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Multilocus phylogeny, diagnosis and generic revision of the Guiana Shield endemic suckermouth armoured catfish tribe Lithoxini (Loricariidae: Hypostominae)

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With > 450 species, Hypostominae is the most species-rich subfamily of suckermouth armoured catfishes (Loricariidae). Both morphological and molecular analyses of Hypostominae strongly support monophyly of ten distinctively small-bodied (< 9 cm standard length), highly depressed (maximal head depth > 7.2 times in standard length) species comprising two currently valid genera (*Exastilithoxus* and *Lithoxus*) that are restricted to fast-flowing, rocky-bottomed rivers and streams draining the Guiana Shield in northwestern South America. We describe the tribe Lithoxini for this clade and present a multilocus phylogeny for eight Lithoxini species, including type species of all nominal genera and subgenera. Based on morphological and molecular data, we resurrect and redescribe the previously synonymized subgenus *Paralithoxus* for a strongly supported clade of eight species that are restricted to rivers south and east of Venezuela and have seven to nine interdorsal plates and five rows of plates on the caudal peduncle. We also erect the new genus *Avalithoxus* for *Lithoxus jantjae*, a species that is narrowly endemic to the Ventuari River immediately upstream of Salto Tencua and is unique in Hypostominae for having only 12 branched caudal-fin rays. *Lithoxus* is restricted to *Lithoxus lithoides* from the Essequibo and Correntyne rivers in Guyana and Suriname. A redescription of *Paralithoxus bovallii* based on recently collected specimens and an identification key to all four genera are also provided. Geographical distributions of Lithoxini clades suggest that geological uplift and geodispersal via stream capture played important roles in promoting vicariance and allopatric speciation.

ADDITIONAL KEYWORDS: *Ancistrus bovallii* – *Avalithoxus* – *Exastilithoxus* – Guyana – *Paralithoxus* – Venezuela.

INTRODUCTION

The Neotropical-endemic suckermouth armoured catfish family Loricariidae contains > 970 species (Eschmeyer & Fong, 2018), making it the most species-rich family of catfishes and fifth most species-rich family of vertebrates. Approximately half of all loricariids in ~40 genera compose the subfamily Hypostominae, which is broadly distributed across tropical South and southern Central America. Such broad taxonomic and geographical diversity, combined with large-scale discordance between Hypostominae phylogenies based on molecular vs. morphological data (e.g. Armbruster, 2004, 2008; Lujan *et al.*, 2015), significantly challenge efforts to revise the taxonomy of Hypostominae subclades comprehensively. Nonetheless, some Hypostominae subclades are consistently well supported by both data sets. One such clade is the *Lithoxus* clade (*sensu* Lujan *et al.*, 2015), which contains the genera *Exastilithoxus* and *Lithoxus*. Isbrücker (1980]) had previously proposed the subtribe Lithoxina for these genera, but this taxon was never formally described or diagnosed, leaving it largely ignored in subsequent taxonomic literature.

The distinctively small-bodied and depressed suckermouth catfish genus *Lithoxus* was first erected as a brief mention in a key by Eigenmann (1910), with

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a subsequent full description and designation of the then new *Lithoxus lithoides* as the type species by Eigenmann (1912). Since its erection, the boundaries of *Lithoxus* have been blurred by erection of the morphologically similar genus *Exastilithoxus* by Isbrücker and Nijssen (in the paper by Isbrücker, 1979; type species *Pseudacanthicus* (*Lithoxus*) *fimbriatus* Steindachner, 1915) and by a short-lived attempt to subdivide *Lithoxus* into two subgenera by Boeseman (1982). Boeseman (1982) used external morphological characteristics to argue that *Lithoxus* should be subdivided into the monotypic subgenus *Lithoxus* and a new subgenus, *Paralithoxus*, in which Boeseman placed four species from rivers draining the Guiana Shield east of Venezuela. *Ancistrus bovallii* Regan, 1906 was designated as the type species for *Paralithoxus*, and in the same publication Boeseman described three new *Paralithoxus* species: *Paralithoxus pallidimaculatus*, *Paralithoxus planquettei* and *Paralithoxus surinamensis*. Eight years later, Nijssen & Isbrücker (1990) dissolved *Paralithoxus* back into *Lithoxus* with their description of *Lithoxus stocki*, arguing without phylogenetic evidence that continued recognition of *Paralithoxus* would unjustifiably complicate taxonomy and necessitate the description of several additional genera. Subsequently described *Lithoxus* species that would have fitted Boeseman's definition of *Paralithoxus* include *Lithoxus boujardi* Muller & Isbrücker, 1993, *Lithoxus jariensis* Silva *et al*., 2017, and *Lithoxus raso* Silva *et al*., 2017. In contrast to this serial expansion of *Lithoxus*, only one species has been added to the genus *Exastilithoxus* since its erection: *Exastilithoxus hoedemani* Isbrücker and Nijssen, 1985. *Exastilithoxus*, which also includes several known but undescribed species (Lujan *et al.*, 2015), primarily differs from *Lithoxus* (including *Paralithoxus*) by having digitate papillae around the margin of the oral disc (Fig. 1).

Lujan (2008) revisited the question of *Paralithoxus* validity with his description of *Lithoxus jantjae*, a species that appeared to reinforce morphological differences that Boeseman (1982) highlighted between *Lithoxus* and *Paralithoxus*. In contrast to all putative members of *Paralithoxus*, *L. jantjae* shares with *L. lithoides* the presence of four branched anal-fin rays (vs. five in *Paralithoxus*) and five interdorsal plates (vs. seven or eight in *Paralithoxus* species having an adipose fin). *Lithoxus jantjae* also lacks digitate papillae around the oral disc margin. At that time, the genus *Lithoxus*, including putative *Paralithoxus* species, was believed to be a monophyletic genus sister to *Exastilithoxus* based on results of Armbruster's (2004) morphology-based phylogenetic analysis of the Loricariidae, thus no recommendation was made to resurrect *Paralithoxus* at that time.

Intrageneric phylogenetic analyses of *Lithoxus* and *Exastilithoxus* began with Lujan *et al.* (2015), who analysed two mitochondrial (16S and *Cytb*) and three nuclear gene regions (*RAG1*, *RAG2* and *MyH6*) spanning *L. lithoides*, *L. jantjae*, *Exastilithoxus fimbriatus*, several undescribed *Exastilithoxus* species, and three species that fit Boeseman's (1982) definition of *Paralithoxus* (*Lithoxus pallidimaculatus*, *Lithoxus planquettei* and *L.* cf. *stocki*). Lujan *et al.* (2015) confirmed overall monophyly of the *Lithoxus* clade containing these species, plus respective monophyly of *Exastilithoxus* (including *E. fimbriatus* and several undescribed species) and a clade consistent with the putative subgenus *Paralithoxus*. However, *L. lithoides* and *L. jantjae* did not form their own clade. *Lithoxus lithoides* was sister to *Exastilithoxus*, and the position of *L. jantjae* was unresolved. Also, tissues of the type species of *Paralithoxus* (*Ancistrus bovallii* Regan, 1906) were not available at that time, so the taxonomic status of *Paralithoxus* was not tested. A more restricted molecular analysis of six *Lithoxus* (*Paralithoxus*) species based only on the mitochondrial *COI* marker was recently published by Silva *et al*. (2017), but interspecific relationships were poorly resolved, and this analysis omitted specimens of the true *Lithoxus bovallii*. Thus, a more robust phylogenetic hypothesis and taxonomic revision that includes the type species of *Paralithoxus* is still needed.

Lithoxus bovallii was originally described by Regan (1906) based on specimens received from Dr Carl Bovallius, a Swedish naturalist who travelled extensively across the Guiana Shield highlands near the borders between Brazil, Guyana and Venezuela. Bovallius also resided along the upper Potaro River above Kaieteur Falls, where he ran the Essequibo Exploration Company (Eigenmann, 1909: 34). Unfortunately, the type locality that Regan listed for *L. bovallii* (the 'Kaat River, tributary to the Treng River, Upper Potaro, British Guiana') does not match any currently recognized stream or river in or near the upper Potaro River. Moreover, fieldwork in the upper Potaro River in 1998 and 2014 by J.W.A. and others (Hardman *et al.*, 2002) and in 2005 and 2011 by N.K.L. and others failed to yield any specimens assignable to the genus *Lithoxus*. Given the apparent absence of *Lithoxus* from the Potaro River itself, and the similarity in spelling between the 'Treng River' locality given by Regan and the 'Ireng River' whose headwaters border those of the Potaro, it has long been assumed that the upper Ireng River was the true type locality for *L. bovallii* (e.g. Eschmeyer, Fricke & van der Laan, 2017). However, little scientific fieldwork had been conducted in the upper Ireng River, leaving open the question of where *L. bovallii* was originally collected. A January 2016 expedition to the upper Ireng by N.K.L. and J.W.A. (Lujan, 2017) confirmed this as the type locality for *L. bovallii* (Fig. 2) and yielded a large series of *L. bovallii* specimens, tissues and live photographs that form part of the basis of the present study.

