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TITLE

GENETIC DIVERGENCE AND SPECIES DELIMITATION IN BIOMPHALARIA SNAILS USING COI AND ITS1 MARKERS: A MULTI-ALGORITHM APPROACH5

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ABSTRACT

The genus Biomphalaria contains medically significant snail species that serve as intermediate hosts for Schistosoma mansoni and exhibits complex patterns of genetic divergence and speciation. Morphological identification methods struggle to distinguish cryptic species, making molecular techniques essential for accurate species-level classification. Reliable species identification is essential for understanding schistosomiasis epidemiology, improving disease surveillance and biodiversity conservation efforts. We aim to assess genetic divergence among Biomphalaria populations using COI and ITS1 markers and delimit operational taxonomic units (OTUs) to identify potential cryptic species and genetic lineages. We constructed a database by collecting sequences from NCBI, and snails from the Fiocruz-MG Medical Malacology Collection and the UNM Museum of Southwestern Biology. DNA was extracted, COI and ITS1 genes were partially sequenced, aligned using MAFFT, and phylogenetic relationships were reconstructed using maximum likelihood and Bayesian inference. Identification and delimitation of species were performed using DNA barcoding methodology, applying the BOLD Identification Criteria and Best Close Match approaches, and five species delimitation algorithms: GMYC, bPTP, ABGD, ASAP, and haplotype networks (TCS). Divergence metrics were calculated for each species using DnaSPv.6. Integrating morphological identification, PCR/RFLP analyses and phylogenetic reconstructions, 29 of the 415 COI sequences required taxonomic revisions. Phylogenetic reconstructions resolved well-supported clades for most species, except for the cryptic species pairs B. choanomphala/B. sudanica, B. straminea/B. kuhniana, B. cousini/B. amazonica, and B. peregrina/B. oligoza, which BOLD and BCM analyses also failed to distinguish. In the genetic divergence analyses, both markers corroborated the patterns of genetic proximity observed within the cryptic species complexes. In pairwise comparisons between species, B. schrammi had the highest average nucleotide divergence, suggesting significant evolutionary isolation. Species delineation into OTUs revealed multiple groups within each nominal species, suggestive of high intraspecific diversity and complexity not fully recognized by traditional morphological taxonomy. The presence of mixed groups containing sequences from closely related species suggests the possibility of hybridization or ongoing gene flow between these species. Although the bPTP algorithm identified 43.8% of the groups with medium to high statistical support (>0.7), the mixed-species groups tended to have lower support values. TCS analyses revealed haplotype exclusivity for most species, but also indicated potential hybridization. Our multi-algorithm approach reveals the complex patterns of genetic diversity and potential presence of cryptic species in the genus underscoring the need for integrative taxonomic revisions that combine multiple approaches.

KEYWORDS

Biomphalaria; Schistosomiasis; Genetic Divergence; Cryptic Species; Molecular Taxonomy.

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