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Fylogeneze vybraných rodů gekonů Mediteránu
a přilehlých oblastí

Phylogeny of selected genera of geckos in the Mediterranean
and adjacent regions

Disertační práce

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OBSAH

ABSTRAKT	5
ABSTRACT	6
ÚVOD	7
KAPITOLA 1	21
Phylogeny and taxonomy of the Middle Eastern geckos of the genus <i>Cyrtopodion</i> and their selected relatives	
Jan Červenka, Lukáš Kratochvíl a Daniel Frynta	
<i>Zootaxa</i> (2008) 1931: 25-36	
KAPITOLA 2	34
Phylogenetic relationships of the gecko genus <i>Carinatogekko</i> (Reptilia: Gekkonidae)	
Jan Červenka, Daniel Frynta a Lukáš Kratochvíl	
<i>Zootaxa</i> (2010) 2636: 56-94	
KAPITOLA 3	41
Generic reassignment and validity of the recently described species <i>Cyrtopodion dehakroense</i>	
Jan Červenka a Lukáš Kratochvíl	
<i>Herpetology Notes</i> (2010) 3: 135-137	
KAPITOLA 4	45
Revisiting the taxonomy and biogeography of <i>Bunopus spatalurus</i> (Reptilia; Gekkonidae) from the Arabian Peninsula	
Philip de Pous, Nefeli Paschou, Jan Červenka, Lukáš Kratochvíl, Tomáš Mazuch, Jiří Šmíd, Luis Machado, Marc Simó-Riudalbas, Delfi Sanuy a Salvador Carranza	
<i>Rukopis v přípravě</i>	
ZÁVĚR	88

ABSTRAKT

Disertační práce, jejímž základem jsou tři již publikované práce a jeden rukopis, je zaměřena na fylogenezi vybraných rodů gekonů z oblasti Mediteránu a oblastí přilehlých. Cílovou skupinu taxonů spojuje z morfologického hlediska absence přísavných lamel na prstech a z hlediska historie výzkumu skupiny také jejich předpokládaná blízká fylogenetická příbuznost. Na problematiku studovanou v této práci byly aplikovány především metody molekulární fylogenetiky s využitím sekvenčních dat získaných z mitochondriálních genů. Morfologické znaky, u ještěřů tradičně používané, byly zkoumány zejména v souvislosti s ekologií studovaných forem. Práce umožnila první podrobnější náhled na fylogenezi zkoumaných taxonů. Ze zjištěných výsledků vyplývá, že rod *Cyrtopodion*, dříve považovaný za monofyletický, monofylem není, neboť rody *Bunopus* a *Agamura* tvoří jeho vnitřní skupiny. Jeho podrod *Mediodactylus* tvoří monofylum, nikoli však blízce příbuzné ostatním zástupcům rodu a byl povýšen na samostatný rod *Mediodactylus*. Enigmatický a doposud poměrně málo prozkoumaný rod *Carinatogekko* tvoří se všemi svými zástupci vnitřní skupinu rodu *Mediodactylus*. Na základě vnější morfologie a ekologických charakteristik bylo též doporučeno přeradit recentně popsáný druh *Cyrtopodion dehakroense* z Pákistánu do rodu *Mediodactylus*. Fylogenetickou analýzou sekvenčních dat, doplněnou modelováním ekologických nik, byl podpořen druhový status obou známých poddruhů druhu *Bunopus spatalurus*.

ABSTRACT

This Ph.D. thesis is composed of three published articles and one manuscript, and is focused on the phylogenetic relationships of selected species of geckos from the Mediterranean and surrounding areas. The group of geckos of interest shares the common characteristic of an absence of adhesive lamellas on their toes. Historically, it was assumed that these species were closely related. Molecular-phylogenetic approaches were used in order to reveal the phylogenetic relationships within this group, especially using the sequential data from mitochondrial genes. Morphological characteristics commonly used in lizards were studied in connection with the ecology of the group. This thesis provides the first more detailed view of the phylogeny of the studied species. The results show that the genus *Cyrtopodion*, previously considered as monophyletic, in fact is not monophyletic as the genera *Bunopus* and *Agamura* represent its inner groups. *Mediodactylus*, the subgenus of *Cyrtopodion*, forms monophylum but is not closely related to the other members of the genus and so was reclassified as the independent genus. The enigmatic and yet so far very poorly studied genus *Carinatogecko* was discovered to be the inner group of the genus *Mediodactylus*. The recently described species *Cyrtopodion dehakroense* was therefore transferred to the genus *Mediodactylus* based on ecological and morphological data. Phylogenetic analysis of sequence data enhanced by ecological niche modelling supported the specific status of the two recognized subspecies of *Bunopus spatalurus*.

ÚVOD

Gekoni (Gekkota) tvoří značně morfologicky i ekologicky diverzifikovanou skupinu zahrnující přes jednu šestinu z celkového počtu více než 9000 druhů šupinatých plazů (Squamata). Recentně se dělí na sedm čeledí (Gamble et al. 2008), z nichž se zástupci čtyř těchto čeledí vyskytují v Mediteránu a okolních oblastech. Středomoří je dnes chápáno jako významný „hot spot“ palearktické druhové diverzity, například i s ohledem na předpokládaná glaciální refugia mnoha evropských taxonů (např. Blondel a Aronson 1999). Objasnění fylogeneze taxonů vyskytujících se v současnosti v Mediteránní oblasti je jedním z nezbytných výchozích kroků pro pochopení a interpretaci řady fylogeografických scénářů, a také pro poznávání biodiverzity daného území. Disertační práce se soustředí na rekonstrukci fylogeneze vybraných rodů takzvaných palearktických „nahoprstých“ gekonů (gekonů bez přísavných lamel na prstech) z Mediteránu a přilehlých oblastí.

Gekoni postrádající na prstech adhezivní lamely tvoří významnou složku noční herpetofauny na většině lokalit svého výskytu, často velmi aridních (Leviton et al. 1992). Svým výskytem zasahují od Severní Afriky přes Blízký Východ a jihozápadní Asii až po severní Indii a úpatí Himálaje, na severu se vyskytují až ve Střední Asii. Z hlediska své ekologie obývají tito gekoni jako skupina širokou škálu biotopů. Je možno je nalézt na skalách, písčítých, sprašových či kamenitých površích, ale také na stromech, některé druhy žijí i synantropně (Anderson 1999).

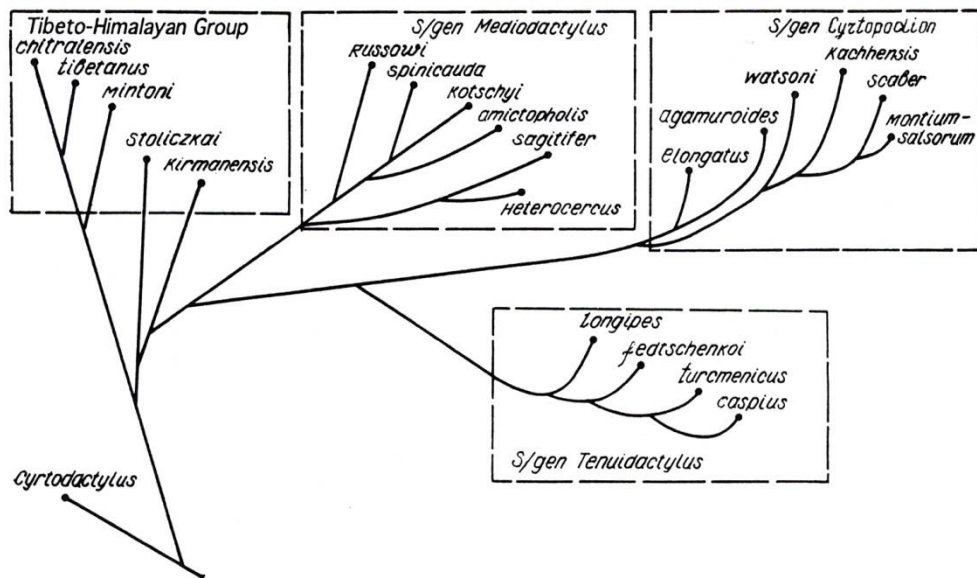
Označení „nahoprstí“ gekoni zahrnuje v tradičním pojetí spíše sběrnou skupinu rodů na základě podobné morfologie prstů. Jejich fylogenetická příbuznost však byla a je i v současnosti stále předmětem výzkumu a dosud není zcela vyjasněna. Gamble et al. (2012) například ve své práci ukazují, že v rámci gekonů došlo nejméně jedenáctkrát k vyvinutí přísavných lamel a devětkrát k jejich ztrátě. Usuzovat proto na fylogenetickou příbuznost na základě pouze tohoto jednoho znaku tak může být zavádějící. Do skupiny „nahoprstých“ gekonů byly řazeny především rody: *Agamura* Blanford, 1874; *Alsophylax* Fitzinger, 1943; *Bunopus* Blanford, 1874; *Carinatogekko* Golubev & Szczerbak, 1981; *Crossobamon* Boettger, 1888; *Cyrtodactylus* Gray, 1827; *Cyrtopodion* Fitzinger, 1843; *Stenodactylus* Fitzinger, 1826; *Tropicolotes* Peters, 1880; úplný výčet rodů včetně dnes již synonymizovaných shrnují například Bauer et al. (2013). Předkládaná disertační práce je zaměřena na rody *Agamura*, *Bunopus*,

Carinatogeo a především druhově bohatý rod *Cyrtopodion*, tj. rody tradičně považované za fylogeneticky blízce příbuzné (např. Szczerbak a Golubev 1986).

Taxonomie samotného rodu *Cyrtopodion* prošla v minulosti právě vzhledem k vysokému počtu zahrnutých druhů (původně více než 40 druhů) poměrně složitým vývojem. Tehdy známé druhy rodu byly původně řazeny do kosmopolitního rodu *Gymnodactylus* Spix, 1825 (např. Annandale 1906; Smith 1935). Underwood (1954) vyčlenil na základě odlišného uspořádání prekloakálních pórů starosvětské druhy z rodu *Gymnodactylus*, do kterého zařadil nadále jen druhy novosvětské. Rozdělení rodu však nebylo obecně akceptováno a Szczerbak a Golubev (1977) například následně vymezili v rámci původního rodu *Gymnodactylus* podrody *Cyrtodactylus* a *Mediodactylus* Szczerbak & Golubev, 1977, kam zahrnuli euroasijské zástupce. Do podrodu *Cyrtodactylus* zařadili druhy ze střední a jižní Asie, do podrodu *Mediodactylus* pak druhy z oblasti Středomoří, odlišující se absencí femorálních pórů samců a dalších znaků na folidóze. Kluge (1983) shledal rod *Cyrtodactylus*, tak jak jej definoval Underwood (1954), polyfyletickým a vyčlenil z něj tropické zástupce s přítomným druhým ceratobranchiálním obloukem, které přeřadil do nově jím ustanoveného rodu *Nactus* Kluge, 1983.

Szczerbak a Golubev (1984) pak již přejali rod *Cyrtodactylus* jak jej redefinoval Kluge (1983). Na základě odlišného tvaru prstů a dalších znaků na folidóze z něj však byly do nového rodu *Tenuidactylus* Szczerbak & Golubev, 1984 odděleny palearktické druhy s podrody *Mediodactylus*, *Mesodactylus* a *Tenuidactylus*, část druhů z Tibetu-Himálajské oblasti nicméně nebyla zařazena do žádného podrodu (Szczerbak a Golubev 1984). Böhme (1985) a Kluge (1985) shodně poukázali, že pro takto vymezený rod *Tenuidactylus* má prioritu název *Cyrtopodion* a označení rodu *Tenuidactylus* a podrodu *Mesodactylus* je nutno považovat za mladší synonyma jména *Cyrtopodion*. Tato nomenklatorická úprava pak byla již v následných pracích akceptována a například Szczerbak a Golubev (1986) dále uvažují rod *Cyrtopodion* s podrody *Cyrtopodion* (= *Mesodactylus*), *Mediodactylus* a *Tenuidactylus*.

Ve stejné shrnující práci předložili také Szczerbak a Golubev (1986) jeden z náhledů na možnou fylogenezi v rámci rodu *Cyrtopodion* (viz Obr. 1). Předkládaný pohled na fylogenezi dané skupiny však byl založen pouze na morfologickém a areálovém srovnání zkoumaných druhů a nebyl podpořen žádnou fylogenetickou analýzou. Přesto i tento srovnávací přístup může, a jak se ukázalo dále, také poskytl, poměrně dobrý náhled na fylogenezi uvnitř rodu.



Obr. 1 Předpokládané příbuzenské vztahy uvnitř rodu *Cyrtopodion* (převzato ze Szczerbak a Golubev 1986).

Anderson (1999) rozdělil jemu dostupné druhy rodu *Cyrtopodion* na základě vnější morfologie a znaků na folidóze na čtyři potenciálně, avšak ne nutně monofyletické skupiny, více či méně odpovídající jednotlivým podrodům dle Szczerbak a Golubev (1984, 1986). Podrod *Cyrtopodion* tak zhruba koresponduje se skupinou *scabrum* a *agamuroides*, podrod *Tenuidactylus* se skupinou *caspius* a podrod *Mediodactylus* se skupinou *kotschy*. Pro jednotlivé skupiny také identifikoval určité zjevné synapomorfie, které je charakterizují. Skupina *scabrum* nebyla určena žádnou synapomorfii, její zástupci sdílí spíše primitivnější znaky celého rodu. Skupina *agamuroides* sdílí tmavě pigmentované peritoneum a pro skupinu *caspius* by měla být typická přítomnost prekloakálních a femorálních pórů u samců a také přítomnost jednoho až šesti subpostfemorálních tuberkulů. Skupina *kotschy* by se pak měla vyznačovat charakterem ocasních tuberkulů (šest na každém ocasním kroužku), které tvoří terminální řadu, ale jsou rozmístěny podél středu každého ocasního segmentu (Anderson 1999).

Z rodu *Cyrtopodion* se nejvýrazněji vyčleňuje právě podrod *Mediodactylus* sdružující druhy převážně z oblasti Mediteránu (Szczerbak a Golubev 1977). Výlučnost

forem řazených do tohoto podrodu naznačuje i kladistická analýza alozymových dat (Macey et al. 2000), kdy zkoumané druhy z podrodu *Mediodactylus* tvořily samostatnou skupinu, oddělenou od ostatních zástupců rodu *Cyrtopodion*. Autory bylo proto navrženo vyčlenění podrodu *Mediodactylus* jako samostatného rodu. Z celého rodu je nejpodrobněji prozkoumaným druhem nyní již *Mediodactylus kotschy* (Steindachner, 1870) s více než třiceti poddruhy (viz např. Uetz a Hošek 2014), které jsou dále členěny do několika skupin (např. Baran a Gruber 1981, 1982). Příbuznost jednotlivých poddruhů *M. kotschy* byla určena na základě morfologických znaků, molekulární data byla použita až poměrně nedávno (Kasapidis et al. 2005). Tato studie však zůstala omezena na studium fylogeneze uvnitř druhu a například vztah jednotlivých poddruhů k populacím *Mediodactylus heterocercus* (Blanford, 1874) na styku areálů obou druhů zůstal nejasný.

Přibližně ve dvou dekádách po roce 1980 bylo popsáno asi deset nových druhů rodu *Cyrtopodion* (*Cyrtodactylus*) z Pákistánu a Afghánistánu (viz například recentní seznam druhů Khan 2004). Jejich příbuznost s druhy z Blízkého Východu a Mediteránu však není uspokojivě objasněna. Popisy některých druhů naznačují příbuznost s druhy podrodů *Cyrtopodion*, případně *Tenuidactylus*, většina těchto nových druhů je ale spíše členy blíže nespecifikované Tibeto-Himálajské skupiny. Khan (2003) se pokusil tuto komplikovanou situaci vyřešit ustanovením nových rodů *Altigekko*, *Indogekko* a *Siwaligekko*, vzhledem k absenci fylogenetické analýzy byl však přínos k objasnění komplexní situace v rámci diverzity rodu v této oblasti jen částečný.

Gekony rodu *Bunopus* uvažují Szczerbak a Golubev (1977, 1986) jako blíže příbuzné rodu *Cyrtopodion*. Recentně jsou do rodu *Bunopus* řazeny čtyři druhy: *Bunopus blanfordii* Strauch, 1887; *Bunopus crassicauda* Nikolsky, 1907; *Bunopus spatalurus* Anderson, 1901; *Bunopus tuberculatus* Blanford, 1874. Pouze u druhu *B. spatalurus* byly dosud rozlišovány dva morfologicky odlišitelné poddruhy, a to sice *Bunopus spatalurus spatalurus* Anderson, 1901 a *Bunopus spatalurus hajarensis* Arnold, 1980.

Rod *Agamura* byl původně považován za dobře definovaný a monotypický s jediným druhem *Agamura persica* (Duméril, 1856). Do rodu byl však později zařazen druh *Agamura femoralis* (Smith, 1933), který byl ale posléze přesunut do nového rodu *Rhinogekko* de Witte, 1973 zastoupeného druhem *Rhinogekko missonei* (de Witte, 1980). Szczerbak a Golubev (1986) následně synonymizovali rod *Rhinogekko* s rodem *Agamura* spolu se zařazením druhu *Cyrtopodion gastrophole* (Werner, 1917) do rodu

Agamura. Anderson (1999) však vzhledem k předpokládané blízké příbuznosti druhů *C. gastrophole* a *Cyrtopodion agamuroides* (Nikolsky, 1900) tomuto řazení oponuje, přestože také usuzuje na relativně blízkou příbuznost těchto dvou druhů k rodu *Agamura*. Synonymizace rodu *Rhinogecko* s rodem *Agamura* tak dosud nebyla, především z důvodu nedostatku podpory ve fylogenetických datech všeobecně přijata (recentně např. Bauer et al. 2013).

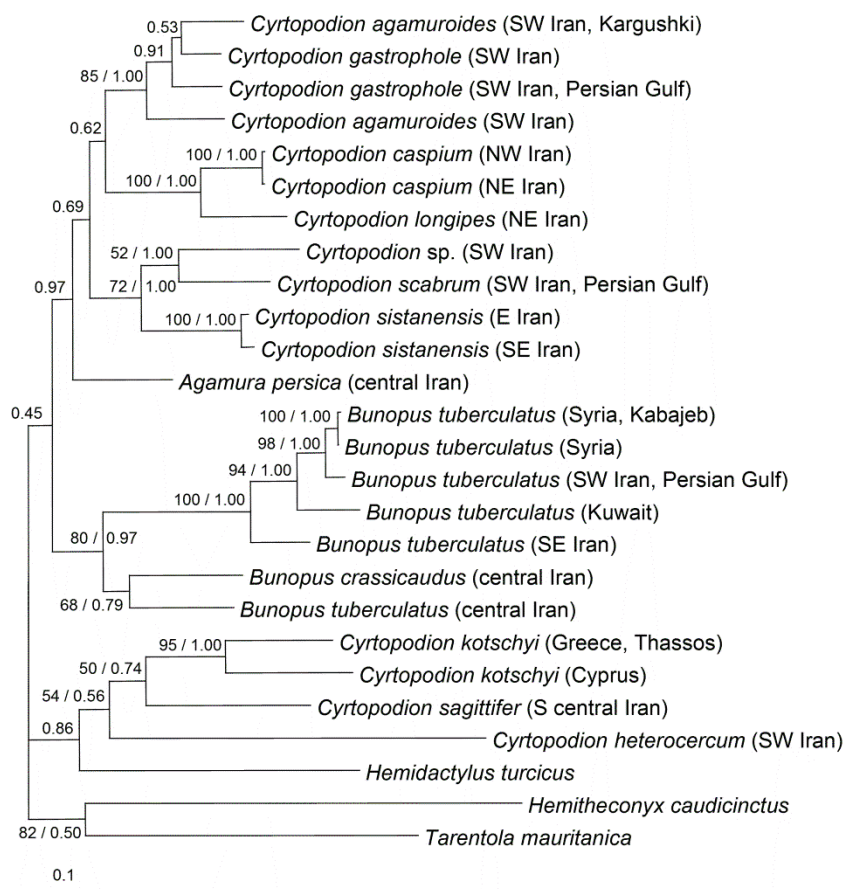
Dosavadní značně nedostatečné znalosti fylogeneze a z toho také částečně vyplývající neustálenost v taxonomii výše jmenovaných rodů (*Agamura*, *Bunopus* a *Cyrtopodion sensu lato*) spolu s možností přístupu k unikátní kolekci zástupců těchto rodů ve sbírkách Přírodovědecké fakulty Univerzity Karlovy v Praze, vyústily k vypracování studie, která tvoří první kapitolu této práce. Cílem bylo především na základě fylogenetické kladistické analýzy sekvenčních dat prozkoumat monofylii jednotlivých podrodů rodu *Cyrtopodion*, případně jeho potenciálně monofyletických skupin druhů dle Andersona (1999). Pro analýzu byly použity částečné sekvence dvou mitochondriálních genů (pro 12S rRNA a cytochrom *b*), které jsou ve fylogenetice ještě tradičně používané jako molekulární markery. Mitochondriální DNA byla použita také vzhledem k již vyššímu stáří dostupného materiálu. Do studie bylo zahrnuto celkem 13 druhů (forem) studovaných gekonů.

Výsledky analýzy upozornily primárně na skutečnost, že rod *Cyrtopodion sensu lato* se svými třemi podrody není monofyletický (viz Obr. 2). Rody *Agamura* a *Bunopus* se ukázaly být příbuznější zkoumaným zástupcům ze skupin *agamuroides*, *caspium* a *scabrum* více než skupině *kotschyi*, odpovídající podrodu *Mediodactylus*. Odlišnost druhů podrodu *Mediodactylus* byla již dříve podpořena analýzou alozymových dat (Macey et al. 2000) a některé následné publikace jej také jako rod přijaly (např. Szczerbak 2003). Naše práce tak potvrdila rodový status podrodu *Mediodactylus*.

V rámci rodu *Cyrtopodion sensu stricto* se podařilo podpořit monofylii jednotlivých skupin dle Andersona (1999) a s výjimkou podrodu *Cyrtopodion* také monofylii jednotlivých podrodů rozlišených v práci Szczerbak a Golubev (1984). Složitější výsledky vzhledem k druhové koherenci uvnitř skupiny *agamuroides* naznačily, že se zde jedná patrně o druhový komplex. Tento předpoklad se ukázal jako oprávněný vzhledem k recentním popisům několika nových druhů, právě z okruhu *C. agamuroides* a *C. gastrophole* (např. Nazarov et al. 2009 a Ahmadzadeh et al. 2011).

Na základě použitých sekvencí se nicméně nepodařilo osvětlit fylogenetickou pozici jednotlivých linií vůči sobě navzájem. V recentní revidující, a co se druhového

záběru týče, rozšiřující studii použili Bauer et al. (2013) pro svou analýzu již nukleární geny a podpořili rodový status všech tří dřívějších podrodů rodu *Cyrtopodion* spolu s patřičnými nomenklatorickými úpravami. Ve stejné práci bylo také odhadnuto stáří celé skupiny zhruba na 55 milionů let, což posouvá její evoluční původ dále do minulosti než k často dříve uvažované souvislosti s vyzdvižením Íránského plata před 5 až 10 miliony let (např. Macey et al. 1998). Použití nukleárních genů tak mohlo vzhledem k možné saturaci mitochondriálních genů vést k přesnější fylogenetické hypotéze.



Obr. 2 Fylogenetický strom založený na částečných sekvencích genů pro 12S rRNA a cytochrom *b*, vytvořený pomocí Bayesovské analýzy. Hodnoty u jednotlivých nodů představují hodnoty bootstrappingu z analýzy metodou maximální parsimonie / posteriorní pravděpodobnosti z Bayesovské analýzy (převzato z Červenka et al. 2008).

Dříve předpokládanou bližší fylogenetickou příbuznost, a také teoreticky očekávatelnou z důvodu podobné vnější morfologie, rodu *Agamura* s druhy ze skupiny *agamuroides*, se potvrdit nepodařilo. Nicméně ani použití nukleárních genů nebylo v upřesnění fylogenetické pozice rodu úspěšnější (Bauer et al. 2013). Pozice rodu *Agamura* je tak stále chápána jako nejasná, je však zřejmě blízce příbuzná studované skupině gekonů s výjimkou rodu *Mediodactylus*.

Fylogenetické řazení dosud dobře podpořeného rodu *Bunopus* do příbuzenstva gekonů rodu *Cyrtopodion sensu stricto* se na základě mitochondriálních sekvencí nepodařilo ověřit. Stejně tak analýza nukleárního *C-mos* genu (Han et al. 2004), tuto skutečnost nepotvrdila. Bauer et al. (2013) však při použití většího počtu genů umístili rod *Bunopus* do blízkosti rodu *Agamura*. Naše analýza nicméně naznačila, že široce rozšířený druh *B. tuberculatus* může být parafyletický vzhledem k druhu *B. crassicauda*. Některé populace prvního jmenovaného z centrálního Íránu se zdají být příbuznější druhu *B. crassicauda*, což dokládají odlišnosti v sekvencích dosahující mezidruhových rozdílů.

