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

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Abstract

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

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

Contents

Introduction

Pigmentation patterns are known to play several important roles in fishes, being involved both in cryptic and aposematic coloration (e.g. Breder 1946, Haas 1976, Endler 1978, 1988, Armbruster & Page 1996), in gluing social aggregates (Harden Jones 1963, Hemmings 1966, Whitney 1969) or in social interactions, most importantly including courtship behaviour (Tinbergen 1952, Barlow 1963, Haas 1976, Ehrlich et al. 1977, Endler 1983, Long & Houde 1989, Hulscher-Emeis 1992). Through these important roles, variation in pigment patterns is subject to natural and sexual selection. However, the specific pigment pattern featured by a fish population/species is primarily dependent on the developmental processes producing the adult phenotypes.

A great deal of detailed information on the ontogeny of pigment patterns can be found in various larval fish identification guides and other taxonomic literature (e.g. Dawson 1964, Kortmulder & Poll 1981, Rauchenbacher et al. 1990), but the use of pigmentation characters in phylogenetic studies is seen very rarely (Dawson 1964, Kortmulder 1972, 1982, Kortmulder & Poll 1981, Mabee 1995, Kullander 1998), and even less common are pigmentation characters based on ontogenetic transformations of pigmentation patterns (Mabee 1995).

Pigmentation patterns in vertebrates have become an important model for those interested in mechanisms of pattern formation, in both theoretical (e.g. Murray 1981a,b) and experimental fields (e.g. Epperlein & Löfberg 1990). The history of proposed relationships between ontogeny and phylogeny has been particularly well reviewed (Gould 1977, Nelson & Platnick 1981, Rieppel 1985) and the correspondence of these two processes sharing a temporal dimension continues to be a fascinating issue.

Ontogeny provides critical information for recognizing homologies, of pigment patterns or any other characters, and its usefulness is uncontested (e.g. Brooks & McClennan 1991, Harvey & Pagel 1991, Wray & Raff 1991).

All vertebrate pigment cell types (except those in the retina) are derived from the neural crest, a paired strip of tissue that separates from the dorsal edges of the neural groove as it forms the neural tube (DuShane 1934, 1935). The neural crest cells are a unique feature found solely in vertebrates and nowhere else in the animal kingdom. Once formed, neural crest cells disperse from their position along the neural tube and migrate widely throughout the embryo. This migration occurs in an anterior-to-posterior wave, with cell precursors migrating along several pathways (e.g. Jesuthasan 1996, Loring & Erickson 1987, Raible et al. 1992, Vaglia & Hall 2000). Neural crest cells first migrate deep inside the body, and these cells differentiate into many lineages, including Schwann cells, sympathetic neurons, adrenal chromafin cells, branchial cartilage cells, odontoblasts, dermis and cephalic armor cells, sensory capsules, parts of the neurocranium and also chromatophores (Raible & Eisen 1994, 1996). Subsequently neural crest cells also start to migrate beneath the epidermis (Jesuthasan 1996). In contrast to the neural crest cells in the medial pathway, laterally migrating cells have been observed to develop only into chromatophores (Raible & Eisen 1994, but see Collazo et al. 1994).

In fishes and amphibians, pigment cell precursors can differentiate into four pigment cell types: melanophores (pigmented darkly with melanin); xanthophores (yellow to orange pigment cells); erythrophores (orange to red pigment cells); or iridophores (pigment cells that are reflective due to structured guanin or other similar crystals (Bagnara & Hardley 1973). Mechanisms of both cell fate determination and cell migration play a role in generating

pigment patterns. The ultimate pattern an organism exhibits may be affected by a variety of additional factors, including interactions between pigment cell types (Epperlein & Löfberg 1990, Parichy 1996a), the composition of the extracellular matrix (Epperlein 1982, Parichy 1996b), and the migration of lateral line primordia (Parichy 1996b). Finally, rates and timing of growth have also been implicated in the determination of pigment patterns from both theoretical and experimental standpoints (Murray 1981b, Murray et al. 1990). Variation between species in pigment patterns may be due to changes in any of the above processes (e.g. Parichy 1996b), or in other, unidentified factors.

In systematics, pigmentation patterns are traditionally most often used for species identification, while their use as characters in phylogenetic studies has been more limited. The problem is most certainly in the approach that morphologists adopt, since most coloration characters in phylogenetic studies are taken from adult pigmentation patterns. Here the constituting homologous elements are often most difficult to recognize, their developmental basis being probably more blurred by selection and adaptation than at any other developmental stage. In fish systematics, there is a wealth of focus and information available on the numerous slight differences in color pattern in very closely related and otherwise also very similar species, but as soon as more inclusive clades are being compared, coloration characters very quickly disappear from our character lists. The obvious reason is that phylogenetic studies are mostly undertaken using preserved museum material, and complete developmental series are rare in museum collections. With having no understanding of the development of the coloration patterns, any chance of interlinking different coloration patterns is very soon lost and no justifiable homology statements can any longer be made.

Cichlids are one of the groups for which it is feasible to obtain developmental series for most species. Field collection of developmental series is facilitated by the extensive parental care, making identifications of the juveniles straightforward, and an even easier source are developmental series specifically bred under aquarium conditions. It would thus seem that cichlids are an ideal group for the exploration of the famous diversity of coloration patterns. Yet, there is virtually nothing known about the ways in which the various patterns are generated.

Many behavioral and evolutionary aspects of this coloration variation have been addressed (e.g. Knight & Turner 1999, Barlow 2000, Seehausen et al. 1999), but few studies have focused on the development of cichlid coloration patterns (Balon 1959, 1960, Beeching et al. 2002).

This article is part of our ongoing revision of the Neotropical cichlid tribe Heroini Kullander, 1998. Heroine cichlids feature a substantial variation in coloration patterns, but this wealth of different patterns has been mostly beyond the reach of systematists, because mechanisms generating this diversity were not understood. In this article we will describe the mechanisms forming the adult coloration patterns, focusing on barring patterns. Our approach towards individual homologization of vertical body bars will be described and shown that it can explain a great part of the diversity of heroine barring patterns. The topic of extracting phylogenetic information from coloration ontogenies will be only touched upon here and will be the main focus of a subsequent paper, where it will be confronted with other characters.

Heroines have only quite recently been proposed as a monophyletic group (Kullander 1996, 1998), and their morphological diagnosis is still weak (see Kullander 1996, 1998). Despite the recency of their formal diagnosis, these fishes have always been considered as a very important component of neotropical fish communities, especially so in Middle America, where they have by far the greatest diversity and are one of the dominant groups of aquatic organisms.

Heroines belong among the least explored groups of Neotropical fishes, especially so in terms of their phylogenetic structure and evolutionary history. This is quite surprising, since heroines have one of the most interesting distribution patterns among Neotropical fishes, reaching from northern Mexico, south on both slopes of Middle and Central America, through most of South America, in both the cis- and trans Andean parts, down to the Rio de La Plata region in Argentina. On top of this, they also include a few species on the Greater Antilles (Cuba and Hispaniola). One of the reasons for their lagging behind is their complicated taxonomical history. As stressed above, heroines have been formally recognized only quite recently (K u l l a n d e r , op. cit.) and until the revision of *Cichlasoma* (sensu R e g a n 1905) in the early eighties (K u l l a n d e r 1983), most heroine species have been included in this catch-all genus. *Cichlasoma* (sensu K u l l a n d e r 1983) today includes only 12 very similar allopatric South American species, while the majority of former *Cichlasoma* was left in a nomenclatural limbo, where almost two thirds of the species formerly included in the catch-all genus were not assigned to any genus, and the monophyly of some of the remaining genera has not been convincingly demonstrated. The unplaced heroines are referred to as *'Cichlasoma'* following K u l l a n d e r ' s suggestion (1983) until their phylogenetic placement has been stabilized and new names are proposed.

Several published studies have recently focused on the heroine cichlids using sequences of the mitochondrial cytochrome b gene in order to shed light on the nomenclature and biogeographic history (R o e et al. 1997, M a r t i n & B e r m i n g h a m 1998, H u l s e y et al. 2004).

Heroines as understood today fall into several major lineages (unpublished results, see also F a r i a s 1999, 2000, 2001), of which all but one are exclusively South American groups. These South American groups are comprised of several specialized and morphologically very distinct genera that have been long recognized, although their relationships were never clear. The other five-sixths of the species in the tribe Heroini belong to the remaining lineage, called here the Mesoamerican lineage, which includes all the Middle American and Antillean species as well as some South American groups (the genera *Caquetaia*, *´Caquetaia´ umbriferum*, *Heroina*, the *´Cichlasoma´ festae* group and the *´Cichlasoma´ facetum* group). The taxonomic situation of the exclusively South American lineages is in good order, while the above described taxonomical and nomenclatural problems are mostly typical for the Mesoamerican lineage. Today, the genus *Cichlasoma*, once grouping almost all heroines, belongs to a separate monophyletic tribe Cichlasomatini, which is the sister group to Heroini (K u l l a n d e r 1998, F a r i a s et al. 1998, 1999, 2000, 2001). Heroines, together with cichlasomatines and geophagines, encompass a vast majority of Neotropical cichlid diversity and heroines are the second largest cichlid group in the neotropics after the geophagines (139 species according to K u l l a n d e r 2003).

Material and Methods

The ontogenetic series forming the base of our study came from three sources. The first subset of species has been bred and the growth series raised in laboratory conditions, whereas the coloration ontogenies of the remaining species are based on preserved museum material. Breeding stock for *Aequidens patricki*, '*Aequidens*' *pulcher*, '*Aequidens*' *rivulatus*, *Amphilophus citrinellus*, *Amphilophus xiloanesis*, *Astatheros longimanus*, *Astatheros robertsoni*, *Cichlasoma amazonarum*, *Cichlasoma bimaculatum*, '*Cichlasoma*' *facetum*, '*Cichlasoma*' *festae,* '*Cichlasoma*' *salvini*, '*Cichlasoma*' *scitulum*, '*Cichlasoma*' *urophthalmum*, *Cryptoheros nigrofasciatus*, *Cryptoheros sajica*, *Cryptoheros spilurus,* '*Cichlasoma*' *octofasciatum*,

Herichthys carpintis, *Heros* cf. *notatus*, *Heros severus, Herotilapia multispinosa*, *Parachromis managuensis*, *Paratheraps breidohri*, *Paratheraps fenestratus*, '*Paratheraps*' *regani*, *Vieja maculicauda* and *Vieja synspila* were acquired from the pet trade.

Melanophore pattern development of *Acarichthys heckelii*, *Acaronia nassa*, *Aequidens rondoni*, *Aequidens tetramerus*, '*Aequidens*' cf. *pulcher* "Colombia", '*Cichlasoma*' *atromaculatum*, *Bujurquina vittata*, *Cichlasoma boliviense*, *Cichlasoma dimerus*, *Gymnogeophagus setequedas*, *Krobia sp.*, *Laetacara* sp. "orangenflossen", *Laetacara thayeri*, *Nandopsis ramsdeni*, *Nandopsis tetracanthus* and *Satanoperca jurupari* is based on museum specimens housed at NRM (Swedish Museum of Natural History).

A third part of developmental information was extracted from published aquarium hobbyist literature and the photographic collection of Uwe W e r n e r .

Live fish were maintained in single species groups of 6 to 20 individuals and allowed to freely form pairs. Pairs were left to spawn in the group, but after spawning the remaining members of the group were removed. In cases where parents fought with each other or where on previous spawnings the eggs or juveniles were eaten, embryos and larvae were raised separately from the adults. Coloration ontogenies of all species are based on at least two successive spawning of two different parents.

Embryos and larvae were raised all in one tank per spawning and fed brine shrimp nauplii twice daily. The aquarium room was maintained on a 14-h light / 10-h dark schedule at 28ºC.

Specimens were usually sampled and preserved first at 3 days post fertilization, then at the first day of free-swimming, then on the 3rd to 4th day, on the 7th day, and then at about one week intervals until the formation of the adult coloration pattern. At least five individuals of every species were examined and photographed per each sampling. The specimens were fixed in 10% buffered formalin before photographing. After fixation, they were transferred to 70% ethanol. For some species in the original stages of our study, specimens could not be preserved because of high larval or juvenile mortality or low numbers of specimens, but the developmental stage of coloration pattern formation has been documented on photographs.

Specimens were observed using an Olympus SZX9 dissecting microscope and photographed using a Nikon digital camera fitted to the dissecting microscope. Representative specimens of chosen species have been drawn using a camera lucida attachment to illustrate the ontogeny of melanophore pattern development in each of the species. Only melanophores are depicted in the drawings. Bars are numbered in tail to head direction (i.e. first bar is always the most posterior).

Results

A d u l t m e l a n o p h o r e p a t t e r n d e v e l o p m e n t

Melanophores become visible during their migration from the neural crest over lateral surface of the body and yolk sac. They establish themselves in four distinct lines (Fig. 1), as has been also demonstrated for *Danio* and *Tanichthys* (M i l o s & D i n g l e 1978a,b, K e l s h et al. 1996, M c C l u r e 1999, Quigley & Parichy 2002). Dorsoventraly these lines are referred to as:

1) the dorsal melanophore line (nomenclature of lines adopted from M c C l u r e 1999), formed of a paired concentration of melanophores in the posterior head region, migrating posteriorly along the dorsal body border, on both sides of the base of the dorsal fin.

2) the septal melanophore line – in the horizontal myoseptum (between the hypaxial and epaxial musculature).

3) the abdominal melanophore line – overlying the abdominal cavity and continuing caudally.

4) the ventral melanophore line – originating from the ventral part of the yolk sac (the yolk sac stripe of K e l s h et al. 1996) and continuing along the ventral surface of the abdomen caudally along the ventral body border.

The two midlateral lines (i.e. the septal and the abdominal melanophore lines) are located deeper inside the body, exactly as in zebrafish (see Fig. 1 in K e l s h et al. 1996), while the dorsal and ventral stripes lie close to the body surface, as do the later developing vertical bars. The next general step in cichlid coloration ontogeny is the replacement of the longitudinal stripes by vertical bars, which also constitute the final coloration elements in most cichlid species.

Cichlid vertical bars form through disruption of horizontal melanophore lines (Fig. 1a,b). The disrupted melanophore lines resemble a series of longitudinal blotches. Melanophores from the disrupted melanophore lines then start to migrate dorsaly and ventraly, widening the blotches dorsoventrally (Fig. 1b,c). This vertical migration continues for about two weeks when horizontal lines become finally replaced with vertical bars.

In some species, the abdominal line persists for a much longer time and becomes much more pigmented before it is finally disrupted and incorporated into vertical bars. This is a typical condition of many heroines.

The transformation from longitudinal lines into vertical bars happens through interconnection of the dorsal (A, B, C, D in Fig. 1B, C) and ventral blotches with the midlateral blotches. This interconnection is later accompanied by resegmentation of the dorsal and ventral blotches. As the dorsal blotches become incorporated into the developing bars through vertical migration of melanophores, the bars pass through an X-like form in the case of the postanal bars and Y-like form in the case of abdominal bars (Fig. 1). Migrating melanophores resegment the dorsal blotches and these become incorporated into the bars as their dorsal portions. Midlateral blotches always form at the intersection of the X-like bars.

Homologous pattern elements

Our homologization procedure rests on the observation that in most cichlid species examined in our study, the dorsal melanophore line becomes disrupted into four, obviously homologous blotches (A, B, C, D in Fig. 1). The anterior blotch (A) is always situated at the anterior insertion of the dorsal fin and the posterior at the posterior insertion of the dorsal fin, and this is not much dependent on the length of the dorsal fin or body proportions, which are quite similar in the larvae and small juveniles. Vertical bars form in all species in identical positions when compared to the four dorsal blotches, and the situation is shown in Figure 1. There are nine developing homologous bars in all species examined. Adult bar numbers do differ among species, and there thus have to be irregularities in the development of bars creating the various bar numbers, and such irregularities can indeed be observed when following the ontogenies. Bars 1, 8, and 9 do not show any variation in development among the examined species. Other bars do show variation, but as stressed above, they develop in identical positions when compared to the dorsal melanophore line blotches. Increase and decrease in the number of bars can thus be attributed to observed divisions or fusions of individual bars. In this way, we can individually determine which bar becomes divided or which bars become fused and thus distinguish non-homologous numbers of adult bars. In some species with derived coloration ontogenies, the blotches may be obliterated or presumably lost, but additional landmarks can still be used for homologization.

The nine developing bars are located very conservatively on the fish. The positions are much more conservative during early ontogeny and can be later quite modified due to changes in body proportions or changes in the development of the coloration patterns themselves. Bars 8, 6, 4 and 3 develop intersegmentally between the dorsal blotches, while bars 7, 5, and 2 develop in alignment with the dorsal blotches (see Fig. 1). More specifically, the anteriormost bar (9) runs through the eye and develops from a separate pigmented area on the head anteriorly from dorsal blotch A, the next one (8) develops intersegmentally between the pigmented area of bar 9 and between the anteriormost dorsal blotch A and is always located on the opercular series, number 7 develops ventraly from blotch A and runs between the opercular cleft and the insertion of the pectoral fin, and number 6 develops between dorsal blotches A and B posteriorly from the insertion of the pectoral fin. Number 5 is the next bar, developing ventraly from dorsal blotch B, followed by the usually most pigmented bar number 4, which develops intersegmentally between the two middle dorsal blotches B and C and is centered above the vent at the anterior insertion of the anal fin. Bar number 3 develops intersegmentally between the posterior two dorsal blotches (C and D) and ventraly from blotch D develops the last body bar number 2. In most species, this bar (2) is located at the posterior insertion of the dorsal and anal fins. The posteriormost bar number 1 is always on the base of the caudal fin, in most species dividing into two bars, known as the caudal peduncle bar and the caudal base bar, this latter one often carrying the caudal fin spot. Bars two, three, four and five show most developmental variation and are responsible for most of the diversity in number of adult bars. Bar two develops at the posterior insertion of the anal and dorsal fins, but in some species it becomes divided into two bars, which creates confusion about the homology of the bars above the anal fin. In some species the third bar becomes divided into two, producing the same adult bar pattern with the same number of adult bars. These two situations are clearly not homologous and can only be distinguished using developmental information. Bars number four and five also become divided in some species, further confusing bar homologization without ontogenetic information available. In some geophagines, bars number two and three on the contrary fuse and thus decrease the total number of bars. All combinations are possible and so a simple mechanism of bar division/fusion can produce quite an extensive range of adult bar numbers and positions.

Changing body proportions during development can further complicate the picture, as for example species with long caudal peduncles will have a different position of bar number 2 than will have those with short ones.

There are most certainly one or maybe two additional primary bars developing on the anterior parts of the head between the eyes and further down towards the snout, but these are virtually impossible to study using the methods we have used. The most important problem is that the head is in this area very heavily pigmented and always turns very dark when the juveniles are even minimally disturbed (as during photography or fixation itself).

The arrangement of the dorsal blotches (A, B, C, D) is best seen in our developmental series raised in aquaria, for which we also have complete developmental series. Follow Fig. 1 and for some examples, refer to the ontogenies of *Heros* (Fig. 2b), *Herotilapia multispinosa* (Fig. 3a–f), *Cryptoheros sajica* (Fig. 4b–e), *Cryptoheros nigrofasciatus* (Fig. 5 c–f), *Parachromis managuensis* (Fig. 7c,d), *Herichthys carpintis* (Fig. 15c), or *´Aequidens´ pulcher* (Fig. 24a–d).

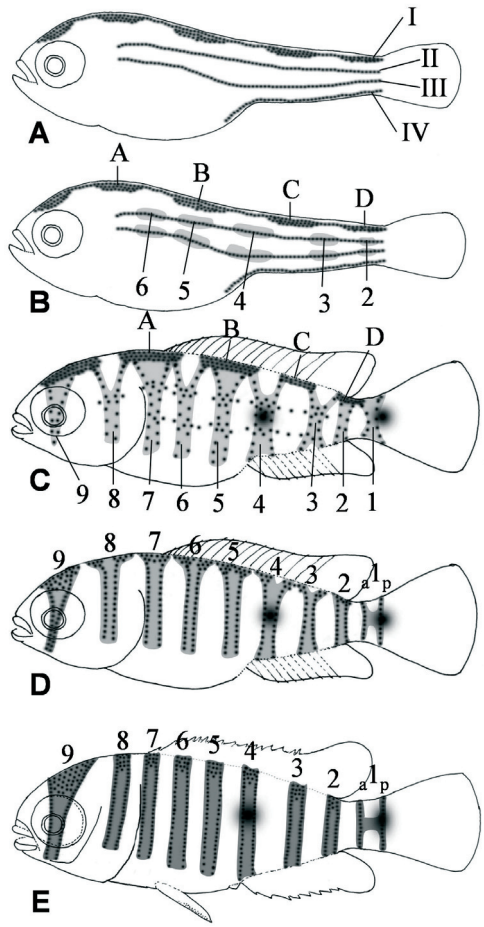


Fig. 1. Simplified model ontogenetic series showing crucial steps during transformation of horizontal migration lines into vertical bars. A. Four melanophore migration lines (I–IV) become established on the body. B. Migration lines start to disrupt into blotches, the dorsal migration line (I) disrupts into four longitudinal blotches (A,B,C,D) which are conservatively arranged on the body (see text). Melanophores from the horizontal lines start to migrate dorsaly and ventraly. Dorsal and ventral migration of melanophores from the two middle midlateral lines (II and III) shown in light gray, ventral migration of melanophores from the four dorsal blotches shown with dots. C. Continued vertical migration of melanophores leading to resegmentation of the four dorsal blotches. D,E. Incorporation of the resegmented dorsal blotches into the vertical bars.

