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Taxonomic investigations on Naididae: *Pristina aequiseta* Bourne, 1891 and *Pristina foreli* (Piguet, 1906) (Annelida, Oligochaeta, Naididae) are distinct species

Short title: Investigations on Naididae

Keywords: Annelida, Oligochaeta, Naididae, *Pristina*, karyology, cocoon, sexual reproduction, chaetae, CO1

Abstract *Pristina aequiseta* and *Pristina foreli* are two species of Naididae (Oligochaeta, Annelida) that are regarded as synonymous by some authors. We investigated the morphological characters, the chromosomes and the CO1-gene of specimens from different locations. Additionally, clones of both species were held under laboratory conditions for years. The occurrence of thick chaetae in segment IV in *P. aequiseta* and different proboscis morphology of both species are constant characters. Although chromosomes are difficult to investigate, the two species differ from each other, having 34 (*P. aequiseta*) or 48 (*P. foreli*) chromosomes. Specimens of both species occur in different clades in the CO1-tree and are separated clearly by p-distances. We conclude that both are valid species. In addition, we report the occurrence of cocoons in a culture of *P. aequiseta*, indicating sexual reproduction. This is the first proof of sexual reproduction in this species that predominantly reproduces asexually by paratomy.

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Introduction

The taxon Naididae includes slender, usually freshwater oligochaetes, which reproduce primarily asexually by paratomy, a special form of fission (Fig. 1A). Species are grazers as well as particle feeders, predators, commensals and parasites. We use here the name "Naididae" as introduced by Ehrenberg (1828) (as Naidina). In some recent publications, "Naididae" is used as including Naididae in Ehrenberg's sense and Tubificidae (formerly described by Vejdovský 1876) (see, e.g., Erséus & Gustavson 2002, Erseús et al. 2008).

The differentiation of species is often difficult in some genera of the family. Erroneous identifications and problematic taxonomic allocations are familiar problems in the taxon Naididae. One such case is the genus *Pristina*.

The essential characters of *Pristina* Ehrenberg, 1828 are as follows: The animals have no eyes and the prostomium has either a proboscis or this is absent. Partially present coelomocytes give the animals a whitish or pale yellow appearance. The dorsal chaetae usually begin in segment II (few exceptions such as *P. machrochaeta* exist, see Naidu 1963), hair chaetae exist. In some of the segments III to VI septal glands appear. The extension of the esophagus to the stomach is in segment VII or VIII. The stomach is pear-shaped or spindle-shaped with intra-cellular canals (Fig. 1C). The nephridia begin in segment IX. In those species where gonads are present, testes and spermathecae occur in segment VII, the ovaries and atria are in segment VIII. The taxon is spread worldwide (e.g. Naidu 1963, Nemec & Brinkhurst 1987, Fauna Europaea 2010). Information on synonyms is found in Brinkhurst & Wetzel (1984).

Two worldwide species with a broad distribution in Europe are *Pristina aequiseta* Bourne, 1891 and *P. foreli* (Piguet, 1906). After their description, these two species have experienced a vivid history reflecting the problems to distinguish them from each other. For *P. aequiseta* Michaelsen (1909) already named the enlarged hook chaetae in the segment IV and/or V as essential distinguishing character and named them "giant chaetae" (in the literature sometimes enlarged hook chaetae, enlarged ventral chaetae, ventral large chaetae or thick chaetae) (see also Sperber 1948). Hempelmann (1923) investigated difficulties in the differentiation of the two species with respect to the giant chaetae. He conducted extensive experiments with two *Pristina aequiseta* populations and found that the giant chaetae in the mentioned segments appeared with different percentages in worms of an advanced age and during the entire year. The temperature had no influence on their appearance. Hempelmann supposes the giant chaetae evolve from genital chaetae. He additionally remarks that both species can easily be distinguished by their different head shapes.

Besides the giant chaetae Sperber (1950) named as characters for *P. aequiseta* one or two dorsal very well pinnate hair chaetae (120–270 µm long; Fig. 4C,D), as well as one to two bifid and slightly crooked dorsal needle chaetae (30–69 µm long) in the

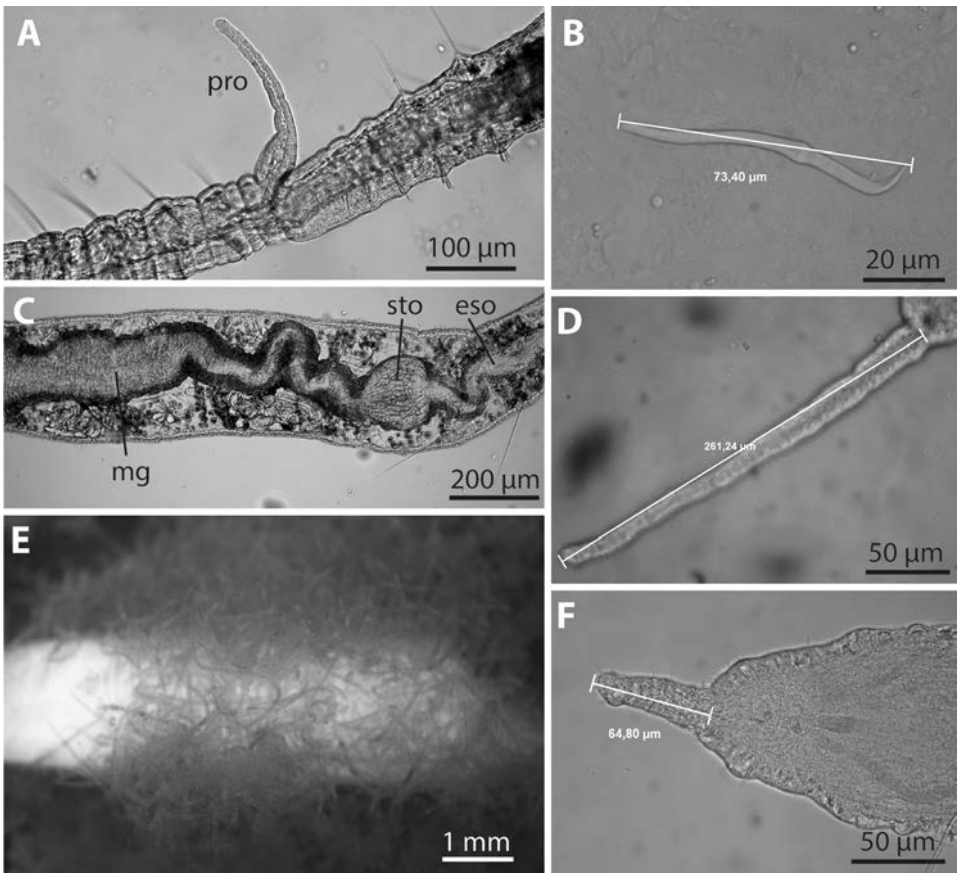

Fig. 1. A. Paratomy in *Pristina aequiseta*, two zooids are attached to each other, the daughter zooid can be recognized by the proboscis (pr). B. Thick seta from segment IV from a *P. aequiseta* from Bornhöveder See to show the way of measurement. C. Stomach with internal canals (sto), between esophagus (eso) and midgut (mg) in *P. aequiseta*. D. Very long proboscis of *P. aequiseta* showing mode of measurement. E. Culture of *P. foreli* on a grain of rice in water. F. Short proboscis of *P. foreli*.

bundle, these are also present in *P. foreli*. Sperber (1948) reported cases, where *P. aequiseta* lacks the diagnostic giant chaetae and Kasprzak & Szczesny (1976) stress the great variability within the entire taxon *Pristina*. Harman (1979) as well as Loden & Harman (1980) assumed that the variability of chaetae is caused by environmental factors and they therefore synonymized *P. foreli* with *P. aequiseta*. In laboratory cultures Loden & Harman (1980) found descendants of a clone of *P. aequiseta* where individuals lost or regenerated their giant chaetae in correlation to the ion concentration in artificial well water. As a result of this study, *P. foreli* disappears to some extent from the subsequent literature and is, for example, neglected in databases such as GenBank or Fauna Europaea (2010), although it is still included in the data base ITIS (2010) (taxonomic standard number 68881 with the taxonomic status "valid"). Milligan (1997) repeats that *P. foreli* (and *P. evelinae*) are synonyms of *P. aequiseta*

and, hence, the presence of giant chaetae for *P. aequiseta* is no longer necessary to identify the taxon. *Pristina foreli* is regarded as a valid species e.g. by Van Haaren & Soors (2013).

Asexual reproduction seems to be the main mode of reproduction for *Pristina aequiseta* and *P. foreli*. Indications for sexual reproduction, e.g. genital chaetae or cocoons have not been documented (Hempelmann 1923, Bely & Wray 2001).

This vivid history of the species *P. aequiseta* and *P. foreli* motivated us to approach the question whether these are distinct species or synonyms. We used a broader approach, investigating specimens from different locations according to their chaetal morphology, their karyotype and their "barcoding gene" cytochrome oxidase 1 (CO1).

Material and Methods

Material

Pristina worms were obtained from two different freshwater lakes in northern Germany (Hamburg, Schleswig-Holstein, Niedersachsen), a freshwater tidal flat of the river Elbe (Fährmannssand, north of Hamburg) and from a freshwater pond near Rovinj, Croatia (see Tab. 1). Additionally, specimens of unknown origin were obtained from aquaria in the Zoological Institute of the University Hamburg. Specimens were cultured at room temperature in mineral water (Vovic ©, Danone Waters, Germany) and fed on uncooked rice or wheat grains (Fig. 1E). Identification took place with the aid of Sperber (1948) and Brinkhurst & Jamieson (1971).

Table 1. Locations for collection of *Pristina aequiseta* and *P. foreli* specimens.

