

## DNA barcoding and integrative taxonomy of *Macrobiotus hufelandi* C.A.S. Schultze 1834, the first tardigrade species to be described, and some related species

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### Abstract

Within the framework of a DNA barcoding project on tardigrade species, a study was carried out on *Macrobiotus hufelandi* C.A.S. Schultze 1834, the first formally described tardigrade species. We used samples collected from the type locality and additional material from other European sites containing species of the “*M. hufelandi* group”. The study was performed by integrating morphological, karyological and molecular (mt-DNA *cox1*) information and comparing these data with morphological data from the type material. Several species from this group were found in the type locality of *M. hufelandi* (near Freiburg, Black Forest, Germany) and these were all barcoded. One was *M. hufelandi*, the other two were: *Macrobiotus sandrae* Bertolani & Rebecchi 1993 (originally described from the same locality), and *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari in press (type locality Andalo, Italy), all with interspecific genetic distances of more than 19%. A fourth cryptic species, which had the same morphology as *M. hufelandi* but a genetic distance of 6.7%, was not described as a new taxon but named *M. cf. hufelandi* sp.1 for this study. *Macrobiotus sandrae* and *M. vladimiri* were also present (and barcoded) in Italy (Alps). Additional individuals (animals and eggs) were also found, and barcoded, in Italy (Apennines) and Switzerland that belonged to the haplogroup *Macrobiotus cf. hufelandi* sp. 1. These data together with other recent studies on tardigrade DNA barcoding represent a starting point for further studies on tardigrade biogeography, phylogeography and diversity.

**Key words:** tardigrades, *Macrobiotus sandrae*, *Macrobiotus vladimiri*, DNA, *cox1*

### Introduction

In the first half of the 19<sup>th</sup> century Carl August Sigismund Schultze (1834) provided the first formal description of a tardigrade species. He dedicated that species to Christoph Wilhelm von Hufeland, the inventor of the term “Makrobiotik”, naming it *Macrobiotus Hufelandii*. Currently, according to the International Zoological Nomenclature Code (1999), the species must be indicated as *Macrobiotus hufelandi* C.A.S. Schultze, 1834 (including the initials of the authority name, because M. Schultze also exists as an authority in describing tardigrades). C.A.S. Schultze (1834) described and illustrated only the adult animal and did not include or observe eggs. The egg morphology was later described and illustrated by Doyère (1840). This species is still valid and probably the most cited among tardigrades, even though, in our opinion, very often without sufficient in-depth taxonomic analysis. In the last fifty years several similar species have been described and grouped in the so-called “*Macrobiotus hufelandi* group”. As a matter of fact, it is not a well defined group, but it certainly includes *Macrobiotus* specimens with two (rarely three) macroplacoids and one microplacoid, and eggs with inverted goblet-shaped processes, or similar. Some species of this group have been examined in depth from a morphological and morphometric point of view (Biserov 1990a, b; Bertolani & Rebecchi 1993), considering both animals and especially the ornamented egg shell morphology. In particular, Bertolani & Rebecchi (1993), examining new material from mosses collected at the type locality of *M. hufelandi* (near Freiburg, Black Forest, Germany), and namely by St. Ulrich, re-described the species and defined a neotype from that locality. From this same locality they described a second species from the same group, *Macrobiotus sandrae* Bertolani & Rebecchi 1993.