



Updating the description and taxonomic status of *Brachionus sessilis* Varga, 1951 (Rotifera: Brachionidae) based on detailed morphological analysis and molecular data

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Abstract

Brachionus sessilis Varga, 1951 is an epizoic rotifer living exclusively on cladocerans of the genus *Diaphanosoma*. Current taxonomic knowledge relies solely on limited morphological information, whereas there is no type material. Here, we aim to resolve issues concerning its morphology and taxonomy using both morphological and genetic characters on material sampled from Lake Balaton (Hungary), as well as Lake Doirani (Greece) that was selected for comparison purposes. Biometrical analysis was based on extensive lorica measurements. Phylogenetic reconstruction was based on DNA sequence information of the mitochondrial cytochrome c oxidase subunit I (COI) and 16S rRNA gene regions as well as of the nuclear internal transcribed spacer 1 (ITS1). Well-supported evidence for substantial differentiation of *B. sessilis* from its closest phylogenetic relatives supports its species-rank status. Our phylogenetic analysis suggests a highly supported clade encompassing *B. sessilis* and another epizoic rotifer, namely *B. rubens*.

Key words: taxonomy, species delimitation, DNA barcoding, biometry, *Brachionus*, epizoism, phylogenetic trait conservatism

Introduction

Rotifera is a phylum of microscopic organisms commonly found in freshwater environments throughout the world (Segers 2007). *Brachionus* Pallas, 1766 is one of the most speciose genera of the phylum (Ahlstrom 1940), largely known for its use in aquaculture as food to fish larvae (Lubzens 1987). Recognizing species boundaries in *Brachionus* rotifers has proven to be a challenging task even in routine microscopic observations. On the one hand, *Brachionus* species are renowned for their great phenotypic variability which has been partly attributed to a high degree of plasticity (Segers 2007). As a consequence, markedly different morphological variants can be found within the same species (Ahlstrom 1940). On the other hand, remarkable interspecific similarity also exists as in the case of the *B. plicatilis* complex of cryptic species in which several morphologically similar albeit phylogenetically distinct lineages have been identified (Gómez *et al.* 2002; Suatoni *et al.* 2006). These characteristics, along with other taxonomic difficulties typical of the phylum, such as the dearth of taxonomically important morphological characters (Ahlstrom 1940), the deficiency of comprehensive descriptions including analysis of biometry or geometric morphometrics (Koste & Shiel 1989; Adams *et al.* 2004), the improper use of infraspecific rank names and a long list of synonyms (Harring 1913; Segers 2007) pose major taxonomic impediments to the taxonomists dealing with the systematics of *Brachionus* rotifers.

Accurate species delineation is fundamental in order to explain patterns of biological diversity, understand population genetic processes, detect ecological divergence and ultimately assess the ways in which ecosystems function. Traditional species delimitation has been based on morphological comparisons in which phenotypically

topotypic material and a formal description we also offer a basis on which the recognition and report of *B. sessilis* is facilitated.

Complementary to the biometrical measurements, this work uses molecular data (for the first time) to clarify previous taxonomic ambiguities regarding the taxonomic status of *B. sessilis* in relation to the *B. plicatilis* group and *B. urceolaris*. Strong support is obtained for substantial phylogenetic divergence between *B. sessilis* and *B. rotundiformis*, which once was considered as its closest relative (Varga 1951). In fact, *B. sessilis* clusters with high confidence outside the *B. plicatilis* complex of cryptic species (Fig. 3). Our results firmly indicate that *B. sessilis* is certainly not a subspecies of *B. urceolaris* (Fig. 3, Suppl. Figure 1, 2, 3, 4) but is rather to be treated as a distinct species, in concordance with the latest morphology-based taxonomic re-assessments (Segers 2007; Jersabek *et al.* 2012).

Our molecular data reveal *B. rubens*, also an epizoic rotifer (May 1989; Vanjare *et al.* 2010) as the closest relative of *B. sessilis*. Previously, *B. rubens* had also been classified as a variety *B. urceolaris* var. *rubens* (Koste 1978). However, the results of our study (Fig. 3, Suppl. Figures, Suppl. Tables 2, 3, 4) support that *B. sessilis* and *B. rubens* are strongly differentiated from each other as well as from their next closest relatives, *B. urceolaris* and *B. quadridentatus*. Consequently, we suggest that *B. sessilis* and *B. rubens*, should be treated as species-level taxa and not at sub- or infrasubspecific levels.

Interestingly, the closest relatives *B. sessilis* and *B. rubens* are both epizoic unlike their sister clade *B. urceolaris/B. quadridentatus* (Fig. 3). *B. rubens* is often found on the carapace of *Daphnia* species (May 1989). Other *Brachionus* rotifers are also known to be epizoic, for instance *B. caudatus* (Chandra & Kameswara 1976; Sharma 1979), *B. variabilis* (Ahlstrom 1940) and *B. novaezealandiae* (May 1989). These other taxa could not be incorporated into the combined dataset due to the lack of sequence data for the markers used. Regardless, the case of *B. sessilis* and *B. rubens* can be an example of phylogenetic trait conservatism – the tendency of closely related clades to preserve similar ecological traits (Cooper *et al.* 2010; Wiens *et al.* 2010) – with regard to epizoism. A recent study pointed at the eco-evolutionary implications of host-epibiont interactions showing that epizoism of *B. rubens* on *D. magna* can have considerable effects on life history traits of the host, promoting micro-evolutionary responses against rotifer infections (Pauwels *et al.* 2014). Under this perspective, phylogenetic trait conservatism offers a promising prism under which the addition of more taxa to the current dataset could provide valuable insights as to the ecological implications and the mode of evolution of epizoic life in *Brachionus* rotifers.

Conclusions

Our study resolves taxonomic uncertainties surrounding the species-rank status of *B. sessilis*. Sequence analysis of *B. sessilis* rotifers (for the first time) and other relatives provides evidence that *B. rubens*, also epizoic, is its closest phylogenetic relative, whereas *B. urceolaris* is placed as a sister clade to these two species. We present a more detailed description of the species using samples from Lake Balaton (Hungary) where it was originally described, and provide topotypic material to conclude the species description. The current work provides a robust scheme for species delineation and fills a critical gap in rotifer systematics.

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