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Andreas Schmidt-Rhaesa [a] & Preben Kristensen [b]
[a] Zoomorphology and Systematics, University Bielefeld , Bielefeld, Germany
[b] Hirtshals, Denmark
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Horsehair worms (Nematomorpha) from the Baltic island Bornholm (Denmark), with notes on the biology of *Gordius albopunctatus*

ANDREAS SCHMIDT-RHAESA[1] & PREBEN KRISTENSEN[2]

[1]*Zoomorphology and Systematics, University Bielefeld, Bielefeld, Germany, and* [2]*Hirtshals, Denmark*

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Abstract
Two species of horsehair worms (Nematomorpha), *Gordius albopunctatus* and *Gordionus violaceus*, are reported from eight streams on the Baltic island Bornholm. Males can be easily distinguished from each other by differing features in the posterior end, but females resemble each other and can be distinguished reliably only using scanning electron microscopy. Aquarium experiments showed that larvae and praepupae of *Pomatophylax cingulatus* (Trichoptera) are the host for *G. albopunctatus*, which is one of the few cases in which nematomorphs develop completely in aquatic animals. Both nematomorph species occur sympatrically in almost all streams, but *G. albopunctatus* prefers northeastern streams and *G. violaceus* western streams. This might be due to the distribution of hosts, because *P. cingulatus* is more abundant in northeastern streams.

Keywords: *Denmark, horsehair worms, host, morphology, Nematomorpha, SEM, Trichoptera*

Introduction

No nematomorph species has been recorded from Denmark, except for a brief record assigned with the name *Gordius aquaticus* by Wesenberg-Lund (1937). Because about 40 species have been recorded from Germany and seven species from the rest of Scandinavia (see Schmidt-Rhaesa 1997), the absence of records from Denmark seems to be due to lack of sampling. We report here collections of nematomorphs from eight streams on the Baltic island Bornholm. One species, *Gordionus violaceus* (Baird, 1853), is widespread in Central Europe and has been described in detail elsewhere (Schmidt-Rhaesa 2000, 2001a). The second species, *Gordius albopunctatus* Müller, 1927, has not yet been documented by scanning electron microscopy (SEM). Traditionally, cuticular sections are removed from the worm and investigated under the light microscope. However, several structures are so delicate that they cannot be documented reliably by light microscopy and SEM has become

Correspondence: Andreas Schmidt-Rhaesa, Zoomorphology and Systematics, University Bielefeld, PO Box 100131, 33501 Bielefeld, Germany. Email: a.schmidt-rhaesa@uni-bielefeld.de

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the standard method for documentation in nematomorphs. In the present case, the use of SEM is of great help, because cuticular patterns of the two species, although belonging to different genera, are easy to confuse in light microscopical investigations.

All nematomorphs live in water, but the majority of those living in freshwater parasitize as adults terrestrial arthropod hosts (Schmidt-Rhaesa 2001b). This switch from the aquatic to the terrestrial environment is probably performed using intermediate hosts such as insects with aquatic larvae but terrestrial adults. However, there are few records that indicate that some species might develop completely in freshwater. Heinze (1941) reports that *Gordius albopunctatus* parasitizes the aquatic larval stages of the caddis fly (Trichoptera) *Potamophylax latipennis* (Curtis, 1834) and other Limnephilidae. *Gordius villoti* has been reported to parasitize *Allogamus auricollis* (Valvassori et al. 1988). Such "holoaquatic" development appears to be exceptional among freshwater nematomorphs. We can present here further data on the association of *Gordius albopunctatus* and the caddis fly *Potamophylax cingulatus*.

Material and methods

Free-living, adult specimens of horsehair worms were collected by eye between 15 August and 4 September 2003. A total of 132 worms was collected (108 males and 24 females; see Table I). Eleven additional specimens were variously incomplete and could not be determined. Streams (see Table I for names; Figure 3) are distributed over the whole island. Specimens were stored in ethanol (70%). From all females and 10 males, about 1 mm long pieces were removed from the midbody region and prepared for SEM. From all males, the posterior end was also prepared. Pieces were dehydrated in an increasing ethanol series, critically point dried in a Bal-Tec CPD 030, and sputtered with gold in a Bal-Tec SCD 005. Observation took place under a Hitachi SEM 450 under 15 kV.