Odlišnost fylogenetické linie, nyní již rodu *Mediodactylus*, vůči ostatním zástupcům rodu *Cyrtopodion sensu stricto* nás dovedla k úvaze nad fylogenetickým postavením málo známého endemického rodu *Carinatogeko* z úpatí Zagrosu Íráku a Íránu, jehož několik jedinců se shodou okolností podařilo ve sbírkách Přírodovědecké fakulty nalézt. V době našeho výzkumu bylo o gekonech rodu *Carinatogeko* známo velmi málo informací. Ačkoliv od doby popisu rodu uplynulo již téměř 30 let, byly druhy známy prakticky jen z typových lokalit. Jen ojediněle se vyskytly publikace rozšiřující znalosti o zástupcích rodu a doplňující případně další lokality výskytu (např. Nazari-Serenjeh a Torki 2008). Výsledky našeho výzkumu shrnuje druhá kapitola předkládané disertační práce.

Golubev a Szczerbak (1981) ustanovili rod *Carinatogeko* jako nový rod a zahrnuli do něj dva druhy, *Carinatogeko aspratilis* (popsán původně jako *Bunopus aspratilis*, Anderson 1973) a *Carinatogeko heteropholis* (popsán dříve jako *Tropicolotes heteropholis*, Minton et al. 1970). Historie poznání tohoto rodu je tak dobrým dokladem komplikovaných představ o fylogenetických vztazích v rámci studované skupiny „nahoprstých“ gekonů Blízkého Východu a pro celou skupinu velmi typická. Autoři ve stejné práci totiž navíc na základě morfologických znaků zařadili rod *Carinatogeko* do sesterské pozice k druhu *Mediodactylus heterocercus*, dříve

Cyrtopodion heterocercum, jako vnitřní skupinu podrodu *Mediodactylus* (jak jej chápe Szczerbak a Golubev 1986). Nynější rod *Mediodactylus* se tak stal vlastně parafyletickým.

Za použití sekvencí stejných genů jako v předchozí práci zabývající se především rodem *Cyrtopodion* (viz první kapitola disertační práce), se nám podařilo fylogenetickou analýzou jasně prokázat zařazení gekonů rodu *Carinatogeocho* dovnitř rodu *Mediodactylus* a rod *Carinatogeocho* tak synonymizovat s rodem *Mediodactylus*. Výsledky analýzy sekvencí byly v souladu i s vnější morfologií a charakterem folidózy těchto gekonů. Rod *Mediodactylus* může být charakterizován zřejmou synapomorfii převzatou z práce Anderson (1999) upozorňující na charakter ocasních tuberkulů, které v počtu šesti na každém segmentu netvoří jejich terminální linii, ale jsou umístěny uprostřed těchto segmentů. Tento znak vystihuje také zástupce rodu *Carinatogeocho*. Pro druhy z rodu *Carinatogeocho* je typická výrazná kýlnatost prakticky všech šupin těla a rod byl také původně na základě tohoto znaku definován (Golubev a Szczerbak 1981). Tento konkrétní znak se však jeví jako pouhé rozšíření určité evoluční tendence patrné pro rod *Mediodactylus* jako celek. Kýlnaté šupiny určitých okrsků těla jsou totiž běžné pro mnohé druhy rodu, příkladem může být *M. heterocercus* nebo *Mediodactylus sagittifer* (Nikolsky, 1900).

Při našem studiu sbírkového materiálu z rodu *Carinatogeocho* nastaly určité komplikace s přesnou druhovou identifikací. Na základě analýzy klíčových znaků folidózy definujících jednotlivé druhy rodu nebylo možné identifikovat nám dostupné jedince, a v práci byli proto označeni jako *Carinatogeocho* cf. *heteropholis*. Morfologická odlišení obou druhů totiž mohla být vzhledem k malému počtu dosud prozkoumaných jedinců založena pouze na drobných rozdílech v charakteru folidózy s neúplným náhledem na celkovou morfologickou plasticitu druhu. Ve skutečnosti by se tak mohlo jednat o druh jeden. Nedlouho po publikaci našich výsledků ohledně přeřazení zástupců/zástupce rodu *Carinatogeocho* do rodu *Mediodactylus* byly popsány dva nové druhy, *Mediodactylus stevenandersoni* (Torki, 2011) a *Mediodactylus ilamensis* (Fathinia, Karamiani, Darvishnia, Heidari & Rastegar-Pouyani, 2011), v době svého popisu řazené do rodu *Carinatogeocho* (Torki 2011 a Fathinia et al. 2011a). První jmenovaný druh se zdá být velmi podobný již dříve popsaným druhům *M. aspratilis* a *M. heteropholis*. Nicméně, ani popis nového druhu neumožnil jasně identifikovat námi zkoumané jedince a Torki (2011) se domnívá, že se v našem případě jedná o blíže neidentifikovanou formu z okruhu *M. heterocercus*. Celkový charakter folidózy námi

prozkoumaných jedinců však tuto možnost vylučuje. Klíčové znaky definující nový druh *M. stevenandersoni* jsou naneštěstí poměrně nejednoznačné, protože určující mezidruhové rozdíly jsou často na úrovni sousedících intervalů v kontinuu konkrétního morfologického znaku. Možný mezidruhový překryv klíčových znaků pak spíše nechtěně naznačily i další publikované práce (např. Fathinia et al. 2011b, Karamiani a Rastegar-Pouyani 2011, Sadeghi a Torki 2011). Nelze proto s určitostí vyloučit, že se v případě druhů *M. aspratilis*, *M. heteropholis* a *M. stevenandersoni* nejedná o jeden druh. Objasnění této situace by mohla poskytnout analýza sekvenčních dat, která však nejsou pro všechny tyto druhy k dispozici. Podobně by u druhého nově popsánoho druhu (*M. ilamensis*), vzhledem k jeho podobnosti se zástupci rodu *Bunopus*, mohla být analýza sekvencí nápomocná s jeho taxonomickým zařazením. V tomto případě je však dle znaků vnější morfologie zjevné, že *M. ilamensis* skutečně platným novým druhem je.

Při popisu nových druhů pouze na základě morfologických znaků je obvyklé doplnění popisné studie o komparativní analýzu klíčových morfologických znaků nového druhu s potenciálně zaměnitelnými druhy vyskytujícími se s novým druhem v sympatrii, případně s možným areálovým překryvem. V každém případě je však důležité do srovnání důsledně zahrnout také druhy potenciálně, nebo i reálně fylogeneticky příbuzné. V publikaci tvořící třetí kapitulu poukážeme právě na opomenutí tohoto typu, které pak může přinést nemalé taxonomické komplikace v poznávání druhové diverzity.

Masroor (2009) popsal v rodě *Cyrtopodion* na základě morfologických znaků nový arborealní druh z jižního Pákistánu. Popis nového druhu však plně odpovídal rozšířené definici rodu *Mediodactylus* převzaté z práce Anderson (1999), především pak sdílel možnou synapomorfii rodu, charakterizovanou počtem a umístěním ocasních tuberkulů na jednotlivých segmentech ocasu. Ačkoli Masroor (2009) ve své práci odlišil nový druh od rodu *Mediodactylus* pomocí několika znaků, podrobnější prozkoumání deklarovaných rozdílů ukázalo v původním srovnání určitá pochybení. Popisovaný nový druh, *Cyrtopodion dehakroense* Masroor, 2009, je tak jednoznačně příslušníkem rodu *Mediodactylus* a spadá do něj mimo jiné i ekologicky. Arborealita není pro zástupce rodu *Cyrtopodion* typická, u druhů rodu *Mediodactylus* je však poměrně častá (např. Anderson 1999, Werner 1993). Vnější podobnost s na stromy silně asociovaným druhem *M. sagittifer* z íránské části Balúčistánu se ukázala být až zarážející. Popis

a přiložené fotografie druhu, nyní již *Mediodactylus dehakroense* (Masroor, 2009), údaje z literatury (Szczerbak a Golubev 1986) a nám dostupná data sbírkových jedinců *M. sagittifer* byla následně podrobena pečlivému srovnání. Na základě zjištěných faktů se ukázalo, že nelze vyloučit příslušnost obou forem k jedinému druhu *M. sagittifer*. Nicméně, vzhledem ke skutečnosti, že typové lokality obou druhů jsou vzdáleny zhruba 800 km, vyžaduje definitivní rozhodnutí o druhové příslušnosti podrobnější studii, nejlépe doplněnou analýzou molekulárních znaků.

Zaznamenaná morfologická odlišnost forem, doposud chápaných jako poddruhy, se při bližším zkoumání s využitím fylogenetické analýzy sekvenčních dat, může ukázat jako významnější, než se dříve předpokládalo. Příkladem může být gekon druhu *Bunopus spatalurus* z jihu Arabského poloostrova, jehož taxonomií a biogeografií se zabývá studie, která je ve formě rozpracovaného rukopisu obsahem čtvrté kapitoly.

Poddruh tohoto druhu, *B. s. hajarensis*, byl popsán z oblasti severovýchodního Ománu, respektive z pohoří Hadžar a ostrova Masíra, na základě zřetelných morfologických odlišností od poddruhu nominálního (Arnold 1980). Areál rozšíření poddruhu *B. s. spatalurus* byl pak ohraničen pohořím Dafáru a pokračoval dále na území Jemenu. Celkově disjunktního rozšíření druhu *B. spatalurus* si všiml, vzhledem k absenci nálezů z nížinných oblastí mezi dvěma výše zmíněnými horskými celky, již Arnold (1980). Fylogenetickou analýzou částečných sekvencí mitochondriálního genu pro 12S rRNA se nám podařilo odhalit značnou odlišnost obou poddruhů s genetickými vzdálenostmi plně odpovídajícími rozdílu mezi dobře oddělenými druhy. Analýza haplotypů jaderného *C-mos* genu pak ukázala, že oba poddruhy nesdílejí žádný haplotyp tohoto genu. V práci proto navrhuje povýšit poddruh *B. s. hajarensis* na úroveň druhu *Bunopus hajarensis*. V rámci druhu *B. hajarensis* byly dále analýzou mitochondriálních sekvencí odhaleny tři monofyletické linie, které však navzájem sdílejí některé haplotypy jaderného *C-mos* genu. Přesnější taxonomické zařazení těchto linií tak bude vyžadovat zevrubnější studii, zahrnující větší počet prozkoumaných jedinců i lokalit.

Rozšiřující analýza modelování distribuce vhodných klimatických podmínek pro výskyt druhů *B. hajarensis* a *B. spatalurus*, zahrnující recentní klimatické podmínky, ale i podmínky z posledního glaciálního maxima, ukázala také zajímavý výsledek. Ačkoli je mezi klimatickými nikami obou druhů signifikantní rozdíl, podobnost jejich nik je značná. Oba druhy vyžadují relativně chladnější a vlhčí klima. Jejich recentní

výskyt je zřejmě proto vázán na horská pásma (Hadžar, Dafár) a ve vysoce aridních pouštních nížinných oblastech se nevyskytují. Modely distribuce druhů sice naznačily v době posledního maximálního zalednění možnost většího rozšíření než je recentní, ovšem i v té době patrně existovalo území s nevyhovujícími klimatickými podmínkami v oblasti nynějšího centrálního Ománu, které tak pravděpodobně přispělo k prostorovému oddělení obou druhů.

Disertační práce tedy nejen že přispívá k poznání o fylogenetické příslušnosti několika rodů „nahoprstých“ gekonů, ale snaží se i upozornit na druhovou komplexitu této velmi diverzifikované skupiny šupinatých plazů. Zároveň poukazuje na to, jak nesnadná je v určitých případech identifikace a správné taxonomické zařazení druhů pouze na základě morfologických znaků a jak jsou v této problematice molekulární analýzy klíčové.

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KAPITOLA 1

Phylogeny and taxonomy of the Middle Eastern geckos of the genus *Cyrtopodion* and their selected relatives

Jan Červenka, Lukáš Kratochvíl a Daniel Frynta

Zootaxa (2008) 1931: 25-36

Phylogeny and taxonomy of the Middle Eastern geckos of the genus *Cyrtopodion* and their selected relatives

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Abstract

Representatives of the gekkonid genus *Cyrtopodion* and related genera form a highly diversified group of Palearctic geckos, whose taxonomy is still unstable and phylogeny little known. This study tries to improve this situation by attempting to reconstruct the phylogeny based on two molecular markers (partial sequences of mitochondrial cytochrome *b* and 12S rRNA genes) in the Middle Eastern species of the genus *Cyrtopodion* and members of the genera *Agamura* and *Bunopus*. The results support the monophyly of the species groups within the genus *Cyrtopodion* as defined by Anderson (1999), but relationships between these groups remain largely unresolved. The *kotschy* group (= subgenus *Mediodactylus*) is not closely related to the rest of the genus *Cyrtopodion* and should be treated as an autonomous genus. *Agamura persica* forms a monophyletic group with *agamuroides* + *caspium* + *scabrum* species groups. The genus *Bunopus* does not seem to be closely related to this clade. We briefly discuss the implications of our findings and stress the importance of further molecular studies in Palearctic geckos.

Key words: *Agamura*, angular-toed, *Bunopus*, lizards, mtDNA

Introduction

The representatives of the gekkonid genus *Cyrtopodion* form one of the most diversified groups of Palearctic geckos (Szczerbak & Golubev 1986). The majority of the species inhabits arid regions from the eastern Mediterranean, through the Middle East and south-west Asia to northern India and the slopes of the Himalayas. Their northern range reaches Central Asia. Geckos of the genus *Cyrtopodion* are found on a variety of substrates in diverse environments including rocks, trees and sandy, loess or stone biotopes, with some species living even in synantropy. Individual species differ not only in ecology, but also in external morphology, especially in body size and shape. Ecological and morphological diversity of Palearctic geckos, together with their high abundance in many regions makes this group an interesting model for research in such fields as ecomorphology or evolutionary ecology. However, progress in these fields and the interpretations of evolutionary scenarios are prevented by unknown phylogeny and taxonomy of the group.

The history of the delimitation of the genus *Cyrtopodion* and its unstable systematics includes many competing views and mirrors complex relationships among taxa involved. Briefly, the species of the genus were assigned to the cosmopolitan genus *Gymnodactylus* (e.g. Annandale 1906; Smith 1935) in the past. Underwood (1954) split the genus *Gymnodactylus*, and incorporated most of its Old World species into the genus *Cyrtodactylus*. His classification was not universally accepted and some European researchers still understood

the genus *Gymnodactylus* in the original cosmopolitan concept (e.g. Szczerbak & Golubev 1977). Szczerbak and Golubev (1977) moved the Eurasian species of the original genus *Gymnodactylus* into the subgenera *Cyrtodactylus* and *Mediodactylus* differing in the presence of femoral pores in males, the number of subdigital lamellae and other characters of pholidosis. Kluge (1983) considered the genus *Cyrtodactylus* as defined by Underwood (1954) to be polyphyletic and separated the tropical species possessing the second ceratobranchial arch into the genus *Nactus*. Szczerbak and Golubev (1984) accepted the genus *Cyrtodactylus* as redefined by Kluge (1983). They separated the Palearctic species into the genus *Tenuidactylus* based on the differences in toe shape and other characters of pholidosis. Böhme (1985) and Kluge (1985) pointed out the priority of the name *Cyrtopodion* Fitzinger (1843) over its younger synonym, *Tenuidactylus*. Subsequent studies (e.g. Szczerbak & Golubev 1986; Anderson 1999) thus used the generic name *Cyrtopodion*.

A variety of attempts to classify the more than 40 species of *Cyrtopodion* as so understood were undertaken. Szczerbak and Golubev (1984) divided the species of *Tenuidactylus* (now *Cyrtopodion*) into three subgenera (*Mediodactylus*, *Mesodactylus* and *Tenuidactylus*). Certain species from the so-called Tibeto-Himalayan group were not classified into any subgenus. After the work of Böhme (1985) and Kluge (1985), they started to use the subgeneric names *Cyrtopodion* (= *Mesodactylus*), *Mediodactylus* and *Tenuidactylus* (Szczerbak & Golubev 1986). Szczerbak (1988) elevated the individual subgenera to generic level. This taxonomical modification is generally not followed and all species are still assigned to the single genus *Cyrtopodion* (Anderson 1999; Uetz & Hallermann 2007). Nevertheless, cladistic analysis of allozymic data revealed high divergence of the subgenus *Mediodactylus*, and thus supported its generic status (Macey *et al.* 2000).

Anderson (1999) sorted the geckos of the genus *Cyrtopodion* based on external morphology and pholidosis not into subgenera, but into four species groups of unverified monophyly. He attempted to identify apparent synapomorphies of each species group. However, the material of some known species was not available to him. According to Anderson (1999), the *scabrum* group possesses primitive characters of the genus *Cyrtopodion*. The identified synapomorphies of the remaining groups are: darkly pigmented peritoneum (*agamuroides* group); presence of both femoral and precloacal pores in males, presence of one to six subfemoral tubercles (*caspium* group); caudal tubercles do not form a terminal row (there are six of them on each annulus), but are spread around the middle of each caudal segment (*kotschyi* group). The species groups defined by Anderson more-or-less correspond to the subgenera of Szczerbak and Golubev (1984, 1986), i.e. subgenus *Cyrtopodion* = *scabrum* + *agamuroides* group, subgenus *Tenuidactylus* = *caspium* group, subgenus *Mediodactylus* = *kotschyi* group.

During the last two decades, several new species of the genus *Cyrtopodion* (*sensu lato*) were described from the circum-Himalayan region, mostly from Pakistan (e.g. Khan 1980, 1991, 1993a, 1993b, 2001; Khan & Tasnim 1990; Khan & Baig 1992; Baig 1998; Krysko *et al.* 2007), which illustrates the high diversity of the gekkonid fauna in this region. Generic designation of these species and their relationship to the species from the Middle East mentioned above is not clear. Some descriptions suggest a relationship to the subgenera *Cyrtopodion* and *Tenuidactylus*, but more frequently they are members of the unclassified Tibeto-Himalayan group. Some attempt to bring order to the complex situation in this region was made by Khan (2003b), who established new genera *Altigekko*, *Indogekko* and *Siwaligekko* (reviewed in Khan 2004).

Two other gekkonid genera, *Agamura* and *Bunopus*, are traditionally supposed to be closely related to the genus *Cyrtopodion*. As was shown by Kluge (1983), all three genera share the absence of the second ceratobranchial arch together with some other Palearctic geckos. The geographical distribution of these genera is partially overlapping (e.g. Anderson 1999). According to Szczerbak and Golubev (1977, 1986), the genus *Bunopus* is closely related to the genus *Cyrtopodion*. Only three species are recently assigned into genus *Bunopus* (*Bunopus crassicaudus* Nikolsky, *Bunopus spatulurus* Anderson, and *Bunopus tuberculatus* Blanford; Leviton *et al.* 1992). The genus *Agamura* is considered to be monotypic, the only representative being *Agamura persica* (Duméril). Another species, *Agamura femoralis* Smith, was later reclassified to the genus *Rhinogekko* represented by *Rhinogekko missonei* de Witte (de Witte 1980). The genus *Rhinogekko* was then

synonymized with the genus *Agamura* by Szczerbak and Golubev (1986), and *Cyrtopodion gastrophole* (Werner) was included in the genus *Agamura* in the same study. Synonymization of the genus *Rhinogecko* with *Agamura* has not been generally accepted (e.g. Khan 2003a; cf. to the recent checklists: Rösler 2000, Kluge 2001). Anderson (1999) maintained the genus *Rhinogecko*, but kept *C. gastrophole* in the genus *Cyrtopodion* because of its expected close relationship to *Cyrtopodion agamuroides* (Nikolsky). Nevertheless, Anderson (1999) did not reject the idea of the close affinity between these two species and the genus *Agamura*.

Here, we present the molecular analysis of the relationship among members of *Agamura*, *Bunopus* and particularly the Middle-Eastern group of the genus *Cyrtopodion*. Our specific aim was to test the phylogenetic coherence (monophyly) of the subgenera/groups and their mutual relationships.

Material and methods

Material

In total, 23 samples of 13 species (forms) from the collections of the Faculty of Science, Charles University in Prague were included in the analysis (Tab 1). The forms were determined according to the keys and descriptions in Blanford (1874); Anderson (1973); Minton *et al.* (1970); Szczerbak & Golubev (1977, 1986); Anderson (1999); Baig (1998); Szczerbak (1978); Duda & Sahi (1978a, 1978b); Golubev & Szczerbak (1981a, 1981b, 1983); Leviton & Anderson (1984); Khan (1988, 1989, 1991, 1993a, 1993b, 1997, 2001, 2003a); Khan & Tasnim (1990); Nazarov & Rajabizadeh (2007). One form, here assigned to *Cyrtopodion* sp., did not concur morphologically with any described form. It was found close to the type locality of *Cyrtopodion brevipes* (Blanford), but it does not correspond to the original description of either this or other species.

The classification of the analyzed species was adopted from Anderson (1999) as it is the latest available summarizing publication. The studied species of the genus *Cyrtopodion* were divided into species groups as follows: *agamuroides* group (*C. agamuroides* and *C. gastrophole*), *caspium* group (*C. caspium* and *C. longipes*), *kotschyi* group (*C. kotschyi*, *C. heterocercum* and *C. sagittifer*), *scabrum* group (*C. scabrum*, *C. sistanensis* and *Cyrtopodion* sp.). Two species of the genus *Bunopus* (*B. crassicaudus* and *B. tuberculatus*) and *Agamura persica* were also included, while *Hemidactylus turcicus* (Linnaeus), *Tarentola mauritanica* (Linnaeus) and *Hemitheconyx caudicinctus* (Duméril) were used as outgroups for the analyses of DNA sequences. Partial sequences of the outgroup species for 12S rRNA and cytochrome *b* genes were obtained from GenBank, NCBI, for details see Table 1.

DNA extraction, amplification and sequencing

The total DNA from alcohol preserved samples (tongue or regenerated tail-tips) was isolated using DNeasy® Tissue Kit (Qiagen) based on the manufacturer's instructions. Taking into consideration the relatively old material and the aim of our work, mtDNA genes were selected for the analyses. Two segments, the partial sequences of the genes for cytochrome *b* and 12S rRNA, were amplified via PCR using following primers: 12S rRNA - L1091 (5' - AAA CTG GGA TTA GAT ACC CCA CTA T - 3') and H1478 (5' - GAG GGT GAC GGG CGG TGT GT - 3') (Kocher *et al.* 1989, as modified by Honda *et al.* 1999), cytochrome *b* - L14841 (5' - AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA - 3') and H15149 (5' - AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A - 3') (Kocher *et al.* 1989). PCR reaction for the 12S rRNA gene was carried out in 50 µl and contained 10 µl of DNA, 5 mM MgCl₂, 0.6 mM dNTP mix, 8 pmol of primer L1091, 6.75 pmol of primer H1478, 1x PCR buffer (Fermentas) and 5U of *Taq* DNA Polymerase (Fermentas). Reaction conditions were: 93°C for 120s, then 41 cycles of 93°C for 60s, 52°C for 60s and 72°C for 60s, final extension 72°C for 10 min. Cytochrome *b* reaction was also carried out in 50 µl and contained 10 µl of DNA, 5 mM MgCl₂, 0.6 mM dNTP mix, 30 pmol of each primer, 1x PCR buffer (Fermentas) and 5U of *Taq*

TABLE 1. List of species used in this study including locality, length and GenBank Accession numbers of obtained sequences of 12S rRNA and cytochrome *b* genes.

