Zootaxa 5437 (1): 001–014
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5437.1.1
http://zoobank.org/urn:lsid:zoobank.org:pub:873EED65-BAD8-49CB-A23D-C86F4289E742

Two new species of *Achaeta* (Enchytraeidae, Oligochaeta) from afforested post-mining and post-fire sites in Poland

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Abstract

Achaeta (Enchytraeidae, Oligochaeta, Clitellata) is a genus of small, soil-dwelling annelids, peculiar by the absence of chaetae in all species. Here two new species of this genus are described from afforestation sites in southern Poland. Descriptions are based on the investigation of living and preserved material. The species are also characterized at the DNA level, using fragments of the COI and ITS genes, sequenced from the holotypes and paratypes. The worms are not longer than 4–6 mm, with about 30 to 35 segments, and with medium-large male and female reproductive organs. *Achaeta florens* **sp. nov.**, discovered at a post-mining site, has four segmental pyriform glands, an oesophageal appendage restricted to segment V, a clitellum with distinct transverse rows and dorsal and ventral interruption, and spermathecae with blossom-like fields of vesicular gland cell apices around the ventrally located ectal pores. The species is without peculiar characters but the combination of characters is diagnostic. Its morphological distinction from *A. nurmineni*, described from the Indian subcontinent, is difficult due to the poor description of that species; *A. nurmineni* is therefore considered a *species inquirenda*. *Achaeta gemmata* **sp. nov.**, discovered in high abundance at a post-fire site, is without pyriform glands but with conspicuous, gem-like lateral papillae in all preclitellar segments. The papillae are modified and enlarged epidermal gland cells that protrude into the body cavity. The species is without oesophageal appendage and has a dorsally closed clitellum and lateral spermathecal pores. It is superficially similar to *Achaeta camerani*, found at the same site, but can be distinguished based on sexual and non-sexual characters. A comparison of COI sequences showed 100% identity with a specimen from Sweden, erroneously identified as *A.* cf. *brevivasa*. Genetic distance between the two new species based on COI differences is 20%, distances to other species of *Achaeta* with publicly available COI sequences range from 16% to 26%.

Key words: Taxonomy, soil fauna, soil biodiversity, Clitellata

Introduction

Achaeta Vejdovský, 1878 is a cosmopolitan genus of mostly soil-dwelling annelids with currently 48 species. Seven species of this genus are listed in the comprehensive monograph on Polish enchytraeids (Kasprzak 1986a): *A. affinis* Nielsen & Christensen, 1959, *A. bohemica* (Vejdovský, 1879) sensu Nielsen & Christensen (1959), *A. camerani* (Cognetti, 1899), *A. danica* Nielsen & Christensen, 1959, *A. eiseni* Vejdovský, 1878, *A. seminalis* Kasprzak, 1972, and *A. vesiculata* Nielsen & Christensen, 1959. Two further records of Polish *Achaeta* species include *A. bulbosa* Nielsen & Christensen, 1961 (Kasprzak 1986b) and *A. parva* Nielsen & Christensen, 1961 (Kapusta *et al.* 2003). [For a comment on the identity of *A. bohemica* sensu Nielsen & Christensen and *A. vesiculata*, see Schmelz & Collado (2010)]. Here we describe two further species of *Achaeta*, new to science, from Poland, and we provide DNA barcodes (COI and ITS) of the holotypes and some paratypes of these species. Since the description of *Achaeta*

Accepted by D. Marchán: 12 Mar. 2024; published: 11 Apr. 2024

seminalis Kasprzak, 1972, this is the first time that new species of *Achaeta* are described from this country. They were discovered in samples from a postmining area in southern Poland, in the framework of an ongoing project that investigates soil-forming processes and carbon accumulation in reclaimed forest soils containing pyrogenic and fossil carbon residues and the role of soil biota in these processes.

Material and methods

Study site. Studies are conducted at two localities in southern Poland. The first area of research is situated in the Rudziniec Forest District (50°17'N, 18°25'E), regenerated after fire. The area experienced a huge fire in August 1992, affecting 9062 ha, mostly forestland, causing a tree mortality of > 75% and significant soil exposure. After the fire, the area was regenerated by afforestation mainly with pine (*Pinus sylvestris* L.) but also with larch (*Larix decidua* Mill.), birch (*Betula pendula* Roth,) and oak (*Quercus robur* L.). The soil in this area is a sandy to sandy-loamy podzol with pH (H_2O) about 4.2. The second area of research is situated in the sand pit Szczakowa located in the Upper Silesia region (N50°14' E19°23'). Here a disturbed area covers 3100 ha with an excavated depth of 5–25 m, as a result of open-strip lignite mining. Afforestation was preceded by mineral fertilization (70–120–120 kg NPK ha^{-1}) and cultivation of lupine as green manure (Pietrzykowski *et al.* 2017). Soils in this area mainly belong to the initial ones such as Arenosols, with sandy-loamy to sandy texture and pH (H_2O) 4.6–5.0 (Woś *et al.* 2023).

Methods. Sampling took place in May and June 2023. Replicate samples for enchytraeid extraction were taken with a soil corer, surface area 16.6 cm^2, depth 0–10 cm. The animals were extracted using a modified version of O'Connor's (1967) hot/wet funnel technique. In acid soil samples, pH of extraction water was adjusted with drops of HCl. Worms were investigated and photographed light-microscopically *in vivo* using a microscope with interference (Nomarski) contrast optics and integrated photographic equipment. Details of methods of *in vivo* investigation of these worms are described in Schmelz (2003) and in Schmelz and Collado (2010). After investigation, specimens were fixed either in hot Bouin's fluid and stored after 24 h in 70% ethanol for morphological investigation, or fixed in 70% ethanol for 12 h and stored in 100% ethanol for DNA sequencing. Three specimens of each species, intended for holotype and paratype designation, were selected for DNA barcoding. Animals were cut in halves. The posterior end was used for DNA extraction, the anterior end was maintained as physical voucher. Specimens fixed in Bouin's fluid were stained with paracarmine, dehydrated, and whole-mounted between two coverslips in Canada Balsam. The DNA vouchers were whole-mounted in the same way, but left unstained. Drawings were made with the help of a drawing tube (whole mounts) or as freehand sketches (living worms).

Total genomic DNA was extracted from the whole worms using TIANamp Micro DNA Kit (Tiangen Biotech, Beijing, China). COI genes and ITS regions were amplified by PCR using the following primer pairs: for COI, HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') and LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') (Folmer *et al*. 1994); for ITS, ETTS1 (5′-TGC TTA AGT TCA GCG GGT-3′) and ETTS2 (5′-TAA CAA GGT TTC CGT AGG TGAA-3′) (Kane & Rollinson 1994). PCR was performed in a final volume of 25 μL including: 2 μL genomic DNA, 1 μL primers, 12.5 μL 2 ×Hieff™ PCR Master Mix and 8.5 μL ddH_2O. The thermal profile consisted of 5 min at 94 °C for initial denaturation, followed by 35 cycles (30 sec for denaturation, 30 sec at 52 °C in the case of COI and ITS, 1 min for extension) and with a final step of 10 min at 72 °C for final extension. DNA sequencing was successful in five out of the six selected specimens.

