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Relationships among the Neotropical Candirus (Trichomycteridae, Siluriformes) and the evolution of parasitism based on analysis of mitochondrial and nuclear gene sequences

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ABSTRACT

Phylogenetic relationships among the trichomycterid catfishes are investigated for the first time using molecular sequence data. Data derived from mitochondrial and nuclear DNA sequences for representatives of 17 genera were analyzed to test previous hypotheses of relationships among trichomycterid subfamilies, the monophyly of the subfamily Stegophilinae, and the monophyly and relationships among the genera of parasitic members of the family. We analyzed 2325 aligned base-pairs from mitochondrial 12S, 16S, ND4 ($tRNA^{His}$ $tRNA^{Ser}$), and the nuclear histone H3 gene for representatives of 10 of 12 stegophiline and 3 of 4 vandelliine genera, plus 10 outgroup taxa selected to represent the range of subfamilial diversity. Maximum parsimony and likelihood approaches resolved a monophyletic semiparasitic Stegophilinae as the sister-group of the obligate hematophagous Vandelliinae. At the level of subfamilies, the pattern of relationships of the parasitic members among the remainder of the family is fully congruent with the most recent hypothesis of relationships for trichomycterids based exclusively on morphological data. Within stegophilines, our results differ from multiple previous morphological studies in recovery of (1) *Haemomaster* and *Ochmacanthus* as sister-taxa, (2) the morphologically plesiomorphic *Pareidon microps* nested within a relatively distal part of the tree topology, (3) *Apomatoceros* as sister to *Henonemus*, rather than to the morphologically similar *Megalocentor*. These result indicate that parasitism arose once and was unreversed within the Trichomycteridae. Survey of diet and feeding morphology among trichomycterids suggests that the semiparasitic lifestyle of the members of the Stegophilinae was retained in the enigmatic *Pareiodon microps*, despite reversal to the generalized trichomycterid condition of the associated morphological specializations found in all other stegophilines. These results further support the reconstruction of semiparasitism, rather than blood feeding, for the shared common ancestor of the parasitic Trichomycteridae.

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1. Introduction

The Neotropical Trichomycteridae (pencil or parasitic catfishes) is a dominant component of the South American ichthyofauna and widely distributed throughout the major river drainage basins of the continent, from Costa Rica to Patagonia, and in all types of freshwater habitats from flooded lowland forest to high-elevation streams of the Andes. The family is demonstrably monophyletic (de Pinna, 1998) and includes 207 species arranged in 41 genera and eight subfamilies (Ferraris, 2007). Most trichomycterid species are moderately small (to 100 mm standard length) generalist predators of small invertebrates, but members of two subfamilies, the Vandelliinae (four genera, nine species) and Stegophilinae (13 genera, 31 species), are exclusively parasitic. Vandelliines are hematophagous and parasitize the gills of larger fishes (Kelley and Atz, 1964), while the stegophilines feed on mucus, scales, and flesh. Both are popularly known as "candiru" or "carnero", although the infamy surrounding the penetration of the human urethra by vandelliines (Gudger, 1930; de Pinna and Britski, 1991) is restricted to a single species, *Vandellia cirrhosa*. Vandelliines most typically attack a branchial artery or vein of the host using highly specialized dagger-like teeth, whereby they ingest a blood meal followed by disengagement from the host (Machado and Sazima, 1983). The diet of stegophilines, in contrast, is much broader and these fishes feed on the scales and mucus of larger fishes (Baskin et al., 1980; Winemiller and Yan, 1989), with some species known to ingest skin and pieces of flesh (Lüling, 1984; de Pinna and Wosiacki, 2003). As the host individual is negatively impacted but not consumed or killed, such feeding habits are properly regarded as parasitic (Price, 1980; Machado and Sazima, 1983).

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Although 40 species of candirus have been described, our knowledge of their diversity, classification, and biology are poor. Hypotheses for the phylogenetic relationships among trichomycterid catfishes date back to Baskin (1973), who first proposed, based on analysis of morphological features using explicit cladistic methodology, the monophyly of all but one of the established subfamilies (i.e., Trichomycterinae) and offered a scheme of relationships for many of the included genera. Subsequent studies of trichomycterid relationships (summarized in de Pinna, 1998 and in Fig. 1) have relied exclusively on morphological data. Both Baskin (1973) and de Pinna (1998) provided morphological characters to support a sister-group relationship of vandelliines and stegophilines, with the subfamily Tridentinae as their sister-taxon. Proposals for the phylogenetic relationships within the candiru subfamilies are restricted to those of Baskin (1973) and Schmidt (1993), who differ in their respective hypotheses for the relationships among vandelliine genera. do Nascimiento and Provenzano (2006) provided character evidence to support a suprageneric clade within Stegophilinae and a sister-group relationship between *Acanthopoma* and *Henonemus*. de Pinna and Britski (1991) argued for a sister-group relationship between the stegophilines *Megalocentor* and *Apomatoceros* based on the uniquely-derived absence of opercular odontodes in representatives of those genera. All of these proposals have been based exclusively on morphological data, and none other than Baskin (1973) have included a large representation of the included taxa.

