Molecular Phylogeny of Acerentomidae (Protura), with Description of Acerentuloides bernardi sp. nov. from North America

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Molecular phylogeny of Acerentomidae (Protura), with description of Acerentuloides bernardi sp. nov. from North America

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Abstract

The closely related genera Acerentulus Berlese, 1908 and Acerentuloides Ewing, 1921 are represented in the USA by only 3 species: Acerentulus confinis (Berlese, 1908), Acerentulus christensoni (Ewing, 1940), and Acerentuloides americanus (Ewing, 1924). Study of proturan material from North America yielded a new species belonging to Acerentuloides. The new species is characterized by the absence of seta s4d on the head, presence of a minute apical median seta on abdominal legs II and III, unique shape of maxillary gland with distal row of clustered globules, presence of setae modified as short, thickened sensilla (sds on head, β1 and δ4 on foretarsus, setae P4 on metanotum and A2 on thoracic sternite, and accessory setae on tergites and sternites I–VI), absence of seta P1a on abdominal tergites I–VI, foretarsal sensillum b longer than c, and presence of psl pores only on tergite VI. Molecular sequences for several Acerentomidae, including A. confinis, were used to develop hypotheses about species relationships within Acerentomidae.

Key Words: Acerentulus; Podolinella; Indiana; USA; DNA barcoding; phylogeny

Resumen

Los géneros Acerentulus Berlese, 1908 y Acerentuloides Ewing, 1921, muy relacionados entre sí, están representados en Estados Unidos por sólo 3 especies: Acerentulus confinis (Berlese, 1908), Acerentulus christensoni (Ewing, 1940) y Acerentuloides americanus (Ewing, 1924). El estudio del material de proturas de Norteamérica resultó en una nueva especie perteneciente a Acerentuloides. La nueva especie se caracteriza por la ausencia de seta s4d en la cabeza, la presencia de una seta muy corta en el apéce central de las patas abdominales II y III, la forma única de la glándula maxilar con una hilera distal de glóbulos agrupados, la presencia de setas modificadas como sensilas cortas y gruesas (sds en cabeza, β1 y δ4 en tarso anterior, setae P4 en el metanotum y A2 en esternon torácico y setas accesorias en tergitos y esternitos I–VI), ausencia de seta P1a en tergitos abdominales I–VI, sensillum b de primer tarso más largo que c, y la presencia de poros psl solamente en el tergito VI. Se utilizaron secuencias moleculares para varios Acerentomidae, incluyendo A. confinis, para desarrollar la hipótesis sobre las relaciones de especies dentro de Acerentomidae.

Palabras Clave: Acerentulus; Podolinella; Indiana; Estados unidos; códigos de barras de ADN; filogenia

The history of the work on the genus Acerentuloides [Entognatha: Protura: Acerentomidae], especially of its type species, Acerentuloides americanus (Ewing, 1924), is worth examining. The genus was originally described by Ewing (1921) with Acerentuloides bicolor Ewing, 1921 as its type species. The species A. americanus was originally described by Ewing (1924) as Acerentomon americanum (Ewing, 1924). Acerentomon americanum was transferred to the genus Acerentulus after revision of Ewing’s type material of Protula (Hilton 1943; Bonet & Tuxen 1960). Tuxen (1963) created for this species the new genus Amenterulus Tuxen, 1963 with the type species Amenterulus americanus (Ewing, 1924), despite the fact that type material was lost and Tuxen had doubts about the validity of the new genus (Tuxen 1964, p. 225). Nosek & McEwan Kevan (1984, p. 59), studying Canadian specimens of A. americanus, re-established the generic name of Acerentuloides and synonymized it with Amenterulus, with the type species Amenterulus americanus.

The genus Acerentuloides contains only the single species A. americanus, distributed in the USA and Canada (Szeptycki 2007). The morphologically similar genus Acerentulus comprises 49 species (Szeptycki 2007; Wu & Yin 2007; Shrubovych et al. 2012, 2014b; Galli & Capurro 2013) and is distributed worldwide, but in North America only 2 species are known: Acerentulus confinis (Berlese, 1908) and Acerentulus christensoni (Ewing, 1940). In the present paper, we describe a second species of Acerentuloides, provide a generic diagnosis, and discuss the phylogenetic position of the genus Acerentuloides, subfamily Berberentulinae and family Acerentomidae within Acerentomata.