Genetic distances (uncorrected p-distances) were calculated using MEGA X (Kumar *et al.* 2018).

Terminology in the species descriptions follows Schmelz (2003) and Schmelz & Collado (2010). Paired reproductive organs are often described in the singular form. Dimensions are usually approximate due to the soft-bodied nature of the animals. Holotypes and paratypes are deposited at the Nature Education Centre of the Jagiellonian University, Gronostajowa 5, 30-387 Kraków, Poland, acronym CEPUJ (catalogue numbers in the following preceded by "CEP-DZ"). Further reference specimens will be deposited at the Natural History Museum, London; the rest will remain in the collection of the first author. NCBI accession numbers of sequenced gene fragments are shown in Table 1.

TABLE 1. *Achaeta florens* and *Achaeta gemmata* spp. nov.: NCBI accession numbers of DNA sequences, together with type status and collection numbers of specimens. CEPUJ: Nature Education Centre of the Jagiellonian University, Gronostajowa 5, 30-387 Kraków, Poland. COI: cytochrome c oxidase subunit 1 (CO1) gene, partial cds; mitochondrial. ITS: internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.

Species name	Type status	CEPUJ	COI	ITS
Achaeta florens **sp. nov.**	holotype	CEP-DZ-149401-N	PP079494	PP084616
Achaeta florens **sp. nov.**	paratype	CEP-DZ-149402-N	PP079493	PP084615
Achaeta florens **sp. nov.**	paratype	CEP-DZ-149403-N	PP079495	PP084617
Achaeta gemmata **sp. nov.**	holotype	CEP-DZ-149414-N	PP079496	PP084618
Achaeta gemmata **sp. nov.**	paratype	CEP-DZ-149415-N	-	PP084619

Abbreviations used in text and figures: am, spermathecal ampulla; ct, connecting tube of spermatheca; dp, dorsal papilla in I; dv, dorsal blood vessel; ep, ectal pore of spermatheca; er, ental reservoir of spermatheca; fix, dimensions taken from fixed specimens; fp, female pore; ga, prostomial ganglion; hp, head pore; lp, lateral papilla; mp, male pore; oa, oesophageal appendage; ov, ovary; pg, pyriform gland; pp, prostomial papilla; te, testis; viv, dimensions as observed in living specimens; vn, ventral nerve cord; vp, ventral papilla.

Achaeta florens sp. nov.

(Figures 1, 2)

Holotype. CEP-DZ-149401-N, adult specimen, anterior 16 segments, unstained whole-mount, with DNA sequences COI PP079494, ITS PP084616.

Type locality. Poland, Upper Silesia, Szczakowa, reclaimed post-mining sand pit afforested with oak (*Quercus rubra* L.), 50°14’28.8”N 19°23’36.2”E; soil type Arenosol, sandy loam and loamy sand texture, pH (H_2O) 4.6–4.8.

Paratypes. CEP-DZ-149402-N, adult specimen, anterior 21 segments, unstained whole mount, with DNA sequences COI PP079493, ITS PP084615. CEP-DZ-149404-N, adult specimen, anterior 17 segments, unstained whole mount, with DNA sequences COI PP079495, ITS PP084617. CEPU: CEP-DZ-14905-N to CEP-DZ-14913-N, 10 specimens (7 adult, 3 subadult), complete, stained whole mounts. All from type locality.

Further material. 11 specimens (6 adult, 4 subadult, 1 juvenile), stained whole mounts, in the 1st author’s collection. 55 specimens, investigated and identified *in vivo*, a subset preserved in 100% ethanol. All from type locality.

Diagnosis. Body length 4–6 mm; up to 35 segments; pyriform glands dorsally and ventrally; oesophageal appendage small in V; pharyngeal glands without secondary lobes; two pairs preclitellar nephridia, at 6/7, 7/8; clitellum interrupted dorsally and ventrally, separate transverse rows, hyalocytes in irregular longitudinal rows dorso-laterally; seminal vesicle absent; male glands small and separate; spermatheca length c. 2 segments, ectal pores ventrally, each surrounded by flower-like field of vesicular gland cell apices.

Description. Thin, comparatively active and agile *Achaeta*-worms, forming irregular coils and curls in water when alive. Body length 4–6 mm (viv); 4.9–6.3 mm (fix). Body diameter 0.13 mm (viv, 1 ind.), up to 0.2 mm at XII (viv); fixed specimens: 0.08–0.13 mm (V) 0.08–0.16 mm (XII) 0.09–0.11 mm (XX). Adults with 31–35 segments (N=13). Body wall thin, diameter 10–12 µm, cuticle thin (< 1µm). Pyriform glands (Fig. 1B,C, 2D) dorsally and ventrally, dorsals from II, length 1/3 to 1/2 body diameter, ventrals from III, smaller. Other epidermal gland cells inconspicuous.

Brain (Fig. 1A) rounded posteriorly, 76–90 µm long, 30–40 µm wide (fix), ventral nerve cord (Fig. 1C, 2D,E) ganglionic. One pair of prostomial ganglia in front of brain; three pairs of papillae laterally and anteriorly, as inner thickenings of body wall (Fig. 1A). Pharyngeal glands (Fig. 1C) in IV–VI, all united dorsally, no secondary lobes. Oesophageal appendage (Fig. 1C) unpaired in V, no canal in IV. Dorsal blood vessel from VII. Intestine widening gradually. Chloragocytes (Fig. 2A) conspicuous, large, diameter c. 20 µm, filled with yellow-brown to brown vesicles. Midgut par tumida in 1/2XVIII–XXVI, extending over 6–7 segments. Two pairs preclitellar nephridia (Fig. 1C, 2A), at 6/7, 7/8. Anteseptale with coils of canal, as long as wide, funnel in oblique position, postseptale c. 2x as long as anteseptale, gradually merging into thin and elongate efferent duct; no terminal vesicle. Coelomocytes (Fig. 2F) pale flat discs, with outer circumferal and inner radial lines, finely vesicular, outer margin hyaline.

