

# Description of a new species of *Acerentulus* Berlese 1908 (Protura: Acerentomata: Acerentomidae) with its barcode sequence and a key to the *confinis* group

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**Abstract.** *Acerentulus charrieri* n. sp. is described from France. This species belongs to the *confinis* group, which is characterized by a long foretarsal sensillum *a*. *Acerentulus charrieri* n. sp. is characterized by possessing of 8 anterior setae on abdominal tergite VII, presence of seta *P1a* and absence of seta *P3a* on abdominal tergite VII, foretarsal sensillum *b* longer than *c*, posterior position of seta  $\delta 4$  on foretarsus and absence of pores on sternite IV. The new species is similar to *A. gigas*, which was also described from France, and *A. apuliacus*, described from Italy. The mitochondrial cytochrome c-oxidase subunit I COI sequence (barcode) of the new species is provided.

**Résumé.** Description d'une nouvelle espèce *Acerentulus* Berlese 1908 (Protura : Acerentomata : Acerentomidae) avec sa séquence de barcode et une clé du groupe de *confinis*. *Acerentulus charrieri* n. sp. est décrite de France. Elle appartient au groupe *confinis* avec de longues sensilles *a* sur les tarsi de la première paire de pattes. *Acerentulus charrieri* n. sp. est caractérisée par la présence de 8 soies antérieures sur le tergite abdominal VII, la présence des soies *P1a* et l'absence des soies *P3a* sur le tergite abdominal VII, sensille *b* plus longue que *c* et position postérieure des soies  $\delta 4$  sur les tarsi de la première paire de pattes, l'absence de pores sur le sternite abdominal IV. La nouvelle espèce est proche d' *A. gigas*, elle aussi décrite de France, et de *A. apuliacus*, décrite d'Italie. Le gène mitochondrial de la sous-unité I de la cytochrome oxydase, COI (barcode) de la nouvelle espèce est joint.

**Keywords:** Chaetotaxy, porotaxy, DNA barcoding, COI, France.

To date, the genus *Acerentulus* Berlese 1908 comprises 46 species worldwide (Szeptycki 2007, Wu & Yin 2007). The type species *A. confinis* (Berlese 1908) has a cosmopolitan distribution, but most of data must be confirmed (*A. confinis* probably is a collection of closely related species in places other than Europe). More than half of the species (23) have been reported only from their type localities. Most of the species (40) have a Palaearctic distribution, mostly in Central and Southern Europe with the exception of six species described from Russia, Japan and China. The remaining six species are known from other parts of world: one from Australia, one from New Zealand, two from South America (Argentina) and one from North America (Mexico, USA). Eleven *Acerentulus* spp. are mentioned from France. Four of these species (*A. cassagnai* Nosek 1969, *A. corzeanus* Szeptycki 1997, *A. gigas* Szeptycki 1997, *A. proximus* Szeptycki 1997) are known only from their type localities. The present

paper contains the description of a new species from France, including its barcode (COI) sequence since DNA barcoding. An identification key to the *confinis*-group species worldwide is also provided.

## Material and methods

**Material.** Holotype female (No. 28.1): France, Cherier, Bois Vague, 45°59'01" N 03°50'45" E, 1030 m elev., 16.IV.2011, coll. G. Charrier and C. Schneider; 8 female and 2 male paratypes (No. 28.2–28.5 and 6611) same data as holotype, extracted from peat in woodland with Berlese-Tullgren funnel into 95% ethanol. All specimens are mounted as microscopic slides in Faure (Dunger & Fiedler 1989) or Marc André II medium. The holotype, 5 female and 1 male paratypes (slides No. 28.1–28.5) are deposited in the collection of the State Museum of Natural History, Lviv, Ukraine (SMNH). One female and 1 male paratypes (No. 6611) are deposited in the collection of the Institute of Systematics and Evolution of Animals, Cracov, Poland (ISEA), 2 female paratypes (No. MNHN-EA010059 and MNHN-EA010064) are deposited in the Museum of Natural History, Paris (MNHN).