Adult coloration pattern development in the tribe Heroini

Adult pattern development in *Heros* (Fig. 2)
We have examined two species of the genus *Heros* (*H.* cf. *notatus*, *H. severus*) and both species have identical indistinguishable ontogenies. In the early, completely transparent eleuterembryos, the four longitudinal lines of migratory chromatophores are not easily seen because of the embryo's narrow tail region (Fig. 2a). The four lines are nevertheless clearly developed in the next stage (Fig. 2b), where the process of their fragmentation into longitudinal blotches is under way. Developing bars are clearly visible in the third step (Fig. 2 c–f) as faint, bend vertical bands of chromatophores interconnecting the longitudinal blotches from the second step (Fig. 2 b). Developing bars now resemble V-like structures in the abdominal region and head region and X-like structures in the tail region. These V- and X-like bars later fuse along their midline and produce the adult bars (Fig. 2 f–i). Typically for *Heros*, the fourth developing bar (4) does not fuse and produces two adult bars. The first larval bar also fuses into one adult bar, probably due to the short caudal peduncle, as also observed in ontogenies of other species with very short caudal peduncles. Thus the final number of adult bars in *Heros* is 8 body and tail bars (1, 2, 3, 4a, 4p, 5, 6, 7), one opercular bar (8) and the suborbital stripe bar (9; not fused above the eye, as in many cichlids). Sometimes the 7th and 8th bars fuse to produce only one observable bar in the opercular area of adult *Heros* specimens.

Adult pattern development in *Herotilapia multispinosa* (Fig. 3)
The developmental pattern of *Herotilapia multispinosa* is also easy to follow as in the case of *Heros*. Small eleuterembryos in the yolk sac stage develop the four migration lines, from

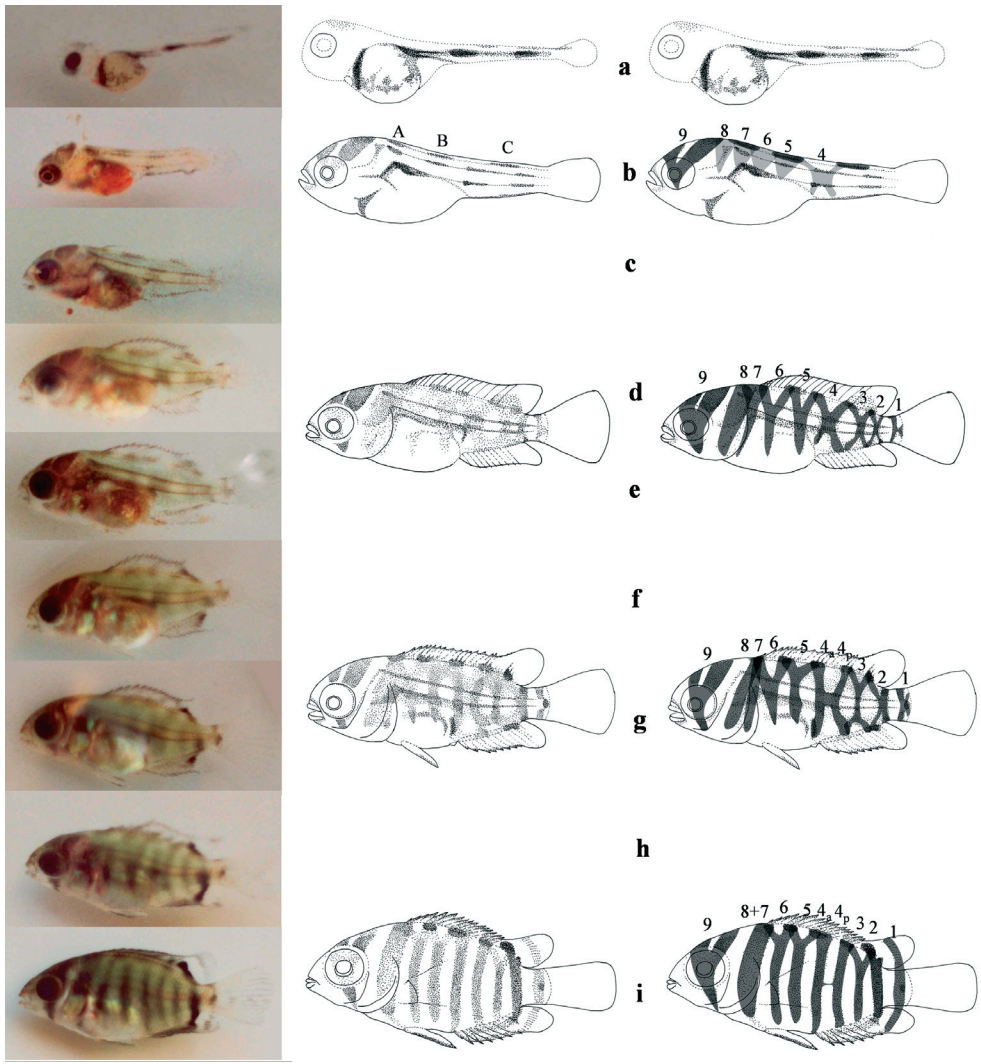


Fig. 2. Color pattern development in *Heros* cf. *notatus*. (a) 4,5 mm TL; (b) 6 mm TL; (c) 8 mm TL; (d) 9 mm TL; (e) 11 mm TL; (f) 13 mm TL; (g) 14 mm TL; (h) 17 mm TL; (i) 24 mm TL.

which the dorsal line already becomes fragmented at free-swimming (Fig. 3a). As typical for most heroines, the abdominal line/stripe is the most heavily pigmented of the four horizontal migration lies (see descriptions of following heroines and also discussion). As described in methods, vertical bars form in conserved positions in relation to the blotches in the fragmented dorsal migration line. This is very easily observed in *Herotilapia*, especially the resegmentation of the dorsal blotches and the X-like shape of the developing bars. Vertical migration of chromatophores connecting adjacent blotches (Fig. 3 b–e) and resegmentation of dorsal blotches (Fig. 3 e–h) are clearly visible in the developmental series of *Herotilapia*. The development of bars in *Herotilapia* features no exceptions to the model producing the most common homologous bar number of eight body and tail bars (1a, 1p, 2, 3, 4, 5, 6, 7), one opercular bar (8) and the suborbital stripe bar (9). Two important things are to be noted.

First, although we name bar number 9 the suborbital stripe bar, there is no developing stripe below the eye since the bar does not reach there during development. This is different from the situation just described for *Heros* and it will be discussed in discussion, as it is a feature distinguishing most heroines from the rest of Neotropical cichlids. Second, although the total number of bars is the same as in *Heros*, not all of the bars correspond to each other in the two genera (i.e. not all the bars are developmentally speaking homologous structures).

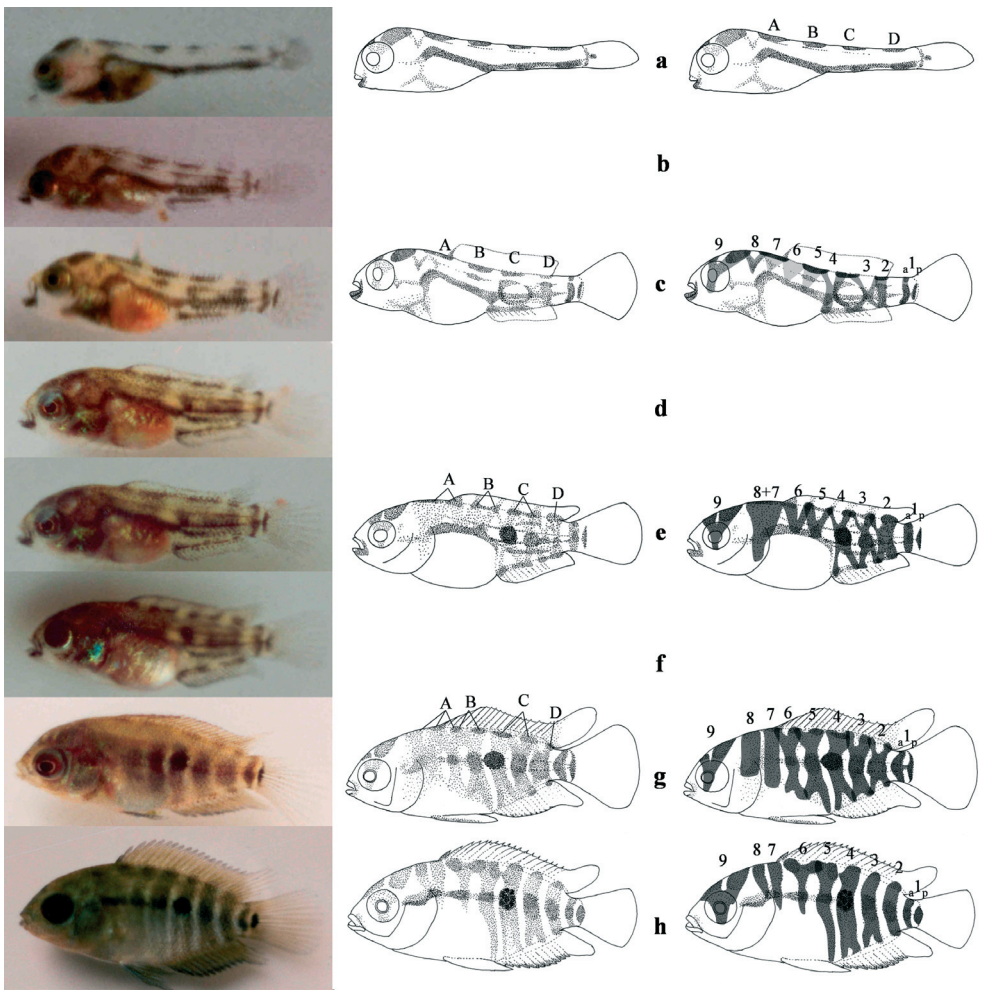


Fig. 3. Color pattern development in *Herotilapia multispinosa*. (a) 6 mm TL; (b) 7 mm TL; (c) 8 mm TL; (d) 9–10 mm TL; (e) 10 mm TL; (f) 12 mm TL; (g) 18 mm TL; (h) 25 mm TL.

Adult pattern development in *Cryptoheros* (Figs 4, 5, 6)

The name *Cryptoheros* is the latest addition to the messy nomenclature of heroines. The name is supposed to contain small Central American heroine species with an elevated number of anal fin spines (A l l g a y e r 2001) previously known as *Archocentrus*. Despite the very vague diagnosis of the group we follow the usage of the name, since the type species of *Archocentrus* (*A. centrarchus*) seems to be more related to the type species of *Amphilophus* (*A.*

labiatus) (unpublished results; see also Martin & Bermingham 1998, Hulsey et al. 2004). We have studied three species of *Cryptoheros*.

In *Cryptoheros sajica* the four migration lines become fragmented soon after free-swimming and the process of larval bar formation starts comparatively early (Fig. 4b). Again there are the four blotches into which the dorsal line disrupts as in all heroines, but here there are two bars developing from the dorsal blotch C (Fig. 4 d). The posterior one of the two bars is clearly bar number 3. Bar number 4 develops in its typical position intersegmentally between blotches B and C. We thus interpret the additional bar as the anterior portion of bar number 3 (hence 3a and 3p) but an alternative explanation is also possible. Our model is perfectly symmetrical, with bars alternatingly developing segmentally (7, 5, 2) and intersegmentally (8, 6, 4, 3) compared to the dorsal blotches A–C. But there is one irregularity, where between bars 3 and 4 there is usually no bar developing in the vast majority of the species, even though it would make the model even more symmetrical. The position of the additional bar in *Cryptoheros* (the bar also develops in the two other *Cryptoheros* species studied) thus fits this explanation well. In any case, this bar is a unique for *Cryptoheros* and one of the two species of *Nandopsis* among the almost 40 species studied, comprising a large diversity of at least the subfamily Cichlasomatinae. None of

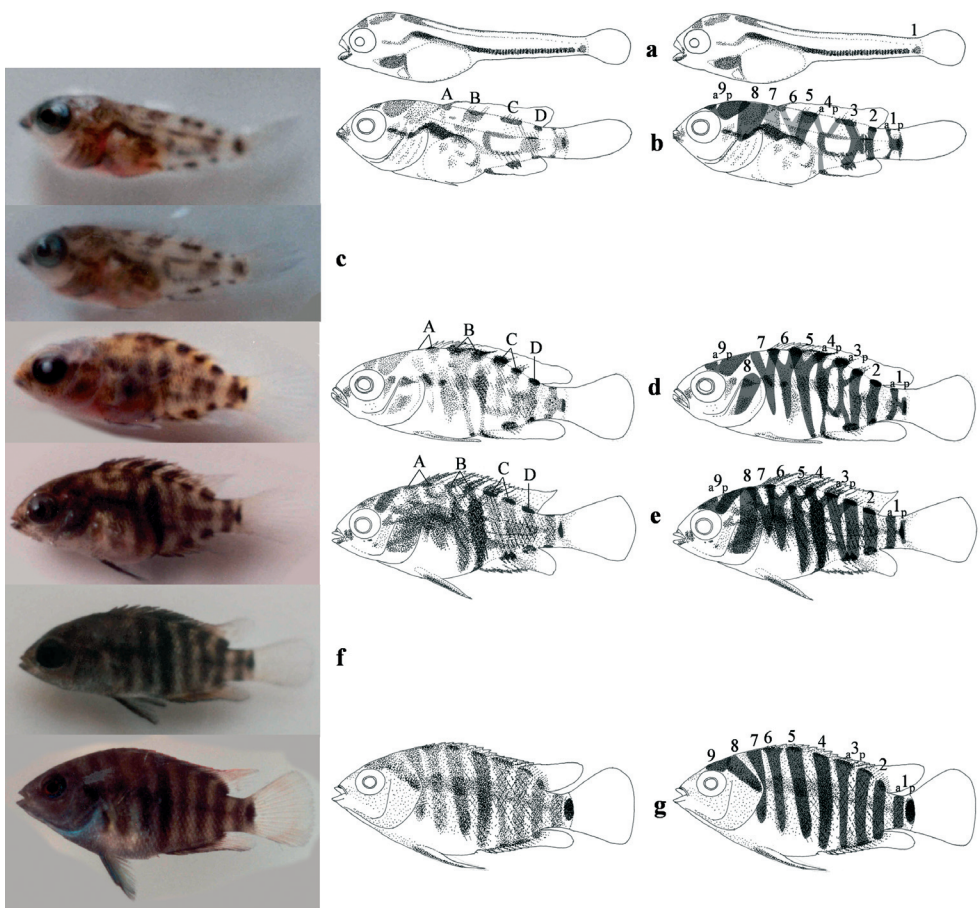


Fig. 4. Color pattern development in *Cryptoheros sajica*. (a) 5 mm TL; (b) 6 mm TL; (c) 6 mm TL; (d) 11–12 mm TL; (e) 14 mm TL; (f) 20 mm TL; (g) 35–40 mm TL.

the three geophagines develops this bar either. The adult bar number in *Cryptoheros sajica* is thus 9 adult bars on the body and tail (1a, 1p, 2, 3a, 3p, 4, 5, 6, 7), one opercular bar (8) and the orbital bar (9). The same homologization of bars applies also to *Cryptoheros nigrofasciatus* and *Cryptoheros spilurus*, even though specific details of the ontogenies do differ.

In the second studied species of *Cryptoheros* (*C. nigrofasciatus*) larvae retain the larval migration lines and especially the abdominal stripe longer than *Cryptoheros sajica,* well after they are free-swimming and the development of vertical bars is also postponed. The abdominal stripe is again the most pigmented area on the body. In the next step, the dorsal and later also the midlateral lines become fragmented into blotches of chromatophores (Fig. 5 c–f). The four dorsal blotches are well visible (Fig. 5 c–e) as is their resegmentation and incorporation into the bars (Fig. 5 e–g). As in *Cryptoheros sajica*, a supernumerary bar develops from dorsal blotch C (Fig. 5 e–g). We refer to this bar as 3a as in *Cryptoheros sajica* (see above).

Specific details make the species ontogenies of *Cryptoheros nigrofasciatus* and *Cryptoheros sajica* easily recognizable and species specific, especially in the first developmental

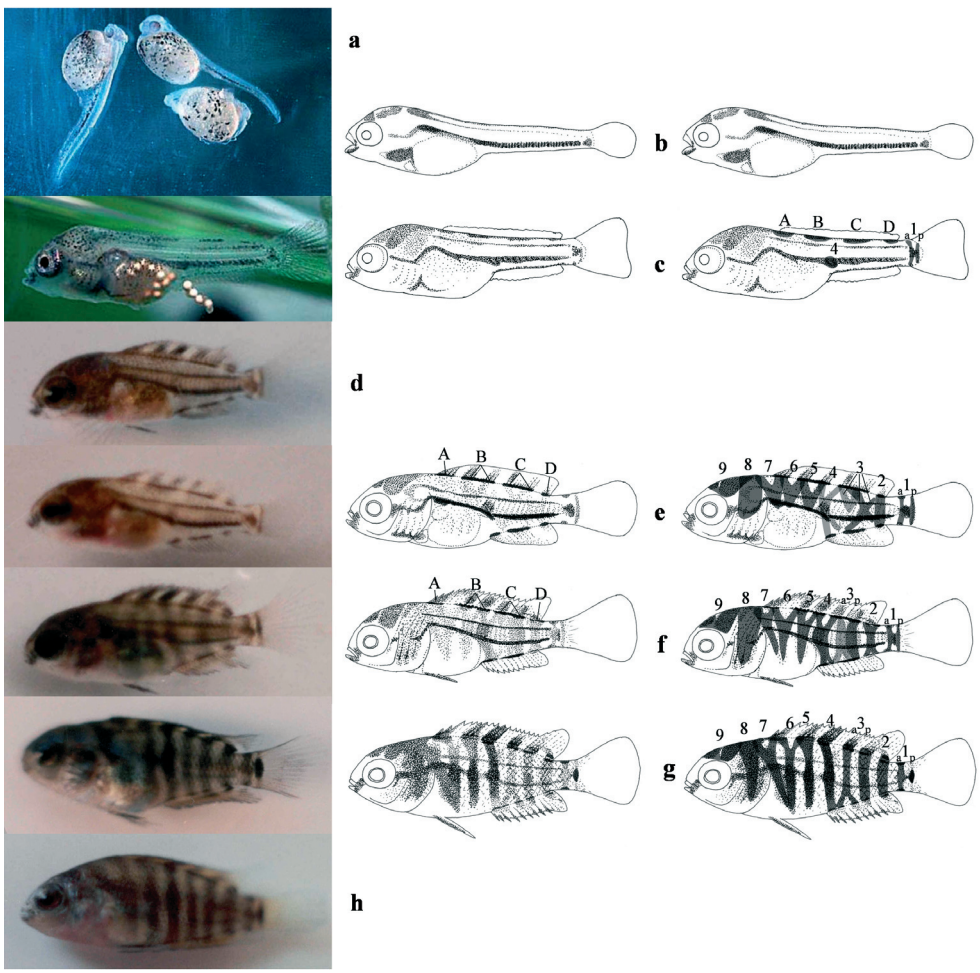


Fig. 5. Color pattern development in *Cryptoheros nigrofasciatus*. (a) 5 mm TL; (b) 7 mm TL; (c) 8 mm TL; (d) 10 mm TL; (e) 11mm TL; (f) 15 mm TL; (g) 18 mm TL; (h) 19 mm TL.

stages (cf. Fig. 4b,c in *C. sajica* and Fig. 5c,d in *C. nigrofasciatus*). Juvenile *Cryptoheros nigrofasciatus* retain the ventral lateral line for much a longer time and the developing bars are quite diffuse, while *Cryptoheros sajica* loose the line much earlier, developing bars are better circumscribed and the species develops a specific longitudinal horseshoe pigmented structure connecting the midlateral lines above the anterior half of the anal fin (Fig. 4c). This structure is made of the midlateral blotch of the fourth developing bar connected with two longitudinal blotches in the midlateral lines. Exactly the same specific detail is developed in the extremely similar ontogenies of *Cryptoheros nanoluteus*.

