

# **An unpublished first cladistic analysis of the Chiapas catfish (*Lacantunia enigmatica*) among all catfishes and two anonymous reviews**

The following unpublished web based document supplements our description of *Lacantunia enigmatica*: Rodiles-Hernández, R., D. A. Hendrickson, J. G. Lundberg & J. M. Humphries. 2005. *Lacantunia enigmatica* (Teleostei: Siluriformes) a new and phylogenetically puzzling freshwater fish from Mesoamerica. *Zootaxa* 1000: 1-24 (2005).

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## **Background:**

Prior to the submission and recent publication in *Zootaxa* of the description of *Lacantunia enigmatica* the authors submitted manuscripts on the same subject first to Science magazine and then to Nature. The authors felt that the first published notice of the discovery of this remarkable fish would be of general interest in itself and also for its enigmatic evolutionary and biogeographic implications. Both of the earlier manuscripts included a first cladistic analysis of *Lacantunia enigmatica* among all catfishes building on morphological characters used by Mo (1991), de Pinna (1993), some other sources and our own data. Rui Diogo's 2005 book on catfishes with his large character set was published after our study was done. The receiving editor at Science rejected the first manuscript without review. The Nature editor sent the manuscript out for review, the two anonymous reviews returned were not favorable, and the editor rejected the paper. The external reviews for Nature were especially attentive to aspects of our character set, cladistic analysis and results.

With a concern for the timely description of the Chiapas catfish, we removed the cladistic analysis from the manuscript published in *Zootaxa*. Nevertheless, we continue to feel that our analysis has merit and contains useful information, and that it will be of general interest to ichthyologists concerned with the phylogeny of catfishes and especially the data upon which phylogenetic hypotheses are based. So too could be the

anonymous reviews for their corrections, additions and differing perspectives.

Accordingly, we present below,

- A copy of our manuscript submitted to Nature but not published.
- Copies of the parts of the Supplemental Information submitted to Nature that detail the cladistic analysis including Methods, Character descriptions, Nexus file with Character by taxon matrix, Results including a strict consensus tree, and References.
- Copies of the two anonymous reviews and one comment by the first author Rodiles.

**An editable copy of the character descriptions, Nexus file and character by taxon matrix is available from John Lundberg at [lundberg@acnatsci.org](mailto:lundberg@acnatsci.org).**

Clearly, the inference of the tree of catfish life is a complex problem that will require several investigators working independently and collaboratively with different taxon sets and character sources. To achieve the satisfactory result in phylogenetic inference that the community of catfish specialists seeks (the tree of catfish life), both the taxon sample of catfishes and the sample of characters should be as large and diverse and informative as possible. Thus, we hope that the following will encourage a mutually beneficial exchange of information and ideas about catfish taxa and characters and ultimately an advancement of our understanding of siluriform phylogeny.

# Unpublished Manuscript and summary cladogram submitted to Nature.

## Discovery of a phylogenetically puzzling fish from Mesoamerica

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Catfishes (Order Siluriformes) form a globally diverse, mostly freshwater clade of >2700 valid species that continues to yield about 45 new species annually<sup>1,2</sup>. Whereas most newly found catfishes are small representatives of established genera found in poorly explored tropical waters, we describe a remarkable new species from southern México's Río Usumacinta<sup>3,4</sup>, a watershed thought to be well surveyed. Not only is its large size (0.5 m) as surprising as the fact that this new taxon was overlooked by science until now, but our phylogenetic analysis of 290 morphological characters demonstrates that it is not a member of any of the order's 35 known living or 2 fossil families<sup>2</sup>. Though new finds of deep phylogenetic lines of any living vertebrates are rare<sup>5</sup>, this new species clearly represents an ancient family-level lineage of perhaps early Tertiary age. Given its deep phylogenetic position and provenance in one of the world's most historically complex biotas<sup>6,7</sup>, discovery of this enigmatic new family emphasizes how little we know about Mesoamerica's biotic legacy and conservation value. Additionally, once its phylogenetic relationships are clarified, geologists and biogeographers may find that this fish sheds new light on the highly complex yet poorly understood and hotly debated regional geologic history<sup>8</sup>.

Acinopterygii Cope, 1887  
Teleostei sensu Patterson and Rosen, 1977  
Siluriformes sensu Chardon, 1968  
Siluroidei sensu Grande, 1987  
Lacantuniidae *fam. nov.\**  
Lacantunia *gen. nov.\**  
Lacantunia enigmatica *gen. et sp. nov.\**

\*Authorship of new taxa: Rodiles-Hernández, Hendrickson & Lundberg.

Etymology. Lacantún for the river in México, gender feminine, and enigmatic for the unexpected discovery, obscure relationships and origin.

Common name: "Chiapas Catfish" or "Bagre de Chiapas".

Holotype. El Colegio de la Frontera Sur (ECO-SC; San Cristóbal de las Casas), ECO-SC 3859, adult male, 427 mm Standard Length (SL), collected by RRH, November, 2002 (Fig. 1a-c).

Type Locality. Río Lacantún, Río Usumacinta basin, Selva Lacandona, Reserva de la Biosfera Montes Azules (16°08'05"N, 90°55'19"W), Chiapas, México (Fig. 1d).

Paratypes and other specimens. All from Río Usumacinta basin, listed in Supplementary Data.

Diagnosis. The family, genus and species are uniquely distinguished from other siluriforms by five autapomorphies. 1) Fifth infraorbital expanded, boomerang-shaped, anteriorly convex, with long, bone-free sensory canal continuing posteriorly from it to prominent sphenotic process (Fig. 2a, b). Primitively in catfishes the infraorbital sensory canal is almost completely surrounded by thin ossicles separated by narrow gaps, the largest infraorbital bone (fifth or sixth) posterior to eye is simple and anteriorly concave, and the sphenotic process is small or lacking<sup>9,10</sup>.

2) Margin of skull thickened along frontals and adjacent lateral ethmoids and sphenotics at origins of much enlarged adductor mandibulae (jaw) and levator arcus palatini (hyoid arch) muscles (Fig. 2b; Supplementary Fig. S1). Also, skull medial to muscle origins severely constricted, flat, lacking crests and fossae. Most catfishes have smaller jaw and hyoid arch muscles with limited cranial attachments<sup>9,11</sup> and broader, arched skulls across frontals and sphenotics. Other catfishes with enlarged jaw and hyoid arch muscles (e.g. diplomystids, modern ictalurids, most cetopsids, some amblycipitids, bagrids, clariids and heptapterids) have different muscle arrangements and attachment sites on skull<sup>12-15</sup>.

3) Pair of cone-shaped "pseudo-pharyngobranchial" bones at anterior tips of enlarged accessory cartilages medial to first and second epibranchials (Fig. 3a). Many catfishes have small paired accessory cartilages medial to epibranchials<sup>13</sup>, but without ossification. The "pseudo-pharyngobranchial" bones and accessory cartilages of *Lacantunia* are not homologous with first or second pharyngobranchials that are primitively retained in few siluriforms as rod-shaped bones anterior and parallel to their companion epibranchials<sup>12</sup>.

4) Hypertrophied, axe-shaped uncinat process on third epibranchial (Fig. 3a). Primitively the third epibranchial of catfishes lacks an uncinat process. Some catfishes have a low process or, if enlarged, a process of markedly different shape<sup>16</sup>.

5) Gas bladder with paired spherical, unencapsulated diverticulae protruding from anterodorsal wall, each extending dorsad before anterior limb of fourth transverse process, lateral to first centrum and anterior limb of tripus, and posterior to occiput and ossified Baudelot's ligament (Fig. 3b). Diverticulae walls of tough connective tissue containing silvery guanine crystals as main gas bladder chamber; without heavy vascularization. Catfishes primitively lack gas bladder diverticulae, though a few have different unpaired or multiple diverticulae posterior or lateral to main bladder<sup>17</sup>.

See Supplementary Information for additional descriptive and measurement data and images. Movies illustrating anatomical detail of bony skeleton from high-resolution X-ray computed tomography (HRXCT) available online: [www.nature.com/nature](http://www.nature.com/nature), <http://clade.acnatsci.org/catfishbone> and [www.digimorph.org](http://www.digimorph.org).

The major phylogenetic lines of Siluriformes are unevenly resolved (Fig. 4, Supplementary Fig. S3). South American Diplomystidae are the sister-group to all other catfishes or Siluroidei<sup>12,18</sup>. The North American fossil Hypsidoridae<sup>18</sup> form a deep clade subtending the remaining extant catfishes. The Neotropical extant Cetopsidae may also be among the most basal catfish lines<sup>14</sup>. The many remaining catfishes are placed in 31 mostly well supported, monophyletic families, yet few multi-family groups<sup>19</sup>. Phylogenetic resolution is high within many families<sup>16,19</sup>, whereas most family interrelationships remain to be determined.

Our phylogenetic analysis (See Supplementary Information for Methods, Data and Supplementary Fig. S3) based on 290 morphological characters varying across a sample of all catfish families confirms that *Lacantunia* is not a basal catfish within or below Diplomystidae. Instead, the "Chiapas Catfish" shares

the derived characters uniting the Siluroidei<sup>18</sup>. Within Siluroidei, *Lacantunia* shares the synapomorphies uniting non-hypsidorid catfishes<sup>18</sup>. Furthermore, the apomorphic development of interdigitating coracoid symphyseal sutures places *Lacantunia* with non-cetopsid Siluroidei<sup>14,16</sup>.

