RESEARCH ARTICLE

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# Comparative analysis of the mitogenomes of two *Corydoras* (Siluriformes, Loricarioidei) with nine known *Corydoras*, and a phylogenetic analysis of Loricarioidei

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## Abstract

*Corydoras* is a speciose catfish genus from South America with widely investigated phylogenetic and evolutionary relationships. The complete mitogenomes of *C. aeneus* and *C. paleatus* were sequenced, assembled, and annotated using next-generation sequencing. The genome arrangements, gene contents, genome structures, base compositions, evolutionary features, codon usage, and tRNA structures of the two mitogenomes were compared and analyzed with nine published mitogenomes of *Corydoras*. Phylogenetic analysis was performed using concatenated nucleotide sequences with 13 protein-coding genes and two rRNAs with 44 mitogenomes of Siluriformes. These results provide information on the mitogenomes of eleven *Corydoras* species and evolutionary relationships within the suborder Loricarioidei, which may be applicable for further phylogenetic and taxonomic studies on Siluriformes and Loricarioidei.

## Keywords

## Introduction

Fish mitochondrial DNA shares characteristics with other vertebrate mitochondrial DNA (Anderson et al. 1981; Manchado et al. 2007; Xu et al. 2011), e.g., small molecular weight, simple structure, and compact arrangement. It exists in the form of a covalently closed circular supercoil structure and contains heavy and light chains. The genetic material can be replicated, transcribed, and translated independently from the nuclear DNA in the cell. With few exceptions, fish mitochondrial DNA comprises 13 protein-coding genes (PCGs), 22 transfer RNA genes, two ribosomal RNA genes, original region of light-strand replication, and control region (D-loop) (Ojala et al. 1981; Gadaleta et al. 1989; Wolstenholme 1992 Simon et al. 1994; De Rijk et al. 1995). The mitochondrial DNA mutates rapidly, nearly 10-fold faster than the nuclear DNA, and the fragment length and evolution rate differ for each gene, providing molecular evidence for studying different species (Brown et al. 1979; Pesole et al. 1999). In addition, mitochondrial DNA is highly heterogeneous and harbors the genetic characteristics associated with maternal traits (O'Brien 1971; Michot et al. 1990; Bartlett and Davidson 1991; Meyer 1993; Beheregaray and Sunnucks 2001; Liu et al. 2002; Yoshizawa and Johnson 2003). Hence, mitochondrial DNA can be used to identify fish groups at the molecular level and explore geographic distribution, species origin, and species differentiation (Avise et al. 1987; Kai et al. 2002; Hrbek et al. 2007). As fish are a large group with a complex origin in the vertebrate subphylum, studies on their phylogenetic and evolutionary relationships performed using traditional morphological methods often provide limited information. With advances in biotechnology, complete mitochondrial genome sequences have been determined as a useful tool to study the phylogeny and phylogeography of fish (Bermingham and Avise 1986; Xu et al. 2020).

*Corydoras* Lacépède, 1803, belongs to the order Siluriformes, suborder Loricarioidei, family Callichthyidae. *Corydoras* contains 175 valid species, which makes it the most species-rich genus of the family Callichthyidae (Lima and Britto 2020; Tencatt et al. 2021). The body of these fish is covered with bone plates, and the pectoral and dorsal fins have hard spines that can be used for protection. In addition, *Corydoras* can use the back end of their intestines, which is rich in blood vessels, to obtain oxygen from air taken in at the water surface, enabling survival under environmental stress, such as drought or insufficient dissolved oxygen content in water. *Corydoras* catfish are benthic omnivorous fish (Moreira et al. 2016b, 2017; Liu et al. 2019b, 2019c; Saitoh et al. 2003). Typically, *Corydoras* is active only during feeding, and otherwise hide while resting. *Corydoras* is primarily distributed in South America. Most species of *Corydoras* gather in the middle and lower reaches of the river where the current is relatively gentle, whereas a few live in the upper reaches of the river in rapids (Saitoh et al. 2003; Liu et al. 2019c). *Corydoras* is also valuable as an ornamental fish. Some phylogenetic relationships in *Corydoras* remain unclear. The number of species reported in relevant articles is small, which is not sufficient to reflect the phylogenetic variety of the genus *Corydoras* (Alexandrou et al. 2011; Lujan et al. 2015; Roxo et al. 2019). Therefore, a comprehensive understanding of the relationships between different species of *Corydoras* is essential.

In this study, the complete mitogenomes of two species of *Corydoras* (Bronze corydoras *C. aeneus* Gill, 1858 and peppered corydoras *C. paleatus* Jenyns, 1842) were sequenced, assembled, and annotated. The genome organization, gene contents, repeat sequences, and tRNA structures of the eleven mitogenomes were compared and analyzed in combination with nine published mitogenomes of *Corydoras* (Saitoh et al. 2003; Moreira et al. 2016a, 2017; Liu et al. 2019a, b, c, d; Chen et al. 2020; Lv et al. 2020). Determining the similarities and differences in gene orders, genetic structures, base compositions, evolutionary features, and codon usage can provide molecular insights into the taxonomic and phylogenetic characteristics of the order Siluriformes. Based on these data, and those obtained from the NCBI database, we examined the phylogenetic relationships among species in the suborder Loricarioidei. We also evaluated the mitogenomes of eleven species of *Corydoras* and evolutionary relationships within the suborder Loricarioidei, thereby providing a valuable basis for further evolutionary studies on Siluriformes and Loricarioidei.

