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Diversity and evolution of the Middle American cichlid fishes (Teleostei: Cichlidae) with revised classification

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Accepted 19.ii.2016.

Published online at www.senckenberg.de/vertebrate-zoology on 4.iv.2016.

Abstract

We present a taxonomically complete and topologically robust molecular phylogeny of the Middle American heroine cichlids based on which we review their diversity and genus-level systematics. In order to ascertain the diversity of the group and its phylogeny we have used three nested taxon sampling analyses of the concatenated nDNA/mtDNA datasets and additionally to these analyses we present a summary of the results of a new Next Generation Sequencing-generated nuclear phylogeny based on a data set of ~ 140,000 informative characters. The NGS ddRAD phylogeny has a species-level sampling covering virtually all species (including the enigmatic *Cichlasoma microlepis* DAHL, 1960) with multiple sequenced specimens per species. Based on our results the Middle American heroine cichlids are made up of three main clades. The three clades (the herichthyines, the amphiphines, and the astatheroines) are however not each other sister groups since they are interspersed with South American (*Australoheros*, *Caquetaia*, *Chocoheros*, *Heroina*, *Mesoheros*) and Antillean (*Nandopsis*) genera and they represent probably two separate colonization events of Middle America from South America, probably via the Antilles. Our study reveals many cases of cytonuclear discordance and/or introgressive hybridization both at the genus and deeper levels stressing the importance to study the nuclear and mitochondrial phylogenetic signals independently and not solely in concatenated analyses. We have found that a great majority of morphological characters are ecologically correlated and that they form only a limited number of functionally-determined combinations – i.e. ecomorphs. We have found five main cranial ecomorphs but only two postcranial ecomorphs (the lotic and lentic ecomorphs, plus the undifferentiated ancestral character combination). The cranial and postcranial ecomorphs are not combined completely randomly having produced thirteen modular whole-body ecomorphs. Both the cranial and postcranial ecomorphs, and even their combinations, have evolved repeatedly in the Middle American cichlids in the same habitats both in sympatry as well as in allopatry. Our analyses of the diversity of Middle American cichlid clade support the existence of 31 genera in Middle America (plus six in South America and one in the Greater Antilles) as separate evolutionary lineages occupying separate adaptive zones. Nine new genera are described here for species and species groups that have lacked a genus level name to this day or were associated with other unrelated genera. We also review the species level diversity based on the mtDNA cytb gene *population-level* analysis. Furthermore, we provide a new biogeographical analysis of the group which explains their evolutionary history and demonstrates that biogeography is a much better indicator of evolutionary relationships in this fish group than are most morphological characters due to their ecological correlation.

Key words

Adaptive radiation, Central America, cytonuclear discordance, introgressive hybridization, freshwater fishes, new genera, putative new species.

Discovery consists of looking at the same thing as everyone else and thinking something different.

ALBERT SZENT-GYORGYI (1985)

This review is dedicated to the many enthusiastic cichlidophiles who are obtaining information of great value to professional ichthyologists and who are the true lovers of these remarkable fishes.

1. Introduction

Middle America has a very diverse cichlid fauna. This is especially evident when considering the small size of Middle America compared to tropical South America (only ca 15 % of the latter). Virtually all cichlid species in Middle America with the exception of only two (one geophagine and one cichlasomatine, both only in Panamá – Costa Rica) belong to one lineage, the heroine cichlids (*sensu* KULLANDER, 1996, 1998, 2003). At present there are 124 valid heroine cichlid species in Middle America (plus three in the Greater Antilles) out of the 184 valid species of all Neotropical heroine cichlids. The species diversity in the largest South American genus (*Australoheros*, currently with 27 species) is however highly exaggerated (as many as 15 recently described species are probably synonyms; ŘÍČAN *et al.*, 2011) and the total of heroine diversity is thus even more in favour of the Middle American species. The high diversity of the Middle American cichlids is due to two areas in Middle America which are hyper-diverse in cichlid fishes. These are the Usumacinta ichthyological province (*sensu* BUSING, 1985) with 46–56 valid species (depending on the treatment of subspecies in the ‘*Heros*’ *urophthalmus* group), followed by the San Juan province (19 riverine species plus 10 crater-lake endemics). Geographically the limits of the Middle American cichlid lineages are not completely sharp since the fauna mixes with more South American lineages (e.g. *Caquetaia*, *Mesoheros*) in eastern Panamá (ŘÍČAN *et al.*, 2013).

The heroine cichlid fishes have produced in Middle America a much wider spectrum of morphological and ecological diversity (small and large pickers-predators, specialized piscivores, molluscivores, detritivores, substrate-sifters, plant-eaters, algae-scrapers, cavity-suckers, and also rheophilics and euryhaline species) than in South America. The only morphology that is uniquely South American is the laterally flattened deep-bodied one and the only uniquely South American behaviour is mouthbrooding, both present in the Amazonian heroine cichlids (i.e. *Pterophyllum*, *Mesonauta*, *Uaru*, *Heros* in the former and *Heros* in the latter).

The large morphoecological diversity of the Middle American cichlids is reflected in their extremely complicated genus-level classification. Morphological diagnosability of genera in Middle American heroine cichlids is very difficult due to a weak morphological phylogenetic signal caused by the lack of informative non-convergent characters and conflicts within the morphological partition and with molecular phylogenies of the group (ŘÍČAN *et al.*, 2008). This is a general problem of cichlid systematics which is plagued with a paucity of phylogenetically informative morphological characters (STIASSNY, 1991), especially at the lowest taxonomic levels. The weak morphological phylogenetic signal and the conflicts with the molecular phylogenies are due to rampant morphological convergence among cichlids caused by the enormous ecological versatility of the group (which has undergone

frequent adaptive modifications associated with trophic ecology, habitat use, reproductive biology, and behaviour) and evident across the whole spectrum of the Neotropical Cichlidae and for Cichlidae in general (e.g. DUNZ & SCHLIEWEN, 2013; GALIS & METZ, 1998; KORNFIELD & SMITH, 2000; LÓPEZ-FERNÁNDEZ *et al.*, 2013; PIÁLEK *et al.*, 2012; RÜBER & ADAMS, 2001; ŘÍČAN *et al.*, 2008; STIASSNY, 1991; STIASSNY & MEYER, 1999; WINEMILLER *et al.*, 1995).

The lack of clear diagnostic characters in the morphological data sets (KULLANDER, 1998; ŘÍČAN *et al.*, 2008) has led to the situation that most Neotropical cichlid genera were diagnosed using combinations (putatively unique) of character states. Such diagnoses can however be problematic since the putatively unique combinations have to be of apomorphic character states in order to diagnose monophyletic groups and this is difficult to achieve without a phylogenetic analysis. Virtually all Neotropical cichlid genera were additionally diagnosed prior to the first morphological (KULLANDER, 1998) and molecular phylogenetic analyses (FARIAS *et al.*, 1999, 2000; MARTIN & BERMINGHAM, 1998; ROE *et al.*, 1997). This has led to the situation that several Neotropical cichlid genera are diagnosed using plesiomorphies and the genera are thus paraphyletic or even polyphyletic as suggested by molecular phylogenetic analyses (see e.g. MUSILOVÁ *et al.*, 2008, 2009; ŘÍČAN *et al.*, 2008, 2013; this study).

The most influential morphological phylogeny and resulting classification published for the Neotropical Cichlidae to date is that of KULLANDER (1998). This study has unfortunately specifically omitted the Middle American cichlids. Another problematic issue with this study is that it cannot be taken as a phylogenetic test of the monophyly of genera and hence a foundation for a formal classification, since in most cases predefined genera were input as terminal taxa (as one OTU) into the phylogeny. The phylogeny of KULLANDER (1998) nevertheless shows the same limited phylogenetic performance as do other morphological data sets published for the Neotropical Cichlidae (e.g. MUSILOVÁ *et al.*, 2009; ŘÍČAN *et al.*, 2008) and also contains very little hierarchical (phylogenetic) information (KULLANDER, 1998) and is thus not a robust hypothesis for the relationships and the classification of the Neotropical Cichlidae.

Morphological convergences are evidently very strong also among the Middle American heroine cichlids since all phylogenetic studies employing molecular data (e.g. CONCEIRO PÉREZ *et al.*, 2007; MARTIN & BERMINGHAM, 1998; ROE *et al.*, 1997; ŘÍČAN *et al.*, 2008, 2013) have provided well resolved and well supported phylogenetic reconstructions that are in strong disagreement with the already conflicting morphological classifications. Many of the presently valid genera and their taxonomic composition thus do not reflect the phylogenetic relationships of the Middle American heroine cichlids. In fact six of the largest genera or species groups (*sensu* KULLANDER, 2003; i.e. *Amphilophus*, *Archocentrus*, *Paraneotroplus*, *Theraps*, *Tomocichla* and *Vieja*) are found non-monophyletic in all studies employing molecular data and an

even larger number of species are unplaced and referred to as ‘*Cichlasoma*’ or ‘*Heros*’. Together, these seven non-monophyletic groupings (including the *Petenia* - *Caquetaia* controversy) account for no less than 70 species, a sizeable chunk of the Middle American cichlid diversity of 120+ species. The conflicts between existing classifications (e.g. BUSSING, 1998; KULLANDER, 2003; MILLER *et al.*, 2005; STAWIKOWSKI & WERNER, 1998) and recently recovered phylogenetic relationships (e.g. CONCEIRO PÉREZ *et al.*, 2007; ŘÍČAN *et al.*, 2008, 2013) are thus massive.

The well resolved and well supported molecular phylogenies show that the Middle American cichlids are made of two phylogenetically independent yet geographically and temporally synonymous colonizations from South America and the Antillean cichlids (*Nandopsis*) were also part of this colonization (ŘÍČAN *et al.*, 2013). The term Middle American cichlids can thus only be used in a geographical context, not in a phylogenetic sense. The discovery that the Middle American cichlids are clearly divided into two to three distinct clades has been the turning point that has greatly altered our perception of their genus level diversity. Several of the largest traditionally recognized genera have their species placed in both of the two main clades (e.g. *Amphilophus*, ‘*Cichlasoma*’, *Theraps*, *Tomocichla*, or *Vieja sensu* KULLANDER, 2003). Such taxonomic discrepancies clearly need review as the suprageneric clades are well supported and recovered independently in both mitochondrial and nuclear DNA markers (ŘÍČAN *et al.*, 2008).

The two main clades of Middle American cichlids are the amphilophines and the herichthyines (ŘÍČAN *et al.*, 2013). The third postulated clade (astatheroines *sensu* ŘÍČAN *et al.*, 2008) is in ŘÍČAN *et al.* (2013) rather a basal paraphyletic stem-group of the herichthyines. The crown-group herichthyines are virtually endemic to the Usumacinta province (Atlantic slope of México – Guatemala), while amphilophines originated and have the highest diversity in the San Juan ichthyological province, centred around the great Rio San Juan and the Great lakes of Nicaragua (BUSSING, 1985; CONCEIRO PÉREZ *et al.*, 2007; ŘÍČAN *et al.*, 2013). The basal South American genera include *Australoheros*, *Heroina* and *Caquetaia*, while *Mesoheros* is either the result of secondary colonization from Middle America or is a native South American clade connected to the Middle American herichthyines through extinct relatives (ŘÍČAN *et al.*, 2013).

The recently recovered molecular phylogenetic relationships among all species of Middle American cichlids with demonstrably monophyletic assemblages call for a review of their classification in order to facilitate communication and further study. To facilitate the discussion of Middle American cichlid diversity, we provide here a short overview of the previous attempts to classify these fishes based on morphological data. The diversified Middle American cichlids have contained vexing taxonomic and systematic problems even before their phylogenetic relationships became ascertained with molecular data. The main problem lies with the generic treatment, which remains “both chaotic and frustrating” (KULLANDER,

1996; MILLER 1996; MILLER *et al.*, 2005; ŘÍČAN *et al.*, 2008, 2013). Throughout the 20th century most species have traditionally been assigned to *Cichlasoma* SWAINSON. REGAN (1905, 1906, 1908) in an early attempt to classify these fishes, divided that genus into a number of informal groups or sections to which he applied generic-group names. These units became reasonably established and many can be delineated or diagnosed with varying degrees of ease. REGAN’s names (with some subsequent modifications) have been widely employed, sometimes denoting discrete genera and other times designing subgenera or informal groups within *Cichlasoma* (e.g. CONCEIRO PÉREZ *et al.*, 2007; CONKEL, 1993; MILLER, 1996; MILLER & TAYLOR, 1984; ŘÍČAN *et al.*, 2008, 2013; STAWIKOWSKI & WERNER, 1998).

In 1983, KULLANDER restricted *Cichlasoma* to a small group of South American species, leaving generic assignment of Middle American cichlids (and some South American cichlids as well) classified previously as *Cichlasoma* in limbo (see also KULLANDER, 1996; KULLANDER & HARTEL, 1997; STIASNY, 1991). Lacking a sound phylogeny with demonstrably monophyletic assemblages on which to base a classification, some workers have hesitated to recognize “sections” of REGAN (1905, 1906, 1908) in formal classification. One suggestion was to classify those Middle American cichlids with no generic name available as ‘*Cichlasoma*’, with quotes indicating their uncertain systematic placement (KULLANDER, 1983), in the expectation that additional studies would soon clarify their systematic status. Alternatively, complete disassociation with *Cichlasoma* might have been accomplished by placing all species in one genus, e.g. *Heros* HECKEL or *Herichthys* BAIRD & GIRARD, 1854 (BURGESS & WALLS, 1993), which would however not convey the very large diversity of the group.

KULLANDER (1996) himself after a time of stasis and uncertainty rejected his own approach to classify most Middle American cichlids as ‘*Cichlasoma*’ and described a new genus *Heroina* KULLANDER, 1996 for a single species from the Andean piedmont in NW Amazonia. In terminating the wide scale use of ‘*Cichlasoma*’ KULLANDER (1996) further provided a list of available names for diagnosable groups and started to use them. Quite a number of species however still continued to be called ‘*Cichlasoma*’ as no alternative name was available. In the same publication KULLANDER (1996) also divided cichlasomine cichlids into two groups, the large-scaled cichlasomines and the small-scaled heroines (after *Heros* HECKEL, 1840). Both cichlasomines and heroines were later elevated to tribe status (Cichlasomatini and Heroini) within the subfamily Cichlasomatinae (KULLANDER, 1998). The name Heroini has been widely used since 1998 but technically Heroini KULLANDER, 1998 is a junior synonym of Therapsini ALLGAYER, 1989. The latter name was published in cichlid ‘hobby literature’ which was probably the reason for it having been overlooked and it has never been used since its original publication. But the name has been published in accordance with the ICZN and appears to fulfil all criteria of

availability relevant for family group names published before 2000. The name Therapsini was proposed only cursorily in the text (ALLGAYER, 1989) but the explicit intension to establish a new nominal taxon applies only to names published after 1999. In the present study we use the cichlasomatine and heroine names only as informal categories within the subfamily Cichlasomatinae KULLANDER, 1998.

KULLANDER (1996, 2003) provided the following available generic names for Middle American and related heroine cichlids (major revisionary works are cited for each genus if available) which have been used since (e.g. CONCEIRO PÉREZ *et al.*, 2007; LÓPEZ-FERNÁNDEZ *et al.*, 2010, 2013; MARTIN & BERMINGHAM, 1998; ROE *et al.*, 1997; ŘÍČAN *et al.*, 2008, 2013):

Amphilophus AGASSIZ, 1859 (*Amphilophus froebelii* AGASSIZ = *Heros labiatus* GÜNTHER; see BUSSING & MARTIN, 1975);
Archocentrus GILL, 1877 (*Heros centrarchus* GILL & BRANSFORD; reviewed by SCHMITTER-SOTO, 2007a);
Astatheros PELLEGRIN, 1904 (*Heros heterodontus* VAILANT & PELLEGRIN = *Heros macracanthus* GÜNTHER; reviewed by BUSSING & MARTIN, 1975);
Caquetaia FOWLER, 1945 (*Caquetaia amploris* FOWLER = *Petenia myersi* SCHULZ; reviewed by KULLANDER, 1996);
Chuco FERNÁNDEZ-YÉPEZ, 1969 (*Cichlasoma milleri* MEEK = *Heros microphthalmus* GÜNTHER);
Herichthys BAIRD & GIRARD, 1854 (*Herichthys cyano-guttatus* BAIRD & GIRARD; reviewed by KULLANDER, 1996; species treated by TAYLOR & MILLER, 1983 and DE LA MAZA-BENIGNOS & LOZANO-VILANO, 2013);
Herotilapia PELLEGRIN, 1904 (*Heros multispinosus* GÜNTHER; monotypic);
Heroína KULLANDER, 1996 (*Heroína isonycterina* KULLANDER, 1996; monotypic);
Hypsophrys AGASSIZ, 1859 (*Hypsophrys unimaculatus* AGASSIZ = *Heros nicaraguensis* GÜNTHER; see KULLANDER & HARTEL, 1997; reviewed by CHAKRABARTY & SPARKS, 2007 and SCHMITTER-SOTO, 2007a);
Nandopsis GILL, 1862 (*Centrarchus tetracanthus* VALENCIENNES; reviewed by CHAKRABARTY, 2006b);
Neetroplus GÜNTHER, 1867 (*Neetroplus nematopus* GÜNTHER; reviewed by CHAKRABARTY & SPARKS, 2007 and synonymized with *Hypsophrys* AGASSIZ);
Parachromis AGASSIZ, 1859 (*Parachromis gulosus* = *Heros managuensis* GÜNTHER; see KULLANDER, 1996 and KULLANDER & HARTEL, 1997);
Paraneetroplus REGAN, 1905 (*Paraneetroplus bulleri* REGAN; reviewed by ALLGAYER, 1988);
Paratheraps WERNER & STAWIKOWSKI, 1987 (*Paratheraps breidohri* WERNER & STAWIKOWSKI);
Petenia GÜNTHER, 1862 (*Petenia splendida* GÜNTHER; reviewed by KULLANDER, 1996; monotypic);
Theraps GÜNTHER, 1862 (*Theraps irregularis* GÜNTHER; reviewed by ALLGAYER, 1989);
Thorichthys MEEK, 1904 (*Thorichthys ellioti* MEEK = *Heros maculipinnis* Steindachner; reviewed by MILLER & NELSON, 1961; see also MILLER & TAYLOR, 1984);

Tomocichla REGAN, 1908 (*Tomocichla underwoodi* REGAN = *Cichlasoma tuba* MEEK; species reviewed by BUSSING, 1976);

Vieja FERNÁNDEZ-YÉPEZ, 1969 (*Vieja panamensis* FERNÁNDEZ-YÉPEZ = Yépez = *Cichlosoma maculicauda* REGAN; see ALLGAYER, 1991).

Several other workers contributed to the classification of Middle American cichlids concurrently with KULLANDER (see citations for major reviews in the above section of available generic names). Distinct positions among these workers deserve the late R. R. MILLER, the foremost authority on Mexican freshwater fishes (see MILLER, 1996, MILLER *et al.*, 2005), W. A. BUSSING, the foremost authority on Central American freshwater fishes (see BUSSING, 1998), and R. STAWIKOWSKI & U. WERNER, the authors of the widely recognized best compendium of Neotropical cichlids (STAWIKOWSKI & WERNER, 1998).

MILLER *et al.* (2005) noted that notations in quotes (i.e. ‘*Cichlasoma*’) are clumsy and most importantly, they do not adequately demonstrate the known diversity of Middle American cichlids, and thus also started to use the available generic names put forward by KULLANDER (1996), although with sometimes different composition of the genera.

The latest formal classification of all Middle American cichlids is by KULLANDER (2003). New or available genera described after KULLANDER (1996, 2003) are (by date of description):

Cryptoheros ALLGAYER, 2001 (*Heros spilurus* GÜNTHER, 1862; treated as a synonym of *Archocentrus* GILL, 1877 by KULLANDER, 2003);
Australoheros ŘÍČAN & KULLANDER, 2006 (*Chromis facetus* JENYNS, 1842);
Amatitlania SCHMITTER-SOTO, 2007a (*Heros nigrofasciatus* GÜNTHER, 1867);
Bussingius SCHMITTER-SOTO, 2007a (*Cichlosoma septemfasciatum* REGAN, 1908);
Panamius SCHMITTER-SOTO, 2007a (*Neetroplus panamensis* MEEK & HILDEBRAND, 1913);
Rocio SCHMITTER-SOTO, 2007a (*Heros octofasciatus* REGAN, 1903);
Nosferatu DE LA MAZA-BENIGNOS *et al.*, 2015 (*Herichthys pame* DE LA MAZA-BENIGNOS & LOZANO-VILANO, 2015; treated as a synonym of *Herichthys* BAIRD & GIRARD, 1854 by MEJÍA *et al.*, 2015);
Maskaheros McMAHAN & PILLER, 2015 (*Vieja argentea* ALLGAYER 1991; McMAHAN *et al.*, 2015);
Kihnichthys McMAHAN & MATAMOROS, 2015 (*Vieja ufermanni* ALLGAYER, 2002; McMAHAN *et al.*, 2015);
Cincelichthys McMAHAN & PILLER, 2015 (*Neetroplus bocourti* VAILLANT & PELLEGRIN, 1902; McMAHAN *et al.*, 2015);
Rheoheros McMAHAN & MATAMOROS, 2015 (*Heros lenti-ginosus* STEINDACHNER, 1864; McMAHAN *et al.*, 2015);
Oscura McMAHAN & CHAKRABARTY, 2015 (*Cichlasoma heterospilum* HUBBS, 1936; McMAHAN *et al.*, 2015);

Chiapaheros McMAHAN & PILLER, 2015 (*Cichlasoma grammodes* TAYLOR & MILLER, 1980; McMAHAN *et al.*, 2015);
Mesoheros McMAHAN & CHAKRABARTY, 2015 (*Heros festae* BOULENGER, 1899; McMAHAN *et al.*, 2015);
Trichromis McMAHAN & CHAKRABARTY, 2015 (*Heros salvini* GÜNTHER, 1862; McMAHAN *et al.*, 2015).

The above listed names have almost obliterated the need to use the cumbersome ‘*Cichlasoma*’ or ‘*Heros*’ to refer to species without generic names. Some species and species groups, predominantly among the amphiphines, however still remain without an available genus name. The majority of the newly described genera (especially those by DE LA MAZA-BENIGNOS *et al.*, 2015 and McMAHAN *et al.*, 2015) have however come from studies characterized by the use of superficial characters without proper homologization and context and complete lack of analysis or discussion of the morphological characters that would establish the polarity of the characters, their variability between and within the putative genera or their usefulness in diagnosing the various new Middle American cichlid genera. These studies thus basically consisted of applying names to clades recovered in mtDNA dominated molecular phylogenies.

Our goal in the present paper is to 1) review all the morphological characters used in the genus descriptions and in the reconstructions of the classification and phylogeny of Middle American cichlids and their usefulness for classification and phylogeny; 2) summarize the current state of molecular-based phylogenetic knowledge on Middle American cichlids and contrast these mtDNA dominated phylogenies available to date with a new robust NGS-generated nuclear phylogeny of the group; 3) analyse the morphological characters in a functional ecological context to see whether and which characters are or are not correlated with ecological and life-history characteristics and which characters are still useful for the recognition of evolutionary unique genera; 4) derive a revised classification of the Middle American heroine cichlids based on this multidimensional analysis rather than based on naming molecular clades without any analysis of the morphological characters.

2. Material and Methods

2.1. Molecular methods

We have used three nested taxon sampling analyses in the present study. (1) The first taxon sampling has a complete coverage across the putative genus-level diversity of the studied group and includes eight molecular markers and 53 ingroup species (*genus-level sampling*). We have used this taxon sampling to compare phylogenetic signals of nuclear and mitochondrial DNA. (2) The second data set has its taxon sampling extended to a com-

plete species-level coverage including 107 ingroup species (*species-level sampling*) and is based on seven molecular markers. It has the same taxon sampling (except undescribed species or species described during preparation of this paper) as that of ŘÍČAN *et al.* (2013). The novel nuclear genome NGS ddRAD dataset also has a comparable species-level sampling. This ddRAD dataset has been used to study morphological character evolution. (3) Finally, in order to ascertain the species diversity of the Middle American heroine cichlids we have extended the molecular matrix of the *species-level analysis* with multiple mtDNA cytb sequences per species that cover the whole distribution areas of all species. We refer to this analysis as the *population-level sampling* and it includes a total of 903 terminals with 445 new cytb sequences representing all described and putative ingroup species (see Supplementary material 1). The sequencing of the cytb gene from the new material and the ddRAD data were generated by LP and KD while OŘ collected all the 445 newly sequenced specimens that also include all specimens used for the ddRAD analysis (and a larger number of so far not sequenced specimens) in the field between 2002 and 2014.

The concatenated molecular data set includes seven molecular markers (eight including the nuclear S7 intron 2 in the smallest *genus-level* taxon-sampling analyses), four mitochondrial (cytochrome *b* [cyt *b*], NADH dehydrogenase subunit 4 [ND4], cytochrome *c* oxidase subunit I [COI], 16S) and three nuclear (RAG1, RAG2, S7 intron 1 [S7i1]), which all have a representative coverage across the genus-level diversity of the studied group. The cyt *b* gene is always sampled for all terminal taxa. The final aligned and concatenated molecular data matrix with seven markers has 5914 bp. Molecular data for this dataset are taken from various phylogenetic studies published on the Middle American heroine cichlids. See Appendix 1 in ŘÍČAN *et al.* (2013) for the sources of the data and the character and taxon sampling table. For molecular laboratory protocols see ŘÍČAN *et al.* (2008, 2013).

Phylogenomic analysis using ddRAD sequencing. The double digest Restriction-site Associated DNA Sequencing (ddRADseq; PETERSON *et al.* 2012) method was used to acquire a sufficient amount of nuclear markers. The principle of ddRADseq is based on digesting of genomic DNA with two different restriction enzymes followed by ligation of individually bar coded sequencing adaptors to the obtained DNA fragments, and precise size-selection of appropriate fraction of fragments for sequencing.

RADseq library preparation. 240 samples representing 105 species of the Middle American cichlid clade (Supplementary Material 1; plus 18 outgroup species including four heroines, four cichlasomatines, four geophagines and *Retroculus* plus *Cichla*) were processed together into one final ddRAD library. The library preparation followed a slightly modified protocol of PETERSON *et al.* (2012). 300 ng of genomic DNA from each individual (extracted by standard column chromatography based kits) was digested with two restriction enzymes, SphI and MluCI in one 30 µl reaction. P1 and P2 “flex” adapt-

ers (PETERSON *et al.*, 2012) were ligated in a 40 µl reaction with 100 ng of the digestion product. The total volume of 48 ligation products differing in adapter barcode were pooled together into a “sublibrary” and five sublibraries in total were prepared; the order of samples was randomized between and within sublibraries. Automated size selection of a fraction of 276–324 bp separately from each sublibrary was performed on Pippin Prep laboratory platform using Pippin Prep 2010 kit. PCR amplification with primers bearing the multiplexing indices and Illumina flowcell annealing regions was done in several 50 µl reactions (separately for each sublibrary). PCR products were purified on AMPure XP beads, and combined in equimolar ratios to compose the final library. Sequencing was performed on an Illumina HiSeq 2000/2500 (2 lanes, 100 and 125 cycles P/E) in the EMBL Genomic Core Facility, Heidelberg, Germany.

Bioinformatic processing of sequenced tags. Basic characteristics of obtained reads were reviewed in FastQC v0.10.1 (ANDREWS, 2010). Barcode sorting and quality filtering of raw reads were performed in *process_radtags*, a pipeline component of Stacks v1.19 (CATCHEN *et al.*, 2011). Two different strategies were used for assembling of the obtained RAD sequences: a de-novo assembling, and assembling based on a reference genome: 1. In the de-novo approach, *denovo* pipeline of Stacks v1.34 was used to find homologous loci between individuals and call for SNPs to build a phylogenetic matrix. Different combinations of parameters (m, minimum number of identical reads to create a stack, i.e. potential locus within an individual; M, number of mismatches between loci when processing a single individual; n, number of mismatches between loci when building a catalogue of different individuals) were used. 2. Alternatively, RAD sequences were first aligned onto the genome of *Oreochromis niloticus* GCA_000188235.1 (<http://www.ensembl.org>) using Bowtie 2 assembler (v2.2.4; LANGMEAD & SALZBERG, 2012; local alignment with ‘very sensitive’ set of otherwise default parameters), and followingly processed in the *ref_map* pipeline implemented in Stacks v1.34. In both approaches, extracting of SNPs into a phylogenetic matrix was processed in *population* component of the pipeline with several different sets of parameters (p, minimum number of individuals a homologous locus must be present; m, minimum stack depth for each individual). Obtained matrix in PHYLIP format was processed in a Linux script (written by LP) assigning names to all individuals and transforming the data also into FASTA and NEXUS formats.

PhyML 3.0 (GUINDON & GASCUEL, 2003) was used to reconstruct maximum likelihood (ML) phylogenetic trees from the SNP data. Analyses were run with optimization of equilibrium frequencies and using the GTR+G substitution model. To evaluate statistical branch supports, a PhyML-implemented aLRT (approximate likelihood ratio test; ANISIMOVA & GASCUEL, 2006) non-parametric bootstrap support based on Shimodaira-Hasegawa-like procedure was computed. For the purpose of ML analyses SNP matrices were built exclusively from homozy-

gote (fixed) sites masking thus within-individual polymorphism; only sites with m parameter ≥ 7 , and p $\geq 50\%$ were used.

2.2. Morphological methods

Morphological characters targeted primarily those used as putatively diagnostic in published genus descriptions. Our morphological data set however includes also characters used in all species descriptions and also novel characters. The morphological data set, as well as the list of taxonomical studies from which these characters were taken, and the morphological material examined was published by ŘÍČAN *et al.* (2008). All these morphological characters were studied by OŘ using the specimens of Middle American cichlids and outgroup taxa housed at NRM (Stockholm, Sweden), MNCN (Madrid, Spain), and for the present study also using specimens collected in the field for the molecular samples. In total 1106 museum (NRM and MNCN) specimens of all species (except ‘*Cichlasoma*’ *microlepis* and ‘*Heros*’ *margaritiferus*) were studied for external characters, using x-rays for osteological and internal meristic characters (all specimens were x-rayed) and 194 specimens representing all described and putative genera were cleared and stained for the study of anatomical characters. Partial dissections primarily (but by far not entirely) of species not available for study in the NRM and MNCN collections were done with specimens collected by OŘ and housed in the Ichthyological Collections of the Faculty of Science (University of South Bohemia [ICFS], České Budějovice, Czech Republic).

The novel characters used in the character matrix of ŘÍČAN *et al.* (2008) were coloration pattern characters. These were studied based on 1) literature (STAWIKOWSKI & WERNER, 1998), 2) the personal photographic archives of OŘ and JN, 3) the personal photographic archive of UWE WERNER, and 4) specimens of virtually all species that were caught and observed by OŘ in the field both outside and during the breeding season. Coloration pattern characters were additionally studied in an ontogenetic perspective based on 1) the personal photographic archive of JN and UWE WERNER, 2) a specifically designed study of the ontogeny of coloration patterns conducted between 1997 and 2007 by OŘ (part of these results were published by ŘÍČAN *et al.*, 2005, 2008) and 3) from various stages of development of juveniles that were caught while being guarded by their parents in the field between 2002 and 2014. Thanks to these efforts we have available complete ontogenetic series of coloration pattern development of all genus-level taxa and for the vast majority of Middle American cichlid species (but also all other heroine cichlids, all cichlasomatine cichlid genera and all geophagine cichlid genera; some results were published by ŘÍČAN *et al.*, 2005). From part of these studies a morphological character matrix of 94 characters published by ŘÍČAN *et al.* (2008) was constructed.

2.3. Ecology and ecomorphology of the Middle American cichlids

In this review, we are striving for a functional approach in the analysis and interpretation of morphological characters because a mere listing of differences and similarities between taxa as character states without the regard to their overall context reflecting their way of life (ecology, life-history) can be very simplistic and/or misleading, resulting in scoring as homologous characters and character states that are only superficial similarities, or that are ancestral. Cichlids are well known for their adaptive capabilities, and many morphological characters historically interpreted as synapomorphies of various taxa could well be merely reflections of the same adaptations to similar or identical ecological conditions.

Ecological characteristics of Middle American cichlid habitats and the species food preferences were studied in laboratory following the methodology of WINEMILLER *et al.* (1995) and in the field during several field trips led by OR between years 2002 and 2014 using the voucher specimens of the collected DNA tissue samples. The observations were compared with the information contained in the compendium on the biology of Middle American cichlids (STAWIKOWSKI & WERNER, 1998), with all primary literature summarized in this book (the most significant being WINEMILLER *et al.*, 1995; CONKEL, 1997; KONINGS, 1989) and with subsequent works including HULSEY (2006), SORIA-BARRETO & RODILES-HERNÁNDEZ (2008), COCHRAN-BIEDERMAN & WINEMILLER (2010). Species were divided into ecomorphotypes based on their food preferences (their predominant diet) and food gathering mode (feeding mode) using this data and literature comparisons. LÓPEZ-FERNÁNDEZ *et al.* (2013) provided a functional multidimensional PCA analysis of the morphological diversity of Neotropical cichlids. We compared our ecomorphological classification of Middle American cichlids with this analysis that is based solely on body shape characterization. We also compared the Middle American cichlid ecomorphological diversity with that of the South American geophagine cichlids.

2.4. Phylogenetic methods

Phylogenetic methods follow ŘÍČAN *et al.* (2008, 2013). We have here additionally used Bayesian methods to analyse morphological and combined morphological-molecular data sets. Bayesian inference (BI) were conducted with a Metropolis-coupled Markov chain Monte Carlo algorithm (ALTEKAR *et al.*, 2004) as implemented in MRBAYES version 3.1.2 (HUELSENBECK & RONQUIST, 2001; RONQUIST & HUELSENBECK, 2003). MODELTEST 3.06 (POSADA & CRANDALL, 1998), and PAUP* version 4.0b10 (SWOFFORD, 2003) were used to estimate the best-fitting substitution models for DNA data. Morphological characters were treated as unordered, with standard discrete model assuming Γ -shaped rate variation and variable coding bias. Model parameters were unlinked

across partitions. Two independent runs of combined analysis with 10 Markov chains each were conducted for 5,000,000 generations with a sample frequency of 100 (heating 0.2).

The seven used molecular markers (eight in the *genus-level* taxon sampling) were analysed using the same five data-partition scheme as in ŘÍČAN *et al.* (2013). Mitochondrial and nuclear markers were analysed separately, both divided into coding and non-coding, with mitochondrial coding loci divided by codon position (first plus second versus third). The partition scheme was thus as follows: (1) cytb+ND4+COI pos. 1&2, (2) cytb+ND4+COI pos. 3, (3) 16S, (4) RAG1+RAG2, and (5) S7i1+2.

An optimal model of evolution for each data partition according to Akaike's information criterion was selected using MrModelTest 2.2 (NYLANDER, 2004) and PAUP* 4.0b10 (SWOFFORD, 2003). The Bayesian analysis using the Markov chain Monte Carlo (MCMC) simulation was run with unlinked parameters (except for branch length and topology) for 10 million (30 million in the *population-level sampling*) generations with trees sampled and saved every 1000 generations. Two independent analyses, each comprising two runs with eight chains, were performed to compare results of independent analyses. The analyses were run at the then freely available Bioportal server (<http://www.bioportal.uio.no/>). The same parameters were also used for the combined morphological – molecular Bayesian analyses. The first 25% of trees from each run were discarded as burn-in. Convergence of the runs was estimated with the use of: (1) diagnostic criteria produced by the 'sump' command in MrBayes; (2) graphical visualization and diagnostics in Tracer 1.5.0 (RAMBAUT & DRUMMOND, 2007). The remaining trees were used for reconstruction of the 50% majority-rule consensus tree with posterior probability (PP) values of the branches.

Maximum parsimony analyses (MP) were run in PAUP* version 4.0b10 (SWOFFORD, 2003) as described in ŘÍČAN *et al.* (2008). Robustness of clades was assessed here using bootstrapping in MP and posterior probabilities in BI.

2.5. Character evolution

Character evolution has been studied by mapping morphological characters on molecular phylogenetic trees as implemented in Mesquite (MADDISON & MADDISON, 2004) and WinClada (NIXON, 2002) using the unambiguous-only optimization.

For diagnoses of genera in this group of fishes that features very few clear-cut and unique morphological characters we have used an approach that tries to look at deeper homologies that go beyond the directly observable by using a functional and developmental approach in the analysis and interpretation of morphological characters.

We have divided the morphological character matrix (ŘÍČAN *et al.*, 2008) into four logical parts that describe 1) cranial morphology, 2) postcranial morphology, and

3) coloration pattern ontogeny and 4) adult coloration including breeding coloration and life-history. We have then mapped the individual characters of these data partitions on 1) the multilocus molecular phylogeny with the *species-level sampling* and 2) on the newly generated ddRAD topology. Based on this mapping we have reconstructed the common ancestor of all Middle American cichlids for all characters.

We have then compared character states at all nodes to the common ancestor. The changes between the genera and the common ancestor could be used to diagnose the genera but they can still be nonhomologous and in the studied group are highly homoplastic (ŘÍČAN *et al.*, 2008). We have at this stage looked for correlations between characters i.e. for combinations of characters and their changes (states). Identical combinations of character changes are far less likely to be nonhomologous and are thus a better tool to diagnose unique genera than single character diagnoses. We have also during this stage looked for correlations between individual characters and character combinations with ecological characters.

The coloration pattern characters have been studied using an even deeper homology approach since they were studied in an ontogenetic perspective (see ŘÍČAN *et al.*, 2005 for partial results). Characters were thus scored not only in adults but throughout all stages of development and in this ways many novel and often unique characters for Middle American cichlids (and Neotropical cichlids in general) were found. These ontogenetic studies revealed an unexpected diversity of coloration pattern developmental pathways that have additionally given us understanding of how the diversity of the adult coloration patterns form throughout development; the developmental knowledge has also shown us that many similar adult coloration patterns are only superficial similarities that lack any developmental homology. These ontogenetic coloration characters are unique among all characters (morphological or adult coloration characters) in that they are much less variable within genera and are diagnostic for genera and even deeper lineages where all other non-molecular characters are virtually uninformative. The unique developmental pathways are directly used for diagnoses of genera in combination with other character complexes from the three additional morphological data partitions.

2.6. Biogeographical methods

Diversity and distributional data were obtained by combining our field work data (2002–2014) with published information and museum records (the most important being the STRI collection in Panamá, the NRM collection in Stockholm and the MNCN collection in Madrid). Additional major works with distributional information beyond publications listed in Appendix 1 in ŘÍČAN *et al.* (2008, 2013) included ANGULO *et al.* (2013) and MATAMOROS *et al.* (2009, 2012). A diversity map using 143 river basins in Middle America was compiled from this data.

As terminal distribution units for biogeographic analyses we are using cichlid endemic areas (CEAs). We have identified 28 CEAs in Middle America using the Phylogenetic Analysis of Endemism (PAE) based on localities (*sensu* ROSEN, 1988; in our case using river basins). We have in parallel to PAE also made a delimitation of the CEAs by hand. Both methods have found the same 28 units with practically the same borders, which in the PAE analysis were in several cases poorly resolved because of limitations of the PAE analysis (see ROSEN, 1988).

Delimitation of CEAs using the Phylogenetic Analysis of Endemism (PAE) based on localities (*sensu* ROSEN, 1988; in our case using river basins) using the *species-level* taxon sampling phylogeny was done by taking into account the proposed modifications of a PAE data matrix by ROSEN (1988), which are: 1) Any taxon common to all the localities or present in only one of them must be eliminated from the analysis as it is not informative; 2) The localities that show scarce presence must be eliminated because low diversity is interpreted in the analysis as primitive; 3) The localities that present exactly the same taxa must be considered as a unique analysis unit (CRISCI *et al.*, 2003; ROSEN, 1988).

ROSEN (1988) in general and our observations of distributional limits of Middle American cichlids in particular demonstrate that areas of endemism are in some regions difficult to establish, as there generally exist taxa that pass beyond the defined limits. In our case of Middle American cichlids many CEAs have very straightforward borders (e.g. within the Usumacinta ichthyological province or between the San Juan and Bocas CEAs), but some areas do have borders of a filter-barrier type (e.g. the border between the San Juan and Chiapas-Nicaragua ichthyological provinces). Most resulting CEAs have two or more endemic species, but some peripheral (in two cases with a massive area) which do not influence the analysis have only one endemic species.

The CEAs are more fine-scaled but have the same main boundaries as the 12 Ichthyological provinces (IPs) of ŘÍČAN *et al.* (2013), which were based on MILLER (1966), BUSSING (1976, 1985), MILLER *et al.* (2005), SMITH & BIRMINGHAM (2005) and which are in agreement with MATAMOROS *et al.* (2015) except where the latter study differs from all the previous studies (e.g. in the wrong delimitation of the San Juan area vs. the Bocas). Our CEAs differ in some areas from MATAMOROS *et al.* (2015) because we characterize the group-specific endemism of only one fish group and because MATAMOROS *et al.* (2015) have not implemented into their PAE analysis the necessary modifications of a PAE data matrix proposed by ROSEN (1988; see above).

Reconstruction of ancestral areas for all nodes of the phylogenetic tree using the CEAs as terminal geographical units was done using the event-based Bayesian statistical dispersal–vicariance analysis (S-DIVA; implemented in RASP 2.0; YU *et al.*, 2011). Distributions of all terminals at the level of CEAs were input into S-DIVA.

For the S-DIVA analyses we used trees from BEAST runs with 25% trees discarded as burn-in. The probabil-

ity of ancestral areas for nodes was then plotted on the majority-rule consensus tree derived from MCMC. The analyses were carried out using a number of different the ‘maxareas’ options in S-DIVA up to the maximum number of areas in the analysis.

2.7. Generic classification

We strive in the present study for a balanced and stable solution to generic classification. We follow the proposed taxon naming criteria (TNCs) of VENCES *et al.* (2013) and try to eliminate both the objective and subjective reasons for the instability of classifications. The guidelines for classifications expressed as TNCs were ordered by VENCES *et al.* (2013) based on the order of importance. The *Primary TNCs* includes: 1) Monophyly. Monophyly is the first, and the only strict taxon-naming criterion; 2) Clade Stability, i.e., the monophyly of a clade to be named as taxon should be as strongly supported as possible by various methods of tree inference, tests of clade robustness, and different data sets; 3) Phenotypic Diagnosability, i.e., ranked supraspecific taxa should be those that are phenotypically most conspicuous (although in phenotypically cryptic groups of organisms it can be warranted to name taxa based on molecular differences alone); The *Secondary TNCs* include: 4) Time banding, i.e. evolutionary age as a criterion; 5) Biogeography; 6) Adaptive zone, i.e. a clade occupying a distinctive adaptive zone should be assigned to a ranked category and thus named as Linnaean taxon; and finally the *Accessory TNCs* include: 7) Manageable units (species-rich taxa can be advantageous if they are phenotypically homogeneous, phenotypically diverse are more manageable when partitioned into multiple genera); 8) Community consensus.

Diagnoses of genera are based on the possession of unique characters, character states or a unique apomorphic character state combination. We however also propose new genera for long-isolated and in DNA characters unique monotypic lineages that have ancestral morphological character combinations because such taxa would otherwise remain lingering in the taxonomical limbo because they are undiagnosable using morphological characters only.

3. Results

— Phylogeny

3.1. Sequence quality, species determination, and comparison with previous molecular phylogenies

The phylogenetic analyses of the Middle American heroine cichlids in this study confirm in the broad the

results of previous studies. There are however several notable differences between the studies of ŘÍČAN *et al.* (2008, 2013), CONCEIRO PÉREZ *et al.* (2007), HULSEY *et al.* (2004, 2010, 2011), HIGHAM *et al.* (2007) and between ARBOUR & LÓPEZ-FERNÁNDEZ (2014), LÓPEZ-FERNÁNDEZ *et al.* (2010, 2013), CHAKRABARTY (2006a), CHAKRABARTY & ALBERT (2011), MATAMOROS *et al.* (2015), McMAHAN *et al.* (2010, 2015).

These differences are based on our analysis due to species misidentifications and/or contaminations, or simply the use of wrong sequences in the cited studies. We have discovered these incorrect sequences by initial first-step analyses which analysed all molecular markers separately by including all available sequences from GenBank (as was done also by ŘÍČAN *et al.*, 2013) and in the second step by comparisons of the sequences with the population-level sampling analysis based on specimens from multiple localities collected by us in the field. We have found in these analyses quite a substantial number of sequences that were clear and striking cases of species and even genus misidentification (ŘÍČAN *et al.*, 2013: Appendix S1; at the deepest-levels we have found sequences referred to be from amphiphine species that are actually from misidentified species of the herichthyines or basal South American genera and vice versa). There were more than ten such terminals that were either species misidentifications and/or laboratory contaminations (*‘Heros’ calobrensis*, *Theraps heterospilus*, *Trichromis salvini*, *‘Heros’ urophthalmus*, *‘Heros’ wesseli*, *Australoheros facetus*, *Cryptoheros myrnae*, *Cryptoheros nanoluteus*, *Herichthys labridens*, *Nandopsis tetracanthus*, *Thorichthys aureus*, *Thorichthys helleri*) and several published studies involved these and thus resulted in flawed phylogenies. The problematic sequences virtually all came from the study of LÓPEZ-FERNÁNDEZ *et al.* (2010; same dataset also used in LÓPEZ-FERNÁNDEZ *et al.*, 2013 and in several other studies including the taxonomic revision of McMAHAN *et al.*, 2015). In these studies some of these mixed-up terminals appear in incorrect phylogenetic positions (e.g. as successive outgroups of the amphiphines in the case of *Cryptoheros nanoluteus* and *Trichromis salvini* or deeply within the amphiphines – *Australoheros facetus*), which has spawned heated discussions in cichlid meetings and forums e.g. about the close relationships and confusing biogeography of *Australoheros* within Middle American cichlids refuted by ŘÍČAN & KULLANDER (2006) and ŘÍČAN *et al.* (2008, 2013). Especially the wrong phylogenetic position and biogeographic interpretations of *Australoheros* is a point worth stressing. Many other mixed-up terminals are in the correct phylogenetic position and could only be detected in individual gene analyses. All these visible and invisible mixed-up terminals however do have artificial branch lengths and these factors have to a large extent flawed the topology, node supports, and especially the age estimates within the whole of the Middle American cichlids in the study of LÓPEZ-FERNÁNDEZ *et al.* (2013; see ŘÍČAN *et al.*, 2013). Recently McMAHAN *et al.* (2010) have added to the list of species for which wrong sequences have been used in

the case where they excluded *Paraneetroplus nebuliferus* from *Paraneetroplus* on the basis of a DNA sequence from '*Heros*' *sieboldii*, a species found not in México but in Costa Rica (PAEPKE *et al.*, 2014).

Before we describe the phylogenetic relationships and resulting taxonomical adjustments of the Middle American cichlids we would like to point out these problematic issues found in some previous studies. The message from this study is that concatenating sequences from different specimens (often with unknown origin) is potentially dangerous by producing chimerical phylogenies. In this study we overcome these problems by using for both the mtDNA and nDNA phylogenies multiple specimens and for the nDNA additionally a newly developed ddRAD method, relying almost solely on wild-caught specimens. Conflicts between mitochondrial and nuclear DNA described in Results are thus not these cases of species misidentifications but likely reflect real biological phenomena mostly often referable to natural hybridization events.

3.2. Bayesian phylogenetic analyses

Bayesian phylogenetic analyses in independent runs for each taxon-sampling dataset converged well, in the divisions of the *population-level* dataset convergence was more difficult but enough runs were obtained where parameters scored effective sample size (ESS) values of 200 or higher (as reported in Tracer 1.5.0 for four well-converged combined runs with a 25% burn-in). The 5-partition analysis had the highest ESS scores (using a 25% burn-in) and is shown in the final trees (figs. 1–3). Bayesian runs of the *species-level* analysis received strong support for most nodes as judged from posterior probability values (fig. 2). The topology of the population-level analyses has lower support at the basal nodes, but the topology is at equivalent nodes virtually identical to the *species-level* analysis (see figs. 2–3).

3.3. Molecular phylogeny using *genus-level sampling*: comparing phylogenetic signals in molecular datasets

The molecular matrix including both mitochondrial (mtDNA) and nuclear genes (nDNA) with *genus-level sampling* analyzed using Bayesian inference (BI) is shown in fig. 1. The analysis of nDNA (fig. 1A) revealed the existence of three suprageneric clades with high posterior probability (the amphiphines, the herichthyines, and the astatheroines) plus three separate genera (*Australoheros*, *Nandopsis*, and *Caquetaia*). *Australoheros* is the sister-group of the remaining groups, while *Nandopsis* and *Caquetaia* are possible sister-groups in a clade that also includes the amphiphines. The herichthyines and the astatheroines are the successive sister-groups to this clade.

The basal relationships between the main clades in the mtDNA analysis are weakly supported (as opposed to

the analysis based on nDNA), with strong support only for the amphiphines and the crown-group herichthyines (fig. 1B). The astatheroines are found nested among the weakly supported herichthyines, the basal-most lineage is *Nandopsis* (instead of *Australoheros*), and *Australoheros* and *Caquetaia* are weakly supported successive sister-groups of the amphiphines.

The combined analysis of the nuclear and mitochondrial genes (fig. 1C) again recovers strongly supported amphiphines, well supported herichthyines which in this analysis include also the well supported monophyletic astatheroines, and the three separate genera are found without statistical support in a clade with the amphiphines.

All molecular analyses with the genus-level taxon sampling support the monophyly of the following genera: *Amphilophus* (including *Archocentrus* in mtDNA and combined analyses), *Astatheros*, *Australoheros*, *Caquetaia* (including *Heroína*), *Cryptoheros*, *Herichthys* (represented by only one species in these analyses, but its monophyly is well established by other analyses; e.g. ŘÍČAN *et al.*, 2008), *Hypsophrys*, *Nandopsis*, *Parachromis*, *Paraneetroplus* (including *Paratheraps* and *Vieja*), *Theraps* (including *Chuco* and some *Vieja*), and *Tomocichla*.

There are two additional strongly supported supraspecific clades recovered by all analyses in the same phylogenetic position that were recently or that we here elevate to new genera (the '*Heros*' *festae* group, the '*Heros*' *wesseli* group, the '*Heros*' *sieboldii* group). There are additionally three unique species with long evolutionary branches that are consistently found in isolated phylogenetic position in all the analyses (*Cichlasoma calobrense* MEEK & HILDEBRAND, *Cichlasoma grammodes* TAYLOR & MILLER, and *Heros salvini* GÜNTHER). The first is here described as a new genus and the latter two were recently described as new monotypic genera.

Our analyses reveal several species and genera that have conflicting positions in the nDNA and mtDNA topologies (*cf.* fig. 1A and B). Among the amphiphines *Heros istlanus* JORDAN & SNYDER is in the nDNA topology the sister-group of *Amphilophus trimaculatus*, while in the mtDNA topology it is the sister-species of *Heros beani* JORDAN, in both cases with the highest posterior probability. *Petenia splendida* is in the nDNA topology the sister-group of *Parachromis*, while in the mtDNA it is the sister-group of '*Heros*' *urophthalmus*, in both cases again with the highest posterior probability. '*Heros*' *urophthalmus* is in the nDNA topology a basal amphiphine together with '*Heros*' *beani*. Among the herichthyines *Heros lentiginosus* STEINDACHNER is in the nDNA analysis found as a basal-most *Paraneetroplus*, while in mtDNA analysis as the basal-most *Theraps*, in both cases with the highest posterior probability. Some of these cases are probably more than just mtDNA introgressions and are possibly species of hybrid origin since they have unique and very easily distinguishable morphologies among the Middle American heroine cichlids (e.g. '*Heros*' *istlanus*).

There are also conflicting positions between the nDNA and mtDNA topologies at the genus level. The positions of *Trichromis*, *Thorichthys*, *Mesoheros* and *Tomocichla* are different between the nDNA and mtDNA topologies. Finally, there are a few novel relationships in the combined molecular analysis (fig. 1C) found in neither of the nDNA and mtDNA analyses; the most striking of these regard the genera *Cryptoheros* and *Tomocichla*. *Cryptoheros* is in individual mtDNA and nDNA analyses found as a strongly monophyletic genus, while the combined molecular analysis (as well as the combined molecular-morphological analysis; see below) reconstructs it as paraphyletic to *Hypsophrys* (*C. spilurus* is a strongly supported sister-group of *Hypsophrys*). *Tomocichla* is in individual mtDNA and nDNA analyses found as either the sister group of *Rocio* or *Mesoheros*, while in the combined molecular analysis (as well as the combined molecular-morphological analysis) it is the sister genus of *Herotilapia*.

3.4. Combined molecular-morphological phylogenies

The combined morphological-molecular matrices with both taxon samplings (*genus-level* and *species-level*) were also analysed with Bayesian inference (BI). The analyses resulted in topologies that were (except for branch-lengths) identical to the corresponding molecular analyses stressing the limited phylogenetic influence of the morphological character partition of the resulting topologies (see fig. 1D for the combined *genus-level* molecular-morphological phylogeny).

3.5. Molecular phylogenies using *species-level* sampling

The *species-level* sampling matrix including concatenated mitochondrial (mtDNA) and nuclear genes (nDNA) was analysed using Bayesian inference (BI; fig. 2). The topology and the very high support values are in agreement with ŘÍČAN *et al.* (2013) and with the *genus-level* analysis (fig. 1).

Contrary to the individual nDNA and mtDNA *genus-level* analyses (fig. 1A and B) but in agreement with the combined molecular *genus-level* analysis (fig. 1C) the *species-level* analysis (fig. 2) does not support the monophyly of the genus *Cryptoheros*. The *species-level* analysis also does not support the monophyly of the genus *Astatheros* (contrary to all *genus-level* analyses; fig. 1).

In agreement with the *genus-level* analyses the monophyly of the newly proposed genera, as well as of the postulated monotypic genera is well supported (see above). Also in agreement with the former analyses is the paraphyly of *Caquetaia* to *Heroina isonycterina* and of *Amphilophus* to *Archocentrus centrarchus*.

3.6. Molecular phylogeny using *population-level* sampling

The concatenated mtDNA/nDNA molecular matrix with *species-level* sampling has been extended by enlarging the mtDNA cytb partition to 903 taxa (*population-level* sampling) and was again analysed with Bayesian inference (BI; fig. 3). This is in terms of taxon sampling and locality coverage the largest analysis of Middle American heroine cichlids published to date, with multiple sequences per species from many localities. We use this mtDNA-based dataset to verify species status of virtually all species throughout their complete native ranges. The analysis confirms the existence of several putatively new species of cichlids in Middle America (pers. obs., see also e.g. STAWIKOWSKI & WERNER, 1998) and at the same time calls the validity of several described species in question.

In total nine putatively new species are recovered in the mtDNA-based analysis with times of divergence from their sister species being in all cases quite considerable, ranging between 1.2 and 9.9 My (mean 4.8 My; see ŘÍČAN *et al.*, 2013 for the molecular-clock dates). All the putative new species except one (the recently described *Herichthys pame* DE LA MAZA-BENIGNOS & LOZANO-VILANO, 2013) are allopatric (parapatric) in regard to their sister species or their previously associated species. *Herichthys pame* is a molariform-toothed molluscivorous species sympatric with its sister-species the piscivorous *Herichthys steindachneri* (this species pair also shows the smallest time divergence of 1.2 My). Most of these potentially new species have already been mentioned in (aquarium hobby) literature as likely candidates for a separate species status (e.g. STAWIKOWSKI & WERNER, 1998).

3.7. Novel nDNA molecular phylogeny based on reduced genome representation (ddRAD analysis) using *species-level* sampling

Two Illumina paired-end sequencing lanes comprising 240 individuals resulted in 760.0 million 100/125 bp sequenced fragments of which 745.1 million (98.0 %) passed through the procedure of barcode sorting and raw reads quality filtering (with default Stacks setting). Discarded reads contained: ambiguous barcodes, 1.8 mil.; low quality reads, 6.4 mil.; ambiguous restriction sites, 5.7 mil.; reads containing adapter sequence, 1.0 mil. Sequenced individuals were represented in average by $3,104,602 \pm 1,569,224$ SE QC-passed reads per sample (single and pair-end reads in total). Processing of RAD tags in Stacks resulted in a catalogue containing 4,361,747 and 16,239,056 prospectively homologous loci based on de-novo and reference-genome approaches, respectively.

SNP extraction resulted in a set of phylogenetic matrices varying in size from ca. 45,000 to 370,000 variable characters, depending on selected approach and setting of parameters in *populations* pipeline component (p, 120–216; m, 3–10). Both assembling strategy and matrix size have very little effect on the tree topology; relevant values of branch supports slightly grow with the matrix size. The topology shown in fig. 5 is a consensus topology based on both de-novo and reference-mapping analyses on the genome of *Oreochromis niloticus*. The individual analyses will be provided elsewhere (PIÁLEK *et al.*, in preparation) but overall they provided well resolved and well supported phylogenies with virtually all nodes at and above the species level having a bootstrap support of 1. One of the trees analysed using Maximum likelihood based on reference-mapping assembly and including 140,000 characters (SNPs) is shown in Supplementary Material 4.

The ddRAD analyses in general supported the results of the concatenated *genus-level* nDNA analysis (fig. 1A) and additionally provided much better resolution. The analyses have also confirmed and even extended (due to a much better taxon sampling) the conflicts between mtDNA and nDNA shown in the genus-level analysis (fig. 1A, B). Figures 4 and 5 compare the phylogenetic signals of mtDNA and nDNA.

Among the herichthyines the ddRAD analysis confirmed virtually all nodes from fig. 1A. The separate genus status of the genera *Chiapaheros*, *Trichromis* and *Mesoheros* was confirmed with high probability, as are their phylogenetic relationships based on the nDNA concatenated analysis (fig. 1A). *Herichthys* is the sister-group of the monophyletic *Theraps-Paraneetroplus* clade (contrary to concatenated mtDNA/nDNA). *Rheoheros* is not part of *Theraps sensu stricto* (*T. irregularis*) but forms a separate genus which is the sister group of the *Paraneetroplus* clade. The proposed genus *Cincelichthys* (*C. bocourti*, *C. pearsei*) was in agreement with the nDNA *genus-level* analysis (fig. 1A; contrary to mtDNA and concatenated mtDNA and nDNA) found in the ddRAD analysis not as a sister-group of *Theraps* (*sensu* McMAHAN *et al.*, 2015), but within *Theraps* and we thus for the former *T. intermedius* group use the available generic name *Chuco*. In the nDNA and mtDNA *genus-level* analyses (fig. 1A, B) the alternative phylogenetic positions of *Cincelichthys* are without statistical robustness, nor is the mtDNA dominated topology in the concatenated species-level phylogeny (fig. 2) statistically robust, but the ddRAD position within *Theraps sensu* McMAHAN *et al.* (2015) has the highest possible support value of 1. The newly proposed genus *Kihnichthys* was not available for the ddRAD study, but its close phylogenetic relationship with *Cincelichthys* in the concatenated nDNA/mtDNA phylogeny (fig. 2 and 3A) and monophyly in morphological phylogeny (see below) together with lack of diagnostic characters against *Cincelichthys* make it questionable whether they are two separate diagnosable genera. The putative sister-group of *Rheoheros* (described as the separate genus *Oscura*; *O. heterospila*) was represented

in the ddRAD analysis by only one juvenile specimen and it is the sister-group of *Vieja sensu stricto* (*V. maculicauda* and *V. melanura*; see Supplementary Material 4). This placement thus refutes the separate genus *Oscura* but this possible synonymy with *Vieja* needs to be tested with more than one specimen. The newly proposed genus *Maskaheros* is the sister-group of *Paraneetroplus*.

