Molecular Phylogenetics and Evolution 109 (2017) 321–336

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Multilocus molecular phylogeny of the ornamental wood-eating catfishes (Siluriformes, Loricariidae, *Panaqolus* and *Panaque*) reveals undescribed diversity and parapatric clades

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ARTICLE INFO

Article history:
Received 21 September 2016
Revised 29 December 2016
Accepted 30 December 2016
Available online 5 January 2017

Keywords:
Neotropics
Undescribed species
L-numbers
Biogeography
Western Amazon
Introgression

ABSTRACT

Approximately two-dozen species in three genera of the Neotropical suckermouth armored catfish family Loricariidae are the only described fishes known to specialize on diets consisting largely of wood. We conducted a molecular phylogenetic analysis of 10 described species and 14 undescribed species or morphotypes assigned to the wood-eating catfish genus *Panaqolus*, and four described species and three undescribed species or morphotypes assigned to the distantly related wood-eating catfish genus *Panaque*. Our analyses included individuals and species from both genera that are broadly distributed throughout tropical South America east of the Andes Mountains and 13 additional genera hypothesized to have also descended from the most recent common ancestor of *Panaqolus* and *Panaque*. Bayesian and maximum likelihood analyses of two mitochondrial and three nuclear loci totaling 4293 bp confirmed respective monophyly of *Panaqolus*, exclusive of the putative congener '*Panaqolus*' *koko*, and *Panaque*. Members of *Panaqolus sensu stricto* were distributed across three strongly monophyletic clades: a clade of 10 generally darkly colored, lyretail species distributed across western headwaters of the Amazon Basin, a clade of three irregularly and narrowly banded species from the western Orinoco Basin, and a clade of 11 generally brown, broadly banded species that are widely distributed throughout the Amazon Basin. We erect new subgenera for each of these clades and a new genus for the morphologically, biogeographically and ecologically distinct species '*Panaqolus*' *koko*. Our finding that perhaps half of the species-level diversity in the widespread genus *Panaqolus* remains undescribed illustrates the extent to which total taxonomic diversity of small and philopatric, yet apparently widely distributed, Amazonian fishes may remain underestimated. Ranges for two *Panaqolus* subgenera and the genus *Panaque* overlap with the wood-eating genus *Cochliodon* in central Andean tributaries of the upper Amazon Basin, which appear to be a global epicenter of wood-eating catfish diversity.

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1. Introduction

Approximately two-dozen species in the Neotropical suckermouth armored catfish family Loricariidae are the only described fish species known to specialize on diets consisting largely of wood, which they scrape from dead submerged logs using specialized spoon-shaped teeth and force-maximizing jaws (Lujan et al., 2011; Lujan and Armbruster, 2011). Until recently, the most taxonomically comprehensive phylogenetic hypotheses for the Loricariidae (Armbruster, 2004, 2008) suggested that wood-eating evolved only twice: once in the genus *Cochliodon* Heckel in Kner 1854 and once in the genus *Panaque* Eigenmann & Eigenmann 1889 (both in the subfamily Hypostominae). However, multiple molecular phylogenetic studies (Hardman, 2005; Cramer et al., 2011; Lujan et al., 2015a) have found consistent evidence of paraphyly among putative subclades within the genus *Panaque sensu* Armbruster (2004). Specifically, these studies have found that wood-eating species in the genus *Panaqolus* Isbrücker & Schraml 2001 – then recognized as a subgenus of *Panaque* that was also

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http://dx.doi.org/10.1016/j.ympev.2016.12.040
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known as the *Panaque dentex* group (Schaefer and Stewart, 1993) – were distantly related to wood-eating species retained in the genus *Panaque*. Moreover, all three major wood-eating clades – *Cochliodon*, *Panaque*, and *Panaqolus* – were independently nested within clades consisting predominantly of non-wood eating species (Lujan et al., 2015a). This has led to the current hypothesis that wood-eating dietary specializations have evolved at least three times within the Loricariidae.

To further complicate our understanding of wood-eating fish evolution, the most recent and comprehensive molecular phylogenetic analysis of the subfamily Hypostominae (Lujan et al., 2015a) found little support for a close relationship between the enigmatic Guiana Shield species *Panaqolus koko* and other members of the genus *Panaqolus*. Since *P. koko* shares a putatively wood-eating jaw morphology with other members of *Panaqolus*, this left open the possibility that *P. koko* represented a fourth independent origin of wood-eating. Indeed, differences in the morphology of *P. koko* (Fisch-Muller et al., 2012) and its restricted geographic distribution in the upper Maroni River of French Guiana – which is well outside the range of any other known member of the genus *Panaqolus* – support the distinctiveness of this species. However, the exact placement of *P. koko* with respect to *Panaqolus sensu stricto* remains unresolved, and gut contents of *P. koko* have not previously been examined.

To date, various studies have examined the species-level taxonomy of *Cochliodon* (or the *Hypostomus cochliodon* group; Armbruster, 2003; Hollanda-Carvalho and Weber, 2004), *Panaqolus* (Schaefer and Stewart, 1993; Chockley and Armbruster, 2002), and *Panaque* (Lujan et al., 2010), but these studies have thus far been restricted to morphological analyses alone and only Schaefer and Stewart (1993) and Armbruster (2003) included phylogenetic hypotheses. Moreover, in the case of *Panaqolus*, Schaefer and Stewart (1993) examined only five of the 22 putative species or morphotypes examined in this study and one species (*Panaqolus dentex*) not examined in this study. We use molecular phylogenetic methods to investigate relationships within *Panaqolus* and *Panaque*, both of which are widespread within the Orinoco and Amazon basins, with *Panaque* also occurring west of the Andes in the Magdalena and Maracaibo basins. Adult *Panaque* can reach almost one meter in total length (60 cm SL) and are widely distributed in large river channel habitats (Lujan et al., 2010). In contrast, the genus *Panaqolus* rarely exceeds 15 cm SL and is most commonly encountered in medium-sized piedmont rivers of the Andes and the Brazilian and Guiana shields (Lujan et al., 2011, 2013a; Cramer and Rapp Py-Daniel, 2015; Cramer and de Sousa, 2016; Tan et al., 2016). Biogeographical patterns within these genera may therefore be highly complementary and informative of the hydrogeographic history of South America – particularly the vast expansion of piedmont habitats that likely occurred during Late Miocene tectonic uplifts of the Andes Mountains (Wesselingh and Hoorn, 2011).

Both *Panaqolus* and *Panaque* contain boldly patterned species that are highly coveted by aquarists and are collected and exported in large numbers to supply the global aquarium fish trade. Species in the genus *Panaqolus* are popularly known as ‘clown plecos’, and those in *Panaque* as ‘royal plecos’. Within these groups, vivid popular names convey some of the striking color diversity of these groups, including: ‘goldstripe pleco’, ‘tiger pleco’, ‘orange spot pleco’, ‘blue-eyed pleco’, ‘flash pleco’, and ‘watermelon pleco’. The interest of aquarists in distinctive color morphs has driven an intense search for new diversity in many rivers still unstudied by ichthyologists, leading to the discovery and exportation of dozens of new, distinctive color morphs and species unknown to science from throughout tropical South America. Unfortunately, voucher specimens from many of these populations remain scarce or absent in scientific collections, largely precluding their comprehensive examination and taxonomic description by ichthyologists.

In the absence of such descriptions, and in order to market and track distinctive populations and species-specific life history information without interfering with scientific taxonomy, aquarists have assigned each geographic color morph a unique alphanumeric code known as an L-number (i.e., Loricariidae numbers; Stawikowski, 1988; Dignall, 2014). To maximize the diversity of taxa included in this study, we worked with aquarists to obtain tissues from as many putatively undescribed species of *Panaqolus* and *Panaque* as possible, and have combined these in our analyses with all but one currently recognized *Panaqolus* species and all but two recognized *Panaque* species. Photos alone serve as vouchers for many of the undescribed ornamental species in our analyses, although the aquarists with whom we collaborated have themselves collected many of the morphotypes in our study and can therefore provide reliable locality data. Of course, this has not precluded us from also including a wide range of museum-vouchered specimens.

Our goals are to (1) examine phylogenetic relationships among a comprehensive set of both described and undescribed *Panaqolus* and *Panaque* species and color morphs, (2) examine morphological and biogeographical correlates of the well-supported clades that we find, (3) describe new subgenera for clades that have both strong statistical support and clearly diagnostic morphological and biogeographical patterns, and (4) to conduct a more robust reevaluation of the hypothesis that there has been a fourth independent origin of wood-eating in the enigmatic Maroni River species ‘*Panaqolus*’ *koko*, including both new phylogenetic and gut contents data. Even if our phylogenetic and taxonomic appraisal must remain incomplete pending the availability of scientifically vouchered specimens, we believe our results are still a valuable contribution to ongoing taxonomic and evolutionary studies and to conservation efforts of two largely unstudied yet diverse, widespread and commercially exploited fish groups.

2. Methods

2.1. Taxon sampling

We sampled most densely within the three tribe-level clades Hypostomini, *Peckoltia* Clade, and *Hemiancistrus* Clade (*sensu* Lujan et al., 2015a; Table 1), which respectively contain the wood-eating genera *Cochliodon*, *Panaqolus*, and *Panaque*, and comprise a single strongly monophyletic clade nested within the subfamily Hypostominae (Lujan et al., 2015a). We included in our analyses all but one currently valid species of *Panaqolus* (Fig. 1; missing *Panaqolus dentex* from the Huallaga River basin in Peru), all but two currently valid species of *Panaque* (missing *Panaque suttonorum* from the Lake Maracaibo basin in Venezuela and *P. titan* from the Napo River basin in Ecuador), and representatives of every other currently valid genus hypothesized to have descended from the most recent common ancestor of *Panaqolus* and *Panaque* (n = 14).

We also sampled broadly outside of these clades (Table 1), including representatives of five of six other tribe-level clades within Hypostominae (*sensu* Lujan et al., 2015a), four of five other loricariid subfamilies (Delturinae, Neoplecostominae, Loricariinae, and Lithogeninae) and three of five other families within the suborder Loricarioidei (Astroblepidae, Callichthyidae, and Trichomycteridae).

2.2. Tissue and DNA sources

Newly generated sequence data (Table 1) were obtained from tissue samples or DNA extracts collected by the authors or provided by the Academy of Natural Sciences of Drexel University in

Table 1
Loci sequenced, voucher catalog number and country and river drainage of origin for the tissue samples analyzed in this study. Boxes demarcate sequences concatenated from conspecific individuals. Taxa are listed in indented groupings according to family, subfamily, and tribe (if described) or tribe-level clade (if undescribed), with tribe-level clades following Lujan et al. (2015a). 'Type specimen' indicates that a voucher was either part of the type series for that species or was collected from at or near the type locality.