Materials and Methods

Material of the new species was collected in the framework of a soil biota study in post-mining sites in the USA (Frouz et al. 2013). Samples were collected from litter and rhizosphere of deciduous forest with dominant black locust tree (Robinia pseudoacacia L.; Fabaceae) and red oak (Quercus rubra L.; Fagaceae). The forest patch is a 16-yr-old forest patch is a 16-yr-old

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re-growth after coal mining in the Patoka River National Wildlife Refuge and Management Area near the Hugh Boyd Fishing Pier & Wildlife Observation Deck, near Oakland City, Gibson County, Indiana. Specimens of A. confinis were collected from leaf litter at the base of white oak in woods in Calhoun, McMinn County, Tennessee (35.295833°N, 84.748056°W), on 6 Aug 2013 by E. C. Bernard and K. J. Whitlock. The specimens were extracted from soil and moss samples with a Berlèse–Tüllgren funnel into 95% ethanol. Specimens were mounted on microscope slides in Marc André II medium (Massoud 1967) and were examined with a compound microscope with differential interference contrast optics at magnifications ranging from 250× to 1000×. Drawings were made with a drawing tube. Specimens for scanning electron microscopy (SEM) observations were preserved in 100% ethanol before critical point drying (Emitech K850, Quorum Technologies Ltd, Laughton, England) and sputter gold coating, and imaged with a Jeol JFC-1200 (JEOL Ltd, Tokyo, Japan) scanning electron microscope.

All synonyms, systematics, and literature data up to 2006 are given in Szeptycki (2007). Head seta designations follow Rusek et al. (2012); terminology for body chaetotaxy and porotaxy follows Szeptycki (1988, 1993) and Shrubovych (2014), body indices (PR, CF, BS, TR, EU) follow Tuexen (1964). Abbreviations used in the description are as follows: Abd = abdominal segments, al = anterolateral pore, cp = clypeal pore, fp = frontal pore, ps1 = posterolateral subpore, psm = posterosubmedial pore, spm = sternal posterosmedial pore, Th = thoracic segments.

In addition to the morphological description, the DNA barcode sequences were amplified to enhance the description (Stevens et al. 2011). DNA was extracted from 95% ethanol preserved specimens with a Qiagen DNeasy tissue extraction kit (Qiagen, Hilden, Germany). The voucher specimens were then retrieved, further cleared in lactic acid solution for approximately 24 h, and mounted on microscope slides in Marc André II mounting medium. Polymerase chain reaction (PCR) for DNA amplification was carried out in a 25µL volume reaction using the TotalPrep spin kit (BioSprint; Izolyte, UK) and Mastermix 5x reagent (MP Biomedicals, Santa Ana, California). The thermocycler program consisted of an initial denaturation step at 94 °C for 2 min, 5 amplification cycles with a 45 °C annealing temperature (94 °C for 40 s, 45 °C for 40 s, 72 °C for 1 min), 30 cycles with a 51 °C annealing temperature, and a final extension step at 72 °C for 5 min.

The PCR amplification and sequencing were carried out with primers newly designed for Protura: LCOProt (5'–TTT CTA CTA ATC ATA AGG ATA TTG G-3') and HCOProt (5’–TAN ACY TCW GGR TGS CGA AAR AAT TA–3'). We also selected 3 other loci to amplify for the phylogenetic analyses: 18S rRNA and 28S rDNA (regions D1–D2 and D3–D6) nuclear ribosomal DNA. The 18S rRNA loci were PCR amplified in 3 overlapping fragments using primer pairs 1F + 5R, 3F + 185Bif, and 5F + 9R (e.g., Giribet et al. 2004). For the 28S rDNA regions, details are described in Schneider & D’Haese (2013) and Stevens & D’Haese (2014), and several primer pairs were used combining Collembola and Protura specific primers (some newly designed for the present work): 28S-C1c (5’–ACC CGC YWA ATT TAA GCA T-3') and 28S-Ap (5’–CGT GTT TCA AGA CGG GYC-3') for 28S rDNA D1–D2; and 28S-Ap (5’–GRC CGC TCT TAA ACG-3') and 28S-D4DSR (5’–GTT ACA CAC TCT TTA AGC GA-3') for 28S rDNA D3–D6.

The PCR products were sequenced by Eurofins (Paris, France). Resulting chromatograms were interpreted using the program Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, Michigan) or CodonCode Aligner 6.0.2 (CodonCode Corporation, Centerville, Massachusetts). Sequences were manually checked for misreadings of the chromatograms.

Phylogenetic affinities were analyzed by comparing the obtained COI, 18S rRNA, 28S rDNA D1–D2, and 28S rDNA D3–D6 sequences with sequences of other members of Acerentomidae published in previous works (Shrubovych et al. 2012, 2014b,c, 2016; Resch et al. 2014) completed with published sequences (Bu & Wu 2012; Bai & Bu 2013) and other unpublished sequences found in BOLD (Table 1). Other Acerentomata genera (Neaconceedium [Protentomidae], Protreutomon [Protentomidae], and Hesperentomon [Hesperentomidae]) were used to root the trees (see Table 1). Some of the COI sequences downloaded from GenBank or the Barcode of Life Data System (BOLD) were shorter than the barcode 658 bp so the missing ends of the alignment were completed with Ns. Sequences were aligned, for each locus independently, using MUSCLE v3.8.31 with default parameters (Edgar 2004).