In this study, we offer the first comprehensive treatment of phylogenetic relationships of trichomycterid catfishes based on DNA sequence data. Use of DNA sequence data obtained from trichomycterid representatives in previous studies of inter-familial phylogenetics of catfishes is limited to that of Alves-Gomes et al. (1995), who included one trichomycterid species, Shimabukuro-Dias et al. (2004) and Hardman (2005), who each included two trichomycterid species, and that of Sullivan et al. (2006), who included four trichomycterid species. We focus specifically on the relationships among the parasitic candirus of the subfamilies Stegophilinae (Fig. 1A) and Vandelliinae (Fig. 1B) in an effort to test the monophyly of those groups and offer limited tests of the monophyly of some of the included genera. We further test the scheme of relationships among the trichomycterid subfamilies proposed by Baskin (1973) and de Pinna (1998; Fig. 1C) based on morphological data. We combine these results with broad survey of the morphology of feeding structures and gut contents among diverse trichomycterid representatives in an examination of the evolution of parasitism within the Trichomycteridae.

2. Materials and methods

2.1. Taxon sampling

We obtained DNA sequence data for 26 trichomycterid specimens, representing six subfamilies, 17 genera and 21 species. The

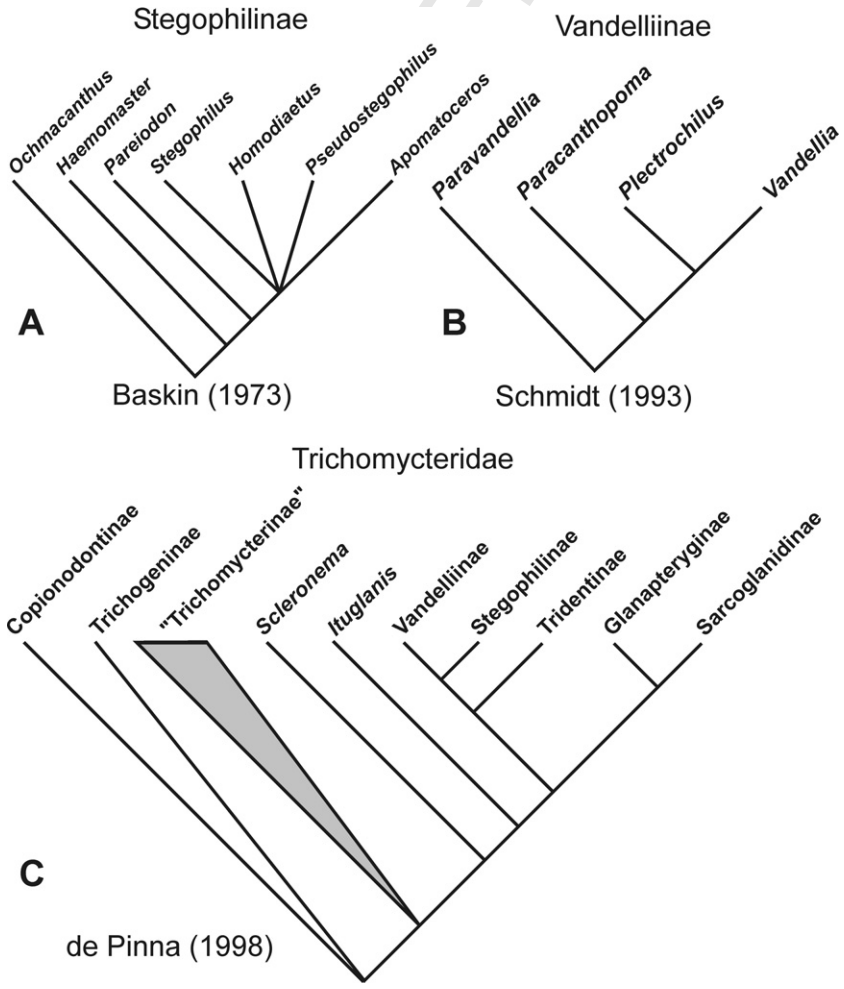

Fig. 1. Comparison of previous hypotheses of relationships among Trichomycteridae. (A) Stegophilinae, Baskin (1973); (B) Vandelliinae, Schmidt (1993); (C) Trichomycteridae, de Pinna (1998).

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ingroup included representatives of 10 of 12 genera of Stegophilinae (*Acanthopoma, Apomatoceros, Haemomaster, Henonemus, Homodiaetus, Megalocenthor, Ochmacanthus, Parastegophilus, Pareiodon,* and *Pseudostegophilus; Schultzichthys* and *Stegophilus* unavailable) plus three of the four genera of Vandelliinae (*Vandellia, Plectrochilus, Paravandellia; Paracanthopoma* unavailable). Six of the 13 ingroup genera were represented in the analyses by multiple individuals. Outgroup taxa included representatives of one of four genera of Tridentinae (*Tridens* sp.), one of six genera of Sarcoglanidinae (*Sarcoglanis simplex*), one of four genera of Glanapteryginae (*Typhlobelus*), one of six genera of Trichomycterinae (*Trichomycterus*). Specimens were either collected by us or were provided by colleagues. In all cases, tissues (fin clips, liver, or muscle) were sampled from specimens field preserved in 70% ethanol, with tissues subsequently transferred to 95% ethanol for long-term storage at −80 °C. Voucher specimens were fixed in formalin and transferred to 70% ethanol. Tissue, GenBank, and voucher specimen numbers for all taxa examined are listed in Table 1.

