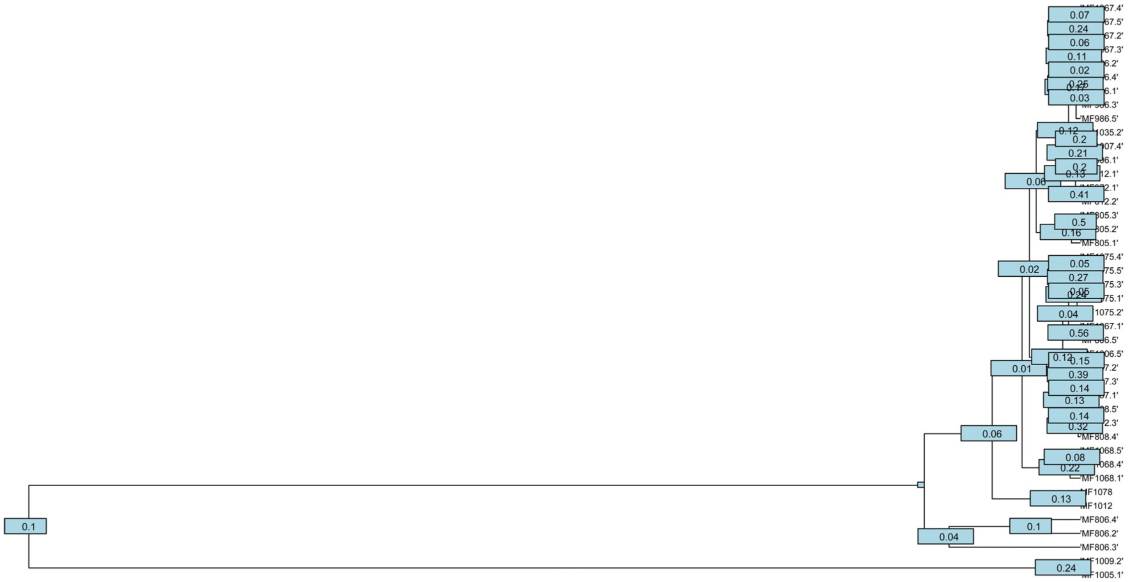
```
	method: single

	likelihood of null model:	130.5965

	maximum likelihood of GMYC model:	132.1996

	likelihood ratio:	3.206187

	result of LR test:	0.201273n.s.


	number of ML clusters:  10

	confidence interval:	1-13


	number of ML entities:  24

	confidence interval:	1-41


	threshold time: -0.03618664


   GMYC_spec sample_name

1          1   'MF612.3'

2          1   'MF808.5'

3          2   'MF807.1'

4          2   'MF807.3'

5          2   'MF807.2'

6          2  'MF1006.5'

7          3   'MF806.5'

8          3  'MF1067.1'

9          4  'MF1075.1'

10         4  'MF1075.3'
```

11	4	'MF1075.5'
12	4	'MF1075.4'
13	5	'MF805.2'
14	5	'MF805.3'
15	6	'MF612.2'
16	6	'MF972.1'
17	7	'MF612.1'
18	7	'MF806.1'
19	8	'MF1007.4'
20	8	'MF1035.2'
21	9	'MF986.3'
22	9	'MF986.1'
23	9	'MF986.4'
24	9	'MF986.2'
25	10	'MF1067.3'
26	10	'MF1067.2'
27	10	'MF1067.5'
28	10	'MF1067.4'
29	11	'MF1005.1'
30	12	'MF1009.2'
31	13	'MF806.3'
32	14	'MF806.2'
33	15	'MF806.4'
34	16	MF1012
35	17	MF1078
36	18	'MF1068.1'
37	19	'MF1068.4'

38	20	'MF1068.5'
39	21	'MF808.4'
40	22	'MF1075.2'
41	23	'MF805.1'
42	24	'MF986.5'

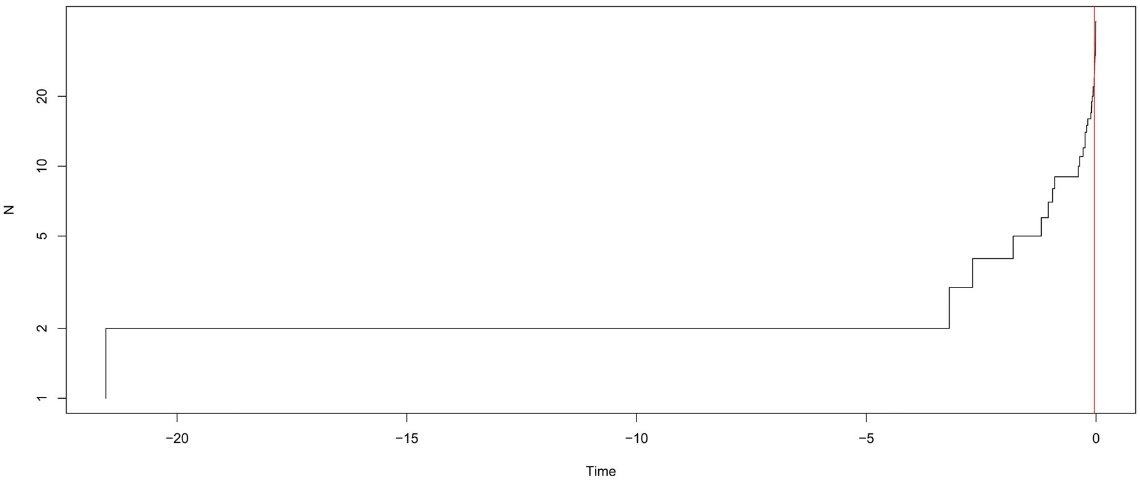
GMYC species delimitation analysis using ITS2 genes

Support



GMYC species delimitation analysis using ITS2 genes

Treshold



Part 2: Contributions to the systematics of aquatic beetles of Greater Antilles

CHAPTER 3

Deler-Hernández A., F. Cala-Riquelme and Fikáček M. 2013. Description of a new species of *Phaenonotum* from eastern Cuba (Coleoptera: Hydrophilidae: Sphaeridiinae). *Acta Entomologica Musei Nationalis Pragae* 53(2): 615-622.

ACTA ENTOMOLOGICA MUSEI NATIONALIS PRAGAE
Published 15.xi.2013 Volume 53(2), pp. 615–622 ISSN 0374-1036
http://zoobank.org/urn:lsid:zoobank.org:pub:B94AF43D-E483-4EB7-B1B6-C48E6492F66A

Description of a new species of *Phaenonotum* from eastern Cuba (Coleoptera: Hydrophilidae: Sphaeridiinae)

Albert DELER-HERNÁNDEZ[1,2], Franklyn CALA-RIQUELME[2]
& Martin FIKÁČEK[1,3]

[1] Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44 Praha 2, Czech Republic; e-mail: adeler1982@yahoo.com

[2] Departamento de Zoología, Centro Oriental de Ecosistemas y Biodiversidad, Enramadas 601 esquina Barnada, Santiago de Cuba, 90100, Cuba

[3] Department of Entomology, National Museum, Kunratice 1, CZ-148 00 Praha 4, Czech Republic; e-mail: mfikacek@gmail.com

Abstract. A new species of the genus *Phaenonotum* Sharp, 1882, *P. delgadoi* sp. nov., is described from the Nipe-Sagua-Baracoa mountain range in eastern Cuba. The new species is diagnosed from other species of the genus, and compared in detail with *P. exstriatum* (Say, 1835) and *P. minor* Smetana, 1978. All specimens of the new species were collected by sifting forest leaf litter far from any known water source and the species is therefore likely terrestrial.

Resumen. La nueva especie perteneciente al género *Phaenonotum* Sharp, 1882, *P. delgadoi* sp. nov., es descrita, para el sistema montañoso Nipe-Sagua-Baracoa situado en el oriente de Cuba. La nueva especie es diagnosticada a partir de otras especies del género, y se compara en detalles con *P. exstriatum* (Say, 1835) y *P. minor* Smetana, 1978. *Phaenonotum delgadoi* sp. nov. fue recolectada tamizando hojarasca de bosques, alejados de cualquier fuente de agua, por lo que parece ser una especie terrestre.

Key words. Coleoptera, Hydrophilidae, Coelostomatini, new species, Cuba, Caribbean, Neotropical Region

Introduction

The hydrophilid genus *Phaenonotum* Sharp, 1882 currently contains 18 species, 17 of which occur in the New World and one in the Afrotropical Region (Equatorial Guinea: Bioko Island) (Hansen 1999, Short & Fikáček 2011). In Cuba and other Caribbean islands, the genus is represented by the single species *P. exstriatum* (Say, 1835), which is otherwise widespread in North and Central America (Smetana 1978). The species is mainly collected at margins of standing waters or at light (Spangler 1981; Short, pers. comm. 2013). Recent fieldwork in Cuba initiated by the Cuban-Canadian-Czech team has focused on the invertebrate fauna of the rainforest and pluviselva leaf litter, resulting in the discovery of new species of various beetle families, surprisingly including a new terrestrial species of *Phaenonotum*. The aim of this study is to describe this species and provide the data on its distribution and biology.

Materials and methods

Specimens used for this study were collected by sifting leaf litter in various kinds of forests in eastern Cuba. Beetles were extracted from the samples using Berlese funnels and Winkler traps, and preserved in 95% ethanol.

Habitus photographs were taken using Canon EOS 550D digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS5. Photographs of genitalia were taken using Canon EOS 1100D digital camera attached to Olympus BX41 compound microscope and subsequently combined in Helicon Focus software. SEM micrographs of the holotype of the new species were taken using a Hitachi S-3700N environmental electron microscope at the Department of Paleontology, National Museum in Prague. General morphological terminology follows Smetana (1978), Archangelsky (1989) and Hansen (1991). We used specimens of *Phaenonotum* deposited in BMNH (including the types of species described by D. Sharp) and NMPC for comparative purposes. The present paper is a continuation of the study of the hydrophilid fauna of Cuba led by the first author (see Deler-Hernández & Delgado 2010, Deler-Hernández et al. 2013).

Examined specimens are deposited in the following collections:

BSC-E	Departamento de Zoología, Centro Oriental de Ecosistemas y Biodiversidad, Santiago de Cuba, Cuba (A. Deler-Hernández);
BMNH	The Natural History Museum, London, U.K. (M. Barclay);
CMN	Canadian Museum of Nature, Ottawa, Canada (R. Anderson);
DZRJ	Coleção Entomológica Prof. José Alfredo Pinheiro Dutra, Instituto de Biologia, Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil (B. Clarkson);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček);
SEMC	Division of Entomology, University of Kansas Natural History Museum, Lawrence, USA (A. Short).

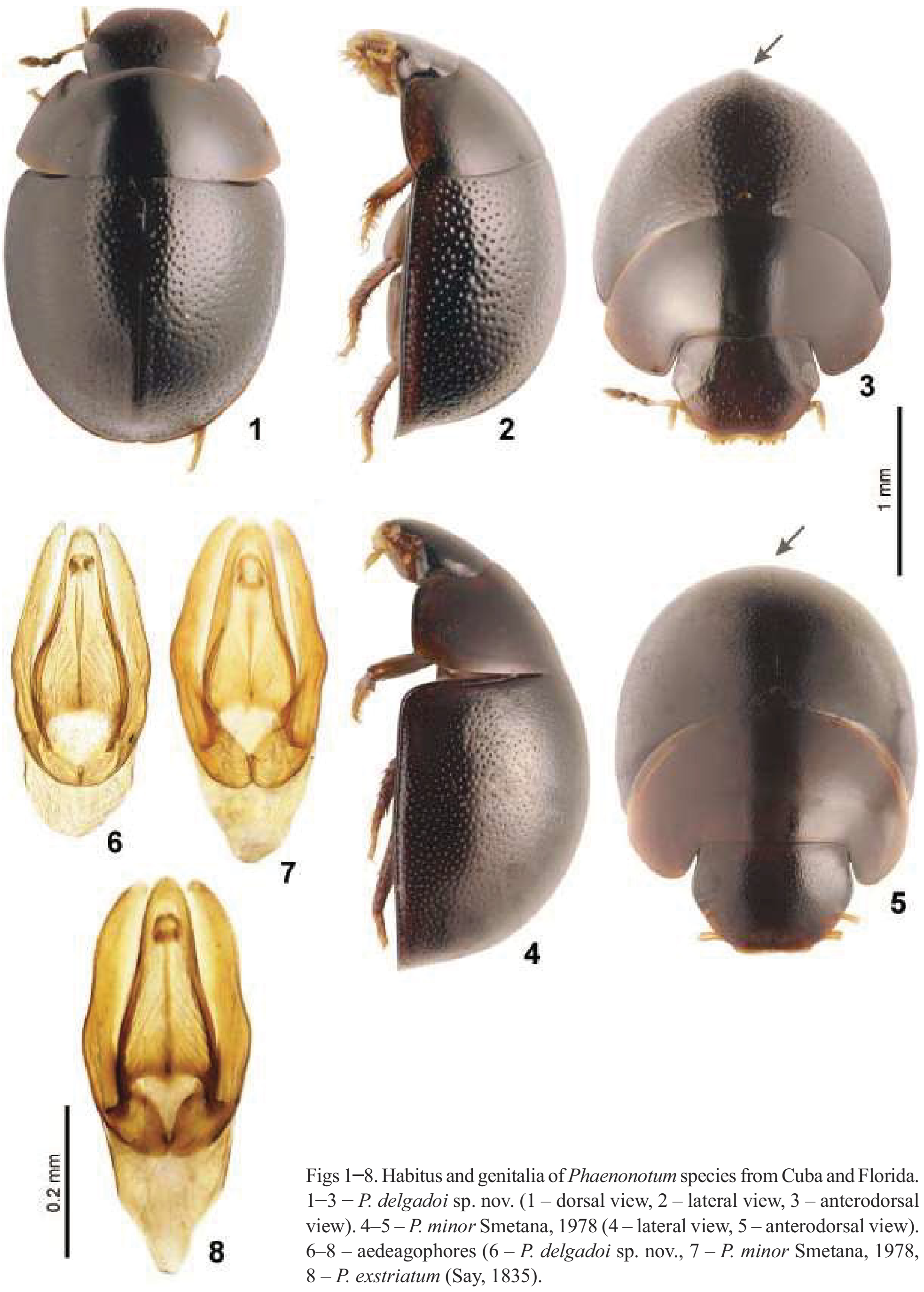
Taxonomy

Phaenonotum delgadoi sp. nov.

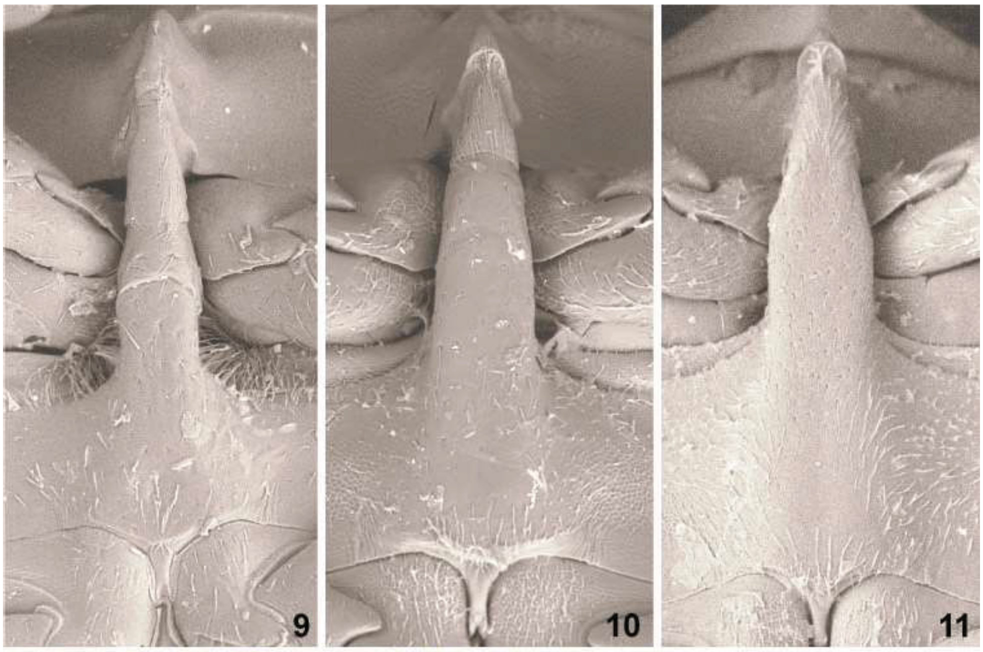
(Figs 1–8, 9–14, 15)

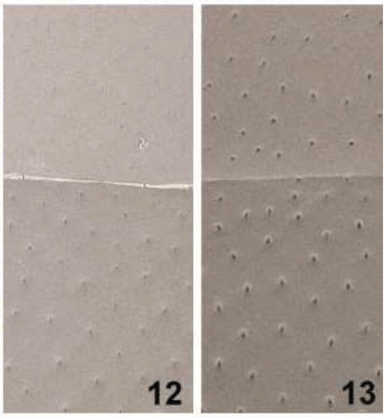
Type locality. Cuba, Guantánamo Province, Baracoa Municipality, El Yunque, Finca La Delicia, 20.313ºN 74.574ºW, 200 m a.s.l.

Type material. Holotype: ♂, ‘CUBA: Guantánamo: El Yunque, Finca La Delicia, 01.ii.2012, R. Anderson leg. 20.313ºN 74.574ºW, elevation 200 m [printed] / Holotype, Phaenonotum delgadoi sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]’ (NMPC). Paratypes (14 spec.): **CUBA: Holguín province:** PN Pico Cristal, Frank País, road to Pico Cristal, ca. 565 m a.s.l., 20°34′12.61″N 75°25′42.17″W, 11.v.2013, R. Anderson leg. (2013-007) (5 spec., BSC-E, SEMC, NMPC, DZRJ). **Guantánamo province:** same data as holotype (5 spec., BMNH, CMN, NMPC,



Figs 1–8. Habitus and genitalia of *Phaenonotum* species from Cuba and Florida. 1–3 – *P. delgadoi* sp. nov. (1 – dorsal view, 2 – lateral view, 3 – anterodorsal view). 4–5 – *P. minor* Smetana, 1978 (4 – lateral view, 5 – anterodorsal view). 6–8 – aedeagophores (6 – *P. delgadoi* sp. nov., 7 – *P. minor* Smetana, 1978, 8 – *P. exstriatum* (Say, 1835).





Figs 9–14. Morphology and habitat of *Phaenonotum*. 9–11 – meso-metaventral process (9 – *P. delgadoi* sp. nov., 10 – *P. minor* Smetana, 1978, 11 – *P. exstriatum* (Say, 1835)), 12–13 – detail of pronotum and elytron surface of Cuban *Phaenonotum* (12 – *P. delgadoi* sp. nov., 13 – *P. exstriatum* (Say, 1835)), 14 – habitat and type locality of *P. delgadoi* sp. nov.: Finca La Delicia, El Yunque, Baracoa, Guantánamo Province (photo by M. Fikáček, 4th July 2012).

NHMW); El Yunque, 3.2 km SW of Campismo, 20º19′N 74º34′W, ca. 150 m a.s.l., 13.vi.2012, Deler-Hernández leg. (3 spec., BSC-E, NMPC); La Melba, 2 km NW of Tetas de Julia, ca. 350 m a.s.l., 20°28′20□N 74°46′43□W, 15/18. ii.2004, without collector (El 26) (1 spec., NMPC).

Description. Habitus as in Figs 1–3. Body size 2.2–2.5 mm (2.3 mm in the holotype). Body form oval in dorsal view, highly convex in lateral view (Fig. 2). Dorsal surface dark brown to reddish brown. Antennae and maxillary palpi testaceous. Head and pronotum slightly paler than elytra. Lateral and posterolateral margins of pronotum and lateral margins of elytra paler than the disc. Ventral surface reddish brown. Leg reddish, tarsomeres paler in coloration. Head with fine and sparse setiferous punctures. Pronotum with punctures of same size as on head. Elytral punctation (Figs 1–3, 12) strongly impressed, much coarser than the pronotal and head punctation. Pronotum wider than long and very convex. Epipleura very broad throughout. Meso- and metaventral processes fused into a common keel, mesoventral process arrow-head shaped, elevated anteromedian portion of metaventrite ca. as long as mesoventral process, with a transverse subanterior ridge and basal cavities bearing dense pubescence (Fig. 9). Profemora with long sparse pubescence in basal 0.75. Meso- and metafemora with very sparse and short pubescence only. All tarsi with long setae on ventral surface. Aedeagus (Fig. 6) 0.4 mm long, with median lobe slightly shorter that parameres, basal region rounded, apical region elongate and stout. Parameres moderately wide and indistinctly sinuate in median region. Phallobase short, without distinct manubrium.

Differential diagnosis. *Phaenonotum delgadoi* sp. nov. differs from *P. exstriatum* (which is the only other *Phaenonotum* species known in Cuba) by the smaller body size (*P. exstriatum* is 2.3–4.0 mm long), head and pronotum with very fine, nearly obsolete punctures being in contrast to very coarsely punctate elytra (whole dorsal surface bears uniform, moderately coarse punctuation in *P. exstriatum*), the slender meso-metaventral process with basal pubescent cavities (stout and broad and without dense basal pubescence in *P. exstriatum*), and the shape of the aedeagus (phallobase is short and parameres are nearly continually arched on outer margin in *P. delgadoi* sp. nov. whereas the phallobase is long and parameres are distinctly sinuate externally in *P. exstriatum* (compare Figs 4 and 5).

The new species is unique among other known *Phaenonotum* by its small highly convex body (Fig. 2), extremely coarse elytral punctation being in contrast to very weak pronotal one (Figs 1–3, 12), and the presence of the pubescent cavities on the base of the meso-metaventral keel (Fig. 9). It may be also easily recognized by slightly elevated elytral suture in posterior half making the elytra arched at the suture (best seen when examined in anterodorsal or anterolateral view, see Fig. 3; all other species have evenly convex elytra). By the size, highly globular body and big difference between the size of punctation on the pronotum and elytra, it is most similar to *P. minor* Smetana, 1978 from the southeast USA, but differs from it by much more coarse elytral punctuation (compare Figs. 2 and 4), elytra arched at suture posteriorly (compare Figs 3 and 5), the presence of the pubescent cavities at the base of meso-metaventral keel (compare Figs 9 and 10) and the morphology of the aedeagus (compare Figs 6 and 7). *Phaenonotum delgadoi* sp. nov. resembles *P. apicale* Sharp, 1882, *P. collare* Sharp, 1882, *P. flavitarse* (Kirsch, 1871), *P. globulosum* (Mulsant, 1844) and *P. rotundulum* Sharp, 1882 by the very minute pronotal punctation, but easily differs from all these species by the above unique characters.

Fig. 15. Known distribution of *Phaenonotum delgadoi* sp. nov. in Cuba.

Etymology. The new species is dedicated to our excellent friend Dr. Juan Antonio Delgado Iniesta (Universidad de Murcia, Spain) for his contribution and help to the senior author's studies on aquatic beetles.

Habitat. All available specimens of the new species were collected from wet rainforest and pluviselva litter (e.g., Fig. 14). No collecting site was situated near water.

Distribution. Based on the material available to us, *Phaenonotum delgadoi* sp. nov. is a Cuban endemic species currently known only from the eastern part of the island (Holguín and Guantánamo provinces) (Fig. 15). All known localities are situated in the Nipe-Sagua-Baracoa mountain range which is considered the main center of diversity in Cuba and the Caribbean as a whole (CENAP 2004, TOLEDO et al. 2011).

Discussion

The knowledge on the Neotropical Coelostomatini remains very limited both concerning the proportion of described species, information on their taxonomy and morphology, and the data on biology and distribution. Most described species are actually known only from their historical original descriptions (e.g. SHARP 1882, 1887; SCOTT 1912; ORCHYMONT 1937, 1943). The only recent additions are the descriptions of two new *Phaenonotum* from USA (Florida, Alabama; SMETANA 1978) and Venezuela (ARCHANGELSKY 1989) and the revision of the genus *Phaenostoma* Orchymont, 1937 (GUSTAFSON & SHORT 2010). The recently described genus *Badioglobus* Short, 2004 from Costa Rica was also originally assigned to the Coelostomatini (SHORT 2004), but new data suggest it not a member of that clade (SHORT & FIKÁČEK 2013). Examination of museum collections and material accumulated during recent fieldwork, moreover, reveals that the majority of Neotropical Coelostomatini remain undescribed (M. Fikáček, unpubl. data; A. E. Z. Short, pers. comm. 2013).

Phaenonotum was usually considered as morphologically rather uniform genus containing species associated with various aquatic habitats, much in the way the better known genera *Coelostoma* Brullé, 1835 and *Phaenostoma* Orchymont, 1937 (e.g. HANSEN 1991). Our discovery of an aberrant *P. delgadoi* sp. nov. in Cuba indicates that *Phaenonotum* actually includes species of diverse morphology and biology. The comparison of the meso-metaventral morphology of *P. delgadoi* sp. nov. with that of *P. exstriatum* and *P. minor* we made in this contribution revealed surprising differences and illustrates the morphological diversity of the genus quite well. Our new species was always collected from leaf litter during both rainy and dry seasons, never in association with aquatic habitats. It is hence likely terrestrial, which may be the reason for the aberrant morphology. In a similar way, *P. caribense* Archangelsky, 1989 also clearly differs from other species of *Phaenonotum* (including *P. delgadoi* sp. nov.) by its meso-metaventral morphology and the tiny body size (with its 1.5 mm it is actually easy to confuse with an omicrine), and is also a terrestrial species inhabiting leaf litter based on the samples collected recently in Ecuador and Venezuela (Fikáček, unpubl. data; Short, pers. comm. 2013). Alternatively, the aberrant morphology of *P. delgadoi* sp. nov. and *P. caribense* may have nothing to do with the life style and may actually indicate that *Phaenonotum* in our current understanding is not a monophyletic taxon. Therefore, further studies are necessary to understand the diversity and taxonomy of *Phaenonotum* and the Neotropical Coelostomatini in general.

Acknowledgements

We are deeply grateful to the authorities and personnel of the Cuban Ministry of Science, Technology and Environment (CICA-CITMA) and the Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF) for providing access to protected areas under their control, to Robert Anderson, Andrew Smith (Canadian Museum of Nature, Ottawa) and Guangyang Zhang (School of Life Sciences, Arizona State University) for considerable help during the field work and for providing us with the specimens collected by them, to Maxwell Barclay (BMNH) for his assistance during the visit of MF to the Natural History Museum, London, and to Andrew Short (SEMC) and Sergey Ryndevich (Baranovichy, Belarus) for critical comments on the manuscript. The study was supported by the grant SVV-2013-267 201 to ADH and by the Ministry of Culture of the Czech Republic (DKRVO 2012 and DKRVO 2013/12, National Museum, 00023272) to MF. This research received support from the SYNTHESYS Project http://www.synthesys.info/ which is financed by European Community Research Infrastructure Action under FP7 Integrating Activity Programme.

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CHAPTER 4

Deler-Hernández A., Fikáček M. and F. Cala-Riquelme. 2013. A review of the genus *Berosus* Leach of Cuba (Coleoptera: Hydrophilidae). *Zookeys* 273: 73-106.

ZooKeys 273: 73–106 (2013)
doi: 10.3897/zookeys.273.4591
www.zookeys.org

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A review of the genus *Berosus* Leach of Cuba (Coleoptera, Hydrophilidae)

Albert Deler-Hernández[1], Martin Fikáček[2,3], Franklyn Cala-Riquelme[1]

1 *Departamento de Zoología, Centro Oriental de Ecosistemas y Biodiversidad, Enramadas 601 esquina Barnada, Santiago de Cuba, 90100, Cuba* **2** *Department of Entomology, National Museum, Kunratice 1, CZ-148 00 Praha 4, Czech Republic* **3** *Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44 Praha 2, Czech Republic*

Corresponding author: *Albert Deler-Hernández* (albert@bioeco.ciges.inf.cu)

Academic editor: *C. Majka* | Received 28 December 2012 | Accepted 3 February 2013 | Published 28 February 2013

Citation: Deler-Hernández A, Fikáček M, Cala-Riquelme F (2013) A review of the genus *Berosus* Leach of Cuba (Coleoptera, Hydrophilidae). ZooKeys 273: 73–106. doi: 10.3897/zookeys.273.4591

Abstract

The Cuban fauna of the genus *Berosus* Leach, 1817 is reviewed based on newly collected material as well as historical and type specimens. Nine species are recognized, including three recorded from Cuba for the first time: *B. infuscatus* LeConte, 1855, *B. interstitialis* Knisch, 1924 (= *B. stribalus* Orchymont, 1946 **syn. n.**) and *B. metalliceps* Sharp, 1882. Only one of the nine Cuban species, *B. chevrolati*, remains endemic to Cuba, as two other species previously considered as endemic to Cuba are recorded from elsewhere: *B. quadridens* from Mexico and Central America and *B. trilobus* from the Dominican Republic. Notes on biology and Cuban distribution are provided for all nine species. *Berosus quadridens* Chevrolat, 1863, **stat. restit.** is removed from synonym with *B. truncatipennis* and considered a valid species.

Resumen

La fauna cubana de *Berosus* Leach, 1817 es revisada a partir de nuevo material colectado así, como de material tipo e histórico. Se reconocen un total de nueve especies, incluyendo tres nuevos registros: *B. infuscatus* LeConte, 1855, *B. interstitialis* Knisch, 1924 (= *B. stribalus* Orchymont, 1946, **syn. n.**) y *B metalliceps* Sharp, 1882. Solo una de las nueve especies cubanas, *B. chevrolati*, permanece como endémica, las otras dos especies originalmente consideradas como endémicas han sido registradas fuera de Cuba: *B. quadridens* presente en México y América Central y *B. trilobus* en República Dominicana. Se ofrecen datos sobre la biología y la distribución de todas las especies cubanas. *Berosus quadridens* Chevrolat, 1863, **stat. restit.** es destituida como sinónimo de *B. truncatipennis* y considerada como una especie válida.

Keywords

Hydrophilinae, Berosini, taxonomy, new synonymy, new records, Caribbean, Neotropical region, identification key

Palabras clave

Hydrophilinae, Berosini, taxonomía, nuevo sinónimo, nuevo registro, Caribe, región Neotropica, clave de identificación

Introduction

The hydrophilid genus *Berosus* Leach, 1817 is the largest genus in family Hydrophilidae, containing more than 270 species distributed worldwide (Hansen 1999, Short and Fikáček 2011) and inhabiting various types of standing and slowly running waters (Oliva and Short 2012). The genus has been little studied in the Caribbean and in Cuba specifically, and the current knowledge is based primarily on occasional collecting events and historical records. Chevrolat (1863) described three species which are until now considered Cuban endemics: *Berosus trilobus* Chevrolat, 1863, *B. quadridens* Chevrolat, 1863 and *B. aculeatus* Chevrolat, 1863 (the name of the latter was later changed to *B. chevrolati* Zaitzev, 1908 due to the homonymy). Gundlach (1891) provided short redescriptions of these species and few additional records. Another supposedly endemic species, *B. stribalus* Orchymont, 1946, was described later by Orchymont (1946). Spangler (1973, 1981) recorded *B. undatus* (Fabricius, 1792) for the first time from Cuba and provided additional records on the five Cuban species. Hansen (1999) only listed four species of *Berosus* from Cuba. Finally, Peck (2005) published the most complete checklist of Cuban Coleoptera with data on their distribution; in this work he listed seven species of *Berosus*. Except of the published works, an unpublished thesis by Van Tassell (1966) contains additional data on Cuban *Berosus*, which we also adopt here.

In this paper we provide a review of the Cuban fauna of *Berosus* containing redescriptions of the three of four species described as Cuban endemics (*B. chevrolati*, *B. quadridens* and *B. trilobus*), we synonymize the fourth supposedly endemic species *B. stribalus* with a widely distributed Caribbean *B. interstitialis*, provide identification key and illustrations of all Cuban species and notes on their distribution and bionomics based on newly collected material. Three species are newly recorded for the Cuban fauna.

Materials and methods

This study is mainly based on the material collected during the field survey of Cuban aquatic beetles conducted between 2008 to 2012 by A. Deler-Hernández, Y. S. Megna and F. Cala-Riquelme. The survey was mainly focused on eastern Cuba, but several areas of western Cuba were also sampled. In total, the samples from 170 localities have been collected, of which only 40 sites yielded *Berosus*. Specimens were collected with aquatic nets and preserved in 70%–95% ethanol. Except of this material, we also used the following sources of information: *i)* recently collected specimens provided to us by some Cuban colleagues; *ii)* material deposited in the zoological collection of the Instituto de Ecología y Sistemática in La Habana, Museo de Historia Natural "Charles T. Ramsden", Universidad de Oriente in Santiago de Cuba, National Museum in Prague and the Division of Entomology of the University of Kansas in Lawrence; and *iii)* literature records (Chevrolat 1863; Gundlach 1891; Van Tassell 1966; Spangler 1973, 1981; Hansen 1999; Peck 2005). In the systematic section we provide detailed descriptions and differential diagnoses for three species originally described as Cuban endemics (*B. chevrolati*, *B. quadridens* and *B. trilobus*), for remaining species we only include a short diagnosis summarizing the most important diagnostic characters.

Habitus photographs were taken using Canon D-550 digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS2. Photographs of genitalia were taken using Nikon Coolpix P6000 digital camera attached to Olympus BX41 compound microscope and subsequently combined with Helicon Focus software. Line drawings were traced from the photographs taken using a Canon PowerShot A620 camera attached to a Zeiss Stemi 2000-C stereomicroscope or with the same equipment as for taking the habitus photographs. Dissections of male genitalia and mounting techniques follow those used by Oliva and Short (2012). Complete label data are provided for type specimens, data of additional material are listed in an adapted form; our notes to the label data are in square brackets [ ]; and it is added the catalogue number for each vial of the Cuban material deposited in BSC-E. General morphological terminology follows Hansen (1991) and Komarek (2004), special terminology concerning *Berosus* follows Oliva (1989) and Oliva and Short (2012).

Examined material is deposited in the following collections:

BSC-E	Departamento de Zoología, Centro Oriental de Ecosistemas y Biodiversidad, Santiago de Cuba, Cuba (A. Deler-Hernández);
CZACC	Colección Zoológica, Instituto de Ecología y Sistemática, La Habana, Cuba (I. Fernández);
CZCTR	Museo de Historia Natural "Charles Ramsden", Facultad de Ciencias Naturales, Universidad de Oriente, Santiago de Cuba, Cuba (C. T. Ramsden historical collection) (M. Soto);
IRSNB	Institut Royal des Sciences Naturelles de Belgique, Brussels, Belgium (P. Limbourg);
KSEM	Division of Entomology, University of Kansas Natural History Museum, Lawrence, USA (A. Short);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček);
MNHN	Museum National d'Histoire Naturelle, Paris, Frances (Bedel collection) (A. Mantilleri).

Checklist of the Cuban species of *Berosus*

(asterisk indicates the species newly recorded for Cuba)

Berosus chevrolati Zaitzev, 1908
Berosus exiguus (Say, 1825)
**Berosus infuscatus* LeConte, 1855
**Berosus interstitialis* Knisch, 1924
=*Berosus stribalus* Orchymont, 1946, **syn. n.**
**Berosus metalliceps* Sharp, 1882
Berosus peregrinus (Herbst, 1797)
Berosus quadridens Chevrolat, 1863 **stat. restit.**
Berosus trilobus Chevrolat, 1863
Berosus undatus (Fabricius, 1792)

Systematics

Genus *Berosus* Leach, 1817
http://species-id.net/wiki/Berosus

Diagnosis. Adults are mostly medium-sized, elongate, and strongly convex. Coloration of the body is brown to yellowish-brown, with or without dark spots on the pronotum and elytra. The head is strongly flexed down, eyes are protuberant, and antennae have 7 antennomeres. The elytral apex is entire or produced into one or two spines. The mesoventral process is usually laminar. The male protarsi are widened and have four tarsomeres; those of females have five tarsomeres. The middle and hind tibiae and tarsi bear a fringe of long natatory setae. Abdominal ventrite 5 has a rectangular (or less frequently semicircular) emargination posteriorly.

Among Cuban hydrophilid genera, *Berosus* may be easily identified by large globular eyes, scutellum longer that wide and middle and hind tibiae and tarsi with well developed fringe of long natatory setae.

Species treatments

Berosus chevrolati Zaitzev, 1908
http://species-id.net/wiki/Berosus_chevrolati
Figures 1a–g, 11, 12a

Berosus aculeatus Chevrolat, 1863: 207 (primary homonym of *Berosus aculeatus* LeConte, 1855). – Gundlach 1891: 48 (diagnosis and distribution).

Berosus (s.str.) *chevrolati* Zaitzev, 1908: 358 (replacement name for *B. aculeatus* Chevrolat, 1863). – Van Tassell 1966: 169 (unpublished PhD thesis: redescription, identification key). – Spangler 1981: 155 (diagnosis and distribution). – Hansen 1999: 84 (catalogue). – Peck 2005: 48 (checklist). – Epler 2010: 12.24 (notes on distribution).

Type locality. Cuba.

Type material examined. Holotype: female (MNHN): "aculeatus / Ch. Cuba // this must be / Chevr. unique type of aculeatus / PJS [= P. J. Spangler] 1966".

Additional material examined. CUBA: **Santiago de Cuba**: 3 exs. (dry-mounted) (NMPC): Dos Caminos, stream, 20°11'2.50"N, 75°46'17.7"W, 150 m a.s.l., 01.viii.2008, leg. A. Deler-Hernández., 1 ex. (dry-mounted) (BSC-E): El Vivero, 1.6 km E of Dos Caminos, 20°11'2.50"N, 75°46'17.7"W, 150 m a.s.l. Guaninicú river, 20–21.vi.2012, leg. Deler-Hernández & Fikáček; 1 ex. (in alcohol) (BSC-E): La Maya, Cuatro Caminos, remanso [backwater] 20°07'58"N, 75°34'01"W, 150 m a.s.l, 24.i.2008, leg. Y. S. Megna, 00019.

Published Cuban records: Pinar del Río: Quemado de Pineda (Spangler 1981). **Matanzas**: Este de Matanzas [Eastern Matanzas], Río Canimar (Gundlach, 1891). **Camagüey**: Río El Manantiales (Spangler 1981). **Sancti Spíritus**: Río Caburny (Spangler 1973); Arroyo Vega Grande (Spangler, 1973). **Santiago de Cuba**: Contramaestre, Pozo Caliente, Río Contramaestre (Spangler 1981); II Frente, Arroyo Jarahueca (Spangler 1981); II Frente, Sabanilla, Río Mayarí (Spangler 1981); Río Ceiba (Spangler 1981); III Frente, Matías, Río Mogote (Spangler 1981). **Guantánamo**: La Tinta, Río Baracoa (Spangler 1981); Baracoa, Río Miel (Spangler 1973); Niceto Peréz, Arroyo de los Berros (Spangler 1981).

Diagnosis. Small, widely elongate species, body length 3.6-4.6 mm. Head dark, metallic; pronotum pale, with median unpaired narrow black longitudinal spot mesally, pronotal punctation not darkened; elytra pale with irregular small dark spots in posterior half of elytral intervals. Elytral apices each without subapical tooth. Mesoventral process highly laminar, square-shaped, with large anterior and posterior teeth. Abdominal ventrite 1 with median keel throughout its length. Emargination of abdominal ventrite 5 rectangular with a median teeth. Median lobe of the aedeagus with short basal projection and rounded apex in lateral view.

Differential diagnosis. *Berosus chevrolati* resembles *B. trilobus* (with which it may even co-occur) by the small strongly punctate body, metallic head, presence of an unpaired dark spot on the pronotum, mesoventrite with hooded anterior tooth, median keel developed throughout abdominal ventrite 1, emargination of abdominal ventrite 5 rectangular with single median tooth and the median lobe of the aedeagus with long basal lobe projecting far posteriad and enlarged apical portion in lateral view. It differs from *B. trilobus* by the narrow central dark spot on the pronotum (dark spot is large and trilobate in *B. trilobus*), elytra more evenly convex (subapical area of each elytra forms a bump in *B. trilobus*), short basal projection of the median lobe (long in *B. trilobus*) and, rounded apex of the median lobe in lateral view (apex is beak-shaped in lateral view in *B. trilobus*).

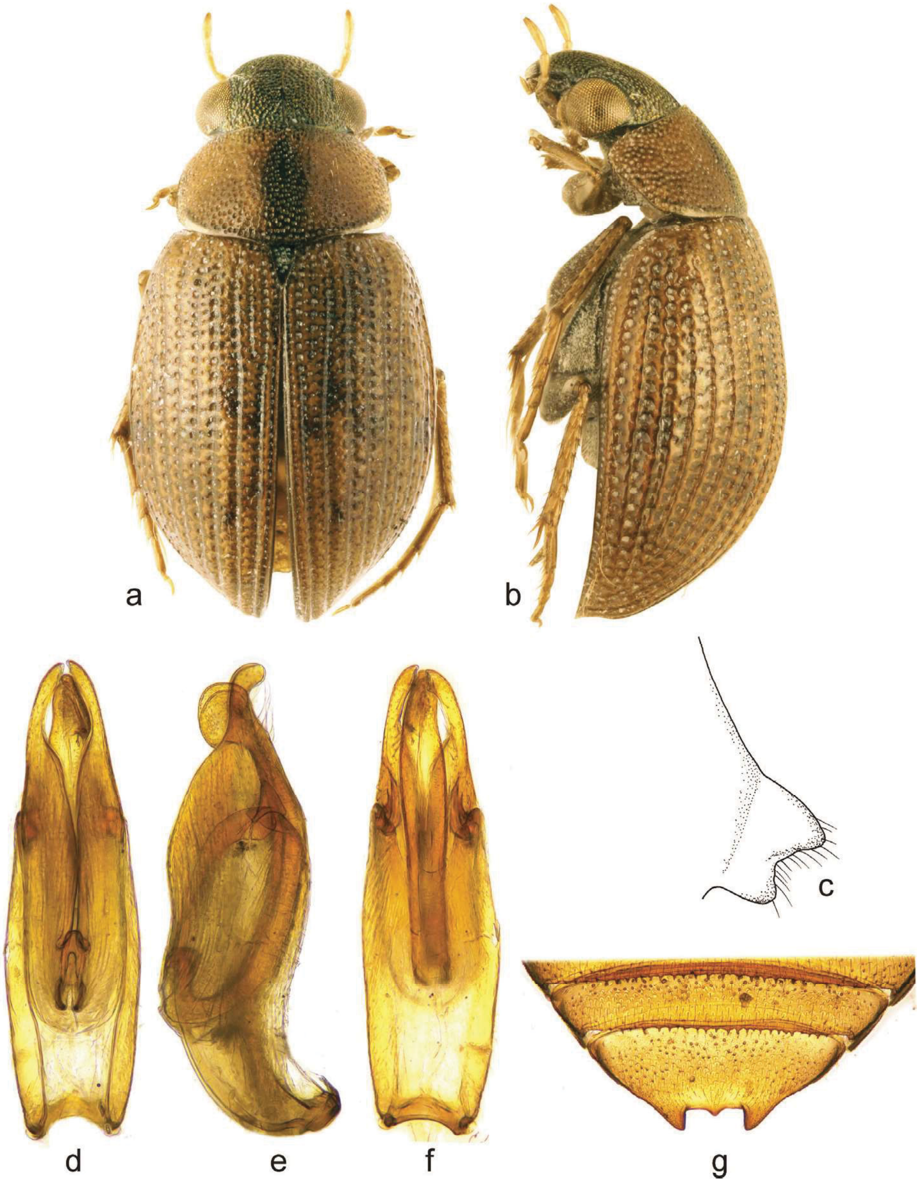

Figure 1. *Berosus chevrolati* Zaitzev, 1908. **a** habitus in dorsal view **b** habitus in lateral view **c** mesoventral process in lateral view **d-f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

Redescription. Habitus as in Figs 1a, b. Body length 3.4–4.6 mm. Body short and wide, moderately convex. Head black with metallic sheen, labrum black. Antennae testaceous. Maxillary palpi testaceous with palpomere 4 brown at apex. Pronotum testaceous with a central elongate metallic spot. Scutellum black with metallic sheen. Elytra testaceous with small brown spots without discrete borders. Pro-, meso- and metafemora testaceous, basal portion of metafemora sometimes slightly darker.

Head with moderately large and rounded punctures. Pronotum with punctures of the same size as on head. Scutellum with a few deeply impressed punctures slightly smaller than those on the pronotum. Elytral striae well-impressed. Interstriae with small and shallow punctures, irregular long setae on posterior half of elytra; spine-like setae absent. Elytral apices entire and rounded, of same shape in males and females. Mesoventral process highly raised, square-shaped, with hood-like anterior tooth, posterior tooth moderately large (Fig. 1c). Metaventral process wide, slightly raised, square-shaped, with large, deep glabrous rhomboid median depression; posterolateral angles raised and rounded, posteromesal projection carinate. Abdominal ventrite 1 with median carina throughout its length. Abdominal ventrite 5 with deep rectangular emargination, bearing a broad median tooth (Fig. 1g). Basal pubescence on basal 0.7 of meso- and of metafemora, the margin between pubescent and bare portions sinuate. Protarsus of male with adhesive soles on the first basal tarsomeres, first and second tarsomere distinctly thickened, third tarsomere very slightly thickened, fourth tarsomere elongate, almost as long as tarsomeres 1-3 combined. Claws moderately long, slender, arcuate.

Male genitalia (Figs 1d–f): Phallobase ca. 0.6× total length of aedeagus. Parameres in lateral view wide basally, apically projecting into rounded apex slightly bent ventrad, bearing a row of subapical setae ventrally. Median lobe C-shaped in lateral view; basal projection short, directing apicad; apex wide and rounded in lateral view.

Distribution. Currently only known from Cuba. Spangler (1981) recorded this species from several localities across the island, but all new material is from two sites in Santiago de Cuba province.

Habitat. We collected *B. chevrolati* along the margins of lowland streams and in isolated pools along these streams, in both cases having clear to turbid water and abundant organic matter (Fig. 11a). This species is found at low altitudes (ranging from sea level to ca. 160 m a.s.l.) situated in the Central Valley (Valle Central). *Berosus chevrolati* is frequently associated with *B. trilobus* in those habitats. Spangler (1981) also collected the species in standing waters.

Berosus exiguus (Say, 1825)

http://species-id.net/wiki/Berosus_exiguus
Figures 2a–g, 11

Hydrophilus exiguus Say, 1825: 189.

Berosus exiguus (Say). – Van Tassell 1966: 145 (unpublished PhD thesis: redescription, identification key, recorded from Cuba). – Testa and Lago 1994: 26 (diagnosis, bionomic and distribution notes, identification key). – Hansen 1999: 86 (catalogue). – Peck 2005: 48 (checklist). – Epler 2010: 12.19 (identification key, taxonomic notes). – Fernández et al. 2010: 28 (checklist).

For complete references and synonymy see Hansen (1999).

Type locality. USA: Virginia, Chincoteague Island.

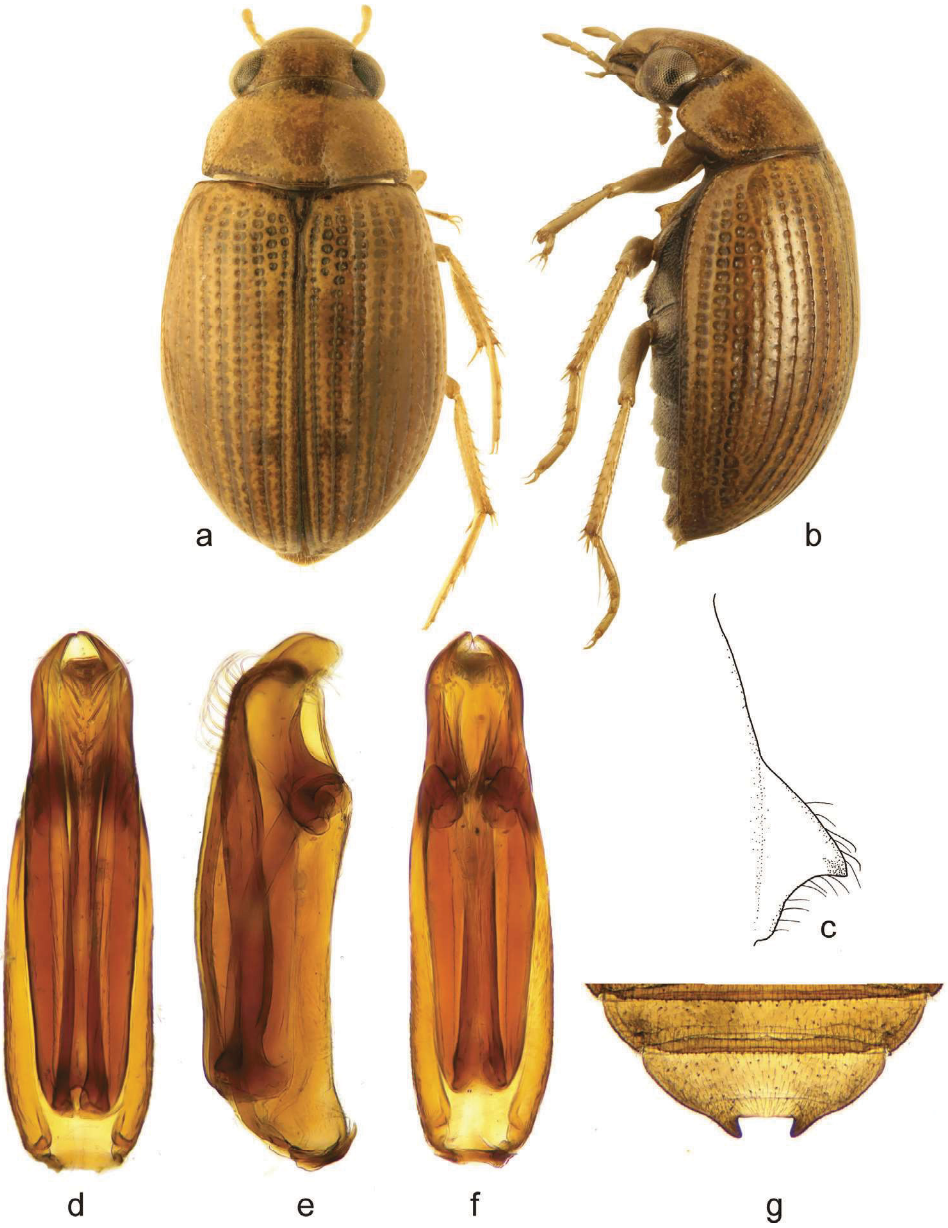

Figure 2. *Berosus exiguus* (Say, 1825). **a** habitus in dorsal view **b** habitus in lateral view **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

Material examined. CUBA: **Isla de la Juventud**: 1 ex. (in alcohol) (BSC-E): Punta del Este, Laguna Cayamás, 21°33'43"N, 82°33'18"W, 3 m a.s.l., 23.v.2006, leg. Y. S. Megna, 00165. **Camagüey**: 2 exs. (in alcohol) (BSC-E): Nuevitas, Cayo Sabinal, Laguna permanente [permanent pool], 21°38'6.1"N, 77°10'8.2"W, 5 m a.s.l., 06.v.2010, leg. O. Bello, 00153; 2 exs. (dry-mounted) (NMPC): Cayo Sabinal, permanent lagoon, 21°38'6.1"N, 77°10'8.2"W, 5 m a.s.l., 06.v.2010, leg. Y. Torres. **Santiago de Cuba:** 1 ex. (in alcohol) (BSC-E): San Miguel de Parada, Laguna temporal [temporal pool], 20°11'2.50"N, 75°46'17.7"W, 1 m a.s.l., 29.v.2009, leg. A. Deler-Hernández, 00136; 4 exs. (in alcohol) (BSC-E): San Miguel de Parada, Laguna temporal [temporal pool], 20°11'2.50"N, 75°46'17.7"W, 1 m a.s.l., 05.ix.2009, leg. A. Deler-Hernández, 00151; 2 exs. (dry-mounted) (NMPC): San Miguel de Parada, temporal lagoon, 20°11'2.50"N, 75°46'17.7"W, 1 m a.s.l, 05.ix.2009, leg. A. Deler-Hernández.

Published Cuban records: Pinar del Rio: unspecified locality (Van Tassell 1966: 149, Map 21).

Diagnosis. Habitus as in Figs 2a, b. Body length 3.0–3.7 mm. Head testaceuos, pronotum testaceous without median darker spots, punctation not darkened, elytra testaceous with irregularly arranged ill-defined slightly darker spots. Elytral apices entire and rounded in both sexes. Mesoventral process highly laminar, triangular in shape, anterior tooth large projecting posteriad (Fig. 2c). Abdominal ventrite 1 with median keel developed on basal half only. Emargination of abdominal ventrite 5 rectangular, without teeth (Fig. 2g) (in non-Cuban specimens, a very small medial tooth is present: Testa and Lago 1994). Aedeagus (Figs 2d–f) with median lobe only slightly shorter than parameres, with apex curved ventrad, bearing two series of long setae on dorsal surface.

Distribution. Eastern USA (from New York to Florida, westwards reaching to Illinois, Indiana, Mississippi and Oklahoma), Bahamas (Young 1953; Hansen 1999; Peck 2005) and Cuba. In Cuba, it is known from the central and eastern region.

Habitat. *Berosus exiguus* is mainly restricted to brackish waters in coastal regions. Cuban specimens have been collected in temporary brackish pools with clear water, abundant organic detritus on the bottom and associated aquatic riparian vegetation.

Berosus infuscatus LeConte, 1855

http://species-id.net/wiki/Berosus_infuscatus

Figures 3a–g, 11

Berosus infuscatus LeConte, 1855: 365: – Van Tassell 1966: 248 (unpublished PhD thesis: redescription, identification key). – Testa and Lago 1994: 26 (diagnosis, bionomic and distribution notes, identification key). – Epler 2010: 12.21 (identification key, taxonomic notes).

For complete synonymy and references see Hansen (1999).

Type locality. USA: “middle and southern States, e.g. New Orleans”.

Material examined. CUBA: **Isla de la Juventud**: 1 ex. (in alcohol) (BSC-E): Punta del Este, Laguna temporal [temporal pool], 21°33'43"N, 82°33'18"W, 1 m a.s.l., 21.v.2006, leg. Y. S. Megna, 00160. **Pinar del Río**: 1 ex. (in alcohol) (BSC-E): Guanahacabibes, 21°54'26"N, 84°39'14"W, 3 m a.s.l., 20.iii.2003, leg. Y. S. Megna and

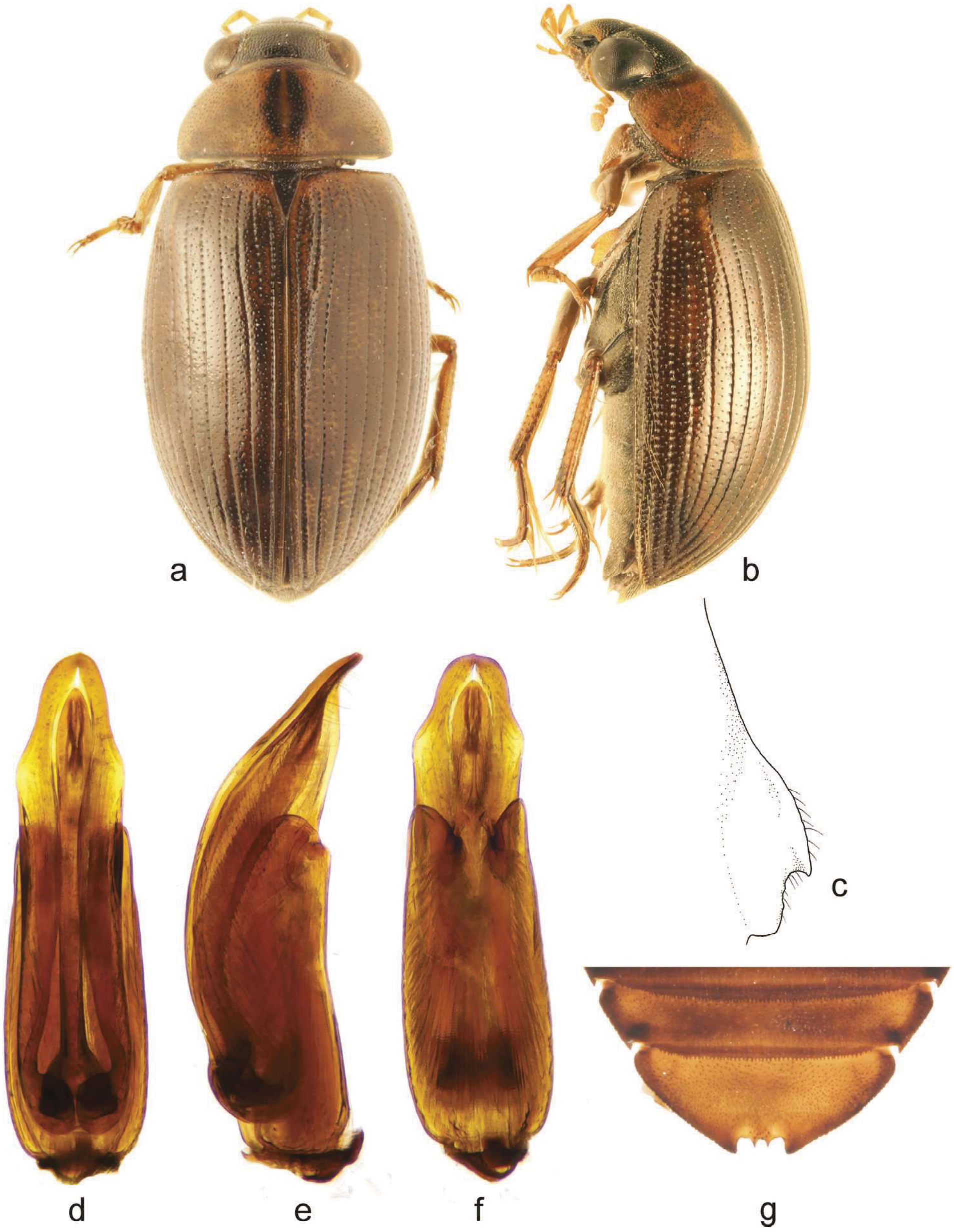

Figure 3. *Berosus infuscatus* LeConte, 1855. **a** habitus in dorsal view **b** habitus in lateral view **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

O. Bello, 00176. **Camagüey**: 2 exs. (dry-mounted) (NMPC): Cayo Sabinal, lagoon, 21°38'6.1"N, 77°10'8.2"W, 5 m a.s.l., 06.v.2010, leg. Y. T. Cambas. **Las Tunas:** 1 ex. (in alcohol) (BSC-E): Palancón, 21°00'N, 76°54'W, 100 m a.s.l., 04.viii.2004, leg. Y. S. Megna, 00017. **Granma**: 1 ex. (in alcohol) (BSC-E): Cauto Cristo, Laguna permanente-I [permanent pool-I], 20°33'33.1"N, 76°28'44"W, 44 m a.s.l., 04.i.2005, leg. L. Chávez, 00175. **Guantánamo**: 1 ex. (dry-mounted) (CZACC): [no locality and date] leg. C. T. Ramsdem.

Diagnosis. Habitus as in Figs 3a, b. Body length 5.5–6.0 mm. Head metallic black with paler anterior margin of clypeus; pronotum pale, with a pair of closely associated dark narrow longitudinal spots mesally, elytra brownish with indistinct irregularly arranged slightly darker spots. Head and pronotum with very distinct mesh-like microsculpture on interstices. Elytral apices entire and rounded in both sexes. Mesoventral process laminar, anterior tooth large, projecting posteriad (Fig. 3c). Abdominal ventrite 1 with median keel developed only between metacoxae. Emargination of abdominal ventrite 5 rectangular, with two sharp medial teeth (Fig. 3g). Aedeagus (Figs 3d–f) with median lobe slightly shorter than parameres, arched in lateral view. Parameres sinuate on lateral margin subapically.

Distribution. USA (Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Louisiana, Mississippi, Missouri, North Carolina, Texas, Wisconsin), Mexico (Young 1953; Hansen 1999) and Cuba. The above specimens represent the first record of *B. infuscatus* from Cuba and the West Indies.

Habitat. Cuban specimens of *B. infuscatus* have been collected among submerged aquatic vegetation in sun-exposed brackish permanent and temporary lagoons in coastal zones with turbid water and muddy/stony bottoms.

***Berosus interstitialis* Knisch, 1924**

http://species-id.net/wiki/Berosus_interstitialis
Figures 4a–g, 11

Berosus tessellatus Fletiaux and Sallé 1889: 337 (secondary homonym of *Enoplurus tessellatus* Motschulsky, 1859).

Berosus (s.str.) *interstitialis* Knisch, 1924: 270. – Van Tassell 1966: 191 (unpublished PhD thesis: redescription, identification key). – Epler 2010: 12.24 (taxonomic notes).

Berosus stribalus Orchymont, 1946: 13. **Syn. n.** – Spangler 1981: 156 (taxonomic and distribution notes). – Fernández et al. 2010: 28 (checklist).

Type locality. Guadeloupe, Grande Terre and Trois-Rivières.

Type material examined. *Berosus tessellatus*: Not examined. Type specimens were not found on our request for loan in MNHN. Instead, we examined the specimens identified as *B. tessellatus* and *B. interstitialis* deposited in coll. d'Orchymont and coll. Knisch in IRSNB (see below).

Berosus stribalus: Holotype: male (IRSNB): "[male sign] / Cuba 10. K / S. of Pinar Rio / Sep. 12-23 '13 // A. d'Orchymont det. / Berosus (s.str.) / stribalus m. // Type". Paratype: 1 spec. (IRSNB): "St. / Domin- / go // Berosus / striatus / Say // coll. Orch. // A. d'Orchymont det. / Berosus (s.str.) / stribalus m. // Para- / type".

Additional material examined. CUBA: Isla de la Juventud: 4 exs. (dry-mounted) (CZACC): vii.1960 [no locality and collector indicated]; 8 exs. (in alcohol) (BSC-E):

Punta del Este, laguna temporal [temporal pool], 21°33'43"N 82°33'18"W, 1 m a.s.l., 21.v.2006, leg. Y. S. Megna, 00180; 8 exs. (in alcohol) (BSC-E): Laguna Cayamás, 21°33'43"N, 82°33'18"W, 3 m a.s.l., 23.v.2006, leg. Y. S. Megna, 00178. **Pinar del Río**: 57 exs. (dry-mounted) (CZACC): Lomas de Soroa, v/vi.1963, [no collector indicated]; 1 ex. (in alcohol) (BSC-E): Viñales, arroyo [stream], 22°33'36.35"N, 83°49'59"W, 170 m a.s.l., 18.iv.2012, leg. A. Deler-Hernández, 00146. **Artemisa**: 8 exs. (dry-mounted) (CZACC): Laguna Ariguanabo, vi.1963, [no collector indicated]. **Mayabeque**: 1 ex. (dry-mounted) (CZACC): Jibacoa, littoral on north coast, v.1962, [no collector indicated]. **Matanzas**: 1 ex. (dry-mounted) (CZACC): Playa Larga, iv.1965, [no collector indicated]; 1 ex. (dry-mounted) (CZACC): Bacunayagua, vi.1940 [no collector indicated]. **Camagüey**: 1 ex. (in alcohol) (BSC-E): Sierra de Cubitas, Río El Roble, 21°32'53.23"N, 77°46'42.31"W, 55 m a.s.l., 14.iv.2012, leg. A. Deler-Hernández, 00148. **Las Tunas**: 1 ex. (in alcohol) (BSC-E): Amancio, Comunales, laguna permanente [permanent pool], 20°49'59"N, 77°32'32"W, 34 m a.s.l., 04.x.2008, leg. Y. S. Megna, 00179; 1 ex. (in alcohol) (BSC-E): La Fé, laguna temporal [temporal pool], 20°49'17.7"N, 77°34'40.8"W, 50 m a.s.l., 18.xii.2008, leg. Y. S. Megna, 00147. **Granma**: 2 exs. (in alcohol) (BSC-E): Cauto Cristo, laguna permanente [permanent pool], 20°33'33.1"N, 76°28'44"W, 44 m a.s.l., 04.i.2005, leg. L. Chávez, 00150; 1 ex. (dry-mounted) (NMPC): Cauto Cristo, permanent lagoon 20°33'33.1"N, 76°28'44"W, 44 m a.s.l., 04.i.2005, leg. L. Chávez. **Santiago de Cuba**: 1 ex. (in alcohol) (BSC-E): Guamá, La Mula, laguna permanente [permanent pool], 19°58'33.6"N, 76°46'4.8"W, 4 m a.s.l., 20.vi.2008, leg. A. Deler-Hernández, 00018. **Guantánamo**: 1 ex. (dry-mounted) (CZCTR): Guantánamo, San Carlos [at light], 20°26'22"N, 74°42'31"W, 160 m a.s.l., 18.vii.1915, leg. C. T. Ramsden; 1 ex. (dry-mounted) (CZCTR): San Carlos [at light], 20°26'22"N, 74°42'31"W, 160 m a.s.l., 24.viii.1917, leg. C. T. Ramsden; 1 ex. (in alcohol) (BSC-E): San Antonio del Sur, Macambo, Río Macambo, 20°03'26.9"N, 74°44'15"W, 4 m a.s.l., 24.x.2008, leg. A. Deler-Hernández; 1 ex. (in alcohol) (BSC-E): Baracoa, Nibujón, laguna temporal [temporal pool], 20°30'8.6"N, 74°38'88"W, 8 m a.s.l., 03.ii.2010, leg. A. Deler-Hernández, 00149. 1 ex. (dry-mounted) (IRSNB): [without precise locality]: "Cuba / Gundlach // 1541 / 977 // Kniž det. / interstitialis". **GUADELOUPE:** 1 ex. (dry-mounted) (IRSNB): "Guadeloupe / coll. A. d'Orchymont // Berosus s.str. / tessellatus / Fleut. & Salle // A. d'Orchymont det." [based on attached note, d'Orchymont compared this specimen with one of the types of *B. tessellatus* from the collection of Fleutiaux which is currently lost and not available for reexamination; the specimen is a male, but has the abdomen destroyed by a dermestid larva]; 1 ex. (dry-mounted) (IRSNB): Trois Riviéres, leg. Dufau. **PUERTO RICO:** 1 ex. (dry-mounted) (IRSNB): [without detailed locality data], leg. Moritz. **VIRGIN ISLANDS:** 2 exs. (dry-mounted) (IRSNB): Saint Thomas, leg. C. Felsche.

Published Cuban records: Pinar del Río: Entronque de Manuel Sanguili (Spangler 1981). **Isla de la Juventud**: Laguna Base Julio Antonio Mella (Spangler 1981). **Holguín**: Gibara, Arroyo Landivar at Finca Pozón (Spangler 1981). **Santiago de Cuba**: Matías (Spangler 1981).