Taxa	Tissue #	Type specimen	Type species	# of loci	16S	Cytb	RAG1	RAG2	Myh6	Voucher Cat #	Country	Drainage
Trichomycteridae												
Vandellia sp.	V5509			2	X		X		$\boxed{\text{X}}$	AUM 43867	Venezuela	Orinoco River
Callichthyidae												
Corydoradinae												
Corydoras panda	T12932			4	X	X	X	X		ROM 94924	unknown	
Corydoras stenocephalus	T12839			5	X	X	X	X	X	ROM 90345	Bolivia	Mamoré River
Astroblepidae												
Astroblepus sp.	CH146			5	X	X	X	X	X	MUSM uncataloged	Peru	Huallaga River
Astroblepus sp.	CH161			5	X	X	X	X	X	MUSM uncataloged	Peru	Huallaga River
Loricariidae												
Lithogininae												
Lithogenes villosus	T17140			5	X	X	X	X	X	ROM 95075	Guyana	Potaro River
Lithogenes villosus	T9048			4	X	X		X	X	AUM 62934	Guyana	Potaro River
Delturinae												
Hemipsilichthys gobio	T14765			4	X	X	X		X	MCP 42452	Brazil	Pirapetinga River
Loricariinae												
Harttiini												
Cteniloricaria platystoma	T06287			4	X	X	X	X		ROM 85921	Guyana	Essequibo River
Farlowellini												
Farlowella vittata	V5314			4	X	X	X	X		AUM 42218		
Sturisoma cf. *monopelte*	T06853			5	X	X	X	X	X	ROM 86207	Guyana	Rupununi Rive
Loricariini												
Rineloricaria fallax	G5063			5	X	X	X	X	X	AUM 44444	Guyana	Essequibo River
Hypoptopomatinae												
Neoplecostomini												
Pareiorhaphis steindachneri	Genbank									Genbank		
Hypostominae												
Chaetostoma Clade												
Chaetostoma bifurcum	T13602	*		5	X	X	X	X	X	ROM 93687	Ecuador	Esmeraldas River
Chaetostoma dermorhynchum	T14258			5	X	X	X	X	X	ROM 93656	Ecuador	Pastaza River
Chaetostoma fischeri	T9026	*		5	X	X	X	X	X	STRI 7604	Panama	Chagres River
Chaetostoma n.sp. Meta L445	T12930			5	X	X	X	X	X	ROM 94925	Colombia	Meta River
Chaetostoma vasquezi	T09945	*		5	X	X	X	X	X	AUM 53812	Venezuela	Caura River
Dolichancistrus carnegiei	6650			4	X	X	X	X		ANSP 189598	Colombia	Magdalena River
Transancistrus santarosensis	T13980	*		5	X	X	X	X	X	ROM 93798	Ecuador	Santa Rosa River
Ancistrini												
Ancistrus clementinae	T13829	*		5	X	X	X	X	X	ROM 93737	Ecuador	Guayas River
Ancistrus ranunculus	B1500	*		5	X	X	X	X	X	ANSP 199525	Brazil	Xingu River
Corymbophanes kaiei	T12637			5	X	X	X	X	X	ROM 89856	Guyana	Potaro River
Dekeyseria pulchra	V5296			5	X	X	X	X	X	AUM 44110	Venezuela	Atabapo River
Dekeyseria scaphirhyncha	T09540			5	X	X	X	X	X	AUM 54309	Venezuela	Ventuari River
Guyanancistrus brevispinis	SU01-121	*	†	5	X	X	X	X	X	MHNG 2621.073	Suriname	Nickerie River
Hopliancistrus tricornis	T9017		†	5	X	X	X	X	X	AUM 39853	unknown	
Lasiancistrus schomburgkii	P6125			5	X	X	X	X	X	AUM 45548	Peru	Marañon River
Lasiancistrus tentaculatus	T09686			5	X	X	X	X	X	AUM 53895	Venezuela	Ventuari River
Lithoxancistrus orinoco	T09663		†	5	X	X	X	X	X	AUM 54439	Venezuela	Ventuari River
Lithoxancistrus yekuana	T9004	*		5	X	X	X	X	X	AUM 39473	Venezuela	Ventuari River
Neblinichthys brevibracchium	T06068	*		5	X	X	$\boxed{\text{X}}$	X	X	ROM 83692	Guyana	Mazaruni River
Neblinichthys echinasus	T06066	*		4	X	X	X	X		ROM 83692	Guyana	Mazaruni River
Paulasquama callis	T06189	*	†	5	X	X	X	X	X	ROM 83784	Guyana	Mazaruni River
Pseudolithoxus dumus	T09512	*		5	X	X	X	X	X	ANSP 190757	Venezuela	Ventuari River
Pseudolithoxus tigris	T09376	*	†	5	X	X	X	X	X	AUM 57674	Venezuela	Orinoco River
Pseudolithoxus stearleyi	V5533	*		5	X	X	X	X	X	AUM 43872	Venezuela	Soromoni River
Pseudancistrus Clade												
Pseudancistrus nigrescens	G5942	*		5	X	X	X	X	X	AUM 45299	Guyana	Essequibo River
Lithoxus Clade												
Exastilithoxus fimbriatus	V049	*	†	5	X	X	X	X	X	AUM 36632	Venezuela	Caroni River
Exastilithoxus n.sp. Ventuari	T09667	*		5	X	X	X	X	X	AUM 54450	Venezuela	Ventuari River
Lithoxus jantjae	T9019			5	X	X	X	X	X	AUM 39475	Venezuela	Ventuari River
Lithoxus lithoides	T412		†	4	X		X	X	X	AUM 37922	Guyana	Essequibo River

(continued on next page)

Lithoxus pallidimaculatus	T9021			5	X	X	X	X	X	AUM 50410	Suriname	Maroni River
Acanthicus Clade												
Acanthicus hystrix	5900			5	X	X	X	X	X	UFRO-ICT uncatalogued	Brazil	Madeira River
Leporacanthicus triactis	T09826	*		5	X	X	X	X	X	AUM 54030	Venezuela	Ventuari River
Megalancistrus barae	T9045			5	X	X	X	X	X	photo only	Brazil	São Francisco
Pseudacanthicus leopardus	G5089	*		5	X	X	X	X	X	AUM 44440	Guyana	Essequibo River
Hemiancistrus												
'Baryancistrus' beggini	T09392	*		5	X	X	X	X	X	AUM 54990	Venezuela	Orinoco River
'Baryancistrus' beggini	T09393			5	X	X	X	X	X	AUM 54990	Venezuela	Orinoco River
'Baryancistrus' beggini	V5424			4	X	X		X	X	AUM 42145	Venezuela	Orinoco River
'Baryancistrus' demantoides	T09361	*		5	X	X	X	X	X	ROM 93339	Venezuela	Orinoco River
'Baryancistrus' demantoides	T09334			5	X	X	X	X	X	ROM 93339	Venezuela	Orinoco River
'Baryancistrus' demantoides	V026			5	X	X	X	X	X	AUM 39228	Venezuela	Orinoco River
'Hemiancistrus' guahiborum	V096			3	X	$\boxed{\text{X}}$	X		X	AUM 39239	Venezuela	Orinoco River
'Hemiancistrus' guahiborum	V097			2	X		X			AUM 39239	Venezuela	Orinoco River
'Hemiancistrus' guahiborum	T09949			3	X	X			X	ROM 94545	Venezuela	Orinoco River
'Hemiancistrus' guahiborum	T09400			3	X		X		X	AUM 57677	Venezuela	Orinoco River
'Hemiancistrus' subviridis	T09437	*		5	X	X	X	X	X	AUM 54456	Venezuela	Orinoco River
'Hemiancistrus' subviridis	T09609			5	X	X	X	X	X	ROM 93588	Venezuela	Orinoco River
'Hemiancistrus' subviridis	T09248			3	X	X			X	ROM 94149	Venezuela	Orinoco River
Baryancistrus chrysolomus	B1505	*		4	X	X	X		X	INPA uncataloged	Brazil	Xingu River
Baryancistrus chrysolomus	B1506	*		4	X	X	X		X	INPA uncataloged	Brazil	Xingu River
Baryancistrus niveatus	HLF1288		†	5	X	X	X	X	X	INPA uncataloged	Brazil	Iriri River
Baryancistrus niveatus	HLF1405			5	X	X	X	X	X	INPA uncataloged	Brazil	Iriri River
Baryancistrus niveatus	B1984			4	X	X	X		X	INPA uncataloged	Brazil	Xingu River
Baryancistrus niveatus	B1985			4	X	X	X		X	ANSP 199623	Brazil	Xingu River
Baryancistrus n.sp. L142	T17420			5	X	X	X	X	X	ROM 95253	Brazil	Tapajós River
Baryancistrus xanthellus	B1490	*		5	X	X	X	X	X	ANSP 199528	Brazil	Xingu River
Baryancistrus xanthellus	B2163			5	X	X	X	X	X	ANSP 193086	Brazil	Xingu River
Baryancistrus xanthellus	B2064			3	X	X			X	INPA uncataloged	Brazil	Xingu River
Hemiancistrus medians	6948	*	†	5	X	X	X	X	X	ANSP 187122	Suriname	Marowijne River
Panaque cf. *armbrusteri*	B2189			4	X	X	X		X	ANSP 193093	Brazil	Xingu River
Panaque cf. *armbrusteri*	B2188			3	X	X	X			ANSP 193093	Brazil	Xingu River
Panaque cf. *armbrusteri*	BR936			5	X	X	X	X	X	MNRJ 15209	Brazil	Tocantins River
Panaque cf. *armbrusteri*	BR1024			4	X	X	X	X		MNRJ 15238	Brazil	Tocantins River
Panaque bathyphilus	P6269			5	X	X	X	X	X	AUM 45503	Peru	Marañon River
Panaque bathyphilus	8241			4	X	X		X	X	UFRO-ICT 17666	Brazil	Madeira River
Panaque bathyphilus	6000			5	X	X	X	X	X	UFRO-ICT 13109	Brazil	Madeira River
Panaque bathyphilus	4172			4	X	X	X	X		UFRO-ICT 6383	Brazil	Madeira River
Panaque bathyphilus	P6279			5	X	X	X	X	X	AUM 45503	Peru	Marañon River
Panaque bathyphilus	T07132			5	X	X	X	X	X	ROM 88352	Peru	Iquitos
Panaque bathyphilus	T07191			3		X	X	X		ROM 88920	Peru	Iquitos
Panaque cochliodon	T14628	*		5	X	X	X	X	X	photo only	Colombia	Magdalena River
Panaque nigrolineatus	T09018	*	†	5	X	X	X	X	X	AUM 53764	Venezuela	Apure River
Panaque nigrolineatus	T10799			5	X	X	X	X	X	ROM 91268	Colombia	Meta River
Panaque n.sp. Ariari	T17418			5	X	X	X	X	X	ROM 95251	Colombia	Ariari River
Panaque schaeferi	T9023	*		5	X	X	X	X	X	INHS 55408	Peru	Solimões River
Panaque schaeferi	6651			5	X	X	X	X	X	UFRO-ICT 13162	Brazil	Madeira River
Panaque schaeferi	6003			3	X	X	X			UFRO-ICT 13146	Brazil	Madeira River
Panaque schaeferi	5997			4	X	X	X		X	UFRO-ICT 13152	Brazil	Madeira River
Panaque schaeferi	6649b			5	X	X	X	X	X	UFRO-ICT 13162	Brazil	Madeira River
Panaque schaeferi	6002			2	X			X		UFRO-ICT 13152	Brazil	Madeira River
Panaque schaeferi	T9043			5	X	X	X	X	X	MUSM 39426	Brazil	Purus River
Panaque schaeferi	T9044			3	X	X	X			MUSM 39427	Brazil	Purus River
Parancistrus nudiventris	B1526	*		5	X	X	X	X	X	ANSP 199530	Brazil	Xingu River
Parancistrus nudiventris	B1520			5	X	X	X	X	X	ANSP 199529	Brazil	Xingu River
Parancistrus nudiventris	B2086			5	X	X	X	X	X	ANSP 193002	Brazil	Xingu River
Parancistrus nudiventris	B2052			5	X	X	X	X	X	INPA uncatalogued	Brazil	Xingu River
Parancistrus nudiventris	B2050			4	X	X	X		X	ANSP 193072	Brazil	Xingu River
Spectracanthicus punctatissimus	B1496		†	5	X	X	X	X	X	ANSP 199539	Brazil	Xingu River
Spectracanthicus punctatissimus	B1495			5	X	X	X	X	X	ANSP 199539	Brazil	Xingu River
Spectracanthicus punctatissimus	B2061			4	X	X	X		X	ANSP 193020	Brazil	Xingu River
Spectracanthicus punctatissimus	B2059			5	X	X	X	X	X	ANSP 193020	Brazil	Xingu River
Spectracanthicus punctatissimus	B2068			4	X	X	X		X	ANSP 193013	Brazil	Xingu River
Spectracanthicus punctatissimus	B2069			5	X	X	X	X	X	ANSP 193013	Brazil	Xingu River
Spectracanthicus punctatissimus	B2080			4	X	X	X		X	ANSP 193013	Brazil	Xingu River

