UNIVERZITA KARLOVA V PRAZE
PŘÍRODOVĚDECKÁ FAKULTA
KATEDRA ZOOLOGIE

Phylogeny and biogeography of Neotropical and African riverine cichlids: multilocus phylogenetic methods in the evolutionary studies

Disertační práce

Zuzana Musilová

Školitel: RNDr. Jindřich Novák, Ph.D.

Konzultant: Mgr. Oldřich Říčan, Ph.D.

Praha 2011

Prohlašuji, že všichni spoluautoři jsou řádně uvedeni a že můj podíl na jednotlivých publikacích zařazených do disertační práce není menší než odpovídá pořadí a počtu autorů. Souhlasím s veřejným vystavením disertační práce v knihovně. Prohlašuji také, že jsem nepředložila práci ani její podstatnou část k získaní jiného nebo stejného akademického titulu.

7. ledna 2011 Zuzana Musilová

Tímto bych chtěla poděkovat všem, kteří jakkoliv přispěli ke vzniku této práce. Hlavní dík určitě patří mému školiteli Jindřichu Novákovi za iniciaci studia neotropických cichlid. Děkuji také Oldřichu Říčanovi za věcné rady a konzultace. Děkuji všem slušným lidem z laboratoře v Liběchově za pozitivní pracovní atmosféru. Také děkuji všem, kdož se podíleli na vzniku manuskriptů a jejichž jméno je uvedeno na konci jednotlivých textů. V neposlední řadě bych chtěla poděkovat mé rodině za zázemí a zejména Robertu Tropkovi za pomoc při finalizaci a smysluplné komentáře.

Contents:

Appendix I

- ŘÍČAN, O., **MUSILOVÁ, Z.**, MUŠKA, M., NOVÁK, J., 2005: Development of Colour Patterns in Neotropical Cichlids (Perciformes: Cichlidae: Cichlasomatinae). *Folia Zoologica*, 54: 1-46 **(IF = 0,5)**

Appendix II

- KRAJÁKOVÁ, L., **MUSILOVÁ, Z.**, KALOUS, L., 2011: Cytogenetická analýza a evoluce karyotypu u jihoamerických cichlid tribu Cichlasomatini, Bulletin VÚRH Vodňany 46 (4): 13-21).

Appendix III

- MUSILOVÁ, Z., 2010, **Blue acaras (genus *Andinoacara*) from Colombia and other southamerican countries**. Deutsche Cichliden Gesellschaft – Infomationen. 2010/1

Appendix IV

- MUSILOVÁ, Z., 2010, Rozporuplné akary: akara potoční (*Andinoacara rivulatus*) a její příbuzní z Peru, Ekvádoru a Kolumbie. **[Inconsistent acaras: green terror fish (*A. rivulatus*) and its relatives from Peru, Ecuador and Colombia?]**, Akvaristik. 2010/2.

Appendix V

- MUSILOVÁ, Z., 2006, Akary – co jsou vlastně zač? **[Acaras – what they are?]**, Akvatera fórum, 2006/2

Appendix VI

- MUSILOVÁ, Z., RICAN, O., NOVÁK, J. - **Phylogeography of the neotropical cichlid genus *Andinoacara* from northern South America** – poster presented in Cichlid Science 2010 in Basel, Switzerland (August, 2010)

Appendix VII

- MUSILOVÁ, Z., KALOUS, L., PETRTÝL, M., HOLÍKOVÁ, P. - **Angola headwaters: the white spot on the Serranochromis phylogeographic map** – poster presented in XIII European Congress of Ichthyology in Lithuania (September, 2009)

Appendix VIII

- MUSILOVÁ, Z. AND JANKO, K. - **Incongruence in phylogenetic signals: real conflict or insufficient taxon sampling? -** poster presented in Congress of Society for Molecular Biology and Evolution (SMBE) in Barcelona (June, 2008)

Aims and scopes of the thesis

The thesis comprises from the five main parts: three of them are published papers, the rest two are manuscripts prepared for submitting to the scientific journals. The first two are published phylogenetic studies of the cichlasomatine cichlids based on **(1)** molecular characters, and **(2)** both morphological and molecular data with the description of a new genus *Andinoacara*. The next unpublished manuscript **(3)** is the more detailed comprehensive phylogeography of the two non-relative genera (including *Andinoacara*s) of the trans-Andean cichlids. Including all valid species from the majority of their areals it reconstructed the ancestral area of both genera in the Choco region, Colombia, and revealed the directions of their distribution spreading. The forth **(4)** is the already published description of the new species *Andinoacara stalsbergi* from Peru combining both morphological and phylogenetical approaches and including the detailed phylogeny of the genus *Andinoacara*. The last **(5)** unpublished manuscript is the phylogeographical study of the cichlid genus *Serranochromis* from the headwaters of the totally unknown Central Angola. It showed several evidences of the faunal exchange among the adjacent river systems. Lastly, the thesis is supplemented by several appendices including some additional results produced during the cichlid studies, several popular papers, and some posters presented in international conferences.

Introduction

Cichlids as Gondwanian elements

Cichlid fishes represent one of the most speciose perciform families with more than 1600 valid species up to date (Froese & Pauly, 2010) with additional several hundreds of species estimated to be described (Stawikowski & Werner, 1998). The cichlids are typical element with Gondwanian ditribution, i.e. their distribution pattern was influenced by the Gondwana break-up during the Cretaceous (Sparks and Smith, 2004, Murray, 2001). Recently, they are distributed throughout South and Central America, West Indies, Africa, Levant, Syria, Iran, India, Sri Lanka, and Madagascar (Stiassny, 1991, Nelson, 1994, Murray, 2001) with the most of their diversity concentrated in the Sub-Saharan Africa and the Neotropics.

The origin of cichlids has been considered to lie in Madagascar. The Malagasy cichlids are presented by two lineages, one sharing with the Indian cichlids and one endemic to Madagascar (Sparks, 2008). These lineages were recognised as two basal assemblages in performed molecular studies focused on a cichlid phylogeny (Farias et al., 1999, Farias et al., 2001), however the Malagasy lineages were just poorly represented in the data sets of above cited studies. The more intensive taxon sampling in the basal lineages revealed that the two Malagasy groups of cichlids are sister clades, although very distant. Together, they represent the sister clade to the African–Neotropical cichlids (Sparks and Smith, 2004, Sparks, 2008). While only few tens of species are distributed in Malagasy lineages, the major portion of the cichlid diversity lies within the African and Neotropical clades representing the two sister monophyletic groups (Froese and Pauly, 2010).

Concerning the separation of the Neotropical and African cichlids, there are two alternative hypotheses. The first hypothesis of the continental distribution suggests that the common ancestor of the African–Neotropical clade is dated to 100-120 mya (Spark and Smith, 2004), as the lineage divergence had to predate the African and South American continent separation (Farias et al., 1999, Spark and Smith, 2004). The second hypothesis proposes the over-ocean distribution later after the continent separation, in ~ 55 Mya. The ancestor would have to reach the South American continent by the ocean streams and along the zone of ocean islands (Murray, 2001). The main reason supporting the second hypotheses is the lack of fossil material from the period of 50 – 100 Mya (Murray, 2001). Unfortunately, both hypotheses are still very poorly supported by any conclusive study.

Phylogeny of cichlids

The present view on the cichlids as the two monophyletic clades was not thought untill the molecular methods came to phylogeny studies. Previous morpohology-based phylogenetic studies on the Neotropical cichlids (Kullander, 1998, Stiassny, 1991) placed congruently the African genus *Heterochromis* directly inside the neotropical clade. This was, however, rejected by all following molecular studies (e.g., Farias et al, 1999, Farias et al, 2001, Lopez-Fernandez, 2010, Sparks and Smith, 2004, Smith et al., 2008), and the neotropical cichlids has been definitely found as a monophyletic unit.

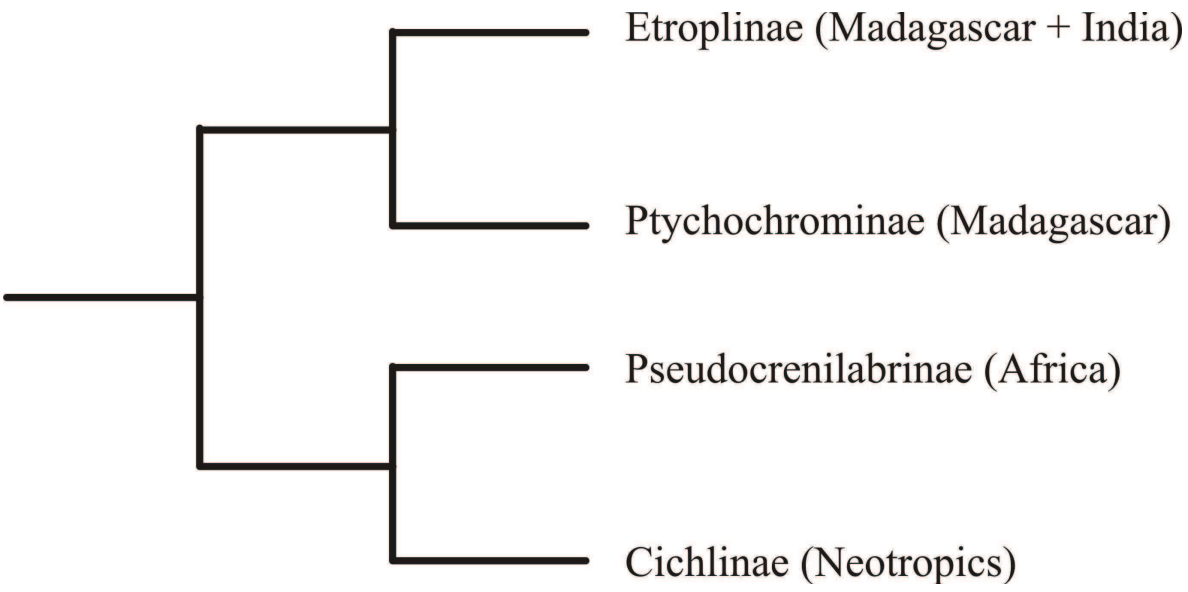


Fig. 1 – Schematic phylogeny of cichlid subfamilies. Adopted and simplified from Sparks and Smith, 2004.

Research on the African riverine cichlids

Obviously, the most of research focus performed on African cichlids is oriented to the lacustrine species flocks from the Malawi, Tanganjika and Victoria lakes (e.g., Strumbauer et al., 2010, Koblmuller et al., 2008, Genner et al., 2007), although riverine cichlids has been also intensively studied covering the major river systems in sub-Saharan Africa (Markert et al, 2010, Katongo, 2005). In contrast to the Neotropics, in Africa large portion of biodiversity still remains hidden for any broader survey, because of the regions with persistent and long-lasting limited accessibility (i. e. tropical rainforest region in Congo, inland parts of Angola). (Musilová et al, in prep.).

Evolution of the Neotropical cichlids

The rate of the Neotropical cichlids diversity exploration is quite constant, covering representatives of most known genera/forms and biogeography areas. Actually, more than 50 new species of neotropical cichlids were described during last five years (2006-2010; Froese and Pauly, 2010). Generally, the survey is still ongoing, every year several new cichlid species are continuously described.

The molecular phylogenetic studies of the Netropical cichlids (Cichlinae) are running since late 90´s, when the first 40 species were genetically analysed using mitochondrial markers (Farias et al., 1999, Roe et al., 1997). The subsequent studies revealed more detailed phylogenies leading to our present level of knowledge by enlarging the taxon sampling and including more genetic markes (Farias et al, 2000, Farias et al, 2001, Sparks and Smith, 2004, Lopez – Fernandez, 2005, Perez et al., 2007, Smith et al., 2008, Musilová et al, 2008, Říčan et al, 2008, Musilová et al., 2009, Lopez-Fernandez, 2010).

The major part of the Neotropical cichlid diversity is concentrated in the three species-rich lineages: Geophagini, Cichlasomatini, and Heroini. Further, there are four smaller lineages resulting mainly in the basal positions of the Neotropical cichlids and including just few species (See Fig. 2). While the monophyly of these lineages is strongly supported in most of the analyses (Lopez-Fernandez, 2010), the deeper phylogeny describing the relationships among lineages is not yet well establishedand further research is required focusing mainly on these basal lineages.

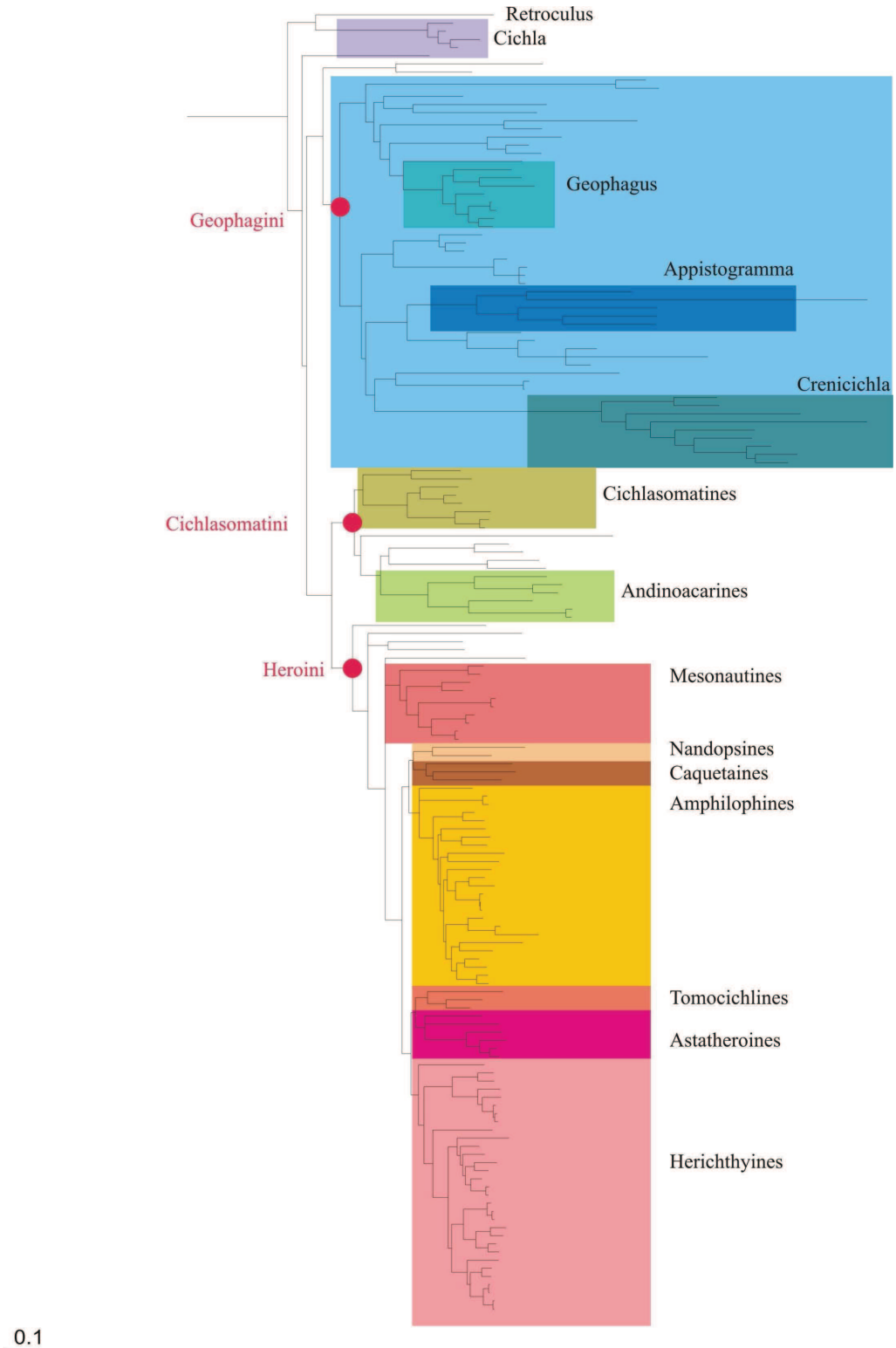


Fig. 2 – Phylogeny of neotropical cichlids presented in Lopez-Fernandez, 2010. Three species-rich tribes marked by red circle. Representative genera or generic assemblages highlighted.

In closer view, the tribes Heroini with mainly Central American distribution, and Cichlasomatini with South American distribution represent the groups best covered by the

robust phylogenetic studies (Perez, 2007, Říčan et al., 2008, Musilová et al., 2008, Musilová et al., 2009) including the representative sampling of all genera or species groups. In contrast, within the tribe Geophagini, large amount of the species is still missing in any analysis, especially from the species-rich genus *Apistogramma*, representing one of the potential radiation lineages (Lopez-Fernandez, 2010).

The reliable and robust reconstruction of phylogeny is not necessary only for taxonomic and systematic purpose, but it is essential also for the further evolutionary interpretation of the biogeography patterns, selected morphology traits or behavioral or evolutionary-developmental studies (Avise, 2001). The robustness of any molecular phylogeny depends directly 1) on sufficient selection of genetic markers, but also 2) on the taxon sampling covering the representative portion of the biodiversity involved in the investigated groups.

Recent development of South American geography

The uplift of Andes represents the geological event that strongly influenced the composition of the river systems as well as the distribution patterns of fauna. Although the uplift initiated already in the Cretaceous (Lundberg et al., 1998), its major effect to the continental geological changes was found during the Tertiary (Antonelli et al., 2009). The Andes were not uplifted as a one continuous ridge. Northern and Central Andes were separated in the recent Ecuador/Peru area by the Western Andean Portal up to Miocene (Antonelli et al., 2009) (Fig. 3). This portal formerly represented the connection from the South American continent to the Pacific and possible marine incursion could occur here (Santos et al., 2009). From the recent estuaries of Amazon in Brazil, Paraná in Argentina, and the Maracaibo lake in Venezuela, other marine incursions into the continental inland occurred periodically during the water level fluctuation (Lundberg et al., 1998, Montoya-Burgos, 2003). Existence of the other large water assemblage – ancient lake Pebas in the recent Western Amazon – is dated to 17-11 mya (Antonelli et al., 2009). It is not clear if it represented the residual of the former marine incursion or if it was strictly freshwater formation (Antonelli et al., 2009) (see Fig. 3).

The river systems of South America changed significantly during last 10-20 my (Lundberg et al., 1998, Santos et al., 2009). The Amazon river system was originally represented only by a short river flowing to the recent estuary, and a larger portion of the recent Amazon tributaries flowing in the opposite direction to a different river system, Paleo-Amazon-Orinoco (Fig. 3). This large river was flowing northward along Andes and it existed

at least since the Cretatious/Tertiary boundary, when the first Andean ridge appeared as a western barrier. The recent Amazon river was formed about 8 mya, when the ongoing uplift definitely disrupted the existence of the previous formations. The Orinoco river basin had the very similar development as Amazon (Lundberg et al., 1998).

Fig. 3 – The marine incursions in Amazon, Paraná and Maracaibo estuaries and Western Amazon Portal with the hypothetical connection between Pacific and the cis-Andean South America, the early Miocene. The Paleo-Amazon-Orinoco river system existed from the Cretaceous/Tertiary till 8 mya, when the recent Amazon was formated. Position of the large lake of Pebas during the middle Miocene. Adapted from Lundberg et al, 1998 and Santos et al., 2009.

Cichlid biodiversity patterns in South America emphasizing the study area

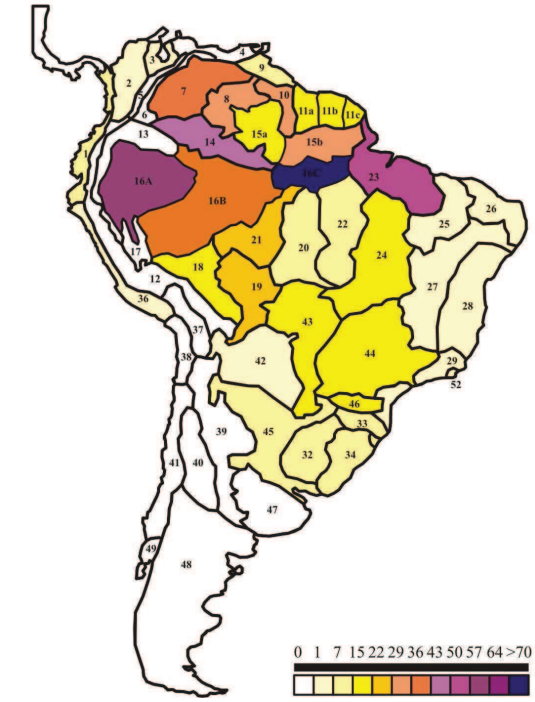
For visualisation of the patterns in the recent distribution of riverine cichlids I focused on the composition of the communities in the Freshwater Ecoregions (herein 'ecoregins';

Abell et al., 2008). The ecoregions were defined as suitable units for any following biogeographic and ecological analyses. This huge study proposed the ecoregions in all continents, based on the known distribution information from all freshwater fishes. For the biogeographic analyses presented in this thesis, the biogeographic regions were proposed with respect to the ecoregion units (i.e. respecting defined borders), although some of the ecoregions comprises huge area and for more detailed biogeography reconstruction in cichlids could not be suitable. The all available distribution data of the Neotropical cichlids was collected from literature and web (mainly from Froese and Pauly, 2010, Stawikowski and Werner, 1998, Stawikowski and Werner, 2004, Koslowski, 2002, Romer, 2006) and summarised through the ecoregions. See Fig 4-6 for the distribution patterns of all species of the South American cichlids.

The majority of the Neotropic cichlid diversity lies in the cis-Andean region, with the richest regions in western Amazon, Rio Negro, eastern Amazon and Orinoco. See Figs. 4-6. An important part of the biodiversity occurs also in Central America (not presented herein), where the radiation of Heroine cichlids occurred (Lopez-Fernandez et al., 2010).

The trans-Andean region covering the Pacific slope river drainages in South America represents the relatively species-poor region (Musilová et al., 2008). Representatives of four cichlid groups are found there totalling about 15 species. Two genera are widely distributed through the region and the other two occur just in the northernmost part of Choco. The trans-Andean organisms could be suitable for the biogeography studies, as the Andean uplift represents one of the major geological events impacting distribution patterns in South America. Study of this region could help during the reconstruction of contacts and colonisations 1) between the two slopes of Andes and 2) between South and Central America. Last but not least, the biodiversity in the trans-Andean region could be covered more completely than in the much larger and species-richer Amazonia.

A) cichlids - valid species (373 species)



B) cichlids - valid species + undescribed forms (561 species)

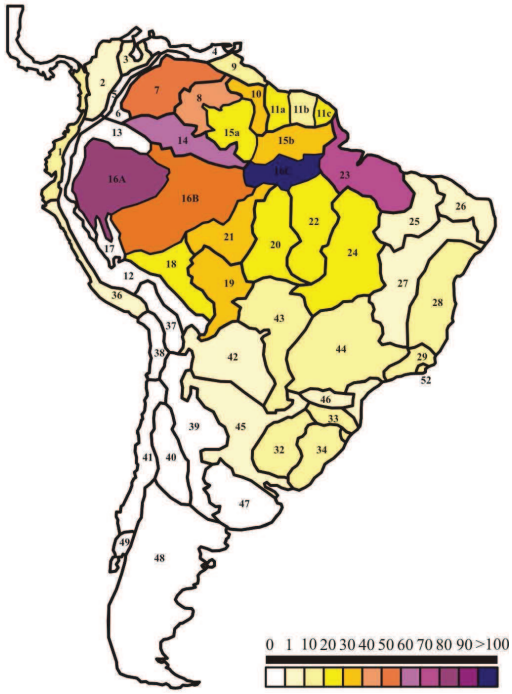
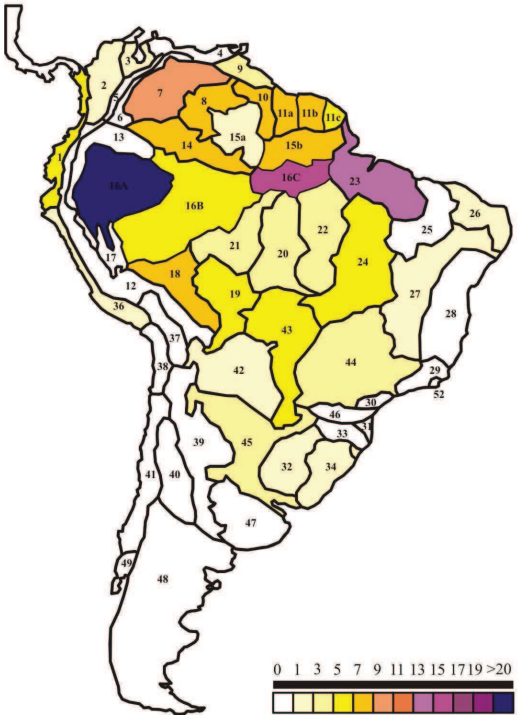


Fig. 4 - Diversity of the Neotropical cichlids in the ecoregions. A) data of all valid cichlid species, B) data of all valid species + underscribed forms. Ecoregion definition from Abell et al., 2008. Numbers in regions correspond to ecoregion number. The colour of ecoregion corresponds with the number of species as shown in the line below the map.

C) Cichlasomatini



D) Heroini

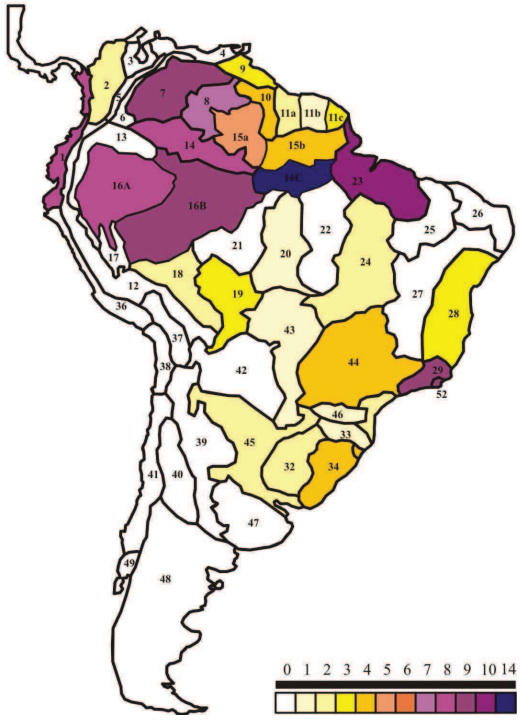


Fig. 4, cont. – C) the diversity of Cichlasomatines, D) diversity of Heroines through South America; the major portion of heroine cichlids is however concentrated in Central America

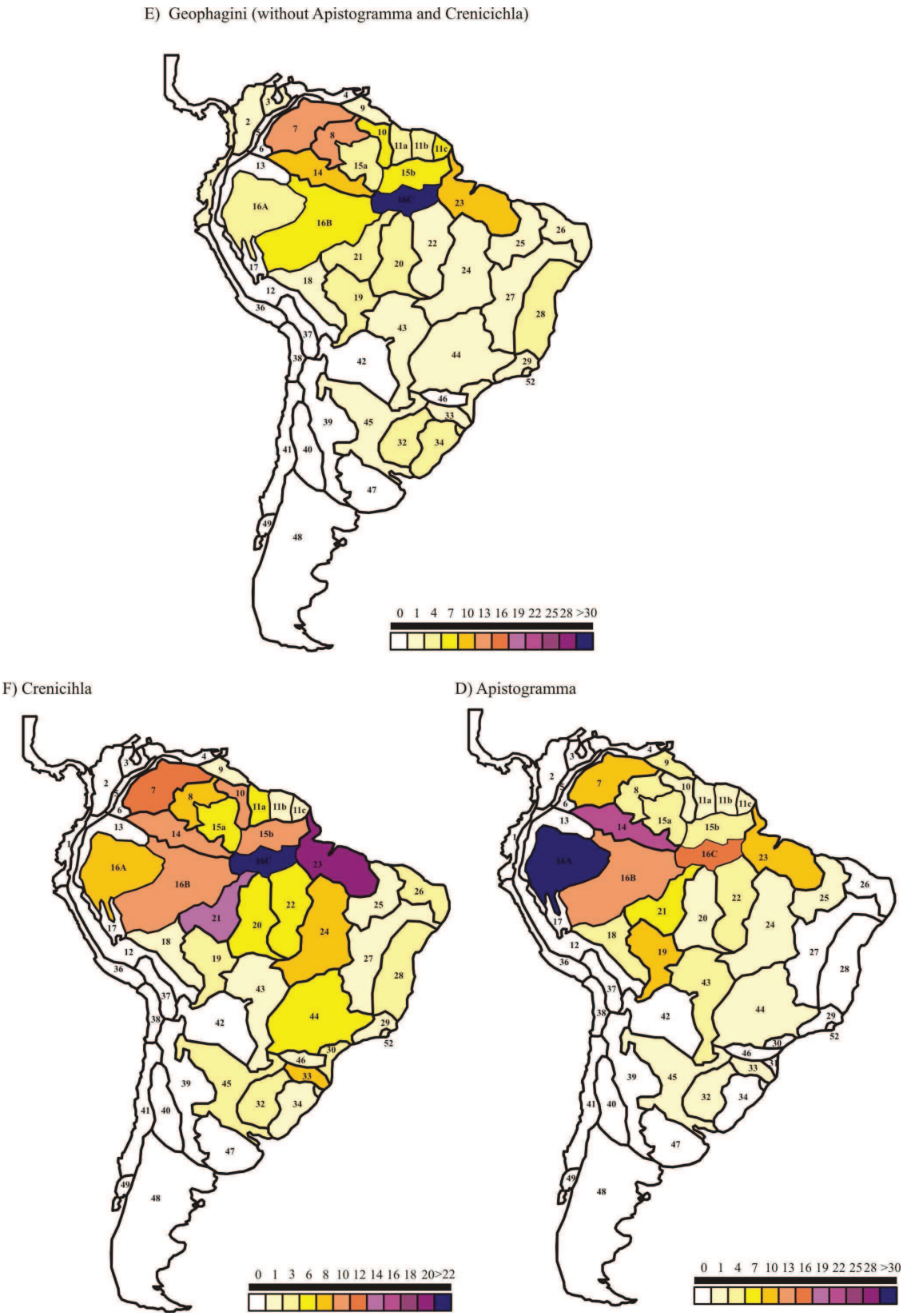


Fig. 4. cont. – E) the diversity of Geophanes without the species-rich genera Apistogramma and Crenicichla, F) diversity of the genus Crenicichla, and G) Apistogramma.

References:

Antonelli A, Nylander JAA, Persson C, et al. 2009 Tracing the impact of the Andean uplift on Neotropical plant evolution. *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA,* 106, 9749-9754.

Avise, J 2001 *Phylogeography: The History and Formation of Species* Harvard University Press, Cambridge, London.

Farias, I. P., Orti, G., Sampaio, I., Schneider, H., Meyer, A. 1999. Mitochondrial DNA Phylogeny of the Family Cichlidae: Monophyly and Fast Molecular Evolution of the Neotropical Assemblage. *JOURNAL OF MOLECULAR EVOLUTION*, 48: 703-711.

Farias, I.P., Orti G., Sampaio, I., Schneider, H., Meyer, A. 2001 The Cytochrom b Gene as a Phylogenetic Marker: The Limits of Resolution for Analyzing Relationships Among Cichlid Fishes. *JOURNAL OF MOLECULAR EVOLUTION*, 53: 89-103

Froese, R., Pauly, D. 2010: *FishBase*. www.fishbase.org (accessed December, 2010).

Genner MJ, Seehausen O, Lunt DH, et al. 2007 Age of cichlids: New dates for ancient lake fish radiations. *MOLECULAR BIOLOGY AND EVOLUTION,* 24: 5.

Katongo C, Koblmuller S, Duftner N, et al. 2005 Phylogeography and speciation in the Pseudocrenilabrus philander species complex in Zambian Rivers. *HYDROBIOLOGIA,* 542: 221-233.

Koblmuller, S., Schliewen, U.K., Duftner, N., Sefc, K.M., Katongo C., Sturmbauer, C. 2008 Age and spread of the haplochromine cichlid fishes in Africa. *MOLECULAR PHYLOGENETICS AND EVOLUTION*, 49: 153-169.

Koslowski I. 2002 *Die Buntbarsche Südamerikas 02. Apistogramma und Co*. Ulmer (Eugen).

Kullander, S.O. 1998. A Phylogeny and Classification of the South American Cichlidae (Teleostei: Perciformes). Pp. 461-498. In: MALABARBA L. R., REIS R. P., LUCENA Z. M., LUCENA C. A. S. (Eds.): Phylogeny and Classification of Neotropical Fishes. Porto Edipucrs, Alegre, Brazil.

Lopez-Fernandez, H., Honeycutt, R.L., Winemiller, K.O. 2005 Molecular phylogeny and evidence for an adaptive radiation of geophagine cichlids from South America (Perciformes : Labroidei). MOLECULAR PHYLOGENETICS AND EVOLUTION 34: 227-244.

Lopez-Fernandez H, Winemiller KO, Honeycutt RL 2010 Title: Multilocus phylogeny and rapid radiations in Neotropical cichlid fishes (Perciformes: Cichlidae: Cichlinae). *MOLECULAR PHYLOGENETICS AND EVOLUTION,* 55: 1070-1086.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton B., Malabarba, M. C. S. L., Wesselingh, F. 1998: The stage for Neotropical fish diversification: a history of tropical South American rivers. Pp. 13-48. In: MALABARBA L. R., REIS R. P., LUCENA Z. M., LUCENA C. A. S. (Eds.): Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brazil. 603 pp.

Markert JA, Schelly RC, Stiassny MLJ 2010 Genetic isolation and morphological divergence mediated by high-energy rapids in two cichlid genera from the lower Congo rapids. *BMC EVOLUTIONARY BIOLOGY*, 10: 149.

Murray, A.M. 2001 The fossil record and biogeography of the Cichlidae (Actinopterygii: Labroidei). *BIOLOGICAL JOURNAL OF THE LINNEAN SOCIETY* 74: 517-532.

Musilová, Z., Říčan, O., Janko, K., Novák, J. 2008 Molecular phylogeny and biogeography of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae), Molecular phylogenetics and evolution, 46:2, 659-672.

Musilová, Z., Říčan, O., Novák, J., 2009a Phylogeny of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus, *JOURNAL OF ZOOLOGICAL SYSTEMATICS AND EVOLUTIONAIRY RESEARCH*, 47: 234-247

Musilová, Z., Schindler, I., Staeck, W. 2009b Description of Andinoacara stalsbergi sp. n. (Teleostei: Cichlidae: Cichlasomatini) from Pacific coastal rivers in Peru, and annotation on the phylogeny of the genus. *VERTEBRATE ZOOLOGY*, 59: 131-141.

Nelson JS 1994 *Fishes of the world*. John Wiley & Sons, INC. Canada.

Perez GAC, Rican O, Orti G, et al. 2007 Phylogeny and biogeography of 91 species of heroine cichlids (Teleostei : Cichlidae) based on sequences of the cytochrome b gene. *MOLECULAR PHYLOGENETICS AND EVOLUTION,* 43: 91-110.

Rican O, Zardoya R, Doadrio I 2008 Phylogenetic relationships of Middle American cichlids (Cichlidae, Heroini) based on combined evidence from nuclear genes, mtDNA, and morphology. *MOLECULAR PHYLOGENETICS AND EVOLUTION,* 49: 941-957.

Roe KJ, Conkel D, Lydeard C 1997 Molecular systematics of middle American cichlid fishes and the evolution of trophic-types in 'Cichlasoma (Amphilophus)' and 'C-(Thorichthys)'. *MOLECULAR PHYLOGENETICS AND EVOLUTION,* 7: 366-376.

Romer, U. 2006 *Baensch/Mergus Cichlid Atlas, Vol. 2* Mergus Verlag GmbH.

Santos JC, Coloma LA, Summers K, et al. 2009 Amazonian Amphibian Diversity Is Primarily Derived from Late Miocene Andean Lineages. *PLoS BIOLOGY,* 7: 448-461.

Smith WL, Chakrabarty P, Sparks JS 2008 Phylogeny, taxonomy, and evolution of Neotropical cichlids (Teleostei : Cichlidae : Cichlinae). *CLADISTICS,* 24: 625-641.

Sparks JS 2008 Phylogeny of the cichlid subfamily Etroplinae and taxonomic revision of the Malagasy cichlid genus Paretroplus (Teleostei : Cichlidae). *BULLETIN OF THE AMERICAN MUSEUM OF NATURAL HISTORY,* 314: 5-151.

Sparks, J.S., Smith, W.L. 2004 Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). *CLADISTICS* 20: 501-517.

Stawikowski, R., Werner, U. 1998 *Die Buntbarsche Amerikas. Band 1.* Eugen Ulmer GmbH and Co., Stuttgart, Germany.

Stawikowski, R., Werner, U., 2004 Die Buntbarsche Amerikas, Band 3: Erdfesser Hecht und Kammbuntbarsche. Eugen Ulmer Verlag.

Stiassny, M.L.J. (1991) *Phylogenetic intrarelationships of the family Cichlidae: An overview. Cichlid Fishes: Behaviour, Ecology and Evolution* (ed. By Keenleyside, M.H.E.), pp. 1-35. Croom-Helm, London,

Sturmbauer, C., Salzburger, W., Duftner, N., Schelly, R., Koblmuller, S. 2010 Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data. *MOLECULAR PHYLOGENETICS AND EVOLUTION*, 57: 266-284.

Musilová Z., Říčan O., Janko K., Novák J.

2008

Molecular phylogeny and biogeography of the Neotropical cichlidfish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae)

Molecular Phylogenetics and Evolution **46: 659-672**

Scientific paper

To whom it may concern

I declare that Zuzana Musilova performed significant portion of work on our manusript:

MUSILOVÁ, **Z.**, ŘÍČAN, O., JANKO, K. AND NOVÁK, J., 2008 - Molecular phylogeny and biogeography of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae), *Molecular phylogenetics and evolution,* 46:2, 659-672.

In behalf of co-author,

In České Budějovice, 6.1.2011 Oldřich Říčan

Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution 46 (2008) 659–672
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Molecular phylogeny and biogeography of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae)

Zuzana Musilová [a,b,*], Oldřich Říčan [b,c], Karel Janko [b], Jindřich Novák [c]

[a] Department of Zoology, Charles University in Prague, Viničná 7, 128 44 Praha, Czech Republic
[b] Institute of Animal Physiology and Genetics of the Academy of Sciences of the Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic
[c] Department of Zoology, Faculty of Sciences, University of South Bohemia, Branišovská 31, 37005 České Budějovice, Czech Republic

Received 31 May 2007; revised 5 October 2007; accepted 11 October 2007
Available online 22 October 2007

Abstract

We have conducted the first comprehensive molecular phylogeny of the tribe Cichlasomatini including all valid genera as well as important species of questionable generic status. To recover the relationships among cichlasomatine genera and to test their monophyly we analyzed sequences from two mitochondrial (16S rRNA, cytochrome *b*) and one nuclear marker (first intron of S7 ribosomal gene) totalling 2236 bp. Our data suggest that all genera except *Aequidens* are monophyletic, but we found important disagreements between the traditional morphological relationships and the phylogeny based on our molecular data. Our analyses support the following conclusions: (a) *Aequidens* sensu stricto is paraphyletic, including also *Cichlasoma* (CA clade); (b) *Krobia* is not closely related to *Bujurquina* and includes also the Guyanan *Aequidens* species *A. potaroensis* and probably *A. paloemeuensis* (KA clade). (c) *Bujurquina* and *Tahuantinsuyoa* are sister groups, closely related to an undescribed genus formed by the '*Aequidens*' *pulcher*–'*Aequidens*' *rivulatus* groups (BTA clade). (d) *Nannacara* (plus *Ivanacara*) and *Cleithracara* are found as sister groups (NIC clade). *Acaronia* is most probably the sister group of the BTA clade, and *Laetacara* may be the sister group of this clade. Estimation of divergence times suggests that the divergence of Cichlasomatini started around 44 Mya with the vicariance between coastal rivers of the Guyanas (KA and NIC clades) and remaining cis-andean South America, followed by evolution of the *Acaronia–Laetacara*–BTA clade in Western Amazon, and the CA clade in the Eastern Amazon. Vicariant divergence has played importantly in evolution of cichlasomatine genera, with dispersal limited to later range extension of species within genera.

Keywords: Molecular phylogeny; Cichlids; Cichlidae; Cichlasomatini; South America; Biogeography; Penalized likelihood; Partitioned Bremer support; Cytochrome *b*; 16S rRNA; S7; DIVA; Vicariance; Dispersal

1. Introduction

Neotropical cichlids are extremely varied in morphology, behaviour and ecology (Lowe-McConnell, 1991) despite comprising fewer species than their relatives in African lakes. Since the early 20th century, when Regan (1905a,b,c, 1906a,b) completely revised the group, the number of species and genera has increased considerably. Kullander (2003) summarizes the cichlid diversity in the Neotropics as including 54 genera and 407 species. These numbers are likely to increase significantly, as there still are many undescribed species and genera (e.g. Kullander and Ferreira, 2006; Kullander and Lucena, 2006; López-Fernández et al., 2006; Říčan and Kullander, 2006).

The cichlid subfamily Cichlasomatinae has one of the most complex taxonomic histories among cichlid groups in the Neotropics and no other group of Neotropical cichlids has witnessed so many taxonomic changes and new descriptions at the genus level. The subfamily Cichlasomatinae was formally diagnosed by Kullander (1998) and today includes species placed during the 19th and 20th cen-

* Corresponding author. Address: Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic.
E-mail address: zuzmus@email.cz (Z. Musilová).

1055-7903/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2007.10.011

tury mainly in the genera *Cichlasoma* and *Aequidens*. Kullander (1998) also divided the subfamily into two tribes, the Heroini and the Cichlasomatini. Since this seminal work, both the monophyly and division of the subfamily have been supported by independent data (Farias et al., 1999, 2000, 2001; Sparks, 2004; Marescalchi, 2005). Cichlasomatine species have mostly been associated with the genus *Aequidens*, while most heroines have been placed in the catchall genus *Cichlasoma*. Since Kullander (1998) the genus *Cichlasoma* has been placed in the tribe Cichlasomatini, while most of the species previously constituting *Cichlasoma* are now in the tribe Heroini (type genus *Heros* Heckel 1840). Kullander's studies (1983a, 1986; Kullander and Nijssen, 1989) rejected the classification of Regan (1905a,b) based predominantly on the number of anal fin spines. Regan (op. cit.) placed Neotropical cichlids into two groups, one with more than three spines in the anal fin (nearly all placed in former *Cichlasoma* and most of them distributed in Central America), and the other one with three anal fin spines, distributed in many genera (and subfamilies according to current classification).

At present, the Neotropical cichlid fish tribe Cichlasomatini Kullander, 1998 comprises 69 valid species placed in 10 valid genera (*Aequidens*, *Bujurquina*, *Cichlasoma*, *Cleithracara*, *Ivanacara*, *Krobia*, *Laetacara*, *Nannacara*, *Tahuantinsuyoa* and *Acaronia*). The generic assignment of several species is questionable (e.g. '*Aequidens*' *potaroensis*, '*Aequidens*' *paloemeuensis*, '*Aequidens*' *hoehnei*; Kullander, 1998) and some species groups likely represent unnamed genera (i.e. the '*Aequidens*' *pulcher* group and '*Aequidens*' *rivulatus* group; Kullander, 1998). Species of the genera *Aequidens*, *Bujurquina*, *Krobia*, *Cleithracara* and *Laetacara* were formerly placed in the catchall genus *Aequidens* previous to the taxonomic revisions of Kullander (1986) and Kullander and Nijssen (1989), which excluded them from *Aequidens*. In addition to its current twelve species, *Cichlasoma*, another catchall group, contained also the majority of Mesoamerican cichlids according to Regan (1905b). These have been later classified as the tribe Heroini (Kullander, 1998). A revision of the Mesoamerican Heroini formerly placed in *Cichlasoma* is still pending, but recent studies have contributed to that goal (Roe et al., 1997; Martin and Bermingham, 1998; Hulsey et al., 2004; Chakrabarty, 2006; Říčan and Kullander, 2006; Concheiro Pérez et al., 2007). The South American ex-*Cichlasoma* (now Heroini) have a stable genus-level taxonomy (*Caquetaia* and *Heroina*—Kullander, 1996; *Australoheros*—Říčan and Kullander, 2006). The genus *Nannacara* established by Regan (1905a) remained without intrageneric changes until 2007, when Römer and Hahn (2007) described and separated the genus *Ivanacara* for two species of *Nannacara*. The genus *Acaronia* has been variously assigned to Heroini (Stiassny, 1991) or a separate tribe Acaroniini (Kullander, 1998). These placements based on morphology are refuted by all molecular analyses, which clearly place *Acaronia* among the Cichlasomatini (Farias et al., 1999, 2000, 2001; Sparks and Smith, 2004; Marescalchi, 2005; Concheiro Pérez et al., 2007).

The distribution of Cichlasomatini covers most of cisandean South America and to a lesser extent also transandean South America including lower Central America. Therefore, their distribution area covers biogeographically crucial regions such as both Andean slopes, the Amazon basin and the old geological formations (the Guyana and Brazilian shields). Cichlasomatini are thus an ideally suited model group for the study of historical biogeography and evolutionary processes in the Neotropics, especially in combination with their sister group, the predominantly mesoamerican Heroini.

Kullander (1998) presented the first morphology-based phylogeny which was not able to convincingly determine relationships between and within the genera of Cichlasomatini. No robust tests of the relationships among genera or their monophyly have been performed to date. Using molecular markers and extensive taxonomical sampling we evaluated for the first time the monophyly of cichlasomatine genera and their relationships. Our results contribute to the ongoing investigation of the World's richest (Neotropical) freshwater biota, its diversity and biogeographic history.

2. Materials and methods

2.1. Taxon sampling

Representative species of all valid genera of the tribe Cichlasomatini as well as the genus *Acaronia* were included in this study. We have strived to include multiple morphologically and geographically distant species for each genus to have as representative sampling as possible. The taxon sampling includes 47 OTUs representing 41 species for all three genes studied, i.e. the mitochondrial genes for cytochrome *b* (cyt *b*) and 16S rRNA and the nuclear intron in the ribosomal S7 gene. Specimens were wild-caught and obtained from ornamental-fish importers with reliable locality data, aquarium populations were used as well (Table 1). Representative species of Heroini and Geophagini were used as outgroup taxa.

2.2. DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted from a fin clip (approx. 5×5 mm) using Dneasy® Tissue Kit (Qiagen), following the manufacturer's protocol. The polymerase chain reactions (PCRs) used 1 μl of DNA as templates. Primers used in the study are the following: for the 16S rRNA gene we used the forward mtD-32 (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) and the reverse mtD-34 (5′-CGC CTG TTT AAC AAA AAC AT-3′; both Marescalchi, 2005). The S7 intron primers S7RPE-X1F (5′-TGG CCT CTT CCT TGG CCG TC-3′) and S7RPE-X2R (5′-AAC TCG TCT GGC TTT TCG CC-3′) were from Chow and Hazama (1998). The following combinations of cyt *b* prim-

Table 1
Species examined

Taxon name	Source/locality	Collection number	Accession number		
			16S	cyt *b*	S7
Geophagus brasiliensis	Aquarium stock	ICCU 0700	AF049016	AF370659	EU199082
Thorichthys meeki	Aquarium stock	ICCU 0701	AY279669	U88860	EU199083
'*Aequidens*' *biseriatus*	Rio Atrato, Colombia	ICCU 0702	EF432902	EF432958	EF432994
'*Aequidens*' *coeruleopunctatus*	Panama	ICCU 0703	EF432905	EF432957	EF432997
'*Aequidens*' *pulcher* AKV	Aquarium stock	ICCU 0704	EF432889	EF432944	EF432981
'*Aequidens*' cf. *pulcher* "*Venezuela*"	Cunaviche, Los Llanos, Venezuela	ICCU 0705	EF432888	EF432928	EF432980
'Aequidens' cf. *pulcher* "Rio Chirgua"	Rio Chirgua, Carabobo, Venezuela	ICCU 0706	EF432890	EF432934	EF432982
'*Aequidens*' *pulcher* "Trinidad"	Trinidad	ICCU 0707	EF432887	EF432943	EF432979
'*Aequidens*' *rivulatus*	Rio Guayaquil, Ecuador	ICCU 0708	EF432885	EF432935	EF432977
'*Aequidens*' *rivulatus* AKV	Aquarium stock	ICCU 0709	EF432886	EF432935	EF432978
'*Aequidens*' sp. "Maracaibo"	Maracaibo, Venezuela	ICCU 0710	EF432904	EF432955	EF432996
Acaronia nassa	Peru	ICCU 0711	EF432897	EF432937	EF432989
Aequidens diadema	Tobogán, Orinoco, Venezuela	ICCU 0712	EF432880	EF432930	EF432972
Aequidens chimantanus	Rio Caroní, Venezuela	USBZC 1011	EF432884	EF432938	EF432976
Aequidens metae	Pto Ayacucho, Venezuela	ICCU 0713	EF432882	EF432927	EF432974
Aequidens patricki	Aquarium stock	ICCU 0714	EF432876	EF432913	EF432968
Aequidens potaroensis	Las Claritas, Venezuela	USBZC 1010	EF432870	EF432917	EF432962
Aequidens sp. "Atabapo"	Rio Atabapo, Colombia	ICCU 0715	EF432881	EF432924	EF432973
Aequidens sp. "Jenaro Herrera"	Jenaro Herrera, Peru	ICCU 0716	EF432912	EF432956	EF433004
Aequidens sp. Jarú	Rio Jaru, Rondonia, Brazil	ICCU 0717	EF432878	EF432926	EF432970
Aequidens tetramerus "Venezuela"	Las Claritas, Venezuela	ICCU 0718	EF432893	EF432929	EF432985
Aequidens tetramerus "Rio Negro"	Rio Negro, Brazil	ICCU 0719	EF432879	EF432936	EF432971
Aequidens tetramerus "Peru"	Peru	ICCU 0720	EF432911	EF432949	EF433003
Aequidens tubicen	Rio Trombetas, Brazil	ICCU 0724	EF432883	EF432945	EF432975
Bujurquina vittata	Chaco, Argentina	ICCU 0726	EF432892	EF432951	EF432984
Bujurquina peregrinabunda	Aquarium stock	ICCU 0727	EF432907	EF432954	EF432999
Bujurquina syspilus	Aquarium stock	ICCU 0728	EF432906	EF432952	EF432998
Bujurquina sp. "Maicurú"	Maicurú	ICCU 0729	EF432908	EF432953	EF433000
Cichlasoma amazonarum	Iquitos, Peru	ICCU 0730	EF432875	EF432914	EF432967
Cichlasoma bimaculatum	Rio Tacutu, Bonfim, Brazil	ICCU 0731	EF432874	EF432925	EF432966
Cichlasoma cf. *araguaiense*	Rio Xingu, Altamira, Brazil	ICCU 0732	EF432877	EF432923	EF432969
Cichlasoma dimerus	Rio Paraguay, Paraguay	ICCU 0733*	EF432872	EF432941	EF432964
Cichlasoma orinocense	Orinoco estuary, Venezuela	ICCU 0734	EF432873	EF432922	EF432965
Cichlasoma cf. *pusillum*	South America, exact locality unknown	ICCU 0735	EF432871	EF432916	EF432963
Cleithracara maronii	Aquarium stock	ICCU 0736	EF432901	AY050614	EF432993
Krobia cf. *guianensis*	Aquarium stock	ICCU 0737*	EF432910	EF432933	EF433002
Krobia sp. "Oyapock"	Oyapock River, French Guyana	ICCU 0738	EF432868	EF432932	EF432960
Krobia sp. "Xingu"	Rio Xingu, Cachoeira Parati, Brazil	ICCU 0739	EF432869	EF432931	EF432961
Laetacara curviceps	Aquarium stock	ICCU 0740	EF432895	EF432918	EF432987
Laetacara dorsigera	Aquarium stock	ICCU 0741	EF432894	EF432919	EF432986
Laetacara sp. "Buckelkopf"	Aquarium stock	ICCU 0742	EF432896	EF432940	EF432988
Laetacara thayeri	Aquarium stock	ICCU 0744	EF432909	AY050608	EF433001
Ivanacara adoketa	Aquarium stock	ICCU 0745	EF432903	EF432946	EF432995
Nannacara anomala	Aquarium stock	ICCU 0746	EF432898	AY050618	EF432990
Nannacara aureocephalus	Aquarium stock	ICCU 0747*	EF432899	EF432939	EF432991
Nannacara taenia	Aquarium stock	ICCU 0749	EF432900	EF432921	EF432992
Tahuantinsuyoa macantzatza	South America, exact locality unknown	ICCU 0750	EF432891	EF432915	EF432983

The asterisk (*) in the collection number marks the sample, where only the fin tissue sample was obtained. The accession numbers beginning with EF and EU mean original sequences from this study. Different letters are sequences downloaded from GenBank. ICCU, Ichthyological collections of Charles University in Prague; USBZC, University of South Bohemia-Zoological Collections.

ers were used: forward Glu-DGL (5′-TGA CTT GAA RAA CCA YCG TTG-3′; Palumbi et al., 1991), Fish-CytB-F (5′-ACC ACC GTT GTT ATT CAA CTA CAA GAA C-3′) and reverse H15149 (5′-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3′; Kocher et al., 1989), H15915 (5′-AAC TGC CAG TCA TCT CCG GGT TAC AAG AC-3′; Irwin et al., 1991), Cb6b.H (GGA ATT CAC CTC TCC-3′; Martin and Bermingham, 1998) and TrucCytB-R (5′-CCG ACT TCC GGA TTA CAA GAC CG-3′). The PCR volume of 25 μl contained PPP master mix (Top-Bio) including $MgCl_2$ (2.5 μM), both forward and reverse primers (0.4 μM) and 1 μl of DNA sample. The reactions for all fragments consisted of an initial denaturation step of 94 °C (2 min), followed by 35–40 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at 46–50 °C for cyt *b*, 48 °C for 16S rDNA and 60 °C for the S7 intron and extension at 72 °C for 1 min. The terminal extension was at 72 °C for 8 min. The reac-

tions were performed on thermo-cyclers PTC-200 (MJ Research) and iCyclerTM Thermal Cycler (Bio-Rad).

PCR products were purified using the QIAquick® PCR Purification Kit (Qiagen) and directly used as a template for the sequencing reaction using the ABI PRISM® BigDye™ Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems). Sequences were read on the 3130 Genetic Analyser (Applied Biosystems, Hittachi) automatic sequencer.

2.3. Phylogenetic analyses

Phylogenetic analyses included 47 taxa sequenced for two mitochondrial genes (16S rRNA gene, cyt *b* gene) and one nuclear marker (first intron in the S7 gene). All obtained sequences were submitted to GenBank (Accession Nos. EF432868–EF433004).

Sequences were aligned using the Clustal W program with the default settings as implemented in the BioEdit software package (Hall, 1999) and then were manually edited. The fragment of 16S rRNA was aligned with respect to its secondary structure (Moyer et al., 2004). The amino acid translation of the cyt *b* sequences was examined for stop codons. Each gene was analyzed separately before a combined analysis was performed in order to explore topological differences and possible sources of conflict between the signals of individual genes. Additionally we performed saturation test in cytochrome *b* dataset using PAUP* 4.0b10 (Swofford, 2002).

Maximum parsimony (MP) analyses were performed in PAUP under the heuristic search with 500 random additions, holding 10 trees in memory in each step. In the next step we ran a search on the saved trees to find all the shortest trees (command: *hsearch addseq = random hold = 10 nchuck = 5 chuckscore = 1 nreps = 1000; hsearch start = current nchuck = 0 chuckscore = 0*). Branch support was estimated with 1000 bootstrap replicates and also using Partitioned Bremer Support (PBS; Baker and DeSalle, 1997) performed in PAUP* using constraint topologies and the converse constraints command. The PBS reveals the support for each node from every gene separately and was used to pinpoint possible sources of conflict between datasets at individual nodes, since it is a commonly used measure of congruence in the genetic signal (e.g. Wahlberg et al., 2005).

Maximum likelihood (ML) analyses were conducted in PAUP* under the evolutionary model that best fit the analyzed sequence dataset. The model was selected using Modeltest version 3.7 and the hierarchical likelihood (hLRT) criterion (Posada and Crandall, 1998). Bootstrap analysis with 1000 replications was performed in the software PhyML (Guindon and Gascuel, 2003).

Bayesian analyses were performed in MrBayes, version 3.1 (Ronquist and Huelsenbeck, 2003). The Bayesian tree was inferred using the models suggested by Modeltest. We analyzed every gene separately, as well as in the combined dataset. The analyses were performed with 1,000,000 (genes separately) to 2,500,000 (combined dataset) generations, sampling every 100 trees using two parallel runs with four chains. The final burnin was 40% of sampled trees (i.e. 40,000 from the run of 1,000,000 generations). The combined dataset was partitioned into loops and stems in the case of the 16S rRNA gene and codon positions in the cytochrome *b* gene. Each analysis was replicated to ensure that convergence was reached.

Monophyly of genera and higher clades was additionally tested by the approximately unbiased test (AU test; Shimodaira, 2002) as implemented in the software Consel (Shimodaira and Hasegawa, 2001). This test assesses the significance of differences in likelihood scores between the best maximum likelihood (ML) tree and the tree constructed under the user-defined constraints. We thus constructed the tree with the contrained monophyly of selected clades and tested whether there is significant difference against the best ML tree. The likelihood scores of constrained and unconstrained trees were then compared by AU test.

We also applied the incongruence length difference partition homogeneity test (ILD; Farris et al., 1995) as implemented in PAUP to assess whether there are significant topological differences between individual gene-trees before combining all loci into single phylogenetic analysis. This test was run under 1000 replicates with the randomly selected characters from dataset (Farris et al., 1995).

2.4. Dating of divergences and biogeography

Since our dataset deviated from clocklike behaviour, we could not use standard molecular clock estimation. Instead, we have performed the Penalized likelihood analysis (Sanderson, 2002) in R8S software (Sanderson, 2003) which allows to estimate absolute substitution rates and divergence times under a relaxed molecular clock using truncated Newton (TN) algorithm.

We used ML topology with branch length as input tree for the analyses. The penalty function was set to additive and cross validation was used to assess the most appropriate value of smoothing. We have used geological dating to calibrate the penalized likelihood tree. As a point of the geological calibration we used the final separation of Paraná–Amazon drainages (10–11.8 Mya; Lundberg et al., 1998; Montoya-Burgos, 2003) evident in the phylogeny of the genera *Cichlasoma* and *Bujurquina*.

The geographic distribution of individual species was obtained from the Neodat II and Fishbase (www.fishbase.org) databases (Froese and Pauly, 2006) and from Stawikowski and Werner (1998). The studied samples are coded as present in eight ichthyological provinces of South America (Hubert and Renno, 2006). Dispersal–vicariance analysis as implemented in DIVA version 1.1 (Ronquist, 1997) was used to explain the biogeographic history of cichlasomatine cichlid fishes in South America in terms of vicariance, dispersal and extinctions. This method places one or more ancestral areas (depending upon the con-

straints imposed) at each internal node so as to minimize the costs associated with dispersal and extinction events (Ronquist, 1997).

3. Results

3.1. Phylogenetic analyses

The final alignment of 47 taxa consisted of a 615 bp fragment of the 16S rRNA, the complete cyt *b* gene (1111 bp) and 510 bp of the first intron in the S7 gene (2236 bp in total). The ILD test allowed combining all data partitions in a single dataset ($p = 0.09$). We found slight tendency of saturation for cytochrome *b* gene in the third codon position. Nevertheless, when the third position was excluded from analyses, the tree topology remained generally unchanged. The only difference was lower resolution for some nodes.

Results of maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses (BI) of the combined dataset are shown in Fig. 1. The analyses resulted in very similar topologies, different only in the position of the genus *Krobia* and of the *Nannacara–Ivanacara–Cleithracara* clade. The ML and BI topologies are almost identical including estimated branch lengths, differing only in the position of *B. vittata–B. peregrinabunda* and *A. patricki–C. cf. araguaiense*.

Analyses of individual gene partitions also resulted in very similar topologies (not shown), with conflicts limited to nodes with low support. Possible sources of conflict between the data partitions were further studied by analyzing the PBS results, which found five points of conflict (see Fig. 1). All of these are nodes having low bootstrap support and four nodes also differ between the MP and ML (BI) topologies: (1) in the MP topology, *Krobia* sp. "Xingu" is the basal most *Krobia*, while in ML (BI) it is *Aequidens potaroensis*; (2) in the MP topology *Cichlasoma* is monophyletic, while in BI *Aequidens patricki* is nested as a sister species to *Cichlasoma,* (in this case, ML shows the same result as MP, see Fig. 1); (3) MP topology groups

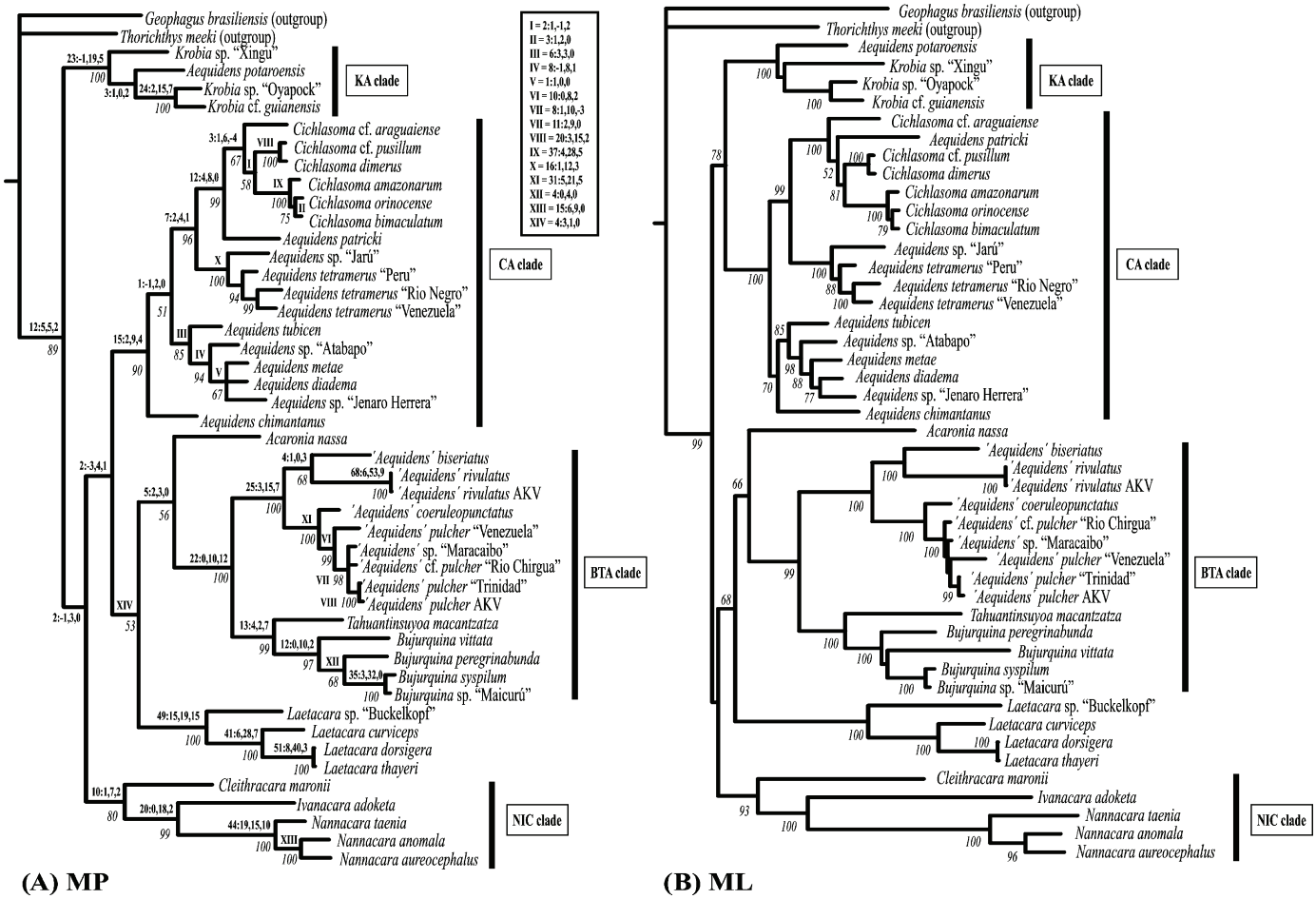


Fig. 1. Results of phylogenetic analyses of the 47 taxon dataset including 2236 bp of three genetic markers (16S rRNA, cyt *b*, first intron in S7 gene). The maximum parsimony (MP) analysis (A) is a strict consensus of six trees (L = 3930; CI = 0.3143; RI = 0.6356). The maximum likelihood (ML) analysis (B) has a log-likelihood score of −20249.37366. (C) Bayesian inference tree. Numbers below nodes represent bootstrap support (1000 replications) in the MP and ML analyses and posterior probabilities in the BI analysis. Numbers above nodes in the MP tree show Bremer support values and partitioned Bremer Support in the following order: BS: 16S, cyt *b*, S7. The roman numbers in the MP tree link to the table above the tree. There are the BS and PBS values of several nodes because of limited space in the tree figure. The ML and BI topologies differ only in two terminal nodes (position of *B. vittata–B. peregrinabunda* and position of *A. patricki–C. cf. araguaiense*).