Other Río Usumacinta catfishes represent three families: Ictaluridae, Heptapteridae and Ariidae, so discovery of a new catfish belonging to any of these families would be unsurprising. Such is, however, not the case (Supplementary Fig. S3 Legend). *Lacantunia* resembles ictalurids more than it does heptapterids or ariids, but, *Lacantunia* lacks the synapomorphies uniting crown group Ictaluridae<sup>9,11</sup>, as well as the synapomorphies that place the fossil genus *Astephus*, an early Tertiary North American catfish, as the sister lineage to crown group Ictaluridae<sup>9,11</sup>. Equally, *Lacantunia* possesses none of the synapomorphies of Heptapteridae<sup>13</sup> or Ariidae<sup>20</sup>.

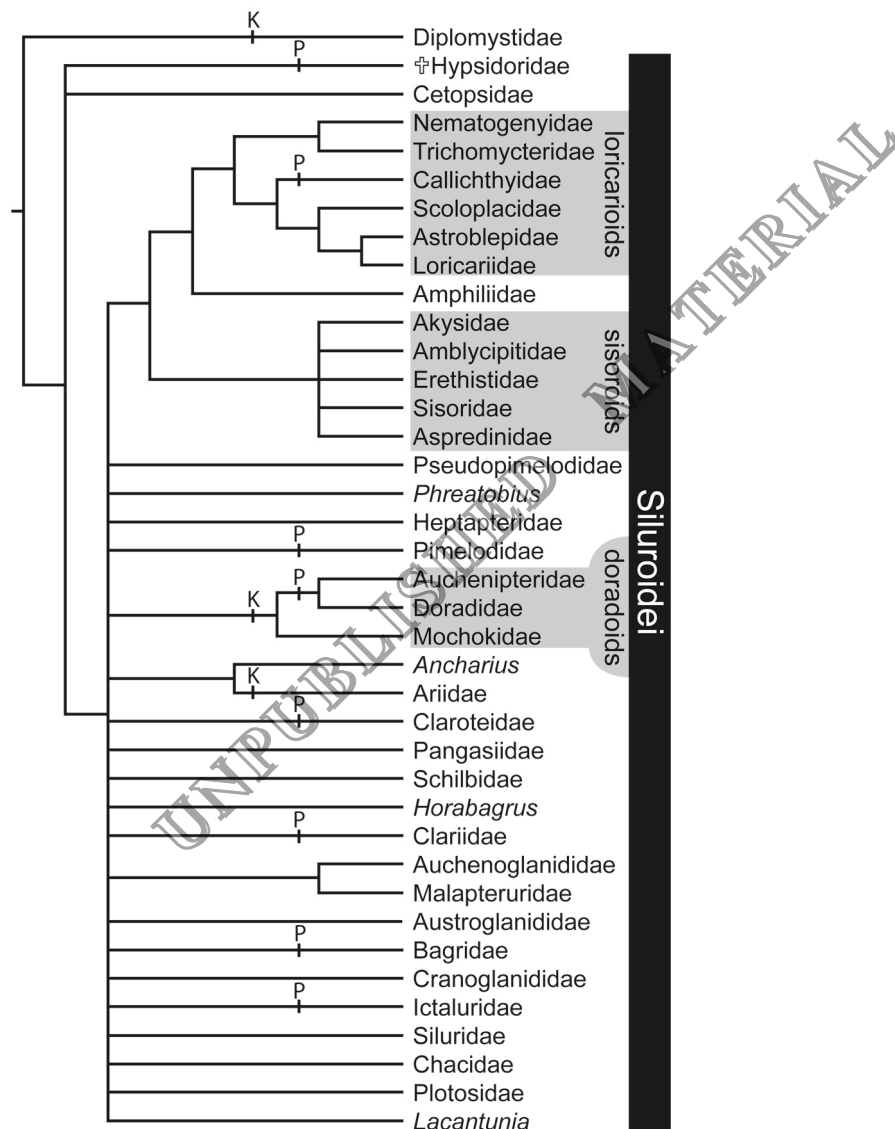


Fig. 4. Summary of higher-level siluriform phylogenetic relationships based on maximum parsimony analysis of 290 morphological characters (Supplementary Fig. 3). The tree is simplified where possible by collapsing multiple terminal taxa into their monophyletic families. Although the strict consensus analysis did not resolve Hypsidoridae and Cetopsidae among Siluroidei, these plesiomorphic families are depicted

as subtending remaining siluroids following Grande<sup>18</sup> and de Pinna & Vari<sup>14</sup>. Letter labels mark lineages judged by fossil evidence to be at least as old as late Cretaceous (K) or Paleocene-Eocene (P)

*Lacantunia* shares several derived characters with various non-hypsidorid siluroids. For examples, an anteromesially expanded palatine bone and cartilage (Fig. 2, Supplementary Fig. S2) is shared with African Claroteidae and South American Cetopsidae, and nasal barbels on the posterior nares (Fig. 1) are present in several Old World families and Ictaluridae. However, maximum parsimony analysis does not identify any of these as unambiguous synapomorphies for placing the “Chiapas Catfish” within or as the sister lineage to any recognized siluroid subgroup.

Our analysis, therefore, places *Lacantunia* above diplomystids, hypsidorids and cetopsids, but as unresolved among the remaining monophyletic subgroups of Siluroidei. As shown by late Campanian to early Maastrichtian fossils of Diplomystidae, Ariidae and Doradoidei<sup>21,22</sup>, diversification of modern catfishes was well underway by late Cretaceous (Fig. 4). Fossils also demonstrate that by at least Paleocene or Eocene several other catfish families and higher groups had originated: extinct Hypsidoridae, and modern Callichthyidae, Pimelodidae, Clariidae, Claroteidae, Bagridae, and Ictaluridae<sup>15,18,21-23</sup>. Significantly, some of these indicate coexistence of related “ghost” lineages yet unrecorded by fossils. For example, late Paleocene fossils of *Corydoras*, a living callichthyid genus, imply coeval or prior origins of confamilial genera and of other groups of Loricarioidei<sup>22,24</sup>. A comparable early Tertiary age seems likely for distinctive, yet unresolved, siluroid lineages such as *Lacantunia* if these are basal relatives to any single or multi-family clades.

Mesoamerica is famous for its complex yet elusive biogeographic<sup>7,25</sup> and geologic<sup>8</sup> histories. The biota of Central America and México is largely composed of plants and animals with separate extralimital affinities and differing inferred ages of origin<sup>7,25</sup>. Biogeographers have drawn on the region's rich biota to illustrate or postulate post Late Cretaceous vicariance, endemic diversification and dispersal from North and South America, Caribbean islands and suspect accretionary terranes<sup>6-8,26-28</sup>. Nowhere is this broad biotic mix better exemplified than in the Río Usumacinta, where a highly endemic, apparently long-isolated, aquatic biota consists of species with North American affinity (e.g. ictalurid catfishes, catostomid suckers, dermatemyd turtles) living alongside others with South American relationships (heptapterid catfishes, characids, gymnotids, cichlids), as well as some with deeper Afro-Indo-southeast Asian affinities (genus *Ophisternon* of Synbranchidae<sup>29</sup>). The Usumacinta also harbors several resident freshwater representatives of Atlantic coastal groups (ariids, gobioids), and many species belonging to rich endemic clades such as poeciliids<sup>3</sup>, with deeper and less obvious geographic affinities.

*Lacantunia enigmatica* thus adds a fourth catfish clade to the Mesoamerican aquatic fauna. This biogeographically puzzling species shows no close relationship to any North or South American or marine taxon, as do the other Usumacinta siluriforms. Without a clear and geographically proximate relative, we propose that *Lacantunia* represents an ancient and relatively basal siluroid lineage from which intermediate members have disappeared. The phylogenetic resolution needed for more definitive dating and biogeographic placement of *Lacantunia* will require additional evidence, including both molecular and additional morphological characters.

As enigmatic as any evolutionary question raised by *Lacantunia* is its late ichthyological discovery. How was such a large and conspicuous species missed until now? Whatever the reasons, the “Chiapas Catfish” reminds us that the most basic scientific inventory of Earth's biodiversity is woefully incomplete. Unfortunately, this fascinating and geographically restricted taxon is threatened by forest removal, proposed hydroelectric reservoirs, and introductions of non-native taxa. Conservation of this unique organism, situated as it is in the Mesoamerican Biodiversity Hotspot<sup>30</sup>, should be a high priority for regional natural resource biodiversity conservation and management planners.

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## Supplementary Information – phylogenetic parts only.

### Phylogenetic Analysis Methods

We assessed the phylogenetic placement of *Lacantunia* in two ways. First, the new catfish was examined for possession of previously reported diagnostic synapomorphies of monophyletic siluriform clades at and above the family level. The clades and principal references are: Diplomystidae<sup>3</sup>; Siluroidei<sup>4</sup>; Hypsidoridae<sup>4,5</sup>; Cetopsidae<sup>6</sup>; Amphiliidae<sup>7</sup>; Loricarioidei<sup>8-10</sup>; Nematogenyidae<sup>8,9</sup>; Trichomycteridae<sup>8,9</sup>; Callichthyidae<sup>11</sup>; Scoloplacidae<sup>10</sup>; Astroblepidae<sup>10</sup>; Loricariidae<sup>12,13</sup>; Sisoroidei<sup>14,15</sup>; Akysidae<sup>14</sup>; Amblycipitidae<sup>14,16</sup>; Erethistidae<sup>14</sup>; Sisoridae<sup>14</sup>; Aspredinidae<sup>14,17</sup>; Pseudopimelodidae<sup>18,19</sup>; Heptapteridae<sup>1,20</sup>; Pimelodidae<sup>21,22</sup>; Doradoidei<sup>9,23,24</sup>; Mochokidae<sup>25,26</sup>; Doradidae<sup>27</sup>; Auchenipteridae<sup>24</sup>; Ariidae<sup>25,28</sup>; Clariidae<sup>29</sup>; Schilbidae<sup>25,26</sup>; Pangasiidae<sup>26</sup>; Claroteidae<sup>25</sup>; Auchenoglanidae<sup>25</sup>; Malapteruridae<sup>26,30</sup>; Bagridae<sup>25,31</sup>; Australoglanididae<sup>32</sup>; Cranoglanididae<sup>32</sup>; Ictaluridae<sup>33-35</sup>; Siluridae<sup>36,37</sup>; Chacidae<sup>38,39</sup>; Plotosidae<sup>26</sup>.