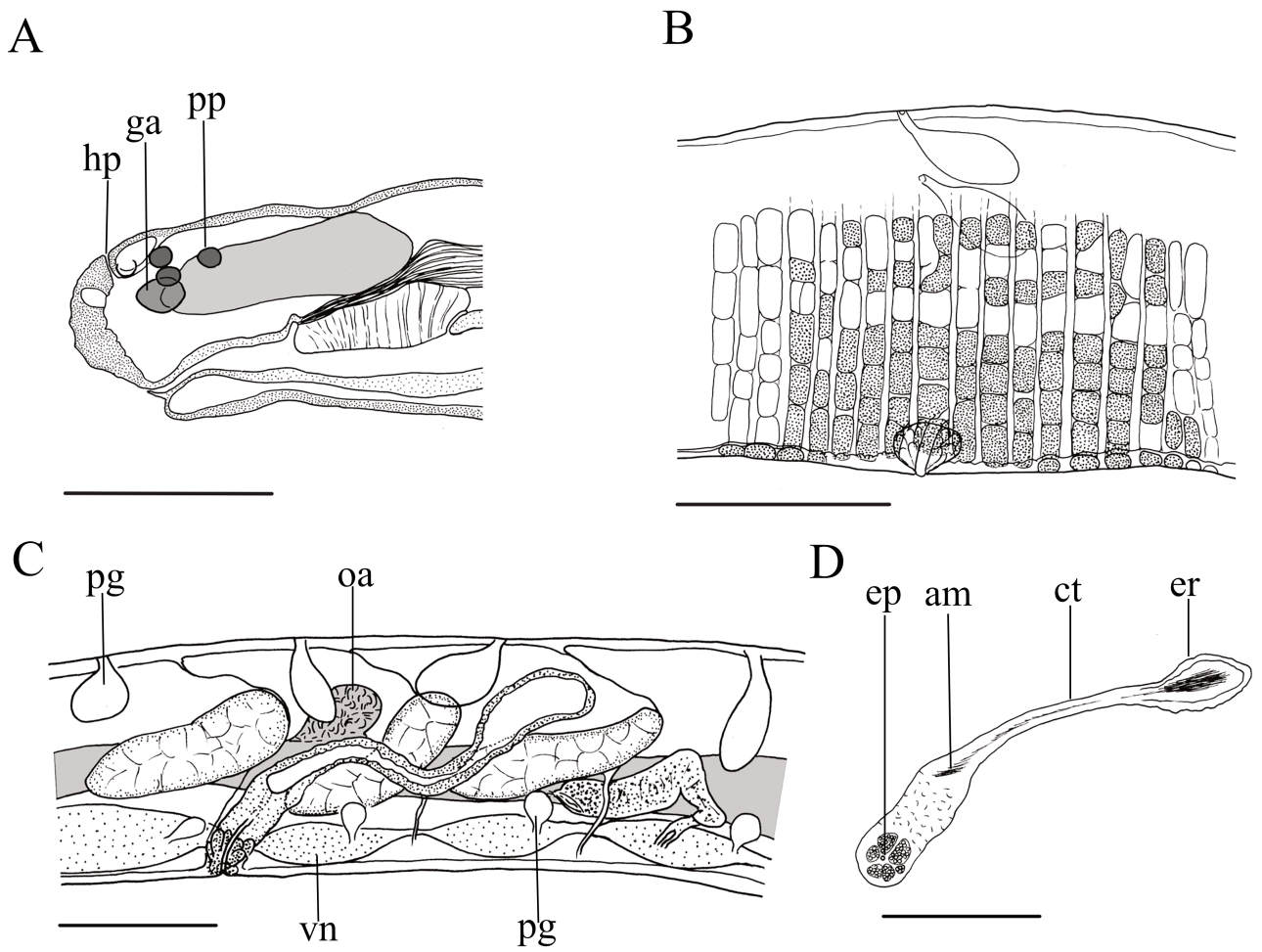

FIGURE 1. *Achaeta florens* **sp. nov.**, drawing from whole mounted holotype (A–C) and living non-type specimen (D). **A.** Head region, lateral view, with epidermal papillae (dark-grey), prostomial ganglion (medium-grey), and head pore. **B.** Clitellar region, lateral view. **C.** Segments IV–VII, lateral view. In the original, the ental part of the spermatheca lies between pharyngeal glands and the oesophagus. **D.** Spermatheca, free-hand drawing.

Clitellum (Fig. 1B, 2E) in XII–1/2XIII, interrupted dorsally and ventrally, dorsal interruption wider than ventral one, wider than distance of dorsal pyriform glands, ventral interruption anteriorly as wide as male pore distance, narrowed posteriorly to one gland cell width; clitellum cells in 18 or 19 conspicuously separate transverse rows; hyalocytes mostly in dorsal half, two irregular longitudinal rows on each side; at dorsal borders of clitellum, more granulocytes than hyalocytes; at ventral border only granulocytes. Hyalocytes 18–24 µm long and 12–13 µm wide, granulocytes ca. 14 µm long and 9 µm wide (fix).

Testis in XI, ovary inXII. Seminal vesicle absent, spermatogonial cysts in XI. Sperm funnels (Fig. 2B) cylindrical, not longer than body diameter, c. 3–4x as long as wide (ca. 120 µm by 30–40 µm) (106–140 µm long and 26–36 µm wide, fix), collar almost as wide as funnel body. Spermatozoa 30–35 µm, heads 11–15 µm. Vas deferens often in dense regular spirals or coils, diameter 7–8 um. Male pores (Fig. 2E) on body surface, no area glareosa. Male glandular bulbs (Fig. 1B, 2E) oval, longer than wide (32–50 um by 20–31um), pierced centrally by vas deferens; glands separate, distance as wide as or slightly wider than width of ventral nerve cord or male glandular bulbs.