2.2. DNA extraction, amplification, and sequencing

Total DNA was extracted using a Qiagen DNEasy tissue extraction kit following the manufacturer's protocol. Target genes included three mitochondrial genes (12S rDNA, 16S rDNA, ND4) and one

Table 1
Taxa, specimens examined, tissue number, and GenBank numbers for the representatives of the Trichomycteridae analyzed in this study. Institutional abbreviations follow Leviton et al. (1985); "cs" denotes material cleared and stained for visualization of bone and cartilage.

Taxon	Voucher	# Spec.	Tissue #	GenBank Accession #			
				12S	16S	ND4	H3
Ingroup							
Stegophilinae							
Acanthopoma annectens	ANSP181146	2	t889	FJ744612	FJ744638	FJ744661	–
			t890	FJ744611	FJ744637	FJ744660	–
Apomatoceros alleni	ANSP181148	1	t894	FJ744617	FJ744643	FJ744666	–
	INHS52723	3 (1 cs)					
Haemomaster venezuelae	ANSP185147	1	t923	FJ744623	FJ744647	FJ744673	FJ744691
	AMNH10194	2 (1 cs)					
Henonemus punctatus	ANSP178174	1	t900	FJ744620	FJ744645	FJ744669	FJ744689
	INHS54706	2 (1 cs)					
Homodiaetus anisitsi	MCP40532	1	–	FJ744618	FJ744644	FJ744667	FJ744688
	MCP35109	1	–	FJ744619	–	FJ744668	–
Megalocentor echthrus	ANSP181150	1	t882	FJ744615	FJ744641	FJ744664	FJ744686
	AMNH94438	1					
Ochmacanthus orinoco	ANSP185146	1	t922	FJ744622	–	FJ744671	–
	ANSP185141	1	t926	FJ744621	–	FJ744670	FJ744690
	ANSP163018	1 cs					
Ochmacanthus sp.	ANSP187117	1	t974	FJ744623	FJ744646	FJ744672	–
	ANSP180010	2 (1 cs)					
Parastegophilus sp.	ANSP180490	1	t915	FJ744616	FJ744642	FJ744665	FJ744687
Pareiodon microps	ANSP181152	2	t884	FJ744609	FJ744635	FJ74465	FJ744684
			t885	FJ744610	FJ744636	FJ744659	–
	ANSP181151	2 (1 cs)					
Pseudostegophilus nemurus	ANSP180466	2	t913	FJ744613	FJ744639	FJ744662	FJ744685
			t914	FJ744614	FJ744640	FJ744663	–
	AMNH15486	1 cs					
	UF131110	2 (1 cs)					
Schutzichthys sp.	ANSP180496	2					
	ANSP136018	2 (1 cs)					
Vandelliinae							
Paravandellia oxyptera	ANSP180885	1	t921	FJ744626	FJ744649	FJ744675	FJ744692
Vandellia sanguinea	ANSP179813	1	t919	FJ744628	FJ744651	FJ744677	FJ744694
	ANSP185152	3 (1 cs)					
Vandellia cirrhosa	ANSP180838	1	t907	FJ744629	FJ744652	FJ744678	FJ744695
	AMNH20497	1 cs					
	UF77839	3 (1 cs)					
Plectrochilus sp.	ANSP181109	1	t887	FJ744627	FJ744650	FJ744676	FJ744693
Outgroups							
Trichomycterinae							
Trichomycterus areolatus	MCMI1370	1	lf185.1	FJ744606	FJ744632	FJ744655	FJ744681
	UMMZ215386	2 (1 cs)					
Trichomycterus corduvensis	MCMI1371	1	lf175.1	FJ744607	FJ744632	FJ744656	FJ744682
Trichomycterus cf. guianensis	ANSP179111	1	t917	FJ744608	FJ744634	FJ744657	FJ744683
Sarcoglanidinae							
Sarcoglanis simplex	ANSP179212	1	t871	FJ744630	FJ744653	FJ744679	FJ744696
Glanapteryginae							
Typhlobelus guacamaya	AMNH232974	1	–	FJ744631	FJ744654	FJ744680	–
	AMNH232994	1 cs					
Tridentinae							
Tridens melanops	MCZ156639	1 cs					
Tridens sp.	ANSP185221	1	t898	FJ744625	FJ744648	FJ744674	–
Tridentopsis cahuali	AMNH223161	5 (1 cs)					
Tridentopsis pearsoni	INHS37169	2 (1 cs)					

nuclear gene (histone H3). Sequences of the mitochondrial genes were amplified by the polymerase chain reaction (PCR) with the following primers: rRNA 12S L1091, 5′-AAACTGGGATTAGATACCCCACTAT-3′ and 12S H1478, 5′-GAGGGTGACGGGCGGTGTGT-3′ (Kocher et al., 1989); 16S a-L 5′-CGCCTGTTTATCAAAAAC-3′, 16S b-H 5′-CCGGTCTGAACTCAGATCACGT-3′ (Palumbi et al., 1991); ND4 H3 L11935 5′-CCAAAAGCACACGTAGAAGC-3′, H12857 5′-ACCAAGAGTTTTGGTTCCTA-3′ (Palumbi et al., 1991). Sequences of the nuclear gene histone H3 fragment were amplified by the primers H3a-L 5′-ATGGCTCGTACCAAGCAGACVGC-3′, and H3b-H 5′-ATATCCTTRGGCATRATRGTGAC-3′ (Colgan et al., 1998). Double-stranded amplifications were performed in 25-μL reactions containing one Ready-To-Go PCR bead (Amersham Biosciences), 1.00–1.25 μL of each primer, and 2–5 μL of DNA. Amplifications for all fragments were carried out in 35–40 cycles, with initial denaturation for 5 min at 94 °C, denaturation for 45–60 s at 94 °C, annealing for 45–60 s at 45–55 °C, extension for 1–2 m at 72 °C, and an additional terminal extension at 72 °C for 6 m. The nucleotides were sequenced on an ABI 3700 automated DNA sequencer. Contigs were built in Sequencher 4.8 (Gene Codes, Ann Arbor, MI) using DNA sequences from the complementary heavy and light strands. Sequences were edited in Sequencher and Biodedit (Hall, 1999).

