Morphological and Genetic Analysis of the 
Acerentomon doderoi Group (Protura: 
Acerentomidae) with Description of A. 
christiani sp. nov

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Abstract

Acerentomon christiani sp. nov. is described from Vienna, Austria. The new species is a member of the “doderoi” group, characterized by the presence of seta x on tergite VII. It is most similar to A. gallicum, A. brevisetosum and A. tenuisetosum, but differs from these species in the length of foretarsal sensillum c and certain other chaetotaxic measurements and indices. In addition to the morphological description, the DNA barcoding region of the mitochondrial cytochrome c oxidase subunit 1 gene (COI) and the 28S ribosomal RNA of the new species are provided. The morphological characters and the barcode of the new species are discussed in comparison to those of other Acerentomon spp. of the “doderoi” group is given.

Introduction

Until recently, the taxonomy of Protura was based exclusively on morphology. Many characters are inconspicuous and difficult to recognize, often making a reliable species determination problematic even for long-experienced experts (for review see [1]). However, it has been convincingly demonstrated [2] that molecular barcodes are an additional important and useful tool in the taxonomy of these minute soil arthropods. Use of barcodes has revealed that morphological taxonomy is reflected very closely by the molecular data. This result is of special importance since the reproductive biology of proturans remains enigmatic to date (for review see [3]). As a consequence it is not possible to check the described morphospecies from a biospecies perspective. Therefore, whenever possible each new species description of Protura should include a molecular characterization by barcodes as well as the description of morphological characters.

The genus Acerentomon Silvestri, 1907 presently comprises 38 species [1], [4], [5]), all of which have a West Palaearctic distribution (Europe and northern Africa). Most species are...
recorded solely from their type localities. Thirteen species of the genus *Acerentomon* have been reported from Austria ([6]). In soil samples from the Leopoldsberg (near Vienna) we found an undescribed species of *Acerentomon* together with *A. italicum* Nosek, 1969, both belonging to the “doderoi” group sensu Nosek (1973) [7]. The present paper contains a description of the new species from Austria, along with its barcoding sequence, and an identification key for all *Acerentomon* species of the *doderoi* group.

**Materials and Methods**

**Ethics Statement**

The species used in this study are neither CITES-species nor endangered species according to regional Red Lists. Our sampling permission was RU5-BE-939/001-2013.

**Nomenclature Acts**

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “http://zoobank.org/”. The LSID for this publication is: urn:lsid:zoobank.org:pub:1F2DDC05-5E40-4127-AB12-FC089F40EEAE. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS.

**Morphological Approach**

All specimens were mounted on microscopic slides in Faure medium or Marc Andre II medium. Head setae are labeled as in [8]. Body chaetotaxy is given as in [9] with the following changes: setae A0 and P0 are labeled as Ac and Pc; chaetotaxy of tergite VIII is labeled as in [10]. Terminology for body porotaxy follows [11] and [12]. Abbreviations used in the description are as follows: Abd.—abdominal segments, Th.—thoracic segments, *psm* = posterosubmedial, *psl* = posterosublateral, *al* = anterolateral, *sam* = sternal anteromedial, *sc* = sternal central, *spm* = sternal posteromedial, *spsm* = sternal posterosubmedial, *spsl* = sternal posterosublateral pore.

