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Phylogeny and historical biogeography of trans-Andean cichlid fishes (Teleostei: Cichlidae)

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Abstract

We reconstruct the historical biogeography of cichlid fishes endemic to the trans-Andean region of NW South America. DNA sequences were used to study historical biogeography of the cichlid genera *Andinoacara* (Cichlasomatini) and *Mesoheros* (Heroini). Two event-based methodological approaches, parsimony-based Statistical Dispersal-Vicariance Analysis (S-DIVA) and likelihood-based Dispersal-Extinction Cladogenesis (DEC in Lagrange) were used for ancestral-area reconstructions. Molecular clock analysis of the whole group of Neotropical Cichlidae (using mtDNA and nucDNA markers) was calibrated using BEAST by six known cichlid fossils. The historical biogeography of both studied trans-Andean cichlid genera is best explained by a series of vicariance events that fragmented an ancestrally wider distribution. Both genera have a highly congruent vicariant historical biogeography in their shared distribution in the Colombian-Ecuadorian Choco. The Andean uplift and formation of the Central American isthmus strongly impacted the distribution patterns of the freshwater ichtyofauna in the NW Neotropics as suggested by the historical biogeography of the two studied cichlid groups. Despite strong congruence in their historical biogeography the two studied cichlid lineages (part of the tribe Cichlasomatini and Heroini, respectively) have highly different evolutionary substitution rates in the studied mtDNA cytb marker.

Key words

Andean uplift, *Andinoacara*, *Mesoheros*, Central American isthmus, DEC, Lagrange, cichlid dating.

Introduction

Cichlid fish diversity in trans-Andean South America

The core of trans-Andean South America (the Pacific slope river drainages of South America plus the Magdalena and Maracaibo river regions in the Caribbean drainage) is formed by the Tumbes-Choco-Magdalena (TCM) region. This is one of the 25 biodiversity hotspots (Reid, 1998; Myers *et al*., 2000), and Choco further represents one the wettest regions on the planet. The TCM region, extending from eastern Panamá to north-western Perú, is bordered by two additional biodiversity hotspots, Mesoamerica in the northwest, and the Tropical Andes (in terms of global biodiversity levels the richest region) in the east.

Compared with the high biodiversity of terrestrial animals and plants, and with the great watersheds on the Atlantic (cis-Andean) side of South America (SA), the

freshwater fish fauna of trans-Andean SA is relatively sparse. The TCM hotspot has about 250 species of freshwater fish (nearly half of which are endemic) in 54 families (Mittermeier *et al*., 2005). In total, representatives of four cichlid groups (*Andinoacara* Musilová, Říčan & Novák 2009, *Mesoheros* Mcmahan & Chakrabarty 2015 in McMahan *et al.* 2015, *Caquetaia* Fowler 1945, and *Geophagus* Heckel 1840) are distributed in the TCM region and only two of these genera are distributed throughout the whole TCM region (*Andinoacara* and *Mesoheros*). *Mesoheros* is endemic to the TCM region (bordered in the south by the Tumbes River drainage in NW Perú), while *Andinoacara* distribution includes also the Magdalena basin and reaches even further in trans-Andean SA (Lake Maracaibo) and also into cis-Andean northern SA (in the Orinoco River drainage and on Trinidad), and in the south almost to Lima in central Peru.

Andinoacara belongs to the tribe Cichlasomatini and presently includes eight valid species (Musilová *et al*., 2009a, b; one of them described recently – Wijkmark *et al*., 2012). Due to its distribution range it represents one of the most suitable cichlid genera for historical biogeography studies of trans-Andean SA related to the uplift of the Andes and formation of the Central American isthmus. The present study includes all of the valid *Andinoacara* species and three additional lineages with uncertain taxonomic status.

Mesoheros is a representative of the tribe Heroini (sister taxonomic group to Cichlasomatini) and is related to a subgroup of Middle American cichlid fishes (Říčan *et al*., 2013). Samples from the whole distribution area of the group except for south-central Pacific Colombia (putative *M. gephyrus*) are included in this study.

Historical Biogeography of trans-Andean South America

NW South America represents a region with a high level of geological activity where large changes have occurred in the geological past. The uplift of Andes initiated already in the Late Cretaceous (Lundberg *et al*., 1998) but was most prominent in the second half of the Cenozoic and has heavily impacted the geological and biological history of South America (Lundberg *et al*., 1998; Antonelli *et al*., 2009). It significantly reshaped the river systems on the whole continent including the large proto-Orinoco-Amazon system (Rodriguez-Olarte *et al*., 2011), and 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 configuration of the major river basins occurred quite recently, between 10 and 3 Mya, when e.g. trans-Andean SA was separated from the Amazon, and when Orinoco became independent from the paleo-Magdalena and the Amazon river (except for two adjacent connections still persistent to this day). Lake Maracaibo, the former delta of the proto-Orinoco-Amazon system, was formed by rise of the Merida Andes in Late Pliocene (Rodriguez-Olarte *et al*., 2011). Such intense watershed reshaping can strongly impact the species distribution as well as promote the speciation process (Joyce *et al*., 2005). Additionally, species distribution patterns were also affected by concurrent periodical marine incursions into the continent (Lundberg *et al*., 1998; Montoya-Burgos, 2003). Lastly, the incipient formation of the Panama isthmus in the Miocene/Pliocene (followed by its break up and renewed formation around 3 Mya; Coates *et al*., 2004) played a major role in the colonization of Central America by freshwater fishes (Bermingham & Martin, 1998; Smith & Bermingham, 2005; Říčan *et al*., 2013).

Prior to ca. 10 Mya contacts between cis- and trans-Andean regions would have been enabled by at least two connections. First, the uplift of the Northern Andes (Western, Central and Eastern Cordilleras in Colombia and Merida Andes in Venezuela) was incomplete and included also marine incursions until 11.8 Mya (Lundberg *et al*., 1998). Second, the Western Andean portal, a connection and also putative marine incursion into the continent between the Northern and Southern Andes at the current Ecuadorian-Peruvian border, probably persisted up to 13 – 11 Mya (Antonelli *et al*., 2009).

Distribution patterns in Neotropical fishes

Generally, allopatric speciation promoted by vicariance is the most common way of fish speciation in the Neotropical region and this is surprisingly true even for the Amazon, despite the presence of only few obvious hard barriers (Albert & Reis, 2011). The mountainous Northern South America is a typical example of a region with distribution barriers represented by several mountains ranges. The uplift of the four northern Andean mountain chains (Western, Central and Eastern Cordillera in Colombia, and the Merida Andes in Venezuela) during last 10 My strongly impacted the biota inhabiting the original continuous area of the recent Magdalena, Maracaibo and trans-Andean drainages. Many species of freshwater fishes in this region went extinct here but have (or their close relatives have) survived in the larger river systems of neighbouring South America, such as the Amazon or Orinoco (Rodriguez-Olarte *et al*., 2011). There are only a few genera shared across more river systems in Northern South America, and both dispersal and vicariance theories were used to explain these distribution patterns (Rodriguez-Olarte *et al*., 2011). Cichlids (i. e. genus *Andinoacara*) represent one of the lineages widely distributed throughout virtually all of NW South America. Most of the cichlid species in this region have allopatric (i.e. non-overlapping) distribution areas, except for the trans-Andean Choco region where basal species of two *Andinoacara* lineages live in sympatry (and syntopy; ZM and OG, pers. obs.).

The main goal of the present study

This is the first detailed study of cichlids from trans-Andean and NW South America focusing on their species diversity and historical biogeography. Previous studies considered the whole tribes Cichlasomatini and Heroini and have provided the biogeographical overview on a broader scale (Musilová *et al*., 2008; Říčan *et al*., 2013). The historical biogeography approach is used herein to reconstruct ancestral areas and the evolutionary histories by distinguishing between vicariance and dispersal events during evolution of the two cichlid genera. Further, the historical biogeography of the two cichlid genera is dated using molecular clocks calibrated by fossils in a wider all-Neotropical cichlid context. Lastly, we interpret the historical biogeography of the cichlid fishes in comparison with the geological history of NW South America.

Material and Methods

Material used in this study

The material of more than 150 specimens of eight *Andinoacara* species and three *Mesoheros* species was collected through museum collections, university collections, via aquarium trade importers and several private collections of aquarium hobbyists including our own for the specific task of the present study. Samples were only obtained from trusted aquarium hobbyists or importers who carry pure lineages with known localities of origin and these were verified independently with multiple samples. Finally, samples from previous studies from GenBank were used to complement the data sets (Table 1).

Our sampling covers virtually the whole distribution areas of both cichlid groups. The areas not represented in our data sets are the Colombian basins Dagua, Patia and Mira from departments Valle de Cauca, Cauca and Nariño with a remaining complicated geopolitical situation.

DNA sequencing and phylogenetic analyses

The mitochondrial cytochrome b gene was amplified using the primers FishCytB-F and TrucCytB-R from Sevilla *et al*. (2007). The process of DNA isolation, amplification and sequencing, as well as sequence editing and alignment was identical to the protocol used in Musilová *et al.* (2009a) and Concheiro-Pérez *et al*. (2007). All obtained sequences in this study were submitted to GenBank (KJ127312 – KJ127457 for *Andinoacara* and KJ127458 – KJ127474 for *Mesoheros*).