	Location	**Substrate**	**Coordinates**
P. aequiseta	Pond in Rovinj (Croatia)	dead organic substances	unknown
	Barumer See, near Barum, Niedersachsen, Germany	sandy sediment	N53.35229°, E 10.41363°
	Fährmannssand, freshwater tidal flat near Wedel, Schleswig-Holstein	Fine sand with dead organic substances	several stations in the area N53.59703°; E962231°
P. foreli	Barumer See, near Barum, Niedersachsen, Germany	Sediment with dead organic substances	N53.35229°, E 10.41363°
	Fährmannssand, freshwater tidal flat near Wedel, Schleswig-Holstein	Fine sand with dead organic substances	several stations in the area N53.59703°; E962231°

Morphology of chaetae and proboscis

All identification were carried out on living or freshly killed animals. After removing them from their biotope, the worms were predetermined under the dissecting microscope, arranged in homogeneous groups and then checked again under the compound microscope using 400x or 1000x magnification. Specimens showing clear characters of either of the two species were taken as starting point for cultures. Variability in number and length of chaetae was measured in random specimens among these clones. Further chaetotaxonomical investigations were carried out in the course of the karyological investigation, because the chaetae were brought by squeezing in a level position. Chaetotaxonomical identification was also performed in the specimens used for molecular analyses. Worms whose characters could not be determined in vivo unambiguously were divided with the scalpel (normally after the first ten front segments). Anterior segments of the animal were fixed, dyed, squeezed and the chaetae were surveyed. The remaining, generally bigger posterior part of the naidid was transferred either in 99.8% ethanol or was processed immediately.

The linear measurements of the chaetae were carried out as described by Sperber (1948) (see Fig. 1B). Measurements were taken on specimens from different populations. Ventral chaetae in a bundle were investigated in segment II. The measurements were analyzed with statistical methods (average determination, t – test or variance analysis as well as Post Hoc Test Bonferoni) using the software SPSS® 14.0 (SPSS Inc. Chicago).

Phalloidin staining and immunohistochemistry

Specimens were fixed in 4 % paraformaldehyde (PFA) in 0.1 M phosphate buffered saline (PBS, pH 7.3) overnight at 4 °C. Fixation was stopped by washing with 0.1 M PBS. For phalloidin staining, specimens were incubated in 2 µL of a 25.5 µmol/l phalloidin-TRITC (Sigma) stock solution in 200 µL incubation buffer (0.5 % bovine serum albumin, 6 % normal goat serum and 1 % Triton X-100 in 0.1 M PBS) overnight on ice. Staining was completed by several washing steps in 0.1 M PBS (pH 7.3). Subsequently specimens were mounted on microscope slides using LMScope Confocal-UV-Matrix® or CitiFluor™ AF1. All preparations were investigated under a Leica TSC-SPE confocal microscope with a 532 nm laser line and an emission bandpass filter set to 570 – 615 nm.

Karyological methods

Karyologically investigated were 11 specimens of *P. aequiseta* from a pond near Rovinj (Croatia), 57 animals from a culture of the Bornhöveder See, 31 individuals

from the Barumer See, 72 *P. foreli* from a culture of the Zoological Institute of the University Hamburg, 12 specimens from the freshwater mudflat Fährmannssand and 41 specimens from a culture from the Barumer See.

Because Naididae are rarely found as mature individuals, somatic chromosomes had to be investigated primarily. Increased mitotic activity is found in the budding zone and in the posterior segments (pygidial growth zone, teloblastic production of new somites) (Jelinek 1974). From preceding investigations (Jelinek 1974, Christensen 1980) it was known that naidids have small and many chromosomes, causing difficulty to count and distinguish them. Specimens were pretreated with colchicine (O'Mara 1939), which prevents the development of the mitotic spindle and therefore interrupts the cycle of the nuclear division in the somatic metaphase. The determined animals were placed with a fine needle in a 0.25% colchicine solution made with local water for 2 – 30 hours, according to the robustness of the specimen. A better dissemination of the chromosomes was reached by a hypotonic treatment for 10 to 15 minutes in distilled water under microscopic check (Jelinek 1974). In addition, the treatment of *Pristina* specimens in distilled water accelerated defecation, which is advantageous for the following preparation.

The specimens were then fixed 15 minutes in ethanol-acetic acid (3:1) and dyed (10 minutes) in orcein-acetic acid. Staining was carried out with a solution of approx. 1.1% orcein in 50% acetic acid. Preparations on microscopical slides were bordered after squeezing immediately with coverslip varnish. The preparations could be kept at ˜8 °C in the fridge for two to four weeks.

Investigation took place with a microscope (Leitz-Dialux or Zeiss Neofluar). Evaluable objects were photographed with a digital camera (Leitz EC3). Interpretation and counting of chromosomes was a combined approach using the photos, the microscopic original and drawings. Employing these operations chromosomes could often only be optically isolated and concretely located to distinct metaphase plates.

In many cases squeezing of *P. aequiseta* produced artifacts, which are probably due to unequal hardening of tissue with ethanol-acetic acid. Therefore, a number of preparations were performed with simultaneous fixation and dyeing with orcein-acetic acid, which improved the spreading of tissue on the slide. The quantity of countable somatic metaphases in the taxon *Pristina* was low and the chromosomes were hard to distinguish. These problems were present in all investigated specimens of the taxon *Pristina*.

Barcoding

Genomic DNA was extracted with the DNeasy® Blood and Tissue Kit (Quiagen) from fresh catches as well as from individuals from laboratory cultures. The standard pri-

mers LCO1490 and HCO2198 (Folmer et al. 1994) as well as the primer COI-E developed by Bely & Wray (2004) did not yield good results in the following PCR. Therefore, new primers JCO1 and JCO2 were designed according to proposals of the program Primer3:

JCO1 [5 ' TAGGAGTATGAGCAGGAATAGTAG 3 ' (24-mer, Tm = 59.3°C)]

JCO2 [5 ' TATGTGCTACAATATGTGAGATTAC 3 ' (25-mer, Tm = 56.4°C)]

(All primers were purchased from Eurofins, mwg operon-®.)

Reproducible results which delivered clear single bands in the agarose gel were received after several tests and run as a rule according to the following protocol:

1. Initial DNA – denaturing:	94°C, 3 min
2. DNA – denaturing:	94°C, 1 min
3. Primer – annealing, according to template:	44 – 56°C, 1 min 35 cycles
4. Chain extension (polymerization):	72°C, 1 min
5. Fill – up of DNA:	72°C, 7 min
6. Termination:	4°C, up to removal

PCR was performed with the thermocycler "MyCycler™ Thermal Cycler 580BR6363", from BioRad (Munich, Germany) as well as "Primus 25 advanced" from Peqlab (Erlangen, Germany). Gel electrophoresis of DNA was carried out in 1.5% TAE – agarose gel in horizontal electrophoresis chambers (BioRad® Subcell GT Mini). It was dyed with ethidiumbromid (5 mg / ml $_{H_2O}$ ethidiumbromid, Sigma, E – 1510), the bands of DNA were analyzed with the help of UV – transluminators (model NU 72, Urhammer).

Sequencing of cleaned PCR products was carried out by Macrogen (Seoul, Korea). The obtained partial CO I – sequences were aligned with ClustalW and visually corrected. Sequences were analyzed with maximum parsimony, distance methods, maximum likelihood and Bayesian inference. For comparison, sequences from Genbank were used. Five sequences of *Pristina foreli* were deposited in Genbank under the accession numbers MK249360-MK249364 and four sequences *of P. aequiseta* under the numbers MK249365- MK249368.

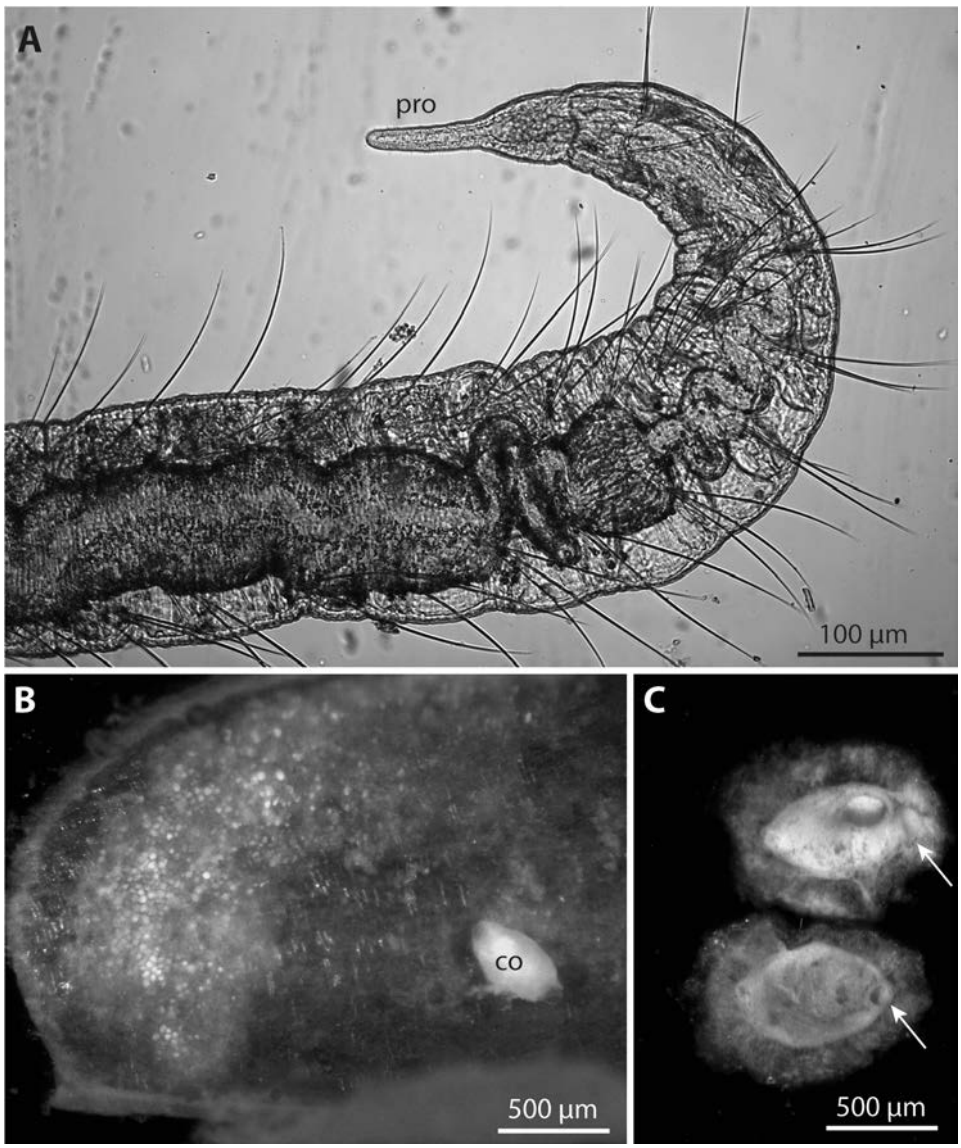

Fig. 2. *Pristina foreli*. A. Entire anterior end with proboscis (pro). B. Cocoon (co) attached to the bran of a grain of wheat). C. Two cocoons. Arrows point at the opening for emergence. The large opening in the upper cocoon is caused by unknown reasons.

