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Astacopsidrilus hibernicus sp. nov. (Phreodrilidae, Oligochaeta, Annelida) from Irish peatlands

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Abstract

The discovery of a large and flourishing population of Phreodrilidae in terrestrial peatlands in northwest Ireland was surprising on two counts: these oligochaete worms are usually aquatic and most of the species occur in the Southern Hemisphere. The phreodrilids were discovered in a project that targeted Enchytraeidae, therefore methods adapted to the investigation of enchytraeids could be applied, including the study of living animals and properly fixed whole mounts. DNA sequencing was also performed. All worms identified here belong to one species, new to science, and placed in the genus *Astacopsidrilus*, because of the ventral position of the spermathecal pores and the opening of the female funnels inside the spermathecal vestibule. *Astacopsidrilus hibernicus* **sp. nov.** is mainly distinguished by thick segmental cushions of epidermal gland cells on the dorsal side of the posterior body half. Male sexual organs and spermathecae are comparatively small and without the often-observed bizarre modifications common in species of this family. DNA sequencing yielded a fragment of the 16S rRNA gene. This is the first description of a phreodrilid species from Europe; the few previous recordings of this family in Ireland and the United Kingdom had been left unidentified.

Keywords: Clitellata, Irish fauna, taxonomy, Enchytraeidae

Introduction

Phreodrilidae are a small family of aquatic oligochaetes mainly distributed in the Southern Hemisphere; southern Australia and New Zealand harbour most of the species. The global distribution, which includes South America, Africa, the Arabian Peninsula, Sri Lanka, and subantarctic islands, indicates a Gondwanan origin of the family (Martin *et al.* 2008). Phreodrilids are mainly cool-adapted freshwater species; when recorded from warmer environments, their habitat is groundwater (Martínez-Ansemil *et al.* 2002; Pinder 2008). There are also records from acid (dystrophic) peatland waters (Pinder & Brinkhurst 1997), from brackish water (Pinder & Erséus 2000), and from beech forest litter (Stout 1958)—the latter being the only record thus far documenting the presence of phreodrilids in a terrestrial habitat. Records of this family outside the former Gondwana landmass are very scarce, and they are based on a very limited number of specimens: *Insulodrilus ryuteki* Martin & Ohtaka, 2008 from the profundal of Lake Biwa, Japan (one specimen); *Insulodrilus* sp. from the Thames estuary in London, U.K. (Pinder *et al.* 2013) (eight specimens), and one unidentified phreodrilid specimen from a small stream in Northern Ireland (Gunn *et al.* 2003); all specimens were amputees, i.e. the posterior end of each specimen was missing.

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Schmelz *et al.* (2015) reported an unexpectedly large and flourishing population of Phreodrilidae in terrestrial peatlands in northwest Ireland. More than 100 specimens of phreodrilids were sampled in the framework of the project *BOGLAND: Sustainable Management of Peatlands in Ireland* (Renou-Wilson *et al.* 2011), which included the study of invertebrate taxa (Wisdom 2010) at intact, damaged and restored peatland sites. Among the targeted groups, which included mites, beetles and spiders, were Enchytraeidae, which are abundant and dominant in peatlands not only in Ireland (Healy 1979; Healy & Bolger 1984). Enchytraeids are obtained for study by extracting them from soil samples in the laboratory, and they are then identified *in vivo* because the taxonomically important structures, mostly soft-bodied internal organs, are best seen in living animals. Suitable specimens may later be fixed in Bouin's fluid, stained, and whole-mounted for more detailed investigation (see Schmelz & Collado 2010 for details). This procedure was also applied to the phreodrilids found in the samples, which allowed an investigation in greater detail than would have been possible with ethanol-preserved specimens. All specimens belonged to one species, which is described as new to science in this paper. It was first considered to belong to *Insulodrilus* Brinkhurst, 1965 (Schmelz *et al.* 2015); its placement here in *Astacopsidrilus* Goddard, 1909 is explained further below.

Material and methods

Sampling began in October 2008 with a preliminary screening of the oligochaete fauna at three different peatland sites and was extended in May 2009 to a systematic sampling of 11 different peatland sites (Fig. 1A). The sites were located in fens, raised bogs, and blanket bogs. Sampling at each site was limited to the upper 5 cm of soil. Extraction, investigation and identification of worms in each sample was completed at the University of A Coruña, Science Faculty. Worms were extracted from soil samples using a modified version of O'Connor's (1967) wet/heat funnel device, then stored in water for a maximum of 3 days. Specimens were then studied *in vivo* and as stained whole mounts using dissecting microscope and light microscope with differential interference contrast (Nomarski) optics, following protocols as outlined in Schmelz and Collado (2010).

The dominant species in all samples was *Cognettia sphagnetorum* (Vejdovský, 1878). However, this species was recently split into several species based on DNA-sequences, of which one has retained the name *sphagnetorum* (Martinsson & Erséus 2015, there as *Chamaedrilus sphagnetorum*). It is most probably this species that was present in the peatland samples; it is congruent with the morphological "variant B" in Chalupský (1992) and Schmelz and Collado (2010: 81f.)—a form that prefers moist to wet habitats. Phreodrilids were found in large numbers in samples collected from two sites, Knockmoyle and Owenanirragh. Knockmoyle, the type locality for *Astacopsidrilus hibernicus* **sp. nov.**, is a state-owned site at Bellacorick, Co. Mayo, 1,198 hectares in area (Fig. 1B). It has extensive pool systems, flushes, shrub and grassland communities, dominated by the fen sedges *Schoenus nigricans*, *Eriophorum angustifolium* and *Rhynchospora fusca* (Cyperaceae). Owenanirragh (Fig. 1C) is an isolated area of Atlantic blanket bog, located 6 km NE of Glenamoy, Co. Mayo—approx.14 km NNW of the type locality. The dominant plant species is *Schoenus nigricans*. Restored sites, dominated by *Molinia caerulea*, *Calluna* sp. and *Rubus* sp., are present as well, although these sites were not sampled for oligochaetes.

