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Editorial

Since 1994, the International Symposia on Enchytraeidae have been held in every two years. The Newsletter on Enchytraeidae is a series of proceedings, which from issue Nº 4 present the scientific output of this meeting and traditionally edited by the attendance of the organizers of the conference.

The Newsletter includes contributions presented at the 12th International Symposium on Enchytraeidae held at the Limnological Institute of the Centre for Ecological Research, Tihany, Hungary, 27-29 June 2016.

In total, seventeen participants from ten countries attended the symposium. During the three days, twelve oral and five poster presentations were given and discussed. As usual at these Symposia, a workshop provided the opportunity for the participants to study and practice the identification of specimens of enchytraeids supervised by Rüdiger M. Schmelz and Klára Dózsa-Farkas.

The papers in this volume cover a wide range of basic and applied research on terrestrial enchytraeids, and they contribute valuable information to the knowledge of taxonomy, ecology, and ecotoxicology of this Oligochaete family. All papers included in this volume have been critically evaluated by two reviewers. Referees' and all authors' efforts are highly appreciated to make these proceedings possible.

The symposium was organized by Gergely Boros and kindly hosted by the Limnological Institute of the Centre for Ecological Research of the Hungarian Academy of Sciences. Special thanks are due to Réka Ádám, István Egervári, Bence Kovács and László Somay, who did a lot of work behind the scenes to make the symposium comfortable for all participants.

The 13th International Symposium on Enchytraeidae will take place in Versailles, France, hosted by INRA and organized by Céline Pelosi.

Gergely Boros

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Description of *Enchytronia pygmaea* sp. n. (Enchytraeidae, Clitellata), a very small enchytraeid in European soils

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Abstract. A new and very small European species of terrestrial enchytraeids is described, *Enchytronia pygmaea* sp. nov. (Enchytraeidae, Oligochaeta). It differs from all enchytraeids known so far in the chaetal pattern: lateral bundles have 2 chaetae from segment II to V, 0 from segment VI to XII–XV, and only 1 chaeta in lateral postclitellar bundles; ventral bundles have 2 chaetae. A further peculiarity is the presence of only 1 pair of preclitellar nephridia. The species must be considered as widespread as it is recorded here from 17 different localities distributed over seven European countries ranging from the Atlantic to the Mediterranean zone.

Keywords. New species, Oligochaeta, Annelida, soil biodiversity, taxonomy.

INTRODUCTION

A new and very small species of Enchytraeidae is described. It was discovered in the course of various large-scale sampling campaigns carried out in Europe during the last 30 years. The species must be considered widespread as it is recorded here from 17 different localities distributed over seven European countries ranging from the Atlantic to the Mediterranean zone. It is easily identified due to a chaetal pattern that has hitherto not been found in any species of enchytraeids.

MATERIAL AND METHODS

Soil samples were extracted for enchytraeids, using either cold or hot wet extraction. Enchytraeids were identified and investigated in vivo. A representative sample of specimens was fixed in hot Bouin's fluid, stained, and whole-mounted. Other specimens from several localities, the type locality included, were preserved in 96% or 100% ethanol, for future DNA sequencing. Mode of description follows Schmelz & Collado (2010). Geographical coordinates are expressed in decimal degrees, with southern latitudes and western longitudes given as negative numbers (GPS coordinates). "BDF" refers to permanent soil monitoring sites in Germany ("Boden-Dauerbeobachtungs-Flächen"). Specimens found by the second author were extracted from soil samples that had been taken by other workers and been sent to him.

RESULTS

Enchytronia pygmaea sp. nov.

(Figures 1–2)

Material examined. Holotype. ZMH OL 14530, adult specimen, stained whole mount; Germany, city of Hamburg, urban lawn Amsinckpark, German permanent soil monitoring site (BDF-HH), 53.36719; 9.56378, 54 m asl, pH (CaCl$_2$) 4.3, leg. U. Graefe, 29 IV 2013.

Paratypes. ZMH OL 14531–35, 5 specimens, adult, stained whole mounts, same data as holo-

type. ZMH OL 14536, 10 specimens, preserved in 96% ethanol, from type locality; leg. U. Graefe, 26 IV 2016.

Further material. Germany, type locality: 10 specimens, preserved in 96% ethanol, from type locality; leg. U. Graefe, 26.IV.2016. 139 specimens, mostly adult, extracted from soil samples of type locality, leg. U. Graefe, 20.X.1992 and 5.X.2012, not preserved.

Germany, other localities: 5 specimens, adult, stained whole mounts: North Rhine-Westphalia, Lippetal/Lippborg (project "Vergleichsuntersuch-ungen Alternativer Landbau" der Landesanstalt für Ökologie), 51.39546; 8.02009, arable land, sandy soil, pH 6.0–6.4; leg. U. Graefe, 6.V.1986. Further specimens from same locality investigated *in vivo* 1986–1989, not preserved. – 1 specimen, adult, Schleswig-Holstein, BDF-SH 23 Bokhorst (permanent soil monitoring site), 54.03473; 9.24003, arable land, sandy soil, pH (CaCl$_2$) 5.8; leg. U. Graefe, 21.V.1996, not preserved. – 16 specimens, adult, Schleswig-Holstein, BDF-SH 26 Bad Bramstedt (permanent soil monitoring site), 53.55297; 9.55244, grassland, sandy soil, pH (CaCl$_2$) 4.9–5.6; leg. U. Graefe, 29.XI.1998 and 2.XI.2005, not preserved. – 1 specimen, adult, Brandenburg, Komturei Lietzen (ZALF field trial reduced tillage), 52.29451; 14.21344, 48 m asl, arable land, sandy soil, pH (KCl) 5.1; leg. U. Graefe, 1.XI.2005, not preserved. – 2 specimens, adult, Schleswig-Holstein, BDF-SH 09 Schuby (permanent soil monitoring site), 54.32087; 9.26005, 15 m asl, arable land, sandy soil, pH (CaCl$_2$) 5.3; leg. U. Graefe, 4.XI.2009, not preserved. – 2 specimens, adult, Brandenburg, Mün-cheberg (ZALF permanent field trial V 140), 52.31018; 14.07192, arable land, sandy soil; leg. U. Graefe, 13.X.2010 and 19.IX.2013, not preserved. – 4 specimens, adult, Schleswig-Hol-stein, BDF-SH 24 Bornhöved (permanent soil monitoring site), 54.32087; 9.26005, 38 m asl, arable land, sandy soil, pH (CaCl$_2$) 5.4; leg. U. Graefe, 25.IX.2015, not preserved. – 22 speci-mens, adult, Schleswig-Holstein, BDF-SH 22 Hindorf (permanent soil monitoring site), 53.59597; 9.08497, 20 m asl, grassland, sandy soil, pH (CaCl$_2$) 4.7; leg. U. Graefe, 20.X.2016, not preserved. – 1 specimen, juvenile, stained whole mount: Naturpark Eifel (Natural Reserve), near Erkensruhr, 50.54022; 6.33821, 508 m asl, old-growth beech forest on brown soil, pH 3.29, R.M. Schmelz (project Edaphobase II) X.2014. Six further specimens from the same and an adjacent site, investigated *in vivo*, not preserved.

Estonia: 14 specimens, adult, Tartu County, Kaagvere (research area UA-II), 58.20272; 26.54215, 45 m asl, mixed pine-spruce forest, loamy sand on sandy loam, WRB soil classi-fication Dystric Albic Glossic Fragic Retisol, pH (KCl) 4.3; leg. U. Graefe, 28.VIII.2012, not pre-served.

Denmark: Three specimens, 2 subadult, 1 ju-venile, stained whole mounts: Jutland, Foulum, 56.483; 9.567, 53 m asl, experimental grassland of Arhus University; leg. R.M. Schmelz, 12.V.2011. Four further specimens collected from same site, two at same date, two in X.2010, not preserved.

The Netherlands: Three specimens, adult, in 100% ethanol: Dennenkamp, 2.028611; 5.800556, 50 m asl, extensively managed grassland (project EcoFINDERS), pH 5.4–5.6; leg. R.M. Schmelz, 26 XII 2011. Further 71 specimens, not preserved. Further 12 specimens from an adjacent grassland site (Nieuw Reemst, 52.042500; 5.774722, pH 4.6), not preserved.

France: One specimen, adult: Pays de la Loire, Dép. Vendée, 46.758333; -1.867194, 18 m asl, small Atlantic oak forest plot (80x100 m, 9 km to the coast, *Quercus robur*) on loamy sand, pH data unavailable (project EcoFINDERS), RMQS site ("Réseau de Mesures de la Qualité des Sols", French Monitoring Network for Soil Quality); leg. R.M. Schmelz, 11.III.2013. Further 6 specimens from same locality, not preserved. – 1 specimen, stained whole mount: Auvergne-Rhône-Alpes, Dép. Cantal, 45.10875; 2.530472, 944 m asl, steep, south-exposed mountain grassland on clay loam, pH data unavailable (project EcoFIN-DERS), RMQS site; leg. R.M. Schmelz, 7.I.2013.

Italy: Three specimens, adult, stained whole mounts: 2 specimens, adult, in 100% ethanol; Sardinia, Berchidda, 40.822920; 9.279786 (2 sites within a radius of ca. 50 m), 335 m asl, oak wood, pH 5.8–6.4 (project EcoFINDERS); leg. R.M. Schmelz, 19.V.2012. Further 7 specimens from same locality, not preserved.

Portugal: One specimen, juvenile: stained whole mount; Olaia, 40.1706; -8.716, 7 m asl, oak wood on a small hill in flood plain near the coast, soil type and pH unknown; leg. R.M. Schmelz, 18.V.2014. Four further specimens from same locality, not preserved.

Diagnosis. Very small enchytraeids, adults *ca.* 1.5–2 mm long *in vivo*, 17–19 segments, chaetal formula 2,0 – (0),1 : 2 – 2, chaetae absent laterally from VI to XII–XV; from XIII–XVI on only 1 chaeta per bundle, brain rounded posteriorly, pharyngeal glands united in IV, V, separate and elongate in VI, oesophageal appendages and intestinal diverticula absent, dorsal blood vessel from XI with peristomial anterior bifurcation, one pair preclitellar nephridia at 7/8, clitellum only lateral, male reproductive organs small, spermathecae with ectal gland, non-glandular ectal duct, small ampulla without diverticula, ental ducts united proximally.

Description. Slow body movements. Colour transparently whitish-pale with gut-contents shining through. Body dimensions: length 1.5–1.9 mm (*viv*), 1.3–1.45 mm (*fix*), juveniles down to 0.5 mm (*viv*); diameter 0.05–0.07 mm (*viv*), *fix*: 0.06–0.1 at V, 0.07–0.12 at XII, 0.06–0.09 at XV. Holotype (*fix*): length 1.4 mm, diameter 0.1 mm at V, 0.1 mm at XII, 0.07 mm at XV. Segment number 17–19. Chaetae. Formula 2,0 – (0),1 : 2 – 2. Laterally two chaetae from II–V, absent from VI to XII–XV; from XIII–XVI on, 1 chaeta per bundle. Ventrally 2 chaetae per bundle, absent in XII. Chaetae straight with weak ental hook, ectally pointed; length range 10–26 µm, diameter ca. 2 µm; chaetae shorter anteriorly (*ca.* 15 µm) than posteriorly (up to 20–26 µm), and shorter laterally than ventrally, *e.g.* (1 specimen): chaetae increasing in size from 10 µm (II) to 15 µm (V)

laterally, and from 16 µm (II) to 20 µm (V) ventrally. Lateral postclitellar chaetae near clitellum smallest (10 µm), caudal ventral chaetae largest (20–26 µm). Prostomium short, with thickened frontal epithelium. Head pore not ascertained, probably at 0/I. Epidermal gland cells not seen. Body wall *ca.* 3–4 µm thick, *i.e.* comparatively thin, cuticle inconspicuous, mostly < 1 µm. Brain rounded posteriorly, extending into III, twice as long as wide. Pharyngeal pad small, almost as high as long, roughly spherical, often below brain. Pharyngeal glands united in IV and V dorsally, primary ventral lobes in V; glands separate in VI, larger than in IV or V, compact, variable in shape, not separated into dorsal and ventral lobe, often incised halfway. Holotype with postero-dorsal projections and small postero-ventral projections into VII. No oesophageal appendages, no intestinal diverticula. Chloragocytes from IV, dense layer from VII, cell diameter *ca.* 16 µm, equalling chaetal lengths; vesicles conspicuously greenish-brown, diameter 1–2 µm, more than twice the diameter of the coelomocyte vesicles. Oesophagus narrow; transition into intestine marked as a spindle-shaped widening, together with a more or less abrupt widening of gut lumen; no canals or furrows distinguished; widening beginning at about 1/2 VI – 1/2 VII; here long cilia in movement observed. Midgut: Pars tumida in XIV–XVI, occupying 1–2 segment lengths; no Cejkaian tubules. Dorsal blood vessel from XI, anterior bifurcation in peristomium. Two pairs of commissural vessels seen in IV; ventral blood vessel from IV on. One pair of preclitellar nephridia, at 7/8. Anteseptale with parts of nephridial body, no constriction at septum, funnel in obliquely upright position, postseptale *ca.* 2x as long as anteseptale; efferent duct as a narrowed continuation of postseptale. Windings of canal dense and conspicuous, down to nephridioporus. No terminal vesicles. Postclitellar nephridia in many positions, *e.g.* at 13/14, 14/15, 15/16, 17/18, paired and unpaired. Coelomocytes one type, mucocytes. Cells flat, almost disc-shaped, slightly longer than wide, length *ca.* 20 µm, completely and homogeneously filled with small, conspicuous and slightly brownish vesicles, neither opaque not translucent in transmitted light, pro-

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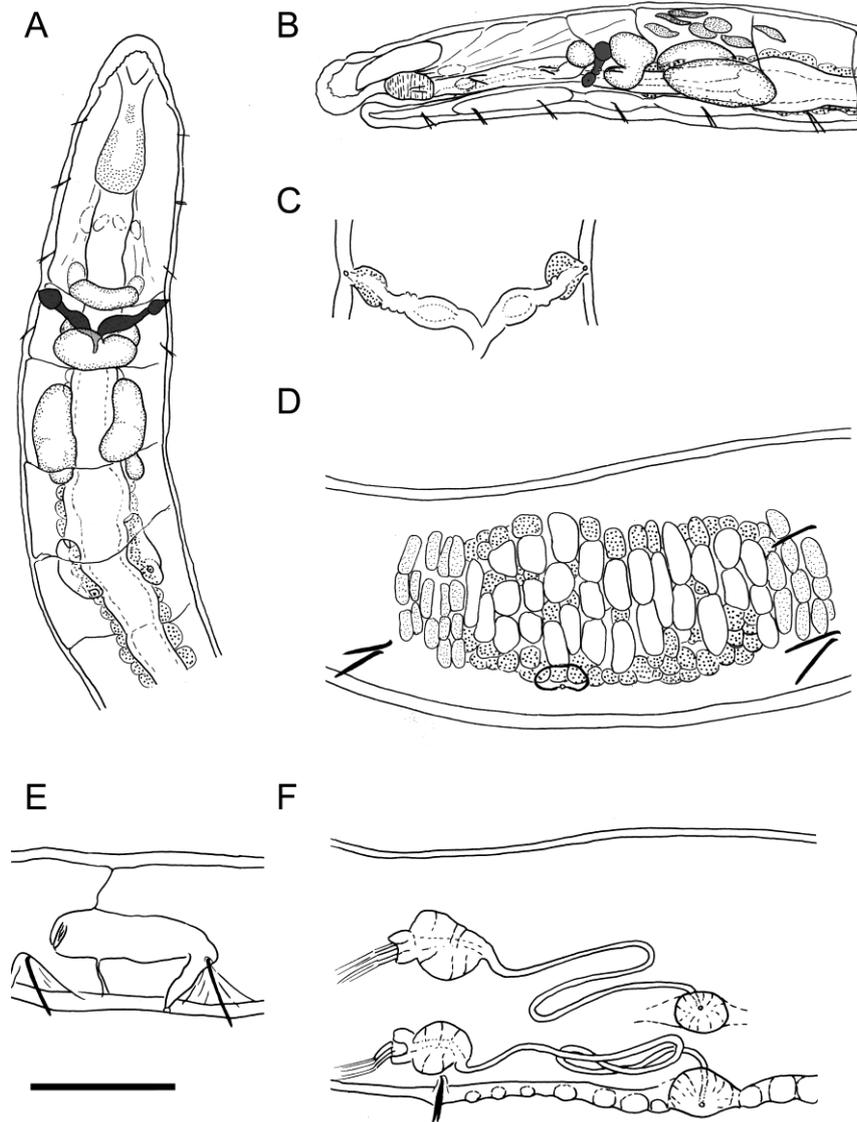
Figure 1. *Enchytronia pygmaea* sp. nov. A = Anterior end, dorsal view, holotype. Spermathecae shaded grey. Punctation in brain indicate region of perikarya. The pharyngeal pad is hidden below the brain. B = Anterior end, lateral view, paratype. Spermathecae shaded grey. Pharyngeal pad below brain. Coelomocytes shown in VI–VII. C = Spermathecae, dorsal view, holotype. D = Clitellum, lateral view, non-type specimen from Sardinia, Italy. E. Preclitellar nephridium, lateral view. F = Gonadal region, latero-ventral view. Roman numerals: segments. Scale bar = 100µm (A–B), 50 µm (C–F).

viding a slight tinge of the cell. Vesicles distinctly smaller than chloragocyte vesicles, estimated diameter 0.5 µm. Cells brownish *in vivo*, with rose tint in the paracarmine-stained whole mounts. In fixed specimens, cells in oblique position often lemon-shaped, apparently because of flat cell margins.

Clitellum only laterally developed, *i.e.* absent dorsally and ventrally; dorsal margin one longitudinal cell row above lateral chaetae, ventral margin at level of male pores; longitudinal extension from septum 11/12 to chaetae of XIII; cells in *ca.* 11 separate rows; inconspicuous in most specimens; anterior and posterior margins as

well as cell types not well-distinguished; gland cells about twice as high (*ca.* 5 µm) as adjacent epidermis (*ca.* 2 µm). When fully developed, up to 12 µm high, hyalocytes inflated, granulocytes not distinguishable. Seminal vesicle absent, few cysts dorsally in XI. Spermatozoa *ca.* 30 µm long, heads 12–15 µm long. Sperm funnel small, length *ca.* 40 µm, less than 1/2 body diameter, collar conspicuous, about as high as wide (15 µm), funnel body flattened, about as wide as long, maximum diameter equalling length (*ca.* 25 µm), minimum diameter almost equalling collar diameter. Vas deferens comparatively short, in 1–2 wide loops, diameter *ca.* 5 µm, estimated length 2x body diameter. Male pore surrounded by a

small lens-shaped gland, about twice as high as body wall, length 25 µm, *ca.* 1.5x as long as wide or high. Male pores apparently on body surface, no bursa or bursal slits distinguished. No subneural glands. Spermathecae united mid-dorsally, ectal gland rounded, sessile, diameter *ca.* 15 µm; ectal duct half as wide as ectal gland, 1–2x as long as ampulla (depending on state of contraction), not glandular but outline wavy due to adhering cell nuclei, ampulla inconspicuous, spherical or ellipsoid, diameter *ca.* 11 µm, sperm not distinguished in living and preserved specimens. Ental ducts shorter than ectal ducts, uniting mid-dorsally. One large egg at a time, occupying more than 1 segment length.
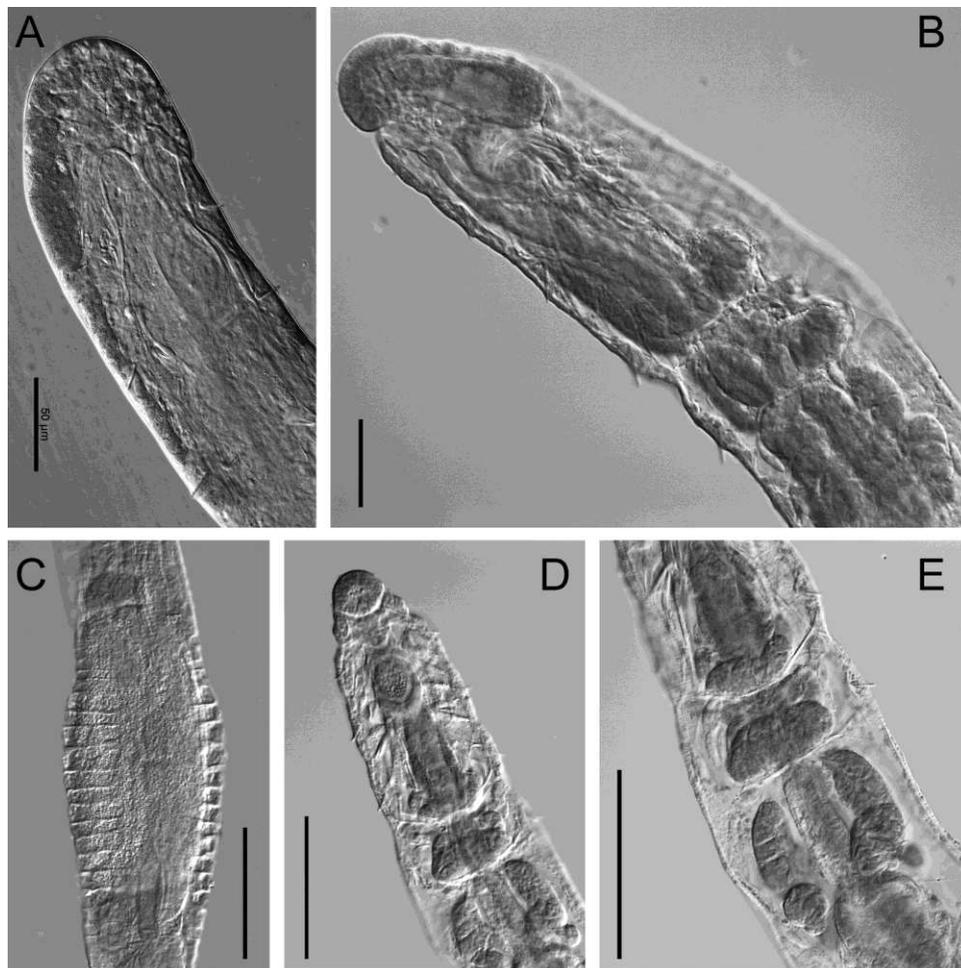
Figure 2. *Enchytronia pygmaea* sp. nov. A = Anterior body region, dorsal view, living non-type specimen from type locality, strongly flattened to show peristomial bifurcation of dorsal blood vessel. B = Anterior body region, latero-dorsal view, paratype. C = Clitellar region, dorso-lateral view, paratype. D = Anterior body region, ventral view, holotype. E = Anterior body region, ventral view, focusing plane in dorsal body region, holotype.

DISCUSSION

Species comparison and generic placement

We place this species in the genus *Enchytronia* Nielsen & Christensen, 1959, because of the shape and arrangement of chaetae lacking laterally in a number of preclitellar segments, the peristomial bifurcation of the dorsal blood vessel, the overall shape of the spermathecae, and the only laterally developed clitellum. It disagrees with the generic diagnosis – and with all species included in this genus so far – by the posteriorly rounded brain (incised in the other species), the presence of only one lateral chaeta in postclitellar positions (2 in the other species), and in the presence of only one pair of preclitellar nephridia.

As an alternative, the new species could be placed in *Marionina* Michaelsen, 1890, *sensu auctorum*. However, *Marionina* is a heterogeneous and polyphyletic assemblage of species (Rota *et al.* 2008, Schmelz & Collado 2008). Furthermore, the new species is much more similar to the type species of *Enchytronia* (*Enchytronia parva* Nielsen & Christensen, 1959) than to the type species of *Marionina* (*Pachydrilus georgianus* Michaelsen, 1888) considering morphology, distribution and habitat: *Enchytronia parva* is a small mineral soil dweller with palearctic distribution; it has a maximum of two, distally straight chaetae per bundle, chaetae often missing laterally in some segments before clitellum, the nephridial anteseptale is with coils of canal, and spermathecae are fused entally. *Marionina georgiana*, on the other hand, is a marine littoral subantarctic species with up to 6 (or even 7) sigmoid chaetae per bundle in asymmetric fan-shaped arrangement, all positions occupied; the nephridial anteseptale consists of the funnel only, and spermathecae are separate entally (Rota *et al.* 2008, Schmelz & Collado 2008).

From the remarks above it is obvious that the new species has to be compared with species of *Enchytronia* and *Marionina*. The pattern of chaetae is unique in Enchytraeidae, and few species are as small as or even smaller than *E. pygmaea*. The most apparent attribute of the new species is the presence of lateral chaetae in only 4 preclitellar segments, from II–V, a character shared by *Marionina minutissima* Healy, 1975, and *Enchytronia oligosetosa* Sesma & Dózsa-Farkas, 1993, two species of likewise small body size. However, *M. minutissima* is distinguished from the new species by the absence of lateral postclitellar chaetae, a posteriorly incised brain and the presence of yellow-brown epidermal glands on each segment. *E. oligosetosa* differs in having 2 chaetae per bundle laterally in postclitellar segments and a pair of lateral intestinal diverticula in VI. The number of preclitellar nephridia is not explicitly described in either species. *Marionina clavata* Nielsen & Christensen, 1961 has a similar chaetal formula: 2,0 – 1 : 2 – 2, but lateral preclitellar chaetae are present in 7 segments, from II to VIII, and not in 4, from II to V, as in *E. pygmaea*. Further differences from the new species are a pair of oesophageal appendages in IV, dorsally developed clitellum, and a posteriorly incised brain, among other characters. The presence in *E. pygmaea* of only one pair of preclitellar nephridia is unique among species of *Enchytronia* and *Marionina*, but this character is unknown in many species of these two genera. It is known, however, in the two other similarly small enchytraeid species known to-date: *M. eleonorae* Rota, 1995 (15–17 segments, body length 0.9–1.4 mm) and *M. deminuta* Rota, 2013 (16–21 segments, 0.9–1.4 mm) have preclitellar nephridia at 7/8 and 8/9; furthermore, they differ from the new species in the presence of two chaetae in all lateral postclitellar bundles, among many other characters.

In general, *E. pygmaea* is most easily identified because of the unique chaetal pattern, which shows up well even in fixed material. Among the preserved specimens, only those from Italy have lateral postclitellar chaetae throughout from XIII, in all other specimens, the holotype included, some postclitellar segments are without lateral chaetae. However, the state of this character is unknown in the specimens that were investigated only *in vivo*.

