Enchytraeid assemblages (Annelida: Clitellata: Enchytraeidae) of two old growth forests in the Porcupine Mountains (Michigan, USA)

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Abstract

Very little is known about enchytraeid assemblages in North American soils. It is difficult to assess to what extent present-day assemblages include introduced species that originally did not occur on the continent. Old growth forests are among the few preserved original habitats that provide the opportunity to study a presumably pristine soil fauna. In May 2011 soil samples were taken in two old growth forest stands on Podzols (Spodosols) in the Porcupine Mountains (Michigan, USA) differing in elevation, soil pH and stand composition (sugar maple vs. hemlock-sugar maple). Enchytraeids were extracted by the wet funnel method and identified alive. Species composition and representation, vertical distribution and density were assessed. In the maple stand, mean enchytraeid density was $32,100 \pm 9,000$ individuals/m² ($\pm$ SE), 74% of individuals being present in the upper 3 cm of soil. Six enchytraeid taxa were present, in four cases their identity was ascertained to species level. *Cognettia sphagnetorum* was predominant, comprising at least 82% of all individuals. The occurrence of *C. sphagnetorum* in North America had been hitherto known only from a tentative record from Florida. In the hemlock-sugar maple stand (with less acidic soil), mean enchytraeid density was $35,900 \pm 7,300$ ind./m² ($\pm$ SE) and the vertical distribution was more even. Also the representation of the 8 taxa identified was more even than in the maple stand. The genus *Marionina* was dominant (63% of all individuals) and represented by 1 or 2 species. *M.* cf. *minutissima* is reported for the first time from North America, but may also represent a new species.

Keywords Enchytraeidae | community structure | forests | old growth | North America

1. Introduction

Whereas enchytraeids (Annelida: Clitellata: Enchytraeidae) of North America received some attention around the turn of the 20th century, resulting in a number of species descriptions and faunistic records, few studies devoted to that group have been conducted in North America since then, in particular when studies on marine and marine littoral species are excluded. Studies dealing with terrestrial, soil-dwelling enchytraeids were mostly conducted by researchers from abroad staying for a limited period of time. Most focused on taxonomy and faunistics and were conducted in Canada or on Canadian material (Dash 1970, Dash & Cragg 1972, Nurminen 1973a, 1973b, Tynen et al. 1991, Rota & Brinkhurst 2000, Christensen & Dózsa-Farkas 2006). One exception was

the study of Healy (1989, 1996) from the western part ('panhandle') of Florida. Another exception were the numerous papers on the distribution and various aspects of the biology of the 'ice-worm', *Mesenchytraeus solifugus*, from glacier and snow-fields in Alaska and Mt. Rainier, Washington (see Hartzell & Shain 2009 and references therein), but these present a special case with no relevance to the present paper. A few ecological papers dealt with gross community parameters such as density and biomass, with little, if any, taxonomic resolution (van Vliet et al. 1995, 1997, 1998, Laganière et al. 2009, Waldrop et al. 2012). Enchytraeids had been also mentioned in earlier studies by Bird (1930) and Dirk-Edmunds (1947), however, the methods used by these authors neither allowed a reliable assessment of abundance nor did the published papers include

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any taxonomic information below family level. Only a single study (Dash & Cragg 1972) presented data on the species composition of entire enchytraeid assemblages. Not a single publication included information about the percentage representation of individual species, although some information about representation based on biomass (not abundance) can be derived from the data published by Dash & Cragg (1972). In contrast, information on the community structure of enchytraeid assemblages is widely available from many European countries, where enchytraeids have been studied most intensively. Many changes due to environmental factors changing in space or time do not occur at the level of higher taxa but rather at the species level: for instance, overall densities of a family such as Enchytraeidae might stay approximately the same while the species composition or percentage representation of species can change substantially (Holmstrup et al. 2012). Therefore, information on the community structure presents an essential baseline for studies of community changes due to changes of their habitat. These changes can be caused by such processes as global warming or invasions of exotic plant or animal species. All these processes can be currently observed in North America. One example of particular importance for soil biota is the invasion of exotic earthworms, in part into areas hitherto completely devoid of native earthworm species (e.g. Bohlen et al. 2004). These were shown to affect the structure of enchytraeid assemblages much more than overall density or biomass (Schlaghamerský et al. submitted). North America has also undergone large-scale changes in land-use over a rather short time period since European colonization, in particular the transformation of natural woodlands and grasslands into pastures and arable soil. Unfortunately, for many such cases, the baseline from which the effects of such changes could be assessed has never been established: the 'original' community structures of soil organisms in natural habitats have never been described for most taxonomic groups, including enchytraeids. No assessment of the degree by which the native North American enchytraeid fauna has been 'enriched' or displaced by introduced species has been made. As shown by Gates (1976), potworms are easily transported with live plants in soil (they are not called potworms by accident). An early introduction of some species during the European colonization of North America is therefore probable (another means of transport would have been, as in the case of earthworms, soil material used as dry ballast on ships). However, the penetration of exotic enchytraeids into remote areas not used for agriculture seems less probable than, for instance, that of earthworms, which are often accidentally introduced by sport anglers (Holdsworth et al. 2007, Keller et al.

2007). As a Holarctic distribution of many species is rather probable and supported by current data, at best molecular methods might shed some light on the origin of enchytraeid species shared by the Nearctic and Palearctic regions.

The present study is a small contribution towards closing the outlined gap in our knowledge, reporting data on enchytraeid assemblages from old-growth northern temperate forests in the Porcupine Mountains of the Upper Peninsula of Michigan in the northern Midwest of the USA.