Maximum likelihood (ML) analyses were performed using the partitioned ML method in RaxML (Stamatakis 2014) through the graphical interface raxmlGUI v1.5 (Silvestro & Michalak 2012). A thorough ML tree search was conducted with the GTR + f + I model through 10 runs, and nodal branch support was evaluated with 1,000 bootstrap replicates (+BS brl). Identical sequences were not omitted for the analysis. Parsimony under dynamic homology was performed using TOY v5.1.2 (Wheeler et al. 2015) for 9 gap:transversion:transition Sankoff matrices ranging from 1:1:1 to 16:4:1 (for a detailed procedure, see Schneider & D’Haese 2013). All trees were visualized using Figtree v1.4.2 (Rambaut & Drummond 2010).

Results

The genus Acerentuloides is characterized by 2 pairs of A-setae on the mesonotum and metanotum; 3 setae on the 2nd and 3rd pair of abdominal legs, of which the apical median seta is minute; well developed labial palpus with terminal tuft of setae and broadened sensillum; maxillary gland with small, smooth, globular vesicle or granulated appendix on the calyx and with several small globules in distal part; some setae modified as short, thickened sensilla (sd5 on head; β1 and δ4 on foretarsus; setae P4 on metanotum; M2 on pro sternum, and A2 on thoracic sterna; and accessory setae on tergites and sternites I–VI); 6 setae on sternite VIII; anterior position of seta P3 on abdominal tergites II–VI; developed striate band with clearly visible parallel-sided striae. Foretarsal sensillum t1 is claviform, sensillum t3 is long and lancet-like (Bonet & Tuexen 1960) or finger-like, sensillum b’ is present, the base of sensillum d is closer to base of c than to base of e, and the position of sensillum a’ is close to the base of t1.

Acerentuloides bernardi Shrubovych sp. nov., 2016 (Figs. 1–34; Table 2)

TYPE MATERIAL

The type materials were deposited in the collection of the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences (ISEA), Cracow, Poland, and in the collection of the State Museum of Natural History (SMMH) of the National Academy of Sciences of Ukraine, L’viv, Ukraine.

HOLOTYPE 1 female (ISEA 6647) USA: Indiana, Oakland City, Hugh Boyd, Patoka River National Wildlife Refuge and Management Area, 9-V-2008, latitude 38.3539°N, longitude 87.3156°E, elevation 133 m, deciduous growth with dominant black locust tree (Robinia pseudoacacia L.; Fabaceae) and red oak, sample of litter and rhizosphere, collected by J. Tajovsky & J. Frouz.

PARATYPES 2 females (ISEA 6648, SMNH 89.1), data same as holotype.
Table 1. List of investigated proturans and accession numbers of gene sequences used in the phylogenetic analysis (a dash indicates that the gene sequence was not available).

