

Using Morphology to Test DNA-Based Phylogenetic Relationships within the Guiana Shield Catfish Tribe Lithoxini (Siluriformes: Loricariidae)

Jonathan W. Armbruster¹, Lauren Greene¹, and Nathan K. Lujan²

As DNA-based phylogenetic analyses have exploded, historically phenotype-based evolutionary hypotheses throughout the tree of life have been rewritten. However, rarely are DNA-based phylogenetic hypotheses tested via the reanalysis of phenotypic data. Skeletons representing all four recognized genera of the Guiana Shield endemic suckermouth armored catfish clade Lithoxini were examined to test a recently published DNA-based phylogenetic hypothesis using morphological evidence. Phylogenetic analysis of 54 mostly osteological characters yielded a single most parsimonious tree of 90 steps that was congruent with the molecular hypothesis: (*Avalithoxus*, ((*Exastilithoxus*), (*Lithoxus*, *Paralithoxus*))). Lithoxini was a well-supported clade with 20 synapomorphies, as was each of the genera within this clade. *Avalithoxus jantjæ*, which was originally described in *Lithoxus*, was found to be missing the unique synapomorphies of *Lithoxus* + *Paralithoxus*: a spoon-shaped, ventrally oriented process on the metapterygoid and a process on the preoperculo-hyomandibular ridge.

THE Lithoxini is a recently described tribe of suckermouth armored catfishes (Loricariidae) containing four genera: *Avalithoxus*, *Exastilithoxus*, *Lithoxus*, and *Paralithoxus* (Lujan et al., 2018). Each of these genera is relatively species-poor, with one species each in *Avalithoxus* and *Lithoxus*, two in *Exastilithoxus* (excluding several known but undescribed species), and eight in *Paralithoxus* (excluding several undescribed; Eschmeyer et al., 2018). All species of Lithoxini are small (<65 mm SL) and dorsoventrally flattened (>7.2 times in standard length). Unlike most other members of the Hypostominae, members of the Lithoxini are primarily invertivores, although seeds were present in the gut of the *Avalithoxus* examined here. The stomach is enlarged in *Lithoxus* and *Paralithoxus*, and they are thought capable of breathing air (Armbruster, 1998). Given their small size, how dorsoventrally flattened they are, and the large size of their eggs, female *Lithoxus* typically carry only a few ripe eggs in each ovary (Armbruster, 1998).

Until recently, *Exastilithoxus* and *Lithoxus* were the only lithoxin genera recognized as valid, with all species of *Avalithoxus* and *Paralithoxus* being recognized in *Lithoxus*. Lujan et al. (2015, 2018) found, based on genetic data, *Lithoxus sensu lato* (and *sensu* Nijssen and Isbrücker, 1990; Armbruster, 2004, 2008) paraphyletic, with the more narrowly focused 2018 study finding *Avalithoxus* to be sister to all other Lithoxini and *Exastilithoxus* sister to *Lithoxus* + *Paralithoxus*. Lithoxini was the best supported clade in morphological phylogenies (Armbruster, 2004, 2008), and it was hypothesized that phylogenetic analysis of variation in morphological characters could reinforce the DNA-based hypothesis of Lujan et al. (2018).

MATERIALS AND METHODS

Specimens were cleared and stained using the methods of Taylor and Van Dyke (1985). Characters from Armbruster (2004) were used as a starting point for further observations; these characters are denoted by '2004-character number' in character descriptions, and complete descriptions of the characters can be found in that paper. New characters are described in detail here for a total of 54 new (21) and old (33)

characters (Table 1). All characters were coded as unordered. Names of skeletal characteristics follow Schaefer (1987) and Geerinckx et al. (2007) and for body plate rows follow Schaefer (1997).

Outgroups for the analysis included taxa from clades found to be closely related to the Lithoxini in further, unpublished analyses based on the molecular hypothesis of Lujan et al. (2015) and included *Lithoxancistrus yekuana*, *Neblinichthys yaravi*, and '*Pseudancistrus*' *pectegenitor*. Characters were coded numerically in Mesquite v3.04 (Maddison and Maddison, 2015) and analyzed under Maximum Parsimony in PAUP* v4.0a (Swofford, 2002) using an exhaustive search. The tree found was saved as a tree file, and character changes were examined in Mesquite with the relationships of outgroups to one another and the ingroup configured per the molecular phylogeny of Lujan et al. (2015). A bootstrap analysis was performed in PAUP* using 10,000 replicates of a Branch and Bound search. Bremer Decay Index values (Bremer, 1988) were calculated by assessing trees with an exhaustive search in PAUP* at successive steps higher than the most parsimonious tree until all nodes collapsed with the value calculated as the number of steps in the tree where a clade collapsed minus the number of steps in the most parsimonious tree.

Character descriptions

Branchial arch.—1. Length of accessory process of ceratobranchial 1 (2004-7). (0) process absent (or nearly so). (1) shorter than one-half length of main body. (2) same length as main body.

Loricariids often have an accessory process of the first ceratobranchial. In most hypostomines, it is as long or longer than the main body of the ceratobranchial (state 0), while it is reduced in the Lithoxini. In most of the Lithoxini, the process is present as a narrow structure half or less the length of the main body of the ceratobranchial (state 1). In *Exastilithoxus fimbriatus*, the process is absent (coded as state 0), but the ceratobranchial is slightly wider at this point.

2. Width of accessory process of CB1 (2004-8). (0) absent. (1) thin. (2) wide.

¹ Department of Biological Sciences, Auburn University, 101 Rouse Life Sciences Building, Auburn, Alabama 36849; Email: (JWA) armbrjw@auburn.edu; and (LG) leg0023@auburn.edu. Send reprint requests to JWA.

² Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, ON, M1C 1A4, Canada; Email: nklujan@gmail.com.

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Table 1. Character states for species in the analysis.

Species	Character states
<i>Avalithoxus jantjæ</i>	110010000111200010000111021120120001111011000010101101
<i>Exastilithoxus fimbriatus</i>	01001000111100001010011101101100110010111111012100111
<i>Lithoxus lithoides</i>	1100100001110110111001110111202100011111111111000101
<i>Paralithoxus planquettei</i>	11001100011101001100011111100012000120110111111100100
<i>Paralithoxus surinamensis</i>	110011000111110111100111111020110001201101111111200100
<i>Paralithoxus bovallii</i>	110011000111110101001111111000110001201101111111200100
<i>Paralithoxus cf. bovallii</i>	110010000111110111001111111000100001201101111111200100
<i>Lithoxancistrus yekuana</i>	221100210000200110110000000020100012000110100110210000
<i>Neblichthys yaravi</i>	221100210000201100010000000000220012000110000100110000
<i>'Pseudancistrus' pectegenitor</i>	221100210100100100110000000000100212000110100100210000

See Character 1. *Exastilithoxus* is coded as state 1 because the bone is slightly wider at the point where the process would be.