Spectracanthicus punctatissimus	B1521			4	X	X	X		X	ANSP 199538	Brazil	Xingu River
Spectracanthicus punctatissimus	B2151			5	X	X	X	X	X	ANSP 193076	Brazil	Xingu River
Spectracanthicus punctatissimus	B2174			5	X	X	X	X	X	ANSP 193092	Brazil	Xingu River
Spectracanthicus punctatissimus	B1980			4	X	X	X		X	ANSP 199620	Brazil	Xingu River
Spectracanthicus punctatissimus	B1979			3	X		X		X	ANSP 199624	Brazil	Xingu River
Spectracanthicus zuanoni	B1982			4	X	X	X		X	ANSP 199619	Brazil	Xingu River
Spectracanthicus zuanoni	B2172			5	X	X	X	X	X	ANSP 193095	Brazil	Xingu River
Spectracanthicus zuanoni	B1515			4	X	X	X		X	ANSP 199537	Brazil	Xingu River
Spectracanthicus zuanoni	B2116			2		X	X			ANSP 193047	Brazil	Xingu River
Hypostomini												
'Hemiancistrus' meizospilos	T14750			4		X	X	X	X	MCP 40168	Brazil	Chapecó River
'Hemiancistrus' punctulatus	T14754			3		X	X		X	MCP 40946	Brazil	Carreiro River
'Hemiancistrus' votuoro	T14766			3	X	X	X			MCP 44181	Brazil	Passo Fundo River
Hypostomus (Coch.) macushi	T07038	*		5	X	X	X	X	X	ROM 85939	Guyana	Essequibo River
Hypostomus (Coch.) pyrineusi	T10377			5	X	X	X	X	X	AUM 51394	Peru	Madre de Dios River
Hypostomus (Coch.) taphorni	T07074	*		5	X	X	X	X	X	ROM 86352	Guyana	Essequibo River
Hypostomus (Hyp.) rhantos	T09530	*		5	X	X	X	X	X	AUM 54306	Venezuela	Ventuari River
Pterygoplichthys gibbiceps	P4893			5	X	X	X	X	X	AUM 42131	Venezuela	Casiquiare River
Peckoltia Clade												
'Hemiancistrus' landoni	T13601			4	X	X	X	X		ROM 93688	Ecuador	Esmeraldas Rivr
'Hemiancistrus' landoni	T13836	*		5	X	X	X	X	X	ROM 93738	Ecuador	Guayas River
'Hemiancistrus' landoni	T13837			5	X	X	X	X	X	ROM 93738	Ecuador	Guayas River
'Spectracanthicus' immaculatus	T1385	*		4	X	X	$\boxed{X}$	X	X	ANSP 194670	Brazil	Xingu River (mouth)
'Spectracanthicus' immaculatus	T1387	*		4	X	X	$\boxed{X}$	X	X	ANSP 194670	Brazil	Xingu River (mouth)
Ancistomus feldbergae	B2071			5	X	X	X	X	X	INPA uncatalogued	Brazil	Iriri River
Ancistomus feldbergae	B2072			5	X	X	X	X	X	ANSP 193012	Brazil	Iriri River
Ancistomus feldbergae	B2178			5	X	X	X	X	X	ANSP 193088	Brazil	Bacaja River
Ancistomus feldbergae	B2181			5	X	X	X	X	X	ANSP 193088	Brazil	Bacaja River
Ancistomus snethlageae	T17383			5	X	X	X	X	X	ROM 95302	unknown	
Aphanotorulus emarginatus	B2046			4		X	X	X	X	ANSP 199645	Brazil	Xingu River
Aphanotorulus squalinus	T09528			5	X	X	X	X	X	AUM 54305	Venezuela	Ventuari River
Hypancistrus contradens	T09355	*		5	X	X	X	X	X	ANSP 190815	Venezuela	Orinoco River
Hypancistrus contradens	T09407			4	X	X	X	X		AUM 54993	Venezuela	Orinoco River
Hypancistrus contradens	V062			5	X	X	X	X	X	AUM 39241	Venezuela	Orinoco River
Hypancistrus debilittera	T09279	*		5	X	X	X	X	X	AUM 53528	Venezuela	Orinoco River
Hypancistrus debilittera	T09280			5	X	X	X	X	X	ROM 94150	Venezuela	Orinoco River
Hypancistrus furunculus	T09278			4	X	X	X	X		ROM 94150	Venezuela	Orinoco River
Hypancistrus furunculus	T09440			5	X	X	X	X	X	AUM 54463	Venezuela	Orinoco River
Hypancistrus furunculus	V028	*		5	X	X	X	X	X	AUM 39225	Venezuela	Orinoco River
Hypancistrus lunaorum	T09562	*		5	X	X	X	X	X	ROM 92224	Venezuela	Orinoco River
Hypancistrus lunaorum	V118			5	X	X	X	X	X	AUM 39247	Venezuela	Orinoco River
Hypancistrus n.sp. Xingu L174	B2141			5	X	X	X	X	X	ANSP 193084	Brazil	Xingu River
Hypancistrus n.sp. Xingu L174	B2142			5	X	X	X	X	X	ANSP 193084	Brazil	Xingu River
Hypancistrus vandragti	T09307			5	X	X	X	X	X	AUM 54408	Venezuela	Orinoco River
Hypancistrus vandragti	T09367			4	X	X	X	X		AUM 54408	Venezuela	Orinoco River
Hypancistrus vandragti	T09490	*		5	X	X	X	X	X	ROM 93324	Venezuela	Ventuari River
Isorineloricaria spinosissima	T13692	*	†	5	X	X	X	X	X	ROM 93722	Ecuador	Guayas River
Isorineloricaria spinosissima	T13694			3	X			X	X	ROM 93722	Ecuador	Guayas River
Isorineloricaria spinosissima	T13764			4	X	X	X	X		ROM 93065	Ecuador	Guayas River
Panafilus albivermis	P27						X	X		photo only	Peru	
Panafilus cf. *albivermis*	PE08-754			5	X	X	X	X	X	MHNG 2710.077	Peru	Ucayali River
Panafilus cf. *albivermis*	PE08-842			5	X	X	X	X	X	MHNG 2710.083	Peru	Ucayali River
Panafilus cf. *albivermis*	PE08-755			5	X	X	X	X	X	MHNG 2710.077	Peru	Ucayali River
Panafilus albomaculatus	P18			3		X	X	X		photo only	Peru	Marañon River
Panafilus albomaculatus	P6121			5	X	X	X	X	X	AUM 45502	Peru	Marañon River
Panafilus albomaculatus	P6147			5	X	X	X	X	X	AUM 45502	Peru	Marañon River
Panafilus n.sp. Huallaga L351	P17			5	X	X	X	X	X	photo only	Peru	Huallaga River
Panafilus n.sp. Madeira	6376			4	X	X		X	X	UFRO-ICT 5497	Brazil	Madeira River
Panafilus n.sp. Madeira	6684			4	X	X		X	X	UFRO-ICT 5497	Brazil	Madeira River
Panafilus n.sp. Moa L453	P25			2	X				X	photo only	Brazil	Moa River
Panafilus n.sp. Napo L466	P14			4	X	X	X	X		photo only	Peru	Napo River
Panafilus n.sp. Ucayali L425	P19			5	X	X	X	X	X	photo only	Peru	Ucayali River
Panafilus n.sp. Ucayali L425	P20			3	X	X	X			photo only	Peru	Ucayali River
Panafilus nix	4170			5	X	X	X	X	X	UFRO-ICT 6384	Brazil	Madeira River
Panafilus nix	5999			5	X	X	X	X	X	UFRO-ICT 13132	Brazil	Madeira River
Panafilus nix	7645			4		X	X	X	X	UFRO-ICT 19646	Brazil	Madeira River

Panafilus nix	7647			4	X	X	X		X	INPA 39606	Brazil	Madeira River
Panafilus nocturnus	MUS773			4	X	X		X	X	MHNG 2726.063	Bolivia	Purus River
Panafilus nocturnus	P26			3	X	X		X		photo only	Peru	Huallaga River
Panafilus nocturnus	P6126	*		4	X	X	X	X	X	AUM 45500	Peru	Marañon River
Panafilus nocturnus	P6127			3	X		X	X		AUM 45500	Peru	Marañon River
Panaqoco maccus	T09009	*		5	X	X	X	X	X	AUM 53768	Venezuela	Guanare River
Panaqoco maccus	T09016			5	X	X	X	X	X	ROM 94129	Venezuela	Guanare River
Panaqoco n.sp. Orinoco L448	P22			2	X	X				photo only	Venezuela	Orinoco River
Panaqoco n.sp. Tomo L465	P29			1	X					photo only	Venezuela	Tomo River
Panaqolus changae	T660						X	X		ANSP 181097	Peru	Itaya River
Panaqolus claustellifer	G07258			2			X	X		AUM 47717	Guyana	Tacutu River
Panaqolus claustellifer	G5183	*		5	X	X	X	X	X	AUM 44721	Guyana	Tacutu River
Panaqolus claustellifer	P16			3	X	X	X			photo only	Guyana	Tacutu River
Panaqolus gnomus	P6128	*	†	5	X	X	X	X	X	AUM 45501	Peru	Marañon River
Panaqolus gnomus	P6129			5	X	X	X	X	X	AUM 45501	Peru	Marañon River
Panaqolus n.sp. Amazon L397	P13			3	X	X	X			photo only	Brazil	
Panaqolus n.sp. Curua Una	P24			1		X				photo only	Brazil	Curua-Una River
Panaqolus n.sp. Itaya L459	P12			4	X	X	X	X		photo only	Peru	Itaya River
Panaqolus n.sp. Negro L169	P21			1	X					photo only	Brazil	Negro River
Panaqolus n.sp. Tocantins L002	P15			5	X	X	X	X	X	photo only	Brazil	Tocantins River
Panaqolus n.sp. Ucayali L206	P11			4	X	X	X	X		photo only	Peru	Ucayali River
Panaqolus n.sp. Ucayali L206	PE08-749			5	X	X	X	X	X	MHNG 2710.076	Peru	Ucayali River
Panaqolus n.sp. Ucayali L206	PE08-752			4	X	X	X	X		MHNG 2710.077	Peru	Ucayali River
Panaqolus n.sp. Ucayali L206	PE08-839			4	X	X	X	X		MHNG 2710.082	Peru	Ucayali River
Panaqolus n.sp. Ucayali L206	PE08-840			5	X	X	X	X	X	MHNG 2710.082	Peru	Ucayali River
Panaqolus n.sp. Xingu L398	598			5	X	X	X	X	X	LIA_M 0598	Brazil	Xingu River
Panaqolus n.sp. Xingu L398	P23			2	X	X				photo only	Brazil	Xingu River
Panaqolus purusiensis	4652			2	X				X	UFRO-ICT17720	Brazil	Purus River
Panaqolus purusiensis	4654			3	X			X	X	UFRO-ICT17720	Brazil	Purus River
Peckoltia furcata	P6200			5	X	X	X	X	X	AUM 45593	Peru	Marañon River
Peckoltia braueri	T06465	*		5	X	X	X	X	X	ROM 86240	Guyana	Takutu River
Peckoltia compta	T10774			3	X		X		X	ROM 91263	Brazil	Tapajós River
Peckoltia compta	T10775			5	X	X	X	X	X	ROM 91263	Brazil	Tapajós River
Peckoltia lineola	T09831	*		5	X	X	X	X	X	AUM 54033	Venezuela	Ventuari River
Peckoltia lineola	T09832			5	X	X	X	X	X	ROM 94334	Venezuela	Ventuari River
Peckoltia lujani	T09143			5	X	X	X	X	X	ANSP 190894	Venezuela	Orinoco River
Peckoltia lujani	T09144			5	X	X	X	X	X	ROM 93352	Venezuela	Orinoco River
Peckoltia n.sp. Madeira L210	T14753			2			X		X	MCP 35628	Brazil	Madeira River
Peckoltia n.sp. Orinoco L147	T17381			4	X		X	X	X	ROM 95301	Venezuela	Orinoco River
Peckoltia pankimpuju	P6233	*		4	X	X	X	X		AUM 45595	Peru	Marañon River
Peckoltia relictum	CH157			5	$\boxed{\text{X}}$	X	X	X	X	MUSM 44256	Peru	Huallaga River
Peckoltia relictum	P6099	*		4	X	X	X	X		AUM 45531	Peru	Marañon River
Peckoltia relictum	P6100	*		4	X	X	X	X		AUM 45531	Peru	Marañon River
Peckoltia sabaji	B1969			5	X	X	X	X	X	ANSP 199615	Brazil	Xingu River
Peckoltia sabaji	B2175			4	X	X	X		X	ANSP 193089	Brazil	Xingu River
Peckoltia sabaji	T09602			5	X	X	X	X	X	ANSP 191152	Venezuela	Orinoco River
Peckoltia sabaji	T09719			5	X	X	X	X	X	AUM 53577	Venezuela	Orinoco River
Peckoltia sabaji	T12928			4	X	X	X	X		photo only	unknown	
Peckoltia vittata	8973			1		X				UFRO-ICT8282	Brazil	Madeira River
Peckoltia vittata	10514		†	3	X	X			X	photo only	Brazil	Madeira River
Peckoltia vittata	B1507			4	X	X	X	X		ANSP 199531	Brazil	Xingu River
Peckoltia vittata	B2152	*	†	5	X	X	X	X	X	ANSP 193078	Brazil	Xingu River
Peckoltia wernekei	T09533			5	X	X	X	X	X	AUM 54314	Venezuela	Orinoco River
Peckoltia wernekei	T09534			5	X	X	X	X	X	AUM 54314	Venezuela	Orinoco River
Peckoltichthys bachi	9220			3	X	X	X			UFRO-ICT17328	Brazil	Madeira River
Peckoltichthys bachi	P6196			3	X		X	X		AUM 45592	Peru	Marañon River
Peckoltichthys bachi	P6254		†	5	X	X	X	X	X	AUM 45592	Peru	Marañon River
Scobinancistrus aff. *pariolispos* L082	B1518			4		X	X	X	X	ANSP 199534	Brazil	Xingu River
Scobinancistrus aff. *pariolispos* L082	B2113			5	X	X	X	X	X	ANSP 193045	Brazil	Xingu River
Scobinancistrus aureatus	B2115			3		X	X		X	ANSP 193044	Brazil	Xingu River
Scobinancistrus aureatus	B2153			4	X	X	X		X	ANSP 193075	Brazil	Xingu River
Scobinancistrus aureatus	B2193			4	X	X	X	X	X	ANSP 193094	Brazil	Xingu River
Scobinancistrus pariolispos	B2088		†	4	X	X	X		X	ANSP 193006	Brazil	Xingu River
Pseudoqolus koko	GF00-115	*		4	X	X	X	X		MNHN 2011-0013	French Guiana	Maroni River