A total of 285 larvae of *Potamophylax cingulatus* (Stephens, 1837) was collected in the stream Kobbeå on 3 July 2004 and kept in aquaria to check for emerging nematomorphs. The experiment was terminated on 20 September 2004. Additional 125 larvae/praepupae

Table I. Number of males and females of *Gordionus violaceus* and *G. albopunctatus* in different streams and at different collection times.

	Gordionus violaceus		*Gordius albopunctatus*		
Location	Males	Females	Males	Females	Other[a]
Nydams Å, 31 March 1998	–	–	–	–	1♀
Kobbeå, 1999	–	?	–	?	
Kobbeå, 15 August 2003	1	–	10	–	2
Melsted Å, 17 August 2003	1	–	5	4	2
Bobbeå, 18 August 2003	–	1	19	1	2
Døndal Å, 18 August 2003	2	–	12	1	–
Gyldenså, 18 August 2003	–	–	14	1	1
Blykobbe Å, 18 August 2003	–	–	1	–	–
Blykobbe Å, 21 August 2003	5	–	2	2	1
Læså, 21 August 2003	4	–	3	4	2
Kobbeå, 24 August 2003	–	–	22	5	–
Byå, 26 August 2003	4	5	3	–	–
Kobbeå, 4 September 2003	–	–	8	1	1

[a]Specimens or fragments not able to be determined.

and 11 pupae of *P. cingulatus* were collected from Kobbeå on 28 July 2004 and were dissected.

Single specimens of *Gordius albopunctatus* and *Gordionus violaceus* were deposited in the Zoological Museum of the University of Copenhagen (Denmark) under the accession numbers ZMUC-NEP-1 to ZMUC-NEP-4 (*Gordius albopunctatus*) and ZMUC-NEP-5 to ZMUC-NEP-6 (*Gordionus violaceus*).

Results

All collected specimens of Nematomorpha belonged to either of the two species, *Gordionus violaceus* and *Gordius albopunctatus*. One additional female from a collection in Nydams Å (31 March 1998) was devoid of cuticular characters and could represent a third species.

Gordius albopunctatus

The posterior end of *G. albopunctatus* males is bifurcated into two lobes (Figure 1B). Between the ventral cloacal opening and the beginning of the bifurcation is a semicircular