Species	Collection number	Latitude	Longitude	Altitude (m above sea level)	Locality	12S rRNA sequence length	GenBank Accession No. 12S rRNA	Cytochrome <i>b</i> sequence length	GenBank Accession No. cytochrome <i>b</i>	Citation
<i>Agamura persica</i> (Duméril)	R\IRA\1201	32°42' N	55°22' E	1150	central Iran, Karavansaraj-ye-Shah Abbasi	395 bp	EU589153	307 bp	EU589176	
<i>Bunopus crassicaudus</i> Nikolsky	R\IRA\1193	32°42' N	55°22' E	1150	central Iran, Karavansaraj-ye-Shah Abbasi	396 bp	EU589154	307 bp	EU589177	
<i>Bunopus tuberculatus</i> Blanford	SUR 084				Syria, Kabajeb (Deir ez-Zur)	395 bp	EU589155	307 bp	EU589178	
<i>Bunopus tuberculatus</i> Blanford	R\IRA\1160	29°38' N	50°26' E	10	SW Iran, Persian Gulf, Bandar-e-Gonaveh	395 bp	EU589156	307 bp	EU589179	
<i>Bunopus tuberculatus</i> Blanford	REPT\SUR\347				Syria	395 bp	EU589157	307 bp	EU589180	
<i>Bunopus tuberculatus</i> Blanford	R\IRA\1044	34°52' N	58°52' E	958	central Iran	397 bp	EU589158	307 bp	EU589181	
<i>Bunopus tuberculatus</i> Blanford	9014	22°44' N	60°50' E	100	SE Iran, Pir Sohrab	394 bp	EU589159	307 bp	EU589182	
<i>Bunopus tuberculatus</i> Blanford	9016				Kuwait	394 bp	EU589160	307 bp	EU589183	
<i>Cyrtopodion agamuroides</i> (Nikolsky)	REPT\IRA\897	26°04' N	57°18' E	11	SW Iran, Kargushki	392 bp	EU589161	307 bp	EU589184	
<i>Cyrtopodion agamuroides</i> (Nikolsky)	REPT\IRA\940	28°55' N	52°31' E	1412	SW Iran, Firuz Abad	395 bp	EU589162	307 bp	EU589185	
<i>Cyrtopodion caspium</i> (Eichwald)	R\IRA\1013	36°40' N	52°38' E	13	NW Iran, Babol Sar	396 bp	EU589163	307 bp	EU589186	
<i>Cyrtopodion caspium</i> (Eichwald)	R\IRA\1189	36°16' N	60°32' E	750	NE Iran, Bazzangan	396 bp	EU589164	307 bp	EU589187	
<i>Cyrtopodion gastrophole</i> (Werner)	REPT\IRA\579	29°39' N	50°27' E	32	SW Iran, Persian Gulf, Bandar-e-Gonaveh	394 bp	EU589165	307 bp	EU589188	
<i>Cyrtopodion gastrophole</i> (Werner)	REPT\IRA\866	27°38' N	56°13' E	500	SW Iran, Khos Hangan	396 bp	EU589166	307 bp	EU589189	
<i>Cyrtopodion heterocercum</i> (Blanford)	REPT\IRA\538	34°24' N	47°26' E	1300-1600	SW Iran, Bisotun	390 bp	EU589167	307 bp	EU589190	
<i>Cyrtopodion kotschyi</i> (Steindachner)	GR001	40°45' N	24°33' E		Greece, Thassos	386 bp	EU589168	307 bp	EU589191	
<i>Cyrtopodion kotschyi</i> (Steindachner)	CY001	35°20' N	33°04' E		Cyprus	391 bp	EU589169	307 bp	EU589192	
<i>Cyrtopodion longipes</i> (Nikolsky)	R\IRA\1030	36°16' N	60°32' E	750	NE Iran, Bazzangan	397 bp	EU589170	307 bp	EU589193	
<i>Cyrtopodion longipes</i> (Nikolsky)	REPT\IRA\846	25°30' N	58°56' E	5	S central Iran, Karti	389 bp	EU589171	307 bp	EU589194	
<i>Cyrtopodion sistanensis</i> (Heyden)	REPT\IRA\578	29°39' N	50°27' E	32	SW Iran, Persian Gulf, Bandar-e-Gonaveh	390 bp	EU589172	307 bp	EU589195	
<i>Cyrtopodion sistanensis</i> Nazarov & Rajabizadeh	REPT\IRA\122	29°46' N	59°52' E	1270	E Iran, Nosrat Abad	391 bp	EU589174	307 bp	EU589197	
<i>Cyrtopodion sistanensis</i> Nazarov & Rajabizadeh	REPT\IRA\827				SE Iran, Arq-e-Bam	391 bp	EU589175	307 bp	EU589198	
<i>Cyrtopodion</i> sp.	REPT\IRA\904	26°04' N	57°18' E	11	SW Iran, Kargushki	392 bp	EU589173	307 bp	EU589196	
<i>Hemidactylus tureticus</i> (Linnaeus)					Canary Islands (Spain), Gran Canaria, Las Palmas	405 bp	AF363568	684 bp	AF364319	Carranza <i>et al.</i> , 2002
<i>Hemithecomys caudicinctus</i> (Duméril)					not given	399 bp	AY140989	428 bp	AY141007	Vicario <i>et al.</i> , 2003
<i>Tarentola mauritanica</i> (Linnaeus)	BEV.2136				Algeria, Abdelmaleh Rahmd	402 bp	AF363576	684 bp	AF364327	Carranza <i>et al.</i> , 2002

DNA Polymerase (Fermentas). Reaction conditions were: 93°C for 120s, then 41 cycles of 93°C for 60s, 46°C for 60s and 72°C for 60s, final extension 72°C for 10 min. Amplification products of an expected size of about 400bp for the 12S rRNA gene fragment and 300bp for the cytochrome *b* fragment were gel purified using QIAquick® Gel Extraction Kit (Qiagen) according to the manufacturer's instructions.

Both segments were sequenced directly using ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) and the original primers. Where necessary, a set of internal primers for 12S rRNA gene designed for this purpose was used: 12SINTF1 (5' - AGA GAT GGG CTA CAT TTT CTA - 3'), 12SINTR1 (5' - TAG AAA ATG TAG CCC ATC TCT - 3'), 12SINTF2 (5' - CCT AGA GGA GCC TGT CCT A - 3'), 12SINTR2 (5' - TAG GAC AGG CTC CTC TAG G - 3'). Sequencing was performed on an ABI PRISM 3100-Avant Genetic Analyzer at the Laboratory of DNA sequencing, Faculty of Science, Charles University in Prague. All obtained sequences were deposited in GenBank (for Accession numbers see Table 1).

Phylogenetic analysis

Sequences of cytochrome *b* were aligned manually. Translation to protein (using MEGA 2.1, Kumar *et al.* 2001) did not reveal any stop codons or gaps. Sequence of 307bp was shortened by the initial nucleotide to reach the first codon position. Partial sequences of 12S rRNA gene were aligned based on the secondary structure of 12S rRNA (Kjer 1995; Titus & Frost 1996; Hickson *et al.* 1996). Before alignment, identical decamers (GATCATCTAG) were inserted before and after each homologous stem, which enforced aligning of conservative motifs (Wiens & Hollingsworth 2000). The alignment was performed using ClustalX 1.81 (Thompson *et al.* 1997; Chenna *et al.* 2003). Three alignments differing in gap opening penalty values (GOP = 5, 10 and 15) were constructed. Other alignment parameters were set as follows: gap extension penalty (GEP) 0.1, delay divergent sequences 40%, DNA transition weight 0.5. Inserted decamers were removed from the sequences after the completion of the alignment. Small manual corrections reflecting secondary structure of 12S rRNA were carried out (Wiens & Hollingsworth 2000). Final alignment out of the three mentioned alternatives was processed by the "culling" method (Gatesy *et al.* 1993; Leaché & Reeder 2002), i.e. only the non-ambiguous positions were taken into account.

The incongruence length difference test (Farris *et al.* 1994, 1995) performed in PAUP* 4.0b10 (Swofford 2002) with 1000 heuristic searches did not reject congruence between the data from both genes ($P = 0.125$, gap coded as fifth base). Therefore, the sequences of both genes were combined in further phylogenetic analyses. Parsimony-uninformative characters were excluded from the incongruence length difference test following Wiens and Hollingsworth (2000), although e.g. Cunningham (1997a, 1997b) recommended exclusion of only the invariant characters.

Phylogenetic relationship among studied forms were estimated by maximum parsimony (MP) and Bayesian analysis (BA), using PAUP* 4.0b10 (Swofford 2002) for MP and MrBayes 3.1 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) for BA. Gaps was coded as a fifth base and heuristic search was conducted using 100 random sequence addition replicates and TBR branch-swapping in MP. The robustness of trees was assessed by nonparametric bootstrap analysis (Felsenstein 1985) with 1000 heuristic pseudoreplicates. For Bayesian analysis, the best fit model of nucleotide substitution was determined for both genes via the hierarchical likelihood ratio test implemented in Modeltest 3.6 (Posada & Crandall 1998). General time reversible model (Tavaré 1986) with gamma distributed rates of variable sites (GTR + Γ) was selected as the best fit model for the 12S rRNA gene. A transversional model with a proportion of invariant sites and gamma distributed rates of variable sites (TVM + I + Γ) was selected for cytochrome *b*. This is a submodel of GTR + I + Γ and was input into the program as such. The Bayesian analysis was initiated using a separate model for each partition (12S rRNA and cytochrome *b* fragments) and used one heated and three cold Markov chains estimated for 10^6 generations, with every 100^{th} tree being sampled. The number of sampled trees prior to stationarity of log-likelihood scores (burn-in value) was determined graphically. A majority consensus tree was

generated from the remaining trees after discarding the burn-in trees. The frequency of individual clades in the consensus tree was represented by their posterior probabilities (Huelsenbeck & Ronquist 2001).

Results

Genetic distances (p distances) among individuals of the studied species together with three species of the outgroup for each gene (12S rRNA and cytochrome *b*) are shown in Table 2. Average genetic divergences of both genes between studied species are above 16% for 12S rRNA and 22% for cytochrome *b*, with maxima of 21% and 33%, respectively. Average distances between ingroup taxa and the outgroup species are comparable, i.e. 20% for 12S rRNA and 26% for cytochrome *b*.

The main result of the analyses was the well-supported monophyly of most of the species groups defined by Anderson (1999), i.e. *agamuroides*, *caspium*, *scabrum* and *kotschyi* groups, as well as of the genus *Bunopus* (Fig. 1). The *agamuroides* group (two species, two representatives of each were used) seems to be monophyletic, even if the specimens identified as *C. agamuroides* and *C. gastrophole* were not reciprocally monophyletic. The *scabrum* group (three species, two representatives of one of them) is also well-supported. BA revealed a close relationship between *Cyrtopodion* sp. and *C. scabrum*. Both specimens of *C. sistanensis* were placed in a single clade, sister to *Cyrtopodion* sp. + *C. scabrum*. The support of the *kotschyi* group (three species, two forms of one of them) was weak in both MP and BA; only the monophyly of *C. kotschyi* from Cyprus and Greece was clearly supported. All seven studied individuals from two species of *Bunopus* form a clade with very high support. The position of internal branches inside the genus *Bunopus* is the same in both MP and BA. *B. crassicaudus* and *B. tuberculatus* from the central Iran form a clade with weak support in both BA and MP.

In contrast to the good resolution of the species groups, the phylogenetic relationship among them remains unresolved. The position of *Agamura persica* in BA indicated that the genus *Cyrtopodion* (*sensu stricto*) is not monophyletic. This species was placed as the sister to the clade consisting of *agamuroides*, *caspium* and *scabrum* group, albeit without significant support. The *kotschyi* group (i.e. subgenus *Mediodactylus*) did not form part of the same clade with the remaining species groups of the genus *Cyrtopodion*.

Discussion

Mitochondrial cytochrome *b* and 12S rRNA genes are the traditional markers for phylogenetic studies of lizards (e.g. Vicario *et al.* 2003; Whiting *et al.* 2003; Brandley & de Queiroz 2004; Austin *et al.* 2004). In our study, we used partial sequences of both genes but failed to obtain a fully resolved phylogeny of the studied group of geckos. 12S rRNA gene is characterized by the presence of highly conservative motifs of secondary structure as well as regions with high substitution rate and the occurrence of indels precluding indisputable alignment of sequences (Simon *et al.* 1994). On the other hand, the alignment of sequences of cytochrome *b* is obvious, but in our case, this gene shown relatively high saturation level, following p distances of ingroup forms vs. outgroups (see Tab. 2) and saturation tests (not shown). Another limiting factor for the current analysis might be the insufficient length of used sequences. However, partial sequence of only the cytochrome *b* gene was successfully used in the resolution of even deep phylogenetic divergences in other squamate reptiles (e.g. relationships among snake families; Lawson *et al.* 2004). It is possible that the age of particular lineages within the groups of geckos studied herein is considerably higher than in other groups, or that the examined geckos possess much higher rate of molecular changes. Relatively high genetic distances can be found in both genes even within a single species. For example, Kasapidis *et al.* (2005) revealed very high genetic distances

TABLE 2. Uncorrected genetic distances (*p* distances) of 12S rRNA (lower triangular matrix) and cytochrome *b* (upper triangular matrix)

Legend: 1 - *Agamura persica* (central Iran), 2 - *Bunopus crassicaudus* (central Iran), 3 - *Bunopus tuberculatus* (Syria, Kabajeb), 4 - *Bunopus tuberculatus* (SW Iran, Persian Gulf), 5 - *Bunopus tuberculatus* (Syria), 6 - *Bunopus tuberculatus* (central Iran), 7 - *Bunopus tuberculatus* (SE Iran), 8 - *Bunopus tuberculatus* (Kuwait), 9 - *Cyrtopodion agamuroides* (SW Iran, Kargushki), 10 - *Cyrtopodion agamuroides* (SW Iran), 11 - *Cyrtopodion* sp. (SW Iran), 12 - *Cyrtopodion sistansensis* (E Iran), 13 - *Cyrtopodion sistansensis* (SE Iran), 14 - *Cyrtopodion caspium* (NW Iran), 15 - *Cyrtopodion caspium* (NE Iran), 16 - *Cyrtopodion gastrophole* (SW Iran, Persian Gulf), 17 - *Cyrtopodion gastrophole* (SW Iran), 18 - *Cyrtopodion heterocercum* (SW Iran), 19 - *Cyrtopodion kotschy* (Greece, Thassos), 20 - *Cyrtopodion kotschy* (Cyprus), 21 - *Cyrtopodion longipes* (NE Iran), 22 - *Cyrtopodion scabrum* (SW Iran, Persian Gulf), 23 - *Cyrtopodion sagittifer* (S central Iran), 24 - *Hemitheconyx caudicinctus*, 25 - *Hemidactylus turcicus*, 26 - *Tarentola mauritanica*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1		0.1732	0.2386	0.2353	0.2353	0.1765	0.2092	0.2386	0.1699	0.1569	0.1830	0.1863	0.1895	0.1993	0.1961	0.1699	0.1667	0.2680	0.2059	0.1830	0.1928	0.1732	0.1928	0.2549	0.1993	0.2549
2	0.1406		0.2484	0.2386	0.2451	0.1765	0.2386	0.2484	0.2026	0.1797	0.2190	0.2157	0.2026	0.2190	0.2157	0.2190	0.1732	0.3007	0.2320	0.2124	0.2059	0.1863	0.2353	0.2941	0.2255	0.2908
3	0.1369	0.1247		0.0556	0.0033	0.2516	0.1667	0.1275	0.2516	0.2549	0.2320	0.2549	0.2647	0.2908	0.2876	0.2386	0.2451	0.3301	0.2647	0.2614	0.2810	0.2647	0.2941	0.3072	0.2712	0.3203
4	0.1338	0.1248	0.0060		0.0523	0.2418	0.1634	0.1438	0.2386	0.2451	0.2353	0.2680	0.2712	0.2810	0.2778	0.2549	0.2484	0.3333	0.2647	0.2418	0.2614	0.2484	0.2908	0.3105	0.2745	0.3007
5	0.1369	0.1247	0.0000	0.0060		0.2484	0.1667	0.1242	0.2484	0.2516	0.2288	0.2516	0.2614	0.2876	0.2843	0.2353	0.2418	0.3268	0.2614	0.2582	0.2778	0.2614	0.2908	0.3039	0.2680	0.3170
6	0.1190	0.1004	0.1036	0.1036		0.2190	0.2549	0.2026	0.1863	0.2157	0.2026	0.2026	0.2092	0.2222	0.2190	0.2059	0.1993	0.2974	0.2026	0.2026	0.2320	0.2124	0.2255	0.2908	0.2222	0.2941
7	0.1528	0.1285	0.0425	0.0425	0.1220		0.1830	0.1993	0.2222	0.2124	0.2190	0.2288	0.2712	0.2680	0.2288	0.2288	0.2092	0.3301	0.2549	0.2418	0.2418	0.2255	0.2876	0.2843	0.2516	0.2974
8	0.1339	0.1309	0.0302	0.0272	0.0302	0.1126	0.0425		0.2386	0.2451	0.2451	0.2614	0.2549	0.2843	0.2810	0.2353	0.2320	0.3333	0.2843	0.2418	0.2614	0.2516	0.3007	0.3072	0.2843	0.3366
9	0.0942	0.1275	0.1426	0.1395	0.1426	0.1186	0.1553	0.1486		0.1569	0.1634	0.1667	0.1797	0.2059	0.2026	0.1536	0.1373	0.2778	0.1961	0.1830	0.1895	0.1634	0.2190	0.2810	0.1928	0.2484
10	0.1002	0.1215	0.1486	0.1455	0.1486	0.1339	0.1674	0.1546	0.0485		0.1895	0.1928	0.2026	0.1830	0.1797	0.1340	0.1503	0.2516	0.1928	0.1667	0.1895	0.1830	0.2059	0.2876	0.2059	0.2582
11	0.1274	0.1340	0.1518	0.1578	0.1518	0.1371	0.1493	0.1578	0.1212	0.1364		0.1765	0.1797	0.2288	0.2255	0.1699	0.1928	0.2680	0.2288	0.2026	0.2026	0.1797	0.2288	0.2810	0.2026	0.2614
12	0.1124	0.1374	0.1493	0.1462	0.1493	0.1375	0.1499	0.1584	0.0882	0.1065	0.1153		0.0327	0.2288	0.2320	0.1699	0.1601	0.2745	0.1993	0.2026	0.2157	0.1503	0.1993	0.2908	0.1797	0.2582
13	0.1154	0.1343	0.1462	0.1432	0.1462	0.1406	0.1468	0.1553	0.0913	0.1095	0.1183	0.0030		0.2288	0.2255	0.1797	0.1569	0.2908	0.2124	0.2124	0.2092	0.1503	0.2092	0.2974	0.1830	0.2582
14	0.1033	0.1125	0.1214	0.1244	0.1214	0.1005	0.1463	0.1214	0.0910	0.1062	0.1213	0.1158	0.1188		0.0033	0.2190	0.2157	0.3105	0.2124	0.2418	0.1601	0.2157	0.2484	0.2680	0.2124	0.2320
15	0.1033	0.1125	0.1214	0.1244	0.1214	0.1005	0.1463	0.1214	0.0910	0.1062	0.1213	0.1158	0.1188	0.0000		0.2157	0.2124	0.3137	0.2124	0.2386	0.1569	0.2124	0.2484	0.2680	0.2092	0.2320
16	0.0941	0.1275	0.1335	0.1305	0.1335	0.1095	0.1462	0.1396	0.0212	0.0515	0.1212	0.0912	0.0943	0.0910	0.0910		0.1209	0.2614	0.1895	0.1961	0.1830	0.2157	0.2745	0.1797	0.2614	
17	0.0910	0.1276	0.1456	0.1426	0.1456	0.1216	0.1583	0.1517	0.0182	0.0485	0.1182	0.0911	0.0941	0.0849	0.0849	0.0182		0.2810	0.2190	0.2157	0.1732	0.1667	0.2092	0.2680	0.1993	0.2810
18	0.1945	0.2054	0.2103	0.2133	0.2103	0.1990	0.2147	0.2164	0.1734	0.1858	0.1919	0.2009	0.2039	0.1852	0.1852	0.1762	0.1705		0.2810	0.2810	0.3007	0.3137	0.2582	0.2876	0.3464	
19	0.1670	0.1898	0.1859	0.1828	0.1859	0.1715	0.1873	0.1767	0.1491	0.1706	0.1737	0.1735	0.1765	0.1486	0.1486	0.1549	0.1523	0.1763		0.1601	0.2288	0.2353	0.1961	0.2647	0.2157	0.2712
20	0.1791	0.2015	0.2072	0.2072	0.2072	0.2016	0.2180	0.2041	0.1765	0.1921	0.1891	0.2070	0.2101	0.1667	0.1667	0.1793	0.1738	0.1459	0.1246		0.2255	0.2157	0.2092	0.2876	0.2222	0.2582
21	0.1334	0.1277	0.1395	0.1425	0.1395	0.1126	0.1367	0.1274	0.1090	0.1301	0.1397	0.1370	0.1400	0.0513	0.0513	0.1000	0.1060	0.1891	0.1589	0.1775		0.2059	0.2418	0.2876	0.1993	0.2516
22	0.1246	0.1558	0.1339	0.1399	0.1339	0.1435	0.1558	0.1521	0.1125	0.1186	0.0880	0.1185	0.1216	0.1462	0.1462	0.1156	0.1185	0.1917	0.2009	0.2101	0.1642		0.2288	0.2810	0.2190	0.2582
23	0.1454	0.1527	0.1556	0.1556	0.1556	0.1410	0.1723	0.1618	0.1405	0.1559	0.1687	0.1711	0.1741	0.1396	0.1396	0.1406	0.1377	0.1495	0.1500	0.1408	0.1651	0.1680		0.2549	0.2484	0.2843
24	0.1856	0.2114	0.1981	0.2012	0.1981	0.1831	0.2142	0.2044	0.1765	0.1854	0.1973	0.2161	0.2161	0.1827	0.1827	0.1760	0.1697	0.2231	0.2168	0.2226	0.1982	0.2193	0.2235		0.2778	0.2974
25	0.2061	0.2078	0.2193	0.2253	0.2193	0.2109	0.2292	0.2283	0.1978	0.2042	0.1944	0.2220	0.2250	0.2030	0.2030	0.2008	0.1980	0.2067	0.1975	0.1975	0.2158	0.2160	0.1771	0.2190		0.2255
26	0.1798	0.1562	0.1830	0.1861	0.1830	0.1714	0.1961	0.1953	0.1767	0.1768	0.1885	0.1980	0.1980	0.1647	0.1647	0.1765	0.1767	0.2169	0.2076	0.2165	0.1893	0.2194	0.2083	0.2134	0.2040	

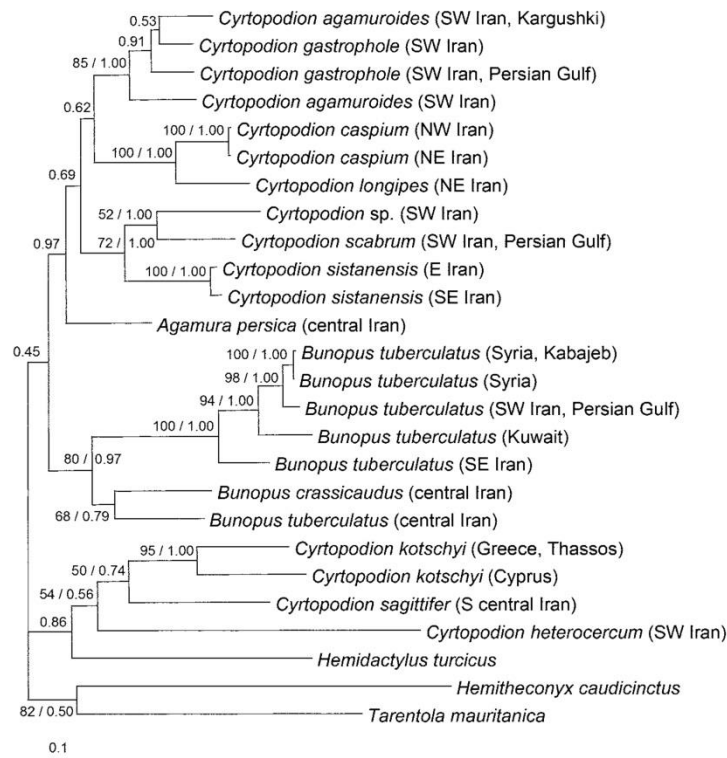


FIGURE 1. Phylogenetic tree based on partial sequences of 12S rRNA and cytochrome *b* genes constructed by Bayesian inference. Scores at nodes represent bootstrap values (for MP) if higher than 50% / posterior probability of BA.

among populations of *Cyrtopodion kotschyi* (Steindachner) and concluded that its intraspecific differentiation started during Miocene (about 10 Mya). Old divergences, consistent with those found here, were suggested by Gamble *et al.* (2008) across the whole clade Gekkota. On the other hand, the genetic distances for 12S rRNA gene between *C. longipes* and *C. caspium*, members of *caspium* group (= subgenus *Tenuidactylus*) are relatively small in comparison with other species analyzed in this study (Tab. 2) and more recent speciation within some lineages can be thus anticipated as well.

Although it is now clear that further molecular data, preferably sequences of conservative nuclear genes will be needed to better resolve deep splits, the present study brings several insights into the phylogeny and taxonomy of the group. Our study supports the non-monophyly of the genus *Cyrtopodion* (*sensu lato*) as recently used (e.g. Anderson 1999; Uetz & Hallermann 2007). The genus *Agamura* seems to be more closely related to the remaining *Cyrtopodion* species than the members of the subgenus *Mediodactylus* (= *kotschyi* group). The distinction of the *Mediodactylus* clade was also supported by the cladistic analysis of allozyme data (Macey *et al.* 2000). Thus, the subgenus *Mediodactylus* should be excluded from the genus *Cyrtopodion* and elevated to the generic status. The name *Mediodactylus* has been already used in generic position in some recent publications (e.g. Szczerbak 2003; Ananjeva *et al.* 2006).

Within the genus *Cyrtopodion* (*sensu stricto*), our results clearly supported monophyly of the species groups defined by Anderson (1999) and principally also the monophyly of the subgenera recognized by Szczerbak and Golubev (1984), except for their subgenus *Cyrtopodion*. Nevertheless, the relationship between *scabrum* group and *agamuroides* group forming it remains unresolved. The position of the subgenus *Tenuidactylus* (= *caspium* group) within the genus *Cyrtopodion* remains unclear as well. It should be noted, that the morphological characters of the species examined in this study do not always match Anderson's

(1999) synapomorphies of species groups. *Cyrtopodion* sp., probably a new, currently undescribed species nested within the *scabrum* group possesses femoral pores, which should be a synapomorphy of the *caspium* group. Recently described *Cyrtopodion sistanensis*, syntopic with *Cyrtopodion scabrum*, resembles *Cyrtopodion watsoni* (Murray), but its localities are distant from the known distribution of this species (Szczerbak & Golubev 1986). This species clustered with *scabrum* group, but exhibited subpostfemoral tubercles suggested by Anderson (1999) as another synapomorphy of the *caspium* group. Therefore, the status of femoral pores and subpostfemoral tubercles as synapomorphic characters of *caspium* group is questionable.