Philadelphia, PA, USA (ANSP), the Auburn University Museum Fish Collection in Auburn, AL, USA (AUM), the Coleções Zoológicas e Laboratórios Integrados, Universidade Federal de Rondônia, Porto Velho, Brazil (UFRO-ICT), the Muséum d'histoire naturelle of the City of Geneva, Switzerland (MHNG), the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos in Lima, Peru

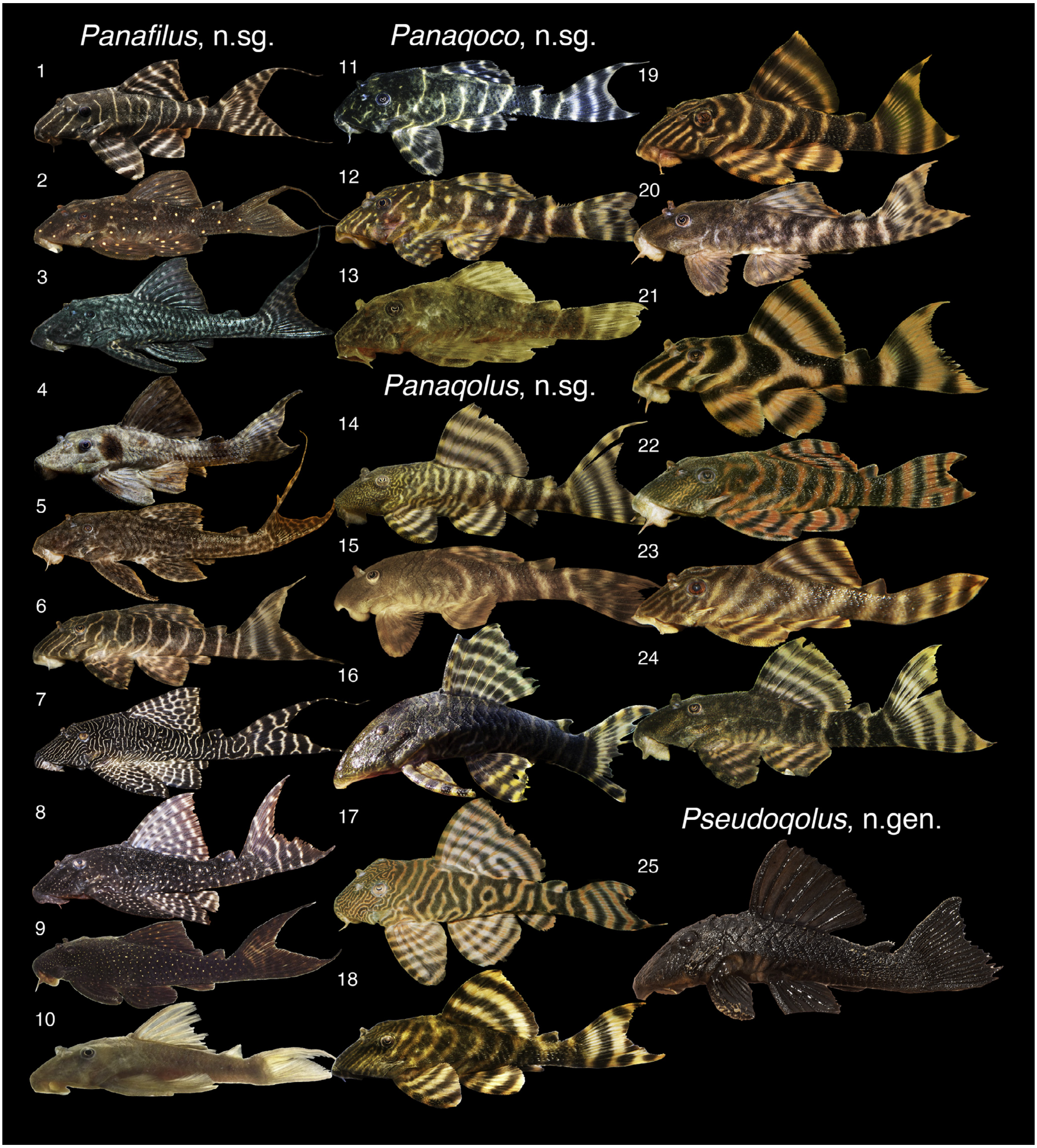

Fig. 1. Voucher specimens examined in this study, from the new subgenus *Panafilus* (lyretail clown plecos): (1) *Pf. albivermis*, (2) *Pf. albomaculatus*, (3) *Pf. nocturnus*, (4) *Pf.* n. sp. Huallaga L329, (5) *Pf.* n.sp. Huallaga L351, (6) *Pf.* n.sp. Ucayali L425, (7) *Pf.* n.sp. Moa L453, (8) *Pf.* n.sp. Napo L466, (9) *Pf. nix*, (10) *Pf.* n.sp. Madeira; new subgenus *Panaqoco* (Orinoco clown plecos): (11) *Pc. maccus*, (12) *Pc.* n.sp. Tomo L465, (13) *Pc.* n.sp. Orinoco L448; new subgenus *Panaqolus* (the tiger clown plecos): (14) *Pq. changae*, (15) *Pq. gnomus*, (16) *Pq. purusiensis*, (17) *Pq.* n.sp. Curua Una, (18) *Pq.* n.sp. Tocantins L002, (19) *Pq.* n.sp. Negro L169, (20) *Pq.* n.sp. Ucayali L206, (21) *Pq.* n.sp. Branco L306, (22) *Pq.* n. sp. Amazon L397, (23) *Pq. tankei*, (24) *Pq.* n.sp. Itaya L459; and new genus *Pseudoqolus*: (25) *Ps. koko*.

(MUSM), the Royal Ontario Museum in Toronto, Canada (ROM), or obtained through the ornamental fish trade. Voucher specimens (Table 1) were identified via either direct examination of specimens or via photograph. Many species or morphotypes of *Panaqolus* are only available through the ornamental fish trade and tissues (fin clips) of these species were obtained non-lethally from living specimens for which the river drainage of origin was known directly from the collector. Only photo vouchers are available for these tissues (Fig. 1). Given the allopatric distributions of most *Panaqolus* and *Panaque* species and morphotypes, and the distinctive color and/or tooth patterns of most sympatric species, we believe that identifications made via combinations of geography, color pattern, and gross external morphology are robust, even if the color pattern and external morphology of some specimens were only examined via photographs.

2.3. Molecular markers, DNA extraction, amplification, and sequencing

Molecular phylogenetic methods followed those of Lujan et al. (2015a). In brief, we amplified and sequenced a fragment of the mitochondrial 16S (~600 bp) and cytochrome *b* (~1150 bp) genes as well as the nuclear RAG1 (~1020 bp), RAG2 (~950 bp) and MyH6 (~660 bp) genes for a total of 4293 aligned base pairs. Each fragment was amplified using previously published primers (Li et al., 2007; Lujan et al., 2015a). Whole genomic DNA was extracted from fin or muscle tissues preserved in 95% ethanol following manufacturer's instructions for the DNeasy Blood & Tissue Kit (Qiagen N.V., Venlo, Netherlands). Fragment amplifications were performed following the methods of Lujan et al. (2015a). Post-PCR cleanup of all loci, was achieved by running the entire volume of PCR product on a 1% agarose gel with 0.01% SYBR® Safe DNA gel stain (LTI: Life Technologies Inc., Carlsbad, CA). The band corresponding to the target locus was cut from the gel and the target PCR product extracted by centrifuge filtration through the top of a P-200 pipette filter tip in a labeled 1 mL snap-top tube (5 min at 15,000 rpm). Forward and reverse sequencing reactions followed the manufacturer's recommendations for sequencing on an Applied Biosystems™ 3730 DNA analyzer (LTI).

2.4. Sequence assembly, alignment, and phylogenetic inference

Sequence data were assembled, edited, aligned, and concatenated following the methods of Lujan et al. (2015a). PartitionFinder (v1.1.1, Lanfear et al., 2012) was used to determine codon-specific models of molecular evolution for each gene under the Bayesian information criterion (BIC). A generalized time reversible model with proportion of invariable sites estimated and rate heterogeneity of the remainder being modeled by a gamma distribution (GTR + I + Gamma) was determined to be the best model of molecular evolution for 16S (all sites), Cyt *b* (all sites), the first two codon positions of RAG1 and RAG2, and the first and third codon positions of MyH6. A GTR model with rate heterogeneity of all sites being modeled by a gamma distribution (GTR + Gamma) was determined to be the best model of molecular evolution for the third codon positions of RAG1 and RAG2 and the second codon position of MyH6. All data partitions were unlinked with rates free to vary across partitions. Under this partitioning scheme, phylogenetic analyses of the concatenated alignment of 4293 base pairs were conducted using Bayesian inference and maximum likelihood methods with *Vandellia* sp. (Trichomycteridae) designated as the outgroup.