The astatheroines form in the ddRAD phylogeny a strongly supported separate clade (as in the nDNA genus-level analysis; fig. 1A). The phylogenies based on concatenated data (figs. 1C, D & 2) were thus dominated by the mtDNA partition (figs. 1B & 4–5). *Herotilapia* is the basal lineage of the astatheroines, followed by *Tomocichla*. *Astatheros* is non-monophyletic with *Rocio* nested within.

Most new groupings discovered by the ddRAD analysis are found within the complicated amphiphine clade radiation. *Hypsophrys sensu lato* is found within *Cryptoheros sensu* SCHMITTER-SOTO (2007a, b). *Cryptoheros sensu stricto* is the sister-group of *Hypsophrys sensu lato* and *Amatitlania* plus the rest of *Cryptoheros* (subgen. *Bussingius*), which is however paraphyletic to *Amatitlania*. *Cryptoheros panamensis* forms a separate lineage unrelated to this clade (*Panamius* SCHMITTER-SOTO, 2007b).

Amphilophus is monophyletic in the wide sense suggested by the genus-level and species-level concatenated analyses, i.e. including also *A. istlanus* and *A. trimaculatus* (as terminal sister-species). *Archocentrus centrarchus* is, contrary to mtDNA (figs. 1B & 2), recovered as the sister group of *Amphilophus*.

A separate clade is formed by ‘*Heros*’ *sieboldii* plus ‘*Heros*’ *underwoodi* and ‘*Heros*’ *tuyrensis*, as in all other DNA analyses (figs. 1–3), but ‘*Heros*’ *wesseli* is not a sister group of this clade (as suggested in fig. 1) and makes up a separate isolated lineage, which is surprisingly the sister-group of *Petenia splendida*.

Another discovery of the ddRAD analysis among the amphiphines is the firm sister-group relationship (yet with long branches) of ‘*Heros*’ *calobrensis* and ‘*Heros*’ (*Panamius*) *panamensis*. ‘*Heros*’ *calobrensis* thus clearly also represents an evolutionarily independent separate genus.

The amphiphines are in the ddRAD analyses divided into two clades, one of which appears to have an allopatric vicariance pattern. All amphiphine species from the Pacific slope of Costa Rica and Panamá form one clade (the Isthmian clade; ‘*Heros*’ *calobrensis*, ‘*Heros*’ *panamensis*, ‘*Heros*’ *sieboldii*, ‘*Heros*’ *underwoodi* and ‘*Heros*’ *tuyrensis*), which is the sister-clade of *Amphilophus* with some of the northernmost Middle American species on the Pacific slope of México (*A. istlanus*, *A. trimaculatus*).

The ddRAD analysis also groups ‘*Heros*’ *beani* with ‘*Heros*’ *urophthalmus*. The clade has strong support but shows very long divergence of the two species. Both these species have different sister-groups in mtDNA and concatenated phylogenies, ‘*Heros*’ *beani* being the sister-group of ‘*Heros*’ *istlanus* (*Amphilophus istlanus*

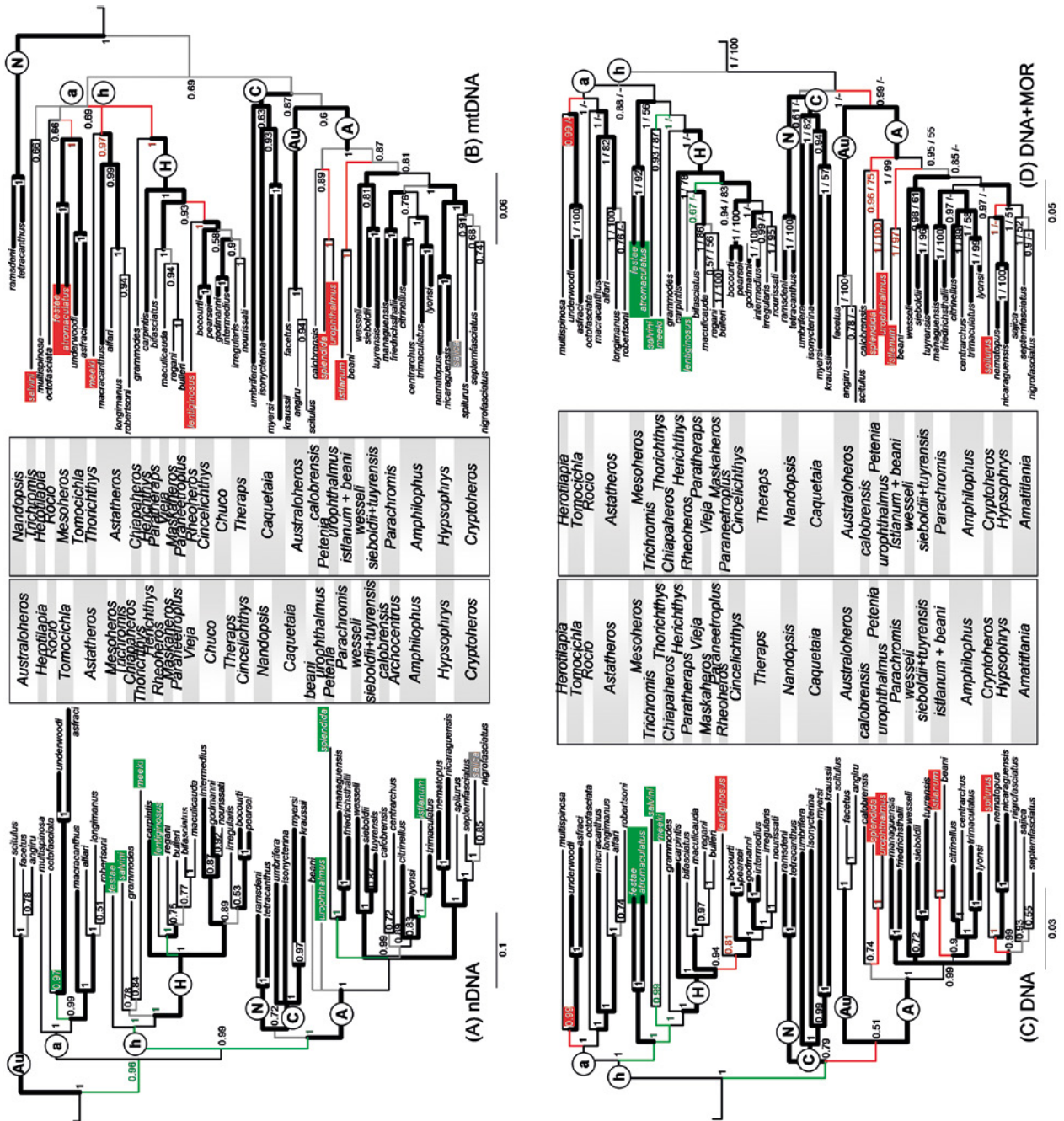


Fig. 1. Phylogenetic relationships of Middle American and related heroine cichlids using *genus-level sampling* analysis based on (A) nuclear DNA (nDNA: S7i1 and 2, RAG1, RAG2), (B) mitochondrial DNA (mtDNA: cyt b, ND4, COI, 16S), (C) combined DNA (DNA) and (D) combined DNA+morphology datasets. The trees shown are 50% majority-rule consensus trees obtained with Bayesian inference analysed as five data partitions (six with morphology), i.e. (1) cyt b+ND4+COI pos. 1&2, (2) cyt b+ND4+COI pos. 3, (3) 16S, (4) RAG1+RAG2, and (5) S7i1+2 (see Methods). Node support shows posterior probabilities (PP) followed by bootstrap support from maximum parsimony analysis in the combined DNA+morphology analysis. Outgroup taxa are the same three non-heroine cichlids as in fig. 2. Nodes and branches shown with thick lines are found in all analyses. Taxa and nodes in green and red colour show conflict between nDNA (green) and mtDNA (red). Main clade abbreviations are as follows: A: amphiphilines; a: astatheroines; Au: *Australoheros*; C: *Caquetaia*; H: crown-group herichthyines; h: herichthyines; N: *Nandopsis*.

based on nDNA), and ‘*Heros*’ *urophthalmus* the sister-group of the sympatric *Petenia splendida* (long separated sister-group of ‘*Heros*’ *wesseli* in nDNA). ‘*Heros*’ *beani* plus ‘*Heros*’ *urophthalmus* are quite similar generalized predators that we place into a single new genus (see below).

Finally at the genus level, the ddRAD analysis enabled us to study the phylogenetic relationships of the enigmatic species described as *Cichlasoma microlepis* DAHL, 1960. We have been able to obtain gill tissue from the alcohol-preserved holotype and sequence it using the ddRAD analysis. The resulting position of *Cichlasoma*

microlepis DAHL, 1960 is the sister-group of the recently erected genus *Mesoheros*. The phylogenetic position appears to be very robust (probability of 1) despite a much lower number of sampled SNPs due to the age of the preserved specimen. Interestingly *Cichlasoma microlepis* DAHL, 1960 is the only heroine (apart from *Caquetaia*) that falls within the distribution area of *Mesoheros*. *Cichlasoma microlepis* DAHL, 1960 is poorly known but very dissimilar morphologically from *Mesoheros* and we are thus elevating it to a separate genus on its own.

Among the putative new species indicated by the mtDNA-dominated population-level phylogeny (fig. 3) five are supported as separate species from previously known species by the nDNA ddRAD analysis (fig. 5). These are *Herichthys pame*, *Herichthys tepehua*, *Thorichthys* sp. aff. *maculipinnis*, *Cryptoheros cutteri* and ‘*Heros*’ *underwoodi* (shown by green colour in fig. 3). Two of the cytb-based putative new species are not supported by the ddRAD analysis (*Thorichthys helleri* and *Astatheros alfari* are strongly monophyletic in the latter analysis; shown with grey colour in fig. 3). These two taxa (populations) are thus likely cases of mtDNA introgression. Two of the putative new species (*Astatheros* sp. aff. *robertsoni*, *Panamius* sp. aff. *panamensis*) were not available for testing with the ddRAD analysis.

On the other hand our mtDNA based molecular data cast doubt on the separate species status of ten presently valid species (fig. 3). Four of these species, or at least their geographical borders (*Cryptoheros spilurus* vs. *Cryptoheros chetumalensis*) remain unsupported also in the ddRAD analysis (shown by red colour; *Herichthys pratinius*, *Amatitlania siquia*, *Amatitlania kanna*). Two species

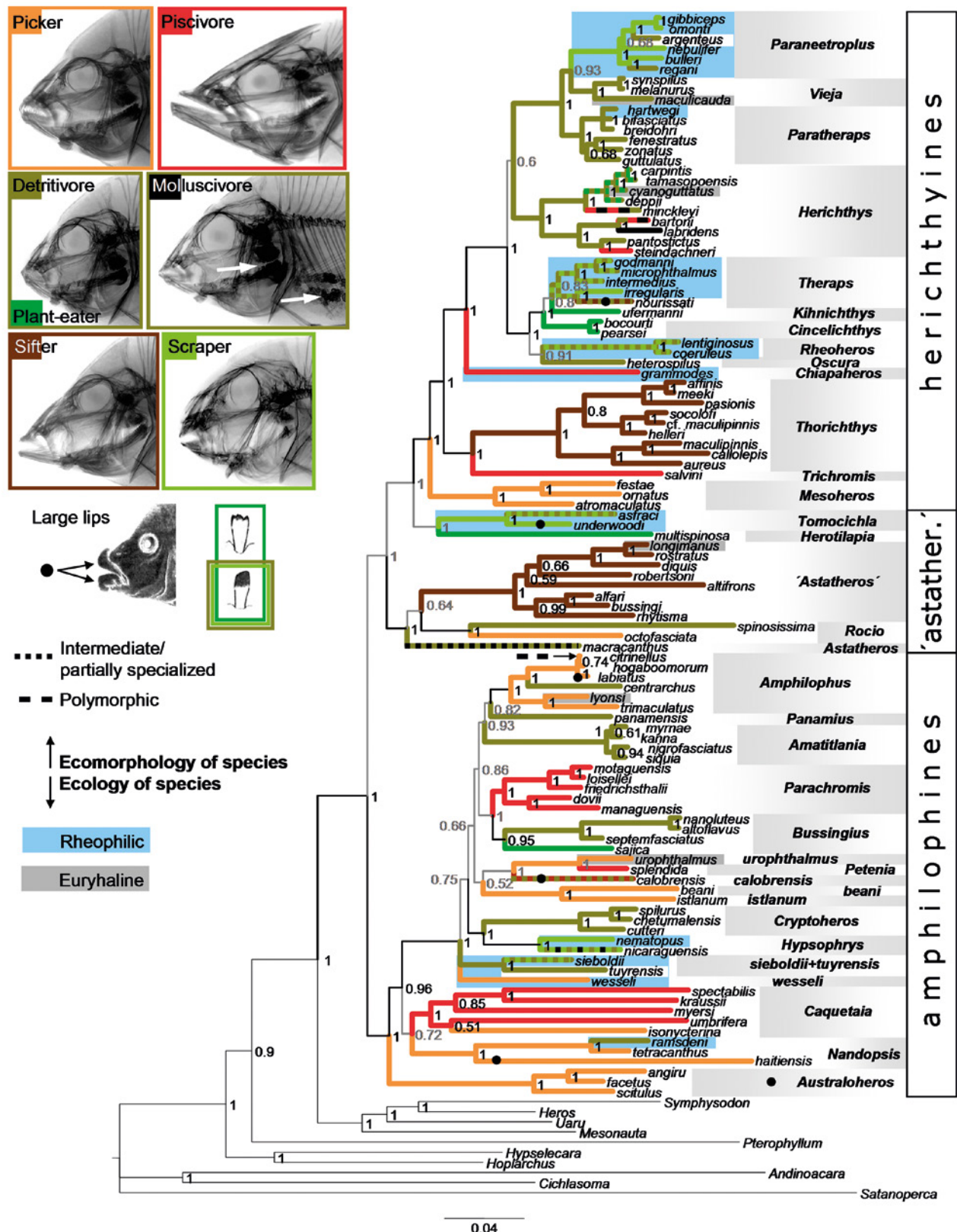
were not available for testing in the ddRAD analysis (also shown by red colour; *Thorichthys affinis*, *Amatitlania altoflava*). The remaining four species are on the other hand monophyletic or do not infringe in the monophyly of other species in the ddRAD analysis and do thus appear to be good species contrary to the mtDNA dominated phylogeny (shown by grey branches and black letters; *Vieja hartwegi*, *Vieja breidohri*, *Herichthys carpintis*, *Paraneetroplus omonti*). The last named species is distinctive in overall body coloration from *P. gibbiceps*, but shows near zero-length branches in both mtDNA and ddRAD analyses. Similar near zero-length branches are however also found within the genus *Herichthys* separating several established or recently described species. *Vieja hartwegi*, *Vieja breidohri* and *Herichthys carpintis* thus do carry introgression of trans-specific mtDNA or alternatively do show incomplete ancestral lineage sorting.

The nuclear genome ddRAD analysis also recovers different relationships of other species compared to the mtDNA-based phylogeny. *Amatitlania myrnae* is not found within the *Amatitlania nigrofasciata* lineage (contrary to mtDNA), but is firmly the sister species of the very similar *A. septemfasciata*. *Amatitlania sajica* is based on the ddRAD topology not a basal species of *Bussingius* (fig. 3) but a sister-group of the *A. nigrofasciata* lineage (fig. 5). Additional differences in relationships among the amphiphine species were described above.

Among the herichthyines the nDNA ddRAD analysis demonstrates a conflicting phylogeny compared to mtDNA for the following species: *Herichthys labridens* (*H. bartoni* vs. rest of *labridens* group), the genus *Rheoheros* (the *Paraneetroplus* vs. the *Theraps* clade), the genus

→ **Fig. 2.** Phylogenetic relationships of heroine cichlids using *species-level sampling* analysis based on mitochondrial DNA (mtDNA: cyt b, ND4, COI, 16S) and nuclear DNA (nDNA: S7i1, RAG1, RAG2). The tree topology is a 50% majority-rule consensus tree obtained with Bayesian inference analysed as five data partitions (see fig. 1 and Methods). Support values (PP) are shown to the right of the nodes. Branches that show conflict between mtDNA and nDNA or that are weakly supported are in grey colour. The combination of this data set with the morphological data partition of ŘÍČAN *et al.* (2008) or CHAKRABARTY (2007) does not change the topology (not shown). The scale bar represents the average number of substitutions per site. Species morphoecologies are shown on the branches. The ecomorphs are represented by the x-radiographs of heads of the following species: Picker: *Mesoheros atromaculatus*, Piscivore: *Petenia splendida*, Detritivore: *Herichthys cyanoguttatus* (Molluscivore: *Herichthys labridens*), Sifter: *Astatheros rostratus*, Scraper: *Neetroplus nematopus*. White arrows in the molluscivorous ecomorph x-radiograph show massive pharyngeal jaws and visible snail shell remains. The ecomorphological classification in this figure is based on our review of own data and published information of stomach content analyses and modes of food intake. The morphoecological classification is not based on morphological characters. These are studied and their correspondence to the here presented ecomorphologies is explored in figs. 7–13. Note the repeated evolution of the ecomorphologies in the main clades of the Middle American cichlids.

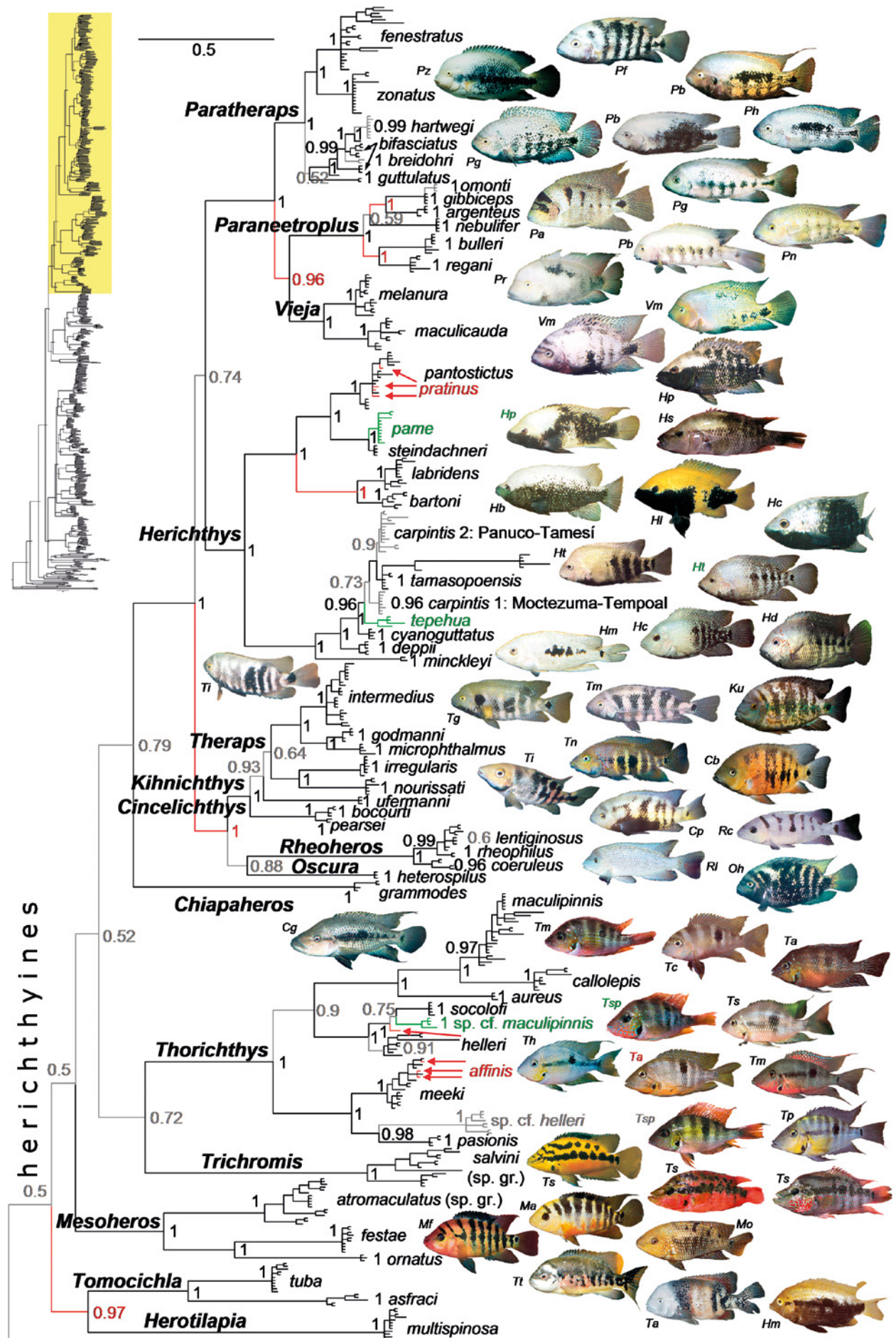
Fig. 3 (pp. 18–19). Phylogenetic relationships of Middle American heroine cichlids using the 903 terminal taxon *population-level sampling* analysis based on mitochondrial DNA (mtDNA: cyt b, ND4, COI, 16S) and nuclear DNA (nDNA: S7i1, RAG1, RAG2). The population-level information is based on the cytb marker only, while the skeleton of the tree is based on the multilocus species-level sampling dataset (fig. 2). The first part of the figure shows relationships within herichthyines, the second part within the amphiphines and astatheroines (both highlighted in the inset tree). Other parts of the tree are omitted (see Supplementary material 2). The tree shown is a 50% majority-rule consensus tree obtained with Bayesian inference analysed as five data partitions (see figs. 1–2 and Methods). Support values (PP) are shown to the right of the nodes. Putative new species are shown in green. Conflicts with existing classification and conflicts between nDNA and mtDNA are shown in red (*cf.* figs. 1 & 4). Weakly supported nodes are in grey colour. The scale bar represents the average number of substitutions per site. Each species is also shown by a photograph of a specimen in breeding coloration accompanied by a two-letter species-name acronym. See Supplementary material 1 for the specimen sampling of the Middle American species.

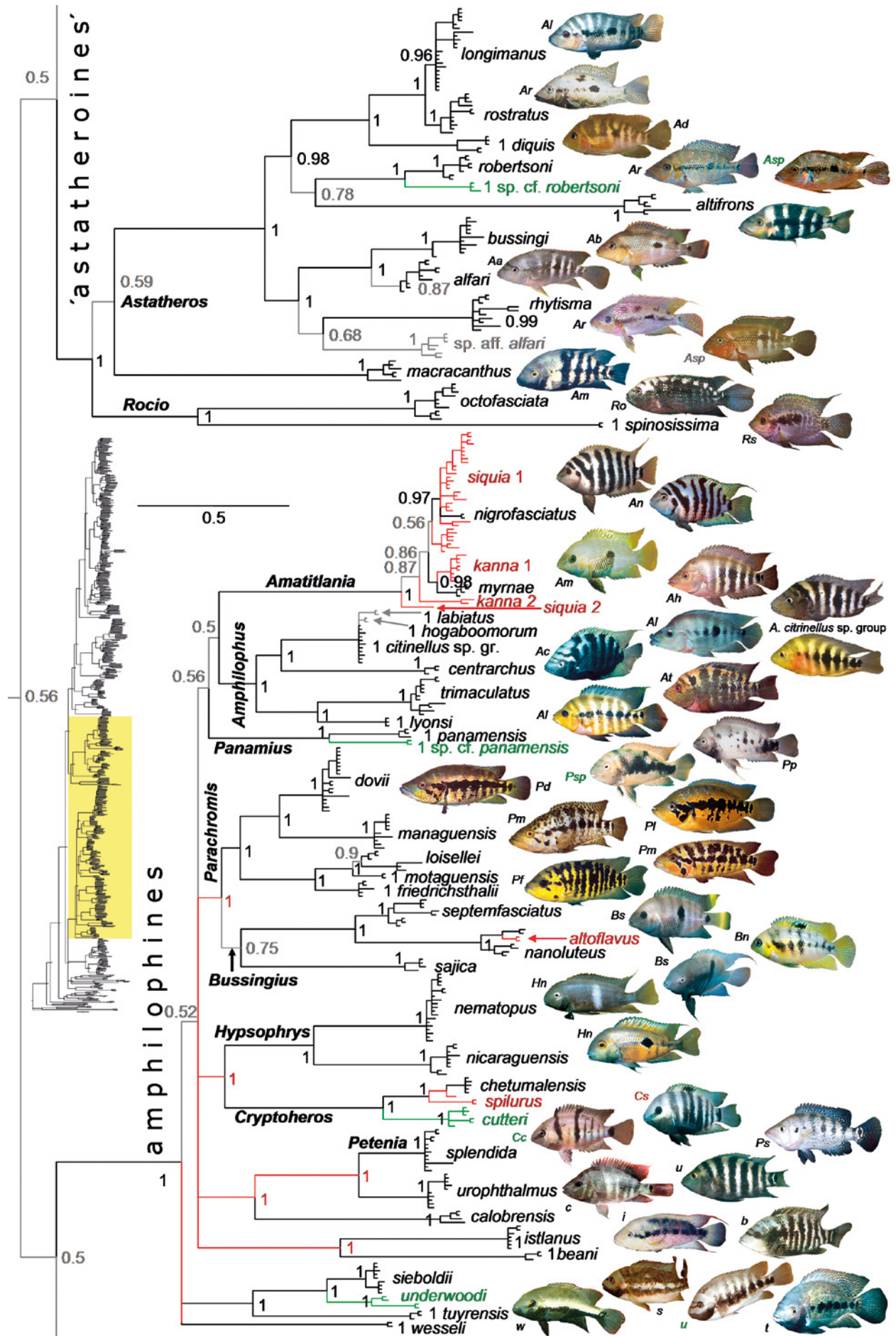


Cincelichthys (within *Theraps sensu stricto* vs. sister to), *Maskaheros argenteus*, and *M. regani*.

All the above described conflicts strongly demonstrate that mtDNA and nDNA phylogenetic signals have to be analysed first separately and only then in combination (if there is no conflict), because conflict between the

two partitions will remain in the combined analysis and the resulting phylogeny is most often dominated by the (non-organismal) mtDNA topology because it has more informative characters. Virtually all studies published on the Middle American cichlids with the exceptions of ŘÍČAN *et al.* (2008, 2013) have not analysed conflicts be-





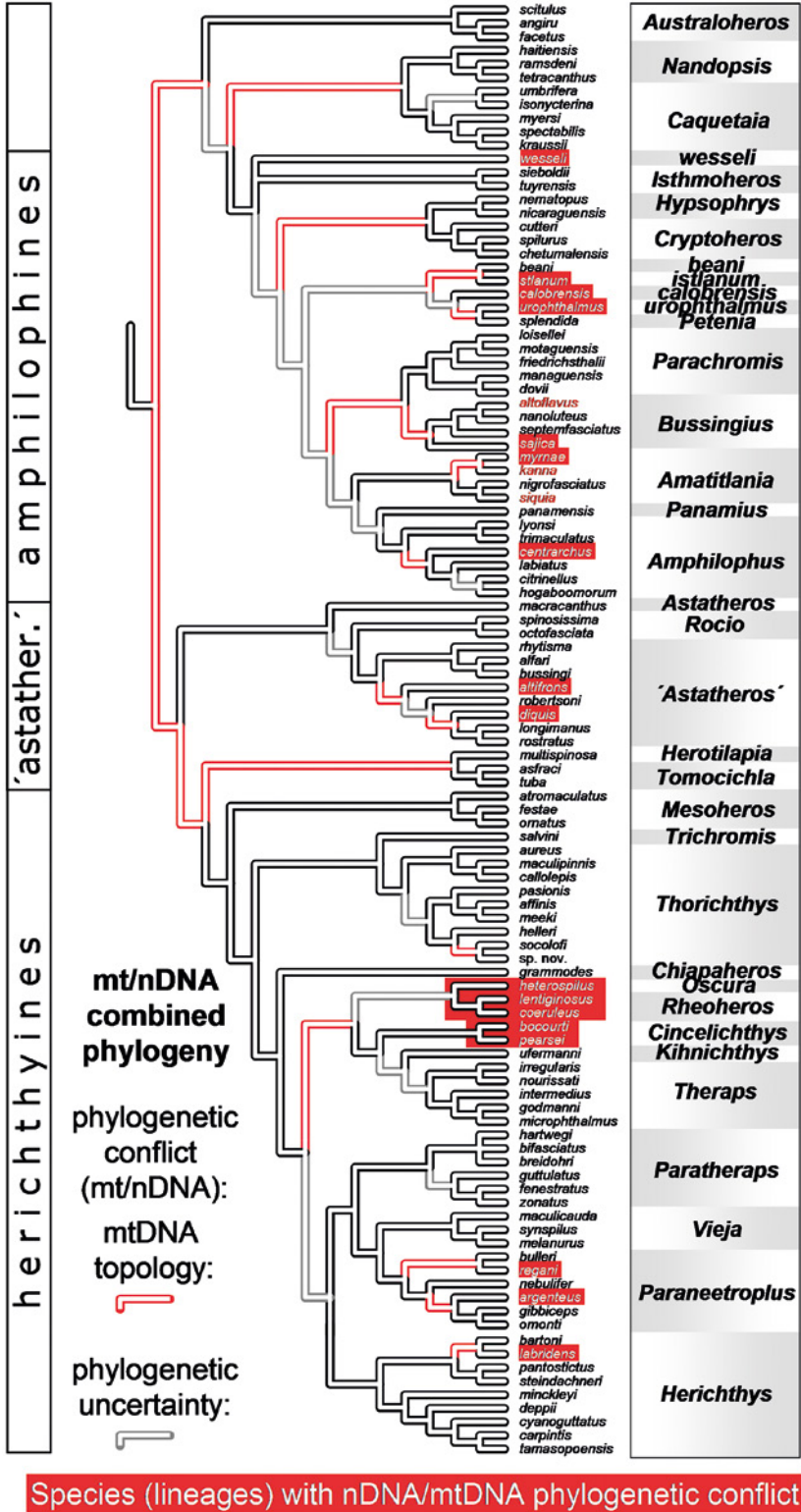


Fig. 4. Summary topology of phylogenies based on concatenated mt/nDNA datasets (figs. 1–3). In red are shown conflicts between the mtDNA and nDNA phylogenetic signals.

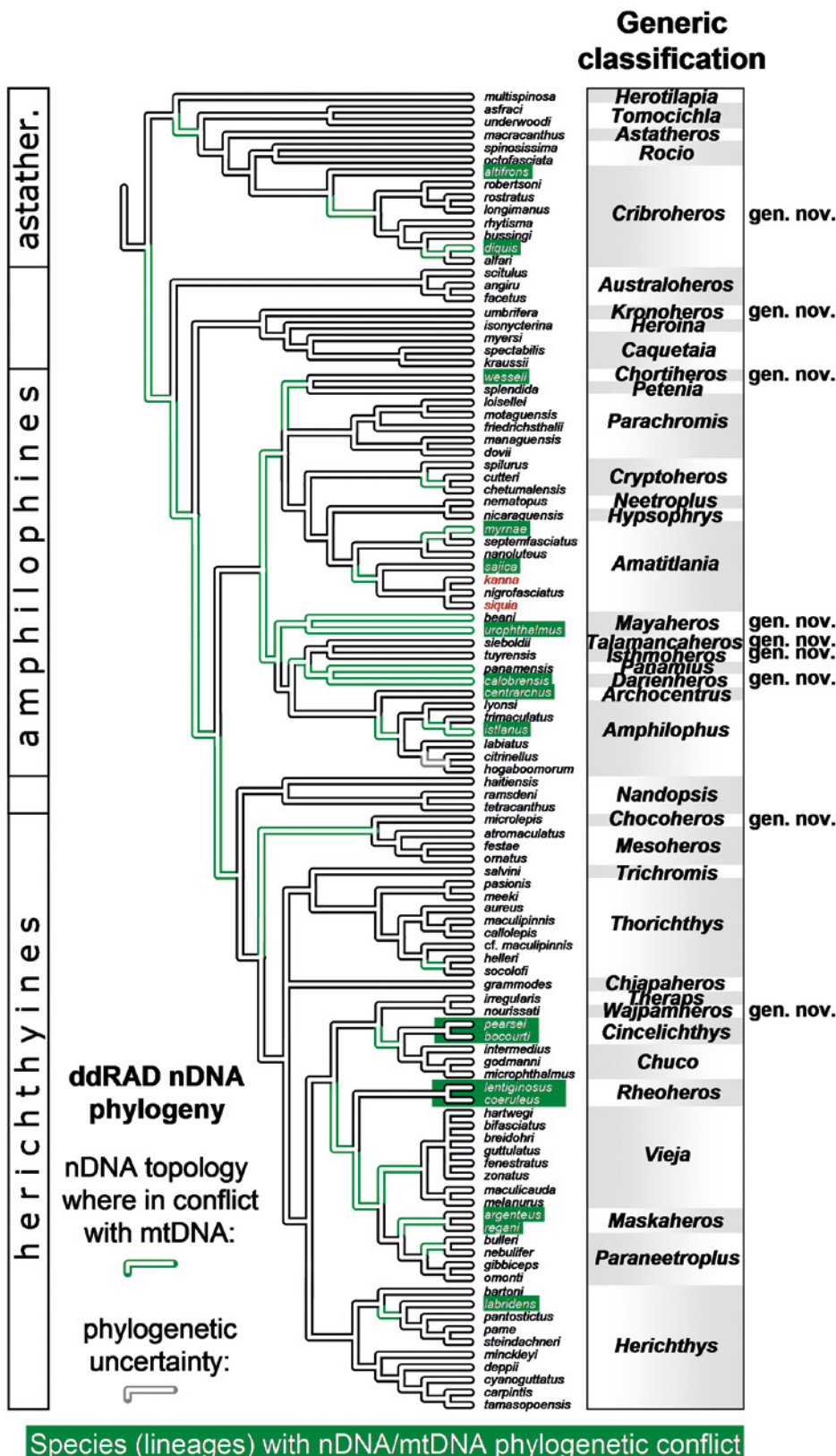


Fig. 5. Summary topology of the novel nDNA phylogeny based on ddRAD-sequencing with proposed classification. The topology shown is a consensus topology based on both de-novo and reference-mapping analyses. The analyses provided almost identical, well resolved and well supported phylogenies with virtually all nodes at and above the species level having a bootstrap support of 1. One of the trees analysed using Maximum likelihood based on reference-mapping assembly onto the genome of *Oreochromis niloticus* and including ~140,000 characters (SNPs) is shown in Supplementary Material 4. Approximate branch lengths are shown in the summary topology to give impression of the evolutionary distances between genera. In green is shown the nDNA topology for nodes that show nDNA/mtDNA conflict (see figs. 1–3). This summary nDNA ddRAD topology is used to study character evolution throughout the present study.

tween mtDNA and nDNA and even in those studies that have the resulting topologies still represent a mixture of mtDNA and nDNA phylogenetic signals (as demonstrated in figs. 1–2). Where there is a conflict between mtDNA and nDNA both may be true evolutionary histories, but their combined analysis does not represent any of them. We have overcome this problem in the present study by sampling enough nDNA informative characters (SNPs) using the ddRAD analysis and the approach of combining nDNA with mtDNA to gain robustness of phylogenetic hypotheses has become obsolete.

— Systematics

All treated genera belong to the subfamily Cichlasominae KULLANDER, 1998. Morphological methods follow KULLANDER (1983, 1986, 1996) and also REGAN (1905, 1906, 1908) for cranial and jaw proportions. All counts given in the diagnoses are modal values. Coloration and coloration pattern ontogenetic characters follow methodology of ŘÍČAN *et al.* (2005). Direction of change and apomorphy of character states is based on ingroup and outgroup comparisons through mapping of all morphological characters on the nDNA ddRAD consensus topology (Fig. 5). In this way the common ancestor of Middle American cichlids was reconstructed and apomorphic changes from this reconstructed ancestor are used for generic diagnoses (see chapter 3.12.).

3.8. Descriptions of new genera of South and Middle American cichlids

— caquetaines

Genus *Kronoheros* gen. nov. ŘÍČAN & PIÁLEK

Type species: *Cichlasoma umbriferum* MEEK & HILDEBRAND, 1913: 88. Type locality: Río Cupe, Cituro, Panamá. Holotype: FMNH 7598.

Included species. *Kronoheros umbriferus* (MEEK & HILDEBRAND, 1913)

Diagnosis. At present a monotypic genus diagnosed by a piscivorous cranial morphology and ancestral postcranial morphology. This character combination is only found in *Caquetaia* and *Kronoheros* among heroine cichlids. Most similar in this regard to *Caquetaia*, from which distinguished by having body, head and mouth much wider (vs. distinctly laterally compressed which is an apomorphy of *Caquetaia*). The piscivorous-adapted cranial morphology is highly developed, but to a slightly lesser extent than in *Caquetaia* by having the ascending arm of premaxilla reaching only to the vertical through the posterior margin of the eye. In all characters thus of ancestral morphology compared to *Caquetaia*, but the phylogenetic position of *Heroína* between *Kronoheros* and *Caquetaia* and the distinctive lentic morphology of

Heroína precludes inclusion of *Kronoheros* into *Caquetaia*. All three genera share some aspects of piscivorous cranial morphology (least developed and secondarily reduced in *Heroína*) including tip of the lower jaw projects distinctly in front of the upper jaw; maxilla extends below the eye; transverse arm of premaxilla with an oblong posteriad process (shared with *Caquetaia* and *Petenia*); long to very long premaxillary ascending process reaching to the vertical from the posterior margin of the eye; pointed conical teeth without a second cusp, but teeth in the lower jaw are small, short, without very distinctly enlarged symphyseal teeth (shared with *Caquetaia*, in *Petenia* the teeth are even much smaller and these small teeth are also found in the upper jaw; *Heroína* is reverted to the ancestral condition of large teeth with distinctly enlarged symphyseal teeth); ethmoethmoid ligament (lateroethmoid-premaxillary ligament); reduced anteroventral palatine wing and slightly exposed median palatovomerine ligament; fold of lower lip continuous; second lower lip prominent; lateral band L-type coloration pattern ontogeny.

Distribution. North-western South America and Pacific slope eastern Panamá in the Atrato and Magdalena River drainages in Colombia, and Tuira and Chucunaque River drainages in Panamá.

Etymology. Named after Kronos (Greek: Κρόνος, *krónos*), the leader of the first generation of Titans (the descendants of Uranus and Gaia) of Greek mythology that preceded the Olympian deities, in combination with the old cichlid genus name *Heros* (meaning hero). Gender: masculine. The name is given after the leader of the Titans because *Kronoheros* attains the largest body size among Neotropical cichlids.

Notes. The Panamanian and Colombian populations of *Kronoheros umbriferus* likely represent two distinct species distinguished by coloration patterns (STAWIKOWSKI & WERNER, 1998). Mitochondrial DNA *cytb* gene (CONCHEIRO PÉREZ *et al.*, 2007) shows substantial separation (~ 6 My; ŘÍČAN *et al.*, 2013) supporting two separate species.

— chocoheroines

Genus *Chocoheros* gen. nov. ŘÍČAN & PIÁLEK

Type species: *Cichlasoma microlepis* DAHL, 1960: 480. Type locality: Pavarandó, Río Baudó, Colombia. Holotype: ICNMHN 95.

Included species. *Chocoheros microlepis* (DAHL, 1960).

Diagnosis. This very little known genus is similar to other non-specialized substratum sifters (*Darienheros calobrensis*, *Wajpamheros nourissati*, *Astatheros macracanthus*, *Nandopsis ramsdeni*) in thick lip structure, long preorbital distance, eye positioned relatively high on head and slightly inferior mouth. Diagnosed from all Middle American and related heroine cichlid species by

the autapomorphically high number of scales along lateral line (45 as opposed to 50–52 in the original description; vs. less than 35).

Etymology. Named after the Embera-Wounaan indigenous tribe, also known as the Chocó, a name derived from their language, in combination with the old cichlid genus name *Heros* (meaning hero). Chocó is also the well known biogeographic area and area of endemism encompassing the distribution of the genus. The name is to be understood as the hero of the Choco. Gender: masculine.

Distribution. *Chocoheros* is endemic to the single river basin of Rio Baudó on the Pacific side of NW South America in Colombia.

Notes. *Chocoheros microlepis* has never been collected since its original description despite several efforts (KULLANDER, pers. comm., MALDONADO-OCAMPO, pers. comm.). STALSBERG in 1990 (pers. comm. in STAWIKOWSKI & WERNER, 1998) observed while snorkelling in one of the tributaries of the Rio Baudó silvery cichlids that evaded all efforts of their capture. The silvery coloration and the obviously good swimming capacity agree with the many parallel horizontal silvery lines on the body of the type specimens and with the long caudal peduncle and large caudal fin. OŘ has obtained a small amount of gill tissue from the alcohol-fixed and alcohol-preserved holotype specimen of *Chocoheros microlepis* in 2003 from Prof. PLUTARCO CALA (ICNMFH, Bogotá, Colombia). We have never succeeded in amplifying DNA using classical Sanger sequencing (neither for mtDNA nor for nDNA loci) from the tissue despite DNA being present in the sample. The ddRAD protocol has fortunately provided a substantial amount of SNP data that enabled us to reconstruct the phylogenetic position of the species with high probability (Supplementary Material 4). *Chocoheros* is the sympatric sister genus of *Mesoheros*, which inhabits the whole Choco plus some adjacent areas while *Chocoheros* is endemic to a single river basin within the Choco. *Chocoheros* is however not included in most of our detailed morphological and life-history analyses in this study because these traits are virtually unknown. The difference in scale counts between the original description and our study is due to a different method of counting scales that was not clearly explained in the original work.

— astatheroines

Genus *Cribroheros* gen. nov. ŘÍČAN & PIÁLEK

Type species: *Heros rostratus* GILL in GILL & BRANSFORD, 1877: 181. Type locality: Lake Nicaragua. Syntypes: USNM 16872(9).

Included species. *Cribroheros alfari* (MEEK, 1907); *Cribroheros altifrons* (KNER, 1863); *Cribroheros bussingi* (LOISELLE, 1997); *Cribroheros diquis* (BUSSING, 1974); *Cribroheros longimanus* (GÜNTHER, 1867); *Cribroheros rhytisma* (LÓPEZ, 1983); *Cribroheros robertsoni* (REGAN, 1905); *Cribroheros rostratus* (GILL, 1877).

Diagnosis. *Cribroheros* is a genus combining a horizontal substratum-sifting cranial morphology with an ancestral (plesiomorphic) postcranial, further diagnosed by intermediate (interrupted-line) I-type coloration ontogeny. This character combination is unique among Middle American cichlids. Distinguished from *Thorichthys* with the same substratum-sifting cranial morphology by ancestral postcranial morphology (vs. lentic postcranial with increased numbers of anal fin spines) and by lacking an ocellated spot on the opercle used for signaling. Distinguished from *Astatheros* and *Darienheros calobrensis* by its horizontal substratum-sifting cranial morphology (vs. detritivorous/molluscivorous or less specialized sifting cranial morphology) with a produced snout, a deep to very deep preorbital region associated with an anteroposteriorly deep lachrymal, fine, needle-like, tightly spaced teeth that are scarcely increasing in size towards symphysis (there is a trend to this condition but not all species of *Cribroheros* have this tooth type completely developed vs. large, widely spaced and towards symphysis in size increasing teeth), and by the presence of a frenum on the lower lip (vs. absent in both genera). Additionally distinguished from *Astatheros* by anterior and posterior palatovomerine ligaments inserting onto the palatine in close proximity; second lower lip small (absent in *Astatheros*); presence of iridescent blue spots and vermiculations on the sides of head (absent in *Astatheros*); ancestral number of caudal vertebrae (vs. decreased to 15; present also in *C. longimanus*); ancestral or elevated number of dorsal fin spines (vs. decreased to 15); ancestral number of anal fin spines at the genus level (vs. decreased to 5; present also in *C. altifrons* and *C. diquis*); ancestral number of scale rows between anterior margin of anal fin and lateral line (8 vs. elevated number to 10); 15 vs. 18 transverse scale rows in total; decreased number of scale rows between upper lateral line and the base of anterior part of soft dorsal (2.5 vs. 3.5); decreased number of scale rows between bases of pelvic and pectoral fins (4–5). Additionally distinguished from *Darienheros calobrensis* by the intermediate (interrupted-line) I-type coloration ontogeny (vs. the amphiphilophine lateral stripe L-type coloration ontogeny). Compared to most Middle American heroines (which have three abdominal bars) *Cribroheros* usually only has two abdominal bars in adult coloration (similar to *Astatheros*).

Etymology. Name derived from the Latin noun *cribrum* which means sieve (verb *cribrō* meaning to sift or sieve), in combination with the old cichlid genus name *Heros* (meaning hero). The name is an allusion to the substratum sifting feeding strategy. Gender: masculine.

Distribution. *Cribroheros* is distributed over most of Middle America, from western Panamá to southern México, on both Atlantic and Pacific slopes. The *alfari* group is restricted to Central America south of the Río Aguán in Honduras on the Caribbean slope (the San Juan and Bocas ichthyological provinces), the *rostratus* group is here sympatric but reaches further to the north into south-

ernmost North America up to the Río Papaloapán (the Usumacinta ichthyological province; also Caribbean – Atlantic slope). *Cribroheros diquis* and *C. altifrons* are distributed on the Pacific slope in the Chiriqui ichthyological province.

Notes. The separation of *Cribroheros* and *Astatheros* into two genera is justified based on both phylogenetic relationships and morphological distinctiveness. Some concatenated nDNA/mtDNA phylogenies and all nDNA ddRAD phylogenies find *Rocio* nested between the genera *Astatheros* and *Cribroheros*. *Rocio* cannot be included into *Astatheros* due to completely different cranial and postcranial morphology and coloration patterns and *Astatheros* and *Cribroheros* thus have to be separated into two genera, easily diagnosed by many morphological characters. The substratum-sifting feeding habits of species in *Cribroheros* are best developed in the *C. rostratus* group, since these species have an elevated number of gill rakers on the lower part of anterior arch (> 14) and produced snouts with a very deep preorbital and longer lower jaw. The sister group relationship of the two most derived species (*C. longimanus* and *C. rostratus*) is additionally supported by unique thin lower jaw teeth, which are not increasing in size anteriorly, and teeth in 2nd (and 3rd) rows as large as 1st row teeth and also very slender. The *C. alfari* group is diagnostic by increased number of dorsal fin spines (17). The *C. rostratus* group additionally has an increased number of pectoral fin rays (15).

'Heros' calobrensis (*Cichlasoma calobrense* MEEK & HILDEBRAND, 1913) often included in *Amphilophus* or *Astatheros* is recovered here as a separate genus within the amphilophines.

Heros margaritifer Günther, 1862 is known only from the Holotype collected in Lago Petén in Guatemala. The species is generally placed into *Astatheros* (*Cribroheros* in the present study; included in *Amphilophus* in KULLANDER, 2003) but this has never been tested using phylogenetic analysis. Several specimens have been collected on various occasions that have been attributed to *Heros margaritifer*. All these specimens possess a strange combination of characters (<http://www.cichlidae.com/species.php?id=486>; <http://www.cichlidae.com/forum/viewtopic.php?f=4&t=8602>) which include; five dentary pores (otherwise only known from *Thorichthys*) vs. four in all other genera; large anteriorly in size increasing oral jaw teeth vs. teeth fine, needle-like and scarcely increasing in size towards symphysis (otherwise only known from some species of *Cribroheros* and *Thorichthys*); an indistinct midlateral blotch (vs. usually prominent in *Cribroheros* and *Thorichthys*), caudal blotch elongated onto peduncle (vs. confined to caudal fin base). In addition to this character combination which does not fit any here recognized genus the individual specimens referred to *Heros margaritifer* Günther, 1862 differ substantially from each other in body shape and coloration patterns and the best current hypothesis is that all these specimens are hybrids between a *Thorichthys* species (five dentary

pores; i.e. *T. affinis* based on distribution) and some yet unspecified species. MORGENSTERN (2015) has very reasonably argued that the character combination suggests as the second parental species *V. melanura*.

— herichthyines

Genus *Wajpamheros* gen. nov. ŘÍČAN & PÁLEK

Type species: *Theraps nourissati* ALLGAYER, 1989: 12, fig. p. 13. Type locality: petite rivière à 1 km avant le village “La Pera” sur la route de Palenque vers Ojo de Agua, Chiapas (Mexique). Holotype: MNHN 1989-583.

Included species. *Wajpamheros nourissati* (ALLGAYER, 1989).

Diagnosis. *Wajpamheros* is a monotypic genus with a unique combination of substratum-sifting cranial morphology with a lotic postcranial morphology. All other Middle American substratum-sifting cichlids have an ancestral or lentic postcranial morphology. *Wajpamheros* is similar to other herichthyine genera in the *Theraps* clade in having double vertical bars four and five. *Wajpamheros* is very similar to *Theraps* (*T. irregularis*), its sister genus in coloration patterns but is distinguished by its substratum-sifting cranial morphology including a much longer head and produced snout with a long preorbital area, thick lips, deeper body and shorter caudal peduncle.

Etymology. Name derived from the Maya Chol dialect word wajpam which means ‘to have mud on one’s face’, in combination with the old cichlid genus name *Heros* (meaning hero). The name is an allusion to the substratum sifting feeding strategy during which mouth and lips are inserted into soft substrates (hence mud on the face). Gender: masculine.

Distribution. *Wajpamheros* is distributed in the Usumacinta river basin in México and Guatemala.

Notes. Because of its substratum sifting ecomorphology *Wajpamheros* has been included into *Astatheros* (then *Amphilophus*) in some previous classifications. *Wajpamheros* is however a herichthyine sharing coloration patterns and coloration ontogeny with the *Theraps* clade and it is the only substratum sifter in the *Theraps-Paraneetroplus* clade. It lives syntopically with other more specialized substratum sifters of *Thorichthys* and one species of *Cribroheros* (*C. robertsoni*).

— amphilophines

Genus *Darienheros* gen. nov. ŘÍČAN & NOVÁK

Type species: *Cichlasoma calobrense* MEEK & HILDEBRAND, 1913: 90. Type locality: Río Calobre, Panamá. Holotype: FMNH 7600.

Included species. *Darienheros calobrensis* (MEEK & HILDEBRAND, 1913).

Diagnosis. *Darienheros calobrensis* has been referred to *Astatheros* or *Amphilophus*, but the species shows a combination of characters not seen in any of the two genera, and is in our analyses supported as a separate lineage of heroine cichlids. Diagnosed by its apomorphic character combination of thick lips, long preorbital distance, eye positioned relatively high on head and slightly inferior mouth. *Darienheros* however shares this character combination with other non-specialized substratum sifters (*Chocoheros microlepis*, *Wajpamheros nourissati*, *Astatheros macracanthus* [partial molluscivore], *Nandopsis ramsdeni*). Distinguished from *Cribroheros* by lacking the frenum in the lower lip (also absent in *A. macracanthus*) and from *Astatheros* and *Cribroheros* in having the longitudinal stripe (L-type) coloration ontogeny (ancestral for the amphilophines vs. the interrupted I-type coloration development in *Astatheros* and *Cribroheros*). Distinguished from *Chocoheros* by having an ancestral number of scales along lateral line (vs. 45). Distinguished from *Wajpamheros* by not having an elevated number of abdominal and caudal vertebrae, and scales along lateral line. Distinguished from *Nandopsis* by not having vertical bars dissolved into the irregular pattern of blotches in adults and by lacking divisions of vertical bars three or four (present in juveniles of *Nandopsis*). Distinguished from *Amphilophus* by lacking its apomorphic features having the ancestral count of 13 abdominal vertebrae (vs. 14); only one pterygiophore anteriorly from the first haemal spine (vs. two); only six anal spines vs. more than seven; second lower lip small (vs. large); the anteroventral palatine wing not reduced and the median palatovomerine ligament not exposed (as present in some *Amphilophus*). *Darienheros* is the only genus in the Isthmian clade of amphilophines that has the ancestral breeding coloration of dominant vertical bars.

Etymology. Named after the Darién area between Panamá and Colombia in combination with the old cichlid genus name *Heros* (meaning hero). The name Darién originates from the language spoken by the indigenous Cueva, an Indian tribe that lived in the Darién region of eastern Panamá and that were destroyed by the conquistadores during the 16th century (ROMOLI, 1987). The name is to be understood as the hero of the Darién. Gender: masculine.

Distribution. *Darienheros* is distributed in the Darién area of eastern Panamá.

Notes. *Darienheros* has been previously included in *Amphilophus* or *Astatheros*, but it does not share a diagnosis with any of these genera, and is also not closely related to any of them (figs. 1–5). In mtDNA phylogeny it is the sister-group of *Mayaheros urophthalmus* plus *Petenia splendida*, while in the nDNA phylogeny it remains in proximity to *M. urophthalmus* in the same clade, where it is however more closely related to all other Isthmian taxa (*Panamius*, *Talamancaheros*, *Isthmoheros*) being the sister-group of *Panamius panamensis*. BEHRE'S records

(1928) of *D. calobrensis* from Bocas del Toro, in western Panamá (Bocas ichthyological province) are erroneous - she reports the presence of a lower jaw frenum in those fish, and the fishes can therefore be referred to one or more of the *Cribroheros* species that occur in the area.

Genus *Talamancaheros* gen. nov. ŘÍČAN & NOVÁK

Type species: *Heros sieboldii* KNER, 1863: 223. Type locality: Panamá an der Südseeseite. Syntypes: (11) NMW 22012 (1).

Included species. *Talamancaheros sieboldii* (KNER, 1863), *Talamancaheros underwoodi* (REGAN, 1906).

Diagnosis. A monophyletic genus with the cranial morphology of a scraper and a lotic postcranial morphology. *Talamancaheros* was previously classified in several genera with the same ecomorphology (*Tomocichla*, *Theraps*, *Paraneetroplus*). *Talamancaheros* also is similar to the genera in the *Theraps-Paraneetroplus* clade in breeding coloration composed of distinctive black coloration of the ventral portion of the head, but not of body, which is pale to white ventrally as in *Tomocichla*. The breeding colours are thus unlike any amphilophines except for *Panamius*. *Talamancaheros* is distinguished from all similar genera (except *Panamius*) by being an amphilophine, which is supported by the lateroethmoid-premaxillary ligament and lateral band L-type coloration ontogeny. Further diagnosable by: second lower lip missing; teeth pointed conical without second cusp (*contra* BUSSING, 1976), with tip labiolingually flattened. Distinguished from *Tomocichla* by lateral band type (L-type) coloration ontogeny (vs. unique blotch-type i.e. B-type in *Tomocichla*, *Rocio* and *Mesoheros*) by lateroethmoid-premaxillary ligament (vs. palatine-premaxillary ligament) and by presence of frenum on lower lip (absent in *Tomocichla*). This is a unique character combination among all non-predatory heroine cichlid genera. The breeding coloration of *Talamancaheros* is different from that of *Tomocichla*. Characters that were used to group *Talamancaheros sieboldii* with *Tomocichla tuba* (e.g. *sensu* BUSSING, 1976 and also *Theraps* or *Paraneetroplus*) have evolved in parallel and are not synapomorphic. These characters include: long caudal peduncle including 5 vertebrae; anal spines 4–5; elevated number of pectoral fin rays (16); elevated number scales along lateral line (32–33).

Etymology. Genus name composed from the word Talamanca, referring to the Talamanca mountains of lower Central America, in combination with the old cichlid genus name *Heros* (meaning hero). Gender: masculine. The name is to be understood as the hero of the Talamanca mountains.

Distribution. *Talamancaheros* occurs on the Pacific slope of the Talamanca mountains of southern Central America (Costa Rica and western Panamá).

Notes. We restrict *Talamancaheros sieboldii* to populations from Panamá, while populations previously refer-

red to this species from Costa Rica we treat as a separate species *Talamancaheros underwoodi* (REGAN, 1906). *Herichthys underwoodi* REGAN, 1906 is the oldest available name for the Costa Rican species, *Cichlasoma punctatum* MEEK, 1909, *Cichlasoma frontale* MEEK, 1909 and *Theraps terrabae* JORDAN & EVERMANN, 1927 are junior synonyms. The last species level revision (BUSSING, 1975) placed all these nominal taxa in the synonymy of *Cichlasoma sieboldii*. BUSSING found no substantial differences between Costa Rican and Panamanian population but they are different in adult and especially breeding coloration patterns (e.g. STAWIKOWSKI & WERNER, 1998; see photos in fig. 3), the most important characters distinguishing closely related cichlid species. The genetic divergence between the two species is considerable. Using the molecular clock calibration of ŘÍČAN *et al.* (2013) the two species have diverged 4.5 Mya.

The sister group of *Talamancaheros* is *Isthmoheros tuyrensis* (MEEK & HILDEBRAND) and each belongs to a different ecological and morphological type: *Isthmoheros tuyrensis* is a high-bodied detritivore-herbivore that inhabits slow-moving waters (previously placed into *Vieja* among the herichthyines), while *Talamancaheros* are rheophilic scrapers. Despite different morphoecology both genera share a similar tooth morphology and semi-herbivorous diet (KONINGS, 1989; CONKEL, 1993). Both genera are recovered as a separate long-isolated lineage within the amphiloophines, based on the ddRAD analyses in a monophyletic clade of all other Isthmian amphiloophines which are its sister-group (*Darienheros* and *Panamius*).

Genus *Isthmoheros* gen. nov. ŘÍČAN & NOVÁK

Type species: *Cichlasoma tuyrense* MEEK & HILDEBRAND, 1913: 89.
Type locality: Río Tuyra, Boca de Cupe, Panamá. Holotype: FMNH 7599.

Included species. *Isthmoheros tuyrensis* (MEEK & HILDEBRAND, 1913)

Diagnosis. A monotypic genus with cranial morphology of a detritivore and a lentic postcranial morphology. *Isthmoheros* was previously classified in the lentic detritivore genus *Vieja*. *Vieja* is however a herichthyine genus, while *Isthmoheros* is an amphiloophine, related to other Isthmian amphiloophine genera. Diagnosed from its sister-genus *Talamancaheros* by detritivore cranial morphology and lentic postcranial morphology with and obscure breeding coloration (vs. scraping cranial and lotic postcranial morphology with a white and black breeding coloration). Diagnosed from other amphiloophine detritivores (*Cryptoheros*, *Amatitlania*, *Archocentrus*, *Panamius*, *Hypsophrys*) by much larger body size (250 mm SL vs. < 150 mm SL), by having much fewer anal fin spines (6–7 vs. > 8; except for *Panamius* with 6), by having 14 (vs. 13) abdominal vertebrae, and by having 32 (vs. < 30 except *Hypsophrys*) scales along lateral line. Further diagnosed by: second lower lip missing; teeth pointed conical without second cusp, with tip labiolingually flattened, lateral band L-type coloration ontogeny.

cal without second cusp, with tip labiolingually flattened, lateral band L-type coloration ontogeny.