Distribution and habitat

(Figure 3)

Up to now, *E. pygmaea* was found in Germany, Denmark, The Netherlands, France, Portugal, Italy, and Estonia. The distribution of the new species can hence be characterized as widespread in Europe. The species is a mineral soil dweller and seems to prefer sandy soils without restriction to a specific land-use type (forest, grassland, arable land). In terms of humus forms the known locations can be classified as Mull sites ranging from moderately to slightly acid soil conditions (Graefe & Schmelz 1999). It was often but not exclusively found in deeper strata (below 5 cm from surface), similar to *Enchytronia parva*. It was abundant at the type locality and at the Netherlands sites; these are all extensively used grassland sites.
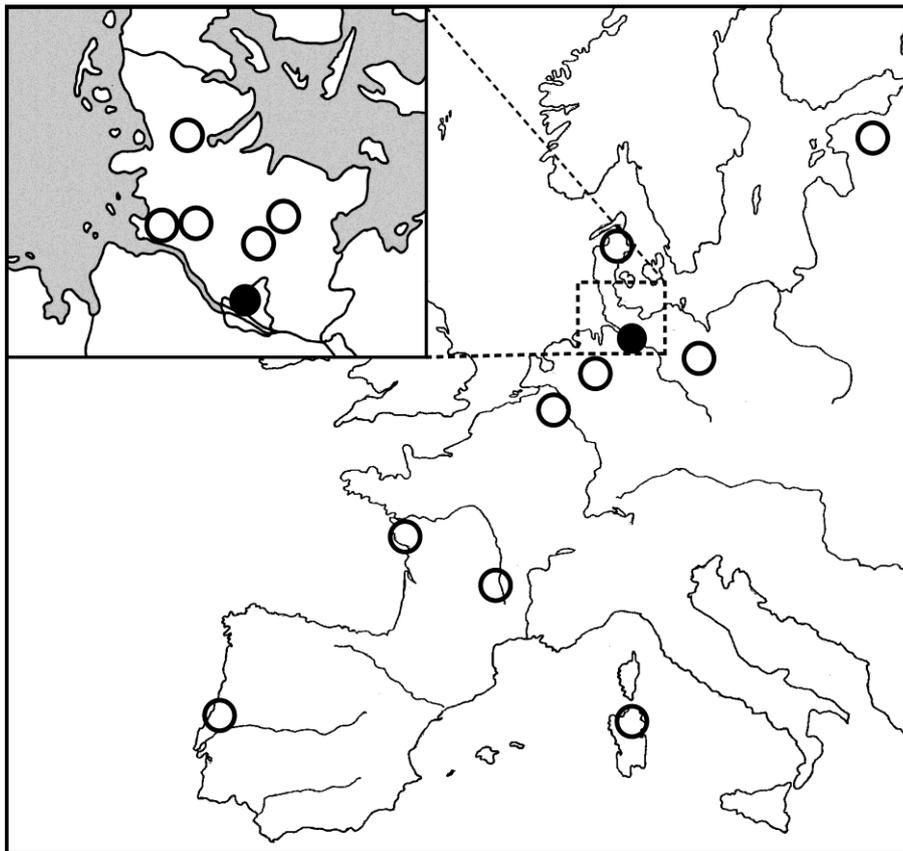
Figure 3. Localities with finds of *Enchytronia pygmaea* sp. nov. in Europe, and especially Schleswig-Holstein (top left). The type locality (filled circle) is shown twice.

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Feeding spectrum of a dominant splash zone enchytraeid, *Mesenchytraeus bungei* Michaelsen, 1901 (Annelida: Oligochaeta), in Lake Baikal (East Siberia)

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Abstract. The first data on feeding spectrum of Baikal enchytraeids are given. Previously we found that *Mesenchytraeus bungei* Michaelsen, 1901 dominates in abundance in splash zone Oligochaeta community of Bolshie Koty Bay, Lake Baikal. Feeding spectrum of *M. bungei* was investigated in summer (partially also in spring and autumn) of 2010–2012 and 2015. Our study is based on pellet content analyses of more than 200 *M. bungei* specimens. Diverse components in different ratio were found during the investigation period, namely green algae, diatoms, higher plants debris, various animal remains, some minor components and sediment particles. All the pellet content was subdivided into phytogenous and animal material, unidentified matter and sediment particles. Phytogenous material appeared to be a dominant component of pellets (up to 87%) in almost all cases. Our analysis showed that *M. bungei* is a saprophage with a preference to phytogenous detritus.

Key words. Feeding, splash zone, Enchytraeidae, *Mesenchytraeus bungei*, Lake Baikal

INTRODUCTION

Aquatic oligochaetes (Annelida: Oligochaeta) are easily found at the bottom of different water bodies and play an essential role in self-purification and organic matter decomposition processes. Investigation of oligochaetes' feeding behavior is necessary in order to assess their significance for food chains; nevertheless, most of facts on it are ambiguous and contradictory. The way of feeding is inseparably related to their lifestyle. For instance, phytophilic worms living among the aquatic vegetation feed like grazers (Monakov 1998), using periphyton (*e.g*., diatoms and other unicellular algae) with organic sediment particles as a food. Mostly it concerns representatives of the Family Naididae. They are capable of selective digestion of ingested particles (Timm 1987). Species of the genus *Chaetogaster* can attack and ingest tiny invertebrates (Monakov 1998, Čekanovskaya 1962).

The tubificids, lumbriculids and aquatic enchytraeids belong to the ground-dwelling oligo-chaetes (Čekanovskaya 1962). It has been considered for a long time that such worms just pass sediment particles through their intestine without any selection, but many studies disproved this point of view (Brinkhurst & Austin 1979, Rodriguez *et al.* 2001, Poddubnaya 1961).

Soil enchytraeids could be considered as well-studied ones in terms of their trophism. But before the work of O'Connor (1967) one could find only scattered data for different species of soil enchytraeids until the above author had surveyed all known facts on their feeding behavior. Soil enchytraeids feed on diverse items: decaying leaf litter, bacteria, fungi, nematodes (Dózsa-Farkas 1976). When cultivated, they can utilize oats, yeast, algae and even dead bodies of lumbriculids and arthropods (Briones & Ineson 2002). Several authors have shown that enchytraeids demonstrated evident preference to microfungi (Dash *et al.* 1980) but, vice versa, others have denied it (Standen & Latter 1977).

On contrary to soil enchytraeids, feeding of the aquatic ones is almost unknown (Timm 1987).

We could find some information on the subject for marine enchytraeids only, since representatives of the family are a dominant component of marine meiobenthos (Giere 2009). Giere (1975) examined a diet of some dominant marine enchytraeid species in details. According to the author, marine enchytraeids can feed on diatoms (*Marionina subterranea* Knöllner, 1935), bacteria [*M. spicula* (Leuckart, 1847), *Lumbricillus lineatus* (Müller, 1774)] and decaying aquatic plants and macrophytes [*L. rivalis* (Levinsen, 1884), *L. lineatus* and *Enchytraeus albidus* Henle, 1837].

There is a marked lack of information on the trophism of freshwater enchytraeid species, despite the fact they are often to be found in lacustrine littoral (Lindegaard *et al*. 1994) and profundal (Timm 1996) zone. The data on the feeding of the Baikalian Enchytraeidae are also lacking. That is one of the reasons why our investigation focuses on the study of feeding of the *Mesenchytraeus bungei* Michaelsen, 1901.

The second reason is that the species is widely distributed for the whole lake splash zone. Recently we have started an investigation of the Lake Baikal splash zone (Timoshkin *et al.* 2012b). This zone is an above-water part of the littoral, which is subject to wave action (an analogue of marine supralittoral). So far there was only one special work focused on the coastal zone (Veinberg & Kamaltynov 1998). Our and previous studies showed Baikal Lake splash zone as unique one with quite specific hydrodynamic and temperature regime. We revealed this zone has some characteristic peculiarities, for instance there is the highest level of detritus accumulation here (Timoshkin *et al.* 2012a).

It seems remarkable that taxocenoses of the Baikalian splash zone are dominated by a single or 2–3 species. For example, the Oligochaeta community here is dominated by *M. bungei* (Zvereva *et al.* 2012). It comprised up to 92% of the total number of oligochaetes. Though, the identity of this mass enchytraeid species and the species, which was described by Michaelsen in

1901, one could call into a question. *M. bungei* was mentioned in Čekanovskaya 1962, Veinberg & Kamaltynov 1998, and Semernoy, 2004 without any revision. The only attempt to redescribe it was made by Timm (2003) on a single available specimen differed from the Michaelsen's original description. So now the species evidently needs a revision (Timm 2003). Nevertheless, the authors still believe the species under study is a single mass species. Our supposition is supported by preliminary molecular analyses (unpublished data). Although there is a taxonomic problem, it seems reasonable to investigate such dominant species from ecological point of view to reveal their role for the whole lake ecosystem. Obtained results could be regarded as the first data on feeding habits of Baikal enchytraeids.

MATERIALS AND METHODS

Lake Baikal, located in the central part of Asia (eastern Siberia) on the border of Irkutsk Region and the Republic of Buryatia, Russia. Our investigation was carried out at the Limnological Institute field station in Bolshie Koty Bay. The bay is slightly jutting into the land near the village of the same name, located 20 km north-east of Listvyanka village, South Baikal (Fig. 1).

Investigation was conducted in the different months of 2010–2012 and 2015. There are two routine methods for oligochaetes' feeding pattern study: (1) stomach content analyses (Dash *et al.* 1980, McElhone 1979, Poddubnaya 1961) and (2) pellet content examination (Rodriguez *et al.* 2001). The latter method seemed to us more appropriate after practicing both of these approaches.

The present study was based on pellet content analyses of *M. bungei*, collected on the beach opposite to Limnological Institute field station (Fig. 1). Specimens were sampled in the splash zone, at the area between the water edge and 0.5 m upward. A total of 207 specimens were examined.

We chose worms of the largest size group (3–4 cm long) for our analysis. They were kept in Petri dishes with 3 ml of bottled Baikal water in the
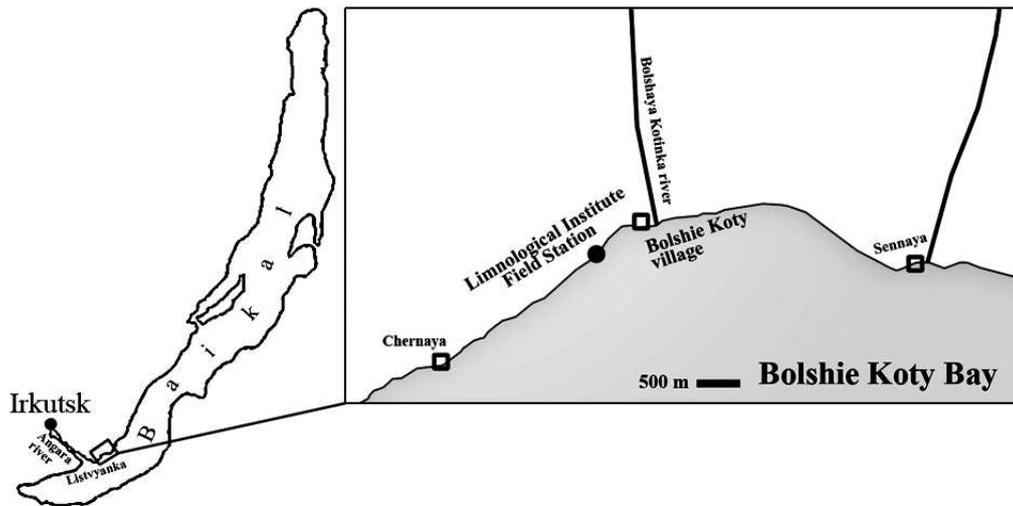
Figure 1. Sampling site location (big black dot) in Bolshie Koty Bay, Lake Baikal

fridge individually for 24 hours. Then pellets from the dishes were mounted on slides. We examined the slides under a microscope and estimated the percentage contribution of each food component in 10–20 microscopic fields per slide. The similar method of estimation was implied by Poddubnaya (1961) for the intestine content of tubificids.

For estimation we subdivided all pellet content into three basic groups: (1) phytogenous material *sensu lato* or pellet phyto-component (it consists of algae, macrophytes and land plant remains), (2) animal material and (3) unidentified matter (Fig. 3). Fungi and Cyanobacteria from pellets were mentioned separately from phytogenous material. Besides the foregoing components we found sediment particles, but they could not be considered as a food item.

Pellet content was analyzed with Olympus CX21FS1 microscope, with 4x, 10x, 20x and 40x magnifications. Photos were made by means of Olympus C-3040 zoom (3.3 MPx) camera and digital camera ToupCam FMA050 with microscope lens adapter.

RESULTS

The earliest season we obtained the data for was **March** of 2012 when the splash zone was yet mostly frozen. At that season diatom algae consti-

tuted the pellets bulk (Fig. 2a). We found both single cells and conglomerates as a mix of sediment particles, diatom cells and their fragments. Diatoms were presented by *Cymbella*, *Navicula*, *Cocconeis*, *Didymosphenia*, *Synedra* (*S. acus* and *S. ulna*), *Diatoma* and other genera. Conifer needles and various filamentous green algae (*e.g. Ulothrix zonata* (Web. & Mohr) Küetz., *Mougeotia* spp., *Spirogyra* spp.) occurred in the pellets. Cyanobacteria and fragments of arthropod chitinous exoskeletons were scarce. Unfortunately, such an "early" data were available only for one year.

In **June** of 2010–2012 the worms utilized basically filaments of the green macroalga *U. zonata*. In 2010 it was almost the single algal species (Fig. 2b), but in 2011 with the predominance of *Ulothrix* a considerable part was contributed by *Tetraspora* and remains of land plants (Fig. 2c). In **June** of 2012 *Ulothrix* with another green and diatom algae, and unidentified plant remains comprised up to 80% of pellet content (Fig. 2d). Among the diatoms *Cocconeis*, *Hannaea*, *Gomphonema*, *Navicula* were found as well. We also identified pine pollen and sponge spicules in a small amount.

In **July** of 2010–2011 the phyto-component of pellets was more diverse, unless *U. zonata* still played an important role. In 2010 the diatom
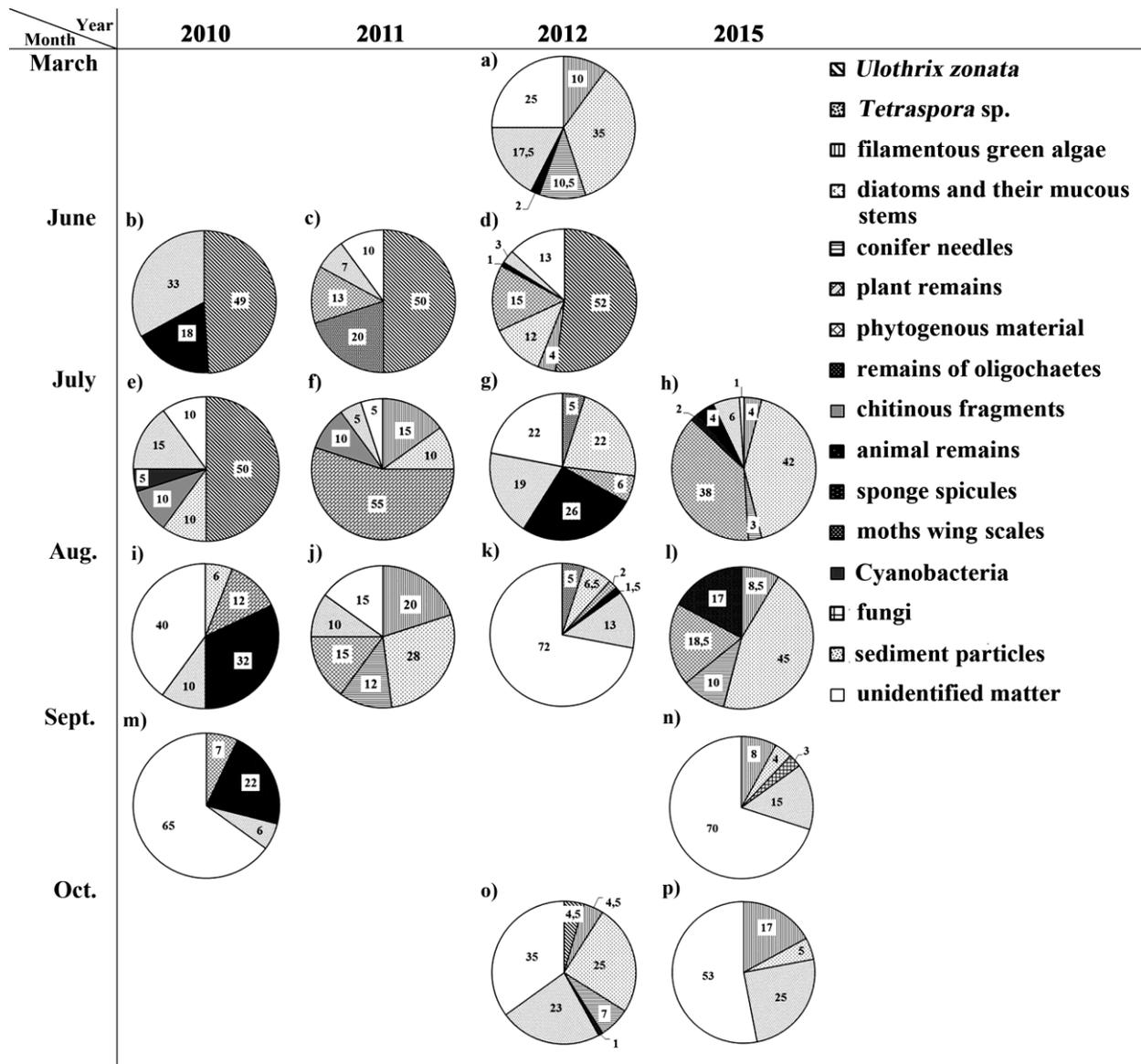
Figure 2. *M. bungei* pellet content in different months of 2010–2012 and 2015. Data are shown as a percentage (%)

algae *Gomphonema* and *Didymosphenia* were added to *Ulothrix*, while their mucous stems occurred even more frequently than diatom cells themselves (Fig. 2e). We also marked presence of attached infusoria *Vorticella* sp. (or solely their stems) and Cyanobacteria in a small amount. In 2011 phyto-component dominated in the pellets: *U. zonata*, land plant remains, diatoms (*Gomphonema*, *Cymbella* and *Fragilaria*) and their mucous stems (Fig. 2f). In **July** of 2012 we observed a rare case, when animal material in pellets was

comparable with phyto-material in percentage (Fig. 3c). The most substantial contribution among animal material was made by chitinous remains of arthropods and scales of moth wings (Fig. 2g). The phyto-component was represented by mixture of diatoms (*Hannaea*, *Cocconeis*, *Aulacoseira*, *Synedra*, *Didymosphenia*, *Navicula*), *Tetraspora*, and semidigested plant cells matrix. In **July** of 2015 there was a strong predominance of phyto-component (87%) in the pellet content (Figs. 2h, 3d). Mucous stems of diatoms were a

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biggest part of pellet masses (42%). We observed a significant amount of semidigested plant cell matrix (38%), remains of green filamentous algae, conifers needles. Among the animal component moth wing scales played a significant role. The minor components included sponge spicules, pine pollen, Cyanobacteria.

Interestingly, besides semidigested *U. zonata* in **July** of 2010–2012 and 2015 we found in the worm pellets lots of «newborn» *Ulothrix* filaments, which began to grow up and had a size of a few cells. In 2015 one third of *M. bungei* specimens had growing few-celled *Ulothrix* zoospores in their pellets.

M. bungei food spectrum in **August** was highly variable. In 2010 the enchytraeids fed on animal material (Fig. 3a). We found fragments of oligochaete bodies with chaetae of the own

species, *M. bungei*, in the pellets (Fig. 2i). In the pellets collected in **August** of 2011, phyto-material dominated (70% – Fig. 3b). Mucous stems of *Didymosphenia* spp. comprised about 26% of the pellet content (Fig. 2j). Diatoms of the genera *Cymbella* and *Cocconeis* were permanent components of the pellet mass. Also we found remains of oligochaete bodies. In **August** of 2012 there was an uncharacteristic case (Figs. 2k, 3c): the biggest part of the pellets was formed by unidentifiable detritus (72%) and sediment particles (13%). The rest of pellet content consisted of phyto-material, namely diatoms and their mucous stems and various plant remains; animal material and fungal hyphae (1.5%). Also we noticed in average 2 germinating *Ulothrix* zoospores with the length of 3–12 cells per every analyzed *M. bungei* specimen. As minor components were identified pine pollen and sponge spicules.
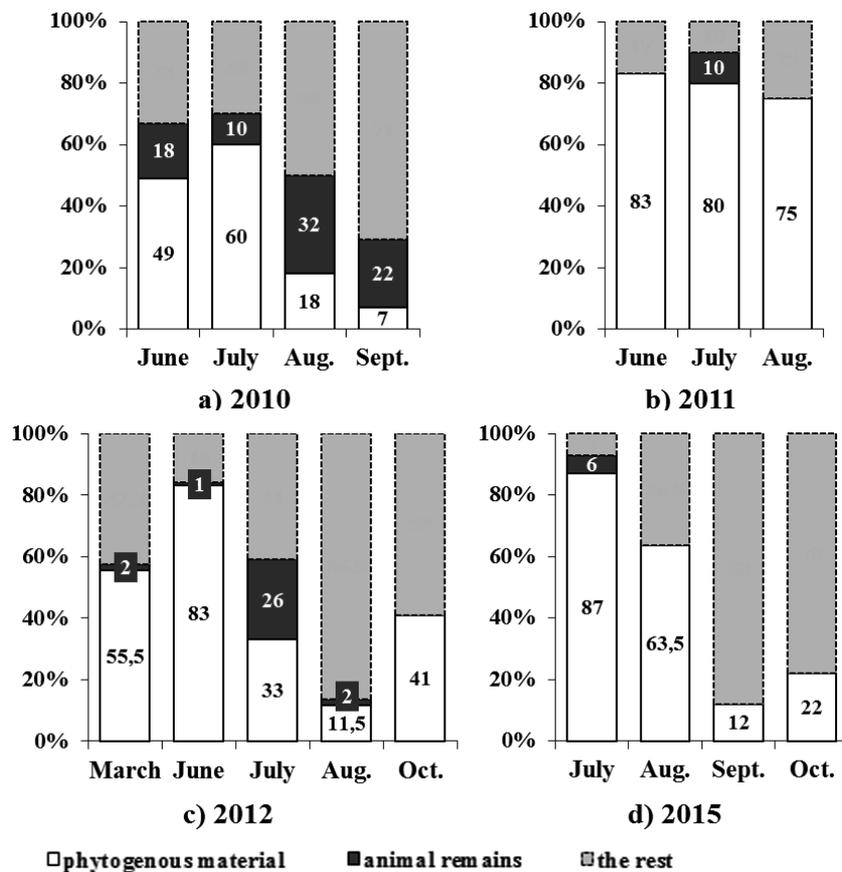
Figure 3. The change in plant and animal material contribution in *M. bungei* pellet content in 2010–2012 and 2015. Such scarce components as Fungi and Cyanobacteria were included into "the rest" category

In **August** of 2015 phyto-component prevailed in pellet content (Fig. 3d). Mainly it was mucous stems of *Didymosphenia*, green filamentous algae (*Spirogyra* spp.) and other algal and plant remains (Fig. 2l). Additionally, we found fungal hyphae, sponge spicules, moth scales, sedentary infusoria *Vorticella* (1–2 specimens per worm), colonial algae *Volvox*, and pine pollen.

In **September** of 2010 the pellet content was performed by oligochaete bodies' remains with *M. bungei* chaeta along with a larger part of unidentified matter (Fig. 2m). In **September** of 2015 unidentified matter dominated (70%) in *M. bungei* pellets (Fig. 2n). Various filamentous algae (including *Spirogyra* spp.) and diatom mucous stems were as a phyto-component with a small percentage contribution. We found a little amount of fungi (3%), sponge spicules, pine pollen.

In **October** of 2012 besides unidentified matter and sediment particles there was almost solely phytogenous material (40% – Fig. 3c). Such components as mucous stems of *Didymosphenia*, other diatoms (*Cocconeis*, *Cymbella*, *Hannaea*, *Navicula*, *Synedra*), green filamentous algae (*Ulothrix*, Cladophoraceae), conifer needles remains contributed a biggest part in pellet content (Fig. 2o). We observed Cyanobacteria presumably of the genus *Phormidium* in a small amount (1%). In **October** of 2015 we marked only phytogenous material (22%) together with sediment particles and unidentified matter in the enchytraeids' pellets. The phytogenous material was represented by different green filamentous algae remains and mucous stems of diatoms (Fig. 2p). As minor components were found moth scales, sponge spicules, pollen, animal remains (chitinous exosceletons), and *Eudorina*-like colonial algae.

DISCUSSION

All the non-predatory oligochaetes could be roughly subdivided into three groups: (1) "detritus feeders", (2) "sand swallowers", and (3) "diatom eaters" (Giere 1975). The enchytraeid *M. bungei* apparently could be attributed to a detritophage with phytogenous detritus preference. This Baikal oligochaete occupies an ecological niche, which is similar with enchytraeids *L. lineatus*, *L. rivalis* and *E. albidus*. These enchytraeids also prefer to gather in high numbers around or inside of detritus masses (O'Connor 1967). In laboratory cultures they are able to transform fresh *Fucus* spp. and *Zostera marina* Linnaeus into dark-brown amorphous fecal masses in a short time (O'Connor 1967).

Dózsa-Farkas (1998) carried out a special investigation of enchytraeid fauna in detritus accumulations on the shallow Lake Balaton shores (Hungary). She found that the detritus accumulation biotope is poor in terms of enchytraeid species number in comparison with soil. Although species richness was less in the shore detritus than in soil, enchytraeids yielded there a maximum value of $217,900 \pm 10,872$ inds. m^{-2}. In the case of Lake Baikal detritus accumulations we can assume a maximum number about 40,000 inds. m^{-2} (Timoshkin *et al.* 2012a). Such species of enchytraeids evidently have to play a significant ecological role in consuming and transforming a tremendous mass of organic matter thrown on the shores of lakes (Dózsa-Farkas 1998) and seas (O'Connor 1967).

Researchers' opinions concerning the detritus as a food item is rather contradictory. In the former studies detritus and algae were considered as a primary food source for oligochaetes (Giere 1975). The previous (Giere 1975) and our original detritus attractiveness tests showed that the overwhelming majority of enchytraeids concentrated in detritus layer and surrounding ground layers.