DNA was extracted from 95% ethanol preserved specimens with a Qiagen DNeasy tissue extraction kit (digestion overnight and re-suspension in 120  $\mu$ l of the elution buffer). The specimens were retrieved after three hours of digestion in buffer+proteinase k and the extraction was carried on as ad-

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Accepté le 23 février 2012

vised by the manufacturer. Specimens from which DNA was extracted were further cleared in lactic acid solution for ~24 h and mounted on microscope slides in Marc André II mounting medium. DNA amplification was carried out in 25 µl volume reaction with Amersham Bioscience puReTaq Ready-To-Go PCR Beads. The thermocycler program consisted of an initial denaturing 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 step at 72 °C for 5 min. PCR amplification and sequencing were carried out with primers designed for *Collembola* (Greenslade et al. 2011): LCO1490col (5' - WYT CDA CWA AYC RYA ARG AYA TYG G - 3') and HCO2198col (5' - TAN ACY TCN GGR TGN CCR AAR AAT CA - 3').

## Results.

### *Acerentulus charrieri* n. sp.

#### Table 1, figs 1-24.

**Diagnosis.** It is a large species within *Acerentulus* genus. Foretarsal sensillum *a* long, seta  $\delta 4$  in distal position. Seta *P1a* absents on tergites I-VI, presents on tergite VII. Seta *P3a* absents on tergites I-VII. Seta *P4a* on tergite VII on membrane between tergite and laterotergite. Sternites I-IV without pores, sternites V-VI with 1+1 pores.

**Description.** Head with long setae, dorsally with three pairs of sensilliform setae (*ap*, *pp* and *ls*). Additional setae absent (fig. 1). Pseudoculus circular, with very short posterior extension, PR = 16 (fig. 2). Maxillary palps with slender sensilla, lateral longer than dorsal one (fig. 3). Labial palps well developed, with slender basal sensillum (fig. 4). Maxillary gland with large calyx, long posterior filament and simple posterior dilation, CF = 4.0-4.7 (fig. 5). Foretarsus (figs. 6-7) with long sensillum *a*, reaching to base of seta *y3*; *b* longer than *c*, surpassing the base of seta *y3*; *t1* claviform, *t2* slender and long, *t3* leaf-like. All other sensilla slender. Base of sensillum *d* closer to *c* than to *e*; *a'* situated distally from *t1* insertion. Relative length of sensilla:  $t3 < t1$

$< a' < (c = d = c') < (g = b') < (e = f < b < t2 < a)$ . Setae  $\beta l$  and  $\delta 4$  shorter and thicker than other  $\delta$ -setae, with rounded apices (figs. 8-9). Seta  $\delta 4$  slightly distal to base of *c'* (fig. 8). Claw long, without inner tooth, empodial appendage long. BS = 0.3-0.4, TR = 4.2-4.3, EU = 0.2.

Formula of chaetotaxy given in Table 1. Length ratio of pronotal setae 1 : 2 as 2.7-3.2 : 1. Setae *Pla* and *P2a* on meso- and metanota as short gemmate microchaetae (figs. 10-11); *P2a* close to *P3*. Length ratio of *Pl* : *P2* on mesonotum as 1 : 1.3-1.4. Mesonotum with *sl* and *al* pores, metanotum with *sl* pores only. Thoracic sterna without pores (fig. 12). Seta *A2* on thoracic sterna and seta *M2* on prosternum short, sensilliform.