A Bayesian Markov chain Monte Carlo (MCMC) search of tree space was conducted using MrBayes (v3.2.3; Ronquist and Huelsenbeck, 2003) on the CIPRES supercomputing cluster (Miller et al., 2010). MrBayes was programmed to run for 35 million generations using two sets of four chains (1 cold, 3 hot, with default temperature parameter), sampling every 9000 trees with the first 25% of trees (968) being discarded as burn-in, thus generating a total of 2916 trees from which posterior probabilities were calculated. The Bayesian search was determined to have reached stationarity when likelihood values of the cold chains began randomly fluctuating within a stable range and when effective sample sizes for all metrics exceeded 200 as determined by the program Tracer (v1.6; Rambaut et al., 2007). Maximum likelihood analysis was conducted using RAxML-HPC2 (v8.1.11; Stamatakis, 2014), also on the CIPRES supercomputing cluster, programmed to first conduct 1500 independent runs with random starting trees to search for the best tree and then generate bootstrap support values based on a 1000 replication search of tree space. To evaluate the influence of mitochondrial loci on our results, we also conducted separate maximum likelihood analyses on respective alignments of mitochondrial vs. nuclear loci using RAxML-HPC2 parameterized as in the full analysis.

Table 2

Support values for each of the *Peckoltia* Clade nodes in Fig. 2, derived from Bayesian inference (BI) and maximum likelihood (ML) optimality criteria. Numbers in italics indicate BI < 0.90; numbers in bold indicate ML < 60.

Node	BI	ML	Clade	Node	BI	ML	Clade
1	1.00	99		***30***	*0.50*	**–**	
2	1.00	96		***31***	*0.51*	**35**	
3	*0.89*	68		***32***	*0.52*	**44**	
4	1.00	99		33	0.98	77	*Peckoltia sabaji*
5	1.00	69		*34*	*0.64*	63	
6	*0.59*	72		35	1.00	99	*Peckoltia relictum*
7	0.97	88		*36*	*0.61*	60	
8	1.00	91	Parallel-jawed *Panafilus*	*37*	*0.68*	59	
9	1.00	90	*Panafilus* n.sg.	38	0.94	61	
10	*0.87*	82		39	0.99	99	Orinoco *Peckoltia*
11	1.00	100	*Panaqoco* n.sg.	***40***	–	**–**	
12	*0.87*	71		41	1.00	77	*Peckoltia*
13	*0.52*	**12**		***42***	*0.84*	**26**	
14	–	–		43	0.97	63	
15	*0.56*	86		***44***	–	**–**	
16	1.00	90		***45***	–	**–**	
17	0.91	**56**		***46***	–	**–**	
18	*0.84*	83		47	1.00	100	Orinoco *Hypancistrus*
19	*0.78*	**56**		48	1.00	100	*Hypancistrus*
20	*0.62*	**–**		49	1.00	99	
21	1.00	86	*Panaqolus* n.sg.	50	1.00	98	
22	1.00	92	Genus *Panaqolus*	***51***	*0.71*	**51**	
23	1.00	100	*Scobinancistrus*	52	0.96	72	
24	–	**–**		53	1.00	100	*Aphanotorulus*
25	1.00	99	*Ancistomus*	54	1.00	97	
26	1.00	76		55	1.00	100	'*Hemiancistrus*' *landoni*
27	*0.71*	**–**		56	0.98	88	*Peckoltia* Clade
28	0.99	95		57	1.00	100	
29	0.96	81		58	1.00	99	

Table 3
Support values for each of the *Hemiancistrus* Clade nodes in Fig. 3, derived from Bayesian inference (BI) and maximum likelihood (ML) optimality criteria. Numbers in italics indicate BI < 0.90; numbers in bold indicate ML < 60.

Node	BI	ML	Clade	Node	BI	ML	Clade
1	*0.68*	65		12	1.00	100	*Baryancistrus*
2	1.00	99		13	1.00	100	
3	1.00	99		***14***	*0.58*	**39**	
4	1.00	100		15	1.00	100	
5	–	**49**		16	1.00	100	
6	*0.74*	**46**		17	0.99	68	Upper Orinoco species
7	1.00	100	*Panaque*	18	1.00	67	
8	1.00	92	*Spectracanthicus*	*19*	*0.88*	63	
9	1.00	100		20	1.00	100	
10	1.00	99		21	1.00	99	
11	1.00	100					

2.5. Presentation of phylogenetic results

Complete results of the Bayesian and maximum likelihood analyses, including results of the separate maximum likelihood analyses of mitochondrial and nuclear loci, are presented as supplementary files. Manuscript figures were trimmed of all outgroup taxa and were based on results of the Bayesian analysis; however, node support values from both the Bayesian and full maximum likelihood analyses are provided in Tables 2 and 3. We also provide Bayesian posterior probability (i.e., Bayesian inference = BI) and maximum likelihood (ML) bootstrap support values for each node discussed in the text.

2.6. Undescribed and incertae sedis taxa

Given the utility and generally standardized application of so-called ‘L-number’ codes (Stawikowski, 1988; Dignall, 2014), we have adopted them throughout this manuscript as a means of referring to species or geographically defined morphotypes that have not yet been scientifically described. Our study also includes several species that are currently recognized as members of genera that this and previous molecular phylogenetic analyses have revealed to be paraphyletic. For these species, the genus name in general usage is still provided but this name is placed in single quotation marks if the species is separated from the clade that includes the type species for the genus. Tribe-level clade names follow Lujan et al. (2015a) in which undescribed tribes are named by an included genus plus capitalized ‘Clade’.

2.7. Gut contents analysis of ‘Panaqolus’ koko

The entire gastrointestinal (GI) track of a single specimen of *‘Panaqolus’ koko* (paratype: MHNG 2723.089, 88.3 mm SL) was removed, measured, and examined via 50× dissecting scope at several points along its length by opening holes in the GI tract, and removing and visually examining the gut contents. These results are compared with previous observations by Schaefer and Stewart (1993) and German (2009) that the gut contents of *Panaqolus dentex*, *P. gnomus*, *P. nocturnus*, and *P. purusiensis* (i.e., four of the 11 described species in the genus) contain wood particles.

3. Results

3.1. Deep relationships, Fig. 2, Table 2

As previously found by Lujan et al. (2015a), statistical support for monophyly of the clade containing all wood-eating (and many non-wood eating) genera was strong (Node 57: BI: 1.0, ML: 100). Statistical support for monophyly of the clade containing the sister tribes Hypostomini and the *Peckoltia* Clade was also strong (Node 58: BI: 1.0, ML: 99), as was support for the tribe Hypostomini, containing the wood-eating genus *Cochliodon* and the non-wood eating genera *Hypostomus* and *Pterygoplichthys* (SI Figs. 1 and 2: BI: 1.0, ML: 96). As expected, deep relationships were poorly resolved in our analysis of mitochondrial data alone (SI Fig. 3). However, the composition of major clades and their node support values were similar in our analysis of nuclear data alone (SI Fig. 4), with the exception that *Pterygoplichthys gibbiceps* formed a well-supported clade (ML: 90) with *‘Hemiancistrus’ landoni* in the nuclear analysis.

3.2. Generic relationships within the Peckoltia Clade, Fig. 2, Table 2

Support for monophyly of the *Peckoltia* Clade was much stronger in this study (Node 56: BI: 0.98, ML: 88) than in Lujan et al. (2015a: BI: 0.73, ML: 52), although both studies found the clade to contain the currently valid genera *Ancistomus*, *Aphanotorulus*, *Hypancistrus*, *Isorineloricaria*, *Peckoltia*, *Peckoltichthys*, *Panaqolus*, and *Scobinancistrus*, as well as the *incertae sedis* species ‘*Spectracanthicus*’ *immaculatus* and ‘*Hemiancistrus*’ *landoni*. All respectively valid, non-monotypic genera within the *Peckoltia* Clade were found to be strongly monophyletic (BI: >0.99, ML: >75). *Hypancistrus* contained the monotypic genus and species *Micracanthicus vandragti*, which is therefore treated herein as a member thereof. Intergeneric relationships within the *Peckoltia* Clade largely paralleled those of Lujan et al. (2015a), with only two exceptions: First, ‘*Spectracanthicus*’ *immaculatus* and *Isorineloricaria spinosissimus* were no longer found to be sister to each other but rather successive sister lineages to all other *Peckoltia* Clade genera exclusive of *Aphanotorulus* and ‘*Hemiancistrus*’ *landoni*. Second, ‘*Panaqolus*’ *koko* was no longer found to form a polytomy with *Panaqolus*, *Peckoltia* and *Scobinancistrus* + *Ancistomus*, but was consistently (though weakly) supported as sister to a clade containing all these other genera (Node 42: BI: 84, ML: 26). Within this later clade, *Ancistomus* and *Scobinancistrus* were found to form a strongly monophyletic clade (Node 26: BI: 1.00, ML: 76), with this clade being weakly and ambiguously supported as sister to *Panaqolus* (Node 27: BI: 0.71, ML: –). With the exception of the already mentioned change in position of *Pterygoplichthys gibbiceps*, the topology of relationships from the full analysis was similar to that found in our nuclear analysis (SI Fig. 4); however, node support values were much lower when based only on nuclear data. The mitochondrial analysis (SI Fig. 3) also yielded a monophyletic *Panaqolus* exclusive of *‘P.’ koko*. Interestingly, the mitochondrial analysis found *‘P.’ koko* to be sister to a clade containing the majority of Amazon Basin *Peckoltia* (i.e., exclusive of *Peckoltia pankimpuju* and a clade of upper Orinoco *Peckoltia*), although support for these relationships was generally very weak.