Second, a maximum parsimony cladistic analysis was conducted using a data set with 80 terminal taxa scored for 290 morphological characters. The data were assembled from previous studies (see above and Table 2) plus original observations. Terminal taxa were scored as missing for character states not verifiable by published or original observation of specimens. Following the leads of Mo<sup>25</sup> and de Pinna<sup>26</sup>, the terminal taxa represent the living catfish families and the extinct family Hypsidoridae. The taxonomic scope of the terminals ranged from individual species to families, where entities above the species level are usually based on examination of more than one species (species and specimens listed in references above). We added *L. enigmatica* to the data set. We did not attempt to include the highly fragmentary nominal fossil family Andinichthyidae. The fossil Hypsidoridae was scoreable on 182 or 62.8% of the characters. Unlike de Pinna<sup>26</sup> we did not reconstruct a hypothetical ancestral taxon for rooting the phylogenetic tree. Instead, we designated Diplomystidae as the monophyletic outgroup (sister clade) to all other catfishes (Siluroidei).

The data (Table 2) were analyzed using the parsimony criterion implemented in PAUP\* 4.0b10. Characters were equally weighted and multistate characters were unordered. Heuristic tree searches were conducted with tree bisection-reconnection (TBR) branch swapping in random stepwise addition of taxa replicated 10 times. The strict consensus tree was computed for all of the shortest MP trees and used as the



basis for assessing clade support with Bremer decay indices implemented in MacClade 4.05 and PAUP\* (Fig. 3).

Supplementary Table S2

Table S2. Character and character state descriptions and codes for phylogenetic analysis, and sources.

1. Odontodes<sup>41</sup>: Absent = 0 Present = 1.
2. Mental barbels<sup>42</sup>: Absent = 0; Present = 1.
3. Nasal barbels associated with anterior nostril<sup>26,43</sup>: Absent = 0; Present = 1.
4. Posterior nasal barbel with elastocartilage core<sup>43</sup>: Absent = 0; Present = 1.
5. Cephalic lateralis tubes in skin of snout, cheek & nape<sup>21,26</sup>: Simple, unbranched = 0; Dendritic = 1.
6. Lateralis canal form<sup>24,26</sup>: Straight = 0; Sinusoidal along body = 1.
7. Lateralis canal bones<sup>24,26,44</sup>: Simple = 0; Expanded as single series of scutes = 1.
8. Lips<sup>26</sup>: Simple, not expanded = 0; Expanded as oral disk = 1.
9. Urogenital pore in adult males located at distal tip of anterior margin of anal fin<sup>24,26</sup>: Absent = 0; Present = 1.
10. Anteriorly recurved process "mesethmoid hook" from ventrolateral corner of mesethmoid<sup>1,26</sup>: Absent = 0; Present = 1.
11. Ossified ethmoid plate form<sup>21,26</sup>: Rounded posteriorly or not as state 1 = 0; Parallel sides with abruptly-rounded to nearly right-angled corners = 1.
12. Mesethmoid cornua<sup>10,26</sup>: Prominent = 0; Reduced or absent = 1.
13. Rostral plate<sup>10,26,41,45</sup>: Absent = 0; Present = 1.
14. Mesethmoid ossification<sup>26</sup>: Complete = 0; Only at distal portion of lateral cornua = 1.
15. Anterior portion of ethmoid cartilage<sup>26</sup>: Near anterior margin of lateral ethmoids = 0; Extended anteriorly, forming horizontal shelf between lateral ethmoid & mesethmoid cornua = 1; Larger, roughly quadrangular shelf = 2.
16. Lateral ethmoid wing<sup>1,18,26</sup>: Present as a spine-like wing of membrane bone projecting beyond the palatine condyle = 0; Lateral ethmoid wing absent = 1.
17. Lateral ethmoid facet for palatine<sup>18,26</sup>: A foreshortened inverted triangle = 0; Elongate forming an articular band = 1.
18. Lateral ethmoid facet for palatine<sup>25,26</sup>: Scarcely separate from lateral ethmoid body = 0; Produced laterally, distal cartilage not connected to ethmoid cartilage = 1.
19. Ethmoid cartilage latero-posterior extension<sup>26</sup>: Present = 0; Absent = 1.
20. Posterior margin of lateral ethmoid<sup>26</sup>: Straight, parallel to anterior edge of orbitosphenoid & abruptly angled at midline = 0; Broadly curved in ventral view, forming convex arc = 1.
21. Anterior profile of lateral ethmoid & associated cartilage<sup>26</sup>: Rounded & convex = 0; Lateral ethmoid truncated; profile transverse relative to long axis of neurocranium or concave = 1.
22. Orientation of lateral ethmoid articular facet including cartilaginous & bony parts for palatine<sup>26</sup>: Lateral = 0; Anterolateral or anterior = 1.
23. Superficial ophthalmic nerve foramen<sup>26,43</sup>: Small & inconspicuous = 0; Evident foramen bounded laterally by a bridge of bone between frontal & lateral ethmoid = 1; Much enlarged fenestra = 2.
24. Exit of infraorbital sensory canal from skull<sup>33,43</sup>: Sphenotic = 0; Frontal = 1.
25. Cranial fontanels<sup>10,26,43</sup>: One or both fontanels open in adults = 0; Both fontanels closed in adults = 1.
26. Supraoccipital<sup>6</sup>: Extended as posterior process even if short and forms free posterior border of skull margin = 0; Abruptly truncated posteriorly & isolated from skull margin = 1.
27. Lateral ethmoid - sphenotic<sup>26,36</sup>: Separate & remote = 0; Contacting each other by extensions lateral to frontal = 1.
28. Temporal region bones<sup>10,26,46</sup>: Pterotic, posttemporo-supracleithrum separate = 0; Pterotic, posttemporo-supracleithrum fused = 1.
29. Pterotic extent<sup>10,26</sup>: Not covering cleithrum = 0; Dorsally covering dorsal process of cleithrum = 1.
30. Pterotic - Frontal<sup>25,26</sup>: No contact = 0; Pterotic sutured with frontal = 1.
31. Dorsal end of supraclathrum<sup>26</sup>: Overlays pterotic or exoccipital and exposed dorsally = 0; Overlain by posterolateral shelf of pterotic = 1.
32. Epioccipital<sup>24,26</sup>: Not in skull roof = 0; Extending onto skull roof = 1.

33. Epioccipital posterior process<sup>25,26,42</sup>: None = 0; Posterior laminar process, often sutured to elements of anterior vertebrae. = 1.
34. Vomerine teeth<sup>26,33,43</sup>: Absent = 0; Present = 1.
35. Vomer-palatine articulation<sup>26</sup>: Present = 0; Absent = 1.
36. Vomer<sup>10,18,26,38</sup>: Present = 0; Absent = 1.
37. Extra-vomerine "palatine," "pterygoid" toothplates<sup>4,26,47</sup>: Absent = 0; Present = 1.
38. Vomer<sup>3</sup>: T-shaped = 0; Broad rhomboidal = 1.
39. Vomer with large crescentic head<sup>25,31</sup>: Absent = 0; Present = 1.
40. Orbitosphenoid-lateral ethmoid joint<sup>25,26</sup>: Synchondral = 0; Strongly sutured = 1.
41. Fenestra between lateral ethmoid & orbitosphenoid<sup>25,26</sup>: Present & large = 0; Reduced or absent = 1.
42. Anterior portion of orbitosphenoid<sup>26</sup>: Broad = 0; Strongly narrowed = 1.
43. Orbitosphenoid<sup>38</sup>: Single = 0; Paired = 1.
44. Utriculus<sup>26</sup>: Not enlarged = 0; Moderately enlarged = 1, Much enlarged occupying a bulla formed by prootic, pterotic & exoccipital = 2.
45. Vagus foramen<sup>25</sup>: Small or moderate = 0; Enlarged = 1.
46. Transcapular (Baudelot's) process<sup>26</sup>: Bony or ligamentous connection with basioccipital or exoccipital = 0; Linked to anterior part of Weberian complex = 1.
47. Short lateral processes of basioccipital-exoccipital extend to ossified Transcapular process<sup>48</sup>: Absent = 0; Present = 1.
48. Lateral ethmoid-palatine ligament<sup>10,26</sup>: Absent = 0; Present = 1.
49. First infraorbital<sup>25,26,49</sup>: Loosely or not connected to lateral ethmoid = 0; Thickened, in contact or sutured to lateral ethmoid = 1
50. Last posterior infraorbital<sup>26</sup>: Tubular = 0; Plate forming a lateral shelf of cranial roof = 1.
51. Infraorbital latero-sensory canal<sup>26</sup>: Curved & apart from lateral ethmoid = 0; Adpressed to lateral margin of lateral ethmoid = 1.
52. Infraorbital latero-sensory canal<sup>26</sup>: Curved = 0; Straight for most of its length = 1.
53. Infraorbital bone number<sup>18</sup>: Five bones = 0; Six = 1; > Six = 2; Four = 3.
54. Infraorbital 5 (This study): Rod or comma shaped = 0; Boomerang shaped & posteriorly concave = 1.
55. Opercle-interopercle joint<sup>26</sup>: Loose with simple bone margins = 0; Overlapping articulation = 1.
56. Opercle shape<sup>26</sup>: Broadly triangular = 0; Narrow posteriorly = 1.
57. Articular facet of opercle<sup>26</sup>: Short = 0; Produced = 1.
58. Interopercle<sup>10,26</sup>: Present = 0; Absent = 1.
59. Interoperculo-mandibular ligament<sup>26</sup>: To anterior tip of interopercle = 0; To dorsal tip of interopercle = 1.
60. Interopercle<sup>26</sup>: Shallow & elongated = 0; Expanded dorso-ventrally = 1.
61. Interoperculo-mandibular ligament<sup>10,26,50</sup>: Present = 0; Absent = 1.
62. Interopercle pit<sup>26</sup>: Absent = 0; Mesial face with deep pit = 1.
63. Premaxillary mobility<sup>10,26</sup>: None or limited = 0; Highly movable = 1.
64. Premaxillary bone dorsal process near symphysis<sup>10,26</sup>: Absent = 0; Present = 1.
65. Lateral margin of premaxilla<sup>26</sup>: Straight or convex = 0; Bifurcated or strongly concave, with pointed corners = 1.
66. Premaxilla width<sup>26</sup>: Broad, passing beyond mesethmoid cornua = 0; Extremely narrow, not extending posteriorly beyond margin of mesethmoid cornua = 1.
67. Lateral tip of premaxilla<sup>26</sup>: Straight or convex = 0; pointed = 1.
68. Premaxillary dorsolateral processes<sup>26</sup>: Absent = 0; Single claw-shaped process on anterior border = 1; Enlarged bifurcate process = 2.
69. Maxillary bone head<sup>26,43</sup>: Palatine condyle unpaired = 0; palatine condyles paired = 1.
70. Maxillary barbel orientation<sup>24,26</sup>: Abducted posteriorly = 0; Abducted laterally & dorsally = 1.
71. Maxillary bone foramen<sup>26</sup>: Absent = 0; Present = 1.
72. Maxilla size<sup>26</sup>: much larger than anterior cartilage of palatine = 0; Small, about the size of anterior cartilage of palatine = 1.
73. Maxillary shaft<sup>26</sup>: Slightly concave or flat against maxillary barbel core = 0; A complete or nearly complete tube around maxillary barbel core = 1.
74. Maxillary shaft shape<sup>26</sup>: Straight or curved posteriorly when abducted = 0; Curved anteriorly when abducted = 1.
75. Maxillary teeth<sup>3,4,26,42</sup>: Present = 0; Absent = 1.

