Evolutionary morphology of trichomycterid catfishes: about hanging on and digging in

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Abstract

The catfishes (Siluriformes) comprise a particularly diverse teleost clade, from a taxonomic, morphological, biogeographical, ecological and behavioural perspective. The Neotropical Trichomycteridae (the "parasitic" catfishes) are emblematic of this diversity, including fishes with some of the most specialized habits and habitats among teleosts (e.g. hematophagy, lepidophagy, miniaturization, fossorial habitats, altitudinal extremes). Relatively little information is available on general trichomycterid morphology, as most work so far has concentrated on phylogenetically informative characters, with little concern about general descriptive anatomy. In this paper we provide a synthesis of new and previously-available data in order to build a general picture of basal crown group trichomycterid morphology and of its main modifications. We focus on the evolutionary morphology in two relatively distal trichomycterid lineages, *i.e.* the hematophagous Vandelliinae and the miniature, substrate dwelling Glanapteryginae. New evidence is discussed in relation to the evolution of the opercular system as well as morphological modifications in miniature species exhibiting an interstitial life style.

Introduction

A recent review of described trichomycterid species has totaled up to 207, classified in eight subfamilies: Trichogeninae, Copionodontinae, Trichomycterinae, Tridentinae, Stegophilinae, Vandelliinae, Glanapteryginae and Sarcoglanidinae (de Pinna 1998, Ferraris 2007) (Fig. 1). Currently, only the Trichomycterinae are considered to be paraphyletic (de Pinna 1998), and already were considered an artificial group of taxa since the first survey by Baskin (1973). Still, some synapomorphies may support its monophyly (Arratia 1990a). The closest relative is considered to be the monotypic Nematogenyidae, but had been included as a separate subfamily within the Trichomycteridae (Eigenmann 1918a). However, the absence of some distinct trichomycterid synapomorphies, as well as it most probably being a sistergroup to all other loricarioids (*i.e.* Trichomycteridae, Callichthyidae, Scoloplacidae, Astroblepidae and Loricariidae), justifies its separation (Baskin 1973, Sullivan et al. 2006).

FIG 1

As reviewed by de Pinna (1998), trichomycterid catfishes have occupied a range of trophic niches, living in very distinct hydrodynamic conditions. Insectivorous feeding is common in trichomycterids, but also algivory (*Copionodon*), lepidophagy (*e.g. Pseudostegophilus*), muciphagy (*Ochmacanthus alternus*) and hematophagy have been reported (Eigenmann 1918b, Baskin et al. 1980, Winemiller 1989, de Pinna 1992). The hydrodynamic environment may range from stagnant water in marshes (*e.g. Listrura nematopteryx*) or water holes (*Silvinichthys* and some *Trichomycterus*) to strong currents in rivers at high elevations (*e.g. Trichomycterus yuska*, living at elevations of 4000 m, and *T. chungaraensis* found at 4500 m), being pelagic swimmers (*e.g. Trichogenes longipinnis*) to benthic foragers, in some cases even living submerged in the sediment and leaflitter (*i.e.* infauna, found in Glanapteryginae and Sarcoglanidinae) (Britski & Ortega 1983, de Pinna 1988, Arratia 1990a, Romero & Paulson 2001, Fernández & de Pinna 2005, Schaefer et al. 2005). Some trichomycterids are even believed to be able to cling onto vertical faces of waterfalls, or “elbowing” their way between rocks in fast running water, using opercular and interopercular spines (Eigenmann 1918a).

One feature that distinguishes Trichomycteridae from all other catfishes, and even other teleosts, is the presence of a highly modified opercular system, involving the interopercular and opercular bone. It is believed that this system, being equipped with enlarged integumentary teeth, enables the elbowing locomotion in basal trichomycterids and has facilitated the ectoparasitic clinging to hosts and into gill cavities in stegophiline and vandelliine taxa. The morphology of this system has thus proven to show a substantial amount of variation, and thus its usefulness as taxonomical and phylogenetically relevant characters.

Over the years, the major focus on trichomycterids has been on understanding the species diversity and the phylogenetic relationships between lineages. This has yielded a large amount of information on biometry, distribution, coloration and osteology. However, because of the interest in osteology to support hypotheses of affinities, a holistic approach to the skeletal anatomy is frequently lacking. Exceptions are papers providing data on complete neuro- and viscerocrania, however, represented in a disarticulated manner (de Pinna 1989a,b, 1992, Datovo & Landim 2005). Some comparative studies of partial systems of the skull (including lateral line system) have been performed, providing some relevant data on differences between trichomycterids and nematogenyids (as well as other loricarioids) (Schaefer 1988, Arratia & Huaquín 1995, Arratia 2003). The most complete comparative study of trichomycterid osteology has been provided by Baskin (1973). A representation of trichomycterid osteology of articulating elements (thus illustrating couplings and articulations) has been given for *Silvinichthys* (Arratia 1998). Information on the ontogeny of the cranial skeleton in trichomycterids is restricted to that of the suspensorium (Arratia 1990b,1992). Published information on muscle systems, however, is almost non-existant for trichomycterids but has been published for *Nematogenys inermis* (Diogo et al. 2006). Some accounts have been made on the adductor mandibulae complex and opercular muscles in trichomycterids, without providing a complete overview of the musculo-skeletal anatomy of the head or postcranial system (Howes 1983, de Pinna 1992,1998). Despite all this information, an overview of the musculo-skeletal system of the head is lacking for trichomycterids. Also, even though legendary for its opercular system with enlarged integumentary teeth, no understanding of the detailed anatomy or its functioning exists. As such, an interpretation of the observed specialisations, whether synapomorphic or not, in an evolutionary (adaptive) context is currently lacking. With this paper, we want to provide a basis for understanding the evolutionary morphology of trichomycterids by compiling existing evidence in literature, as well as new data from dissections, CT-scanning and serial histological sections to meet three goals: (1) give a more integrated overview of the morphology of the musculo-skeletal system of a basal crown group trichomycterid, (2) provide a detailed study of the complex anatomy of the opercular system in order to understand its functioning in hematophagous vandelliines, and

(3) provide information on aspects of body elongation in trichomycterids in relation to a fossorial habit.

Material and methods

For this study, specimens were used from representatives of all major trichomycterid subfamilies and a nematogenyid. Specimens used for CT-scanning were: *Nematogenys inermis* (Guichenot, 1848) (ANSP 180477, 45.7 mm SL), *Copionodon pecten* de Pinna, 1992 (MZUSP 93281, 47.6 mm SL), *Trichomycterus punctulatus* Valenciennes, 1846 (in Cuvier & Valenciennes 1846) (ANSP 180733, 71.3 mm SL), *Scleronema operculatum* Eigenmann, 1917 (ANSP 168843, 18.5 mm SL), *Ituglanis amazonicus* (Steindachner, 1882) (ANSP 160591, 36.2 mm SL), *Sarcoglanis simplex* Myers and Weitzman, 1966 (ANSP 179212, 16.1 mm SL), *Pygidianops cuao* Schaefer, Provenzano, de Pinna and Baskin, 2005 (AMNH 232970, 19.3 mm SL), *Apomatoceros alleni* Eigenmann, 1922 (ANSP 181148, 99.4 mm SL), *Vandellia* cf *plazai* (AMNH 43119, 53.1-54.5 mm SL) and *Vandellia cirrhosa* Valenciennes, 1846 (in Cuvier & Valenciennes 1846) (ANSP 178176, 112.7 mm SL). Specimens were scanned at the modular micro-CT setup of the Ghent University (Masschaele et al. 2007; http://www.ugct.ugent.be). The raw data were processed and reconstructed using the in-house developed CT software Octopus (Vlassenbroeck et al. 2007).

Specimens of *Trichomycterus guianense* (Eigenmann, 1909) were used for clearing and staining (Taylor & Van Dyke 1985) and dissecting (ZMA 116.891, 63.8-70.8 mm SL). Muscle fibers were stained with iodine (Bock & Shear 1972). One specimen of *T. guianense* (ZMA 116.891, 40 mm SL) and one specimen of *Vandellia* cf. *plazai* (AMNH 43119, 53.1 mm SL) were used for serial histological sectioning. Those specimens were embedded in Technovit 7100 (Heraus, Kulzer) and sections of 5µm were made of the complete head using a Leica Polycut equipped with metal knives, coated with wolfram carbide. Sections were stained with toluidin, and photographed digitally at high resolutions using a Colorview8 camera mounted on a Polyvar light microscope. Several images were taken at high magnification for a single section, and stitched into one image using the Multiple Image Align function in Analysis (SIS) software package.

Three dimensional graphical reconstructions were made based on the CT-data and histological sections, using VG Studiomax (Volume Graphics, Heidelberg) and Amira 3.1 (Mercury Computer Systems, Villebon), respectively. Rendering and visualisation of reconstructed volumes was done in Amira. Modelling using these 3D volumes was done using Rhino3D and Bongo (McNeel, Seattle).