The third *Cryptoheros* species studied, C. *spilurus*, shows an intermediate rate of disruption of the abdominal stripe. The four blotches in the dorsal migration line are clearly visible and make bar homologization straightforward. As in *Cryptoheros sajica* and *Cryptoheros nigrofasciatus*, *C. spilurus* develops the additional bar ventraly from dorsal blotch C and as in

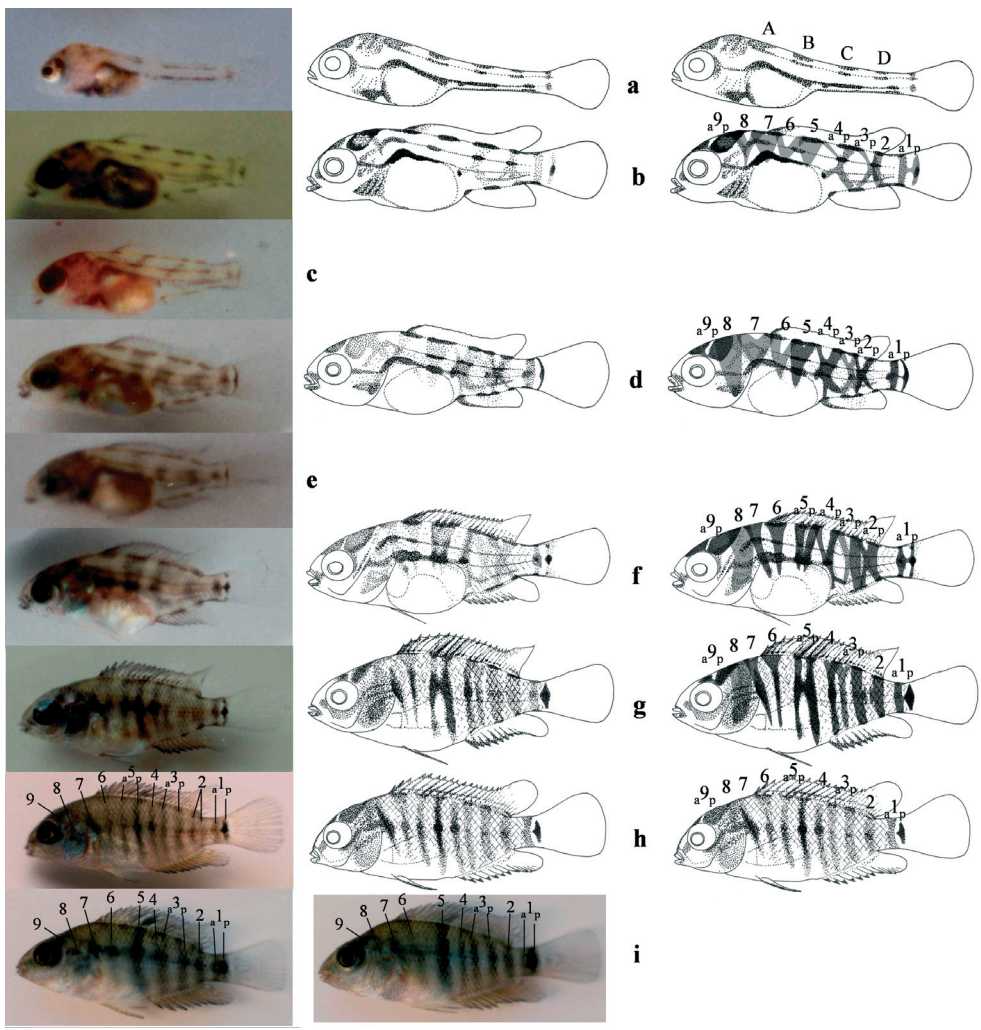


Fig. 6. Color pattern development in *Cryptoheros spilurus*. (a) 6–7 mm TL; (b) 9 mm TL; (c) 10–11 mm TL; (d) 11mm TL; (e) 11mm TL; (f) 14 mm TL; (g) 16–20 mm TL; (h) 22–24 mm TL; (i) 25–29 mm TL.

the two species the development of this bar is postponed. The fifth developing bar divides into two bars (Fig. 6 f–h). The total number of adult bars is thus 10 body and tail bars (1a, 1p, 2, 3a, 3p, 4, 5a, 5p, 6, 7), and as always one opercular (8) and one orbital bar (9). This bar pattern is unique for this species among the species studied. At larger sizes, in some specimens the fifth bar does fuse into one bar, while in others it will stay unfused. There is thus variation in the number of bars in this species. What remains a distinguishing fact is that the most pigmented bar in *Cryptoheros spilurus* is the fifth bar (whether divided or not), while in the vast majority of heroines, the dominant bar is the fourth bar. Obviously, the dominant bar in *Cryptoheros spilurus* is not homologous to the dominant bar in *Cryptoheros sajica* or *C. nigrofasciatus*, and it would thus be erroneous to use this bar as a landmark for homologizing bars in these species in case no ontogenetic information was available.

We would like to stress here that *Cryptoheros spilurus* is rather a species complex than a single species and that these species do differ in the number and position of bars. At least three forms are known. Such differences between closely related species do also occur among species of the *´Cichlasoma´ facetum* group (unpublished results; see below).

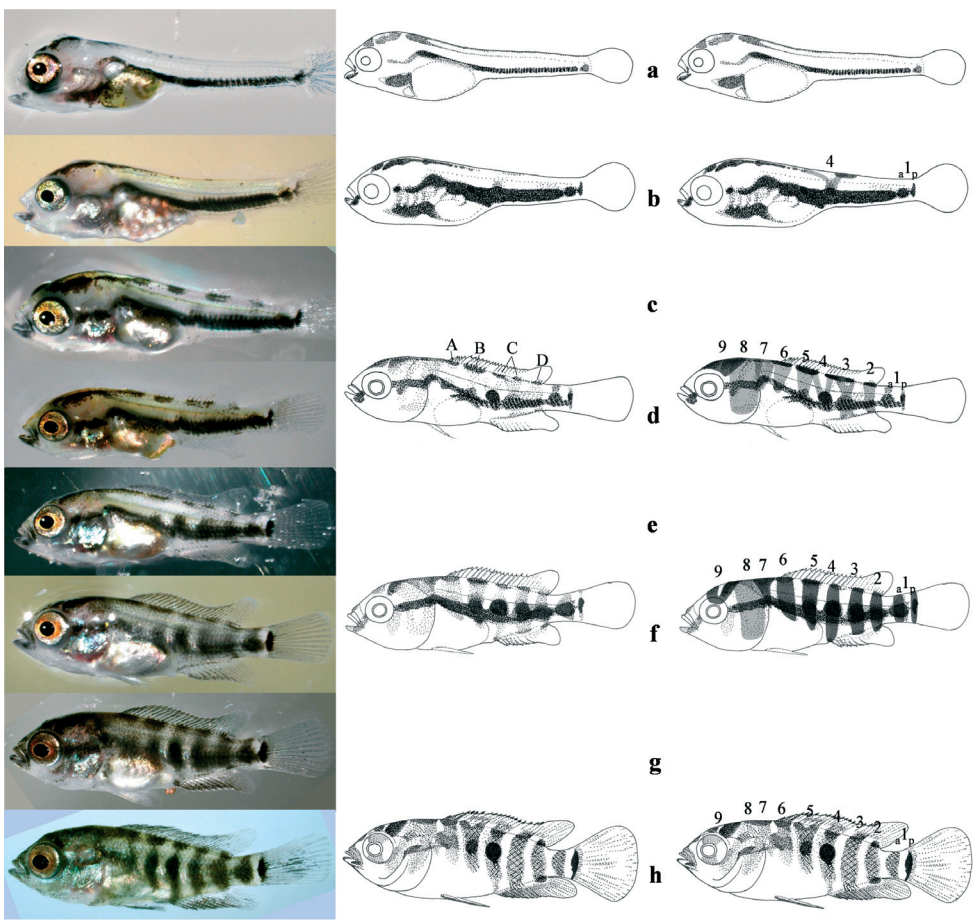


Fig. 7. Color pattern development in *Parachromis managuensis*. (a) 7–9 mm TL; (b) 10 mm TL; (c) 11 mm TL; (d) 11–12 mm TL; (e) 14 mm TL; (f) 15 mm TL; (g) 17 mm TL; (h) 18–20 mm TL.

Adult pattern development in *Parachromis* (Fig. 7)

We have studied one species of the genus *Parachromis* (*P. managuensis*) but all five species contained in the genus have identical coloration ontogenies (pers. obs.). The coloration ontogeny in *Parachromis* is dominated by the persistent and heavily pigmented abdominal line. Typically, after free-swimming of the larvae the abdominal line becomes even more heavily pigmented, and the line stays much longer than in the previously described species. The four dorsal blotches are well visible and bars form first as blotches in the abdominal line (Fig. 7c–g). Gradually as the bars grow vertically, the abdominal line looses intensity and becomes disrupted and incorporated into the developing bars, as do the dorsal blotches. The fourth bar features the most prominent midlateral blotch throughout the development of the coloration pattern. The ontogeny results into the most common heroine bar pattern, i.e. eight body and tail bars (1a, 1p, 2, 3, 4, 5, 6, 7), one opercular bar (8) and one orbital bar (9).

Adult pattern development in hybrids between *Cryptoheros nigrofasciatus* and *Parachromis managuensis*.

Hybrids between various species of heroines are readily produced in aquarium conditions and some of the more bizarre (called parrots) are pricey fishes. We have produced hybrids between *Cryptoheros nigrofasciatus* and *Parachromis managuensis* and we use them here to demonstrate a few points about

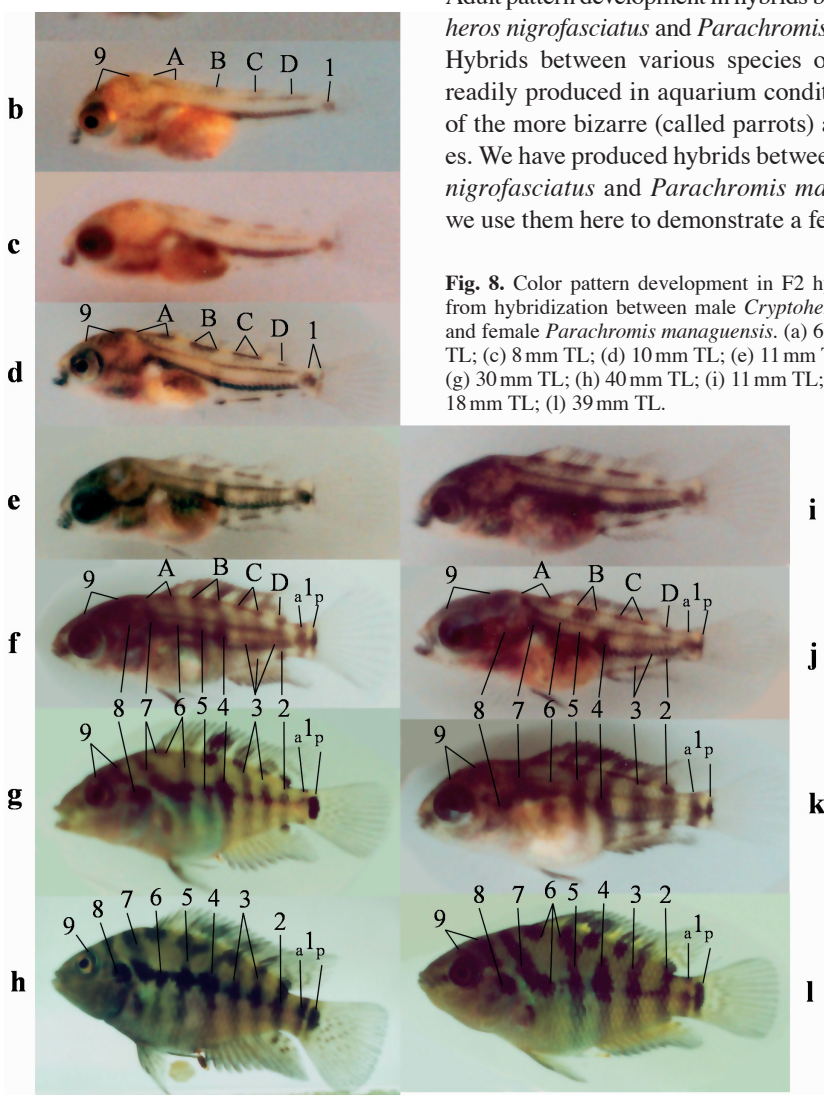


Fig. 8. Color pattern development in F2 hybrids originating from hybridization between male *Cryptoheros nigrofasciatus* and female *Parachromis managuensis*. (a) 6 mm TL; (b) 7 mm TL; (c) 8 mm TL; (d) 10 mm TL; (e) 11 mm TL; (f) 14 mm TL; (g) 30 mm TL; (h) 40 mm TL; (i) 11 mm TL; (j) 14 mm TL; (k) 18 mm TL; (l) 39 mm TL.

coloration pattern development. The two parental species are genetically closely related despite quite some differences in size and morphology (M a r t i n & B e r m i n g h a m 1998, H u l s e y et al. 2004), and thus the possibility of their hybrids is not surprising. The main differences in coloration ontogenies between *Cryptoheros nigrofasciatus* and *Parachromis managuensis* are the longer persisting and much more dominant abdominal stripe in *Parachromis managuensis* and the presence of an additional bar (3a) in *Cryptoheros nigrofasciatus* developing ventraly from dorsal blotch C. The hybrids in F1 generation as well as in successive generations (we have now F4 generation) do all show variation in the number of bars, as predicted from the differences in the parental species and the variation occurs within the same clutch. The variation seems to be of [an all or nothing kind], because a specimen either develops the additional bar (Fig. 8 e–h) or it does not (Fig. 8 i–l), but there are no intermediates, e.g. with a weak or underdeveloped additional bar. Overall the ontogeny, as well as the grown fishes, have more the likeness of *Cryptoheros nigrofasciatus* than of *Parachromis managuensis.*

Adult pattern development in *Amphilophus* (Fig. 9) and *´Cichlasoma´ urophthalmum* (Fig. 10). We have bred three species of the genus *Amphilophus* (*A. citrinellus*, *A. xiloaensis*, *A. trimaculatus*). The coloration ontogenies of *A. citrinellus* and *A. xiloaensis* are indistinguishable,

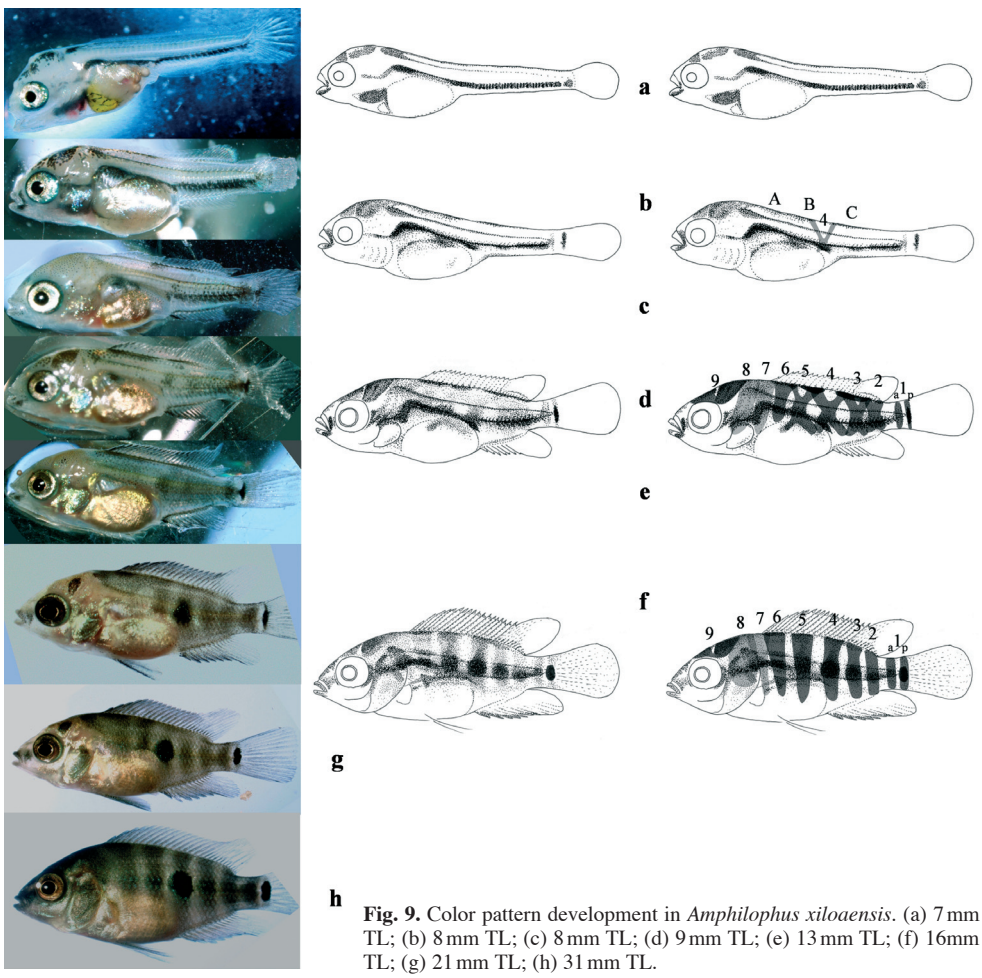


Fig. 9. Color pattern development in *Amphilophus xiloaensis*. (a) 7 mm TL; (b) 8 mm TL; (c) 8 mm TL; (d) 9 mm TL; (e) 13 mm TL; (f) 16mm TL; (g) 21 mm TL; (h) 31 mm TL.

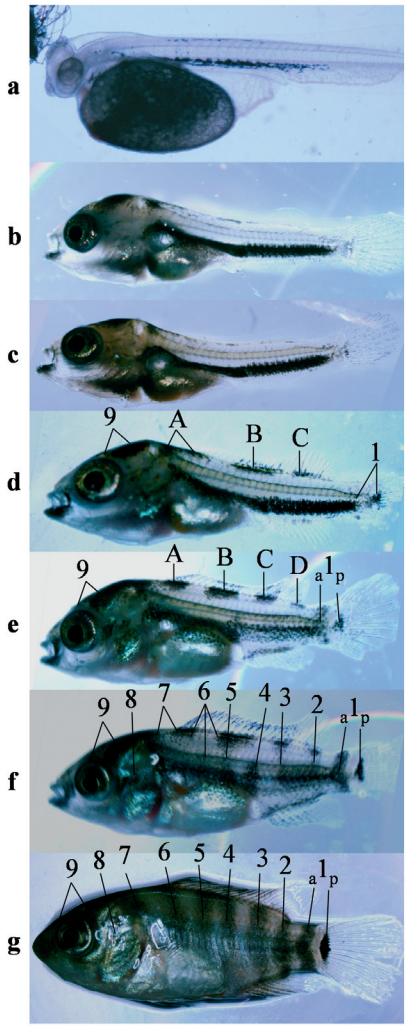


Fig. 10. Color pattern development in *´Cichlasoma´ urophthalmum*. (a) 4 mm TL; (b) 7 mm TL; (c) 8 mm TL; (d) 10 mm TL; (e) 13 mm TL; (f) 15 mm TL; (g) 30 mm TL.

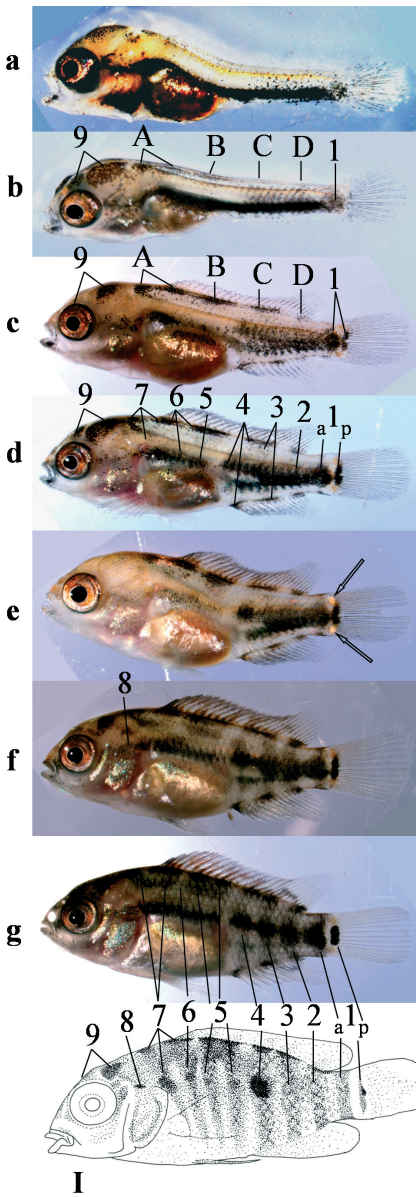
while the ontogeny of *A. trimaculatus* closely resembles that of *´Cichlasoma´ urophthalmum* (see below; Fig 10). The main difference is that the ontogenies of *A. trimaculatus* and *´Cichlasoma´ urophthalmum* do still feature clearly developed dorsal blotches, while the two *Amphilophus* species have them very much obliterated, which is the apomorphic state. The dominant structure in the ontogenies of *A. citrinellus* and *A. xiloaensis* is the midlateral blotch in the fourth bar (Fig. 9f–h). This blotch is also the dominant element in both *A. trimaculatus* and *´Cichlasoma´ urophthalmum*, but at larger sizes than shown in the figures. The abdominal stripe is dominant of the ontogenies of all four species, as was the case in *Parachromis*. The manner of development in the bars is also similar, with bars developing from the midlateral blotches. Adult bar homologization is again of the most common type, as in *Parachromis managuensis*, with eight flank and tail bars (1a, 1p, 2, 3, 4, 5, 6, 7). The very faint and diffuse way of formation of the bars is not attributable to the fact that we have worked with the xanthoric form of *Amphilophus citrinellus*, because the normally pigmented *Amphilophus xiloaensis* features indistinguishable and also very weakly pigmented early stages of coloration development (Fig. 9 b–e). This faint pigmentation ontogeny seems to be characteristic for the labiatus species group of *Amphilophus*, since *Amphilophus trimaculatus* ontogeny is much more similar to *Parachromis* and thus heavily pigmented (pers. obs.).

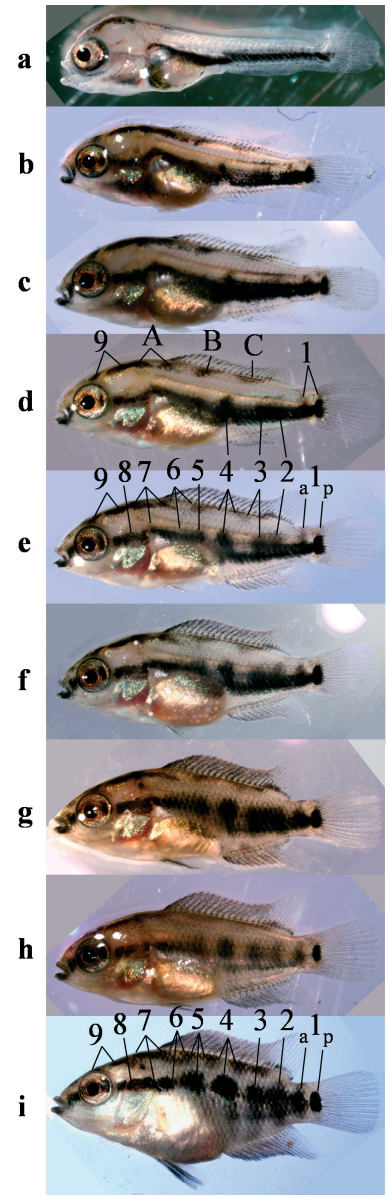
Adult pattern development in *´Cichlasoma´ salvini* (Fig. 11) and *´Cichlasoma´ facetum* (Fig. 12).