76. Maxilla form<sup>26</sup>: Attenuated & elongated, expanding or narrowing gently = 0; Expanding, then narrowing abruptly in leaf-like shape = 1.
77. Distally expanded maxilla<sup>3-5</sup>: Yes = 0; Reduced = 1.
78. Long medial maxillary process<sup>3-5</sup>: Yes = 0; Reduced = 1.
79. Maxilla position<sup>3-5</sup>: In margin, i.e. biting surface, of upper jaw = 0; Excluded from margin of upper jaw = 1.
80. Jaw teeth form<sup>10,26</sup>: Simple conical = 0; Bifid = 1.
81. Cusps of jaw teeth asymmetrical<sup>10,12,26</sup>: Conic or equally bifid = 0; Asymmetrically bifid = 1.
82. Coronoid process form<sup>4,21,26</sup>: Shallow = 0; Deep & steep sided = 1.
83. Mandibular symphysis<sup>10,26</sup>: Tightly bound = 0; Loosely tied = 1.
84. Meckel's cartilage<sup>25,26</sup>: Simple rod on mesial face of lower jaw = 0; With an ascending portion along coronoid process = 1.
85. Coronomeckelian bone<sup>25,26</sup>: Present = 0; Absent = 1.
86. Rod-like elastic cartilage linking coronoid process of lower jaw with region just ventral to maxillary barbel base<sup>26</sup>: Absent = 0; Present = 1.
87. Meckel's cartilage posterior limit<sup>26</sup>: To about level of coronoid process = 0; Extending posteriorly much further beyond limit of dentary anguloarticular in coronoid process = 1.
88. Posterior end of anguloarticular<sup>26</sup>: Short & straight posterior process = 0; Process directed dorsally, fitting into a well-defined concavity on quadrate = 1.
89. Coronomeckelian bone<sup>26</sup>: Small & not projecting beyond dorsal border of lower jaw, or otherwise not as state 1 = 0; Elongated to project beyond dorsal border of lower jaw & directed posteriorly = 1.
90. Meckel's cartilage length<sup>26</sup>: Longer than coronoid region of mandible = 0; Shorter than coronoid region of mandible = 1.
91. Meckel's cartilage size<sup>25,26</sup>: Larger than thin disk between dentary & anguloarticular = 0; Reduced to a thin cartilaginous disk between dentary & anguloarticular = 1.
92. Articular condyle<sup>26</sup>: Shallow concave condyle for quadrate = 0; Anguloarticular expanded into spoon-like structure, partly surrounding anterior facet of quadrate = 1.
93. Tooth-bearing surface of lower jaw<sup>26</sup>: Flat or slightly convex = 0; In a deep, anterior concavity of the dentary = 1.
94. Ossified coronoid process<sup>26</sup>: Present = 0; Process absent or cartilaginous only = 1.
95. Articulation of coronomeckelian bone<sup>26</sup>: Mainly to Meckel's cartilage = 0; Firmly attached or fused to dentary & anguloarticular = 1.
96. Mandibular latero-sensory canal<sup>10,26</sup>: Present = 0; Absent = 1.
97. Mandibular latero-sensory canal<sup>26</sup>: Narrow = 0; Enlarged to occupy about one-third of bone = 1; Greatly enlarged to occupy about half of bone = 2.
98. Palatine anterior head<sup>26,43</sup>: Single = 0; Double = 1.
99. Palatine size & shape<sup>26,36,42,51</sup>: Rod-like = 0; Small nodule = 1.
100. Palatine shape anteriorly<sup>25,26</sup>: Rod-like = 0; depressed, expanded, much wider than posterior portion = 1.
101. Palatine posterior cartilage<sup>25,26</sup>: Present = 0; Absent = 1.
102. Posterior portion of palatine<sup>26</sup>: Center of bone & cartilage not compressed & deep = 0; both bone & distal cartilage laterally compressed, dorso-ventrally expanded = 1.
103. Anterior cartilage of palatine<sup>26</sup>: Short, rounded = 0; Enlarged, ca. 50% of bone length = 1.
104. Posterior limb of palatine<sup>26</sup>: Rod-like or oval in section = 0; Depressed, expanded lateromesially = 1.
105. Anterior distal cartilage of palatine<sup>26</sup>: Limited to anterior tip = 0; Extended mesially = 1.
106. Anterior cartilage of palatine<sup>26</sup>: Free of covering bone = 0; Covered laterally by sheet of superficial ossification = 1.
107. Posterior limb of palatine<sup>26</sup>: Slightly widening or not markedly tapering to a small cartilaginous tip = 0; markedly narrowing to small distal cartilage = 1.
108. Relative lengths of bony palatine arms<sup>26</sup>: Pre- & post-articular arms about equal = 0; Post-articular arm of bony palatine shorter than prearticular = 1.
109. Orientation of facet on palatine for lateral ethmoid<sup>26</sup>: Directed posteromesially = 0; Directed anteromesially = 1.
110. Orientation of posterior cartilage of palatine<sup>26</sup>: Posteriorly = 0; Posterolaterally = 1.
111. Bony posterior end of palatine<sup>26</sup>: Single conical tip = 0; Dorsoventrally bifurcated = 1.
112. Posterior ossification center of palatine<sup>26</sup>: Single = 0; Double = 1.

