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Research Article

Phylogeny of the Genera with Oromandibular Discs in the Subfamily Labeoninae (Teleostei: Cyprinidae) with Descriptions of Two New Genera

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The aim of this study was to understand the systematic relationships between certain fish genera with oromandibular discs in the Labeoninae. At the same time, this was an opportunity to clarify the taxonomic status of certain species in the genera *Ageneiogarra*, *Garra*, *Placocheilus*, and other taxa distributed in China. Morphological comparison and molecular analysis were used in the study. The results showed that the genera with oromandibular discs had no recent common ancestor and that their oromandibular discs were the result of convergent evolution in different evolutionary lineages. Due to the homogeneity of their habitats, these fish have evolved oromandibular discs with similar appearance and structure. Both morphological and molecular evidence suggests that (1) *Placocheilus cryptonemus* distributed in the Nu-Jiang (the Upper Salween River Basin), Yunnan, China, represent a new genus, here described as *Pseudoplacocheilus*, and (2) *Garra micropulvinus* occurring in the Panlong-He (the upper portion of the Lo River, a branch of the Red River Basin) from Wenshan, Yunnan, represents another new genus, here described as *Supradiscus*.

1. Introduction

The cyprinid taxon Labeoninae is a subfamily of the Cyprinidae (Cypriniformes) [1]. The Labeoninae has limited distribution in tropical and subtropical regions in Asia and Africa [2]. To date, 52 valid genera and 525 valid species of this subfamily are recorded, accounting for 29.41% of the fish species in the Cyprinidae [1]. Some genera within the Labeoninae, including *Ageneiogarra*, *Ceratogarra*, *Discocheilus*, *Discogobio*, *Garra*, *Placocheilus*, *Sinigarra*, and *Vinagarra*, have a developed lower lip, which extends backwards to form a complete oromandibular disc structure [2–6].

The genus *Garra* Hamilton [7] was first described 200 years ago, but new species are still being discovered [8–14], and 192 valid species of *Garra* are currently recognised [15]. The identification and classification of *Garra* species are still almost entirely dependent on external morphological characteristics, which undoubtedly increases the complexity of the classification and the uncertainty of the results. Yang et al. [5, 16] and Zheng et al. [17, 18] constructed a phylogenetic tree of the Labeoninae based on molecular data, and their results confirmed the fact that *Garra* was not a monophyletic group. The findings of Yang et al. [5] were highly believable because they used nine molecular markers and 34 genera and 142 species of the cyprinid tribe Labeonini, including 31 species of *Garra*. At that time, 19 species of *Garra* and four species of *Placocheilus* were recorded from China [2, 19]. However, the study materials of Yang et al. [5] only included eight species of *Garra* and one species of *Placocheilus* found in China. Therefore, their research results were inevitably limited with respect to the taxonomic and phylogenetic relationships of the members of the Labeonini with Chinese distribution.

With further molecular and morphological studies, some species or groups have been removed from *Garra*. For example, the genus *Ageneiogarra* was restored by Yang et al. [5], and the new genera *Vinagarra* and *Ceratogarra* were split

from *Garra* by Nguyen and Bui [3] and Kottelat [6], respectively, on the basis of morphological characters. Unfortunately, the *Eschmeyer's Catalog of Fishes* database arbitrarily assigned several species of the genus *Garra* to *Ageneiogarra* and *Vinagarra* in the absence of morphological and molecular evidence [15]. The results of this designation are dubious and unconvincing.

Several genera in the Labeoninae, including *Ageneiogarra*, *Garra*, *Placocheilus*, and *Vinagarra*, have an oromandibular disc, the structure of which is complex and varied. From our long-term study of these fish, we observed that the structures of the oromandibular discs seen in these genera are inconsistent in pattern within the same genus, and the taxonomic status of some of the species therefore remains in doubt. This is a barrier to understanding the interspecific relationships, evolution, fish diversity, and formation of these genera.

In this study, we wanted to explore the classification of the genera and species in the Labeoninae with oromandibular discs by combining morphological and molecular evidence. We sampled five molecular markers from *Garra*, *Placocheilus*, and other Chinese taxa in the Labeoninae. Meanwhile, based on published genetic data from the Labeoninae, the phylogenetic relationships within the subfamily were reconstructed. This work will deepen our understanding of the taxonomy and structure of the Labeoninae and will inform further studies into species and generic classification in this complex group.

2. Materials and Methods

Samples of *Garra* and *Placocheilus* from China and Vietnam were collected, mainly from the type localities. A large number of Labeoninae species were also observed in the fields and markets of Sichuan, Guizhou, Guangxi, and Yunnan provinces in China. The fish specimens used for this study were collected in accordance with the Chinese Laboratory Animal Welfare and Ethics animal welfare laws. All specimens were collected as part of routine surveys. After being anaesthetised, the right pelvic fin or caudal peduncle muscle was cut and stored in 95% alcohol, then the alcohol was changed once, and the samples were stored in a −20°C refrigerator for DNA extraction. All fished individuals were euthanized by immersion in 95% alcohol, or in 10% formalin for morphological examination.

2.1. Morphological Methods and Specimens. This study mainly focused on the few genera in the Labeoninae having oromandibular discs, including *Ageneiogarra*, *Ceratogarra*, *Discogobio*, *Discocheilus*, *Garra*, *Placocheilus*, *Sinigarra*, and *Vinagarra*. The identification and terminology of the elements of the oromandibular disc follow Zhang and Zhou [4], Zhang et al. [20], and Nebeshwar and Vishwanath [21]. We used the subtribe in the classification of groups under the Labeoninae. Of course, this does not conform to the principle of taxonomic hierarchy and is only for the convenience of comparison with the results of Yang et al. [5].

Measurements followed Nebeshwar and Vishwanath [21], and Kottelat [22]. All measurements were taken point to point with digital calipers connected directly to a data recording computer, and data were recorded to the nearest 0.1 mm. Measurements and counts were made on the left side of individuals whenever possible. In the account below, we give the following specimen information: catalogue number, total number of individuals examined, standard length, collection locality, and river basin. Abbreviations listed in the text and tables are as follows: ex., example; Co., county; HL, head length; Pref., prefecture; Prov., province; and SL, standard length. The suffixes -Jiang and -He can both mean either river or stream in Mandarin Chinese.

A total of 1,244 specimens belonging to 13 genera and 47 species were morphologically examined (Appendix S1). The specimens examined in this study were deposited at the Museum of Southwest Forestry University (SWFU), Kunming, Yunnan, China; the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences, Kunming, Yunnan, China; or the Institute of Hydrobiology (IHB), Chinese Academy of Sciences, Wuhan, Hubei, China.

2.2. Experimental Materials and Molecular Biology Methods. DNA of 23 species from eight genera was newly sequenced for our study. Combined with DNA sequences downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov), a total of 68 species in 21 genera were compared in this study, with 16 genera and 63 species of Labeoninae selected as the ingroup. Five genera and five species from the Gobionidae and Acheilognathidae and the subfamily Acrossocheilinae, Cyprininae, and Smiliogastrinae in the Cyprinidae were chosen as the outgroup. Of the Labeoninae samples, 31 species from the genus *Garra* were included, as well as four species from the genus *Placocheilus*. Of the included taxa, 15 *Garra* species and the four *Placocheilus* species have a Chinese distribution. The genetic samples from *Garra* were collected in East Asia, Southeast Asia, South Asia, and Africa, basically covering the whole distribution of *Garra*. The species names of the downloaded DNA sequences were retained as uploaded without any changes (Appendix S2).

2.2.1. DNA Extraction and PCR. All samples (muscles or fins) were preserved in alcohol, and genomic DNA was extracted using a tissue DNA kit (Omega, USA), following the manufacturer's protocols. In this study, three mitochondrial genes, i.e., cytochrome oxidase subunit 1 (COI), cytochrome b (Cyt *b*), and NADH dehydrogenase subunit 4 (ND4), and two nuclear genes, i.e., interphotoreceptor retinoid-binding protein (IRBP) and recombination activating protein 1, exon 3 (RAG1), were amplified. The primers and PCR protocols of COI, Cyt *b*, IRBP, and RAG1 followed Yang et al. [5]. The primers for ND4 were as follows: Fd15869-ND4, 5′-CHGARCCRRTTA DYCGRCAACG-3′, and Rd13626-ND4, 5′-AHCCTCATR WYAKTTCYGGHTTTG-3′. The reaction mixture (50 μL) contained 5 μL of DNA template (10 pmol/μL), 2 μL of each primer (10 pmol/μL), and 12.5 μL of PCR MasterMix (Bio-Teke, China). Reactions were carried out for 35 cycles, each involving a predenaturation step at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 90 s, before a final extension step at 72°C for 8 min. All primers and PCR products were purified and sequenced

(Applied Biosystems ABI 3730xl 96-capillary DNA analyser) by Sino Geno Max Co., Ltd. The sequences were deposited in GenBank (accession numbers are listed in Appendix S2).

