Acta Zoologica Academiae Scientiarum Hungaricae 59(2), pp. 143–156, 2013

HRABEIELLA PERIGLANDULATA (ANNELIDA: "POLYCHAETA") – DO APPARENT DIFFERENCES IN CHAETAL ULTRASTRUCTURE INDICATE THE EXISTENCE OF SEVERAL SPECIES IN EUROPE?

Dózsa-Farkas, K.[1] and Schlaghamerský, J.[2]

[1]*Eötvös Loránd University, Department of Systematic Zoology and Ecology*
H-1117 Budapest, Pázmány Péter sétány 1/C, Hungary; E-mail: kdozsafarkas@gmail.com

[2]*Masaryk University, Faculty of Science, Department of Botany and Zoology*
Kotlářská 2, CZ-611 37 Brno, Czech Republic; E-mail: jiris@sci.muni.cz

Hrabeiella periglandulata (Annelida: "Polychaeta"), one of only two known exclusively terrestrial non-clitellate annelids ("polychaetes"), has been found at an increasing number of sites in Europe and has also recently been reported from Korea. Until recently it was known only from a zone from southern Sweden across central Europe to central Italy. Looking at SEM micrographs, the ultrastructure of the unique chaetae of *Hrabeiella periglandulata* appeared to differ rather markedly among different populations, which gave rise to the question of whether they all belonged to a single species. To resolve this question, specimens from a total of 17 sites were collected, ranging from Spain in the West to Romania in the East. Specimens from these populations were processed by two different methods and examined using SEM. The results obtained indicate that apparent differences, as published for some Italian and German populations, can be caused by even small differences in the fixation procedure preceding the SEM examination. Such causes might lead to false conclusions when judging morphological differences visible by SEM within taxonomical studies. We conclude that chaetal morphology and ultrastructure point at the existence of only one species of *Hrabeiella* that is widely distributed. We present 19 previously unpublished localities for the species (not fully overlapping with the 17 collection sites used in our SEM examination), including the first records for four countries: Spain, Denmark, Hungary, and Romania.

Keywords: *Hrabeiella periglandulata*, Polychaeta, structure of chaetae, SEM investigation

INTRODUCTION

Hrabeiella periglandulata Pižl et Chalupský, 1984, is one of only two truly and exclusively terrestrial non-clitellate annelids, the other being *Parergodrilus heideri* Reisinger, 1925. Both species are found in soils, the former preferring the upper mineral layer of slightly acidic forest and meadow soils, the latter the litter layer of moderately acidic forest soils (see Graefe & Schmelz 1999, Graefe & Beylich 2003, and authors cited below with regard to distribution data). The systematic position of *H. periglandulata* has been an enigma and has

not yet been fully resolved, despite numerous investigations touching this matter (e.g. Struck & Purschke 2005, Struck *et al.* 2008, Rousset *et al.* 2007, Zrzavý *et al.* 2009). The interest in both species has been also driven by the fact that many questions regarding annelid and clitellate phylogeny remain open. They resemble terrestrial clitellates to some extent, but lack several of their characters, foremost a clitellum, making them interesting models for considerations of convergent adaptations to life in soil (Purschke 1999), but also potential basal members of a hypothetical lineage within the paraphyletic "Polychaeta" that may lead towards Clitellata (Purschke *et al.* 2000, Purschke 2003, Jördens *et al.* 2004). Within these studies it was convincingly shown that *H. periglandulata* and *P. heideri* were not closely related (Rota 1998, Rota *et al.* 2001, Jördens *et al.* 2004). Based on an ultrastructural examination, Purschke (2003) suggested that *H. periglandulata* might be the sister group of the Clitellata, but the molecular study of Jördens *et al.* (2004) did not provide any definitive support for this hypothesis. Nevertheless, Christoffersen (2012) included *H. periglandulata* as the sister group of Euclitellata within Clitellata sensu lato. Rousset *et al.* (2007) questioned the notion of a close relationship of *H. periglandulata* to Clitellata or Aphanoneura, whereas Zrzavý *et al.* (2009) confirmed the notion of a taxon (Clitellatomorpha) including *H. periglandulata* and Aphanoneura as one sister group and the true Clitellata as the other.

Pižl and Chalupský (1984) described *H. periglandulata* from South Bohemia, i.e. the south-west of the Czech Republic (= Czechia). Until recently it was only known from Europe, ranging from southern Sweden (Erséus & Rota 1998) in the North to central Italy in the South (Rota & Lupetti 1996, 1997, Ascher *et al.* 2012) and from Germany in the West (Jans & Römbke 1989, Graefe 1989, 1990, 1993*a, b,* Purschke 1999, Haag *et al.* 2009), across western Austria (Bauer 2003, 2004) to south-eastern Poland (Dumnicka & Rożen 2002) and south-eastern Czechia (Schlaghamerský & Šídová 2007, 2009) in the East. Recently, the species was also found in Korea (Dózsa-Farkas & Hong 2010), indicating a much wider distribution than had been assumed before. Investigations under the light microscope did not show any conspicuous morphological differences between populations, the chaetae resembling a trident with the three prongs interconnected by a thinner lamina into a small shovel- or ice-spoon-shaped blade. However, investigations of external morphology by scanning electronic microscope (SEM) revealed striking differences in the ultrastructure of the chaetae. Jans and Römbke (1989) presented some SEM photographs with a brief description of chaetal morphology. They found the ectal blades of the chaetae to be curved, sail-shaped, and scaly, with three apical tips (according to Jans & Römbke 1989, Rota & Luppetti 1996, call this shape "flame-like") and a joint-like base. Rota and Lupetti (1996), in contrast, found chaetae resembling a handled brush: a "shaft, which is devoid of a nod-