Etymology. Genus name composed from isthmos, meaning narrow passage or neck of land, in combination with the old cichlid genus name *Heros* (meaning hero). Gender: masculine. The name is to be understood as the isthmian hero, as it is the only eastern Isthmian genus that has its sister-genus on the opposite side of the Isthmus in western Panamá-Costa Rica.

Distribution. *Isthmoheros* is endemic to the pacific slope of eastern Panamá.

Notes. The sister genus of *Isthmoheros* is *Talamancaheros* and each belong to a different ecological and morphological type (see Notes under *Talamancaheros*). *Isthmoheros tuyrensis* is a high-bodied detritivore-herbivore that inhabits slow-moving waters and was previously placed into *Vieja* among the herichthyines.

Genus *Chortiheros* gen. nov. ŘÍČAN & DRAGOVÁ

Type species: *Theraps wesseli* MILLER, 1996: 180, fig. 1. Type locality: Río Belleaire at CA 13 bridge (near Entelina), 8 km south of the Río Jutiapa crossing, in the Río Papaloteca drainage, Departamento de Atlantida; 15° 41' 30" W. Lat., 86° 30' N. Long. Holotype: UMMZ 231103.

Included species. *Chortiheros wesseli* (MILLER, 1996)

Diagnosis. A monotypic amphiloophine genus with a unique combination of an elongated highly adapted rheophilic body with a generalized (ancestral) cranial morphology and with an isolated distribution in Honduras. All other genera and species of Middle American cichlids with a highly rheophilic body have either scraping or piscivorous cranial morphologies. Diagnosed by high longitudinal meristics (elevated number of caudal vertebrae [> 17]; elevated number of caudal peduncle vertebrae [> 4]; elevated number of scales along lateral line [32]; elevated number of scales in lower lateral line [13]); absence of vertical bars on sides; a continuous lateral stripe from the upper end of the opercle along midbody to the base of caudal fin, the stripe also continues between the eye and mouth; and unique breeding colours unlike any other amphiloophines, very similar to genera of crown-group herichthyines (especially *Herichthys*), with lower body and head uniformly black, contrasting with upper head and upper body completely white. Further diagnosable by: elevated number of dorsal spines (> 17); elevated number of pectoral fin rays (15). Distinguished from the superficially similar *Theraps* (where originally described) by the breeding coloration, by the tip of lower jaw projecting in front of upper jaw (vs. lower jaw shorter in *Theraps*); mouth terminal, positioned relatively high on head (vs. mouth subterminal positioned low on head in *Theraps*) and large and wide (vs. small and narrow); lacking the elevated

number of abdominal vertebrae (13 vs. 15 in *Theraps*), pointed conical teeth (vs. teeth with labiolingually flattened tips in *Theraps*).

Distribution. *Chortiheros* is endemic to fast-flowing waters of the Río Papaloteca, Río Cangrejal and Río Danto in the northern Caribbean coast of Honduras.

Etymology. Named after the *Ch'orti'* people, an indigenous Maya people of southeastern Guatemala, northwestern Honduras, and northern El Salvador, after whom also the Chortis Block as one of the main geological components of Middle America is named and to whose northern part the new genus is the oldest and most isolated endemic lineage. The name Chorti is combined with the old cichlid genus name *Heros* (meaning hero). The genus name is to be understood as the hero of the Chortis. Gender: masculine.

Notes. The monotypic genus includes a rheophilic elongated cichlid originally described, with reservation, as a *Theraps* (*Theraps wesseli* MILLER, 1996), but based on our results it is not closely related to that genus, not even to any other herichthyines, but more similar and also more closely related to the amphiphine *Isthmoheros* and *Hypsophrys* (figs. 1–5). Based on the ddRAD analysis it is a very long separate sister-group of the piscivorous *Petenia splendida* (fig. 5). MILLER (1996) himself placed the species into *Theraps* only with reservation. Based on our results this species is the best adapted rheophilic amphiphine and it is allopatric and not immediately closely related to the other rheophilic species among the amphiphines (*Talamancaheros*, *Neetroplus*).

Genus *Mayaheros* gen. nov. ŘÍČAN & PÍÁLEK

Type species: *Heros urophthalmus* GÜNTHER, 1862: 291. Type locality: Lake Peten. Syntypes: (3) BMNH 1864.1.26.74–77.

Included species. *Mayaheros aguadae* (HUBBS, 1936), *Mayaheros alborus* (HUBBS, 1936), *Mayaheros amarus* (HUBBS, 1936), *Mayaheros beani* (JORDAN, 1889), *Mayaheros cienagae* (HUBBS, 1936), *Mayaheros conchitae* (HUBBS, 1936), *Mayaheros ericymba* (HUBBS, 1936), *Mayaheros mayorum* (HUBBS, 1936), *Mayaheros stenozoneus* (HUBBS, 1936), *Mayaheros trispilus* (HUBBS, 1935), *Mayaheros troschelii* (STEINDACHNER, 1867), *Mayaheros urophthalmus* (GÜNTHER, 1862), *Mayaheros zebra* (HUBBS, 1936).

Diagnosis. A monophyletic group of heroine cichlids of very generalized morphology, best diagnosed by a colour pattern of well developed evenly spaced bars virtually without midlateral blotches along midlateral line and also without a dominant midlateral blotch; distinct zebra-like breeding colours with black bars on a light background; lateral band (L-type) coloration pattern ontogeny; tip of the lower jaw projects distinctly in front of the upper jaw; maxilla extends to below the eye; reduced anteroventral wing and exposed median palatovomerine ligament; teeth pointed conical without second cusp on premaxil-

lary and mandibular teeth of the 1st series; fold of lower lip continuous; second lower lip prominent. None of the above characters nor their combination is however unique, these being some of the most generalized cichlids in Middle America.

Etymology. Named after the native Mesoamerican Maya people whose ancestral distribution includes most of the native area of the *M. urophthalmus* group and which was very likely the ancestral area of the whole genus. The name is combined with the old cichlid genus name *Heros* (meaning hero). Gender: masculine. The name is to be understood as the hero of the Maya people.

Distribution. *Mayaheros* has a disjunct distribution, with the *M. urophthalmus* group being found in the Usumacinta ichthyological province in the Atlantic drainages of southeastern México (Veracruz, Oaxaca, Chiapas, Tabasco, Campeche, Yucatán, Quintana Roo), Belize and eastern Guatemala, reaching also into Honduras, while *M. beani* is the northern-most cichlid in the Pacific drainages of Middle America in México.

Notes. Morphologically this is clearly the most ancestral genus of the amphiphines, grouping two disjunct and relict species groups. Their sister-group relationship has only been found in the ddRAD analysis. Even before the ddRAD analysis was available the nDNA (fig. 1) however already suggested that both *M. beani* and *M. urophthalmus* are very basal amphiphines. The phylogenetic relationships of both species groups are compromised in the mtDNA and combined mtDNA/nDNA phylogenies, where *M. beani* is the sister-species of *Amphilophus istlanus* (which is probably a hybrid species between the lineages of *M. beani* and *A. trimaculatus*) and *M. urophthalmus* is the sister-species of *Petenia splendida*. HUBBS (1935, 1936) described several, mostly geographically isolated subspecies of *Mayaheros urophthalmus*, often based on a limited number of specimens. KULLANDER (2003) elevated all the subspecies by HUBBS (1935, 1936) and all synonyms to species, however without any revision of the material of HUBBS or any additional material. BARRIENTOS-MEDINA (2005) provided such an analysis in his M.Sc. thesis and he proposes the elevation of nine subspecies (*M. aguadae* [HUBBS, 1936]; *M. alborus* [HUBBS, 1936]; *M. amarus* [HUBBS, 1936]; *M. cienagae* [HUBBS, 1936]; *M. conchitae* [HUBBS, 1936]; *M. ericymba* [HUBBS, 1936]; *M. mayorum* [HUBBS, 1936]; *M. trispilus* [HUBBS, 1935]; *M. zebra* [HUBBS, 1936]) to species status and additionally proposes the existence of another eight new species. *M. stenozoneus* (HUBBS, 1936) and *M. troschelii* (STEINDACHNER, 1867) were not included in the study of BARRIENTOS-MEDINA (2005). The range of *M. urophthalmus sensu stricto* is limited to the lake Petén Itzá and contiguous zones, being substituted in Yucatán Peninsula, in additional parts of México, Guatemala and Honduras by morphologically similar species, endemic and restricted in their distribution.

3.9. Revised diagnoses of South and Middle American heroine cichlid genera

Genera are ordered geographically (South America, Antilles, Middle America) and within Middle America by main clades and then in phylogenetic order of branching within clades.

Notes on the recently published revision of genera of the herichthyine cichlids by McMAHAN *et al.* (2015). McMAHAN *et al.* (2015) recently described eight new genera in a study that was supposed to be a revision of the herichthyine cichlids. The study has unfortunately solely provided genus level names but nothing else. The genera are based solely on a mtDNA dominated phylogeny that used several wrong sequences (see Introduction) and the study has not brought any new nor solid information, is completely devoid of any methodology and includes (striking) shortcomings and technical flaws. All the newly described genera were diagnosed by extremely vague diagnoses based only on superficial and superficially described characters. There was no analysis or discussion of the morphological characters, no homologization or character mapping and no context for the characters that would establish polarity and reconstruct evolution of the characters. There was no analysis of their variability between and within the putative genera or their usefulness in diagnosing the various new Middle American cichlid genera. Examined specimens are misidentified (including two figured ones: fig. 5 is *Paraneetroplus nebuliferus*, not *P. bulleri*, fig. 7 is *Herichthys pantostictus*, not *H. steindachneri*). In the key four mandibular pores are indicated for *Thorichthys*, five for all other herichthyines, the opposite is correct. There are no synonymies given for generic names, type species are given without author names and are given with the current genus name instead in the original combination with author and date. No authorship and date is given for included species in the systematic section. Several diagnoses of existing genera are ‘mutilated’ versions (they omit several important characters) of previously already existing diagnoses (e.g. *Thorichthys* by MILLER & NELSON, 1961; *C. grammodes* by TAYLOR & MILLER, 1980). The study is of a highly substandard quality and represents deplorable ‘gut feeling’ taxonomy. Below we re-diagnose all these described genera (those that are supported by our results) because the diagnoses of McMAHAN *et al.* (2015) do not diagnose any of them.

South American genera

Genus *Australoheros* ŘÍČAN & KULLANDER, 2006

Type species: *Chromis facetus* JENYNS, 1842. Type by original designation. Gender: masculine.

Included species. *Australoheros acaroides* (HENSEL, 1870); *Australoheros angiru* ŘÍČAN, PIÁLEK, ALMIRÓN & CASCIOTTA, 2011; *Australoheros autochthon* (GÜNTHER, 1862); *Australoheros aut-*

rani OTTONI & COSTA, 2008; *Australoheros barbosa* OTTONI & COSTA, 2008; *Australoheros capixaba* OTTONI, 2010; *Australoheros facetus* (JENYNS, 1842); *Australoheros forquilha* ŘÍČAN & KULLANDER, 2008; *Australoheros guarani* ŘÍČAN & KULLANDER, 2008; *Australoheros charrua* ŘÍČAN & KULLANDER, 2008; *Australoheros ipatinguensis* OTTONI & COSTA, 2008; *Australoheros kaaygua* CASCIOTTA, ALMIRÓN & GÓMEZ, 2006; *Australoheros macacuensis* OTTONI & COSTA, 2008; *Australoheros macaensis* OTTONI & COSTA, 2008; *Australoheros mattosi* OTTONI, 2012; *Australoheros minuano* ŘÍČAN & KULLANDER, 2008; *Australoheros montanus* OTTONI, 2012; *Australoheros muriae* OTTONI & COSTA, 2008; *Australoheros paraibae* OTTONI & COSTA, 2008; *Australoheros perdi* OTTONI, LEZAMA, TRIQUES, FRAGOSO-MOURA, LUCAS & BARBOSA, 2011; *Australoheros ribeirae* OTTONI, OYAKAWA & COSTA, 2008; *Australoheros robustus* OTTONI & COSTA, 2008; *Australoheros sanguineus* OTTONI, 2013; *Australoheros saquarema* OTTONI & COSTA, 2008; *Australoheros scitulus* (ŘÍČAN & KULLANDER, 2003); *Australoheros taura* OTTONI & CHEFFE, 2009; *Australoheros tavaresi* OTTONI, 2012; *Australoheros tembe* (CASCIOTTA, GÓMEZ & TORESANI, 1995); *Australoheros ykeregua* ŘÍČAN, PIÁLEK, ALMIRÓN & CASCIOTTA, 2011.

Diagnosis. ŘÍČAN & KULLANDER (2006).

Distribution. Southern tropical and temperate South America in the La Plata river basin and the coastal drainages of Argentina, Paraguay, Uruguay and southern Brazil. The southernmost heroine genus with the southernmost species among the subfamily Cichlasomatinae (*A. facetus*).

Notes. At present the largest genus among heroine cichlids but the number of the extremely similar species in the genus in SE coastal Brazil is probably highly overestimated and unprecedented in any other fish group from the area (ŘÍČAN *et al.*, 2011). DNA data with which to test the species diversity and phylogenetic relationships in SE coastal Brazil are so far unavailable. Phylogenetic relationships of the species in the La Plata river basin based on nDNA ddRAD data are shown in Supplementary Material 4 and based on mtDNA are in ŘÍČAN *et al.* (2011).

Genus *Heroína* KULLANDER, 1996

Type species: *Heroína isonycterina* KULLANDER, 1996. Type by original designation. Gender: feminine.

Included species. *Heroína isonycterina* KULLANDER, 1996

Diagnosis. KULLANDER (1996).

Distribution. South America in the western Amazon in the Napo (Ecuador), Caquetá (Colombia), and Tigre, Corrientes, and Pastaza (Peru) River basins.

Notes. The monotypic invertebrate-picker *Heroína isonycterina* is in all molecular phylogenies placed inside the piscivorous *Caquetaia* (*sensu* KULLANDER, 1996, i.e. including *Kronoheros*), i.e. in our classification between the piscivorous *Kronoheros* and *Caquetaia*. There are two possible scenarios for the explanation of the non-piscivo-

rous external cranial morphology and lentic deep-bodied postcranial morphology of *Heroina*. Either there were two independent origins of the piscivorous morphology (with *Heroina* retaining a more ancestral morphology) or there was one gain and one loss (in *Heroina*) of piscivory. The latter is the more plausible scenario, since *Heroina* has a strange combination of characters that do not suggest that it is a genus with an ancestral morphology. While its superficial head morphology is that of a generalized picker with short jaws, its jaw suspension anatomy is that of a highly specialized piscivore (KULLANDER, 1996; pers. obs.). Its palatinum and associated ligaments are similar only to the specialized piscivores *Caquetaia*, *Kronoheros*, *Petenia*, *Parachromis* (KULLANDER, 1996) and *Amphilophus trimaculatus* (pers. obs.). These facts thus demonstrate that *Heroina* is a piscivore turned into a generalized picker.

Genus *Caquetaia* FOWLER, 1945

Type species: *Caquetaia amploris* [= *Petenia myersi* SCHULZ, 1944] FOWLER, 1945. Type by original designation. Gender: feminine.
Included species. *Caquetaia kraussii* (STEINDACHNER, 1878); *Caquetaia myersi* (SCHULTZ, 1944); *Caquetaia spectabilis* (STEINDACHNER, 1875).

Diagnosis. A monophyletic group of heroine cichlids combining ancestral postcranial morphology with a piscivorous cranial morphology: transverse arm of premaxilla with an oblong posteriad process (shared with *Petenia* and *Kronoheros*); long to very long premaxillary ascending process reaching beyond a vertical from the posterior margin of the eye (shared with *Petenia*); pointed conical teeth without a second cusp, teeth in upper jaw large but in lower jaw small, short, without distinctly enlarged symphyseal teeth (shared with *Kronoheros*, in *Petenia* the teeth are even much smaller and these small teeth are also found in the upper jaw); lower jaw tip distinctly projecting in front of upper jaw tip; maxillary extending to below the eye; ethmoethmoid ligament (lateroethmoid-premaxillary ligament); reduced anteroventral palatine wing and exposed median palatovomerine ligament, increased number of cheek scale series (8). Further diagnosed by: slightly increased number of scales in the lower lateral line (11–13); slightly decreased number of scales between bases of pectoral and pelvic fins (5–4); fold of lower lip continuous; second lower lip prominent. Diagnosed from *Kronoheros* by laterally strongly compressed head and body (vs. rounded in cross-section).

Distribution. Amazonian, Orinocoan (introduced), NW trans-Andean South America.

Notes. MILLER (e.g. MILLER *et al.*, 2005) considered *Caquetaia* synonymous with *Petenia*. All molecular studies support two completely unrelated genera (one South American and one in northern Middle America). The cranial morphology of both genera is strikingly similar

but the postcranial morphology is highly distinct, with a lotic morphology in *Petenia* and ancestral morphology in *Caquetaia*.

Genus *Mesoheros* McMAHAN & CHAKRABARTY in McMAHAN *et al.*, 2015

Type species. *Heros festae* BOULENGER 1899. Type by original designation. Gender: masculine.

Included species. *Mesoheros atromaculatus* (REGAN, 1912), *Mesoheros festae* (BOULENGER, 1899), *Mesoheros geophyrus* (EIGENMANN, 1922), *Mesoheros ornatus* (REGAN, 1906)

Diagnosis. *Mesoheros* is a monophyletic genus of heroine cichlids of a very generalized morphology closely resembling the reconstructed ancestor of the Middle American cichlid clade except for coloration pattern ontogeny. Diagnosed by unique coloration pattern development, which is very similar to the situation in *Tomocichla*. The superficially similar coloration ontogeny of *Rocio* is not homologous (fig. 7; ŘÍČAN *et al.*, 2005), and can be easily distinguished by presence of bar 4 at initial stages of the ontogeny of *Rocio*, while its development is uniquely postponed in both *Mesoheros* and *Tomocichla*. *Mesoheros* is distinguished from *Tomocichla* and unique among all Neotropical cichlids by having a postponed development of bars 3 and 4 (only 4 in *Tomocichla*), and is also unique in having large juveniles with remains of dorsal blotches in dorsal portions of bars 3 and 5. Coloration pattern ontogeny (as in *Tomocichla* and *Rocio*) does neither start with a longitudinal stripe (the ancestral condition), nor with the interrupted longitudinal stripe (the ancestral situation in herichthyines; see ŘÍČAN *et al.*, 2005 and this study), but with two large blotches, one in the anterior and one in the posterior part of the larval body. Similarly as in *Tomocichla* and *Rocio*, the second ontogenetic bar is in subadult coloration divided into two bars. *Mesoheros* can be distinguished from *Tomocichla* by being plesiomorphic in all characters except coloration pattern ontogeny and the two genera are morphologically highly dissimilar. Further characterized by: slightly elevated number of pectoral fin rays (15); pointed conical teeth without a second cusp; fold of lower lip continuous; second lower lip small; median palatovomerine ligament slightly exposed.

Distribution. *Mesoheros* is endemic to the Pacific side of NW South America in Colombia, Ecuador and northernmost Peru and to the Atlantic coast of eastern Panamá. No other heroine cichlids are found south of the San Juan drainage in the trans-Andean region of Colombia, Ecuador and Peru.

Notes. The diagnosis of *Mesoheros* by McMAHAN *et al.* (2015) lacks any diagnostic characters. While the genus is indeed highly generalized with an ancestral cranial and postcranial morphology it has a highly apomorphic coloration pattern ontogeny and subadult coloration.

Antillean genera

Genus *Nandopsis* GILL, 1862

Type species: *Centrarchus tetracanthus* POEY [= *Centrarchus tetracanthus* VALENCIENNES, 1831]. Type by monotypy. Gender: feminine.

Synonyms: *Parapetenia* REGAN, 1905a: 324. Type species: *Acara adspersa* GÜNTHER, 1862. Type by subsequent designation. Type designated by EIGENMANN (1910: 476). Gender: feminine.

Included species. *Nandopsis haitiensis* (TEE-VAN, 1935); *Nandopsis ramsdeni* (FOWLER, 1938); *Nandopsis tetracanthus* (VALENCIENNES, 1831), † *Nandopsis woodringi* (COCKEREL, 1924)

Diagnosis. A monophyletic group of heroines with generalized morphology, lateral band L-type coloration pattern ontogeny, characteristic adult coloration pattern lacking vertical bars and the midlateral and caudal base blotch in adult coloration; adult coloration instead dominated by irregularly spaced blotches on a pale background (the lost vertical bars become visible and dominant only in breeding coloration as in most other heroine cichlids). Further diagnosed by: long caudal peduncle (including > 4 vertebrae; reversed to < 3 in *N. ramsdeni*); slightly decreased number of caudal vertebrae (16 to 15); decreased number of dorsal (15) and anal spines (4; reversed in *N. ramsdeni* to > 5); slightly increased number of cheek scale series (7); slightly increased number of scale rows between anterior margin of dorsal fin and lateral line (6–7); slightly increased number of scale rows between anterior margin of anal fin and lateral line (9); pointed conical teeth with second cusp on premaxillary and mandibular teeth of the 1st series; fold of lower lip continuous; second lower lip prominent; maxillary extending to below the eye (reversed in *N. ramsdeni*).

Distribution. Greater Antilles (Cuba and Hispaniola).

Middle American genera

— astatheroines

Genus *Herotilapia* PELLEGRIN, 1904

Type species: *Heros multispinosus* GÜNTHER, 1867. Type by monotypy. Gender: feminine.

Included species. *Herotilapia multispinosa* (GÜNTHER, 1867)

Diagnosis. A monotypic genus of heroine cichlids combining a herbivorous adapted cranial morphology and teeth with a lentic postcranial morphology. Diagnosable by combination of the unique teeth with labiolingually flat tricuspid tips and the not unique elevated counts of dorsal and anal fin spines (XVIII–XIX and XI–XII), as stated in the original description.

Distribution. *Herotilapia* is distributed in the San Juan ichthyological province of Costa Rica and Nicaragua.

Notes. *Herotilapia* includes only one nominal species, *Herotilapia multispinosa*, a small herbivorous cichlid with an extensive range in Central America, often found in stagnant bodies of water as oxbow lakes and swamps, and is the only heroine capable of breeding in these extreme environments (BAYLIS, 1974). *Herotilapia* is characterized by unique teeth morphology (REGAN, 1905). *Herotilapia* has been traditionally considered as closely related to the sympatric *Archocentrus*, mainly based on sharing an elevated number of anal spines. These two species were seen as having arisen from a common ancestor by trophic specialization (KONINGS, 1989). SCHMITTER-SOTO (2007a) recently even synonymized *Herotilapia* with *Archocentrus*. The synonymization is based on our results unjustified, as the two genera are not closely related. The phylogeny of SCHMITTER-SOTO (2007a,b) has a very sparse taxon sampling and does not include molecular characters, whose strong signal is against the synonymy. The morphological proximity of the two genera is expressed in our morphological analyses (ŘÍČAN *et al.*, 2008), but both mitochondrial and nuclear DNA reject a close relationship. *Herotilapia multispinosa* is not even part of the amphiphilophine radiation where *Archocentrus* is the sister group of *Amphilophus*. Based on molecular phylogeny *Herotilapia* is the sister group of all other astatheroines which include *Tomocichla*, *Rocio*, *Astatheros* and *Cribroheros*. *Rocio* is the morphologically most similar to *Herotilapia* supporting the results of our analyses.

Genus *Tomocichla* REGAN, 1908

Type species: *Tomocichla underwoodi* REGAN, 1908. Type by monotypy. Gender: feminine.

Included species. *Tomocichla asfraci* ALLGAYER 2002, *Tomocichla tuba* (MEEK, 1912)

Diagnosis. *Tomocichla* combines a scraping/biting short jawed cranial morphology with a lotic postcranial morphology. Diagnosed by unique development of coloration patterns, which is closely similar only to the situation in *Mesoheros* (see above) and also resembles *Rocio*. Distinguished from *Mesoheros* in having ontogenetic bar 3 present at initial stages of coloration ontogeny, and from *Rocio*, in the postponed development of bar 4 (shared with *Mesoheros*). The following meristic characters are diagnostic for *Tomocichla*: elevated number of abdominal vertebrae (15); elevated number of soft dorsal rays (13–14); slightly increased number of anal fin rays (9–10); Further diagnosed by: fold of lower lip continuous; palatine-premaxillary ligament; second lower lip missing; teeth with labiolingually flat unicuspid tips without distinct second cusp.

Distribution. *Tomocichla* has a continuous geographical distribution on the Atlantic side of southern Central America, from Bocas del Toro in western Panamá to southern Nicaragua.

Notes. Our definition of *Tomocichla* differs from the traditional view of the genus in that we do not find any support for the inclusion of *Talamancaheros sieboldii*. Note that the original diagnosis of *Tomocichla* (REGAN, 1908) is very brief and superficial stating that it is distinguished from *Herichthys* in having pelvic fins distinctly set off from pectoral fins. *Tomocichla*, in our view, includes two known species, *Tomocichla tuba* and *T. asfraci*, which are strongly supported as sister groups in all of our analyses. *Tomocichla tuba* (MEEK, 1912) was believed to be the sister group of *Talamancaheros sieboldii*, and the two were assumed to have arisen by geographic isolation at both sides of the Central American cordillera (BUSSING, 1976). This scenario remains unsupported by our results, and *Talamancaheros sieboldii* also completely lacks the diagnostic coloration ontogeny of *Tomocichla*, instead possessing lateral band L-type coloration ontogeny. The adult body shapes and coloration patterns of *Tomocichla* and *Talamancaheros* are similar only superficially and are nonhomologous. The type species of *Tomocichla* is known and considered valid as *Tomocichla tuba*, because *Tomocichla underwoodi* REGAN, 1908 became a junior secondary homonym of *Herichthys underwoodi* REGAN, 1906 (synonymized with *Cichlosoma sieboldii* by REGAN, 1908) and subsequently assigned to *Paraneotroplus* when MEEK (1914) synonymized *Tomocichla underwoodi* with *Cichlasoma tuba* MEEK 1912, assigning both to the genus *Cichlasoma*. According to article 59.3 of the International Code for Zoological Nomenclature (ICZN 1999) a secondary homonym is permanently invalid, even if the taxa (*underwoodi* and *tuba*) are no longer considered to be congeneric, because the replacement name (*tuba*) is in use. The other species in the genus is *Tomocichla asfraci* ALLGAYER, 2002 from the Caribbean slope of western Panamá (Bocas del Toro), often informally assigned to *Theraps* (the microphthalmus group) for which we find no support.

Genus *Rocio* SCHMITTER-SOTO, 2007a

Type species: *Heros octofasciatus* REGAN, 1903. Type by original designation. Gender: feminine.

Included species. *Rocio gemmata* CONTRERAS-BALDERAS & SCHMITTER-SOTO, 2007a; *Rocio ocotal* SCHMITTER-SOTO, 2007a; *Rocio octofasciata* (REGAN, 1903); *Rocio spinosissima* (VAILLANT & PELLEGRIN, 1902)

Diagnosis. A monophyletic group of heroine cichlids combining ancestral picker/generalized predator cranial morphology with a lentic postcranial morphology. *Rocio* is a long isolated lineage of Middle American heroines with most of the unique diagnostic characters found in coloration ontogeny: different from the majority of heroines in lacking any traces of the abdominal line during coloration development, the line only being visible in eleutherembryos (see ŘÍČAN *et al.*, 2005); coloration ontogeny distinctive by late development of ontogenetic bar 3; all other ontogenetic bars already dorsoventrally con-

tinuous in very early developmental stages; the three anterior body bars form as already dorsoventrally fused and additionally are initially fused into one large pigmented area which only later divides into three bars; second ontogenetic bar divides into two bars (i.e. three bars present posteriorly from the midlateral spot in adults); unique among Middle American heroines in developing a suborbital stripe during ontogeny; the timing of development of the suborbital stripe is accelerated, already developed at free-swimming, and is lost in juvenile fishes and absent from adult fishes as in all Middle American heroines. The timing of development of the suborbital stripe is intermediate between the Amazonian heroines and all the other Middle American heroines. Further diagnosable by: elevated number of anal fin pterygiophores anteriorly from the first haemal spine (2); elevated number of anal fin spines (> 8); elevated number of dorsal fin spines (> 17); pointed conical teeth without a second cusp; fold of lower lip interrupted (frenum); second lower lip small; maxilla extending to below the eye.

Distribution. *Rocio* has a large distribution area in the Atlantic drainages of Middle America covering the whole Usumacinta ichthyological province between the Actopán river north of Veracruz to the lower Ulua drainage (Honduras) and including most of the Yucatán peninsula.

Notes. *Rocio octofasciata* has been previously included in the sections *Parapetenia* (REGAN, 1905), based on dentition, or *Archocentrus* (REGAN, 1906), based on its high anal fin spine counts. As in case of *Herotilapia*, the species has never been recovered as related to *Archocentrus*. The species has many unique morphological features and deserves a separate generic status. *Rocio*, based on our results, also includes the much less well known *Heros spinosissimus* VAILLANT & PELLEGRIN, 1902 which shares the here presented generic diagnosis.

Genus *Astatheros* PELLEGRIN, 1904

Type species: *Heros macracanthus* GÜNTHER, 1864b = [*Heros (Cichlasoma) heterodontus* VAILLANT & PELLEGRIN, 1902]. Type by monotypy. Gender: masculine.

Included species. *Astatheros macracanthus* (GÜNTHER, 1864)

Diagnosis. *Astatheros* is a genus combining a detritivorous/molluscivorous cranial morphology with an ancestral slightly meristically shortened postcranial, further diagnosed by intermediate (interrupted-line) I-type coloration ontogeny. This character combination is unique among Middle American cichlids. Distinguished from *Cribroheros* and *Darienheros* (both of which have been previously placed in *Astatheros*) by decreased (to 15) number of caudal vertebrae (vs. ancestral); decreased (to 5) number of anal fin spines (vs. ancestral); decreased number (to 15) of dorsal fin spines (vs. ancestral or elevated decreased number); elevated number (to 10) of

scale rows between anterior margin of anal fin and lateral line (vs. ancestral number of 8 rows; also 18 vs. 15 transverse scale rows in total); increased number of scale rows between upper lateral line and the base of anterior part of soft dorsal (3.5 vs. 2.5); ancestral number of scale rows between bases of pelvic and pectoral fins (vs. decreased to 4–5 rows); herichthyine-type like breeding coloration. Additionally distinguished from *Cribroheros* in lacking the close proximity of insertion of the anterior and posterior palato-vomerine ligaments onto the palatine; second lower lip absent (small in *Cribroheros*); absence of iridescent blue spots and vermiculations on the sides of head (present in *Cribroheros*). Additionally distinguished from *Darienheros* by the intermediate (interrupted-line) I-type coloration ontogeny (vs. the amphilophine lateral stripe coloration ontogeny). Compared to most Middle American heroines (which have three abdominal bars) *Astatheros* usually only has two abdominal bars in adult coloration (similar to *Cribroheros*).

Distribution. *Astatheros macracanthus* is distributed along the Pacific versant of northern Middle America (the Chiapas-Nicaragua ichthyological province).

Notes. The separation of *Astatheros* and *Cribroheros* into two genera is justified based on both phylogenetic relationships and morphological distinctiveness. Some concatenated nDNA/mtDNA phylogenies and all nDNA ddRAD phylogenies find *Rocio* nested between the genera *Astatheros* and *Cribroheros*. *Rocio* cannot be included into *Astatheros* due to completely different cranial and postcranial morphology and coloration patterns and *Astatheros* and *Cribroheros* thus have to be separated into two genera, easily diagnosed by many morphological characters.

Astatheros often has oral teeth showing a large amount of wear with tips completely missing (sometimes worn but other times looking like broken-off). The original diagnosis of *Astatheros* was based on the worn teeth of *Heros heterodontus* (currently a synonym of *Astatheros macracanthus*). MORGENSTERN (pers. comm.) has not found these worn teeth in two syntypes of *A. macracanthus* which show numerous conical, rather slender teeth only slightly increasing in size towards symphysis, thus resembling type D teeth. Whether this is an indication for ecophenotypic variation within the species or for *Astatheros* being not monotypic, cannot be said at present. MILLER (1966) and BUSSING & MARTIN (1975) recognized three species within the group (here *Astatheros*), but they were synonymized by MILLER (1976) without explanation.

One species often included in *Astatheros* (*Cichlasoma calobrense* MEEK & HILDEBRAND, 1913) is recovered here as a separate genus (*Darienheros*) within the amphiloophines.

— amphiloophines

Genus *Petenia* GÜNTHER, 1862

Type species: *Petenia splendida* GÜNTHER, 1862. Type by monotypy. Gender: feminine.

Included species. *Petenia splendida* GÜNTHER, 1862.

Diagnosis. A piscivorous heroine genus diagnosed by both head morphology as well as by lotic postcranial morphology (compare with *Caquetaia*) with increased number of abdominal vertebrae (14); increased number of caudal vertebrae (> 18); very long caudal peduncle (including > 6 vertebral centra); increased number of anal fin pterygiophores anterior of the first haemal spine (modally 2); slightly decreased number of anal spines (5) compensated by increase in anal ray number (> 9); slightly decreased number of dorsal spines (15); bases of soft parts of dorsal and anal fins without scales; slightly increased number of cheek scale series (7); increased number of scales along lateral line (32); highly increased number of perforated scales in the lower lateral line (16); increased number of scales between lower lateral line and anterior insertion of on anal fin (10; transverse scale rows 18); increased number of scale rows between bases of pectoral and pelvic fins (8); increased number of gill rakers on 1st ceratobranchial (10); transverse arm of premaxilla with an oblong posteriad process (also in *Caquetaia* and *Kronoheros*); very long premaxillary ascending processes, reaching much beyond a vertical from the posterior margin of eye; pointed conical teeth without a second cusp, but teeth very small, short, strongly hooked, without distinctly enlarged symphyseal teeth (also in *Caquetaia*); upper jaw teeth rows increased in number (10); lower jaw tip distinctly projecting in front of upper jaw tip; maxillary extending to below the eye; lateroethmoid-premaxillary ligament; reduced anteroventral palatine wing and exposed median palatovomerine ligament; lower lip fold continuous; second lower lip prominent; amphiloophine-type (L-type) coloration ontogeny.

Distribution. *Petenia* occurs over southeastern México and in Guatemala in Atlantic slope drainages of the Usumacinta ichthyological province.

Notes. See *Caquetaia*.

Genus *Parachromis* AGASSIZ, 1859

Type species: *Parachromis gulosus* [= *Heros managuensis* GÜNTHER, 1867] AGASSIZ, 1859. Type by monotypy. Gender: masculine.

Included species. *Parachromis dovii* (GÜNTHER, 1864); *Parachromis friedrichsthalii* (HECKEL, 1840); *Parachromis loisellei* (BUSSING, 1989); *Parachromis managuensis* (GÜNTHER, 1867); *Parachromis motaguensis* (GÜNTHER, 1867).

Diagnosis. *Parachromis* is a predatory heroine genus diagnosed by both cranial as well as postcranial characters. The cranial morphology is predatory-piscivorous,

the postcranial morphology includes aspects of both lentic (high fin spine meristics, rather short caudal peduncle) and lotic ecomorphologies because ancestrally *Parachromis* is a short lentic genus, with elongation only secondary and most so in the most piscivorous species (*P. dovii* and *P. managuensis*; the *managuensis* group). The genus is diagnosed in cranial morphology by long premaxillary ascending processes, reaching through a vertical from the middle of eye to vertical from posterior margin of eye (*managuensis* group); tip of lower jaw projecting distinctly in front of tip of upper jaw; maxillary extending to below the eye; ethmoethmoid ligament (lateroethmoid-premaxillary ligament); reduced anteroventral palatine wing and exposed median palatovomerine ligament; symphyseal 1–2 teeth in lower jaw reduced in size. In postcranial morphology it is diagnosed by slightly increased number of caudal vertebrae, especially in *P. managuensis* and *P. dovii* (18); increased number of anal fin pterygiophores anteriorly from the 1st haemal spine (> 2); of anal spines (> 7 ; > 8 in the *friedrichsthalii* group); of dorsal spines (> 17 ; > 18 in the *friedrichsthalii* group); of cheek scale rows (> 7 ; > 8 in the *managuensis* group); of scales along lateral line (> 31 ; > 33 in the *managuensis* group); lateral band L-type coloration pattern ontogeny; posteriorly inclined suborbital stripe running between the eye and the corner of the suboperculum, often divided to form a separate blotch on the suboperculum; in some species the blotch is even ocellated, as are in this case other blotches on the head which distinguishes it from *Thorichthys* and *Trichromis* where always only the one blotch is present and ocellated. Further diagnosed: teeth pointed conical, without second cusp on premaxillary and mandibular teeth of the 1st series; fold of lower lip continuous; second lower lip prominent.

Distribution. The distribution range of *Parachromis* extends over most of Middle America, from southeastern México to western Panamá, predominantly in Atlantic drainages.

Notes. The genus *Parachromis* was created for *Parachromis gulosus* AGASSIZ, 1859, a senior synonym of *Heros managuensis* GÜNTHER, 1867 (KULLANDER & HARTEL, 1997). KULLANDER & HARTEL (1997) suggested that *Parachromis gulosus* should be treated as *nomen oblitum*, given the widespread usage of GÜNTHER's name for the species. The monophyly of the genus *sensu* KULLANDER (2003) is well supported in our analyses.

Genus *Cryptoheros* ALLGAYER, 2001

Type species: *Heros spilurus* GÜNTHER, 1862. Type by original designation. Gender: masculine.

Included species. *Cryptoheros chetumalensis* SCHMITTER-SOTO, 2007a; *Cryptoheros cutteri* (FOWLER, 1932); *Cryptoheros spilurus* (GÜNTHER, 1862).

Diagnosis. *Cryptoheros* combines a small mouthed-detritivorous cranial morphology with a lentic postcranial.

Diagnosed by small to very small body size (< 100 mm SL), by a distinctive mouth and oral jaw morphology typical for the detritivorous/herbivorous ecomorphology, i.e. oral jaws short, subequal or lower jaw shorter than upper, the tooth-bearing part of the lower jaw strongly labiolingually shortened making it short and wide rather than long and narrow, lower jaw teeth thus placed in an almost straight transverse line (relative to the body axis) vs. in a parallel line with the body axis (as in predators), jaw teeth relatively delicate, pointed, conical but at the tip labiolingually flattened with relatively large teeth also in 2nd and 3rd rows, fold of lower lip interrupted (frenum); second lower lip present. This head morphology is shared with *Amatitlania* and *Hypsophrys* and is unique among Middle American cichlids in combination with high unpaired fin meristic values: increased number of anal pterygiophores anteriorly from the 1st haemal spines (2–3); elevated number of anal spines (7–9) compensated by decreased number of anal fin rays (< 8); elevated number of dorsal fin spines (17–19). Distinguished from the similar genera *Hypsophrys* and *Neetroplus* in having ancestral numbers of vertebrae (vs. elevated number of 14 abdominal and > 17 caudal), scale rows, and a lentic postcranial morphology (deep bodies with short caudal peduncles). Coloration pattern ontogeny (shared with *Amatitlania*, *Hypsophrys* and *Neetroplus*) is of the lateral band type but characteristic in early disruption of a weakly developed midlateral line and by three adult body bars posterior from the midlateral blotch due to division of ontogenetic bar 3 into two bars. Diagnosed from the morphologically very similar genus *Amatitlania* solely by coloration patterns and geographical distribution. *Cryptoheros* has compared to *Amatitlania* one vertical bar more in the abdominal part of body due to division of the ontogenetic bar 5 into two separate bars (in a small minority of specimens the bars can be fused as in *Amatitlania*). The posterior branch of bar 5 (or if fused the whole bar 5) is the most pigmented and dominant of all the vertical bars in *Cryptoheros*, while in *Amatitlania* bar 4 is the dominant one. The coloration pattern in *Cryptoheros* is apomorphic to *Amatitlania*. Diagnosed from *Archocentrus* by the sexual dimorphism in size, coloration or courtship behaviour (vs. absent in all forms), by rounded (pointed) snout, by lacking the strongly laterally compressed body, by having the division of ontogenetic bar three, and by absence of two large blotches on the opercular series.

Distribution. The distribution range of *Cryptoheros* is limited to the Caribbean slope of Honduras, Guatemala and eastern Yucatán of México.

Notes. Separate genus status of *Cryptoheros* and *Amatitlania* is necessary because *Hypsophrys* and *Neetroplus* are nested between *Amatitlania* and *Cryptoheros*. The genus *Cryptoheros* was described by ALLGAYER (2001) to include all species traditionally placed into *Archocentrus* except for *A. centrarchus* and the *C. nigrofasciatus* group. A separate evolutionary position from *A. centrarchus* is supported by our results. The monophyly of *Crypto-*

heros sensu ALLGAYER (2001) and *sensu* SCHMITTER-SOTO (2007a) is however not supported in any of our molecular analyses (it always includes *Hypsophrys nicaraguensis* and *Neetroplus nematopus*) except those analyses that have a limited taxon sampling (fig. 1). All species classified as the subgenus *Bussingius* SCHMITTER-SOTO, 2007a do not belong to *Cryptoheros* but are a paraphyletic stem-group of *Amatitlania* SCHMITTER-SOTO, 2007a. The subgenus *Panamius* SCHMITTER-SOTO, 2007a of *Cryptoheros* (*P. panamensis*) is elevated here to a separate genus, as it is unrelated to *Cryptoheros*, but rather belongs to the Isthmian clade of amphiphines.

Genus *Amatitlania* SCHMITTER-SOTO, 2007a

Type species. *Heros nigrofasciatus* GÜNTHER, 1867. Type by original designation. Gender: feminine.

Synonyms: *Bussingius* SCHMITTER-SOTO, 2007a: 41. Type species. *Cichlosoma septemfasciatum* REGAN, 1908. Type by original designation. Gender: masculine.

Included species. *Amatitlania altoflava* (ALLGAYER, 2001); *Amatitlania coatepeque* SCHMITTER-SOTO, 2007a; *Amatitlania kanna* SCHMITTER-SOTO, 2007a; *Amatitlania myrnae* (LOISELLE, 1997); *Amatitlania nanolutea* (ALLGAYER, 1994); *Amatitlania nigrofasciata* (GÜNTHER, 1867); *Amatitlania sajica* (BUSSING, 1974); *Amatitlania septemfasciata* (REGAN, 1908); *Amatitlania siquia* SCHMITTER-SOTO, 2007a.

Diagnosis. *Amatitlania* combines a small mouthed-detritivorous cranial morphology with a lentic postcranial. Diagnosed by small to very small body size (< 100 mm SL), by a distinctive mouth and oral jaw morphology typical for the detritivorous/herbivorous ecomorphology, i.e. oral jaws short, subequal or lower jaw shorter than upper, the tooth bearing part of the lower jaw strongly labiolingually shortened making it short and wide rather than long and narrow, lower jaw teeth thus placed in an almost straight transverse line (relative to the body axis) vs. in a parallel line with the body axis (as in predators), jaw teeth relatively delicate, pointed, conical but at the tip labiolingually flattened with relatively large teeth also in 2nd and 3rd rows, fold of lower lip interrupted (frenum); second lower lip present. This head morphology is shared with *Cryptoheros* and *Hypsophrys* and is unique among Middle American cichlids in combination with high unpaired fin meristic values: increased number of anal pterygiophores anteriorly from the 1st haemal spines (2–3); elevated number of anal spines (7–9) compensated by decreased number of anal fin rays (< 8); elevated number of dorsal fin spines (17–19). Distinguished from the similar genera *Hypsophrys* and *Neetroplus* in having ancestral numbers of vertebrae (vs. elevated number of 14 abdominal and > 17 caudal) and scales and a lentic postcranial morphology (deep bodies with short caudal peduncles). Coloration pattern ontogeny (shared with *Cryptoheros*, *Hypsophrys* and *Neetroplus*) is of the lateral band type but characteristic in early disruption of a weakly developed midlateral line (I-type) and by three adult body bars posterior from the midlateral blotch due to division of ontogenetic bar 3 into two bars. Diagnosed

from the morphologically very similar genus *Cryptoheros* solely by coloration patterns and geographical distribution. *Cryptoheros* has compared to *Amatitlania* one vertical bar more in the abdominal part of body due to division of the ontogenetic bar 5 into two separate bars (in a small minority of specimens the bars can be fused as in *Amatitlania*). The posterior branch of bar 5 (or is fused the whole bar 5) is the most pigmented and dominant of all the vertical bars in *Cryptoheros*, while in *Amatitlania* bar 4 is the dominant one (in juveniles of all species and in adults of most species; in adults of some species all bars can be of the same intensity – e.g. in the *nigrofasciatus* group). The coloration pattern in *Amatitlania* is plesiomorphic compared to *Cryptoheros*. Diagnosed from *Cryptoheros* by sexually ripe females which in all *Amatitlania* species develop a patch of iridescent golden to copper-red scales on abdominal sides and anterior flank, usually accompanied (or replaced in *A. septemfasciata* and *A. sajica*) by a bluish to intense peacock-blue throat, chest and lower part of abdomen. This is not just an intensification of the normal colors but a novel color trait not found in males or in this form in any other genera of Middle American cichlids. This is a derived character state and a synapomorphy of *Amatitlania* diagnosing it from *Cryptoheros*. It is associated with active female courtship. Diagnosed from *Archocentrus* by the sexual dimorphism in size, coloration or courtship behaviour (vs. absent in all forms), by rounded (vs. pointed) snout, by lacking the strongly laterally compressed body, by having the division of ontogenetic bar three, and by absence of two large blotches on the opercular series.

Distribution. The distribution range of *Amatitlania* extends over most of Middle America, from the southern Pacific slope of México, Pacific slope of Guatemala, El Salvador and both slopes of eastern Honduras to westernmost Panamá on both slopes.

Notes. Separate genus status of *Amatitlania* and *Cryptoheros* is necessary because *Hypsophrys* and *Neetroplus* are nested between *Amatitlania* and *Cryptoheros*. *Amatitlania* was proposed as a separate genus for the *nigrofasciata* group by SCHMITTER-SOTO (2007a). This species group is however nested within the paraphyletic *Cryptoheros sensu* SCHMITTER-SOTO (2007a). All species classified as the subgenus *Bussingius* of *Cryptoheros* by SCHMITTER-SOTO (2007a) do not belong into *Cryptoheros* but are a paraphyletic stem-group of *Amatitlania* SCHMITTER-SOTO, 2007a. The separate species status or at least the species boundaries are questionable for *Amatitlania altoflava* (ALLGAYER, 2001), *Amatitlania siquia* SCHMITTER-SOTO, 2007a and *Amatitlania kanna* SCHMITTER-SOTO, 2007a.

Genus *Hypsophrys* AGASSIZ, 1859

Type species. *Hypsophrys unimaculatus* [= *Heros nicaraguensis* GÜNTHER, 1864b] Agassiz, 1859. Type by monotypy. Gender: feminine.

Synonyms: *Copora* FERNÁNDEZ-YÉPEZ, 1969: 4. Type species. *Heros nicaraguensis* GÜNTHER, 1864b.

Included species. *Hypsophrys nicaraguensis* GÜNTHER, 1864b.

Diagnosis. *Hypsophrys* is the largest in body size (> 150 mm SL) among the small-sized amphiphine detritivores (*Amatitlania*, *Cryptoheros*, *Archocentrus*, *Panamius*). *Hypsophrys* combines a detritivorous/molluscivorous cranial morphology with an elongated postcranial morphology that includes character states of both the lotic and lentic ecomorphs. Diagnosed by a distinctive mouth and oral jaw morphology typical for the detritivorous ecomorphology, i.e. oral jaws short, subequal or lower jaw shorter than upper, the tooth bearing part of the lower jaw strongly labiolingually shortened making it short and wide rather than long and narrow, lower jaw teeth thus placed in an almost straight transverse line (relative to the body axis) vs. in a parallel line with the body axis (as in predators), jaw teeth relatively delicate, pointed, conical but at the tip labiolingually flattened with relatively large teeth also in 2nd and 3rd rows, fold of lower lip interrupted (frenum); second lower lip present. This head morphology is shared with *Amatitlania* and *Cryptoheros* and is unique among Middle American cichlids in combination with high unpaired fin meristic values: increased number of anal pterygiophores anteriorly from the 1st haemal spines (2–3); elevated number of anal spines (7–9) compensated by decreased number of anal fin rays (< 8); elevated number of dorsal fin spines (17–19). *Hypsophrys nicaraguensis* is derived from ancestrally short and deep-bodied cichlids (as are *Cryptoheros* and *Amatitlania*). *Hypsophrys* (and its sister genus *Neetroplus*) differs from this morphology by having an increased number of both abdominal (14) and caudal vertebrae (> 17); increased number of caudal peduncle vertebrae (4); elevated number of scales along lateral line (32–33); elevated number of scales in the upper lateral line (22). *Hypsophrys* is thus similar to other rheophilic species (e.g. *Theraps*, *Talamancaheros*, *Tomocichla*). Coloration pattern ontogeny is however different from all these genera, being of the lateral band type but characteristic in early disruption of a weakly developed midlateral line (I-type) and by three adult body bars posterior from the midlateral blotch due to division of ontogenetic bar 3 into two bars. *Hypsophrys* shares most character states with *Neetroplus*. *Hypsophrys* is diagnosed from *Neetroplus* by its different and separate ecomorphology (detritivore-molluscivore vs. algae scraper), the prominent, narrow longitudinal band (similar to that in *Chortiheros* but not continued onto snout and usually faded in ripe males) on a pale golden background without vertical bars and a truncate to slightly emarginate caudal fin with pointed corners (vs. truncate with rounded corners to subtruncate). These characters are unique in the *Hypsophrys-Neetroplus-Cryptoheros-Amatitlania* clade. The pale, golden adult coloration without vertical bars of *Hypsophrys* is associated with the open habitat sand/mud-dwelling niche as are the among Middle American cichlids uniquely non-adhesive eggs and associated

reproduction where pits or holes are excavated for egg deposition (*Neetroplus* on the other hand is strictly associated with rocky habitats). The non-adhesive eggs are otherwise among Neotropical cichlids present only in the most advanced geophagine mouthbrooders which are also strictly associated with a sand-dwelling niche.

Distribution. *Hypsophrys* is a Central American genus found in the San Juan ichthyological province on both slopes of Nicaragua and Costa Rica.

Notes. We find very strong support for *Hypsophrys nicaraguensis* and *Neetroplus nematopus* being sister species, both in separate and combined analyses of morphological and molecular data. Our results however do not support the inclusion of both species in one genus *Hypsophrys* (*sensu* CHAKRABARTY & SPARKS, 2007 and SCHMITTER-SOTO, 2007a). Both species differ strikingly in cranial and teeth morphology, in preferred food and food gathering modes, in niche, in breeding coloration and in the uniquely non-adhesive eggs of *Hypsophrys*. A two genus classification is more in line with the classification of the Middle American cichlids based on cranial and postcranial ecomorph combinations proposed in the present study.

Genus *Neetroplus* GÜNTHER, 1867

Type species. *Neetroplus nematopus* GÜNTHER, 1867.

Included species. *Neetroplus nematopus* GÜNTHER, 1867. Type by monotypy. Gender: masculine.

Diagnosis. *Neetroplus* combines a scraping cranial morphology with highly specialized oral teeth with an elongated postcranial that includes character states of both the lotic and lentic ecomorphs. *Neetroplus* has autapomorphic oral jaws and teeth, which are truncated, incisor-like, labiolingually flattened. *Neetroplus* is in addition characterized by a unique change between nonbreeding and breeding coloration (the latter becomes the inverse of the former, with a dark, almost black body with a white bar in front of the anal fin). Additionally diagnosed from *Hypsophrys* by the absence of the second lower lip (vs. present). Most other characters are shared with *Hypsophrys*, *Amatitlania*, *Cryptoheros*, *Archocentrus* and *Panamius* including very small body size (< 150 mm SL; except *Hypsophrys*), a distinctive mouth and oral jaw morphology with oral jaws short, subequal or lower jaw shorter than upper, the tooth bearing part of the lower jaw strongly labiolingually shortened making it short and wide rather than long and narrow, lower jaw teeth thus placed in an almost straight transverse line (relative to the body axis) vs. in a parallel line with the body axis (as in predators), fold of lower lip interrupted (frenum); second lower lip present. This head morphology is unique among Middle American cichlids in combination with high unpaired fin meristic values: increased number of anal pterygiophores anteriorly from the 1st haemal spines (2–3); elevated number of anal spines (7–9) compensated by decreased number of anal fin rays (< 8);

elevated number of dorsal fin spines (17–19). *Neetroplus nematopus* is derived from ancestrally short and deep-bodied cichlids (as are *Cryptoheros* and *Amatitlania*). *Neetroplus* (and its sister genus *Hypsophrys*) differs from this morphology by having an increased number of both abdominal (14) and caudal vertebrae (> 17); increased number of caudal peduncle vertebrae (4); elevated number of scales along lateral line (32–33); elevated number of scales in the upper lateral line (22). *Neetroplus* is thus similar to other rheophilic species (e.g. in *Theraps*, in *Isthmoheros*, *Tomocichla*). Coloration pattern ontogeny is however different from all these genera, being of the lateral band type but characteristic in early disruption of a weakly developed midlateral line (I-type) and by three adult body bars posterior from the midlateral blotch due to division of ontogenetic bar 3 into two bars.

Distribution. *Neetroplus* is a Central American genus found in the San Juan ichthyological province on the Caribbean slopes of Nicaragua and Costa Rica.

Notes. See under *Hypsophrys*. *Neetroplus panamensis* MEEK & HILDEBRAND, 1913 and its undescribed sister-species are based on both nDNA and mtDNA not part of *Neetroplus*. They represent a separate long isolated genus (*Panamius*).

Genus *Archocentrus* GILL in GILL & BRANSFORD, 1877

Type species. *Heros centrarchus* GILL in GILL & BRANSFORD, 1877. Type by monotypy. Gender: masculine. First described as sub-genus of *Heros* (*Archocentrus*).

Included species. *Archocentrus centrarchus* GILL in GILL & BRANSFORD, 1877

Diagnosis. A monotypic genus of heroine cichlids combining a sharp-mouthed detritivorous cranial morphology with a lentic postcranial morphology. In association with what is apparently a special type of detritivory *Archocentrus* has a peculiar and very likely autapomorphic elongated gill raker structure (described and figured in SCHMITTER-SOTO, 2007b) and gill raker number (12–17, modally 14 on first ceratobranchial) that approaches or even exceeds that of specialized sifters. *Archocentrus* is similar to its sister genus *Amphilophus* by L-type longitudinal stripe coloration pattern ontogeny (*contra* *Cryptoheros*, *Amatitlania*, and *contra* the more generalized ontogeny in *Herotilapia*); increased number of anal fin pterygiophores anterior of the first haemal spine (> 2 in *Amphilophus*, 4 in *Archocentrus*) and thus proportionally increased number of anal fin spines; increased number of dorsal spines (17); lateroethoid-premaxillary ligament; large second lower lip; teeth pointed, conical, without second cusp on premaxillary and mandibular teeth of the 1st series. Distinguished from its sister genus *Amphilophus* by ancestral number of abdominal vertebrae (13 vs. 14), by much higher number of anal fin spines (modally 10 in *Archocentrus*, 7 in *Amphilophus*), by much rounder body shape, and by much smaller adult size (< 150 mm

SL vs. 200–300 mm SL). Diagnosed from *Cryptoheros* and *Amatitlania* by lacking sexual dimorphism in size, coloration or courtship behaviour (vs. very strong with highly active females), by pointed (vs. rounded) snout, strongly laterally compressed body (the most similar body shape among Middle American heroines to South American heroines, especially to *Heros*), by lacking the division of ontogenetic bar three, and by presence of two large blotches on the opercular series, the lower one a remnant of the suborbital stripe.

Distribution. *Archocentrus* is distributed in the San Juan ichthyological province on the Caribbean slope of Nicaragua and Costa Rica.

Notes. Several species groups of Middle American cichlids are characterized by highly elevated anal fin spine numbers and the accompanying lentic postcranial morphology. This ecomorph combination has previously been used as a basis of character diagnosis that has grouped these species into one (*Archocentrus*) or a few supposedly closely related genera. We here classify these species in five separate genera; *Archocentrus*, *Rocio*, *Herotilapia*, *Cryptoheros* and *Amatitlania*. These genera are monophyletic, not closely related (except *Cryptoheros* and *Amatitlania*), and their high anal fin spine number is an adaptive correlate of the lentic postcranial.

KULLANDER (2003) and MILLER *et al.* (2005) have placed all these species except *Herotilapia* into *Archocentrus*. SCHMITTER-SOTO (2007a,b) has placed only those species with the highest anal fin spine numbers (*Rocio spinosissima*, *Herotilapia*, *A. centrarchus*) into *Archocentrus*.

Archocentrus sensu KULLANDER 2003 (i.e. including also *Cryptoheros*, *Amatitlania* and *Panamius*) is not found as monophyletic and is rejected by our data. *Archocentrus* is in all mtDNA dominated molecular and combined molecular-morphological analyses nested inside *Amphilophus* while the nuclear ddRAD analysis places it as a monotypic sister genus of *Amphilophus*.

SCHMITTER-SOTO (2007a) synonymized *Herotilapia* with *Archocentrus*, largely based on meristic characters. Some (not all) of our morphological analyses show *A. centrarchus* as the sister group of *Herotilapia*. This sister group is found only in analyses with ordered meristic characters, while unordered analyses do not show this grouping. Thus only when meristic characters receive higher weights (as in the ordered analysis) is this group recovered. All molecular phylogenies reject this grouping and were specifically disregarded by SCHMITTER-SOTO (2007a,b). Neither *Herotilapia* nor *Cryptoheros* are synonymous with *Archocentrus*.