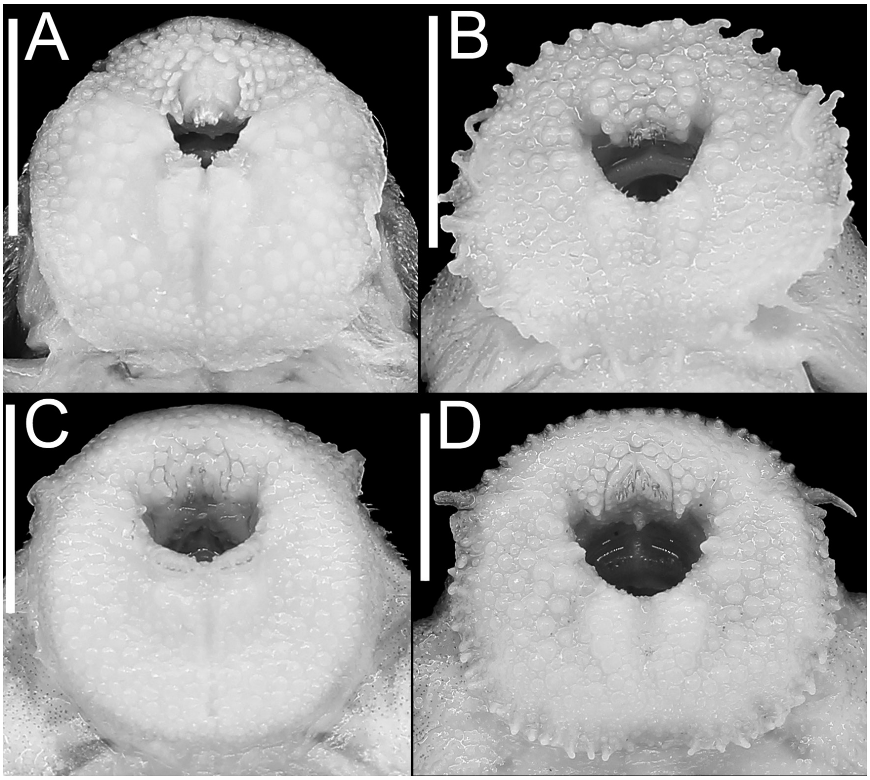

Figure 1. Oral discs of: A, *Avalithoxus jantjae*, AUM 39478; B, *Exastilithoxus fimbriatus*, MCNG 44872; C, *Lithoxus lithoides*, AUM 28018; and D, *Paralithoxus bovallii*, AUM 67174. Scale bars represent 5 mm.

Our goals for this study are as follows: (1) to describe and diagnose the tribe Lithoxini; (2) to re-examine molecular phylogenetic relationships within this clade based on the addition of more taxa and sequence data; (3) to update the genus-level classification of Lithoxini species based on combinations of molecular and morphological data, including the resurrection of *Paralithoxus* and the description of one new genus; and (4) to redescribe *L. bovallii* based on recently collected specimens from the upper Ireng River.

MATERIAL AND METHODS

Taxon sampling

We sampled most densely within the *Lithoxus* clade (*sensu* Lujan *et al.*, 2015; or subtribe Lithoxina *sensu* Isbrücker, 1980; tribe Lithoxini herein) and focus our results and discussion on patterns within this clade. Lineages from throughout the clade's geographical range (Fig. 2), including eight of the clade's 12 recognized species (67%), were examined. However, only five species were represented by individuals that were phenotypically and geographically close enough to type specimens and localities for us to be certain that they represented the named lineage: *E. fimbriatus*, *L. bovallii*, *L. jantjae*, *L. lithoides* and *L. planquettei*. In the absence of a comprehensive taxonomic revision of this group, and given the potential for cryptic, allopatric diversity to go unrecognized in small-bodied, philopatric species (Fusinatto *et al.*, 2013), we refer to the three additional species that are not from or near type localities as *Lithoxus* cf. *pallidimaculatus*, *Lithoxus* cf. *stocki* and *Lithoxus* cf. *surinamensis*. Finally, we include several populations of *Exastilithoxus* and *Lithoxus*/*Paralithoxus* that we suspect are undescribed species within widespread species groups. These are identified as 'sp. nov. aff.', followed by the most closely related species name and the river drainage from which they were collected, which is a conservative approach that minimizes the proliferation of names while conveying the likely occurrence of unrecognized diversity, the need for additional study and the populations/species that need comparison. Missing from our analyses are the species *Exastilithoxus hoedemani*, *L. jariensis* and *Lithoxus raso* (respectively from the Negro, Jari and Amapa drainages in northern Brazil), and *L. boujardi* from the Approuague and Oyapock rivers in French Guiana.

Tissue and DNA sources

Newly generated sequence data (Tables 1, 2) were obtained from tissue samples or DNA extracts

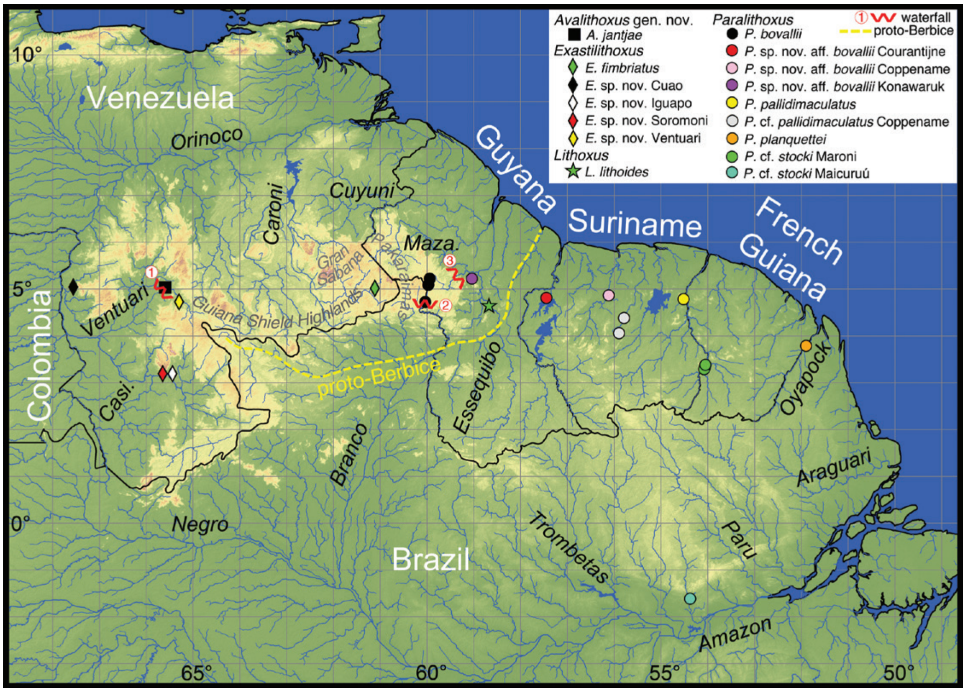

Figure 2. Geographical distribution of specimens examined in our molecular phylogenetic analyses. Yellow dashed line represents the hypothesized route of the main channel of the historical proto-Berbice River (McConnell, 1968). Red numbers refer to the following waterfalls: 1, Salto Tencua; 2, Orinduik Falls; 3, Kaieteur Falls. Abbreviations: Casi., Casiquiare; Maza., Mazaruni.

collected by the authors or provided by the Academy of Natural Sciences of Drexel University in Philadelphia, PA, USA (ANSP), the Auburn University Museum Fish Collection in Auburn, AL, USA (AUM), the Pontifícia Universidade Católica do Rio Grande do Sul, Museu de Ciências e Tecnologia (MCP), the Royal Ontario Museum in Toronto, Canada (ROM), or obtained directly from collectors Christian Cramer (University of Marburg) and Karsten Schönherr (independent aquarium fish hobbyist). Voucher specimens (Table 2) were identified either by direct examination of specimens or by photograph of the source specimen.

MOLECULAR MARKERS AND DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Molecular phylogenetic methods followed those of Lujan *et al.* (2015a) except that a region of the NADH dehydrogenase 2 (*ND2*) gene was added to this analysis. In brief, we amplified and sequenced a fragment of the mitochondrial 16S (515 bp), cytochrome *b* (1050 bp) and *ND2* (1032 bp) genes as well as the nuclear *RAG1* (1019 bp), *RAG2* (867 bp) and *MyH6* (657 bp) genes for a total of 5,140 aligned base pairs. Fragments were amplified using combinations of previously published primers (*ND2*: Arroyave, Denton & Stiassny, 2013; all others: Lujan *et al.*, 2015). Whole genomic DNA was extracted from fin or muscle tissues preserved in 95% ethanol following either manufacturer's instructions for the DNeasy Blood & Tissue Kit (Qiagen N.V., Venlo, Netherlands) or standard laboratory protocols for salt extraction and ethanol precipitation. Fragment amplifications were performed following the methods of Lujan *et al.* (2015a). Post-PCR cleanup of all loci was achieved by either 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) or by adding ExoSap-IT™ (Applied Biosystems Co., Foster City, CA) and following manufacturer's instructions. For samples that were gel purified, 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 15000 rpm). Forward and reverse sequencing reactions either followed the manufacturer's recommendations for sequencing on an Applied Biosystems™ 3730 DNA analyzer (LTI) at the Royal Ontario Museum or were conducted by staff at The Centre for Applied Genomics at The Hospital for Sick Children (SickKids) in Toronto, ON, Canada. All new sequence data generated for this study have been uploaded to GenBank under the following accession numbers: MH490817–MH490827 (16S), MH490828–MH490838 (cytb), MH490850–MH490865 (ND2), MH490839–MH490849 (MyH6), MH490866–MH490877 (RAG1), MH490878–MH490886 (RAG2).