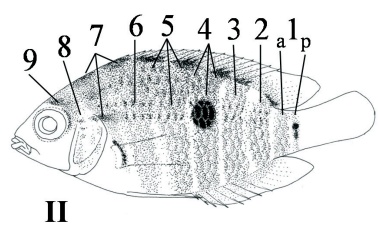
Both species show ontogenies very similar to those described for the *Parachromis*-like species (Figs 2–10). The abdominal line and the midlateral blotch are dominant, the dorsal blotches are somewhat diffuse but still well developed. The ontogeny shown in the case of *´Cichlasoma´ salvini* (i.e. very similar to *Parachromis*, *´Cichlasoma´ urophthalmum*, *´Cichlasoma´ facetum*, *Herotilapia multispinosa* and *Amphilophus*) is probably the most common single type of coloration ontogeny among heroine cichlids. It is present in indistinguishable detail in a variety of species. It is positively present in *´Cichlasoma´istlanum*, *´Cichlasoma´grammodes*, *´Cichlasoma´ umbriferum*, *Petenia splendida*, *Hypsophrys nicaraguensis*, *´Cichlasoma´ panamense*, *Neetroplus nematopus* and *Nandopsis* (see below). Most of the named species develop the usual eight flank and tail bars (1a, 1p, 2, 3, 4, 5, 6, 7).

Fig. 11. Color pattern development in *´Cichlasoma´ salvini*. (a) 6 mm TL; (b) 9 mm TL; (c) 10 mm TL; (d) 11 mm TL; (e) 13 mm TL; (f) 14 mm TL; (g) 15 mm TL; (h) 15 mm TL; (i) 21 mm TL.

Fig. 12. Color pattern development in *´Cichlasoma´ facetum*. (a) 5,5 mm TL; (b) 7 mm TL; (c) 9,5 mm TL; (d) 10,5 mm TL; (e) 11 mm TL; (f) 13,5 mm TL; (g) 17 mm TL. I – *`C.´ sp.* "Forquilha" MCP 13389, 20 mm TL; II – *´C.´ scitulum* NRM 40063E, 47 mm TL.







A certain similarity exists between the ontogenies of *´Cichlasoma´ salvini*, *´Cichlasoma´ facetum* and *Herotilapia multispinosa*, in which the abdominal line posteriorly from the dominant midlateral blotch in the fourth bar partially divides into two lines, the lower portion being bend ventraly (Fig. 2 d–f; Fig. 11 e–i; Fig. 12 c–g).

Species (including no less than six undescribed species; unpubl. res.) in the *´Cichlasoma´ facetum* group show variation in the number of abdominal bars, with some species (e.g. *´C.´ scitulum*) always developing only three bars (5, 6, 7; Fig. 12 II), while other species develop four abdominal bars (e.g. *´Cichlasoma´ facetum*, Fig. 12 a–g; *´Cichlasoma´* sp. "Forquilha", Fig. 12 I). The dividing bar is bar number 5 and different species also differ in the proportion of individuals featuring this division. While about 90% of specimens of *´Cichlasoma´facetum* do have it, in *´Cichlasoma´* sp. "Forquilha" only about 50% of adult specimens posses the division, but it is present in all juveniles. The proportion of individuals with a divided fifth bar seems to be specific for a species and the variation also seems to be phylogeneticaly informative, as it agrees with the topology of the cladogram constructed for the species (unpubl. res.).

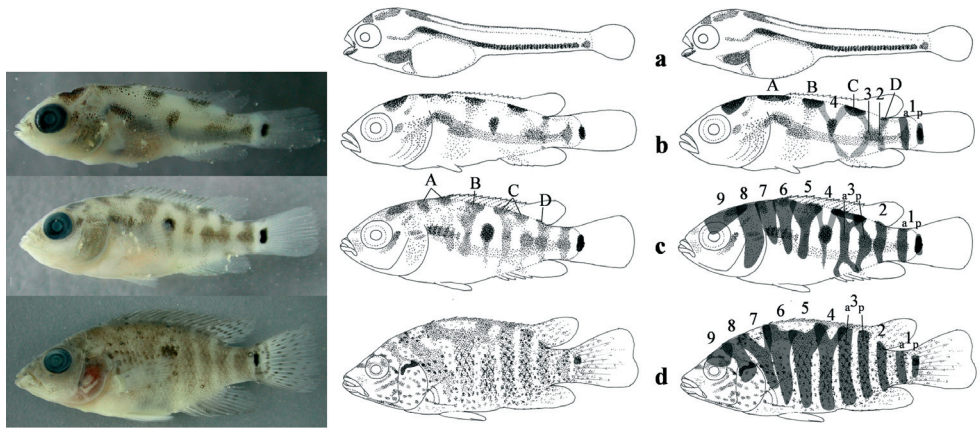


Fig. 13. Color pattern development in *Nandopsis tetracanthus*. (a) 7 mm TL; (b) 11 mm TL; (c) 13,5 mm TL; (d) 75 mm TL.

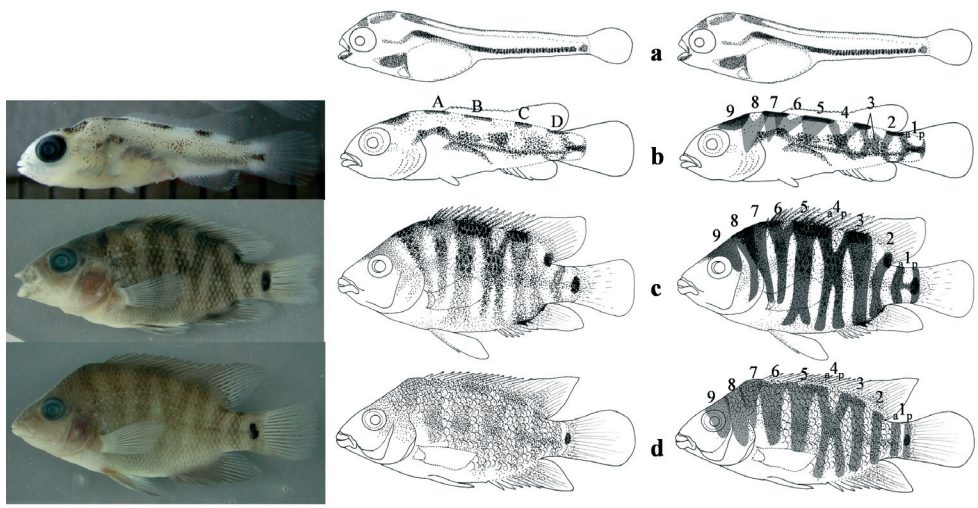


Fig. 14. Color pattern development in *Nandopsis ramsdeni*. (a) 7 mm TL; (b) 10 mm TL; (c) 45 mm TL; (d) 90 mm TL.

Adult pattern development in *Nandopsis* (Figs 13, 14).

The early ontogenies of all four *Nandopsis* species (*N. tetracanthus*, *N. haitiensis*, *N. vombergi, N. ramsdeni*) follow the most common developmental, pathway as described above, but later stages do differ between the two species studied in more detail (*N. tetracanthus*, *N. ramsdeni*). While in *N. tetracanthus* it looks like there is an additional bar developing from the dorsal blotch C (Fig. 13 c) as in *Cryptoheros*; see above), in *N. ramsdeni* it seems that the fourth bar divides into two bars (Fig. 14 c). The series of both species are incomplete and have been taken in the wild in Cuba, so our interpretation of the situation is only preliminary. Not considering individual bar homologies, all *Nandopsis* species do have one bar more above the anal fin than is the usual model situation of eight tail and body bars. *Nandopsis ramsdeni* differs in many aspects of its morphology from the remaining three species (probably including a different bar formula), but molecular data indicate its close relationships to the other Cuban *Nandopsis*, *N. tetracanthus* to the exclusion of the Hispaniolan species (unpubl. res.).

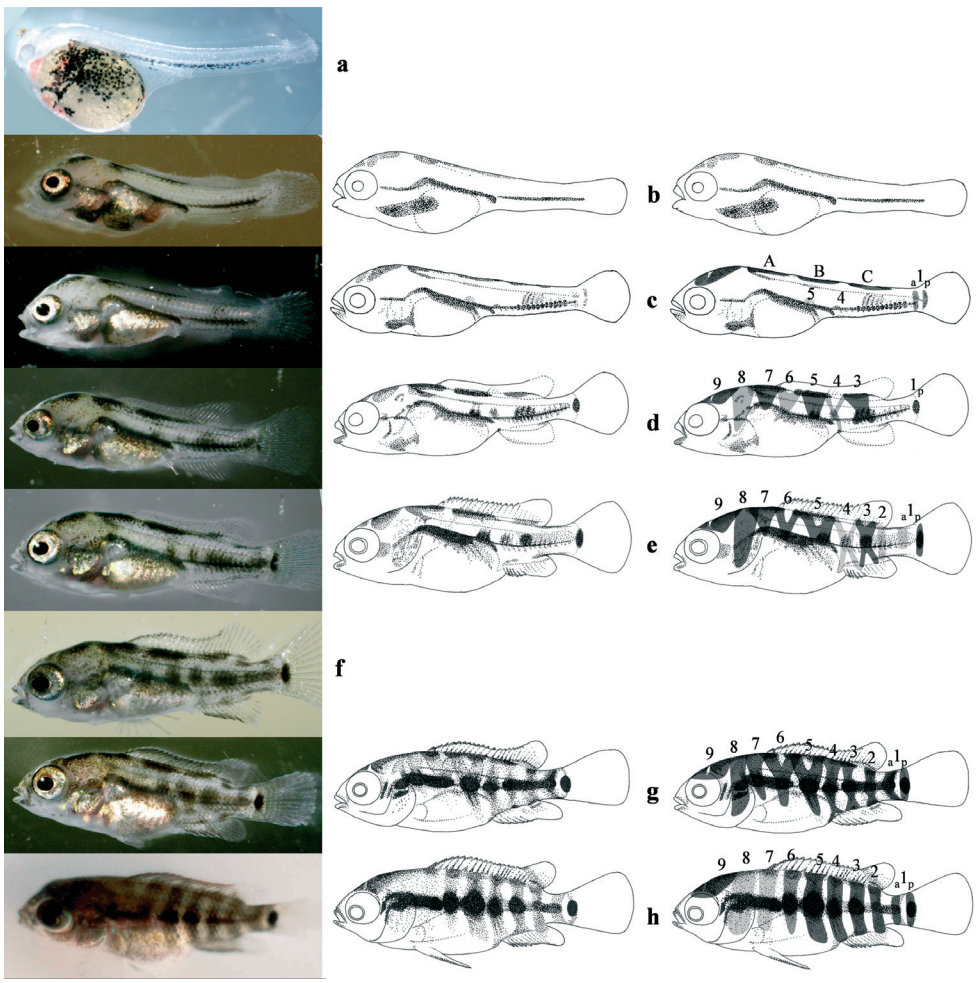


Fig. 15. Color pattern development in *Herichthys carpintis*. (a) 4 mm TL; (b) 6 mm TL; (c) 8 mm TL; (d) 9 mm TL; (e) 9–10 mm TL; (f) 13 mm TL; (g) 14 mm TL, (h) 16–18 mm TL.

Adult pattern development in *Herichthys* (Fig. 15)

Herichthys species are also characteristic by a dominant and long lasting abdominal stripe, but specific details are more similar to the situation in *Theraps*, *Chuco* and *Paratheraps* than to the *Parachromis*-like species described to this point. Early stages in the development of *Herichthys* are characteristic in that the abdominal stripe becomes divided into two parts before there are any signs of vertical bars. The division is sometimes not complete, but the stripe is always at least very narrow in the area above the anterior insertion of the anal fin (Fig. 15 b–d). Linked with this feature is also the observation that the usually heavily developed midlateral blotch of the fourth bar is less pigmented then the two neighboring blotches (Fig. 15 f; compare with the situation *Parachromis*, *Nandopsis*, *Amphilophus*, or *Herotilapia*). These two features have been observed in all *Herichthys* species for which we have some developmental information (*H. carpintis, H. cyanoguttatus, H. bartoni, H. labridens, H. minckleyi*). The interruption of the abdominal stripe in larvae about one week old is also known in *Theraps* and *Chuco* species and in *Paratheraps breidohri* (see below).

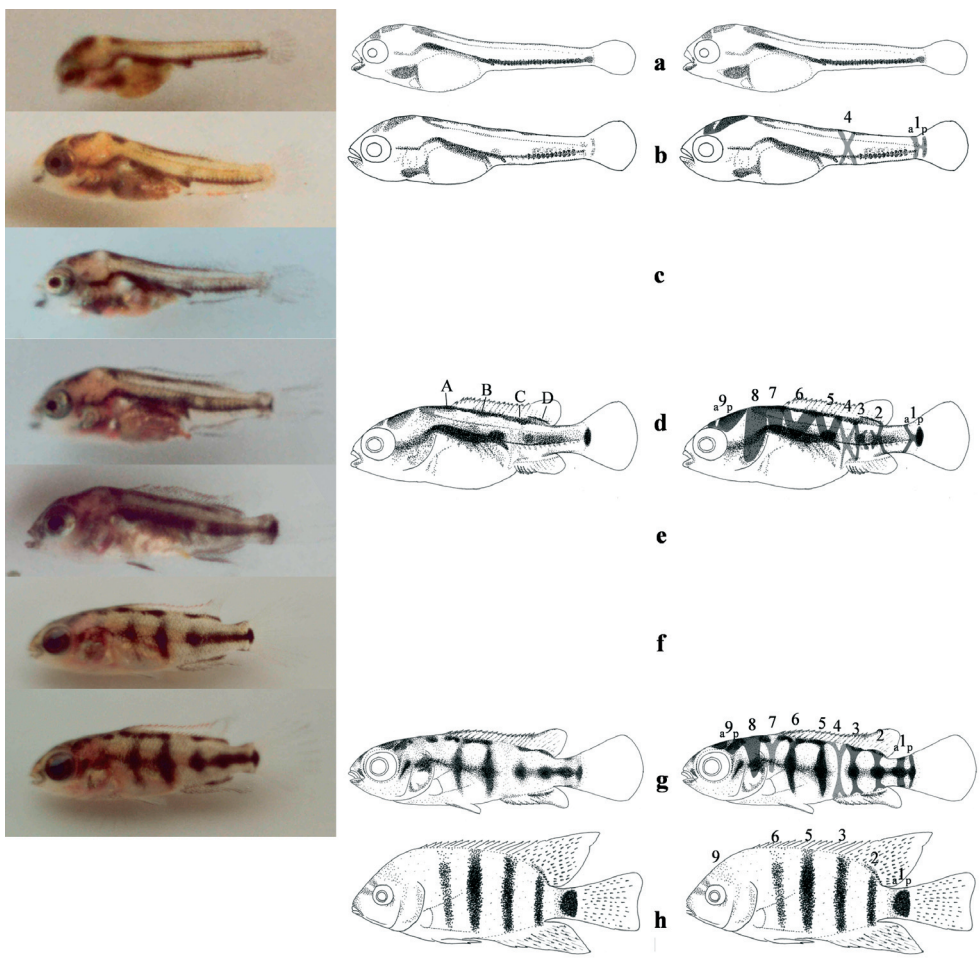


Fig. 16. Color pattern development in *´Paratheraps´ regani*. (a) 8 mm TL; (b) 9 mm TL; (c) 11 mm TL; (d) 13 mm TL; (e) 14 mm TL; (f) 18 mm TL; (g) 20 mm TL; (h) 35 mm TL.

The rest of the development is in general similar as in *Parachromis* or e.g. *Amphilophus* also with the same adult bar homologization. There are thus eight adult body and tail bars (the anteriormost body bar is the least visible one). Contrary to *Parachromis* or e.g. *Amphilophus*, the largest and most distinct midlateral blotch in adults of *Herichthys* species belongs to the fifth bar, not to the fourth as is usual in heroines (viz. discussion of this blotch above). The same situation is found in *Theraps* (*T. lentiginosus*, *T. irregularis*). Among the *´Cichlasoma´* species, the typically interrupted line (Fig. 15 b–d) is also present in the north Mexican *´Cichlasoma´ beani*.

Adult pattern development in *´Paratheraps´ regani* (Fig. 16)
´Paratheraps´ regani also features the interruption of the abdominal stripe above the anterior insertion of the anal fin (Fig. 16 c–e) and to even a higher extent than *Herichthys*. Traces of the developing fourth bar are visible in one stage (Fig. 16 d,e), but there are no signs of the fourth bar midlateral blotch and the fourth bar itself very soon disappears as well (Fig. 16 f) and is completely missing in the adult coloration pattern (Fig. 16 h). The anteriormost body bar (7) is also missing from the adult coloration (note that this bar is also very little developed in *Herichthys*; see above). The abdominal line is more pigmented than in *Herichthys* throughout the ontogeny and also disappears in later stages. There is also only one big blotch in the place of the two bars on the caudal peduncle, and this is because the divided first bar (1) in late stages again fuses into one blotch.

Adult pattern development in *Paratheraps* (Fig.17) and *Vieja* (Figs 18, 19)
Vieja and *Paratheraps* species develop a very intensively pigmented, dominant and long lasting abdominal stripe. *Paratheraps breidohri* similarly to *´Paratheraps´ regani* does not develop this hypertrophied abdominal stripe, and shows the more ancestral condition. Also postponed is the formation of bars. The adult bar pattern in all four species (*P. breidohri*, *P. fenestratus*, *V. synspila*, *V. maculicauda*) is of the common formula of six body bars (2, 3, 4, 5, 6, 7; the tail bar no. 1 is usually developed undivided as one wide bar). The ontogenetic series of *Paratheraps fenestratus* do show the typical interruption of the abdominal line and the postponed development of the fourth bar (Fig. 17 d,e), but subadult *P. breidohri* as well as *P. fenestratus* do have the fourth bar developed, contrary to the situation in *´P.´ regani*. The adult coloration patterns of most *Paratheraps* species are nevertheless highly modified, and only traces of bars are visible. The bars are usually best visible in breeding coloration. In the *Vieja* species (*V. maculicauda*, *V. synspila*) the abdominal line is very wide, intensive and long lasting, the extreme situation among heroine cichlids. Development of vertical bars is only hinted, and no developing bars can usually be observed in the abdominal region (Figs 18, 19). It thus seems that the postponing of bar formation due to prolonged persistence of the abdominal stripe has reached its maximum in *Vieja* species among the heroines. There are also at best only traces of the dorsal line being disrupted into the four dorsal blotches as is the case in the majority of species examined. Adults of *Vieja* species do not show any bars, and compared to *Paratheraps* species, the abdominal line is restricted to the postanal part of body only. Another synapomorphy of *Vieja* is best seen in adult *Vieja maculicauda*, where there is a wide diffuse bar in front of the insertion of the anal fin. It is homologous to the fourth body bar. In *Vieja melanura* and *Vieja synspila*, this bar is not a continuous structure, but is formed of a pigmented area in front of the insertion of the anal fin and vertically aligned with it below the dorsal fin. It is clearly interpreted as the wide diffuse bar of *Vieja maculicauda*, interrupted along the body midline. In adult *Paratheraps*, the abdominal stripe runs over the entire

body, but it is bent to a much more ventral position, running anteriorly towards the insertion of the pectoral fin. *Paratherpas breidohri* has only the plesiomorphic upper positioned adult abdominal stripe. *Paratheraps bifasciatus* has the abdominal stripe divided into two as the name suggests and this is also true for *Paratheraps gutulatus* and *P. hartwegi*, while *Paratheraps fenestratus* has already only the lower part developed. The presence of both the upper and lower parts of the abdominal stripe is probably a synapomorphy of part of *Paratheraps* (i.e. without *P. breidohri*).

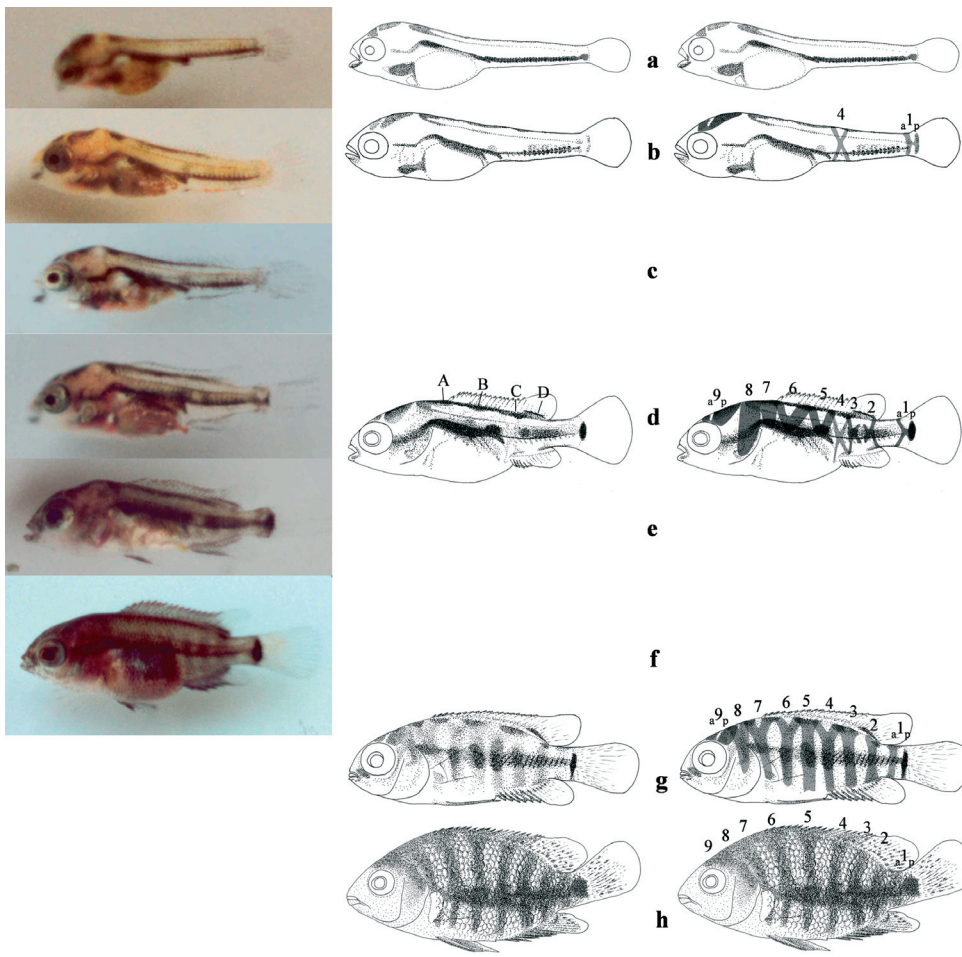


Fig. 17. Color pattern development in *Paratheraps breidohri* (a–f) and *Paratheraps fenestratus* (g, h). (a) 8 mm TL; (b) 9 mm TL; (c) 11 mm TL; (d) 13 mm TL; (e) 14 mm TL; (f) 17 mm TL; (g) 18 mm TL; (h) 59 mm TL.