2. Materials and methods

2.1. Study area and sites

The study sites were situated in the Porcupine Mountains on the southern shore of Lake Superior on the Upper Michigan Peninsula, Michigan, USA (Fig. 1). The climate of Upper Michigan is humid continental. Summers are short and cool; average July temperatures are 19.1°C at Marquette near Lake Superior. Winters are long and cold; average January temperatures are -7.5°C at Marquette. The mean annual frost-free period near Lake Superior is ca 120 d. Annual precipitation averages 800–900 mm over Upper Michigan and is fairly evenly distributed throughout the year (Frelich & Lorimer 1991). The Porcupine Mountains Wilderness State Park contains 14,500 ha of primary or virgin forest (and some previously logged areas). Elevations range from 182 m at the surface of Lake Superior to ca 600 m 5 km inland (Frelich & Lorimer 1991). Bedrock types in the Porcupine Mountains are shale and sandstone near Lake Superior, and amygdaloidal basalt, granite and Copper Harbor Conglomerate at higher elevations (Frelich & Lorimer 1991). The soils are mostly Spodosols (Podzols), i.e., Typic and Alfic Haplorthods according to US Soil Taxonomy (Scharenbroch & Bockheim 2007). The principal vegetation type in the area are closed-canopy northern mesic forests with sugar maple (*Acer saccharum*) dominating most of the forest inland from Lake Superior and mixing extensively with hemlock (*Tsuga canadensis*) closer to the lake shore. Lesser amounts of yellow birch (*Betula alleghaniensis*), red maple (*Acer rubrum*), northern red oak (*Quercus rubra*) and basswood (*Tilia americana*) occur throughout the area. Originally lacking native earthworms, one can observe an ongoing invasion of lumbricid species, including *Lumbricus terrestris*, even in the primeval forest stands (pers. obs.). On May 12, 2011 two forest stands differing in elevation and stand composition were
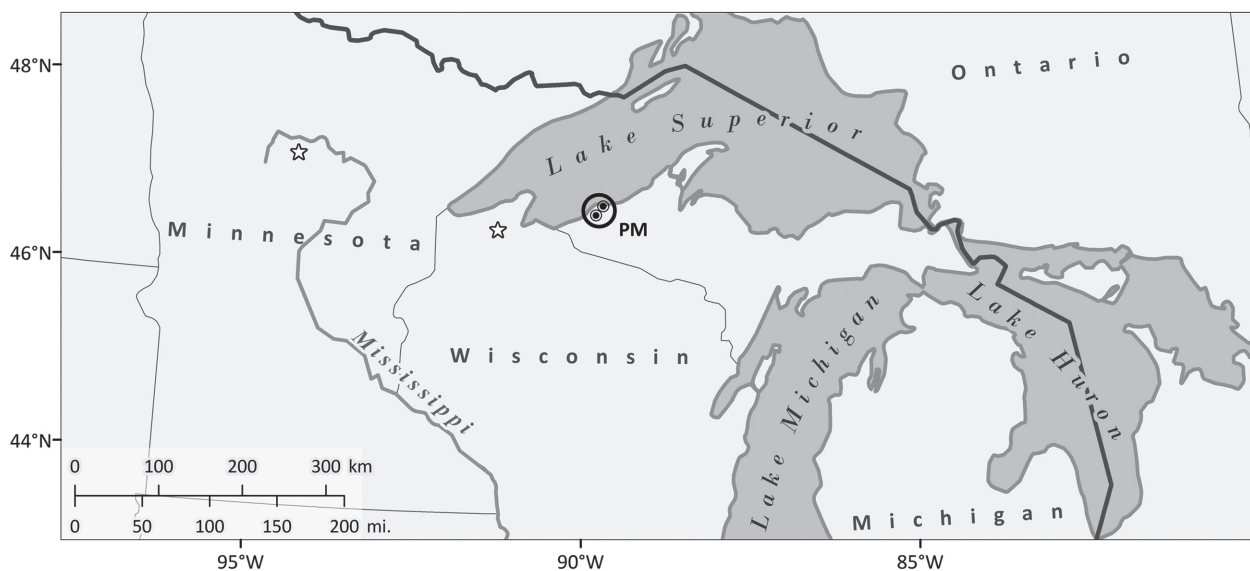
Figure 1. Location of the Porcupine Mountains (PM) with the two study sites (black dots in circle) in the Upper Peninsula of Michigan and the two closest sites (asterisks) where terrestrial enchytraeid assemblages were studied (white asterisks: northern Minnesota – Ottertail Peninsula on Leech Lake, Chippewa National Forest; northern Wisconsin – vicinity of Tower Lake within the Rainbow Lake Wilderness, Chequamegon-Nicolet National Forest).

sampled. One sampling site was situated ca 550 m south-west of the lookout tower at Summit Peak (595 m a.s.l.) in a sugar maple stand (46°44'45.280"N, 89°46'28.556"W) with some ironwood (*Ostrya virginiana*) and black cherry (*Prunus serotina*) in the undergrowth (Lee E. Frelich, pers. comm.). The site had never been logged, but the present stand originated after a natural windthrow in 1953. Soil was rocky, with a pH (H$_2$O) of the upper 12 cm of 3.95 (mean of two composite samples). The forest floor appeared unaffected by earthworm activity and no traces of earthworm presence were observed. The second sampling site was an old-growth maple-hemlock stand along the southern part of the Overlook Trail (46°48'34.74"N, 89°41'7.86"W). Hemlock made up ca. 70 % of the tree storey, complemented by sugar maple and some interspersed yellow birch, basswood, northern red oak and red maple present within ca. 30 m of the sampled area. Soil pH (H$_2$O) of the upper 12 cm was 4.21 (mean of three composite samples). Earthworms and traces of their activity were observed in the stand.

2.2. Sampling and sample processing

At both sites, soil samples were taken at random positions within an area of 5 m × 5 m, using a split soil corer of 4.8 cm in diameter (18 cm^2 surface area). Four and three soil cores were analysed from the Summit Peak and Overlook Trail sites, respectively. The sampling depth was 12 cm, litter layer included (sampling to greater depths than 12 cm was mostly prevented by the high density of large stones, although one core at the Summit Peak site was taken down to 14 cm). Enchytraeids were extracted from these layers by a modified O'Connor wet funnel extraction as developed for the quantitative extraction of enchytraeids (24 h without heating with subsequent heating of the soil surface up to 44°C within 4 h; for a comparison of extraction efficiency of the original O'Connor method and some modifications see Kobetičová & Schlaghamerský 2003). Prior to extraction, the soil was kept cool during transportation and stored in a refrigerator at ca. 4°C. Extracted enchytraeids were kept in water-filled Petri dishes at the same temperature. As tap water in the lab was above pH 7, the water used in the extraction and keeping of enchytraeids was adjusted to ca. pH 4, the approximate soil pH of the two sites, adding HCl solution to a mixture of tap water and deionized water. Temperature control and pH adjustments were adopted to minimize enchytraeid mortality. Enchytraeids were identified under a light microscope to species or at least to genus (the latter in particular in cases of juveniles, injured or dead specimens), except a few specimens already decomposing at the time of examination.