Seta *P2a* on tergite I of same shape as *Pla* and *P2a* on nota, *A5* as short, slim linear microchaeta (fig. 13). Accessory setae on tergites II-VI sensilliform, relatively thick, on tergite VII slightly thinner (fig. 14). Seta *P3a* absent on tergites I-VII, seta *P4a* on tergite VII on isolated sclerite of membrane between tergite and laterotergite (fig. 15). Seta *P3* on tergites II-V anterior to other P-setae, on tergites VI-VII in the row of P-setae (fig. 16). Tergites V-VI with a transverse anterior line, tergite VII with two lines. Pores *psm* on tergites I-VII, *al* on tergites II-VII, *psl* on tergites VI-VII (figs. 15-16). Abdominal legs with 4, 3, 3 setae (fig. 18). Accessory setae on sternites I-VI sensilliform, slightly shorter than on tergites (4 and 6 µm respectively), on sternite VII same shape and length as on tergite VII (fig. 14). Sternites II-IV with a transverse anterior line, sternites V-VII with two transverse lines (figs. 18-19). Sternites I-IV without pores (figs. 17-18). Sternites V-VI with 1+1 pores, anterior to *P1* setae, sternite VII with single medial pore posteriorly (fig. 19).

Abdominal segment VIII with distinct striate band; tergite and sternite each with a transverse row of small teeth; tergite with a few teeth posteriorly in central position (figs. 20-21). Comb VIII with 7-8 regular teeth (fig. 22). Pore *psm* without accompanying teeth. Posterior margin of sternite VIII and laterotergites smooth. Seta *la* on tergite IX same length as seta *l*. Seta *2a* on tergites IX and X shorter than other setae. Posterior part of tergite XI with weak striation. Sternites IX-XII smooth. Dorsal lobe of abdomen XII with single median pore, ventral

3' - AACTCTTTACTTTCTTTTAGGGCTGTGATCGGGCATGGTGGGGCTATCTC  
 TAAGACTTTTAATTCGTAGCGAGCTCTCCTCCCCGGGGGAGTCATTGGG  
 GATGATCAAATTTTAAACGTATTGTTACTGCTCATGCCTTTGTAATAAT  
 TTTTTTTATGGTTATACCAATCTTAATGGGGGGTTTGAAATTTGGATAA  
 TCCCCCTAATGCTGAGAAGCCCCGGATATGGCCTTCCCACGAATGAATAAC  
 TTAAGATTTTGGCTACTCCCCCGCTGCCTTTTTCCTAATCTTAGAAG  
 ATTAGTTGAATCTGGAGTGGGGACAGGGTGAACCTGCCTACCCCCCTTTAG  
 CGGGGTAGAGGGACACTCAGGCCCTTCGGTAGACTTGGCTATCTTTTCT  
 TTACATTTAGCAGGGGCCTCCTCTATATGGGGGGCTGTTAACTTTATCAC  
 AACAGTTCTTAACATACGCCCTAATATATATCTTTGCCTAATACTTCTC  
 TCTTTAGCTGGTTCGGTACTAATCACTGCTGTCCTTCTTTTACTTTTCTTA  
 CCAGTTTLAGCCGCTGCTATCACGATACTCTTGACGGACCGAAACTTAAA  
 TACATCTTTTTCAGCCCCTCAGGAGGGGGGACCCTATTTATTCCAGC  
 ATCTTTTC- 5'

The base composition of the DNA barcode sequence is 21.4% A, 34.7% T, 23.4% C and 20.5% G (A+T = 56.1%). In comparison, base composition of *Sinentomon erythranum* Yin, 1965 barcode sequence is 18.8% A, 50.5% T, 11.1% C and 19.5% G (A+T = 69.3%) (Chen et al. 2011).

lobe with 1+1 anterolateral pores. Hind margin of dorsal and ventral lobes smooth. Male squama genitalis with 6+6 setae (fig. 23). Female squama genitalis with long distal prolongation of stylus and short, forked acrostyli (fig. 24).

Body measurements (9 adults) (in  $\mu\text{m}$ ): maximum body length 1350, head 155–160, pseudoculus 10, posterior part of maxillary gland 33–40, pronotal setae *l* 41–45, 2 14–16, mesonotal setae *P1* 38–40, *P2* 50–53, *M* 13–15, foretarsus 125–130, claw 28–30, empodial appendage 5–6.