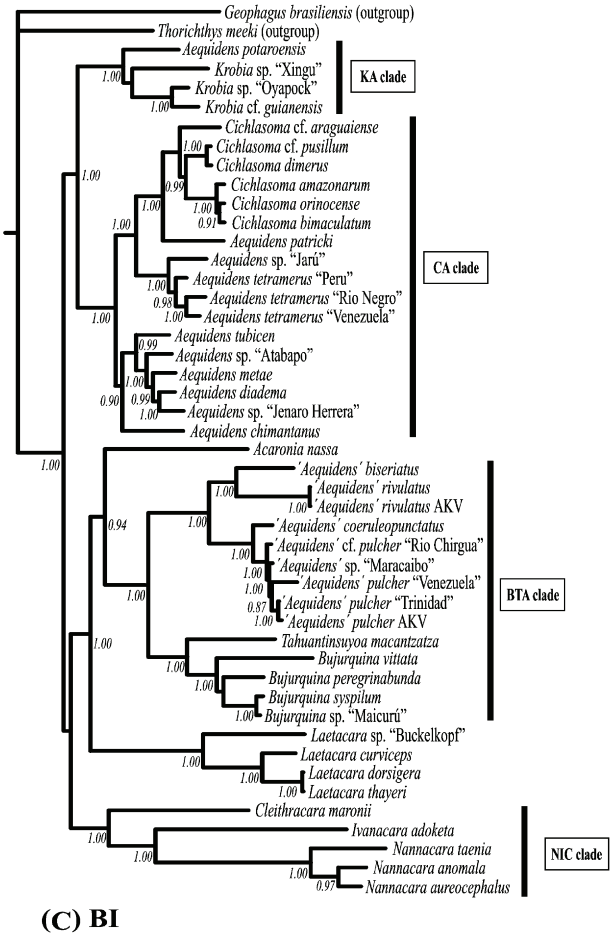


Fig. 1 (*continued*)

the CA and BTA–*Acaronia*–*Laetacara* clades to the exclusion of the NIC clade, while the ML (BI) topology groups the NIC clade with the BTA–*Acaronia*–*Laetacara* clade to the exclusion of the CA (and KA) clade; (4) the MP and ML (BI) topologies differ in the position of the KA clade (see below for explanation of clade acronyms).

3.1.1. Monophyly of individual genera

With the exception of the genera *Tahuantinsuyoa*, *Ivanacara*, *Acaronia* and the monotypic *Cleithracara*, our taxon sampling includes multiple species per genus and thus enables us to test their monophyly. Monophyly of all genera except *Aequidens* is supported. The monophyly of *Aequidens* was rejected by the AU test ($p = 0.000005$). The genus *Cichlasoma* is nested within *Aequidens*. Furthermore, *Aequidens potaroensis* is not an *Aequidens*, but a member of the genus *Krobia* (Fig. 1). Even after exclusion of *A. potaroensis* is monophyly of *Aequidens* still rejected by our data (AU test $p = 0.005$). ML analysis (Fig. 1B) additionally did not recover a monophyletic *Cichlasoma* since *Aequidens patricki* was placed as a basal *Cichlasoma* to the exclusion of *Cichlasoma* cf. *araguaiense*.

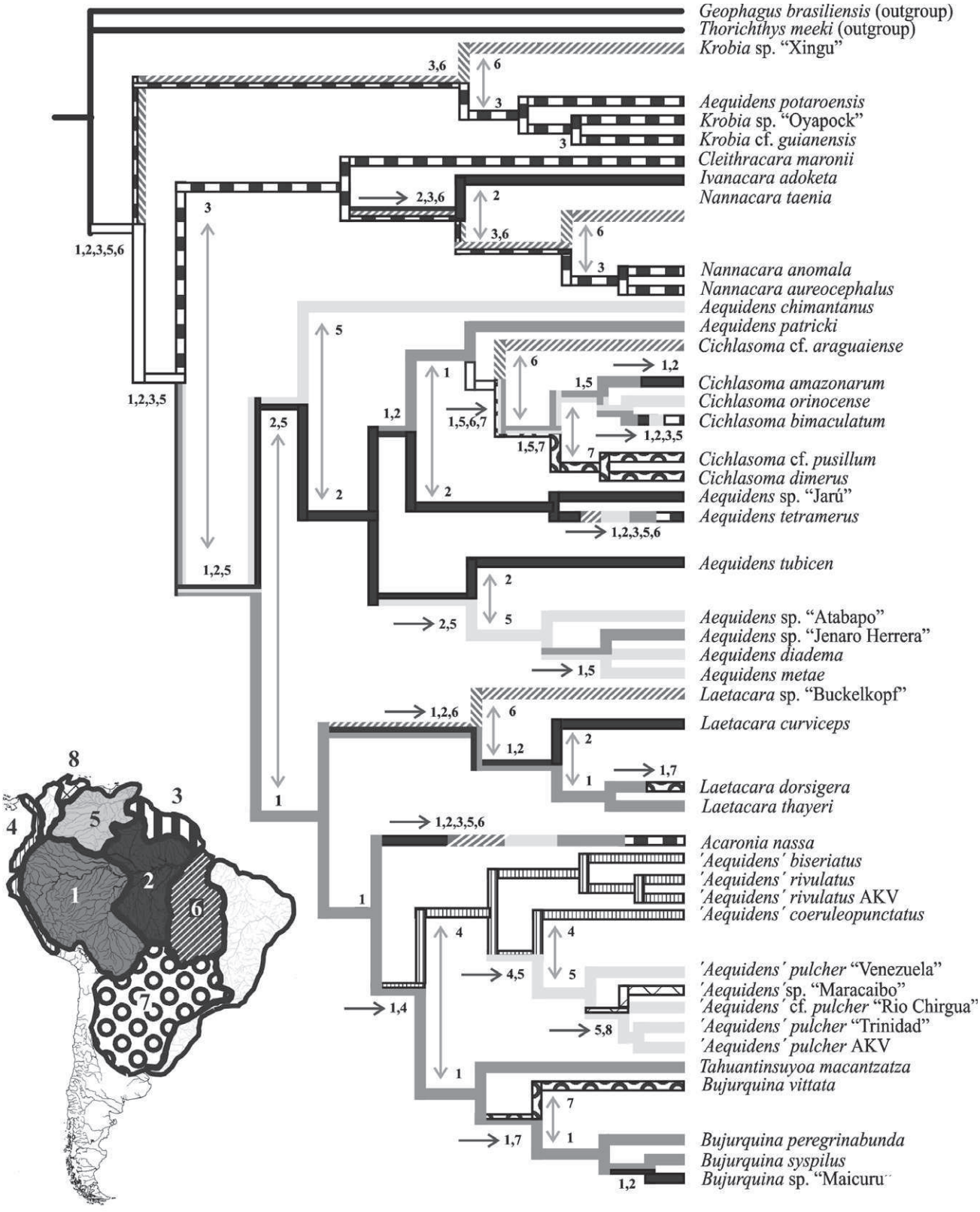
Both the '*Aequidens*' *pulcher* group and the '*Aequidens*' *rivulatus* group were monophyletic and strongly supported sister taxa, unrelated to *Aequidens*. These two species groups thus clearly represent an unnamed genus, justified also on morphological grounds (OŘ unpublished results).

3.1.2. Relationships of cichlasomatine genera

We recovered four suprageneric clades among cichlasomatine cichlids with strong support (Fig. 1). One clade includes the genera *Bujurquina*, *Tahuantinsuyoa* and the '*Aequidens*' *pulcher*–'*Aequidens*' *rivulatus* group (termed here the BTA clade). The second clade, referred to as the CA clade, includes *Aequidens* (excluding *A. potaroensis*) and *Cichlasoma*. The third clade (NIC clade) includes *Nan-*

Fig. 2. Dispersal–vicariance analysis (DIVA) of the biogeography of cichlasomatine cichlids in South America. (A) DIVA of the maximum parsimony (MP) tree (see Fig. 1A). (B) DIVA of the maximum likelihood (ML) tree using the same dataset. Three samples of *Aequidens tetramerus* are shown as one branch. ML and BI topologies (see Fig. 1) are almost identical, resulting in very similar mapping of biogeography. The map shows the Neotropical biogeographic provinces based on Hubert and Renno (2006). Representatives of eight provinces were included in the analyses. Two provinces marked with white color in the map (Magdalena River basin in Colombia and Atlantic coast of Brazil and Uruguay) show South American ichthyological provinces from which we have no samples analyzed. Branches with more than three ancestral areas are marked by white color.

nacara, *Ivanacara* and *Cleithracara*. Since *Krobia* also includes *Aequidens potaroensis*, we refer to it as the KA clade (*Krobia–Aequidens potaroensis* clade). The genera *Acaronia* and *Laetacara* form successive sister groups to the BTA clade, but the relationships are only weakly supported, except in the Bayesian analysis (Fig. 1C). Further-



(A) MP

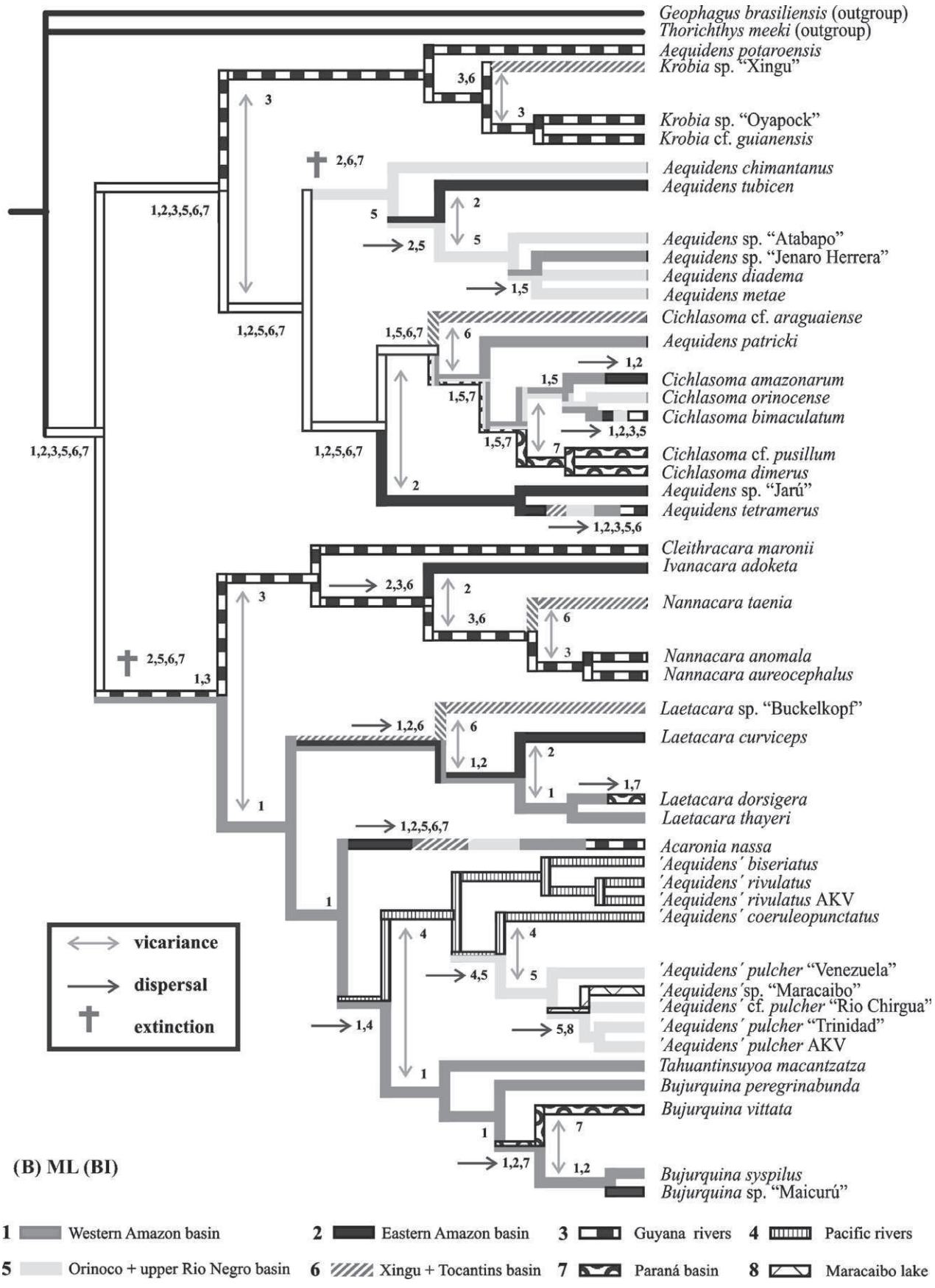


Fig. 2 (*continued*)

more, the combined dataset analyzed with ML and BI methods places the CA and KA clades as sister groups (Fig. 1B and C), while the MP analysis places the KA clade as the basal most cichlasomatine clade (Fig. 1A). Again only the BI analysis found statistical support for the relationships of the KA clade.

3.2. Biogeography and dating of cichlasomatine diversification

Biogeography of cichlasomatine cichlids has been studied using dispersal–vicariance analysis (DIVA; Ronquist, 1997) applied to the MP and ML (BI) topologies. The ML and BI topologies differ in only two terminal nodes, and thus the ML topology only was used for the DIVA (the two terminal nodes in the BI analysis being in agreement with those in the MP analysis). Due to differences at basal nodes between the MP and ML (BI) topologies the DIVA resulted in slightly different ancestral areas and biogeographical interpretations. Both topologies show a high degree of vicariant events at basal nodes, with dispersal events largely limited to terminal nodes. The MP topology shows cis-andean South America excluding the La Plata drainage as ancestral for the Cichlasomatini, while the ML (BI) topologies include the La Plata drainage.

In the 45 nodes of the fully resolved phylogeny, DIVA of the MP topology shows 17 vicariant events, while on the ML (BI) topology there is one less (16). See Fig. 2 for the DIVA results and Fig. 3 for dating of cichlasomatine phylogeny and of biogeographical events.

Cichlasomatine divergence started at around 44 Mya (penalized likelihood estimation = 44.74 Mya) with a vicariance between the Guyana rivers and cis-andean South America (Orinoco, Western and Eastern Amazon, and in the ML (BI) also the Tocantins–Xingu drainages and the La Plata). This is the oldest vicariant event. Both MP and ML (BI) topologies show this vicariance. In the MP topology it is a single event, while in the ML (BI) there are two parallel vicariances between these areas (Figs. 2 and 3). The Guyana rivers fauna is thus supported as very old with a long independent history, which is also demonstrated by the highest number of endemic genera per biogeographic province (see below).

The MP topology shows as a second event the vicariance between Western Amazon and Orinoco–Eastern Amazon, while the ML (BI) topology shows an extinction in Orinoco–Eastern Amazon plus the Tocantins–Xingu and La Plata. Leaving aside the mechanism Western Amazon is in both MP and ML (BI) topologies reconstructed as the ancestral area of the *Acaronia–Laetacara*–BTA clade (at ca 38 Mya). Both MP and ML (BI) topologies also show colonization of trans-andean South America from the Western Amazon at around 30 Mya, followed by vicariance between the two areas (and within the ancestor of Western Amazonian *Bujurquina–Tahuantinsuyoa* and trans-andean '*Aequidens*'). Both topologies also reconstruct a dispersal of '*Aequidens*' into the Orinoco and the Maracaibo from trans-andean South America between 7 and 11 Mya, as well as a dispersal of *Bujurquina* into the La Plata. *Laetacara* has in both reconstructions colonized the Eastern Amazon–Tocantins–Xingu areas, later diverging by vicariance. *Acaronia* has similar to *Laetacara* extended its distribution from the Western Amazon.

Reconstructions in the *Aequidens–Cichlasoma* clade (CA clade) are more different between the MP and ML (BI) topologies due to the different position of the Orinoco–Guyanan endemic *A. chimantanus* and the interchanging positions of *A. patricki* and *C. araguaiense*. The MP topology shows a vicariance between *A. chimantanus* and all remaining species, while the ML (BI) reconstruction is more complex, with exctinction in the Eastern Amazon, Tocantins–Xingu and La Plata. Both reconstructions however agree on extensive dispersal and vicariance between the Eastern Amazon, Western Amazon and Orinoco–upper Rio Negro (in the *A. tubicen–A. metae* clade). A better taxon sampling in this clade is needed to resolve the incongruences between the MP and ML (BI) reconstructions. The widespread *A. tetramerus* is under the current taxon sampling reconstructed in both scenarios as ancestral in the Eastern Amazon, later having dispersed into all cis-andean South America except the La Plata. *A. tetramerus* populations have a long independent history, the oldest split being ca 7 Mya old, and it is thus quite probable that several independent species are hidden under the name. *Cichlasoma* plus *A. patricki* have diverged from *A. tetramerus* and *A.* sp. "*Jaru*" through vicariance between the Eastern Amazon and rest of cis-andean South America (or alternatively between East and West Amazon in the MP reconstruction). As in the case of the *A. tubicen–A. metae* clade dispersal and vicariance within cis-andean South America have been extensive in the *Cichlasoma* plus *A. patricki* clade. The oldest vicariance is the Xingu–Tocantins (*C. araguainse*; around 16 Mya), followed by the La Plata (*C. pusillum*, *C. dimerus* and *C. portalegrense*). The rest of *Cichlasoma* is Western Amazonian–Orinocoan, from where it also secondarily colonized the Guyana rivers (*C. bimaculatum*).

In general the biogeographic analysis shows that vicariance has been the main factor responsible for the generic phylogeny of the Cichlasomatini and that genera have secondarily extended their distributions through dispersal. Very few exctinctions are required to reconcile the dispersal–vicariance reconstruction. Biogeographic interpretations at more inclusive levels within genera have to await a better taxon sampling. However at the generic level our taxon sampling is extensive, and except in the case of *Aequidens* the ancestral areas of genera will probably remain as reconstructed in this study.

4. Discussion

4.1. Phylogeny of Cichlasomatini

The systematics of cichlasomatine cichlids at the genus level has received much attention (see Kullander, 1998

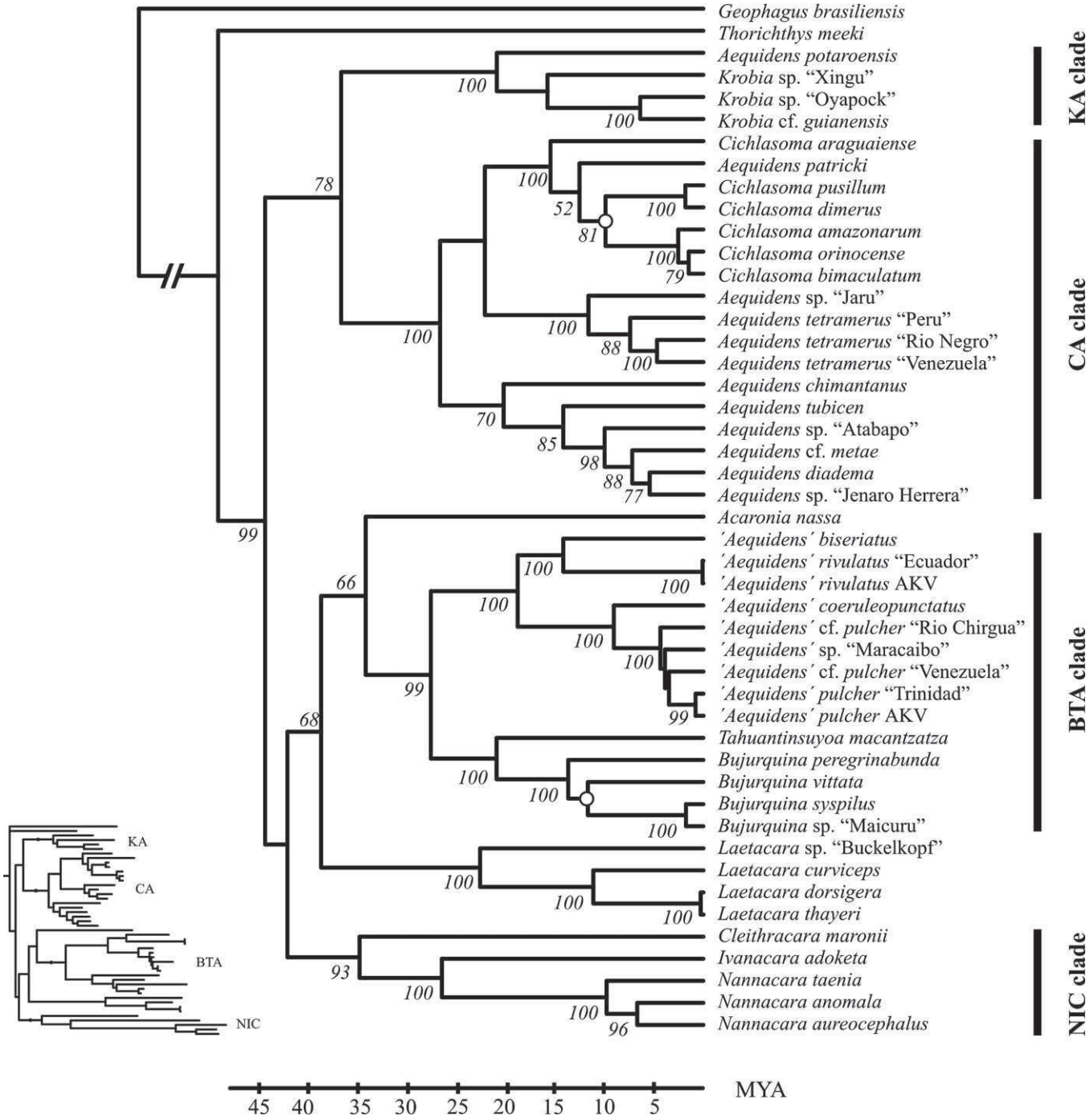


Fig. 3. Dating of cichlasomatine phylogeny (ML topology) using the Penalized likelihood analysis as implemented in R8S software (Sanderson, 2003) calibrated with geological dating (see Section 2).

for a review) and our results support the monophyly of all but one genus. According to Kullander (1998), *Cichlasoma* and *Aequidens* are very similar and well-diagnosed genera. While our data support their close relationship, the genus *Aequidens* appears to be paraphyletic (even after excluding *A. potaroensis*), which is in agreement with indications already present in Marescalchi (2005) or Říčan and Kullander (2006). The paraphyly of *Aequidens* relative to *Cichlasoma* has never been postulated on morphological grounds. Some previous studies (Kullander, 1983b) suggested the inclusion of two Guyanan *Aequidens* species (*A. potaroensis* and *A. paloemeuensis*) into *Krobia*. Kullander (op. cit.) already hypothesized the relation of these species to *Krobia*, although he did not include them into this genus (Kullander and Nijssen, 1989). He subsequently omitted them from his phylogenetic analysis (Kullander, 1998). Our data clearly suggest the inclusion of the Guyanan river species (represented herein by *A. potaroensis*) into

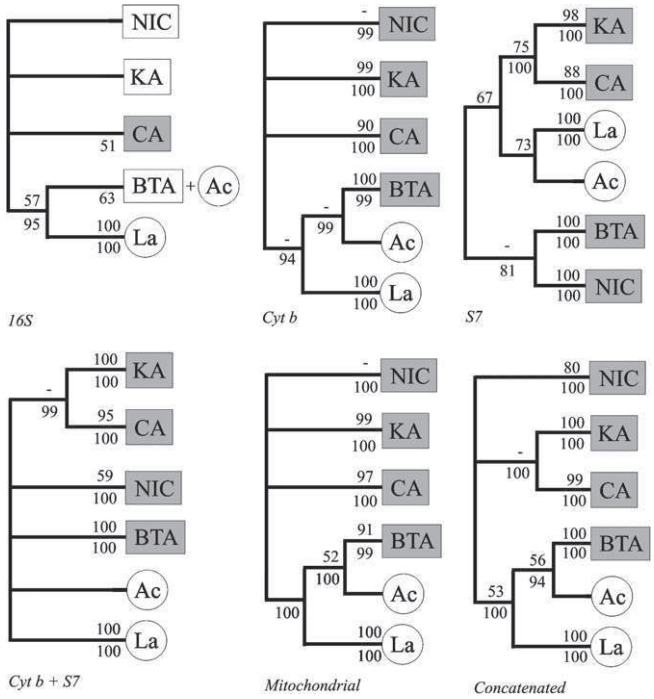


Fig. 4. Topologies observed in the particular markers. Each clade is shown if at least one of the Bayesian or the MP analyses supported its monophyly. Resolved nodes are marked only if they appeared in both MP and Bayesian analyses or in one of them with the bootstrap value >50 or posterior probability value >80. Numbers above branch correspond to bootstrap values in MP analyses, numbers below branches to Bayesian posterior probabilities (×100). Clade abbreviations are as follows: BTA (*Bujurquina*, *Tahuantinsuyoa*, '*Aequidens*'), CA (*Cichlasoma, Aequidens*), KA (*Krobia, Aequidens potaroensis*), NIC (*Nannacara*, *Ivanacara* and *Cleithracara*), La (*Laetacara* genus), Ac (*Acaronia nassa*). The BTA + Ac grouping in the 16S analysis depicts a position of *Acaronia* within the BTA clade. White clades in the 16S analysis mark their unresolved topology.

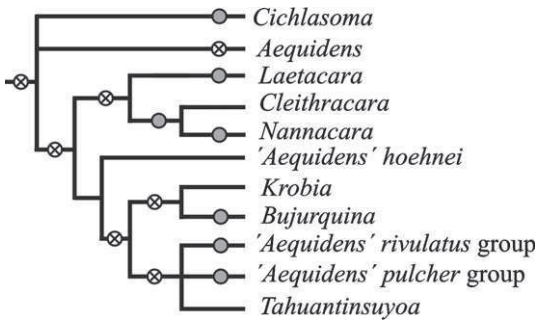


Fig. 5. Comparison of the morphological phylogeny of cichlasomatines (Kullander, 1998) and our molecular study. Gray circles mark congruence in topology between the two studies, white crossed circles mark conflicts. All tested genera except *Aequidens* are supported as monophyletic in both studies, but relationships between genera are mostly different and in conflict. The monophyly of the genera *Tahuantinsuyoa*, *Krobia* and *Cleithracara* was not tested since only one valid species per genus analyzed. '*Aequidens*' *hoehnei* was not included in our molecular analysis. The genus *Acaronia* is not shown as it was considered to a separate tribe Acaroniini by Kullander (1998). Our results place it firmly among cichlasomatines. Tree modified from Kullander (1998).

the genus *Krobia*. *Aequidens paloemeuensis* was not included in our analysis, but according to Kullander and Nijssen (1989), *A. potaroensis* and *A. paloemeuensis* are sister species. Our data did neither support nor reject the justification of the newly established genus *Ivanacara*. Although the species *I. adoketa* (formerly *Nannacara adoketa*) resulted as a sister species to other *Nannacara* species with the genetic uncorrected *p*-distance of about 20% (19.4–20.8%) from the rest of species belonging to the NIC clade, we lacked the other member of the genus, *I. bimaculata,* to test the monophyly of *Ivanacara* and *Nannacara* in our analyses (Fig. 4).

Three suprageneric clades are supported by all our analyses. These are the *Aequidens* (*Cichlasoma*) clade (CA), the *Bujurquina–Tahuantinsuyoa–*'*Aequidens*' clade (BTA) and the *Cleithracara–Nannacara–Ivanacara* clade (NIC). *Acaronia* is in all analyses the sister group of the BTA clade, and Laetacara is the sister group of this clade (Fig. 4). Statistical support for this grouping is however much lower than in the previous cases. The genus *Krobia* and the NIC clade are basal lineages in the cichlasomatine phylogeny. *Krobia* is either the sister group of the CA clade (ML and BI analyses) or the basal most lineage of cichlasomatines (MP analysis). The NIC clade is similarly either the sister group of the BTA–*Acaronia–Laetacara* clade (ML and BI), or of all clades except the KA clade (MP). As can be seen, support decreases and disagreements increase towards the more inclusive nodes. Examination of our data suggests that the weak support and differences in basal nodes between MP and ML (BI) analyses are a combination of lack of phylogenetic signal and character conflict (the latter not being significant and may be the result of the former).

The above-described suprageneric relationships of cichlasomatine cichlids are very different from those reported by Kullander (1998) based on phylogenetic analysis of morphological characters. Our results agree with the morphology-based phylogeny of Cichlasomatini of Kullander, 1998; see Fig. 5) in only one point, i.e. the close relationship of *Nannacara* + *Ivanacara* and *Cleithracara* (herein the NIC clade). All other suprageneric relationships are different. There is however very good agreement with other studies based on molecular markers (Farias et al., 1999, 2000, 2001; Marescalchi, 2005; Concheiro Pérez et al., 2007). In agreement with these studies and contrary to Kullander (1998) also the genus *Acaronia* is a part of the Cichlasomatini, while Kullander (op. cit.) placed it as intermediate between Cichlasomatini and Heroini. While Kullander (op. cit.) placed the genera *Krobia* and *Bujurquina* as sister groups, our analyses find them only distantly related. *Bujurquina* is together with *Tahuantinsuyoa* and the '*Aequidens*' *pulcher–*'*Aequidens*' *rivulatus* group part of the strongly supported BTA clade. This topology agrees well with life history traits and biogeography, since *Tahuantinsuyoa* and *Bujurquina* are strongly supported sister groups, with sympatric distribution and a mouthbrooding behaviour, which is unique among cichlasomatine cichlids (Stawikowski and Werner, 1998). The sister clade of these two Western Amazonian genera is the '*Aequidens*' *pulcher–*'*Aequidens*' *rivulatus* group, distributed both in cis-

and trans-andean northern South America. *Krobia* is either related to *Aequidens–Cichlasoma* (ML and BI analyses), or the basal most genus among Cichlasomatini (MP analysis). *Laetacara* is in all our analyses the sister group of the BTA clade. None of our analyses thus supports its relationship to the NIC clade (*Nannacara*, *Ivanacara*, *Cleithracara*) as found in Kullander (1998; see Fig. 5).

4.2. Biogeography and dating of divergence

Cichlasomatini and Heroini have wide and more or less complementary distributions. While the latter are distributed throughout the Neotropics from Argentina up to Mexico, being mostly represented in Mesoamerica and covering also trans-andean South America, western Amazonia and the Paraná–Paraguay–São Francisco drainages, the former group appears strictly in South America, with only one species ('*Aequidens*' *coeruleopunctatus*) present in lower Central America. The distribution of both tribes is to some extent complementary also in South America as heroines are mostly restricted to northern and western Amazonia, trans-andean South America and the Paraná–Paraguay–São Francisco drainages (Brasilian shield) (Concheiro Pérez et al., 2007), while cichlasomatines are well represented in eastern Amazonia and especially on and around the Guyana shield (eastern Amazonia, Guiana rivers and Orinoco) where heroines are almost completely absent.

The divergence between the Guyana rivers and rest of South America is well reflected in our biogeographic analyses, being the oldest vicariant event in the phylogeny of Cichlasomatini. The origin of the genera *Krobia* and the NIC clade (*Nannacara*, *Ivanacara*, *Cleithracara*) dates back to this old vicariance event dated at around 44 Mya. The area was later additionally colonized by species of several other genera (e.g. *Cichlasoma bimaculatum*, *Acaronia nassa*, *Aequidens tetramerus*). Dating of these secondary invasions is hard to ascertain from our data and taxon sampling, but source areas for the colonizations can be pinpointed. In case of *Cichlasoma* it was Western Amazon–Orinoco plus Upper Rio Negro (*C. taenia* from Trinidad and Orinoco delta further supporting this route was not present in our analysis), in the case of *Acaronia* it was the Western Amazon (probably also including Orinoco plus Upper Rio Negro, as *A. vultuosa* present there was not included in our analysis), and in case of *Aequidens tetramerus* it was the Eastern Amazon based on the present taxon sampling. However a much better locality sampling of this widespread species group (see above) is needed to reconcile its biogeography. The first two species thus colonized the Guyana rivers probably through river anastomosis in the Orinoco delta area during a period of lower sea levels, while *Aequidens* might have dispersed through the low lying Rupununi area between Guyana and Brazil or through river anastomosis in the present day Amazon delta following the change of the Amazon outlet to the east between the Guyana and Brazilian shields (Lundberg et al., 1998), or even through the same route as *Cichlasoma* and *Acaronia*. It is not possible to rule out even independent invasions into the Guyana rivers through several routes by the *A. tetramerus* superspecies.

The Guyana shield, an area divided into three areas of endemism (Orinoco– Upper Rio Negro, Eastern Amazon and the coastal Guyana rivers), harbours the highest level of endemism and diversity among cichlasomatine cichlids (eight genera, i.e. *Cleithracara*, *Krobia, Nannacara, Ivanacara, Acaronia*, *Aequidens*, *Cichlasoma* and marginally *Laetacara*). The former four are endemic or have their centre of distribution in the Guyana shield. On the contrary, only five genera, none of them endemic (*Cichlasoma*, *Aequidens*, *Laetacara*, *Bujurquina*, *Krobia*; latter three only marginally), are found in the much larger Brazilian shield. Whole Western Amazonia possesses five genera (*Bujurquina*, *Aequidens*, *Cichlasoma*, *Laetacara*, *Tahuantinsuyoa*) and only one of them is endemic (*Tahuantinsuyoa*; *Bujurquina* is nearly endemic). Western Amazonia has however based on our results played importantly in the evolution of Cichlasomatini, as the whole BTA clade (*Bujurquina–Tahuantinsuyoa–*'*Aequidens*') plus *Acaronia* and *Laetacara* have their ancestral area in Western Amazonia. From Western Amazonia, this clade has colonized trans-andean South America (30 Mya) and also the La Plata (10–12 Mya). In contrast to the Western Amazonian clade, the *Aequidens–Cichlasoma* clade is better represented in eastern South America (in Eastern Amazon and Orinoco plus Upper Rio Negro), which is also its reconstructed ancestral area in the DIVA based on the MP topology (Fig. 2A). The oldest species of this clade in the Orinoco province is *A. chimantanus*, in MP topology DIVA reconstruction the basal most species of the whole clade.

The two largest clades of Cichlasomatini thus have complementary and largely exclusive distributions in South America, one (the BTA clade plus *Acaronia* and *Laetacara*) in the Western Amazon, the other (CA clade) in Eastern Amazon. Orinoco plus Upper Rio Negro is an intermediate area, today still connected to the Eastern Amazon through several points (the best known being the Casiquiare), while previously being the northern portion of the Western Amazon previous to the final rise of the Andes (Lundberg et al., 1998).

The cichlasomatine fauna of the Xingu–Tocantins province is supported as being old and independent for a long time (e.g. Hubert and Renno, 2006). Within *Krobia* and *Nannacara* it has vicariant relationships to the Guyana Rivers province (ca. 15 Mya in *Krobia* and ca. 10 Mya in *Nannacara*), while in *Laetacara* and *Cichlasoma* with Amazonia in the former (around 22 Mya) and most of cis-andean South America in the latter (around 16 Mya).

The dating of age and initial divergence within Cichlasomatini is placed around 44 Mya, which is in good agreement with the independently estimated age of heroines, the cichlasomatine sister group (Concheiro Pérez et al., 2007). Heroines very likely colonized Mesoamerica at least 16–24 Mya (at least 10 Mya prior to the final emergence of the Isthmus of Panama) in agreement with other secondary

freshwater fishes (Concheiro Pérez et al., 2007; Hrbek et al., 2007). Although the colonization of Mesoamerica by the only cichlasomatine species (‘*Aequidens*’ *coeruleopunctatus*) likely followed the same route from trans-andean South America (Figs. 2 and 3), it happened much more recently, after the separation from the remaining species at around 10 Mya (Fig. 3). This is in agreement with Bussing’s (1976, 1985) postulate that cichlasomatines belong to the New Southern Element and heroines to the Old Southern Element of the Central American freshwater ichthyofauna. The Heroini have radiated in at least two lineages in Mesoamerica already at around 16–24 Mya (Concheiro Pérez et al., 2007). Based on our biogeographic analyses, ‘*Aequidens*’ has been present in trans-andean South America as long as the Heroini (ca. 28 Myr; Figs. 2 and 3), and the time discrepancy between the heroine and cichlasomatine colonization of Central America thus cannot be explained using the available studies and is worthy of further study.

All six ‘*Aequidens*’ species are primarily trans-andean, including the putative sister group of ‘*A.*’ *pulcher* (*A. latifrons*), which was not available for analyses. ‘*Aequidens*’ *pulcher* is thus the only species of the group that is secondarily found also in cis-andean South America (Orinoco river Basin in Venezuelan llanos and on Trinidad). Based on our analyses this secondary dispersal into cis-andean South America and vicariance from trans-andean relatives is dated at ca. 10 Mya (Figs. 2 and 3). This dating is well in agreement with the final rise of the easternmost chain of the Andes in Venezuela, isolating the Maracaibo drainage from surrounding coastal areas and from the Llanos (dated by Lundberg et al., 1998 at ca. 10–11.8 Mya).

The internal phylogenetic structure of cichlasomatine cichlids contrasts with the Heroini, where the genera are vaguely defined and except for monotypic ones they are not supported as monophyletic (Concheiro Pérez et al., 2007). Cichlasomatine genera, on the other hand, are well differentiated as suggested by phylogenetic support for all but one (*Aequidens*) genus. Internodes are much longer among cichlasomatine cichlids compared to heroines (cf. Concheiro Pérez et al., 2007) suggesting completely different cladogenetic histories. While at least the Mesoamerican heroine diversity is probably the result of repeated radiations (Concheiro Pérez et al., 2007; OŘ unpublished results), cichlasomatines seem to be shaped primarily by interspaced vicariant events. Studies comparing the macroevolution of the two tribes are thus potentially extremely informative on Neotropical biogeography and faunal evolution.

We have provided the first detailed molecular phylogenetic study of Cichlasomatini cichlids and found patterns of diversification that are very different from those in their sister group—the Heroini cichlids (Concheiro Pérez et al., 2007). Our data allowed us to solve the systematic position of all genera belonging to this tribe and their large scale biogeography in South America. Furthermore, we offer a comparison with the morphology-based phylogenetic hypothesis of this cichlid assemblage.

Acknowledgments

We thank Rainer Stawikowski, Lutz Krahnefeld, Ingoma Kratz, Antonín Prouza, Aqua Ferrytale and Hobby Zoo Tillmann for help with obtaining several samples. Thanks are also to Vendula Šlechtová, Ingo Schindler, Petr Ráb, Ivan Čepička, J. Andres López and two anonymous reviewers for the interesting discussion and for all comments significantly improving the manuscript. Daniel Frynta encouraged the initial stage of the study. Thanks to Petr Janšta, Lukáš Kubička, Zuzana Starostová, Milan Kaftan and Miroslav Švátora for their help with the fish manipulation. We also thank M. Noren (Stockholm) for providing the TrucCytB-R primer. We would like to thank the Biodiversity Research group for providing us laboratory background.

This work was supported by the Grant Agency of Charles University in Prague (GAUK 182/2004/B-BIO/PrF and GAUK 139407), Hlávka foundation and the foundation of the Centre for Biodiversity LC06073 (MSMT) to Z.M., and by the MSM6007665801 grant to OŘ. The Institute of Animal Physiology and Genetics receives continuous support from IRP IAPG (No. AV0Z50450515).

References

Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. Syst. Biol. 46 (4), 654–673.

Bussing, W.A., 1976. Geographical distribution of the San Juan ichthyofauna of Central America with remarks on its origin and ecology. In: Thorson, T.B. (Ed.), Investigations of the Ichthyofauna of Nicaraguan Lakes. University of Nebraska, Lincoln, pp. 157–175.

Bussing, W.A., 1985. Patterns of distribution of the Central American ichthyofauna. In: Stehli, F.G., Webb, S.D. (Eds.), The Great American Biotic Interchange. Plenum Publishing, pp. 453–473.

Chakrabarty, P., 2006. Systematics and historical biogeography of Greater Antillean Cichlidae. Mol. Phylogenet. Evol. 39 (3), 619–627.

Chow, S., Hazama, K., 1998. Universal PCR primers for S7 ribosomal protein gene introns in fish. Mol. Ecol. 7, 1255–1256.

Concheiro Pérez, G.A., Říčan, O., Ortí, G., Bermingham, E., Doadrio, I., Zardoya, R., 2007. Phylogeny and biogeography of 91 species of heroine cichlids (Teleostei: Cichlidae) based on sequences of the cytochrome *b* gene. Mol. Phylogenet. Evol. 43, 91–110.

Farias, I.P., Ortí, G., Sampaio, I., Schneider, H., Meyer, A., 1999. Mitochondrial DNA phylogeny of the family Cichlidae: monophyly and fast molecular evolution of the Neotropical assemblage. J. Mol. Evol. 48, 703–711.

Farias, I.P., Ortí, G., Meyer, A., 2000. Total evidence: molecules, morphology, and the phylogenetics of cichlid fishes. J. Exp. Zool. 288, 76–92.

Farias, I.P., Ortí, G., Sampaio, I., Schneider, H., Meyer, A., 2001. The Cytochrom *b* gene as a phylogenetic marker: the limits of resolution for analyzing relationships among Cichlid fishes. J. Mol. Evol. 53, 89–103.

Farris, J.S., Kallersjo, M., Kluge, A.G., 1995. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.

Froese, R., Pauly, D., 2006. FishBase. Published by author. <http://fishbase.com/search.php/>.

Guindon, S., Gascuel, O., 2003. PhyML - A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52, 696–704.

Hall, T.A., 1999. Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. 41, 95–98.

Hrbek, T., Seckinger, J., Meyer, A., 2007. A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. Mol. Phylogenet. Evol. 43, 986–998.

Hubert, N., Renno, J.F., 2006. Historical biogeography of South American freshwater fishes. J. Biogeogr. 33, 1414–1436.

Hulsey, C.D., de Leon, F.J.G., Johnson, Y.S., Hendrickson, D.A., Near, T.J., 2004. Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. Mol. Phylogenet. Evol. 31 (2), 754–764.

Irwin, D.M., Kocher, T.D., Wilson, A.C., 1991. Evolution of the cytochrome b gene in mammals. J. Mol. Evol. 32, 128–144.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial—DNA evolution in animals—amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Kullander, S.O., 1983a. A Revision of the South American Cichlid genus *Cichlasoma*. Swedish Museum of Natural History, Stockholm, Sweden.

Kullander, S.O., 1983b. Taxonomic Studies on the Percoid Freshwater Fish Family Cichlidae in South America. PhD. thesis, Stockholm.

Kullander, S.O., 1986. Cichlid fishes of the Amazon River Drainage of Peru. Swedish Museum of Natural History, Stockholm, Sweden.

Kullander, S.O., 1996. *Heroina isonycterin* a, a new genus and species of cichlid fish from western Amazonia, with comments on cichlasomine systematics. Ichthyol. Explor. Freshwaters 7, 149–172.

Kullander, S.O., 1998. A Phylogeny and Classification of the South American Cichlidae (Teleostei: Perciformes). In: Malabarba, L.R., Reis, R.P., Lucena, Z.M., Lucena, C.A.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brazil, pp. 461–498.

Kullander, S.O., 2003. Family Cichlidae (Cichlids). In: Reis, R.E., Kullander, S.O., Ferraris, C.J., Jr. (Eds.), Check List of the Freshwater Fishes of South and Central America. Edipucrs, Porto Alegre, pp. 605–654.

Kullander, S.O., Nijssen, H., 1989. The Cichlids of Surinam. Teleostei: Labroidei. E.J. Brill, Leiden, The Netherlands.

Kullander, S.O., Ferreira, E.J.G., 2006. A review of the South American cichlid genus *Cichla*, with descriptions of nine new species (Teleostei: Cichlidae). Ichthyol. Explor. Freshwaters 17, 289–398.

Kullander, S.O., Lucena, C.A.S., 2006. A review of the species of *Crenicichla* (Teleostei: Cichlidae) from the Atlantic coastal rivers of southeastern Brazil from Bahia to Rio Grande do Sul States, with descriptions of three new species. Neotrop. Ichthyol. 4, 127–146.

López-Fernández, H., Taphorn, D.C., Kullander, S.O., 2006. Two new species of *Guianacara* from the Guiana Shield of Eastern Venezuela (Perciformes: Cichlidae). Copeia 2006 (3), 384–395.

Lowe-McConnell, R.H., 1991. Ecology of cichlids in South American and African waters, excluding the African Great Lakes. In: Keensleyside, M.H.A. (Ed.), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman Hall, London, Great Britain, pp. 60–85.

Lundberg, J.G., Marshal, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L., Wesselingh, F., 1998. The stage for Neotropical fish diversification: a history of tropical South American rivers. In: Malabarba, L.R., Reis, R.P., Lucena, Z.M., Lucena, C.A.S. (Eds.), Phylogeny and Classification of Neotropical Fishes. Edipucrs, Porto Alegre, Brazil, pp. 13–48.

Marescalchi, O., 2005. Karyotype and mitochondrial 16S gene characterizations in seven South American Cichlasomatini species (Perciformes, Cichlidae). J. Zool. Syst. Evol. Res. 43, 22–28.

Martin, A.P., Bermingham, E., 1998. Systematics and evolution of lower central American Cichlids inferred from analysis of cytochrome *b* gene sequences. Mol. Phylogenet. Evol. 9, 192–203.

MontoyaBurgos, J.I., 2003. Historical biogeography of the catfish genus *Hypostomus* (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. Mol. Ecol. 12, 1855–1867.

Moyer, G.R., Burr, B.M., Krajewski, C., 2004. Phylogenetic relationships of thorny catfishes (Siluriformes: Doradidae) inferred from molecular and morphological data. Zool. J. Linn. Soc. 140, 551–575.

Palumbi, S., Martin, A.P., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The Simple Fool's Guide to PCR, Version 2.0. Univ. of Hawai i Press, Honolulu.

Posada, D., Crandall, C.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Regan, C.T., 1905a. A revision of the fishes of the American cichlid genus *Acara*, *Nannacara*, *Acaropsis* and *Astronotus*. Ann. Mag. Nat. Hist. 15, 329–347.

Regan, C.T., 1905b. A revision of the fishes of the American cichlid genus *Cichlosoma* and of the allied genera. Ann. Mag. Nat. Hist. 7 (15), 60–67, 225–243, 316–340, 433–445.

Regan, C.T., 1905c. A revision of the fishes of the South-American cichlid genera *Crenacara*, *Batrachops*, and *Crenicichla*. Proc. zool. Soc. Lond. 1905, 152–168.

Regan, C.T., 1906a. A revision of the South American cichlid genera *Retroculus*, *Geophagus*, *Heterogramma*, and *Biotoecus*. Ann. Mag. Nat. Hist. 17 (7), 49–66.

Regan, C.T., 1906b. A revision of the fishes of the South American cichlid genera *Cichla*, *Chaetobranchus*, and *Chaetobranchopsis*, with notes on the genera of American Cichlidae. Ann. Mag. Nat. Hist. 17 (7), 230–239.

Říčan, O., Kullander, S.O., 2006. Character and tree-based delimitation of species in the *'Cichlasoma' facetum* group (Teleostei, Cichlidae) with the description of a new genus. J. Zool. Syst. Evol. Res. 44, 136–152.

Roe, K.J., Conkel, D., Lydeard, C., 1997. Molecular systematics of middle American cichlid fishes and the evolution of trophic-types in '*Cichlasoma* (*Amphilophus*)' and '*C*-(*Thorichthys*)'. Mol. Phylogenet. Evol. 7, 366–376.

Römer, U., Hahn, I., 2007. *Ivanacara* gen. n. (Teleostei: Perciformes, Cichlasomatini)—a new genus of cichlids from the Neotropis. In: Römer, U. (Ed.), Cichlid Atlas, volume 2, Natural History of South American Dwarf Cichlids, Part 2. Mergus Verlag GmbH, Melle, Germany, pp. 1190–1197.

Ronquist, F., 1997. Dispersal–vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46 (1), 195–203.

Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Sanderson, M.J., 2002. Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. Mol. Biol. Evol. 19, 101–109.

Sanderson, M.J., 2003. R8S: inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19, 301–302.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 51, 492–508.

Shimodaira, H., Hasegawa, M., 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17, 1246–1247.

Sparks, J.S., 2004. Molecular phylogeny and biogeography of the Malagasy and South Asian cichlids (Teleostei: Perciformes: Cichlidae). Mol. Phylogenet. Evol. 30, 599–614.

Sparks, J.S., Smith, W.L., 2004. Phylogeny and biogeography of cichlid fishes (Teleostei: Perciformes: Cichlidae). Cladistics 20, 501–517.

Stawikowski, R., Werner, U., 1998. Die Buntbarsche Amerikas. Band 1. Eugen Ulmer GmbH and Co., Stuttgart, Germany.

Stiassny, M.L.J., 1991. Phylogenetic intrarelationships of the family Cichlidae: an overview. In: Keensleyside, M.H.A. (Ed.), Cichlid Fishes: Behaviour, Ecology and Evolution. Chapman Hall, London, Great Britain, pp. 1–35.

Swofford, D.L., 2002. PAUP*. Phylogenetic analysis using parsimony (and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Wahlberg, N., Brower, A.V.Z., Nylin, S., 2005. Phylogenetic relationships and historical biogeography of tribes and genera in the subfamily Nymphalinae (Lepidoptera: Nymphalidae). Biol. J. Linn. Soc. 86, 227–251.

Musilová Z., Říčan O., Novák J.

2009

Phylogeny of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus

Journal of Zoological Systematics and Evolutionary Research **47: 234-247**

Scientific paper

To whom it may concern

I declare that Zuzana Musilova performed significant portion of work on our manusript:

MUSILOVÁ, Z., ŘÍČAN, O., AND NOVÁK, J., 2009 - Phylogeny of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus. Journal of Zoological Systematics and Evolutionairy research, 47: 234-247 (IF = 1.791) -141.

In behalf of co-author,

In České Budějovice, 6.1.2011 Oldřich Říčan

Accepted on 1 December 2008
J Zool Syst Evol Res doi: 10.1111/j.1439-0469.2009.00528.x

[1]*Department of Zoology, Faculty of Science, Charles University in Prague, Praha, Czech Republic;* [2]*Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic;* [3]*Laboratory of fish genetics, Institute of Animal Physiology and Genetics of the Academy of Sciences of Czech Republic, Liběchov, Czech Republic*

Phylogeny of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus

Z. Musilová[1,3], O. Říčan[2,3] and J. Novák[2]

Abstract

Phylogenetic relationships among cichlasomatine cichlids were studied using an extensive taxon sampling and both morphological and molecular data sets. A new genus, *Andinoacara* n. gen. with six species (*A. pulcher-rivulatus* group of previous authors) from trans-andean South America and NW cis-andean South America, is described based on results of phylogenetic and diagnosability analyses and tests of alternative topologies Our results demonstrate that cichlasomatine cichlid diversity is divided into five principal lineages composed of eleven genera and three suprageneric clades: the [(*Bujurquina*, *Tahuantinsuyoa*), (*Andinoacara*) (BAT) clade; the (*Cleithracara*, (*Nannacara*, *Ivanacara*)] clade (NIC) plus *Laetacara* and *'Aequidens' hoehnei*; and the (*Aequidens*, *Cichlasoma*) clade, where *Aequidens* is paraphyletic to *Cichlasoma*. Two former *Aequidens* species are additionally transferred into *Krobia* (*K. potaroensis*, *K. paloemeuensis*). *'Aequidens' hoehnei* probably represents a unique evolutionary lineage and would thus qualify for a separate generic status. Molecular data are yet not available for this species and its generic status requires further study. Relationships between the three suprageneric clades and between *Acaronia* and *Krobia* could not be convincingly resolved with our data set of two mitochondrial (16S and cyt *b*) and two nuclear (S7 and RAG1) molecular markers and 96 morphological characters.

Key words: Cichlasomatini – *Andinoacara* – *Aequidens* – phylogeny – cichlids – cytochrome *b*

Introduction

Cichlasomatine cichlids are one of the major South American cichlid groups and are among the most common small cichlids in most habitats. The sister group of the South American cichlasomatine cichlids are the predominantly Mesoamerican heroine cichlids (Kullander 1998; Farias et al. 2001). The subfamily Cichlasomatinae groups both cichlasomatine and heroine tribes and has one of the most complex taxonomic histories among Neotropical cichlids (see Kullander 1998; Miller et al. 2005 and references therein). Progress in deciphering the taxonomy and generic diversity of the group has been slow, and only recently gained momentum due to increased phylogenetic resolution derived from molecular markers (Farias et al. 1999, 2000, 2001; Marescalchi 2005; Říčan and Kullander 2006; Concheiro Pérez et al. 2007; Musilová et al. 2008). Solid insight into the phylogeny of the subfamily Cichlasomatinae would be an essential prerequisite for the stabilization of the confusing taxonomy.

The diversity of the tribe Cichlasomatini among cichlasomatine cichlids has been until the 1980's grouped into four genera – *Cichlasoma*, *Aequidens*, *Nannacara* and *Acaronia*. Kullander (1983a) recognized *Cichlasoma* as an unnatural catch-all group in demonstrating that: (1) the type species and several newly described species of *Cichlasoma* are related to *Aequidens*, (2) most of the species previously placed in *Cichlasoma* are unrelated and many of them also lack an alternative generic allocation (referred to as *'Cichlasoma'* by Kullander 1983a,b). All of the former *Cichlasoma* were later placed in the newly proposed tribe Heroini with several other genera (Kullander 1986, 1996). Kullander (1986) and Kullander and Nijssen (1989) also revised the genus *Aequidens*, from which they erected the genera *Cleithracara*, *Krobia*, *Laetacara*, *Bujurquina* and *Tahuantinsuyoa*. Kullander (1983a, 1986) himself stated that the generic classification of cichlasomatine cichlids is not final and the first morphological phylogenetic study of cichlasomatine cichlids (Kullander 1998) showed that at least two additional groups are not part of the present genus *Aequidens* and could have a generic status (the *'Aequidens' pulcher-rivulatus* group and *'Aequidens' hoehnei*).

Musilová et al. (2008) have provided the first molecular phylogenetic hypothesis for cichlasomatine cichlids including all recognized genera and also representatives of unplaced taxa. Monophyly of all recognized genera except *Aequidens* has been supported. *Aequidens* was shown to include (1) species belonging instead into *Krobia* and (2) one well supported yet unnamed genus (the *'Aequidens' pulcher-rivulatus* group). The genus *Aequidens* itself was found paraphyletic with *Cichlasoma* as its inner group. Results at the generic level except for Aequidens were otherwise mostly congruent with morphological phylogeny (Kullander 1998). Relationships between genera yet differed markedly from the morphological phylogeny of Kullander (1998).

The aim of this paper is to: (1) Provide a morphological phylogeny of cichlasomatine cichlids which would include a much more detailed taxon sampling to test monophyly of genera; (2) Expand the molecular data set of Musilová et al. (2008) with another more conservative nuclear gene to address phylogeny at deep nodes in the cichlasomatine evolution and reconcile the morphological and molecular phylogenetic signals; and (3) Review the generic classification of cichlasomatine cichlids.

Material and Methods

Taxonomic sampling

We studied 72 cichlasomatine species for which 96 morphological characters were sampled and this data set is accompanied by four molecular markers (two mitochondrial; cyt *b* and 16S; and two nuclear; S7 intron 1 and RAG1) with a 42 species taxon sampling. Representatives of all putative and established cichlasomatine genera were included

Table 1. Species examined in this study

Taxon name	RAG1	Taxon name	RAG1
Geophagus brasiliensis	EU706360	*Bujurquina peregrinabunda*	EU706399
Thorichthys meeki	EU706361	*Bujurquina syspilus*	EU706405
Andinoacara biseriatus	EU706395	*Bujurquina* sp. 'Maicurú'	EU706400
Andinoacara coeruleopunctatus	EU706398	*Cichlasoma amazonarum*	EU706369
Andinoacara pulcher AKV	EU706381	*Cichlasoma bimaculatum*	EU706368
Andinoacara cf. *pulcher* 'Venezuela'	EU706382	*Cichlasoma* cf. *araguaiense*	EU706371
Andinoacara cf. *pulcher* 'Rio Chirgua'	EU706383	*Cichlasoma dimerus*	EU706366
Andinoacara pulcher 'Trinidad'	EU706408	*Cichlasoma orinocense*	EU706367
Andinoacara rivulatus	EU706379	*Cichlasoma* cf. *pusillum*	EU706365
Andinoacara rivulatus AKV	EU706380	*Cleithracara maronii*	EU706394
Andinoacara sp. 'Maracaibo'	EU706397	*Krobia* cf. *guianensis*	EU706402
Acaronia nassa	EU706390	*Krobia potaroensis*	EU706364
Aequidens diadema	EU706374	*Krobia sp.* 'Oyapock'	EU706362
Aequidens chimantanus	EU706378	*Krobia sp.* 'Xingu'	EU706363
Aequidens metae	EU706376	*Laetacara curviceps*	EU706388
Aequidens patricki	EU706370	*Laetacara dorsigera*	EU706387
Aequidens sp. 'Atabapo'	EU706375	*Laetacara* sp. 'Buckelkopf'	EU706389
Aequidens sp. 'Jenaro Herrera'	EU706403	*Laetacara thayeri*	EU706401
Aequidens sp. Jarú	EU706372	*Ivanacara adoketa*	EU706396
Aequidens tetramerus 'Venezuela'	EU706386	*Nannacara anomala*	EU706391
Aequidens tetramerus 'Rio Negro'	EU706373	*Nannacara aureocephalus*	EU706392
Aequidens tubicen	EU706377	*Nannacara taenia*	EU706393
Bujurquina vittata	EU706385	*Tahuantinsuyoa macantzatza*	EU706384

Newly added taxa (i.e. not available for previous study Musilová et al. 2008):	col. no	16S	cyt *b*	S7	RAG1
Andinoacara sp. 'Silbersaum' (Peru)	*ICCU 0751*	EU706351	EU706352	EU706353	EU706404
Bujurquina sp. 'Chazuta' (Peru)	*ICCU 0765*	EU706354	EU706355	EU706356	EU706406
Bujurquina sp. 'Pilcopata' (Peru)	*ICCU 0766*	EU706357	EU706358	EU706359	EU706407

Samples already used in previous study (Musilová et al. 2008) are mentioned in the upper part with the accession number for newly obtained RAG1 sequence from GenBank.
Newly added species in this study mentioned below with their locality data, collection number and accession numbers for all four markers studied herein.

in the phylogenetic analyses with the exception of '*Aequidens*' *hoehnei*, which was not available for molecular study. The cyt *b* – 16S–S7 gene data set included sequences from Musilová et al. (2008). The nuclear gene RAG1 is newly sequenced in this study to provide more resolution and support at deeper nodes. The morphological data set was based on an extensive review of literature coupled with a study of museum specimens (Appendices S1–S3). Locations of the specimens used in this study for the RAG 1 gene are the same as in Musilová et al. (2008) and the locations of newly sequenced species are placed in Table 1. Geophagine and heroine cichlids were used as outgroup taxa.

Morphological methods

Morphological characters are taken from two sources. In total, 96 characters are included. The first part of the characters is taken from the study of Kullander (1998), so far the only study of cichlasomatine cichlids using morphological characters. These are characters 32–75 (see Appendix S2). The remaining characters were obtained in this study, both from literature (mainly Allgayer 1983; Casciotta 1998; Kullander 1983a,b, 1984a,b, 1986, 1987, 1991, 1995, 1997, 1998; Kullander and Nijssen 1989; Kullander and Prada-Pedreros 1993; Kullander and Ferreira 1991; Inger 1956) as well as from study of museum specimens. Characters from Kullander (1998) are predominantly internal anatomical characters, while those from this study (characters 1–31 and 76–96) deal mostly with external morphology (including meristic and shape characters), coloration patterns (studied in an ontogenetic perspective; see Říčan et al. 2005), and behaviour. Kullander (1998) studied a much smaller number of taxa compared with the present study. We have included Kullander's characters only for those species that he actually examined (thus not artificially inflating support for monophyly of genera), so that characters introduced in the present study make it possible to test the monophyly of genera.

Coding of morphological characters follows recommendations of Campbell and Frost (1993) and Wiens (1995, 1999). Qualitative characters were coded using the majority approach. Some characters, which showed more discernible states, were coded using the scaled coding (Campbell and Frost 1993) under the assumption that traits pass through a polymorphic stage between absence and fixed presence. The scaled method is advantageous in that it allows polymorphisms to act as synapomorphies.

Quantitative characters were coded using a modified gap weighting (GW) method of Thiele (1993). Thiele's implementation of GW involves finding, for a given character, the mean value of the trait in each species in the analysis, the range of mean species values among taxa (i.e. the species with the greatest mean value and the species with the lowest), and then dividing this range into smaller ranges or segments equal to the maximum number of character states allowed by the phylogenetic software program (i.e. 32 for PAUP*). We used a less fine-grained spacing, thus having in most cases < 32 states. States are then assigned to species based on these ranges. Evolving from low to high mean trait values (or vice versa) therefore requires passing through many intermediate states and requires many steps, whereas smaller changes in trait values involve fewer state changes and fewer steps. An important advantage of the gap-weighting method is that it incorporates information on the distance between states, weighting the changes according to the difference between mean species values (hence the name).