ulus, supports distally a flattened, quadrangular cushion, which gives off on one side numerous, densely packed 'bristles'". Thus it seemed questionable whether all of the investigated populations did indeed belong to a single species or if there were at least two distinct species within Europe. Already Rota and Lupetti (1996) suggested that a reinvestigation of topotypes of *H. periglandulata* was "needed to clarify the extent of variation of chaetal morphology and to establish whether the Italian or the German populations might be recognized as a separate species rather than a variant of the Bohemian ones". Purschke (1999) investigated specimens from northern Germany and found chaetae similar to the Italian specimens under SEM. However, he suggested that the apparent differences between chaetae of different populations could be the effect of different handling of the specimens prior to their examination by SEM (Purschke 1999).

In the present study, we try to answer the question of the species-identity of European *Hrabeiella* populations, based on the investigation of the chaetal ultrastructure (as shown by SEM) of specimens from several European populations (including the type locality), using various fixation procedures. We also present previously unpublished localities for the species from seven European countries, including first records for the fauna of four countries, thus substantially expanding the known range of *Hrabeiella* within Europe. Our study was triggered by the first finds of *Hrabeiella periglandulata* in Hungary, which we investigated under the light microscope as well as by SEM, based on our initial examinations the latter showed a chaetal ultrastructure resembling that described by Jans and Römbke (1989), but as shown below this case was not that straightforward.

MATERIALS AND METHODS

Hrabeiella specimens collected in Hungary (in the Kőszeg and in the Bakony Mts), and Romania (Gutin Mts) were extracted from soil samples by the wet funnel method (O'Connor 1962). They were kept in water, examined and measured alive under the light microscope. The important morphological structures were noted, drawn and photographed with a Zeiss Axio Imager.A2 microscope, using DIC (differential interference contrast) illumination and an AxioCam MRc 5 (Zeiss) digital camera with Axiovision software. Subsequently some specimens were preserved in 70% ethanol and some were permanently mounted in Euparal on a microscope slide. Twenty-one specimens were then prepared for scanning electron microscopy by dehydration through an ethanol series (96%, absolute), critical-point dried (CO_2), mounted on stubs, and sputter-coated with gold-palladium. Scanning electron micrographs were taken in the Hungarian Natural History Museum, Budapest, with a Hitachi SN 2600 scanning electron microscope.

To obtain a more general overview of the variability of chaetal morphology in *Hrabeiella*, additional specimens from populations across Europe (ranging from north-western Spain to north-western Romania) were collected by the authors or obtained from fellow

Table 1. Collecting sites of specimens used for the SEM investigation and other, hitherto unpublished, localities where *Hrabeiella periglandulata* was recorded. Set in bold are countries for which the listed records are the first published ones, geographic coordinates of hitherto unpublished localities, and collectors of material for those sites from which specimens were used for our SEM investigation.

Country	Locality (situation and habitat)	Collector and collecting date
Spain	Galicia, Prov. Coruña, Sobrado dos Monxes, **43°02′12″N, 08°00′33″W**, ca 530 m a.s.l.; pasture near a lake	**R. M. Schmelz**; April 1994
Spain	Galicia, Prov. Coruña, west of Cesuras, near River Mendo: **43°10′36″N, 08°10′27″W**, ca 214 m a.s.l.	**R. M. Schmelz**; Oct. 2007
Denmark	Eastern Jutland, Hestehaven Forest near Aarhus (former IBP study area), **ca 56°17′14″N, 10°28′24″E**, ca 15 m a.s.l.; beech forest	**R. M. Schmelz**; Nov. 1998
Germany	Waldpark Falkenstein near Hamburg; **53°33′54″N, 9°45′56″E**, ca 20 m a.s.l.; landscape park with mixed forest (deciduous trees and pine)	**U. Graefe**; 31 July 1972
Germany	Amsinckpark, Hamburg; **53°36′06″N, 9°56′36″E**, ca 20 m a.s.l.; lawn within a landscape park.	U. Graefe; 20 Oct. 1992, 1. Nov. 2002
Germany	Eifel Mts. near Aachen, Soil Monitoring Site "BDF NRW Lammersdorf Buche"; ca **50°39′57″N, 6°14′57″E**, ca 450 m a.s.l., deciduous forest (beech, oak) with interspersed spruce	**U. Graefe**; 2009 (first record from 22 Oct. 2004)
Germany	Lehndorf-Watenbüttel near Braunschweig (land of the Thünen-Institute), **52°17′02″N, 10°26′36″E**, 85 m a.s.l.; oak-beech wood	**J. Schlaghamerský**; 16 July 2010
Austria	Salzburg state, permanent soil monitoring plot (Bodendauerbeobachtungsfläche 2) near Sankt Koloman, **ca 47°39′ N, 13°11′ E**, 1005 m a.s.l.; grassland	R. Bauer; 12 Sept. 2002
Austria	Eastern Austria, Rax Mountains, **47°43′01″ N, 15°46′32″E**, 1563 m a.s.l.; spruce forest	**J. Farkas**; 15 May 2012
Czechia	NW-Bohemia, Bohemian Switzerland Nat. Park, alluvium of the stream Brtnický potok; **50°56′01″N, 14°24′19″E**, 313 m a.s.l.; open maple-ash stand on sandy alluvial deposits	**J. Schlaghamerský**; 3 Oct. 2009 & 22 June 2010
Czechia	NW-Bohemia, Bohemian Switzerland Nat. Park, Häuschengrund ravine; **50°52′36″N, 14°22′21″E**, 324 m a.s.l.; sandy alluvial deposits along a small, intermittent brook in a spruce forest	**J. Schlaghamerský**; 3 Oct. 2009