For the analysis on patterns of body elongation in fossorial and other trichomycterids, the following samples were used for measuring standard length (SL, mm) and head width (HW, mm) using digital callipers (0.01 mm accuracy) (in total 177 specimens): *Ammoglanis diaphanus* (CAS 225387), *Apomatoceros alleni* (ANSP 109804; SMNH 1984317.4111); *Apomatoceros* cf. *alleni* (INPA 8388); *Branchioica* sp. 1 (FL94-70); *Branchioica* sp. 2 (JGL66-93); *Copionodon* sp. (MZUSP 48962; UMMZ 231762); *Eremophilus mutisii* (UMMZ 179261); *Glanapteryx* sp. (Guanare 21679; 48277); *Homodiaetus haemomyzon* (Provenzano, 1982); *Homodiaetus maculatus* (ANSP 93977); *Ituglanis amazonicus* (ANSP160591; UF 33476 Colombia, Rio Meta); *Ituglanis metae* (ANSP 128599; ANSP 131641); *Listrura nematopteryx* (27-IV-95 de Pinna; BMNH 1990.11.26 1-6); *Malacoglanis gelatinosus* (INHS 28281); *Nematogenys inermis* (ANSP 84197; CAS 27796); *Ochmacanthus alternus* (VEN 24-July-89); *Paracanthopoma* sp. (ANSP 149547; 149548); *Paracanthopoma* sp. (AMZ94.126); *Paracanthopoma parva* (KONIG Belg. 10.121); *Paravandellia* sp. (Guanare 21814); *Plectrochilus machadoi* (Hohn 1955); *Pygidianops cuao* (AMNH 232970; AMNH 232978; AMNH 232995; AMNH 233036; MBUCV 29905; MBUCV 30917); *Pygidianops magoi* (AMNH 233706; AMNH 279; AMNH 5979; MBUCV 31035; MBUCV 31036; MBUCV 31037); *Sarcoglanis simplex* (SAS 01-3; AMNH uncat; ANSP 179212); *Scleronema operculatum* (FMNH 58520); *Scleronema* sp. (MCP 38344, MCP 37043); *Stegophilus* sp. (MUMSM Lima 13775); *Stegophilus punctatus* (MUMSM Lima 20594); *Trichogenes* sp. (MNRJ 13809; MZUSP 39034); *Trichomycterus* sp. (DJS-14-79; FMC 2-19608); *Trichomycterus mondolfi* (MBUCV -V-3993); *Tridens* sp. (ZMA 114.218); *Tridensimilis* sp. (SMNH 1984312.4114 (new species); ZMA 109.247; NRM SOK 1984334.4165); *Tridentopsis pearsoni* (MG 2236.42); *Tridentopsis* cf. *pearsoni* (aquarium (Larenberg)); *Typhlobelus guacamaya* (AMNH 103; AMNH 232974; MBUCV 29904; MBUCV 30006); *Typhlobelus lundbergi* (AMNH 233709; AMNH 233710; MBUCV 279; MBUCV 31040); *Typhlobelus* sp. (AUM 35802); *Vandellia cirrhosa* (Cruise #: E-2B-78; No data - Baskin 1982).

Results and discussion

The primary goal of this study is to compile the available evidence from literature and new observations, to give an overview of what a basal representative of the crown group trichomycterid (thus its common ancestor) would look like, and to focus on some evolutionary transformations in morphology in derived clades. With respect to bone terminology, we follow homology-based terminology wherever supported by proper comparative studies, instead of historically rooted terminology (see Harrington 1955, Schultze 2008). As such, we use ‘parietals’ and ‘postparietals’ instead of ‘frontals’ and ‘parietals’, respectively, thus reflecting taxic homology (for a recent discussion on concepts of homology, see Wiley 2008). As dealing with catfishes, this then involves the acceptance of the presence of parietals and postparieto-supraoccipitals.

Recent phylogenetic analysis of Siluriformes (de Pinna 1992, Diogo 2005, Hardman 2005, Sullivan et al. 2006), as well as a historical overview (de Pinna & Starnes 1990), show that no consensus yet exists about the position of the Nematogenyidae with respect to Trichomycteridae and/or other Loricarioidea. Considering the supported evidence that within true trichomycterids (thus excluding nematogenyids) the Trichogeninae and Copionodontinae take a most basal position, it could be suggested that the ancestral trichomycterid morphology must correspond to what *Nematogenys inermis,* copionodontines and *Trichogenes longipinnis* have in common (de Pinna 1992). However, the rather distinct habitus of *Trichogenes longipinnis*, related to being a unique trichomycterid as a permanent mid-water swimmer, suggests that this lineage may not represent the basal trichomycterid conditions and lifestyle. Also, recent discoveries of a copionodontine equipped with opercular odontodes (unique for this subfamily) suggest a necessary reevaluation of the current hypotheses of these basal trichomycterids (de Pinna 2008). The morphology of a basal but true trichomycterid (further referred to in the text as the common ancestor to the crown group trichomycterid, Fig. 1, clade C) may thus be illustrated by a representative of the speciose genus *Trichomycterus* (in the current absence of a phylogenetic hypothesis of Trichomycterinae intrarelationships). Using this morphology as a reference, this study focuses on specialisations on that design that typifies the two derived lineages: Tridentinae-Stegophilinae-Vandelliinae and Sarcoglanidinae-Glanapteryginae (Fig. 1, clade F). As a case study, the specialisation of the opercular system is discussed in Vandelliinae and the specialisations related to fossorial habits in miniature trichomycterids are dealt with, respectively.

Ancestral morphology of crown group trichomycterids

Based on the phylogenetic hypothesis followed in this paper (Fig. 1), the Trichomycterinae *s.s.* (excluding *Scleronema*, *Ituglanis* and some *Trichomycterus*) should be considered as best candidates reflecting the basal morphology of the crown group of the trichomycterids (de Pinna 1989a, Arratia 1990a). Initially, Trichomycterinae were considered problematic because of their “widespread distribution, high endemism, high intraspecific variation, and its apparent lack of synapomorphies” (Arratia 1990a). The morphology of such a hypothetical ancestor to the crown group trichomycterids should thus include synapomorphies of Trichomycteridae, but not synapomorphies of Trichomycterinae and all subsequent lineages within this crown group. The following description is based on these phylogenetic characters described in literature, as well as the study of the cranial osteology of *T. punctulatus* (Fig. 2B-D, 3) and *T. guianense*, and myology of *T. guianense* (Fig. 4). As current knowledge on trichomycterid myology, as well as within-trichomycterine phylogenetic relationships, is non-existent, it is at this point impossible to verify to what degree these species chosen are the best representatives or not. However, we believe this approach is the most fruitful one to take at this time.

FIG 2

Habitus

The ancestral crown group trichomycterids show a slightly elongated body, with a dorso-ventrally flattened head and laterally compressed caudal region (Fig. 2A). Eyes small, laterodorsally positioned on the head. Mouth subventrally positioned, with toothed upper and lower jaws. Head bearing three pairs of barbels, i.e. a nasal (at anterior nostril), a maxillary and a pair of shorter rictal barbels (the latter being an extra pair of barbels attached to the maxillary ones) (Baskin 1973). Anterior nostril bordered posteriorly by the nasal barbel, posterior nostril tubulous. Pectoral fins lacking a pectoral spine. Short dorsal fin has its origin slightly posterior to that of the pelvic ones, whereas the short anal fin starts slightly posterior to the base of the caudal dorsal fin ray. Pectoral and dorsal fin spines absent, hence also the associated spine locking mechanism (Alexander 1965). The head bearing a complete supraorbital canal with four pores, whereas the infraorbital canal interrupted at the suborbital level (both anterior and posterior part bear external pores) (Arratia 1998). Preopercular canal atrophied, bearing one pore. Body lateral line also atrophied to its anteriormost part, with only a few external pores. Skin also bearing cellular projections (epidermal and dermal papillae and epidermal micropapillae) as well as numerous sensory organs, such as neuromasts, taste buds (two types, especially in the barbels and upper lip), and ampullary organs. Skin covered with superficial neuromasts only in adults, where five pit lines can be distinguished on the head (rostral line, infraorbital line (also interrupted suborbitally), anterior line, middle line, and supratemporal accessory line) and four trunk pit lines (dorsal, subdorsal, middle and ventral pit lines) (Arratia & Huaquin 1995: fig. 11). Up to five different cell types composing the epidermis have been distinguished (Martinez et al. 1998). Enlarged integumentary teeth covering the opercular and interopercular region, which arise very early during ontogeny (Arratia 1990b).

Skull

Neurocranium - The skull in trichomycterids in general is somewhat wedge shaped, with the rostral end being tapered (Fig. 2D). Mesethmoid T-shaped, with a straight anterior margin, supporting plate-like and toothed premaxillaries. Maxillaries small, bearing two articulatory facets for the articulation with the rostral tip of the autopalatine and fixed to the supporting cartilages of the maxillary and rictal barbels. Toothless, T-shaped vomeral bone, posteriorly enclosed dorsally and laterally by the parasphenoid, and forming an articulation with the autopalatine (as well as a large ligament connecting both) (Fig. 2E). This autopalatine, which is rather plate-like and bears a posterior (non-cartilaginous) process, posteriorly articulates with the lateral ethmoid as well. Dorsal to the nasal sac, a tubular nasal bone is present. Already from the cartilaginous precursor on, the autopalatine shows a hooked shape in *Trichomycterus areolatus* (Arratia 1990b: fig. 3). Lateral ethmoid encloses a large olfactory foramen, ventrally bordered by a bony flange that forms the articulation for the autopalatine. Caudally, the lateral ethmoids form a synchondrosis with the orbitosphenoid. The latter is separated by a large foramen from the sphenotic-pterosphenoid-prootic complex (see below) and encloses two small foramina. Arratia (1998) suggested that both the trigemino-facial and optic nerves would pass through this large foramen (Arratia 1998: fig. 8C). However, serial sections of *Trichomycterus guianense* showed that the trigemino-facial complex passes through it, whereas the optic nerve passes through the ventral, small foramen enclosed by the orbitosphenoid (the upper orbitosphenoid foramen is transversed by a vein) (Figs. 2B**,**C, 3A).