With the exception of mineral sediment particles, which may comprise up to 33%, the rest of *M. bungei* pellet content is detritus of different origin. In the present study we subdivided pellet content into three basic groups: (1) phytogenous material *s. l.* (algae, macrophytes, and plant remains), (2) animal material and (3) unidentified matter (Fig. 3). Additionally, sometimes we found fungal hyphae and Cyanobacteria, but in a quite small amounts. It should be emphasized that it is rather difficult to judge about fresh plant material

in enchytraeids feeding, because often it is hard to separate it from already decaying material. Although, such widespread enchytraeid species as *L. lineatus* and *E. albidus* are supposed to prefer exactly thin-walled inner plant cells (Giere 1975). For example, in July of 2015 we observed lots of semidigested matrix of fresh algal cells.

Phytogenous material has been quantitatively predominating in comparison with the other types of detritus during all investigation period (Fig. 3); its percentage varied in the range of 7–87%. Also phytogenous detritus appeared to be more diverse. It was performed by green algae (i.e., *U. zonata*, *Tetraspora*, unidentified Cladophoracea, *Spirogyra*). *Ulothrix* should be mentioned especially, as it was a permanent component of the oligochaete pellet content in June and July. Sometimes it made up to 100% of *M. bungei* pellet content. Even when there was *Ulothrix* in the shore detritus accumulations just in a small amount, it was certainly found in the pellets of *M. bungei*. In this case we can state that the latter species demonstrates some kind of nutritional selectivity. The fact of *Ulothrix* zoospores germination in the enchytraeid pellets undoubtedly requires a special study to reveal the role of *M. bungei* in *U. zonata* distribution.

Remains of land plants play also an essential role in the nutrition of this oligochaete species. Fragments of leaf external tissue and conifer needle remains can often be found in *M. bungei* pellets. Diatom algae could be indicated as an important part of the pellet phyto-component. It should be marked, that they are characteristic component of gut content of oligochaetes from various families. For instance, widespread *Stylaria lacustris* (Linnaeus, 1767) assimilates diatoms *Asterionella*, but does not consume green algae *Scenedesmus* (Streit 1978). *Chaetogaster diastrophus* (Gruithuisen, 1828) prefers certain diatom genera among the other food items (McElhone 1979). Some authors noted, that tubificids also use diatom algae as a food, and *Lumbriculus variegatus* (Müller, 1774) may digest diatoms but not green algae (Timm 1987). Analyses of gut content of dominant marine

interstitial enchytraeid *M. subterranea* showed that it exclusively fed on pennate diatoms (Giere 1975).

Diatoms' thecae are a regular component in pellets of *M. bungei*. The genera *Didymosphenia*, *Gomphonema*, *Cocconeis*, *Hannaea*, *Cymbella*, *Navicula*, *Fragelaria*, *Synedra*, and *Aulacoseira* were found most frequently. We often observed *Cocconeis* in quite large amounts in pellet content as it is an epiphyte of filamentous green algae. Mucous stems of diatoms should be mentioned specifically. These stems permanently occur in the pellet content of splash zone enchytraeids. Due to the high number of *Didymosphenia* in detritus accumulation in 2015 the mucous stems were observed during July–October and contributed 4–45% of *M. bungei* pellet content (Fig. 2 h, l, n, p).

As for animal material, it rarely constituted more than 30% in *M. bungei* pellet content (Fig. 3). The biggest contribution was made by large fragments of arthropod (crustaceans and insects) chitinous exoskeleton. Remains of oligochaetes (with *M. bungei* chaetae) also were a significant component among animal material of pellets. Finding of *M. bungei* remains in their own pellets is rather intriguing phenomena, which is hard to explain by their possible predatory behavior. Although, authors noted that necrophaging is not unknown among enchytraeids (O'Connor 1967, Čekanovskaya 1962).

During the whole period of studying we found various minor components of the pellet content: Cyanobacteria, pine pollen, moth scales, sponge spicules, colonial green algae (*Volvox* and *Eudorina*), attached infusoria *Vorticella*, and fungal hyphae. Besides, we observed parasitic rotifers at a resting (cyst-like) stage. More often they were noticed being dead, but in some cases we watched them alive and rarely free-floating. In this paper we do not discuss this fact in details.

It is known that a rich microflora grows on the particles of decaying organic matter (Monakov 1998). Certainly feeding on microorganisms could

explain the preference of rotten phytogenous material such as "ripe" coastal detritus accumulations (Timoshkin *et al.* 2012a). Hence the question appears: do enchytraeids utilize larger detritus particles themselves as a food, or the primary source of their feeding are microorganisms.

In literature there are arguments both for (Dash *et al.* 1980, 1981, O'Connor 1967), and against (Latter 1977, Latter & Howson 1978, Standen & Latter 1977, Toutain *et al.* 1982) the statement that detritus debris in the majority of cases serves only like a substratum for microorganisms and stays more or less untransformed, but bacteria and fungi are main food source to be directly consumed. Detritus was not recorded in the alimentary tracts of marine enchytraeid *M. spicula*. There was only brownish amorphous mass, which appears to be an agglomeration of bacteria (Giere 1975). Bacterial food is available to oligochaetes even in sand biotopes. Not only small, but also large oligochaete specimens can partially or entirely live on bacteria. That was shown in experiments with lacustrine and soil species (Giere 1975).

On the other hand, could a bacterial biomass serve as sufficient food source to sustain oligochaete populations? Even despite the great rates of bacterial growth, it seems to be doubtful that oligochaetes are able to get required energy with feeding on bacteria exclusively (Giere 1975). Nielsen (1961) has calculated that population of soil enchytraeids with density of 50,000 inds. m^{-2} for surviving for 1 year need to utilize 30–40 g m^{-2} of bacteria.

Latter (1977) demonstrated, that *Cognettia sphagnetorum* (Vejdovský, 1878) grew better in axenic cultures, rather than in cultures with microorganisms. Studies of Toutain *et al.* (1982) indicated that soil enchytraeids were feeding on plant remains like saprotrophs. Giere & Hauschildt (1979, cited in Gelder 1984) experimentally proved that a suitable food source for *L. lineatus* was algae, but not bacterial films. Additionally, investigation of digestive enzymes of some tropic soil enchytraeids revealed the presence of cellulase, which theoretically gives them an ability to consume a phytogenous material

(Dash *et al.* 1981). Thus mixed diet (microorganisms, living/dead plants) seems to be more expectable at least for oligochaete macrofauna (Giere 1975).

We can conclude that to solve the complicated question on a real food source for oligochaetes, one would need to combine traditional techniques of feeding examination with analyses of stable isotopes. Such analyses are commonly implied in feeding biology, but not yet on enchytraeids (Briones & Ineson 2002). Unfortunately, in literature we can find only a few cases of investigation of the feeding of Enchytraeidae with stable isotopes and mostly it is considered only in the context of a large soil ecosystem trophic net (Schmidt *et al.* 2004). There is only one special work devoted to soil enchytraeids feeding using radiocarbon techniques (Briones & Ineson 2002). The results of ^{14}C carbon dating showed that enchytraeids mainly assimilated organic matter (leaf litter), about 5–10 years old (Briones & Ineson 2002).

To summarize, we could state that *M. bungei* is a saprophage like many soil enchytraeids (Toutain *et al.* 1982). Our results demonstrate that it feeds on phytogenous detritus, what is also characteristic to soil representatives of the Family Enchytraeidae (Briones & Ineson 2002). We also suppose that the species has a feeding preference to filamentous green algae, especially to *U. zonata*. Our research supposed to be logically continued with stable isotopes analyses of *M. bungei* to specify its trophic status and establish what kind of material it assimilates primarily. Usually it is difficult to interpret stable isotopes data without information on ecology of feeding. Our results can be regarded as the first data on feeding of the Baikalian enchytraeids.

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On a collection of enchytraeids (Oligochaeta) from first order streams in São Paulo State, Brazil

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Abstract. A collection of 21 ethanol-preserved specimens of enchytraeids collected in first order streams in São Paulo State, Brazil, was whole-mounted on slides and investigated under light microscope. Eight species taxa were distinguished, belonging to *Achaeta*, *Guaranidrilus*, and *Marionina*. Five species could be named, one of them tentatively: *Achaeta neotropica* Černosvitov, 1937, *Achaeta singularis* Schmelz, 2008, *Guaranidrilus oiepe* Righi, 1974, *Marionina argentea* (Michaelsen, 1889) s.l., *Marionina* cf. *seminuda* Xie & Rota, 2001. Three further unnamed species taxa of *Guaranidrilus* were distinguished; two of them may be new species. The presence of sexually mature specimens of *A. singularis* allowed an emendation of the original description which was based on juvenile specimens. The rod-shaped crystals in the coelom of *A. singularis* are similar to raphides in plants and sponges, and may consist of calcium oxalate. The material is deposited in the Zoological Museum of the University of São Paulo, Brazil.

Keywords. Annelida, Clitellata, freshwater biodiversity, South America, raphides.

INTRODUCTION

Enchytraeids are frequently found in samples from South American freshwater sediments (Brinkhurst & Marchese 1989, Gorni & Alves 2008). In small streams with oxygen-rich water up to 30–50% of the oligochaete fauna may consist of enchytraeids (Rodrigues *et al.* 2015, 2016). However, species are rarely identified because of the lack of identification guides and taxonomic expertise in South America (Brown *et al.* 2013). Furthermore, enchytraeids are best identified *in vivo*, whereas the other aquatic oligochaetes are identified on fixed material. As a result, while 171 species and subspecies of other microdrile oligochaete families have been counted in South American freshwaters (Christoffersen 2007), not more than 7 species of Enchytraeidae have been recorded from freshwater sediments (Table 1).

Here we report on a small collection of ethanol-preserved enchytraeids sampled in first-order forest streams of the State of São Paulo, southeastern Brazil. Sexually adult specimens that could be identified to species are described and illustrated. The investigation profited from previous work on terrestrial enchytraeids in the neighbouring Paraná State in the framework of the SOLOBIOMA project (Römbke *et al.* 2007, Schmelz *et al.* 2008, 2009, 2011). It turned out that almost half of all specimens belonged to a species discovered during that project: *Achaeta singularis* Schmelz, 2008 (Schmelz *et al.* 2008). It had been described based on juvenile specimens, and the presence of adults in this collection allowed to complement the original description with details of the reproductive organs. This species is peculiar by rod-shaped crystals in the coelom. They are re-described and their possible function is discussed. Furthermore, a single adult specimen of *Marionina argentea* sensu lato is described. The remaining specimens in the collection are characterized briefly.

Table 1. List of enchytraeid species identified from freshwater sediment samples in South America, based on Christoffersen (2009) and original literature.

Species	Reference	Habitat, comments
Buchholzia appendiculata (Buchholz)	Martínez-Ansemil & Giani 1986	small stream in Bolivia
Enchytraeus buchholzi Vejdovský	Christoffersen 2009	Argentina, Peru, Bolivia, Venezuela. Species complex.
Enchytraeus gordioides Černosvitov	Černosvitov 1941	sediment of a small stream in Loreto, Argentina
Fridericia bisetosa (Levinsen)	Botea 1987	Venezuela, stream and phreatic sediment. Records doubtful.
Hemienchytraeus loksai Dózsa-Farkas	Černosvitov 1939	Peru, Lake Lagunilla, as *H. stephensoni* (see Schmelz & Collado 2007)
Henlea perpusilla Friend	Martínez-Ansemil & Giani 1986	small stream in Bolivia
Globulodrilus riparius (Bretscher)	Martínez-Ansemil & Giani 1986	small stream in Bolivia, as *Marionina riparia*
Lumbricillus lineatus (Müller)	Černosvitov 1939	shore of Lake Titicaca, as *Pachydrilus lineatus*

MATERIAL AND METHODS

The collection contained 21 ethanol-preserved specimens of enchytraeids in 18 vials, collected from river sediments in the State of São Paulo, Brazil. Most of the material (17 specimens) was collected in first order streams in the "Parque Estadual Campos do Jordão", São Paulo State, Brazil, in different sampling campaigns of aquatic oligochaetes, from 2006 to 2008, by Ana Emiliar Siegloch. Methods and some results are available in Gorni & Alves (2008). The remaining four specimens are from other sites in São Paulo State. Sampling took place within the framework of the project BIOTA/FAPESP, "Research Program on Biodiversity Characterization, Conservation, Restoration and Sustainable Use" (www.biota.org.br). Geographical coordinates were not available for all sampling localities.

The specimens were passed through an ethanol-xylene dehydration series and whole-mounted in Canada balsam between two coverslips to allow investigation from both sides. The coverslip preparation was framed with an aluminium slide, the so-called Cobb slide used in nematology (Neuhaus *et al.* 2017). Microscopical investigation was carried out at x40 to x400 magnification. For the redescription of *A. singularis*, the type series was consulted as well. In the descriptions, taxonomically important details of the morphology that could not be observed due to the mode of fixation are indicated by "not distinguished". All material is in the possession of the Zoological Museum of the University of São Paulo, Brazil (MZUSP, #1232 – #1249).

RESULTS

Identification

Eight species taxa of three genera were distinguished: two species of *Achaeta*, three species of *Guaranidrilus*, and two species of *Marionina*. Five species could be named, one of them tentatively: *Achaeta neotropica* Černosvitov, 1937, *Achaeta singularis* Schmelz, 2008, *Guaranidrilus oiepe* Righi, 1974, *Marionina argentea* (Michaelsen, 1889) s.l., and *Marionina* cf. *seminuda* Xie & Rota, 2001. In the other cases, only the genus or the family could be assigned.

Comments on species and specimens

Achaeta singularis Schmelz, 2008

(Figures 1–2)

Achaeta singularis Schmelz. Schmelz, Collado & Römbke 2008: 26–28, Fig. 6, Table 4.

Material examined. Ten specimens, 4 adult, 3 subadult, 3 juvenile, São Paulo State, Parque Estadual Campos do Jordão, collected by Ana Emiliar Siegloch. MZUSP #1239, 1 adult specimen, Parque Estadual Campos do Jordão - Campo do Meio, 22°41'56.6"S, 45°29'16.8"W. 21-08-2006. MZUSP #1240, 1 subadult specimen, Parque Estadual Campos do Jordão – Camhambora, 22°42'29.3"S, 45°30'6.7"W, 07-10-2007. MZUSP #1242, 1 adult specimen, Parque Estadual Campos do Jordão – Mimosinha, 22°41'29.3"S, 45°27'36"W, 08-10-2007. MZUSP #1243, 1 subadult and 1 juvenile specimen, Parque Estadual Campos do Jordão - Serrote, 22°39'39"S, 45°26'58.4"W, 20-08-2006. MZUSP #1244, 1 subadult specimen, Parque Estadual Campos do Jordão – Casguilho, 22°40'20.6"S, 45°27'53"W, 07-10-2007. MZUSP #1245, 1 adult specimen, record data as in #1240. MZUSP #1247, 1 juvenile specimen, record data as in #1239. MZUSP #1248, 1 juvenile specimen, record data as in #1242. MZUSP #1249, 1 adult specimen, record data as in #1240.

Further material for comparison, not included in this description: type series, 7 specimens (MZUSP # 1370: holotype, MZUSP #1371: paratypes).

Description. (The description is based on adult and subadult specimens unless stated otherwise.) Worms stout with tapering body ends. Largest adult specimen 8 mm long, diameter 0.45 mm at V, 0.6 mm at XI, 0.48 mm at XX. Remaining adult and subadult specimens 5–6 mm long and up to 0.4–0.55 mm wide. Juveniles 3.5–4 mm long, 0.3–0.4 mm wide. Segment numbers 25–30 in adult and subadult specimens, juveniles with 18–22 segments. Paired prostomial ganglia and inner prostomial papillae present. Body wall very thin, usually 6 μm thick, 8–10 μ in some regions of the largest adults, longitudinal muscle layer ca. 2.5–3 μm thick, not thicker than outer layer of epidermis and ring muscles. Pygidium flattened dorso-ventrally, rectal muscles inserting laterally, no ventral nerve cord. Epidermal gland cells not seen, pyriform glands absent. Brain incised anteriorly and posteriorly. Ventral nerve cord gang-

lionated from II on, ganglia of II–IV not fused, ganglia ovoid, rounded, separate from each other by elongate connectives free of perikarya. Towards growth zone in caudal segments, connectives with a narrow line of ventral perikarya, and ganglia increasingly fused into a medullar strand with segmental bulges (the ganglion precursors). Pharyngeal glands all united dorsally and with ventral lobes, dorsal connection usually wide, in VI sometimes narrow. Oesophageal appendages in V, dorso-laterally of oesophagus, paired, outline irregular. Chloragocytes as a thin, continuous layer from VI on, absent in X. Intestinal diverticula absent, gut widening more or less gradually in preclitellar segments, pars tumida inconspicuous, circumferal. Dorsal blood vessel from VII in juveniles and adults. Preclitellar nephridia at 6/7 and 7/8, *ca.* 4x as long as wide, c. 220 μm long and 60 μm wide in largest adult, compact and without constriction at septum. Anteseptale short, truncate, postseptale slightly tapering towards ectal pore; terminal vesicle present; epidermis thickened around ectal pore. Postclitellar nephridia from 11/12, of similar shape and size as in preclitellar region, those of hindmost 5–7 segments increasingly reduced in size towards pygidium. Nephridia unpaired or absent in several segments of the mid-body region. Coelomocytes three types: (1) flat, filled with pale vesicles, length *ca.* 25–40 μm; (2) flat, pale, matrix hyaline, cell perimeter brimmed, *ca.* 25 μm; (3) completely filled with packages of crystalline, needle-like bodies; needles pointed at both ends, length of packages c. 25 μm, thickness varying but never exceeding length.

Clitellum girdle-shaped, cells in *ca.* 24 separate or dense rows. Hyalocytes dorsally and laterally, alternating with granulocytes. Hyalocyte diameter *ca.* 15–25 μm, granulocyte diameter *ca.* 10–15 μm, height max. 20–30 μm; cells thickened around male pores. Rows of border cells (*i.e.* the 3–4 most anterior and most posterior rows, devoid of hyalocytes) interrupted mid-ventrally. Transverse row at the level of male pores wider than adjacent rows, situated in the middle of clitellum (ca. 12th row). Testis in IX, ovary in X in all specimens. Gonads unpaired. In adults large
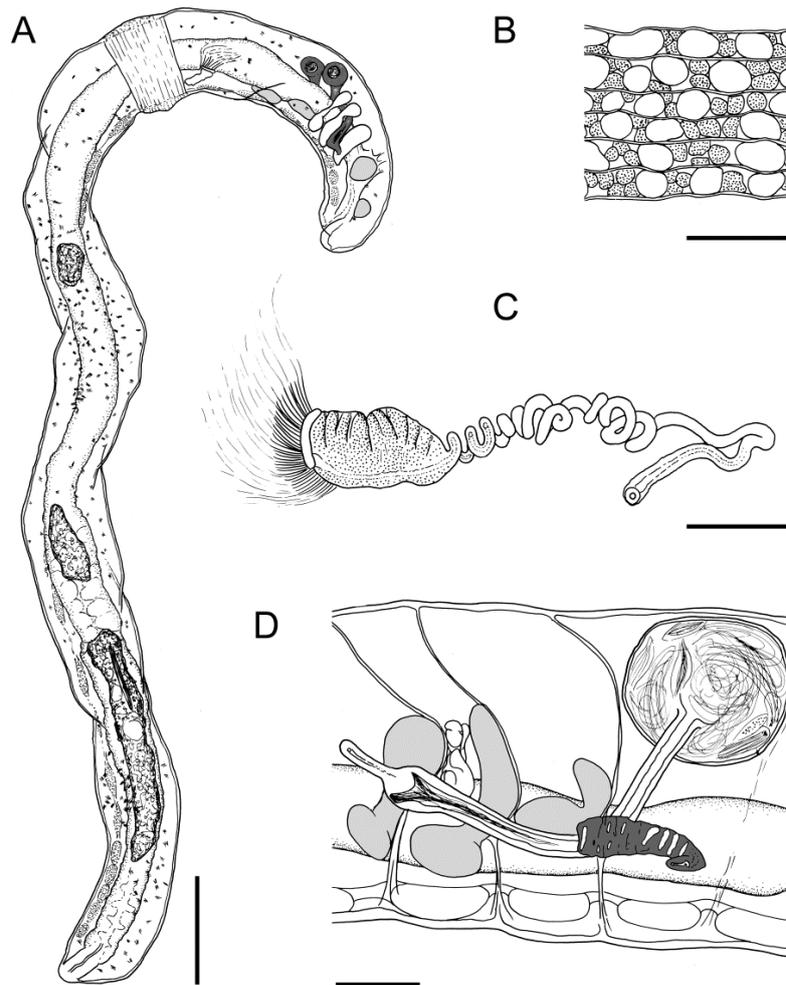
Figure 1. *Achaeta singularis* Schmelz, 2008. A. MZUSP #1243, adult specimen. Oocyte, postclitellar nephridia, and dorsal blood vessel not shown. Dark-grey: spermathecae. Light-grey, from right to left: brain, pharyngeal pad, preclitellar nephridia. Coelom with raphides, intestine with three pellets of ingested organic and inorganic matter (sand grains up to 100 μm diameter). Circumferal midgut pars tumida between 2nd and 3rd pellet. Perkarya of last five segments non-ganglionic. B. MZUSP #1243, detail of clitellum dorsolaterally, head region to the top. The second row from the top is the one that encloses the male pores. C. MZUSP #1243, male efferent apparatus. D. MZUSP #1245, segments IV–VII, lateral view, with pharyngeal glands (light-grey), oesophageal appendage in V, nephridium 6/7 (dark-grey), ventral nerve cord, and spermatheca. 2nd preclitellar nephridium at 7/8 not shown (specimen damaged here).

masses of developing and mature sperm present dorsally in IX. One specimen with a paired bulge anteriad of septum 8/9 dorsally, bulge unpaired in another specimen (seminal vesicle?). Spermatozoa about 140 μm long, heads *ca*. 40 μm long. Sperm funnels small, length less than half of body diameter, about twice as long as wide (*e.g*., 130 μm long, 70 μm wide); funnel body cylindrical, tapering towards vas deferens; collar apparently of varying width: as wide as funnel body, or up to

twice as wide (*ca*. 100 μm), judging from masses of sperm attached in parallel. Vasa deferentia tightly and irregularly coiled, of equal diameter throughout (*ca*. 7 μm), barely distinguishable near male pore; coil extending posteriad without loops. Male pores ventrally on body surface, widely separate, without surrounding glands, inconspicuous. Pores in the middle of clitellum. No further accessory glands seen. Spermathecae with short ectal duct and large ampulla; ampulla subdivided

into ectal part, connecting tube and ental reservoir. Ectal pores lateral; ectal glands absent; ectal duct short, in V, length *ca*. 60 μm, diameter ca. 18 μm, connection with ampulla thickened (ental bulb). Ampullar ectal part in V, dilated (diameter 50–60 μm) and with distinct lumen, sperm arranged here in parallel with nuclei oriented ectad. Ampullar ectal part gradually narrowing into connecting tube with distinct walls and lumen, diameter *ca*. 25–30 μm; connecting tube widening more or less abruptly in VII into thin-walled, spherical or egg-shaped ental reservoir, length ca. 140 μm, diameter 100–120 μm.

Remarks. Comparison with the original description. This redescription of *Achaeta singularis* enhances the original description, which was based on juvenile material, confirms most of the characters as originally described, and clarifies several doubtful points. In the following we list the peculiar and species-distinguishing characters of *A singularis* as given in Schmelz *et al.* (2008: 27) and compare them with our material: (1) Short brain with deep anterior and posterior concavities. Confirmed. (2) Ganglia of segments II–IV not fused into a suboesophageal ganglion. Confirmed. (3) Anterior ganglia rounded like ovoid bodies, not subdivided into an anterior and a posterior lobe. Confirmed. (4) Posterior ganglia fused into a common medullar strand. Confirmed, but less pronounced in subadult and adult specimens, where ganglia can always be distinguished even though they are in close contact with each other (Fig. 1A). (5) Presumably small body size and low segment number. Not confirmed. Adult specimens have the size and segment number in the medium range for species of *Achaeta* (comp. Schmelz & Collado 2010). The juvenile specimens of the type series measure 2 mm in length and 0.12–0.15 in body diameter, segment number 14–24. (6) Pyriform glands absent. Confirmed. (7) Oesophageal appendages present in V. Confirmed. (8) All pharyngeal glands united dorsally. Confirmed. (9) Secondary ventral pharyngeal gland lobes absent. Confirmed. (10) Nephridia at 6/7 and 7/8. Confirmed. (11) Dorsal blood vessel originating in preclitellar region. The origin was observed always in VII in juvenile and adult

specimens alike, and serves hence as a taxonomic character in all age groups. The origin may vary in other species: In the European *A. camerani,* juveniles have the origin in VII and adults in VIII (Schmelz & Collado 2010). (12) Spermathecal ectal pores in lateral position. Confirmed. Furthermore the girdle-shape of the clitellum and the wide spacing of the male pores is confirmed. The vas deferens is coiled and not straight (as seen in one paratype specimen). The originally described prostomial ganglia and prostomial papilla could not be investigated due to the poor mode of fixation.

The details of the reproductive system added here support the hypothesis in Schmelz *et al.* (2008) that *A. singularis* belongs to an ancient lineage within *Achaeta*. Girdle-shape of the clitellum, the uniform distribution of hylaocytes, and the absence of glands near the orifice of the spermatheca and of the male efferent duct are most likely plesiomorphies in the genus. Contrasting characters in other species of *Achaeta* (and hypothesized apomorphies) are: Clitellum interrupted dorsally and ventrally, hyalocytes concentrated into dorso-lateral longitudinal rows, spermathecal ectal duct swollen into a glandular mass, glands around male pore (see, *e.g.*, Schmelz & Collado 2010). The hypothesis was originally based on the absence of a suboesophageal ganglion (see above, point 2).

In the following we comment on two further peculiarities: the forward shift of the gonadal region by two segments, and the presence of rod-shaped crystals in the coelom. Both characters, while present in only some specimens of the type series, are present in all specimens of the new collection.

Displacement forwards of the gonadal region. The gonadal region is displaced two segments forwards in all specimens of the present collection, with testis in IX instead of XI, and ovary, male pores and clitellum in X instead of XII. In the type series this shift ahead is present only in one paratype specimen (Schmelz *et al.* 2008); the other six type specimens have the gonadal region in the usual position (XI–XII). A displacement

forwards of the gonadal region by one segment occurs in several species of enchytraeids, especially in *Achaeta*, where hitherto seven species are known to share this character. A shift ahead by *more* than one segment, however, is – with one exception – only found in taxa with the faculty to reproduce asexually by fragmentation (in the genera *Cognettia/Chamaedrilus*, *Buchholzia* and *Enchytraeus*). The only exception is *E. gordioides* Černosvitov, 1942 (Table 1), but this species may be fragmenting as well, considering its similarity with the fragmenting *E. bigeminus* Nielsen & Christensen, 1963 and *E. dudichi* Dózsa-Farkas, 1995. Fragmentation, if not directly observed, can be inferred from incomplete front or rear ends in many specimens. It is unknown in *Achaeta*, and the material of *Achaeta singularis* gives no indication of fragmentation either: all specimens are complete anteriorly and posteriorly. This seems to indicate a new type of variation in the family: a shift of the gonadal region ahead by more than one segment in a species with exclusively sexual reproduction.