We used the between-state scaling (Wiens 2001) to weight quantitative multistate characters (i.e. those coded with the modified GW method; see above) against each other. This weighting scheme assigns transformations between species with fixed, adjacent values of meristic variables (e.g. 13 to 14 vertebrae or five to six anal spines) the same weight in all GW coded characters. The more fixed steps a multistate quantitative character expresses the more information in contains, but all multistate characters are a-priory weighted 1:1 in

doi: 10.1111/j.1439-0469.2009.00528.x

this method. To weight quantitative multistate characters against qualitative characters we used the between-character scaling (Wiens 2001). All characters are thus in effect weighted 1:1 to each other irrespective of their method of coding. Changes in binary variables (0–1) thus have the same weight as the whole transformation series of a multistate character.

Examined material is in Appendix S1, character description is in Appendix S2 and the character matrix is in Appendix S3.

Molecular methods

The mitochondrial cytochrome *b* and 16S rRNA genes and the nuclear first intron in S7 gene were used from Musilová et al. (2008). We have in this study additionally amplified and sequenced the conservative nuclear RAG1 gene. DNA was extracted from small pieces of muscle or gill (10–25 mg) using the DNeasyTM Tissue Kit (Qiagen, Valencia, CA, USA). We used forward primer 5′-CTGAGCTGCAGTCAGTACCATAAGATGT-3′ and reverse primer 5′-CTGAGTCCTTGTGAGCTTCCATRAAYTT-3′ (both Grande et al. 2004) for amplification of 1481 bp product. PCR condition consisted of an initial denaturation step of 94°C (2 min), followed by 36 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min. The terminal extension was at 72°C for 8 min. PCR products were purified by QIAquick® PCR Purification Kit (Qiagen), and directly sequenced with the PCR primers using the BigDye™ Terminator Cycle Sequencing Kit v.1.1 (Applied Biosystems, Foster City, CA, USA), and following manufacturer's instructions. Sequencing reaction products were cleaned with DyeEx 2.0 Spin Kit (Qiagen), and run on ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Chromatograms were assembled and checked by eye for potential mistakes using SeqMan II of the DNASTAR software package (http://www.dnastar.com). Edited sequences were aligned using Clustal W as implemented in BIOEDIT software package (Hall 1999). Stems and loops were identified in the 16S rRNA sequences by alignment and homologization with secondary structure of ribosomal RNA of catfishes (Moyer et al. 2004) and alignment was checked to correspond with the stem-loop positions.

All newly obtained sequences for RAG1 as well as sequences for 16S rRNA, cytochrome *b* and S7 intron of species newly added to data set, were submitted to GenBank (Accession Nos EU706351–EU706408). See Table 1 with the list of species.

Phylogenetic analyses

Phylogenetic analyses of the morphological data set separately or combined with nucleotide sequences (four genetic markers) were performed using maximum parsimony (MP) as implemented in PAUP* (Swofford 2002). The following heuristic search strategy was applied: we first extensively sampled the tree space using 1000 random sequence additions and keeping 10 trees per search (commands in PAUP*: hsearch addseq = random nchuck = 10 chuckscore = 1 nreps = 1000). In the next step, we run a search on the saved trees to find all the shortest trees (commands in PAUP*: hsearch start = current nchuck = 0 chuckscore = 0). Robustness of clades was assessed using bootstrapping (1000 pseudo-replications in PAUP*) with the same approach as in the MP searches, with five random sequence additions per bootstrap replication, and saving 10 trees from each random sequence addition. Data exploration further included Bremer support and partitioned Bremer support (PBS) to assess congruence or conflict between the data partitions at each node of interest. Bremer analyses were run with the same parameters as MP searches in PAUP*.

Phylogenetic analyses of the nuclear and mitochondrial sequence data were performed using Bayesian Inference (BI) as implemented in MrBayes 3.0 (Huelsenbeck and Ronquist 2001). The best-fit model for the different genes was selected with Modeltest 3.06 (Posada and Crandall 1998) using the Akaike information criterion.

Bayesian analysis was performed using two independent runs of four Metropolis-coupled chains (MCMC) of 10 million generations each, to estimate the posterior probability distribution. The combined sequence matrices were partitioned per gene fragment, and independent model parameters were estimated for each partition. Separated genes were analysed in runs of four million generations. Topologies were sampled every 100 generations, and majority-rule consensus trees were estimated after discarding the first 25% generations. Robustness of clades was assessed using Bayesian posterior probabilities.

Tests of alternative topologies

To statistically test the significance of our results against competing taxonomies (as found in the literature) and alternative phylogenies, we performed statistical tests of alternative topologies. The tests used MP and included the compare-2 T-PTP test, the Templeton test and the Shimodaira-Hasegawa (SH) as implemented in PAUP* and using default settings. The compare-2 T-PTP test has been run with 500 random additions.

Results

Results are summarized in Figs 1–5 and in Table 2. List of morphological characters is in Appendix S2, matrix of morphological characters is in Appendix S3.

Morphological phylogenetic analyses

Analyses of the morphological matrix have been performed with two alternative approaches dealing differently with multistate characters coded with the GW method (see Materials and Methods; Fig. 1). In one analysis (Fig. 1a), all multistate characters are unordered, in the second (Fig. 1b), those coded with the GW method are ordered. Analysis with all multistate characters unordered (L = 1563, N = 1206, CI = 0.46, RI = 0.72) had higher values of consistency (CI) and retention (RI) indices than the partially ordered analysis (L = 2560, N = 144, CI = 0.32, RI = 0.68). All established genera except *Bujurquina* are supported as monophyletic in both analyses (in case of *Aequidens* only with exception of *A. chimantanus*). Monophyly of (1) *Bujurquina*, (2) *Aequidens* and (3) the *′Aequidens′ pulcher* group is supported only in the unordered analysis (Fig. 1a). Two suprageneric clades are also recovered in both analyses: the {*′Aequidens′ hoehnei*, [*Laetacara*, (*Cleithracara* (*Nannacara* –- *Ivanacara*))]} clade and the (*Bujurquina*, *Tahuantinsuyoa*, *′Aequidens′ pulcher-rivulatus* group) clade.

Molecular phylogenetic analyses

Results of our molecular data set support all suprageneric clades of Musilová et al. (2008), but slightly modify relationships among them, with strong support for the branching pattern in the BI analysis (Fig. 2). The endemic lineage of the Guiana highlands (the NIC or *Nannacara – Ivanacara – Cleithracara* clade) is found as the basal-most group of cichlasomatine cichlids. The {[((*Bujurquina*, *Tahuantinsuyoa*), *′Aequidens′ pulcher-rivulatus* group), *Acaronia*], *Laetacara*} clade is the sister group of the [(*Aequidens – Cichlasoma*), *Krobia*] clade, among which *Aequidens* is paraphyletic to *Cichlasoma*. The analysis supports the independent generic status of the *′Aequidens′ pulcher-rivulatus* group, which is also supported by all molecular markers independently (Fig. 2). Only *Aequidens* is thus not supported as a monophyletic genus.

Agreement between the morphological (Fig. 1) and molecular (Fig. 2) phylogeny is thus very good at the generic level (the only exception being *Aequidens*). At the suprageneric level, the *Cleithracara*, *Nannacara – Ivanacara* clade (NIC clade in Musilová et al. 2008) and the *Bujurquina*, *Tahuantinsuyoa*, *′Aequidens′ pulcher-rivulatus* clade (BTA clade in Musilová et al. 2008) are found in both analyses. Conversely, *Laetacara* is not supported by molecular characters as a close relative of

doi: 10.1111/j.1439-0469.2009.00528.x

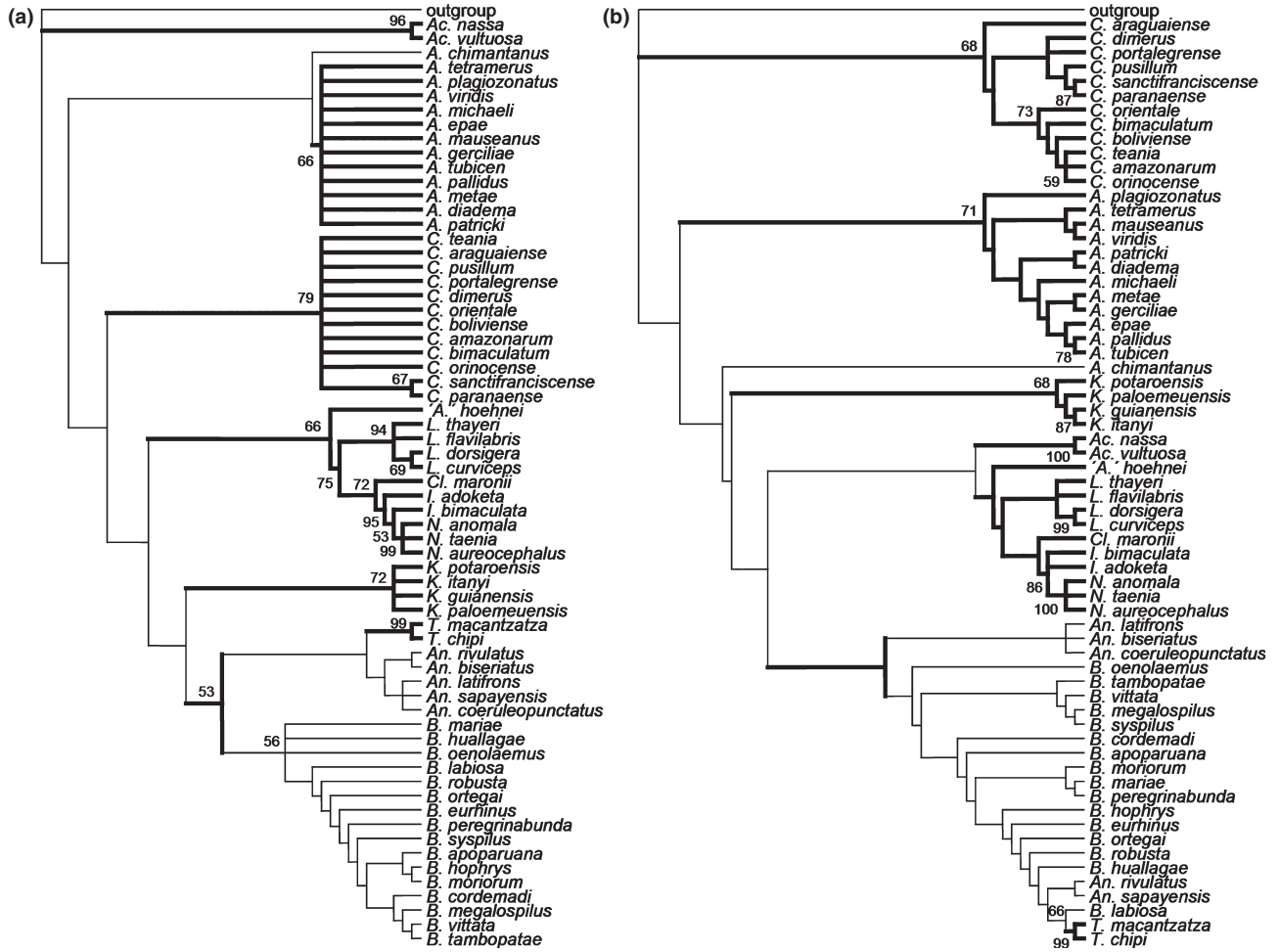


Fig. 1. Phylogeny based on morphological characters. (a)Analysis with all multistate characters unordered (L = 1563, *N* = 1206, CI = 0.46, RI = 0.72). (b) Analysis with multistate characters coded with the gap weighting method ordered (L = 2560, *N* = 144, CI = 0.32, RI = 0.68). Agreement between the two topologies shown with bold lines. Values represent bootstrap support (*N* = 1000). Genus name abbreviations: *A.* = *Aequidens, Ac.* = *Acaronia, An.* = *Andinoacara, B.* = *Bujurquina, C.* = *Cichlasoma, Cl.* = *Cleithracara, I.* = *Ivanacara, K.* = *Krobia*, *L.* = *Laetacara*, *N.* = *Nannacara*, *T.* = *Tahuantinsuyoa.*

the *Cleithracara*, *Nannacara* – *Ivanacara* (NIC) clade as found in morphological analyses.

Combined morphological-molecular phylogenetic analyses

Full taxon sampling and limited overlap between data partitions
Combined analysis of morphological and molecular data with full taxon sampling had limited overlap between morphological and molecular partitions, but nevertheless resulted in a resolved phylogeny (Fig. 3). The support values are low except for the generic level, and the phylogenetic hypothesis is also different based on treatment of ordered versus unordered multistate characters. The analysis with all multistate characters unordered resulted in more equally parsimonious trees (Fig. 3a; L = 5566, *N* > 15 000, CI = 0.37, RI = 0.67), but has higher CI and RI indices compared with the partially ordered analysis (Fig. 3b; L = 6545, *N* = 62, CI = 0.33, RI = 0.66), and is also more in agreement with the molecular analysis (Fig. 2) at the genus level. Both analyses support monophyly of all genera except *Aequidens* (see above), and the monophyly of the *'Aequidens' pulcher-rivulatus* group and of *Bujurquina* are supported only in the all-unordered analysis (Fig. 3a). Importantly, both analyses also support the {*'Aequidens' hoehnei*, [*Laetacara*, (*Nannacara* –- *Ivanacara, Cleithracara*)]} clade, in agreement with the morphological phylogeny (Fig. 1), but contrary to molecular phylogeny (Fig. 2), which finds *Laetacara* as unrelated. *'Aequidens' hoehnei* is a separate lineage.

We have performed PBS analyses on this data set to assess character conflict and agreement between data partitions, but the results are inconsistent, widely different even in the total contribution of individual data partitions and probably the reason is too much missing data partitions. Hence, we do not present the PBS results herein, but we report a PBS analysis on the matrix with reduced taxon sampling (see below).

Reduced taxon sampling and full overlap between data partitions
The reduced taxon sampling data set is the only way for us to combine both molecular and morphological data partitions without having too much missing information in our analyses. We have again performed two analyses differing in the treatment of potentially ordered characters. The all-unordered analysis (Fig. 4a; L = 4888, *N* = 1, CI = 0.38, RI = 0.66) still has slightly higher CI and RI indices, as well as a higher overall Bremer support (972 versus 965; L = 5487, *N* = 1, CI = 0.37, RI = 0.65; Fig 4b, see Table 2 for node support

doi: 10.1111/j.1439-0469.2009.00528.x

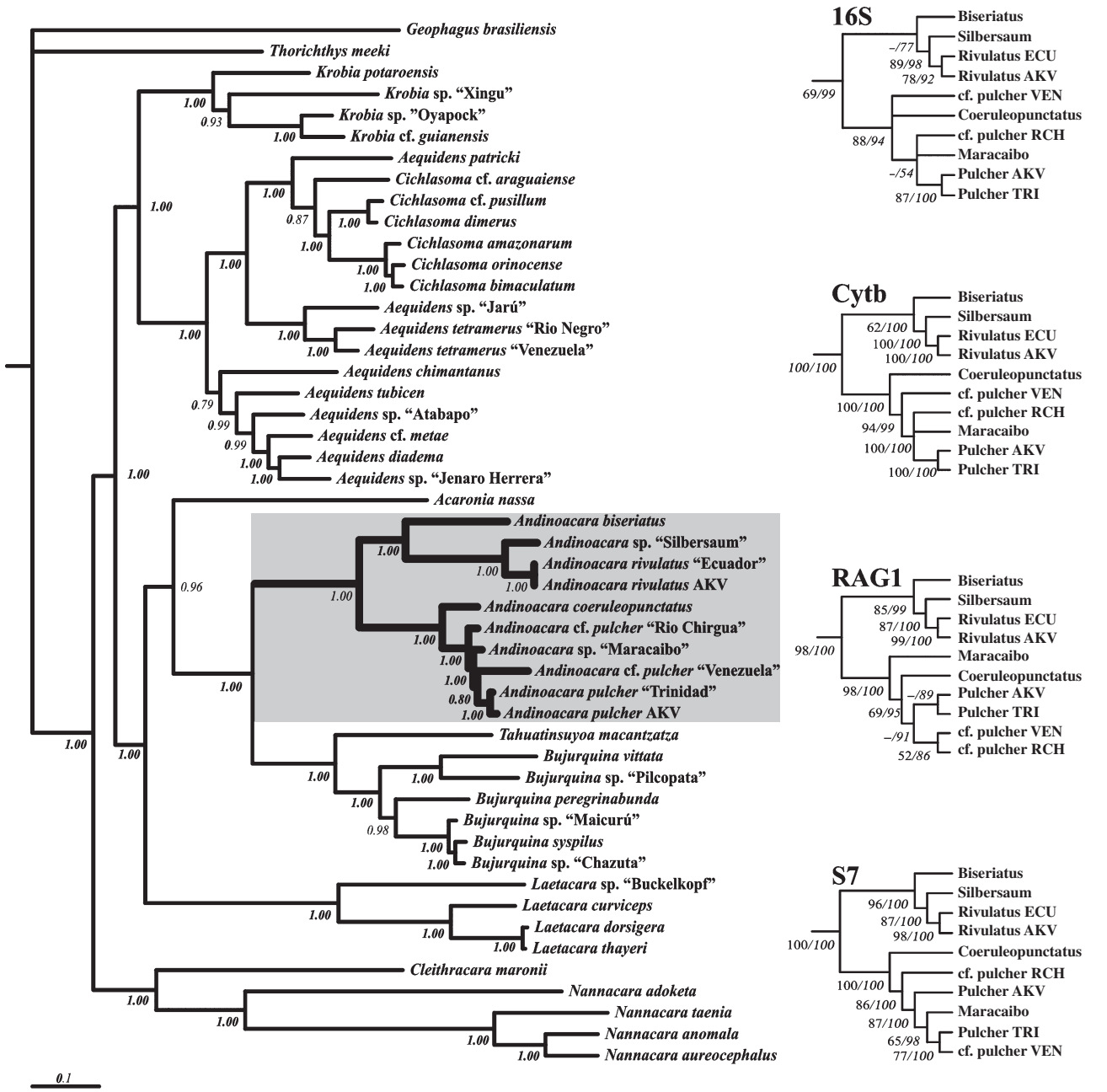


Fig. 2. Phylogeny based on 3718 molecular characters of four genes (16S, cyt b, RAG1, S7) using Bayesian inference. Analysis was performed with two parallel runs of 10 000 000 generations. The newly proposed genus *Andinoacara* is highlighted and its support and relationships of included species are shown in the individual trees to the right. These phylogenetic trees presented insets for each molecular marker were analysed separately by 4 000 000 generations. Values in nodes represent Bayesian posterior probabilities; values in inset trees represent (1) the MP bootstrap support ($N = 1000$) and (2) posterior probabilities ×100.

and PBS) and is thus internally more congruent. Composition of genera and of suprageneric clades is the same as in the molecular analysis with the exception of *Laetacara* being related to *Cleithracara* plus *Nannacara – Ivanacara*. Relationships between the main clades are yet different in the two analyses, demonstrating limits of resolution of our phylogenetic hypotheses. A considerable factor is however character conflict. Morphology strongly supports the placement of *Laetacara* with *Nannacara – Ivanacara – Cleithracara clade* (Figs 1, 3 and 4), while molecular characters strongly support a different topology (Fig. 2). Morphology yet prevails in the combined analyses (Figs 3 and 4).

On the taxonomic level most of our phylogenetic analyses are conclusive, and support the *'Aequidens' pulcher-rivulatus* group as a separate lineage, strongly supported by molecular and combined analyses, with marginal support also in morphological analyses with all characters unordered (Fig. 1a). We thus propose the *'Aequidens' pulcher* group as a new genus related to the *Bujurquina – Tahuantinsuyoa* clade. *'Aequidens' hoehnei* is also in all analyses supported as a separate lineage among cichlasomatine cichlids. But the situation is less clear-cut, since we have not been able to study this rare species for molecular characters. Based on our results it is the sister group of the *Laetacara, Cleithracara*, *Nannacara – Ivanacara* clade

doi: 10.1111/j.1439-0469.2009.00528.x

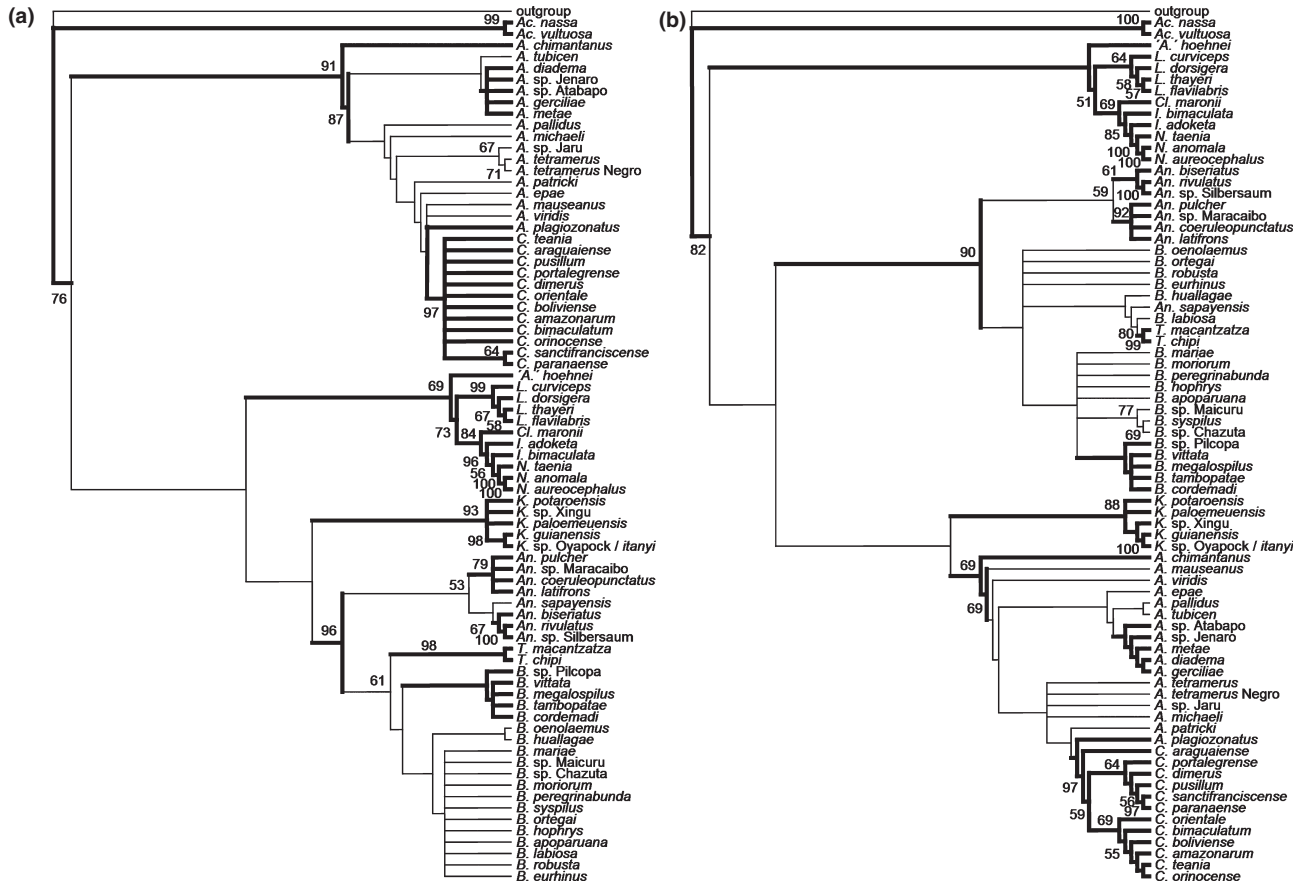


Fig. 3. Phylogeny based on combined analysis of morphological and molecular data (16S, cytb, S7, RAG1) with full taxon sampling and limited overlap between partitions (*Phase 1*). (a) Analysis with all multistate characters unordered (L = 5566, $N > 15\,000$, CI = 0.37, RI = 0.67). (b) Analysis with multistate characters coded with the gap weighting method ordered (L = 6545, $N = 62$, CI = 0.33, RI = 0.66). Agreement between the two topologies shown with bold lines. Values represent bootstrap support ($N = 1000$). Genus name abbreviations as in Fig. 1.

(which yet has a conflict between morphological and molecular data in regard of the inclusion of *Laetacara* into this clade; see Figs 2 and 4). We have additionally examined the phylogenetic position of *'Aequidens' hoehnei* under two different taxon samplings, removing from our analyses either *Laetacara* or *Cleithracara* plus *Nannacara – Ivanacara* (not shown). In both cases *'Aequidens' hoehnei* is placed in the same position in the phylogenetic tree, either as the sister group of *Laetacara* (if *Cleithracara* plus *Nannacara – Ivanacara* are deleted), or *Cleithracara* plus *Nannacara – Ivanacara* (vice versa). This demonstrates that the position of *'Aequidens' hoehnei* is not an artifact, but that it shares characters with both groups in question. While its position for the moment remains tentative, our analyses demonstrate that it represents a separate genus at least based on morphological characters.

Character analyses

The combined morphological – molecular phylogenies (Figs 3 and 4) have been used to study character evolution using the software Mesquite (Maddison and Maddison 2004). The aim of these analyses was to test diagnosability of all established genera and two separate lineages (the *'Aequidens' pulcher-rivulatus* group and *'Aequidens' hoehnei*). Autapomorphies and apomorphic character combinations have been obtained in this way for all genera and were specifically used to formulate diagnoses of the newly proposed genera (with addition of characters known to be autapomorphic and not included in the phylogenetic character matrix). Results of the diagnosability analysis are shown in Fig. 5. Based on the diagnosability analyses all genera as supported in this study except *Aequidens* are diagnosable (either on their own by unique characters and/or by combinations of apomorphic character states, or in comparison against closely related genera). *Aequidens* should best be synonymized with *Cichlasoma*, as it is paraphyletic and undiagnosable on its own. *Ivanacara* is based on our results paraphyletic to *Nannacara*, and our diagnosability analysis also recovered only very few characters shared by the *Ivanacara* species to the exclusion of *Nannacara*. Our molecular sampling is yet not complete for *Ivanacara* genus and support for it's paraphyly is also very low and a final decision about the generic status of *Ivanacara* thus awaits further study (the same applies to *'Aequidens' hoehnei*; see above). The highest degree of morphological change among cichlasomatines is found in the *Laetacara – Cleithracara – Nannacara – Ivanacara* clade and in *Acaronia*. The other genera are in comparison rather generalized morphologically.

Taxonomy

Andinoacara n. gen.

'*Aequidens*' *pulcher* group (Kullander 1998)

'*Aequidens*' *rivulatus* group (Kullander 1998)

'*Aequidens*' *pulcher* group (Musilová et al. 2008)

doi: 10.1111/j.1439-0469.2009.00528.x

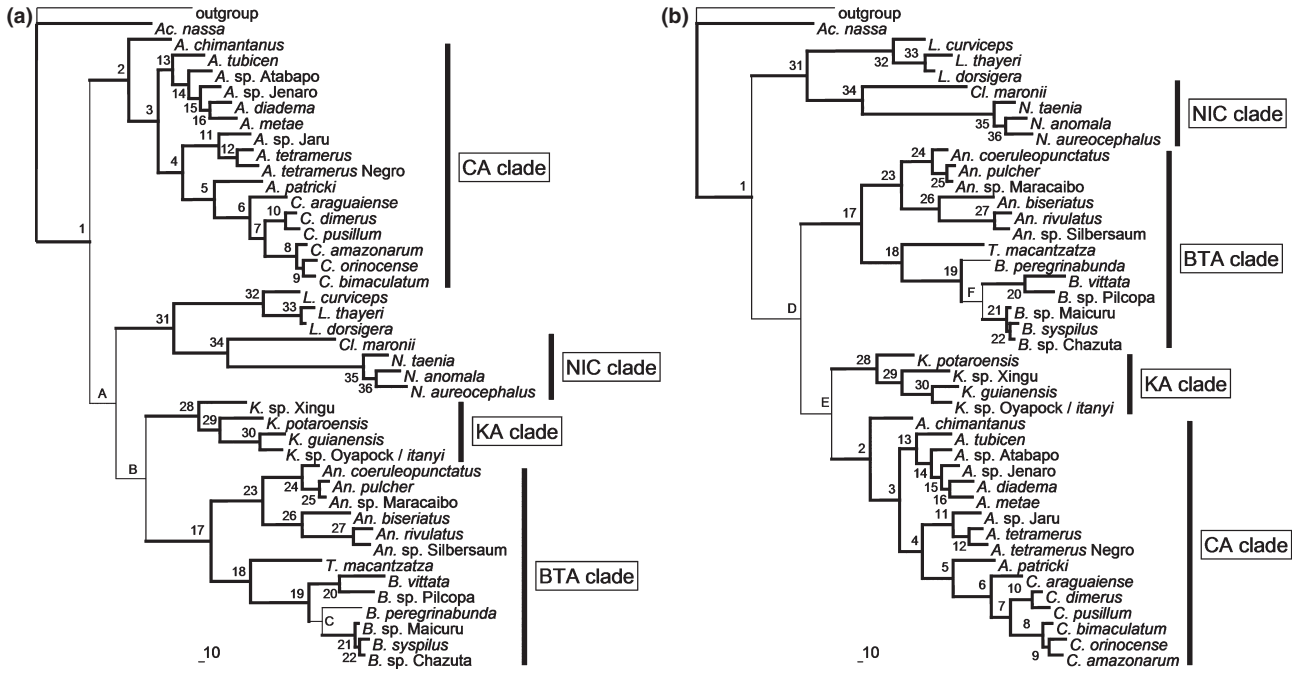


Fig. 4. Phylogeny based on combined analysis of morphological and molecular data (16S, cytb, S7, RAG1) with a reduced taxon sampling and complete overlap between morphological and molecular partitions (*Phase 2*). Branch lengths are shown. (a) Analysis with all multistate characters unordered (L = 4888, *N* = 1, CI = 0.38, RI = 0.66). The total Bremer support of the tree is 972 (PBS: Rican, Kullander, 16S, cytb, S7, RAG1: 19, 163, 111, 541, 65, 75). (b) Analysis with multistate characters coded with the gap weighting method ordered (see character matrix for details; L = 5487, *N* = 1, CI = 0.37, RI = 0.65). The total Bremer support of the tree is 965 (PBS: Rican, Kullander, 16S, cytb, S7, RAG1: 116, −24, 173, 387, 190, 123). Agreement between the two topologies shown with bold lines. Values represent node numbers. Refer to Table 1 for node support. Genus name abbreviations as in Fig. 1.

Type species. *Acara latifrons* Steindachner, 1878

Contained species. *Cichlosoma biseriatum* Regan, 1913, *Acara coeruleopuncta* Kner, 1863, *Acara latifrons* Steindachner, 1878, *Cychlasoma pulchrum* Gill, 1858, *Chromis rivulata* Günther, 1860, *Acara sapayensis* Regan, 1903, undescribed tentative species *Andinoacara* sp. Silbersaum.

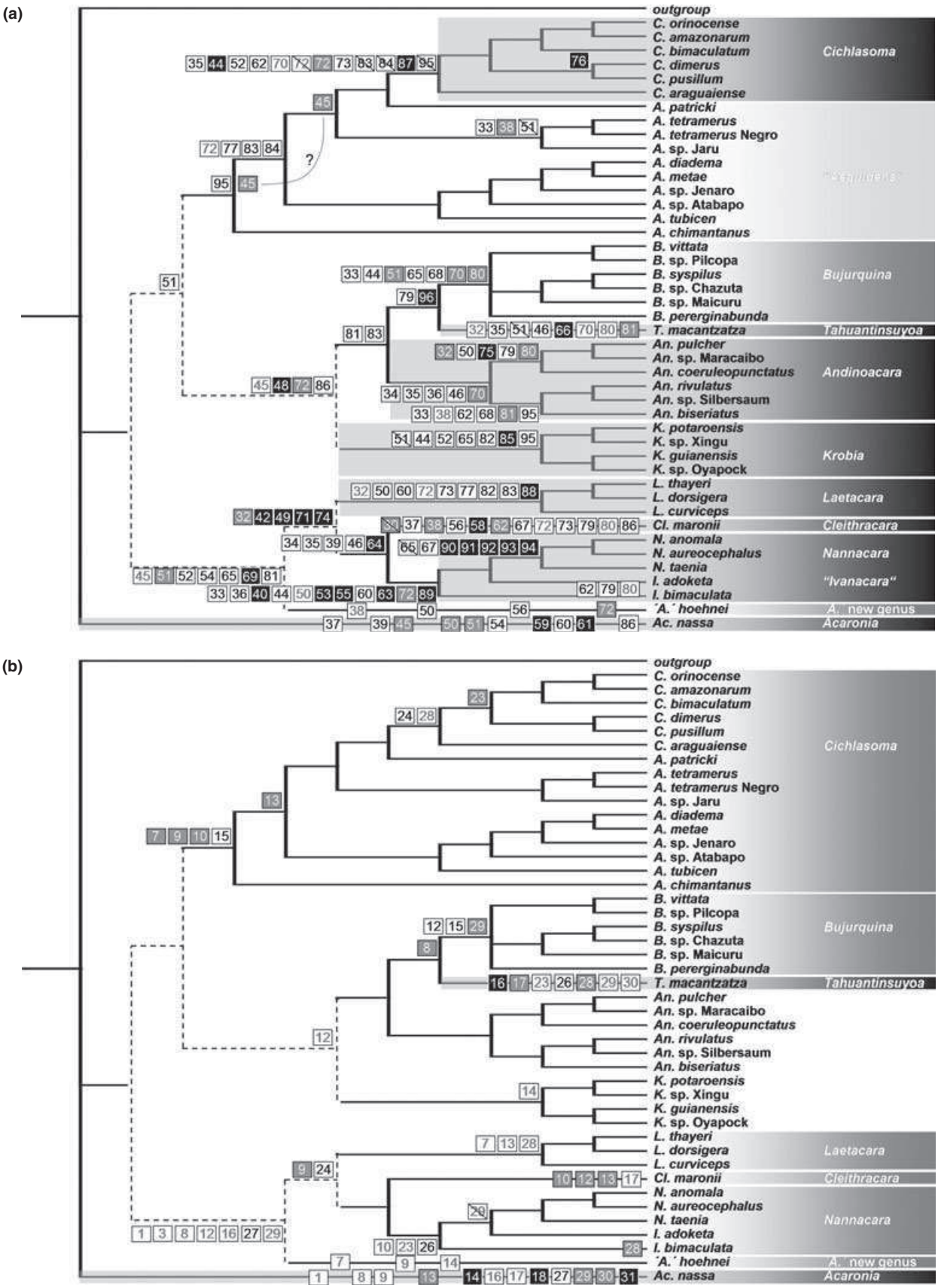
Diagnosis. Small to moderate sized (8–30 cm TL) South American cichlids with American type lips, six preoprecular and four dentary lateralis foramina, minute or absent interarcual cartilage. Similar to the closely related *Bujurquina* and *Tahuantinsuyoa* in having enlarged lateralis foramina, uniserial predorsal scale pattern, notched dorsal margin of anterior ceratohyal with laminar ledges bordering arterial groove (forming a bony canal in *A. rivulatus*), only three anal fin spines, modally six ceratobranchial gill rakers on first arch, opalescent spots and vermiculations on head, body and fins, the posterior part of the midlateral stripe inclined dorsally towards the insertion of the posterior border of the dorsal fin. Distinguished from similar genera by a combination of characters: absent parhypural spine; angle of the uncinate process of epibranchial 1 relative to the main axis of the bone very wide (versus narrow angle); epibranchial 1 with a posterodorsal laminar expansion with sharp angle (versus absent); caudal opening of the posterior myodome narrow (versus wide in *Tahuantinsuyoa* and closed in *Bujurquina*); caudal fin rounded (versus subtruncate-truncate) with brightly colored edges (also egde of dorsal fin); caudal-fin accessory lateral lines modally present and running between rays V4 and V5, and D1 and D2; < 8 scales in the lower lateral line (versus more than 8); continuation of band on head obliquely forwards across nape in some species; anteriorly inclined supraorbital stripe (or missing); prominent suborbital stripe and nearly midbasal caudal spot; *Aequidens* type breeding coloration (pale vertical bars bordering the midlateral blotch); substrate spawning non-oral brooders (versus mouthbrooders). Additionaly distinguished from *Bujurquina* in having modally 8 (versus 7) anal fin rays, < 12 (versus 12–13) abdominal vertebrae, globular interarcual cartilage (versus absent) and last basapophysis present on first to third caudal vertebra (versus on the last abdominal vertebra). Additionaly distinguished from *Tahuantinsuyoa* in modally having < 13 caudal vertebrae (versus more than 14) and having the first hemal arch on the first caudal vertebra (versus on the last abdominal vertebra).

Description. Largest examined specimen 133.3 mm SL.

Shape. Range in parentheses is range of average values in individual species. Head length 29–37% of SL (33–35%), snouth legth 6–14% of SL (8–10%), body depth 24–51% of SL (38–48%), orbital diameter 7–15% of SL (10–13%), head width 16–23% of SL (18–21%), interorbital width 8–16% of SL (11–14%), preorbital width 5–11% of SL (7–9%), caudal peduncle depth to length 45–100% (53–85%), pectoral fin length 25–44% of SL (31–41%), ventral fin length 25–52% of SL (27–42%), last dorsal fin spine length 14–23% of SL (15–21%).

Fins. Dorsal fin. XIII–XVI (XIV–XV; range of modal values for species in parentheses), 9–13 (10–11), Anal fin. III, 7–10 (8), Pectoral fin. 12–15. Unpaired fins naked.

doi: 10.1111/j.1439-0469.2009.00528.x



doi: 10.1111/j.1439-0469.2009.00528.x

Scales. Scales along midlateral line 22–25, L1. 11–18 (15–17), L2 scales 6–10 (7–9), predorsal scales 7–13 (7–10), cheek scale series 2–4 (2–3).

Gill rakers. Ceratobranchial gill rakers externally on lower portion of first arch 5–10.

Vertebrae. 12 abdominal, 13–14 caudal vertebrae, 2–4 caudal peduncle vertebrae.

Etymology. Named after the Andes mountain chain and the name *Acara*, used historically for cichlid fishes. History of the genus has been shaped by the Andes mountains and this cichlid group is distributed on both cis- and trans-andean slopes and in the intervening valleys of the three arms of the Northern Andes. The name *Acara* has been variously used as both femininum and masculinum. We use it as masculinum following *Acara tetramerus* Heckel, 1840, which is the type of the genus *Aequidens*, from which we elevate the *'Aequidens' pulcher* species group to generic status.

Distribution. North-western trans-andean South American cichlids with one species reaching into lower Central America (*A. coeruleopunctatus*) and one or more species into cis-andean South America in the Orinoco drainage (*A. pulcher*). Southern-most report is 50 km south from Lima (Peru; Stawikowski and Werner 1998), the southern-most cichlid fish record in trans-andean South America. Northern distribution border is reached in the southern pacific drainages of Costa Rica (Río Santiago-Tabasara and Río Bubi drainages; Bussing 1998). Easternmost distribution is in Venezuela and Trinidad (see Fig. 6).

Notes. *Andinoacara* species were previously placed within the genus *Aequidens* Eigenmann & Bray, 1894. Kullander (1986) erected the genus *Bujurquina* for similar species placed also in *Aequidens* and additionally described the genus *Tahuantinsuyoa*, which is, based on our results, the sister group of *Bujurquina*. The species placed here in *Andinoacara* were left in *Aequidens* in Kullander (1986) and in all subsequent studies (summary in Kullander 2003). Kullander (1983b)in his PhD thesis mentioned that species of *Andinoacara* could be placed into a separate genus (he proposed the name *Coeruleocara*, but later never used it, and specifically stated that all names introduced in his dissertation are disclaimed for the purpose of Zoological nomenclature – ICZN). We demonstrate that *Andinoacara* species are not synonymous with *Aequidens* and have to be placed in a different genus. *Andinoacara* does not present clear unique distinguishing characters or character states from the closely related *Tahuantinsuyoa* and *Bujurquina*, but is distinguished from these by character combination (see Fig. 5). An important distinguishing character from the two genera *Tahuantinsuyoa* and *Bujurquina* is behaviour, because both are larvophilic biparental mouthbooders, while *Andinoacara* are substrate spawning non-oral brooders as all other Cichlasomatini. Distribution is also a good distinguishing feature. *Andinoacara* is a trans-andean South American genus, while *Tahuantinsuyoa* and *Bujurquina* are mostly western Amazonian taxa. The phylogeny of the clade dates back to a vicariance caused by the Andes (Musilová et al. 2008).

Discussion

In this study, discordances were found in the relationships between the genera based on morphology (Kullander 1998) and molecular characters (Musilová et al. 2008). The first molecular phylogeny of cichlasomatines (Musilová et al. 2008) supported the taxonomy established by Kullander (1998, 2003 and references therein) only in the generic level. Contrarily, deeper phylogeny presented by these two studies was conflicting a lot. For this reason we have decided to explore both morphological and molecular data sets simultaneously in the present study.

Fig. 5. Diagnosability analyses. Morphological characters have been mapped onto the total evidence phylogenetic analysis with the addition of *'Aequidens' hoehnei* and both *Ivanacara* species (*'Aequidens' hoehnei* and *Ivanacara bimaculata* are missing molecular information, all other species have both morphological and molecular data partitions). The characters have been mapped using Mesquite (Maddison and Maddison 2004). Multistate characters coded with the gap weighting method have been ordered (as in Fig. 4b) which is justified by the logic of this coding method and is also better for interpreting apomorphies in the multistate characters (especially those coded with the gap weighting GW method; see Materials and Methods). The analysis had the following results: L = 5889, N = 2, CI = 0.36, RI = 0.65. (a) Diagnosability analysis based on qualitative characters (characters 23–96; see Appendix S2). (b) Diagnosability analysis based on quantitative characters (characters 1–31). Legend to character boxes: White number in a black box: the character is unique for the group (in case of multistate characters – (b) – is this coding applied when the ancestral state for cichlasomatines is on one extreme of the character range and the given group is unique for being at the opposite extreme of the range); Black number in a white box: the character is apomorphic for the group, but is shared with one or more other groups (in case of mutistate characters – (b) – it is applied in a situation where the ancestral state for cichlasomatines is on one extreme of the character range and the given group is apomorphic in lying on the opposite extreme of the range, but this apomorphy is not unique); The two other codings are only applied to multistate characters in the situation that the ancestral state for cichlasomatines is the middle of the characters range. White number in a grey box is for apomorphies at the upper extreme of the characters range and the character state is not autapomorphic for the given group. Grey number in a white box is for apomorphies at the lower extreme of the characters range and the character state is not autapomorphic for the given group. Shading of taxa demonstrates level of diagnosability (shading towards black for genera with unique characters; shading towards grey for genera with a unique combination of characters but lacking unique characters). Interrupted lines demonstrate lack of resolution and support at basal nodes in the cichlasomatine phylogeny. In (a) *Aequidens* is the only non-diagnosable genus (and it is also paraphyletic to the well diagnosable *Cichlasoma*). In (b) *Krobia* and *Andinoacara* are not diagnosable [both genera are diagnosable in (a)] and *Aequidens* is diagnosable only when synonymized with *Cichlasoma*. Based on the diagnosability analyses all genera as proposed in this study except *Aequidens* are diagnosable (either on their own by unique characters and⁄or by combinations of apomorphic character states, or in comparison against closely related genera). *Aequidens* should best be synonymized with *Cichlasoma*, as it is paraphyletic and undiagnosable on its own. *Ivanacara* is based on our results paraphyletic to *Nannacara*. Our molecular sampling is yet not complete for this genus and support for it's paraphyly is also very low and a final decision about the generic status of *Ivanacara* thus awaits further study. The highest degree of morphological change among cichlasomatines is found in the *Laetacara – Cleithracara – Nannacara – Ivanacara* clade and in *Acaronia*. The other genera are in comparison rather generalized morphologically. The question mark in (a) show the uncertain position of character 45, because of missing data.

doi: 10.1111/j.1439-0469.2009.00528.x

Table 2. Character support for combined analyses with reduced taxon sampling (see Fig. 4)

			PBS									PBS					
			Morphology		Molecular partitions							Morphology		molecular partitions			
	Boot	BS	This study	Kullander	16S	cytb	S7	RAG1		Boot	BS	This study	Kullander	16S	cytb	S7	RAG1
Node 1	80	24	−10	30	1	7	−3	−1	Node 1	73	16	−20	20	4	−1	10	3
Node 2	100	22	−5	10	2	17	−2	0	Node 2	98	20	6	−5	5	7	5	2
Node 3	96	20	6	10	−1	13	−6	−1	Node 3	99	31	5	0	5	22	−2	1
Node 4	95	12	0	0	2	5	1	4	Node 4	91	15	9	−10	4	1	5	6
Node 5	94	13	0	0	2	11	0	0	Node 5	90	14	2	0	2	10	0	0
Node 6	100	39	21	15	1	5	−4	1	Node 6	100	48	23	5	6	9	2	4
Node 7	88	5	0	0	3	1	1	1	Node 7	93	8	1	0	2	5	0	1
Node 8	100	39	0	0	6	31	1	1	Node 8	100	41	2	0	5	32	1	1
Node 9	54	1	−2	0	1	2	0	0	Node 9	53	2	4	0	0	−2	0	0
Node 10	100	24	−3	0	3	22	0	2	Node 10	100	23	−3	0	3	21	0	2
Node 11	100	27	0	0	2	25	−1	1	Node 11	100	27	0	0	2	25	−1	1
Node 12	100	18	0	0	4	11	0	3	Node 12	100	19	0	0	4	12	0	3
Node 13	90	5	−1	0	3	3	0	0	Node 13	82	8	6	−9	5	0	3	1
Node 14	94	7	0	0	−1	10	−2	−1	Node 14	76	6	−8	0	3	16	−5	0
Node 15	61	1	0	0	1	0	0	0	Node 15	55	2	0	0	1	1	0	0
Nod 16e	59	1	0	0	1	4	−4	0	Node 16	52	1	0	0	1	4	−4	0
Node 17	100	39	1	13	2	10	9	4	Node 17	100	38	0	5	3	13	12	5
Node 18	91	13	0	−15	6	8	6	8	Node 18	87	13	7	−20	9	−2	10	9
Node 19	100	21	0	5	0	14	2	0	Node 19	90	18	−4	5	4	14	2	−3
Node 20	100	23	0	0	7	15	2	−1	Node 20	100	25	12	−10	11	6	6	0
Node 21	100	34	−5	0	3	33	2	1	Node 21	100	31	0	0	4	26	1	0
Node 22	88	3	0	0	0	1	1	1	Node 22	90	3	0	0	0	1	1	1
Node 23	100	43	−1	0	1	27	10	6	Node 23	100	33	0	0	6	11	10	6
Node 24	100	35	0	5	5	16	5	4	Node 24	100	35	7	−5	5	13	9	6
Node 25	100	17	0	0	1	12	5	−1	Node 25	100	15	7	−10	1	6	10	1
Node 26	82	8	0	0	−1	5	3	1	Node 26	94	13	12	−10	3	−3	7	4
Node 27	100	64	8	0	2	50	2	2	Node 27	100	83	12	10	8	38	9	6
Node 28	100	34	−1	8	3	22	2	2	Node 28	100	27	12	−10	0	11	9	5
Node 29	/	4	0	0	2	4	−2	0	Node 29	/	1	7	−10	2	−5	5	2
Node 30	100	34	−3	2	6	16	6	6	Node 30	100	31	0	0	5	13	7	6
Node 31	85	27	0	25	4	−1	−1	0	Node 31	88	26	−22	40	2	−10	9	7
Node 32	100	117	7	15	14	53	16	12	Node 32	100	123	27	−3	18	44	23	15
Node 33	100	46	−4	0	7	41	1	1	Node 33	98	25	−18	−10	9	36	5	3
Node 34	94	33	−1	15	−1	5	7	8	Node 34	66	11	−20	20	1	−5	9	6
Node 35	100	91	21	5	20	14	18	13	Node 35	100	89	21	0	22	15	18	13
Node 36	100	13	0	0	2	10	0	1	Node 36	100	14	7	−10	3	6	5	3
Node A	/	5	−5	10	−1	8	−5	−2	Node D	73	27	5	10	1	9	1	1
Node B	/	5	−5	10	−1	8	−5	−2	Node E	/	1	7	−10	2	−5	5	2
Node C	75	5	1	0	0	3	0	1	Node F	/	2	10	−7	2	−7	3	0
Total	3326	972	19	163	111	541	65	75	Total	3248	965	116	−24	173	387	190	123
In % Unord		100	1.95	16.76	11.41	55.65	6.68	7.71	In % Ord		100	12.02	−2.48	17.92	40.1	19.68	12.74

Left half of table is for unordered analysis (see Fig. 4a), right half for matrix containing ordered characters (see Fig. 4b).
First column in each half of table shows bootstrap support (Boot; Nreps = 1000), remaining columns show Bremer support (BS) and partitioned Bremer support (PBS; six data partitions: MOR: this study, Kullander (1998); MOL: 16S, cytb, S7, RAG1).
Lower two lines show totals of bootstrap and BS (PBS) analyses, which can be interpreted as overall support for each cladogram.
Based on this the unordered analysis (left half, Fig. 4a) is more internally consistent.

Phylogeny of cichlasomatine cichlids based on morphological and molecular characters

Our morphological phylogeny supports the monophyly of all cichlasomatine genera with the exception of *Aequidens* sensu Kullander (2003) and *Ivanacara* (sensu Römer and Hahn 2007). In most respects our analyses thus support the revisionary studies by Kullander (1986) and Kullander and Nijssen (1989). The least supported genera in morphological characters are (1) *Bujurquina* and (2) the newly proposed genus *Andinoacara*, where each of them lack unique characters, but are nevertheless diagnosable by combinations of apomorphic character states (Fig. 5). Both are monophyletic and strongly supported in all molecular analyses, in all combined morphological – molecular analyses with a full overlap between data partitions, and in all other analyses with multistate morphological characters unordered (Fig. 1a). The only conflict is thus with the multistate quantitative morphological characters, which when ordered receive effectively higher weights and thus overweight qualitative morphological characters. Based on combined analyses many of these characters need to be interpreted as convergent and as depicting the meristic and plastic variation within the genera.

The genus *Aequidens* was found as paraphyletic to *Cichlasoma* in both the previous (Musilová et al. 2008) and the present molecular study (Fig. 2). In morphological data is

doi: 10.1111/j.1439-0469.2009.00528.x

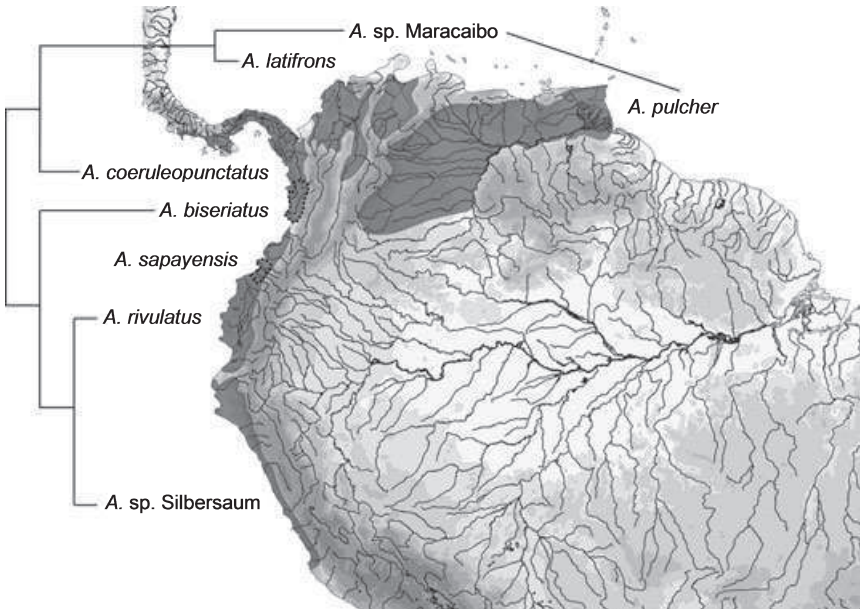


Fig. 6. Map showing distribution of the newly proposed genus *Andinoacara*. The genus is originally trans-Andean South American (i.e. distributed west of the Andes). The sister group of *Andinoacara* (*Bujurquina* plus *Tahuantinsuyoa*) are ancestrally a western Amazonian lineage. The separation of *Andinoacara* from the *Bujurquina – Tahuantinsuyoa* lineage corresponds to a vicariance caused by the Andean orogeny (see Musilová et al. 2008). The *A. rivulatus* group is limited to the ancestral area, while the *A. pulcher* group has one species in the ancestral area (*A. coeruleopunctatus*, which also reaches into southern Costa Rica in lower Central America) and the rest of the group has secondarily colonized cis-Andean South America in the Orinoco drainage; *A. latifrons* in the Cauca valley (between the Western and Central Andean chains) and in the Magdalena valley (between the Central and Eastern chains), *A.* sp. Maracaibo in the Maracaibo valley (within two bifurcations of the Eastern chain) and *A. pulcher* in the extensive Orinoco llanos and on the island of Trinidad in the Orinoco delta area.

Aequidens monophyletic (Fig. 1; with the exclusions of the Guianan species belonging to *Krobia* instead; see below), but combined morphological and molecular phylogenies do again recover *Aequidens* as paraphyletic to *Cichlasoma* with high support (Figs 3 and 4). Morphological analyses recover the two genera as monophyletic, but in combined analyses, where *Aequidens* is paraphyletic, a conflict between morphological and molecular partitions is weak or absent (see nodes 2–5 in Figs 4a and b and in Table 2). Morphological characters do not reject the paraphyly of *Aequidens* s. str. in respect to *Cichlasoma* (see also Fig. 5).

Two species from the Guiana drainages formerly placed tentatively in *Aequidens* (Kullander 2003) are in all our morphological, molecular and combined analyses strongly supported as part of the genus *Krobia* (*Krobia potaroensis*, *Krobia paloemeuensis*).

In agreement with previous morphological phylogeny (Kullander 1998) and with molecular phylogenies (Musilová et al. 2008; this study) the genera *Nannacara, Ivanacara* and *Cleithracara* are found as closely related (*Ivanacara* is, yet, in our analyses paraphyletic to *Nannacara*). Both Kullander (1998) and our morphological phylogeny (Fig. 1) support a close relationship of *Laetacara* with the *Nannacara – Ivanacara – Cleithracara* clade. This group is yet not supported by molecular characters, which find *Laetacara* as the sister group to BTA clade (*Bujurquina – Tahuantinsuyoa – Andinoacara*) (Fig. 2). Morphological characters overweight molecular characters in the total evidence analyses (Figs 3 and 4), but the conflict cannot be interpreted as based only on molecular data (see PBS results). In the analysis with all multistate morphological characters unordered (node 31 in Fig. 4a; Table 2 left), there is virtually no conflict between morphological and molecular partitions. The conflict is only present in the analysis with ordered multistate characters (node 31 in Fig. 4a; Table 2 right), and in this case both morphological as well as part of molecular characters (cyt *b*) are conflicting the node. The conflict is thus not between data partitions but in treatment of ordering of multistate morphological characters.

Morphological phylogeny further supports a close relationships between *′Aequidens′ hoehnei* and the {*Laetacara*, [*Cleithracara* (*Nannacara – Ivanacara*)]} clade. Kullander's (1998) morphological phylogeny supports the same placement, but only in analysis without a-posteriori weighting, which Kullander (1998) did not present as his final phylogenetic hypothesis. We have unfortunately been unable to study this rare species for molecular characters and its phylogenetic position thus remains tentative, based only on morphological characters. It yet presents a completely unique character combination and is both in terms of phylogenetic relationships and diagnosability not attributable to any established cichlasomatine genus. Statistical tests of alternative topologies (Table 3) yet could not reject the possibility that *′Aequidens′ hoehnei* belongs to any of the above genera (*Laetacara*, *Cleithracara* or *Nannacara – Ivanacara*), probably only due to lack of molecular data.

Finally, morphological (with unordered quantitative morphological characters (see Results), molecular and combined phylogenies, diagnosability analyses (Fig. 5) and tests of alternative topologies (Table 3) support a close relationship and separate generic status of the genera *Bujurquina*, *Tahuantinsuyoa* and the newly named genus *Andinoacara* (the BTA clade). Support for the monophyly of *Bujurquina* and *Andinoacara* is weak in morphological data (Fig. 1), but very strong in the molecular data (Fig. 2) and in combined analyses (Figs 3 and 4) with unordered quantitative morphological characters (see Results).

doi: 10.1111/j.1439-0469.2009.00528.x

Table 3. Tests of alternative topologies for newly proposed genera and taxonomic rearrangements

Test no.	Tested genus/topology	Test result	Taxonomic solution	Tested data set (length difference)	Test		
					Compare-2	Templeton	Kishino-Hasegawa
1	*Aequidens* monophyletic	Rejected	Synonymize with *Cichlasoma*	Comb. data (35)	0.0021	$< 0.0001^{1}$	0.0196^{1}
2	*A: potaroensis* part of *Aequidens – Cichlasoma*	Rejected	Part of *Krobia*	Comb. data (36)	0.034^{1}	0.0070^{1}	0.0086^{1}
3a	*A: pulcher-rivulatus*group part of *Bujurquina*	Rejected	*Andinoacara* n. gen.	Comb. data (29)	0.008^{1}	0.0017^{1}	0.0283^{1}
3b	*A: rivulatus group* sister to *Bujurquina - Tahuant.*	Rejected	*Andinoacara* n. gen.	comb. data (38)	0.03^{1}	0.0182^{1}	0.0118^{1}
4a	*A: hoehnei* part of *Namacara – Ivanacara*	Not rejected	Generic status uncertain	Comb. data, but mol. data missing (43)	0.002^{1}	0.0954	0.0894
4b	*A: hoehnei* part of *Cleithracara*	Not rejected	Generic status uncertain	Comb. data, but mol. data missing (43)	0.002^{1}	0.6251	0.0719
4c	*A: hoehnei* part of *Laetacara*	Not rejected	Generic status uncertain	Comb. data, but mol. data missing (18)	0.138	0.8011	0.4014
4d	*A: hoehnei* part of *Krobia* (Kullander 1983b)	Rejected	Not congeneric with *Krobia*	Comb. data, but mol. data missing (51)	0.002^{1}	0.3424	0.0433^{1}
5	*Ivanacara* monophyletic	Not rejected	*Ivanacara*	Comb. data (3), but mol. data missing in *I. bimaculata*	0.144	0.4862	0.0804

'Part of' is tested as 'sister group of'. Alternative topologies have been tested on the same combined morphological – molecular data set as show in Fig. 5. The tests (1) reject monophyly of *Aequidens* in respect to *Cichlasoma* (see Discussion) and also reject placement of *'Aequidens' potaroensis* into the *Aequidens – Cichlasoma* lineage (test 2). All tests (3a and 3b) support the separate generic status of *Andinoacara* n. gen. (the *'Aequidens' pulcher – rivulatus* group of previous authors). The rare and enigmatic *'Aequidens' hoehnei* is in all morphological and in combined analyses with molecular data recovered as the sister group to the NIC clade (*Namacara – Ivanacara – Cleithracara*) plus *Laetacara* and phylogenetic analyses are thus supportive of its separate generic status, which is also justified based on the diagnosability analyses (see Fig. 5 and Discussion). We have not yet been able to obtain samples for molecular data analysis of this species and most tests of alternative topologies are thus insignificant. We thus cannot reject the possibility that *'Aequidens' hoehnei* is congeneric with *Namacara – Ivanacara, Cleithracara* or *Laetacara*. Morphological data are however strong enough to reject its placement in *Krobia* (sensu Kullander 1983b). Despite paraphyly of Ivanacara to Nannacara in our phylogenetic analyses we cannot reject its monophyly (sensu Römer and Hahn 2007; test 5). [1] = tested topologies are significantly rejected.

Our separate and combined analyses yet do not provide a robust phylogenetic hypothesis for the relationships between the three main clades (*Bujurquina, Tahuantinsuyoa*, *Andinoacara*) versus (*'Aequidens' hoehnei*, *Laetacara*, *Cleithracara*, *Nannacara – Ivanacara*) versus (*Aequidens – Cichlasoma*) and between *Krobia* and *Acaronia*.

Generic diversity of cichlasomatine cichlids

Based on the results of our study cichlasomatine cichlids are divided into five lineages and eleven genera. Major changes to previous classification (Kullander 2003) are made to the genus *Aequidens*. One new genus is described from within *Aequidens* sensu Kullander (2003) in the present study (*Andinoacara* n. gen.) and one remains putative pending study of molecular data (*'Aequidens' hoehnei*). Both are unrelated to *Aequidens*. *Andinoacara* n. gen. is the trans-andean sister group of the western Amazonian *Bujurquina* plus *Tahuantinsuyoa*, while *'Aequidens' hoehnei* is likely the sister group of [*Laetacara*, *Cleithracara* (*Nannacara – Ivanacara*)]. We also transfer two former *Aequidens* species into *Krobia* (*K. potaroensis*, *K. paloemeuensis*). Even with these adjustments *Aequidens* still remains paraphyletic to *Cichlasoma*.

To provide taxonomical continuity and based on our results we divide the BTA (*Bujurquina*, *Tahuantinsuyoa*, *Andinoacara*) clade into three genera. Without the previous work of Kullander (1986) who established the genera *Bujurquina* and *Tahuantinsuyoa* some authors may as well have classified the BTA clade as one genus. But historical and taxonomical continuity are not the only considerations for our taxonomic decision. Three macro evolutionary traits distinguish *Andinoacara* from *Bujurquina* plus *Tahuantinsuyoa*: (1) *Andinoacara* has a independent history from *Bujurquina* plus *Tahuantinsuyoa* (see molecular phylogeny, Fig. 2); (2) it also has a disjunct distribution from the two genera (see above and Fig. 6); and (3) *Bujurquina* plus *Tahuantinsuyoa* are mouth breeding species, while *Andinoacara*, like the rest of cichlasomatine cichlids are substratum breeding species (with some possible exceptions in *Aequidens*). Apart from these macro evolutionary considerations, also the diagnosability analysis (Fig. 5) demonstrates that having the BTA clade as representing one genus is not the most accurate solution. Despite rather strong support in morphological trees (Fig. 1) the BTA clade has very few clear-cut or even unique characters (Fig. 5). The morphological diversity is rather strongly clustered within the group, and would actually slightly better conform to a four genus classification scheme (in splitting *Andinoacara* into the pulcher group and the rivulatus group). Our proposed classification is thus not over splitting.

Some of the most interesting taxonomic questions in cichlasomatine cichlids further remain within the genus *Aequidens*. *Aequidens* is composed of several phylogenic lineages successively more closely related to *Cichlasoma*. The basal-most lineage is the Guiana highland endemic from Venezuela, *A. chimantanus*. The second lineage are *Aequidens* species with distinct facial markings, which are yet also distinctly developed in *A. patricki* (western Amazonia), the closest relative of *Cichlasoma* (without any facial markings). The last group is the type lineage of *Aequidens* (*A. tetramerus*, *A.* sp. Jaru), again without facial markings. The diversity of species within *Aequidens* s. str. is yet large, and only a small portion of the species has so far been studied using molecular markers. Before any further taxonomic changes are made to *Aequidens* it is necessary to have

doi: 10.1111/j.1439-0469.2009.00528.x

a robust phylogeny of all included species using both molecular (nuclear and mitochondrial) and morphological characters.