FIG 3

Skull roof is formed by the paired parietals and an unpaired postparieto-supraoccipital. Initially, they enclose a large fontanel, which becomes interrupted by the parietal part enclosing the epiphysial bridge of the chondrocranium (as found in younger specimens) and eventually splits into an anterior and posterior fontanel, enclosed by the parietal and postparieto-supraoccipital, respectively (Fig. 2D). Side walls of the braincase comprise the pterotic and sphenotic bone, but the latter being fused to both the pterosphenoid and prootic, forming a large complex extending to the ventral side of the skull. The sphenotic part of this complex forms, together with the parietal, an orbital process through which the infraorbital canal enters the skull roof. The parasphenoid anteriorly encloses the vomeral processes and is caudally wedged into the basioccipital, without showing distinct lateral wing-like processes. The basioccipital, lacking anterior membranous outgrowths (as its presence is considered a synapomorphy of Trichomycterinae by Arratia 1990a), is fused to the swimbladder capsule (the fusion with exoccipitals, as observed in most (but not all) trichomycterids but absent in copiononodontines and trichogenines, may thus not be the ancestral condition for the crown group trichomycterids, depending on the condition in basal trichomycterines) (de Pinna 1998: p. 302). This swimbladder capsule has a small lateral opening and shows no fusion with the neurocranium at the level of the skull roof. There it contacts the postparieto-supraoccipitals and the small epioccipitals (= epiotics). Exoccipitals show no median connection (as in other loricarioids) (Chardon, 1968). In between the lateral extensions of the swimbladder capsule and the pterotics, lies the small posttemporo-supracleithral bone, thus enclosing a triangular cavity. Intercalarium absent.

The posttemporo-supracleithral bone encloses the postotic canal as it exits the pterotic bone. Other canal enclosing bones are: sphenotic part of the sphenotic complex (otic canal), and parietal and nasal bone (supraorbital canal). Only the posterior part of the infraorbital canal, interrupted at the suborbital level, is enclosed in an infraorbital bone before it enters the sphenotic (e.g. *T. rivulatus*, in: Arratia, 1998: fig. 3b) (Fig. 3C). A small antorbital bone is also present, lying next to the autopalatine, but does not enclose the infraorbital canal. Instead, it is connected to the nasal barbel cartilage (a condition also observed in other catfishes, and thus an argument for its homology with the antorbital instead of the lacrimal) (Adriaens et al. 1997). Medio-ventral to the eye lies a spike-like bone, which is connected ligamentously to both the antorbital (anteriorly) and a process on the parietal (posteriorly). As it seems to be an ossification of this ligament, as well as it lacks any sensory canal, this bone has been considered being a sesamoid supraorbital bone (‘tendon bone supraorbital’ of Arratia 1998, and ’fronto-lacrimal tendon bone’ of Sarmento-Soares et al. 2006). If it would be a true tendon bone, it could be related to its connection to the oblique eye muscles (see below).

Mandibula, suspensorium, hyoid and opercular series – Lower jaw short and robust (interconnected by cartilage), bearing on the dentary (actually a complex of the mentomeckelium and true dentary) a series of villiform teeth running up to the rostral base of the coronoid process (Fig. 2B). This process, onto which the major part of the adductor mandibulae complex inserts (see below), is formed by a dental part and an angular part. A ligament connects the process to the maxillary bone (Fig. 4A-D), as well as there is a distinct strip of cartilaginous tissue connecting both bones. A coronomeckelium is lacking. The angular is part of a complex, formed by a fusion with the articular (forming the articulatory facet with the suspensorium) and retroarticular bones. The retroarticular part bears a distinct process, from the mandibulo-interopercular and mandibulo-hyoid ligaments originate (Fig. 4, 6B). The quadrate, which forms a synchondrosis with both the metapterygoid and the hyomandibula, is connected to the preopercular bone by connective tissue. A posterior membranous outgrowth of the quadrate contacts an anterior outgrowth of the hyomandibula, forming a large insertion surface for suspensorial and jaw muscles (Arratia 1990b: fig. 8). The metapterygoid bears a plate-like extension, around a perichondral core, which is connected to the autopalatine through a ligamentous sheet. A sesamoid entopterygoid, and other pterygoid bones, are absent (Arratia 1990b). The hyomandibula bears a cartilaginous articulatory ridge, which articulates with a cartilaginous strip enclosed by the autosphenotic and prootic components of the sphenotic complex, and the pterotic, but additionally shows a bone-to-bone contact between the anterior bony extension of the hyomandibula and the sphenotic complex (Fig. 2B,C). The preopercle has lost its sensory canal (a tube-like cavity could be observed in the preopercle in *Trichomycterus*, including that of its branches, but a sensory canal is lacking (Fig. 3B). This is believed to be linked to structural reinforcements of the bone for the opercular odontode system (Baskin, 1973), but see below (*Evolutionary morphology of the opercular apparatus*).

FIG 4

The interhyal has been assumed to get lost during later ontogeny (Arratia 1990b). However, serial sections in both *Trichomycterus guianense* and *Vandellia* cf. *plazai* (which show fully ossified skulls) indicate the presence of a cartilaginous-like tissue strip that is continuous with both the ceratohyal and hyomandibular cartilage (Fig. 3B, 7A,B). Whether this structure is indeed homologous to the interhyal of other catfishes, still needs to be verified in a comparative study. The hyoid bar comprises three ossifications, *i.e.* ventral hypohyal, anterior ceratohyal and posterior ceratohyal. A large parurohyal ligamentously couples the sternohyoid muscle to the hyoid bar, at the level of the ventral hypohyals (Fig. 4G) (Arratia & Schultze 1990).

The opercle is a solid bone (thus no longer plate-like as in nematogenyids), bearing a caudally extended plate onto which a set of enlarged integumentary teeth are attached

(Fig. 2C). In ancestral crown group trichomycterids, the number of opercular teeth is higher compared to that in derived lineages (Baskin 1973). The articulatory facet for the articulation with the hyomandibula bears a distinct lateral projection (Fig. 8A). Rostroventrally, the opercle has a pointed anteroventral process that is ligamentously connected to the interopercle. This connection has been observed in trichomycterids by other authors, as a single ligament (Arratia 1990b, de Pinna 1992, 1998, Arratia 1998). However, as is described below, this actually involves a complex of three ligaments between the latter bones, as well as many additional ligaments that are present interconnecting opercle, interopercle, hyoid bar and suspensorium (Fig. 6A,B). Being a synapomorphy for Trichomycterinae, a ball and socket joint between the opercle and preopercle can be considered to be absent in ancestral crown group trichomycterids (Arratia 1990a). The interopercle is also a massive bone, bearing numerous enlarged integumentary teeth at its ventral to ventrocaudal face (Fig. 2C). Together with the opercle, suspensorium and associated muscles, it forms a highly specialised system for moving these teeth when fixing themselves to the substrate in fast flowing rivers (or even elbowing with them) (observed in the wild by one of us, JNB). The interopercle is connected by a total of seven ligaments to: the opercle (3), preopercle (2), hyomandibula (1), and posterior ceratohyal (1). These ligaments most probably have a triple function (for a discussion on the mechanism, see below): powering the movements of the interopercle (being coupled to moving elements), guiding its complex movements, and securing the opercular system against excessive movements and subsequent dislocations.

Branchial basket – In the basal condition, as observed in *Trichomycterus guianense*, all gill arch elements as found in generalised siluriforms are present: three separate basibranchial elements (first one incorporated in parurohyal complex, second and third ossified, fourth and fifth fused into posterior copula) (Adriaens & Verraes 1998), three separate hypobranchials (all ossified), five ceratobranchials (all ossified, fifth one bearing toothed lower pharyngeal jaw), four epibranchials and two infrapharyngobranchials (III and IV, the latter supporting the upper pharyngeal tooth plate) (Fig. 2F). The third ceratobranchial can be considered to be without a posterior notch, which is unique to trichomycterines (Arratia 1990a).

Cranial musculature

Even though very little is known about trichomycterids myology, at least one synapomorphic character of crown group trichomycterids involves a novelty: the protractor operculi (de Pinna 1998) ('preopercular muscle' of Howes 1983: fig. 12). As comparative data is thus lacking, the following description of ancestral crown group trichomycterid myology is based on dissections and serial histological sections of *T. guianense* (Fig. 4). Branchial muscles, however, are not included in this paper.

Lower jaw and palatine muscles: The cheeks are completely covered by massive jaw adductor muscles, as well as the protractor operculi (see below). Ventrally, an A1-OST (this terminology follows Gosline 1989, and Diogo & Chardon 2000) runs from the preopercle to the angular part of the coronoid process (Fig. 4A,B). Dorsal to it lies the largest part, the A2A3'. Both serial sections and dissections yielded no clear evidence for the presence of an A2 being separated from an A3', even though Diogo (2006) did observe it in *Nematogenys inermis*. According to Diogo & Chardon (2000), the A3 of *Diplomystes* also comprises a part lying lateral to the levator arcus palatini. As such, the largest jaw adductor muscle part, lying lateral to the levator arcus palatini, is considered the complex A2A3'. The superficial fibers, corresponding to the A2-part, show a bipennate configuration, with its long tendon anteriorly fusing with the connective tissue of the A1-OST before inserting on the mandibula. Subsequently, a dorsal A2A3'α and ventral A2A3'β is distinguished. Caudally, the muscle originates from the preopercle and hyomandibula, ventral and caudal to that of the deeper A3". This A3", lying medial to the levator arcus palatini, originates from the membranous plate of the quadrate, thereby partially covering both the extensor tentaculi and adductor arcus palatini (Fig. 4C). Rostrally, the fibers insert on the coronoid process (both medial face of angular part and a caudal fossa on dental part), medial to the A2A3'. There is no Aω.

