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An integrative description of *Macrobotus paulinae* sp. nov. (Tardigrada: Eutardigrada: Macrobiotidae: *hufelandi* group) from Kenya

DANIEL STEC¹, RADOSLAV SMOLAK², ŁUKASZ KACZMAREK^{3,4} & ŁUKASZ MICHALCZYK^{1,*}

¹Department of Entomology, Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland

²Department of Ecology, Faculty of Humanities and Natural Sciences, Presov University, 17 Novembra 1, 081 16 Presov, Slovakia

³Department of Animal Taxonomy and Ecology, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61-614 Poznań, Poland

⁴Prometeo Researcher, Laboratorio de Ecología Natural y Aplicada de Invertebrados, Universidad Estatal Amazónica, Campus Principal Km 2.1/2 via a Napo (Paso Lateral) Puyo, Pastaza, Ecuador

*Correspondence: E-mail: LM@tardigrada.net

Abstract

In this paper we describe *Macrobotus paulinae*, a new species of the *hufelandi* group from the Kenyan highlands. In addition to the traditional taxonomic description, aided with morphometrics as well as light and scanning microscopy imaging, we also provide nucleotide sequences of three nuclear (18S rRNA, 28S rRNA, ITS-2) and one mitochondrial (COI) DNA fragment of the new species. The sequences allowed not only a more accurate description but also provided an independent verification of the taxonomic status of *Ma. paulinae* sp. nov. Such integrative approach requires a considerable number of individuals and eggs, which we have partially subsidised by employing an *in vitro* culture of the new species. Our analyses revealed that *Ma. paulinae* sp. nov. is most similar to *Macrobotus madegassus* Maucci, 1993 and *Macrobotus modestus* Pilato & Lisi, 2009, however it differs from these species, as well as from all other known species of the *hufelandi* group, by the presence of seven paired dorso-lateral patches of cuticular granulation and the presence of chorionic filaments growing out of terminal discs of egg processes. *Macrobotus paulinae* sp. nov. is an example of a species with a miniaturised buccal apparatus (*i.e.* with reduced peribuccal lamellae and oral cavity armature, and stylet supports inserted on the buccal tube more anteriorly than in typical *Ma. hufelandi* group species), and it therefore resembles two recently described two-macropalcoided *Minibiotus* species: *Mi. acadianus* Meyer & Domingue, 2011 and *Mi. julianae* Meyer, 2012. The re-examination of the type material for these two species confirmed that they are equipped with peribuccal lamellae and therefore we transfer them to the genus *Macrobotus*, specifically to the *hufelandi* group.

Key words: 18S rRNA, 28S rRNA, barcoding, COI, *in vitro* culture, DNA extraction protocol, ITS-2, *Macrobotus madegassus*, *Macrobotus modestus*, *Macrobotus acadianus* comb. nov., *Macrobotus julianae* comb. nov., *Minibiotus*, taxonomy

Introduction

The Phylum Tardigrada consists currently of *ca.* 1,200 taxa (Guidetti & Bertolani 2005; Degma & Guidetti 2007; Degma *et al.* 2009–2015) that inhabit terrestrial and aquatic (both freshwater and marine) environments throughout the world (Nelson *et al.* 2015). Although, first studies on African tardigrades were conducted over a hundred years ago (Murray 1907, 1913), until now only 46 papers were published and *ca.* 160 tardigrade taxa (Kaczmarek *et al.* 2006) reported from this continent. In fact, the tardigrade fauna in the majority of the African regions/countries is completely unknown. Tardigrades from Kenya (formerly reported as part of the British East Africa, BEA) have been recorded on four occasions, by Murray (1913), Haspelslagh (1982), De Smet & Balfort (1990), and Schill *et al.* (2010). Murray (1913) found four species in the Kikuyu region and another twelve taxa from undefined localities in the BEA, whereas Haspelslagh (1982) reported eight species from water bodies on the Mt. Kenya. De Smet & Balfort (1990) recorded three species on the Mt. Kenya, however none were new records for the country. The most recent report, by Schill *et al.* (2010), describes a single new *Paramacrobotus* species.