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First records of Mermithidae (Nematoda) parasitic in Plecoptera

Abstract - The first record of Nematoda Mermithidae in adults and larvae of two *Protonemura* species (Plecoptera Nemouridae) and of *Isoperla rivulorum* (Pictet) (Plecoptera Perlodidae) from North Italy and Bavaria (Germany) is presented. The malformed genitalia of several parasitically castrated adults are described and illustrated.

Riassunto - *Primi reperti di Mermithidae (Nematoda) endoparassiti di Plecoptera.*
Viene segnalata per la prima volta la presenza di Mermithidae (Nematoda) nell'addome di adulti e di ninfe di due specie di *Protonemura* (Plecoptera Nemouridae) e di *Isoperla rivulorum* (Pictet) (Plecoptera Perlodidae), raccolte rispettivamente nell'Italia settentrionale ed in Baviera (Germania). I genitali malformati di alcuni adulti, deformità causate da castrazione parassitaria, sono descritti e illustrati.

Key-words: Parasitism, stoneflies, parasitic castration, Nematoda, malformation.

INTRODUCTION

The phylum Nematoda includes, besides free living species, a large share of taxa parasitic in plants or animals. Many of these parasites are serious pests of great economic and/or medical importance. In contrast, the Nematoda Mermithidae whose juvenile stages are insect parasites have been considered as agents of biological control (Lacey & Undeen, 1987; Poinar, 1981) of insect pests, especially blackflies (Diptera Simuliidae).

The free-living adults of Mermithidae are found in soil or at the bottom of water bodies. They have no functional gut and live on resources acquired during their juvenile stages. The way of infestation of insect larvae by young worms is variable, but the worm larvae always live in the body cavity of their hosts. Larval Mermithidae also lack a continuous, functional digestive tract and acquire material from their hosts through their body surface only. The worms grow to a relatively large size, eventually occupying much of the host's body. Typically, hosts survive but the parasites leave virtually no space and probably also little material for the development of the host's genitalia. Parasitic castration is a typical consequence of mermithid parasitism (Adler et al., 2004; Crosskey, 1990). Late instar parasites exit from their hosts shortly before or after the hosts's metamorphosis and then spend several weeks until their own metamorphosis occurs (Grandi, 1951; Imms, 1970; Hartwich, 1984; Adler et al., 2004). Many insect orders are affected, but the present examples are the first records in Plecoptera.

MATERIAL STUDIED

Protonemura caprai (Aubert, 1954): Italia, Appennino Emiliano, Parma, torrente Lecca,

1,500 m a.s.l., upstream from Bedonia: 1♂ (nematode extracted), 6 July 1976; 2♂, 1♀ 12 June 1977; 3♂, 1♀, 27 June 1977; 2♂ (a nematode extracted from each), 3 Aug. 1977; sweepnet collections by C. Ravizza, in his collection, some individuals also in coll. P. Zwick.

Unidentifiable *Protonemura* sp., Germany, Bavaria, Nationalpark Berchtesgaden, spring source 1f at Herrenroit: 1♂, 5 July 1996; 2♂, 26 July 1996, one still containing the nematode (fig. 1); 1♂, 16 Aug. 1996; 1♂, 4 Oct. 1996; emergence trap and sweepnet collections by I. Schrankel, in coll. Gerecke; 2 parasitized larvae, Nationalpark Berchtesgaden, BGL 258, untere Rotwasserquelle, 1875m, 10.9.1999, 5,1°C, 168 µS/cm, leg. and coll. R. Gerecke.

Isoperla rivulorum (Pictet), Italy, Rhaetian Alps, Trentino, Peio, torrente Noce Bianco, 1900-2000m, 29.8.1989, 1♂, leg. et det. C. Ravizza, in coll. Zwick.

RESULTS

The two parasitized larvae from Rotwasserquelle are most probably *P. lateralis* (Pictet, 1836) (according to the key by Raušer, 1956). Wing-pads differ a little from shapes typical of particular instars (Zwick, 2003), the specimens seem to be slightly under-average last instar larvae. Because the body was ruptured at the base of the abdomen total body size could not be measured. Head capsule width (HCW) was determined instead and was 0.93 and 0.95 mm, respectively. Long nematodes protruded through the slit in the otherwise apparently empty abdomen of each larva. One larva was left untouched, the other contained two worms approximately 20 and 23 mm long, respectively, and about 0.15 mm thick, corresponding to a common volume of about 0.75 mm^3.

