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Integrative taxonomy allows the identification of synonymous species and the erection of a new genus of Echiniscidae (Tardigrada, Heterotardigrada)

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Abstract

The taxonomy of tardigrades is challenging as these animals demonstrate a limited number of useful morphological characters, therefore several species descriptions are supported by only minor differences. For example, *Echiniscus oihonnae* and *Echiniscus multispinosus* are separated exclusively by the absence or presence of dorsal spines at position B^d. Doubts were raised on the validity of these two species, which were often sampled together. Using an integrative approach, based on genetic and morphological investigations, we studied two new Portuguese populations, and compared these with archived collections. We have determined that the two species must be considered synonymous with *Echiniscus oihonnae* the senior synonym. Our study showed generally low genetic distances of *cox1* gene (with a maximum of 4.1%), with specimens displaying both morphologies sharing the same haplotype, and revealed character B^d to be variable. Additionally, a more detailed morphological and phylogenetic study based on the 18S gene uncovered a new evolutionary line within the Echiniscidae, which justified the erection of *Diploechiniscus* **gen. nov.** The new genus is in a sister group relationship with *Echiniscus* and is, for the moment, composed of a single species.

Key words: *Diploechiniscus* **gen. nov.**, *Diploechiniscus oihonnae* **comb. nov.**, DNA barcoding, 18S, phylogeny, morphology

Introduction

Currently the phylum Tardigrada comprises *c.* 1000 described species (Guidetti & Bertolani, 2005), with regular new additions. These microscopic metazoans have a limited number of taxonomically useful morphological characters. As a consequence, species descriptions are sometimes based on minor differences that are not always easy to confirm. Only recently has α -taxonomy been combined with genetic data (Guidetti *et al.*, 2005, 2009; Møbjerg *et al.*, 2007; Jørgensen *et al.*, 2007, 2011; Cesari *et al.*, 2009, 2011; Guil & Giribet, 2009; Schill *et al.*, 2010; Bertolani *et al.*, 2010, 2011a, 2011b).

An example of one of the minor morphological differences that has separated two species can be found in the absence or presence of a spine at position B^d (for a schematic representation of spines and appendices in several Echiniscidae, see Fig. 1), which differentiates *Echiniscus oihonnae* Richters, 1903 from *Echiniscus multispinosus* Cunha, 1944b within the heterotardigrade genus *Echiniscus* (for the classification of dorsal plates, spines and filaments see also Ramazzotti & Maucci, 1983 and Kristensen, 1987). In describing *E. multispinosus*, Cunha (1944b) also noted a difference in size, i.e. slightly smaller dimensions with respect to *E. oihonnae*. Moreover, specimens have been reported with spine B^d on only one side (found in Norway moss) together with numerous