The sequences were aligned with ClustalW (Thompson et al., 1994) and alignments refined manually using Bioedit. Each gene fragment was aligned independently. A total of 2325 aligned base-pairs from the four gene fragments were combined and analyzed simultaneously under a total-evidence approach (Eernisse and Kluge, 1993; Nixon and Carpenter, 1996) employing equal weights following Frost et al. (2001). Gaps were considered as informative characters. Eleven fragments could not be successfully amplified and/or sequenced and were coded as missing data in the analysis. Maximum parsimony (MP) analyses were performed using TNT 1.1 (Goloboff et al., 2003) and employed TBR branch-swapping with five rounds of tree-fusing and implementation of the parsimony ratchet with 20 iterations per replicate. Maximum Likelihood (ML) analyses were performed using GARLI v.0.96 (http://www.nescent.org/wg_garli/Main_Page) using default parameter settings and the GTR+γ model, based on results from best-fit model selection (AIC criterion = 8298.282; LnL = −4140.14) using FindModel (http://www.hiv.lanl.gov). Multiple independent runs (n = 25) were performed and log-likelihood scores examined to ensure that analyses were not trapped in local optimal topologies. To estimate the robustness of the support for recovered nodes in both the MP and ML analyses, 1000 bootstrap replicates (Felsenstein, 1985) were computed employing 10 random taxon-addition sequences and TBR branch-swapping. We also computed jackknife percentage values for each node based on 1000 replications and 10 random addition sequences per replicate. The topologies were examined using Winclada (Nixon, 2002) and MacClade 4.0 (Maddison and Maddison, 2000).

3. Results

The combined dataset consist of 2325 aligned base-pairs: 501 from 12S, 611 from 16S, 879 from ND4, and 334 from H3. Of these, 1692 base-pairs were parsimony informative. The MP analysis of the complete dataset resulted in a single most-parsimonious tree with a length of 3057 steps, a consistency index 0.56, and a retention index 0.56 (Fig. 2). ML results were uniform among the 25 independent runs, with log-likelihood scores ranging from −16402.5057 (best; Fig. 3) to −16403.4559. Both MP and ML trees are shown rooted on *Trichomycterus areolatus*. The MP and ML topologies are identical except for (1) the grouping of *Tridens* as the sister-group of *Sarcoglanis* plus *Typhobelus* in the MP tree, versus sister to the clade inclusive of Stegophilinae plus Vandelliinae in the ML tree, and (2) the grouping of *Haemomaster venezuelae* as the sister-taxon of *Ochmacanthus* in the ML tree, versus *H. venezuelae* as the sister-taxon of all other Stegophilinae in the MP tree. The parasitic members of the Trichomycteridae were recovered as monophyletic in both trees. Both Stegophilinae and Vandelliinae were each resolved as monophyletic, with strong support in both trees.

Relationships within both Stegophilinae and Vandelliinae were fully resolved, but only three of four intergeneric nodes (same nodes in both trees) were well supported. Although intrageneric taxon sampling was limited, we find no evidence to reject the monophyly of any of the genera included in the ingroup in this analysis. We find strong support for a suprageneric assemblage of stegophilines that includes *Homodiaetus, Megalocentor, Parastegophilus, Henonemus, Apomatoceros, Pseudostegophilus, Acanthopoma* and *Pareidon*. Nested within that clade is a less-inclusive clade (minus *Homodiaetus*) of the remaining stegophiline genera that was also well supported in both analyses. Both topologies recovered *Henonemus* as sister to *Apomatoceros*, with *Pseudostegophilus* representing the sister-group of that clade, and *Pareiodon* as sister to *Acanthopoma*; however, only the latter sister-group pair was well supported in all analyses. Relationships among Vandelliinae were strongly supported, wherein *Vandellia* was recovered as the sister-group of *Plectrochilus*, and that clade the sister-group to *Paravandellia*.

Relative branch lengths for those lineages receiving moderate to high levels of nodal support were rather uniform in the ML tree, with a few notable exceptions. The independent branches leading to *Paravandellia* and to *Haemomaster* are roughly four to seven times longer than the next longest branches. Except among species of *Ochmacanthus*, relatively few if any base-pair changes were observed between congeners. The uncorrected pair-wise average sequence divergence between species of *Ochmacanthus* was low (0.033 between two species of *O. orinoco*, 0.048 between *O. orinoco* t926 and *O.* sp.; 0.068 between *O. orinoco* t922 and *O.* sp.). Average overall uncorrected pair-wise sequence divergence among all included taxa was 0.189.