## Materials and methods

### Sample collection and identification

Single specimens of *C. aeneus* and *C. paleatus* were collected from the temple of Confucius flower and wood fish market, Nanjing city, Jiangsu province, China (32°0'27.1"N, 118°50'11.5"E) in June 2020 and identified based on their morphological characteristics, according to the latest taxonomic classification of fish (Popazoglo and Boeger 2000; Huysentruyt and Adriaens 2005a, b). Their geographic data and specific origins were unknown. All fresh tissues were immediately stored at -80 °C in 95% ethanol until DNA extraction. Total DNA was extracted from the muscle tissue using a TIANamp Marine Animals DNA Kit DP324 (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. DNA integrity and purity were evaluated by 1% agarose gel electrophoresis, and DNA purity was determined with a NanoDrop 2000 (NanoDrop Technologies, Wilmington, DE, USA). DNA concentrations were quantified using a Qubit$^R$ 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). To ensure the accuracy of morphological identification, COI primers were designed based on the latest DNA barcoding database (NCBI and FishBase) and were amplified, sequenced, and compared. The COI sequences are provided in the Suppl. material 1. The results of the sequence alignment verify the accuracy of the morphological identification.

### Genome sequencing and assembly

Next-generation sequencing was performed to determine the complete mitogenome sequence of the two species of *Corydoras*. The DNA libraries were sequenced on an Illumina sequencing platform by Novogene Co., Ltd. (Beijing, China). Briefly, the total DNA genome was quantified and fragmented into 250-base pair (bp) fragments using a Covaris M220 ultrasonic crushing system (Woburn, MA, USA) followed by whole-

genome shotgun sequencing. According to the manufacturer's instructions, a library was constructed based on two indices using an Illumina TruSeq DNA PCR-Free HT kit (San Diego, CA, USA). An Illumina Novaseq 6000 platform was used for sequencing of 150 paired-end reads approximately 4 Gb in size. Clean reads were generated as previously described, and the remaining high-quality reads were assembled using SPADES V3.15.2 (Bankevich et al. 2012) (http://cab.spbu.ru/software/spades/) and SOAPDENOVO2 V2.01 (Luo et al. 2012) software. The preliminary assembly results were compared with the NT database, and looped sequences annotated as mitochondrial genomes were screened. CAP3 was used to merge the splicing results from the two software programs, and the assembly results were compared with those of related species using MUMMER v3.23 (Delcher et al. 2003). The mitogenome composition was confirmed, and a complete, high-quality map of the mitochondrial genome was obtained.

## Genome annotation and analysis

The tRNA genes were verified using tRNASCAN-SE V1.3.1 (Lowe and Eddy 1997) with default settings for the vertebrate mitochondrial genetic code. The software, which integrates multiple analysis tools, can identify 99% of the tRNA genes with a very low number of false positives and predict the secondary structure of tRNAs. Protein-coding regions were re-identified using GLIMMER V3.0 (Ingram et al. 2009), and manual comparisons were performed using the SEQMAN program of LASERGENE V7.1 (Burland 2000) (DNAStar, Inc., Madison, WI, USA) based on the PCGs of nine species of *Corydoras* and translated into putative proteins via GenBank. The non-coding RNAs were verified using RFAM V12.0 (Griffiths-Jones et al. 2003) and INFERNAL V1.1 (Nawrocki and Eddy 2013). The rRNA genes were assumed to extend to the boundaries of flanking genes, similar to the homologous regions of other published mitogenomes of *Corydoras* in GenBank. The MITOS WebServer (http://mitos2.bioinf.uni-leipzig.de/index.py) and MitoFish (Iwasaki et al. 2013) (http://mitofish.aori.u-tokyo.ac.jp/) online tools were used for the final annotation of the entire mitogenome sequence of the two species of *Corydoras*, and the annotated mitogenomes were compared with nine published mitogenomes of *Corydoras*. Base compositions, genetic distances, and relative synonymous codon usage values were determined using MEGA V7.0 (Kumar et al. 1994). A graph comparing the relative synonymous codon usage was drawn using PHYLOSUITE V1.2.2 (Zhang et al. 2020). Strand asymmetry was analyzed using the formula: AT-skew = $(A - T)/(A + T)$. The numbers of non-synonymous (Ka) and synonymous (Ks) substitutions and the ratio of Ka/Ks and nucleotide diversity for the nine species of *Corydoras* were calculated using DNASP 5.1 (Librado and Rozas 2009). The MitoFish (http://mitofish.aori.u-tokyo.ac.jp/) online tool was used to generate circular mitogenome maps.

## Phylogenetic analysis

Phylogenetic trees for the eleven mitogenomes of *Corydoras* within the family Callichthyidae and Suborder Loricarioidei were constructed by aligning 13 PCGs and two

rRNA sequences with those of 42 species of Loricarioidei, 29 species from Loricariidae, and one species from Trichomycteridae (Table 1). The mitogenomes of *Pterocryptis cochinchinensis* (Resende et al. 2016) and *Silurus asotus* (Nakatani et al. 2011) (accession no. NC_027107.1 and NC_015806.1, respectively, suborder Siluroidei) were included as outgroups to root the Loricarioidei tree. All operations were performed in PHYLOSUITE V1.2.2 (Zhang et al. 2020) software package. The nucleotide sequences of 13 PCGs from 44 mitogenomes were aligned in batches with MAFFT V7.313 (Katoh and Standley 2013) (https://mafft.cbrc.jp/alignment/server/) using the