| Species name                     | 28S rDNA D3–D6 | 28S rDNA D1–D2 | COI  | 18S rRNA | Family                      | Subfamily                  |
|----------------------------------|----------------|----------------|------|----------|-----------------------------|---------------------------|
| Proturentomon chinense           | EU557257       | EU557257       | HQ882814 | EU557244 | Protentomidae               | Protentominae             |
| Neoconelium brychytarsum         | EU557259       | EU557259       | HQ882816 | EU557245 | Protentomidae               | Condeeolina               |
| Neoconelium dolichotarsus        | EU557258       | EU557258       | HQ882815 | —         | Protentomidae               | Condeeolina               |
| Hesperentomon nanshanensis       | —              | —              | —     | KF530828 | Hesperentomidae             | Hesperentominae           |
| Hesperentomon pectigastrolum      | EU557255       | EU557255       | HQ882812 | EU557242 | Hesperentomidae             | Hesperentominae           |
| Hesperentomon yangi               | —              | —              | —     | KF530824 | Hesperentomidae             | Hesperentominae           |
| Acerella muscorum                | HQ882811       | HQ882811       | HQ882825 | HQ882809 | Acerentomidae               | Acerellinae               |
| Acerella muscorum                | —              | —              | —     | —         | Acerentomidae               | Acerellinae               |
| Acerentomon carpaticum PROTA003  | PROTA003-15    | PROTA003-15    | PROTA003-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon carpaticum PROTA006  | PROTA006-15    | PROTA006-15    | PROTA006-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon carpaticum PROTA007  | PROTA007-15    | PROTA007-15    | PROTA007-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon dispar PROTA001       | PROTA001-15    | PROTA001-15    | PROTA001-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon dispers PROTA009      | PROTA009-15    | PROTA009-15    | PROTA009-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon dispers PROTA023      | PROTA023-15    | PROTA023-15    | PROTA023-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon dispers PROTA024      | PROTA024-15    | PROTA024-15    | PROTA024-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon dispers PROTA025      | PROTA025-15    | PROTA025-15    | PROTA025-15 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon italicum PROATA052   | PROAT052-12    | PROAT052-12    | PROAT052-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon maius PROAT001        | PROAT001-12    | PROAT001-12    | PROAT001-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon maius PROAT005        | PROAT005-12    | PROAT005-12    | PROAT005-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon maius PROAT17         | PROAT17-12     | PROAT17-12     | PROAT17-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon maius PROAT24         | PROAT24-12     | PROAT24-12     | PROAT24-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon microthinus HQ8828    | HQ882810       | HQ882810       | HQ882819 | HQ882808 | Acerentomidae               | Acerentominae             |
| Acerentomon microthinus JQ728012  | —              | —              | —     | —         | Acerentomidae               | Acerentominae             |
| Acerentomon christiani PROAT026  | PROAT026-12    | PROAT026-12    | PROAT026-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon christiani PROAT027  | PROAT027-12    | PROAT027-12    | PROAT027-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon christiani PROAT040  | PROAT040-12    | PROAT040-12    | PROAT040-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon christiani PROAT055  | PROAT055-12    | PROAT055-12    | PROAT055-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon christiani PROAT064  | PROAT064-12    | PROAT064-12    | PROAT064-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon christiani PROAT071  | PROAT071-12    | PROAT071-12    | PROAT071-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon scop microthinus PROAT022 | PROAT022-12 | PROAT022-12 | PROAT022-12 | —         | Acerentomidae               | Acerentominae             |
| Acerentomon sp. JX963616          | —              | —              | —     | —         | Acerentomidae               | Acerentominae             |
| Filientomon takanawanum          | EU557261       | EU557261       | HQ882822 | EU557249 | Acerentomidae               | Acerentominae             |
| Huashantulus huashanensis        | EU557262       | EU557262       | HQ882821 | EU557248 | Acerentomidae               | Acerentominae             |
| Yamatenotomon guoi YHYH45        | —              | —              | —     | —         | Acerentomidae               | Acerentominae             |
| Yamatenotomon guoi YHYH46        | —              | —              | —     | —         | Acerentomidae               | Acerentominae             |
| Yamatenotomon guoi YHYH47        | —              | —              | —     | —         | Acerentomidae               | Acerentominae             |
| Acerentulus charrieri cs59 ABPE002-17 | ABPE002-17 | —              | —     | JQ411216 | ABPE002-17                 | Berberentulinae           |
| Acerentulus charrieri cs64 ABPE003-17 | ABPE003-17 | —              | —     | JQ411217 | ABPE003-17                 | Berberentulinae           |
| Acerentulus confinis p029 ABPE004-17 | —              | ABPE004-17     | —     | ABPE004-17 | Acerentomidae               | Berberentulinae           |
| Acerentulus noeli csp06 ABPE005-17 | ABPE005-17     | ABPE005-17     | —     | KJ210840 | ABPE005-17                 | Berbereentulinae          |
| Acerentulus noeli csp13 ABPE006   | —              | —              | —     | KJ210841 | —                           | Berbereentulinae          |

*Extension after the species name corresponds to the specimen voucher code.*
Habitus typical for members of Acerentomidae (Fig. 24). Head setae short, cephalic seta I3 setiform (Figs. 1 and 2). Setae sds5 thick, sensilliform (Figs. 1 and 3). Setae sds4 and d6 absent, length ratio of posterior setae d7:sd7 as 1.0:1.5 (Fig. 1). Pseudoculus circular, with short posterior extension, PR = 14 (Fig. 2). Maxillary palpus apically with tuft of setae and 4 single setae, basal sensilla slender, subequal in length (Fig. 4). Labial palpus well developed with 4-branched api
cal tuft and sausage-shaped basal sensillum (Figs. 5 and 25). Maxi
lary gland with small elongated and weakly granulated calyx and
small globular vesicles on calyx, long posterior filament with row
of small globules posteriorly ending in bilobed dilation, CF = 3.7
(Fig. 6).