3. Accessory process on first epibranchial (2004-14). (0) absent. (1) present.

4. Posterior shelf of epibranchial 4 (2004-17). (0) absent. (1) present.

5. Hypobranchial 1 (2004-23). (0) short and stout. (1) elongate.

6. Hypobranchial 1. (0) oriented at less than 30°. (1) oriented at greater than 45°.

In most *Paralithoxus*, hypobranchial 1 is rotated so that it is oriented at an angle of 45° or greater relative to the sagittal plane (state 1) vs. 30° or less in all other taxa (state 0).

7. Interhyal position (2004-26). (0) on or behind hyomandibula. (1) contacts cartilaginous section between the hyomandibula and quadrate. (2) contacts only bony part of quadrate.

8. Upper pharyngeal tooth plate (2004-30). (0) round, teeth uniformly distributed. (1) with a mesial shelf and raised, rounded area, teeth restricted to bulbous area and posterior edge.

9. Urohyal. (0) rounded or concave distally. (1) pointed distally.

The urohyal has lateral arms that are usually rounded or concave distally (state 0), while the arms are pointed in *Exastilithoxus* (state 1).

10. Lateral edge of posterohyal (2004-32). (0) pointed. (1) forming a half cylinder.

Suspensorium.—11. Posterior part of the hyomandibula beyond opercle (2004-40). (0) not well developed. (1) developed into a shelf.

12. Preoperculo-hyomandibular ridge (2004-48). (0) continuous. (1) ridge branches.

13. Levator arcus palatine crest (2004-44). (0) absent. (1) short. (2) tall.

14. Process on preoperculo-hyomandibular ridge (2004-47). (0) absent. (1) present.

15. Posterior region of hyomandibula greatly deflected (2004-42). (0) absent. (1) present.

16. Hyomandibula with crenulations dorsally. (0) absent. (1) present.

In most of the Lithoxini, the dorsal surface of the hyomandibula anterior to the condyle with the cranium is smooth (state 0), but in all *Paralithoxus* (except *P. planquettei* [Boeseman, 1982]) and the outgroup, there are pointed regions or crenulations in the hyomandibula (state 1).

17. Metapterygoid sutured to lateral ethmoid (2004-50). (0) absent. (1) present.

Armbruster (2004) used a more restricted definition of this character. Here, *Neblichthys*, *'Pseudancistrus' pectegenitor*, and *Paralithoxus bovallii* have the anterior portion of the metapterygoid with a ligamentous connection to the lateral ethmoid (state 0), while *Lithoxancistrus* and the remainder of the Lithoxini have a distinct suture between the metapterygoid and lateral ethmoid (Fig. 1A, D).

18. Metapterygoid anterior process (2004-58). (0) pointed (Fig. 1A, B). (1) spoon-shaped, angled ventrally (Fig. 1C, D).

19. Metapterygoid condyle with strong ridge proceeding anteroventrally (2004-57). (0) absent. (1) present.

20. Metapterygoid channel (2004-52). (0) absent or with slight furrow (Fig. 1C, D). (1) present (Fig. 1B).

21. Longitudinal ridge on lateral portion of quadrate (2004-68). (0) absent. (1) present.

Armbruster (2004) used a broader definition of this character for any slight ridge along the length of the quadrate. Here, it is defined as a tall ridge that is very plainly observed. In most species examined, the quadrate is either flat or with a slight, rounded area along the length of the quadrate (state 0), while the quadrate has a distinct, thin crest in *Paralithoxus* sp. nov. aff. *bovallii* (Konawaruk) and *P. bovallii* (state 1).

22. Dentary tooth cups reduced. (0) absent (Fig. 2A, C). (1) present (Fig. 2B).

In most Hypostominae, the dentary tooth cup is a large structure that runs nearly the length of the mandible (state 0). In the Lithoxini, the dentary tooth cup is considerably smaller and more mesially displaced (state 1).

23. Premaxilla reduced to oval bone with few teeth. (0) absent. (1) present.

In most Hypostominae, the premaxilla is a large, almost square bone with its longest dimension in the transverse plane (state 0). In the Lithoxini, the premaxilla is reduced in size and oval, with the longest dimension in the sagittal plane (state 1).

24. Maxilla shape (2004-71). (0) narrow throughout length (Fig. 3B). (1) wide distally (Fig. 3A, C).

25. Maxilla maximum width. (0) less than two times minimum width (Fig. 3A, B). (1) greater than three times minimal width (Fig. 3C).

In most Hypostominae, the maxilla is an almost straight bone of relatively equal width along its entire length. In the Lithoxini, the maxilla is slightly wide proximally, narrows in the middle, then expands outwards into a rounded end (character 24). In most Hypostominae, the distal end of the maxilla is less than two times as wide as its narrowest section (state 0), while the distal end is at least three times wider than the narrowest part in *Paralithoxus* (state 1).