2.2.2. Molecular Data Analyses. We used DAMBE 5.3.48 to conduct a saturation test [23]. Alignment of the five molecular sequences was performed using the Clustal W algorithm in MEGA 7 [24], with manual checks for inconsistencies. Aligned sequences were analysed using maximum likelihood (ML), neighbour joining (NJ), and Bayesian inference (BI) methods. ML and NJ analyses were performed using MEGA 7. The ML tree topologies were inferred using the general time reversible (GTR) model. Nodal support for the NJ phylogram was calculated using 1,000 bootstrap replicates. For the NJ analysis, we conducted heuristic searches (1,000 runs) using Kimura's 2-parameter (K2P) model. The software PhyloSuite v. 1.2.3 was used to concatenate the five sequences into a combined dataset [25, 26]. The evolutionary models of ML and BI trees were provided by the Partition Finder 2 program in the PhyloSuite v1.2.3 software [25, 26]. BI analyses were conducted in MrBayes 3.2.6 [27], and the generalized time reversible model (nst = 6), gamma-distributed rate variation, and the proportion of invariable positions (GTR+G+I) for the combined dataset of five sequences were used. We ran four simultaneous Markov chain Monte Carlo simulations for 2,000,000 generations, sampling every 1,000 generations, and the first 25% of samples were discarded as burn-in. The phylogenetic trees were visualized and edited using FigTree v. 1.4.2 [28].

The state of morphological characteristic of oromandibular disc was transformed into "0, 1" dataset. For example, the anteromedian fold was present (1) or absent (0). Picante package was used to examine the similarity of oromandibular disc on the phylogenetic tree of species [29]. The Blomberg K statistics was used to determine the phylogenetic signal of oromandibular disc. If $K > 1$, it showed that oromandibular disc had high degree of phylogenetic signals. If $K < 1$ and closed to 0, it showed that character evolution tended to be random; that is, it meant convergence adaptation [30].

3. Results

3.1. Morphological Comparisons of the Oromandibular Disc in Different Taxa. Typical oromandibular disc structures of Labeoninae fish include the rostral cap (rc), upper lip (ul), upper jaw (uj), lower jaw (lj), anteromedian fold (amf), central callous pad (ccp), anterolateral lobe (all), lateroposterior flap (lf), and the pleated papilliferous fold (ppf, this fold extends from the inner side of the anterolateral lobe to the groove between the lower jaw and the anteromedian fold on each side). The comparison of the morphological characteristics of genera with oromandibular discs in the Labeoninae demonstrated that the morphological characteristics of the oromandibular discs diverged widely, as shown below.

(1) The anteromedian fold is either present or absent:

  (a) Anteromedian absent (Figure 1(a))

  (b) Anteromedian fold present (Figures 1(b), 1(c), 1(d), 1(e), 1(f), 1(g), 1(h), 1(i), and 1(j))

(2) The anteromedian fold can have one of the four shapes:

  (a) Pocket shaped: the two ends of the anteromedian fold are joined with the central callous pad and cannot be distinguished from each other, and the posterior edge of the anteromedian fold is curved and free and covers the central callous pad. The anteromedian fold resembles a pocket with the opening facing backwards (pob) (Figure 1(b))

  (b) Horseshoe shaped: the two ends of the anteromedian fold are curved backwards and inwards, forming a regular or irregular horseshoe shape surrounding the central callous pad. The two ends of the anteromedian fold are joined with the central callous pad and are not free (Figures 1(c), 1(d), and 1(e))

  (c) Crescent shaped: the two ends of the anteromedian fold are curved backwards and not inwards; the ends of the fold are slightly pointed and are not free, forming a crescent to the front of the central callous pad (Figures 1(f) and 1(h))

  (d) Arcuate: the anteromedian fold is curved and becomes arcuate. Its two ends are not pointed and extend to both sides, but not backwards. The ends of anteromedian fold can be further divided into two states:

    (i) The lateral ends of the anteromedian fold are free (Figure 1(g))

    (ii) The lateral ends of the anteromedian fold are not free (Figures 1(i) and 1(j))

(3) The relationship between the anteromedian fold and the anterolateral lobe has two states:

  (a) Joined: the lateral ends of the anteromedian fold extend and are joined with the anterolateral lobe, and the papillae on the anteromedian fold extend to the anterolateral lobe. There is no pleated papilliferous fold (ppf) (Figures 1(b) and 1(f))

  (b) Separated: the lateral ends of the anteromedian fold are separated from the anterolateral lobe by the pleated papilliferous fold (ppf) that does not affect the distribution of the papillae (Figures 1(c), 1(d), 1(e), 1(g), 1(h), 1(i), and 1(j))

(4) The surface of the rostral cap has papillae that are arranged neatly, and the surface can be described as having one of the two states according to the morphology of the margin of the rostral cap:

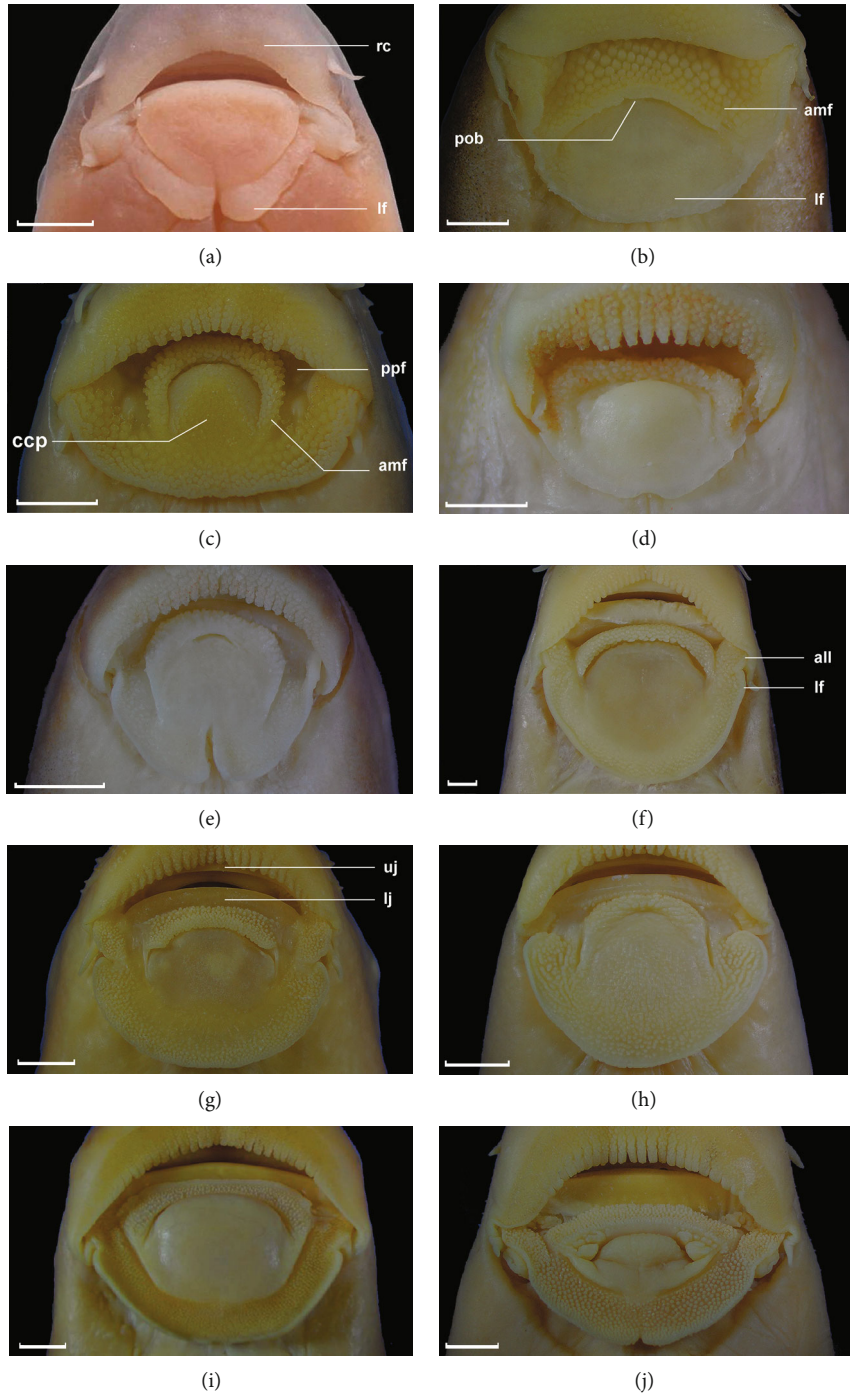

Figure 1: Comparisons of oral morphology among the genera of Labeoninae that have oromandibular discs. (a) *Sinigarra napoensis*: IHB201001008, holotype, 87.3 mm SL, Napo Co., Guangxi Prov.; (b) *Discocheilus wui*: SWFU0411039, 54.2 mm SL, Dalitang, Guangnan Co., Yunnan Prov.; (c) *Discogobio laticeps*: SWFU0506048, topotype, 67.3 mm SL, Lianhuan, Zhenfeng Co., Guizhou Prov.; (d) *Ceratogarra fasciacauda*: SWFU1503047, 106.3 mm SL, Menglun Town, Mengla Co., Yunnan Prov.; (e) *Vinagarra findolabium*: SWFU0412045, holotype, 65.1 mm SL, Niuluohe, Jiangcheng Co., Yunnan Prov.; (f) *Garra qiaojiensis*: SWFU0101001, 50.8 mm SL, Daju, Tengchong City, Yunnan Prov.; (g) *Placocheilus caudofasciatus*: SWFU0506002, 69.9 mm SL, Daheishan, Lvchun Co., Yunnan Prov.; (h) *Pseudoplacocheilus cryptonemus*: SWFU1501025, 74.9 mm SL, Mangkuan Town, Longyang District, Yunnan Prov.; (i) *Ageneiogarra imberba*: SWFC0607006, 103.8 mm, SL, Weixin Co., Yunnan Prov.; (j) *Supradiscus micropulvinus*: SWFC 0111011, holotype, 107.0 mm SL, Gaji, Xichou Co., Yunnan Prov. (all: anterolateral lobe; amf: anteromedian fold; ccp: central callous pad; lf: lateroposterior flap; lj: lower jaw; pob: a pocket with an opening backward; ppf: pleated papilliferous fold; rc: rostral cap; uj: upper jaw).

(a) Noncrenulated: the margin of rostral cap is not divided and not split into tassels (Figure 1(b))

(b) Crenulated: the margin of rostral cap is divided into tassels, and each tassel has two to three rows of papillae (Figures 1(a), 1(c), 1(d), 1(e), 1(f), 1(g), 1(h), 1(i), and 1(j))

(5) The posterior shape of the oromandibular disc has three states:

(a) Bilobed: the left and right lateroposterior flaps are not joined at the posterior margin of the oromandibular disc and are divided into two distinct lobes (Figures 1(a) and 1(e))

(b) Shallowly notched: the left and right lateroposterior flaps are mostly joined and have a shallow notch at the posterior margin (Figures 1(d) and 1(j))

(c) Complete and smooth: the left and right lateroposterior flaps are completely joined, and the posterior margin of the oromandibular disc is complete and smooth

(6) The shape of the central callous pad has two states:

(a) Entire: according to the shape of the central callous pad, this can be further divided into two states:

(i) Without a boundary: there is no boundary between the posterior margin of the central callous pad and the lateroposterior flap, and they are joined (Figures 1(b), 1(c), 1(d), 1(e), 1(f), 1(g), and 1(h))

(ii) With a distinct boundary: there is a distinct boundary between the posterior margin of the central callous pad and the lateroposterior flap (Figures 1(a) and 1(i))

(b) Stacked: in the middle of the central callous pad, there is a shallow transverse constriction dividing the central callous pad into anterior and posterior parts. There are many small fleshy buds between the sides of the central callous pad and the anteromedian fold, and the central callous pad appears to be stacked. There is a distinct boundary between the central callous pad and the lateroposterior flap, and the surface of the lateroposterior flap is densely covered with papillae (Figure 1(j))

(7) The relationship between the anteromedian fold and the central callous pad can be described as having three states:

(a) Separated by a distinct slit (Figures 1(b), 1(g), 1(i), and 1(j))

(b) Separated only by a deeper slit in the centre, with shallow constrictions on both sides (Figure 1(e))

(c) Separated entirely by a narrow constriction (Figures 1(c), 1(d), 1(f), and 1(h))

(8) The relationship between the rostral cap and the anterolateral lobe can be described as having two states:

(a) The rostral cap and the anterolateral lobe are not connected, and the anterior margin of the anterolateral lobe is free. This can be further divided into two states:

(i) Opposite: the lateral ends of the rostral cap are opposite the anterior end of the anterolateral lobe, but they are not connected (Figure 2(a))

(ii) Overlapping: the lateral ends of the rostral cap overlap the dorsal surface of the anterolateral lobe (Figure 2(b))

(b) The rostral cap and the anterolateral lobe are connected. This can be in two ways:

(i) Direct connection: the lateral ends of the rostral cap connect directly to the anterior edge of anterolateral lobe that is not free (Figures 2(c) and 2(e))

(ii) T-shaped connection: the lateral ends of the rostral cap connect directly to the dorsal surface of the anterolateral lobe in a T-shaped connection, and the anterior edge of the anterolateral lobe is free (Figures 2(d) and 2(f))

(9) The relationship between the anterolateral lobe and the lateroposterior flap is diverse and has several states:

(a) Outer edge notched: the outer edge of the oromandibular disc, between the anterolateral lobe and the lateroposterior flap, is notched, and these can be clearly distinguished. This state can be further divided into three states according to the papillae distributed on the surface of the anterolateral lobe and the lateroposterior flap:

(i) No papillae on the surface (Figure 1(a))

(ii) Papillae distribution interrupted. The distribution of papillae on the surface of the junction between the anterolateral lobe and the lateroposterior flap is interrupted (Figure 1(g))

(iii) Papillae distribution continuous. The distribution of the papillae is continuous on the surface of the junction between the anterolateral lobe and the lateroposterior flap (Figures 1(d), 1(f), and 1(i))

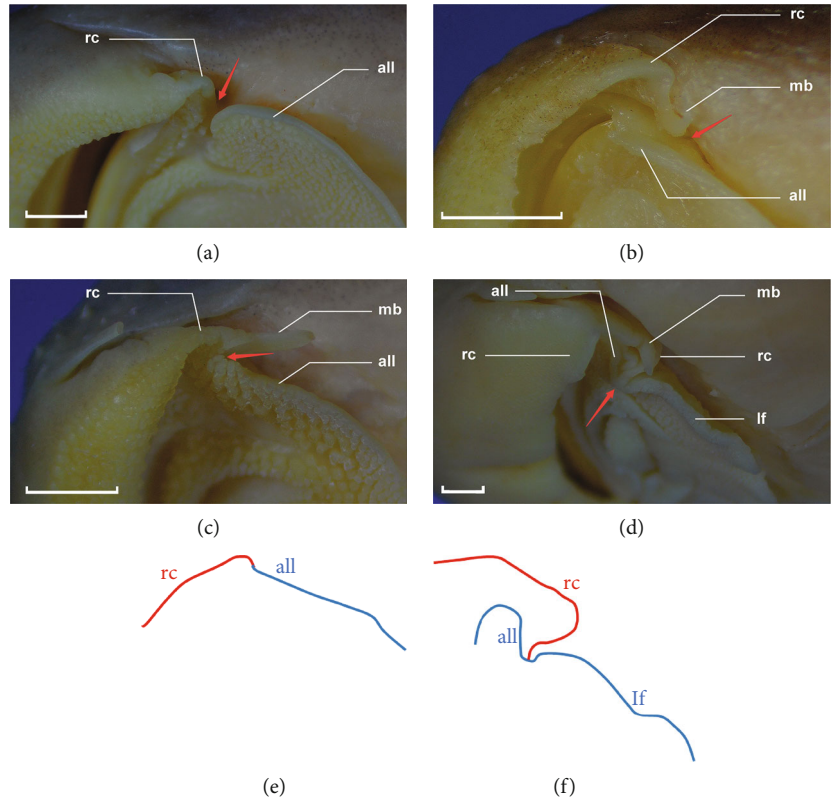

Figure 2: Comparison of the connection between the rostral cap and the anterolateral lobe among the genera in Labeoninae with oromandibular discs. (a) *Pseudoplacocheilus cryptonemus*: SWFU1501025, 74.9 mm SL, Mangkuan Town, Longyang District, Yunnan Prov.; (b) *Vinagarra findolabium*: SWFU0412045, holotype, 65.1 mm SL, Niuluohe, Jiangcheng Co., Yunnan Prov.; (c, e) *Discogobio laticeps*: SWFU0506048, topotype, 67.3 mm SL, Lianhuan, Zhenfeng Co., Guizhou Prov.; (d, f) *Supradiscus micropulvinus*: SWFC 0111011, holotype, 107.0 mm SL, Gaji, Xichou Co., Yunnan Prov. (all: anterolateral lobe; lf: lateroposterior flap; mb: maxillary barbell; rc: rostral cap).

(b) Outer edge complete and smooth: the outer edge between the anterolateral lobe and the lateroposterior flap is complete and smooth. The distribution of papillae is continuous on the surface of the junction between the anterolateral lobe and the lateroposterior flap (Figures 1(b), 1(c), 1(e), 1(h), and 1(j))

A comparison of the morphological characters of the oromandibular disc in six genera in the Labeoninae is summarized in Table 1.