3. Results

The total numbers of enchytraeids extracted from the analysed soil cores were 232 specimens from the Summit Peak site and 195 specimens from the Overlook

Trail site. No other 'microannelids' were present in the samples. In the maple stand at Summit Peak, 74 % of the specimens were present in the upper 3 cm of soil (L, F and partially H-layers of the organic horizon) and only 10 % were in the mineral soil in 6–12 cm depth (Fig. 2). In the hemlock-maple stand along the Outlook Trail, enchytraeid distribution along the vertical profile was more even, with the highest percentage (45 %) in the 3–6 cm layer (transition from H-layer to mineral soil); 20 % were present at depths between 6 and 12 cm (Fig. 3).

In the maple stand at Summit Peak, mean enchytraeid density was 32,100 ± 9,000 individuals/m² (± SE; rounded to the nearest hundred) and 6 enchytraeid taxa were identified (Fig. 4). *Cognettia sphagnetorum* (Vejdovský, 1878) was the most dominant species, comprising at least 82 % of individuals. Another 6 % were *Cognettia* specimens that could not be identified to species, for instance regenerating rear and mid fragments or individuals with injuries preventing a clear observation of the number and arrangement of pharyngeal glands (of the non-regenerated fragments, only those including the head were counted; all presented percentages were calculated based on all specimens identified at least to genus). About 6 %
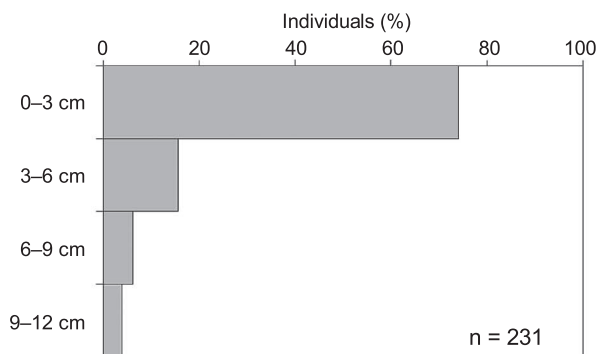

Figure 2. Vertical distribution of enchytraeids in the upper 12 cm of soil (four 3-cm layers extracted) at the Summit Peak site (sugar maple stand).
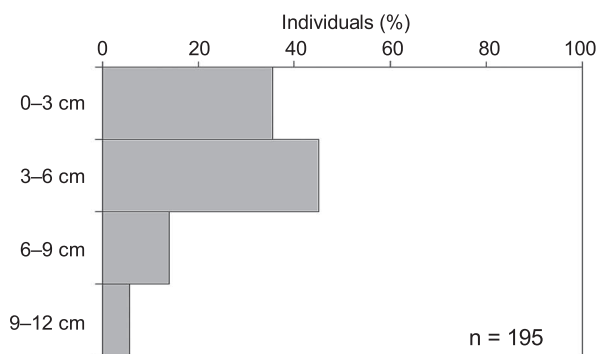

Figure 3. Vertical distribution of enchytraeids in the upper 12 cm of soil (four 3-cm layers extracted) at the Overlook Trail site (hemlock-sugar maple stand).

were assigned to a hitherto undescribed *Marionina* species (*Marionina* sp. 1) similar to *M. minutissima* Healy, 1975. All other taxa were even less abundant: 8 specimens of *Bryodrilus* sp., probably *B. ehlersi* Ude, 1892, two specimens of *Enchytraeus norvegicus* Abrahamsen, 1969, another three *Enchytraeus* juveniles possibly representing another species (counted above as a separate taxon; see taxonomic comments below) and a single, immature specimen of *Mesenchytraeus* sp. (see taxonomic comments) were present.

At the Overlook Trail site, mean enchytraeid density was 35,900 ± 7,300 ind./m² (± SE; rounded to the nearest hundred). There were more species (8) and these were more evenly represented in the assemblage than at Summit Peak (Fig. 5). The genus *Marionina* was most dominant, comprising 63 % of all individuals and represented by 1 or 2 species of small body size. A high proportion of specimens died soon after extraction and started to decompose, so that the two similar species potentially present could often not be discriminated. By far more dominant was a species initially identified as *Marionina minutissima* (20 % of all enchytraeids were identified as this species, whereas the occurrence of the similar *Marionina* sp. 1, found at the Summit Peak site, could neither be confirmed nor excluded). However, differences in observed characters in comparison with the species description (Healy 1975) led to some doubt about the true species identity – see taxonomic remarks below. *Cognettia sphagnetorum* accounted for one fourth of the assemblage (26 %), and also *C. glandulosa* (Michaelsen, 1888) was present (4 %; another 4 % of *Cognettia* individuals could not be discriminated). The genus *Mesenchytraeus* was represented by four juveniles and one adult, its identity was not ascertained. Another two genera – *Bryodrilus* and *Fridericia* – were represented by single juveniles.