Diagnosis. Habitus as in Figs 4a, b. Body length 5.0–5.3 mm. Head uniformly dark, metallic green; pronotum pale with a pair of closely aggregated longitudinal nar-

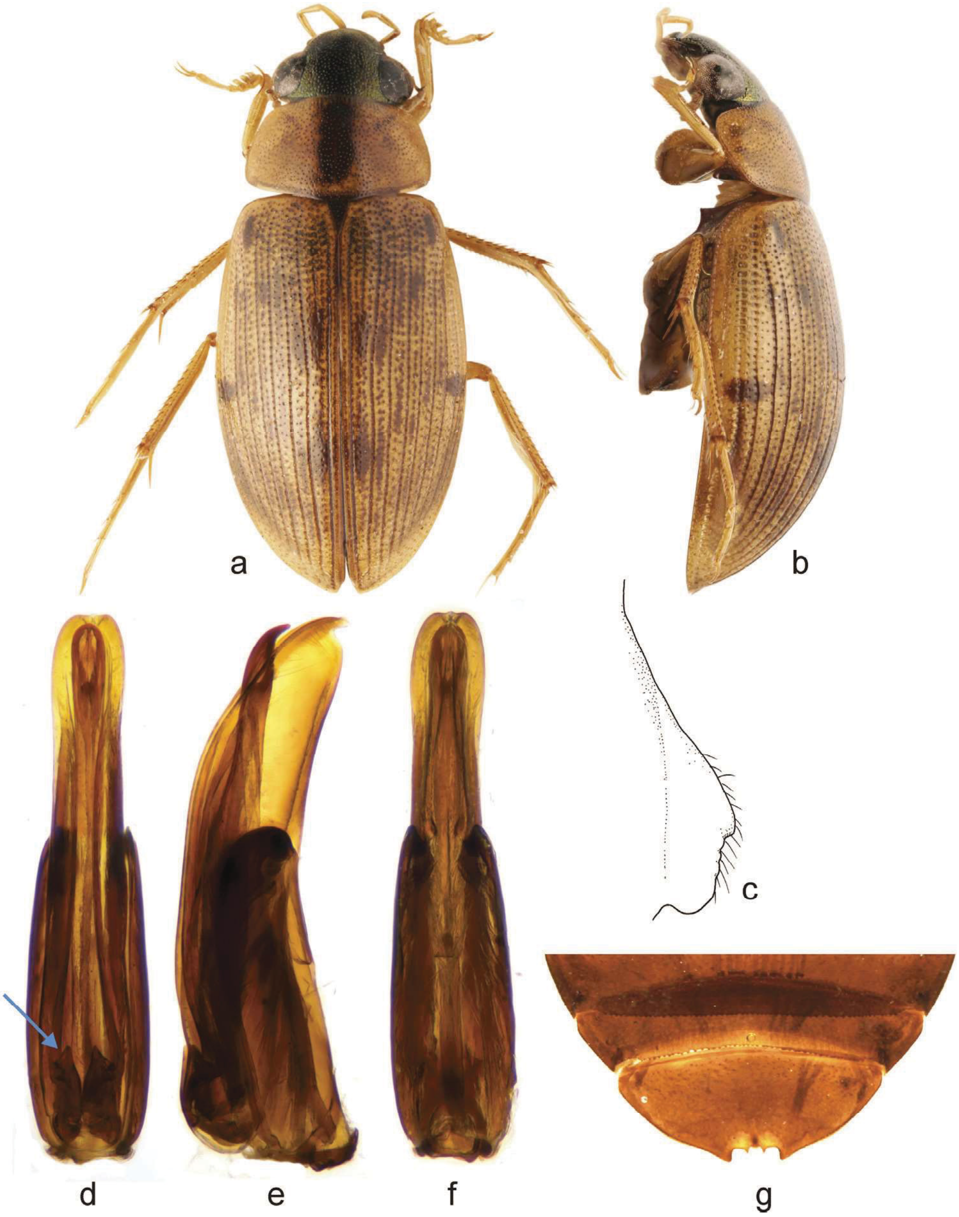

Figure 4. *Berosus interstitialis* Knisch, 1924. **a-b** habitus of the holotype of *B. stribalus* Orchymont, 1946 (**a** dorsal view **b** lateral view) **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

row dark spots mesally; elytra pale with darkened punctation and with dark spots in anterior and posterior third of intervals 1 and 2, in humeral area and at midlength of intervals 7-9, plus with variable number of spots on remaining intervals. Elytral apices entire in both sexes. Mesoventral process laminar, with small anterior tooth projecting ventrad, nearly straight middle portion and rounded posterior part (Fig. 4c). Abdominal ventrite 1 with median keel developed only between metacoxae. Emargination of ventrite 5 deep, subrectangular, with two slender medial teeth (Fig. 4g), not showing sexual dimorphism. Aedeagus (Figs 4d–f) strongly compressed from sides; parameres ca. 2× as long as phallobase, wide throughout in lateral view except for tooth-like apex; bases of the parameres in dorsal view with characteristic basal teeth.

Taxonomic note. The synonymy of *Berosus stribalus* with *B. interstitialis* was first proposed in an unpublished thesis by Van Tassell (1966: 302). The reasons for the synonymy were not explained, and Cuba (i.e. type locality of *B. stribalus*) was not even mentioned in the distribution of *B. interstitialis* in the taxonomic part of the thesis. We were not able to examine the types of *B. tessellatus* from the collection of Fleutiaux in MNHN as the specimens were not found. We therefore examined the specimens identified as *B. tessellatus* and *B. interstitialis* deposited in IRSNB, including one male from Guadeloupe (type locality of *B. tessellatus*) bearing the note that it was compared with the types of *B. tessellatus* by A. d'Orchymont. Comparison of these specimens with the types of *B. stribalus* and with newly collected Cuban specimens revealed that they all specimens agree in the diagnostic characters mentioned above, including the characteristic shape of the aedeagus and a characteristic tooth on the base of each paramere. We may therefore confirm the unpublished synonymy proposed by Van Tassell (1966) and consider *B. stribalus* as a junior subjective synonym of *B. interstitialis*.

Habitat. Cuban specimens were collected mainly in standing waters as well as in isolated pools along streams and rivers in the lowlands. The localities are usually exposed to sun and have turbid water, muddy bottom, submerged vegetation and are rich in organic matter.

Distribution. Widely distributed Caribbean species, so far recorded from the Bahamas, Haiti Guadeloupe, Virgin Islands Puerto Rico and Cuba (Van Tassell 1966, Orchymont 1946, Epler 2010, this paper). The species is here recorded for the first time from Cuba, due to the synonymy of *B. stribalus* with *B. interstitialis.*

Berosus metalliceps **Sharp, 1882**

http://species-id.net/wiki/Berosus_metalliceps
Figures 5a–g, 11

Berosus metalliceps Sharp, 1882: 83. – Van Tassell 1966: 150 (unpublished PhD thesis: redescription, identification key). – Epler 2010: 12.24 (taxonomic and distribution notes).

For complete synonymy and references see Hansen (1999).

Type locality. México: Tres Marías Island.

Material examined. CUBA: **Camagüey**: 1 ex. (dry-mounted) (NMPC): Cayo Sabinal, permanent lagoon, 21°38'6.1"N, 77°10'8.2"W, 5 m a.s.l., 06.v.2010, leg. Y. Torres.

Figure 5. *Berosus metalliceps* Sharp, 1882. **a–b** habitus of the only known Cuban specimen (**a** dorsal view **b** lateral view) **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

Diagnosis. Habitus as in Figs 5a, b. Body length 4.5 mm. Head metallic black, pronotum pale without dark spots, elytra pale with dark stripes on elytral series and slightly darker spot in posterior third of interval 1. Elytral apices entire and rounded.

Mesoventral process laminar, triangular in shape, anterior tooth projecting posteriad (Fig. 5c). Abdominal ventrite 1 with median keel developed only between metacoxae. Emargination of abdominal ventrite 5 rectangular, without tooth median (Fig. 5g). Aedeagus (Figs 5d–f) with median lobe much longer than parameres, with enlarged spatula–shaped apex in ventral view, sinuate on dorsal face in lateral view. Parameres simple, rounded apically, phallobase ca. 0.3× total length of aedeagus.

Distribution. USA (California), Mexico, Bahamas (Young 1953; Hansen 1999) and Cuba. The above specimen represents the first record of *B. metalliceps* from Cuba.

Habitat. The Cuban specimen was collected in the highly exposed brackish permanent lagoon with muddy bottom.

Berosus peregrinus (Herbst, 1797)

http://species-id.net/wiki/Berosus_peregrinus

Figures 6a–g

Hydrophilus peregrinus Herbst, 1797: 314.

Berosus peregrinus (Herbst); LeConte (1855: 364, transferred to *Berosus*). – Van Tassell 1966: 163 (unpublished PhD thesis: redescription, identification key). – Smetana 1988: 50 (diagnosis, recorded from Cuba). – Hansen 1999: 91 (catalogue). – Peck 2005: 48 (checklist). – Epler 2010: 12.20 (identification key).

Type locality. “North America”.

Material examined. CUBA: no material examined. **USA: Texas:** 1 ex. (dry-mounted) (NMPC); 1 ex. (in alcohol) (BSC-E): Maverick Co., Rt. 277 at Tequesquite Creek, large creek [AS-03-011], 31.viii.2003, leg. A. E. Z. Short.

Published Cuban records: Cuba: without specified locality (Smetana 1988). **Pinar del Río:** without specified locality (Peck 2005).

Diagnosis. Habitus as in Figs 6a, b. Body length 4.1–5.2 mm. Head metallic black, pronotum pale with two small submedian dark spots anteriorly, elytra pale with rather sharply defined dark spots on intervals 1-2 and in humeral area. Elytral apices entire and rounded. Mesoventral process laminar, triangular in shape, anterior tooth large, projecting posteriad (Fig. 6c). Abdominal ventrite 1 with median keel developed only between metacoxae. Emargination of abdominal ventrite 5 rectangular with a single median broad and short tooth (Fig. 6g). Aedeagus (Figs 6d–f) with median lobe slender, pointed at apex, parameres shorter than median lobe, very wide in lateral view, narrowing into sharply pointed apex bearing tuft of setae apically. Phallobase long, ca. 0.6× total length of aedeagus.

Distribution. Canada (Nova Scotia, Ontario, Quebec), USA (from New York and Pennsylvania to Florida, Louisiana, Mississippi and Texas, westward at least to Arizona, Illinois, Indiana and Wisconsin) (Hansen 1999), and Cuba. In Cuba, *B. peregrinus* has been recorded only from Pinar del Río (without exact locality) by Peck (2005). We did not collect this species in our survey.

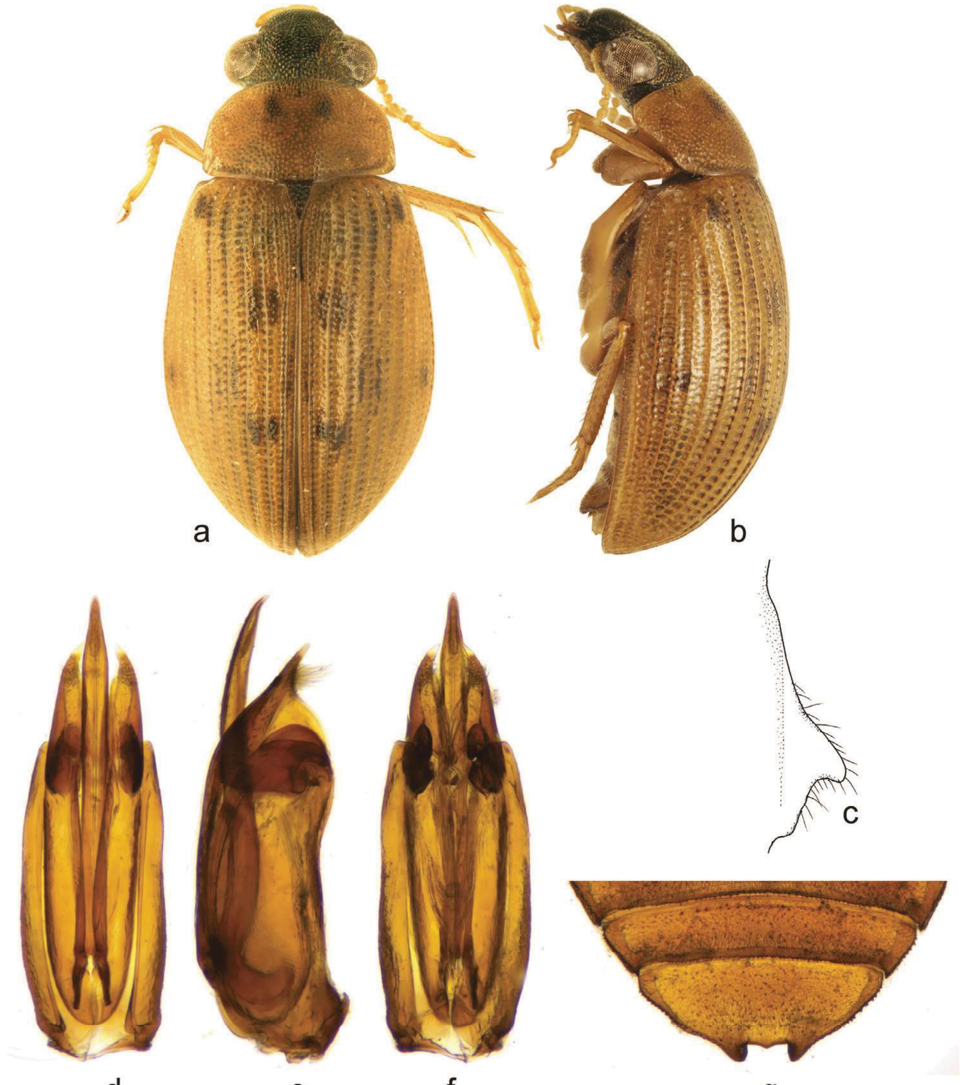

Figure 6. *Berosus peregrinus* (Herbst, 1797), specimen from USA, Texas. **a** habitus in dorsal view **b** habitus in lateral view **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

Berosus quadridens Chevrolat, 1863, stat. restit.

http://species-id.net/wiki/Berosus_quadridens

Figures 7a–g, 8e–h, 11

Berosus (*Anchialus*) *quadridens* Chevrolat, 1863: 206.

Berosus quadridens: – Gundlach 1891: 47 (diagnosis and distribution). – Zaitzev 1908: 357. – Mouchamps 1963: 121 (synonymized with *B. truncatipennis* Castelnau

1840). – Van Tassell 1966: 56 (unpublished PhD thesis: redescription, identification key). – Spangler 1981: 156 (diagnosis and distribution). – Hansen 1999: 82 (as synonym of *B. truncatipennis*). – Peck 2005: 48 (checklist). – Deler-Hernández and Cala-Riquelme 2010: 73 (diagnosis, distribution, identification key).

Type locality. Cuba.

Type material. Not examined.

Additional material examined CUBA: **Pinar del Río:** 1 ex. (dry-mounted) (IRSNB): S of Pinar del Rio, 12/23.ix.1913. **Isla de la Juventud**: 7 exs. (in alcohol) (BSC-E): Punta del Este, Laguna temporal [temporal pool], 21°33'43"N, 82°33'18"W, 1 m a.s.l., 21.v.2006, leg. Y. S. Megna, 00142. **Granma**: 3 exs. (in alcohol) (BSC-E): Cauto Cristo, Laguna permanente-I [permanent pool-I], 20°33'33.1"N, 76°28'44"W, 44 m a.s.l., 04.i.2005, leg. L. Chávez, 00087; 1 ex. (in alcohol) (BSC-E): Cauto Cristo, Laguna permanente-I [permanent pool-I], 20°33'33.1"N, 76°28'44"W, 44 m a.s.l., 13.vi.2004, leg. L. Chávez, 00174. **Santiago de Cuba**: 1 ex. (in alcohol) (BSC-E): Palma Soriano, Monte Barranca, 20°20'13.5"N, 76°1'11.6"W, 203 m a.s.l., 05.xii.2007, leg. A. Deler-Hernández and B. Téllez, 00052. **MEXICO**: **Sinaloa**: 1 ex. (dry-mounted) (IRSNB): Los Mochis Station, x.1921 leg. R. V. van Zwaluwenburg. **Veracruz**: 2 exs. (dry-mounted) (IRSNB): without more detailed locality, leg. Höge. **GUATEMALA:** 4 exs. (dry-mounted) (IRSNB): Paso Antonio, 400 ft., leg. Champion. **NICARAGUA: Chinandega:** 1 ex. (dry-mounted) (IRSNB): Posoltega, 06.v.1984, UV light, leg. Algodon. **COSTA RICA**: **Guanacaste**: 10 exs. (dry-mounted) (KSEM, NMPC): 11.5 km W of Cañas, 15 m a.s.l., HG light by ditch/field [AS-04-026], leg. A. E. Z. Short & D. J. Lebbin; 1 ex. (dry-mounted) (NMPC): Highway 1, 13.1 km SW of Liberia, roadside ditch/pools, 16.vi.2003, leg. A. E. Z. Short.

Published Cuban records: Cuba: **Isla de la Juventud**: Laguna Base Julio Antonio Mella (Spangler 1981). **Matanzas: Cárdenas** (Gundlach 1891). **Holguín**: Gibara, Arroyo Landivar at Finca Pozón (Spangler 1981).

Diagnosis. Large elongate species, body length 6.2–6.7 mm. Head testaceous with darker central part of clypeus and frons; pronotum pale, with a pair of vaguely defined narrow black longitudinal spots mesally, pronotal punctation darkened; elytra pale with dark elytral striae, interval punctation and variable number of larger dark spots on elytral intervals. Elytral apices each with a large subapical tooth, sutural angle sexually dimorphic, rounded in males, sharply pointed in females. Mesoventral process highly laminar, subtriangular in shape, anterior tooth weakly developed. Abdominal ventrite 1 with median keel developed only between metacoxae. Emargination of abdominal ventrite 5 deeply and narrowly excised in males, shallowly semicircular in females. Aedeagus large, with joint parameres pointed apically, with subbasal tuft of setae on dorsal surface, ventral membranous lobes minute, median lobe slender and long.

Differential diagnosis. *Berosus quadridens* is easily distinguishable from *B. truncatipennis* by the relatively larger and more sclerotized aedeagus having stouter and relatively longer phallobase, by ventral face of parameres bearing subbasal tuft of setae

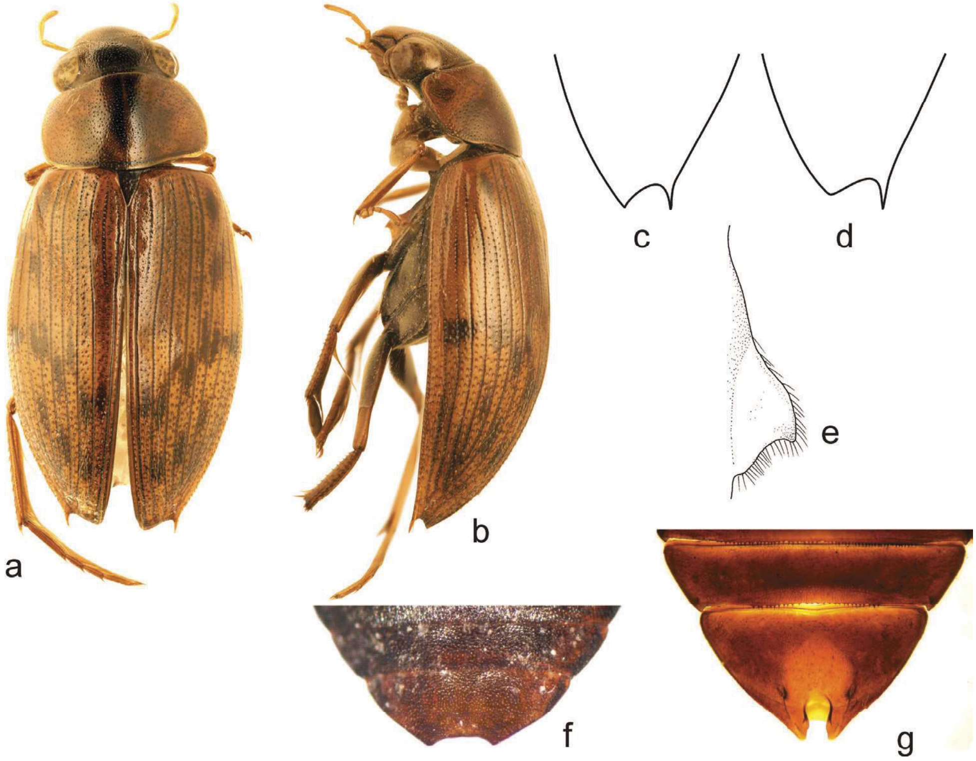

Figure 7. *Berosus quadridens* Chevrolat, 1863. **a** habitus in dorsal view **b** habitus in lateral view **c** apex of female elytron **d** apex of male elytron **e** mesoventral process in lateral view **f–g** abdominal ventrite 5 (**f** female **g** male).

(Fig. 8g) (whereas bearing a series of setae (Fig. 8c) in *B. truncatipennis*), by relatively longer and narrower median lobe and minute membranous dorsal projections of the parameres (Figs 8e, f, h) (in contrast to moderately large ones present (Figs 8a, b, d) in *B. truncatipennis*). The aedeagus of *B. quadridens* may resemble that of *B. megaphallus* by its large size and presence of subbasal tuft of setae on ventral face of the paramere, but both species distinctly differ by the size and proportions of the phallobase (ca. half as long as the whole aedeagus and very robust in *B. megaphallus*; ca. third as long as the whole aedeadus and less robust in *B. quadridens*) and by the proportions of the ventral membranous lobe of the paramere (minute in *B. quadridens*, nearly as long as paramere in *B. megaphallus*). In general, the aedeagus of *B. quadridens* looks like an enlarged aedeagus of *B. truncatipennis* on the first view, whereas that of *B. megaphallus* clearly differs from both *B. truncatipennis* and *B. quadridens* by the general proportions of its parts. We failed to find any realiable external differences between *B. truncatipennis* and *B. quadridens*; Van Tassell (1966) indicates the differences in the shape of the apical portion of elytra – these were found rather constant in shape in examined specimens of *B. quadridens*, but seem to be very variable in examined specimens of *B. truncatipennis*

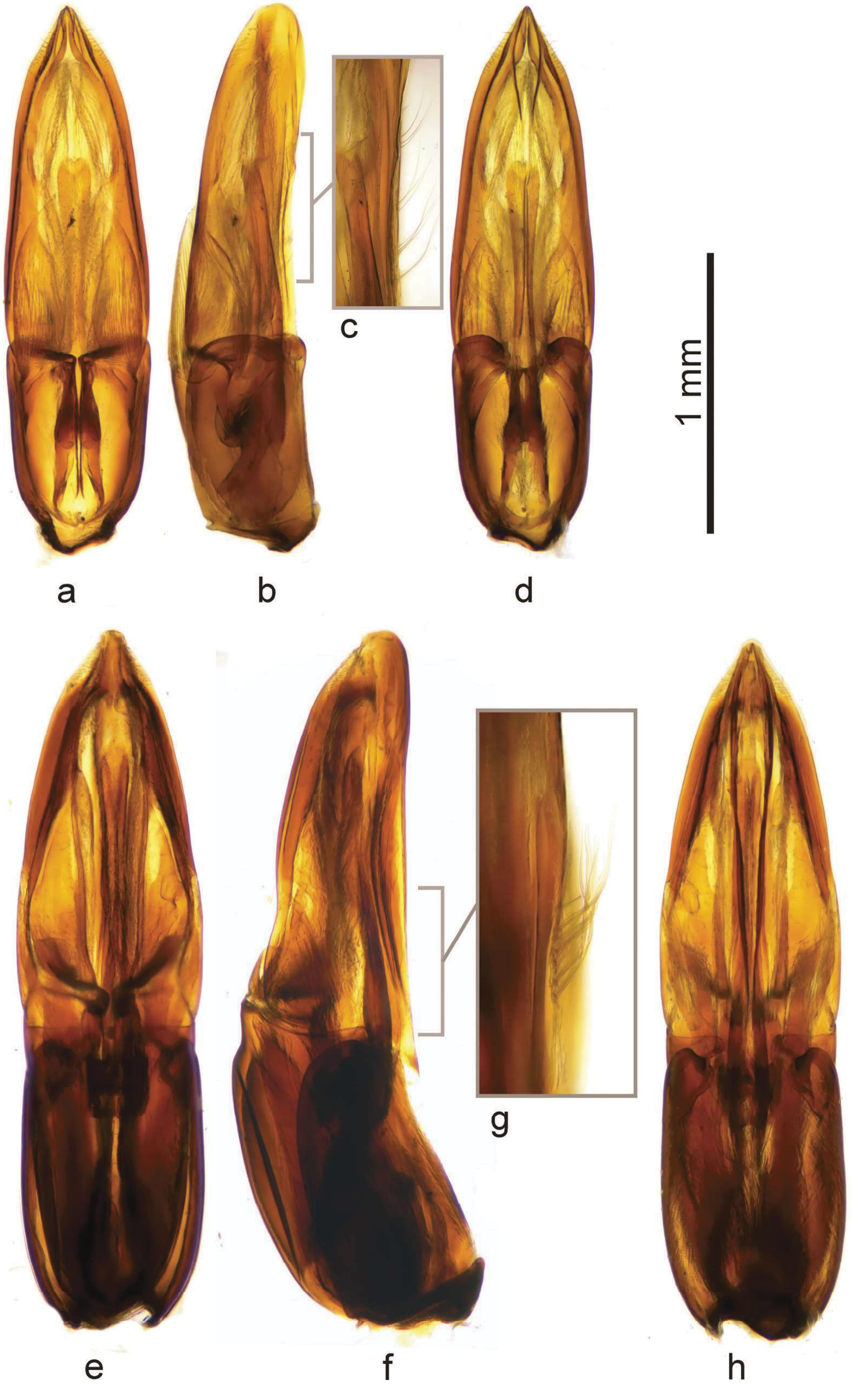

Figure 8. Comparison of the aedeagus of *Berosus truncatipennis* Castelnau, 1840 (**a–d**) and *B. quadridens* Chevrolat, 1863 (**e–h**). **a,e** dorsal view **b, f** lateral view **c, g** detail of setae of parameres **d, h** ventral view. Both aedeagi shown to scale.

and the character seems to be therefore unrealiable for distinguishing both species at the moment. Based on the differences mentioned above, we confirm that *B. quadridens* is a valid species, distinct from *B. truncatipennis*.

Redescription. Habitus as in Figs 7a, b. Body length 6.2–6.7 mm. Head, labrum and antennae testaceous. Maxillary palpi testaceous with palpomere 4 dark at apex. Pronotum testaceous with two metallic black central spots developed throughout pronotum. Scutellum testaceous. Elytra testaceous with oblique series of dark brown spots in posterior half and laterally, interval punctuation and elytra striae darkened. Pro- and mesofemora testaceous; metafemora with pubescent portion brown, glabrous portion testaceous.

Head and pronotum with punctures moderately fine and rounded. Elytral striae narrow well impressed. Interstriae fine and flat, bearing spine-like setae on posterior half of elytra. Scutellum with few impressed punctures. Elytral apices bidentate, each bearing a projection on sutural angle and subapically; shape sexually dimorphic, with sutural angle rounded in males (Fig. 7d), sharply pointed in females (Figs 7c). Mesoventral process highly laminar, triangular in shape, anterior tooth barely visible, followed by a convex and smooth ridge (Fig. 7e). Metaventral process raised, triangular in shape, with elongate and deep glabrous median depression; posterolateral angles produced into triangular laminae, posterior projection pointed. Abdominal ventrite 1 with median carina only between metacoxae and with large, deep, rounded lateral depressions. Abdominal ventrite 5 with a deep rounded emargination without tooth in males (Fig. 7g), in females with semicircular apical notch (Fig. 7f). Basal pubescence of meso- and metafemora covering basal two thirds of femoral length, borderline between pubescent and glabrous portion sinuate on mesofemur, straight on metafemur. Protarsus of male with adhesive soles on the two basal tarsomeres, protarsomeres 1-2 thickened, tarsomere 1 longer than tarsomere 2, tarsomere 3 elongate; tarsomere 4 elongate, as long as tarsomeres 1-3 combined. Claws long, slender and curved.

Male genitalia (Figs 8e–h): Phallobase robust, ca. 0.4× as long as whole aedeagus, slightly widening basad in lateral view. Parameres joint mesally, together forming a dish-like structure surrounding median lobe; apical portion rounded in lateral view, pointed in ventral view; ventral portion of each paramere with minute membranous lobe; dorsal face of each paramere with a tuft of setae situated subbasally. Median lobe stick-shaped, reaching to apical 0.75 of paremeres.

Taxonomic comments. Described from Cuba, *Berosus quadridens* was considered endemic to the island, whereas the continental form was supposed to represent the widely distributed South American species *B. truncatipennis* (e.g., Zaitzev 1908, Knisch 1924). Based on two females from Cuba (one of which we reexamined in this study), Mouchamps (1963) synonymized *B. quadridens* with *B. truncatipennis*. This was questioned by Van Tassell (1966) who followed the unpublished opinion of J. Balfour-Browne and considered *B. quadridens* as a species separate from *B. truncatipennis* occurring not only in Cuba, but also in Central America. The thesis by Van Tassell (1966), and therefore the revalidation of *B. quadridens*, remained unpublished and was only adopted without any explanatory comments in the catalogue of Cuban beetles by Peck (2005). Hansen (1999) considered *B. quadridens* as a dubious synonym of *B. truncatipennis* pending revision (Hansen 1999). Oliva (1989) considered the size and proportions of the genitalia of *B. truncatipennis* as geographically variable, being larger and wider in subtropical areas. Recently, Oliva & Short (2012) described the specimens with the large aedeagus from Venezuela and Guyana as a separate species *B. megaphallus* Oliva & Short, 2012, but the identity of the Central American and Caribbean specimens remained unsolved.

We were not able to examine the unique type of *B. quadridens* from "Cuba", as it was not found in MNHN after our loan request in 2012. A single species of Cuban *Berosus* matching the original description by Chevrolat (1863) was found in Cuba in our survey; no closely related or similar species was recorded from Cuba. We therefore do not have doubts that the Cuban specimens examined correspond to Chevrolat's (1863) understanding of *B. quadridens*. Moreover, Van Tassell (1966) mentioned that J. Balfour-Browne has examined the type of *B. quadridens* and found it to be conspecific with Central American specimens identified previously as *B. truncatipennis*. This corresponds with our findings, as we found that all examined Central American specimens of "*B. truncatipennis*" are conspecific with the Cuban ones, and clearly differ from the South American species (see Diagnosis above for diagnostic characters).

By confirming the separate species status of *B. quadridens*, the originally widely understood *B. truncatipennis* is shown to consist of three species: the widely distributed South American *B. truncatipennis*, *B. quadridens* confined to the Caribbean and Central America, and *B. megaphallus* known so far from Venezuela and Guyana. In the material from IRSNB we examined for this study, we have found few specimens from Bolivia (Río Beni) and southern Peru (Ica) which male genitalia are extremely similar to those of *B. quadridens* by their large size, strong sclerotization and relatively longer phallobase; however, they seem to differ from *B. quadridens* by the presence of the series of setae on the paramere (as in *B. truncatipennis*) and the dorsal membranous lobe of the paramere being ca. as long as in *B. megaphallus* (examined only in the Bolivian specimen, indistict in dissected Peruan ones). We suppose that these specimens may represent yet another undescribed species of the formerly broadly understood *B. truncatipennis*.

Habitat. The Cuban specimens examined in the present work were collected in highly exposed freshwater pools with turbid water, muddy bottom and without cover vegetation. Gundlach (1891) also reports this species from permanent ponds in the Matanzas Province.

Distribution. Based on the specimens examined for this study, we may confirm the occurrence of *B. quadridens* for Mexico, Guatemala, Nicaragua, Costa Rica and Cuba. Van Tassell (1966) also maps one record from Panama, but does not cite label data. In Cuba, the species is known from the western (including Isla de la Juventud special municipality) and eastern regions.

Berosus trilobus **Chevrolat, 1863**

http://species-id.net/wiki/Berosus_trilobus
Figures 9a–g, 11, 12a–b

Berosus trilobus Chevrolat, 1863: 207. – Gundlach 1891: 47 (diagnosis and distribution). – Spangler 1973: 354 (distribution). – Spangler 1981: 155 (diagnosis and distribution). – Hansen 1999: 95 (catalogue). – Peck 2005: 48 (checklist). – Epler 2010: 12.24 (notes on distribution).

Type locality. Cuba.

Type material examined. Holotype: female (MNHN): "Berosus / trifidus / Chv. Cuba / … [illegible] // von / G. Hemiosus / Sharp [= of the genus Hemiosus Sharp] // this must be / Chev. unique / type of trilobus / 1966 / PJS [= P. J. Spangler]".

Additional material examined. CUBA: **Sancti Spíritus**: 50 exs. (in alcohol) (BSC-E): Topes de Collantes, El Nueve, Río Caburny, 21°55'50"N, 80°00'59"W, 539 m a.s.l., 29.vi.2010, leg. A. Deler-Hernández. **Camagüey**: 19 exs. (in alcohol) (BSC-E): Sierra de Cubitas, Río El Roble, 21°32'53.23"N, 77°46'42.31"W, 55 m a.s.l., 14.iv.2012, leg. A. Deler-Hernández, 00144. **Holguín**: 6 exs. (in alcohol) (BSC-E): Jardín Botánico, Arroyo [stream], 20°51'46.8"N, 76°13'22,8"W, 84 m a.s.l., 07.xii.2008, leg. A. Deler-Hernández, 00074. **Granma**: 7 exs. (in alcohol) (BSC-E): Parque Nacional Turquino, La Platica, 20°00'33.80"N, 76°53'38.47"W, 800 m a.s.l., 29.iii.2012, leg. A. Deler-Hernández, 00143; 12 exs. (dry-mounted) (NMPC): Turquino NP, around La Platica, 20°0.7'N, 76°53.4'W, 880 m a.s.l. [MF24], 25-26. vi.2012 leg. A. Deler-Hernández and M. Fikáček. **Santiago de Cuba**: 6 exs. (in alcohol) (BSC-E): Campo Rico-II, Río Indio, 19°59'54.5"N, 75°32'4.6"W, 150 m a.s.l., 15.ix.2003, leg. A. Deler-Hernández and F. Cala-Riquelme, 00046; 4 exs. (in alcohol) (BSC-E): Gran Piedra, El Olimpo, Arroyo [stream], 20°00'33"N, 75°40'13"W, 820 m a.s.l., 04.viii.2005, leg. A. Deler-Hernández, 00016; 1 ex. (in alcohol) (BSC-E): II Palmas, La Cubana, Laguna temporal-II [temporal pool-II], 20°3'15.48"N, 76°8'3.12"W, 320 m a.s.l., 02.xii.2005, leg. Y. S. Megna, 00086; 30 exs. (in alcohol) (BSC-E): Palma Soriano, Arroyo [stream], 20°06'05"N, 75°58'44"W, 130 m a.s.l., 16.ii.2005, leg. K. Blanco, 00047; 5 exs. (in alcohol) (BSC-E): Guamá, La Mula, Río Turquino, 19°56'57"N, 76°45'36"W, 8 m a.s.l., 21.vi.2005, leg. Y. S. Megna, 00085; 6 exs. (in alcohol) (BSC-E): Guamá, Los Morones, Río Turquino, 19°58'33.6"N, 76°46'4.8"W, 200 m a.s.l., 18.vi.2008, leg. A. Deler-Hernández, 00006; 2 exs. (in alcohol) (BSC-E): San Luis, Dos Caminos, El Vivero, Laguna permanente [permanent pool], 20°11'2.50"N, 75°46'17.7"W, 150 m a.s.l., 01.viii.2008, leg. A. Deler-Hernández, 00028; 3 exs. (in alcohol) (BSC-E): San Luis, Dos Caminos, El Vivero, Río Guaninicú, 20°11'2.50"N, 75°46'17.7"W, 150 m a.s.l., 01.viii.2008, leg. A. Deler-Hernández, 00029; 31 exs. (dry-mounted) (NMPC): El Vivero, 1.6 km E of Dos Caminos, 20°10.8'N, 75°46.4'W, ca. 150 m a.s.l. [MF18], 20–21.vi.2012, leg. A. Deler-Hernández and M. Fikáček; 5 exs. (in alcohol) (BSC-E): Loma del Gato,

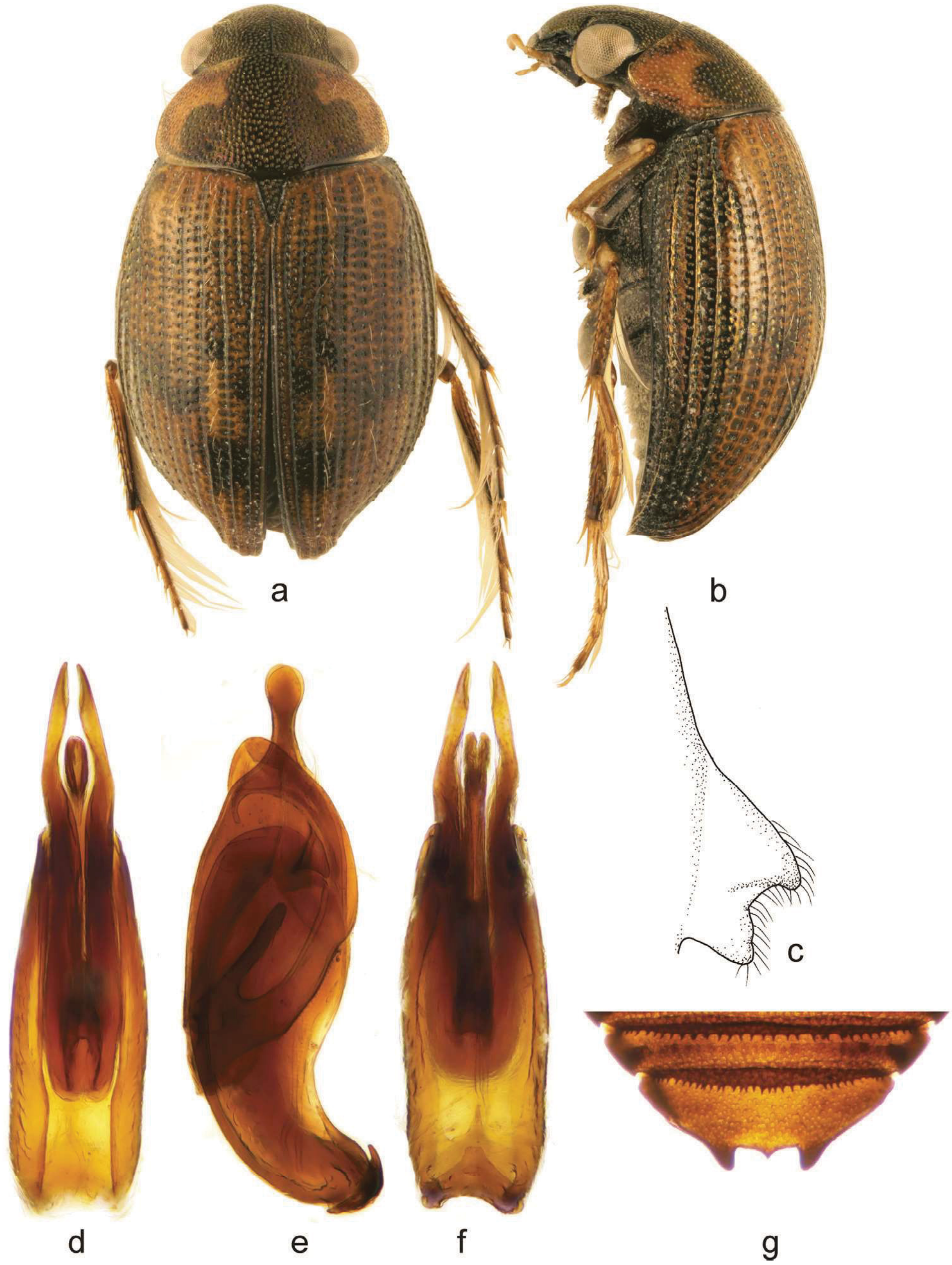

Figure 9. *Berosus trilobus* Chevrolat, 1863. **a** habitus in dorsal view **b** habitus in lateral view **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** abdominal ventrite 5.

Chan-Chan, Arroyo [stream], 19°58'27.4"N, 75°53'22.2"W, 353 m a.s.l., 27.vi.2009, leg. A. Deler-Hernández, 00118; 3 exs. (in alcohol) (BSC-E): La Redonda, Río Sevilla, 20°00'54.3"N, 75°45'45.6"W, 15 m a.s.l., 17.v.2009, leg. A. Deler-Hernández, 00154. **Guantánamo**: 36 exs. (in alcohol) (BSC-E) Imías, Yacabo Abajo, Río Yacabo Abajo, 20°06'05"N, 74°69'00"W, 5 m a.s.l., 24.x.2008, leg. A. Deler-Hernández and S. Muñiz, 00091; 20 exs. (in alcohol) (BSC-E): San Antonio del Sur, Macambo, Río Macambo, 20°03'26.9"N, 74°44'15.82"W, 4 m a.s.l., 25.x.2008, leg. A. Deler-Hernández and S. Muñiz, 00055; 16 exs. (in alcohol) (BSC-E): Baracoa-Maisí, Río Yumurí, 20°17'47.76"N, 74°17'39.5"W, 5 m a.s.l., 27.i.2010, leg. A. Deler-Hernández and R. Correa, 00152; 96 exs. (in alcohol) (BSC-E): Baracoa, Yunque, Río Duaba, 20°19'54.40"N, 74°34'9.08"W, 70 m a.s.l., 31.i.2010, leg. A. Deler-Hernández, 00171; 27 exs. (dry-mounted) (NMPC): El Yunque, 2.5-3.3 km SW of campismo popular, 20°19.4'N, 74°34.2'W, ca. 80-100 m a.s.l., 10.vi.2012 [MF02], leg. A. Deler-Hernández and M. Fikáček; 61 exs. (dry-mounted) (NMPC, KSEM): El Yunque, "La Cascada", ca. 2.1 km SW of campismo, 20°19.9'N, 74°34'W, ca. 60 m a.s.l. [MF07], 12-13.vi.2012, leg. F. Cala-Riquelme, A. Deler-Hernández and M. Fikáček; 2 exs. (dry-mounted) (NMPC): El Yunque, 3.2 km SW of campismo, right tributary of Duaba river, 20°19'N, 74°34'W, ca. 150 m a.s.l. [MF09], 13.vi.2012; leg. A. Deler-Hernández and M. Fikáček; 14 exs. (dry-mounted) (NMPC): El Yunque, in/around campismo popular, 20°20.4'N, 74°32.9'W, ca. 40 m a.s.l. [MF05], 10-13.vi.2012, leg. M. Fikáček; 20 exs. (dry-mounted) (NMPC): PN Alejandro de Humboldt, ca. 1.7 km NW of Santa María, 20°32'N, 74°43'W, ca. 50 m a.s.l. [MF13], 16.vi.2012, leg. A. Deler-Hernández and M. Fikáček; 18 exs. (in alcohol) (BSC-E): Baracoa, Jamal, 20°17'13.9"N, 74°25'33.6"W, 40 m a.s.l., 09.ii.2010, leg. R. Correa, 00169; 1 ex. (in alcohol) (BSC-E): Baracoa, Cabacú, Laguna permanente [permanent pool], 20°19'14"N, 74°28'58"W, 10 m a.s.l., 04.iii.2010, leg. R. Correa, 00170; 6 exs. (in alcohol) (BSC-E) 6 exs. (dry-mounted) (NMPC): Baracoa, Cabacú, Laguna permanente [permanent pool], 20°19'14"N, 74°28'58"W, 10 m a.s.l., 16.iii.2010, leg. R. Correa , 00164; 1 ex. (in alcohol) (BSC-E): La Marsella, Río Guaso, 20°26'22"N, 74°42'31"W, 60 m a.s.l., 26.i.2004, leg. Y. S. Megna , 00173; 3 exs. (in alcohol) (BSC-E): Baracoa, Loma de los Guineos, Arroyo [stream], 20°19'38.38"N, 74°35'35.37"W, 530 m a.s.l., 07.iv.2012; leg. A. Deler-Hernández, 00177. **Without precise locality**: 2 exs. (dry-mounted) (NMPC): "O. Koechin / Cuba // Collectio / Dr. Jureček / H. Jurečková"; 1 ex. (dry-mounted) (MNHN): "1542 / 1798". **DOMINICAN REPUBLIC:** 25 exs. (dry-mounted) (KSEM, NMPC): near Hato Mayor, creek off Ruta 103, 02.xi.2000, leg. A. E. Z. Short.

Published Cuban records: Cuba: without specified locality (Gundlach, 1891). **Pinar del Río**: Quemado de Pineda (Spangler 1981). **Sancti Spíritus**: Río Caburny near Topes de Collantes (Spangler 1973); Arroyo Vegas Grande near Topes de Collantes (Spangler 1973). **Camagüey**: Río El Manantiales (Spangler 1981). **Holguín**: Arroyo Jarahueca (Spangler 1981). **Santiago de Cuba**: II Frente, Sabanilla, Arroyo La Poa (Spangler 1981); II Frente, Arroyo Jarahueca (Spangler 1981); Contramaestre, Pozo Caliente, Río Contramaestre (Spangler 1981); II Frente, Sabanilla, Río Mayarí (Spangler 1981); II Frente, Río Ceiba affl. Río Mayarí (Spangler 1981); III Frente, Río Brazo Seco (Spangler 1981); III Frente, Matías, Río Mogote (Spangler 1981). **Guantánamo**: Maisí, La Tinta, Río Baracoa (Spangler 1973, 1981); Niceto Pérez, Sierra de

Canasta, Arroyo de los Berros (Spangler 1981); Río Miel at Baracoa (Spangler 1973); Baracoa, Yumurí, Río Yumurí (Spangler 1981).

Diagnosis. Small widely elongate species, body length 3.2–3.7 mm. Head dark, metallic; pronotum pale laterally, with large trilobite central dark spot, pronotal punctation not darkened laterally; elytra pale with dark intervals 8-10 and large transverse dark spots on posterior half of elyttral intervals 1-7. Elytral apices without subapical tooth, bumpy along suture subapically. Mesoventral process highly laminar, rectangular with large anterior and posterior teeth. Abdominal ventrite 1 with median keel throughout its length. Emargination of abdominal ventrite 5 rectangular with a median tooth. Median lobe of the aedeagus with long basal projection and beak-like apex in lateral view.

Differential diagnosis. For diagnostic characters and difference from *B. chevrolati*, see the latter species.

Redescription. Habitus as in Figs 9a, b. Body length 3.2–3.7 mm. Body short and wide, moderately convex in lateral view. Labrum black, dorsum of head melanic with strong metallic luster. Antennae testaceous. Maxillary palpi testaceous with palpomere 4 dark at apex. Pronotum testaceous with unpaired metallic black spot, the spot expanding laterad posteriorly, and hence trilobite in general shape. Elytra testaceous with small ill-defined dark brown spots on disc and, a broad metallic dark area throughout lateral portion. Pro-, meso- and metafemora with pubescent portion dark brown, glabrous portion testaceous.

Head with moderately large and rounded punctures. Pronotum with punctures slightly larger than on head. Scutellum with few impressed punctures. Elytral striae distinctly impressed; intervals flat and wide, irregular long setae on elytra; spine-like setae absent. Elytral apices entire and rounded in both sexes; highly bumpy along suture, depressed laterally on sides. Mesoventral process raised, rectangular in shape, with hood-like anterior tooth, posterior tooth large (Fig. 9c). Metaventral process widely rectangular, with large and deep elongate glabrous median depression; posterolateral portions bulge-like, posterior projection pointed. Abdominal ventrite 1 with median carina throughout the length. Abdominal ventrite 5 with rectangular emargination bearing broad and sharp median tooth (Fig. 9g). Meso- and metafemora with pubescence covering basal 0.7 of total length, borderline between pubescent and glabrous portion sinuate. Protarsus of male with adhesive soles on tarsomeres 1-2, tarsomeres 1-2 distinctly thickened, tarsomere 3 elongate; tarsomere 4 2× as long as tarsomere 3. Claws long, slender, slightly arched.

Male genitalia (Figs 9d-f). Phallobase ca. 0.7× total length of aedeagus. Parameres in lateral view wide basaly, narrowing subapically and apically projecting into rounded apex, lacking setae. Median lobe G-shaped in lateral view, with long basal projection directing apicad; apex wide, beak-shaped in lateral view.

Distribution. Dominican Republic and Cuba. The species was until now considered as Cuban endemic (e.g., Hansen 1999, Peck 2005), although Van Tassell (1966) mentioned specimens from the Dominican Republic. We are here confirming the occurrence of the species in the Dominican Republic based on recently collected specimens deposited in KSEM.

Habitat. In our survey, the specimens of *B. trilobus* were collected usually in streams and rivers with stony or sandy bottom, clear water and with or without aquatic vegetation (Figs 11a, b), although once it has also been collected in a temporary pool with stony-muddy bottom, abundant organic matter, turbid water and rich submerged vegetation. *Berosus trilobus* is found in elevations ranging from sea level to ca. 850 m a.s.l.

Berosus undatus (Fabricius, 1792)

http://species-id.net/wiki/Berosus_undatus
Figures 10a–i, 11

Hydrophilus undatus Fabricius, 1792: 185.
Berosus undatus (Fabricius, 1792). Synonymy: Gemminger and Harold 1868: 485. – Van Tassell 1966: 74 (unpublished PhD thesis: redescription, identification key). – Spangler 1981: 156 (diagnosis and distribution). – Hansen 1999: 82 (catalogue). – Peck 2005: 48 (checklist). – Epler 2010: 12.24 (notes on distribution). – Deler-Hernández and Cala-Riquelme 2010: 73 (diagnosis, distribution, identification key).
For complete synonymy and references see Hansen (1999).

Type locality. “America meridionalis”.

Material examined. CUBA: **Las Tunas**: 1 ex. (in alcohol) (BSC-E): Las Cuarenta, 20°00'9.72"N 76°57'48.6"W, 100 m a.s.l., 27.xi.2004, leg. Y. S. Megna, 00045. **Granma**: 2 exs. (in alcohol) (BSC-E): Cauto Cristo, Laguna permanente-II [permanent pool-II], 20°33'33.1"N, 76°28'44"W, 44 m a.s.l., 06.iii.2004, leg. L. Chávez, 00053. **Santiago de Cuba**: 4 exs. (in alcohol) (BSC-E): Laguna Juraguá, 19°56'30.8"N, 75°40'21.3"W, 22 m a.s.l, 17.ix.2003, leg. Y. S. Megna, 00044; 1 ex. (in alcohol) (BSC-E); 3 exs. (dry-mounted) (NMPC): Palma Soriano, Monte Barranca, 20°20'13.5"N, 76°1'11.6"W, 203 m a.s.l., 05.xii.2007, leg. A. Deler-Hernández, 00054; 1 ex. (dry-mounted) (NMPC): Palma Soriano 20°06'05"N, 75°58'44"W, 130 m a.s.l. 01.v.2005, leg. K. Blanco; 2 exs. (in alcohol) (BSC-E): La Maya, Los Reinaldos, Laguna temporal [temporal pool], 20°11'12"N, 75°31'43"W, 100 m a.s.l., 17.iii.2006, leg. Y. S. Megna, 00088. **Guantánamo:** 1 ex. (in alcohol) (BSC-E): Imías, Yacabo Abajo, Laguna temporal [temporal pool], 20°03'33.1"N, 74°42'29.9"W, 6 m a.s.l., 24.x.2008, leg. A. Deler-Hernández and S. Muñiz, 00060; 3 exs. (in alcohol) (BSC-E): San Antonio del Sur, Macambo, río Macambo, remanso [backwater], 20°03'26.9"N, 74°44'15.82"W, 4 m a.s.l., 25.x.2008, leg. A. Deler-Hernández and S. Muñiz, 00059.

Published Cuban records: Santiago de Cuba: Laguna Juraguá (Spangler 1981); Siboney (Spangler 1981). **Holguín**: Gibara, La Aguada (Spangler 1981).

Diagnosis. Habitus as in Figs 10a, b. Body length 6.3–7.2 mm. Head metallic black; pronotum pale with a pair of closely arranged elongate longitudinal black spots mesally, pronotal punctuation darkened; elytra pale with darkened striae and interval punctuation, plus with larger elongate dark spots on posterior half of intervals 1-4 and at midlength of intervals 8-10. Elytral apices with subapical spine in male (Fig. 10g),

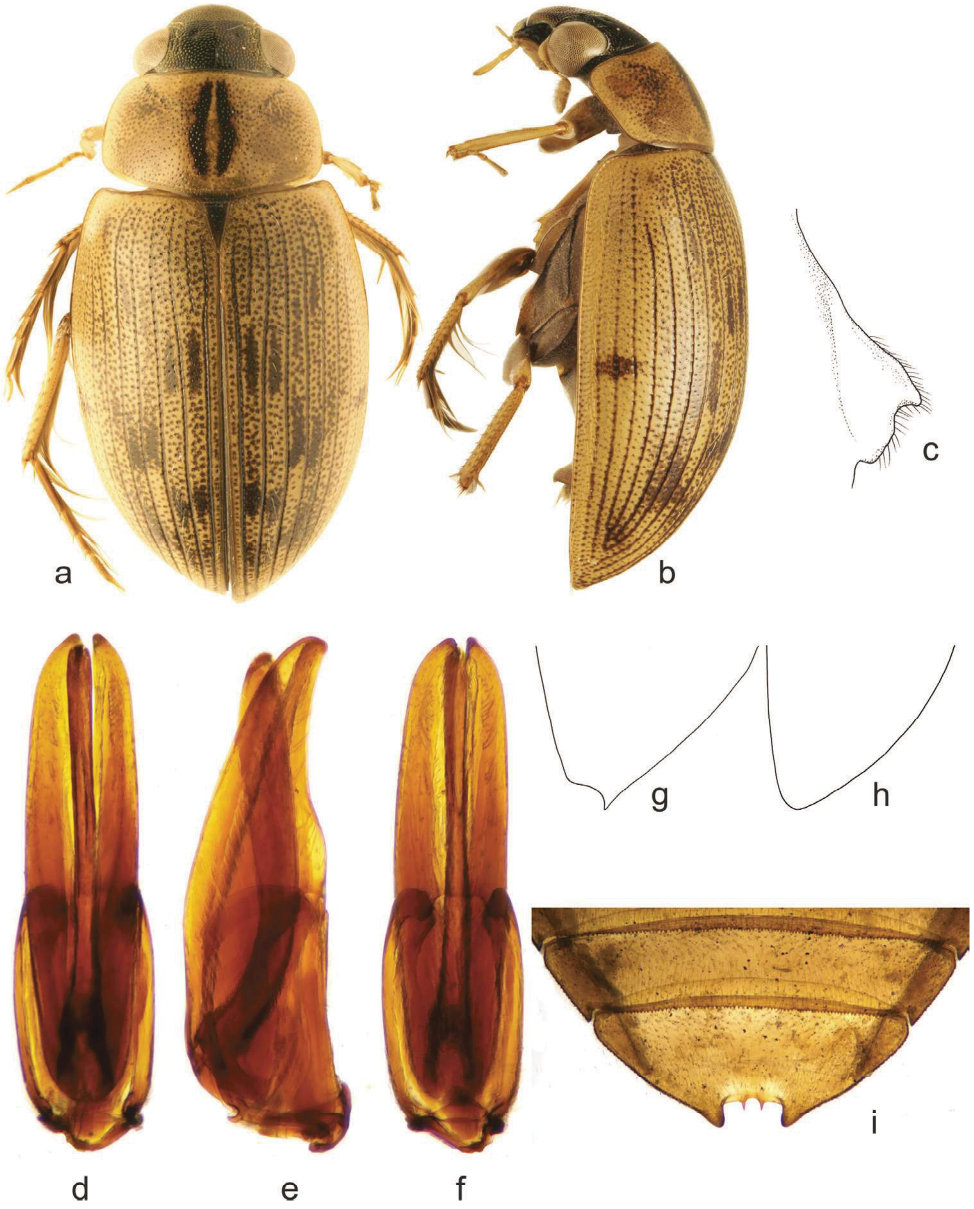

Figure 10. *Berosus undatus* (Fabricius, 1792). **a** habitus in dorsal view **b** habitus in lateral view **c** mesoventral process in lateral view **d–f** aedeagus (**d** dorsal view **e** lateral view **f** ventral view) **g** apex of male elytron **h** apex of female elytron **i** abdominal ventrite 5.

entire and rounded in female (Fig. 10h). Mesoventral process lowly laminar with large tooth directed posteriad (Fig. 10c). Abdominal ventrite 1 with median keel developed only between metacoxae. Emargination of abdominal ventrite 5 rectangular, with two broad and short medial teeth (Fig. 10i). Aedeagus (Figs 10d-f) with median lobe ca.

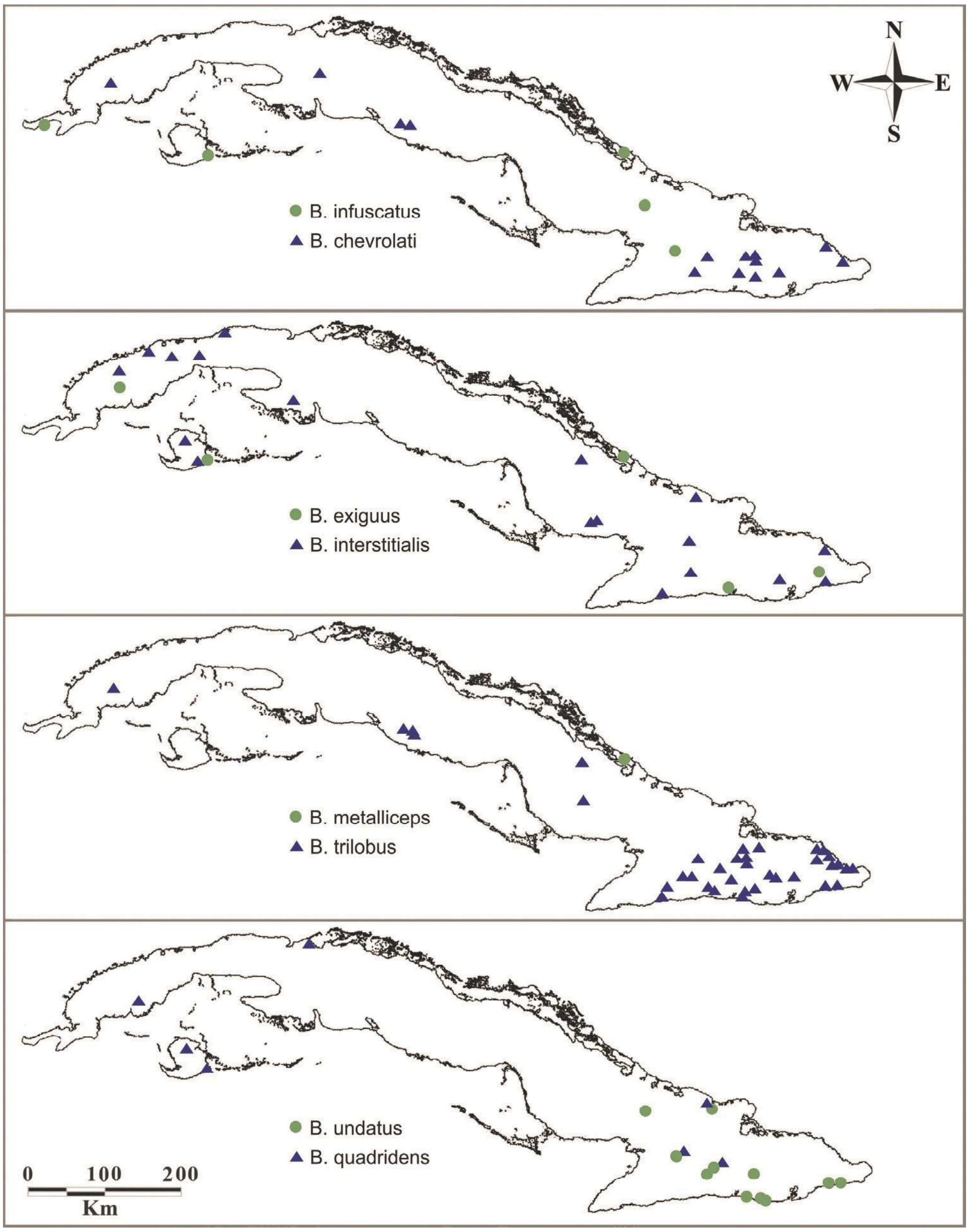

Figure 11. Known distribution of *Berosus* species in Cuba (includes our as well as historical records).

as long as parameres, lateral margins of parameres subparallel except apically; median lobe narrow in dorsal view, slightly wider in lateral view.

Distribution. USA (Texas), Mexico, Lesser Antilles (Hansen, 1999) and Cuba.

Habitat. Cuban specimens were collected in permanent and temporary pools as well as from running waters with clear or turbid water, having or lacking aquatic vegetation.

Figure 12. Localities of Cuban *Berosus* **a** Deler-Hernández collecting *B. chevrolati* and *B. trilobus* in a deep pool on side of a lowland river in Dos Caminos (June 2012) **b** locality of *B. trilobus* near Topes de Collantes (June 2010).

Key to identification of Cuban *Berosus*

1 First abdominal ventrite carinate along its entire length or large part of it. Small to medium sized species (3.0-4.6 mm). Pronotum testaceous, with unpaired median black spot (Figs 1a, 9a) or without any dark spots (Fig. 2a)... **2**

– First abdominal ventrite carinate only basally. Medium sized to large species (4.1-7.2 mm). Pronotum pale or testaceous with a pair of median black spots (Figs 3a, 4a, 6a, 7a, 10a), or pale without dark spots (Fig. 5a).................... **4**

2 Head black. Pronotum and elytra with dark spots. Dorsal surface coarsely punctate. Mesoventral process subquadrate. First abdominal ventrite with median carina throughout the length. Median lobe of the aedeagus without subapical dorsal series of setae.. **3**

– Head testaceous; pronotum and elytra without dark spots (Fig. 2a). Mesoventral process triangular (Figs 2b-c). First abdominal ventrite with median carina reaching the midlength. Aedeagus with median lobe bearing two series of long setae setae subapically on dorsal surface (Fig. 2e) .... ***B. exiguus*** **(Say)**

3 Dark pronotal spot large and trilobate, narrow anteriorly and very wide posteriorly (Fig. 9a). Subapical area of each elytron forming a bump (Figs 9a-b). Apex of the median lobe beak-shaped in lateral view, basal projection of the median lobe long (Fig. 9e)..***B. trilobus*** **Chevrolat**

– Dark pronotal spot narrow, situated mesally, not widened posteriad (Fig. 1a). Subapical area of each elytron without a distinct bump (Figs 1a-b). Apex of the median lobe rounded in lateral view, basal projection of the median lobe short (Fig. 1e). .... ***B. chevrolati*** **Zaitzev**

4 Head metallic black to black. First abdominal ventrite without lateral depressions, emargination of abdominal ventrite 5 rectangular, without distinct sexual dismorphism. Parameres separated from each other. Elytral apex with or without subapical spine... **5**

– Head testaceous (except mesally in some cases, Figs 7a–b). First abdominal ventrite with lateral depressions; emargination of abdominal ventrite 5 deeply or shallowly circular, sexually dimorphic (Figs 7f–g). Parameres joined mesally into a common dish-like structure (Figs 8e–h). Elytral apex with subapical spine in both sexes (Figs 7c–d)........................***B. quadridens*** **Chevrolat**

5 Pronotal disc without spots (Fig. 5a) or with small submesal anterior spots (Fig. 6a), never with a pair of mesal elongate large dark spots throughout the pronotal length. Apical emargination of abdominal ventrite 5 without tooth (Fig. 5g) or with a single medial tooth (Fig. 6g). Median lobe longer that parameres... **6**

– Pronotal disc with a pair of narrow elongate metallic central black spots. Apical emargination of abdominal ventrite 5 with two medial teeth. Median lobe shorter that parameres.. **7**

6 Elytral striae distinctly darkened, elytral disc without numerous darker spots (Figs 5a–b). Apical emargination of abdominal ventrite 5 without median

tooth (Fig. 5g). Median lobe of the aedeagus very long, spatulate apically in dorsal view, sinuate in lateral view (Figs 5d–f)............. ***B. metalliceps*** **Sharp**

– Elytral series not darkened, each elytron with several darker spots on the disc (Figs 6a-b). Apical emargination of abdominal ventrite 5 with a broad and short median tooth (Fig. 6g). Median lobe of the aedeagus slender and apex, pointed in dorsal view, slightly arcuate in lateral view (Figs 6d–f) .... ***B. peregrinus*** **(Herbst)**

7 Body size less than 6.0 mm. Elytral apices entire (without subapical spines) in both sexes. Phallobase longer than a half of total length of the aedeagus **8**

– Body size more than 6.3 mm. Elytral apices sexually dimorphic, with subapical spine in males (Fig. 10g) and rounded in females (Fig. 10h). Phallobase shorter than a half of the total length of the aedeagus (Figs 10d–f)... ***B. undatus*** **(Fabricius)**

8 Pronotum without mesh-like microsculpture on interstices. Mesoventral process with very small tooth (Fig. 4c). Posterolateral angles of metaventral process triangular. Aedeagus narrow, lateral margins of parameres subparallel, base of each paramere with a conspicuous tooth (Figs 4d–f) .***B. interstitialis***

– Pronotum with strong mesh-like microsculpture on interstices. Mesoventral process with larger tooth (Fig. 3c). Posterolateral angles of metaventral process with rounded laminae. Parameres sinuate along lateral margins, base of parameres without conspicuous teeth (Figs 3d–f)..................... ***B. infuscatus***

Acknowledgements

We thank Yoandri S. Megna (Universidad de Oriente, Santiago de Cuba) for his help during the field collecting and sharing of some specimens for this study, Andrew Short (Kansas University, Lawrence, U.S.A.) for his help with identification of the specimens, providing the literature, valuable discussions and numerous corrections of this manuscript, and Bruno Clarkson Mattos (Universidade do Brasil, Rio de Janeiro, Brasil) for numerous corrections of the text and comments on the morphology of male genitalia. We are deeply grateful to the authorities and personnel of the Cuban Ministry of Science, Technology and Environment (CITMA) and the Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF) for providing access to protected areas under their control. The work of M. Fikáček was partly supported by the Ministry of Culture of the Czech Republic (DKRVO 2012 and DKRVO 2013/12, National Museum, 00023272), the visit of the first author to Prague was supported by the institutional resources of the Ministry of Education, Youth and Sport of the Czech Republic. Open access to this paper was supported by the Encyclopedia of Life (EOL) Open Access Support Project (EOASP).

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CHAPTER 5

Deler-Hernández A., F. Cala-Riquelme and Fikáček M. 2014. A review of the genus *Oosternum* Sharp of the West Indies (Coleoptera: Hydrophilidae: Sphaeridiinae). *Deutsche Entomologische Zeitschrift* 53(2): 615-622**.**

Dtsch. Entomol. Z. 61 (1) 2014, 43–63 | DOI 10.3897/dez.61.7566

PENSOFT. museum für naturkunde

A review of the genus *Oosternum* Sharp of the West Indies (Coleoptera: Hydrophilidae: Sphaeridiinae)

Albert Deler-Hernández[1,†], Franklyn Cala-Riquelme[2,‡], Martin Fikáček[1,3,§]

1 *Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44 Praha 2, Czech Republic*
2 *Departamento de Zoología, Centro Oriental de Ecosistemas y Biodiversidad, Enramadas 601 esquina Barnada, Santiago de Cuba, 90100, Cuba*
3 *Department of Entomology, National Museum, Kunratice 1, CZ-148 00 Praha 4, Czech Republic*

† *http://zoobank.org/6FA44B89-67C1-47D0-ADC9-77EC2A1DD61B*
‡ *http://zoobank.org/63AF3355-3A67-46D8-9D96-4170DDDCA51E*
§ *http://zoobank.org/E1A02744-41A2-4B9C-A127-B78F0D812637*

http://zoobank.org/6BB87610-5563-4032-96CF-62E0AC578B1E

Corresponding author: *Albert Deler-Hernández* (adeler1982@gmail.com)

Received 21 March 2014
Accepted 02 May 2014
Published 30 May 2014

Academic editor:
James Liebherr

Abstract

The representatives of the genus *Oosternum* Sharp, 1882 occurring in the West Indies are revised. Ten species are recorded, of which seven are here described as new: *Oosternum andersoni* **sp. n.** (Cuba), *O. bacharenge* **sp. n.** (Dominican Republic), *O. cercyonoides* **sp. n.** (Jamaica), *O. insulare* **sp. n.** (Jamaica), *O. luciae* **sp. n.** (Saint Lucia), *O. megnai* **sp. n.** (Cuba) and *O. pecki* **sp. n.** (Dominican Republic). Diagnoses and detailed distributional data are also provided for *O. sharpi* Hansen, 1999 (widespread throughout both Greater and Lesser Antilles), *O. latum* Fikáček, Hebauer & Hansen, 2009 (endemic to St. Vincent) and an undescribed species from the Bahamas. A key to the West Indian *Oosternum* is provided and important diagnostic characters are illustrated. The West Indian fauna of *Oosternum* contains representatives of five different species groups and likely originated by multiple independent colonizations from the American continent. Within the study region, the highest diversity is known from the Greater Antilles, where two endemic species each in Cuba, Jamaica, and Hispaniola. The populations of *O. sharpi* were found to consist exclusively of females on all islands with the exception Puerto Rico.