Based on the results of the phylogenetic (Figs 1–4) and diagnosability analyses (Fig. 5), and the results of the statistical tests of alternative topologies (Table 3), the most accurate solution at the present state of knowledge would be to synonymize *Aequidens* with *Cichlasoma*. *Cichlasoma* is well diagnosable, while *Aequidens* is not and is in all morphological characters simply ancestral to *Cichlasoma* (quite many characters actually show a gradual transformation within *Aequidens* towards *Cichlasoma*; Fig. 5). The alternative, that of splitting *Aequidens* into several genera, does not find much support in the presented morphological characters (but many more characters could probably be found in a more detailed study focusing on the *Aequidens – Cichlasoma* question).

The alternative, that of splitting *Aequidens* into several genera, would at the present moment need to result in the establishment of at least four separate genera based on our analyses of a limited number of taxa (Fig. 4), but the paraphyly of *Aequidens* could be much more extensive as suggested by the more inclusive analysis (Fig. 3). If this should prove to be true the number of small genera would grow excessive and would not be justifiable. Molecular trees also suggest that such genera would be separated by very short internodes, suggesting recent speciations, which are better treated as within generic. Most indications thus for the present moment suggest to synonymize *Aequidens* with *Cichlasoma* but additional more detailed studies are clearly needed.

Römer and Hahn (2007) described the genus *Ivanacara* for two species traditionally placed into *Nannacara* (*N. adoketa*, *N. bimaculata*). None of our analyses supports division of *Nannacara* into two genera, the proposed genus *Ivanacara* being in the morphological phylogeny paraphyletic to the rest of *Nannacara* or the topology was unresolved (Figs 1 and 3). Nevertheless, this paraphyly is weakly supported in our analyses (bootstrap 53 and 56; see Figs 1a and 3b respectively) and we thus continue using the name *Ivanacara*. Unfortunately, one of two *Ivanacara* species, *I. bimaculata,* has not been available to us for molecular analyses. Both *Ivanacara* species need to be included in future studies to finally resolve the generic status of *Ivanacara*.

Contrasting diversity and evolution of cichlasomatine and heroine cichlids

The two sister tribes of cichlasomatine cichlids (Cichlasomatini and Heroini) differ greatly in their generic diversity and evolutionary history. While both groups have comparable species diversity, heroine cichlids include a much larger number of lineages, restricted or monotypic genera and also a much larger spectrum of body forms, ecologies and adaptations (Concheiro Pérez et al. 2007; Říčan et al. 2008). The different evolutionary histories of cichlasomatines and heroines are well reflected in the different structuring of diversity i.e. the high number of isolated small lineages and fast divergences in large clades in heroines compared with the well clustered diversity of cichlasomatines. These alternative evolutionary histories are also well reflected in the degree in which morphological diversity agrees with molecular phylogeny. In cichlasomatines there is a very good agreement between morphological and molecular phylogenetic trees, while in heroines the morphological trees have very little resemblance to molecular trees (Chakrabarty 2007; Říčan et al. 2008), despite a similar number of characters sampled and the same coding techniques and computing algorithms used in both groups. The differences thus appear to have a biological meaning.

The biogeographical setting of cichlasomatine and heroine diversification has also been intriguingly rather different. Most cichlasomatine lineages evolved in different corners of the Amazon basin and especially in and around the Guiana highlands (Musilová et al. 2008), while most heroine evolution is centered on Mesoamerica (plus Greater Antilles), the Andes and the Brazilian highlands (Říčan and Kullander 2006; Concheiro Pérez et al. 2007 Říčan et al. 2008). The only common area of distribution of cichlasomatines and heroines (except for the highly mixed lowland Amazonia) are thus the slopes of the Andes, with both groups having diversified mainly elsewhere. The different evolutionary areas and local conditions obviously played important roles in the evolution of each of the two sister groups. With the known phylogeny of both tribes the subfamily Cichlasomatinae opens itself as an ideal model to a wealth of both micro- and macroevolutionary studies.

Zusammenfassung

Phylogenie der neotropischen Cichlasomatini (Teleostei: Cichlidae) auf Grund morphologischer und molekularer Daten, mit der Beschreibung einer neuen Sattung

Die phylogenetischen Beziehungen der cichlasomatinen Cichliden wurden anhand einer umfangreichen Artenauswahl mittels morphologischer und molekularer Datensätze ermittelt. Die neue Gattung, *Andinoacara* n. gen. (von früheren Autoren als *A. pulcher-rivulatus* Gruppe bezeichnet) mit sechs Arten aus dem westandinen Südamerika und dem nordwestlichen ostandinen Südamerika wird basierend auf phylogenetischen und diagnostischen Analysen sowie Tests alternativer Topologien beschrieben. Unsere Ergebnisse zeigen, dass die Vielfalt der cichlasomatinen Cichliden in fünf prinzipiellen Linien mit elf Gattungen und drei Kladen (über Gattungsebene) aufgeteilt ist. Das sind: der ((*Bujurquina*, *Tahuantinsuyoa*), *Andinoacara*) Kladus (BAT); der (*Cleithracara*, (*Nannacara*, *Ivanacara*)) Kladus (NIC) mit *Laetacara* und *'Aequidens' hoehnei* sowie der (*Aequidens*, *Cichlasoma*) Kladus. Zwei ehemalige *Aequidens*-Arten (*K. potaroensis*, *K. paloemeuensis*) werden in die Gattung *Krobia* überführt. *'Aequidens' hoehnei* stellt wahrscheinlich eine einzigartige evolutionäre Linie dar, die eine eigenständige Gattung rechtfertigt. Molekulare Daten liegen hierfür jedoch nicht vor, sodass weitere Untersuchungen notwendig sind. Die verwandtschaftlichen Beziehungen der drei Kladen (oberhalb Gattungsebene) und zwischen *Acaronia* und *Krobia* konnten mit unseren Datensätzen aus den zwei mitochondrialen (16S and cyt *b*) und den zwei nuklearen (S7 and RAG1) molekularen Markern und den 96 morphologischen Merkmalen nicht überzeugend geklärt werden.

Acknowledgements

We thank Sven Kullander (NRM, Stockholm) for access to specimens under his curation. We would like to thank Rainer Stawikowski, Lutz Krahnefeld and Ingomar Kranz for their help with obtaining several samples. We also thank Ingo Schindler for the help with second language abstract. Thanks to Ori for locality information. Financial support was provided by a scholarship from Stockholm University (to OŘ), grant from the SYNTHESYS program for Access to the Swedish Museum of Natural History (Stockholm, Sweden; to OŘ) within the European Community programme "Improving the Human Research Potential and the Socio-Economic Knowledge Base", by the MSM6007665801 and the GAČR 206/08/P003 grants to OŘ; and GAUK 139407 and foundation of the Centre for Biodiversity LC06073 (MSMT) and IRP FAPPZ, CZU No. MSMT 604670901 to ZM. The Institute of Animal Physiology and Genetics receives continuous support from IRP IAPG (No. AV0Z50450515). Two anonymous reviewers significantly improved an early version of the manuscript.

doi: 10.1111/j.1439-0469.2009.00528.x

References

Allgayer R (1983) Nannacara aureocephalus, espèce nouvelle de Guyane française (Pisces, Cichlidae). Rev Fr Cichlidoph **33:**13–16.

Bussing WA (1998) Peces de las Aguas Continentales de Costa Rica. Supplement to Revista de Biologia tropical 46:2. Universidad de Costa Rica, pp 468.

Campbell JA, Frost DR (1993) Anguid lizards of the genus *Abronia*: revisionary notes, description of four new species, a phylogenetic analysis, and key. Bull Am Mus Nat Hist **216:**1–121.

Casciotta JR (1998) Cichlid-fishes from la Plata basin in Argentina: *Laetacara dorsigera* (Heckel), *Bujurquina vittata* (Heckel), and *'Cichlasoma' facetum* (Jenyns) (Perciformes: Labroidei). Neotrópica **44:**23–39.

Chakrabarty P (2007) A morphological phylogenetic analysis of Middle American cichlids with special emphasis on the section ''Nandopsis'' sensu Regan. Miscellaneous Publications, Museum of Zoology, University of Michigan, **198:**1–31.

Concheiro Pérez GA, Říčan O, Ortí G, Bermingham E, Doadrio I, Zardoya R (2007) Phylogeny and biogeography of 91 species of heroine cichlids (Teleostei: Cichlidae) based on sequences of the cytochrome *b* gene. Mol Phylogenet Evol **43:**91–110.

Farias IP, Ortí G, Sampaio I, Schneider H, Meyer A (1999) Mitochondrial DNA phylogeny of the family Cichlidae: monophyly and fast molecular evolution of the Neotropical assemblage. J Mol Evol **48:**703–711.

Farias IP, Ortí G, Meyer A (2000) Total evidence: molecules, morphology, and the phylogenetics of cichlid fishes. J Exp Zool **288:**76–92.

Farias IP, Ortí G, Sampaio I, Schneider H, Meyer A (2001) The Cytochrom b gene as a phylogenetic marker: the limits of resolution for analyzing relationships among Cichlid fishes. J Mol Evol **53:**89–103.

Grande T, Laten H, Lopez JA (2004) Phylogenetic relationships of extant esocid species (Teleostei : Salmoniformes) based on mophological and molecular characters. Copeia **4:**743–757.

Hall TA (1999) Bio Edit: Biological sequence alignment editor v 5.0.9., http://www.mbio.ncsu.edu/BioEdit/bioedit.html

Huelsenbeck JP, Ronquist F (2001) MrBayes: Bayesian inference of phylogeny. Bioinformatics **17:**754–755.

Inger RF (1956) Notes on a collection of fishes from southeastern Venezuela. Fieldiana Zool **34:**425–440.

Kullander SO (1983a) A Revision of the South American Cichlid genus Cichlasoma. Swedish Museum of Natural History, Stockholm, Sweden.

Kullander SO (1983b) Taxonomic Studies on the Percoid Freshwater Fish Family Cichlidae in South America. PhD. thesis, Stockholm University.

Kullander SO (1984a) Une nouvelle espèce d'Aequidens des bassins des Rios Aguaytia et Pachitea au Pérou: *Aequidens patricki* n. sp. (Teleostei: Cichlidae). Rev Fr Aquariol **11:**1–6.

Kullander SO (1984b) Cichlid fishes from the La Plata basin. Part V. Description of Aequidens plagiozonatus sp. n. (Teleostei, cichlidae) from the Paraguay River system. Zool Scr **13:**155–159.

Kullander SO (1986) Cichlid fishes of the Amazon River Drainage of Peru. Swedish Museum of Natural History, Stockholm, Sweden.

Kullander SO (1987) Cichlid fishes from the La Plata Basin. Part VI. Description of a new Bujurquina species from Bolivia. Cybium **11:**195–205.

Kullander SO (1991) *Tahuantinsuyoa chipi*, a new species of cichlid fish from the Rio Pachitea drainage in Peru. Cybium **15:**3–13.

Kullander SO (1995) Three new cichlid species from southern Amazonia: *Aequidens gerciliae*, *A. epae* and *A. michaeli*. Ichthyol Explor Freshw **6:**149–170.

Kullander SO (1996) *Heroina isonycterina*, a new genus and species of cichlid fish from Western Amazonia, with comments on cichlasomine systematics. Ichthyol Explor Freshw **7:**149–172.

Kullander SO (1997) *Aequidens mauesanus*, a new species of cichlid fish from the Amazon basin, Brazil. Ichthyol Explor Freshw **7:**377–383.

Kullander SO (1998) A phylogeny and classification of the South American Cichlidae (Teleostei: Perciformes). In: Malabarba LR, Reis RE, Vari RP, Lucena ZMS, Lucena CAS (eds), Phylogeny and classification of Neotropical fishes. Edipucrs, Porto Alegre, pp. 461–498.

Kullander SO (2003) Cichlidae. In: Reis RE, Kullander SO, Ferraris SJ (eds), Check list of the freshwater fishes of South and Central America. Edipucrs, Porto Alegre, pp. 605–654.

Kullander SO, Ferreira EJG (1991) A new *Aequidens* species from the Rio Trombetas, Brasil [sic], and redescription of *Aequidens pallidus* (Teleostei, Cichlidae). Zool Scr **19:**425–433.

Kullander SO, Nijssen H (1989) The Cichlids of Surinam. E. J. Brill, Leiden.

Kullander SO, Prada-Pedreros S (1993) *Nannacara adoketa*, a new species of cichlid fish from the Rio Negro in Brazil. Ichthyol Explor Freshw **4:**357–366.

Maddison WP, Maddison DR (2004) Mesquite: A modular system for evolutionary analysis. Version 1.05. http://mesquiteproject.org.

Marescalchi O (2005) Karyotype and mitochondrial 16S gene characterizations in seven South American Cichlasomatini species (Perciformes, Cichlidae). J Zoolog Syst Evol Res **43:**22–28.

Miller RR, Minckley WL, Norris SM (2005) Freshwater Fishes of Mexico. University of Chicago Press, Chicago, pp 652.

Moyer GR, Burr BM, Krajewski C (2004) Phylogenetic relationships of thorny catfishes (Siluriformes: Doradidae) inferred from molecular and morphological data. Zool J Linn Soc **140:**551–575.

Musilová Z, Říčan O, Janko K, Novák J (2008) Molecular phylogeny and biogeography of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae). Mol Phylogenet Evol **46:**659–672.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics **14:**817–818.

Říčan O, Kullander SO (2006) Character- and Tree-based delimitation of species in the *'Cichlasoma' facetum* group (Teleostei, Cichlidae) with the description of a new genus. J Zool Syst Evol Res **44:**136–152.

Říčan O, Musilová Z, Muška M, Novák J (2005) Development of coloration patterns in Neotropical cichlids (Perciformes: Cichlidae: Cichlasomatinae). Folia Zool **54:**Monograph–1.

Říčan O, Zardoya R, Doadrio I (2008) Phylogenetic relationships of Middle American cichlids (Cichlidae, Heroini) based on combined evidence from nuclear genes, mtDNA, and morphology. Mol Phylogenet Evol **49:**941–957.

Römer U, Hahn I (2007) *Ivanacara* gen. n. (Teleostei: Perciformes, Cichlasomatini) – a new genus of cichlids from the Neotropis. In: Römer U. (ed.), Cichlid Atlas, volume 2, Natural History of South American Dwarf Cichlids, Part 2. Mergus Verlag GmbH, Melle, Germany, pp. 1190–1197.

Stawikowski R, Werner U (1998) Die Buntbarsche Amerikas. Eugen Ulmer Verlag, Stuttgart, Germany.

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Thiele K (1993) The holy grail of the perfect character: the cladistic treatment of morphometric data. Cladistics **9:**275–304.

Wiens JJ (1995) Polymorphic characters in phylogenetic systematics. Syst Biol **44:**482–500.

Wiens JJ (1999) Polymorphism in systematics and comparative biology. Annu Rev Ecol Syst **30:**327–362.

Wiens JJ (2001) Character analysis in morphological phylogenetics: problems and solutions. Syst Biol **50:**688–699.

Author's address: Zuzana Musilová, Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, 128 44 Praha, Czech Republic, E-mail: zuzmus@email.cz.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Material used in this study.

Appendix S2. Morphological characters.

Appendix S3. Morphological character matrix.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

doi: 10.1111/j.1439-0469.2009.00528.x

Musilová Z., Říčan O., Novák J., Janšta P., Gahura O., Říčanová Š., Escobar U.R.

Phylogeny and historical biogeography of the trans-Andean cichlid fishes

prepared for submission to *Journal of Biogeography*

Manuscript

To whom it may concern

I declare that Zuzana Musilova performed significant portion of work on our manusript:

Zuzana Musilová, Oldřich Říčan, Jindřich Novák, Petr Janšta, Ondřej Gahura, Štěpánka Říčanová, Ursula Ramírez Escobar - **Phylogeny and historical biogeography of the trans-Andean cichlid fishes.**

In behalf of co-author,

In České Budějovice, 6.1.2011 Oldřich Říčan

Phylogeny and historical biogeography of the trans-Andean cichlid fishes

Zuzana Musilová[1,2)*], Oldřich Říčan[3)], Jindřich Novák[3)], Petr Janšta[2)], Ondřej Gahura[2)], Štěpánka Říčanová[3)], Ursula Ramírez Escobar[4)]

1) Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AV ČR v.v.i., 277 21 Liběchov, Czech Republic,

2) Department of Zoology, Faculty of Sciences, Charles University in Prague, 128 44 Praha 2, Czech Republic

3) Department of Zoology, Faculty of Science, University of South Bohemia, 370 05, Ceske Budejovice, Czech Republic

4) Instituto de Genética, Universidad Nacional de Colombia, Entrada Calle 53 con Cra 37, Edificio 426, Bogotá, Colombia

** corresponding author, e-mail: zuzmus@gmail.com*

Abstract

The Andean uplift strongly impacted the ichtyofauna distribution patterns in South America. While the majority of neotropical cichlid biodiversity is found throughout Amazon, several lineages colonized the trans-Andean region (i. e. the pacific slope of Andes). DNA sequences of cytochrome b were used as a marker for the phylogeographic study of two cichlid genera, *Andinoacara* and the *´Heros´ festae* group with the mainly trans-Andean distribution. Two approaches, S-DIVA and DEC analyses, were used for biogeography scenario reconstruction. The Choco region in Colombia was found as a most probable ancestral area, where both *Andinoacara* and *´Heros´ festae* group ancestors originated. The colonisation of cichlid fishes occurred from the Choco both northward to the Central America and southward up to Peru. We studied both genera separately and reconstructed phylogenetic patterns as well as the suggested biogeography scenario were generally in accord.

Keywords

Neotropical , Andes, S-DIVA, Lagrange, cytochrome b, Andinoacara, Heros, ichtyofauna

Introduction

Cichlid fish diversity in the trans-Andean South America

While most Neotropical cichlid species richness is centered in the cis-Andean South America, a lower but indispensable part of the species diversity is found also in Middle America and in the trans-Andean South America (the Pacific slope river drainages of South America) (Froese and Pauly, 2010). Representatives of four cichlid groups (*Andinoacara*, the *´Heros´ festae* group, *Caquetaia*, and *Geophagus*) are distributed in the latter region. Only two genera are truly characteristic of the trans-Andean (*Andinoacara*, the *´Heros´ festae* group), while the rest two (*Caquetaia* and *Geophagus*) are found north from the Rio San Juan drainage (Colombia) only. The former trans-Andean genera cover the distribution from the Rio Atrato drainage in Colombia to Peru, i. e. from the Choco area of endemism southward to Rio Tumbes in nortern Peru for the *´Heros´ festae* group, and southward to Lima in central Peru for *Andinoacara*; Stawikowski and Werner, 1998).

The genus *Andinoacara* belongs to the tribe Cichlasomatini and presently includes seven valid species (Musilová et al., 2009a, b). Apart from the pacific Colombia, Peru and Ecuador, the genus is also distributed in Lower Central America up to Costa Rica, in Cauca – Magdalena basin in northern Colombia, in the coastal drainages on northern Venezuela and in the Orinoco river system in Colombia, Venezuela and Trinidad. It represents one of the most suitable cichlid genera for historical biogeography studies of the Andean uplift and changes covering all of the region and occuring there during the geological history. There are two sister lineages within *Andinoacara*, i. e. *A. pulcher* group and *A. rivulatus* group (Musilová et al. 2009). The present study includes all of the valid *Andinoacara* species and further two or three lineages with uncertain taxonomic status.

The *´Heros´ festae* group is a representative of the tribe Heroini, related to a subgroup of Middle American cichlid fishes (Concheiro Perez et al., 2007; Říčan et al., 2008). This assemblage is formed by three species, although the situation within *´H.´ atromaculatus* and the status of the nominal species *´H.´ gephyrus* is unclear (Froese & Pauly, 2010). This genus is endemic to the Choco region of trans-Andean South America in Colombia (and easternmost Caribbean Panama), Ecuador and northernmost Peru (Rio Tumbes). Samples from the whole distribution area of the group are included in this study.

Historical Biogeography of trans-Andean South America

South America represents a region with a high level of geological activity and large changes have occurred not so long ago. The uplift of Andes, initiated already in the Late Cretaceous

(Lundberg et al., 1998), but most prominent in the second half of the Cenozoic, has heavily impacted the geological and biological history of South America (Antonelli et al., 2009). It significantly reshaped the river systems on the whole continent including Amazon and Orinoco, as well as the uplift itself formed an important barrier for species distributions in the region of Northern South America and between South and Middle America (Lundberg et al., 1998). The major changes in the configuration of the major river basins occurred quite recently, between 10 and 3 Mya (e.g., Orinoco and the trans-Andean South America were separated from the Amazon). Additionally, the recent species distribution patterns were also affected by concurrent periodical marine incursions (Lundberg et al., 1998; Montoya-Burgos 2003). Last but not least, the incipient formation of the Panamian land bridge in Miocen/Pliocen boundary followed by break up and reformation of new Panamian Isthmus around 3 Mya (Coates & Obando, 1996) played a major role in the following colonization of Central America by freshwater fishes (Martin and Bermingham, 1998).

During last 20 Mya, the contacts between the cis-Andean and trans-Andean regions could have been enabled first by the Western Andean portal - the connection and probable marine incursion into the continent between the Northern and Southern Andes at the current Ecuadorian-Peruvian border that probably persisted during up to 15 Mya, (Santos. et al., 2009). Second, the incomplete uplift of the Northern Andes (Western, Central and Eastern Cordilleras in Colombia and Venezuela) could also affect the recent distribution pattern as the uplift of the Eastern Cordilleras was not completely formed until 11.8 Mya (Lundberg et al., 1998).

The main goal of the present study

Our study is reconstructing the historical biogeography of the two cichlid genera distributed mainly in the trans-Andean region. The ancestral area reconstruction reveals the area, where the studied cichlids originally came from to the trans-Andean region. Further, the vicariance and dispersal estimation reconstructs the possible scenario of colonisations and speciation during the cichlids evolutionary history.

Material and Methods:

The material of more than 150 specimens of seven *Andinoacara* species and three species of the *´Heros´ festae* group was collected via the European and USA aquarium trade importers after the specified task. Further, additional samples were obtained from various European aquarium hobbyists who carry the pure lineages with the known origin localities from the

previous imports (since late 80´s). Finally, sequences from GenBank were used to get more complete data set.

The cytochrome b gene was amplified in this study using the primers FishCytB-F and TrucCytB-R from Sevilla et al., 2007. The process of DNA isolation, amplification and sequencing was identical to as previously described in Musilová et al, 2009. Alternatively, we also used Macrogen service in South Korea (www.macrogen.com), where the unpurified PCR products were sent to. All newly obtained sequences in this study were submitted to GenBank (Accession Nos. XXX, to be completed after submission).

Chromatograms were assembled and checked by eye for potential mistakes and edited sequences were aligned using Clustal W as implemented in BioEdit software package (Hall 1999). Further, we checked the alignment for potential stop codons by translation into the protein sequence.

. We selected the cytochrome b as a marker for this study as it is considered to provide consistent results during phylogenetic analyses, it is an appropriate marker for molecular clock analyses and its general mutation rate is known (van Oppen et al., 1999). Moreover, cytochrome b has been previously used in several studies concerning neotropical cichlids (Concheiro Perez et al., 2007, Martin and Bermingham, 1998) and we could use the published DNA sequences for analyses in this study.

Phylogenetic analyses of mitochondrial sequence data (cytochrome b) were performed using Bayesian Inference as implemented in MrBayes 3.0 (Huelsenbeck & Ronquist, 2001). The best-fit model for the separate genes was selected with jModeltest (Posada, 2008) using the Akaike information criterion. Bayesian analyses were performed using two independent runs of four Metropolis-coupled chains (MCMC) of 5 million generations each, to estimate the posterior probability distribution. Tree topologies were sampled every 100 generations, and majority-rule consensus trees were estimated after discarding the first 25% generations. Robustness of clades was assessed using Bayesian posterior probabilities.

The ultrametric trees for biogeographic analyses were obtained a) by Bayesian evolutionary analyses by sampling trees (BEAST, Drummond & Rambaut, 2007) under the relaxed molecular clock models. The run in BEAST was performed for 5 million generation under the evolutionary model as suggested by jModeltest. Alternatively, b) the consensus tree from Bayesian analyses (MrBayes 3.0, Huelsenbeck & Ronquist, 2001) was used as a template for the Penalised-Likelihood analyses performed in R8S software (Sanderson, 2002).

As the biogeography softwares require fully resolved (bifurcated) tree, we used only unique haplotypes to reconstruct the ultrametric trees. Haplotypes were defined in FABOX sotware (Villesen, 2007).

We used two event-based approaches for the biogeography reconstruction. First, the statistic dispersal – vicariance analyses (S-DIVA, Yu et al., 2010) was performed. This improved version of DIVA (Ronquist, 2001) summarizes the sampled trees from the Bayesian analyses runs, which serve as a source data for the statistics. S-DIVA does not take into account the branch length, thus it works only with the final topology. We used both MrBayes and BEAST data sets to find the ancestral areas and reconstruct the biogeographic scenario of the genera *Andinoacara* and the *´Heros´ festae* group. Last 2000 of sampled trees were used (from 5000) and the pool of 300 random trees of them was analyzed.

Another event-based approach, Dispersal-Extinction-Cladogenesis modelling (DEC analyses) as implemented in Lagrange software (Ree and Smith, 2008) was used for the ancestral area reconstruction. This likelihood method works with the branch lengths, so either the penalized likelihood (PL) topology from R8S software, or the BEAST tree can be used. The polytomies in the PL tree were artificially resolved with zero length branches in Mesquite 2.74 software (Maddison & Maddison, 2010). The on-line configurator for Lagrange software (http://www.reelab.net/lagrange) was used for the input file preparation.

Another difference between two aforementioned approaches is in the penalties for the biogeography events. While DIVA (and S-DIVA) is prefering vicariance by penalizing the dispersal and extinction, the DEC analyses makes no difference among these events.

Because of a polytomy found in the basal node of the *Andinoacara pulcher* group, we performed the biogeographical reconstruction using all three alternative topologies for this node. Another polytomies occured in several distal nodes, hence, only the representants of each species (and region) were selected to the „limited data set“ and the Bayesian analyses was performed with this selection under the same conditions as aforementioned. See Fig. 4, where the clades for selection are marked. These topologies were followingly analysed by the S-DIVA software and the ancestral area was reconstructed for all possible topologies.

We tested the alternative topologies in observed polytomies in S-DIVA approach by changing topology to resolved one in all three possible topologies. However, only penalized likelihood tree (from R8S) and relaxed molecular clock (from BEAST) trees were used for the DEC analyses, because it is not possible simply change topology to alternative, when the analysis requires the branch lengths. Additionally, the BEAST trees from the representative of each clade (the same as for the topology tests by S-DIVA) were used. No alternative

topologies were tested by DEC analyses, however the algorithm in Lagrange take into count the branch length, and the length for the branches in original polytomies was manually set to zero.

Nine distribution areas were used in the genus *Andinoacara* and four in the *´Heros´ festae* group. The historical biogeographical reconstructions were perfomed under two different scenarios: a) all-area connections are allowed („no limitation model"), and b) only the adjacent areas can be connected („stepping-stone model"). A „relaxed stepping-stone model" (i. e. areas connected by two steps were also allowed) was also applied for *Andinoacara* in order of not forcing the settings so strictly (see map C in Fig. 4). In the *´Heros´festae* group only four areas were necessary, and thus only the „strict stepping-stone model" was applied (i.e. only the adjacent areas connected with one step were allowed). See map D in Fig. 4).

The molecular-clock trees from BEAST analyses were used for the dating. Two ways of dating were used in this study. First, the divergence rate of cytochrome b was applied, which is generally considered to be 1% of divergence per 1 million of years (general divergence rate for vertebrates; van Oppen et al., 1999). Second, the geologic timing was applied as presented in the phylogeny of the whole tribe Cichlasomatini (Musilová et al., 2008). Two calibration points in the genera *Cichlasoma* and *Bujurquina* were used representing the Paraná – Amazon separation calibrated to 10 Mya (Musilová et al., 2008; Lundberg et al., 1998). The projection to the timeline was performed, i) ignoring the root in the divergence time method, and assesing the averagre rate between two clades, and ii) using the two previously dated nodes (basals of northern and southern clade) from Musilová et el., 2008 for the geology time projection.

Results

The phylogeny of the two trans-Andean cichlid genera with full taxon sampling was reconstructed. See Fig. 1 and Fig. 6. In *Andinoacara*, there have been revealed two sister lineages, the *A. pulcher* group and the *A. rivulatus* group. These groups were well defined with strong support from posterior probabilities (Fig. 1). Within the groups some polytomies or weakly supported nodes were observed. First, within the *A. pulcher* group, the polytomy included *A*. sp. „Choco" from the Colombian pacific slope, *A. coeruleopunctatus* (Central America) and the rest of the clade, i. e. *A. pulcher-A. latifrons* from Colombia and Venezuela. The lineage of *A*. sp. „Choco" was found as a basal lineage of the group in the Bayesian analyses, although with very low support. On the contrary, in the relaxed molecular clock

analysis performed in BEAST, this lineage resulted as sister to *A. coerueopunctatus* from Central America. Second, in the *A. rivulatus* group was found the very stable pattern (see Figs. 1 and 2), although the basal position of *A. biseriatus* was not strongly supported (0.98 by the posterior probability in MrBayes in the full sample data set, 0.89 of pp in the haplotype data set, and only 0.68 in the „limited data set" where all lineages are represented only by one sample; not shown). The cytochrome b divergence in the basal node of *Andinoacara* is 14.1% of uncorrected p-distance. While in the *A. rivulatus* group, old divergences were found (13.3% and 12.5% of p-distance), the *A. pulcher* group represented clade with much lower divergences (the deepest node is represented by 5.7%; see Fig. 2).

The phylogenetic reconstruction of the *´Heros´ festae* group found all three included species as monophyletic with the strong support (Fig. 6). *´H. ´atromaculatus* represents the northern clade sister to the well supported southern clade of *´H.´festae* + *´H.´ornatum*. The ancestral divergence was dated to 7.3% of uncorected p-distance (Fig. 3, B).

The observed ditribution patterns in both *Andinoacara* and the *´Heros´ festae* group were closely similar. In both genera, two sister clades were present, one with the distribution in the southern and one in the northern part of the areal (see maps in Fig. 3). In *Andinoacara,* the *A. pulcher* group represents the Northern clade of *Andinoacara*, distributed from the Colombian Choco to the North, including Central America and the Orinoco river system. Complementary, the *A. rivulatus* group covers the distribution from the Colombian Choco to the Pacific slopes of Ecuador and Peru with the Chocoan *A. biseriatus* as a basal. In both Northern and Southern clades, the Choco region represents a basal lineage, although in the Northern clade weekly supported.

In the *´Heros´ festae* group, the Northern clade is represented by *´H.´ atromaculatus* distributed from Colombian Choco up to the Eastern Panama. The Southern clade was represented by *´H.´ ornatus* and *´H.´festae* distributed through the Ecuadorian pacific slope up to northern Peru.

In the Santiago river system (Western Ecuador) a well separated and diverged lineage was found in both *Andinocara* (Fig. 1) and the *´Heros´festae* group (Fig. 6), represented by the endemic *Andinoacara sapayensis* and *´Heros´ ornatus*, respectively. Contrarily, in the rest of the pacific Ecuadorian river systems, i. e. Esmeraldas, Daule, Manabi-Guayas, and the rivers of Golfo de Guayaquil, almost no genetic structure was found in both *A. rivulatus* and *´H.´ festae*. See map in Fig 3.

The relaxed molecular clock performed in BEAST, and the Bayesian phylogenetic analyses from MrBayes resulted in slightly different patterns in the basal node and within the

A. pulcher group. The following biogeography analyses were thus performed on both trees (and alternative topologies, where basal polytomy was found). The differences in pattern are caused by the different algorithm of Bayesian analyses, while BEAST force the final tree to be ultrametric applying molecular clock model, the branch length in MrBayes is not limited.

The biogeographic analyses were applied for the reconstruction of the ancestral areas and of the vicariance – dispersal events. In both *Andinoacara* (Fig. 5) and the *´Heros´ festae* group (Fig. 7), the Colombian Choco was revealed as the region most probably included in the ancestral area, although the situation in the *Andinoacara* was not so clear due to the polytomy in the basal node of the *A. pulcher* group. In both cases, several vicariance and dispersal events occured.

In the *´Heros´ festae* group four areas for the biogeography analyses were used. It was 1) Panama (Carribean slope), 2) Choco (Colombia) including Atrato, San Juan, Baudó and small coastal creeks, 3) the Santiago river system (North-Western Ecuador), and 4) the rest of the Ecuadorian pacific rivers + Northern Peru. For the basal node (ancestor) of the *´Heros´ festae* group the ancestral area of Choco – Santiago was reconstructed (Fig.7) followed by a vicariance between the Northern (*´H.´ atromaculatus* – Choco + Panama) and Southern (*´H.´ ornatus*, *´H.´ festae* – Santiago + Ecuador + Peru) clades. In the Northern clade (*´H.´ atromaculatus*) the dispersal northward occured up to Panama, followed later by another vicariance between Choco and Panama. In the southern clade, the southward dispersal up to northern Peru was followed by a vicariance between Santiago and the rest of Ecuador + northern Peru. Aforementioned results were found under the stepping-stone model parameters (only adjacent areas allowed as ancestral areas). Under the no-limitation model, where all combination of areas were allowed, a similar pattern was observed except of the basal node, where an unresolved ancestral area was suggested (see Fig. 7, below the tree).

The situation in the biogeographic reconstruction of *Andinoacara* is more complicated due to the aforementioned polytomy. As the polytomy in the basal nodes could strongly impact the results of the biogeographical analyses, we performed the analyses on all three possible topologies (see Fig. 5). In either case three vicariance events occurred during the history in the southern "*A. rivulatus* group" clade. First, a vicariance occurred between Choco and the southern area(s), when the Chocoan *A. biseriatus* separated. A second vicariance separated *A. sapayensis* distributed in the Santiago river system from the rest of Ecuador and Peru. A third vicariant event occurred between *A. rivulatus* and *A. stalsbergi* and separated the Ecuadorian fishes from Peruvian. At least one dispersal event occurred in the southern clade. See Fig. 5 for detail. The biogeographic reconstruction of the northern (*A. pulcher*) clade

differs based on the topology used for the analysis, but in all cases, four vicariance events occured during the evolution. The possible ancestral area for the basal node of the genus *Andinoacara* was suggested in all three topologies identically: four alternative combinations with almost the same probability (very slightly differing among topologies, see Fig. 5). The Colombian Choco was present in all of the suggested areas, and in the three of them Eastern Panama was found. The Santiago river system resulted in the two of the ancestral combinations. If no-limitation model was applied (i.e. all combinations of the areas were allowed), the resolution of the basal nodes became much more inclusive: the root resulted as completely unresolved, and the basal nodes of both *A. pulcher* and *A. rivulatus* groups resulted with more potential areas. The rest of nodes remained independent on the model applied for the biogeography analysis. The DIVA results are not shown as being almost identical to the S-DIVA results.

The DEC analysis (Tab. 2) also supported the S-DIVA results, offering sometimes even more resolved results for *Andinoacara*. The results of the DEC analyses were very consistent among the different model settings – the stepping-stone models and maximum areas allowed in the ancestral areas. Colombian Choco was found as the most probable ancestral area for the root in three of four runs under the different models (stepping-stone vs. no-limitation) and different data sets (haplotypes vs. limited data set; see Material and Methods). In the *´Heros´ festae* group, the different model setting did not affected the analyses and both models produced the uniform results. See Tab. 2.

The general accord was observed comparing results of both biogeogaphic approaches, i.e. S-DIVA and DEC analyses, applied on the genetic data of the *´H.´ festae* group.

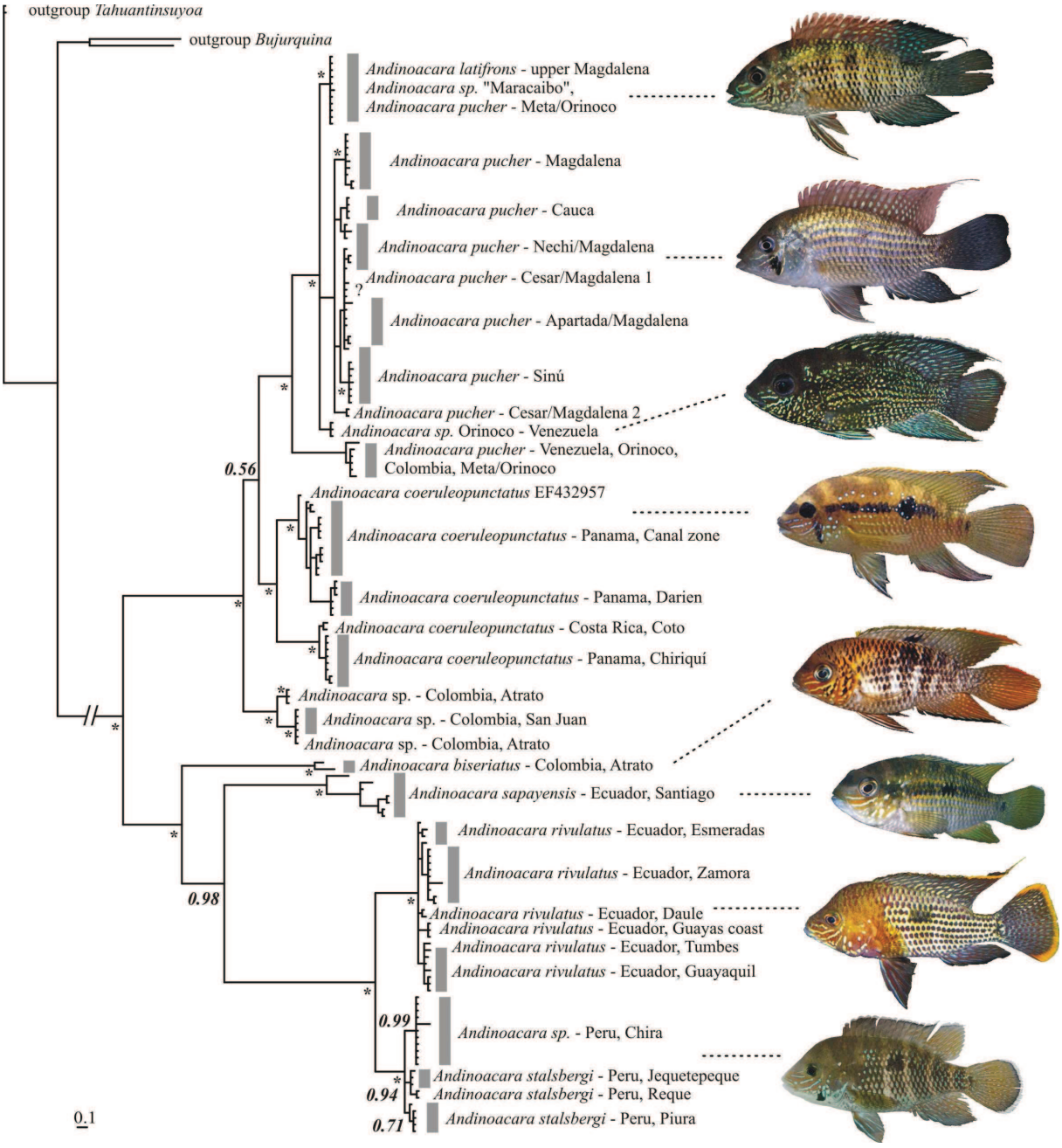


Figure 1: Bayesian tree of *Andinoacara*. Result of Bayesian analyses with 5 million generation based on cytochrome *b* data set including samples from 7 valid *Andinoacara* species. Asterisk marks nodes with posterior probability support equal to 1.00.

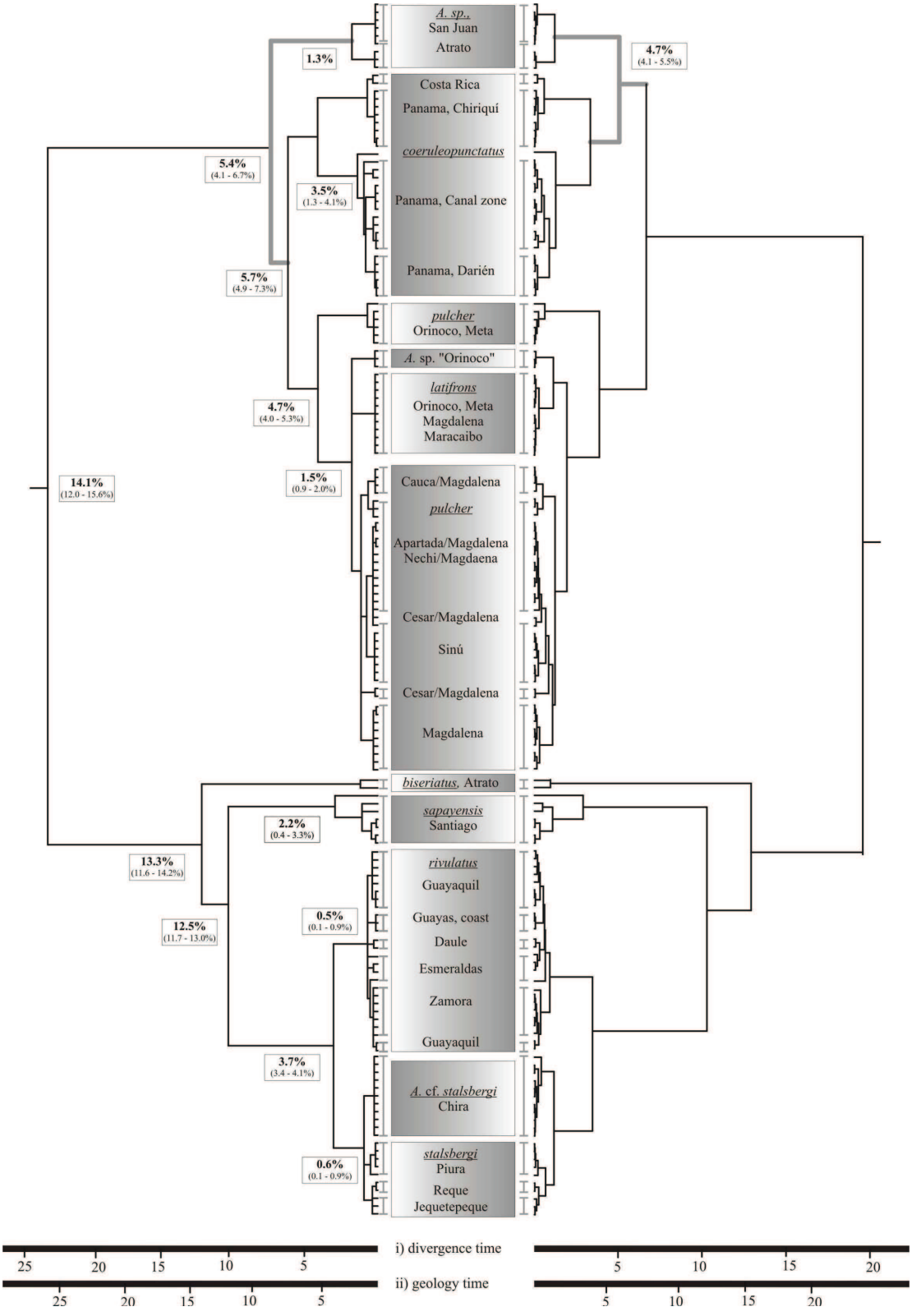


Figure 2: A) Bayesian tree resulted from following Penalized-Likelihood analysis in R8S and B) relaxed molecular clock tree from BEAST analyses. Number in the nodes represent the genetic divergence among the clades (p-distance). Molecular dating in Mya based on i) constant divergence rate of cytochrome b, and ii) geological dating adopted from Musilová et al., 2008

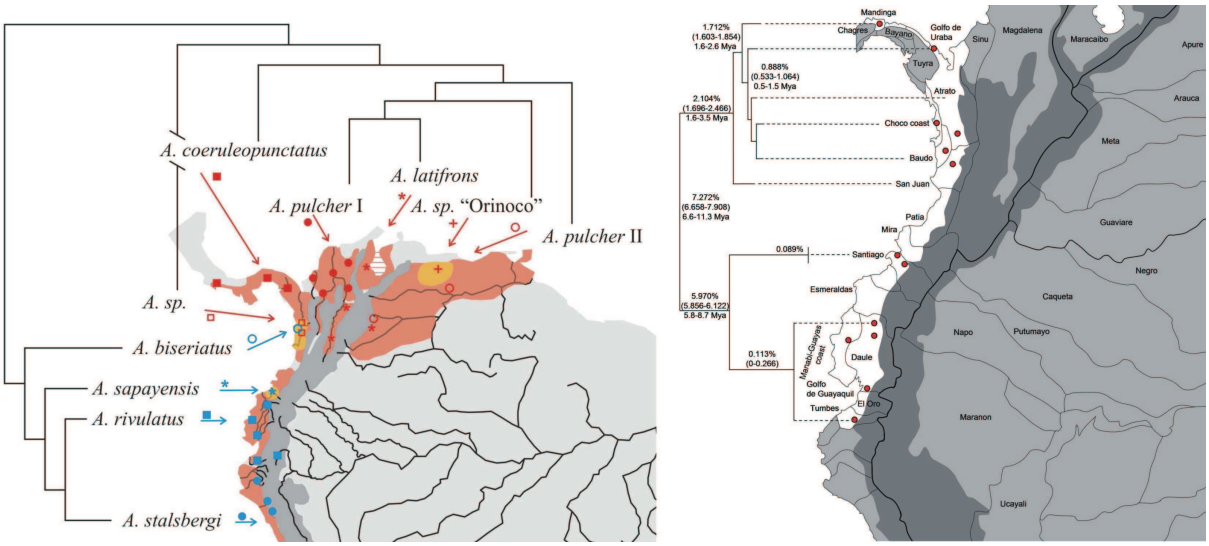


Figure 3: Schematic tree placed to the map of distribution of a) *Andinoacara* and b) *´Heros´ festae* group

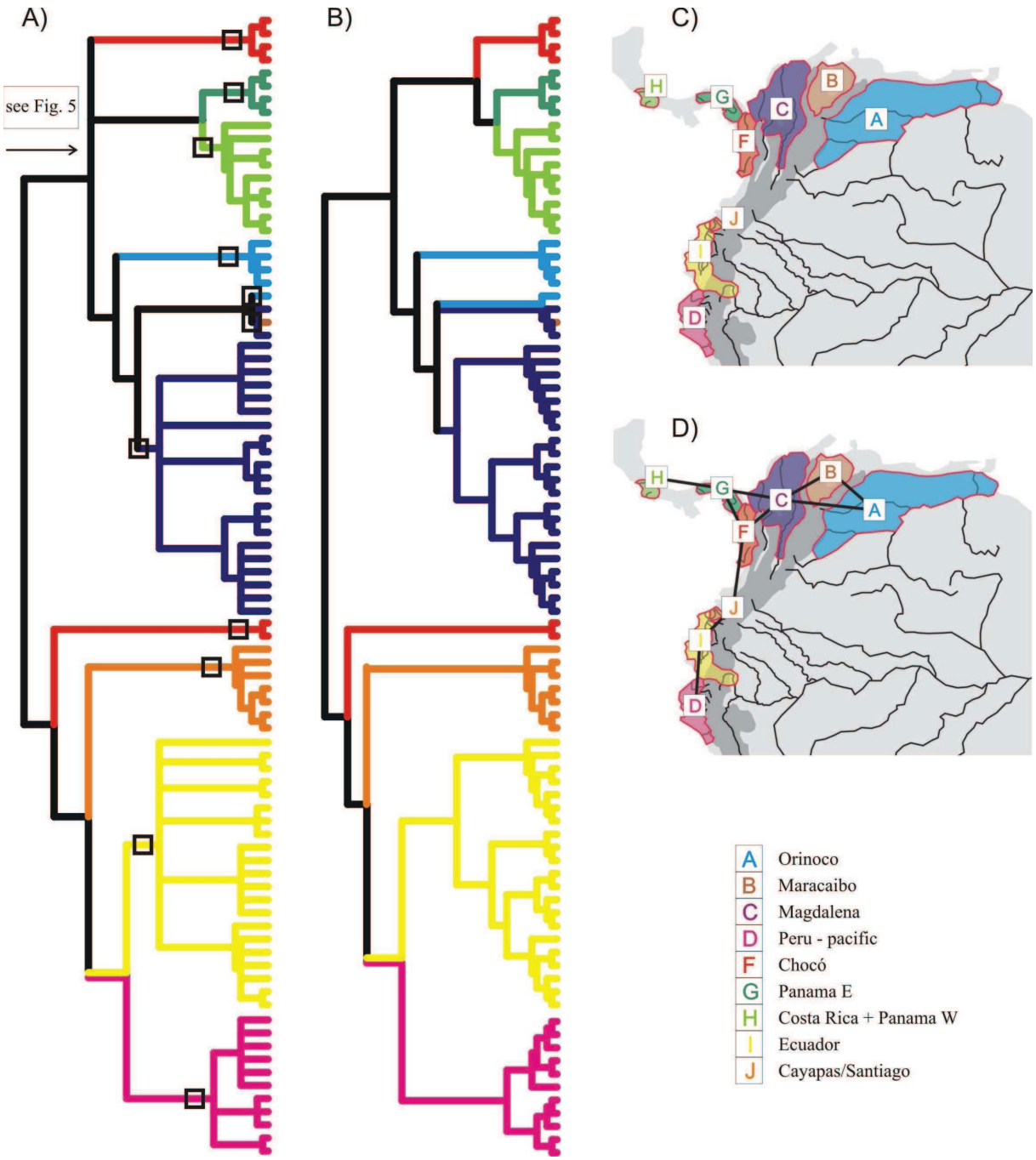


Figure 4: *Andinoacara* phylogenetic trees (topologies only) of the haplotype dataset with mapped biogeographic areas. A) Bayesian tree, B) BEAST tree. C) Map of areas tested in biogeographic analyses (fig. 5), D) connection of areas for the stepping-stone model. In the Bayesian tree (A) polytomy in the basal node of *A. pulcher* group was found; squares in the tree mark the monophyletic clades, from which one representant was used for the biogeography test of all three alternative topologies. (See Fig. 5).

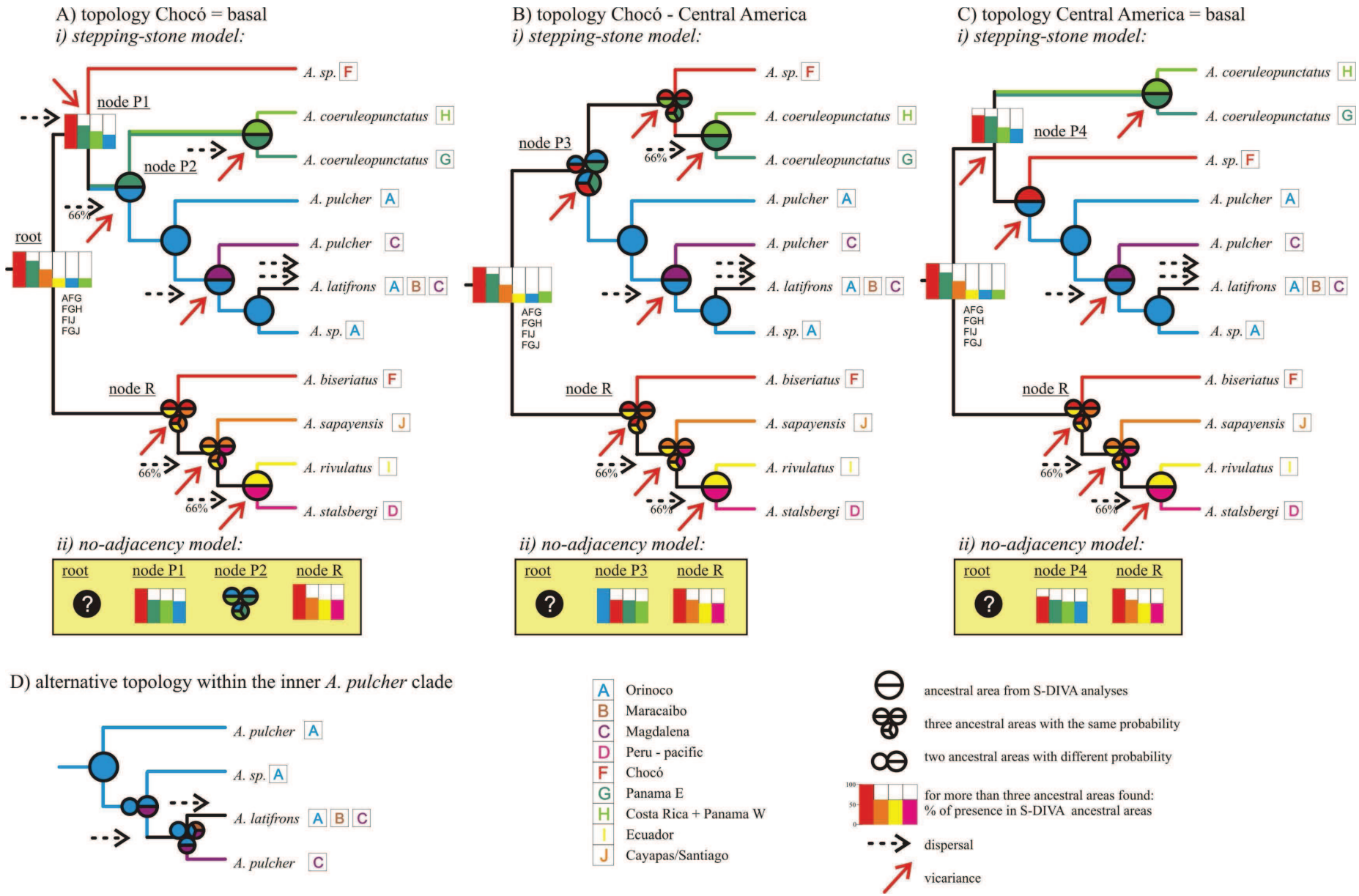

A) topology Chocó = basal
i) stepping-stone model:
A. sp. F
node P1
A. coeruleopunctatus H
A. coeruleopunctatus G
node P2
A. pulcher A
66%
A. pulcher C
root
A. latifrons A B C
AFG
FGH
FIJ
FGJ
A. sp. A
node R
A. biseriatus F
A. sapayensis J
66%
A. rivulatus I
66%
A. stalsbergi D
ii) no-adjacency model:
root
node P1
node P2
node R
B) topology Chocó - Central America
i) stepping-stone model:
A. sp. F
A. coeruleopunctatus H
node P3
66%
A. coeruleopunctatus G
A. pulcher A
A. pulcher C
A. latifrons A B C
AFG
FGH
FIJ
FGJ
A. sp. A
node R
A. biseriatus F
A. sapayensis J
66%
A. rivulatus I
66%
A. stalsbergi D
ii) no-adjacency model:
root
node P3
node R
C) topology Central America = basal
i) stepping-stone model:
A. coeruleopunctatus H
node P4
A. coeruleopunctatus G
A. sp. F
A. pulcher A
A. pulcher C
A. latifrons A B C
AFG
FGH
FIJ
FGJ
A. sp. A
node R
A. biseriatus F
A. sapayensis J
66%
A. rivulatus I
66%
A. stalsbergi D
ii) no-adjacency model:
root
node P4
node R
D) alternative topology within the inner A. pulcher clade
A. pulcher A
A. sp. A
A. latifrons A B C
A. pulcher C
A Orinoco
B Maracaibo
C Magdalena
D Peru - pacific
F Chocó
G Panama E
H Costa Rica + Panama W
I Ecuador
J Cayapas/Santiago
ancestral area from S-DIVA analyses
three ancestral areas with the same probability
two ancestral areas with different probability
100
50
0
for more than three ancestral areas found:
% of presence in S-DIVA ancestral areas
dispersal
vicariance

Figure 5: Biogeographic analyses on three alternative topologies in the basal node of *Andinoacara* phylogeny. A) topology Chocó – basal: the Chocoan clade as a basal in *A. pulcher* group; this topology resulted from Bayesian analyses including all samples (see Fig. 1), B) topology Chocó – Central America: the chocoan clade and cetral-american clade resulted as sister clades within *A. pulcher* group; this topology was supported by BEAST analyses based on both all-haplotypes and selected-taxa data sets; C) topology Central America – basal; the central-american clade as a basal in *A. pulcher* group; D) alternative topology found in the inner node of *A. pulcher* group, as resulted by BEAST analysis; i) stepping-stone model of biogeography history – only areas with one or two steps allowed in S-DIVA (see map in Fig. 4), ii) no-adjacency model – no limitation by areas excllusion in the S-DIVA analyses; stepping stone model results mapped on the tree, nodes with different results among the models are showed below the trees in yellow squares; symbols in trees: circle in node – hypothetical ancestral area as suggested by S-DIVA analyses, colors correspond to areas, more colors = area includes more regions; more circles = more alternative ancestral areas for the node, circles of the same size = same probability of suggested areas in the node; different size of circles = different probability of areas, larger circle = more probable hypothesis; bar graph in node = more then four alternative ancestral areas found for the node, bars represent % cover of areas in the proposed combinations suggested as ancestral ranges (and weighted by probability value from S-DIVA analyses). Black dashed arrow = dispersal event, red arrow = vicariance event. For areas in the map see Fig.4.

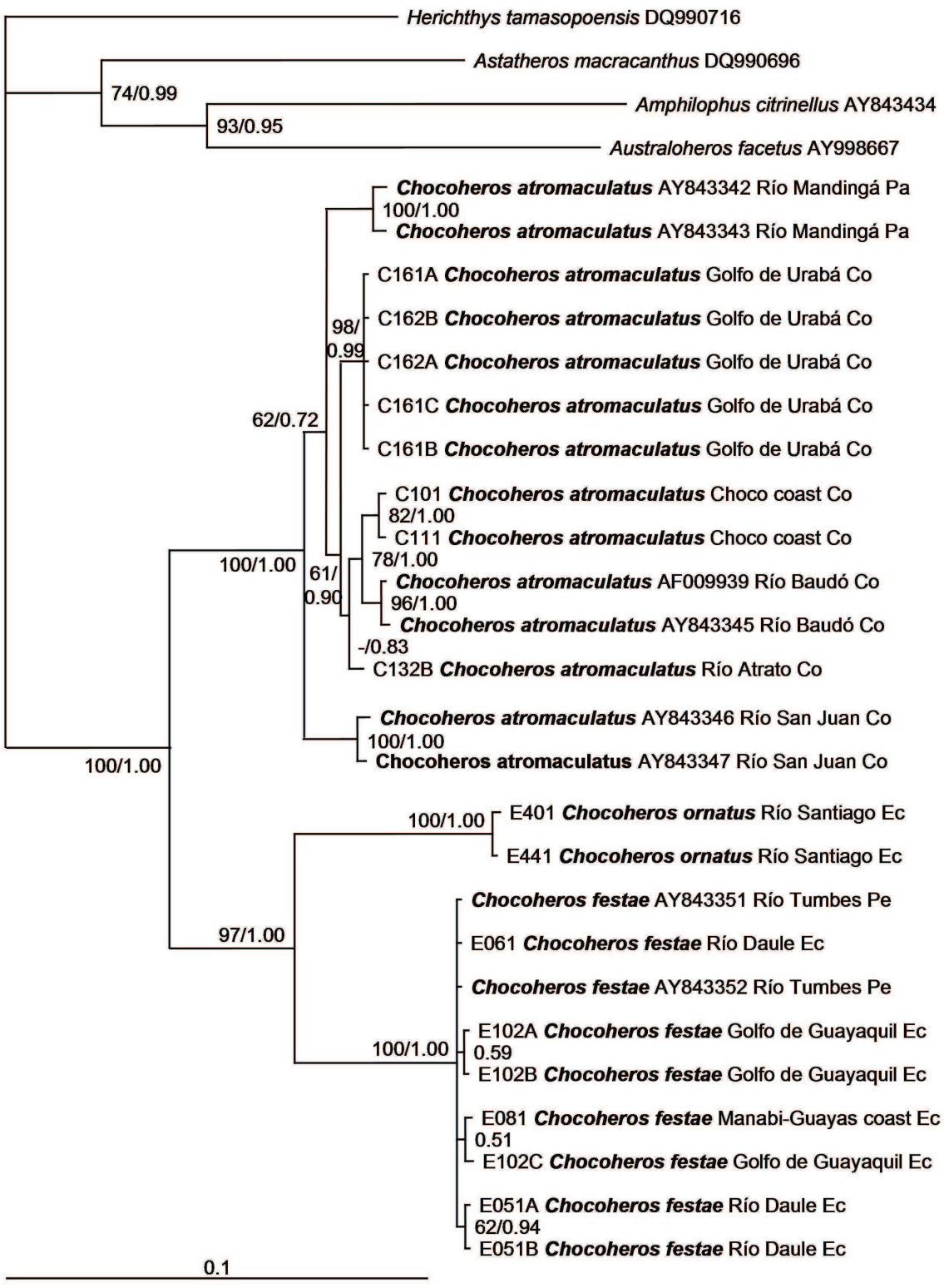


Figure 6: Bayesian phylogeny of the *´Heros´ festae* group based on cytochrome b.

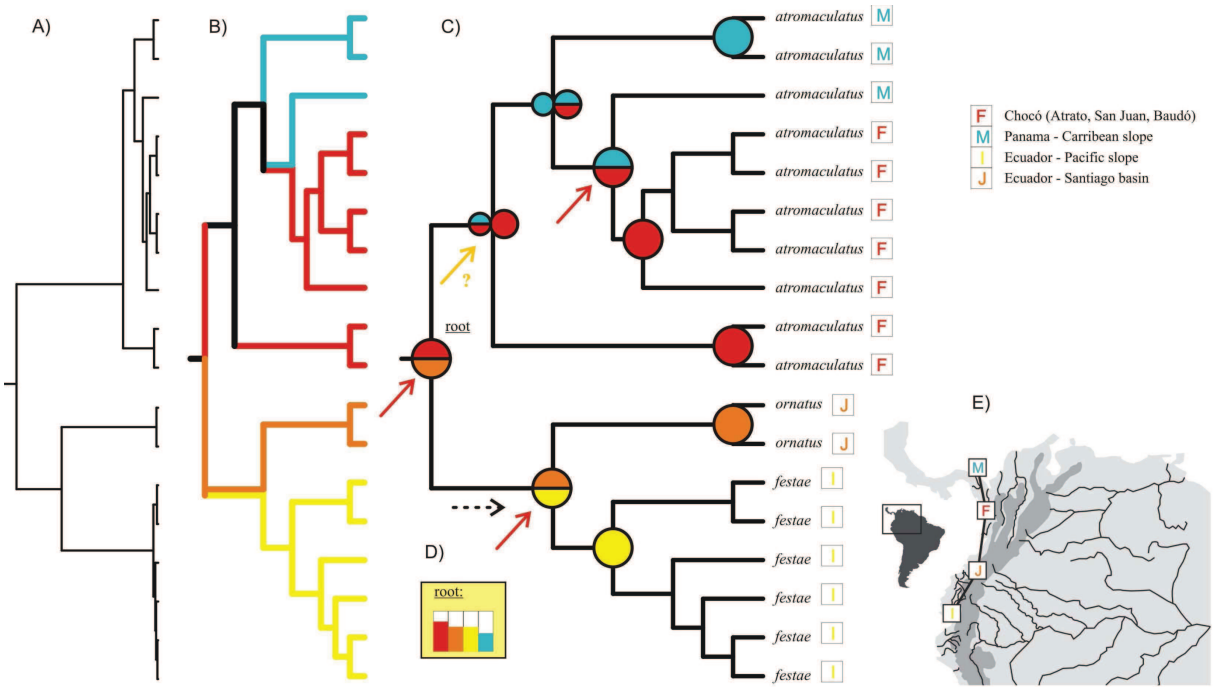


Figure7: Biogeography reconstruction in the genus Chocoheros: A) Relaxed molecular clock tree of haplotypes analyzed in BEAST. B) Topology with distribution areas mapped on the tree. C) Biogeographic scenario, ancestral area reconstruction by S-DIVA, under the stepping-stone model (only connected areas allowed); D) different ancestral area in the root of tree resulted from the analysis without adjacency limitation; for graphic symbols see legend in Fig. 5. E) map with areas and marked connection for stepping-stone model.