3.1. Taxonomic remarks

Segments are given in Roman numerals. Chaetal formulae often refer to single worms and Arabic numerals put in brackets indicate occurrence of the given number of chaetae in single or very few bundles only; the dash indicating segment XII, i.e. the position of the clitellum, is put in brackets when chaetae were present in XII in the respective position (laterally or ventrally); Roman numerals (and their ranges) given in brackets behind individual Arabic numerals show in which segments the corresponding numbers of chaetae were present.
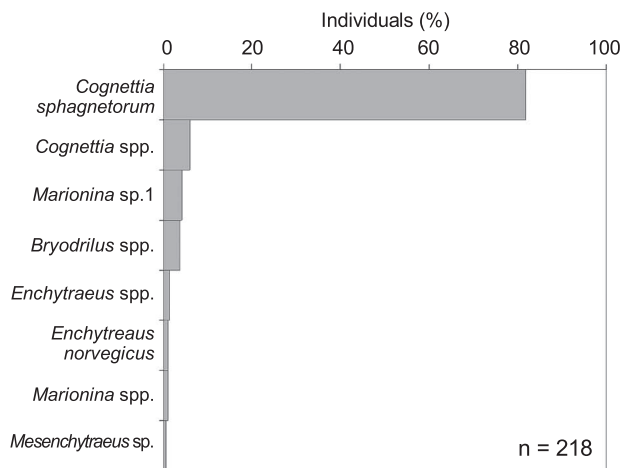
Individuals (%)

Figure 4. Dominance of enchytraeid taxa at the Summit Peak site (sugar maple stand).
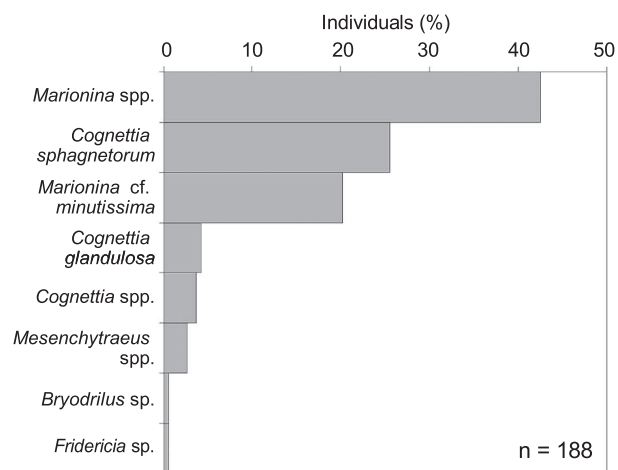
Individuals (%)

Figure 5. Dominance of enchytraeid taxa at the Overlook Trail site (hemlock-sugar maple stand).

Bryodrilus cf. *ehlersi* Ude, 1892

In total 9 specimens, mostly juvenile, only one at the Overlook Trail site. Probably only one species. Largest observed specimen (subadult, sexual organs not fully developed) ca. 11 mm long (live), with 48 segments. Dorsal vessel originating at IX/X; intestine widening at VII/VIII; dorsal and ventral pair of oesophageal appendages in VI, without lumen (or lumen very small); 7 pairs of preclitellar nephridia at IV/V–X/XI (only 6 at IV/VI–IX/XI in one juvenile); posterior margin of brain incised; 3 pairs of dorsally separate pharyngeal glands, with ventral lobes stretching anteriad; coelomocytes discoid, small (in vivo ca 12–15 µm in diameter); chaetae sigmoid, without nodulus, arrangement within bundle resembles an asymmetric fan; formula: 2, 3, (1) – 2, 3, 4 : (3), 4, 5, 6 – 3, 4, 5, 6 (largest two specimens, neither fully mature, had 1 or 2 chaetae dorso-laterally in XII).

Identity with *B. novaescotiae* Bell, 1962 was excluded foremost due to the position of the first nephridia, because the spermatheca was not fully developed in the single subadult specimen available in the present study: in *B. novaescotiae* nephridia start at V/VI according to Bell (1962) and at VI/VII according to Healy (1996). Taking into account the low number of specimens, none of which was fully mature, the observed characters are in fair agreement with the redescription of *B. ehlersi* by Cech et al. (2012), however, there were some differences (in parentheses characters according to these authors): posterior margin of brain incised (straight or slightly rounded), oesophageal appendages almost totally lacking a lumen (small lumen), slightly smaller coelomocytes (15–30 µm in vivo).

Cognettia glandulosa (Michaelsen, 1888)

Only 8 juvenile specimens (including some fragments of more or less advanced regeneration), without any traces of sexual organs; largest specimen 16 mm long (live) with 52 segments; primary and secondary pharyngeal glands (the latter ventral, arranged posteriad to the dorsal ones), mostly 4 pairs of primary pairs (dorsally separate), in some specimens only 3, in some 5 (the last pair usually small, sometimes only a single gland present unilaterally), usually 2–3 pairs of secondary pharyngeal glands posteriad of the primary ones (i.e. in V–VII), but in the largest specimen a full set of 5 primary and 5 secondary pharyngeal gland pairs present; first preclitellar nephridia usually at IX/X; chaetae: preclitellar lateral bundles usually with 2 (often in II and from V backwards) or 3 (often in III-IV) chaetae, elsewhere 3 chaetae per bundle. Although no identification based on sexually mature specimens was possible (these are always exceedingly rare in this species), based on morphology I have no doubt about the identity of the collected specimens as the combination of characters is unique for this species: anterior chaetal bundles of 2 and 3 chaetae, high but variable number of pharyngeal glands with distinct secondary ones, first nephridia far back (the latter two characters are typical of species reproducing by fragmentation). Three whole-mounted specimens are in the author's collection.

Cognettia sphagnetorum (Vejdovský, 1878)

In total, 180 juvenile specimens (including fragments of more or less advanced regeneration); largest specimen 14 mm long (live); maximal segment number 46; lateral chaetal bundles with only 2 chaetae in anterior segments

at least up to VI, in some specimens as far back as X and XI, elsewhere 3 chaetae per bundle; pharyngeal glands dorsally not merged, only primary ones, mostly 4 pairs, in some specimens only 3, in some 5 (the last pair usually smaller, sometimes only a single gland present unilaterally); coelomocytes not granulate; first preclitellar nephridia usually at VIII/IX, in a few cases at VII/VIII or IX/X. All observed specimens resembled one specific form of the species, i.e. type A according to Chalupský (1992) – see also Schmelz & Collado (2010). Thus, although no identification based on sexually mature specimens was possible, I have no doubt about the identity of the collected specimens. Five whole-mounted specimens and a number of specimens stored in 99 % ethanol are in the author's collection.