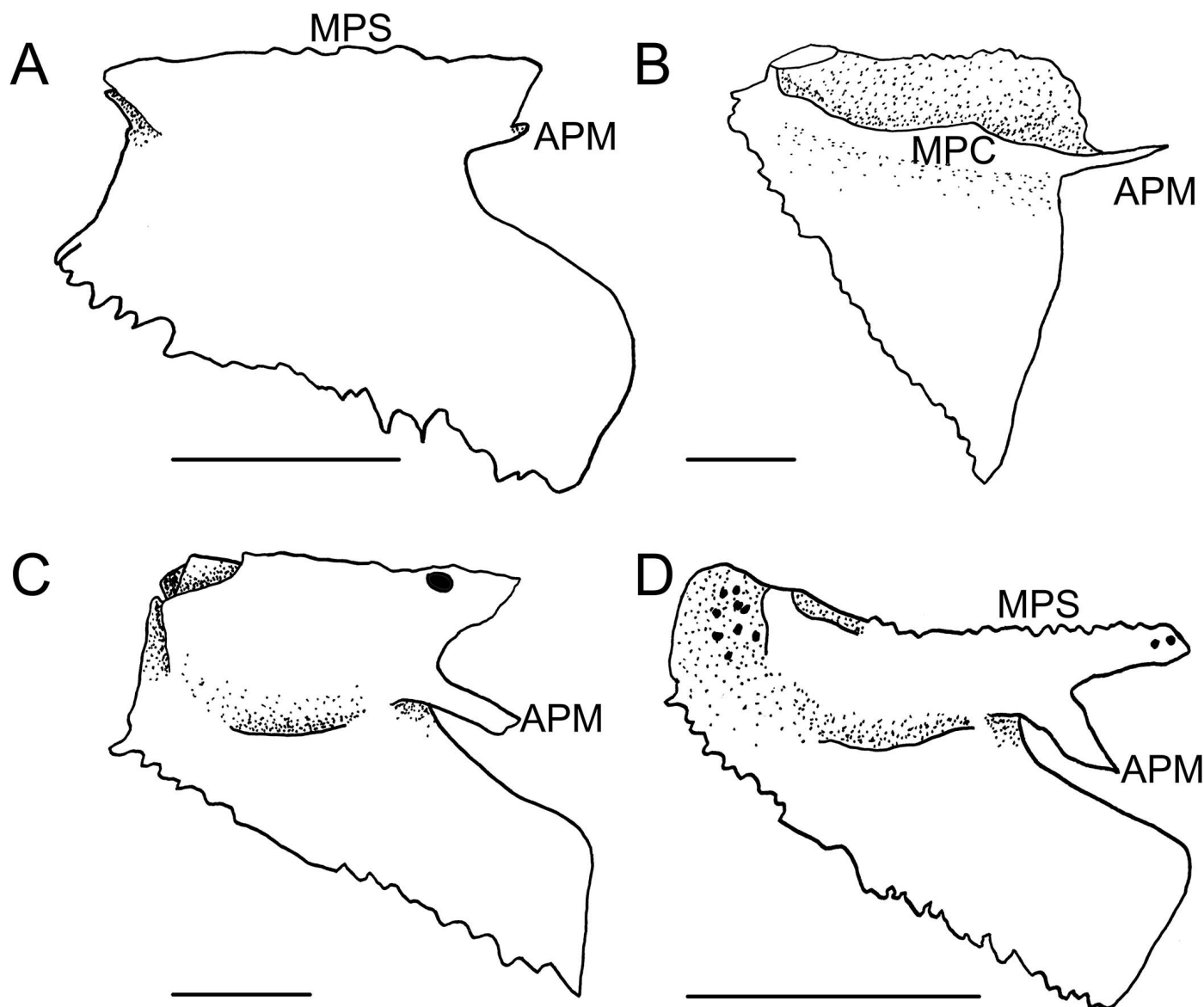


Fig. 1. Right metapterygoids, lateral view of (A) *Avalithoxus jantjæ* AUM 39478, (B) *Neblinichthys yaravi* AUM 36699, (C) *Paralithoxus bovallii* AUM 67127, (D) *Paralithoxus* sp. nov. aff. *bovallii* (Konawaruk) AUM 35549. APM, anterior process of metapterygoid; MPC, metapterygoid channel; MPS, metapterygoid suture to lateral ethmoid. Scale = 1 mm.

26. Bulbous process on anguloarticular for attachment of adductor mandibulae. (0) present, large (Fig. 2B). (1) present, reduced to thin process (Fig. 2C). (2) absent (Fig. 2A).

Morphology of the dentary bone is complex and variable in loricariids. In most Hypostominae, there is a large, bulbous area on the anguloarticular located above the cartilaginous section between the anguloarticular and dentary to which the adductor mandibulae connects (state 0). This raised area is much smaller in the Lithoxini (state 1) and completely lost in *Avalithoxus* (state 2).

27. Fenestra of anguloarticular. (0) absent (Fig. 2B). (1) present (Fig. 2A, C).

The anguloarticular is generally a conical bone that does not have any large perforations (state 0). In the Lithoxini, the anguloarticular is roughly rectangular with a large mesial fenestra (state 1).

28. Process of anguloarticular anterolateral to quadrate condyle. (0) short (Fig. 2B, C). (1) long (Fig. 2A).

In most Hypostominae, the articulating condyle of the anguloarticular is preceded by a short process that extends

laterally to the condyle (state 0). In *Avalithoxus jantjæ* and *Lithoxus lithoides*, the process is very long (state 1).

Opercular series.—29. Plates between suprapreopercle and opercle. (0) present, large. (1) present, small, not much larger than a single odontode. (2) absent.

The suprapreopercle is a canal-bearing plate located ventrally to the opercle. Generally, there are large plates present between the suprapreopercle and the exposed portion of the opercle (state 0), but there are only tiny plates in *Exastilithoxus* that are not much wider than the bases of their odontodes (state 1). There are no plates present (state 2) in *Lithoxancistrus*, *Avalithoxus*, *Paralithoxus surinamensis*, and *Lithoxus lithoides*.

30. Preopercle width. (0) greater than five times canal width. (1) less than four times canal width.

The preopercle in most Hypostominae is a robust bone that at its widest point is at least five times the width of the preopercular canal or greater (state 0). In *Exastilithoxus*, the