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 genetic evolution 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 10 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. Bayesian analyses were performed for the "full data set", where all samples were included and also for the "haplotype data set" for unique haplotypes.

Molecular clock and fossil calibration of Neotropical cichlids

Since no fossil is known for *Andinoacara* and only one fossil is known for a related genus of *Mesoheros* (†*Nandopsis woodringi*), we have used an external dating approach. First we have reconstructed the phylogenetic tree for all neotropical cichlids based on published data (see Appendix S1 and Figure S1). This "Neotropical cichlid tree" reconstructed by Bayesian Evolutionary Analyses by Sampling Trees (BEAST, Drummond & Rambaut, 2007) was then calibrated by all known cichlid fossils, for which both the phylogenetic position and a non-ambiguous age estimate are available (Appendix S1). Based on this "Neotropical cichlid tree" we estimated the age of both *Andinoacara* and *Mesoheros* root nodes and we used these estimates as secondary calibration points for the molecular clock analyses of both studied groups. The detailed genus-level ultrametric phylogenetic trees were obtained by BEAST analyses under the relaxed molecular clock models. The run in BEAST was performed for 10 million generation under the evolutionary model as suggested by jModeltest (for detailed parameters see Appendix S1).

Additionally, divergence rates of cytochrome b were calculated as the uncorrected p-distances based on haplotypes for each node in PAUP software (Swofford, 2003).

Historical biogeography analyses

We performed historical biogeography analyses by two approaches, the parsimony-based Statistical Dispersal – Vicariance Analyses (S-DiVA, Yu *et al*., 2010) and the maximum-likelihood based Dispersal-Extintion-Cladogenesis model (DEC) in Lagrange (Ree & Smith, 2008). The goal of the analyses was to reconstruct vicariance/dispersal scenarios for both *Andinoacara* and *Mesoheros*, as well as putative ancestral areas of both genera and all

Table 1. Samples used in the phylogenetic study with the collection code of the DNA voucher. For the sequences downloaded from GenBank, the accession number (GB no.) is mentioned.

Species	collection code or GenBank acc. no.	locality (if known)
outgroups for genus *Andinoacara:*		
Aequidens metae Eigenmann 1922	EF432927	unknown / previous studies
Aequidens diadema (Heckel 1840)	EF432930	unknown / previous studies
Bujurquina huallagae (Kullander 1986) "Chazuta"	EU706355	unknown / previous studies
Bujurquina peregrinabunda (Kullander 1986)	EF432954	unknown / previous studies
Bujurquina sp. "Pilcopata"	EU706358	unknown / previous studies
Bujurquina sp. Maicuru	EF432953	unknown / previous studies
Bujurquina syspilus (Cope 1872)	EF432952	unknown / previous studies
Cichlasoma amazonarum Kullander 1983	EF432914	unknown / previous studies
Cichlasoma dimerus (Heckel 1840)	EF432941	unknown / previous studies
Krobia potaroensis (Eigenmann 1912)	EF432917	unknown / previous studies
Krobia xinguensis Kullander 2012	EF432931	unknown / previous studies
Tahuantinsuoya macantzatza Kullander 1986	EF432915	unknown / previous studies
ingroup:		
Andinoacara sp. "Choco"	Atra021	Certegui, Rio Atrato, Colombia
Andinoacara sp. "Choco"	Atra022	Certegui, Rio Atrato, Colombia
Andinoacara sp. "Choco"	Atra023	Certegui, Rio Atrato, Colombia
Andinoacara sp. "Choco"	SaJu021	Rio San Juan, Colombia
Andinoacara sp. "Choco"	SaJu023	Rio San Juan, Colombia
Andinoacara sp. "Choco"	SaJu024	Rio San Juan, Colombia
Andinoacara sp. "Choco"	Atra0914	Rio Atrato, Colombia
Andinoacara coeruleopunctatus (Kner 1863)	CoRi03	Rio Coto, Costa Rica
Andinoacara coeruleopunctatus (Kner 1863)	CoRi04	Rio Coto, Costa Rica
Andinoacara coeruleopunctatus (Kner 1863)	Chir01	Chiriqui, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Chir031	Chiriqui, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Chir033	Chiriqui, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Pan02	Chiriqui, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Pan03	Chiriqui, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Dar03	Darien, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Dar061	Darien, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Dar062	Darien, Panama
Andinoacara coeruleopunctatus (Kner 1863)	Dar07	Darien, Panama
Andinoacara coeruleopunctatus (Kner 1863)	aqua03	unknown
Andinoacara coeruleopunctatus (Kner 1863)	P01	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P02	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P04_1	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P04_2	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P06_2	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P07_1	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P07_2	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P07_3	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P07_4	Canal zone, Panama
Andinoacara coeruleopunctatus (Kner 1863)	P08	Canal zone, Panama
Andinoacara pulcher (Gill 1858) I	Meta09k	Villavicencio, Rio Meta (Orinoco), Colombia
Andinoacara pulcher (Gill 1858) I	Ae05	Los Llanos, Rio Orinoco, Venezuela
Andinoacara pulcher (Gill 1858) I	Ae04	Los Llanos, Rio Orinoco, Venezuela
Andinoacara pulcher (Gill 1858) I	Meta02c	Rio Meta (Orinoco), Colombia
Andinoacara pulcher (Gill 1858) I	Meta03c	Rio Meta (Orinoco), Colombia
Andinoacara sp. "Orinoco"	aqua06	Rio Cuchivero (Orinoco), Venezuela
Andinoacara sp. "Orinoco"	ICCU0706	Rio Chirgua, Orinoco, Venezuela
Andinoacara sp. "Orinoco"	Orisp	Orinoco, Venezuela
Andinoacara pulcher (Gill 1858) II	ICCU0710	Maracaibo, Venezuela
Andinoacara pulcher (Gill 1858) II	upMag08A	Neiva, upper Magdalena, Colombia
Andinoacara pulcher (Gill 1858) II	Meta04c	Rio Meta (Orinoco), Colombia
Andinoacara pulcher (Gill 1858) II	upMag05H	Honda, upper Magdalena, Colombia
Andinoacara pulcher (Gill 1858) II	Meta10k	Villavicencio, Rio Meta (Orinoco), Colombia
Andinoacara latifrons (Steindachner 1878)	Cauc04	Rio Cauca (Magdalena), Colombia

Table 1 continued.

Species	collection code or GenBank acc. no.	locality (if known)
Andinoacara latifrons (Steindachner 1878)	Cauc05	Rio Cauca (Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	loMag02	lower Magdalena, Colombia
Andinoacara latifrons (Steindachner 1878)	loMag04	lower Magdalena, Colombia
Andinoacara latifrons (Steindachner 1878)	loMag051	lower Magdalena, Colombia
Andinoacara latifrons (Steindachner 1878)	loMag053	lower Magdalena, Colombia
Andinoacara latifrons (Steindachner 1878)	loMag054	lower Magdalena, Colombia
Andinoacara latifrons (Steindachner 1878)	loMag055	lower Magdalena, Colombia
Andinoacara latifrons (Steindachner 1878)	Nech01	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Nech02	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Nech03	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Nech04	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Nech072	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Nech073	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Nech074	Rio Nechi (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Sinu012	Rio Sinu, Colombia
Andinoacara latifrons (Steindachner 1878)	Sinu013	Rio Sinu, Colombia
Andinoacara latifrons (Steindachner 1878)	Sinu014	Rio Sinu, Colombia
Andinoacara latifrons (Steindachner 1878)	Sinu08	Rio Sinu, Colombia
Andinoacara latifrons (Steindachner 1878)	Sinu09	Rio Sinu, Colombia
Andinoacara latifrons (Steindachner 1878)	Jorg031	Rio San Jorge (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Jorg032	Rio San Jorge (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Jorg033	Rio San Jorge (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Jorg034	Rio San Jorge (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Jorg041	Rio San Jorge (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Jorg13	Rio San Jorge (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Cesa02	Rio Cesar (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Cesa03	Rio Cesar (lower Magdalena), Colombia
Andinoacara latifrons (Steindachner 1878)	Cesa08	Rio Cesar (lower Magdalena), Colombia
Andinoacara biseriatus (Regan 1913)	Atra01kra	Rio Atrato, Colombia
Andinoacara biseriatus (Regan 1913)	Atra01	Certegui, Rio Atrato, Colombia
Andinoacara sapayensis (Regan 1903)	E01_1	Rio Santiago, Ecuador
Andinoacara sapayensis (Regan 1903)	E19_1	Rio Santiago, Ecuador
Andinoacara sapayensis (Regan 1903)	E41_1	Rio Santiago, Ecuador
Andinoacara sapayensis (Regan 1903)	E42_1	Rio Santiago, Ecuador
Andinoacara sapayensis (Regan 1903)	E43_1	Rio Santiago, Ecuador
Andinoacara sapayensis (Regan 1903)	E44_2	Rio Santiago, Ecuador
Andinoacara sapayensis (Regan 1903)	Sapa01	Rio Santiago, Ecuador
Andinoacara rivulatus (Günther 1860)	RiGU	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	Qu02	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	Qu03	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	Qu04	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	Qu05	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	E02_1	Esmeraldas, Ecuador
Andinoacara rivulatus (Günther 1860)	E03_1	Esmeraldas, Ecuador
Andinoacara rivulatus (Günther 1860)	E04_1	Esmeraldas, Ecuador
Andinoacara rivulatus (Günther 1860)	E05_2	Esmeraldas, Ecuador
Andinoacara rivulatus (Günther 1860)	E09_1	Guayas coast, Ecuador
Andinoacara rivulatus (Günther 1860)	E09_1B	Guayas coast, Ecuador
Andinoacara rivulatus (Günther 1860)	E09_1C	Guayas coast, Ecuador
Andinoacara rivulatus (Günther 1860)	E06_2	Daule, Ecuador
Andinoacara rivulatus (Günther 1860)	E07_1	Daule, Ecuador
Andinoacara rivulatus (Günther 1860)	E10_1	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	E11_1	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	E12_1	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	E13_1	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	E17_1	Rio Zamora, Ecuador
Andinoacara rivulatus (Günther 1860)	E11_1C	Guayaquil. Ecuador
Andinoacara rivulatus (Günther 1860)	ASJ7	Rio Zamora, Ecuador
Andinoacara rivulatus (Günther 1860)	E17_1	Rio Zamora, Ecuador