Ventrally, the left and right mandibula are connected to each other by two muscles: the anterior and posterior intermandibularis (Fig. 4E). The anterior one lies rostrally, at the level of the mandibular symphysis. The posterior one runs more caudally, inserting on a median fascia onto which the fibers of the anterior interhyoideus muscle attach also, thereby ventrally covering those interhyoideus fibers. These two muscles are generally considered to form a complex protractor hyoidei, where in many cases the distinction can only be made during early ontogeny (e.g. Adriaens & Verraes 1997) (but see Geerinckx & Adriaens 2007 for a discussion on the homology of this muscle in catfishes).

As a retractor tentaculi is absent, only the extensor tentaculi acts onto the maxillary barbel. In *T. guianense*, it is a large muscle that appears to be bipennate (a large tendon runs from the posterior tip of the autopalatine caudodorsally) (Fig. 4D). The origin of this muscle is spread over the lateral ethmoid and orbitosphenoid, whereas the fibers insert both onto the tendon as well as directly onto the dorsal face of the autopalatine (posterior to its articulation with the lateral ethmoid). Considering the double articulation with the neurocranium, a contraction of this extensor may only result in a dorsal tilting of the autopalatine (however, kinematics are unknown).

Hyoid and suspensorial muscles: The anterior interhyoid muscle originates from the posterior ceratohyals (at the synchondrosis with the anterior ceratohyals) and runs anteromedially to insert onto a median fascia. In *Trichomycterus guianense*, the anterior part is flanked laterodorsally by flanges of cartilaginous-like tissue that is attached to the ventrolateral face of the hypohyals and anterior ceratohyals (Fig. 3D). The posterior interhyoid muscle comprises the differentiated hyohyoideus inferior and posterior, where in the latter an abductor and a series of adductor muscles can be distinguished (Fig. 4E). The inferior hyohyoideus muscle originates from the posterior ceratohyals (close to the synchondrosis with the anterior ceratohyals), and has its anterior part covered ventrally by the anterior interhyoid muscle. The hyohyoideus abductor, originating from the ventral side of the first three branchiostegals (visible in the histological sections), runs medially with its anterior fibers merging with the posterior fibers of the inferior hyohyoideus muscle (Fig. 4D). Both muscles thus insert onto a large median fascia, which has two distinct anterior tendons that insert onto the ventral hypohyals (Fig. 3D). The hyohyoideus abductor and adductor series are only separated from each other anteriorly (posterior fibers run as one sheet between the median aponeurosis and the opercle). The anterior part of the hyohyoideus adductor forms a series of small muscles running between the consecutive branchiostegal rays. The last branchiostegal ray is connected to the opercle through both an adductor muscle slip, as well as a distinct strip of cartilaginous tissue (comparable tissue as the one flanking the hyoid bar anteriorly) (Fig. 3D). The muscle slip inserts into a ventral cavity of the opercle. Caudally, an additional muscle slip connects the opercle to this cartilaginous-like tissue strip (‘m-hh-ad’ in Figs. 4B, 6A). Whether this should be considered as a differentiation of the hyohyoideus adductor remains to be studied.

A distinct sternohyoid muscle connects the pectoral girdle (at the level of the cleithrum, where left and right cleithral bones no longer form a syndesmosis in the ancestral crown group trichomycterids) and hypaxial musculature to the hyoid bar. The muscle runs ventral to the cleithrum, attaching onto a myocomma separating it from the hypaxials, but also has a small muscle slip attaching on the dorsal face of the cleithrum (Fig. 4F,G). Rostrally, the paired muscle fits into the cavity formed by the parhypural bone, with its median process lying in the median aponeurosis. As in other catfishes, due to the ligamentous connection between parhypural and hypohyals, this muscle must induce a hyoid retraction during contraction.

Two muscles interconnect the suspensorium with the neurocranium. The levator arcus palatini is a large, bipennate muscle, connecting the lateral face of the suspensorium (at the level of the hyomandibula) to the neurocranium (its tendon originates at the level of the orbital process of the sphenotic complex and parietal) (Fig. 4B). The adductor arcus palatine lies posterior to the extensor tentaculi, and connects the orbitosphenoid and sphenotic complex to the medial face of the hyomandibular membranous plate (Fig. 4D). Posterior, the muscle shows a bipennate organisation, with a distinct tendon inserting onto the hyomandibula. A separate adductor hyomandibulae could not be observed (also lacking in *Nematogenys*) (Diogo et al. 2006).

Opercular muscles: Coupled to the specialised morphology of the opercle, and its mechanical coupling to the interopercle, distinct opercular muscles are present in *T. guianense*. Presumably related to this opercular apparatus, and its functioning (see below), is the presence of a novel muscle: the protractor operculi (of which the homology still remains unclear). This large muscle originates onto the lateral face of the opercle (Fig. 4A), inserting onto a small lateral process of the preopercle. The muscle fibers run laterally to and at the same level of the opercular articulation with the hyomandibula. Compared to most other catfishes, the dilatator operculi has become substantially enlarged, thus being the largest opercular muscle in *Trichomycterus*. It comprises two distinct bundles, anteriorly separated by the levator arcus palatini (Fig. 4B). The medial bundle has its origin as far as halfway the eye, attaching onto the parietals and running posteriorly beneath the orbital process where it merges with the lateral bundle. The lateral bundle originates from this orbital process, and converges together with the medial one onto a distinct tendon that inserts onto a dorsal process on the opercle (Fig. 4B). Posterior to the dilatator lies the levator operculi, connecting the pterotic to the dorsomedial face of the opercle.

Superficially, this levator is covered by a distinct ligamentous sheet that connects the opercle and pterotic (Fig. 4A). Medioventral to the levator runs the adductor operculi, also originating onto the pterotic but inserting onto the medial face of the opercle (Fig. 4B,C).

Eye muscles: Both obliquus muscles originate on the ventral side of sesamoid supraorbital bone, and are attached to connective tissue surrounding the bone. All rectus muscles converge posteriorly, onto a connective tissue sheet separating the supraorbital branch of the trigemino-facial nerve complex (running into the nasal barbel) from the infraorbital branches.

Evolutionary morphology of the opercular apparatus

As mentioned above, the basal condition of the opercular apparatus of crown group trichomycterids differs from that of the typical catfish condition (as observed in *Diplomystes* and *Nematogenys*) in the presence of a solid interopercular bone bearing elongated integumentary teeth (Fig. 2C). In these two taxa, ligamentous connections exist between the opercle and interopercle, between interopercle and lower jaw and between interopercle and posterior ceratohyal (Schaefer 1988, Diogo et al. 2006). Three opercular muscles attach onto the opercle, with the dilatator being the largest one and the levator inserting onto the lateral face of the opercle (adductor typically teleostean) (de Pinna 1998). The condition found in ancestral crown group trichomycterids, as explained above, is quite different from this. Most striking features are: massive opercular bone also bearing a set of enlarged integumentary teeth; opercle with distinct anteroventral process fitting into a dorsomedial cavity of interopercle and attached to it through a complex of ligaments; and novel opercular muscle claimed to protract the opercle (‘m. protractor operculi’) (Fig. 4A). The robustness of the opercular bones is attained ontogenetically, from small, plate-like precursors (Arratia 1990b). Also the suspensorium seems to be fortified, as the loss of the preopercular sensory canal is believed to be linked to its supportive role of the solid opercle bone (Baskin 1973). However, this sensory canal independently got lost in scoloplacids and some loricariids, where in the case of ancistrine loricariids that also posses a highly specialised erectile spine apparatus (but different from the trichomycterid apparatus), the preopercle does still enclose a sensory canal (Geerinckx et al. 2005, Geerinckx & Adriaens 2006). The latter at least shows that strengthening the suspensorium for supporting an opercularly driven integumentary teeth system does not necessarily imply the loss of the sensory canal. Additionally, our observations that canals remain in the bone, but without a sensory canal, seem to support this (Fig. 3B).

The adaptive nature of this specialised opercular system in ancestral crown group trichomycterids, as mentioned in the introduction, is believed to be related to living in torrential rivers, and thereby allowing the fish to move over hard substrate or even climb waterfalls (Eigenmann 1918a, Arratia 1983). Even though strongly interconnected by what is generally assumed as a single ligament, the interopercle and opercle still retain independent mobility up to some degree (de Pinna 1992). Moving the enlarged and ventrally directed interopercular integumentary teeth could thus allow an ‘elbowing’ behaviour. The presence of these enlarged teeth on the interopercle only, such as in *Copionodon*, may support this behaviour to be ancestral to Trichomycteridae (but see de Pinna, 2008). In general, trichomycterid evolution is characterised by a decrease in the size of the interopercular teeth, where relatively large ones can be found in *Copionodon, Scleronema*, *Ituglanis* and *Trichomycterus* (Fig. 5B,C,D) and a reduced number can be observed in some stegophilines (*e.g. Apomatoceros*) (Fig. 5E), or then may even be absent in some glanapterygines (*e.g. Glanapteryx, Typhlobelus*, *Pygidianops*) (Fig. 5H), some stegophilines (*e.g. Megalocentor*) and some Sarcoglanidinae (*e.g. Sarcoglanis*) (Fig. 5G) (Eigenmann 1922, Baskin 1973, de Pinna 1992). The ancestral condition for the crown group trichomycterids involves the presence of enlarged opercular teeth also, which may indicate a more versatile use of the opercular system for holding on to a substrate and thus became exapted for holding on to living objects. There seems to be no clear correlation between the loss of interopercular teeth and opercular ones, as the opercular ones are for example absent in the above mentioned *Apomatoceros* but present in *Sarcoglanis* (only *Glanapteryx* and *Pygidianops* may lack them on both bones) (de Pinna 1989b) (Fig. 5H). On the other hand, both bones generally bear large and numerous integumentary teeth in the clade giving rise to the parasitic stegophilines and vandellines, where especially in the latter ones, the holding on even seems to have become specialised into lodging into branchial cavities of large fishes.