**Table 1.** Information on 44 Siluriformes species evaluated in the study.

| No. | Suborder | Family | Taxa | GenBank accession no. | Length (bp) | Location/Reference |
|---|---|---|---|---|---|---|
| 1 | Loricarioidei | Callichthyidae | *Corydoras aeneus* | MZ571336 | 16604 | This study |
| 2 | | | *Corydoras agassizii* | MN641875.1 | 16538 | Lv et al. 2020 |
| 3 | | | *Corydoras arcuatus* | NC_049096.1 | 16177 | Liu et al. 2019d |
| 4 | | | *Corydoras duplicareus* | NC_049095.1 | 16632 | Liu et al. 2019a |
| 5 | | | *Corydoras nattereri* | KT239008.1 | 16557 | Moreira et al. 2016a |
| 6 | | | *Corydoras paleatus* | MZ571337 | 16320 | This study |
| 7 | | | *Corydoras panda* | NC_049097.1 | 16398 | Liu et al. 2019b |
| 8 | | | *Corydoras rabauti* | NC_004698.1 | 16711 | Saitoh et al. 2003 |
| 9 | | | *Corydoras schwartzi* | KT239007.1 | 15671 | Moreira et al. 2017 |
| 10 | | | *Corydoras sterbai* | NC_048967.1 | 16520 | Liu et al. 2019c |
| 11 | | | *Corydoras trilineatus* | NC_049098.1 | 15359 | Chen et al. 2020 |
| 12 | | | *Hoplosternum littorale* | KX087170.1 | 16262 | Parente et al. 2018 |
| 13 | | Loricariidae | *Ancistomus snethlageae* | KX087166.1 | 16464 | Moreira et al. 2017 |
| 14 | | | *Ancistrus cryptophthalmus* | MF804392.1 | 16333 | Lv et al. 2020 |
| 15 | | | *Ancistrus multispinis* | KT239006.1 | 16539 | Moreira 2018 |
| 16 | | | *Ancistrus temminckii* | NC_051963.1 | 16439 | Meng et al. 2021 |
| 17 | | | *Aphanotorulus emarginatus* | KT239019.1 | 16597 | Moreira et al. 2017 |
| 18 | | | *Baryancistrus xanthellus* | KX087167.1 | 16167 | Moreira et al. 2017 |
| 19 | | | *Dekeyseria amazonica* | KX087168.1 | 16409 | Moreira 2018 |
| 20 | | | *Hemipsilichthys nimius* | KT239011.1 | 16477 | Moreira et al. 2017 |
| 21 | | | *Hisonotus thayeri* | KX087173.1 | 16269 | Moreira et al. 2017 |
| 22 | | | *Hypancistrus zebra* | KX611143.1 | 16202 | Magalhães et al. 2017 |
| 23 | | | *Hypoptopoma incognitum* | NC_028072.1 | 16313 | Moreira et al. 2016b |
| 24 | | | *Hypostomus affinis* | KT239013.1 | 16330 | Moreira et al. 2017 |
| 25 | | | *Hypostomus ancistroides* | NC_052710.1 | 16422 | Rocha-Reis et al. 2020 |
| 26 | | | *Hypostomus francisci* | NC_045188.1 | 16916 | Pereira et al. 2019 |
| 27 | | | *Hypostomus plecostomus* | NC_025584.1 | 16562 | Liu et al. 2016 |
| 28 | | | *Kronichthys heylandi* | KT239014.1 | 16632 | Moreira et al. 2017 |
| 29 | | | *Loricaria cataphracta* | KX087174.1 | 16831 | Moreira et al. 2017 |
| 30 | | | *Loricariichthys castaneus* | KT239015.1 | 16521 | Moreira et al. 2017 |
| 31 | | | *Loricariichthys platymetopon* | KT239018.1 | 16521 | Moreira et al. 2017 |
| 32 | | | *Neoplecostomus microps* | KX087175.1 | 16523 | Moreira et al. 2017 |
| 33 | | | *Otocinclus affinis* | MT323116.1 | 16501 | Zhang et al. 2021 |
| 34 | | | *Pareiorhaphis garbei* | KX087178.1 | 16630 | Moreira et al. 2017 |
| 35 | | | *Parotocinclus maculicauda* | KX087179.1 | 16541 | Moreira et al. 2017 |
| 36 | | | *Peckoltia furcata* | KX087180.1 | 16497 | Moreira et al. 2017 |
| 37 | | | *Pterygoplichthys anisitsi* | KT239003.1 | 16636 | Parente et al. 2017 |
| 38 | | | *Pterygoplichthys disjunctivus* | NC_015747.1 | 16667 | Nakatani et al. 2011 |
| 39 | | | *Pterygoplichthys pardalis* | KT239016.1 | 16822 | Moreira et al. 2017 |
| 40 | | | *Schizolecis guntheri* | KT239017.1 | 16611 | Moreira et al. 2017 |
| 41 | | | *Sturisomatichthys panamensis* | NC_045877.1 | 16526 | Ren et al. 2019 |
| 42 | | Trichomycteridae | *Trichomycterus areolatus* | AP012026.1 | 16657 | Nakatani et al. 2011 |
| 43 | Siluroidei | Siluridae | *Pterocryptis cochinchinensis* | NC_027107.1 | 16826 | Resende et al. 2016 |
| 44 | | | *Silurus asotus* | NC_015806.1 | 16593 | Nakatani et al. 2011 |

codon alignment mode. The results were optimized using MACSE V2.03 (Ranwez et al. 2018). The nucleotide sequences of two rRNAs were aligned using the online tool MAFFT with default settings. Ambiguously aligned regions were removed via GBLOCKS 0.91 b with default settings. The resulting alignments were concatenated into a single dataset with PHYLOSUITE. The best partition schemes and optimal substitution models were selected by MODELFINDER (Kalyaanamoorthy et al. 2017) with the greedy algorithm and Bayesian information criterion (Watanabe 2013). The best substitution models applied to each partition are listed in Suppl. material 1: Table S1. Phylogenetic trees were constructed using two inference methods: maximum likelihood (ML) and Bayesian inference (BI). ML analyses were performed with IQ-TREE V1.6.8 with the models selected for each partition, and 1,000 bootstrap replicates were used to estimate node reliability. Bayesian analyses were performed using MRBAYES V3.2.6 (Huelsenbeck and Ronquist 2001). One million generations of two independent runs were performed with four chains and sampling trees every 100 generations. The initial 25% of trees generated prior to reaching stable log-likelihood values were discarded as burn-in. The remaining trees were used to calculate the Bayesian posterior probabilities. The resulting phylogenetic trees and gene orders were visualized and edited using iTOL (Letunic and Bork 2016).