Table 1 (continued)

Country	Locality (situation and habitat)	Collector and collecting date
Czechia	S-Moravia, Brno municipal forest: Holedná Game Park at Jundrov; 49°12′17″N, 16°32′38″E, 300 a.s.l.; mixed forest (pine, oak, beech)	**J. Schlaghamerský**; April 2010
Czechia	Locus typicus: South Bohemia, meadow on Věncová hora between the villages Krnín, Dolní Třebonín and Zlatá Koruna; 48°51′26″N, 14°23′37″E, 550 m a.s.l.; meadow at forest edge	**J. Schlaghamerský**; 27 April 2010
Hungary	Western Hungary, Kőszeg Mts. (foothills of the Alps), near Velem; **47°22′N, 16°29′E**, ca 600 m a.s.l.; pine forest	**K. Dózsa-Farkas**; 24 April 1985
Hungary	Western Hungary, Kőszeg Mts. (foothills of the Alps), near Velem; **47°21′07″N, 16°29′09″E**, 430 m a.s.l.; mixed forest (hornbeam-oak-beech-pine)	**K. Dózsa-Farkas**; 6 March 2009
Hungary	Western Hungary, Kőszeg Mts., Steier Houses, **47°21′10″N, 16°28′30″E**, 563 m a.s.l.; mixed forest (pine and deciduous trees)	**G. Szövényi**; 22 Jan. 2011
Hungary	Bakony Mts., near Nyirád; **47°01′21″N, 17°23′34″E**, 209 m a.s.l.; oak forest (Turkey oak with some hornbeam, with *Pteridium aquilinum* in undergrowth)	K. Dózsa-Farkas & J. Farkas; 8 May 2008
Hungary	Bakony Mts., Hárskút, Esztergáli-Valley, **47°10′58″N, 17°48′49″E**, 490 m a.s.l.; meadow	K. Dózsa-Farkas & J. Farkas; 8 May 2008
Hungary	Bakony Mts., Öreg Szarvad Graben; **47°17′13″N, 17°42′54″E,** 450 m a.s.l.; deciduous forests	**G. Boros & A.Kunszeri**; 14 Nov. 2009
Romania	Gutin Mts., (Munții Gutâi; Eastern Carpathians), near Mogosa (ski resort) and lake (lacul) Bodi, **47°40′52″N, 23°47′14″E**, 698 m a.s.l.; beech forest and a clearing	**K. Dózsa-Farkas**; 17 Sept. 2005
Romania	Apuseni Mts., Mărişel; **46°40′06″N, 23°08′43″E**, 900 m a.s.l.; hornbeam-beech forest	**Cs. Csuzdi, J. Kontschán, V.V. Pop & Zs.Ujvári**; 24 Oct. 2009

researchers from other countries, who either submitted fixed specimens (some already collected many years ago) or soil samples (Table 1). The investigated populations included the locus typicus of *Hrabeiella periglandulata* at Věncova hora (South Bohemia, Czechia). Unfortunately, we did not succeed in obtaining live specimens from Tuscany, Italy, despite many attempts. In those cases in which live specimens were obtained by one of the authors (those listed as such in Table 1 and from the Rax Mountains, Austria), these were also examined under the light microscope. Twenty-two specimens preserved in 96% ethanol or, in a few cases (specimens from the Spanish sites), in Bouin solution or formaldehyde were prepared for the SEM as described above.

To clarify the question if different fixation procedures were responsible for apparent differences in chaetal ultrastructure, further live specimens from the Kőszeg Mountains, Hungary, were collected and eight of those were treated following the procedure as described by Rota and Lupetti (1996), namely: the specimens were fixed in Karnovsky's fluid for 1 h, washed twice in cacodylate buffer (pH 7.4) and postfixed in a cacodylate–buffered 0.5% osmium tetroxide solution (at 4 °C, for 90 min). For SEM, after rinsing twice in cacodylate buffer, they were dehydrated in ethanol, critical-point dried, mounted on aluminium stubs and sputter-coated with gold. They were examined and photographed with a Hitachi S-2360N scanning electron microscope at the Department of Plant Anatomy of Eötvös University, Budapest.

RESULTS

New recordings

Below we list (from West to East) 19 so far unpublished records (localities) of *Hrabeiella periglandulata* from Europe (Table 1). These include the first country records for Spain, Denmark, Hungary and Romania. The site from western Austria presented below is based on an unpublished report of Bauer (2004), the records from Hamburg (Amsinckpark and Waldpark Falkenstein) on the unpublished report of Graefe *et al.* (2003) and on additional information received from U. Graefe (in lit. 2012). European localities with known occurrence of *H. periglandulata* (previously published or presented here) are shown in Fig. 1. One locality from Bohemian Switzerland (Czechia) and the Amsinckpark in Hamburg (Germany) had been previously shown in a distribution map (Schlaghamerský & Šídová 2009), but without locality names or any further details, these are therefore shown with the symbol for "previously published sites" in Fig. 1 but listed as new localities in Table 1.