**Chaetal variability.** Asymmetrical absence of *M1* and asymmetrical duplication of *A2* on prosternum (1 specimen).

**Etymology.** *Acerentulus charrieri* is named after Gaspard Charrier, fellow entomologist student at the Muséum National d'Histoire Naturelle, who helped to collect these specimens.

**Molecular Analysis.** A 658bp fragment of the COI gene (DNA barcode) was amplified and sequenced from two different specimens (MNHN-EA010059 and MNHN-EA010064). The two sequences were identical. The sequences have been deposited into the GenBank Database under accession number JQ411216 and JQ411217.

Sequence:

### Differential diagnosis

*Acerentulus charrieri* n. sp. belongs to the *Acerentulus confinis* species group (Nosek 1973). The 21 *Acerentulus* species from this group are characterized by a long foretarsal sensillum *a* that nearly reaches the base of seta *y3*, and sensillum *b* shorter or equal to the length of *c*. The new species does not fit this

definition perfectly since sensillum *b* is slightly longer than *c*. However, it cannot be included in the *Acerentulus traegardhi* species group, which is characterized by sensillum *b* longer than *c*, because this group has a clearly shorter sensillum *a* (reaching nearly the base of seta *y2*) and very long sensillum *b*, which surpasses the base of claw. In the presence of 8 anterior setae on tergite VII and seta *Pla* on tergite VII, the new species is similar to *A. abni*, *A. apuliacus*, *A. confinis*, *A. gigas*, *A. silvanus* and *A. sinensis* (Nosek 1973, Rusek & Stumpp 1988, Szeptycki 1991, Wu & Yin 2007). The general morphology of the foretarsus (sensillum *a* reaching to base of seta *y3*) of the new species places it closer to *A. apuliacus*, *A. confinis* and *A. gigas*. In *A. abni*, *A. silvanus* and *A. sinensis*, sensillum *a* does not reach base of seta *y3*. All these species are characterised by the presence of setae *P3a* on abdominal tergite VII with the exception of the new species and *A. apuliacus*, described from Southeast Italy. The new species differs from *A. apuliacus* by its significantly longer foretarsus (127 and 87  $\mu\text{m}$  respectively), slender sensilla *a* and *a'*, length of sensilla *b* and *c* (in the new species *b* is longer than *c*, in *A. apuliacus* these sensilla are of equal length) and by the distal position seta  $\delta 4$  on foretarsus (in *A. apuliacus* sensilla *a* and *a'* thick and seta  $\delta 4$  proximal to base of *c'*). Only four species within the *confinis*-group are characterized the distal position of foretarsal seta  $\delta 4$ : *A. charrieri* n. sp., *A. gigas*, *A. confinis maderensis*