Discussion

The phylogeographic reconstruction of the two cichlid groups with the mostly trans-Andean distribution in South America was performed. The *Andinoacara* data set covered by this study represents the most comprehensive sampling of this genus covering all valid species (and, additionally, represented by much more then one specimen), and also the two or three lineages with the uncertain taxonomic status. In ´Heros´ festae group, the most comprehensive sampling was analyzed as well, including all three species.

The phylogenetic reconstruction of the genus *Andinoacara* was highly consistent with the previous studies. Within *Andinoacara*, two sister lineages were found, *A. pulcher* group and *A. rivulatus* group corresponding to the published phylogenies (Kullander, 1998; Musilová et al., 2008; Musilová et. al. 2009a; Musilová et al., 2009b). On the other hand, no detailed phylogeny of *´Heros´ festae* group has been previously published. Our

comprehensive study showed the monophyly of the group previously suggested in few samples within large Heroine phylogeny (Concheiro Perez, 2007).

The Choco region in the Colombian Pacific was found as the most probable ancestral area for the both cichlid genera involved in this study. Choco alone or in a combination with the adjacent Santiago region in Ecuador, or with the Panama Isthmus, was suggested as the exclusive ancestral area. The dispersion patterns of the both genera were also consistent as they were spreading both to the North and the South. In *Andinoacara*, the dispersal from Choco to the Ecuadorian rivers, via Santiago occurred around 13 Mya (dated by divergence in cytochrome b). In ´H.´ festae group, the divergence between Santiago and Choco was dated around 5 Mya. The Choco region is also considered as one of the most important centers of species diversity and endemism in the Earth (Sarkar et al. 2009). It is the crucial hot-spot not only for the cichlids but also for many other freshwater organisms. Therefore, the patterns of historical dispersion could be similar on more taxa than the two studied genera.

The both studied groups colonized Central America during their evolutionary history. Three waves of colonisation were reported based on different freshwater fishes (Martin & Bermingham, 1998). The youngest colonisation occurred recently, in Pleistocene, the second one corresponds with the Panama Isthmus rise, 3 Mya, while the oldest colonisation (Late Miocene) predates it. The *´Heros´ festae* group represents the basal lineage of herichthyines, the heroine group, which colonized Central America between 16-20 Mya (Concheiro Perez, 2007). This is even much older colonisation than the oldest wave suggested by Martin and Bermingham, 1998). However, divergence between Panama and Choco within the *´H.´ atromaculatus* populations is much lower (about 2% of p-distance) and shows a more recent independent colonization of the Panama Isthmus by the *´H.´ festae* group. It corresponds with the Pleistocene migration wave reported by Martin and Bermingham, 1998. Cichlasomatines colonized Central America later than Heroines. It was previously dated around 10 Mya (Musilová, et al., 2008). Although the final rise of Panama Isthmus 3 Mya enables massive colonisation (second wave in Martin and Bermingham, 1998), there are the faunistical evidences, that several connection existed before and the possible colonisation events occurred between the second and first colonisation waves (Martin & Bermingham, 1998). This could be the case of *Andinoacara* which colonised the Central America 4,7 Mya, or 6-8 Mya in the geological dating.

The cichlid genus *Andinoacara* represents one of the most suitable models for the biogeographical study of the definitive revealing of the Andean uplift role in history of biodiversity. Its sister clade, genera *Bujurquina* and *Tahuantinsuyoa* (Musilová et al., 2009),

is distributed in the cis-Andean region generally in the western half of the South American continent (Kullander, 1986; Stawikowski & Werner, 1998). Although distributed in lowlands, they can be found mainly along the Andes ridge (Western Amazon in Peru and Ecuador, upper Madeira basin in Bolivia, and westernmost tributaries of Orinoco in Colombia). According the phylogeny, the Andean uplift should create an important distribution barrier for the ancestors of the whole *Andinoacara-Buurquina-Tahuantinsuyoa* group. Hypothetically, the ancient colonisation of the trans-Andean region could occur followed by the speciation of both genera each on the opposite slope of the Andes. In this study, the ancestor of all *Andinoacara*s was dated to be older than 20 My old (Fig. 2), and the common ancestor of the two sister vicariant clades of *Bujurquina* and *Andinoacara* was previously dated to 25 - 30 Mya (Musilová et al, 2008). In that time the hypothetic connection between the cis- and trans-Andean region existed, presented by Western Andean portal. It represented the marine incursion from the West through the Central / Northern Andes low altitude boundary (Antonelli et al., 2009; Santos et al., 2009). The ancestor of *Andinoacara* and *Bujurquina* predates the closing of the Western Andean Portal (it persisted till 13-11 Mya; Antonelli et al, 2009) and the potential colonisation by this way would be thus possible. However, the biogeographic reconstruction showed the opposite direction of the historical dispersion from Choco to the south by both *Andinoacara* and the *´Heros´ festae* group. It means that the Western Andean portal would not be the colonisation route to the trans-Andean region for the fresh water fishes for a relatively long time before its closing.

Our study revealed the colonisation both to the north and the south of the two cichlid genera from the Choco region, one of the diversity hot-spots. We also showed that the Western Andean Portal would not be used as migration corridor between the trans- and cis-regions for a relatively long time before its closing. As it could be the historical endemic centre of some fresh water fauna, the colonisation patterns could be more common through different taxa. We also better specified the patterns of the Central American colonisation by frashwater fishes.

Acknowledgement

We would like to thank to Lukáš Kubička, Rainer Stawikowski, Ingomar Kratz, Alf Stalsberg, Ingo Schindler, Wolfgang Staeck, Lutz Krahnefeld, Jens Gottwald, Leonel Calderón, Martha´s Bodega in Bogota, Roman Papík, Miloš Kroupa, Jeff Rapps, Antonín Prouza, Aqua Ferrytale and Hobby Zoo Tillmann for their help during sample collection. We thank to Robert Tropek for valuable comments to the manuscript. Part of this work was carried out by

using the resources of the Computational Biology Service Unit from Cornell University. Financial support was provided for ZM by GAUK 139407, Deutsche Cichliden Gesellschaft, Expedicni fond and the foundation of the Centre for Biodiversity LC06073 (MSMT). The Institute of Animal Physiology and Genetics receives continuous support from IRP IAPG (No. AV0Z50450515) The work was supported by MSM 6007665801 (MSMT), 206/08/P003 (GAČR) and Deutsche Cichliden Gesellschaft for OŘ.

Literature

Abell, R., Thieme, M.L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B. Mandrak, N., Balderas, S.C., Bussing, W., Stiassny, M.L.J., Skelton, P., Allen, G.R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E., Olson, D., Lopez, H.L., Reis, R.E., Lundberg, J.G., Perez, M.H.S. & Petry, P. (2008) Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *Bioscience*, **58,** 403-414.

Antonelli, A., Nylander, J.A.A., Persson, C. & Sanmartin, I. (2009) Tracing the impact of the Andean uplift on Neotropical plant evolution. *PNAS,* **106,** 9749-9754.

Bermingham, E. & Martin, A.P. (1998) Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology*, **7,** 499-517.

Froese, R. & Pauly, D. (2010) *FishBase*. www.fishbase.org, version (09/2010).

Greenwood, P.H. (1984). The haplochmine species (Teleostei, Cichlidae) of the Cunene and certain other Angolan rivers. *Bulletin of the British Museum (Natural History)*, **47**, 187-239.

Coates, A.G. & Obando, J.A. (1996). The geologic evolution of the Central American Isthmus. *Evolution and Environment in Tropical America* (ed. by Jackson, J.B.C., Budd, A.F. and Coates, A.G.), p. 21-56. University of Chicago, Chicago.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology,* **7**, 214

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, **17**, 754-755.

Kullander, S.O. (1986) *Cichlid fishes of the Amazon River drainage of Peru*. Swedish Museum of Natural History, Stockholm, Sweden.

Kullander, S.O. (1998) A Phylogeny and Classification of the South American Cichlidae

(Teleostei: Perciformes). Phylogeny and Classification of Neotropical Fishes, p. 461-498 (ed. by Malabarba, L.R., Reis, R.P., Lucena, Z.M. & Lucena, C.A.S.). Edipucrs, Porto Alegre, Brazil.

Lundberg, J.G., Marshall, L.G., Guerrero, J., Horton, B., Malabarba, M.C.S.L., Wesselingh, F. (1998) The stage for Neotropical fish diversification: a history of tropical South American rivers. *Phylogeny and Classification of Neotropical Fishes*, p. 13-48. (ed. by Malabarba, L.R., Reis, R.P., Lucena, Z.M. & Lucena, C.A.S.). Edipucrs, Porto Alegre, Brazil.

Maddison, W.P. & Maddison, D.R. (2010) *Mesquite: a modular system for evolutionary analysis*. Version 2.73 http://mesquiteproject.org

Montoya-Burgos, J.I. (2003) Historical biogeography of the catfish genus Hypostomus (Siluriformes: Loricariidae), with implications on the diversification of Neotropical ichthyofauna. *Molecular Ecology*, **12**, 1855-1867.

Perez, G.A.C., Říčan, O., Orti, G., Bermingham, E., Doadrio, I. & Zardoya, R. (2007) Phylogeny and biogeography of 91 species of heroine cichlids (Teleostei : Cichlidae) based on sequences of the cytochrome b gene. *Molecular Phylogenetics and Evolution,* **43,** 91-110.

Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Phylogenetics and Evolution*, **25**, 1253-1256.

Říčan, O., Zardoya, R. & Doadrio, I. (2008) Phylogenetic relationships of Middle American cichlids (Cichlidae, Heroini) based on combined evidence from nuclear genes, mtDNA, and morphology. *Molecular Phylogenetics and Evolution,* **49,** 941-957.

Sanderson, M.J. (2002) Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Molecular Biology and Evolution*, **19**, 101-109.

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4-14.

Ronquist, F. (2001) *DIVA version 1.2. Computer program for MacOS and Win32*. Evolutionary Biology Centre, Uppsala University. Available at http://www.ebc.uu.se/systzoo/research/diva/diva.html.

Santos, J.C., Coloma, L.A., Summers, K., Caldwell, J.P., Ree, R., Cannatella, D.C. (2009) Amazonian Amphibian Diversity Is Primarily Derived from Late Miocene Andean Lineages. *PLoS BIOLOGY*, **7,** 448-461.

Sarkar, S., Sanchez-Cordero, V., Londono, M.C. & Fuller, T. (2009) Systematic conservation assessment for the Mesoamerica, Choco, and Tropical Andes biodiversity hotspots: a preliminary analysis. *Biodiversity and Conservation*, **18**, 1793-1828.

Sevilla, R.G., Diez, A., Noren, M., Mouchel, O., Jerome, M., Verrez-Bagnis, V., van Pelt, H., Favre-Krey, L., Krey, G. & Bautista, J.M. (2007) Primers and polymerase chain reaction conditions for DNA barcoding teleost fi sh based on the mitochondrial cytochrome b and nuclear rhodopsin genes. *Molecular Ecology Notes*, **7**, 730-734.

van Oppen, M.J.H., Willis, B.L. & Miller, D.J. (1999) Atypically low rate of cytochrome b evolution in the scleractinian coral genus Acropora. *Proceedings of the Royal Society of London Series B*, **266**, 179-183.

Villesen, P. (2007) FaBox: an online toolbox for fasta sequences. *Molecular Ecology Notes*, **7**, 965-968.

Yu, Y., Harris, A.J. & He, X. (2010) S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution*, **56**. Retreived from http://www.biomedsearch.com/nih/S-DIVA-Statistical-Dispersal-Vicariance/20399277.html

Musilová Z., Schindler I., Staeck W.

2009

Description of Andinoacara stalsbergi sp. n. (Teleostei: Cichlidae:Cichlasomatini) from Pacific coastal rivers in Peru, and annotationson the phylogeny of the genus

Vertebrate Zoology **59: 131-141**

Scientific paper

Ingo Schindler
Warthestr. 53a
12051 Berlin
Deutschland / Germany

Berlin, 06. 01. 2011

To whom it may concern

I declare that Zuzana Musilová performed significant portion of work on our manusript:

MUSILOVÁ, Z., SCHINDLER, I. AND STAECK, W., 2009 - Description of *Andinoacara stalsbergi* sp. n. (Teleostei: Cichlidae: Cichlasomatini) from Pacific coastal rivers in Peru, and annotation on the phylogeny of the genus. Vertebrate Zoology, 59(2): 131- 141.

Ingo Schindler

Description of *Andinoacara stalsbergi* sp. n. (Teleostei: Cichlidae: Cichlasomatini) from Pacific coastal rivers in Peru, and annotations on the phylogeny of the genus

Zuzana Musilová [1,2], Ingo Schindler [3] & Wolfgang Staeck [4]

[1] Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, 128 44 Praha, Czech Republic
zuzmus(at)email.cz
[2] Laboratory of fish genetics, Institute of Animal Physiology and Genetics of the Academy of Sciences of Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic
[3] Warthestr. 53a, 12051 Berlin, Germany
ingoschindler(at)web.de
[4] Auf dem Grat 41a, 14195 Berlin, Germany

Received on September 28, 2009, accepted on October 19, 2009.
Published online at www.vertebrate-zoology.de on December 11, 2009.

> Abstract

Andinoacara stalsbergi sp. n. is described from the drainages of trans-andean rivers and lakes at the Peruvian Pacific coast where this species occurs between Río Chira (Depto. Piura) in the north and Río Pisco (Depto. Ica) in the south. It is distinguished from its sister species *A. rivulatus* by the possession of a conspicuous white margin of both the dorsal and caudal fin and scales with light centres and contrasting dark marginal lines forming a fine reticulate pattern on the body sides. Studies based on molecular data confirm the status of *Andinoacara stalsbergi* sp. n. and reveal its phylogenetic relationships to its congeners. The reconstruction of the phylogeny within the genus *Andinoacara* results in the existence of two clades: one with *A. stalsbergi* sp. n., *A. rivulatus*, *A. sapayensis* and *A. biseriatus* and another with the remaining species.

> Kurzfassung

Andinoacara stalsbergi sp. n. wird aus den Einzugsgebieten von Flüssen und Seen an der peruanischen Pazifikküste beschrieben. Dort ist die Art zwischen dem Río Chira (Depto. Piura) im Norden und dem Río Pisco (Depto. Ica) im Süden verbreitet. Von der Schwesterart *A. rivulatus* unterscheidet sie sich durch einen auffälligen weißen Saum entlang der Rücken- und der Schwanzflosse und dunkel geränderte Schuppen mit hellem Zentrum, die auf den Körperseiten ein Netzmuster bilden. Die Analyse molekularer Daten bestätigt den Status von *Andinoacara stalsbergi* sp. n. und zeigt die phylogenetischen Beziehungen zu den übrigen Gattungsmitgliedern. Die Rekonstruktion der Stammesgeschichte in der Gattung *Andinoacara* hat die Existenz von zwei Verwandtschaftslinien zum Ergebnis: eine mit den Arten *A. stalsbergi* sp. n., *A. rivulatus*, *A. sapayensis* und *A. biseriatus* sowie eine zweite mit den übrigen Arten.

> Key words

Systematic, ichthyology, ecology, reproductive behaviour, Cichlidae, Cichlasomatini, new species, Peru, *Andinoacara*, *Aequidens*, blue acara.

Introduction

The South American cichlid genus *Andinoacara* Musilová, Říčan & Novák, 2009 belonging to the tribe Cichlasomatini is one of the genera which were recently described as the result of analyses of morphological and molecular data and ensuing nomenclatural revisions. This genus involves six species previously placed in the genus *Aequidens* Eigenmann & Bray, 1894, viz. *Cichlosoma biseriatum* Regan, 1913, *Acara coeruleopunctata* Kner, 1863, *Acara latifrons* Steindachner, 1878, *Cychlasoma pulchrum* Gill, 1858, *Chromis rivulata* Günther, 1860 and *Acara sapayensis* Regan, 1903.

Kullander (1983) demonstrated that *Aequidens* was an unnatural catch-all group. He listed the six species mentioned above among the *Aequidens* species which ought to have been placed into a separate

Tab. 1. Primers used for molecular analyses in this study.

	F	R	citation
12S rRNA	AAAAAGCTTCAAACTGGGATT AGATACCCCACTAT	TGACTGCAGAGGGTGACGGGC GGTGTGT	Kocher *et al*. (1989)
16S rRNA	CCGGTCTGAACTCAGATCACG	CTGTTTAACAAAAACAT	Marescalchi (2005)
cytochrome b	ACCACCGTTGTTATTCAACTACAAGAA	CCGACTTCCGGATTACAAGACCG	Sevilla *et a*l. (2007)
ND 4	TGGAGCTTCTACGTGRGCTTT	CAAAACCTTAATCTYCTACAATGCT	Arévalo *et al*. 1994, Bielawsky *et al*. (2002)
RAG 1	CTGAGCTGCAGTCAGTACCATAAGATGT	CTGAGTCCTTGTGAGCTTCCATRAAYTT	Grande *et al*. (2004)
rhodopsin	GCAAGCCCATCAGCAACTTCCG	TGCTTGTTCATGCAGATGTAGA	Chen *et al*. (2003)
Tmo-4C4	CGGCCTTCCTAAAACCTCTCATTAAG	GTGCTCCTGGGTGACAAAGTCTACAG	Farias *et al*. (1999)
S7 intron 1	TGGCCTCTTCCTTGGCCGTC	AACTCGTCTGGCTTTTCGCC	Chow & Hazama (1998)

genus and introduced the term '*Aequidens*' *pulcher* group for them because of lack of an alternative generic allocation. In addition, he later (Kullander 1991) used the term '*Aequidens*' *rivulatus* group for '*Aequidens*' *rivulatus* and undescribed forms (cf. Stawikowski & Werner 1998) closely related to this species, and eventually showed that the '*Aequidens*' *pulcher-rivulatus* group could have a generic status (Kullander 1998). Musilová *et al*. (2008) confirmed that the '*Aequidens*' *pulcher-rivulatus* group represents a well supported yet unnamed genus. In their study of the phylogenetic relationships among cichlasomatine cichlids Musilová *et al*. (2009) tentatively listed '*Aequidens*' sp. "Silbersaum" as an undescribed species among the members of the '*Aequidens*' *pulcher-rivulatus* group, for which they established the new genus *Andinoacara*.

The species provisionally referred to as '*Aequidens*' sp. "Silbersaum" in Europe or as "Green Terror" in the USA has been known both in the aquarium trade and the popular literature for about forty years (Lüling 1972; Staeck & Linke 1985). In the older ichthyological and aquaristic literature it was treated as a form of *A. rivulatus* (e. g. Regan 1905, Everman & Radcliffe 1917, Eigenmann 1922, Lüling 1972, Werner 1983). The formal description of this cichlid is one subject of this paper. In addition the phylogeny within the genus *Andinoacara* is discussed on the basis of a set of molecular data. This is the first molecular phylogenetic analysis including all valid nominal species of *Andinoacara*.

Material and Methods

Type specimens were fixed in formalin and later transferred into 75% ethanol. The holotype and paratypes are deposited in the fish collection of the Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde, (MTD F).

The techniques for taking measurements and meristic data follow those described in Kullander (1986) and Kullander & Nijssen (1989). Measurements were made with an electronic digital caliper reading to the nearest 0.1 mm. Figures in brackets after counts indicate the number of specimens examined with that condition. Terminology and methods of measurements of jaws and teeth follow Casciotta & Arratia (1993). Scale rows are numbered as described by Kullander (1990). Nomenclature of colour patterns follows Kullander (1983, 1991). Vertical bars are numbered from the caudal fin to the snout as described by Kullander & Silfvergrip (1991). According to this approach the caudal spot is counted as bar 1 (homolog to bar 1p in Říčan *et al*. 2005). The description follows the general format used by Kullander (1991).

In the phylogenetic analysis 11 species or forms of *Andinoacara* were studied. We analyzed specimens of seven nominal species, viz. *Andinoacara biseriatus*, *A. coeruleopunctatus*, *A. latifrons*, *A. pulcher*, *A. rivulatus*, *A. sapayensis*, *A. stalsbergi* and four apparently undescribed forms. All specimens used in the molecular analyses were obtained from direct imports to specialized aquarium trade companies in Europe. We studied 2–4 specimens of each species or form, except for *A. coeruleopunctatus* and *A. sapayensis*, as only one individual of these species was available.

In the molecular study eight genetic markers were sequenced, and a data set with the total length of 5627 bp was obtained. Both mitochondrial (16S rRNA, 12S rRNA, cytochrome *b*, ND 4) and nuclear coding (RAG1, rhodopsin, Tmo-4C4) and non-coding (intron 1 in S7 gene) markers were used. We analyzed a data set of eight genes with the exception of *A. sapayensis* because sequences of only two genes of this species were available. The set of primers is listed in Tab. 1.

PCR condition consisted of an initial denaturation step at 94 °C followed by the extension of DNA at

72°C. The annealing temperature of the genes differed: 48 °C for 12S rRNA and 55 °C for rhodopsin, ND 4 and Tmo-4C4. Sequences of the four genes 16S rRNA, cytochrome b, S7 intron 1 and RAG1 were used from previous phylogenetic studies of the tribe Cichlasomatini (Musilová *et al*. 2008, Musilová *et al*. 2009). In case of *A. sapayensis* we followed conditions for PCR described in the previous studies. Sequences of genes newly obtained for this study (12S rRNA, ND 4, rhodopsin, Tmo-4C4) and sequences of *A. sapayensis* (16S rRNA and cytochrome *b*) are available in GenBank.

The substitution model for phylogenetic analysis was suggested by using jModeltest software (Posada, 2008) with Bayesian information criterion (BIC). Phylogenetic analyses of the nuclear and mitochondrial sequence data were performed using Bayesian methods (MrBayes software, Huelsenbeck & Ronquist 2001) with two parallel runs of 20 million generations, each run with 4 chains. Independent model parameters were estimated for each gene partition during Bayesian analysis. Topologies were sampled every 1000 generations and the final results are based on 75% of the obtained trees (15000 trees).

Abbreviations

BIC	Bayesian information criterion; method used in molecular analyses for choosing an apropriate model of molecular evolution
E1	Row of scales in the horizontal series directly above the longitudinal row including the lower lateral line
MTD F	Senckenberg Naturhistorische Sammlungen Dresden, Museum für Tierkunde, Fischsammlung
ND 4	NADH dehydrogenase subunit 4, mitochondrial gene for enzyme from respiratory complexes
PCR	Polymerase chain reaction, method used for amplification of selected gene markers
RAG1	Recombination activating gene, subunit 1, nuclear genetic marker
SL	Standard length
TL	Total length
12S rRNA	Mitochondrial genetic marker coding ribosomal RNA (part of mitochondrial ribosomes).
16S rRNA	Mitochondrial genetic marker coding ribosomal RNA (part of mitochondrial ribosomes)

Andinoacara stalsbergi sp. n.

Figs. 1–5, Tables 2–3

Holotype. MTD F 31782, adult female, 103.0 mm SL, Peru, Depto. Ica, Rio Pisco, 13º 43' 26" S, 75º 58' 59" W; *leg*. A. Stalsberg, 2008.
Paratypes. MDT F 31783–31794, 12 ex., 25.3-68.0 mm SL, Peru, Depto. Lambayeque, vicinity of Chiclayo; licensed and confirmed import for aquarium trade, *don*. Musilová. MDT F 31795–31797, 3 ex., 98.9–113.0 mm SL, Peru, Depto. Piura, few km north of Piura, Laguna Ñapique, 5º 30' 27" S, 80º 42' 40" W; *leg*. A. Stalsberg, 2008. MTD F 31798–31801, 4 ex., 89.6–108.2 mm SL, data like holotype. Not catalogued, 1 ex., 73.3 mm SL, alizarin red stained and dissected, data like holotype.

Diagnosis. A species of the *Andinoacara rivulatus* group. It is most similar to *A. rivulatus* with which it shares the comparatively large size (TL > 200 mm in males), the colour pattern of the cheeks and a light vertical stripe anterior and posterior to the rectangular midlateral spot. It is readily distinguished from this species by specific colour characteristics, viz. having (1) a conspicuous white margin in both the dorsal and caudal fin and (2) on the body sides scales with light centres and contrasting dark marginal lines forming a fine reticulate pattern.

Description. Refer to Figs. 1–4 for general appearance and colour pattern. Morphometric data of eight specimens (89.6–113.0 mm SL) are summarised in Tab. 2. Counts from 12 specimens (65.8–113.0 mm SL), osteological characters from a dissected specimen (73.3 mm SL).

Body moderately deep (body depth 42–49% of SL) and laterally compressed. Snout round, moderately long. Jaws isognathus. Lips moderately thick. Interorbital area convex. Anterior dorsal head profile straight, on nape curved; ventral contours less arched. Pre-pelvic and abdominal contour straight or slightly concave. Dorsal-fin base almost straight. Caudal peduncle with straight dorsal and ventral edge. In frontal aspect outline of body elliptic with rounded nape and chest.

Uniserial predorsal scale pattern. Cheek scales in 3 series. Dorsal, anal, pelvic and pectoral fins naked. Caudal-fin base densely scaled. Scales in E1 row 24(2) or 25(10). Scales on upper lateral line 15(1), 16(2) or 17(9), on lower lateral line 8(1), 9(4) or 10(7), including 1 or 2 on caudal fin base.

Soft portion of anal and dorsal fin pointed, but not produced, reaching anterior caudal fin in adult specimens. Caudal fin round or slightly subtruncate; caudal fin length about $^1/_4$ to almost $^1/_3$ of SL. Pelvic fins rounded, extending to anus. Pectoral fin rounded,

Fig. 1. *Andinoacara stalsbergi* sp. n., holotype, female 103 mm SL, MTD F 31782.

Fig. 2. Adult female of *Andinoacara stalsbergi* sp. n., from the vicinity of Chiclayo (Depto. Lambayeque), TL 116 mm. Live specimen photographed in aquarium.

with 13–14 rays. Dorsal fin XIII.12(1), XIII.13(1), XIV.11(3) or XIV.12(7). Anal fin III.8(8), III.9(3) or III.10(1).

On first gill arch 2 or 3 small gill rakers on epibranchial, 1 in the angle and 9–10 (n=5) externally on ceratobranchial. Fourth ceratobranchial with 5 tooth plates and 3 to 7 teeth on each plate. Lower pharyngeal tooth plate (Fig. 5) robust, moderately long (width of bone 83–85% of its length; n=2), with well-ordered teeth; length of dentigerous area 85% of its width; 14–17 teeth in posterior row (n=2), 6–9 teeth in median row (n=2); teeth obviously lost were also counted. Oral jaw teeth conical with recurved tips. In upper jaw hemiseries 15–22 outer row teeth and in lower jaw hemiseries 17–23. Length of dentigerous arm of premaxilla shorter than length of ascending arm (premaxillary ascending arm length/dentigerous arm length ≈ 1.6); width of the ascending arm about 15% of its length.

Fig. 3. **T**opotypic female of *Andinoacara stalsbergi* sp. n. showing breeding colour pattern in aquarium. Photo: A. Stalsberg.

Fig. 4. Adult male of *Andinoacara stalsbergi* sp. n. showing neutral colour pattern in aquarium.

Lower jaw comparatively high (anguloarticular depth about 72% of length, coulter area depth about 43% of anguloarticular length). Coulter area deeper than its length (coulter area depth/coulter area width ≈ 1.3). Dorsal margin of hyoid more or less straight, without a deep notch.

Colouration in life. Based on observations on specimens kept in aquarium and photos taken immediately after capture. Forehead, nape and pre-dorsal part of dorsum uniformly greyish or light brown. On the body sides each scale with iridescent or metallic green centre and contrasting dark brown marginal line. The dark scale margins form a fine reticulate pattern which is particularly prominent in adult specimens. Cheeks with two to four narrow oblique opalescent green lines and several small buccal dots of the same colour. Dark cheek spot in the corner of the preopercle usually vis-

Tab. 2. Body proportions of *Andinoacara stalsbergi*. Measurements of holotype (MTD F 31782) and seven paratypes (MTD F 31795–31797, MTD F 31798–31801) in percent of SL (except SL in mm); min = lowest value, max = highest value, mean = arithmetic mean, sd = standard deviation.

	min	max	mean	sd
Standard length	89.6	113.0	100.8	8.2
Body depth	42.0	48.9	44.8	2.60
Head length	32.3	36.0	34.6	1.27
Eye diameter	6.7	8.2	7.6	0.47
Interorbital width	12.0	14.1	13.0	0.80
Preorbital depth	8.6	11.1	10.0	0.70
Predorsal length	41.4	47.7	44.0	2.30
Prepelvic length	40.7	45.3	41.9	1.72
Preanal length	65.3	73.2	69.8	2.25
Dorsal-fin base length	52.9	60.4	56.3	2.54
Anal-fin base length	20.1	23.9	21.5	1.47
Pectoral-fin length	26.8	30.4	28.5	1.35
Peduncle depth	15.3	17.9	16.6	0.78
Peduncle length	14.0	15.5	14.9	0.80

ible only during brood care. Lips, lower region of preopercle and gill cover iridescent green. Iris golden.

No horizontal lateral band. Midlateral spot black, squarish or rectangular, extending dorso-ventrally from $^3/_4$ of El scales 8–10, 9–11 or 9–12 to all of these scales in E2 and E3 row above. Anterior and posterior to midlateral spot with contrasting narrow vertical white stripe which fades in dorsal and ventral region. Sometimes with three ill-defined wide dark vertical bars and narrow light interspaces behind the midlateral spot. Usually no caudal spot (if visible: small, vertically extended, positioned on level of lower lateral line).

Dorsal fin grey, with narrow dark submarginal band and white lappets forming a conspicuous white margin; soft part with iridescent streaks on the membranes. Anal fin grey with blackish margin and small iridescent green dots and short lines. Caudal fin grey, with darker distal region, a conspicuous white posterior margin and a pattern of tiny greenish dots. Pelvic fins grey, darker along anterior margin, with green first interradial membrane and greenish dots or short streaks inwardly. Pectoral fins hyaline and colourless. After spawning and during parental care both sexes develop a very dark, almost blackish colouration with two contrasting white vertical stripes anterior and posterior to midlateral spot.

Colouration in alcohol. Based on holotype with notes on paratype specimens. Body sides grey, darker on nape and back. Scales on sides with narrow black margin. Snout and cheeks grey. Lips dark grey. Gill cover, preopercle and branchiostegal region dark grey. Two or three dark preorbital stripes and several irregular short streaks or dots. Supraorbital markings indistinct and faint. Suborbital stripe reduced to a short dark marking (often masked by dark head sides) in the corner of the preopercle.

No continious lateral band. Bar 1 (caudal spot) narrow, prominent and blackish, in the centre of the caudal-fin base, not reaching ventral or dorsal edges of it; bar 2 on caudal peduncle; bar 3 on anterior part of caudal peduncle between posterior rays of dorsal fin and anal fin, in smaller specimens (< 70 mm SL) often split into two parallel vertical bars; bar 4 and 5 fused in adult specimens, but separate in smaller ones; bar 6 darker than the other bars, straight, not vertically split, on both sides with contrasting narrow light margin; bar 7 and 8 indistinct, usually fused. Midlateral spot black, on upper half of bar 6.

Dorsal fin dark grey with light marginal band and light grey streaks in its soft part. Anal fin grey with darker marginal band and light grey streaks in posterior portion. Caudal fin grey, with light grey streaks in posterior portion and contrasting light distal margin. Pelvic fins dark grey. Pectoral fins hyaline.

Sexual dimorphism. There are no obvious external sex differences in fin length or intensity of colour pattern. However, observations under aquarium conditions revealed that there is a distinct size difference between males and females and that in addition dominant males develop a prominent nuchal hump.

Geographical distribution. *Andinoacara stalsbergi* is distributed in trans-andean rivers and lakes at the Peruvian Pacific coast. The distribution of this species in the Pacific slope of western Peru between Río Chira (Depto. Piura) in the north and the Río Pisco (Depto. Ica) in the south is well documented (cf. STAWIKOWSKI

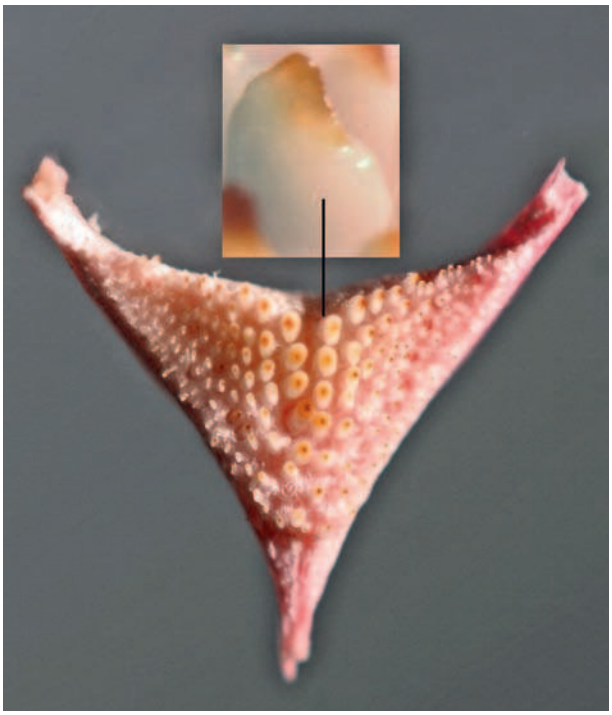


Fig 5. Lower pharyngeal tooth plate of *A. stalsbergi* sp. n. (73.3 mm SL).

& Werner, 1998). Collecting sites confirmed by Stalsberg (personal communication) were (from the south to the north) Río Pisco, Río Cañete (Lunahuana: 13º01'57''S, 76º12'02'' W), Río Mala, Río Lurin, Laguna Ñapique (05º30'27" S, 80º42'40" W), Lago San Ramón, Río Piura, Quebrada Carneros, Quebrada Onda, Río Pidregal and Quebrada Samana (tributary to Río Chira). Additional collecting sites are the Laguna de Végueta (Lüling 1973) and Pacasmayo (Everman & Radcliffe 1917). In the vicinity of Tumbes (Depto. Tumbes) in the extreme north of Peru (Río Tumbes and Río Zarumilla) and in the adjacent Pacific slope of Ecuador this species is replaced by another species of the *A. rivulatus* group, which is also known in popular aquarium literature as "*Aequidens*" sp. "Goldsaum" and treated as *A. rivulatus* by Stawikowski & Werner (1998) and Kullander (2003).

Ecological notes. Lüling (1973) published a detailed description of the Laguna de Vegueta (approx. 11° 00' S, 77° 08' W), a collecting site of *Andinoacara stalsbergi*. The banks of this brackish lake situated close to the shoreline of the Pacific were partly covered with aquatic and submerged terrestrial vegetation (*Hydrocotyle bonariensis* and *Bacopa monnieri*). The associated fish fauna included *Bryconamericus peruvianus, Lebiasina bimaculata* (Characidae), *Poecilia reticulata* (Poeciliidae) *Dorminator latifrons* (Eleotridae). Water data collected in November 1970: pH 7.7; electrical conductivity 4280 μS/cm; total and temporary hardness 6.7 °dH. Lüling (1973) advanced the hypothesis that the Characidae and Poeciliidae are an important source of food for the cichlids.

Stalsberg, who repeatedly caught this cichlid between 1994 and 2008 in different years at many localities, collected additional ecological data at several collecting sites (from the south to north): (1) Depto. Ica: Río Pisco near Independencia (13º43'25" S, 75º58'59" W). At the collecting site the rather clear river was approx. 10 m wide. Its bottom was covered with sand and rocks. There was no submerged vegetation. Water data: water temperature 25.5 ºC, pH 8.3, total hardness > 40 °dH, temporary hardness 7 dH. (2) Depto. Piura: Laguna Ñapique about 60 km south of Piura (05º30'27" S, 80º42'40" W). Water data collected in August: water and air temperature 26 ºC, pH 9.2, total hardness >30 °dH, temporary hardness 4 ºdH. The water of the Lake was turbid and its bottom sandy. (3) Depto. Piura: Laguna San Ramón about 40 km south of Piura. Water data: water temperature 28.2 ºC, pH 9.0, total hardness 26 °dH, temporary hardness 5 ºdH, electrical conductivity 1700 μS/cm. The associated fish fauna included *Tilapia* sp. (Cichlidae) and *Bryconamericus peruanus* (Characidae). (4) Depto. Piura: Quebrada Saman, tributary to Río Chira at Pueblo Mallares. Water data: water temperature 22.5 ºC, air temperature 27 °C, pH 8.2, total hardness 31 °dH, temporary hardness 15 ºdH, electrical conductivity 1850 μS/cm. (5) Depto. Piura: Río Pidregal in the northwest of Sullana. Water data collected in August: water temperature 24 ºC, air temperature 28 ºC, pH 8.3, total hardness 15 °dH, temporary hardness 13 ºdH, electrical conductivity 270 μS/cm.

The available ecological data reveal that *Andinoacara stalsbergi* is well adapted to very alkaline and hard water rich in dissolved minerals and even tolerates brackish water (Lüling 1973).

Reproductive behaviour. Observations under aquarium conditions showed that *Andinoacara stalsbergi* is a monogamous substratum spawner and that both sexes share in all the duties of brood care. The female, however, is usually the more active partner as long as the pair cares for eggs or larvae, while the male defends the spawning territory against intruders. Like most other open brooders these cichlids deposit their eggs on a horizontal surface. At 27 °C hatching occurs about two days postspawning, and the fry attempt swimming seven days thereafter. The male and female fish practice long-term biparental defense of their mobile fry. A detailed description of the reproductive behaviour was published by Werner (1983).

Etymology. Named in honour of Alf Stalsberg (Tjodalyng, Norway), the collector of the holotype, in recognition of his longstanding commitment to increase the knowledge about cichlid fishes.

Fig. 6. Adult male of *Andinocara rivulatus* from Río Tumbes photographed in aquarium.

Discussion

Andinoacara stalsbergi is most closely related to *A. rivulatus* (Fig. 6), its sister species. Both differ from other species of *Andinoacara* by the lack of conspicuous dark nape markings (versus distinct dark supraorbital marks in species of both the *A. pulcher* group and of the related genera *Bujurquina* and *Tahuantinsuyoa*; cf. KULLANDER 1986, 1991), larger size (adult males > 150 mm TL versus < 150 mm TL in the previously mentioned taxa; cf. EIGENMANN 1922) and a trend towards more gill rakers (usually 9 or 10 rakers on ceratobrachial of outer gill arch versus usually < 9 in the remaining species of *Andinoacara*; cf. REGAN 1905, 1913, EIGENMANN 1922).

Although *A. stalsbergi* is very similar to *A. rivulatus* in morphometric and meristic data, general appearance and colouration of its head, these two allopatric species can readily be distinguished for distinctive specific colour patterns: *Andinoacara stalsbergi* has light scale centres and dark scale edges forming a reticulate pattern on the flanks (versus light scale edges and dark scale centres forming a pattern of horizontal lines in *A. rivulatus*) and a prominent white margin of the dorsal and caudal fin (versus a broad orange margin in *A. rivulatus*). The genetic differences between *A. stalsbergi* and its congeners (Tab. 3) provide additional arguments for its taxonomical separateness according to the evolutionary species concept (WILEY 1978).

Andinoacara biseriatus listed as a member of the *pulcher* group by KULLANDER (1998) and included in the *rivulatus* group by STAWIKOWSKI & WERNER (1998) clustered as the basal sister taxon of the *rivulatus-stalsbergi* clade (Fig. 7). This species differs from *A. stalsbergi* by the possession of dark rimmed scales on its nape (versus scales without dark posterior rim), a dark dot in the centre of each scale on body and opercle (versus dark edges, but no dark dots in *A. stalsbergi*), a caudal fin with a narrow reddish margin (versus caudal fin with broad whitish margin), a lateral spot positioned more dorsally than in *A.stalsbergi,* an additional spot (more prominent in females) in the dorsal fin above the bar with lateral spot (versus no such spot in *A. stalsbergi*) and, according to REGAN (1913), frequently 2 rows of scales on its cheek (versus 3 rows of scales in *A. stalsbergi*).

In the result of the phylogenetic analysis *Andinoacara sapayensis* clustered as a sister taxon of the rivulatus-stalsbergi clade. Adult specimens of *Andinoacara stalsbergi* differ significantly from *A. sapayensis* by the possession of only 3 bars between midlateral spot and caudal spot (versus four bars; compare Fig. 1-3 with Plate 31, Fig. 1 in EIGENMANN 1922), by the number of gill rakers on first outer gill arch (8–10 in *A. stalsbergi* versus <8 in *A. sapayensis*; cf. REGAN 1905, EIGENMANN 1922) and the lack of distinct dark supraorbital marks (versus possession of prominent supraorbital marks in *A. sapayensis*).

The molecular studies confirm the status of *Andinoacara stalsbergi* and reveal its phylogenetic relationships to its congeners (Fig. 7). The reconstruction of the phylogeny within the genus *Andinoacara* results in the existence of two clades. One consists of three valid species (i. e. *A. latifrons*, the type species

Tab. 3. Uncorrected p-distance between *Andinoacara stalsbergi* sp. n. and the two closely related species *A. rivulatus* (sister species) and *A. biseriatus* (1st & 2nd column). Average distance between nominal species of *Andinoacara,* including *A. stalsbergi* sp. n. in 3rd column.

	A. stalsbergi vs. *A. rivulatus*	*A. stalsbergi* vs. *A. biseriatus*	average *Andinoacara*
total (8 genes)	1.6%	5%	4.5%
12S rRNA	0.8%	2.6%	1.6%
16S rRNA	0.8%	2.7%	2%
cytochrome b	3.9%	14.1%	11.4%
NADH 4	4.3%	12.8%	13%
RAG 1	0.3%	0.3%	0.7%
rhodopsin	0%	0.7%	0.7%
Tmo-4C4	0.2%	1%	1.5%
S7 intron 1	1.6%	1.4%	2.5%

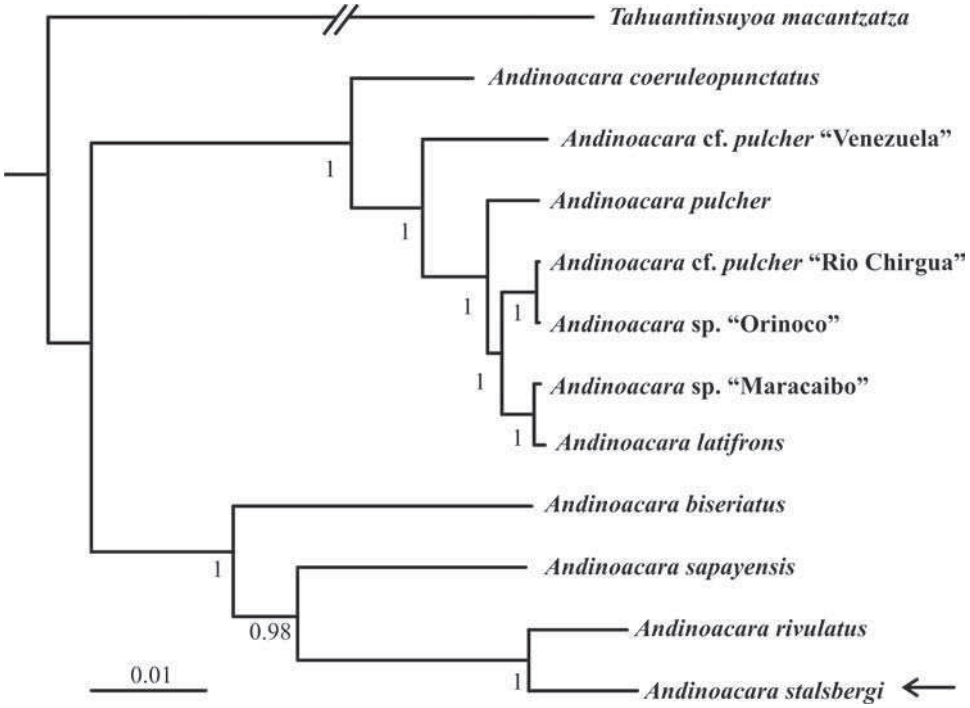


Fig. 7. Phylogenetic relationships within the genus *Andinoacara* based on a data set of eight genes (16S rRNA, 12S rRNA, cytochrome b, ND 4, S7 intron 1, RAG1, Rhodopsin, Tmo-4C4). Analysis was performed by using MrBayes software with two parallel runs of 20 million generations and sampling every 1000 trees. The model parameters suggested by jModeltest were applied. The phylogenetic position of *Andinoacara stalsbergi* sp. n. is marked by the indicator.

of *Andinoacara, A. coeruleopunctatus* and *A. pulcher*) and several possibly undescribed forms: *Andinoacara* sp. „Rio Chirgua" and *A.* sp. „Orinoco" representing separate lineages of fishes living in Venezuela and differing from *A. pulcher* and *A. latifrons* in their colouration. This group of „blue acaras" was referred to as „*Aequidens*" sp. „Orinoco" in Stawikowski & Werner (1998). But despite considerable differences, further morphological studies are required to confirm their status as new species. *Andinoacara* sp. "Maracaibo" (Stawikowski & Werner 1998) is endemic to the drainages of Lago de Maracaibo in western Venezuela and represents another undescribed form with limited distribution. However, in the results of our analyses we found only minor genetic difference between this form and *A. latifrons* living in eastern Colombia. Morphological comparisons of both are still missing because of the lack of material.

The specimens of *A. pulcher* used in our analyses were collected in Trinidad. They are genetically identical with *A. pulcher* obtained from Czech and German aquarium trade. Eventually *A.* sp. "Venezuela" appears to represent another form of „blue acara" with uncertain taxonomic position, for conclusive evidence is missing as only few specimens were available.

The second clade of *Andinoacara* is formed by *A. biseriatus*, *A. sapayensis, A. rivulatus* and *A. stalsbergi*. The two latter species clustered in our analyses as sister species, but are well distinguishable from each other on the basis of molecular data.

The two clades defined above correspond to the '*Aequidens*' *pulcher* group and the '*Aequidens*' *rivulatus* group respectively, previously distinguished by Kullander (1998). The only exception is *A. biseriatus* as it was considered by Kullander (1998) as a species of the '*A.*' *pulcher* group, but, by contrast, clustered as a basal species in the second clade, (i.e. with *A. sapayensis, A. rivulatus* and *A. stalsbergi*) in the results of our study of molecular phylogeny.

The genetic distance among particular species of *Andinoacara* (Tab. 3) varies in the different loci used in the molecular analysis. The average distance between the valid nominal species of *Andinoacara* was found in the range between 0.7% and 13% of uncorrected p-distance. The distance between *A. stalsbergi* and its sister species *A. rivulatus* varies up to 4.3% in different genes (Tab. 3).

Acknowledgements

Part of this work was carried out by using the resources of the Computational Biology Service Unit from Cornell University. Financial support was provided for the first author by GAUK 139407, Deutsche Cichliden Gesellschaft, and the foundation of the Centre for Biodiversity LC06073 (MSMT). The Institute of Animal Physiology and Genetics receives continuous support from IRP IAPG (No. AV0Z50450515). We are indebted to Alf Stalsberg for providing study material, photos and information on his collecting sites and thank Axel Zarske (MTD) for depositing the material in his institute.

References

Arevalo, E.; Davis, S.K. & Sites, J.W. (1994): Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. – Systematic Biology, **43**: 387–418.

Bielawsky, J.; Brault, A. & Gold, J. R. (2002): Phylogenetic relationships within the genus *Pimephales* as inferred from ND4 and ND4L nucleotide sequences. – Journal of Fish Biology, **61**: 293–297.

Casciotta, J. R. & Arratia, G. (1993): Jaws and teeth of American Cichlids (Pisces: Labroidei). – Journal of Morphology, **217**: 1–36.

Chen, W. J.; Bonillo, C. & Lecointre, G. (2003): Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. – Molecular Phylogenetics and Evolution, **26**: 262–288.

Chow, S. & Hazama, K. (1998): Universal PCR primers for S7 ribosomal protein gene introns in fish. – Molecular Ecolology, **7**: 1255–1256.

Grande, T.; Laten, H. & Lopez, J.A. (2004): Phylogenetic relationships of extant esocid species (Teleostei: Salmoniformes) based on mophological and molecular characters. – Copeia, **2004**: 743–757.

Eigenmann, C. H. (1922): The fishes of western South America, Part I. The fresh-water fishes of northwestern South America, including Colombia, Panama, and the Pacific slopes of Ecuador and Peru, together with an appendix upon the fishes of the Rio Meta in Colombia. – Memoirs of the Carnegie Museum, **9**: 1–346, Pls. 1–38.

Evermann, B. W. & Radcliffe, L. (1917): The fishes of the west coast of Peru and the Titicaca Basin. – Bulletin of the United States National Museum, No. **95**: 1–166.

Farias, I. P., Ortí, G.; Sampaio, I.; Schneider, H. & Meyer, A. (1999): Mitochondrial DNA phylogeny of the family Cichlidae: monophyly and fast molecular evolution of the Neotropical assemblage. – Journal of Molecular Evolution, **48**: 703–711.

Huelsenbeck, J. P. & Ronquist, F. (2001): MrBayes: Bayesian inference of phylogeny. – Bioinformatics, **17**: 754–755.

Kocher, T. D.; Thomas, W. K.; Meyer, A.; Edwards, S. V.; Paabo, S.; Villablanca, F. X. & Wilson, A. C. (1989): Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. – Proceedings of the National Academy of Sciences of the United States of America, **86**: 6196–6200.

Kullander, S. O. (1983): A revision of the South American Cichlid genus *Cichlasoma* (Teleostei: Cichlidae). – Swedish Museum Natural History. Stockholm, 296 pp.

Kullander, S. O. (1986): Cichlid fishes of the Amazon River Drainage of Peru. – Swedish Museum Natural History. Stockholm, 431 pp.

Kullander, S. O. (1990): *Mazarunia mazarunii* (Teleostei: Cichlidae), a new genus and species from Guyana, South America. – Ichthyological Exploration of Freshwaters, **1**: 3–14.

Kullander, S. O. (1991): *Tahuantinsuyoa chipi*, a new species of Cichlid fish from the Rio Pachitea drainage in Peru. – Cybium, **15**: 3–13.

Kullander, S. O. (1998): A phylogeny and classification of the South American Cichlidae (Teleostei: Perciformes). In: Malabarba, L. R.; Reis, R. E.; Vari, R. P.; Lucena, Z. M. S.; Lucena, C. A. S. (eds.): Phylogeny and classification of Neotropical fishes, 461–498. – EDIPUCRS, Porto Alegre, Brazil.

Kullander, S. O. (2003): Family Cichlidae (Cichlids). In: Reis, R. E., S. O. Kullander & C. J. Ferraris, Jr. (eds.): Check List of the Freshwater Fishes of South America and Central America: 605–654. – EDIPUCRS, Porto Alegre, Brazil.

Kullander, S. O. & Nijssen, H. (1989): The Cichlids of Surinam. – Leiden. Brill, 256 pp.

Kullander, S. O. & Silfvergrip, A. M. C. (1991): Review of the South American cichlid genus *Mesonauta* Günther

(Teleostei, Cichlidae) with descriptions of two new species. – Revue Suisse de Zoologie, **98**: 407–448.

Lüling, K. H. (1972): *Aequidens rivulatus*. – Tatsachen & Informationen aus der Aquaristik, **6**, No. 19: 2–3.

Lüling, K. H. (1973): Die Laguna de Vegueta an der Küste Mittelperus und ihre Fische, insbesondere *Aequidens rivulatus* (Guenther 1859). – Zoologische Beiträge, Neue Folge, **19**: 93–108.

Marescalchi, O. (2005): Karyotype and mitochondrial 16S gene characterizations in seven South American Cichlasomatini species (Perciformes, Cichlidae). – Journal of Zoological Systematics & Evolutionary Research, **43**: 22–28.

Musilová, Z.; Říčan, O.; Janko, K. & Novák, J. (2008): Molecular phylogeny and biogeography of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae: Cichlasomatinae). – Molecular Phylogenetics and Evolution, **46**: 659–672.

Musilová, Z.; Říčan, O. & Novák, J. (2009): Phylogeny of the neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus. – Journal of Zoological Systematics and Evolutionary Research, **47**: 234–247.

Posada, D. (2008): jModelTest: Phylogenetic model averaging. – Molecular Biology and Evolution, **25**: 1253–1256.

Regan, C. T. (1905): A revision of the fishes of the South-American cichlid genera *Acara*, *Nannacara*, *Acaropsis*, and *Astronotus*. – Annals and Magazine of Natural History, Series 7, **15**: 329–347.

Regan, C. T. (1913): The fishes of the San Juan River, Colombia. – Annals and Magazine of Natural History, Series 8, **12**: 462–473.

Říčan, O.; Musilová, Z.; Muška, M. & Novák, J. (2005): Development of coloration patterns in neotropical cichlids (Teleostei: Cichlidae: Cichlasomatinae). – Folia Zoologica, **54**, Monograph 1: 1–46.

Sevilla, R. G.; Diez, A.; Noren, M.; Mouchel, O.; Jerome, M.; Verrez-Bagnis, V.; van Pelt, H.; Favre-Krey, L.; Krey, G. & Bautista, J.M. (2007): Primers and polymerase chain reaction conditions for DNA barcoding teleost fish based on the mitochondrial cytochrome b and nuclear rhodopsin genes. – Molecular Ecology Notes, **7**: 730–734.

Staeck, W. & Linke, H. (1985): Amerikanische Cichliden II: Große Buntbarsche. – Tetra Verlag. Melle. 165 pp.

Stawikowski, R. & Werner, U. (1998): Die Buntbarsche Amerikas. Band 1. – Ulmer, Stuttgart, 540 pp.

Werner, U. (1983): Silbersaumbuntbarsch, *Aequidens rivulatus*. – Das Aquarium, **17** (7): 355–360.

Wiley, E. O. (1978): The evolutionary species concept reconsidered. – Systematic Zoology, **27**: 17–26.

Musilová Z., Kalous L., Petrtýl M., Chaloupková P.

Phylogeography of the cichlid genus *Serranochromis* in the Angola headwaters region: evidence of the faunal contact between the Cuanza and Okavango-Zambezi systems

prepared for submission to *Journal of Biogeography*

Manuscript

To whom it may concern

I declare that Zuzana Musilova performed significant portion of work on our manusript:

Zuzana Musilová, Lukáš Kalous, Miloslav Petrtýl, Petra Chaloupková - **Phylogeography of the cichlid genus *Serranochromis* in the Angola headwaters region: evidence of the faunal contact between the Cuanza and Okavango-Zambezi systems.**

In behalf of co-authors,

In Praha, 6.1.2011 Lukáš Kalous

Phylogeography of the cichlid genus *Serranochromis* in the Angola headwaters region: evidence of the faunal contact between the Cuanza and Okavango-Zambezi systems

Zuzana Musilová[1;2;3], Lukáš Kalous[3], Miloslav Petrtýl[3], Petra Chaloupková [4]

1) Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AV ČR v.v.i., 277 21 Liběchov, Czech Republic, E-mail: zuzmus@gmail.com

2) Department of Zoology, Faculty of Sciences, Charles University in Prague, 128 44 Praha 2, Czech Republic

3) Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 165 21 Praha 6 – Suchdol, Czech Republic

4) Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, 165 21 Praha 6 – Suchdol, Czech Republic

Abstract:

Present study brings the first molecular work on cichlids from the headwaters region of the central Angola. The evidence of faunal exchance among upper Cuanza and upper Okavango was found by reconstruction of phylogeography of the cichlid genus *Serranochromis*. The similar result was further shown by another cichlid genus *Tilapia* and also by catfish genus *Clarias* from the Bie plateau. DNA sequences of CO1, NADH2, D-loop and S7 intron were used for the phylogeographic reconstruction. Genetic data of the Angolan serranochromine cichlids were joined to the available DNA sequences from the previous African cichlid studies and this significantly enlarged the cichlid sampling area in Africa. This study should be considered as a first step in a biodiversity survey in the poorly investigated region and, any therefore, any strongly supported conclusion can be hardly formulated about the distribution patterns observed here.

Keywords

Africa, cichlids, Kwanza, faunal exchange, fishes, CO1

Introduction:

The most important factors for the distribution of freshwater organisms are natural barriers such as oceans or mountain ranges that divide the individual river basins and also waterfalls that prevent the exchange of species between the upper and lower sections of the rivers (Balon, 1974; Balon & Stewart, 1983; Berra, 2001). These biogeographic barriers can be considered as the dominant factor in determining the composition of the regional ichthyofauna (Avise, 2000). Further, the geological events change the appearance of the river systems and have strong impact to the recent distribution patterns of the freshwater ichthyofauna. Many river systems, although recently separated, share significant portion of fauna due to their connection in past (Lévêque, 1997). Earth movements in the post-Miocene have caused greater separation of river basins throughout Africa (Haddon & McCarthy, 2005; Stankiewicz & de Wit, 2006) and promoted the speciation process. Because of these tectonic activities during the past 20 million years, African rivers are generally characterised by much more rapids and waterfalls than other rivers in the world. As a result, there are no bigger rivers with unimpeded access to the interior of the continent (Lévêque, 1997).

The river Cuanza (Kwanza) represents together with westward flowing rivers of the Angola distinct Freshwater ecoregion (Abell et al., 2008). This region covers a narrow coastal plain and a steep escarpment rising to an altitude of more than 1000 m (Hughes & Hughes, 1992) and belongs to the most poorly understood drainages in Africa (Thieme et al., 2005; Skelton, 2001). In contrast, upper Cuanza is considered as part of the Zambezian Headwaters ecoregion based on species relativeness to the Zambezi and Cunene basins (Abell et al., 2008; Trewavas, 1973), and also according to few old records (Nichols & Boulton, 1927; Flower, 1930).

The Bié Plateau represents the headwater region with tributaries belonging to the five important large river systems (Congo, Zambezi, Okavango, Cuanza and Cunene). Rivers with tributaries belonging to all aforementioned basins are found in a relatively short distance (ca 200 km) of the plateau area. The river systems of the Bié plateau are recently well separated, although the geological activity in this region is still considered to be high (Guiraud et al., 2010).

African cichlids can be considered as enormously studied group of fishes, with the research focus mainly on the lacustrine assemblages of the Malawi, Tanganjika and Victoria lakes (Strumbauer et al., 2010; Koblmuller et al., 2008; Genner et al., 2007), but also the genetic research on the riverine cichlids has been intensively running covering the major river

systems in sub-Saharan Africa (Markert et al., 2010; Katongo, 2005; Stiassny1991). In contrast, there is no record of cichlid fishes from the central Angola since 1975 due to the lack of any field work area caused by long term civil war in the whole area. Generally, the rivers of Angola belong among the least faunistically surveyed regions in the African continent and only few older studies regarding ichthyofauna are available (Poll, 1967; Trewavas, 1936, 1973; Roberts, 1975; Nichols & Boulton, 1927; Greenwood, 1984).

Serranochromine cichlids represent mainly riverine predators with mouthbreeding reproduction behaviour. They are largely distributed from South Africa up to the Congo basin in the north in rivers of both Atlantic and Indian Ocean river systems. The genus *Serranochromis* contains 10 valid species (Froese & Pauly, 2010), three of them were previously reported from Angola (Poll, 1965). Possible radiation of serranochromine cichlids is documented from a lake formation found in the Middle Zambezi river system during Pleistocene (Joyce et al, 2005).

Overview studies covering large regions with different level of fish exploration and using molecular methods could bring information about the true biodiversity richness. Unfortunately, all modern works up to now dealing with molecular genetics of the African riverine cichlids always miss the region of the Bié Plateau in the central Angola (see for example maps in Koblmueller et al., 2008; Joyce et al., 2005).

This study comprises the first molecular analyses including fishes from central Angola (the Bié Plateau) and the first molecular study on the Angolan cichlids at all. The main goal of this study is to perform molecular phylogeography of the three fishes genera living in Bié plateau region, central Angola. Followingly, this study brings evidences of the faunal exchange among river systems in poorly investigated region, where the contact zone of Cunene, Okavango and Cuanza river systems exists.

Material and methods:

Field collecting:

The material of the serranochromine cichlids was preferentially collected in the field during years 2006 to 2009 (see Supp. 1). Other samples of cichlid genus *Tilapia*, and catfish genus *Clarias* were also included in this study. The specimens were caught mainly by seine net, rod or hand net in both smaller creeks and larger rivers in the Bié Plateau, Angola. We collected samples from three river systems, i.e. Kubango (Okavango), Kwanza (Cuanza) and Kunene (Cunene), totally from seven localities (one locality could be represented by more collection sites; see fig. 1). All specimens were photographed, fin-clipped for DNA analyses

and selected voucher specimens were fixed in the formaldehyde and stored in the collection of University of Life Sciences Prague, Czech Republic under the numbers as mentioned in Suppl. 1.

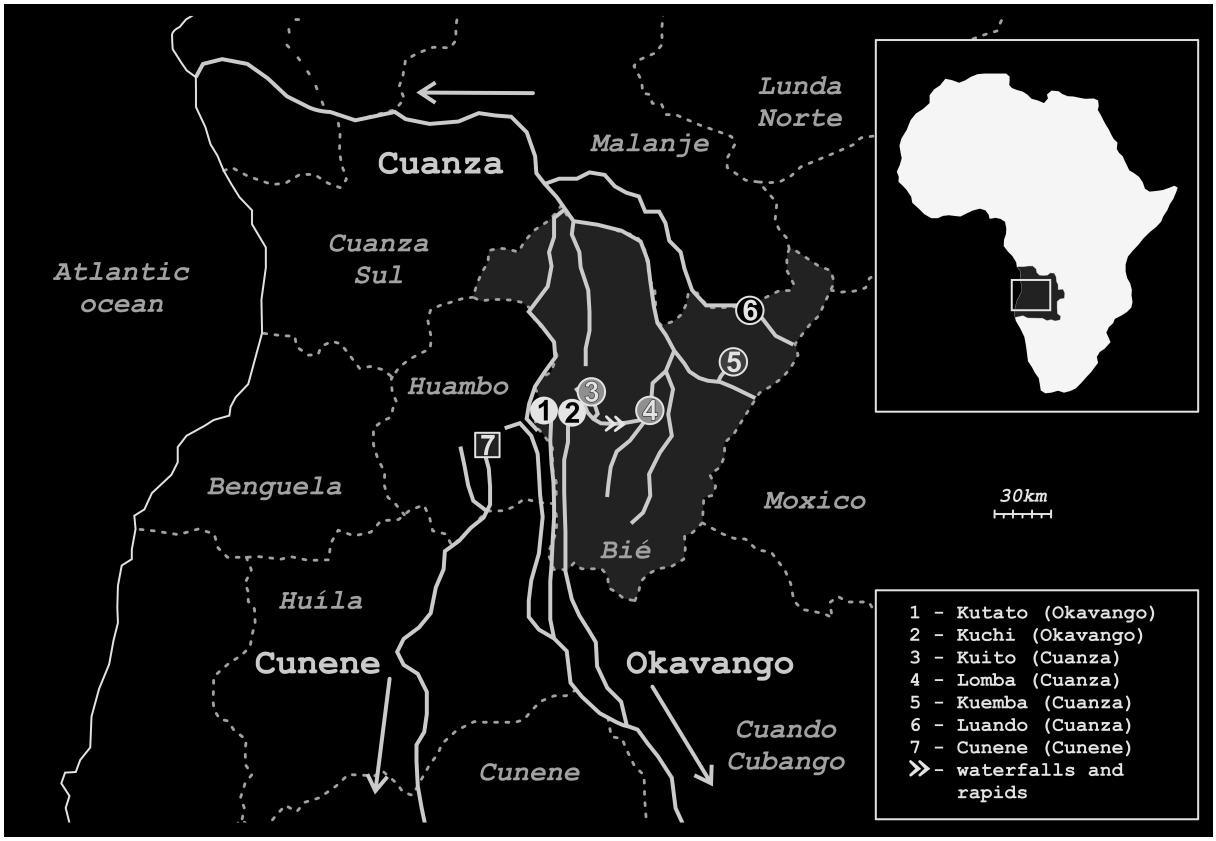


Fig. 1 – Sampling localities in the Bie province (highlighted by grey color) in the central Angola with the river systems scheme. Localities could represent more collection sites.