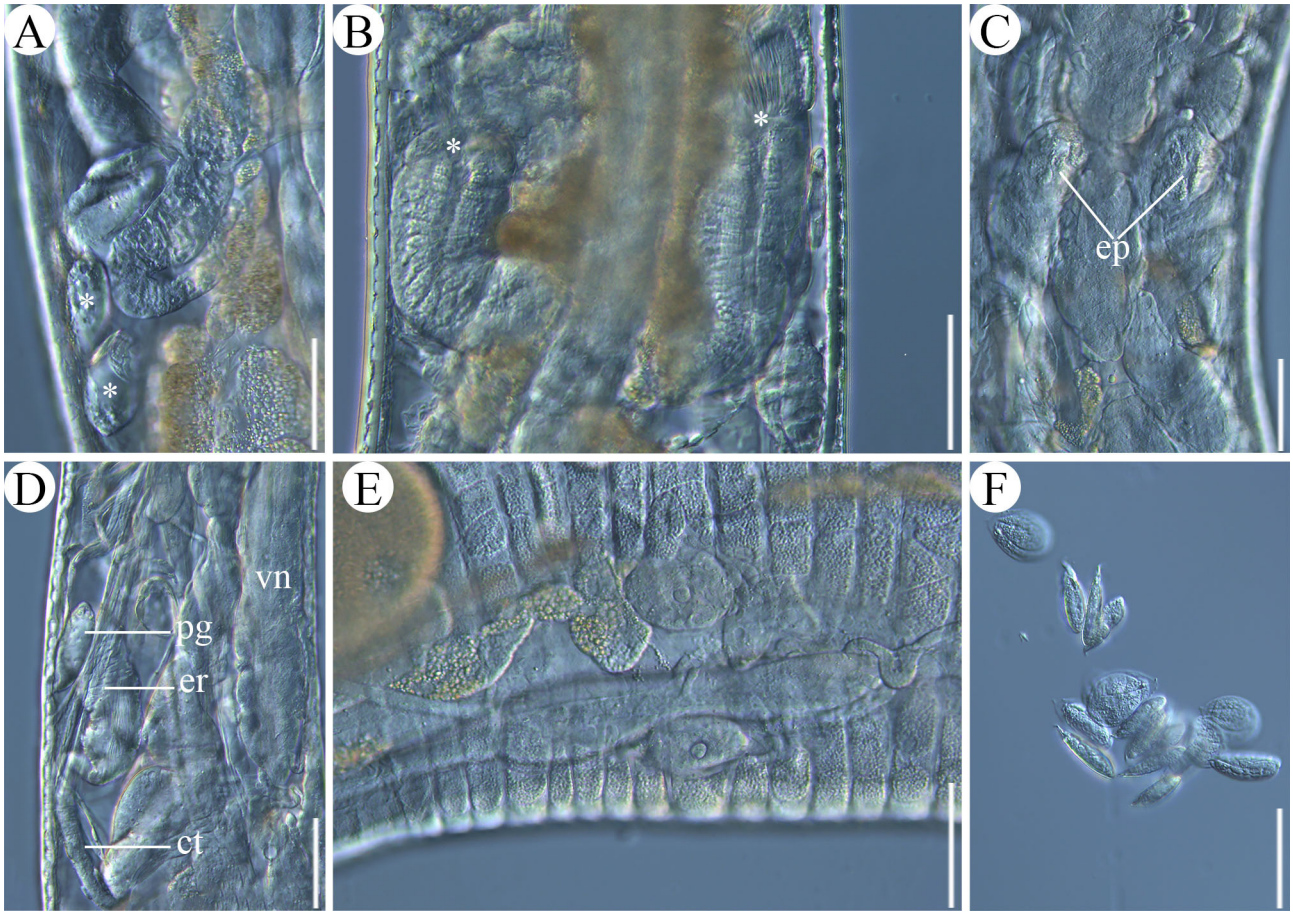

FIGURE 2. *Achaeta florens* **sp. nov.**, photographs from living specimens. **A.** Nephridium at 6/7, ventro-lateral view. Asterisks: Dorsal pyriform glands (ventral pyriform glands out of focu). **B.** Sperm funnels on both sides of the intestine. Asterisks: collar. **C.** Segments IV, V, ventral view, with ectal part of spermathecae. **D.** Segment IV, ventro-lateral view. The free-ending ental part of the spermatheca (ct, er) is pushed forwards. **E.** Segment XII, ventro-lateral view, showing ventral borders of clitellum and male glandular bulbs with central male pores. Ventral nerve cord slightly out of focus. **F.** Coelomocytes, extruded from the body.

Spermathecae (Fig. 1C,D, 2C,D) free, extending into VI (VII), sometimes confined to IV–V or IV, depending on bends or tortions; spermathecal pores ventrally, each surrounded by a blossom-like crown of c. 6 vesicular gland cell apices (Fig. 1D, 2C); ectal part massive, diameter c. 35–40 um, >2x as long as wide, also vesicular but less conspicuously, entally spermatozoa in oblique position; connecting tube narrowed, ental reservoir as wide as ectal part; sperm in ampulla, connecting tube and ental reservoir.

Etymology. Named after the blossom-like arrangement of glandular vesicles around the spermathecal ectal pores (Fig. 1D, 2C).

Remarks, morphology. There are currently 10 nominal species of *Achaeta* with four segmental pyriform glands, eight from Europe (Schmelz & Collado 2010) and two from India (Prabhoo 1966, Dash & Thambi 1978). In Europe, *A. affinis* and *A. danica* are in the size range of the new species. They differ from the new species in the oesophageal appendage, which has a canal in IV, and in the clitellum, with hyalocytes arranged in baguette-like longitudinal packages. Furthermore, the spermathecae in *A. danica* are usually longer (extending into IX); in *A. affinis*, they are absent.

Outside Europe, the new species is most similar to *A. nurmineni* Dash & Thambi, 1978, described from southern Orissa, India, in body length (4–6 mm), segment number [24–30–(34)], clitellum in 18–20 transverse rows, absence of a seminal vesicle and short spermathecae. A species comparison is made difficult by the lack of information on several taxonomically important characters: oesophageal appendage, position of preclitellar nephridia, clitellum dorsally and ventrally. Other characters are doubtfully described or illustrated and need reinvestigation: the large

pyriform glands are said to be in lateral position (not in dorsal position as usual) and Figure 12 shows spermathecal ectal pores in lateral position (not in ventral position as in all other *Achaeta* species with four segmental pyriform glands). The spermathecae are described as sac-like, and the coelomocytes are oval to spindle-shaped and with granular structure; these details may or may not serve as characters to distinguish the species. There is no record after the original description, and whereabouts of the type specimens are unknown. We consider *A. nurmineni* Dash & Thambi, 1978 as a *species inquirenda*.

The blossom-like crown of intracellular vesicles around the spermathecal pores was new to us when we first saw it but in the meantime we have also seen it in *Achaeta aberrans*, Nielsen & Christensen, 1961, a species with 6 segmental pyriform glands and lateral spermathecal ectal pores, and in *Achaeta bohemica* (Vejdovský, 1879), a species with 2 segmental pyriform glands and secondary pharyngeal gland lobes in V and VI. In fact, this new species of *Achaeta* lacks any kind of particular feature, and is distinguished only by a combination of characters.

Remarks, molecular comparison. Currently 42 COI sequences of specimens identified as *Achaeta* are publicly available. Uncorrected p-distances were calculated based on COI sequences, and clear genetic gaps were observed between our new species and the other 23 species records, which represent 16 different species of *Achaeta*, some of them unnamed (Table 2), with interspecific distances ranging from 16.4% to 26.1%. Intraspecific distances were not observed; the three sequences of COI and ITS, respectively, obtained from the holotype and two paratypes are identical.

Achaeta gemmata sp. nov.

(Figures 3, 4)

Holotype. CEP-DZ-149414-N, adult specimen, anterior 23 segments, unstained whole-mount, with DNA sequences COI PP079496, ITS PP084618.

Type locality. Poland, Rudziniec Forest District, forest regenerated after fire, pine stand (*Pinus sylvestris* L.), 50°18’09.5”N 18°24’47.7”E, soil type podsol, pH 3.8 (H_2O).

Paratypes. CEP-DZ-149415-N, adult specimen, anterior 15 segments, unstained whole-mount, with DNA sequence ITS PP084619. CEP-DZ-149416-N to CEP-DZ-149430-N, 15 specimens (8 adult, 3 subadult, 4 juvenile), mostly complete (3 amputees), stained whole mounts. 33 specimens (5 adult, 17 subadult, 11 juvenile), stained whole mounts, in the 1st author’s collection. From type locality or adjacent sites overgrown by *Larix decidua, Betula pendula,* and *Quercus robur* L.; soil type podsols, sandy and loamy-sandy texture, pH (H_2O) 3.7–4.8.

Further material. A total of c. 1700 specimens, investigated and identified *in vivo*, from type locality or adjacent sites. Of these: 33 specimens (5 adult, 17 subadult, 11 juvenile), stained whole mounts; 9 specimens, fixed in Bouin’s fluid, preserved in 70% ethanol; c. 130 specimens, preserved in 100% ethanol; from type locality or adjacent sites. Material in the 1st author’s collection.