Table 1 continued.

Species	collection code or GenBank acc. no.	locality (if known)
Andinoacara rivulatus (Günther 1860)	E18_1B	Rio Zamora, Ecuador
Andinoacara rivulatus (Günther 1860)	E18_1C	Rio Zamora, Ecuador
Andinoacara rivulatus (Günther 1860)	E18_2u	Rio Zamora, Ecuador
Andinoacara rivulatus (Günther 1860)	E20_1	Rio Zamora, Ecuador
Andinoacara rivulatus (Günther 1860)	E20_1B	Rio Zamora, Ecuador
Andinoacara cf. *stalsbergi*	E14_1B	Rio Chira, Peru
Andinoacara cf. *stalsbergi*	E14_1D	Rio Chira, Peru
Andinoacara cf. *stalsbergi*	E14_2A	Rio Chira, Peru
Andinoacara cf. *stalsbergi*	E14_2B	Rio Chira, Peru
Andinoacara cf. *stalsbergi*	E14_1	Rio Chira, Peru
Andinoacara cf. *stalsbergi*	E15_1	Rio Chira, Peru
Andinoacara cf. *stalsbergi*	E16_1	Rio Chira, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2009	SIL01	Rio Reque, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2010	Je01b	Rio Jequetepeque, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2011	Je03_1	Rio Jequetepeque, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2012	Je03_2	Rio Jequetepeque, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2013	Pi03b	Rio Piura, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2014	Pi04b	Rio Piura, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2015	Pi05b	Rio Piura, Peru
Andinoacara stalsbergi Musilová, Schindler & Staeck 2016	Pi09b	Rio Piura, Peru
outgroups for 'Heros' festae group		
Herichthys tamasopoensis Artigas Azas 1993	DQ990716	unknown / previous studies
Astatheros macracanthus (Günther 1864)	DQ990696	unknown / previous studies
Amphilophus citrinellus (Günther 1864)	AY843434	unknown / previous studies
Australoheros facetus (Jenyns 1842)	AY998667	unknown / previous studies
ingroup:		
Mesoheros atromaculatus (Regan 1912)	AY843346	Rio San Juan, Colombia
Mesoheros atromaculatus (Regan 1912)	AY843347	Rio San Juan, Colombia
Mesoheros atromaculatus (Regan 1912)	AY843342	Rio Mandinga, Panama
Mesoheros atromaculatus (Regan 1912)	AY843343	Rio Mandinga, Panama
Mesoheros atromaculatus (Regan 1912)	C132B	Rio Atrato, Colombia
Mesoheros atromaculatus (Regan 1912)	AF009939	Rio Baudo, Colombia
Mesoheros atromaculatus (Regan 1912)	AY843345	Rio Baudo, Colombia
Mesoheros atromaculatus (Regan 1912)	C101	Choco coast, Colombia
Mesoheros atromaculatus (Regan 1912)	C111	Choco coast, Colombia
Mesoheros atromaculatus (Regan 1912)	C161A	Golfo de Uraba, Colombia
Mesoheros atromaculatus (Regan 1912)	C161B	Golfo de Uraba, Colombia
Mesoheros atromaculatus (Regan 1912)	C161C	Golfo de Uraba, Colombia
Mesoheros atromaculatus (Regan 1912)	C162A	Golfo de Uraba, Colombia
Mesoheros atromaculatus (Regan 1912)	C162B	Golfo de Uraba, Colombia
Mesoheros ornatus (Regan 1905)	E401	Rio Santiago, Ecuador
Mesoheros ornatus (Regan 1905)	E441	Rio Santiago, Ecuador
Mesoheros festae (Boulenger 1899)	E061	Rio Daule, Ecuador
Mesoheros festae (Boulenger 1899)	AY843351	Rio Tumbes, Peru
Mesoheros festae (Boulenger 1899)	AY843352	Rio Tumbes, Peru
Mesoheros festae (Boulenger 1899)	E102A	Guayaquil, Ecuador
Mesoheros festae (Boulenger 1899)	E102B	Guayaquil, Ecuador
Mesoheros festae (Boulenger 1899)	E081	Guayas coast, Ecuador
Mesoheros festae (Boulenger 1899)	E102C	Guayaquil, Ecuador
Mesoheros festae (Boulenger 1899)	E051A	Rio Daule, Ecuador
Mesoheros festae (Boulenger 1899)	E051B	Rio Daule, Ecuador

nodes. We used S-DiVA for the datasets of both studied cichlid groups providing detailed biogeographic information (up to 10 distribution regions were allowed in order to allow for complete vicariance, see below). Such data set was however too large for the DEC analysis and it did not converge. We further applied both S-DiVA and DEC analyses on the genus *Andinoacara* with larger-scale biogeographic units (four "super regions") to specifically address the question about the role of trans-Andean region in the evolution of this genus.

For both analyses, we used only unique haplotypes to reconstruct the ultrametric trees to approximate the assumption of fully resolved (bifurcated) tree required by biogeographic softwares. Haplotypes were defined in the FABOX software (Villesen, 2007) and the trees were reconstructed by both MrBayes and BEAST methods. The fully bifurcated trees served then as inputs for the biogeography analyses.

The S-DiVA represents an event-based approach for the reconstruction of historical biogeography and it summarizes the reconstructions across a sampled pool of trees from the Bayesian analysis runs. In S-DiVA we analysed the data sets from four different runs of BEAST to find the ancestral areas and to reconstruct the biogeographic scenario of the genera *Andinoacara* and *Mesoheros*. The last 5000 of sampled trees from each BEAST run were used (from the total of 10000 sampled trees) for the S-DiVA analyses. Similarly, the MrBayes consensus tree as well as its 5000 trees randomly sampled from the MrBayes run .t files served as an input for S-DiVA.

The DEC model analysis in Lagrange was performed using the haplotype MrBayes and BEAST trees, similar as for the S-DiVA analysis. The input file for Lagrange software (Ree & Smith, 2008) was generated on the online web configurator (http://www.reelab.net/lagrange/configurator/index) requiring the ultrametric tree and the distribution matrix. We performed the analysis for different settings considering the maximum number of areas allowed in the ancestral areas (from 2 to 5 having four bigeographic regions + outgroup).

The following distribution regions were used for coding of biogeographical analyses in *Andinoacara* and in *Mesoheros*: A – Orinoco, B – Maracaibo, C – Magdalena, D – Tuira (eastern Panamá – Pacific slope), E – Chiriqui (SW Costa Rica plus W Panama – Pacific slope), F – Choco (Colombia), G – Cayapas/Santiago system (Ecuador, Pacific slope), H – Ecuador + Rio Tumbes (Pacific slope), I – Peru (Pacific slope), J – Chagres (eastern Panamá – Caribbean slope) (see also maps in Fig 2 and 3). Fishes from the genus *Andinoacara* are found in nine (A–I) from these ten regions and *Mesoheros* distribution covers four regions (F, G, H, J).

In the more widely distributed genus *Andinoacara* we additionally performed a biogeographic analysis with larger "super regions" (more akin to the wider freshwater ecoregions sensu Abell *et al*. (2008)). These "super regions" were: TR – trans-Andean region of South America (corresponds to the aforementioned regions F, G, H and I), MG – Magdalena basin (= region C), OR – Orinoco & Maracaibo basin (= regions A and B) and CA – Central America (= regions D and E).

All biogeographic analyses were run as unconstrained (connections between all areas were allowed) and the maximum of the areas allowed in the ancestral area corresponding to the number of regions + 1 (outgroup), i.e. 10 and 5, respectively, not to introduce any bias in *a priori* constraining the parameters. Further, analyses with different set of allowed maxareas was performed to test the data under different assumptions.