FIG 5

Opercular system in ancestral crown group trichomycterids: mobile but constrained: Apart from being solid, the interopercular bone in trichomycterids also shows some distinct specialisations (Fig. 9). Anteriorly it bears a forked anterolateral process that is dorsally continued into a transverse crest, running medially (crest not present in *Copionodon*, Fig. 9B). This crest then extends up to the base of an anteromedial process, where both are connected to the main body of the interopercle. Along its ventral and caudal margin, this body supports the interopercular integumentary teeth. This is a condition observed in *Trichomycterus*, but as mentioned above, quite some variation exists in other lineages. A more dorsally directed process, presumably homologous to this anterolateral process and the transverse crest, is also observed in copionodontines (de Pinna 1992: fig. 10) (Fig. 9B). The anterolateral process in *Trichomycterus guianense* is connected to the lower jaw through a large ligament (Fig. 6B). In between this anterolateral and anteromedial processes, the proximal head of the hyoid bar is articulating with the interopercle, as well as the hyoid bar is connected to the anteromedial process by a distinct ligament. As was already suggested for *Ituglanis* as well, this contact presumably functionally couples hyoid bar movements to interopercular movements (Sarmento-Soares et al. 2006: p. 314) (see below). The dorsal face of the corpus of the interopercle then provides a gliding surface for the bifurcated anteroventral process of the opercle, with this bifurcation being common for trichomycterids (with exception of some vandelliines) (de Pinna 1992). The third component involved, is the suspensorium, where the hyomandibula provides a distinct articulatory facet for the opercle, laterally flanked by a bony process (Fig. 8A) (an additional articulation for the opercle on the preopercle is found only in trichomycterines) (Arratia 1990a).

FIG 6

Based on this it seems that the interopercle is a highly mobile element, able to move with respect to the opercle and suspensorium. However, movements seem to be constrained by an extraordinary large number of ligaments confined to this region, additional to the one already mentioned connecting the interopercle to the hyoid bar. In *Trichomycterus guianense* the interopercle itself is connected by three ligaments to the opercle (Fig. 6A,B): (1) an anterolateral ligament, running from the lateral tip of the anteroventral process of the opercle to the lateral face of the anterolateral process of the interopercle, (2) an anteromedial ligament, running from the medial tip of the anteroventral opercular process to the interopercular transverse crest, and (3) a posterior ligament, running from the caudal face of the opercular anteroventral process to the dorsal face of the caudal interopercular corpus (up to the base of the integumentary teeth). Two ligaments connect the interopercle to the preopercle: (1) a rostrocaudal one, running from the transverse crest to the ventral face of the preopercle, and (2) an anterolateral one, running from the dorsal face of the interopercular anterolateral process to the ventral face of the preopercle. An additional ligament connects the interopercle to the suspensorium, at the level of the hyomandibula. It is an anterior ligament, running from the posterior face of the transverse crest to the ventral face of the hyomandibula. Apart from this, the opercle is also connected to the hyomandibula through a connective tissue sheet (Fig. 6B), as well as it is connected to the lateral face of the neurocranium (Fig. 4A). Both the cartilaginous strip and a ligament interconnect the posterior ceratohyal with the hyomandibula, as well as the ceratohyal is also connected by a ligament to the anterior branchiostegal rays.

So how could this opercular system function to allow the alleged elbowing? Input force presumably comes from two sources: muscles acting onto the opercle and those acting onto the hyoid bar. Considering the mediodorsal direction of the levator and adductor operculi muscle fibres, as well as the shape of the operculo-hyomandibular articulatory facet, these opercular muscles will presumably induce an adduction-elevation of the post-articulatory part of the opercle. The enlarged, rostrally directed dilatator operculi will then assist in elevation. The resulting force could then trigger a ventrocaudal and lateral movement of the opercular anteroventral process. This will in turn make the posterior part of the interopercle follow in the same direction, thus pushing the integumentary teeth out and down. The same movement would be induced if the hyoid bar would be abducted and retracted, an action generally triggered by the sternohyoid muscle (Aerts 1991). Because of the fact that the hyoid bar makes a substantial angle with the plane of the hyomandibular articulation, the abduction component could be substantial (Fig. 2E) (de Visser & Barel 1996). The interopercular transverse crest may than actually serve as a strut for the hyoid bar to transfer the abduction and retraction forces, whereas the ligaments interconnecting them would prevent dislocations.

This would be the power phase, where the recovery phase would require less powerful muscles. The hyohyoideus adductor muscles could then restore the opercle to its starting position, thereby pulling in the interopercle. A restoring of the hyoid bar to its starting position would then be accomplished by muscles acting upon it (intermandibularis posterior, interhyoideus anterior and hyohyoideus inferior) and mandibulo-hyoid ligament involved in mouth closing (Van Wassenbergh et al. 2005). The complex set of ligaments would then couple all opercular and hyoid movements to interopercular ones.

Even though being quite surely involved in the opercular system movements, the role of the protractor operculi muscles is hard to predict because of the position of its line of action being at the same level as the operculo-hyomandibular articulation. It could assist in abducting the postarticulatory part of the opercular bone, however, considering its fibers running adjacent to that articulation, this would be quite inefficiently. Depending on its rotational condition, the protractor could induce a ventral (when line of action lies below the articulation) or dorsal (when above the articulation) movement of the postarticulatory part. Thus a protraction may not necessarily be the actual function of this ‘protractor’. This, however, needs to be confirmed by more detailed modelling or function analyses on muscle activities and kinematics.

Opercular system in vandellines: equipped to hang on: One lineage of trichomycterids show a gradual increase in structural and behavioural specialisations towards “parasitism ” (for a discussion on the appropriateness of this term for these fishes, see Spotte 2002), i.e. the clade of Tridentinae-Stegophilinae-Vandelliinae (Fig. 1, clade G). Tridentinae are alleged to show some degree of parasitism, because of their miniature size and ventral mouth with specialised dentition on upper and lower jaw (de Pinna 1989a, Azpelicueta 1990). Field observations or stomach contents to support this are currently lacking, however some recent field observations indicated specimens of *Tridentopsis* being associated with piranha’s in the Rio Cuyabeno (Aguarico River Basin, Eastern Ecuador) (Stewart, pers. comm.). Stegophilines, on the other hand, have been demonstrated to be ectoparasitic mucus feeding and scale eating fishes, adhering themselves to host fish species (Baskin et al. 1980, Winemiller 1989). Some of them even posses a highly specialised oral jaw apparatus with numerous rows of long, needle-like teeth (Baskin 1973), perhaps used for this feeding (e.g. *Apomatoceros alleni*) (Fig. 5E) (Eigenmann 1918a). One stegophiline (*Pareiodon microps*), however, feeds by taking bites of flesh from other fishes (von Ihering, 1940, in: Spotte, 2002). Vandelliines then are specialised hematophagous feeders, engulfing blood from lacerated gill filaments in large fish while anchored into the gill cavity (Kelley & Atz 1964, Spotte et al. 2001). Because of their unique hematophagous feeding behaviour, but especially their possibly accidental entering orifices of other animals and even human, candirú are probably the most legendary fish that brings up the most vivid imaginations (Gudger 1930a,b, Vinton & Stickler 1941, Spotte 2002, Zuanon & Sazima 2004). Not many fish made it into journals on human urology (Herman 1973). Lots of speculations have been made on both the feeding and anchoring apparatus (including the alleged but dubious retraction of vomeral teeth) (Spotte 2002), however, no solid data on the detailed anatomy or functioning of both apparatuses has been provided to corroborate this. Several synapomorphies of the Vandelliinae have been postulated, of which some may be linked to this parasitic behaviour: (1) claw-like teeth on distal end of premaxilla (possibly for slashing the gill filaments of the host), (2) dentaries well separated along the midline (function unknown), and (3) reduction of the branchial basket, including loss of functional upper and lower pharyngeal jaws (i.e. lack of the 5th ceratobranchial in *Vandellia* and the loss of both the 4th and 5th ceratobranchials in *Branchioica*, its absence may be linked to being of no use when feeding on blood) (Baskin 1973, de Pinna 1998). Also at the level of the digestive system, specialisations have occurred to allow a temporary storing of a large volume of blood, as well as they apparently have managed to deal physiologically with excessive volumes of water and food of pure protein contents (Spotte 2002). A buoyancy or respiratory function of the digestive tract (especially the stomach), as observed in some trichomycterids, is also unknown in stegophilines and vandelliines (Gee 1976, Cala 1987).