Archocentrus (sensu KULLANDER, 2003) was believed to be one of the best natural groups of heroines in pre-cladistics works even though it was supported mostly just by the high anal fin meristics and small size. The similarity of *Archocentrus* to the genera *Cryptoheros* and *Amatitlania* is solely in the high anal fin spine number, in most other characters *Archocentrus* is very similar to its sister genus

Amphilophus. The homology of high number of anal fin spines is, based on our results, highly questionable. Additionally, the number of anal fin spines is an adaptive character in the lentic character complex. The patterning of unpaired fin meristics in *A. centrarchus* is different from *Amatitlania* and *Cryptoheros*, which in turn is more similar to (and also more related to) *Hypsophrys* and *Neetroplus*. *Archocentrus centrarchus*, together with *Herotilapia* and the little known *Rocio spinosissima*, is the only heroine having more than 10 anal fin spines (*Cryptoheros* and *Amatitlania* have modally 8–9 anal fin spines). The patterning of the unpaired fins (dorsal and anal) is in general concerted and species with increased numbers of dorsal fin (D) spines also tend to have elevated numbers of anal fin (A) spines. Moreover, numbers of the abdominal vertebrae and D spines also tend to be correlated. In *Cryptoheros*, *Amatitlania*, *Hypsophrys* and *Neetroplus* the increase in A fin spine number is also followed by increase in D fin spine number. *Hypsophrys* plus *Neetroplus* have modally 18–19 D spines with 14 abdominal vertebrae; *Cryptoheros* and *Amatitlania* have modally 17–18 D spines with 12–13 abdominal vertebrae. The increase in number of abdominal vertebrae thus increases the number of D spines as the two are strictly intertwined. In *A. centrarchus*, however, the increase in the number of A spines is not followed by increase in D spines (> 10 A spines and only the ancestral count of 16 [17] D spines). Importantly, *Herotilapia*, unlike *A. centrarchus*, has the correlated unpaired fin patterning (as does the *Hypsophrys* clade), with 18–19 D spines coupled with > 10 A spines. *A. centrarchus* thus has a unique D-A fin patterning among Middle American cichlids, not homologous to the situation in the *Hypsophrys* clade and *Herotilapia*, which have a plesiomorphic patterning. Additionally, the patterning in *Herotilapia* is based on phylogenetic evidence closer to *Rocio* than to *A. centrarchus*. Also supporting our argument is the fact that while all species with high number of A spines have also high number of anal pterygiophores in front of the first haemal spine, *A. centrarchus* seems to have added more A spines anteriorly than in the posterior region (as in the *Hypsophrys* clade or *Herotilapia*). *Archocentrus centrarchus* is the only heroine with more than 3 (modally 4) anal pterygiophores anteriorly from the first haemal spine. All other heroines with elevated anal meristics have maximally 3 anterior pterygiophores, and the increase in number of spines also occurred posteriorly at the transition between anal spines and rays. The homology of the main supporting character of high anal fin spine count of *Archocentrus sensu* KULLANDER (2003) or *sensu* SCHMITTER-SOTO (2007a) is thus flawed by our analyses. Our results instead suggest that the high anal fin meristics of *A. centrarchus* have evolved independently from the *Hypsophrys* clade and *Herotilapia*. As a result of this *Cryptoheros* and *Amatitlania* are surprisingly supported as related to *Hypsophrys* and *Neetroplus* by elevated numbers of D spines. The number of A spines is an important separating character for heroines (vs. other Neotropical cichlids), but that seems to be the limit of its resolution. Genera with high anal fin spine numbers are

not found closely related to each other (see *Rocio*, *Herotilapia*, *Archocentrus*, *Cryptoheros*, *Amatitlania*) and the increased number of anal fin spines is correlated with the lentic ecomorphology (negative correlate of the length of the caudal peduncle). For phylogenetic purposes, the number of D spines seems to be more stable and in agreement with phylogeny.

Genus *Amphilophus* AGASSIZ, 1859

Type species. *Amphilophus froebelii* AGASSIZ, 1859 [= *Heros labiatus* GÜNTHER, 1864a]. Type by monotypy. Gender: masculine.

Synonyms: *Erythrichthys* MEEK, 1907b: 118. Type species. *Heros citrinellus* GÜNTHER, 1864b. Type by original designation. Gender: masculine. Originally *Cichlasoma* (*Erythrichthys*).

Curraichthys FERNÁNDEZ-YÉPEZ, 1969: [3]. Type species. *Heros lobocheilus* GÜNTHER, 1868 [= *Heros labiatus* GÜNTHER, 1864a]. Type species by original designation. Gender: masculine.

Included species. *Amphilophus amarillo* STAUFFER & MCKAYE, 2002; *Amphilophus astorquii* STAUFFER, MCCRARY & BLACK, 2008; *Amphilophus chanco* STAUFFER, MCCRARY & BLACK, 2008; *Amphilophus citrinellus* (GÜNTHER, 1864); *Amphilophus flaveolus* STAUFFER, MCCRARY & BLACK, 2008; *Amphilophus globosus* GEIGER, MCCRARY & STAUFFER, 2010; *Amphilophus hogaboomorum* (CARR & GIOVANNOLI, 1950); *Amphilophus istlanus* (JORDAN & SNYDER, 1899); *Amphilophus labiatus* (GÜNTHER, 1864); *Amphilophus lyonsi* (GOSSE, 1966); *Amphilophus sagittae* STAUFFER & MCKAYE, 2002; *Amphilophus superciliosus* GEIGER, MCCRARY & STAUFFER, 2010; *Amphilophus tolteca* RECKNAGEL, KUSCHE, ELMER, & MEYER, 2013; *Amphilophus trimaculatus* (GÜNTHER, 1867); *Amphilophus viridis* RECKNAGEL, KUSCHE, ELMER, & MEYER, 2013; *Amphilophus xiloensis* STAUFFER & MCKAYE, 2002; *Amphilophus zaliosus* (BARLOW, 1976).

Diagnosis. A monophyletic group of morphologically very generalized heroine cichlids representing the ancestral amphilophine morphology and showing a similar coloration of well-developed, evenly spaced bars with the midlateral stripe disrupted into a series blotches both anteriorly and posteriorly from the midlateral blotch. Diagnosed by L-type coloration pattern ontogeny and increased number of abdominal vertebrae (14); ancestrally increased number of anal fin pterygiophores anterior of the first haemal spine (modally 2) and thus ancestrally proportionally increased number of anal spines (but reduced in *A. lyonsi* to 5 and to 4 in *A. istlanus*); slightly increased number of dorsal spines (17); lateroethoid-premaxillary ligament. These, and additional characters also distinguish *Amphilophus* from *Astatheros*: large second lower lip (vs. absent); reduced anteroventral palatine wing (vs. enlarged or normal palatine wings); transverse scale rows 15 (17) compared to 18 in *Astatheros* (scale rows between anterior margin of anal fin and lateral line < 8(9) compared to > 10); longer premaxillary process extending to the vertical from middle of the eye; anal spines > 7(6) vs. < 5; dorsal spines > 17(16) vs. < 15; *Amphilophus* species have three clearly developed abdominal bars, while *Astatheros* species develop only two wide abdominal bars as adults (the two bars anterior from the midlateral blotch are fused into one bar in *Astatheros macracanthus*). Further diagnosed by: teeth pointed, conical, without second cusp on premaxillary and mandi-

bular teeth of the 1st series; decreased number of cheek scale series (4–5).

Distribution. *Amphilophus* is distributed in the San Juan ichthyological province on the Caribbean slope of Nicaragua and Costa Rica and on the Pacific slope of Honduras, Guatemala and México, in the Chiapas-Nicaragua ichthyological province, and reaching into the Balsas river basin in the north (*A. istlanus*) and into the Chiriqui ichthyological province of Costa Rica in the south (*A. lyonsi*).

Notes. The genus *Amphilophus* was erected for *Amphilophus froebelii* AGASSIZ, 1859 (*nomen oblitum*; = *Heros labiatus* GÜNTHER, 1864a), as type species. *Astatheros* was considered a junior synonym of *Amphilophus* (KULLANDER, 2003). ROE *et al.* (1997) showed non-monophyly of *Amphilophus sensu lato* and resurrected the genus *Astatheros*. Our phylogeny and morphological data agree with the concept of two separate genera. *Astatheros* is additionally retained only for the type species and the other species are treated as a separate genus *Cribroheros* due to non-monophyly of *Astatheros sensu lato*.

Several species of amphilophines (*Heros trimaculatus* GÜNTHER, 1867, *Cichlasoma lyonsi* GOSSE, 1966, and *Heros istlanus* JORDAN & SNYDER, 1899) are in all our analyses recovered as part of *Amphilophus*. LOISELLE (1980) already proposed inclusion of *Amphilophus trimaculatus* into *Amphilophus*: based on general morphology, female breeding dress and behaviour virtually indistinguishable from *Amphilophus* of the *A. labiatus* group. *Amphilophus istlanus* is most likely a species of hybrid origin with the *Amphilophus trimaculatus* lineage on one side and *Mayaheros beani* on the other (including mtDNA) as suggested by the strong conflict between nuclear and mitochondrial DNA, the in some respects unique morphology and coloration of *A. istlanus* that deviates from other *Amphilophus*, and by the geographical distribution between the two likely parental species (or their ancestors). We are including *Amphilophus istlanus* into *Amphilophus* based on the prevailing nDNA signal.

TAYLOR & MILLER (1980) and KONINGS (1989) proposed a sister group relationship between *Heros istlanus* JORDAN & SNYDER, 1899 and *Cichlasoma grammodes* TAYLOR & MILLER, 1980, a grouping that is recovered in some of our morphological analyses. Also *Heros beani* JORDAN, 1889 is found as closely related in morphological characters. The morphology-based sister group relationship between *Heros istlanus* JORDAN & SNYDER, 1899 and *Cichlasoma grammodes* TAYLOR & MILLER, 1980 is strongly rejected by nDNA and mtDNA and none of the *Amphilophus* species is found closely related to *Chiapaheros grammodes*, which is a member of the herichthyines.

Genus *Panamius* SCHMITTER-SOTO, 2007a; new status

Type species. *Neetroplus panamensis* MEEK & HILDEBRAND, 1913: 90. Holotype. FMNH 7601, 79 mm SL, S. E. MEEK & S. F. HILDEBRAND, Feb. 2, 1911. Type locality. Río “Mandingo”

[Mandinga] at “Bas Obispo”, Canal Zone, Panama. Type by monotypy. Gender: masculine.

Included species. *Panamius panamensis* (MEEK & HILDEBRAND, 1913).

Diagnosis. *Panamius* combines a detritivorous/scraping cranial morphology with apomorphic oral teeth with a lentic postcranial morphology. *Panamius* has apomorphic truncated, labiolingually flattened incisor-like oral teeth similar to *Neetroplus*, but plesiomorphic to that genus in having tips rounded (pointed in juveniles), their biting edges not forming a line. *Panamius* is also distinct from *Neetroplus* in having a lentic postcranial (vs. lotic). *Panamius* is distinct from the otherwise cranially (except oral teeth) and postcranially (except fin meristics) similar *Cryptoheros* and *Amatitlania* by marked apomorphic change in breeding coloration which is not an intensification of the normal coloration (i.e. vertical bars, as is the common state in cichlids) but presents a novel pattern, different from *Neetroplus*. The breeding coloration and some aspects of non-breeding coloration as well as body shape is most similar to *Talamancaheros* (see fig. 3). The body shape is only superficially similar since *Talamancaheros* has a lotic complex of characters characterizing its postcranial while *Panamius* has a lentic complex of characters. *Panamius* is plesiomorphic to *Cryptoheros*, *Amatitlania* and *Neetroplus* in not having elevated fin-spine meristics. In *Panamius* the paired fins seldom or never extend posteriorly to the first anal-fin spine (because of low number of anteriorly shifted anal-fin spines), the anal fin has only six anal-fin spines (vs. seven or more) and the dorsal fin as few as 16 spines (vs. always 17 or more).

Distribution. Endemic to the canal zone and pacific slope of eastern Panamá.

Notes. We elevate the subgenus *Panamius* SCHMITTER-SOTO, 2007a to genus status. The elevation of *Panamius* to genus level is justified by its distinct morphological, molecular and biogeographical differentiation from *Cryptoheros* and *Neetroplus*, the two genera where it was previously classified with reservation. It is the only species previously associated with *Cryptoheros* that is found east of the Panamá canal boundary zone between the western and eastern part of the Isthmus of Panamá. It is found related (in the nDNA ddRAD analysis) to all other eastern Isthmian Middle American amphilophine species (*Isthmoheros*, *Talamancaheros*, *Darienheros*). *Panamius* probably includes two species (fig. 3), which is confirmed both by two unique breeding dresses as well as by a considerable separation in time in nDNA/mtDNA phylogeny (4.9 My based on ŘÍČAN *et al.*, 2013).

— herichthyines

Genus *Chiapaheros* McMAHAN & PILLER in McMAHAN *et al.*, 2015

Type species. *Cichlasoma grammodes* TAYLOR & MILLER, 1980. Type by original designation (also monotypic). Gender: masculine.

Included species. *Chiapaheros grammodes* (TAYLOR & MILLER, 1980)

Diagnosis. *Chiapaheros* is a monotypic piscivorous genus combining a large-head and large-mouth with an elongated lotic (rheophilic) postcranial. Diagnosed by elevated meristic characters (scales, caudal vertebrae, fin rays), unique in having the highest number (19–20) of transverse scale rows among the Middle American heroine cichlid clade, unique brass coloured vermiculations on face, and large caniniform teeth at jaw symphysis. Further diagnosed by: increased number of caudal vertebrae (> 17); long caudal peduncle including > 5 vertebrae; elevated number of: dorsal fin spines (> 17); compensated by low number of soft dorsal rays (< 9), cheek scales series (7–8), scales along lateral line (32), scale rows between anterior margin of dorsal fin and lateral line (7), scales rows between bases of pelvic and pectoral fins (8); teeth conical, pointed, strongly recurved; fold of lower lip continuous; second lower lip small, thin; jaws almost subequal in length, lower jaw tip only slightly projecting in front of the upper jaw; mouth large, but maxillary not reaching below the eye (morphology of palatine and its ligaments unknown); L-type coloration pattern ontogeny.

Distribution. *Chiapaheros* is endemic to the high elevation Río Grande de Chiapa valley in the Sierra de Chiapas, Chiapas, México and Huehuetenango in Guatemala.

Genus *Trichromis* McMAHAN & CHAKRABARTY in McMAHAN *et al.*, 2015

Type species. *Heros salvini* GÜNTHER, 1862. Type by original designation (also monotypic). Gender: masculine.

Included species. *Trichromis salvini* (GÜNTHER, 1864)

Diagnosis. *Trichromis* combines a predatory cranial morphology with a lentic postcranial morphology. A monotypic genus of Middle American heroine cichlids, diagnosed by a distinct karyotype of $2N = 52$ and coloration and meristic characters. Karyotypes are otherwise rather uniform among Neotropical cichlids (THOMPSON, 1979) and especially heroine cichlids which virtually all have $2N = 48$ except for South American *Caquetaia kraussii* ($2N = 50$) and *Trichromis*. The proportion of metacentric-submetacentric to subtolocentric-telocentric chromosomes is ancestrally in heroines 6:42 while it is 28:24 in *Trichromis* (THOMPSON, 1979). A unique coloration pattern of *Trichromis* includes an ocellated eye spot (mainly developed in females) on the suboperculum like in its sister genus *Thorichthys* (here in both sexes) and a more or less fused series of midlateral blotches forming a lateral stripe, combined with a dorsal horizontal stripe of fused blotches (formed from dorsal portions of interrupted ver-

tical bars) running below the dorsal fin. Females develop a large blotch in the dorsal fin resembling a third stripe. This blotch is also often developed in males, which is quite unusual among Neotropical cichlids. This unique melanin pattern is combined with a striking combination of yellow and red ground colour, the extent and intensity of which varies among populations. Vertical bars are mostly incorporated into the two horizontal stripes and almost missing from lower portions of body, especially in breeding colours which are dominated by the horizontal stripes, which is a very uncommon situation among heroine cichlids. Both sexes with opalescent vermiculations on the face. Further diagnosed by: rather short caudal peduncle including only two vertebrae; increased number of anal pterygiophores anteriorly from the first haemal spine (> 2); increased number of anal spines (7–8); slightly increased number of dorsal spines (17); decreased number of scales along lateral line (< 28); few scales between upper lateral line and base of anterior part of soft dorsal (< 2); few transverse scale rows (< 13); teeth pointed conical with a small second cusp; symphyseal pair of teeth in the lower jaw small; mouth small, sharp, not reaching below the eye; fold of the lower lip continuous; second lower lip prominent; reduced anteroventral palatine wing and exposed median palatovomerine ligament; L-type coloration pattern ontogeny (as in *Chiapaheros*, despite being herichthyine cichlids).

Distribution. *Trichromis* is distributed on the Atlantic slope of México (Veracruz, Oaxaca, Chiapas, Tabasco, Campeche, Yucatán, Quintana Roo), Belize and Guatemala in the Usumacinta ichthyological province.

Notes. *Trichromis* is a herichthyine, the sister-group of *Thorichthys*. It is not an amphiphilophine as recovered by McMAHAN *et al.* (2015) and several other studies that used the same dubious sequences. *Trichromis* was described on the basis of a completely erroneous phylogeny that was based either on wrongly determined specimens, chimerical concatenation of markers, or contamination during lab work. We have already previously to the description of *Trichromis* reported that some of the used sequences are identical with *Cryptoheros* (ŘÍČAN *et al.*, 2013: Appendix 1).

Genus *Thorichthys* MEEK, 1904

Type species. *Thorichthys ellioti* MEEK, 1904 (= *Heros maculipinnis* STEINDACHNER, 1864). Type by original designation. Gender: masculine.

Included species. *Thorichthys affinis* (GÜNTHER, 1862), *Thorichthys aureus* (GÜNTHER, 1862), *Thorichthys callolepis* (REGAN, 1904), *Thorichthys helleri* (STEINDACHNER, 1864), *Thorichthys maculipinnis* (STEINDACHNER, 1864), *Thorichthys meeki* BRIND, 1918, *Thorichthys passionis* (RIVAS 1962), *Thorichthys socolofi* MILLER & TAYLOR, 1984.

Diagnosis. *Thorichthys* combines a substratum-sifting cranial morphology with a lentic postcranial morphology. This combination is unique among Middle American

heroine cichlids. *Thorichthys* is in detail well diagnosed by five dentary pores (unique reversal among the heroine cichlids); an ocellated eye spot on the suboperculum (lost in *T. callolepis*); red-orange-yellow intermandibular region, lower part of head and belly; truncate to lunate caudal fin with lobes typically produced into filaments in adults; substratum-sifting cranial morphology with a produced snout, a deep to very deep preorbital region associated with an anteroposteriorly deep lachrymal, fine, needle-like, tightly spaced teeth that are scarcely increasing in size towards symphysis (there is a trend to this condition but not all species of *Thorichthys* have this tooth type completely developed); unscaled bases of soft portion of anal and dorsal fin; 12 abdominal vertebrae (reversed to 13 in *T. callolepis*); proportionally long caudal peduncle (including 3–4 vertebrae); more than two anal fin pterygiophores anteriorly from the first haemal spine; elevated number of anal fin spines ((7)8–9); decreased number of dorsal fin elements (spines plus rays < 26); reduced number of scale series between upper lateral line and base of anterior part of soft dorsal (< 2.5); elevated number of gill rakers on the lower part of 1st arch (10–15); reduced number of pectoral fin rays (13; reversed to 14 in *T. callolepis*); fold of lower lip continuous; second lower lip prominent; pointed conical teeth without second cusp on premaxillary and mandibular teeth of the 1st series; palatine-premaxillary ligament; head and anterior part of body usually with prominent blue spots; characteristic coloration ontogeny with well developed pigmentation centres, early obliterated midlateral line, and dominant midlateral blotch in juveniles (and adults).

Distribution. *Thorichthys* is distributed over the Atlantic slope of southern México, Guatemala, and Belize.

Notes. Support for *Thorichthys* is very high in all data sets. All species are small to medium-sized sifter feeders with mouth in an inferior position and a pronounced snout. All but one species share a diagnostic ocellus in the posteroinferior margin of the operculum. *Thorichthys* has often been regarded as closely related to *Astatheros* (*sensu* ROE *et al.*, 1997; here as *Cribroheros*) because of similar trophic morphology and substrate-sifting behaviour (BUSSING & MARTIN, 1975). Most, but not all, of our morphological analyses indeed recover this grouping, but it is rejected by molecular data and combined analyses.

Genus *Herichthys* BAIRD & GIRARD, 1854

Type species. *Herichthys cyanoguttatus* BAIRD & GIRARD, 1854.
Type by monotypy. Gender: masculine.

Synonyms. *Nosferatu* DE LA MAZA-BENIGNOS *et al.*, 2015: 210.
Type by original designation. Gender: masculine.

Included species. *Herichthys bartoni* (BEAN, 1892); *Herichthys carpintis* (JORDAN & SNYDER, 1899); *Herichthys cyanoguttatus* BAIRD & GIRARD, 1854; *Herichthys deppii* (HECKEL, 1840); *Herichthys labridens* (PELLEGRIN, 1903); *Herichthys minckleyi* (KORNFIELD & TAYLOR, 1983); *Herichthys molango* DE LA

MAZA-BENIGNOS & LOZANO-VILANO, 2013; *Herichthys pame* DE LA MAZA-BENIGNOS & LOZANO-VILANO, 2013; *Herichthys pantostictus* (TAYLOR & MILLER, 1983); *Herichthys steindachneri* (JORDAN & SNYDER, 1899); *Herichthys tamasopoensis* ARTIGAS AZAS, 1993.

Diagnosis. *Herichthys* is in many respects the most plesiomorphic genus of the herichthyine crown-group. *Herichthys* combines an unspecialized detritivorous cranial morphology (lower jaw shorter, oral tooth morphology etc.) with an ancestral/slightly lentic postcranial morphology. The genus has ancestral meristics (13 abdominal vertebrae; 16 caudal vertebrae [decreased to 15 in the cyanoguttatus group]; 16 dorsal fin spines [secondarily 15 in one species and 17 in one species]; < 14 pectoral fin rays [vs. 15 or more in other crown-group herichthyines]; and numbers of scale rows) and other ancestral characters (e.g. development of midlateral blotches), but shares with other crown-group herichthyines the apomorphic Li-type coloration ontogeny, herichthyine-type breeding colours and development of the dominant midlateral blotch in both 4th and 5th ontogenetic bars (instead of in the 4th as ancestral for heroine cichlids), tooth morphology (teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed, tip labiolingually flattened), and also weakly scaled bases of dorsal and anal fins. The genus has the shortest anal fin among heroines (together with *Nandopsis*), containing ancestrally less than 14 elements (5 anal spines, secondarily 4 in *H. bartoni* and 7 in *H. deppii*). Further diagnosed by: high number of caudal peduncle vertebrae (> 4; secondarily reduced in some species of the cyanoguttatus group); low number of dorsal fin rays (10–11); fold of lower lip subcontinuous (interrupted in *H. labridens*); second lower lip small. The labridens group has the most typical herichthyine-type breeding colours, characteristic by complete lack of intensified bars but replaced by sharply demarcated black (most of body) and pale areas (typically upper part of head and anterior body).

Distribution. *Herichthys* represents the northernmost group of Neotropical cichlids and is restricted to the Atlantic drainages of México and southernmost Texas, ranging from Veracruz to the Río Bravo/Grande river basin.

Notes. The genus *Herichthys* was redefined by KULLANDER (1996) to include also species previously classified as part of REGAN'S (1905) section "*Parapetenia*" (the *H. labridens* group). KULLANDER also excluded the *bocourti* species group (*Cincelichthys*; see below), classified as *Herichthys* since REGAN (1905). Our results strongly support the monophyly of *Herichthys sensu* KULLANDER. The diagnosis based on breeding coloration provided by KULLANDER (1996) is however not correct, since it is diagnostic for all the crown-group herichthyines and not for *Herichthys* only.

There are two main morphological types representing clades within *Herichthys* plus two more basal species. The *H. cyanoguttatus* group has spatulate teeth, semi-

herbivorous diet (except *H. minckleyi* which lacks the spatulate teeth and is trophically polymorphic without a herbivorous morph), and blue opalescent body markings. The other morphological type are species which also have labiolingually flattened teeth, but only on their tips which are pointed (the *H. labridens* group, *H. bartoni* and *H. minckleyi*). The *H. labridens* group has a unique synapomorphy of a naked red area in the dorsal part of the axil of the pectoral fin. The species in the *H. labridens* group are ancestrally detritivorous, one species (*H. steindachneri*) is however a piscivore and several species are variable (*H. pantostictus*) or polymorphic (*H. minckleyi*, *H. bartoni*). The *H. labridens* group plus *H. bartoni* was recently proposed to be elevated to a separate genus (*Nosferatu*) named after and supposedly diagnosed by its tooth morphology. We have demonstrated that this tooth morphology is ancestral for the *Theraps-Paraneetroplus* clade and also for *Herichthys*, where it is also present in the basal species of the *H. cyanoguttatus* group (the polymorphic species *H. minckleyi*). The diagnosis is thus invalid diagnosing a paraphyletic group (i.e. based on a plesiomorphic character state). We are thus not using the genus in agreement with MEJÍA *et al.* (2015).

Herichthys includes the polymorphic *Herichthys minckleyi*, a species endemic to Cuatro Ciénegas de Caranza in Coahuila, México. This species has three very distinct sympatric morphs: a molluscivorous, a detritivorous, and a piscivorous (KORNFIELD *et al.*, 1982; KORNFIELD & TAYLOR, 1983).

Although the monophyly of *Herichthys* has strong support, the actual number and identity of the species of *Herichthys* is far from clear (ŘÍČAN *et al.*, in prep.), and several of these ‘varieties’ have recently been described as species (*H. molango*, *H. pratinus*, *H. tepehua*). *Herichthys pratinus* DE LA MAZA-BENIGNOS & LOZANO-VILANO, 2013 is considered here a junior synonym of *Herichthys pantostictus* (TAYLOR & MILLER, 1983) because the species lacks any morphological distinguishing characters (ŘÍČAN *et al.*, in prep.) and because mtDNA (cyt *b*) and nDNA (ddRAD) place it within the phylogeographic structure of *Herichthys pantostictus*.

Herichthys bartoni shows signatures of hybridization with *H. labridens* – mtDNA is closest to *H. labridens*, in nDNA it is the sister-group of the rest of the clade.

The *Theraps-Paraneetroplus* clade of herichthyines

The *Theraps* clade

Genus *Theraps* GÜNTHER, 1862

Type species. *Theraps irregularis* GÜNTHER, 1862. Type by monotypy. Gender: masculine.

Included species. *Theraps irregularis* GÜNTHER, 1862

Diagnosis. *Theraps* combines a scraping cranial morphology with a lotic postcranial morphology (*Theraps* is the most elongated and streamlined genus of Middle American cichlids). *Theraps* is similar to other herichthyine genera in the *Theraps* clade (*Wajpamheros*, *Cinzelichthys*, *Kihnichthys*, *Chuco*) in having double vertical bars four and five. Diagnosed by: Li-type coloration ontogeny; herichthyine-type breeding colours (with black limited to the ventral portion of head, body background all white); dorsal and anal fins almost scaleless at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed but often blunt from scraping, implanted almost horizontally, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip absent; increased longitudinal meristics: elevated number of abdominal vertebrae (15); highest number of caudal vertebrae among heroine cichlids (19; rarely 18); very long caudal peduncle including 6–7 vertebrae; elevated number of scales along lateral line (33). The autapomorphically high numbers of caudal vertebrae and caudal peduncle vertebrae distinguish *Theraps* from all other Middle American cichlid genera including *Paraneetroplus*.

Distribution. *Theraps* is distributed in SE México and Guatemala in the Usumacinta river basin and a few records are from the neighbouring Polochic river basin.

Notes. *Theraps* is restricted to a monotypic genus because other species previously associated with it (e.g. MILLER *et al.*, 2005) do not form a monophylum with it. The genus *Chuco* is revalidated to include three species previously placed in *Theraps*, the genus *Rheoheros* includes yet other species previously placed in *Theraps*, and *Paraneetroplus* is also an unrelated rheophilic genus. Two additional species previously placed in *Theraps* form separate genera among the distantly related amphiphilic cichlids (*Talamancaheros*, *Chortiheros*) and both are distributed outside the Usumacinta ichthyological province.

The *Theraps-Paraneetroplus* clade is surprisingly young considering its high number of species (based on time estimates from ŘÍČAN *et al.*, 2013; see Discussion). The *Theraps-Paraneetroplus* clade is one of the most difficult clades in terms of a formal classification into genera. Within the clade repeated cases of parallel evolution of the lotic scrapers and the lentic detritivore-herbivores have occurred in the tight biogeographical setting of the Usumacinta ichthyological province, in our new biogeographic analysis most likely within the single Usumacinta river basin. Most species-groups of the *Theraps-Paraneetroplus* clade are thus at least partially sympatric and have probably evolved in parapatry or sympatry. The *Theraps-Paraneetroplus* clade is also very homogeneous morphologically, sharing an elongated abdominal part of the body with 15 vertebrae, ontogenetic coloration patterns, and even the (among Middle American cichlids) highly variable number of anal fin spines is almost uniform in the *Theraps-Paraneetroplus* clade (either the ancestral count of 6 or 5 in a few species). MILLER *et al.* (2005) classified all the lotic species as *Theraps* and all the lentic species (plus the *T. intermedius* group) as *Vieja*. This two genera concept is however completely

polyphyletic as it just represents two repeatedly evolved ecomorphs. We agree with McMAHAN *et al.* (2015) in splitting the clade into several genera and in our analysis they correspond to repeated cases of evolution of lentic detritivore-herbivore (*Cincelichthys-Kihnichthys*, *Oscura*, *Vieja*, *Maskaheros*) and the lotic scraper (*Theraps*, *Chuco*, *Rheoheros*, *Paraneetroplus*) plus one monotypic genus (*Wajpamheros*) for a lotic substratum sifter.

The problematic issue with the study by McMAHAN *et al.* (2015) is, that the genera were described without any analysis of the morphological characters. The morphological characters used in the diagnoses are those characters correlated with ecology and most of the supposedly diagnostic characters were thus taken from coloration patterns. The coloration patterns however were also not studied in any systematic way and the coloration pattern characters were thus extremely vaguely defined, without statements of homology, and the coloration pattern characters that were proposed are at best of species-level value. McMAHAN *et al.*'s diagnoses are thus based on minor differences in coloration patterns which are either ecomorph correlates (e.g. the caudal fin blotch), ubiquitous in the group (the low lying lateral stripe), or complete misperceptions of the coloration patterns (e.g. "freckled appearance" of *Rheoheros*). Wherever possible we therefore provide homologous coloration pattern characters in the re-diagnoses of the genera proposed by McMAHAN *et al.* (2015).

Another problem resulting from the intended revision of the *Theraps-Paraneetroplus* clade by McMAHAN *et al.* (2015) is the phylogeny upon which it was directly based. The phylogeny lacks support (posterior probability in Bayesian analysis below 95%) at many nodes connecting the supposed genera (*Oscura-Rheoheros*, *Theraps*, *Theraps-Kihnichthys*). In addition, as we have demonstrated here, the topology is dominated by the mtDNA signal (the non-monophyly of the *Theraps-Paraneetroplus* clade versus *Herichthys*, the sister-group relationship of *Oscura-Rheoheros* to *Theraps-Cincelichthys-Kihnichthys*). The mtDNA dominance of the tree is made obvious by the *cyt b* gene being the only partition represented in all species. The ddRAD phylogeny that we present here represents the first strong nuclear phylogenetic signal and species-tree phylogeny of the Middle American cichlids.

Genus *Cincelichthys* McMAHAN & PILLER in McMAHAN *et al.*, 2015

Type species. *Neetroplus bocourti* VAILLANT & PELLEGRIN, 1902. Type by original designation. Gender: masculine.

Included species. *Cincelichthys bocourti* (VAILLANT & PELLEGRIN, 1902); *Cincelichthys pearsei* (HUBBS, 1936).

Diagnosis. *Cincelichthys* combines a biting detritivorous/herbivorous cranial morphology with a lentic postcranial morphology. *Cincelichthys* is similar to other herichthyine genera in the *Theraps* clade (*Wajpamheros*, *Kihnichthys*, *Theraps*, *Chuco*) in having double vertical

bars four and five. Also similar to most other herichthyine genera in the *Theraps-Paraneetroplus* clade (except the two most streamlined, i.e. *Theraps* and *Rheoheros*) in having a ventrally located lateral stripe (located usually well below the level of the lower lateral line vs. just above in all other [where present] Middle American cichlids). Diagnosed by: oral teeth truncated labiolingually flattened (shared only with *Kihnichthys* among morphologically similar species); Li-type coloration ontogeny; herichthyine-type breeding colours that are however much obscured due to lack of a white background (as in all lentic species of the *Theraps-Paraneetroplus* clade except *Maskaheros*); dorsal and anal fins weakly scaled at their bases; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip absent; increased longitudinal meristics: elevated number of abdominal vertebrae (15) but ancestral number of caudal vertebrae (16) and only slightly elongated caudal peduncle including 4 vertebrae; elevated number of scales along lateral line (31). Additionally distinguished from similarly built *Vieja* (except in tooth morphology and in coloration – i.e. division of bars four and five) in having ancestral number of caudal vertebrae (16 vs. reduced to 15).

Distribution. *Cincelichthys* species are allopatrically distributed in SE México and Guatemala in the Usumacinta ichthyological province in the Usumacinta (*C. pearsei*) and Polochic (*C. bocourti*) river basins.

Notes. *Cincelichthys* and *Kihnichthys* have among the *Theraps* clade a unique tooth morphology. *Kihnichthys* however does not have distinguishing characters from *Cincelichthys* except for coloration pattern details of species-level value. We have not been able to include *Kihnichthys* in the nuclear ddRAD phylogeny because we presently lack a good sample for DNA analysis but concatenated mtDNA/nDNA phylogenies do not reject monophyly of both genera (which appear paraphyletic but without support to *Theraps*, *Wajpamheros* and *Chuco*; figs. 2 & 3A) and morphological characters do support monophyly of both genera (fig. 6). *Kihnichthys* and *Cincelichthys* are likely congeneric and their separate genus status has to be tested with nuclear DNA data.

Genus *Kihnichthys* McMAHAN & MATAMOROS in McMAHAN *et al.*, 2015

Type species. *Vieja ufermanni* ALLGAYER, 2002. Type by original designation (also monotypic). Gender: masculine.

Included species. *Kihnichthys ufermanni* (ALLGAYER, 2002).

Diagnosis. *Kihnichthys* combines a biting/herbivorous cranial morphology with a lentic postcranial morphology. *Kihnichthys* is similar to other herichthyine genera in the *Theraps* clade (*Wajpamheros*, *Cincelichthys*, *Theraps*, *Chuco*) in having double vertical bars four and five. Also similar to most other herichthyine genera in the *Theraps-*

Paraneetroplus clade (except the two most streamlined, i.e. *Theraps* and *Rheoheros*) in having a ventrally located lateral stripe (located usually well below the lateral line vs. a midlateral stripe in all other [where present] Middle American cichlids). Diagnosis is identical to *Cinzelichthys*: oral teeth truncated labiolingually flattened (shared only with *Cinzelichthys* among morphologically similar species); Li-type coloration ontogeny; herichthyine-type breeding colours that are however much obscured due to lack of a white background (as in all lentic species of the *Theraps-Paraneetroplus* clade except *Maskaheros*); dorsal and anal fins weakly scaled at their bases; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip absent; increased longitudinal meristics: elevated number of abdominal vertebrae (15) but ancestral number of caudal vertebrae (16) and only slightly elongated caudal peduncle including 4 vertebrae; elevated number of scales along lateral line (31). Additionally distinguished from similarly built *Vieja* (except in tooth morphology and in coloration – i.e. division of bars four and five) in having ancestral number of caudal vertebrae (16 vs. reduction to 15).

Distribution. *Kihnichthys* is distributed in SE México and Guatemala in the Usumacinta river basin.

Notes. *Kihnichthys* does not have distinguishing characters from *Cinzelichthys* except for coloration pattern details of species-level value. We have not been able to include this species in the nuclear ddRAD phylogeny but concatenated mtDNA/nDNA phylogenies do not reject monophyly of both genera (which appear paraphyletic but without support to *Theraps*, *Wajpamheros* and *Chuco*; figs. 2 & 3A) and morphological characters do support monophyly of both genera (fig. 6). *Kihnichthys* and *Cinzelichthys* are likely congeneric and their separate genus status has to be tested with nuclear DNA data.

Genus *Chuco* FERNÁNDEZ-YÉPEZ, 1969

Type species. *Cichlasoma milleri* [= *Heros microphthalmus* GÜNTHER, 1862] MEEK, 1907. Type species by original designation. Gender: neuter.

Included species. *Chuco godmanni* (GÜNTHER, 1862); *Chuco intermedium* (GÜNTHER, 1862); *Chuco microphthalmus* (GÜNTHER, 1862).

Diagnosis. *Chuco* combines a scraping/biting cranial morphology with a lotic (but rather deep-bodied) postcranial morphology. *Chuco* is similar to other herichthyine genera in the *Theraps* clade (*Wajpamheros*, *Cinzelichthys*, *Kihnichthys*, *Theraps*) in having double vertical bars four and five. In *Chuco* except *C. microphthalmus* the low-lying lateral stripe and the dominant (one or two) vertical bar form an „L“-like dominant marking on the flank. Diagnosed by: Li-type coloration ontogeny; herichthyine-type breeding colours (with black ventral portion of head and anterior body); dorsal and anal fins

weakly scaled at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold subcontinuous or variable within *C. microphthalmus*; second lower lip absent; increased longitudinal meristics: elevated number of abdominal vertebrae (15) but ancestral number of caudal vertebrae (16) and only slightly elongated caudal peduncle including 4 vertebrae; elevated number of scales along lateral line (31–32). Distinguished from genera in the *Paraneetroplus* clade and from *Rheoheros* by having double vertical bars four and five; distinguished from *Theraps* in being more deep-bodied with a shorter postcranial and caudal peduncle; distinguished from *Cinzelichthys* in being less deep-bodied with a longer caudal peduncle and especially in lacking the specialized tooth morphology; distinguished from *Wajpamheros* in lacking substratum-sifting cranial morphology.

Distribution. *Chuco* is distributed in the Usumacinta ichthyological province in SE México and Guatemala with allopatric species in the Usumacinta/Grijalva (*C. intermedium*), Polochic (*C. godmanni*) and Motagua (*C. microphthalmus*) river basins.

Notes. *Chuco* is revalidated here to include three species previously placed in *Theraps*. *Theraps* is restricted here to a monotypic genus because other species previously associated with it (including *Chuco*; e.g. MILLER *et al.*, 2005) do not form a monophylum with it.

The *Paraneetroplus* clade

Genus *Rheoheros* McMAHAN & MATAMOROS in McMAHAN *et al.*, 2015

Type species. *Heros lentiginosus* STEINDACHNER, 1864. Type by original designation. Gender: masculine.

Included species. *Rheoheros coeruleus* (STAWIKOWSKI & WERNER, 1987); *Rheoheros lentiginosus* (STEINDACHNER, 1864).

Diagnosis. *Rheoheros* combines a scraping cranial morphology with a lotic postcranial morphology. The mouth is very narrow and the symphyseal 1–2 pairs in both jaws are very distinctly enlarged with strongly curved tips, producing a forceps-like dentition used to scrape invertebrates encrusted in the rocks, hiding in the crevices or under them. Distinguished from other rheophilic genera in the *Theraps-Paraneetroplus* clade (*Theraps*, *Paraneetroplus*) by this mouth and tooth morphology and by having slightly less caudal vertebrae and caudal peduncle vertebrae; from *Paraneetroplus* additionally in having a normal midlaterally located lateral stripe and a less deep body; from *Theraps* additionally in having a completely white head and body in breeding coloration (vs. black ventral portion of head and anterior body) and in lacking double vertical bars four and five. Diagnosed by: Li-type coloration ontogeny; highly advanced nearly totally white-dominated

herichthyine-type breeding colours (with black limited to the ventral portion of an otherwise almost completely white head and body); dorsal and anal fins almost scaleless at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip absent; increased longitudinal meristics: elevated number of abdominal vertebrae (15); elevated number of caudal vertebrae (17–18); long caudal peduncle including 4–5 vertebrae; elevated number of scales along lateral line (34).

Distribution. *Rheoheros* is distributed in SE México and Guatemala in the Usumacinta and Grijalva river basin.

Notes. *Rheoheros* was previously included in *Theraps*, but monophyly of *Theraps* is compromised by the phylogenetic position of the morphologically different genera *Cincelichthys* and *Kihnichthys*. Additionally *Rheoheros* is based on nDNA the sister-group of the *Paraneetroplus* clade (*Paraneetroplus*, *Maskaheros*, *Vieja*). Mitochondrial DNA-dominated phylogenies place *Rheoheros* as the sister-genus of *Oscura* and then this clade is the sister-group of the *Theraps* clade (*Theraps*, *Cincelichthys*, *Kihnichthys*, *Chuco*). The diagnosis of *Rheoheros* by McMAHAN *et al.* (2015) includes no apomorphic character states, in our analysis it can be diagnosed by characters in combination plus the apomorphic mouth and teeth and the nearly all-white breeding coloration (the degree, but not the details, are shared with *Paraneetroplus* and *Maskaheros*). *Rheoheros* is morphologically closest to the reconstructed common ancestor of the whole *Theraps-Paraneetroplus* clade. *Rheoheros* has no “freckled markings” (McMAHAN *et al.*, 2015) on body, it has the most plesiomorphic coloration pattern composed of a combination of vertical bars and midlateral blotches, with no vertical bars divided or fused, with the plesiomorphic dorsally placed lateral band (Supplementary material 3) with some spotting (which it shares with *Maskaheros*).

Genus *Oscura* McMAHAN & CHAKRABARTY in McMAHAN *et al.*, 2015

Type species. *Cichlasoma heterospilum* HUBBS, 1936. Type by original designation (also monotypic). Gender: feminine.

Included species. *Oscura heterospila* (HUBBS, 1936);

Diagnosis. *Oscura* combines a biting/detritivorous cranial morphology with a lentic postcranial morphology. *Oscura* is similar to other lentic morphology herichthyine genera in body shape and the ventrally located lateral stripe (*Vieja*, *Cincelichthys*, *Kihnichthys*, *Maskaheros*). Distinguished from *Cincelichthys* and *Kihnichthys* in lacking double vertical bars four and five (but one of the bars appears to be divided), and in lacking the spatulate tooth morphology. Distinguished from the very similar *Vieja* in lacking the unique and fixed combination of 15

abdominal and 15 caudal vertebrae and instead having 14 abdominal and 16 caudal vertebrae and in having the shortest caudal peduncle in the *Theraps-Paraneetroplus* clade. Distinguished from *Maskaheros* in having developed vertical bar four (visible throughout ontogeny, in juveniles and in adult breeding coloration) (vs. lacking in all stages of development and all colorations) and in completely different breeding coloration (more obscure with black vertical bars and black ventral portion of body vs. pure white without black ventral portion of body and without vertical bars). Diagnosed by only plesiomorphic character states compared to other lentic herichthyine genera: Li-type coloration ontogeny; obscured herichthyine-type breeding colours (with black ventral portion of head and anterior body); dorsal and anal fins weakly scaled at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold continuous; second lower lip absent; slightly elevated number of abdominal vertebrae (14) but ancestral number of caudal vertebrae (16), shortest caudal peduncle of all genera in the *Theraps-Paraneetroplus* clade (less than 2 vertebrae contained in the caudal peduncle); ancestral number of scales along lateral line (30).

Distribution. *Oscura* is distributed in SE México and Guatemala in the Usumacinta/Grijalva and Candelaria river basins.

Notes. *Oscura* is similar to *Vieja* but is distinguished by plesiomorphic vertebrae numbers, caudal peduncle length and coloration details. The diagnosis of *Oscura* by McMAHAN *et al.* (2015) relied solely on coloration pattern characters of, at-best, a species-level value and includes no apomorphic character states. Mitochondrial dominated phylogenies place it as the sister genus of the rheophilic *Rheoheros* (without or on the margin of statistical support). We have been able to include only one juvenile specimen of *Oscura* in the nuclear ddRAD phylogeny and it appears as the sister-group of *Vieja sensu stricto* (*V. maculicauda* and *V. melanura*). The cytb DNA sequence of this specimen is however different from the other specimens of *Oscura* (CONCHEIRO PÉREZ *et al.*, 2007) and is nested within *V. bifasciata* (Supplementary Material 1 and 2) and not as the sister-group of *Rheoheros*. Whether *Oscura* is the sister-genus of *Rheoheros* or is a synonym of *Vieja* remains to be fully tested with nuclear DNA data with more specimens.

Genus *Vieja* FERNÁNDEZ-YÉPEZ, 1969

Type species. *Vieja panamensis* [= *Cichlosoma maculicauda* REGAN, 1905a] FERNÁNDEZ-YÉPEZ, 1969. Type by original designation. Gender: feminine.

Synonyms. *Paratheraps* HOHL, 1988. Type species. *Paratheraps breidohri* WERNER & STAWIKOWSKI, 1987. Type species by secondary designation. Gender: masculine.

Included species. *Vieja bifasciata* (STEINDACHNER, 1864); *Vieja breidohri* (WERNER & STAWIKOWSKI, 1989); *Vieja fenestrata* (GÜNTHER, 1860); *Vieja guttulata* (GÜNTHER, 1864); *Vieja hartwegi* (TAYLOR & MILLER, 1980); *Vieja maculicauda* (REGAN, 1905); *Vieja melanura* (GÜNTHER, 1862); *Vieja zonata* (MEEK, 1905).

Diagnosis. *Vieja* combines a short jawed biting/detritivorous cranial morphology with a lentic postcranial morphology. *Vieja* is similar to other lentic morphology herichthyine genera in body shape and the ventrally located lateral stripe (*Cincelichthys*, *Kihnichthys*, *Oscura*, *Maskaheros*). Distinguished from *Cincelichthys* and *Kihnichthys* in lacking double vertical bars four and five and in lacking the spatulate tooth morphology. Distinguished from the very similar *Oscura* in having the unique and fixed combination of 15 abdominal and 15 caudal vertebrae (vs. 14+16 vertebrae) and in having a longer (but still rather short) caudal peduncle. Distinguished from *Maskaheros* in having developed vertical bar four (visible throughout ontogeny, in juveniles and in adult breeding coloration) (vs. lacking in all stages of development and all colorations) and in completely different breeding coloration (more obscure with black vertical bars and black ventral portion of body vs. pure white without black ventral portion of body and without vertical bars). Diagnosed by: Li-type coloration ontogeny; obscured herichthyine-type breeding colours (with black ventral portion of head and anterior body); dorsal and anal fins weakly scaled at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip absent; elevated number of abdominal vertebrae (15) but lowered number of caudal vertebrae (15) producing a unique combination of 15+15 vertebrae among the Middle American cichlids, shorter caudal peduncle than all genera in the *Theraps-Paraneetroplus* clade except (*Oscura*) (including 2–3 vertebrae; less than 2 vertebrae in *Oscura*); elevated number of scales along lateral line (31).

Distribution. *Vieja* is ancestrally distributed in SE México and Guatemala in the Usumacinta ichthyological province. *Vieja maculicauda* has naturally colonized most of Caribbean-slope rivers in their lowest reaches all the way to the Panamá canal. *Vieja maculicauda* is one of the few Middle American cichlids routinely found in brackish-water conditions. *Vieja guttulata* and some populations of *V. zonata* have colonized the Pacific slope Chiapas-Nicaragua ichthyological province.

Notes. *Vieja* in previous classifications included most of the deep-bodied large-sized lentic species of Middle America. These are now included in separate genera *Cincelichthys*, *Kihnichthys*, *Maskaheros*, *Chuco*, *Oscura* and one species is now placed among the amphiphiline clade as yet another separate genus (*Isthmoheros*; *I. tuyenensis*). None of these species forms a monophyletic group with *Vieja*. The diagnosis of *Vieja* by McMAHAN

et al. (2015) relied virtually solely on coloration pattern characters of, at-best, species-level value and these do not distinguish it from other lentic genera in the *Theraps* clade. The large caudal peduncle blotch is the result of shortening of the caudal peduncle and as such is found in all lentic-detritivorous genera. The low-running lateral stripe is found in all species of the *Theraps-Paraneetroplus* clade except for the very streamlined genera *Theraps* and *Rheoheros* (here it is thus very likely not homologous to other Middle American cichlids due to the very slender body). Reference to *Vieja* (and other genera) having bicuspid teeth is erroneous (no species of Middle American cichlids has bicuspid teeth, though some species and genera have a small second subapical cusp on the lingual side of the oral teeth). We treat *Paratheraps* HOHL, 1988 as a synonym of *Vieja* since it is monophyletic with *Vieja* in nDNA phylogeny and represents the same ecomorph combination.

Genus *Maskaheros* McMAHAN & PILLER in McMAHAN *et al.*, 2015

Type species. *Vieja argentea* ALLGAYER, 1991. Type by original designation. Gender: masculine.

Included species. *Maskaheros argenteus* (ALLGAYER, 1991); *Maskaheros regani* (Miller, 1974).

Diagnosis. *Maskaheros* combines a biting/detritivorous cranial morphology with a meristically elongated but deep-bodied postcranial morphology with a short caudal peduncle. The postcranial morphology is thus intermediate between the lentic of *Vieja* and the lotic of *Paraneetroplus* in agreement with the intermediate phylogenetic position of *Maskaheros* between the two genera. *Maskaheros* is similar to other lentic morphology herichthyine genera in body shape and the ventrally located lateral line (*Vieja*, *Cincelichthys*, *Kihnichthys*, *Oscura*). Distinguished from all these lentic genera in lacking the vertical bar four in all stages of development and all colorations (vs. visible throughout ontogeny, in juveniles and in adult breeding coloration) and in completely different breeding coloration (shared with *Paraneetroplus*; pure white with only a small area of black on the ventral portion of head and without vertical bars vs. more obscure with black vertical bars and black ventral portion of head and body). Distinguished from the *Theraps* clade in lacking double vertical bars four and five and in lacking the spatulate tooth morphology of *Cincelichthys* and *Kihnichthys*. Distinguished from *Vieja* in lacking the unique and fixed combination of 15 abdominal and 15 caudal vertebrae and instead having 14 abdominal and 17 caudal vertebrae. Diagnosed by: Li-type coloration ontogeny; pure white herichthyine-type breeding colours (without black ventral portion of head and anterior body); dorsal and anal fins almost scaleless at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip

absent; slightly elevated number of abdominal vertebrae (14) and elevated number of caudal vertebrae (17), but short caudal peduncle with only 3 contained vertebrae; elevated number of scales along lateral line (32).

Distribution. *Maskaheros* species are allopatrically distributed in SE México and Guatemala in the Usumacinta ichthyological province in the Usumacinta (*M. argenteus*) and Coatzacoalcos (*M. regani*) river basins.

Notes. *Maskaheros* has previously been classified in the genus *Vieja*, but it is the sister group of *Paraneetroplus* with intermediate morphology between *Vieja* and *Paraneetroplus*. Young to subadult *M. regani* are very similar to adult *Rheoheros* (the basal genus of the *Paraneetroplus* clade) in head and body shape and in coloration (except for the lack of distinct vertical bars). Both *Maskaheros* species show introgression of mtDNA from *Paraneetroplus*. The diagnosis of *Maskaheros* by McMAHAN *et al.* (2015) is unsatisfactory and lacks apomorphic character states, the main distinguishing character of two bars between eyes is a plesiomorphy of all cichlids and the generic name is thus quite inappropriate.

Genus *Paraneetroplus* REGAN, 1905

Type species. *Paraneetroplus bulleri* REGAN, 1905a: Type by monotypy. Gender: masculine.

Included species. *Paraneetroplus bulleri* REGAN, 1905; *Paraneetroplus gibbiceps* (STEINDACHNER, 1864); *Paraneetroplus nebuliferus* (GÜNTHER, 1860); *Paraneetroplus omonti* ALLGAYER, 1988.

Diagnosis. *Paraneetroplus* combines a scraping cranial morphology with a lotic (very long body and caudal peduncle but with a much deeper head than *Theraps* or *Rheoheros*) postcranial morphology. Distinguished from other rheophilic genera in the *Theraps-Paraneetroplus* clade (*Theraps*, *Rheoheros*) in having a rather ventrally located lateral stripe and a much deeper head. Distinguished additionally from *Theraps* in lacking double vertical bars four and five. Diagnosed by: Li-type coloration ontogeny; herichthyine-type breeding colours (with black limited to the ventral portion of head and body in some species); dorsal and anal fins almost scaleless at their bases; teeth with second cusp on premaxillary and mandibular teeth of the 1st series; teeth pointed but often blunt from scraping, implanted almost horizontally, tip of teeth labiolingually flattened; premaxillary ascending process not reaching to the vertical from eye; lower lip fold interrupted; second lower lip absent; increased longitudinal meristics: elevated number of abdominal vertebrae (15–16) and caudal vertebrae (18); very long caudal peduncle including 5–6 vertebrae; elevated number of scales along lateral line (34). All of these elevated meristic character states are shared with *Theraps*.

Distribution. *Paraneetroplus* species are allopatrically distributed in SE México in the Usumacinta ichthyo-

logical province in the Grijalva (*P. gibbiceps*, *P. omonti*), Coatzacoalcos (*P. bulleri*) and Papaloapán (*P. nebulifer*) river basins. *Paraneetroplus* is the only genus in the *Theraps-Paraneetroplus*-clade with no species in the Usumacinta river basin.

Notes. The distinguishing character of teeth orientation between *Theraps* and *Paraneetroplus sensu* McMAHAN *et al.* (2015) is invalid, both genera have teeth set more or less horizontally as already observed by MILLER *et al.* (2005) prior to our analysis of the teeth morphology in Middle American cichlids. The teeth orientation is another correlate of the scraping cranial ecomorph.

Review of morphological characters used in Middle American cichlids

3.10. Re-examination of published morphological characters – morphological phylogenies reflect ecomorphologies, not phylogenetic relationships

Morphological characters are hypotheses of homology (primary homology) and only primary homologies can ever be considered as secondary homologies (synapomorphies). Synapomorphies are shared homologous apomorphic character states derived from a phylogenetic analysis and their apomorphic nature is most often determined by outgroup comparisons, which have to be extensive in order to capture the variation and polarity of the putative characters across the outgroup.

The homology of morphological characters is however in most cases much more difficult to establish compared to most molecular characters, because unlike the digital nature (discrete, discontinuous representations of information) of molecular sequence characters morphological characters are of an analogue nature (continuous representation of information with a varying feature). Because of this (and other reasons) few morphological characters are clear-cut and they thus have to be formulated and interpreted in such a way that independent researches will agree on the same information content. Second, characters have to be interpreted within some context (e.g. ecological), because characters should ideally be selectively neutral (this applies both to molecular and especially to morphological characters). Selectively neutral characters have a much bigger chance to be truly homologous and free from convergence or parallelism. Third, many characters are correlated because they are often used to describe parts of a larger whole. In a phylogenetic analysis they then (because of their larger number) potentially overweight independent characters leading to an incorrect phylogenetic hypothesis. Fourth, the coding of the characters has to account for natural variation, which is rarely done especially for anatomi-

cal characters which are very often studied using single specimens. Morphological characters formulated for phylogenetic analysis thus are not all differences and similarities between taxa that we can observe. Morphological similarities and differences have to be carefully analysed prior to phylogenetic analysis to establish their homologous nature. Characters formulated in such a way that they cannot be taken to represent homology cannot serve as phylogenetically informative morphological characters.

Re-examination of the phylogenetic analysis, characters and character state distributions reported in the first morphological phylogeny dedicated to the Middle American cichlids (CHAKRABARTY, 2007) in our much wider sample of species and specimens revealed many points that are stressed in the above considerations.

First of all, all mentions by CHAKRABARTY about characters being synapomorphies of heroines are meaningless given his taxon-sampling, because the outgroup comparisons were limited to only two very distant species and the outgroup comparisons thus missed all phylogenetically basal Amazonian heroines (*Hoplarchus*, *Hypselecara*, *Pterophyllum*, *Mesonotua*, *Symphysodon*, *Uaru*, *Heros*) and all cichlasomatines (the sister group of heroines). Our re-examination of the characters using these wider outgroup comparisons shows that none of the proposed characters is a synapomorphy of heroines.

Second and directly connected with the first point, CHAKRABARTY (2007) used only two species of phylogenetically very distant and morphologically very specialized piscivores (*Crenicichla* and *Cichla*) as outgroups for comparisons and for rooting of the morphological phylogeny. This resulted in the ingroup being rooted with predatory species with the most advanced heroine piscivore (*Petenia splendida*) as the most basal examined heroine and the sister-group to the remaining examined heroines, and with the well homogeneous genus *Parachromis* recovered as paraphyletic because of the influence of the poorly chosen outgroup.

Third, the strong influence of the two distantly related piscivorous outgroup taxa on the ingroup phylogeny of the studied heroines (and the attraction of the most highly adapted piscivore as the most ancestral heroine) clearly demonstrates that a large part of the character matrix are not selectively neutral putative homologies (and hence synapomorphies) but merely very similar but convergent adaptations towards the same feeding mode (namely piscivory). This demonstrates that the vast majority of the character matrix merely reflects ecomorphological similarity.

Out of the 89 characters used by CHAKRABARTY (2007) only 78 are parsimony informative (are variable within the ingroup and are not limited to a single taxon). Characters 2, 18, 21, 24, 30, 58, 72, 78, 80, 83, 87 are thus uninformative.

Among the informative characters the following are, according to our re-examination either the result of wrong observation and/or interpretation, fail to comply with the criterion of primary homology (this includes

characters coded with two states that divide a natural continuum of variation), are inadequately explained to be reproducible (e.g. character 7, what represents the common vertical plane in all species?), or are the result of variation in size between examined specimens etc. (characters 1–9, 11–15, 17, 19, 25–26, 49–56, 58–59, 62, 67–68, 71, 84–89). Some examples follow: Very good examples of the artificial division of a natural continuum into two states which lacks any foundation of homology are characters 88, maximum body size below or above 15 cm, or character 89, overall body depth below or above 50% SL. Some examples of erroneous characters follow: Character 19. All heroine species scored by CHAKRABARTY (2007) as having a palato-vomerine articulation are wrongly scored (the state is absent). All heroine cichlids examined by us (this study) or by KULLANDER (1996) always lack a palato-vomerine articulation. Character 49. All heroine cichlids (in fact all cichlids at least during their development) have two bands across the forehead. Not all show it in preserved state, but all show it during some coloration change (e.g. aggression, breeding coloration, sleeping coloration etc.) and during coloration ontogeny (ŘÍČAN *et al.*, 2005). This character is thus a nonsense. It has however been used in a later study by the same author (McMAHAN *et al.*, 2015) to diagnose a new genus (*Maskaheros*).