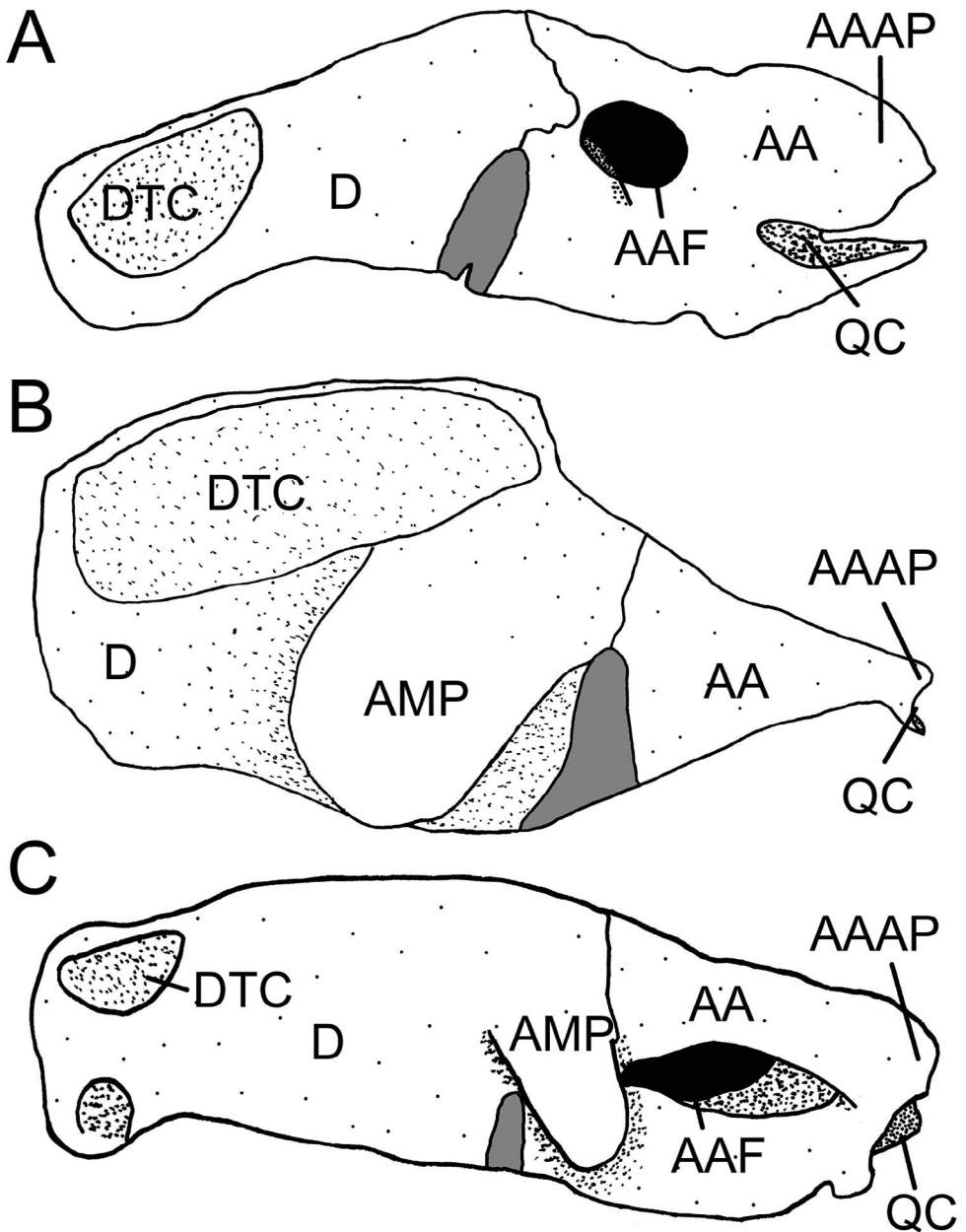


Fig. 2. Right mandible, roughly dorsal view of (A) *Avalithoxus jantjæ* AUM 39478, (B) *Neblinichthys yaravi* AUM 36699, (C) *Paralithoxus bovallii* AUM 67127. AA, anguloarticular; AAF, anguloarticular fenestra; AAAP, anguloarticular anterior process; AMP, adductor mandibulae process; D, dentary; DTC, dentary tooth cup; QC, area quadrate condyle connects. Scale = 1 mm; gray shading indicates cartilage.

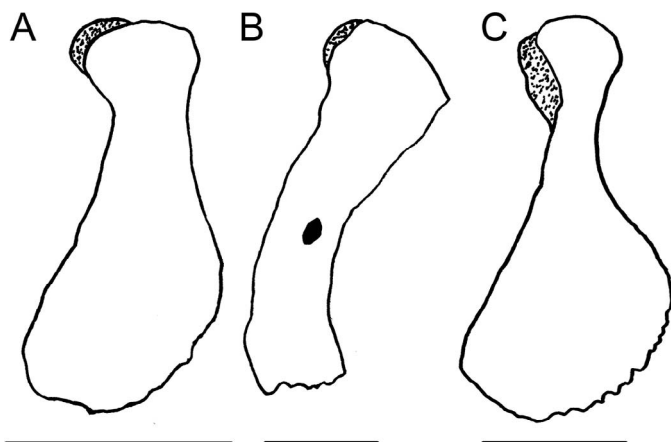


Fig. 3. Right maxilla, dorsolateral view of (A) *Avalithoxus jantjæ* AUM 39478, (B) *Neblinichthys yaravi* AUM 36699, (C) *Paralithoxus bovallii* AUM 67127. Scale = 1 mm.

preopercle is greatly reduced with its widest point less than four times the width of the canal (state 1).

31. Length of posterior process of opercle. (0) long (Fig. 4B). (1) short (Fig. 4A, E). (2) nearly absent (Fig. 4C, D).

The portion of the opercle posterodorsal to the opercular condyle is generally long, reaching at least halfway (state 1) to almost all of the way (state 0) to the joint between the hyomandibula and the compound pterotic (state 0). In *Neblinichthys* and *Lithoxus*, this dorsal section is restricted to the portion of the opercle sutured to the hyomandibula (state 2; see character 32).

32. Suture of opercle to hyomandibula behind condyle (based on 2004-76). (0) absent (Fig. 4B). (1) short (Fig. 4C, E). (2) long (Fig. 4A, D).

Schaefer (1986) described the presence of a connection of the opercle to the hyomandibula posterior to the opercular condyle, which exists as a suture where folds in the opercle interdigitate with folds in the hyomandibula. This suture is absent in *Lithoxancistrus* and '*Pseudancistrus*' *pectegenitor* (state 0). In most Lithoxini, the sutured length is about

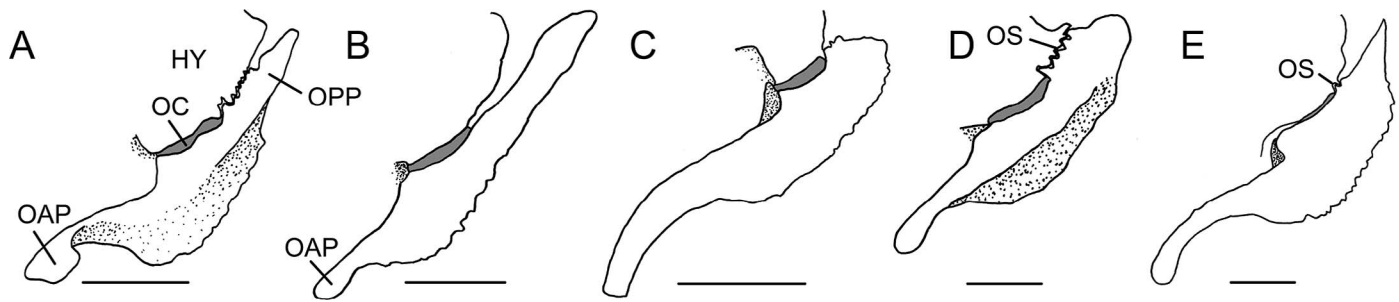


Fig. 4. Right opercle, medial view of (A) *Avalithoxus jantjæ* AUM 39478, (B) *Exastilithoxus fimbriatus* MCNG 44872, (C) *Lithoxus lithoides* AUM 39040, (D) *Neblinichthys yaravi* AUM 36699, (E) *Paralithoxus bovallii* AUM 67127. HY, hyomandibula; OAP, opercle anterior process; OC, opercular condyle of hyomandibula; OPP, opercular posterior process; OS, opercular suture to hyomandibula. Scale = 1 mm; gray shading indicates cartilage.

equal to the length of the folds (state 1) and in *Avalithoxus*, *Neblinichthys*, and *Paralithoxus planquettei*, the sutured length is longer than the length of the folds (state 2).