Diagnosis. Body length 4.5–5 mm; up to 38 segments; pyriform glands and oesophageal appendage absent; pharyngeal glands with secondary lobes in V and VI; two pairs preclitellar nephridia, at 6/7, 7/8; clitellum interrupted ventrally, complete dorsally, cells in conspicuous longitudinal rows; dorsal rows consisting of hyalocytes and interspersed granulocytes; lateral and ventrolateral rows with granulocytes only; last ventral row in longitudinal line with male pores; seminal vesicle absent; male glands small and widely separate; spermatheca length c. 1.5 segments, ectal pores laterally.

Description. Body length (fix) 4.4–4.9 mm. Body diameter (fix) 0.11–0.25 mm; 0.11–0.15 mm at V, 0.15–0.25 at XII, 0.14–0.17 mm at XX. Segment number 31–38 (N=11; 32–34, eight specimens, 31, 35, 38, one specimen each).

Pyriform glands absent, lentiform glands enlarged to ellipsoid cushion-like intracoelomic papillae in anterior segments: dorsally one pair in I anteriorly of brain (Fig. 3A, 4A), laterally one pair per segment in II to X (Fig. 3A, 4G); ventrally one pair per segment (Fig. 3A,B), first pair in III, IV or V, last preclitellar pair in XI, only slightly enlarged; behind clitellum from XIII on, reducing in size in following segments, absent in posterior body half. In living specimens, dorsal papillae in I and lateral papillae conspicuous, ventral papillae inconspicuous. Papillae longer than wider than high. Size of lateral papillae varying among specimens, and often also within a specimen; maximum length 32–50 µm, minimum length 18–32 µm, mean length 27–34 µm. Length within an individual varying, e.g., between 50 µm and 32 µm or 32 µm and 20 µm. Ventral papillae 21–25 µm long and 14–20 µm wide.

Papillae not developed in all positions. Additionally, 2 pairs of segmental lentiform cells dorsally, inconspicuous, only seen in whole mounted specimens: one pair closely behind septum, the other pair at level of lateral papillae.

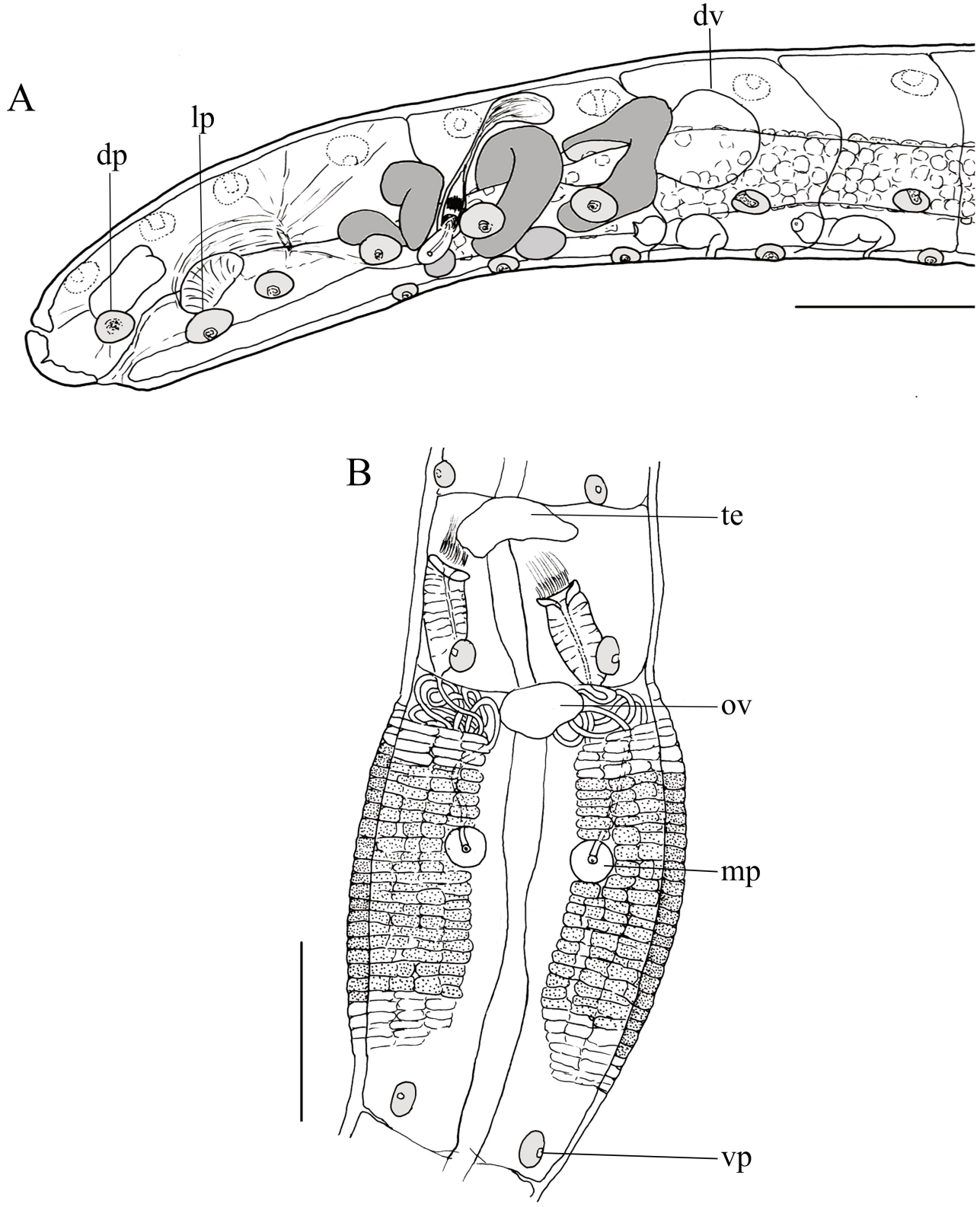

FIGURE 3. *Achaeta gemmata* **sp. nov.**, drawings from whole mounts. **A.** Anterior end, segments 0–VIII, latero-dorsal view. In this specimen, ventral papillae begin at IV. Pharyngeal glands shaded dark-grey. **B.** Clitellar region, ventral view.

Brain (Fig. 3A, 4A) about 1.7x as long as wide, posteriorly slightly convex, sometimes straight, length 80–90 µm (viv), 70–73 µm, fix. Ventral nerve cord ganglionic from V. Pharyngeal glands (Fig. 3A, 4C) all widely connected dorsally, in V and VI no subdivision into dorsal and ventral lobes; secondary ventral glands in V, VI. Oesophageal appendage absent or not distinguishable light-microscopically. Dorsal blood vessel from VII. Intestine widening gradually, no intestinal diverticula. Pars tumida of midgut in region XXIX–XXXIV, in 3.5–4 consecutive segments. Two pairs of preclitellar nephridia (Fig. 3A, 4B), at 6/7, 7/8; anteseptale with coils of canal, bulky, postseptale 1.5x as long as anteseptale, septal constriction slight or absent, postseptale gradually merging into short and thick efferent duct, terminal vesicle absent. Postclitellar nephridia from 15/16 or further back, of similar shape but postseptale more slender and stretched than in preclitellar nephridia. Coelomocytes (Fig. 4D) pale, flat, disc-shaped, with varying amounts of refractile granules in the centre, periphery often as a flat hyaline frame; cells almost as wide as long, length 30–35 µm.