113. Orientation of lateral ethmoid-palatine articulation<sup>26</sup>: Dorsoventral = 0; Mesiolateral = 1.
114. Mesethmoid-maxillary ligament<sup>10,26</sup>: Absent = 0; Present = 1.
115. Mesethmoid-premaxillary ligaments<sup>10,26</sup>: Absent = 0; One ligament = 1; Two ligaments = 2.
116. Retractor tentaculi muscle<sup>10,26,43,52</sup>: Absent = 0; Present = 1.
117. Adductor mandibulae linked to premaxilla<sup>10,26,46</sup>: No = 0; Muscle in part inserts on ligamentum primordium near premaxilla = 1; "Retractor premaxillae" subdivision inserts on premaxilla = 2.
118. Retractor tentaculi muscle origin<sup>10,26</sup>: Suspensorium = 0; Skull = 1.
119. Geniohyodeus muscle<sup>10,26</sup>: Undivided = 0; Bilaterally subdivided = 1.
120. Adductor mandibulae mesial subdivision inserting on premaxilla<sup>10,26,46,50</sup>: Absent = 0; Present = 1.
121. Adductor hyomandibularis process on hyomandibula<sup>26,43</sup>: Not enlarged = 0; Hypertrophied = 1.
122. Hyomandibula-metapterygoid joint<sup>26</sup>: Simple overlap or suture = 0; "Lap" joint with depressions = 1.
123. Condyle for opercle on hyomandibula<sup>26</sup>: Directed posteriorly = 0; Directed ventrally = 1.
124. Cranial facet of hyomandibula<sup>26</sup>: Long = 0; Short < 20% of longest side of hyomandibular = 1.
125. Levator operculi process on hyomandibula<sup>1,18,20</sup>: Small to moderate = 0; Hypertrophied = 1.
126. Hyomandibula pierced by a large mandibular nerve foramen (This study): Absent = 0; Present = 1.
127. Hyomandibular cartilage<sup>10,26</sup>: Absent = 0; Present = 1.
128. Quadrate - metapterygoid joint<sup>26</sup>: Partly cartilaginous = 0; Without intermediate layer of cartilage = 1.
129. Hyomandibula-metapterygoid contact<sup>18,20</sup>: Present above quadrate = 0; None, quadrate with free dorsal edge = 1.
130. Dorsal extension of preopercle<sup>26</sup>: Short = 0; dorsally to near dorsal border of hyomandibula = 1.
131. Dorsal margin of metapterygoid<sup>12,26</sup>: No contact with lateral ethmoid = 0; Contacting lateral ethmoid = 1.
132. Metapterygoid<sup>18,26</sup>: About as broad as long = 0; Foreshortened twice or more as broad as long, its dorsal fourth or more deflected inward & usually subtended by a ridge or crest = 1.
133. Metapterygoid<sup>24,26</sup>: Expanded & laminar = 0; A conical ossification with little or no laminar extension = 1.
134. Metapterygoid-palatine ligament<sup>10,26</sup>: Poorly differentiated sheet of connective tissue = 0; Discrete ligament = 1.
135. Endopterygoid<sup>26</sup>: Elongate irregular plate = 0; L-shaped with anterior process directed laterally = 1.
136. Endopterygoid & ectopterygoid form<sup>18,21,53</sup>: If present, otherwise = 0; Endopterygoid broad & ectopterygoid comma shaped = 1.
137. Pterygoethmoid ligament<sup>10,26</sup>: Pterygoids attached to ethmovomerine area = 0; Absent or not attached to cranium = 1.
138. Adductor mandibulae muscle origin<sup>4,33,45</sup>: Restricted to cheek & suspensium = 0; Spread onto skull roof = 1.
139. Conformation of cheek-muscle attachment sites (This study): Not as in state 1 = 0; Skull roof lateral margin thickened & emarginated, except for large sphenotic spine, muscle not extending onto skull roof. Present = 1.
140. Basibranchial 1<sup>26</sup>: slightly expanded & convex = 0; expanded & flat anteriorly = 1.
141. Basibranchials 1, 2, 3<sup>38</sup>: Not unusually small = 0; Reduced in size = 1.
142. Basibranchial 2 shaft<sup>26</sup>: Cylindrical = 0; Expanded laterally, with strongly convex lateral margins = 1.
143. Basibranchial 4<sup>26</sup>: Longer than broad = 0; Broader than long = 1.
144. Posterior extension of basibranchial 4<sup>26</sup>: Very short posterior extension = 0; Greatly elongated tip reaching midlength of ceratobranchial 5 = 1.
145. Accessory basibranchial cartilage located between basibranchials 3 & 4<sup>24,26</sup>: Absent = 0; Present = 1.
146. All basibranchial & hypobranchial elements<sup>26</sup>: Partly ossified = 0; Unossified = 1.
147. Hypobranchials<sup>26</sup>: Present ossified or cartilaginous = 0; Absent or very reduced even as cartilages = 1.
148. Lateral cartilage of hypobranchial 1<sup>26</sup>: Shorter than its bone = 0; Enlarged = 1.
149. Hypobranchial 1<sup>26</sup>: With posterior cartilage. = 0; Without posterior cartilage = 1.
150. Hypobranchial 1<sup>26</sup>: Ossified = 0; Unossified = 1.
151. Hypobranchials 1 & 2<sup>26</sup>: Not unusually narrow, width > 10% length = 0; Extremely narrow = 1.
152. Hypobranchial 2<sup>26</sup>: Straight or slightly convex anteriorly = 0; With anterior concavity = 1.
153. Hypobranchial 2<sup>26</sup>: With posterior cartilage = 0; Lacking posterior cartilage = 1.
154. Hypobranchial 2<sup>26</sup>: Ossified = 0; Unossified = 1.
155. Hypobranchial 3<sup>26</sup>: Ossified = 0; Unossified = 1.

156. Hypobranchials 3<sup>26</sup>: Separated on midline = 0; United mesially by continuous cartilage, surrounding basibranchial 2 = 1.
157. Hypobranchial 3<sup>26</sup>: No lateral process = 0; Lateral process directed anteriorly or anterolaterally = 1.
158. Gill rakers of ceratobranchial 1 and usually 2<sup>26</sup>: Double row = 0; Single row = 1.
159. Lateral cartilage of ceratobranchial 3<sup>26</sup>: No or small process = 0; Strongly extended as a process, supporting basket of gill-filaments = 1.
160. Lateral process on anterior cartilage of ceratobranchial 4<sup>26</sup>: Absent = 0; Present = 1.
161. Basibranchial 4<sup>26</sup>: Without lateral expansions = 0; With lateral expansions near contact with proximal cartilaginous tip of ceratobranchial 4 = 1.
162. Anterior cartilage of ceratobranchial 4<sup>26</sup>: Anteriorly broad = 0; Anteriorly narrowed to point = 1.
163. Cartilaginous head of ceratobranchial 4<sup>26</sup>: Not overlapping hypobranchial 3 = 0; Overlapping posterior part or fitting into a concavity = 1.
164. Cartilaginous head of 4th ceratobranchial<sup>26</sup>: Meets basibranchial 4 at oblique angle = 0; Elongate, running parallel to lateral sides of basibranchial 4 = 1.
165. Ceratobranchial 4 anterior cartilage<sup>26</sup>: Lateral process at midlength = 0; Lateral process located distally = 1.
166. Ceratobranchial 4<sup>26</sup>: Lacking a tooth patch = 0; Tooth patch present = 1.
167. Ceratobranchial 5<sup>26</sup>: Distal cartilage unossified = 0; Distal cartilage with posterior ossification = 1.
168. Ceratobranchial 5 teeth<sup>26</sup>: Widespread = 0; Limited to mesial margin of bone = 1.
169. Ceratobranchial 5 teeth<sup>26</sup>: On flat or little thickened surface = 0; On a bony platform = 1.
170. Mesial ends of epibranchials 1 & 2<sup>26</sup>: At same mesial level as anterior tip of pharyngobranchial 3 = 0; Laterally remote from anterior tip of pharyngobranchial 3 = 1.
171. Mesial ends of epibranchials 1 & 2<sup>26</sup>: Not surpassing anterior tip of pharyngobranchial 3 = 0; anteriorly covering anterior tip of pharyngobranchial 3 = 1.
172. Posterior margin of epibranchials 1 & 2<sup>26</sup>: Lacking expansions = 0; Expanded posterolaterally = 1.
173. Anterior margin of epibranchial 1<sup>26</sup>: Lacking expansions = 0; Irregular expansion at mesial end = 1.
174. Epibranchials 1 & 2<sup>26</sup>: Slightly or not expanded mesially = 0; Expanded at mesial end = 1.
175. Distance between mesial tips of epibranchials 2 & 3<sup>26</sup>: About equal to distance between epibranchials 3 & 4 = 0; Much larger at least three times than that between epibranchials 3 & 4 = 1.
176. Mesial tip of epibranchial 2<sup>26</sup>: Not contacting epibranchial 1 = 0; Abutting against posterior margin of epibranchial 1 = 1.
177. Uncinate processes on epibranchials 3 & 4<sup>26</sup>: With cartilages at tips = 0; Without cartilages = 1.
178. Uncinate process of epibranchial 3<sup>26</sup>: Turned mesially = 0; Turned laterally = 1.
179. Epibranchial 3 articulation<sup>26</sup>: With pharyngobranchial 4 = 0; curved anteriorly, articulating directly with pharyngobranchial 3 = 1.
180. Basibranchial 3<sup>26</sup>: Simply truncated = 0; Expanded posteriorly, fan-like = 1.
181. Epibranchial 3 uncinat process<sup>26</sup> (This study): Elongate & narrow = 0; Broader than main axis of bone & triangular = 1; Distinctively elevated = 2.
182. Epibranchial 3 uncinat process<sup>26</sup>: gently curved & with a rounded distal margin = 0; very elongate, pointed & straight = 1.
183. Epibranchial 3 uncinat process<sup>26</sup>: not strongly deflected = 0; directed strongly mesially = 1.
184. Epibranchial 1<sup>26</sup>: Irregularly curved or nearly straight = 0; Elongate S-shape = 1.
185. Epibranchial 4 uncinat process<sup>26</sup>: Absent = 0; Well-defined = 1; Extremely large = 2.
186. Epibranchial 4 width<sup>26</sup>: Wider than other epibranchials = 0; As narrow, or narrower than, epibranchial 3 = 1.
187. Epibranchial 4<sup>26</sup>: Posterior margin evenly convex = 0; Posterior margin pointed = 1.
188. Epibranchial 5<sup>26</sup>: Cartilaginous remnant independent = 0; Cartilaginous remnant fused to distal cartilage of ceratobranchial 4 = 1.
189. Epibranchial 5<sup>26</sup>: Cartilaginous = 0; Partly ossified. = 1.
190. Epibranchial 5 vestige<sup>26</sup>: Present = 0; Absent = 1.
191. Remnant of epibranchial 5<sup>26</sup>: Simple rod = 0; Expanded distally = 1.
192. Pharyngobranchial 1<sup>26</sup>: Slender rod parallel to epibranchial = 0; Strongly curved dorsally at distal end = 1.
193. Pharyngobranchial 1<sup>26</sup>: Present = 0; Absent = 1.
194. Pharyngobranchial 2<sup>26</sup>: When present reaches to mesial ends of epibranchials 1 & 2 & pharyngobranchial 1 = 0; Reaches more mesially than associated bones = 1.