Key Words

Coleoptera
Hydrophilidae
Megasternini
new species
island
endemics
Caribbean Region
Neotropical Region

Introduction

The West Indies (i.e. the Caribbean islands off the continental shelf; Bond 1993) is a region generally known for its high species diversity and endemism. At the same time, large part of its natural habitats were destroyed and it is therefore considered as one of the world's biodiversity hotspots (Mittermeier et al. 2005). Although a large number of biodiversity studies have been conducted in the area, surprisingly little is known about the diversity of insects and other invertebrates. Cryptic leaf-litter inhabiting taxa are among those for which available information is especially limited, although a very high diversity is expected based on available data from the continental Central America (e.g., Anderson and Ashe 2000) and preliminary data available from several projects currently running in the Carribean islands (e.g., Cala-Riquelme 2013; Cala-Riquelme and Agnarsson in press; Deler-Hernández et al. 2013; R. Anderson, pers. comm.).

In the present contribution we examine the West Indian fauna of the hydrophilid beetle genus *Oosternum* Sharp, 1882. The genus contains minute beetles generally inhabiting leaf-litter of various types of tropical forests. Although it also occurs in the Oriental Region, the genus is especially diverse in the Neotropical Region, from where 15 species are presently described (Fikáček 2009;

Fikáček and Hebauer 2009; Fikáček et al. 2009; Makhan 2009; Short and Fikáček 2011) and additional ca. 45 species are awaiting description (M. Fikáček unpubl. data). Based on the distribution data of the species groups which were already revised in detail (see aforementioned references), the genus includes both widely distributed species as well as those with very limited ranges, and seems therefore an ideal model group to study the biogeography and endemism of the leaf-litter invertebrates of the West Indian islands. This motivated us to perform its detailed taxonomic revision.

Available data from the West Indies were very scarce prior to this study. A single widespread species, *O. sharpi* Hansen, 1999, was recorded from Cuba and Puerto Rico (Peck 2005; Spangler 1981, in both cases as *O. costatum* Sharp, 1882) and the supposed single island endemic *O. latum* Fikáček, Hebauer & Hansen, 2009 was described recently from the Saint Vincent Island of the Lesser Antilles (Fikáček et al. 2009). For this study we have accumulated the material from 15 islands of both the Greater and Lesser Antilles, resulting from our own recent field work in Cuba and Jamaica and from several large projects by the Canadian and US entomologists. The results of the taxonomic revision of this material are summarized below.

Materials and methods

A total of 280 specimens from 13 countries (15 islands) of the West Indies were examined in this study. Most specimens were collected by sifting leaf litter in various kinds of forests with subsequent extraction using Berlese funnels and Winkler traps. Part of this material was collected during our recent expeditions to various parts of Cuba and Jamaica. The remaining material was examined from the Canadian and US collections, most importantly the personal collection of Professor Stewart Peck (SBPC) and the West Indian Beetle Fauna Project Collection (WICP).

Habitus photographs were taken using Canon EOS 550D digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS5. Drawings of male genitalia are based on photographs taken using Canon EOS 1100D digital camera attached to Olympus BX41 compound microscope and subsequently combined in Helicon Focus software. Scanning electron micrographs of the holotypes of the new species were taken using Hitachi S-3700N environmental electron microscope at the Department of Paleontology, National Museum in Prague. General morphological terminology follows Hansen (1999) and Fikáček et al. (2009).

Examined specimens are deposited in the following collections:

BMNH	The Natural History Museum, London, U.K. (M. Barclay);
BSC-E	Departamento de Zoología, Centro Oriental de Ecosistemas y Biodiversidad, Santiago de Cuba, Cuba (F. Cala-Riquelme);
CMN	Canadian Museum of Nature, Ottawa, Canada (R. Anderson);
CNC	Canadian National Collection, Ottawa, Canada (A. Smetana);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček);
SBPC	Stewart Peck Personal Collection, Ottawa, Canada;
SEMC	Division of Entomology, Biodiversity Institute, University of Kansas, Lawrence, USA (A. Short);
WIBF	West Indian Beetle Fauna Project Collection, Montana State University, Bozeman, Montana (M. Ivie).

Checklist of the West Indian species of *Oosternum*

Oosternum andersoni sp. n.	Cuba
Oosternum bacharenge sp. n.	Hispaniola: Dominican Rep.
Oosternum cercyonoides sp. n.	Jamaica
Oosternum insulare sp. n.	Jamaica
Oosternum latum Fikáček, Hebauer & Hansen, 2009	Saint Vincent
Oosternum luciae sp. n.	Saint Lucia
Oosternum megnai sp. n.	Cuba
Oosternum pecki sp. n.	Hispaniola: Dominican Rep.
Oosternum sharpi Hansen, 1999	widespread in the West Indies
Oosternum sp.	Bahamas: Andros Is.

Key to species of West Indian *Oosternum*

1 Median portion of prosternum elevated and demarcated from lateral portions (Figs 35–38). Antennal grooves of prothorax with acute lateral projection (see Fikáček 2009, Fig. 13) 2

– Median portion of prosternum not elevated and demarcated from lateral portions (Figs 29–33). Antennal grooves of prothorax rounded to subangular, without acute lateral projection 5

2 Pronotal punctation consisting of punctures of two very different sizes (Figs 22, 27). Each paramere bearing a single seta apically (Figs 55, 60) 3

– Pronotal punctation uniform, all punctures of the same size (Figs 23, 24). Each paramere bearing two short setae apically (Figs 56, 57) 4

3 Pronotum without microsculpture on interstices (Fig. 22). Metaventrite crenulate behind mesocoxal cavities (Fig. 42). Lateral membrane in apical portion of the median lobe very narrow and without setae (Fig. 55) .... *O. insulare*

– Pronotum with fine mesh-like microsculpture on interstices (Fig. 27). Metaventrite not crenulate behind mesocoxal cavities (Fig. 47). Lateral membrane in apical portion of the median lobe wide and with a series of fine setae (Fig. 60) .... *O. sharpi*

4 Body widest at elytral base (Fig. 9). Pronotum very convex, not forming a continuous curve with elytra in lateral view (Fig. 10). Lateral membrane only present in apical fifth of the median lobe and lacking setae (Fig. 56) .... *O. latum*

– Body widest ca at midlength (Fig. 11). Pronotum moderately convex, forming a continuous curve with elytra in lateral view (Fig. 12). Lateral membrane reaching midlength of the median lobe and bearing a series of fine setae (Fig. 57) ... . *O. luciae*

5 Punctures of pronotum increasing in size towards posterior margin, smaller and transverse anteriorly and large and rounded posteriorly (Fig. 26). Preepisternal plate of mesoventrite subrhomboid, largely overlapping over anterior portion of the metaventrite (Fig. 46). Aedeagus as in Fig. 59 .... *O. pecki*

– Pronotal punctation uniform, size of punctures not increasing posteriad. Preepisternal plate of mesoventrite drop-like or suboval, only slightly overlapping over the anterior margin of the metaventrite (Figs 39–41, 45) 6

6 Elytral interval 2 narrower and lower than interval 3 subapically. Preepisternal elevation of the mesothorax narrower, 2.0–2.8× as long as wide. Smaller species, body length smaller than 1.5 mm 7

– Elytral interval 2 of the same width and height as interval 3 subapically. Preepisternal elevation of the mesothorax wide, 1.7× as long as wide. Large species, body length 1.9–2.0 mm .... *O. cercyonoides*

7 Anterolateral ridges of metaventrite meeting mesally and projecting posteriad (Fig. 40). Apical portion of the median lobe bottle-shaped, widely rounded at apex (Fig. 54). General coloration dark brown (Figs 3, 4) .... *O. bacharenge*

– Anterolateral ridges of metaventrite not meeting mesally (Figs 39, 45). Apical portion of the median lobe gradually narrowing, acute at apex (Figs 53, 58) 8

8 Lateral margin of the pronotum sinuate. Preepisternal elevation of mesoventrite wider, 2.0× as long as wide (Fig. 39). Median lobe very wide, not reaching the level of parameral apices (Fig. 53) .... *O. andersoni*

– Lateral margin of the pronotum arcuate. Preepisternal elevation of mesoventrite narrower, 2.3× as long as wide (Fig. 45). Median lobe narrow, slightly overlapping the parameral apices (Fig. 58) .... *O. megnai*

Taxonomy

Oosternum andersoni sp. n.

http://zoobank.org/FEEF4A2B-C113-4209-8551-25BE9943D63D
http://species-id.net/wiki/Oosternum_andersoni

Figs 1–2, 19, 29, 39, 53, 61

Type-locality. Cuba, Santiago de Cuba Province: Santiago Municipality, 20.011°N, 75.673°W, 550 m, Km 7 of the road to Gran Piedra.

Type-specimens (144 spec.). Holotype male, dry mounted, with genitalia mounted in Euparal on a microslide pinned below the specimen. Original label: "CUBA: Santiago de Cuba: Km 7 of the road to Gran Piedra, 26.i.2012, R. Anderson leg., 20.011°N, 75.673°W, elevation 550 m [printed] / Holotype, Oosternum andersoni sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]" (NMPC). Paratypes: **CUBA:** Pinar del Río province, Sierra del Rosario, Rangel, ca. 15 Km S. Cinco Pesos, 30.v.1990, M. A. Ivie leg., berlese from deep log and leaf litter (2 spec., WIFP). Cienfuegos province: Cumanayagua municipality, 2 Km E of Mayarí, ca. 842 m a.s.l., 21.96651°N, 80.11497°W, 18.v.2013, R. Anderson leg (2013-017) (29 spec., NMPC); Cumanayagua municipality, uphill road to Pico San Juan, ca. 1086 m a.s.l., 21.9881°N, 80.1464°W, 19.v.2013, R. Anderson leg (2013-022) (37 spec., NMPC); Cumanayagua municipality, Aguacate, Gruta Mengoa, near of Río Cabagan, ca. 620 m a.s.l., 21°55'52.43"N, 80°5'4.60"W, 20.v.2013, R. Anderson & A. Deler-Hernández leg (CF6) (53 spec., NMPC, CMN, BMNH, SEMC, SBPC). Holguín province: Mayarí municipality, Pinarito, PN La Mensura-Piloto, ca. 410 m a.s.l., 20.41598°N, 75.82008°W, 12.v.2013, R. Anderson leg (13 spec., NMPC). Santiago de Cuba province: Km 7 of the road to Gran Piedra, ca. 550 m a.s.l., 20.011°N, 75.673°W, 26.i.2012, R. Anderson leg. (2012-007) (5 spec., BSC-E, NMPC); 23.v.2013, R. Anderson leg. (2013-031) (4 spec., NMPC).

Differential diagnosis. Body widest ca at midlength. Lateral margin of pronotum weakly sinuate. Pronotal punctation uniform in size, moderately dense, consisting of small, rasp-like punctures. Pronotal interstices without microsculpture. Mesal part of prosternum not divided from lateral portions. Lateral margin of antennal grooves rounded. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 2× longer than wide. Interstices of median part of metaventrite without microsculpture. Anterolateral ridges of metaventrite not meeting together mesally. Parameres 1.2× longer than phallobase, bearing two short setae apically. Median lobe shorter than parameres, widely oval in shape, acute at apex. Membranous lateral projections of median lobe absent.

Description. ***Habitus.*** Body elongate oval, gradually narrowing posteriad; total leght/total width ratio = 1.7. Length: 1.25–1.29 mm, length of holotype: 1.37 mm; width: 0.73–0.77 mm, width of holotype: 0.75 mm.

Coloration. Coloration of dorsal side brown to dark brown, elytra darker than pronotum, head dark brown. Ventral side brown. Femora and tibiae brown, tarsi,

Figures 1–12. Habitus of *Oosternum* from West Indies. **1–2.** *Oosternum andersoni* sp. n., lenght = 1.25 mm (1. dorsal view, 2. lateral view). **3–4.** *Oosternum bacharenge* sp. n., lenght = 1.40 mm (3. dorsal view, 4. lateral view). **5–6.** *Oosternum cercyonoides* sp. n., lenght = 1.95 mm (5. dorsal view, 6. lateral view). **7–8.** *Oosternum insulare* sp. n., lenght = 1.40 mm (7. dorsal view, 8. lateral view). **9–10.** *Oosternum latum* Fikáček, Hebauer & Hansen, 2009, lenght = 1.50 mm (9. dorsal view, 10. lateral view). **11–12.** *Oosternum luciae* sp. n., lenght = 1.41 mm (11. dorsal view, 22. lateral view).

antennomeres 1–6 and mouthparts yellowish, antennal club pale reddish brown.

Head. Clypeus with sparse punctation consisting of fine rounded punctures, each puncture bearing fine decumbent seta; setae pale; interstices without microsculpture; anterior margin of clypeus slightly concave. Frons with dense punctation consisting of coarse rounded punctures, punctures of same shape medially and laterally; interstices with fine mesh-like microsculpture. Eyes moderately large. Mentum 1.8× wider than long, anterior margin slightly emarginate; anteromedian part not distinctly impressed; with sparse punctation, punctation consisting of minute, nearly indistinct punctures bearing minute setae; interstices with very fine microsculpture, opaque. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.5× longer than palpomere 3. Antenna with 9 antennomeres; scapus shorter than antennomeres 2–6 combined.

Prothorax. Pronotum evenly convex forming continuous curve with elytra in lateral view. Lateral margin weakly sinuate; with narrow marginal bead. Pronotal punctation uniform in size, moderately dense; slightly sparser than that on frons, consisting of small, rasp-like punctures, slightly sparser laterally than medially; all punctures bearing long setae; interstices without microsculpture. Transverse row of punctures on posterior margin of pronotum absent. Median portion of prosternum not elevated and demarcated from lateral portions, median carina of prosternum narrow, projecting more anteriad mesally than anterior margin of median portion, with anterior portion elevated into small tooth in lateral view. Postero-mesal projection with deep notch. Antennal groves moderately large. Lateral margin of antennal grooves rounded.

Mesothorax. Scutellar shield bearing a few small punctures; interstices without microsculpture. Elytral series 1–5 arising basally, series 6–9 joint subbasally. Serial punctures small, transverse, sparsely arranged, with minute setae (indistinct under binocular microscope). Interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex, intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 2× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation slightly overlapping over anterior margin of metaventrite; median part flat; bearing sparsely arranged shallow setiferous punctures; interstices without microsculpture.

Metathorax. Metaventrite distinctly longer than preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture; punctation of median portion consisting of small rounded punctures, interstices without microsculpture, shiny. Anterolateral ridges bent posteriad along lateral margin of metaventrite, concave laterally, not meeting together and bent posteriad mesally. Anterior margin of metaventrite not crenulate.

Figures 13–18. Habitus of *Oosternum* from West Indies. **13–14.** *Oosternum megnai* sp. n., lenght = 1.49 mm (13. dorsal view, 14. lateral view). **15–16.** *Oosternum pecki* sp. n., lenght = 1.50 mm (15. dorsal view, 16. lateral view). **17–18.** *Oosternum sharpi* Hansen, 1999, length = 1.37 mm (17. dorsal view, 18. lateral view).

Abdomen. Ventrite 1 with additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Male genitalia. Aedeagus 0.5 mm long, parameres 1.2× longer than phallobase. Parameres continuously narrowing apicad, bearing two short setae apically. Phallobase wide, 1.4× longer than wide. Median lobe slightly shorter than parameres, widely oval in shape, slightly narrowing at apex. Membranous lateral projections of median lobe absent. Gonopore absent.

Etymology. The new species is dedicated to our friend Robert S. Anderson (Canadian Museum of Nature, Ottawa), a very enthusiastic entomologist and collector of the type specimens.

Distribution. *Oosternum andersoni* sp. n. is a Cuban endemic species currently known from the western, central and eastern parts of the island (Fig. 61).

Habitat. Most specimens were sifted from wet leaf litter in evergreen montane forests.

Oosternum bacharenge sp. n.

http://zoobank.org/AFDE881F-D1DB-419C-874D-C373DC6E36B7
http://species-id.net/wiki/Oosternum_bacharenge
Figs 3–4, 20, 30, 40, 54, 61

Type-locality. Dominican Republic, Hatomayor province: Parque Nacional Los Haitises, 19°1’4.26”N, 69°37’17.24”W, 250 m, W of Sabana de la Mar.

Type-specimens (1 spec.). Holotype dry mounted, with genitalia mounted in Euparal on a microslide pinned below the specimen. Original label: “DOM REP: Hatomayor province, Par. Nac. Los Haitises W. of Sabana de la Mar, 01.iv.1992, M. A. Ivie, D. S. Sikes & W. Lanier, 19°1’4.26”N, 69°37’17.24”W, elevation 250 m, [printed] / Holotype, Oosternum bacharenge sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]” (WIFP).

Diagnosis. Body widest ca. at midlength. Lateral margin of pronotum angulate. Pronotal punctation uniform in size, moderately dense consisting of small rasp-like punctures. Pronotal interstices without microsculpture. Mesal part of prosternum not divided from lateral portions. Lateral margin of antennal grooves rounded. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 as convex as adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate narrow, suboval, 2.8× longer than wide. Interstices of median part of metaventrite without microsculpture, shiny. Anterolateral ridges of metaventrite meeting together and bent posteriad mesally. Parameres 0.8× as long as phallobase, bearing two short setae apically. Median lobe ca. as long as parameres, nearly parallel-sided, bottle-shaped apically. Membranous lateral projections of median lobe absent.

Description. ***Habitus.*** Body elongate oval, gradually narrowing posteriad; total length /total widht ratio = 1.6. Length: 1.4 mm; width: 0.84 mm.

Coloration. Coloration of dorsal side of head, pronotum and elytra dark brown. Ventral side brown.

Head. Clypeus with sparse punctation consisting of fine punctures, each puncture bearing fine decumbent seta; setae pale; interstices without microsculpture; anterior margin of clypeus slightly concave. Frons with moderately dense punctation, consisting of coarse, crescent-like punctures, punctures of same shape medially and laterally; interstices without microsculpture. Eyes moderately large. Mentum 1.8× wider than long, anterior margin slightly emarginate; anteromedian part not distinctly impressed; with sparse punctation consisting of small and isolated, nearly indistinct punctures bearing minute setae; interstices with very fine microsculpture, opaque. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.5× longer than palpomere 3. Antenna with 9 antennomeres; scapus shorter than antennomeres 2–6 combined.

Prothorax. Pronotum evenly convex forming continuous curve with elytra in lateral view. Lateral margin of pronotum angulate, with narrow marginal bead. Pronotal punctation uniform in size, moderately dense, slightly denser than that on frons, consisting of small, rasp-like punctures similar on whole surface of pronotum; all punctures bearing long setae; interstices without microsculpture. Median portion of prosternum not elevated and demarcated from lateral portions, median carina of prosternum narrow, projecting more anteriad mesally than anterior margin of median portion, with anterior portion elevated into small tooth in lateral view. Postero-mesal projection with deep notch. Antennal groves moderately large. Lateral margin of antennal grooves rounded.

Mesothorax. Scutellar shield bearing a few tiny punctures; interstices without microsculpture. Elytral punctures coarse, shallowly impressed, rounded to slightly transverse. Serial punctures small; transverse; sparsely arranged, with minute setae (indistinct under binocular microscope). Interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex, intervals 5, 7 and 9 as convex as adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate narrow, suboval, 2.8× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation slightly overlapping over anterior margin of metaventrite; median part flat; bearing sparsely arranged shallow setiferous punctures; interstices without microsculpture.

Metathorax. Metaventrite distinctly longer than preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture; punctation of median portion sparse, consisting of minute punctures, interstices without microsculpture, shiny. Anterolateral ridges bent posteriad along lateral margin of metaventrite, concave laterally, meeting together and proyecting posteriad mesally. Anterior margin of metaventrite not crenulate.

Abdomen. Ventrite 1 with additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Male genitalia. Aedeagus 0.48 mm long, parameres 0.8× longer than phallobase. Parameres continuously narrowing apicad, bearing two short setae apically. Phallobase wide, 2× longer than wide. Median lobe ca. as long as parameres, nearly parallel-sided, with wide apical projection. Membranous lateral projections of median lobe absent. Gonopore absent.

Etymology. The new species is dedicated to the Dominican radio station El Bacharenge, a source of the Caribbean music for the first author during his studies in the Czech Republic.

Distribution. *Oosternum bacharenge* sp. n. is a Hispaniolan endemic species currently only known from the type locality in the northeastern part of the Dominican Republic (Hato Mayor province) (Fig. 61).

Habitat. Based on the label data, the type specimens was collected in litter between tree buttresses in the rain forest.

Oosternum cercyonoides sp. n.

http://zoobank.org/4B4F624C-6044-4B16-A829-E7F699B70567
http://species-id.net/wiki/Oosternum_cercyonoides
Figs 5–6, 21, 31, 41, 50, 61

Type-locality. Jamaica, St. Thomas P. Portland Gap: 18°1’44.76”N, 76°30’22.40”W, 1676 m.

Type-specimens (5 spec.). Holotype female. Original label: “JAMAICA: St. Thomas P. Portland Gap, 17.xii.1972/01.i.1973, S & J. Peck, elevation 5500′, cloud for., dung & carrion tr. [printed] / Holotype, Oosternum cercyonoides sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]” (CNC). Paratypes: **JAMAICA**: St. Thomas P. Portland Gap: same data as holotype (4 spec., CNC, BSC-E, NMPC).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum angulate. Pronotal punctation uniform in size, moderately dense consisting of small rasp-like punctures. Pronotal interstices without microsculpture. Mesal part of prosternum not divided from lateral portions. Lateral margin of antennal grooves subangulate. Elytral interval 2 of the same width as interval 3, as high as intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 as convex as adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 1.7× longer than wide. Interstices without microsculpture, shiny. Anterolateral ridges of metaventrite not meeting together of median part of metaventrite mesally.

Description. ***Habitus.*** Body elongate oval, gradually narrowing posteriad; total length /total width ratio = 2.5. Length: 1.9–1.95 mm, length of holotype: 1.9 mm; width: 0.75–0.78 mm, width of holotype: 0.78 mm.

Coloration. Coloration of dorsal side brown to dark brown, elytra darker than pronotum, head dark brown. Ventral side brown. Femora and tibiae brown, tarsi and mouthparts yellow.

Head. Clypeus with sparse punctation consisting of small rounded punctures, each puncture bearing fine decumbent seta; setae pale; interstices without microsculpture; anterior margin of clypeus truncate. Frons with dense punctation consisting of coarse, impressed rounded punctures, punctures of same shape medially and laterally; interstices without microsculpture. Eyes moderately large. Mentum 1.8× wider than long, anterior margin slightly emarginate; anteromedian part not distinctly impressed; with sparse punctation, consisting of small, nearly indistinct punctures bearing minute setae; interstices with very fine microsculpture, opaque. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.2× longer than palpomere 3.

Prothorax. Pronotum evenly convex forming continuous curve with elytra in lateral view. Lateral margin of pronotum angulate, with narrow marginal bead. Pronotal punctation uniform in size, moderately dense, as dense as that on frons, consisting of small rasp-like punctures similar on whole surface of pronotum; all punctures bearing long setae; interstices without microsculpture. Median portion of prosternum not elevated and demarcated from lateral portions, median carina of prosternum narrow, not projecting more anteriad mesally than anterior margin of median portion, with anterior portion elevated into small tooth in lateral view. Postero-mesal projection with deep notch. Antennal grooves moderately large. Lateral margin of antennal grooves subangulate.

Mesothorax. Scutellar shield bearing a few small rasp-like punctures; interstices without microsculpture. Elytral series 1–5 and 8 arising basally, series 6–7 and 9 joint subbasally. Serial punctures small; transverse; sparsely arranged; with minute setae (indistinct under binocular microscope). Interval 2 of the same width as interval 3, as high as intervals 1 and 3, reaching elytral apex, intervals 5, 7 and 9 as convex as adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 1.7× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation slightly overlapping over anterior margin of metaventrite; median part flat; bearing sparsely arranged shallow setiferous punctures; interstices without microsculpture.

Metathorax. Metaventrite distinctly shorter than preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture; punctation of median portion consting of sparsely arranged but large rasp-like setiferious punctures, interstices without microsculpture, shiny. Anterolateral ridges bent posteriad along lateral margin of metaventrite, concave laterally, not meeting together mesally. Anterior margin of metaventrite not crenulate.

Abdomen. Ventrite 1 with additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Etymology. The species name is derived from the name of the megasternine genus *Cercyon* Leach, 1817, reflecting the *Cercyon*-like appearance of this new species.

Distribution. *Oosternum cercyonoides* sp. n. is a Jamaican endemic currently only known from the type locality in the Blue Mountains, i.e. the highest mountain massif in the eastern part of the island (Fig. 61).

Habitat. Based on the label data, the specimens were collected using baited pitfall traps in the montane cloud forest.

Oosternum insulare sp. n.

http://zoobank.org/725652DA-DD49-4A9C-867F-077D6788AB5F
http://species-id.net/wiki/Oosternum_insulare
Figs 7–8, 22, 35, 42, 55, 61

Type-locality. Jamaica: Cockpit Country, 18°20'51.7"N, 77°38'29.7"W, 250 m, trail to upper Windsor Cave.

Type-specimens (10 spec.). Holotype male, dry mounted, with genitalia mounted in Euparal on a microslide pinned below the specimen. Original label: "JAMAICA, Cockpit Country, trail to upper Windsor Cave, 11.xi.2013, ca. 250 m a.s.l., 18°20'51,7"N, 77°38'29,7"W, F. Cala-Riquelme leg. [printed] / Holotype, Oosternum insulare sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]' (NMPC). Paratypes: **JAMAICA:** Cockpit Country: same data as holotype (5 spec., NMPC, CMN, SEMC, BMNH). Trelawny, 5 mi. N. Alberttown, 30.xii.1972, S&J. Peck leg. Ber. 250 (3 spec., CNC). Portland: 1 mi W Ecclesdown, 18°5'33.08"N, 76°30'3.64"W, 10.viii.1974, S & J. Peck leg., 1500′ (1 spec., CNC).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum angulate. Pronotal punctation uniform in size, dense, consisting of large rounded punctures. Pronotal interstices without microsculpture. Mesal part of prosternum divided from lateral portions by oblique sharp ridges. Lateral margin of antennal grooves with acute projection. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 1.8× longer than wide. Median elevate portion of metaventrite nearly reaching lateral margins, interstices of median part of metaventrite with very fine microsculpture, opaque. Anterolateral ridges of metaventrite not meeting together mesally. Parameres 0.7× longer than phallobase, bearing a single seta apically. Median lobe ca. 1.5× longer than parameres, slight narrowing from base towards apex, with minute apical projection. Membranous lateral projections of median lobe absent.

Description. ***Habitus.*** Body elongate oval, gradually narrowing posteriad; total length /total width = 1.6. Length: 1.3–1.4 mm, length of holotype: 1.4 mm; width: 0.78–0.84 mm, width of holotype: 0.84 mm.

Coloration. Coloration of dorsal side reddish brown. Ventral side dark to reddish brown. Femora and tibiae dark brown, tarsi, mouthparts and antennomeres 1–6 yellowish.

Head. Clypeus with sparse punctation consisting of fine puncture, each puncture bearing fine decumbent seta; setae pale; interstices without microsculpture; anterior margin of clypeus slightly concave. Frons with dense punctation consisting of coarse rounded punctures, punctures of same shape medially and laterally; interstices without microsculpture. Eyes moderately large. Mentum 1.9× wider than long, anterior margin slightly emarginate; anteromedian part deeply impressed in contrast to lateral portions; with sparse punctation, punctation consisting of small, nearly indistinct punctures bearing minute setae; interstices with very fine microsculpture, opaque. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.2× longer than palpomere 3. Antenna with 9 antennomeres; scapus shorter than antennomeres 2–6 combined.

Prothorax. Pronotum evenly convex, slightly more convex than elytra in lateral view; lateral margin angulate, with narrow marginal bead. Pronotal punctation consisting of two types of punctures, large rounded without seta and smaller transverse with long seta; interstices without microsculpture. Transverse row of punctures on posterior margin of pronotum hardly defined. Median portion of prosternum elevated and demarcated from lateral portion. Median carina of prosternum narrow, projecting more anteriad mesally than anterior margin of median portion, straight in lateral view. Median portion of prosternum 1.2× wider than long, postero-mesal projection with shallow notch. Pair of deep pits next to ridge delimiting median portion of prosternum present. Antennal grooves moderately large. Lateral margin of antennal grooves with acute projection.

Mesothorax. Scutellar shield bearing a few small, round punctures; interstices without microsculpture. Elytral series 1–6 arising basally, series 7–9 subbasally. Serial punctures small; transverse; sparsely arranged, with minute setae (indistinct under binocular microscope). Interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex, intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 1.8× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation slightly overlapping over anterior margin of metaventrite; median part flat, bearing densely arranged, large, shallow setiferous punctures; interstices with very fine microsculpture, opaque.

Metathorax. Metaventrite ca. as long as preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture reaching nearly to lateral margin; punctation of median portion sparse consisting of small, rounded punctures, interstices without microsculpture, shiny. Anterolateral ridges bent posteriad along lateral margin of metaventrite, angulate laterally, not meeting together mesally. Anterior margin of metaventrite crenulate.

Abdomen. Ventrite 1 with additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Male genitalia. Aedeagus 0.57 mm long, parameres 0.7× longer than phallobase. Parameres continuously narrowing apicad, bearing a single seta apically. Phallobase narrow, 3× longer than wide. Median lobe ca. 1.5× longer than parameres, slight narrowing from base towards apex, with minute apical projection. Membranous lateral projections of median lobe absent. Gonopore absent.

Etymology. The species name is the manuscript name used in an unpublished revision of the genus *Oosternum* by M. Hansen – it was the only West Indian endemic in the manuscript, hence the highlighting of the fact that it is the island endemic.

Distribution. *Oosternum insulare* sp. n. is the Jamaican endemic currently known from three localities throughout the island, all situated in the altitudes around 500 m a.s.l. (Fig. 61).

Habitat. Specimens of *O. insulare* were collected from leaf litter of the well-preserved semi-deciduous forest in the karst area.

Oosternum latum Fikáček, Hebauer & Hansen, 2009

http://species-id.net/wiki/Oosternum_latum
Figs 9–10, 23, 36, 43, 56, 61

Oosternum latum Fikáček, Hebauer & Hansen, 2009: 34.

New material examined (21 spec.). **SAINT VINCENT AND GRENADINAS:** Hermitage Forest, E. of Spring Village, ca. 360 m a.s.l., 13°14'53.04"N, 61°12'47.49"W, 16–27.viii.2006, S & J. Peck leg., forest FIT (06-105) (10 spec., SBPC, NMPC), 15-27.viii.2006, S & J. Peck leg., clearing FIT trap, (06-101B) (1 spec., SBPC), 16–27.viii.2006, S & J. Peck leg., forest edge Malaise, (06-104) (10 spec., SBPC, NMPC).

Diagnosis. Body widest on base of elytra. Lateral margin of pronotum angulate. Pronotal punctation uniform in size, sparse, consisting of small, rasp-like, weakly impressed punctures. Pronotal interstices with microsculpture. Mesal part of prosternum divided from lateral portions by oblique sharp ridges. Lateral margin of antennal grooves with acute projection. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices opaque, with very fine microsculpture. Preepisternal plate wide, suboval, 2× longer than wide. Interstices of median part of metaventrite without microsculpture, with subpentagonal slightly elevate median portion. Anterolateral ridges of metaventrite not meeting together mesally. Parameres 1.2× longer than phallobase, bearing two short setae apically. Median lobe longer than parameres, projecting slightly farther than parameres, nearly parallel-sided basally, narrowing apicad in apical 0.2. Membranous lateral projections of median lobe present.

Description. For complete description see Fikáček et al. (2009).

Distribution. *Oosternum latum* is the endemic of the Saint Vincent Island (Fig. 61). The specimens examined for this study represent the first precisely localized material available for the species, as only the island name is mentioned in the locality label of the type specimens (Fikáček et al. 2009).

Habitat. Specimens of *O. latum* examined here were collected in a lower montane rain forest with mixed forest plant compositions. A portion of the specimens were collected using flight intercept traps, and others using a Malaise trap.

Oosternum luciae sp. n.

http://zoobank.org/DEF8AEF2-93DB-449D-BFB6-7D0157A566BB
http://species-id.net/wiki/Oosternum_luciae
Figs 11–12, 24, 37, 44, 57, 61

Type-locality. Saint Lucia, Barre de L´Isle: 13.9368°N, 60.9593°W, 340 m.

Type-specimens (1 spec.). Holotype male, dry mounted, with genitalia mounted in Euparal on a microslide pinned below the specimen. Original label: "ST LUCIA: Barre de L´Isle, 25/28.v.2009, E. A. Ivie, 13.9368°N, 60.9593°W, elevation 340 m, [printed] / Holotype, Oosternum luciae sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]" (WIFP).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum weakly sinuate. Pronotal punctation uniform in size, moderately dense consisting of small, transverse punctures. Pronotal interstices with microsculpture. Mesal part of prosternum divided from lateral portions by oblique sharp ridges. Lateral margin of antennal grooves with acute projection. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate narrow, drop-like, 2.6× longer than wide. Interstices of median portion of metaventrite with strong mesh-like microsculpture on the whole surface. Anterolateral ridges of metaventrite not meeting together mesally. Parameres 1.2× longer than phallobase, bearing two short setae apically. Median lobe slightly longer than parameres, nearly parallel-sided basally, narrowing apicad in apical 0.4. Membranous lateral projections of median lobe present, with series of long setae on each side.

Description. ***Habitus.*** Body widely oval, strongly narrowing posteriad; total leght /total width ratio = 1.3. Length: 1.41 mm; width: 0.87 mm.

Coloration. Coloration of dorsal side of head, pronotum and elytra dark brown. Ventral side brown.

Head. Clypeus with sparse punctation consisting of fine, each puncture bearing fine decumbent seta; setae pale; interstices with fine microsculpture; anterior margin of clypeus slightly concave. Frons with moderately dense punctation consisting of small, shallowly impressed rounded to slightly transverse punctures, punctures of same shape medially and laterally; interstices with fine mesh-like microsculpture. Eyes moderately large. Mentum 1.9× wider than long, anterior margin slightly emarginate; anteromedian part deeply impressed in contrast to lateral portions; with sparse punctation, punctation consisting of small, nearly indistinct punctures bearing minute setae; interstices with fine microsculpture consisting of transverse ridges. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.2× longer than palpomere 3. Antenna with 9 antennomeres; scapus shorter than antennomeres 2–6 combined. Evenly convex.

Prothorax. Pronotum forming continuous curve with elytra in lateral view; lateral margin weakly sinuate, with narrow marginal bead. Pronotal punctation uniform in size, moderately dense, as dense as that on frons consisting of small, transverse punctures similar on whole surface of pronotum; punctures with minute setae intermixed among those bearing long setae; interstices with microsculpture. Transverse row of punctures on posterior margin of pronotum absent. Median portion of prosterum elevated and demarcated from lateral portins; median carina of prosternum narrow, projecting more anteriad mesally than anterior margin of median portion, with anterior portion elevated into small tooth in lateral view. Median portion of prosternum 1.8× wider than long, postero-mesal projection with shallow notch. Pair of deep pits next to ridge delimiting median portion of prosternum present. Antennal grooves moderately large. Lateral margin of antennal grooves with acute projection.

Mesothorax. Scutellar shield bearing few minute rounded punctures; interstices without microsculpture. Elytral punctures coarse, shallowly impressed rounded to slightly transverse. Serial punctures small; transverse; sparsely arranged, with minute setae (indistinct under binocular microscope). Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate narrow, drop-like, 2.6× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation slightly overlapping over anterior margin of metaventrite, median part flat, bearing densely arranged, large, shallow setiferous punctures; interstices with very fine microsculpture, opaque.

Metathorax. Metaventrite ca. as long as preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture; punctation of median portion moderately dense, consisting of large, sharply impressed round setiferous punctures, interstices with strong mesh-like microsculpture on the whole surface. Anterolateral ridges bent posteriad along lateral margin of metaventrite, concave, laterally not meeting together mesally. Anterior margin of metaventrite not crenulate.

Abdomen. Ventrite 1 with additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Male genitalia. Aedeagus 0.44 mm long, parameres 1.2× longer than phallobase. Parameres continuously narrowing apicad, bearing two short setae apically. Phallobase narrow, 1.9× longer than wide. Median lobe slightly longer than parameres, nearly parallel-sided basally, narrowing apicad in apical 0.4. Membranous lateral projections of median lobe present. Gonopore present.

Etymology. The species name is derived from the woman's name Lucia, referring to the presence of this species in the Saint Lucia island.

Distribution. *Oosternum luciae* sp. n. is an endemic of Saint Lucia island and currently known only from the type locality (Fig. 61).

Habitat. Based on the label data, the type specimen was collected using an UV light trap.

Oosternum megnai sp. n.

http://zoobank.org/E11CD275-C61D-42D2-BBDD-D8258ED20B73
http://species-id.net/wiki/Oosternum_megnai
Figs 13–14, 25, 32, 45, 51, 58, 61

Type-locality. Cuba, Granma Province: PN Turquino, 20°0.9'N, 76°53.3'W, slope below Pico Naranjo ca. 0.4 km N of La Platica (by air), 950 m.

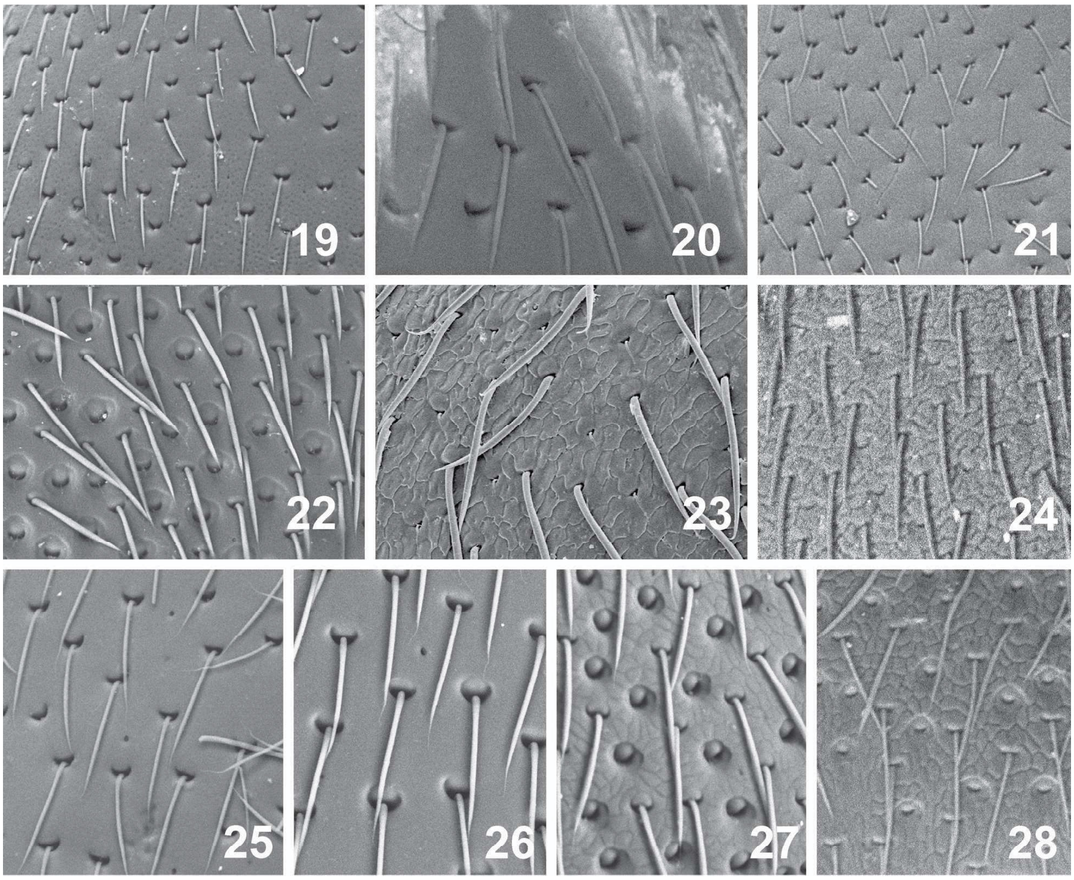
Type-specimens (13 spec.)**.** Holotype male, dry mounted, with genitalia mounted in Euparal on a microslide attach to the specimens. Original label: "CUBA: Granma: PN Turquino, slope below Pico Naranjo ca. 04 km N of La Platica (by air), 24.vi.2012, Deler-Hernández & Fikáček leg. 20°0.9'N, 76°53.3'W, elevation 950 m, MF23 [printed] / Holotype, Oosternum megnai sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]" (NMPC). Paratypes **CUBA:** Granma province: same data as holotype (12 spec., NMPC, CMN, SEMC, BSC-E, BMNH).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum angulate. Punctation uniform in size, moderately dense, consisting of small, rasp-like punctures. Pronotal interstices without microsculpture. Mesal part of prosternum not divided from lateral portions. Lateral margin of antennal grooves rounded. Elytral interval 2 narrower than interval 3, as high as intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 as convex as adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate narrow, drop-like, 2.3× longer than wide. Interstices of median part of metaventrite without microsculpture. Anterolateral ridges of metaventrite not meeting together mesally. Parameres 0.8× longer than phallobase, bearing two long setae apically. Median lobe longer than parameres, oval in shape, with small apical projection. Membranous lateral projections of median lobe absent.

Description. ***Habitus.*** Body elongate oval, gradually narrowing posteriad; total length/total width ratio = 1.9. Length: 1.47–1.49 mm, length of holotype: 1.32 mm; width: 0.75–0.82 mm, width of holotype: 0.8 mm.

Coloration. Coloration of dorsal side brown to dark brown, head, pronotum and elytra dark brown. Ventral side light brown. Femora, tibiae, tarsi and antennomeres and light brown, antennal club dark brown.

Head. Clypeus with moderately dense punctation consisting of fine punctures, each puncture bearing

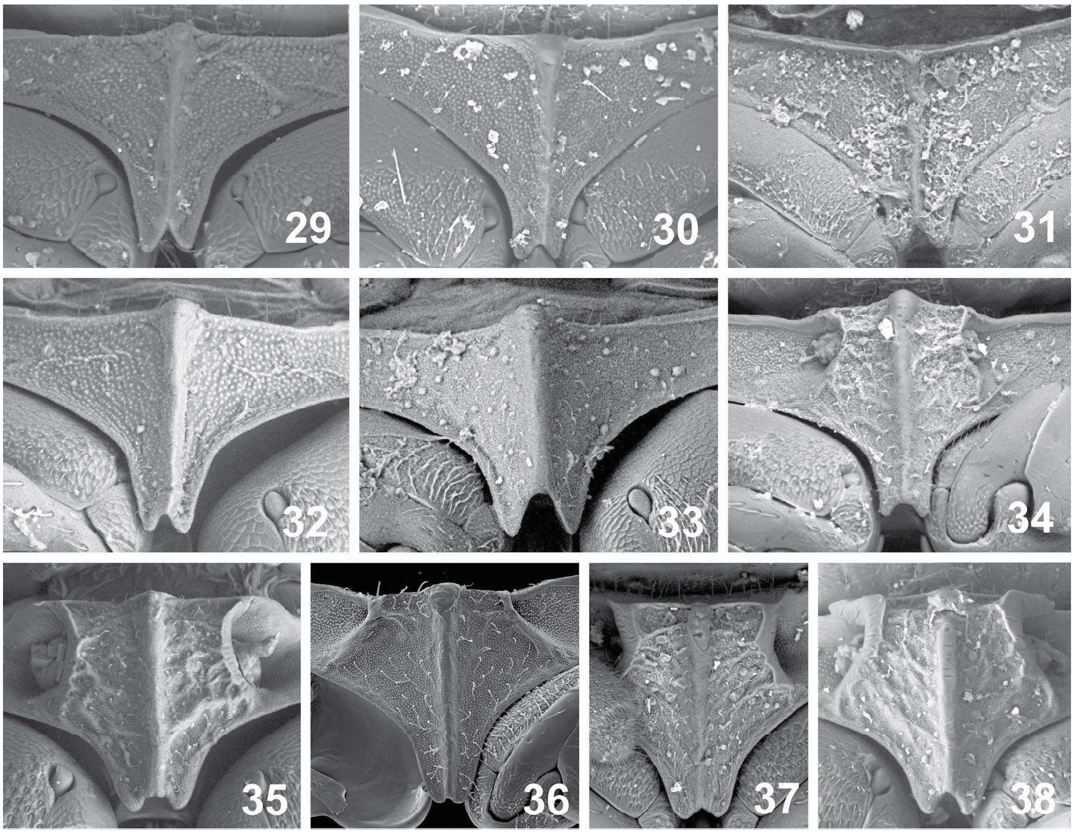


Figures 19–28. Pronotal punctation and microsculpture. **19.** *Oosternum andersoni* **20.** *O. bacharenge* **21.** *O. cercyonoides* **22.** *O. insulare* **23.** *O. latum* **24.** *O. luciae* **25.** *O. megnai* **26.** *O. pecki* **27.** *O. sharpi* **28.** *Oosternum sp.* (Bahamas).

fine decumbent seta; setae pale; interstices without microsculpture; anterior margin of clypeus truncate. Frons with dense punctation consisting of small, punctures of same shape medially and laterally; interstices without microsculpture. Eyes moderately large. Mentum 1.8× wider than long, anterior margin deeply emarginate; anteromedian part not distinctly impressed; with sparse punctation, punctation consisting of minute, nearly indistinct punctures bearing minute setae; interstices with fine microsculpture consisting of transverse ridges. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.2× longer than palpomere 3. Antenna with 9 antennomeres; scapus shorter than antennomeres 2–6 combined.

Prothorax. Pronotum evenly convex forming continuous curve with elytra in lateral view. Lateral margin of pronotum angulate; with narrow marginal bead. Pronotal punctation uniform in size, moderately dense, as dense as that on frons; consisting of small rasp-like punctures, slightly sparser laterally than medially; all punctures bearing long setae; interstices without microsculpture. Median portion of prosternum not elevated and demarcated from lateral portions, median carina of prosternum narrow, projecting more anteriad mesally than anterior margin of median portion, with anterior portion elevated into small tooth in lateral view. Postero-mesal projection with shallow notch. Antennal grooves moderately large. Lateral margin of antennal grooves rounded.

Mesothorax. Scutellar shield bearing a few small, rasp-like punctures; interstices without microsculpture. Elytral series 1–5 and 8 arising basally, series 6–7 and 9 joint subbasally. Serial punctures small; transverse; sparsely arranged, with minute setae (indistinct under binocular microscope). Elytral interval 2 narrower than interval 3, as high as intervals 1 and 3, reaching elytral apex, intervals 5, 7 and 9 as convex as adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate narrow, drop-like, 2.3× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation slightly overlapping over anterior margin of metaventrite; median part flat; bearing densely



Figures 29–38. Median portion of prosternum. **29.** *Oosternum andersoni* **30.** *O. bacharenge* **31.** *O. cercyonoides* **32.** *O. megnai* **33.** *O. pecki* **34.** *Oosternum sp.* (Bahamas) **35.** *O. insulare* **36.** *O. latum* **37.** *O. luciae* **38.** *O. sharpi*.

arranged, large, shallow setiferous punctures; interstices without microsculpture.

Metathorax. Metaventrite distinctly longer than preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture; punctation of median portion sparse consisting of small rounded punctures, interstices without microsculpture, shiny. Anterolateral ridges bent posteriad along lateral margin of metaventrite, concave laterally, not meeting together mesally. Anterior margin of metaventrite not crenulate.

Abdomen. Ventrite 1 with additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Male genitalia. Aedeagus 0.52 mm long, parameres 0.8× longer than phallobase. Parameres continuously narrowing apicad, bearing two long setae apically. Phallobase narrow, 2.1× longer than wide. Median lobe longer than parameres, oval in shape, with small apical projection. Membranous lateral projections of median lobe absent. Gonopore absent.

Etymology. The new species is dedicated to our excellent colleague and friend Yoandri S. Megna (Universidad de Oriente, Santiago de Cuba, Cuba).

Distribution. *Oosternum megnai* sp. n. is the Cuban endemic species currently known only from the type locality in the southeastern part of the island (Granma province) (Fig. 61). The locality is situated in the Sierra Maestra mountain range which is considered one of the main centers of diversity in Cuba (CENAP 2004).

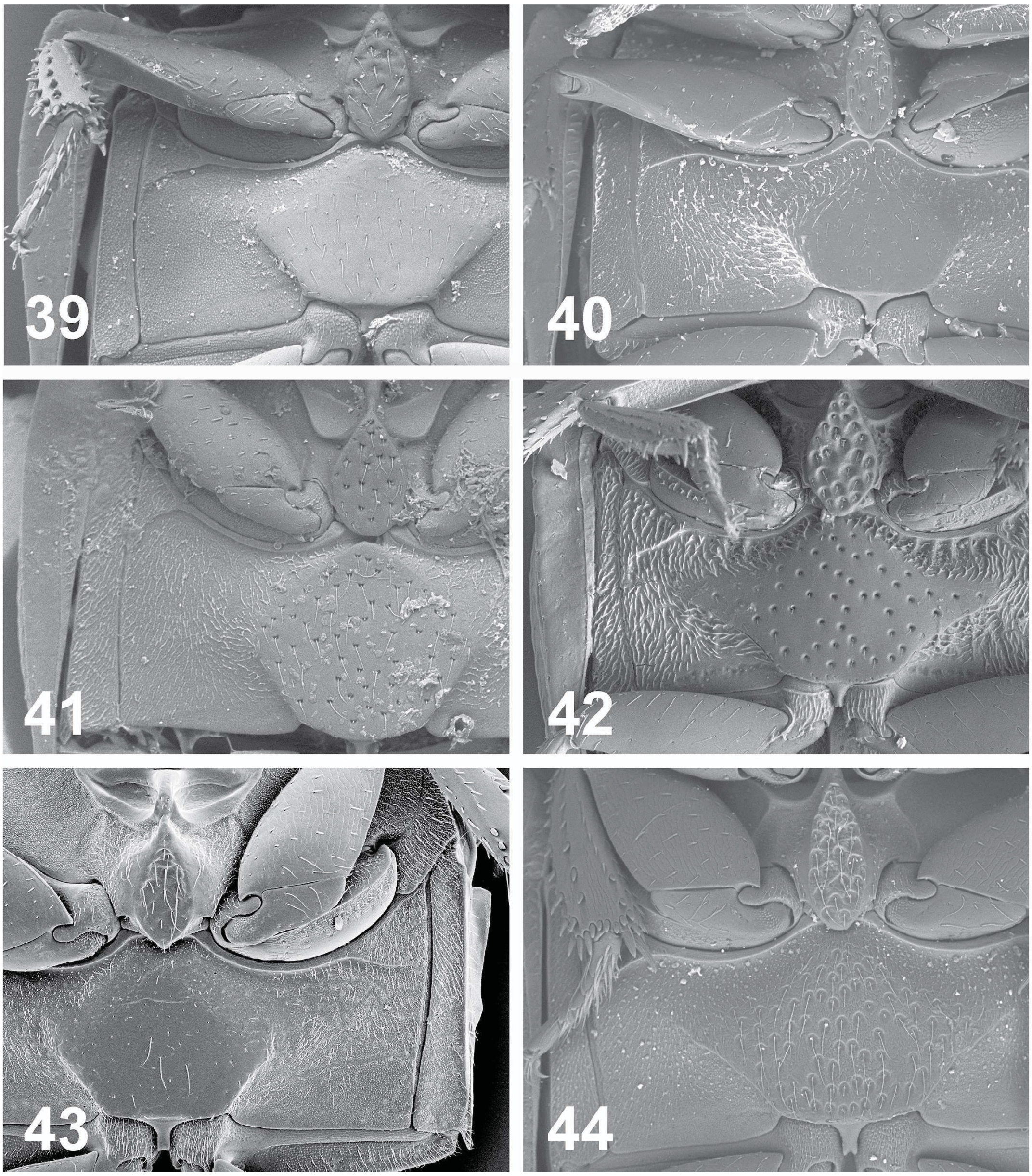
Habitat. Specimens of *O. megnai* were collected in dry leaf litter in the secondary forest.

Oosternum pecki sp. n.

http://zoobank.org/425E2398-6C4F-4966-A966-E7B1DD0C8207
http://species-id.net/wiki/Oosternum_pecki
Figs 15–16, 26, 33, 46, 49, 52, 59, 61

Type-locality. Dominican Republic, Barahona: 17°59'06.85"N, 71°12'57.29"W, 7 Km NW Paraiso, 200 m.

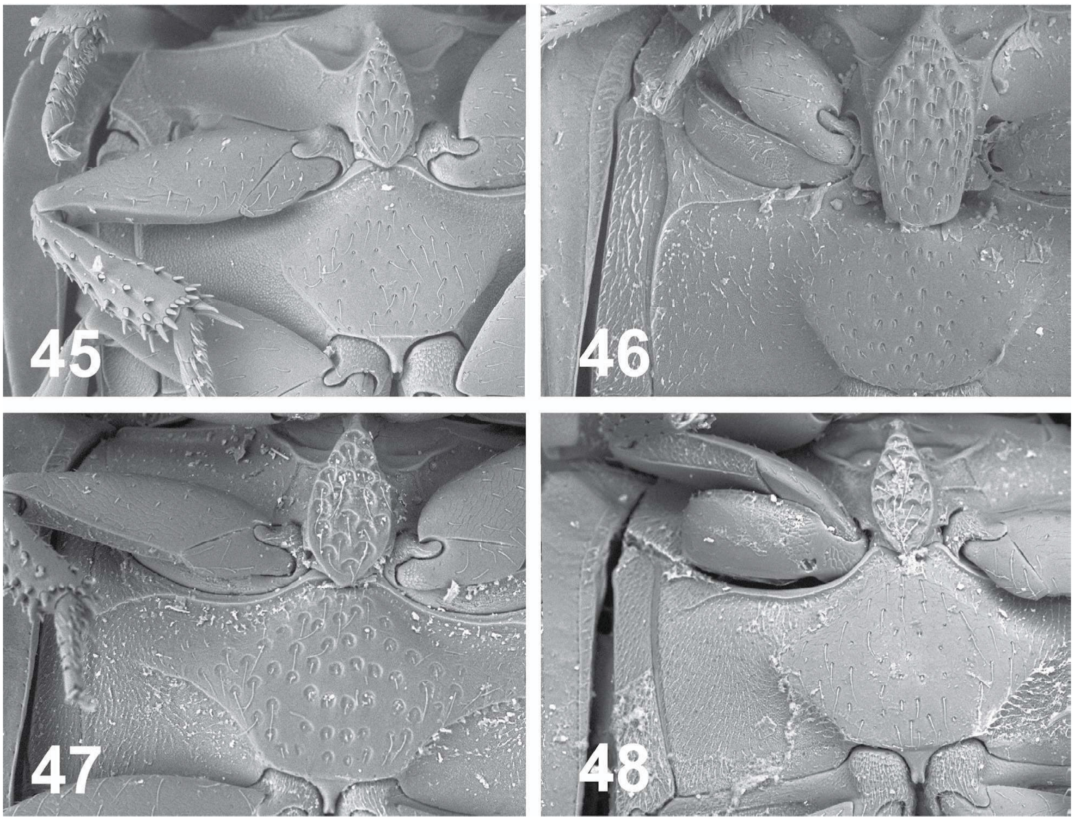
Type-specimens (19 spec.). Holotype male, dry mounted, with genitalia mounted in Euparal on a microslide pinned below the specimen. Original label: "DOM REP: Barahona: 7 Km NW Paraiso, 27.xi/04.xii.1991, Masner & Peck, elevation 200 m, rainforest remnant, intercept tp. [printed] / Holotype, Oosternum pecki sp. n., Deler-Hernández & Fikáček det. 2013 [red, printed]" (CNC). Paratypes: **DO-**



Figures 39–44. Meso- and metaventrite plate. **39.** *Oosternum andersoni* **40.** *O. bacharenge* **41.** *O. cercyonoides* **42.** *O. insulare* **43.** *O. latum* **44.** *O. luciae*.

MINICAN REPUBLIC: Barahona: same data as holotype (18 spec., CNC, NMPC, BSC-E, CMN, SEMC).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum angulate. Pronotal punctation double-sized, dense consisting of moderately large round and rasp-like punctures. Pronotal interstices without microsculpture. Mesal part of prosternum not divided from lateral portions. Lateral margin of antennal grooves rounded. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, subrhomboid, 2.1× longer than wide, largely overlapping over metaventrite. Interstices of median part of metaventrite without microsculpture. Anterolateral ridges of metaventrite not meeting together mesally. Parameres 1.1× longer than phallobase, bearing two long setae apically. Median lobe ca. as long as parameres, nearly parallel-sided basally,



Figures 45–48. Meso- and metaventrite plate. **45.** *O. megnai* **46.** *O. pecki* **47.** *O. sharpi* **48.** *Oosternum sp*.

narrowing apicad in apical 0.2, apex rounded. Membranous lateral projections of median lobe absent.

Description. ***Habitus.*** Body elongate oval, gradually narrowing posteriad; total lenght/total width ratio = 1.6. Length: 1.45–1.5 mm, length of holotype: 1.41 mm; width: 0.89–0.97 mm, width of holotype: 0.89 mm.

Coloration. Coloration of dorsal side reddish brown. Ventral side brown to reddish brown. Femora, tibiae and antennal club pale reddish brown, tarsi and antennomeres 1–6 yellowish.

Head. Clypeus with sparse punctation consisting of fine punctures, each puncture bearing fine decumbent seta; setae pale; interstices without microsculpture; anterior margin of clypeus truncate. Frons with dense punctation consisting of small punctures of same shape medially and laterally; interstices without microsculpture. Eyes moderately large. Mentum 1.9× wider than long, anterior margin slightly emarginate; anteromedian part not distinctly impressed; with sparse punctation, punctation consisting of minute, nearly indistinct punctures bearing minute setae; interstices with fine microsculpture consisting of transverse ridges. Submentum without poriferous disc-like fields. Maxillary palpus with palpomeres 2 and 4 ca. 1.2× longer than palpomere 3. Antenna with 9 antennomeres; scapus shorter than antennomeres 2–6 combined.

Prothorax. Pronotum evenly convex, forming continuous curve with elytra in lateral view. Lateral margin of pronotum angulate, with narrow marginal bead. Pronotal punctation double-sized, dense, slightly denser than on frons, consisting of transverse punctures anteriorly and large round punctures posteriorly; all punctures bearing long setae; interstices without microsculpture. Median portion of prosternum not elevated and demarcated from lateral portions, median carina of prosternum narrow projecting more anteriad mesally than anterior margin of median portion, with anterior portion elevated into small tooth in lateral view. Postero-mesal projection with deep notch. Antenal grooves moderately large. Lateral margin of antennal grooves rounded.

Mesothorax. Scutellar shield bearing a few small rasp-like punctures; interstices without microsculpture. Elytral series 1–5 and 8 arising basally, series 6–7 and 9 joint subbasally. Serial punctures small; transverse; sparsely arranged, with minute setae (indistinct under binocular microscope). Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex, intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, subrhomboid, 2.1× longer than wide, widely attached to metaventrite; posterior part of preepisternal elevation much overlapping over anterior margin of metaventrite; median part flat; bearing densely arranged, large, shallow setiferous punctures; interstices without microsculpture.

Metathorax. Metaventrite distinctly shorter than preepisternal elevation of mesothorax, median portion markedly differing from lateral portion in punctation and microsculpture; punctation of median portion sparse, consisting of small rounded punctures, interstices without microsculpture, shiny. Anterolateral ridges bent posteriad along lateral margin of metaventrite, concave laterally, not meeting together mesally. Anterior margin of metaventrite not crenulate. Lateral margin with additional slightly concave ridge.

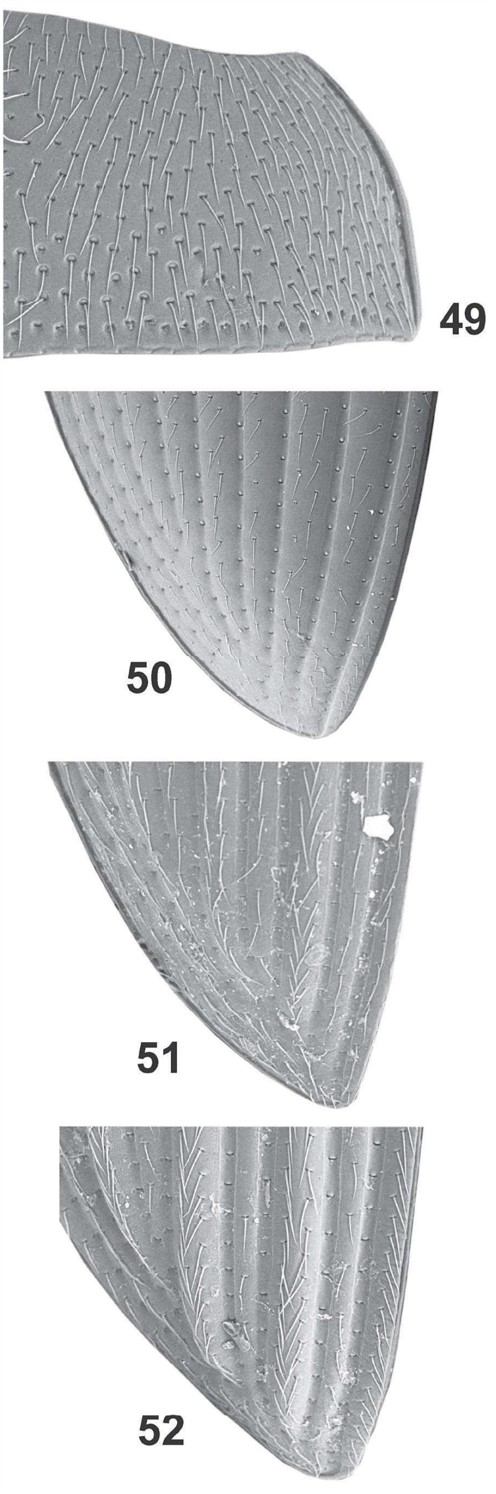
Abdomen. Ventrite 1 without additional longitudinal ridges laterally. Ventrites 2–5 without longitudinal ridges; posterior margin of all ventrites lacking denticles.

Male genitalia. Aedeagus 0.57 mm long, parameres 1.1× longer than phallobase. Parameres continuously narrowing apicad, bearing two long setae apically. Phallobase wide, 1.6× longer than wide. Median lobe ca. as long as parameres, nearly parallel-sided basally, narrowing apicad in apical 0.2, apex rounded. Membranous lateral projections of median lobe absent. Gonopore present.

Etymology. The new species is dedicated to Professor Stuart Peck (Carleton University, Canada) whose collecting trips accumulated a huge material of the West Indian Hydrophilidae, including many *Oosternum* specimens used for this study.

Distribution. *Oosternum pecki* sp. n. is a Hispaniolan endemic species currently known only from the type locality situated in the southern part of the Dominican Republic (Barahona province) (Fig. 61).

Habitat. Based on the label data, the type specimens were collected in a remnant of the rain forest using a flight intercept trap.



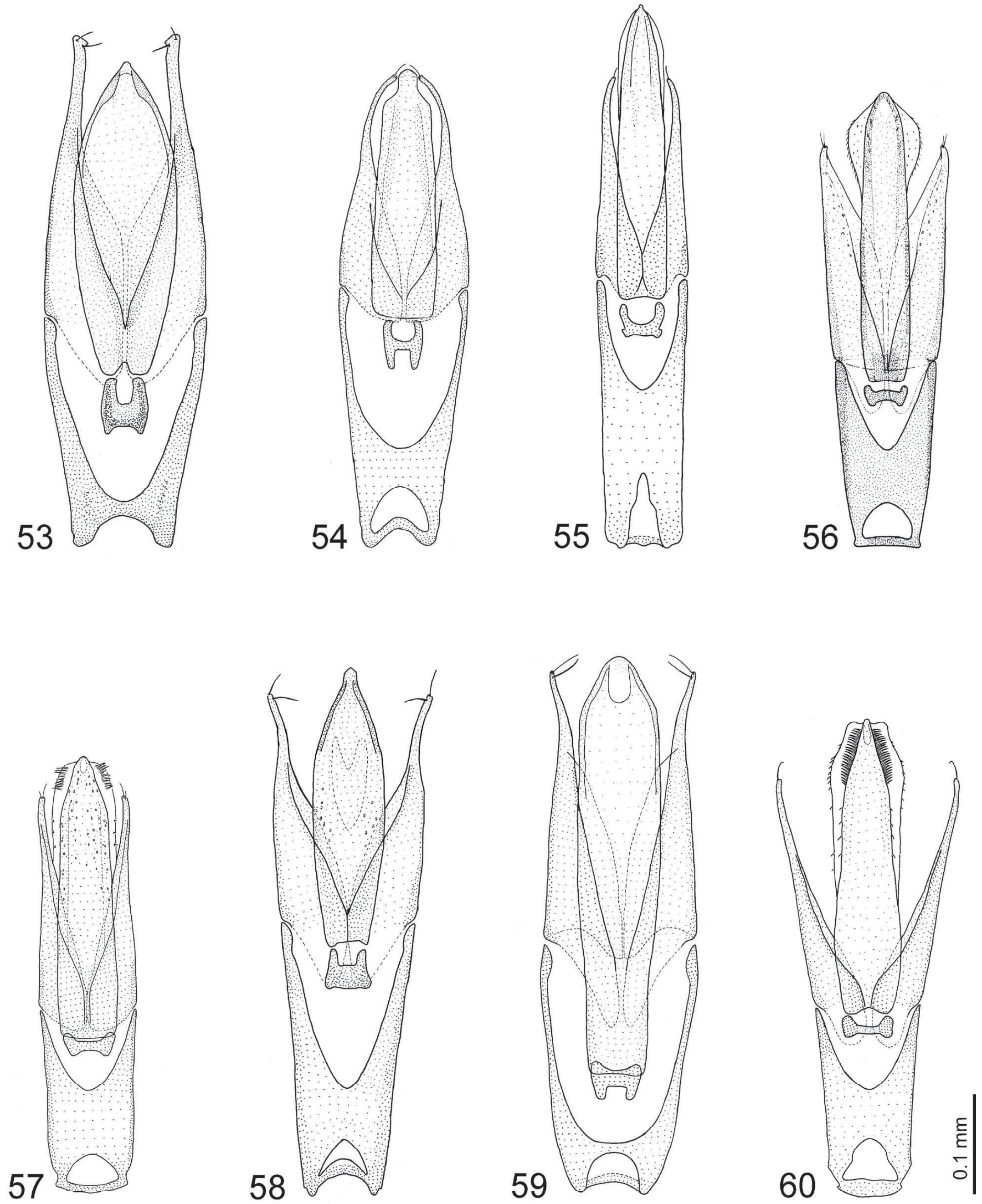
Figures 49–52. Additional diagnostic characters of West Indian *Oosterum*. (**49**) Pronotal punctation of *O. pecki*. (**50–52**) Apical portion of elytra **50.** *O. cercyonoides* **51.** *O. megnai* **49, 52.** *O. pecki*.