Results

Phylogeny of trans-Andean cichlids

The genus *Andinoacara* is divided into two well supported clades (the *A. pulcher* group = the northern clade, and *A. rivulatus* group = the southern clade; Fig. 1a, 2). Within the northern clade (*A. pulcher* group), three species and two undescribed lineages were found as monophyletic clades. The lineage *A.* sp. "Choco" distributed in the pacific-coast basins is either basal clade of whole northern clade (MrBayes analysis) or sister group to the Central American *A. coeruleopunctatus* (Kner 1863); (BEAST analysis). *Andinoacara pulcher* (Gill 1858), *A. latifrons* (Steindachner 1878) and *A.* sp. "Orinoco" represent the crown group of the northern clade.

The southern clade (*A. rivulatus* group) is represented by four well defined species. The basalmost is *A. biseriatus* (Regan 1913) from the pacific basins of Colombia (Atrato, San Juan), followed by *A. sapayensis* (Regan 1903) from northern Ecuador and then the sister species *A. rivulatus* (Günther 1860) and *A. stalsbergi* Musilová, Schindler & Staeck 2009 from Ecuador and Peru, respectively (Fig. 1). *A. blombergi* Wijkmark, Kullander & Salazar 2012 clusters non-monophyletically within the *A. rivulatus* populations.

In *Mesoheros* all three studied species were found as monophyletic with strong support (Fig. 1b) and *M. atromaculatus* (Regan 1912) from Atrato, San Juan, and neighbouring river drainages represents the northern sister group to the well supported southern clade of *M. ornatus* (Regan 1905) from northern Ecuador and southern Colombia and *M. festae* (Boulenger 1899) from the rest of Ecuador and northernmost Peru.

Distribution patterns of trans-Andean cichlids

The observed distribution patterns in both cichlid genera (*Andinoacara* and *Mesoheros*) are highly congruent. In both genera, two sister clades are present, one with the distribution in the southern and one in the northern part of the area. The three species of *Mesoheros* can be considered as corresponding to the three southern *Andinoacara rivulatus* group species when excluding *A. stalsbergi* (the only Neotropical cichlid in its southernmost trans-Andean area). The northern clades of both genera have made it into Central America, where they, however, are not sympatric (unlike in Choco), with *M. atromaculatus* limited to the southernmost isthmus on the Caribbean slope while *A. coeruleopunctatus* to the Pacific slope up to southern Costa Rica (Figs 2 and 3).

In both genera there is an old divergence between the Santiago river system (Western Ecuador) and the rest of Ecuador (+ Peru) (Fig. 1, 2 and 3). The Santiago river system in Western Ecuador (together with neighbouring southern Colombia) represents therefore an important

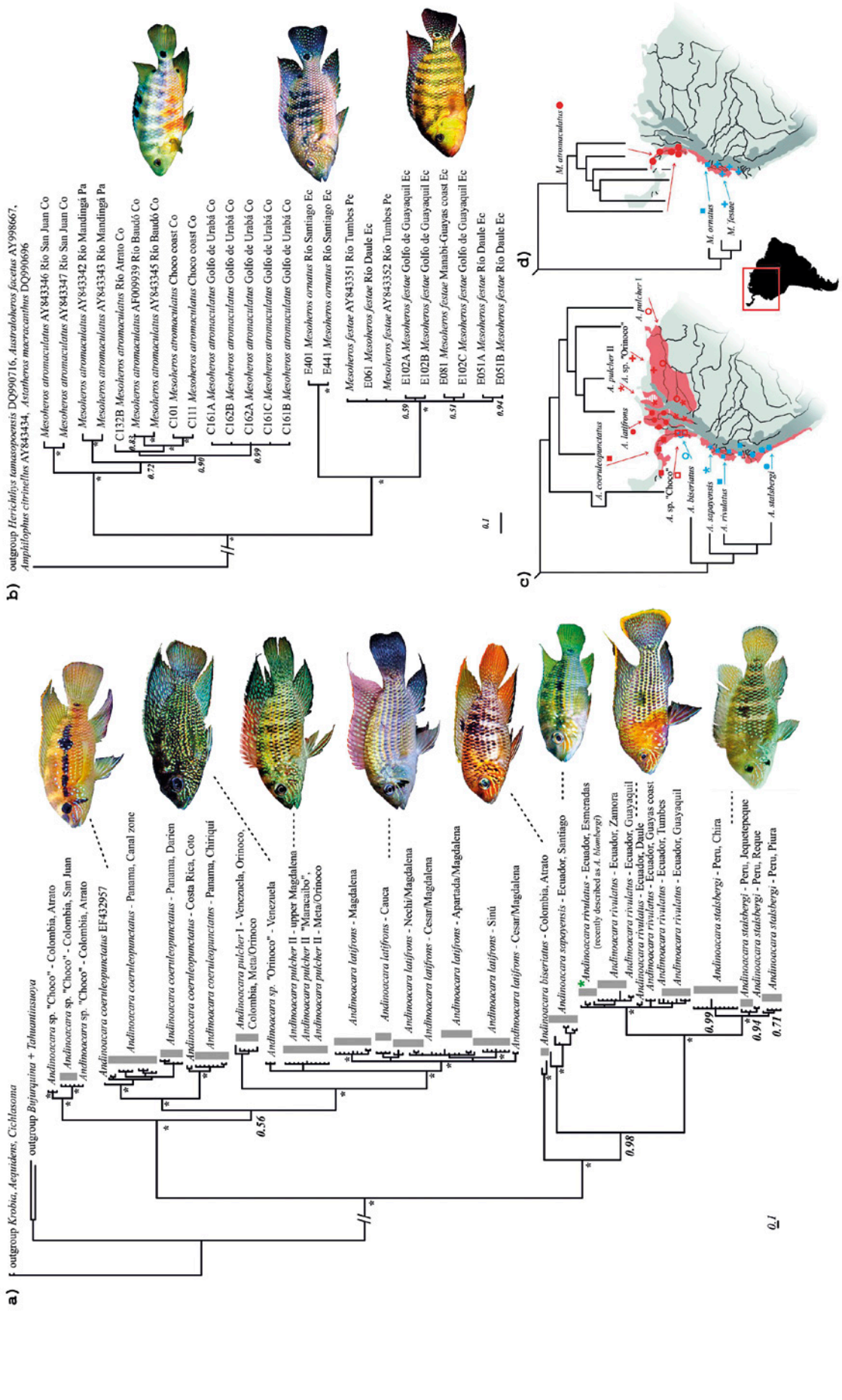


Fig. 1. Phylogenetic relationships of trans-Andean cichlids with distributions and localities. **A)** Bayesian tree of the genus *Andinoacara*. Result of Bayesian analyses in MrBayes with 10 million generation based on cytochrome *b* "full data set" including samples from 7 valid *Andinoacara* species and three putative undescribed species. Position of the recently described questionable species *A. blombergi* within the *A. rivulatus* marked by a green star symbol. **B)** Bayesian phylogeny of the *Mesoheros* genus based on cytochrome b "full data set". MrBayes run of 10 million generations; numbers in the nodes represent the Bayesian posterior probability values for the nodes. Asterisks mark nodes with posterior probability support equal to 1.00. **C+D)** distribution maps of both cichlid genera with their schematic phylogenies, c) *Andinoacara* and d) *Mesoheros*. Blue symbols on the map represent the Southern clade and red symbols the Northern clade in both genera.