FIG 7

Compared to the configuration found in *Trichomycterus*, the opercular system seems highly specialised in *Vandellia* (Figs. 5F, 6C,D, 7B,C, 9E). The interopercle is relatively much broader, especially posteriorly and thus provides posteriorly a broader support surface for the enlarged and numerous integumentary teeth (Fig. 5F). All the interopercular processes, as found in *Trichomycterus*, are present in *Vandellia*, however, the transverse crest has shifted posteriorly (Fig. 9C,E). The anterolateral process is also pointed in *Vandellia* and is quite solid. The cavity, into which the posterior ceratohyal fits, is also well developed but now a true articulation is present (thus with a cartilaginous-like facet on both the interopercle and posterior ceratohyal) (Fig. 7B). This already suggests an improved mechanical coupling of hyoid bar and interopercle. Also at the level of the complex ligaments, some specialisations have occurred in this region. First, no less than four additional ligaments are found (adding the total up to 13) (Fig. 6C,D): (1) an anteromedial ligament connecting the interopercle with the preopercle, running medially to the antero-lateral ligament, (2) a posterior ligament connecting the interopercle with the hyomandibula, (3) a ligament between the interopercle and the anterior branchiostegal ray, and (4) a ligament between the posterior ceratohyal and the preopercle. Some of these ligaments were already noticed by Arratia (1990b: fig. 11C). Additionally, two ligaments running in a rostrocaudal direction (rostrocaudal interoperculo-preopercular ligament and anterolateral interoperculo-opercular ligament) have become relatively elongated, compared to the situation in *Trichomycterus*.

FIG 8

The opercle has a more slender anteroventral process, and bears a distinct dorsal process at the anterior face of the plate supporting the integumentary teeth (this plate being penetrated by a foramen) (Fig. 5F). A distinct difference with *Trichomycterus* is that the opercular articulation with the hyomandibula is now flanked both laterally and medially by bony struts of the opercle, thus constraining the mobility of the joint (Fig. 8B). Also the hyomandibular process of this articulation now shows a more bilateral distribution of cartilaginous tissue (instead of only lateral as in *Trichomycterus*). The hyomandibula bears a distinct foramen (running rostrocaudally), through which passes the hyoideomandibular nerve branch, as well as the fibers of the levator arcus palatini insert into it. The preopercle is well developed, lacking a sensory canal. Especially distinct is the presence of a large lateral process (Fig. 7B1, green arrowhead), covering the anterolateral process of the interopercle. This process may assist in guiding the interopercular pro- and retraction.

So, what does this all tell us about the functioning of this opercular system in *Vandellia*, and can the observed differences be related to the undoubtedly increased functional demands that it has to meet when anchoring itself into a branchial cavity while feeding? Taking into consideration the bilateral flanking of the operculo-hyomandibular articulation with bilateral distribution of articulatory cartilage on the hyomandibular process, and the shifted orientation of the adductor and levator operculi muscles (now running caudodorsally, instead of mediodorsally), modelling suggested that the mobility of the opercular bone is restricted to a sagittal plane (so no adduction of the postarticulatory part and thus abduction of its anteroventral process). Contraction of the large dilatator operculi muscle, inserting onto the enlarged dorsal process, will supplement this movement. This will restrict interopercular movements to being pulled ventrally and caudally, where the lateral process of the preopercle could assist in guiding this retraction. The elongated ligaments, running in such a rostrocaudal direction, thus allow a more extended caudal and ventral displacement of the interopercle. The coupling with hyoid bar depression or retraction could then result in a restricted abduction of the interopercle (as it may be prevented to do excessive abductions due to the mentioned constrained mobility of the opercle), or even a rotation around its length axis (the presence of a true articulation could indicate this). These movements would then largely push down and back the interopercular integumentary teeth, and maybe rotate them outward during this power stroke. Opercular teeth would then simultaneously move upward and away from the interopercular ones, which could allow a powerful anchoring if both sets of teeth would initially be inserted into the soft tissue lining the branchial cavity of a host. The subsequent dislodging of the teeth during a recovery stroke may thus also require a powerful muscle input. Several muscle configurations seem to support this: (1) the A1-OST now directly connects the anteroventral side of the interopercle with the lateral face of the coronoid process (Fig. 6C), and will thus protract the interopercle when the mandibula is kept closed; (2) the hyohyoideus adductor muscle seems to have differentiated, splitting off two additional muscle slips (running from the medioventral face of the opercle anteriorly towards the branchiostegal ray); and (3) the line of action of the enlarged protractor operculi muscle seems to run rather ventral to the operculo-hyomandibular articulation, and would thus allow a more powerful protraction of the anteroventral process of the opercle. Also, the elongated rostrocaudal ligaments could allow storage of more strain energy during the power stroke, which would be released during the recovery stroke (thus protracting the interopercle). However, again this needs to be confirmed by additional functional analyses.

FIG 9

Specialisations to a meiobenthic life style

The habits and structure of catfishes reflect their adaptation to a bottom dwelling, substrate oriented existence, although many taxa exhibit a secondary midwater habit (Alexander 1965). A large basal siluriform clade, the Loricarioidea, has few taxa that show this secondary trend (e.g. the callichthyid *Dianema* and the basal trichomycterid *Trichogenes*). In fact, not a single species of loricariid, astroblepid or scoloplacid has a midwater habit (Schaefer pers. com.). In stark contrast, loricarioids have developed extremes of substrate orientation, seen particularly in the Trichomycteridae. Species of Glanapteryginae and Sarcoglanidinae actually live in the substrate, i.e. digging into sand and gravel, a microhabitat that some species perhaps may never deliberately leave (Schaefer et al. 2005). Such fossorial habits are not uncommon in teleost fishes (e.g. heterocongrids, moringuids, or gobiids are known to burrow) (Nelson, 2006), but are far from common in catfishes. Exceptions known to live submerged in the substrate are some clariids (Adriaens et al. 2002). Other trichomycterids are well known for their parasitic habits, as mentioned above.

An examination of trichomycterids in light of their phylogeny (Fig. 1) reveals several distinct morphological trends associated with these extremes of substrate orientation in digging species but also in the parasitic ones. Several of the evolutionary trends that Giere (1993) identified among various meiofaunal invertebrate taxa can also be seen among both these trichomycterids: reduced body size, loss of pigment with translucent skin, body elongation with increased number of vertebrae, reduction of appendages, increased surface area of the body (Baskin et al. 2004). Some of these trends are particularly marked among the digging (interstitial) Glanapteryginae (*Pygidianops* and *Typhlobelus*) and Sarcoglanidinae (*Sarcoglanis* and perhaps others) as pointed out by

Schaefer et al. (2005). Of course not all meiofauna invertebrates and not all burrowing and parasitic trichomycterids show all of these trends. But the presence of these trends found in diverse invertebrates and in trichomycterids as well as other burrowing vertebrates also (Withers 1981; Adriaens et al. 2002), reinforces the idea that these may reflect evolutionary responses to selective pressures typical for a substrate inhabiting life style. Although these trichomycterids may not meet the arbitrary criteria of being meiofaunal, (passing through a 0.5mm mesh sieve) their sharing of several common evolutionary trends with true meiofauna invertebrates shows that they are sharing similar adaptation pressures associated with the interstitial microhabitat. These trichomycterids and their relatives that are intermediate in habitat occupancy (i.e. leaf litter inhabitants such as *Stenolicmus*, *Glanapteryx* and *Listrura*, and miniature non-trichomycterids such as scoloplacids) can elucidate the various ways these adaptations may have arisen, and help in the understanding of their significance in the interstitial habitat.

Body Form: Body elongation appears to be a common trend in the parasites and diggers (clade F in Fig. 1). Figure 10 shows variation in the ratio of body length versus head width in a phylogenetic context. For the entire clade A all seven basal genera show the primitive, non-elongate condition: *Nematogenys, Copionodon, Trichogenes, Eremophilus, Trichomycterus, Scleronema* and *Ituglanis*. A 99% Bonferroni Multiple Comparisons Test shows that these genera are not different from each other statistically at the 99.0% confidence level. Clade F contains the parasitic subfamilies (clade G - Tridentine, possible blood feeders; Stegophilinae, most scale and mucus feeders; Vandellinae, blood feeders) and the digging-in subfamilies (clade I – Glanapteryginae and Sarcoglanidinae, leaf litter and burrowing habitat). All of the clade F subfamilies with the exception of the Vandellinae have all genera (i.e. Sarcoglanidinae) or some genera that are statistically indistinguishable from the basal non-elongate genera (*Malacoglanis* is not included in the statistical analysis because only one specimen was available). The statistical analysis shows that in the Tridentinae only *Tridensimilis* is more elongate. Given the very poor state of our knowledge of the habits of the tridentines, little can be inferred from this. Among the stegophilines only *Apomatoceros*, a scale feeder, is elongate. Other stegophiline genera retain the basal condition. Baskin (1973) pointed out that *A. alleni* appears to have the most specialized feeding apparatus among the scale and mucus feeding stegophilines. Among the Vandellinae all genera are elongate and apparently all feed on blood from the gills of other fishes. Larger body size genera such as *Vandellia*, *Plectrochilus* and *Paravandellia* only insert their heads into the host gill chamber, while smaller genera *Branchioica* and *Paracanthopoma* fully enter the gill chamber and may stay there for extended periods (Baskin 1973, Spotte 2002). Small size in this subfamily appears to be a primitive condition based on the phylogeny in Baskin (1973), and all species appear to be elongate relative to the basal taxa.