Oosternum sharpi Hansen, 1999

http://species-id.net/wiki/Oosternum_sharpi
Figs 17–18, 27, 38, 47, 60, 61

Oosternum costatum Sharp, 1882: 113 (secondary homonym of *Megasternum costatum* LeConte, 1855).
Oosternum sharpi Hansen, 1999: 242 (replacement name).

Material examined (65 spec.). **CUBA:** Cienfuegos province: Cumanayagua municipality, Jardín Botánico de Cienfuegos, Soledad, ca. 73 m, 22°7'18.44"N, 80°19'35.26"W, 21.v.2013, A. Deler-Hernández leg., horse excrements (3 spec., NMPC); 01.v.1950, Acc. No 49672 (4 spec., WIFP); Mina Carlota, ca. 470 m, 22.0667°N, 80.1667°W, 06.v.1950, Acc. No 49681 (1 spec., WIFP). Camagüey province: Sierra de Cubitas municipality, Hoyo de Bonet, Res. Ecol. "Limones-Tuabaquey", ca. 90 m a.s.l., 21°36'32.51"N, 77°47'5.30"W, 14.iv.2012, F. Cala-Riquelme & N. Duperré leg., (CU-20) (1 spec., NMPC). Granma



Figures 53–60. Aedeagus. **53.** *Oosternum andersoni* **54.** *O. bacharenge* **55.** *O. insulare* **56.** *O. latum* **57.** *O. luciae* **58.** *O. megnai* **59.** *O. pecki* **60.** *O. sharpi*.

province: PN Turquino track to Pico Turquino, ca. 900 m a.s.l., 20°0.5'N, 76°53.6'W, 29.iii.2012, F. Cala-Riquelme & N. Duperré leg., (CU03) (15 spec., NMPC). Santiago de Cuba province: San Luis municipality, El Vivero 1.6 Km E of Dos Caminos, ca. 150 m a.s.l., 20°10.8'N, 76°46.4'W, 20–21.vi.2012, A. Deler-Hernández & M. Fikáček leg., (MF18) (3 spec., NMPC); Km 7 road to Gran Piedra, ca. 550 m a.s.l., 20.011°N, 75.673°W, 26.i.2012, R. Anderson leg., (2012–007) (1 spec., NMPC). Guantánamo province: El Yunque, 0.5–1.0 Km W of campismo popular, ca.

40–50 m a.s.l., 20°20.1N 74°33.6W, 10.vi.2012, A. Deler-Hernández & M. Fikáček leg., (MF01) (1 spec., NMPC). **HAITI:** Port-au-Prince: 18°31’1.33”N, 72°20’7.42”W, 22.v.1950, Acc # 49650, H. B. Mills colr. (4 spec., WIFP). **DOMINICAN REPUBLIC:** Barahona: 7 Km NW Paraiso, ca. 200 m a.s.l., 17°59’06.85”N, 71°12’57.29”W, 27.xi/04.xii.1991, Masner & Peck leg., (91–341) (2 spec., SBPC); 1–04.xii.2012, Masner & Peck leg (91–358) (1 spec., SBPC). **JAMAICA:** Try Good Hope: ca. 145 m a.s.l., 18°25’N, 77°41’W, 17.viii.1966, H. F. Howden leg., (1 spec., SBPC); Trelawny Par. Nr. Windsor Caves, 18°20’59.21”N, 77°38’56.11”W, 24.iii.1991, T. K. Phillip and L. Gerofsky leg., berlese stream-side leaves (1 spec., WIFP). **PUERTO RICO:** 3.5 Km S Cabo Rojo, Cueva Tuna, ca. 25 m, 18°02’N, 67°09’W, 14.vi.1974, S. Peck leg., (1 spec., SBPC); Aguas Buenas, near Aguas Buenas Cave, ca. 250 m a.s.l., 18°15’N, 66°07’W, 07–17.v.1973, S. Peck leg., (1 male, SBPC); 2 km S Mamayes (Palmer), ca. 70 m, 18°21’13.63”N, 65°46’36.05”W, 25.IX.1987, M. Ivie, ex. old lawn debris (3 spec., WIFP); Isla Verde, vii/viii.1957, I. Fox lgt, light trap (1 spec., WIFP); El Verde, ca 250 m, 18°19’27.35”N, 65°49’26.90”W, M. A. Ivie lgt, at light (1 spec., WIFP). **BARBADOS:** Colescave: 1 Km SSE Welchman Hall, ca. 200 m a.s.l., 13°11’N, 59°34’W, 22–26.ii.1979, S. Peck leg., (3 spec., SBPC); Welchman Hall Gully, ca. 270 m a.s.l., 13°11.74’N, 59°34.60’W, 26.v.2006, S & P. Peck leg (06–60) (1 spec., SBPC). **TRINIDAD & TOBAGO:** Curupe: ca. 13 m a.s.l., 10°38’21.96”N, 61°24’35.91”W 05.xii.1977, W.R.M. Mason leg (1 spec., SBPC). **SAINT LUCIA:** Vieux Fort, ca. 3 m a.s.l., 13°43’18.68”N, 60°56’58.86”W, 12.vii.2007, S & P. Peck leg (07–60) (1 spec., SBPC). **SABA:** Neth Antil, ca. 384 m a.s.l., 17°37’43.45”N, 63°13’47.93”W, 20.V.2008, D. S. Sikes & M. A. Ivie leg, berlese litter/lawn clipping, (1 spec., WIFP). **DOMINICA:** Springfield Estate, ca. 330–360 m a.s.l., 15°20.796’N, 61°22.142’W, 30.v/16.vi.2004, S & P. Peck leg (04–86) (1 spec., SBPC). **SAINT VINCENT AND GRENADINAS:** Esmerald Valley Hotel, E. of Layou, ca. 20 m a.s.l., 13°12’N, 61°14.8’W, 27–29.viii.2006, S & J. Peck leg., forest edge UV, (06–123) (6 spec., SBPC); Buccament. Esmerald Valley Hotel, ca. 20 m a.s.l., 13°12’36.18”N, 61°13’47.74”W, 10–20.vi.2007, S & P. Peck leg (07–12) (1 spec., SBPC), Bequia island, Hamilton, ca. 25 m a.s.l., 13°00’30”N, 61°14’W, 01.viii.2008, S. Peck & M. de Silva leg (08–75) (1 spec., SBPC). **GUADELOUPE:** BT Malendure, Petite Trace, Fond Ravine, ca. 3 m a.s.l., 16°10.44’N, 61°46.7’W, 21.v.2012, S. Peck leg., forest edge UV, (12–31) (2 spec., SBPC). **VIRGIN ISLAND:** St. John, Dennis Bay, ca. 5 m, 18°21’7.08”N, 64°46’29.86”W, 16.vi.1980, (1 spec., WIFP); Maho Bay, ca 30 m, 18°21’41.92”N, 64°44’32.81”W, 12.iii.1984, W. B. colr, under trees near road, (1 spec., WIFP); St. Thomas, ca. 50 m, 18°21’20.93”N, 64°55’31.20”W, 05.viii.1984, (1 spec., WIFP).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum angulate. Pronotal punctation double-sized, dense consisting of moderately large, round punctures intermixed with smaller transverse punctures. Pronotal interstices with microsculpture. Mesal part of prosternum divided from lateral portions by oblique sharp ridges. Lateral margin of antennal grooves with acute projection. Elytral interval 2 narrower than interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 2× longer than wide. Interstices of median part of metaventrite with very fine microsculpture, opaque. Anterolateral ridges not meeting together mesally. Parameres 1.2× longer than phallobase, bearing a single seta apically. Median lobe longer than parameres, narrowing towards apex. Membranous lateral projections of median lobe large, bearing long setae on each side.

Distribution. *Oosternum sharpi* is a common species distributed throughout the West Indies. We are recording it from all islands of the Greater Antilles (Cuba, Jamaica, Hispaniola and Puerto Rico) as well as from 9 islands of the Lesser Antilles. The species is otherwise widely distributed in southern USA, Central America and northern part of the South America, and was also introduced to the Azores (Orchymont 1940, Svensson 1973), Hawaiian islands (Hansen 1995), Ghana (Smetana 1978) and Sri Lanka (Hansen 1995). All specimens but one examined by us from the West Indian islands were females. The only male known from the West Indies is from Puerto Rico.

Habitat. Specimens of *O. sharpi* are often collected from dry leaf litter of secondary forests or other secondary types of vegetation (including bamboo stands in agricultural areas), and are also found in cow and horse dung in the lowlands or occasionally attracted to UV light.

Oosternum sp.

Figs 34, 48

Material examined (1 spec.): **BAHAMAS:** Andros Island, Forfar Field Station, 10 m, 24°53’50.81”N, 77°55’54.29”W, 10/15.vii.1983, J. Peacock leg., at light (1 spec., WIFP).

Diagnosis. Body widest ca at midlength. Lateral margin of pronotum angulate. Pronotal punctation double-sized, dense consisting of moderately large, round punctures intermixed with smaller transverse punctures. Pronotal interstices with microsculpture. Lateral margin of antennal grooves with acute projection. Elytral interval 2 narrower that interval 3, lower than intervals 1 and 3, reaching elytral apex. Elytral intervals 5, 7 and 9 distinctly higher than adjacent intervals. Elytral interstices shiny, without microsculpture. Preepisternal plate wide, drop-like, 2× longer than wide. Interstices of median part of metaventrite with very fine microsculpture, opaque. Anterolateral ridegs of metaventrite not meeting together mesally, indistinct laterally.

Comment. The single examined specimen is very similar to *O. sharpi* but differs from it by the relatively smaller preepisternal plate of the mesothorax, central portion of the metaventrite with much finer punctation and the

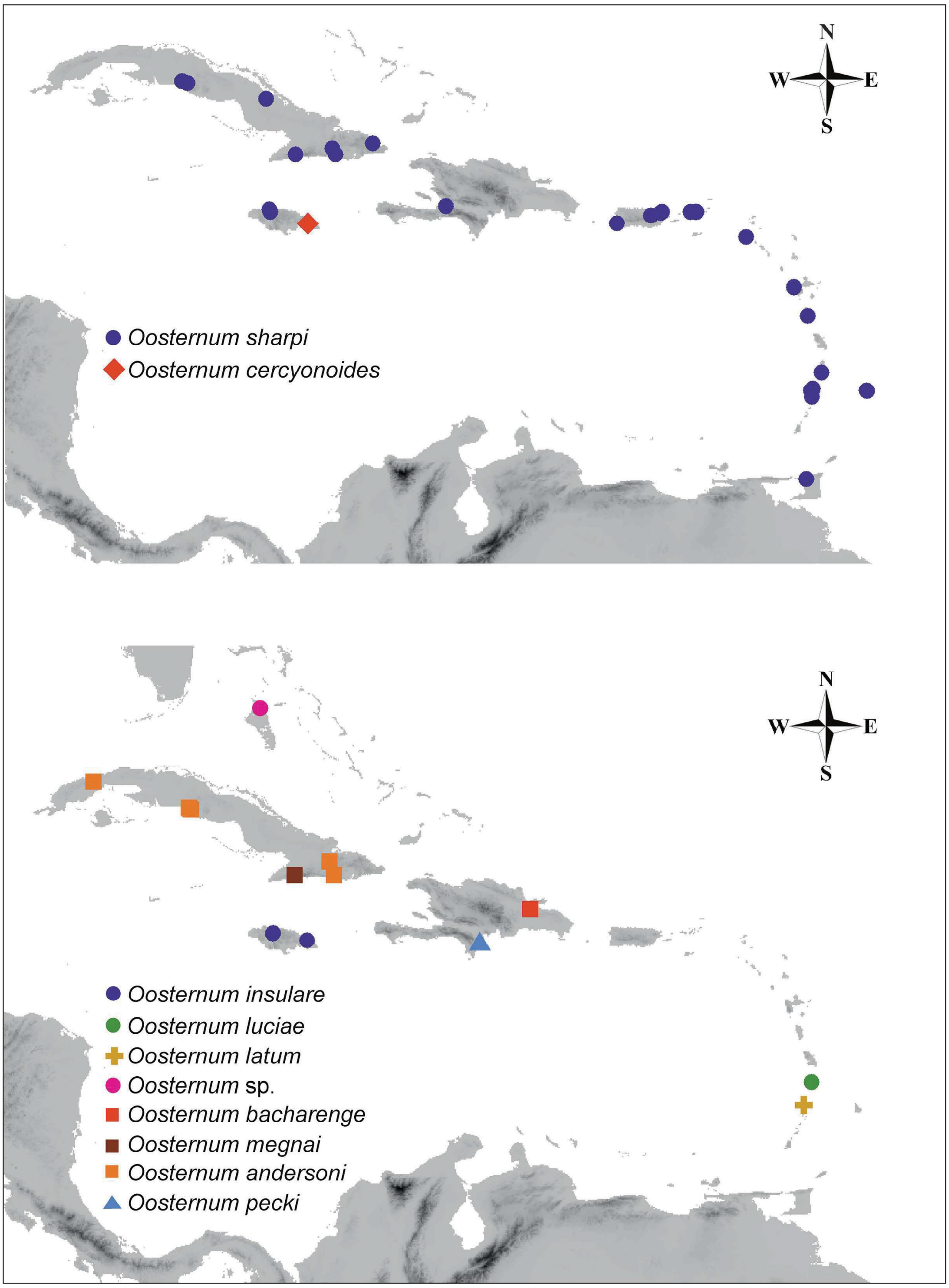


Figure 61. Distribution of the representatives of the *Oosternum* from West Indies. Shape of the symbols indicate the species group which the respective species belongs to (see the Discussion for details).

laterally incomplete anterolateral ridge (Fig. 48). However, since only a single female is available, we refrain from describing it as a new species, pending the discovery of additional specimens and ideally males.

Discussion

Composition of the West Indian fauna of *Oosternum*. Ten species groups of *Oosternum* were defined by Fikáček et al. (2009), using a set of arbitrarily selected characters (prosternal morphology, the form the pronotum, elytral morphology and the morphology of the metaventrite). These groups were aimed to divide the genus into smaller parts facilitating the species-level revision. Their phylogenetic significance has never been tested, yet they are the only available proxy of the internal structure of *Oosternum* at the moment. Moreover, additional characters exclusively correlating with some of the groups or their combinations were subsequently found (Fikáček 2009), which possibly indicates that at least some of these groups are candidates for monophyletic clades.

Representatives of five different species groups were found in the West Indies. Four species, the widely distributed *O. sharpi*, the endemic *O. insulare* and *O. luciae*, and the undescribed species from the Bahamas, are members of the *O. sharpi* species group (indicated by circles in Fig. 61). One species, *O. latum*, is a member of the *O. aequinoctiale* group (Fikáček et al. 2009; cross-shaped symbol in Fig. 61), which seems to be morphologically very close to the *O. sharpi* group based on the medially differentiated prosternum, pronotal punctation consisting of two intermixed types of punctures (one with long seta, one with extremely reduced seta) and antennal grooves with acute lateral projection. Further five species represent the groups without differentiated median portion of prosternum. They key out as members of *Oosternum* group C (in case of *O. cercyonoides*) and of the *O. pubescens* group (in case of *O. andersoni*, *O. bacharenge*, *O. megnai* and *O. pecki*) using the key to groups by Fikáček et al. (2009), as they differ in the morphology of the elytral intervals. However, the close relationship of the West Indian species with *O. pubescens* (LeConte, 1855) seems rather improbable, as the latter species is unique among *Oosternum* by possessing a partially differentiated median portion of prosternum (see Fig. 250 in Smetana 1978, under the name *Cercyon pubescens*). In contrast, all West Indian species bear a simply carinate prosternum. Moreover, *O. pecki* seems rather isolated from the remaining West Indian species based on its unique morphology of the meso- and metaventrite (preepisternal plate largely overlapping over metaventrite, metaventrite with an additional lateral ridge along the lateral margin) as well as by the character of the pronotal punctation (shape of the punctures changes from anterior margin to the posterior one). We therefore tentatively consider the West Indian species with simple prosternum as members of three species groups: *O. cercyonoides* and *O. pecki* each represent a separate species group (marked by a rhomboid and a triangle in Fig. 61, respectively), whereas *O. andersoni*, *O. bacharenge* and *O. megnai* are extremely similar to each other and form the group depicted by squares in Fig. 61.

Biogeography of the West Indian *Oosternum*. Nine of the ten *Oosternum* species occurring in the West Indies are thusfar endemic to the region. All of them are single-island endemics. The highest diversity is found in the Greater Antilles, where six endemic species were found, two on each island (Cuba, Jamaica, Hispaniola) except for Puerto Rico, from where no endemic species is known. In the Lesser Antilles, only two endemic species are known from the southern part of the island arc; one from each Saint Lucia and Saint Vincent islands.

Based on the assignment to the tentative species groups discussed above, the fauna of the Greater Antilles clearly shows a composite character, hosting representatives of five different species groups. Each Jamaican endemic species belongs to a different species group, and the same is the case of Hispaniola. This seems to indicate that the fauna of these islands resulted from multiple independent colonizations. A different situation is found in Cuba, where both endemic species, *O. andersoni* and *O. megnai*, are morphologically very similar and very likely closely related. They are moreover very similar to *O. bacharenge* from Hispaniola. It is hence probable that the three species are closely related. The geological block that today forms the northern part of Hispaniola was originally connected to that of eastern Cuba until the Early to Middle Miocene when it separated (Graham 2003). Hence, *Oosternum bacharenge* may have originated by vicariance after the separation of northern Hispaniola from Cuba. In Cuba, the two endemic species were never collected syntopically: *Oosternum andersoni* is a highland species widespread throughout the island, whereas *O. megnai* is endemic to the western part of the Sierra Maestra Mts. The type locality lies at the slope of Pico Turquino, i.e. the highest Cuban mountain. We failed to find the species in the central and eastern parts of Sierra Maestra despite our recent intensive collecting effort in these areas, which confirms that *O. megnai* is very likely a very local endemic. To understand the reasons for the within island split of *O. andersoni* and *O. megnai*, it would be necessary to date the age of the split. However, a local split along the elevation gradient seems currently as the most probable explanation.

Although data from other islands of the Greater Antilles are more limited than those from Cuba, the separation along the altitudinal gradient is likely also present in Jamaica, where *O. cercyonoides* is known from the highest mountain range only (Blue Mountains in eastern Jamaica, the type locality at 1600 m a.s.l.), whereas *O. insulare* is known from localities at around 500 m a.s.l. across the island. In this case, the species are however not related to each other, but the different environmental requirements may have facilitated their coexistence in the island after two independent colonizations. The composite character of the Hispaniolan fauna may on the other hand reflect the composite geological origin of the island. The current island consists of two originally separate blocks. The northern one was connected to eastern Cuba until the Early/Middle Miocene (as discussed above), whereas the southern one originally formed a separate island and was connected with the northern one in the Middle Miocene (Graham 2003). One Hispaniolan endemic species is known from the former northern island (*O. bacharenge*), the other from the former southern island (*O. pecki*) and they are moreover not closely related to each other. The probable origin of *O. bacharenge* was already discussed. *Oosternum pecki* may have originally been the sole endemic in the small island of Southern Hispaniola, resulting from the dispersal from the continent or another West Indian island.

The endemics of the Lesser Antilles are most probably not closely related, and are both known from the volcanic island in the south of the island arc (*O. luciae* from St. Lucia, *O. latum* from St. Vincent), i.e. those which are rather close to the South American continent. The taxonomy and distribution is known in detail at least for the *O. aequinoctiale* group to which *O. latum* belongs. All species of this group but *O. latum* are restricted to South and Central America, and none of them, including the otherwise very widespread *O. aequinoctiale* (Motschulsky, 1855), does not occur in the West Indies (Fikáček et al. 2009). It seems hence probable that *O. latum* colonized the volcanic island of St. Vincent from South America. Same may be true for *O. luciae* which also has its relative species only in South America (Fikáček, unpubl. data) and is not related to any other West Indian species.

Unbalanced sex ratio of West Indian populations of *O. sharpi*? *Oosternum sharpi* is the only species of the genus that is widespread in the West Indies and also the only one that is not endemic – it is also widespread in the continental Central and South America and in the southern USA (this material was not studied in detail in this study). Surprisingly, the vast majority of the West Indian specimens of this species examined by us (64 of 65 specimens) are females. No males were found in most islands, with the only exception of Puerto Rico, from which the only West Indian male of the species is known. Even through this may be accidental due to a small number of specimens collected in most collecting events, it still stands in contrast to most other West Indian *Oosternum* species treated here in which males were found despite the limited number of collected specimens. It is also in a strong contrast to the continental populations of *O. sharpi*, in which males are frequent (M. Fikáček, unpubl. data). Additional collecting is needed to test whether the absence of males in most islands is just a collecting bias, or whether some island population of *O. sharpi* may be parthenogenetic. The latter possibility would however correspond with the wide distribution of the species in the West Indies as well as with the fact that *O. sharpi* is the only species of the genus which was introduced outside of the Neotropical Region (as a single female is able to establish a new population in parthenogenetic species).

Acknowledgements

We are deeply grateful to the authorities and personnel of the Cuban Ministry of Science, Technology and Environment (CICA-CITMA) and the Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF) for providing access to protected areas under their control, to Robert Anderson (Canadian Museum of Nature, Ottawa) and the project Paleobiogeography of the Caribbean for considerable help during our field work, and to S. Peck (Carletton University) and M. Ivie (Montana State University) for the possibility to examine the specimens from the collections under their custody. We specially acknowledge the help of Andrew Short (SEMC) and the anonymous reviewer for their valuable comments on the manuscript and careful correction of our English text. The study was supported by the grant SVV-2013-267 201 to ADH and by the Ministry of Culture of the Czech Republic (DKRVO 2014/13, National Museum, 00023272) to MF.

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CHAPTER 6

Deler-Hernández A. and Fikáček M. 2016. Redescriptions and lectotype designations of Central American species of *Phaenonotum* Sharp (Coleoptera, Hydrophilidae) based on the type material from the David Sharp collection. *ZooKeys* 579: 83–98.

ZooKeys 579: 83–98 (2016)
doi: 10.3897/zookeys.579.7748
http://zookeys.pensoft.net

RESEARCH ARTICLE

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Redescriptions and lectotype designations of Central American species of *Phaenonotum* Sharp (Coleoptera, Hydrophilidae) based on the type material from the David Sharp collection

Albert Deler-Hernández[1], Martin Fikáček[1,2]

1 *Department of Zoology, Faculty of Science, Charles University in Prague, Vinicná 7, CZ–128 44,Praha 2, Czech Republic* **2** *Department of Entomology, National Museum, Cirkusova 1740, Praha 9 – Horni Pocernice, CZ–193 00, Czech Republic*

Corresponding authors: *Albert Deler-Hernández* (adeler1982@gmail.com); *Martin Fikáček* (mfikacek@gmail.com)

Academic editor: *M. Michat* | Received 9 January 2016 | Accepted 7 March 2016 | Published 11 April 2016

http://zoobank.org/A897CC9B-42EF-4443-B48B-289686BA9555

Citation: Deler-Hernández A, Fikáček M (2016) Redescriptions and lectotype designations of Central American species of Phaenonotum Sharp (Coleoptera, Hydrophilidae) based on the type material from the David Sharp collection. ZooKeys 579: 83–98. doi: 10.3897/zookeys.579.7748

Abstract

In order to understand the identity of the Central American species of the genus *Phaenonotum* Sharp, 1882, the type specimens of the species described by Sharp (1882) deposited in the David Sharp collection in the Natural History Museum in London have been re-examined. The following species are redescribed: *Phaenonotum apicale* Sharp, 1882, *P. collare* Sharp, 1882, *P. dubium* Sharp, 1882 (confirmed as junior synonym of *P. exstriatum* (Say, 1835)), *P. laevicolle* Sharp, 1882, *P. rotundulum* Sharp, 1882 and *P. tarsale* Sharp, 1882. Lectotypes are designated for *P. apicale*, *P. collare*, *P. rotundulum* and *P. tarsale*. External diagnostic characters and morphology of male genitalia are illustrated. A table summarizing diagnostic characters allowing the identification of the species is provided.

Resumen

Para entender la identidad de las especies del género *Phaenonotum* Sharp, 1882 de América Central, se han reexaminado los especímenes tipo de las especies descritas por Sharp (1882) depositadas en la colección de David Sharp, del Museo de Historia Natural en Londres. Las especies redescritas son: *Phaenonotum apicale* Sharp, 1882, *P. collare* Sharp, 1882, *P. dubium* Sharp, 1882 (confirmado como sinónimo más reciente de *P. exstriatum* (Say, 1835)), *P. laevicolle* Sharp, 1882, *P. rotundulum* Sharp, 1882 y *P. tarsale* Sharp, 1882. Se designan lectotipos para las especies *Phaenonotum apicale*, *P. collare*, *P. rotundulum* y *P. tarsale*. Se ilustran los caracteres diagnósticos y la morfología de los genitales. Una tabla resumen con los caracteres diagnósticos para facilitar la identificación de las especies es ofrecida.

Keywords

Coelostomatini, morphology, Neotropical region, Sphaeridiinae, taxonomy

Palabras clave

Coelostomatini, morfología, Neotrópico, Sphaeridiinae, taxonomía

Introduction

The genus *Phaenonotum* Sharp, 1882 was described by the British specialist on water beetles, David Sharp, in his treatment of the Central American hydrophilid fauna in the famous *Biologia Centrali–Americana*. Based on material from Mexico, Nicaragua, Guatemala, Costa Rica and Panama available to him, Sharp (1882) recognized and described six species of that genus, and also recognized that the North American species *Cyclonotum exstriatum* (Say, 1835) is congeneric. A few other species originally described in other genera were later assigned to *Phaenonotum* by other authors (Knisch 1924; Orchymont 1937) and few additional species were described subsequently from Brazil (Orchymont 1937, 1943), Argentina (Bruch 1915), Venezuela (Archangelsky 1989), U.S.A (Smetana 1978) and Cuba (Deler-Hernández et al. 2013). In addition, the monotypic genus *Hydroglobus* Knisch, 1921 from Argentina was considered a part of *Phaenonotum* by Archangelsky (1991), but this was not followed by subsequent authors (see e.g. Clarkson et al. 2014 for diagnostic characters between *Hydroglobus* and *Phaenonotum*). At present, *Phaenonotum* seems to occur exclusively in the Neotropical and southern Nearctic Region from where 18 species are described (Hansen 1999; Deler-Hernández et al. 2013). The identity of the only non-American species, *P. africanum* Régimbart, 1907 from the island of Bioko in Guinean Gulf, Africa, is unclear and the species needs to be re-examined.

Despite being frequently collected, *Phaenonotum* species were never properly revised, and only the fauna of North America and Argentina (partly) were treated in details by modern authors (Smetana 1978; Archangelsky 1991). Hence, no information on morphology of the species or identity of their types was published for the majority of species after their original descriptions, which makes the identification of newly collected material almost impossible. The only species for which types were reexamined and redescriptions and/or illustrations published are *P. argentinense* Bruch, 1915, *P. regimbarti* Bruch, 1915, and *P. exstriatum* (Say, 1835) and its synonyms (Smetana 1978; Archangelsky 1991). In addition, the lectotype of *P. laevicolle* Sharp, 1882 was designated by Smetana (1976), but without providing any information about the identity of that species. Of the recently described species, photos of the habitus and genitalia, and some details on morphology of *P. minor* Smetana, 1978 were published by Deler-Hernández et al. (2013). The assignment of *P. caribense* Archangelsky, 1989 to *Phaenonotum* was found questionable based on preliminary molecular data (A. Deler-Hernández & V. Sýkora, unpubl. data).

In the course of the review of *Phaenonotum* from the Greater Antilles, it was necessary to study the identities of the Central American species of the genus described by D. Sharp in order to confirm or exclude their occurrence in the Caribbean islands. The type series of all species described by Sharp and deposited in the Natural History Museum in London were therefore re-examined. To facilitate future studies, the results of these studies are summarized in the present paper, providing the redescriptions and illustrations of the species examined. In needed cases, the lectotypes have been designated in order to fix the identity of the species for future studies.

Material and methods

Habitus photographs were taken using Canon EOS 550D digital camera with attached Canon MP-E65mm f/2.8 1–5× macro lens, and subsequently adapted in Adobe Photoshop CS5. Drawings of male genitalia are based on photographs taken using Canon EOS 1100D digital camera attached to Olympus BX41 compound microscope and subsequently combined in Helicon Focus software. Scanning electron micrographs of lectotypes were taken using Hitachi S-3700N environmental electron microscope at the Department of Paleontology, National Museum in Prague, using the uncoated specimens in low vacuum regime. Morphological terminology follows Smetana (1978), Archangelsky (1989, 1991) and Deler-Hernández et al. (2013).

Part of the specimens including the lectotypes were dissected, their genitalia were mounted in an alcohol soluble Euparal resin on a small piece of glass attached to the same pin as the specimen.

All lectotypes designated were labeled with the following red label: "Lectotype [or Paralectotype] / *Phaenonotum* / species-name with author and year of description / des. Deler-Hernández".

Under each species listed as material examined label data are given verbatim between quotes (" "), each line of text is separated by a slash with spaces on both sides (/) and the information of each label is separated by double slashes with space on both sides (//). Other data are in square brackets ([ ]).

Examined specimens are deposited in the following collections:

BMNH	The Natural History Museum, London, U.K. (M. Barclay);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček).

Taxonomy

Phaenonotum apicale **Sharp, 1882**
Figures 1a, 2a, 3a, 4a

Phaenonotum apicale Sharp, 1882: 98.

Type material examined. Lectotype (hereby designated): male (BMNH): "Phaenonotum / apicale Var. / D.S. / Guatemala City. / 5000 ft. Salvin. // Guatemala City.

Champion. // B.C.A. I. 2. / Phaenonotum / apicale, Sharp. // Sharp Coll. 1905.-313." The specimen was re-mounted to a new label, with abdomen glued separately and aedeagus embedded in Euparal slide attached below the specimen. **Paralectotype:** female (BMNH): "Phaenonotum / apicale / Type / D.S. / Chontales, Nicaragua / Janson. // Chontales, / Nicaragua. / Janson. // B.C.A. Col. I. 2. / Phaenonotum / apicale, / Sharp. // Sharp Coll. / 1905.-313.".

Other material examined. 1 unsexed specimen (BMNH): "Phaenonotum / apicale Var.? / David. Chiriqui / Champion // David, / Panama / Champion. // B.C.A. Col. I. 2. / Phaenonotum / apicale, / Sharp. // Sharp Coll. / 1905.-313."; 1 unsexed specimen (BMNH): "Cuernavaca, / Morelos. / Hoge. // B.C.A. Col. I. 2. / Phaenonotum / apicale, / Sharp. // apicale / var, [hand written]"; 1 unsexed specimens (BMNH): "Tejupilco, Mex. / Temescaltepec / 18.vi.1933 [hand written] // H. E. Hilton, / R. L. Usinger / Collectors"; male (BMNH): "Tejupilco, Mex. / Temescaltepec / 18.vi.1933 [hand written] // H. E. Hilton, / R. L. Usinger / Collectors // Phaenonotum [hand written] / apicale Sharp [hand written] / J. Balfour-Brown det.".

Type locality (following lectotype designation). Guatemala City, 5000 feet [= 1525 m a.s.l.], Guatemala.

Redescription. Habitus as in Figs. 1a and 2a. Body length 2.9–3.2 mm (lectotype: 2.9 mm). Body form oval in dorsal view (Fig. 1a), elytra uniformly convex in lateral view (Fig. 2a). Dorsal surface dark brown (Fig. 1a). Antennae and maxillary palpi testaceous. Ventral surface reddish. Leg reddish, tarsomeres yellowish. Head and pronotum with fine and sparse punctures. Elytral punctation strongly impressed, coarser than pronotal and head punctation. Pronotum wider than long and convex. Epipleura very broad throughout. Meso- and metaventral processes fused into a common keel; mesoventral process arrow-head shaped with a distinct hood, as wide as metaventral process basally, metaventral process slender, parallel-sided, length of metaventrite medially (including metaventral process) ca. four times longer than mesoventral process; metathoracic discrimen indistinct (Fig. 3a). Profemora with long sparse pubescence in basal 0.75. Meso- and metafemora with very sparse and short pubescence only. All tarsi with long setae on ventral surface. Aedeagus (Fig. 4a) 0.4 mm long, with median lobe reaching apices of parameres; basal portion of median lobe angulate laterally, apical portion strongly narrowing; shape of the gonopore oval. Parameres wide and curved in median region. Phallobase not examined in detail.

Comments on lectotype designation. Sharp (1882) mentions specimens from two localities: Nicaragua: Chontales and Guatemala: Guatemala City, but without specifying the number of specimens. In the Sharp collection, there are two specimens standing under the name of *P. apicale*, one from each locality mentioned, and both corresponding with the data in the original description. We hence consider both as syntypes. The specimen from Guatemala City is the only male, and thus is designated here as lectotype, despite it appearing to be slightly teneral. Otherwise, there are four specimens from localities not corresponding to those given in the original description, which we do not consider as a part of the type series (see Other material examined).

Phaenonotum collare **Sharp, 1882**

Figures 1b, 2b, 3b, 4b

Phaenonotum collare Sharp, 1882: 99.

Type material examined. Lectotype (hereby designated): male (BMNH): “Phaenonotum / collare D.S. // Chontales, / Nicaragua. / Janson. // B.C.A. I. 2. / Phaenonotum / collare, / Sharp. // Sharp Coll. 1905.-313.”. The specimen was dissected, its abdomen is glued separately and the aedeagus is embedded in Euparal slide attached to the same pin. **Paralectotypes:** 1 female, 1 unsexed (BMNH): “Phaen / Cyclonotum / collare D.S. / Type / Chontales. Nicaragua. / Janson // B.C.A. I. 2. Phaenonotum / collare, Sharp. // Sharp Coll. 1905.-313.”; “Phaenonotum / collare D.S. / Chontales. Nicaragua. / Janson / B.C.A. I. 2. Phaenonotum / collare, Sharp. / Sharp Coll. 1905.-313.”.

Other material examined. unsexed specimen (BMNH): “Phaenonotum / collare Var. / D.S. / El Zumbador / 2500 ft. Champion // El Tumbador, / Guatemala. / Champion. // B.C.A. I. 2. / Phaenonotum / collare, / Sharp. // Phaenonotum collare [hand written]”.

Type locality (following lectotype designation). Chontales, Nicaragua.

Redescription. Habitus as in Figs. 1b and 2b. Body length 3.5–3.9 mm (lectotype: 3.9 mm). Body form oval in dorsal view (Fig. 1b), elytra less convex anteriorly and more convex posteriorly in lateral view (Fig. 2b). Dorsal surface brown (Fig. 1b). Antennae and maxillary palpi testaceous. Ventral surface reddish brown. Leg reddish, tarsomeres yellowish. Head with coarse and strongly impressed punctures. Pronotum with fine punctures, but sparser than head punctation. Elytral punctation (Fig. 1b) strongly impressed, punctures of the same size as on head and as coarse as head punctations. Epipleura very broad throughout. Meso- and metaventral processes slender and fused into a common keel; mesoventral process arrow-head shaped with an distinct hood, slightly wider than apex of metaventral process basally, metaventral process slender, nearly parallel-sided, only indistinctly narrowing anteriad, length of metaventrite medially (including metaventral process) ca. four time longer than mesoventral process; metathoracic discrimen distinct, forming a shallow impression basally (Fig. 3b). All tarsi with long setae on ventral surface. Aedeagus (Fig. 4b) 0.4 mm long, with median lobe reaching apices of parameres; basal portion of median lobe rounded laterally, apical portion widely rounded, median lobe narrowing towards apex; shape of the gonopore transversely oval. Parameres wide and slightly sinuate in median region. Phallobase as long as wide.

Comments on lectotype designation. Sharp (1882) mentions specimens from two localities: Nicaragua: Chontales and Guatemala: El Tumbador, without specifying the numbers of specimens. Specimen(s) from Guatemala are assigned to the “var. *paulo angustior*” [= a little narrower], which excludes them from the type series based on Article 72.4.1 of the Code (ICZN 1999). In the Sharp collection, there are four specimens under the name *Phaenonotum collare*, three of which correspond to the

Figure 1. Habitus of type specimens (dorsal view) and original type labels. **a** *Phaenonotum apicale* Sharp (lectotype) **b** *Phaenonotum collare* Sharp (lectotype) **c** *Phaenonotum dubium* Sharp (lectotype) **d** *Phaenonotum laevicolle* Sharp (lectotype) **e** *Phaenonotum rotundulum* Sharp (lectotype) **f** *Phaenonotum tarsale* Sharp (lectotype).

Nicaraguan specimens mentioned in the original description, and one corresponding with “var. *paulo angustior*”. Only the specimens from Nicaragua are considered as part of the type series, and one of them, a dissected male, is designated as a lectotype, in order to fix the identity of the species for future studies.

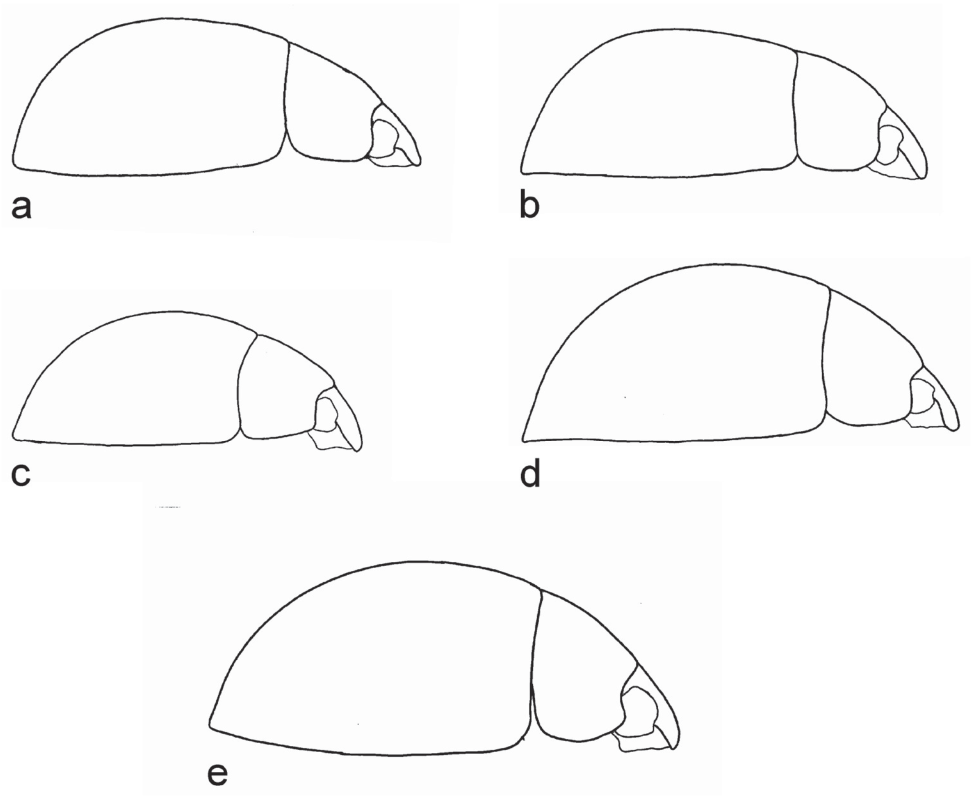

Figure 2. Habitus (lateral view). **a** *Phaenonotum apicale* Sharp (lectotype) **b** *Phaenonotum collare* Sharp (lectotype) **c** *Phaenonotum laevicolle* Sharp (lectotype) **d** *Phaenonotum rotundulum* Sharp (lectotype) **e** *Phaenonotum tarsale* Sharp (lectotype).

Phaenonotum dubium Sharp, 1882 (= *P. exstriatum* (Say, 1835))

Figures 1c, 4c

Hydrophilus exstriatus Say 1835: 171. Trasferred to *Phaenonotum* by Sharp (1882: 98).
Phaenonotum dubium Sharp, 1882: 98. Synonymized with *P. exstriatum* by Smetana (1978: 14).
For complete synonymy of *P. exstriatum* see Hansen (1999).

Type material examined. Lectotype (designated by Smetana 1978: 14): male (BMNH): “Phaen / ~~cycl~~onotum / dubium / D.S. / S. Geronimo. / Guatemala / Champion // B.C.A. I. 2. / Phaenonotum / dubium, / Sharp. // S. Geronimo. / Guatemala / Champion. // LECTO- / TYPE [round label with purple margins]”. **Paralectotype:** 1 unsexed specimen (BMNH): “Phaen / Cyclonotum / dubium / Types / D.S. / S. Geronimo. / Guate- / mala. Champion. // B.C.A. I. 2. / Phaenonotum / dubium, / Sharp. // San Geronimo. / Vera Paz. / Champion. // Type / H. T. [round label with red margins]”.

Other type material. Sharp (1882) also examined specimens from Mexico: Cordova, Vera Cruz, Oaxaca and Costa Rica: Cache, all of which have to be considered as paralectotypes. We did not examine these specimens.

Additional material examined. male (dry-mounted) (NMPC): **CUBA**: **Granma Prov**: Cauto Cristo, Río Cauto, El Sitio, 01.v.2005, Coll. L. Chaves. male (dry-mounted) (BMNH): **HAITI**: Port au Prince, 1.iii.1908, Coll. Dr. M. Cameron, B. M. 1936-555. male (dry-mounted) (BMNH): **JAMAICA**: Kinstong, 16.ii.1908, Coll. Dr. M. Cameron. male (dry-mounted) (BMNH): **USA**: Delaware (ABTC000175) (NMPC).

Type locality. San Geronimo, Guatemala.

Redescription. Habitus as in Fig. 1c. Body length 3.5–3.7 mm (lectotype: 3.5 mm). Body form oval in dorsal view (Fig. 1c), elytra convex in lateral view. Dorsal surface dark brown (Fig. 1c). Antennae and maxillary palpi testaceous. Pronotum slightly paler than elytra. Ventral surface reddish brown. Leg reddish, tarsomeres yellowish. Head with fine and sparse punctures. Pronotum with punctures of same size as on head. Elytral punctation strongly impressed, much denser than on pronotum and head. Epipleura very broad throughout. Meso- and metaventral processes fused into a common keel; mesoventral process arrow-head shaped with an distinct hood, as wide as metaventral process basally, metaventral process slender, parallel-sided, length of metaventrite medially (including metaventral process) ca. four times longer than mesoventral process; metathoracic discrimen indistinct (Fig. 3f). Profemora with long sparse pubescence in basal 0.75. All tarsi with long setae on ventral surface. Aedeagus (Fig. 4c) 0.4 mm long, with median lobe reaching apices of parameres or nearly so; basal portion of median lobe nearly straight laterally, apical portion widely rounded, median lobe nearly of the same width throughout; shape of the gonopore transversely oval. Parameres strongly sinuate in median region. Phallobase as long as wide (Fig. 4d).

Comments on synonymy. Examined type specimens of *P. dubium* morphologically correspond with specimens of *P. exstriatum* listed in "Additional material examined" in all characters, including morphology of the aedeagus and meso-metaventral process. Hence, we confirm that *P. dubium* is a junior synonym of *P. exstriatum*, as proposed by Smetana (1978).

***Phaenonotum laevicolle* Sharp, 1882**

Figures 1d, 2c, 3c, 4e

Cyclonotum globulosum Mulsant, 1844: 167 (ascribed to Klug). ["Amérique méridionale"] (cf., Orchymont, 1937). Transferred to *Phaenonotum* by Knisch (1924: 114).

Phaenonotum laevicolle Sharp, 1882: 99. Considered as synonym of *P. globulosum* by Orchymont (1937: 241). Synonymy not confirmed by subsequent authors.

Type material examined. Lectotype (designated by Smetana 1976: 213): male (BMNH): "Phaenonotum / laevicolle / Type / D.S. / Cordova Mex Sallé. // B.C.A. I. 2. / Phaenonotum / laevicolle, / Sharp. // Sharp Coll. / 1905.-313.". **Paralectotype:**

male (BMNH): "Cubilguitz / Vera Paz. / Champion. // B.C.A. I. 2. / Phaenonotum / laevicolle, / Sharp".

Other type material. Sharp (1882) also examined specimens from Nicaragua: Chinandega, Managua and Chontales, all of which have to be considered as paralectotypes. We did not examine these specimens.

Type locality (following lectotype designation). Cordova, Mexico.

Redescription. Habitus as in Figs 1d and 2c. Body length 2.5–2.7 mm (lectotype: 2.7 mm). Body form oval in dorsal view (Fig. 1d), elytra evenly convex in lateral view (Fig. 2c). Dorsal surface brown (Fig. 1d). Antennae and maxillary palpi testaceous. Ventral surface reddish brown. Leg reddish, tarsomeres yellowish. Head with fine and sparse punctures. Pronotum with punctures of same size as on head. Elytral punctation strongly impressed, much coarser than pronotal and head punctation. Epipleura very broad throughout. Meso- and metaventral processes fused into a common keel; mesoventral process arrow-head shaped with narrow hood, its base narrower than apex of metaventrite; metaventral process stout, slightly widened subapically, length of metaventrite medially (including metaventral process) ca. three times longer than mesoventral process (Fig. 3c). All tarsi with long setae on ventral surface. Aedeagus (Fig. 4e) 0.5 mm long, with median lobe not reaching apices of parameres; basal portion of median lobe nearly straight laterally, apical portion widely rounded, median lobe narrowing towards apex; shape of the gonopore transversely subtriangular. Parameres wide and curved in median region. Phallobase not examined in detail.

Comments on synonymy. Orchymont (1937) considered *P. laevicolle* as a junior synonym of *P. globulosum* described from Colombia, based on the study of the type specimens of both taxa. However, he only compared external characters used for diagnosis of *Phaenonotum* species at that time (i.e. dorsal punctation, length of tarsi), and did not study ventral morphology and male genitalia, which are crucial characters for species identification. Smetana (1976) reexamined the types of *P. laevicolle* including genitalia, but he did not provide any comments on the synonymy proposed by Orchymont (1937), he neither studied the types of *P. globulosum*. For that reason, the synonymy of *P. laevicolle* with *P. globulosum* needs to be confirmed by future studies.

***Phaenonotum rotundulum* Sharp, 1882**

Figures 1e, 2d, 3d, 4f

Phaenonotum rotundulum Sharp, 1882: 100.

Type material examined. Lectotype (hereby designated): male (BMNH): "Phaenonotum rotund– / ulum. / Type D.S. / S. Geronimo. Guatema– / la. Champion // San Geronimo, / Vera Paz. / Champion. // B.C.A. I. 2. / Phaenonotum / rotundulum, / Sharp. // Sharp Coll. / 1905.-313. // Type / H.T. [round label with red margins]." We remounted the specimens, the abdomen is glued separately, and the aedeagus is embedded in a Euparal slide attached to the same pin. **Paralectotypes:** 1 unsexed specimen

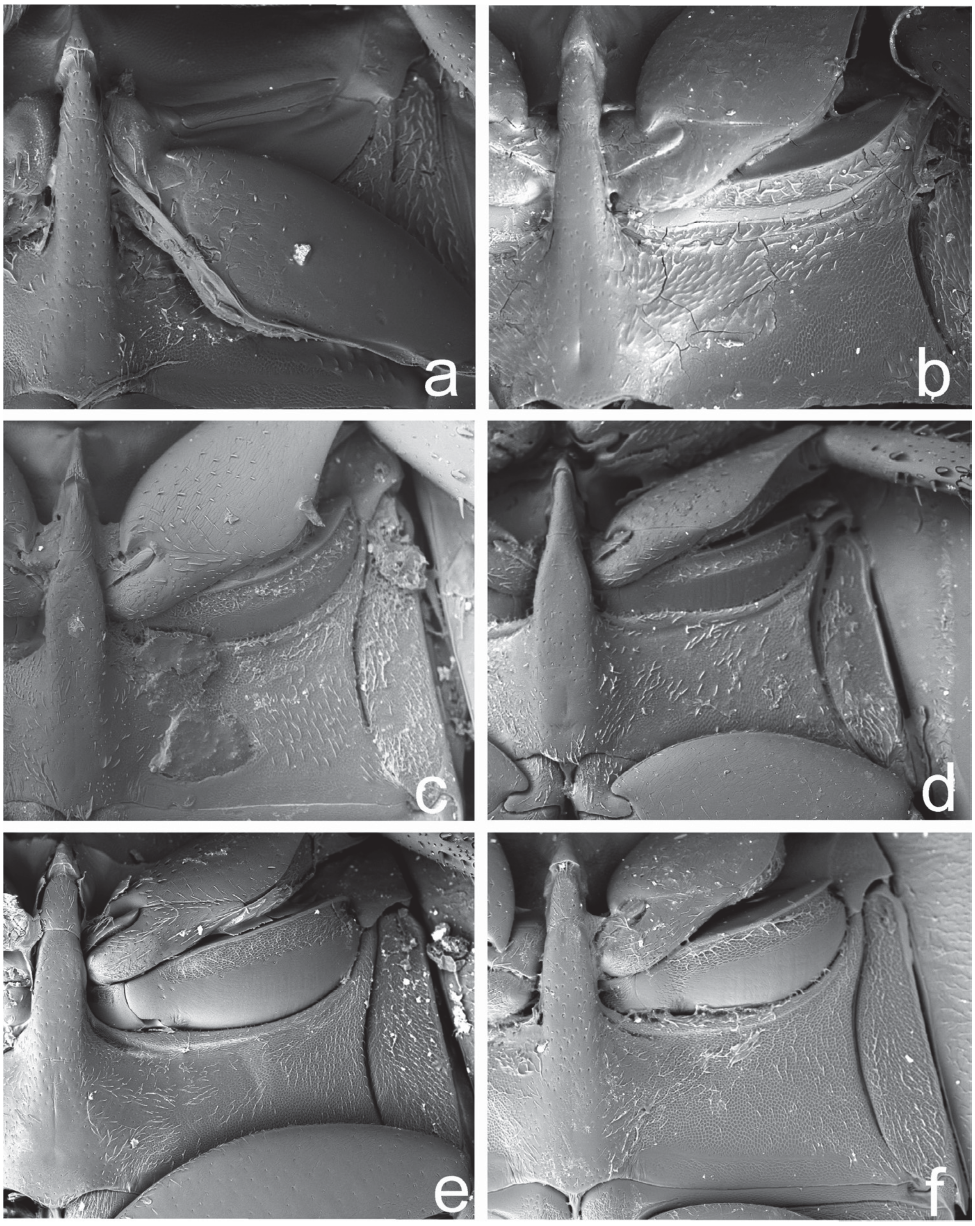

Figure 3. Meso-metaventral process. **a** *Phaenonotum apicale* Sharp (Lectotype) **b** *Phaenonotum collare* Sharp (Lectotype) **c** *Phaenonotum laevicolle* Sharp (Lectotype) **d** *Phaenonotum rotumdulum* Sharp (Lectotype) **e** *Phaenonotum tarsale* Sharp (Lectotype) **f** *Phaenonotum exstriatum* (Say).

(BMNH): "Phaenonotum / rotundulum / D.S. / El Zumbador. / 2500 ft. Guate– / mala. Champion. // El Tumbador, / Guatemala. / Champion. // B.C.A. I. 2. / Phaenonotum / rotundulum, / Sharp. // Sharp Coll. / 1905.-313.". 1 unsexed specimen

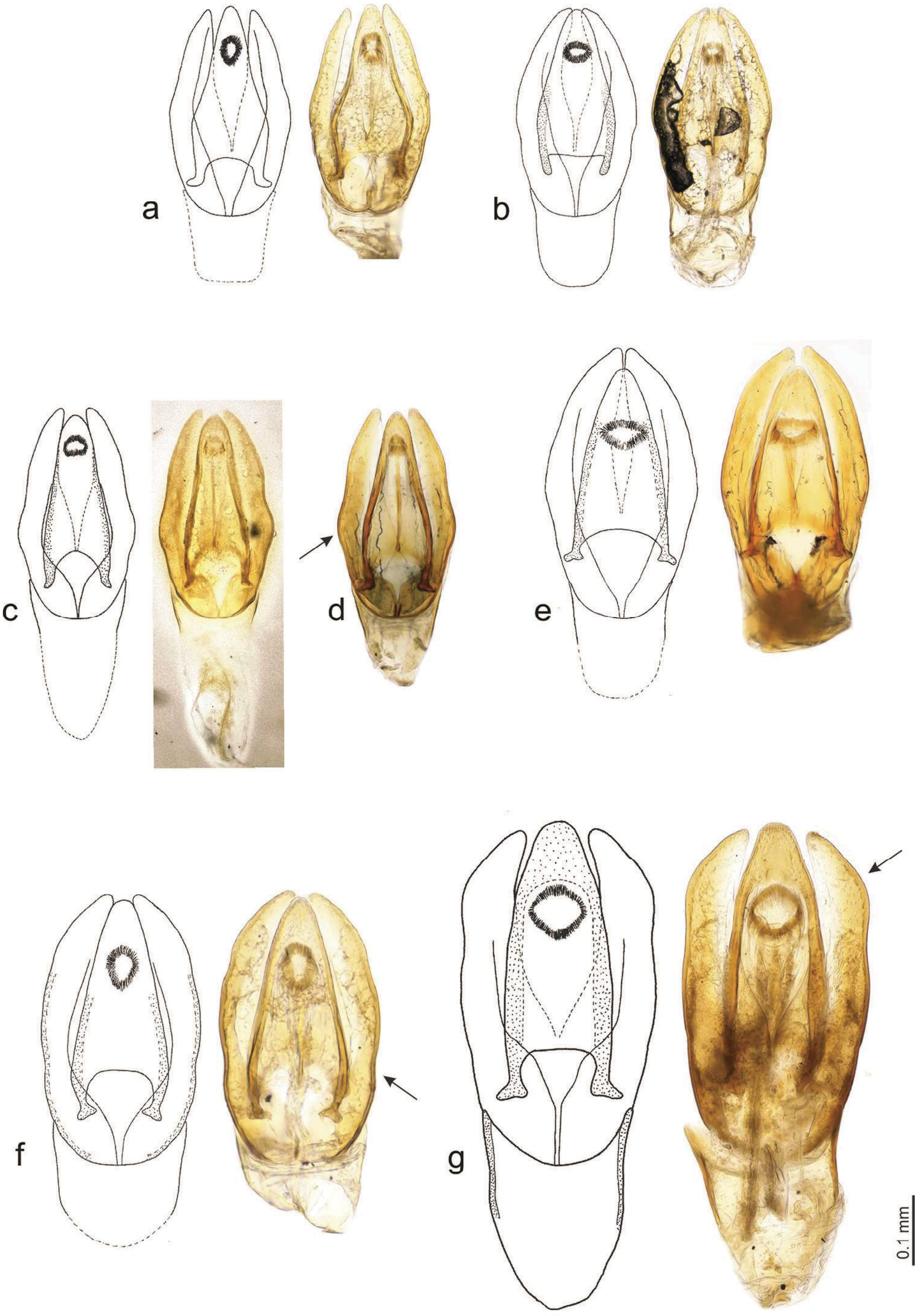

Figure 4. Aedeagus. **a** *Phaenonotum apicale* Sharp (Lectotype) **b** *Phaenonotum collare* Sharp (Lectotype) **c** *Phaenonotum dubium* Sharp (Lectotype) **d** *Phaenonotum exstriatum* (Say) (specimen from Haiti) **e** *Phaenonotum laevicolle* Sharp (Lectotype) **f** *Phaenonotum rotundulum* Sharp (Lectotype) **g** *Phaenonotum tarsale* Sharp (Lectotype).

(BMNH): "Phaenonotum / rotundulum / D.S. / Chacoj. Guatema / la. Champion // Chacoj, / R. Polochic, / Guatemala. / Champion // B.C.A. Col. I. 2. / Phaenonotum / rotundulum, / Sharp.". 2 females (BMNH): same label data as the lectotype.

Other type material. Sharp (1882) also examined specimens from Mexico: Cordova, Toxpam, Guatemala: San Juan, San Joaquin, Zapote, and Panama: Volcan de Chiriqui, 4000 to 6000 feet, all of which have to be considered as paralectotypes. We did not examine these specimens.

Type locality (following lectotype designation). San Geronimo, Guatemala.

Redescription. Habitus as in Figs 1e and 2d. Body length approximately 2.8–3.3 mm (lectotype: 3.3 mm). Body form oval in dorsal view (Fig. 1e), elytra highly and evenly convex in lateral view (Fig. 2d). Dorsal surface reddish brown (Fig. 1e). Antennae and maxillary palpi testaceous. Ventral surface reddish brown. Leg reddish, tarsomeres yellowish. Head with fine and sparse punctures. Pronotum with punctures of same size as on head. Elytral punctation strongly impressed, much coarser than on pronotum and head. Epipleura very broad throughout. Meso- and metaventral processes fused into a common keel; mesoventral process arrow-head shaped with indistinct hood, its base as wide as apex of metaventral process, metaventral process wide basally, strongly narrowing anteriad and hence triangular in shape, length of metaventrite medially (including metaventral process) ca. three time longer than mesoventral process; metathoracic discrimen weakly developed (Fig. 3d). Profemora with long sparse pubescence in basal 0.75. All tarsi with long setae on ventral surface. Aedeagus (Fig. 4f) 0.5 mm long, with median lobe not reaching apices of parameres; basal portion of median lobe curved laterally, apical portion widely rounded, median lobe narrowing towards apex; shape of the gonopore oval. Parameres slightly sinuate in median region. Phallobase not examined in detail.

Comments on lectotype designation. Our request to borrow the Sharp specimens of *P. rotundulum* resulted in the receipt of the above five specimens. These specimens, however, clearly represent only a smaller part of the type series, as many other localities were mentioned in the original description by Sharp (1882). All specimens examined agree with the data provided in the original description, and hence are clearly part of the type series. In order to fix the identity of the species for future studies, we are designating the dissected male labeled as "Type" as the lectotype of *P. rotundulum*.

Phaenonotum tarsale Sharp, 1882

Figures 1f, 2e, 3e, 4g

Phaenonotum tarsale Sharp, 1882: 98.

Type material examined. Lectotype (hereby designated): male (BMNH): "Phaenonotum / tarsale D.S. / Panama. // B.C.A. Col. I. 2. / Phaenonotum / tarsale, / Sharp. // Panama. // Sharp Coll. / 1905.-313." We remounted the specimen on a new label, with abdomen glued separately and aedeagus embedded in Euparal slide attached on the same pin. **Paralectotypes:** 1 male, 2 unsexed specimens (BMNH): same label data as the lectotype.

Type locality (following lectotype designation). Panama.

Redescription. Habitus as in Figs 1f and 2e. Body length 4.7–4.8 mm (lectotype: 4.8 mm). Body form oval in dorsal view (Fig. 1f), elytra highly and evenly convex in lateral view (Fig. 2e). Dorsal surface dark brown (Fig. 1f). Antennae and maxillary palpi testaceous. Pronotum slightly paler than elytra. Ventral surface reddish brown. Leg reddish, tarsomeres yellowish. Head with fine and sparse punctures. Pronotum with punctures of same size as on head, but slightly more sparsely than the head. Elytral punctation strongly impressed, much denser than on pronotum and head. Epipleura very broad throughout. Meso- and metaventral processes fused into a common keel; mesoventral process arrow-head shaped, very wide basally, slightly hooded apically, its base slightly wider than apex of metaventral process, metaventral process stout, parallel-sided, length of metaventrite medially (including metaventral process) ca. three time longer than mesoventral process; metathoracic discrimen weakly developed (Fig. 3e). Profemora with long sparse pubescence in basal 0.75. All tarsi with long setae on ventral surface. Aedeagus (Fig. 4g) 0.7 mm long, with median lobe slightly overlapping apices of parameres; basal portion of median lobe nearly straight laterally, apical portion widely rounded, median lobe nearly of the same width throughout; shape of the gonopore rounded. Parameres slightly sinuate in median region. Phallobase slightly longer than wide.

Comments on lectotype designation. Our request to borrow the Sharp specimens of *P. tarsale* resulted in the receipt of the above four specimens, all of them corresponding with the original description and clearly part of the type series. In order to fix the identity of the species for future studies, we are designating the dissected male as the lectotype of *P. tarsale*.

Discussion

The identification of species of *Phaenonotum* is a difficult task, due to the similarity of the species and the complicated process of finding relevant morphological characters. This may explain the absence of keys to *Phaenonotum* species. Studies on *Phaenonotum* from Central America, together with preliminary studies on this genus in the Caribbean and South America (Deler-Hernández, unpublished data) show that reliable identification is possible based on several external morphological characters, especially the morphology of the meso-metaventral process. This structure exhibits some variation between species, especially in the shape of the metaventral process, the width of the mesoventral process, and the "size" of the apical hood of the mesoventral process (Table 1; Fig. 3; figs 10–12 in Deler-Hernández et al. 2013; figs 230–231 in Smetana 1978). Male genitalia, though very similar at first view, provide the most important characters for species identification, such as the shape of the apex and the base of the median lobe, the shape and position of the gonopore, and the shape of the external margin of the parameres (Fig. 4). Body size is also helpful in some cases, allowing the

Table 1. Diagnostic characters of the *Phaenonotum* species described by D. Sharp.

	P. apicale	***P. collare***	***P. exstriatum* (= *P. dubium*)**	***P. laevicolle***	***P. rotundulum***	***P. tarsale***
Total body length	2.9–3.2 mm	3.5–3.9 mm	3.5–3.7 mm	2.5–2.7 mm	2.8–3.3 mm	4.7–4.8 mm
Shape of mesoventral process	arrow-head shaped with a distinct wide hood	arrow-head shaped with a distinct wide hood	arrow-head shaped with a distinct wide hood	arrow-head shaped with distinct narrow hood	arrow-head shaped with indistinct hood	arrow-head shaped, slightly hooded apically
Base of mesoventral process	as wide as apex of metaventral process	slightly wider than apex of metaventral process	as wide as apex of metaventral process	narrower than apex of metaventral process	as wide as apex of the metaventral process	slightly wider than apex of metaventral process
Metaventral process	slender, subparallel-sided	slender, subparallel-sided	slender, subparallel-sided	stout, slightly widened subapically	stout, wide basally, narrowing apically	stout, parallel-sided
Aedeagus: length of parameres	0.4 mm	0.4 mm	0.4 mm	0.5 mm	0.5 mm	0.7 mm
Aedeagus: length of median lobe	reaching apices of parameres	reaching apices of parameres	reaching apices of parameres or nearly so	not reaching apices of parameres	not reaching apices of parameres	slightly overlapping apices of parameres
Aedeagus: basal region of the median lobe laterally	angulate	rounded	nearly straight	nearly straight	slightly curved basally	nearly straight
Aedeagus: apical region of the median lobe	strongly narrowing	widely rounded	widely rounded	widely rounded	widely rounded	widely rounded
Aedeagus: shape of the parameres	wide and curved in median region	wide and slightly sinuate in median region	strongly sinuate in median region	wide and curved in median region	slightly sinuate	slightly sinuate
Aedeagus: shape of the gonopore	oval	transversely oval	transversely oval	transversely subtriangular	oval	rounded

separation of species with rather similar male genitalia. Traditional characters used by previous authors (e.g. Sharp 1882; Smetana 1978), i.e. the dorsal coloration and punctation of pronotum and elytra, are insufficient for a reliable identification, although may be helpful when used in combination with those of the meso-metaventral elevation and the aedeagus.

Acknowledgements

We are indebted to Christine Taylor and Maxwell Barclay (both BMNH) for the opportunity to study the type specimens from the D. Sharp collection under their curatorship. The authors wish to thank Bruno Clarkson (Universidade Federal do Rio de Janeiro) and Miguel Archangelsky (LIESA – Universidad Nacional de La Patagonia) for comments and suggestions to improve the manuscript, as well as Robert S. Anderson (Canadian Museum of Nature, Ottawa) for improving the English. The study was supported by grant SVV 260 313/2016 to ADH and by the Ministry of Culture of the Czech Republic (DKRVO 2016/14, National Museum, 00023272) to MF.

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CHAPTER 7

Deler-Hernández A. and Fikáček M. 2016. Larval morphology and chaetotaxy of three Caribbean *Berosus* Leach, 1817 with revised adult diagnosis of *Berosus undatus* (Fabricius, 1792) (Coleoptera: Hydrophilidae). *Aquatic Insect* 37(2): 99-113.

AQUATIC INSECTS, 2016
VOL. 37, NO. 2, 99–113
http://dx.doi.org/10.1080/01650424.2015.1137324

Larval morphology and chaetotaxy of three Caribbean *Berosus* Leach, 1817 with revised adult diagnosis of *Berosus undatus* (Fabricius, 1792) (Coleoptera: Hydrophilidae)

Albert Deler-Hernández[a] and Martin Fikáček[a,b]

[a]Department of Zoology, Faculty of Science, Charles University in Prague, Czech Republic; [b]Department of Entomology, National Museum in Prague, Czech Republic

ABSTRACT

The morphology and head chaetotaxy of third instar larva of the endemic Cuban *B. chevrolati* Zaitzev, 1908 are described. We provided the diagnosis of third instar larvae of *B. exiguus* (Say, 1825) and *B. cf. undatus* (Fabricius, 1792). This study is based on specimens collected in Cuba and identified by associations with adults. Larvae of *B. exiguus* and *B. cf. undatus* agree well with previously described larvae of *Berosus*. In contrast, *B. chevrolati* represents an unusual morphotype, characterised by narrow and strongly projecting nasale and well-developed right epistomal lobe. We also revise the adult diagnosis of *B. undatus*, which was incorrectly provided by Deler-Hernández, Fikáček, and Cala-Riquelme (2013), and provide additional characters to distinguish this species from *B. interstitialis* Knisch, 1924 and *B. infuscatus* LeConte, 1855.

ARTICLE HISTORY
Received 8 July 2015
Accepted 21 December 2015

KEYWORDS
Coleoptera; Hydrophilidae; *Berosus*; immature stages; larval morphology; Cuba; Neotropical region

Introduction

With more than 270 species distributed worldwide, *Berosus* Leach, 1817 is the most species-rich genus of the family Hydrophilidae (Short and Fikáček 2011, 2013); however, the number of species with described larvae is still low. Described larvae mostly belong to European and North American species (e.g., Bøving and Henriksen 1938; Archangelsky 1994, 1997), and a few Asian, Neotropical and Australian species also have described larvae (Archangelsky 2002; Watts 2002; Minoshima and Hayashi 2015; Rodriguez, Archangelsky, and Torres 2015). In the Caribbean Region, nine species of *Berosus* have been recorded, all of them are found in Cuba, (Hansen 1999; Deler-Hernández, Fikáček, and Cala-Riquelme 2013), but only the larva of the widespread species *B. peregrinus* (Herbst, 1797) is known; larvae of no other Caribbean species have been described until now.

The aim of this study is to describe third instar larva of the endemic Cuban species *B. chevrolati* Zaitzev, 1908 and to offer a diagnosis on the morphology of larval stage of *B. exiguus* (Say, 1825) and *B. cf. undatus* (Fabricius, 1792). We also provide an update of

CONTACT Albert Deler-Hernández ✉ adeler1982@gmail.com; delerhea@natur.cuni.cz

the diagnosis of *Berosus undatus* (Fabricius, 1792) recently published in the review of Cuban *Berosus* (Deler-Hernández et al. 2013).

