



# Molecular and morphological evidence for two new species of terrestrial planarians of the genus *Microplana* (Platyhelminthes; Turbellaria; Tricladida; Terricola) from Europe

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## Abstract

Three species of terrestrial planarian were found in a garden in Yorkshire, *Microplana scharffi* (Graff 1896), *M. terrestris* (Müller 1774) and a third *Microplana* species similar in size and shape to *M. terrestris* but brown (khaki) rather than grey/black. Mitochondrial cytochrome oxidase I sequences show that the three are distinct species, but that the sequences of the khaki specimens are identical with a GenBank sequence under the name "*Microplana terrestris*". Photomicrographs of the copulatory apparatus of several specimens of each species, including type material, are presented which confirm that the three species are distinct and show the variation within each species. The khaki species is named as *Microplana kwiskea* n. sp. Specimens from Spain relating to the GenBank "*Microplana terrestris*" sequence have been examined and are not of that species, nor are they *M. kwiskea*. They are considered to be a further species, *M. groga* n. sp.

**Key words:** Cytochrome oxidase I; *cox1*; COI; *Microplana scharffi*; *Microplana groga*; *Microplana kwiskea*; *Microplana terrestris*; Rhynchodemidae; Microplaninae

## Introduction

During the course of observations in a domestic garden in Yorkshire, UK, three species of terrestrial planarian were found (McDonald and Jones 2007). Two of these, *Microplana scharffi* (Graff 1896) and *M. terrestris* (Müller 1774), are well known and widely distributed across Europe (Minelli 1977; Jones 1998; 2005). *Microplana scharffi* (Fig. 1a) is up to 7 cm long. Its colour varies, dependent on recent prey, from cream/yellow to pink or even greyish, with a small dark patch anteriorly (McDonald and Jones 2007). *Microplana terrestris* (Fig. 1b & c, lower) is up to about 2 cm long and is normally uniformly dark grey, almost black, dorsally and laterally but with a pale ventral creeping sole. The third species found is of similar size and shape to *M. terrestris* but brown (khaki) (Fig. 1b & c, upper) and was referred to as "khaki *Microplana*" by McDonald and Jones (2007). Also found were specimens again similar to *M. terrestris* but with non-uniform "granular" dark and pale grey pigmentation (Fig. 1d). Both the khaki and granular specimens were clearly distinct from *M. scharffi*, but we were unsure whether they were colour variations of *M. terrestris* or different species. Other brown species have been previously described from Europe, e.g. *Rhynchodemus henrici* Bendl 1908, *R. attemsi* Bendl 1909 (these were synonymised by Minelli 1977) and *R. carli* Fuhrmann 1914. The GenBank sequence under the name "*M. terrestris*" originates from one of the "reddish to brown" specimens found near Barcelona, Spain, and identified as *M. terrestris* by Mateos *et al.* (1998). One of us (HDJ) had earlier collected similar khaki specimens from other localities (near Southport, Lancashire, the site of the studies in Jones *et al.* 1998; 2001, and in Manchester) but they had not been identified.

In order to identify the khaki and granular specimens, specimens of these and of *M. scharffi* and *M. terrestris* were collected from the same site for comparative purposes. Taxonomy and identification of this group is classically based on general morphology but particularly that of the copulatory apparatus. Thus specimens were partially sectioned to compare morphological details with published descriptions and with type material. The remaining portions of the same specimens were used for DNA extraction to compare with each other and with existing sequences in GenBank. The use of the same specimens for morphological and molecular studies ensures that we can be certain of our identification and the attribution of the genetic sequences. Since the main issue was the identity of *M. terrestris* and the only available GenBank sequence apparently of that species (AF178318) is the mitochondrial cytochrome oxidase 1 (*cox1* or COI) sequence, that sequence was determined for each of our specimens. One problem with type material, published descriptions and GenBank data is that most are based on a single specimen. Obviously there is a single holotype (or neotype) and there may or may not be further paratype material. The majority of GenBank sequences (at least for the Platyhelminthes) are from a single specimen of each species. Thus there is little or no information on individual variation within a species for either the morphology (which may in any case vary with maturity, fixation and storage) or the