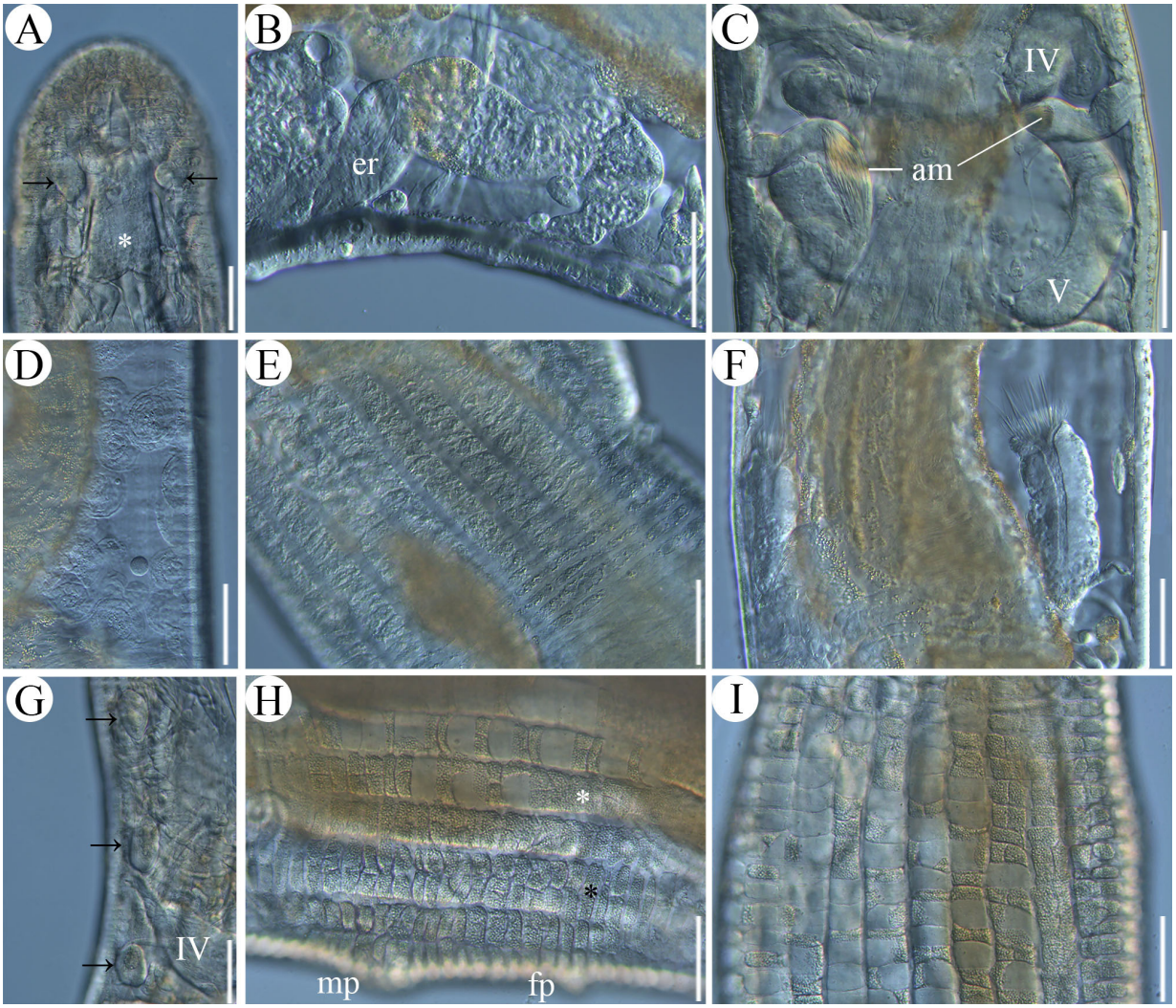

FIGURE 4. *Achaeta gemmata* **sp. nov.**, photographs from living specimens. **A.** Head region, dorsal view, showing prostomial papillae (arrows) antero-laterally of brain (asterisk). **B.** Nephridium at 7/8; the funnel is covered by the ental reservoir (er) of the spermatheca. **C.** Spermathecae between pharyngeal glands of IV and V, dorsal view, both ectal pores focused. **D.** Coelomocytes. **E.** Clitellum, dorso-lateral view, as seen in a subadult specimen. **F.** Sperm funnels on both sides of the intestine. **G.** Lateral papillae (arrows) of segments III–V. **H.** Clitellum, lateral view. White asterisk: First lateral row with alternating hyalocytes and granulocytes. Dark asterisk: incipient double row of granulocytes. **I.** Clitellum, dorso-lateral view. Note the absence of a mid-dorsal interruption.

Clitellum from XII–1/2XIII, saddle-shaped, no dorsal interruption; cells in conspicuous longitudinal rows; dorsal rows consisting of hyalocytes and interspersed granulocytes (Fig. 4I); lateral and ventrolateral rows with granulocytes only (Fig. 3B, 4H); last ventral row in longitudinal line with male pores (Fig. 3B).

Testis in XI, ovary in XII (Fig. 3B). Seminal vesicle absent, spermatogonial cysts free in XI dorsally. Spermatozoa 36 µm, heads 14 µm. Sperm funnels (Fig. 4F) small, about 2–2.5x as long as wide, 60–100 µm by 26–40 µm, collar almost as wide as funnel body. Vas deferens often coiled in dense regular spirals, diameter 6–7 µm. Male pores (Fig. 3B) on body surface, widely distant, surrounded by area glareosa. Male glandular bulb spherical, diameter 25–35 µm (fix), pierced centrally by vas deferens; distance of bulbs 1.5–2x bulb diameter.

Spermathecae (Fig. 3A, 4C) short, extending into VI, rarely VII; often confined to V. Ectal pores lateral. Ectal duct slightly longer than wide, 20–24 µm by 14–20 µm (fix, N=6); ampulla 24–34 µm wide (fix, N=6), separated by a constriction from ectal duct in fixed specimens; spermatozoa placed obliquely in ampullar lumen; connecting tube thin, ental reservoir spherical, 30–36µm wide (fix, N=4).

Etymology. Named after the conspicuous lateral papillae. Due to their serial arrangement and easy recognition they appear as true gems when large numbers of specimens have to be identified to species level.

Remarks, morphology. This new species is most similar to *Achaeta camerani*, a common species in Northern and Central Europe (Schmelz & Collado 2010) and with additional records from NW Spain (Collado & Martínez-Ansemil 1996) and Japan (Nakamura & Christensen 1978). Both species often occurred together at the same plot or even in the same soil sample. They agree in (1) body size, (2) absence of pyriform glands, (2) presence of secondary pharyngeal gland lobes in V and VI, (3) preclitellar nephridia at 6/7, 7/8, (4) absence of a seminal vesicle, (5) short spermathecae with lateral ectal pores, and, most conspicuously, in (6) the saddle-shaped clitellum, without dorsal interruption and with cells arranged in distinct longitudinal rows.