The situation within the *agamuroides* group seems to be more complicated, as the species *C. agamuroides* itself might be a complex of species, most probably paraphyletic with respect to *C. gastrophole*. According to genetic divergences, the close relationship of *C. gastrophole* and *Agamura* (supposed by Szczerbak & Golubev 1986) was not supported. This could be caused by rapid molecular evolution within the hypothetical *Agamura-agamuroides-gastrophole* clade. The long limbs that are a putative synapomorphy of this clade could be also explained as convergence of the individual species. Because of the unresolved position of the enigmatic genus *Agamura*, we are inclined to believe that *Agamura* is a discrete genus closely related to the genus *Cyrtopodion* (*sensu stricto*, represented by *agamuroides*, *caspium* and *scabrum* species groups).

The phylogenetic hypothesis of a close relationship between *Cyrtopodion* and *Bunopus* has not been supported by our study. Neither did the phylogenetic analysis of the gekkotan lizards based on sequences of the nuclear *C-mos* gene (Han *et al.* 2004) support the close relationship of *Cyrtopodion*, represented by *Cyrtopodion elongatus* (Blanford), and *Bunopus*, represented by *Bunopus tuberculatus* Blanford. Consequently, the genus *Bunopus* is probably not closely related to the *Agamura-Cyrtopodion* clade. Our analysis revealed that the widely distributed *B. tuberculatus* might be paraphyletic with respect to *B. crassicaudus*. Genetic divergences between *B. tuberculatus* from central Iran and other populations of this species were similar to those between populations of *B. tuberculatus* and *B. crassicaudus*.

In summary, the present study provides basic insight into the phylogeny and taxonomy of important, but somewhat neglected taxa of Palearctic geckos. We hope that it will stimulate further research leading to deeper understanding of this speciose and ecologically and phenotypically highly diversified group.

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KAPITOLA 2

Phylogenetic relationships of the gecko genus *Carinatogekko* (Reptilia: Gekkonidae)

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Phylogenetic relationships of the gecko genus *Carinatogekko* (Reptilia: Gekkonidae)

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Abstract

The gecko genus *Carinatogekko* comprises two species endemic to the foothills of the Zagros Mountains, in Iran and Iraq. Both species are poorly known and to date they are known only from very few records. The phylogenetic affiliation of the genus has been only hypothesized. The aim of this study was to clarify the phylogenetic relationships of the genus by analyzing partial sequences of the mitochondrial 12S rRNA and cytochrome *b* genes. All phylogenetic analyses performed support placing the genus *Carinatogekko* within the genus *Mediodactylus*. Taxonomic relevance of both species of the genus *Carinatogekko* based on morphological characters is discussed along with nomenclatural implications of our findings.

Key words: *Cyrtopodion*, lizards, *Mediodactylus*, mtDNA, phylogeny

Introduction

The geckos of the genus *Carinatogekko* Golubev et Szczerbak, 1981, endemics of the foothills of the Zagros Mountains of Iran and Iraq, remain enigmatic and poorly known lizards despite being described nearly 40 years ago. The history of their discovery and classification is rather complicated and quite typical for geckos of the Middle East. The genus *Carinatogekko* currently includes two species. The *Carinatogekko* geckos have long been known only from the type localities with additional localities discovered only recently (e.g., Nazari-Serenjeh & Torki 2008). The earliest known specimen of *Carinatogekko heteropholis* (Minton, Anderson et Anderson, 1970), an immature female from scrub oak firewood forest of northeastern Iraq, was first erroneously determined by Reed and Marx (1959) as *Alsophylax persicus* Nikolsky, 1903. Minton *et al.* (1970) described the same individual as the holotype of the new species *Tropicolotes heteropholis*. The second representative of the genus, *Carinatogekko aspratilis* (Anderson, 1973), was originally placed in *Bunopus* as *B. aspratilis*. The description was based on two tentatively immature female specimens from the southwestern Zagros foothills of Iran. Golubev and Szczerbak (1981) erected the genus *Carinatogekko* with the two above mentioned species. The diagnosis of the genus provided by Anderson (1999) is: all scales with the exception of intermaxillaries, nasals, chin shield and upper and lower labials strongly keeled; three nasal scales contact nostrils; digits weakly angularly bent, clawed, not dilated, not webbed nor ornamented, with keeled transverse subdigital lamellae; dorsal pholidosis heterogeneous, small juxtaposed scales intermixed with tubercles; pupil vertical; tail segmented, caudal tubercles with bases in middle of each segment, not in contact with one another, separated from posterior margin of segment by ring of scales. A diagnosis was also provided by Szczerbak and Golubev (1986).

Surprisingly, Golubev and Szczerbak (1981), the authors of the genus *Carinatogekko*, themselves hypothesized that a possible phylogenetic position of the genus *Carinatogekko* was inside their subgenus

Mediodactylus of the genus *Cyrtopodion* (as understood in Szczerbak & Golubev 1986). They hypothesised that the genus *Carinatogecko* could be closely related to *Cyrtopodion heterocercum* (Blanford, 1874). Szczerbak (1988) elevated the subgenus *Mediodactylus* to generic level, which was later confirmed by the phylogenetic analysis of allozyme data (Macey *et al.* 2000) and mitochondrial markers (Červenka *et al.* 2008). The genus *Mediodactylus* is characterized by the apparent synapomorphy used by Anderson (1999) to describe the *kotschy* group of the genus *Cyrtopodion*: caudal tubercles do not form a terminal row (there are six of them on each annulus), but they are spread around the middle of each caudal segment. The hypothesis regarding the phylogenetic position of *Carinatogecko* inside *Mediodactylus* was based on morphological characters without any cladistic analysis. This suggested phylogenetic position would mean that the genus *Mediodactylus* is paraphyletic with respect to *Carinatogecko*, as mentioned by Anderson (1999). Due to the absence of further studies, the nomenclature of Golubev and Szczerbak (1981) has been adopted in all subsequent publications involving Middle Eastern geckos (e.g., Szczerbak & Golubev 1986; Leviton *et al.* 1992; Anderson 1999; Uetz *et al.* 2010).

Recently, while investigating phylogenetic relationships of *Cyrtopodion* geckos based on molecular markers (Červenka *et al.* 2008), we discovered several specimens in our university collections that possessed the keeled pholidosis characteristic of the genus *Carinatogecko*. Using the key to the genus *Carinatogecko*, we were unable to determine the species. The specimens display characteristics of both species in the genus, but based on pholidosis of the forelimbs, they appear closer to *Carinatogecko heteropholis*. In order to clarify the phylogenetic position of the genus *Carinatogecko*, we expanded the DNA sequence data compiled in our previous study and conducted a phylogenetic analysis to resolve the position of this enigmatic genus.

Material and methods

The studied material included five specimens of *Carinatogecko* cf. *heteropholis* (one male, one female, three juveniles) preserved in alcohol and stored in the collections of the Faculty of Science, Charles University in Prague. The locality of the animals was designated as the road near Khorramabad, Lorestān Province, Iran, with coordinates 33°25'N, 48°12'E. Voucher specimens numbers are R\IRA\1123-25 and R\IRA\1139-40. R\IRA\1140 was used for molecular analyses. Partial sequences of the 12S rRNA and cytochrome *b* genes were obtained using the same protocol as described in Červenka *et al.* (2008) and deposited in GenBank, NCBI. Further phylogenetic analyses were performed using sequences previously published in Červenka *et al.* (2008), GenBank Accession Nos. EU589153–EU589175 (12S rRNA), EU589176–EU589198 (cytochrome *b*). Sequences of cytochrome *b* (307 bp) were aligned manually and translation to protein (using MEGA version 4, Tamura *et al.* 2007) did not reveal any stop codons or insertion or deletions. All sequences of 12S rRNA gene were aligned using ClustalX 2.0.12 (Larkin *et al.* 2007) with default parameters and also using progressive multiple alignment as implemented in the Probabilistic Alignment Kit, PRANK (Löytynoja & Goldman 2005). The sequences of both genes were combined for subsequent phylogenetic analyses in two alignments (cytochrome *b* + 12S rRNA-ClustalX and cytochrome *b* + 12S rRNA-PRANK). The probabilistic alignment can perform better in the case of sequences with more indels. Phylogenetic relationships were inferred by Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The best fit model of nucleotide substitution for BI was chosen to be GTR + I + Γ for both the alignments of 12S rRNA gene sequences as per the Akaike information criterion (AIC) in Modeltest 3.7 (Posada & Crandall 1998). The TVM + I + Γ model, which is a specific case of GTR + I + Γ , was selected for cytochrome *b*, therefore, the latter was used in the BI analysis. The Bayesian analysis was initiated using a separate model for each partition (12S rRNA and cytochrome *b* fragments) and used one heated and three cold Markov chains. The analysis was conducted for 4×10^6 generations, with every 100th tree being sampled. Stationarity of log-likelihood scores was determined graphically and a majority consensus tree was generated from the remaining trees after discarding the burn-in trees. The frequency of individual clades in the consensus tree was represented by posterior probabilities (Huelsenbeck & Ronquist 2001).

Both combined alignments were also analyzed using maximum parsimony (MP) method as implemented in PAUP* 4.0b10 (Swofford 2002). The gaps were coded as a fifth base. A heuristic search was conducted

using 100 random sequence addition replicates and tree bisection-reconnection branch swapping. Clade support was assessed by nonparametric bootstrap analysis (Felsenstein 1985) with 1000 pseudoreplicates.

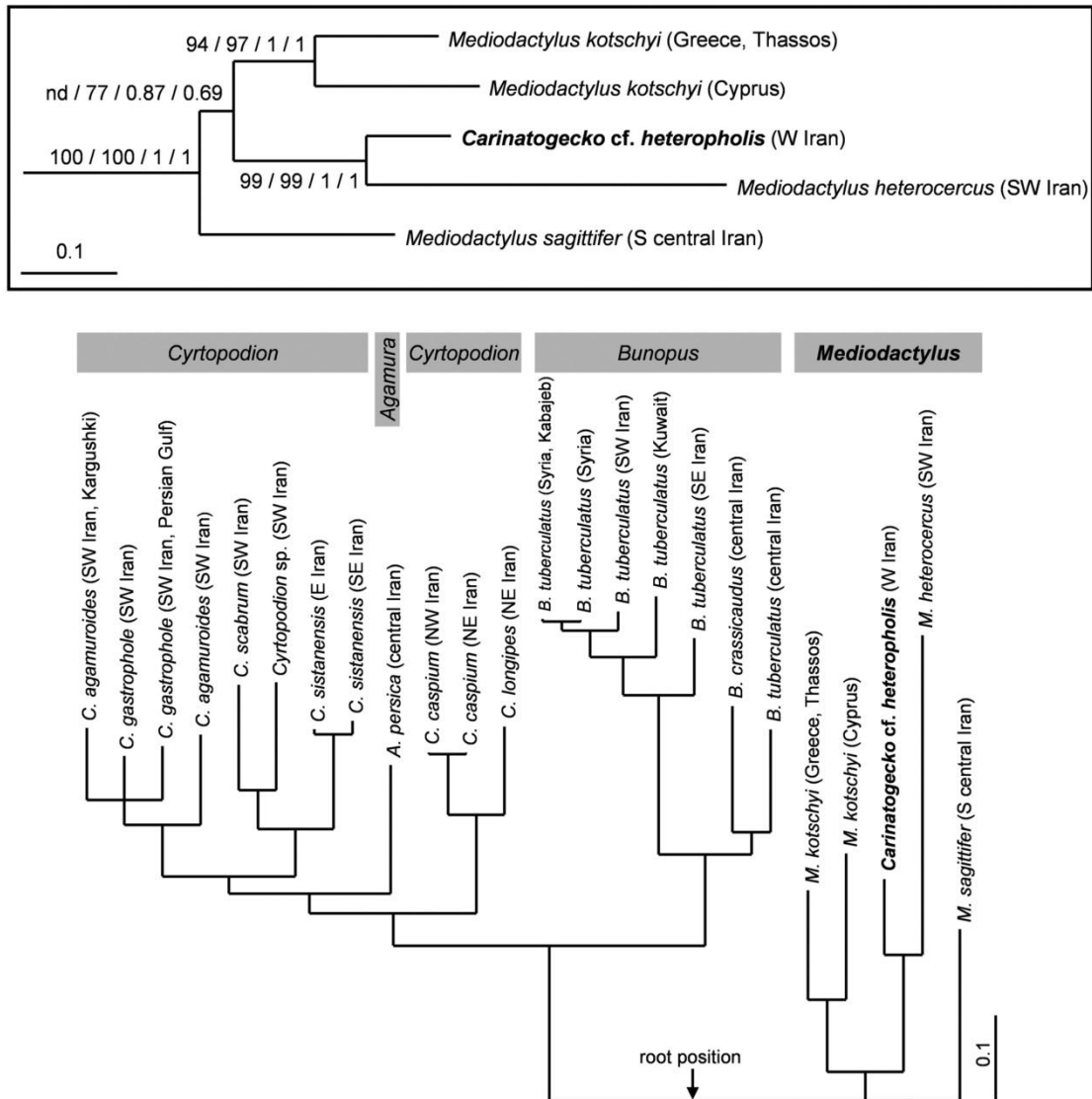


FIGURE 1. Phylogenetic tree based on partial sequences of the cytochrome *b* and 12S rRNA (PRANK alignment) genes constructed by Bayesian inference (topology only, see Červenka *et al.* 2008 for further information). In the box, detailed view of *Mediodactylus* clade, scores at nodes represent: MP bootstrap values – ClustalX alignment / MP bootstrap values – PRANK alignment / BI posterior probability – ClustalX alignment / BI posterior probability – PRANK alignment; nd indicates branch which is not present in respective tree.

Results

The partial sequences of *Carinatogecko* cf. *heteropholis* totaled 387 bp for the 12S rRNA gene (submitted to GenBank, GQ354886) and 307 bp for the cytochrome *b* gene (GenBank, HM140839). All phylogenetic analyses inferred *Carinatogecko* cf. *heteropholis* having a congruent position as a sister taxon to

Mediodactylus heterocercus nested within the *Mediodactylus* clade (see Fig. 1). Genetic distances for 12S rRNA gene (*p* distances, shown in Table 1) among species of this clade indicate even closer relationships between *Carinatogecko* cf. *heteropholis* and *Mediodactylus heterocercus* than between two representatives of *Mediodactylus kotschyi* (Steindachner, 1870).

TABLE 1. Uncorrected genetic distances (*p* distances) of 12S rRNA gene alignments – ClustalX (below diagonal), PRANK (above diagonal).

	<i>C. cf. heteropholis</i>	<i>M. heterocercus</i>	<i>M. kotschyi</i> (Greece, Thassos)	<i>M. kotschyi</i> (Cyprus)	<i>M. sagittifer</i>
<i>C. cf. heteropholis</i>	—	0.1212	0.1826	0.1944	0.1675
<i>M. heterocercus</i>	0.1212	—	0.2129	0.1826	0.1876
<i>M. kotschyi</i> (Greece, Thassos)	0.1852	0.2129	—	0.1452	0.1716
<i>M. kotschyi</i> (Cyprus)	0.1996	0.1826	0.1452	—	0.1808
<i>M. sagittifer</i>	0.1753	0.1949	0.1810	0.1861	—

Discussion

The phylogenetic analyses of mitochondrial genes (12S rRNA and cytochrome *b*) clearly placed the genus *Carinatogecko* inside the genus *Mediodactylus*. This finding corresponds with the phylogenetic hypothesis of Golubev and Szczerbak (1981) in the original description of the genus *Carinatogecko*. This relationship is also supported by the morphology of these geckos. The primary synapomorphy of the genus *Mediodactylus* adopted from Anderson (1999), i.e., caudal tubercles not forming a terminal row (six of them on each annulus) but spread around the middle of each caudal segment, unambiguously characterizes the genus *Carinatogecko* too. The typical cryptic color pattern on dorsum and tail of the *Mediodactylus* species, resembling several undulating, M- or V-shaped dark transverse bars, accompanied by light or white venter, is also shared by *Carinatogecko* species (see Fig. 2). Several species of the genus *Mediodactylus* possess keeled scales on some body parts: e.g., *Mediodactylus sagittifer* (Nikolsky, 1900) or *M. heterocercus* (Szczerbak & Golubev 1986; Anderson 1999; pers. obs.). The strongly keeled pholidosis of *Carinatogecko*, which was formerly considered a synapomorphy of this genus thus seems to represent completion of a trend shared with its close relatives. The distribution of the geckos of the genus *Carinatogecko*—endemics of the Zagros foothills—further confirms the phylogenetic relationships suggested herein as all their known localities fall within geographic range of the genus *Mediodactylus* (e.g., Szczerbak & Golubev 1986; Anderson 1999; Červenka & Kratochvíl 2010).

We had problems with species determination for the *Carinatogecko* specimens examined because they display a mixture of characteristics of both described species of the genus. Four key morphological characters discriminate between the species *Carinatogecko heteropholis* and *Carinatogecko aspratilis* (Golubev & Szczerbak 1981; Szczerbak & Golubev 1986; Anderson 1999). The first character is the size of the scales in the middle of the back, which should be smaller than the abdominals in *C. aspratilis* and more or less equal to the abdominals in *C. heteropholis*. Unfortunately, there seems to be a typographic error in Anderson (1999), where the stated relative size of the scales is reversed relative to the publications of Szczerbak and Golubev (Golubev & Szczerbak 1981; Szczerbak & Golubev 1986). Abdominals of the specimens we examined are more or less equal in size to the scales on the back. Due to the heterogeneous nature of dorsal pholidosis (cf. Fig. 2), however, it is difficult to decide which scales around tubercles should be compared. Also, it should be noted that small dorsal scales of juvenile individuals are quite homogeneous and relatively smaller in comparison with those of adults. The second key character is the shape of caudal tubercles, which should be pointed with enlarged posterior facet in *C. aspratilis*, but flattened in *C. heteropholis*. In our material, caudal tubercles are rather sharp with an enlarged posterior facet. Nevertheless, the holotype of *C. heteropholis* is not optimally preserved (cf. the photo in Minton *et al.* 1970), and, in such conditions, the nature of scales could be

easily misinterpreted. The third key character is the presence (in *C. aspratilis*) or absence (in *C. heteropholis*) of identical tubercles on forelimbs as on dorsum. We consider that our individuals lack those tubercles on the forelimbs. We observed only a single large scale, but not an actual tubercle on a leg in one out of five specimens. The last key character (the number of subdigital lamellae on the fourth toe), does not contribute to unambiguous species identification. *C. aspratilis* is reported to have 17–18 lamellae and *C. heteropholis* 15 lamellae, but our specimens have 16–17. In the original description of *C. aspratilis*, 17–19 lamellae are mentioned (Anderson 1973). We can conclude, moreover, that separation of the two species of the genus *Carinatogekko* was based on subtle differences in pholidosis found during inspection of only a few available specimens (Anderson 1999). Therefore, we question the validity of the two species within the genus. *C. aspratilis* could be a junior synonym of *C. heteropholis*, but due to the low number of known specimens and hence poorly understood morphological variability, the final resolution requires further study.

In summary, phylogenetic analyses confirm the suggestion of Golubev and Szczerbak (1981) and place *Carinatogekko* cf. *heteropholis* inside the genus *Mediodactylus*. Regardless of the number of species in the genus *Carinatogekko*, the generic name *Mediodactylus* should be used for these geckos to avoid rendering the latter genus paraphyletic.

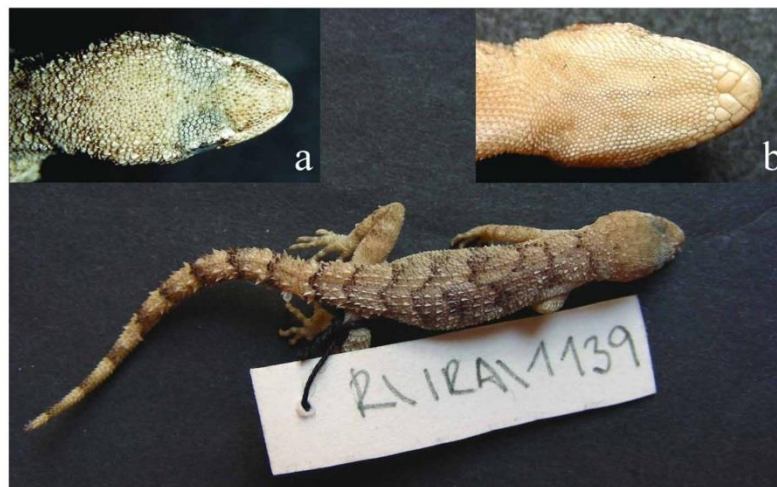


FIGURE 2. *Carinatogekko* cf. *heteropholis* (REPT/IRA/1139); head in detail: a—dorsal view, b—ventral view.

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KAPITOLA 3

Generic reassignment and validity of the recently described species *Cyrtopodion dehakroense*

Jan Červenka a Lukáš Kratochvíl

Herpetology Notes (2010) 3: 135-137

Generic reassignment and validity of the recently described species *Cyrtopodion dehakroense*

Jan Červenka and Lukáš Kratochvíl*

Abstract. The new arboreal gecko species from Pakistan, *Cyrtopodion dehakroense* Masroor, 2009, was recently described. Here we document that based on external morphology (meristic and measurable morphological characters), this form is a member of the genus *Mediodactylus*. Moreover, the newly described species is morphologically and ecologically very similar to *Mediodactylus sagittifer*, which suggests that both forms could be conspecific.

Keywords. Gecko, Middle East, Pakistan, taxonomy.

The taxonomy of the genus *Cyrtopodion sensu lato* Fitzinger, 1843 has been substantially revised in recent years (for a brief history of understanding of the species content of the genus see e.g., Krysko et al. 2007; Červenka et al. 2008). For example, Khan (2003) attempted to resolve the complex situation in circum-Indus species of the genus *Cyrtopodion* using morphological characters and separated some species into three newly established genera (*Altigekko*, *Indogekko*, *Siwaligekko*). Possible phylogenetic relationships of these genera were suggested by Khan (2009). The analyses of allozymic data (Macey et al. 2000) and of the sequences of mitochondrial markers (Červenka et al. 2008) supported monophyly and the full generic status of *Mediodactylus* Szczerbak et Golubev, 1977, which was previously treated as a subgenus of *Cyrtopodion* (e.g., Szczerbak and Golubev 1986). Inclusion of *Mediodactylus* into the genus *Cyrtopodion* would make the genus *Cyrtopodion* paraphyletic. Several new species of the genus *Cyrtopodion sensu lato* have been recently discovered (e.g., Krysko et al. 2007; Nazarov and Rajabizadeh 2007; Masroor 2008).

Masroor (2009) described the new arboreal species *Cyrtopodion dehakroense* from southern Pakistan based on several morphological characters. The description contains comparison of this form with species of the genus *Cyrtopodion sensu lato* known in Pakistan.

Nevertheless, morphology of the new species perfectly fits in Anderson's (1999) definition of the genus *Mediodactylus* (assigned by him as "the *kotschyi* group" of the genus *Cyrtopodion*): "caudal tubercles, six to each annulus, do not form terminal row, but are distributed around middle of each caudal segment; no subfemoral tubercles; dorsal tubercles strongly keeled, trihedral, larger than interspaces; peritoneum unpigmented; 13–23 lamellae under fourth toe; preanal pores only; adults usually less than 55 mm snout-vent length." The number and position of tubercles on each caudal segment could be taken as the main synapomorphy of the genus *Mediodactylus*. According to the photos in Masroor (2009), *C. dehakroense* also possesses this character.

The author differentiates the new species from geckos of the genus *Mediodactylus* by few characters: "dorsal tubercles (strongly keeled versus smooth), transverse rows of enlarged subcaudals (2 versus 1), precloacal pore counts (4 versus 4–6) and TL/SVL ratio (0.73–0.75 versus 0.69–0.80)" (Masroor 2009). However, keeled dorsal tubercles are typical for the genus *Mediodactylus* (e.g., Anderson 1999; Szczerbak and Golubev 1986). The nature of transverse rows of enlarged subcaudals is variable in the genus *Mediodactylus*. For example, *Mediodactylus kotschyi* (Steindachner, 1870) has only one row, *Mediodactylus sagittifer* (Nikolsky, 1900) has two rows, and there is no clear continual row of subcaudals in *Mediodactylus heterocercus* (Blanford, 1874). Neither of the other two mentioned characters have specific discriminative value, because the states given for *C. dehakroense* form subsets of those reported for the genus *Mediodactylus*. We can specify that

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Figure 1. *Mediodactylus sagittifer* from Iranian Baluchestan. Photo by Milan Kaftan.

Table 1. The comparison of the measurable and meristic characters of *Cyrtopodion dehakroense* and *Mediodactylus sagittifer*. All measurements are given in mm.