33. Length of shaft of opercle to evertible cheek plates. (0) long (Fig. 4A, C–E). (1) short (Fig. 4B).

In Hypostominae with evertible cheek plates, the opercle rotates on the opercular condyle and pushes a modified cluster of cheek plates that support long odontodes out from the head. In general, the length of the opercle from the condyle to the evertible cheek plates is fairly long, occupying half or greater the maximum length of the opercle (state 0). In *Exastilithoxus*, this shaft of the opercle is greatly reduced (state 1) such that it is much shorter than the dorsal section of the opercle (character 30).

34. Size of area with odontodes on opercle. (0) wide, in those with evertible cheek plates it is greater than two times the width of the shaft. (1) narrow, less than two times shaft. (2) absent.

In Hypostominae with evertible cheek plates, the opercle is generally reduced in size when compared to those species that lack evertibility, but it remains as a fairly stout bone and its width can generally be assessed by examining the width of the exposed portion of the opercle that supports odontodes. This area is generally two or more times the width of the shaft of the opercle leading to the evertible cheek plates (state 0). In *Exastilithoxus*, the opercle is greatly reduced in length and width, with the dentigerous area of the opercle less than two times the width of the shaft and the entire bone scarcely changing in width along its entire length (state 1). The opercle does not support odontodes in '*Pseudancistrus*' *pectegenitor* (state 2).

Cranium.—35. Baudelot's ligament (2004-93). (0) short, rounded ridge. (1) tall shelf.

36. Mesethmoid disk (2004-100). (0) absent. (1) reduced. (2) developed.

37. Ventral process of sphenotic (see 2004-116). (0) thin (Fig. 5C). (1) wide (Fig. 5A). (2) absent (Fig. 5B).

The sphenotic in most Hypostominae consists of a mesial oval area and a narrow ventrolateral process (not much wider than the laterosensory canal that runs through it) that forms the posterior border of the orbit (state 0). In most of the Lithoxini, the ventrolateral process is very wide (state 1) and still forms most of the posterior border of the orbit. In *Paralithoxus*, the process is lost with most of the posterior border of the orbit formed from infraorbital 6 (state 2).

38. Mesial extent of sphenotic. (0) well mesial to orbit (Fig. 5B, C). (1) about as mesial as orbit (Fig. 5A).

The mesial extent of the sphenotic is normally well medial to a longitudinal line through the dorsal rim of the orbit

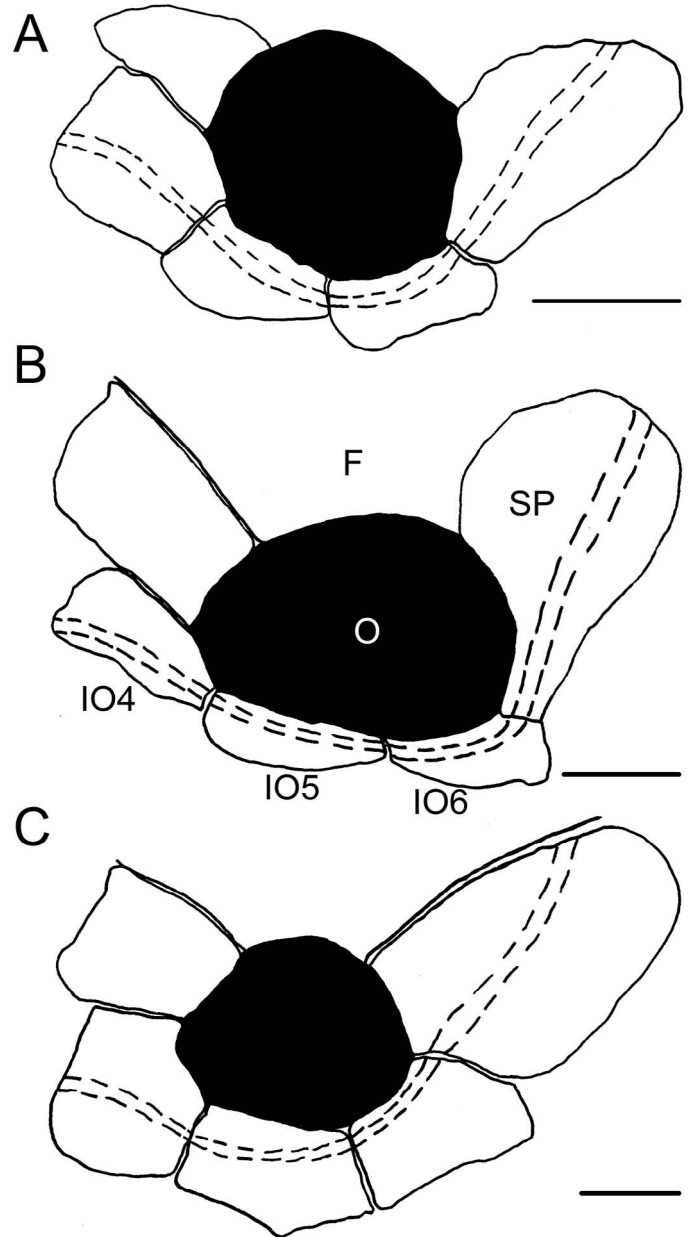


Fig. 5. Sphenotic and orbital region, dorsolateral view of (A) *Avalithoxus jantjæ* AUM 39478, (B) *Neblinichthys yaravi* AUM 36699, (C) *Paralithoxus bovallii* AUM 67127. A and C, left side, B, right side but reflected. F, frontal; IO, infraorbital; O, orbit; SP, sphenotic. Scale = 1 mm.