Differences of *A. camerani* are based on own observations but confirm the details given in Graefe (1980), Chalupský (1992), and Dózsa-Farkas and Felföldi (2017): (1) Oesophageal appendage in V; (2) no conspicuously enlarged papillae; (3) coelomocytes larger, with a thinner margin, and with more granules in the centre; (4) dorsal blood vessel from VIII (in adult specimens); (5) spermathecae smaller, confined to V; (6) no clitellar gland cells in front of and behind the male pores; (7) longitudinal gland cell rows of clitellum with either hyalocytes or granulocytes, not both types in one row: dorsally two longitudinal rows of hyalocytes on each side, each separated by a longitudinal double row of granulocytes.

The first three of these differences are recognizable already in juvenile specimens. Of these the oesophageal appendage, present in *A. camerani* and absent in *A. gemmata* **sp. nov.**, is most useful for the separation of these two species, whereas differences in papillae and coelomocytes are not always clearcut: lateral papillae can be small and inconspicuous in juveniles of *A. gemmata* **sp. nov.**, whereas the lateral lense-shaped epidermal gland cells of *A. camerani*, found in exactly the same location as the papillae in *A. gemmata* **sp. nov.**, may be inflated to resemble rudimentary or incipient papillae. Considering the coelomocytes, there is overlap in the thinness of the outer margin and the degree of inner granulation.

This new species belongs to the group of *Achaeta* species without pyriform glands. All species of this group have spermathecal pores in lateral position (*vs*. ventral in most of the species with pyriform glands) and most of them have lentiform epidermal gland cells in segmentally fixed and repetitive positions. These cells vary among species in number, colouration, and size. Segmental series of enlarged lentiform gland cells with conspicuous inner bulges of the body wall or protrusions beneath it, called papillae in this paper, are described from three further species, *A. antefolliculata* Dózsa-Farkas & Boros, 2005, *A. hanagarthi* Schmelz, 2008, and *A. paranensis* Schmelz, 2008. In all of them, they are developed in different positions:

A. antefolliculata, described and known so far only from Hungary, has one pair each of enlarged lentiform gland cells laterally in III, IV, V, VI, and one dorsal pair in I: this dorsal shift in I is also seen in *A. gemmata* **sp. nov.** (Fig. 3A). These glands appear to be as large as the lateral papillae in *A. gemmata* **sp. nov.** Further differences to the new species are segment number (20–21), only one pair of secondary pharyngeal gland lobes, in V, a smaller spermatheca, which is always confined to V, and clitellar glands cells in transverse rows with a narrow dorsal interruption. *Achaeta hanagarthi* Schmelz, 2008 and *A. paranensis* Schmelz, 2008, both originally described and so far known only from Brazil, have enlarged lentiform gland cells only in ventral, but not in lateral, position; these glands are similar in shape and size to the ventral glands in *A. gemmata* **sp. nov.**, which are notably smaller than the lateral ones. Further differences to the new species are as follows: *A. hanagarthi*: no secondary pharyngeal gland lobes, 1st nephridium at 7/8, clitellum widely open dorsally; *A. paranensis*: oesophageal appendage present, large in V, with canal in IV, intestinal diverticulum in VII, only one pair of preclitellar nephridia, at 5/6.

TABLE 2. Species records of *Achaeta* spp. with publicly available COI-DNA barcodes, and their genetic differences to the holotypes of the two new species, calculated as uncorrected p-distances based on COI-DNA sequence differences. Only one sequence per species record is presented here. Distance between the two new species is 2.0. *: Specimen misidentified, see text.

	Specimen ID	Genbank Accession N.º	Country	Reference	*Achaeta florens* sp. nov.	*Achaeta gemmata* sp. nov.
Achaeta aberrans Nielsen & Christensen, 1961	CE875	GU902030.1	Sweden	Erséus *et al*. 2010	0,19	0,18
Achaeta affinis Nielsen & Christensen, 1959	919	KY583145.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,19	0,20
Achaeta affinis Nielsen & Christensen, 1959	none	GU453383.1	Denmark	Christensen & Glenner 2010	0,20	0,19
Achaeta bibulba Graefe, 1989	CE1206	GU902031.1	Sweden	Erséus *et al*. 2010	0,20	0,21
Achaeta bibulba Graefe, 1989	e115	MT159573.1	Russia	Lebedev *et al*. (unpublished)	0,19	0,21
Achaeta bifollicula Chalupský, 1992	CE1035	GU902032.1	Sweden	Erséus *et al*. 2010	0,19	0,16
Achaeta bohemica (Vejdovský, 1879)	885	KY583141.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,19	0,18
Achaeta cf. bohemica (Vejdovský, 1879)	CE1766	GU902033.1	Italy	Erséus *et al*. 2010	0,16	0,18
Achaeta brevivasa Graefe, 1980	CJJ92	ON419115.1	China	Chen *et al*. 2022	0,18	0,16
Achaeta cf. brevivasa Graefe, 1980	CE1234	GU902034.1	Sweden	Erséus *et al*. 2010	0,20	0,00*
Achaeta camerani (Cognetti, 1899)	903	KY583144.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,22	0,21
Achaeta camerani (Cognetti, 1899)	CE790	GU902035.1	Sweden	Erséus *et al*. 2010	0,22	0,21
Achaeta danica Nielsen & Christensen, 1959	901	KY583142.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,19	0,16
Achaeta cf. *danica* Nielsen & Christensen, 1959	860	KY583134.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,19	0,20
Achaeta iberica Graefe, 1989	CE1051	GU902036.1	Sweden	Erséus *et al*. 2010	0,20	0,17
Achaeta macroampullacea Dózsa-Farkas *et al*., 2018	1091	MG252131.1	Korea	Dózsa-Farkas *et al*. 2018	0,21	0,17
Achaeta tothi Dózsa-Farkas & Felföldi, 2017	882	KY583140.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,21	0,19
Achaeta unibulba Graefe, Christensen & Dózsa-Farkas, 2005	851	KY583130.1	Hungary	Dózsa-Farkas & Felföldi 2017	0,20	0,19
Achaeta unibulba Graefe, Christensen & Dózsa-Farkas, 2005	CE812	GU902037.1	Sweden	Erséus *et al*. 2010	0,20	0,19
Achaeta sp.	1143	MH124585.1	Korea	Nagy *et al*. (unpublished)	0,18	0,17
Achaeta sp.	BIOUG08058-B10	MF544984.1	Canada	deWaard *et al*. 2019	0,26	0,22
Achaeta sp.	812_E7	LT905364.1	Switzerland	Vivien *et al*. 2017	0,18	0,17
Achaeta sp.	1021_E7	LT903839.1	Switzerland	Vivien *et al*. 2017	0,20	0,19

The lentiform glands in *Achaeta etrusca* Rota, 1995 may also belong here (see Rota 2015) even though a figure (Rota 2015, Fig. 4A) suggests that they are less enlarged and more similar to the ordinary lentiform gland cells such as found in *A. pannonica* Graefe, 1989*, A. iberica* Graefe, 1989*, A. diddeni* Graefe, 2007*,* and *A. brevivasa* Graefe, 1980. All lentiform gland cells that we consider as enlarged have two different compartments: a non-staining central region which connects with the body surface, and a strongly staining peripheral region which extends into the body cavity. It is mainly this staining region which is responsible for the increase in size of the lentiform gland cells. These two regions are also distinguishable in living organisms, as an inner and outer circle (comp. Fig. 4G). Schmelz *et al.* (2008) refer to enlarged and non-enlarged cells as "type I" cells and "type II" cells, respectively, but the difference between them is probably only one of degree.