195. Pharyngobranchial 2<sup>25,26</sup>: Present = 0; Absent = 1.
196. Pharyngobranchial 3<sup>26</sup>: Straight, concave or gently convex laterally = 0; Strongly protruded laterally = 1.
197. Pharyngobranchial 3<sup>26</sup>: Short anterior process = 0; Very elongated anterior part = 1.
198. Pharyngobranchial 3<sup>26</sup>: Wide posteriorly = 0; Of nearly uniform width = 1.
199. Pharyngobranchial 3<sup>26</sup>: Smooth mesial lamina = 0; Mesial lamina present = 1.
200. Pharyngobranchial 4<sup>26</sup>: Unossified = 0; Ossified = 1.
201. Paired "pseudo-pharyngobranchial" bones at anterior tips of much-elongated accessory cartilages medial to epibranchials 1 (This study): Absent = 0; Present = 1.
202. Upper pharyngeal toothplate<sup>26</sup>: Close to overlying epibranchial = 0; Far from overlying epibranchial = 1.
203. Upper pharyngeal toothplate-pharyngobranchial 4 joint<sup>26</sup>: Without bony expansions = 0; Two struts of bone = 1.
204. Dorso-mesial border of upper pharyngeal tooth plate<sup>26</sup>: Without processes = 0; Ridge of bone partly covering ventral border of last pharyngobranchial = 1.
205. Gill filament ossifications<sup>26</sup>: None = 0; Present along proximal part of ceratobranchial = 1.
206. Internal support of gill filaments<sup>26</sup>: Simple without long branches = 0; With long transverse secondary branches = 1.
207. Gill filaments & skeleton of epibranchials & ceratobranchials 2 & 3<sup>26</sup>: Unmodified = 0; Feather-like forming basket-shaped structure = 1.
208. Arborescent organ at distal margin of epibranchials 2 & 4<sup>26</sup>: Absent = 0; Present = 1.
209. Gill rakers<sup>26</sup>: Present = 0; Absent = 1.
210. Gill rakers on branchial arch 1<sup>26,54</sup>: < 54 = 0; > 55 = 1.
211. Gill rakers<sup>26,54</sup>: Not unusually elongated = 0; Elongated as long as or longer than gill filaments on branchial arch 1. = 1.
212. Dorsal hypohyal<sup>18,21,53</sup>: Present = 0; Absent = 1.
213. Anterior-posterior ceratohyal joint<sup>4,5</sup>: Synchronal = 0; Sutural = 1.
214. Interhyal<sup>25</sup>: Small rod-like or nodular bone = 0; With an enlarged wing = 1.
215. Posterior part of urohyal<sup>25,26</sup>: With dorsal & horizontal laminae = 0; Directed ventrally, lacking horizontal & dorsal laminae = 1.
216. Posterior limb of transverse process 4<sup>1,18,26</sup>: Laterally slender or otherwise expanded = 0; Laterally expanded above swimbladder & notched once to several times = 1.
217. Transverse processes 4, 5<sup>21,26</sup>: Not or incompletely conjoined = 0; Sutured to their lateral edges = 1.
218. Neural arch of complex centrum sutured to exoccipital<sup>24,26</sup>: No = 0; Yes = 1.
219. Os suspensorium<sup>24,26</sup>: Bony shaft thickened distally = 0; Reduced to a disc-shaped nodule = 1.
220. Müllerian ramus<sup>26,55</sup>: Absent = 0; Present = 1.
221. Wall of swimbladder ossified<sup>25,26,46</sup>: No = 0; Yes = 1.
222. "Lateral bone" between proximal dorsal radial 2 & vertebra 6<sup>10,26,45</sup>: Absent = 0; Present = 1.
223. Ventral side of complex centrum<sup>26</sup>: Without flattened anterior processes = 0; Flat paired processes near anterior end = 1.
224. Flat processes described in character 200<sup>26</sup>: Absent or as described above = 0; Extremely elongated, partly surrounding ventral region of swimbladder & forming part of ventral wall of swimbladder capsule = 1.
225. Vertebra 5<sup>26</sup>: With single transverse process = 0; With secondary transverse process directed laterally. = 1.
226. Transverse process 4<sup>26</sup>: Expanded compared to posterior transverse processes but undivided or shallowly concave laterally = 0; With a well-defined posterior arm = 1; Anterior & posterior arms deeply divided to compound centrum = 2.
227. Weberian neural spine lamina<sup>26</sup>: Concave or low = 0; Dorsal margin straight = 1.
228. Ventral side of complex centrum<sup>26</sup>: Without extra processes at midlength = 0; Extra ventral process at midlength on complex centrum = 1.
229. Vertebral centrum 5<sup>26</sup>: Normal intervertebral joint to complex centrum = 0; Sutured to complex centrum. = 1.
230. Lamellar bone on fifth vertebra<sup>4,5</sup>: Absent = 0; Present = 1.
231. Supracarinalis muscle forms elastic spring muscle<sup>23</sup>: Absent = 0; Present = 1.
232. Centra 5-6<sup>18</sup>: Normal intervertebral joint = 0; Sutured = 1.

233. Vertebral parapophyses<sup>10,26</sup>: Present = 0; Absent = 1.
234. Neural spine of seventh vertebra<sup>26</sup>: Not enlarged = 0; Enlarged & oriented vertically = 1.
235. Precaudal vertebrae articulations<sup>26</sup>: Normal intervertebral joints = 0; Connected by complex system of articulating processes & associated ligaments = 1.
236. Parapophyses of anterior free vertebrae<sup>26</sup>: Short, about equal in length to centrum width = 0; Elongate, twice or more as long as centrum width = 1.
237. Elongated posterior neural spines<sup>29</sup>: Absent = 0; Present = 1.
238. Ribs attachment<sup>25,26</sup>: To ventral face of parapophyses = 0; To dorsal face of parapophyses = 1.
239. Rib on vertebra 6<sup>10,26</sup>: Articulating with parapophysis = 0; Articulating in concave socket on centrum = 1.
240. Pleural ribs<sup>26</sup>: Unmodified = 0; Contorted basally, wrapping around neck of vertebral parapophyses = 1.
241. Humerovertebral ligamen<sup>14</sup>: Absent = 0; Present = 1.
242. Limbs of dorsal-fin lepidotrich 1<sup>23</sup>: Not unusually large = 0; Elongate, thick & recurved with insertion of dorsal fin depressor muscle = 1.
243. Dorsal-fin spine sexually dimorphic, seasonally enlarged & with additional spinulation in males<sup>24,26</sup>: Absent = 0; Present = 1.
244. Dorsal spine medial ridges<sup>14</sup>: Absent = 0; Present = 1.
245. Dorsal fin<sup>36,42</sup>: Present = 0; Absent = 1.
246. Anterior & middle nuchal plates<sup>23,24,48</sup>: In series & with overall triangular shape = 0; Anterior nuchal plate surrounded by anterior limbs of middle nuchal plate, & overall quadrangular = 1.
247. Ossified ligament between second dorsal-fin spine radial & rib of sixth vertebra<sup>24,26</sup>: Absent = 0; Present = 1.
248. Dorsal-fin radials 1, 2<sup>23</sup>: Not jointed to vertebrae = 0; Tightly jointed to vertebrae 4-5-6 = 1.
249. Dorsal-fin proximal radials 3-7<sup>18,21,53</sup>: Separated = 0; In broad contact = 1.
250. Dorsal-fin distal radials<sup>36</sup>: Present = 0; Absence = 1.
251. Dorsal procurent rays of caudal fin<sup>25,26</sup>: Few = 0; Extended anteriorly = 1.
252. Principal upper caudal-fin rays<sup>4,26,56</sup>: 8 = 0; 7 or fewer = 1.
253. Second ural centrum<sup>15,56</sup>: Reduced = 0; Developed = 1.
254. Anal-fin pterygiophores<sup>36</sup>: Normal teleostean alignment = 0; Shifted posterodorsally = 1.
255. Anal fin-caudal fin<sup>42</sup>: Separate = 0; Anal fin elongated & confluent with caudal = 1.
256. Medial interdigitation of cleithra<sup>7</sup>: Absent = 0; Present = 1.
257. Symphysis of coracoids<sup>5,6,36,42</sup>: Scarcely articulated or weakly sutured = 0; Coarsely interdigitating sutures = 1.
258. Mesocoracoid<sup>26,42</sup>: Present = 0; Absent = 1.
259. Retractor temporalis muscle<sup>25,31</sup>: Absent = 0; Present = 1.
260. Pectoral spine segments<sup>14</sup>: Normal = 0; Oblique = 1.
261. Ossified Baudelot's transscapular ligament<sup>26,36</sup>: Rounded in cross-section = 0; Anteroposteriorly compressed = 1.
262. Large posttemporal-supracleithrum with process & fossa<sup>25,31</sup>: Absent = 0; Present = 1.
263. Subpterotic process on supracleithrum<sup>43</sup>: Absent = 0; Present = 1.
264. External anterior process of basipterygium<sup>26</sup>: Distal cartilage a small rod-like cap = 0; Distal cartilage expanded = 1.
265. Internal & external anterior arms of basipterygium<sup>26</sup>: Distal cartilages separate = 0; Distal cartilages united, forming an anterior ring-like structure anterior to each basipterygium = 1.
266. Anterior processes of basipterygium<sup>26</sup>: Present = 0; Undifferentiated from main part of bone = 1.
267. Distal cartilage of external anterior arm of basipterygium<sup>26</sup>: Simple anteriorly directed rod = 0; Lateral extension directed posteriorly = 1.
268. External anterior arm of basipterygium<sup>26</sup>: Narrows or expands evenly = 0; Extremely expanded distally = 1.
269. Anterior arms of basipterygium<sup>26</sup>: With cartilaginous tips = 0; Without a cartilaginous tips = 1.
270. Basipterygium dorsal surface<sup>26</sup>: Lacking processes = 0; With a vertical lamina forming posteriorly directed process = 1.
271. Ventral surface of basipterygium<sup>26</sup>: Lacking processes = 0; With a flange along internal anterior process = 1.