**Molecular Approach**

To obtain COI and 28S (region D2–D3) sequences, genomic DNA was extracted from complete animals applying a non-destructive extraction method (NDE) [13]. Protocols for DNA extraction, amplification, and sequencing of 41 published individuals from the genus *Acerentomon* are given in [2]. In the present study, DNA of 27 individuals was extracted by means of an NDE-method with the Blood & Tissue Kit (Qiagen). After DNA extraction the cuticle was transferred to 100% EtOH, before wholemounts were prepared. Thermocycler profiles differed for the amplification of the COI and 28S rDNA fragment: initial denaturation of 30 sec, 30 cycles of 1 min at 94°C, 1 min at 46°C (COI) / 48°C (28S rDNA) and 1 min (COI) / 1.5 min (28S rDNA) at 68°C and a final extension step for 5 min at 68°C. Each PCR reaction consisted of 2 μl DNA, 5 μl PCR buffer (5x containing 18 mM MgCl2: BioLabs OneTaq), 0.7 μl dNTP (10 mM each; BioLabs Desoxynucleotide Solution Mix), 0.7 μl each primer (10 μM, VBC Biotech), 0.1 μl Polymerase (BioLabs OneTaq), 10.8 μl ddH2O. A
second PCR-repeat, applying the same conditions, was used to increase the yield of DNA. PCR products were purified using GeneJET PCR Purification Kit (Thermo Scientific) and eluted in 20 μl with ddH2O. Sequencing reaction was performed at LGC Genomics, Germany. Different primer pairs were needed to successfully amplify the COI and 28S rDNA fragments (Table 1). In this study we successfully sequenced 13 sequences of the COI, and 27 sequences of the 28S rDNA. All sequences from the study of [2] are deposited at the Barcode of Life Data Systems (BOLD) under the project name PROTAT. New sequences are deposited at BOLD under the project name PROTA (Table 2). The entire dataset of the manuscript can be downloaded at: http://www.boldsystems.org/index.php/MAS_Management_OpenDataSet?datasetcode=DS-2016ADOD

Alignment and NJ tree based on K2P distances were performed using MUSCLE, as implemented in Mega v. 6.0 [14]. COI and 28S rDNA were analyzed separately. The reliability of trees was assessed with 1000 bootstrap replicates.

Results

Systematics of the Genus Acerentomon

Protura of the genus Acerentomon Silvestri, 1907 are characterized by the presence of three pairs of anterior setae on the mesonotum and four pairs of anterior setae on the metanotum, well developed maxillary palps with an apical tuft of setae and basal sensilla, a striate band with distinct striae, a claviform foretarsal sensillum t1, a leaf-like sensillum t3, a very long seta δ4 which contrasts with the length of other δ-setae, and the absence of sensillum b'. The second and third abdominal legs usually carry two setae differing in length (apical seta half the length of subapical seta). The maxillary gland possesses a small calyx and a short distal part. The genus has been subdivided into four groups, which differ in the presence or absence of seta x on tergite VII, and seta Pla on sternite VIII ([7], [10]). However, a cladistic analysis performed on 36 Acerentomon species using 24 morphological characters supports a split into only two main groups: “doderoi” and “aceris” [15]. Of these the “doderoi” group is characterized by the

Table 1. List of primers used in the present study.

| Locus   | primer cocktail | Primer  | direction | Sequences (3'-5')                           |
|---------|-----------------|---------|-----------|---------------------------------------------|
| COI     | no              | LCO1490 | forward   | ggtcaacaaatcataaagaagatttgg                  |
|         | no              | LepF1   | forward   | attcaacaaatcataaagaagatttgg                  |
|         | no              | DiplR1  | reverse   | gcataaatattgdgtgc                           |
|         | yes             | GlomF1  | forward   | Prot F4/1 + CamF1 (1:1)                     |
|         | no              | ProtF4/1| forward   | ctactaaactaataargaataggg                    |
|         | no              | CamF1   | forward   | ctactaaactaataargaataggg                    |
|         | yes             | GlomR1  | reverse   | JapR1 + CamR1 + ProtR1 (1:1:1)              |
|         | yes             | LCO, LepF1| forward   | LCO + LepF1 (1:1)                           |
|         | no              | JapR1   | reverse   | taaacttcdgggtgbcacaaagatc                   |
|         | no              | CamR1   | reverse   | taaacttcdgggtgbcacaaagatc                   |
|         | no              | ProtR1  | reverse   | taaacttcdgggtgbcacaaagatc                   |
| 28S rDNA| no              | D2a     | forward   | gattacgcagaagalc                            |
|         | no              | D2aProt | forward   | gtaccgagaggaagttg                           |
|         | no              | D3b     | reverse   | tcggaagagaacgacta                           |
|         | no              | D3bProt2| reverse   | gaaagaactaatgacact                           |
|         | no              | D3bProt2| reverse   | ctactaaactaataargaatc                       |