Among 221 adult specimens of *P. caprai* collected along the mountain brooklet Lecca (Ravizza & Ravizza Dematteis, 1978) were a few with malformations of the external genitalia. All these specimens hosted within their abdomen a long thread-like entangled mermithid. A similar case in an unidentified species of *Protonemura* from Herrenroit is illustrated (fig. 1). The distinct small anterolateral sclerites on each sternite and the mal-

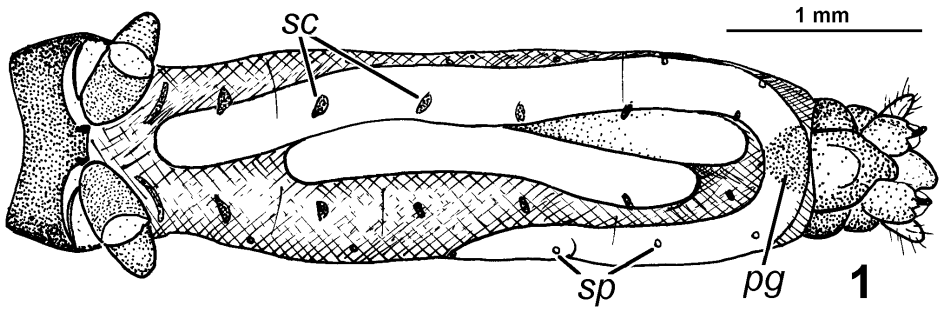
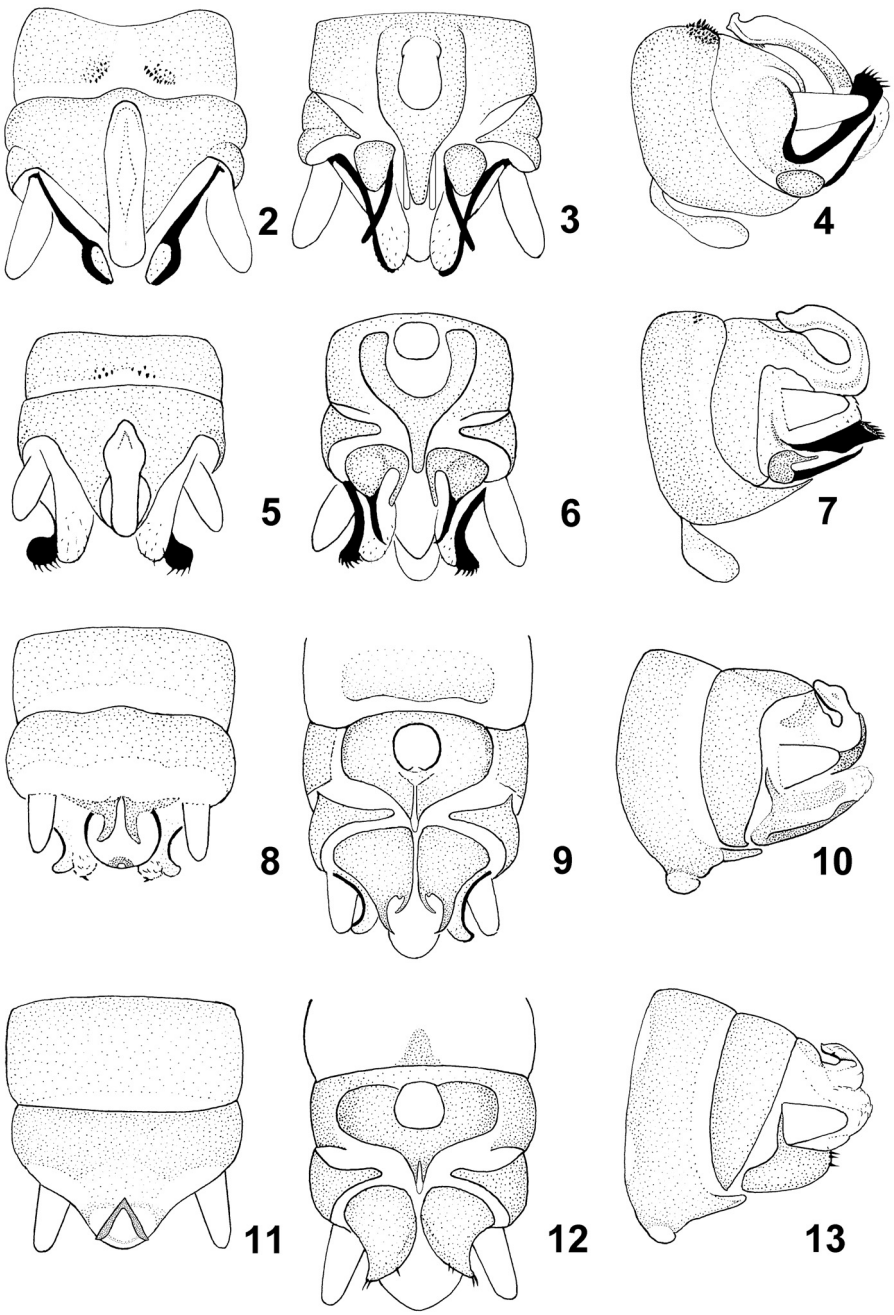