DNA sequencing was carried out at the Department of Biological and Environmental Sciences, University of Gothenburg, Sweden. DNA was extracted from amputated posterior ends of four specimens, using QuickExtract DNA Extraction Solution 1.0 (Epicentre, Madison, WI, USA). The extracts were then used to run PCR for Cytochrome C Oxidase C subunit I (COI) (following Folmer *et al.* 1994), and a shorter part of 16S using the primers Ann16SF and Ann16SR following Sjölin *et al.* (2005). Sequencing was conducted by MWG Eurofins Operon (Edersberg, Germany), sequences were assembled in Genious 6.1.8 (https://www.geneious.com, Kearse *et al.* 2012), and deposited in GenBank.

In the description, *ectal* and *ental* parts of a structure are oriented towards the periphery and the center of the body, respectively. Type material is deposited at the British Museum of Natural History (NHMUK ANEA) and at the National Museum of Ireland (NMINH). Further reference material is in the first author's personal collection.

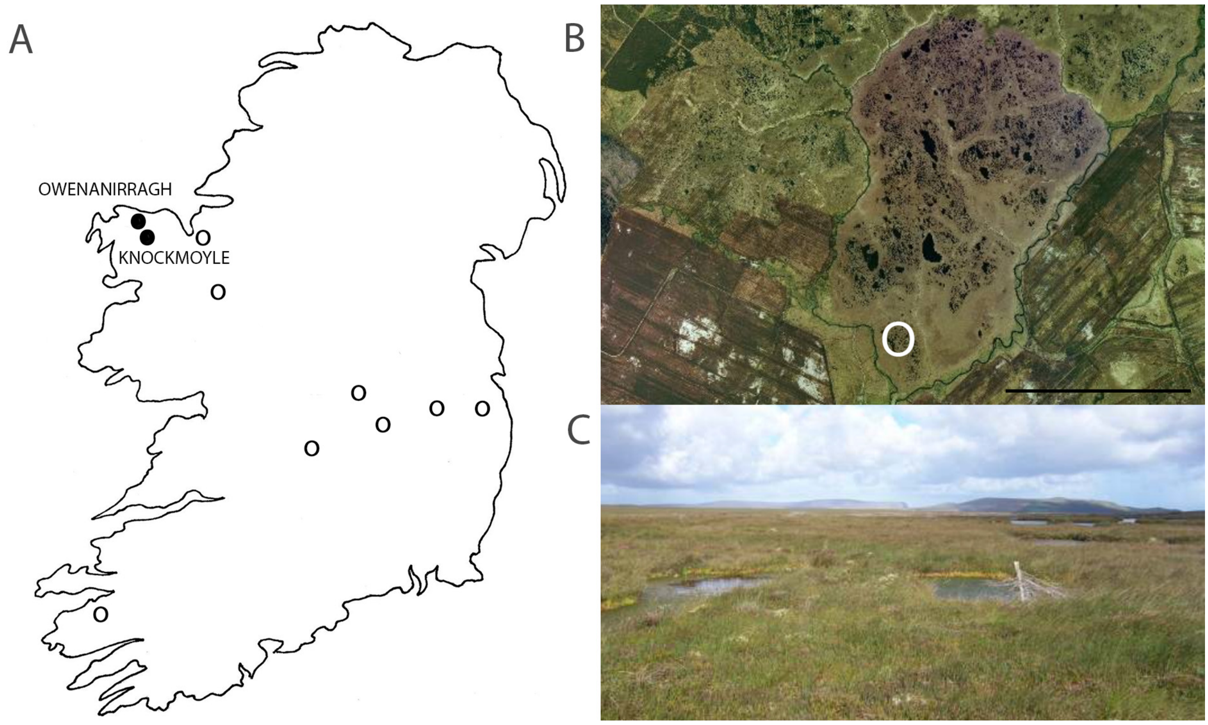

FIGURE 1. *Astacopsidrilus hibernicus* **sp. nov.**, sampling localities. **A.** Map of Ireland with localities of bogs and fens sampled for invertebrates in the BOGLAND project. Filled circles: Sites with *Astacopsidrilus hibernicus* **sp. nov.** The two sites are 14 km apart. The third site where this species was found (Bellacorick, 1 specimen) is too close to Knockmoyle, the type locality, to be indicated separately. Open circles: Sites without that species. **B.** Aerial photograph of the type locality, the Atlantic blanket bog at Knockmoyle, Co. Mayo. White circle: Sampling site. Scale bar: 1 km. **C.** View of the site at Owenanirragh. **B**,**C** from Wisdom (2010).

Abbreviations used in Figures 2, 3

aa atrium apically (blind-ending), **ab** atrium basally (connecting with vas deferens, closer to male pore), **br** brain, **ch** chloragocytes, **cy** spermatogonial cysts in different stages of sperm development, **eg** epidermal gland cells, **ff** female funnel, **ga** ganglia on pharyngeal pad, **gc** clitellum, granulocytes, **hc** clitellum, hyalocytes, **im** intracoelomic dorso-ventral muscle strands, **ne** nephridium, **ov** ovary, **pe** penis, **pm** dorsoventral intra-prostomial musculature, **pp** pharnyngeal pad, **rm** retractor muscles of pharyngeal pad (Fig. 2A,B) or penis and spermathecal vestibule (Fig. 3A), **sa** spermathecal ampulla, **sf** sperm funnel, **st** spermathecal sperm trap, **sv** spermathecal vestibule, **te** testis, **vn** ventral nerve cord.

Results

Astacopsidrilus hibernicus Schmelz, sp. nov.

(Figures 2, 3)

Holotype. NHMUK ANEA 2019.7340, adult specimen, fixed in hot Bouin's fluid, stained with Paracarmin, whole-mounted in Canada-Balsam between two coverslips. Type locality: Ireland, Knockmoyle, Co. Mayo, 54°09'N, 9°34'W. Habitat: Atlantic blanket bog. Soil sample taken by R. Wisdom, Oct. 2008.

Paratypes. NHMUK ANEA 2019.7341–7346, 6 specimens (4 adult, 2 juvenile), same data as holotype. NMINH 2019.3.1–3.6, 6 specimens (4 adult, 2 juvenile), one per slide and accession number, same data as holotype.