Only some of the specimens have a forward shift of the gonadal region (the holotype is not among them), and up to now there are only two morphs, one with male pores in X and one with male pores in XII. The resulting possibility that *A. singularis* presents, in fact, two different species should be tested with DNA sequencing methods.

In this context we have to notify an error in a figure within the original description of *Achaeta singularis* (Schmelz *et al.* 2008). In Figure 6A, testis and ovary are shown in X and XI, respectively, counting the ganglia. Reinvestigation of the paratype specimen from which the drawing was made (MZUSP #1371) revealed that, in fact, they are situated in XI and XII, respectively, in accordance with the text of the original description.

Raphides in the coelom of *A. singularis*. Bundles of microscopic, needle-like crystals are present in the coelom of all specimens of the São Paulo State material (Fig. 2), and also in some of the type specimens (Schmelz *et al.* 2008), the holotype included (Schmelz, pers. obs.). These crystals are named here "raphides" (Greek raphidos = needle), in accordance with the use of this term in the biological literature. Raphides occur in sponges, fungi, and plants, and they consist mostly of calcium oxalate (plants, fungi) (Arnott 1995, Horner & Wagner 1995) or aragonite (sponges) (Hooper & Van Soest 2006). In *A. singularis* each bundle of raphides is about 8 µm wide and 4 µm thick. They are inclusions of coelomocytes, because most bundles are surrounded by a membrane, which also encloses a nucleus, closely attached to the needles either laterally or apically, at one side of the needle tips (comp Fig. 2C, bottom). The crystals do no disappear after clearing with xylene. As to their chemical composition, it may be calcium oxalate. Raphides are common in plants, and most raphides in plants consist of calcium oxalate. Shape and size of raphides in *A. singularis* are similar to the crystals in plants (comp. Coté 2009, Saadi & Modal 2011). Their function is usually considered to be a repellent against predation. We hypothesized a similar function in *A. singularis* (Schmelz *et al.* 2008), but this would imply that possible predators are able to distinguish between different species of enchytraeids. Further possible functions are related to calcium metabolism. Calcium is rare in tropical soils, and oxalate, abundantly produced by fungi, binds calcium. A capacity to ingest and to metabolize oxalate to bind calcium has been suggested for a Nearctic earthworm (*Arctiostrotus* sp.) (Spiers *et al.* 1986), and the North American earthworm *Diplocardia mississippiensis* Smith may selectively feed on fungi to derive calcium via the fungal oxalates (Lachnicht & Hendrix 2001). A similar function as a calcium-binding device may be assumed for the oxalate crystals in *A. singularis*, which may result from fungal grazing as well. On the other hand, the target may not be calcium but oxalate. In the strongly acid soil or freshwater sediment, a surplus of oxalate may be assimilated, increasing acidity in the coelomic fluid. Low pH is a problem for soil biota in the tropics (Lavelle *et al.* 1995). The binding of oxalate by calcium in the coelomocytes of *A. singularis* may therefore be a mechanism to regulate the pH in the body fluid, similar to one of the functions of the so-called calciferous glands in earthworms.
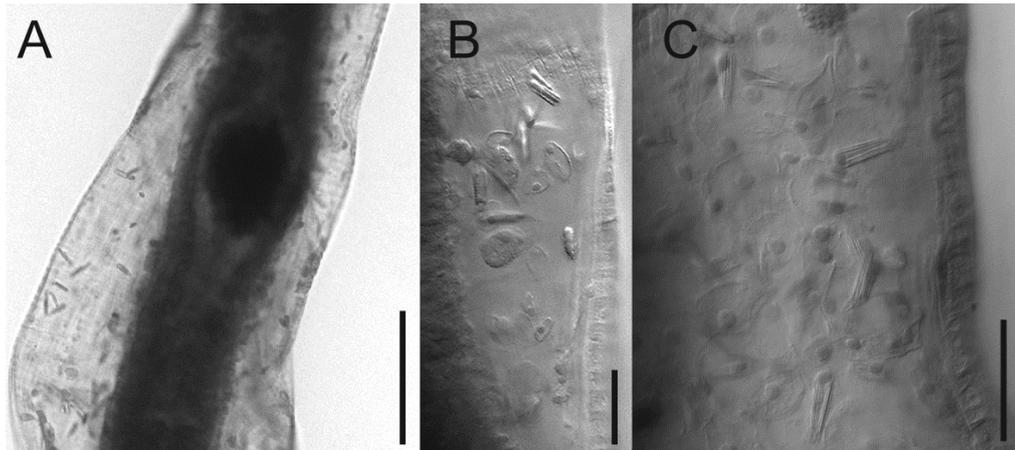
Figure 2. *Achaeta singularis* Schmelz, 2008. MZUSP #1243, micrographs of coelomocytes with and without raphides, at different magnifications. Scale bars = 200 μm in A, 50 μm in B, C.

Raphides are also present in sponges (Hooper & Van Soest 2006), here as aragonite crystals, and with a different shape. To our knowledge, raphides are unknown in other animal taxa. With more material sampled in the future, the chemical nature of raphides in *A. singularis* may be determined with certainty.

Raphides were present in only three specimens (out of seven) of the type series and therefore excluded from the list of species-distinguishing characters (Schmelz *et al.* 2008). However, their presence in all specimens of our collection here suggests that raphides should be included as a taxonomic character for *A. singularis*. All specimens of the type series are small juveniles, and it may take some time during growth and maturation to metabolize or to store calcium oxalate in the coelomocytes. We have observed a similar case in some European specimens of *Marionina argentea* (Michaelsen) sensu lato, a complex of semi-aquatic species (Rota 2013), characterized by strongly refractile bodies in the coelomocytes. Presence of these bodies is so constant that it is a diagnostic character of the species complex, but some very small specimens are without these bodies (Schmelz, pers. obs.). It seems that, as in *A. singularis*, these bodies are metabolites that are not yet present immediately after hatching of the worms from the cocoon. The chemical compo-

sition of the refractile bodies in *Marionina argentea* is unknown; but they are not calcium crystals, because they disappear during clearing with xylene.

Achaeta neotropica Černosvitov, 1937

Achaeta neotropica Černosvitov, 1937b: 154–157, Figs 16–26.
Achaeta neotropica Cern.—Righi 1974: 127–129, Figs 1–6; Christoffersen 1979: 153–154, Figs 1–10; Righi 1981: 427. Schmelz *et al.* 2008: 14–21, Fig. 4.
?*Achaeta becki* Schmelz & Collado, 2005: 49–57, Figs 1–2, Table 1.

Material examined. MZUSP #1235, 1 juvenile specimen, Parque Estadual Campos do Jordão - Serrote, 22°39'39"S, 45°26'58.4"W, 20-08-2006, col.: Ana Emilia Siegloch.

Description. Length 3 mm, chaetae and pyriform glands absent, oesophageal appendages not seen, cuticle thick, anterior nephridia at 6/7 and 8/9, absent at 7/8, with terminal vesicle.

A. neotropica is possibly a complex of species (Schmelz *et al.* 2008). The specimen was identified mainly on account of the peculiar distribution of preclitellar nephridia.

Marionina argentea (Michaelsen, 1889) sensu lato

(Figure 3, Table 3)

Enchytraeus argenteus Michaelsen, 1889: 15–16, Fig. 6.
Pachydrilus (*Marionina*) *argenteus* (Michaelsen). Černosvitov 1937a: 293.
Marionina argentea (Michaelsen). Nielsen & Christensen 1959: 113–114, Figs 138–141. Chalupský 1992: 145, Fig. 15. Rota 1995: 220. Schmelz & Collado 2010: 100, Fig. 39A.
Marionina argentea (Michaelsen) sensu lato. Rota 2013: 136.

Material examined. MZUSP #1233, 1 adult specimen on a slide with 2 specimens (the other one being an unidentified juvenile specimen of enchytraeids, possibly *Hemienchytraeus* sp., see below). Ubatuba, Rio da Pipoca, 23º24'1.7"S, 45º6'47.9"W, 05-10-2007, col.: Ana Emilia Siegloch.

Description. Body length 1 mm, diameter c. 0.07 mm (0.07 mm in V, 0.09 mm in XI, 0.06 mm in XX). 20 segments. Body wall thin, mostly *ca.* 3 µm thick. Prostomium and first two segments strongly contracted, with deep external transverse furrows (fixation artefact). Prostomial epithelium with frontal recess, location of head pore unknown. Chaetae two per bundle, absent laterally at II, straight with ental hook, ectally slightly bent, length *ca.* 22 µm in anterior segments, 25 µm posteriorly. Pharyngeal glands dorsal in IV and V, elongate and ventral in VI. Brain shape unknown due to lateral position of worm, ventral nerve cord medullar, wide and lobed in II–IV. Shape of nephridia not distinguished; lobed tissue seen in nephridial positions in VIII and IX. Coelomocytes present, shape and size unknown. Dorsal blood vessel origin in XI or 1/2 XII, anterior bifurcation not visible. Gonadal region shifted one segment forward: Testes in X, male gland in XI, clitellum in XI–1/2 XII. Clitellum saddle-shaped, ventral border roughly at level of male glands; cells in *ca.* 14 dense, transverse rows, granulocytes *ca.* 8 µm wide, hyalocytes *ca.* 12 µm wide, roughly alternating with tendency to form longitudinal rows. Ventral borders of clitellum formed by one longitudinal row of hyalocytes on each side; each line interrupted by one granulocyte dorsally of male gland (Fig. 3). Seminal vesicle absent, developing sperm present dorsally in X. Sperm funnel and vas deferens not distinguished. Male glandular bulb spherical, not larger than clitellar gland cells, diameter 8–10 µm. Spermatheca small, consisting of short ectal duct and globular ampulla, the latter smaller than pharyngeal gland lobes, connected with oesophagus; further details (*e.g.* glands) not distinguished.
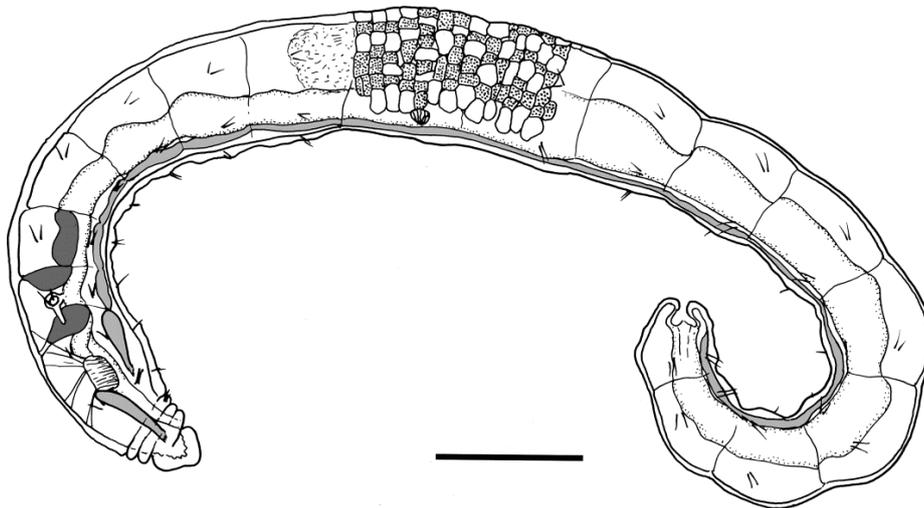
Figure 3. *Marionina argentea* (Michaelsen, 1889) *sensu lato*. Light-grey: brain and ventral nerve cord. Dark-grey: pharyngeal glands.

Remarks. Although several details were not distinguishable – brain shape, texture of coelomocytes, spermathecal glands, nephridia, sperm funnels – this specimen is safely assigned to *Marionina argentea* (Michaelsen, 1889) due to the peculiar chaetal pattern, together with small body size, low segment number, medullar ventral nerve cord, shape of the pharyngeal glands, small size of the reproductive organs, and low amounts of developing sperm. *Marionina argentea* (Michaelsen, 1889) sensu auctorum was recently split into four species by Rota (2013) and a fifth species was added in Martin *et al.* (2015) (Table 3). Among these five species under the umbrella of *M. argentea* sensu lato, the single specimen described here is most similar to *M. deminuta* Rota, 2013 (Table 3) by the small body size, the forward shift of the gonadal region by one segment, the small size of the male gland and the almost identical pattern of clitellar gland cells (compare Fig. 3 with Fig. 3B,C in Rota 2013), here especially the ventro-lateral row of hyalocytes, interrupted by one granulocyte at the level of the male gland. Hence, this specimen belongs either to *M. deminuta* or to a new species. This is the first record of *Marionina argentea* sensu lato from South America.

Marionina cf. *seminuda* Xie & Rota, 2001

Material examined. MZUSP #1237, 1 juvenile specimen, Parque Estadual Campos do Jordão - Celestina, 19-08-2006, col.: Ana Emilia Siegloch.

Description. Body length 2 mm, diameter 0.09 (V), 0.075 (XII), 0.08 (XX). 22 segments. Chaetae straight ectally, ental hook almost absent, 2 per bundle, laterals absent, ventrals from III (= absent in II). Cuticle conspicuous, 1.5 μm thick. Brain apparently indented posteriorly. Ventral nerve cord medullar. Pharyngeal glands in IV, V with ventral lobes and unpaired dorsal lobe, in VI elongate, separate ventral lobes. No oesophageal appendages or intestinal diverticula seen. Preclitellar nephridia at 7/8, 9/10. Dorsal blood vessel from XII. Coelomocytes floppy, length 10–20 μm, with pale blurred vesicles, coagulated masses of cells in anterior segments, but most of coelom empty. Precursors of testis and ovary in XI and XII, respectively.

The peculiar distribution pattern of the chaetae is known only from *M. seminuda* Xie & Rota, 2001, a terrestrial species described from China. The other details of this juvenile specimens also agree with the description of *M. seminuda*, which hence may be a peregrine species. Sexually mature material is necessary to confirm the identification.

Guaranidrilus oiepe Righi, 1974

Guaranidrilus oiepe Righi, 1974: 140–141, Figs 32–40. Righi 1981: 427–428. Coates & Diaz 1988: 779–780, Fig. 2.

Material examined. MZUSP #1232, 1 adult specimen, Picinguaba - Riacho Sabesp, Sao Paulo State, Brazil, 04-10-2007, col.: Ana Emilia Siegloch.

Description. Body length 4 mm, diameter 0.14 mm (V), 0.16 mm (XII, XX), 29 segments. Two chaetae per bundle absent at XII, ventral chaetae ca. 32 : 3 μm, caudal chaetae *ca.* 56 : 5 μm; *i.e.*, caudal chaetae almost twice as large as anterior chaetae. Epidermal gland cells present, pattern not distinguished. Body wall thin (*e.g.*, 8 μm dorsally in XVI), cuticle 1.5–2 μm thick. Secondary lobes of pharyngeal glands in VI. Oesophageal appendages absent. Intestinal diverticula in VII, directed anteriad only, no posterior projection. Dorsal blood vessel from XI or XII. Preclitellar nephridia 2 pairs, at 8/9 and 9/10. Coelomocytes sparse, with very fine, regular, blurred vesicles.

Clitellum saddle-shaped, well-developed, cell height dorso-laterally 16 μm, on dorsal half hyalocytes and granulocytes, ventro-laterally only granulocytes, *ca.* 33 dense rows; hyalocytes contagious. Seminal vesicle absent. Sperm length *ca.* 50 μm, heads *ca.* 20 μm. Sperm funnel small, cylindrical, 30 μm long, 20 μm thick, collar high, not wider than funnel body. Vas deferens in tight isodiametric coils. Male pores on body surface, no male glands distinguished. Spermatheca small, elongate, bent in V, extending into VI. No accessory glands seen.

This specimen agrees with the description of *G. oiepe* Righi, 1974, except for the body size (length 5.7–8.5 mm, diameter ca. 0.25 at XII, *ca.* 0.2 in rest of body, 32–34 segments). However, a subsequent record of the species (Righi 1981) gives dimensions that agree better with our specimen (length 3.5–4.1 mm, diameter *ca.* 0.14 mm at XII, *ca.* 0.13 mm in rest of body, 31–32 segments). Number of preclitellar nephridia is unknown in *G. oiepe*, only the presence of the 1st nephridium at 8/9 is originally described. Apart from *G. oiepe*, only *G. hoeferi* Schmelz *et al.* 2011 has preclitellar nephridia in 8/9 and 9/10, in other species the 1st nephridium is placed more anteriorly. *G. hoeferi* differs by huge spermathecae and seminal vesicle, among other characters. *G. oiepe* was originally found in decaying wood inside humus-rich soil in Minas Gerais, and there are two more records from Brazil, from the States Rio de Janeiro (Righi 1975) and Mato Grosso (Righi 1981); this is the first record from São Paulo State.

Guaranidrilus sp. 1

Material examined. MZUSP #1234, 1 subadult specimen, Núcleo Santa Virgínia (São Luis do Paraitinga, SP), Ponte Três Paus, 31-10-2008, col. Ana Emilia Siegloch.

Description. Length 3 mm, 26 segments. Two chaetae per bundle, 60 μm long in caudal segments, 26 μm anteriorly. No epidermal gland cells seen. Cuticle 1.5–2 μm thick. Brain incised posteriorly. Pharyngeal glands all united dorsally. Presence or absence of oesophageal appendages not ascertained. Intestinal diverticula in VII, filling out segment. Dorsal blood vessel from IX or further back. Preclitellar nephridia at 6/7–8/9. Coelomocytes 12–18 μm long, filled with brown granules, cells very conspicuous against rest of body. Clitellum girdle-shaped, in c. 35 rows. Compact mass of cysts in XI dorsally. Spermatozoa short: *ca.* 40 μm long, heads *ca.* 10 μm, or longer. Sperm funnels small, 32 μm : 16 μm, collar as wide as funnel body. Vasa deferentia in tight isodiametric coils, male pores on body surface, no "penial bulb", clitellum thickened around male pores. Spermathecae stump-like and very short, *ca.* 30 μm long, diameter 10 μm, possibly extending into VI.

Remarks. This specimen probably belongs to a new species. It is conspicuous by the strong-brown coelomocytes and the girdle-shaped clitellum. The only species of *Guaranidrilus* currently known with a girdle-shaped clitellum is *G. cingulatus* Schmelz *et al.*, 2011, a species with pale coelomocytes.

Guaranidrilus sp. 2

Material examined. MZUSP #1236, 1 juvenile specimen, Parque Estadual Campos do Jordão - Campo do Meio, 22°41'56.6"S, 45°29'16.8"W, 27-04-2006. MZUSP #1241, 1 juvenile specimen, Ubatuba - Rio do Veio, 23°21'35"S, 44°47'0,4"W, 03-10-2007. Col. Ana Emilia Siegloch.

Description. Length 3 mm. Chaetae *ca.* 50 μm in caudal segments, 25 in anterior segments (MZUSP #1236), or 26 μm anteriorly, 36 μm posteriorly (MZUSP #1241). Cuticle thin (< 1 μm). Secondary pharyngeal gland lobes in V and VI. Oesophageal appendages paired anteriorly in VI, small. Intestinal diverticula in VII. Preclitellar nephridia 3 pairs, from 6/7 to 8/9. Coelomocytes small, ca. 10 μm long.

Remark. The two juvenile specimens of *Guaranidrilus* sp. 2 differ in the length of the chaetae and may belong to different species.

Guaranidrilus sp. 3

Material examined. MZUSP #1246, one adult specimen. Parque Estadual Campos do Jordão - Serrote, 22°39'39"S, 45°26'58.4"W, 15-02-2007, col. Ana Emilia Siegloch.

Description. Length 7 mm, diameter 0.2–0.25 mm. 36 segments. Head pore on prostomium. Chaetae 2 throughout, anteriorly ca. 40–45 μm long, in caudal segments 60–65 μm. Epidermal gland cells at least 6 rows dorsally in anterior segments; shape not distinguished. Body wall *ca.*

15–18 μm thick dorsally, cuticle *ca*. 2.5 μm thick, longitudinal muscle layer 8–10 μm. Septa not conspicuously thickened. Pharyngeal glands with secondary ventral lobes in VI (possibly also in V, small). Oesophageal appendages absent. Intestinal diverticula extending over 3 segments, VII–IX, with anterior and posterior projections. Dorsal blood vessel from XI. Preclitellar nephridia 4 pairs, 6/7–9/10. Coelomocytes concentrated at body ends, length *ca*. 30–50 μm, 1.5–2x as long as wide, with very fine blurred vesicles.

Clitellum saddle-shaped, *ca*. 33 rows, midventral interruption narrower than distance of male pores. Maximum cell height 18 μm dorsally and 14 μm ventrally around male pores. Seminal vesicle absent, few cysts dorsally in XI. Spermatozoa present on sperm funnel, length *ca*. 85 μm, heads 32 μm. Sperm funnel pear-shaped, *ca*. 60 μm long, *ca*. 1.5x as long as wide, tapering at both ends, the tapering being more pronounced ectally, towards vas deferens; collar narrower than funnel body. Vas deferens wound in tight isodiametric coils. Male pores on body surface, male glands small or absent, no "penial bulb". Spermatheca small, without sperm, extending into anterior region of VI only, length *ca*. 170 μm, ectal duct 16 μm wide, ental reservoir 24 μm wide, transition from ectal duct to ampulla inconspicuous. No accessory glands seen. Two mature eggs.

Remarks. The specimen agrees with most details of the original description of *Guaranidrilus glandulosus* Černosvitov, 1937 (Černosvitov 1937b), notably the large intestinal diverticula, so far unique in the genus (in other species the diverticula extend over 1 or 2 segments). However, *G. glandulosus* has large male glands ("bulbes péniales") around the male pores, so this specimen most probably belongs to a new species. Furthermore, *G. glandulosus* is incompletely known and in need of revision, because one important character is controversial: Oesophageal appendages are present in VI according to the original description ("peptonéphridies"), but they appear to be absent in the type material (Coates & Diaz 1988).

Enchytraeidae gen. sp.

Material examined. MZUSP #1233, one juvenile specimen, Ubatuba – Rio da Pipoca, 23°24'1.7"S, 45°6'27.9"W, 05-10-2007. MZUSP #1238, two juvenile specimens, Pindamonhangaba - Cachoeira Nilceia, 22°45'25.6"S, 45°28'38.2"W, 28-09-2007. Col. Ana Emilia Siegloch.

Remarks. The three juvenile specimens have two strongly sigmoid chaetae per bundle and a medullar nerve cord. They may belong to *Hemienchytraeus*, but genus-diagnostic characters (*e.g.* oesophageal appendages) were not distinguished.

DISCUSSION

Achaeta singularis, *Guaranidrilus oiepe* and *Marionina argentea* sensu lato are new records for the State of São Paulo, and *M. argentea* is also new for South America. The latter, very common in Europe, is a complex of semi-aquatic species, often found in moist soil, at river banks, but also in river sediments (Rota 2013). As was mentioned above, among the five species currently distinguished within *M. argentea* sensu lato, the specimens resemble most *M. deminuta* Rota, 2013.

Better knowledge of freshwater enchytraeids in South America faces three challenges: First, the species diversity of enchytraeids in South America is still largely unexplored (Schmelz *et al.* 2013) and any sampling may result in collecting undescribed species. Second, taxonomic work with enchytraeids requires the investigation of living specimens. Third, identification of the aquatic species requires also knowledge of the terrestrial species, because many species commonly considered as terrestrial may survive or even reproduce in aquatic habitats, provided that no oxygen-deficiency occurs. In Europe, enchytraeids are species-rich and abundant in the ecotone between soil and freshwater (*e.g.*, Erséus *et al.* 2005, Plum & Filser 2005, Beylich & Graefe 2007). It will be highly interesting to sampling this ecotone in tropical and subtropical Brazil and to compare the fauna with the aquatic and the

terrestrial one. For example, *A. singularis* was originally found on "terra firme" but it makes up almost half of the specimens from the freshwater samples. A sampling campaign along the moisture gradient from soil to freshwater should reveal the true moisture preferences of this species that was considered to be a remnant of the old autochthonous forest (Schmelz *et al.* 2008).

Another point of interest will be the percentage of native *vs*. peregrine species in the aquatic enchytraeid assemblage. Among all taxa identified here, *M. argentea* and *M.* cf. *seminuda* may be considered as peregrine species, all others are likely native species.

Table 2. List of enchytraeid specimens, with MZUSP accession numbers, sampling sites, and identification results.

MZUSP accession numbers	Sampling locality	Identification result
MZUSP 1232	Picinguaba - Riacho Sabesp	*Guaranidrilus oiepe* Righi, 1974
MZUSP 1233a,b	Ubatuba – Rio da Pipoca	a: *Marionina argentea* s.l. (Michaelsen, 1889) b: Enchytraeidae gen. sp.
MZUSP 1234	Núcleo Santa Virgínia (São Luis do Paraitinga, SP) Ponte Tres Paus	*Guaranidrilus* sp. 1
MZUSP 1235	Parque Estadual Campos do Jordão - Serrote	*Achaeta neotropica* Černosvitov, 1937
MZUSP 1236	Parque Estadual Campos do Jordão - Campo do Meio	*Guaranidrilus* sp. 2
MZUSP 1237	Parque Estadual Campos do Jordão - Celestina	*Marionina* cf. *seminuda* Xie & Rota, 2001
MZUSP 1238 a,b	Pindamonhangaba - Cachoeira Nilceia	a, b: Enchytraeidae gen. sp.
MZUSP 1239	Parque Estadual Campos do Jordão - Campo do Meio	*Achaeta singularis* Schmelz, 2008
MZUSP 1240	Parque Estadual Campos do Jordão - Camhambora	*Achaeta singularis*
MZUSP 1241	Ubatuba - Rio do Veio	*Guaranidrilus* sp. 2
MZUSP 1242	Parque Estadual Campos do Jordão - Mimosinha	*Achaeta singularis*
MZUSP 1243a,b	Parque Estadual Campos do Jordão - Serrote	*Achaeta singularis*
MZUSP 1244	Parque Estadual Campos do Jordão - Casguilho	*Achaeta singularis*
MZUSP 1245	Parque Estadual Campos do Jordão - Camhambora	*Achaeta singularis*
MZUSP 1246	Parque Estadual Campos do Jordão - Serrote	*Guaranidrilus* sp. 3
MZUSP 1247	Parque Estadual Campos do Jordão - Campo do Meio	*Achaeta singularis*
MZUSP 1248	Parque Estadual Campos do Jordão - Mimosinha	*Achaeta singularis*
MZUSP 1249	Parque Estadual Campos do Jordão - Camhambora	*Achaeta singularis*

Table 3. Comparison of specimen MZUSP #1233, identified as *Marionina argentea* s.l., with known species of the *Marionina argentea* (Michaelsen, 1889) species complex. Literature data from Rota (2013) and Martin *et al.* (2015).

