



An example of problems associated with DNA barcoding in tardigrades: a novel method for obtaining voucher specimens

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Abstract

We have in recent papers revealed that an integrative taxonomy approach helps to solve taxonomic problems in tardigrades. However, whole tardigrades are required for DNA work, which leaves no hologenophore voucher specimens with adult morphology. Using a novel methodology for the Tardigrada, we introduce the practice of collecting high quality maximum magnification light microscopy images of recently thawed animals to act as hologenophore voucher specimens of animals later used for DNA barcode sequencing. Within the framework of a DNA barcoding project on tardigrades, we collected a moss sample from the type locality of *Macrobiotus terminalis* Bertolani & Rebecchi, 1993 (Castelsantangelo, Central Apennines, Italy), a species of the “*Macrobiotus hufelandi* group”. Within the moss sample we found several animals and eggs with a morphology that corresponded to the original description of *M. terminalis*, while others were attributable to *Macrobiotus macrocalix* Bertolani & Rebecchi, 1993. In this study, molecular (*cox1* mtDNA) analyses demonstrated no intraspecific variability in *M. terminalis* from the type locality but very large interspecific differences when compared with *M. macrocalix* and GenBank data for other species within the *M. “hufelandi* group”. There was also a large difference between our *M. terminalis* sequences and the GenBank data of a specimen attributed to the same species. The GenBank sequence originated from a population in the Northern Apennines, whose morphology appeared to be like that of the specimens of the *locus typicus*. This confirmed the importance in utilising material from the type locality for linking molecular data to the species’ morphological characters. Our paper underlines the importance of an integrative taxonomy in species diagnoses and demonstrates a scenario where morphological observations alone are not always sufficient. Lastly, this work adds reliable information to the sequence reference library that provides a useful building block for further studies on similar and related tardigrade taxa.

Key words: Tardigrada, *Macrobiotus terminalis*, *cox1*, integrative taxonomy

Introduction

DNA barcoding is a technique used to discriminate biological entities through the analysis of a short fragment of the mitochondrial cytochrome c oxidase subunit I (*cox1*). It relies on the assumption that the genetic distance between species exceeds the variation scored within species (Hebert *et al.* 2003), and is especially useful in establishing a species-level identification by comparing newly analysed specimens with a reference library of sequences of known species (Casiraghi *et al.* 2010). There is a growing interest in DNA barcoding in the scientific community (Mitchell 2011), even though controversy still arises regarding barcoding and taxonomy (for example see Ebach 2011). It is important to note that DNA barcoding does not focus on building a tree-of-life or on doing DNA taxonomy, but rather it focuses on producing a universal molecular identification key based on strong taxonomic knowledge that should be embedded in the barcode reference library. In order to gain more comprehensive taxonomic information, it is now common practice that both morphological and molecular data will be simultaneously analysed, developing what has been coined as “integrative taxonomy” (Teletchea 2010).

In the phylum Tardigrada, DNA barcoding represents a recent approach for helping to solve taxonomic problems, and within the framework of the “MoDNA project” (involving the DNA barcoding of tardigrades) we are combining this with morphological data to form an integrative taxonomy of tardigrades. To date we have considered a number of species from the so-called “*Macrobiotus hufelandi* group”, focusing our attention both on the