## Results and discussion

### Genome structure and organization

The complete mitogenomes of *C. aeneus* and *C. paleatus* comprising 16,604 and 16,593 bp, respectively, were submitted to GenBank (accession nos. MZ571336 and MZ571337, respectively) (Fig. 1, Table 2). The two mitogenomes were circular and contained 37 mitochondrial genes (13 PCGs, 22 tRNA genes, and two rRNA genes)
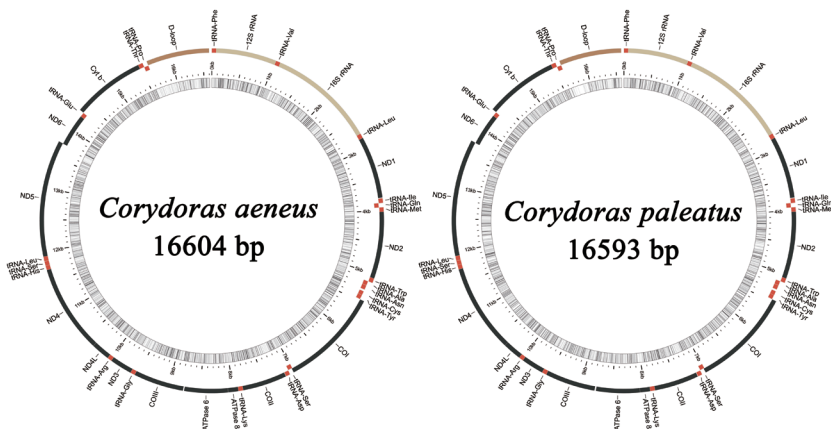


**Figure 1.** Gene maps of the two newly sequenced *Corydoras* species.

**Table 2.** Characteristic features of *Corydoras aeneus* and *Corydoras paleatus* mitogenomes (+ denotes heavy strand; - denotes light strand).

| Feature | Position | | | | Length (bp) | | Start codons | | Stop codons | | Anticodon | Strand | Intergenic nucleotides | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C. aeneus | | C. paleatus | | C. aeneus | C. paleatus | C. a | C. p | C. a | C. p | | | C. a | C. p |
| | From | to | From | to | | | | | | | | | | |
| tRNA-Phe | 1 | 68 | 1 | 68 | 68 | 68 | | | | | GAA | + | 0 | 0 |
| 12S rRNA | 69 | 1014 | 69 | 1013 | 946 | 945 | | | | | | + | 0 | 0 |
| tRNA-Val | 1015 | 1086 | 1014 | 1085 | 72 | 72 | | | | | TAC | + | 0 | 0 |
| 16S rRNA | 1087 | 2757 | 1086 | 2753 | 1671 | 1668 | | | | | | + | 0 | 0 |
| tRNA-Leu | 2758 | 2832 | 2754 | 2828 | 75 | 75 | | | | | TAA | + | 0 | 0 |
| ND1 | 2833 | 3804 | 2829 | 3800 | 972 | 972 | ATG | ATG | TAG | TAG | | + | 8 | 8 |
| tRNA-Ile | 3813 | 3884 | 3809 | 3880 | 72 | 72 | | | | | GAT | + | -2 | -2 |
| tRNA-Gln | 3883 | 3953 | 3879 | 3949 | 71 | 71 | | | | | TTG | - | -1 | -1 |
| tRNA-Met | 3953 | 4022 | 3949 | 4018 | 70 | 70 | | | | | CAT | + | 0 | 0 |
| ND2 | 4023 | 5067 | 4019 | 5063 | 1045 | 1045 | ATG | ATG | T | T | | + | 0 | 0 |
| tRNA-Trp | 5068 | 5139 | 5064 | 5134 | 72 | 71 | | | | | TCA | + | 1 | 1 |
| tRNA-Ala | 5141 | 5209 | 5136 | 5204 | 69 | 69 | | | | | TGC | - | 1 | 1 |
| tRNA-Asn | 5211 | 5283 | 5206 | 5278 | 73 | 73 | | | | | GTT | - | 30 | 31 |
| tRNA-Cys | 5314 | 5380 | 5310 | 5377 | 67 | 68 | | | | | GCA | - | -1 | -1 |
| tRNA-Tyr | 5380 | 5449 | 5377 | 5446 | 70 | 70 | | | | | GTA | - | 1 | 1 |
| COI | 5451 | 7010 | 5448 | 7007 | 1560 | 1560 | GTG | GTG | AGG | AGG | | + | -13 | -13 |
| tRNA-Ser | 6998 | 7068 | 6995 | 7065 | 71 | 71 | | | | | TGA | - | 4 | 4 |
| tRNA-Asp | 7073 | 7141 | 7070 | 7138 | 69 | 69 | | | | | GTC | + | 4 | 6 |
| COII | 7146 | 7836 | 7145 | 7835 | 691 | 691 | ATG | ATG | T | T | | + | 0 | 0 |
| tRNA-Lys | 7837 | 7910 | 7836 | 7909 | 74 | 74 | | | | | TTT | + | 1 | 1 |
| ATPase 8 | 7912 | 8079 | 7911 | 8078 | 168 | 168 | ATG | ATG | TAA | TAA | | + | -10 | -10 |
| ATPase 6 | 8070 | 8753 | 8069 | 8752 | 684 | 684 | ATG | ATG | TAA | TAA | | + | 17 | 21 |
| COIII | 8771 | 9554 | 8774 | 9557 | 784 | 784 | ATG | ATG | T | T | | + | 0 | 0 |
| tRNA-Gly | 9555 | 9626 | 9558 | 9629 | 72 | 72 | | | | | TCC | + | 0 | 0 |
| ND3 | 9627 | 9975 | 9630 | 9978 | 349 | 349 | ATG | ATG | T | T | | + | 0 | 0 |
| tRNA-Arg | 9976 | 10045 | 9979 | 10048 | 70 | 70 | | | | | TCG | + | 0 | 0 |
| ND4L | 10046 | 10342 | 10049 | 10345 | 297 | 297 | ATG | ATG | TAA | TAA | | + | -7 | -7 |
| ND4 | 10336 | 11716 | 10339 | 11719 | 1381 | 1381 | ATG | ATG | T | T | | + | 0 | 0 |
| tRNA-His | 11717 | 11786 | 11720 | 11789 | 70 | 70 | | | | | GTG | + | 0 | 0 |
| tRNA-Ser | 11787 | 11853 | 11790 | 11856 | 67 | 67 | | | | | GCT | + | 1 | 1 |
| tRNA-Leu | 11855 | 11927 | 11858 | 11930 | 73 | 73 | | | | | TAG | + | 0 | 0 |
| ND5 | 11928 | 13754 | 11931 | 13757 | 1827 | 1827 | ATG | ATG | TAA | TAA | | + | -4 | -4 |
| ND6 | 13751 | 14266 | 13754 | 14269 | 516 | 516 | ATG | ATG | TAA | TAA | | - | 0 | 0 |
| tRNA-Glu | 14267 | 14335 | 14270 | 14337 | 69 | 68 | | | | | TTC | - | 2 | 3 |
| Cyt b | 14338 | 15475 | 14341 | 15478 | 1138 | 1138 | ATG | ATG | T | T | | + | 0 | 0 |
| tRNA-Thr | 15476 | 15548 | 15479 | 15550 | 73 | 72 | | | | | TGT | + | -2 | -2 |
| tRNA-Pro | 15547 | 15616 | 15549 | 15618 | 70 | 70 | | | | | TGG | - | 0 | 0 |
| D-loop | 15617 | 16604 | 15619 | 16593 | 988 | 975 | | | | | | | 0 | 0 |