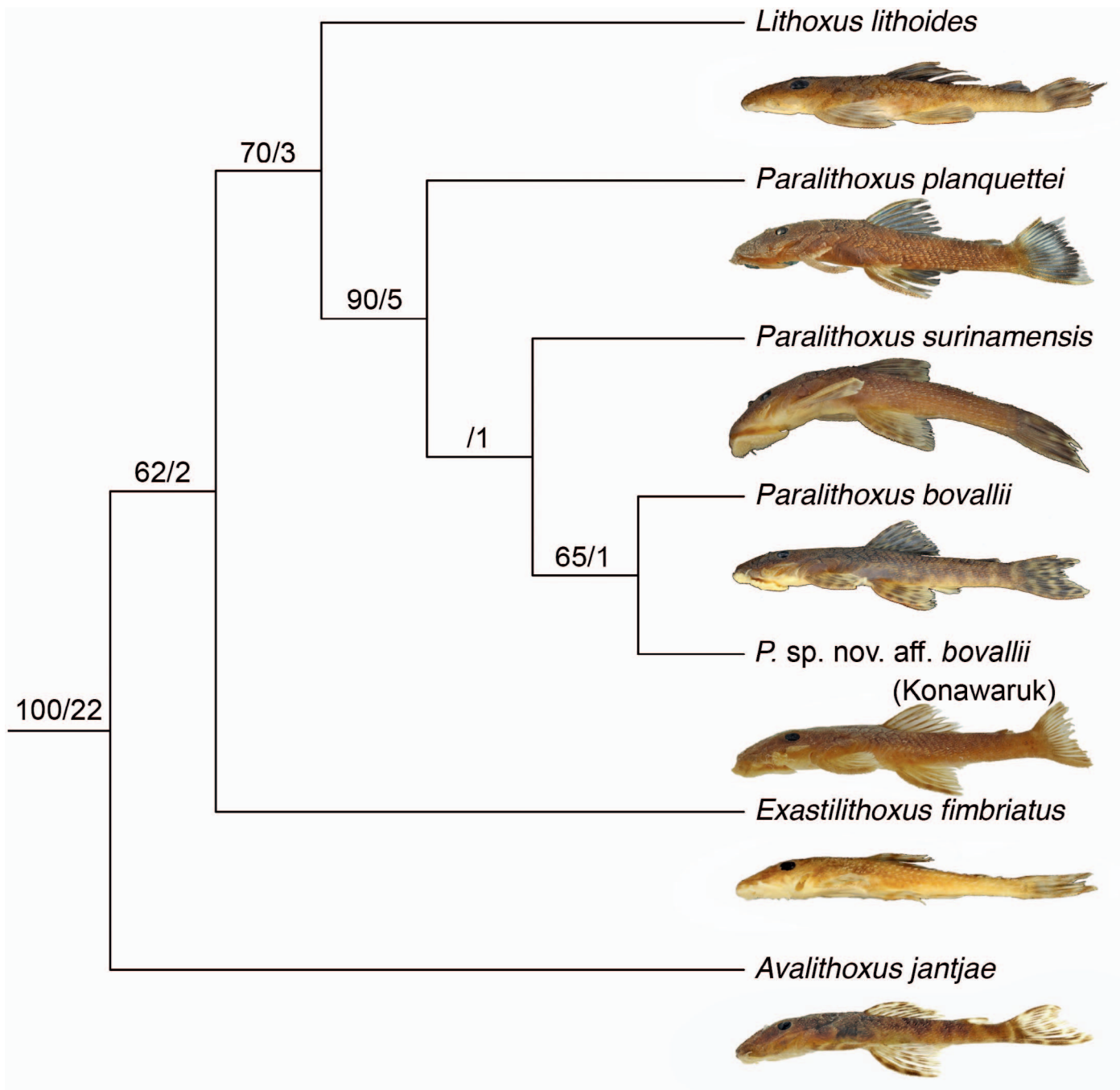


Fig. 6. Single most parsimonious tree of 90 steps, CI = 0.722, numbers above branches are bootstrap values/Bremer decay indices. *Avalithoxus jantjiae* AUM 39478 (31.9 mm SL), *Exastilithoxus fimbriatus* AUM 36668 (23.6 mm SL), *Lithoxus lithoides* AUM 39040 (43.0 mm SL), *Paralithoxus bovallii* AUM 67127 (51.7 mm SL), *P. sp. nov. aff. bovallii* (Konawaruk) AUM 35549 (42.6 mm SL), *P. planquettei* AUM 50395 (47.0 mm SL), and *P. surinamensis* AUM 51737 (34.7 mm SL). Photos by JWA.

(state 0). In *Avalithoxus* and *Lithoxus*, the mesial extent of the sphenotic does not surpass a longitudinal line through the dorsal rim of the orbit (state 1).

Axial skeleton.—39. Bifid hemal spines (2004-122). (0) absent. (1) present.

40. Distal margin of the rib of the sixth vertebral centrum (2004-128). (0) about same width as shaft. (1) flared so that the tip is much wider than the shaft.

41. Width of end of transverse process of Weberian complex centrum. (0) less than half maximum width. (1) greater than half maximum width.

The swim bladder of loricariids is encapsulated by the compound pterotic plus the supraoccipital and the widened and lengthened transverse process of the Weberian complex centrum. Plesiomorphically (and in *Paralithoxus*), the distal end of the transverse process is narrow, less than half the width of its widest point (state 0), while the distal end is wider than half the width in all other species examined here (state 1).

Fins.—42. Elongate transverse processes of the first anal-fin pterygiophore. (0) absent. (1) present.

The first anal-fin pterygiophore of most Hypostominae is a broad, roughly rectangular (longest along axial plane), platelike structure (state 0). In the Lithoxini, the base of the

pterygiophore is greatly elongated laterally, forming pointed processes (state 1).

43. Transverse process of first anal-fin pterygiophore. (0) does not extend posterior to first anal-fin ray. (1) extends posterior to first anal-fin ray.

In most Hypostominae, the platelike section of the first anal-fin pterygiophore has posterior flaps that generally reach to parallel with or beyond the base of the first anal-fin ray (state 0). These flaps are absent in *Avalithoxus* with the lateral processes in character 42 being straight posteriorly (state 1).

44. Adductor fossa of pectoral girdle (2004-153). (0) deep to midline. (1) nearly flat anteromesially.

45. Shape of cleithrum (2004-155). (0) rectangular. (1) trapezoidal.

In most Hypostominae, the cleithrum and coracoids form a roughly rectangular structure ventrally (state 0). In the Lithoxini (except *Avalithoxus*), the cleithrum is expanded anteriorly, and the anterolateral margins of the cleithrum are angled mesially forming a trapezoid (state 1).

46. Cleithrum with posterolateral foramen. (0) present. (1) absent.

Avalithoxus and *Exastilithoxus* have a fairly large foramen posterolaterally on the cleithrum that can be seen in the ventral view (state 0), while all other species examined here lack the foramen (state 1). One specimen of *Paralithoxus bovallii* has one foramen on one side, but the species was coded as state 1.

47. Extent of exposed cleithral process (2004-157). (0) large. (1) reduced.

48. Anterolateral process of pelvic basipterygium (based on 2004-168). (0) not fused to anteromesial processes. (1) with a posteromedial process that extends posteriorly to contact anteromesial process. (2) fused to anteromedial process.

In most Hypostominae, the anterolateral and anteromesial processes of the pelvic basipterygium are separate (state 0), while they are fused in *Exastilithoxus* (state 2). *Lithoxus* and *Paralithoxus* likely have an intermediate state where the anterolateral process has a posteromedial process that extends posteriorly to meet, but not fuse with, the anteromesial process (state 1).