3.2. Phylogenetic Relationship. A total of 346 Cyt *b*, COI, ND4, RAG1, and IRBP gene sequences were obtained in this study, and the final length of the concatenated alignment was 5052 bp. The sequence consisted of 2,726 conservative sites, 2,281 variant sites, and 393 reduced information sites. The mean base composition of the sequence was $A = 28.3$ %, $T = 26.6\%$, $C = 25.8\%$, $G = 19.3\%$, $A + T = 54.9\%$, and $G + C = 45.1\%$. *Barbonymus gonionotus*, *Barbus leonenis* (now as *Enteromius leonensis*), *Acrossocheilus monticola*, *Acheilognathus typus*, and *Gobio gobio* were used as outgroups. Phylogenetic analyses employing BI, ML, and NJ methods yielded identical topologies for the main clades and could be divided into four tribes: Labeonini (tribe A), "Semilabeonini" (tribe B), "Osteochilini" (tribe C), and Garrini (tribe D) (quotation marks denote a lack of formal description). The ML, NJ, and BI trees included the same species within each tribe and only minor differences at the terminals. The relationships among the tribes of NJ tree was (((D, B) C) A), and the value of posterior probability was lower. The relationships among tribes of ML tree was (((D, C) B) A), which is consistent with the BI tree. However, the value of posterior probability of BI tree was the highest. Therefore, only the phylogenetic tree of BI is presented in Figure 3, and the results of remaining two analyses were placed in the supplementary materials (Figure S1 and S2). Now, the phylogenetic tree of BI gives the following important information:

(1) Clade A contained all species in the subfamily Labeoninae and all genera in the Labeoninae included in our study

(2) The species originally placed in *Garra* did not aggregate in the same clade but were dispersed throughout four different clades (E, H, M, and R). In clade E, all species except for *Placocheilus dulongensis* (clade I) were *Garra* species and were distributed in the Mekong River and basins to the west (including

Table 1: Comparisons of the main morphological oromandibular disc characters among certain genera of the Labeoninae.

Genus	Anteromedian fold present or absent	Shape of anteromedian fold	Relationship between anteromedian fold and anterolateral lobe	Margin of rostral cap	Posterior shape of oromandibular disc	Shape of central callous pad, and relationship between central callous pad and lateroposterior flap	Relationship between anteromedian fold and central callous pad	Relationship between rostral cap and anterolateral lobe	Relationship between anterolateral lobe and lateroposterior flap
Ageneiogarra	Present	Arcuate, lateral ends not free	Separated	Crenulated	Complete and smooth	Entire, with a distinct boundary	Separated by a distinct slit	Connected with T-shaped connection	Outer edge notched, papillae distribution continuous
Ceratogarra	Present	Horseshoe shaped	Separated	Crenulated	With a shallow notch	Entire, connected without a boundary	Separated entirely by a narrow constriction	Connected with T-shaped connection	Outer edge notched, papillae distribution continuous
Discocheilus	Present	Pocket shaped	Joined	Non-crenulated	Complete and smooth	Entire, connected without a boundary	Separated by a distinct slit	Connected with direct connection	Outer edge smooth and complete, papillae distribution continuous
Discogobio	Present	Porseshoe shaped	Separated	Crenulated	Complete and smooth	Entire, connected without a boundary	Separated entirely by a narrow constriction	Connected with direct connection	Outer edge smooth and complete, papillae distribution continuous
Garra	Present	Prescent shaped	Joined	Crenulated	Complete and smooth	Entire, connected without a boundary	Separated entirely by a narrow constriction	Connected with T-shaped connection	Outer edge notched, papillae distribution continuous
Placocheilus	Present	Arcuate, lateral ends free	Separated	Crenulated	Complete and smooth	Entire, connected without a boundary	Separated by a distinct slit	Connected with T-shaped connection	Outer edge notched, papillae distribution interrupted
***Pseudoplacocheilus*, new genus**	Present	Crescent shaped	Separated	Crenulated	Complete and smooth	Entire, connected without a boundary	Separated entirely by a narrow constriction	Unconnected, lateral end of rostral cap opposite anterior end of anterolateral lobe	Outer edge smooth and complete, papillae distribution continuous
Sinigarra	Absent	—	—	Crenulated	Bilobed	Entire, with a distinct boundary	—	Connected with direct connection	Outer edge notched, no papillae on surface
***Supradiscus*, new genus**	Present	Arcuate, lateral ends not free	Separated	Crenulated	With a shallow notch	Stacked, with a distinct boundary	Separated by a distinct slit	Connected with T-shaped connection	Outer edge smooth and complete, papillae distribution continuous
Vinagarra	Present	Horseshoe shaped	Separated	Crenulated	Bilobed	Entire, connected without a boundary	Separated only by a deeper slit in the centre, with shallow constrictions on both sides	Unconnected, lateral end of rostral cap overlapping dorsal surface of anterolateral lobe	Outer edge smooth and complete, papillae distribution continuous

The different colours of text in each column represent different character states. Similarly, text of the same colour indicates that a character has the same state in different genera.

Africa). The two species of *Garra* in clade H were distributed in the Mekong River, and these species have now been transferred to *Ceratogarra*. Clade M contained four species of *Garra* that were only distantly related to the *Garra* species in clade D. The four species of *Garra* in clade M fell into clade N (including three species) and clade O (containing *G. findolabium* only)

(3) The four species of *Placocheilus* did not converge in the same clade but were dispersed throughout three different clades (I, Q, and S). Moreover, *Placocheilus dulongensis* of clade I was genetically distant from *Placocheilus caudofasciatus*, the type species of genus *Placocheilus*, nor did *P. cryptonemus* share a recent common ancestor with *P. caudofasciatus*

(4) The subfamily Labeoninae could be divided into four taxa, naming them as tribes Labeonini, Garrini, "Semilabeonini," and "Osteochilini" (Figures 3 and 4)

3.3. Phylogenetic Signal. The result of phylogenetic signal analysis showed that the Blomberg K statistic was 0.009511728 ($P < 0.01$), indicating that the morphological characteristics of oromandibular disc tended to evolve randomly.

3.4. Description of New Taxa. Based on the results of our morphological comparisons and molecular analysis, we confirmed that *Placocheilus cryptonemus*, distributed in the Nu-Jiang (the Upper Salween River Basin), Yunnan, China, represents a new genus that has not been described scientifically. This genus is named here *Pseudoplacocheilus* n. g. (Figure 4(b)). Furthermore, *Garra micropulvinus*, which is distributed in the Panlong-He (the upper portion of Lo River, a branch of the Red River Basin) in Wenshan, Yunnan, China, represents another new genus that has not been described scientifically, here named *Supradiscus* n. g. (Figure 4(b)).

3.4.1. Pseudoplacocheilus, New Genus. LSID urn:lsid:http://zoobank.org/:act:CAAAB274-A69F-4190-AA8C-AE053135BF12

Type Species. *Placocheilus cryptonemus* Cui & Li 1984 [31]: 110–112, figure 1 (type locality: Laowo, Yunlong Co. (now belongs to Lushui City), Yunnan, China).

Diagnosis. *Pseudoplacocheilus* is distinguished from other Labeoninae genera with an oromandibular disc by the following combination of characteristics: anteromedian fold present (vs. anteromedian fold absent in *Sinigarra*), anteromedian fold crescent shaped (vs. horseshoe shaped in *Discogobio*, *Ceratogarra*, and *Vinagarra*), rostral cap crenulated (vs. noncrenulated in *Discocheilus*), posterior margin of lower lip smooth and complete (vs. with a median notch in the posterior margin in *Sinigarra*, *Ceratogarra*, *Vinagarra*, and *Supradiscus*), with a constriction only in the middle part between the posterior margin of the anteromedian fold and the central callous pad (vs. with a shallow groove in *Discogobio*, *Ceratogarra*, and *Garra*; with a deep groove in *Vinagarra*, *Ageneiogarra*, *Placocheilus*, and *Supradiscus*; and with a pocket opening backwards in *Discocheilus*), the lateral terminal of the anteromedian fold not

FIGURE 3: Bayesian phylogeny of the Labeoninae inferred from COI, Cyt b, ND4, IRBP, and RAG1 sequences. Numbers at the nodes show the posterior probability (PP) values. The species of subfamily Labeoninae were divided into four taxa, naming them as tribes Labeonini, Garrini, "Semilabeonini," and "Osteochilini."

free (vs. free in *Placocheilus*), and rostral cap unconnected to the anterolateral lobe (vs. connected in *Sinigarra*, *Discogobio*, *Ceratogarra*, *Garra*, *Ageneiogarra*, *Placocheilus*, *Supradiscus*, and *Discocheilus*). The characters distinguishing *Pseudoplacocheilus* from the other genera in the Labeoninae are summarized in Table 1.