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| Ind.ID | Species name               | Sampling location | COI (BOLD) | 28S (BOLD) |
|--------|---------------------------|-------------------|------------|------------|
| HP006  | Acerentomon cf. italicum  | Leopoldsberg      | -          | PROAT091-13|
| HP010  | Acerentomon sp.           | Leopoldsberg      | -          | PROAT092-13|
| HP013  | Acerentomon sp.           | Twimberger Graben | PROAT005-12| PROAT005-12|
| HP014  | Acerentomon sp.           | Twimberger Graben | PROAT006-12| PROAT006-12|
| HP042  | Acerentomon maius         | Twimberger Graben | PROAT001-12| PROAT001-12|
| HP044  | Acerentomon maius         | Twimberger Graben | PROAT015-12| PROAT015-12|
| HP045  | Acerentomon maius         | Twimberger Graben | PROAT016-12| PROAT016-12|
| HP046  | Acerentomon maius         | Twimberger Graben | PROAT017-12| PROAT017-12|
| HP047  | Acerentomon maius         | Twimberger Graben | -          | PROAT096-13|
| HP052  | Acerentomon maius         | Twimberger Graben | PROAT018-12| PROAT018-12|
| HP058  | Acerentomon maius         | Twimberger Graben | PROAT019-12| PROAT019-12|
| HP059  | Acerentomon maius         | Twimberger Graben | PROAT020-12| PROAT020-12|
| HP061  | Acerentomon carpaticum    | Twimberger Graben | PROAT022-12| PROAT022-12|
| HP067  | Acerentomon cf. maius     | Twimberger Graben | PROAT024-12| PROAT024-12|
| HP075  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT025-12| -          |
| HP076  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT026-12| PROAT026-12|
| HP077  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT027-12| PROAT027-12|
| HP079  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT029-12| -          |
| HP085  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT030-12| PROAT030-12|
| HP086  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT031-12| PROAT031-12|
| HP087  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT032-12| PROAT032-12|
| HP089  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT033-12| PROAT033-12|
| HP090  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT034-12| PROAT034-12|
| HP096  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT035-12| PROAT035-12|
| HP099  | Acerentomon christiani sp. nov. | Leopoldsberg | -          | PROAT100-13 |
| HP100  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT036-12| PROAT036-12|
| HP101  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT037-12| PROAT037-12|
| HP102  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT038-12| PROAT038-12|
| HP103  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT039-12| -          |
| HP106  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT040-12| PROAT040-12|
| HP107  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT041-12| PROAT041-12|
| HP108  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT042-12| PROAT042-12|
| HP132  | Acerentomon italicum      | Leopoldsberg      | PROAT052-12| PROAT052-12|
| HP135  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT054-12| PROAT054-12|
| HP136  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT055-12| PROAT055-12|
| HP144  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT063-12| PROAT063-12|
| HP145  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT064-12| PROAT064-12|
| HP146  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT065-12| PROAT065-12|
| HP147  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT066-12| PROAT066-12|
| HP150  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT069-12| PROAT069-12|
| HP152  | Acerentomon christiani sp. nov. | Leopoldsberg | PROAT071-12| PROAT071-12|
| 1558_PROTA | Acerentomon dispar      | Trebesiner Weg   | PROTA001-15| PROTA001-15|
| 1561_PROTA | Acerentomon dispar    | Trebesiner Weg   | PROTA002-15| PROTA002-15|
| 1562_PROTA | Acerentomon carpaticum | Slovakia         | PROTA003-15| PROTA003-15|
| 1563_PROTA | Acerentomon carpaticum | Slovakia         | PROTA004-15| PROTA004-15|
| 1564_PROTA | Acerentomon carpaticum | Slovakia         | -          | PROTA005-15|
| 1575_PROTA | Acerentomon carpaticum | Slovakia         | PROTA006-15| PROTA006-15|

(Continued)
presence of seta \( x \) on tergite VII, and comprises a total of 21 species. Two *Acerentomon* spp. described after the study can be assigned to the *aceris* group.