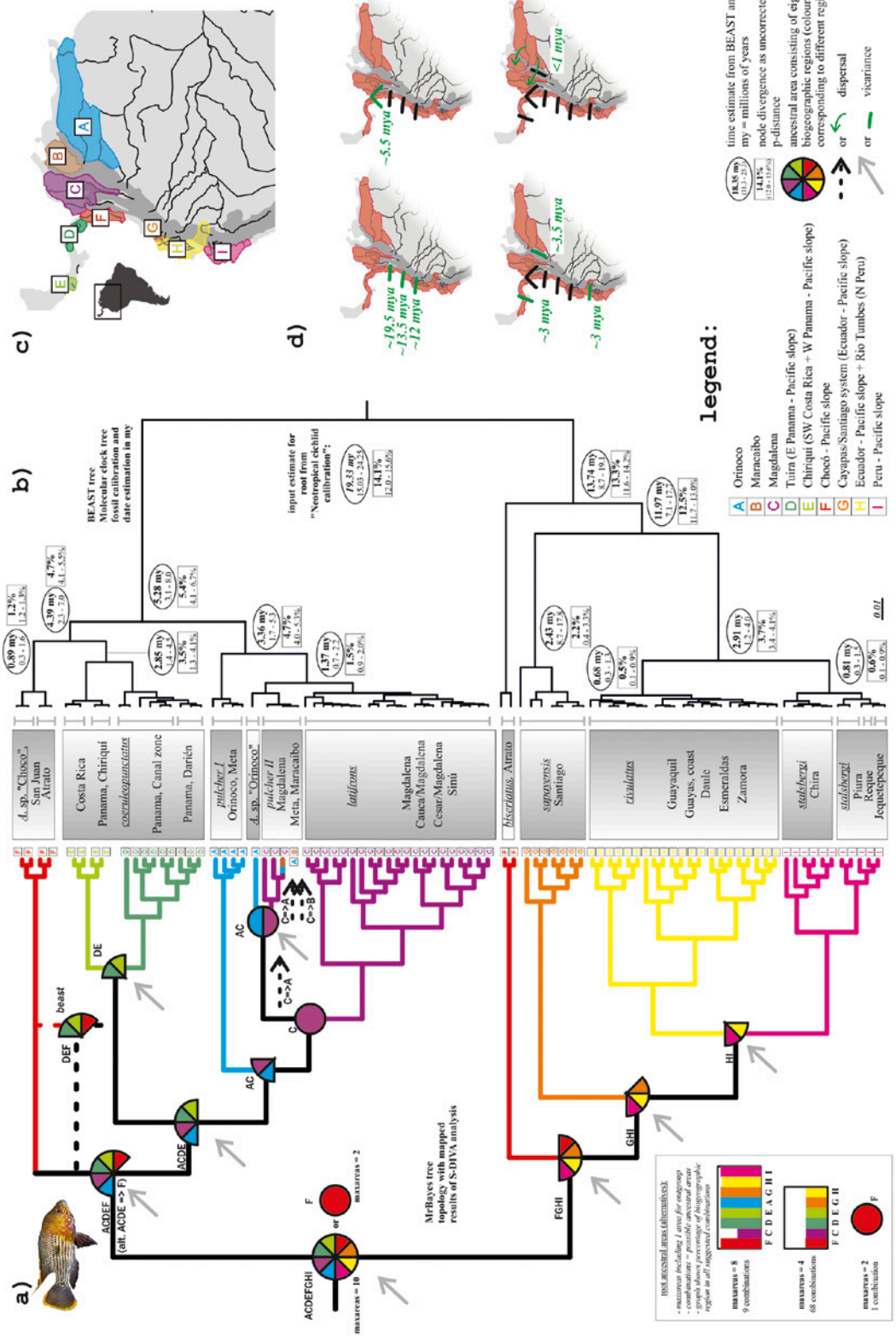


Fig. 2. Historical biogeography and molecular clock dating of *Andinoacara*. **A)** Biogeographic analysis of the "haplotype data set". Ancestral areas as well as vicariance and dispersal events were reconstructed for the nodes using the S-DIVA analysis. The topology shown is derived from a MrBayes haplotype analysis. The BEAST based topology (where in disagreement) is shown by the dashed line. Branch colors correspond to areas of endemism in C). Letters and graphics at nodes show ancestral areas as suggested by S-DIVA analyses. Gray arrows show vicariant events, black dashed arrows show dispersal events. **B)** Relaxed molecular clock tree from BEAST analysis. Numbers in squares show the genetic divergence for particular nodes (uncorrected p-distance) in %. The interval covers the lowest and highest distance observed. Numbers in circles show estimated ages in millions of years (My). The 95% highest posterior density (HPD) interval is shown in brackets. Only values above one My, or values for basal nodes of each particular species are shown. The input age value of the root for the molecular clock dating in BEAST is derived from the "Neotropical cichlid tree" (Appendix S1) and is shown at the root of the tree. **C)** Map of areas of endemism used in the biogeographic analysis. **D)** Graphical illustration of the vicariances and dispersals with timing shown on a map.

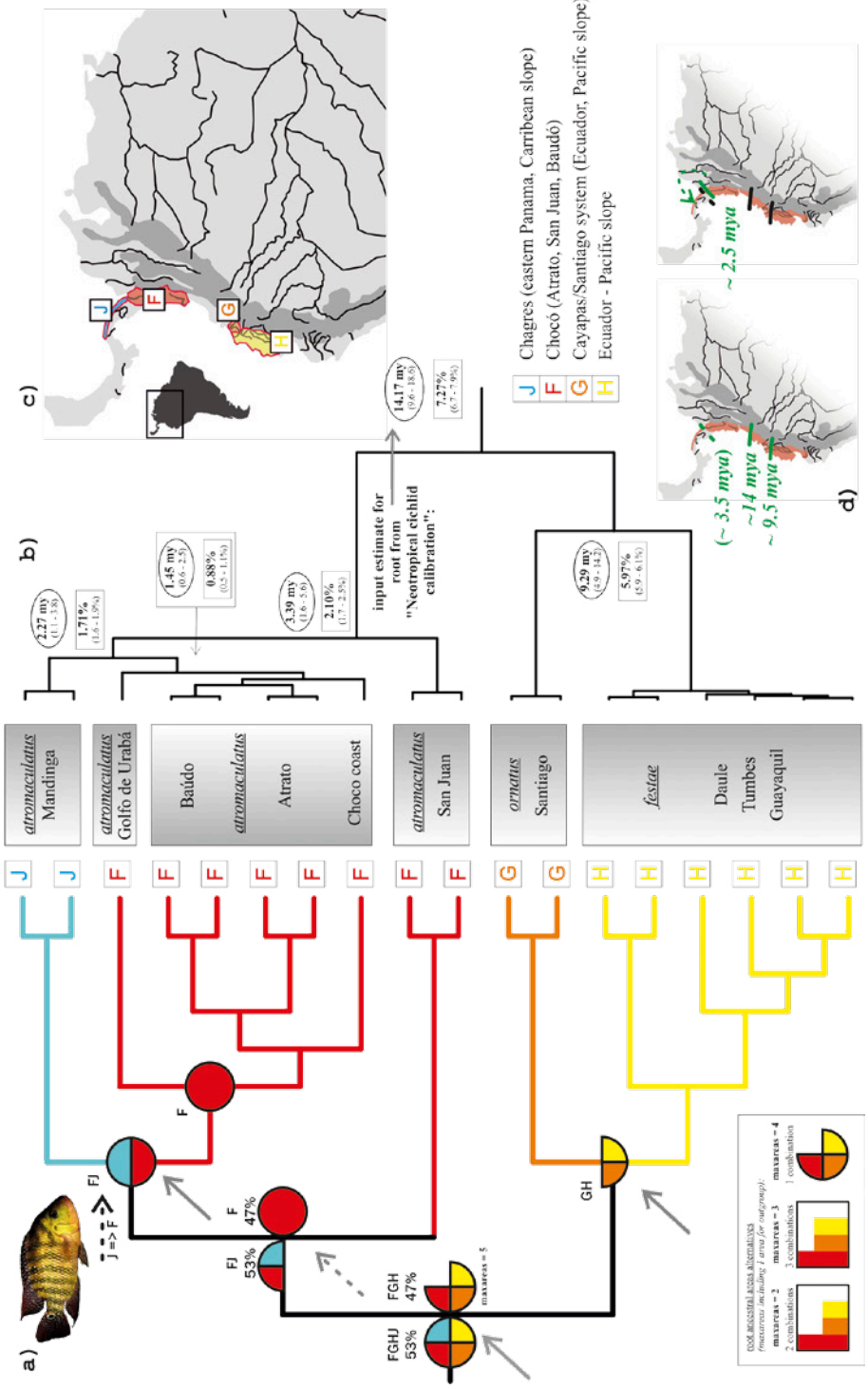


Fig. 3. Historical biogeography and molecular clock dating of the *Mesoheros* genus using the "haplotype data set" **A)** Bayesian topology (MrBayes) and biogeographic reconstruction from S-DiVA mapped on the tree. Colours of the branches correspond to the areas of endemism in the map in C. **B)** Relaxed molecular clock tree from BEAST. Numbers in squares show the genetic divergence for particular nodes (uncorrected p-distance) in %. The interval covers the lowest and highest distance observed. Numbers in circles show estimated ages in millions of years (My). The 95% highest posterior density (HPD) interval is shown in brackets. For additional details see legend to Fig. 2B. **C)** Distribution map with areas of endemism. **D)** Graphical illustration of the vicariant and dispersal events with timing shown on the map. For graphic symbols see legend in Fig. 2.

area of endemism hosting separated endemic species (i.e. *Andinoacara sapayensis* and *Mesoheros ornatus*). The rest of the Ecuadorian pacific river systems, i. e. Esmeraldas, Daule, Manabi-Guayas, and the rivers of Golfo de Guayaquil have on the contrary very low genetic structure within both *A. rivulatus* and *M. festae* (Figs 1, 2 and 3).

Historical biogeography and molecular clock of trans-Andean cichlid fishes

The relaxed molecular-clock analysis based on the Neotropical cichlids data set estimated the age of *Andinoacara* to be 19.33 million years (My), and of *Mesoheros* 14.17 My (Appendix S1). These time estimates were then used as secondary calibration points for the individual *Andinoacara* and *Mesoheros* molecular clock estimates. The estimated ages within the studied groups are shown in Fig. 2 for *Andinoacara* and in Fig. 3 for *Mesoheros*.