Source	<i>C. dehakroense</i>		<i>M. sagittifer</i>
	Masroor 2009	Szczerbak & Golubev 1986	own data
Number of examined males	7	3	2
Number of examined females	1	2	2
Head width	5.9–6.6	-	5.7–6.8
Snout-eye distance	3.8–4.3	-	2.7–3.0
Orbital diameter	2.0–2.2	-	2.6–3.2
Trunk length	13.9–15.1	-	12.7–14.1
Snout-vent length	32.6–36.0	24.1–31.9	30.2–32.8
Supralabials	10–13	9–10	10–11
Infralabials	7–9	7–8	7–8
Postmentals	3	-	3
Interorbital scales	16–18	14–15	15–19
Longitudinal rows of tubercles	10–12	-	11–12
Scales around dorsal tubercles	10–12	12–13	11–14
Paravertebral tubercle rows	22–24	-	23–25
Midventrals	92–102	83–88	89–100
Scales across belly	21–25	16–19	18–20
Precloacal pores	4	4	4
Lamellae under digit IV Manus	12–16	-	15–18
Lamellae under digit IV Pes	18–23	18–19	17–20

the counts of preloacal pores vary within the genus *Mediodactylus* from two to six (e.g., Szczerbak and Golubev 1986; Anderson 1999).

The new form fits well into the genus *Mediodactylus* also ecologically. Arboreality is not typical for members of the genus *Cyrtopodion*; however, it is not unusual for some members of the genus *Mediodactylus*. For example, Werner (1993) documented arboreality in Israeli populations of *Mediodactylus kotschy orientalis* (Štěpánek, 1937) and *M. sagittifer* is a typical arboreal species (Anderson 1999; pers. obs.; Figure 1). The species *M. sagittifer*, known only from Iranian Baluchestan, inhabits an environment very similar to that described for *C. dehakroense*. Although the type localities of these two forms are about 800 km apart, the morphological similarity between them is striking. Anderson (1999) compiled the detailed diagnosis of *M. sagittifer*: “Dorsal tubercles oval, keeled; diameter of ear opening smaller than half longitudinal diameter of eye; anterior pair of enlarged postmentals in contact; no subfemoral tubercles; males with 2–4 preanal pores; subcaudal scales one head-width behind vent small, keeled, not forming large plates; 14–16 abdominal scales across middle of belly (9–12 scales in a distance across belly equal to length of snout).” This diagnosis seems to be consistent with the description of *C. dehakroense*. We find broad overlap in values of the majority of meristic and measurable morphological characters reported for *C. dehakroense* and *M. sagittifer* in published literature and in our own data (summarized in the Table 1). Out of the only three characters which do not overlap, two (snout-eye distance and orbital diameter) are plastic characters varying with age. Moreover, character variability is further enhanced by very small size and thus small measurement repeatability and accuracy, especially when taken by different persons. The third character, number of scales across belly, depends on personal choice from where to start counting ventrals, distinguishing them from laterals, and the differences observed between *C. dehakroense* and *M. sagittifer* are minimal.

Based on morphological and ecological similarities, we thus cannot exclude the possibility that *C. dehakroense*

is conspecific with *M. sagittifer*. The data accumulated to date do not support the validity of the newly described species. Nevertheless, more detailed studies preferably with molecular markers will be needed to make a final decision.

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KAPITOLA 4

Revisiting the taxonomy and biogeography of *Bunopus spatulurus* (Reptilia; Gekkonidae) from the Arabian Peninsula

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Rukopis v přípravě

Revisiting the taxonomy and biogeography of *Bunopus spatulurus* (Reptilia; Gekkonidae) from the Arabian Peninsula

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ABSTRACT

The southern Arabian Peninsula provides an interesting area to study historical biogeography due to high reptile species richness and endemism, and interesting geological history of this region; however, studies on biodiversity patterns of this area are still rare. A notable pattern, yet not fully understood, is the divergence in reptile fauna between north (Hajar Mountains) and south (Dhofar Mountains) Oman. In this study, we use a molecular phylogenetic analysis using partial sequences of the *12S* mtDNA, a haplotype network of the nuclear *cmos* gene, and species distribution modelling (SDM) for the present and past (Last Glacial Maximum) climatic conditions, to uncover the taxonomy and biogeography of the gecko *Bunopus spatulurus*. This species contains two subspecies (*B. spatulurus spatulurus* and *B. s. hajarensis*) distributed in southern Arabia. The results of the phylogenetic and networks analyses show that the two subspecies form two well-supported reciprocally monophyletic groups that do not share any alleles in the *cmos* nuclear gene. The level of genetic divergence in the *12S* between *B. s. spatulurus* and *B. s. hajarensis* is very high ($13 \pm 2\%$). The level of genetic variability within *B. s. hajarensis* is also very high and contains three clades with geographical structure across the Hajar Mountains. Due to morphological differences, high level of genetic differentiation in the *12S* mitochondrial gene, and the results of the phylogenetic and *cmos* haplotype network analysis, we elevate *Bunopus spatulurus hajarensis* to the species level, *Bunopus hajarensis* **stat. nov.** Arnold, 1980. The niche overlap analysis reveals significant differences between niches of the species, but at the same time it shows their higher than random similarity. The SDM indicates a large potential distribution for *B. hajarensis* extending well beyond the current distribution range and shows that the range of *B. spatulurus* has climatic conditions suitable for *B. hajarensis* as well. However, at the same time, the SDMs indicate a gap with unsuitable climatic conditions between ranges of both species and suggest that this gap was already present during the Last Glacial Maximum. This longer-lasting gap between areas with suitable climatic conditions could be the cause of separation ranges of *B. spatulurus* and *B. hajarensis*.

INTRODUCTION

Deserts encompass a large portion (12%) of the Earth's land surface and are important for understanding global biodiversity patterns. While deserts are thought to have relative low species richness compared to other biomes, they are often inhabited by many specialized species and clades possessing a wide array of unique adaptations to arid conditions. For example, lizard communities in deserts might be richer compared to warm temperate and tropical regions and they can contain as many as 70 different species co-occurring at single localities (Pianka 1973; Rabosky *et al.* 2011). In the past, extensive work on desert reptiles has mainly focused on Australian, South African and North American deserts (e.g. Pianka 1986), while fewer studies exist for the Arabian deserts (e.g. Anderson 1896; Haas 1957; Arnold 1972; Arnold 1980; Arnold 1986; Gasperetti 1988; Schätti & Gasperetti 1994; Schätti & Desvoignes 1999; Carranza & Arnold 2012; Gardner 2013). Recently, however, an increasing number of taxonomic, biogeographic and phylogenetic studies have focused on lizards inhabiting these deserts (e.g. Carranza & Arnold 2012; Metallinou *et al.* 2012; Šmíd *et al.* 2013a). In the last decade, taxonomic studies have drastically increased the number of species inhabiting the Arabian desert, while ongoing phylogenetic studies continue to identify new species, especially in the southern Arabian Peninsula (Babocsay 2004; Busais & Joger 2011; Carranza & Arnold 2012; Šmíd *et al.* 2013b; Metallinou & Carranza 2013; Vasconcelos & Carranza in press) as predicted by Ficetola *et al.* (2013). Likewise, several studies have revealed high levels of intraspecific genetic structure of Arabian lizards, mostly in species inhabiting mountainous areas such as the Hajar Mountains in northern Oman and the UAE and the mountains in southern Oman and Yemen (e.g. Carranza & Arnold 2012; Šmíd *et al.* 2013a), but also in species occurring in lowlands (Metallinou *et al.* 2012). As reported previously (Carranza & Arnold 2012), within Oman, two biodiversity rich areas with high levels of endemism are recognized: the Hajar Mountains in northern Oman and the Dhofar Mountains in southern Oman and eastern Yemen. These two mountainous regions have their own unique and complex geological histories (reviewed in Carranza & Arnold 2012 and Gardner 2013; see also Arnold 1977, 1980 and other articles in the same volumes) but share distinct climatic conditions and vegetation that differentiate them from the much more arid surrounding lowland desert. Although the Hajar and the Dhofar Mountains are inhabited by somewhat distinct reptile communities with unique endemic species (e.g. *Hemidactylus*, *Asaccus*

geckos) and even genera (*Omanosaura*) (see Arnold 1986; Arnold & Gardner 1994; Carranza & Arnold 2012), several species occur in both regions while being absent from the lowland areas between them (*Bunopus spatalurus*, *Chalcides ocellatus* and *Trachylepis tessellata*; see Gardner 2013). The existence of this North-South pattern with a gap in between was previously highlighted by Arnold (1980), who stated that "The present Survey also underlines the importance of the poorly known dry lowland country that lies between Dhofar and the mountains of northern Oman. Investigation of this may throw light on how the faunal differentiation between the two regions it separates has evolved and been maintained. It might also clarify some specific systematic problems such as the relationship of the two subspecies of *Bunopus spatalurus* and the status of the two kinds of *Echis carinatus* found in Arabia". Years later, Babocsay (2004) showed that this gap was probably responsible for the speciation event separated *Echis omanensis* (restricted to the Hajar Mountains) and *Echis coloratus* (from Dhofar in south Oman across the south and southwestern Arabian Peninsula and up to northern Egypt (see Arnold *et al.* 2009).

At the time when Arnold wrote this note (1980), it was difficult to analyze such observed biogeographic patterns and uncover their causes. Nowadays, the rise of new powerful statistical techniques and Geographic Information Systems (GIS) together with the availability of high resolution environmental data, provide ideal tools to revisit this still rather neglected but interesting biodiversity pattern in southeast Arabia. As an example, the development and application of correlative species distribution models (SDM; Guisan & Zimmermann 2000; Guisan & Thuiller 2005) such as Maxent (Phillips *et al.* 2006) has received much attention in conservation biology (e.g. Peterson *et al.* 2011) and evolutionary biology (Kozak *et al.* 2008; Chan *et al.* 2011) and it is increasingly being used to solve taxonomic questions (Koch *et al.* 2013; Ahmadzadeh *et al.* 2013). Among the species exhibiting this notable North-South distribution pattern, *Bunopus spatalurus* provides an interesting example, as previously mentioned by Arnold (1980). This species is widely distributed in the southern Arabian Peninsula, ranging from western Yemen to the mountains of northern Oman and the UAE, but with some areas of absence (Sindaco & Jeremčenko 2008; Gardner 2013). Arnold (1980) described the subspecies *Bunopus spatalurus hajarensis* from the Hajar Mountain range and Masirah Island based on clear morphological differences. However, despite some recent phylogenetic studies on Palearctic naked-toed geckos (Červenka *et al.* 2008; Bauer *et al.* 2013), no molecular studies have yet addressed the status of *Bunopus spatalurus*.

This is possibly the result of the difficulty in finding specimens compared with the relatively abundant *B. s. hajarensis* (Haas & Battersby 1959; Arnold 1980; Gardner 2013) as well as the current political instability in Yemen that obstructs fieldwork. Clarifying the phylogenetic position of *B. s. spatalurus* and *B. s. hajarensis* should provide important insights into Arabian reptile biogeography in general, while in the future, it would also contribute to clarifying the taxonomy of the potentially non-monophyletic genus *Bunopus* (Pyron *et al.* 2013).

In this study, we used a combination of species distribution modelling and a molecular phylogeny of *Bunopus spatalurus* to explore its taxonomy and biogeography. Specifically, we aimed to explore the role of past climatic conditions and the potential existence of biogeographic barriers in central Oman on contemporary genetic structuring.

MATERIALS AND METHODS

Molecular samples, DNA extraction and amplification

A total of 32 specimens of *Bunopus spatalurus* including four *B. s. spatalurus* and 28 *B. s. hajarensis* from 30 localities across its entire distribution range in Arabia were included in the phylogenetic analyses (Fig. 1). A list of all the specimens with their taxonomic identification, sample code, voucher code, country, and corresponding geographical distribution data and GenBank accession numbers for the two sequenced genes is presented in Supplementary Table S1. Preliminary analyses (data not shown) indicated that the two subspecies form a highly supported monophyletic group within the Palearctic naked-toed geckos but as a result of the uncertainty of its sister taxa relationship and especially of the high level of genetic divergence between all the species of naked-toed geckos, which interfered with the intraspecific analysis, we decided not to include any outgroups in our analyses and use Bayesian methods for inferring the root of the phylogenetic tree (Huelsenbeck *et al.* 2002; see below). The apparent polyphyletic status of the genus *Bunopus* (Pyron *et al.* 2013) and the phylogenetic position of *B. spatalurus* within the Palearctic naked-toed geckos will be assessed elsewhere.

Genomic DNA was extracted from ethanol-preserved tissue samples using the standard high salt method (Sambrook *et al.* 1989). Two genetic markers were PCR-amplified and sequenced: one mitochondrial fragment of the gene encoding the

ribosomal 12S rRNA (*12S*; primers 12SaGekko – 5' CAAACTAGGATTAGATACCCTACTATGC 3' and 12SbGekko – 5' GAGGGTGACGGGCGGTGTGTAC 3') and one nuclear fragment of the gene encoding the oocyte maturation factor Mos (*cmos*; primers FUF and FUR – Gamble *et al.* 2008). PCR conditions used for the amplification of the *12S* mitochondrial fragment and the nuclear gene *cmos* were the same as in Šmíd *et al.* (2013a).

Sequence analysis

Geneious v. R6.1.6 (Biomatters Ltd.) was used for assembling and editing the chromatographs. Heterozygous positions for the nuclear coding gene fragment were identified based on the presence of two peaks of approximately equal height at a single nucleotide site in both strands. The nuclear coding fragment was translated into amino acids and no stop codons were observed. DNA sequences were aligned using MAFFT v.6 (Kato & Toh 2008) with the options maxiterate 1000 and localpair. Phased sequences of the *cmos* gene were used for the network analysis. SEQPHASE (Flot 2010) was used to convert the input files, and the software PHASE v. 2.1.1 to resolve phased haplotypes (Stephens *et al.* 2001). Default settings of PHASE were used except for phase probabilities that were set as ≥ 0.7 (see Harrigan *et al.* 2008). Uncorrected mean genetic distances between and within groups for the mitochondrial gene fragment were calculated using MEGA 5 (Tamura *et al.* 2011), using the *p*-distance model.

Phylogenetic and network analyses

Best-fitting models were inferred for *12S* using jModeltest v.0.1.1 (Posada 2008) under the Akaike information criterion (AIC) (Akaike 1973). Phylogenetic analyses of the *12S* mitochondrial gene were performed using Bayesian (BI) analysis in BEAST v.1.6.1 (Drummond & Rambaut 2007). Analyses were run three times for 5×10^7 generations with a sampling frequency of 10 000. Models and prior specifications applied were as follows (otherwise by default): model of sequence evolution for the *12S* HKY+G+I (see above); coalescent constant size process of speciation; random starting tree; alpha Uniform (0, 10); fix mean rate of molecular clock model to 1. Convergence was assessed by examining the posterior trace plots and by confirming sufficient effective sample sizes for all parameters in Tracer v1.5 (Rambaut & Drummond 2007). The results of the individual runs were combined in LogCombiner discarding 10% of the

samples and the ultrametric tree was produced with TreeAnnotator (both provided with the BEAST package). Nodes in the phylogenies were considered strongly supported if they received a posterior probability (pp) support values ≥ 0.95 (Huelsenbeck & Rannala 2004). Genealogical relationships in the *cmos* nuclear gene were assessed with a haplotype network inferred using statistical parsimony as implemented in the program TCS v.1.21 (Clement *et al.* 2000). Phased sequences were used (see above) and a connection limit of 95% was applied.

Species distribution modelling

A total of 82 distribution records of *Bunopus spatalurus* (*B. s. spatalurus*, n = 16; *B. s. hajarensis*, n = 66) were assembled from literature and fieldwork (Supplementary Figure S1 and Table S2). Bioclimatic variables were downloaded from the WorldClim database version 1.4 (Hijmans *et al.* 2005) at a resolution of 2.5 arc minutes (nearly 5x5 km). Past climate data for the Last Glacial Maximum (LGM) were obtained from the WorldClim database as well at a similar resolution. Two general atmospheric circulation models (GCM) were used to generate the LGM scenarios: the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC). Collinearity of the initial variables was measured by Pearson's correlation coefficient in ENMtools 1.3 (Warren *et al.* 2010). A total of ten bioclimatic variables, all of which had a correlation degree lower than 0.75 (Pearson's coefficient), were retained. Selection of predictor variables was based on ecological understanding of the species and represented mean temperature values as well as temperature variation and precipitation regimes considered relevant for Squamata (Clusella-Trullas *et al.* 2011).

In a preliminary session, we generated species distribution models (SDM) for each taxon using the presence/background algorithm Maxent, version 3.3.3k (Phillips *et al.* 2006). It has been shown that Maxent produces high quality predictions that are more often corroborated than those of other predictive models (e.g. Hernandez *et al.* 2006; Giovanelli *et al.* 2010). We subsequently used null-models to test for significance of the SDM (Raes & ter Steege 2007) by generating 100 null distributions of random points in the study area using ENMtools (Warren *et al.* 2010) with the number of random points equal to the actual number of distribution records used for SDM. The null-models were created and assessed following Raes & ter Steege (2007). Based on these results, only the SDMs of *B. s. hajarensis* performed statistically significantly

better than random and *B. s. spatalurus* was therefore excluded from further analysis. To tune the SDMs for *B. s. hajarensis*, the jackknife procedure was implemented in Maxent with the remaining ten bioclimatic variables to find the best set of predictor variables following a parsimony approach (de Pous *et al.* 2011) based on the average (AUC) test of ten replicates. The final set of variables used for the *B. s. hajarensis* SDMs consisted of seven bioclimatic variables: Annual Mean Temperature (BIO1), Max Temperature of Warmest Month (BIO5), Temperature Annual Range (BIO7), Mean Temperature of Wettest Quarter (BIO8), Annual Precipitation (BIO12), Precipitation of Warmest Quarter (BIO18) and Precipitation of Coldest Quarter (BIO19). For the final SDMs we varied the level of regularization in Maxent by using ten different fixed values of the β parameter (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10) in a criterion-based model selection framework (Warren & Seifert 2011) as implemented in ENMtools 1.3 (Warren *et al.* 2010). We compared competing models using the AICc as suggested by Warren & Seifert (2011) and found $\beta = 3.0$ as the appropriate level of regularization. Maxent was subsequently used with the following settings (convergence threshold = 0.00001, maximum number of iterations = 500 and $\beta_j = 3.0$), while partitioning the geographical records between training and test samples.

Several studies have recently addressed the importance of selecting pseudo-absence or background locations in SDM (Phillips *et al.* 2009; VanDerWal *et al.* 2009; Anderson & Raza 2010; Giovanelli *et al.* 2010). Moreover, some of these studies reported that using very large areas for model calibration, especially if the species is absent from these areas, can result in serious ramifications for predictions and performance of SDMs (VanDerWal *et al.* 2009; Anderson & Raza 2010; Giovanelli *et al.* 2010; Elith *et al.* 2011; Barve *et al.* 2011; Saupe *et al.* 2012). Therefore, we followed the suggestion of VanDerWal *et al.* (2009) and used an exploratory analysis to define the most appropriate calibration region. Final models were calibrated in a background region that encompassed all known localities and included areas that have been accessible to the species via dispersal over relevant time periods (Supplementary Figure S2). Subsequently, models were projected onto a larger area. The average of ten pseudo-replicated models with randomly selected test samples was used to produce SDMs, which were plotted in logistic format. The final models were reclassified in ArcGIS 10 (ESRI) into binary presence-absence maps using the maximum training sensitivity plus specificity threshold (MTSPS), which maximizes the sum of sensitivity (proportion of actual positives that are correctly identified) and specificity (proportion

of negatives that are correctly identified) and has been shown to produce highly accurate predictions (Liu *et al.* 2005; Jiménez-Valverde & Lobo 2007). All models were tested with receiver operating characteristics (ROC) curve plots, which plot the true-positive rate against the false-positive rate. The average area under the curve (AUC) of the ROC plot of ten models was taken as a measure of the overall fit of each model. Comparisons of the environmental variables used for projection to those used for training the model were made using visual interpretation of multivariate similarity surface (MESS) pictures and the most dissimilar variable (MoD) (Elith *et al.* 2010).

Quantifying niche overlap

In order to quantify the degree of ecological differentiation between *B. s. spatalurus* and *B. s. hajarensis*, we employed a multivariate analysis framework proposed by Broennimann *et al.* (2012) implemented in R (R Development Core Team 2008), using the same climate variables, distribution records and background as for SDM. Following this framework we computed multivariate environmental niche overlaps between *B. s. spatalurus* and *B. s. hajarensis* employing the two best performing ordination techniques (Broennimann *et al.* 2012): (1) Principal Component Analysis (PCA) calibrated on the entire environmental space of the study area (termed PCA-env; Broennimann *et al.* 2012), and (2) Ecological Niche Factor Analysis (ENFA) (Hirzel *et al.* 2002). The framework by Broennimann *et al.* (2012) implements a modified niche similarity and niche equivalency tests *sensu* Warren *et al.* (2008) and calculates niche overlap for pairs of species using Schoener's *D* (Schoener 1970).

RESULTS

Molecular analyses

The dataset used for the phylogenetic analysis of the *12S* mitochondrial gene consisted of an alignment of 396 base pairs (bp) (87 variable sites) for four *Bunopus s. spatalurus* and 28 *B. s. hajarensis* (Supplementary Table S1). The results of the BI analysis are presented in Fig. 2 and show that the two subspecies form two well-supported reciprocally monophyletic groups. Within *Bunopus s. spatalurus*, the two samples from loc. 30 in Yemen are sister to the two samples from locs. 28–29 in Dhofar, Oman. Within *B. s. hajarensis*, three geographically structured clades can be recognized: clade 1, sister to the other two clades and restricted to the northeastern tip of Oman, in the

isolated massifs of the Jebel Khamis and Jebel Qahwan and surrounding areas and in Masirah Island (locs. 23–27 in Fig. 1); clade 2, distributed across the Eastern Hajars and the Jebel Akhdar (locs. 15–22 in Fig. 1); and clade 3, distributed from the western foothills of the Jebel Akhdar, across the Western Hajars and up to the northernmost tip of the Hajar mountain range (Musandam Peninsula, Oman) (locs. 1–14 in Fig. 1). Although monophyly of each of these three clades is well supported, the resolution of the phylogenetic relationship among these clades is not supported (Fig. 2). The uncorrected genetic distances (*p*-distance) for the *12S* mitochondrial gene among the 32 samples included in the phylogenetic analysis are presented in Supplementary Table S3. The level of genetic divergence in the *12S* between the two subspecies of *B. spatalurus* is $13 \pm 2\%$. The genetic divergence within *B. s. spatalurus* is only $2 \pm 0.6\%$, while it is $4.5 \pm 0.7\%$ for *B. s. hajarensis*. The genetic divergence for the three clades of *B. s. hajarensis* is $6.9 \pm 1.2\%$ between clades 1 and 2; $6.6 \pm 1.2\%$ between clades 1 and 3; and $6.2 \pm 1.1\%$ between clades 2 and 3. The level of genetic variability within each one of the three clades is $0.5 \pm 0.3\%$ for clade 1, $1.7 \pm 0.4\%$ for clade 2, and $1.1 \pm 0.3\%$ for clade 3.

The nuclear *cmos* haplotype network analysis clearly shows that the two subspecies of *B. spatalurus* do not share a single haplotype (Fig. 3). The three haplotypes revealed in *Bunopus s. spatalurus* are separated by a maximum of two mutations. The high level of genetic variability within *B. s. hajarensis* in the *12S* mitochondrial gene, with clades 1–3 diverging by a *p*-distance above 6% in all three comparisons (see above), is also apparent in the *cmos* nuclear gene, for which 12 haplotypes separated by 1–4 mutational steps were recovered (see Fig. 3). Despite the high level of genetic variability at both mitochondrial and nuclear levels and the geographic coherence of the three clades of *B. s. hajarensis* across its distribution range (Fig. 3), all three clades share *cmos* nuclear haplotypes. Clade 1 includes two private haplotypes, clade 2 is represented by two private haplotypes, clade 3, which is represented in total by six haplotypes, has five private haplotypes (see Fig. 3).

Species distribution modelling and niche overlap

Maxent produced SDMs of moderate predictive accuracy (following Swets 1988), according to the average test AUC for the present *B. s. hajarensis* models (0.858 ± 0.063). The SDMs also performed statistically significantly better than random ($p < 0.05$). The present (Fig. 4) and past (Fig. 5) SDMs for *B. s. hajarensis* reveal large

suitable areas in southern Arabia that have remained stable and extend well beyond the current distribution range in northern Oman and Masirah Island. The SDMs also predict suitable climatic conditions in large parts of the range of *B. s. spatalurus*, indicating relative similar environmental conditions in these areas. This is reflected by the SDMs of *B. s. hajarensis*, which correctly predict 53.3% of the *B. s. spatalurus* distribution records in the present (Fig. 4) and up to 80% (MIROC) for the past models (Fig. 5). Although the SDMs predict overall stability in most areas in the southern Arabian Peninsula, a remarkable gap in areas with suitable condition is evident between the distribution ranges of the two subspecies. This gap remains present despite the increased area with suitable conditions in the LGM.