Foretarsus with sensillum b’ present, t1 claviform, t3 long and finger-like, all other sensilla slender and parallel-sided (Figs. 9, 10, and 26). Sensillum a reaching base of t2. Sensillum b longer than c, extending past base of sensillum e. Sensillum c reaching base of sensillum e. Sensillum b inserted proximally to c, sensillum d inserted between sensilla c and t2. Sensillum a’ short, extending past base of t2 (Fig. 26). Sensillum b’ reaching base of sensillum c’, apex of sensillum c’ not reaching base of claw. Length formula of sensilla: t1 < t3 < (a′ = b′) < (g = e) < (a = d) < b < f. Setae b1 and d4 modified, sensilliform, shorter than other 6-setae (length of b1 = 4 μm, d4 = 6 μm). Pores on foretarsus near base of sensillum t3 and between bases of sensillum c and seta a3. Claw short, without inner tooth, empodial appendage short. BS = 0.6, TR = 3.6, EU = 0.12.

Formula of chaetotaxy given in Table 2. Setae on nota strongly differing in length. Pronotal seta 1 twice the length of seta 2 (Fig. 11). Meso- and metanotal setae P1a and P2a very short, oblong (Fig. 27), P2a situated close to P3, seta P5 gemmate (Figs. 11 and 12). Length ratio of mesonotal setae P1: P2 = 1.0:1.6. Setae P4 on metanotum a short sensillum, length 3 μm (Fig. 12). Meso- and metanota with sl and al pores (Figs. 11 and 12). Thoracic sternum without pores. Setae A2 and M2 on prosternum and seta A2 on thoracic sternum short, sensilliform; length of prosternal A2 = 3 μm, of prosternal M2 = 2 μm (Figs. 28 and 29), of meso- and metasternal A2 > = 1 μm (Figs. 13–15).

Setae A5 and P2a on tergite I, setae P2a and P4a on tergites II–VI short and sensilliform, their length 3 μm (Figs. 16, 17, and 30). Accessory setae P1a, P2a, and P4a on tergite VII setiform, longer, length 4 μm (Fig. 18). Setae P3 on tergites I–VI inserted anteriorly to other setae of P-row (Fig. 17), P3 on tergites I and VII level with other setae of P-row (Figs. 16 and 18). Tergites VII with connecting line in anterior part (Figs. 17 and 18). Pores psm present on tergites I–VII between setae P1 and P2, and close to seta P1a on tergite VII, al pores on tergites II–VII, psl pores on tergite VI only (Figs. 16–18).

Abdominal legs with 4, 3, 3 setae. Subapical setae on 2nd and 3rd pairs of abdominal legs nearly twice the length of apical lateral seta (21 and 12 μm, respectively). Apical median seta minute, length 1 μm (Figs. 21 and 31). Accessory setae P1a on sternites I–VII sensilliform, on sternite VII setiform, short, P1a length on sternites II–VII 3 μm (Figs. 20, 22, 23, and 31–33). Sternites VII–VIII with a connecting line in anterior region (Figs. 22 and 23). Sternites II–V with asymmetrical pspsm pore (Fig. 20). Sternite VI with pspsm pores, 2 near each other and 1 close to base of seta P1 (Figs. 22 and 32); sternite VII with pspsm pore (Fig. 23) or in 1 paratype with asymmetrical pspsm pore between setae P1 and P1a (Fig. 33).

Abdominal segment VIII with well-developed striate band (Figs. 19 and 34). Pore psm without accompanying teeth (Fig. 19). Posterior margin of sternite VIII and laterotergites smooth (Figs. 23 and 34). Comb on tergite VIII with 8–9 short teeth (Fig. 7). Sternites IX–XII with smooth hind margin; setae 1 about one-third length of setae 2 (Figs.

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**Table 1.** (continued) List of investigated proturans and accession numbers of gene sequences used in the phylogenetic analysis (a dash indicates that the gene sequence was not available).

| Species name | Family | Subfamily | GenBank Accession Numbers |
|--------------|--------|-----------|--------------------------|
| Acerentulus noeli | Acerentomidae | Berberentulinae | ABPE008-17, ABPE009, ABPE010-17, E2577280, E2577281, KJ395311, KJ395312, KJ395313, KJ395314 |
| Acroyrus pyreneicus | Acerentomidae | Berberentulinae | ABPE008-17, ABPE009, ABPE010-17, E2577280, E2577281, KJ395311, KJ395312, KJ395313, KJ395314 |
| Andinentulus rapoporti | Acerentomidae | Berberentulinae | ABPE008-17, ABPE009, ABPE010-17, E2577280, E2577281, KJ395311, KJ395312, KJ395313, KJ395314 |
| Baculentulus densus | Acerentomidae | Berberentulinae | ABPE008-17, ABPE009, ABPE010-17, E2577280, E2577281, KJ395311, KJ395312, KJ395313, KJ395314 |
| Gracilentulus maijiawensis | Acerentomidae | Berberentulinae | ABPE008-17, ABPE009, ABPE010-17, E2577280, E2577281, KJ395311, KJ395312, KJ395313, KJ395314 |
| Nosekiella sinensis | Acerentomidae | Berberentulinae | ABPE008-17, ABPE009, ABPE010-17, E2577280, E2577281, KJ395311, KJ395312, KJ395313, KJ395314 |

*Extension after the species name corresponds to the specimen voucher code.*
23 and 34). Segment XII with medial pore on dorsal lobe and pair of sternal anterolateral pores.