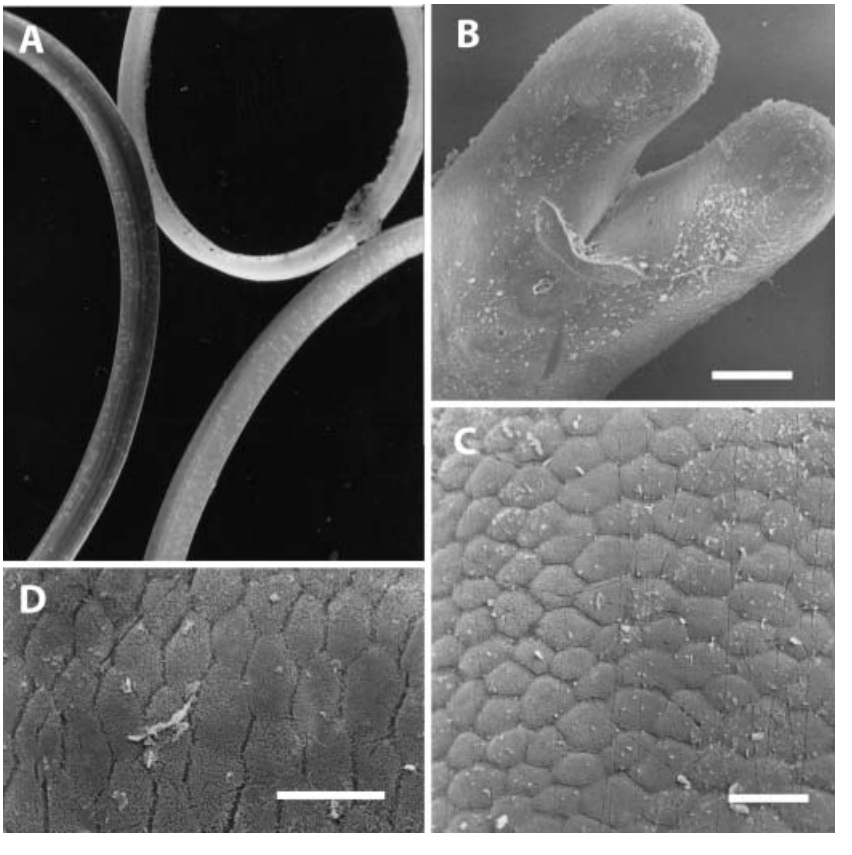

Figure 1. *Gordius albopunctatus*. (A) Details of three males with different coloration, white spots are not visible on the lightest specimen (top); (B) ventral view of male posterior end showing tail lobes, cloacal opening, and postcloacal crescent; (C, D) cuticle with regular polygonal areoles. (B–D) SEM. Scale bars: 100 μm (B); 20 μm (C, D).

cuticular fold, the so-called postcloacal crescent (Figure 1B), which is characteristic for the genus *Gordius*. The body colour varies in both sexes between very light brown and medium brown. In all but the lightest male specimens, abundant white spots can be recognized (Figure 1A). In females, the posterior end is round and the cloacal opening is terminal. The cuticle is in both sexes structured into adjoining, polygonal to rounded areoles (Figure 1C, D). Between these areoles, no further cuticular structures can be recognized.

Gordionus violaceus

Males of *G. violaceus* are easy to distinguish from *G. albopunctatus* by the absence of the postcloacal crescent in the posterior end (Figure 2A, B). Instead, anterolaterally of the cloacal opening are paired rows of cuticular bristles and posterior of the cloacal opening are short spines extending on to the inner side of the tail lobes (Figure 2A, B). The posterior end of the females is similar to *G. albopunctatus*. The colour varies between light and medium brown, but white spots are not present. The cuticle contains rounded areoles which are separated from each other by an interareolar groove (Figure 2C). Each areole is surrounded by short cuticular bristles (Figure 2C, D).

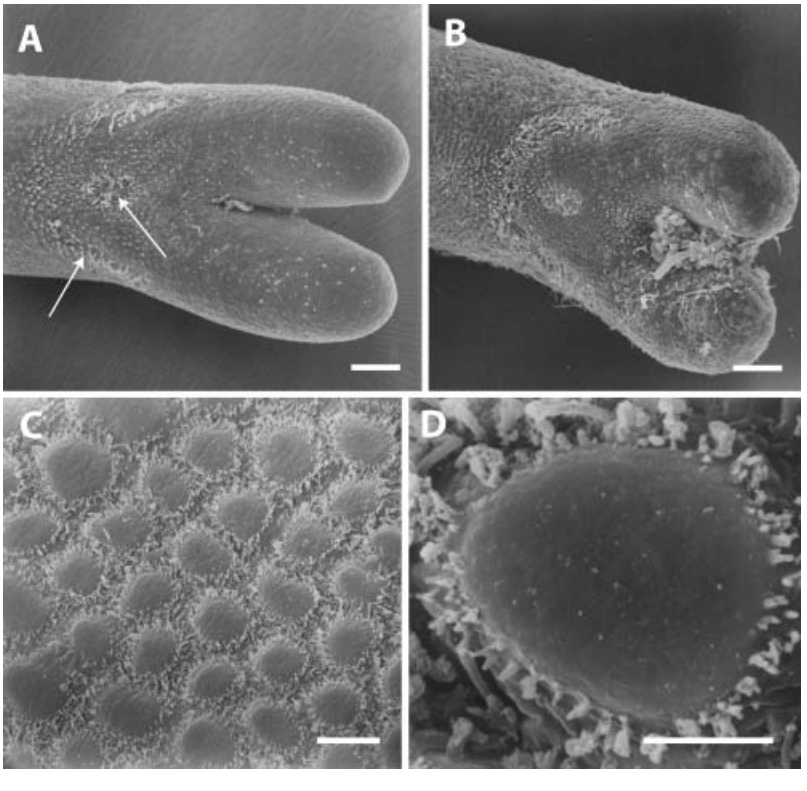

Figure 2. *Gordionus violaceus*. (A, B) Ventral view of male posterior end showing tail lobes, cloacal opening surrounded by spines, and anterolateral rows of bristles (arrows in A); (C) cuticle with rounded areoles which are surrounded by short bristles; (D) magnification of one areole. All SEM. Scale bars: 50 µm (A, B); 10 µm (C); 5 µm (D).