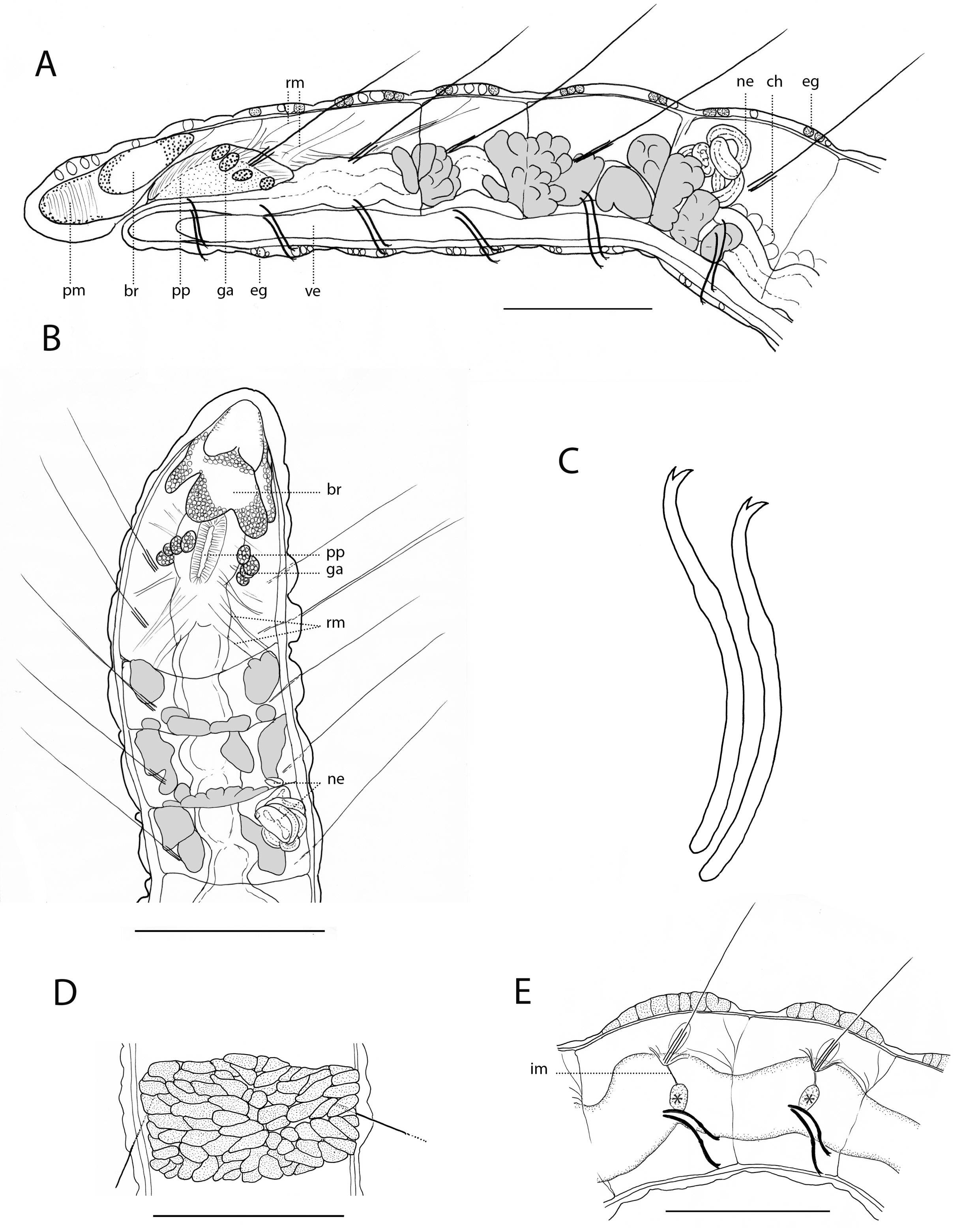

FIGURE 2. *Astacopsidrilus hibernicus* **sp. nov.**, drawings from stained whole mounts. **A.** Anterior body, lateral view. The nephridium in VII is on the right side, the nephrostome therefore hidden behind the pharyngeal glands (shaded grey). **B.** Anterior body, dorsal view. Circles in brain and pharyngeal ganglia represent perikarya, their size being approximately accurate. **C.** Ventral chaetae of VII. **D.** Dorsal cushion of epidermal gland cells in a posterior segment, dorsal view. **E.** Posterior body region, 2.5 segments, lateral view, with dorsal cushions of epidermal gland cells. Asterisks: parachaetal bodies. All scale bars: 100 µm.