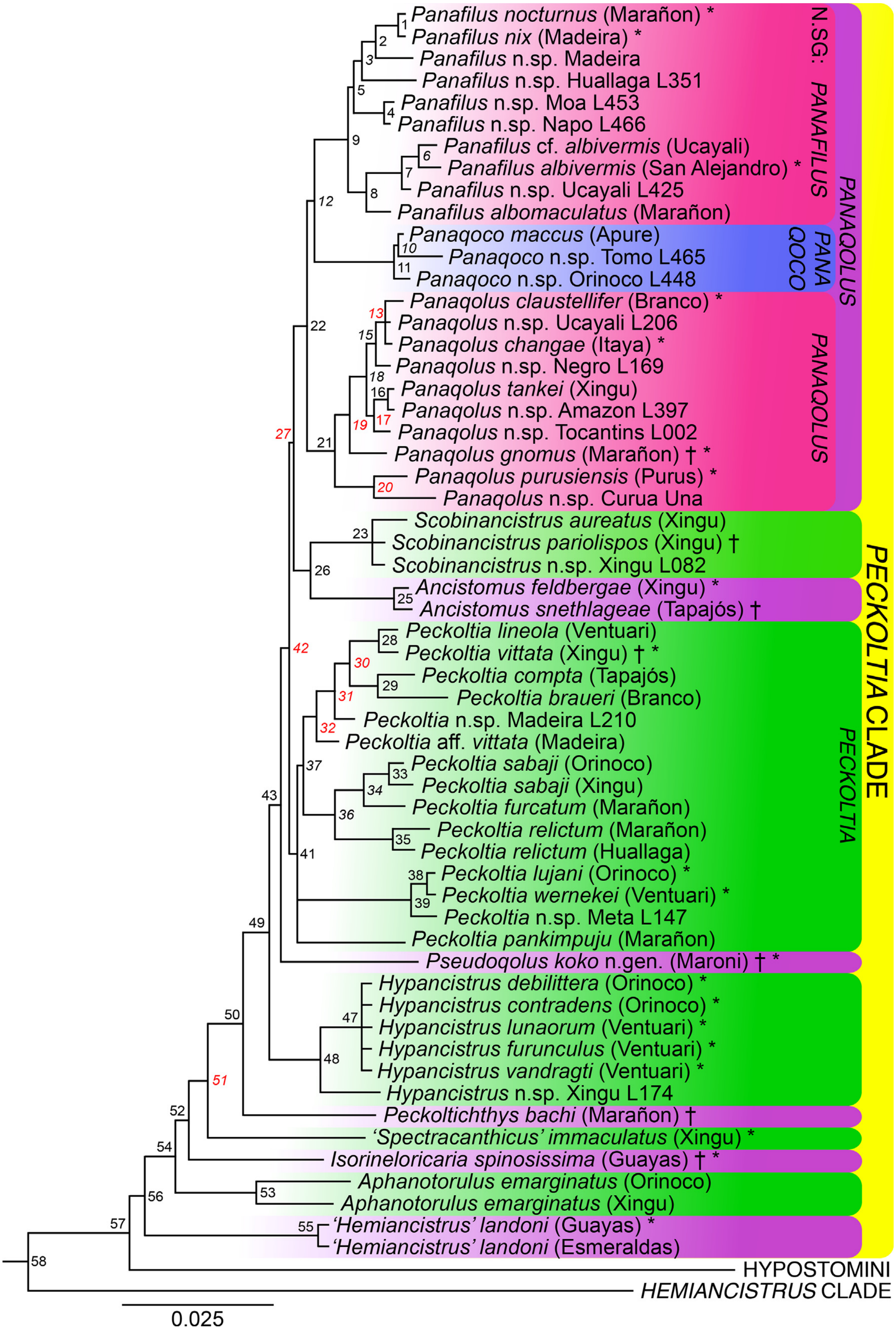

Fig. 2. Phylogenetic relationships of taxa within the *Peckoltia* Clade (Loricariidae, Hypostominae), including the new genus (n.gen.) *Pseudoqolus* and new subgenera (N.SG) *Panafilus* (lyretail clown plecos), *Panaqoco* (Orinoco clown plecos), and *Panaqolus* (tiger clown plecos), based on Bayesian analysis of a 4293 base pair alignment consisting of two mitochondrial (16S, Cyt *b*) and three nuclear loci (RAG1, RAG2, MyH6). Node numbers correspond to Bayesian posterior probability (BI) and maximum likelihood (ML) support values in Table 2. Numbers in italics indicate BI < 0.90; numbers in red indicate ML < 60. Samples taken from at or near the type locality for a given species are indicated by an asterisk (∗) and specimens representing species that are types for their genus are indicated by a cross (†).

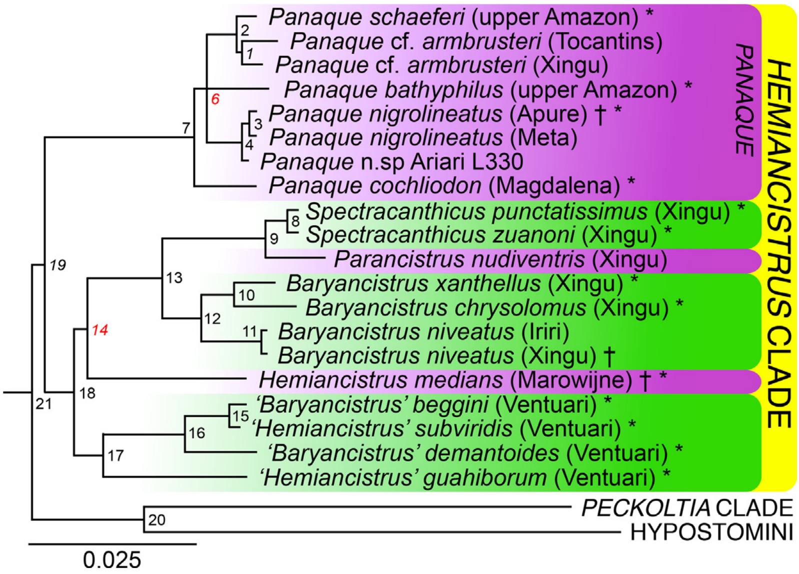

Fig. 3. Phylogenetic relationships of genera within the *Hemiancistrus* Clade (Loricariidae, Hypostominae) based on Bayesian analysis of a 4293 base pair alignment consisting of two mitochondrial (16S, Cyt *b*) and three nuclear loci (RAG1, RAG2, MyH6). Node numbers correspond to Bayesian posterior probability (BI) and maximum likelihood (ML) support values in Table 3. Numbers in italics indicate BI < 0.90; numbers in red indicate ML < 60. Samples taken from at or near the type locality for a given species are indicated by an asterisk (∗) and specimens representing species that are types for their genus are indicated by a cross (†).

3.3. Species relationships within Panaqolus, Fig. 2, Table 2

The genus *Panaqolus* was only found to be strongly monophyletic (Node 22: BI: 1.0, ML: 92) with the exclusion of *P. koko*. *Panaqolus* species and morphotypes were clustered into three strongly monophyletic clades designated herein as subgenera because of their correlated morphological and biogeographical characteristics (see Section 4). Three small-bodied species from the Orinoco River (hereafter: 'Orinoco clown plecos') were found to be strongly monophyletic (Node 11: BI: 1.0, ML: 100) and moderately supported as sister (Node 12: BI: 0.87, ML: 71) to a well-supported clade (Node 9: BI: 1.0, ML: 90) of upper Amazon Basin species that are distinguished by having unbranched principal caudal-fin rays elongated as filaments (Fig. 1; hereafter: 'lyretail clown plecos').

Within the clade of lyretail clown plecos, three (or possibly four) Andean piedmont species (*Panaqolus albomaculatus*, *P. albivermis* San Alejandro, *P.* cf. *albivermis* Ucayali, and *P.* n.sp. Ucayali L425) were found to be strongly monophyletic (Node 8: BI: 1.0, ML: 91) and sister to a well-supported clade containing all other species (Node 5: BI: 1.0, ML: 69). Intriguingly, the clade containing *P. albomaculatus*, *P. albivermis*, and *P.* n.sp. L425 is further distinguished by having rows of elongate mandibular teeth that are aligned parallel with the longitudinal body axis – a condition that is unique within the Loricariidae. Within this clade, *P. albomaculatus* from the Marañon River was sister to a well-supported clade (Node 7: BI: 0.97, ML: 88) containing *P. albivermis* from the San Alejandro River, a morphologically similar population from the nearby Ucayali River (*P.* cf. *albivermis* Ucayali) and the morphologically distinct *P.* n.sp. L425 (also from the Ucayali River). Within the clade containing all other lyretail species having more typically angled mandibular tooth rows, two undescribed species from the respective Moa and Napo river drainages were found to form a strongly monophyletic clade (Node 4: BI: 1.0, ML: 99) that was sister to a moderately supported clade (Node 3: BI: 0.89, ML: 68) containing *P. nocturnus*, *P. nix* and two putatively undescribed species from the Madeira and Huallaga river drainages. Within this latter clade, the putatively undescribed species *P.* n.sp. Huallaga L351 was found to be sister to a well-supported clade containing the remaining three species (Node 2: BI: 1.0, ML: 96). Within this last clade, *P. nix* and *P. nocturnus* were well-supported as sister species (Node 1: BI: 1.00, ML: 99).

Together, the Orinoco clown plecos plus the lyretail clown plecos were found to be sister to a third strongly monophyletic clade (Node 21: BI: 1.0, ML: 86) of boldly banded species distributed throughout the Amazon Basin (hereafter: 'tiger clown plecos'). Within the clade of tiger clown plecos, a weakly supported clade (Node 20: BI: 0.62, ML: –) of two sister species (*P. purusiensis* from the Purus River and *P.* n.sp. from the Curua Una River) was found to be sister to a weakly supported clade containing all other species (Node 19: BI: 0.78, ML: 56). Within the latter clade, *P. gnomus* from the Marañon River was found to be sister to a moderately supported clade containing all other species (Node 18: BI: 0.84, ML: 83). Relationships within the latter clade were weakly and/or ambiguously supported. Bayesian analysis found this clade to comprise moderate to weakly monophyletic sister clades that are respectively restricted to the lower Amazon River and its southern tributaries (Node 17: BI: 0.91) and the upper Amazon River and its northern tributaries (Node 15: BI: 0.56). The lower Amazon Basin clade contained one described and two putatively undescribed species that are respectively distributed within the lower Amazon River itself (*P.* n.sp. L397), and its southern tributaries the Xingu River (*P. tankei*) and Tocantins River (*P.* n.sp. L002), with the Amazon and Xingu species being strongly supported as monophyletic (Node 16: BI: 1.0, ML: 90). Relationships within the upper Amazon Basin clade, comprising species from the Branco (*P. claustellifer*), the Negro (*P.* n.sp. L169), the Itaya (*P. changae*) and the Ucayali (*P.* n.sp. L206) were all weakly supported in the Bayesian (BI: <60) and maximum likelihood (SI Fig. 2) analyses. Mitochondrial data supported the same three major clades within *Panaqolus*, although relationships within these clades differed (SI Fig. 3). *Panaqolus* intergeneric relationships were very weakly supported when only nuclear data were examined (SI Fig. 4); however, the same individuals were still found to be part of a single monophyletic clade exclusive of *P. koko*.

3.4. Generic relationships within the Hemiancistrus Clade, Fig. 3, Table 3

Statistical support for monophyly of the *Hemiancistrus* Clade increased slightly in this study (Fig. 3, Node 19: BI: 0.88, ML: 63) from that of Lujan et al. (2015a; BI: 0.70, ML: 59). Both studies found this clade to comprise the valid genera *Hemiancistrus*, *Baryancistrus*, *Panaque*, *Parancistrus*, and *Spectracanthicus* plus a group of four *incertae sedis* species from the upper Orinoco (‘*Baryancistrus*’ *beggini*, ‘*B.*’ *demantoides*, ‘*Hemiancistrus*’ *guahiborum*, ‘*H.*’ *subviridis*). Of these genera, *Baryancistrus sensu stricto* (exclusive of upper Orinoco species) and *Panaque* were found to be strongly monophyletic (Nodes 7 and 12: BI: 1.0, ML: 100), as was the group of upper Orinoco *incertae sedis* species (Node 17: BI: 0.99, ML: 68).

The clade containing *Spectracanthicus* + *Parancistrus* was also strongly monophyletic (Node 9: BI: 1.0, ML: 100); however, neither genus was monophyletic in any of our analyses (SI Figs. 1–3). Both Bayesian and maximum likelihood analyses of the full dataset found the *Spectracanthicus* + *Parancistrus* clade to comprise a strongly monophyletic clade of three *Parancistrus nudiventris* individuals (SI Fig. 1 and 2; BI: 1.0, ML: 100) that was sister to a strongly monophyletic clade (SI Figs. 1 and 2; BI: 1.0, ML: 92) of interleaved *Parancistrus nudiventris* (n = 2), *Spectracanthicus punctatissimus* (n = 12), and *S. zuanoni* (n = 4) individuals. These relationships were largely paralleled in our analysis of nuclear data only (SI Fig. 4), whereas mitochondrial data found the *Hemiancistrus* Clade genera to form two separate clades that were not sister to each other: a clade of Amazonian and eastern Guiana Shield (*H. medians*) genera (including a similarly interleaved clade of *Parancistrus* and *Spectracanthicus*), and a clade of *Panaque* plus upper Orinoco ‘*Hemiancistrus*’ and ‘*Baryancistrus*’.

3.5. Species relationships within Panaque, Fig. 3, Table 3

Five described and one undescribed species were found to be included within the strongly monophyletic genus *Panaque* (Node 7: BI: 1.0, ML: 100). Within this clade, our sole trans-Andean species, *Panaque cochliodon* from the Magdalena River basin, was weakly supported as sister to an East Andean clade of all other Amazon and Orinoco Basin species (Node 6: BI: 0.74, ML: 46). Within the latter clade, a clade of Orinoco Basin species was strongly supported as monophyletic (Node 4: BI: 1.0, ML: 100), as was the widespread Amazonian clade of *Panaque schaeferi* (upper Amazon and Amazon mainstream) and *P.* cf. *armbrusteri* (Xingu and Tocantins rivers; Node 2: BI: 1.0, ML: 99). Within this latter clade, we found moderate support for monophyly of *P.* cf. *armbrusteri* (Node 1: BI: 0.68, ML: 65). Although several *P. bathyphilus* individuals from the Marañon and Madeira rivers were found to be strongly monophyletic (SI Figs. 1 and 2: BI: 1.0, ML: 100), our analyses were inconclusive regarding this species’ phylogenetic position within the clade of East Andean *Panaque*. Relationships within *Panaque* were generally poorly supported by nuclear data (SI Fig. 4), but similar and well supported by mitochondrial data (SI Fig. 3).