	Marionina argentea (Michaelsen, 1889) s. l. MZUSP #1233	*Marionina argentea* (Michaelsen, 1889) s.s. (Rota 2013)	*Marionina mendax* Rota, 2013	*Marionina mimula* Rota, 2013	*Marionina deminuta* Rota, 2013	*Marionina sambugarae* Schmelz, 2015
Body length	1 mm	1.8–2.4 mm	1.5–2.4 mm	1.5–1.7 mm	0.9–1.4 mm	1.5 mm
Chaetae, length	22–25 µm	16–32 µm	19–42 µm	18–38 µm	16–29 µm	28–38 µm
Pharyngeal glands in VI: dorsal lobe	absent	present	absent	present	present	present
Gonadal region	X–XII	XI–XIII	XI–XIII	XI–XIII	X–XII or XI–XIII	XI–XIII
Clitellum dorsally	present	present	present	present	present	absent
Clitellum, ventral margin	hyalocytes	granulocytes	granulocytes	unknown	hyalocytes	granulocytes
Male glandular bulb length	10 µm	29–37 µm	16–27 µm	32–48 µm	17–19 µm	20–32 µm

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First records of *Parergodrilus heideri* ("Polychaeta": Parergodrilidae) and *Hrabeiella periglandulata* ("Polychaeta": Hrabeiellidae) from Slovakia and new records of both species from Czechia

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Abstract. In the course of a study on microannelids of spring fens and adjacent grasslands in the Western Carpathians (within Czechia and Slovakia), conducted in spring and autumn of 2015 and 2016, the two terrestrial, soil-dwelling polychaetes *Parergodrilus heideri* and *Hrabeiella periglandulata* were recorded for the first time in Slovakia at six sites and one site, respectively. The find of *P. heideri* in the eastern part of Slovakia represents the second eastern-most published record within its known European range (only a recent record from Estonia lies further in the east). Within Czechia, *P. heideri* (hitherto known from a single site in the north of Bohemia) was found at four sites and *H. periglandulata* at three sites, all in north-eastern Moravia close to the Polish and Slovak borders.

Keywords. Annelida, grassland, fen, wetland, Western Carpathians, soil fauna.

INTRODUCTION

Only two truly soil-dwelling non-clitellate annelids are known, *i.e. Parergodrilus heideri* Reisinger, 1925 and *Hrabeiella periglandulata* Pižl & Chalupský, 1984. Non-clitellate means that they have been traditionally placed in the taxon Polychaeta, which is, however, considered paraphyletic and therefore given in-between quotation marks elsewhere in this text. Also members of the Aeolosomatidae (formerly placed within the clitellate "Oligochaeta", now as one of the two taxa constituting Aphanoneura, also within "Polychaeta") are found in soil. However, these aeolosomatid species are apparently semiaquatic, being active in the water-filled soil pores, and the very same species are more frequently found in aquatic habitats. This is not the case with the above-mentioned species, though one of them, *P. heideri*, has also been found in water-logged soil and ground water (Martínez-Ansemil & Parapar 2009, Stoch *et al.* 2011). After some confusion about its taxonomic position in the years after its description (Reisinger 1925), *P. heideri* was placed in the small family Parergodrilidae (Reisinger 1960) together with one other species, *Stygocapitella subterranea* Knöllner, 1934 (living in sediments of the marine littoral and in coastal groundwater).

There is no close phylogenetic relationship between the Parergodrilidae and *H. periglandulata* (Jördens *et al.* 2004). *H. periglandulata* had been listed in scientific works without assignation to any family, until Christoffersen (2012) formally erected the monotypic family Hrabeiellidae. For an up-to-date view on the phylogenetic position (yet not fully resolved) of the above-mentioned taxa see Weigert and Bleidorn (2016).

Both species were originally described from Central Europe: *P. heideri* from the vicinity of Graz in Styria, Austria (Reisinger 1925), *H. periglandulata* from South Bohemia, Czechia (Pižl & Chalupský 1984). Since then our knowledge about their distribution has increased substantially. An overview of the occurrence of *P. heideri* in Europe was given by Rota *et al.* (2010). The species has been found from north-eastern Spain

in the west to northern Croatia in the east and from southern Sweden in the north to southern Italy. Outside of Europe it was recorded in Korea (Dózsa-Farkas & Hong 2010) and within North America in the states of Minnesota and Wisconsin, USA (Schlaghamerský & Frelich 2012). An overview of our current knowledge on the occurrence of *H. periglandulata* in Europe was published by Dózsa-Farkas & Schlaghamerský (2013), showing a distribution from north-western Spain in the west to north-western Romania in the east and from southern Sweden in the north to central Italy in the south. This work furthermore rejected ideas about the presence of more than one species in Europe, to be discriminated based on chaetal ultrastructure. Outside of Europe, *H. periglandulata* was also found at a site in Korea (Dózsa-Farkas & Hong 2010). The publication of further finds of both species outside of Europe by the first author of the present paper is under preparation.

In the present paper we report on the first records of both species in Slovakia. In the case of *P. heideri* this is based on finds at multiple localities. Whereas *H. periglandulata* had been reported from five sites in three areas of Czechia, four of which are located in Bohemia and one in the eastern part of the country, in Moravia (Schlaghamerský & Šídová 2009; Dózsa-Farkas & Schlaghamerský 2013), *P. heideri* was hitherto known from a single locality in Czechia, more exactly the Giant Mountains (Krkonoše) in northern Bohemia (this single published record, lacking details on the locality, was rather hidden in a paper about the fauna of Enchytraeidae and Parergodrilidae of Sweden: Chalupský 1992). In the present paper we report on three additional sites of occurrence for each of the two species within Czechia, this time in eastern Moravia, close to the Slovak and Polish border. The fact that we found both species at several sites and in considerable numbers also allows us to present some additional information and thoughts on the species' habitat preferences.

MATERIAL AND METHODS

Specimens of both species were found during a large scale sampling of annelids of non-forest micro-wetlands (spring fens of different levels of pH and mineral richness) and adjacent grasslands (meadows or pastures) in the Western Carpathians, conducted in 2015 and 2016 in the easternmost part of Czechia and a substantial part of Slovakia. This included 21 pairs of wetland and grassland in Slovakia, each sampled once in spring (April − May) and once in autumn (September − October). Microannelids were sampled by a cylinder-shaped steel corer with a circular area of 17 cm^2 to the depth of 9–15 cm (depending of the penetrability of the sediments or soil). Five cores were taken at each site-habitat combination at each sampling date. The obtained cores were subdivided into 3-cm thick layers. These were put in plastic bags and kept cool during transport and subsequent storage in the laboratory (at 4 °C). Annelids were extracted by wet funnel extraction without heating (48 h, cooling by a water bath, retrieval of a first batch of extracted annelids and water replacement after the first 24 hours), sorted out under a preparatory binocular microscope, stored in water-filled Petri dishes (at 4 °C, for a short time), and identified under a compound laboratory microscope. Due to the high number of enchytraeids in the samples, the processing of grassland soil cores from a given site was stopped when at least 140 microannelid specimens from at least five soil cores, covering both the spring and autumn seasons, were extracted and identified. Microannelid numbers in the wetland samples were substantially lower and thus all fen sediment cores were processed. The absolute numbers reported here for the two habitats, fen and grassland, are therefore not based on the same sampling effort.

RESULTS

The geographic situation of the sites where either of the two species was recorded is presented in Figure 1. For the geographic coordinates and further details about the localities and the numbers of recorded specimens see Table 1.

Parergodrilis heideri was found at six sites in Slovakia (31 specimens in total, all females) from the Kysuce Highlands in the north-west of the
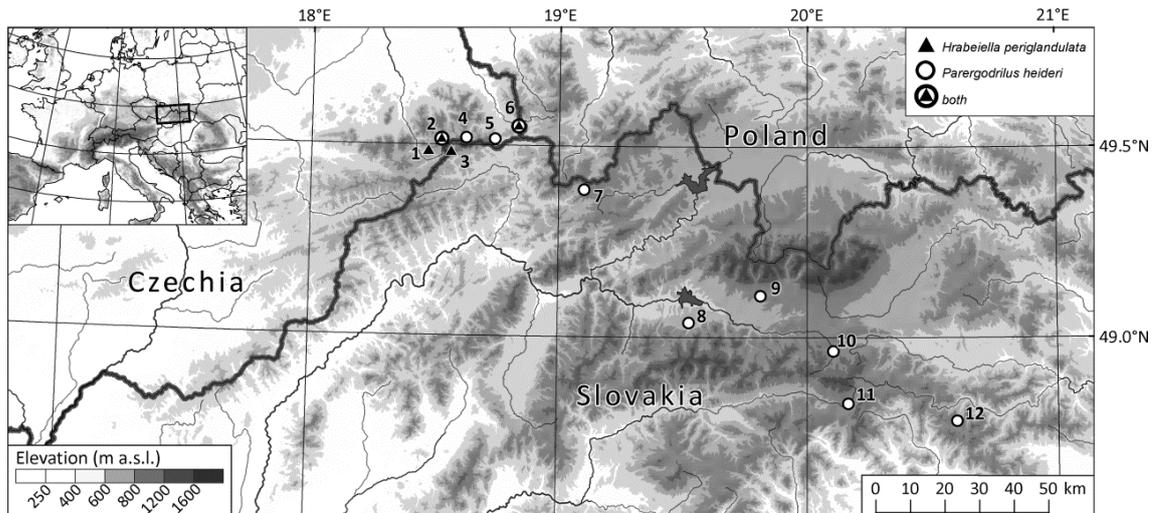
Figure 1. Map with localities of new records of *Parergodrilus heideri* (Parergodrilidae) and *Hrabeiella periglandulata* (Hrabeiellidae) in the Western Carpathians

country to the Volov Hills in the east. Furthermore we found *P. heideri* also at four sites in north-eastern Moravia, Czechia (15 specimens in total), one of which was situated in the Silesian Beskids (Slezské Beskydy) in the north-eastern corner of Czechia just at the border to Poland and the other two in the Moravian-Silesian Beskids (Moravskoslezské Beskydy), *ca* 1.6 km north and 2.5 km north-west of the border to Slovakia, respectively. Of the total number of *P. heideri* specimens, 31 were found in fen sediments (68% in the 0–3 cm layer, 26% in the 3–6 cm layer) and 15 in grassland soil (87% in the 0–3 cm layer, 13% in the 3–6 cm layer).

Hrabeiella periglandulata was found at three sites in Czechia (16 specimens, some with yolky eggs, several dead upon examination), situated in the Silesian and Moravian Beskids and at a single site within Slovakia (4 specimens), the latter situated in the Turzovka Highlands (Turzovská vrchovina), part of the western Beskids within the Outer Western Carpathians, *ca* 1 km east of the border between Czechia and Slovakia. Specimens were exclusively obtained from soil samples, *i.e.* none from fen sediments. Vertical distribution in the upper 15 cm was rather even, with a moderate drop with depth (25% both in the 0–3 cm and 3–6 cm layers, 20% in the 6–9 cm layer, 15% both in

the 9–12 cm and 12–15 cm layers). The site in Slovakia differed from those in Czechia in that it was not a true grassland but a logged area of spruce forest, with some standing dead spruce trees and logging stumps, and rather sparsely afforested with young saplings of silver fir (*Abies alba*) and beech (*Fagus sylvatica*). The ground floor was overgrown with grass (predominantly *Avenella flexuosa*) and common bilberry (*Vaccinium myrtillus*), partially also mosses, with visible traces of red deer grazing. In general, the upper 3–6 cm of the soil profile were made up of an upper layer of decomposing grass and a lower layer of spruce needles in a more advanced stage of decomposition (F- and H-layers), followed by mineral soil, sometimes including rotten pieces of wood. The depth of the organic layer derived from forest litter varied, in some samples reaching down to ca 10 cm depth.

DISCUSSION

Neither of the two species has been reported from Slovakia before. In particular in the case of *H. periglandulata*, this might seem surprising, as there are records from all neighbouring countries, except the Ukraine, and also from north-eastern Romania (Dózsa-Farkas & Schlaghamerský 2013). The closest previously published site of

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occurrence is situated in southern Poland (Dumnicka & Rożen 2002), *i.e.* the western-most part of Niepołomice forest east of Cracow (Kraków), *ca* 135 km to the north-east from the present site. The closest locality in Czechia known prior to the present article was from a municipal forest of the city of Brno, 150 km to the west-southwest (Schlaghamerský & Šídová 2009).

To our knowledge, *P. heideri* has neither been reported from Poland, nor from Lithuania, Latvia, Belarus, Ukraine or Russia. However, it was recently found in Estonia (Kõlli *et al.* 2015), which has shifted the eastern border of its known distribution area within Europe substantially to the east. From Czechia it had been reported only from the north of Bohemia (Chalupský 1992), close to the Polish border, whereas there were so far no records from Moravia. There is also no published record from Hungary, despite intensive research on soil-dwelling microannelids conducted there. However, in 2012 it was found in the Kőszeg Mountains close to the Austrian border (unpublished record, Dózsa-Farkas, pers. com.). There are no records from the Balkans. Our find of *P. heideri* at Teplá Voda in the Volov Hills (eastern Slovakia) thus represents the second eastern-most published record within its known European range. Taking into account the species' confirmed occurrence in other regions of the Holarctic, *i.e.* eastern Asia and North America, the lacking records from many regions within Europe are most probably an effect of the lack of studies on soil-living microannelids (or, more generally, semiaquatic soil fauna).

Both *H. periglandulata* and *P. heideri* have been mostly found by enchytraeid researchers using some type of wet extraction from soil. Dry extraction methods, such as those used for microarthropods, do not allow to obtain intact specimens. Furthermore, tiny specimens belonging to taxa that are not the focus of the given researcher usually go unnoticed. Even in the case of studies on enchytraeids, the approach might differ among researchers. Whereas some have been paying attention to the "odd worm" in their samples, others might not have bothered spending time on spe-

cimens not belonging to their group of interest. A fair number of studies on enchytraeids have been conducted in Poland, but few in the countries east of it. Before the present study Slovakia also remained almost unexplored in terms of its enchytraeid fauna, the only more comprehensive inventory being published by the Polish researcher Kasprzak (1986) on enchytraeid assemblages of forest sites in the Little Carpathians (Malé Karpaty), a small mountain range in the south-west of the country (see also Chalupský 1988).

Originally, *P. heideri* had been found in organic soil layers of montane beech forests and it was reported to avoid waterlogged soils (Reisinger 1925). In contrast, Rota *et al.* (2010) suggested that it might spread through waterlogged soils along hydrographic systems. Enchytraeids had been studied at four montane beech-fir forests in the Moravian-Silesian Beskids, rather close to the new localities of *P. heideri* presented above, but *P. heideri* was not found there (Schlaghamerský 2012). It has to be said that the scope of that study was rather limited in terms of the numbers of soil cores taken and the numbers of microannelids obtained. However, *P. heideri* was also not found during studies of enchytraeids and other microannelids in 10 grasslands and three broadleaved forests in the White Carpathians, including two montane beech forests, both close to the border to Slovakia (Schlaghamerský & Kobetičová 2006, Schlaghamerský *et al.* 2007, Tajovský *et al.* 2008, Schlaghamerský 2010). *Hrabeiella periglandulata* was also not encountered during these studies.

This shows that albeit both polychaetes are surely more common than shown by the limited number of records, they are by no means omnipresent. Also in the present study both species were only found in a limited subset of the sampled sites. Records of *P. heideri* obtained during the present study confirm that it occurs indeed in terrestrial as well as semiaquatic habitats. It is worth mentioning that many enchytraeid individuals belonging to species that are generally considered terrestrial were also extract-

ed from the studied fen sediments. The presence of *P. heideri* in substrates of pH values around 7 or even above is in line with the characterization given by Graefe & Schmelz (1999), but we found the species also in soils with a pH as low as 4.6. As the numbers of evaluated soil or sediment cores from fens and grasslands were not identical, a direct evaluation of its habitat preferences (fen vs. grassland) is not possible. In the case of *H. periglandulata*, however, its complete lack from the wetland portions of the investigated sites indicates that this species avoids waterlogged substrate, though another reason might be the avoidance of higher pH values. Our fens were generally rich in minerals and of higher pH than the soils of the adjacent terrestrial habitats. This correlation of pH and moisture levels in our set of sites hampers a comparison of pH preferences between the two species.

Vertical distribution of both species in the soil or sediment profile probably matches the distribution of organic matter and, in particular in the case of the fen sediments, the availability of oxygen, but data on these variables are not available for the individual layers.

Table 1. Overview of localities, given from west to east, where *Parergodrilus heideri* (Parergodrilidae) and *Hrabeiella periglandulata* (Hrabeiellidae) were recorded in 2015 or 2016, with information on each site (habitat type and pH – two values correspond to records from two habitat types) and the numbers of specimens obtained (N *P. h.* and N *H. p.*, respectively, two values correspond to records from the two habitat types); numbering of localities corresponds to site numbers in Figure 1, CZ and SK shows if the site is located in Czechia or Slovakia.

Locality	Latitude (N)	Longitude (E)	Geomorphological Mesoregion	Habitat	pH (H$_2$O)	N *P.h*	N *H.p*
1) Podgruň (CZ)	49°29'10"	18°28'23"	Moravian-Silesian Beskids (Moravskoslezské Beskydy)	Pasture	4.5	-	5
2) Obidová (CZ)	49°31'04"	18°31'26"	Moravian-Silesian Beskids (Moravskoslezské Beskydy)	Mineral-poor fen with calcitolerant *Sphagnum* spp./meadow	7.7/4.3	2/0	0/10
3) Jančíkovci (SK)	49°29'29"	18°33'00"	Turzov Highland (Turzovská vrchovina)	Forest clearcut with grass, moss and bilberry	3.8	-	4
4) Kyčmol (CZ)	49°30'47"	18°37'27"	Moravian-Silesian Beskids (Moravskoslezské Beskydy)	Pasture	4.6	1	-
5) Vřesová stráň (CZ)	49°30'16"	18°44'44"	Moravian-Silesian Beskids (Moravskoslezské Beskydy)	Pasture	5.31	1	-
6) Bukovec (CZ)	49°32'57"	18°51'30"	Moravian-Silesian Beskids (Moravskoslezské Beskydy)	Mineral-rich fen/ wet pasture	8.5/5.5	9/2	0/1
7) Vychylovka (SK)	49°23'03"	19°06'05"	Kysuce Highland (Kysucká vrchovina)	Tall herb meadow	6.3	1	-
8) Chrastě at Dúbrava (SK)	49°02'14"	19°31'31"	Tatras: Liptov Basin (Liptovská kotlina)	Mineral-rich fen	7.4	11	-
9) Potôčky (SK)	49°06'29"	19°48'44"	Tatras: Tatra Foothills (Tatranské podhorie)	Dry pasture	4.9	1	-
10) Liptovská Teplička (SK)	48°57'47"	20°06'16"	Low Tatras (Nízke Tatry)	Mineral-rich fen	7.5	4	-
11) Meandre Hrona (SK)	48°49'34	20°09'51"	Low Tatras (Nízke Tatry)	Drier margin of alluvial meadow	6.6	7	-
12) Tichá Voda (SK)	48°46'50"	20°35'50"	Volov Hills (Volovské vrchy)	Mineral-poor fen with calcitolerant *Sphagnum* spp./ adjacent dry grassland	6.7/4.9	5/2	-

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How to deal with cryptic species in Enchytraeidae, with recommendations on taxonomical descriptions

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Abstract. During the 12th International Symposium on Enchytraeidae, held in Tihany, Hungary (27–29 June 2016), the participants discussed cryptic species, *i.e.,* species that are morphologically so similar that they are classified as the same species (Bickford *et al.* 2007), and how to deal with them taxonomically. Here we summarise the discussion together with a few additional comments, and we give recommendations for species descriptions in Enchytraeidae.

Keywords. Annelids, morphospecies, sequencing.

INTRODUCTION

Species are basic biological units, and the first step in the exploration of biodiversity. Species are also entities of generalisation: information from different studies of individuals of the same species can be generalised to that species, but not necessarily for a more inclusive taxon, *e.g.*, a genus or a family. However, for this generalisation it is important that the specimens are correctly identified to species, and species are correctly delimited, to avoid, for example, that several species differing in various properties, *e.g.* ecological or physiological, are included under the same name. Both correct identification of previously named species, and the naming and description of new taxa are crucial steps for describing the biota of the world, and also to ensure that scientists mean the same thing when using a species name. Taxonomic names are also needed to link species to data, produced in different studies, so that they can be related in various analyses. If data (*e.g.* ecological, morphological, and molecular) cannot be linked to formal species

and well-referenced names, these data will lose much of their value. The proper naming and description of species is therefore essential.

Enchytraeids have traditionally been studied alive using light microscopy, and the morphological investigation of whole worms, either alive or fixed, is still the basis for the identification of specimens and descriptions of new species. However, with the introduction of widely accessible molecular methods, notably the sequencing of DNA 'barcode' fragments and refined analytical tools, a new standard set of data has become available to recognize and to delimit species. DNA sequences often confirmed the distinctions drawn between morphologically defined species (Klinth *et al*. 2017, De Wit & Erséus 2010), but in some cases they showed that species described on the basis of morphological differences are in fact synonyms (Dózsa-Farkas *et al.* 2012). More importantly, they also revealed the existence of cryptic species, *i.e*. species that, so far, cannot be differentiated with morphology-based methods (Martinsson & Erséus 2014, Matamoros *et al.* 2012).

It is important to note that cryptic species have been known in enchytraeids for more than half a century, based on karyology (Christensen 1961), protein patterns (Brockmeyer 1991, Christensen *et al.* 1992, Schmelz 2003, Westheide & Graefe 1992) or other techniques, but they were never formally recognized and described, with the notable exception of *Enchytraeus crypticus* Westheide & Graefe, 1992. A list in Collado *et al.* (2012) contains 40 enchytraeid species as candidates for species complexes; they include almost all commonly cited species. Formal recognition of cryptic species has increased with the establishment of DNA sequencing as standard taxonomic method. There are currently four described truly cryptic species-pairs in Enchytraeidae (Table 1) and we expect many more to come. Cryptic species cannot be distinguished using the traditional and widely-used method of studying the morphology using light microscopy. Therefore, a discussion on how cryptic species should be treated was held during the symposium.

During the discussion, the participants agreed that cryptic species are distinct evolutionary lineages, which deserve recognition in a classificatory system. There is a growing body of evidence that cryptic species may differ in ecological and physiological properties, and therefore the separation of cryptic lineages within morphospecies can be important when such species are used as models in ecology, ecotoxicology and physiology (see Feckler *et al.* 2013, Römbke *et al.* 2016). ('Morphospecies' is used here to denote mainly *named* species, described and identified in the traditional way, using morphological characters. Our use differs from the one in ecology, where 'morphospecies' often means morphologically distinguishable but *unnamed* species of unknown identity.) It was also agreed that a morphospecies that comprises an assemblage of cryptic species still deserves recognition even though it cannot be considered, due to reproductive barriers within, as one biological species any more. The reasons are not only practical but also biological: The assemblage of cryptic species (*i.e.* the morphospecies) may form a monophyletic group and may have common ecological properties that are different from the rest of the species in the genus. Morphospecies that turn out to be polyphyletic assemblages, however, should be abandoned. It should be noted that this consensual opinion differs from previous practice, where either the cryptic species or the morphospecies was discarded (Christensen 1961, Sturmbauer *et al.* 1999, Gustafsson *et al.* 2009, James *et al.* 2010).

However, opinions differed as to how cryptic lineages should be recognised. Two options included the use of informal categories:

− Maintain the species name of the morphospecies and denote the cryptic species appending a series of alphanumerical codes to the name of the morphospecies;
− Give full species rank to the cryptic species and denote the morphospecies with the old name plus an epitheton like "*sensu lato*", or "species group" or "species complex".

Table 1. Cryptic species pairs in Enchytraeidae. Included are also species pairs with morphological differences inconclusive or difficult to access.

	Habitat	Type of difference	Morphological differences
Enchytraeus variatus Bouguenec & Giani, 1987 *Enchytraeus crypticus* Westheide & Graefe, 1992	compost, soil	Isozyme patterns, total protein patterns (Brockmeyer 1991) DNA-RFLPs (Schlegel et al. 2009) CIE, crossed immuno-electrophoresis (Gabrich et al. 1991) RAPD-PCR (Schirmacher et al. 1998)	Ultrastructure of spermatozoa (Westheide et al. 1991)
Grania bekkouchei Prantoni, De Wit & Erséus, 2016 *Grania cryptica* Prantoni, De Wit & Erséus, 2016	marine sediment	DNA sequences (Prantoni et al. 2016)	none
Chamaedrilus/Cognettia* sphagnetorum (Vejdovský, 1878) *Chamaedrilus pseudosphagnetorum* Martinsson, Rota & Erséus, 2015a	soil	DNA sequences (Martinsson and Erséus 2014, Martinsson et al. 2015b)	none
Grania ovitheca Erséus, 1977 *Grania occulta* De Wit & Erséus, 2010	marine sediment	DNA sequences (De Wit and Erséus 2010)	none
Enchytraeus bigeminus Nielsen & Christensen, 1963 *Enchytraeus japonensis* Nakamura, 1993	compost, soil	Isozyme patterns, Total protein patterns (Schmelz et al. 2000)	Male sexual glands (species with predominantly asexual repoduction) (Schmelz et al. 2000)
Chamaedrilus/Cognettia glandulosus (Michaelsen, 1888) *Chamaedrilus varisetosus* Martinsson & Erséus, 2015b	soil, aquatic sediments	DNA sequences (Martinsson and Erséus 2014, Martinsson et al. 2015a)	Body size, chaetal numbers (Martinsson et al. 2015a)

* Priority of *Cognettia* or *Chamaedrilus* awaits ruling by the International Commission on Zoological Nomenclature, see http://www.iczn.org, Case 3689.