Sequence assembly, alignment and phylogenetic inference

Sequence data were assembled, edited, aligned, and concatenated following the methods of Lujan *et al.* (2015). PartitionFinder (v1.1.1; Lanfear *et al.*, 2012) was used to determine codon-position-specific models of molecular evolution for each gene under the Bayesian information criterion (BIC). For the Bayesian phylogenetic analysis using MrBayes (v3.2.3; Ronquist & Huelsenbeck, 2003), an Hasegawa, Kishino and Yano (HKY) model with rate heterogeneity being modelled by a gamma distribution (HKY+G) was determined to be the best model of molecular evolution for the second positions of *Cytb* and *ND2* and the first positions of *RAG1*, *RAG2* and *MyH6*. An HKY model with proportion of invariable sites estimated (HKY+I) was determined to be the best model of molecular evolution for the third position of *ND2* and the second positions of *MyH6*, *RAG1* and *RAG2*. A Kimura, 1980 (K80) model with rate heterogeneity being modelled by a gamma distribution (K80+G) was determined to be the best model of molecular evolution for the third positions of *RAG1*, *RAG2* and *MyH6*. A symmetrical (SYM) model with rate heterogeneity being modelled by a gamma distribution (SYM+G) was determined to be the best model of molecular evolution for 16S and the first position of *Cytb*. A general time reversible (GTR) model with rate heterogeneity being modelled by a gamma distribution (GTR+G) was determined to be the best model of molecular evolution for the third positions of *Cytb* and the first position of *ND2*. All data partitions were unlinked, with rates free to vary across partitions. For the maximum likelihood phylogenetic analysis using RAxML (v8.0.0; Stamatakis, 2014), GTR+G was determined to be the best model of molecular evolution for all positions. All analyses were rooted using *Lithogenes villosus* as the designated outgroup.

A Bayesian Markov chain Monte Carlo (MCMC) search of tree space was conducted using MrBayes programmed to run for 5 million generations using two sets of eight chains each (one cold, seven hot, with default temperature parameter), sampling

Table 1. Summary of valid Lithoxini genera and species

Species		Author	Year	Holotype	Paratypes	Country	Drainage
Avalithoxus gen. nov.							
A. jantjae	•	(Lujan)	2008	MCNG 55349	10	Venezuela	Ventuari (OD)
Exastilithoxus Isbrücker & Nijssen, 1979							
E. fimbriatus	•	(Steindachner)	1915	NMW 44418	–	Venezuela	Caroni (OD)
E. hoedemani		Isbrücker & Nijssen	1985	INPA 506	2	Brazil	Negro (AD)
Lithoxus Eigenmann, 1910							
L. lithoides	•	Eigenmann	1912	FMNH 53557	181	Guyana	Essequibo (NWA)
Paralithoxus Boeseman, 1982							
P. boujardi		(Muller & Isbrücker)	1993	MNHN 1992–1321	6	French Guiana	Approuague (NWA)
P. bovallii	•	(Regan)	1906	BMNH 1905.11.1.43	6 (syn)	Guyana	Ireng (AD)
P. jariensis		(Silva, Covain, Oliviera & Roxo)	2017	MZUSP 123131	89	Brazil	Jari (AD)
P. pallidimaculatus	•	Boeseman	1982	RMNH 28368	3	Suriname	Suriname (NWA)
P. planquettei	•	Boeseman	1982	RMNH 28304	12	French Guiana	Comté (NWA)
P. raso		(Silva, Covain, Oliviera & Roxo)	2017	MZUSP 123132	7	Brazil	Amapa (NWA)
P. stocki	•	(Nijssen & Isbrücker)	1990	IRSNB 639	41	French Guiana	Maroni (NWA)
P. surinamensis	•	Boeseman	1982	RMNH 28361	55	Suriname	Suriname (NWA)

Species included in our molecular phylogenetic analysis are designated with a bullet (•). Institutional abbreviations follow Sabaj (2016). Drainages indicated are those for the type locality only plus the following regional watershed abbreviations: AD, Amazon Drainage; NWA, Northwestern Atlantic coast of South America; OD, Orinoco Drainage. Other abbreviation: syn, syntype.

Table 2. Loci sequenced, voucher catalogue number, country and river drainage of origin for all tissue samples analysed in this study

Taxa	Tissue number	Type specimen	Type status	Number of loci	16S	*Cytb*	*ND2*	*RAG1*	*RAG2*	*MyH6*	Voucher catalogue number	Country	Drainage
Lithogeninae													
Lithogenes villosus	T17140		tt	5	X	X		X	X	X	ROM 95075	Guyana	Potaro
Loricariinae													
Ctenilorricaria platystoma	T06288			5	X	X	X	X	X		ROM 85921	Guyana	Essequibo
Hypostominae													
Lithoxus clade													
Avalithoxus jantjae	T9020	*	ht	6	X	X	X	X	X	X	AUM 39475	Venezuela	Ventuari
Exastilithoxus fimbriatus	V049	*	tt	5	X	X		X	X	X	AUM 36632	Venezuela	Caroni
Exastilithoxus sp. nov.	T09165			4	X			X	X	X	AUM 56685	Venezuela	Cuao
Exastilithoxus sp. nov.	V5561			6	X	X	X	X	X	X	AUM 43923	Venezuela	Iguapo
Exastilithoxus sp. nov.	V5536			4	X	X		X	X		AUM 43875	Venezuela	Soromoni
Exastilithoxus sp. nov.	T09667			6	X	X	X	X	X	X	AUM 54450	Venezuela	Ventuari
Lithoxus lithoides	T412		tt	5	X		X	X	X	X	AUM 37922	Guyana	Essequibo
Paralithoxus bovallii	AUF10022	*	tt	6	X	X	X	X	X	X	AUM 67059	Guyana	Ireng
Paralithoxus bovallii	AUF10147	*	tt	6	X	X	X	X	X	X	AUM 67127	Guyana	Ireng
Paralithoxus cf. *bovallii*	KS004			5	X	X	X	X		X	Photo voucher	Suriname	Coppename
Paralithoxus cf. *bovallii*	KS017			6	X	X	X	X	X	X	Photo voucher	Suriname	Courantijne
Paralithoxus cf. *bovallii*	T18089			4	X		X	X		X	ROM 96809	Guyana	Konawaruk
Paralithoxus cf. *pallidimaculatus*	T22559			6	X	X	X	X	X	X	ROM 101457	Suriname	Saramacca
Paralithoxus cf. *surinamensis*	T9021			6	X	X	X	X	X	X	AUM 50410	Suriname	Marowijne

Table 2. *Continued*

Taxa	Tissue number	Type specimen	Type status	Number of loci	16S	*Cytb*	*ND2*	*RAG1*	*RAG2*	*MyH6*	Voucher catalogue number	Country	Drainage
Paralithoxus cf. *stocki*	6907			6	X	X	X	X	X	X	ANSP 189139	French Guiana	Maroni
Paralithoxus cf. *stocki*	49999			6	X	X	X	X	X	X	MCP 49999	Brazil	Maicurú
Paralithoxus planquettei	T9040			6	X	X	X	X	X	X	Uncatalogued	French Guiana	Oyapock
Hypostominae outgroups													
‘*Pseudancistrus*’ *pectegenitor*	T09465	*		5	X	X		X	X	X	ANSP 190755	Venezuela	Ventuari
‘*Pseudancistrus*’ *sidereus*	T09506			5	X	X		X	X	X	ANSP 190756	Venezuela	Ventuari
Lithoxancistrus orinoco	T09663		tt	5	X	X		X	X	X	AUM 54439	Venezuela	Ventuari
Lithoxancistrus yekuana	T9004	*		5	X	X		X	X	X	AUM 39473	Venezuela	Ventuari
Neblinichthys brevibracchium	T06067	*		5	X	X		X	X	X	ROM 83692	Guyana	Mazaruni
Neblinichthys echinasus	T06066	*		4	X	X		X	X		ROM 83692	Guyana	Mazaruni
Paulasquama callis	T06189	*	pt	5	X	X		X	X	X	ROM 83784	Guyana	Mazaruni
Pseudancistrus asurini	B1509	*		5	X	X		X	X	X	ANSP 199533	Brazil	Xingu
Pseudancistrus barbatus	85.1	*	tt	5	X	X		X	X	X	MHNG 2653.059	French Guiana	Maroni
Pseudancistrus corantijniensis	JMB1	*		5	X	X		X	X	X	MHNG 2672.092	French Guiana	Corentyne
Pseudancistrus depressus	JMB2	*		5	X	X		X	X	X	MHNG 2651.069	Guyana	Essequibo
Pseudancistrus sp. nov.	T14764			3		X		X		X	MCP 46103	Brazil	Branco
Pseudancistrus nigrescens	G5942	*		6	X	X	X	X	X	X	AUM 45299	Guyana	Essequibo

Taxa are listed in indented groupings according to subfamily. ‘Type status’ indicates that a voucher was either part of the type series for that species (ht, holotype; pt, paratype) or was collected from at or near the type locality (tt, topotype). Specimens for which no type status is given had no type status.

every 666 trees with the first 25% of trees (1,877) being discarded as burn-in, thus generating a total of 5630 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 500 as determined by the program Tracer (v1.6; Rambaut, Drummond & Suchard, 2007). Maximum likelihood analysis was conducted locally using RAxML programmed first to conduct 500 independent runs with random starting trees to search for the best tree and then to generate bootstrap support values based on a 2000 replication search of tree space.