4. Discussion

4.1. Subfamilial relationships

Among the previously established hypotheses for the relationships among trichomycterid catfishes, our results are fully congruent with those statements of subfamilial relationships based on morphological data. Thus, our molecular data are in broad agreement with previous studies, beginning with that of Eigenmann (1918) and Myers (1944) and subsequently confirmed via explicit cladistic methodology by Baskin (1973) and de Pinna (1998), which have established the monophyly of the candiru subfamilies Stegophilinae and Vandelliinae. Baskin (1973) was first to propose a sister-group relationship between Stegophilinae and Vandelliinae. He recognized a monophyletic "Vandelliinae group" within the Trichomycteridae that also included Tridentinae as the sister-group of the candiru subfamilies, but he also argued that the evidence for the latter hypothesis was not particularly strong. In addition to parasitism as a synapomorphy for Stegophilinae plus Vandelliinae, Baskin (1973) proposed that these two subfamilies share derived conditions of the mesethmoid conua, maxillary and rictal barbels, restricted gill openings, and branchiostegal membrane lacking a free edge. Of these five characters supporting sister-group status for the candiru subfamilies, the latter three characters do not appear to involve morphological modifications associated with the evolution of parasitism. Regarding Baskin's characters, de Pinna (1998: Fig. 10) instead argued that the candiru

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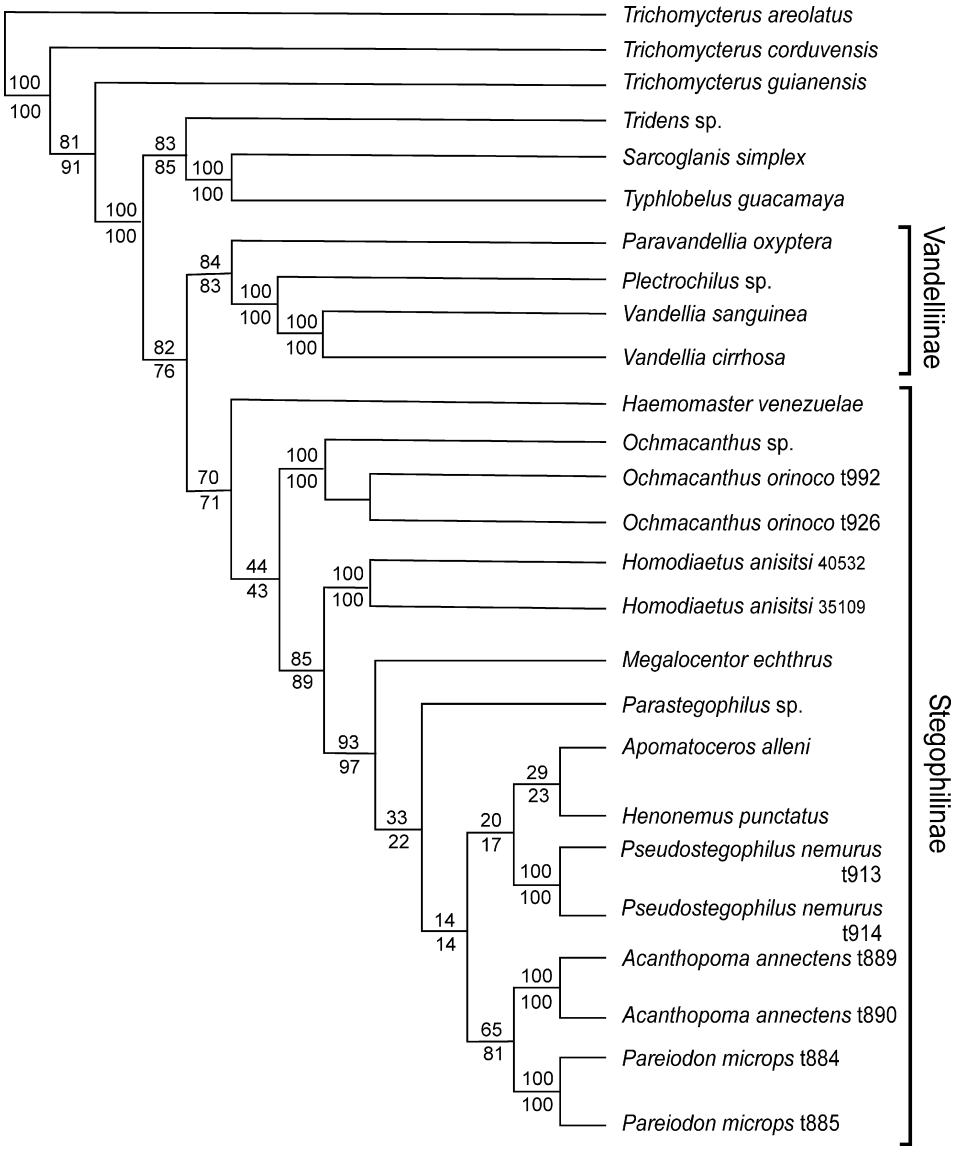

Fig. 2. Tree obtained from maximum parsimony analysis. Numbers above and below branches are bootstrap and jackknife values, respectively. Taxon identifiers as represented in Table 1.

subfamilies uniquely share only parasitism and the mesethmoid condition, but added the presence of a median premaxilla to the list of synapomorphies uniting Stegophilinae and Vandelliinae, at the exclusion of the Tridentinae. Our ML results are consistent with this hypothesis (although node support is relatively weak at the base of the tree), but our MP analysis are not and instead grouped the sole Tridentinae with Sarcoglanidinae plus Glanapteryginae with strong support (Fig. 2).