DNA analyses:

Four genes were amplified in this study for all serranochromine cichlids collected in Bié, Angola. Three of the studied markers were mitochondrial, CO1 (primers: forward - 5´-TCA ACC AAC CAC AAA GAC ATT GGC AC-3´ and reverse 5´-TAG ACT TCT GGG TGG CCA AAG AAT CA-3´, from Hubert et al., 2008), D-loop (primers: forward 5´-CCT ACT CCC AAA GCT AGG ATC-3´ and reverse 5´- TGC GGA GAC TTG CAT GTG TAA G -3´ from Joyce et al., 2005) and NADH2 (primers: forward 5´-CTA CCT GAA GAG ATC AAA A-3´ and reverse 5´-CGC GTT TAG CTG TTA ACT AA-3´ from Kocher, 1995) and one was nuclear, i.e. S7 first intron (primers: forward 5´-TGG CCT CTT CCT TGG CCG TC-3´ and reverse 5´-AAC TCG TCT GGC TTT TCG CC-3´ from Chow & Hazama, 1998).

Additionally, the genes of CO1 and S7 were amplified for the samples of *Tilapia* and *Clarias* using the aforementioned primers.

DNA was extracted from small pieces of muscle or gill (10–25 mg) using the DNeasy™ Tissue Kit (Qiagen, Valencia, CA, USA). PCR condition consisted of an initial denaturation step of 94°C (2 min), followed by 36 cycles of denaturation at 94°C for 1 min, annealing at 50°C (D-loop), 52°C (CO1), 55°C (NADH2) or 59°C (S7 intron) for 1 min and extension at 72°C for 1 min. The terminal extension was at 72°C for 10 min. PCR products were then purified by QIAquick PCR Purification Kit (Qiagen), and directly sequenced with the PCR primers using the BigDye® Terminator Cycle Sequencing Kit v.1.1 (Applied Biosystems, Foster City, CA, USA) following manufacturer´s protocol. Sequencing reaction products were cleaned with DyeEx 2.0 Spin Kit (Qiagen), and run on ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Alternatively, we used Macrogen service in South Korea (www.macrogen.com), where the unpurified PCR products were sent by mail. Chromatograms were assembled and checked by eye for potential mistakes and edited sequences were aligned using Clustal W as implemented in BioEdit software package (Hall 1999). All obtained sequences were submitted to GenBank (Accession Nos XXX; to completed after submission). See Suppl. 1 with the list of all samples and localities.

Phylogenetic analyses:

We prepaired two sequence data sets of the serranochromine cichlids: 1) the first data set represents all specimens collected in Angola and was based on the four genes used in this study; 2) the second data set includes also sequences of the genus *Serranochromis* (and relative genera) from the previous studies (Katongo et al., 2007; Joyce et al., 2005; Verheyen et al, 2003) downloaded from GenBank and is based on D-loop sequences.

Sequences of the mitochondrial CO1 gene for *Tilapia* and *Clarias* present the two additional separated data sets analyzed by phylogenetic methods.

Phylogenetic analyses of the nuclear and mitochondrial sequence data were performed using Bayesian Inference as implemented in MrBayes 3.0 (Huelsenbeck & Ronquist, 2001). The best-fit model for each gene separately was selected by jModeltest (Posada, 2008) using the Bayesian information criterion. Bayesian analysis was performed using two independent runs of four Metropolis-coupled chains (MCMC) of five million generations each, to estimate the posterior probability distribution. The combined sequence matrices were partitioned per gene fragment, and independent model parameters were estimated for each partition. Tree topologies were sampled every 100 generations, and majority-rule consensus trees were

estimated after discarding the first 30% generations. Robustness of clades was assessed using Bayesian posterior probabilities.

Tests of alternative topologies:

We performed statistical tests of alternative topologies in the serranochromine cichlids using Likelihood Ratio Test (LRT) to statistically test the significance of our results against the hypothetic bigeographic scenario of the river systems separation. We tested observed topology with the topologies constructed under the constraints where a) all samples collected in the Cuquema river system were forced into one clade, b) all samples belonging to the Okavango river system were forced to be together and c) both Cuquema and Okavango samples constrainted into monophyletic clades. All the phylogenetic analyses (both constrainted and unconstrainted) were performed in MrBayes under the aforementioned conditions and using command „constraint".

Results:

We sequenced 41 specimens belonging to four species of serranochromine cichlids, 22 specimens of *Tilapia sparrmanii* and 9 specimens of *Clarias theodorae* from central Angola. We reconstructed phylogenetic relationships of the serranochromine cichlids sampled in Angola (Fig. 3). Further, we combined newly obtained sequences with the previous analyses in the haplochromine cichlids (Katongo et al., 2007; Joyce et al., 2005; Verheyen et al., 2003) and we reconstructed the phylogenetic relations to check the position of the Angolan samples within the other serranochromines (Fig. 2).

Based on the larger phylogeny results including other haplochromines from the GenBank source, we found that Angolan serranochromine cichlids used in this study belong to four monophyletic clades corresponding to species. See Fig. 2 for the phylogenetic position of the clades within the resulted tree. We found separate lineage of *Serranochromis macrocephalus* from the upper Cuanza and the upper Okavango, which represent monophyletic clade among other serranochromine fishes.

Further, we reconstructed the phylogenetic tree based on CO1 gene and S7 intron (*Tilapia* only) in other fish of genera *Tilapia* and *Clarias*. In both genera, the observed distribution pattern did not correspond to the recent river system separation and it shows the evidence of ichtyofauna communication.

The phylogeography of the species *Serranochromis macrocephalus* shows the pattern also corresponding to the recent river system separation, similarly to *Tilapia* and *Clarias*

results. We thus performed the test of the alternative topologies where the both Cuanza and Okavango specimens were constrainted each into monophyletic clades. These three alternative topologies (i. e. with Okavango monophyletic, Cuanza monophyletic and both river systems monophyletic) were rejected by Likelihood Ratio test in all cases (not shown).

Based on the observed pattern in all three tested genera, evidence of the faunal exchange was found between neighbouring (and geographically very close) tributaries of Okavango and Cuanza river systems. Fig. 4 shows in detail the observed distribution pattern of *S. macrocephalus* and the potential hypothesis of the waterfall as a barrier for the species dispersal.

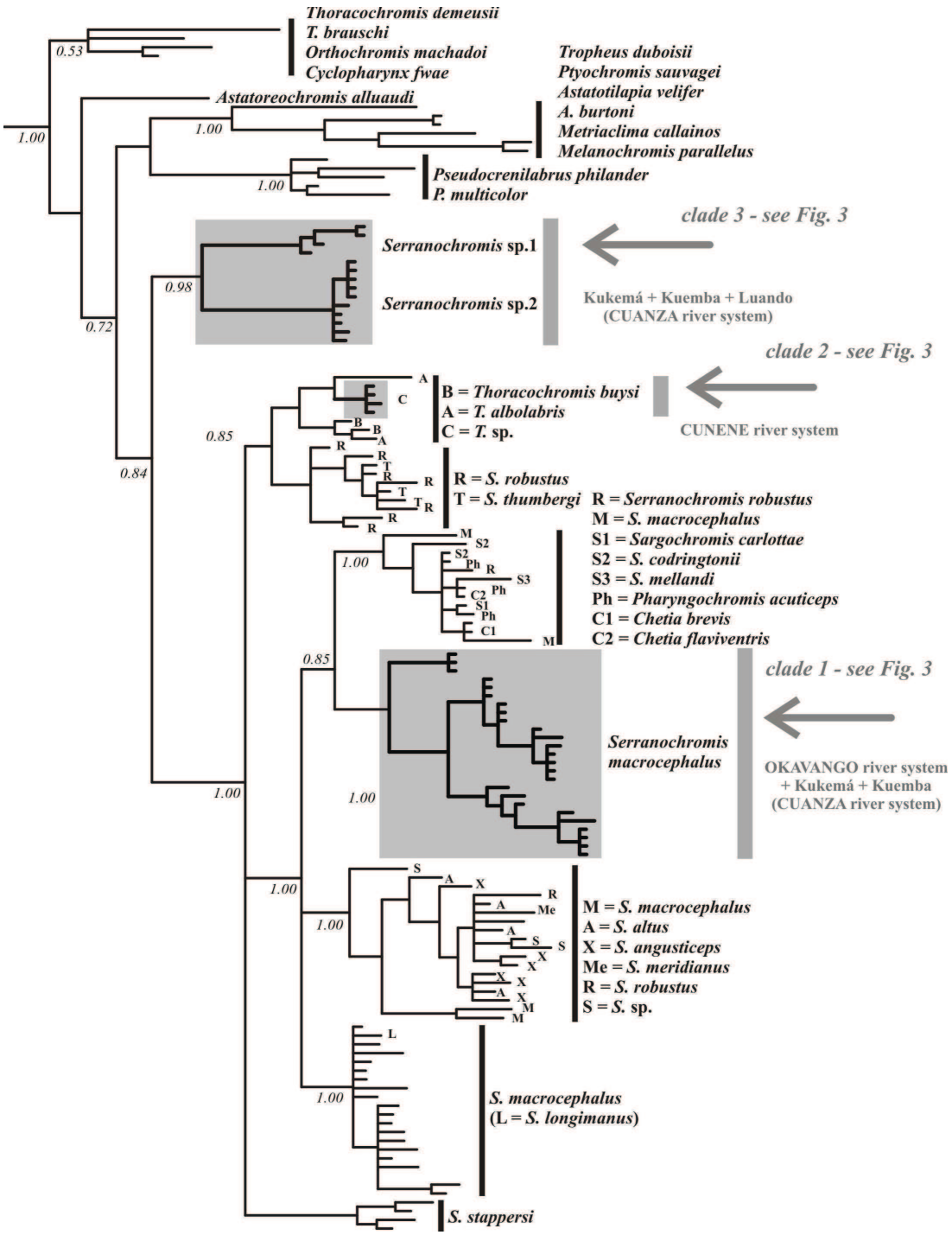


Fig. 2 – Bayesian phylogenetic tree based on the sequences of mitochondrial D-loop. The angolan cichlids joined to the sequence data from the previous studies on cichlids (available on GenBank). The grey squares highlighting the clades mark original samples of the seranochromine cichlids collected for this study in the Bié province, Angola. The rest of sequences originate from other studies dealing with the African cichlids. MrBayes run of five million generations. The letter M marks the position of *S. macrocephalus* from GenBank in the tree. The arrow shows three clades of our samples clustering among the rest of GenBank samples.

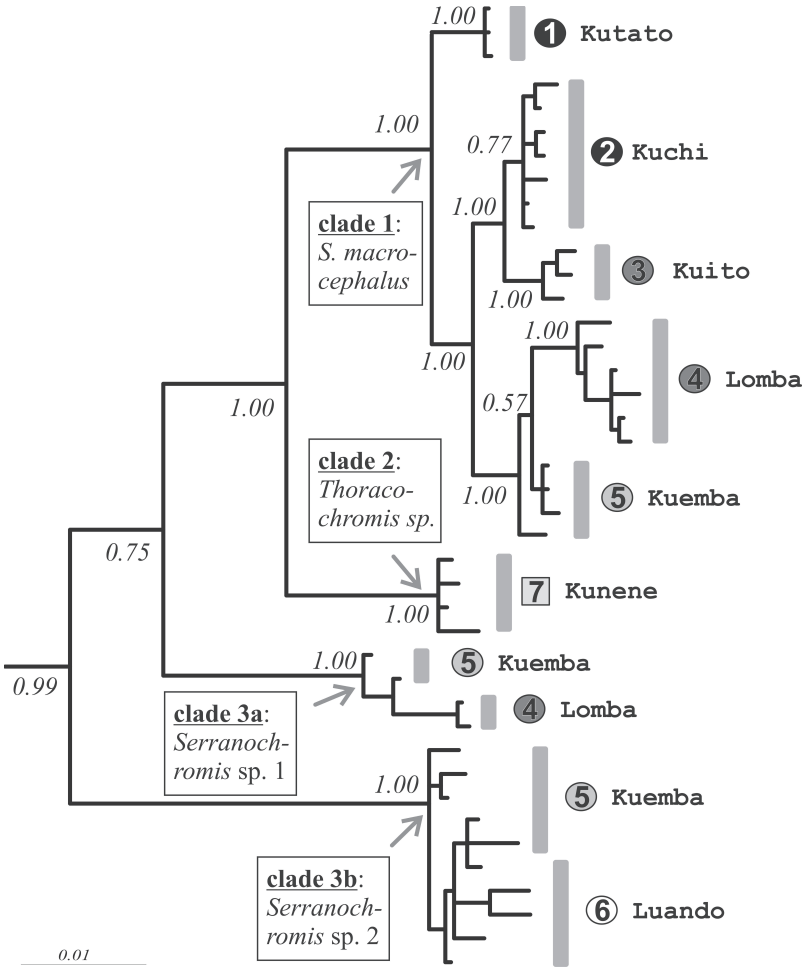


Fig. 3 – Phylogenetic tree of serranochromine cichlids collected in the central Angola. The tree is based on the sequences of the four genes (d-loop, NADH2, COI and the first intron in S7). MrBayes run for 5 million generations.

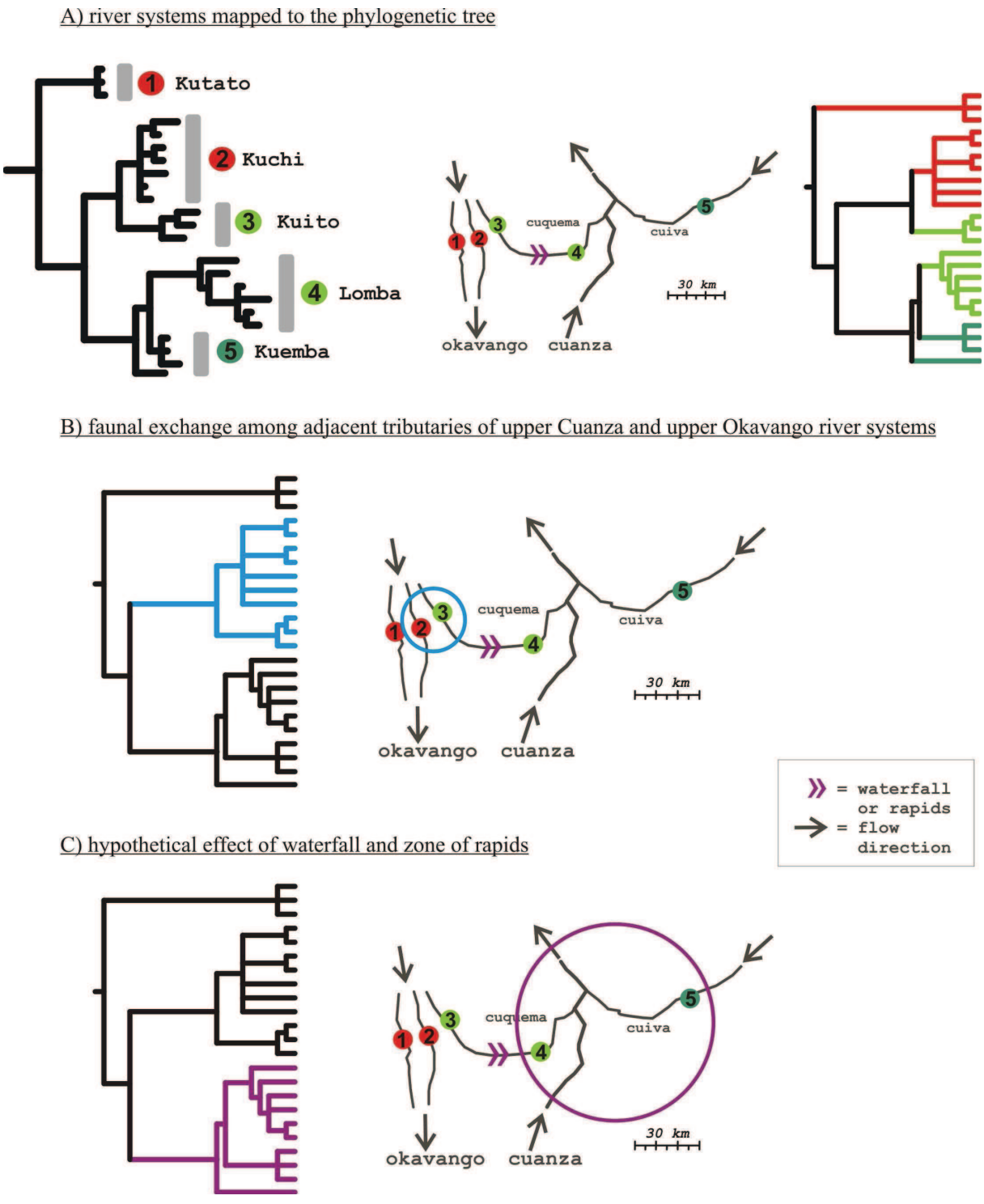


Fig. 4 – Biogeographic scheme of the observed pattern in cichlid species *Serranochromis macrocephalus*. Clade extracted from the Bayesian tree, see Fig. 3.

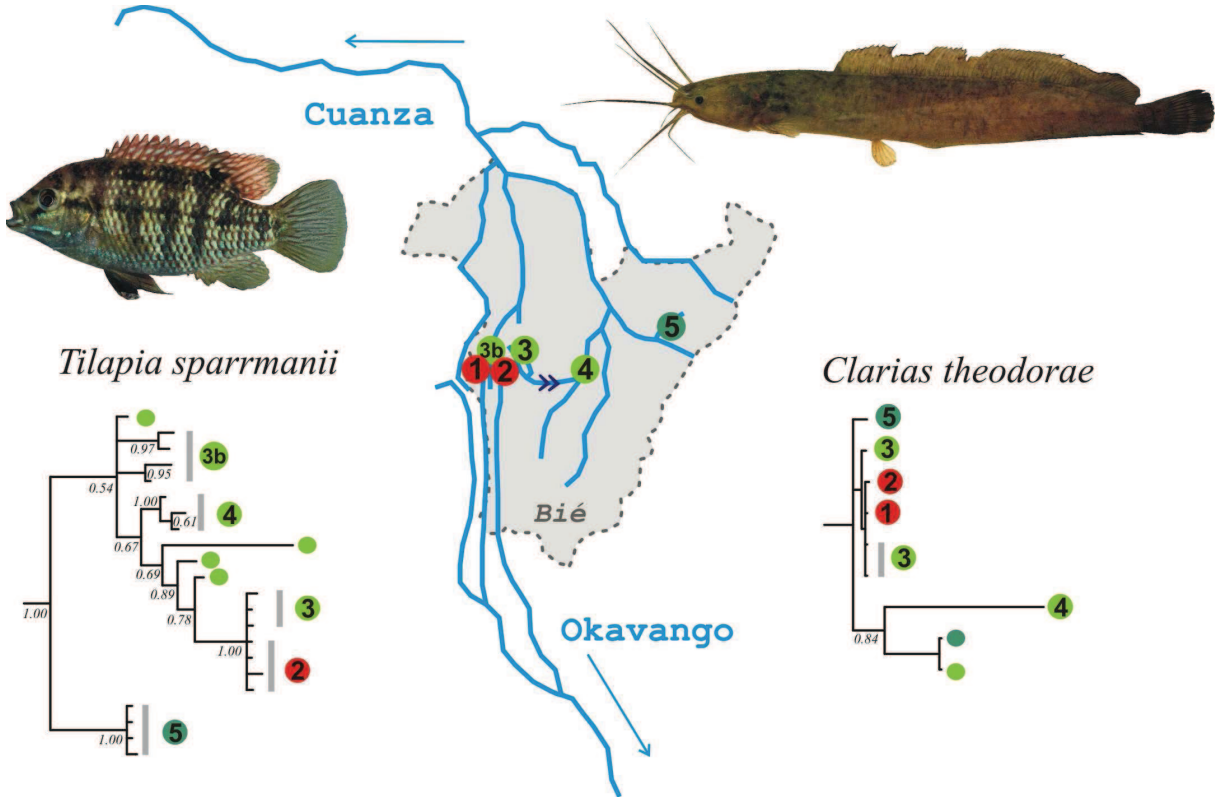


Fig. 5 – Evidence of faunal contacts at other fish species from the Bié province, the cichlid *Tilapia sparrmanii* and the catfish *Clarias theodorae*.

Discussion:

The material of the Angolan serranochromine cichlids as well as the cichlid genus *Tilapia* and catfish genus *Clarias* were genetically analyzed using molecular phylogenetics approach.

The material presented in this study is highly unique, as since 1975 no ichthyological collectings could occure throughout Angola (except of the coastal regions) and up to now no study dealing with African cichlids (Katongo et al., 2007; Joyce et al., 2005; Verheyen et al., 2003) included samples from the central Angola. Our study hence significantly enlarges the sampling area of the freshwater fishes groups in Africa.

We found that the Angolan samples of *Serranochromis macrocephalus* represent the separate lineage among the other serranochromines analysed in the previous studies (Katongo et al., 2007; Joyce et al., 2005). On the other hand, sequences of the same species *S. macrocephalus* from Zambia (Katongo et al., 2007) seem nor to be most relative to the Angolan clade neither to form its own monophyletic clade when analysed together with more serranochromines. Based on the D-loop analysis, the sequences of these Zambian specimens

are placed in several clades of related species and also genera. There is no more sequenced genes available to check independently the observed pattern. We cannot make any conclusion about the GenBank sequences herein, however, there is evidence about huge sharing of mitochondrial DNA among serranochromines due to their rapid evolution (Joyce et al., 2005). There is evidence of the large Pleistocene lake (palaeo-Makgadikgadi) present in the recent middle Zambezi, and the serranochromine cichlids are thought to undergo the radiation there (Joyce et al., 2005).

We found the evidence of faunal exchange between the two geographicaly adjacent rivers belonging to the different river systems, i.e. river Cuchi (Okavango basin) and river Cuquema (Cuanza basin). The same pattern was observed in *Serranochromis*, as well as in *Tilapia* and *Clarias*. Although the river systems in the Bié plateau are recently well separated, the geological activity in this region is still relatively extraordinary (Guiraud et al., 2010). Further, the plateau landscape does not provide effective barrier, especially in the periodical high water levels during the geological time (Hubert & Renno, 2006). Similar study on freshwater fishes of the Guyana plateau in South America shows the evidence of repeated faunal contacts between the adjacent river systems there (Hubert & Renno, 2006).

The human impact to the distribution pattern is not very probable, as the area is not very populated and no manipulation with these fish was noticed. There have not been any aquaculture activities since now although fishing activities are common along larger rivers (Hughes & Hughes, 1992; Kalous, 2009).

The phylogeographic pattern in *Serranochromis macrocephalus* could be also interpreted as effected by some barrier for distribution. There is a waterfall and a rapid zone present in the river Cuquema (Cuanza system) and fish from up and down of this zone belong to the different clades, i.e. they were closely related to the fish from more distant localities from different sub-system (river Cuiva/Cuanza). In contrast, this barrier had no effect in case of *Tilapia sparrmanii*. Recently, ongoing research in Congo river fishes showed, that the rapids form a effective distribution barrier strongly influencing the observed genetic pattern (Markert et al., 2010). However, more intensive studies of the Bie plateau region is necessary to test the rapids effect to the fauna distribution as our data set is to low to uncover it. The diversity of this region is still almost unknown, and any strongly supported conclusion can be hardly formulated about the distribution patterns observed here untill the knowledge improve.

Conclusions:

Present study brings the first molecular work on the Angolan fishes. The evidence of the faunal exchange among the upper Cuanza and the upper Okavango was found by the phylogeography reconstruction of the cichlid genus *Serranochromis*. The similar result was further shown by the another cichlid genus *Tilapia* and by the catfish genus *Clarias* from the Bie plateau. We also joined the genetic data of the Angolan serranochromine cichlids to the available DNA sequences from the previous African cichlid studies and this significantly enlarged the sampling area of cichlids in Africa. This study should be considered as a first step in the biodiversity survey in the poorly investigated region and no strong conclusion can be yet formulated about the distribution patterns in fishes of central Angola.

Acknowledgement:

We would like to thank to Vladimír de Abreu Lima, Hynek Ciboch, Zdeňka Horajsová, Blába, Honza, Martin Lošťák, the Czech Embassy in Luanda and Angolan students from Kuito for their help in the field. We thank to Robert Tropek for the comment improving the manuscript. Part of this work was carried out by using the resources of the Computational Biology Service Unit from Cornell University which is partially funded by the Microsoft Corporation. The work was supported by the Official Development Cooperation Programme of the Czech Republic specifically by the project "Poradenství v oblasti chovu ryb a drubeže, Angola" No. MZe/B/2. Further support was realised within the Centre for Biodiversity Research LC06073 (MSMT), IRP IAPG AVOZ50450515, GAUK 129510 and IRP FAPPZ, CZU MŠMT 6046070901. The fieldwork was performed under the permit of the Provincial Government of the province Bié.

Supporting information:

Supp. 1 - List of all samples and collection locality information

Supp. 2 - Images of the studied cichlid fishes (*Serranochromis macrocephalus*, *Thoracochromis* sp. and *Serranochromis* sp.1 + *S.* sp.2)

Biosketches:

Zuzana Musilová is a PhD. candidate at the Charles University in Prague, Czech Republic perfoming her studies mainly in the Laboratory of Fish Genetics, the Czech Academy of Sciences. She is interested in molecular genetics and biogeography of fishes, with special focus on cichlids.

Lukáš Kalous is an assitant professor of the Department of Zoology and Fisheries at the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague. His research is focused on ichtyology and aquaculture in tropics and subtropics and also the impact of human activities on the water environment and fishes.

Miloslav Petrtýl currently works as an assistant of the Department of Zoology and Fisheries at the Faculty Agrobiology, Food and Natural Resources of the Czech University of Life Sciences Prague. His research is focused on ichthyology, freshwater aquaculture, hydrobiology and involves the use of digital photography in biology, computer image analysis and geometric morphometrics.

Petra Chaloupková is a Vice Director for the International Relations, research and teaching staff at the Institute of Tropics and Subtropics, Czech University of Life Sciences Prague. She is interested in the project management and marketing.

References:

Abell, R., Thieme, M.L., Revenga, C., Bryer, M., Kottelat, M., Bogutskaya, N., Coad, B. Mandrak, N., Balderas, S.C., Bussing, W., Stiassny, M.L.J., Skelton, P., Allen, G.R., Unmack, P., Naseka, A., Ng, R., Sindorf, N., Robertson, J., Armijo, E., Higgins, J.V., Heibel, T.J., Wikramanayake, E., Olson, D., Lopez, H.L., Reis, R.E., Lundberg, J.G., Perez, M.H.S. & Petry, P. (2008) Freshwater ecoregions of the world: A new map of biogeographic units for freshwater biodiversity conservation. *Bioscience*, **58,** 403-414.

Avise, J.C. (2000) *Phylogeography: The History and Formation of Species*. Cambridge, MA: Harvard University Press.

Balon, E.K. (1974) Fishes from the edge of Victoria Falls: demise of a physical barrier for downstream invasions. *Copeia*, **1974**, 643-660.

Balon, E.K. & Stewart, D.J. (1983) Fish assemblages in a river with unusual gradient (Luongo, Africa-Zaire system), reflections on river zonation, and description of another species. *Environmental Biology of Fishes*, **9,** 225-252.

Berra, T.M. (2001) *Freshwater Fish Distribution*. Academic Press.

Chow, S. & Hazama, K. (1998) Universal PCR primers for S7 ribosomal protein gene introns in fish. *Molecular Ecolology*, **7**, 1255-1256.

Froese, R. & Pauly, D. (2010) *FishBase***.** www.fishbase.org, version (09/2010).

Genner, M.J., Seehausen, O., Lunt, D.H., Joyce, D.A., Shaw, P.W., Carvalho, G.R. & Turner G.F. 2007 Age of cichlids: New dates for ancient lake fish radiations. *Molecular Phylogenetics and Evolution*, **24,** 1269-1282.

Greenwood, P.H. (1984). The haplochmine species (Teleostei, Cichlidae) of the Cunene and certain other Angolan rivers. *Bulletin of the British Museum (Natural History)*, **47**, 187-239.

Guiraud, M., Buta-Neto, A. & Quesne, D. (2010) Segmentation and differential post-rift uplift at the Angola margin as recorded by the transform-rifted Benguela and oblique-to-orthogonal-rifted Kwanza basins. *Marine and Petroleum Geology*, **27,** 1040-1068.

Haddon, I.G. & McCarthy, T.S. (2005) The Mesozoic-Cenozoic interior sag basins of Central Africa: The Late-Cretaceous-Cenozoic Kalahari and Okavango basins. *Journal of African Earth Sciences*, **43**, 316-333.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, **41**, 95-98.

Hubert, N. & Renno, J.F. (2006) Historical biogeography of South American freshwater fishes. *Journal of Biogeography*, **33,** 1414-1436.

Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J.B., April, J. & Bernatchez, L. (2008) Identifying Canadian Freshwater Fishes through DNA Barcodes. *PLoS ONE*, **3,** e2490.

Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.

Hughes, R.H. & Hughes, J.S. (1992) *A Directory of African Wetlands*. Gland, Switzerland & Cambridge, UK: IUCN/Nairobi, Kenya: UNEP/Cambridge, UK: WCMC.

Joyce, D.A., Lunt, D.H., Bills, R., Turner, G.F., Katongo, C., Duftner, N., Sturmbauer, C. & Seehausen. O. (2005) An extant cichlid fish radiation emerged in an extinct Pleistocene lake. *Nature,* **435,** 90-95.

Kalous, L. (2009) Chov ryb v provincii Bié: zkušenosti a perspektiva. *Twapandula* (ed by Holíková, P., Kalous, L., Petrtýl, M. and Trefil, P.) pp. 39-43 Czech University of Life Sciences Prague, Prague.

Katongo, C., Koblmuller, S., Duftner, N., Makasa, L. & Sturmbauer, C. (2005) Phylogeography and speciation in the Pseudocrenilabrus philander species complex in Zambian Rivers. *Hydrobiologia*, **542,** 221-233.

Katongo, C., Koblmuller, S., Duftner, N., Mumba, L. & Sturmbauer, C. (2007) Evolutionary history and biogeographic affinities of the serranochromine cichlids in Zambian rivers. *Molecular Phylogenetics and Evolution*, **45,** 326-338.

Koblmuller, S., Schliewen, U.K., Duftner, N., Sefc, K.M., Katongo C. & Sturmbauer, C. (2008) Age and spread of the haplochromine cichlid fishes in Africa. *Molecular Phylogenetics and Evolution*, **49,** 153-169.

Kocher, T.D., Conroy, J.A., McKaye, K.R., Stauffer, J.R., Lockwood, S.F. (1995) Evolution of NADH dehydrogenase in East African cichlid fish. *Molecular Phylogenetics and Evolution*, **4**, 420-432.

Lévêque, C. (1997) *Biodiversity dynamics and conservation. The freshwater fishes of tropical Africa*. Cambridge: Cambridge University Press, Inc.

Markert, J.A., Schelly, R.C. & Stiassny, M.L.J. (2010) Genetic isolation and morphological divergence mediated by high-energy rapids in two cichlid genera from the lower Congo rapids. *BMC Evolutionary Biology*, **10,** 149.

Nichols, J.T. & Boulton, R. (1927) Three new minnows of the genus Barbus, and a new characin from the Vernay Angola Expedition. *American Museum Novitates,* **264,** 1-8.

Poll, M. (1967) Contribution à la faune ichthyologique de l´Angola. *Lisbon: Diamang Publicações Culturais*, **75**.

Posada, D. (2008) jModelTest: Phylogenetic Model Averaging. *Molecular Phylogenetics and Evolution,* **25,** 1253-1256.

Roberts, T.R. (1975) Geographical distribution of African freshwater fishes. *Zoological Journal of the Linnean Society*, **57**, 249-319.

Skelton, P. (2001) *A complete guide to the freshwater fishes of southern Africa, 2nd Edition.* Cape Town, South Africa: Struik Publishers.

Stankiewicz, J. & de Wit, M.J. (2006) A proposed drainage evolution model for Central Africa - Did the Congo flow east? *Journal of African Earth Sciences*, **44,** 75-84.

Stiassny, M.L.J. (1991) Phylogenetic intrarelationships of the family Cichlidae: An overview. *Cichlid Fishes: Behaviour, Ecology and Evolution* (ed. By Keenleyside, M.H.E.), pp. 1-35. Croom-Helm, London,

Sturmbauer, C., Salzburger, W., Duftner, N., Schelly, R. & Koblmuller, S. (2010) Evolutionary history of the Lake Tanganyika cichlid tribe Lamprologini (Teleostei: Perciformes) derived from mitochondrial and nuclear DNA data. *Molecular Phylogenetics and Evolution*, **57**, 266-284.

Thieme, M.L., Abell, R., et al. (2005) *Freshwater Ecoregions of Africa and Madagascar: A Conservation Assessment*. Washington, D.C., USA: Island Press.

Trewavas, E. (1936) Dr. Karl Jordan's expedition to South-West Africa and Angola: The freshwater fishes. *Novitates Zoologicae*, **40,** 63-74.

Trewavas, E. (1973) A new species of cichlid fish of rivers Quanza and Bengo, Angola, with a list of the known Cichlidae of these rivers and a note on Pseudocrenilabrus natalensis" Fowler. *Bulletin of the British Museum (Zoology)*, **25**, 27-38.

Verheyen, E., Salzburger, W., Snoeks, J. & Meyer, A. (2003) Origin of the superflock of cichlid fishes from Lake Victoria, East Africa. *Science*, **300,** 325-329.

Supplementary material:

Supp. 1 - List of all samples and collection locality information

Sample No.	species	collection date	locality No.	locality name	system	GPS
C70	*Serranochromis macrocephalus*	30.10.2007	1	Rio Cutato, mainroad Kuito-Huambo	Okavango	S 12 34 13.6 E 016 29 30.5
C71	*Serranochromis macrocephalus*	30.10.2007	1	Rio Cutato, mainroad Kuito-Huambo	Okavango	S 12 34 13.6 E 016 29 30.5
C74	*Serranochromis macrocephalus*	30.10.2007	1	Rio Cutato, mainroad Kuito-Huambo	Okavango	S 12 34 13.6 E 016 29 30.5
K01	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K03	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K05	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K07	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K16	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K21	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K28	*Serranochromis macrocephalus*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
B51	*Serranochromis macrocephalus*	22.10.2007	3	Kuito env., Rio Cuquema	Cuanza	S12 28 14.9 E16 49 26.7
Z05	*Serranochromis macrocephalus*	16.10.2008	3	Kuito env., Rio Cuquema	Cuanza	S12 28 14.9 E16 49 26.7
Z09	*Serranochromis macrocephalus*	17.10.2008	3	Kuito env., Rio Cuquema	Cuanza	S12 28 14.9 E16 49 26.7
C05	*Serranochromis macrocephalus*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C11	*Serranochromis macrocephalus*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C16	*Serranochromis macrocephalus*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C17	*Serranochromis macrocephalus*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C27	*Serranochromis macrocephalus*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C31	*Serranochromis macrocephalus*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C50	*Serranochromis sp. 2*	31.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
C52	*Serranochromis sp. 2*	31.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
V33	*Serranochromis macrocephalus*	6.5.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
V35	*Serranochromis macrocephalus*	6.5.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z52	*Serranochromis sp. 1*	24.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z54	*Serranochromis sp. 1*	24.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z55	*Serranochromis sp. 1*	24.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z56	*Serranochromis sp. 1*	24.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z80_1	*Serranochromis macrocephalus*	february 2009	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z80_2	*Serranochromis macrocephalus*	february 2009	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Cu03	*Serranochromis sp. 2*	february 2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Cu05	*Serranochromis sp. 2*	february 2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Cu06	*Serranochromis sp. 1*	february 2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z35	*Serranochromis sp. 1*	22.10.2008	6	Luando village, Rio Luando	Cuanza	S11 35 33.4 E18 28 10.3
Z36	*Serranochromis sp. 1*	22.10.2008	6	Luando village, Rio Luando	Cuanza	S11 35 33.4 E18 28 10.3
Z37	*Serranochromis sp. 1*	22.10.2008	6	Luando village, Rio Luando	Cuanza	S11 35 33.4 E18 28 10.3
Z38	*Serranochromis sp. 1*	22.10.2008	6	Luando village, Rio Luando	Cuanza	S11 35 33.4 E18 28 10.3
Z39	*Serranochromis sp. 1*	22.10.2008	6	Luando village, Rio Luando	Cuanza	S11 35 33.4 E18 28 10.3
Z19	*Thoracochromis sp.*	19.10.2008	7	Rio Cunene, mainroad Kuito-Huambo	Cunene	S12 45 40.9 E15 47 22.2
Z20	*Thoracochromis sp.*	19.10.2008	7	Rio Cunene, mainroad Kuito-Huambo	Cunene	S12 45 40.9 E15 47 22.2
Z21	*Thoracochromis sp.*	19.10.2008	7	Rio Cunene, mainroad Kuito-Huambo	Cunene	S12 45 40.9 E15 47 22.2
Z23	*Thoracochromis sp.*	19.10.2008	7	Rio Cunene, mainroad Kuito-Huambo	Cunene	S12 45 40.9 E15 47 22.2
K159	*Tilapia sparrmanii*	15.10.2008	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
K160	*Tilapia sparrmanii*	15.10.2008	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 59
Z02	*Tilapia sparrmanii*	16.10.2008	3	Kuito env., Rio Cuquema	Cuanza	S12 26.559 E16 54.385
Z08	*Tilapia sparrmanii*	16.10.2008	4	Kuito env., Rio Cuquema	Cuanza	S12 28 14.9 E16 49 26.7
Z10	*Tilapia sparrmanii*	18.10.2008	5	Kuito env., Rio Cuquema	Cuanza	S12 26.559 E16 54.385
Z11	*Tilapia sparrmanii*	19.10.2008	3b	Chinguar village, mainroad Kuito-Huambo	Cuanza	S12 33 26.4 E16 18 45.9
Z12	*Tilapia sparrmanii*	19.10.2008	3b	Chinguar village, mainroad Kuito-Huambo	Cuanza	S12 33 26.4 E16 18 45.9
Z13	*Tilapia sparrmanii*	19.10.2008	3b	Chinguar village, mainroad Kuito-Huambo	Cuanza	S12 33 26.4 E16 18 45.9
Z14	*Tilapia sparrmanii*	19.10.2008	3b	Chinguar village, mainroad Kuito-Huambo	Cuanza	S12 33 26.4 E16 18 45.9
Z51	*Tilapia sparrmanii*	24.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
Z60	*Tilapia sparrmanii*	24.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 32.1 E18 05 48.7
N8	*Tilapia sparrmanii*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
K09	*Tilapia sparrmanii*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K10	*Tilapia sparrmanii*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K17	*Tilapia sparrmanii*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
K18	*Tilapia sparrmanii*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
Til8	*Tilapia sparrmanii*	May, 2007	3	Kuito env., Rio Cuquema	Cuanza	S12 25.542 E16 49.113
Cu01	*Tilapia sparrmanii*	May, 2008	5	dried specimen from Kuemba village	Cuanza	-
Cu02	*Tilapia sparrmanii*	May, 2008	5	dried specimen from Kuemba village	Cuanza	-
Z87	*Tilapia sparrmanii*	October, 2008	?	dried specimen from Kuito village	Cuanza	-
Kw02	*Tilapia sparrmanii*	October, 2008	?	dried specimen from Kuito village	Cuanza	-
Kw06	*Tilapia sparrmanii*	October, 2008	?	dried specimen from Kuito village	Cuanza	-
M01	*Clarias theodorae*	16.10.2008	3	Kuito env., Rio Cuquema	Cuanza	S12 26.559 E16 54.385
M02	*Clarias theodorae*	16.10.2008	3	Kuito env., Rio Cuquema	Cuanza	S12 26.559 E16 54.385
M47	*Clarias theodorae*	23.10.2008	5	Kuemba village, Rio Cuiva	Cuanza	S12 09 17.6 E18 05 59.2
M44	*Clarias theodorae*	24.10.2008	?	dried specimen from Kuemba village	Cuanza	-
M46	*Clarias theodorae*	30.10.2008	?	dried specimen from Kuito village	Cuanza	-
B21	*Clarias theodorae*	18.10.2007	3	Nequilo village	Cuanza	S12 36.289 E17 04.139
C30	*Clarias theodorae*	25.10.2007	4	Lomba village, Rio Cuquema	Cuanza	S12 30 55.2 E017 25 58
K13	*Clarias theodorae*	1.11.2007	2	Rio Kuchi, mainroad Kuito-Huambo	Okavango	S12 31 52.2 E 016 41 46.1
C82	*Clarias theodorae*	30.10.2007	1	Rio Cutato, mainroad Kuito-Huambo	Okavango	S 12 34 13.6 E 016 29 30.5

Supp. 2 - Images of the studied cichlid fishes (*Serranochromis macrocephalus*, *Thoracochromis* sp. and *Serranochromis* sp.1 + *S.* sp.2)

a) *Serranochromis macrocephalus*

b) *Serranochromis* sp. 1

c) *Serranochromis* sp. 2

d) *Thoracochromis* sp.

Conclusion

This thesis presents the molecular phylogeny studies performed on the Neotropical and African cichlids. **1)** The original research focus started with the neotropical cichlid tribe Cichlasomatini, where the first comprehensive phylogeny was reconstructed based on three molecular markers (Musilová et al.,. 2008). This study defined the phylogenetic pattern and opened the question of the unstudied species group of *´Aequidens´*. **2)** Later, further focus was focused on the more detailed phylogenetic investigation of Cichlasomatini and more robust phylogeny was reconstructed including addition of the morphological characters. As a taxonomic result, the genus *Andinoacara* was described as a new name for *´Aequidens´* group (Musilová et al.,. 2009a). **3)** The ongoing research focused on the genus *Andinoacara* brought further results, when the new species *Andinoacara stalsbergi* was recognised both morphologically and genetically, and subsequently described (Musilová et al., 2009b). Naturally, more questions appeared during the more detailed discover of *Andinoacara* evolutionary history. As a next step, the comprehensive study on *Andinoacara* produces the **4)** phylogeography presented here as Musilová et al., (unpublished manuscript). Aditionally, the other syntopic and unrelated 'Heros' group was investigated for better results interpretation. Interesting biogeography patterns were found in that work including the ancestral area and colonisation events within the both genera. **5)** The possibility of joining the team working in Africa allowed to apply the skills in the poorly investigated region and the first phylogeography study of Angolan cichlids was the result (Musilová et al., in prep). Among the phylogenetic pattern, the evidence of faunal contacts between different river systems was found as a most interesting conclusion.

Appendices: The results of two different projects related to cichlid fishes are involved as Appendices. It is followed by the presentations of the preliminar/ongoing results in scientific congresses, and the popularisation works as the essential part of the PhD. study.

Appendix I.

Říčan O., Musilová Z., Muška M., Novák J.

2005

Development of coloration patterns in Neotropical cichlids (Teleostei : Cichlidae : Cichlasomatinae)

Folia Zoologica **54 (Monogr. 1): 1-46**

Scientific paper

this work was published as a voluminous monograph,
therefore it is presented just the first page and abstract here

International Journal of Vertebrate Zoology

FOLIA ZOOLOGICA

ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

DEVELOPMENT OF COLORATION PATTERNS IN NEOTROPICAL CICHLIDS (TELEOSTEI: CICHLIDAE: CICHLASOMATINAE)

Oldřich ŘÍČAN, Zuzana MUSILOVÁ, Milan MUŠKA and Jindřich NOVÁK

54 **2005**

Monograph 1

INSTITUTE OF VERTEBRATE BIOLOGY

MONOGRAPHS

Oldřich Říčan[1], Zuzana Musilová[2], Milan Muška1 and Jindřich Novák[1]

[1] *Department of Zoology, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic; e-mail: oldrichr@yahoo.com*

[2] *Department of Zoology, Faculty of Sciences, Charles University, Viničná 7, CZ-128 44 Praha 2, Czech Republic; e-mail: zuzka.musil@email.cz*

ŘÍČAN O., MUSILOVÁ Z., MUŠKA M. & NOVÁK J. 2005: Development of coloration patterns in Neotropical cichlids (Teleostei: Cichlidae: Cichlasomatinae). *Folia Zool. 54 (Monogr. 1): 46 pp.*

Abstract

We present a developmental study focusing on the development of coloration patterns in a subgroup of Neotropical cichlids, the subfamily Cichlasomatinae. Based on the presented coloration ontogenetic series of 40 species we show that developmental information is a necessary prerequisite for any serious attempts in understanding adult coloration patterns. The center of our contribution is a detailed description of coloration ontogenies in a selected sample of cichlids and their discussion in a much wider taxonomical sampling. The pigmentation pattern ontogeny is specifically used to determine developmental homology of individual vertical bars. Early ontogeny is documented from the onset of the free-swimming period, which is also used as a point of reference for possible heterochronic shifts as presented here. A single universal process is responsible for the transformation of longitudinal melanophore migration lines into vertical bars, which form the dominant elements of adult coloration of most cichlids. Adult vertical bars vary interspecificaly in their numbers, whereas their ontogenetic precursors are stable in number across all surveyed species. The diversity of adult barring patterns is produced by differential fusions of a conserved number of developing bars, from which the different taxon specific numbers of adult bars develop. The possibility of determining individual homology of cichlid vertical bars is a prerequisite for the use of coloration pattern characters in cichlid phylogenetic studies. Several ontogenetic characters are formulated as synapomorphic at various systematic levels.

Key words: ontogeny, color patterns, cichlidae, neotropics, phylogeny

Appendix II.

Krajáková L., Musilová Z., Kalous L.

2010

Cytogenetická analýza a evoluce karyotypu u jihoamerických cichlid tribu Cichlasomatini

Bulletin VÚRH Vodňany **46 (4): 13-21**

Scientific paper

CYTOGENETICKÁ ANALÝZA A EVOLUCE KARYOTYPU U JIHOAMERICKÝCH CICHLID TRIBU CICHLASOMATINI

CYTOGENETIC ANALYSIS AND KARYOTYPE EVOLUTION IN SOUTH AMERICAN CICHLIDS OF THE TRIBE CICHLASOMATINI

L. KRAJÁKOVÁ[1], Z. MUSILOVÁ[1,2], L. KALOUS[1]

[1]Česká zemědělská univerzita v Praze, Fakulta agrobiologie potravinových a přírodních zdrojů, Katedra zoologie a rybářství, 165 21 Praha 6 – Suchdol, krajakova@af.czu.cz

[2]Ústav živočišné fyziologie a genetiky AVČR v.v.i., Laboratoř genetiky ryb, 277 21 Liběchov

ABSTRACT

We present our cytogenetic data of South American cichlids (tribus Cichlasomatini) that were consequently consulted with phylogenetic analysis of other authors. Nuclei suspensions for this study were obtained by different methods of chromosome preparation, then dropped on slides and stained by the solution of Giemsa - Romanowski. Obtained metaphases were investigated by microscope and recorded by digital camera connected with image analysing software. Karyotypes were constructed only in samples with good metaphases and cytogenetic data (chromosome number, karyotype) and they were consequently applied to known phylogenetic tree. We concluded that common ancestor had 2n = 48 chromosomes. Change of chromosome number occurred at least in seven evolution events; six times the number of chromosome increased and once decreased. The increase of chromosome number was observed in group of Nannacara and Cleithracara (Cleithracara maronii 2n = 50). In the second clade of Bujurquina – Tahuantinsuyoa – Andinoacara – Laetacara – Acaronia we found a reduction of chromosome number (Bujurquina 2n = 44; 40, Laetacara 2n = 44; 38 and Tahuantinsuyoa 2n = 30). From available data we are still not able to formulate final hypothesis of karyotype evolution within the tribus Cichlasomatini. Anyway, present study is the first cytogenetic overview of the tribe.

Klíčová slova: *Cytogenetika, jihoamerické cichlidy, Cichlosomatini, evoluce karyotypu*
Keywords: *Cytogenetics, South American cichlids, Cichlosomatini, evolution of caryotype*

ÚVOD

Cytogenetická analýza karyotypu je dostupnou a relativně levnou metodou, jak získat informace o organizaci struktur dědičné informace, tedy o počtu a stavbě chromozomů v jádře buňky. Jedná se tak o signál, který molekulární genetika a sekvenace DNA nemůže odhalit, a proto může být vhodným doplňkem při studiu evoluce dané skupiny (Pisano a kol., 2007). Tyto informace je možné porovnat mezi jednotlivými druhy, přesto o jihoamerických cichlidách (tribus Cichlasomatini) není v literatuře mnoho cytogenetických informací. Konkrétně existují dvě cytogenetické studie: od autorů Thompson (1979) a Marescalchi (2005). Z těchto studií vyplývá, že variabilita chromozomového uspořádání je větší, než se předpokládalo. Studované rody byly původně považovány za uniformní v počtu chromozomů.

Ukazuje se, že zástupci rodů *Aequidens*, *Cichlasoma* a *Krobia* mají 48 chromozomů (2n = 48), což je doposud považováno za ancestrální stav. Naopak rody *Bujurquina* (2n = 44; 40), *Laetacara* (2n = 44; 38), *Nannacara* (2n = 44) a *Cleithracara* (2n = 50) se v počtech chromozomů liší.

Cílem této studie je popsat cytogenetické charakteristiky druhů jihoamerických cichlid, které dosud nebyly z tohoto pohledu zkoumány, a následnou fylogenetickou interpretací zároveň rozšířit znalosti o možném průběhu evoluce karyotypu v rámci tribu Cichlasomatini.

MATERIÁL A METODIKA

Do studie bylo zahrnuto 8 druhů z 6 různých zemí (1. *Andinoacara biseriatus* – Kolumbie, 2. *Andinoacara* cf. *pulcher* „Rio Chirgua" – Venezuela, 3. *Andinoacara rivulatus* – Ekvádor, 4. *Aequidens tubicen* – Peru, 5. *Cichlasoma amazonarum* – Brazílie, 6. *Cichlasoma boliviense* – Bolivie, 7. *Krobia* sp.„Xingu" – Brazílie, 8. *Tahuantinsuyoa macantzatza* – Peru. Většina ryb použitých v této studii pochází z původního místa jejich výskytu a byla zakoupena prostřednictvím specializovaných firem v České republice nebo v Německu. Všechny ryby byly chovány v akváriích na ČZU a PřF UK v Praze. Od každého druhu byli použiti dva jedinci ke stanovení počtu chromozomů, pro sestavení karyotypu pak byla vybrána jedna nejlepší metafáze.

K získání chromozomových preparátů byly použity tři metody, a to: 1) preparace chromozomů z regenerátu ploutve dle M. Völkera, 2) preparace chromozomů z embryí dle M. Völkera a 3) kultivace fibroblastů *in vitro*.

Metody preparace chromozomů z regenerátu ploutve dle M. Völkera a kultivace fibroblastů in vitro byly použity u všech vyjmenovaných druhů, metoda preparace chromozomů z embryí dle M. Völkera byla použita u druhů *Cichlasoma amazonarum* a *Cichlasoma boliviense*, které se přirozeně vytíraly v akváriích, k jednotlivým preparacím bylo použito postupně pět snůšek od každého druhu.

Metoda preparace chromozomů z regenerátu ploutve dle M. Völkera je založena na principu regenerace buněk v místě poškození po odstranění části tkáně, v tomto případě se jednalo o ocasní ploutev. Rybě byla odstřižena malá část ocasní ploutve (pásek o šířce cca 1 mm). Ocasní ploutev se nechala regenerovat 4–7 dní podle regenerační aktivity. Po uplynulém časovém úseku byl regenerát odstřižen a vložen na 2–3 hodiny do Petriho misky s kultivačním roztokem skládajícího se z 14,3 ml zásobního roztoku (7,48 g NaCl; 0,18 g KCl; 0,2 g $CaCl_2$; 0,016 g $NaHCO_3$ a 1000 ml destilované vody), 85,7 ml destilované vody a 0,025 g kolchicinu (Serva). Během této doby probíhalo dělení buněk, ale pouze do stadia metafáze, ve které kolchicin zastavuje buněčné dělení. Po té byly přidány 3–4 kapky fixačního roztoku (100% CH_3OH a 100% CH_3COOH, 3 : 1), který se nechal působit 30 minut v chladničce při 4 °C. V této fázi došlo k usmrcení všech buněk. Poté byl z Petriho misky veškerý roztok odstraněn a byl přidán pouze fixační roztok, který působil dalších 30 minut. Odstranění starého a přidání čerstvého fixačního roztoku bylo opakováno dvakrát. Následně byl regenerát přenesen na síťku a zakápnut 45–50 µl 20% kyseliny octové. Ta způsobila rozbití jaderných membrán a rozvolnění chromozomů, což je důležité pro následné pozorování na podložním sklíčku. Poté byla tkáň regenerátu jemně rozrušena o síťku pinzetou a z druhé strany síťky byla automatickou pipetou Nichirio 200 odsáta suspenze buněk do mikrozkumavky typu Eppendorf. Suspenze byla následně nanesena na podložní skla (Menzel GmbH) zahřátá na teplotu 45 °C na topné plotýnce. Suspenze byla na sklo nakápnuta a po 20 sekundách nasáta zpět. Tímto způsobem byly na jednom podložním skle vytvořeny tři kapky. Díky teplotě skel se vytvořil v místě kapky jemný film s buňkami. Skla se nechala vychladnout při pokojové teplotě v laboratoři.

Metoda preparace chromozomů z embryí, byla též optimalizována Völkerem (2006).

Do připraveného roztoku kolchicinu (Serva) (0,25 g a 50 ml vody z akvária) v Petriho miskách bylo umístěno po 3–4 embryích, které byly získány z přirozeného výtěru ryb v akváriu. Embrya byla takto kultivována 4 hodiny při pokojové teplotě. Během této doby probíhalo dělení buněk, ale pouze do stadia metafáze, ve které kolchicin zastavuje buněčné dělení. Do zkumavek byl připraven roztok citronanu sodného (0,8%), ve kterém byla embrya ponechána hypotonickému působení po dobu 40 minut při laboratorní teplotě. Poté byla tekutina ze zkumavek slita a bylo přidáno 5 ml fixativa (100% CH_3OH a 100% CH_3COOH, 3 : 1). Embrya byla provzdušněna pomocí Pasteurovy pipety a umístěna do chladničky (4 °C) na 20 minut. V této fázi došlo k usmrcení všech buněk. Tento krok byl opakován ještě dvakrát, s tím že v posledním kroku byly zkumavky ponechány v chladničce 24 hodin. Po 24 hodinách byla embrya osušena a umístěna do mikrozkumavky typu Eppendorf s 40% kyselinou octovou, která způsobila rozbití jaderných membrán a rozvolnění chromozomů pro následné pozorování na sklíčku. Zde byla embrya rozmělněna pomocí plastové tyčinky. Poté byla suspenze kapána na předehřátá (45 °C) podložní skla (Menzel GmbH) stejným způsobem jako v předchozí metodě preparace chromozomů z regenerátu.

Kultura fibroblastů byla založena z malého vzorku tkáně (cca 2 x 2 mm), který byl rybě odebrán z hřbetní ploutve. Případně byl použit odstřižek ocasní ploutve, který byl odebrán při využití metody preparace z regenerátu ploutví. Odebraný kousek tkáně z hřbetní nebo ocasní ploutve byl umístěn do roztoku PBS 0,5 ml (10 g NaCl, 0,25 g KCl, 0,25 g KH_2PO_4, 1,44 g $Na_2HPO_4.12H_2O$, 1000 ml H_2O) a 0,5 ml antibiotické–antimykotické směsi (Sigma-Aldrich) v mikrozkumavce typu Eppendorf na 60 minut. Poté byla tkáň umístěna do roztoku 1,5 ml PBS + 0,5 ml antibiotické–antimykotické směsi v mikrozkumavce typu Eppendorf na 60 minut. Ve „flow-boxu" byla tkáň postupně oplachována ve třech Petriho miskách s roztokem kompletního media složeného z 80 ml Liebovitz media L15 (Sigma-Aldrich) a 20 ml bovinního sera (Baria). Dále byl přidán 1 ml antibiotické–antimykotické směsi (Sigma-Aldrich) a 1 ml glutaminu (Sigma-Aldrich). Takto připravené medium bylo přefiltrováno speciálními filtry (Millex Millipore) a uchováváno ve sterilní nádobě v chladničce při 4 °C. V poslední Petriho misce byla tkáň ponechána hodinu. Následně byla tkáň rozstřihána na několik menších kousků. Takto získané kousky tkáně byly pomocí očkovací kličky přeneseny na dno kultivační lahvičky (Nunc). Po 20 minutách, kdy došlo k přilnutí tkáně ke dnu kultivační lahvičky, byly přidány 3 ml kompletního media. Fibroblasty byly kultivovány při 28,5 °C v termostatu. Každý třetí den probíhala výměna kompletního media. Celková doba kultivace byla přibližně 14 dní, podle vizuální kontroly růstu fibroblastů pod inverzním mikroskopem. Do kultivačních lahviček byly přidány 3 kapky 0,1% kolchicinu (Serva), který se nechal působit po dobu 4 hodin v termostatu při 28,5 °C. Poté byly dvakrát přidány a odsáty 2 ml PBS. K takto ošetřeným fibroblastům byl přidán 1 ml PBS a 0,7 ml 0,1% trypsinu (Sigma-Aldrich). Celková doba působení trypsinu byla 2 minuty, pak byl jeho účinek zastaven přidáním kultivačního media. Očkovací kličkou byly ze dna kultivační nádobky seškrabány narostlé fibroblasty, které byly spolu s kultivačním roztokem převedeny do zkumavky. Suspenze byla centrifugována 10 minut při 1200 otáčkách a laboratorní teplotě. Poté byl supernatant odsát a k buňkám usazeným na dně zkumavky bylo přidáno 5 ml KCl (5,6 g/l, 37 °C). Doba hypotonického působení KCl byla 8 minut. Po centrifugaci (10 min/1200 otáček) byl supernatant odsán a přistoupilo se k fixaci (100% CH_3OH, 100% CH_3COOH, 3 : 1) po dobu 20 minut. Proces fixace byl třikrát opakován a poté byla suspenze nakapána na vyčištěné podložní sklo (Menzel GmbH). Barvení bylo prováděno po dobu 10 minut ve 3% roztoku Giemsa Romanovski (Dr. Kulich Pharma) v Sorensově pufru (4,5 g KH_2PO_4, 4,7 g Na_2PO_4, 1000 ml destilované vody; pH = 6,8). Poté byly preparáty důkladně opláchnuty destilovanou vodou.

Všechny preparáty byly prohlíženy na mikroskopu Olympus AX-70 při zvětšení 1000x za použití imerzního oleje. Vybrané metafáze byly vyfoceny CDD kamerou (Olympus; DP30BW). Obraz byl zpracován v programu Microimage. Vlastní počítání chromozomů v metafázích probíhalo ručně nebo pomocí programu Microimage. Sestavování karyotypu bylo provedeno ve specializovaném programu Ikaros (Metasystems).

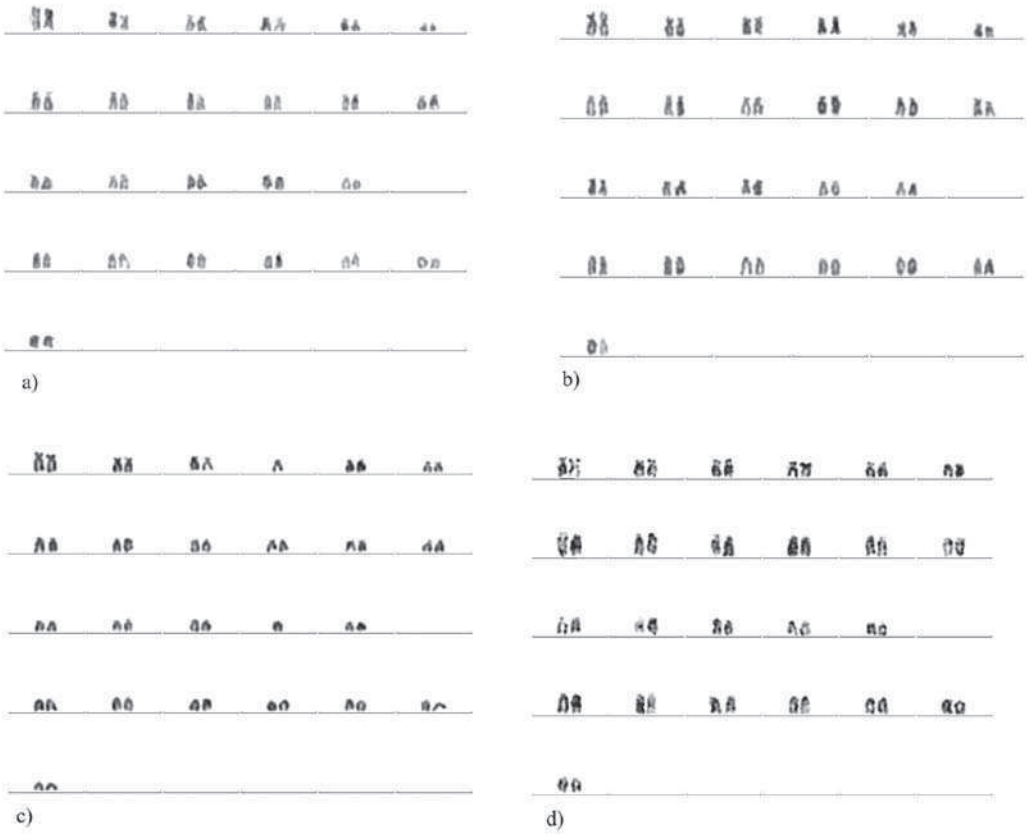
Chromozomy byly klasifikovány dle Levana a kol. (1964) na základě délky chromozomových ramen a polohy centromery do těchto kategorií: metacentrické (m), submetacentrické (sm), subtelocentrické (st) a akrocentrické (a). Získané výsledky cytogenetické analýzy byly posouzeny s ohledem na známé příbuzenské vztahy zkoumaných druhů. Zjištěné počty chromozomů byly zaneseny na fylogenetický strom. Použit byl publikovaný fylogenetický strom (Musilová a kol., 2009), který byl sestaven na základě dat získaných molekulárně genetickými metodami, a to sekvenací jak jaderných (RAG 1 a S7), tak mitochondriálních (Cytb, 16S) genů. Na strom byla zanesena i data získaná z publikací Marescalchi (2005), Feldberg a kol. (2003) a Thompson (1979) (obr. č. 2). Dále byly na fylogenetickém stromu vyznačeny evoluční události ve změně počtu chromozomů (zvýšení/redukce počtu) a pravděpodobný počet chromozomů společných předků s použitím kriteria maximální parsimonie (tj. aby celkový počet evolučních změn schopných vysvětlit rozložení znaků u reálných studovaných druhů byl co nejmenší; Flegr, 2005). Pro zanesení byla využita optimalizace ACCTRAN, která předpokládá, že evoluční změny se udály co nejpozději, tj. co nejblíže koncovým taxonům ve fylogenetickém stromu, a tedy co nejdále od jeho kořenů (Agnarsson a Miller, 2008). Poslední vstupní informace je předpoklad, že redukce počtu chromozomů je častější jev než zvýšení počtu (Imai a kol., 1986). Ke zvýšení počtu chromozomů dochází rozpadem, naopak snížení počtu se může odehrávat fúzí či hromadnou fúzí.