Chaetal morphology

The morphology of specimens under the light microscope was identical with the earlier descriptions, therefore only a few characteristic photos are presented here (Figs 2A–D).

The SEM analysis of Hungarian and the other European specimens dehydrated in an ethanol series and sputter-coated with gold-palladium showed that chaetae of *H. periglandulata* can be found in segments II–XV, distributed mostly in two ventro-lateral and two ventral bundles per segment, each containing 2–3 chaetae (Figs 3A, C, D, 4A). A closer examination reveals that the chaetae were not completely uniform: The posterior chaetae and those of the (medio-) ventral pairs were shovel-shaped (Fig. 4C), the ventro-lateral ones more elongated and pointed (Fig. 3B, 4B). Our photos were compared with the results of Jans and Römbke (1989), Rota and Lupetti (1996) and Purschke (1999), and it was observable, that they resembled mostly Jans' and Römbke's (1989, p. 161. Fig. 4) results, and did not show the very different "handled brush" (consisting of parallel fibres) described by Rota and Lupetti (1996, p.

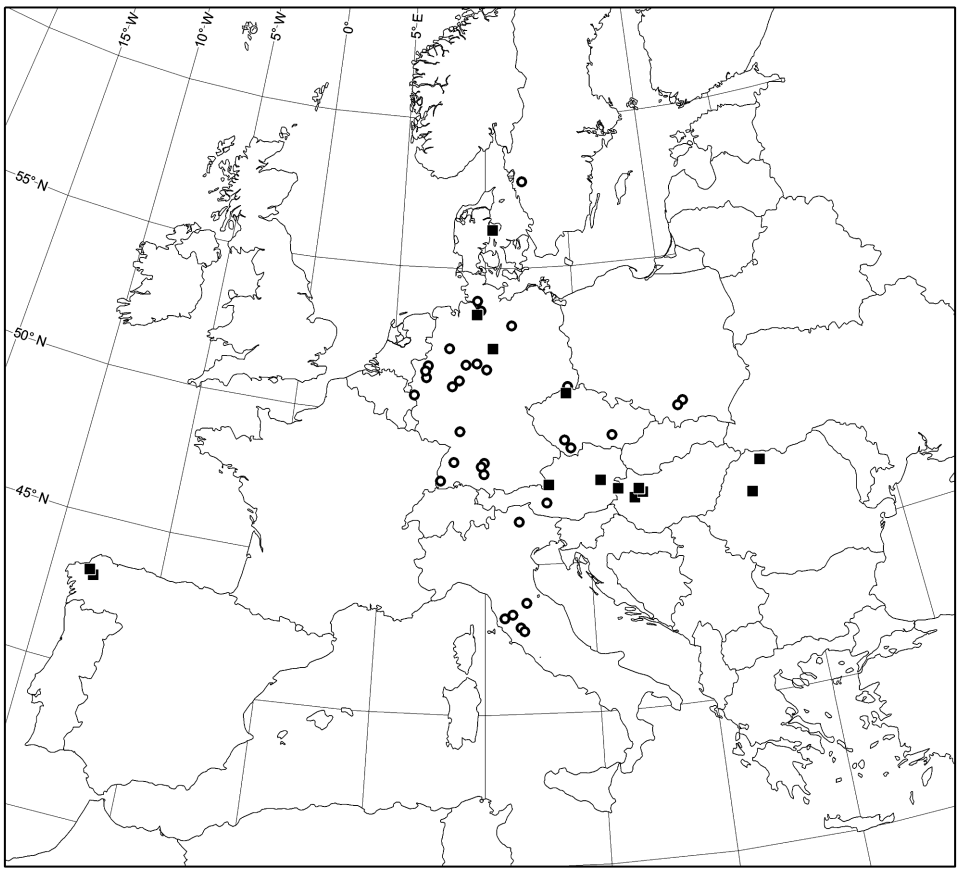

Fig. 1. Distribution of *Hrabeiella periglandulata* in Europe based on records previously published (circles) or reported in this study (squares).

232, Fig. 8) and also found by Purschke (1999. p 3. Fig 1F). The examination of the chaetal ultrastructure of specimens from the other European populations included in the study (Table 1) showed the same chaetal morphology in all investigated specimens with only small differences among them (Figs 3B–D, 4A, B). The observed chaetal morphology was very similar to that shown by the SEM photographs of Jans and Römbke (1989). This was also true for the specimens from Spain, the most remote and also the furthest south of the populations investigated.

However, the SEM analysis of Hungarian specimens fixed by the method of Rota and Lupetti (1996) showed a chaetal ultrastructure that to a fair extent resembled the one found in Italian specimens by Rota and Lupetti (1996) and in specimens from northern Germany by Purschke (1999). Although not identical, it was apparent that the two flattened laminae bending towards each other were not built up by sheets but in all cases by thin fibres, clinging to each other, that were emerging from flattened quadrangular cushions (Fig. 5A, B). In several cases, their real structure was more clearly visible at the beginning or at the end of the chaetae (Fig. 4D, 5C, D). Also here, we observed that the ventro-lateral chaetae were somewhat more elongate and pointed than the ventral ones.