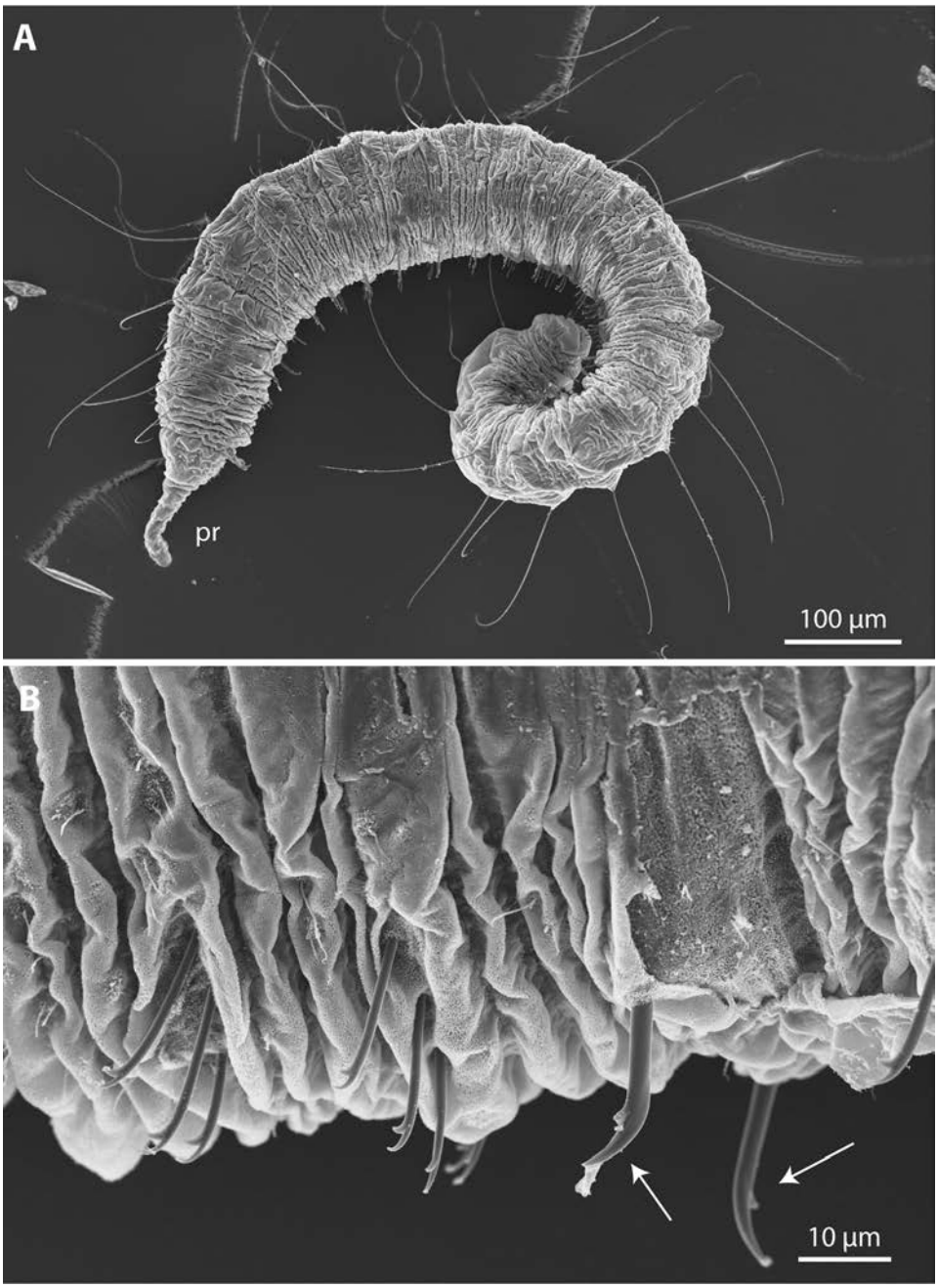

Fig. 3. *Pristina aequiseta,* scanning electron microscopy. A. Entire animal from dorsal, with proboscis (pr). B. Giant chaetae in segment IV (arrows), the anterior segments III and II are to the left.

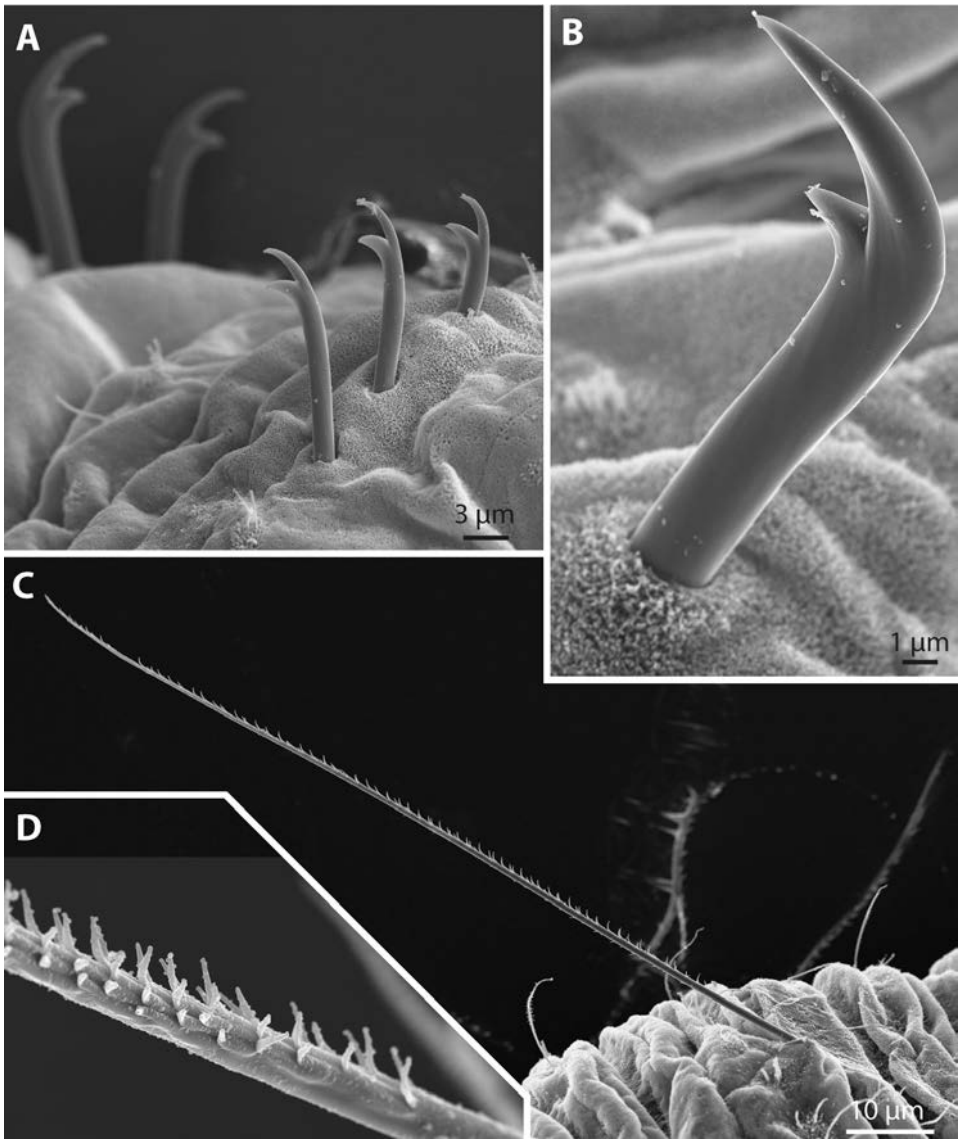

Fig. 4. Chaetae in *Pristina aequiseta*, scanning electron microscopy. A. Ventral chaetae, with giant chaetae of segment IV in the background. B. Magnification of a giant chaeta. C. Dorsal pinnated hair chaeta. D. Fine structure of the pinnate hair chaeta.