and one D-loop. The position of each gene in the mitogenome was identical to that in other species of *Corydoras* (Saitoh et al. 2003; Moreira et al. 2016a, 2017; Liu et al. 2019a, b, c, d; Chen et al. 2020; Lv et al. 2020). One of the 13 PCGs (ND6) and eight tRNAs (tRNA-Ala, tRNA-Cys, tRNA-Glu, tRNA-Asn, tRNA-Pro, tRNA-Gln, tRNA-Ser(TGA), and tRNA-Tyr) were encoded by the light chain (-), whereas the other 28 genes, including 12 PCGs, 14 tRNAs, two rRNAs, and one D-loop, were encoded by the heavy chain (+) (Fig. 1, Table 2). The 44 mitogenomes of Siluriformes (Nakatani et al. 2011; Liu et al. 2016; Moreira et al. 2016b, 2018; Resende et al. 2016;

Magalhães et al. 2017; Parente et al. 2017; Parente et al. 2018; Pereira et al. 2019; Ren et al. 2019; Rocha-Reis et al. 2020; Meng et al. 2021; Zhang et al. 2021) used in this study were compared, and the gene composition and order were consistent (Suppl. material 1: Fig. S1). The nucleotide composition of the two entire mitogenomes was as follows: *C. aeneus* A = 5417 (32.63%), T = 4299 (25.89%), G = 2451 (14.76%), C = 4437 (26.72%) and *C. paleatus* A = 5380 (32.42%), T = 4282 (25.81%), G = 2481 (14.95%), C = 4450 (26.82%). The two mitogenomes (values for *C. aeneus* followed by values for *C. paleatus*) had high A+T contents of 58.52% and 58.23% (Suppl. material 1: Table S2), including 58.08% and 57.67% in PCGs, 56.97% and 57.04% in tRNA genes, 59.70% and 59.10% in 16S rRNA, 55.30% in 12S rRNA, and 67.51% and 68.21% in the D-loop, respectively, which agrees with the typical base bias of fish mitogenomes (Gadaleta et al. 1989; Manchado et al. 2007; Xu et al. 2011). The overall AT and GC skew values in the entire mitogenome of *C. aeneus* were 0.115 and -0.288 and in *C. paleatus* were 0.114 and -0.284, respectively. The GC skew value of the eleven mitogenomes of *Corydoras*, except for tRNA, was slightly negative (-0.014 to -0.288), showing a higher occurrence of C than of G. In contrast, AT skew value, except for the second codon position, was slightly positive (0.028 to 0.379), showing a higher content of A than of T. The K2P genetic distances of the eleven mitogenomes of *Corydoras* were all less than 0.12 (Suppl. material 1: Table S3). *C. nattereri* and *C. sterbai* and *C. nattereri* and *C. trilineatus* showed the largest K2P genetic distances among the eleven species of *Corydoras*.

## Protein-coding genes

The 13 PCGs of the two new mitogenomes and those of the previously published nine mitogenomes of *Corydoras* contained COI–COIII, ND1–ND6, ND4L, two AT-Pases, and one Cyt-b, similar to that in other Siluriformes (Nakatani et al. 2011; Liu et al. 2016; Moreira et al. 2016b; Resende et al. 2016; Magalhães et al. 2017; Parente et al. 2017; Moreira 2018; Parente et al. 2018; Pereira et al. 2019; Ren et al. 2019; Rocha-Reis et al. 2020; Meng et al. 2021; Zhang et al. 2021). The total lengths of PCGs in the eleven mitogenomes of *Corydoras* were 11,400–11,414 bp, accounting for 67.84–69.24% of the entire mitogenome. Similar to the mitogenomes of other species of Loricarioidei, ND5 and ATPase 8 were largest (1,827 bp) and smallest (168 bp), respectively. Most PCGs stringently start with an ATG start codon, except for all COIs, which start with GTG, *C. nattereri* COIII (Moreira et al. 2016a) which starts with GCA, and *C. schwartzi* COII (Moreira et al. 2017), which starts with CCA (Suppl. material 1: Table S4). Most PCGs are stringently terminated by the stop codon TAR (TAA/TAG) or an incomplete stop codon T, except for all COIs, which terminate with AGG and *C. schwartzi* ATPase 6 and *C. nattereri* ND3, which terminate with TA. The presence of a truncated stop codon is common among vertebrate mitochondrial genes and is thought to be introduced by posttranscriptional poly-adenylation.