Enchytraeus norvegicus Abrahamsen, 1969

Two specimens only, one adult (3.5 mm long, 25 segments) and one subadult; numerous coelomocytes full of refractile granules, dark in transmitted light; 3 pairs preclitellar nephridia at VII/VIII–IX/X; chaetae: 2 (–) 2, 3 : 3, (2) – 3; spermatheca with small glands covering short portion of ectal duct near its orifice; small testis sac; small sperm funnel (37 μm long), body width 210 μm under slight pressure of the cover slip.

Enchytraeus spp.

Three specimens. Two juveniles with coelomocytes with rather few refractile granules, thus less dark in transmitted light than in third specimen; chaetae: 2 (–) 2, 3 : 3, 2 – 3, 2; 4 pairs of preclitellar nephridia at VI/VII–IX/X (therefore most probably not *E. norvegicus*); such specimens would be usually assigned to *E. buchholzi* Vejdovský, 1879 (species complex – see Schmelz & Collado 2010). Third, larger specimen (23 segments, ca. 4.5 mm) with very dark coelomocytes in transmitted light; chaetae: 2 (–) 2, 3 : 3 – 3; accidentally destroyed at the beginning of its examination, possibly *E. norvegicus*.

Fridericia sp.

One juvenile specimen (ca. 2 mm long, 18 segments); two straight, rather slender chaetae per bundle throughout; oesophageal appendages rather short, unbranched; coelomo-mucocytes elongate, with few scattered refractile granules, coelomo-lenticytes tiny; 5 preclitellar pairs of nephridia at VI/VII–X/XI.

Marionina cf. *minutissima* Healy, 1875 (Fig. 6)

38 specimens at the Outlook Trail site, in another 80 specimens dead shortly after extraction the chaetal arrangement was not always clearly visible but probably they belonged to the same species (some possibly to the similar *Marionina* sp. 1 – see below). Three stained whole-mounts of specimens originally fixed in hot 4 % formaldehyde solution (1 adult with egg, 1 (sub)adult without egg, 1 juvenile) and several specimens in pure ethanol are in the author's collection. The character description below is complemented by a comparison with characters described for *M. minutissima* given in square brackets behind the corresponding character descriptions; 'H:' stands for Healy (1975).

Description based on live observation of three adult specimens (2.2–2.5 mm long, with 20–25 segments, one yolky egg occupying up to 3 segments) and 8 subadult specimens (1.5–2.8 mm long, 21–25 segments) and observation of the three whole-mounted specimens (which were part of the set of live specimens observed; adult 1.45 mm, subadult 1.43 mm long). Chaetae straight, thin and pointed, with ental hooks (Fig. 6E); laterally in II 12 μm, in IV 16 μm, ventrally in VII and VIII 18 μm long, measured in whole-mounted specimens [H: length about 12 μm]; chaetal formula: 2 (II–V), 0 – 0 : 2 – 2, in a few cases lateral chaetae up to IV or VI; another subadult specimen (22 segments) with irregularities of chaetal arrangement: 1 (II), 0 (III), 1 (IV–V, unilaterally), 2 (V, unilat.), 0 – 0 : 0 (II), 1 (III, unilat.–IV), 2 (III, unilat., V–XI) – 2. Body width in XII: 72–110 μm (live; measured in three non-depressed, large juveniles), 103 μm (measured in one whole-mounted adult with elevated clitellum). Body width in XI (measured in whole-mounted adult): 82 μm. Brain with rounded or straight posterior margin, possibly with small concave stretch in the middle; according to free hand drawing (Fig. 6D) ca 2.2× longer than wide, measured in whole-mounted specimen only 1.7× longer (length 53 μm) [H: 'Brain about 2½ times longer than broad and incised posteriorly']; In all specimens (as far as recorded): 3 pairs of pharyngeal glands, first two dorsally merged, third dorsally separate, elongate, second and third pair with ventral lobes in anterior position [H: 'three pairs...each is divided into four lobes']; 2 pairs of preclitellar nephridia at VII/VIII and VIII/IX; not incised at septum, anteseptale rather large, with parts of nephridial body, terminal position of efferent duct (anterior nephridia observed) [H: 'efferent duct arising postero-ventrally in the anterior region, terminally in posterior segments']; orifice small, in some specimens a small dilatation of the efferent duct close to its orifice observed (Fig. 6C); coelomocytes small, oval, pale with fine grizzle, somewhat smaller than (ventral?)
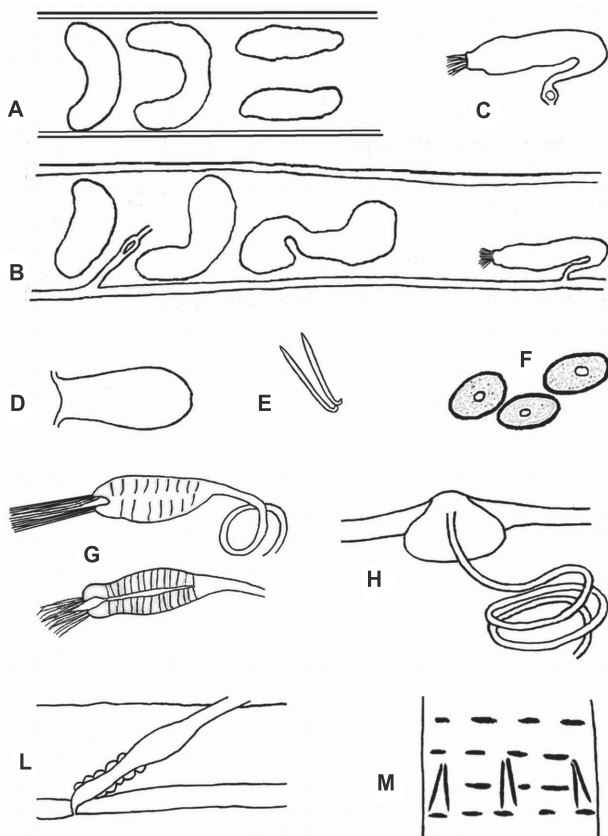
Figure 6. Drawings (free hand, based on live observation) of characters of *Marionina* cf. *minutissima*: **A** – pharyngeal (= septal) glands in dorsal view, **B** – pharyngeal glands and first nephridium in lateral view, **C** – nephridium (preclitellar), **D** – brain, **E** – chaetal bundle, **F** – coelomocytes, **G** – spermfunnel (based on two different observations), **H** – male copulatory organ and seminal duct in lateral view, **L** – spermatheca, **M** – arrangement of cutaneous glands and chaetae in an anterior segment in latero-ventral view.