Adult pattern development in *Astatheros* (Figs 20, 21) and *Thorichthys*

We have not been able to examine the coloration ontogeny of the type species of *Astatheros* (*A. macracanthus*), but two species of the longimanus group of *Astatheros* (*A. longimanus*, *A. robertsoni*) show nearly identical ontogenies, and these are also very similar to ontogenies of other species of *Astatheros* (alfari group) and also to *Thorichthys*. All these species start the free-swimming period with already disrupted horizontal lines (Figs 20a, 21a), the

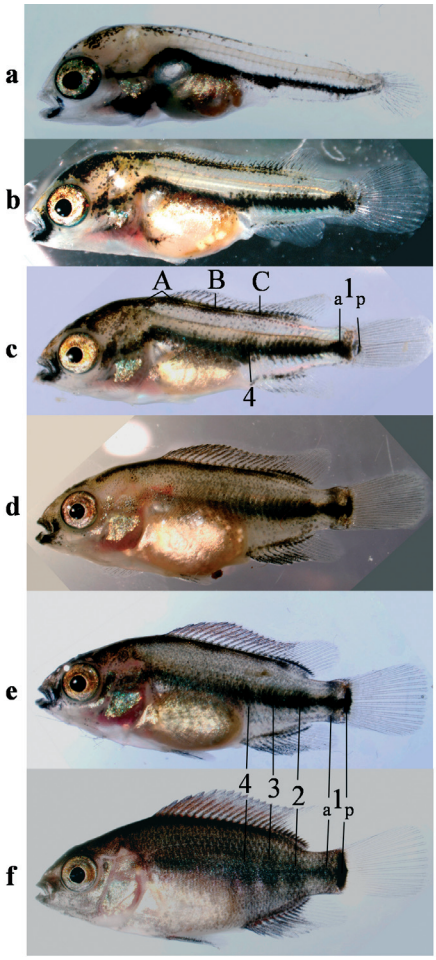


Fig. 18. Color pattern development in *Vieja synspila*. (a) 6 mm TL; (b) 8,5 mm TL; (c) 10 mm TL; (d) 14 mm TL; (e) 15 mm TL; (f) 19,5 mm TL.

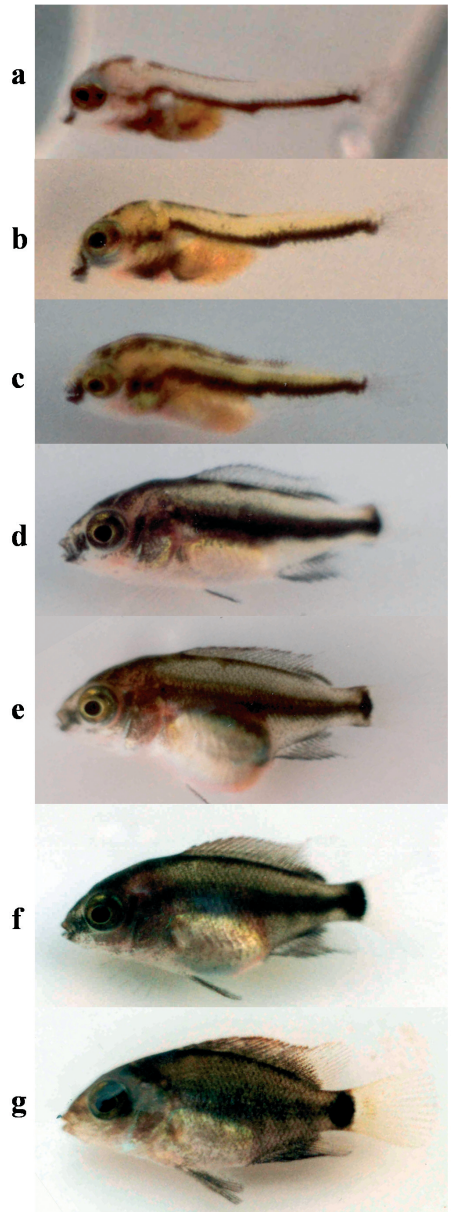


Fig. 19. Color pattern development in *Vieja maculicauda*. (a) 8 mm TL; (b) 9–10 mm TL; (c) 10–11 mm TL; (d) 15–16 mm TL; (e) 18 mm TL; (f) 21–24 mm TL; (g) 34 mm TL.

four blotches originating from the disruption of the dorsal line (A, B, C, D) are well developed. The early interrupted abdominal line should be considered as apomorphic for *Astatheros* and *Thorichthys*, because the majority of heroine species start ontogenies with an uninterrupted abdominal line. But this type of ontogeny with an uninterrupted and persistent abdominal line is a speciality of heroines, and other groups, e.g. cichlasomatines do start ontogeny with a pattern much more similar to *Astatheros* or *Thorichthys*. The final word has to await a firm establishment of the phylogenetic position of these two groups among the heroines.

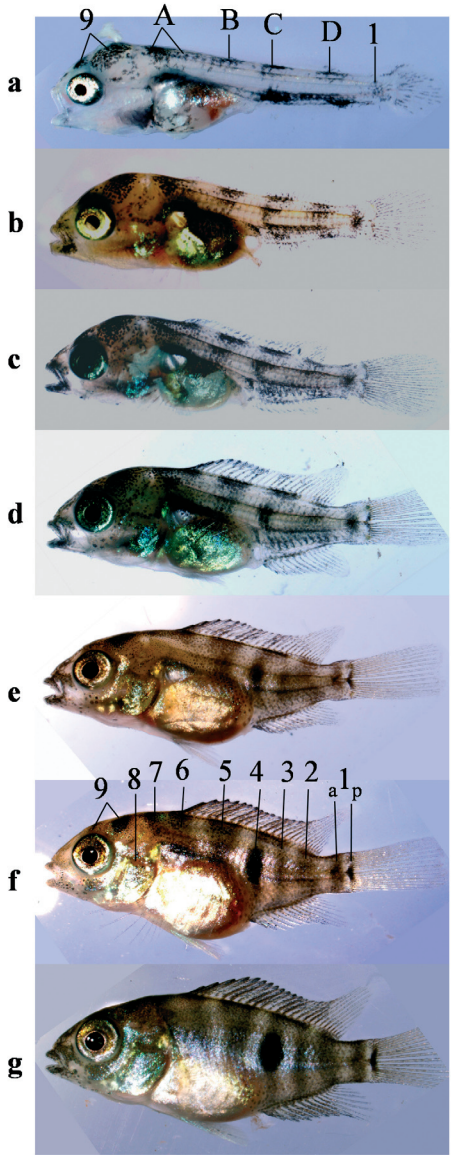


Fig. 20. Color pattern development in *Astatheros longimanus*. (a) 6 mm TL; (b) 7 mm TL; (c) 8 mm TL; (d) 10 mm TL; (e) 12 mm TL; (f) 14 mm; (g) 15 mm.

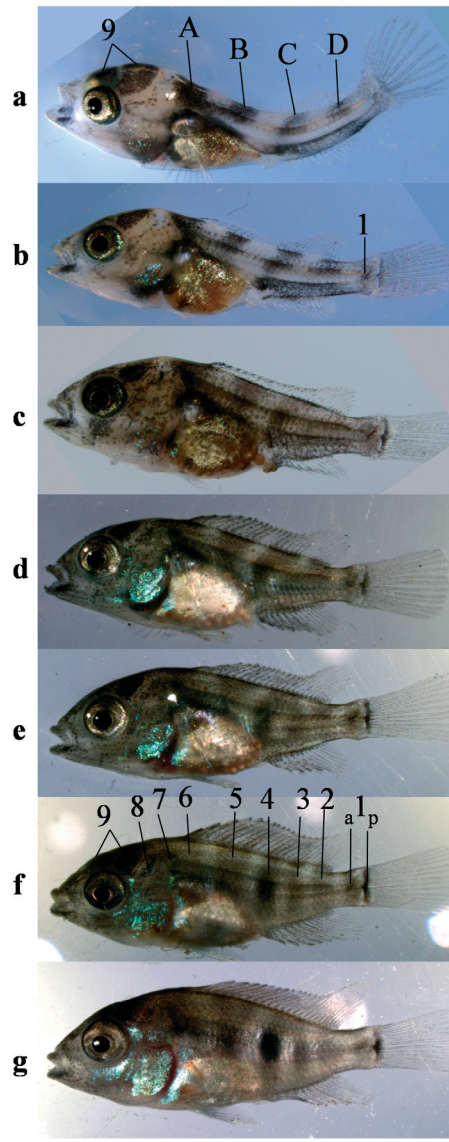


Fig. 21. Color pattern development in *Astatheros robertsoni*. (a) 7 mm TL; (b) 8 mm TL; (c) 9 mm TL; (d) 10 mm TL; (e) 12 mm TL; (f) 15 mm TL; (g) 18 mm TL.

Further development of bars in *Astatheros* and *Thorichthys* proceeds classically by vertical migration of melanophores producing the most common bar formula of eight body and tail bars (1a, 1p, 2, 3, 4, 5, 6, 7). The late stages of development in *Astatheros* and *Thorichtys* look very similar to the situation in *Amphilophus*, with the dominant and large midlateral blotch, but this color pattern develops through a different ontogenetic pathway compared to *Amphilophus* (see Fig. 9 f–h).

Adult pattern development in *´Cichlasoma´ octofasciatum* (Fig. 22)

The last two heroine groups, whose ontogenies we present, show very modified, distinctive and group specific coloration ontogenies. It is interesting that the adult pigmentation patterns, though distinctive, are not as profoundly different from the majority of heroines as are their juvenile pigmentation patterns. The pigment pattern ontogenies of these two species are in some features similar (as is the adult bar pattern homologization) but distinctive in others, and it only remains to be tested by an independent data set with cladistic methods whether they are independently gained or whether the similarities are homologous. There is one character which is unique for these two species among the studied taxa. In early larvae of both *´Cichlasoma´ octofasciatum* and *´Cichlasoma´ atromaculatum*, the two anterior dorsal migration line blotches (A, B) are fused into a single large blotch and the bars originating from these blotches develop as one. The bars of this area gain their independence only much later in the development. This condition is to be considered as apomorphic, compared with both heroines and cichlasomatines (see below). This state is also present in the two species

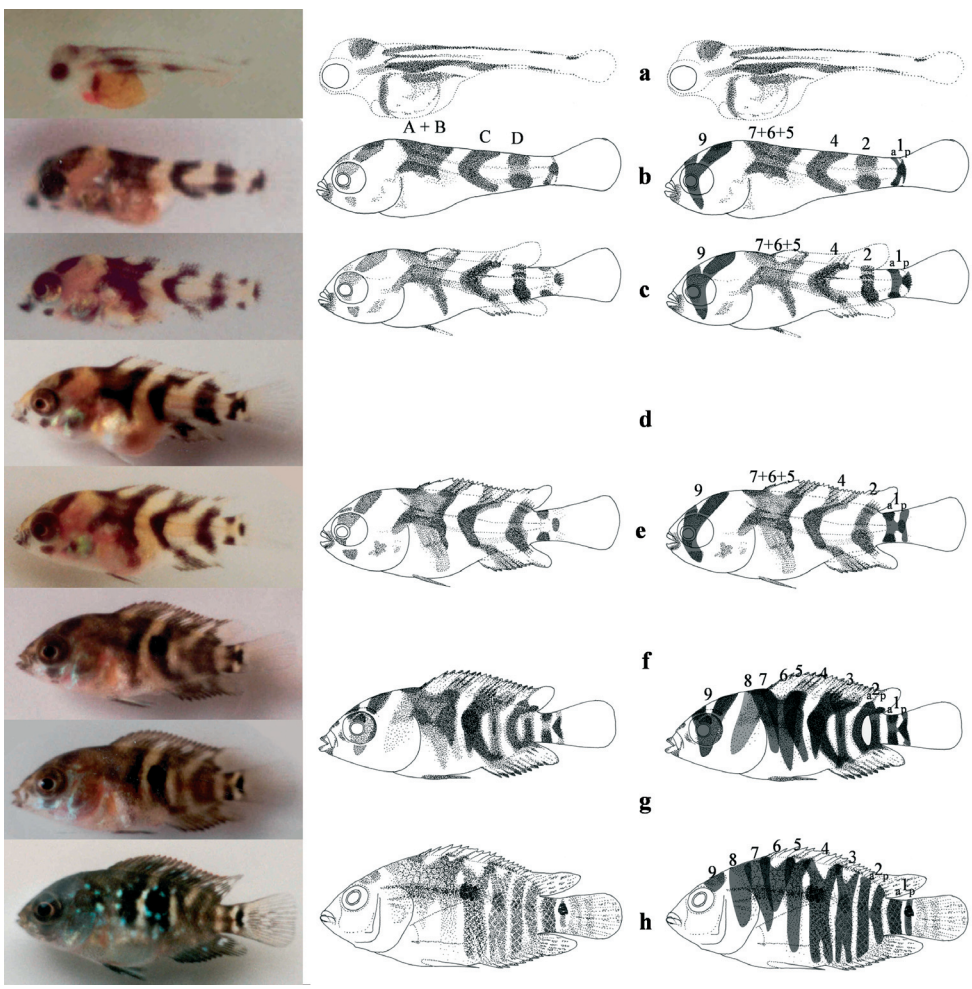


Fig. 22. Color pattern development in *´Cichlasoma´ octofasciatum*. (a) 4,5 mm TL; (b) 6–7 mm TL; (c) 8 mm TL; (d) 11mm TL; (e) 12–13 mm TL; (f) 18 mm TL; (g) 20 mm TL; (h) 30–50 mm TL.

of the genus *Tomocichla* (*T. underwoodi* (syn. *tuba*) and *T. asfraci*). An important difference between *´C.´ octofasciatum* and *´C.´ atromaculatum* is that *´Cichlasoma´ octofasciatum* develops the suborbital stripe in larger larvae and juveniles (Fig. 22 b–g; cf. with *´C.´ atromaculatum*), which is among the studied heroines only known from *Heros*, where it is also part of the adult pigmentation pattern. The presence of the suborbital stripe in adults is primitive for heroines, developed only in the South American genera *Heros*, *Uaru*, *Symphysodon*, *Mesonauta*, *Pterophyllum*, *Hypselecara*, and *Hoplarchus*, while all other heroines have obliterated it completely from their ontogenies (see Figs 2–21 and 23).

´Cichlasoma´ octofasciatum develops the conservative larval pigmentation pattern of four migration lines, which is shown in Fig. 22 a in the yolk sac stage, but this pattern is lost very soon and is transformed into a distinctive pigmentation pattern worn by the larvae when they start to swim for the first time (Fig. 22 b). This larval pigmentation pattern is composed of four pigmented areas on the body sharply contrasting with the unpigmented rest of the body. The posteriormost blotch is easily identified as the blotch from which the first bar will develop, later dividing into two parts (1a, 1p). The second, very intensive, wide stripe in the larval pattern is based on its position at the posterior insertion of the dorsal and anal fins identified as the second bar (2), already developed and fused with the fourth dorsal blotch (D). This bar later divides into two bars (Fig. 22f–h). The next intensively pigmented bar is the fourth bar (4), which also in later stages develops the for heroines typical midlateral blotch (Fig. 22 f–h). The third bar (3) is completely missing from the early larval stage (Fig. 22 b) and develops only later in the area between the second and fourth larval bars as expected (Fig. 22 e–h). The three anterior body bars (5, 6, 7) develop from the anterior heavily pigmented area of the first developmental stage (Fig. 22 b), which includes the two anterior dorsal blotches A and B. The subadult bar homologization is shown in the drawings of stages g and h (Fig. 22 g, h) and there are nine body and tail bars (1a, 1p, 2a, 2p, 3, 4, 5, 6, 7).

A phenomenon worth stressing is the developmental sequence of the suborbital stripe, since *´Cichlasoma´ octofasciatum* is the only known Mesoamerican heroine that develops a suborbital stripe during its ontogeny. We discuss its implications in the discussion. Here it is important to stress that the suborbital stripe starts to develop very early, it is present already at the size of 7–8 mm at the age of few days after free-swimming, but it disappears in juveniles larger than 18–20 mm TL, and is not developed in adults (Fig. 22 c–h; see the developmental timing of the bar in cichlasomatines).

Adult pattern development in the *´Cichlasoma´ festae* group (Fig. 23)

Whether *´Cichlasoma´ atromaculatum* develops the conservative larval pattern of four migration lines is unknown. The homologization of the adult bar pattern is the same as in *´Cichlasoma´ octofasciatum*, also the earliest larval pattern is similar, but there are also differences. The smallest specimens available feature a superficially similar pigmentation pattern as the early larvae of *´Cichlasoma´ octofasciatum* (Fig. 23 b cf. with Fig. 22 b). Fig. 23 a shows the first developmental stage in *´Cichlasoma´ festae*, a closely related species to *´Cichlasoma´ atromaculatum*. The pattern in the two species of the *´Cichlasoma´ festae* group is the same, being only different in that the stage in *´Cichlasoma´ atromaculatum* is a later one compared to *´Cichlasoma´ festae* (cf. sizes of specimens in Fig. 23 a, b). The same juvenile pattern as in *´Cichlasoma´ atromaculatum* (Fig. 23 b) is developed in the two *Tomocichla* species (*T. underwoodi*, *T. asfraci*). The major difference between the early juveniles of *´Cichlasoma´ octofasciatum* and those of the *´Cichlasoma´ festae* group are in the absence of the fourth bar in the earliest stages of *´Cichlasoma´ atromaculatum* (cf. Figs 22 b and 23 b).

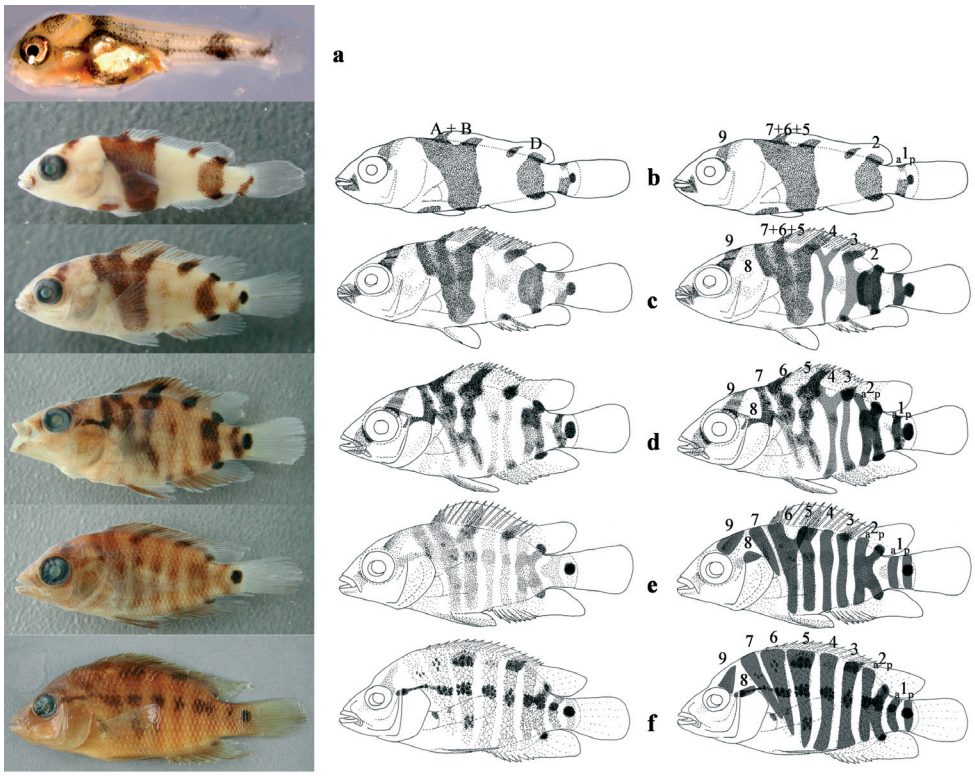


Fig. 23. Color pattern development in *´Cichlasoma´ atromaculatum*. (a) 5 mm TL (*´C.` festae*); (b) 11 mm TL; (c) 17 mm TL; (d) 22 mm TL; (e) 24 mm TL; (f) 55 mm TL.