Etymology. The generic name *Pseudoplacocheilus* is a combination of the Greek prefix *pseudo-* (fake, phony) and *Placocheilus*, a generic name used for a group of Labeoninae, alluding to the fact that the morphology of its gular disc is very similar to the external shape of *Placocheilus*, although the fine structure is not exactly the same. Gender: masculine.

There is currently only one known species in the genus *Pseudoplacocheilus*, *Pseudoplacocheilus cryptonemus* (Cui & Li, 1984), which is found in the Nu-Jiang, Upper Salween River Basin, Yunnan Prov., China [31].

(1) *Pseudoplacocheilus cryptonemus* (Cui & Li 1984)

Placocheilus cryptonemus Cui & Li 1984 [31]: 110–112, figure 1 (type locality: Laowo, Yunlong Co. (now belongs to Lushui City), Yunnan).

Material Examined. SWFU 0103348–51, 4 ex., topotype, 50.4–55.0 mm SL, Laowo, Lushui City, Nujiang Pref., Yunnan Prov., China; SWFU 1501021–31, 11 ex., 58.8–84.1 mm SL, Mangkuan, Longyang District, Baoshan City, Yunnan Prov., China.

Distribution. Known only from the Nu-Jiang, Yunnan Prov., China (upper Salween River) (Figure 4(b)).

3.4.2. Supradiscus, New Genus. LSID urn:lsid:http://zoobank.org/:act:02FC0E4A-EC13-4056-AE55-EF9B78EBA1C3

Type Species. *Garra micropulvinus* Zhou, Pan & Kottelat 2005 [32]: 451–452, figure 7 (type locality: Gaji in Xichou Co., Wenshan Pref., Yunnan, China).

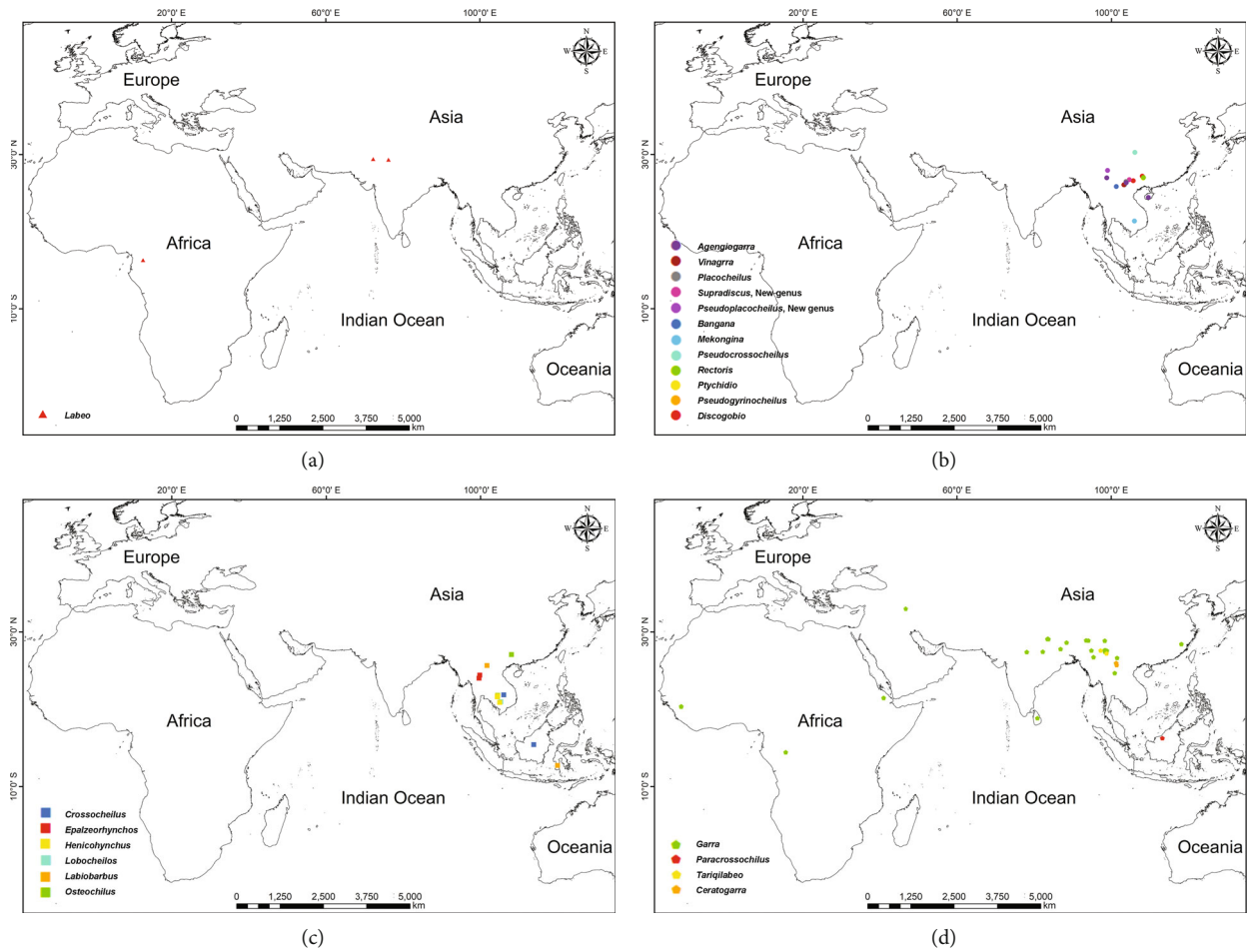

FIGURE 4: Map showing distribution of four subtribes of Labeoninae in the world. (a) Distribution of tribe Labeonini. (b) Distribution of tribe "Semilabeonini" (containing two new genera). (c) Distribution of tribe "Osteochilini." (d) Distribution of tribe Garrini. The sequence of genera on each tribe is consistent with the sequence appearing on the same subtribe in Figure 3.

Diagnosis. *Supradiscus* is distinguished from other Labeoninae genera with an oromandibular disc by the following combination of characteristics: central callous pad composed of multiple stacked parts (vs. unstacked and complete in all other genera), anteromedian fold present (vs. anteromedian fold absent in *Sinigarra*), anteromedian fold crescent shaped (vs. horseshoe shaped in *Discogobio*, *Ceratogarra*, and *Vinagarra*), rostral cap crenulated (vs. noncrenulated in *Discocheilus*), posterior margin of lower lip with a median notch (vs. smooth and complete in *Discogobio*, *Pseudoplacocheilus*, *Garra*, *Ageneiogarra*, *Placocheilus*, and *Discocheilus*), anteromedian fold with a pocket opening backward (vs. with a shallow or deep groove in *Discogobio*, *Ceratogarra*, *Pseudoplacocheilus*, and *Garra*). The characters distinguishing *Supradiscus* from the other genera of the Labeoninae are summarized in Table 1.

Etymology. The generic name *Supradiscus* is a combination of the Greek prefix *supra-* (above, over) and Greek *discos* (disc), alluding to the mouth having an overlapping disc. Gender: masculine.

Two species are currently known from the genus *Supradiscus*, *Supradiscus micropulvinus* (Zhou, Pan & Kottelat, 2005), found in the Panlong-He, Yunnan, China, in the upper portion of the Lo River, a tributary of Red River Basin [32], and *Supradiscus incisorbis* (Zheng, Yang & Chen 2016), which is found in Napo Co., Guangxi, China, in Zuojiang River drainage, a tributary of the Pearl River Basin [33].

(1) *Supradiscus incisorbis* (Zheng, Yang & Chen 2016)

Garra incisorbis Zheng, Yang & Chen 2016 [33]: 299-303, figure 4 (type locality: Pohe in Napo Co., Baise City, Guangxi, China).

Ageneiogarra incisorbis: Nebeshwar & Vishwanath 2017 [21]: 43; Tangjitjaroen et al. 2023 [4]: 388.

Material Examined. *Holotype*: KIZ 2013003383, 68.2 mm SL, China: Guangxi: Pohe (23°18′56″N, 105°56′16″E) from Zuo-Jiang River, a tributary of the Pearl River in Napo Co.; J.-H. Lan, 19 May 2013. *Paratype*: KIZ 201300300-3304, 3384–3385, 7, 47.1–79.5 mm SL; data as for holotype.

Distribution. Now known only from the Zuo-Jiang River, a tributary of the Pearl River Basin, Guangxi Prov., China [33].

Remark. Nebeshwar & Vishwanath [21], Tangjitjaroen et al. [4] designed *Garra incisorbis* as *Ageneiogarra incisorbis*. However, neither of the two papers presented strong morphological or molecular evidence to support their idea, but only

made inferences based on the results of Yang et al. [5]. Although we did not have molecular evidence, our morphological comparison strongly supported that the oromandibular disc structures of *Garra incisorbis* was consistent with that of *Garra micropulvinus*. Therefore, we considered that these two species should be placed in the new genus *Supradiscus*.

