

# TESTING THE EFFECTIVENESS OF A CYTOCHROME C OXIDASE SUBUNIT 1 BARCODE REFERENCE LIBRARY BASED ON VOUCHER SPECIMENS OF AN ENTIRE RIVER DRAINAGE IN THE NEOTROPICS

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## RESUMO

There is a growing trend toward producing barcode libraries as references in biodiversity studies, once it allows for applications in various areas of research. The Tramandaí River basin is home to 102 reported fish species, with a notable difference in species composition between the ichthyofauna of the highland rivers and those found in the lowlands. We conducted a DNA barcoding analysis of the Tramandaí River basin, including 93 freshwater fish species (92% of the composition). We recovered 596 base pairs from 570 samples. All data were obtained from DNA extracted from voucher specimens that are permanently available for review in scientific collections. Among the 93 species analyzed, barcode data allowed for the individual identification of 95% of the species. Exceptions included the three species of *Odontesthes* and the two species of *Pareiorhaphis*. If the 2% genetic distance for species recognition (BOLD initiative) is applied, only 81 species are recovered, excluding the two species of *Microglanis* and five species of the *Astyanax-Psalidodon* clade. The highest intraspecific p-distance was 0.04 in *Oligosarcus robustus* and 0.02 in *Gymnogeophagus rhabdotus* and *Loricariichthys anus*, suggesting the presence of cryptic species. Our barcode analysis led to the discovery of two species not previously recorded in the area, *Hoplisoma longipinne* and *Hoplias misionera*, thereby extending the distribution of these species to a coastal river drainage. In conclusion, the lack of divergence in COI sequences cannot be considered the sole criterion for species recognition. Barcode inventories are valuable for: uncovering hidden diversity and providing records of previously undetected species; for detecting potential cases of cryptic species for further investigation; in provide initial information on population structure and candidate species for further phylogeographic studies. All these advantages of barcode inventories are

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valid only when supported by accurate taxonomic identification of samples based on voucher specimens deposited in permanent collections.

**Palavras-chave:** Fish species, Collection, COI, BOLD, Rio Grande do Sul.