Characters 50 to 56 refer to coloration patterns. From our expertise (ŘÍČAN *et al.*, 2005; this study) it seems that all were interpreted from preserved specimens without any knowledge of coloration pattern variability of live fishes and none of them is homologous nor represents a situation anywhere near the complex coloration pattern reality of cichlids. Character 50. State 1. An ocellated spot is only present in *Thorichthys* and in *Trichromis*. The other types of spots coded as states 2 and 3 (4 is wrong, see state 1) are non-homologous within the states. Heroines are diagnosed by the loss of the suborbital stripe from both adult coloration and from ontogeny (ŘÍČAN *et al.*, 2005). Some species and genera that are all predators-piscivores have secondarily reverted to the state of having a suborbital stripe (which can become disrupted into blotches), but it is a neomorph, present only in adult coloration pattern but not in the ontogeny (ŘÍČAN *et al.*, 2005). The only genus of Middle American heroines that does have the ‘ancestral’ condition (a reversal) is *Rocio*, which also has a very distinct coloration ontogeny of all coloration pattern characters (ŘÍČAN *et al.*, 2005). Character 51. State 1 is not homologous, part of *Herichthys* have a unique red-magenta axillar blotch on a naked (scaleless) area behind the base of the pectoral fin that is not homologous to the remaining spots, which are also non-homologous within the remaining species. Character 52 does not relate to coloration but to body shape and hence a relative change in the position of the lateral line in respect to the much more stably positioned midlateral blotch. The two main character states describing the position of the midlateral spot in heroine cichlids were given by ŘÍČAN *et al.* (2005). Character 53 lacks any homology, see ŘÍČAN *et al.* (2005). Character 62. Scales are counted along their natural lines,

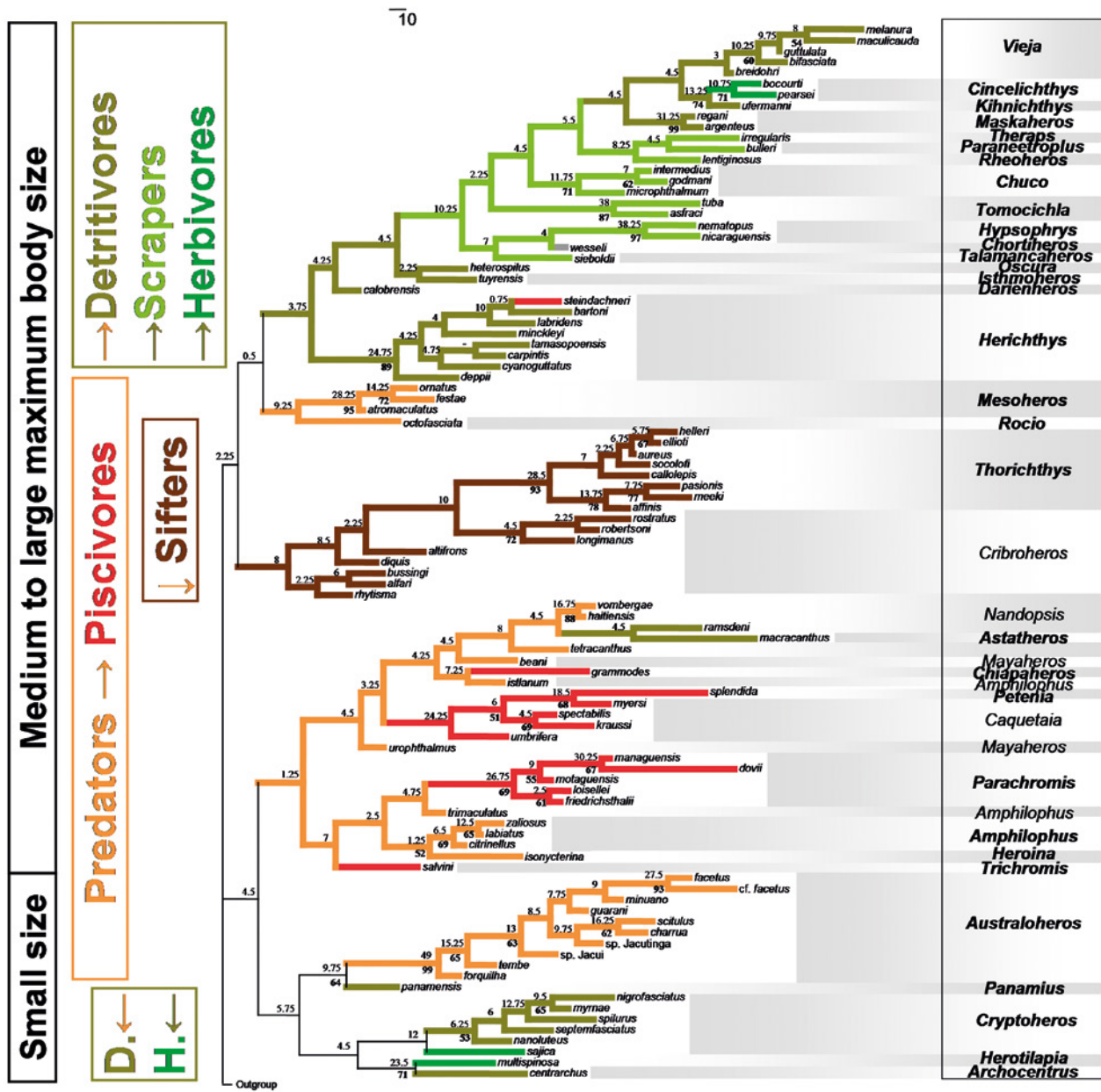


Fig. 6. Phylogeny based on morphological characters from ŘÍČAN *et al.* (2008) with proposed classification. Taxa in bold are monophyletic in the morphological analysis. Colours show morphoecological classification based on figs. 2 & 7. The morphological topology groups species mostly in agreement with the ecomorphological classification, while molecular topologies show ecomorphologies to have evolved much more repeatedly (*cf.* fig. 2).

not along some imaginary and arbitrary axes (e.g. KULLANDER, 1983, 1986). When this is realized then the upper and lower lateral lines are found in all species to be offset by vertical scale rows, and some species indeed show an overlap between the two scale rows. We have with this way of counting found a completely different distribution of character states than CHAKRABARTY (2007). Even so, many species which CHAKRABARTY codes in his system with state 0 should in our examination have clearly state 1 (e.g. *Astatheros macracanthus*). Character 67. The putative reversals to state 0 are artificial, in all Middle American cichlid species the male has a nuchal hump during breeding (the extent of it varies greatly, but males can be recognized by their head profile). Character 84. We have

not been able to find any structure coded with state 0 that would be similar to the illustration of the character in any of the species. Character 85. We cannot find any natural break in this continuum that was coded as two states.

Based on our re-examination, about half of the characters in the matrix by CHAKRABARTY (2007) are clearly not selectively neutral but are correlated with ecology (ecomorphological characteristics). The following are all ecomorphological characters: 1–9, 11–16, 22, 27–29, 31–40, 57, 60–61, 63, 66, 69–73–79, 81, 83. Many of the characters are also correlated with each other (see next point). Some of the most widely used and best-observable characters correlated with ecology are discussed in the next chapter.

Only very few characters in the matrix of CHAKRABARTY (2007) have passed the criteria for morphological characters described in the introduction and additionally do not appear to be immediately correlated with ecology (e.g. characters 10, 20, 23, 41–47).

The second morphological phylogeny of Middle American cichlids was published by ŘÍČAN *et al.* (2008). SCHMITTER-SOTO (2007b) provided a morphological phylogeny for only a small subset of Middle American cichlids.

ŘÍČAN *et al.* (2008) have similarly to other authors (e.g. CHAKRABARTY, 2007; SCHMITTER-SOTO, 2007b) analysed their morphological characters without any considerations of their possible correlation with ecology or life-history. ŘÍČAN *et al.* (2008) were aware that many characters probably are correlated with ecology, but at that time they have strived to provide a sufficiently large character matrix to provide enough resolution in the analysis of a large number of terminals. Their idea was that a sufficiently large character matrix could provide some approximation of a true phylogeny. This has however failed for the same reason as stated above for the characters of CHAKRABARTY (2007). In the present study we show that virtually all of the widely used characters in Middle American cichlid systematics are clearly correlated with ecology and life history, and that so few characters remain which are not correlated that these are insufficient to provide a resolved morphological phylogeny. We demonstrate this correlation using the morphological phylogeny of ŘÍČAN *et al.* (2008; fig. 6) which is correlated with ecomorphology to the same extent as the morphological phylogeny of CHAKRABARTY (2007) described above.

In the following chapters we provide the analysis of the ecomorphological diversity in Middle American heroine cichlids including more detailed discussions of the important characters previously used in the Middle American cichlids.

Ecomorphological diversity

3.11. Ecomorphologies of Middle American heroine cichlids

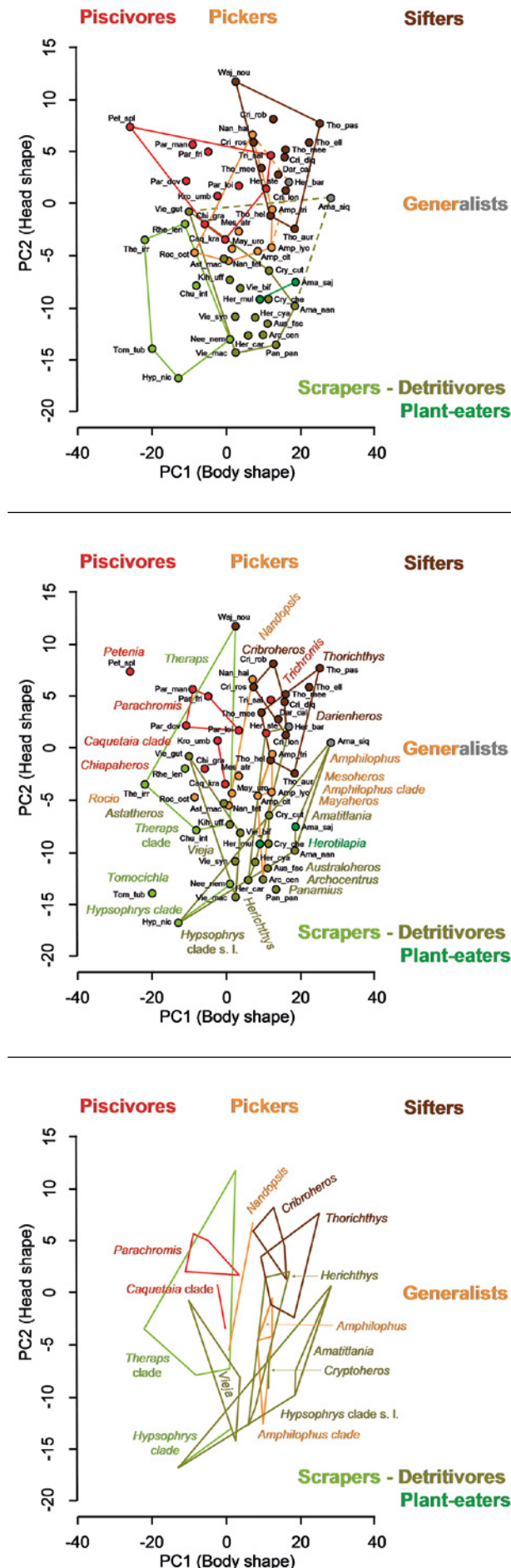
Our analysis of Middle American cichlids based on their food preferences (their predominant diet) and feeding mode (how they take in the food) resulted in their division into five main ecomorphs (fig. 2) with additional subcategories. These five major ecomorphs are the: 1) Pickers (Generalists). These feed predominantly on invertebrates and the food is taken in by active picking of individual food items. 2) Predators-Piscivores. These feed mostly on fishes, the less specialized predators on small fishes (e.g. poeciliids) and large invertebrates.

The following ecomorphs on the other hand take in substantial amounts of sediment and/or decomposing or living plant (vegetable) material which can form

a substantial to major part of their diet. When opportunity arises they of course also take in animal prey. Three main ecomorphs can be recognized here: 3) Substratum sifters. These probably look exclusively for animal prey, but for prey hidden in a soft (sand, silt) substrate of the bottom. They take in, in a shovel-like horizontal motion, mouthfuls of the substrate and actively sort the prey items from it in the buccal cavity. Most of the substrate is then expelled through the mouth and gill openings, but substantial amounts are swallowed and found in stomach contents. 4) Detritivores. This is the broadest category (together with the generalists) with several subcategories. There are the detritus-pickers, often small-sized small-mouthed species with a combined diet of small invertebrates and fine detritus particles (e.g. *Cryptoheros*, *Amatitlania*, *Archocentrus centrarchus*, *Rocio spinosissima*). Then there are the usually much larger-bodied larger-mouthed true detritivores, which also include molluscivores as a specialization. Both of the size categories of the detritivores have developed true plant-eaters, species that regularly feed on living vegetation (both higher plants and algae). Another type of detritivores is what we consider to be less specialized or alternative sifters (we call them vertical sifters). These are species conforming to the larger-bodied larger-mouthed detritivores which take in detritus by submerging their mouths and often frontal parts of heads into the substrate (e.g. *Darienheros calobrensis*, *Nandopsis ramsdeni*, *Wajpamheros nourisati*; these also include molluscivores, e.g. *Astatheros macracanthus*, *Herichthys labridens*, *Herichthys minckleyi*). 5) Scrapers (Biters). These are species that feed on periphyton, most often in fast flowing waters. They take in both animal and vegetable food particles that make up the periphyton. This ecomorph also includes species with peculiar food items (e.g. *Tomocichla tuba*) that are found in the periphyton (nematodes) as well as those not found naturally as part of the periphyton such as fruits, a feeding ecology otherwise unknown in Middle American cichlid fishes.

The ecomorphs that include in part or in full vegetable food in their diets (sifters, detritivores, scrapers-biters) are among the heroine clade of Neotropical cichlids exclusively found in Middle America. All South American genera related to the Middle American cichlids are either generalists or predators.

These same five major ecomorphs are also independently recovered in the principal component analysis (PCA) of LÓPEZ-FERNÁNDEZ *et al.* (2013; fig. 7) that is however based solely on shape characters of the head and body. Our empirical classification of the fishes based on their food preferences and feeding mode is thus independent but at the same time in very good agreement with the analysis of head and body shapes. Below we provide yet another analysis that studies the evolution of the morphological characters that make-up the shape patterns. In the previous chapter we have already found most of the morphological characters to be correlated with ecology, demonstrated also by the fact that the five main ecomorphs show very good correspondence to clades in the



morphological phylogenies of CHAKRABARTY (2007) and ŘÍČAN *et al.* (2008; fig. 6; see above). In the following chapters the most important morphological characters are studied in detail.

The five major ecomorphs among Middle American heroine cichlids derived from these independent analyses are the generalized pickers, the predators-piscivores, the substratum sifters, the scrapers and the detritivores (figs. 2, 6–8). The predominantly carnivorous pickers, predators-piscivores and the substratum sifters have rather long to very long heads and jaws (the lower jaw is longer than the upper) and differ mainly in body shape (figs. 7, 8, 9A, B). Similarly the predominantly herbivorous scrapers and detritivores with short heads and jaws (the lower jaw is shorter than the upper) also differ mainly in body shape. The predominantly rheophilic (figs. 7–8) scrapers have generally longer and lower bodies than the short and deep-bodied detritivores (figs. 7, 8, 9C, D).

Figure 8 shows examples of the respective ecomorphologies. We examine the correlations of individual morphological character with the five main ecomorphs in the following chapters.

Evolution of morphology

3.12. Reconstructing the ancestor of Middle American cichlids

By mapping individual morphological characters from the morphological matrix of ŘÍČAN *et al.* (2008; with some modifications; see Methods) onto the ddRAD phylogeny (fig. 5) we have reconstructed the morphology and also life-history of the ancestor of the Middle American cichlid clade (including also the Antillean *Nandopsis* and the South American *Australoheros*, *Caquetaia*, *Heroina*, *Kronoheros*, *Mesoheros* and *Chocoheros*). This ancestral morphology is used to study the evolution of morphological characters and their correlation with the five major ecomorphs.

Fig. 7. Principal component analysis of head and body shape. Analysis is redrawn from fig. 3 in LÓPEZ-FERNÁNDEZ *et al.* (2013). A. Separation into five main ecomorphs. Head shape separates Piscivores, Predators and Sifters from Scrapers, Detritivores-Herbivores. Body shape separates Piscivores from Sifters with Predators being intermediate, and it also separates Scrapers from Detritivores-Herbivores (Scrapers are more elongated and streamlined compared to Detritivores-Herbivores). B. Morphospace variability within clades and genera. C. As in B without names and dots and with monotypic genera omitted from the figure. Variability within clades and genera is predominantly along the cranial axis (except in the *Hypsophrys* clade *sensu lato*, i.e. including also *Cryptoheros* and *Amatitlania*) with much more homogeneous body stressing the head as the main source of variability within genera and hence adaptation.

The distribution of the reconstructed ancestral morphology within the phylogeny is quite extensive (reaching within the herichthyines always to the node connecting *Mesoheros*, often to the node connecting *Chiapaheros*, in some characters even beyond; in the amphiloophines at least to the node where the two subclades meet but often all the way to *Mayaheros* and *Amphilophus*; and deepest within the astatheroines where including all suprageneric nodes and in the postcranial reaching within *Cribroheros*, signifying that most morphological changes have happened only with the evolution of the extant genera or even within genera, and several genera (*Australoheros*, *Cribroheros*, *Amphilophus*, *Mayaheros*, *Mesoheros*, *Nandopsis*) actually have either the cranial (figs. 9–13, 20–21) or postcranial morphology (figs. 14–19, 20–21) or life history traits (figs. 22–29) of the ancestor. This signifies delayed morphological transformation that has only happened after the colonization of Middle America while the colonization appears to have been carried out by a morphologically ancestral and uniform morphotype, in two separate colonization waves (the amphiloophines plus the herichthyines, and the astatheroines).

The reconstructed ancestor (figs. 9–29) was cranially of the generalized picker ecomorphology (figs. 9–13), and postcranially also of a generalized ancestral morphology characterized (figs. 14–19, 20–21) by: 29 vertebrae (13 abdominal and 16 caudal), 2.5–3 caudal peduncle vertebrae, one anal pterygiophore anteriorly from the first haemal spine, 6 anal fin spines, 8–9 anal fin rays, 16 dorsal fin spines, 11 dorsal fin rays, 30 scales along lateral line, 20 to 21 scales in the upper lateral line, 16 transversal scale rows, 5 scale rows between anterior margin of dorsal fin and upper lateral line, 8 scale rows between anterior margin of anal fin and lower lateral line, 5 series of scales on the cheek, 7 to 8 ceratobranchial gill rakers on the first arch, 14 pectoral fin rays.

In cranial characters the reconstructed ancestor is most similar to *Australoheros*, *Mayaheros*, *Amphilophus*, *Nandopsis* or *Mesoheros* (fig. 9). In postcranial characters the reconstructed ancestor is most similar to *Cribroheros*, *Darienheros* and *Mesoheros* (figs. 14–15), and with very few changes also to *Nandopsis*, *Caquetaia*, *Kronoheros* and *Mayaheros* (all depart from the ancestral morphology only in having a marginally shortened spinous part of the dorsal fin), and *Australoheros* (ancestrally minor shortening of the caudal peduncle). These genera are thus the most morphologically plesiomorphic. It is interesting to notice that the most plesiomorphic genera include all the South American genera of the Middle American clade of heroine cichlids (*Australoheros*, *Caquetaia* and *Kronoheros* except for cranial characters and *Heroina* except for unpaired fins, *Darienheros*, *Mesoheros*), the only Antillean genus (*Nandopsis*), but only three of the Middle American genera (*Amphilophus*, *Cribroheros*, *Mayaheros*). Morphological change has clearly been proportionally larger after colonization of Middle America than in the South American – Antillean ancestral area.

The ancestral distribution of the life-history traits reconstructs an equally widely topologically distributed

ancestor. In the life history traits the common ancestor had the longitudinal-stripe type (the L-type) of coloration pattern development (except for the astatheroines clade where the interrupted line I-type was ancestral; fig. 23), was characterized by dominant vertical bars in normal and breeding coloration (figs. 25–26), lived on soft substrates or sandy bottoms (i.e. in rather calm waters; fig. 26), had large numbers of eggs (above 500) that were of a medium to small size (1.7 mm; figs. 27–28), reached a maximum body size of around 200–250 mm SL (fig. 28), and had a moderate to strong separation of the parental role during reproduction and parental care (fig. 29). Several life history traits (unlike the morphological characters where the ancestor is uniform) show that the astatheroine ancestor was possibly a slightly different type of fish from the ancestor of the amphiloophines and herichthyines (e.g. coloration pattern development, maximum adult body size, parental role division) in agreement with its more isolated phylogenetic position and possibly a separate colonization event.

3.13. Evolution of ecomorphologies

Both morphological (fig. 6) and molecular (fig. 9) phylogenies reconstruct the generalized pickers as the ancestral ecomorph.

Specialized piscivory has evolved independently several times, once in South America (in the *Caquetaia-Heroina-Kronoheros* ancestor), and four times at the genus level in Middle America, twice among the amphiloophines (*Petenia*, *Parachromis*) and twice among the herichthyines (*Trichromis*, *Chiapaheros*) plus once within the genus *Herichthys* (*H. steindachneri*) (figs. 2, 7, 9). Interestingly specialized piscivory was once lost (in *Heroina isonycterina*). Even more interestingly the herichthyine piscivores have only reached a lower grade of specialization because they did not develop the lateroethmoid-premaxillary ligament (associated with advanced predators and specialized piscivores; see below). They also did not develop the very specialized condition with the very delicate lateroethmoid-premaxillary ligament (this is only found in specialized piscivores among the amphiloophines plus *Caquetaia* and *Kronoheros*). In all but one case piscivores have evolved through the series from pickers to more generalized predators to piscivores. The interesting exception is that of *Herichthys steindachneri*, where a piscivore has evolved from a sympatric detritivore/molluscivore (*H. pame*) (fig. 9).

Substratum sifting has evolved five times independently in Middle American cichlids (*Thorichthys*, *Cribroheros*, *Darienheros*, *Chocoheros*, *Wajpamheros*; figs. 2, 6, 9) with two instances of well adapted horizontal sifters (several species within *Thorichthys* and *Cribroheros*; see below). The substratum sifters have in three cases probably evolved from generalized pickers (*Thorichthys*, *Cribroheros*, *Chocoheros*), in one case (*Darienheros*) from detritivores and in one case from periphyton scrapers (*Wajpamheros nourissati*) (fig. 9).

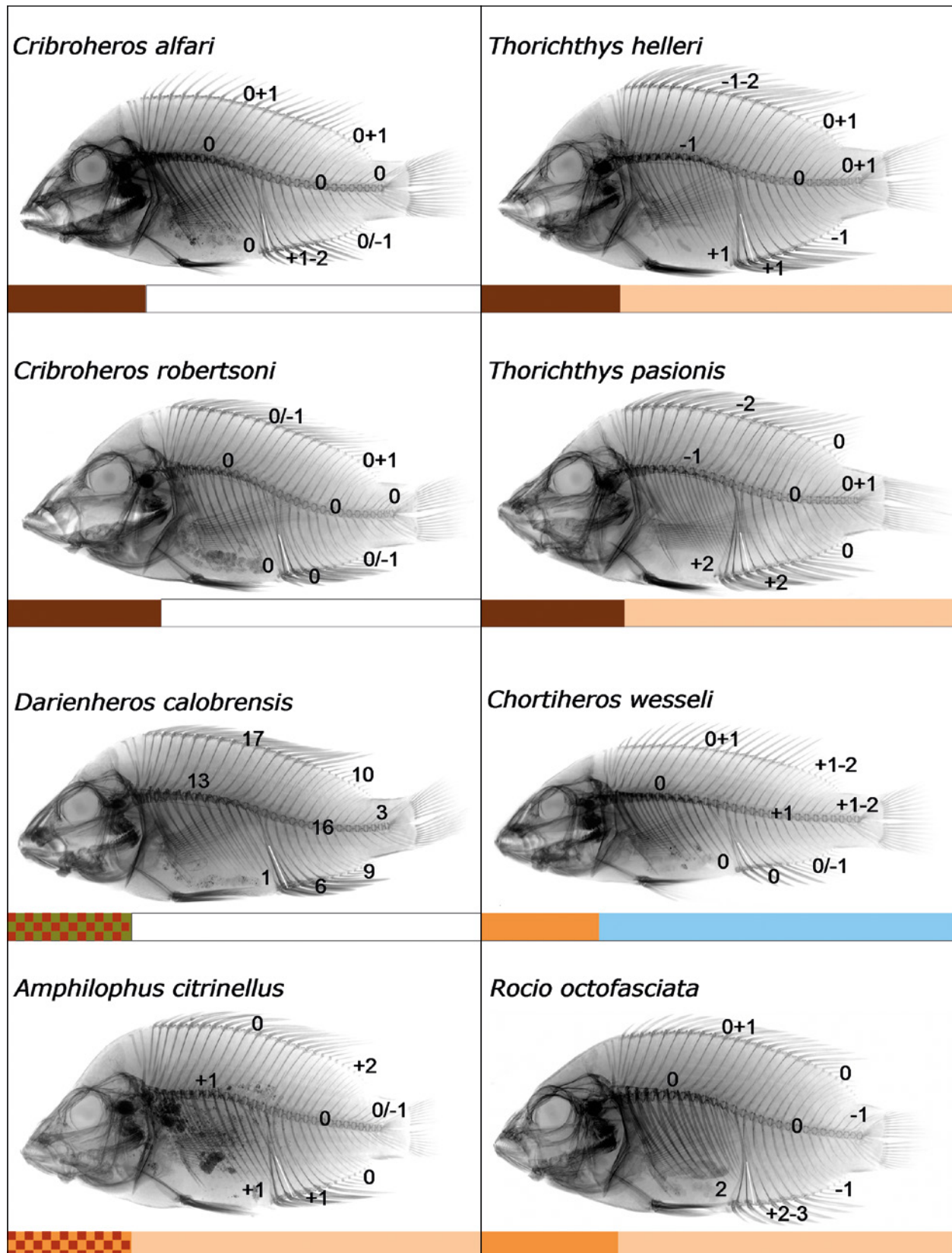


Fig. 8a.

Fig. 8 (pp. 52–55). X-ray photographs of representative species showing diversity of ecomorphologies in the Middle American cichlid clade. Numbers on fish bodies show change in meristic characters from the reconstructed ancestor of the Middle American cichlid clade. The colours of the bars below the head and below the body show cranial (figs. 9–13) and postcranial (figs. 14–19) ecomorphs, respectively (the ecomorph combinations are as in figs. 20–21). *Darienheros calobrensis* is shown with direct values of meristic characters as one of the genera closest to the common ancestor, other species show absolute change in meristic values compared to the reconstructed common ancestor.

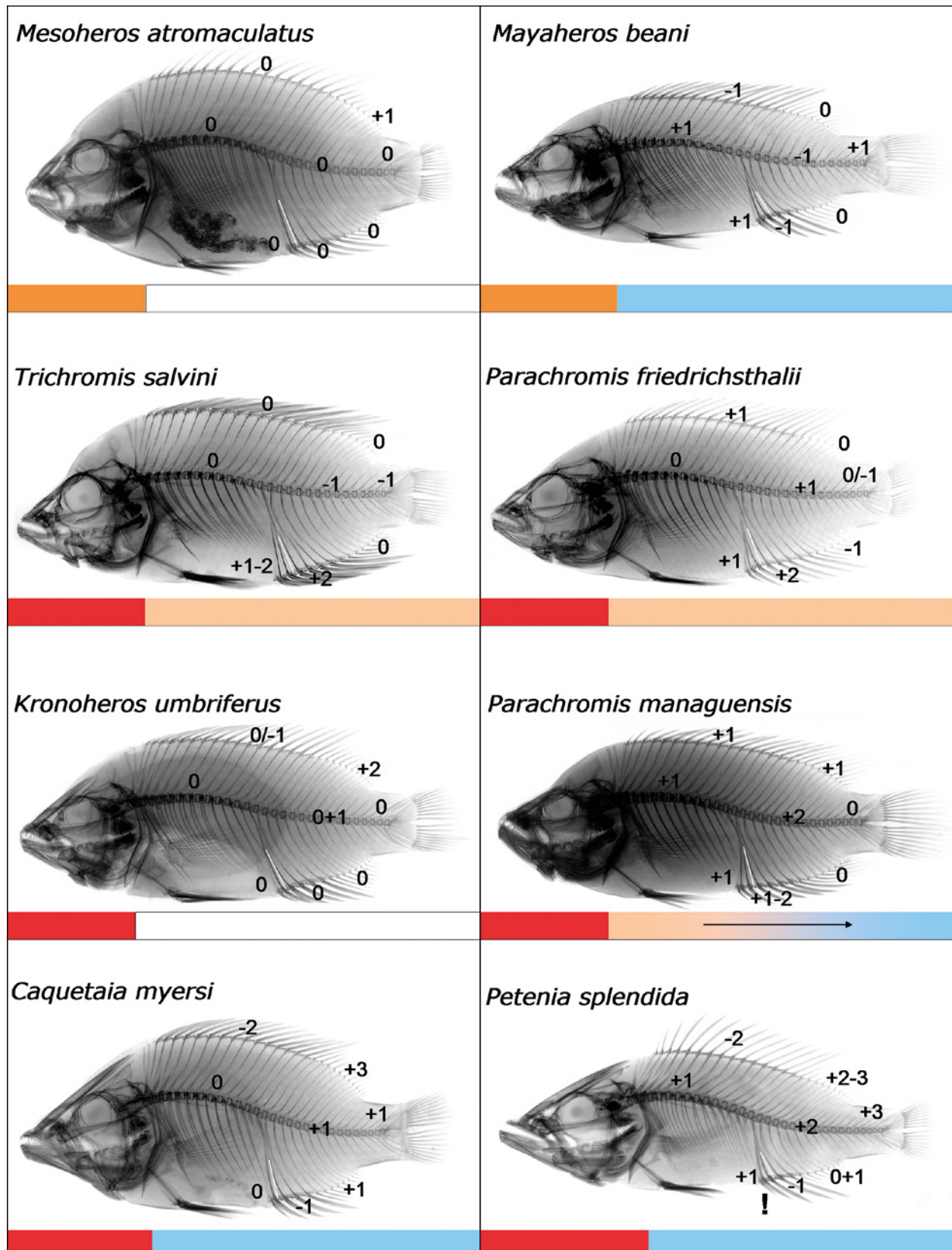


Fig. 8b.

Herbivory (either strict or in the form of lentic detritivores and rheophilic periphyton scrapers) shows the highest number of independent cases of evolution, often quite complex and with intertwined ecomorphologies (especially between lentic detritivores and rheophilic

scrapers). There are three independent cases of virtually strict herbivory (either algae as in *Herotilapia* and *Neetroplus*, or macrovegetation in *Cinzelichthys*), seven cases of independent evolution of rheophilic periphyton scrapers (*Tomocichla*, *Neetroplus*, *Talamancaheros*,

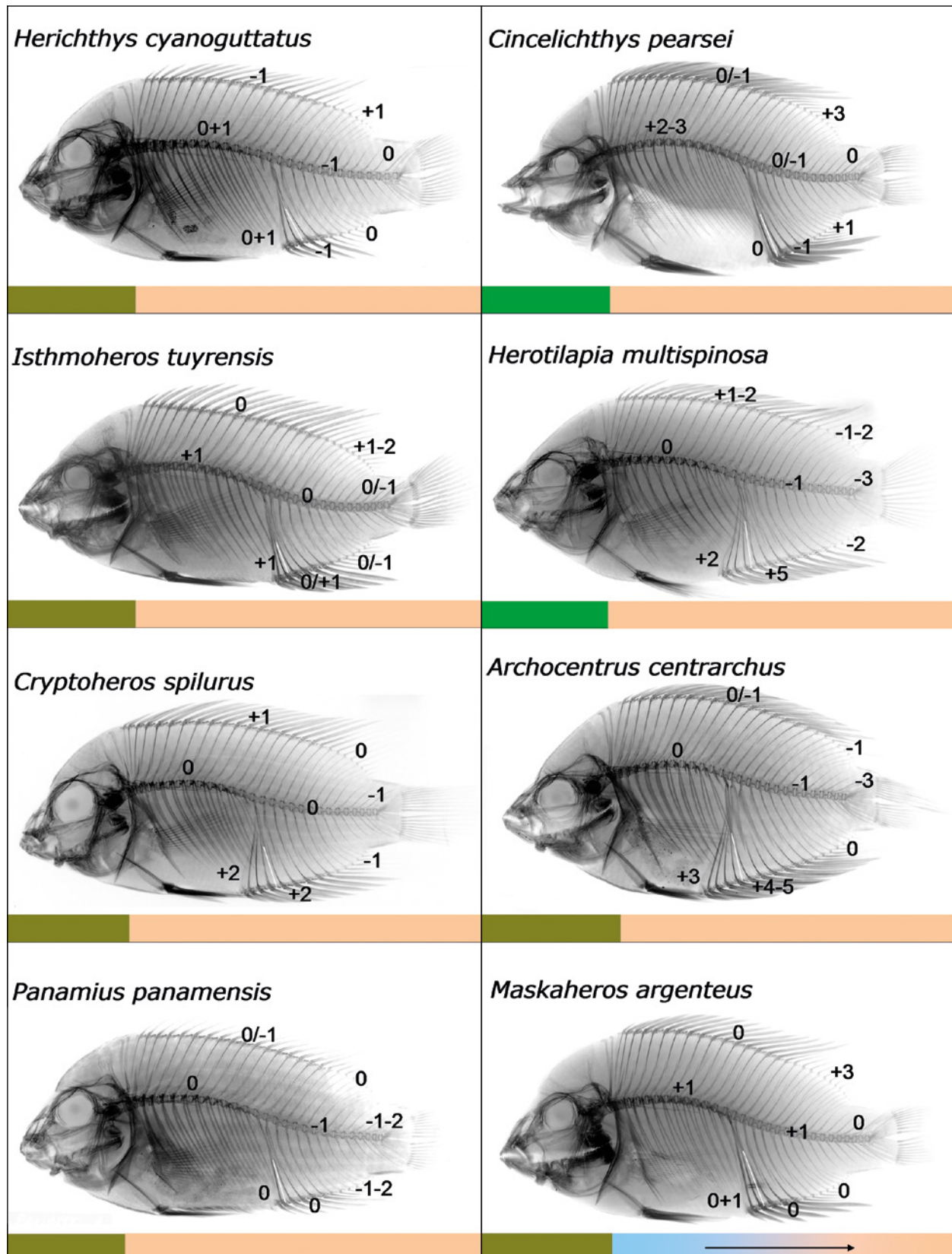


Fig. 8c.

Theraps, *Chuco*, *Rheoheros*, *Paraneetroplus*), nine cases of independent evolution of lentic detritivores (*Amatitlania*, *Archocentrus*, *Cryptoheros*, *Herotilapia*, *Cincelichthys*, *Maskaheros*, *Vieja*, *Isthmoheros*, *Panamius*), two cases of independent evolution of lotic detritivores (*Hyp-*

sophrys, *Herichthys*) and one case of evolution of a post-cranially ancestral detritivore (*Astatheros*).

The evolutionary trajectories among the herbivores are the most complex among the Middle American cichlids. Herbivory in general has in Middle American cich-

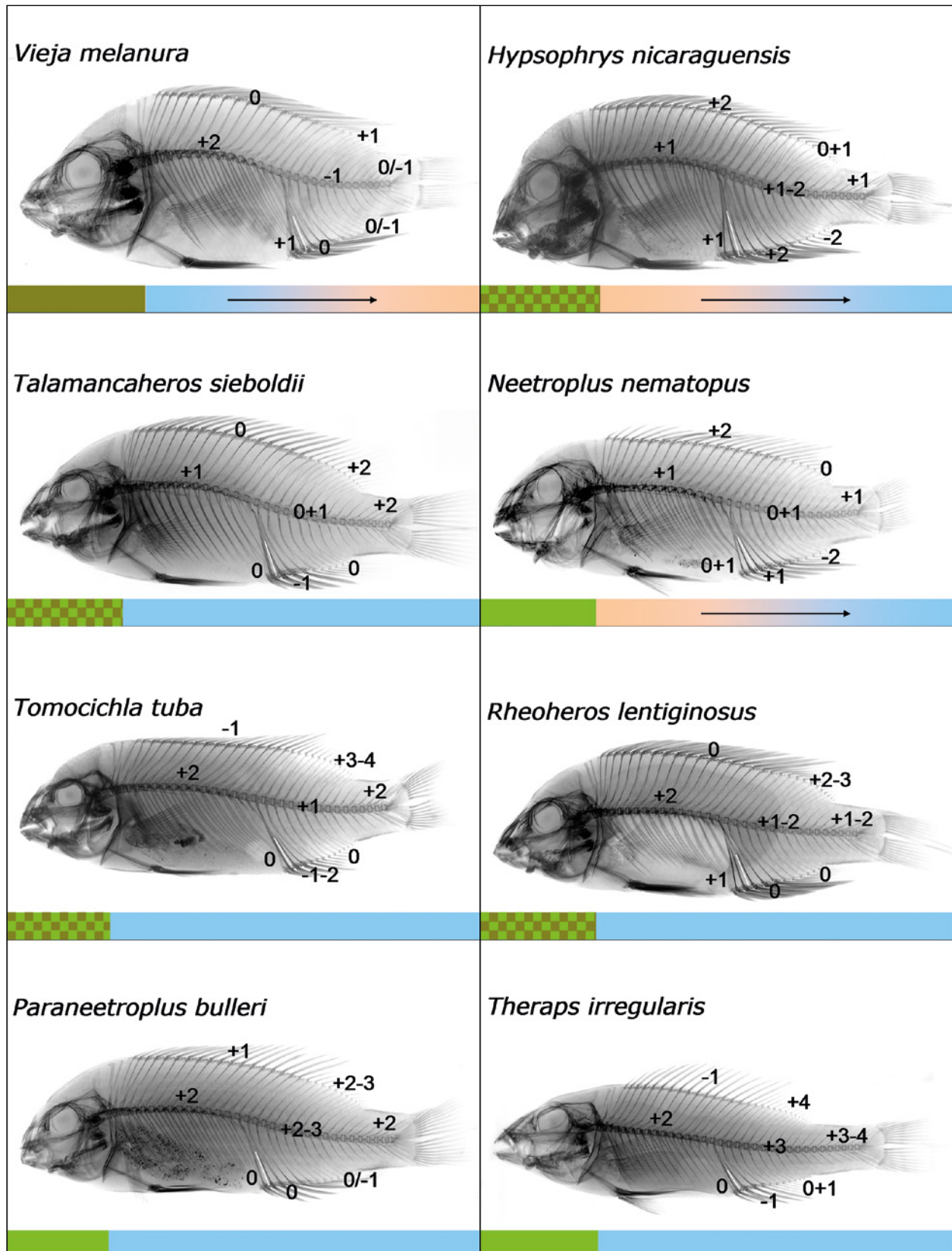


Fig. 8d.

lids evolved from the ancestral generalized pickers (fig. 9). The two main types of herbivorous ecomorphs (the lentic detritivores and the rheophilic scrapers) appear to have also evolved one from the other and vice versa. The *Theraps* clade appears to have been ancestrally a rheo-

philic scraper with macrovegetation herbivory evolving from this ecomorph (*Cincolichthys*, *Kihnichthys*), while the *Paraneetroplus* clade, *Isthmoheros*, *Hypsophrys*-*Neetroplus*, and *Tomocichla* appear to have evolved from lentic detritivores (fig. 9). In *Herichthys* the more herbiv-

orous species have also evolved from detritivores. The lotic scraper *Rheoheros* is possibly the sister-group of the lentic detritivorous *Oscura*.

3.14. Repeated evolution of ecomorphologies

Each of the three main clades of the Middle American cichlids (the amphiphilines, the herichthyines, the astatheroines) has evolved a large ecomorphological spectrum (figs. 2, 9–21). Repeated evolution of the morphoecologies has thus occurred within all three clades and additionally even within some genera (e.g. *Herichthys*, *Nandopsis*). The morphoecological non-homogeneity of monophyletic clades is well evident when mapped onto the phylogeny (figs. 2 & 9) and even better when viewed in the multivariate PCA analysis (fig. 7). Most genera (that are in the PCA analysis represented by multiple species) and clades are stretched along the head shape axis but cover a relatively narrow band along the body shape axis. This demonstrates that the head shape and hence feeding habits are much more variable within clades than is body shape.

3.15. Evolution of cranial characters

Virtually all cranial morphological characters show concerted evolution and are directly associated with feeding and hence the respective ecomorph and are thus not independent phylogenetically informative morphological characters (see above). Below we analyse in detail the most important characters used in Middle American cichlid classification which are however at the same time the best correlates of ecomorphology.

The main ecomorphs are well identifiable by dental characteristics (figs. 9–10). Based on our examination of all heroine genera and virtually all species in Middle America the oral teeth can be divided into nine types (that cover all the separate tooth characters in CHAKRABARTY, 2007 and in ŘÍČAN *et al.*, 2008).

All ecomorphs except the horizontal substratum sifters have jaw teeth that increase in size towards the symphysis. The sifters have jaw teeth that are subequal in length throughout the jaws and the teeth are slender and peg-like (more so in *Cribroheros* than in *Thorichthys*).

Generalists-predators-piscivores have large robust pointed teeth, in the piscivores developed into distinct enlarged symphyseal canines in the upper jaw that correspond to a smaller symphyseal pair in the lower jaw. A very specialized piscivorous dentition is found in *Petenia splendida* which has secondarily very small and numerous teeth more similar to the condition in the sifters (*Caquetaia* and *Kronoheros* have the normal piscivorous dentition with teeth slightly decreased in size in the lower jaw).

All partially or predominantly herbivorous species have teeth that are labiolingually flattened to some de-

gree. The lowest flattening is in detritivores, where only the tip of the teeth is flattened and which is the only ecomorph that retains sharp tips of the teeth. Similar teeth but with a blunt tip are characteristic of (algae) scrapers and also of plant-eaters that eat leaves or fruit (as does *Tomocichla tuba*). Very specialized scrapers have whole teeth flattened (best developed in *Neotroplus nematopus*). *Herotilapia multispinosa* has a unique type of wholly flattened teeth with three terminal cusps that it used for biting-off filamentous algae.

A type teeth (fig. 10A) are the small autapomorphic specialized teeth in both upper and lower jaw of the piscivorous *Petenia splendida*. *Caquetaia* and *Kronoheros* have slightly decreased teeth only in the lower jaw.

B type teeth (fig. 10B) are found only in piscivores and the most advanced predators (*Parachromis*, *Caquetaia*, *Kronoheros*, *Chiapaheros*, *Amphilophus trimaculatus*, *Mayaheros urophthalmus* and *Trichromis*). They are characterized as robust, pointed, conical, widely spaced large teeth with a distinctly enlarged anteriormost canine in the upper jaw and smaller anteriormost canine followed by the biggest canine in the lower jaw. *Caquetaia* and *Kronoheros* have this type of teeth only in the upper jaw.

C type teeth (fig. 10C) are found in all generalized predators and undifferentiated omnivores (*Amphilophus*, *Archocentrus*, *Mesoheros*, *Astatheros* [often with high amount of wear in this species; see fig. 10, type C*], *Australoheros*, *Mayaheros beani*, *Amphilophus istlanus*, *Rocio*, *Heroína*, *Nandopsis tetracanthus*, *N. haitiensis*, *Chortiheros* – here the symphyseal teeth are in some specimens peg-like, and *Darienheros*) and as such are a rather broad category. They are characterized as robust, pointed, conical teeth that gradually increase in size towards the symphysis, without the adaptations of the B type teeth.

D type teeth (fig. 10D) are only found in the specialized sifters (in *Thorichthys* only in *T. affinis*, *T. meeki*, and *T. pasionis*, while remaining species have C type teeth; in *Cribroheros* only in *C. rostratus*, *C. robertsoni*, and *C. longimanus*, while remaining species of *Cribroheros* have C type teeth). The same taxonomic distribution does have the shape of the palatine and lacrymal bones (see below). The teeth are characterized as slender, conical, pointed teeth that do not or only very slightly increase in size towards symphysis. The remaining species of *Thorichthys* and *Cribroheros* have normal omnivore-predator type C teeth as do *Darienheros* and *Chocoheros* which are also less specialized substratum sifters (figs. 2, 6, 9).

E type teeth (fig. 10E) are only found in detritivores and omnivorous generalists. They are characterized as robust, pointed conical but widened laterally and at the tip labiolingually flattened teeth. These are together with type C the most common tooth type, present in the majority of detritivores-omnivores among herichthyines including all genera in the *Paraneotroplus* clade (*Paraneotroplus*, *Maskaheros*, *Vieja*, *Rheoheros*, *Oscura*), most genera in the *Theraps* clade (*Theraps*, *Chuco*), *Tomocichla asfraci* (*Tomocichla tuba* has in some specimens E and in some F type teeth), *Isthmoheros*, *Talamancaheros*, *Nandopsis*

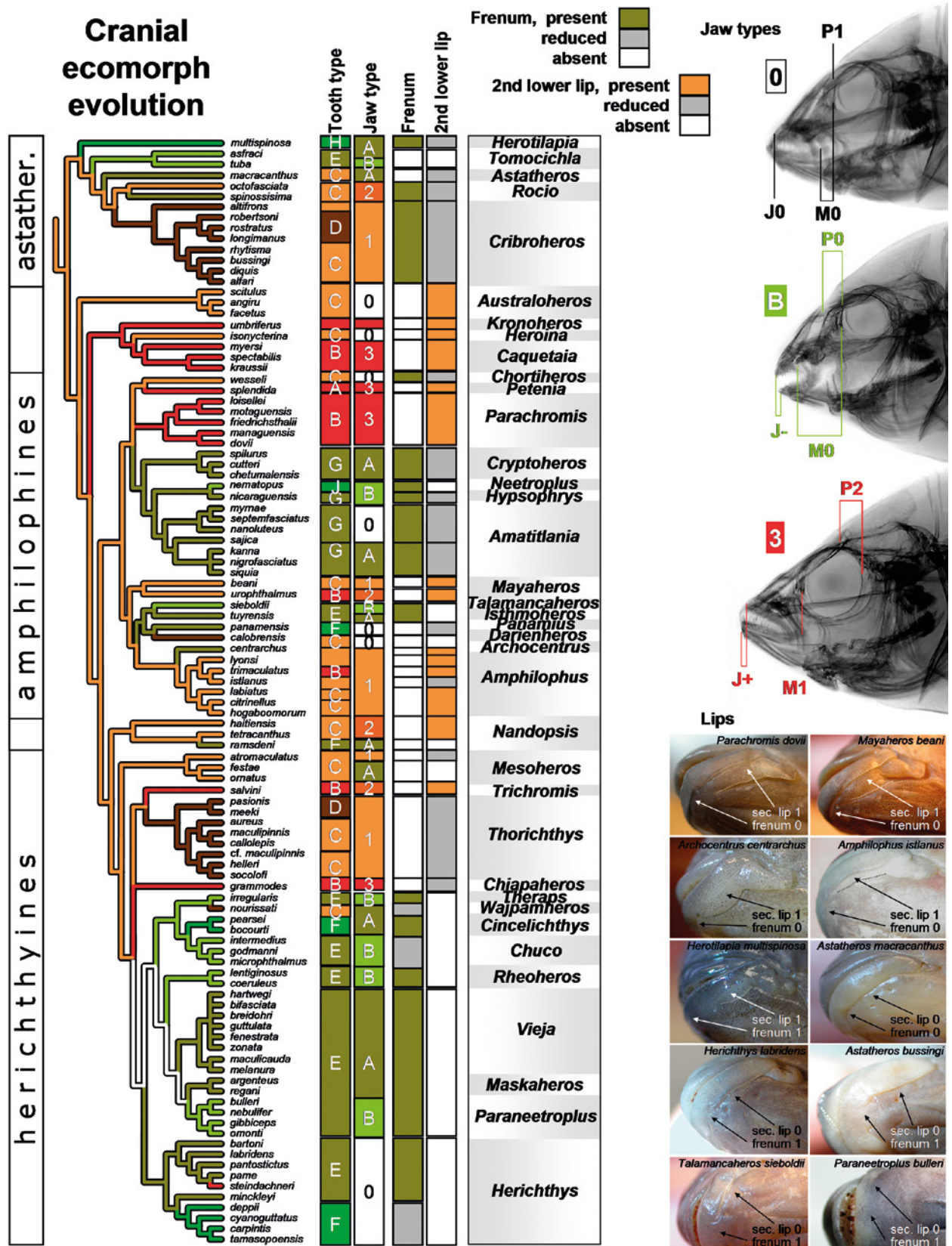


Fig. 9. ddRAD topology with cranial ecomorphs mapped on the tree topology and four mapped cranial characters (tooth type, jaw type, frenum on lower lip, 2nd lower lip). The jaw type is a character complex of correlated characters (upper/lower jaw length, maxillary and premaxillary length compared to the eye). Jaw type, frenum and 2nd lower lip shown on the figures to the right, tooth types are shown in fig. 10. The x-radiographs show the head morphology of the following species (top to bottom): *Chortiheros wesseli*, *Talamancaheros sieboldii*, *Parachromis managuensis*.

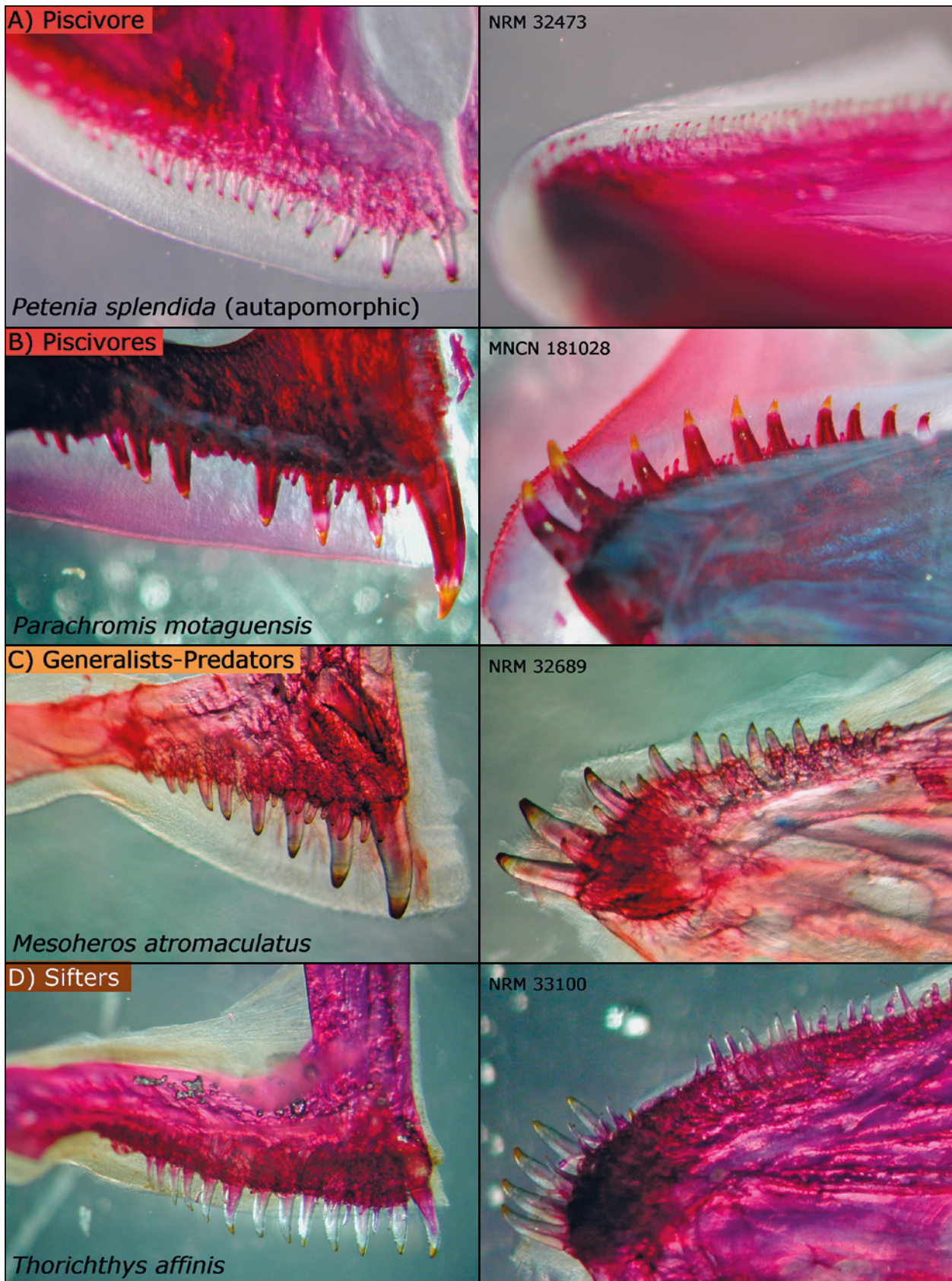


Fig. 10a.

Fig. 10 (pp. 58–60). Tooth type diversity and classification into types A–J in the Middle American cichlid clade. Fig. 10 (I) shows the development of the 2nd cusp on oral teeth as a result of the flattening of the tip of the tooth (teeth shown in lateral view, labial side on the left, lingual side on the right).

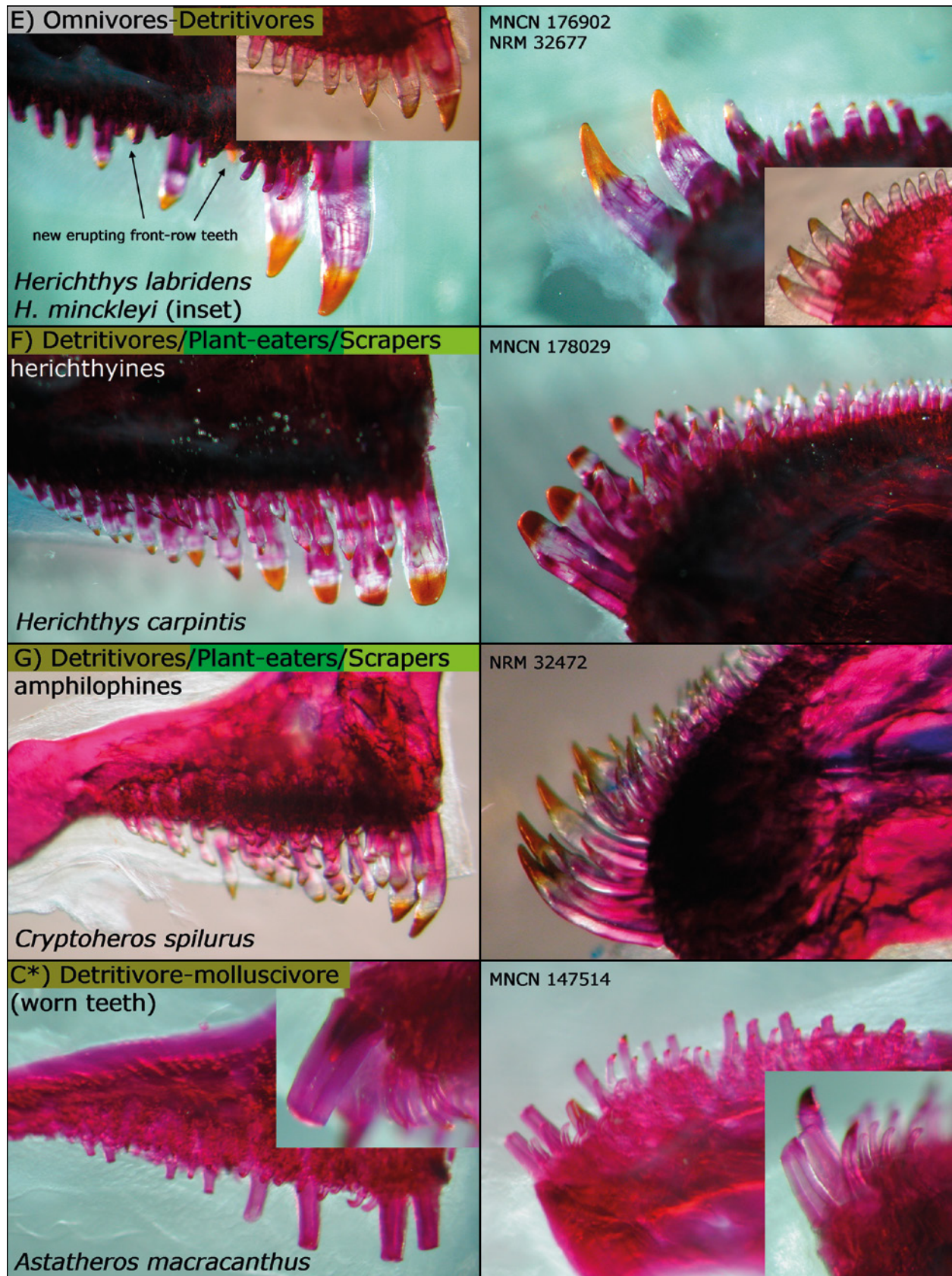


Fig. 10b.

ramsdeni, all species in the *Herichthys labridens* group plus *Herichthys minckleyi*. This teeth morphology was proposed as diagnostic for the proposed genus *Nosferatu*

DE LA MAZA-BENIGNOS *et al.* (2015), but our examinations show that it is plesiomorphic within the broader context of *Herichthys sensu lato* and very common out-

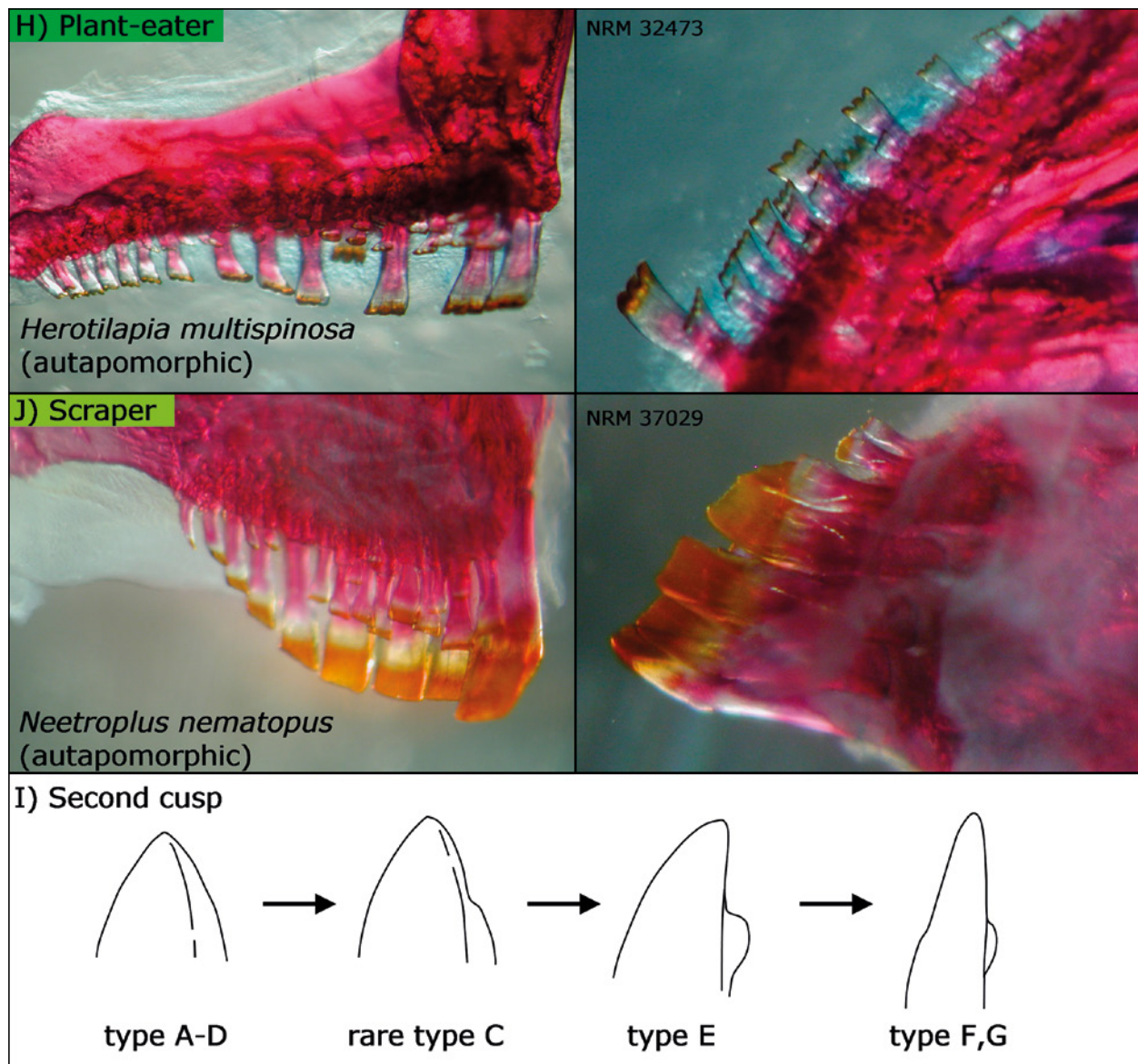


Fig. 10c.

side of *Herichthys sensu lato* and thus cannot be used for diagnosis of a single genus. *Wajpamheros* has somewhat worn type E teeth with rounded tips (similar but less extremely worn as in the C* type teeth of *Astatheros*) suggesting a similar food-gathering/processing mechanism by oral jaws as in *Astatheros macracanthus*, and not as in true sifters (type D teeth). The damage to the frontal jaw teeth in both species based on our field observations of food gathering suggests taking-in of coarse (sand) material together with the buried food items (mainly molluscs in *Astatheros*).