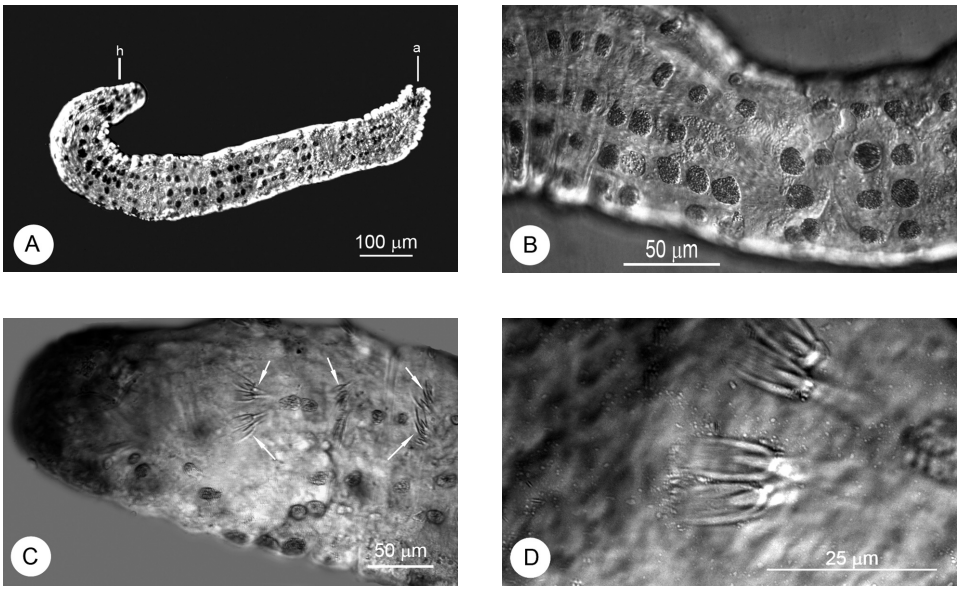

Fig. 2. Light microscope micrographs of *Hrabeiella periglandulata* specimens from Hungary (Bakony Mts), in vivo: A = entire specimen (a = anus, h = head), B = the characteristic epidermal glands, dorsal view, C–D: Light microscope micrographs of chaetae (specimens from Gutin Mts, Romania), in vivo: C = the first three rows of chaetae marked with arrows, D = two groups of chaetae.

DISCUSSION

Our study shows that *Hrabeiella periglandulata* has a much wider distribution in Europe than previously reported. The increasing number of known localities indicates that *H. periglandulata* is a rather common inhabitant of grassland and forest soils from lowland to montane-subalpine elevations. We found that *Hrabeiella* specimens from mutually distant populations from the western-most one in north-western Spain to the eastern-most one in north-western Romania have virtually the same ultrastructure of chaetae. We were not able to fully reproduce the brush-like state of the chaetae, with the fibres completely separated, as found by Rota and Lupetti (1996) and Purschke (1999). Even when we followed their fixation method, the fibres of which the chaetal blades were composed were more or less sticking together. Nevertheless, based on the results obtained, we are convinced that the degree to which the fibres are free, showing a brush-like structure, or stick together and be-

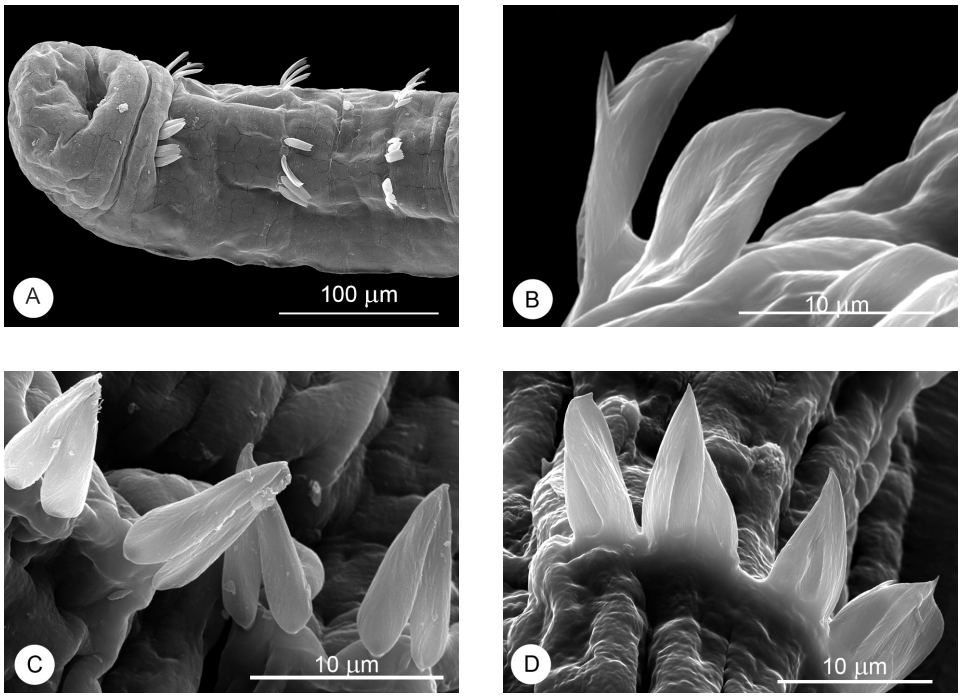

Fig. 3. *Hrabeiella periglandulata*, chaetae, SEM micrograph (the worms were fixed in ethanol, the specimen from Spain fixed in Bouin solution): A = anterior part of body with three rows of chaetae of a specimen from Bakony Mts, Hungary (ventro-lateral view), B = lateral chaetae in the second row (specimen from Brno, Czech Republic), C = chaetae in the second row (ventral view, specimen from Věncová hora (locus typicus), Czech Republic), D = chaetae in the fourth row (ventral view, specimen from Coruña province, Spain).