Both MESS and MoD pictures indicate conditions in several areas outside the training range. For example, most areas in the north (outside the present distribution range of *B. s. spatalurus*) as well as parts of the mountains in western Yemen have areas with no-analog climatic conditions. The gap between northern and southern Oman has remained relative stable with most areas having similar climatic conditions, while some areas have no-analog values for Annual Precipitation (BIO12) and Precipitation of Coldest Quarter (BIO19) (see Supplementary Figures S3 and S4).

The environmental space occupied by *B. s. hajarensis* and *B. s. spatalurus* as determined by PCA-env and ENFA is shown in Fig. 6A,B (Supplementary Figures S5 and S6). Niche overlap between the subspecies is low ($D = 0.328$) for PCA-env to very limited for ENFA ($D = 0.087$) (Rödder & Engler 2011). The niche equivalency hypothesis was rejected ($p = 0.02$) for both PCA-env and ENFA, revealing significant differences between niches of the subspecies. The randomization test of background similarity, however, shows that niches of these two subspecies are significantly different than would be expected given the underlying environmental differences between the regions they inhabit ($p = 0.02$ in both directions) only for PCA-env (Fig. 6A). The niche overlap for ENFA, however, falls within the 95% confidence interval of the null distributions, leading to non-rejection of the null hypothesis of retained niche similarity (Fig. 6B). This result of relative similar niches is consistent with the large overlap predicted by the SDMs.

Taxonomic account

As already stated by Arnold (1980) in the original description of *B. s. hajarensis*, the two subspecies of *B. spatalurus* present some differences at the morphological level.

Bunopus s. hajarensis is differentiated from *B. s. spatalurus* by its smaller size (up to 50 mm of SVL compared with up to 67 mm in *B. s. spatalurus*); by the presence in the dorsum of neck and body of about eight (at mid-body) and six (between the hind legs) longitudinal rows of irregular, enlarged scales with a strong medial keel that increases in height to the posterior border, compared with unkeeled or feebly keeled dorsal scales in *B. s. spatalurus* (Fig. 7A,B,C,D); strongly keeled scales also on the dorsal part of the hind limbs, compared with unkeeled or feebly keeled dorsal scales in *B. s. spatalurus* (Fig. 7G,J); presence of protruding (convex) scales on top of the head, compared with flattened scales in *B. s. spatalurus* (Fig. 7E,H); presence of a pair of enlarged scales, each situated laterally to the posterior section of the mental, compared with no clearly differentiated chin shields in *B. s. spatalurus* (Fig. 7K–N). As a result of the morphological differences, the high level of genetic differentiation in the *12S* mitochondrial gene ($13 \pm 2\%$), and the results of the phylogenetic and *cmos* haplotype network analysis (Figs. 2 and 3; Supplementary Table S3), we elevate *Bunopus spatalurus hajarensis* to the species level, *Bunopus hajarensis* **stat. nov.** Arnold, 1980.

DISCUSSION

By upgrading *Bunopus hajarensis* to the specific status, we add a new species to the already long and varied list of endemic reptiles of the Hajar Mountains, which include one snake (*Echis omanensis*), one endemic genus of lacertid lizards with two endemic species (*Omanosaura jayakari* and *O. cyanura*), four species of the genus *Asaccus* (*A. montanus*, *A. platyrhynchus*, *A. gallagheri* and *A. caudivolvulus*), two species of *Pristurus* (*P. gallagheri* and *P. celerrimus*) and three *Hemidactylus* (*H. luqueorum*, *H. hajarensis*, and *H. endophis*, although the latter is known only from a single museum preserved specimen and therefore might be extinct) (see Gardner 2013). Moreover, ongoing phylogeographic research in this mountain range suggests that the level of genetic variability in some other taxa, like for instance the diurnal gecko *Pristurus rupestris* and the nocturnal geckos of the genera *Ptyodactylus* and *Asaccus* is much higher; including several undescribed species. The old age and specific situation of the Hajar Mountains, surrounded by the sea in the northwest, north and east and by a very large arid desert in the south and west (Edgell 2006), have probably played a crucial role in the origin and maintenance of its unique reptile fauna (Arnold

& Gallagher 1977). The Hajar mountain range, being generally strongly arid with poor vegetation, contains some rather isolated highland areas like the Jebel Akhdar and much lower areas like the Western Hajars with very different climatic conditions, which probably played and still plays a very important role in the speciation dynamics of most of the lineages. One of the best examples are the geckos of the genus *Asaccus*, with *A. platyrhynchus* being restricted to the Jebel Akhdar massif, *A. caudivolvulus* distributed across the Western Hajars, and *A. gallagheri* distributed across the whole mountain range and found in sympatry with the other two species (Arnold & Gardner 1994; Papenfuss *et al.* 2010). Another *Asaccus* endemic species, *A. montanus*, is restricted to the highlands of the Jebel Akhdar, but, as suggested by phylogenetic analyses using morphological and molecular data, it is most probably the result of an independent colonization from Iran (Arnold & Gardner 1994; Papenfuss *et al.* 2010). Similar patterns are found in the genus *Pristurus*, with *P. gallagheri* being restricted to the Jebel Akhdar and *P. celerrimus* being found in the Jebel Akhdar but also across the Western Hajars and up to the Musandam Peninsula (Arnold 2009), although these two species are not sister (Papenfuss *et al.* 2009). The two extant *Hemidactylus* present almost non-overlapping distribution ranges within the massif. While *Hemidactylus luqueorum* is restricted to the highlands of the Jebel Akhdar, its sister species, *H. hajarensis*, is only found in the lowland areas of the Jebel Akhdar and across the Eastern Hajars (Carranza & Arnold 2012). The present work on *Bunopus hajarensis* reveals a high level of genetic diversity in both mtDNA (*12S*) and nDNA (*cmos*) genes. As a result of the apparent similar ecology and morphology of all specimens of *B. hajarensis* across its distribution range (Arnold 1980; pers. observ.), the different clades are most probably the result of allopatric isolation caused by a combination of past geographic and climatic events (see above). At the moment, a case analogous to *Bunopus hajarensis* with the presence of three highly divergent clades across the Hajar Mountain range has not been reported yet, but preliminary data on some other groups (Papenfuss *et al.* 2010), and especially ongoing studies using molecular phylogenies for all the reptile endemics of this massif indicate that this could be a common pattern. Additional studies in other taxa will help to understand processes that have shaped the distribution patterns of the taxa of the Hajar Mountains.

Although the mitochondrial and nuclear results presented in Figs. 2 and 3 and in Supplementary Table 2 seem to suggest that *B. hajarensis* in the Hajar Mountains can represent a species complex, more detailed phylogenetic and morphological analyses

will be necessary to solve this issue. The most divergent clade (clade 1) includes specimens from the extreme northeast of Oman and a population in Masirah Island. These populations seem to be isolated by the extremely arid Sharqiyah Sands, a sand dune desert with no records of *B. hajarensis* (Arnold 1980; Gallagher & Arnold 1988; Gardner 2013; pers. observ.) and with no predicted suitable areas for this species either at present (Fig. 4) or in the recent past (LGM) (Fig. 5A,B). According to our phylogenetic analyses (Fig. 3) and *p*-distance values (Supplementary Table 3), the two specimens from Masirah Island (both genetically identical) are differentiated from the specimens from locs. 23–24 (Fig. 1) by a genetic divergence of 0.9%. These results, together with the widespread distribution range of *B. hajarensis* in Masirah Island (pers. observ.), suggest that the population from Masirah Island may be established by natural colonization instead of a human-mediated introduction (*cf.* to lizards of the genus *Chamaeleo*; Gardner 2013). Nevertheless, more sampling in extreme northeast Oman will be necessary in order to assess the real level of genetic differentiation between *B. hajarensis* from this area and from Masirah Island. Other taxa, like for instance the amphibian *Duttaphrynus dhufarensis*, and the reptiles *Telescopus dhara dhara*, *Spalerosophis diadema cliffordii*, *Platyceps rhodorachis rhodorachis*, and *Pseudotrapelus sinaitus* seem to present a similar disjunct distribution to *B. hajarensis* concerning Masirah Island (Gardner 2013). Geomorphological studies and dating estimates of the presence of endemic sand dune specialist taxa like *Stenodactylus sharqiyahensis* suggest that the Sharqiyah Sands have been in place for a relatively long period, which could date back to the Middle Miocene to Plio-Pleistocene (Radies et al. 2004; Metallinou & Carranza 2013). If the connection between the populations of *B. hajarensis* from the Hajar Mountains and Masirah Island had been cut off at that time, we would expect that the level of genetic variability would be maintained despite increasing sampling in extreme northeastern Oman.

The present and LGM SDMs of *B. hajarensis* show a large potential distribution in southern Arabia extending well beyond the current known distribution range and into most of the range of *B. spatalurus*. Interestingly, the models indicate a large gap between the species' distributions ranges in the middle of Oman, as well as in the Sharqiya Sands region between Masirah Island and the Hajar Mountains in northern Oman. The existence of this gap was highlighted by Arnold (1980) (see Introduction). As the SDMs of *B. spatalurus* were excluded from the present study due to extremely low performance, we are unable to verify if the gap is important for this species as well.

However, as the results indicate that the niches of both species are similar, it is likely that this area is also unsuitable for *B. spatalurus*. The potential distribution of *B. hajarensis* during the LGM (Fig. 5A,B) is larger compared to the present (Fig. 4). Although, to our knowledge, no studies have used paleodistribution models in Arabian biogeography research yet, this pattern is in agreement with studies from North Africa, where wetter and cooler annual climatic conditions during the LGM increased the potential distributions of several species (e.g. de Pous *et al.* 2011, 2013). Several studies on terrestrial habitats indicate that LGM climatic conditions in Arabia were typified by lower temperatures compared to present. For example, Weyhenmeyer *et al.* (2000) used concentrations of atmospheric noble gases dissolved in groundwaters from northern Oman to study changes in temperature and moisture during the Late Pleistocene (15 000 to 24 000 years before present). These authors showed that average ground temperature was 6.5 °C lower than today. At present, *B. hajarensis* is mainly distributed in mountainous areas that have lower temperatures in comparison to surrounding lowland areas. According to extensive fieldwork, *B. hajarensis* is largely absent from these lowlands despite some records in the foothills of the Hajar Mountains and on Masirah Island. It is therefore assumed that a combination of rocky microhabitat and cooler and wetter climatic conditions confine the species to mountainous areas. During the LGM, preferred climatic conditions were more widespread resulting in a larger potential distribution area in southern Arabia.

As indicated in the results, most areas within the distribution have remained stable and relatively few areas have no-analog climatic conditions. The areas with variables outside the training range are mainly located in the northern Arabia outside the present distribution range of *B. hajarensis* and *B. spatalurus*. However, the MESS and MoD pictures also show some areas with no-analog climate conditions within the distribution range (Supplementary Figures S3 and S4) and predictions in these areas should be treated with caution (Elith *et al.* 2010). The consistent gap between northern and southern Oman has few areas with no-analog conditions for the CCSM, whereas they occurrence increases for the MIROC. The most dissimilar variables in the gap area are Annual Precipitation (BIO12) and Precipitation of Coldest Quarter (BIO19). As the Maxent response curves (not shown) indicate a higher probability of presence with increased precipitation, it is more likely that these areas were more suitable for *B. hajarensis* during the LGM. Altogether, we believe that no-analog conditions have played a minor role in the present study.

The present study indicates that the combination of molecular and species distribution modelling, including analyses of niche overlap, are very powerful tools for systematic studies, providing multiple lines of evidence, which increase the robustness of the taxonomic conclusions (Ahmadzadeh *et al.* 2013; Koch *et al.* 2013). Future studies comparing the patterns of the several endemic reptiles of the Hajar Mountains using multiple lines of evidence will be very valuable in order to have a better understanding of the patterns and processes that have shaped their unique diversity and distribution.

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Figure captions

Figure 1. Map of localities of examined material in this study. Circles indicate samples used in the genetic analyses (red = *Bunopus spatalurus*, blue = *B. hajarensis*), with different blue colors of *B. hajarensis* representing the distinct clades as shown in Fig. 2. A list of all the specimens with their taxonomic identification, sample code, voucher code, country, and corresponding geographical distribution data and GenBank accession numbers for the two sequenced genes is presented in Supplementary Table S1.

Figure 2. Bayesian tree of *Bunopus spatalurus* and *B. hajarensis* inferred using the *12S* mtDNA gene. A list of all the specimens, sample code, voucher code, country, and corresponding geographical distribution data and GenBank accession numbers for the two sequenced genes is presented in Supplementary Table S1.

Figure 3. Haplotype network of the phased sequences of the nuclear marker *cmos*. Phase probabilities were set as ≥ 0.7 . A list of all the specimens, sample code, voucher code, country, and corresponding geographical distribution data and GenBank accession numbers for the two sequenced genes is presented in Supplementary Table S1.

Figure 4. Potential species distribution model of *Bunopus hajarensis* for the present based on the MTSPS threshold. The available distribution data for both species are indicated (white dots = *B. hajarensis*; white triangles = *B. spatalurus*).

Figure 5. Potential species distribution model of *Bunopus hajarensis* for the Last Glacial Maximum based on the MTSPS threshold. (A) MIROC and (B) CCSM. The available distribution records for both species are indicated (white dots = *B. hajarensis*; white triangles = *B. spatalurus*).

Figure 6. Niches of *Bunopus hajarensis* and *B. spatalurus* based on PCA-env (A) and ENFA (B). (A) The niches of both species are displayed on a multi-dimensional scale represented by the first two axes of a principal component analyses (PCA) summarizing the entire study area. (B) The x-axis shows marginality and the y-axis specialization. In both figures the grey shadings reflect the density of the occurrences of each species by cell. The dashed and solid contour lines indicate 50% and 100% of the available background environment. The significance of the equivalency (1) and similarity tests is shown (2 with a and b indicating directions) (ns, non-significant, * $p < 0.05$).

Figure 7. Pictures of (A) *Bunopus spatulurus* (TMHC 2013.10.404) and (B) *Bunopus hajarensis* (TMHC 2013.10.407). Close up detail of the dorsal scales of (C) *Bunopus spatulurus* (TMHC 2013.10.404) and (D) *Bunopus hajarensis* (TMHC 2013.10.408). Details of the dorsal side of head (E), lateral side of head (F), and dorsal side of the right hind limb (G) of *Bunopus spatulurus* (TMHC 2013.10.404); of the dorsal side of head (H), lateral side of head (I) of *Bunopus hajarensis* (TMHC 2013.10.407), and of the dorsal side of the right hind limb (J) of *Bunopus hajarensis* (TMHC 2013.10.407). Underside of head (gular region showing the arrangement of mental scale and chin shields) of (K) *Bunopus spatulurus* (TMHC 2013.10.405), (L) *Bunopus spatulurus* (TMHC 2013.10.404), (M) *Bunopus hajarensis* (TMHC 2013.10.407), (N) and *Bunopus hajarensis* (TMHC 2013.10.409).

Data for *Bunopus spatulurus* specimens TMHC 2013.10.404 and TMHC 2013.10.405 are presented in Supplementary Table 1. *Bunopus hajarensis* TMHC 2013.10.407, 3 km S. of Al-Hamra, Nizwa, Oman (23.057444 57.287778, 671 m a.s.l.); TMHC 2013.10.408, same data as TMHC 2013.10.407; TMHC 2013.10.409, Surrounding of Jebel Shams Resort, Oman, 23.207861 57.199833, 1983 m a.s.l.