Material and methods

We examined a total of six larvae of *Berosus* from Cuba in this study, all collected in association with adults (for details see under each species). All larvae were preserved in 70% ethanol directly in the field. The methods of larval studies followed those described by Minoshima and Hayashi (2011). The terminology and format, used in the description of external morphology and chaetotaxy of the larval head, followed those proposed by Archangelsky (1997), Fikáček, Archangelsky, and Torres (2008), Byttebier and Torres (2009), Minoshima and Hayashi (2011, 2015) and Rodriguez et al. (2015). We understand 'nasale' as the median portion of anterior margin of the head (clypeolabrum) between epistomal lobes which bears the setae of gFR1 (see also Minoshima and Hayashi 2011). Due to the low number of larval specimens examined, we were not able to examine all details of some body parts. The mentum was broken during preparation in larvae of *B. chevrolati* and *B. exiguus* and, therefore, was not illustrated and described for these species. Some sensilla (e.g., maxillary MX27 and sensilla originally placed on ligula which is reduced in *Berosus*) are difficult to see and their proper examination usually requires examination of multiple specimens and dissections of the respective body parts, which was impossible with the limited amount of specimens available to us. For this reason, we were not able to decide whether some tiny sensilla are simply overlooked or truly absent in the examined specimens. In these cases, we refer to the respective sensilla as 'not observed' in the chaetotaxy descriptions.

Drawings of the larvae were made with the help of a camera lucida attached to an Olympus BX41 compound microscope, based on temporary glycerine slides of the larvae cleared in 10% KOH solution. Habitus photographs of the larvae and adults of *B. undatus* were taken using a Canon EOS 550D digital camera with attached Canon MP-E65mm f/2.8 1−5× macro lens, and subsequently edited in Adobe Photoshop CS5. All larval and adult material examined in this study was deposited in the collection of the Department of Entomology, National Museum in Prague, Czech Republic (NMPC).

The following abbreviations were used in descriptions of larval chaetotaxy: AN: antenna; FR: frontale; gAN: group of antennal sensilla; gAPP: group of sensilla on inner appendage of maxilla; gFR: group of sensilla on frontale; gLA: group of sensilla on labium; gMX: group of sensilla on maxilla; LA: labium; MN: mandible; MX: maxilla; PA: parietale; SE: sensorium.

Larval descriptions

Berosus chevrolati Zaitzev, 1908

(Figures 1A, 2, 3, 8A)

Material examined

Two larvae of third instar (NMPC), 'Cuba, Santiago de Cuba Prov., III Frente, La Mandarina, 02.III.2006, leg. Y. S. Megna'.

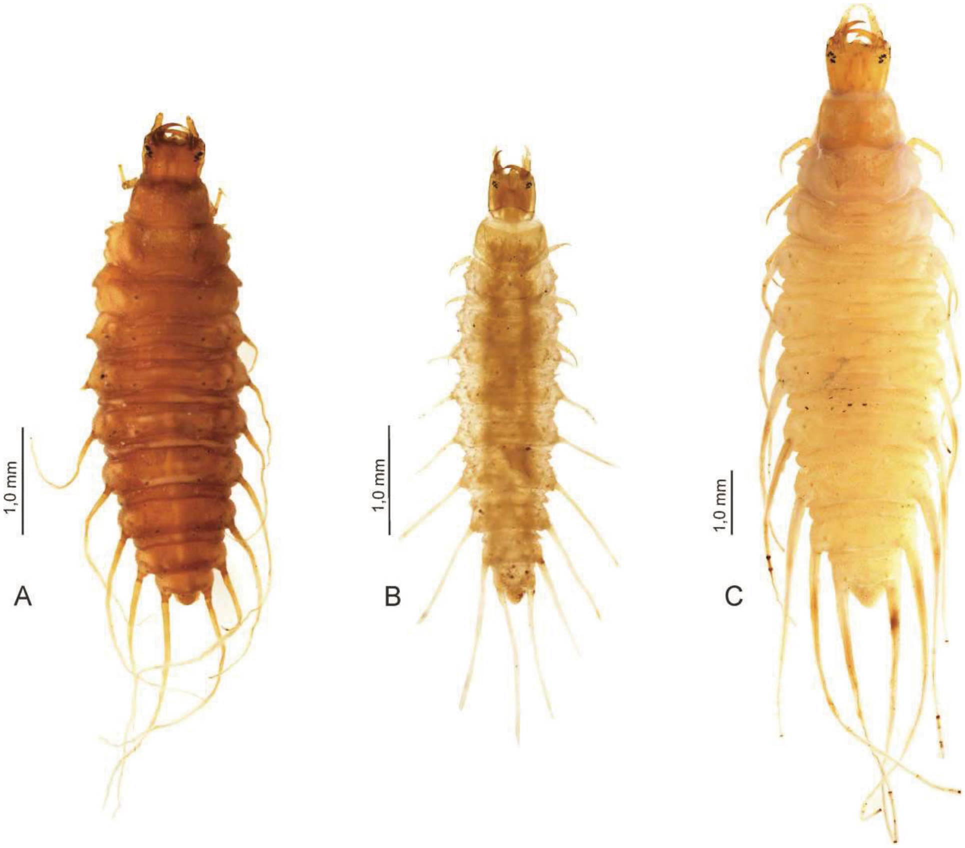

Figure 1. (Colour online) Habitus of the third instar larva: (A) *Berosus chevrolati* Zaitzev, 1908; (B) *B. exiguus* (Say, 1825); (C) *B. cf. undatus* (Fabricius, 1792).

Identification of the larvae

Larvae of *B. chevrolati* examined for this study were collected at margins of a stream with clear water and abundant organic material. This kind of habitat is typically inhabited by *B. chevrolati* and *B. trilobus* Chevrolat, 1863, the latter species is usually much more abundant in Cuba (Deler-Hernández et al. 2013). Although the locality has been sampled many times between the years 2003 and 2006, not a single adult specimen of *B. trilobus* was collected, only adults of *B. chevrolati* were found. Hence, we identified the examined larvae by association with adults as belonging to *B. chevrolati*.

Differential diagnosis

The larvae of *B. chevrolati* show all diagnostic characters of *Berosus* (see Minoshima and Hayashi 2015), but differ from all other *Berosus* with known larvae (including *Berosus* species treated below) in the narrow projecting nasale bearing the four median setae of gFR1, the lateral setae of gFR1 situated between nasale and epistomal lobes, the large well-developed right epistomal lobe, basal external face of the maxillary stipes with a small projection, and seta FR12 very short. It may also be distinguished from other Cuban larvae of *Berosus* by the left epistomal lobe bearing 10 rather stout and curved setae (gFR2, Figure 2C.)

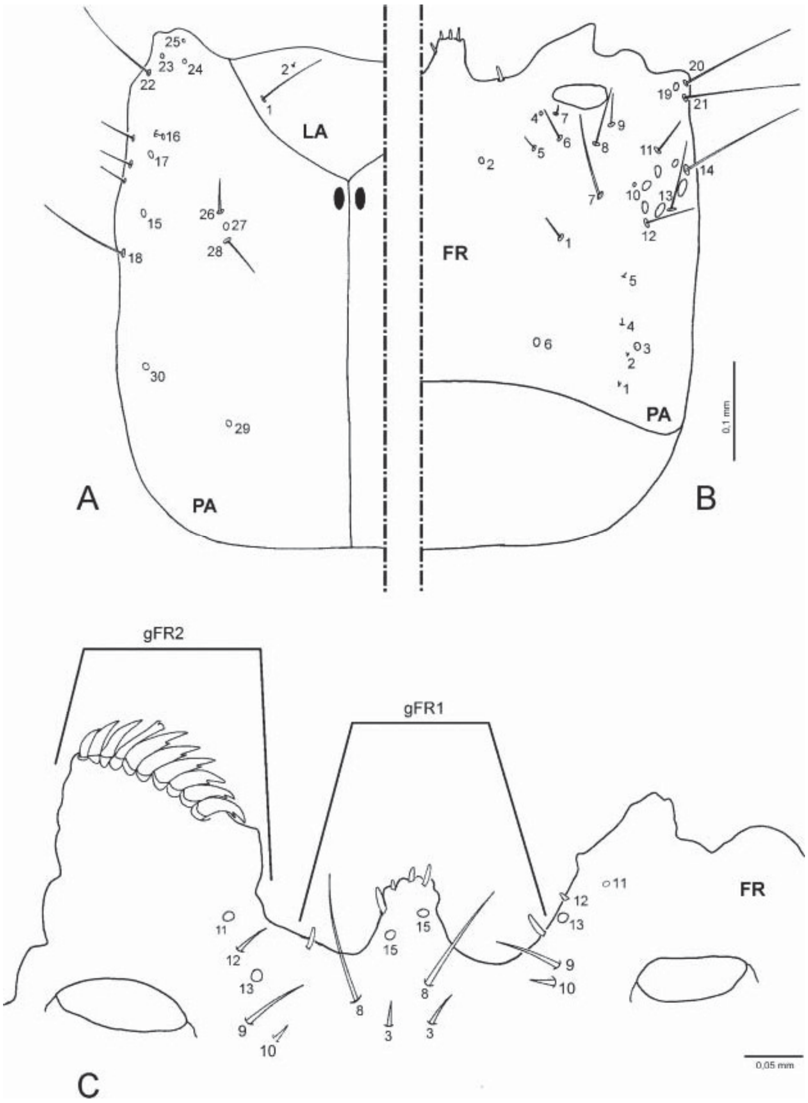

Figure 2. *Berosus chevrolati* Zaitzev, 1908, third instar larva: (A) head capsule, ventral view; (B) head capsule, dorsal view, chaetoraxy of nasal area omitted; (C) anterior margin of head capsule dorsal view.

Description of the third instar larva

General morphology: body moderately stout (Figure 1A). Colour yellow, with sclerotised parts light brown. Total body length about 4.6 mm.

Head (Figures 2A and 2B): head capsule subquadrate; cervical sclerites small. Frontal lines not visible. Six stemmata present on each anterolateral corner of head capsule. Clypeolabrum strongly asymmetrical (Figure 2C). Nasale with large but narrow median projection bearing four stout setae and another stout seta present on each side of median projection (between nasale and epistomal lobes). Lateral epistomal lobes present on both sides: left lobe very strongly projecting anteriorly; right epistome with an asymmetrical projection (Figure 2C).

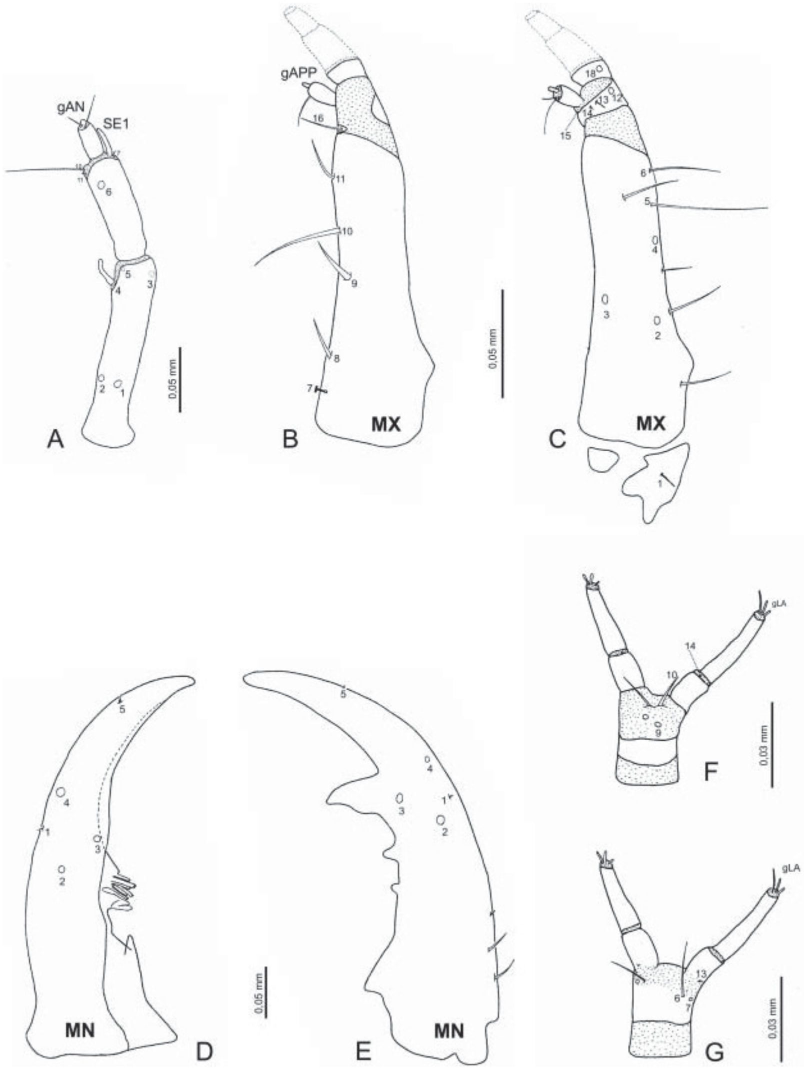

Figure 3. *Berosus chevrolati* Zaitzev, 1908, head appendages of the third instar larva: (A) right antenna, dorsal view; (B) right maxilla, dorsal view; (C) left maxilla, ventral view; (D) left mandible, dorsal view; (E) right mandible, dorsal view; (F) labium, dorsal view (G) labium, ventral view.

Antenna (Figure 3A) three-segmented, long and slender. Antennomere 1 longer than antennomeres 2 and 3 combined. Antennomere 3 shortest.

Mandibles (Figures 3D and 3E) asymmetrical. Right mandible with three inner teeth, distal one larger than the other two (Figure 3E). Left mandible with three teeth, basal tooth sharp and pointing to apex, subbasal tooth stout, bilobed and pointing medially, distal tooth with a comb of six long projections (Figure 3E); dorsal surface of left mandible with a basal groove.

Maxilla (Figures 3B and 3C) six-segmented (including cardo), longer than antenna. Cardo divided in two sclerites, one smaller and oval in shape, the second one irregular in shape (Figure 3C). Stipes longest, its inner face without numerous cuticular spine-like projections, external face with small projection basally. Palpomeres 3 and 4 not observed as they are broken in examined larvae.

Labium (Figures 3F and 3G): submentum fused to head capsule, wide; mentum subtrapezoidal; prementum subquadrate. Ligula absent. Labial palpi two-segmented, well sclerotised, slightly longer than mentum and prementum combined; palpomere 1 shorter than palpomere 2.

Thorax: thoracic membrane covered with fine cuticular pubescence. Pronotum wider than head capsule. Pronotal plate large, covering whole dorsal part of prothorax, subrectangular in shape, with posterior margin rounded and fine sagittal line. Mesonotum with one pair of subtriangular sclerites on anterior margin. Metanotum without sclerites. Meso- and metathorax each longer than prothorax; mesothorax with two pair of lateral tubercles, anterior smaller than posterior; metathorax with pair of lateral tubercles at about mid-length. Legs long, slender, visible in dorsal view, five-segmented (Figure 8A).

Abdomen: 10 segmented, covered with fine densely arranged cuticular projections; segments 1−7 each with pair of tracheal gills, tapering towards apex, subdivided by transverse fold, segments 1−5 similar in shape, each of them longer than segments 6 and 7. Segments 1−3 with one submedian and two sublateral small tubercles. Segments 4−7 with one submedian and one sublateral small tubercles. Spiracular atrium absent. Segment 8 with oval dorsal plate; segment 9 bilobed. Sclerotised ring at base of tracheal gills absent.

Chaetotaxy of head

Frontale (Figure 2B) with primary sensilla only. Short seta FR1 on mid-length of frontale, pore FR2 more anteriorly and medially, seta FR3 short and placed anteriorly close to long seta FR8, two setae (shorter FR5 and longer FR6) and one pore (FR4) near base of each antenna; setae FR7 very short, close of antennal socket. Group of five sensilla placed on area between nasale and epistomal lobes (Figure 2C): minute seta FR10, rather long seta FR9, two pores (FR13 on anterior margin and FR11 near epistome) and one small stout seta FR12. Pore FR15 on median part of nasale. Nasale with group of six stout, short setae (gFR1): four setae on median projection, one stout seta on each side of median projection on anterior margin between nasale and epistomal lobes. Left epistomal lobe with group of about 10 stout setae densely arranged on anterior margin (gFR2).

Parietale (Figures 2A and 2B) with a group of four setae (PA1 and PA2 minute, PA4 and PA5 short), pore PA3 between PA2 and PA4, pore PA6 on basal third of parietale; long setae PA7 located between setae FR1 and PA8 close to frontal line, setae PA8 and PA9 located posterior to antennal socket, pore PA10 in ocular area; anterolateral corner of epicranium with two long setae (PA20 and PA21) and one pore (PA19). Ventral surface with one long seta (PA22) and three pores (PA23, PA24, PA25) near mandibular articulation; two setae (PA16 and PA18), PA16 located quite distant from PA18, was broken in the examined larvae, PA18 near mid-length, pore PA17 close to setae PA16, pore PA15 posteriorly of PA17; two long setae (PA26 and PA28) and one pore (PA27) ventrally on median part of parietale; two pores (PA29 and PA30) on basal third of parietale.

Antenna (Figure 3A): antennomere 1 with five pores, two (AN1 and AN2) on basal portion of dorsal surface, two (AN3 and AN5) on distal margin of antennomere and AN4 on inner face of intersegmental membrane. Antennomere 2 with one dorsal pore (AN6) near distal margin of antennomere; setae AN7, AN10 and AN11 and sensorium (SE1) on intersegmental membrane between antennomeres 2 and 3: AN10 long seta, AN7 and

AN11 minute setae. Antennomere 3 with group of apical sensilla (gAN) on apical membranous area.

Mandibles (Figures 3D and 3E) with five primary sensilla and three secondary setae on basal half along external margin. One minute seta (MN1) laterally at about mid-length, three pores (MN2-4) forming a triangular group at mid-length, MN4 close to external margin, MN2 near to external margin, MN3 close to base of apical retinacular tooth; one minute seta (MN5) subapically. MN6 not observed in the examined specimens.

Maxilla (Figures 3B and 3C): cardo with one moderately long seta (MX1). Stipes ventrally with three pores (MX2, MX3 and MX4) laterally at about mid-length (Figure 3C); external margin of ventral face with two long setae (MX5 and MX6) and four secondary setae; inner margin of dorsal face with row of five setae: MX7 broken in examined larvae, MX8 and MX9 stout, short, MX10 hair-like, MX11 short. Palpomere 1 dorsally with one long basal seta (MX16) on inner face, with one pore (MX12) and two small setae (MX13 and MX14) lateroventrally, MX13 longer than MX14. Basal membrane of inner appendage with small pore (MX15) on ventral surface, pore on dorsal surface (MX17) not observed. Inner appendage with a group of sensilla (gAPP). Palpomere 2 with one pore (MX18) ventrally on median part of distal margin of sclerite. Chaetotaxy of palpomeres 3-4 not observed.

Labium (Figures 2A, 3F, 3G): submentum with two pairs of setae, LA1 long, LA2 minute (Figure 2A). Chaetotaxy of mentum not examined, (broken during preparation in examined larvae). Prementum with one pore (LA9) at mid-length and one long seta (LA10) on dorsal surface; one long seta (LA6) and one pore (LA7) close to external margin on ventral surface; sensilla LA11 and LA12 not observed due to limited number of specimens examined; palpomere 1 with one minute seta (LA13) in basal portion on ventral surface and one pore (LA14) on dorsal face of membranous area between palpomeres 1-2; apical portion of palpomere 2 with group of sensilla (gLA).

***Berosus exiguus* (Say, 1825)**

(Figures 1B, 4A, 5, 8B)

Material examined

One larva of third instar (NMPC), ‘CUBA, Santiago de Cuba Prov., San Miguel de Parada, 29.V.2008, leg. A. Deler-Hernández, temporal lagoon, 20°11'2.50″;N 75°46'17.7″W, 1 m a.s.l.’.

Identification of the larvae

The examined larvae were collected in brackish water in a coastal region, in a temporary pool with clear water, abundant organic detritus on the bottom and rich aquatic riparian vegetation. Based on an extensive inventory of Cuban *Berosus* performed to date (Deler-Hernández et al. 2013), the coastal brackish pools in Cuba are inhabited only by *B. exiguus*. Hence, we identified the examined larvae by association with adults as belonging to *B. exiguus*.

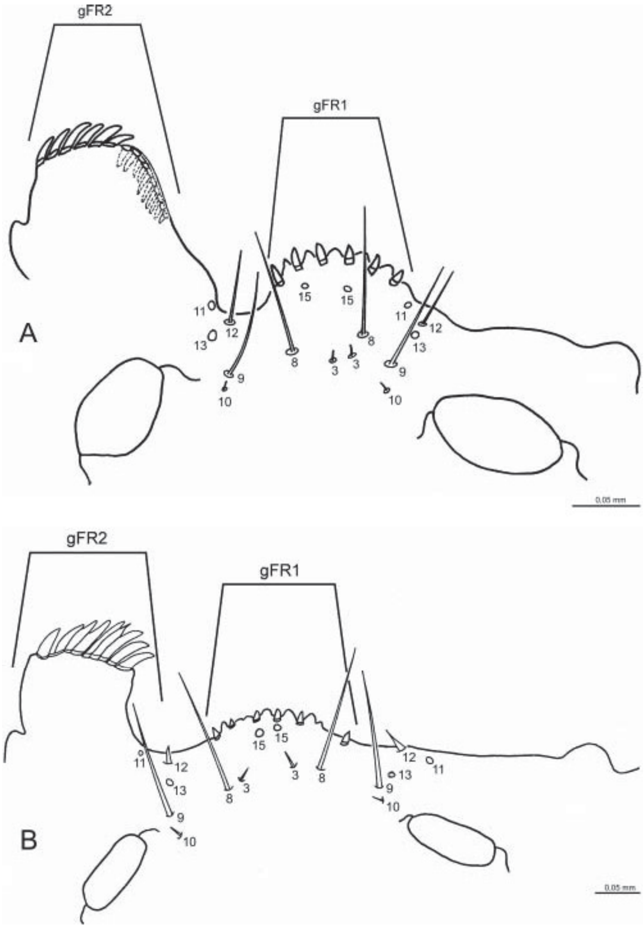

Figure 4. Anterior margin of head capsule, third instar larva: (A) *Berosus exiguus* (Say 1825); (B) *Berosus cf. undatus* (Fabricius 1792).

Differential diagnosis

The larvae of *B. exiguus* can be recognised from the other Caribbean *Berosus* larvae treated here as well as other known larval *Berosus* by the following combination of characters: nasale with a median projection bearing six stout setae (Figure 4A); left epistomal lobe (gFR2) with 16 stout and curved setae (Figure 4A); right mandible bearing three teeth, with basal tooth inconspicuous, median tooth stout and rounded at apex, and distal tooth bigger, stout and bifid (Figure 5D); apex of left mandible with inner face slightly serrate; stipes of maxilla with external face with small projections at mid-length; seta FR12 long.

Berosus cf. undatus **(Fabricius, 1792)**

(Figures 1C, 4B, 6, 7, 8C)

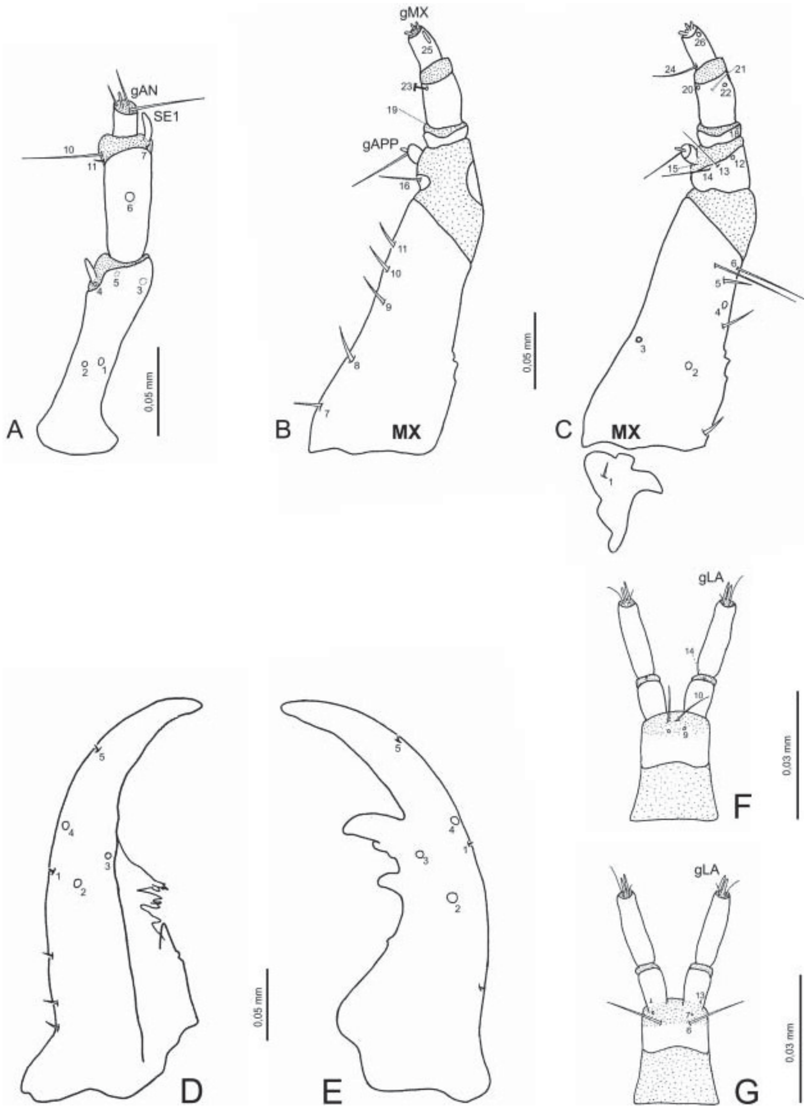

Figure 5. *Berosus exiguus* (Say, 1825), head appendages of the third instar larva: (A) right antenna, dorsal view; (B) right maxilla, dorsal view; (C) left maxilla, ventral view; (D) left mandible, dorsal view (E) right mandible, dorsal view; (F) labium, dorsal view; (G) labium, ventral view.

Material examined

Two larvae of third instar (NMPC), 'Cuba, Guantánamo Prov., Ímias, 24.X.2008, leg. A. Deler-Hernández and S. Muñiz, temporal lagoon, 20°06'05″N 74°69'00″W, 5 m'; one larvae of third instar (NMPC), 'Cuba, Granma Prov., Cauto Cristo, 06.III.2004, leg. L. Chavéz, permanent lagoon, 20°33'33.1″N 76°28'44″W, 5 m'.

Identification of the larvae

The examined larvae were collected in permanent and temporary pools with turbid water, and rich aquatic vegetation. Only adults of *B. undatus* were collected at these localities.

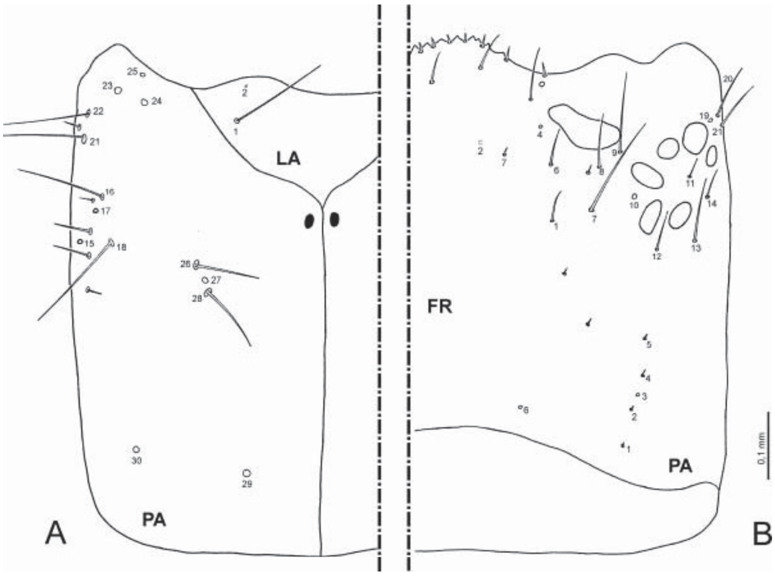

Figure 6. *Berosus cf. undatus* (Fabricius, 1792), head capsule of the third instar larva: (A) ventral view; (B) dorsal view, chaetoraxy of nasal area omitted.

However, as multiple species occur in this kind of habitat in Cuba, and *B. infuscatus* LeConte, 1855 was found in other pools nearby, the identification of the examined larvae is tentative.

Differential diagnosis

The larvae of *B. cf. undatus* can be recognised from the other Caribbean *Berosus* larvae treated here as well as other known larval *Berosus* by the following combination of characters: nasale weakly projecting, bearing six stout setae (Figure 4B); left epistomal lobe (gFR2) with nine stout and curved setae (Figure 4B); right mandible bearing three teeth, basal and median teeth small and sharp, distal tooth bigger, and slightly sharp (Figure 7D); left mandible with inner face slightly serrate (Figure 7E); cardo asymmetrical with two projections (Figure 7C); mentum subtrapezoidal (Figure 7F).

Corrections of the review of adult Cuban *Berosus*

Deler-Hernández et al. (2013) provided a review of the genus *Berosus* of Cuba based on adults, with diagnoses and illustrations of all species, summaries of their distribution and an identification key. After publication of the paper, we found a mistake in the figures and diagnosis of *B. undatus* caused by incorrect identification of the female specimen available to us at that time (the female we examined actually belonged to *Berosus interstitialis* Knisch, 1924). Here, we are providing a correction of the respective parts and illustrations concerning *B. undatus*. In addition, we provide the updated respective part of the identification key, into which we included also the previously overlooked characters of the microsculpture of the pronotum and elytra allowing for an easy identification of *B. undatus*, *B. interstitialis* and *B. infuscatus*.

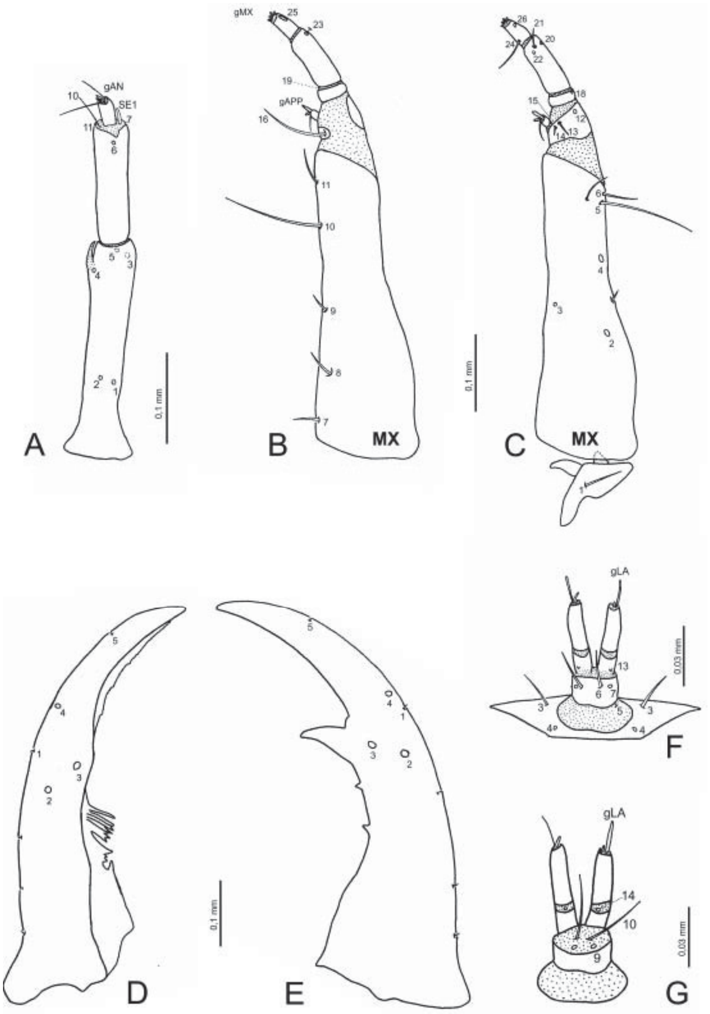

Figure 7. *Berosus cf. undatus* (Fabricius, 1792), head appendages of the third instar larva: (A) right antenna, dorsal view; (B) right maxilla, dorsal view; (C) left maxilla, ventral view; (D) left mandible, dorsal view; (E) right mandible, dorsal view; (F) labium, ventral view (G) labium, dorsal view.

Berosus undatus (Fabricius, 1792)

(Figure 9)

Revised adult diagnosis

Habitus as in Figure 9A and 9B. Body length 6.3–7.2 mm. Head dark, with iridescent metallic reflection; pronotum pale with a pair of closely arranged elongate longitudinal black spots medially. Pronotum with microsculpture on interstices. Elytra without microsculture. Elytral apices with subapical spines in both sexes (Figure 9C and 9D). Mesoventral process lowly laminar, with large posteriorly directed tooth. Abdominal ventrite 1 with median carina developed only between metacoxae. Emargination of abdominal

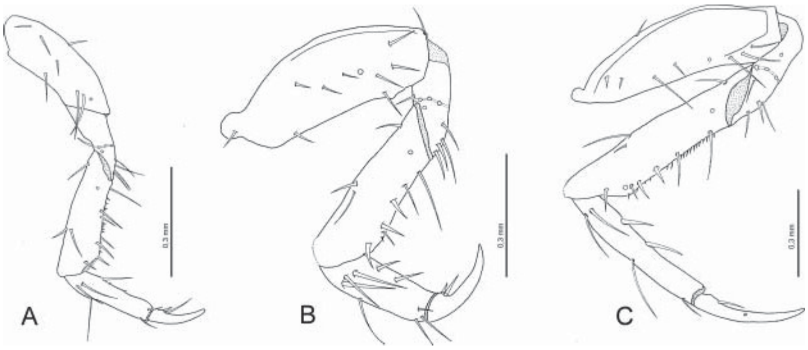

Figure 8. Mesothoracic legs, anterior view: (A) *Berosus chevrolati* Zaitzev, 1908; (B) *Berosus exiguus* (Say, 1825); (C) *Berosus cf. undatus* (Fabricius, 1792).

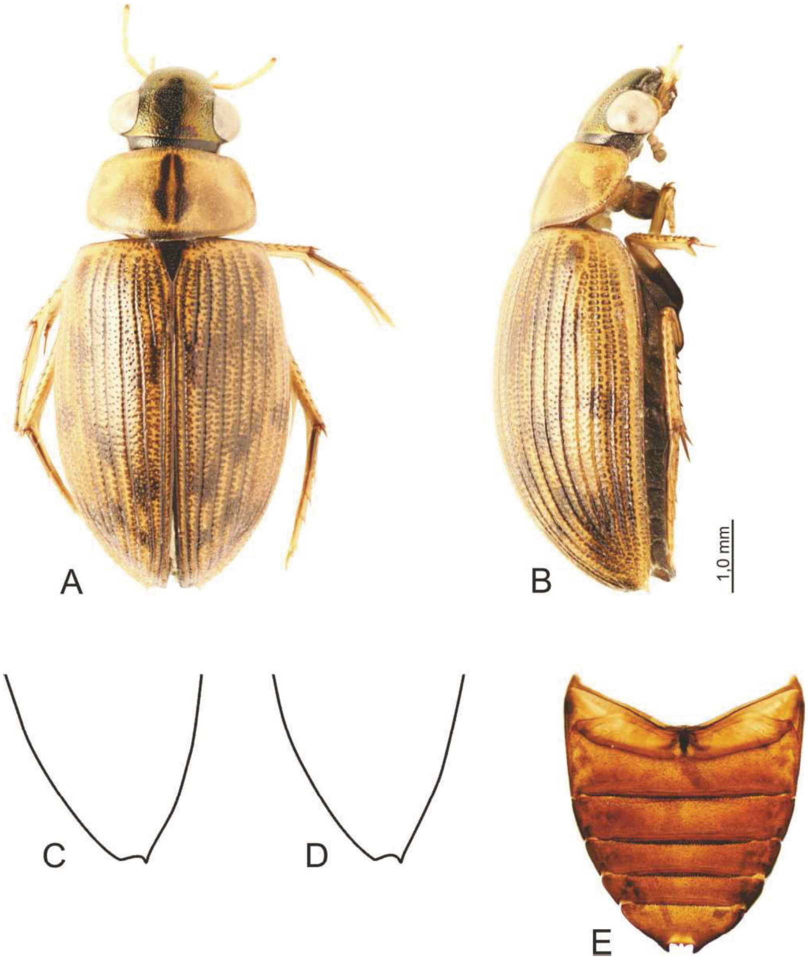

Figure 9. (Colour online) *Berosus undatus* (Fabricius, 1792), adult characters: (A) habitus, dorsal view; (B) habitus, lateral view; (C) apex of male elytron; (D) apex of female elytron; (E) abdominal ventrites.

ventrite 5 rectangular, with two broad and short medial teeth (Figure 9E). Aedeagus (see Figure 10d−f in Deler-Hernández et al. (2013)) with median lobe about as long as parameres, lateral margins of parameres subparallel, except apically; median lobe narrow in ventral view, slightly wider in lateral view.

Revised key to identification of Cuban Berosus

The following couplets need to be corrected in the key provided by Deler-Hernández et al. (2013):

7 Elytral apices entire (without subapical spines) in both sexes. Pronotal punctation uniform, all punctures of the same size. Phallobase longer than a half of total length of aedeagus……………………………………………………………………**8**

\- Elytral apices with subapical spine in both sexes (Figure 9C and 9D). Pronotal punctation consisting of punctures of two different sizes. Phallobase shorter than one half total length of aedeagus…………………………………… ***B. undatus*** **(Fabricius, 1792)**

8 Mesh-like microsculpture on interstices absent on disc of pronotum, elytra without microsculture. Mesoventral process with very small tooth. Posterolateral angles of metaventral process triangular. Aedeagus narrow, lateral margins of parameres subparallel, base of each paramere with a conspicuous tooth ……………………………………………***B. interstitialis*** **Knisch, 1924**

\- Pronotum with strong mesh-like microsculpture on interstices, elytra microreticulate in females but without apparent microreticulation in males. Mesoventral process with larger tooth. Posterolateral angles of metaventral process with rounded laminae. Parameres sinuate on lateral margins, base of parameres without conspicuous teeth……………………………………***B. infuscatus*** **LeConte, 1855**

Discussion

The larvae of the three Cuban species of *Berosus* treated above agree with other known larvae of *Berosus* in most characters. They may be easily assigned to *Berosus* by the general habitus, with abdominal segments bearing lateral tracheal gills. The other diagnostic characters of *Berosus* also present in these larvae: clypeolabrum asymmetrical, left epistomal lobe strongly projecting forward, bearing several stout and curved setae, and mandibles strongly asymmetrical, with the left one bearing dorsal groove and combs of projections on inner teeth (Figure 3D). Larvae of *B. exiguus* and *B. cf. undatus* also agree with all other described *Berosus* larvae in most other characters. In contrast, larvae of Cuban endemic *B. chevrolati* differ from all known larvae of *Berosus* worldwide in the morphology of the clypeolabrum: (1) right epistomal lobe present (absent in all other *Berosus* with known larvae); (2) median portion of the nasale narrow and projecting far anteriad (wide and lowly projecting in other *Berosus*), (3) the projecting portion of nasale bears four setae only, the lateral two are situated on non-projecting portion of the nasale (all six setae are situated in projecting portion of nasale in other *Berosus*). Hence, the larva of *B. chevrolati* described here represents an unusual morphotype of larval *Berosus*. Adults of *B. chevrolati* (and also of the Caribbean endemic *B. trilobus*) are rather unusual amongst other *Berosus* in the small, highly sculptured body, morphology of the aedeagus, and the form of the mesoventral carina (see Deler-Hernández et al. (2013) for details). Hence, we suppose that the unusual larval morphology of *B. chevrolati* may also be of phylogenetic importance. A phylogenetic study of the genus *Berosus* is in preparation (B. Clarkson, personal communication, 2015) and the relevance of the larval morphology of *B. chevrolati* may be discussed later, after the results of this study are known.

Until now, the presence versus absence of the right epistomal lobe was considered as an easy character to distinguish *Berosus* larva (right epistomal lobe absent) from the rather similar larvae of *Laccobius* Erichson, 1837 and *Oocyclus* Sharp, 1882 (right epistomal lobe always present and large), especially in taxa with aberrant morphology in which the presence or absence of tracheal gills cannot be used (M. Fikáček and Y. Minoshima, personal observations). However, the discovery of the larva of *B. chevrolati* shows that the presence/absence of the right epistomal lobe cannot be used to diagnose *Berosus* from the laccobiine genera, as some *Berosus* larvae also bear the right epistomal lobe. On the other hand, the head chaetotaxy works well to differentiate between *Berosus* and these Laccobiini, as it remain unmodified even in morphologically unusual larvae of both groups (M. Fikáček and Y. Minoshima, personal observations). Thus, in cases of morphologically aberrant larvae of *Laccobius* and *Oocyclus,* characteristics of chaetotaxy should be used to distinguish them from *Berosus* larvae.

Acknowledgements

The authors wish to thank Bruno Clarkson (Universidade Federal do Rio de Janeiro) and the other anonymous reviewer for comments and suggestions to the manuscript, as well as Robert S. Anderson (Canadian Museum of Nature, Ottawa) for improving the English.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The study was supported by the grant [SVV 260 313/2016] to Albert Deler-Hernández and by the Ministry of Culture of the Czech Republic [DKRVO 2016/14, National Museum, 00023272] to Martin Fikáček.

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CHAPTER 8

Deler-Hernández A. and J. A. Delgado. 2017. The Hydraenidae of Cuba (Insecta: Coleoptera) II: Morphology of preimaginal stages of six species and notes on their biology. *Zootaxa* 4238 (4): 451–498.

Zootaxa 4238 (4): 451–498
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4238.4.1
http://zoobank.org/urn:lsid:zoobank.org:pub:C82909F6-9264-45D1-A04F-43656263A4D6

The Hydraenidae of Cuba (Insecta: Coleoptera) II: Morphology of preimaginal stages of six species and notes on their biology

ALBERT DELER-HERNÁNDEZ[1] & JUAN A. DELGADO[2]

[1] *Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44 Praha 2, Czech Republic.*
E-mail: adeler1982@gmail.com

[2] *Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad de Murcia, 30100, Murcia. Spain.*
E-mail: jdelgado@um.es

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Abstract

Preimaginal stages of the six species of Hydraenidae presently known from Cuba were obtained by rearing adults in the laboratory. Eggs of *Hydraena perkinsi* Spangler, 1980, *H. decui* Spangler, 1980 and *H. franklyni* Deler-Hernández & Delgado, 2012 are described and illustrated for the first time. The first instar larva of *Gymnochthebius fossatus* (LeConte, 1855) is redescribed, adding some new remarkable morphological characters including what could be the first abdominal egg-burster reported for this family. All larval instars of *H. perkinsi*, *H. guadelupensis* Orchymont, 1923 and *Ochthebius attritus* LeConte, 1878 are described and illustrated for the first time, with a special emphasis on their chaetotaxy. The second instar larva of *G. fossatus* along with first and third instar larvae of *H. decui* and *H. franklyni* are also studied for the first time. The pupal morphology and vestiture of a species belonging to the genus *Hydraena* are described for the first time, based on the pupa of *H. perkinsi.* Biological notes for several preimaginal stages of the studied species are also given.

Key words: Coleoptera, Hydraenidae, preimaginal stages, egg, larva, pupa, chaetotaxy, Cuba, Neotropical Region

Resumen

En este trabajo se estudian los estados preimaginales de las seis especies de la familia Hydraenidae actualmente conocidas en Cuba. Los huevos de *Hydraena perkinsi* Spangler, 1980, *H. decui* Spangler, 1980 e *H. franklyni* Deler-Hernández y Delgado, 2012 se describen y figuran por vez primera. La larva del primer estadio de *Gymnochthebius fossatus* (LeConte, 1855) se redescribe y se señala la presencia de algunos rasgos interesantes como lo que parece ser el primer diente ovir-

Accepted by P. Perkins: 23 Jan. 2017; published: 7 Mar. 2017

ruptor abdominal descrito en una larva de esta familia. Igualmente se describen los tres estadios larvarios de *H. perkinsi*, *Hydraena guadelupensis* Orchymont, 1923 y *Ochthebius attritus* LeConte, 1878 haciéndose una especial mención a su patrón quetotáxico. El segundo estadio de *G. fossatus*, así como el primer y el tercer estadio larvario de *H. decui* e *H. franklyni* también son estudiados y descritos. Por vez primera se describe en detalle la morfología y la distribución de las proyecciones cuticulares de una especie del género *Hydraena* en fase de pupa, basándose esta descripción en la pupa de *H. perkinsi.* También son aportadas algunas notas sobre la biología de todos estos estados preimaginales.

Palabras clave: Coleoptera, Hydraenidae, estados preimaginales, huevos, larvas, pupas, quetotaxia, Cuba, Región Neotropical

Introduction

Members of the beetle family Hydraenidae are common inhabitants of diverse aquatic and semiaquatic habitats in the Nearctic and Neotropical Regions. Six species are presently known from the island of Cuba (Deler-Hernández & Delgado 2012) although at least two additional undescribed species are known to us. In contrast to adults, whose knowledge in the New World has been greatly enhanced due mainly to the comprehensive works of Perkins (1980, 2011), information on preimaginal stages is rather scarce and biological information is sporadic. In addition to some descriptions offered in the pioneering work of Richmond (1920), only a few recent works have focused on the larval morphology of a number of American species. Richmond (1920) obtained eggs of *Gymnochthebius fossatus* (LeConte, 1855) (cited by him as *Ochthebius tuberculatus* LeConte, 1878) as well as from *Hydraena pennsylvanica* Kiesenwetter, 1849 and *Limnebius discolor* Casey, 1900, describing and illustrating the egg envelope of the two first-mentioned species. He also provided information on the egg-laying habits and timing of hatching for all these species, in conjunction with descriptions of first instar larvae of *G. fossatus* and *H. pennsylvanica*. Without any new significant contribution for decades, this account on eggs and larvae remained the main information source on immature Hydraenidae for the New World as well as for many general treatises on aquatic Coleoptera (Bøving & Henriksen 1938; Bertrand 1954; Leech & Chandler 1956; Spangler 1991).

Perkins (1980) offered the first detailed description of some larval structures, focusing especially on the shape and chaetotaxy of the labrum, antennae, urogomphi and distal abdominal segments of the Nearctic species *Hydraena circulata* Perkins, 1980, *Limnebius alutaceus* (Casey, 1886), *Limnebius arenicolus* Perkins, 1980, *Ochthebius tubus* Perkins, 1980 and *Ochthebius gruwelli* Perkins, 1980. Before this major work on the Nearctic fauna, preimaginal stages of several species were previously studied also by Perkins (1972) as a part of his unpublished MSc thesis. Larvae of four species of *Ochthebius* reared by him in laboratory: *Ochthebius interruptus* LeConte, 1852, *Ochthebius lineatus* LeConte, 1852, *G. fossatus* (cited in the text as *Ochthebius fossatus*) and *Ochthebius puncticollis* LeConte, 1852, along with one unidentified species of *Hydraena* and two of *Limnebius* were studied. From these species, seven larval structures were selected to be described and figured: labrum, clypeus, tibia, claw, terminal abdominal segments, urogomphi and antennae. Egg morphology and biological remarks of *O. interruptus* and *O. puncticollis* were also presented.

Preimaginal stages of the Neotropical fauna of Hydraenidae have received rather less attention, although the few described species have been studied in more detail. Delgado *et al.* (1997a) described the third instar larvae of *Hydraena particeps* Perkins, 1980 and Delgado & Archangelsky (2005) provided a thorough description of the larvae of *Gymnochthebius jensenhaarupi* (Knisch, 1924). In both works, a special emphasis was made in the study of larval chaetotaxy, whereas no information was offered about eggs or pupae. Delgado *et al.* (1997b) performed a short study presenting preliminary data on the life cycle of three species of Nicaraguan *Hydraena*, addressing especially *H. particeps*. In the case of Cuba, Spangler (1973) found two hydraenid larvae in the margins of a stream, near Topes de Collantes Natural Landscape Protected Area. One of them was judged to be an immature of *G. fossatus* by association with the single hydraenid species known at that time from Cuba: *Ochthebius nitiduloides* Orchymont, 1943. A second larva was an unidentified species of *Hydraena*. Unfortunately, neither of them were described.

As in many other families of Coleoptera, it is obvious that comprehensive works on immature stages are needed not only to find significant traits that could serve as diagnostic features for accurate identifications, but also to gain a better understanding of aspects of the natural history. On this background, our objective was to describe the external morphology of preimaginal stages obtained *ex ovo* from all the species of Hydraenidae occurring in Cuba as a new step in our aim to understand the biodiversity and biology of all the members of that family in this Caribbean island. The present paper is partially based on the MSc thesis of the first author.

Materials and methods

Source of material. To ensure an accurate identification, immature stages used in this study were obtained by rearing the adults in the laboratory. Rearing techniques used by us follow those described by Delgado *et al*. (1996b) and Deler-Hernández & Delgado (2011). Adults were captured in the field at several localities in East Cuba (listed in "material examined" sections under each species) between years 2009 and 2012, transported alive to our lab at Santiago de Cuba and transferred there to small plastic aquaria (Fig. 1a). In each aquarium, water of the same habitat where the adults were collected, along with small stones covered with algae, decaying or rotten leaves and substrate of the sampling locality were included to simulate natural conditions. These recipients were prepared at least two weeks before the start of the rearing, in order to insure that no eggs or larvae were present. The aquaria were placed in a shaded place, illuminated by natural light and at room temperature. During the rearings, aquaria were checked daily and larvae were extracted from them. Pupae were removed from aquaria immediately after detection, while eggs were only obtained at the end of some rearings. During this period, instar stage was determined by body parameters observable to the naked eye, mainly body length and shape. After several unsuccessful attempts to rear *Hydraena decui* Spangler, 1980 and *Hydraena franklyni* Deler-Hernández & Delgado, 2012 in our lab at Santiago de Cuba (44 m.a.s.l., 20°2'40.70"N 75°46'48.58"W), rearing experiments were relocated to a highland biological station (1100 m.a.s.l, 20°0'37.52"N 75°38'12.20"W) close to the natural localities where these species occur (Deler-Hernández & Delgado 2012). Due to logistic reasons, these aquaria were only checked at one- or two-week intervals. Although rearing experiments were conducted to obtain material for morphological descriptions, information on some other biological aspects was also collected when possible.

Preparation and study of specimens. Larvae and pupae were taken with a fine brush from aquaria and directly preserved in 70% ethanol. Eggs and pupae were preserved in the same medium. Most parts of the larvae and pupae examined were cleared in lactic acid for several days and examined as whole mount preparations. A few larvae were detached, macerated in 10% KOH and stained with chlorazol black. Observations, drawings and photographs were made using either a microscope Nikon Eclipse 80i or a Nikon Eclipse E600. The material studied is deposited in the following collections: (CDUM) Departamento de Zoología, Universidad de Murcia, Murcia, Spain (J. Delgado) and (NMPC) National Museum, Prague, Czech Republic (M. Fikáček).

Morphometrics. Some measurements were taken for eggs, larvae and pupae. Four measurements were taken for eggs: EL (egg length): length measured along the major axis of egg (considering it as an ellipsoid); EW (egg width): length measured along one of the minor axes of egg; EEL (egg envelope length): measured as the maximum length of the egg envelope (because the margins are irregular, and frequently are obscured by substrate particles, this value must be approximated); EEW (egg envelope width): measured as the maximum width of the egg envelope (also an approximate value). In larvae, two measurements were taken: total body length: full body length measured from the anterior margin of labrum to the posterior end of segment X, and head cephalic width: maximum width of cephalic capsule in dorsal view excluding stemmata. In pupae, total body length was measured as the distance from anterior margin of prothorax, excluding cuticular projections, to the distal end of segment X, excluding urogomphi. All data were analysed using measures of central (arithmetic mean) and dispersion (standard deviation: SD) tendency. Measurements were carried out on light microscope images using Nikon NIS-Elements software version 3.03.

Chaetotaxy. A series of useful but different setal nomenclatural systems have been proposed to describe the chaetotaxic pattern in larval Coleoptera (Alarie 1991, 1995; Alarie & Michat 2007; Ashe & Watrous 1984; Ashe 1986; Bousquet & Goulet 1984; Fikáček *et al*. 2008; Kovarik & Passoa 1993; May 1994; Marvaldi 2003; Michat *et al*. 2007; Wheeler 1990), although a unifying descriptive method is still wanting. The terminology and format used here follows that of previous hydraenid larval descriptions published by us (Delgado & Soler 1996a–b, 1997a–d; Delgado *et al*. 1997a, 1998; Delgado & Palma 1998, 2004; Delgado & Mutsui 2000; Delgado 2003; Delgado & Archangelsky 2005). Our setal labeling system considers three types of setae: a) primary setae, and two types of additional ones: b) sub-primary and c) secondary setae. Primary setae are a set of setae that occur in all instars (and all species studied by us so far) with a stable pattern. These setae have a fixed number and position on each morphological region and can be easily homologized among different species and higher taxa. They are the single set of setae present in the first instar larva. Additional setae occur only in subsequent instars. Sub-primary setae are present in second and third instars, and also in a constant number and approximate position. Subprimay setae are considered a complement of the pattern of primary setae observed in first instars. Frequently, in a given species some subprimary setae may be absent, but always lacking in the same position and in all individuals of this species. Conversely, secondary setae are setae that may occur, or not, in a particular specimen. Among the individuals of a species these setae are remarkably variable in number and often occur in great number and at random positions. As the three types of setae have the same aspect, the presence of secondary setae obscures the identification and labeling of primary and subprimary ones. In our labeling system, primary setae are arranged in series or rows throughout sclerotized plates, anatomical fields or appendages and are designated by a combination of one or more letters and a number. The former letter (or letters) represents the relative position of the set or row on a sclerite (A: anterior, L: lateral, D: discal, P: posterior, etc.) or refers to a sclerite or appendage (Prehy: prehypopleuron; M: mandible, Cdo: cardo, etc.). Clusters of setae are frequently arranged into pairs of distinct series or rows, along the margins or disc of a sclerite, either vertically (e.g. bordering the lateral margin of a sclerite and parallel to its median line) or horizontally oriented (i.e. bordering either the anterior or posterior margins of a sclerite). The latter number of each labeled seta refers to the relative position on its set or row, with increasing consecutive values backward (in vertical rows), laterad from median axis (in horizontal rows) or distad (in some appendages and articles). Subprimary setae are assigned to the nearest set of primary setae and are labeled as a primary seta, adding between letters and number either the prime symbol (') or the double prime symbol (''). Secondary setae are not labeled when they occur. Being aware of the reflections exposed in Solodovnikov (2007) about the description and real nature of the different pores observed on the larval cuticle in Coleoptera, we have decided to keep the same labels for these pores as in previous descriptions, in order to facilitate comparisons.

Results

Description of the immature stages of *Hydraena perkinsi* Spangler, 1980

Egg (Figs. 1b–d)**. Material examined**. **CUBA**: 2 eggs (CDUM): "Santiago de Cuba, La Redonda, Río Sevilla, 20º00'54.3"N 75º45'45.6"W, 15 m, 9.xii.2009, coll.: A. Deler-Hernández"; same locality: 6 eggs (CDUM), 27.xi.2010; 13 eggs (CDUM), 3.xii.2011; 4 eggs (CDUM): "San Luis, Dos Caminos, El Vivero, Laguna temporal, 20º11'2.50"N 75º46'17.7"W, 150 m, 06.ix.2011, coll.: A. Deler-Hernández". Starting dates for rearing not available.

Description. Elongate, ellipsoidal, with round poles (Figs. 1b–d). Color white to yellowish. Chorion thin, smooth, without any trace of chorionic sculpture observed at 400X. Each egg is concealed by an external membrane or protective coat but it can be perfectly seen and measured by transparency. EL: 0.44±0.02 mm (mean±SD.; n= 12); EW: 0.17±0.01 mm (n= 11); ELL: 0.65±0.04 mm (n= 3); EEW: 0.38±0.02 mm (n= 3).

Biological remarks. Eggs were laid in the aquaria attached to a variety of submersed objects such as stones, algae, recipient walls and, especially, decaying leaves. Eggs were protected by a whitish covering layer extending beyond the limits of the chorion to form an irregular flange, which confers the whole a "fried egg-shaped" appearance (Fig. 1b). This covering coat seems to be a hardened mass of froth or spumaline produced by the female, which presumably serves to protect the egg and likely used for attaching it to the substrate. This external coat is normally scattered with substrate particles and even filamentous material. Females normally laid eggs singly (Fig. 1b) but, in some cases, two or more eggs are deposited following a linear pattern, with their major axes similarly oriented (Fig. 1d). In other cases, some eggs can be observed disposed in batches (Fig. 1c). In this last case, the chaotic arrangement of the cluster suggests that these batches are most probably the result of random egg-laying activities of one or several females than a deliberate behavior. Unfortunately, no data were obtained about the number of eggs laid by a single female during our study. The egg stage lasted 3 to 5 days in our rearing experiments at a temperature of 30–35ºC. After hatching, a longitudinal tearing of the chorion can be seen at the main body axis of the egg, through which the larva has exited the egg (see Fig. 1e).

Larva

First instar larva (Figs. 2b; 3–6; 28e). **Material examined**. **CUBA**: 4 larvae (CDUM): "Santiago de Cuba, Humedal San Miguel de Parada, Río Cobre, Punta de Sal, 20°0'56.40"N 75°52'15.14"W, 3 m, starting rearing 08.vii.2009, larvae collected 13.vii.2009, coll.: A. Deler-Hernández"; same locality: 1 larva (CDUM), starting rearing 08.vii.2009, larva collected 02.viii.2009; 1 larva (CDUM), starting rearing 01.ix.2009, larva collected

17.ix.2009; 2 larvae (CDUM): “Santiago de Cuba, La Redonda, Río Sevilla, 20º00’54.3”N 75º45’45.6”W, 15 m, starting rearing 19.xii.2009, larvae collected 27.xii.2009, coll.: A. Deler-Hernández”; same locality: 2 larvae (CDUM), starting rearing 19.xii.2009, larvae collected 07.i.2010; 4 larvae (CDUM), starting rearing 19.xii.2009, larvae collected 13.i.2010; 1 larva (CDUM), starting rearing 19.i.2010, larva collected 23.i.2010; 7 larvae (NMPC), starting rearing 27.xi.2010, larvae collected 19.xii.2010; 1 larva (NMPC), starting rearing 27.xi.2010, larva collected 30.xii.2010; 4 larvae (NMPC), starting rearing 11.xii.2010, larvae collected 24.xii.2010.

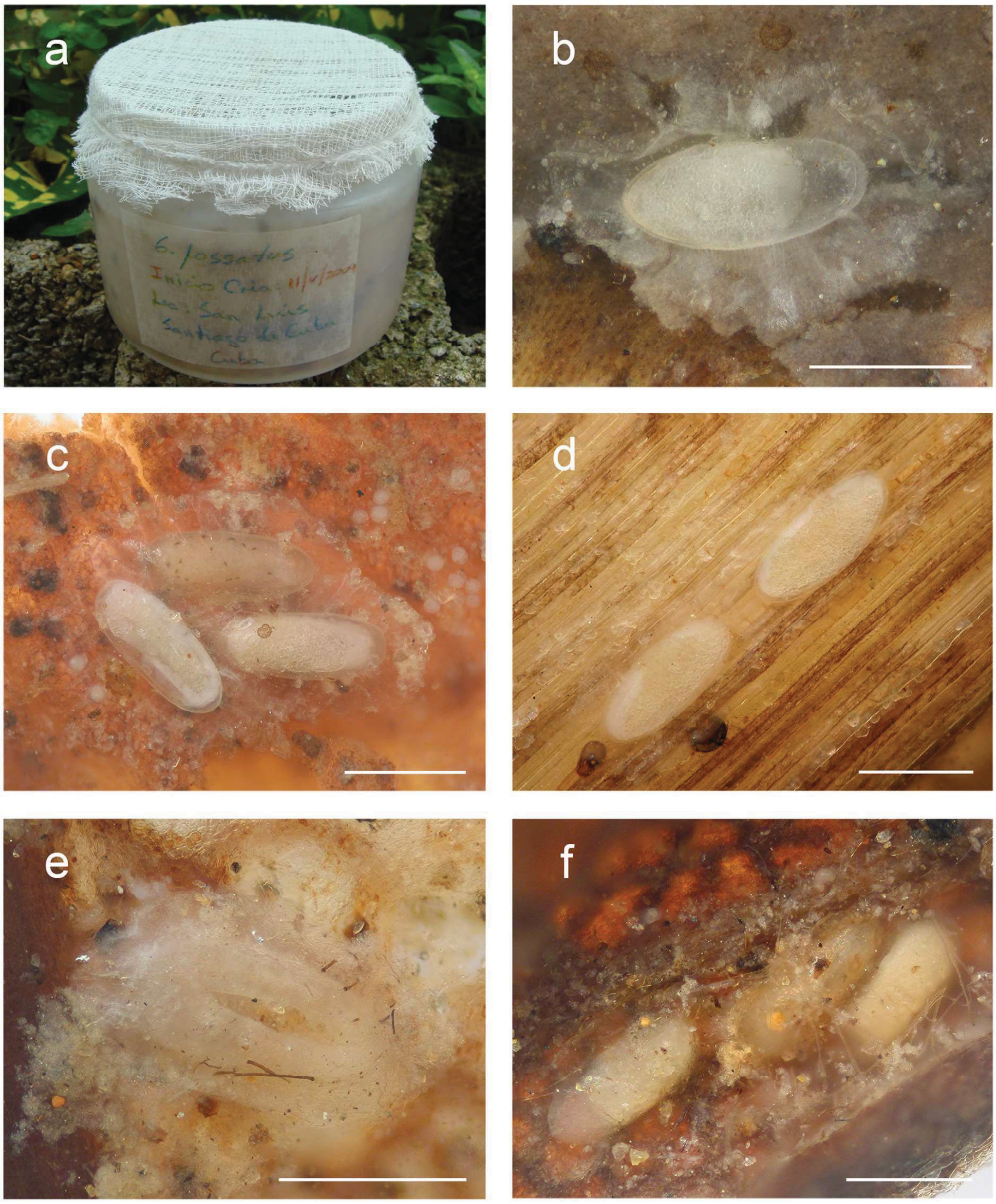

FIGURE 1. Example of one of the plastic boxes used during rearing experiences (a) and eggs of three Cuban *Hydraena*: b, *H. perkinsi* from La Redonda; c–d, same species from Sevilla river; e, *H. franklyni* from type locality; f, *H. decui* from Gran Piedra. Note that the embryo is clearly visible through the chorion in some eggs. Scale bars: 0.3 mm.

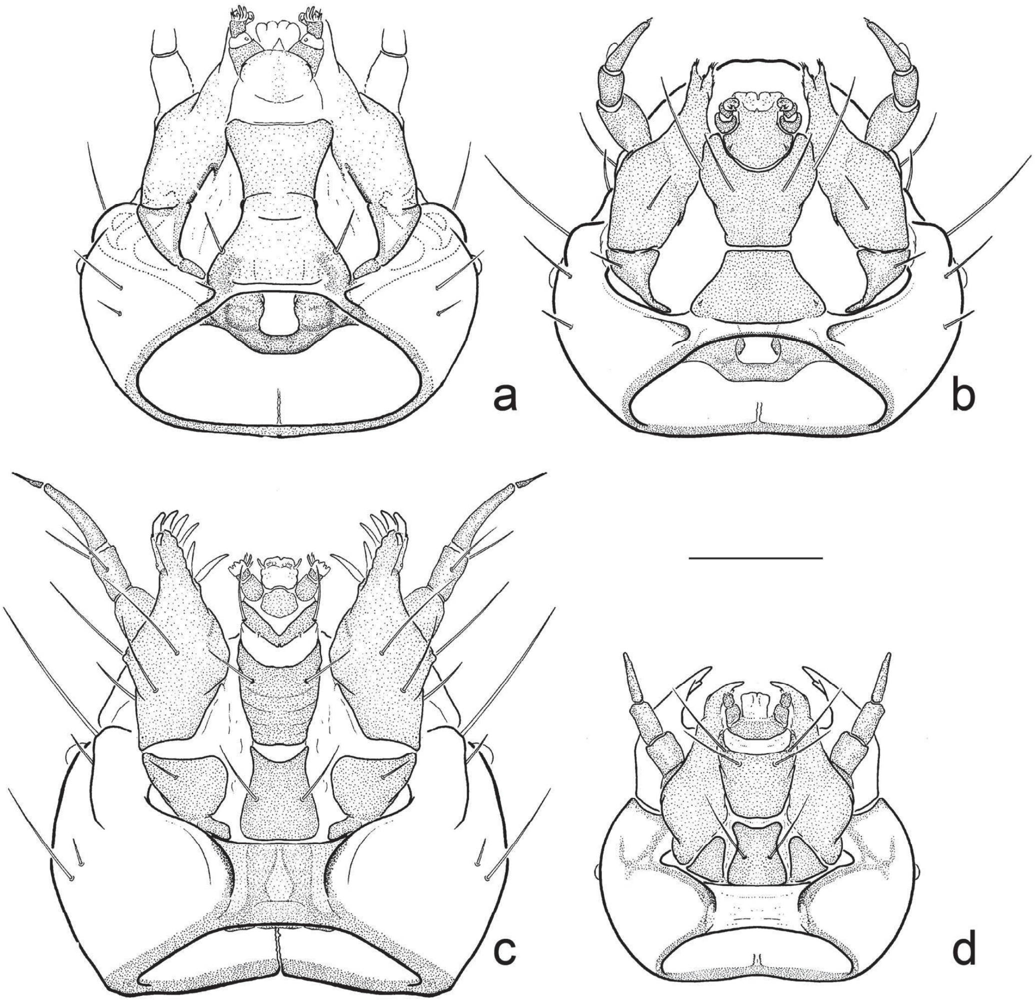

FIGURE 2. Head capsule in ventral view showing shape and position of paired posterior tentorial pits of: a, *H. guadelupensis*; b, *H. perkinsi*; c, *O. attritus*; d, *G. fossatus*. Scale bar: 0.1 mm.

Description. General aspect as in Fig. 3. Total body length: 1.20±0.16 mm (mean±SD.; n= 13). General body form elongate and slender. Head as wide as thorax, abdomen approximately parallel-sided and only slightly tapered posteriorly. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous areas grayish. Maximum head width: 0.19±0.005 mm (mean±S.D.; n= 12). Head somewhat hypognathous, slightly emarginated posteriorly, globose in lateral view. Ecdysial line Y-shaped, epicranial stem long, frontal arms V-shaped connecting distally with base of antennae. Head capsule (Fig. 4a) divided into three regions: a medial frontal area bordered by the frontal arms and two epicranial plates, located behind and laterad to the frontal arms. Gula distinct but remarkably narrow, closing the cephalic capsule ventrally, almost fused to submentum anteriorly and bordered laterally by short tentorial pits (Fig. 2b). Occipital foramen divided into two parts by tentorial bridge (see Fig. 10c). Epicranial plates each with five globose stemmata, forming a dorsolateral cluster behind the base of antennae (Fig. 4b).

FIGURE 3. *Hydraena perkinsi*, first instar larva in dorsal and lateral view. Scale bar: 0.3 mm.