come less visible, depends on differences in the fixation process. The apparent differences in chaetal ultrastructure of *Hrabeiella* specimens from different European populations, published by the above-mentioned authors or obtained by us, can be considered artefacts. Purschke (1999) had already hypothesized that the dissimilarities described by Jans and Römbke (1989) in comparison with the chaetal morphology found by Rota and Lupetti (1996) and himself were due to fixation artefacts rather than to genetic differences. We also believe that the brush-like structure is closer to the true structure than the scaly one with all fibres sticking together. However, one can not exclude the possibility that some fixation procedures eliminate adhesive properties of these fibres. Generally, fixation in alcohol may obscure many surface properties of the object. Unfortunately, most specimens available to us had been fixed in alcohol and a comparison across Europe therefore had to be based on these specimens. Only the Spanish specimens had been fixed either in Bouin or in formaldehyde solution, but we did not observe any difference between the

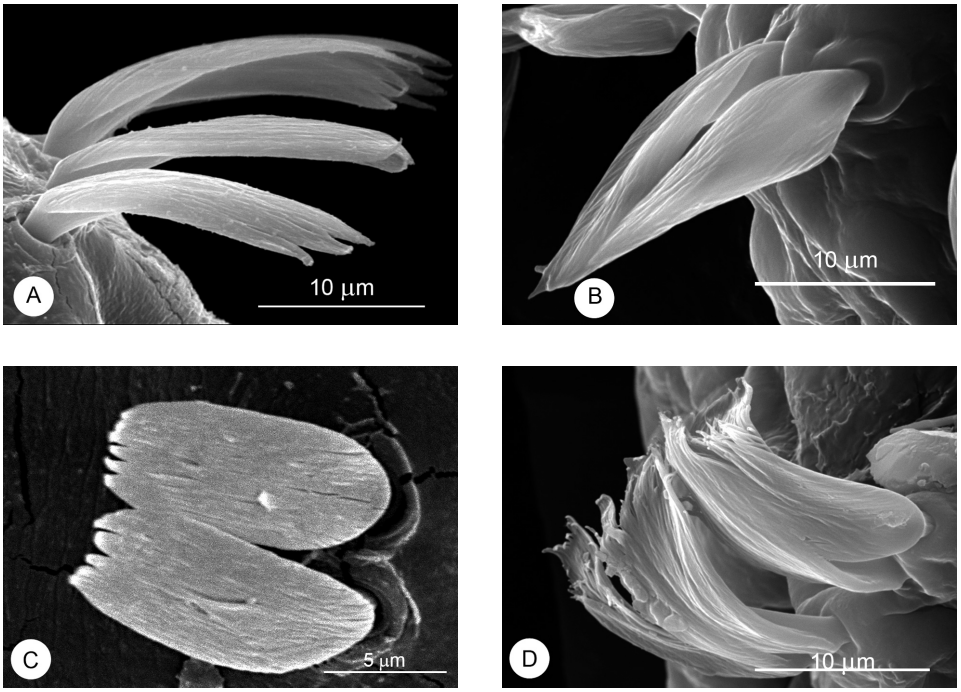

Fig. 4. SEM micrographs of *Hrabeiella periglandulata* chaetae showing a different shape (the worms were fixed only in ethanol): A = Lateral chaetae of a specimen from Hungary. B = Lateral chaetae in the third row of a specimen from north-western Czechia. C = Ventral chaetae of the last rows of a specimen from Hungary (Bakony Mts). D = Chaetae in the fifth row of a specimen from south-western Czechia (locus typicus, Věncová hora).