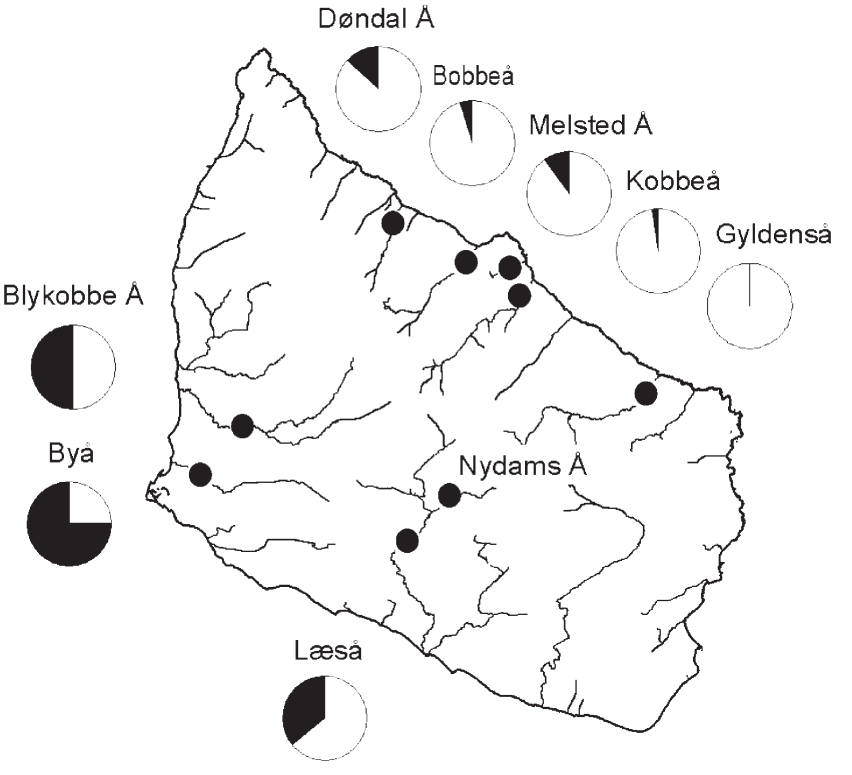

Figure 3. Outline of the Baltic island Bornholm with location of investigated streams and relation of species found in those streams. White, *Gordius albopunctatus*; black, *Gordionus violaceus*.

Distribution patterns

Both species, *Gordionus violaceus* and *Gordius albopunctatus*, occur sympatrically in all streams except Gyldensså, where only *G. albopunctatus* was collected (see Table I; Figure 3). In some streams (Kobbeå, Melsted Å, Bobbeå, and Døndal Å) *G. albopunctatus* dominates clearly. In the remaining three streams, Blykobbe Å, Læså, and Byå, relatively few specimens were collected, but both species are about equally abundant or *G. violaceus* dominates (as in Byå).

Relation of *Gordius albopunctatus* to *Potamophylax cingulatus*

From the 285 larvae of *Potamophylax cingulatus* kept in aquaria, a total of 24 specimens of *Gordius albopunctatus* emerged. This is a parasitation rate of 8.4%, 17 specimens were males, six females, and one specimen remained unsexed. Dissections of 125 larvae, praepupae, and pupae of *P. cingulatus* resulted in six specimens which were not further determined but left *in situ* (Figure 4). Five of the specimens were white in colour and one was medium brown, indicating an advanced developmental stage. The parasitation rate for this experiment was 4.6%.

Figure 4. Six dissected larvae and praepupae of *Pomatophylax cingulatus* (Trichoptera), parasitized by *Gordius albopunctatus* (note that worms are white in colour except for the one to the right which is light brown).