Fig. 1. *Protonemura* sp., malformed male specimen containing a parasitic mermithid nematode. Ventral view of metathorax and abdomen; pg, pregenital plate; sc, sclerite patch; sp, spiracle.



Figs 2-13. *Protonemura caprai,* male abdominal tips in dorsal, ventral and lateral views. 2-4 - normal male; 5-13 - three malformed males.

formed external genitalia show this to be a male, while a weak pregenital plate suggests a female. There were neither gonads nor fat body, and only a rudiment of the empty gut was visible on the dorsal side. A convoluted worm (or worms) extending the abdomen considerably can be seen through the transparent body wall, from all directions. The ventral view shown is the one leaving most worm-free space in the stonefly abdomen.

The secondary genitalia of the *Protonemura* specimens are malformed, the anomalities are variously severe, but similar in the two samples. Several specimens from Torrente Lecca are illustrated (figs 5-13, 15, 16). In comparison with normal males (figs 2-4) and females (fig. 14) of *P. caprai*, the following modifications are observed.

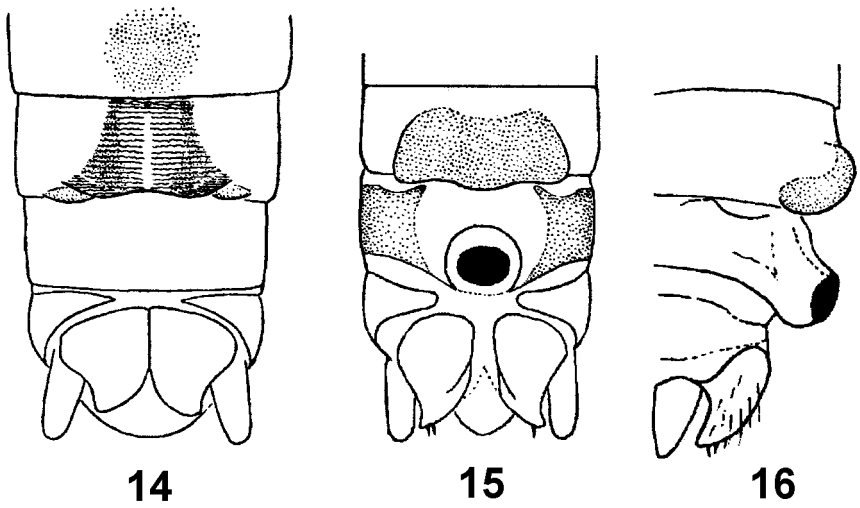
MALES. Spinule patches on tergite 9 weak to absent (figs 5, 8, 11). Even when the epiproct on tergite 10 is large (figs 5, 7) it lacks the specific details of shape and sclerotization. However, more frequently it is strongly reduced in size, sometimes to an insignificant, largely membraneous curved cone (figs 8, 10, 11, 13). A normal male sternite 9 (fig. 3) is developed into a subgenital plate which bears at the base a ventral lobe of specific fine structure needed for drumming (Gnatzy & Rupprecht, 1972). The caudal portion of a normal sternite 9 is greatly elongated, finger-shaped and bears the male gonopore at the tip. In the malformed males the ventral vesicle has neither the typical semi-erect position on the sternite nor the differentiated fine structure; in extreme cases only a membraneous knob without peduncle remains. The prolonged caudal part of the subgenital plate is short and thin (figs 9, 12).

The paraprocts of normal males are prolonged and composed of several lobes. Middle and outer lobes possess specifically shaped sclerites bearing long sclerotized processes that provide excellent means for species identification. In the abnormal males, secondary male structures on the paraprocts are more or less reduced. In the extreme, the inner paraproct lobes normally forming a guide for the long tip of the subgenital plate (which is the functional penis) disappear and only some coarse setae remain at the tip of the paraproct which resembles a normal female paraproct.