The new species described in this paper belongs to the "doderoi" group of *Acerentomon* species in the sense of [15] and is characterized by the presence of seta \( x \) on tergite VII and a pair of posterior setae \( P1a \) on sternite VIII, presence of seta \( Pc \) on tergite VII and sternite VII, absence of seta \( P3a \) on tergite VII, long mesonotal and metanotal setae \( P1a \) that are longer than \( P1 \), long foretarsal sensillum \( c \), short and slender sensillum \( b \), and broadened sensillum \( a \). The new species is most similar to *A. gallicum* Ionescu, 1933 in chaetotaxy and measurements. A detailed description is given below.

*Acerentomon christiani* sp. nov. Shrubovych & Resch

**Morphological description**

**Characters for diagnosis:** Head setae long, setiform, not modified. Posterior margin of head with seta \( d7 \) slightly shorter than seta \( sd7 \). Additional seta \( d6 \) present (Fig 1A). Rostrum long, LR 3.4–3.8 (Fig 1B). Pseudoculus longer than broad, PR 16–17 (Fig 1C). Sensilla of maxillary palp nearly equal in length (Fig 1D). Labial palps well developed, with broad basal sensillum (Fig 1E). Canal of maxillary gland with distinct thickening in posterior part and simple posterior dilation, 1.4–1.6 times the length of the pseudoculus, CF 10.0–11.9 (Fig 1F).

Chaetotaxy formula given in Table 3. Setae on nota differing distinctly in length (Fig 2A). Length ratio of pronotal setae \( 1:2 \) as 2:1. Seta \( M \) on meso- and metanota longer than seta \( A2 \), which are approximately 50 and 30 \( \mu \)m, respectively. Accessory setae \( P1a \) and \( P2a \) setiform,
Fig 1. *Acerentomon christiani* sp. nov. (A) Head. (B) Rostrum. (C) Pseudoculus. (D) Maxillary palp. (E) Labial palp. (F) Maxillary gland. (G) Acrostylus. (H) Comb. (I) Foretarsus, exterior view. (J) Foretarsus, interior view. Arrows indicate pores. All figures are of holotype. Scale bars: 20 μm.

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differing in length; P1a slightly longer than P1, P2a one-third the length of P1a (Fig 2A). Setae P3a and P4 subequal in length, short, setiform; P5 a small sensillum. Length ratio of P1:P1a:P2 on mesonotum as 0.9:1:1.4. Pronotum lacking pores. Mesonotum with 2+2 pores (al, sl) (Fig 2A). Metanotum with 1+1 sl pores. Prosternum with seta M2, meso- and metasterna without A1 setae (Fig 2F and 2G). Setae A2 and M2 on prosternum and A2 on meso- and metasterna setiform. Lateral margin of meso- and metasternum with distinct coxal incision (Fig 2G). Prosternum lacking pores; meso- and metasterna usually with three or four closely adjacent sternal central pores (sc), situated posterior to seta Ac (Fig 2G).

Sensillum t1 claviform; t3 leaf-like; a sword-shaped; b slender, nearly setiform; other sensilla parallel-sided (Fig 11 and 1J). Sensillum a long, reaching base of sensillum d; b very short, its apex not reaching base of γ3; c very long, longer than a and three times longer than b, its base proximal to base of sensillum b and its apex reaching the base of t3; sensillum e located halfway between bases of sensilla d and f (Fig 11). Sensilla a’ and c’ long. Sensillum a’ located distal to level of t2 insertion, close to the base of c’ (Fig 1). Seta β1 setiform, longer than δ1 setae, δ4 setiform, very long in comparison with other δ-setae (Fig 1J). Claw long and slender, with small inner tooth. Empodial appendage short. Relative length of foretarsal sensilla: t1 = b < t3 < t2 < c < a < (c = f) < (d = g) < (a’ = c’). BS 0.5; TR 3.0–3.1; EU 0.1–0.2. Pores present near base of sensilla c and t3.

Accessory setae on tergite I differing in length: seta P1a slightly shorter than P1, seta P2a one-third the length of P2, equal in length to P3, P4 and P5 setae (Fig 2B). Accessory setae on tergites II-VII setiform, nearly equal in length, about half the length of the principal setae (Fig 2C).