Discussion

White spots are the name-giving character for *Gordius albopunctatus*. This character has, however, to be treated with care. It is present in several species of the genus *Gordius* (e.g. *G. mülleri* Heinze, 1933, *G. villoti* Rosa, 1882; see Schmidt-Rhaesa 1997). In some species, white spots can be present or absent (*Gordius aquaticus* Linnaeus, 1758, see Schmidt-Rhaesa 1997; *Gordius robustus*, see Schmidt-Rhaesa et al. 2003). In *Gordius albopunctatus*, however, white spots seem to be present in all male specimens, although they are not recognizable in few very light specimens. White spots occur exclusively in males, which has already been mentioned by Müller (1927). What is characteristic for *G. albopunctatus* is the combination of white spots with polygonal areoles on the cuticle. Most species of the genus *Gordius* are devoid of areoles.

Gordionus violaceus is an abundant species in Central Europe. It also seems to be quite polymorphic, because several transitional stages of the cuticular pattern have been found between what has originally been described as *G. violaceus* and *G. wolterstorffii* (Schmidt-Rhaesa 2001a). This polymorphism could be confirmed from several locations (Schmidt-Rhaesa 2000; also A. Schmidt-Rhaesa, unpublished results). In Bornholm, we could find in the specimens investigated only the ''pure'' cuticular pattern of *G. violaceus* with areoles surrounded completely by short bristles.

In the light microscope, the short bristles are not always clearly recognizable and the cuticular patterns of *G. violaceus* and *G. albopunctatus* appear quite similar. As males are easily distinguishable by their different posterior ends, females might be confused when SEM is not applied.

With a total of 125 males and 30 females, there is a strong bias in sexes. Such bias has been reported several times (e.g. Linstow 1891; Mühldorf 1914; Schmidt-Rhaesa et al. 1998), while other authors report a similar amount of males and females (e.g. May 1919; Müller 1927). The reasons for such bias, when present, are not clear, but different temporal occurrence or different drifting velocities downstream may be possible

explanations. As males are generally more active than females, another suggestion is that males are easier to observe while females might be overlooked.

Although both species occur in almost all streams on Bornholm, there is a bias in occurrence of the two species. The streams in which *Gordius albopunctatus* dominates are located in the northeastern part of the island (Kobbeå, Melsted Å, Bobbeå, and Døndal Å) while *Gordionus violaceus* dominates in western streams (Blykobbe Å, Læså, and Byå) (see Figure 3). The reason for this is yet unknown, but one hypothesis that should be tested next is that it has to do with the distribution of hosts.

The aquarium experiments have shown that *Potamophylax cingulatus* (Stephens, 1837) is a host for *Gordius albopunctatus* which supports Heinze's (1941) report of another *Potamophylax* species as hosts of *G. albopunctatus*. Therefore, *G. albopunctatus* is exclusively known from aquatic hosts, while almost all other species develop in terrestrial hosts (see Schmidt-Rhaesa 2001b). Schmidt-Rhaesa (2005) has shown that parasitic stages of nematomorphs are white in colour, but become darker towards the end of their parasitic period, probably in relation to the replacement of a thin larval cuticle by a solid adult cuticle.

There are few data on parasitation rates from freshwater nematomorphs. Baker (1985) found a rate of 28.7% in an undetermined nematomorph parasitizing the millipede *Ommatoiulus moreletii*. Thorne (1940) found infection rates of *Gordius robustus* parasitizing the cricket *Anabrus simplex* decreasing with distance to a pond, but his values are over 50%. The infection values of *Gordius albopunctatus* in *Potamophylax cingulatus* are more within the range reported for marine nematomorphs, here the infection rate is between 0.4 and 7.86 (Arvy 1963; Nielsen 1969).

Four of the streams located on the northeastern part of the island (Døndal Å, Bobbeå, Kobbeå, and Gyldenså) are permanent streams, never drying out completely, while Melsted Å can occasionally dry out for a short period of time at extreme dry summer periods. However, all these five streams have a good stock of Limnephilidae, predominantly *Potamophylax cingulatus*. This species has been reported from clear, oxygen-rich aquatic environments (Otto 1971, 1976; Higler and Solem 1986). The stream stations in the western part of Bornholm, included in this survey, are very often dry in periods of the summer, and the Limnephilidae are much rarer in those streams than in the northeastern streams.

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