PRESENTATION OF PHYLOGENETIC RESULTS

Complete results of the Bayesian and maximum likelihood analyses are presented as Supporting Information (Figs S1, S2). The manuscript phylogeny Figure 3 was trimmed of outgroup taxa and built from results of the Bayesian analysis; however, node support values from both the Bayesian and concatenated maximum likelihood analyses are provided. 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.

MORPHOMETRICS

Measurements were made with digital callipers to the nearest 0.1 mm. Measurements and counts of bilaterally symmetrical features were made preferentially from the left side and followed Armbruster (2003), with the exception that mouth length was the entire length of the oral disc extending to the snout tip and that premaxillary length was preferentially measured on the right side because this was easier. Counts follow Armbruster *et al.* (2000). Names of skeletal characteristics follow Schaefer (1987) and Geerinckx, Brunain & Adriaens (2007), and names of plate rows follow Schaefer (1997). For fin ray counts, Arabic numbers refer to numbers of branched fin rays and Roman numerals refer to unbranched fin rays, with upper case indicating rays ossified as spines and lower case indicating flexible rays. Numbers of specimen with a given count reported parenthetically. Institutional abbreviations follow Sabaj (2016). Morphometric and meristic data were examined in JMP (v.11.0; SAS Institute, 2013).

TAXONOMIC ACCOUNTS

LITHOXINI TRIB. NOV.

Composition: This taxon is established for the clade containing the described genera *Exastilithoxus* and *Lithoxus* and for the newly resurrected or described genera *Avalithoxus* and *Paralithoxus* (Fig. 3).

Diagnosis: Externally, Lithoxini can be diagnosed from all other Loricariidae except *Leporacanthicus* by having a distinctly round and flat oral disc, in which the anterior lip does not fold ventrally and the mandibular barbels are anterolaterally positioned and directed (Fig. 1; vs. typically ovoid oral disc, with an anterior lip that folds ventrally and mandibular barbels that are

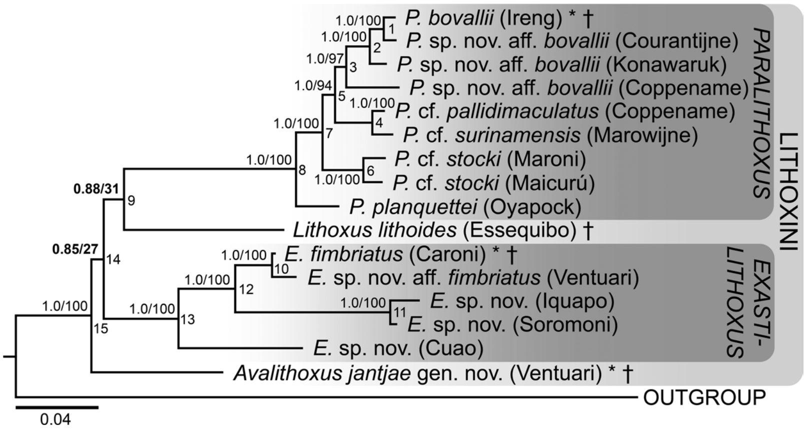

Figure 3. Phylogenetic relationships within Lithoxini (Loricariidae: Hypostominae), including ***Avalithoxus* gen. nov.** and the resurrected and elevated genus *Paralithoxus* Boeseman, 1982. Relationships are based on a Bayesian analysis of a 5140 bp alignment consisting of three mitochondrial (16S, *Cytb* and *ND2*) and three nuclear loci (*RAG1*, *RAG2* and *MyH6*; Table 2). Numbers before each node correspond to Bayesian posterior probability and maximum likelihood support values. Numbers after each node correspond to the node numbers given in the text. *The specimen used to represent this species was a holotype, paratype or topotype (see Table 2). †This species is the type for its genus.

Table 3. Characteristics interspecifically variable within *Paralithoxus*, summarized from direct observation, Boeseman (1982), Muller & Isbrücker (1993), Nijssen & Isbrücker (1990) and Silva *et al*. (2017)

Characteristic	*P. boujardi*	*P. bovallii*	*P. jariensis*	*P. pallidimaculatus*	*P. planquettei*	*P. raso*	*P. stocki*	*P. surinamensis*
Adipose fin	Present	Present	Present	Absent	Present	Present	Present	Absent
White spots on body	Small	Absent	Absent	Small	Absent	Large	Absent	Absent
Paired-fin bars	Absent	Present	Present	Present	Absent/faint	Present	Absent	Present
White margin of caudal fin	Present	Absent	Absent	Absent	Absent	Absent	Present	Absent
Enlarged odontodes on anterodistal margin of pectoral-fin spine	Present	Absent	Present	Absent	Absent	Present	Present	Absent
Predorsal plates	3(4)	5(6,7)	4*	5	4	4*	4	6
Interdorsal plates	8	(7)8	7–9*	n.a.	7	7*	7	n.a.

Asterisks (*) indicate corrected values that differ from those in the original description and have been confirmed via personal correspondence with the authors. Plate counts are given as modes, with less common variations from the mode in parentheses. Abbreviation: n.a., not applicable.

laterally positioned and posterolaterally directed); from all non-Hypostominae loricariids plus *Corymbophanes*, *Hypostomus* and most *Pterygoplichthys* by having a well-developed cluster of evertible cheek odontodes on each side of the head; from all other members of the Hypostominae except *Hypancistrus*, *Leporacanthicus*, *Panaqolus*, *Panaque*, *Pseudacanthicus*, *Pseudoqolus*, *Scobinancistrus* and *Spectracanthicus* by having < 13 teeth in either the dentary or the premaxilla; from *Hypancistrus*, *Leporacanthicus*, *Panaqolus*, *Panaque* *Pseudacanthicus*, *Pseudoqolus*, *Scobinancistrus* and *Spectracanthicus* by having an extremely depressed body with maximum head depth > 7.2 times in standard length (SL; vs. < 7 times; head depth measured from immediately posterior to the supraoccipital to a point directly ventral on the ventral surface); from *Leporacanthicus*, *Panaque*, *Pseudacanthicus*, *Pseudoqolus*, *Scobinancistrus* and *Spectracanthicus* by growing no larger than 60 mm SL (vs. > 90 mm SL); from *Panaqolus*, *Panaque*, *Pseudoqolus* and *Scobinancistrus* by having flattened, acute tooth cusps (vs. spoon shaped or broadly truncate); and from *Leporacanthicus* by having all teeth approximately coequal in length (vs. two enlarged and elongate premaxillary teeth much longer than dentary teeth).

Cladistically, Armbruster (2004) found 23 mostly internal osteological changes along the branch leading to Lithoxini, including many reversals. These changes included the following: a decrease in the length and width of the accessory process of ceratobranchial 1 (characters 7-1, 8-1), loss of the accessory process of epibranchial 1 (14-0), loss of the posterior shelf of epibranchial 4 (17-0), elongation of hypobranchial 1 (23-1), loss of the interhyal (26-0), reversal to a round upper pharyngeal tooth plate with evenly distributed teeth (30-0), reversal to a pointed lateral edge of the posterohyal (32-0), incorporation of the posterior process of the hyomandibula into the main body of the hyomandibula (41-1; unique to the clade), loss of the lateral wall of the metapterygoid channel (52-1), longitudinal ridge on the quadrate (68-1), dentaries forming an acute angle (69-1), a bowling pin-shaped maxilla (71-1), reduction in the size of the metapterygoid disc (100-1), presence of bifid hemal spines (122-1), first neural spine anterior to first dorsal-fin pterygiophore (125-1), reduced exposure of the cleithral process (157-1), reversal to curved anterolateral processes of the pelvic basipterygium meeting or nearly meeting at the midline (167-0), loss of the posteroventral ridge of the pelvic basipterygium (173-0) and the presence of enlarged teeth (205-2). Lithoxini was among the best-supported clades described by Armbruster (2004) with a Bremer decay index (Bremer, 1988) of 17.

Phylogenetic relationships within Lithoxini (Fig. 3): Molecular data strongly support monophyly of Lithoxini (node 15: BI, 1.0; ML, 100) and its inclusion of the respectively strongly monophyletic genera *Exastilithoxus* (node 13: BI, 1.0; ML, 100) and *Paralithoxus* (node 8: BI, 1.0; ML, 100) and the monotypic genera and species *L. lithoides* and *Avalithoxus jantjae* gen. nov. Relationships among these lineages were weakly supported, albeit topologically consistent. Both Bayesian and maximum likelihood analyses found weak support for *A. jantjae* to be sister to a clade containing all other species (node 14: BI, 0.85; ML, 27), and within this latter clade *Exastilithoxus* was consistently found to be sister to a clade in which *L. lithoides* was sister to *Paralithoxus* (node 9: BI, 0.88; ML, 31).