F type teeth (fig. 10F) are found only in detritivores, scrapers, and some herbivores. They are directly derived from type E and are characterized as robust, laterally widened and labiolingually flattened truncated (tips are not pointed) teeth. Juveniles of species with this type of teeth have the E type teeth. They are present in specialized detritivores and herbivores/scrapers among the crown-group herichthyines (*Herichthys cyanoguttatus*

group, *Cincelichthys*, *Kihnichthys*). Scrapers in the genera *Theraps* and *Paraneetroplus* have very worn and blunt symphyseal teeth of the E type almost approaching the F type condition for symphyseal teeth. F type teeth are also found in the amphilophine *Panamius*.

G type teeth (fig. 10G) are also found in detritivores, scrapers, and some herbivores, but they differ from the E type teeth by being delicate. They are pointed as E type teeth, conical but at the tip flattened. Unlike the E type teeth the G type teeth are relatively large teeth also in 2nd and 3rd rows. The G type teeth are only found in the small-sized amphilophine detritivores (*Cryptoheros*, *Amatitlania*) and from these derived detritivores/molluscivores (*Hypsophrys*). The delicate nature of the G type teeth compared to the ecologically equivalent and morphologically similar E type teeth is most probably due to the much smaller body size of the amphilophine detritivores compared to the generally large herichthyine detritivores.

H type teeth (fig. 10H) are the autapomorphic tricuspid teeth of the herbivorous filamentous-algae eating *Herotilapia*.

J type teeth (fig. 10J) are the autapomorphic spatulate truncated teeth of the algae-scraping *Neetroplus*.

The secondary cusp on the anterior jaw teeth of heroine cichlids (fig. 10I) has been used to diagnose the group (CASCIOTTA & ARRATIA, 1993a,b) or its subgroup (KULLANDER 1996; CHAKRABARTY 2007). The second cusp has however also been reported from geophagines (KULLANDER 1986, 1996). Our examination of virtually all heroine species and their teeth makes it clear that the secondary cusp is a direct consequence of the labiolingual flattening of the tip of the teeth as demonstrated in the drawing (lateral aspect of teeth, left to right labiolingual orientation). The lingual side of all tooth types (except types H and J; see fig. 10I) has a ridge that continues to the tip of the tooth in conical teeth without a flattened tip. When the tip is flattened to various degrees the ridge terminates subapically with a resulting secondary cusp whose size depends on the degree of tip-flattening. Type E, F and G teeth have the ridge excavated also from the direction of the tooth root resulting in the clearest secondary cusps (especially in the large robust E type teeth). On the other hand species with completely labiolingually flattened whole teeth (e.g. types H and J) lack the secondary cusp altogether (secondarily most often). The secondary cusp is thus not a separate character, it is a direct reflection of the apical shape of the teeth.

Oral jaws. The oral jaws are the quintessential expression of ecomorphology. They determine the size and position of the mouth and also the whole head shape. The shapes and relative sizes of the individual bones making up the oral jaws are intertwined and cannot be separated into individual morphological characters. All variation in the oral jaws among the Middle American and related heroine cichlids makes up only five (six) combinations. Based on proportions, lengths and shapes (Fig. 9) we termed these combinations 0 (ancestral; fig. 9), 1 (pickers, generalized predators), 2 and 3 (specialized predators and piscivores; 2 and 3 only differ in the amount of elongation of the premaxillary process), A (detritivores; mouth terminal due to upper and lower jaw being of the same length in front), and B (scrapers – biters with a sub-terminal mouth and a shorter lower jaw; fig. 9).

Lips. Lip morphology is also very well correlated with the ecomorphs (fig. 9). The second lower lip (character 71 in CHAKRABARTY, 2007; character 32 in ŘÍČAN *et al.*, 2008) is not an expansion of the ventral fold of lower lip (*contra* CHAKRABARTY, 2007). It is a separate, soft, wrinkled structure that follows the posteroventral border of the lower jaw. It is well correlated with ecology, being best developed only in predators-piscivores possibly as a result of larger jaw protractability. There is a loss of the structure in detritivores and herbivores, with generalized pickers being intermediate in having small second lower lips. The illustration in fig. 11 in ŘÍČAN &

KULLANDER (2008) demonstrates correlation of the second lower lip with ecomorphs within the genus *Australoheros*, where the two semi-detritivore species (*A. tembe* and *A. forquilha*; and also in *A. ykeregua* then considered as *A. forquilha*) lack the structure while generalized pickers (*A. facetus*, *A. minuano*, *A. guarani*) have it best developed (there is no predator-piscivore in the genus *Australoheros*). Similar correlation with ecomorphs is found also within Middle American cichlids. The best development of the second lower lip is found in specialized predators-piscivores (*Caquetaia*, *Kronoheros*, also *Heroina*, *Petenia*, *Parachromis*, *Mayaheros urophthalmus*, *Amphilophus trimaculatus*, *Trichromis*) with only one exception (*Chiapaheros*) where the structure is only slightly developed (note that as described above, herichthyine piscivores also in other characters, including the palatine ligaments, have not reached the degree of adaptation seen in amphilophine piscivores or in *Caquetaia* and *Kronoheros*). All generalized pickers have the structure also well developed (*Australoheros* without detritivore species, *Nandopsis*, *Mayaheros beani*, *Amphilophus*); with the exceptions only of *Amphilophus istlanus*, *Rocio octofasciata* and *Mesoheros*, in which it is slightly developed (in *M. atromaculatus*). *Mesoheros festae* and *M. ornatus* lack the structure and the lack of it correlates in this genus with the proportional length of jaws (*M. atromaculatus* has a longer lower jaw while the other two species have it shorter). It is absent from all detritivores-scrapers-herbivores among the herichthyines and the astatheroines (the *Paraneetroplus* clade, the *Theraps* clade, *Herichthys* – in some species slightly developed, *Tomocichla*, *Astatheros*). *Herotilapia* shows a moderate development. Amphilophine detritus-pickers (less specialized detritivores than the numerous herichthyine species), detritivores and herbivores have on the other hand a slightly developed second lip (all *Cryptoheros*, *Amatitlania*, *Hypsophrys*) but it is absent from *Neetroplus* and *Isthmoheros*. The second lower lip thus among detritivores-herbivores has the same distribution as do E and G type teeth. This is strong evidence for functional-ecological connection. The second lower lip is only slightly developed in sifters (*Cribroheros*, *Thorichthys*) regardless of their tooth type. It is so small that it is hidden from lateral view being covered by the overhanging lower lip. In the sifter-detritivore *Wajpamheros* the second lower lip is also slightly developed, and is hidden from lateral view below the hypertrophied lower lip. It is completely absent in *Darienheros* with a similar mouth and lip structure.

Thick lips. Thick lips are among the Middle American riverine cichlids (and the related South American *Australoheros*) associated with a shorter lower jaw (as in detritivores but unlike horizontal sifters where lower jaw is longer) and a relatively large preorbital distance (as in sifters). This character combination is found in *Darienheros*, *Chocoheros*, *Wajpamheros*, *Astatheros*, *Nandopsis ramsdeni*, *Cribroheros altifrons*, riverine *C. rostratus*, *Australoheros tembe*, *A. ykeregua*, *A. forquilha*, *Herich-*

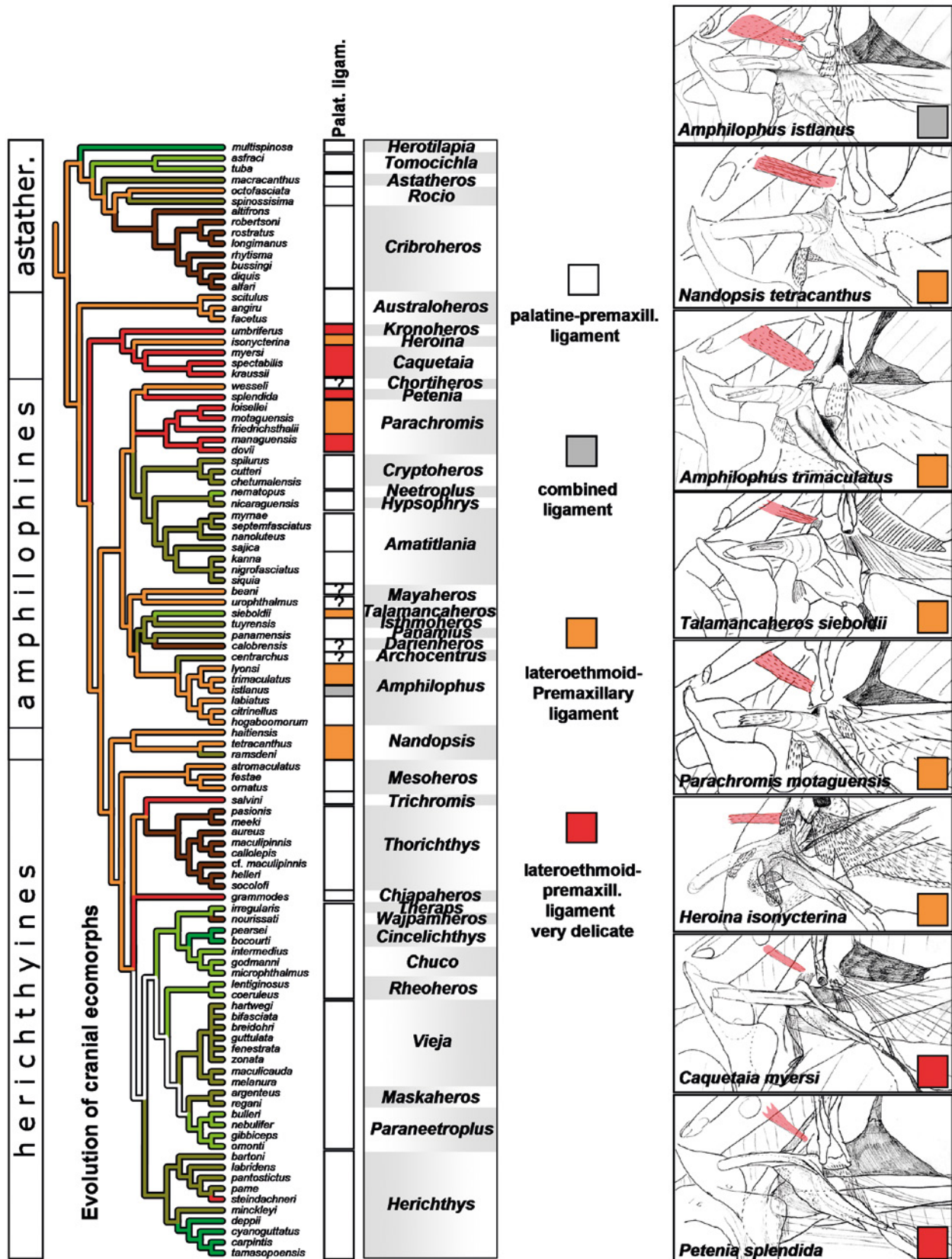


Fig. 11. Mapping of the palatine-premaxillary ligament's two types of insertion onto the ddRAD topology. The branches of the ddRAD topology show cranial ecomorph evolution. The apomorphic character state showing insertion onto the vomer/ethmoid (i.e. lateroethmoid-premaxillary ligament) is correlated with the predatory-piscivorous cranial ecomorph. The very delicate lateroethmoid-premaxillary ligament is only found in the most specialized piscivores.

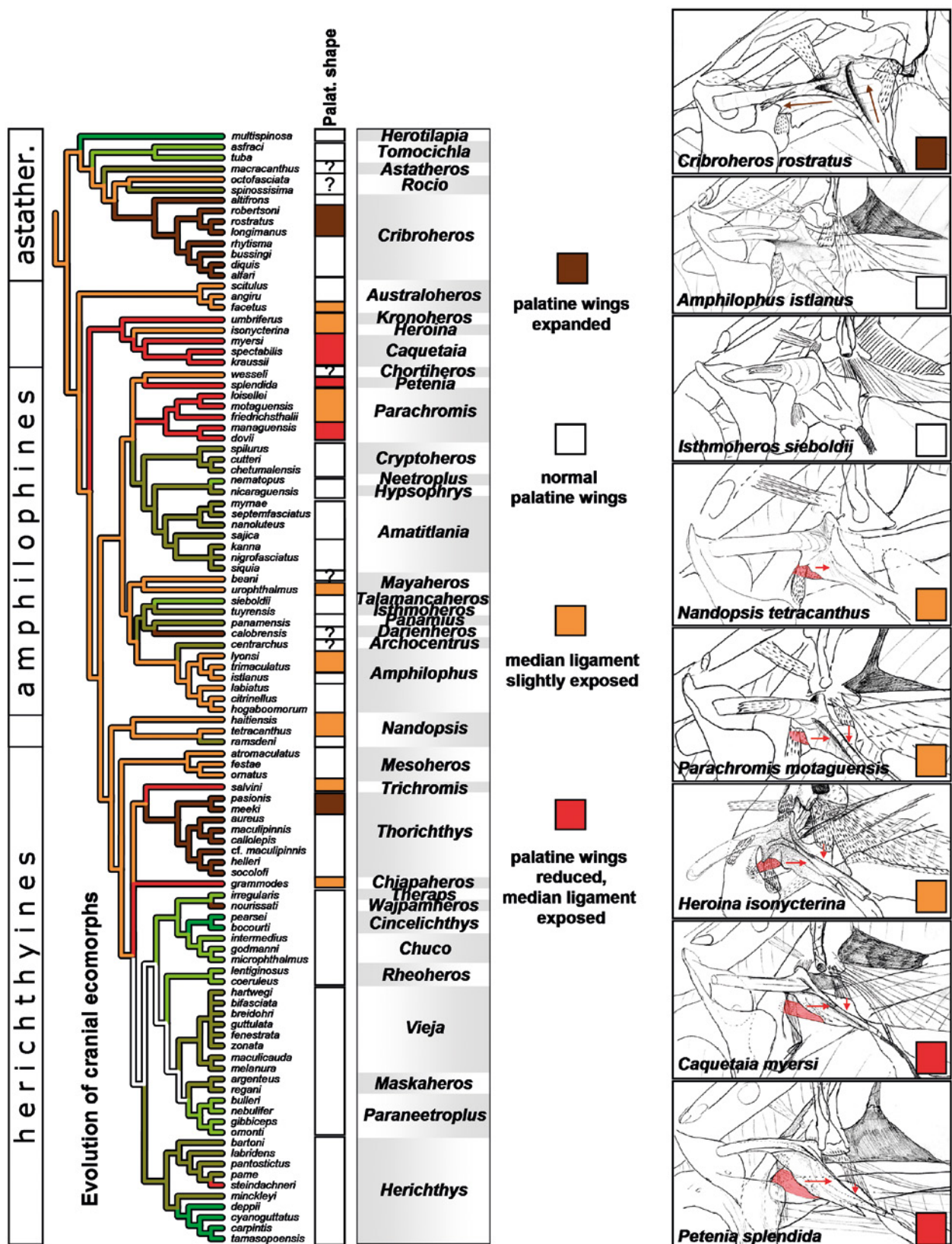


Fig. 12. Mapping of the palatine shape and the exposure of the median ligament onto the ddRAD topology. The branches of the ddRAD topology show cranial ecomorph evolution. Palatine wings expansion is only found in the most specialized substratum-sifters, palatine wings reduction (with a slight exposure of the median ligament) is only found in predators/piscivores. Strong palatine wings reduction with full exposure of the median ligament is only found in the most specialized piscivores.

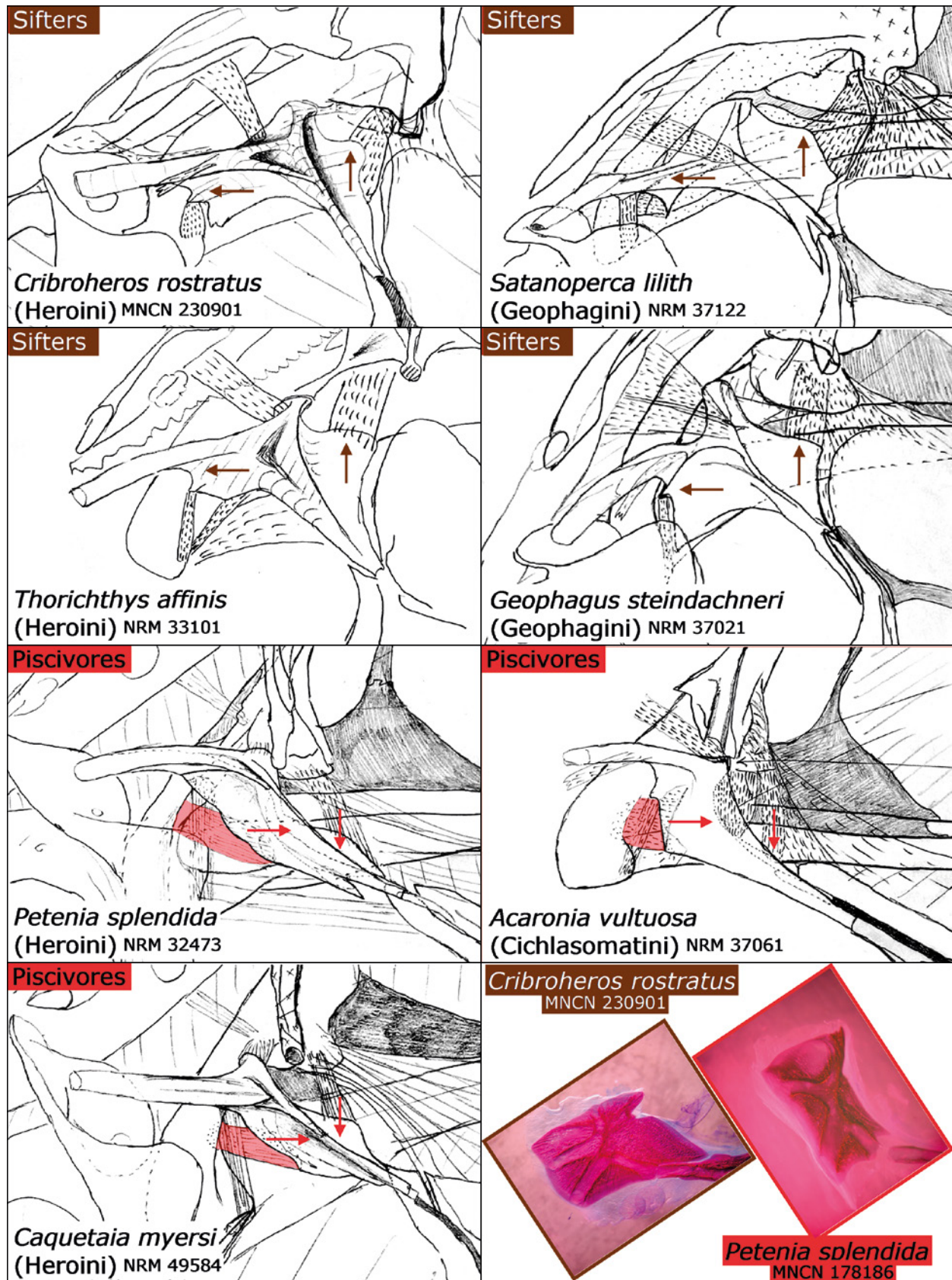


Fig. 13. Palatine and lachrymal bone shape association with piscivores and sifters across Neotropical cichlids.

thys labridens, one morph of *H. bartoni*, one morph of *H. minckleyi*. ARTIGAS-AZAS (1994a,b, 1996; pers. com.) and our own field observations show that at least in the listed *Herichthys* species and in *Wajpamheros* the character complex is used for vertical sifting. Thick lips in *Amphilophus labiatus* are placed on a different mouth structure (lower jaw longer, head more predatory-looking) and resemble those in some South American *Crenicichla* (*C. tendybaguassu* and *C. tuca*; PIÁLEK *et al.*, 2015) and otherwise only found in African lacustrine cichlids. There are some speculations about the function but the seemingly most plausible explanation is that this type of large lips facilitates suction of invertebrates from small cracks or pockets in rocks. It is therefore a specialization of the picker/generalized predator ecomorph. Since the specialized horizontal sifters (*Cribroheros*, *Thorichthys*; see above) do not have thick lips we can speculate that either these are two distinct ecologies (see below) or that past some degree of osteological and myological adaptation for sifting thick lips (as a protection of the jaws and teeth?) are not necessary any longer. The alternative explanation that the two types are possibly distinct sub-ecomorphs is supported by difference in habitats, because the ‘unspecialized’ sifters with the large lips live often in habitats with much faster flowing water and much coarser substrates while the specialized sifters are strictly associated with much finer substrates (mud/silt/sand) and more lentic habitats.

Frenum. The development of the interrupted lower lip (also called a frenum, fig. 9; character 70 in CHAKRABARTY, 2007, character 31 in ŘÍČAN *et al.*, 2008) is very tightly but inversely correlated with the development of the second lower lip and is thus found in the opposite spectrum of ecomorphs. Species with a well developed second lower lip all lack a frenum. Those detritivores-scrappers that do have only a slightly developed second lower lip (previous character) (e.g. *Hypsophrys*, *Cryptoheros*, *Amatitlania*) do fit into the distribution of the present character and because they are detritivores they have the frenum. Exceptions to the general pattern (species without or with a very small second lower lip that lack a frenum) are *Astatheros*, *Mesoheros*, *Wajpamheros*, *Darienheros*, *Chuco*, *Tomocichla*. All these taxa possess thickened lips and the former four taxa (as described above) represent a partially separate ecology of unspecialized sifters (which is intermediate between detritivores and sifters). The frenum on the lower lip is thus tightly associated with all ecomorphs that feed from the substrate (detritivores, sifters – except *Thorichthys*) and in our view the frenum is a clever adaptation for detritivorous feeding because the smooth ventral side enables smooth movement within the sediment without the sediment becoming trapped in the groove behind the lower lip. Interestingly some species that are ancestrally derived from detritivores but are e.g. herbivores or scrapers secondarily nearly lack the frenum (e.g. *Chuco* or the *Herichthys cyanoguttatus* group, where it is intermediate with variation between specimens) which shows that they have no need for it.

This supports a direct adaptive value of the frenum for detritivores and substratum-sifters.

Palatine bone and ligaments. The shape of the palatine bone and its ligaments (figs. 11–13) has been proposed (KULLANDER, 1996) to be one of the best and phylogenetically most informative morphological characters. Our analysis shows that similarly to the jaws, teeth and lips these characters are also a direct reflection of feeding and hence ecomorphology. Specialized predators and piscivores (except those among the herichthyines; see above) have developed independently the lateroethmoid-premaxillary ligament (fig. 11). In the most specialized forms the ligament is additionally so very delicate as to be on the verge of being functionally lost. The lateroethmoid-premaxillary ligament is largest and best developed in predators, but not in the most specialized piscivores (*Petenia*, *Caquetaia*) in which it is very delicate. Only the delicate nature of the ligament (and not the ligament itself) can be used to diagnose highly advanced piscivory and mouth protractility. The shift of the attachment of the ligament was thus not associated with piscivory (*contra* KULLANDER, 1996).

The lateroethmoid-premaxillary ligament has the same taxonomical distribution as type 2 and 3 jaws, the very delicate lateroethmoid-premaxillary ligament has the same distribution as the most piscivory-adapted type 3 oral jaws (*cf.* figs. 9 & 11).

The shape of the palatine bone is strongly correlated with ecology, showing two different shapes in two ecomorphological extremes, the predators-piscivores vs. the most specialized horizontal substratum sifters (fig. 12). All piscivores and most predators (except the two most generalized, i.e. *Australoheros* and *Mesoheros*) have the palatine wings reduced and thus exposed the below lying median ligament. On the other hand the most specialized substratum sifters (in *Cribroheros* and *Thorichthys*) have the palatine wings expanded. The specialized algae scraper *Neotroplus nematopus* and filamentous algae feeder *Herotilapia multispinosa* additionally have each a different and autapomorphic shape of the palatine.

The same shape of the palatine bone associated with piscivores and sifters in the Middle American and related heroine cichlids is also found in the unrelated but ecologically equivalent geophagine and cichlasomatine cichlids of South America. In figure 13 we show that the expanded palatine wings are typical for Neotropical cichlid substratum-sifters in general, as are the reduced palatine wings and the exposed median ligament for Neotropical cichlid predators-piscivores. In the same way the shape of the overlying lachrymal bone is directly associated with the shape of the palatine bone (fig. 13) and again only reflects a given ecomorph (sifter vs. piscivore) and is not a phylogenetically informative character. The expanded wings of the palatine are directly associated with a deep lachrymal bone, which is in all cases present only in very specialized sifters, both among the heroine cichlids (*Cribroheros*, *Thorichthys*) and also among the geophagines (*Geophagus sensu stricto*, the

Geophagus steindachneri group, *Satanoperca*). On the other extreme reduced palatine wings and an exposed median ligament are similarly associated with a narrow lachrymal and this combination is always found in specialized predators-piscivores among the heroines and in our example also among the cichlasomatines (*Acaronia*; *Acaronia* is the only predatory cichlasomatine genus). These shape changes are thus not synapomorphies as suggested by KULLANDER, 1996, they are parallel adaptations to the same life style. *Acaronia* additionally lacks the palatovomerine articulation which was proposed by KULLANDER (1996) as distinguishing cichlasomatines (present) from heroines (absent). This postulated synapomorphy of heroines as opposed to the cichlasomatines (formulated by KULLANDER (1996) as anteroventral palatine wing not articulating with the vomerine shaft and being posteriorly displaced relative to the head of the vomer) is thus invalidated by *Acaronia* (and also *Laetacara*; OR pers. obs.).

Pharyngeal jaws. Pharyngeal jaws are not reviewed in the present study because reviews for Middle American cichlids (e.g. HULSEY, 2006; HULSEY *et al.*, 2006) and for cichlids in general (e.g. BURRESS, 2015; BURRESS *et al.*, 2013 for Neotropical cichlids) are very numerous and all studies are reporting on the strict correlation between the pharyngeal jaws morphology and the preferred food source.

Oral and pharyngeal jaws, teeth, lip structure and head shape are thus not phylogenetically informative characters but direct reflections of the associated feeding ecomorphology. *Herichthys* is an especially notable genus for the study of the morphological and ecological cranial correlates since it is the only genus where piscivores have developed from detritivores (*H. steindachneri*, one morph of *H. minckleyi*). The polymorphic *Herichthys* species (*H. bartoni*, *H. minckleyi*) are especially illustrative for the changes of cranial characters in dependence on the preferred food source.

3.16. Evolution of postcranial characters

Postcranial characters that have been used in the classification and phylogeny of Middle American cichlids are in their majority meristic or shape characters. The meristics and morphometrics are the same characters expressed in a different way. LÓPEZ FERNÁNDEZ *et al.* (2013) have studied the morphometrics, while we, in the present study, prefer to use the meristic characters (as was done in ŘÍČAN *et al.*, 2008) because they offer easier homologization and separation into character-states for mapping onto phylogenetic trees.

For all species the meristic values of all studied postcranial characters have been mapped onto the ddRAD phylogeny (selected characters are shown in figs. 14–17) and we have thus reconstructed the common ancestor of the Middle American and related cichlids (see above) as well as the evolution of each character based on this character optimization. The postcranial charac-

ters show an equally topologically widely distributed ancestor as the cranial characters (*cf.* fig. 9 with figs. 14–17). As in the cranial characters, most changes in the postcranial characters have thus taken place late in the evolution of the group, coinciding with the divergence of the genera or even occurring within the genera because several are postcranially ancestral (figs. 14–17). The longitudinal meristic characters (number of vertebrae, length of caudal peduncle, number of scales) are in general positively correlated, and also their evolution as judged from their optimization on the cladogram is concerted (figs. 16–17). The characters are thus clearly not independent.

One postcranial meristic character has however been treated in all previous studies as independent and has been among the most important characters used in the classification of the Middle American cichlids. This character is the number of anal fin spines. The heroine cichlids are the only clade of Neotropical cichlids that has more than three anal fin spines in all species. So far no one has proposed an explanation for this apomorphy. The ancestral and most common Neotropical anal fin spine number is three, while the ancestral count for the Middle American and related heroine cichlids is based on our character optimization six anal fin spines. There are species and genera in this clade that have four or five anal fin spines (fig. 17), but these low counts are among the Middle American and related heroines apomorphic (reductive) changes. The most interesting observation regarding the number of anal fin spine number is however that it also is not an independently evolving character but that it is well correlated with other postcranial characters, but negatively. This negative correlation is very strong and it is best visible when compared with the length of the caudal peduncle (fig. 17). In virtually all Middle American cichlids an increase in the number of anal fin spines is accompanied by shortening of the caudal peduncle, while elongation of the caudal peduncle is virtually always accompanied by a decrease in the number of anal fin spines. The only exception at the species level are the terminal herichthyines (*Herichthys*, the *Theraps-Paraneotroplus* clade) where the anal fin spine number is almost conserved. But even in this conservative group the anal fin spines number has been reduced twice from six to five in correspondence with elongation of the caudal peduncle (fig. 17). The length of the caudal peduncle and the number of anal fin spines are thus two opposite ends on one axis. On one side is the trajectory towards streamlined, elongated species with long caudal peduncles and low numbers of anal fin spines, on the other is the trajectory to short and high bodies with short caudal peduncles but elevated numbers of anal fin spines. All postcranial characters thus fit into only two categories, which we call the lotic and lentic postcranial ecomorphs (figs. 19–20).

That the strong negative correlation of the anal fin spines number is best evident in comparison with the length of the caudal peduncle is in our opinion not by chance. We believe that since there are only two postcra-

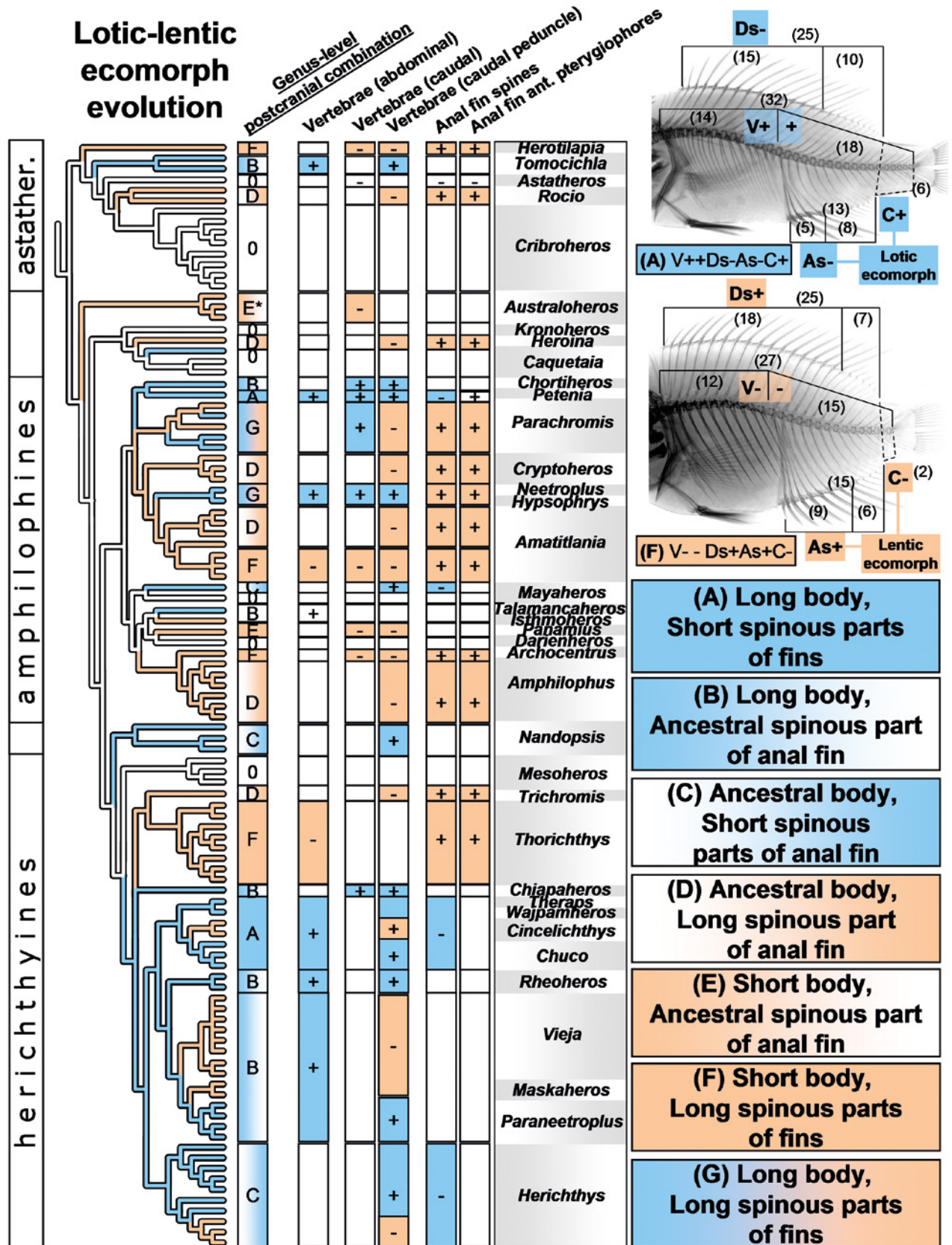
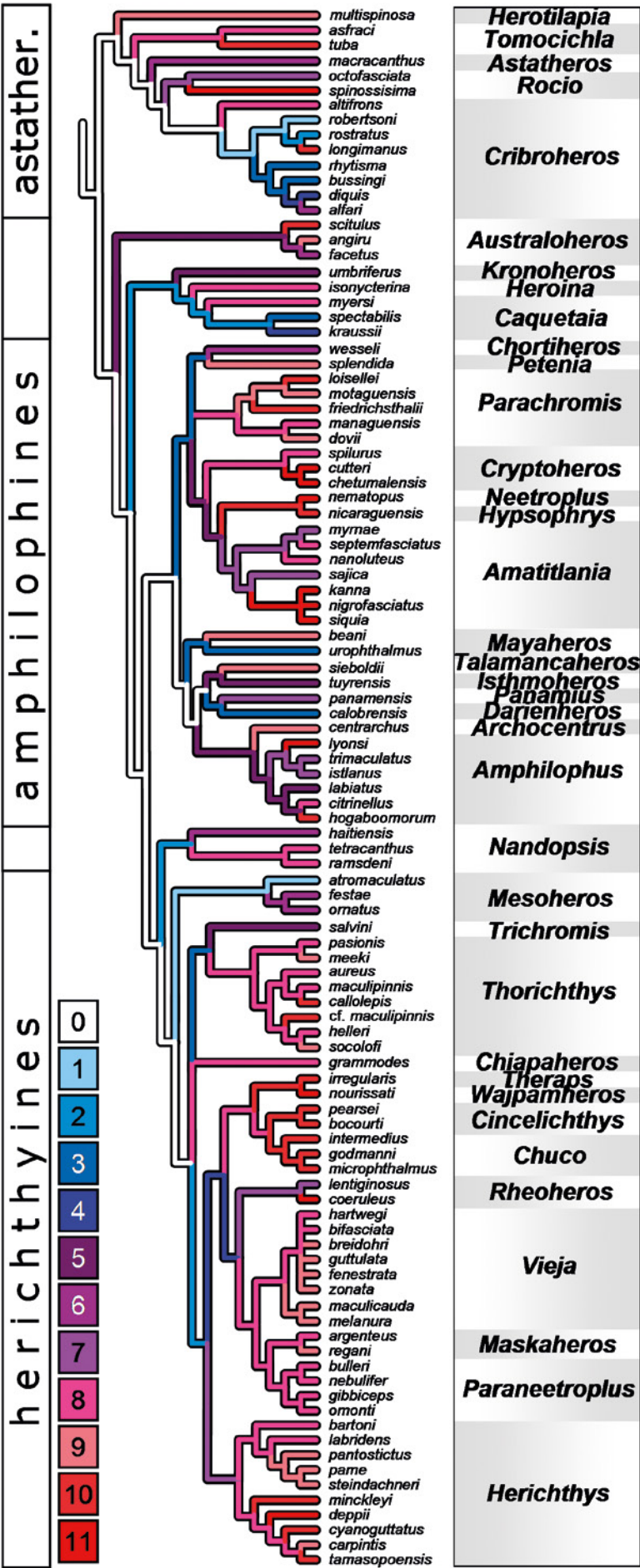


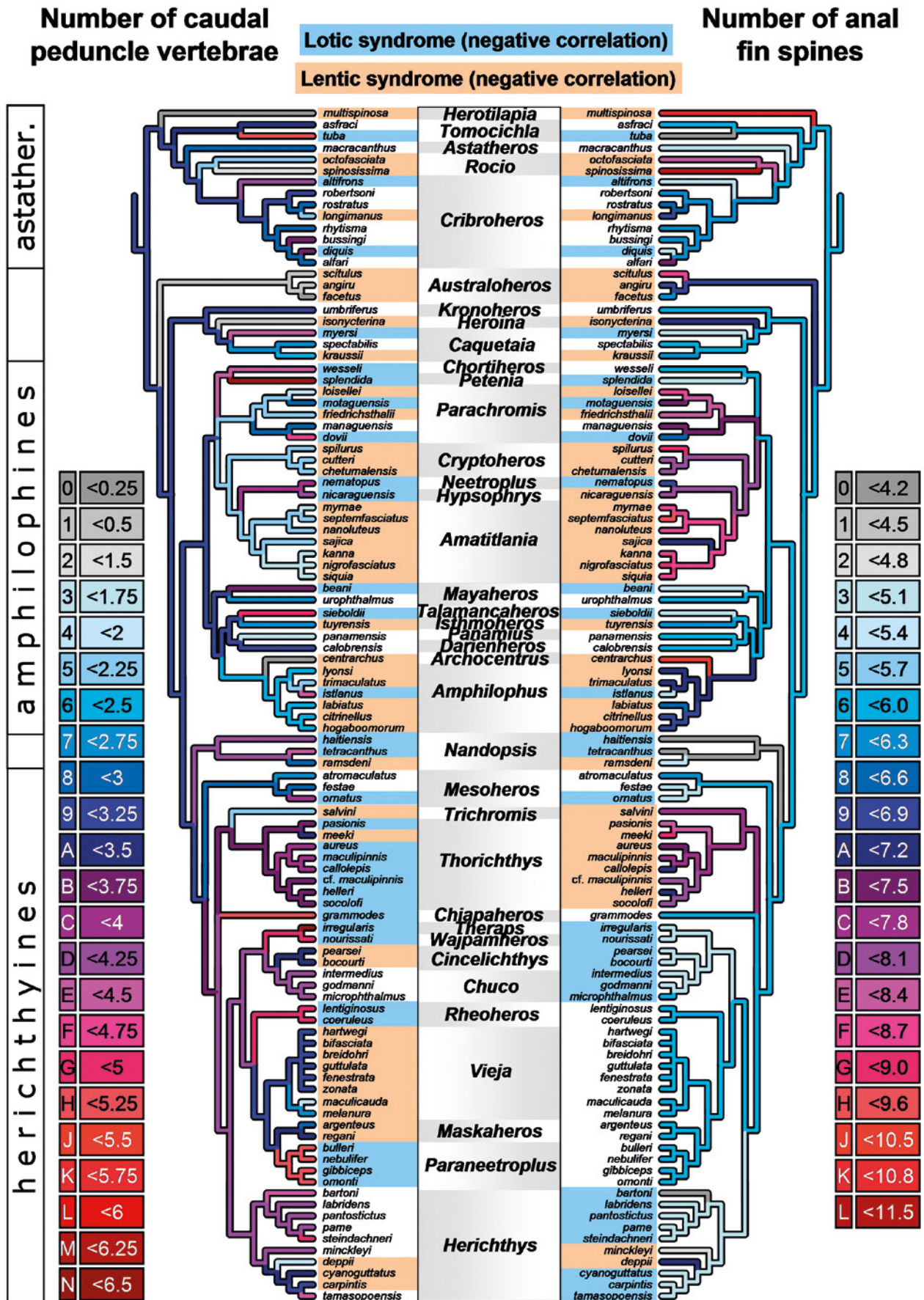
Fig. 14. Mapping of postcranial characters and the evolution of postcranial ecomorphs on the ddRAD topology. The mapped characters are shown at the insert postcranial radiographs. Mapped characters show relative change to the reconstructed ancestor of the Middle American cichlid clade. Blue colours show elongated postcranials (i.e. with more vertebrae, e.g. rheophilics or predators), brown colours show shortened postcranials (e.g. lentic habitat fishes). All combinations of characters are coded with a letter code.



← Fig. 15. Mapping of postcranial character complexes. The tree shows dissimilarity at the species level to the reconstructed ancestor in 11 postcranial characters. The mapped characters are numbers of: abdominal vertebrae, caudal vertebrae, total number of vertebrae, caudal peduncle vertebrae, anal fin spines, anal fin rays, total anal fin elements, anal fin anterior pterygiophores, dorsal fin spines, dorsal fin rays, total number of dorsal fin elements. Note the wide distribution of the reconstructed ancestor with zero changes and the many genera additionally connected by only a few (1–3) changes.

→ Fig. 16. Mapping of vertebral count on the left and lateral line scale count on the right at the species level using the ddRAD topology. This is an example of positively correlated meristic characters. In the same way are e.g. dorsal fin elements positively correlated with vertebral numbers (not shown). Abdominal and caudal vertebrae are also positively correlated, as are caudal peduncle vertebrae and total number of vertebrae (*cf.* figs. 16–17). The numbers in the coloured legends show the actual values, which were mapped using character states (0–K for the number of vertebrae, 0–8 for the number of scales). The mean values of each character for each species were mapped. The two characters show high correlation and also concerted evolution. Increase in values of both characters characterizes the lotic syndrome (part of the lotic ecomorph), while decrease in values of both characters characterizes the lentic syndrome (part of the lentic ecomorph; *cf.* figs. 14, 18–21).





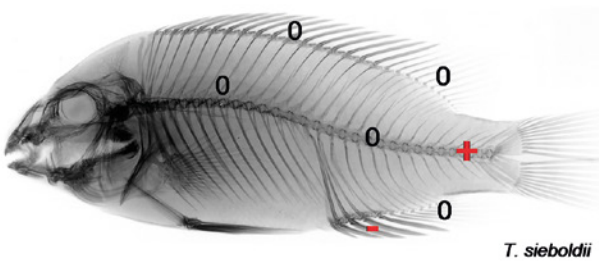
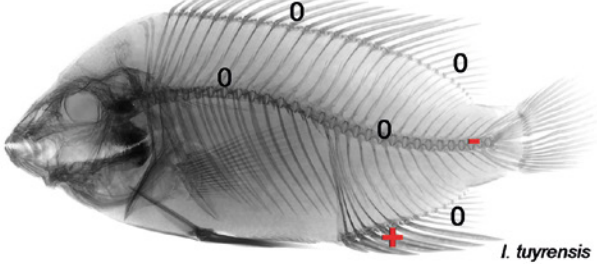
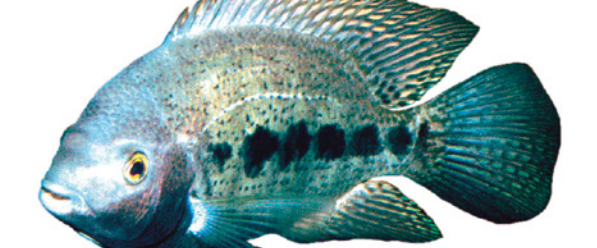
Lotic-lentic ecomorph dichotomy	
Lotic ecomorph Body elongated Caudal peduncle elongated Anal fin spine-count reduced Breeding coloration white Mouth inferior Periphyton scrapers	Lentic ecomorph Body deep Caudal peduncle shortened Anal fin spine-count increased Breeding coloration obscure Mouth terminal Detritivores (herbivores)
<i>Talamancaheros</i>  <i>T. sieboldii</i>	<i>Isthmoheros</i>  <i>I. tuyrensis</i>
 <i>T. underwoodii</i>	 <i>I. tuyrensis</i>

Fig. 18. The lotic-lentic ecomorph dichotomy and some of the associated characters (see figs. 14, 16–17). An example of this ecomorph dichotomy is given for the amphipoline sister genera *Talamancaheros* and *Isthmoheros*.

nial ecomorphs (i.e. evolutionary trajectories; except for the third undifferentiated ancestral ecomorphology) both probably confer an adaptive advantage. The long caudal peduncle (and reduced numbers of anal fin spines) is strongly associated with all rheophilic species (figs. 20–21) and also predominantly with piscivores, which are all fishes that need to be efficient fast swimmers. The short caudal peduncle is on the other hand found predominantly in detritivores that live in lentic habitats. These do not need to be and because of the structure of their habitats

often cannot be fast and enduring long-distance swimmers. We believe that the high number of anal fin spines might offer some adaptive advantage as an antipredatory mechanism (i.e. increasing non-palatability by increased pungency), since otherwise the correlation with a short caudal peduncle is hard to explain. The dorsal fin spine numbers are not correlated with the anal fin spine numbers but with the other postcranial meristics (i.e. number of vertebrae, especially abdominal vertebrae) and thus cannot be, in evolutionary terms, influenced indepen-

← **Fig. 17.** Mapping of caudal peduncle vertebral count on the left and the number of anal fin spines on the right at the species level using the ddRAD topology. The number of anal fin spines is a negatively correlated meristic character. Caudal peduncle vertebrae number is positively correlated with the number of caudal vertebrae, with the total number of vertebrae and hence with the number of dorsal fin elements or with number of scales along lateral line (cf. figs. 16–17). The numbers in the coloured legends show the actual values, which were mapped using character states (0–N for the number of caudal peduncle vertebrae, 0–L for the number of anal fin spines). The mean values of each character for each species were mapped. The two characters show high negative correlation between each other and also concerted evolution. Increase in the number of caudal peduncle vertebrae accompanied by decrease in the number of anal fin spines characterizes the lotic syndrome (part of the lotic ecomorph), decrease in the number of caudal peduncle vertebrae accompanied by increase in the number of anal fin spines characterizes the lentic syndrome (part of the lentic ecomorph; cf. figs. 14, 16 and 18–21). Note that the number of anal fin spines is highly conserved in the *Theraps-Paraneotroplus* clade.

Lotic-lentic ecomorph dichotomy (example of repeated evolution of the dichotomy in the herichthyine <i>Theraps-Paraneetroplus</i> clade)	
similarity $\updownarrow$ relatedness $\longleftrightarrow$	
Lotic ecomorph Body elongated Caudal peduncle elongated Caudal peduncle blotch small Breeding coloration white Mouth inferior Periphyton scrapers	Lentic ecomorph Body deep Caudal peduncle shortened Caudal peduncle blotch large Breeding coloration obscure Mouth terminal Detritivores (herbivores)
<i>Paraneetroplus</i>  <i>P. bulleri</i>	<i>Vieja</i>  <i>V. bifasciata</i>
<i>Chuco</i>  <i>C. microphthalmus</i>	<i>Cincelichthys</i>  <i>C. uffermanni</i>
<i>Rheoheros</i>  <i>R. coeruleus</i>	<i>Oscura</i>  <i>O. heterospila</i>

Fig. 19. The lotic-lentic ecomorph dichotomy and some of the associated characters (see figs. 14, 16–17) in an example from the herichthyine cichlids in the *Theraps-Paraneetroplus* clade. The lotic-lentic ecomorph dichotomy has evolved several times in parallel in one river basin (Usumacinta river basin) in the *Theraps-Paraneetroplus* clade (see figs. 2–3, 9). All lotic species were previously classified as one genus *Theraps* (e.g. MILLER *et al.*, 2005), and all the lentic species as the genus *Vieja*. Such classification of this clade thus reflects the two ecomorphs, not monophyletic genera.

dently by selection for defensive purposes. The elevated numbers of anal fin spines in Middle American cichlids are thus predominantly correlated with the detritivores or herbivores, exactly those feeding ecomorphs that are unique to Middle America. It is tempting to speculate on this deduction and think of alternative ways of protection for detritivorous or herbivorous non-heroin cichlids in South America. Our hypothesis is that these species simply cannot reduce their active escape options (i.e. shorten their caudal peduncles beyond a certain point) to the degree the Middle American detritivores or herbivores can. The increased number of anal fin spines present in the ancestor of all heroin cichlids could well have been a pre-adaptation for the evolution of so many detritivores and herbivores among the Middle American cichlids.

Significantly all the species of Middle American cichlids that have high numbers of anal fin spines but are not detritivores (or herbivores) (e.g. the predatory *Parachromis*, *Trichromis*, or the scraper *Neetroplus*) are based on our character reconstructions evolved from the lentic ecomorph prior to their shifting to the lotic ecomorph and to the alternative cranial adaptations (figs. 14, 17, 20). This also means that the postcranial skeleton is more conservative than the cranial skeleton (which evolves faster) because it carries for a longer time vestiges of past adaptations.

3.17. Diversity of Middle American and related heroin cichlids as combinations of cranial and postcranial ecomorphs

We have discovered that morphological characters in the Middle American cichlid clade are strongly correlated and that their combinations are best classified as five cranial and two (plus the undifferentiated ancestor) postcranial ecomorphs. The question is how and to which degree the cranial and postcranial ecomorphs are correlated and interdependent and whether all or only certain combinations of cranial and postcranial ecomorphs are found. In figures 20 and 21 we explore this question. The strictest correlation between cranial and postcranial ecomorphs is found in the scrapers and detritivores (herbivores). The first are always found in combination with a lotic postcranial, while the latter almost always with a lentic postcranial. The other cranial ecomorphs show free combinations between the cranial and postcranial ecomorphs (even among the most specialized predators-piscivores).

We found thirteen (of fifteen possible) combinations of cranial and postcranial ecomorphs in the cichlid group studied. Only three of them are occupied by a single genus each (*Thorichthys*, *Astatheros*, *Wajpamheros*). The most repeated combination by far is the lentic detritivore (herbivore) ecomorph combination.

The diversity of the studied cichlid group can thus be expressed as combinations of cranial and postcranial ecomorphs having produced 13 genus-level combinations. Among the generalists, the predators-piscivores, and the

sifters the genera are always (at least ancestrally) allopatric (fig. 21; see below). The situation is different among the most numerous detritivores and scrapers, where different genera often are sympatric and closely related genera are also ancestrally sympatric. Diagnosing and delimiting genera among the detritivores and scrapers is thus more complicated than in the other ecomorphs.

Biogeography and the cranial-postcranial ecomorph combinations are thus sufficient to diagnose genera among all generalists, all predators-piscivores, all sifters, and some scrapers and detritivores. These short and easy diagnoses follow:

Caquetaia: piscivores with an ancestral postcranial, South America east of the Andes (except *C. kraussii* which is also in NW South America west of the Andes);

Kronoheros: piscivores with an ancestral postcranial, NW South America west of the Andes up to eastern Panamá;

Trichromis: predator/piscivore with a lentic postcranial, Usumacinta ichthyological province of northern Middle America;

Parachromis: predators/piscivores with an ancestrally lentic (secondarily lotic) postcranial, Central America (except two species in northern Middle America);

Petenia: piscivore with a lotic postcranial, Usumacinta ichthyological province of northern Middle America;

Chiapaheros: piscivore with a lotic postcranial, upper Grijalva river basin of northern Middle America;

Chortiheros: generalist with a lotic postcranial, Caribbean Honduras;

Nandopsis: generalists with a lotic postcranial, Greater Antilles;

Australoheros: generalists with a lentic postcranial, southern tropical South America;

Heroína: generalist with a lentic postcranial, western Amazon of South America;

Amphilophus: generalists with a lentic postcranial, Central America and Pacific slope of northern Middle America;

Rocio: generalists with a lentic postcranial, Usumacinta ichthyological province of northern Middle America;

Mesoheros: generalists with an ancestral postcranial, Choco of NW South America and Caribbean slope of eastern Panamá;

Mayaheros: generalists with an ancestral postcranial, northern Middle America;

Cribroheros: sifters with an ancestral postcranial, Central America (except one species in northern Middle America);

Darienheros: sifter with an ancestral postcranial, eastern Pacific slope Panamá;

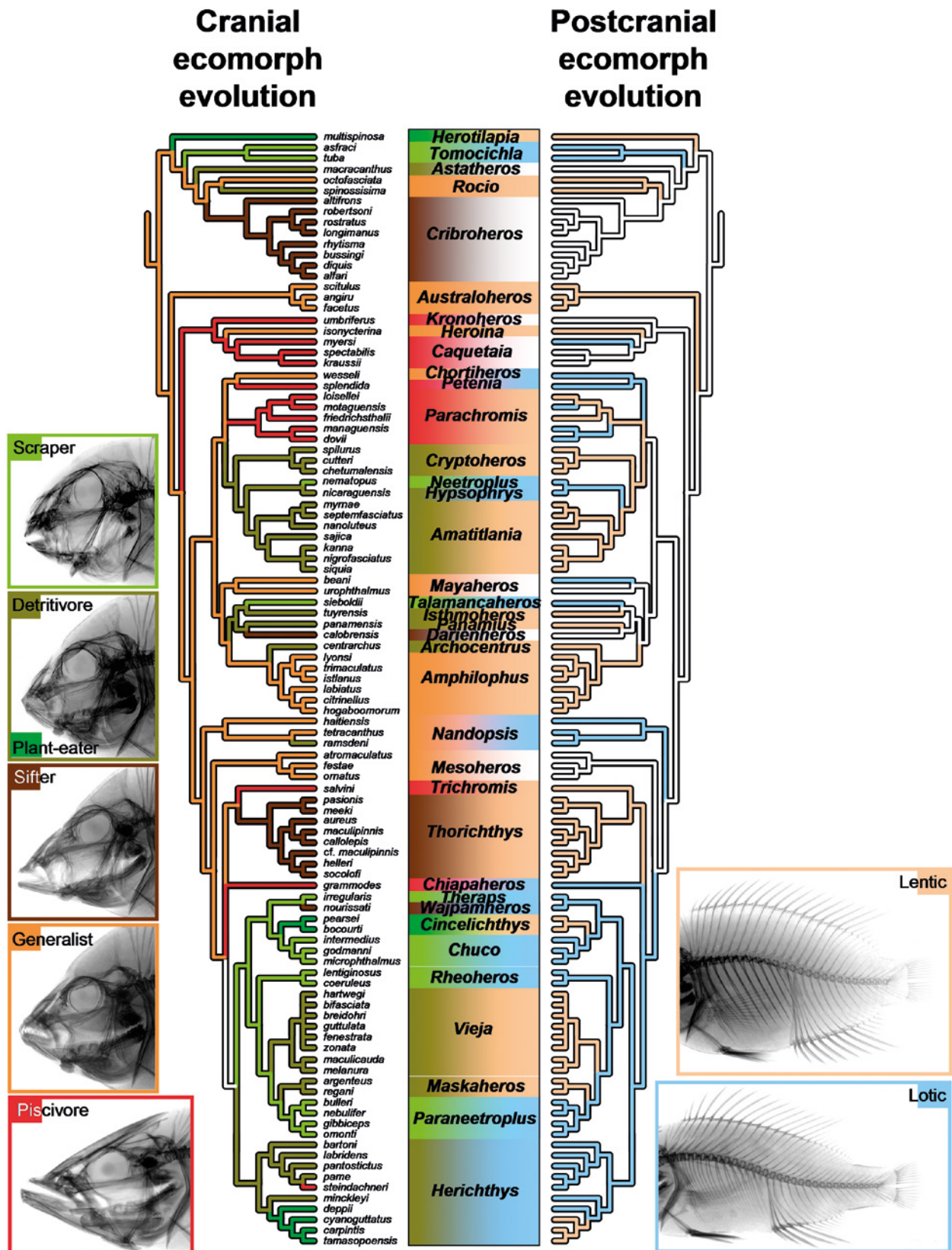


Fig. 20. Combinations of cranial and postcranial ecomorphs and corresponding generic classification of the Middle American cichlid clade.

