Conference Abstract

DNA barcoding in recognition of *Gammarus* flock diversity and distribution in the ancient Lake Ohrid

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Abstract

Lake Ohrid, located on the Balkan Peninsula at the Albanian-Macedonian border, is the oldest European lake (1.3-1.9 My old) and one of the world's smallest ancient lakes. Taking into account the size of the lake and its biodiversity, it harbors the highest level of endemism, especially within amphipod crustaceans (ca. 90%) with the endemic *Gammarus* species flock. Our previous studies upon this flock have shown a substantial decoupling between molecular and morphological diversity, existence of cryptic species and puzzling speciation history. In order to explore sources of observed diversity, in the current study we are investigating ecological preferences of the species within the flock, based on their distribution in depth gradient, in relation to molecular diversity based on DNA barcoding.

In the study over 200 barcodes were generated and combined with 173 previously published. The specimens were collected from all depth ranges of Lake Ohrid as well as from springs located on or near the banks of the lake.

Within the species flock, 13 BIN's were identified, 12 previously known and one newly recognized, representing separate lineage and putatively a new species. Two of the flock species were found only in the springs: *G. sketi* and *G. cryptosalemaai*. *G. sketi*, previously

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found only in springs on the southern banks of Lake Ohrid, has now also been discovered in springs in its north-eastern part. Both species show low haplotypic diversity. All remaining species were recorded from the depth between 20 and 60 meters, that is characterized by the highest ecological diversity with different types of substrates: stones, macrophytes, abundant Dreissena shells as well as sand and silt. Among them G. sywulai, G. macedonicus, G. cryptoparechiniformis, G. lychnidensis, G. ochridensis, G. parechinifromis were found exclusively within this depth range. The three latter species represent single BIN and share haplotypes, at the same time this BIN has the highest number of haplotypes in comparison to others. The remaining species found on this depth represent separate BINs with different levels of haplotype diversity. Only G. lychnidensis, G. stankokaramani and G. solidus were found below the depth of 60 meters, in a quite homogenous environment dominated by silt. In the deepest parts of the lake, between 260 and 290 meters, only G. solidus was found. This species is represented only by three haplotypes while G. stankokaramani is characterized by multiple haplotypes partially shared with G. lychnidensis. The shared haplotype represents the only G. lychnidensis occurrences on the depths below 60 meters.

Summarizing, the highest abundance of BINs, species and haplotypes was recovered from the most ecologically diversified depth range of the lake (20 to 60 meters). This suggests that ecological heterogeneity could be the main driver of *Gammarus* species flock diversification in the ancient Lake Ohrid. Due to the complex pattern of morphological diversity, DNA barcoding proved to be the best if not the only method in identification of the species flock diversity.

Keywords

Amphipoda - cox I - cryptic species - DNA barcoding - Gammarus - ancient lake

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