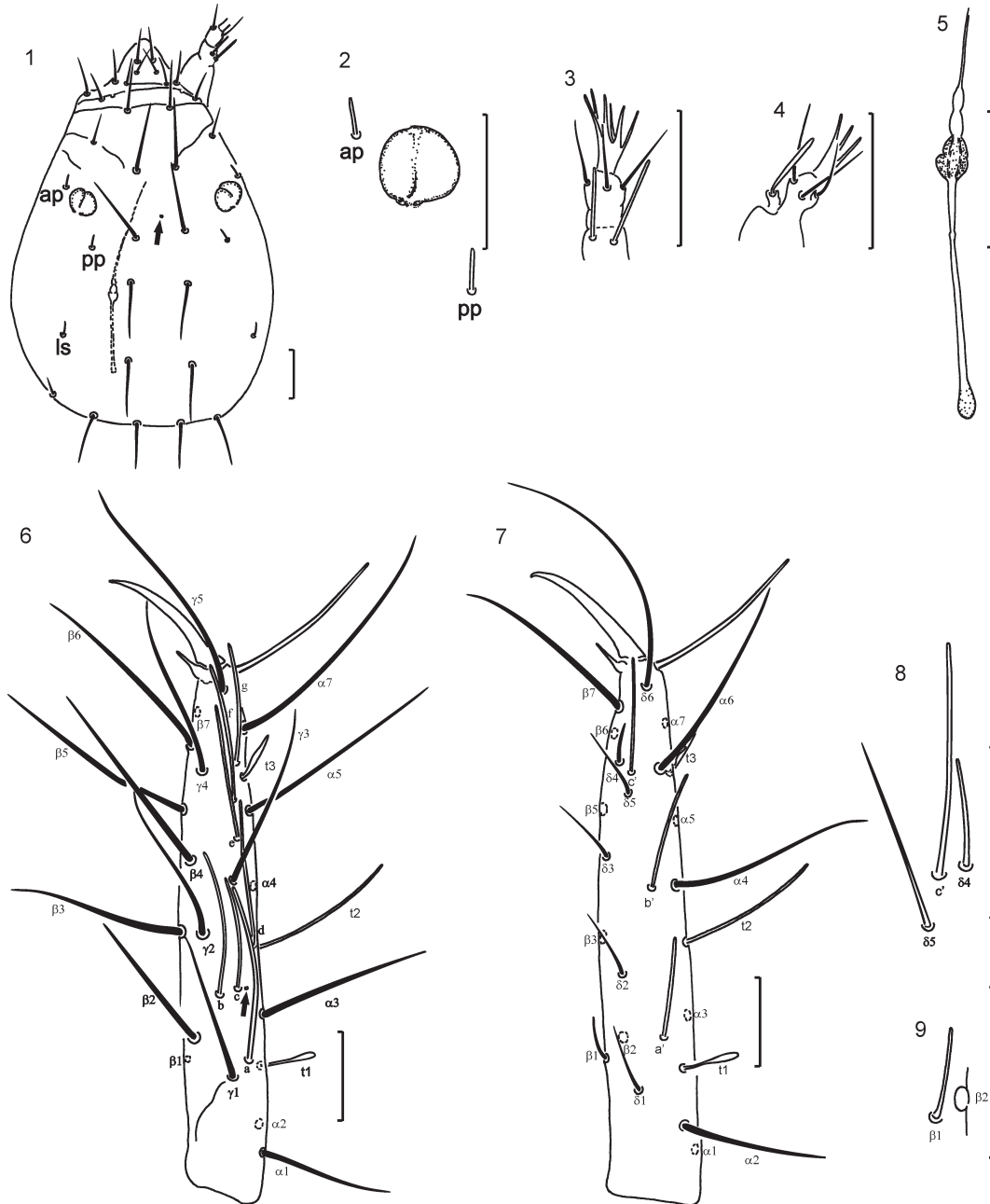
**Table 1.** Body chaetotaxy of *Acerentulus charrieri* n. sp.

	Dorsal		Ventral	
	Setae	Formula	Setae	Formula
Th. I	1, 2	4	A1, 2, M1, 2 P1, 2, 3	$\frac{4+4}{6}$
Th. II	A2, 4, M P1, 1a, 2, 2a, 3, 3a, 4, 5	$\frac{6}{16}$	Ac, 2, 3, M P1, 3	$\frac{5+2}{4}$
Th. III	A2, 4, M P1, 1a, 2, 2a, 3, 3a, 4, 5	$\frac{6}{16}$	Ac, 2, 3, 4, M P1, 3	$\frac{7+2}{4}$
Abd. I	A1, 2, 5 P1, 2, 2a, 3, 4	$\frac{6}{10}$	Ac, 2 P1, 1a	$\frac{3}{4}$
Abd. II–III	A1, 2, 5 P1, 2, 2a, 3, 4, 4a, 5	$\frac{6}{14}$	Ac, 2 Pc, 1a, 2	$\frac{3}{5}$
Abd. IV–V	A1, 2, 5 P1, 2, 2a, 3, 4, 4a, 5	$\frac{6}{14}$	Ac, 2 P1, 1a, 2, 3	$\frac{3}{8}$
Abd. VI	A1, 2, 4, 5 P1, 2, 2a, 3, 4, 4a, 5	$\frac{8}{14}$	Ac, 2 P1, 1a, 2, 3	$\frac{3}{8}$
Abd. VII	A2, 3, 4, 5 P1, 1a, 2, 2a, 3, 4, 4a, 5	$\frac{8}{16}$	Ac, 2 P1, 1a, 2, 3	$\frac{3}{8}$
Abd. VIII	A1, 4, 5 P1, 2, 2a, 3, 3a, 4, 4a, 5	$\frac{6}{16}$	1, 2 1a	$\frac{4}{2}$
Abd. IX	1, 1a, 2, 2a, 3, 4	12	1, 2	4
Abd. X	1, 2, 2a, 3, 4	10	1, 2	4
Abd. XI	1, 3, 4	6		6
Abd. XII		9		6