chaetae [H: 'oval, coarsely granular, approximately equal to the length of the setae']. In one specimen several (ca. 4) transverse rows of elongated cutaneous glands per segment in anterior segments conspicuous (Fig. 6M), in most specimens not discernible [H: 'Four or five rows of elongated, yellow-brown cutaneous glands on each segment, very conspicuous in most specimens although in some individuals they may be scarcely visible.']. Chloragogenous tissue from VI, dense from VII (only recorded in few specimens). Oesophagus widening into intestine at XI/XII. Origin of dorsal vessel not always recorded (many specimens were dead upon examination), observed from X to XIII, in a fixed and mounted adult specimen visible in XIII [H: 'Dorsal vessel originating in XIII.']. In (sub)adult specimens: spermatheca with rather indistinct ampulla about mid-way between ectal orifice and oesophagus, communicates separately with the oesophagus [in agreement with H: 'The ampulla of the spermatheca is oval and thick-walled, not sharply distinguished from the ectal duct...Spermathecae

communicate separately with the oesophagus'], no ectal gland, but small glands on ectal duct from orifice to ampulla noted in live specimens (Fig. 6L), however, in one of the whole-mounted ones, in which the spermathecae are well visible, one or two small glands at the ectal orifice, whereas the outline of the ectal duct appears otherwise almost smooth [H: 'Ectal duct stout with a narrow canal and without gland at the orifice'; Healy's drawing –Fig. 5 H– can be interpreted as depicting small glands along the entire ectal duct]; sperm funnel elongate (length 28 μm in whole-mounted specimen), length : width ≈ 2 : 1, with not very distinct, somewhat narrower collar [H: 'Sperm funnel two or three times longer than wide with a narrow collar about half the width of the funnel']; male copulatory organ small and compact (Fig. 6H) [H: 'Penial bulb small and compact'].

Despite a number of differences in comparison with the species description of *M. minutissima* (shape of brain, ventral lobes of first pair of pharyngeal glands, length of chaetae, position of efferent duct of preclitellar nephridia, possibly origin of dorsal vessel and ectal glands of spermatheca), I hesitate to describe a new species based on them. The overall impression and many characters are so much in agreement (foremost the body size and segment number, chaetal shape and formula, cutaneous glands –when visible– and the male copulatory organ) that I tend to attribute the observed differences to erroneous observation either of Healy (1975) or of myself (as far as notes from live observations are concerned). To some extent they might also present intraspecific variation at the population level, as the species was described from Ireland and thus the populations are geographically very far apart. It has to be noted that the type locality is a grassland with a soil pH of 4.9. A comparison with the type material or topotypes of *M. minutissima* and, finally, a molecular analysis of specimens from both populations would be required to reach a final decision about the true species identity of the population in the Porcupine Mountains.

Marionina sp. 1

In total 9 specimens at the Summit Peak site. (Sub) adult specimens ca 4 mm long, with 22–25 segments; chaetae: 0 (II), 2 (III–V or VI), 0 – 0 : 2 – 2; dorsal vessel originating in XII or XIII, with anterior bifurcation in I ('prostomial'), oval coelomocytes pale or with light grizzle; 2 pairs preclitellar nephridia in two adjacent segments between VI/VII and VIII/IX; posterior margin of brain rounded; intestine gradually widening in XI; three pairs of pharyngeal glands with anteriad ventral lobes, first two dorsally merged, third elongate; sperm

funnel small, ca 50 µm long), body width 149 µm in XI. I found the species also earlier at study sites in northern Minnesota and Wisconsin; the species will be described as new to science in a separate paper.

Mesenchytraeus sp.

In total 6 specimens, one of which at the Summit Peak site. Three specimens preserved in pure ethanol in the author's collection.

One adult (yolky egg in XIV–XVI): 13 mm long, 39 segments, body wide; chaetae sigmoid with nodulus, none of extraordinary size, formula: 2, 3 – 3, 4, (5), 3, 2 : 4, 5, 6 – 4, 5, 6, 7, (3, 2); pharyngeal glands: two pairs of primary glands at IV/V and V/VI, two pairs secondary ones in VI and VII; small coelomocytes of typical shape (broad spindle), with refractile granules, dark in transmitted light; preclitellar nephridia from VII/VIII; brain short, posterior margin convex, slightly incised; 2–4 transverse rows (1–2 well pronounced) of cutaneous glands per segment in anterior body part; spermatheca with 1–2 (?) diverticula with wide lumen, connected to oesophagus, no glands at ectal duct observed.

The single juvenile/subadult from the Summit Peak site (3.8 mm, 30 segments, of whitish, inconspicuous colouration) had spermathecae (which seem to develop very early in *Mesenchytraeus*), but these were probably not fully developed (wide lumen, communicating with oesophagus, no diverticula). Pharyngeal glands similar to above specimen, but with three pairs of secondary glands in VI, VII and VIII; chaetae sigmoid with nodulus: 2 (II–X), 1 – 1, (2) : 2 (II), 3 (III–VII), 2 (from VIII); coelomocytes of the shape of a broad spindle, matrix with fine grizzle; 5 pairs preclitellar nephridia at VI/VII–X/XI; dorsal vessel originating in XI; spermathecae with wide lumen, without diverticula, separately communicating with oesophagus, most probably not fully developed.

The four juveniles from the Outlook Trail site were 3–3.2 mm long, had 30–31 segments and coelomocytes of typical shape as above, with some large refractile granules. Two pairs of primary pharyngeal glands were present at IV/V and V/VI, secondary ones either only in VI or in V and VI (in two specimens). Chaetal formula: 2 – 2 : 3 – 3; brain short, posterior margin convex; preclitellar nephridia in VI/VII–X/XI.