4.2. Relationships among Vandelliinae

Our study included three of the four genera of Vandelliinae; only *Paracanthopoma* was unavailable. Our results are nevertheless fully congruent with the two previous hypotheses of relationships among vandelliines based on morphological data. Schmidt (1993), in general agreement with Baskin (1973) and de Pinna and Britski (1991), discussed eleven synapomorphies in support of the monophyly of the Vandelliinae. The most notable of these is blood parasitism, the presence of a median premaxilla, and premaxillary dentition reduced in extent and individual teeth robust, sharp, and claw-like. Among vandelliines, both the morphological and molecular-based phylogenetic hypotheses place *Paravandellia* as the sister-group of a clade composed of *Plectrochilus* plus *Vandellia*. Our results involve strong node support for this scheme of relationships in both MP and ML analyses. Schmidt (1993) argued for sister-group status between *Paracanthopoma* and *Plectrochilus* plus *Vandellia* on the basis of loss of median premaxillary teeth, proximal end of premaxilla and ethmoid cornua both forked, reduced numbers of dentary teeth, and interopercular odontodes directed posterior.

4.3. Relationships among Stegophilinae

All members of the Stegophilinae share three derived features of the skull, jaw suspensorium, and pectoral skeleton (de Pinna, 1998: Fig. 10, node 10). Except for *Pareiodon microps*, all Stegophilinae also share a relatively wide mouth opening in the form of a crescent-shaped disk. Within Stegophilinae, Baskin (1973) recognized a "*Haemomaster*-group", consisting of *Haemomaster, Pareiodon, Stegophilus* (*Henonemus* considered a synonym), *Pseudostegophilus,*

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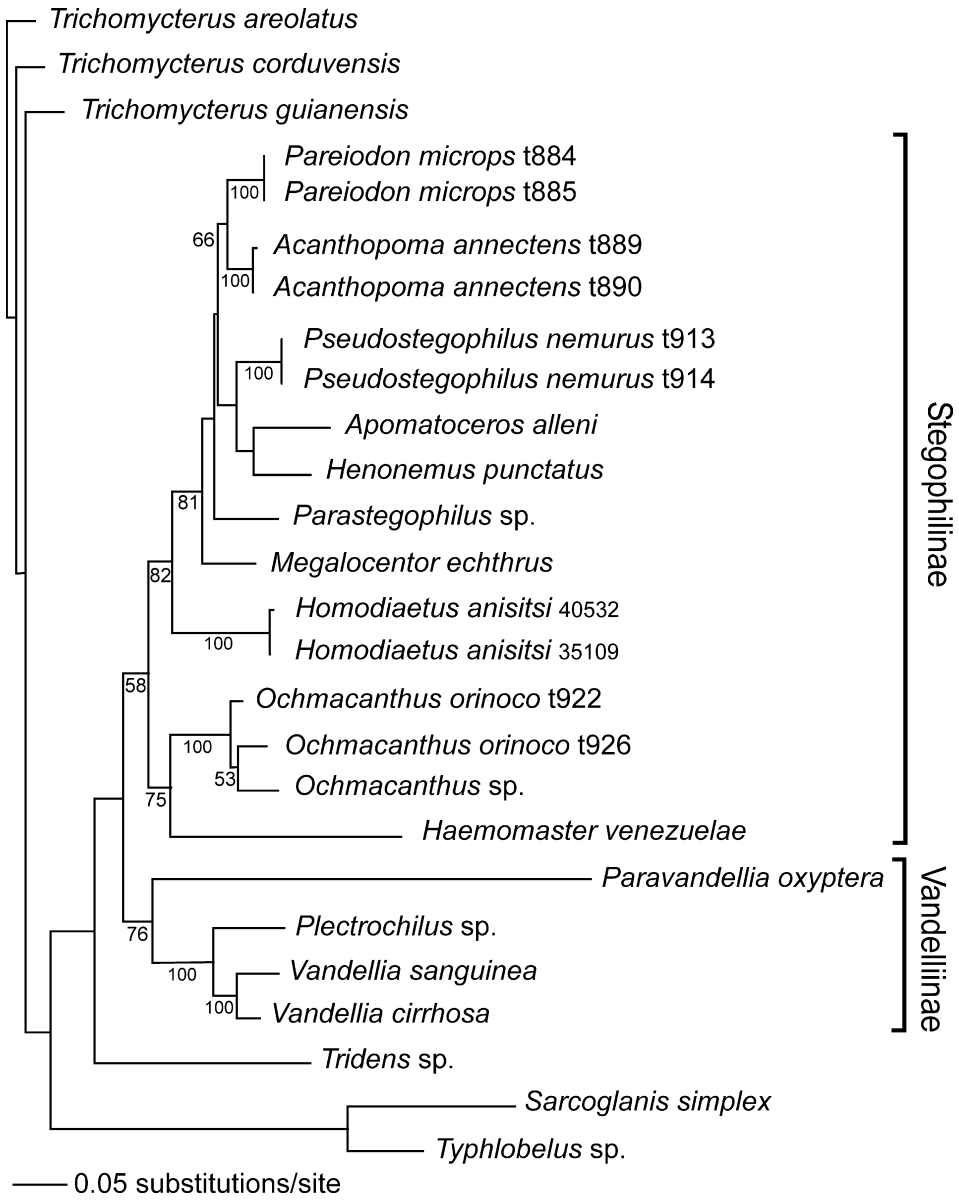

Fig. 3. Tree obtained from maximum likelihood analysis. Length of Ln likelihood = −16402.5057. Numbers above nodes indicate support at or above the 50% level in the majority-rule consensus tree. Taxon identifiers as represented in Table 1.