At the basal node within *Exastilithoxus*, an undescribed species from the Cuao River in southern Venezuela was found to be sister to a strongly supported clade (node 12: BI, 1.0; ML, 100) containing *E. fimbriatus*, from headwaters of the Caroni River on the eastern Venezuelan Gran Sabana plateau, and three undescribed lineages from tributaries of the upper Orinoco in southern Venezuela. Intriguingly, *E. fimbriatus* was strongly supported as sister (node 10: BI, 1.0; ML, 100) to an undescribed lineage from the far upper Ventuari River. Both these species originate from streams atop the Guiana Shield escarpment that are > 450 km apart from east to west (Fig. 2). Despite this disjunct distribution and great distance, these lineages exhibited only ~2.4% *Cytb* sequence divergence. The two remaining, undescribed *Exastilithoxus* lineages, respectively from the Iguapo and Soromoni rivers, were also strongly monophyletic (node 11: BI, 1.0; ML, 100). The Iguapo and Soromoni rivers are lower-elevation tributaries that enter the right bank of the upper Orinoco River upstream of the Casiquiare Canal and have mouths that are < 25 river km apart (Fig. 2). Despite the geographical proximity of these localities and the hydrological connectivity of these habitats via the Orinoco River main channel, the Iguapo and Soromoni rivers drain opposite (east–west) sides of Cerro Duida [elevation, 2358 m above sea level (a.s.l.)], and their *Exastilithoxus* populations also exhibit ~2.4% *Cytb* sequence divergence. All *Exastilithoxus* lineages were restricted to drainages west of the Essequibo River watershed (Fig. 2).

At the basal node within *Paralithoxus*, *P. planquettei* (from the Oyapock River along the border between French Guiana and Brazil) was sister to a strongly supported clade (node 7: BI, 1.0; ML, 100) containing species distributed from the Maroni (French; Dutch: Marowijne) River in the northeast to the Ireng River, which is > 650 linear km to the west of the Maroni, and the Maicurú River, which is > 650 linear km to the south of the Maroni (Fig. 2). Within this latter clade, a clade containing *Paralithoxus stocki* morphotypes from the geographically distant and disjunct Maroni and Maicurú rivers was found to be strongly monophyletic (node 6: BI, 1.0; ML, 100), albeit with ~4.9% *Cytb* sequence divergence between lineages. The *P. stocki* clade was found to be sister to a strongly supported clade (node 5: BI, 1.0; ML, 94) containing all remaining species. Within this last clade, a clade of *P. pallidimaculatus* morphotypes from the Saramacca and Marowijne drainages in Suriname was sister to a strongly supported clade (node 3: BI, 1.0; ML, 97) containing *P. bovallii* morphotypes. Within the *P. bovallii* clade, a lineage from the Coppename River in Suriname was sister to a strongly supported clade (node 2: BI, 1.0; ML, 100) containing an undescribed species from the Konawaruk River in Guyana, and the strongly supported clade (node 1: BI, 1.0; ML, 100) containing *P. bovallii* topotypes from the Ireng River and *P.* sp. nov. aff. *bovallii* from the Courantijne River. The undescribed Courantijne River lineage exhibited ~3% *Cytb* and 1.1% *ND2* sequence divergence from topotypic *P. bovallii*. The undescribed Konawaruk River species exhibited ~2.8% *ND2* sequence divergence from both topotypic *P. bovallii* and the undescribed species from the Courantijne (*Cytb* was not obtained from the Konawaruk lineage).

AVALITHOXUS GEN. NOV.

urn:lsid:zoobank.org:act:68E963BF-A8CA-437C-BE9C-DD593968E34D

Type species: Lithoxus jantjae Lujan, 2008: 414.

Diagnosis: Avalithoxus can be diagnosed from other members of Lithoxini by having 12 branched caudal-fin rays (vs. 14 or, rarely, 13), four branched anal-fin rays (vs. five in *Paralithoxus*), five interdorsal plates (vs. six to nine in *Exastilithoxus* and *Paralithoxus*), at most low hemispherical papillae around the oral disc margin (Fig. 1; vs. digitate papillae in *Exastilithoxus*), and a maximum of 25 hypertrophied cheek odontodes (mode, 18; range, 16–25) vs. a maximum of 21 in *Lithoxus* (mode, 12; range, 5–21).

Etymology: Avalithoxus is a portmanteau of the Latin word *ava*, meaning 'grandmother', and the genus name *Lithoxus*. It refers to both the apparently basal position of this lineage and the matronym of the type species, which honoured Jeanne Lujan, mother of N.K.L.

Composition: Avalithoxus currently includes only *Avalithoxus jantjae* (Lujan, 2007).

Geographical range: Avalithoxus is known only from ~180 m a.s.l. in the Ventuari River, immediately upstream of Salto Tencua in the central region of Amazonas State, Venezuela (Fig. 2). Repeated sampling of habitats immediately downstream of Salto Tencua, at ~130 m a.s.l., in 2004 and 2010, and ~50 linear km further upstream (130 m higher elevation) in 2010, yielded no specimens of *Avalithoxus*. At our most upstream sampling points in the Ventuari River, near the Yekuana community of Cacuri, *Avalithoxus* was entirely replaced by an undescribed species of *Exastilithoxus* that we found in the present study to be sister to *E. fimbriatus*. Across the ~50 linear km between Salto Tencua and Cacuri, there is a 130 m drop in river elevation and a long series of high-energy rapids that might act as a barrier or intergrade zone between these two Lithoxini lineages.

Table 4. Morphometrics of *Paralithoxus bovallii* based on measurements of specimens collected in 2016

Interlandmarks	Measurement	Mean	SD	Minimum	Maximum	*N*
1–20	SL (mm)	47.8		33.0	61.8	24
Percentage of SL						
1–10	Predorsal L	46.6	1.6	43.0	48.9	24
1–7	Head L	34.6	1.8	29.5	37.5	24
7–10	Head–dorsal L	11.9	1.3	8.1	15.1	24
8–9	Cleithral W	29.9	3.4	21.9	34.3	24
1–12	Head–pectoral L	26.3	6.9	1.8	31.1	24
12–13	Thorax L	23.2	2.4	18.6	29.5	24
12–29	Pectoral spine L	26.2	5.1	6.9	33.0	23
13–14	Abdominal L	22.5	1.7	18.6	24.9	24
13–30	Pelvic spine L	22.8	4.3	4.9	29.0	24
14–15	Postanal L	32.7	4.8	24.9	53.1	24
14–31	Anal fin spine L	10.4	1.6	4.5	12.6	24
10–12	Dorsal–pectoral L	26.3	1.4	23.9	29.1	24
10–11	Dorsal spine L	20.5	1.3	17.3	22.8	24
10–13	Dorsal–pelvic D	14.5	1.4	11.3	17.4	24
10–16	Dorsal–fin base L	18.0	1.1	16.2	20.1	24
16–17	Dorsal–adipose L	22.1	4.3	2.9	26.1	24
17–18	Adipose-spine L	6.2	2.3	3.6	15.6	24
17–19	Adipose–upper caudal L	12.2	2.3	9.3	18.0	24
15–19	Caudal peduncle D	8.8	0.7	7.6	9.9	24
15–17	Adipose–lower caudal D	17.3	2.2	14.1	22.1	24
14–17	Adipose–anal D	20.7	1.6	16.8	24.1	24
14–16	Dorsal–anal L	10.2	0.7	8.8	11.6	24
13–16	Pelvic–dorsal D	19.2	1.5	16.9	22.9	24
Percentage of HL						
5–7	Head–eye L	44.4	3.2	39.8	51.3	24
4–5	Orbit diameter	14.6	1.7	11.2	19.0	24
1–4	Snout L	52.7	3.2	48.6	59.8	24
2–3	Internares W	11.3	1.1	9.2	13.8	24
5–6	Interorbital W	38.9	4.7	27.2	45.2	24
7–12	Head D	56.6	3.4	50.0	66.8	24
1–24	Mouth L	54.5	4.2	46.5	61.2	24
21–22	Mouth W	50.0	3.8	38.6	56.6	24
22–23	Barbel L	11.0	2.5	5.6	15.0	24
25–26	Dentary tooth cup L	6.5	0.6	5.3	7.4	20
27–28	Premaxillary tooth cup L	7.8	1.3	4.2	10.4	20

Interlandmarks are the points between which each measurement was made (from Armbruster, 2003). Abbreviations: D, depth; HL, head length; L, length; *N*, number of specimens examined; SL, standard length; W, width.

PARALITHOXUS BOESEMAN, 1982: 46.

Type species: Ancistrus bovallii Regan, 1906: 96.

Diagnosis: Paralithoxus can be diagnosed from other members of Lithoxini by having at most low hemispherical papillae around the oral disc margin (Fig. 1; vs. digitate papillae in *Exastilithoxus*), 14 (rarely 13) branched caudal-fin rays (vs. 12 in *Avalithoxus*), five branched anal-fin rays (vs. four in *Avalithoxus* and *Lithoxus*), seven to nine interdorsal plates (vs. five in *Avalithoxus* and *Lithoxus*), and five rows of plates on the caudal peduncle (vs. three in *Avalithoxus* and *Lithoxus*).

Etymology: Boeseman (1982) did not provide an etymology for *Paralithoxus*, but it can be assumed that the original Greek meaning of the prefix *para*- (i.e. beside; next to, near, from; against, contrary to) was intended to distinguish this clade from *Lithoxus* while still alluding to their close relationship.

Composition: See Tables 1 and 3.