(2) *Supradiscus micropulvinus* (Zhou, Pan & Kottelat 2005)

Garra micropulvinus Zhou, Pan & Kottelat 2005 [32]: 451–452, figure 7 (type locality: Gaji in Xichou Co., Wenshan Pref., Yunnan, China).

Material Examined. *Holotype*: SWFC 0111011, 107 mm SL, China: Yunnan: Gaji (23°09.216′N, 104°27.383′E) from Panlong-He (a small branch of the Yuan-Jiang) in Xichou Co.; 885 m asl; W. Zhou and X. F. Pan, 23 Nov. 2001. *Paratypes*: SWFC 0111001-0111010, 0111012-0111029, 26, 63.5–133.0 mm SL; same data as for holotype.

Distribution. Now known only from the Panlong-He, Yunnan, China, the upper portion of the Lo River, a tributary of the Red River Basin [32].

4. Discussion

4.1. Taxonomic Status of Some Taxa. The molecular markers we used in this study were consistent with or identical to those used by Yang et al. [5] and Zheng et al. [18]. Moreover, we added several species of *Garra* and *Placocheilus* that are only found in China. Our results therefore clarify certain lineages with unclear evolutionary relationships in the results of Yang et al. [5] and Zheng et al. [18], or disrupt some of the original lineages suggested by these two studies. Based on the results of our morphological comparison and phylogenetic tree, we discuss the following issues.

(1) Taxonomic status of the genus *Ceratogarra*: clade H contains a pair of sister species, *Garra fasciacauda* and *G. cambodgiensis*, that are distributed only in the Mekong River Basin. This result is consistent with the molecular tree of Yang et al. [5] (figure 1 part A). Kottelat [6] designated these two species as a new genus, *Ceratogarra*. Our molecular and morphological results support the validity of *Ceratogarra*. Meanwhile, our results provide new evidence for distinguishing between *Ceratogarra* and *Garra* on the basis of the morphological characters of the oromandibular disc (Table 1)

(2) Taxonomic status of the genus *Garra*: clade E contains *Garra lamta*, the type species of the genus *Garra* (type designated by Bleeker 1863: 192) [34], meaning that the taxa in clade E continue to carry the name *Garra*. In subclade I, *Garra tengchongensis* is a sister species to *Placocheilus dulongensis*. Yang et al. [5] (figure 1 part A) and Zheng et al. [18] (figure 2) sampled only *Garra tengchongensis* and not *Placocheilus dulongensis*. The relationship between *Garra tengchongensis* and *Placocheilus dulongensis* cannot be determined from their results. We examined the type specimens of *P. dulongensis* and found that the morphological characteristics of its oromandibular disc were entirely consistent with those of *Garra*. Both morphological and molecular results therefore support the transfer of *P. dulongensis* from *Placocheilus* to the genus *Garra*, as *Garra dulongensis* (Chen, Pan, Xiao & Yang) [35]. Interestingly, the *Garra* species in clade E are only distributed in the Mekong River and basins to the west (including Africa), and none of the species formerly named *Garra* in east of the Mekong River are closely related to or share a recent common ancestor with the taxa in clade E

(3) Taxonomic status of the species of *Placocheilus*: both Yang et al. [5] (figure 1 part A) and Zheng et al. [18] (figure 2) used only samples of *P. cryptonemus*, while we included four species of *Placocheilus* in our study. Our results show that these four species do not converge in one clade but are scattered across three clades, I, Q, and S. Clade Q contains *P. caudofasciatus* and *P. robustus*, which are clearly distinguished from each other in the tree and should therefore continue to be regarded as two independent species. *Placocheilus cryptonemus* falls into a separate clade, clade S, and does not aggregate with the other species of *Placocheilus* at all, apparently sharing no recent common ancestor with clade Q. The morphological characteristics of the oromandibular disc of *P. cryptonemus* are significantly different from those of *P. caudofasciatus* or *P. robustus*. Therefore, *P. cryptonemus* represents an independent genus. This new genus, *Pseudoplacocheilus*, is described for the first time in this paper. We have to emphasize that, at present, there is no evidence to determine whether or not *Placocheilus bibarbatus* Nguyen [36] and *P. imbarbatus* Nguyen [36] are indeed species of *Placocheilus*, as the original descriptions were too simplistic. Therefore, their taxonomic status remains uncertain and should be treated with caution. The taxonomic status of these two taxa should be given research attention in the future

(4) Taxonomic status of *Garra micropulvinus*: the results of Yang et al. [5] (figure part A) and Zhang et al. [18] (figure 2) were in this case similar, with both showing that *Garra imberba* and *G. micropulvinus* formed a clade of sister species/groups. Both of these species were collected from Wenshan, Yunnan, China. Yang et al. [5] (figure 1 part A) designated this clade as *Ageneiogarra*. When we added further samples of these species, the lineage relationships broke down. Our results show that *Garra micropulvinus* forms a sister group to *Placocheilus* (clade Q), but that the oromandibular disc of *Garra micropulvinus* is significantly different from that of *Placocheilus* as well as from those of *Garra* and *Ageneiogarra*. Thus, *Garra micropulvinus* represents a separate taxon, which we describe here as the genus *Supradiscus*. After comparing the oromandibular discs, we consider

that *Garra incisorbis* Zheng, Yang & Chen [33] should also be assigned to the genus *Supradiscus* and is named here *S. incisorbis* (Zheng, Yang & Chen, 2016) [33]

(5) Taxonomic status of the genus *Ageneiogarra*: clade M contains the sister clades N and O. Clade O contains as yet only a single species, *Garra findolabium* (= *Vinagarra findolabium*). Neither Yang et al. [5] (figure 1 part A) nor Zheng et al. [18] (figure 2) sampled *G. findolabium*. If we consider only the phylogenetic tree, it seems reasonable to consider these two sister groups as the same genus. However, the morphological characteristics of the oromandibular disc of the two sister groups are significantly different. Therefore, we do not think that these two clades should be simply combined into one genus. However, the *G. findolabium* in clade O should be renamed *Vinagarra findolabium*. In the phylogenetic tree of Yang et al. [5] (figure 1 part A), the samples of *Garra imberba* and *G. micropulvinus* from Wenshan, Yunnan, China, formed sister groups. Because *G. imberba* was the type species of the genus *Ageneiogarra*, they designated this group as *Ageneiogarra*. When we added more *Garra* species to our analysis, we found that *G. imberba* and *G. micropulvinus* are not sister species. However, clade N contains *G. hainanensis*, *G. imberba*, and *G. nujiangensis*. Following the reasoning proposed by Yang et al. [5], we tentatively named clade N as the genus *Ageneiogarra*. The taxonomic status of this genus is, however, somewhat complex and should be discussed in detail in a different study

(6) Taxonomic status of the genus *Vinagarra*: *Garra findolabium* (= *Vinagarra findolabium*) formed an independent clade, O, in our phylogenetic tree. Moreover, its oromandibular disc has unique morphological characteristics, including a distinct anteromedian fold, and a lateroposterior flap that does not join to the posterior margin of the oromandibular disc, instead showing two flaps. The validity of the genus *Vinagarra* is not in doubt. According to Fricke et al. [15], four valid species are known from the genus *Vinagarra*, all of them with a distribution in the Black River Basin (or Song Da in Vietnamese, a tributary of the Red River Basin). *Vinagarra findolabium* (Li, Zhou & Fu) [37] is found in a branch of the Lixian-Jiang (the upper portion of the Black River), Jiangcheng Co., Puer Pref., Yunnan, China, while the other three species are concentrated in the Black River in Lai Chau Prov., Northern Vietnam, next to Yunnan, China. *Vinagarra laichowensis* (Nguyen & Doan) [38] was recorded from Phong Tho District, Lai Chau Prov., Vietnam, and *Vinagarra elongata* Nguyen & Bui [39] and *V. tamduongensis* Nguyen & Bui [39] both occur in Tam Duong District, Lai Chau Prov., Northern Vietnam. *Vinagarra findolabium* is distinguished from the type species of the genus *V. laichowensis*, by having an irregular dark patch on its caudal fin base that extends to half the length of the caudal fin [33, 34]. The validity of the taxonomic status of the other two species of *Vinagarra* found in Lai Chau, Vietnam, was difficult to determine as we did not have the opportunity to examine types or topotypes. It will be interesting to discover whether the *Vinagarra* species have really diverged so strongly within such a narrow geographical range

4.2. Genera with Oromandibular Discs Do Not Form a Natural Group in the Labeoninae. The molecular tree (Figure 3) showed that the genera of Labeoninae with oromandibular discs shared no recent common ancestor. This proved from the molecular tree that similar oromandibular discs were not homologous structures. Therefore, they did not constitute a natural group. As the calculation result of the K statistic was 0.009511728, the evolution of oromandibular disc tended to be random in the phylogenetic tree, suggesting convergence adaptation (Figure 3) [27, 28]. Although the oral morphological characteristics were very similar in these groups, these characters cannot be used for phylogenetic studies because the similar structures of the oromandibular disc were the result of convergent evolution [18].