and *A. berruezanus*. The latter two species differ from the new species in possessing 6 anterior setae and seta *P3a* on tergite VII.

The new species is closer to *A. gigas* than other species in regards of distal position of foretarsal seta  $\delta 4$ , position of seta *P4a* on isolated sclerite of tergite VII

and in majority of measurements. However, *A. charrieri* **n. sp.** differs from *A. gigas* in the absence of seta *P3a* on the abdominal tergites (this seta presents on tergites VI–VII in *A. gigas*), the shape of foretarsal sensillum *a* (slender in the new species and thickened in *A. gigas*) and the longer sensillum *b* and shorter sensilla *a'* and *c'*



**Figures 1–9**  
*Acerentulus charrieri* **n. sp.** **1**, head (ap = anteropseudocular seta, pp = postpseudocular seta, ls = lateral seta); **2**, pseudoculus; **3**, maxillary palp; **4**, labial palp; **5**, canal of maxillary gland; **6**, foretarsus, exterior view; **7**, foretarsus, interior view; **8**, foretarsal sensillum *c'* and seta  $\delta 4$ ; **9**, foretarsal seta  $\beta 1$ . Arrows show pores. Figs. 1–2 -paratype 28.4b, others - holotype. Scale bars: 20  $\mu$ m.





**Key to the *Acerentulus confinis* species group**

1. Tergite VII with 6 anterior setae ..... 2
- Tergite VII with 8 anterior setae ..... 14
2. Tergite VII without *Pla* seta ..... 3
- Tergite VII with *Pla* seta ..... 6
3. Sternite XI with 2+2 setae ..... *A. condei* Nosek 1983
- Sternite XI with 3+3 setae ..... 4
4. Foretarsal sensillum *a* very broad .....  
..... *A. ochsenhausenus* Rusek 1988
- Foretarsal sensillum *a* slender ..... 5
5. Tergite VIII with 16 posterior setae (*Pl* setae present)  
..... *A. gisini* Conde 1952
- Tergite VIII with 14 posterior setae (*Pl* setae absent)  
..... *A. alpinus* Gisin 1945
6. Tergite VII with *P3a* seta ..... 7
- Tergite VII without *P3a* seta ..... 11
7. Tergite I with *Pla* seta ..... *A. berruezanus* Aldaba 1983
- Tergite I without *Pla* seta ..... 8
8. Sternite VII with seta *Pc* ..... *A. xerophilus* Szeptycki 1979
- Sternite VII without seta *Pc* ..... 9
9. Foretarsal sensilla *a*, *b* and *c* long, reaching or surpassing  
base of *e* ..... 10
- Foretarsal sensilla *a*, *b* and *c* shorter, reaching to base of  
seta *y3* ..... *A. confinis maderensis* Tuxen 1982
10. Sensillum *c* clearly longer than *b*, surpassing the base  
of *f* ..... *A. palissai* Nosek 1967
- Sensilla *c* and *b* subequal in length, reaching the base  
of *e* ..... *A. setosus* Szeptycki 1993
11. Sternite XI with 2+2 setae ..... *A. halae* Szeptycki 1997
- Sternite XI with 3+3 setae ..... 12
12. Base of *a'* at level of seta *a3* insertion ..... 13
- Base of *a'* more distally to seta *a3* insertion, at level of  
seta  $\delta 2$  insertion ..... *A. occultus* Szeptycki 1979
13. Foretarsal sensillum *a'* short, reaching to base of *t2*  
..... *A. exiguus* Conde 1944
- Foretarsal sensillum *a'* long, reaching to base of *b'*  
..... *A. carpaticus* Nosek 1967
14. Tergite VII with *Pla* seta ..... 15
- Tergite VII without *Pla* seta ..... *A. terricola* Rusek 1965
15. Tergite VII with *P3a* seta ..... 16
- Tergite VII without *P3a* seta ..... 20
16. Tergite VI with *P3a* seta ..... *A. gigas* Szeptycki 1997
- Tergite VI without *P3a* seta ..... 17
17. Maxillary sensilla of similar shape ..... 18
- Maxillary sensilla different in shape (one setiform, other  
sensilliform) ..... *A. sinensis* Bu&Yin 2007
18. Maxillary sensilla parallel-sided, slender ..... 19
- Maxillary sensilla spindle-shaped .....  
..... *A. confinis* (Berlese 1908)
19. Sensilla *b* and *c* subequal in length, *d* long, passing base  
of *f*, sternites II-III without pores, sternite VI with  
composed pores ..... *A. silvanus* Szeptycki 1991
- Sensillum *b* shorter than *c*, *d* shorter, not passing base  
of *e*, sternites II-III with pores, sternite VI with simple  
pores ..... *A. alni* Szeptycki 1991
20. Sensilla *b* and *c* subequal in length, sensillum *a'* broad,  
foretarsal seta  $\delta 4$  in proximal position .....  
..... *A. apuliacus* Rusek & Stumpp 1988
- Sensillum *b* longer than *c*, sensillum *a'* slim, foretarsal  
seta  $\delta 4$  in distal position ..... *A. charrieri* **n. sp.**