Results

Chaetotaxonomy

Specimens determined as *Pristina aequiseta* were found in the localities Barumer See, Bornhöveder See, the freshwater mudflat Fährmannssand and in a lake near Rovinj. All specimens with giant chaetae in the segment IV (Tab. 2, Fig. 3B, 4A, B)

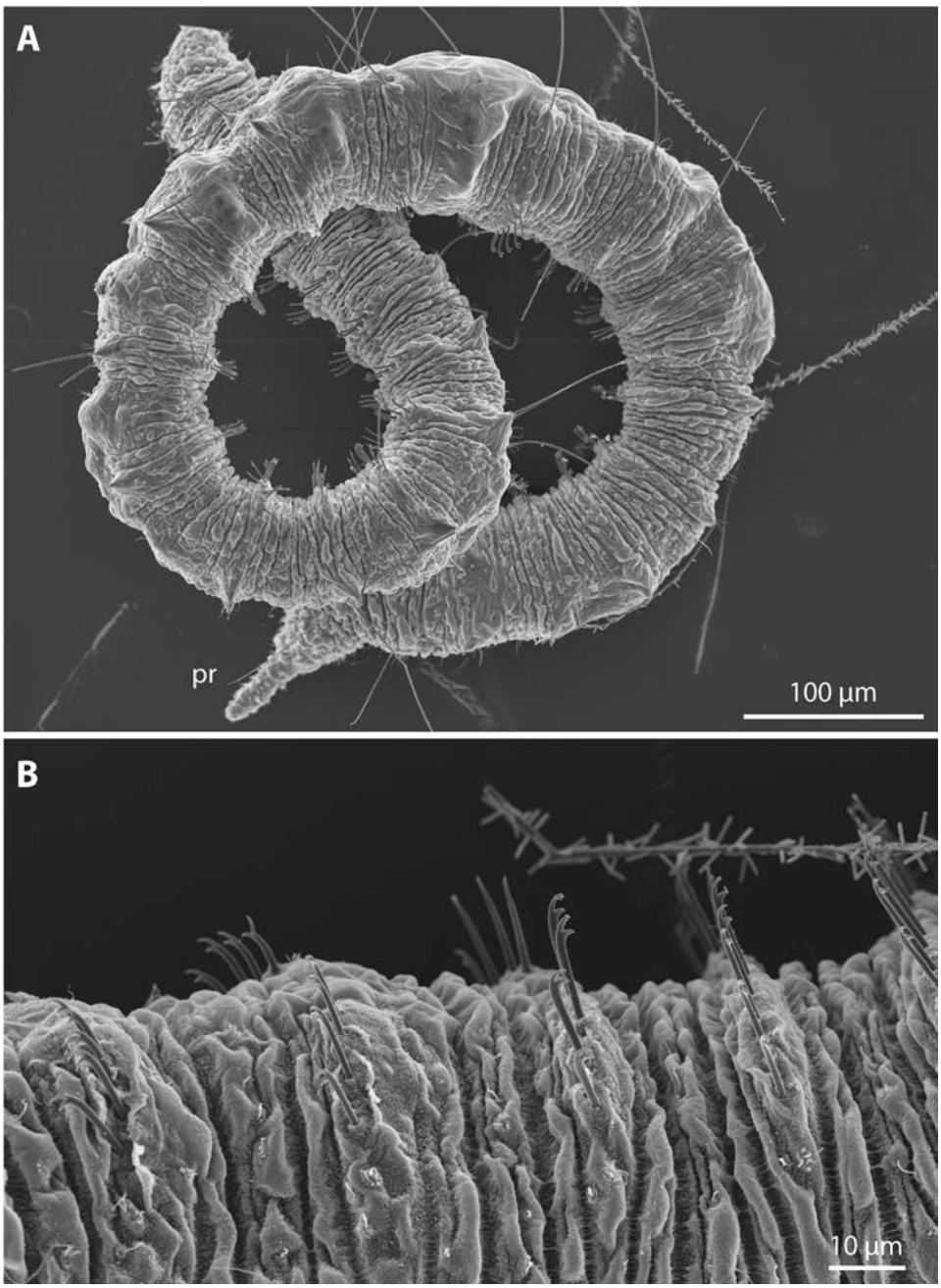

Fig. 5. *Pristina foreli,* scanning electron microscopy. A. Entire animal from dorsal, with proboscis (pr). B. Segments around segment IV showing no giant chaetae.

and a comparably long proboscis (Tab. 3, Fig. 1D, and 3A) were assigned to this species. Cultures were observed for more than seven years (2009 – 2016) under laboratory conditions in isogenetic cultures and maintained these characters consistently.

Specimens determined as *Pristina foreli* were found in the localities Barumer See, the freshwater mudflat Fährmannssand and in aquarium cultures at the Zoological Institute of the University Hamburg. Specimens with a comparably shorter proboscis (Tab. 3, Fig. 1F, 5A, 6A) and no giant chaetae in the segment IV were assigned to this species. In isogenetic laboratory cultures (2009–2016) no changes concerning the giant chaetae occurred over the entire period, but sometimes specimens or even the entire population could be observed with strongly shortened proboscis.

The giant chaetae of *P. aequiseta* occur as two in a bundle and both chaetae in a bundle normally lay close together. One of both chaetae often was not fully developed.

Table 2. Length (in µm) of the giant chaetae in *Pristina aequiseta* from Bornhöveder See (N = 49)

minimum	maximum	mean	standard deviation
59,03	73,66	64,98	3,74

Proboscis morphology

Specimens of *Pristina aequiseta* had a significantly longer proboscis compared to *P. foreli* (Tab. 3, Fig. 1D, F, 3A, 5A, and 6A). The basis of the proboscis of *P. aequiseta* appears to origin from the anterior body by a well-defined and more or less sharp border (Fig. 1D), whereas the shorter proboscis of *P. foreli* appears to taper more gradually from the anterior body (Fig. 1F). In stainings of the proboscis musculature (Fig. 7A-C) no differences were observed between both species. Longitudinal and circular musculatures are continuous with the musculature in the anterior body, only the circular musculature decreases in size.

Table 3. Comparison of the length of the proboscis from *Pristina aequiseta* and *Pristina foreli*.

species	mean length in µm	standard deviation	95% – confidence interval for mean length in µm
Pristina aequiseta (N = 25)	261,36	27,45	250,03 –272,69
Pristina foreli (N = 25)	114,49	23,90	104,62 – 124,35

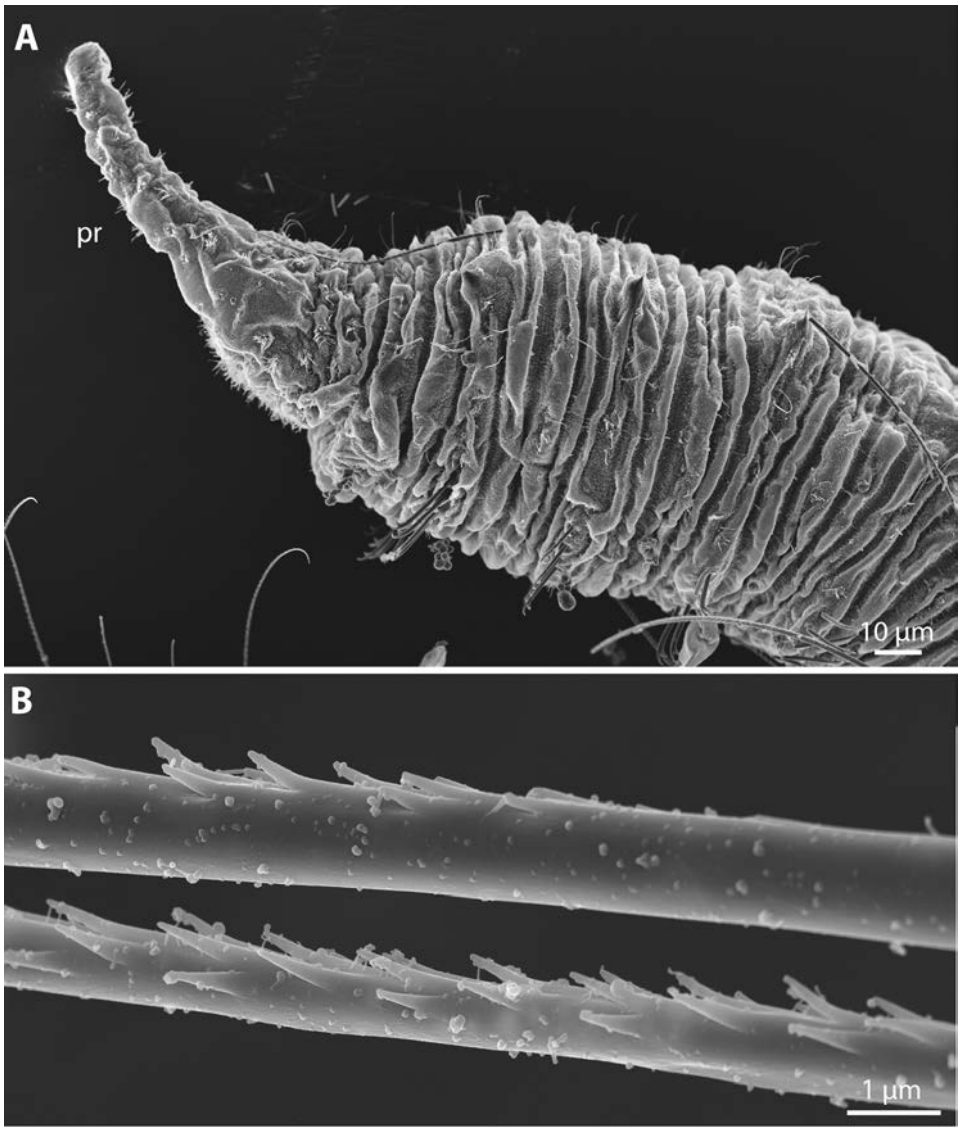

Fig. 6. *Pristina foreli,* scanning electron microscopy. A. Short proboscis (pr). B. Magnification of a dorsal pinnate hair chaeta.