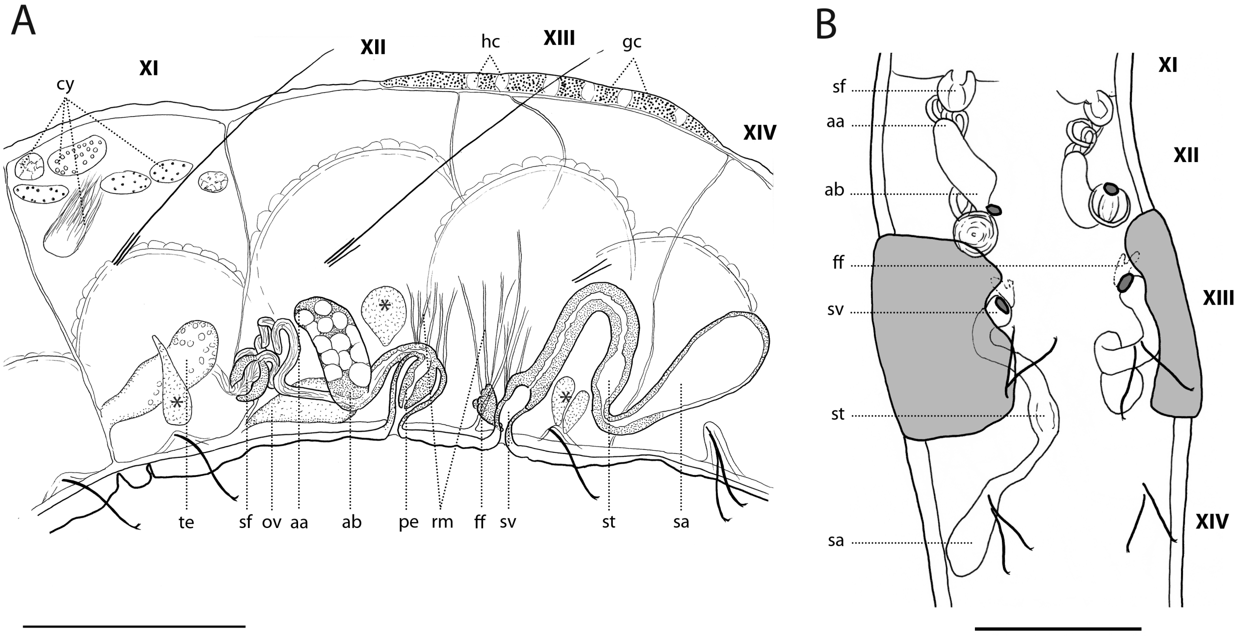

FIGURE 3. *Astacopsidrilus hibernicus* **sp. nov.**, gonadal and clitellar region (segments XI–XIV), drawings from stained whole mounts. **A.** Lateral view. The canal of the spermathecal ectal duct is less conspicuous than shown here. Asterisks: parachaetal bodies. **B.** Ventral view, semi-schematic. Shaded grey: clitellum. Dark-grey: male pores in XII and pores of spermathecal vestibules in XIII. Dotted lines: female funnels. All scale bars: 100 µm.

Additional material. 36 specimens (21 adult or subadult, 15 juvenile), stained whole mounts, same data as holotype.—15 specimens, adult, fixed and preserved in 70% ethanol, Owenanirragh, Co. Mayo, 54°16'N, 9°37'W, Atlantic blanket bog; soil samples taken by R. Wisdom, V 2009. Of these, four specimens processed for DNA sequencing, GenBank accession numbers of 16S rRNA gene sequence fragments MK837018, MK837019, MK837020, MK837021.—Three adult specimens, fixed in Bouin's fluid, preserved in 70% ethanol, Owenanirragh, same sampling data as above.—110 specimens, identified *in vivo*, not preserved, Owenanirragh, same sampling data as above.—One subadult specimen, investigated and identified *in vivo*, not preserved; Bellacorick flush, Co. Mayo, 53°11'N, 6°50'W, fen; soil sample taken by R. Wisdom, V 2009.

Etymology. "hibernicus" (Latin) = Irish.

Diagnosis. Small phreodrilid worms, live body length of adult specimens c. 3–4 mm; length in whole mounts 1.5–3 mm, diameter at V 0.12–0.15 mm, at XIII 0.17–0.23 mm, at XVIII 0.14–0.2 mm; 31–42 segments. Dorsal chaetae from III, one hair per bundle with a pair of support chaetae, hairs 120–180 µm long in IV–VIII, shorter in posterior segments. Ventral chaetae from II, two bifids per bundle with upper tooth half as long and half as thick as lower tooth. In adults, hairs absent in XIII and ventral chaetae in absent XII, present in XIII, not modified. Epidermal gland cells circumferal in anterior segments, forming thick dorsal cushions in posterior segments. Pharyngeal glands in (IV)V–VII. Nephridia unpaired, at VII and X in preclitellar segments, positions variable in postclitellar segments. Clitellum in 1/2 XII–XIII, saddle-shaped. Testes in XI, ovaries in XII. Developing sperm not abundant. Male efferent apparatus comparatively small, with spherical funnel at 11/12, vas deferens entering atrium basally, and short conical penis in XII. Male pores in line with ventral chaetae. Spermathecae comparatively small, confined to XIII or extending into adjacent segment, with ampulla, sperm trap and short vestibule, opening ventrally behind 12/13. Female funnel and duct plesiopore, opening into vestibule, close to body surface. One mature egg present at a time.

Description. Living worms transparent, habitus *Cernosvitoviella*-like. Live body length 3–4 mm, diameter 0.15–0.18 mm; clitellar region conspicuously widened in both living and preserved adult animals. Preserved adult worms 120–150 µm at V, 170–250 µm at XII, 140–180 µm at XVIII (N=19). Segments 30–42 in adults, fewer in juveniles (18–24 counted).

Dorsal chaetae from III, inserting latero-dorsally, above lateral lines; one hair (rarely 2) per bundle, each hair with a pair of minute and thin support chaetae entally, confined in the chaetal sacs. Hairs largest in anterior segments, maximum length 150–190 µm in IV–VIII, width at base c. 2 µm; hairs in posterior segments shorter and thinner, length c. 80–120 µm. Hairs continuously tapering ectad. Support chaetae always parallel to hairs, on opposite sides, confined to chaetal sacs, i.e. not protruding, c. 30–36 µm long. Ventral chaetae 2 per bundle, all alike, sigmoid with nodulus below ectal 1/3, bifid with upper tooth half as long and thick as lower tooth (Fig. 2C); measured lengths 45–52 µm in III–XI and in posterior segments, 30–40 µm in II and in mid-body segments posterior to clitellum. Rarely a supernumerary bundle with 1 or 2 extra-chaetae. Chaetae shifted backwards in a segment, i.e. nearer to posterior than to anterior septum. At sexual maturity ventral chaetae absent in XII, dorsal hair chaetae absent in XIII, but support chaetae still observed in XIII (Fig. 3A). No modified (spermathecal or penial) chaetae.

Prostomium rounded, slightly longer than wide *in vivo*, as long as or shorter than wide in fixed (contracted) specimens, here length c. 50–60 µm. Epithelium with smooth outer and inner surface, prostomial lumen with transverse dorso-ventral musculature at peripheries (Fig. 2A: pm). Proboscis absent. Body wall thin (3–8 µm), consisting of cuticle (visible at x400 magnification, thickness < 1 µm), epidermis, ring muscles (single-layered, continuous) and longitudinal muscles. Lateral lines slightly shifted dorsad. Epidermal gland cells of two types, staining and non-staining. Staining cells oval, hyaline, absent at intersegmental furrows; in anterior segments present dorsally, laterally and ventrally, most dense in anterior 7 segments, here in two segmental rows, one anterior, the other one posterior to the chaetae. Between the rows, at chaetal level, non-staining cells present (Fig. 2A: eg). From VIII on increasingly fewer cells, scattered over body surface, not in rows. In posterior segments, beginning from XV or further back, epidermal gland cells forming thick segmental cushions, conspicuous in living and preserved material; cells densely packed with non-staining vesicles or secretory bodies (Fig. 2D,E). Cushions increasing in size and thickness caudad. Body surface below cuticle and cells staining.