Two further options excluded informal categories and promoted the integration of the taxic diversity into the Linnaean system:

– Maintain the species rank for the morphospecies and use the subspecies rank for the cryptic species.
– Give full species rank for the cryptic species and a supraspecific rank (*e.g.*, subgenus), for the morphospecies.

All of these options have their pros and cons. Using the morphospecies with an alphanumerical code to represent the different cryptic lineages will let us continue using the morphospecies as taxonomical units in inventories, species lists etc., but there is the risk that the knowledge about the cryptic lineages is ignored, as they are not formally recognised and described taxa. Whereas if cryptic lineages were formally described as species, identification of specimens to species based on morphology would become impossible, and specimens could only be identified to species groups or species "*sensu lato*". However, the cryptic lineages would at least be recognised as species, and could thereby be included in counts of biodiversity and be seen as different units. A drawback of this option is the possible confusion caused by the same name used with two different meanings, either *sensu lato* (morphospecies) and *sensu stricto* (cryptic species).

Informal ranks and categories have the advantage of being flexible but the disadvantage of not being regulated, which may promote confusion in the meaning of names; they should therefore be considered only as an interim solution. The preferable full integration of the diversity into the

Linnaean system of names, however, faces other problems: The use of the subspecies category for cryptic species would contradict the traditional concept of subspecies as morphologically distinguishable populations of a species that replace each other geographically (Mallet 2007). In fact, cryptic species fulfil all criteria of being 'species', regardless which species concept is applied here (Bickford *et al.* 2007). On the other hand, a rank elevation of morphospecies to subgenus level would create considerable classificatory and nomenclatural instability: First, it would necessitate a complete reorganization of the classificatory architecture of a genus, because the subgeneric category cannot be applied selectively and hence affects all species of a genus. Second, each morphospecies elevated to subgeneric rank (the type species of the genus excepted) would need a new subgeneric name. The same problems would arise, *mutatis mutandis*, with the elevation of morphospecies to genus rank. To conclude, solutions to this classificatory problem are not straightforward and may differ from case to case.

Another important question is what evidence at the level of genetic markers is necessary to decide whether specimens belong to the same species or to different species, and whether a species is undescribed or not. Traditionally the characters used are morphological, both external and internal, but molecular data are becoming more and more common as the base for taxonomical decisions. Also ecological and physiological data, if available, can aid in the species delimitation process. The most commonly available genetic marker is the mitochondrial gene cytochrome C subunit I (COI) that is used as the barcode for animals (Hebert *et al.* 2003). However, if used alone, COI will often overestimate the number of species, and it should be used with caution and in combination with other data (Dasmahapatra *et al.* 2010). As a broad rule of thumb, in clitellates, if two cluster differ with more than 10% uncorrected genetic distance, *i.e.,* if more than 10% of the nucleotides differ between the two lineages, they are likely to belong to different species, and if they differ with less than 5% they are likely to belong to one species (Rougerie *et al.* 2009, Römbke *et al.* 2016, but see Giska *et al.* 2015,

Martinsson & Erséus 2017 for exceptions). More support is, however, needed in order to make a robust delimitation. Other commonly used markers are the nuclear Histone H3 (H3) and the ribosomal internally transcribed spacer region (ITS) consisting of ITS1, 5.8S and ITS2. H3 has been recommended as a secondary barcode for Enchytraeidae (Schmelz *et al.* 2014), and both H3 and ITS have good discriminatory power and will in most cases separate closely related species.

A third problem with cryptic species was discussed at the symposium: When cryptic species are detected within a morphospecies and are described formally according to nomenclatural rules (ICZN 1999), one lineage should bear the name of the morphospecies, notably the one to which the name-bearing type of the morphospecies belongs. However, finding the correct lineage may be difficult because type material is lost or in a state of preservation that does not allow extraction of DNA; both cases are very common in enchytraeids. A possible solution is to get fresh material from the type locality for sequencing, and in that way tie the name to a genetic lineage. However, in many cases the type localities are vague or missing; in these cases it should be sufficient to use material from the wider area where it can be supposed that the original material was collected. As a further complication, however, more than one cryptic lineage may be present at the type locality or in the wider area. To conclude, the choice of the name-bearing lineage is often a decision based on probabilities, and the task is to raise the probability-level as much as possible. For example, in case that small morphological differences exist between the candidate lineages, the one that fits the original description best should be chosen to bear the name of the old morphospecies. If uncertainty is too high, there remains the radical solution of dismissing the old name as "*nomen dubium*".

RECOMMENDATIONS

Based on the consensus that both morphological characters and molecular markers are important for species-level taxonomy in enchytraeids, we recommend:

1. The erection of new species should include a good morphological description with illustrations of the important taxonomic characters and also a reference to molecular markers that are informative at the species level: at least one, but preferably two markers, one being mitochondrial (*e.g.,* COI), one nuclear (*e.g.,* ITS, H3).

2. DNA sequences should be generated from at least one of the type specimens, preferably the holotype or a syntype specimen, to link the sequence permanently with the name. However, some of the paratypes and other reference specimens should also be sequenced to avoid errors and to allow estimates of variability.

3. When species are erected based on only one set of data (*i.e.,* either morphological characters alone or DNA sequences alone), the retrieval of missing or additional data should be made possible by appropriate fixation and preservation of at least some specimens of the type series. This means, for DNA, at the present state of knowledge, the use of ethanol as preservation liquid at concentrations higher than 70%.

4. Sequencing is also recommended in specimens that form the basis of redescriptions and in those that are elected as neotypes in taxonomic revisions.

5. Of each sequenced specimen, the anterior part of the animal should be retained as a voucher and deposited in a public collection. (In enchytraeids, most of the taxonomically informative structures are located in the anterior body part.)

We understand that it will not always be possible to extract and sequence DNA from the specimens used as the basis for a new species description, *e.g.,* due to fixation methods, old age and bad storage or due to other factors, but whenever possible we recommend that DNA-sequence-data should be included in future descriptions of enchytraeids.

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First record of terrestrial Enchytraeidae (Annelida: Clitellata) in Versailles palace's park, France

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Abstract. France can be qualified as *terra incognita* regarding terrestrial enchytraeids because very little data has been recorded so far in this country. In spring and autumn 2016, enchytraeid communities were investigated in a loamy soil in a meadow located in the park of Versailles palace, France. In total, twenty four enchytraeid species were identified, belonging to six different genera i.e. eleven *Fridericia* species, four *Enchytraeus* species, four *Achaeta* species, two *Buchholzia* species, two *Marionina* species and one *Enchytronia* species. According to the published data, this was one of the highest diversity found in a meadow in Europe.

Keywords. Enchytraeids, Potworms, Soil fauna, Annelids, Oligochaeta, Meadow.

INTRODUCTION

Despite their key role in soils (Didden 1993), enchytraeids (Annelida: Clitellata) are so far poorly studied in many countries worldwide. To our knowledge, and except a few species recorded in Schmelz & Collado (2010), no data have been published on enchytraeid communities in France, *i.e.*, based on a literature search in the ISI Web of Knowledge, using the "All Databases" option, with the formula: '(enchytr* or potworm*) and (France or French) in Topics'.

Although some studies assessed enchytraeid community structures in grasslands, meadows and pastures, studying the influence of grazing (Schlaghamerský *et al*. 2007), liming (Davidson *et al*. 2004), fertilization (Sokołowska & Seniczak 2005) or pesticides (Clements *et al*. 1987, Forsters *et al*. 1992, Martin 1975), most of them only reported a total abundance of enchytraeids (*e.g.* Clements *et al*. 1987, Francini *et al*. 2014, Van Vliet *et al*. 2006), without any details on species diversity. Yet, several authors recommended identifying the individuals at least at the genus level and if possible at the species level (Pelosi & Römbke 2016). Indeed, indicators at the genus or species level appeared to be more sensitive to land use than total abundance. Moreover, taxonomic diversity provides key information on the requirements and sensitivity of the different species to environmental conditions, land use and management.

The objective of this study was to record the diversity of enchytraeids in a meadow in France and to compare it with other data in Europe.

MATERIAL AND METHODS
Study site

Samples were collected in a meadow (about 1 ha, Figure 1.) located in the park of Versailles palace, France (48°48'31''N, 2°05'26''E), which has not been cultivated since 1994. Before that time, it was conventionally cultivated with *e.g.*

Figure 1. Meadow located in the park of Versailles palace.

barley, oats, beans, corn and wheat. The climate is oceanic temperate, with a mean annual temperature of 11 °C and a mean annual rainfall of 660 mm. The soil is a deep Luvisol (FAO soil classification) developed on loess. The homogeneity of the physicochemical characteristics in the experimental site was verified by random sampling a soil core (0–20 cm depth) at seven different locations in the site (Table 1.).

Table 1. Physicochemical characteristics of the soil at the experimental site (n = 7, ± Standard Deviation)

Parameters	Meadow soil
Texture (USDA, 1975)	Loamy soil
Clay (%)	22.9 ± 0.7
Silt (%)	48.0 ± 1.3
Sand (%)	29.1 ± 1.2
pH_{H2O}	7.5 ± 0.2
C_{org} (g kg^{-1})	18.9 ± 1.0
N_{tot} (g kg^{-1})	1.5 ± 0.1
C_{org}/N_{tot}	12.7 ± 0.3
P_2O_5 (g kg^{-1})	0.08 ± 0.01
$CaCO_3$ (g kg^{-1})	23.3 ± 8.1
Cu_{tot} (mg.kg^{-1})	25.2 ± 1.6

Sampling and identification

Ninety-six soil cores were sampled both in April and November 2016, following a regular grid of 60×40 m and using a split soil corer (diameter of 5 cm) at 0–5 cm and 5–10 cm depths. Each sample was transferred separately into a plastic bag and stored at 4°C. Enchytraeids were extracted using wet funnel extractors under a light from incandescent light bulbs. Soil samples were heated up for 3 hours (O'Connor 1959, Kobetičová & Schlaghamerský 2003). All the individuals were kept in Petri dishes in tap water and counted. Adult and sub-adult individuals were identified at the species level under a light microscope (Olympus BX53) up to 400× magnification according to the key of Schmelz and Collado (2010).

RESULTS

In total, twenty four species of enchytraeids were identified, belonging to 6 genera. Species are listed below, with available information on their presence in Europe, habitat and life strategy (based on Graefe & Schmelz 1999 and Schmelz & Collado 2010):

Achaeta bohemica (Vejdovský, 1879): widespread, fresh soils, absent in wet habitats, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.

Achaeta iberica Graefe, 1989: found in Spain, Italy (Schmelz and Collado 2000), and Switzerland (Amossé *et al*. 2016), only known from the original description.

Achaeta pannonica Graefe, 1989: widespread, fresh soils, absent in wet habitats, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.

Achaeta unibulba Graefe, Christensen & Dózsa-Farkas, 2005: widespread.

Buchholzia appendiculata (Buchholz, 1862): common and widespread in neutral to slightly acidic soils, *r/F* (fragmenting)-strategist, litter dweller.

Buchholzia fallax Michaelsen, 1887: widespread.

Enchytraeus buchholzi Vejdovský, 1879: widespread, very common, species complex, not in strongly acidic soils, *r*-strategist, OF (F-horizon dweller) and soil dweller.

Enchytraeus bulbosus Nielsen & Christensen, 1963: widespread, not in strongly acidic soils, *r*-strategist, OF and soil dweller.

Enchytraeus christenseni Dózsa-Farkas, 1992: widespread but not common, wet habitats, not in strongly acidic soils, *r*-strategist, OF and soil dweller.

Enchytraeus lacteus Nielsen & Christensen, 1961: widespread, not in strongly acidic soils, *r*-strategist, OF and soil dweller.

Enchytronia parva Nielsen & Christensen, 1959: common and widespread in neutral to moderately acidic soils, probably a species complex, soil dweller, stress tolerant species adapted to acidity stress.

Fridericia bulboides Nielsen & Christensen, 1959: widespread and common, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller

Fridericia christeri Rota & Healy, 1999: Northern half of Europe, very common in Germany.

Fridericia galba (Hoffmeister, 1843): widespread and very common in Central Europe, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.

Fridericia isseli Rota, 1994: widespread and common.

Fridericia maculata Issel, 1905: widespread, probably a species complex, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.

Fridericia nix Rota, 1995: Italy, Germany.

Fridericia paroniana Issel, 1904: widespread and common, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.

Fridericia tuberosa Rota, 1995: moist soil, widespread.

Fridericia ulrikae Rota & Healy, 1999: Northern and Central Europe.

Fridericia viridula Issel, 1904: found in Ireland, The Netherlands, and Italy.

Fridericia glandifera Friend, 1911: few records in England, Denmark, Germany and France.

Marionina argentea (Michaelsen, 1889): very common and widespread, wet sites also aquatic, slightly salt-tolerant, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, soil dweller.

Marionina communis Nielsen & Christensen, 1959: widespread, fresh soils but not wet, slightly acid to slightly alkaline conditions, never in strongly acid soils, *K*-strategist, OF dweller.

A total of 5 633 and 3 390 individuals were identified in Spring 2016 and in Autumn 2016, respectively. The number of individuals and the percentage of enchytraeid species are presented in Table 2. In Spring, the three most abundant species were *E. buchholzi* (23,6 %), *F. galba* (14,4 %), and *F. isseli* (13,2 %). In autumn, the three most abundant species were *En. parva* (12,7%), *F. christeri* (12,2 %), and *E. buchholzi* (11,8 %).

DISCUSSION

This study, in which twenty four species were found, represents the first record of enchytraeid's community in France. Schmelz & Collado (2010) reported only four to five species identified in France: *Guaranidrilus europaeus* Healy, 1979; *Enchytraeus varithecatus* Bouguenec & Giani, 1987; *Enchytraeus doerjesi* Westheide & Graefe, 1992; *Fridericia glandifera* Friend, 1911 and *Fridericia stephensoni* Moszyński, 1933 (need to be confirmed). A bit more than 200 species are listed in the key for terrestrial enchytraeids of Europe (Schmelz & Collado 2010). Among them, 126 have been sampled in Germany (Römbke *et al.* 2013), and 50 of them can be classified as common for Central Europe (Didden *et al.* 1997).

Table 2. Enchytraeid density (ind.m^{-2}) per species and per season (Spring and Autumn 2016). Nomenclature follows Schmelz & Collado (2010).

Genus	Species	Spring 2016		Autumn 2016	
		No. of individuals	% abundance	No. of individuals	% abundance
Achaeta	*bohemica*	0	0.00	20	0.59
Achaeta	*iberica*	311	5.52	186	5.49
Achaeta	*pannonica*	12	0.21	34	1.00
Achaeta	*unibulba*	13	0.23	28	0.83
Buchholzia	*appendiculata*	238	4.23	120	3.54
Buchholzia	*fallax*	27	0.48	9	0.27
Enchytraeus	***buchholzi***	**1330**	**23.61**	**399**	**11.77**
Enchytraeus	*bulbosus*	97	1.72	29	0.86
Enchytraeus	*christenseni*	32	0.57	0	0.00
Enchytraeus	*lacteus*	2	0.04	0	0.00
Enchytronia	***parva***	441	7.83	**432**	**12.74**
Fridericia	*bulboides*	0	0.00	31	0.91
Fridericia	***christeri***	426	7.56	**412**	**12.15**
Fridericia	***galba***	**813**	**14.43**	397	11.71
Fridericia	*glandifera*	0	0.00	2	0.06
Fridericia	***isseli***	**745**	**13.23**	386	11.39
Fridericia	*maculata*	10	0.18	55	1.62
Fridericia	*nix*	17	0.30	30	0.88
Fridericia	*paroniana*	319	5.66	320	9.44
Fridericia	*tuberosa*	254	4.51	270	7.96
Fridericia	*ulrikae*	152	2.70	102	3.01
Fridericia	*viridula*	62	1.1	23	0.68
Marionina	*argentea*	244	4.33	37	1.09
Marionina	*communis*	3	0.05	4	0.12
	NI	85	1.51	64	1.89
	Total	**5633**	**100 %**	**3390**	**100 %**

When looking at the published literature on enchytraeid communities in grasslands, meadows and pastures, only forty publications could be found. Among them, some were performed under tropical climates, in Brazil (*e.g.* Schmelz *et al.* 2008). Schlaghamerský & Kobetičová (2005) studied Enchytraeidae, Tubificidae, Aeolosomatidae in a field experiment in the Czech Republic during meadow restoration on arable land and in a well-preserved meadow nearby. They found 22 species of Enchytraeidae in the meadow and considered it as a relatively high diversity. The same authors also studied two sites in the White Carpathians (Czechia) at four dates during one year (Schlaghamerský & Kobetičová 2006). Each sites consisted of one meadow and one cattle pasture. They found respectively 16 and 17 enchytraeid species in the two meadows. The high percentages of *Fridericia* spp. in all plots were considered to correspond with their rather low soil acidity. In the present study, despite the neutral pH, we also found many *Fridericia* individuals and eleven out of twenty four species belonged to this genus. To our knowledge, the highest

diversity reported in a meadow was 23 species (Schlaghamerský *et al.* 2007) in White Carpathians (Czechia), with twelve *Fridericia* species. The diversity found in our study was close to these results and thirteen species were in common. Some species such as *F. galba, E. buchholzi* or *M. argentea* appeared to be relatively well represented in the different meadows of the published studies. Contrarily, some others, such as *F. christeri* are more rarely found.

Regarding enchytraeid species distribution in European countries and soil characteristics (pH, soil water and C$_{org}$ contents), the species found in our study are in accordance with the observations of Schmelz & Collado (2010) and Graefe & Schmelz (1999). We found horizontal heterogeneity in the meadow concerning the occurrence of the different species of enchytraeids. This was probably due to the variations in soil properties in the plot (*e.g.* soil moisture, temperature, vegetation). For example, numerous *M. argentea* were found in few soil cores and absent in others. This could be mainly explained by wetter soil conditions in one part of the plot. By contrast, species such as *F. galba*, *F. isseli*, *F. christeri*, *E. buchholzi* or *En. parva* were observed in most of soil samples. Few species were mainly found in the soil layer at 5–10 cm depth such as *Achaeta spp.* or *F. nix*. Those observations confirm Graefe & Schmelz study (1999) as they indicated that *A. bohemica* and *A. pannonica* are known to be soil dwellers. However, no information was found about *F. nix* habitat requirements.

We found a total of five *r*-strategists (*Enchytraeus spp.* and *B. appendiculata*) including one fragmenting species (*B. appendiculata*) among the sampled species. We also observed numerous specimens of *Buchholzia sp.* with two chaetae, which is unusual except for very young specimens just hatched from cocoons and not "born" by fragmentation (Schmelz pers. observ.). DNA test will confirm if a new species of *Buchholzia* has been found.

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Annelids of beech forests on basaltic bedrock: findings from two forest reserves in Germany

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Abstract. The annelid communities (microannelids and earthworms) of woodruff beech forest in two Strict Forest Reserves on moderately base-rich soil (basaltic bedrock) were studied. For the Weiherskopf (Hesse) Strict Forest Reserve, a reference site that was under forest management was included in the investigation. While the investigations at Weiherskopf took place in 2014, the Nonnenstromberg (North Rhine-Westphalia) Strict Forest Reserve was studied already 1991/1993. Site conditions at Weiherskopf and Nonnenstromberg were similar in many respects, apart from the fact that at both Weiherskopf sites several heavy storms had caused extensive windthrow in 1990, resulting in different amounts of deadwood at the sites. Despite this, the species composition was similar at the studied sites (Sørensen Similarity Index between 0.73 and 0.83). At all three sites a species-rich annelid community was present, the majority of species indicating either slightly or moderately acid soil conditions. Among the microannelid species with the highest frequency were *Hrabeiella periglandulata, Enchytronia parva* and *Buchholzia appendiculata*. At all sites, the same humus form (F-mull) was developed, being closely linked to the presence of endogeic and anecic earthworms. Possible distinguishing features between annelid communities on basalt and calcareous bedrock respectively are discussed.

Keywords. Enchytraeidae; earthworms; deciduous forest; humus form; *Dendrobaena pygmaea; Hrabeiella periglandulata*.

INTRODUCTION

Beech forests are an important component of the natural vegetation of Central Europe and currently constitute approximately 15 % of the forested area in Germany (BMEL 2016). According to trophic soil conditions two main groups of beech forests can be distinguished: *1.* forests on oligo- to mesotrophic soils, being poor in bases, with moder humus forms, and *2.* forests on base-rich meso- to eutrophic soils with mull humus forms (Bohn & Gollub 2007). Since the 1980s, investigations on earthworms and microannelids in beech forest soils have focused on oligotrophic, acid forest soils, triggered by concerns about increasing anthropogenic acidification (Beck 1987, Römbke 1989). Some studies include base-rich sites for comparison (Graefe 1990, Schaefer & Schauermann 1990, Schoch-Bösken & Greven 1987), but investigations dealing chiefly with microannelid communities in beech forests on base-rich soils are rather limited (Mellin 1988, Schlaghamerský 2010). Among base-rich soils, those on basaltic bedrock (siliceous) often differ in soil properties like pH from those on limestone and other calcareous parent materials due to the different mineral composition of the parent material. Thus, we distinguish moderately base-rich soils (on basalt) from base-rich soils (on calcareous bedrock) in this study.

Old growth beech forests can display high biodiversity (Assmann *et al.* 2007). Strict forest reserves are, besides core zones of national parks and biosphere reserves, the only areas in Germany, where natural forest development can proceed undisturbed by management and resource use (Wolf & Striepen 2007). More than 700 strict forest reserves have been established in Germany since 1970 to study forest development at minimized human impact.

We investigated microannelids and earthworms of woodruff beech forests in two strict forest reserves with moderately base-rich soils on basaltic bedrock. The Nonnenstromberg (North Rhine-Westphalia) Strict Forest Reserve was investigated already in 1991/1993 with a focus on the impact of soil acidification. At the Weiherskopf (Hesse) Forest Reserve the occurrence of several heavy storms in 1990 caused extensive windthrow. In 2014 the investigation of micro-annelids and earthworms was undertaken as part of a study to assess the possible impact of dead-wood management on the ecosystem. However, this aspect is not within the main focus of the present publication.

The present contribution aims at characterizing the annelid community of beech forests on basaltic parent material concerning *a*) species diversity and typical species composition, and *b*) indicators of soil acidity, life-form types and strategy-types of species. The results enable a comparison with communities of other types of beech forests, *e.g.* on calcareous bedrock.

MATERIAL AND METHODS

Study sites

The Weiherskopf Strict Forest Reserve is situated in the Vogelsberg region in the Federal State of Hesse in Germany. In this area, two sites were investigated that had been affected by severe windthrow 24 years before the investigation. In the strict forest reserve, the deadwood had not been removed (site WK-DW), while deadwood had partly been taken out at the reference site (WK-RF), which is situated about 600 m south of WK-DW outside the strict forest reserve. The forest community is woodruff beech forest (*Galio odorati-Fagenion*); often a variant with wood-barley is dominating (NW-FVA 2015). At both sites, the forest at present forms an irregular pattern of patches with the original beech population (age ≥ 100 years) and patches with young-growth forest, that developed since the windthrow (< 25 years) and consisting apart from beech mainly of Norway maple and ash.

The Nonnenstromberg (NB) Strict Forest Reserve belongs to the Siebengebirge region in the Federal State of North Rhine-Westphalia in Germany. The trees in the old growth beech forest had an age of about 110 years in the year of investigation. The forest community is woodruff beech forest (*Galio odorati-Fagenion*), with woodruff and wood melick dominating.

Information on principal site conditions and soil properties is given in Table 1. The humus form was specified according to the German soil classification (Ad-hoc-AG Boden 2005). An OH horizon, which would be an identifier for moder and mor humus forms, was lacking at most sample points, while an OF horizon was mostly well developed. The humus form was thus classified as F-mull, being an intermediate humus form between L-Mull (OL-layer only) and Moder (OL-, OF- and OH-layers). Information on the soil type was transferred from the German classification to categories of the world reference base for soil resources (IUSS Working Group WRB 2015). As both systems show fundamental differences, translation of soil types is not always consistent. Thus, in Table 1 the soil type is given according to both classification systems.

Sampling

At the WK Strict Forest Reserve 10 sample points for each of the two sites, WK-DW and WK-RF, were selected on the nodes of a regular grid of 100 × 100 m. At the NB Strict Forest Reserve, 8 sample points were selected along a transect of about 200 m at the north-facing slope and 6 sample points each along two transects of about 150 m at the south-facing slope of the central hill (total of 20 sample points). All three transects were running roughly in north-south direction.

The soil faunistic analyses included earthworms and microannelids. Soil samples for microannelids were taken with a split soil corer (diameter 5 cm) to a total sampling depth of 10 cm. The core samples were divided into 4 sub-samples of 2.5 cm thickness to assess the vertical

Table 1. Characterization of the study sites Weiherskopf (WK) and Nonnenstromberg (NB) (Data sources: Bundesanstalt für Landwirtschaft und Ernährung 2007, Landesbetrieb Wald und Holz Nordrhein-Westfalen 2018). Soil types of the German classification transferred according to IUSS Working Group WRB (2015).

	WK	NB
Coordinates	N 50°22'16'' E 9°26'34'' WK-DW N 50°21'34'' E 9°27'05'' WK-RF	N 50°41'8'' E 7°13'12''
Parent material	basalt with loess cover of variable thickness	basalt with loess cover of variable thickness
Height [m.a.s.l.]	310–410 m	240–335 m
Annual precipitation [mm]	969 mm	844 mm
Mean annual temperature [°C]	7.7°C	9.4°C
Vegetation	old-growth forest: *Fagus sylvatica* with few *Quercus petraea;* young-growth forest: mainly *Acer platanoides, Fraxinus excelsior*	old-growth forest: mainly *Fagus sylvatica* with few *Quercus petraea; Tilia cordata* and *Fraxinus excelsior* at steep slopes
Forest community	Woodruff-Beechwood	Woodruff-Beechwood
Strict forest reserve since	1989	1989 (nature protection area since 1965)
pH (KCl)	5.5–5.6 (humus layer) 4.2–4.4 (mineral topsoil)	4.1–4.7 (mineral soil)
pH (H₂O)	6.0–6.1 (humus layer) 5.3–5.6 (mineral topsoil)	5.3–6.3 (mineral soil)
Texture	silt loam	silty clay loam
Soil type (WRB)	Luvisol, Stagnic Luvisol	Cambisol
Soil type (German classification)	Pseudogley-Parabraunerde	Braunerde, partly Pseudogley-Braunerde
Humus form	mainly F-Mull, partly transition to moder	mainly F-Mull, partly transition to moder

distribution of microannelids. The sub-samples were extracted over 48 h by a wet-funnel technique without heating, changing the water once after 12−24 h. The extracted animals were counted and identified in vivo, following the keys of Nielsen & Christensen (1959), Schmelz (2003) and Schmelz & Collado (2010). Microannelid samples were vertically divided at fixed depth levels and not at humus horizon boundaries, as this enables the calculation and comparison of mean abundance per m^2 for these depth levels at all three sites independently from the varying thickness of humus horizons. To link the occurrence of species to specific horizons and to compare humus layer thickness, at the WK sites, for each microannelid core sample the organic hori-

zons, their thickness and their distribution over the above mentioned vertical sub-samples were recorded. For the NB site only the thickness of the humus layer in total was measured.