Similar to most previously sequenced members of Loricarioidei, the AT-skews (0.033 to 0.052) and GC-skews (-0.268 to -0.299) of the PCGs were similar among

the eleven species of *Corydoras* (Suppl. material 1: Table S2). Summaries of the relative synonymous codon usage and the number of amino acids in the annotated PCGs are presented in Suppl. material 1: Figs S2, S3. The PCGs of the eleven mitogenomes of *Corydoras* (Saitoh et al. 2003; Moreira et al. 2016a, 2017; Liu et al. 2019a, b, c, d; Chen et al. 2020; Lv et al. 2020) translate into 3,798–3,802 codons and showed very similar codon usage, excluding the stop codons (26–28 bp). Ile (310.82 ± 2.69 codons), Thr (312.64 ± 2.27 codons), Ala (312.73 ± 3.08 codons), and Leu1 (CUN) (475.45 ± 12.89 codons) were the four most predominant codon families and may be associated with the coding function of the chondriosome. In contrast, Cys (24.91 ± 0.79 codons) and Ser1 (AGN) (52.18 ± 0.83 codons) had the smallest number of codons. A/T rather than G/C bias was observed in the third position, as almost all frequently used codons ended with A/T. The synonymous codon preferences for the eleven species of *Corydoras* were conserved, possibly because of the close relationships among members of this genus.

To reveal the evolutionary pattern of the PCGs, the Ka/Ks, nucleotide diversity, and K2P genetic distance across all mitogenomes of *Corydoras* were calculated for each aligned PCG. The K2P genetic distances of 13 PCGs were all less than 0.12 (Fig. 2a). Among the PCGs detected, ND4 and ATPase 8 showed the largest K2P genetic distance among the eleven species of *Corydoras*, followed by ND2 and ND3. The nucleotide diversity of the 13 PCGs was less than 0.11 (Fig. 2b). ND4 showed the highest nucleotide diversity, whereas COII showed the lowest diversity. To investigate the selective pressure across species of *Corydoras*, the Ka/Ks ratios of the PCGs of each mitogenome were estimated (Fig. 2c). The Ka/Ks value was highest for ND6, followed by ND2; the lowest values were observed for COI, COIII, ND1, and ND4L. All 13 PCGs showed Ka/Ks << 1, suggesting that all PCGs of *Corydoras* evolved under purifying selection.

## tRNAs, ribosomal RNAs, and control region

The total lengths of the 22 tRNA genes ranged from 1,438 (*C. schwartzi*) to 1,561 bp (*C. arcuatus* and *C. panda*), whereas individual tRNA genes typically ranged from 58 to 75 bp. All tRNA genes displayed the expected cloverleaf secondary structures with normal base pairing, except for tRNA-Ser(GCT), which lacked the DHU stem (Suppl. material 1: Fig. S4), forming a loop commonly found in other vertebrates (Ojala et al. 1981; Gadaleta et al. 1989; Wolstenholme 1992). The A+T contents of these tRNAs were 56.55–57.58%. All AT-skew and GC-skew values were slightly positive, indicating a slight bias toward the use of A and G in the tRNAs (Suppl. material 1: Table S2). These rRNA genes are between tRNA-Phe and tRNA-Leu(TAA) and are separated by tRNA-Val. The average total size of the two rRNAs was 2,614 bp, and the average A+T content was 57.89%. Like the tRNAs, all AT-skew values were positive, whereas all GC-skew values were negative, indicating that rRNAs favor C compared to tRNAs in *Corydoras*.

The control region (D-loop), also known as the A+T rich region that contains hypervariable non-coding sequences and regulates the replication and transcription of mitochondrial DNA, is the largest non-coding region and is located between tRNA-
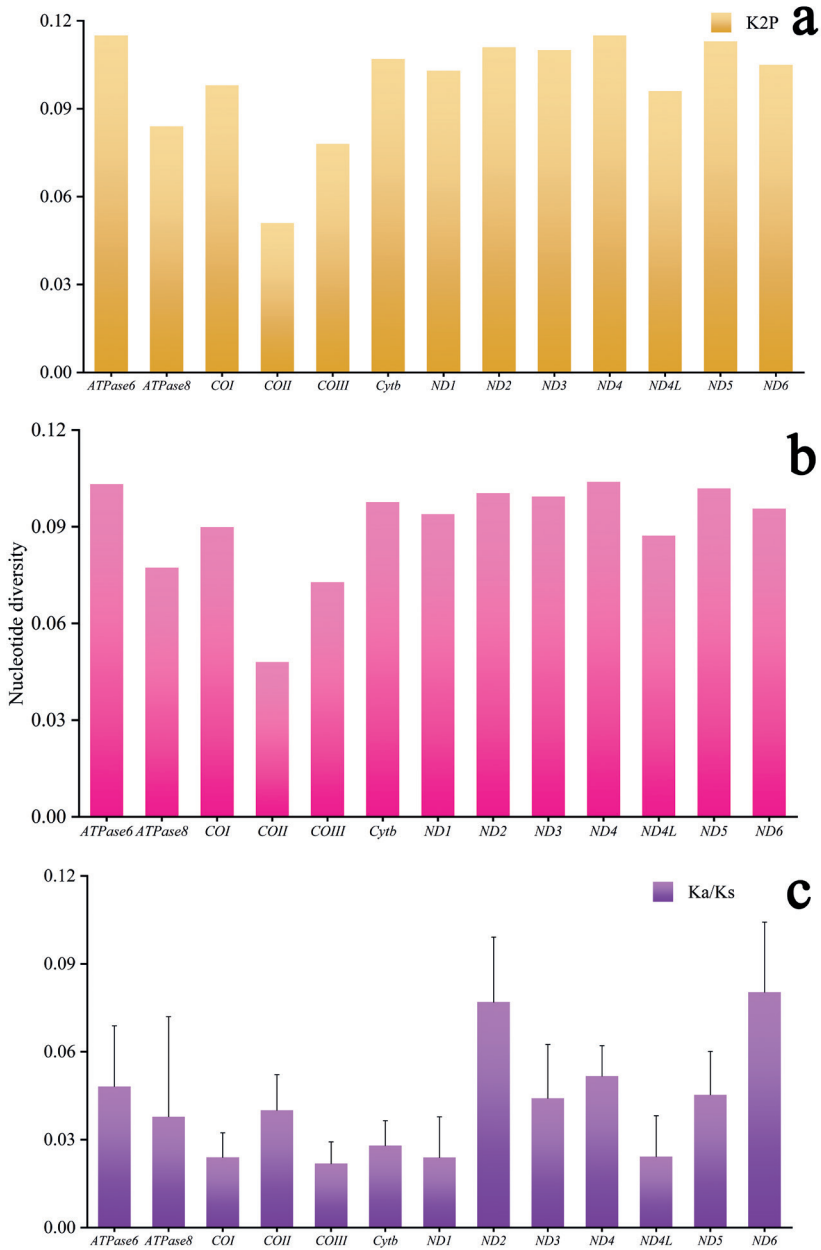
**Figure 2.** K2P genetic distance **a** nucleotide diversity **b** Ka/Ks ratio **c** analyses of protein-coding genes among the eleven *Corydoras* mitogenomes.