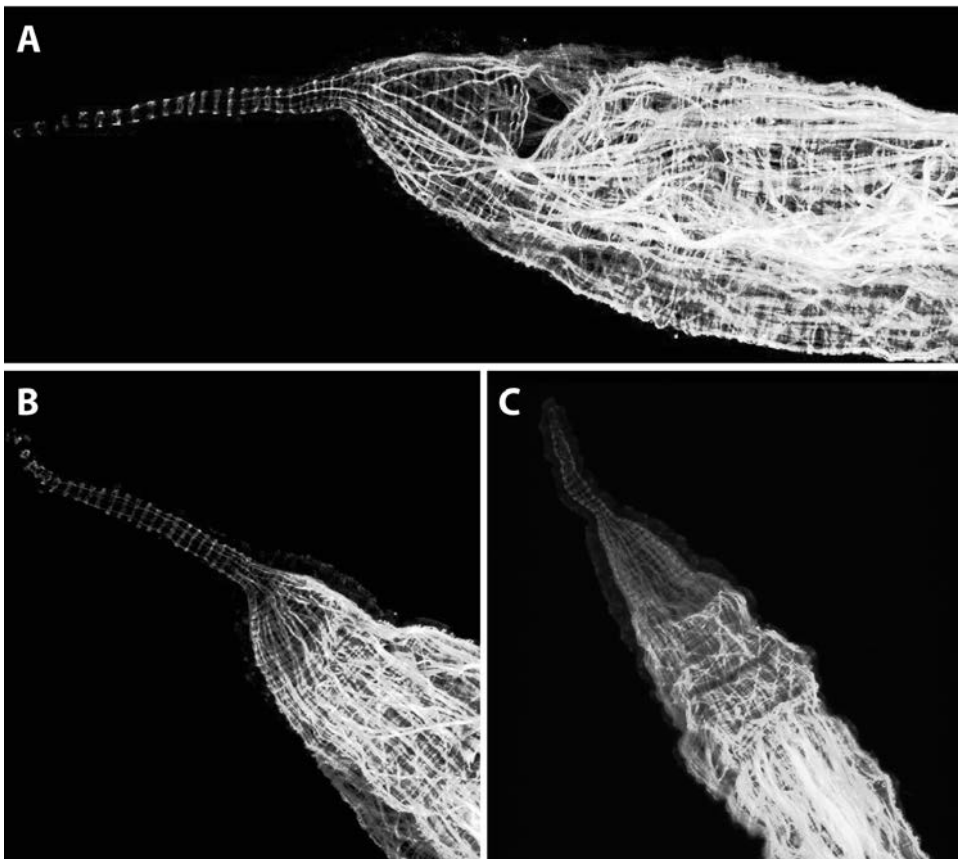

Fig. 7. Musculature of the anterior end and proboscis, phalloidin staining and confocal laser scanning microscopy. A, B. *Pristina aequiseta*. C. *Pristina foreli*.

Karyology

In general, karyological investigations in *Pristina* turn out to be difficult. This is due to problems in squeezing the tissue, as some parts of the tissue tend to form bulges which are hard to squeeze completely flat. Therefore the observation and counting of chromosomes is difficult.

Especially *Pristina aequiseta* was difficult to determine karyologically with the aid of the methods used here. Chromosomes tend to attach together or are difficult to spot reliably due to the tissue problems described above. Careful observation led to a count of a chromosome set of 2n = 34 for *P. aequiseta* (Tab. 4, Fig. 8A, B).

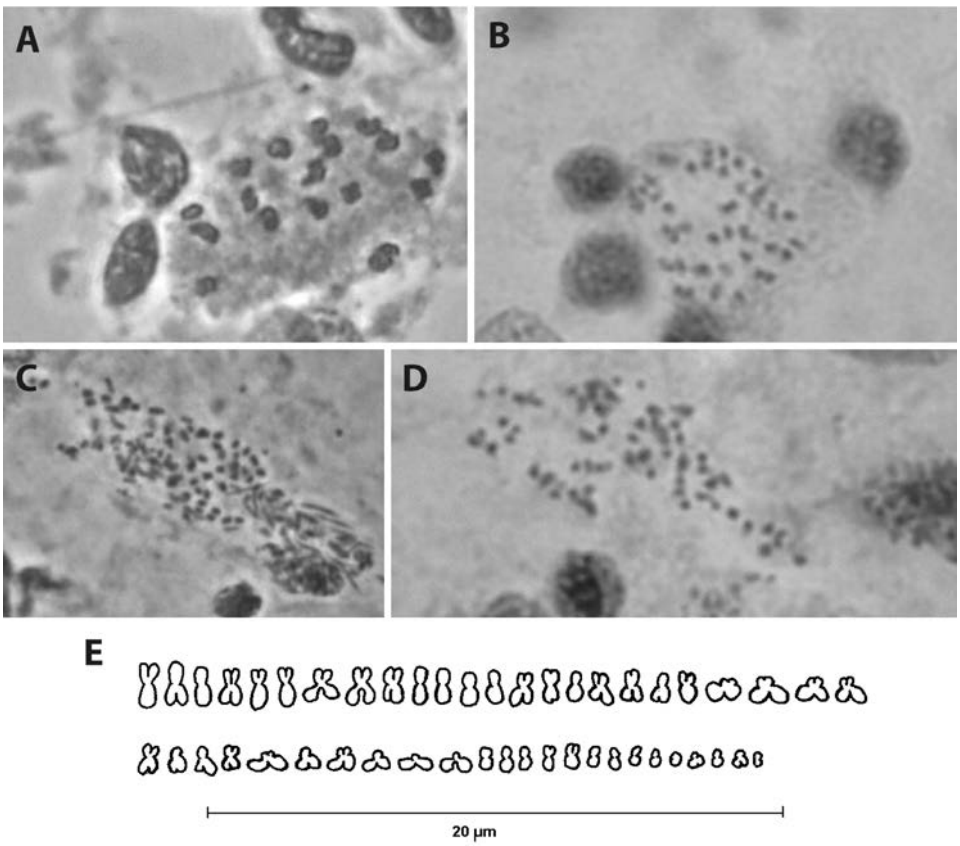

Fig. 8. Karyological methods. A, B. *Pristina aequiseta*, specimens from Bornhöveder See. A. Coelomocyte, somatic metaphase, diplochromosomes. B. Somatic cell, somatic metaphase. Single very small chromosomes. C-E. *Pristina foreli*. C. Somatic chromosomes, specimen from Zoological Institute. D. Somatic chromosomes, specimen from Barumer See. E. Karyogram.

Table 4. Frequency of observed number of somatic chronomosomes (2n) in *P. aequiseta*

Number	30	31	32	33	34
Bornhöveder See	–	2	2	1	17
Barumer See	–	1	1	3	8

For *P. foreli* a somatic chromosome set of 2n = 48 could be counted (Tab. 5, Fig. 8C, D).

Table 5. Frequency of observed number of somatic chromosomes (2n) in *P. foreli*.

Number	46	47	48	49	50
Barumer See	1	1	4	1	1
Zoological Institute	1	3	16	2	1
Fährmannssand	–	–	4	–	1

By comparing the microscopic figure, its photograph and its drawings it was possible to draw an analogous "karyogram" of the somatic chromosome set of *P. foreli* (Fig. 8E). A similar representation for *P. aequiseta* was not successful due to the presence of diplo-chromosomes.

Molecular barcoding

Uncorrected p-distances show slight variability among specimens from one location, stronger values between populations of one species from different locations and very strong values between species. *Pristina aequiseta* shows values of maximally 0.62% in Bornhöveder See and of maximally 0.77% in Fährmannssand. Between these two locations, p-distance is around 8.31%. In comparison of American and German specimens of *P. aequiseta* the p-distance is maximally 11.67%.

For *P. foreli*, uncorrected p-distances between specimens from the Zoological Institute and Fährmannssand are maximally 1.55%.

Between *P. aequiseta* and *P. foreli*, p-distances are maximally 13.6%.

In phylogenetic trees, the German specimens of *P. aequiseta* and *P. foreli* both form separate monophyletic clades with high or reasonably high statistical support (Fig. 9). The sequence from the American *P. aequiseta* is not included into the German *P. aequiseta* specimens, but appears separately.

Additional observation

In the course of the present investigation in the middle of August, 2010, some cocoons were found in an isogenetic culture *of P. aequiseta*, which originally came from the Bornhöveder See (Germany) (Fig. 2B, C). Unfortunately, no mature animals were found, probably due to a strong increase in the number of specimens, in which mature animals could not be found.

Discussion

All three methods applied here support the conclusion that *Pristina aequiseta* and *P. foreli* are separate species. Presences of giant chaetae in segment IV in combination with a comparably long proboscis are good diagnostic characters for *P. aequiseta*. Giant chaetae never occurred in specimens that were assigned to *P. foreli*. Giant chaetae occur early in development and at least one thick chaeta is present in juvenile organisms. The length values measured here (between about 59 and 73 µm) deviate slightly from values given by Sperber (1950) (49–70 µm). The presence of giant chae-

tae in isogenetic cultures of *P. aequiseta* or their absence in isogenetic cultures of *P. foreli* was a constant factor. We did not experimentally change the culture conditions, but there were some variations in water level and related variations in the concentration of ions in the culture. At least under these conditions we never observed the loss or the gain of giant chaetae as hypothesized by Loden & Harman (1980).

Despite the problems with the karyological methods, careful and repeated investigations made it very likely that *P. aequiseta* and *P. foreli* differ considerably in the number of somatic chromosomes (34 in *P. aequiseta* and 48 in *P. foreli*). Hardly any karyological investigations were made before. The undocumented record of McGee & French (1977) that *P. aequiseta* has 12 chromosomes may be due to the problems to count and document the chromosomes in *Pristina* as described above. The number of 48 chromosomes in *P. foreli* is close to the numbers given by Christensen (1980) for Naididae. Also the morphology of the 8–10 larger chromosomes resembles those of other naidids.