Clade I contains all of the burrowing trichomycterids. The sarcoglanines are all either diggers, burrowing through loose sand/gravel substrate, or leaf litter inhabitants. With regard to elongation, none of the sarcoglanines examined here are elongate and are not statistically distinguishable from the basal clades. However all are among the smallest catfishes and teleosts known, and in this respect resemble other meiofaunal animals. Also, both *Sarcoglanis* and *Malacoglanis*, which are strongly burrowing forms, do have large, bluntly shaped heads, thus a strikingly different morphology than that of other burrowing trichomycterids and even most other head-first or tail-first burrowing teleosts (De Schepper et al. 2007a,b). All the Glanapteryginae are also either strong diggers (*Typhlobelus* and *Pygidianops*) in sand/gravel substrates, or are found in both the leaf litter and sand/gravel substrates (*Glanapteryx* and *Listrura*). All are distinctly more elongate and smaller in size than the basal clades. *Pygidianops* and *Typhlobelus* are also among the smallest catfishes and teleosts known. *Typhlobelus,* and to some degree *Pygidianops* and other glanapterygines, have a shovel shaped rostrum or snout, which could be an adaptation for movement through the loose substrate they inhabit. They have only been found in this microhabitat so far.

Reduction of appendages: This trend, found in meiofaunal invertebrates and commonly observed in fossorial species generally (Withers 1981, Greer 1990), is found in only one of the two fossorial trichomycterid clades, i.e. the Glanapteryginae. Its sister clade the Sarcoglanidinae, still shares with other trichomycterids the presence of the paired appendages, with the exception of the occasional loss of pelvic fins (de Pinna, 1989b). All of the glanapterygine species show some degree of fin reduction, and one, *Pygidianops magoi,* has retained only the caudal fin. Not surprisingly all of these species have either reduced or lost the pelvic fins, a feature common in elongate fishes (Adriaens et al. 2002). For example, in *Glanapteryx anguilla*, the most elongate species, the pelvic fins are absent in only some specimens (de Pinna, 1989b). In all other glanapterygine species they are lacking entirely.

Pectoral fins are also reduced or absent in all Glanapteryginae species. When present the greatest number of rays is 3, in *Listrura camposi*. In *L. nematopteryx* there is but a single ray, which is markedly elongate, as is the first ray in *L. camposi* (de Pinna 1988) and in some species of the Sarcoglanidinae. An elongate filament of the first pectoral ray is also commonly found in species of *Trichomycterus* and in *Ituglanis*. A possible association of this feature with a fossorial existence in these species has not yet been investigated.

Both *Listrura* species are the only glanapterygines with both a dorsal and anal fin with fin rays. The dorsal fin is lacking in all others, and the anal is present in only some species. This argues strongly for a basal position for *Listrura* within the Glanaptyginae, as suggested by de Pinna (1989b). It is also notable that *Typhlobelus* and *Pygidianops* have a low median fold of translucent flesh, lacking fin rays extending along most of the body to the caudal fin (Schaefer et al. 2005). A similar structure is present as an elongated adipose fin in some Sarcoglanidinae (*Sarcoglanis* and *Malacoglanis*), preceded by a well developed dorsal fin. This may represent an adaptation in both digging subfamilies for increased surface area, another trend characteristic of invertebrate meiofauna.

In addition, the complete loss of integumentary teeth from both the opercle and interopercle in all glanapterygine genera, except for *Listrura*, could also be considered part of this marked trend in appendage reduction associated with fossorial habits in this clade.

An additional feature of meiofaunal species is pigment reduction. Although live color is poorly known for most of these species, skin pigment reduction is seen quite markedly in the parasitic and digging clades in comparison to the basal clades (Burgess 1989: plates 154-157). In clade F only the stegophilines have well developed pigment patterns and in the majority of the species the dominant coloring is pale with some dark spotting or blotches. Tridentines are similar, but with much less pigment, more pale or white and some may be translucent. Most vandellines also lack dark pigment almost entirely, and *Vandellia* and *Branchioica* have translucent skin in life. Among glanapterygines *Listura* is darkly pigmented, *Glanapteryx* is predominately pale and both *Pygidianops* and *Typhlobelus* have only a few melanophores (Landim & Costa 2002, Schaefer et al. 2005). Both of the latter have translucent skin in life. Among the Sarcoglanidinae examined here, *Ammoglanis* is weakly pigmented. Both *Sarcoglanis* and *Malacoglanis* have only a few melanophores, being almost entirely pale, and *Sarcoglanis* has translucent skin in life. The lack of skin pigment here also corresponds to greater use of the loose sand/gravel habitat by the latter two genera. The pigment reduction in these two clades corresponds strongly to greater use of the loose sand/gravel habitat. Alternatively, reduction in size has also been linked developmentally to a reduction in pigmentation (McClure & McCune 2003), as well as other modular developmental patterns linking pigmentation loss to living in light deprived conditions (whether adaptive or not) (Wilkens 2001, Wilkens & Strecker 2003).

The pattern of development of these evolutionary trends toward the characters associated with meiofauna species to a greater or lesser degree appears to indicate independent trends in each of the various trichomycterid clades, in both parasitic and digging taxa, with each subfamily having its own suite of meiofaunal characters. This further reinforces our confidence in the reality of the clades of parasitic and digging subfamily clades. The occurrence of these same meiofaunal characters in both the parasitic and digging species hints at common selective pressures in both of these seemingly very disparate life styles. Greater knowledge of ecological and behavioral details of these animals may further elucidate this relationship.

FIG 10

Conclusions

Although a basal siluriform lineage, Trichomycteridae clearly show some specialised symplesiomorphic morphological traits, of which the well developed opercular system and associated opercular muscles is the most striking one. Enlarged integumentary teeth are supported by interopercle and opercle, that comprise a sophisticated articulated but mobile system for anchoring to the substrate. This system subsequently provided the basis for an even more specialised opercular system for hanging onto host fishes and finally even in host fishes within the Tridentinae-Stegophilinae-Vandelliinae clade. This clade is also featuring an increasing elongation of the body (relatively to the width), which is even more striking in the Sarcoglanidinae-Glanapteryginae lineage. In this clade, it seems that similar selective pressures (towards body elongated and loss of paired appendages) may have facilitated the occupation of an interstitial niche.

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Captions to the figures

Figure 1: Cladogram of Trichomycteridae (based on de Pinna 1998) (white arrow indicates position of 'basal crown group trichomycterid' as dealt with in the text)**.**

Figure 2: Habitus and cranial osteology of a basal crown group trichomycterid (*Trichomycterus punctulatus*). A. Habitus (ANSP180733) (scale bar 20 mm); **B-F.** 3D-reconstruction based on CT-data: lateral view (B), detail of opercular bones in lateral view (C), dorsal view (D), ventral view (E), detail of branchial elements in ventral view (F) (nasal and infraorbital bone not shown). Abbreviations: **lpj,** lower pharyngeal jaw; **o-ang-c,** angular complex; **o-ant,** antorbital; **o-apal,** autopalatine; **o-bb-III,** basibranchial III; **o-boc,** basioccipital; **o-cb-II,** ceratobranchial II; **o-ch-a,** anterior ceratohyal; **o-ch-p,** posterior ceratohyal; **o-cl,** cleithral bone; **o-den-c,** dentary complex; **o-eoc,** exoccipital; **o-epoc,** epioccipital (epiotic); **o-hb-II, III,** hypobranchials II, III; **o-hh-v,** ventral hypohyal; **o-hm,** hyomandibula; **o-iop,** interopercle; **o-let,** lateral ethmoid; **o-met,** mesethmoid; **o-mp,** metapterygoid; **o-mx,** maxillary; **o-op,** opercle; **o-orb,** orbitosphenoid; **o-par,** parietal; **o-ppar-soc,** postparieto-supraoccipital; **o-pmx,** premaxillary; **o-pop,** preopercle; **o-pt,** pterotic; **o-pt-scl,** posttemporo-supracleithrum; **o-puh,** parurohyal; **o-q,** quadrate; **o-sph-c,** sphenotico-pterosphenoideo-prootic complex; **o-ssob,** sesamoid supraorbital bone; **o-vm,** vomer; **od-op,** integumentary teeth (odontodes) of opercular bone; **p-fr-I,** first pectoral fin ray; **r-br-IV,** branchiostegal ray IV; **sb-c,** swimbladder capsule; **upj,** upper pharyngeal tooth plate.

Figure 3: Details of trichomycterid anatomy (based on histological sections of *Trichomycterus guianense*)**. A.** Detail of orbitosphenoid, showing the passage of the optic nerve through the bone; **B.** Detail of preopercle, showing the cavities lacking a sensory canal (arrowheads); **C.** Infraorbital bone enclosing postorbital part of infraorbital canal; **D.** Detail of anterior part of hyoid bar, showing flange of cartilaginous-like tissue on the anterior ceratohyal (arrow head) (scale bar 200 µm). Abbreviations: **l-mnd-ch,** mandibulo-ceratohyal ligament; **l-mnd-iop,** mandibulo-interopercular ligament; **o-ch-a,** anterior ceratohyal; **o-ch-p,** posterior ceratohyal; **o-iop,** interopercle; **o-orb,** orbitosphenoid; **o-par,** parietal; **ioc,** infraorbital sensory canal; **m-A2A3',** adductor mandibulae A2A3'; **m-ad-ap,** adductor arcus palatini; **m-dil-op,** dilatator operculi; **m-hh-inf,** inferior hyohyoideus; **m-intm-a,** anterior intermandibularis; **m-l-ap,** levator arcus palatini; **n-opt,** optic nerve (fasciculus opticus); **n-tr-fac,** trigemino-facialis nerve complex; **o-hh-v,** ventral hypohyal; **o-hm,** hyomandibula; **o-pop,** preopercle; **o-puh,** parurohyal; **soc,** supraorbital sensory canal; **syn-hh-ch,** synchondrosis between hypohyal and ceratohyal; **t-hh-inf,** inferior hyohyoideus tendon.