Pro and tRNA-Phe in these mitogenomes. Compared with PCGs, the D-loop displayed a higher mutation rate and the highest variation throughout the mitogenome; thus, this region is dominant and can be used to evaluate intraspecies variations. The

D-loops in the eleven species of *Corydoras* were 718−1,218 bp. Compared with the other four regions (entire genome, PCGs, tRNAs, and rRNAs), the control region showed the highest A+T content, ranging from 66.77% to 71.87%. Like the rRNAs, all AT-skew values were positive, and all GC-skew values were negative.

## Phylogenetic analysis

To determine the phylogenetic relationships within the suborder Loricarioidei and family Callichthyidae, we obtained the concatenated nucleotide sequences of 13 PCGs and two rRNAs from 42 species of Loricarioidei. Phylogenetic analyses based on both ML and BI methods revealed same topologies, which also generally agreed with those presented in previous studies (Alexandrou et al. 2011; Lujan et al. 2015; Moreira et al. 2017; Roxo et al. 2019) (Figs 3, 4). These analyses confirmed that the genus *Corydoras* was part of the monophyletic family Callichthyidae.

Both Callichthyidae and Loricariidae were recovered as monophyletic with very high support values (BI posterior probabilities, PP = 1; ML bootstrap, BS = 100). The 44 species of Siluriformes were divided into four major clades corresponding to the families Siluridae Callichthyidae, Trichomycteridae, and Loricariidae. The target
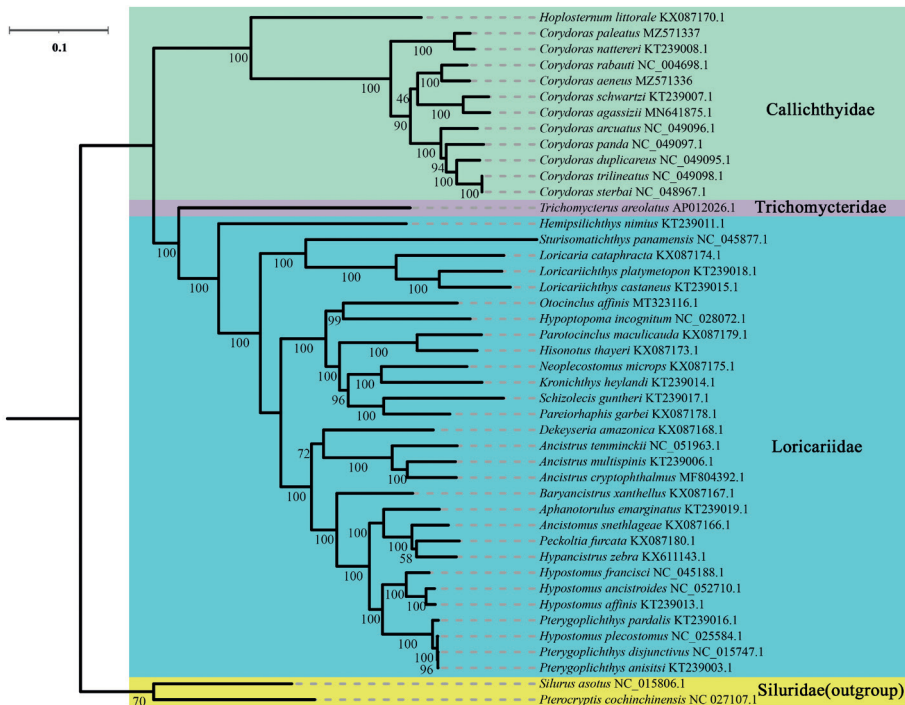