The comparison of DNA-sequences shows that both *P. aequiseta* and *P. foreli* form separate clades. A problem is the sequence GQ355374 of an American specimen, which is identified as *P. aequiseta*, but falls outside both *Pristina* clades. As other American sequences determined as *P. aequiseta* fall within the *P. aequiseta* clade, it is likely that at least GQ355374 is not *Pristina aequiseta*. The measured p-distances are comparably high between populations from different localities, at least in *P. aequiseta*. Although falling into the same clade in the CO1-tree, the American and German populations of *P. aequiseta* show p-values of almost 12%, a value that usually supports the conclusion that they represent different species.

Finally, our record of cocoons in a *P. aequiseta* culture show that sexual reproduction occurs at a low rate in this species. The fact that no mature adults were observed can be explained by the large number of individuals in the culture, from which only a fraction could be investigated in detail.

Acknowledgements

We are very thankful to our colleagues Martin Schwentner and Stephan Henne for their help with Genbank submission, genetic methods (MS) and phalloidin staining (SH). We also thank Renate Walter for her help with scanning electron microscopy. Many thanks also go to Ton van Haaren for very helpful comments on the manuscript.

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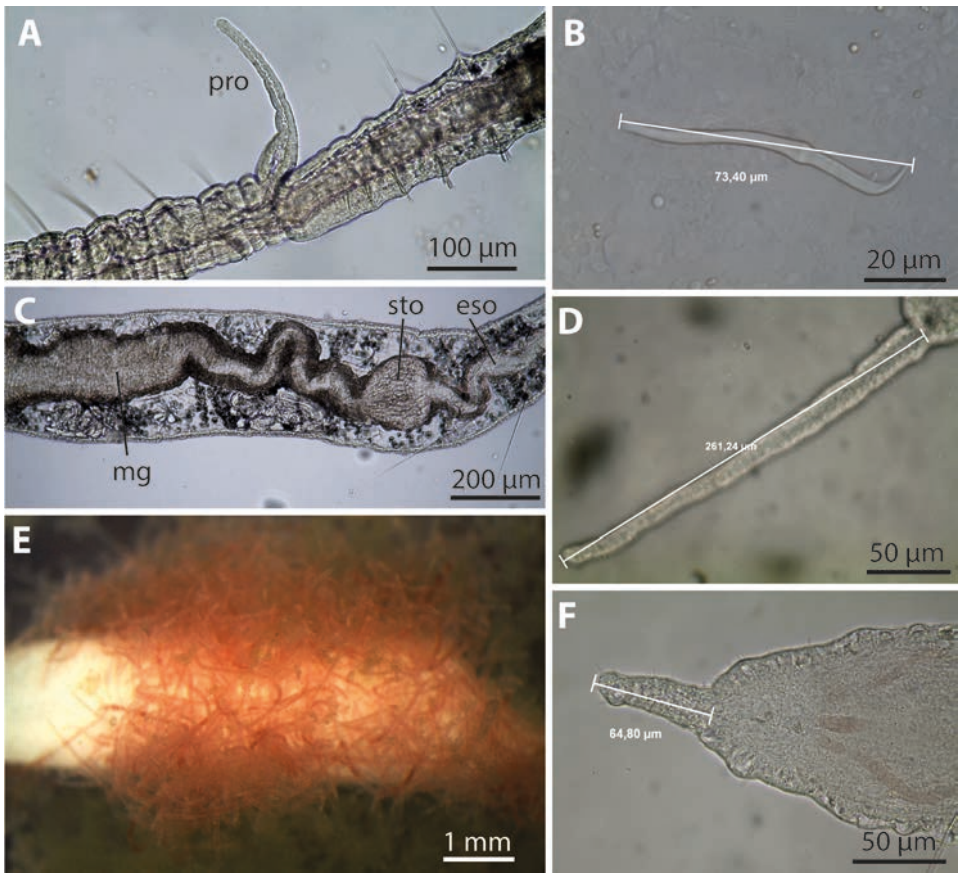

Fig. 1. A. Paratomy in *Pristina aequiseta*, two zooids are attached to each other, the daughter zooid can be recognized by the proboscis (pr). B. Thick seta from segment IV from a *P. aequiseta* from Bornhöveder See to show the way of measurement. C. Stomach with internal canals (sto), between esophagus (eso) and midgut (mg) in *P. aequiseta*. D. Very long proboscis of *P. aequiseta* showing mode of measurement. E. Culture of *P. foreli* on a grain of rice in water. F. Short proboscis of *P. foreli*.

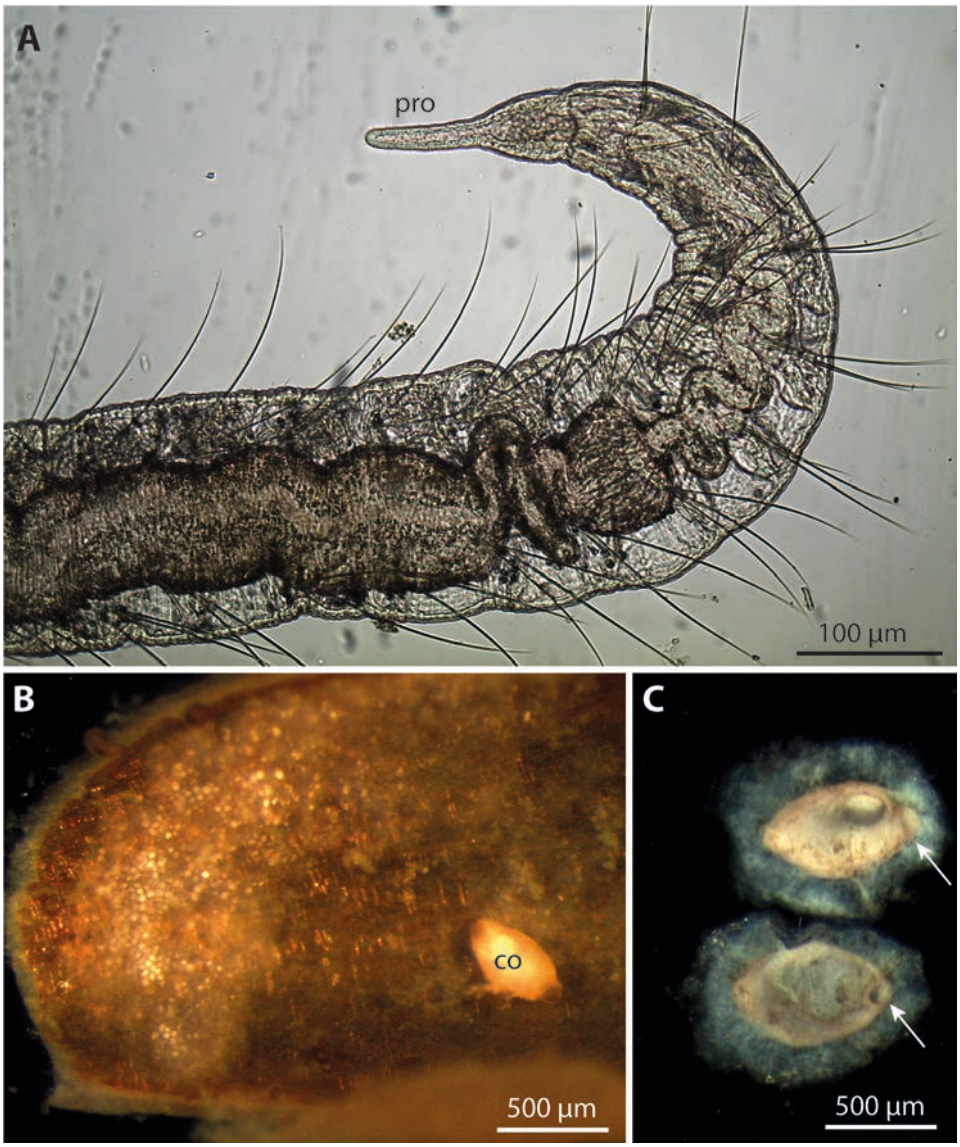

Fig. 2. *Pristina foreli*. A. Entire anterior end with proboscis (pro). B. Cocoon (co) attached to the bran of a grain of wheat). C. Two cocoons. Arrows point at the opening for emergence. The large opening in the upper cocoon is caused by unknown reasons.