49. Fenestra on pelvic basipterygium anterior to cartilaginous section (based on 2004-171). (0) absent. (1) present, rectangular. (2) present, round.

Primitively and in *Lithoxus*, the pelvic basipterygium lacks a fenestra anterior to the medial cartilaginous portion (state 0). In *Neblinichthys*, *Avalithoxus*, *Exastilithoxus*, and *Paralithoxus planquettei*, the fenestra is rectangular, and in *Lithoxancistrus*, '*Pseudancistrus*' *pectegenitor*, and the remainder of *Paralithoxus*, the fenestra is round (state 2).

50. Posteroventral ridge of the pelvic basipterygium (2004-173). (0) absent. (1) present.

51. Number of branched caudal-fin rays. (0) 14. (1) 12.

In all Hypostominae except *Avalithoxus*, there are 14 branched caudal-fin rays (state 0), while *Avalithoxus* has 12 (state 1; Lujan, 2008).

External anatomy.—52. Premaxillary teeth (2004-205). (0) villiform, same size as dentary teeth. (1) stouter and longer than dentary teeth.

In most Hypostominae, the premaxillary and dentary teeth are villiform and are of the same size (state 0). In the Lithoxini, the premaxillary teeth are much stouter and at least twice as long as the dentary teeth (state 1).

53. Lower lip fimbriate (2004-207). (0) no. (1) yes.

In most Hypostominae, the edge of the oral disk is either smooth, crenulate, or with short papillae that are about as long as wide (state 0). In *Exastilithoxus*, the edge of the oral disk has papillae extended into fimbriae that are much longer than wide (state 1; Lujan et al., 2018; fig. 1). Illustrated in Lujan et al. (2018).

54. Number of plate rows on distal portion of caudal peduncle. (0) four to five. (1) three.

Most Hypostominae have five complete (rarely four) rows of plates on the caudal peduncle, and all of the plate rows are continuous to the caudal fin (state 0). In *Avalithoxus*, *Exastilithoxus*, and *Lithoxus*, the midventral and middorsal plate rows end before the adipose fin, leaving three rows of plates on the posterior half of the caudal peduncle (state 1). This can be difficult to discern in some specimens of the Lithoxini, even in cleared and stained specimens.

RESULTS AND DISCUSSION

A single tree of 90 steps and CI = 0.722 was congruent with the relationships found in Lujan et al. (2018). Although the results presented here as well as the basal nodes in Lujan et al. (2018) are relatively weakly supported, congruence between the morphological and molecular analyses reinforces the results and taxonomic decisions of Lujan et al. (2018).

Monophyly of the Lithoxini in this study was supported by 21 characters, many of which are losses likely due to the invertivorous/granivorous diet of the species, such as a reduction of branchial elements that are used to help sift biofilm in loricariids (characters 1-1, 2-1, 3-0, 4-0, 5-1, and 7-0) and changes in the dentigerous elements (pharyngeal and oral jaws) and teeth to capture and process food (characters 8-0, 22-1, 23-1, and 52-1). There are changes to other mechanical elements likely due to diet, but the structures serve an unknown function, such as loss of the lateral wall of the metapterygoid channel (character 20-0) through which the *levator arcus palatini* muscle passes, reduction or loss of a bulbous area on the anguloarticular (character 26-1), the presence of a strangely shaped maxilla that is wide proximally, narrows, and then widens greatly distally (character 24-1), reduction of Baudelot's ligament (character 35-0), and reduction or loss of the mesethmoid disk (character 36-1). The maxilla shape is convergent with that found in *Spectracanthicus murinus* and *Leporacanthicus*, which also have relatively protein-rich, invertivorous diets (Lujan et al., 2012), suggesting that these changes are adaptive and perhaps related to the increased jaw protrusion and dexterity needed to extract invertebrates from crevices and cases (Marrero and Winemiller, 1993). There are also some changes to the hyomandibula, including an expanded posterior portion of the hyomandibula posterior to the now branched preoperculo-hyomandibular ridge (characters 11-1 and 12-1), characters that are shared most notably with *Ancistrus* and *Lasiancistrus* and that may be related to the evertibility of the cheek plates and associated hypertrophied odontodes. *Ancistrus* and *Lasiancistrus* were conclusively shown to be distantly related to Lithoxini by morphology and molecules (Armbruster, 2004, 2008; Lujan et al., 2015). In addition, the first anal-fin pterygiophore has a unique shape in the Lithoxini with long, pointed, laterally directed wings (character 42-1, vs. being basally rectangular).

Avalithoxus was found to be missing the two unique synapomorphies found for *Lithoxus* + *Paralithoxus* in Armbruster (2004): process on the preoperculo-hyomandibular ridge (character 14-1) and a spoon-shaped, ventrally

oriented anterior process of the metapterygoid (character 18-1; Fig. 1). *Avalithoxus* has two unique synapomorphies, a loss of the bulbous region of the anguloarticular (character 26-2; Fig. 2) leaving the mandible as an almost rectangular bone and 12 caudal-fin rays (character 51-1; vs. 14). Only one specimen was available for dissection, and its gut was full of monocotyledonous seeds with the seed coats removed. *Avalithoxus* also has a particularly long anterolateral process of the anguloarticular (character 28-1), which is only shared with *Lithoxus*, and was the only species in the analysis that lacked a widened distal end of the enlarged rib of vertebra 6 (character 40-0), a reversal to the plesiomorphic state. *Avalithoxus* is further supported by several homoplastic characters: the metapterygoid condyle with a strong ridge proceeding from its base (character 19-1), a relatively long suture of the opercle to the hyomandibula (character 32-2; Fig. 4), a small sphenotic whose mesial margin is approximately equal to that of the mesial portion of orbit (character 38-1; Fig. 5), and the lack of a posterior section of the transverse process of the first anal-fin pterygiophore (character 43-0).