Based on the observed characters and the principle of parsimony, it is assumed that all specimens belonged to a single species. The single mature specimen was insufficient to decide on its identity. Species of excessive size, having giant chaetae, spermathecae not attached to the oesophagus or lacking diverticula, can be excluded. The combination of two primary and two secondary pairs

of pharyngeal glands as found in the adult specimen in the above-given positions, a higher number of chaetae per bundle ventrally than dorsally (with no giant chaetae present) and a spermatheca with at least one diverticulum did not match any species known from Europe (Schmelz & Collado 2010). The more variable position of pharyngeal glands as observed in the juveniles could indicate three other species known from Europe with a similar pattern, but in all cases some other characters are not in agreement: *M. straminicolus* Rota, 1995 has coelomocytes with pale vesicles and many more chaetae in the ventral bundles then observed in the present specimens, its dorsal vessel originates in XVI (not in XIV as in the adult above) and first nephridia are located at VI/VII (as in all juveniles above, but not in the adult); *M. beumeri* (Michaelsen, 1886), has ellipsoid coleomocytes with few colourless inclusions only and its adults should have at least 55 segments and 20 mm in length, the dorsal vessel should originate in XVI–XX, nephridia should start at VI/VII; adults of *M. viivi* Timm, 1978 should have at least 59 segments and ventrally up to 9 chaetae per bundle, its dorsal vessel should start in XVI–XVII, nephridia at VI/VII (information on coelomocytes not available). In the present specimens, the arrangement of pharyngeal glands was the same as in specimens of *Mesenchytraeus* sp. found by Healy (1996) in hardwood forests of Florida. Also their spermetheca with two diverticula could match the observed state in the present adult specimen. The Florida specimens, said to resemble *M. beumeri*, were immature and did not allow identification or the description of a new species (Healy 1996). Also in *Mesenchytraeus argentatus* Nurminen, 1973, described from terrestrial and freshwater habitats in the Canadian Arctic archipelago and also reported from the vicinity of Montreal, pharyngeal glands show such an arrangement, but this seems to be a somewhat smaller species and – more importantly – does not have spermathecal diverticula (Nurminen 1973a, 1973b). Neither yellowish colouration as reported in the specimens from Florida, nor an intense white one (in reflected light) as in *M. argentatus* was noted in the specimens from the Porcupine Mountains. Similar to *M. argentatus* is *M. torbeni* Christensen & Dózsa-Farkas, 1999, recorded along the arctic coast of Siberia, which has one spermathecal diverticulum (Christensen & Dózsa-Farkas 1999).

4. Discussion

At two other forest sites (six distinct plots were sampled in total) also situated in the western Great Lakes area (Chequamegon section of the Chequamegon-Nicolet National Forest in northern Wisconsin and Chippewa

National Forest in northern Minnesota, see Fig. 1) and sampled more intensively, 22 and 15 enchytraeid species, respectively, were identified by the present author (Schlaghamerský et al. submitted). Compared to this, the species numbers found in the present study (6 and 8 per site) were very low. To some extent this might have been the consequence of the lower sampling intensity in the present study, but total numbers of identified specimens at the individual plots sampled in Minnesota and Wisconsin ranged from 207 to 462, with the lower values very similar to those in the present study, and most species were recovered even at the plots with low densities. These two sites had an average soil pH (H_2O) of 6.2 and 4.4, respectively, with the first value, belonging to the site with higher species richness, being substantially higher than the pH-values of the two sites sampled in the Porcupine Mountains. In general, enchytraeid species richness decreases with decreasing soil pH, whereas a few acidotolerant or even acidophilic species might develop large populations in very acidic soils (Didden 1993). Furthermore, the assemblages found at the Minnesota and Wisconsin sites had a more even representation of individual species: only in a single plot (at the Wisconsin site) did the percentage of a single species (identical with *Marionina* sp. 1 of the present study) exceed 50 %. The low species richness encountered in the Porcupine Mountains is thus not just due to low sampling intensity but reflects real differences, which are the consequence of differing site conditions. However, due to undersampling, the true species richness of the sites was most probably somewhat higher than shown by this study.

Hitherto, a single study from North America reporting species composition (albeit no percentage representations) for well-defined terrestrial sites has been published: in the Canadian Rocky Mountains, Dash & Cragg (1972) studied enchytraeids in an aspen-willow stand, a fen (with some pine and spruce) and a coniferous stand. Soils were of calcareous origin with a pH of 6.4–6.7. They found 7 species in each of the forest stands and 10 in the fen, which was also the total species number for the entire study area. There was absolutely no species overlap with the sites in the Porcupine Mountains, possibly except *E. buchholzi* (however, there was some overlap with the above-mentioned Wisconsin and Minnesota sites). Total enchytraeid densities ranged from ca 2,000 to ca 21,000 ind./m² in the aspen willow stand and from ca 500 to ca 9,000 ind./m² in the fen (only qualitative date for the coniferous forest available). The geographically closest species-level data about enchytraeids were published by Nurminen (1973a) from Montreal and its vicinity (Quebec, Canada), ca 1300 km to the east of the Porcupine Mountains. However, he reported only finds of individual species at several collection sites (with very little information on these sites). Sampled sites included deciduous forests with sugar maple, ash and beech, where he found *C. glandulosa*, *Achaeta silvatica* Nurminen, 1973, *Fridericia bulbosa* (Rosa, 1887) – invalidated by Schmelz (2003) – and *F. paroniana* Issel, 1904. Additional species were found in mixed balsam fir-eastern white cedar forests: *Achaeta camerani* (Cognetti, 1899), *Buchholzia appendiculata*, *Enchytraeus buchholzi* Vejdovský, 1979, *E. minutus* Nielsen & Christensen, 1961 (now *E. christenseni* Dózsa-Farkas, 1992) and *Fridericia* cf. *caprensis* Bell, 1947 (now *F. pretoriana* Stephenson, 1930). A few other species were reported from non-forest habitats or forests for which explicitly mull was given as the humus form (which was apparently also true for at least some of the fir-cedar forest sites), *Bryodrilus ehlersi* was reported from a mixed cedar swamp (Nurminen 1973a).