Female squama genitalis with short, forked acrostyli (Fig. 8). Males unknown.

Body measurement (3 females): body length 1,100 μm; head 130–134 μm; pseudoculus 9 μm, lever 1 μm; posterior part of maxillary gland 35 μm; head setae d7 = 11 μm, sd7 = 16 μm, l3 = 6 μm; pronotal setae 1 = 25 μm, 2 = 12 μm, mesonotal setae P1 = 19–20 μm, P1a = 1 μm, P2 = 30–31 μm; foretarsus 90 μm, claw 25 μm, empodial appendage 3 μm.

**ETYMOLOGY**

The new species is cordially dedicated to our colleague and eminent zoologist, Prof. Dr. Ernest C. Bernard.

**REMARKS**

The habitus of the new species is very similar to *A. confinis* (Figs. 24 and 35). The species is characterized by absence of seta sd4 and additional seta d6 on head, absence of seta P1a on abdominal tergites I–VI, presence of seta Pc on sternite VII, short sensillum a reaching to...
the base of sensillum t2, long foretarsal sensilla b and f, sensillum c shorter than b, short and slender sensilla a’, b’ and c’, and presence of psl pores on tergite VI only. *Acerentuloides bernardi* sp. nov. is similar to *A. americanus* in the shape of the maxillary gland, presence of a minute apical medial seta on abdominal legs II and III, shape of the comb and the length of foretarsal setae a, b’ and c’ (Ewing 1921, 1940; Bonet & Tuxen 1960). The new species differs in possessing seta Pc on sternite VII and in the shape of foretarsal sensillum t3 (lancet-like in *A. americanus*, finger-like in the new species). Foretarsal sensillum b is longer than c and clearly surpasses the base of seta y3 in the new species, whereas in *A. americanus* sensillum b is shorter and does not reach the base of y3; in Canadian specimens of *A. americanus* sensilla b and c are of equal length (Nosek & McEwan Kevan 1984) and sensillum a’ is longer and extends past the base of sensillum t2. In addition, the new species has a smooth, globular vesicle on the maxillary gland, whereas Canadian specimens of *A. americanus* have a granulated appendix on the calyx (Nosek & McEwan Kevan 1984).

MOLECULAR ANALYSIS

Unfortunately, DNA was not retrieved from specimens of *A. bernardi* sp. nov. However, the sequencing of *A. confinis*, a species from a closely related genus, was successful. A 658 bp fragment of the COI gene (DNA barcode) as well as 1,541 bp of the 18S rRNA and 971 bp of the 28S rDNA D1–D2 were amplified and sequenced from a specimen collected in the USA. To complete the COI data sets previously published (Shrubovych et al. 2012, 2014b, 2014c), other *Acerentulus* and *Andinentulus* species were amplified for 18S rRNA: *Acerentulus charrieri* Shrubovych, Schneider & D’Haese, 2012, *A. noeli* Shrubovych, Schneider & D’Haese, 2014, *A. pyreneicus* Shrubovych, Schneider & D’Haese, 2014, and *Andinentulus rapoporti* (Condé, 1963); 28S rDNA D1–D2: *A. noeli* and *Andinentulus rapoporti* (2 different specimens); 28S rDNA D3–D6: *A. charrieri* (2 different specimens), *A. noeli* (2 different specimens), *A. pyreneicus*, and *Andinentulus rapoporti*. The sequences were deposited in BOLD under accession numbers ABPE002-17-ABPE011-17 (see Table 1).

The concatenated data (COI, 18S rRNA, 28S rDNA D1–D2, and 28S rDNA D3–D6) consist of a total alignment length of 4,438 bp for 55 terminal taxa (27 species). The ML inference and direct optimization parsimony phylogenies all showed consistent agreement among topologies. The only real difference is the position of Nosekiella+Huashanentulus sister group of Acerella in the ML analysis and sister group of the rest of Acerentominae in parsimony. Here we show the ML tree (Fig. 41).