**Figure 3.** Phylogenetic trees of 44 Siluriformes species using concatenated nucleotide sequences of 13 protein-coding genes and two rRNAs using the maximum likelihood method. Numbers in the ML tree represent SH-aLRT support/ultrafast bootstrap support values.
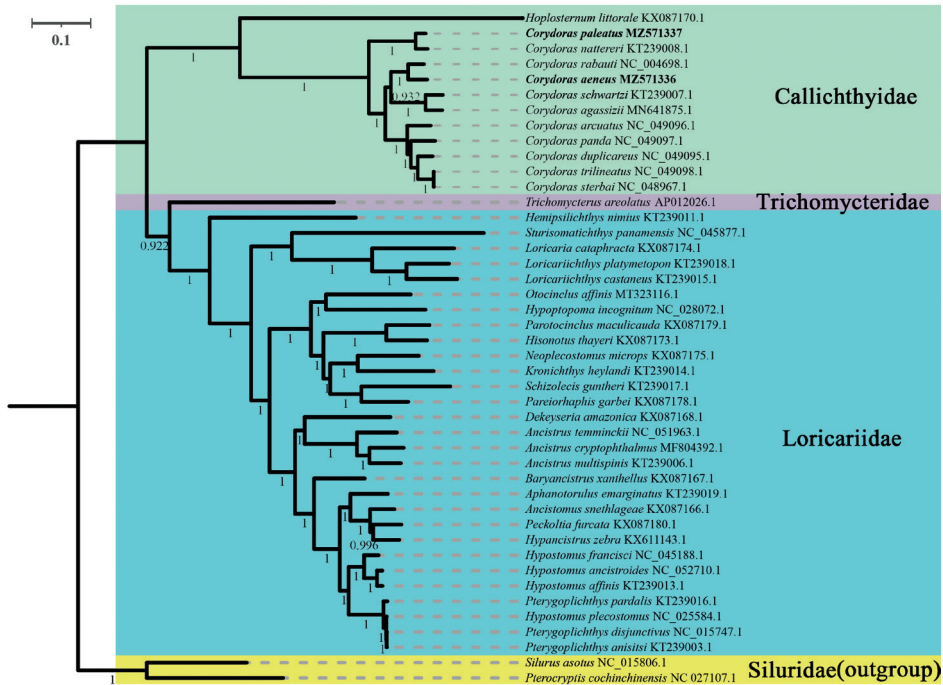
**Figure 4.** Phylogenetic tree of 44 Siluriformes species using concatenated nucleotide sequences of 13 protein-coding genes and two rRNAs via the Bayesian interference method. Applicable posterior probability values are shown.

species *C. aeneus* and *C. paleatus* were clustered into two clades (*C. aeneus* + *C. rabauti*) and (*C. paleatus* + *C. nattereri*) with a high nodal support value (PP = 1; BS = 100). The eleven species of the genus *Corydoras* clustered together quite well [(((*C. aeneus* + *C. rabauti*) + (*C. schwartzi* + *C. agassizii*)) + (*C. arcuatus* + (*C. panda* + (*C. duplicareus* + (*C. sterbai* + *C. trilineatus*))))) + [(*C. paleatus* + *C. nattereri*)]. *Corydoras trilineatus* and *C. sterbai* have short, almost non-existent branch lengths; thus, they are likely the same species. The K2P genetic distances of these two species are 0.000 (Suppl. material 1: Table S3), which verifies that they are the same species. This may be caused by incorrect identification, taxonomic problems (these two species are, in fact, synonymous), and/ or introgressive hybridization. Moreover, in the family Loricariidae, the genera *Ancistrus* and *Loricariichthys* were clustered into monophyletic clades [(*A. cryptophthalmus* + *A. multispinis*) + *A. temminckii*] and (*L. castaneus* + *L. platymetopon*) with a high nodal support value (PP = 1; BS = 100). There was a paraphyletic relationship between the genera *Hypostomus* and *Pterygoplichthys*, [*H. francisci* + (*H. ancistroides* + *H. affinis*), *P. pardalis* + (*H. plecostomus* + (*P. anisitsi* + *P. disjunctivus*))]. Our results demonstrate that the concatenated nucleotide sequences of the 13 PCGs and two rRNAs were useful for determining the phylogenetic relationships of the order Siluriformes. These results can be used to improve classification of the families Callichthyidae and Loricariidae.

## Conclusions

Using next-generation sequencing methods, the complete mitogenomes of the bronze *C. aeneus* and peppered *C. paleatus* were analyzed and compared with those of nine members of *Corydoras*. The complete mitogenomes of *C. aeneus* and *C. paleatus* comprised 16,604 and 16,593 bp, respectively. The two mitogenomes had high A+T contents (58.52% in *C. aeneus* and 58.23% in *C. paleatus*), a phenomenon that agrees with the typical base bias of ichthyic mitogenomes. Our results indicate that the mitogenome features, including genome size, gene content, and gene arrangement, in *Corydoras* are highly conserved. Phylogenetic analysis was performed with 42 species of Loricarioidei and two outgroup species. These analyses confirmed the occurrence of the genus *Corydoras* within the monophyletic family Callichthyidae. The complete mitogenome information, including the gene content, gene orders, genome structure, base compositions, evolutionary features, codon usage, gene arrangement, and phylogenetic analyses, provides a basis for future studies on the population genetic and evolution of *Corydoras* and related groups.

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## Supplementary material 1

**COI sequences of *Corydoras aeneus* and *C. paleatus* Tables S1–S4, Figs S1–S4**

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Explanation note: COI sequences of *Corydoras aeneus* and *C. paleatus*. Table S1. Best substitution models for Bayesian inference (BI) and maximum-likelihood (ML) analyses. Table S2. Summarized mitogenomic characteristics of the eleven *Corydoras* species investigated in this study. Table S3. The K2P genetic distances of the eleven mitogenomes of *Corydoras*. Table S4. Start and stop codons of protein-coding genes in the eleven *Corydoras* mitogenomes. Figure S1. Gene orders of mitogenomes of the studied species. Figure S2. Relative synonymous codon usage of 13 protein-coding genes in the mitogenomes of eleven *Corydoras* species. Figure S3. Codon usage patterns of eleven *Corydoras* mitogenomes. Figure S4. Secondary structures of tRNA-Ser(GCT) in the two newly sequenced *Corydoras* species.

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