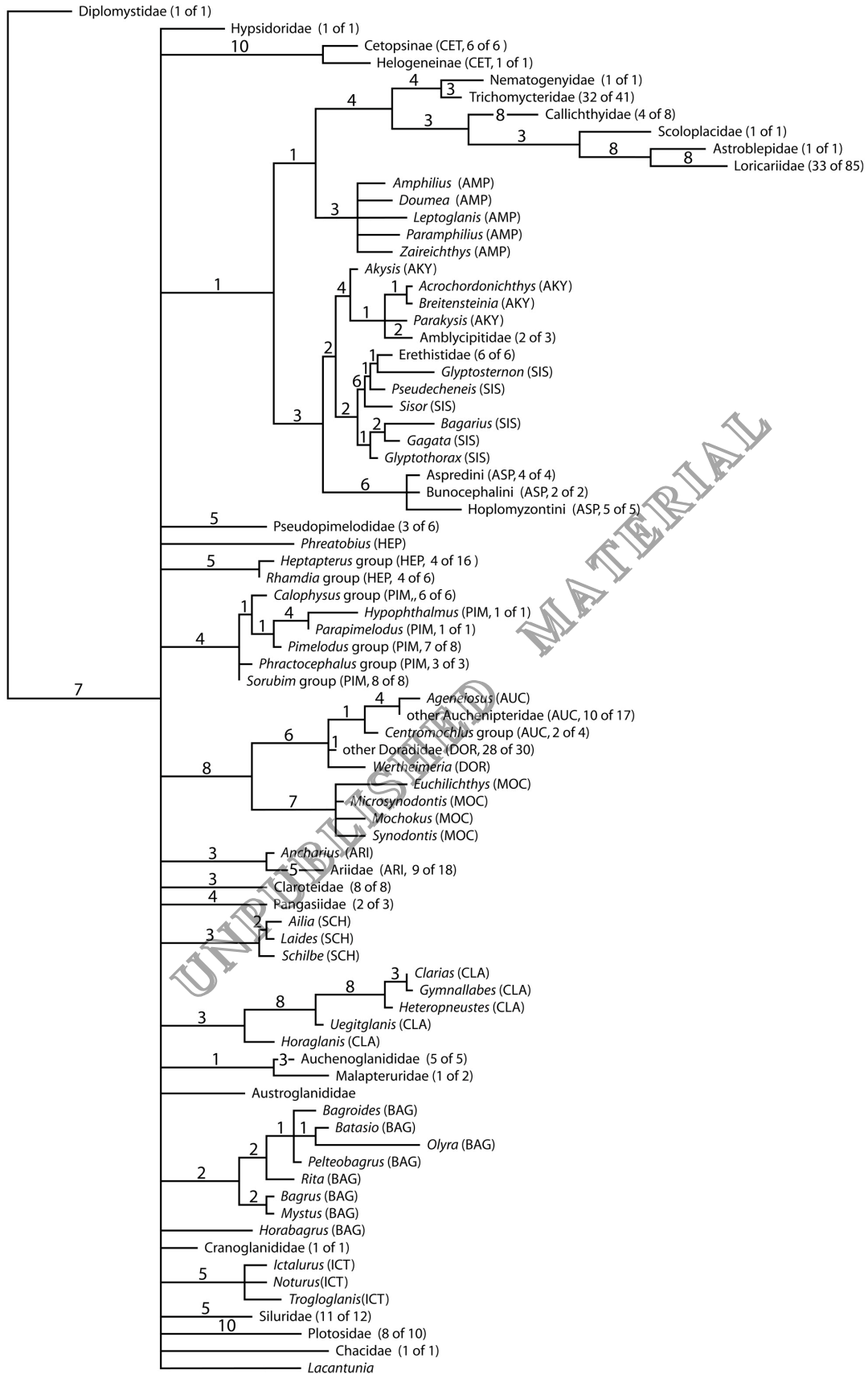






HEP.Heptapterida 00  
HEP.Heptapterida 00  
CLA.Heteropneust 00  
HYP.Hypsidoridae 00  
ICT.Ictalurus 00  
ICT.Noturus 00  
ICT.Trogloglanis 00  
LAC.Lacantunia 10  
LOR.Loricariidae 00  
MAL.Malapterurid 01  
MOC.Euchilichthy 00  
MOC.Microsynodon 00  
MOC.Mochokus 00  
MOC.Synodontis 00  
NEM.Nematogenyid 00  
PAN.Pangasiidae 00  
PIM.Calophysus 00  
PIM.Hypophthalmu 00  
PIM.Parapimelodu 00  
PIM.Phractocephu 00  
PIM.Pimelodus 00  
PIM.Sorubiminae 00  
PLO.Plotosidae 00  
PSE.Pseudopimelo 00  
SCH.Ailiinae 00  
HOR.Horabagrus 00  
SCH.Laides 00  
SCH.Schilbinae 00  
SCO.Scoloplacida 00  
SIL.Siluridae 00  
SIS.Bagarius 00  
SIS.Gagata 00  
SIS.Glyptosterno 00  
SIS.Glyptothorax 00  
SIS.Pseudechenei 00  
SIS.Sisor 00  
TRI.Trichomycter 00  
;  
END;

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## Supplementary Figure S3 Legend: Strict Consensus Tree

Supplementary Fig. S3. Strict consensus of 561,400 maximum parsimony trees of length 690 steps (CI = 0.44; RI = 0.74) resulting from an analysis of 290 unweighted and unordered morphological characters. Bremer (decay) support values are shown above or on stems leading to multi-taxon groups and single terminals that are composites based on more than one genus. Letter codes in parentheses following terminals within a family index their family membership. Numbers in parentheses following terminal taxa show number of genera examined in previous or this study. Abbreviations: CET Cetopsidae, AMP Amphiliidae, AKY Akysidae, SIS Sisoridae, ASP Aspredinidae, HEP Heptaptidae, PIM Pimelodidae, AUC Auchenipteridae, DOR Doradidae, MOC Mochokidae, ARI Ariidae, SCH Schilbiidae, CLA Clariidae, BAG Bagridae, ICT Ictaluridae.

Three synapomorphies place *Lacantunia* within Siluroidei: eight upper principal caudal-fin rays (cf. primitively nine in diplomystids); barbels on the chin (cf. no mental barbels); fifth vertebra sutured to compound second-fourth (Weberian) fused vertebrae and partly covered by lamellar bone (cf. fifth vertebra with non-sutural joints and lacking covering of lamellar bone).

In Siluroidei, *Lacantunia* and non-hypsidorid catfishes share three synapomorphies: reduced distal maxillary arm and short medial maxillary process (cf. in diplomystids and hypsidorids maxillary arm primitively expanded and maxillary process elongated), loss of maxillary teeth (cf. teeth present), and sutures uniting anterior and posterior ceratohyals (cf. synchondral joint between ceratohyals<sup>4,26</sup>)

Also in Siluroidei, *Lacantunia* and non-cetopsid catfishes share the development of interdigitating coracoid symphyseal sutures (cf. primitively in diplomystids and cetopsids coracoid symphyseal sutures not present; although the sutures secondarily lost in Siluridae and some Trichomycteridae)<sup>6,26</sup>.

*Lacantunia* lacks the synapomorphies uniting crown group Ictaluridae<sup>33-35</sup> skull roof covered by jaw adductor muscles attached to prominent sagittal crest (cf. primitively, as in *Lacantunia*, muscles not on skull roof and sagittal crest not developed); infraorbital canal exits frontal bone (cf. canal exits sphenotic); supracleithrum with subpteroic process (cf. process absent).

*Lacantunia* lacks synapomorphies that place the fossil genus *Astephus*, as the sister lineage to crown group Ictaluridae<sup>33,35</sup>: loss of bony posterior process on pelvic girdle (cf. primitively ossified posterior process present as in *Lacantunia*); continuous cartilage along posterior edge of pelvic girdle (cf. cartilage in two parts interrupted by bone); pelvic fin rays seven or more and intraspecifically variable (cf. pelvic fin rays invariably six). We found no characters suggesting a deeper sister group relationship between *Lacantunia* and all Ictaluridae.

*Lacantunia* lacks synapomorphies of Heptapteridae and Ariidae. Heptapterids<sup>20,58</sup> are diagnosed by laterally branched fourth transverse vertebral processes (cf. transverse processes primitively unbranched as in *Lacantunia*); expanded posterodorsal projections of hyomandibula for insertion of levator operculi muscle (cf. process small); hyomandibula and metapterygoid not meeting dorsal to quadrate (cf. hyomandibula and metapterygoid in contact); recurved processes of ventrolateral corners of mesethmoid (cf. processes absent). Ariidae<sup>25,28</sup> have a unique otic capsule with a distended bulla in the prootic, pterotic and exoccipital bones that houses an enlarged utricular otolith (cf. primitively otic bulla absent and otolith small as in *Lacantunia*).

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# Two anonymous reviews of the manuscript submitted to Nature

## Referee #1(Remarks to the Author):

My first impression of the ms. was that authors were so enthusiastic about their results that they forgot to check the text against the abstract because the two are not congruent. The title of the paper calls attention to a phylogenetically puzzling fish, and the abstract insists on presenting the new fish as belonging to a deep phylogenetic line within Siluriformes. However, the results do not support these interpretations. As written, correctly, on page 5 (second paragraph), the "Chiapas Catfish" shares derived characters that unite it with the Siluroidei, but it has an unresolved position among the Siluroidei families (see Fig. 4), so that if the new fish has a puzzling phylogenetic position, we can say the same for most catfish families because we do not know their phylogenetic positions either.

