Supplementary Materials

Materials and Methods

Sample collection

Specimens were collected from the Cailao River, Guangxi Zhuang Autonomous Region, China (Figure 1L). Part of the pelvic fin tip was taken and preserved in 95% ethanol. Specimens were fixed in 10% buffered formalin and then placed in 75% ethanol for longer preservation. The holotype and paratypes are preserved at the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences.

Morphological examinations

Measurements were made with digital calipers point-to-point with an accuracy of 0.1 mm. Counts and measurements followed Kottelat (2001), except for the last two branched dorsal- and anal-fin rays articulated on the same pterygiophore, which counted as one (Chu & Chen, 1989). Predorsal, prepectoral, prepelvic, and preanal lengths followed Zheng et al. (2016). Scales on the caudal-fin base were also included in the lateral line scale count. Oromandibular terms followed Kottelat (2020).

The specimens examined in this study are preserved at the Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences. Abbreviations used in this text: SL, standard length; HL, head length.

DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

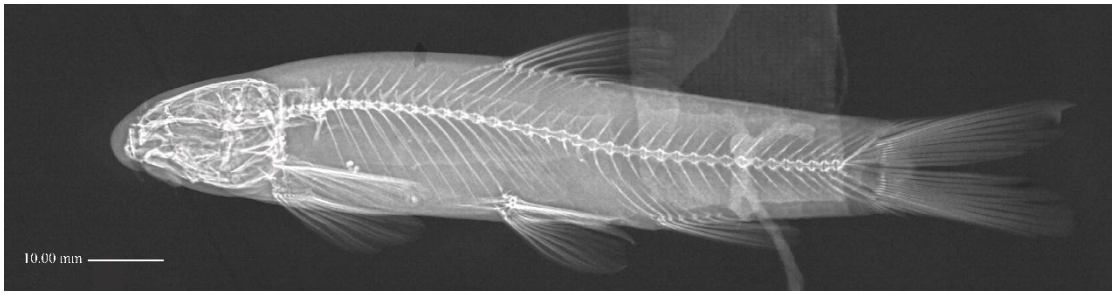
Genomic DNA was extracted from pelvic fin clips preserved in 95% ethanol. Two mitochondrial genes (*COI*, cyt *b*) and one nuclear gene (*Rag 1*) were used in this study. The primers and experimental protocols followed Zheng et al. (2016). The PCR products were sequenced by Tsingke Biotechnology Co., Ltd (Kunming, China). The new species sequences used in this study were deposited in GenBank (Accession Nos. OM810021–30, OM832715–19)

Sequence alignment and phylogenetic analyses

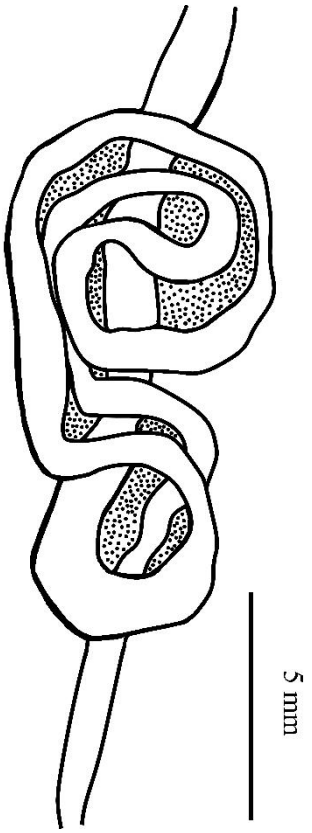
A total of 222 sequences were used, including 15 sequences obtained in this study and other sequences downloaded from the NCBI database. Accession numbers are shown in Table S1. Sequences were aligned using MAFFT v7.313 (Katoh & Standley, 2013) and checked manually for inconsistencies. The aligned sequences of the three genes were concatenated using PhyloSuite v1.2.2 (Zhang et al., 2020). PartitionFinder v2 was used to select the best partitioning scheme (Lanfear et al., 2017) under corrected Akaike information criterion (AICc). Phylogenetic inferences were carried out using maximum-likelihood (ML). IQ-TREE v2.2.0 (Nguyen et al., 2015) was used to conduct ML analysis, with 500 000 ultrafast (Minh et al., 2013) bootstrap replicates. Three different substitution models, i.e., GTR+I+G, GTR+I+G, and SYM+I+G, were applied to different partitions, respectively. All steps were performed using PhyloSuite v1.2.2.