One or two segmental drop-shaped or ellipsoid bodies latero-ventral beneath body wall, extending from ventral chaetae dorsad, with smooth outline and non-staining vesicular content similar to the dorsal epidermal cushions; named here 'parachaetal bodies' (Figs. 2E, 3A). Thin intra-coelomic dorso-ventral muscle strands present, close to inner lateral sides of body wall, extending from ventral chaetae to dorsal chaetae; insertion dorsally below lateral line (Fig. 2E). Septa not closed, developed as dorso-ventral strands open dorsally and ventro-laterally. Coelom almost empty, coelomocytes probably absent, but some cells visible in coelom (detached chloragocytes?), free or attached to coelomic lining, transparent, with vesicles of irregular size and shape.

Brain (Fig. 2A,B: br) with four posterior lobes and three incisions; the outer lobes attached to dorsal side of circumoesophageal connectives. Ventral nerve cord medullar in I–VI, from VII on perikarya increasingly concentrated in mid-segmental region as ganglia.

Pharyngeal pad in 1/2I–1/2III to II–III, longer than wide, as a dome-shaped upfolding of the dorsal epithelium, with retractor muscles attached that extend as far back as into V (Fig. 2A,B: pp). Pharyngeal glands paired, in (IV),V–VII, as more or less compact, racemose aggregations of cells, shape, position and number of aggregations ("lobes") differing among specimens, attached to anterior and/or posterior sides of septa, or to body wall, or free (Fig. 2A,B). Four pairs of pea-shaped ganglia laterally on pharyngeal pad (Fig. 2A,B: ga), arranged in a line from antero-dorsal to postero-ventral, lined by retractor muscles. Pharynx and esophagus densely ciliated; ciliation abruptly less dense (cilia shorter?) at 1/2 VIII, here gut often widened abruptly and with thin walls. Intestinal walls thicker and with inclusions of unknown nature from XIV on.

Nephridia (Fig. 2A,B: ne) unpaired, with small ventro-lateral nephrostome attached to septum, a postseptal thickening and numerous subsequent densely arranged loops with almost no interstitial tissue; nephropore ventro-lateral anterior to ventral chaetae, terminal vesicle absent. Nephridial loops usually confined to the right or the left half of a segment, where the nephroporus is located, i.e. rarely extending into the opposite half, or into an adjacent segment. Location of anterior nephridia always in VII and X, juveniles included. Location of posterior nephridia variable: third nephridium in XVII in 11 out of 20 investigated specimens, further back in the rest (XX–XXVII); nephridia never in adjacent segments, never two nephridia in one segment. Altogether 4 to 8 nephridia in an individual.

Dorsal blood vessel running through all segments, closely attached to gut surface except in foremost segments (point of detachment not seen). One lateral commissure seen in III. Ventral blood vessel not attached to gut. Chloragocytes from VII.

Clitellum (Fig. 3A,B) conspicuous, elevated, saddle-shaped (= absent mid-ventrally), short, beginning immediately behind chaetae of XII, ending at level of septum 13/14. Total extension slightly less than 1.5 segment lengths. Cells in reticulate pattern, more granulocytes than hyalocytes (Fig. 3A: gr, hc). Gonadal region in the usual position, not shifted anteriad; all reproductive organs except clitellum paired.

Testes (Fig. 3A: te) paired in XI, club-shaped, widely separate, attached to posterior face of septum 10/11. Developing sperm not abundant, few cysts floating freely in coelom of XI (Fig. 3A: cy), or further anteriorly. No seminal vesicle. Sperm funnels (Fig. 3A,B: sf) attached to 11/12 ventro-laterally; each funnel an oval body with smooth outline (length c. 20 µm, diameter c. 15 µm), lumen with medial constriction. Vas deferens (Fig. 3A,B) ciliated throughout, densely and irregularly coiled immediately behind funnel, diameter 4 µm. Atrium (Fig. 3A,B: aa, ab) rounded, compact and with smooth outline, length c. 50–60 µm, 2–3x as long as wide, apically blind-ending, basally attached to distal stretch of vas deferens. Atrial walls muscular, atrial lumen densely filled with c. 20, roughly spherical, non-staining gland cells; no lumen distinguished in atrium. No gland cells at junction of atrium with vas deferens. Ectal stretch of vas deferens widened (c. 8 µm), shorter than length of atrium, attached to penis sac apically. Penis (Fig. 3A,B: pe) lemon-shaped, longer than wide, pointed distally, length c. 20 µm, spherical in top view (Fig. 3B), embedded in penial sac with walls 2–3 µm thick. Dorso-ventral retractor muscles inserting at ental roof of penis sacs (Fig. 3A: rm). Male pores in line with ventral chaetae, anterior to ventral clitellum (Fig. 3B).

Ovaries in XII (Fig. 3A: ov). Female funnels (Fig. 3A,B: ff) ventro-laterally at 12/13, adjacently anterior to spermathecal vestibules; female pores close to body surface but still inside vestibules, plesiopore. Spermathecae (Fig. 3A,B: sa, st, sv) simple, slightly longer than body diameter, confined to XIII or extending into XIV (rarely into XII), tube-like, with three thickened areas: vestibule ectally, sperm trap medially and ampulla entally. Vestibule not elongate or enlarged, c. 1.5x as long as wide (e.g. 30 µm by 18 µm), c. 1.5x as wide as duct, ciliated. Dorso-ventral retractor muscles inserting on and near anterior face of vestibule. Duct c. 80 µm long, with smooth outer and undulating, inconspicuous, inner surface, i.e. duct lumen indistinct. Sperm trap slightly wider than duct, with distinct lumen and flat walls, followed by club-shaped ampulla, diameter 20–60 µm. Few spermatozoa seen in sperm trap, ampulla filled with a hyaline yellowish mass, here spermatozoa not distinguished. One mature egg at a time, extending over 2–3 segments, posteriorly into XIV or XV.