3.6. Relative gut length and gut contents of ‘Panaqolus’ koko

Total length of the gastrointestinal tract of the single examined specimen (MHNG 2723.089, 88.3 mm SL) was 660 mm, or 7.5 times standard length (= relative intestine length, or RIL). Gut contents consisted of amorphous detritus and many intact pieces of sponge, some large enough to distend the intestines, as well as aggregations of what appeared to be sponge spicules.

4. Discussion

4.1. Overview

Results of this study and other recent molecular phylogenetic appraisals of the Hypostominae (Lujan et al., 2015a, 2015b) suggest that already-high estimates of species-level diversity within this subfamily may dramatically underestimate true diversity. Hypostominae is already known to be the most species- and genus-rich subfamily within the Loricariidae – itself the fifth most species-rich family of fishes and most species-rich family of catfishes. Current trends suggest that species-level diversity will continue to rapidly expand as ichthyologists combine specimens from increasingly remote drainages throughout tropical South America with increasingly precise molecular methods for inferring species taxonomy and phylogeny. We strive to help bring order to the ongoing proliferation of new species by erecting new subgenera for each of the three major, strongly monophyletic, and biogeographically and morphologically distinct subclades within genus *Panaqolus*.

4.2. New Panaqolus subgenera

4.2.1. Panaqoco, new subgenus

Common name: Orinoco clown plecos.

4.2.1.1. Type species. *Panaque maccus* Schaefer and Stewart, 1993:335, Figs. 18 and 19.

Literature cited: Schaefer, S.A. and Stewart, D.J., 1993. Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. Ichthyological Exploration of Freshwaters 4:309–342.

4.2.1.2. Etymology. *Panaqoco* is a portmanteau of the genus name *Panaqolus* and the drainage name Orinoco. The gender is masculine.

4.2.1.3. Diagnosis. *Panaqoco* is diagnosed from other members of the genus *Panaqolus* by the absence of filamentous extensions on the unbranched principal caudal-fin rays and the absence of either consistent oblique banding on the body or reticulate, wormline patterns across the entire snout, having instead broken, inconsistent banding with occasional spots on the head and body (Fig. 1).

4.2.1.4. Included species. The only currently described species in subgenus *Panaqoco* is the type species *Panaque maccus* Schaefer and Stewart, 1993. Morphotypes commonly referred to as L448 and L465 are also included.

4.2.1.5. Distribution. Species in the subgenus *Panaqoco* are distributed throughout the Orinoco River drainage, with populations primarily concentrated in piedmont habitats in rivers and streams along the lower elevations of the Andes Mountains and the margins of the Guiana Shield.

4.2.2. Panafilus, new subgenus

Common name: lyretail clown plecos.

4.2.2.1. Type species. *Panaque albomaculatus* Kanazawa 1958:327, Fig. 2.

Literature cited: Kanazawa, R.H. 1958. A new species of catfish, family Loricariidae, from Ecuador. Copeia 1958:327–328.

4.2.2.2. Etymology. *Panafilus* is a portmanteau of the genus name *Panaqolus* and the Latin word ‘*filum*’, meaning filament or fiber, in reference to the elongated unbranched principal caudal-fin rays in all members of this subgenus. The gender is masculine.

4.2.2.3. Diagnosis. *Panafilus* is diagnosed from other members of the genus *Panaqolus* by having both unbranched principal caudal-fin rays elongated as filaments extending up to over twice the length of branched caudal-fin rays (vs. unbranched principal caudal-fin rays not elongated as filaments), and by lacking broad and consistent brown bands on the body and fins, having instead either small white, gold, or blue spots, vermiculate markings or irregular and inconsistent narrow bands on a generally black or dark gray base color (Fig. 1).

4.2.2.4. Included species. Four currently described species are included in subgenus *Panafilus*: the type species *Panaque albomaculatus* Kanazawa 1958, *Panaque nocturnus* Schaefer and Stewart, 1993, *Panaqolus albivermis* Lujan, Steele, and Velasquez 2013, and *Panaqolus nix* Cramer and Rapp Py-Daniel, 2015. Morphotypes commonly referred to as L351, L425, L453, and L466 are also included.

4.2.2.5. Distribution. Species in the subgenus *Panafilus* are distributed throughout southwestern tributaries of the upper Amazon Basin, including the Madeira, Ucayali, Huallaga, Marañon, and Napo river drainages. Within these drainages, populations are primarily concentrated in piedmont habitats along the lower elevation flanks of the Andes Mountains, although they can also occur further downstream in main river channel habitats of the western Amazonian lowlands.

4.2.2.6. Dietary ecology. Most members of the subgenus *Panafilus* have upper and lower jaw morphologies similar to those of other members of the genus *Panaqolus* – and indeed other wood-eating genera – consisting of relatively few (<10) and short, spoon-shaped teeth arranged in left and right rows with an angle between them of approximately 90° (Lujan and Armbruster, 2012). This jaw and tooth morphology is strongly associated with the ingestion of wood and the assimilation of cellulosic carbon by members of the genera *Panaqolus* (e.g., *P. nocturnus*, *P. gnomus*), *Panaque* (e.g., *P. bathyphilus*), and *Cochliodon* (*C. pyrineusi*, Lujan et al., 2011).

However, a strongly monophyletic clade within *Panafilus* (Fig. 2, Node 8: BI: 1.0, ML: 88, MP: 7) is distinguished from all congeners by having rows of more elongate dentary teeth that are nearly parallel with the longitudinal body axis. In a study of dietary resource partitioning within a diverse sympatric assemblage of wood-eating catfishes, Lujan et al. (2011) found that one member of this clade – *Panafilus albomaculatus* – had higher nitrogen isotope (^{15}N) values than the four other sympatric wood-eating species listed above. Because ^{15}N enrichment is associated with both trophic level and the amount of protein in a consumers diet (Kelly and Martinez del Rio, 2010), this suggests that the distinctive jaws of this subclade within *Panafilus* are specialized for a more carnivorous diet that is relatively enriched in protein. Moreover, *Panafilus albomaculatus* also had a carbon isotope value closer to that of seston than all other wood-eating species, which all had carbon isotope values closer to that of wood (Lujan et al., 2011). This further suggests that *P. albomaculatus* assimilates less wood carbon than its congeners, and derives energy from a distinctive source such as, perhaps, macroinvertebrate collectors of seston such as crevice-dwelling caddisfly (Trichoptera) and pyralid (Lepidoptera) larvae.

Given these dietary isotope patterns and jaw models suggesting that longitudinally elongate jaws and teeth would be capable of greater protrusion than the angled jaws of other wood-eater species (Lujan and Armbruster, 2012), we hypothesize that these jaws are specialized for the consumption of aquatic invertebrates from within cracks or depressions in the surface of dead wood. Aquatic invertebrates often seek refuge from predation in small spaces along the surfaces of submerged substrates, and the evolution of narrowly protrusible or elongate jaws has occurred both in other invertivorous loricariid genera (e.g., *Leporacanthicus*, *Scobinancistrus*, *Spatuloricaria*) and in many other riverine fish families (e.g., Anostomidae, Apteronotidae, Doradidae, Mormyridae), where such specializations are invariably also associated with the consumption of substrate-dwelling invertebrates (Marrero and Winemiller, 1993; Lujan and Conway, 2015).

4.2.3. Panaqolus, new subgenus

Common name: tiger clown plecos.

4.2.3.1. Type species. *Panaque gnomus* Schaefer and Stewart, 1993:333, Fig. 27.

Literature cited: Schaefer, S.A. and Stewart, D.J., 1993. Systematics of the *Panaque dentex* species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. Ichthyological Exploration of Freshwaters 4:309–342.

4.2.3.2. Etymology. The genus name *Panaqolus* is retained for this subgenus that includes the type species for the genus.

4.2.3.3. Diagnosis. The subgenus *Panaqolus* is diagnosed from other members of its genus by lacking filamentous elongations of the unbranched caudal-fin rays, by having light to dark brown coloration (vs. black to dark gray in *Panafilus*), and by having generally consistent, broad, oblique bands on the body, consistent and distinct bands on the fins, and/or reticulate, wormline patterns covering the entire snout (Fig. 1).

4.2.3.4. Included species. Four currently described species are included in subgenus *Panaqolus*: the type species *Panaque gnomus* Schaefer and Stewart, 1993, *Panaque purusiensis* La Monte 1935, *Panaque changae* Chockley and Armbruster, 2002, and *Panaqolus claustellifer* Tan, Souza and Armbruster 2016. Morphotypes commonly referred to as L002, L169, L206, L397, L398, and L459 are also included.

4.2.3.5. Distribution. Species in the subgenus *Panaqolus* are widely distributed throughout the Amazon Basin including northern tributaries like the Branco and Negro, western tributaries like the Purus, Ucayali, and Itaya, and southern tributaries like the Tapajós, Xingu, and Tocantins. Throughout this region, members of the subgenus *Panaqolus* are often not most abundant in piedmont habitats, but are rather more common in the lower courses of large river channels.

4.3. New genus for 'Panaqolus' koko

4.3.1. Pseudoqolus, new genus

4.3.1.1. Type species. *Panaqolus koko* Fisch-Muller & Covain 2012:184, Figs. 7, 13, 14.

Literature cited: Fisch-Muller, S., Montoya-Burgos, J.I., le Bail, P.-Y., and Covain, R. 2012. Diversity of the Ancistrini (Siluriformes: Loricariidae) from the Guianas: the *Panaque* group, a molecular appraisal with descriptions of new species. Cybium 36:163–191.

4.3.1.2. Etymology. *Pseudoqolus* is a portmanteau of the Greek word *pseudes* meaning false and the genus name *Panaqolus*, indicating that although this genus may look superficially like *Panaqolus*, such an appearance is false.

4.3.1.3. Diagnosis. *Pseudoqolus* can be diagnosed from *Panaqolus* and all other genera in the Hypostominae (*sensu* Lujan et al., 2015a) except *Scobinancistrus* by having bicuspid teeth with a robust, inflexible shaft and broad principal cusp at least four times as wide as secondary cusp (Fisch-Muller et al., 2012; vs. teeth typically unicuspid in *Panaqolus* and slender with principal cusp no more than twice as wide as secondary cusp in all other genera except *Scobinancistrus*). *Pseudoqolus* can be diagnosed from *Scobinancistrus* by having a typically short principal cusp, no more than half of total emergent tooth length (vs. up to two thirds of total emergent tooth length), and by having four to six premaxillary and dentary teeth (vs. rarely more than three).

4.3.1.4. Included species. *Pseudoqolus* contains only the type species *Panaqolus koko* Fisch-Muller & Covain 2012.

4.3.1.5. Distribution. *Pseudoqolus* is known only from the upper Maroni River drainage near Antecume Pata in French Guiana (Fisch-Muller et al., 2012).

4.3.1.6. Phylogenetic position. In the results of this study and that of Lujan et al. (2015a), *Pseudoqolus koko* was found to be included in a strongly monophyletic clade (Fig. 2, Node 43: BI: 0.97, ML: 63) that also included *Panaqolus*, *Peckoltia*, and a strongly monophyletic *Scobinancistrus* + *Ancistomus*. Neither this study nor Lujan et al. (2015a) found any support for *Pseudoqolus koko* to be more closely related to *Panaqolus* than to any of the other three genera in this clade; nor was its exact position relative to these other genera consistently and unambiguously resolved given that monophyly of the clade containing the genera *Ancistomus*, *Panaqolus*, *Peckoltia*, *Scobinancistrus* is weakly supported (Node 42: BI: 0.84, ML: 26).