Figure 4: Cranial musculature in *Trichomycterus guianense (A-D:* lateral view, *E-G:* ventral view*).* Abbreviations: **l-ant-ssob,** ligament between antorbital and sesamoid supraorbital; **l-hh-puh,** hypohyalo-parurohyal ligament; **l-iop-op-p,** posterior interoperculo-opercular ligament; **l-mnd-ch,** mandibulo-ceratohyal ligament; **l-mnd-iop,** mandibulo-interopercular ligament; **l-mx-mnd,** maxillo-mandibular ligament (primordial ligament); **l-op-nc,** operculo-neurocranial ligament; **m-A1-OST,** adductor mandibulae A1-OST; **m-A2A3',** adductor mandibulae A2A3' (α - dorsal part, β - ventral part); **m-A3",** adductor mandibulae A3"; **m-ad-ap,** adductor arcus palatini; **m-ad-op,** adductor operculi; **m-dil-op,** dilatator operculi; **m-epax,** epaxials; **m-ext-t,** extensor tentaculi; **m-hh-ab,** hyohyoideus abductor; **m-hh-ad,** hyohyoideus adductor, main body; **m-hh-ad',**

hyohyoideus adductor, caudal subdivision; **m-hh-inf,** inferior hyohyoideus; **m-hyp,** hypaxials; **m-inth-a,** anterior interhyoideus; **m-intm-a,** anterior intermandibularis; **m-intm-p,** posterior intermandibularis; **m-l-ap,** levator arcus palatini; **m-l-op,** levator operculi; **m-pr-op,** protractor operculi; **m-sh,** sternohyoideus; **m-sh-d,** sternohyoideus, dorsal slip; **o-ang-c,** angular complex; **o-ant,** antorbital; **o-ch-a,** anterior ceratohyal; **o-ch-p,** posterior ceratohyal; **o-cl,** cleithral bone; **o-den-c,** dentary complex; **o-hh-v,** ventral hypohyal; **o-hm,** hyomandibula; **o-iop,** interopercle; **o-let,** lateral ethmoid; **o-met,** mesethmoid; **o-mx,** maxillary; **o-op,** opercle; **o-par,** parietal; **o-ppar-soc,** postparieto-supraoccipital; **o-pop,** preopercle; **o-pmx,** premaxillary; **o-pt,** pterotic; **o-pt-scl,** posttemporo-supracleithrum; **o-puh,** parurohyal; **o-q,** quadrate; **o-sph-c,** sphenotico-pterosphenoideo-prooticum complex; **o-ssob,** sesamoid supraorbital bone; **o-vm,** vomer; **od-op,** integumentary teeth (odontodes) of opercular bone; **prc-cor,** coronoid process; **r-br-VIII,** branchiostegal ray VIII; **t-ext-t,** tendon of extensor tentaculi; **sb-c,** swimbladder capsule.

Figure 5: Cranial skeletal morphology of representatives of nematogenyid and trichomycterid lineages. Graphical 3D-reconstructions based on CT-data (lateral view, left side). **A.** *Nematogenys inermis***; B.** *Copionodon pecten***; C.** *Scleronema operculatum***; D.** *Trichomycterus punctulatus***; E.** *Apomatoceros alleni***; F.** *Vandellia cirrhosa***; G.** *Sargoglanis simplex***; H.** *Pygidianops cuao* (scale bar 1 mm)**.**

Figure 6: Opercular system in basal and specialised Trichomycteridae (graphical reconstruction of opercular region). A-B. *Trichomycterus guianense*: lateral view (left side) of opercular region showing superficial muscles and ligaments (A); detail of complex of ligaments in lateral view (muscles removed) (B); **C-D.** *Vandellia* cf. *plazai*: oblique lateral view (left side) of opercular region showing superficial muscles (C); superficial muscles removed (D). Abbreviations: **cr-iop-tv,** transverse crest of the interopercle; **fc-od-op,** facet for the opercular integumentary teeth; **l-ch-hm,** ceratohyalo-hyomandibular ligament; **l-ch-rbr,** ligament between ceratohyal and branchiostegal rays; **l-iop-hm-a,** anterior interoperculo-hyomandibular ligament; **l-iop-hm-p,** posterior interoperculo-hyomandibular ligament; **l-iop-op-al ,** anterolateral interoperculo-opercular ligament; **l-iop-op-am,** anteromedial interoperculo-opercular ligament; **l-iop-op-p,** posterior interoperculo-opercular ligament; **l-iop-pop-al,** anterolateral interoperculo-preopercular ligament; **l-iop-pop-am,** anteromedial interoperculo-preoperculare; **l-iop-pop-rc,** rostrocaudal interoperculo-preopercular ligament; **l-iop-rbr,** ligament between the interopercle and branchiostegal rays; **l-mnd-ch,** mandibulo-ceratohyal ligament; **l-mnd-iop,** mandibulo-interopercular ligament; **l-op-hm,** operculo-hyomandibular ligament; **l-op-nc,** operculo-neurocranial ligament; **m-A1-OST,** adductor mandibulae A1-OST; **m-A2A3’,** adductor mandibulae A2A3’; **m-dil-op,** dilatator operculi; **m-hh-ad,** hyohyoideus adductor, main body; **m-hh-ad’,** hyohyoideus adductor, caudal subdivision; **m-pr-op,** protractor operculi; **mnd,** mandibula; **o-ch-p,** posterior ceratohyale; **o-hm,** hyomandibula; **o-iop,** interopercle; **o-op,** opercle; **o-pop,** preopercle; **o-q,** quadrate; **prc-op-av,** anteroventral process of opercle.

Fig. 7: Articulation between interopercle and posterior ceratohyal in basal and specialised Trichomycteridae. A.-B. 1: Graphical reconstruction of the complete skull (red line indicates position of histological section on the right, yellow arrow indicates the point of view for the detailed reconstruction); **2:** Detail of interopercle, hyoid bar and opercle (left side, dorsal view with red line indicating position of histological section); **3:** Histological section showing articulation; **A.** *Trichomycterus guianense*; **B.** *Vandellia* cf.

plazai (green arrowhead indicates lateral process of preopercle guiding the interopercle; suspensorium comprises quadrate, hyomandibula and preopercle); **C.** Scheme showing mechanism for spine erection in *Vandellia* (lateral view; green arrowhead indicates lateral process of preopercle guiding the interopercle) (scale bar 200 µm). Abbreviations: **art-ch-iop,** ceratohyalo-interopercular articulatory facet on the posterior ceratohyal; **art-iop-ch,** ceratohyalo-interopercular articulatory facet on the interopercle; **art-op-hm,** operculo-hyomandibular articulatory facet on the opercle; **cr-iop-tv,** transverse crest of the interopercle; **cr-iop-pm,** posteromedial crest of the interopercle; **l-ch-hm,** ceratohyalo-hyomandibular ligament; **l-iop-hm-a,** anterior interoperculo-hyomandibular ligament; **l-iop-op-al ,** anterolateral interoperculo-opercular ligament; **l-iop-pop-rc,** rostrocaudal interoperculo-preopercular ligament; **l-iop-rbr,** ligament between interopercle and branchiostegal rays; **m-pr-op,** protractor operculi; **mnd,** mandibula; **o-ch-a,** anterior ceratohyal; **o-ch-p,** posterior ceratohyal; **o-iop,** interopercle; **o-hm,** hyomandibula; **o-op,** opercle; **o-pop,** preopercle; **prc-iop-al,** anterolateral process of the interopercle; **prc-iop-am,** anteromedial process of the interopercle; **prc-op-av,** anteroventral process of the opercle; **susp,** suspensorium.

Figure 8: Details of opercular articulation in Trichomycteridae. A. Opercular articulatory facet with hyomandibula in *Trichomycterus guianense*, showing lateral extension (white arrow) as well as lateral distribution of articulatory cartilage on hyomandibular articulation; **B.** Same facet in *Vandellia* cf. *plazai*, showing lateral and medial extensions of opercle (white and black arrow, respectively) and bilateral distribution of articulatory cartilage on hyomandibular process (scale bar 200 µm)**.** Abbreviations: **o-hm,** hyomandibula; **o-op,** opercle.

Figure 9: Evolutionary transformations in the interopercle in Nematogenyidae and Trichomycteridae (left interopercle in dorsal view, all scaled to the same interopercular size based on the distance between tip of anteromedial process and distal margin). **A.** *Nematogenys inermis*; **B.** *Copionodon pecten*; **C.** *Trichomycterus punctulatus*; **D.** *Ituglanis amazonicus*; **E.** *Vandellia cirrhosa*; **F.** *Apomatoceros alleni*; **G.** *Sarcoglanis simplex*; **H.** *Pygidianops cuao* (red arrow: position of transverse process; blue arrow: position of hyoid articulation; green arrow: anterolateral process. Abbreviation: **cr-iop-tv**, transverse crest of the interopercle.

Fig. 10: Graph showing variation in degrees of body elongation in Trichomycteridae (expressed as the ratio of standard length versus head width) (bars indicate mean value per genus, error flags indicate min and max values; cladogram below the graph follows that as in Fig. 1, including the codes of the clades).