The earthworms were sampled by formalin extraction in combination with hand-sorting and Kempson extraction (ISO 23611-1, 2006). Formalin extraction was performed on a soil surface area of 0.25 m². For hand-sorting two samples were taken at each sample point using a corer of 250 cm² to a depth of 10 cm. The samples were hand-sorted in the laboratory and subsequently underwent a Kempson extraction to be sure that all individuals were found. Earthworms were fixed in NOTOXhisto™ and determined accord-

ing to Sims & Gerard (1985). Earthworm biomass was determined for fixed animals with gut content. The earthworm results comprise abundance, biomass, species composition and dominance of life form types (epigeic, endogeic, anecic).

Sampling took place in the Weiherskopf (WK) Forest Reserve at the end of April 2014. The Nonnenstromberg (NB) Forest Reserve was investigated in October 1991 (microannelids and earthworms) and April 1993 (earthworms only). At NB the first earthworm sampling was performed without formalin extraction as anecic earthworms had not been expected due to the relatively low pH (NB1991). The results, *i.e.* the occurrence of endogeic earthworms and the presence of mull humus forms, indicated the possible presence of anecic earthworms. Thus a second sampling including also formalin extraction was performed at 10 out of the original 20 sampling points in 1993 (NB1993), as hand-sorting and Kempson extraction are less effective for deep-burrowing species. These 10 sampling points covered the north-facing slope (4 samples) as well as the south-facing slope (6 samples).

Samples were taken irrespective of the dead-wood present to varying degrees at the sample points, *i.e.* we did not sample decaying deadwood or soil below it or in its immediate surroundings at any of the sites.

Data analysis

The results were analyzed with respect to species composition, total abundance and vertical distribution of the community as well as dominance and frequency of species. Functional traits of individual species, as strategy type and acidity indicator group, were assigned according to Graefe & Schmelz (1999). The originally nine-step scale of acidity indicator values presented in Graefe & Schmelz (1999) was here condensed to three acidity indicator groups: indicators of strong acidity (acidity indicator values 1−3), indicators of moderate acidity (acidity indicator values 4−6) and indicators of slight acidity (acidity indicator

value 7). In terms of strategy types, indicators of strong and moderate acidity together are termed as A-strategists, while all k-strategists, *i.e.* persistent species with a low reproduction rate, are indicators of slight acidity. R-strategists are opportunistic species with a high reproduction rate (sexual or by fragmentation) and also belong to the indicators of slight acidity (Graefe & Schmelz 1999).

Statistical analyses were performed with SYSTAT 13. Data were checked for normality with the Shapiro-Wilk Test. Data were not normally distributed in some cases. This was partly due to the occurrence of several sample points with no earthworm findings. Square root transformation did not improve the situation. Thus, differences in abundance and earthworm biomass between sites were checked for significance with the non-parametric Kruskal-Wallis Test, followed by Conover-Inman Test for all pairwise comparisons.

Similarity of species composition was assessed with the Sørensen Similarity Index (Mühlenberg 1989), calculated according to: $QS = 2*C / (A+B)$, where A and B are the numbers of species in sites A and B, respectively, and C is the number of species shared by the two sites. QS ranges between 0 and 1, where 1 represents an identical species composition of two communities. As the index uses just presence / absence data, the species lists for earthworms for NB1991 and NB1993 were pooled.

RESULTS

Species composition and quantitative parameters

At the two sites of the Weiherskopf Strict Forest Reserve the same 9 earthworm species were found. All three life-form types were represented (Table 2). The only anecic species *Lumbricus terrestris* was found with low abundance and frequency. Total earthworm abundance was very similar at both sites. The total earthworm biomass did not differ significantly either $(p < 0.005)$.

The two samplings at the Nonnenstromberg Strict Forest Reserve yielded significantly lower abundance data for earthworms (p < 0.005). The earthworm biomass was on a similar level as at the Weiherskopf sites when, at the second sampling at the Nonnenstromberg site (NB1993), formalin extraction was applied and the anecic species *Lumbricus terrestris* was found. The species found at NB were generally the same ones as at WK, except from *Octolasion tyrtaeum* which was missing at NB.

The species number of microannelids ranged between 27 (NB) and 34 (WK-DW). The majority of species belonged to the family Enchytraeidae, whereas two species, *Hrabeiella periglandulata* and *Parergodrilus heideri* are Polychaeta. The latter only occurred at WK-DW, while *Hrabeiella periglandulata* was present at all three sites with high frequency and abundance (Table 3). Among

the enchytraeid species occurring most frequently and at all three sites were *Buchholzia appendiculata, Stercutus niveus, Enchytronia parva, Enchytraeus christenseni* and *Enchytraeus buchholzi*. Among the frequent species are two that have not been formally described so far, but are known to the authors from investigations on soil monitoring sites in Germany. These are given as *Enchytronia* sp. (sept) and *Achaeta* sp. (dzwi) in Table 3. *Achaeta* sp. (affi) belongs also into this category. This species occurs only in a few samples, but in very high numbers (WK-DW and NB).

The total enchytraeid abundance was similar at WK-RF and NB, but more than twice as high at WK-DW (p < 0.05), although variability at WK-DW was comparatively high due to the agglomerated occurrence of *Achaeta* sp. (affi).

Table 2. Species composition of earthworms at the studied sites. F: frequency, A: abundance, B: biomass. [a, b]: abundance or biomass data with different exponents differ significantly. WK-DW: Weiherskopf with deadwood, WK-RF: Weiherskopf reference site, NB1991: Nonnenstromberg, sampling 1991 without formalin extraction, NB1993: Nonnenstromberg, sampling 1993 with formalin extraction

Site	WK-DW			WK-RF			NB1991			NB1993		
	F	A ind. m^{-2}	B g m^{-2}	F	A ind. m^{-2}	B g m^{-2}	F	A ind. m^{-2}	B g m^{-2}	F	A ind. m^{-2}	B g m^{-2}
epigeic species												
Dendrobaena octaedra (Savigny, 1826)	80%	30.0	2.2	70%	30.0	2.2				20%	4.0	0.4
Dendrobaena pygmaea (Savigny, 1826)	70%	23.6	0.4	60%	15.6	0.3	15%	5.0	0.1			
Dendrodrilus rubidus (Savigny, 1826)	20%	4.0	0.4	20%	2.4	0.3	20%	7.0	0.3	10%	2.0	0.3
Lumbricus rubellus Hoffmeister, 1843	10%	4.0	0.1	10%	2.0	5.5				30%	5.2	3.3
endogeic species												
Aporrectodea caliginosa (Savigny, 1826)	70%	16.8	7.6	90%	42.0	15.4	30%	8.0	1.6	50%	14.4	7.4
Aporrectodea limicola (Michaelsen, 1890)	40%	4.8	0.9	80%	26.8	2.1				30%	2.8	0.3
Aporrectodea rosea (Savigny, 1826)	80%	20.0	2.1	40%	6.0	0.8	10%	3.0	0.1	20%	2.4	0.3
Octolasion tyrtaeum (Savigny, 1826)	100%	36.4	5.4	80%	16.0	2.6						
anecic species												
Lumbricus terrestris Linnaeus, 1758	20%	2.0	4.0	10%	0.4	0.9				40%	3.2	5.6
Total arithmetic mean		141.6[a]	23.1[a]		141.2[a]	30.1[a]		23.0[b]	2.1[b]		34.0[b]	17.7[a]
standard deviation		58.7	20.5		77.9	33.2		36.9	3.6		26.1	20.5
Species number	**9**			**9**			**4**			**7**		

Table 3. Species composition of microannelids at the studied sites. F: frequency, AB: abundance. WK-DW: Weiherskopf with deadwood, WK-RF: Weiherskopf reference site, NB: Nonnenstromberg. AcInd: Acidity indicator group, str: indicator of strong acidity, m: indicator of moderate acidity, sli: indicator of slight acidity. Informal species name with acronym in brackets: Species not formally described, but known to the authors. [a, b]: abundance data with different exponents differ significantly

Site	WK-DW		WK-RF		NB		Ac Ind
	F	AB	F	AB	F	AB	
Enchytraeidae							
Cernosvitoviella atrata (Bretscher, 1903)	20%	7		-	40%	16	x
Cognettia sphagnetorum (Vejdovský, 1878)	40%	13	30%	9	35%	18	str
Achaeta camerani (Cognetti, 1899)	10%	6	10%	48		-	str
Mesenchytraeus pelicensis Issel, 1905		-	10%	1		-	str
Achaeta sp. (affi)	20%	726		-	25%	334	m
Cognettia cognettii (Issel, 1905)	10%	4		-			m
Oconnorella cambrensis (O'Connor, 1963)	20%	112		-	25%	118	m
Achaeta sp. (glin)	40%	79	50%	50		-	m
Enchytraeus norvegicus Abrahamsen, 1969	20%	9	30%	12	30%	17	m
Marionina simillima Nielsen & Christensen, 1959	10%	1		-		-	m
Mesenchytraeus glandulosus (Levinsen, 1884)	30%	5	30%	6	20%	10	m
Enchytronia parva Nielsen & Christensen, 1959	70%	100	90%	77	95%	193	m
Enchytronia sp. (sept)	80%	18	50%	18	65%	42	m
Fridericia striata (Levinsen, 1884)	20%	6		-	60%	24	m
Oconnorella tubifera Nielsen & Christensen, 1959	40%	42	30%	46	15%	115	m
Achaeta bohemica (Vejdovský, 1879)	50%	21	30%	8	75%	92	sli
Achaeta unibulba Graefe, Dózsa-Farkas & Christensen, 2005	10%	1		-	5%	1	sli
Achaeta sp. (dzwi)	70%	107	60%	51	90%	345	sli
Buchholzia appendiculata (Buchholz, 1862)	80%	82	100%	168	60%	151	sli
Enchytraeus buchholzi Vejdovský, 1879	40%	21	60%	18	55%	20	sli
Enchytraeus christenseni Dózsa-Farkas, 1992	100%	72	80%	65	60%	59	sli
Enchytraeus lacteus Nielsen & Christensen, 1961		-		-	5%	1	sli
Enchytronia sp. (holo)	40%	49	10%	1		-	sli
Fridericia benti Schmelz, 2002	40%	8	30%	3		-	sli
Fridericia bisetosa (Levinsen, 1884)	60%	16	60%	25	45%	14	sli
Fridericia connata Bretscher, 1902	40%	12	90%	44	15%	6	sli
Fridericia galba (Hoffmeister, 1843)	30%	11	40%	5	5%	1	sli
Fridericia isseli Rota, 1994		-	20%	7		-	sli
Fridericia maculata Issel, 1905	20%	11	10%	1		-	sli
Fridericia miraflores Sesma & Dózsa-Farkas, 1993	50%	114	50%	14	10%	2	sli
Fridericia cf. nielseni Möller, 1971		-		-	5%	1	sli
Fridericia paroniana Issel, 1904		-	10%	1	15%	4	sli
Fridericia waldenstroemi Rota & Healy, 1999		-	10%	2		-	sli
Fridericia sp. juv.	70%	124	100%	125	90%	63	sli
Henlea nasuta (Eisen, 1878)	10%	5		-		-	sli
Marionina argentea (Michaelsen, 1889)	30%	8	30%	26	5%	1	sli
Mesenchytraeus armatus (Levinsen, 1884)	10%	1		-		-	sli
Stercutus niveus Michaelsen, 1888	80%	51	60%	70	45%	14	sli
Polychaeta							
Hrabeiella periglandulata Pizl & Chalupský, 1984	100%	239	90%	137	90%	175	m
Parergodrilus heideri Reisinger 1925	10%	7		-		-	sli
Total		2088		1038		1837	
Total ind. m^{-2} arithmetic mean		106,341 [a]		52,865 [b]		46,779 [b]	
standard deviation		104,979		30,162		23,341	
Species number		**34**		**28**		**27**	

Acidity indicator groups, strategy types and life-form types

The biomass proportion of the three life-form types of earthworms is illustrated in Figure 1 (left). The percentage of epigeic species was below 30 % at all sites. The proportion of endogeic species was equal at the two WK sites.

For the microannelids, we differentiate between the strategy types r-, A- and k-strategists (Figure 1, centre). In the category r-strategists, species with a potentially high reproduction rate are pooled, *i.e.* most *Enchytraeus* species and *Buchholzia appendiculata*. This group showed a comparatively high percentage at WK-RF, due to a high dominance of *Buchholzia appendiculata* at this site (16%). However, the microannelid community was generally dominated by A-strategist species at all three sites. At WK-DW and NB they represented more than 50% of the species, to which *Achaeta* sp. (affi) contributed the greatest part. At WK-RF k-strategist species reached almost the percentage of A-strategists. To the category A-strategist belong indicators of moderate as well as of strong acidity. Figure 1 (right) differentiates according to the three groups of acidity indicators given in Table 3. It becomes evident that the A-strategists include mainly indicators of moderate acidity here, while indicators of strong acidity had by far the smallest

proportion of the three indicator groups at all investigated sites.

Humus profiles and vertical distribution of microannelids

The humus layer of the soil profile was restricted to the uppermost microannelid sub-sample (0–2.5 cm) at all three sites (Figure 2, data not shown for NB). The microannelid activity showed generally a decline in the deeper sub-samples (figure 3). At site WK-RF the border between humus layer and mineral soil was located within the uppermost sub-sample, where the highest microannelid density was found. At WK-DW and NB the microannelid density was highest in the second depth layer. At these both sites, also the border between humus layer and mineral soil was located slightly deeper than at WK-DW and often coincided with the border between first and second sub-sample. The high abundance in the 2.5–5 cm layer at WK-DW and NB was partly produced by the mass occurrence of *Achaeta* sp. (affi) in this layer in single samples. Some species concentrated in specific horizons: *Buchholzia appendiculata* occurred most numerously in the uppermost layer, which consisted mainly of the organic layer. In contrast, other species, as *Hrabeiella periglandulata* and *Stercutus niveus* occurred predominantly in the mineral topsoil (A-horizon).
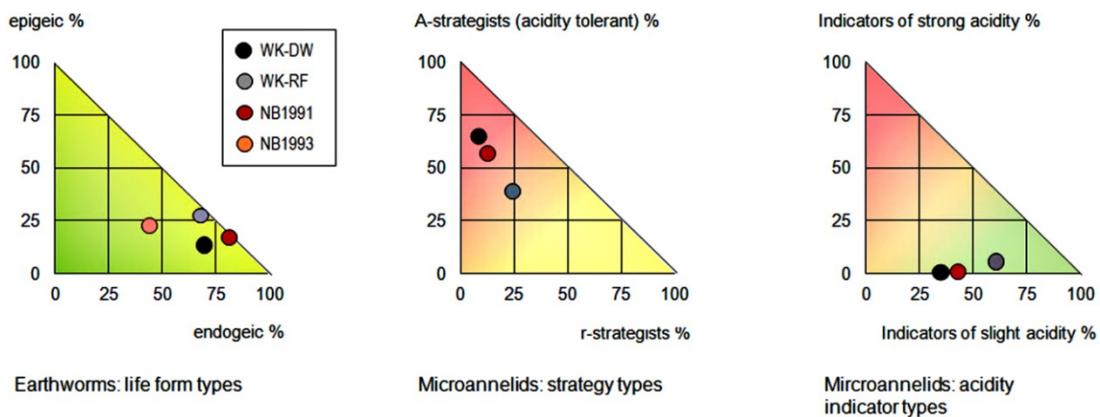
Figure 1. Life-form types (left), strategy types (centre) and acidity indicator types (right). Percentage of biomass (earthworms) or abundance (microannelids). In the diagrams, the parameter complementing the shown values to 100% is: anecic biomass (left), k-strategists (centre) and indicators of moderate acidity (right). Strategy types according to Graefe & Schmelz (1999). WK-DW: Weiherskopf with deadwood, WK-RF: Weiherskopf reference site, NB1991: Nonnenstromberg, sampling 1991 without formalin extraction of earthworms, NB1993: Nonnenstromberg, sampling 1993 with formalin extraction of earthworms
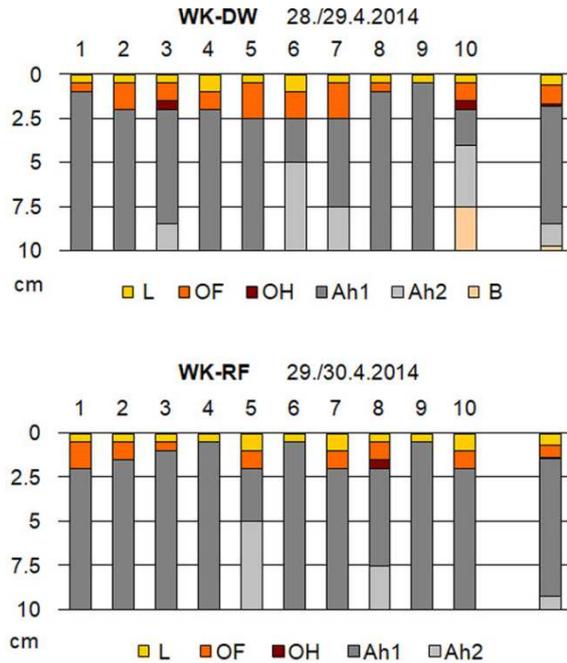
Figure 2. Humus profiles of the two Weiherskopf study sites at the 10 sample points and arithmetic means of horizon thickness (right column). L: undecayed litter, OF: fragmented litter, OH: humified litter, Ah: mineral topsoil, B: subsurface horizon. WK-DW: Weiherskopf with deadwood, WK-RF: Weiherskopf reference site.
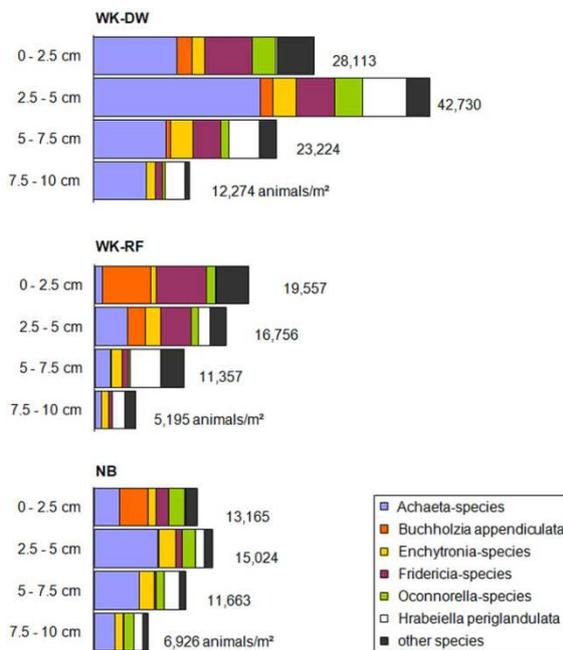
Figure 3. Vertical distribution of microannelids at the study sites. WK-DW: Weiherskopf with deadwood, WK-RF: Weiherskopf reference site, NB: Nonnenstromberg.

Sørensen Similarity Index

The Sørensen Similarity Index was computed for the whole annelid community as well as for microannelids only. Taking into account all annelid species it took values between 0.83 and 0.78, while the exclusion of earthworms from the index yielded slightly lower values (Table 4). While the species spectra of WK-DW / WK-RF and WK-DW / NB overlap almost to the same degree, the similarity between WK-RF and NB was slightly lower.

Table 4. Sørensen Similarity Index of the investigated sites. WK-DW: Weiherskopf with deadwood, WK-RF: Weiherskopf reference site, NB: Nonnenstromberg.

Earthworms and microannelids:	Sørensen Index
WK-DW *vs* WK-RF	0.83
WK-DW *vs* NB	0.82
WK-RF *vs* NB	0.78
Microannelids only:	
WK-DW *vs* WK-RF	0.77
WK-DW *vs* NB	0.79
WK-RF *vs* NB	0.73

DISCUSSION

While the site WK-DW had been strict forest reserve for 25 years at the time of investigation, the Nonnenstromberg strict forest reserve was only established two years before the first investigation at the site NB. However, this site had been part of a nature protection area for several decades before, which also implies reduced human impact. We thus consider the sites WK-DW and NB similar concerning their protection status for ≥ 25 years. Site WK-RF is still under forest management, which includes deadwood removal to some extent. Amounts of deadwood varied strongly horizontally at all sites. At the Weiherskopf sites, windthrow had been patchy, WK-DW being more affected by it than WK-RF. In our

opinion, a correlation of deadwood presence with the zoological data would have required the assessment of deadwood stocks and distribution at each of the sample points, but this was not part of our research contract. Thus, the possible influence of windthrow and deadwood management on species composition, abundance and biomass data of earthworms and microannelids was not explored in detail. As we took soil-samples and did not sample specifically under or in the vicinity of decaying deadwood, we assume that the soil properties were principal determining factors for community composition.

Species composition of earthworms was very similar at the three investigated sites. The much lower earthworm abundance at the Nonnenstromberg site indicates that the habitat conditions might not have been optimal at the sampling occasions in 1991 and 1993. Also comparison with other studies shows that the abundance data for the Nonnenstromberg site were rather low (Bonkowski 1991). Whether soil properties or other factors, as *e.g.* unfavourable weather conditions (drought) have been the reason, is hardly possible to detect more than 20 years after the investigation. When we encounter a very low abundance, this can relate to lower species numbers. Species we would expect might then be seemingly absent, because they were not covered by the sampling, *e.g.* due to sparseness or inactivity. This might have been the case for *Octolasion tyrtaeum* at site NB.

For microannelids the significantly higher abundance at WK-DW is mainly due to high numbers of *Achaeta* sp. (affi) at one sample point. This mass occurrence, which contributed more than one third to the total abundance of this site, was probably caused by the slightly lower pH at this point, as *Achaeta* sp. (affi) is an indicator of moderate acidity. A mass development of this species occurs to a lesser extend also at NB and has been encountered elsewhere as well (Graefe 2004).

With respect to community characterization we consider quantitative parameters, like abundance, that can show high short-term variability, less relevant than parameters linked to the species composition, as proportion of indicator groups or occurrence of functional key species. In this respect, the communities at the three investigated sites shared characteristics that indicate similar soil habitat conditions. As has been repeatedly demonstrated, the characteristics of soil fauna communities are closely linked to humus form development (Jabiol *et al.* 1995, Graefe & Beylich, 2006). At the investigated sites, all three life form types of earthworms were present. Anecic and endogeic earthworm species are considered main actors in the formation of mull humus forms. In addition to these, *Stercutus niveus* among the microannelids is characteristic for the decomposer community type typical for forest soils with mull humus forms according to Graefe (1993). At the studied sites we found a variant of this community type, including a high dominance of endogeic earthworms, along with a reduced presence of anecic earthworms. Further the generally quite rare species *Dendrobaena pygmaea* occurred regularly. Among microannelids a high dominance of *Hrabeiella periglandulata* and the occurrence of several species that are indicators of moderate acidity characterized the community type variant at the studied sites. Given pH values below 5 in the mineral topsoil, indicators of slight acidity, as anecic earthworms and most *Fridericia* species, are near the lower limits of their $pH_{(CaCl2)}$ tolerance range (Graefe & Beylich 2003), even when we consider that measurement in $CaCl_2$-solution would produce slightly higher values than measurement in KCl-solution as used in the present study.

Due to the low frequency of anecic earthworms, the incorporation of organic matter into the soil was limited at the studied sites, resulting in the formation of an OF-layer of partly fragmented organic material. Thus, the main humus form was an F-Mull (Ad-hoc-AG Boden 2005). Although there were patches with a thin OH-horizon, suggesting a transition to moder humus forms, we always found numerous microannelids to a depth of 10 cm, indicating a biologically active A-horizon, being an integral part of mull

humus forms (Jabiol *et al.* 1995). A closer look at the vertical distribution of microannelids in relation to the border between the humus layer and the mineral soil suggests a high decomposition activity just in this border zone. The high dominance of the r-strategist *Buchholzia appendiculata* in the uppermost layer is related to this. The preference of this species for the litter layer (OL- and OF-layer) is well established (Dózsa-Farkas 1992, Graefe & Schmelz 1999). The fact that *Fridericia* species, all of which are considered indicators of only slight acidity, showed the highest dominance in the uppermost layer, could relate to the fact that the organic layer had higher pH-values than the mineral topsoil.

The high species diversity associated with a considerable proportion of species tolerating at least moderate acidification distinguishes the community on basalt from that of limy soils on calcareous bedrock. Along a gradient from basalt to limestone, Schlaghamerský (1998) found a higher species number on basalt than on limestone, with *Cognettia sphagnetorum* and *Enchytronia parva* (indicators of strong and moderate acidity respectively according to Graefe & Schmelz 1999) showing significantly lower densities in the calcareous soil. At the same sites, Bonkowski's (1991) investigations on earthworms yielded a high diversity. The species *Dendrobaena pygmaea* showed a lower biomass on limestone than on basalt. Whether differences in species dominance were due to the lime content or rather differences in other factors could not be ascertained. The fact that soils on calcareous bedrock are frequently shallow and running dry readily may cause problems in relating community characteristics directly to the presence of lime. Our own investigations in the "Hünstollen" Strict Forest Reserve in Lower Saxony on calcareous soil on limestone showed a similarly high annelid diversity as in the current study (26 species), but hardly indicators of moderate acidity (Beylich *et al.* 1995). Notably, the most common species at our current study sites on basalt indicating moderate acidification, *i.e. Enchytronia parva* and *Hrabeiella periglandulata*, did not occur at the "Hünstollen" site. However, Mellin

(1988) found in his investigations in beech forests on limestone a microannelid community extremely rich in species, including also *Enchytronia parva* and other indicators of moderate acidity, although the pH (KCl) was mostly between 5 and 6 and thus slightly higher than at our study sites on basalt. Anyhow, also soils on limestone can show advanced acidification favouring a broad species spectrum comprising species with varying pH preferences. Schoch-Bösken & Greven (1987) found 24−26 species in a beech forest on limestone in the Egge Mountains (Germany), ranging from indicators of strong to slight acidity (mean pH (CaCl$_2$) 4.1−4.3). Apparently, it is difficult to draw a clear line between the annelid community of moderately base-rich soils on basaltic bedrock and the community of base-rich soils on calcareous bedrock. There rather seems to be a smooth transition between both, depending on the degree of acidification of the soil.