DNA-barcoding. All four specimens were successfully sequenced for the shorter 16S fragment (GenBank) accession numbers MK837018, MK837019, MK837020, MK837021), but did not produce any successful COI sequences, possibly due to DNA degradation or primer mismatch. The 16S sequences could not be matched with any published material on GenBank. However, when compared with 16S sequences from Australian phreodrilids and some European Naididae (C. Erséus, unpublished data), the Irish species was clearly a member of Phreodrilidae, but with a genetic distance of about 20 % from the Australian specimens.

Discussion

Generic placement. Among the two subfamilies currently recognized in Phreodrilidae (Pinder & Brinkhurst 1997), the new species belongs to Phreodriloidinae, characterized by a ventral position of spermathecal pores (Brinkhurst 1991). (In the second subfamily, Phreodrilinae, spermathecal pores are located laterally or dorso-laterally.) Within this subfamily we place this species in *Astacopsidrilus* Goddard, 1909 because it is the only genus that includes species where the female pores are located inside the spermathecal vestibules and not separately on the body surface, as is usual in Oligochaeta. In this we follow Pinder & Brinkhurst (1997) who use this character to decide whether a species is to be placed in *Astacopsidrilus* or in the otherwise very similar *Insulodrilus* Brinkhurst, 1965. In a morpho-cladistic analysis (Brinkhurst 1991), the intra-vestibular position of the female pores qualified as an autapomorphy of a monophyletic *Astacopsidrilus*, whereas *Insulodrilus* remained paraphyletic. The two genera in their current circumscription are problematic and in need of revision (Pinder & Brinkhurst 1997; Pinder 2008). A main difficulty here is the fact that the key character to distinguish the genera—the location of the female pore—is unknown in many species; it is not even known in the type species of both genera. (The type species of *Insulodrilus* is *Phreodrilus lacustris* Benham, 1903, that of *Astacopsidrilus* is *Astacopsidrilus notablis* Goddard, 1909). Furthermore the distinction 'inside vs. outside the spermathecal vestibule' is a "character of degree" (Pinder 2008), and often quite subtle, especially when the female pores are located near the opening of the vestibule (compare Pinder & Brinkhurst 1997, Figs. 86, 90 for *Insulodrilus* with Pinder & Brinkhurst 1997, Figs. 149, 164 and Pinder 2003,

Fig. 2 for *Astacopsidrilus*). In the new species, however, its intravestibular position is beyond doubt, as there are only four pores on the body surface of the gonadal region, one pair of male pores and one pair of vestibular pores (Fig. 3B). It should also be mentioned that—comparing all available species descriptions—modified genital chaetae are absent more often in *Astacopsidrilus* than in *Insulodrilus*: we counted 6 out of 11 species, 55%, for *Astacopsidrilus*, and 3 out of 14 species, 21%, for *Insulodrilus*.

On the other hand, the moderate size of the spermathecal vestibule of the new species is in marked contrast to most species of *Astacopsidrilus*, where the vestibules are usually large, deep, and with strong muscular investment that connects them with the dorsal or dorso-lateral side of the body wall. Here, species of *Insulodrilus* are more similar. But this difference is not absolute, as species with large vestibules and strong musculature have been allocated in *Insulodrilus*, e.g., *I. tanganyikae* (Brinkhurst, 1970), while *Astacopsidrilus* includes also species with modest vestibules and poor musculature, e.g., *A. naceri* Giani *et al.*, 1995. Note that vestibular musculature is also present in the new species (Fig. 3A: rm).

[Linguistic side note: We prefer 'vestibules' over the—in our opinion—erroneous latinized plural 'vestibulae', which has become customary in the taxonomic literature on phreodrilids since Brinkhurst (1970). 'Vestibulae' is feminine, while the Latin noun is neuter: 'vestibulum', with the nominative plural 'vestibula' (correctly in Brinkhurst 1965)].

Species separation, character discussion. *Astacopsidrilus hibernicus* **sp. nov.** is among the smallest species of Phreodrilidae described to date. Only *Antarctodrilus micros* Pinder & Brinkhurst, 1997 is similarly small, with up to 34 segments and a recorded body size of 1.4–3.7 mm length and 0.14 mm diameter. This species differs from *A. hibernicus* in dorso-lateral spermathecal pores, sperm sacs extending over several segments, and a forward shift of the gonadal region, among other characters. The smallest species within *Astacopsidrilus* are those with an ectocommensal lifestyle on crayfish—*A. notabilis* Goddard, 1909 (53 segments, length 5.5. mm, width up to 0.65 mm), *A. fusiformis* Goddard, 1909 (46 segments, length 2.8 mm, width 0.6 mm) and *A. jamiesoni* Brinkhurst, 1991 (<50 segments, length up to 4.5 mm, width 0.6 mm). Conspicuous differences to *A. hibernicus* are stoutness of the body (or contractility, if the low length:width ratio is a fixation artefact), much reduced teeth of the ventral chaetae, and much longer atria. *Astacopsidrilus ryuteki* Martin & Ohtaka, 2008 is also small (length 3.1 mm, width up to 0.35 mm, 24 segments, one posteriorly incomplete specimen). Conspicuous differences of this species, found in the profundal of Lake Biwa, Japan, to *A. hibernicus* are presence of spermathecal chaetae, a very long atrium, and dorsal chaetae that resemble the ventral ones. The smallest species in *Insulodrilus* is *I. angela* Pinder, 2008 (c. 6 mm by up to 0.375 mm, segment number unknown). Conspicuous differences to *A. hibernicus* are a long and folded atrium, presence of spermathecal chaetae, and a vestibule with narrow, duct-like lumen.

The fragments of two hitherto unnamed phreodrilid taxa previously recorded from Europe are also very small, but clearly different from *A. hibernicus*. The single amputee (with the posterior body end missing), described in Gunn *et al.* (2003) from a small river in Northern Ireland, is 2.8 mm long, it has spermathecal chaetae and dorsal support chaetae with a flared distal tip. Eight amputees collected in an intertidal area of the upper Thames estuary in London (Pinder *et al.* 2013) are c. 3 mm long and up to 0.25 mm wide; subadult specimens have spermathecal chaetae and an elongate though small spermathecal vestibule. They resemble *Insulodrilus lacustris* (Benham, 1903) or related species (Pinder *et al.* 2013).