Both biogeographic methods (i.e. S-DiVA and DEC-Lagrange) applied within this study were to some extent sensitive to the set parameters, namely to the maximum of areas allowed to be present in the ancestral area ("maxareas"). We ran the analyses with various possible settings, ranging from a large ancestral area (allowing all tested biogeographic regions in maxareas; i.e. nine regions in *Andinoacara* and four regions in *Mesoheros*), through a wide-scale dataset (four "super" regions for *Andinoacara*) to only two areas allowed. See Fig. 4 and ancestral nodes in Fig. 2 and 3. The DEC-Lagrange method was performed only on the wide-scale ("super regions") analysis, because the more complex data sets with fine-scale analysis of nine regions were not converging. There is a justification for the maxareas constraints, such as the non-existence of connection of developing Central America with South America until the Great American Biotic Interchange (and thus its impossible presence in the ancestral area) in the time of lineage origin, or the fact that the maximum of areas occupied by any of recent species is low, e.g. in *Andinoacara* any species is present in just two "super regions" or three biogeographic regions. Such information has been used as a constraining estimation for the maxareas set in several studies, e.g. in Amazonian frogs (Santos *et al*., 2009) or birds (Nylander *et al*., 2008). On the other hand, vicariance is often considered as a null hypothesis in biogeography and any restriction in the number of maxareas may cause the *a priori* rejection of this hypothesis.

Based on our results we thus prefer the unconstrained analyses showing that vicariant events and allopatric speciation were the most important drivers of diversification in both studied groups (*Andinoacara* and *Mesoheros*). The ancestral area of *Andinoacara* likely consists of the whole of its recent distribution except for the Maracaibo region and probably Central America. This latter point represents a potential conflict between our analyses (where Central America is within the ancestral area in virtually all analyses) and the geological development of the Central American isthmus (which was probably not connected to South America until the Pliocene; but see Montes *et al*. 2015 and Discussion).

The following scenario describes a series of predominantly vicariant events that have shaped the present diversity of *Andinoacara*. The first event separated the northern and southern *Andinoacara* clades within the region of the Choco (F) at 19.33 Mya (24.25 – 15.03 Mya). Recently, both lineages live sympatrically in Choco, and therefore secondary dispersal within the region occurred later. The following three vicariant events were reconstructed in the southern clade, the first of which occurred between the Chocoan *A. biseriatus* (F) and the remaining clade (G, H, I) around 13.74 Mya (19.7 – 8.7 Mya), followed by a vicariance separating *A. sapayensis* in the Santiago river (G) from the rest of Ecuador and Peru (G, H) at 11.97 Mya (17.1 – 7.1 Mya), and the third vicariant event occurred between *A. rivulatus* and *A. stalsbergi* and separated the Ecuadorian (H) and Peruvian (I) areas at 2.9 Mya (4.0 – 1.2 Mya; Fig. 2). In the northern clade there are two alternative scenarios for the basal node which either shows a vicariance of the Choco (F) from the rest (D, E, C, A; MrBayes topology), or a vicariance between Choco (F) + Central America (D, E) from Magdalena (C) + Orinoco (A; BEAST topology). The BEAST topology is the one that provides dating of the biogeographical events and based on it the basal vicariance in the Northern clade was dated at 5.28 Mya (3.1 – 8.0 Mya) followed by the separation of Choco (F) from Tuira (D) + Chiriqui (E) at 4.39 Mya (2.3 – 7.0 Mya), and by vicariance between the two Central American provinces, i.e. Tuira (D) and Chiriqui (E) at 2.85 Mya (1.4 – 4.5 Mya). In the Magdalena-Maracaibo-Orinoco clade (*A. latifrons* – *A. pulcher* – *A.* sp. "Orinoco" complex) vicariance between Magdalena (C) and Orinoco (A) occurred at around 3.36 Mya (5.3 – 1.7 Mya; Fig. 2).

Only two dispersals were reconstructed in the *Andinoacara* biogeographic scenario, both within the same lineage of *A. pulcher*. First is the colonization of Maracaibo (B) and the second is the recolonization of Magdalena (C), both from the Orinoco region (A). Both dispersals were found to be recent colonisations and the fishes from Maracaibo, Magdalena and Orinoco basins even share haplotypes in this case (Fig 2).

The biogeographic scenario of *Mesoheros* also has some ambiguity in its resolution (Fig. 3). It requires either four vicariances, or three vicariances and one dispersal event to explain the present distribution pattern (Fig. 3). The difference is in the presence or absence of Chagres (eastern Panama) in the ancestral area, when the first scenario would suggest a completely vicariant explanation, whereas the alternative scenario would assume a later dispersal from the Choco to the Chagres (both reconstructions have a similar probability; 53% vs. 47%). The basal vicariance occurred therefore between the Choco (F) (+ possibly Chagres [J]) and the Santiago (G) + Ecuador (F) around 14.17 Mya (18.6 – 9.6 Mya). The second vicariance was between *M. ornatus* in San-

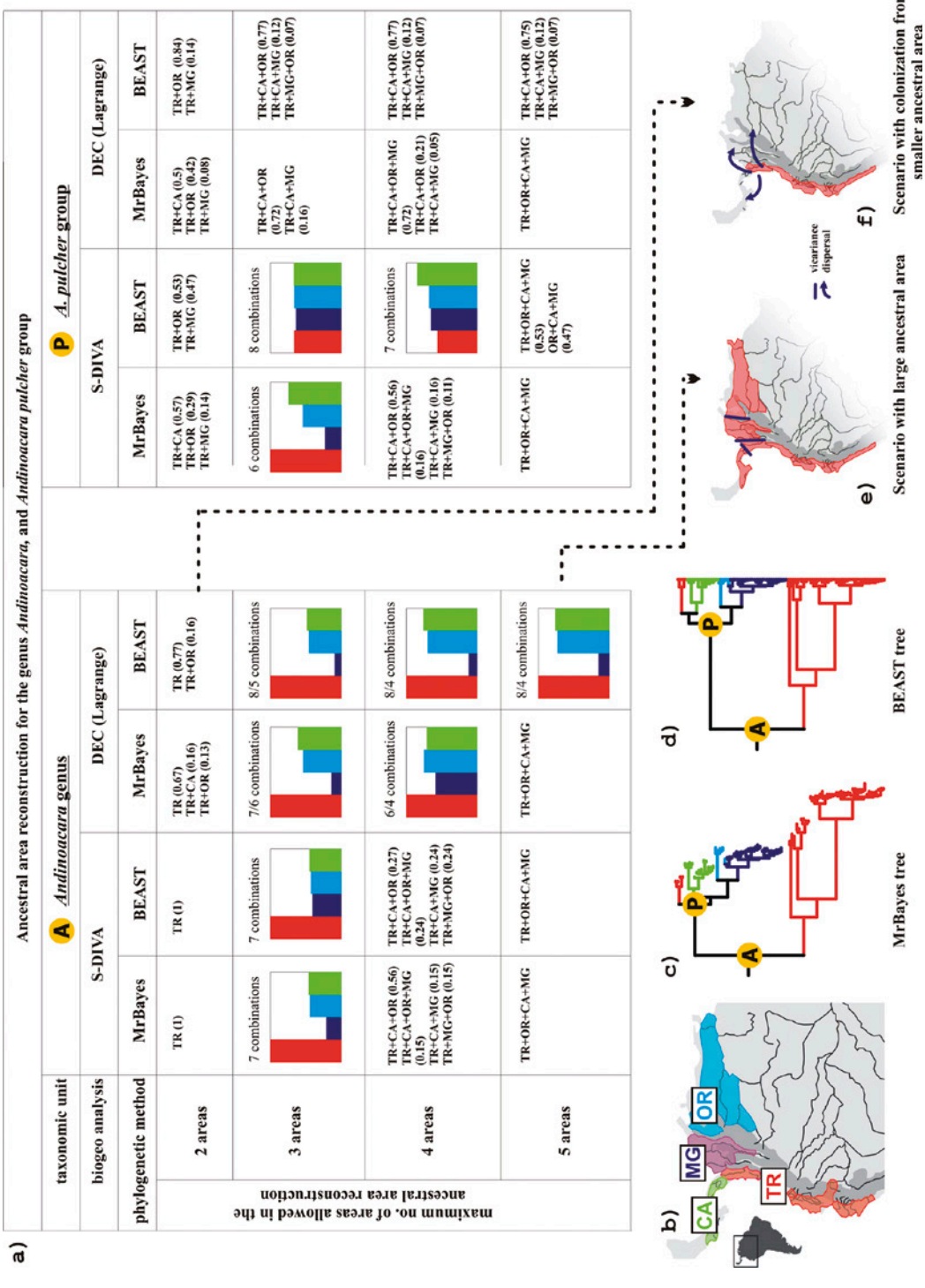


Fig. 4. Comparison of ancestral area reconstruction for the genus *Andinoacara* based on Statistical Dispersal-Vicariance Analysis (S-DIVA) and Dispersal-Extinction-Cladogenesis model (DEC) in Lagrange. The biogeographic areas used here are biogeographic "super" regions (because of limitations of the DEC analysis; map in B) and they are mapped on the phylogenetic trees from MrBayes (C) and BEAST analyses (D). A) Estimated possible ancestral areas shown with their relative probability in the brackets. Only combinations over 5% (0.05) of probability are shown. If more than five combinations were reconstructed, results are presented by the column bars, showing the percentual presence of each region in the estimates of ancestral areas (100% means the region is present in all estimated combinations). E) + F) Different results based on different assumptions – E) a fully vicariant scenario with all regions present in the ancestral area (TR+OR+CA+MG), when the maximum number of areas is not constrained (= 5 areas allowed). F) the ancestral area in trans-Andean region followed by the colonization to the other regions was supported under the constrained settings (= 2 areas allowed); A = ancestor of the whole genus *Andinoacara*. P = ancestor of the *Andinoacara pulcher* group. TR = trans-Andean region, CA = Central America, OR = Orinoco + Maracaibo, MG = Magdalena