The authors state that the new species clearly represents an ancient family-level lineage of perhaps early Tertiary age. There is no evidence to support these statements. The cladogram on Fig. 4 shows that the fish has an unresolved position above the Diplomystidae and the trichotomy formed by Hypsidoridae + Cetopsidae + Siluroidei. As documented in the ms., this is not a basal catfish and the comment on its possible origin in the Early Tertiary is an assumption. Again, on page 7 (second paragraph) the authors argue that the new fish represents an ancient and relatively basal siluroid lineage from which intermediate members have disappeared. However, there is no reason to assume that they have disappeared. Probably, they have not been discovered yet! There are many catfishes known from very restricted geographical areas so this new fish is not an exception.

The importance of the ms., in my opinion, does not depend on the phylogenetic position of the fish, but in the fact that a new fish family (supported by five unambiguous synapomorphies) has just been discovered in southern Mexico. No one is surprised by the discovery of a new species, but the discovery of a new family for any major living clade is a rare finding, and no longer expected in this century.

The ms. is flawed by overstatements and in the coding of some characters. The authors state that they have taken the characters from the literature. However, some of them and their coding should be revised. Examples are:

Diagnosis. - Fifth infraorbital expanded, boomerang-shaped, ..... As shown by Fig. 2A, B, this infraorbital bone looks like an ossicle lacking outgrowths. Thus, infraorbital 5 is not expanded. The word expanded should be removed from the diagnosis and elsewhere.

Following their cladogram, the authors conclude that the major lines of Siluriformes are unevenly resolved, that the North American fossil Hypsidoridae form a deep clade subtending the remaining extant catfishes, and that the extant Cetopsidae may also be among the most basal catfish lineages. The cladogram in Fig. 4 can be read in another way: Hypsidoridae, Cetopsidae and the Siluroidei have unresolved positions and there is no argument (with the exception of being extinct) that Hypsidoridae is more basal than Cetopsidae or vice-versa.

At the end of page 5 it is written that nasal barbels on the posterior nares (Fig. 1) are present in several Old World families and Ictaluridae. Which Old World families?

Diplomystidae is interpreted to have been recorded from the Late Cretaceous. However, the evidence for such assignment is based on fragmentary spines. Lundberg's (1998) criticisms of this assignment are still valid. The features shown by the so-called fossil diplomystids are seen also in some non-diplomystid catfishes. I suggest that the authors add a question mark to the Cretaceous (Fig. 4: K?). Certainly, the catfish fossil record is scarce and incomplete, but what is recorded tells us that there is still a great amount of information that has not been properly understood (e.g., Andinichthys).

## Supplementary information

I did not check the coding for the entire matrix. Instead I checked the coding of Diplomystidae, used as the sister-group of the remaining catfishes, and I selected one family that is known from one living species (Nematogenyidae) and another that it is known by over one hundred species (Trichomycteridae). I detected problems with some of the characters:

Char. 12. Trichomycteridae is coded as having the primitive character state (0); however, members of Trichomycteridae show both character states. It should be coded as 0/1.

Char. 24. Nematogenyidae and Trichomycteridae are coded with a question mark. I suggest that the authors examine the monograph by Arratia and Huaquín on the lateralis system of Diplomystidae and some loricarioids (Bonn. Zool. Monog., 1995).

Char. 43. Why is this character coded with a question mark for Trichomycteridae? There are several papers showing descriptions of the bone and its condition in trichomycterids.

Char. 53. How is this character polarized? What is the criterion for interpreting 5 as the primitive number of infraorbital bones in siluriforms? Nematogenyidae is coded with a question mark. See Arratia and Huaquin (cited above) for illustrations concerning the infraorbital bones.

Char. 82. What is the criterion for interpreting the coronoid process of Diplomystidae as shallow and that of Hypsidoris and Nematogenys as deep? The authors cite Arratia's (1986) monograph on Diplomystidae, but neither that paper nor that on cranial bones in the book "Catfishes" (cited in this ms.) shows a shallow coronoid process for diplomystids.

Char. 94. The character is defined: Ossified coronoid process 26: Present = 0; Process absent or cartilaginous only = 1. The authors took the characters from another paper, but Lundberg is an expert on catfishes and he knows that the coronoid process is formed by two dermal bones (dentary and angular) and that the coronoid process of hardly any catfish is cartilaginous. The dorsal extension of the Mecklian cartilage is a special feature of some siluriforms, but it is not the coronoid process of the lower jaw. I suggest the character be changed or deleted.

Char. 98. Diplomystidae is coded as having a single palatine head. Has Diplomystidae a single palatine head? How do the authors interpret the many specimens that have a doubled head palatine, while in some individuals the double heads have partially fused anteriorly, retaining a foramen between them? I suggest that the authors check specimens, or look at Arratia's (1986) illustrations (paper cited in the ms.) and also Arratia (1992) on the suspensorium of diplomystids and other catfishes. I suggest that the presentation of this character be modified and properly coded for Diplomystidae.

Char. 99 and 100. Both characters refer to the palatine (e.g., rod-like, small nodule, depressed, expanded, etc.) In char. 99 Diplomystidae is coded as having a rod-like palatine. However, in char. 100 it is coded as having a depressed, expanded palatine anteriorly. Is this correct?

Char. 107. How do the authors cut the line for coding Diplomystidae? In the way that the character is presented, diplomystids can be coded as 0 or 1. The presentation of the character states is not clearly defined.

Char. 141 to 155. Basibranchial and hypobranchial bones in diplomystids. The authors should check large specimens and correct the coding of some of the characters. Questions: Is basibranchial 4 ossified in Diplomystidae? Is hypobranchial 3 ossified in Diplomystidae?

Char. 212. Nematogenyidae and Trichomycteridae are coded with question marks. However, Nematogenyidae has dorsal hypohyals, but all trichomycterids that I know of have only ventral hypohyals. The authors should check the literature.

Char. 251. Trichomycteridae should be coded as 0/1 because some species have a large series of dorsal procurent caudal rays. The authors should check the literature.

I suggest that the authors check all the codings because as there are several problems with three of the families, there could be problems elsewhere.

The word Mesoamerica is wrongly used in the title and text. Mesoamerica implies a temporal dimension and is a word created for a specific archaeological division of the past time, not actual time. The authors should obtain information on the proper meaning of Mesoamerica and just use Southern Mexico in the ms.

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First author Rodiles Hernández responds to the last point made by reviewer #1:

The words 'Mesoamerica' and 'Mesoamerican' are currently used in many disciplines such as anthropology, ethnography, and biology. We prefer to use Mesoamerica instead of 'southern Mexico' as the reviewer suggests because it provides a more precise concept of the biogeographic area in which *Lacantunia enigmatica* is found. Commonly held geopolitical views often consider Mexico to be part of North America, but southern Mexico is almost never considered to be part of Central America. Mesoamerica is a valid biogeographic concept that transcends political boundaries.

To confirm current use of 'Mesoamerica' in biology we point to the monumental botanical work in progress: Flora Mesoamericana, UNAM, Missouri Botanical Garden, The Natural History Museum (London) (see <http://www.mobot.org/MOBOT/fm/geninfo.html>). We also point out that the recent massive project, Mesoamerican Biological Corridor (Corredor Biológico Mesoamericano (CBM)), states: "El Corredor Biológico Mesoamericano (CBM) constituye un concepto central del desarrollo para la subregión, integrando la conservación y el uso sostenible de la biodiversidad dentro del marco del desarrollo económico sostenible. El acuerdo para establecer el CBM fue formalmente aprobado por los ministros responsables de los recursos naturales y asuntos ambientales en América Central en febrero de 1997 y oficialmente avalado por los presidentes de la región en su XIX Reunión Cumbre en julio de 1997."(see <http://www.rolac.unep.mx/recnat/esp/CBM/cbm.htm>).

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Referee #2(Remarks to the Author):

This is an interesting paper for two reasons; the discovery of another lineage from the Mesoamerican zone is valuable information; and the manner of the taxonomy-work adds in a nice new dimension in the use of technology to aid osteological descriptions and illustrations. I have little doubt that the authors know what they are dealing with, one of the authors being highly respected in the siluroid world. The new genus and new species detailed appears to be allied or linked to the Ictaluridae, despite the authors' comments, I feel it may be the root to these well-known catfishes. Most of my "unhappiness" with this report is with the tree ... far too much of the tree is unresolved, the comb-like nature of most of the tree suggests too much homoplasy and severely weakens the theory of the authors that their taxon is a new lineage, the authors still use "Lacantunia" and not Lacanthuniidae, genera are mixed within the tree with subfamilies and families the clade "sisoriids" (sic - should be sisoriiids) has a mixture of families and subfamilies (should be sisorioiids then?), having Horabagrus unplaced ... etc. Accepted that this is a maximum parsimony tree and it has been simplified, the fact that various genera like Anchareius, Horabagrus and Phreatobius "unplaced", like Lacantunia begs the question - are these other genera unique lineages as well. The resolution in the sisorioiids and doradoiids suggests that these are deeper lines with the others representing younger lineages as well. I think the tree needs to be "recrunched" and perhaps simplified with some

terminals collapsed and redefined to make resolutions sharper. Similarly, I would have liked to see a character analysis diagram ... I believe the tree needs to be improved to help justify the arguments presented.

The osteological characters detailed and valuable, aided substantially by the high quality computer graphics and IT ... and lend support to the arguments presented. I consider their use of this technology noteworthy and a milestone in systematic work.