Astacopsidrilus hibernicus **sp. nov.** has sperm-related sexual organs (atria, penes, vestibules, spermathecae) that are smaller or shorter than in the other species of the family, and much less convoluted. For example, there is only one other species, *Antarctodrilus micros* Pinder & Brinkhurst, 1997, in which the atrium is no more than 3 times longer than the width, and even in this species it is so big that it occupies most of the segment. In fact, the gonadal region of the new species looks like the 'bauplan' from which all the other variations and sometimes bizarre modifications—at least in *Antarctodrilus*—can be derived. Likewise, the chaetal equipment, which is without peculiarities, can also be considered as the unmodified basic condition in Phreodrilidae. With respect to this 'classical' set of characters (i.e. those commonly considered in species descriptions: chaetae and reproductive organs), and viewed from a phylogenetic perspective, *A. hibernicus* seems to be characterized mainly by plesiomorphies. However, the opposite is possible as well, namely apomorphic reduction of structures.

The truly distinguishing character of this new species is the thick dorsal cushion of epidermal gland cells in posterior body segments (Fig. 2D,E). The two types of epidermal gland cells in the anterior body region are also noteworthy (Fig. 2A) as are the parachaetal bodies between the dorsal and ventral chaetae, attached to the inner face of the body wall (Figs. 2E, 3A). Unfortunately, we have not found any further consideration of these or comparable structures in species descriptions of other Phreodrilidae, so the uniqueness of these features cannot be demonstrated here—they may simply have been overlooked. In fact, parachaetal bodies were detectable *only* in the whole mounts stained with Bouin's fluid. The thick postero-dorsal glandular cushions, however, are as thick and as conspicuous as the clitellum, which is usually described or illustrated. It may therefore be a structure that is truly absent or at least very rare in the rest of Phreodrilidae that have been described to date. Their function is unknown, but their dorsal position—together with the terrestrial habitat of the species (see below)—suggests that these glandular cushions may secrete fluid as a lubrication to suppress desiccation.

Habitat and distribution. A further peculiarity of this new species is the terrestrial habitat. The description of the sites in Wisdom (2010) as well as the occurrence of oribatid and gamasine mites, carabid beetles, and spiders leave no doubt that the sites with *A. hibernicus* are terrestrial and not aquatic. The accompanying oligochaete fauna consisted only of enchytraeids, dominated by *Cognettia sphagnetorum* (Vejdovský, 1878), together with a few specimens of *Cernosvitoviella* sp., *Marionina clavata* Nielsen & Christensen, 1961, *Achaeta affinis* Nielsen & Christensen, 1959, and *Mesenchytraeus glandulosus* Nielsen & Christensen, 1959. Considering the indicator values for enchytraeid species as presented in Graefe & Schmelz (1999), the presence of these enchytraeid species confirms the character of our collecting sites as moist to wet, but terrestrial. Terrestrial phreodrilid species have previously been described from beech litter in New Zealand (Stout 1958). In all of these cases, the microhabitat is probably aquatic: for example, including minute pools or water films around mineral soil and humus particles. Pinder & Brinkhurst (1997) record phreodrilids from Australian peatlands but the sites were definitely aquatic (Pinder, pers. comm.). Sampling at the more terrestrial peat sites will probably yield more terrestrial species of this family in regions of the Southern Hemisphere that are rich in phreodrilid species.

Astacopsidrilus hibernicus **sp. nov.** was found only in the peatlands located in the outer northwest regions of Ireland, even though the other peatland sites sampled during this study (Fig. 1) supported a similar oligochaete fauna—restricted to the enchytraeids *Cognettia sphagnetorum* (the dominant species) and accompanied by *Cernosvitoviella* spp. and a few specimens representing some of the more terrestrial species. Furthermore, *A. hibernicus* was abundant at two localities that are about 14 km apart from each other. This suggests an established population in an extended though clearly confined area.

The implications and alternative explanations for the presence of a taxon (Family Phreodrilidae) in Ireland, whose domain is the Southern Hemisphere ("invasion from down-under or ancient relict?"), have been discussed in Schmelz *et al.* (2015) and need not be repeated here. General aspects of dispersal of phreodrilids are discussed in Pinder and Brinkhurst (1997) and in Martin and Ohtaka (2008). More intensive sampling plus DNA-sequencing may help to find the correct explanation. The species name chosen expresses our opinion that—whatever its origin, travel route or date of arrival—this species should be considered as an established component of the Irish fauna.

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References

Benham, W.B. (1903) On some new species of aquatic Oligochaeta from New Zealand. *Proceedings of the Zoological Society of London*, 1903 (2), 202–232.

Brinkhurst, R.O. (1965) A taxonomic revision of the Phreodrilidae (Oligochaeta). *The Journal of Zoology, London*, 147, 363–386.
https://doi.org/10.1111/j.1469-7998.1966.tb02906.x

Brinkhurst, R.O. (1970) A further contribution towards a study of the aquatic Oligochaeta of Africa. *Revue de Zoologie et Botanie d'Afrique*, 80, 101–108.

Brinkhurst, R.O. (1991) A phylogenetic analysis of the Phreodrilidae (Annelida, Oligochaeta), with a description of a new species. *Canadian Journal of Zoology,* 69, 2031–2040.
https://doi.org/10.1139/z91-284

Chalupský, J. (1992) Terrestrial Enchytraeidae (Oligochaeta) and Parergodrilidae (Polychaeta) from Sweden, with description of a new enchytraeid species. *Zoologica Scripta*, 21, 133–150.
https://doi.org/10.1111/j.1463-6409.1992.tb00316.x

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular marine biology and biotechnology*, 3 (5), 294–299.

Giani, N., Martin, P. & Juget, J. (1995) A new species of Phreodrilidae (Oligochaeta), *Astacopsidrilus naceri* sp. nov., from Morocco (North Africa), with notes on the biogeography of the family. *Canadian Journal of Zoology*, 73, 2375–2381.
https://doi.org/10.1139/z95-277

Goddard, E.J. (1909) Contribution to a further knowledge of Australasian Oligochaeta. Part I. Description of two species of a new genus of Phreodrilidae. *Proceedings of the Linnean society of New South Wales*, 33, 768–793 + Pl. XXIX–XXXI.