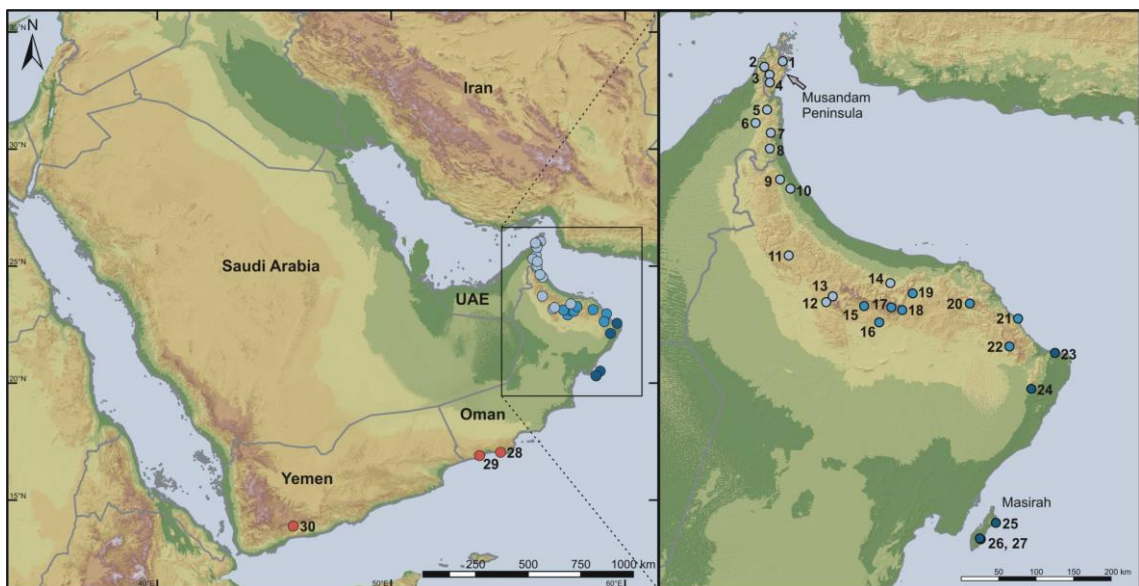


Fig. 1

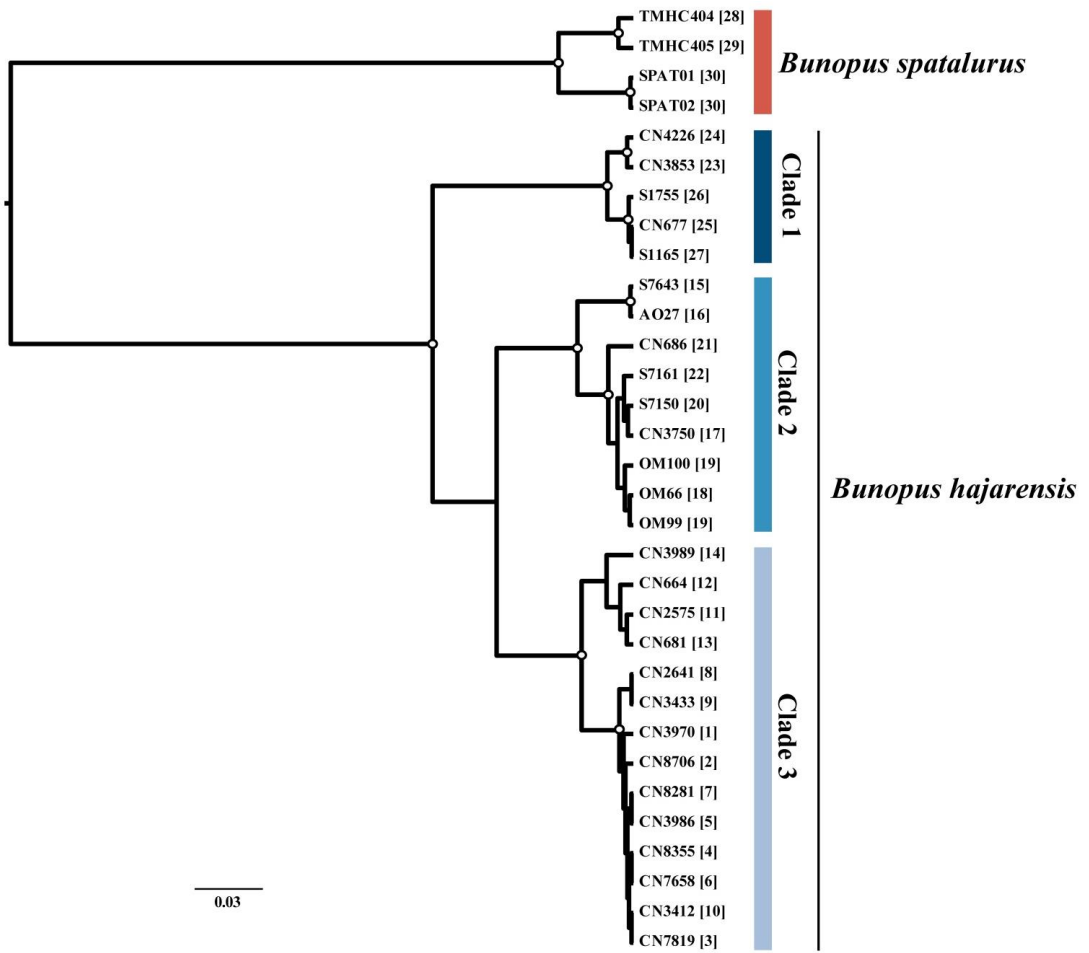


Fig. 2

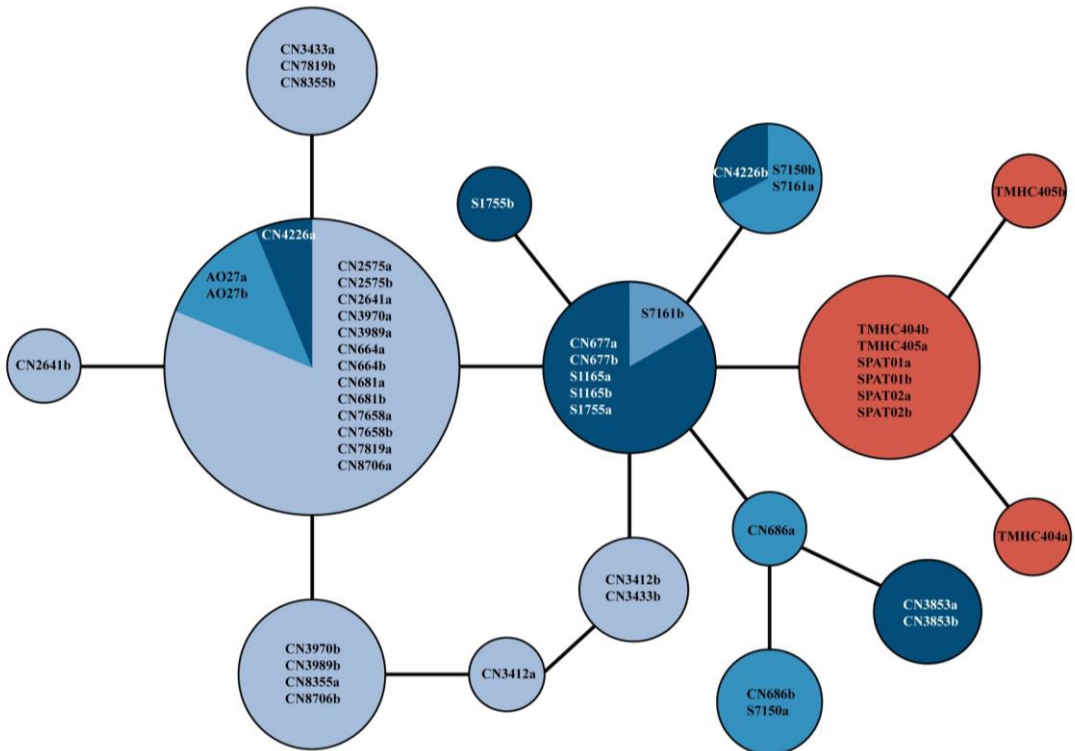


Fig. 3

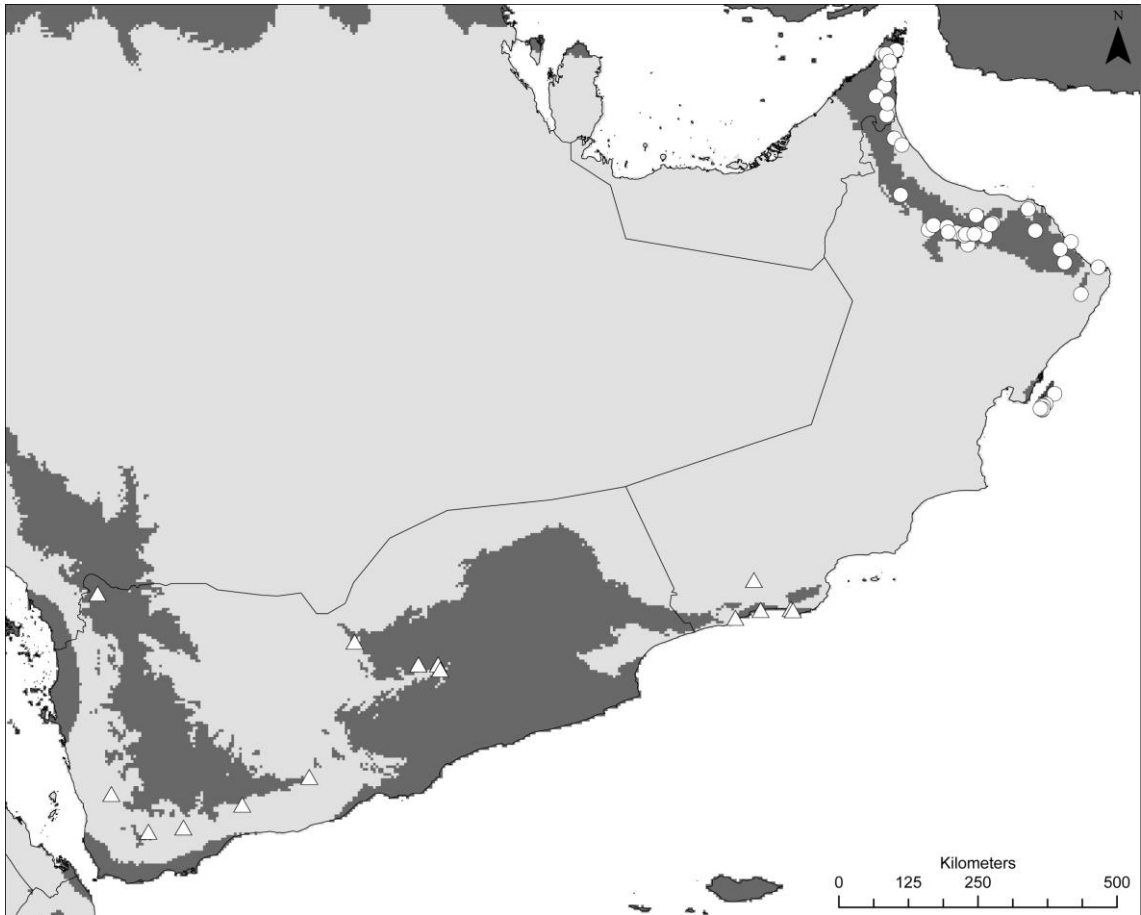


Fig. 4

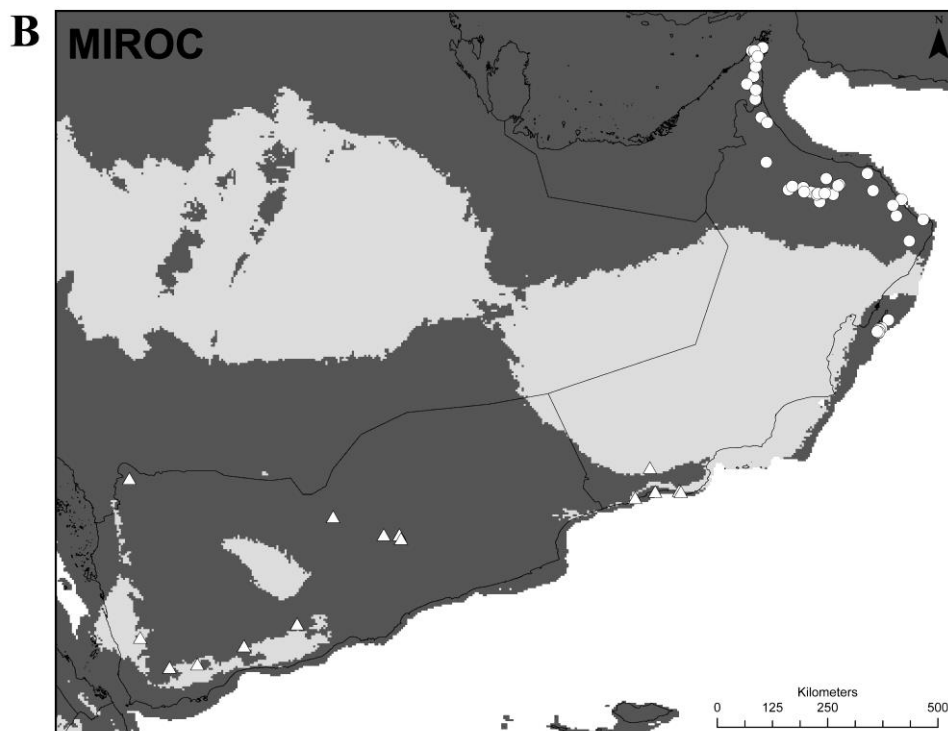
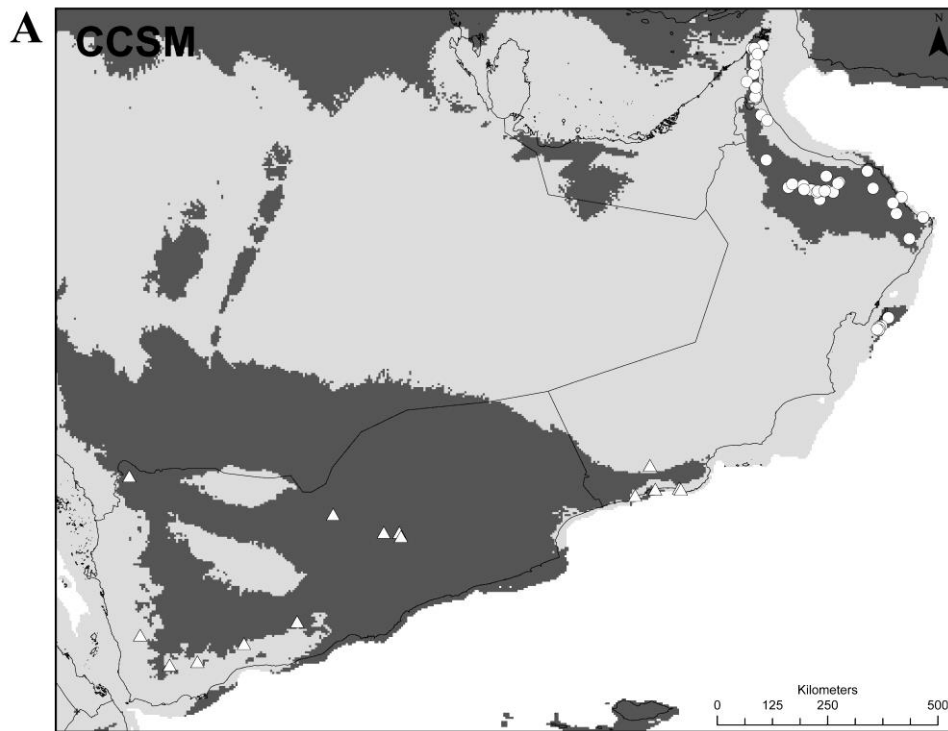


Fig. 5

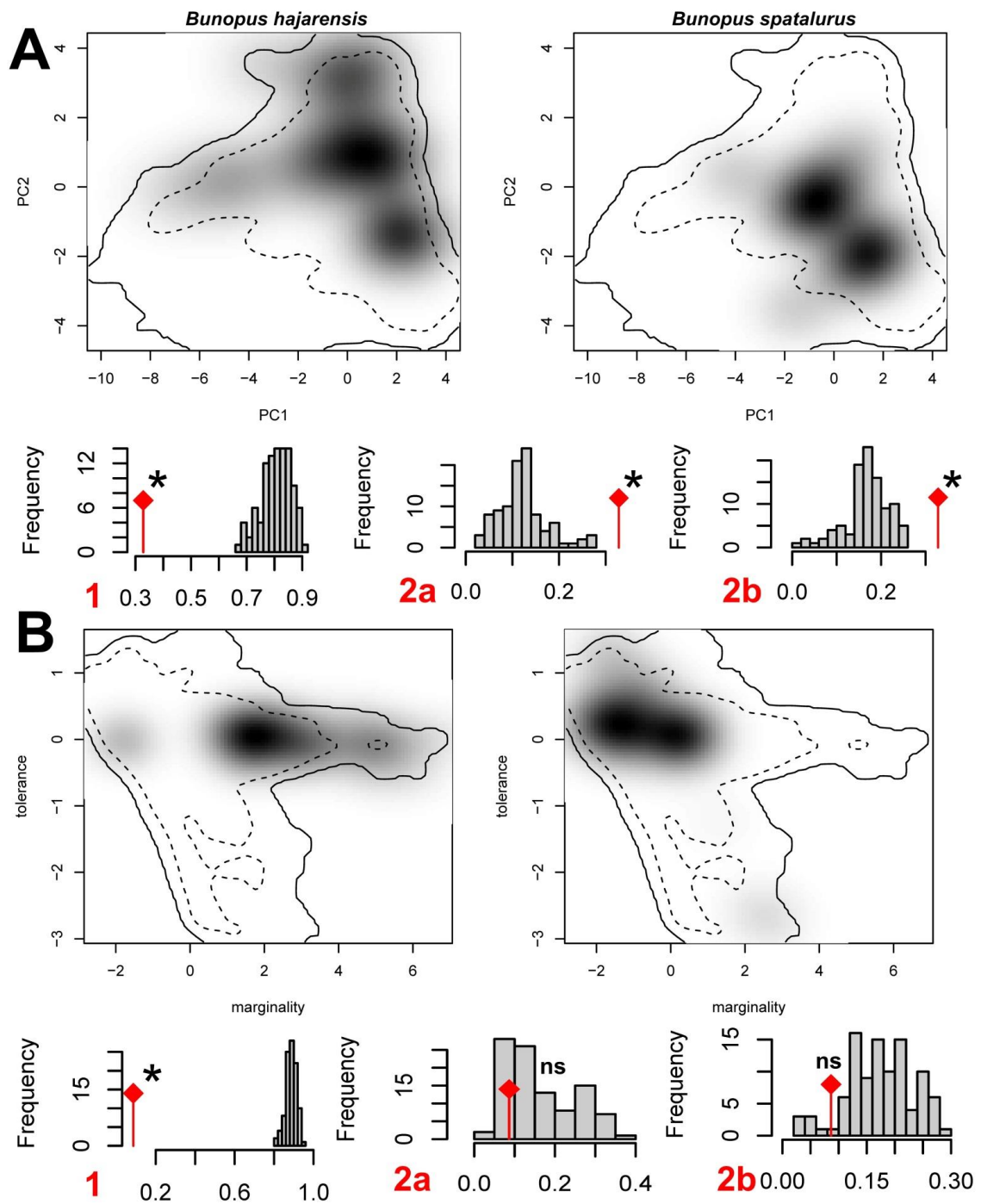


Fig. 6

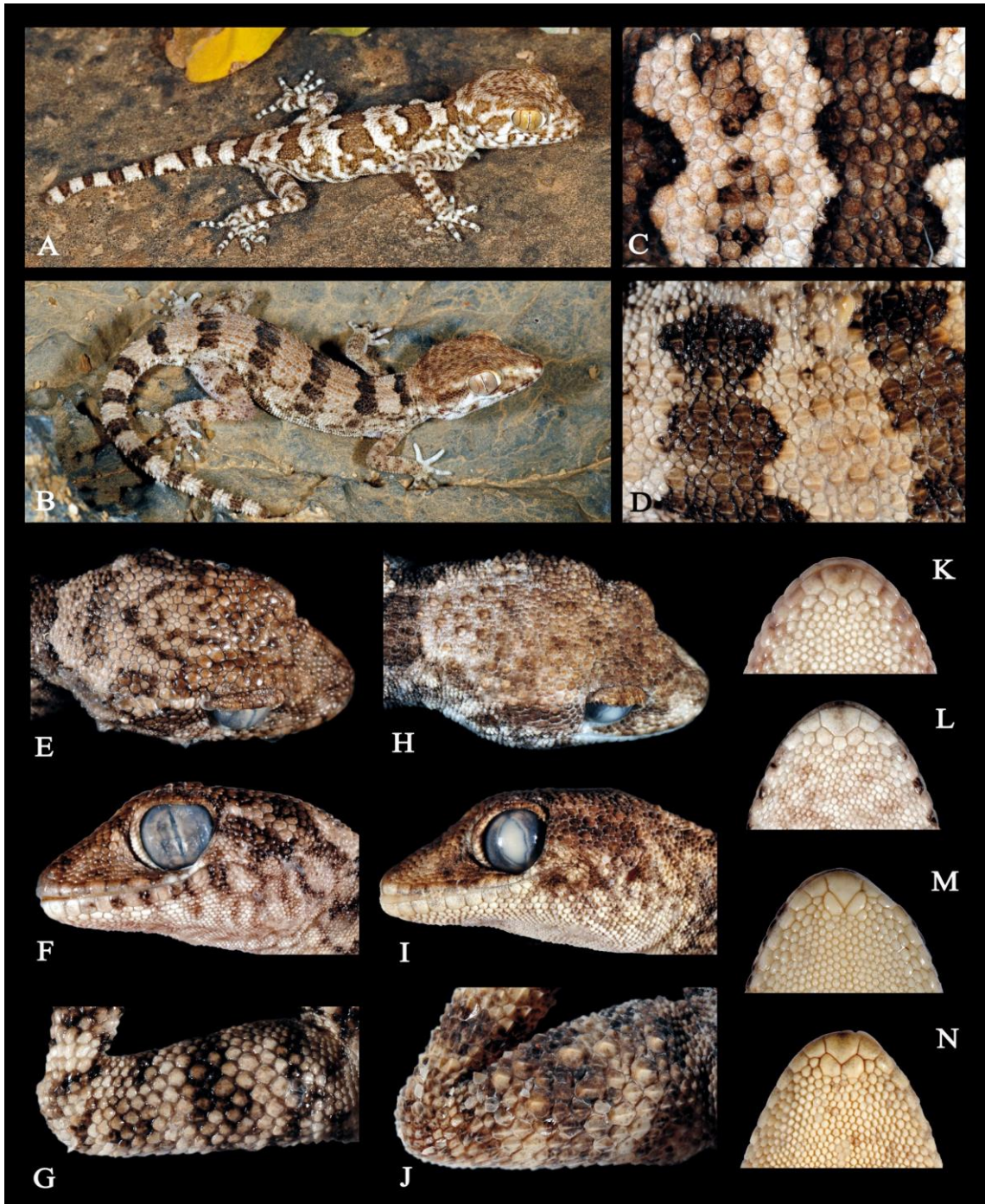


Fig. 7

Supplementary files

Table S1. Specimens included in the molecular analyses, with respective taxonomic identification, sample code, voucher reference, corresponding geographical distribution data (latitude, longitude and country), locality code as shown in the map from Fig. 1 and GenBank accession numbers.

Table S2. Distribution database of *Bunopus spatalurus* and *Bunopus hajarensis* with latitude, longitude, indication of precision and the source.

Table S3. 12S uncorrected genetic distances (p -distances; complete deletion) between all the specimens of *Bunopus spatalurus* and *Bunopus hajarensis* included in the present study. Genetic distances are given in % and are shown below the diagonal. The Standard Errors are shown above the diagonal.

Table S1. Specimens included in the molecular analyses, with respective taxonomic identification, sample code, voucher reference, corresponding geographical distribution data (latitude, longitude and country), locality code as shown in the map from Fig. 1 and GenBank accession numbers.

Species	Sample code	Voucher reference	Latitude	Longitude	Country	Locality code	ITS	cmos
<i>B. spatalurus</i>	SPAT01		13.8770	45.8000	Yemen	30		
<i>B. spatalurus</i>	SPAT02		13.8770	45.8000	Yemen	30		
<i>B. spatalurus</i>	TMHC404		17.0289	54.6665	Oman	28		
<i>B. spatalurus</i>	TMHC405		16.8844	53.7731	Oman	29		
<i>B. hajarensis</i>	CN4226		22.1070	59.3570	Oman	24		
<i>B. hajarensis</i>	CN3853		22.5401	59.6408	Oman	23		
<i>B. hajarensis</i>	CS1755		20.3118	58.7366	Oman	26		
<i>B. hajarensis</i>	CS677		20.4981	58.9306	Oman	25		
<i>B. hajarensis</i>	S1165		20.2995	58.7497	Oman	27		
<i>B. hajarensis</i>	S7643		23.1010	57.3496	Oman	15		
<i>B. hajarensis</i>	AO27		22.9050	57.5300	Oman	16		
<i>B. hajarensis</i>	CN686		22.9492	59.1983	Oman	17		
<i>B. hajarensis</i>	S7161		22.6161	59.0937	Oman	22		
<i>B. hajarensis</i>	S7150		23.1317	58.6189	Oman	20		
<i>B. hajarensis</i>	CN3750		23.0865	57.6762	Oman	17		
<i>B. hajarensis</i>	OM100		23.2543	57.9315	Oman	19		
<i>B. hajarensis</i>	OM66		23.0535	57.8050	Oman	18		
<i>B. hajarensis</i>	OM99		23.2543	57.9315	Oman	19		
<i>B. hajarensis</i>	CN3989		23.3770	57.6644	Oman	14		
<i>B. hajarensis</i>	CN664		23.1498	56.8942	Oman	12		
<i>B. hajarensis</i>	CN2575		23.7102	56.4431	Oman	11		
<i>B. hajarensis</i>	CN681		23.2200	56.9736	Oman	13		
<i>B. hajarensis</i>	CN2641		24.9936	56.2167	UAE	8		
<i>B. hajarensis</i>	CN3433		24.6208	56.3398	Oman	9		
<i>B. hajarensis</i>	CN3970		26.0421	56.3697	Oman	1		
<i>B. hajarensis</i>	CN8706		25.9758	56.1504	Oman	2		
<i>B. hajarensis</i>	CN8281		25.1823	56.2290	UAE	7		
<i>B. hajarensis</i>	CN3986		25.4590	56.1834	UAE	5		
<i>B. hajarensis</i>	CN8355		25.7863	56.2169	Oman	4		
<i>B. hajarensis</i>	CN7658		25.3001	56.0453	UAE	6		
<i>B. hajarensis</i>	CN3412		24.5131	56.4634	Oman	10		
<i>B. hajarensis</i>	CN7819		25.8798	56.2144	Oman	3		

Table S2. Distribution database of *Bunopus spatalurus* and *Bunopus hajarensis* with latitude, longitude, indication of precision and the source.

Species	Latitude	Longitude	Precision	Source
<i>Bunopus hajarensis</i>	22.54	59.64	GPS	Personal database
<i>Bunopus hajarensis</i>	22.11	59.36	GPS	Personal database
<i>Bunopus hajarensis</i>	20.50	58.93	GPS	Personal database
<i>Bunopus hajarensis</i>	20.30	58.75	GPS	Personal database
<i>Bunopus hajarensis</i>	20.31	58.74	GPS	Personal database
<i>Bunopus hajarensis</i>	22.91	57.53	GPS	Personal database
<i>Bunopus hajarensis</i>	23.09	57.68	GPS	Personal database
<i>Bunopus hajarensis</i>	22.95	59.20	GPS	Personal database
<i>Bunopus hajarensis</i>	23.25	57.93	GPS	Personal database
<i>Bunopus hajarensis</i>	23.05	57.80	GPS	Personal database
<i>Bunopus hajarensis</i>	23.25	57.93	GPS	Personal database
<i>Bunopus hajarensis</i>	23.13	58.62	GPS	Personal database
<i>Bunopus hajarensis</i>	22.62	59.09	GPS	Personal database
<i>Bunopus hajarensis</i>	23.10	57.35	GPS	Personal database
<i>Bunopus hajarensis</i>	23.71	56.44	GPS	Personal database
<i>Bunopus hajarensis</i>	24.99	56.22	GPS	Personal database
<i>Bunopus hajarensis</i>	24.51	56.46	GPS	Personal database
<i>Bunopus hajarensis</i>	24.62	56.34	GPS	Personal database
<i>Bunopus hajarensis</i>	26.04	56.37	GPS	Personal database
<i>Bunopus hajarensis</i>	25.46	56.18	GPS	Personal database
<i>Bunopus hajarensis</i>	23.38	57.66	GPS	Personal database
<i>Bunopus hajarensis</i>	23.15	56.89	GPS	Personal database
<i>Bunopus hajarensis</i>	23.22	56.97	GPS	Personal database
<i>Bunopus hajarensis</i>	25.30	56.05	GPS	Personal database
<i>Bunopus hajarensis</i>	25.88	56.21	GPS	Personal database
<i>Bunopus hajarensis</i>	25.18	56.23	GPS	Personal database
<i>Bunopus hajarensis</i>	25.79	56.22	GPS	Personal database
<i>Bunopus hajarensis</i>	25.98	56.15	GPS	Personal database
<i>Bunopus hajarensis</i>	23.10	57.38	GPS	Personal database
<i>Bunopus hajarensis</i>	23.19	57.20	GPS	Personal database
<i>Bunopus hajarensis</i>	25.96	56.20	GPS	Personal database
<i>Bunopus hajarensis</i>	20.30	58.74	GPS	Personal database
<i>Bunopus hajarensis</i>	23.48	58.50	GPS	Personal database
<i>Bunopus hajarensis</i>	20.33	58.79	GPS	Personal database
<i>Bunopus hajarensis</i>	22.54	59.64	GPS	Personal database
<i>Bunopus hajarensis</i>	23.19	57.20	GPS	Personal database
<i>Bunopus hajarensis</i>	22.11	59.36	GPS	Personal database
<i>Bunopus hajarensis</i>	22.54	59.64	GPS	Personal database
<i>Bunopus hajarensis</i>	22.95	59.20	GPS	Personal database
<i>Bunopus hajarensis</i>	24.51	56.46	GPS	Personal database
<i>Bunopus hajarensis</i>	25.98	56.20	GPS	Personal database
<i>Bunopus hajarensis</i>	25.66	56.23	GPS	Personal database
<i>Bunopus hajarensis</i>	20.33	58.79	GPS	Personal database

<i>Bumopus hajarensis</i>	24.51	56.46	GPS	Personal database
<i>Bumopus hajarensis</i>	20.31	58.74	GPS	Personal database
<i>Bumopus hajarensis</i>	20.30	58.75	GPS	Personal database
<i>Bumopus hajarensis</i>	22.62	59.09	GPS	Personal database
<i>Bumopus hajarensis</i>	23.10	57.35	GPS	Personal database
<i>Bumopus hajarensis</i>	22.11	59.36	GPS	Personal database
<i>Bumopus hajarensis</i>	23.05	57.47	GPS	Personal database
<i>Bumopus hajarensis</i>	23.08	57.61	GPS	Personal database
<i>Bumopus hajarensis</i>	23.23	57.90	GPS	Personal database
<i>Bumopus hajarensis</i>	25.86	56.27	GPS	Personal database
<i>Bumopus hajarensis</i>	23.11	57.21	GPS	Personal database
<i>Bumopus hajarensis</i>	20.24	58.73	GPS	Personal database
<i>Bumopus hajarensis</i>	20.25	58.74	GPS	Personal database
<i>Bumopus hajarensis</i>	20.26	58.70	GPS	Personal database
<i>Bumopus hajarensis</i>	22.83	59.02	GPS	Personal database
<i>Bumopus hajarensis</i>	23.08	57.50	GPS	Personal database
<i>Bumopus hajarensis</i>	23.08	57.63	GPS	Personal database
<i>Bumopus spatalurus</i>	13.87	45.80	GPS	Personal database
<i>Bumopus spatalurus</i>	13.87	45.80	GPS	Personal database
<i>Bumopus spatalurus</i>	17.03	54.67	GPS	Personal database
<i>Bumopus spatalurus</i>	16.88	53.77	GPS	Personal database
<i>Bumopus spatalurus</i>	17.01	54.18	GPS	Personal database
<i>Bumopus spatalurus</i>	13.50	44.85	Reference	Schätti and Desvoignes 1999
<i>Bumopus spatalurus</i>	16.50	47.62	Reference	Schätti and Desvoignes 1999
<i>Bumopus spatalurus</i>	14.32	46.88	Reference	Schätti and Desvoignes 1999
<i>Bumopus spatalurus</i>	16.13	48.97	Reference	Schätti and Desvoignes 1999
<i>Bumopus spatalurus</i>	13.43	44.28	Reference	Schätti and Desvoignes 1999
<i>Bumopus spatalurus</i>	16.13	48.65	Reference	Schätti and Desvoignes 1999
<i>Bumopus spatalurus</i>	17.01	54.70	GPS	CAS
<i>Bumopus spatalurus</i>	17.50	54.07	GPS	Personal database
<i>Bumopus spatalurus</i>	17.28	43.46	Google Earth	Schätti and Gasperetti 1994
<i>Bumopus spatalurus</i>	16.05	49.00	Google Earth	Rösler and Wranik 1998
<i>Bumopus spatalurus</i>	14.04	43.68	Google Earth	Schätti 1989

Wranik, H. & Rosler, W. (1998) Beiträge zur Herpetologie der Republik Jemen. 3. Geckos des südlichen Jemen und der Insel Sokotra (Reptilia: Sauria: Gekkonidae). *Zoologische Abhandlungen Staatliches Museum für Tierkunde Dresden*, 21, 113-132.

Schätti, B. (1989) Amphibien und reptilien aus der Arabischen Republik Jemen und Djibouti. *Revue suisse de Zoologie*, 96(4), 905-937.

Schätti, B. & Gasperetti, J. (1994) A contribution to the herpetofauna of Southwest Arabia. *Fauna of Saudi Arabia*, 14, 348-423.

Schätti, B. & Desvoignes, A. (1999) *The herpetofauna of southern Yemen and the Sokotra Archipelago*. Genève, Gilbert-E. Huguët, 179 pp.

Figure S1. Distribution map of *Bunopus hajarensis* and *B. spatalurus* in the southern Arabian Peninsula. The color gradient depicts altitude (green is low and white is high altitude). The source for each record is listed in Supplementary Table S2.

Figure S2. The backgrounds used for calibrating the species distribution models in Maxent as well as the niche overlap analysis (PCA-env and ENFA).

Figure S3. Clamping (A), MESS (B) and MoD (C) pictures of the projected SDMs for the Last Glacial Maximum (CCSM). The clamping picture shows where the prediction is most affected by variables being outside their training range. In the MESS picture areas in red have one or more environmental variables outside the range present in the training data. The MoD picture shows the most dissimilar variable, i.e., the one that is furthest outside its training range.