Neither *Garra* nor *Placocheilus* formed monophyletic groups, and both were highly polyphyletic. The species in these genera did not share a recent common ancestor, and certain morphological characters seen in both genera, and in particular the character of the oromandibular disc, were not the result of evolution within a single lineage, but rather the result of convergent evolution in multiple lineages. Although these morphological characters did not have the systematic significance originally suggested by morphological studies, they could nevertheless exhibit taxonomic significance. This diverse characters of the oromandibular disc are indicative of adaptive radiation in Labeoninae fishes, which frequently live in swift currents. These fish seem to have adapted to their local ecology through the development of a series of superficially similar morphological characteristics. Our study results suggest the possibility of revealing complex patterns of homology through phylogenetics, with the patterns being caused by independent morphological responses to similar ecological conditions [18].

At present, the *Garra* species in the phylogenetic tree are only distributed in the Mekong River and basins to the west (including Africa), and we still cannot confirm whether they form a monophyletic group. If future phylogenetic analyses can be supplemented with many genera and species of Labeoninae from across this region, we would be able to test whether or not the genus *Garra* in its current form is monophyletic.

4.3. Subdivision of the Labeoninae. Tan and Armbruster [40] based their study on phylogenetic and taxonomic publications of the time that use and revise family-group names within the Cypriniformes. They raised Labeonini as subfamily Labeoninae in Cyprinidae, adopted to follow the results of Yang et al.'s [5] classification, and raised their subtribes to tribe level, resulting in four tribes: Labeonini, Garrini,

"Semilabeonini," and "Osteochilini." After comparing the results between Yang et al. [5] and Tan and Armbruster [40], we found that the most significant difference was that some genera were supplemented and only a genus, *Altigena* (="*Bangana*" *lippus* + "*B.*" *tonkinensis* in Yang et al. [5]), was transferred from "Semilabeonini" to Labeonini by Tan and Armbruster [40]. Stout et al. [41] also agreed that Labeoninae could be divided into four tribes, and the division of the tribe was consistent with the results of Yang et al. [5]. However, their analysis did not include representatives of the "Semilabeonini" species group.

Our phylogenetic tree of the Labeoninae (Figure 3) shows that the subfamily can be clearly divided into four taxa, Labeonini, Garrini, "Semilabeonini," and "Osteochilini," which is consistent with the results of Yang et al. [5]. In the molecular tree proposed by Yang et al. [5], the relationships among the subtribes of the tribe Labeonini are as follows: (Labeoina (Semilabeoina (Osteochilina, Garraina))). Due to the addition of some new species/genera in our study, the relationships of some species or genera in "Semilabeonini," as well as the relationships of four taxa, have changed. It turns out that adding more species or genera is necessary to confirm the topology of the reconstructed phylogeny. If more samples are added, the relationships between these four groups in the Labeoninae may become stable, and the credibility of the study results will be improved.

Each of the four tribes has a distinct distribution range. The distributions of the Labeonini and the Garrini basically overlap, with these taxa only found in the Mekong River and basins to the west (including Africa). The "Semilabeonini" are concentrated in the Pearl River and Red River basins and Southwest China. The tribe "Semilabeonini" is largely a "China Clade," because most genera and species included are endemic to China. Most species of the subtribe "Osteochilini" are distributed in the east of the Salween River to the Pearl River Basin, but species can also be found in southwestern China [5]. In order to elucidate the taxonomic hierarchy of these four groups, and the relationships between them, more study genera and species are needed in the analysis.

The research of Yang et al. [5] and Zheng et al. [18] involved a total of 40 valid genera, representing a sample of those recorded at that time. According to their findings, some genera were merged or regrouped, others were restored to validity [5], and new genera were established [6]. Currently, 525 species of Labeoninae in 52 genera are recognised [1]. Phylogenetic relationships based on morphological and molecular analyses have not been assessed in about one-third of these genera. One of the greatest questions we will answer with the addition of these genera is whether the Labeoninae remains a monophyletic group. We hypothesize that with the addition of these genera, the Labeoninae will remain a monophyletic group. There are therefore two possibilities for the addition of these unstudied genera. One possibility is that all remaining genera fall into one of the four currently identified taxa. However, in the second possibility, some genera fall into the four current taxa, while the other genera diverge to form one or more new taxa (subtribes). Of course, the results of the study may also separate new genera, or restore the validity of some previously recorded genera. A great deal of work remains to be done in the study of the subfamily Labeoninae in order to answer these questions.

Data Availability

All data are available in the present manuscript and supplement.

Conflicts of Interest

The authors declare that they have no competing interests.

Acknowledgments

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Supplementary Materials

Supplementary 1. Appendix S1: material examined for morphological comparison of Labeoninae genera with oromandibular discs.

Supplementary 2. Appendix S2: taxa examined in this study with source of each species and GenBank accession numbers for each gene.

Supplementary 3. Figure S1: maximum likelihood (ML) phylogeny of the Labeoninae inferred from COI, Cyt b, ND4, IRBP, and RAG1 sequences. Numbers at the nodes show the posterior probability (PP) values. The species of subfamily Labeoninae were divided into four taxa, naming them as tribes Labeonini, Garrini, "Semilabeonini," and "Osteochilini." Figure S2: neighbour joining (NJ) phylogeny of the Labeoninae inferred from COI, Cyt b, ND4, IRBP, and RAG1 sequences. Numbers at the nodes show the posterior probability (PP) values. The species of subfamily Labeoninae were divided into four taxa, naming them as tribes Labeonini, Garrini, "Semilabeonini," and "Osteochilini."

References

[1] R. Fricke, W. N. Eschmeyer, and J. D. Fong, *Eschmeyer's Catalog of Fishes: Species by Family/Subfamily*, 2024, https://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp.

[2] E. Zhang, P. Q. Yue, and J. X. Chen, "Labeoninae," in *Fauna Sinica. Osteichthyes. Cypriniformes III*, P. Q. Yue, Ed., pp. 171–272, Science Press, Beijing, 2000.

[3] V. H. Nguyen and T. A. Bui, "Description of a new *Vinagarra* n. g. in Cyprinidae which is found in Lai Chau, Viet Nam," *Science and Technology Journal of Agriculture & Rural Development*

(Nông Nghiệp & Phát Triên Nông Thôn), vol. 2009, pp. 52–57, 2009.

[4] E. Zhang and W. Zhou, "*Sinigarra napoense*, a new genus and species of labeonin fishes (Teleostei: Cyprinidae) from Guangxi Province, South China," *Zootaxa*, vol. 3586, pp. 17–25, 2012, https://www.mapress.com/zootaxa/2012/f/zt03586p025.pdf.

[5] L. Yang, M. Arunachalam, T. Sado et al., "Molecular phylogeny of the cyprinid tribe Labeonini (Teleostei: Cypriniformes)," *Molecular Phylogenetics and Evolution*, vol. 65, no. 2, pp. 362–379, 2012.

[6] M. Kottelat, "*Ceratogarra*, a genus name for *Garra cambodgiensis* and *G. fasciacauda* and comments on the oral and gular soft anatomy in labeonine fishes (Teleostei: Cyprinidae)," *The Raffles Bulletin of Zoology*, vol. 357, Supplement, pp. 156–178, 2020.

[7] F. Hamilton, *An account of the fishes found in the river Ganges and its branches*, Archibald Constable, Edinburgh & London, 1822, Often seen as Hamilton-Buchanan or Buchanan-Hamilton; in work as Hamilton formerly Buchanan.

[8] L. Kosygin, B. Shangningam, P. Singh, and U. Das, "*Garra jaldhakaensis*, a new cyprinid fish (Teleostei: Cyprinidae) from West Bengal, India," *Records of the Zoological Survey of India*, vol. 121, no. 3, pp. 325–331, 2021, https://www.recordsofzsi.com/index.php/zsoi/article/view/158415.

[9] M. Zamani-Faradonbe, E. Zhang, and Y. Keivany, "*Garra hormuzensis*, a new species from the upper Kol River drainage in the Persian Gulf basin (Teleostei: Cyprinidae)," *Zootaxa*, vol. 5052, no. 3, pp. 380–394, 2021.

[10] S. Jadhav, M. Karuthapandi, B. Shangningam, D. Jaiswal, and C. S. Shankar, "*Garra deccanensis*, a new species of labeonine fish (Teleostei: Cyprinidae) from Peninsular India," *Ichthyological Exploration of Freshwaters*, vol. 1182, pp. 1–14, 2022, https://pfeil-verlag.de/wp-content/uploads/2024/01/ief-31-3_04_ja.pdf.