Graefe, U. & Schmelz, R.M. (1999) Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. *In:* Schmelz, R.M. & Sühlo, K. (Eds.), *Newsletter on Enchytraeidae No. 6. Proceedings of the 3rd International Symposium on Enchytraeidae, Osnabrück, Germany.* Universitätsverlag Rasch, Osnabrück, Germany, pp. 59–67.

Gunn, R.J.M., Pinder, A. & Walker, B.M. (2003) Phreodrilidae (Annelida: Oligochaeta), a family new to Europe. *The Irish Naturalist's Journal, Dublin*, 27, 315–317.

Healy, B. (1979) Records of Enchytraeidae (Oligochaeta) in Ireland. *Journal of Life Sciences of the Royal Dublin Society,* 1, 39–70.

Healy, B. & Bolger, T. (1984) The occurrence of species of semi-aquatic Enchytraeidae (Oligochaeta) in Ireland. *Hydrobiologia*, 115, 159–170.
https://doi.org/10.1007/BF00027911

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, 28 (12), 1647–1649.
https://doi.org/10.1093/bioinformatics/bts199

Martin, P., Martínez-Ansemil, E., Pinder, A., Timm, T. & Wetzel, M.J. (2008) Global diversity of oligochaetous clitellates ("Oligochaeta"; Clitellata) in freshwater. *Hydrobiologia*, 595, 117–127.
https://doi.org/10.1007/978-1-4020-8259-7_14

Martin, P. & Ohtaka, A. (2008) A new phreodrilid species (Annelida: Clitellata: Phreodrilidae) from Lake Biwa, Japan. *Species Diversity*, 13, 221–230.
https://doi.org/10.12782/specdiv.13.221

Martínez-Ansemil, E., Giani, N. & Sambugar, B. (2002) Oligochaetes from underground waters of Oman with descriptions of two new species of Phreodrilidae (Oligochaeta): *Antarctodrilus arabicus* n. sp. and *Phreodrilus stocki* n. sp. *Contributions to Zoology, SPB Academic Publishing bv, The Hague*, 71, 147–158.
https://doi.org/10.1163/18759866-07104005

Martinsson, S., Rota, E. & Erséus, C. (2015) Revision of *Cognettia* (Clitellata, Enchytraeidae): reestablishment of *Chamaedrilus* and description of cryptic species in the *sphagnetorum* complex. *Systematics and Biodiversity*, 13, 257–277.
https://doi.org/10.1080/14772000.2014.986555

Nielsen, C.O. & Christensen, B. (1959) The Enchytraeidae. Critical revision and taxonomy of European species (Studies on Enchytraeidae VII). *Natura Jutlandica*, 8–9, 1–160.

Nielsen, C.O. & Christensen, B. (1961) The Enchytraeidae. Critical revision and taxonomy of European species. Supplement 1. *Natura Jutlandica*, 10, 1–23.

O'Connor, F.B. (1967) The Enchytraeidae. *In:* Burges, A. & Raw, F. (Eds.), *Soil Biology.* Academic Press, London, pp. 213–257.
https://doi.org/10.1016/B978-0-12-395699-6.50012-2

Pinder, A.M. & Erséus, C. (2000) New Phreodrilidae (Annelilda: Clitellata) from Tasmanian estuaries. *Papers and Proceedings of the Royal Society of Tasmania*, 134, 29–33.
https://doi.org/10.26749/rstpp.134.29

Pinder, A. (2003) New species and records of Phreodrilidae (Annelida: Clitellata) from Western Australia. *Records of the Western Australian Museum*, 21, 307–313.
https://doi.org/10.18195/issn.0312-3162.21(4).2003.307-313

Pinder, A. (2008) Phreodrilidae (Clitellata: Annelida) in north-western Australia with descriptions of two new species. *Records*

of the Western Australian Museum, 24, 459–468.
https://doi.org/10.18195/issn.0312-3162.24(4).2008.459-468

Pinder, A.M. & Brinkhurst, R.O. (1997) Review of the Phreodrilidae (Annelida: Oligochaeta: Tubificida) of Australia. *Invertebrate Taxonomy*, 11, 443–523.
https://doi.org/10.1071/IT95025

Pinder, A.M., Sweeney, P. & Smith, P.R.J. (2013) First confirmed record of the genus *Insulodrilus* (Benham, 1903) (Annelida: Clitellata: Phreodrilidae) in Europe. *BioInvasions Records*, 2, 195–199.
https://doi.org/10.3391/bir.2013.2.3.04

Renou-Wilson, F., Bolger, T., Bullock, C., Convery, F., Curry, J., Ward, S., Wilson, D. & Müller, C. (2011) *BOGLAND: Sustainable Management of Peatlands in Ireland STRIVE Report No. 75*. Environmental Protection Agency, Johnstown Castle, Wexford, 157 pp.

Schmelz, M., Wisdom, R. & Bolger, T. (2015) Phreodrilidae in Irish peatlands: Invasion from down-under or ancient relict? *The Irish Naturalist's Journal*, 34, 101–103.
https://doi.org/10.1080/03323315.2015.1063819

Schmelz, R.M. & Collado, R. (2010) A guide to European terrestrial and freshwater species of Enchytraeidae (Oligochaeta). *Soil Organisms*, 82, 1–176.

Sjölin, E., Erséus, C. & Källersjö, M. (2005) Phylogeny of Tubificidae (Annelida, Clitellata) based on mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution*, 35, 431–441.
https://doi.org/10.1016/j.ympev.2004.12.018

Stout, J.D. (1958) Aquatic Oligochaetes occurring in forest litter. - II. *Transactions of the Royal Society of New Zealand*, 85, 289–299.

Vejdovský, F. (1878) Zur Anatomie und Systematik der Enchytraeiden. *Sitzungsberichte der Königlich Böhmischen Gesellschaft der Wissenschaften*, 1877, 294–304.

Wisdom, R. (2010) *Biodiversity of some of the invertebrate taxa occurring in terrestrial habitats in different types of Irish peatlands. PhD thesis*. University College Dublin, 273 pp.