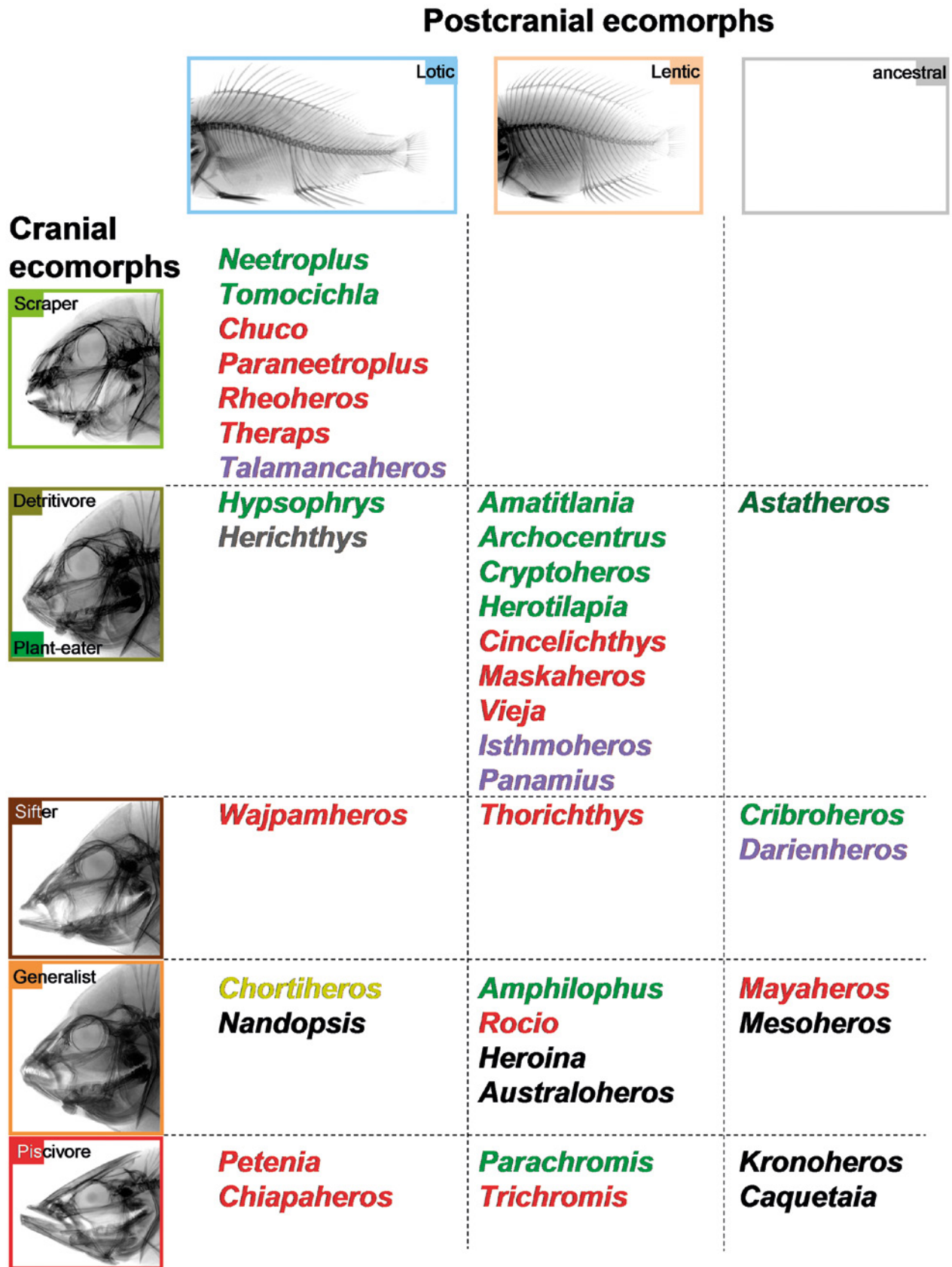


Fig. 21. Thirteen out of fifteen possible combinations of cranial and postcranial ecomorphs encountered in the Middle American cichlid clade and their correspondence to genus level classification. Coloration of genus names shows geographical distribution (or ancestral distribution in case of *Chiapaheros*, *Cryptoheros*, *Mayaheros*, *Paraneetroplus*, and *Talamancaheros*; see fig. 32 for colour codes). Note the two main (red: Usumacinta river basin; green: San Juan river basin) evolutionary centres.

Thorichthys: sifters with a lentic postcranial, Usumacinta ichthyological province of northern Middle America;

Wajpamheros: sifter with a lotic postcranial, Usumacinta ichthyological province of northern Middle America;

Herichthys: detritivores with an ancestral/lotic postcranial, Gulf of México river basins in central and northern México;

Hypsophrys: detritivore with a lotic postcranial, Central America in the San Juan ichthyological province;

Astatheros: detritivore with an ancestral postcranial, Pacific Central America and southern México;

Talamancaheros: scraper with a lotic postcranial, Pacific slope rivers of eastern Costa Rica and western Panamá.

The following detritivores (herbivores) and scrapers cannot be diagnosed just by cranial-postcranial ecomorphs combination plus biogeography and have to be diagnosed by additional characters:

Neetroplus and *Tomocichla*: scrapers with a lotic postcranial, San Juan ichthyological province on the Caribbean slope of Central America from southern Nicaragua to southern Costa Rica (one species, *T. asfraci* in western Panamá);

Theraps, *Chuco*, *Rheoheros* and *Paraneetroplus*: scrapers with a lotic postcranial, northern Middle America in the Usumacinta ichthyological province (*Paraneetroplus* is the only one not found in the Usumacinta river basin, the other three are sympatric);

Amatitlania, *Archocentrus*, *Herotilapia* and *Cryptoheros*: detritivores (herbivores) with a lentic postcranial, Central America predominantly and ancestrally in the San Juan ichthyological province (*Cryptoheros* the only one reaching into northern Middle America into México);

Vieja, *Maskaheros* and *Cincelichthys*: detritivores (herbivores) with a lentic postcranial, northern Middle America in the Usumacinta ichthyological province;

Isthmoheros and *Panamius*: detritivores with an ancestral postcranial, Pacific slope rivers of eastern Panamá.

Evolution of coloration patterns

3.18. Evolution of adult coloration patterns

Adult coloration patterns in Middle American and related cichlids can be broadly divided into four types: 1) vertical bars, 2) midlateral stripe, 3) combination of 1 and 2, and 4) irregular patterns of blotches or dots. Type 4 develops in all cases from one of the previous three main coloration

patterns. All the four main coloration types are accompanied by one or more midlateral blotches, which are found at the intersection of the vertical bars and the midlateral stripe. In several species the midlateral blotches are the sole coloration markings. The main components of the adult coloration patterns (vertical bars, midlateral stripe, midlateral blotches) are rather difficult to homologize across unrelated species from different genera and the usefulness of the coloration patterns has thus in cichlids and in fishes in general been mostly in descriptions and diagnoses of closely related species, not as phylogenetic characters. The difficulty in assessing homology can be easily avoided with the use of ontogenetic information as demonstrated by ŘÍČAN *et al.* (2005).

ŘÍČAN *et al.* (2005) have found that the varied adult coloration patterns are the result of much more conservative ontogenetic pathways. While the number of vertical bars in adult coloration varies significantly, the number of ontogenetic melanophore migration lines (ontogenetic precursors of adult vertical bars) is constant. By following the development of the vertical bars and their numbers from the ontogenetic precursors ŘÍČAN *et al.* (2005) provided a way of establishing homology of the adult coloration patterns. The dominant midlateral blotch in heroine cichlids is thus in all species (or ancestrally for the group) found in the fourth ontogenetic bar. The dominant midlateral blotch is in most species situated approximately above the insertion of the anal fin, but not always, which is due to the elongation or shortening of the anal fin, and also due to divisions of the fourth ontogenetic bar or the missing of the dominant midlateral blotch from adult coloration. On the contrary the dominant midlateral blotch in all other groups of Neotropical cichlids is located in the fifth ontogenetic vertical bar.

The number of adult vertical bars can thus be either determined by following the divisions (or fusions) of the ontogenetic vertical bars, or as a proxy if coloration ontogeny is not known by the location of the dominant midlateral blotch. The vertical bars on the caudal peduncle and caudal fin base are derived from the first ontogenetic vertical bar in all cases (ŘÍČAN *et al.*, 2005). There is usually one bar anteriorly on the caudal peduncle and one caudal fin base blotch, in species with shortened caudal peduncles (see above) there can in some cases only be one large blotch that is then the combination of the two separate bars in other species (i.e. the two arms of the first ontogenetic vertical bar are fused into one adult blotch – or bar). The shape and size of the caudal base blotch without this knowledge and its correlation with caudal peduncle length (fig. 19) was used as a taxonomical character within the *Theraps-Paraneetroplus* clade (McMAHAN *et al.*, 2015).

The number of vertical bars is most changeable in the area between the posterior insertions of the dorsal and anal fins and the dominant midlateral blotch. Several genera and species have here independently increased the numbers of the vertical bars through divisions of the ontogenetic precursors (fig. 22). The reasons for these divisions of vertical bars are not obvious from an adaptive

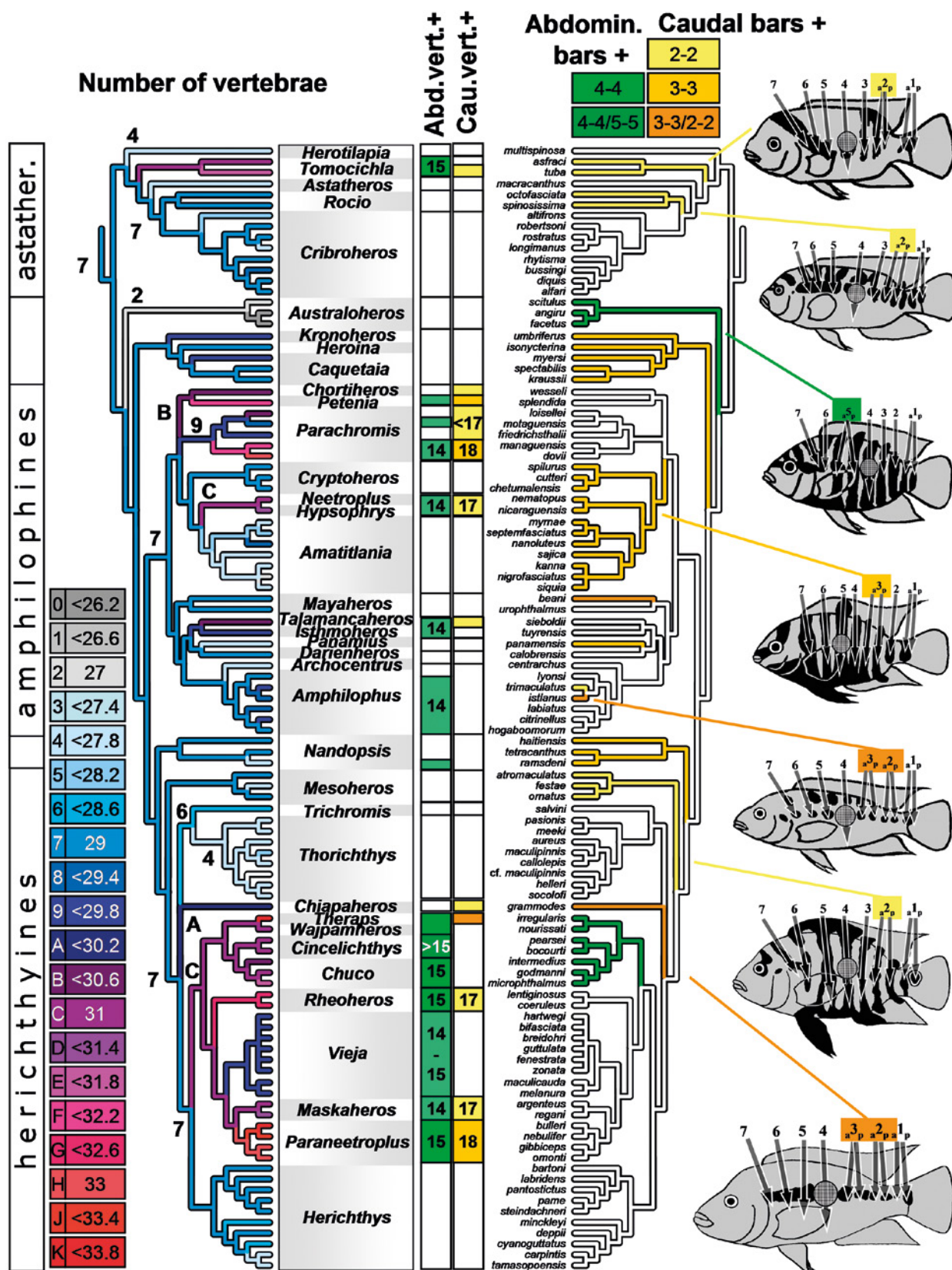


Fig. 22. Comparison between the mapping of the number of vertebrae (left tree) and the number of vertical bars (right tree). In the two columns are shown increases in the numbers of abdominal and caudal vertebrae. There is no evident correlation between increased numbers of vertebrae in a given body zone (abdominal vs. caudal) and increase of vertical bars in the same body zone. The numbers of vertical bars thus appear uncorrelated to the general meristic body patterning.

point of view and our hypothesis thus was that they are the results of elongation of this part of body in the given taxa. Examination of fig. 22 however demonstrates that there is no direct causality between elongation of the caudal (post-anal) part of the body (as judged by the number of vertebrae or scale rows; see fig. 16) and the number of caudal vertical bars, and the same is also true of the abdominal bars. We thus currently have no hypothesis about what is the driving force behind the increase or decrease of the number of vertical bars.

By following the coloration pattern ontogenies we have found that there are four main types of coloration ontogenies among the Middle American and related hercine cichlids. We call these four types based on the starting larval coloration the L-type, the Li-type, the I-type and the B-type (figs. 23, 26–27).

The L-type is characterized by dominance of the midlateral stripe both at the start of the coloration ontogeny as well as during most of its duration. At the opposite extreme is the B-type coloration ontogeny, which altogether lacks any traces of the midlateral stripe from all stages of coloration ontogeny and instead starts with a characteristic blotched pattern that directly proceeds to form the vertical bars of adult coloration.

The Li-type is a modified version of the L-type coloration ontogeny in which the midlateral stripe is already at the start of the coloration ontogeny interrupted in the position of the fourth ontogenetic bar. In this group the development of the fourth ontogenetic bar is thus delayed and the midlateral blotch in the fourth bar is of a similar size as the neighbouring midlateral blotches. The terminal herichthyines with this coloration ontogeny type (*Herichthys*, the *Theraps-Paraneetroplus* clade) thus often do not have a dominant midlateral blotch. The exception is *Rheoheros*, which in coloration patterns is the most plesiomorphic among the terminal herichthyines (Supplementary material 3). The two species of *Maskaheros* (*M. regani*, *M. argenteus*) completely lack the fourth bar and its associated midlateral blotch during the whole ontogeny and in subadult coloration (adult coloration is composed only of a few dorsally located blotches; Supplementary material 3). *Vieja* has secondarily shifted from the Li-type to the L-type by pronounced pigmentation of the midlateral stripe which has obliterated the interruption. These species however still have delayed ontogeny of the fourth ontogenetic bar and midlateral blotch.

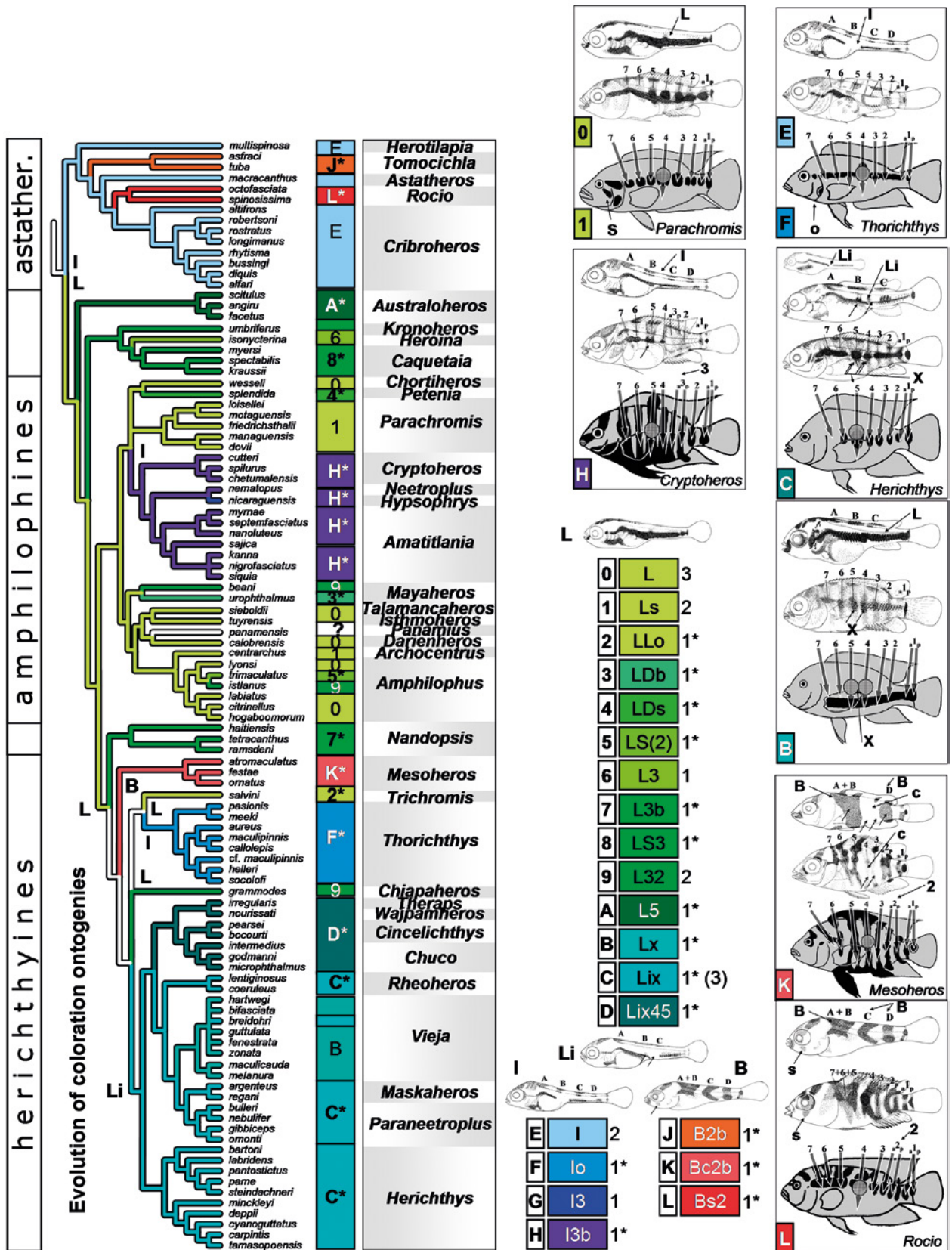
The I-type is an intermediate coloration ontogeny type between the Li-type and the B-type. It lacks a con-

tinuous midlateral stripe in all developmental stages but it also lacks the increased pigmentation of the blotches seen in the B-type coloration ontogeny.

By combining the four main coloration ontogeny types (L, Li, I and B) with the homology of the adult vertical bars (their fusions and divisions) and with other coloration markings (both developmental and adult, e.g. suborbital stripes, opercular blotches etc.) we have identified 21 different coloration patterns (coded as 0–9 and A–L in fig. 23). As can be seen the 21 types are highly diagnostic at and even above the genus level. More information on the coloration pattern ontogenies are found in the following chapter.

A specific and biologically highly important type of the adult coloration is the breeding coloration as an important cue of sexual signalization and partner choice. We provide a visual overview of the diversity of breeding coloration patterns in fig. 3 and the most dramatic breeding coloration pattern changes are demonstrated in figs. 24–25. Most Middle American cichlids and most Neotropical cichlids in general intensify during breeding the contrast between the vertical bars and the background. Intensification of vertical bars is thus the ancestral character state. Several groups of Middle American cichlids depart from this ancestral state. The most distinct breeding coloration pattern change is found in *Herichthys* and in the *Theraps-Paraneetroplus* clade among the herichthyines and in *Talamancaheros*, *Panamius* and *Chortiheros* among the amphilophines. All these genera have a highly contrasting breeding coloration composed of a snow-white (yellow in some species) background and black lower parts of head and body. The extent of the black coloration and its patterning on the white background is group and especially species specific (fig. 24). This breeding coloration type (type 1 in fig. 25) was originally used to diagnose the genus *Herichthys* (e.g. KULLANDER, 1996) but as stated it is more widely distributed. There is a gradient (with species-specific steps) between the possible ancestral version of this breeding coloration type with a large amount of the black coloration on the white background and the most apomorphic condition where some species of *Herichthys* and of the *Theraps-Paraneetroplus* clade are almost completely snow-white without virtually any traces of the black markings (e.g. *Herichthys minckleyi*, *Rheoheros*, *Maskaheros*; figs. 3 & 24). Most of the species with this type 1 breeding coloration do not show intensification of vertical bars (except *Herichthys* s. str., which is thus considered the

→ **Fig. 23.** Mapping of the twenty one different coloration pattern ontogenies found in the Middle American cichlids. Four main ontogenetic types can be discerned based on the coloration of the larval stages immediately after the free-swimming of the larvae; these are the 1) L-type (dominant lateral line), 2) the Li-type (lateral line interrupted in the place of the fourth ontogenetic bar), 3) the I-type (lateral line interrupted in several places), and 4) the B-type (large heavily pigmented blotches dominate the larval coloration). Numbers in the coloration-type codes (e.g. in B2b) show which larval bar is divided. The letter S in the code shows presence of a suborbital stripe (s for less developed than S). The letter x shows codominance of midlateral blotches in ontogenetic bars 4 and 5 (instead dominance of in only one bar). The letter D shows a very strong development (strong pigmentation) of the midlateral stripe in larvae. The letter o shows presence of an ocellated opercular blotch. A second letter L (as in LL) shows a second lateral line in adults (apomorphy of *Trichromis*).



ancestral state; fig. 24) but rather of midlateral blotches or irregularly placed blotches. The breeding coloration of the two amphilophine groups (*Panamius*, *Talamancaheros*) are remarkably similar to each other and in turn to the situation of some species in the *Theraps-Paraneel-*

tropus clade among the herichthyines. *Talamancaheros* was previously placed in either *Theraps* or *Tomocichla*, both unrelated genera with the same ecomorphology. The breeding coloration in *Tomocichla* is however distinct from *Talamancaheros* and hence from the species in the

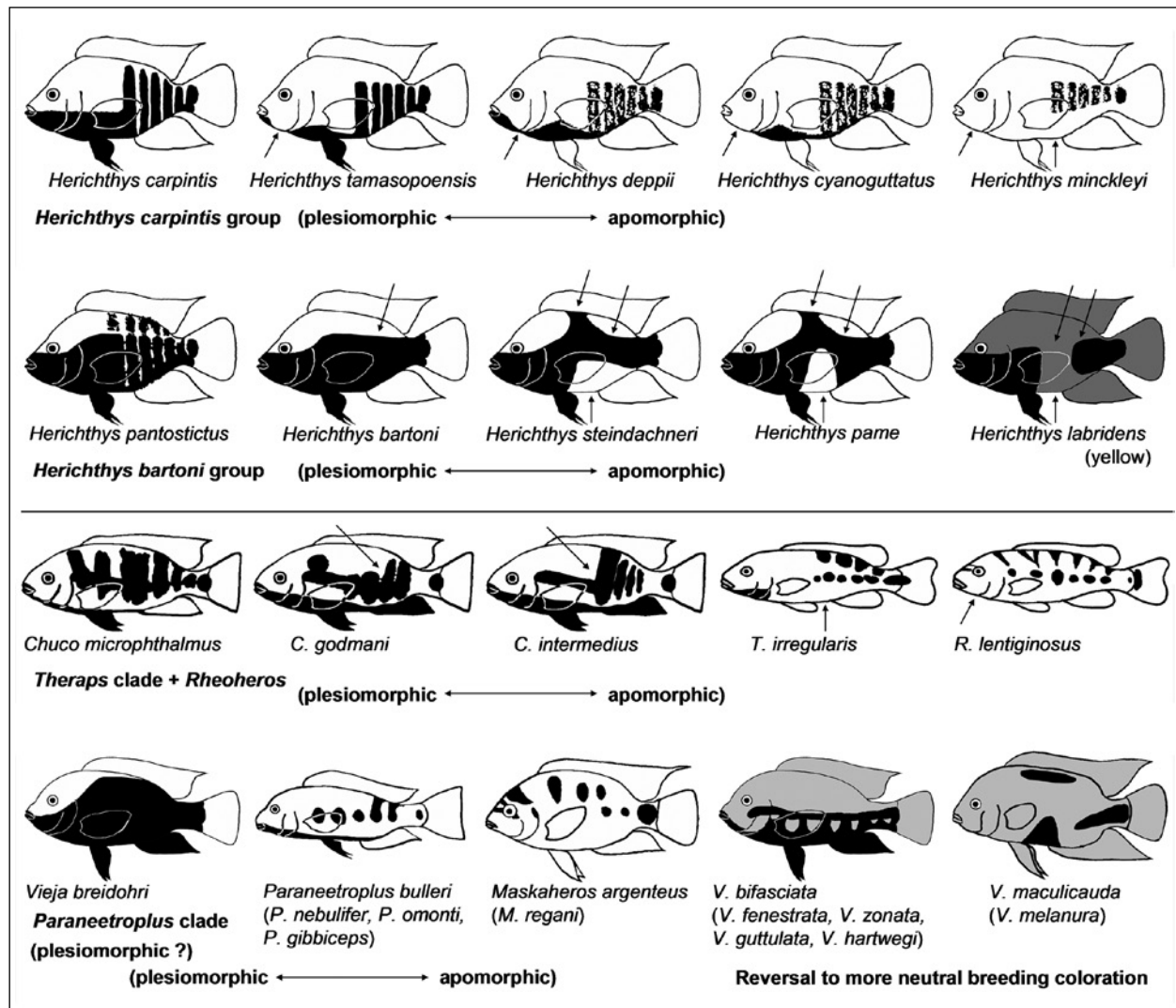


Fig. 24. Diversity of the apomorphic breeding coloration patterns among the crown-group herichthyines. Some variability is associated with the lotic-lentic ecomorphological dichotomy, but most variation is species and genus specific. *Herichthys steindachneri* has a very weakly developed breeding coloration that is however very similar in its pattern to *H. pame*.

Theraps-Paraneetroplus clade, *Panamius* and *Herichthys*. In *Tomocichla* the body coloration does not undergo the striking pure-white / pitch-black coloration change and mostly only the head coloration changes, but here the whole head (in both *Tomocichla* species) turns pure-white and there is thus absent the division between the black ventral part of the head and the white dorsal part of the head. Finally, a unique type of breeding coloration change is found in *Neetroplus* where the breeding coloration (composed of all black head and body with a white midlateral blotch) is the inverse version of the normal coloration.

When looking for a possible explanation for the existence of a unique yet independently evolved breeding coloration pattern change we have noticed that there is a strong correlation between the highly contrasting dorsal pure-white / ventral pitch-black breeding coloration (and also the breeding coloration present in *Tomocichla* and *Neetroplus*) and rheophily. All these cichlids (except *Panamius* and *Herichthys*) are rheophilic fishes. Reduction

of the pure-white background coloration is actually seen several times independently in the *Theraps-Paraneetroplus* clade and in *Isthmoheros-Talamancaheros* in those taxa that have shifted to lentic habitats (figs. 18–19). This observation even strengthens the association of the most distinct breeding coloration with rheophilic and/or very clear-water habitats. *Herichthys* seems to have inherited this breeding coloration from the common ancestor with the *Theraps-Paraneetroplus* clade and the *H. labridens* group has further elaborated on it producing the most beautiful breeding coloration patterns in Middle American cichlids (e.g. *H. labridens* with the autapomorphic yellow instead of white background coloration). Most *Herichthys* are not strictly rheophilic, but many species live in rather fast flowing streams or in clear-water lakes and the whole genus has ancestrally lived in very clear spring-fed waters (only *H. carpintis* is a muddy-water species in most of its distribution) which resemble the rheophilic habitats of the other cichlid groups with the similar breeding coloration.

3.19. Evolution of larval coloration patterns and their possible life history correlates

As we have described above there are four main types of larval coloration in the Middle American and related heroine cichlids. Since there are only two common types of adult coloration (the prevalent vertical bars and the less common lateral band) and one intermediate (the combination of the two) in the studied cichlid group it is interesting to ask why there are four larval coloration types and what is their correspondence to the adult coloration patterns.

The four main larval coloration patterns are very conservatively distributed with regard to phylogeny of the group; indeed they are some of the most conservative characters in the Middle American cichlids. The intriguing question is why are there several different larval coloration patterns at all? Why are the larvae not uniformly coloured in all species? There are several possible reasons that we can think of and we explore these in the following paragraphs.

The four larval types could reasonably well be reduced into two types (blotched type B and all the three lateral band types L, Li and I). What is then the correspondence between these two types (blotches-bars, band-line) and the two adult types (bars, band)? In the simplest scenario the blotches-bars in larval coloration should always result into vertical bars in the adult coloration and vice versa. The situation is not quite as simple, but the blotched-barred larval type indeed results in two of three cases into adults with the vertical bar coloration type (*Tomocichla*, *Mesoheros*; *Rocio* has both a band and bars). The other larval coloration group then includes three degrees of a disruption of the midlateral band (L, Li and I). If the correspondence to adult coloration would be perfect than species with the L-type would have a dominant midlateral band as adults, species of the Li-type would have combination of vertical bars and a midlateral band, and species with the I-type would have predominantly vertical bars. This predicted correspondence is found in the herichthyines and in part in the astatheroines. The correspondence is however almost completely lacking in the amphiphilines (fig. 26).

In more general terms there can possibly be two directions from which selection can operate on the coloration patterns, one being the top-down direction (selection acting on the adult coloration forcing the coloration ontogeny and larval coloration) or vice versa bottom-up direction (selection acting on the larval coloration forcing its effect up to the adult coloration).

In the top-down direction of selection different ecologies could be under different selection regimes. In figure 26 we thus compare the larval coloration patterns with the ecomorphological classification, but do not find any striking correspondence between the two. Changes between lotic and lentic ecomorphs (scrapers vs. detritivores) do not produce a corresponding change in coloration patterns. Specialized predators-piscivores also are not uni-

form in adult coloration, although their majority (including *Amphilophus trimaculatus* as the most predatory *Amphilophus* and *Herichthys steindachneri* as the only piscivorous *Herichthys*; but except *Caquetaia*) tend to have a lateral band as the dominant element in adult coloration. Most of these predatory species are however ancestrally of the L-type of larval coloration, and the correspondence is thus not phylogenetically independent. A correspondence between piscivores and a lateral band coloration has already been proposed in other cichlid groups (SEEHAUSEN *et al.*, 1999). SEEHAUSEN *et al.* (1999) have additionally found that cichlids living in rocky habitats (scrapers in the case of Middle American cichlids) or among vegetation (herbivores) tend to be vertically barred because these habitats are spatially and optically more structured than other habitats (e.g. sand bottoms, open waters and other river habitats). Background matching as a means of predator avoidance (ENDLER, 1978) could thus be one explanation for the association between habitat and vertical bars on the flanks. In the Middle American cichlids this association is not evident (fig. 26), the species with the best development of vertical bars are not associated with either of the two predicted habitats or ecomorphs. SEEHAUSEN *et al.* (1999) tested mainly lacustrine East African species (with few riverine), while virtually all our species are riverine. In our analysis we find very few indications of top-down regulation on the ontogenies of coloration patterns.

In terms of bottom-up regulation of the evolution of coloration patterns the r/K strategy continuum (r/K selection) which relates to the selection of combinations of traits in an organism that trade off between quantity and quality of offspring (MACARTHUR & WILSON, 1967) could be one ultimate determinant by acting on the speed of development. The other could be that larval coloration is under direct selective pressure because the larvae could be forced by selection to match the pattern of the substrate on which they were born and live their larval period. The proximate effects of the r/K selection on the coloration pattern development would be evident in two not necessarily dependent parameters: the size of the eggs and the speed of larval development (ŘÍČAN *et al.*, 2005; see also below for eggs size).

All pigmentation pattern developmental pathways in Middle American cichlids and in fishes in general start with horizontal migration of chromatophores from the cranial region (see refs. in ŘÍČAN *et al.*, 2005) and before hatching from the eggs and before free-swimming of the larvae (the starting point in the classification of our coloration ontogenies) the lateral bands of chromatophores are uniformly found in all species. The larvae with a long persisting lateral band (the L-type larval coloration) thus retain a pre-existing (developmentally initial) coloration pattern. Vertical bars in cichlids then form by the vertical migration of pigment cells that first disrupt the lateral band (the Li and I larval coloration types) and then vertically connect and form adult vertical bars. The blotched (B-type) coloration pattern could thus be viewed as a very precocious coloration pattern where the vertical migration of pigment cells and reduction of the lateral larval

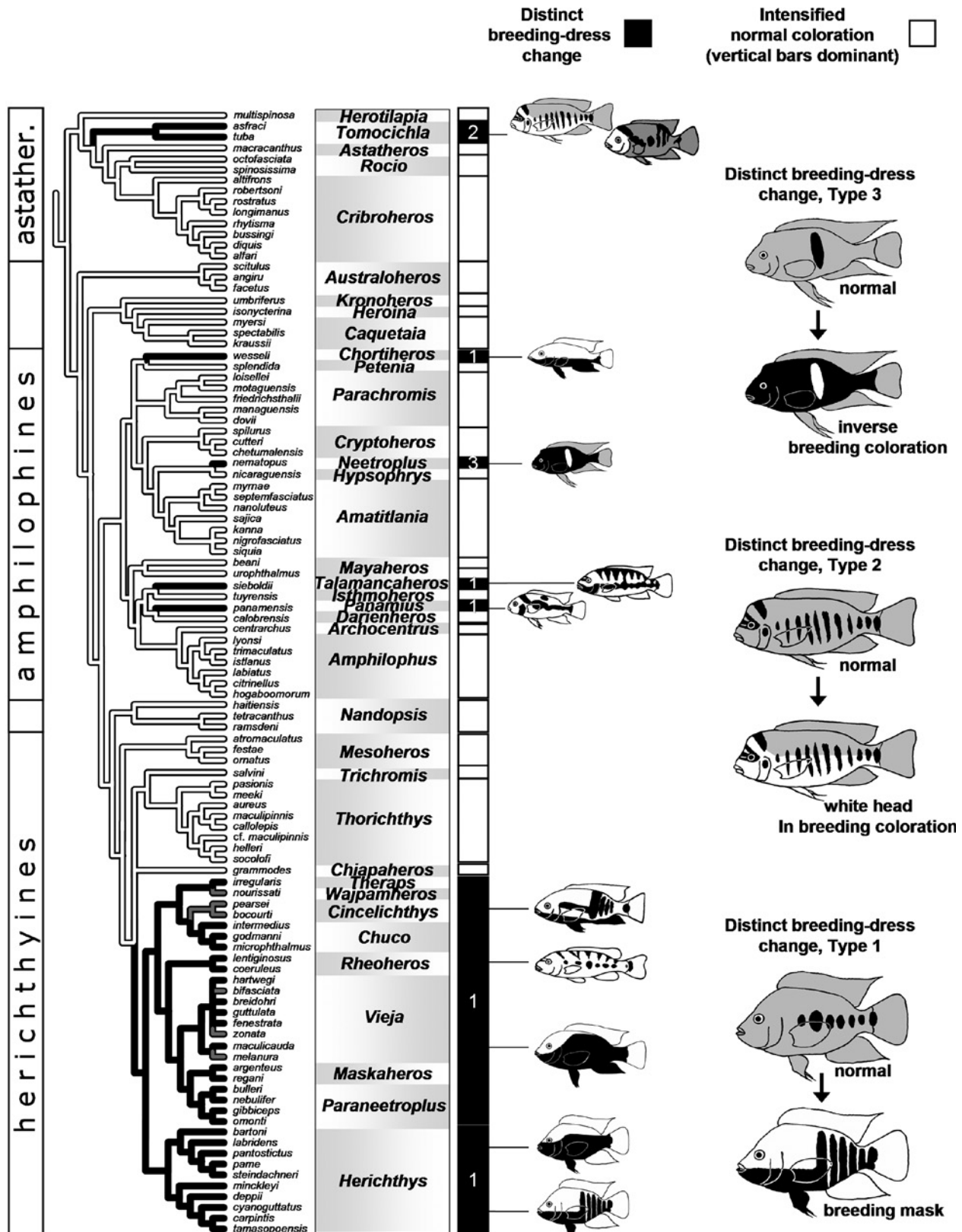


Fig. 25. Diversity and evolution of the apomorphic breeding coloration patterns of types 1-3 among the Middle American cichlids. All genera with such a distinct breeding coloration change have the more apomorphic mother-father family type (fig. 29) with more separation of parental roles between the two sexes. These strongly white-and-black contrasting breeding coloration patterns are also strongly correlated with clear water conditions and in most cases thus with the lotic ecomorphology.

stripe has happened already before the larvae started to swim.

The phylogenetic stability of the larval coloration types seems to speak against the postulated possibility of larval coloration matching the substrate. Also distinct shifts between rocky and sandy (silty) bottoms between the lotic scrapers and lentic detritivores (and herbivores and sifters) has no effect on the larval coloration patterns and coloration ontogenies (fig. 27). The unique blotched type (B-type) larval coloration is found in completely ecomorphologically different genera (*Tomocichla*, *Rocio*, *Mesoheros*) with no shared substrate type.

The ontogenetic timing hypothesis seems more plausible, because it does better fit the phylogenetic stability of the larval coloration types, and also because several characteristics of the coloration ontogenies both within the Middle American cichlids and in Neotropical cichlids in general seem to fit the ontogenetic timing hypothesis (ŘÍČAN *et al.*, 2005).

The time that the eggs take to hatch is dependent mainly on temperature but under standardized laboratory conditions larger eggs tend to take slightly longer to hatch, the span being three to five days between the smallest and largest eggs of Middle American cichlids (STAWIKOWSKI & WERNER, 1998; ŘÍČAN, 1999). Larger eggs thus suggest longer time of development prior to hatching and free swimming. Species with the largest eggs should thus have larvae with the most advanced stages of coloration development at the time of hatching. Egg size is however correlated with adult body size. The largest eggs in Middle American and related South American species are found in many of the rheophilic genera (*Theraps*, *Chuco*, *Neetroplus*, *Tomocichla*; *T. tuba* has absolutely the largest eggs of all Middle American cichlids), but also in *Mesoheros* and in some (but not all) species with very large body sizes (*Parachromis dovii*, see below). When discounting those species where large body size is probably solely responsible for large egg size (*Parachromis dovii*) two of the genera indeed have the most progressed larvae in terms of coloration patterns at the time of hatching (*Tomocichla*, *Mesoheros*). The similar blotched type larvae of *Rocio* have thus probably originated through some other selection because these species have smaller eggs than average and smaller than ancestral ones (figs. 27–28). The ontogeny of *Rocio* has other peculiarities that are otherwise unknown among Middle American cichlids including *Tomocichla* and *Mesoheros* (ŘÍČAN *et al.*, 2005) supporting the phylogenetic non-homology of the B-type larval coloration.

The hypothesis further predicts that the Li and I type larval colorations should be correlated with on average larger eggs than the L-type larval coloration. But two other factors come into play: adult size, and phylogenetic non-independence, since the L-type larval coloration is ancestral for all Middle American and related cichlids except the astatheroines (figs. 23, 26–27). By simply comparing the two larval colorations in their average egg size without taking these other factors into consideration we see that the egg sizes are the same (Li plus I: average and

median both 1.7, min-max 1.4–2.2, SD 0.19; L: average and median both 1.7, min-max 1.4–2.4, SD 0.19). The hypothesis that variability in larval colorations of Middle American cichlids is mainly due to egg size seems to be rejected by our data. Ontogenetic timing shifts without much effect on egg size thus seem a more plausible hypothesis, for the testing of which we however so far lack precise data.

Changes in the speed of development without effect on egg size would predict that species showing a speeded-up ontogeny (here the proxy being coloration pattern ontogeny) could possibly reach maturity sooner and thus at smaller sizes. Species with a slowed-down ontogeny would then reach maturity later, at larger sizes, and body size evolution could be riding on this ontogenetic effect. This hypothesis would predict that species with the L-type larval coloration would on average be larger bodied than species with the Li-type and these in turn larger than species with the even earlier disruption of the lateral line (i.e. I-type). There are of course complications with this simple hypothesis, these being selection pressures on the body size of the adults either acting on fitness (e.g. small bodied species not being able to afford sufficiently large eggs and at the same time their large numbers) and on the body size itself through ecological requirements of the adults (i.e. ecomorphs). Our results seem to support this hypothesis since we have found the predicted adult size differences. The species with the L-type larval coloration have the largest adult body sizes (average 224.5 mm SL, median 220 mm SL, SD 106.8, min–max 77–500 mm SL), the species with the Li-type have intermediate (average 204.4 mm SL, median 200 mm SL, SD 62.3, min–max 102–400), and species with the I-type have smallest adult body sizes (average 130.7, median 137.5, SD 40.3, min–max 70–250 mm SL). This hypothesis however does not work for the special B-type larval coloration, which however has evolved in three separate instances and may not be homologous and not due to the same selection forces.

In summary, we seem to have found support for the interplay between larval coloration types, changes in rate of development, and adult body sizes. Additionally, based on our analysis most ontogenetic coloration pattern characters (divisions and homology of vertical bars, larval coloration types, position and homology of the midlateral blotches, etc.) seem to be relatively selectively neutral, definitely much more so than traditional morphological, osteological or myological character (either cranial or postcranial). Since coloration pattern characters are the main distinguishing characters in cichlid systematics, the study of these characters in an ontogenetic perspective adds very much to their usefulness in phylogenetic and evolutionary applications. Without ontogenetic knowledge coloration pattern characters between dissimilar species (genera) become very difficult to homologize and may thus quickly lose their phylogenetic signal. We thus strongly urge other researchers to study coloration pattern characters with the inclusion of the ontogenetic perspective.

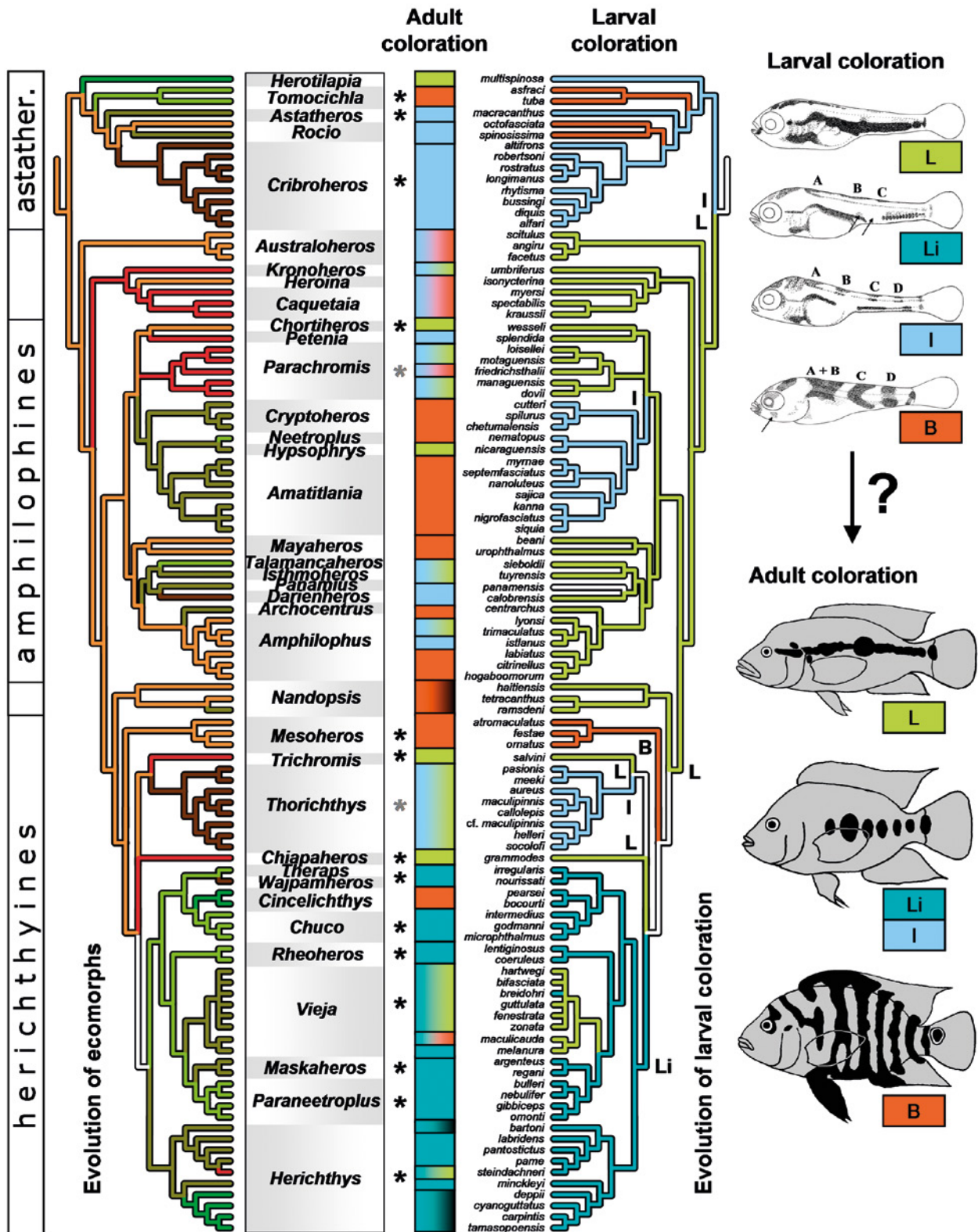


Fig. 26. Comparisons between the mapping of ecomorphologies and coloration pattern ontogenies (larval colorations). There is no visible correlation between the two. There is however noticeable correspondence between larval and adult coloration patterns (except for the amphilophines where this correspondence is mostly lacking). Stars show the genera with correspondence between larval and adult coloration patterns.

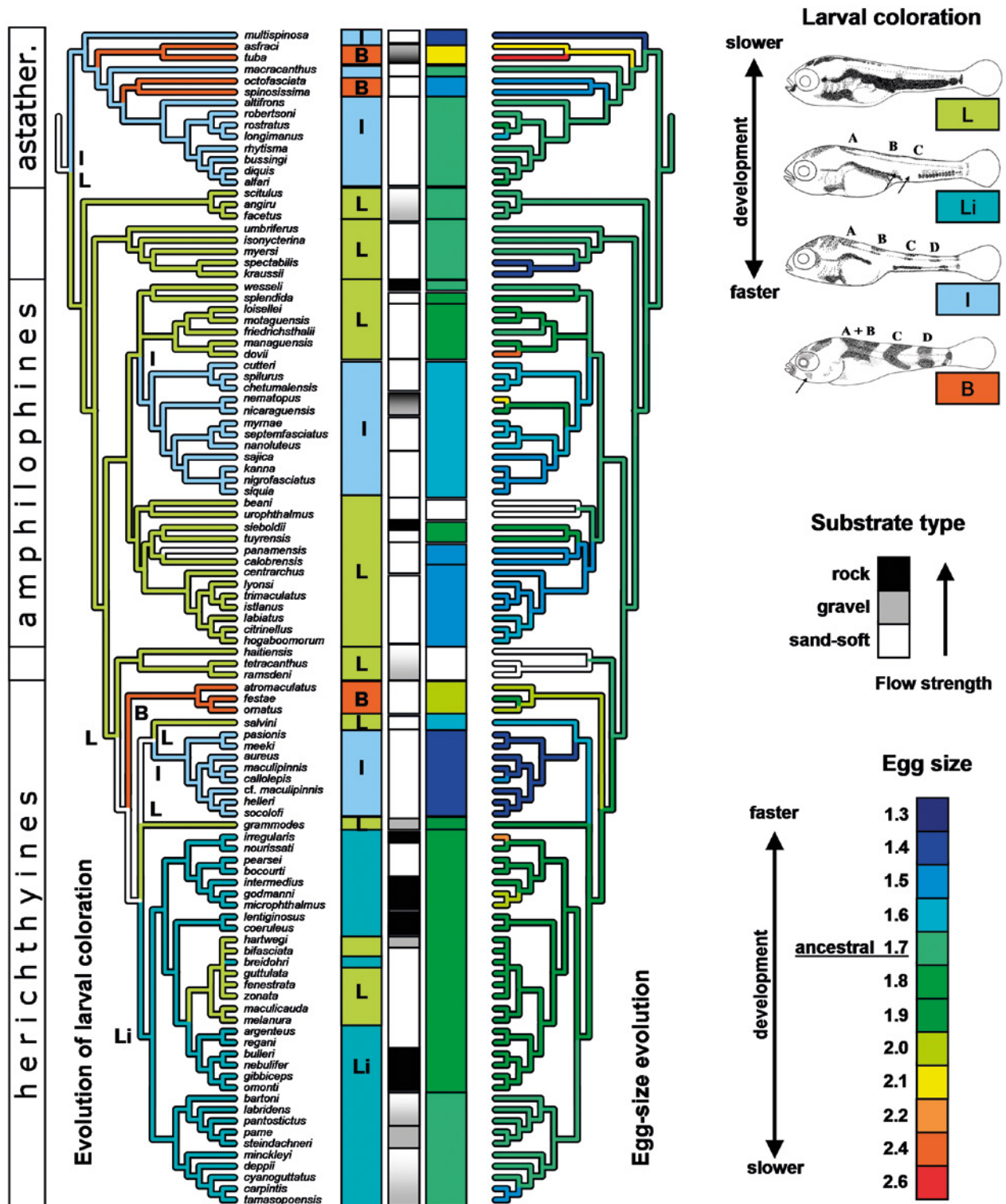


Fig. 27. Comparisons between the mapping of larval coloration, substrate type and the sizes of eggs. There is no visible correlation between the type of larval coloration patterns and substrate type (there thus seems to be no selection for substrate matching by the larvae). There also does not seem to be correlation between the type of larval coloration patterns and egg size (hence larval coloration patterns are not determined by egg size itself but possibly by time of development spend in the egg; see text). Egg size is rather to some extent correlated with adult body size (see text and fig. 28). Egg sizes are averages based on our data and the cichlid egg project (<http://cichlidresearch.com/cichlideggsizedata.html>).

Evolution of life-history traits

3.20. Evolution of body size

The reconstructed ancestor of the Middle American cichlid clade ranged in the 200–250 mm SL size class. This is a medium size within the size range of the group, with the smallest species averaging below 100 mm SL and the fewer largest species around 500 mm SL (fig. 28). Both, the largest species and the smallest, have evolved several times independently and both size extremes are found in Middle America as well as in South America. The smallest size (< 100 mm SL) has evolved two times in the amphiphines, in the common ancestor of *Cryptoheros* – *Hypsophrys*/*Neetroplus* – *Amatitlania*, and in *Archocentrus centrarchus*. The amphiphine genera *Panamius* and *Chortiheros* also are small species below 150 mm SL. Among the herichthyines, *Thorichthys* and *Trichromis* are the genera with the smallest average body size, and the majority of their species are below 150 mm SL. Among the astatheroines *Rocio spinossissima* and *Herotilapia* are small-sized species below 150 mm SL. The smallest heroines in South America are in the genus *Australoheros*. The smallest species among the heroines include generalists, detritus-pickers, algae-eaters and sifters. The largest species among the heroines are on the other hand all specialized predators-piscivores of the genera *Parachromis* (*P. dovii*), *Petenia* (*P. splendida*) and especially *Kronoheros* (*K. umbriferus*).

3.21. Diversity of reproductive strategies

Similarly to the morphological characters the life-history traits related to reproduction form a set of correlated characters. Cichlasomatines and basal Amazonian heroines (*Hypselecara*, *Hoplarthus*, *Pterophyllum*, *Mesonauta*, *Uaru*, *Symphysodon*, *Heros*) have a *parental family* type (ŘÍČAN, 1999; STAWIKOWSKI & WERNER, 1998), which is characterized by almost no division in parental roles between the two sexes. Both sexes are dedicated to the same reproductive activities in very similar intensity. Both sexes clean the breeding substrate, fan (and sometimes clean) the eggs and larvae (in both actions they most often take turns), and guard the free-swimming offspring side by side. Furthermore both sexes are of similar size and equally coloured during breeding and guarding. The males are the slightly more active sex in initiating the pair bonding and the actual spawning (except in *Hypselecara*, *Mesonauta* and *Heros*). The *parental family* type is typical with nesting in the open without looking for natural or building artificial hidden nests.

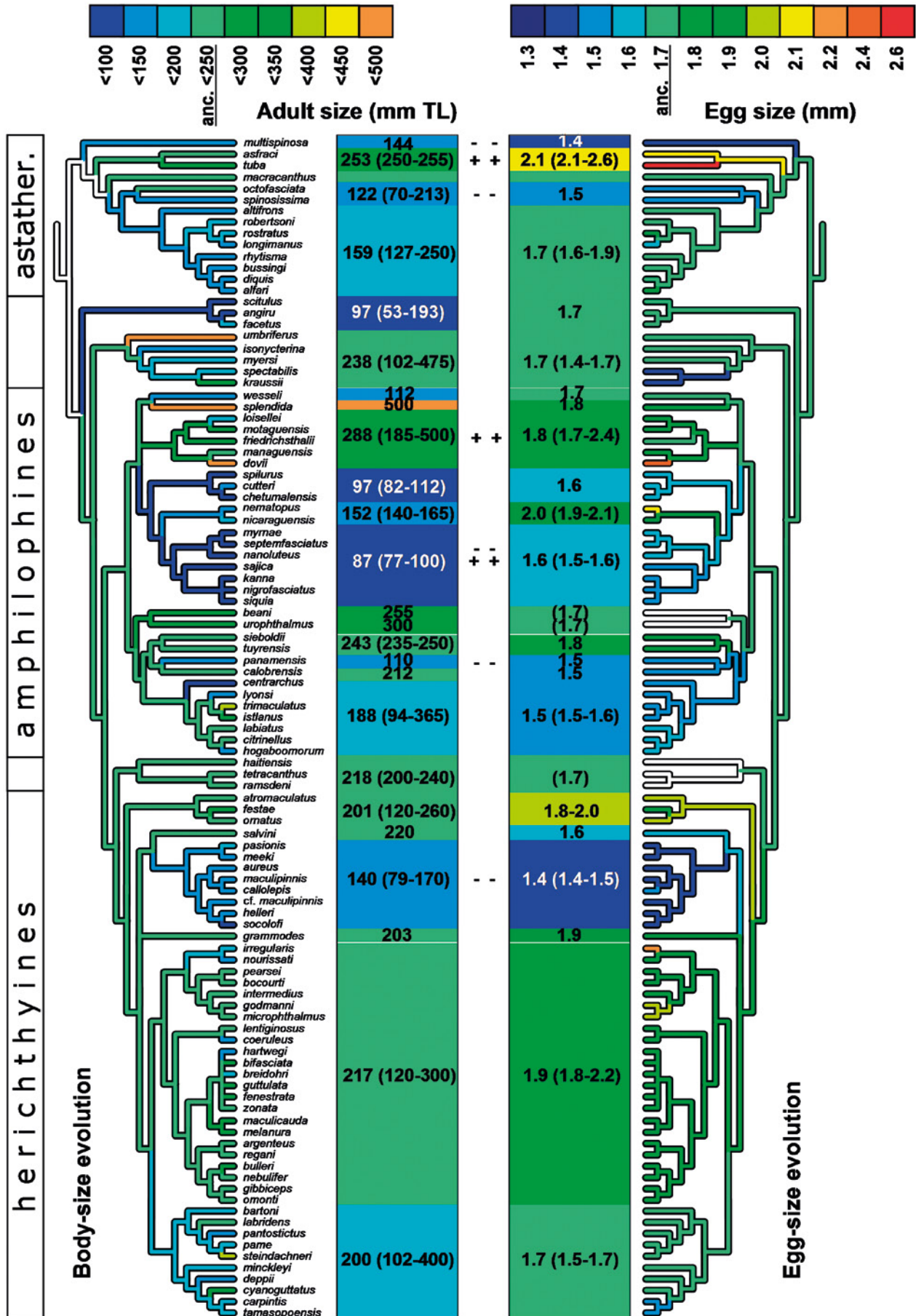
Most groups of Middle American heroine cichlids on the other hand have a *mother-father family* type with pronounced differences in parental roles between the two sexes. All groups in the Middle American cichlids clade have more differentiated parental roles than the cichlasomatines and most basal heroines. The female is of noticeably smaller size, but more colourful and/or more contrastingly coloured than the male. The female is the sex that best develops the species and group-specific breeding dress (see figs. 3, 24–25). The female initiates pair bonding and spawning, chooses the breeding substrate and spot, cleans it or otherwise prepares it for spawning, and spends much more time with the care of the eggs and offspring. In some groups the care of the eggs and offspring is solely the responsibility of the female and the male takes no part. The larger and less colourful male stays on the perimeter of the territory and guards it and the female with offspring from predators and intruders. The male (if ever) develops the breeding coloration only when the offspring start to swim (the female already immediately after spawning). The *mother-father family* type is typical in hidden nesting either by using natural holes, burrows, places under stones or fallen trees and branches or by actively building burrows or nest holes. They also have smaller egg clutches (500–100 eggs vs. 1000–2000) of larger eggs (usually approaching 2 mm).

There is variability in the degree of development of the *mother-father family* type among Middle American heroine cichlids. Amphiphine genera *Amphilophus*, *Amatitlania*, *Cryptoheros*, *Hypsophrys* and *Talamancaheros* are among the most advanced. Less advanced, but also with active females are species of *Parachromis*, which however (correlated to their large size?) have large egg clutches (1000–2000 eggs). Likewise, advanced herichthyines (e.g. *Theraps*, *Chuco*, *Paraneetroplus*) including *Trichromis* and *Chiapaheros* and also the Antillean *Nandopsis* have a *mother-father family* type with highly active, distinctly smaller and more contrastingly coloured breeding females (males are more active in detritivorous species that breed more in the open). *Herichthys* is quite variable (as in most characters including morphology), with highly active males, large clutches, and breeding in the open in e.g. *H. cyanoguttatus* and *H. carpintis*, but highly active females, small clutches, and breeding in natural or artificial burrows and other hidden places in e.g. *H. minckleyi*, *H. bartoni* or *H. labridens*.

The trans-Andean South American heroine cichlid genus *Mesoheros* is the only South American heroine genus with a strongly developed *mother-father family*, which supports its close phylogenetic ties with Middle American genera (ŘÍČAN *et al.*, 2013).

The least advanced genera among Middle American heroine cichlids which have only a slightly more ad-

→ **Fig. 28.** Comparisons between the mapping of body size and egg size. Maximum adult body size (usually the male) is based on STAWIKOWSKI & WERNER (1998), KULLANDER (2003) and the FishBase database. Egg sizes are averages based on our data and the cichlid egg project (<http://cichlidresearch.com/cichlideggsizedata.html>). There is an evident correlation between egg size and adult body size when comparing average egg sizes between the adult size categories (see text).