Divergence time estimation

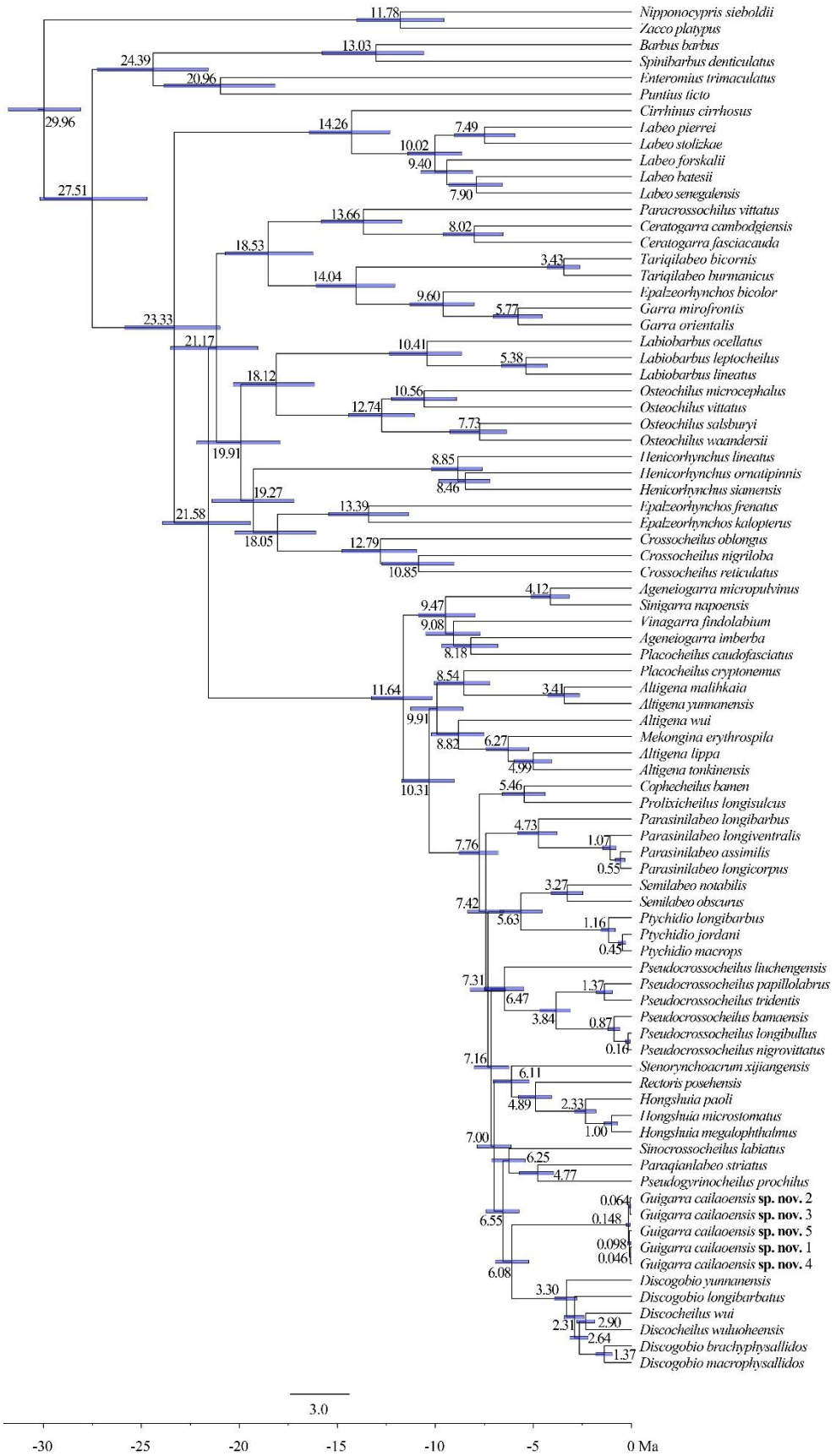
Beast v1.10.4 was used to conduct divergence time analysis, with 10 000 000 generations and sampling every 1 000 generations. Topology of the ML tree was used to infer divergence time and the molecular clock was set as a strict clock. Two calibration points were used, following Tang et al. (2009). Tracer v1.7.2 was used to check that all effective sample size (ESS) values were larger than 200.



Supplementary Figure S1 Radiograph of *Guigarra cailaoensis* sp. nov. holotype KIZ 20210004, 47.0 mm SL



Supplementary Figure S2 Intestine of *Guigarra cailaoensis* sp. nov. paratype KIZ 20210002, 51.6 mm SL.



Supplementary Figure S3 Divergence time of Labeoninae. Nodal numbers denote median divergence time.

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