Discussion

Tuxen created the genus *Amerentulus* Tuxen, 1963 (currently *Acerentuloides* [Ewing, 1921]) on the basis of 2 differentiating peculiarities: the minute apical medial setae on 2nd and 3rd pairs of abdominal legs and the presence of a distal row of globules on the maxillary gland. The species described in the present paper is characterized by the absence of cephalic setae s4d, which is present in all *Acerentulus* species (see Fig. 36, American specimen of type species *A. confinis* [Berlese, 1908]). So far, this characteristic has been observed only in the monotypic genera *Podolinella* Szepycki, 1995 and *Maderentulus* Tuxen, 1963 (Tuxen 1982, fig. 1). Szepycki (1995) stated this characteristic in his generic diagnoses. Therefore, the absence of this seta could be an additional morphological character for *Acerentuloides* or perhaps only for *A. bernardi* sp. nov. The genus *Podolinella* differs from *Acerentuloides* in that abdominal legs II and III have only 2 setae and the labial palpus is reduced to a sensillum and 3 setae. *Maderentulus* differs from *Acerentuloides* in the shape of abdominal legs II and III with 2 setae and shape of foretarsal sensillum t1 (filiform) and t3 (cylindrical). The new species is characterized by having a finger-like foretarsal sensillum t3, which differs from the leaf-like shape of this sensillum in *Acerentulus* species, such as in American specimens of *A. confinis* (Fig. 37).
Figs. 16–23. *Acerentuloides bernardi* sp. nov. 16. Tergite I, right side; 17. tergite VI, right part; 18. tergite VII, right part; 19. tergite VIII; 20. sternite II; 21. abdominal leg of sternite II; 22. sternite VI; 23. sternites VII–IX. Arrows indicate pores (psm = tergal posterosubmedial, psl = tergal posterosublateral, spsm = sternal posterosubmedial, spm = sternal posteromedial). Scale bars: 20 µm.
Porotaxy of the new species is typical for species of *Acerentulus*. The presence of psl pores on tergite VI and absence on tergite VIII may be specific features of *A. bernardi* sp. nov., but the porotaxy of *Acerentulus* species is not well known. The porotaxy of *A. americana* was not described, but Bonet & Tuxen (1960, pp. 283, 285) identified accessory setae P1a on tergites anterior to the posterior setae, exactly where psm pores are located in the new species. Furthermore, Tuxen (1964, p. 225) confirmed that these were dermal glands, not setae.

The genus *Acerentuloides* belongs to the group of Berberentulinae genera sensu Szeptycki (2007) that possess 3 setae on abdominal legs, such as *Acerentulus* Berlese, 1908, *Andinentulus* Tuxen, 1984, *Australentulus* Tuxen, 1967, *Brasilidia* Nosek, 1973, *Najtentulus* Szeptycki & Weiner, 1997, and *Fjellbergella* Nosek, 1978. Within this group *Acerentulus* and *Najtentulus* are characterized by a well-developed labial palpus with an apical tuft of setae (Fig. 38), 6 setae and well-developed striate band on sternite VIII (Fig. 39), and modified sensilliform seta P4 on metanotum (Fig. 40) (Szeptycki & Weiner 1997; Shrubovych et al. 2012, 2014b). *Australentulus* also has a well-developed striate band, but differs in possessing reduced labial palpi and 4 setae on sternite VIII. *Andinentulus*, *Brasilidia*, and *Fjellbergella* also have 6 setae on...
Table 2. Body chaetotaxy of *Acerentuloides bernardi* Shrubovych sp. nov., 2016.

| Segment | Dorsal | Ventral |
|---------|--------|---------|
| Th I    | 4      | 1, 2    |
| Th II   | 6      | A2, 4, M|
|         | 16     | P1, 1a, 2, 2a, 3, 3a, 4, 5 |
| Th III  | 6      | A2, 4, M|
|         | 16     | P1, 1a, 2, 2a, 3, 3a, 4, 5 |
| Abd I   | 6      | A1, 2, 5|
|         | 10     | P1, 2a, 3, 4 |
| Abd II–III | 6    | A1, 2, 5|
|         | 14     | P1, 2a, 3, 4, 4a, 5 |
| Abd IV–V| 6      | A1, 2, 5|
|         | 14     | P1, 2a, 3, 4, 4a, 5 |
| Abd VI  | 8      | A1, 2, 4, 5|
|         | 14     | P1, 2a, 3, 4, 4a, 5 |
| Abd VII | 8      | A1, 2, 4, 5|
|         | 16     | P1, 1a, 2a, 3, 4, 4a, 5 |
| Abd VIII| 6      | A2, 4, 5|
|         | 16     | P1, 2a, 3, 4, 4a, 5 |
| Abd IX  | 12     | 1a, 2a, 3, 4 |
| Abd X   | 12     | 1a, 2a, 3, 4 |
| Abd XI  | 6      | 1, 3, 4 |
| Abd XII | 9      | 1, 2 |

| Formulas | Setal composition | Formulas | Setal composition |
|----------|------------------|----------|------------------|
| Th I     | 4+4              | A1, 2, M1, 2 |
| Th II    | 5+2              | A2, 2, 3, M |
| Th III   | 7+2              | A2, 2, 3, 4, M |
| Abd I    | 3                | Ac, 2    |
| Abd II–III | 3    | Ac, 2    |
| Abd IV–V | 5                | P1, 1a   |
| Abd VI   | 8                | Ac, 2    |
| Abd VII  | 8                | P1, 1a   |
| Abd VIII | 4                | 1, 2     |
| Abd IX   | 4                | P1a      |
| Abd X    | 4                | 1, 2     |
| Abd XI   | 6                | 1, 2     |
| Abd XII  | 6                | 1, 2     |