VÝSLEDKY

U všech osmi zkoumaných druhů se podařilo stanovit počty chromozomů. Druhy *Andinoacara biseriatus*, *Andinoacara* cf. *pulcher* „Rio Chirgua", *Andinoacara rivulatus*, *Aequidens tubicen*, *Cichlasoma amazonarum*, *Cichlasoma boliviense*, *Krobia* sp. „Xingu" měly 2n = 48 chromozomů, pouze druh *Tahuantinsuyoa macantzatza* měl 2n = 30 chromozomů. Karyotypy byly sestaveny u čtyř druhů (obr. č. 1). Jeden karyotyp není kompletní (obr. č. 1c), ale dobře typově zapadá mezi ostatní kompletní karyotypy, proto je uveden.

Obr. 1. *Karyotypy jihoamerických cichlid tribu Cichlasomatini a)* Andinoacara biseriatus*, b)* Aequidens tubicen*, c)* Cichlasoma amazonarum*, d)* Krobia *sp. "Xingu", 1–6 sm, 7–24 st – a.*

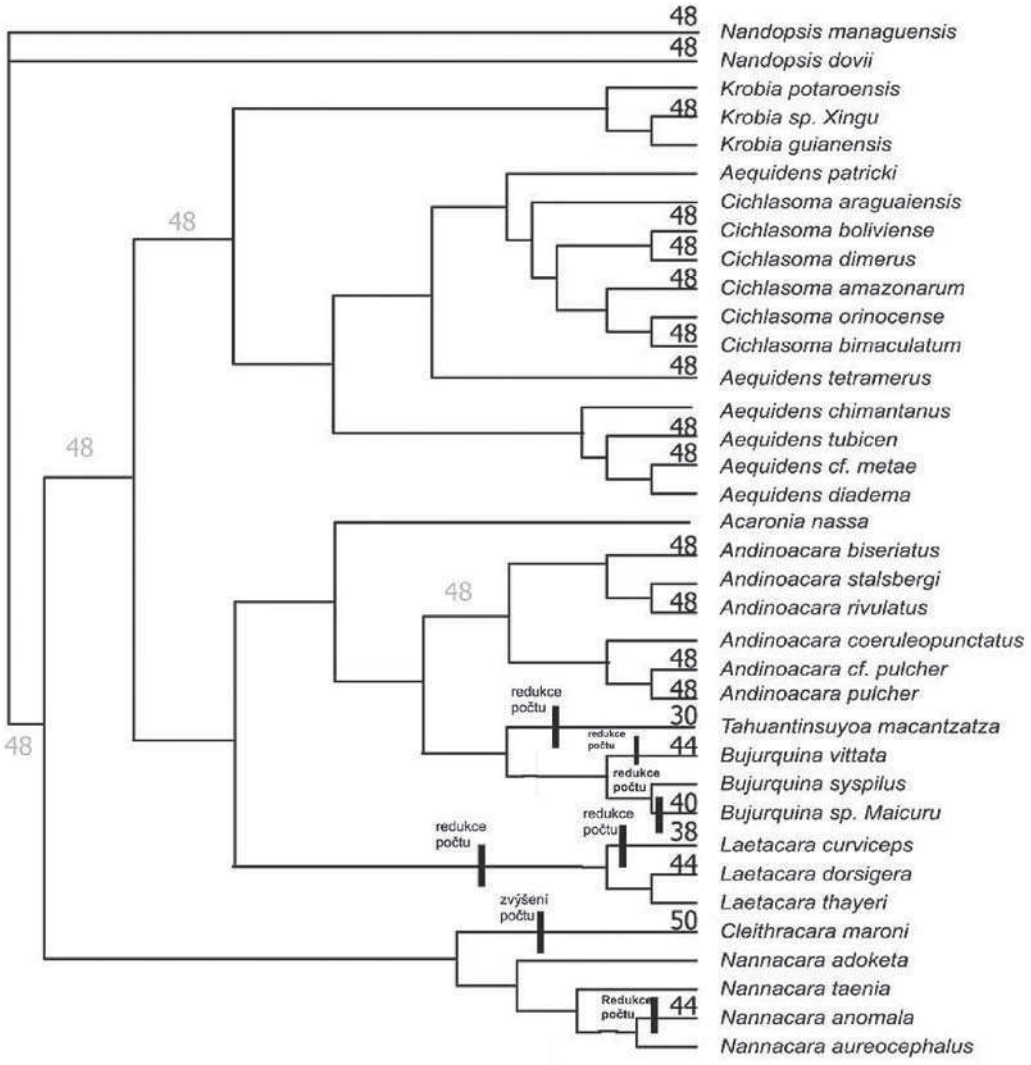
Fig. 1. *Karyotypes of South American cichlids of tribe Cichlasomatini a)* Andinoacara biseriatus*, b)* Aequidens tubicen*, c)* Cichlasoma amazonarum*, d)* Krobia *sp. "Xingu", 1–6 sm, 7–24 st – a.*



Společnou charakteristikou získaných karyotypů je šest párů submetacentrických chromozomů, z toho ve dvou případech (*Aequidens tubicen*, *Andinoacara biseriatus*) je první pár submetacentrických chromozomů výrazně větší než zbývající páry. Na základě získaných výsledků je pravděpodobné, že předek tribu Cichlasomatini měl 2n = 48 chromozomů. Ke změně počtu chromozomů došlo minimálně při sedmi evolučních událostech, z toho jednou ke zvýšení a šestkrát ke snížení počtu chromozomů. Jako stabilní se v počtu chromozomů jeví klastr rodů *Aequidens – Cichlasoma – Krobia*. Dosud všichni zkoumaní jedinci z tohoto klastru mají 2n = 48. U klastru rodů *Nannacara – Cleithracara* došlo ke zvýšení počtu chromozomů u *Cleithracara maroni* a k redukci počtu chromozomů u *Nannacara anomala*. U klastru rodů *Bujurquina – Tahuantinsuyoa – Andinoacara – Laetacara – Acaronia* existuje tendence k postupnému snižování počtu chromozomů, jelikož všechny druhy rodu *Andinoacara* mají 2n = 48, druhy rodů *Bujurquina* mají 2n = 44; 40, druhy rodů *Laetacara* mají 2n = 44; 38 a *Tahuantinsuyoa* má dokonce 2n = 30.

Obr. 2. *Fylogenetický strom (Musilová a kol., 2009) s daty o počtu chromozomů získaných během této práce a dále pak v práci Marescalchi (2005), Feldberga a kol. (2003) a Thompsona (1979). Data pro srovnávací skupinu (rod Nandopsis, tribus Heroini) pochází z práce Salase a Boza (1991). Dále jsou naznačeny hypotetické evoluční události, které mohly změnit počet chromozomů (redukce nebo zvýšení počtu chromozomů) s použitím metody maximální parsimonie za předpokladu vyšší pravděpodobnosti fúze než rozpadu chromozomů a aplikaci optimalizace ACCTRAN (hypotetické změny proběhly co nejdále od kořene stromu). Počet chromozomů u předka byl stanoven s využitím faktu, že původní karyotyp cichlid je 2n = 48 (Feldberg a kol., 2003). Šedá čísla znázorňují počet chromozomů u hypotetického předka.*

Fig. 2. *Fylogenetic tree (Musilová et al., 2009) with the number of chromosomes obtained in this study, and from the Marescalchi (2005), Feldberg et al. (2003) and Thompson (1979) studies. Data for group comparison comes from the study of Salas and Boza (1991). Hypothetical evolutionary events which could change chromosome numbers (increase or reduction) are marked. The method of maximal parsimony was used with a presumption of a higher probability of fusion versus a chromosome break up; the application ACCTRAN optimalization was used (hypothetical changes with the greatest distance from the tree's root). The ancestor chromosome number was determined by the fact that the ancestral karyotype of cichlids is 2n = 48 (Feldberg et al., 2003). Gray numbers indicate the chromosome numbers of a hypothetical ancestor.*



DISKUSE

Důvodem získání malého počtu kvalitních metafází je vlastní metoda regenerátu, která byla optimalizována pro drobné druhy halančíků (Völker, 2006) a pro cichlidy se metodu nepodařilo plně optimalizovat. Rovněž metoda kultivace fibroblastů nepřinesla kvalitní výsledky. Na tématu je tedy třeba nadále pracovat i v budoucnu a popisované výsledky je nutno chápat jako předběžné.

Pokud srovnáme charakteristiky karyotypů, které získala Marescalchi (2005), a charakteristiky získané v této práci, můžeme pozorovat určité rozdíly. Marescalchi (2005) vyhodnotila počet submetacentrických chromozomů v rozmezí 2 až 13 párů chromozomů. Námi získané výsledky ukazují vždy 6 párů submetacentrických chromozomů. Z toho pak vyplývají i rozdílné počty chromozomů v kategorii subtelocentrických až akrocentrických. Rozdíl mezi jedinci stejného druhu (konkrétně *Andinoacara pulcher*) může být způsobený odlišnou lokalitou původu jedinců v práci Marescalchi (2005) a v této práci. Marescalchi (2005) použila ke své studii ryby z akvarijních chovů, naopak v této práci byly použity ryby získané a importované z místa původu. Takovým příkladem je *Andinoacara* cf. *pulcher*, kdy typový materiál (jedinci, podle nichž byl druh popsán) pochází z Trinidadu, zatímco areál rozšíření pokrývá mnohem rozsáhlejší území Venezuely a Kolumbie. Námi použitý jedinec pocházel z řeky Chirgua ve Venezuele. V akvarijních chovech se vyskytuje tento druh již dlouho, ale geografický původ chovaných jedinců není jistý. Není proto možné říci, z které lokality pochází jedinec použitý v práci Marescalchi (2005). Navíc druh *Andinoacara pulcher* je velmi variabilní a podle analýzy DNA se pravděpodobně jedná o více druhů (Musilová a kol., 2009). Dokud nebude vyřešena taxonomická situace v rámci tohoto druhu, nelze vyvodit jednoznačný závěr.

Stabilně se jeví skupina rodů *Aequidens – Cichlasoma – Krobia*, všichni zkoumaní jedinci z této skupiny mají 2n = 48. U skupiny rodů *Nannacara – Cleithracara* došlo ke zvýšení počtu chromozomů u *Cleithracara maroni* a k redukci počtu chromozomů u *Nannacara anomala*. U skupiny rodů *Bujurquina – Tahuantinsuyoa – Andinoacara – Laetacara – Acaronia* se vyskytuje tendence k postupnému snižování počtu chromozomů, všechny druhy rodu *Andinoacara* mají 2n = 48, druhy rodů *Bujurquina* mají 2n = 44; 40 a druhy rodů *Laetacara* mají 2n = 44; 38 a druh *Tahuantinsuyoa* má dokonce 2n = 30. Ke zvýšení počtu chromozomů dochází rozpadem, naopak snížení počtu se může odehrávat fúzí či hromadnou fúzí, pravděpodobnější evoluční událost je fúze chromozomů než jejich rozpad (Imai a kol., 1986).

Pro zanesení počtu chromozomů jednotlivých druhů na jejich fylogenetický strom bylo použita optimalizace ACCTRAN, která předpokládá evoluční události „co nejpozději", tedy co nejdále od kořene stromu. Zvolili jsme tuto optimalizaci, jelikož data o počtu chromozomů jsou nekompletní a použití opačné optimalizace (DELTRAN – událost se udála co nejblíže kořenu stromu) by zanášelo do interpretace výsledků informaci i o druzích, které dosud nebyly zkoumány (Agnarsson a Miller, 2008).

Thompson (1979) tvrdí, že u cichlid se vyskytují dva typy karyotypů: 1) karyotyp „A", který je původní (2n = 48), s malým počtem metacentrických chromozomů, obvyklejší jsou submetacentrické chromozomy a dále nacházíme vysoký počet subtelocentriků a akrocentriků; 2) evolučně odvozený karyotyp „B", ve kterém nacházíme větší počet metacentrických chromozomů, často se také liší samotný počet chromozomů. Pokud porovnáme námi získané metafáze a karyotypy můžeme konstatovat, že většina zkoumaných cichlid majících 2n = 48 chromozomů má karyotyp typu „A". Naopak u druhů s redukovaným počtem chromozomů (2n = 44, 38 nebo 30) se jedná převážně o karyotyp typu „B".

Nejvýraznějším příkladem redukce počtu chromozomů je druh *Tahuantinsuyoa macantzatza*, u kterého došlo k redukci až na 2n = 30. U tohoto druhu se bohužel nepodařilo sestavit karyotyp, ale uvedený počet chromozomů (2n = 30) byl pozorován u dostatečného počtu metafází, kde bylo možno chromozomy alespoň spočítat.

Podle dostupných údajů má většina zástupců jihoamerických cichlid základní karyotyp 2n = 48 (Salas a Boza, 1991, Feldberg a kol., 2003). S ohledem na zjištěné výsledky v této studii je velmi pravděpodobné, že předek tribu Cichlasomatini měl také 2n = 48 chromozómů. Tento počet je též v souladu s prací Marescalchi (2005) a Feldberga a kol. (2003).

SOUHRN

Získali jsme cytogenetická data o jihoamerických cichlidách z tribu Cichlasomatini, která jsme následně porovnávali s fylogenetickými studiemi jiných autorů. Jaderné suspenze byly získány různými metodami chromozomové preparace, nakapány na skla a barveny roztokem Giemsa-Romanowski. Získané metafáze byly pozorovány mikroskopem a zaznamenány pomocí digitální kamery. Karyotypy byly sestaveny pouze z kvalitních metafází. Cytogenetická data (počet chromozomů, karyotyp) byla následně zanesena na známý fylogenetický strom. Ze získaných výsledků je pravděpodobné, že předek tribu Cichlasomatini měl 2n = 48. Ke změně počtu chromozomů došlo minimálně při sedmi evolučních událostech. Z toho jednou ke zvýšení a šestkrát ke snížení počtu chromozomů. Zvýšení počtu chromozomů bylo pozorováno ve skupině *Nannacara* a *Cleithracara* (*Cleithracara maronii* 2n = 50). Ve druhé skupině *Bujurquina – Tahuantinsuoya – Andinoacara – Laetacara – Acaronia* je patrná tendence k postupnému snižování počtu chromozomů. Všechny druhy rodu *Andinoacara* mají 2n = 48, druhy rodů *Bujurquina* mají 2n = 44, 40 a druhy rodů *Laetacara* mají 2n = 44; 38 a *Tahuantinsuyoa* má 2n = 30. Ze získaných dat není zatím možné formulovat finální hypotézu o evoluci karyotypu tribu Cichlasomatini.

PODĚKOVÁNÍ

Autoři děkují oponentům za rozsáhlé a konstruktivní kritické poznámky k rukopisu.

LITERATURA

Agnarsson, I., Miller, J.A., 2008. Is ACCTRAN better than DELTRAN? Cladistics, 24: 1–7.

Feldberg, E., Porto, J.I.R., Bertollo, L.A.C., 2003. Chromosomal changes and adaptation of cichlid fishes during evolution. In: A.L. Val and B.G. Kapoor (Editors), Fish Adaptation. Science Publishers, Inc, Enfield – NH, USA, pp. 285–308.

Flegr, J., 2005. Evoluční biologie. Academia, Praha, 559 pp.

Imai, H.T., Maruyama, T., Gojobori, T., Inoue, Y., Crozier, R.H., 1986. Theoretical bases for karyotype evolution 1. The minimum interaction hypothesis, The American Naturalist, 128: 900–920.

Levan, A., Fredga, K., Sanberg, A.A., 1964. Nomenclature for centromeric position on chromosomes. Hereditas, 52: 201–220.

Marescalchi, O., 2005. Karyotype and mitochondrial 16S gene charakterizations in seven South American Cichlasomatini species (Perciformes, Cichlidae). Journal of Zoological Systematics and Evolutionary Research, 43(1): 22–28.

Musilová, Z., Říčan, O., Novák, J., 2009. Phylogeny of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus. Journal of Zoological Systematics and Evolutionary Research, 47(3): 234–247.

Pisano, E., Ozouf-Costaz, C., Foresti F., Kapoor B. G., (Editors), 2007. Cytogenetics as a tool in fish conservation: the present situation in Europe. In: Fish Cytogenetics. Science Publishers, Enfield, USA, pp. 215–241.

Salas, E., Boza, J., 1991. Comparative cytotaxonomy of 3 species of Cichlasoma (Pisces: Cichlidae) native of Costa Rica. Revista de Biología Tropical, 39(2): 219–224.

Thompson, K.W., 1979. Cytotaxonomy of 41 species of neotropical cichlidae. Copeia, 4: 679–691.

Val, A.L., Kapoor, B.G., (Editors), 2003. Fish Adaption, Chromosomal changes and adaptation of cichlid fishes during evolution. Science Publishers, Inc, Enfield – NH, USA, pp. 285–308.

Völker, M.E., 2006. Karyotype differentiation in *Chromaphyosemion* killifishes (Cyprinodontiformes, Nothobranchiidae): Patterns, mechanisms and evolutionary implications. Ph.D. Thesis.

Appendix III.

Musilová Z.

2010

Blaupunktbuntbarsche (Gattung Andinoacara) aus Kolumbien und weiteren südamerikanischen Ländern

DCG-Informationen **41: 98-108**

Popular paper

Präsident: Dr. Wolfgang Staeck,
Auf dem Grat 41 A, 14195 Berlin,
Telefon und Fax 030/84107818.
E-Mail: praesident@dcg-online.de

Geschäftsführer: Winfried Poesdorf,
Parkstraße 21 a, 33719 Bielefeld,
Telefon und Fax 0521/3369958.
E-Mail: geschaeftsfuehrer-dcg@web.de

Schatzmeister: Michael Schulte,
Heckenweg 18, 32049 Herford,
Telefon 05221/26169.
E-Mail: schatzmeister.dcg@teleos-web.de

Verwaltung der DCG-Bildstelle:
Christian Hofer, Auerbergweg 6,
87600 Kaufbeuren,
Telefon und Fax 08341/9603868.
E-Mail: HoferChristian@t-online.de.

Leiter Geschäftsbereich Versand:
Wolfgang Stösser, Uferstraße 83,
32657 Lemgo, Telefon 05261/72900.

Verwaltung der Literatursammlung:
Prof. Dr. Max Lippitsch, Steingrabenweg 26,
A-8044 Graz, Telefon: 0043-316/392572,
E-Mail: max.lippitsch@uni-graz.at.

Öffentlichkeitsarbeit und gewerbliche Anzeigen: Bernd Kilian, Westpreußenstraße 42, 53119 Bonn, Telefon 0228/669966 (ab 18 Uhr). E-Mail: Kilian.bernd@t-online.de

Mitgliedsbeitrag: Euro 32,–/Jahr,
Euro 25,–/Jahr für Schüler, Studenten.

Alle Zahlungen an die DCG über folgendes Konto:
Sparkasse Bielefeld,
Konto-Nr.: 39818, BLZ: 480 501 61.
IBAN: DE94 4805 0161 0000 0398 18
SWIFT-BIC: SPBIDE3BXXX

Inhaltsverzeichnis:
41. Jahrgang, Heft 5, Mai 2010

Titelbild:
Frau Zuzana Musilová, Inhaberin des Cichliden-Förderpreises 2008, fotografiert Cichliden in einem kleinen Zufluss zum Rio Meta in Kolumbien. Mit finanzieller Unterstützung durch die Deutsche Cichlidengesellschaft konnte eine „Andinoacara-Expedition“ realisiert werden, die überaus erfolgreich war. Das Primärziel der Expedition war, Exemplare der verschiedenen *Andinoacara*-Arten aus allen Gebieten des Verbreitungsgebietes der Gattung zu sammeln. Dabei stand Kolumbien im Mittelpunkt, da es das Zentrum des Verbreitungsareals darstellt. – Foto: Zuzana Musilová

Beilagenhinweis: Dieser Ausgabe der DCG-Informationen liegt DCG-Aktuell, Mai 2010, bei.

Redaktion und Herstellung:
Roland F. Fischer, Fichtelgebirgsstraße 14,
95448 Bayreuth, Telefon 0921/853934,
Fax 0921/7930823,
E-Mail: Fischer.Roland.Bayreuth@t-online.de
Peter Schwer (**DCG-Aktuell**), Paradeisstr. 42d,
82362 Weilheim, Telefon 0881/637509,
E-Mail: DCG-Aktuell@gmx.de
Heinz H. Büscher (**Tanganjikasee**), Salinenstraße 13,
CH-4133 Pratteln, Telefon 0041/61/8214508.
Andreas Spreinat (**Malawisee**), Domänenweg 1,
37079 Göttingen, Telefon 0551/66077.
Anton Lamboj (**Zwergcichliden Südamerikas und Cichliden Westafrikas**), Otto-Glöckel-Straße 42,
A-2486 Pottendorf, E-Mail: chromido08@aon.at.
Lutz Krahnefeld (**Süd- und Mittelamerika)**, Lily-Braun-Straße 25, 12619 Berlin,
Telefon 030/9200537
E-Mail: Lutz.krahnefeld@t-online.de

Manuskripte sind an die Redaktionsanschrift (Roland F. Fischer) einzusenden. Veröffentlichte Manuskripte stellen nicht unbedingt die Meinung der Redaktion dar.

Anzeigenannahme (gewerbliche Anzeigen):
Bernd Kilian, Westpreußenstraße 42, 53119 Bonn,
E-Mail: Kilian.bernd@t-online.de

Druck und Weiterverarbeitung: Limberg-Druck GmbH, Postfach 1249, 41544 Kaarst,
Telefon 02131/668081.

Herausgeber: Deutsche Cichliden-Gesellschaft e.V.,
Winfried Poesdorf, Parkstraße 21 a, 33719 Bielefeld.

DCG-Informationen im Eigenverlag.
Der Verkaufspreis ist durch den Mitgliedsbeitrag abgegolten. ISSN 0724-7435

Blaupunktbuntbarsche (Gattung Andinoacara) aus Kolumbien und weiteren südamerikanischen Ländern

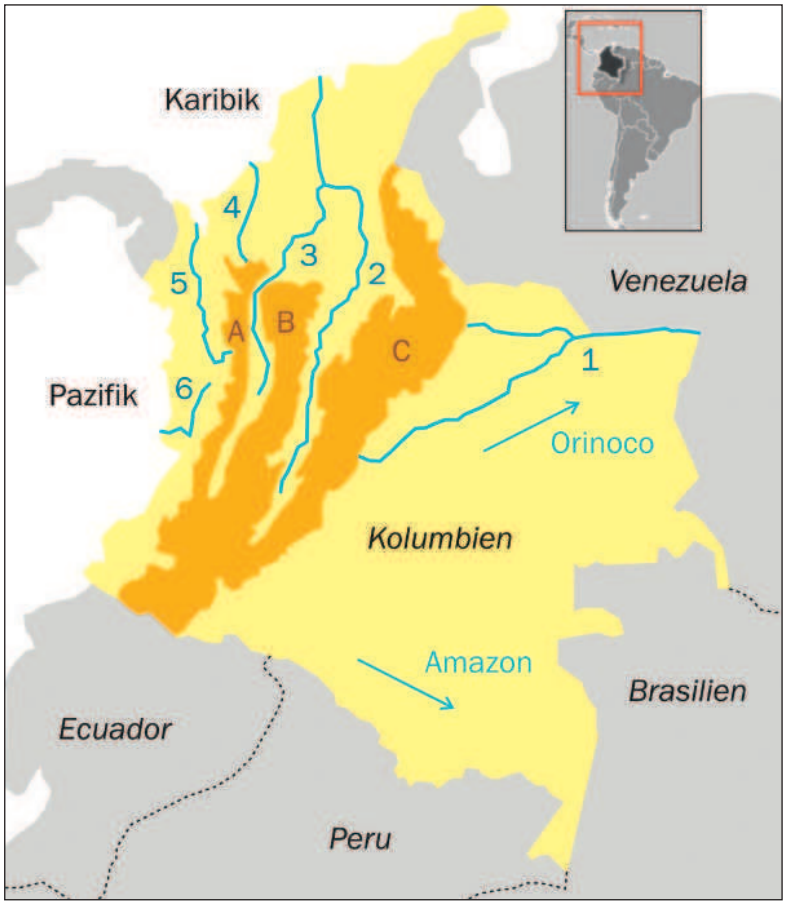
Zuzana Musilová*

Die Gattung Andinoacara

Obwohl die Buntbarsche der „*rivulatus-pulcher*-Gruppe“ eine eigenständige evolutionäre Linie darstellen und nicht besonders nahe mit den eigentlichen *Aequidens* (zum Beispiel *A. metae*, *A. tetramerus*) verwandt sind, wurden sie früher dieser Sammelgattung zugeordnet. Seit der Überarbeitung durch Kullander 1983 wurden sie bis vor kurzem als „*Aequidens*“ (in Anführungszeichen) bezeichnet, um sie von jenen Arten namentlich zu trennen. Heute werden die „Blaugepunkteten“ in ihrer eigenen Gattung *Andinoacara* Musilová, Rícan & Novák, 2009 geführt. Der wissenschaftliche Name der Gattung nimmt darauf Bezug, dass ihre Verbreitung in geologischen Zeiten durch gebirgsbildende Prozesse insbesondere die Auffaltung der Anden, beeinflusst wurde.

Die Gattung *Andinoacara* enthält zurzeit sieben Arten. In Südamerika reicht ihr Verbreitungsgebiet von pazifischen Zuflüssen in Peru und Ekuador über Kolumbien sowie Venezuela bis nach Trinidad (siehe dazu die Karten).

* Übersetzung aus dem Englischen: Ingo Schindler



Flüsse Kolumbiens, in denen wir Andinoacara-Arten sammeln konnten.

1 = Rio Meta (Flusssystem des Orinoko),
2 = Rio Magdalena,
3 = Rio Cauca,
4 = Rio Sinú,
5 = Rio Atrato,
6 = Rio San Juan.

Orangefarbene Flächen kennzeichnen drei kolumbianischen Gebirgszüge:

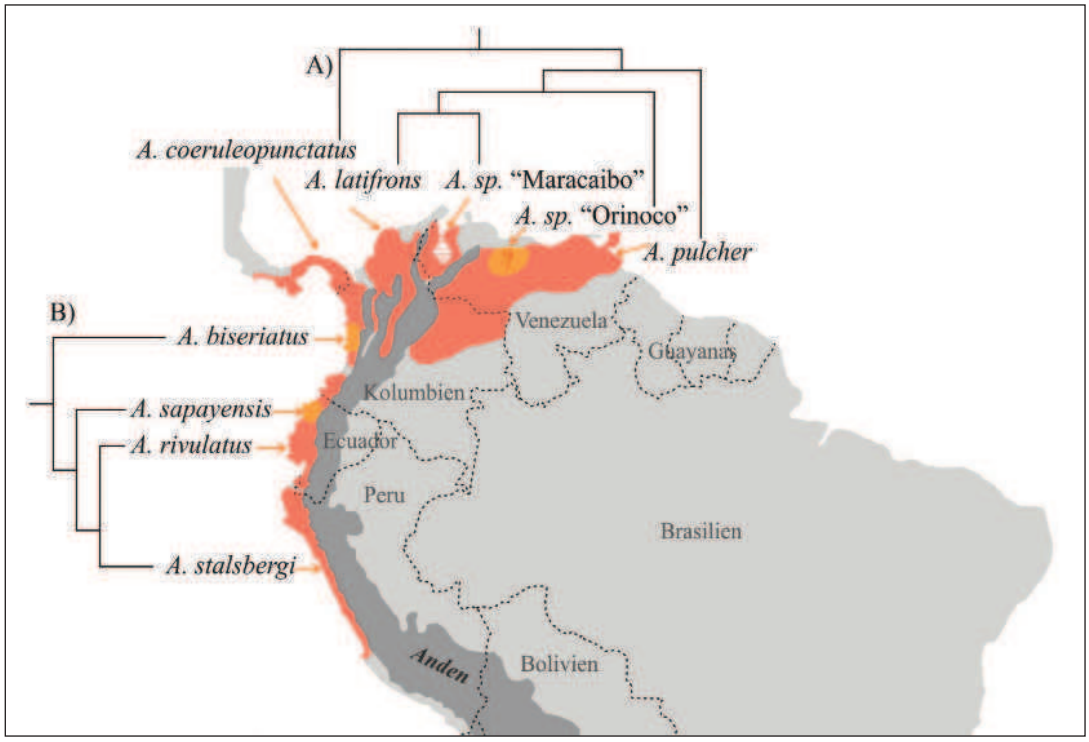
Cordillera occidental (A), Cordillera central (B) und Cordillera oriental (C).

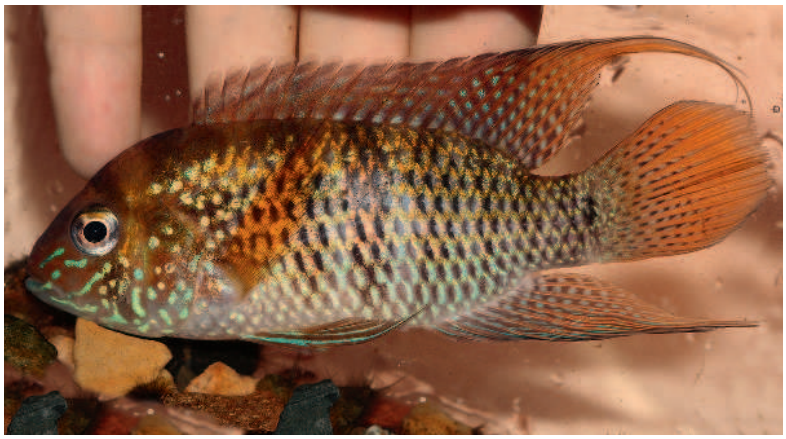
Im Norden des Verbreitungsareals ist *Andinoacara coeruleopunctatus* in Panama und Costa Rica zu finden. Der südlichste Fundort von *Andinoacara stalsbergi* (bisher als „*Aequidens*“ sp. „Silbersaum“ bekannt, vgl. Musilová, Schindler & Staeck 2009) befindet sich an der Pazifikküste Perus etwas südlich von Lima. In Ekuador leben *Andinoacara rivulatus* (A. sp. „Goldsaum“) und *Andinoacara sapayensis*. Nördlich davon sind in Kolumbien mit *Andinoacara biseriatus*, *A. latifrons*, *A. coeruleopunctatus* und *Andinoacara pulcher* (letzterer auch in Venezuela vorkommend) weitere vier *Andinoacara*-Arten verbreitet.

Daneben gibt es in Venezuela noch zwei unbeschriebene Arten. Das sind *A.* sp. „Maracaibo“ aus den Zuflüssen des Maracaibosees (nach Schultz 1949 auch im See selbst) und *A.* sp. „Orinoco“ aus dem mittleren Flusssystem des Orinoko. Von der zuletzt genannten Form habe ich Importfische in Deutschland im Zoofachhandel als „*Aequidens*“ sp. „Rio Cuchivero“ und als „*A*“. sp. „Rio Chirgua“ erhalten. Beide Bezeichnungen nehmen Bezug auf zwei Flüsse in Venezuela. Diese Fische sind ungewöhnlich dunkel getönt. Ihre schwärzliche Färbung wird während des Ablaichens noch intensiver, so dass die Querbalkenzeichnung dann nicht mehr erkennbar ist. Die Fische besitzen eine Vielzahl kleiner, irisierender Pünktchen. Diese Merkmale unterscheiden sie von *Andinoacara pulcher*, der vermutlich nächstverwandten Art, die deutlich weniger dieser glitzernden Pünktchen besitzt.

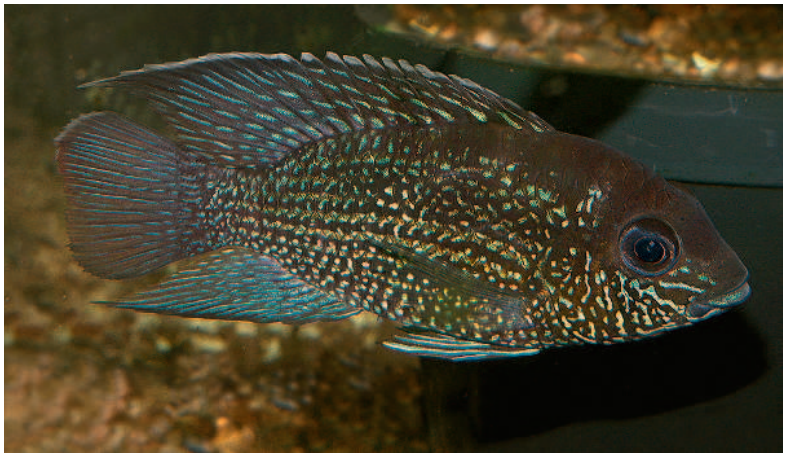
Unser Projekt hatte zum Ziel, Exemplare der verschiedenen *Andinoacara*-Arten aus allen Gebieten des Verbreitungsgebietes der Gattung zu sammeln.

Verbreitung der Andinoacara-Arten. Die Verbreitungsgebiete der einzelnen Arten sind rötlich oder orange gefärbt. Dunkle Linien vor den Artnamen geben die phylogenetischen (evolutionären) Verwandtschaftsverhältnisse wieder; A = A.-pulcher-Gruppe, B = A.-rivulatus-Gruppe. Die evolutionäre Verwandtschaft wurde mittels DNS-Sequenzen erstellt. Die Verbreitung von A. sp. „Orinoco“ ist noch nicht vollständig bekannt. Stawikowski & Werner (1998) betrachten A. sp. „Orinoco“ als eine in Venezuela weit verbreiteten Form, während ich hier unter A. sp. „Orinoco“ ausschließlich die dunkel gefärbte Form verstehe, die nur von wenigen Fundorten bekannt ist (vergleiche dazu die Fotos von A. sp. „Orinoco“ auf Seite 100).





Aus dem Maracaibosee und einigen seiner Zuflüsse im Westen Venezuelas (nahe der Grenze zu Kolumbien) stammt Andinoacara sp. „Maracaibo“.



Weibchen von Andinoacara sp. „Orinoco“. Diese wahrscheinlich noch unbeschriebene Art gehört zur Andinoacara-pulcher-Gruppe.

Andionoacra sapayensis aus dem Westen Ekuadors (Rio Cayapas, nahe dem Rio Sapayo, der Typuslokalität der Art)

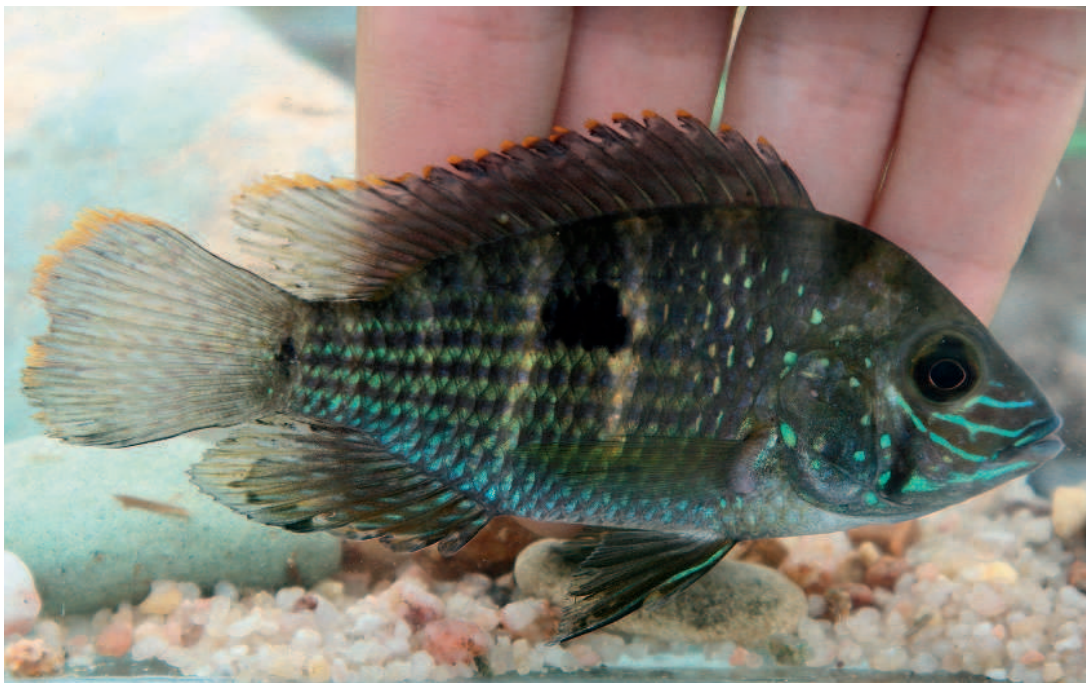
Andinoacara stalsbergi (ehemals bekannt als „Aequidens“ sp. „Silbersaum“) aus Nordperu (Rio Jequetepeque). Dieses Exemplar zeigt einen intensiven Schwarz-Weiss-Kontrast im Rand der Rücken- und der Schwanzflosse.

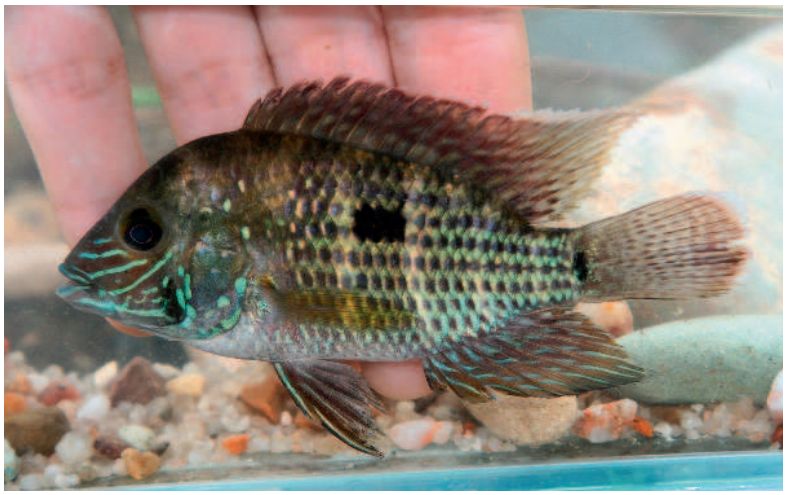
Unten: Andinoacara rivulatus aus dem Südwesten Ekuadors. Innerhalb einer Population kommen Exemplare mit orange oder weiß gefärben Flosssenrändern vor.

Dabei stand Kolumbien im Mittelpunkt, da es das Zentrum des Verbreitungsareals darstellt. Während unserer Feldarbeiten konnten wir Fische in Kolumbien, Ekuador und Peru sammeln. Bis auf *Andinoacara coeruleopunctatus* wurden dabei Exemplare sämtlicher *Andinoacara*-Arten gefangen. Es ist jedoch zu berücksichtigen, dass die Variabilität innerhalb der Arten aus der *pulcher*-Gruppe (insbesondere bei *Andinoacara pulcher*, *Andinoacara latifrons* und *Andinoacara coeruleopunctatus*) noch nicht vollständig dokumentiert ist.

Rio Magdalena: Der größte Strom Kolumbiens

Im Februar 2009 starteten wir unsere Feldarbeiten in Kolumbien, wo wir verschiedene Fundorte aufsuchen wollten. Unser Plan war es, am Rio Magdalena zu beginnen, um dann weiter zum Rio Cauca und anschließend zum Rio Sinú im Nordwesten Kolumbiens zu fahren. Diese Region gehört zu den am dichtesten bevölkerten und sich am schnellsten entwickelnden Gegenden Kolumbiens. Der Rio Magdalena ist der größte Fluss des Landes. Von dort war bisher nur *A. latifrons* bekannt. Wir konn-





Dieser Andinoacara rivulatus mit weißen Flossenrändern stammt aus der gleichen Population wie das Exemplar auf Seite 101.

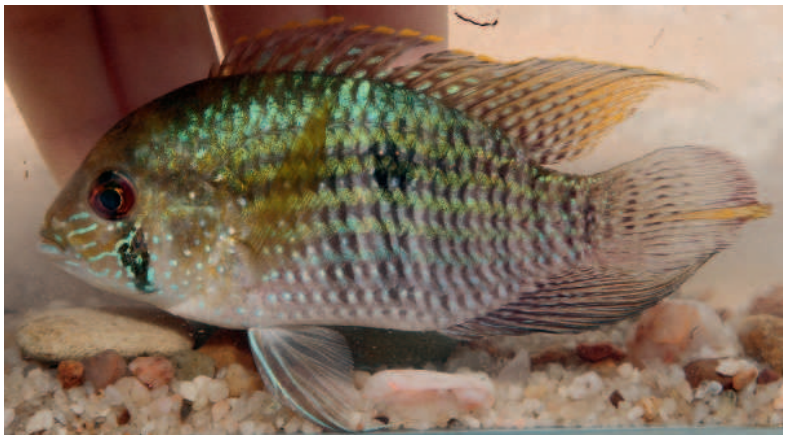
Als Andinoacara coeruleopunctatus wurden auf einer Messe in Duisburg die folgenden beiden Fische angeboten. Leider konnte nur in Erfahrungen gebracht werden, dass es sich dabei um deutsche Nachzuchten handelt. Informationen zur Herkunft der Elternfische waren nicht zu erhalten.

Andinoacara coeruleopunctatus aus dem Sortiment von Zoo Zajac

Jungfisch wie er von Hobby Zoo Tillmann angeboten wurde.

Andinoacara pulcher aus dem Rio Cauca (ein Nebenfluss des Rio Magdalena). Die Fische ähneln A. pulcher aus dem Rio Sinú and dem unteren Rio Magdalena.

Unten:
Andinoacara pulcher wurde im Rio Cesar in der Nähe des Ortes Valledupar mit dem Schleppnetz gefangen. Im Bild zwei weitere Teilnehmer an der „Andinoacara-Expedition“: Petr Jansta and Ondrej Gahura



ten jedoch im mittleren und unteren Flussabschnitt auch *A. pulcher* nachweisen. Allerdings sind die Unterscheidung und Abgrenzung bei diesen beiden Spezies noch nicht ausreichend geklärt.
Wir sammelten in kleinen Bächen, die wir zuvor in detailreichen Karten ausfindig machten, um dann mit öffentlichen Verkehrsmitteln dort hinzufahren. Überraschenderweise funktionierte dies gut, und wir waren beim Fang der Blaupunkt-Buntbarsche sehr erfolgreich. Im System des Rio Magdalena hatten die Bäche klares Wasser und sandigen Bodengrund. Es war daher leicht die Fische bereits aus der Distanz zu beobachten. Der Fang war aber umso schwieriger, da uns die Tiere natürlich auch ausmachen konnten. Wir verwendeten Wurf-, Hand- und Zugnetze. In den Klarwasserbächen gelang es mit dem Wurfnetz jedoch nur bei Nacht Fische zu erbeuten. Während des Tages verwendeten wir das Zugnetz. Wenn zwei Personen gut zusammenarbeiten und das Netz durch den Bach zum Ufer ziehen, um es dann vorsichtig zu schließen, haben die Fische keine Fluchtmöglichkeit.

Dieser Geophagus steindachneri wurde nahe Valledupar im Rio Cesar (Nordkolumbien) gefangen.

Unten:
Sturisoma aureum aus dem Rio Sinú, im Dorf Cereté, Nordwestkolumbien

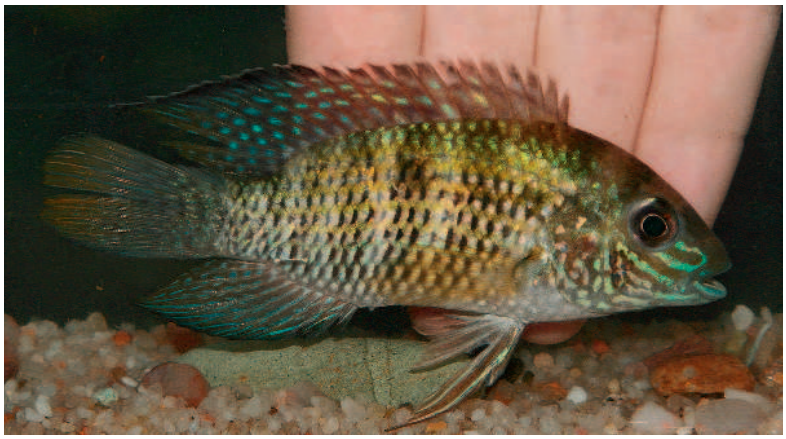
Im Bereich des unteren Rio Magdalena leben *Andinoacara* auch in trüben Bächen und in künstlich angelegten Teichen. Obwohl wir meist kleine Bäche auswählten, hatten wir auch Erfolge beim Fang in den Flüssen zu verzeichnen, so zum Beispiel im etwa zehn Meter breiten Rio Cesar in Nordkolumbien. In einigen Biotopen konnten wir auch andere Cichliden (zum Beispiel „*Geophagus*“ *steindachneri*) und auch einige Loricariiden (unter anderem *Sturisoma aureum*) sammeln.
Die Blaupunkt-Buntbarsche scheinen in dieser Gegend, was die Wasserqualität betrifft, nicht besonders wählerisch zu sein. An einem Tag fingen wir in der kleinen Stadt Namens Cereté, die am Rio Sinú im nordwestlichen Kolumbien liegt. Unter einer Brücke im Zentrum gab es einen kleinen Bach, der über und über mit Müll und alten Reifen verschmutzt war. Es stank fürchterlich. Trotzdem waren dort zahlreiche *Andinoacara* und *Sturisoma* zu finden, und so wurde dieser Fangtag - was die erbeutete Individuenzahl betrifft - der erfolgreichste unserer Reise.

Genetisch betrachtet sind die Fische aus dem Sinú und dem Magdalena (einschließlich Caucá) fast identisch. Dies könnte ein Hinweis darauf sein, dass ein Austausch zwischen den Faunen des Rio Sinú und des unteren Rio Magdalena besteht.

Andinoacara pulcher aus dem mittleren Rio Magdalena

Andinoacara pulcher aus den Zuflüssen des unteren Rio Sinú. Dieser Fisch zeigt viele Glanzpunkte und eine orange gesäumte Rückenflosse. Er ähnelt damit den Exemplaren aus dem unteren Rio Magdalena and Rio Cauca.

Andinoacara pulcher aus dem Rio Meta (einem Nebenfluss des Rio Orinoco) in Kolumbien. Der Fisch besitzt rote Augen und zeigt nur wenige Glanzpunkte und Streifen auf dem Körper. Er sieht Andinoacara pulcher aus Venezuela sehr ähnlich.



Andinoacara cf. pulcher aus dem oberen Rio Magdalena.
Dieser Fisch weist einen schwarz-weißen Rand in der Rücken- und Afterflosse auf.
Diese Zeichnung kann auch bei A. coeruleopunctatus und einigen Arten der Gattung Bujurquina gefunden werden.

Unten:
Eine Tüte voller Andinoacara pulcher aus dem Rio Sinú, Kolumbien

Provinz Chocó auf der pazifischen Seite der Anden

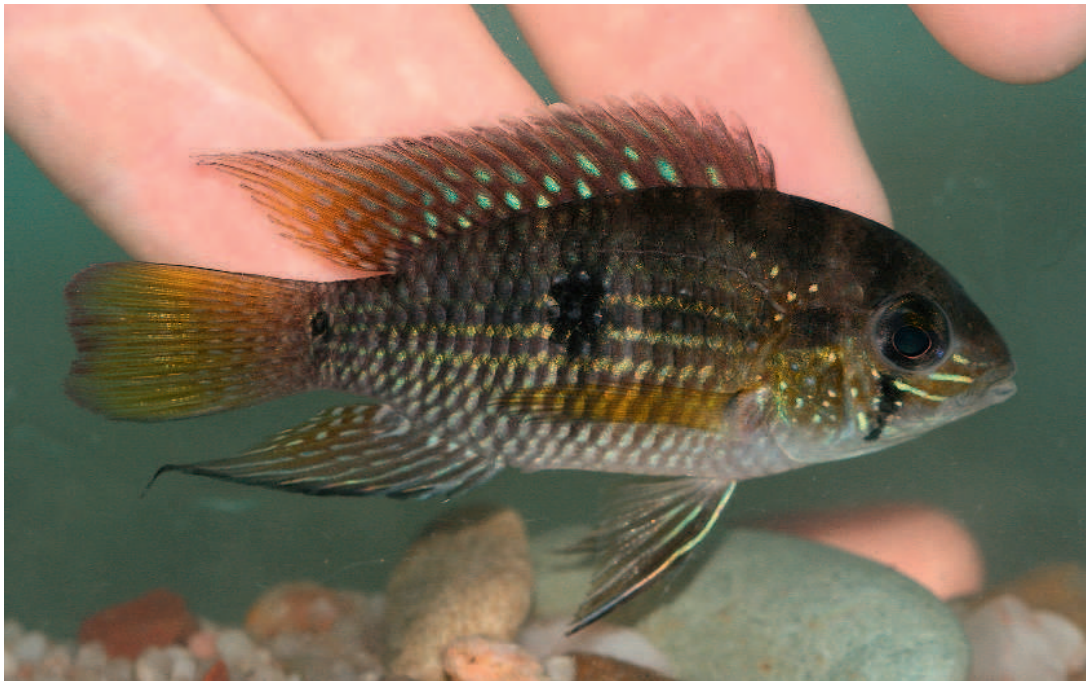
Eines der wichtigsten Ziele unserer Reise war schließlich die Provinz Chocó auf der pazifischen Seite der Anden. In den dortigen Flüssen Atrato, Baúdo und San Juan konnten wir zwei verschiedene *Andinoacara* finden: *Andinoacara biseriatus* ist ausschließlich dort verbreitet. Außerdem lebt in dem Gebiet aber auch noch eine Art aus der *A. pulcher*-Gruppe. Es gibt ferner Berichte sowohl über das Vorkommen von *A. latifrons* als auch über das von *A. coeruleopunctatus* in Chocó. Allerdings ist solchen Äußerungen mit Vorsicht zu begegnen, weil es Unklarheiten in der Bestimmung der betreffenden Exemplare gibt. Wir sammelten einige Fische im Rio Atrato und im Rio San Juan. Beide Formen unterscheiden sich von *A. coeruleopunctatus* aus Panama als auch von *A. latifrons* aus dem oberen Rio Magdalena. Auf der Grundlage von DNS-Daten zeigte sich im Nachhinein, dass die Fische aus dem Chocó den *A. latifrons* aus dem Rio Magdalena sehr ähnlich sind. Dies muss aber noch durch weitere Untersuchungen überprüft werden.

Die Provinz Chocó weist einige Besonderheiten auf. Sie ist flächenmäßig die größte an der Pazifikküste Kolumbiens und wegen der geografischen Gegebenheiten (Ausläufer der Anden im Westen und Pazifik im Osten) durch ein ausgesprochen feuchtes Klima geprägt (tatsächlich befindet sich hier der regenreichste Ort der Welt). Dieser Teil Kolumbiens gehört zu den ärmsten Gegenden und ist wirtschaftlich unterentwickelt. Es ist eine politisch unsichere und wegen der kriegerischen Aktivitäten der Guerillas gefährliche Region. Es gibt nur zwei über weite Teile unbefestigte Straßen, die die Provinz durchqueren. Für die nur 200 Kilometer lange Strecke von Medellín nach Quibdó, der Hauptstadt von Chocó, benötigen die Busse fast 16 Stunden. Im Februar 2009 war eine dieser Straßen gesperrt, weil eine Bus die Böschung hinunter in den Fluss gestürzt war und dabei Teile der Straße beschädigte. Da die andere Straße deshalb die einzige Verbindung des Chocó mit dem Rest der Welt war, wurde sie ungewöhnlich stark frequentiert. Busse, Tanklastwagen und Transporter, die Benzin und Waren hin und wieder zurück bringen, machten die Strecke zu einer stark befahrenen Straße.

Der überwiegende Teil im Chocó ansässigen Bevölkerung ist afrikanischer Abstammung. Aber auch einige indianische Stämme leben dort. Obwohl nur 200 Kilometer von Medellín entfernt, verirren sich Touristen nur sehr selten in diese Gegend, die so wirkt, als wäre man in einer völlig anderen Welt.

Wir begannen unseren Fischfangtag in einem kleinen Dorf am so genannten Playa de Oro (= Goldstrand) am Ufer des Rio San Juan. Das regenreiche Wetter hatte dafür gesorgt, dass die Gewässer anstiegen und über die Ufer traten. Überall gab es jedoch kleine Lagunen und schmale Bäche, so dass es uns möglich war, in diesen kleinen Gewässern Fische zu fangen. Als es für eine Weile aufhörte zu regnen, saßen wir am Ufer eines kleinen Weihers. Es dauerte nur wenige Minuten, bis die ersten *Andinoacara* heran schwammen, um uns zu beobachten. Während des Tages war es schwierig, mit dem Wurfnetz zu fangen, weil die vorsichtigen Fische sofort verschwanden, wenn wir auch nur die kleinste Bewegung mit dem Netz machten. Als der Regen wieder losging, hatten wir mit dem Wurfnetz besseren Erfolg, da uns die Fische nun nicht mehr ausmachen konnten. An den Stellen, an denen wir die *Andinoacara* zuvor gesehen hatten,

Jungfisch einer noch unbeschriebenen Andinoacara-Art aus dem Rio San Juan in Kolumbien. Die Art gehört zur A.-pulcher-Gruppe.



warfen wir das Netz hinein. Auf diese Weise gelang es uns, vier *Andinoacara* cf. *latifrons* zu fangen (von Einigen auch als *A*. sp. „Chocó“ bezeichnet).

Danach zogen wir weiter zum oberen Rio Atrato. Beim Dorf Certeguí konnten wir im dortigen kleinen Bach neben *A*. cf. *latifrons* auch den in Chocó endemischen *A. biseriatus* fangen. Der Bach formte an einer Stelle eine kleine Ausbuchtung mit einer Fläche von zwei bis drei Quadratmeter. Dort fischten wir am Ufer mit schnellen Zügen unserer Handkescher in kolkartigen Vertiefungen, in welche die Fische sofort geflüchtet waren, als wir das Gewässer betraten. Es gelang uns zehn Exemplare zu fangen, bevor ein heftiger Regen begann. Der Wasserstand stieg schnell an, und so mussten wir den Fundort verlassen. Aber zum Glück hatten wir das Hauptziel unseres Projektes erreicht: Wir hatten die Fische aus Chocó gefangen!

Wenn ich die verfügbare Literatur und Informationen (Glaser et al. 1996; Stawikowski & Werner 1998) vergleiche, bin ich mir über die Bestimmung der *Andinoacara*-Arten immer noch nicht sicher, denn einige Arten sind in den Originalbeschreibungen nur mangelhaft definiert. Ich hoffe daher, dass ich in nächster Zeit an einer detaillierten Revision dieser interessanten Gattung arbeiten kann.

Dieses Projekt wurde freundlicherweise durch den Cichliden-Förderpreis 2008 der Deutschen Cichliden-Gesellschaft (DCG), Expedicni fond und GAUK der Tschechischen Republik unterstützt.

Literatur

Glaser, U., F. Schäfer & W. Glaser (1996): Southamerican Cichlids III. Aqualog, Verlag A.C.S., 144 pp.
Kullander, S. O. (1983): A revision of the South American Cichlid genus Cichlasoma (Teleostei: Cichlidae). Swedish Museum Natural History. Stockholm, 296 pp.
Musilová, Z., O. Rícan & J. Novák (2009): Phylogeny of the Neotropical cichlid fish tribe Cichlasomatini (Teleostei: Cichlidae) based on morphological and molecular data, with the description of a new genus. Journal of Zoological Systematics and Evolutionairy research 47: 234–247.
Musilová, Z., I. Schindler & W. Staeck (2009): Description of Andinoacara stalsbergi sp. n. (Teleostei: Cichlidae: Cichlasomatini) from Pacific coastal rivers in Peru, and annotations on the phylogeny of the genus. Vertebrate Zoology 59: 131–41.
Schultz, L. P. (1949): A further contribution to the ichthyology of Venezuela. Proc. U. S. Mus. Washington.
Stawikowski, R. & U. Werner (1998): Die Buntbarsche Amerikas. Band 1. Stuttgart, 540 pp.

Nur im Oberlauf des Rio Atrato und des Rio San Juan (Kolumbien) kommt Andinoacara biseriatus vor. Die Art gehört zur A.-rivulatus-Gruppe.
Im Bild ein junges Männchen wenige Stunden vor dem Laichakt. Ältere Exemplare zeigen eine dunkle, nahezu schwarze Färbung.

Fotos: Zuzana Musilová

Appendix IV.

Musilová Z.

2010

Rozporuplné akary: Akara potoční (Andinoacara rivulatus) a její příbuzní z Peru, Ekvádoru a Kolumbie

Aquaristik **2: 6-11.**

Popular paper

Rozporuplné akary

Text a foto: Zuzana Musilová

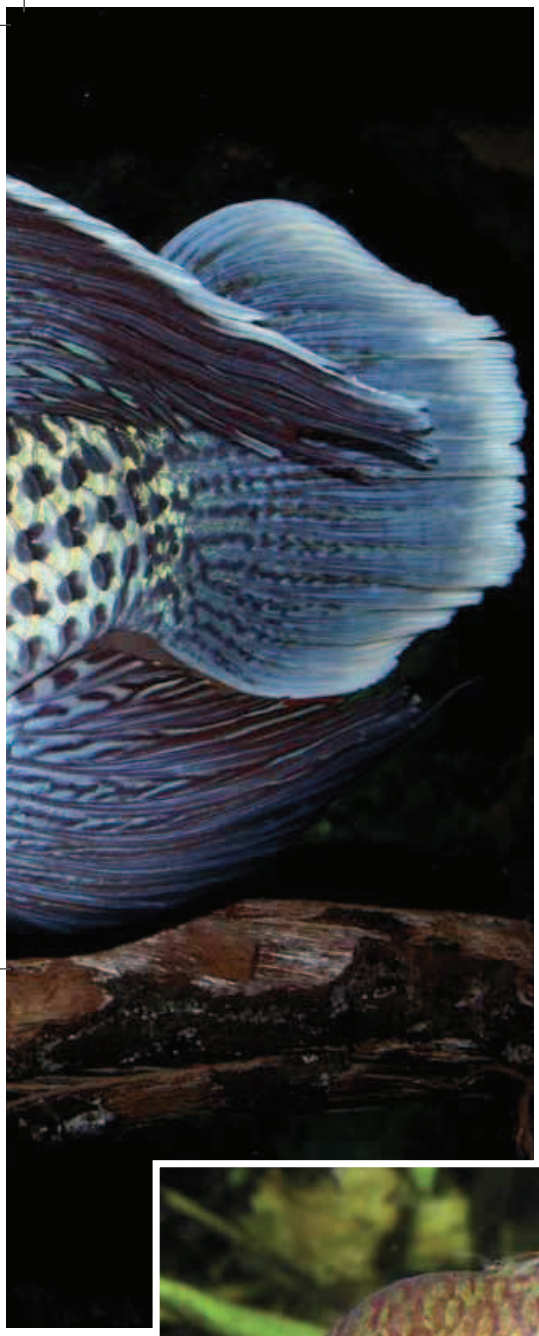
Akara potoční (*Andinoacara rivulatus*) a její příbuzní z Peru, Ekvádoru a Kolumbie

Dospělý samec akary potoční (*Andinoacara rivulatus*) chovatele z Duisburgu, jedinec s bílými lemy

Všeobecně známá a mezi akvaristy velmi oblíbená akara potoční (dříve *'Aequidens' rivulatus*) dostala nedávno nové rodové jméno *Andinoacara*. Byla tak konečně završena malá část výzkumu zapeklité evoluce jihoamerických cichlid.

Kdo by neznal „riviláta"

Rodové jméno *Aequidens* přísluší „pravým" akarám, jako jsou akara kolumbijská (*Aequidens metae*) nebo akara sedlová (*Aequidens tetramerus*). Kvůli taxonomickým nejasnostem byly do tohoto rodu dříve řazeny i nepříbuzné druhy, což byl právě případ akary potoční (*Andinoacara rivulatus*) nebo akary modré (*Andinoacara pulcher*) a jejich příbuzných. Do rodu *Andinoacara* patří celkem sedm platných druhů, z nichž nejznámější dva (akara potoční a modrá) zná skoro každý akvarista.Nejnovějším z této sedmičky je druh *Andinoacara stalsbergi*, vědecky popsaný teprve vloni. Jde o rybu, která se kdysi v 80. letech minulého století dostala do evropských akvárií (včetně českých), aby z nich pak zase vymizela. Pamětníkům možná z té doby utkvělo v mysli její obchodní jméno „Silbersaum". Je to akara velmi podobná akaře potoční (*Andinoacara rivulatus*), od níž se však



na první pohled odlišuje vzorem zbarvení šupin a bílou barvou lemů hřbetní a ocasní ploutve (odtud právě německý název „Silbersaum“ = stříbrný lem). Jenomže se zdá, že vše není tak jednoduché, jak to vypadalo původně.

„Rivulát“ není „rivulát“?

Už nějakou dobu se zejména z Německa ozývají kritické hlasy, že pravou akaru potoční (*Andinoacara rivulatus*) vlastně nikdo pořádně neviděl, ačkoliv se občas – velmi vzácně – objeví u nějakého zaníceného (německého) akvaristy. A to, co kupujeme, chováme a prodáváme, je prý jiný, vědecky nepopsaný druh, jenž by se měl označovat *Andinoacara* sp. „Goldsaum“ (opět německy: „zlatý lem“). Ne, nezahazujte teď tento časopis, nic z toho si zatím není třeba pamatovat. Ale poslechněte si krátkou historii o tom, jak může být někdy těžké zjistit pravdu.

Ve hře tedy máme tři ryby: 1. „Silbersaum“ – nově popsaný druh *Andinoacara stalsbergi*; 2. akara potoční známá z akvárií– „rivulát“ nebo něco jako *Andinoacara* sp. „Goldsaum“; 3. hypotetický „pravý“ druh *Andinoacara rivulatus*. Zatímco první dvě ryby jsou jasně definovány a navzájem odlišitelné, najít informace o rybě č. 3 je obtížné. Vědecky však zatím existují pouze první dva druhy, přičemž vědecké jméno *Andinoacara rivulatus* zatím náleží formě se zlatými lemy ploutví („Goldsaum“), česky akara potoční.