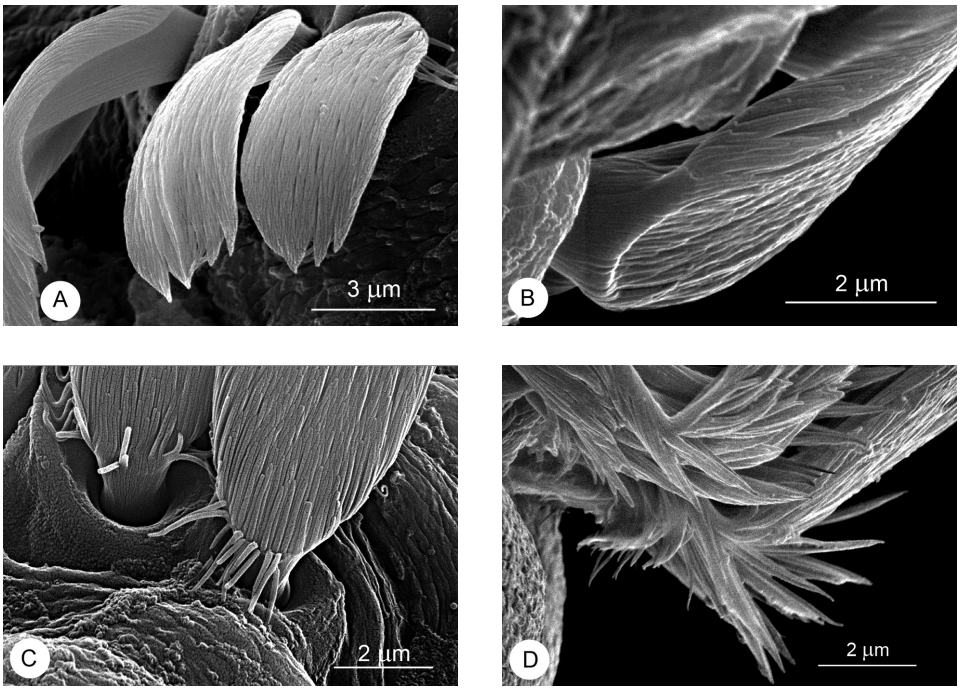

Fig. 5. *Hrabeiella periglandulata* – structure of chaetae with fine fibres visible (second fixation method employed – see Material and Methods), specimens from Kőszeg Mts, Hungary. A–B = The origin of fibres at the quadrangular cushion. C = The fibres more visible at the base, where some are separated from the rest. D = Many of the fibres separated.

appearance of their chaetae and the chaetae of specimens fixed in alcohol (cf. Figs 3C–D). We conclude that based on chaetal morphology alone, all investigated populations can be considered to belong to a single described species, *Hrabeiella periglandulata*, including the Italian population investigated by Rota and Lupetti (1996). Apparent differences in ultrastructure thus might lead to false conclusions when not obtained by exactly the same fixation procedure.

*

Acknowledgements – Many thanks to Dr. Rüdiger M. Schmelz and Dr. Ulfert Graefe for sending fixed specimens, and to Dr. Gergely Szövényi, Balázs Németh, Gergely Boros, and János Farkas for providing soil samples from various localities. We are also grateful to Dr. Károly Bóka, Dr. Jenő Kontschán, Zsolt Újvári, and Dr. Krisztina Buczkó for their help during the SEM analysis, and to the Department of Botany of the Hungarian Natural History Museum and the Department of Plant Anatomy of Eötvös University, Budapest, for permitting the use of their SEM microscopes. Maxwell V. L. Barclay (Natural History Museum, London) kindly checked the English of the final manuscript version. This research was

partially financed by the Hungarian Scientific Research Found (OTKA 49635 and K77999). Three anonymous referees contributed with their critical comments and additional information on the distribution of *H. periglandulata* to the final version of the present study.

REFERENCES

Ascher, J., Sartori, G., Graefe, U., Thornton, B., Ceccherini, M. T., Pietramellara, G. & Egli, M. (2012) Are humus forms, mesofauna and microflora in subalpine forest soils sensitive to thermal conditions? *Biology and Fertility of Soils* **48**: 709–725.

Bauer, R. (2003) Characterization of the decomposer community in Austrian pasture and arable field soils with respect to earthworms and potworms (Annelida: Lumbricidae and Enchytraeidae). *Newsletter on Enchytraeidae* **8**: 41–50.

Bauer, R. (2004) *Bodenzoologische Untersuchungen (Lumbricidae und Enchytraeidae) auf den BDF 1–8, 1996–2004, Endbericht an Amt der Salzburger Landesregierung, Abteilung 4.* [Unpublished report to the Salzburg state government, http://www.salzburg.gv.at/bodenzoologische_untersuchungen_auf_bodendauerbeobachtungsflaechen.pdf. Accessed 24 Sept. 2012.]

Beylich, A. & Graefe, U. (2009) Investigations of annelids at soil monitoring sites in Northern Germany: reference ranges and time-series data. *Soil Organisms* **81**: 175–196.

Christoffersen, M. L. (2012) Phylogeny of basal descendants of cocoon-forming annelids (Clitellata). *Turkish Journal of Zoology* **31**: 95–119.

Dózsa-Farkas, K. & Hong, Y. (2010) Three new Hemienchytraeus species (Enchytraeidae, Oligochaeta, Annelida) from Korea, with first records of other enchytraeids and terrestrial polychaetes (Annelida). *Zootaxa* **2406**: 29–56.

Dumnicka, E. & Rozen, A. (2002) The first record of the terrestrial polychaete Hrabeiella periglandulata Pižl & Chalupský, 1984, in Poland, with a note on anatomy and ecology. *Fragmenta Faunistica, Warszawa* **45**: 1–7.

Erséus, C. & Rota, E. (1998) Polychaetes (bristle worms) on dry land. *Fauna och Flora* **93**: 97–104.

Graefe, U. (1989) Einfluss von saueren Niederschlägen und Bestandeskalkungen auf die Enchytraeidenfauna in Waldböden. *Verhandlungen der Gesellschaft für Ökologie* **17**: 597–603.

Graefe, U. (1990) Untersuchungen zum Einfluss von Kompensationskalkung und Bodenbearbeitung auf die Zersetzerfauna in einem bodensaueren Buchenwald- und Fichtenforst-Ökosystem. *In:* Gehrmann, J. (ed.): *Umweltkontrolle am Waldökosystem*. Forschung und Beratung, Reihe C, Münster **48**: 232–241.

Graefe, U. (1993*a*) Die Gliederung von Zersetzergesellschaften für die standortsökologische Ansprache. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft* **69**: 95–98.

Graefe, U. (1993*b*) Veränderungen der Zersetzergesellschaften im Immissionsbereich eines Zementwerkes. *Mitteilungen der Deutschen Bodenkundlichen Gesellschaft* **72**: 531–534.

Graefe, U. & Beylich, A. (2003) Critical values of soil acidification for annelid species and the decomposer community. *Newsletter on Enchytraeidae* **8**: 51–55.