FEMALES. Both of the present species belong to the *Protonemura lateralis*-group in which normal females (fig. 14) have a small sclerotized patch caudally on sternite 7. On sternite 8 there is a large subgenital plate of specific shape covering the genital opening at the rear margin of segment. Sternite 9 is unmodified, and the paraprocts are short and simple.

One anomalous specimen (figs 15, 16) strongly resembles a female in that it possesses a large sclerotized plate on sternite 8, which reasonably resembles a female subgenital plate. However, there is no pregenital sclerite on sternite 7. The abnormal specimen bears a projecting structure on sternite 9, which has no counterpart in normal females but may represent a male ventral vesicle. The elongate paraprocts with coarse pilosity are also not typically female.

The insect's internal abdominal organs have been pressed against the body wall by the large nematode which is still contained. However, gut and Malpighian tubules are all that is visible through the transparent body wall and thin longitudinal musculature, there is no trace of gonads.



Figs 14-16. *Protonemura caprai*, abdominal tip. 14 - normal female in ventral view (after Aubert, 1954); 15-16 - an apparent intersex in ventral and lateral views.

The cerci of all the anomalous individuals are normal; they are not sexually dimorphic in normal specimens of the species in question.

Unlike in the beforementioned Nemouridae, external appearance and invaginated cuticular secondary male genitalia of the parasitized *Isoperla* are normal. The abdomen of the specimen (wing length 9 mm) is burst, a more than 30 mm long nematode, 0.18 mm thick, is largely exposed. The gut is also projecting through the large opening which permits to inspect the interior of the abdomen. In normal males the abdomen is crowded with large testes, seminal vesicles and efferent ducts, leaving little space for the gut. In the present parasitized male there are only insignificant rudiments of clearly male mesodermal organs, plus fragments of Malpighian tubules in the abdomen.

Discussion

The presence of Mermithidae in several adult stoneflies is the first observation in Plecoptera and justifies the conclusion that malformations of the literally empty specimens collected together with the infected ones have the same cause. Apparently, mermithid parasitism of Plecoptera is not common. For example, the present specimens of *P. caprai* are the only malformed representatives ever taken at the torrente Lecca, during many years of collecting. Evidently, Mermithidae are not found everywhere. For example, among many thousand Nematoda from the Breitenbach, a foothill stream near Schlitz, Germany, studied by the Limnologische Fluss-Station during more than 30 years very few Mermithidae with unknown hosts were discovered (H. Christl, in prep.). Indeed, among hundred thousands of adult Plecoptera taken in emergence traps along the Breitenbach, only a handful of individuals were malformed, for unknown reasons.

Malformation of secondary insect genitalia and castration are some of the typical consequences of parasitism by, for example, *Symbiocladius* Kieffer, 1925 (Diptera Chironomidae; Codreanu, 1939) and also Mermithidae (Adler et al., 2004; Crosskey, 1990). It appears therefore probable that several of the published records of genitalia malformations, suggested gynandromorphs and intersexes (Aubert, 1956, 1958; Klotzek, 1971; Zwick, 1976, 1977) also concern, or at least include, examples of parasitic castration by mermithid Nematoda.

The present *Isoperla* with its rudimentary male gonads is a castrated specimen of normal external appearance, including the secondary male characters on the cuticle of the penis shaft.

In contrast, we had to assign the malformed nemourid adults before us to gender by their appearance but the actual sex of the specimens in question is unknown. There are examples of Nemouridae of predominantly male appearance that contained mature eggs (*Nemurella pictetii* Klapálek, 1900; Zwick, 1973: 176), and, in another case, at the same time parts of male efferent ducts (*Zapada cinctipes* (Banks, 1897); Nebeker & Gaufin, 1966). The cause of the malformations in these cases may have been different from our observations but also in the present cases the genetic gender of the specimens is doubtful. Even if traces of internal genitalia may have been present in a few of the specimens examined here, they were not sufficiently well preserved for study.

The empty abdomen of the dissected *Protonemura* larva provided only half the space required by the parasites but it was so shrivelled that its original length, before removal of the parasites, could no longer be determined. A normal nemourid larva with a head-width of 1 mm is about 5 mm long, half of which is the abdomen, which has a volume of ca 0.77 mm^3 (Zwick, unpublished data). This is almost precisely the space which the worms had occupied, leaving no space for the host's own organs.

Unfortunately, the material before us adds no information on the known limited host specificity of Mermithidae. The worms exit from their hosts in the 4^{th} larval instar and spend time in the benthos before they molt to adulthood. However, only adult male Mermithidae can be identified to species (Kaiser, in litt.).

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