Otherwise, the pigment pattern development of the two species proceeds the same way, i.e. the second bar (2) divides into two adult bars (Fig. 23 c–f) and the third bar (3) develops later than the remaining bars (Fig. 23 c–f), with the difference, that in *´Cichlasoma´ octofasciatum*, the third bar alone develops as last, whereas in *´Cichlasoma´ atromaculatum* both the third and fourth bars develop late (cf. Figs 22 c–g to 23 c–f).

Both species are different from the majority of heroines in that they do not have almost any signs of the abdominal line after free-swimming, but as can be seen from both the smallest *´Cichlasoma´ octofasciatum* as well as *´Cichlasoma´ festae*, the abdominal line is present in stages before free swimming (Figs 22 a and 23 a).

Adult coloration pattern development in the tribe Cichlasomatini

Cichlasomatines develop a unique and apomorphic early larval pattern which is highly uniform across the cichlasomatine taxa that we had a chance to examine. It is present in all recognized cichlasomatine genera.

The early larval coloration pattern of cichlasomatines (see e.g. Fig. 24) is composed from a round blotch at the base of the caudal fin, a blotch in the postanal region of the body, two blotches on the dorsal border of the body and an accompanying blotch placed midlateraly ventral from them and a pair of blotches on the head above the eye. This specific pattern is developed

in all cichlasomatines when their larvae start to swim (Figs 24–36). The dorsal blotches forming from the interrupted dorsal migration line are part of this conserved early juvenile coloration pattern. All four are visible only in the two examined species of *Laetacara* (Figs 35, 36) while all other genera show only the anterior three blotches (A, B, C). Other remains of the four horizontal migration lines are not visible in free swimming juveniles, contrary to the situation in heroines. In cichlasomatines, this conservative larval pattern of four migration lines is developed in only very early larvae with a yolk sac, but then it is very rapidly transformed into the uniform cichlasomatine larval pattern worn by early free-swimming larvae.

In general, not only is the early larval pigmentation pattern of cichlasomatines apomorphic compared to heroines, but also the pigment pattern transformations leading to the adult bar patterns are more modified. There is a general resemblance of the early larval pattern of cichlasomatines to that of the heroine's genera *Astatheros*, *Thorichthys* and also *´Cichlasoma´ octofasciatum* and *´Cichlasoma´ atromaculatum*, particularly in having roughly similar areas of the body heavily pigmented and others devoid of pigmentation. Cichlasomatines also lack the four migration lines even at very small sizes. Even though the first ontogenetic stage of cichlasomatines looks quite similar to that in *´Cichlasoma´ octofasciatum* or in the *´Cichlasoma´ festae* group, the dominant lateral blotch in the first ontogenetic stage of *´Cichlasoma´ festae* is not homologous to the similarly positioned blotch in cichlasomatines. While in *´Cichlasoma´ festae* it is the second bar fused with the dorsal blotch D, in cichlasomatines it is the fourth bar and part of the third bar fused with dorsal blotch C (see Figs 22 and 24). This difference is easily observed in later developmental stages, where in cichlasomatines the second bar forms as the last one (e.g. Fig. 24 a–e), while in the *´Cichlasoma´ festae* group and also in *´Cichlasoma´ octofasciatum*, the second bar is present from the start. The similarity between the initial stages between cichlasomatines and the named heroines is thus non-homologous and based on available information on relationships also convergent rather than ancestral.

Another coloration pattern is entirely unique to the cichlasomatines only. The third bar is from the start anteriorly fused with the fourth bar, and later separates from it through a specific circular pattern as the fourth bar moves into its position above the vent. This circular connection is so well developed only in the cichlasomatines and larval cichlasomatines can be recognized as such based on this structure (Figs 24 d, e; 26 c; 27 a; 30 b, c; 31 b; 34 c; 35 a, b). The distinctive nature of this circular connection is due to the early fusion of the posterior portion of the fourth bar with the anterior portion of the third bar. Less developed versions of this circular connection can also be seen in some heroines, e.g. in *Herotilapia* (Fig. 3 c–d). What is characteristic for cichlasomatines is that it is so heavily developed compared to other pigmented structures and dominates the early developmental stages. Such a fully closed and heavily pigmented circle is almost diagnostic for cichlasomatines. Among all examined cichlasomatine genera it is missing only in *Laetacara* (Figs 36, 37), which has an even more modified coloration ontogeny, as discussed below. In later developmental stages, the third and fourth bars separate and develop into two adult bars in all the genera except *Cichlasoma*, where in most species the fourth bar divides into two bars (Figs 32–35).

Another distinction between the ontogenies of cichlasomatines and heroines is in which bar develops the dominant midlateral blotch. In heroines the most heavily pigmented bar, often carrying the distinct midlateral blotch, is the fourth bar, developing between the dorsal blotches B and C. Also in cichlasomatines, throughout early ontogeny, the dominant bar is the fourth bar, here already developed in the first stages. But in later stages, the dominance shifts

to the fifth bar, and adults carry a midlateral blotch in the fifth bar and not in the fourth bar as do heroines. In the case of *'Aequidens' pulcher*, this transition happens between stages c and f (Fig. 24). In stage c, the dominant bar is still the fourth, whereas in state e it is the fifth bar. There are interesting implications of this observation, which will be detailed in the discussion. The situation in heroines is unique to them while cichlasomatines feature the plesiomorphic situation (see discussion).

The last distinction from Mesoamerican heroines is that cichlasomatines, as the majority of Neotropical cichlids and the South American lineages of heroines, feature a suborbital stripe or blotch as adults. The coloration ontogeny of *'Aequidens' pulcher* (Fig. 24) shows the temporal sequence of its development.

Adult pattern development in the *'Aequidens' pulcher* group (Figs 24, 25)
Most of the details about color pattern development have been described above. The homology of the principal blotches in the early larval pattern has also been discussed above. Note the circular connection between bars three and four typical for cichlasomatines (Fig. 24 d–e) and the sequence of development of the suborbital stripe.

The adult bar pattern is composed of eight body and tail bars (1a, 1p, 2, 3, 4, 5 ,6, 7).

Adult pattern development in *Aequidens* (Figs 26–29)
Most details have been described in the general section about cichlasomatines above, note the typical cichlasomatine color pattern at the onset of the free swimming period (Fig. 26a), the location of the midlateral blotch in bar number five (Figs 26 d, 27 b, 28 b, 29 b) and presence of the suborbital stripe in the same stages. The adult bar pattern in all species except *Aequidens*

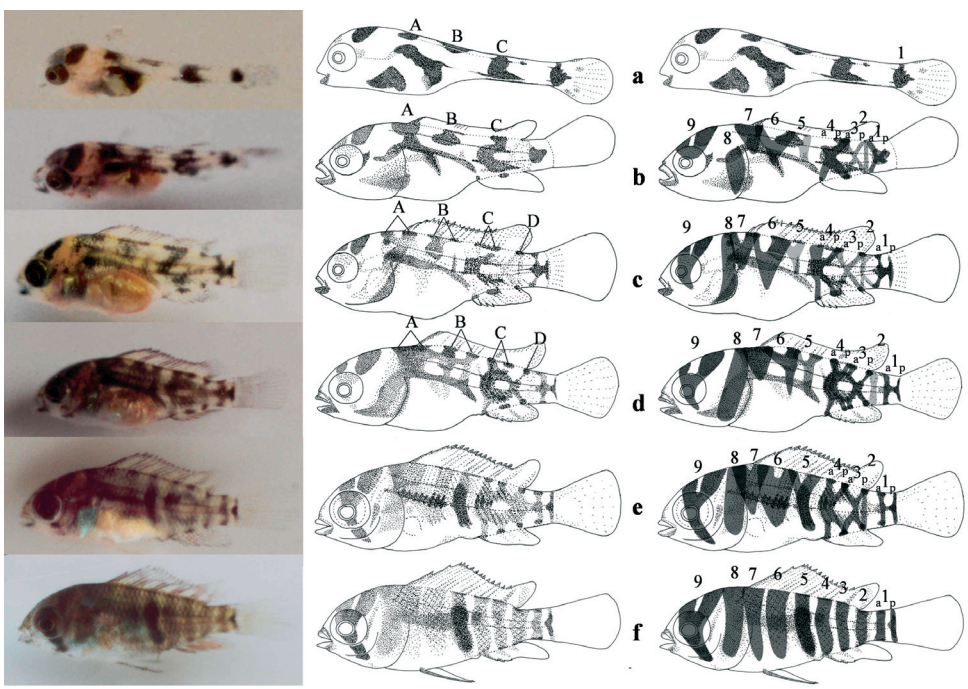


Fig. 24. Color pattern development in *´Aequidens´ pulcher.* (a) 6 mm TL; (b) 8–9 mm TL; (c) 11–12 mm TL; (d) 11–12 mm TL; (e) 17 mm TL; (f) 18–20 mm.

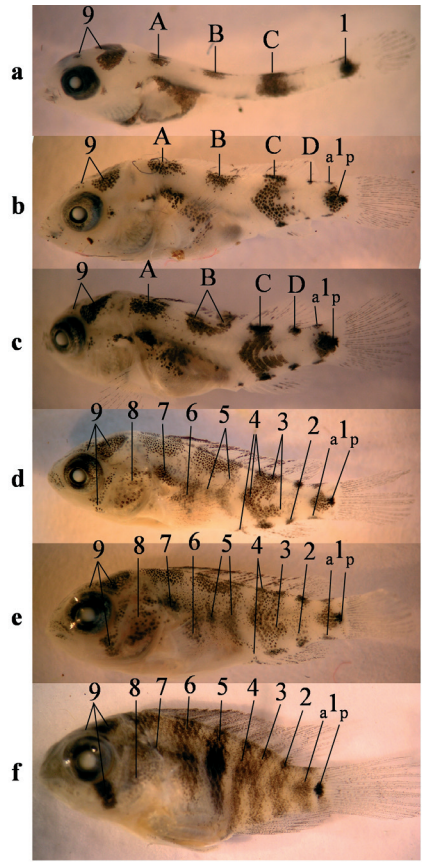


Fig. 25. Color pattern development in hybrids of *´Aequidens´ pulcher* and *´Aequidens´ rivulatus*. (a) 6,5 mm TL; (b) 8 mm TL; (c) 11 mm TL; (d) 12 mm TL; (e) 15 mm TL; (f) 18 mm.

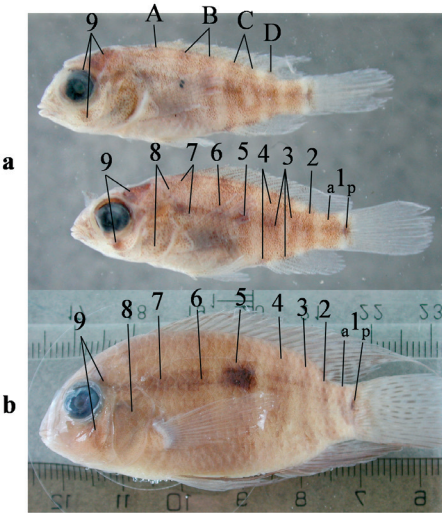


Fig. 27. Color pattern development in *´Aequidens´ cf pulcher* "Colombia". (a) 15 mm TL; (b) 70 mm TL.

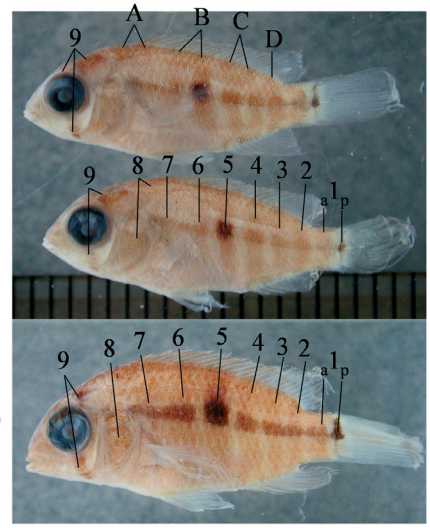


Fig. 28. Color pattern development in *Aequidens tetramerus*. (a) 19,5 mm TL; (b) 30 mm TL.

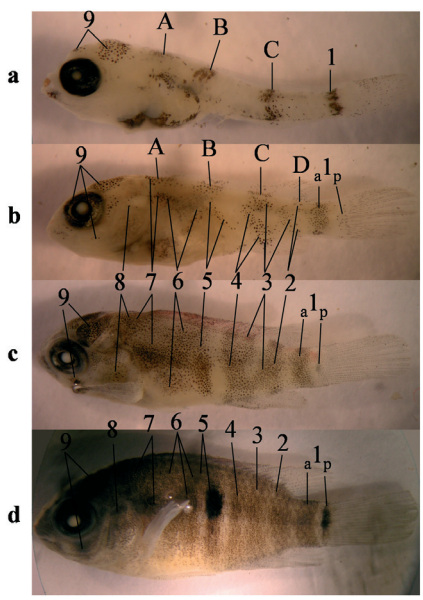
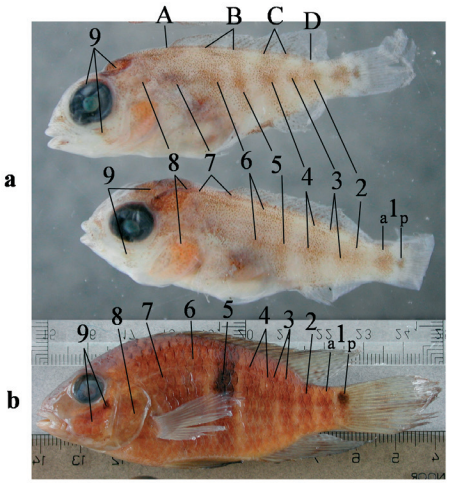


Fig. 26. Color pattern development in *Aequidens patricki*. (a) 6 mm TL; (b) 11 mm TL; (c) 15 mm TL; (d) 21 mm.



rondoni is composed of eight body and tail bars (1a, 1p, 2, 3, 4, 5 ,6, 7).

Adult pattern development in *Bujurquina*, *Acaronia*, and *Krobia* (Figs 30, 31, 32)
The early larval pigmentation pattern in the three genera of *Bujurquina*, *Acaronia*, and *Krobia* is typically cichlasomatine, as is the development of the adult pattern, with the most common bar

Fig. 29. Color pattern development in *Aequidens rondoni*. (a) 16 mm TL; (b) 100 mm TL.

Fig. 30. Color pattern development in *Bujurquina vittata*. (a) 6 mm TL; (b) 16 mm TL; (c) 18 mm TL; (d) 22 mm TL.

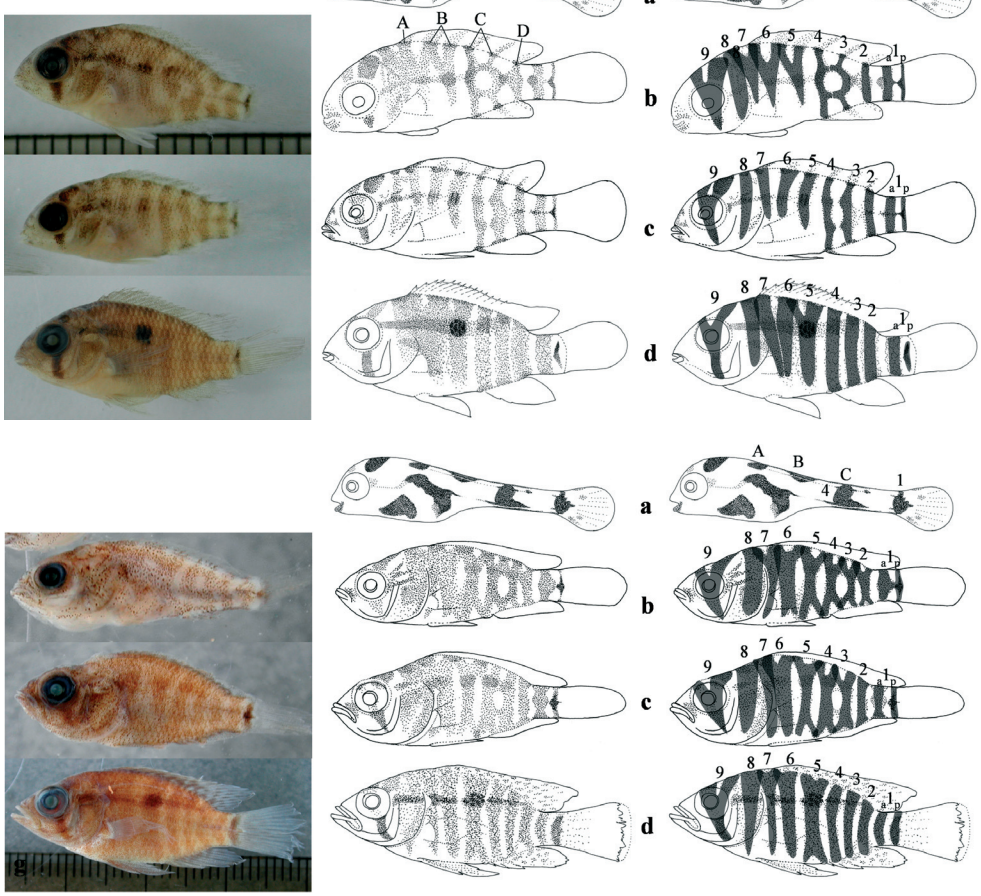


Fig. 31. Color pattern development in *Acaronia nassa*. (a) 6 mm TL; (b) 11 mm SL; (c) 18 mm TL; (d) 37 mm TL.

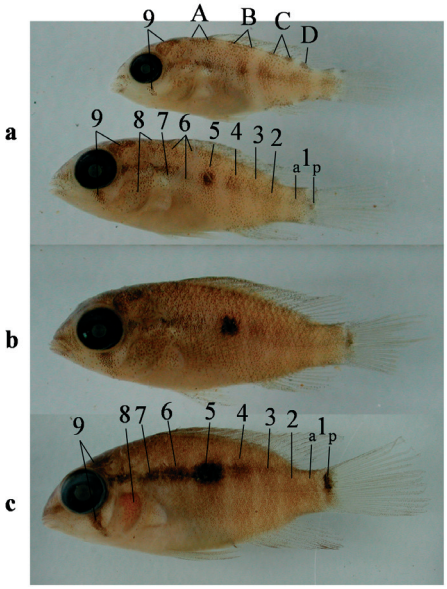


Fig. 32. Color pattern development in *Krobia sp*. (a) 12,5–15 mm TL; (b) 18 mm TL; (c) 30 mm TL.

formula (1a, 1p, 2, 3, 4, 5, 6, 7). Note the typically cichlasomatine circular connection between bars 3 and 4, the strongly developed suborbital stripe, the midlateral blotch positioned in the fifth bar, and also the midlateral stripe inclined dorsaly behind the midlateral blotch, a feature also developed only in these three genera among the studied cichlasomatines.

Adult pattern development in *Cichlasoma* (Figs 33–36)

All *Cichlasoma* species develop the typical cichlasomatine larval melanistic pattern, and also other details of the development agree with what has been written above for cichlasomatines. Some *Cichlasoma* species develop one more body bar than has been described for *'Aequidens' pulcher*, *Aequidens*, *Bujurquina*, *Krobia* or *Acaronia*. In *Cichlasoma*, the fourth bar divides into two adult bars. Probably because of spatial limitations caused by this division, the

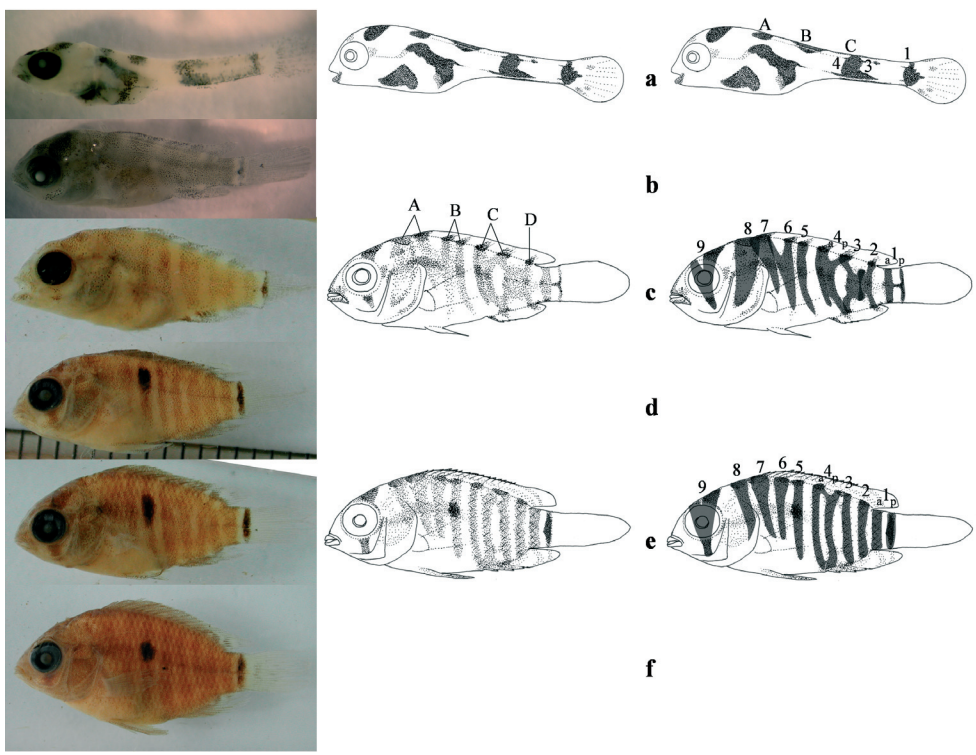


Fig. 33. Color pattern development in *Cichlasoma boliviense*. (a) 6 mm TL; (b) 10 mm TL; (c) 11 mm SL; (d) 16 mm TL; (e) 17 mm TL; (f) 25 mm TL.