*Abd = abdominal segments, Th = thoracic segments.*

*Acerentuloides* is morphologically close to the genera *Acerentulus* and *Najtentulus*, differing from them by the peculiar shape of the maxillary gland and strong reduction of the subapical median seta on abdominal legs II and III. Additionally, it differs from *Najtentulus* by the shape of the foretarsal sensillum t3 (finger-like in *Acerentuloides*, cylindrical in *Najtentulus*). The presence of a vesicle on the maxillary gland is found in only a few genera (*Vesiculentomon* Rusek, 1974, *Nosekientomon* Nosek, 1977, *Callientomon* Yin, 1980, and *Acerella* Berlese, 1909), but they belong to other subfamilies of Acerentomidae (Tuxen 1964; Shrubovych et al. 2014a).

Figs. 35–40. *Acerentulus confinis* (Berlese, 1908), American specimen scanning electron microscopy photographs. 35. Habitus; 36. pseudoculus and cephalic setae sd4 and l3; 37. foretarsus, exterior view: sensilla t1, t3 and a'; 38. labial palpi with apical tuft of setae and basal sensillum (s); 39. sternite VIII; 40. modified seta P4 on metanotum. Scale bars = 300 µm (Fig. 35), 20 µm (Figs. 36 and 38), and 40 µm (Figs. 37, 39, and 40).
Phylogenetic analyses, using both parsimony and ML, strongly support Acerentomidae as monophyletic (Fig. 41). Monophyly of Acerentomidae was already supported by the analysis of 67 morphological characters for 35 species of Acerentomata (Shrubovych 2014). The topology supports a monophyletic position of Berberentulinae. Berberentulinae is a subfamily including many genera (29 genera according to Szeptycki 2007) (Shrubovych 2014). In the present analyses, we included only 4 species of Berberentulinae, therefore more information about genetic data for morphologically differing genera of Berberentulinae is needed (such as Amphiherentulus Tuxen, 1981, Australentulus Tuxen, 1967, Kentyentulus Tuxen, 1961, Silvestridia Bonet, 1942, and Tasmanentulus Tuxen, 1984) to clearly confirm monophyly or even discuss family status for subfamily Berberentulinae.

Within Berberentulinae, species of Acerentulus form a separate and strongly supported taxon, which is sister group to a clade Andinentulus + Baculentulus + Gracilentulus. Within Acerentulus, A. confinis is the sister group of the remaining species of the genus. The Berberentulinae are sister group to a clade comprising all remaining subgroups proposed by Szeptycki (2007), i.e., Acerellinae + Nipponentominae + Acerentominae. Acerentominae are paraphyletic due to the position of the Nosekiella (Nipponentominae) as sister group to Huashanentulus (Acerentominae). François (2003) proposed to create a separate subfamily of Huashanentulinae for genera Huashanentulus Yin, 1980 and Sugaentulus Imadaté, 1978 based on 2 apomorphies: absence of foretarsal sensillum b' and well-developed striate band. These characters also are native for other members of the subfamilies Nipponentominae.

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- Acerentominae are paraphyletic due to the position of Nosekiella (Nipponentominae) as sister group to Huashanentulus (Acerentominae).

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neae, Acerellinae, and Acerentominae sensu Szeptycki (2007). Cladistic analysis, based on morphological characters, shows a separate position of *Huashanentulus* and *Sugaentulus* in a group of *Acerellinae* + *Nipponentominae* + *Acerentominae* genera (François 2003, p. 118, fig. 2, consensus tree). However, these genera do not form a joint clade on the consensus tree. Szeptycki (2007) included these genera in the Acerentominae together with morphologically similar genera (Shrubovyč & Rusek 2010). The genus *Huashanentulus* should be included in the *Nipponentominae* according to our genetic results due to the strongly supported joint clade *Huashanentulus huashanensis* Yin, 1980 with *Nosekiella sinensis* Bu & Yin, 2008. We need additional genetic data for remaining nipponentomine and acerentomine genera to arrive at more comprehensive phylogeographic hypotheses.

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