Regardless of the relatively weak, albeit consistent, support for exclusion of *Pseudoqolus* from the clade containing these four genera, it seems, given the consistently strong monophyly of each constituent genus, that it is unlikely that additional data would shift *Ps. koko* to a position within one of these existing clades. Topological results plus its diagnostic morphological characteristics and non-wood eating diet (see below), justify the erection of a new genus for this species. In addition to the diagnostic morphological characters, *Pseudoqolus koko* is further distinguished from *Panaqolus sensu stricto* by head and body shape differences, including an elongated snout, a small but distinct occipital crest, narrower head and smaller interorbital distance (Fisch-Muller et al., 2012). Osteological analyses might well provide additional characters to reinforce this taxonomic hypothesis.

In their original description of *Pseudoqolus koko*, Fisch-Muller et al. (2012) suggested that *Ps. koko* may likely be introgressed with the sympatric-syntopic species *Peckoltia otali* based on similarity of a 648 bp portion of these two species' mitochondrial cytochrome *c* oxidase I (COI) gene sequence. Between these two species, Fisch-Muller et al. (2012) recorded only five silent transitions in their COI sequence data. To test the hypothesis that historical hybridization may have led to mitochondrial introgression and, therefore, an artificial increase in overall genetic distance between *Ps. koko* and members of the genus *Panaqolus sensu stricto*, we conducted separate maximum likelihood phylogenetic analyses of mitochondrial vs. nuclear data. Relationships recovered in the nuclear analysis (SI Fig. 4) largely paralleled those of our full analysis – consistent with *Ps. koko* representing a new genus – although maximum likelihood bootstrap support values were much lower. However, our mitochondrial analysis (SI Fig. 3) supported the *Peckoltia*-introgression hypothesis of Fisch-Muller et al. (2012) by finding *Ps. koko* to be sister to a clade composed mostly of Amazon Basin species of *Peckoltia* (i.e., exclusive of *Pe. pankimpuju* and a clade of upper Orinoco *Peckoltia*). *Peckoltia otali* was not included in our analysis but our results are consistent with the Fisch-Muller et al. (2012) hypothesis that *Ps. koko* inherited its mitochondrial genome from a co-occurring member of the genus *Peckoltia*.

4.3.1.7. Biogeographical patterns. Regardless of the phylogenetic position of *Pseudoqolus koko* outside of *Panaqolus*, *Peckoltia*, and *Scobinancistrus* + *Ancistomus*, it seems likely that this narrow endemic of the Maroni River, which drains the northeastern slope of the Guiana Shield along the northeastern coast of South America, is sister to a much more species rich and geographically widespread clade distributed predominantly (or entirely) within the Amazon Basin. Such a pattern would make *Ps. koko* one more of a growing list of relatively species-poor Guiana Shield endemic or specialist (*sensu* Lujan and Armbruster, 2011) fish lineages that are sister to much more diverse and geographically widespread Neotropical clades. Other examples include *Hemiancistrus medians*, *Lithogenes*, *Pseudolithoxus*, and the Cichlidae clade of *Guianacara* + *Mazarunia* (López-Fernández et al., 2010). A more extensive discussion of the historical biogeography of the freshwater fishes of the Guiana Shield and their relationships to other Amazonian lineages can be found in Lujan and Armbruster (2011).

4.3.1.8. Relative gut length and gut contents. The *Pseudoqolus koko* relative intestine length (RIL) of 7.5 compares to mean RILs of approximately 11.5 for species of the wood-eating genera *Panaque* and *Panaqolus* (German, 2009). The approximately 35% shorter gastrointestinal tract of *Ps. koko* compared to demonstrably specialized wood-eating taxa is consistent with a hypothesis that this genus and species may be specialized for a more protein rich, non-wood diet (Pouilly et al., 2003). This is also consistent with the gut contents of the single examined individual consisting largely of intact sponge fragments and spicules.

4.4. Paraphyly within the Hemiancistrus clade

This study included more individuals of several *Hemiancistrus* Clade species than the previous study of Lujan et al. (2015a), providing a more thorough evaluation of the monophyly of these species and their genera. Although all species in the *Panaque* and upper Orinoco *'Hemiancistrus'* clade were found to be monophyletic, multiple instances of genus and species paraphyly were observed within the lower Amazon clade of *Baryancistrus sensu stricto*, *Parancistrus*, and *Spectracanthicus*. We discuss these results here and report them in our supplemental figures but have excluded them from our manuscript figures because these taxa and their monophyly were not a primary goal of our study.

First, *Baryancistrus xanthellus* from the lower Xingu River (represented by three specimens having tissue tags B1490, B2064, and B2163; Table 1) was found to be paraphyletic with respect to the distinctive yet undescribed species or color morph *B.* n.sp. L142 from the neighboring Tapajós River (represented by a single specimen). In both our Bayesian and maximum likelihood analyses of the full mitonuclear alignment, *B. xanthellus* formed a monophyletic clade inclusive of *B.* n.sp. L142, but in our analysis of nuclear data alone, one *B. xanthellus* individual (tissue tag: B2064) was found to be part of a well supported (ML: 81) clade with *B. chrysolomus*. All three of these species exhibit highly distinctive color patterns, with *B. xanthellus* juveniles having a dark black base color, distinct golden yellow spots, and golden yellow marginal bands along the dorsal and caudal fins, *B. chrysolomus* having a dark green base color and yellow dorsal- and caudal-fin bands but lacking spots entirely, and *B.* n.sp. L142 having a dark black base color with distinct white spots and no marginal fin bands.

A second complex pattern of paraphyly was observed in the clade containing *Parancistrus nudiventris* (represented by five individuals; Table 1), *Spectracanthicus punctatissimus* (represented by 12 individuals; Table 1), and *S. zuanoni* (represented by four individuals; Table 1). Although this clade as a whole was consistently strongly monophyletic (Fig. 3, Node 9: BI: 1.0, ML: 100), none of the three species within the clade were ever found to be monophyletic, despite all three of them having highly distinctive color patterns and/or body morphologies. The paraphyly of these species persisted throughout all analyses; however, both of our full mitonuclear analyses found a strongly monophyletic (SI Figs. 1 and 2; BI: 1.0, ML: 100) clade of three *Parancistrus nudiventris* individuals that was sister to an also strongly monophyletic clade of all other individuals (SI Figs. 1 and 2; BI: 1.0, ML: 92). We hypothesize, based on these results and on an independent genomic analysis of these taxa by the first author, that this pattern is the result of relatively recent and rapid hybridization among all three species; however, further investigation of this intriguing but clearly complex pattern is needed.

4.5. Reproductive behavior

As with many other members of the subfamily Hypostominae (e.g., *Ancistrus*, *Chaetostoma*, *Pseudancistrus pectegenitor*; Sabaj et al., 1999; Page et al., 1993; Lujan et al., 2007) spawning in the genus *Panaqolus* usually occurs in caves with the male caring for the eggs and early life history stages. Several members of both the subgenera *Panaqoco* and *Panaqolus* are common in the aquarium hobby and spawn relatively easily and frequently in captivity; however, members of the subgenus *Panafilus* have only rarely been spawned in captivity, perhaps due to their larger body size, higher cost, and relative scarcity in the hobby. Likewise, the relatively large-bodied genus *Panaque* has only rarely been spawned in captivity.

4.6. Conservation

Neotropical freshwater fishes face a wide range of conservation threats, the most prominent and severe of which is habitat destruction from hydroelectric dams and in-stream gold mines (Lujan et al., 2013b; Reis et al., 2016; Winemiller et al., 2016). However, given the focus of this paper on species that are exploited for the ornamental fish trade, the potential threat of overfishing should be given special attention. Published data on the commercial exploitation of loricariid catfishes are scarce. In some of the few studies of major freshwater ornamental fisheries in the Neotropics, Moreau and Coomes (2007) and Gerstner et al. (2006) examined harvests in Peru, which – along with Brazil and Colombia – is one of the three largest South American sources of ornamental fishes (Chapman et al., 1997). Moreau and Coomes (2007) found that loricariids comprised approximately 32% of the volume of fishes exported from the Peruvian Amazon, and Gerstner et al. (2006) found that habitats under greatest ornamental fishing pressure near the major fish export center of Iquitos had reduced fish diversity, abundance, and biomass. Ornamental fish harvests have at least the potential to alter community structure and cause local wild population declines, but more finely resolved taxonomies and more data on natural community composition and population density are needed to adequately understand and address such impacts. Our study and other recent research (Alofs et al., 2014) illustrate the significant gaps that exist in our understanding of Neotropical fish diversity, and the extent to which potential threats may be underestimated if such gaps are not addressed.

5. Conclusions

Our finding that approximately half of the species-level diversity in the widespread genus *Panaqolus* may remain undescribed is illustrative of the extent to which total taxonomic diversity of even commercially exploited Amazonian fish lineages may remain underestimated by current taxonomies. Our erection of strongly monophyletic subgenera for the species-rich genus *Panaqolus* should help to facilitate both the conservation and taxonomic description of species by making at least the major clades easier to identify and by restricting the number of congeners that future taxonomists would have to examine to adequately diagnosis new *Panaqolus* species. Moreover, our strong phylogenetic support for large-scale biogeographical influence on the diversification of *Panaqolus* helps to justify and spatially delimit studies by regional researchers with regular access only to collections representing regional diversity.

It is clear from the biogeographical patterns observed in both *Panaqolus* and *Panaque*, as well as the *Cochliodon* group examined elsewhere (e.g., Armbruster, 2003), that Andean affluents of the southwestern Amazon Basin are an epicenter of wood-eating fish diversity, with some drainages having up to five different sympatric but unrelated species of wood-eating catfish coexisting on the same pieces of submerged wood. See Lujan et al. (2011) for a detailed study of trophic resource partitioning in one such diverse assemblage.

Acknowledgements

We gratefully acknowledge our principal foreign collaborators Otto Castillo (MCNG, Venezuela), Oscar Leon Mata (MCNG, Venezuela), Hernán Ortega (MUSM, Peru), and Lucia Rapp Py-Daniel (INPA, Brazil) for ensuring the legal collection and export of specimens; the collection managers and museum workers Erling Holm, Mary Burridge, Marg Zur (ROM), Mark Sabaj Pérez (ANSP), David Werneke (AUM), Dan Wylie (INHS), and aquarium fish importer Oliver Lucanus for generously sharing information, processing specimen loans, and gifting tissues and gDNA extracts; the expedition participants Blanca Rengifo (MUSM), Krista Capps (CU), Alex Flecker (CU), Donovan German (UF), Oscar Leon Mata (MCNG), Mark Sabaj Pérez (ANSP), and David Werneke (AUM) for helping to collect specimens and tissues; and the laboratory technicians Kristen Choffe and Oliver Haddrath (ROM) for helping to generate sequence data. Andreas Tanke provided most of the tissue samples from the undescribed *Panaqolus* species, live photos of aquarium specimens, and contributed with valuable discussions on our group of interest. Funding for this research came from NSF OISE-1064578 (International Research Fellowship) to NKL, NSF DEB-0315963 (Planetary Biodiversity Inventory: All Catfish Species), National Geographic Committee for Research and Exploration grant #8721-09 to NKL, the Coypu Foundation, the Aquatic Critter Inc., and the estate of George and Carolyn Kelso via the International Sportfish Fund. CAC benefited from a DCR fellowship from Brazilian Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and SEPLAN-RO (process 350674/2010-8). Additional funding came from NSF grant DEB 0516831 to K.O. Winemiller, R.L. Honeycutt and HLF, a Conservation Research grant from the Life in Crisis: Schad Gallery of Biodiversity and Museum Volunteers research grants (2009, 2010) from the Royal Ontario Museum to HLF, and Discovery Grants from the Natural Sciences and Engineering Research Council of Canada to HLF. Salary support for NKL provided by NSF DEB-1257813 (the iXingu Project) and the Canada Department of Fisheries and Oceans.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2016.12.040.

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