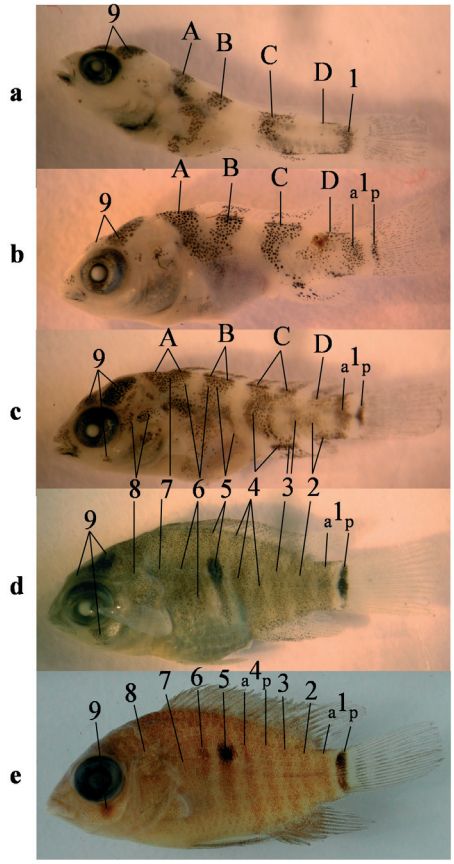


Fig. 34. Color pattern development in *Cichlasoma amazonarum*. (a) 6,5 mm TL; (b) 8 mm TL; (c) 11 mm TL; (d) 18 mm TL; (e) 25 mm TL.

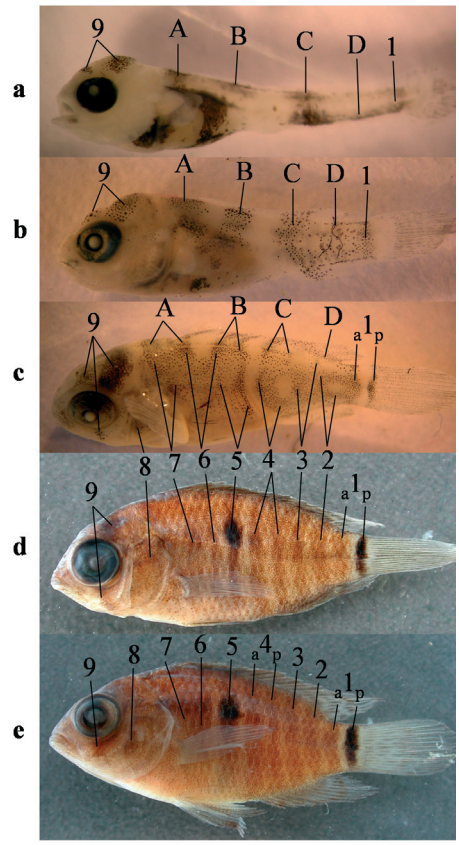
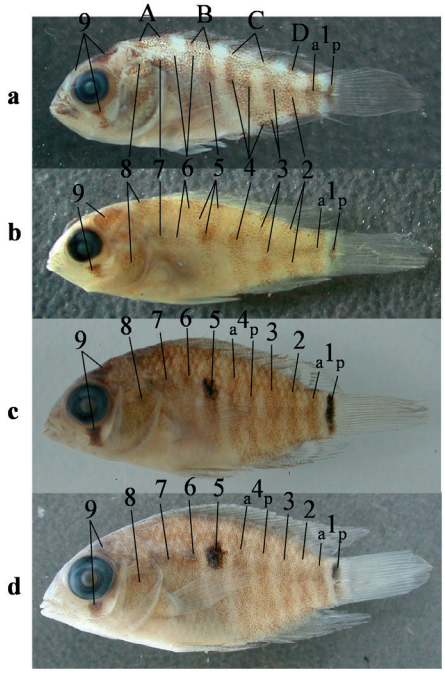


Fig. 35. Color pattern development in *Cichlasoma bimaculatum*. (a) 6 mm TL; (b) 8 mm TL; (c) 10 mm TL; (d) 23 mm TL; (e) 30 mm TL.

circle between the third and fourth bars is postero-anteriorly compressed. The midlateral blotch is in the fifth bar as in all cichlasomatines.

Adult pattern development in *Laetacara* (Figs 37, 38)

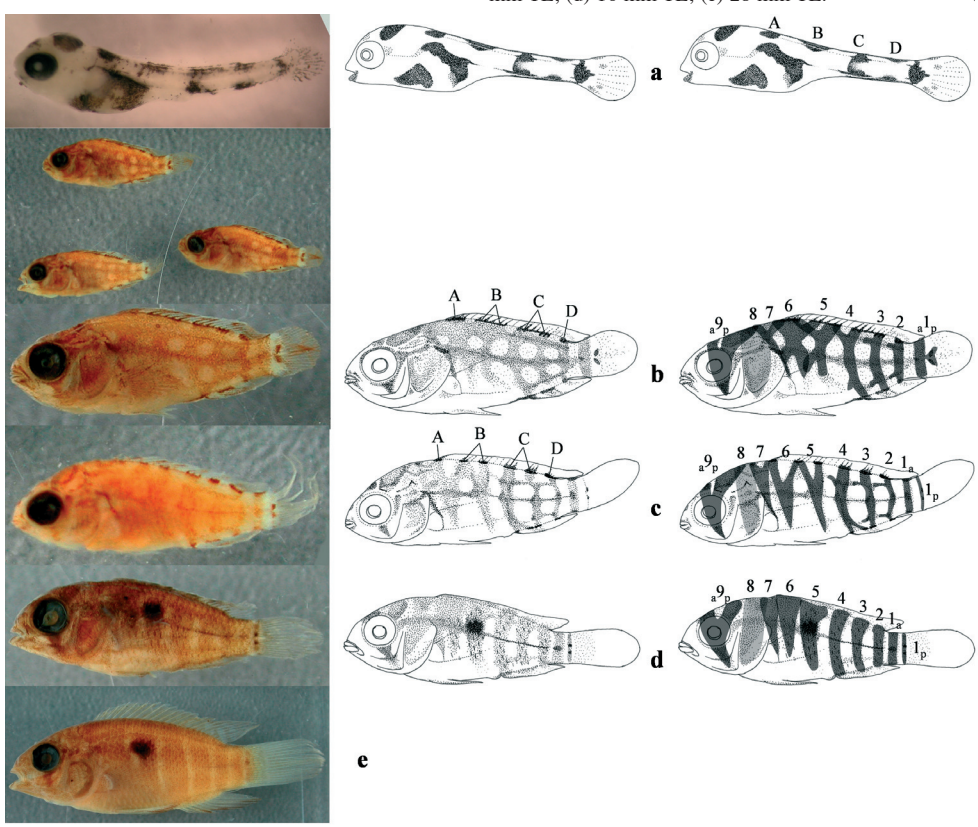
Laetacara species posses a modified coloration ontogeny compared to other examined cichlasomatines. The development starts from the characteristic cichlasomatine early larval pattern, but the posetrior body larval bar starts to develop earlier than in other cichlasomatines and the fourth dorsal blotch (D) is also visible in the first developmental stage, compared to the situation in most cichlasomatines (Figs 37 a, 38 a). The following steps are modified more significantly. The pigmentation pattern can be described as unpigmented circular areas on a dark background (Figs 37 b, 38 b). This pigmented background is actually the body bars merged with each other. It is easy to understand the development of this pattern by observation of the next stage (Fig. 37 c). Compared to other cichlasomatines, *Laetacara* has more developed horizontal pigmentation elements which interconnect the body bars along the dorsal and ventral body borders and also along the body midline. The pattern than resembles a row of X-like structures (the fusing anterior and posterior portions of the



developing bars) which are dorsally, midlaterally and ventrally interconnected. What is then left unpigmented are just these small circles arranged in two rows. In the next developmental step (Fig. 37 d), the horizontal pigmentation retreats and a familiar pattern with bars emerges. Compared to specimens of other cichlasomatines of similar size, *Laetacara* specimens show still the midlaterally running line (Fig. 37 d–e; 38 d–e), whereas e.g. *Acaronia* (Fig. 31), *Bujurquina* (Fig. 30), or *Krobia* (Fig. 32) loose it and develop the adult stripe which runs from the midlateral spot more upwards towards the posterior insertion of the dorsal fin. This pattern develops later also in *Laetacara*. The

Fig. 36. Color pattern development in *Cichlasoma dimerus*. (a) 13 mm TL; (b) 14 mm TL; (C) 20 mm TL; (D) 30 mm TL.

Fig. 37. Color pattern development in *Laetacara* sp. *"orangenflossen"*. (a) 6 mm TL; (b) 11 mm TL; (c) 13 mm TL; (d) 16 mm TL; (e) 28 mm TL.



midlaterally running stripe is a juvenile feature (see above the descriptions of heroine ontogenies) whereas the upwards-directed stripe is, in terms of developmental succession, apomorphic.

Adult coloration pattern development in the tribe Geophagini

Only ontogenetic series from three geophagine species were available to us for study. The mechanism of adult bar formation follows the same rules as in heroines and cichlasomatines with nine developing bars. As in cichlasomatines, the dominant midlateral blotch in adult geophagines (if present; see Fig. 40) is located in the fifth bar, and geophagines as well as cichlasomatines develop the suborbital stripe. All three geophagine species show the same number of adult bars, i.e. seven body and tail bars, which is one bar less than in the common situation in both heroines and cichlasomatines, i.e. eight body and tail bars. In all three species, the 2nd and 3rd bars fuse into a single adult bar (Figs 39 c, 40 g, 41 c).

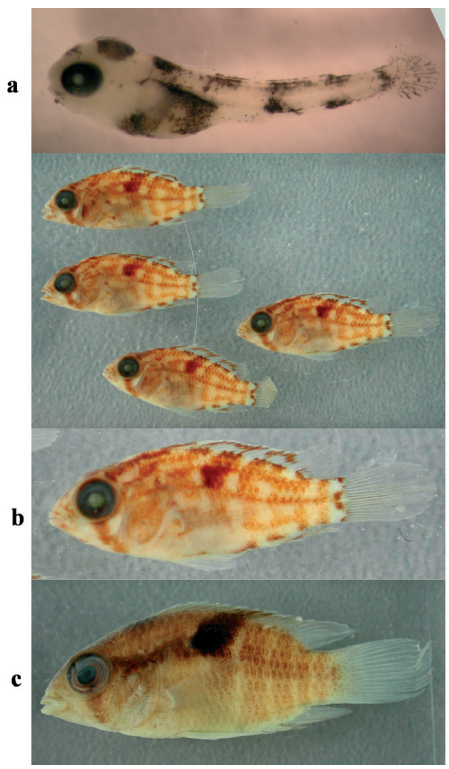


Fig. 38. Color pattern development in *Laetacara thayeri*. (a) 6 mm TL; (b) 16 mm TL; (c) 36 mm TL.

Discussion

Pigment pattern development in the studied cichlid fishes follows a single sequence of events from which different patterns originate. In all species melanoblasts migrate from the neural crest to establish themselves on the body in the form of melanophores in a larval pattern of four lines,

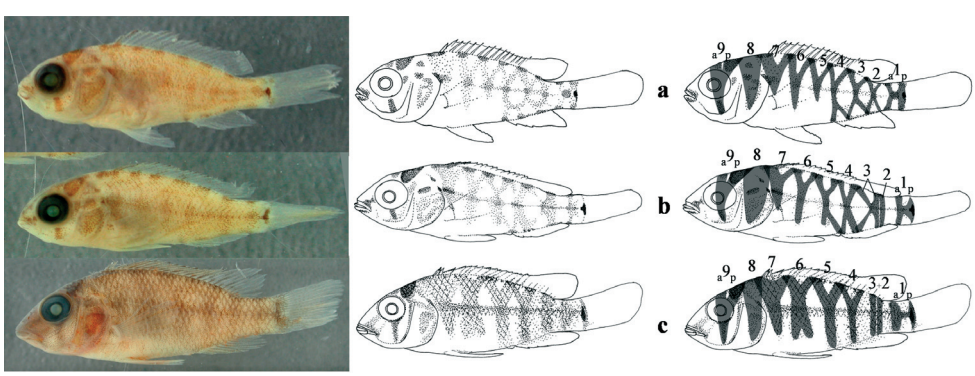


Fig. 39. Color pattern development in *Satanoperca jurupari*. (a) 15 mm TL; (b) 16 mm TL; (c) 29 mm TL.

as has been described for the genera *Danio* and *Tanichthys* (M c C l u r e 1999). In many species this pattern becomes obliterated very early by vertical migration of melanophores, and distinct blotched patterns are generated (e.g. larvae of cichlasomatines, *´Cichlasoma´ octofasciatum* or *´Cichlasoma´ atromaculatum*). Horizontal pigmentation patterns are replaced by vertical bars, which originate from a stable number of precursors that vertically migrate and interconnect the horizontal lines. Various numbers of adult bars are produced by changes in the fusion pattern of these precursors (the developing bars). By following bar fusions, it is possible to assess individual homology of the adult bars, which has been until now impossible and precluded the coding of many coloration pattern characters for systematic purposes. Homologization of adult bars based solely on adult animals is nearly impossible due to changes of bar positions by body proportions and by varying numbers of bars. Our developmental data show that individual bar homology can be assessed through the study of ontogeny in these fishes. Only a study where many species with varying numbers of bars and body proportions are examined can lead to a formulation of the general underlying principle of adult bar formation in these fishes. Studies focusing on individual species ontogenies can be mislead by specific features of the developmental sequence in the studied species and thus not surprisingly our coloration ontogeny of *Cryptoheros nigrofasciatus* departs significantly from the results of a single species study of this species (B e e c h i n g et al. 2002). Our study shows that there are very few bars that would remain constant in development among even this very limited sample of species. There are nine conserved vertical migratory pathways where bars develop, producing the most common ”model” bar pattern. Various bar counts are then possible through fusions and/or divisions of the developing bars. A developing bar can divide into two adult bars (e.g. the fourth body bar in *Heros*, Fig. 1; the second body bar *´Cichlasoma´ octofasciatum*, *´Cichlasoma´ atromaculatum*, Figs 22,23), producing two adult bars from one developing bar. One adult bar can form from two developing bars as in *Satanoperca jurupari*, *Gymnogeophagus setequedas*, and *Acarichthys heckelii* (second and third bars; Figs 39–41).

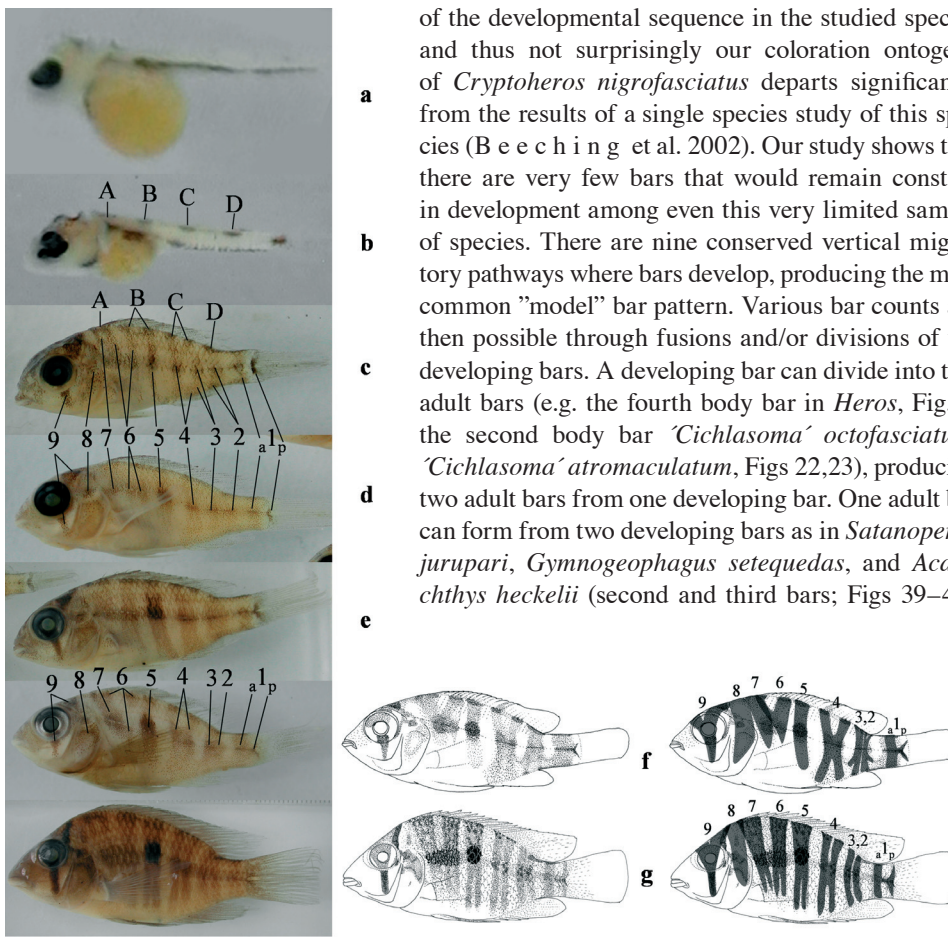


Fig. 40. Color pattern development in *Gymnogeophagus setequedas*. (a) 3 mm TL; (b) 5 mm TL; (c) 14,5 mm TL; (d) 19 mm TL; (e) 21 mm TL; (f) 30 mm TL; (g) 66 mm TL.

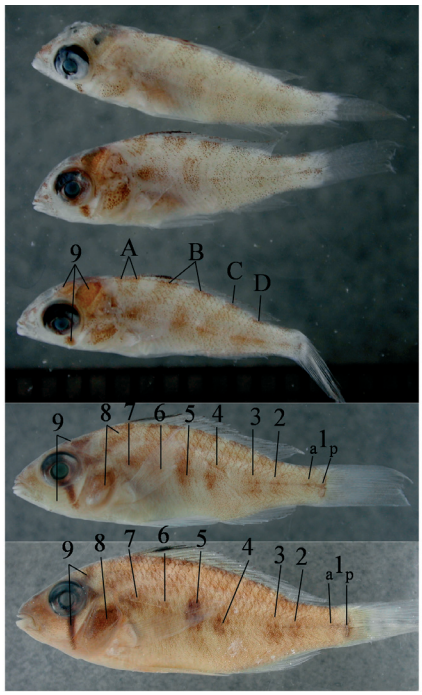


Fig. 41. Color pattern development in *Acarichthys heckelii*. (a) 15 mm TL; (b) 26 mm TL; (c) 42 mm TL.

A larval bar can also be lost during development and be missing from adult coloration, as in *´Paratheraps´ regani* (fourth bar; Fig. 16). Finally bars can be completely obliterated from adult coloration and replaced by other adult coloration patterns (as in most *Paratheraps* species and *Vieja maculicauda* and *Vieja synspila* among the studied species).

Timing shifts seem to be very common in the ontogenies of these fishes. They include to various degrees prolonged persistence of horizontal lines in larvae and juveniles of Mesoamerican heroines, different rates of formation of specific bars (e.g. the late developing third bar in *´Cichlasoma´ octofasciatum*; the late developing second bar in *´Aequidens´ pulcher*; the late developing third and fourth bars in *´Cichlasoma´ atromaculatum*). In the Mesoamerican heroines, where we have examined more species, the third body bar that is missing in adult *´Paratheraps´ regani*, is also very weakly developed throughout its ontogeny, and even though *Herichthys* species develop this adult bar, it is also less developed throughout ontogeny, and the dominant midlateral blotch in adult animals is the one located in the fifth bar, not in the fourth as in all other heroines (Fig. 15 h). All genera related to *Herichthys* (*Theraps*, *Chuco*, *´Paratheraps´, Paratheraps breidohri*) except the most advanced (*Vieja* and *Paratheraps*) do show the characteristic interruption of the abdominal stripe as shown in *Herichthys* (Fig. 15 b–d). This condition is apomorphic for this group of genera and plesiomorphic for *Vieja* and *Paratheraps* (where it has been obliterated by the overdeveloped abdominal line) according to molecular phylogeny (H u l s e y et al. 2004), and also since *Paratheraps breidohri* still shows the typical interrupted condition (Fig. 17 e). In those taxa with the interruption, the midlateral blotch in the developing fourth bar is reduced in size (*Herichthys*, *Theraps*, *Chuco*) or even completely missing (*Paratheraps breidohri*, *´Paratheraps´ regani*). While *Paratheraps breidohri* develops the fourth bar in later stages, it is never developed in *´Paratheraps´ regani*. There is thus evidence for a loss of the fourth bar in *´Paratheraps´ regani* due to a condition that can be traced as ancestral to the whole group of *Herichthys* related genera and that shows variation inside the group. The early interruption of the abdominal stripe is preferred as ancestral for *Paratheraps* and *Vieja*, but none of it can be seen in their ontogenies, since the development of the abdominal stripe has reached its maximum in these genera among heroines. The abdominal stripe is extremely intense and wide and lasts for such a long time, that in most species no bars develop at all, mostly being visible only in breeding dresses. This very intense abdominal stripe is in several lines of evidence not homologous to the situation in *Parachromis* and similar genera (e.g. *´Cichlasoma´ salvini*, *´Cichlasoma´ urophthalmum*). *Parachromis* and similar genera develop a dominant midlateral blotch in the fourth bar, do not pass through the interrupted abdominal line stage, and the non-homology of the dominant midlateral stripes in

Parachromis and similar genera and in *Vieja* or *Paratheraps* is also supported by morphological (unpubl. data.) as well as molecular data (Roe et al. 1997, Martin & Bermingham 1998, Hulsey et al. 2004).