Only one species of *Exastilithoxus* was available for analysis here, so it will remain to be documented if the characters here are found in all *Exastilithoxus*. Although NKL has collected several undescribed species from the upper Orinoco, each of these is represented by only a few specimens. *Exastilithoxus* was originally described based on the presence of fimbriae along the margin of the oral disk (character 53-1). These fimbriae are longer than wide, but some other Lithoxini have similar structures that are shorter (about as long as wide; Lujan et al., 2018: fig. 1). *Exastilithoxus* does have some peculiar characteristics, most notably in the opercle and preopercle, both of which are very narrow (characters 30-1 and 34-1). In addition, the section of the opercle posterior to the hyomandibular condyle is especially long, reaching beyond the joint between the hyomandibula and compound pterotic (character 31-2; Fig. 4), and the portion of the opercle anterior to the hyomandibular condyle is especially short (character 33-1; Fig. 4). *Exastilithoxus* has almost completely lost the accessory process of ceratobranchial 1 (character 1-0, it is present only as a raised area without a flap and reduced in other Lithoxini to a narrow structure less than half the length of the main body of the ceratobranchial, vs. being much wider and the same length or longer than the main body of the ceratobranchial in most other Hypostominae), the presence of pointed arms of the urohyal (character 9-1), very small plates between the suprapreopercle and opercle (character 29-1), complete absence of the mesethmoid disk (character 36-1), and complete fusion of the anterolateral and anteromesial processes of the pelvic basipterygium (character 48-2). *Exastilithoxus* is supported as sister to *Lithoxus* + *Paralithoxus* by the loss or reduction of the levator arcus palatini crest on the hyomandibula (character 13-0 or 1) and a cleithrum that is expanded and flattened anteriorly so that it is trapezoidal (characters 44-1 and 45-1). In addition, the anterolateral processes of the pelvic basipterygium of *Lithoxus* and *Paralithoxus* have posteromedial processes that contact the anteromesial processes (character 48-1), which is likely an intermediate step towards their complete fusion (character 48-2) in *Exastilithoxus*.

Lithoxus and *Paralithoxus* are most notably supported as sister taxa by the presence of a widened, ventrally oriented anterior process of the metapterygoid (character 18-1; Fig. 1) and the presence of a process along the preoperculo-

hyomandibular ridge (character 14-1). The anterior process of the metapterygoid in these taxa is directly pointed at the bulbous region of the anguloarticular. Using polarized light, it appears as if there is a ligamentous connection between the two structures. In addition to these characters, the taxa are supported as sisters by the anterolateral processes of the pelvic basipterygium with posteromedial processes that contact the anteromesial processes (character 48-1).

Paralithoxus is the most species-rich genus of the Lithoxini, and it is supported by the first hypobranchial at an approximately 45° or greater angle with the sagittal plane (character 6-1, lost in *P. sp. nov. aff. bovallii* (Konawaruk)), the distal end of the maxilla being particularly wide (greater than three times the shaft; character 25-1), the sphenotic completely lacking the ventral process (character 37-2), having a particularly narrow distal end of the transverse process of the Weberian complex centrum (character 41-0), and a reversal to four or five rows of plates on the distal portion of the caudal peduncle (character 54-0).

Lithoxus is currently monotypic, and it is supported by the posterior margin of the hyomandibula deflected mesially (character 15-1; this is also seen in *Nebelinichthys* as well as taxa that have particularly strongly evertible cheek plates such as *Ancistrus*; Armbruster, 2004), a particularly long anterolateral process of the anguloarticular (character 28-1; shared with *Avalithoxus*; Fig. 2), a nearly absent posterior section of the opercle (character 31-2; Fig. 4), a small sphenotic whose mesial margin is approximately equal to that of the mesial portion of orbit (character 38-1; shared with *Avalithoxus*; Fig. 5), and loss of the anterior fenestra of the pelvic basipterygium (character 49-0). Although *Lithoxus* shares a couple of characters with *Avalithoxus*, *Avalithoxus* lacks the unique synapomorphies of the clade of *Lithoxus* + *Paralithoxus*. The relatively short mesial extent of the sphenotic is likely due to different reasons in *Lithoxus* and *Avalithoxus*: a small sphenotic in *Avalithoxus* and very large eyes in *Lithoxus*.

Specimens putatively identified as *Paralithoxus bovallii* occur in the type locality in the Ireng River on the border of Guyana and Brazil and the Essequibo, Courantijne, and Coppename rivers. In this study, specimens were included from the Essequibo River basin, and they were found to be osteologically distinct from topotypic *P. bovallii*. This species was identified in Lujan et al. (2018) as *P. sp. nov. aff. bovallii* (Konawaruk). This species differs from *P. bovallii* by having a normally oriented hypobranchial 1 (character 6-0), by having the metapterygoid sutured to the lateral ethmoid (character 17-1 vs. suture lost, character 17-0; one specimen on one side of *P. bovallii* had a slight suture), and loss of the suture between the opercle and hyomandibula (character 32-0; Fig. 4). The two species are supported as sisters by the presence of a tall longitudinal ridge on the quadrate (character 21-1). The Essequibo *P. bovallii* also has a unique metapterygoid. The anterodorsal region of the metapterygoid is offset forwards and very thin compared to that in topotypic *P. bovallii*, and there is a shelf posterior to the metapterygoid condyle (Fig. 1D vs. C). As mentioned above, the metapterygoid is sutured into the lateral ethmoid in the Essequibo species, but true *P. bovallii* have lost the suture. The new Essequibo species is sympatric with *Lithoxus lithoides* in at least the Kurukupari Rapids of the Essequibo River.

Conclusions.—The molecular hypothesis of Lujan et al. (2015) was very different from the first morphology-based hypothesis of relationships among lithoxin genera by Armbruster

(2004). As new technology has increased significantly beyond the number of taxa and genes sampled from the early days of the single gene phylogeny of Montoya-Burgos et al. (1998), it has become clear that the original morphological hypothesis was not correct, and likely suffered from widespread ecomorphological convergence. However, in this study, the molecular and morphological hypotheses were congruent. There are many morphologically variable characters in the Lithoxini, and this clade is related to species that are very different from it in morphology and ecology, with the strong morphological changes leading to this clade making it more likely that phylogenetically informative morphological characters might be distributed across descendent lineages. By focusing on smaller, monophyletic groups, it seems more likely that morphology will provide more reliable phylogenetic signal than trying to spread the characters across all lineages in a group as broad, as old, and as plastic in its morphology as the Loricariidae.

MATERIAL EXAMINED

All specimens examined were cleared and stained. Institutional abbreviations follow Sabaj (2016).

Ingroup: *Avalithoxus jantjæ*, AUM 39478 (1, paratype); *Exastilithoxus fimbriatus*, AMNH 91400 (1), MCNG 44872 (1); *Lithoxus lithoides*, AUM 39040 (2), BMNH 1972.7.17:66–115 (2), USNM 225917 (1); *Paralithoxus bovallii*, AUM 67127 (3); *P. sp. nov. aff. bovallii* (Konawaruk), AUM 35549 (2); *P. planquettei*, AUM 50775 (1); *P. surinamensis*, AUM 50767 (1).

Outgroup: *Lithoxancistrus yekuana*, AUM 39473 (1, paratype); *Neblichthys yaravi* AUM 36699 (2); *'Pseudancistrus' pectegenerator*, AUM 43192 (1, paratype).

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