Geographical range: Paralithoxus is found in rocky riffle and rapids habitats from the Ireng River, along the westernmost border between Guyana and Brazil, in the west, to the Amapa River along the northern

Atlantic Coast of Brazil in the east. *Paralithoxus* is broadly distributed across most north-flowing Guiana Shield drainages between these limits, with the curious exception that it is absent from most of the Essequibo River drainage (everywhere except the Konawaruk River) and apparently also from the Berbice and Demerara rivers (Fig. 2). Given the widespread occurrence of *L. lithoides* throughout much of the Essequibo River drainage, the absence of *Paralithoxus* from most of this basin might be a result of competitive exclusion. *Paralithoxus* is also patchily but broadly distributed across other south-flowing Guiana Shield drainages between these limits (e.g. the Trombetas, Nhamundá and Paru rivers) as is evidenced by the recent description of *Paralithoxus jariensis* (Silva *et al*., 2017) from the Jari River in northeastern Brazil and by reports from European ornamental fish importers of distinctive *Paralithoxus* morphs from the Curuá, Jauaru and Araguari river drainages in Para and Amapa states, northern Brazil (Seidel, 2009).

PARALITHOXUS BOVALLII (REGAN, 1906: 96)
[FIGS 4, 5, TABLE 4]

Ancistrus bovallii Regan, 1906

Lectotype: BMNH 1905.11.1.43, 43.5 mm SL; British Guiana, Kaat River, tributary to the Treng River (sic, now confirmed as a misspelling of the Ireng River), C. Bovallius.

Paralectotypes: BMNH 1905.11.1.44–48 (six, four examined, 33.0–39.6 mm SL), same location as lectotype.

Other specimens examined: All collections Guyana, Region 8 (Potaro-Siparuni): AUM 67039, ten, Ireng River at Orinduik Falls, between upper and lower falls,

Figure 4. *Paralithoxus bovallii*, AUM 67192, 58.0 mm standard length, sexually mature male, Sukwabi Creek, East Fork, downstream of Wotowanda Falls, Region 8, Guyana. Scale bar represents 1 cm.

Figure 5. *Paralithoxus bovallii*, AUM 67080, 49.9 mm standard length, sexually mature female, Ireng River at Orinduik Falls, Region 8, Guyana.

04.72536°N, 060.03852°W, 2 January 2016, GUY 16-01, D. C. Werneke, J. W. Armbruster, N. K. Lujan, M. Ram; AUM 67048, one, Ireng River below lower Orinduik Falls, 04.71898°N, 060.03507°W, 3 January 2016, GUY 16-03, N. K. Lujan, J. W. Armbruster, D. C. Werneke, M. Ram; AUM 67103, 13, Tumong Creek, left-hand tributary of Ireng River, 04.71971°N, 060.01311°W, 6 January 2016, GUY 16-18, D. C. Werneke, N. K. Lujan, J. W. Armbruster, M. Ram; AUM 67119, one, Ireng River at first shoal upriver from split with Sukwabi Creek, 05.07711°N, 059.97423°W, 8 January 2016, GUY 16-23, N. K. Lujan, J. W. Armbruster, D. C. Werneke, M. Ram; AUM 67152, three, Monkey Creek and tributary 1.65 km upstream from mouth of Monkey Creek, 05.03524°N, 059.96504°W, 10 January 2016, GUY 16-28, J. W. Armbruster, D. C. Werneke, M. Ram; AUM 67174, 17, Sukwabi Creek at top of Andu Falls, 05.08955°N, 059.97514°W, 12 January 2016, GUY 16-33, J. W. Armbruster, N. K. Lujan, D. I. Brooks, M. Ram; AUM 67180, 11, Ireng River shoals at mouth of Monkey Creek near Patamona community of Kaibarupai, 05.04398°N, 059.97717°W, 12 January 2016, GUY 16-34, J. W. Armbruster, N. K. Lujan, D. I. Brooks; AUM 67198, one, Ireng River downstream of Kaibarupai, 05.02404°N, 059.97763°W, 14 January 2016, GUY 16-37, D. C. Werneke, J. W. Armbruster, N. K. Lujan, M. Ram, D. I. Brooks; AUM 67206, three, Ireng River at Waipa Landing, 04.93345°N, 059.99514°W, 14 January 2016, GUY 16-39, D. C. Werneke, J. W. Armbruster, N. K. Lujan, M. Ram, D. I. Brooks; AUM 67059, 16, Tumong Creek, left-bank tributary of Ireng River, 04.71388°N, 060.02234°W, 3 January 2016, GUY 16-04, N. K. Lujan, J. W. Armbruster, D. C. Werneke, M. Ram; AUM 67075, two, Ireng River at Orinduik Falls between upper and lower falls, 04.72536°N, 060.03852°W, 4 January 2016, GUY 16-09, D. C. Werneke, J. W. Armbruster, D. I. Brooks, M. Ram, N. K. Lujan; AUM 67080, four, Ireng River at Orinduik Falls about halfway between upper and lower falls, 04.72176°N, 060.03703°W, 4 January 2016, GUY 16-10, D. C. Werneke, J. W. Armbruster, D. I. Brooks, M. Ram, N. K. Lujan; AUM 67127, 33, Sukwabi Creek at top of Andu Falls, 05.08955°N, 059.97514°W, 9 January 2016, GUY 16-25, N. K. Lujan, J. W. Armbruster, D. C. Werneke, M. Ram, D. I. Brooks; AUM 67140, 25, Ireng River shoals at mouth of Monkey Creek near Kaibarupai, 05.04398°N, 059.97717°W, 9 January 2016, GUY 16-27, N. K. Lujan, J. W. Armbruster, D. C. Werneke; AUM 67192, nine, East Fork of Sukwabi Creek downstream of Wotowanda Falls, 05.08867°N, 059.96952°W, 13 January 2016, GUY 16-35, J. W. Armbruster, N. K. Lujan, D. I. Brooks, D. C. Werneke, P. Peters, R. Daniel, local fishermen.

Diagnosis: A summary of interspecifically variable characters within *Paralithoxus* is presented in Table 3. *Paralithoxus bovallii* can be diagnosed from *P. pallidimaculatus* and *P. surinamensis* by having an adipose fin (vs. adipose fin absent), from *P. boujardi*, *P. jariensis*, *P. planquettei*, *P. raso* and *P. stocki* by having five or more predorsal plates (vs. four or, rarely, three), from *P. boujardi*, *P. planquettei*,and *P. stocki* by having paired fins irregularly banded (vs. paired fins uniformly coloured), from *P. jariensis*, *P. planquettei*, *P. raso* and *P. stocki* by having typically eight (rarely seven) interdorsal plates (vs. typically seven), from *P. boujardi*, *P. raso* and *P. pallidimaculatus* by having a uniformly or mottled brown body lacking white spots (vs. body having small, distinct white spots in *P. boujardi* and *P. pallidimaculatus*, or large indistinct spots in *P. raso*), from *P. boujardi* and *P. stocki* by lacking a marginal white caudal-fin band (vs. band present), and from *P. boujardi* and *P. stocki* by lacking enlarged odontodes on the anterodistal margin of the pectoral-fin spines (vs. enlarged pectoral-fin spine odontodes present on males and females).

Description: Morphometrics are presented in Table 4. The largest specimen was 58.0 mm SL. Body depressed; dorsal profile forming slightly convex arc from tip of snout to posterior end of dorsal fin, with apex of arc slightly anterior to dorsal fin; nearly straight from end of dorsal fin to end of adipose-fin membrane, then angled dorsally to caudal fin. Ventral profile somewhat straight from snout tip to anal fin then slightly concave to caudal fin (apex of arc below posterior edge of adipose-fin spine). Caudal peduncle approximately teardrop shaped in cross-section, with ventral surface slightly flattened and wider than dorsal. Body widest at insertion of pectoral fins, narrowest at insertion of caudal fin. Snout rounded in dorsal view.

Eyes small; iris operculum absent. Interorbital surface flat, with modest lateral rise at supraorbital crests. Supraoccipital not elevated. Odontodes along lateral margin of opercle enlarged. Oral disc occupying entire ventral surface of head and extending anterior to snout. Ventral surface of dic covered with low, wide papillae; margin of disc fringed with low triangular papillae (Fig. 1D). Maxillary barbel short and projecting laterally or posterolaterally from anterolateral corners of upper lip (Fig. 1D).

Median plates 24(1), 25(12), 26(9), 27(2). Plates not keeled, but median rows of odontodes slightly larger, with odontodes increasing in size posteriorly in all plate rows. Plates in dorsal series: 5(17), 6(2) or 7(1) predorsal plates; 6(4) or 7(16) plates below dorsal fin; and 7(7) or 8(13) interdorsal plates. Five rows of plates on caudal peduncle; rows difficult to discern from adipose fin to caudal fin. Ventrum from anteroventral margin of snout to anal fin without plates. Evertible cheek plates supporting hypertrophied odontodes; odontodes evertible to ~90° from longitudinal body axis. Hypertrophied cheek odontodes 18–46 (mode, 26). Longest evertible cheek odontode extending to approximately one-half to three-quarters of the length of the opercle (not reaching opercular opening). Slightly enlarged odontodes present along anterodorsal surface of pectoral-fin spine, increasing in length distally. Parietosupraoccipital, compound pterotic, opercle, preopercle, frontal, nasal and infraorbitals supporting odontodes. Tip of snout completely covered in small plates.