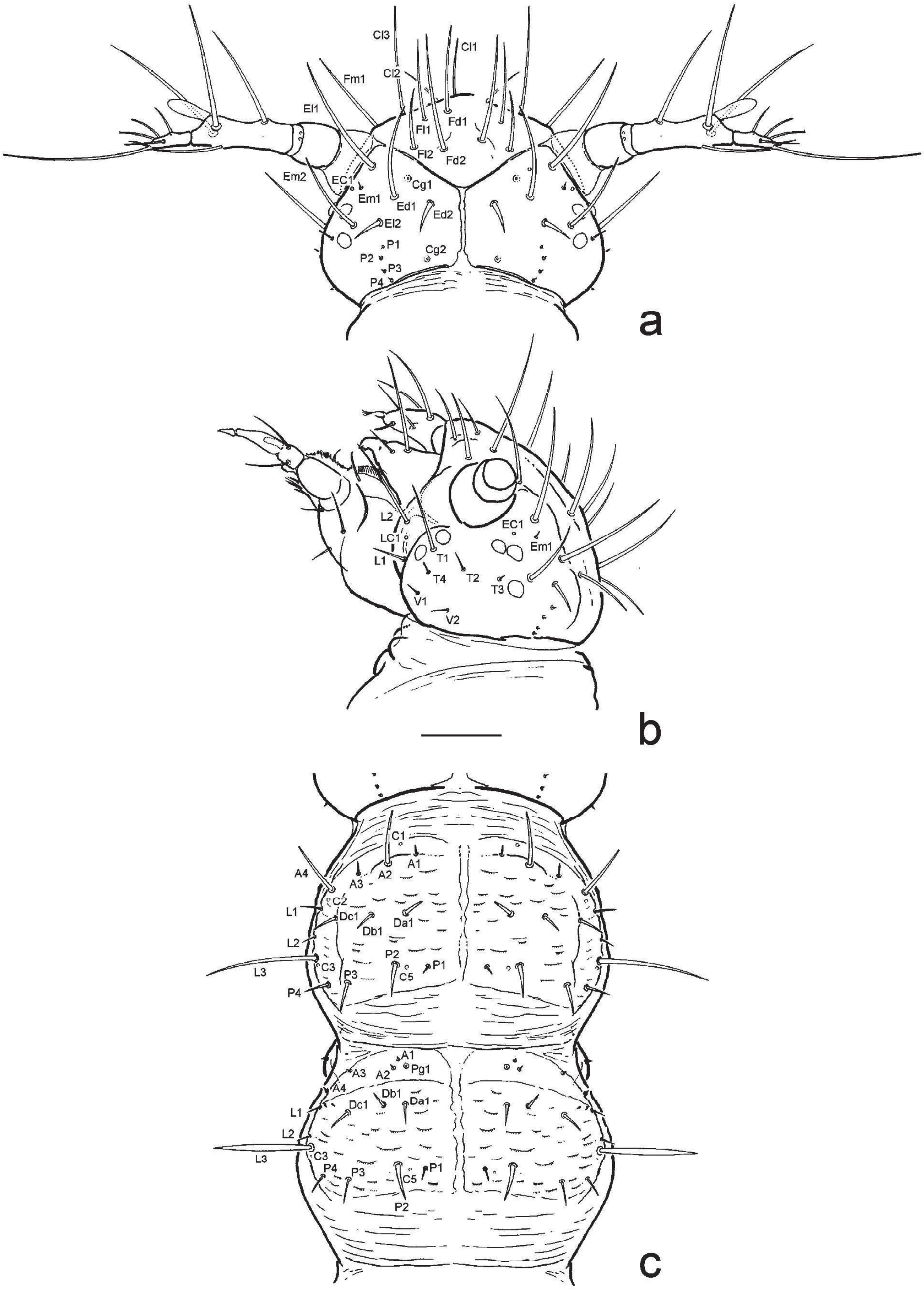

FIGURE 4. *Hydraena perkinsi*, first instar larva: a, head capsule in dorsal view; b, head capsule in lateral view; c, pro- and mesonotum. Scale bar: 0.05 mm.

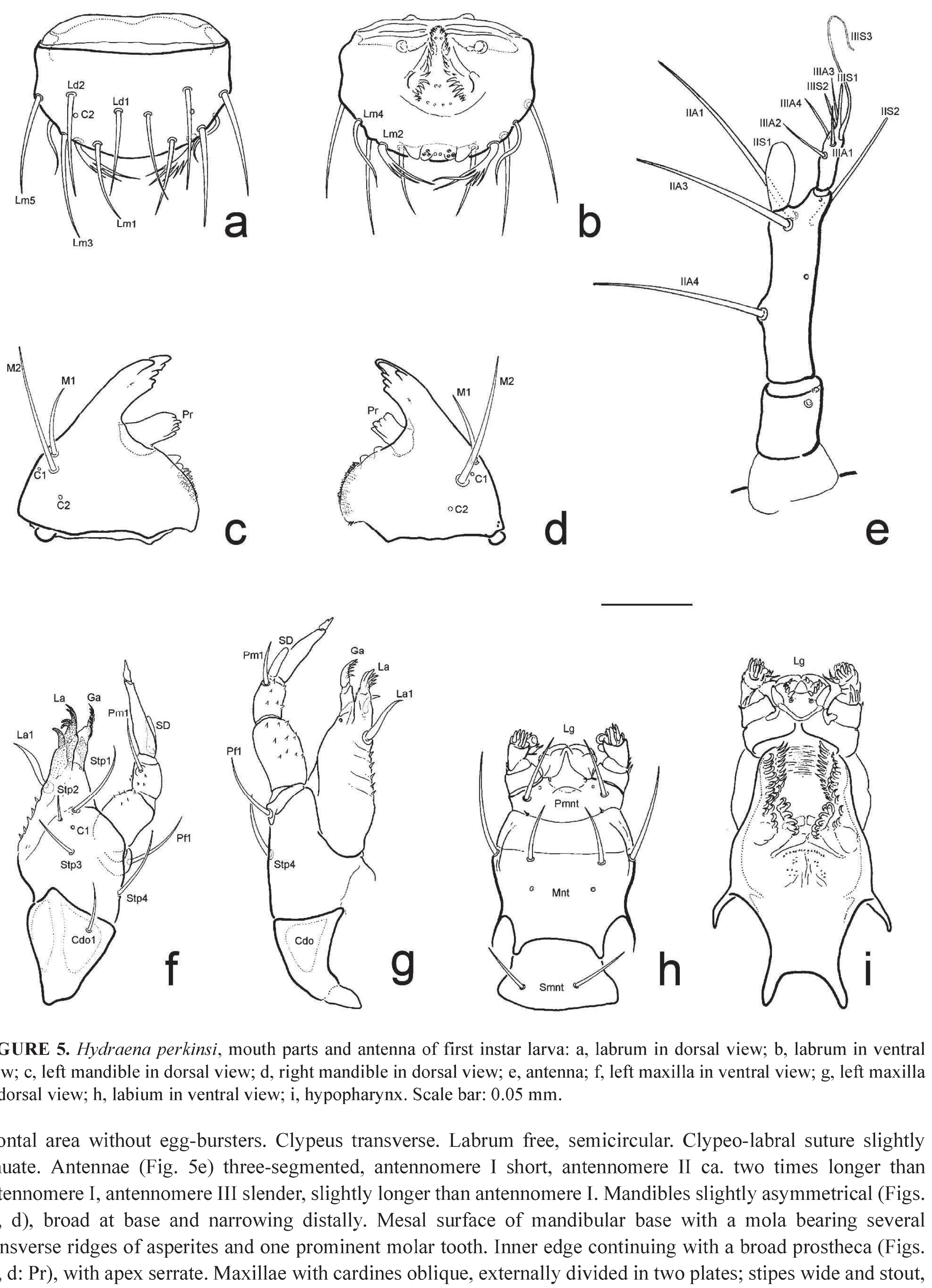

FIGURE 5. *Hydraena perkinsi*, mouth parts and antenna of first instar larva: a, labrum in dorsal view; b, labrum in ventral view; c, left mandible in dorsal view; d, right mandible in dorsal view; e, antenna; f, left maxilla in ventral view; g, left maxilla in dorsal view; h, labium in ventral view; i, hypopharynx. Scale bar: 0.05 mm.

Frontal area without egg-bursters. Clypeus transverse. Labrum free, semicircular. Clypeo-labral suture slightly sinuate. Antennae (Fig. 5e) three-segmented, antennomere I short, antennomere II ca. two times longer than antennomere I, antennomere III slender, slightly longer than antennomere I. Mandibles slightly asymmetrical (Figs. 5c, d), broad at base and narrowing distally. Mesal surface of mandibular base with a mola bearing several transverse ridges of asperites and one prominent molar tooth. Inner edge continuing with a broad prostheca (Figs. 5c, d: Pr), with apex serrate. Maxillae with cardines oblique, externally divided in two plates; stipes wide and stout, mala, distally divided into a fixed galea and a lacinia; galea (Figs. 5f, g: Ga) falcate, spinose along mesal edge; lacinia (Figs. 5f, g: La) fimbriate; maxillary palps three-segmented, segment I long and stout, segment II short, segment III long and slender with a digitiform sensorial appendage (Figs. 5f, g: SD) on external surface situated basally and a small complex sensorial structure on its apex. Labium (Fig. 5h) consisting of three sclerites. Submentum (Smnt) trapezoidal, deeply concave basally, mentum (Mnt) subquadrate and prementum (Pmnt) V-shaped; ligula (Lg) shorter than labial palps, apex bilobed and globose; labial palps two-segmented, directly articulated with prementum. Hypopharynx (Fig. 5i) simple, membranous, clothed with short spicules. Tentorium with identical structure and shape in all larval instars (see description in third instar). Cervical region short, membranous.

Thorax less than a thirdth the length of the abdomen (when the latter completely distended). Prothorax similar in width than meso- and metathorax (Fig. 4c). Dorsal regions with a single sclerite well-developed in the three segments, each with an evident median ecdysial line (Fig. 4c). Lateral areas each with two connate sclerites (see Fig. 7d): a prehypopleuron (episternum) forming a sclerotized lobe anterior to coxal cavity and a posthypopleuron (epimeron). Prothorax laterally also with a small sclerite in each side that bears a spiracle (see Fig. 7c: SP). Mesothoracic spiracles non-functional, reduced to small tubercles. Ventral region (see Fig. 7d) of each thoracic segment consisting of three small sclerites: two lateral presternites and a median posterior sternum. Legs well-developed, long and five-segmented (Fig. 28e).

Abdomen 10-segmented, about two times longer than thorax; segments I to VIII (Fig. 6a), each with a well sclerotized single dorsal plate, longer than wide and with an anterior and a posterior less sclerotized area; lateral regions (Figs. 6c, d), each with two sclerites: a dorsopleural sclerite, which bears a circular annular spiracle (Figs. 6c, d: SP) and a ventropleural sclerite; ventral regions (Fig. 6b) with a single plate, less sclerotized than the dorsal plates. Abdominal segment IX (Figs. 6d, f) without dorsopleural and ventropleural sclerites and bearing a pair of two-segmented urogomphi, not fused to tergite IX; urogomphal bases markedly separated from each other. Urogomphi (Fig. 6e) with segment I (URI) long, segment II (URII) short, distally cup-shaped and with a long apical seta (AS). Abdominal segment X (Figs. 6d, f) visible from above, forming a complete sclerotized ring, distally associated with a membranous region consisting of a ventral sclerotized plate, an anal lobe or pygopod and two well-developed anal hooks (Fig. 6d: AH) projecting downward.

Larval vestiture. Head. Chaetotaxy of head capsule as in Figs. 4a, b. Frontal region (Fig. 4a) on each side with five setae: two frontal dorsal setae (Fd1, Fd2), two frontal lateral setae (Fl1, Fl2) and one frontal marginal seta (Fm1). Clypeus (Fig. 4a) with three setae on each side (Cl1–Cl3). Epicranial regions each with three pores (EC1, Cg1, Cg2) and 10 setae: a row of four minute posterior setae (P1–P4), two epicranial dorsal setae (Ed1, Ed2), two epicranial lateral setae (El1, El2) and two epicranial marginal setae (Em1, Em2), Em1 short. Temporal regions (Fig. 4b), each with four long setae (T1–T4), T1 longest. Lateral regions (Fig. 4b), each with two setae (L1, L2) and one pore (LC1). Ventral regions (Fig. 4b), each with two setae (V1, V2). Labrum (Figs. 5a, b) with two pores (C1, C2) and seven setae on each side: two discal setae (Ld1, Ld2) and five marginal setae (Lm1–Lm5). As in other described species of *Hydraena*, Lm2 and Lm4 situated ventrolaterally and Lm2 pectinate (Fig. 5b). Epipharynx as in Fig. 5b. Antennae as in Fig. 5e, antennomere I with four pores; antennomere II with three long setae (IIA1–IIA3), and two solenidia (IIS1, IIS2), solenidium IIS1 well developed, inserted distally and club-shaped; IIS2 shorter, situated on mesal ventral side of the antennomere; antennomere III, with four setae (IIIA1–IIIA4) and three apical solenidia (IIIS1–IIIS3); solenidium IIIS3 extremely long. Mandibles (Figs. 5c, d) with two pores (C1, C2) and two setae (M1, M2), M2 longer than M1. Maxillae (Figs. 5f, g): cardo with one seta (Cdo1); stipes with four setae (Stp1–Stp4) and one pore (C1); palpifer with a long seta (Pf1); segment II of the maxillary palps with one seta (Pm1); lacinia with one falcate seta (La1).

Thorax. Pronotum (Fig. 4c) with four thoracic pores (C1, C2 C3, C5), and 14 setae on each side. Pore C4 absent. Setae: four in anterior row A (A1–A4), A1 and A3 shorter; three in lateral row L (L1–L3), L3 long; four in posterior row P (P1–P4), P1 shortest; and three discal setae (Da1, Db1, Dc1). Ventral region of prothorax similar in all larval instars (see Fig. 7d): Presternites, each with two setae (Pr1, Pr2); sternite with two pairs of setae St1, St2. Prehypopleurites, each with three small setae (Prehy1–Prehy3), and posthypopleurites with one small setae each (Pohy1). Mesonotum (Fig. 4c) with 14 setae and two pores (C3, C5) on each side. Setae: four minute setae in anterior row A (A1–A4); three discal setae (Da1, Db1, Dc1); three lateral setae in row L (L1–L3), L3 long; four posterior setae in row P (P1–P4). Ventral region of mesothorax (see Fig. 7d): Presternites with one setae (Pr1), and sternites with two pairs of setae (St1, St2). Prehypopleurites, each with two small setae (Prehy1, Prehy2) and posthypopleurites, each with a single small seta (Pohy1). Lateral short mesothoracic tubercles with a seta (S1). Chaetotaxy of metathorax identical to mesothorax except for the absence of setae S1.

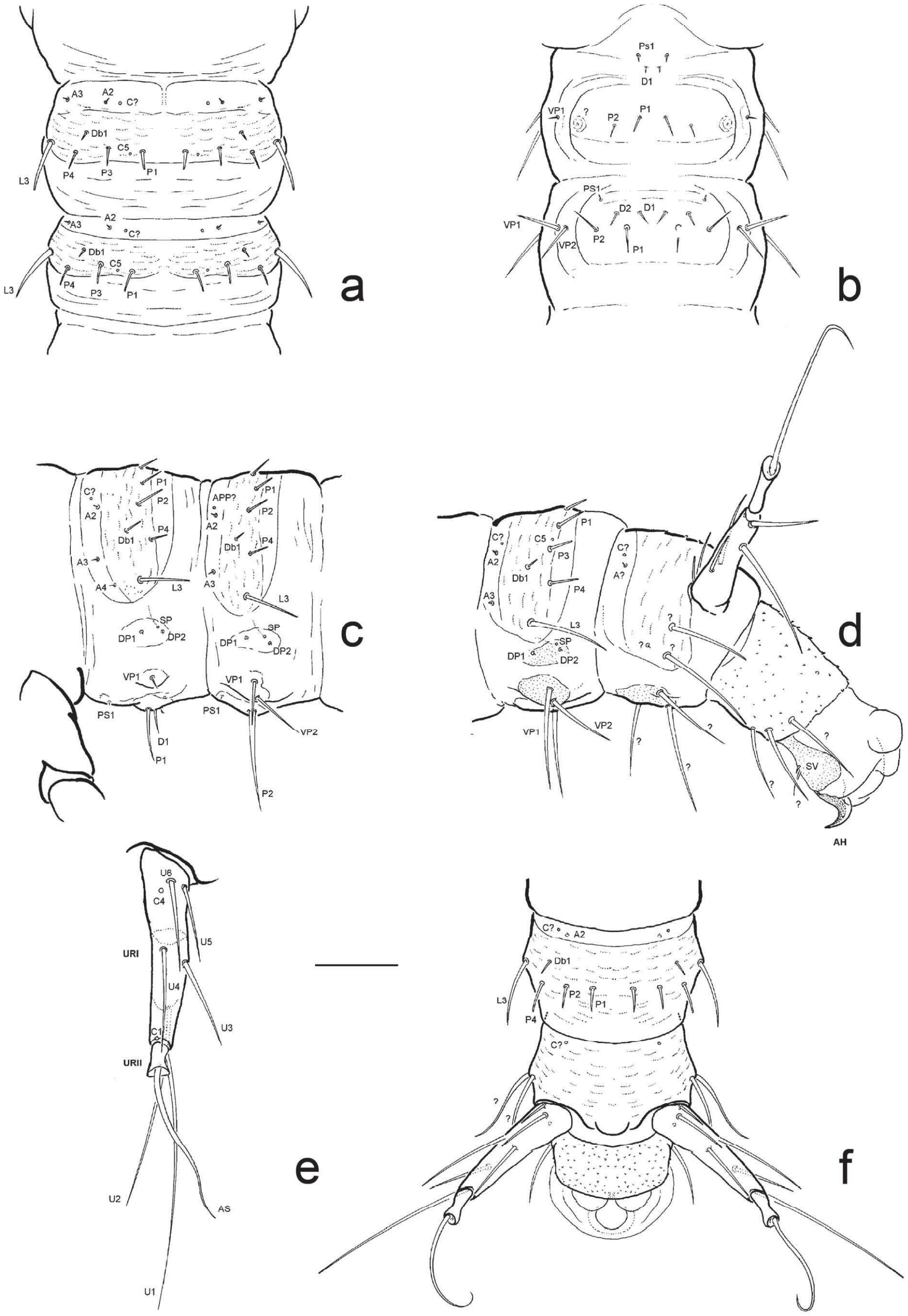

FIGURE 6. *Hydraena perkinsi*, first instar larva: a, abdominal segments I and II in dorsal view; b, same in ventral view; c, same in lateral view; d, abdominal segments VIII to X and pygopod in lateral view; e, left urogomphus in dorsal view; f, abdominal segments VIII to X and pygopod in dorsal view. Scale bar: 0.05 mm.

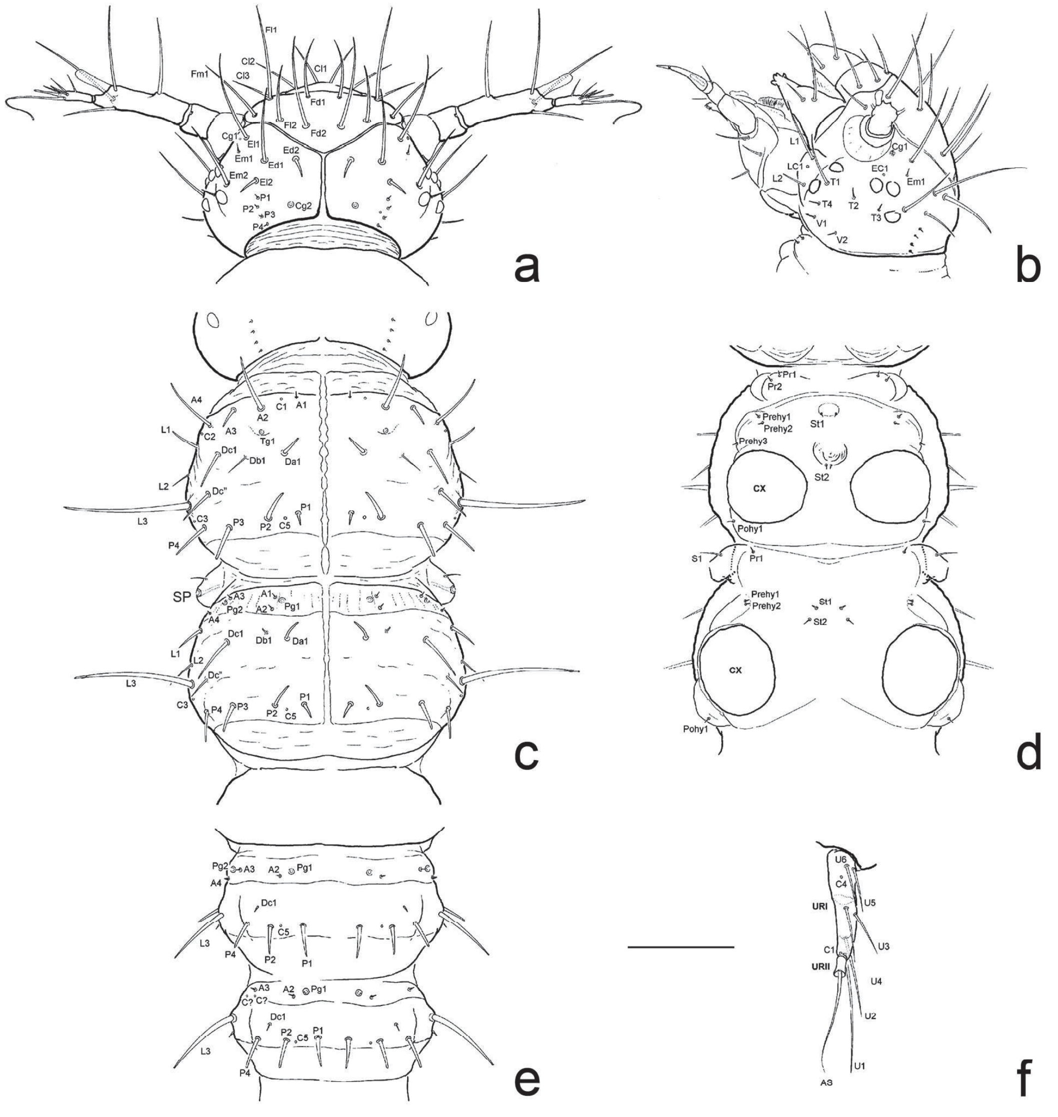

FIGURE 7. *Hydraena perkinsi*, second instar larva: a, head capsule in dorsal view; b, head capsule in lateral view; c pro- and mesothorax in dorsal view; d, same in ventral view; e, abdominal segments I and II in dorsal view; left urogomphus in dorsal view. CX: coxal cavities. Scale bar: 0.1 mm.

Abdomen. Chaetotaxy of tergites I–VIII as in Figs. 6a, c, d. Number of setae and pores reduced with respect to thoracic tergites: setae A1, Db1, Dc1, P3 plus pores C1, C2, C3 absent; pretergal area with a pair of pores (C?). Dorsopleural sclerites of abdominal segment I (Fig. 6c), each with a circular annular spiracle (SP) and two setae (DP1, DP2). Ventropleural sclerites of segment I (Fig. 6c), each with one minute seta (VP1). Ventropleural sclerites of segment II–VIII (Figs. 6c, d), each with two setae (VP1, VP2). Sternite I (Fig. 6b) with one pore (Fig. 6b: ?) and four setae on each side: one presternal seta (PS1), one discal seta (D1) and two posterior setae (P1, P2). Sternites II–VIII (Fig. 6b), with five setae on each side: one presternal seta (PS1), two discal setae (D1, D2) and two posterior setae (P1, P2). Urogomphi with segment I (Fig. 6e: URI) with four pores (C1–C4) and six setae (U1–U6), U5 and U6 short. Tergite IX (Figs. 6d, f) on each side of the pretergal area with one minute setae (A?) and one pore (C?) and two additional pairs of setae on the tergal area. Abdominal segment X (Fig. 6d) with three ventrolateral setae and three spine-like ventral setae on each side (SV).

Second instar larva (Figs. 7; 29a, b)**. Material examined**. **CUBA**: 1 larva (CDUM): "Santiago de Cuba, Humedal San Miguel de Parada, Río Cobre, Punta de Sal, 20°0'56.40"N 75°52'15.14"W, 3 m, starting rearing 08.vii.2009, larva collected 13.vii.2009, coll.: A. Deler-Hernández"; same locality: 1 larva (CDUM), starting rearing 08.vii.2009, larva collected 02.viii.2009; 2 larvae (CDUM): "Santiago de Cuba, La Redonda, Río Sevilla, 20º0'54.3"N 75º45'45.6"W, 15 m, starting rearing 19.xii.2009, larvae collected 27.xii.2009, coll.: A. Deler-Hernández"; same locality: 1 larva (CDUM), starting rearing 19.xii.2009, larva collected 07.i.2010; 1 larva (CDUM), starting rearing 19.xii.2009, larva collected 10.i.2010; 1 larva (CDUM), starting rearing 19.xii.2009, larva collected 13.i.2010; 3 larvae (CDUM), starting rearing 09.i.2010, larvae collected 23.i.2010; 2 larvae (NMPC), starting rearing 27.xi.2010, larvae collected 19.xii.2010; 3 larvae (NMPC), starting rearing 27.xi.2010, larvae collected 23.xii.2010; 1 larva (NMPC), starting rearing 11.xii.2010, larva collected 24.xii.2010; 2 larvae (NMPC): "Santiago de Cuba, San Luis, Dos Caminos, El Vivero, 20º11'2.50"N 75º46'17.7"W, 150 m, starting rearing 20.vii.2011, larvae collected 06.ix.2011, coll.: A. Deler-Hernández".

Description. Total body length: 1.50±0.11 mm (mean±SD.; n= 10). Body form elongate. Head and thorax approximately of same width; abdomen slightly narrower, parallel-sided. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous areas grayish. Maximum head width: 0.24±0.007 mm (mean±S.D.; n= 7). Dorsopleural sclerites of the abdominal segments I and II, as in first instar larvae, not fused to their respective tergites (see Fig. 29a). Dorsopleural sclerites of the abdominal segments III–VI (see Fig. 29a) narrowly fused to their respective tergites. Dorsopleural sclerites of the abdominal segments VII and VIII completely fused to their respective tergites (see Fig. 29b).

Larval vestiture. Similar to that of first instar larva but with some subprimary additional setae on thorax and abdomen. Additional secondary setae absent.

Head. With same setal pattern to that of first instar (Figs. 7a, b). Antennae with SD1 of antennomere II long and cylindrical, not club-shaped as in first instar (compare Fig. 7a with 4a).

Thorax. Pronotal chaetotaxy as in first instar, with one additional subprimary seta (Dc'') and one evident tergal pore (Tg1) on each side (Fig. 7c). Meso- and metanotum similar to pronotum, but with two pairs of conspicuous pretergal pores (Pg1, Pg2) on each segment.

Abdomen. Chaetotaxy of abdominal segments as that of the first instar, but with two pairs of pretergal pores (Pg1, Pg2) on abdominal segment I. Abdominal segments II–VIII, each with one pair of pretergal pores (Pg1). Pores C? located close to the anterior setae A3 (Fig. 7e). Dorsolateral sclerites each with an additional seta (DP'). Sternal setae VP1 of segment I short but not minute as in first instar. Sternite II with a subprimary discal seta (D') on each side. Sternites II to VIII with two subprimary discal setae on each side (D', D''). Chaetotaxy of segments IX, X and urogomphi (Fig. 7f) as in first instar larvae.

Third instar larva (Figs. 8–11; Fig. 30e)

Material examined. **CUBA**: 1 larva (CDUM): "Santiago de Cuba, Humedal San Miguel de Parada, Río Cobre, Punta de Sal, 20°0'56.40"N 75°52'15.14"W, 3 m, starting rearing 08.vii.2009, larva collected 20.vii.2009, coll.: A. Deler-Hernández"; same locality: 1 larva (CDUM), starting rearing 08.vii.2009, larva collected 26.vii.2009; 2 larvae (CDUM), starting rearing 08.vii.2009, larvae collected 05.viii.2009; 5 larvae (CDUM), starting rearing 01.ix.2009, larvae collected 15.ix.2009; 2 larvae (CDUM), starting rearing 01.ix.2009, larvae collected 17.ix.2009; 4 larvae (CDUM): "Santiago de Cuba, La Redonda, Río Sevilla, 20º0'54.3"N 75º45'45.6"W, 15 m, starting rearing 19.xii.2009, larvae collected 07.i.2010, coll.: A. Deler-Hernández"; same locality: 6 larvae (CDUM), starting rearing 19.xii.2009, larvae collected 10.i.2010; 4 larvae (CDUM), starting rearing 19.xii.2009, larvae collected 13.i.2010; 2 larvae (CDUM), starting rearing 19.xii.2009, larvae collected 18.i.2010; 1 larva (CDUM), starting rearing 09.i.2010, larva collected 23.i.2010; 1 larva (MNPC), starting rearing 12.xi.2010, larva collected 12.xii.2010; 4 larvae (NMPC): "Santiago de Cuba, San Luis, Dos Caminos, El Vivero, 20º11'2.50"N 75º46'17.7"W, 150 m, starting rearing 20.vii.2011, larvae collected 15.ix.2011, coll.: A. Deler-Hernández,"; same locality: 2 larvae (NMPC), starting rearing 20.vii.2011, larvae collected 06.ix.2011".

FIGURE 8. *Hydraena perkinsi*, third instar larva in dorsal and lateral view. Scale bar: 0.5 mm.

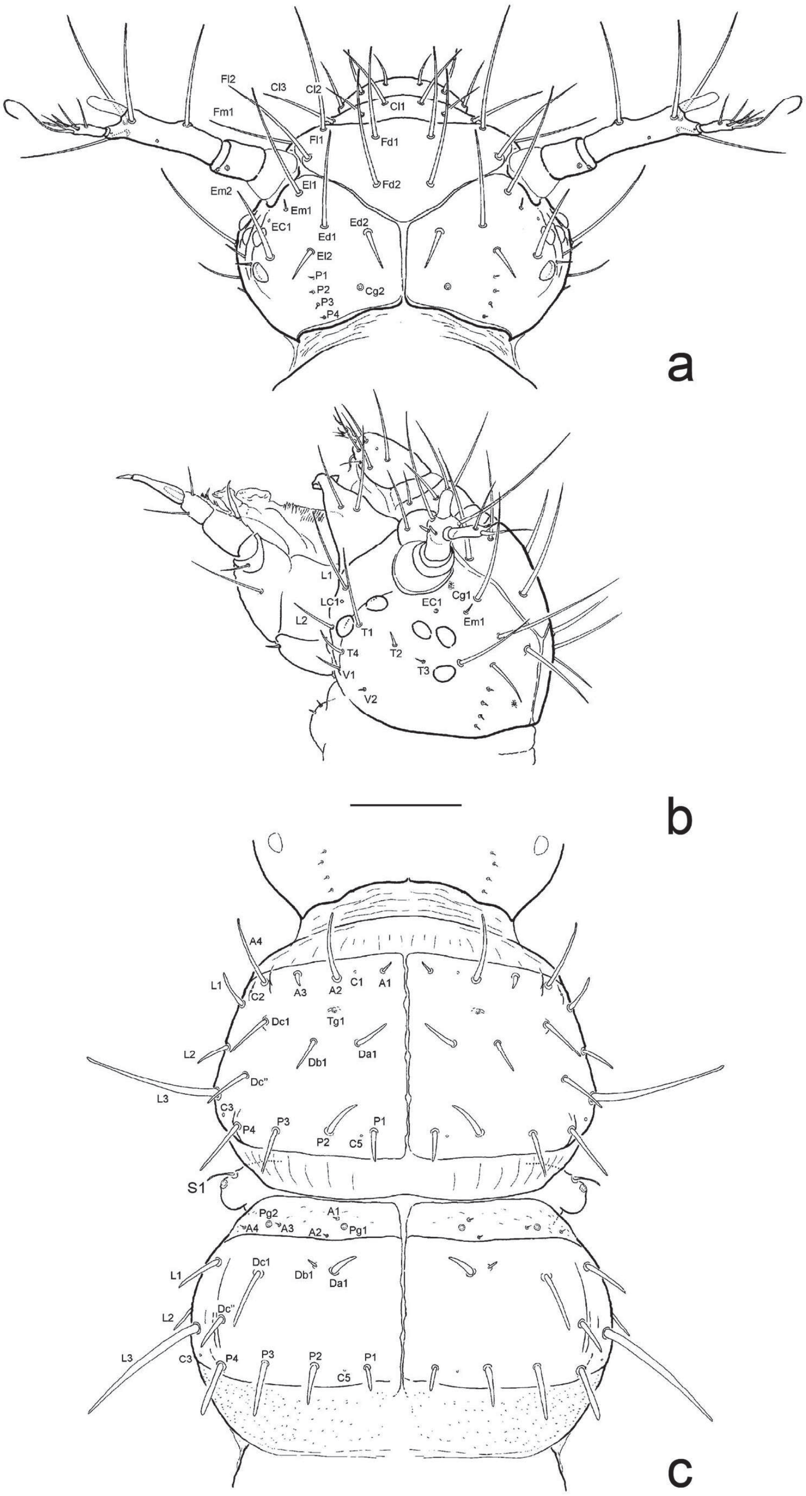

FIGURE 9. *Hydraena perkinsi*, third instar larva: a, head capsule in dorsal view; b, head capsule in lateral view; c, pro- and mesothorax in dorsal view. Scale bar: 0.1 mm.

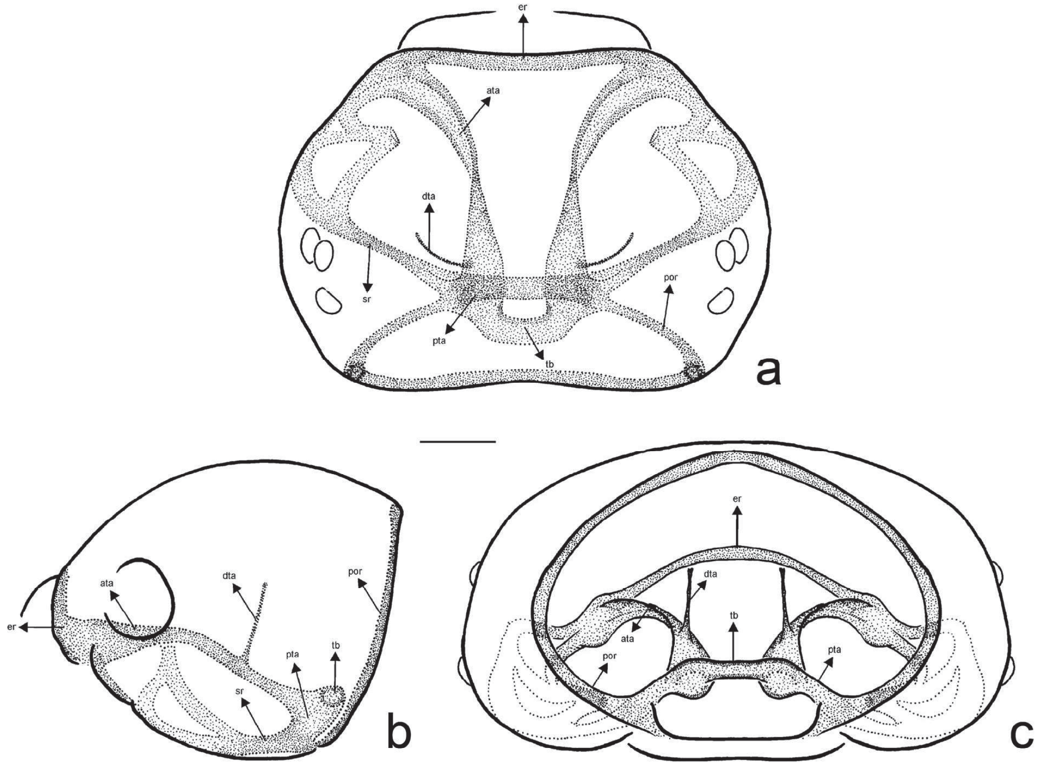

FIGURE 10. *Hydraena perkinsi*, third instar larva. Internal skeletal structures of head capsule: a, dorsal view; b, lateral view; c, posterior view. Scale bar: 0.05 mm.

Description. General aspect as in Fig. 8. Total body length: 2.34±0.20 mm (mean±SD.; n= 14). General body form elongate, dorsally sub-fusiform. Head narrower than thorax. Mesothorax widest than pro- and metathorax, abdominal segments approximately parallel-sided anteriorly and tapering posteriorly. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous areas grayish. Maximum head width: 0.29±0.009 mm (mean±S.D.; n= 12).

Tentorium (Figs. 10a–c) consisting of a pair of posterior tentorial arms originating at the posterobasal region of the head capsule, connected by a tentorial bridge and two anterior arms extending forward to reach the sides of epistomal ridge. From each anterior arm a dorsal tentorial arm extends dorsally. Anterior arms (Figs. 10a–c: ata) wide, dorsolateraly compressed, slightly twisted and strongly united distally to epistomal ridge (Figs. 10a–c: er). Epistomal ridge wide and well developed. Dorsal arms (Figs. 10a–c: dta) slender, slightly enlarged basally, fringed apically and not reaching the epicranium. Tentorial bridge (Figs. 10a–c: tb) wide and short. Posterior arms externally marked by short tentorial pits and basally connected with postoccipital (Figs. 10a–c: por) and subgenal ridges (Figs. 10a, b: sr).

Besides the expected size increase and a moderate change in body form, the most remarkably morphological distinction of third instar larvae with respect to instar II is in segments I to IV in which the dorso- and ventropleural sclerites are fused to their respective tergites and sternites (Fig. 11b). Another remarkable feature refers the shape of many setae, mainly of dorsal series, which are stouter than in previous instars (Fig. 8).

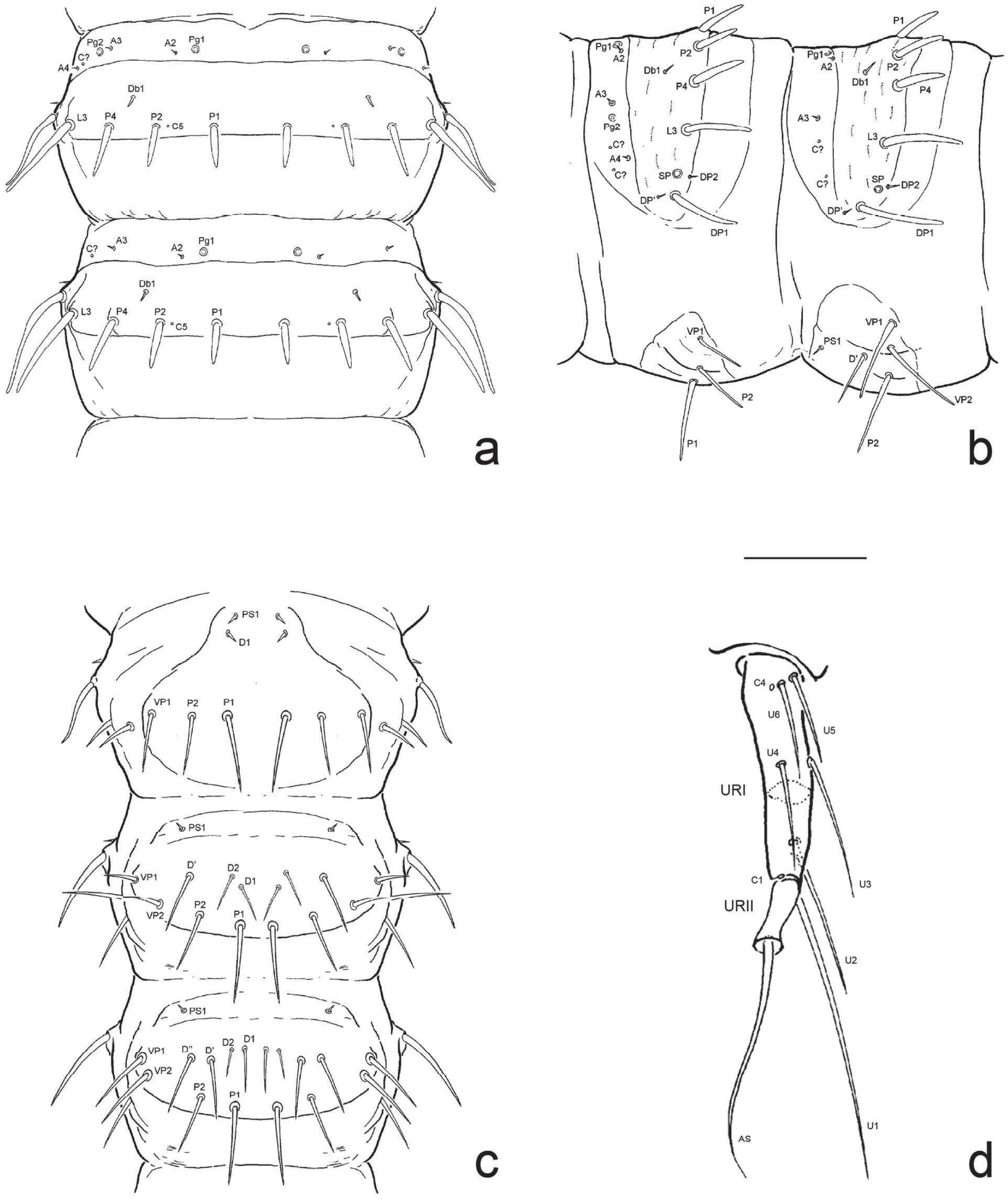

FIGURE 11. *Hydraena perkinsi*, third instar larva: a, abdominal segments I and II in dorsal view; b, same in lateral view; c, abdominal segments I to III in ventral view; d, left urogomphus in dorsal view. Scale bars: 0.1 mm for a–c, 0.05 mm for d.

Larval vestiture. Chaetotaxy identical to that of second instar larvae but with some differences associated to the fusion of abdominal lateral sclerites referred above. Head and thorax as in second instar (Figs. 9a, b); sensory appendage SD1 of antennomere II cylindrical, as in second instar larva. Pronotum and mesonotum as in Fig. 9c. Abdomen with dorsopleural sclerites of segments I to VIII fused with their respective tergites thus, with the pairs of setae DP1, DP2 and DP' plus the spiracles (SP) placed on the lateral margins of a single dorsolateral plate;

similarly, the pair of setae VP1 (abdominal segment I) and VP1 plus VP2 (abdominal segments II to VIII) are placed on the lateral margins of a single lateroventral plate (Figs. 11b, c). Sternal setae VP1 of segment I well developed. Sternite II with an additional subprimary discal seta (D') on each side. Sternites II to VIII with two subprimary discal setae on each side (D', D'') (Fig. 11c). Chaetotaxy of segments IX and X as that of first and second instars. Urogomphi as in Fig. 11d, with same pore and chaetotaxy than in previous instars.

Pupa (Figs. 12–13)

Material examined. 1 male (CDUM): "Santiago de Cuba, La Redonda, Río Sevilla, 20º00'54.3"N 75º45'45.6"W, 15 m, starting rearing 11.xii.2010, pupa collected 12.i.2011, coll.: A. Deler-Hernández"; same locality: 1 male, 5 females (CDUM), starting rearing 11.xii.2010, pupae collected 16.i.2011; 1 male, 4 females (CDUM), starting rearing 11.xii.2010, pupae collected 7.i.2011; 3 females (CDUM), starting rearing 25.viii.2011, pupae collected 27.ix.2011; 1 male (CDUM) "Santiago de Cuba, San Luis, Dos Caminos, El Vivero, Laguna temporal, 20º11'2.50"N 75º46'17.7"W, 150 m, starting rearing 21.viii.2011, pupa collected 15.ix.2011, coll.: A. Deler-Hernández".

Description. Pupa adecticous (without functional mandibles) and exarate (with body appendages free, not fused to body wall). General aspect as in Figs. 12a–c. Elongate. Length: 1.40±0.17 mm (mean±SD.; n= 12); maximum prothorax width: 0.46±0.04 mm (mean±SD.; n= 12). General colouration white to pale yellow in freshly hatched specimens, with eyes becoming darker with age.

Head deflected (Figs. 12b, c), directed ventrally towards thorax, distinctly longer than broad and entirely concealed in dorsal view (Fig. 12a). Maxillary palpi sheaths extremely long, slightly surpassing base of sternite V. Antennal sheaths extended posterolaterally, bordering base of forelegs and reaching base of fore tibiae (Fig. 12c). Prothorax only slightly wider than abdomen. Pronotum wider than long, widest just anterior of middle; distinctly longer than mesonotum and subequal in length to metanotum. Pronotum with anterior corners rounded, not projecting further than anterior border; anterior and lateral margins slightly rounded. Elytral and metathoracic wing sheaths glabrous, the latter slightly longer and with apices reaching ventrally the posterior margin of sternite V (this character can be only seen in specimens with pterothecae not distended laterally). The specimens of *H. perkinsi* used here for illustration are more distended than fresh observed (or better preserved) material, which have the elytral and methatoracic wing sheaths closely fitted against the body. Metaventrite long, glabrous. Legs sheaths with the same color as body, glabrous. Femora protruding beyond body margin, with knees visible in dorsal view; tibiae directed obliquely to middle of body and tarsi oriented parallel to midline. Hind tarsi reaching middle of abdominal sternite VI.

Abdomen with nine fully developed segments and a final tenth considerably reduced, segments only slightly tapering posteriorly. Segments I to VIII transverse, IX trapezoidal and X subquadrate. Length of tergite I nearly the same as II. Abdominal spiracles present dorsolaterally on segment I to VIII. Spiracles of segment I lying on top of very small cuticular elevations. Apparently, only this pair of spiracles is functional (Fig. 13g), the others are reduced and seem atrophied. Functional spiracles uniporous, rounded. Abdominal segment IX and X markedly sexual dimorphic. In males, sternite IX bears a single, central rounded gonotheca and the distal central margin of segment X is clearly bilobed. In females, however, sternite IX bears two rounded, smaller gonothecae and the distal margin of segment X is gently curved, not bilobed. In females, each gonotheca covers externally one of the two subapical tufts of setae of the future adult female gonocoxite (Fig. 13e). In male pupae the single gonotheca is near, but does not fit exactly, with the future adult male sternite X (Fig. 13f).

Pupal vestiture. Pupa ventrally glabrous. Dorsal vestiture consisting of a set of scattered setae, some of them (Fig. 12d) located on domed cuticular bases or tubercles (chalazae) and several rows of long non-articulate, tapered projections, continuous with the cuticle and devoid of setae (spicules) (Figs. 12d, e).

Head with typical setae arranged in five pairs (Fig. 12b): one epicranial, one medial frontal and one frontoclypeal pair and two pairs bordering the base of each ocular area.

Pronotum with eight pairs of chalazae and a single pair of spicules (Fig. 12a). Anterior margin of pronotum with three pairs of chalazae and a pair of very elongate spicules. Lateral and posterior margins of pronotum with two and three pairs of chalazae respectively, pronotal disc with a single pair. Meso- and metanotum each with a pair of chalazae located on what can be seen as two dorsal paramedial rows of projections extending from pronotum to abdominal sternite VIII.

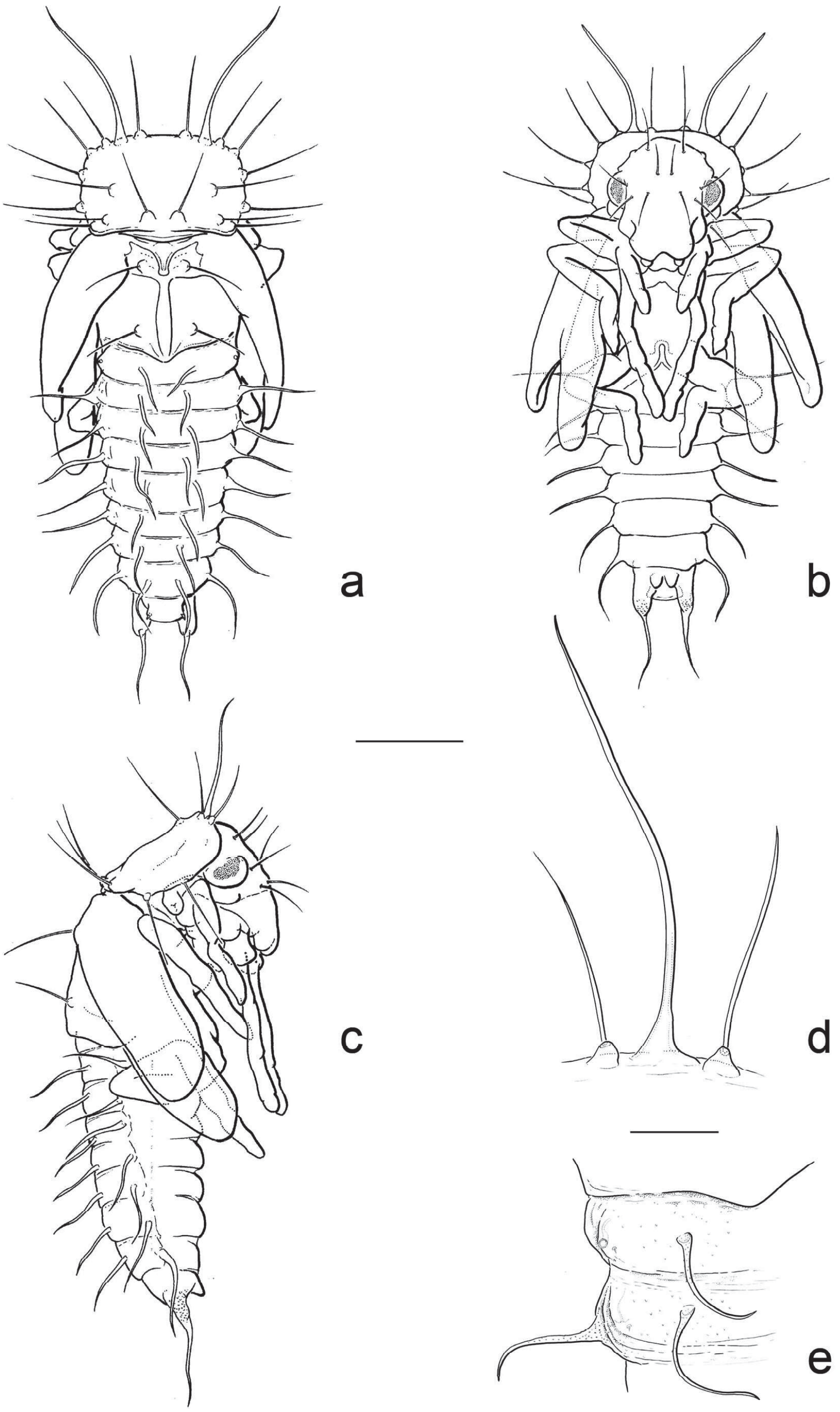

FIGURE 12. *Hydraena perkinsi*, pupa: a, dorsal view; b, ventral view; c, lateral view; d, detail of left anterior margin of prothorax showing two chalazae and an elongate spicule; e, left margin of abdominal segments I and II in dorsal view showing shape and location of spicules and spiracles. Scale bars: 0.3 mm for a–c, 0.1 mm for d–e.

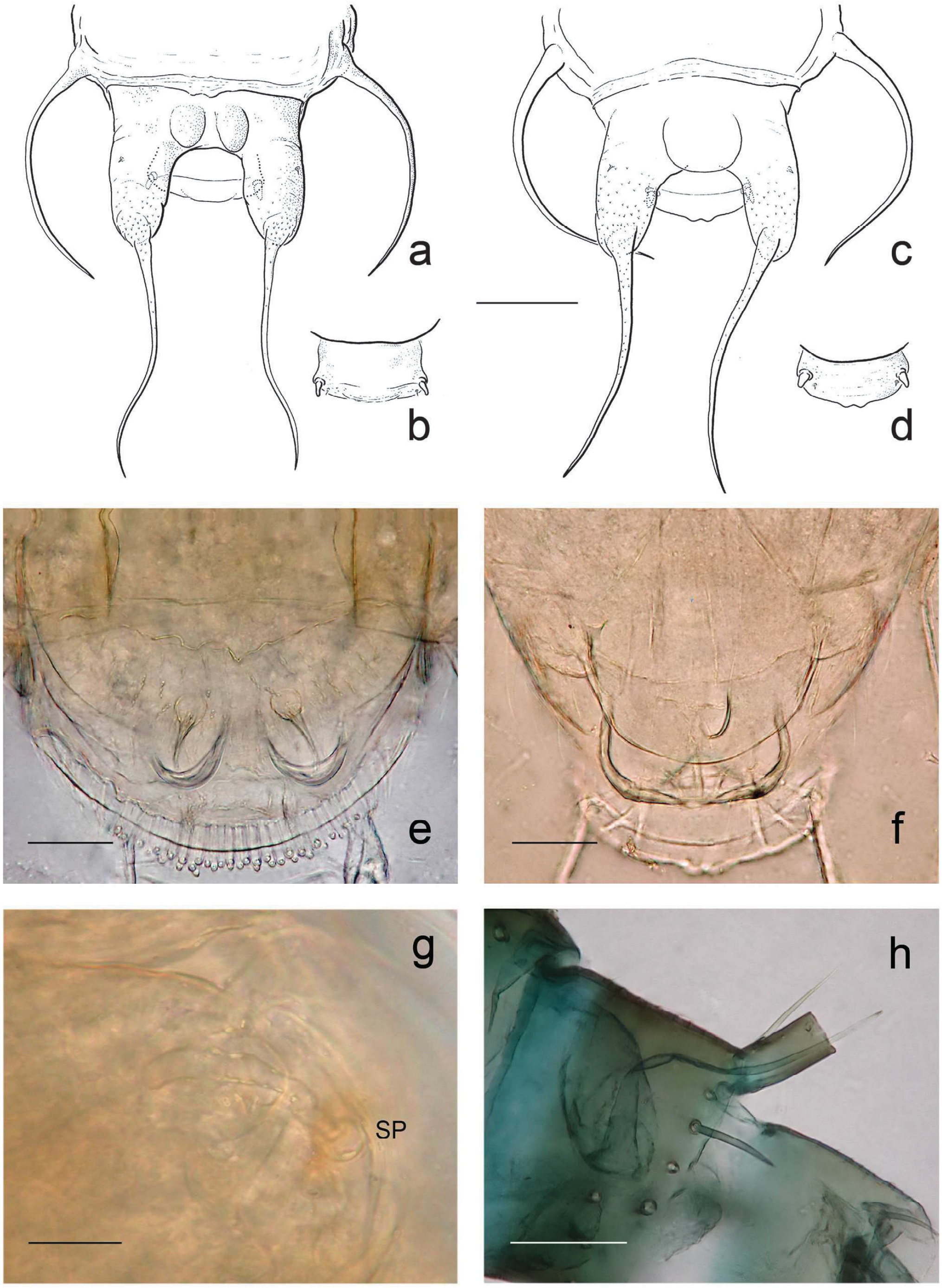

FIGURE 13. *Hydraena perkinsi*, pupa: a, pupal abdominal segment IX and X of female in ventral view showing sexual dimorphism: a, segment IX of female; b, segment X of female; c, segment IX of male; d, segment X of male; e, detail of female gonothecae (subapical tufts of setae of adult gonocoxite seen by transparency); f, detail of male gonotheca (adult male sternite X seen by transparency); g, functional spiracle of abdominal segment I: SP; h, pupal spicule of abdominal segment IX (pupal urogomphus) developing inside larval urogomphus of a third instar larva. Scale bars: 0.1 mm for a–d, 0.03 mm for e–g.

Abdomen with several spicules and setae but devoid of chalazae. Abdominal terga II to VIII, on each side and dorsolaterally with a spicule and a minute seta close to the base of this projection (Fig. 12e). A second pair of spicules is positioned on each segment following the two paramedial rows of projections arranged throughout these seven segments. Tergite I only with a pair of minute setae and the pair of paramedial spicules referred above, without lateral spicules (Fig. 12e). Number, size, shape and location of vestiture elements differ on abdominal segments IX and X with respect to that of anterior ones: In these last segments, the admedial pair of spicules are lacking (Figs. 12a, c). In segment IX (Figs. 13a, c), the base of the lateral pair of spicules is heavily enlarged and spinulose, bearing each a minute seta, serially homologous to that of other tergites. This pair of developed spicules is frequently referred as urogomphi in descriptions of other coleopteran pupae. A spicule is also present on each lateral margin of segment X, but reduced in this segment to a pair of small rudimentary papillae (Figs. 13b, d). A pair of minute setae is also present lying, as in the other segments, close to the rudimentary spicules.

Remarks. Crowson (1981) stated that in Staphylinidae, larval and pupal urogomphi are homologous since the tissue that creates this pair of pupal spicules develops inside the larval urogomphi. We have found that this is also the case in Hydraenidae, as can be seen in Fig. 13h.

Description of immature stages of *Hydraena decui*

Egg (Fig. 1f)

Material examined. **CUBA**: 7 eggs (CDUM): "Santiago de Cuba Prov. Gran Piedra, 20º00'22"N 75º36'59"W, ca. 1144 m a.s.l., 14.iv.2012, coll.: A. Deler-Hernández".

Description. Elongate, ellipsoidal, with round poles (Fig. 1f). Color white. Chorion thin, smooth, without any trace of chorionic sculpture observed at 400X. Each egg is concealed by an external membrane or protective coat but it can be perfectly seen and measured by transparency. EL: 0.37±0.05 mm (mean±S.D, n= 11), EW: 0.18±0.02 mm (n= 11); ELL: 0.59±0.03 mm (n= 3); EEW: 0.32±0.008 mm (n= 3).

Biological remarks. Most eggs were obtained from a single submerged decaying leaf. Eggs were laid along the curling margin of the leaf, in which organic matter and fungal hyphae were also abundant (Fig. 1f).

Larva

First instar larva. Material examined. **CUBA**: 8 larvae (CDUM): "Santiago de Cuba Prov. Gran Piedra, 20º0'22"N 75º36'59"W, ca. 1144 m a.s.l., starting rearing 12.vii.2011, larvae collected 20.vii.2011, coll.: A. Deler-Hernández".

Description and larval vestiture. Total body length: 1.03±0.06 mm (mean±SD.; n= 8); head capsule width: 0.18±0.007 mm (n= 8). This instar is indistinguishable from that of the same instar of *Hydraena guadelupensis* Orchymont, 1923, *H. franklyni* and *H. perkinsi*.

Second instar larva. Unknown.

Third instar larva (Fig. 30d)**. Material examined**. **CUBA**: 3 larvae (CDUM): "Santiago de Cuba Prov. Gran Piedra, 20º00'22"N 75º36'59"W, ca. 1144 m a.s.l., starting rearing 04.iv.2012, larvae collected 12.vii.2012, coll.: A. Deler-Hernández".

Description and larval vestiture. Total body length: 2.05±0.04 mm (mean±SD.; n= 3); head capsule width: 0.26±0.004 mm (mean±SD.; n= 3). This instar is indistinguishable from that of the same instar of *H. franklyni and H. perkinsi*. The antennal solenidium IIS1 is lacking in all the studied species of this instar thus, it is impossible to assure whether this trait have a particular shape or not, as it does in *H. guadelupensis* (see below).

Description of immature stages of *Hydraena franklyni*

Egg (Fig. 1e)

Material examined. **CUBA**: 1 egg (CDUM): "Santiago de Cuba Prov. Gran Piedra, Museo La Isabelica, 20º0'22"N 75º36'59"W, ca. 1144 m a.s.l., 10.v.2012, coll.: A. Deler-Hernández".

Description: Elongate, ellipsoidal, with round poles (Fig. 1e). Color white. Chorion thin, smooth, without any trace of chorionic sculpture observed at 400X. The egg is concealed by an external membrane or protective coat but it can be perfectly seen and measured by transparency. EL: 0.42 mm (n= 1); EW: 0.20 mm (n= 1); ELL: 0.54 mm (n= 1), EEW: 0.32 mm (n= 1).

Biological remarks. A single egg was obtained from a rotten leaf.

Larva

First instar larva. Material examined. **CUBA**: 2 larvae (CDUM): "Santiago de Cuba Prov. Gran Piedra, Museo La Isabelica, 20º00'22"N 75º36'59"W, ca. 1144 m a.s.l., starting rearing 27.xi.2011, larvae collected 10.xii.2011, coll.: A. Deler-Hernández"; same locality: 1 larva (CDUM), starting rearing 10.v.2012, larva collected 14.v.2012.

Description and larval vestiture. Total body length: 1.15±0.08 mm (mean±SD.; n= 3); head capsule width: 0.20±0.005 mm (n= 3). This instar is indistinguishable from that of the same instar of *H. decui*, *H. guadelupensis* and *H. perkinsi*.

Second instar larva. Unknown.

Third instar larva (Fig. 30c**)**. **Material examined**. **CUBA**: 1 larva (CDUM): "Santiago de Cuba Prov. Gran Piedra, Museo La Isabelica, 20º0'22"N 75º36'59"W, ca. 1144 m a.s.l., starting rearing 27.xi.2011, larva collected 28.xii.2011, coll:. A. Deler-Hernández".

Description and larval vestiture. Total body length: 2.63 mm (n=1); head capsule width: 0.30 mm (n= 1). This instar is indistinguishable from that of the same instar of *H. decui* and *H. franklyni*.

Description of immatures stages of *Hydraena guadelupensis*

Egg. Unknown.

Larva

First instar larva. Material examined. **CUBA**: 4 larvae (CDUM): "Santiago de Cuba Prov., Reserva Ecológica Loma del Gato, Chan-Chan, 20º01'47.6"N 76º02'22.5"W, 356 m, starting rearing 27.vi.2009, larvae collected 08.vii.2009, coll.: A. Deler-Hernández".

Description and larval vestiture. Total body length: 1.21±0.05 mm (mean±SD.; n= 4); head capsule width: 0.18±0.002 mm (n= 4). This instar is indistinguishable from that of the same instar of *H. decui*, *H. franklyni*, and *H. perkinsi*.

Second instar larva (Figs. 29A, b). **Material examined**. **CUBA**: 9 larvae (CDUM): "Santiago de Cuba Prov., Reserva Ecológica Loma del Gato, Chan-Chan, 20º01'47.6"N 76º02'22.5"W, 356 m, starting rearing 27.vi.2009, larvae collected 06.vii.2009, coll.: A. Deler-Hernández".

Description and larval vestiture. Total body length: 1.59±0.09 mm (mean±SD.; n= 9); head capsule width: 0.23±0.006 mm (n= 9). Antennal solenidium IIS1 with an apical notch (see Fig. 30b). This trait can be used to differentiate this larva from the other known Cuban *Hydraena*. Interestingly, the notch is absent in the first instar larva. Dorsopleural sclerites of abdominal segments I–II as in first instar larvae, not fused to their respective tergites (see Fig. 29a). Dorsopleural sclerites of the abdominal segments III–VI (see Fig. 29a) narrowly fused to their respective tergites. Dorsopleural sclerites of the abdominal segments VII and VIII completely fused to their respective tergites (see Fig. 29b).

Third instar larva (Figs. 2a; 30b, f). **Material examined**. **CUBA**: 2 larvae (CDUM): "Santiago de Cuba Prov., Reserva Ecológica Loma del Gato, Chan-Chan, 20º01'47.6"N 76º02'22.5"W, 356 m, starting rearing 27.vi.2009, larvae collected 08.vii.2009, coll.: A. Deler-Hernández".

Description and larval vestiture. Total body length: 2.25±0.06 mm (mean±SD.; n= 2); head capsule width: 0.28±0.005 mm (n= 2). As commented above, this instar can be distinguished from the other species by the apical notch of the antennal solenidium IIS1 (Fig. 30b).

Description of the immature stages of *Gymnochthebius fossatus*

Egg. A single oocyte was directly obtained from the end of a female oviduct. Largest diameter of oocyte: 0.36 mm; smallest diameter of oocyte: 0.17 mm. At the time of development, the surface does not shows any trace of corionic sculpture and there are no traces of the protecting covering surface observed in laid mature eggs of other hydraenids.

Larva

First instar larva (Figs. 14; 15a, b; 16–18; 28f). **Material examined**. **CUBA**: 2 larvae (CDUM): "Holguín, Felton, Vuelta Larga, 20°43'7.92"N 75°37'59.19"W, starting rearing 24.iii.2013, larvae collected 02.iv.2013, coll.: A. Deler-Hernández; 2 larvae (CDUM): "Santiago de Cuba Prov., Río Sevilla, La Redonda, 20º0'54.3"N 75º45'45.6"W, 15 m, starting rearing 19.xii.2009, larvae collected 26.xii.2009, coll.: A. Deler-Hernández".

Description. General aspect as in Fig. 14. Total body length: 1.05±0.11 mm (mean±SD.; n= 3). General body form elongate and slender. Head slightly wider than thorax, abdomen approximately parallel-sided and only slightly tapered posteriorly. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous area grayish. Maximum head width: 0.18±0.006 mm (mean±SD.; n= 3). Head protracted, prognathous, slightly declined, subcordate in dorsal view. Ecdysial line Y-shaped, epicranial stem long, frontal arms V-shaped and distally reaching base of antennae. Head capsule divided into three regions: a medial frontal area, bordered by the frontal arms and a pair of epicranial plates located behind and laterad to frontal arms. Gula developed, subquadrate, closing ventrally the cephalic capsule and bordered laterally by elongate tentorial pits (Fig. 2d). Occipital foramen divided into two parts by tentorial bridge. Clypeus transverse. Labrum free, chordate. Clypeo-labral suture slightly sinuate. Frontal area with a pair of reduced, obliquely arranged, serrate egg-bursters (Figs. 16a, b). Epicranial areas each with five stemmata, globose, slightly bigger than in the known larvae of *Hydraena.* Antennae three-segmented, antennomere I short; antennomere II about 4.5x as long as antennomere I, antennomere III, slender, shorter that antennomere II (Fig. 17e). Mandibles slightly asymmetrical, broad at base and narrow at apex. Mesal surface of mandibular base with a mola bearing several transverse ridges of asperites and two prominent premolar teeth. Inner edge continuing with a narrow prostheca (Figs. 17c, d: Pr), fimbriate apically. Maxillae with cardines small, triangular; each with stipes wide, mala stout, distally divided into a fixed galea and a lacinia; galea (Figs. 17f, g: Ga) slender and acuminate; lacinia (Fig. 17f: La) spinose along mesal edge; maxillary palpi three-segmented, segment I stout, segment II long, segment III slender and long with a digitiform sensorial appendage (Fig. 17f: SD) on external surface situated basally and an acuminate complex sensorial structure on its apex. Labium (Fig. 17h) consisting of three sclerites: submentum (Smnt), mentum (Mnt) and prementum (Pmnt); ligula (Lg) shorter than labial palps, apex digitate and well developed; labial palps two-segmented, directly articulated with prementum. Hypopharynx (Fig. 17i) simple, membranous, clothed with short spicules.

Tentorium (Figs. 16c, d) consisting of a pair of posterior tentorial arms originating at the posterobasal region of the head capsule, connected by a tentorial bridge. Each posterior arm connected ventrally with the postoccipital (por) and subgenal (sr) ridges. Anteriorly each posterior arm projected in a narrow anterior arm (ata) reaching the lateral sides of epistomal ridge (er); epistomal ridge wide and developed. From the anterior arms a pair of dorsal arms (dta) extend dorsally not reaching the epicranium, they are remarkably slender, poorly sclerotized and fringed apically. Cervical region short, membranous, dorsally with two small sclerites, not evident in all specimens.

Thorax less than a third the length of the abdomen (when completely distended). Prothorax slightly larger than meso- and metathorax. Dorsal regions with a single sclerite in the three segments, each with an evident median ecdysial suture (Fig. 16e). Lateral areas as in *Hydraena*, with prehypopleural and posthypopleural regions distinct (Fig. 16f). Prothorax laterally also with a small sclerite in each side that bears a spiracle (Fig. 16f: SP). Ventral regions consisting of three small sclerites: two lateral presternites and a median posterior sternite. Mesothorax with a pair of lateral short tubercles bearing each an atrophied spiracle. Legs well-developed, long and five-segmented (Fig. 28f).