tiago (G) and *M. festae* in Ecuador (H) at 9.3 Mya (14.2–4.9 Mya). Within the Northern *M. atromaculatus* clade the oldest node is equivocally reconstructed as a vicariance between the Choco (F) and Chagres (J) or as only including the Choco (see above and Fig. 3), and the node is dated at 3.4 Mya (5.6–1.6 Mya). If not at 3.4 Ma then a definite vicariance between the Choco (F) and Chagres (J) occurred at 2.3 Ma (3.8–1.1 Ma; Fig. 3)

Despite the larger distribution area of *Andinoacara* compared to *Mesoheros*, both groups show congruent biogeography as well as age estimation of events. The geographically and phylogenetically corresponding southern *Andinoacara rivulatus* group and the southern *Mesoheros festae* group show a much more similar age (12 Mya vs. 9.3 Mya) than uncorrected p-distances (12.5% vs. 6.0%).

The molecular clock estimates in both studied genera are thus in better correspondence than the uncorrected molecular divergences, which show a two-fold difference. In the cichlasomatine *Andinoacara* virtually all nodes show a divergence rate of slightly more than 1% per million years (1.03–1.42%, with outliers starting at 0.92%). In the heroine *Mesoheros* on the other hand all nodes show a divergence rate about half that of the cichlasomatine *Andinoacara*, with an average around 0.62% per million years (0.53–0.73%).

Discussion

Phylogeny and notes on the systematics of the two trans-Andean cichlid genera

The phylogenetic reconstruction of the genus *Andinoacara* is consistent with previous studies (Kullander, 1998; Musilová *et al.,* 2008; Musilová *et. al*., 2009a; Musilová *et al.*, 2009b). In case of *Mesoheros* our results are also in agreement with the previous studies (Říčan *et al*., 2013; Mcmahan *et al*., 2015).

The genus *Andinoacara* contains eight valid species and three undescribed lineages (Fig. 1). The status of a recently described species, *A. blombergi* (Wijkmark *et al*., 2012), is questionable based on our results. In this study, we cover all species including also samples from the Esmeraldas drainage in Ecuador from where the putative new species (*A. blombergi*) was described. In our analyses based on the cytochrome b gene the *A. blombergi* samples do not form a unique clade but instead are nested among the other phylogenetically very recent *A. rivulatus* populations from other parts of Ecuador (Figs 1 and 2). *Andinoacara blombergi* was described (Wijkmark *et al*., 2012) solely based on shape differences (head length and interorbital width), but such characters are known as plastic in response to different ecological conditions (e.g. Bouton *et al*., 2002). For example we have found similar variation in the sympatric *M. festae*, where also the lowland populations are more deep-bodied whereas the highland populations are more stream-lined (OŘ pers. obs.).

In order to further test our hypothesis of conspecifity between *A. blombergi* and *A. rivulatus* we have additionally sequenced the nuclear intron S7 (intron 1) for the four samples from the Esmeraldas (*A. blombergi*) as well as for the 23 samples of *A. rivulatus*. This nuclear gene is variable enough to distinguish between most cichlid species including all other *Andinoacara* species (Musilová *et al*., 2008, 2009b; Říčan *et al*., 2008, 2013). However, we found that the Esmeraldas samples (putative *A. blombergi*) share the same haplotype with most of the *A. rivulatus* populations in this nuclear gene (data not shown). Contrarily, difference between sister species *A. rivulatus* and *A. stalsbergi* in this marker amounts to a divergence of 1.6% (Musilová *et al*., 2009b). Based on our mitochondrial results and this nuclear confirmation, we suggest considering *A. blombergi* as a species of questionable status, which should be tested in future by more complex genomic approach based on a substantial sampling of both species.

In both cichlid genera there is also substantial genetic divergence in their northern groups that suggests the potential existence of more species in NW South America than is currently assumed. In *Andinoacara* the only undisputed species in the northern group is Central American *A. coeruleopunctatus*, whose species status is well supported by our results. The remaining northern *Andinoacara* species have been variously classified as *A. pulcher*, *A. latifrons*, or both, however lacking clear differential diagnosis. Our analyses suggest that there are possibly four rather than two species involved. One lineage (*A.* sp. "Choco") is clear-cut since it is a unique clade in mtDNA endemic to the Choco and allopatric from the rest of the clade, or possibly the sister group of the Central American *A. coeruleopunctatus* (Fig. 2). Three additional lineages appear in the region, with mostly allopatric distribution. One lineage is *A. latifrons* from the Magdalena, Sinu and Cauca, a well-supported, unique and also allopatric clade in mtDNA. The rest are populations traditionally most often classified as *A. pulcher*, which are however not monophyletic, but still share the same distribution in the cis-Andean Orinoco drainage plus Maracaibo and also are found in the (upper!) Magdalena. Whether *A. pulcher* is one or more species remains to be studied with additional (genomic) markers as well as with larger sampling effort covering more populations.

Rates of evolution, molecular clock calibration and the age of the cichlids

Since there are no fossils known for the studied genera, we made use of a fossil-based calibration of the whole Neotropical Cichlidae, from which we took secondary calibration points for the detailed molecular clock analyses within both studied groups (see Appendix and Fig S1).

Recently, several studies considering the dating of cichlids were published (e.g. McMahan *et al*., 2013;

Říčan *et al*., 2013; López-Fernández *et al*., 2013; Friedman *et al*., 2013) that have utilized fossils of Neotropical cichlids (for example the three Lumbrera formation fossils, i.e. *Gymnogeophagus eocenicus* Malabarba *et al*., 2010, *Plesioheros chauliodus* Perez *et al*., 2010 and *Proterocara argentina* Malabarba *et al*., 2006; see Fig. S1) and/or other non-cichlid fossils. The age estimates resulting out of the aforementioned studies are in strong disagreement, some of them assuming unlikely interpretations, e.g. in one extreme a huge gap in the fossil record (López-Fernández *et al*., 2013), while in the other extreme most of the known cichlid fossils pre-dating the suggested first cichlid split (Friedman *et al*., 2013). See Appendix S1 for more detailed discussion about cichlid dating. Further, none of the recently or previously published molecular-clock studies provides a reliable estimate of the time frame of evolution of the cichlasomatine *Andinoacara.* Because of the mentioned reasons we have reconstructed our own molecular calibration tree.

Historical biogeography of the trans-Andean cichlids in the context of Andean orogeny

Vicariant events and allopatric speciation were identified as the most important drivers of diversification in both studied cichlid genera (*Andinoacara* and *Mesoheros*). The general biogeographic pattern and molecular clock dating in both genera are highly consistent and include: (1) The basal evolutionary lineages are distributed in the Colombian Pacific-slope Choco; (2) there is a homologous basal division into corresponding southern and northern groups; (3) both genera have very similar ages of the northern and southern groups; (4) both genera have highly corresponding ages and patterns of diversification within the southern lineage (vicariant events between the Choco (F) and the Santiago river system (G), and between the Santiago (G) and rest of Ecuador (H)).

Mountain ranges are the textbook example of a barrier splitting a previously continuous distribution area and thus forcing vicariant evolution and allopatric speciation (Crisci *et al*., 2003). The Andes, and especially their northern part, are geologically extremely complex and yet relatively young mountains, mostly having risen to substantial elevations during the last ~ 20 My (though their uplift started ~ 80 Mya). The Andes represent the most important distributional barrier in the Neotropics and they have caused diversification in most terrestrial groups of animals (reviewed in Antonelli *et al*., 2010) including frogs (Antonelli *et al.,* 2010), geckos (*Gonadotes*; Gamble *et al*., 2008), birds (wrens of the genus *Campylorhynchus*; Piciformes; Moore *et al*., 2006), spider and howler monkeys (*Ateles*, *Alouatta*; Collins & Dubach, 2000; Cortes-Ortiz *et al*., 2003), rodents (*Dactylomys*, *Isothrix*; Patterson & Velazco, 2008) as well as plants (Antonelli *et al*., 2009). *Andinoacara* clearly represents the best cichlid model group for the study of diversification triggered by the Andean orogeny in the whole of tropical South America, especially together with its sister group, i.e. the cis-Andean genera *Bujurquina* Kullander 1986 and *Tahuantinsuyoa* Kullander 1986 (Musilová *et al*., 2009). The distribution and diversity of these cis-Andean cichlid genera is centred mostly along the Andes from Venezuela to Argentina (in the Andean lowland piedmont up to ca 1000 m elevation) and it complements the distribution of its northern counterpart, i.e. genus *Andinoacara*.