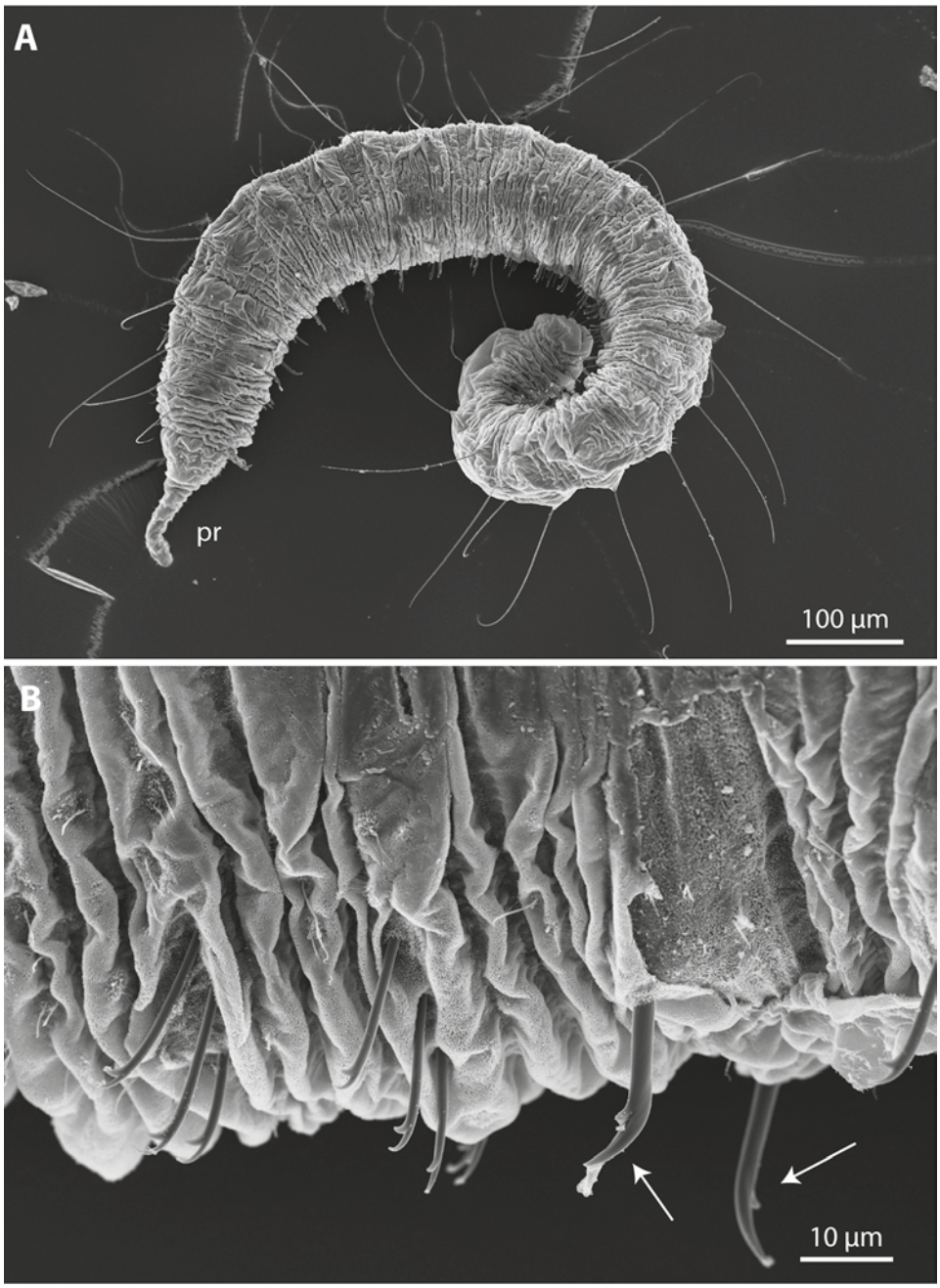

Fig. 3. *Pristina aequiseta,* scanning electron microscopy. A. Entire animal from dorsal, with proboscis (pr). B. Giant chaetae in segment IV (arrows), the anterior segments III and II are to the left.

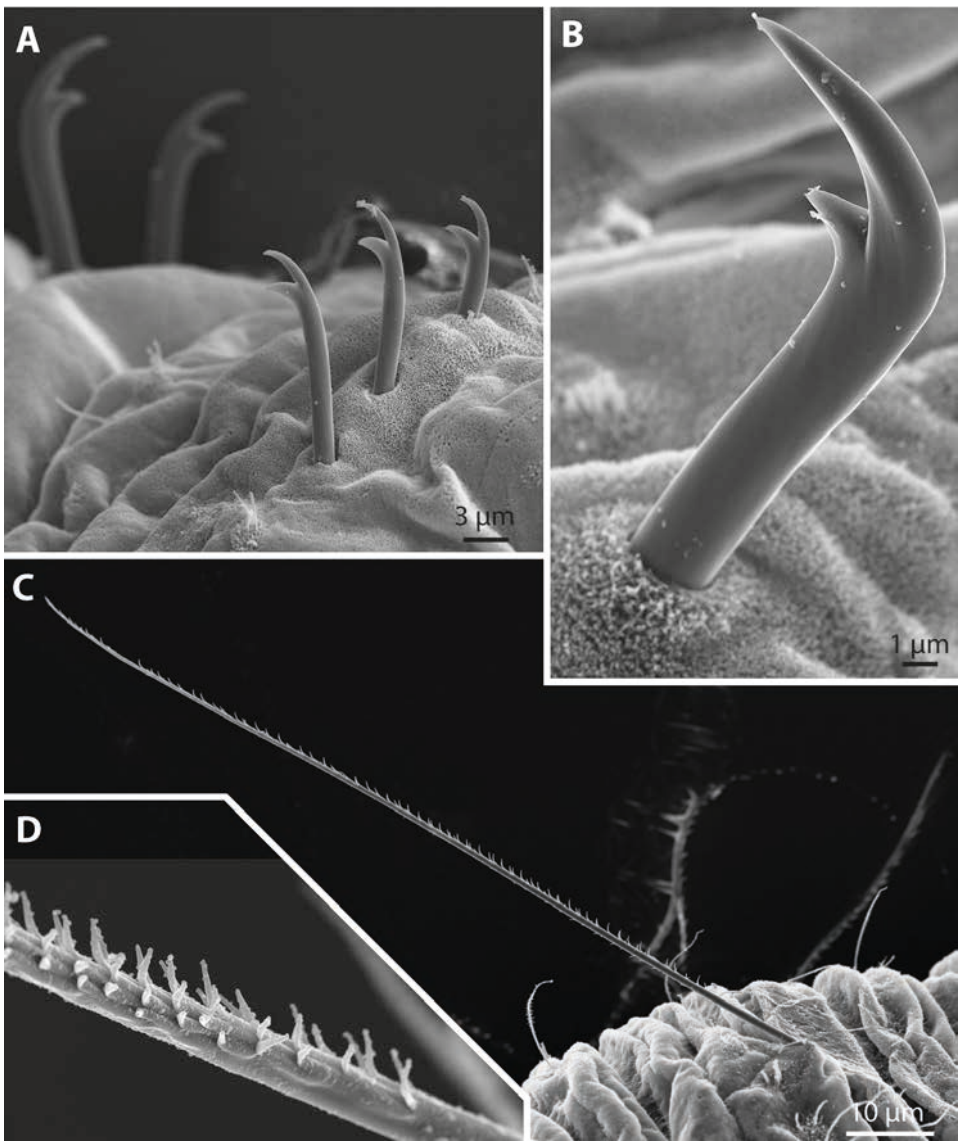

Fig. 4. Chaetae in *Pristina aequiseta*, scanning electron microscopy. A. Ventral chaetae, with giant chaetae of segment IV in the background. B. Magnification of a giant chaeta. C. Dorsal pinnated hair chaeta. D. Fine structure of the pinnate hair chaeta.

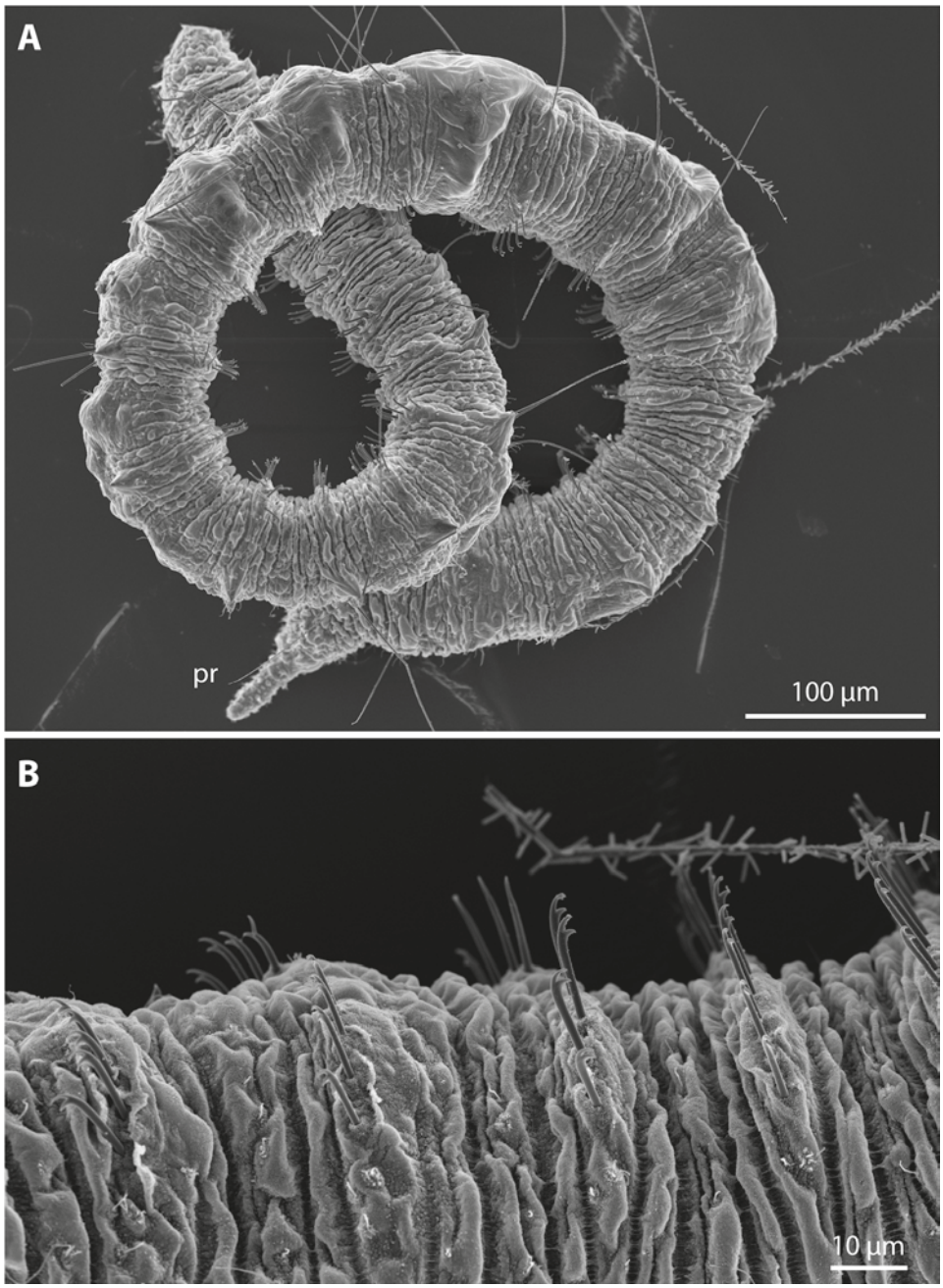

Fig. 5. *Pristina foreli*, scanning electron microscopy. A. Entire animal from dorsal, with proboscis (pr). B. Segments around segment IV showing no giant chaetae.