Jak je poznáme – rozdíly ve zbarvení

Hlavní rozdíly mezi akarou potoční (*Andinoacara rivulatus*) a druhem *Andinoacara stalsbergi* je v kresbě na šupinách: akara potoční má šupiny perleťově modrozelené po většině plochy, s tmavou kulatou skvrnou uprostřed a na zadním konci šupiny. U dospělých samců lze na těle pozorovat jednolitou perleťovou plochu s černými skvrnami nebo pravidelné podélné perleťově pruhy až pravoúhlou mřížku. U druhu *Andinoacara stalsbergi* je tento vzor zbarvení víceméně obráceně: šupiny mají perleťovou skvrnu (byť velkou) v ploše uprostřed a u okraje šupiny (někdy může být přítomen ještě jemný tmavý lem na úplném okraji šupiny). Plošné zbarvení ryby je pak tmavé s perleťovými skvrnami, které jsou oddělené, případně jsou vyskládané střídavě jako tašky na střeše a netvoří nikdy souvislé pruhy. Nejlépe je rozdíl viditelný na fotografiích. Dalším rozlišovacím znakem je pak zbarvení lemů ploutví, kdy u akary potoční je lem většinou (!) oranžový, přičemž u samců na ocasní ploutvi může být velmi silný. U druhu *A. stalsbergi* je zbarvení lemů vždy (!) bílé a lemy jsou

Typická akara potoční (*Andinoacara rivulatus*), jak ji známe z našich akvárií

Akara potoční (*Andinoacara rivulatus*) s přechodným zbarvením lemů – v ocasní ploutvi a zadní části hřbetní ploutve je lem oranžový, zatímco přední část hřbetní ploutve má bílý lem

Andinoacara sapayensis ze severozápadního Ekvádoru je mnohem vzácnější druh než *Andinoacara rivulatus* a *Andinoacara stalsbergi*

Akara potoční (*Andinoacara rivulatus*) odchycená v Ekvádoru – jedinec s typickým oranžovým zbarvením ploutevních lemů

tenké. Často je bílý lem ještě na své vnitřní straně doprovázen tenkým černým proužkem, který ho odděluje od ostatní ploutve. Povšimněte si, prosím, že oranžová barva lemů u akary potoční (*Andinoacara rivulatus*) není ve 100 % případů: existují i jedinci s bílou barvou lemů, a to jak v přírodě, tak i v akváriích, nicméně bílá barva je u tohoto druhu mnohem vzácnější. Navíc lemy nemusí být vždy tak tenké a nikdy nemají na straně k tělu černou linku. Zmiňuji zde barvu lemu také proto, že už pár hodin po zveřejnění vědeckého popisu *Andinoacara stalsbergi* začali někteří lidé na internetu označovat fotografie akar potočních (*Andinoacara rivulatus*) s bílými lemy chybně jako nový druh *Andinoacara stalsbergi*.

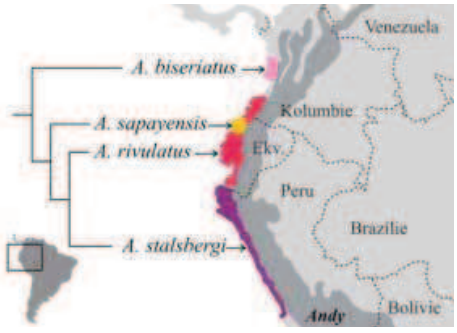
Trocha biogeografie

Akara potoční (*Andinoacara rivulatus*) žije na pacifické straně And v Jižní Americe, konkrétně v Ekvádoru. Její nejbližší příbuzná, nově popsaná akara *Andinoacara stalsbergi*,se vyskytuje jižněji, v Peru. Areály rozšíření obou druhů se nepřekrývají, ale je mezi nimi hranice, která se víceméně shoduje se státní hranicí mezi Peru a Ekvádorem (viz mapa). *Andinoacara rivulatus* a *Andinoacara stalsbergi* jsou dva sesterské druhy (tj. nejpříbuznější), ale do skupiny k nim patří ještě dva vzácné druhy – *Andinoacara sapayensis* z Ekvádoru a *Andinoacara biseriatus* z Kolumbie. Oba se v minulosti sporadicky objevovaly v akváriích.

Lokalita akar potočních (*Andinoacara rivulatus*) ve středním Ekvádoru – odpadní stoka; zjevně nejde o příliš náročné ryby na kvalitu vody

V loňském roce popsali Musilová, Schindler a Staeck akary známé mezi akvaristy pod označením „Silbersaum" jako nový druh *Andinoacara stalsbergi* – na snímku dospělá samice odchycená v přírodě v Peru; od příbuzné akary potoční se liší např. rozdílným zbarvením šupin a přítomností tenkého černého proužku doprovázejícího na vnitřní straně bílý lem v ocasní a hřbetní ploutvi

V Ekvádoru jsme odchytili i akary potoční (*Andinoacara rivulatus*) s bílými lemy ploutví



Mapa rozšíření některých zástupců rodu *Andinoacara*. Graf vlevo od názvů jednotlivých druhů znázorňuje jejich evoluční příbuznost, tj. *Andinoacara rivulatus* a *Andinoacara stalsbergi* jsou sesterské (nejpříbuznější) druhy a do jejich příbuzenstva pak patří druhy další

Vraťme se nyní k hypotetickému „pravému rivulátovi", což by měl být jiný druh podobné akary. Podle stránek norského akvaristy Alfa Stalsberga (na jehož počest byl mimochodem vědecky pojmenován „Silbersaum" jako *A. stalsbergi*) i podle stránek amerického exportéra ryb Jeffa Rappse jde vždy o rybu tmavěji zbarvenou s bílými lemy, ale s různým barevným vzorem šupin, jednou jako u „Goldsauma" (Stalsberg), podruhé naopak spíše jako u „Silbersauma" (Rapps). Některé fotografie těchto „pravých rivulátů" jsou však zcela určitě přerostlí samci vzácnějších „Goldsaumů" s bílými lemy nebo „Silbersaumů". Problém s touto rybou spočívá mimo jiné i v nedostatku informací o jejím původu. Zatímco „Silbersaum" (*Andinoacara stalsbergi*) má jasně definovaný areál (Peru – řeky tekoucí do Pacifiku), zbylé dva druhy/formy mají žít v Ekvádoru, ale bližší vymezení jejich areálu chybí.

Expedice za odpovědí

Vypravili jsme se do Ekvádoru na jaře 2009 zjistit na vlastní oči, jak to tedy je. Ve stejnou dobu vyrazil na expedici i kolega Oldřich Říčan a dohromady jsme nezávisle na sobě nalovili ryby na více než 50 lokalitách, pokrývajících celý areál výskytu těchto druhů. Po celém Ekvádoru jsme nalovili pouze akaru

potoční, kterou známe z akvárií, tj. formu *Andinoacara* sp. „Goldsaum". Kromě toho jsme ale také oba chytili mnohem vzácnější druh *Andinoacara sapayensis*, který žije pouze na malém území Ekvádoru (viz mapa). Vzniká proto otázka, zda někteří akvaristé cestující do Ekvádoru za rybami nepovažují právě tento vzácný druh za „pravého rivuláta"?!? A opět připojím poznámku, že druh *Andinoacara sapayensis* byl také údajně v evropských akváriích v 80. letech chován, nicméně podle několika existujících fotografií z té doby šlo spíše o nějakého příslušníka skupiny modrých akar (komplex *Andinoacara pulcher*).

Andinoacara biseriatus ze západní Kolumbie vyniká krásným zbarvením, přesto se mezi akvaristy objevila v minulosti jenom vzácně

Tři možná vysvětlení

Situace, kterou jsme si popsali v předchozích odstavcích, má tři alternativní vysvětlení:

1. V Ekvádoru žijí pouze dva druhy rodu *Andinoacara*. Jeden je rozšířen po celém území a je to ona známá oranžovolemá akara potoční (*Andinoacara rivulatus*, označovaná také jako *Andinoacara* sp. „Goldsaum"). Druhý druh je vzácnější *Andinoacara sapayensis* a někteří akvaristé jej mylně považují za „pravý" *Andinoacara rivulatus*.

2. V Ekvádoru žijí tři druhy rodu *Andinoacara*: *Andinoacara sapayensis,* *Andinoacara* sp. „Goldsaum" a tajemný *Andinoacara rivulatus*. Ani jedna z našich expedic „pravého rivuláta" neodhalila, ačkoliv jsme lovili i na lokalitách jeho údajného výskytu.

3. V Ekvádoru žijí tři druhy rodu *Andinoacara*: *Andinoacara sapayensis*, *Andinoacara* sp. „Goldsaum" a tajemný *Andinoacara rivulatus*. To, co považujeme my za *Andinoacara sapayensis*, je ve skutečnosti „pravý" *Andinoacara rivulatus*, zatímco *Andinoacara sapayensis* je ještě jiná ryba, kterou jsme nechytili.

Osobně se přikláním k hypotéze č. 1, ale pro definitivní zjištění, která z možností je pravdivá bude nutné ponořit se do původních pramenů, zejména do popisů druhů, a zjistit, jaké konkrétní ryby vlastně tehdy autoři popisovali. Nebude to jednoduché, většina popisů těchto ryb pochází z konce 19. století a někdy obsahuje pouze pár řádků textu bez vyobrazení ryby a přesnějšího uvedení lokality. Každopádně si tím zatím nemusíme lámat hlavu, nazývejme našeho „rivuláta" zatím pořád *Andinoacara rivulatus* a počkejme, čím nás věda zase překvapí.

Lokalita *Andinoacara stalsbergi* mezi rýžovými poli v povodí řeky Jequetepeque v severním Peru

Akvaristé a vědecký výzkum

Akvaristé mohou často pomoci vědeckému výzkumu mnoha unikátními pozorováními. Má-li někdo ze čtenářů jakoukoliv užitečnou informaci týkající se druhů rodu *Andinoacara*, budu velmi ráda, když se mi ozve na e-mail zuzmus@email.cz. Zvláště by mi pomohly dobové fotografie druhu *Andinoacara sapayensis*, případně jakýchkoliv „podivných rivulátů".

Appendix V.

Musilová Z.

2006

Akary - co jsou vlastně zač?

Akva Tera Fórum **2010 (2): 8-16.**

Popular paper

Akary

Názvosloví a chov v akváriu

Akary - co jsou vlastně zač?

Zuzana Musilová
Foto: Zuzana Musilová (ZM), Jaroslav Eliáš (JE), Dieter Hohl (DH) a Jindřich Novák (JN)

Když jsem před časem promýšlela, co všechno napsat do článku o akarách, napadla mě nejprve otázka: „Které ryby jsou akary a které ne?“ Je totiž všeobecně známo, že systematická situace uvnitř americké větve cichlid je nesmírně složitá a prochází neustálým vývojem. Akarami jsou bezpochyby všechny druhy, které dříve náležely do sběrného rodu Aequidens. Tento rod byl však postupně rozdělen do několika rodů nových, a to tak, aby situace co nejlépe odpovídala současným znalostem o skutečné příbuznosti ryb. Dnešní rod Aequidens tedy obsahuje jen část druhů řazených do něj v pojetí předchozím a jako akary označujeme i ryby rodů jiných.

´*Aequidens´ rivulatus*, samec (DH)
Cichlasoma bimaculatum z importu (ZM)
Aequidens chimantanus (DH)

Aequidens tubicen (ZM)

Malý výlet do systematiky

Rozdělování „akar“ probíhalo od 80. let, a to do rodů ***Laetacara***, ***Cleithracara***, ***Krobia*** a méně známých ***Bujurquina*** a ***Tahuantinsuyoa***. V rodu ***Aequidens*** zůstalo dnes kolem 16 druhů.

Několik druhů, mezi nimiž jsou i „akary“ notoricky známé a mezi chovateli běžné, jaksi zbylo a systematické zařazení pro ně zatím nemáme. Jejich prozatímní rodový název je ´***Aequidens***´ (v jednoduchých uvozovkách) a tvoří tři skupiny. Jde o skupinu kolem akary modré (´*Aequidens´ pulcher*), akary potoční (´*Aequidens´ rivulatus*) a skupinu kolem ´*Aequidens´ hoehnei*.

Ještě složitější osud potkal sběrný rod ***Cichlasoma***, k němuž se váže tradiční český název kančík a který původně sdružoval jak středoamerické, tak i jihoamerické ryby. Změny v systematice zde neustále probíhají a za poslední roky bylo ustanoveno více než 20

Aequidens diadema, vzrostlý jedinec z importu (ZM)

nových rodů, především u druhů ze Střední Ameriky. S nezařazenými druhy, či s druhy čekajícími na rodové jméno se pak zachází podobně jako u akar – používá se *´Cichlasoma´* v uvozovkách jako přechodné řešení. Tzv. pravých cichlasom (bez uvozovek) potom zbyl jen zlomek z původního počtu druhů – dnes jich napočítáme dvanáct a najdeme je pouze v Jižní Americe.

Co je nejdůležitějším závěrem naznačených přesunů? *Aequidens* rovná se akara, *Cichlasoma* rovná se kančík, tak hovoří tradice. Jenže to dnes již neplatí. Vznikla nám zde totiž paradoxní situace.

Český název akara přísluší rodu *Aequidens* a po jeho rozpadu i dalším výše zmíněným rodům (*Krobia, Bujurquina, Tahuantinsuyoa, Laetacara, Cleithracara*).
Pravé cichlasomy mají s dnešními kančíky (tj. hlavně se středoamerickými druhy, dnes již klasifikovanými v jiných rodech, či prozatímně jako *´Cichlasoma´* v uvozovkách) jen málo společného. Rod *Cichlasoma* (bez uvozovek) je, jak se zdá, nejpříbuznější právě aequidensům! Pravá *Cichlasoma* nemůže proto zůstat česky kančíkem, ale správně by měla být nazvána také akarou! Však také klasická akvarijní ryba našich dědečků *Aequidens portalegrensis*, dnes pojmenovaná jako *Cichlasoma portalegrensis*, je toho důkazem.

Jen pro úplnost ještě musím zmínit z příbuzenstva akar rod ***Acaronia***, o jehož správné zařazení se vedou spory a který bývá zařazován někdy právě k akarám a jindy zase blíže perleťovkám do podčeledi Geophaginae. A ještě nesmíme zapomenout na rod ***Nannacara***, kterému náleží český název akarka. Ale zanechme již systematiky.

Aequidens cf. *tetramerus*, importované ryby (JN)

Chov v akváriu

Chovat akary, ve smyslu udržet je naživu v dobré kondici, není nic těžkého. Kromě drobných rodů *Nannacara* a *Laetacara* jde většinou o větší ryby dorůstající přes 10 cm, často až 15-20 cm (akara potoční - *´Aequidens´ rivulatus* je největší akarou a dorůstá až 35 cm). Potravně jsou to ryby velmi nenáročné, v přírodě neustále probírají dno a stravují se vším možným, co naleznou. Proto některým druhům postačí i méně kalorické vločkované krmivo. Pochopitelně, chceme-li akary pravidelně množit, je u mnoha druhů naopak velmi důležité krmit kvalitním živým nebo alespoň zmraženým krmením (komáří a pakomáří larvy, kril). Ostatně zkušenosti s chovem těchto ryb má snad každý akvarista. Nemálo jich začínalo s akarou modrou a k jejich spokojenosti nebylo obvykle obtížné dosáhnout i výtěru.

Co se týče chovu akar ve smyslu úspěšně je rozmnožovat, můžeme u některých druhů narazit na určité obtíže. Nezanedbatelnou roli v chovu cichlid totiž hraje jejich vysoce vyvinuté sociální chování, nepředvídatelný výběr jednoho partnera a naopak pro nás nepochopitelné odmítnutí partnera jiného. Pro úspěšný chov je vždy potřeba nasadit do nádrže skupinu okolo osmi jedinců, ve které se sociální hierarchie postupně vyvíjí a partneři mají celkem široký výběr. Stává se poměrně často, že ačkoli jsou akary celkem mírumilovné, čas od času jsou někteří jedinci skupiny zabiti, či alespoň pronásledováni, což vede k výraznému zhoršení jejich kondice. Takové chování nelze příliš předpovídat. K incidentu může dojít u skupiny, která spolu klidně žije již několik měsíců. Máme-li velkou nádrž, je celkem výhodné umístit několik druhů dohromady, agresivita se pak obvykle roztříští.

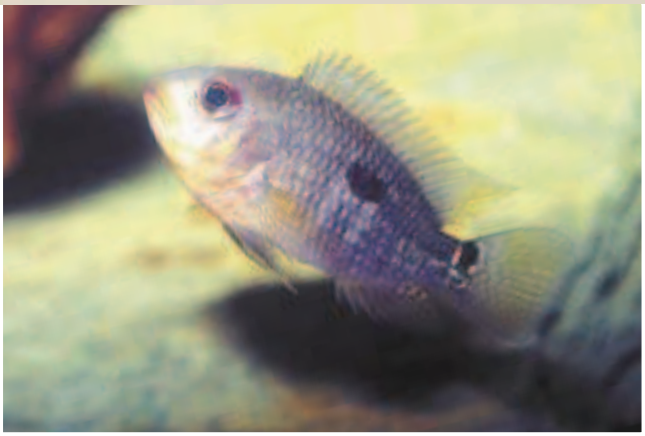
Přimět akary k výtěru je někdy snadnější, jindy obtížné. Pokud se již pár vybral, téměř vždy pomůže jeho oddělení do samostatného akvária. Často je totiž výtěr ve společné nádrži dle názoru rodičů nemožný a nebezpečný. Pokud přece jen k vytření dojde, často následuje sežrání jiker vlastními rodiči, jelikož ti jsou neustále rušeni a neschopni pak déle udržet obhajované teritorium.

Výtěr akar probíhá na pevný podklad, který je předem očištěn. Může to být plochý kámen nebo třeba ▸

Aequidens viridis, pár - sameček nahoře (DH)

Akary Chov v akváriu a přehled druhů

Ryby přírodní populace importované jako *Aequidens* cf. *metae*, patřící pravděpodobně k nepopsanému druhu (ZM)



Aequidens metae, mladý jedinec, dosud bez žlutých znaků, pocházející z akvarijní populace (JN)

keramický květináč vložený do nádrže. Není výjimkou ani výtěr na samotné sklo tvořící dno nádrže. Jiker bývá hodně, u vzrostlých jedinců to může být až 1000 kusů v jednom

Cichlasoma portalegrense, akvarijní populace(JN)

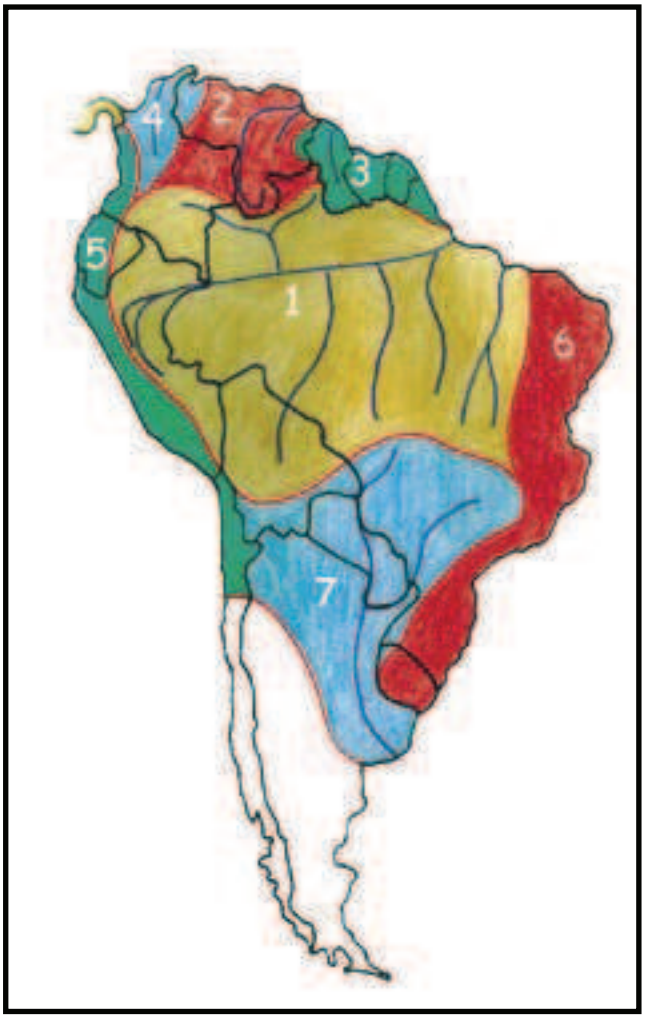
Cichlasoma dimerus (DH)

tření. Koláč jiker potom ochraňují oba rodiče (rodičovská rodina); jednak odhánějí ostatní obyvatele nádrže od třecího místa – teritoria, jednak pečlivě ovívají jikry ploutvemi a vybírají neoplozené a zaplesnivělé jikry.

Obvykle třetí den po vytření se plůdek vykulí a rodiče jej pečlivě přenášejí do jamky v písku, či do jiného vybraného místa, které jim může připadat bezpečné – například za houbu filtru. Celá rodina pak často mění své stanoviště a plůdek bývá přitom přenášen i několikrát denně, minimálně však mezi denním a nočním stanovištěm. Za další tři dny se potěr rozplavává, ale zůstává nadále v péči rodičů – ti ho stále tlamkou přenášejí, zejména pokud chtějí změnit momentální stanoviště a nebo pokud se malá rybka zatoulá nebezpečně daleko od zbytku rodiny.

Je-li pár v nádrži sám, stává se, že žije se svými potomky v souladu až do jejich dospělosti. Ze společné nádrže je potom obvykle nutné potěr - pokud vydrží až do stadia rozplavání - přelovit do jiné nádrže, tedy chceme-li jej odchovat.

Velice zajímavou rozmnožovací strategii mají druhy rodů *Bujurquina* (akvaristé možná znají občas chovanou *B. mariae* – dříve zvanou *Aequidens mariae*) a *Tahuantinsuyoa*. Jsou to totiž tzv. larvofilní tlamovci, kteří se uchýlili ke sběru vylíhlých embryí do tlamky, kde je přece jen pro mláďata nejbezpečněji. Platí za to ovšem omezeným počtem potomků, jakož i omezením vlastního pohodlí během nošení. Plůdek nosí obvykle oba rodiče s tím, že si potomstvo předávají. Tímto způsobem množení trochu připomínají další americké tlamovce – perleťovky např. rodů *Geophagus* a *Satanoperca*, kteří ovšem berou do tlamky vesměs již jikry. Larvofilním



Rybí provincie Jižní Ameriky: 1 = Amazonie, 2 = povodí Orinoka, 3 = řeky Guyanské vysočiny, 4 = povodí řeky Magdalena, 5 = transandská provincie, 6 = řeky východního pobřeží Brazílie, 7 = povodí Paraná; černé čáry = hranice státu, modré čáry = nejdůležitější řeky, červené čáry = hranice rybí provincie

Cichlasoma pusillum z importu patří mezi atraktivně zbarvené zástupce rodu (ZM)

Samice *Cichlasoma bimaculatum* pečující o výtěr. Její tlama je odřená od neustálého přetlačování s partnerem (ZM)

tlamovcem je prý i akara *Aequidens diadema*, nicméně tuto informaci mám ověřenu pouze z jednoho zdroje.

Přehled akar

1. Rod *Aequidens* – kolem 16 druhů a několik dalších dosud nepopsaných. Mezi běžně chované druhy patří akara kolumbijská (*Aequidens metae*) nebo akara *Aequidens tetramerus*, z importů z přírody či zahraničních chovů lze potom občas sehnat *A. patricki* nebo *A. michaeli*. Akary rodu *Aequidens* žijí od severu Jižní Ameriky, od povodí Orinoka a Guyanské vysočiny po systém Amazonky.

2. Rod *Cichlasoma* – 12 druhů, z nichž mezi akvaristy bývají rozšířeny *Cichlasoma dimerus* a *Cichlasoma portalegrense* (znám pod českým názvem kančík pruhovaný, což je ovšem zavádějící název – viz předchozí text). Některé ostatní druhy lze sehnat přes importní firmy (jako např. *C. amazonarum, C. bimaculatum* či *C. pusillum*). Území, které obývají, zahrnuje celou Jižní Ameriku vyjma pacifického pobřeží And (žijí v povodí Amazonky, Orinoka, Paraná, řek Guyanské vysočiny a východní Brazílie).

3. Skupina kolem akary modré *(´Aequidens´ pulcher)* – komplex několika druhů a vědecky nezhodnocených populací. Patří sem *'A.' coeruleopunctatus*, a *'A.' latifrons*, které jsou od sebe a od *´A.´ pulcher* obtížně rozlišitelné a není ani jisté, zda se jedná skutečně o různé druhy. Dále sem patří ještě *´A´. sapayensis*, která je také velmi podobná. Všechny druhy žijí na severu Jižní Ameriky s malým přesahem do Panamy.

4. Skupina kolem akary potoční *(´Aequidens´ rivulatus)*. Akaru potoční lze v České republice sehnat ve dvou „formách" – „Orangesaum" s oranžovým pruhem na ocasní ploutvi a „Rotsaum" s pruhem červeným. Zbarvení lemu ocasní ploutve závisí do značné míry na krmení, zda tu má vliv i dědičnost, zatím není jasné. Kromě tohoto a dalších dvou druhů jsou zde minimálně dvě nové nepopsané formy či druhy, z nichž nejznámější je forma zvaná „Silbersaum" (se stříbrným pruhem na konci ocasní ploutve), pravděpodobně nepopsaný druh. Jako jediní zástupci akar, a jedny z mála cichlid obecně, žijí tyto druhy na opačné straně And, než leží Amazonie – tedy ve vodách pacifického pobřeží Jižní Ameriky.

5. *´Aequidens´ hoehnei* – samostatně vyčleněný druh, který žije v povodí Amazonky.

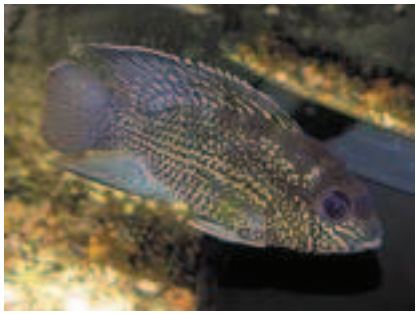
6. Rod *Laetacara* akvaristům netřeba představovat. Čtyři druhy, z nichž dva jsou běžně chovány (*L. dorsiger, L. curviceps*) a další dva (*L. thayeri, L. flavilabris*) se v běžné obchodní síti občas objeví, doplňují dva nepopsané druhy - *Laetacara* sp. ▸

Cichlasoma amazonarum – importovaná samička starající se o rozplavané potomstvo (ZM)

Pár *Cichlasoma amazonarum* z importu pečující o potomstvo, nahoře samička (ZM)

Akary Přehled rodů

„Buckelkopf“ (hrbatá hlava) a *Laetacara* sp. „Orangeflossen” (= oranžové ploutve). Běžnější jsou mezi akvaristy v sousedním Německu. Tyto ryby obývají povodí Amazonky a severní povodí řeky Paraná.



´Aequidens´ cf. *pulcher*, populace z Rio Chirgua (ZM)

´Aequidens´ cf. *pulcher*, akvarijní populace (JN)

Kříženec *´Aequidens´ pulcher* a *´Aequidens´ rivulatus* (ZM)

´Aequidens´ sp. „Silbersaum“, samička (JN)

Krásný pár *´Aequidens´ rivulatus* pečující o jikry. Samice vlevo (DH)

7. Rod *Nannacara* – pět platných druhů. Běžně známý a chovaný druh je akarka zelená (*N. anomala*), vzácněji je chována *N. taenia, N. aureocephalus, N. adoketa a N. bimaculata*. Jsou známy také další dvě dosud nepopsané formy. Akarky žijí v povodí Amazonky a na Guyanské vysočině.

8. *Cleithracara maroni* (akara hnědá) – jediný druh svého rodu. Má velmi mírumilovnou povahu, čímž se liší od ostatních velkých zástupců (*Aequidens, Cichlasoma*). Žije v deltě Orinoka a v řekách na Guyanské vysočině.

9. Rod *Krobia* – dva druhy, z nichž oba byly v minulosti chovány a výjimečně na ně lze narazit i dnes: akara delfínová (*Krobia itanyi*) a *Krobia guianensis*. Existují minimálně další dvě dosud nepopsané formy, chované běžně v Německu nebo v USA. Oba druhy i všechny formy krobií žijí v řekách Guyanské vysočiny.

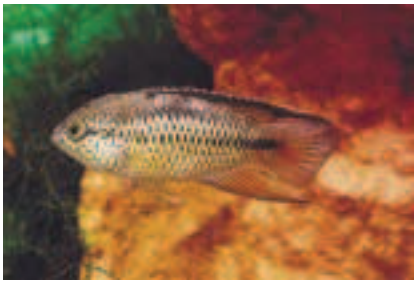
10. Rod *Bujurquina* – sedmnáct druhů (z toho třináct popsaných v 80. letech); známější z akvárií jsou *B. mariae, B. vittata* a *B. syspilus*. Obývají jak povodí Orinoka a Amazonky, tak i řeky Paraná.

Akara zlatá (*Laetacara dorsiger*) v atraktivním třecím zbarvení (JE)

Akara tečkovaná (*Laetacara curviceps*), samec nahoře (JE)

Akara hnědá (*Cleithracara maroni*) (JN)

Akarka *Nannacara taenia* (ZM)



Akarka zelená (*Nannacara anomala*), samec (JE)

11. Rod *Tahuantinsuyoa* – známy jsou dva druhy - *T. macantzatza a T. chipi*, oba z horního toku Amazonky, které se občas dostanou do Evropy s importy. Rod *Tahuantinsuyoa* je velmi blízce příbuzný s rodem *Bujurquina*.

12.* Rod *Acaronia – popsány jsou dva druhy, *A. nassa* a *A. vultuosa*. První z nich žije v Amazonii i v řekách Guyanské vysočiny, druhý naopak můžeme nalézt v povodí Orinoka a na horním toku Rio Negro. *A. nassa* bývá občas dovážena. ■

Krobia sp.„Xingú“ z importu (ZM)

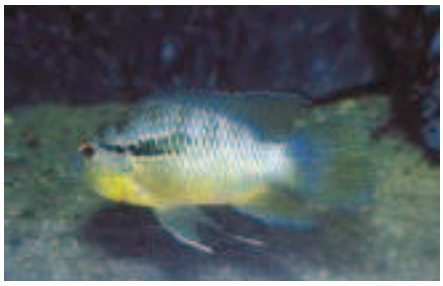
Krobia sp.„Oyapock“ z importu z řeky Oyapock na Guyanské vysočině (ZM)

Literatura

- *Eschmeyer, W. N., 2004: Catalog of Fishes. San Francisco, California Academy of Sciences. (http://www.academy.org/research/ichtyology/catalog)*
- *Kullander, S. O., 1998: A Phylogeny and Classification of the South American Cichlidae (Teleostei: Perciformes). Pp. 461-498. In: Malabarba, L. R. et al. (Eds.) Phylogeny and Classification of Neotropical Fishes. Porto Alegre. Edipucrs.*
- *Stiassny, M. L. J., 1991: Phylogenetic intrarelationships of the family Cichlidae: an overview. Pp. 1 - 35. In: Keenleyside, M. H. A. (Ed.): Cichlid fishes: Behaviour, ecology and evolution. London: Chapman Hall.*

Akary Přehled rodů a *Aequidens patricki*

Mladá ryba druhu *Tahuantinsuyoa macantzatza* z importu (ZM)



Bujurquina sp. Všechny druhy rodu jsou si velmi podobné (JN)

Bujurquina mariae, pár pečující o potomstvo (DH)

Mladá ryba druhu *Acaronia nassa*, krátce po příchodu vzácné zásilky importů z Jižní Ameriky (ZM)

SUMMARY **Acaras – What Sort of Fishes Are They?**

The taxonomic situation is still unsatisfactorily solved in Neotropical Cichlids. A lot of Acaras, classified as *Aequidens* s.l. in the past, are included in genera *Aequidens* (s.s.), *Bujurquina, Tahuantinsuyoa, Krobia, Laetacara* and *Cleithracara*. Remaining species of unclear origin presented probably by three phylogenetical clades being mentioned as *´Aequidens´* (in apostrophes). Similar situation has been solved in the genus *Cichlasoma* (s.l.). Many new genera have been described, species with uncertain systematic position are provisionally classified as *´Cichlasoma´* and twelve species of „true Cichlasomas" (*Cichlasoma* s.s.) being classed with Acaras. The position of the genus *Acaronia* is uncertain.

The breeding of Acaras is quite easy, but problems with aggressive attacks can occure from time to time. Acaras show extremely developed social behavior and it is necessary to bring together the group of about eight individuals for sexual-choice. The parent couple forms a biparental family with nearly equal roles of the male and the female. There is one more specific strategy of breeding so called larvophile mouthbrooding in genus *Bujurquina* and *Tahuantinsuyoa*. Both male and female take the hatched fry into the mouth in last two genera mentioned above.

Zuzana Musilová

Nová akara

Aequidens patricki

Jiří Lánský
Foto: Zuzana Musilová

Tato zajímavá jihoamerická akara pochází z povodí Amazonky v oblasti Peru. Žije zejména v ramenech řek Aguaytía a Pachitea. Vzhledově bych ji přiřadil k podobné u nás chované akaře Aequidens metae. Moje rybky dorostly délky těla do 12 cm, měřeno ke kořeni ocasu, a to nezávisle na druhu pohlaví. Dodnes jsem nenalezl jediný znak, kterým bych mohl odlišit samce od samice (výjimku tvoří samozřejmě období tření, kdy se rybky dají celkem jednoduše odlišit podle tvaru močopohlavní bradavky). Rybky jsou nenáročné, jako ostatně všechny akary, pouze se mi jeví jako relativně placké.

Z časových důvodů jsem se v minulosti musel vzdát mých oblíbených pancéřníčků a dalších sumců a moje malá pěstírnička v činžákovém domě se značně zredukovala. Nechtěl jsem se ale vzdát mého nejoblíbenějšího koníčka a touha nějakou rybu jednou za čas „udělat" zvítězila. Začal jsem hledat nové druhy a orientoval se na méně náročné rybky, jako jsou živorodky, nebo právě chováním velice zajímavé cichlidy. A jelikož jako výkupce akvarijních ryb jsem nechtěl nabourávat

dobré vztahy s chovateli, od kterých pravidelně rybky odebírám, a začít chovat něco z jejich sortimentu, musel jsem si najít rybu, která na českém trhu není nebo dlouho nebyla. Navíc mě vždy velice lákaly méně obvyklé druhy. Nalezl jsem kouzlo i útrapy v chovu různých druhů a forem živorodek a přišel jim i na chuť. Ale stále něco chybělo. Prostě teritoriální chování cichlid a jejich další projevy jsou originální.

Pak jsem narazil v nabídkovém sortimentu na zajímavou a patrně u nás doposud nechovanou hnědo-oranžovou cichlidu *Aequidens patricki*. Neváhal jsem a okamžitě si deset kousků nechal přivézt z Německa. Dovezené rybky byly v dobrém stavu a měřily okolo 6 cm. Vypustil jsem je do dvousetlitrové nádrže se zbytky neonek a dalších drobných teter, se kterými si během pár dnů hravě poradily a zpravily si transportem propadlá bříška.

Voda v nádrži je neupravovaná pražská vodovodní – pH slabě nad 7, celková tvrdost okolo 10 °N. Při krmení cyklopem, hovězím srdcem, patentkami a sušenými krmivy 1x denně a teplotě 22-24 °C rybky očividně rychle rostly. Obzvláště dva jedinci značně přerůstali ostatní a stávali se agresivnějšími jak vůči sobě, tak hlavně k ostatním menším akarám. Ovšem jakýkoli prudší pohyb před akváriem způsobil téměř šokový únik všech ryb do různých dutin a jeskyněk a okamžité přerušení šarvátek.

Aequidens patricki, samec s mláďaty

Přes toto extrémně plaché chování se právě dva největší jedinci vytřeli a snůšku společně bránili před ostatní obsádkou nádrže. Jejich plachost po výtěru opadla jen nepatrně, takže rodiče mnohdy snůšku nakladenou na oblém kameni úplně opustili a vraceli se zpět až po dlouhých minutách. Po několika dnech vyhrabali poblíž snůšky jamku hlubokou 3-4 cm o poloměru cca 4 cm a čerstvě vykulený plůdek okamžitě do tohoto místa přemisťovali. Jelikož jsem samotné tření tehdy nezaznamenal, stále jsem si nebyl stoprocentně jistý, který z rodičů je samec a který samice. Za dalších několik dnů se potěr rozplaval a postupně díky trvající plachosti rodičů ubýval.

Proto jsem se rozhodl k odebrání mladých rybek ze společného akvária a téměř všechny jsem je odsál hadicí do připravené třicetilitrové nádrže. Rybkám jsem držel teplotu okolo 23 °C a krmil je artemií, cyklopem a sušeným krmivem. Jejich růst byl celkem slušný a počet značný, takže záhy následovalo přemístění do dalších větších nádrží. Odchovaných rybek jsem měl přes čtyři sta a začaly problémy s odbytem. První dvě stovky se prodaly relativně jednoduše do všech koutů světa, ale dalších 200 kusů se stalo „stojákem“ a ryby se prodaly až značně přerostlé. To mě od dalších odchovů odradilo a až letos jsem opět nasadil obě ryby znovu do tření.

Přestože jsem tyto dva chovné jedince přemisťoval do různě velikých a odlišně zařízených nádrží, jejich plachost se nikdy výrazně neutlumila. Odchované mladé rybky jsou v klidu do velikosti 2 cm, pak začnou projevovat známky bázlivosti až šokování. S oblibou např. při náhlém rozsvícení mladé vyskakují hromadně z nádrží ven a překonání výšky 10-15 cm pro ně není překážkou opuštění akvária. Je-li na nádrži krycí sklo, mají ryby silně odřené hlavy, což jim na prodejnosti jistě nepřidá. Takže nezbývá, než mít vysoký prostor mezi hladinou a krycím sklem; pak jsou oděrky minimální.

Letos jsem chovný pár nasadil do tření ve stolitrovém akváriu. Přípravy na vlastní tření trvaly téměř čtrnáct dní, pravděpodobně opět díky velké plachosti těchto ryb. Doslova postupně přebagrovaly celé akvárium, když si vždy vyhlédly určitý úsek pro vyhrabání jamky až na samé skleněné dno. Stejně se však po těchto dvou týdnech vytřely na mnou již dávno připravený oblý kámen. Světle žlutých jiker o velikosti 1 mm bylo jistě přes 500 kusů ▸

Aequidens patricki, mladá ryba

Akary Aequidens patricki

a byl jsem rozhodnut všechny mladé v nádrži ponechat s rodiči a pozorovat tyto nádherně vybarvené cichlidy hlídající své mladé.

Aktivnějším jedincem v péči o potomstvo byla ryba nepatrně větší, která je samcem. Poznám ji nejen podle velikosti, ale i protože má roztrženou zadní část hřbetní ploutve. Rozdělení hřbetní ploutve je u těchto rybek celkem běžné a je způsobené šarvátkami mezi odrůstajícími jedinci. Samec byl natolik aktivní, že i čas od času vyhnal od trdliště samici, ovšem velice šetrně, a záhy ji opět pustil do těsné blízkosti. Za dva dny po výtěru začaly akary hloubit v písku opodál třecího kamene jamku. Další den byly vyhloubené dokonce dvě jamky, jedna hluboká až na sklo (v nádrži byla pěticentimetrová vrstva štěrku) a druhá podstatně mělčí. Jikry postupně tmavly a všechny se zdály být oplodněné a zdravé. Čtvrtý den byly jikry již téměř tmavě hnědé, rodiče je stále ovívali svými ploutvemi a bedlivě střežili. Dokonce jejich plachost opadla natolik, že když jsem se přiblížil těsně k čelní stěně nádrže, samec mě krátkými výpady s roztaženými skřelemi zastrašoval, než opět zacouval k trdlišti.

Pátý den od výtěru se plůdek vykulil a oba rodiče jej tlamkou okamžitě přemisťují do mělčího, ale od čelní stěny vzdálenějšího připraveného důlku. Další den jsem objevil třetí vyhloubenou jamku. Byla ze všech největší, štěrk byl vybrán až na skleněné dno a také byla od čelní stěny ze všech nejvzdálenější. Embrya sem již byla přemístěna a rodiče se o ně spokojeně starali, neboť se přestěhováním zbavili dalšího rušícího faktoru, kterým jsem byl já, když jsem je pozoroval. Moc radosti jsem z toho neměl, protože ryby si skutečně vybraly prostor, do kterého jsem téměř neviděl. Odolal jsem nutkání odstranit kořen, za nímž byly ukryty a odměnou mi byla za dalších pět dní přehlídka čerstvě vyvedených rozplavaných mladých společně s rodiči.

Mladých rybiček bylo značné množství, rodiče je brzy vůbec nestačili hlídat, a tak se rozplavaly po celé stolitrové nádrži. Od prvních dnů jsem je krmil živým a mraženým cyklopem a zpočátku přikrmoval žábronožkou. Růst nebyl zvlášť rychlý, mladé 10. den měřily 6 mm, 20. den 8-9 mm, 60. den 25 mm (ke kořeni ocasu); po 120 dnech dosahují první odchovanci v těle prodejní velikosti 3,5-4 cm. Povyrostlý potěr jsem již přikrmoval také patentkou, vločkovým krmením a hovězím srdcem.

Zhruba měsíc a půl od výtěru, to rybky měřily okolo dvou centimetrů, se schylovalo k novému tření rodičů. Odrostlých mladých bylo několik set a všechny plavaly ve stejné nádrži společně s rodiči. Rodiče začali čistit oblíbený kámen, vytvářeli si své teritorium a mladé přestávali do této oblasti pouštět. Jejich agresivita se den ode dne stupňovala, až čtvrtý den skončila pobitím zhruba poloviny potomků, přičemž další čtvrtina na následky agresivního chování rodičů pošla. Nechtěl jsem přijít úplně o všechny odchované rybky, proto jsem jich asi 30 přemístil do jiné nádrže s totožnými parametry vody. Rodiče se přesto již nevytřeli a zbylou stovku mladých nechali dorůst do prodejní velikosti ve své přítomnosti.

Měl jsem tedy možnost porovnat rychlost růstu akar ve dvou různých nádržích, jednou v celkem vysoké koncentraci zarybnění společně s rodiči a podruhé ve stejné nádrži s třetinovým zarybněním bez rodičů. První měsíc rybky rostly stejně, pak byl růst rybek bez rodičů podstatně rychlejší, ale během dalšího měsíce se vše téměř srovnalo a výsledným efektem bylo, že rybky v třetinové koncentraci bez rodičů byly o čtrnáct dní dříve prodejné. Rozdíly v růstu byly tedy téměř zanedbatelné, ba jsem přesvědčen, že mladé s rodiči by vyrostly rychleji, než odlovení jedinci, byl-li by tento pokus uskutečněn ve větší nádrži. V původním akváriu byla přece jen vyšší koncentrace zarybnění – jedna ryba na necelý litr vody.

Závěrem přeji všem nadšeným akvaristům mnoho úspěchů v rozmnožování podobných méně běžných rybek a novinek, protože jedině aktivním přístupem našich chovatelů a objevováním neznámého, či znovu objevováním méně známého, neztratí světoznámá „česká ryba“ své jméno a stále bude na celosvětovém trhu žádána v celé své široké škále. ■

Appendix VI.

Musilová Z., Říčan O., Novák J.

Phylogeography of the Neotropical cichlid genus *Andinoacara* from northern South America

presented in the *Cichlid Science Conference*, Swiss 2010

Conference poster

Phylogeography of the neotropical cichlid genus Andinoacara from northern South America

Zuzana Musilová [1;2;3], Oldřich Říčan[3], Jindřich Novák[3]

e-mail: zuzmus@gmail.com

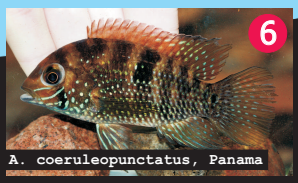
1) Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AV ČR v.v.i., 277 21 Liběchov, Czech Republic,
2) Department of Zoology, Faculty of Sciences, Charles University in Prague, Vinicná 7, 128 44 Praha 2, Czech Republic
3) Department of Zoology, Faculty of Sciences, University of South Bohemia, Branišovská 31, 370 05 Ceské Budejovice, Czech Republic

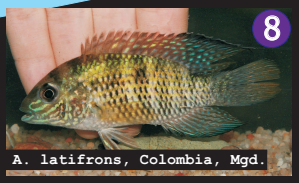

A. pulcher, Colombia, Mgd. 9


A. pulcher, Colombia, Ori. 7


A. pulcher, Colombia, Mgd. 9


A. cf. pulcher, Costa Rica 6


A. coeruleopunctatus, Panama 6


A. latifrons, Colombia, Mgd. 8


A. pulcher, Colombia, Mgd. 9

Results:

Andinoacaras colonised South America from the basins of the Pacific slope to the basins of the Atlantic slope. The crossing of the Andean barrier in the North possibly occurred in 4–8 mya.


A. cf. pulcher, Colombia pac. 5


A. cf. pulcher, Venezuela 8


A. sp. Maracaibo, Venezuela 8


A. pulcher, Colombia, Mgd. 9


A. pulcher, Trinidad


A. biseriatus, Colombia 5


A. sapayensis, Ecuador 4

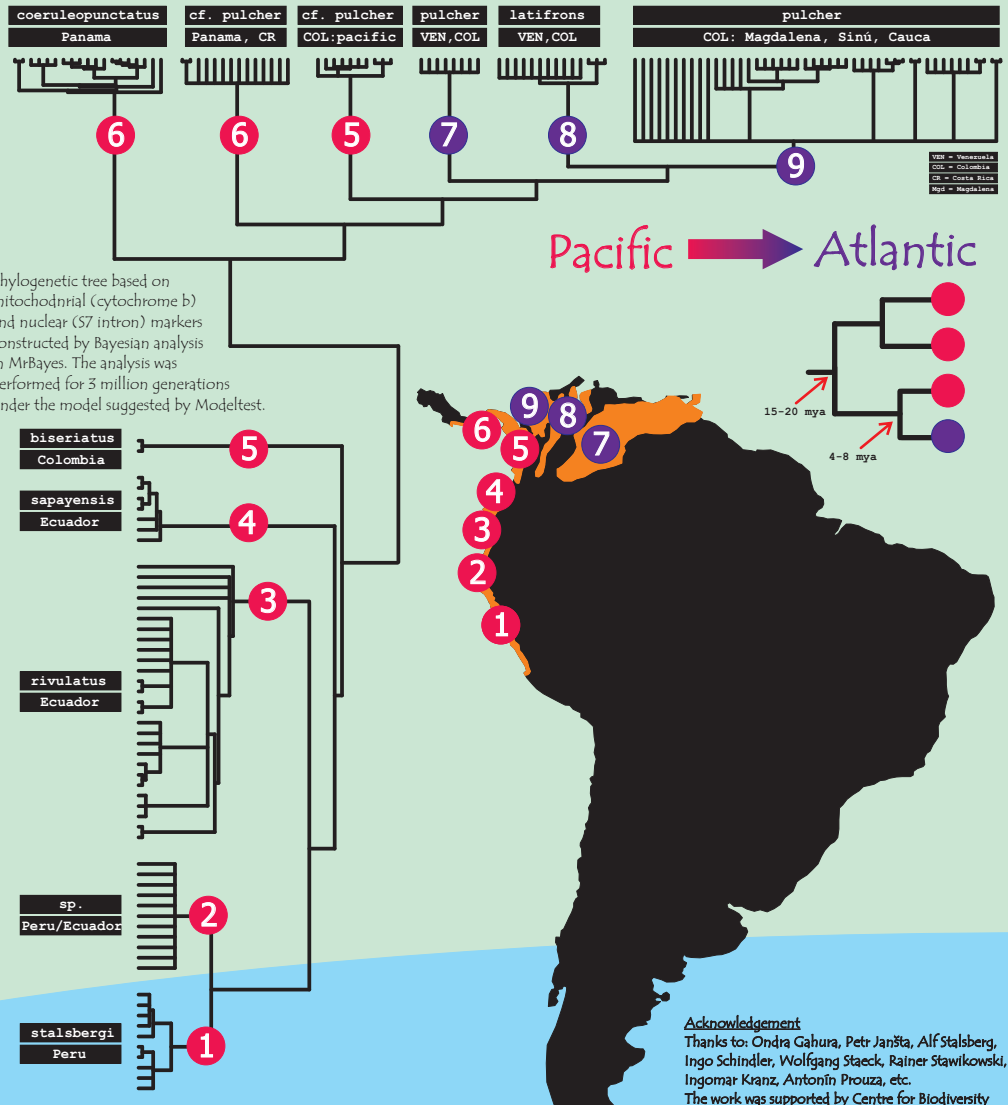

A. rivulatus, Ecuador 3


A. rivulatus, Ecuador 3


A. sp., Peru/Ecuador 2


A. stalsbergi, Peru 1


A. stalsbergi, Peru 1



Acknowledgement
Thanks to: Ondra Gahura, Petr Janšta, Alf Stalsberg, Ingo Schindler, Wolfgang Staeck, Rainer Stawikowski, Ingomar Kranz, Antonín Prouza, etc.
The work was supported by Centre for Biodiversity Research LC06073 (MSMT), IRP IAPG AVOZ50450515 and IRP FAPPZ, CZU MŠMT 6046070901.

Appendix VII.

Musilová Z., Kalous L., Petrtýl M., Holíková P.

Angola headwaters: the white spot on the *Serranochromis* phylogeographic map

presented in the *XIII European Congress of Ichthyology*, Lithuania 2009

Conference poster

Angola headwaters: the white spot on the Serranochromis phylogeographic map

Zuzana Musilová[1;2;3], Lukáš Kalous[2;3], Miloslav Petrtýl[3], Petra Chaloupková[4]

e-mail: zuzmus@gmail.com

1) Laboratory of Fish Genetics, Institute of Animal Physiology and Genetics AV ČR v.v.i., 277 21 Liběchov, Czech Republic,
2) Department of Zoology, Faculty of Sciences, Charles University in Prague, Viničná 7, 128 44 Praha 2, Czech Republic
3) Department of Zoology and Fisheries, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, 165 21 Praha 6, Suchdol, Czech Republic
4) Institute of Tropics and Subtropics, Czech University of Life Sciences Prague, 165 21 Praha 6 Suchdol, Czech Republic

Evidence of Okavango - Cuanza contacts based on different fish species:

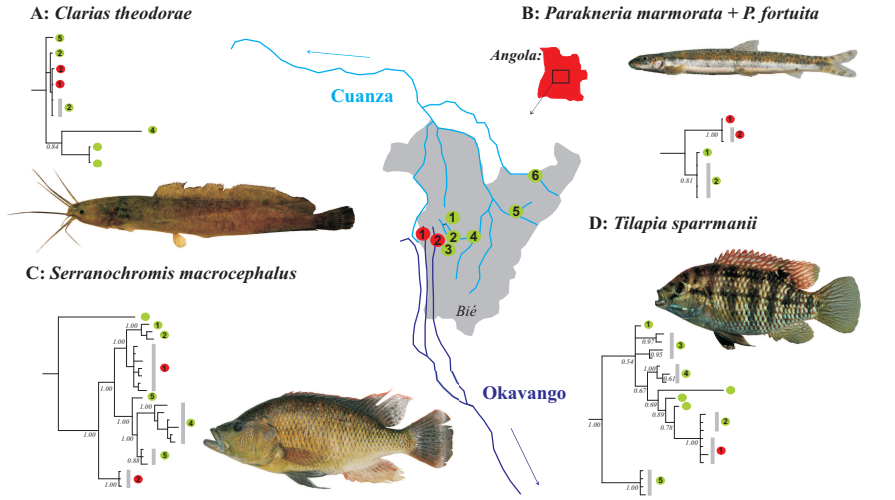


Figure 2: Evidence of Cuanza - Okavango contacts: A) *Clarias theodorae* - sharing haplotypes; based on CO1; B) Two species of *Parakneria* , based on CO1. C) *Serranochromis macrocephalus* - based on CO1, D-loop, NADH2 and S7. D) *Tilapia sparrmanii* - sharing haplotypes, based on C01 and S7 intron.

Although the African cichlids can be considered as enormously studied group of fishes, there is no record of this group from central Angola since 1975 due to lack of any field work in the area. Up to now our project comprises first molecular study on cichlids from Bié Plateau. This Angolan headwater region includes five important river basins in relatively small area and we have collected samples from three of them, i.e. Kubango (Okavango), Kwanza (Cuanza) and Kunene (Cunene). Here, we focused on phylogeographic study of serranochromine cichlids, based on molecular data of mitochondrial D-loop. We included additional sequences from GeneBank, especially from specimens originated from two other river systems missing in our sampling, i. e. Congo and Zambezi.

Serranochromis in Bié:

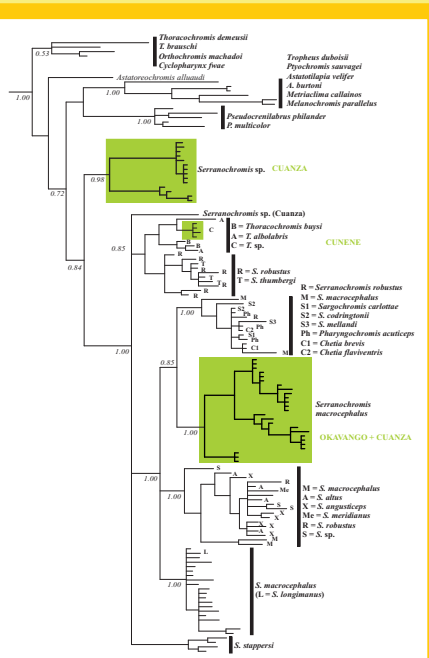
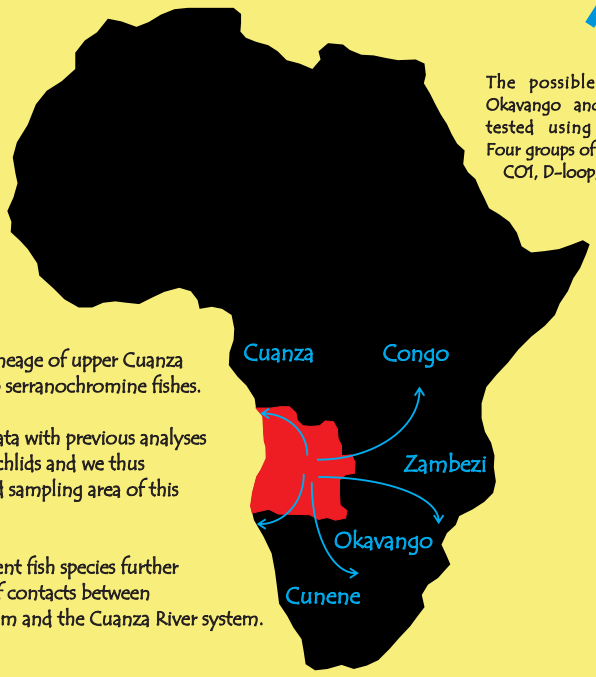


Figure 1: Bayesian phylogenetic tree based on sequences of mitochondrial D-loop. Green square marks samples collected in for this study in Bié province, Angola. Additionally, sequences from other studies available from GenBank were also included in this analysis. MrBayes run for 5 million generations.



The possible contacts of upper Okavango and upper Cuanza were tested using molecular markers. Four groups of fishes were analyzed on CO1, D-loop, NADH2 and S7 genes.

Results:

1) We found separate lineage of upper Cuanza and upper Okavango serranochromine fishes.

2) We combined our data with previous analyses in haplochromine cichlids and we thus significantly enlarged sampling area of this fish group in Africa.

3) Our results on different fish species further show the evidence of contacts between Okavango River system and the Cuanza River system.

Fish & Chicken

Děkujeme / thanks to: Vladimír Lima, Hynek Ciboch, Zdenka Horajsová, Blába, Honza, Martin Lošťák, Czech Ambassy in Luanda, Angolan students from Kuito

The work was supported by Official Development Cooperation Program of The Czech Republic specifically by project "Poradenství v oblasti chovu ryb a drubeže, Angola" No. MZe/B/2. Further support was realized within Centre for Biodiversity Research LC06073 (MSMT), IRP IAPG AVOZ50450515, GAUK 129510 and IRP FAPPZ, CZU MŠMT 6046070901.

Appendix VIII.

Musilová Z., Janko K.

Incongruence in phylogenetic signals: real conflict or insufficient taxon sampling?

presented in the
***Congress of the Society for Molecular Biology and Evolution*,**
Spain 2008

Conference poster

Incongruence in phylogenetic signals: real conflict or insufficient taxon sampling?

Zuzana Musilová[1,2)], Karel Janko[2)]

1) Department of Zoology, Faculty of Science, Charles University in Prague, Viničná 7, 128 44 Praha, Czech Republic
2) Laboratory of fish genetics, Institute of Animal Physiology and Genetics of the Academy of Sciences of Czech Republic, Rumburská 89, 277 21 Liběchov, Czech Republic; zuzmus@email.cz

Taxon sampling in action!!

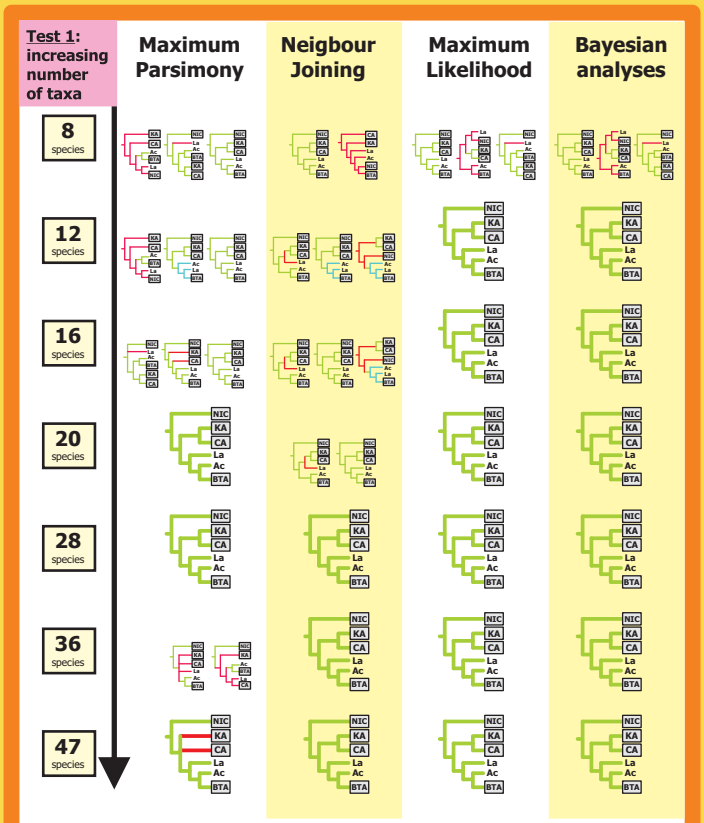


Fig. 1: The starting data set consisted of eight species and others were added until the complete data set reached 47 taxa. We performed in PAUP the MP analysis, NJ analyses under K2P and JC parameters set (slight differences found), ML analyses under the GTR+G+I model (suggested by Modeltest) and under the simplest model (difference only in bootstrap). We performed Bayesian analyses under both models as ML (difference found only in posterior probability).

Introduction

Phylogenetic analyses in Neotropical cichlids from subfamily Cichlasomatinae based on four markers (two mitochondrial - 16S rRNA, cytochrome b, and two nuclear genes - S7, RAG1) revealed several conflicts in results among different methods of analyses. We tested sensitivity of routinely used phylogenetic methods to taxon-sampling changes (i.e. distance analysis Neighbour Joining, Maximum parsimony, Maximum likelihood and Bayesian methods).

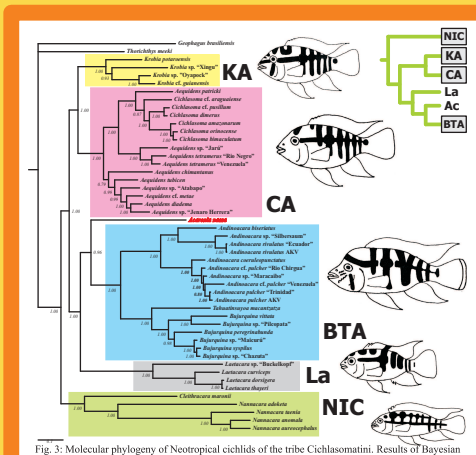


Fig. 3: Molecular phylogeny of Neotropical cichlids of the tribe Cichlasomatini. Results of Bayesian analysis based on data sets of four genetic markers (16S rRNA, cytochrome b, RAG1 and S7 intron). Five generic clades were found in results and one outstanding species *Acaronia*.

Conclusion

In cichlids, Bayesian analyses presented more consistent results than Maximum Parsimony. MP was more sensitive to taxon sampling changes.

Tests of sensitivity of methods to two types of taxon-sampling changes:

1) data sets with increasing number of taxa.

2) data sets with increasing number of fully sampled clades, and only one species in all other clades

We found strong incongruence among phylogenetic methods with increasing number of taxa in our data set. Bayesian method and Maximum likelihood displayed the most stable results in tree topology, while results of Neighbour Joining and Maximum parsimony method were found as incongruent.

Results

Bayes vs. MP:

We tested behaviour of Bayesian (BI) and Maximum Parsimony (MP) analyses with increasing number of included species. In cichlids, BI gave more consistent results than MP. Topologies from Bayesian were stable with 12 and more included species (of 47), while in MP incongruences were found still with 36 species included.

Our cichlid tree consists of 5 generic clades. We performed 30 analyses with 1, 2, 3 or 4 clades fully sampled, while all other clades were represented only by one species. Based on different data sets, Bayesian analyses showed more stable results with 4 different topologies found, while MP resulted in 12 topologies.

Test 2: Fully sampled clades:

Selected clades fully sampled, whereas all other clades are represented just by one species.

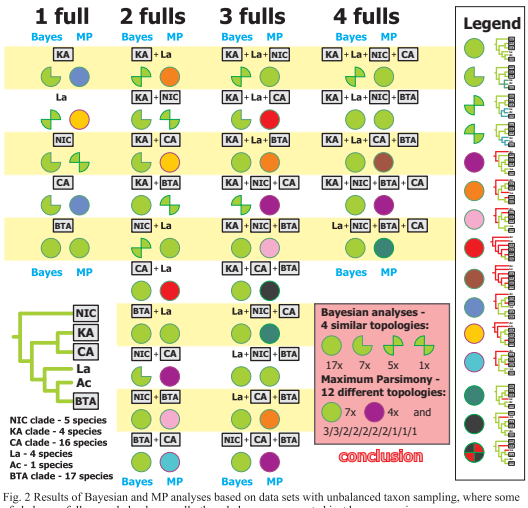


Fig. 2 Results of Bayesian and MP analyses based on data sets with unbalanced taxon sampling, where some of clades are fully sampled, whereas all other clades are represented just by one species.

Acknowledgements:

We would like to thank the financial support from GAUK 139407 and foundation of the Centre for Biodiversity LC06073 (MSMT). The Institute of Animal Physiology and Genetics receives continuous support from IRP IAPG (No. AV0Z50450515).