Graefe, U., Busch, W. & Egberts, B. (2003) *Bodenzoologische Untersuchungen auf der Boden-Dauerbeobachtungsfläche Amsinckpark – Untersuchungsjahr 2002*. [Unpublished report to the City of Hamburg Freie und Hansestadt Hamburg, Behörde für Umwelt und Gesundheit, Amt für Umweltschutz, Geologisches Landesamt]

Graefe, U. & Schmelz, R. M. (1999) Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. *Newsletter on Enchytraeidae* **6**: 59–67.

Haag, R., Stempelmann, I. & Haider, J. (2009) *Bodenbiologische Untersuchungen auf Bodendauerbeobachtungsflächen in Nordrhein-Westfalen im Zeitraum 1995–2007*. Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, Essen. (http://www.lanuv.nrw.de/boden/pdf/Bericht_Bio_BDF_30_11_09.pdf. Accessed 24 Sept 2012)

Jans, W. & Römbke, J. (1989) Funde eines terrestrischen Polychaeten (Annelida) in Wäldern Baden-Würtenbergs. *Carolinea* **47**: 158–162.

Jördens, J., Stuck, T. & Purschke, G. (2004) Phylogenetic inference regarding Parergodrilidae and Hrabeiella periglandulata ('Polychaeta', Annelida) based on 18S rDNA and COI sequences. *Journal of Zoological Systematics and Evolutionary Research* **42**: 270–280.

O'Connor, F. B. (1962) The extraction of Enchytraeidae from soil. Pp. 279–285. *In:* Murphy, P. W. (ed.): *Progress in Soil Zoology*, Butterworths Publishers, London.

Pižl, V. & Chalupský, J. (1984) Hrabeiella periglandulata gen. et sp. n. (Annelida) – A curious worm from Czechoslovakia. *Věstník Československé společnosti zoologické* **48**: 291–295.

Purschke, G. (1999) Terrestrial polychaetes – models for the evolution of the Clitellata (Annelida)? *Hydrobiologia* **406**: 87–99.

Purschke, G., Hessling, R. & Westheide, W. (2000) The phylogenetic position of the Clitellata and the Echiura – on the problematic assessment of absent characters. *Journal of Zoological Systematics and Evolutionary Research* **38**: 165–173.

Purschke, G. (2003) Is Hrabeiella periglandulata (Annelida, "Polychaeta") the sister group of Clitellata? Evidence from an ultrastructural analysis of the dorsal pharynx in H. periglandulata and Enchytraeus minutus (Annelida, Clitellata). *Zoomorphology* **122**: 55–66.

Reisinger, E. (1925) Ein landbewohnender Archiannelide. (Zugleich ein Beitrag zur Systematik der Archianneliden). *Zeitschrift für Morphologie und Ökologie der Tiere* **3**: 197–254.

Rota, E. (1998) Morphology and adaptations of Parergodrilus Reisinger and Hrabeiella Pizl & Chalupský, two enigmatic soil-dwelling annelids. *Italian Journal of Zoology* **65**: 75–84.

Rota, E. & Lupetti, P. (1996) An ultrastructural investigation of Hrabeiella Pižl & Chalupský, 1984 (Annelida). I. Chaetae and body wall organization. *Hydrobiologia* **334**: 229–239.

Rota, E. & Lupetti, P. (1997) An ultrastructural investigation of Hrabeiella Pižl & Chalupský, 1984 (Annelida). II. The spermatozoon. *Tissue & Cell* **29**: 603–609.

Rota, E., Martin, P. & Erséus, C. (2001) Soil-dwelling polychaetes: enigmatic as ever? Some hints on their phylogenetic relationships as suggest by a maximum parsimony analysis of 18S rRNA gene sequence. *Contributions to Zoology* **70**: 127–138.

Rousset, V., Pleijel, F., Rousec, G. W., Erséus, C. & Siddall, M. E. (2007) A molecular phylogeny of annelids. *Cladistics* **23**: 41–63.

Schlaghamerský, J. & Šídová, A. (2007) On a species-poor enchytraeid community of peculiar composition. *Folia Facultatis Scientiarium Naturalium Universitatis Masarykianae Brunensis Biology* **110**: 183–192.

Schlaghamerský, J. & Šídová, A. (2009) Dynamics and vertical distribution of a Hrabeiella periglandulata (Annelida) population in South Moravia, Czech Republic. *Pesquisa Agropecuária Brasileira* **44**: 917–921.

Struck, T. H. & Purschke, G. (2005) The sister group relationship of Aeolosomatidae and Potamodrilidae (Annelida: ''Polychaeta'') – a molecular phylogenetic approach based on 18S rDNA and cytochrome oxidase. *Zoologischer Anzeiger* **243**: 281–293.

Struck, T. H., Nesnidal, M. P., Purschke, G. & Halanych, K. M. (2008) Detecting possibly saturated positions in 18S and 28S sequences and their influence on phylogenetic reconstruction of Annelida (Lophotrochozoa). *Molecular Phylogenetics and Evolution* **84**: 628–645.

Zrzavý, J., Říha, P., Piálek, L. & Janouškovec, J. (2009) Phylogeny of Annelida (Lophotrochozoa): total-evidence analysis of morphology and six genes. *BMC Evolutionary Biology* **9**: 189.

Revised version received November 26, 2012, accepted April 2, 2013, published May 31, 2013