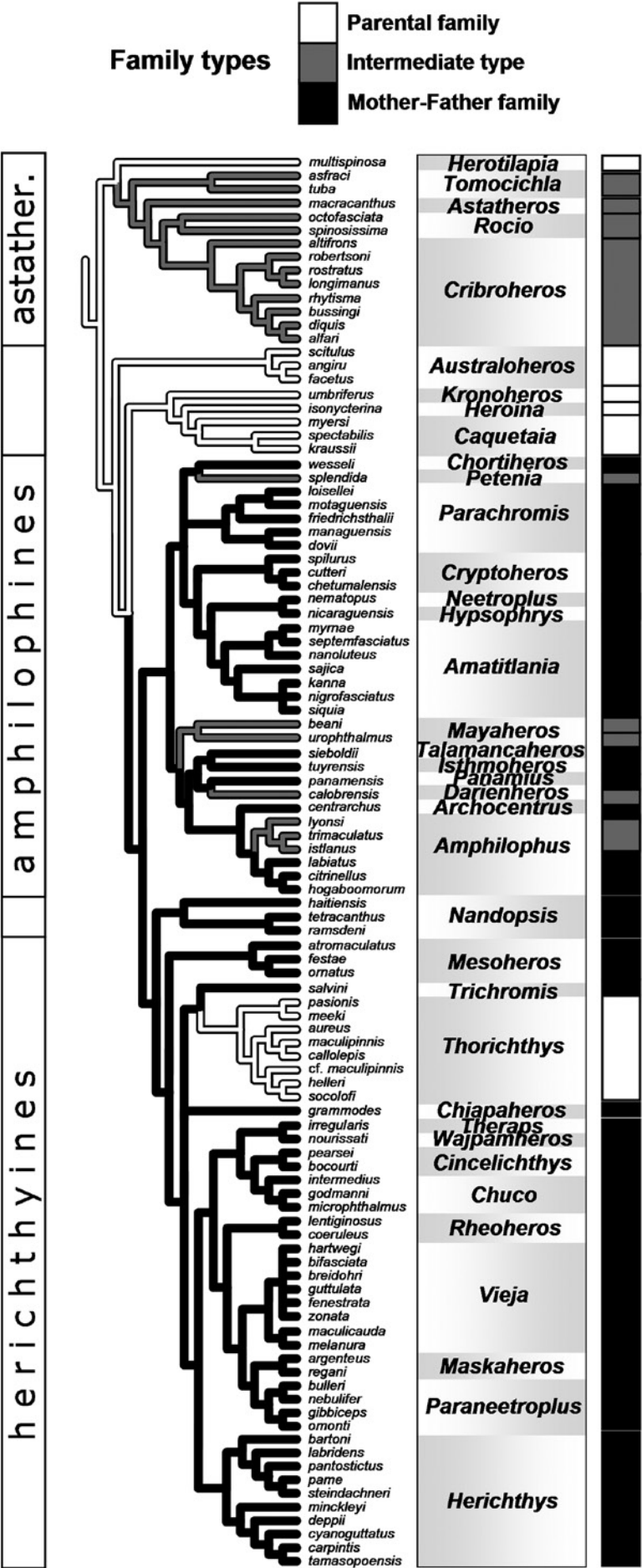


Fig. 29. Mapping of family types. Family types are well correlated with sexual size dimorphism. Males are larger in all species in the Middle American cichlid clade, but size differences are much more pronounced in the *mother-father family* type (males can be several times larger). In the parental family the sexes are virtually indistinguishable in body size and also in coloration.

vanced form of the *parental family* type include *Herotilapia*, *Thorichthys*, and the South American *Caquetaia*.

Herotilapia, which is traditionally placed with *Amatitlania* and *Cryptoheros*, is very different from these genera in having a *parental family* type with low differentiation of sex roles, no size or coloration dimorphism, and with similarly small body size has large egg clutches of 500–1500 eggs. Intermediate between the *parental family* and the *mother-father family* type are *Astatheros*, *Cribroheros*, *Tomocichla* and *Rocio*.

The astatheroines are thus in their ancestral family arrangement quite distinct from the well differentiated *mother-father family* type found predominantly in the amphiphines and herichthyines (and their related South American genus *Mesoheros* and Antillean genus *Nandopsis*). *Thorichthys* is the only genus among this clade that has (secondarily) the “ancestral” *parental family*. It is thus highly different from its clade and especially from its sister genus *Trichromis* which has possibly the most differentiated *mother-father family* with the most active females among all Middle American cichlids.

Sexual dimorphism in size and coloration, egg clutch size or the preferred type of spawning location are thus not independent characters in the heroine cichlids but are all correlates of the degree of division of roles between the sexes during reproduction.

3.22. Evolution of egg size

The above two types of family arrangements with their corresponding differences in the number and size of eggs could be associated with the r/K ecological continuum (r/K selection; see above). Alternatively the number and size of eggs could simply be the result of the maximum adult body size (of the female). In this case there should be a clear correspondence between maximum body size (see above) and the number and/or size of eggs in a clutch. While the data about both the maximum body size (in this case it is the size of the male) and the sizes of eggs are far from optimal, fig. 28 compares the distribution and evolution of the two life history parameters. Judging from casual observation, there does not seem to be a clear correlation between the two parameters. Species with very large eggs (e.g. *Tomocichla*, *Mesoheros*, *Theraps*, *Chuco*, *Neetroplus*, *Parachromis dovii*) include one of the smallest species (*Neetroplus*), one of the largest species (*Parachromis dovii*) and average-sized species. From the opposite point of view most of the largest species do not have the largest eggs (*Kronoheros* and *Petenia splendida* both have ancestral-sized eggs) and the smallest species (*Cryptoheros*, *Amatitlania*, *Australoheros*, *Archocentrus*) also do not have the smallest eggs, but they have smaller than ancestral sized eggs (except *Australoheros*). When comparing average egg sizes between the adult size categories used in figure 28 there is an evident correlation between egg size and adult body size (body size category (mm): egg size [min-max] (mm): < 100: 1.57 [1.4–1.7]; < 150: 1.62 [1.4–2.1]; < 200: 1.74 [1.4–2.2];

< 250: 1.77 [1.5–2.1]; < 300: 1.81 [1.4–2.6]. Larger body sizes than 300 mm have a too low number of species for comparisons. Disproportionally large eggs are thus found in *Hypsophrys/Neetroplus*, *Tomocichla*, *Mesoheros*, *Theraps*, and *Chuco*. These species would thus make likely candidates for K-strategy type. All except *Tomocichla* do agree with this classification since they have the mother-father family type, with highly active females, that are more colourful and much smaller sized, most prefer hidden breeding, and most also have small clutch sizes which are probably signifying the trade-off with increased egg size.

In summary, egg size distribution in Middle American cichlids correlates with body size and also with the r/K selection regime. The above chapters on evolution of coloration patterns and the related life history traits are meant as a summary of what is known with plausible hypotheses formulated about their evolutionary pathways. Tests of these hypotheses are in most cases deferred to subsequent publications when (and if) better life-history data become available.

There is at least one additional factor in connection with judging if a species is underlying r or K selection regime and that is the duration of brood-care. Unfortunately most knowledge about reproduction including brood care in Middle American cichlids comes from studies and observations in aquarium conditions where space limitations curtail the range of natural behaviour of the fishes. There are examples from the wild among the Neotropical cichlids from species with comparatively large eggs that have an extended time of brood care. For example some *Crenicichla* species may care for more than 6 months for their offspring and the offspring is then of an almost similar size to their parents! These protracted cases of parental care can however only be observed in natural conditions in the field and systematic studies have so far not been undertaken on this topic in the field for Middle American cichlids. It would however make a fascinating project and enrich our understanding of the Middle American cichlids. We speculate that the unique periphyton-grazing gregarious *Crenicichla* species (*C. tapii*, *C. hadrostroma*; PIÁLEK *et al.*, 2015) or the gregarious species with large eggs among Middle American cichlids have evolved their gregarious nature through this prolonged maternal care which can now be neatly tested by looking for parent-sibling genetic fingerprints (e.g. using the ddRAD method) in the schools of these cichlids.

Biogeographical evolution

3.23. Biogeographical context of Middle American cichlid diversification

The biogeographical analyses in S-DIVA were carried out using a number of different ‘maxareas’ options up to the maximum number of areas in the analysis. The re-

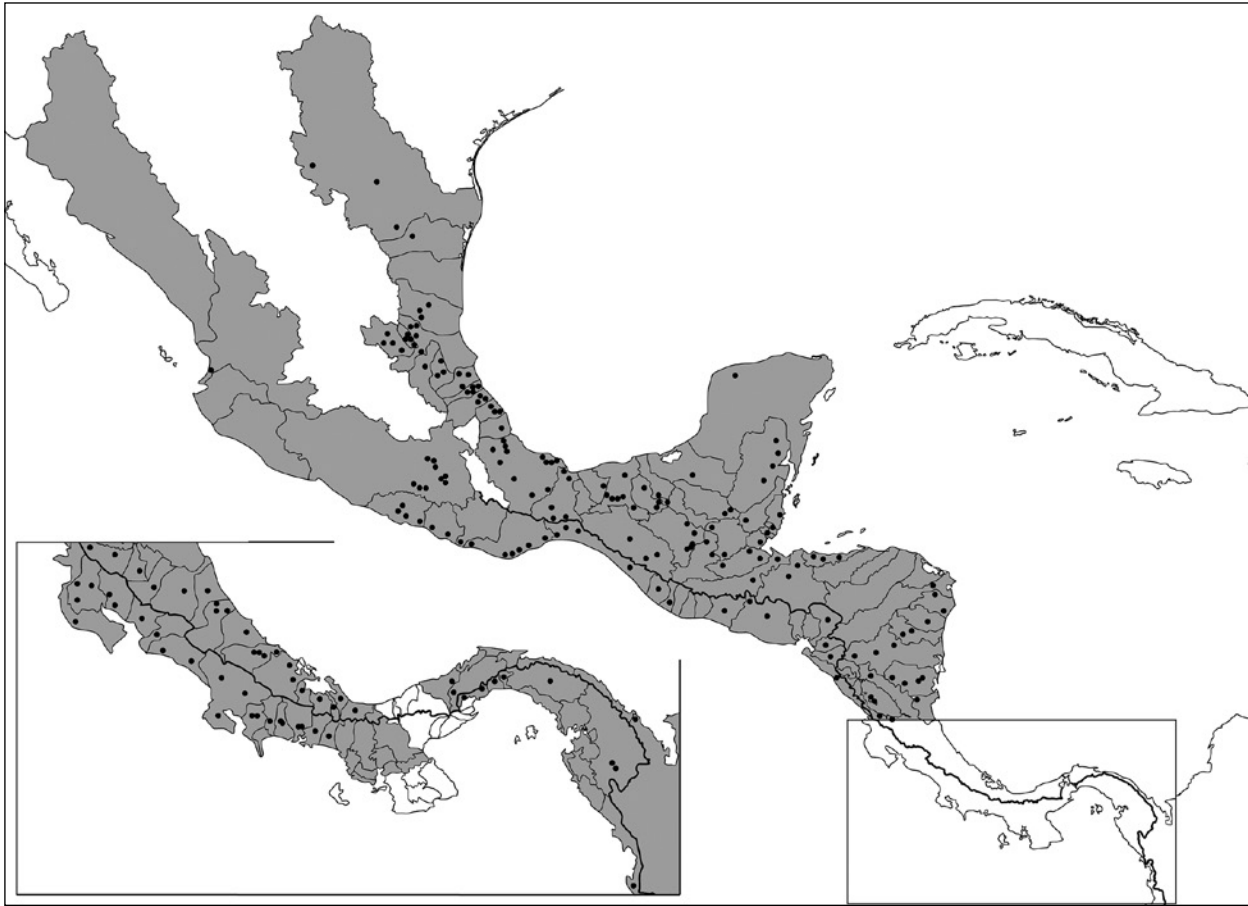


Fig. 30. Study localities of Middle American heroine cichlids in this study (dots) shown on a map of Middle America divided into river basins (see fig. 31). The shaded areas represent the native distribution of heroine cichlids in Middle America. One dot may represent more than one adjacent locality.

sults did not differ from the default ‘maxareas=4’ option because the majority of deep nodes in the reconstructions are dispersal events from two centres of origin (see below). The reported results are those with the default setting. The S-DIVA analysis was done in three steps (corresponding to the three Middle American clades, the herichthyines, the amphiphilines, and the astatheroines) because of the large number of coded areas that can not be analysed in one run.

The two species-richest CEAs of Middle America (the Usumacinta and the San Juan river basins; figs. 31–32; see comments below on the situation regarding the San Juan river basin) are reconstructed as the two ancestral areas of the whole diversification of cichlids in Middle America (fig. 32). The majority of cichlid diversification in Middle America thus appears to be a striking case of localized *in situ* evolution.

The two cichlid evolutionary centres in Middle America are very dissimilar in virtually all aspects of their cichlid evolution. These aspects include 1) the degree to which they have acted as faunal refugia, 2) their role in the colonization of the rest of Middle America, 3) their rate of faunal evolution, and 4) the degree of sympatric speciation and type of morphoecological divergence in their diversification.

The northern evolutionary centre (the Usumacinta river basin and CEA; area A in fig. 32) is based on our results the most diverse, largest and oldest continuously existing large-scale river basin for cichlid fishes in Middle America. The whole Usumacinta ichthyological province (IP) and the northernmost Neotropical Atlantic versant (genus *Herichthys*) were colonized from the Usumacinta river basin. The Usumacinta river is the ancestral area and evolutionary centre of the Middle American herichthyine cichlids (fig. 32). Fifteen crown-group herichthyine cichlids have evolved *in situ* in the Usumacinta river basin. The pace of evolution of the cichlid diversity in the Usumacinta CEA has been gradual (figs. 2–3).

The main ecomorphological dichotomy among genera in the *Theraps-Paraneetroplus* clade in the Usumacinta CEA are deep-bodied lentic detritivores vs. elongated rheophilic periphyton-scrappers (figs. 2–3, 7–9, 19–21). These two ecomorphologies were traditionally treated as two (*Vieja* vs. *Theraps*; fig. 19) or three (*Vieja* vs. *Theraps* and *Paraneetroplus*) distinct genera conforming to the ecomorphological dichotomy, but both ecomorphs have evolved several times in the clade and we classify each independent evolution of the ecomorphology as a separate genus. The ecomorphologically dichotomous genera in the *Theraps-Paraneetroplus* clade are macroscopi-

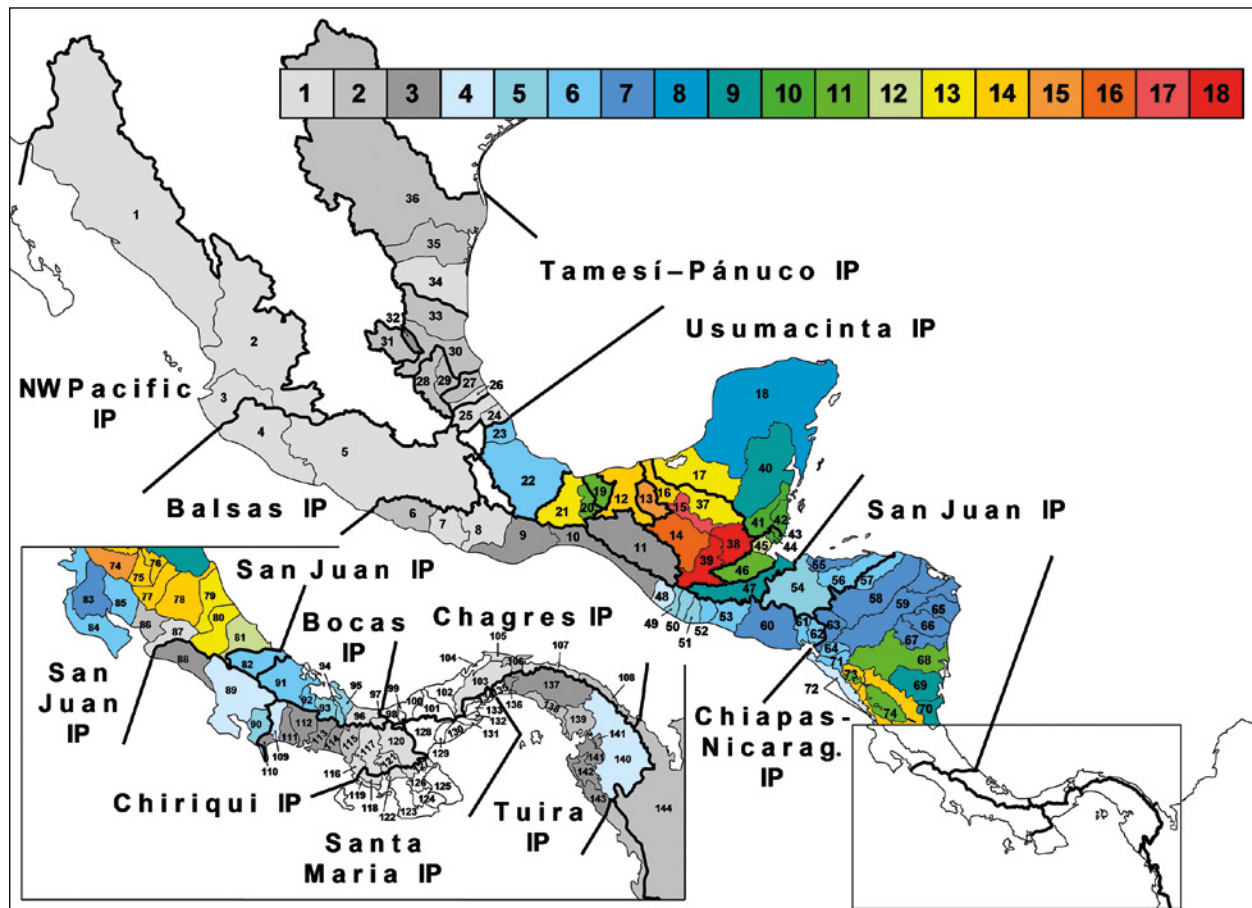
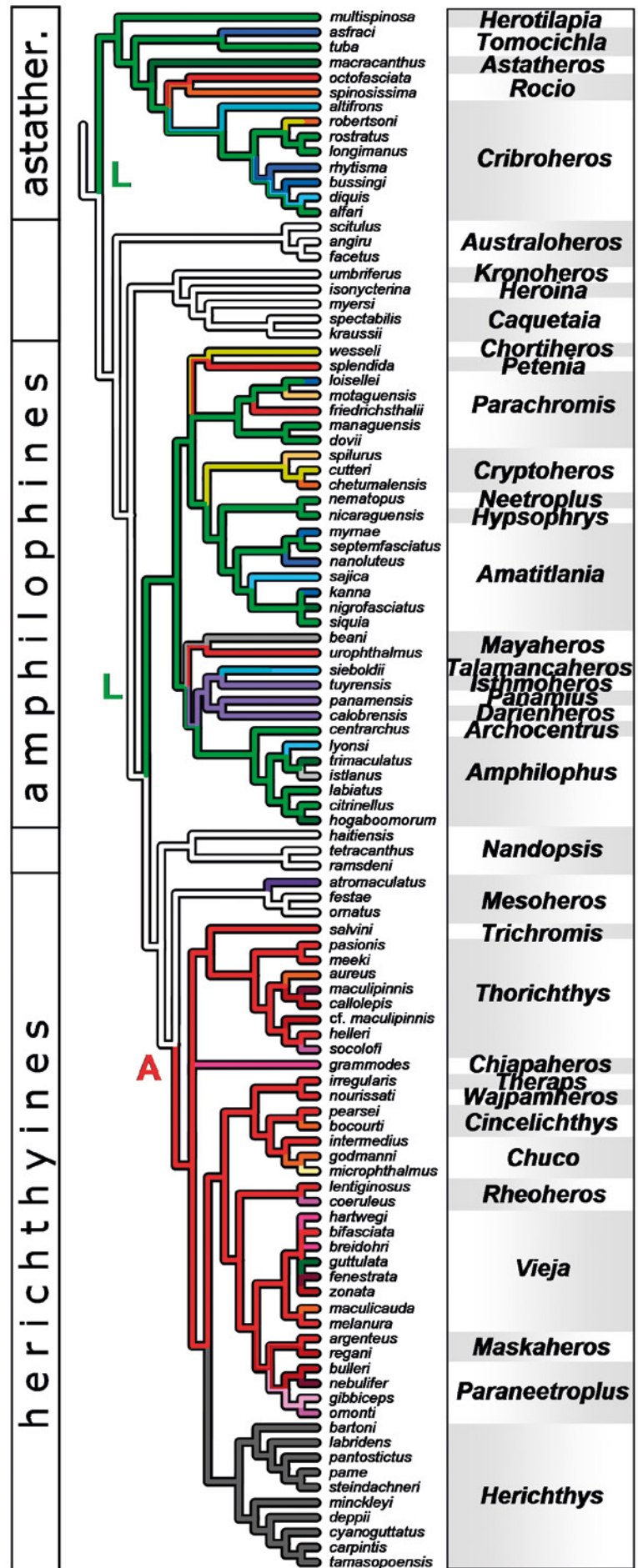
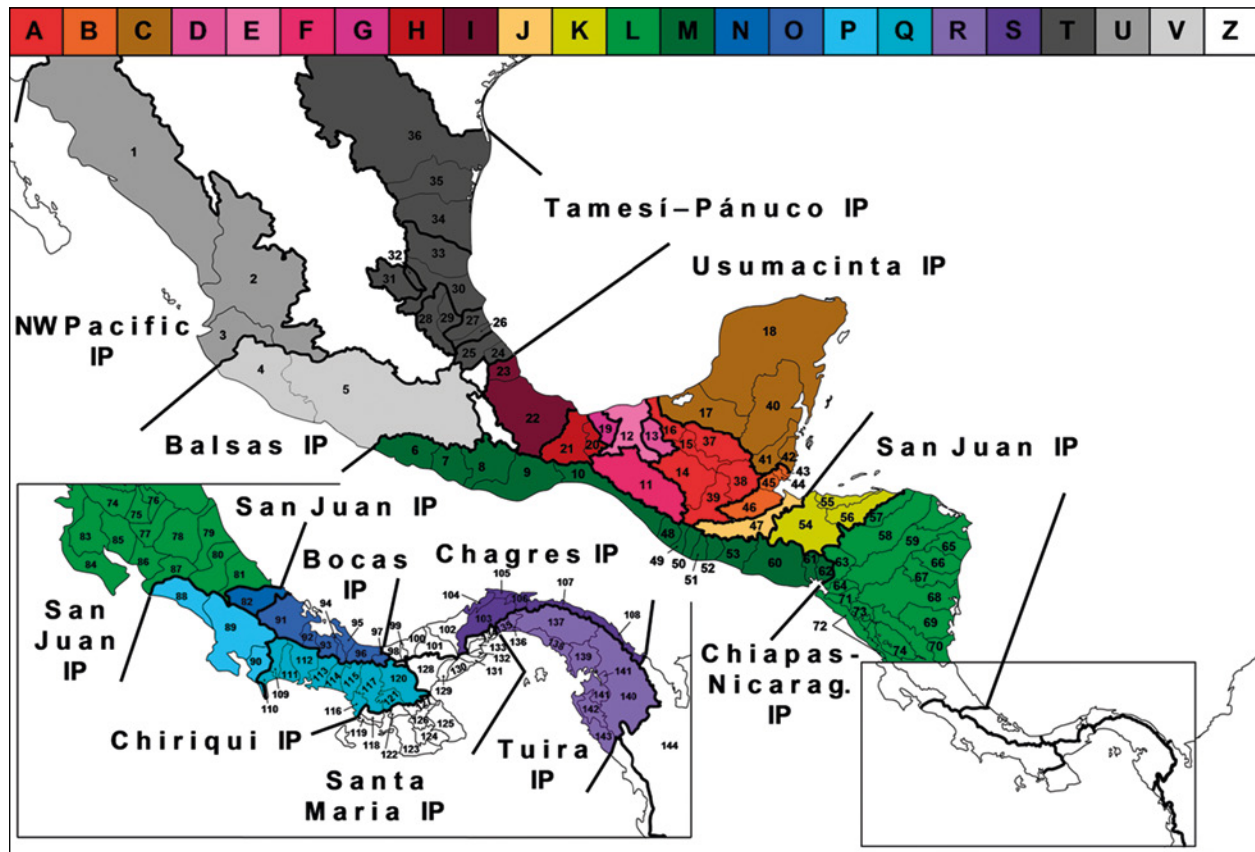


Fig. 31. Species diversity of heroine cichlids across all major drainages of Middle America (shown by numbers from 1 to 143 in map). The coloured scale with numbers at the top of the figure shows the number of heroine cichlid species per river drainage. Solid thick lines in map delimit ichthyological provinces (IPs) of Middle America (see Říčan *et al.*, 2013) and within the IPs areas of cichlid endemism (AEs) in Middle America which have been used as terminal units for the biogeographical analysis in this study. Legend to drainage basins. 1 NW Pacific M P; 2 Río Grande de Santiago M P; 3 Ameca M P; 4 Ameria Coahuylana M P; 5 Balsas M P; 6 Papagayo M P; 7 Sta Catarina M P; 8 Verde M P; 9 Tehuantepec M P; 10 Chiapas Nicaragua MGSN P; 11 Chiapa M A; 12 Mezcalapa Grijalva M A; 13 Tulija M A; 14 Lacantun M A; 15 Upper Usumacinta M A; 16 Lower Usumacinta M A; 17 Candelaria M A; 18 Yucatán M A; 19 Pedregal M A; 20 Uspanapa M A; 21 Coatzacoalcos M A; 22 Papaloapan M A; 23 Actopan M A; 24 Nautla M A; 25 Tecolutla M A; 26 Cazonas M A; 27 Tuxpan M A; 28 Moctezuma M A; 29 Tempoal M A; 30 Panuco M A; 31 Verde M A; 32 Gallinas M A; 33 Tamesi M A; 34 Soto la Marina M A; 35 San Fernando M A; 36 Bravo del Norte M A; 37 San Pedro GM A; 38 Pasion G A; 39 Chixoy Negro GM A; 40 Hondo MB A; 41 Belize GB A; 42 Maya mountains coast B A; 43 Monkey B A; 44 Deep B A; 45 Moho B A; 46 Polochic G A; 47 Motagua GH A; 48 Suchiate MG P; 49 Samala G P; 50 Nahualata G P; 51 Coyolate G P; 52 Maria Linda G P; 53 Paz Chalchuapa GS P; 54 Ulua H A; 55 Papaloteca H A; 56 Aguan H A; 57 Sico H A; 58 Patuca H A; 59 Coco HN A; 60 Lempa SH P; 61 Coascoran SH P; 62 Nacaome H P; 63 Choluteca H P; 64 Negro HN P; 65 Huahua N A; 66 Bambana N A; 67 Prinzapoloka N A; 68 Matagalpa N A; 69 Escondido N A; 70 Indio N A; 71 Estero Real N P; 72 Nicaraguan lakes side N P; 73 Lago Managua N; 74 Lago Nicaragua NC A; 75 Río Frio C A; 76 San Juan NC A; 77 San Carlos C A; 78 Sarapiquí C A; 79 Tortuguero C A; 80 Parismina C A; 81 Matina C A; 82 Sixaola C A; 83 Tempisque C P; 84 Nicoya C P; 85 Bebedero C P; 86 Barranca C P; 87 Tarcoles C P; 88 Pirris C P; 89 Terraba C P; 90 Coto C P; 91 Changuinola P A; 92 Guarumo P A; 93 Guariviara P A; 94 Cricamola P A; 95 Canaveral P A; 96 Int Cuenca 95 P A; 97 Calovebora P A; 98 Int Cuenca 99 P A; 99 Varaguas P A; 100 Int Cuenca 103 P A; 101 Cocle del Norte P A; 102 Int Cuenca 107 P A; 103 Chagres P A; 104 Piedras-Cascajal P A; 105 Claro-Frio P A; 106 Cuango-Mandinga P A; 107 Int Cuenca 121 (Azucar) P A; 108 Playon Chico-Acla P A; 109 Chiriqui Viejo P P; 110 Palo Blanco P P; 111 Escarea-Platanal P P; 112 Chiriqui P P; 113 Estero Salado P P; 114 San Juan-Felix P P; 115 Santiago-Tabasara P P; 116 Bubi P P; 117 Pablo P P; 118 Cate P P; 119 Quebrada Seca P P; 120 Santa Maria P P; 121 San Pedro P P; 122 Ponuga P P; 123 Tebario-Playita P P; 124 Tonosi-Cana P P; 125 Oria-Honda P P; 126 La Villa P P; 127 Parita P P; 128 Cocle del Sur P P; 129 Chorrera-Anton P P; 130 Farallon P P; 131 Chame P P; 132 Sajalices P P; 133 Capira P P; 134 Caimito-Grande P P; 135 Juan Diaz-Cabra P P; 136 Pacora P P; 137 Bayano P P; 138 Int Cuenca 150 P P; 139 Lara P P; 140 Tuira P P; 141 Int Cuenca 160 P P; 142 Samba P P; 143 Int Cuenca 164 P P; 144 Atrato Co A.

Country legend: M—México, G—Guatemala, B—Belize, H—Honduras, S—El Salvador, N—Nicaragua, C—Costa Rica, P—Panamá, Co—Colombia; A—Atlantic (Caribbean) drainage, P—pacific drainage.

Fig. 32 (pp. 92–93). Reconstruction of historical biogeography of the Middle American cichlid clade based on S-DIVA analysis. The ancestral area of each species was prior to this analysis reconstructed using the *population-level* analysis (ŘÍČAN *et al.*, in preparation). The reconstructed ancestral area of each species was then used as input area for the presented biogeographical analysis. The biogeographic reconstruction was however the same also when for each species its whole distribution area was used as input for the analysis. Middle American cichlids form three endemic clades that evolved in just two river basins. Colonization of Middle America is however more complicated and depends on the choice of geographical units used for analyses (ŘÍČAN *et al.*, in preparation). South American and Antillean (*Nandopsis*) genera are shown with white branches. Letter codes A to Z show cichlid endemic areas (CEAs) which were used for the biogeographic analysis: A) Usumacinta river basin; B) Polochic rb; C) Yucatan; D) Tulija rb; E) Lower Grijalva rb; F) Upper Grijalva rb; G) Pedregal rb; H) Coatzacoalcos rb; I) Papaloapan rb; J) Motagua rb; K) Ulua; L) San Juan; M) Chiapas-Nicaragua; N) Sixaola rb; O) Bocas; P) Terraba; Q) Chiriqui; R) Tuira; S) Chagres; T) Tamesí-Pánuco; U) North West Pacific; V) Balsas; Z) South America and Antilles.





cally largely sympatric (either in the Usumacinta or in the lower Grijalva) but at the local scale within each river basin are largely parapatric and rarely syntopic (unlike the amphiplophines in the San Juan IP; see below) with the lotic-scraping ecomorph typically that of the mountains while the lentic-detritivorous/herbivorous ecomorph that of the lowlands (see GPS coordinates of collecting records of species and corresponding ecomorphs in Supplementary material 1). A different, much older and completely sympatric lowland ecomorphological sister-group dichotomy is substratum-sifter vs. predator found at the genus level between *Thorichthys* and *Trichromis*.

The second cichlid evolutionary centre of Middle America is completely different except for the fact that it is also the most diverse river basin in its area, i.e. in Central America. The San Juan river basin is the most diverse part of the much larger San Juan ichthyological province and CEA. Contrary to the Usumacinta river basin in northern Middle America the San Juan river basin is not the largest river basin in Central America. In our analyses we treat the San Juan river basin (river basins no. 73–78 in figs. 31–32) as including also the separate rivers south from it on the Caribbean slope (river basins no. 79–81 in figs. 31–32). The latter river basins have at present sea levels separate mouths from the San Juan river but our faunal sampling of cichlids (they share the same set of species; unlike the river basins north or west from the San Juan river basin, see below; figs. 31–32) and biogeographic results (see below) show that during the majority of the Quaternary they were tributaries of

the San Juan river due to much lower average sea levels throughout that period. We thus treat the San Juan river basin as this palaeogeographical unit (which it was only 12 ka at much lower sea-levels) in agreement with the suggested PAE modification point 3 proposed by ROSEN (1988; see Methods).

The San Juan IP and CEA is the ancestral area from which most of the area of Central and Middle America was colonized by the astatheroines and especially by the amphiplophines (fig. 32). The astatheroines have their ancestral area undoubtedly in the San Juan IP and the amphiplophines most likely in the San Juan IP. We have further tested whether the San Juan river basin is the ancestral area within the San Juan CEA by dividing the IP into three parts; (1) the San Juan river basin; (2) the rest of the northern Caribbean slope of the San Juan CEA termed by geologists the Chortis block; and (3) the Pacific slope part of the San Juan CEA (results not shown). The Chortis block is geologically by far the oldest part of the San Juan CEA and would be expected as ancestral instead of the geologically much younger San Juan river basin. In the astatheroines the ancestral area is undoubtedly the San Juan river basin itself (as delineated here), not the Chortis-block part of the San Juan IP. In the amphiplophines only the northernmost part of the Chortis block in Honduras that is a separate CEA (area K in fig. 32) is among the ancestral areas in one amphiplophine clade. The Chortis block part of the San Juan CEA (in Nicaragua) has no endemic cichlid species and was only colonized at the species level. In the amphiplophines as in

the astatheroines the San Juan river basin itself (not the Chortis-block part of the San Juan IP) is the ancestral area.

The main ecomorphological dichotomy among the sympatric sister clades of amphiloophines in the San Juan CEA are large to very large predators versus small-sized detritivores (*Parachromis* vs. *Cryptoheros-Hypsophrys/Neetroplus-Amatitlania* ancestor), elongated lotic detritivores-scrapers versus small-sized lentic detritivores (*Cryptoheros-Hypsophrys/Neetroplus-Amatitlania* ancestor vs. *Hypsophrys/Neetroplus*), periphyton-scraper versus omnivore-detritivore (*Neetroplus* vs. *Hypsophrys*), and generalized predators-sifters versus small-sized lentic detritivores (*Amphilophus* vs. *Archocentrus*). Unlike the situation with the one main ecomorphological dichotomy in the herichthyines (the *Theraps-Paraneetroplus* clade) in the Usumacinta (and lower Grijalva) river basin the representatives of the ecomorphological dichotomies in the amphiloophines in the San Juan river basin are completely syntopic.

An interesting biogeographical discovery of the ddRAD phylogeny is the existence of an Isthmian clade grouping all amphiloophines cichlids found in eastern Panamá. The group is morphologically quite heterogeneous and dissimilar (the sifter *Darienheros*, the large detritivore *Isthmoheros*, the small detritus-picker/herbivore *Panamius*) and a close relationship of these species was never before hypothesized. The clade must first have colonized eastern Panamá from Central America and has then secondarily populated Central America by *Talamancaheros* which is found in western Panamá and eastern Costa Rica.

4. Discussion

4.1. Repeated evolution of ecomorphologies and a solution for the generic classification of the Middle American cichlid clade

The Middle American heroine cichlids are perhaps the most morphologically and ecologically diverse clade of Neotropical cichlids, with species that specialize on specific resources and habitats (COCHRAN-BIEDERMAN & WINEMILLER, 2010; SORIA-BARRETO AND RODILES-HERNÁNDEZ, 2008; WINEMILLER *et al.*, 1995). WINEMILLER *et al.* (1995) concluded that local fluvial assemblages of Middle American heroine cichlids show greater diversification of functionally significant morphological traits than comparable assemblages of South American or African cichlids.

The morphological phylogenies of Middle American heroine cichlids attest to the strong influence of adaptive characters by grouping ecomorphs as clades (CASCIOITTA & ARRATIA, 1993a,b; CHAKRABARTY, 2007; ŘÍČAN *et al.*, 2008; fig. 6). CASCIOITTA & ARRATIA (1993a,b) and

CHAKRABARTY (2007) demonstrate that even distantly related taxa of the same ecomorph (e.g. piscivores) are grouped in morphological phylogenies together. Their phylogenies group piscivorous heroines (*Petenia*, *Caquetaia*), the one piscivorous cichlasomatine (*Acaronia*), piscivorous geophagines (*Crenicichla*) and basal Neotropical piscivores (*Cichla*) in one clade.

Based on our examination not only the piscivorous morphologies but virtually all morphological characters of the Middle American cichlids are expressions of ecological adaptations (ecomorphological characters). Most morphological characters are thus correlated with ecology, between each other, and thus form repeatedly evolving ecomorphs as supported by the molecular phylogenies. Very few unquestionable synapomorphies of genera and higher clades were thus found among the morphological characters.

Our analyses demonstrate that all Middle American cichlid clade species can be classified into one of the five main cranial (generalized pickers, predators-piscivores, substratum sifters, detritivores-herbivores, and periphyton scrapers) and three postcranial (lotic, lentic, and the ancestral character combination) ecomorphs. Both the cranial and postcranial ecomorphs have independently evolved several times and even their combinations in several cases. Except for the periphyton scrapers and the detritivores-herbivores cranial and postcranial ecomorphs are freely combinable. The scrapers are always found in combination with a lotic postcranial. The detritivores are virtually always found in combination with the lentic ecomorph. The lotic and lentic postcranial ecomorphs are two opposites of an adaptive continuum. On the one hand the lotic postcranial ecomorph is characterized by elongation of the body and the caudal peduncle to increase fast and continued swimming performance while the anal fin shows decrease of the number of fin spines at the same time. The lentic ecomorph on the other hand shows shortening and deepening of the body, shortening of the caudal peduncle which may reduce escape possibilities but this is compensated by increase in the number of anal fin spines (dorsal fin spines are correlated primarily with body length and only secondarily have a common patterning with the anal fin). The increase in the number of anal fin spines that always accompanies the shortening of the caudal peduncle is interpreted here as a possible antipredatory adaptation.

Out of the fifteen possible combinations of the five cranial and three postcranial ecomorphs the diversity of the Middle American cichlid clade can be characterized by thirteen different combinations. The two combinations that were not encountered are lentic periphyton scrapers and postcranially ancestral scrapers. The periphyton scrapers thus clearly require highly adapted postcranials capable of excellent swimming performance. Three cranial-postcranial combinations were each found in only one genus: the lotic substratum sifter *Wajpamheros*, the lentic substratum sifter *Thorichthys*, and the postcranially ancestral detritivore *Astatheros*. These three genera thus have a unique cranial-postcranial ecomorph com-

bination. On the other hand the most commonly found cranial-postcranial ecomorph combinations are the lentic detritivores-herbivores and the lotic scrapers. These two ecomorphs with a substantial amount of vegetable matter in their diet are thus the most genus and species rich cichlids in Middle America.

The correspondence between the number of separate cases of evolution of the ecomorph combinations and generic classification is a debatable matter. There is however an obvious solution for the Middle American cichlid clade and that is to treat all the independent cases of evolution of these ecomorph combinations as independent genera. This solution is in agreement with all the primary TNCs of VENCES *et al.* (2013) and also complies nicely with the secondary TNCs of Adaptive zone and Biogeography, because the ecomorph combinations occupy different adaptive zones and are also in most cases biogeographically justified. Virtually all genera in the Middle American cichlid clade can thus be easily diagnosed by their cranial-postcranial ecomorph combination (Phenotypic diagnosability and Adaptive zone *sensu* VENCES *et al.*, 2013) in conjunction with geographical distribution (biogeography).

Our analyses of the diversity of Middle American cichlid clade support the existence of 31 genera in Middle America (plus six in South America and one in the Greater Antilles) as separate evolutionary lineages occupying separate adaptive zones. Nine new genera are described here for species and monophyletic species groups that are demonstrably unique evolutionary lineages occupying separate adaptive zones

Our classification includes nineteen monotypic genera (in the phylogenetic order of figs. 5, 9–12, 14–17, 20, 22–29, 32: *Herotilapia*, *Astatheros*, *Kronoheros*, *Heroina*, *Chortiheros*, *Petenia*, *Neetroplus*, *Hypsophrys*, *Talamancaheros*, *Isthmoheros*, *Panamius*, *Darienheros*, *Archocentrus*, *Trichromis*, *Chiapaheros*, *Theraps*, *Wajpamheros*, *Oscura*, *Kihnichthys*). These are in all cases (except for *Theraps/Wajpamheros*, *Kihnichthys* and *Neetroplus/Hypsophrys*) lineages with very long independent evolution of more than 10 My (average 18 My, min-max 10.3–25.9 My; *Theraps/Wajpamheros*, *Kihnichthys* and *Neetroplus/Hypsophrys* have 5.4 My, 7.5 My and 8 My, respectively; based on ŘÍČAN *et al.*, 2013). Very long isolation in both space and time is also present between the two lineages of *Mayaheros*, but these are of the same ecomorph combination and we feel that splitting them into two genera is not justified. *Mayaheros* species are the morphologically most plesiomorphic species among the amphiphilophine clade and this genus is not possible to diagnose using an apomorphic morphological character combination, but the phylogenetic position of *Mayaheros* precludes its combination with another genus (the most similar is *Amphilophus*).

The *Theraps-Paraneetroplus* clade is surprisingly young (based on time estimates from ŘÍČAN *et al.*, 2013) considering its high number of species. The average age of genera in the *Theraps-Paraneetroplus* clade is 7.3 My (min–max 5.1–10.6 My). The average age of Middle

American genera outside the *Theraps-Paraneetroplus* clade is 16.2 My (min–max 8–26.3 My).

The average age of monotypic and of non-monotypic genera of Middle American cichlids is the same (13.2 *vs.* 13.6 My; min–max 5.4–26.3 *vs.* 5.1–24.2 My) while the average age of genera among the Middle American cichlid clades is different. In the astatheroines the average is 24.4 My (min–max 21.6–26.3 My), among the amphiphilophines is 12.2 My (min–max 8–18.2 My) and among the herichthyines is 10.6 My (min–max 5.1–20.2 My).

4.2. Biogeography trumps morphology as indicator of phylogenetic relationships

Geographical distribution can often be a better predictor of phylogenetic relationships than is morphological similarity, especially in highly adaptable groups of organisms because natural selection can encourage evolution of parallel forms in allopatry as is the case in the Middle American cichlid clade.

All the here newly described genera among both the amphiphilophines and the herichthyines perfectly fit in their biogeography with their phylogenetic relationships. Among the amphiphilophines *Chortiheros* is the geographically most localized genus and it is the only evolutionary lineage endemic to the reconstructed colonization area of Central America in the north of the San Juan province (ŘÍČAN *et al.*, 2013). Biogeography thus nicely predicts that *Chortiheros* is neither a *Theraps* nor a herichthyine (the herichthyines and the *Theraps-Paraneetroplus* clade are all endemic to the Usumacinta ichthyological province). Similarly *Isthmoheros tuyrensis* is not an extralimital species of *Vieja*, *Talamancaheros sieboldii* is not one of *Theraps* or *Paraneetroplus*, *Darienheros calobrensis* is not one of *Astatheros* or *Criboheros*, *Panamius panamensis* is not one of *Archocentrus* or *Cryptoheros*, but all Isthmian amphiphilophine genera rather form one clade. Similarly the phylogenetic placement of the ancient genera *Trichromis* and *Chiapaheros* as basal genera among the herichthyines is fully in agreement with their biogeography in the Usumacinta ichthyological province of North America. Similarly *Wajpamheros nourissati* is not an *Astatheros* (or *Amphilophus* as in KULLANDER, 2003, MILLER *et al.*, 2005) as would be superficially suggested by its cranial morphology but is indeed the sister group of *Theraps* (it was indeed described originally as *Theraps nourissati*) again in agreement with biogeography. Similar examples in which morphology was misleading and biogeography is a better indicator of phylogenetic relationships are *Rocio spinosissima* (placed in *Archocentrus* by SCHMITTER-SOTO, 2007a,b) or *Nandopsis ramsdeni* (placed in '*Cichlasoma*' by KULLANDER, 2003). One of the longest controversies in Middle American cichlid classification was about the synonymy of *Caquetaia* and *Petenia* and hence the homology of their piscivore-adapted head morphology (see ŘÍČAN *et al.*, 2008). Biogeography again suggested that their head morphology

is adaptive and convergent, in agreement with molecular phylogeny.

4.3. Repeated evolution of ecomorphologies and adaptive radiations

The three main reasons for the past struggle in classifying the Middle American cichlids are repeated evolution of ecomorphologies, reticulate evolution and among the amphiloophines additionally fast adaptive radiation.

Repeated evolution of ecomorphologies and adaptive radiations seem to be the main reasons for the unprecedented diversity of the Middle American heroine cichlid fishes (LÓPEZ-FERNÁNDEZ *et al.*, 2013; ŘÍČAN *et al.*, 2013, this study). The whole diversity is however clearly not the result of a single radiation (contrary to LÓPEZ-FERNÁNDEZ *et al.*, 2013) because the Middle American heroines are not monophyletic, but are the result of two independent colonizations. The three separate diversifications (radiations) took place in only two limited areas and possibly in only two river basins of Middle America (in the Usumacinta and the San Juan ichthyological provinces) while the rest of Middle America is characterized by allopatric evolution (this study and ŘÍČAN *et al.*, 2013). The herichthyine and astatheroine diversifications are not true fast adaptive radiations because we see a slow gradual gain of diversity (this study and ŘÍČAN *et al.*, 2013) while fast adaptive radiation is only evident in the amphiloophines. All three diversifications however took place within the two limited geographical areas and thus likely included diversification in at least partial sympatry fulfilling the criterion of adaptive radiation (SCHLUTER, 2000; SCHLUTER & NAGEL, 1995; see below). The three adaptive radiations in Middle American cichlids are ancient and now completed but on a smaller temporal and spatial scale we still have the chance to study active ongoing adaptive radiations in the small crater lakes of Nicaragua (BARLUENGA *et al.*, 2006; BARLUENGA & MEYER, 2004; GEIGER *et al.*, 2010, 2013; SCHLIEWEN *et al.*, 2006) and in the genus *Herichthys* with its polymorphic species and sympatric species-pairs.

The only other example so far known among the Neotropical cichlids apart from the amphiloophines that also fulfils all criteria of cichlid adaptive radiation are the two *Crenicichla* species flocks of the Iguazu and Uruguay river basins of SE South America (PIÁLEK *et al.*, 2012; BURRESS *et al.*, 2013).

The diversification of the herichthyine cichlids is in their lack of rapid speciation more similar to the most widely publicized “adaptive radiation” in the Neotropical cichlids, that of the geophagine cichlids (LÓPEZ-FERNÁNDEZ *et al.*, 2010, 2013). Several crucial differences however still distinguish these two diversifications and show that the herichthyine cichlids are much closer to the definition of adaptive radiation than are the geophagine cichlids. The differences are 1) the degree of ancestral sympatry, 2) the degree and geographical extent of sympatry of sister species, and 3) the ecomorphological homogeneity

of species within genera. 1) The herichthyines have diversified in situ in partial or complete sympatry in just one small area (the Usumacinta river basin) of Middle America. For the geophagines a biogeographical analysis of their initial diversification has never been published but the present area of sympatry at the genus level is extremely large and represents basically the whole Amazon. This suggests a very large ancestral area. 2) Sister-species (and even sister-genera) among the herichthyines very often occupy the same river basins, which suggests sympatric or parapatric speciation. On the contrary virtually all geophagine sister species are allopatric (with the exception of some *Crenicichla*) occupying different, most often adjacent river basins, which strongly suggests allopatric speciation. These differences suggest that the dominant speciation mode among the geophagines is allopatric speciation, which is not triggered by biological phenomena, and all adaptation thus occurs secondarily and is not the primary trigger of diversification. 3) The geophagines do not show repeated evolution of identical ecomorphologies, most ecomorphologies are restricted to single genera. On the other hand ecomorphologies have evolved repeatedly among the herichthyine cichlids (the genera are not ecomorphologically unique) which supports phenotype-environment correlation and trait utility as the main driving forces.

The diversification of the geophagines has been proposed as an important case of adaptive radiation in the Neotropics even though they do not fulfil one of its important criteria, i.e. rapid speciation (LÓPEZ-FERNÁNDEZ *et al.*, 2013 *contra* LÓPEZ-FERNÁNDEZ *et al.*, 2010). The geophagine cichlids also do not qualify as a species flock (or multiple species flocks) which is the typical form of cichlid adaptive radiations. Our comparisons show that there is nothing special about the diversification of the geophagine cichlids and that Middle American cichlids include much better examples of adaptive radiations with a much higher degree of in situ (or even sympatric) evolution and much faster radiations (the amphiloophines). Since the South American geophagines (and the Middle American herichthyines) do not fulfil the criteria of fast adaptive radiation they should not be associated with this phenomenon.

The Middle American cichlids are very different from the geophagines and from all other Neotropical cichlid clades. They are the only Neotropical cichlids that show so many times repeated evolution of ecomorphologies (classified here as 31 separate genera) and they are the only Neotropical cichlids with such a striking diversity pattern in terms of species/area relationships. The morphospace occupied by the Middle American heroine cichlids is comparable to that of the geophagines despite only ca 15% area available for the diversification of heroines in Middle America compared to tropical South America (e.g. Geophagini). When we leave aside the largest and most modified Neotropical cichlid genus (*Crenicichla*) which occupies about a half of the total morphospace of the geophagines (LÓPEZ-FERNÁNDEZ *et al.*, 2013) the Middle American heroine cichlids actually have the larg-

est diversity of morphologies of all Neotropical cichlid clades (LÓPEZ-FERNÁNDEZ *et al.*, 2013). Nowhere else in the Neotropics can so many closely related yet ecomorphologically so different species be found within single small areas of endemism (river basins) in sympatry as in Middle America. There are two such diversity peak-areas in Middle America (in the Rio Usumacinta with ca 66.000 km² and in the Rio San Juan basin with ca 40.000 km²). Our biogeographical analyses additionally show that these two river basins are the evolutionary centres of the Middle American cichlid diversification (not just depositories or refugia of diversity) and that the Middle American cichlid diversity is primarily derived from evolution in just two river basins.

4.4. Comparisons of the Middle American cichlid diversification to lacustrine diversifications in Africa

The diversity of cichlid fishes in Middle America appears to be more similar to the diversified cichlid faunas of the Great African Lakes than to most riverine cichlid assemblages. This is even strengthened by the fact that compared to most riverine cichlid assemblages in Africa or in South America the Middle American cichlid faunas are not the result of continental-wide cichlid diversity but are the result of two coincident colonizations by one cichlid tribe. Among the Great African Lakes Lake Tanganyika (reviewed, e.g., in KOBLMÜLLER *et al.*, 2008) appears to be the most similar to the Middle American cichlid diversification.

Firstly, the Middle American cichlid diversification with ~ 120 valid species actually approaches the species diversity in Lake Tanganyika (the third most diverse lake after Malawi and Victoria with ~ 200 valid and ~ 250 estimated species) and surpasses radiations in all other lakes in Africa (lakes Kyoga and Edward/George have ~100 and ~ 60 species, respectively; TURNER *et al.*, 2001).

Secondly, the large species diversity in both ecosystems appears to be due to diversification by natural selection of feeding ecomorphologies. The tribes of Lake Tanganyika cichlids and the genera of Middle American cichlids are limited to particular ecological niches. The genera of Lake Tanganyika cichlids and of Middle American cichlids are thus primarily ecomorphologically delineated. The repeatedly evolved ecomorphologies (between but also within the three main clades) in Middle American cichlids which we classify as genera are thus to a large extent equivalent and comparable to repeatedly evolved ecomorphologies (also classified as genera) between (and also within) the tribes of the Tanganyikan cichlids. The spectrum of ecomorphologies in Middle America is of course much lower than in Lake Tanganyika (lacking e.g. the very specialized scale-eaters or mouthbrooders) but it is surprisingly high for riverine cichlids. The Middle American cichlid diversification lacks all the pelagic ecomorphologies (e.g. predators-piscivores as in Hemibatini and Bathybatini, pelagic

plankton-feeders as in Trematocarini, Benthochromini, or Cyprichromini) for obvious limitations of the riverine habitats but the shore-dwelling Lake Tanganyika cichlid diversity is rather comparable to the Middle American riverine cichlid diversity which WINEMILLER *et al.* (1995) found as the most diverse riverine cichlid faunas both in Africa and in the Neotropics. The shore-dwelling Lake Tanganyika cichlids include several scraping groups associated with rocky shores (Eretmodini, Tropheini, Ectodini), sandy/muddy bottom detritivores/sifters (e.g. Limnochromini) and also predators/piscivores (e.g. Boulengerochromini, Cyphotilapiini, Lamprologini) and all these main ecomorphs are also very common and have repeatedly evolved in the Middle American cichlids.

Thirdly, while none of the two diversifications (Lake Tanganyika and Middle America) is monophyletic they are both composed of several independent invasions that were followed by diversification in a circumscribed biogeographical setting, the Lake Tanganyika cichlids in a lacustrine environment and the Middle American cichlids in only two drainage basins. We argue that the three Middle American parallel diversifications are primarily riverine diversifications in two drainage basins, but a lacustrine diversification in the geological past cannot be at present excluded based on scant geological knowledge.

Several authors suggested (reviewed, e.g., in SEEHAUSEN, 2006) that cichlid radiations are usually associated with truly lacustrine conditions. Several indications from the Neotropics (especially the *Crenicichla* species flocks of the Iguazu and Uruguay rivers) including the presented results for the Middle American cichlids however suggest that only the most spectacular and species-richest cichlid radiations are truly lacustrine in origin. Smaller radiations are now known from both smaller lakes and from riverine habitats. The diversified riverine haplochromine (serranochromine) fauna in southern Africa has been suggested to have possibly originated in the paleo-Lake Makgadikgadi (JOYCE *et al.*, 2005), but this argument was heavily based on this supposition that cichlid radiations are usually associated with lacustrine conditions. Our results weaken this association between cichlid radiations and lacustrine conditions.

5. Conclusion

We present a taxonomically complete and topologically robust molecular phylogeny of the Middle American heroine cichlids based on which we review their diversity and genus-level systematics. In order to ascertain the diversity of the group and its phylogeny we have used three nested taxon sampling analyses of the concatenated nDNA/mtDNA datasets and additionally to these analyses we present a summary of the results of a new Next Generation Sequencing-generated nuclear phylogeny based on a data set of ~ 140 000 informative characters.

The NGS ddRAD phylogeny has a species-level sampling covering virtually all species (including the enigmatic *Cichlasoma microlepis* DAHL, 1960) with multiple sequenced specimens per species. Based on our results the Middle American heroine cichlids are made up of three main clades. The three clades (the herichthyines, the amphiphophines, and the astatheroines) are however not each other sister groups since they are interspersed with South American (*Australoheros*, *Caquetaia*, *Mesoheros*) and Antillean (*Nandopsis*) genera and represent two separate colonization events of Middle America from South America, probably via the Antilles. Our study reveals many cases of cytonuclear discordance and/or introgressive hybridization both at the genus and deeper levels stressing the importance to study the nuclear and mitochondrial phylogenetic signals independently and not solely in concatenated analyses. We have analysed all morphological characters previously used in describing the diversity and phylogeny of the Middle American Cichlidae. We have discovered that a great majority of morphological characters are ecologically correlated and that they form only a limited number of functionally-determined combinations – i.e. ecomorphs. This is true for both the cranial as well as the postcranial characters. The cranial ecomorphs are determined by food choice, while the postcranial ecomorphs are determined by habitat characteristics. The long-perused character regarding the anal fin spine number is also part of this ecological determinism of morphological characters as it is strictly negatively correlated with the length of the caudal peduncle. The morphological characters are not only correlated with each other and with ecology, but they also in the majority of cases show concerted evolution. We have found five major cranial ecomorphs but only two postcranial ecomorphs (the lotic and lentic ecomorphs, plus the undifferentiated ancestral character combination). The cranial and postcranial ecomorphs are not combined completely randomly and out of fifteen possible combinations have produced thirteen modular whole-body ecomorphs. Both the cranial and postcranial ecomorphs, and even their combinations, have evolved repeatedly in the Middle American cichlids in the same habitats in sympatry as well as in allopatry. The evolutionary uniqueness of genera thus cannot be determined without the knowledge of phylogeny because of the character dependence with ecomorphs which has been the reason for the long struggle in classifying the Middle American cichlids into genera. In many cases molecularly identified unique evolutionary lineages (genera) have very few characters that enable independent diagnosis of parallel genera. Often the main distinguishing characters are found in coloration patterns (and biogeography), and here especially in the ontogeny of coloration patterns and in breeding coloration. However we have still found some indications that even the breeding dresses are under ecological and not only sexual selection. There is a strong correlation between the clear-water lotic ecomorph and a contrasting white-dominated breeding coloration, while the turbid-water lentic ecomorph is characterized by a more obscure

breeding coloration. This lentic-lotic breeding dress dichotomy exists irrespective of genus-level affinities at the species level. The lentic-lotic dichotomy is most rampant within the terminal herichthyines (the genera in the *Theraps-Paraneetroplus* clade) where the lentic-lotic ecomorph dichotomy was responsible for great confusion in genus-level classification and very likely also for the unparalleled species diversity within this clade due to repeated evolution of the lotic and lentic ecomorphs. On the contrary we have not been able to find ecological correlates for the diversity found in the coloration pattern ontogenies and this character set thus remains the only one known to us that appears free of the ecological determinism dominant in the evolution on the Middle American cichlid fish diversity. Our analysis of the diversity of Middle American cichlid clade supports the existence of 31 genera in Middle America (plus six in South America and one in the Greater Antilles) as separate evolutionary lineages occupying separate adaptive zones. Nine new genera are described here for species and species groups that have lacked a genus level name to this day or were associated with other unrelated genera. We also review the species level diversity of the Middle American cichlids using the mtDNA *cytb* gene *population-level* analysis and the ddRAD analysis. Our biogeographical analysis of the ddRAD phylogeny explains the evolutionary history of Middle American heroine cichlids and demonstrates that biogeography is a much better indicator of evolutionary relationships in this fish group than are most morphological characters due to their ecological correlation.

6. Acknowledgements

We thank the anonymous reviewers and JUAN MIGUEL ARTIGAS AZAS, WILLEM HEIJNS and RICO MORGENTSTERN who have carefully read the manuscript, contributed with many suggestions and corrections and the latter three also provided important samples and also photographs used here. We thank the late GUSTAVO ALCIDES CONCHEIRO PÉREZ for sharing data of specimens housed at STRI (Panamá) and for the generation of the original distribution maps using GIS. We thank Prof. PLUTARCO CALA (ICNMHN, Bogotá, Colombia) for the important sample of *Chocoheros microlepis*. We also thank ZUZANA MUSILOVÁ (University of Basel) for some important samples, and UWE WERNER (Ense-Bremen, Germany) for additional samples and photographs presented in fig. 3. Financial support was provided by a scholarship from Stockholm University (to O.Ř.), grant from the SYNTHESYS program for Access to the Swedish Museum of Natural History (Stockholm, Sweden; to O.Ř.) and to the Museo Nacional de Ciencias Naturales (Madrid, Spain; to O.Ř.) within the European Community program “Improving the Human Research Potential and the Socio-Economic Knowledge Base”, and by the MSM6007665801 and the GAČR 206/08/P003 grants to O.Ř. The NGS ddRAD data analysis in our lab was developed with financial support from the GAČR 14-28518P grant of the Czech Science Foundation to L.P. The access to computing and storage facilities

owned by parties and projects contributing to the National Grid Infrastructure MetaCentrum provided under the program “Projects of Large Infrastructure for Research, Development, and Innovations” (LM2010005) was highly appreciated as well as the access to the CERIT-SC computing and storage facilities provided under the program Center CERIT Scientific Cloud, part of the Operational Program Research and Development for Innovations, reg. no. CZ.1.05/3.2.00/08.0144.

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