For faunistic studies on other animal groups in strict forest reserves, Sørensen values of > 60% are considered as "high" (Dorow 2014). The similarity of the three investigated sites on basalt according to the Sørensen Index is thus quite striking, supporting the conclusion that comparable site conditions led to the development of similar annelid communities. The high similarity also shows that, at least within the given sampling design, differences concerning community composition between the two Weiherskopf sites due to different deadwood management are not traceable. Although the inclusion of the above mentioned studies on basalt or limestone by other researchers into the calculation of Sørensen Indices would have been interesting, it was not undertaken, as the revision of species and description of new species during the last decades makes direct comparison of species lists in particular cases difficult (*e.g.* Schmelz 2003).

Corresponding to the delineated variant of decomposer community, also botanists distinguish within beech forests with mull humus forms (Galio-odorati Fagenion) between communities on moderately base-rich soils as opposed to those on calcareous soils (Ellenberg 1986). Both, the

decomposer community as well as the plant community typical for moderately base-rich soils free of calcium carbonate apparently do not comprise distinct character species, but are characterized by the co-occurrence of indicators of slight and moderate acidity. On the other hand, the decomposer community of forest sites with moder humus forms is characterized by indicator species for strong acidity among the microannelids and the lack of endogeic and anecic earthworms, as outlined by Graefe *et al.* (2002). While reference ranges concerning abundance, biomass and species number for earthworms and microannelids have been published for these acid forest sites with moder humus forms in Beylich & Graefe (2009), no such values were proposed for moderately base-rich and limy sites so far due to the comparatively low number of relevant studies.

According to the Red List of Earthworms Germany (BfN 2016), the species *Dendrobaena pygmaea* is considered very rare, though unthreatened. An enquiry at the public database Edaphobase (Burkhardt *et al.* 2014) rendered only about twenty entries for this species in Germany, mostly in mixed deciduous forests, never in grassland or agricultural sites. We consider this species as one of the few species occurring predominantly in moderately base-rich to base-rich forest soils. The species *Aporrectodea limicola* is classified as rare in the Red List, and endangered to unknown degree, a more precise categorization being impeded by insufficient data.

We conclude that beech forests on basalt with F-mull are associated with a specific type of decomposer community, including also some rare species. Comparison with communities on calcareous bedrock shows considerable overlap in species composition. Characterization of both types could be strengthened by comparative studies.

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Data on the Lumbricidae and Enchytraeidae from six forest sites in Germany and Portugal

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Abstract. The complex interactions between primary production, soil and soil organisms (in particular Lumbricidae and Enchytraeidae) during organic matter decomposition have developed over evolutionary time under specific habitat conditions. Thus, in order to study these processes, four forest sites in Germany (two in southern Hesse, two in eastern Saxony) and two sites in Central Portugal were selected to study both the oligochaete fauna and litter decomposition (almost all of them with a pH <4). Here, the results of the first part of this his work, i.e. the abundance and the species composition of the oligochaete fauna, are described. All samplings were performed using standard ISO-methods, with different temporal intensity. In detail, nine common Central European earthworm species were found at the six study sites plus one unidentified species at a Portuguese site. Abundance was usually low (< 10 Ind/m^2); higher numbers (72.5 and 17.5 Ind/m^2) were detected only at the two Saxonian sites. In total, 53 enchytraeid species were found; 43 of them already described. At four sites densities fell within the expected range for German deciduous forests (20 000 – 80000 Ind/m^2). Finally, notes on the taxonomy and biogeography of several potworm species are given.

Keywords: Annelida, species number, species composition, global climate change

INTRODUCTION

According to recent research on climate-change scenarios, Central European weather will become more extreme, with intermittent very hot and dry summers during the next 50−100 years (Schär *et al*. 2004, Schmidli *et al*. 2007). Tree species currently used in forestry are not adapted to such conditions, thus drought-resistant Mediterranean tree species might be an alternative (Pflug & Brüggemann 2012, Bussotti *et al*. 2015). Litterfall from trees represents a key resource for the below-ground ecosystem, but it is not known whether the complex process of litter decomposition, which depends on the soil organism community (mainly bacteria, fungi and a wide range of soil invertebrates), is or will be impacted by these climatically driven changes in forest composition (Blankinship *et al*. 2011 but see Wall *et al*. 2008). Soil organism communities are adapted in various ways to local conditions, *e.g.* soil properties or climatic factors. As part of the German BiK-F project (www. www.bik-f.de; today belonging to the Senckenberg Museum, Frankfurt), the question was raised whether climate-change driven transformations of forest-tree composition will affect these organisms and their functions, such as their role in litter decomposition. Specifically, one of the research questions is whether soil organisms in Germany, having been adapted to litter from Central European tree species, such as beech (*Fagus sylvatica*) and oaks, *e.g. Quercus pubescens*, can handle litter from southern European trees (mainly oaks). Lumbricid and enchytraeid species were selected as representatives of the whole community due to their important role

in litter decomposition under central European conditions (Blouin *et al*. 2013).

The oligochaete studies were performed at different intensities in six forest sites (two in Saxony [eastern Germany], two in Hesse [western Germany] and two in Central Portugal), representing areas with different climate systems. Thus, the main question of the whole project was: will the local soil organism community be able to decompose "foreign" litter, or will local soil nutrient cycles be disrupted after introduction of the "foreign" trees? In this paper we report on the description of the oligochaete fauna at the six study sites in Germany and Portugal. Abundance and community composition of the fauna are important for the interpretations of the litter decomposition studies which are still in progress.

MATERIAL AND METHODS

In order to address the research question, three geographical regions in Europe were selected, one in the continental and one in the Atlantic region of Germany, and one in the Mediterranean region (Central Portugal). The study sites in eastern Germany are located in the continental climate zone with a mean annual yearly temperature (MAT) average of 8.5°C. These average increases by approx. 2°C when shifting to the sites in western Germany, representing the oceanic climate zone. An additional further 3°C increase in yearly temperature MAT is found at the study sites in Portugal which are influenced by the Mediterranean climate zone (Peel *et al.* 2007). These differences are within the range of predicted temperature increases for Germany and Portugal.

In each region, two study sites with four 20×20 m plots each were selected in order to represent different forest histories and conditions within a climatic region, *i.e.* forests were only replicated within sites but not necessarily between sites of an area. The two eastern German sites are located in the state of Saxony: Viereichen and Ullersdorf, both near Görlitz. They represent two undisturbed deciduous forests, mainly consisting of different species of oaks (mainly *Quercus robur* with an age of about 100 years), hornbeam (*Carpinus betulus*) and few common beech (*Fagus sylvatica*) trees. At Viereichen, rarely small leaf linden (*Tilia cordata*) does occur.

The two south-western German sites are located in the state of Hesse: Rüsselsheim (disturbed mixed forest near human settlements) and Lampertsheim (mixed deciduous forest in the Rhine valley, very sandy soil and dry conditions). These two were most intensively studied. The Rüsselsheim site is originally an open *Pinus sylvestris* forest containing solitary old (>80 years) *Quercus robur* trees. After disturbed by fire in 2008, the area was partly re-planted with *Q. ilex*, *Q. frainetto* and *Q. pubescens*. At Lampertheim, the dominant tree species is also *Q. robur* (*ca*. 110 years old). Saplings of the same oak species as in Rüsselsheim (plus *Q. petraea*) where planted just outside of the undisturbed sampling area (Dorow *et al.* 2012). St. Olaia is a calcareous hill in the coastal plain that has been continuously occupied by forest since the Neolithic times. The vegetation is mainly composed by the tree species *Quercus fagineae* and *Quercion ilicis* and various herb and shrub species (*e.g. Vinca difformis*). Cerdeira has been occupied by forest since about 50 years ago when the area was no longer explored for agriculture. The vegetation is mainly composed by the tree species *Quercus robur*, *Q. pyrenaica*, *Castanea sativa*, *Pinus pinaster* and *Eucalyptus globulus* and the bracken fern (*Pteridium aquilinum*).

The main characteristics of the six study sites and their most important soil properties are given in Table 1. Soils differ strongly in terms of texture, mainly regarding the percentage of sand and silt. The clay content differs only slightly (*i.e.* between 5 and 10%). Except for St. Olaia, which had a pH of 5.0, all other sites had acid soils with pH-values between 3.2 and 3.7. Regarding the organic matter content, a wide range was covered, from 6.2 % in Lampertheim up to 21.3 % at Cerdeira. Despite these differences, the C/N-ratio was not strikingly different, ranging from 13.1 in Ullersdorf and 20.3 in Rüsselsheim. Soil moisture was measured gravimetrically (*i.e.* loss in % weight after drying at 105°C) in parallel to the individual oligochaete samplings. It can change quickly, but the two Hessian sites are certainly drier than the other four sites.

Table 1. Main characteristics and most important soil properties of the six study sites. Mean temperature = mean of daily measures during the study period. Mean annual precipitation (mm/year) data are long-term averages collected from the nearest climate station.

Property	Viereichen	Ullersdorf	Rüsselsheim	Lampertheim	Cerdeira	St. Olaia
Region	Saxony (Germany)		Hesse (Germany)		Central Portugal	
Coordinates	51°22′54” N 14°41′54” E	51°14′15” N 14°50′27” E	49°57′13” N 8°24′54” E	49°35′08” N 8°28′46” E	40°09′43” N 8°19′41” W	40°17′06” N 8°71′52” W
Text. Sand (%)	62.3	19.5	67.8	75.4	35.1	48.9
Text. Silt (%)	32.2	70.2	21.9	19.0	57.2	43.0
Text. Clay (%)	5.5	10.3	10.3	5.6	7.7	8.1
Texture class	Medium silty sand	Weakly loamy silt	Medium loamy sand	Weakly silty sand	Sandy silt	Silty-loamy sand
pH (CaCl$_2$)	3.7	3.2	3.5	3.6	3.5	5.0
Organic Material (%)	9.5	13.4	9.4	6.2	21.3	16.7
C/N	14.9	13.1	20.3	17.1	15.7	13.0
Ø Soil Moisture (%)	30.1	79.5	37.8	30.9	41.8	30.6
Ø Temperature (C°)	8.4	9.9	11.1	7.3	12.8	15.1
Ø Ann. Precipitation	672	635	658	695	958	900

Sampling of soil organisms

Earthworms (Lumbricidae) were sampled according to the ISO standard 23611-1 (2006), *i.e.* by a combination of hand-sorting and formalin extraction. Five randomly selected samples were taken per plot. The two Hessian sites were sampled 2009, twice in spring and autumn each. The remaining four sites (two in Saxony and two in Portugal) were sampled once in April and June 2014, respectively. Each individual worm was morphologically determined using the keys of Graff (1953), Bouché (1972) and Sims & Gerard (1999). The worms were stored in 90% ethanol.

Potworms (Enchytraeidae) were sampled according to the ISO standard 23611-3 (2006) with soil-corers (Ø 5.5 cm). Ten samples were randomly taken per plot. Each sample was divided to litter layer and 0–4 cm mineral soil layer. The Hessian sites were sampled four times between autumn 2008 and spring 2010, while the others were sampled only once, in April or June 2014. Potworms were collected by wet extraction in the laboratory at room temperature, about 20°C. Extraction duration for litter-layer samples was 1–2 days and for mineral soil samples was 3–4 days. Morphological identification was performed using the key and checklist of Schmelz & Collado (2010; 2012).

RESULTS

Abundance, species number and composition of earthworms

All three pairs of sites and, also, the two sites within a region differed considerably in abundance and/or species number (Table 2). In Hesse, very low density (0.4 – 3.4 Ind/m^2) and species richness were found. In Saxony we found considerable differences between the two sites. At Viereichen both abundance and species number were high (80.5 Ind/m^2; 6 species), but at Ullersdorf the respective numbers were clearly lower (17.5 Ind/m^2; 3 species). In Portugal, the differences were even more pronounced; at Cerdeira almost no earthworms were found (0.4 Ind/m^2; 1 species), while at St. Olaia both parameters were much higher (7.9 Ind/m^2; 4 species).

Abundance, species number and composition of enchytraeids

Due to the different sampling efforts at the six sites, it is difficult to compare the results directly, but the number of enchytraeids was in tendency, with two exceptions, in a similar range, i.e. about 40 000 to 50 000 Ind/m^2 (Tables 3 and 4). In Rüsselsheim their average number was almost always lower (about 25 000 Ind/m^2), while at Viereichen on average twice as many enchytraeids were found as at all other sites.

Table 2. Abundance (Ind/m^2), standard deviation (SD), species number and composition of earthworms at the six study sites. Note that earthworms were sampled four times at the two Hessian sites, but only once at the other sites (therefore, mean values are given for Rüsselsheim and Lampertheim). n.d. = not determined due to low numbers

Genus / Species	Vieleichen	Ullersdorf	Rüsselsheim		Lampertheim		Cerdeira	St. Olaia
	Spring 2014	Spring 2014	Autumn 2009	Spring 2010	Autumn 2009	Spring 2010	Spring 2014	Spring 2014
Aporrectodea s.l. sp.	23.0	0.5	-	-	-	-	-	2.5
Aporrectodea caliginosa (Savigny, 1826)	14.5	-	-	-	-	-	-	-
Aporrectodea rosea (Savigny, 1826)	0.5	0.5	-	-	-	-	-	3.0
Lumbricus sp.	12.0	4.5	2.0	-	1.3	-	-	-
Lumbricus rubellus Hoffmeister, 1843	14.0	3.5	0.8	1.2	1.3	0.4	0.4	-
Lumbricus terrestris Linnaeus, 1758	1.5	-	-	-	-	-	-	-
Dendrobaena/-drilus sp.	2.5	0.5	-	-	-	-	-	-
Dendrobaena attemsi (Michaelsen, 1902)	4.0	5.0	-	-	-	-	-	-
Dendrobaena octaedra (Savigny, 1826)	-	-	-	-	0.5	-	-	-
Dendrodrilus rubidus (Savigny, 1826)	0.5	-	-	-	-	-	-	-
Octolasion sp.	-	-	-	-	-	-	-	0.5
Octolasion cyaneum (Savigny, 1826)	-	-	-	-	-	-	-	1.5
Octolasion tyrtaeum (Savigny, 1826)	-	-	-	-	0.3	-	-	-
Unknown species	-	-	-	-	-	-	-	0.5
Undetermined	8.0	3.0	0.5	-	-	-	-	-
Total ± SD.	80.5 ± 32.2	17.5 ± 3.4	3.3 ± 1.7	1.2 n.d.	3.4 ± 2.1	0.4 n.d.	0.4 n.d.	8.0 ± 2.0
Species number per site	6	3	1	1	3	1	1	4

Table 3. Abundance (Ind/m^2) and standard deviation (SD) of enchytraeids at Rüsselsheim and Lampertheim

Year	Season	Hesse (Germany)	
		Rüsselsheim	**Lampertheim**
2008	Autumn	21 520 ± 3 475	75 640 ± 8 286
2009	Spring	41 940 ± 5 841	57 800 ±2 800
2009	Autumn	13 920 ± 2 994	13060 ± 715
2010	Spring	22 700 ± 7 569	48 440 ± 12 517
Average	-	**25 020 ± 11 232**	**48 735 ± 26 324**

Enchytraeid abundance was fairly similar at the two Portuguese sites. At the two Hessian sites, samples were taken four times between the autumn of 2006 and the spring of 2011. Variation in abundance were less pronounced in Rüsselsheim (variation of a factor of three) than in Lampertheim (variation of a factor of six), but no specific pattern was visible.

In total, 53 enchytraeid species were identified (Table 4). Forty-three of them have already been described or are known to science; the remaining ten require descriptions. Not surprisingly, all but one of the undescribed taxa were found at the two Portuguese sites. Enchytraeids of Portugal are poorly known (Schmelz & Collado 2013). The number of enchytraeid species per site varied between 11 (Cerdeira) and 27 (St. Olaia); species numbers at the German sites were in-between. Thirty and 31 species, respectively, were only found at the sites in Germany (four sites) or in Portugal (two sites). Eleven species out of 52 occurred both in Portugal and Germany. Only one species, *Enchytronia parva*, was found at all six sites. At most sites no (Rüsselsheim) or few, *i.e.* 1-4 (Lampertheim, Viereichen, Ullersdorf, and Cerdeira) species occurred which were not found at other sites. The exception is St. Olaia, where 20 species were found which were not found at other sites.

DISCUSSION

Abundance, species number and composition of earthworms

All but one of the earthworm species found are well-known throughout Europe, *i.e.* no species with a restricted regional distribution was de-

tected. The exception was one small worm found at St. Olaia, which was not well enough preserved to be determined. It resembled *Proctodrilus antipai*, a central-European species occurring between central France and the Black Sea (Bouché 1972, Csuzdi & Zicsi 2003).

The low abundances and species numbers at most sites can be explained by the acidic soils with pH-values mainly below four, as indicated by typical acidophilous species of the genera *Dendrobaena/Dendrodrilus* and, partly, *Lumbricus*. The low abundance at the Hessian and Portuguese sites can be explained by site-specific characteristics, *i.e.* very sandy and dry conditions soil in Lampertheim, human disturbance in Rüsselsheim and shallow soil layers, partly on slopes, in Cerdeira. With such low abundances, it is clear that the ecological role of earthworms at these sites is small (*cf.* Blouin *et al.* 2013). The exception is Viereichen with a similarly low pH, but with twice the average earthworm densities reported for German deciduous forests (Jänsch *et al.* 2013). This situation cannot be presently explained.

Abundance, species number and list of enchytraeids

Enchytraeid densities at the six sites varied between 25 000 Ind/m^2 (Rüsselsheim) and almost 100 000 Ind/m^2 (Viereichen). Three out of the four German sites as well as both Portuguese sites are within the "normal" range (51 241 ± 30 677 Ind/m^2) for German deciduous forests, as determined in a recent literature review (Römbke *et al.* 2013). According to the same source, such German deciduous forests "should" harbor 12.4 ± 5.5

Table 4. Abundance (Ind/m^2), standard deviation (SD.), number and composition of enchytraeid species at the six study sites (mean values for the Hesse sites). Species names with four letter codes in brackets indicate forms with new character combinations, probably new species.

Genus / Species	Saxony (Germany)		Hesse (Germany)		Portugal	
	Vier-eichen	Ullers-dorf	Rüssels-heim	Lam-pertheim	Cerdeira	St. Olaia
Achaeta abulba Graefe, 1989	-	80	1050	10480	-	-
Achaeta affinis Nielsen & Christensen, 1959	22940	1000	10920	16600	12960	-
Achaeta bibulba Graefe, 1989	-	-	220	260	-	-
Achaeta bifollicula Chalupský, 1993	-	-	860	80	-	-
Achaeta camerani (Cognetti, 1899)	820	11580	380	1580	-	-
Achaeta unibulba Graefe, Christensen & Dózsa-Farkas, 2005	-	-	-	-	-	60
Achaeta sp. (HEAL)	-	-	-	-	-	600
Achaeta sp. (PAFF)	-	-	-	-	-	2680
Achaeta sp. (PAFU)	-	4560	-	-	-	-
Bryodrilus ehlersi Ude, 1892	-	-	10	170	-	-
Buchholzia appendiculata (Buchholz, 1862)	4640	-	-	-	-	14840
Buchholzia sp. (OLAI)	-	-	-	-	-	1080
Cernosvitoviella cf. *minor* Dózsa-Farkas, 1990	-	-	-	-	-	20
Cognettia cognettii (Issel, 1905)	-	60	-	40	12200	-
Chamaedrilus/ Cognettia chlorophilus * Friend, 1913	7000	17020	5410	6180	-	320
Enchytraeus bigeminus Nielsen & Christensen, 1963	-	-	-	20	-	-
Enchytraeus buchholzi Vejdovsky, 1879	1420	-	-	-	540	3000
Enchytraeus bulbosus Nielsen & Christensen, 1963	-	-	-	-	-	240
Enchytraeus sp. (GRAN)	2020	-	10	90	820	1240
Enchytraeus norvegicus Abrahamsen, 1969	11020	120	70	165	1760	-
Enchytronia parva Nielsen & Christensen, 1959	8440	180	340	2090	1500	840
Enchytronia sp. (MINO)	-	-	-	-	-	120
Enchytronia sp. (TENU)	120	-	-	-	160	-
Enchytronia pygmaea Graefe & Schmelz, 2017						100
Fridericia auritoides Schmelz, 2003	-	-	-	-	-	720
Fridericia benti Schmelz, 2002	60	-	-	-	-	-
Fridericia bisetosa (Levinsen, 1884)	2980	-	-	-	-	1980
Fridericia bretscheri Southern, 1907	-	-	-	-	-	2460
Fridericia brunensis Schlaghamerský, 2008	320	-	30	-	-	-
Fridericia bulboides Nielsen & Christensen, 1959	1220	-	-	180	-	-
Fridericia ciliotheca Schmelz & Collado, 2013	-	-	-	-	-	460
Fridericia connata Bretscher, 1902	240	-	20	-	-	200
Fridericia cylindrica Springett, 1971	-	-	-	-	-	40
Fridericia dura (Eisen, 1879)	620	-	-	-	-	-
Fridericia isseli Rota, 1994	-	-	-	-	-	340
Fridericia larix Schmelz & Collado, 2005	-	-	-	-	-	160
Fridericia monochaeta Rota, 1995	-	-	-	-	800	300
Fridericia paroniana Issel, 1904	-	-	-	-	-	5520
Fridericia ratzeli (Eisen, 1872)	740	-	20	60	-	-
Fridericia striata (Levinsen, 1884)	280	-	-	90	3,120	1780
Fridericia tuberosa Rota, 1995	-	-	-	-	-	240
Fridericia sp. (PANO)	-	-	-	-	1,080	-
Fridericia sp. (PERT	-	-	-	-	-	1980
Marionina argentea (Michaelsen, 1889)	-	-	-	-	-	40
Marionina clavate Nielsen & Christensen, 1961	-	5100	5340	1160	-	-
Marionina filiformis Nielsen & Christensen, 1959	-	-	-	60	-	-

Genus / Species	Saxony (Germany)		Hesse (Germany)		Portugal	
	Vier-eichen	Ullers-dorf	Rüssels-heim	Lam-pertheim	Cerdeira	St. Olaia
Marionina simillima Nielsen & Christensen, 1959	-	-	-	-	5460	-
Marionina sp. (SIM?)	20	-	-	-	-	-
Mesenchytraeus glandulosus(Levinsen, 1884)	40	-	-	-	-	-
Mesenchytraeus pelicensis Issel, 1905	120	80	30	-	-	-
Oconnorella cambrensis(O'Connor, 1963)	-	6680	310	7800	-	-
Oconnorella tubifera (Nielsen & Christensen, 1959)	14360	220	-	1400	-	-
Stercutus niveus Michaelsen, 1888	-	80	-	-	-	-
Abundance (Ind/m^2)	95820 ± 37300	46760 ± 29470	25020 ± 11232	48735 ± 26324	40400 ± 26545	41360 ± 26483
Species number per site	21	13	16	19	11	27

*= *Cognettia sphagnetorum* auct. (partim). See Martinsson *et al.* (2015) for details. The valid genus name is controversial and awaits a ruling by the International Commission on Zoological Nomenclature.

species. Our species number range, 13–21 fits very well to this average richness. To our knowledge, no quantitative studies on enchytraeid abundance and community structure have been carried out at Portuguese forest sites in general so far.

Regarding the biogeographical distribution, it seems that, despite the large geographical distance between the German and Portuguese sites, there is considerable overlap in faunal composition, although the overlap is smaller than in the case of earthworms. The large number of previously known species in Portugal confirmed previous records, reviewed in Schmelz & Collado (2013), who list altogether 32 species for Portugal. Two third of the species have also been recorded in Germany, thus most species belong to both the Portuguese and the German enchytraeid fauna. The high number of species found only in St. Olaia (18), including five species not yet described, indicates that the Portuguese enchytraeid fauna is far from being fully explored. In contrast to Central Europe, many new species were found not only in the species-rich genus *Fridericia* but also in genera such as *Achaeta* or *Enchytronia*. This is especially important when planning to use enchytraeids as indicators of soil quality (i.e. in terms of soil biodiversity). Species already known from other areas can be classified ecologically (*e.g.*, several species found at Cerdeira such as *A. affinis* or *E. norvegicus* are known to be acidophilous).

Five of the six study sites have a pH <4 and the sixth one, St. Olaia, is also acidic, although less so (Table 1), meaning that a relatively small number of acidophilous species (often in high numbers) are expected to occur there. However, it is not clear why at five out of six sites the species number is higher than expected. This may partly be explained by site-specific characteristics which, however, are difficult to identify. For example, in Lampertheim the vegetation pattern is very heterogeneous at a small scale, meaning that open spots and typical forest areas intermingle, providing different ecological niches at small scales. Rüsselsheim, on the other hand, is a forest located close to a densely populated city area, which – after a fire – had partly been afforested about 20 years ago. Thus, it can be considered to represent a disturbed site. However, while this stress may explain the low enchytraeid abundance, it seems to be contradictory to the relatively high species number.

The Cerdeira site is located in a mountainous region, often with shallow (*i.e.* less than 10 cm) soils and steep hills. This means that soils regularly dry up, which may explain the overall low abundance in comparison to such forests in general. A preliminary sampling in summer with dust-dry soil yielded no enchytraeids. Typical species of such acid sites are: *Ac. affinoides*, *Co. cognettii, En. norvegicus* and *Fr. striata*. New geographical elements (to be checked with more

sites) are *Fr. monochaeta*, *Fr.* (PANO), and *Ma. simillima*.

The forest of St. Olaia is the last remnant of a larger forest, being located on a small hill surrounded by agricultural land. The dominance of species from the genera *Enchytraeus* and *Fridericia* indicates neutral soils, which is interesting since the soil pH was measured as being just 5.0. In addition, the absence of *Henlea* species indicates dry conditions. New zoogeographical elements (to be checked ecologically with more sites) are represented by *Fr. auritoides*, *Fr. bretscheri*, *Fr. ciliotheca*, and *Fr. monochaeta*.

Finally, some special remarks on the taxonomy and biogeography regarding the two Portuguese sites can be made. At St. Olaia, *Fr. ciliotheca*, recently described from Coimbra (about 80 km to the west), was found for the first time following its original description. *Fr. larix* was found for the second time following its original description. Thus far it is only known from Ireland (original description) and England (unpublished). Possibly, it is a truly Atlantic species. The record of *Enchytronia pygmaea* at St.Olaia was included in the original description of the species (Graefe & Schmelz 2017).

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