Homodiaetus, and *Apomatoceros*. *Ochmacanthus* was excluded from this suprageneric clade. Baskin (1973) further recognized an unresolved clade comprised of *Stegophilus*, *Pseudostegophilus*, *Homodiaetus*, and *Apomatoceros*, with *Pareiodon* as its sister-group and *Haemomaster* as the sister-group of the clade inclusive of *Pareiodon* (Fig. 1A). Our results are only partly congruent with the scheme of relationships among Stegophilinae proposed by Baskin (1973). Our MP results (Fig. 2) placed *Haemomaster* (not *Ochmacanthus*) as the sister-group of all other Stegophilinae, although the clade excluding *Haemomaster* was not strongly supported. In sharp contrast, our ML results placed *Haemomaster* as the sister-group of *Ochmacanthus* with moderately strong support (Fig. 3), with that clade as sister to all other Stegophilinae. In both our MP and ML analyses, *Pareiodon* was placed in a relatively terminal position within Stegophilinae phylogeny and the sister-group to *Acanthopoma* (the latter genus considered a synonym of *Henonemus* in Baskin (1973)), thereby confirming stegophiline membership for *Pareiodon*.

de Pinna and Britski (1991) erected the genus *Megalocentor* for a new species of scale-eating stegophiline trichomycterid. They argued for a sister-group relationship between *Megalocentor* and *Apomatoceros* on the basis of the unique absence in those genera of the posterior process of the opercle and the associated opercular odontodes, by the close approximation of the hypobranchials along the midline, and by the presence of small paired projections on the dorsal surface of the supraoccipital. Our results do not corroborate de Pinna and Britski's (1991) hypothesis of a sister-group relationship between *Megalocentor* and *Apomatoceros*. In contrast, our results place *Apomatoceros* as the sister-group of *Henonemus*, and strongly support *Megalocentor* as the sister-group of a large suprageneric assemblage that includes the former two genera. do Nascimiento and Provenzano (2006) recognized this same suprageneric assemblage inclusive of *Megalocentor*. Under this scheme of relationships, the derived features shared by *Megalocentor* and *Apomatoceros* that are not also observed in other stegophilines would be most parsimoniously interpreted as independently derived in the latter two genera.

do Nascimiento and Provenzano (2006) argued for a sister-group relationship between *Acanthopoma* and *Henonemus*, whereas

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our results place *Apomatoceros* as the sister-group of the latter. Characters cited by do Nascimiento and Provenzano (2006) in support of the former relationship involve the extension of the lateral line onto the dorsal-fin base, and the regular arrangement of neuromasts along the median caudal-fin rays extending posterior beyond the basal third of the rays. This is a general condition shared among members of the Stegophilinae and also observed in *Pareiodon, Pseudostegophilus, Parastegophilus,* and *Megalocentor*. Consequently, we do not agree that a sister-group relationship between *Acanthopoma* and *Henonemus* is supported by either the morphological or molecular evidence.

We find strong evidence for the continued recognition of *Pareidon microps* as a member of the Stegophilinae. Beginning with the classification of Eigenmann (1918), this very distinctive species was formerly placed in a separate subfamily, the Pareiodontinae. Baskin (1973) noted a number of shared features between *Pareiodon* and other Stegophilinae and further noted that continued recognition of the Pareiodontinae would render the Stegophilinae paraphyletic. Our results agree with Baskin's (1973) assessment, but differ in the relatively derived phylogenetic position of *Pareiodon* within Stegophilinae, rather than having a relatively basal position in Baskin's (1973) phylogeny. Our results place *Pareiodon* as the sister-group of *Acanthopoma* with strong node support in both the MP and ML trees. This finding is somewhat unexpected because *Pareiodon microps* lacks a number of morphological features that occur among all other Stegophilinae. Specifically, *Pareiodon microps* lacks the wide crescent-shaped and ventrally positioned mouth opening that characterizes all other scale feeding stegophilines. In *Pareiodon*, the mouth is small compare to other stegophilines, the opening not crescent-shaped, the mandibular symphysis forming a notch or cleft, resembling the condition observed in the Vandelliinae. In Tridentinae and all other stegophilines, the margin of the mandibular symphysis is either straight or smoothly convex, and does not form a notch or cleft at the midline. The rictal barbel of *Pareiodon* is elongate like that of Tridentinae. *Pareiodon microps* further lacks the median premaxilla and the premaxillary teeth are short and robust, rather than thin and elongate as in all other Stegophilinae. *Pareiodon* has two complete rows of premaxillary teeth and lacks teeth embedded in the fleshy upper lip, in contrast to the presence of four or more rows of premaxillary teeth plus three or more rows of teeth in the fleshy upper lip, as occur in all other stegophilines. Unlike other stegophilines, the eye of *Pareiodon microps* is much smaller, its diameter contained more than twice in snout length, whereas other stegophilines have very large eyes, contained less than once in snout length. The presence in *Pareiodon* of such a large number of plesiomorphic character states relative to other stegophilines would suggest a less-inclusive position for *Pareiodon* within stegophiline phylogeny and perhaps calls into question the exact nature of its feeding biology and status as a parasitic candiru. Baskin (1973:178) went so far as to suggest that "the distinctive feeding apparatus and small eyes of *Pareiodon* may be an indication that its feeding habits are substantially different from those of other stegophilines, and that the large eyes of other stegophilines are related to their parasitic feeding habits".