**Discussion**

Most of the time, DNA extraction from small arthropods result in the complete destruction of the specimen, or in the best cases only the exoskeleton can be saved. Our work goes even beyond that goal inasmuch we achieved to extract the DNA from two specimens while fully preserving internal organs such as *squama genitalis*, maxillary gland etc. These specimens are actually in such a good shape that they are part of the type material. This preserve the fundamental link between molecular sequences and the actual specimens in databases.

Very few mitochondrial sequences are known for proturans. Some sequences for Cytochrome b (Shao *et al.* 1999), COII (Shao *et al.* 2000) and 12s (Carapelli *et al.* 2000) have been published. Until early 2011, no barcode sequence was available for Protura (Jinbo *et al.* 2011, Pass & Szucsich 2011). Since then, Chen *et al.* 2011 published the complete mitochondrial genome of *Sinentomon erythranum*, obviously including the COI sequence. Seldom is known on proturans, their taxonomy and specially their identification is extremely difficult (Pass & Szucsich 2011). In that context, barcoding will be a useful tool for taxonomist in addition to morphology (e.g. Stevens *et al.* 2011). The present work provides the second DNA barcode for a Protura ever published. Barcode sequences of *Acerentulus charrieri* **n. sp.** and *Sinentomon erythranum* are significantly different (pairwise percentage identity: 67.8%) with even *Sinentomon* sequence being three bases shorter. Given the important divergence between the barcode sequences of the two species (belonging to two different orders: Acerentomata and Sinentomata), no doubt that the availability of more mitochondrial loci will allow to expand our Systematics knowledge of Protura and to re-visit Protura position in entognatha. Are they either close to Collembola, the Ellipura hypothesis supported by the classical views using morphology, or close to Diplura, a recent hypothesis supported by nuclear genes analyses (e.g. Giribet *et al.* 2004 and references therein)?

**Acknowledgements.** The authors thank Wanda Maria Weiner for her help in transporting of materials and constructive comments on the earlier version of the manuscript as well as reviewers for

their helpful comments and criticisms. DNA extraction and amplification were carried out in D'Haese's BoEM lab at the MNHN.

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