Prior to the substantial elevation of the Andes the *Andinoacara-Bujurquina-Tahuantinsuyoa* clade (Musilová *et al*., 2008, 2009; Fig. S1) and the herichthyine clade of the heroine cichlids, to which the *Mesoheros* genus belongs (Říčan *et al*., 2013), probably shared a large ancestral area in NW South America (around 30 Mya) which today includes both cis- and trans-Andean South America) or the large area was at least connected through two areas within the developing Andes. One likely connection was the Western Andean portal, originally a marine incursion from the west coast through the Central / Northern Andes low altitude divide (present Ecuador-Peru boundary) into present western Amazonia that persisted till 13 – 11 Mya (Antonelli *et al*., 2009; Santos *et al*., 2009). The better known connection between present cis- and trans-Andean South America was around the northern terminus of the developing Andes (through the mouth of the northward flowing paleo-Amazon before the separation of the Maracaibo, Magdalena and Orinoco) and it terminated at around the same time as the Western Andean portal (12 – 10 Ma) with the final elevation of the Eastern Venezuelan Andes (Lundberg *et al*., 1998; Rodríguez-Olarte *et al*., 2011; Schaeffer, 2011). The ancestral evolution of both studied cichlid genera (Figs 2 and 3) and of their respective evolutionary lineages thus occurred prior to the final closing of both of these cis- and trans-Andean connections.

The oldest (i.e. basal) nodes of both genera differ in both their age and in their ancestral area reconstruction, which is older (ca 20 vs. 14 Ma) and wider (cf. Figs 2 and 3) in *Andinoacara* than in *Mesoheros*. Both differences can be explained with the help of their sister-groups. Based on Říčan *et al.* (2013) the *Mesoheros* genus is either a non-native immigrant from Middle America into Choco or a native group whose ancestral area has (or its sister clades have) been restricted by extinction events. On the other hand, *Andinoacara* clearly is a native NW South American genus both based on its wide reconstructed ancestral distribution and the biogeographical analysis including its sister group, i.e. the *Bujurquina-Tahuantinsuyoa* clade (Musilová *et al*., 2008).

The basal divergence within *Andinoacara* (around 20 Mya) occurred within the area of the Colombian Choco (Fig. 2) while in *Mesoheros* (around 14 Ma) occurred south of the Choco (Fig. 3) and the basal splits are thus not attributable to the same biogeographical event.

In both studied cichlid genera the oldest clear vicariant events occurred in their southern group, possibly in agreement with the older substantial elevation of this central section of the Andes than of the Andes to the north (Lundberg *et al*., 1998; Rodriguez-Olarte *et al*., 2011).

In both cichlid genera the events are homologous having occurred at the same time (around 12 to 9.3 Ma) and in the same area within present Ecuador between the northern Santiago river basin species (*A. sapayensis* and *M. ornatus*) and the rest of Ecuador and Peru to the south (Fig. 2, 3).

The remaining diversification within both *Andinoacara* and *Mesoheros* occurred after the isolation of trans-Andean South America from cis-Andean South America. In both genera the diversification in this period was virtually limited to the northern part of their distribution. In the south there is only the nominotypical species of *Mesoheros* (*M. festae*) with a very shallow molecular diversity (Fig. 3) and only two species of *Andinoacara* (*A. rivulatus* and *A. stalsbergi*; Fig. 2). The northern Andean orogeny has thus generated a higher diversity and also more complicated biogeographical patterns in both genera.

In both cichlid genera their northern groups show complex biogeographical patterns with complex implications for species-level classification (see above). At least four species of *Andinoacara* have evolved here as a direct consequence of the Andean orogeny with each of the Andean chains isolating an endemic species of *Andinoacara* and both studied genera have also colonized lowermost Central America (see below). The Western Andes isolate *A.* sp. Choco, the Central American *A. coeruleopunctatus* and *M. atromaculatus*, the Western and Eastern Andes restrict the distribution of the endemic *A. latifrons*, and the Eastern Andes isolate the cis-Andean *A. pulcher* from the trans-Andean species (Figs 2 and 3). The eastern branch of the Eastern Andes (i.e. Merida Andes) in Venezuela which separates the Maracaibo basin from the Orinoco basin is however not an evident barrier in *Andinoacara* or (more likely) the barrier has been compromised by dispersal (native or artificial) since *A. pulcher* (clade II) is found in both areas without corresponding molecular divergences (Fig. 2). In many other fish groups like stingrays (*Potamotrygon*; Lovejoy *et al*., 1998) or catfishes (*Hypostomus*; Montoya-Burgos, 2003), the lake Maracaibo lineages represent distinct and permanently separated populations after a putative single vicariance event (Lovejoy *et al*., 2010). However, in *Andinoacara*, the Maracaibo population (also referred to as *Andinoacara* sp. "Maracaibo") from the *A. pulcher* group is very likely a result of recent colonization event from the (upper) Magdalena region (based on present sampling effort) or the Orinoco basin, however the exact colonization mechanism and timing is not known and requires further study.

There is one peculiar record of *Andinoacara* in the Amazon basin, namely in the southern Andean region of Ecuador in the Zamora river basin (OŘ, pers. obs.; Alf Stalsberg, pers. comm.; Wijkmark *et al*., 2012). Based on our phylogenetic analysis, the Zamora populations are nested within *A. rivulatus* (Fig. 1). They do not form a monophyletic lineage within *A. rivulatus* because their clade also includes several samples from the adjacent but trans-Andean Ecuadorian Guyaquil basin. The presence of *A. rivulatus* in the cis-Andean region is thus most likely result of a recent colonization (or more likely introduction similar as in *A. pulcher* to Maracaibo; see above) and it is likely not linked to the Andean orogeny.

Finally, both studied genera have made it into Lower Central America even though their distributions there are non-overlapping. *Mesoheros* is found only in eastern Panama on the narrow Caribbean slope, while *Andinoacara* is found only in the Pacific-slope rivers but in a larger area from eastern Panama to SE Costa Rica. In *Andinoacara* Central America is in virtually all biogeographical analyses (both unconstrained and constrained, except for S-DiVA maxareas = 2; Figs 2 and 4) found in the ancestral area of the genus, which suggests an old colonization prior to the final Pliocene consolidation of the Panamanian Isthmus and the coterminal Great American Interchange (GAI; Marshall *et al.*, 1982; Cody *et al*., 2010, Bermingham & Martin, 1998). Several studies have recently shown that e.g. fossil mammals or Middle American cichlids have reached central and eastern Panama already in middle Miocene between 19.5 and 14 Ma (Kirby & MacFadden, 2005; MacFadden, 2006; Kirby *et al*., 2008; Říčan *et al*., 2013) but this dispersal was in the opposite direction, i.e. from North and Middle America to South America. Accordingly to this early dispersal there are also recent geological discoveries (Montes *et al*., 2015) suggesting the potential presence of purely freshwater connection between South America and Panama arc in middle Miocene. Contrary to this, most of the organismal studies (see Říčan *et al*., 2013 for a summary) as well as previous geological studies (e.g. Iturralde-Vinent & MacPhee, 1999; Coates *et al*., 2004; Kirby *et al*., 2008) demonstrate that such early dispersal into Lower Central America from South America was unlikely, because of the eastern Panama - Colombia sea gap (the Darien gap) between Central and South America. Our results for *Andinoacara* suggest that it might be an old lineage within Lower Central America. Contrarily, *Mesoheros* did not colonize Central America in the strict geological sense because it is found only in eastern Panama. The reconstruction of its colonization of this westernmost area is also equivocal, with the inclusion or exclusion of eastern Pacific-slope Panama at the root of the group being equally likely. In this case however both options (3.4 and 2.3 Ma; Fig. 3) fit the known evolution of the Panamian isthmus.

In both cichlid lineages there is an agreement in the vicariance between Panama and Colombia at the Serranía del Darién (2.3 Ma in *Mesoheros* and 4.4 Ma in *Andinoacara*; Figs. 2 and 3), which corresponds to the final completion and recent configuration of the Panamian isthmus and to the age of the Great American Interchange in the Pliocene (Bermingham & Martin, 1998).

To conclude, our study provides the first evolutionary-biogeographic reconstruction of two cichlid groups in trans-Andean South America. We have shown similar evolutionary patterns in these two genera, probably shaped by geological conditions in the past, and we have identified the Colombian Choco as an important region

present in the ancestral area of both genera. We have further found that one cichlid genus (*Andinoacara*) became possibly established in Central America prior to the closure of the Panamian Isthmus. Finally we provide an updated fossil-based molecular-clock dating for all Neotropical cichlids.

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Appendix S1 Molecular clock analysis of Neotropical cichlids: fossil calibration and age estimation.

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