4.4. Evolution of parasitism

The available morphological and molecular evidence is consistent with the hypothesis that parasitism arose once within the Trichomycteridae. Although the two subfamilies of parasitic trichomycterids differ from one another in the mode and method of parasitic feeding, with vandelliines feeding exclusively on blood and stegophilines feeding on scales, skin, and mucus, parasitic feeding has long been regarded as a synapomorphy uniting these two subfamilies. A question posed by the enigmatic stegophiline *Pareidon microps* and its peculiar combination of plesiomorphic and apomorphic features, several of which are thought to be functionally related to the parasitic lifestyle, is whether parasitism among the candiru trichomycterids was unreversed over the course of their evolutionary history. Consideration of the peculiar morphology of *Pareiodon microps*, its small eyes, plesiomorphic jaws and dentition, and the absence of certain key features such as the median premaxilla, led both Baskin (1973) and de Pinna (1998) to regard the evolution of feeding in *Pareiodon* to have diverged substantially from that of other stegophilines. de Pinna (1998) further proposed that *Pareiodon* is specialized for feeding on carrion, an idea we believe can be traced to Goulding (1979, 1980), who reported anecdotal information on *Pareidon* feeding on larger fishes captured by fisherman. Miranda Ribeiro (1951:31) reported carnivory in *Pareiodon microps* taken from living catfishes near Manaus, Brazil. These statements taken together suggest that parasitic feeding habits were reversed in *Pareiodon* to the more ancestral predatory feeding mode that characterizes trichomycterid catfishes generally.

While the molecular results strongly indicate stegophiline membership for *Pareiodon microps*, both the morphological evidence and reports of it feeding behavior (anecdotal and otherwise) suggest that it is not parasitic, but rather is carnivorous, necrophagous, or both. Opportunities to directly observe the feeding of these small secretive fishes in the wild are extremely difficult, and we are aware of no reports of its feeding in the aquarium. Diet of other members of Stegophilinae are restricted to scales (Eigenmann and Allen, 1942; Baskin et al., 1980) and mucus (Roberts, 1972; Machado and Sazima, 1983; Winemiller and Yan, 1989) and is confirmed from examination of gut contents, but reports of scale feeding via direct behavioral observations on these fishes are few (Baskin et al., 1980; Sazima, 1983). Lepidophagous fishes in general do not share a large number of morphological specializations, apart from specialized dentition (Sazima, 1983; Petersen and Winemiller, 1997), suggesting that perhaps an evolutionary shift from carnivory or omnivory to scale feeding is not particularly constrained by the morphology of the feeding apparatus. Among stegophilines, perhaps only mouth shape and position can be added to the list of shared specializations for scale and mucus feeding. Consequently, evolutionary reversal of parasitism in *Pareiodon* is plausible and perhaps supported by the loss of those morphological features associated with scale and mucus feeding in other stegophilines.

There are numerous preserved specimens of *Pareiodon microps* in various museum collections, and our observations of some of this material confirm the occurrence of scale parasitism in that species. We observed the presence of scales inside the gut of *P. microps* ANSP181151, in association with sand and unidentifiable organic debris. Two individual scales (likely of an unidentifiable characiform species) were found in the stomach, one large (5 mm length) and partially digested, and another smaller (3 mm length) with pieces of integument remaining on bother surfaces, suggesting that it was more recently ingested. This observation would appear to confirm the occurrence of scale parasitism for *Pareiodon* at least to a degree, although the inability to identify other organic material in the gut of the specimens available to us does not deny the possibility that its diet is broader than that of other stegophilines. Specialized morphology and modified dentition is not required for lepidophagy in fishes, as scale feeding has been observed in generalized and typically omnivorous characids (Sazima, 1983). Because scale parasitism is indeed part of the feeding repertoire of *Pareiodon microps*, we would infer that facultative parasitism was unreversed in stegophiline trichomycterids and the absence of certain morphological features, such as median premaxilla, large eyes, broad ventral mouth equipped with numerous rows of fine rasping teeth, are independently derived in *Pareiodon* and not indicative of the evolutionary reversal of scale parasitism.

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Sazima (1983) contended that parasitic lepidophagy and mucophagy was the shared condition for the common ancestor of stegophilines and vandelliines, and our results are concordant with that contention, although for different reasons. Sazima (1983) did not base his contention on phylogenetic grounds and instead was likely influenced by the notion that facultative parasitism of stegophilines was an intermediate step in the evolutionary transition to obligate parasitism of vandelliines. Although optimization of the type of parasitism at the ancestral node for the clade inclusive of Stegophilinae plus Vandelliinae is ambiguous, we argue that Sazima (1983) was correct in regarding semiparasitism as the ancestral condition for the candirus because the condition in the immediate sister-group, the Tridentinae, is most similar to that in Stegophilinae. In Tridentinae, the mouth is like a generalist trichomycterine, characterized by long maxillary and rictal barbels, whereas in Stegophilinae the mouth is discoid and the barbels reduced. In Vandelliinae, there are a reduced number of teeth and individual teeth are larger. Tridentinae share with Stegophilinae the depressed head and numerous premaxillary teeth.

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