Apart from the gem-like lateral papillae, the dorsal pattern of the clitellar gland cells is a most conspicuous feature of this new species. The complete coverage with longitudinal rows—i.e., the absence of a mid-dorsal gap—is already distinguishable in subadult specimens (Fig. 4E), but the full pattern with alternating hyalocytes and granulocytes within a row is only seen in fully adult specimens (comp. Fig. 4I and 4E). As a further peculiarity, the most ventral row of granulocytes encloses the male pores, and one or two rows of granulocytes may be widened to form incomplete double rows (Fig. 4H, black asterisk).

Remarks, molecular comparison. Fourty-two COI sequences of 16 species of *Achaeta*, some of them without valid names, are publicly available. According to uncorrected p-distances calculated based on COI sequences (Table 2), high interspecific distances to *A gemmata* **sp. nov.** were observed (15.7%–21.8%), except for one specimen, identified as *Achaeta* cf. *brevivasa* Graefe, 1980 (0%, GenBank accession number: GU902034) by Erséus *et al.* (2010), and collected from Sweden. This specimen should be considered as a misidentified *Achaeta gemmata* **sp. nov.** Unfortunately, there is no voucher of this specimen for a morphological comparison. Chen *et al.* (2022) redescribed *Achaeta brevivasa* collected from China; the specimens correspond well with the original description. The three respective COI sequences (GenBank accession numbers: ON419115–ON419117) differ in 16.4% from that of *Achaeta* cf. *brevivasa* sensu Erséus *et al.* (2010). There are no DNA sequences from the type series of *A. brevivasa* and currently no other specimens identified as *A. brevivasa* with available DNA sequences.

Discussion

Molecular markers. Apart from the differences in COI, clear genetic gaps were also observed for the nuclear marker: Twenty-five ITS sequences belonging to 11 different species of *Achaeta* are publicly available; they were used for calculating uncorrected p-distances (data not shown). Interspecific distances ranged from 16.6% to 39.7%, confirming the results obtained with the mitochondrial marker. Unfortunately no ITS sequence was available for the misidentified specimen of *Achaeta* cf. *brevivasa* (see above).

DNA was sequenced in this study to obtain molecular identifiers, e.g., to facilitate DNA-based identification of soil fauna in the future, and to assure the identity of the species in case that cryptic species occur, a case very common in enchytraeids (Schmelz *et al.* 2017; Martinsson & Erséus 2021). For this reason we made sure that the holotype had DNA sequences. DNA-based species comparison and analysis of phylogenetic relationships are still hampered by the limited number of *Achaeta* species for which DNA sequences are available, therefore the main evidence to erect *A. florens* and *A. gemmata* as new species is based on morphology, and trees based on molecular distances are not presented here: phylogeny is not the topic of this contribution.

Ecology. An analysis of the enchytraeid species assemblages found at the two study sites will be presented elsewhere. Up to now, only one study on post-mining areas in Poland has included enchytraeid species diversity (Józefowska *et al.* 2020). The study sites (combustion waste and reclaimed post-mining sand pit) were similar to those where *Achaeta florens* **sp. nov.** was found, except that they were afforested by various species of alder (*Alnus incana, A. viridis, A. glutinosa*). In Józefowska *et al.* (2020) only a few juvenile specimens of *Achaeta* were found at sites afforested with *Alnus viridis.*

Achaeta florens **sp. nov.** was found only at one location within the post-mining area, a site afforested with red oak. In slightly acid soil (pH 4.7) with sandy loam and loamy sand texture, *Achaeta florens* **sp. nov.** ranked as the second most abundant enchytraeid species, following *Oconnorella cambrensis* (O'Connor, 1963) and preceding species typically found in this area, such as *Cognettia chlorophila* (Friend, 1913), *Marionina clavata* Nielsen & Christensen, 1961, *Enchytraeus norvegicus* Abrahamsen, 1969 and *Enchytronia parva* Nielsen & Christensen, 1959. Its ecological preferences are still unknown.

On the other hand, *Achaeta gemmata* **sp. nov.** was observed in all soil samples taken from post-fire stands. Alongside *Oconnorella cambrensis*, it ranked as the second most abundant enchytraeid species in the surveyed soil, following *Cognettia chlorophila*. *Achaeta camerani* appeared nearly ten times less abundant as *Achaeta gemmata* **sp. nov.**, making the latter the dominant *Achaeta* species at the post-fire investigation sites. The new species was frequently found in high abundance at sites where, thirty years ago, residues from the fire had been removed. However, in post-fire areas where residues had remained, its abundance was lower. Likewise, soil under oak trees had a higher abundance compared to other tree species. This discrepancy might be associated with the soil's acidity; soil with burned residue had a pH approximately 0.35 lower than soil in areas where burned residue had been eliminated. On the other hand, we observed higher mortality of worms that had been extracted from soil with neutral to slightly acid water (pH 5). When pH of the extraction water was adjusted to about 4.0 or slightly lower, mortality was reduced. It seems that *Achaeta gemmata* **sp. nov.** is well-adapted to a narrow range of low pH, but more records, and perhaps experiments, are needed to better describe the ecological preferences of this species with respect to pH and other soil parameters. *Achaeta gemmata* **sp. nov.** appears to be a common species in this region, and the new record of this species from Sweden—based on the identity of COI sequences of "*Achaeta* cf. *brevivasa*" in Erséus *et al.* (2010) and *Achaeta gemmata* **sp. nov.**—suggests that this species has a wider distribution in Europe.

With the description of *Achaeta florens* **sp. nov.** and *Achaeta gemmata* **sp. nov.** there are now eleven species of *Achaeta* that have been recorded from Poland.

Acknowledgements

Research was funded by The National Science Centre, Poland, grant No. 2021/42/E/ST10/00248. Work of J.C. was funded by the Chinese Scholarship Council. Cordial thanks to the staff of the laboratory of GIBE (Grupo de Investigación en Biología Evolutiva) at CICA, Centro de Investigaciones Avanzadas, University of A Coruña, Spain, where large parts of the taxonomic work were carried out. Detailed comments and suggestions of Klára Dózsa-Farkas, Jiří Schlaghamerský and Mårten Klinth greatly helped to improve the manuscript.

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