[11] H. Mousavi-Sabet, S. Eagderi, M. Saemi-Komsari, C. Kaya, and J. Freyhof, "*Garra rezai*, a new species from two widely disjunct areas in the Tigris drainage (Teleostei: Cyprinidae)," *Zootaxa*, vol. 5195, no. 5, pp. 419–436, 2022.

[12] L. Cao, W. D. Nyingi, H. L. Bart Jr., and E. Zhang, "Species of the cyprinid genus *Garra* in Mount Kenya, East Africa: species delineation, taxonomy and historical biogeography," *Zoologica Scripta*, vol. 52, no. 6, 2023.

[13] S. Surachita, B. R. Chowdhury, and S. K. Palita, "*Garra laishrami*, a new species of cyprinid fish (Teleostei: Cyprinidae) from the Godavari River drainage, Eastern Ghats of Odisha, India," *Ichthyological Exploration of Freshwaters*, vol. 1179, 2023https://pfeil-verlag.de/wp-content/uploads/2023/07/ief-1179-www.pdf.

[14] W. Tangjitjaroen, Z. S. Randall, S. Tongnunui, D. A. Boyd, and L. M. Page, "Species of *Garra* (Cyprinidae: Labeoninae) in the Salween River basin with description of an enigmatic new species from the Ataran River drainage of Thailand and Myanmar," *Zootaxa*, vol. 5311, no. 3, pp. 375–392, 2023.

[15] R. Fricke, W. N. Eschmeyer, and R. Van der Laan, "Eschmeyer's catalog of fishes: genera, species, references," 2024. https://researcharchive.calacademy.org/research/ichthyology/catalog/SpeciesByFamily.asp.

[16] L. Yang and R. L. Mayden, "Phylogenetic relationships, subdivision, and biogeography of the cyprinid tribe Labeonini (sensu Rainboth, 1991) (Teleostei: Cypriniformes), with comments on the implications of lips and associated structures in the labeonin classification," *Molecular Phylogenetics and Evolution*, vol. 54, no. 1, pp. 254–265, 2010.

[17] L. P. Zheng, J. X. Yang, X. Y. Chen, and W. Y. Wang, "Phylogenetic relationships of the Chinese Labeoninae (Teleostei, Cypriniformes) derived from two nuclear and three mitochondrial genes," *Zoologica Scripta*, vol. 39, no. 6, pp. 559–571, 2010.

[18] L. P. Zheng, J. X. Yang, and X. Y. Chen, "Phylogeny of the Labeoninae (Teleostei, Cypriniformes) based on nuclear DNA sequences and implications on character evolution and biogeography," *Current Zoology*, vol. 58, pp. 837–850, 2012.

[19] X. Y. Chen, "Checklist of Fishes of Yunnan," *Zoological Research*, vol. 34, no. 4, pp. 281–343, 2013.

[20] E. Zhang, S. P. He, and Y. Y. Chen, "Revision of the cyprinid genus *Placocheilus* Wu, 1977 in China, with description of a new species from Yunnan," *Hydrobiologia*, vol. 487, pp. 207–217, 2002.

[21] K. Nebeshwar and W. Vishwanath, "On the snout and oromandibular morphology of genus *Garra*, description of two new species from the Koladyne River basin in Mizoram, India, and redescription of *G. manipurensis* (Teleostei: Cyprinidae)," *Ichthyological Exploration of Freshwaters*, vol. 28, no. 1, pp. 17–53, 2017, https://pfeil-verlag.de/wp-content/uploads/2017/07/ief28_1_03.pdf.

[22] M. Kottelat, *Freshwater Fishes of Northern Vietnam*, World Bank, Washington, DC, 2001.

[23] X. Xia and P. Lemey, "Assessing substitution saturation with DAMBE," in *The Phylogenetic Handbook*, P. Lemey, Ed., pp. 611–626, Cambridge University Press, Cambridge, UK, 2009.

[24] S. Kumar, G. Stecher, and K. Tamura, "MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets," *Molecular Biology and Evolution*, vol. 33, no. 7, pp. 1870–1874, 2016.

[25] R. Lanfear, P. B. Frandsen, A. M. Wright, T. Senfeld, and B. Calcott, "Partition Finder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses," *Molecular Biology and Evolution*, vol. 34, no. 3, pp. 772-773, 2017.

[26] D. Zhang, F. Gao, I. Jakovlić et al., "PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies," *Molecular Ecology Resources*, vol. 20, no. 1, pp. 348–355, 2020.

[27] F. Ronquist, M. Teslenko, P. van der Mark et al., "MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space," *Systematic Biology*, vol. 61, no. 3, pp. 539–542, 2012.

[28] A. Rambaut, "FigTree 1.4.2 software," in *Institute of Evolutionary Biology*, University of Edinburgh, Edinburgh, 2014.

[29] S. W. Kembel, P. D. Cowan, M. R. Helmus et al., "Picante: R tools for integrating phylogenies and ecology," *Bioinformatics*, vol. 26, no. 11, pp. 1463-1464, 2010.

[30] S. P. Blomberg, T. Garland, and A. R. Ives, "Testing for phylogenetic signal in comparative data: behavioral traits are more labile," *Evolution*, vol. 57, no. 4, pp. 717–745, 2003, http://www.jstor.org/stable/3094610.

[31] G. H. Cui and Z. Y. Li, "Description of a new cyprinid fish of the subfamily Barbinae from China," *Acta Zootaxonomica Sinica*, vol. 9, no. 1, pp. 110–112, 1984.

[32] W. Zhou, X. F. Pan, and M. Kottelat, "Species of *Garra* and *Discogobio* (Teleostei: Cyprinidae) in Yuanjiang (Upper Red River) drainage of Yunnan Province, China with description

of a new species," *Zoological Studies*, vol. 44, no. 4, pp. 445–453, 2005, https://zoolstud.sinica.edu.tw/Journals/44.4/445.pdf.

[33] L. P. Zheng, J. X. Yang, and X. Y. Chen, "*Garra incisorbis*, a new species of labeonine from Pearl River basin in Guangxi, China (Teleostei: Cyprinidae)," *Ichthyological Exploration of Freshwaters*, vol. 26, no. 4, pp. 299–303, 2016, https://pfeil-verlag.de/wp-content/uploads/2016/07/ief26_4_02.pdf.

[34] P. Bleeker, "Systema Cyprinoideorum revisum," *Nederlandsch Tijdschrift voor de Dierkunde*, vol. 1, pp. 187–218, 1863.

[35] Z. M. Chen, X. F. Pan, H. Xiao, and J. Yang, "A new cyprinid species, *Placocheilus dulongensis*, from the upper Irrawaddy system in northwestern Yunnan, China," *Zoologischer Anzeiger*, vol. 251, no. 3, pp. 215–222, 2012.

[36] V. N. Nguyen and S. V. Ngô, "Freshwater Fishes of Vietnam," in *Vol. 1. Carp family (Cyprinidae)*, Agriculture Publishing House, Hanoi, 2001.

[37] F. L. Li, W. Zhou, and Q. Fu, "*Garra findolabium*, a new species of cyprinid fish (Teleostei: Cypriniformes) from the Red River drainage in Yunnan, China," *Zootaxa*, vol. 1743, pp. 62–68, 2008, https://www.mapress.com/zootaxa/2008/f/z01743p068f.pdf.

[38] V. N. Nguyen and L.-H. Doan, *Some data on composition, origin, and distribution of cyprinid species in northern Vietnam*, First Scientific Seminar of the Fisheries Division, 1969, in Vietnamese. [A reproduction of the original is in Kottelat 2001:123 et seq. For nomenclatural decisions see Kottelat 2001:113 et seq.; for translation of original descriptions see Kottelat 2001: 116-122. For a full translation see Nguyen & Doan 2007.].

[39] V. N. Nguyen and T. A. Bui, "Description of two new species of genus *Vinagarra* Hao & Anh 2009 (Cyprinidae) found in Lai Chau," *Science and Technology Journal of Agriculture & Rural Development (Nông Nghiệp & Phát Triên Nông Thôn)*, vol. 2010, pp. 54–59, 2010.

[40] M. Tan and J. W. Armbruster, "Phylogenetic classification of extant genera of fishes of the order Cypriniformes (Teleostei: Ostariophysi)," *Zootaxa*, vol. 4476, no. 1, pp. 6–39, 2018.

[41] C. C. Stout, M. Tan, A. R. Lemmon, E. M. Lemmon, and J. W. Armbruster, "Resolving Cypriniformes relationships using an anchored enrichment approach," *BMC Evolutionary Biology*, vol. 16, no. 1, p. 244, 2016.