Dorsal fin II,7; dorsal-fin spinelet V-shaped, dorsal-fin locking mechanism functional, dorsal-fin origin approximately equidistant from snout and insertion of dorsal spine of caudal fin; tip of dorsal fin very distant from adipose spine when adpressed. Adipose fin with single azygous preadipose plate, adipose-fin membrane generally directed straight ventrally from spine tip, but may extend slightly posteriorly. Caudal fin I,13,I(1) or I,14,I(23); caudal fin slightly emarginate, with lower lobe longer than upper. Procurrent caudal-fin spines appearing as plates, becoming larger posteriorly; 3(3) or 4(21) dorsal procurrent caudal-fin spines and 2(1) or 3(23) ventral procurrent caudal-fin spines. Pectoral fin I,6; pectoral-fin spine reaching slightly beyond base of pelvic-fin spine when adpressed ventral to pelvic fin; pectoral-fin spine thin. Pelvic fin i,5; unbranched pelvic-fin ray reaching approximately half of base of anal fin when adpressed. First branched rays of dorsal, caudal, pectoral and pelvic fins slightly longer than unbranched rays or spines. Anal fin i,5. Urogenital papilla distinct.

Teeth bicuspid with deep division between cusps, medial cusp longer than lateral; three to six left premaxillary teeth (mode five), and three to eight left dentary teeth (mode five); premaxillary teeth smallest laterally, with longest tooth usually the second or third from the midline, longest teeth two to four times the length of the smallest. Dentary teeth considerably shorter than premaxillary teeth, only slightly emergent from flesh around dentary; dentary teeth about half the width of premaxillary teeth.

Live coloration: Mottled light to dark brown dorsum, fins indistinctly and irregularly banded, no more than five indistinct bands on light brown base, abdomen and ventrum of anteromedial caudal peduncle translucent white, with melanophores dispersed around outer margin and concentrated around anal-fin origin, oral disc pale yellow.

Preserved coloration: Dorsum dark brown, with large, tan blotches behind head and occasionally faint spots on head. Tan blotches appearing as light saddles from middle of dorsal fin to posterior caudal peduncle; number of saddles variable, dark interspaces sometimes broken by thin, tan blotches. Tan areas not contiguous with each other but sometimes contiguous with tan ventrum of caudal peduncle. All fins except anal dark, with elongate spots centred on spines and rays (interspaces and membranes tan to hyaline). Anal fin tan, with small dark spot located at the middle of anterior fin rays.

Sexual dimorphism: External body shape differences are subtle (Figs 4 vs. 5). Males have a narrow, tubular genital papilla. Females have a wide, flat genital papilla (longer than males), with short, thin, posteromedial projection. One mature male examined (Fig. 4) with very large, flat testes, and one mature female examined (Fig. 5) with approximately six large eggs per ovary.

Range: Known only from the upper Ireng River along the western border between Guyana and Brazil (Fig. 2), although morphologically similar species occur patchily within the Essequibo River drainage (e.g. Konawaruk River) in Guyana and in the Nickerie and Sipalawini rivers in Suriname.

KEY TO GENERA OF LITHOXINI

1a. Oral disc surrounded by outwardly radiating digitate papillae; papillae may extend > 2 mm beyond the opaque oral disc margin (Fig. 1B) or be largely embedded within a thin (translucent when living) membrane surrounding the opaque oral disc .... *Exastilithoxus*

1b. Oral disc margin lacking distinctly digitate papillae, although low hemispherical papillae may be present (Fig. 1A, C, D) 2

2a. Twelve branched caudal-fin rays .... *Avalithoxus jantjae*

2b. Fourteen (rarely 13) branched caudal-fin rays 3

3a. Four branched anal-fin rays; five interdorsal plates; three rows of plates on caudal peduncle; adipose-fin membrane extending posteriorly beyond posterior projection of tip of adipose-fin spine .... *Lithoxus lithoides*

3b. Five branched anal-fin rays; seven or eight interdorsal plates (when adipose fin present); five rows of plates on caudal peduncle; adipose-fin membrane extending ventrally from tip of adipose-fin spine or adipose fin absent .... *Paralithoxus*

DISCUSSION

The Lithoxini clade exhibits relatively subtle overall interspecific morphological diversity, although there is unambiguous, taxonomically informative body shape and meristic variation across the four genera recognized herein. *Avalithoxus*, *Exastilithoxus* and *Paralithoxus* are all relatively robust, with *Avalithoxus* having a caudal peduncle that is narrower and shallower, with only three rows of plates, and *Exastilithoxus* and *Paralithoxus* having five caudal-peduncle plate rows. *Lithoxus* is flatter than all three other genera and is wider anteriorly, with a very narrow and shallow caudal peduncle having only three plate rows. *Lithoxus* also has eyes that are considerably larger than in *Avalithoxus*, *Exastilithoxus* and *Paralithoxus*, and elongated pectoral-fin spines that nearly reach the vent when adpressed ventral to the pelvic fin (vs. generally not past the base of the pelvic fin). The presence of 12 branched caudal-fin rays in *Avalithoxus* is unique among hypostomines, with all others possessing 14 (Armbruster, 2004).

Despite such gross intergeneric morphological variation, all Lithoxini members appear to be ecologically equivalent, small-bodied, benthic algivores and invertivores throughout their ranges. The geographically broad and generally allopatric distributions of Lithoxini lineages throughout the Guiana Shield, combined with their limited dispersal ability, make them a valuable system from which to infer the complex geological and hydrological history of the Guiana Shield. This study makes progress towards this goal by generating a revised phylogenetic hypothesis for Lithoxini that is improved by an additional 1032 bp of *ND2* sequence data for all major lineages, and by including several lineages from parts of the clade's range that have not been examined previously. Although basal nodes within Lithoxini remain weakly supported, the phylogeny nonetheless suggests that the narrowly endemic genus and species *A. jantjae* was isolated from the lineage giving rise to all other Lithoxini lineages early in the history of the clade. Given the isolation of *A. jantjae* to reaches of the Ventuari River immediately upstream of the ~50 m high Salto Tencua (~130 m a.s.l. at the bottom of the falls and 180 m a.s.l. at the top; Fig. 2), it is plausible that this node originated as a result of geological uplift of the Guiana Shield, which has occurred during at least four phases since the Late Cretaceous–Palaeocene (Lujan & Armbruster, 2011). Given the allopatric distributions of *Exastilithoxus* and *Paralithoxus* on opposite (NW vs. SE) slopes of the Guiana Shield highlands in southeastern Venezuela, western Guyana and northern Brazil (Fig. 2), it also seems likely that geological uplift played an important role in the initial isolation of these lineages.

Evidence for uplift-mediated vicariance early in the history of the Lithoxini is bolstered by other loricariid clades that exhibit similarly allopatric distributions on opposite sides of the Guiana Shield highlands. For example, the clade consisting of *Lithoxancistrus*, from the Orinoco River basin west of the Pakaraima Mtns, is sister to a strongly supported clade of three species (*Paulasquama callis*, *Neblinichthys brevibracchium* and *Neblinichthys echinasus*) from the upper Mazaruni River east of the Pakaraima Mountains (Lujan *et al.*, 2015). Also, the mostly eastern Guiana Shield and northern Brazilian Shield clade of *Guyanancistrus* + (*Corymbophanes* + *Hopliancistrus*) is sister to the upper Orinoco (western Guiana Shield) clade of *Dekeyseria* (Lujan *et al.*, 2015).

Although evidence of early, large-scale vicariance attributable to geological uplift is growing as phylogenetic analyses expand, less clear are patterns of smaller-scale, more recent vicariance resulting from stream capture events, which may result from Plio-Pleistocene geological uplift and/or shifts in the prevailing slope (Gibbs & Barron, 1993). It has previously been hypothesized that the relatively recent (Pleistocene) break-up of a major palaeodrainage known as the proto-Berbice heavily influenced the complicated biogeographical patterns among more recent lineages in the western part of Lithoxini's range (Lujan & Armbruster, 2011). Geologists hypothesize that the proto-Berbice drained the southern slope of the western Guiana Shield in an easterly direction, through the modern Rupununi Savannas, and then northeast into the Atlantic Ocean via the mouth of the modern Berbice River (Fig. 2) from the Late Cretaceous or Early Palaeogene (> 60 Mya) to the Plio-Pleistocene (< 5 Mya; McConnell, 1959; Sinha, 1968; Berrangé, 1975; Crawford, Szelewski & Alvey, 1985). During this time frame, the proto-Berbice is thought to have united many of the modern headwaters of right-bank Orinoco River tributaries that now drain to the north of the Guiana Shield. The proto-Berbice could therefore have spanned much of the modern distributions of *Avalithoxus*, *Exastilithoxus* and *Lithoxus* lineages, in addition to the *P. bovallii* clade. Thus, the relatively recent break-up of the proto-Berbice presents a plausible mechanism for the close genetic similarity between geographically highly disjunct lineages, such as *E. fimbriatus* from the upper Caroni River, *Exastilithoxus* sp. nov. aff. *fimbriatus* from the upper Ventuari River (Fig. 2; > 450 km apart) and members of the broader *P. bovallii* clade (Figs 2, 3).