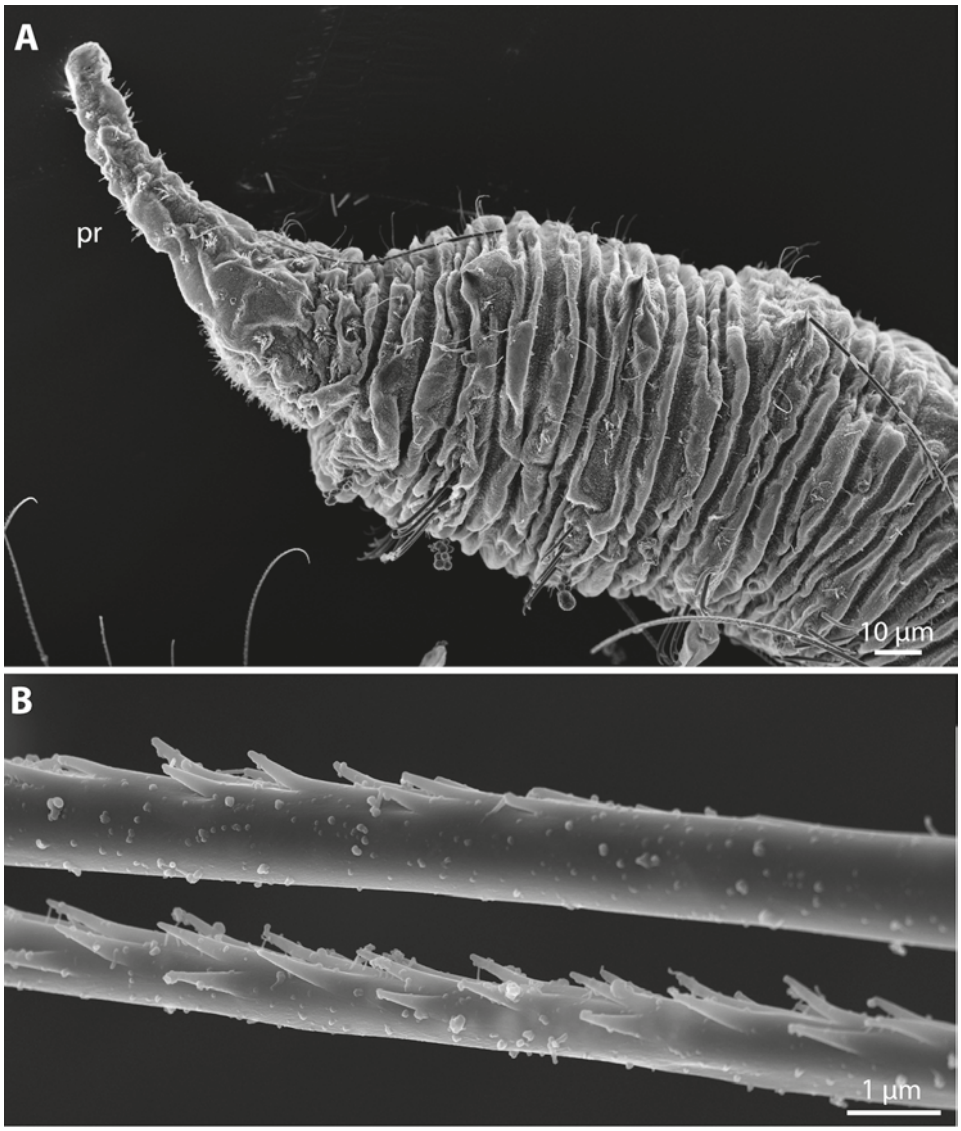

Fig. 6. *Pristina foreli,* scanning electron microscopy. A. Short proboscis (pr). B. Magnification of a dorsal pinnate hair chaeta.

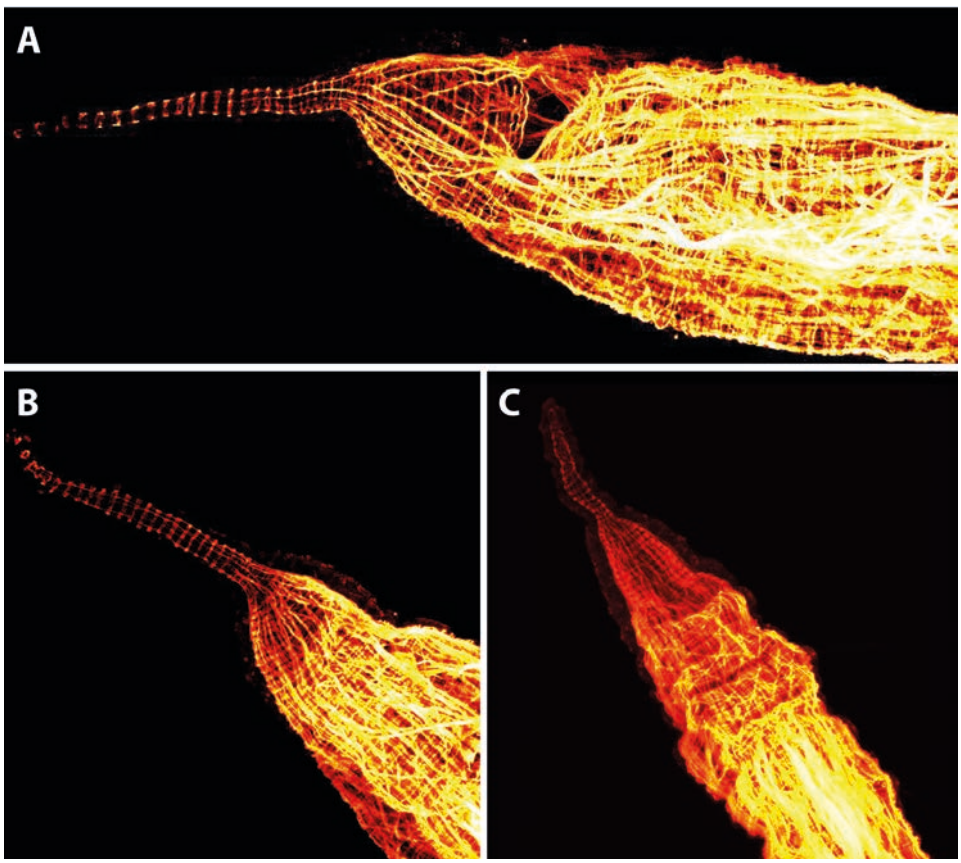

Fig. 7. Musculature of the anterior end and proboscis, phalloidin staining and confocal laser scanning microscopy. A, B. *Pristina aequiseta*. C. *Pristina foreli*.

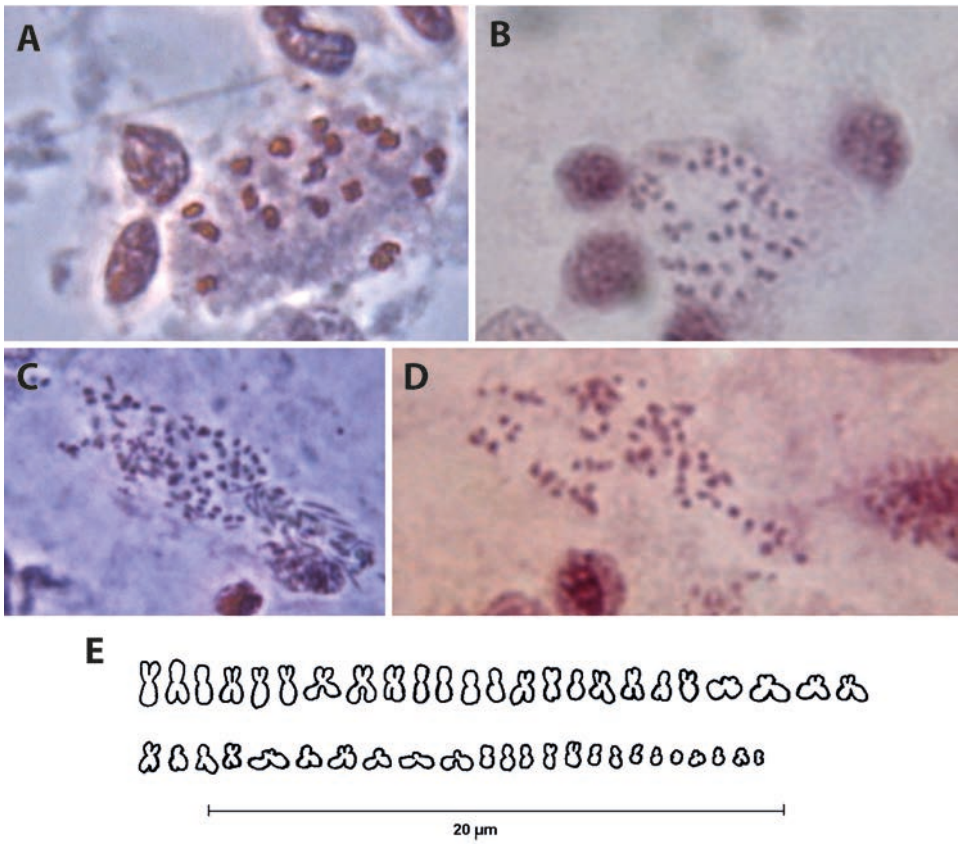

Fig. 8. Karyological methods. A, B. *Pristina aequiseta*, specimens from Bornhöveder See. A. Coelomocyte, somatic metaphase, diplochromosomes. B. Somatic cell, somatic metaphase. Single very small chromosomes. C-E. *Pristina foreli*. C. Somatic chromosomes, specimen from Zoological Institute. D. Somatic chromosomes, specimen from Barumer See. E. Karyogram.