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ScienceDirect
Zoologischer Anzeiger 248 (2009) 101–118
Zoologischer Anzeiger
www.elsevier.de/jcz

Description of a neotype for *Asellus aquaticus* Linné, 1758 (Crustacea: Isopoda: Asellidae), with description of a new subterranean *Asellus* species from Europe

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Received 3 March 2009; accepted 3 March 2009
Corresponding Editor: S. De Grave

Abstract

Asellus aquaticus is one of the most common and well-studied freshwater macroinvertebrates in Europe, but its current taxonomic description is inadequate. Therefore, a neotype is designated and described to allow a comparison with a newly described and illustrated species, *Asellus kosswigi* sp. n. While several troglomorphic *Asellus* species are known from Japan, this is the first subterranean species of the genus in Europe. It is morphologically, as well as genetically, distinct from all other, local, surface and subterranean populations. Its species status is confirmed by its syntopic occurrence with *Asellus aquaticus* without any sign of gene flow.

Keywords: Asellidae; *Asellus aquaticus*; Neotype; *Asellus kosswigi* sp. n.; Speciation; Phylogeny; Morphology; Troglobiont

1. Introduction

Although *Asellus aquaticus* Linnaeus, 1758 is one of the most common and widespread freshwater crustaceans in Europe there is no accurate description of the species available. The original description of '*Oniscus aquaticus*' was "*Oniscus cauda rotundata, stilis bifurcis*" from "*aquis puris*". Racovitza (1919) provided a new but still incomplete description, based on material from France, Great Britain and Slovenia. Since then many authors have tried to clarify the systematics of *A. aquaticus*; with all references until 1951 summarized by Gruner (1965, p. 96). Because morphometric data, as well as data about the morphological variation within the species were inadequate in previous descriptions, a re-analysis of the name-bearing type appears necessary in order to provide a more objective description. However, the type material from Linnaeus, appears to be lost, as is any specific information on the type locality. Our search for the name-bearing specimen was in vain, it not being located in the Linnaeus collection in the Museum of Evolution (Uppsala), nor the Swedish Museum of Natural History (Stockholm). Neither was it present in Racovitza's collection, kindly provided to us by G. Magniez. Therefore it is imperative to designate a neotype for this important taxon, the surrounding area of Uppsala being the historically most justified locality.

Several troglomorphic species in the genus *Asellus* Geoffroy, 1762 are known all described from Japan, a centre of speciation of this genus (Matsumoto 1963; Henry and Magniez 1970). As all these 12 endemic species are subterranean, this high level of endemism can be attributed to the reduced dispersal abilities associated

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doi:10.1016/j.jcz.2009.03.001

with this mode of life (Trontelj et al. 2008). In contrast, all European populations are believed to belong to a single species (Birštejn 1951). *A. aquaticus* inhabits a wide array of different freshwater habitats, including the subterranean (Sket 1994). Nevertheless, as the morphology of *A. aquaticus* is highly variable, many subspecies and forms have been described throughout its range (summarized in Sket 1994). While most of the range is inhabited by the nominotypical *A. a. aquaticus*, five additional subspecies have been described from the Dinaric Region of the western Balkan Peninsula (Racovitza 1925; Karaman 1952; Sket 1965). These subspecies predominantly inhabit surface waters of isolated karst basins. Only *A. aquaticus cavernicolus* Racovitza, 1925 and *A. a. cyclobranchialis* Sket, 1965 from the northwestern Dinaric Region are subterranean and highly (the former) or slightly (the latter) troglomorphic (Sket 1994). Previous molecular studies have dealt with their relationship to surface populations (Verovnik et al. 2003, 2004) and their origin (Verovnik et al. 2005).

The troglomorphic population living in the subterranean part of the river Reka/Timavo (cave Grotta di Trebiciano/Labodnica near Trieste/Trst in NW Italy) has been attributed to *A. a. cavernicolus* (Stammer 1932; Stoch 1984; Sket 1994), but was shown to be morphologically unique (Prevorčnik et al. 2004) and genetically distinct from other Dinaric Karst populations (Verovnik et al. 2003, 2004, 2005), on the basis of six specific RAPD fragments (Verovnik et al. 2003), and monophyly by nuclear and mitochondrial DNA sequences (Verovnik et al. 2004, 2005).

Troglomorphic individuals can be occasionally found in a resurgence of the Reka/Timavo River together with animals belonging to the nominotypical subspecies (Stoch 1984). After several attempts, specimens of both taxa were collected from the resurgence enabling testing for potential gene flow and/or reproductive isolation and description of a new subterranean species of *Asellus*.

2. Material and methods

2.1. Morphology

Body length, length and width of the head, pereomere V and the pleotelson were measured under a dissecting microscope, using an ocular micrometer. After dissection, pereopods I, IV and VII from one side of the body were heat-treated in a KOH solution, dyed with chlorazole black E and mounted in glycerine-gelatine on slides together with antennae I, II, pleopods, uropods and pleotelson, for examination and drawing under a compound microscope with camera lucida. Drawings were measured using a Genius graphic tablet (GT-1212B Series) and the Windows-supported program MERE (by G. Sket). Alternatively, specimens from Sweden were photographed and measured using a Sony DXC-390P digital camera mounted on a stereomicroscope or microscope (depending on the size of the structure), and measured with analySIS® 3.1. The remains of the dissected specimens were transferred to 70% ethanol for storage. In one of the specimens from both type localities, all appendages were dissected and prepared for drawing. All pereopods, as well as the trunk were heat-treated and dyed as described above, and then mounted in glycerine-gelatine on one slide, alongside the rest of dissected specimen (mouth appendages, antennae, pleopods, uropods).

In males of both species, 91 morphometric characters (listed in Prevorčnik et al. 2004, Appendix II) were recorded describing body proportions (trunk, appendages), characterization of cuticular and sense structures (number and length of spines, setae, aesthetascs). In antenna II only the last two articles of the antennal basis (fourth and fifth) were included in the total antennal length together with the length of the flagellum. In pereopods IV and VII, length of the coxa was not included in the total pereopod length. Additionally, pigmentation of the body and eyes was recorded, as well as the number of spines on the capitulum of pleopod II endopodite. In females, 9 morphometric characters describing body proportions (trunk, appendages), and the state of pigmentation were recorded.

A single reported value in the description is based on the holotype or neotype, a range of values (in parenthesis) refers to the males from the type material.

2.2. Molecular phylogenetic analysis

Sixteen population samples of *Asellus* were included in the molecular analysis (Table 1), including the neotype population of *A. a. aquaticus*, the newly described species *Asellus kosswigi*, *A. aquaticus* populations sampled from the Adriatic drainage, and populations from other parts of Europe. All material was collected with a dip net and preserved in 96% ethanol. *A. kosswigi* was collected from the type locality in the cave Grotta di Trebiciano/Labodnica in NW Italy and from the resurgence of the river Reka/Timavo (details in descriptive part) where two females were found among *A. a. aquaticus* specimens. *Asellus hilgendorfii* Bovallius, 1886 from Japan, *Baikaloasellus* sp. from Siberia, and *Proasellus coxalis* Dollfus, 1892 from Slovenia were used as out-groups for the phylogenetic analysis.

Total DNA was extracted from specimens preserved in 96% ethanol using the GenElute Mammalian Genomic DNA minprep kit from Sigma-Aldrich. For each of the 122 specimens (including the out-group taxa) an approximately 700 bp fragment of the first subunit of cytochrome oxidase mitochondrial gene (COI) was