Abdomen 10-segmented, about two times longer than thorax; segments I to VIII (Figs. 18a, c, d), each with a well sclerotized single dorsal plate, longer than wide and bordered anterior and a posteriorly by a less sclerotized area; lateral regions (Figs. 18c, d), each with two sclerites: a dorsopleural sclerite, which bears a circular annular spiracle (Figs. 18c, d: SP) and a ventropleural sclerite; ventral regions (Fig. 18b) with a single plate, less sclerotized than the dorsal plates. Abdominal segment IX (Fig. 18d) devoid of dorsopleural and ventropleural sclerites and bearing a pair of two-segmented urogomphi, not fused to tergite IX; urogomphal bases markedly separated from each other. Urogomphi (Fig. 18f) with segment I (URI) long, segment II (URII) short, distally rounded and with a long apical seta (AS). Abdominal segment X (Fig. 18d) visible from above, forming a complete sclerotized ring,

FIGURE 14. *Gymnochthebius fossatus*, first instar larva in dorsal and lateral view. Scale bar: 0.3 mm.

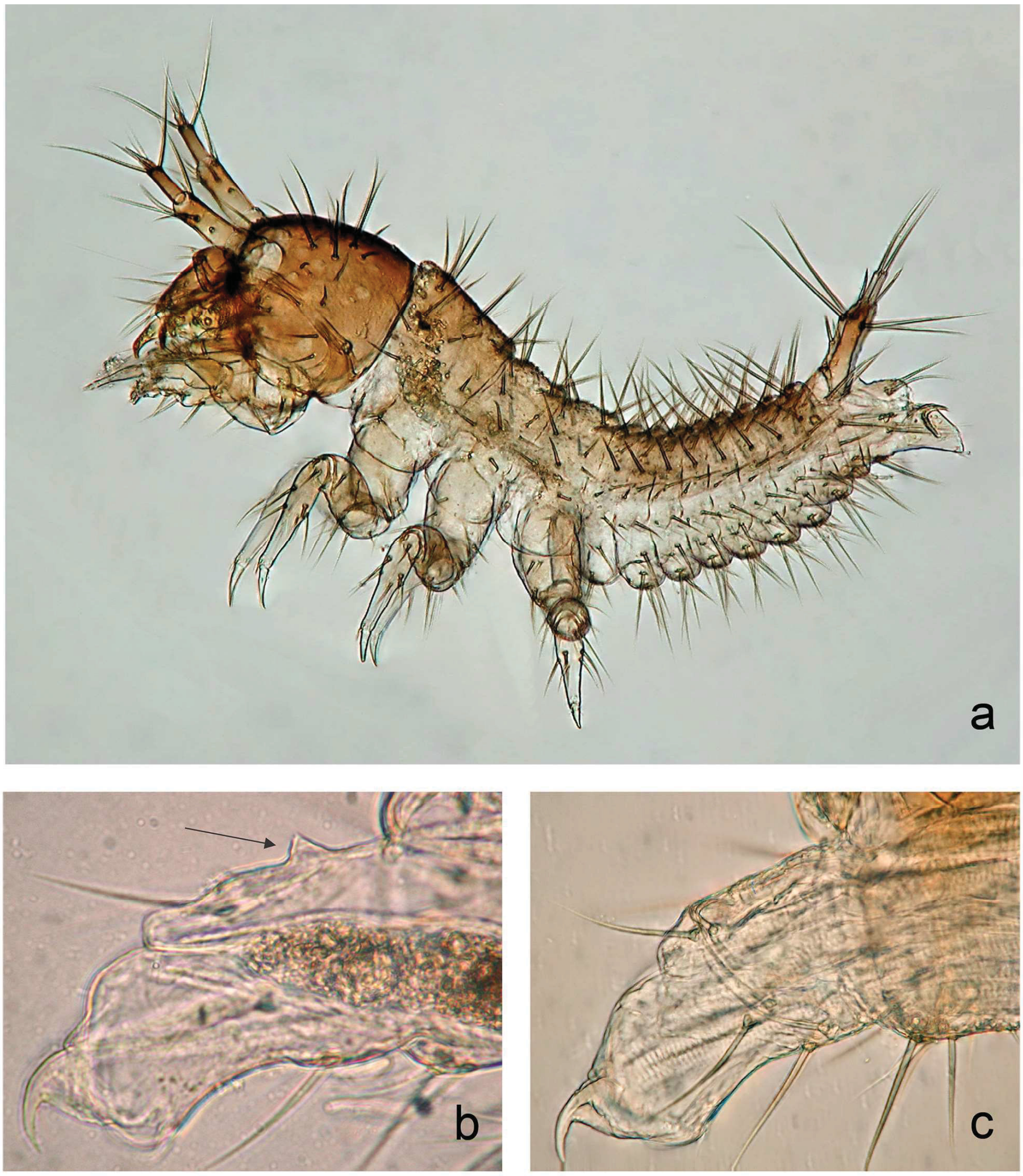

FIGURE 15. *Gymnochthebius fossatus*: a, fresh hatching first instar larva in lateral view; b, abdominal segment X of first instar larva with dorsal egg-burster (arrow); c, abdominal segment X of second instar larva.

distally associated with a membranous region consisting of a ventral sclerotized plate, an anal lobe and two well-developed anal hooks (AH) projecting downward. Abdominal segment X (Fig. 14; 15b; 18d: EB) with a mid-dorsal projection (see discussion).

Larval vestiture. Head. Chaetotaxy of head capsule as in Figs. 16a, b. Frontal region (Fig. 16a) on each side with one pore (FC1) and five setae: two frontal dorsal setae (Fd1, Fd2), two frontal lateral setae (Fl1, Fl2) and one frontal marginal seta (Fm1). Clypeus (Fig. 16a) with three setae on each side (Cl1–Cl3). Epicranial regions (Fig. 16a) each with three pores (EC1, Cg1, Cg2) and 10 setae: a row of four minute posterior setae (P1–P4), two epicranial dorsal setae (Ed1, Ed2), two epicranial lateral setae (El1, El2) and two epicranial marginal setae (Em1, Em2). Temporal regions (Fig. 16b), each with four long setae (T1–T4), T1 longest. Lateral regions (Fig. 16b), each with two setae (L1, L2) and one pore (LC1). Ventral regions (Fig. 16b), each with two setae (V1, V2). Labrum (Figs. 17a, b) with two pores (C1, C2) and seven setae on each side: two discal setae (Ld1, Ld2) and five marginal setae (Lm1–Lm5), Lm2 (Fig. 17b) multifid. Epipharynx as in Fig. 17b. Antennae as in Fig. 17e, antennomere I with four pores; antennomere II with three long setae (IIA1–IIA3), and two solenidia (IIS1, IIS2), solenidium IIS1 long, slender, situated on the distal end of antennomere II; IIS2 short, conical, situated on mesal ventral side of the antennomere II; antennomere III, with four setae (IIIA1–IIIA4) and three apical solenidia (IIIS1–IIIS3). Mandibles (Figs. 17c, d) with two pores (C1, C2) and two setae (M1, M2). Maxillae (Figs. 17e, f): cardo with one seta (Cdo1); stipes with four setae (Stp1–Stp4) and one pore (C1); palpifer with a long seta (Pf1); segment II of the maxillary palps with two setae (Pm1, Pm2); lacinia with six setae (La1–La6) and a blade-like projection on mesal edge.

Thorax. Pronotum (Fig. 16e) with five thoracic pores (C1–C5), one tergal pore (Tg1) and 14 setae on each side. Setae: four in anterior row A (A1–A4), A1 short; three in lateral row L (L1–L3), L3 long; four in posterior row P (P1–P4), P2 long; and three discal setae (Da1, Db1, Dc1). Ventral region of prothorax (Fig. 16f): Presternites, each with two setae (Pr1, Pr2); sternite with two pairs of setae (St1, St2). Prehypopleurites, each with three small setae (Prehy1–Prehy3), and posthypopleurites with one seta each (Pohy1). Mesonotum (Fig. 16e) with 14 setae and three pores (C3, C4, C5) on each side. Setae: four minute setae in anterior row A (A1–A4); three discal setae (Da1, Db1, Dc1); three lateral setae in row L (L1–L3), L3 long; four posterior setae in row P (P1–P4). Ventral region of mesothorax (Fig. 16f): sternite with two pairs of setae (St1, St2). Prehypopleurites (Fig. 16f), each with two small setae (Prehy1, Prehy2) and posthypopleurites, each with a single long seta (Pohy1). Lateral short mesothoracic tubercles with a seta (S1). Chaetotaxy of metathorax identical to mesothorax except for the absence of setae S1.

Abdomen. Chaetotaxy of tergites I–VIII as in Figs. 18a, c, d. Number of setae and pores reduced with respect to thoracic tergites: setae Db1, Dc1, P2 plus pores C1, C2 and C4 absent. Dorsopleural sclerites of abdominal segment I (Figs. 18c, d), each with a circular annular spiracle (SP) and two setae (DP1, DP2). Ventropleural sclerites of segment I (Figs. 18b, c), each with one seta (VP1). Ventropleural sclerites of segment II–VIII (Figs. 18c, d), each with two setae (VP1, VP2). Sternite I (Fig. 18b) with one pore and four setae on each side: one presternal seta (PS1), one discal seta (D1) and two posterior setae (P1, P2). Sternites II–VIII (Figs. 18b, e), with an additional pair of discal setae (D2). Urogomphi with segment I (Fig. 18f: URI) with four pores (C1–C4) and six setae (U1–U6), U5 and U6 short.

Remarks. Upon hatching, the first instar of *G. fossatus* shows a very short abdomen (Fig. 15a) but it enlarges in the next hours, once the larva starts to feed. The abdominal sternal setae D2 are located remarkably close to the basal margin of each sternal plate (Fig. 18b). This feature has been only observed in the first instar larvae of *Gymnochthebius* and led Delgado & Archangelsky (2005) to label this pair of setae as part of the posterior row, thus considering that the larvae of *G. jensenhaarupi* had three pairs of posterior setae (P1 to P3). This feature should be reassessed in future phylogenetic analysis using larval chaetotaxy.

Second instar larva (Figs. 15c; 19–20; 29c, d)**. Material examined**. **CUBA**: 6 larvae (CDUM): "Holguín, Felton, Vuelta Larga, 20°43'7.92"N 75°37'59.19"W, starting rearing 24.iii.2013, larvae collected 02.iv.2013, coll.: A. Deler-Hernández".

Description. General aspect as in Fig. 19. Total body length: 1.69±0.15 mm (mean±SD.; n= 6). General body form elongate. Head and thorax approximately same width, abdomen slightly narrower, parallel-sided. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous area grayish. Maximum head width: 0.25 mm±0.005 (mean±S.D.; n= 6). External morphology similar to that of first instar but lacking cephalic egg-bursters. Dorsopleural sclerites of the abdominal segments I–V, as in first instar larvae, not fused to their respective tergites (see fig. 29c). Dorsopleural sclerites of abdominal segments VI–VIII fused to their respective tergites (see Fig. 29d).

Larval vestiture. Similar to that of first instar larva but with some subprimary additional setae on thorax and abdomen. Additional secondary setae absent.

Head. With same setal pattern to that of first instar (Fig. 20a). Antennae as in Fig. 20b.

Thorax. Pronotal chaetotaxy with the same primary setae as in first instar and with five additional subprimary setae on each side: Da', Db', Dc', Da'', Db'' (Fig. 20c). Meso- and metanotum similar to pronotum, but with setae of row A minute and a pair of tergal pores (Fig. 20c).

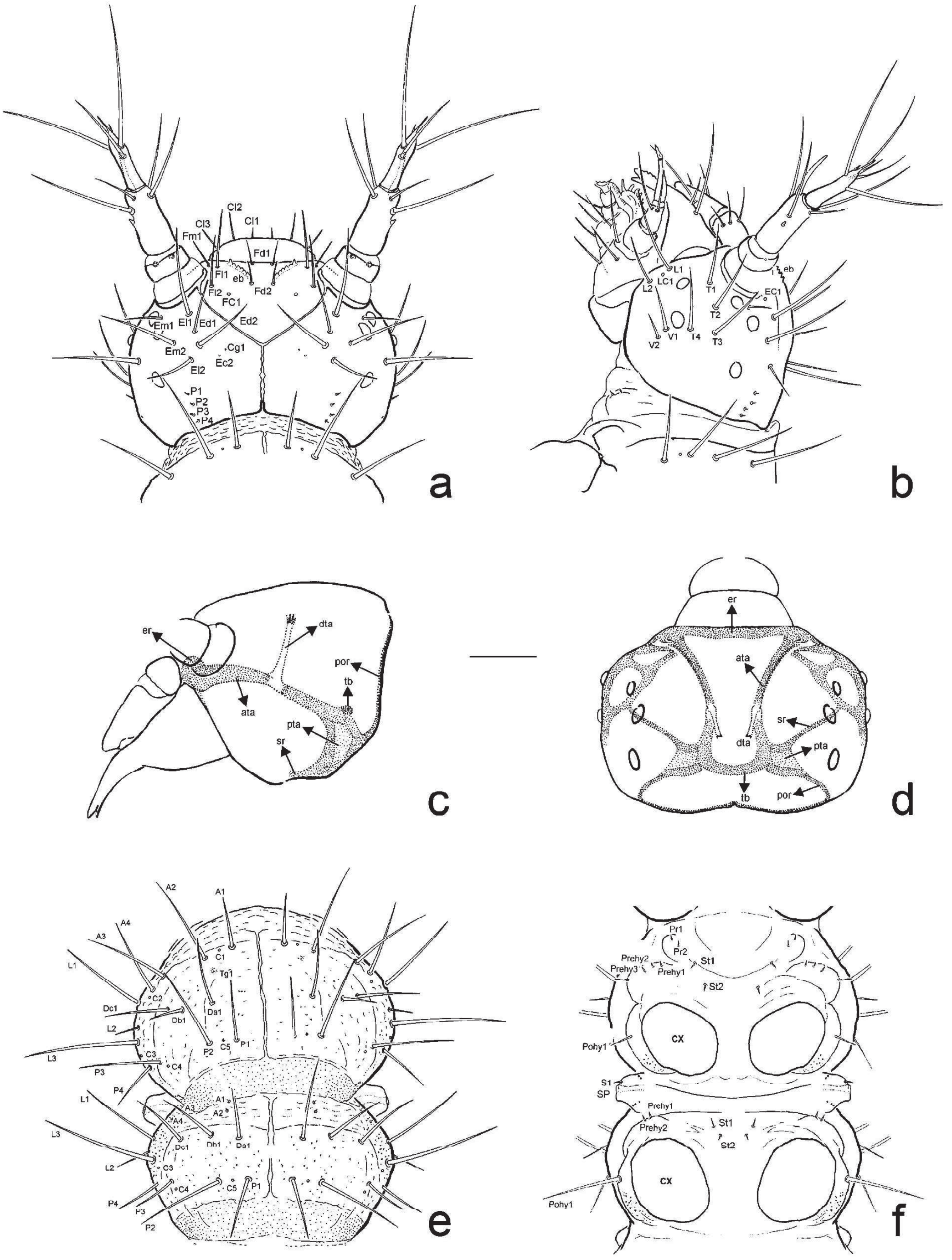

FIGURE 16. *Gymnochthebius fossatus*, first instar larva: a, head capsule in dorsal view; b, head capsule in lateral view; c, internal skeletal structures of head capsule in lateral view; d, same in dorsal view; e, pro- and mesothorax in dorsal view; f, same in ventral view. CX: coxal cavities. Scale bar: 0.05 mm.

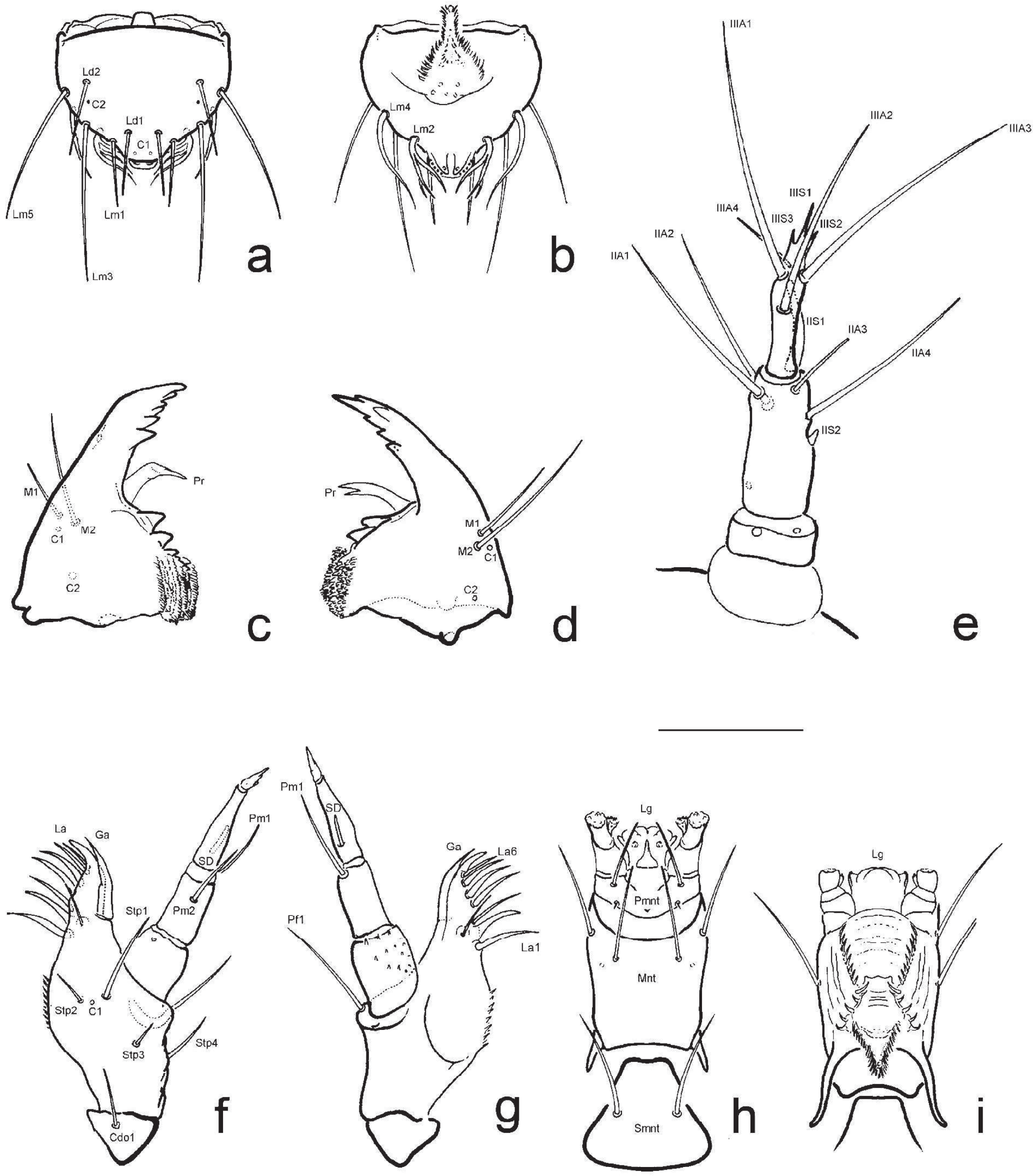

FIGURE 17. *Gymnochthebius fossatus*, first instar larva. Mouthparts and antenna: a, labrum in dorsal view; b, labrum in ventral view; c, left mandible in dorsal view; d, right mandible in dorsal view; e, antenna; f, left maxilla in ventral view; g, left maxilla in dorsal view; h, labium in ventral view; i, hypopharynx. Scale bar: 0.05 mm.

Abdomen. Chaetotaxy of abdominal segments I–VIII as that of the first instar, but without the pairs of setae A1. Abdominal dorsopleural sclerites with a pair of additional subprimary setae (DP') (Fig. 20f). Sternite II with a subprimary discal seta (D') on each side. Sternites II to VIII with two subprimary discal setae on each side (D', D''). Discal setae D2, as in first instar, close to the basal margin of each sternite (Fig. 20d). Chaetotaxy of segments IX and X as in first instar. Urogomphi as in Fig. 20e; poro- and chaetotaxy similar to that of first instar.

Third instar larva. Unknown.

Pupa. Unknown.

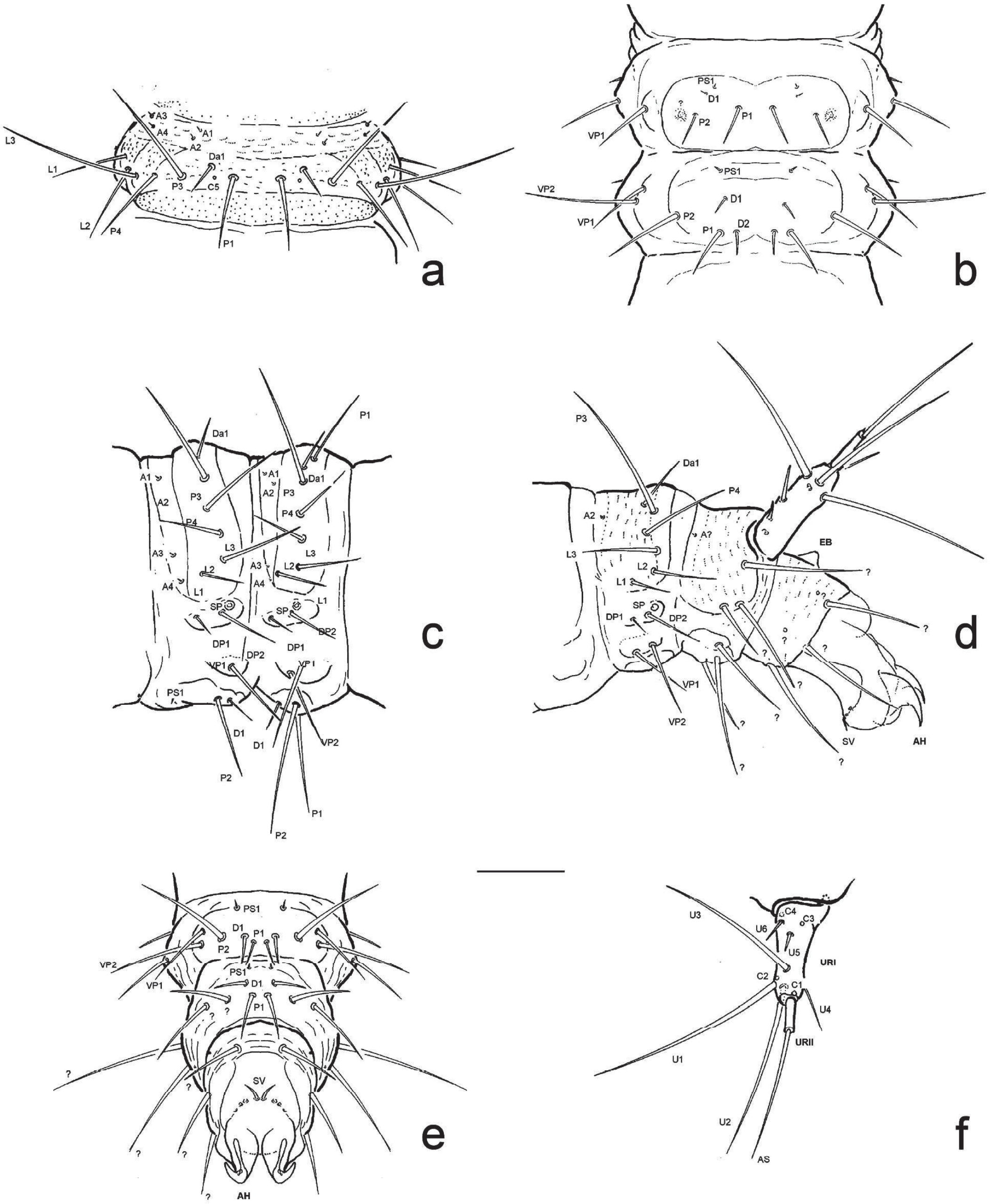

FIGURE 18. *Gymnochthebius fossatus*, first instar larva: a, abdominal tergite I; b, abdominal tergite I and II; c, abdominal segments I and II in lateral view; d, abdominal segments VIII to X and pygopod in lateral view; e, same in ventral view; f, left urogomphus in dorsal view. Scale bar: 0.05 mm.

FIGURE 19. *Gymnochthebius fossatus*. Second instar larva in dorsal and lateral view. Scale bar: 0.3 mm.

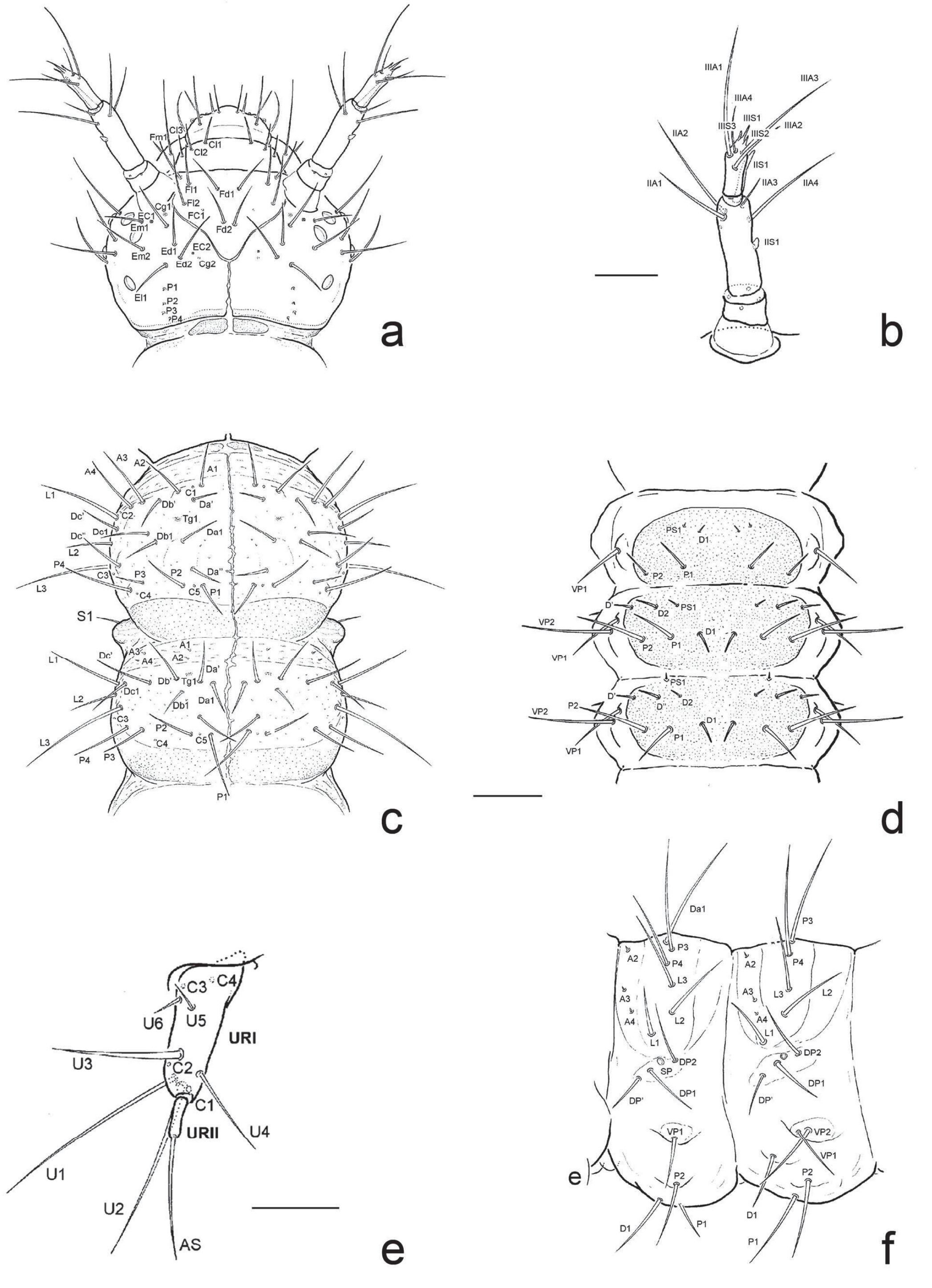

FIGURE 20. *Gymnochthebius fossatus*, second instar larva: a, head capsule in dorsal view; b, antenna; c, pro- and mesothorax in dorsal view; d, abdominal segments I to III in ventral view; e, left urogomphus in dorsal view; f, abdominal segments I and II in lateral view. Scale bars: 0.05 mm.

Description of the immature stages of *Ochthebius attritus*

Egg. Unknown.

Larva

First instar larva (Figs. 2c; 21–24). **Material examined**. **CUBA**: 2 larvae (CDUM) "Santiago de Cuba Prov., Humedal San Miguel de Parada, Laguna Temporal, 20°1'6.93"N 75°52'8.76"W, 1 m, starting rearing 29.v.2009, larvae collected 03.vi.2009, coll.: A. Deler-Hernández"; same locality: 1 larva (CDUM), starting rearing 29.v.2009, larva collected 04.vi.2009; 2 larvae (CDUM), starting rearing 29.v.2009, larvae collected 06.vi.2009; 4 larvae (CDUM), starting rearing 29.v.2009, larvae collected 07.vi.2009; 4 larvae (CDUM), starting rearing 29.v.2009, larvae collected 11.vi.2009; 1 larva (CDUM), starting rearing 29.v.2009, larva collected 13.vi.2009; 1 larva (CDUM), starting rearing 29.v.2009, larva collected 15.vi.2009; 4 larvae (CDUM), starting rearing 29.v.2009, larvae collected 16.vi.2009; 2 larvae (MNPC), starting rearing 29.v.2009, larvae collected 21.vi.2009; 1 larva (CDUM), starting rearing 15.vii.2009, larva collected 31.vii.2009; 6 larvae (MNPC), starting rearing 15.vii.2009, larvae collected 06.viii.2010; 9 larvae (MNPC), starting rearing 15.vii.2009, larvae collected 09.viii.2009; 6 larvae (CDUM), starting rearing 15.vii.2009, larvae collected 10.viii.2009; 4 larvae (CDUM), starting rearing 15.vii.2009, larvae collected 12.viii.2009; 1 larva (MNPC), starting rearing 15.vii.2009, larva collected 16.viii.2009; 6 larvae (MNPC), starting rearing 03.xi.2010, larvae collected 22.xi.2010.

Description. General aspect as in Fig. 21. Total body length: 1.12±0.16 mm (mean±SD.; n= 18). General body form elongate and slender. Head slightly wider than thorax, abdomen approximately parallel-sided and only slightly tapered posteriorly. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous areas grayish. Maximum head width: 0.22±0.007 mm (mean±S.D.; n= 11). Head protracted, prognathous, slightly declined and subchordate in dorsal view. Ecdysial line Y-shaped, epicranial stem long, frontal arms V-shaped connecting distally with base of antennae. Head capsule divided into three regions: a medial frontal area bordered by the frontal arms and two epicranial plates, located behind and laterad to the frontal arms. Gula well developed, subquadrate, closing the cephalic capsule ventrally; almost fused to submentum anteriorly and bordered laterally by elongate tentorial pits (Fig. 2c). Occipital foramen divided into two parts by tentorial bridge. Epicranial plates each with five globose stemmata, forming a dorsolateral cluster behind the base of antennae. Frontal area with two pairs of egg-bursters (Figs. 22a, b); anterior pair well developed, oblique, serrate and projecting over the clypeus; posterior pair small, triangular. Clypeus transverse, with two dentiform projections at its distal margin. Labrum free, semicircular. Clypeo-labral suture slightly sinuate. Antennae three-segmented (Fig. 23e), antennomere I wide, antennomere II ca. two times longer than antennomere I, antennomere III slender, shorter than antennomere I. Mandibles slightly asymmetrical, broad at base and narrowing distally. Mesal surface of mandibular base with a mola bearing several transverse ridges of asperites and one prominent molar tooth. Inner edge continuing with a slender prostheca (Figs. 23c, d: Pr), apex fimbriate. Maxillae with cardines oblique, externally divided in two plates; stipes wide and stout, mala, distally divided into a fixed galea and a lacinia; galea (Fig. 23f: Ga) falciform; lacinia (Fig. 23g: La) spinose along mesal edge; maxillary palp three-segmented, segment I stout, segment II cylindrical, segment III slender, with an elongate digitiform sensorial appendage (Fig. 23g: SD) on external surface situated basally and an acuminate complex sensorial structure on its apex. Labium (Fig. 23h) consisting of three sclerites. Submentum (Smnt) subtrapezoidal, mentum (Mnt) subquadrate and prementum (Pmnt) V-shaped; ligula (Lg) shorter than labial palps, apex digitate and well developed; labial palp two-segmented, directly articulated with prementum. Hypopharynx (Fig. 23i) simple, membranous, clothed with short spicules. Tentorium similar in all larval instars (see description in third instar larva). Cervical region short, membranous, bearing dorsally two small but distinct sclerites.

Thorax less than a third the length of the abdomen (when the latter completely distended). Prothorax wider than meso- and metathorax. Dorsal regions with a single sclerite well-developed in the three segments, each with an evident median ecdysial suture (Fig. 22c). Lateral areas each with two connate sclerites: a prehypopleuron forming a sclerotized lobe anterior to coxal cavity and a posthypopleuron. Prothorax laterally also with a small sclerite in each side that bears a spiracle (Fig. 22d: SP). Mesothoracic spiracles non-functional, reduced to small tubercles. Ventral region (Fig. 22d) of each thoracic segment consisting of three small sclerites: two lateral presternites and a median posterior sternum. Legs well-developed, long and five-segmented (see Fig. 28g).

FIGURE 21. *Ochthebius attritus*, first instar larva in dorsal and lateral view. Scale bar: 0.3 mm.

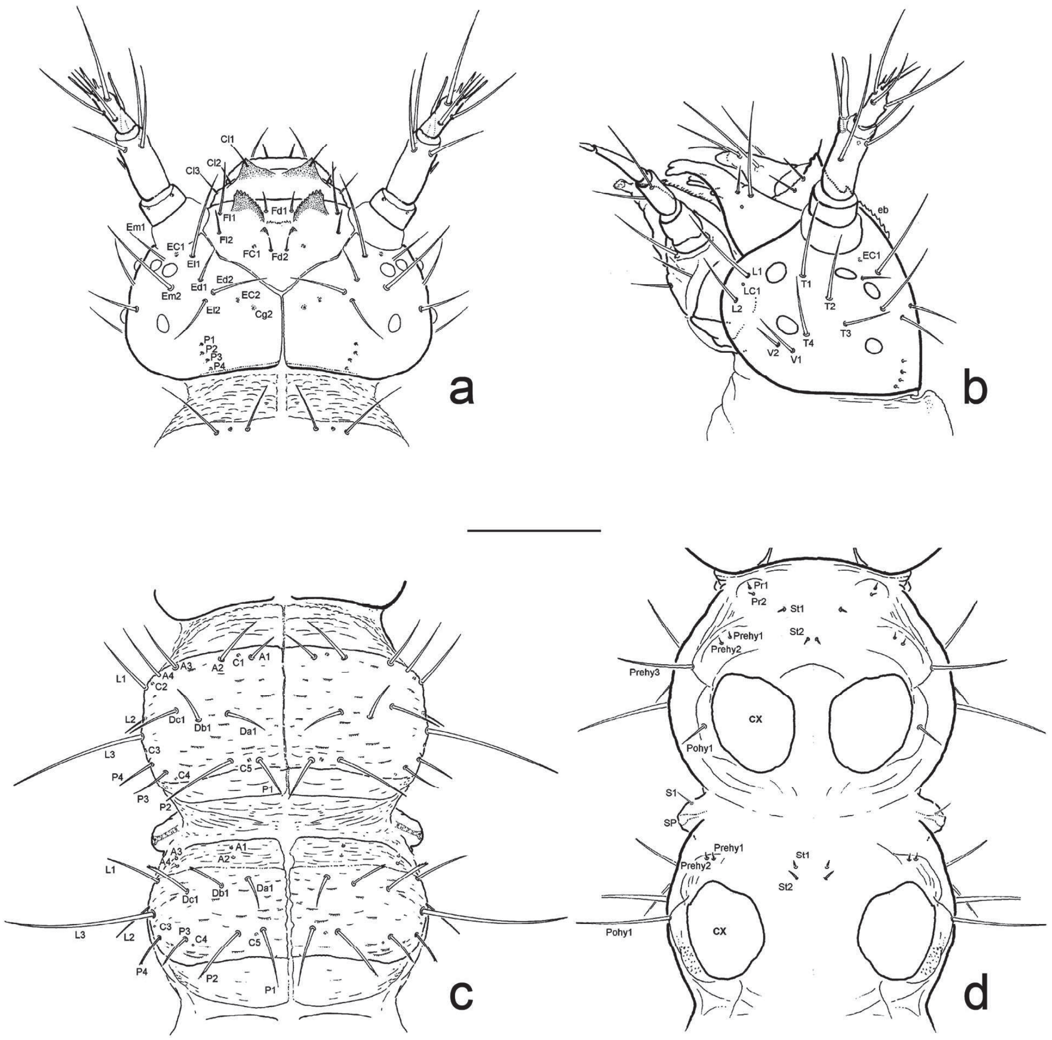

FIGURE 22. *Ochthebius attritus*, first instar larva: a, head capsule in dorsal view; b, same in lateral view; c, pro- and mesothorax in dorsal view; d, same in ventral view. CX: coxal cavities. Scale bar: 0.1 mm.

Abdomen 10-segmented, about two times longer than thorax; segments I to VIII (Fig. 24a), each with a well sclerotized single dorsal plate, longer than wide and with an anterior and a posterior less sclerotized area; lateral regions (Fig. 24c), each with two sclerites: a dorsopleural sclerite, which bears a circular annular spiracle (Fig. 24c: SP) and a ventropleural sclerite; ventral regions (Fig. 24b) with a single plate in each segment, less sclerotized than dorsal plates. Abdominal segment IX (Fig. 24e) without dorsopleural and ventropleural sclerites and bearing a pair of two-segmented urogomphi, not fused to tergite IX and markedly close from each other basally. Urogomphal segment I (Fig. 24d: URI) long, segment II (URII) short, rounded distally and with a long apical seta (AS). Abdominal segment X (Fig. 24e) visible from above, forming a complete sclerotized ring, distally associated with a membranous region consisting of a ventral sclerotized plate, an anal lobe and two well-developed anal hooks (AH) projecting downward.

Larval vestiture. Head. Chaetotaxy of head capsule as in Figs. 22a, b. Frontal region (Fig. 22a) on each side with one pore (FC1) and five setae: two frontal dorsal setae (Fd1, Fd2), two frontal lateral setae (Fl1, Fl2) and one frontal marginal seta (Fm1). Clypeus (Fig. 22a) with three setae on each side (Cl1–Cl3). Each seta Cl1 borne from one of the clypeal projections mentioned above. Epicranial regions (Fig. 22a) each with four pores (EC1, EC2, Cg1, Cg2) and 10 setae: a row of four minute posterior setae (P1–P4), two epicranial dorsal setae (Ed1, Ed2), two epicranial lateral setae (El1, El2) and two epicranial marginal setae (Em1, Em2). Temporal regions (Fig. 24b), each with four long setae (T1–T4). Lateral regions (Fig. 22b), each with two setae (L1, L2) and one pore (LC1). Ventral regions (Fig. 22b), each with two setae (V1, V2). Labrum (Figs. 23a, b) with two pores (C1, C2) and seven setae on each side: two discal setae (Ld1, Ld2) and five marginal setae (Lm1–Lm5). Epipharynx as in Figs. 23b. Antennae as in Fig. 23e; antennomere I with four pores; antennomere II with four long setae (IIA1–IIA4), and two solenidia (IIS1, IIS2), solenidium IIS1 well developed, situated on the distal end of antennomere II; IIS2 small, peg-like, situated on mesal ventral side of the antennomere; antennomere III, with four setae (IIIA1–IIIA4) and three apical solenidia (IIIS1–IIIS3). Mandibles (Figs. 23c, d) with two pores (C1, C2) and two setae (M1, M2. Maxillae (Figs. 23f, g): cardines, each with one seta (Cdo1); stipes with four setae (Stp1–Stp4) and a pore (C1); palpifer with a long seta (Pf1); segment II of the maxillary palps with two setae (Pm1, Pm2); lacinia with five setae on mesal edge (La1–La5) and a blade-like cuticular projection.

Thorax. Pronotum (Fig. 22c) with five thoracic pores (C1–C5), and 14 setae on each side. Setae: four in anterior row A (A1–A4), A1 shortest; three in lateral row L (L1–L3), L3 long; four in posterior row P (P1–P4), P2 long; and three discal setae (Da1, Db1, Dc1). Ventral region of prothorax (Fig. 22d): Presternites, each with two setae (Pr1, Pr2); sternite with two pairs of setae (St1, St2). Prehypopleurites, each with three setae (Prehy1–Prehy3), Prehy3 extremely long compared with other two Prehy setae and posthypopleurites with one setae each (Pohy1). Mesonotum (Fig. 22c) with 14 setae and three pores (C3, C4, C5) on each side. Setae: four minute setae in anterior row A (A1–A4); three discal setae (Da1, Db1, Dc1); three lateral setae in row L (L1–L3) and four posterior setae in row P (P1–P4). Ventral region of mesothorax (Fig. 22d): sternites with two pairs of setae (St1, St2). Prehypopleurites (Fig. 22d), each with two setae (Prehy1, Prehy2) and posthypopleurites (Fig. 22d), each with a single long seta (Pohy1). Lateral short mesothoracic tubercles bearing the spiracles with a seta (S1). Chaetotaxy of metathorax identical to mesothorax except for the absence of setae S1.

Abdomen. Chaetotaxy of tergite I as in Fig. 24a. Number of setae and pores reduced with respect to thoracic tergites: setae A1, Db1, Dc1, P2 plus pores C1, C2 and C4 absent. Dorsopleural sclerites of abdominal segment I–VIII (Fig. 24c), each with a circular annular spiracle (SP) and two setae (DP1, DP2). Ventropleural sclerites of segment I (Fig. 24c), each with one seta (VP1). Sternite I (Fig. 24c) with one pore (Fig. 24c: ?) and four setae on each side: one presternal seta (PS1), one discal seta (D1) and two posterior setae (P1, P2). Chaetotaxy of tergites II–VIII (Fig. 24c) similar to that of tergite I but without setae A4. Chaetotaxy of sternites II–VIII (Fig. 24b) similar to that of sternite I but with a second pair of discal setae (D2). Ventropleural sclerites of segments II–VIII (Fig. 24c), each with two setae (VP1, VP2). Urogomphi with segment I (Fig. 24c: URI) with four pores (C1–C4) and six setae (U1–U6), U5 and U6 short. Tergite IX (Fig. 24e) on each side of the pretergal area with one minute setae (A?) and three additional pairs of setae of unknown homology on the tergal area. Abdominal segment X (Fig. 24e) with three ventrolateral setae and three spine-like setae on each side (SV) associated to the membranous area which bears the anal hooks.

Second instar larva (Figs. 25; 29e, f)**. Material examined**. **CUBA**: 2 larvae (CDUM): "Santiago de Cuba Prov., Humedal San Miguel de Parada, Laguna Temporal, 20°1'6.93"N 75°52'8.76"W, 1 m, starting rearing 29.v.2009, larvae collected 11.vi.2009, coll.: A. Deler-Hernández"; same locality: 2 larvae (CDUM), starting rearing 29.v.2009, larvae collected 13.vi.2009; 3 larvae (MNPC), starting rearing 29.v.2009, larvae collected 15.vi.2009; 1 larva (CDUM), starting rearing 29.v.2009, larva collected 16.vi.2009; 1 larva (CDUM), starting rearing 29.v.2009, larva collected 21.vi.2009; 1 larva (CDUM), starting rearing 15.vii.2009, larva collected 03.viii.2009; 3 larvae (MNPC), starting rearing 15.vii.2009, larvae collected 06.viii.2009; 3 larvae (MNPC), starting rearing 15.vii.2009, larvae collected 09.viii.2009; 2 larvae (CDUM), starting rearing 15.vii.2009, larvae collected 10.viii.2009; 8 larvae (CDUM), starting rearing 15.vii.2009, larvae collected 12.viii.2009; 4 larvae (MNPC), starting rearing 15.vii.2009, larvae collected 16.viii.2009; 2 larvae (CDUM), starting rearing 25.ix.2009, larvae collected 14.x.2009.

Description. Total body length: 1.93±0.15 mm (mean±SD.; n= 7). Body form elongate. Head and thorax approximately of same width; abdomen slightly narrower, parallel-sided. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous areas grayish. Maximum head width: 0.28±0.009 mm (mean±S.D.; n= 9). Dorsopleural sclerites of abdominal segments I–V, as in first instar, not fused to their respective tergites (see Fig. 29e).

Dorsopleural sclerites of abdominal segment VI fused to their respective tergites in some specimens. Dorsopleural sclerites of abdominal segments VII and VIII completely fused to their respective tergites (see Fig. 29f) in all specimens.

Larval vestiture. Similar to that of first instar larva but with some subprimary additional setae on thorax and abdomen. Additional secondary setae absent.

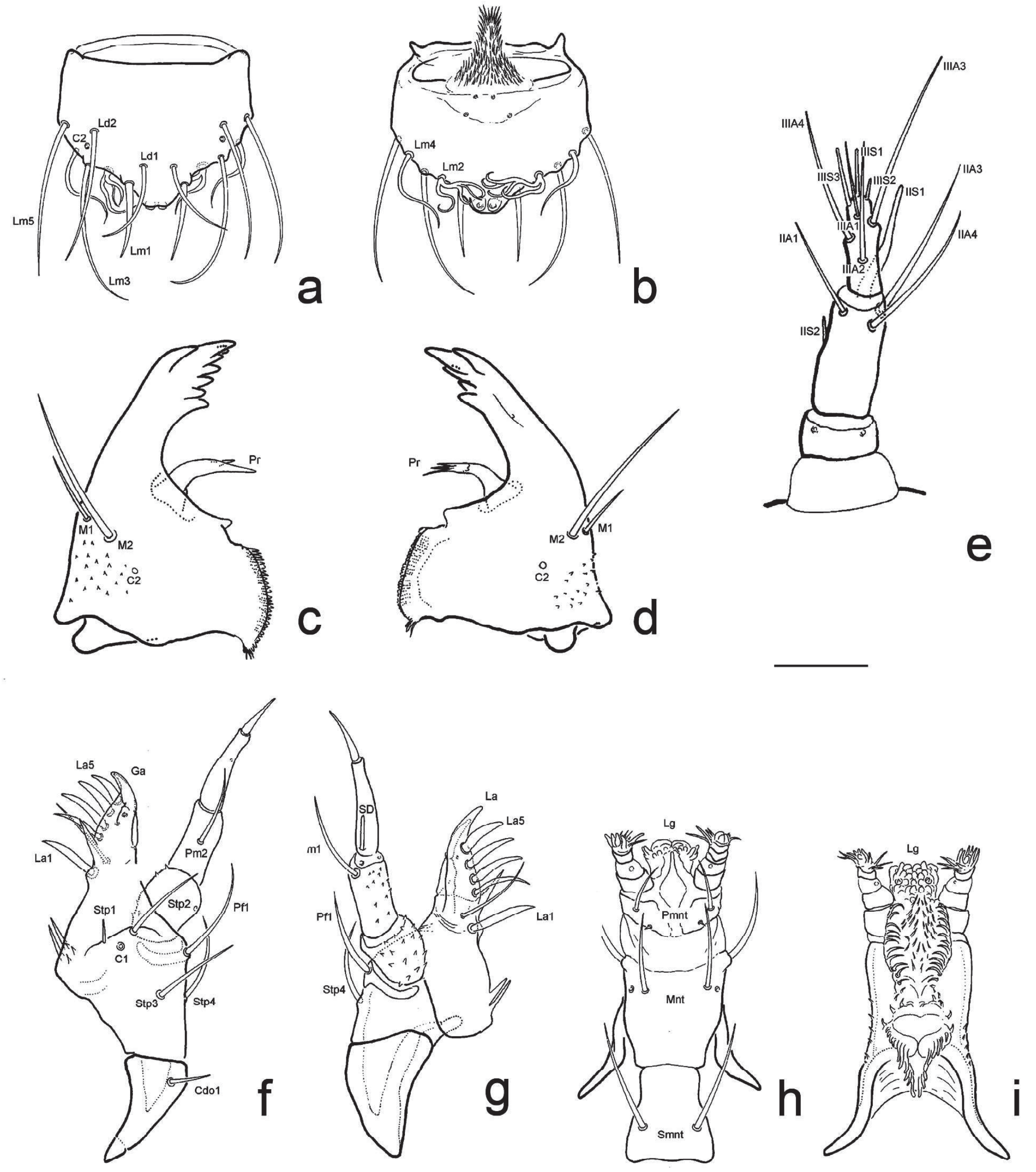

FIGURE 23. *Ochthebius attritus*, first instar larva. Mouthparts and antenna: a, labrum in dorsal view; b, labrum in ventral view; c, left mandible in dorsal view; d, right mandible in dorsal view; e, antenna; f, left maxilla in ventral view; g, left maxilla in dorsal view; h, labium in ventral view; i, hypopharynx. Scale bar: 0.05 mm.

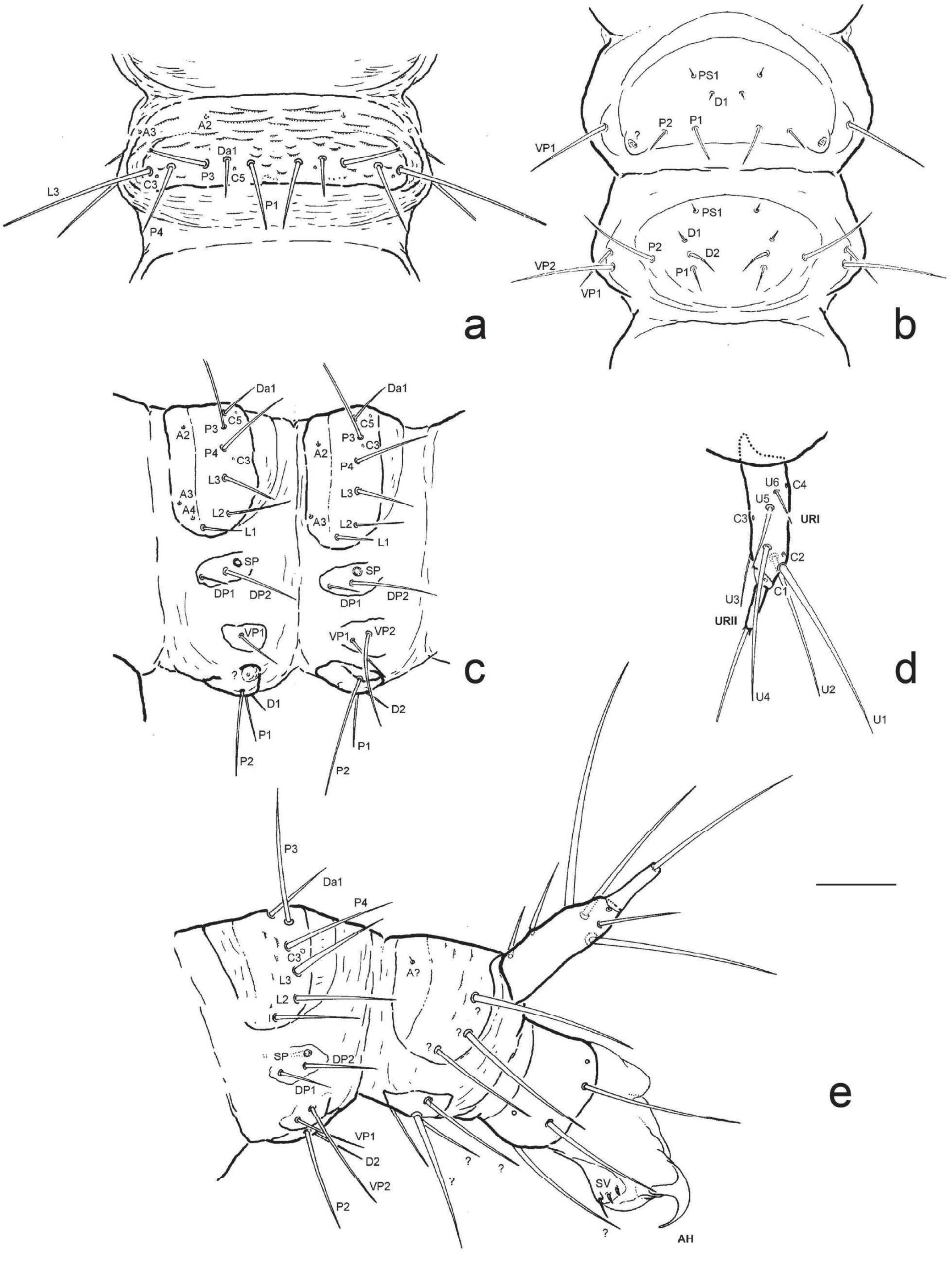

FIGURE 24. *Ochthebius attritus*, first instar larva: a, abdominal segment I in dorsal view; b, abdominal segments I and II in ventral view; c same in lateral view; d, left urogomphus in dorsal view; e, abdominal segments VIII to X and pygopod in lateral view. Scale bars: 0.05 mm.

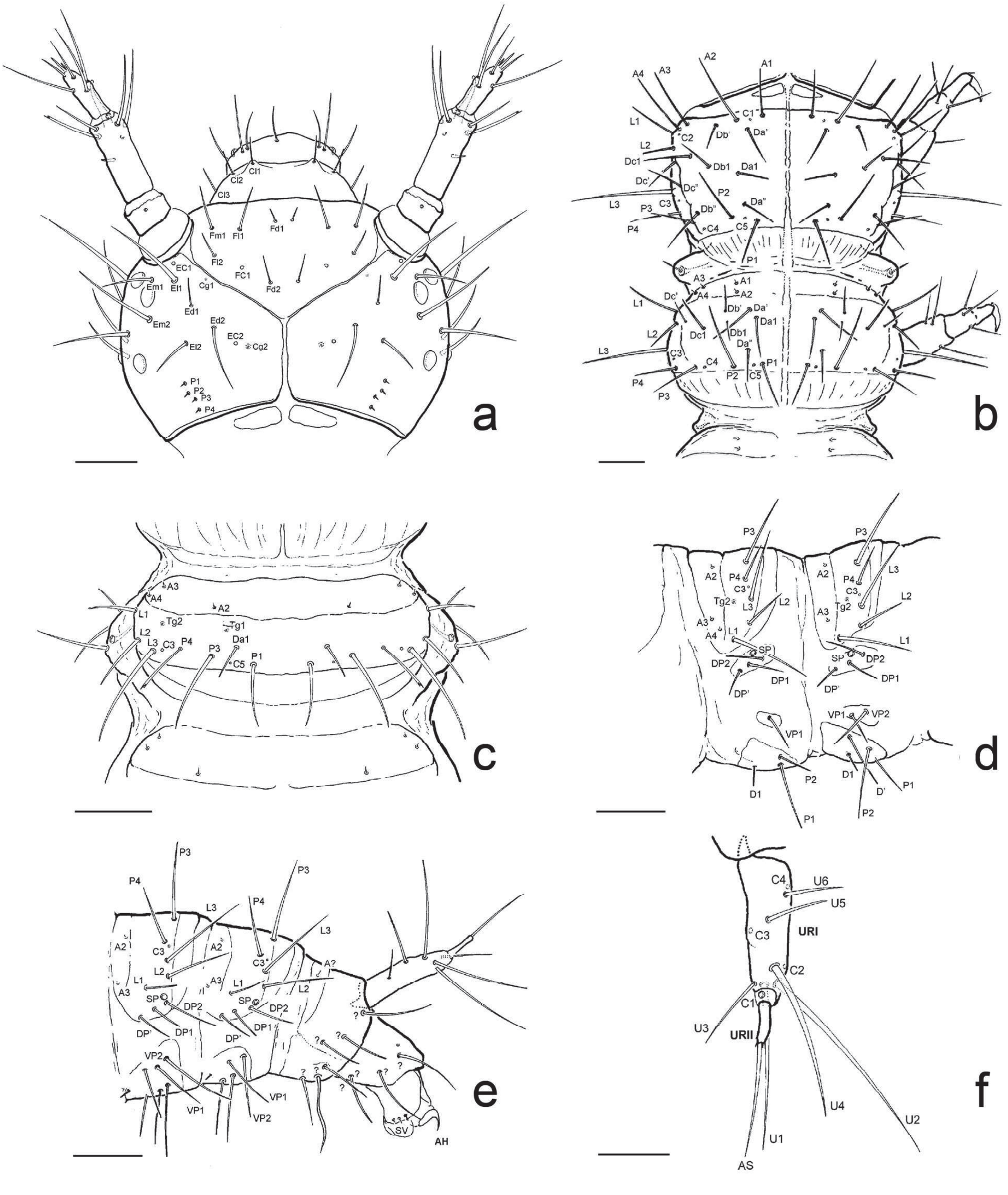

FIGURE 25. *Ochthebius attritus*, second instar larva: a, head capsule in dorsal view; b, pro- and mesothorax in dorsal view; c, abdominal segment I in dorsal view; d, abdominal segments I and II in lateral view; e, abdominal segments VIII to X and pygopod in lateral view; f, left urogomphus in dorsal view. Scale bar: 0.1 mm for a–e, 0.05 mm for f.

Head. With same setal pattern to that of first instar (Fig. 25a).

Thorax. Pronotal chaetotaxy as in first instar, with four additional pairs of subprimary setae (Da', Db', Dc', Da''). Meso- and metanotum with same subprimary setae as pronotum (Fig. 25b).

Abdomen. Chaetotaxy of abdominal segments as that of first instar, but with two pairs of tergal pores (Tg1, Tg2) on abdominal segment I (Fig. 25c). Abdominal dorsopleural sclerites of segments I–VIII, each with an additional seta DP' (Figs. 25d, e). Sternite II with a subprimary discal seta (D') on each side. Sternites II to VIII with two subprimary discal setae on each side (D', D''). Chaetotaxy of segments IX and X as in first instar larvae. Urogomphi as in Fig. 25f, with same poro- and chaetotaxy than in first instar.

FIGURE 26. *Ochthebius attritus*, third instar larva in dorsal and lateral view. Scale bar: 1 mm.

Third instar larva (Figs. 26–27; 28a–d, g). **Material examined**. **CUBA**: 2 larvae (CDUM): "Santiago de Cuba Prov., Humedal San Miguel de Parada, Laguna Temporal, 20°1'6.93"N 75°52'8.76"W, 1 m, starting rearing 29.v.2009, larvae collected 07.vi.2009, coll.: A. Deler-Hernández"; same locality: 2 larvae (CDUM), starting rearing 29.v.2009, larvae collected 13.vi.2009; 1 larva (CDUM), starting rearing 29.v.2009, larva collected 18.vi.2009; 1 larva (CDUM), starting rearing 29.v.2009, larva collected 20.vi.2009; 3 larvae (CDUM), starting rearing 15.vii.2009, larvae collected 03.viii.2009; 1 larva (CDUM), starting rearing 15.vii.2009, larva collected 10.viii.2009; 1 larva (CDUM), starting rearing 15.vii.2009, larva collected 12.viii.2009; 9 larvae (CDUM, MNPC), starting rearing 15.vii.2009, larvae collected 16.viii.2009; 2 larvae (CDUM), larvae collected 25.ix.2009; 1 larva (MNPC), starting rearing 15.ix.2011, larva collected 20.x.2011.

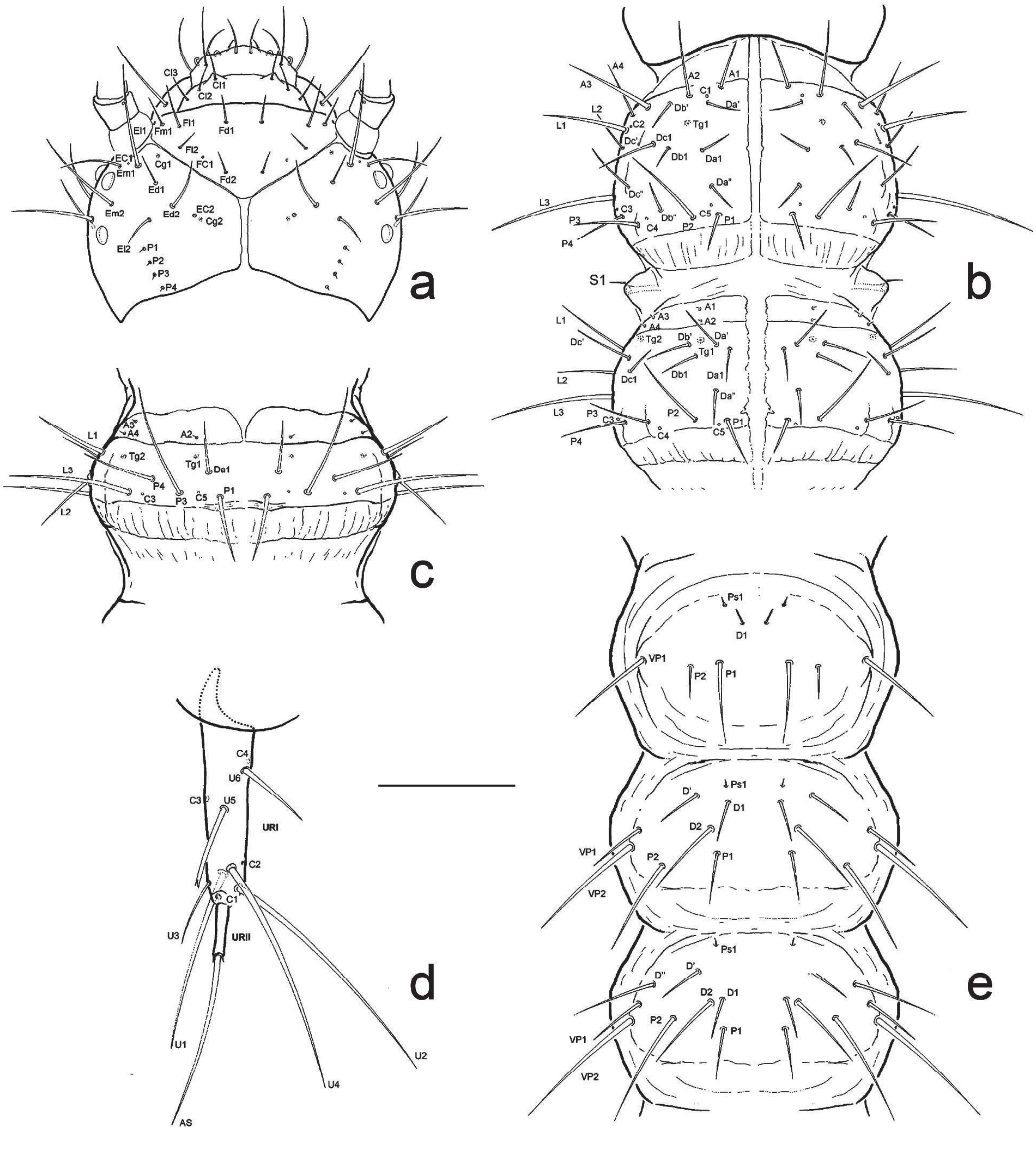

FIGURE 27. *Ochthebius attritus*, third instar larva: a, head capsule in dorsal view; b, pro and mesothorax in dorsal view; c, abdominal segment I in dorsal view; d, left urogomphus in dorsal view; e, abdominal segments I to III in ventral view. Scale bar: 0.1 mm.

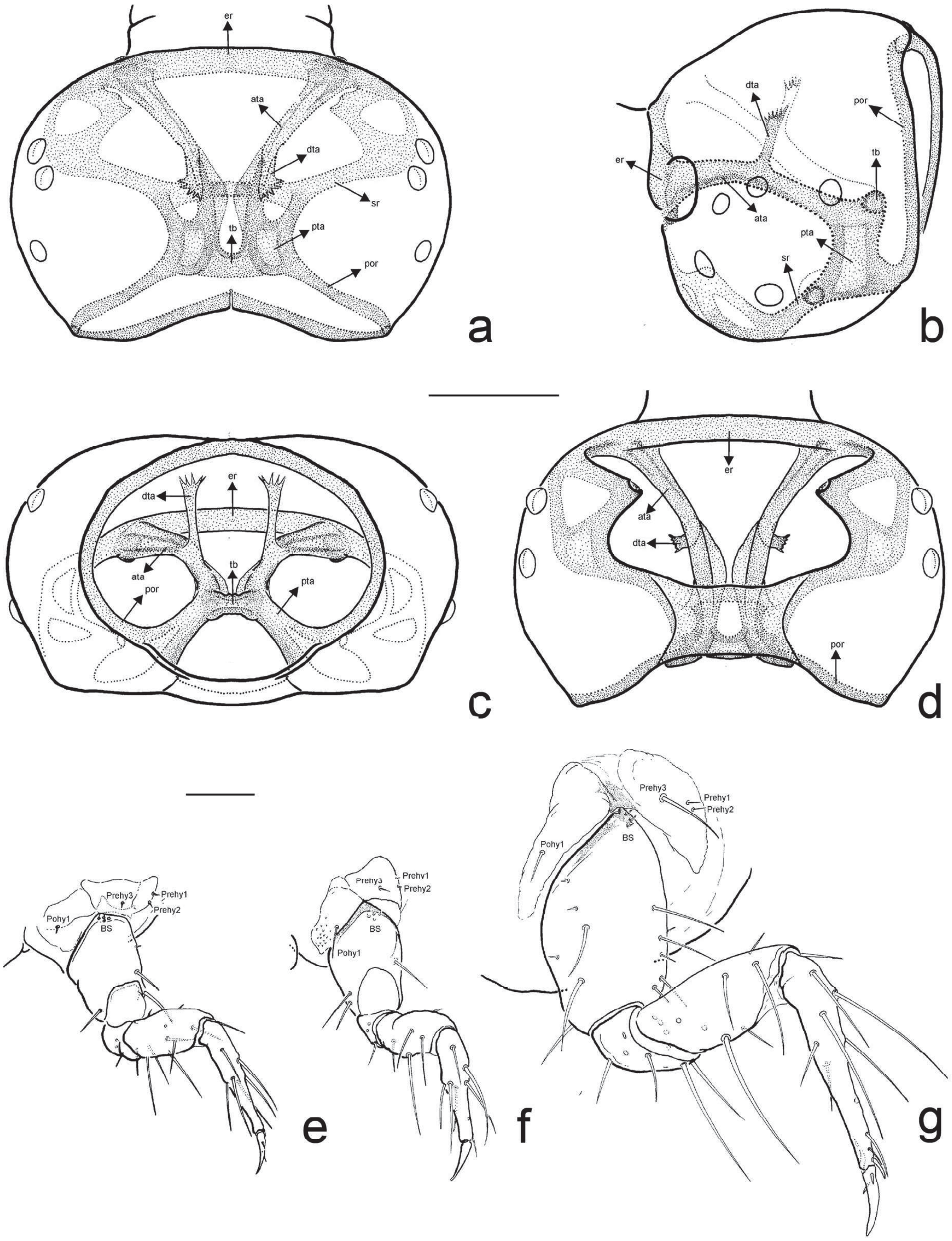

FIGURE 28. Internal skeletal structures of head capsule of *Ochthebius attritus* and right larval prolegs of three different species: a, tentorium in dorsal view; b, lateral view; c, posterior view; d, ventral view; e, proleg of *H. perkinsi* (first instar); f, proleg of *G. fossatus* (first instar); g, proleg of *O. attitus* (third instar). Scale bars: 0.05 mm.