For the lack of data on North American sites comparable in terms of vegetation, soil type and soil pH, some studies from Central European sites shall be used for comparison. In a study of montane beech-fir old growths, conducted by the present author with similarly low sampling intensity (6–12 soil cores of similar size per site), 2–11 species were found in the individual forest stands based on 37–153 identified specimens (Schlaghamerský 2012). Soil reaction was similar to the present study, with pH (H_2O) ranging from an extremely low value of 2.8 at the species-poorest site (based on 133 specimens, i.e. the second highest number of enchytraeids analysed per site; *Cognettia* spp. had an percentage representation of ca 94 %, mostly due to *C. sphagnetorum*) to 4.8 at the site with highest species richness (with the lowest enchytraeid density and thus based on 37 specimens only; *Cognettia* spp.: ca 13 %). Densities at a given sampling date ranged from 1,667 to 13,039 individuals/m² (they were rather low compared to those found in many other studies – see Didden (1993) – but enchytraeid densities are subject to large variation in the course of the year). A long-term study in a mature beech forest on oligotrophic Cambisol yielded 13 enchytraeid species (the most dominant species were *C. sphagnetorum* with 38.5 % and *Marionina clavata* with 36.6 %) and a mean density of ca. 46,000 ind./m² (Römbke 1989). In Podzol soil of a comparable soil pH under spruce, Schlaghamerský (2002) found up to 6 species per site and high dominances of two species, *C. sphagnetorum* and *M. clavata*, with mean annual enchytraeid densities of ca. 38,000–59,000 ind./m² at the three sites studied. In a more continental setting with lower precipitation levels, Makulec (1983) found 11 and 8 enchytraeid species, respectively, in an oak-hornbeam forest on Cambisol and a pine-oak forest on Podzol.

C. sphagnetorum made up for ca. 95 % of enchytraeid individuals in both forests. Mean enchytraeid densities were ca 21,000 and 18,000 ind./m^2, respectively. This comparison indicates that the enchytraeid assemblages encountered in the Porcupine Mountains are – under the given environmental conditions – not unusual in terms of species richness, community structure or total enchytraeid densities (for species composition see further below).

In terms of enchytraeid densities, also a few studies from other North American forests are available. In the rather mild and very humid climate of the southern Appalachians, van Vliet et al. (1995) found in montane stands of red oak and rhododendron (*Rhododendron maximum*) similar mean annual densities (ca 30,000 ind./m^2) as in the present study. Unfortunately, soil pH of the study sites is not given in their paper, and taxonomic information is limited to a list of genera present: *Achaeta*, *Bryodrilus*, *Cognettia*, *Fridericia*, *Marionina*, *Mesenchytraeus*, *Guaranidrilus* and *Hemienchytraeus*; the latter two are representative of warm climates, see Healy (1989). According to Knoepp et al. (2011), hardwood forests in this research area have a soil pH (CaCl$_2$) of 4.4 in the upper 10 cm (measured in H$_2$O it would be probably higher by ca. 0.5). Another study from North America, giving enchytraeid abundance data (but no taxonomic information) based on a reliable extraction method, reports on black spruce (*Picea mariana*) stands with patches of trembling aspen (*Populus tremuloides*) on Grey Luvisols in Western Quebec, Canada (Laganière et al., 2009). Soil pH values were similar to those measured at the Porcupine Mountains sites, but enchytraeid densities were only studied in the FH-layer and much lower than in the present study: the single sampling in August yielded mean densities of only 865–962 ind./m^2 at the three sites studied.

An assemblage strongly dominated by *C. sphagnetorum*, as found at Summit Peak, is typical for many sites in Europe, but mostly of coniferous forests (Schlaghamerský, 2002). Also the presence of the other genera and species is in agreement with the high organic content of a rather acidic soil. For a long time *C. glandulosa*, a species typical of water-logged soil (Graefe & Schmelz 1999), had been the only member of the genus reported from North America. Its most frequent and abundant congener in Europe, *C. sphagnetorum*, seemed to be lacking (Nurminen, 1973a). Healy (1989, 1996) tentatively reported *C. sphagnetorum* from western Florida, not being entirely sure because she had only a few juvenile specimens to her disposal. Also in the present study, only juveniles and regenerating fragments were encountered, but this resembles the normal state at European sites because

this species reproduces predominantly asexually by fragmentation and adult specimens are very rare. The characters observed in a very high number of live specimens (and a few fixed ones) did not give any reason to doubt their species identity (moreover, all were in agreement with a single form of the species, see taxonomical remarks above). This is, thus, the first incontestable record of *C. sphagnetorum* from North America. Furthermore, the situation of the site does not invite speculation about a potential introduction of *C. sphagnetorum*. It is worth mentioning that only *C. glandulosa* was found at the sites investigated by the present author in northern Wisconsin and Minnesota (Schlaghamerský et al. submitted), in a prairie soil in Nebraska (Schlaghamerský 2013, this volume). In the Porcupine Mountains, however, it was much rarer than *C. sphagnetorum* at the Overlook Trail site and not found at the Summit Peak site at all.

What is somewhat surprising is the lack of the genus *Achaeta* at the Porcupine Mountain sites. The genus seems to be generally rare in North America, but was found for instance in the above-mentioned site in northern Wisconsin (Schlaghamerský et al. submitted) as well as (several species) in the environs of Montreal (Nurminen 1973a).

The record of *Marionina minutissima* is the first for North America, however, as explained above, the possibility that the encountered population is not conspecific with the European one cannot be fully excluded. A high dominance of a small-bodied *Marionina* species in acidic soils such as the one found at the Overlook Trail site has been also found at some European sites (see Schlaghamerský 2002). Despite the outlined similarities, North American enchytraeid assemblages of forest sites are by no means identical with European ones. The study shows that there is still much to learn about North American enchytraeid fauna.

5. Acknowledgements

not have been possible without the continuing support of my Czech employer, Masaryk University (Faculty of Science) and my home department.

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