Figure S4. Clamping (A), MESS (B) and MoD (C) pictures of the projected SDMs for the Last Glacial Maximum (MIROC). The clamping picture shows where the prediction is most affected by variables being outside their training range. In the MESS picture areas in red have one or more environmental variables outside the range present in the training data. The MoD picture shows the most dissimilar variable, i.e., the one that is furthest outside its training range.

Figure S5. Niches of *Bunopus hajarensis* and *B. spatalurus* based on PCA-env. The niches of both species are displayed on a multi-dimensional scale represented by the first two axes of a principal component analyses (PCA) summarizing the entire study area, with the grey shadings reflecting the density of the occurrences of each species by cell. The dashed and solid contour lines indicate 50% and 100% of the available background environment. The correlation circle (bottom left) shows climatic variables contribution on the two axes of the PCA as well as the percentage of inertia explained by the two axes.

Figure S6. Niches of *Bunopus hajarensis* and *B. spatalurus* based on ENFA. The x-axis shows marginality and the y-axis specialization. The grey shadings reflect the density of the occurrences of each species by cell. The dashed and solid contour lines indicate 50% and 100% of the available background environment. The correlation circle (bottom left) shows the contribution of the climatic variables contribution to the two axes.

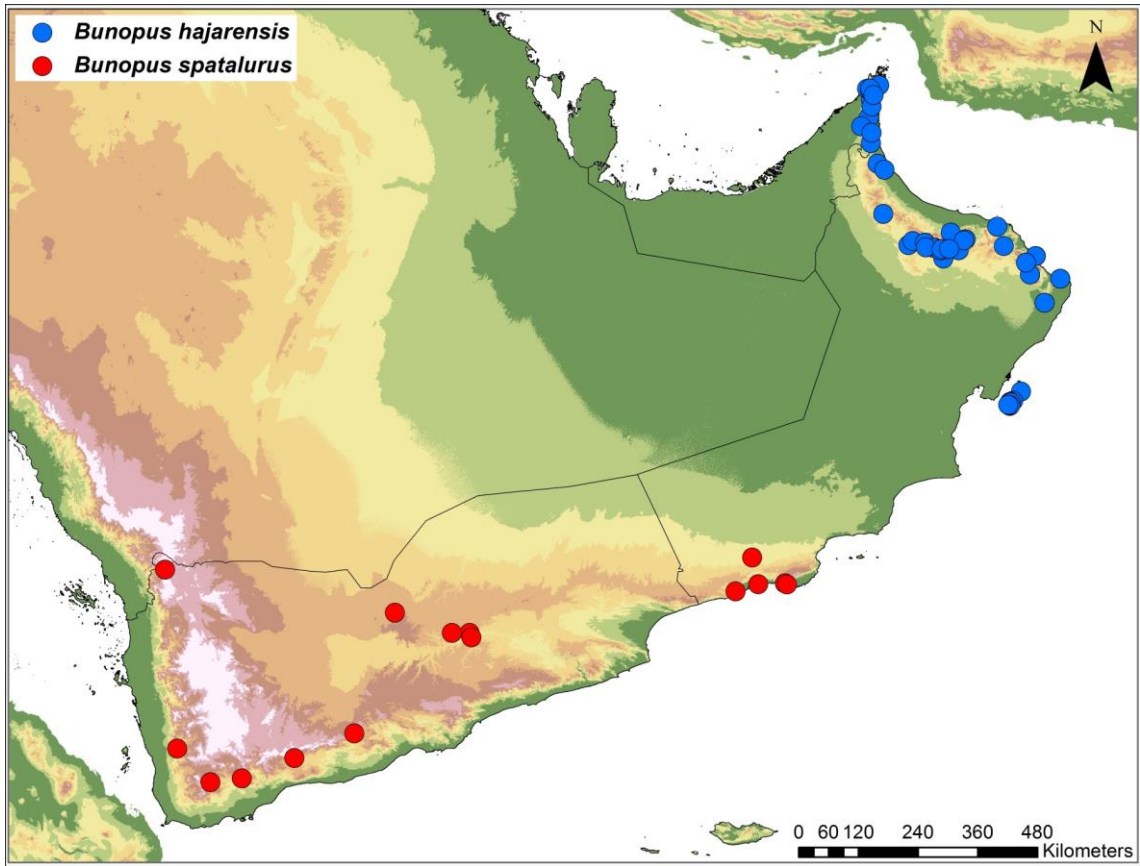


Fig. S1

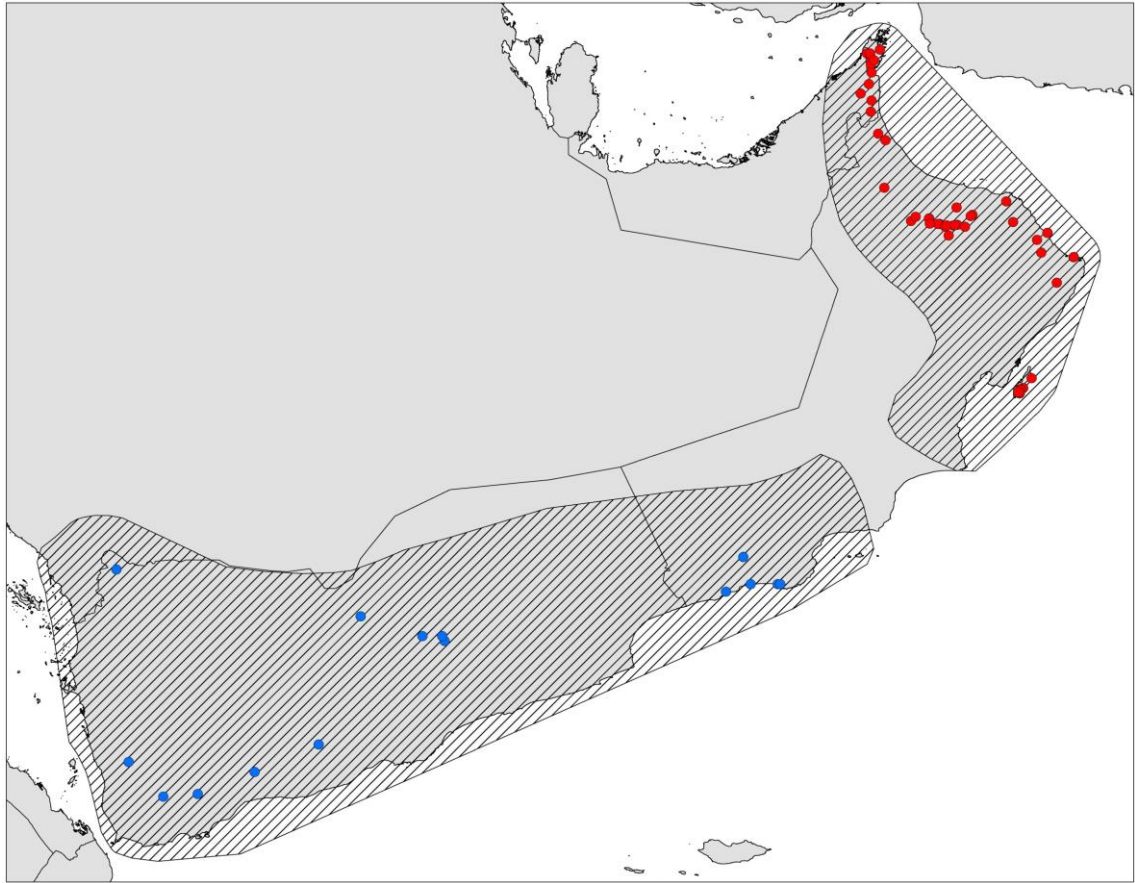


Fig. S2

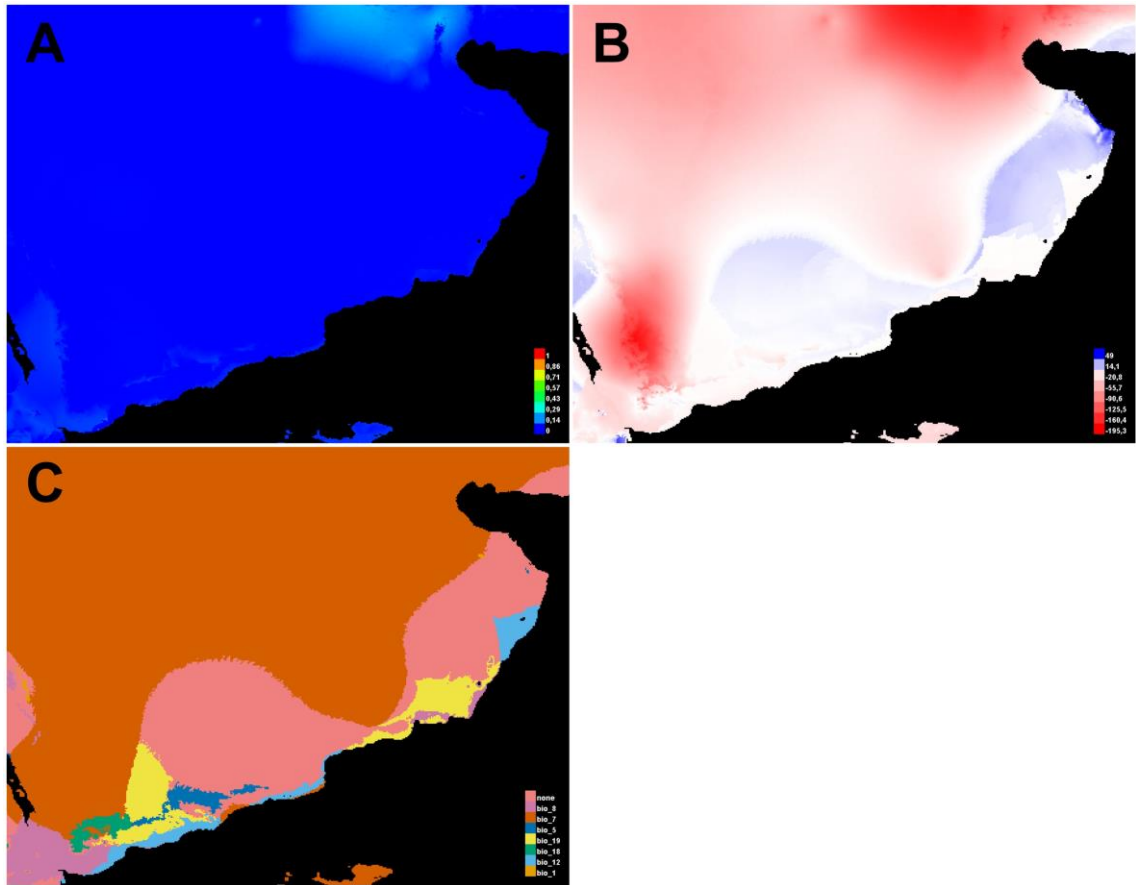


Fig. S3

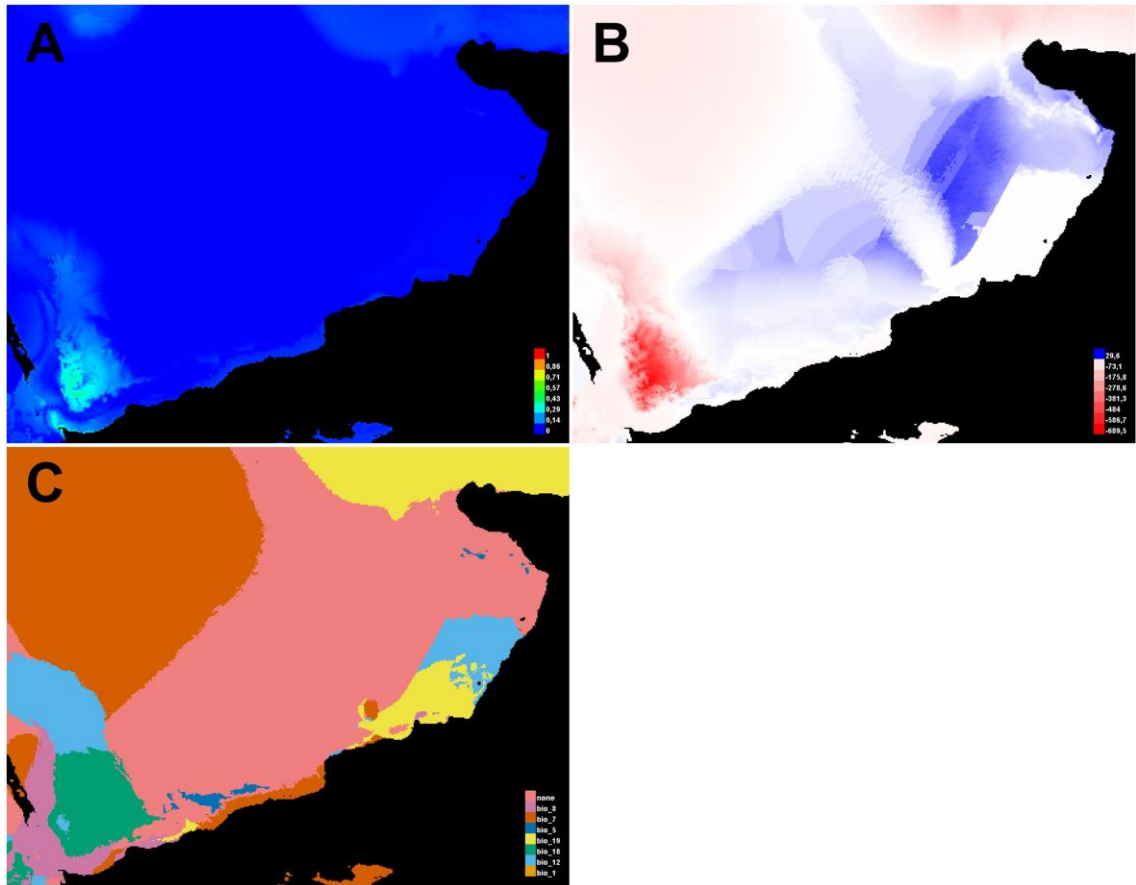


Fig. S4

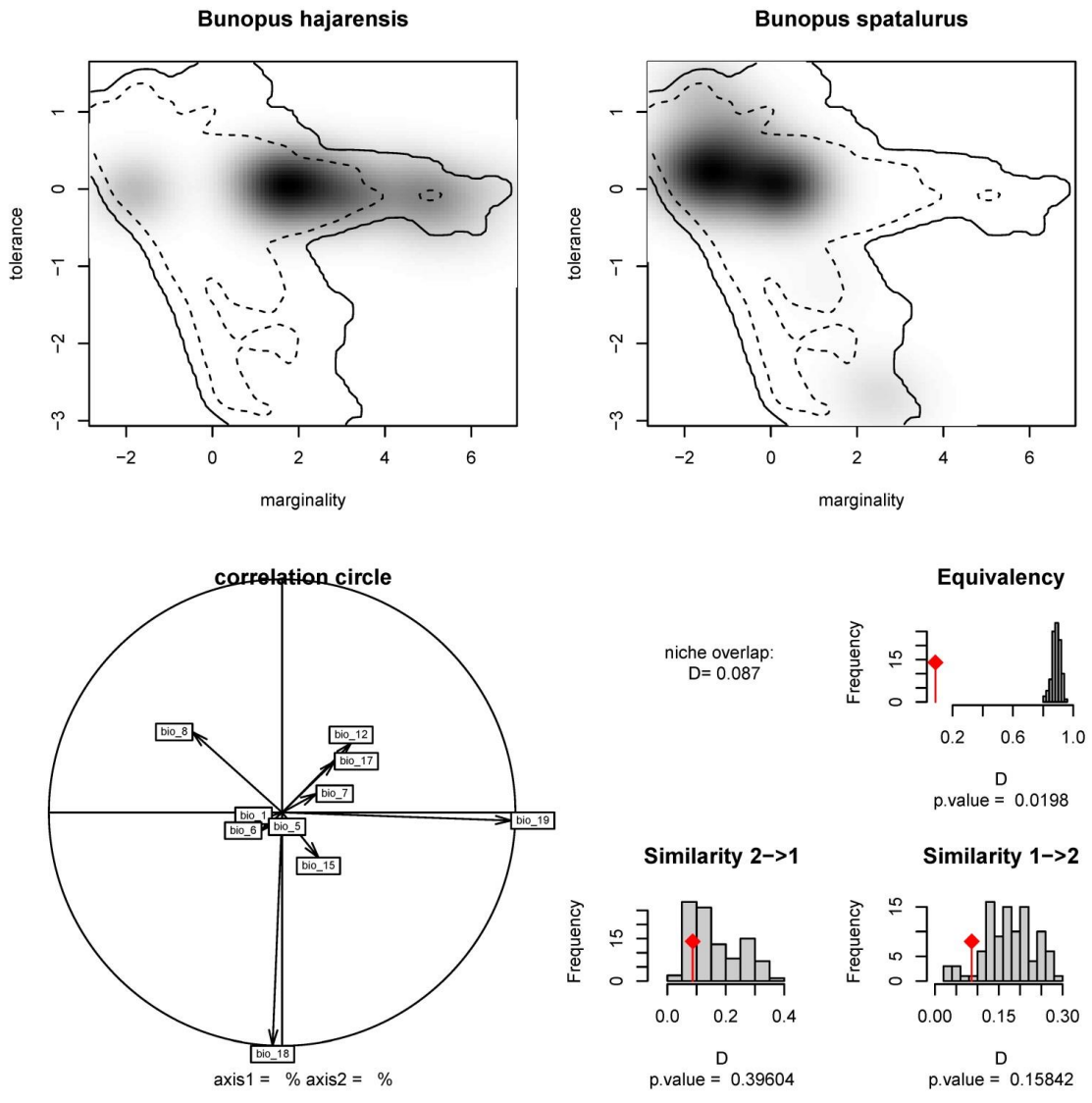


Fig. S5

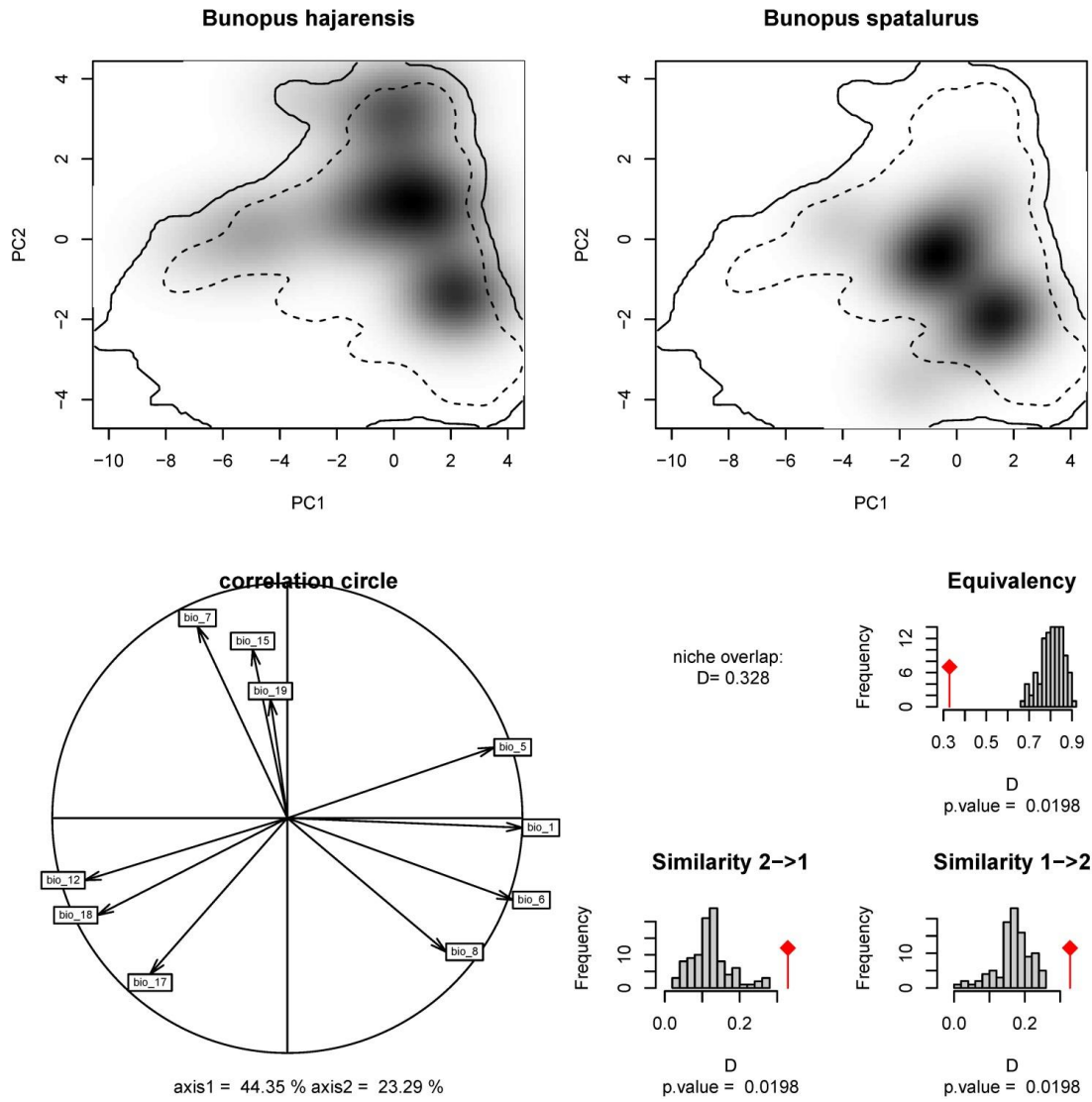


Fig. S6

ZÁVĚR

Poznání fylogeneze skupiny „nahoprstých“ gekonů představuje jeden z mnoha dílčích cílů studia evoluce fauny Mediteránu a Palearktu vůbec. Tato disertační práce ukázala, že diverzifikovaný a druhově velmi bohatý rod *Cyrtopodion sensu lato* není monofyletický, protože rody *Bunopus* a *Agamura* tvoří jeho vnitřní skupiny. Byla potvrzena výjimečnost monofyletické linie dřívějšího podrodu *Mediodactylus* a tento byl povýšen na rodovou úroveň. Málo známý a enigmatický rod *Carinatogeocko* byl synonymizován s rodem *Mediodactylus* a byla diskutována validita druhů dříve do tohoto rodu řazených. Důležitost důsledné komparativní analýzy při popisu nových druhů pouze na základě morfologických znaků byla demonstrována na případu popisu nového druhu z Pákistánu. Fylogenetická analýza sekvencí spolu s doplňujícím modelováním ekologických nik prokázala, že morfologicky odlišné poddruhy druhu *Bunopus spatalurus* jsou ve skutečnosti dobře vymezenými druhy. Taxonomické změny vyplývající z našeho výzkumu byly již povětšinou obecně akceptovány (viz například aktuální druhové seznamy Uetz a Hošek 2014 nebo Speybroeck et al. 2012).

Aktivnější terénní výzkum v posledních letech vedl nejen k dokumentaci nových lokalit výskytu již známých druhů, především z oblasti Íránu (např. Mahroo et al. 2013, Rastegar-Pouyani et al. 2009 a 2010, Yousefkhani et al. 2012), ale i k popisu řady nových druhů (např. Nazarov et al. 2011 a 2012, Nazarov a Poykarov 2013, Shi a Zhao 2011). Celkový počet v současnosti známých druhů studované skupiny gekonů tak jistě nepředstavuje číslo konečné. Nárůst v počtu druhů lze očekávat také díky stále snazší dostupnosti metod molekulární fylogenetiky, které umožňují rozlišit i druhy morfologicky málo rozrůzněné. Význam molekulárních znaků, především pak sekvencí při studiu fylogeneze nelze přeceňovat, jejich přínos je ovšem podstatný. S rozvíjejícími se sekvenčními metodami, s jejich lepší dostupností a zvyšující se rychlostí navíc bude možné analyzovat fylogenetické vztahy do daleko větší hloubky na mnoha lokusech, případně i porovnávat sekvence celých genomů. Znalost fylogeneze pak umožní následné analýzy evoluce různých ekologických charakteristik či rekonstrukce jejich ancestrálních stavů nejen této velmi diverzifikované skupiny gekonů.

Klíčovým prvkem poznání celkového obrazu evoluce dané skupiny pak zůstává a v budoucnu i nadále zůstávat bude především dostupnost materiálu. Přestože muzea po celém světě shromažďují obrovské a stále neprobádané množství materiálu pro studium druhové diverzity, nemusí být materiál již v odpovídající kvalitě pro

molekulární studie. Je tedy nezbytné provádět i nadále terénní výzkum, sběr materiálu a monitoring rozšíření jednotlivých druhů přímo na lokalitách jejich výskytu. To však do značné míry komplikuje i nestálá politická situace v oblastech, kam zasahují známé areály studovaných druhů. V obsáhlé a shrnující práci (Bauer et al. 2013) se autorům podařilo podpořit rodový status všech tří dřívějších podrodů rodu *Cyrtopodion*, materiál některých potenciálně zásadních zástupců skupiny však nebyl z výše uvedených důvodů dostupný ani jim.

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