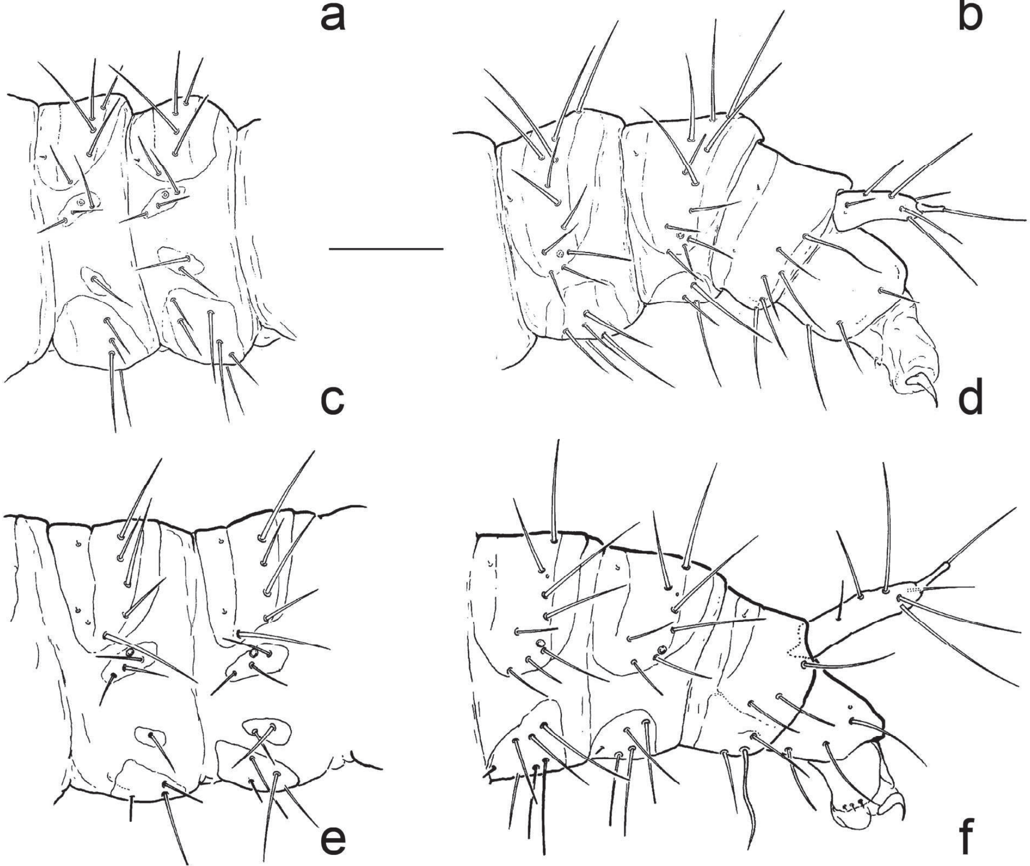

FIGURE 29. Abdominal segments of second instar larvae, in lateral view: a–b, segments I–III and VII–VIII of *H. guadelupensis*; c–d, segments I–II and VII–X of *G. fossatus*; e–f, segments I–II and VII–X of *O. attritus*. Scale bar: 0.1 mm.

Description. General aspect as in Fig. 26. Total body length: 2.58±0.31 mm (mean±SD.; n= 14). General body form elongate, dorsally sub-fusiform. Head narrower than thorax. Mesothorax wider than pro- and metathorax, abdominal segments approximately parallel-sided anteriorly and tapering posteriorly. More or less straight in lateral view and subcylindrical in cross-section. Head moderately sclerotized, light brown. Thorax and abdomen feebly sclerotized, light brown, membranous areas grayish. Maximum head width: 0.33±0.008 mm (mean±S.D.; n= 9). Tentorium (Figs. 28a–d) consisting of a pair of posterior tentorial arms originating at the posterobasal region of the head capsule, connected by a tentorial bridge and two anterior arms extending anteriorly to reach the sides of epistomal ridge. From each anterior arm a dorsal tentorial arm extends dorsally. Anterior arms (Figs. 28a–d: ata) wide and strong, fused distally to epistomal ridge (Figs. 28c, d: er). Epistomal ridge well developed. Dorsal arms

(Figs. 28a–d: dta) stout, well sclerotized and not reaching the base of epicranium. Tentorial bridge (Figs. 28b, c: tb) wide and long. Posterior arms externally marked by long tentorial pits and basally connected with postoccipital (Figs. 28a–d: por) and subgenal ridges (Fig. 28a: sr).

Besides the expected size increase and a moderate change in body form, the most remarkably morphological distinction of third instar larvae with respect to instar II is in segments I to IV in which the dorso- and ventropleural sclerites are fused to their respective tergites and sternites.

Larval vestiture. Chaetotaxy identical to that of second instar larvae but with some differences associated to the fusion of abdominal lateral sclerites referred above. Head and thorax as in second instar (Figs. 27a, b). Chaetotaxy of abdominal tergite I as in Fig. 27c. Abdomen with dorsopleural sclerites of segments I to VIII fused with their respective tergites thus, with the pairs of setae DP1, DP2 and DP' plus the spiracles (SP) placed on the lateral margins of a single dorsolateral plate; similarly, setae VP1 (abdominal segment I) and VP1 plus VP2 (abdominal segments II to VIII) are placed on the lateral margins of a single lateroventral plate. Sternal setae VP1 of segment I well developed (Fig. 27e). Sternite II with an additional subprimary discal seta (D') on each side. Sternites II to VIII with two subprimary discal setae on each side (D', D'') (Fig. 27e). Chaetotaxy of segments IX and X as that of first and second instars. Urogomphi as in Fig. 27d.

Pupa. A single pupa of this species was obtained but could not be studied because it was in poor condition.

Discussion

The preimaginal stages of the studied species here show the typical morphology already known for the family (Jäch *et al.* 2016). However, several results deserve some comments especially those that expand the described morphological repertoire of the preimaginal stages of Hydraenidae.

Descriptions of hydraenid eggs are rare. Our results are in accordance with previous information on a few Palearctic species treated in Jäch *et al.* (2016), and suggest again that *Hydraena* females do not use silk strands to protect their eggs. Whether hydraenid females produce silk to protect their eggs has been a source of some conjecture for years. Information on presumed silk threads in eggs of some American Hydraenidae comes from the old work of Richmond (1920), which described and figured a loosely but continuous web of silk fibers deeply attached to eggs in *H. pennsylvanica* and *G. fossatus*. Most probably, the classical and now-abandoned group relationship between Hydraenidae and Hydrophilidae led Richmond to wrongly homologize the protecting coating of the hydraenid eggs with the silky hydrophilid cocoon. However, it is important to note that Richmond could likely observe some fibrous material around the eggs examined by him, similar to that referred by us in Fig. 1f. As stated above, the covering coat of hydraenid eggs can be scattered with substrate particles and vegetal fibers obtained from the habitat were they are laid. It cannot be therefore discarded at present that this material could be used intentionally by some females to either reinforce or camouflage of the egg envelope. Moreover, some filamentous algae and fungal hyphae grow on eggs obtained in lab conditions. In our opinion, the presence of all this filamentous material around some eggs can be a feasible explanation for the error in illustration produced by Richmond. Except for the silk strands layer, the egg of *H. pennsylvanica* illustrated by Richmond (1920) is remarkably similar to that of the species described here and especially to that of *H. perkinsi*.

Our study allows an almost complete description of the external morphological characters of the larvae for the known Cuban species of the family. Larvae of the three instars and the three genera can be distinguished using the following keys.

Key to the instar larvae of Hydraenidae occurring in Cuba

1. Frontal sclerite of head capsule with egg-bursters (Figs. 16a, b; 22a, b) . . . . *first instar larva*
1' Frontal sclerite of head capsule without egg-bursters (Fig. 4a) 2
2. Lateral abdominal areas of segments I to XVII with dorso- and ventrolateral sclerites clearly differentiated from dorsal and ventral sclerites. Pronotum only with three discal setae. Maximum body width at the level of head capsule . . . . *first instar larva*
2' Abdominal dorso- and ventrolateral sclerites not clearly differentiated from dorsal and ventral sclerites in some segments; at least abdominal segments VI to VIII bearing only dorsal and ventral sclerotized plates. More than three setae on pronotal disc. Thoracic segments with approximately same width as head capsule or wider 3

3. Abdominal dorso- and ventrolateral sclerites clearly differentiated, not fused with their respective dorsal and ventral sclerites, at least in the two first abdominal segments. Thoracic segments of approximately same width as head capsule .... *second instar larva*
3’ Abdominal segments bearing only dorsal and ventral sclerotized plates. Maximum body width at the level of pro- or mesothorax .... *third instar larva*

Key to the Cuban genera and species of Hydraenidae larvae

1. Urogomphi with the distance between their bases equal to or longer than the mid-width of their basal segment (URI). Urogomphal segment URII cup-shaped distally (Fig. 6e). Dorsal thoracic pores C4 absent (Figs. 4c; 7c; 9c). Mesothorax with setae of the posthypopleurites (Pohy 1) short (Fig. 7d) .... 2 (*Hydraena*)
1’ Urogomphal bases of segments URI contiguous. Urogomphal segment URII evenly rounded distally (Figs. 18f; 27d). Dorsal thoracic pores C4 present (Figs. 16c; 20c; 22c; 25b; 27b). Mesothorax with setae of the posthypopleurites (Pohy 1) long (Figs. 16f; 22d) 3
2. Antennal solenidium IIS2 of second and third instar larvae with a distal notch (Fig. 30b) .... *H. guadelupensis*
2’ Antennal solenidium IIS2 of second and third instar larvae evenly rounded, without a distal notch (Fig. 30a) .... remaining species of *Hydraena*
3. Clypeus with two pointed projections at the level of setae Cl1 (Figs. 22a; 25a; 27a). Antennal solenidium IIS2 peg-like (Fig. 23e). Frontal area of head capsule of first instar with four egg-bursters (Fig. 22a) ..... *Ochthebius* (a single species: *attritus*)
3’ Clypleus without pointed projections at the level of setae Cl1. Antennal solenidium IIS2 conical (Fig. 17e). Frontal area of head capsule of first instar with two egg-bursters .... *Gymnochthebius* (a single species: *fossatus*)

The six species studied here express the typical larval hydraenid traits described in previous works, especially those of the genus *Hydraena*. The larvae of *G. fossatus* and *O. attritus* present nevertheless some novel remarkable features with an interesting morphology and a functional role still open to question. As already pointed out, Richmond (1920) described what he judged to be the first instar larva of *G. fossatus*. The length offered by him for this instar: 1.345 mm, slightly differs from that offered by us for the same instar: 1.05±0.11 mm. This mismatch can be due to the remarkable length variation largely known in the first instar larvae of this family (Jäch *et al*. 2016) and already commented by us for this species. Newly hatched larvae are short, with most abdominal segments contracted (Fig. 15a) but they progressively enlarge with time as larvae start to eat. As we have pointed out in its description, the first stage of *G. fossatus* bears on its head capsule a pair of egg-busters, which Richmond did not describe as such. This larger size of Richmond’s larva, along with the absence of egg-bursters can lead one to think that what this author really described was a second instar. However, Richmond (1920) mentioned that in his specimens the “epistoma is apparently marked off from the frons by an irregular group of minute tubercles”. This observation coincides pretty well with the shape of egg-bursters in this species (Fig. 16a). In our opinion, there can be no doubt that Richmond is referring to this important cephalic trait. However, what Richmond definitely overlooked in his description of this instar was the presence of a small spine on the tenth abdominal segment. This trait has never been observed in any other hydraenid larvae described before, and could be considered as the second egg-burster type known in the family. The trait is present in all first instars studied by us and, as can be seen in Fig. 15b, it is clearly absent in the second instar (Fig. 15c). The role of this unusual conical tooth as an egg-burster is suggested therefore by its exclusive presence on the tenth abdominal segment of first instars. However, and since eclosion has not been observed to date in this species, alternative or complementary functions for this spine during hatching cannot be discarded. For example, in first instar larvae of Bruchidae a similar spine on the first abdominal segment has been proposed either as a fulcrum used by the larva to emerge from the egg or as a device to help rotation inside eggs before eclosion (Pfaffenberger & Johnson 1976). On the other hand, the absence of this tiny tooth in the first instar larva of *G. jensenhaarupi* (Delgado & Archangelsky 2005) also raises the question whether this character is an exclusive trait of *G. fossatus* or it varies within the genus and, in both cases, why?

Another interesting character not described before in a larva of Hydraenidae consists of the remarkably dentate clypeal distal margin described for the larva of *O. attritus* (Figs. 22a; 25a, 27a). The shape and location of these dentate projections are congruent also with the shape and functional position of an egg-burster. But we consider that this could not be necessarily the case, as this feature is present in all instars of this species.

After describing the different larvae of the genus *Hydraena* presently known in Cuba, we have found that although the four taxa vary in size, there is too much overlap between members of the same instar to recognize each species solely on the basis of body length or head capsule width measurements (see Table 1). Three of them share in common the external morphological characters commented in the previous section for each instar stage, and their chaetotaxy shows also a remarkably close similarity. As shown in Figs. 30 c–f the shape of the head does not display any clear difference, and this is also the case for the shape of mouthparts and urogomphi. All these characters are unreliable for differentiating the Cuban specimens, and probably this could be the rule when studying closely related species in this genus. In the case of *H. guadelupensis* we have found a single distinguishing character in the shape of the antennal solenidium IIS1 in the second and third instars. This solenidium has a distal notch (Fig. 30b) not present in the same sensillum of the other three *Hydraena* (Fig. 30a). But, apart for this subtle difference, no other significant trait has been found and its first instar is indistinguishable from the other three studied species.

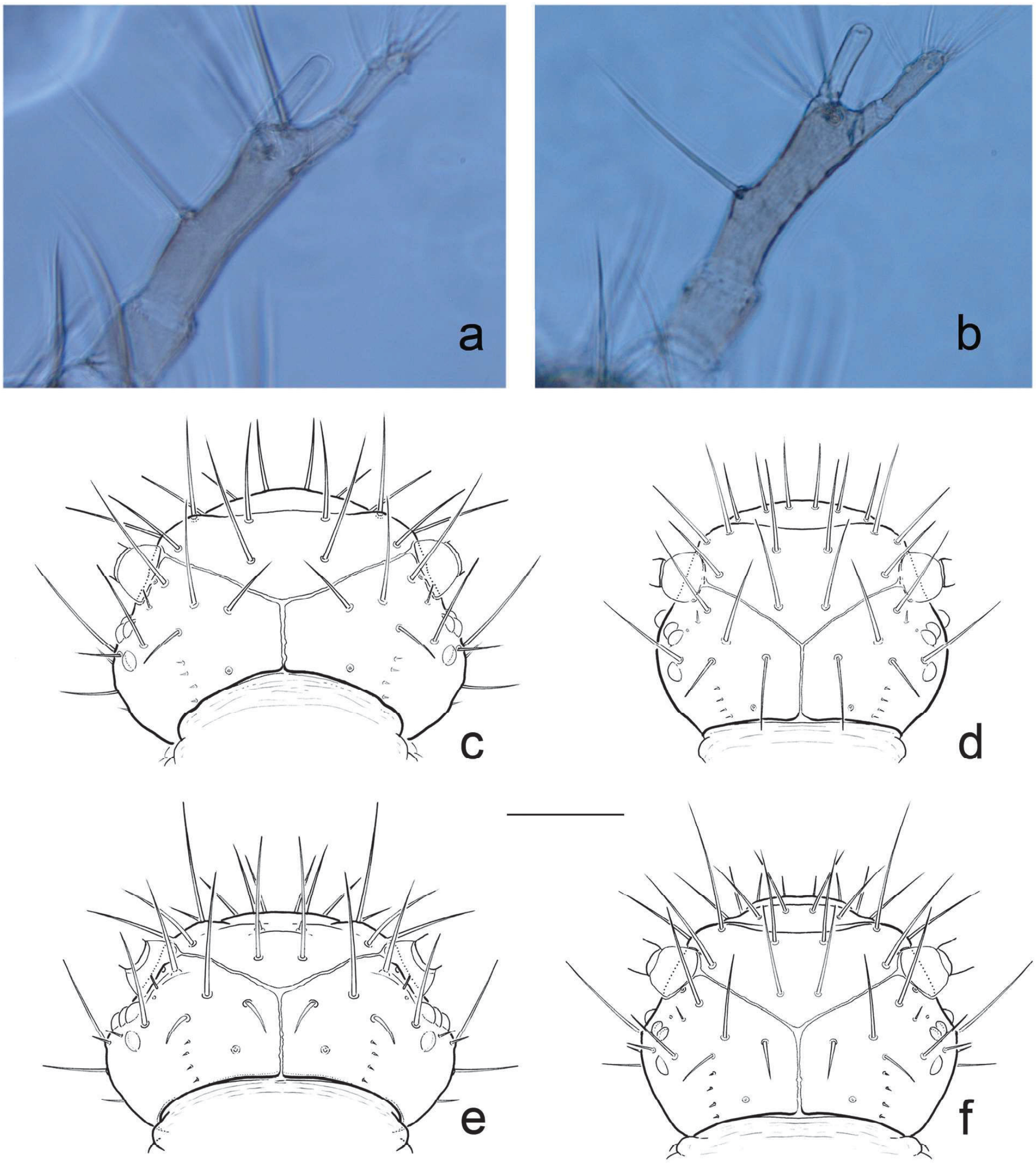

FIGURE 30. Antennae and head capsules in dorsal view of third instar larvae of *Hydraena*: a, antenna of *H. perkinsi*; b, antenna of *H. guadelupensis*; c, head capsule of *H. franklyni*; d, *H. decui*; e, *H. perkinsi*; f, *H. guadelupensis*. Scale bar: 0.1 mm.

In Hydraenidae the most incompletely known preimaginal phase is undoubtedly the pupal stage. A few pupae have been previously described in the genus *Ochthebius* but none in the genus *Hydraena*. Bøving & Henriksen (1938) described the pupa of *Ochthebius minimus* (Fabricius, 1792) (as *Ochthebius impresus* (Marsham, 1802)), Jacquin (1956) described that of *Ochthebius quadricollis* (Mulsant, 1844) and shortly after Beier & Pomeisl (1959) dealt with *Ochthebius colveranus* Ferro, 1979 (studied as *Ochthebius exsculptus* (Germar, 1824)). Unfortunately, these descriptions are insufficiently detailed to be useful in comparative studies, mainly because a thorough account of the pupal vestiture has not been recorded in these works. As in the case of larvae, the distribution, number and distribution of each cuticular projection could be useful in some extent as diagnostic characters. We hope that the set of traits offered here for the pupa of *H. perkinsi* will inspire future investigations in order to assess its utility to differentiate pupae at least to generic level.

TABLE 1. Biometric characteristics (all measurements are in mm) of Cuban Hydraenidae at different developmental stages. Number in parentheses indicates number of studied individuals. L1, first instar; L2, second instar; L3, third instar.

Taxa	Egg	L1	L2	L3	Pupa
	EL±SD EW±SD ELL±SD EEW±SD	Total body length±SD Max. head width±SD	Total body length±SD Max. head width±SD	Total body length±SD Max. head width±SD	Total body length±SD
H. perkinsi	0.44±0.02 (12) 0.17±0.01 (11) 0.65±0.04 (3) 0.38±0.02 (3)	1.29±0.16 (13) 0.19±0.005 (12)	1.50±0.11 (10) 0.24±0.007 (7)	2.34± 0.20 (14) 0.29±0.009 (12)	1.40±0.17 (12)
H. decui	0.37±0.05 (11) 0.18±0.02 (11) 0.59±0.03 (3) 0.32±0.008 (3)	1.03±0.06 (8) 0.18±0.007 (8)	—	2.05±0.04 (3) 0.26±0.004 (3)	—
H. franklyni	0.42 (1) 0.20 (1) 0.54 (1) 0.32 (1)	1.15±0.08 (3) 0.20±0.005 (3)	—	2.63 (1) 0.30 (1)	—
H. guadelupensis	—	1.21±0.05 (4) 0.18±0.002 (4)	1.59±0.09 (9) 0.23±0.006 (9)	2.25±0.06 (2) 0.28±0.005 (2)	—
G. fossatus	—	1.05±0.11 (3) 0.18±0.006 (3)	1.69±0.15 (6) 0.25±0.005 (6)	—	—
O. attritus	—	1.12±0.16 (18) 0.22±0.007 (11)	1.93±0.15 (7) 0.28±0.009 (9)	2.58±0.31 (14) 0.33±0.008 (9)	—

Acknowledgements

We are deeply grateful to the authorities and personnel of the Gran Piedra, Loma del Gato and San Miguel de Parada Protected Areas for providing access. The authors wish to thank Miguel Archangelsky (LIESA – Universidad Nacional de La Patagonia) and an anonymous reviewer for comments and suggestions to improve the manuscript. We are indebted with Robert S. Anderson (Canadian Museum of Nature, Ottawa) for his valuable comments on the draft and careful correction of our English grammar. We are also deeply grateful to Philip Perkins (Museum of Comparative Zoology, Cambridge) for taking the time to provide quite valuable comments and suggestions. The study was supported by the grants SVV 260 313/2016, The Rufford Foundation (15144-1) and Idea Wild to ADH.

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CHAPTER 9

Deler-Hernández A., Fikáček M. and J. A. Delgado. 2017. The Hydraenidae of Cuba (Insecta: Coleoptera) III. Description of two new hygropetric species *Hydraena* Kulgelann. *Zootaxa* 4250 (5): 434–446.

Zootaxa 4250 (5): 434–446
http://www.mapress.com/j/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.4250.5.2
http://zoobank.org/urn:lsid:zoobank.org:pub:9FEDE52A-7950-449A-B4FB-0859055F1923

The Hydraenidae of Cuba (Insecta: Coleoptera) III. Description of two new hygropetric species of *Hydraena* Kugelann

ALBERT DELER-HERNÁNDEZ[1], MARTIN FIKÁČEK[1, 2] & JUAN A. DELGADO[3]

[1]*Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-128 44, Praha 2, Czech Republic. E-mail: adeler1982@gmail.com*

[2]*Department of Entomology, National Museum, Cirkusová 1740, Praha 9 – Horní Počernice, CZ-193 00, Czech Republic. E-mail: mfikacek@gmail.com*

[3]*Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad de Murcia, 30100, Murcia. Spain. E-mail: jdelgado@um.es*

Abstract

Two new species of the genus *Hydraena* Kugelann, 1794 collected from hygropetric habitats in eastern Cuba are described: *Hydraena* (*Hydraenopsis*) *blancae* **sp. nov.** from the Nipe-Sagua-Baracoa mountain range, and *Hydraena* (*Hydraenopsis*) *matthiasi* **sp. nov.** from the Sierra Maestra mountain range. Both species, especially the latter, are closely related to *Hydraena* (*Hydraenopsis*) *franklyni* Deler-Hernández & Delgado, 2012. Diagnostic characters for both new species are provided and illustrated; habitat information and distributional data are also included. An updated key to Cuban species of *Hydraena* is provided. With this study, the number of species of *Hydraena* known from Cuba raises to six.

Key words: Coleoptera, Hydraenidae, *Hydraena*, eastern Cuba, new species

Resumen

Dos nuevas especies del género *Hydraena* Kugelann, 1794 recolectadas en ambientes higropétricos del oriente de Cuba son descritas: *Hydraena* (*Hydraenopsis*) *blancae* **sp. nov.** del sistema montañoso Nipe-Sagua-Baracoa, e *Hydraena* (*Hydraenopsis*) *matthiasi* **sp. nov.** del sistema de la Sierra Maestra. Ambas especies, en especial la segunda de ellas, están estrechamente relacionadas a *Hydraena* (*Hydraenopsis*) *franklyni* Deler-Hernández y Delgado, 2012. Se ofrecen e ilustran caracteres diagnósticos para ambas nuevas especies, junto con datos sobre hábitat y distribución. Se presenta una clave actualizada para las especies de *Hydraena* de Cuba. Con este estudio la fauna del género *Hydraena* de Cuba aumenta a seis especies.

Palabras clave: Coleoptera, Hydraenidae, *Hydraena*, Cuba Oriental, nueva especie

Introduction

Based on the recent review by Deler-Hernández & Delgado (2012), the family Hydraenidae is represented in Cuba by six species, four of which belong to the genus *Hydraena* Kugelann, 1794: *Hydraena* (*Hydraenopsis*) *guadelupensis* Orchymont, 1923, *Hydraena* (*Hydraenopsis*) *decui* Spangler, 1980, *Hydraena* (*Hydraenopsis*) *perkinsi* Spangler, 1980 and the more recently described *Hydraena* (*Hydraenopsis*) *franklyni* Deler-Hernández & Delgado, 2012. The discovery of new species was a result of an extensive survey of aquatic habitats across the main mountain ranges of eastern Cuba, started by the first author in 2008. The first new species was found living among mosses on humid boulders at the Gran Piedra massif in the Sierra Maestra Range, being the first hygropetric species of the family Hydraenidae known from Cuba.

Habitats formed by a thin layer of water seeping over the surface of rocks are referred to as hygropetric or madicolous. These aquatic habitats have recently become a surprising source of water beetle diversity (Miller 2012), with numerous new genera and species discovered during the past few years (Perkins 2005, 2006; Short & García 2010). The presence of *H. franklyni* in such kind of environment attracted our interest in further sampling

Accepted by P. Perkins: 7 Mar. 2017; published: 7 Apr. 2017

hygropetric habitats in Cuba, where they had previously received very little attention. Our collecting effort has resulted in the discovery of two additional new undescribed hygropetric species of *Hydraena,* bringing the total number of species of *Hydraena* known from Cuba to six.

The aim of this study is to describe and name the two new species, as well as to provide data on their distribution and biology. We also include an updated key to all known Cuban species of *Hydraena*. This paper is a further contribution to the study of the hydraenid fauna of Cuba led by the first author (see Deler-Hernández & Delgado 2011, 2012, 2017).

Material and methods

Specimens used for this study were collected in naturally forested areas in two of the largest mountain systems in eastern Cuba: Sierra Maestra Range in the south and Nipe-Sagua-Baracoa Range in the north (Fig. 9). Collecting sites were found by a careful examination of rock walls covered by films of water and apparently wet during long periods of time, based on the presence of a thin layer of algae and mats of mosses. Specimens were found hidden in cracks and moss mats and collected by hand-picking, aided by soft forceps. All specimens collected were immediately preserved in 95% ethanol.

Habitus photographs were taken with the aid of a Nikon DS-U2 unit Camera attached to a Leica MZ95 stereomicroscope. Shots taken at different focal planes were stacked using Combine ZP (Hadley 2010) and resulting images were further enhanced using Adobe Photoshop 5.0 software. SEM micrographs were taken using a Hitachi S-3700N environmental scanning electron microscope at the Department of Paleontology, National Museum in Prague. Specimen examinations, drawings, and photographs were made using either a microscope Nikon Eclipse 80i or a Nikon Eclipse E600. General morphological terminology follows Perkins (1980, 2011) and Jäch *et al*. (2016).

The material examined has been deposited in the following collections:

CDUM	Departamento de Zoología, Universidad de Murcia, Murcia, Spain (J. Delgado);
IBE	Institute of Evolutionary Biology, Barcelona, Spain (I. Ribera);
MCZ	Museum of Comparative Zoology, Cambridge, Massachusetts, U.S.A. (P. Perkins);
NHMW	Naturhistorisches Museum, Wien, Austria (M. Jäch);
NMPC	National Museum, Prague, Czech Republic (M. Fikáček)

DNA was extracted by Ignacio Ribera from paratypes of both new species sent to IBE by us, and it will be used for his research. Although we are not using molecular data in the descriptions of the two new species, we refer to GenBank accession numbers of the sequences generated and submitted by I. Ribera for both species.

Taxonomy

Hydraena (*Hydraenopsis*) *blancae* Deler-Hernández, Fikáček & Delgado, new species
(Figs 1–3; 7a, b; 8d)

Type locality. Cuba, Holguín Province, Frank País Municipality, Pico Cristal National Park, 20.57341ºN 75.4223219ºW, 503 m.

Type material. Holotype (male): **Cuba. Holguín**, Pico Cristal National Park, road to Pico Cristal, 20.57341ºN 75.4223219ºW, 503 m, 12.v.2013, coll., A. Deler-Hernández, [printed] / "HOLOTYPE, HYDRAENA blancae, Det:. A. Deler-Hernández et al. 2016, Hygropetric habitat" [red printed] (NMPC). **Paratypes**: 10 specimens with same data as for the holotype: 4 males, 5 females dry-mounted (CDUM, NHMW, MCZ); 1 male, DNA and genitalia extracted, specimen dry-mounted after extraction (IBE/DNA voucher IBE-AN507). GenBank accession number LT627658.

Descriptions. Male. Habitus as in Fig. 1. *Body* length (taken from anterior margin of labrum to elytral apex) about 1.33−1.40 mm. *Colour*: Head (dorsal) dark brown to black; pronotal disc dark brown; anterior angles of pronotum paler, yellowish brown; elytra brown, with lateral margin, including the apical region, paler; maxillary palpi, antennae and legs pale brown to testaceous; distal half of terminal maxillary palpomere darkened (Fig. 1).

Head. Labrum moderately excised anteriorly; lobes evenly rounded. Clypeus with fine and sparse punctures. Frons finely sparsely punctuate. Clypeal and frontal punctures denser on lateral areas than medially; interstices shining.

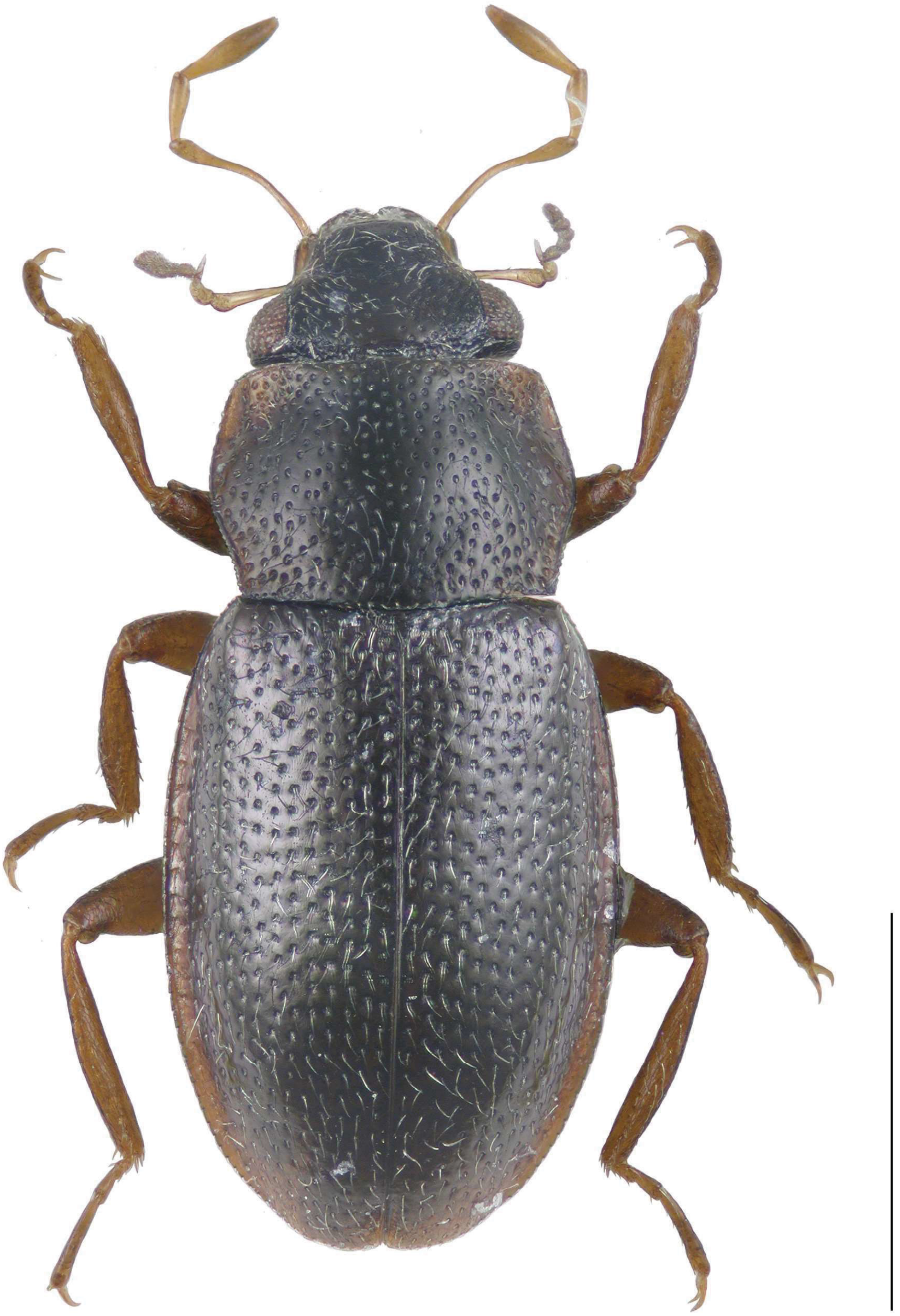

FIGURE 1. Habitus of *Hydraena blancae* (holotype). Scale bar 0.5 mm.

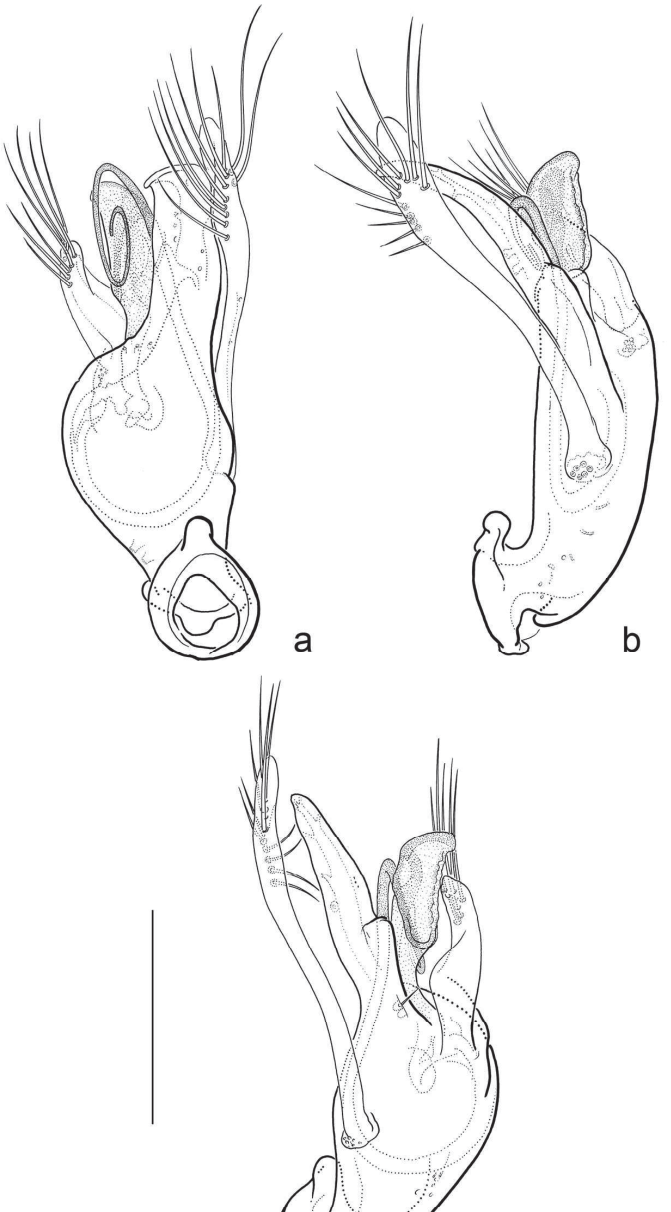

FIGURE 2. *Hydraena blancae* (holotype): aedeagus in (a) ventral (b) lateral, and (c) dorsolateral views. Scale bar 0.1 mm.

Thorax. Pronotum wider than long, widest near middle; anterior margin straight; anterior angles rounded, sides margined; straight and convergent to anterior angle, sinuate and convergent to posterior border; sculpture of pronotum almost obsolete, only anteroexternal foveolae distinct; punctures moderately deeply impressed, of similar size to those of frons, slightly sparser on disc; interstices shining. Elytra elongate oval, lateral explanate margin moderately developed, not reaching apex; apices in dorsal view separately rounded; with 14–15 (10 between suture and shoulder) rows of impressed punctures; rows 5 to 7 (taken from suture) somewhat irregular; punctures round, of approximately same size as on pronotum; interstices not elevated, shining. Mesoventrite with internal and external carinae slightly divergent posteriad; median carina broad, not reaching base of intercoxal process, similar in width to external and internal carinae; intercoxal process narrow, sides nearly parallel; apex blunt, width at apex approximately 0.3 distance between internal and median carinae. Base of mesocoxal cavities angulate. Metaventrite with plaques well developed, oval, subparallel; width of each plaque approximately twice of intercoxal process width; plaques separated by a plaque width. Legs moderately short and stout; protibiae broad, with a small spine on inner face near apex (Fig. 7a); metatibiae not modified (Fig. 7b).

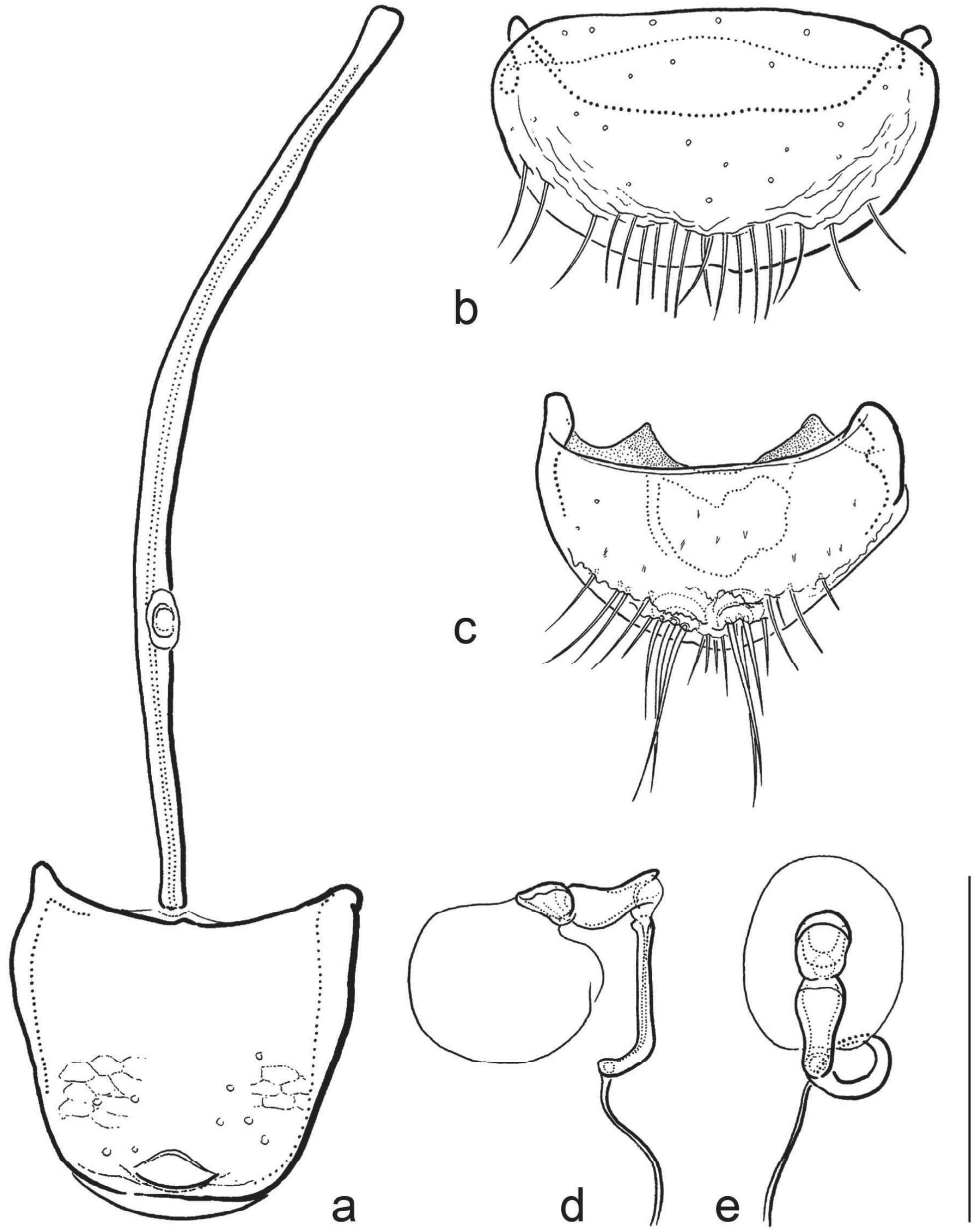

FIGURE 3. *Hydraena blancae* male (holotype) and female (paratype) terminalia: a, male terminal sternite and spiculum; b, female tergite X; c, gonocoxite; d–e, spermatheca. Scale bar 0.1 mm.

Abdomen. Terminal sternite (ventrite VII) and spiculum gastrale as in Fig. 3a; terminal sternite subquadrate, with anterior margin concave and posterior margin rounded; spiculum moderately long and curved, not firmly connected with sternite. Aedeagus as in Figs 2a–c; phallobase symmetrical; main piece stout, short and globose in lateral and dorsolateral views (Figs 2b–c); with two distinct distal processes: a median gonopore-bearing process (flagellum) distinctly coiled (Fig. 2a) and a lateral laminar process; distal end of main piece projected in a curved laminar piece offering the impression of a third process; right paramere long and slender, inserted more proximally than left one; left paramere short and distinctly widened.

Female. Similar to male in shape and size; pro- and mesotibiae not modified; gonocoxite as in Fig. 3c, suboval, longer than wide, outer plate with lateroposterior setae and two tufts of subapical setae; inner plate slightly exposed basally, cavea large and irregular; tergite X as in Fig. 3b, transverse, suboval, hyaline margin slightly emarginated medially; subapical fringe with few and moderately long setae; spermatheca as in Figs 3d–e, with proximal (caudal) portion cylindrical and curved basally, central portion enlarged and distal (cranial) portion small; spermathecal gland smooth and isodiametric; spermathecal duct sinuous and slender.

Differential diagnosis. *Hydraena blancae* shares with *H. franklyni* and *H. matthiasi* a similar body shape and dorsal coloration, but can be differentiated from them by its smaller size, less marked (almost obsolete) pronotal foveolae (Fig. 1), unmodified metatibiae in males (Fig. 7b), short and broad median carina of the thoracic mesoventrite, base of the mesocoxal cavities angulate and the shape of metaventral plaques (Fig. 8d). The shape of the aedeagus indicates a close relationship with *H. franklyni* and *H. matthiasi*, but they can be differentiated by the shape of the distal laminate process of the main piece and the median gonopore bearing process, which is coiled in *H. blancae* and straight in the other two species.

Etymology. This new species is dedicated to Blanca Delgado, daughter of J.A.D. and a future enthusiastic naturalist.

Distribution. Currently known only from the type locality (Pico Cristal National Park) in eastern Cuba (Fig. 9).

Habitat. The type material was collected on sunny, exposed, wet rock faces (seepages) within a well preserved rainforest at 503 m a.s.l.

***Hydraena* (*Hydraenopsis*) *matthiasi* Deler-Hernández, Fikáček & Delgado, new species**

(Figs 4–6; 7c, d; 8b, e)

Type locality. Cuba, Granma Prov., Turquino National Park, Aguada de Joaquín, 20.01447ºN 76.84065°W, 1359 m.

Type material. **Holotype** (male): **Cuba**. **Granma**, Turquino National Park, Aguada de Joaquín, 20.01447ºN 76.84065°W, 1359 m, 27.ix.2014, coll., A. Deler-Hernández, Hygropetric habitat [printed] / "HOLOTYPE, HYDRAENA matthiasi, Det:. A. Deler-Hernández et al. 2016 [red printed] (NMPC). **Paratypes**. 17 specimens with same data as for the holotype: 7 males, 4 females dry-mounted (NMPC, CDUM, NHMW, MCZ); 1 male, 2 females in alcohol (NMPC); 2 females in alcohol (IBE); 1 female, DNA extracted and dry-mounted after extraction (IBE/DNA voucher IBE-AN508). GenBank accession number LT627659.

Descriptions. Male. Habitus as in Fig. 4. *Body* length (taken from anterior margin of labrum to elytral apex) about 1.40–1.43 mm. *Colour*: Head (dorsal) dark brown to black; pronotal disc dark brown; anterior angles and lateral half of anterior margin of pronotum slightly paler; elytra dark brown, with lateral margin, including the apical region, paler; maxillary palpi, antennae and legs pale brown to testaceous; distal half of terminal maxillary palpomere darkened.

Head. Labrum moderately excised anteriorly; lobes evenly rounded. Clypeus with fine and sparse punctures. Frons finely sparsely punctuate. Clypeal and frontal punctures denser on lateral areas than medially; interstices shining.

Thorax. Pronotum distinctly wider than long, widest near middle; anterior margin straight; anterior angles rounded, sides finely denticulate, slightly curved and convergent to anterior angle, sinuate and convergent to posterior border; anteroexternal and posteroexternal foveolae marked, internal foveolae less evident; punctures moderately impressed, of similar size to those of frons; interstices shining. Elytra elongate oval, lateral explanate margin moderately developed, not reaching apex; apices in dorsal view separately rounded; with 14–15 (10 between suture and shoulder) rows of impressed punctures, rows quite regular; punctures round, moderately impressed, of approximately same size as on pronotum; interstices not elevated, shining to matt. Mesoventrite with

FIGURE 4. Habitus of *Hydraena matthiasi* (holotype). Scale bar 0.5 mm.

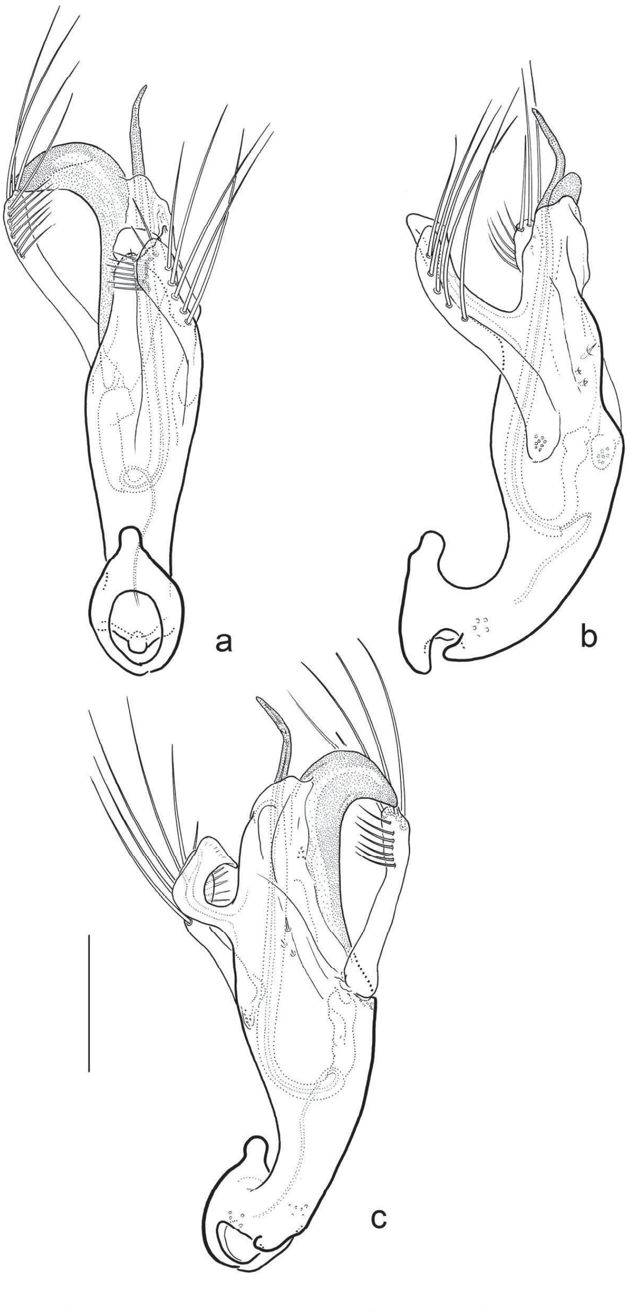

FIGURE 5. *Hydraena matthiasi* (holotype): aedeagus in (a) ventral (b) lateral, and (c) dorsolateral views. Scale bar 0.1 mm.

internal and external carinae slightly divergent posteriad; median carina short and thin, not reaching base of intercoxal process, remarkably thinner than external and internal carinae; intercoxal process narrow, sides nearly parallel; apex blunt, width at apex approximately 0.3 distance between internal and median carinae. Base of mesocoxal cavities rounded. Metaventrite with plaques well developed, straight, slightly converging anteriorly; width of each plaque 1.5 width of intercoxal process; plaque separated approximately twice plaque width. Legs moderately short and stout; protibiae broad, with a small spine on inner face near apex (Fig. 7c); metatibiae arcuate, enlarged apically (Fig. 7d).

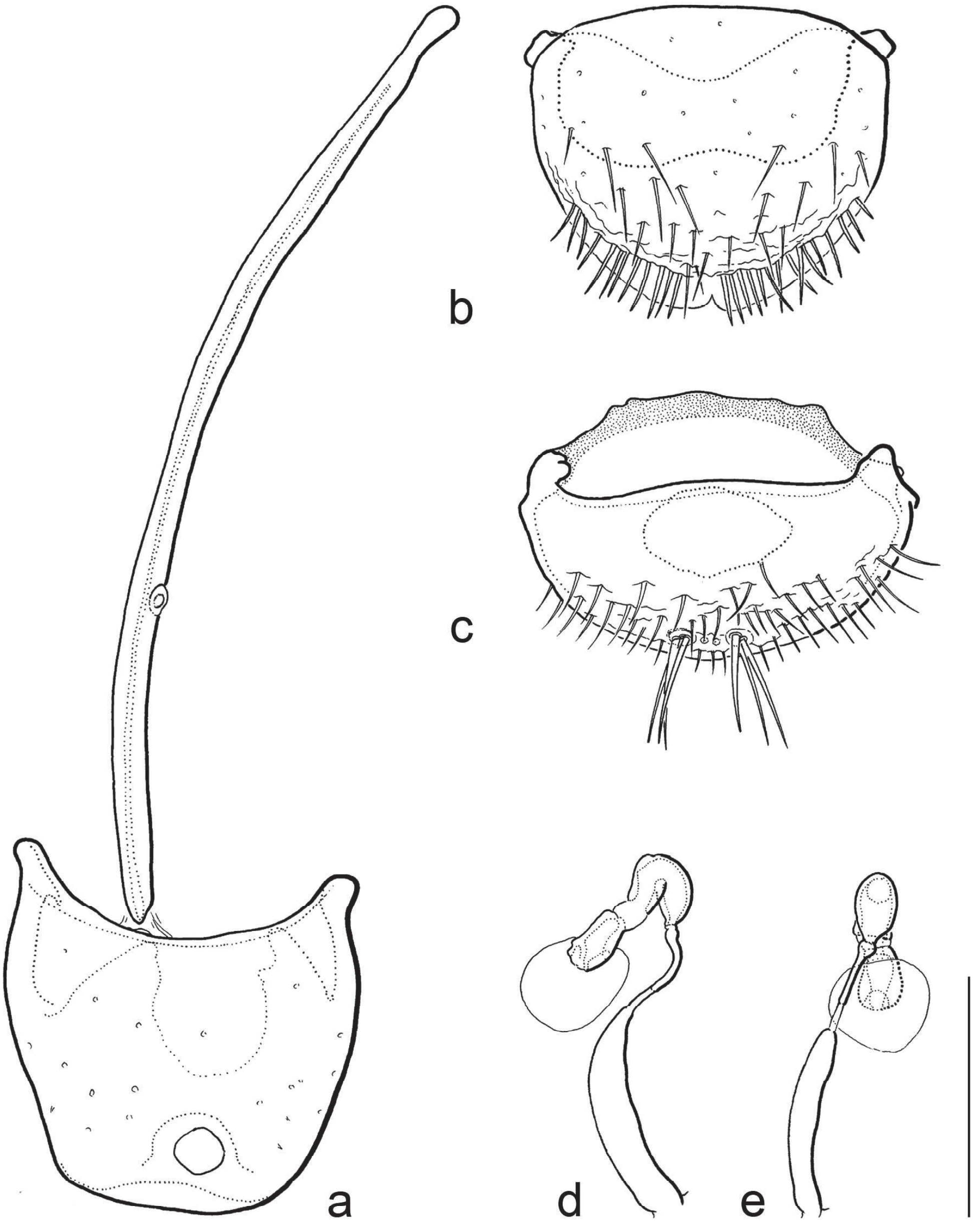

FIGURE 6. *Hydraena matthiasi* male (holotype) and female (paratype) terminalia: a, male terminal sternite and spiculum; b, female tergite X; c, gonocoxite; d–e, spermatheca. Scale bar 0.1 mm.

Abdomen. Male terminal sternite and spiculum gastrale as in Fig. 6a; terminal sternite subtrapezoidal, with anterior margin concave, posterior margin rounded and lateral margins sinuate; spiculum moderately long and curved, not firmly connected with sternite. Male genitalia as in Figs 5a–c; main piece long, only slightly dilated distally both in lateral and dorsolateral views (Figs 5b–c) but not in ventral view (Fig. 5a); with two distinct processes at the apex: a slender, elongate gonopore-bearing process (flagellum) and a falcate laminar process; a subdistal lateral crooked projection of main piece (Figs 5a-c) offers the impression of a third process; parameres inserted near midlenght of main piece, left paramere relatively long and slender, left shorter.

Female. Similar to male in shape and size; pro- and metatibiae not modified; gonocoxite as in Fig. 6c, semicircular, transverse, condyles well developed, outer plate with trichoid setae on disc and lateroposterior areas and two tufts of subapical setae, inner plate distinctly exposed basally, cavea suboval; tergite X as in Fig. 6b, suboval, with hyaline margin emarginated medially; spermatheca as in Figs 6d–e, with proximal portion slightly curved, central portion enlarged and strongly curved and distal portion long; spermathecal gland smooth and rounded; spermathecal duct wide.

Differential diagnosis. *Hydraena matthiasi* is externally very close to *H. blancae* and *H. franklyni* with similar body coloration and shape. However, it can be externally distinguished from *H. blancae* by its slightly larger size and by modified male metatibiae (Figs 7b, d). The shape of the metaventral plates can be also useful to distinguish both species, being wider in *H. blancae* (Fig. 8d) than in *H. matthiasi* (Fig. 8e) and, the base of the mesocoxal cavities rounded (Fig. 8e). Furthermore, *H. matthiasi* is easily distinguished from *H. blancae* by the morphology of their aedeagi (Figs 2, 5), and their male and female terminalia (Figs 3, 6).

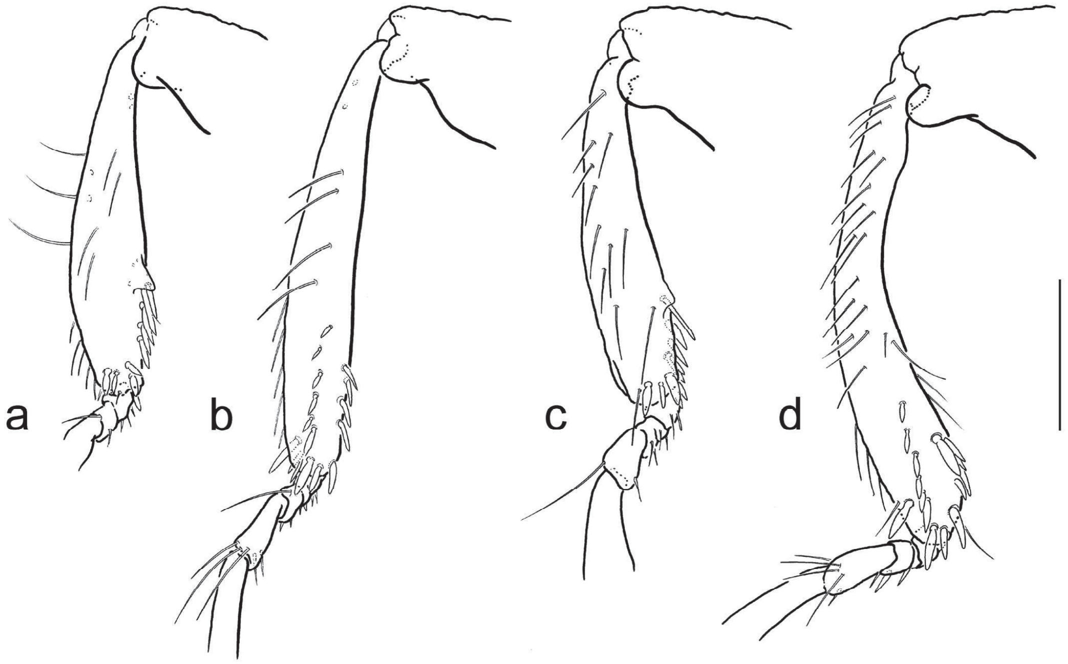

FIGURE 7. *Hydraena blancae* (paratype male): a, protibia; b, metatibia. *Hydraena matthiasi* (paratype male): c, protibia; d, metatibia. Scale bar 0.1 mm.

The evident similarities in the shape of the male and female genitalia indicate a very close relationship between *H. matthiasi* and *H. franklyni*. They can only be reliably separated by a close examination of the male aedeagus. In ventral view, the apical portion of the right process of the distal lobe is clearly more angulate in *H. franklyni* (see fig. 34 in Deler-Hernández & Delgado 2012), than in *H. matthiasi* (Fig. 5c). In dorsolateral view, this angulation is not so evident but the main piece of *H. franklyni* is clearly more globose (Figs 8a–b). The subdistal lateral process of the main piece is slender and less twisted in *H. matthiasi* and the flagellum is also slender in *H. matthiasi* (Figs 8a–b). A less evident but useful character is the length of the mesoventral median carina, which reaches the base of the intercoxal process in *H. franklyni* (Fig. 8c) being shorter, not reaching the base of the intercoxal process in *H. matthiasi* (Fig. 8e).

Etymology. The new species is dedicated to Matthias Seidel, fellow co-worker of the first author at Charles University and a very enthusiastic entomologist specializing on Scarabaeidae.

Distribution. Currently only known from the type locality (Turquino National Park) in eastern Cuba (Fig. 9).

Habitat. Hygropetric. *Hydraena matthiasi* has been collected on wet rock faces in a well preserved cloud forest at 1359 m a.s.l.

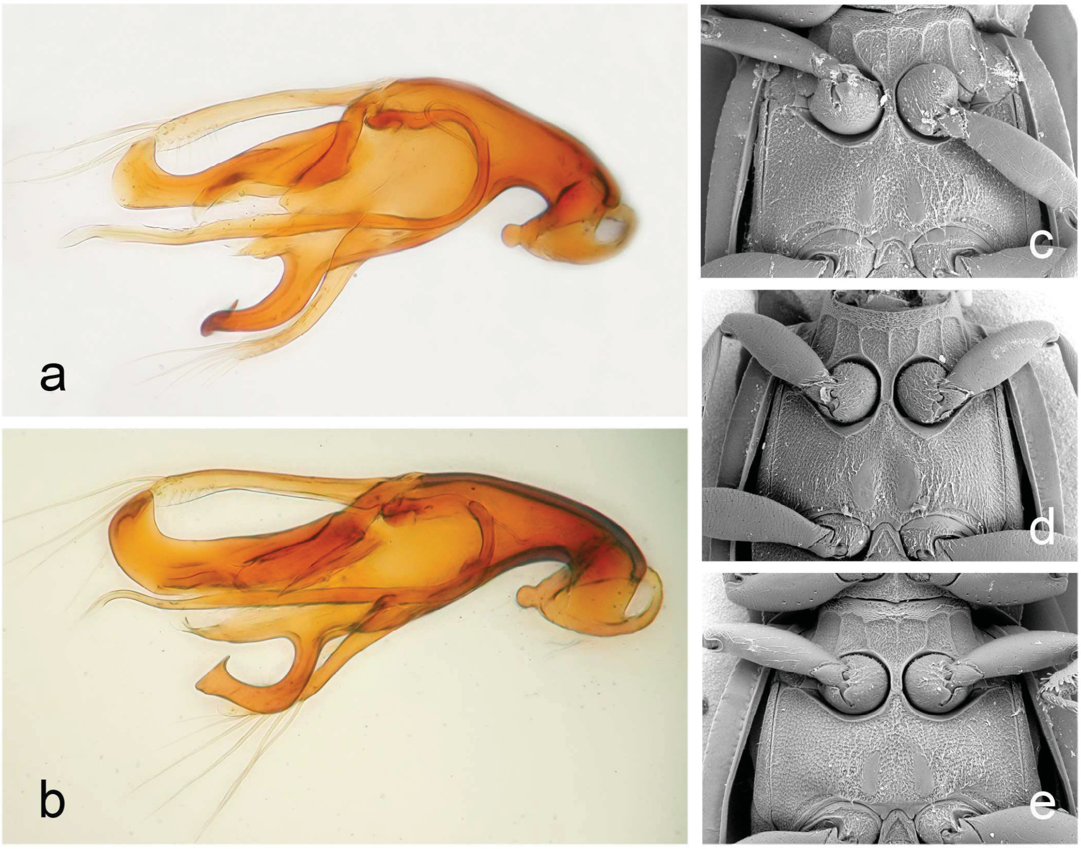

FIGURE 8. Aedeagi in dorsolateral views: a, *Hydraena franklyni*; b, *Hydraena matthiasi*. Thoracic venter (SEM images): c, *Hydraena franklyni*; d, *Hydraena blancae*; e, *Hydraena matthiasi*.

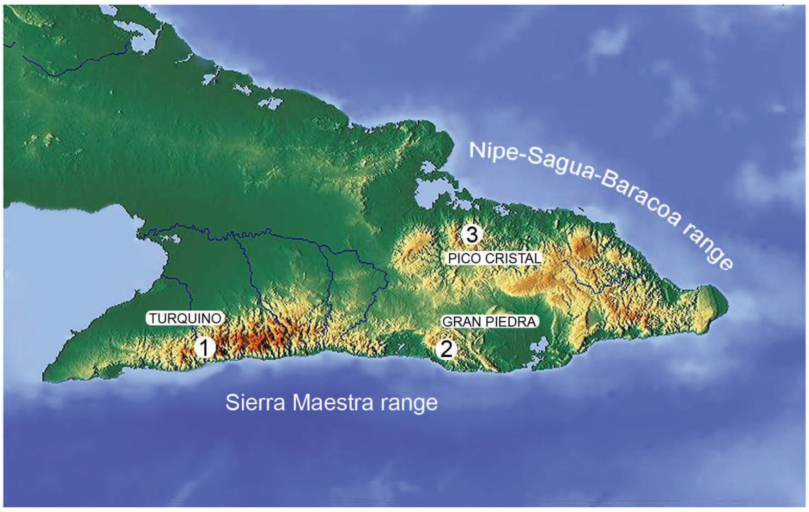

FIGURE 9. Geographical distribution: *Hydraena matthiasi* (1); *Hydraena franklyni* (2); *Hydraena blancae* (3).

Updated key to the *Hydraena* species from Cuba

1. Pronotum clearly bi-coloured. Disc dark brown to black, anterior and posterior margins light brown to testaceous. Lateral pronotal sides evenly curved, not angulate at middle 2
\- Pronotum with disc and posterior margin of approximately the same colour; sometimes with anterior angles paler. Lateral pronotal sides angulate at middle 3
2. Posterointernal foveolae of pronotum generally distinct. Aedeagus as in Deler-Hernández & Delgado (2012: figs 31, 32) .... *Hydraena guadelupensis*
\- Posterointernal foveolae of the pronotum absent. Aedeagus as in Deler-Hernández & Delgado (2012: figs 36, 37) .... *Hydraena perkinsi*
3. Total body length ≥ 1.3 mm. Colour dark brown to black 4
\- Total body length < 1.3 mm. Colour reddish-brown.......... *Hydraena decui*
4. Internal foveolae shallowly impressed but evident, posteroexternal foveolae marked. Length around 1.4 mm. Males with metatibiae arcuate. Median carina of mesoventrite thinner than external and internal carinae (Figs 8c, e) 5
\- Internal and posteroexternal foveolae almost obsolete. Length around 1.3 mm. Males with metatibiae not modified. Median carina of mesoventrite broad, of similar width to that external and internal carinae (Fig. 8d) .... *Hydraena blancae*
5. Internal carina of mesoventrite reaching base of intercoxal process (Fig. 8c). Male aedeagus in dorsolateral view as in Fig. 8a, with distal half of main piece strongly dilated and distal end of main piece broad and evenly curved ..... *Hydraena franklyni*
\- Internal carina of mesoventrite not reaching base of intercoxal process (Fig. 8e). Male aedeagus in dorsolateral view as in Fig. 8b, with distal half of main piece moderately dilated and distal end of main piece slender and c-shaped. . *Hydraena matthiasi*

Discussion

Hygropetric habitats have been reported rarely as collecting sites for Neotropical Hydraenidae. As stressed by Perkins (2011), the vast majority of *Hydraena* species from South America are found at stream margins, with only a small proportion being pond specialists. In fact, based on present knowledge, this seems to be the pattern for the entire hydraenid fauna of the New World. Hygropetric species of American *Hydraena* are apparently rare, and only a few species—such as *Hydraena* (*Hydraenopsis*) *malkini* Perkins, 1980 (see Perkins 2011)—together with some Ochthebiinae as *Ochthebius* (*Ochthebius*) *mexcavatus* Perkins, 1980 and *Gymnochthebius bartyrae* Perkins, 1980, have been collected in this type of habitat (Perkins 1980). The few records of some species of *Hydraena* collected in clearly hygropetric habitats have been regarded by Perkins (1980) as a secondary use of those habitats due to overpopulation or dry periods. The species of a single genus—*Parhydraenida* Balfour-Browne, 1975—seem to exploit these habitats efficiently in this part of the world (Perkins 1980).

Hydraena franklyni and the two new species described here appear to be restricted to hygropetric habitats in the highlands of eastern Cuba (Fig. 9). However, hygropetric habitats are among the least studied habitats associated with tropical ecosystems (Short 2009), and numerous authors have suggested that they may harbor many undescribed species and new records. Our findings are in agreement with this statement. The discovery of two new madicolous species highlights the potential of these habitats for the faunistic diversity of *Hydraena* in Cuba, and probably in many other islands of the Caribbean Sea. As stressed by Deler-Hermández & Delgado (2012) the scarcity of suitable aquatic habitats in some Caribbean islands has likely influenced the colonization of novel habitats as a survival strategy for members of the family Hydraenidae. We hope that additional sampling and further research may ultimately result in new information that may reveal the role that hygropetric habitats have played in the diversification of the genus *Hydraena* in Cuba.

Acknowledgements

We are indebted to Ricardo L. Palma (Museum of New Zealand, Wellington, New Zealand) for his revision of the English text and his many suggestions, and to Miguel Archangelsky (LIESA—Universidad Nacional de La Patagonia) for comments and suggestions to improve the manuscript. We thank members of the 2013 Canadian-Cuban Expedition (Robert Anderson and Andrew Smith from the Canadian Museum of Nature, Ottawa, Canada, Guanyang Zhang from Arizona State University, Tempe, U.S.A., and Franklyn Cala-Riquelme from Universidad de Bogotá, Bogotá, Colombia) for their great help during field work. We are deeply grateful to the authorities and personnel of the Pico Cristal and Turquino National Parks for providing access to both protected regions and, especially, for sharing invaluable local knowledge. We thank Ignacio Ribera (Institut de Biología Evolutiva, Barcelona, Spain) for fruitful discussion regarding our preliminary results based on molecular data. This study was supported by Grant SVV 260 434/2017 and The Rufford Foundation (15144-1) to A.D.H., and by the Ministry of

Culture of the Czech Republic (DKRVO 2017/14, National Museum, 00023272) to M.F. The stay of A.D.H. at the Universidad de Murcia in 2016 was partly supported by the Mobility Fund of Charles University.

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