The continuous and long lasting abdominal stripe, shown in the majority of heroine species examined here, is with the current knowledge best interpreted as ancestral for heroines (or at least Mesoamerican heroines) based both on additional ontogenetic information which we have for all genera and also based on published molecular phylogenies (Roe et al. 1997, Martin & Bermingham 1998, Hulsey et al. 2004). Apomorphic are thus deviations from this long lasting abdominal stripe developmental scheme (e.g. its even longer persistence as in *Vieja* or *Paratheraps*, or its early disappearance as in *Astatheros*, *Thorichthys*, or *´Cichlasoma´ octofasciatum* and the *´Cichlasoma´ festae* group). Definitely apomorphic are the developmental series of *´Cichlasoma´ octofasciatum*, of the *´Cichlasoma´ festae* group, and also of *Tomocichla* (*T. tuba* and *T. asfraci*). Also the early disruption of the abdominal line in *Cryptoheros* are to be considered apomorphic, at least based on the phylogenetic position of these species, being nested inside a group of species with ontogenies similar to *Parachromis* or *´Cichlasoma´ urophthalmum* (Hulsey et al. 2004). As the most ancestral situation should be considered the ontogenetic series where a post-free swimming abdominal line is accompanied by the presence of well developed dorsal blotches (A, B, C, D; e.g. *Nandopsis*, *Herotilapia*), while the more apomorphic condition is the one where the dorsal blotches become less and less developed or do not develop at all (e.g. *Amphilophus*).

Heroines seem to be unique among Neotropical cichlids in the possession of a midlateral blotch in the fourth bar. In all other tribes, the adult midlateral blotch is developed in the fifth bar, and is thus developmentally not homologous to the midlateral blotch of heroines. This is a new synapomorphy for heroines. An answer to the question why do heroines retain a midlateral blotch homologous to the juvenile blotch while other tribes do develop a blotch in the adjacent bar can only be tentative at the present time. Heroine ontogenies are ancestrally slowed down compared to the situation in other tribes, indicated for example by the prolonged persistence of the abdominal stripe, by the start of bar formation at larger sizes and age, by the loss of the suborbital stripe (see below). Our explanation would thus be that the juvenile midlateral blotch simply persists into adulthood, since heroines due to slowed down ontogeny never reach the switch point, where dominance would be shifted to the fifth bar. In the developmental series of *´Aequidens´ pulcher* (Fig. 24) there is in stages c–e a shift from the dominance of the fourth bar to the dominance of the fifth bar. The same situation occurs in all cichlasomatines and also in other tribes. But the situation is more complex than a simple shift in timing. Not all heroines do develop a midlateral blotch, but those that do develop it constantly in the fourth bar. There is one exception, and that are the genera related to *Herichthys* (see above). Also in cichlasomatines and other tribes (e.g. geophagines) not all species/genera develop a midlateral blotch, but again those that do develop it in the fifth bar. This stability probably indicates a deeply rooted and conserved mechanism.

There also seem to be interesting timing shifts in the development of the suborbital stripe. We believe to have observed three different timing types of the suborbital stripe development. The ancestral developmental sequence among Neotropical cichlids is that featured by cichlasomatines and geophagines among the taxa that we present here. The same sequence is also present in all other Neotropical cichlid subfamilies/tribes and in most tribes of heroines except in the Mesoamerican lineage. This ancestral type of the suborbital stripe ontogeny is characteristic by the following timing. The suborbital stripe develops relatively late compared to the development of other bars, usually at about 12–15 mm TL, and persists into adult coloration.

In heroines, there are two basic developmental sequences. In South American heroines the development starts at the same stage or earlier (at about 11 mm SL in Heros; Fig. 2) and some taxa among these groups, as for example *Heros*, *Mesonauta* or *Uaru* loose the suborbital stripe in subadult developmental stages. *Pterophyllum* on the contrary has a well developed suborbital stripe in adults (pers. obs.). Two observations are thus notable. First, this paraphyletic stem-group of the heroines shows a shift towards an earlier start of development of the suborbital stripe, and second, some taxa loose the stripe before they reach full sizes, while other taxa keep the stripe for all their lives. The variation actually seems to be hierarchically ordered, since based on molecular evidence (H u l s e y et al. 2004), *Pterophyllum* is the most basal heroine and it is also one of the taxa which show a completely ancestral, non-heroine, developmental sequence of the suborbital stripe. On the other pole of this stem-group, the genera *Heros*, *Uaru*, *Symphysodon* or *Mesonauta*, the likely sister group of the Mesoamerican heroines (H u l s e y et al. 2004), loose the stripe before fully grown. A complete loss of any traces of the suborbital stripe in all developmental stages is what sets the Mesoamerican heroines apart from all Neotropical cichlid groups. The condition is clearly apomorphic, which gains even more support from the above described shift towards this condition inside the stem-group heroines. This developmental sequence devoid of any traces of the suborbital stripe is present in more than 95% of the species and genera of Mesoamerican heroines. The only Mesoamerican heroine groups that have as adults a true suborbital stripe are the genera *Caquetaia* (especially *C. myersi*), *Petenia* (*P. splendida*) and also *Parachromis* and *Amphilophus trimaculatus.* In all these species, the suborbital stripe develops very late in ontogeny, no sooner than at about 50 mm TL and it is thus very much postponed compared to the situation in all South American cichlids as well as to South American heroines. Since molecular data (H u l s e y et al. 2004) do not support a close relationship between *Caquetaia* and the remaining species, and also since *Petenia* is the sister group of *´Cichlasoma´ urophthalmum*, a species without any suborbital stripe, the suborbital stripe reappeared more than once among Mesoamerican heroines. It is interesting to note, that as it seems the suborbital stripe reappeared repeatedly among heroines, but only among the highly piscivorous groups. *Caquetaia* (especially *C. myersi*), *Petenia* and *Parachromis* are the most piscivorous genera among heroines and are also the only species with developed suborbital stripes. There thus seems to be correlation between the presence of a suborbital stripe and piscivorous feeding habits in heroines.

The only Mesoamerican heroine known to develop a suborbital stripe during its ontogeny is *´Cichlasoma´ octofasciatum*. Compared to *Heros*, the suborbital stripe develops even earlier, is present already at the size of 7–8 mm at the age of few days, but gets also lost even earlier, in juveniles larger than 18–20 mm TL, and is not developed in adults (Fig. 22). It would thus seem that *´Cichlasoma´ octofasciatum* follows directly the trend leading to Mesoamerican heroines. The phylogenetic position of *´Cichlasoma´ octofasciatum* complicates this interpretation a bit. The exact phylogenetic position of *´Cichlasoma´ octofasciatum* is not known today and also no sister group is known (see H u l s e y et al. 2004). What we do know, is that *´Cichlasoma´ octofasciatum* is strongly supported as being a Mesoamerican heroine and that it is one of the basal lineages inside the group (H u l s e y op. cit.). All other basal lineages of Mesoamerican heroines are without any traces of the suborbital stripe, and the available phylogenetic hypotheses also seem to indicate that the sister group of all Mesoamerican heroines is the Antillean genus *Nandopsis* (unpubl. results), whose developmental series are also devoid of a suborbital stripe (see Figs 13, 14). The best interpretation thus seems to be that in *´Cichlasoma´ octofasciatum* the suborbital stripe is also a reacquired feature, because the hypothetical ancestor would be without any suborbital stripe. This unique coloration

character is a good synapomorphy for this yet undescribed genus (probably including more than one similar species; pers. obs.).

In conclusion, contrary to published studies, e.g. centrarchids (M a b e e 1995), in neotropical cichlids there is a way how to determine individual bar homology and the way to do so is using other coloration elements (i.e. the blotches in the disrupted migration lines) accompanied by their position on the body. As in centrarchids (M a b e e op. cit.), no other landmark, such as fin position alone or scale row position can be used for bar homologization. The coloration ontogenies are very stable and series from separate spawnings are undistinguishable. The only variation detected was in the case of hybrids between *Parachromis managuensis* and *Cryptoheros nigrofasciatus*. We propose several new characters supporting the monophyly of Mesoamerican heroines, i.e. (i) the apomorphic absence of the suborbital stripe, which is also lost from ontogeny in this group of species, (ii) the presence of a midlateral blotch in the fourth bar instead in the fifth as in the ancestral condition of all other Neotropical cichlid groups, and (iii) the apomorphic persistent abdominal line which is also interpreted as ancestral to Mesoamerican heroines. Several apomorphies are also proposed for genus level groups, such as the unique ontogenies of *´Cichlasoma´ octofasciatum*, the similar but distinct ontogeny of the *´Cichlasoma´ festae* group, that of *Tomocichla*, that of *´Paratheraps´ (`P.` regani*, *´P.´ argenteus*), those of the *Astatheros longimanus* and *Astatheros alfari* groups and *Thorichthys*. All these unnamed groups supported by distinctive coloration ontogenies would be best treated as separate genera, an issue that will be the focus of our next paper. The genus *Cryptoheros*, here represented by three species, is unique in the possession of a divided third bar forming two adult bars (possibly also present in *Nandopsis*). To our knowledge, this is the only unique trait of *Cryptoheros* that is not associated with the small sizes of these species. The close relationship of *Herichthys* with *Theraps*, *Chuco*, *´Paratheraps´*, *Paratheraps*, and *Vieja* is also confirmed by coloration ontogenetic characters.

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LITERATURE

ALLGAYER R. 2001: Description d'un genre nouveau, *Cryptoheros*, d'Amérique centrale et d'une espèce nouvelle du Panama (Pisces: Cichlidae). *L'an Cichlidé (Edited by Association France Cichlid) 2001 (1): 13–20.*

ARMBRUSTER J. W. & PAGE L. M. 1996: Convergence of a cryptic saddle pattern in benthic freshwater fishes. *Environ. Biol. Fishes 45: 249–257.*

BAGNARA J.T. & HADLEY M.E. 1973: Chromatophores and color change: the comparative physiology of animal pigmentation. *Prentice-Hall, Englewood Cliffs, NJ.*

BALON E.K. 1959: Die Entwicklung der Texas-Cichlide (*Herichthys cyanoguttatus* Baird et Girard) nach dem Schlüpfen. *Zool. Anz. 162: 339–355.*

BALON E.K. 1960: Vývoj *Cichlasoma nigrofasciatum* (Guenther) v embryonálnej perióde života (Embryonic development of *Cichlasoma nigrofasciatum* (Guenther)). *Acta Soc. Zool. Bohem. 24(3): 199–214 (in Slovak with English summary).*

BARLOW G.W. 1963: Ethology of the Asian teleost *Badis badi*s. II. Motivations and signal value of the colour patterns. *Anim. Behav. 11: 97–105.*

BARLOW G.W. 2000: The cichlid fishes: Natures grand experiment in evolution. *Perseus Publishing.*

BREDER C.H. 1946: An analysis of the deceptive resemblances of fishes to plant parts with critical remarks on protective coloration. *Bull. Bingham. Oceanog. Coll. 10: 1–49.*

BROOKS D.R. & MCCLENNAN D.A. 1991: Phylogeny, ecology and behavior: a research program in comparative biology. *University of Chicago Press, Chicago.*

COLLAZO A., FRASER S.E. & MABEE P.M. 1994: A dual embryonic origin for vertebrate mechanoreceptors. *Science 264: 426–430.*

DAWSON C.E. 1964: A revision of the West Atlantic flatfish genus *Gymnachirus* (naked soles). *Copeia 1964: 646–665.*

DUSHANE G.P. 1934: The origin of pigment cells in amphibia. *Science 80: 620–621.*

DUSHANE G.P. 1935: An experimental study of the origin of pigment cells in amphibia. *J. Exp. Zool. 72: 1–31.*

EHRLICH P.R., TALBOT F.H., RUSSELL B.C. & ANDERSON G.R.V. 1977: The behavior of chaetodontid fishes with special reference to Lorenz's ''poster coloration'' hypothesis. *J. Zool. Lond. 183: 213–228.*

ENDLER J.A. 1978: A predator's view of animal color patterns. *Evol. Biol. 11: 319–364.*

ENDLER J.A. 1983: Natural and sexual selection on color patterns in poeciliid fishes. *Environ. Biol. Fish. 9: 173–190.*

ENDLER J.A. 1988. Frequency dependent predation, crypsis and apo-sematic coloration. *Philos. Trans. R. Soc. Lond B, Biol. Sci. 319: 505–524.*

EPPERLEIN H.H. 1982: Different distribution of melano-phores and xanothophores in early tailbud and larval stages of *Triturus alpestri*s. *Roux's. Arch. Dev. Biol. 191: 19–27.*

EPPERLEIN H.H. & LÖFBERG J. 1990: The development of the larval pigment patterns in *Triturus alpestris* and *Ambystoma mexicanum*. *Springer-Verlag, Berlin.*

FARIAS I.P., ORTÍ G. & MEYER A. 2000: Total evidence: Molecules, morphology, and the phylogenetics of cichlid fishes. *J. Exp. Zool. 288: 76–92.*

FARIAS I.P., ORTÍ G., SAMPAIO I., SCHNEIDER H. & MEYER A. 1999: Mitochondrial DNA phylogeny of the family Cichlidae: Monophyly and fast molecular evolution of the Neotropical assemblage. *J. Mol. Evol. 48: 703–711.*

FARIAS I.P., ORTÍ G., SAMPAIO I., SCHNEIDER H. & MEYER A. 2001: The *cytochrome b* gene as a phylogenetic marker: the limits of resolution for analyzing relationships among cichlid fishes. *J. Mol. Evol. 53: 89–103.*

FARIAS I.P., SCHNEIDER H. & SAMPAIO I. 1998: Molecular phylogeny of Neotropical cichlids: the relationships of cichlasomines and heroines. In: Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S & Lucena C.A.S. (eds), Phylogeny and classification of Neotropical fishes. *Edipucrs, Porto Alegre*: *499–508.*

GOULD S.J. 1977: Ontogeny and phylogeny. *Belknap Press, Cambridge MA.*

HAAS R. 1976: Sexual selection in *Nothobranchius guentheri* (Pisces, Cyprinodontidae). *Evolution 20: 614–622.*

HARDEN JONES F.R. 1963: The reaction of fish to moving back-grounds. *J. Exp. Biol. 40: 437–446.*

HARVEY P.A. & PAGEL M.D. 1991: The comparative method in evolutionary biology. *Oxford University Press, Oxford.*

HEMMINGS C.C. 1966: Olfaction and vision in fish schooling. *J. Exp. Biol. 45: 449–464.*

HULSEY C.D., GARCÍA DE LEÓN F.J., SÁNCHEZ JOHNSON Y., HENDRICKSON D.A. & NEAR T.J. 2004: Temporal diversification of Mesoamerican cichlid fishes across a major biogeographic boundary. *Mol. Phyl. Evol. 31: 754–764.*

HULSCHER-EMEIS T.M. 1992: The variable colour patterns of *Tilapia zilii* (Cichlidae), integrating ethology, chromatophore regulation and the physiology of stress. *Neth. J. Zool. 42: 525–560.*

JESUTHASAN S. 1996: Contact inhibition-collapse and pathfinding of neural crest cells in the zebrafish trunk. *Development 122: 381–389.*

KELSH R.N., BRAND M., JIANG Y.J., HEISENBERG C.P., LIN S., HAFFTER P., ODENTHAL J., MULLINS M.C., VAN EEDEN F.J.M., FURUTANI-SEIKI M., GRANATO M., HAMMERSCHMIDT M., KANE D.A., WARGA R.M., BEUCHLE D., VOGELSANG L. & NUESSLEIN-VOLHARD C. 1996: Zebrafish pigmentation mutations and the processes of neural crest development. *Development 123: 369–389.*

KNIGHT M.E. & TURNER G.F. 1999: Reproductive isolation among closely related Lake Malawi cichlids: Can males recognize conspecific females by visual cues? *Anim. Behav. 58: 761–768.*

KORTMULDER K. 1972: A comparative study in colour patterns and behaviour in 7 Asiatic *Barbus* species; a progress report. *Behaviour Suppl. 19: 1–331.*

KORTMULDER K. 1982: Etho-ecology of seventeen *Barbus* species (Pisces, Cyprinidae). *Neth. J. Zool. 32: 144–168.*

KORTMULDER K. & POLL R.J.V.D. 1981: The juvenile and adult pigment patterns of *Barbus lateristiga* Cuv. and Val. 1842, *B. titteya* (Deraniyagala 1929) and *B. naranyani* Hora 1927 (Pisces, Cyprinidae), and their taxonomic value. *Neth. J. Zool. 31: 453–465.*

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

KULLANDER S.O. 2003: Cichlidae. In: Check list of the freshwater fishes of South and Central America. *Edipucrs, Porto Alegre.*

KULLANDER S.O. 1996: *Heroina isonycterina*, a new genus and species of cichlid fish from Western Amazonia, with comments on cichlasomine systematics. *Ichthyological Exploration of Freshwaters 1996, 7: 149–172.*

KULLANDER S.O. 1983: Revision of the South American cichlid genus *Cichlasoma. Swedish Museum of Natural History, Stockholm, 1983.*

LONG K.D. & HOUDE A.E. 1989. Orange spots as a visual cue for female mate choice in the guppy *(Poecilia reticulat*a). *Ethology 82: 316–324.*

LORING J.F. & ERICKSON C.A. 1987. Neural crest cell migratory pathways in the trunk of the chick embryo. *Dev. Biol. 121: 220–236.*

MABEE P.M. 1995: Evolution of pigment pattern development in centrarchid fishes. *Copeia 1995: 586–607.*

MARTIN A.P. & BERMINGHAM E. 1998: Systematics and Evolution of Lower Central American Cichlids Inferred from Analysis of Cytochrome b Gene Sequences. *Mol. Phyl. Evol. 9: 192–203.*

MCCLURE M. 1999: Development and evolution of melanophore patterns in fishes of the genus *Danio* (Teleostei: Cyprinidae). *J. Morph. 241: 83–105.*

MILOS N. & DINGLE A.D. 1978A: Dynamics of pigment pat-tern formation in the zebrafish, *Brachydanio reri*o. I. Establishment and regulation of the lateral line mela-nophore stripe during the first eight days of development. *J. Exp. Zool. 205: 205–216.*

MILOS N. & DINGLE A.D. 1978b: Dynamics of pigment pat-tern formation in the zebrafish, *Brachydanio reri*o. II. Lability of lateral line stripe formation and regulation of pattern defects. *J. Exp. Zool. 205: 217–224.*

MURRAY J.D., DEEMING D.C. & FERGUSON M.W.J. 1990: Size-dependent pigmentation-pattern formation in embryos of *Alligator mississippiensis*: time of initiation of pattern generation mechanism. *Proc. R. Soc. Lond. B. 239: 279–293.*

MURRAY J.D. 1981a: On pattern formation mechanisms for lepidopteran wing patterns and mammalian coat markings. *Phil. Trans. R. Soc. London, B. Biol. Sci. 295: 473–496.*

MURRAY J.D. 1981b: A pre-pattern formation mechanism for animal coat markings. *J. Theor. Biol. 88: 161–199.*

NELSON G. & PLATNICK N. 1981: Systematics and biogeography: Cladistics and vicariance. *Columbia Univ. Press, New York.*

PARICHY D.M. 1996a: When neural crest and placodes collide: interactions between melanophores and the lateral lines that generate stripes in the salamander *Ambystoma tigrinum tigrinum* (Ambystomatidae). *Dev. Biol. 175: 283–300.*

PARICHY D.M. 1996b: Pigment patterns of larval sala-manders (Ambystomatidae, Salamandridae): The role of the lateral line sensory system and the evolution of pattern forming mechanisms. *Dev. Biol. 175: 265–282.*

QUIGLEY I.K. & PARICHY D.M. 2002: Pigment pattern formation in Zebrafish: A model for developmental genetics and the evolution of form. *Microscopy research and technique 58: 442–455.*

RAIBLE D.W. & EISEN J.S. 1994: Restriction of neural crest cell fate in the trunk of the embryonic zebrafish. *Development 120: 495–503.*

RAIBLE D.W. & EISEN J.S. 1996: Regulative interactions in zebrafish neural crest. *Development 122: 501–507.*

RAIBLE D.W., WOOD A., HODSDON W., HENION P.D., WESTON J.A. & EISEN J.S. 1992: Segregation and early dispersal of neural crest cells in the embryonic zebrafish. *Dev. Dyna. 195: 29–42.*

RAUCHENBACHER M., KALLMAN K. & MORIZOT D. 1990: Monophyly and geography of the Rio Panuco basin swordtails (Genus *Xiphophorus*) with descriptions of four new species. *Am. Mus. Nov. 2975: 1–41.*

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

RIEPPEL O. 1985: Ontogeny and the hierarchy of types. *Cladistics 1: 34–246.*

ROE K.J., CONKEL D. & LYDEARD C. 1997: Molecular Systematics of Middle American Cichlid Fishes and the Evolution of Trophic-Types in '*Cichlasoma* (*Amphilophus*)' and '*C. (Thorichthys)*'. *Mol. Phyl. Evol. 7: 366–376.*

SEEHAUSEN O., MAYHEW P.J. & VAN ALPHEN J.J.M. 1999: Evolution of colour patterns in East African cichlid fish. *J. Evol. Biol. 12: 514–534.*

TINBERGEN N. 1952: The curious behavior of the stickleback. *Sci. Am. 187: 22–26.*

VAGLIA J.L. & HALL B.K. 2000: Patterns of migration and regulation of trunk neural crest cells in zebrafish. *Int. J. Dev. Biol. 44: 867–881.*

WHITNEY R.R. 1969: Schooling of fishes relative to available light. *Trans. Am. Fish. Soc. 98: 497–504.*

WRAY G.A. RAFF R.A. 1991: The evolution of developmental strategy in marine invertebrates. *Trends. Ecol. Evol. 6: 45–50.*