The continuing expansion of phylogenetic analyses of the Lithoxini, including more taxa and localities and the development of a time-calibrated phylogenetic hypothesis, offer a valuable opportunity to shed light on the complex hydrological and topographic history of the Guiana Shield which is one of the most biodiverse regions on Earth.

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REFERENCES

Armbruster, JW. 2003. *Peckoltia sabaji*, a new species from the Guyana Shield (Siluriformes: Loricariidae). *Zootaxa* **344**: 1–12. doi:10.11646/zootaxa.344.1.1

Armbruster, JW. 2004. Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. *Zoological Journal of the Linnean Society* **141:** 1–80.

Armbruster JW. 2008. The genus *Peckoltia* with the description of two new species and a reanalysis of the phylogeny of the genera of the Hypostominae (Siluriformes: Loricariidae). *Zootaxa* **1822:** 1–76.

Armbruster JW, Hardman M. 1999. Redescription of *Pseudorinelepis genibarbis* (Loricariidae: Hypostominae) with comments on behavior as it relates to air-holding. *Ichthyological Exploration of Freshwaters* **10:** 53–61.

Armbruster JW, Page LM. 1996. Rediscription of *Aphanotorulus* (Teleostei: Loricariidae) with description of one new species, *A. ammophilus*, from the Rio Orinoco basin. *Copeia* **2:** 379–389.

Armbruster JW, Sabaj MH, Hardman M, Page LM, Knouft JH. 2000. Catfish of the genus *Corymbophanes* (Loricariidae: Hypostominae) with description of one new species: *Corymbophanes kaiei*. *Copeia* **2000:** 997–1006.

Arroyave J, Denton JS, Stiassny ML. 2013. Are characiform fishes Gondwanan in origin? Insights from a time-scaled molecular phylogeny of the Citharinoidei (Ostariophysi: Characiformes). *PLoS ONE* **8:** e77269.

Berrangé JP. 1975. The geomorphology of southern Guyana with special reference to the development of planation surfaces. *Anais Décima Conferência Geológica Interguianas* **1:** 804–824.

Boeseman M. 1968. The genus *Hypostomus* Lacépède, 1803, and its Surinam representatives (Siluriformes, Loricariidae). *Zoologische Verhandelingen, Leiden* **99:** 1–89.

Boeseman M. 1982. The South American mailed catfish genus *Lithoxus* Eigenmann, 1910, with the description of three new species from Surinam and French Guyana and records of related species (Siluriformes, Loricariidae). *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen - Series C: Biological & Medical Sciences* **85:** 41–58.

Bremer K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42:** 795–803.

Crawford FD, Szelewski CE, Alvey GD. 1985. Geology and exploration in the Takutu Graben of Guyana and Brazil. *Journal of Petroleum Geology* **8:** 5–36.

Eigenmann, CH. 1910. Catalogue of the fresh-water fishes of tropical and south temperate America. Reports of the Princeton University expeditions to Patagonia 1896–1899. *Zoology*. **3:** 375–511.

Eigenmann, CH. 1912. The freshwater fishes of British Guiana, including a study of the ecological grouping of species, and the relation of the fauna of the plateau to that of the lowlands. *Memoirs of the Carnegie Museum* **5:** 1–578.

Eigenmann CH. 1909. Reports on the expedition to British Guiana of the Indiana University and the Carnegie Museum, 1908. Report no. 1. Some new genera and species of fishes from British Guiana. *Annals of the Carnegie Museum* **6:** 4–54.

Eschmeyer WN, Fong JD. 2018. Species by Family/Subfamily. (http://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp). Electronic version accessed 11 April 2018.

Eschmeyer WN, Fricke R, van der Laan R, eds. 2017. *Catalog of fishes: genera, species, references*. Available at: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Fusinatto LA, Alexandrino J, Haddad CF, Brunes TO, Rocha CF, Sequeira F. 2013. Cryptic genetic diversity is paramount in small-bodied amphibians of the genus *Euparkerella* (Anura: Craugastoridae) endemic to the Brazilian Atlantic forest. *PLoS One* **8:** e79504.

Geerinckx T, Brunain M, Adriaens D. 2007. Development of the osteocranium in the suckermouth armored catfish *Ancistrus* cf. *triradiatus* (Loricariidae, siluriformes). *Journal of Morphology* **268:** 254–274.

Gibbs AK, Barron CN. 1983. The Guiana Shield reviewed. *Episodes* **1983:** 7–14.

Hardman M, Page LM, Sabaj MH, Armbruster JW, Knouft JH. 2002. A comparison of fish surveys made in 1908 and 1998 of the Potaro, Essequibo, Demerara, and coastal river drainages of Guyana. *Ichthyological Exploration of Freshwaters* **13:** 225–238.

Isbrücker IJH. 1979. Descriptions préliminaries de nouveaux taxa de la famille des Loricariidae, poissons-chats cuirassés néotropicaux, avec un catalogue critique de la sous-famille nominale (Pisces, Siluriformes). *Revue française d'Aquariologie* **5:** 86–117.

Isbrücker IJH, Nijssen H. 1985. *Exastilithoxus hoedemani*, a new species of mailed catfish from Rio Marauiá, Est. Amazonas, Brazil (Pisces, Siluriformes, Loricariidae). *Spixiana* **8:** 221–229.

Isbrücker IJH. 1980. Classification and catalogue of the mailed Loricariidae (Pisces, Siluriformes). *Verslagen en Technische Gegevens, Instituut voor Taxonomische Zoöogie, Universiteit van Amsterdam* **22:** 1–181.

Lanfear R, Calcott B, Ho SYW, Guindon S. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analysis. *Molecular Biology and Evolution* **29:** 1695–1701.

Li C, Ortí G, Zhang G, Lu G. 2007. A practical approach to phylogenomics: the phylogeny of ray-finned fish (Actinopterygii) as a case study. *BMC Evolutionary Biology* **7:** 44.

Lujan NK. 2008. Description of a new *Lithoxus* (Siluriformes: Loricariidae) from the Guayana Highlands with a discussion of Guiana Shield biogeography. *Neotropical Ichthyology* **6:** 413–418.

Lujan NK. 2017. *Ein vergessener Harnishwels in einer vergessenen Welt. Pages 56–61 Amazonas*. Münster, Germany: Natur und Tier - Verlag GmbH.

Lujan NK, Armbruster JW. 2011. The Guiana Shield. In: Albert JS, Reis RE eds. *Historical biogeography of neotropical freshwater fishes*. Berkeley, CA: University of California Press, 211–224

Lujan NK, Armbruster JW, Lovejoy NR, López-Fernández H. 2015. Multilocus molecular phylogeny of the suckermouth armored catfishes (Siluriformes: Loricariidae) with a focus on subfamily Hypostominae. *Molecular Phylogenetics and Evolution* **82:** 269–288.

McConnell RB. 1959. Fossils in the North Savannas and their significance in the search for oil in British Guiana. *Timehri: Journal of the Royal Agricultural and Commercial Society of British Guiana* **38:** 65–85.

McConnell RB. 1968. Planation surfaces in Guyana. *Geographical Journal* **134:** 506–520.

Muller S, Isbrucker IJH. 1993. *Lithoxus boujardi* (Siluriformes, Loricariidae), une espece nouvelle du bassin de l'Approuague, Guyane Francaise. *Cybium* **17:** 71–76.

Nijssen H, Isbrücker IJH. 1990. *Lithoxus stocki*, a species new to science of ancistrin loricariid catfish from the Maroni River drainage, with a comparison of the primary type specimens of the six species of *Lithoxus* (sy.: *Paralithoxus*) (Pisces, Siluriformes, Loricariidae). *Bijdragen tot de Dierkunde* **60:** 327–333.

Rambaut A, Drummond AJ, Suchard M. 2007. *Tracer v1.6*. Available at: http://tree.bio.ed.ac.uk/software/tracer/

Regan CT. 1906. Notes on some loricariid fishes, with descriptions of two new species. *Annals and Magazine of Natural History, Series 7* **17:** 94–98.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Sabaj MH. 2016. *Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an online reference. Version 6.5* (16 August 2016). Washington, DC: American Society of Ichthyologists and Herpetologists. Available at: http://www.asih.org/

Schaefer SA. 1987. Osteology of *Hypostomus plecostomus* (Linnaeus), with a phylogenetic analysis of the loricariid subfamilies (Pisces: Siluriformes). *Contributions in Science, Natural History Museum of Los Angeles County* **394:** 1–31.

Schaefer SA. 1997. The neotropical cascudinhos: systematics and biogeography of the *Otocinclus* catfishes (Siluriformes: Loricariidae). *Proceedings of the Academy of Natural Sciences of Philadelphia* **148:** 1–120.

Seidel I. 2009. Aquaristisch neue Harniscorden Braziliens (II). *Datz* **12:** 30–31.

Silva GSC., Covain R, Oliviera C, Roxo FF. 2017. Description of two new species of Lithoxus (Hypostominae: Loricariidae) from rio Jari and rio Amapá basins, Brazilian Guiana Shield. *Zootaxa* **4347:** 151–168.

Sinha NKP. 1968. *Geomorphic evolution of the Northern Rupununi Basin, Guyana*. McGill University Savanna Research Project, Savanna Research Series 11. Montreal: McGill University.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30:** 1312–1313.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Figure S1. Complete results of the full Bayesian phylogenetic analysis.
Figure S2. Complete results of the full maximum likelihood phylogenetic analysis.