### Table 3. Body chaetotaxy of Acerentomon christiani sp. nov.

| Segment | Dorsal | Ventral |
|---------|--------|---------|
|         | Formula | Setal composition | Formula | Setal composition |
| Th. I   | 4 1, 2  | 4+4 A1, 2, M1, 2 |         |                   |
|         | 4 1, 2  | 6 P1, 2, 3       |         |                   |
| Th. II  | 8 A2, 3, 4, M | 5+2 Ac, 2, 4, M |         |                   |
|         | 16 P1, 1a, 2, 2a, 3, 3a, 4, 5 | 4 P1, 3 |         |                   |
| Th. III | 10 A2, 3, 4, 5, M | 7+2 Ac, 2, 3, 4, M |         |                   |
|         | 16 P1, 1a, 2, 2a, 3, 3a, 4, 5 | 4 P1, 3 |         |                   |
| Abd. I  | 6 A1, 2, 3 | 3 Ac, 2        |         |                   |
|         | 14 P1, 1a, 2, 2a, 3, 4, 5 | 4 P1, 1a |         |                   |
| Abd. II | 10 A1, 2, 3, 4, 5 | 5 Ac, 2, 3 |         |                   |
|         | 16 P1, 1a, 2, 2a, 3, 4, 4a, 5 | 5 P1, 1a, 2 |         |                   |
| Abd. III| 10 A1, 2, 3, 4, 5 | 7 Ac, 1, 2, 3 |         |                   |
|         | 16 P1, 1a, 2, 2a, 3, 4, 4a, 5 | 5 P1, 1a, 2 |         |                   |
| Abd. IV-VI | 10 A1, 2, 3, 4, 5 | 7 Ac, 1, 2, 3 |         |                   |
|         | 16 P1, 1a, 2, 2a, 3, 4, 4a, 5 | 8 P1, 1a, 2, 3 |         |                   |
| Abd. VII| 12 A1, 2, 3, 4, 5, x | 5 Ac, 2, 3 |         |                   |
|         | 17 P1, 1a, 2, 2a, 3, 4, 4a, 5 | 9 P1, 1a, 2, 3 |         |                   |
| Abd. VIII| 8 A1, 2, 4, 5 | 4 1, 2        |         |                   |
|         | 16 P1, 2a, 3, 4, 4a, 5 | 2 1a         |         |                   |
| Abd. IX | 14 1, 1a, 2, 2a, 3, 3a, 4 | 4 1, 2       |         |                   |
| Abd. X  | 10 1, 2, 2a, 3, 4 | 4 1, 2      |         |                   |
| Abd. XI | 6 1, 3, 4 | 6            |         |                   |
| Abd. XII| 9            | 6            |         |                   |

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Fig 2. Acerentomon christiani sp. nov. (A) Pronotum and mesonotum (left side). (B) Tergite I (left side). (C) Tergite VII (right side). (D) Tergite VIII (right side). (E) Hind margin of sternite XII. (F) Prosternum. (G) Mesosternum. (H) Stermite II (right side). (I) Stermite III (right side). (J) Anterolateral structures on tergite VI. (K) Anterolateral structures on tergite VII. (L) Hind margin of laterotergite VIII. (M) Stermites VII–IX. (N) Male sguama genitalis. Arrows indicate pores (ali = anterolateral, sl = sublateral, psm = posterosubmedial, psl = posterosublateral, sam = sternal anteromedial, sc = sternal central, spm = sternal posteromedial, spsm = sternal posterosubmedial, spsl = sternal posterosublateral pore). Figure N; paratype Cat. N 840+HP 136, other figures are of holotype. Scale bars: 20 μm.

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Tergite VII with seta $Pc$ and seta $x$ (Fig 2D). Tergite I with 1+1 $psm$ pores only (Fig 2B). Tergites II – VII with three pairs of pores ($psm$, $psl$ and $al$) (Fig 2C and 2M). Tergites I – VII anteriorly with two parallel cuticular lines (Fig 2C and 2D). Pleural structures not developed on tergites I – V; on tergite VI 20–22 teeth present anterior to pore $al$, on tergite VII some distinct teeth near to pore $al$ (Fig 2 and 2K).

Abdominal appendages with 4, 2, 2 setae. Apical seta of abdominal legs II and III less than half the length of subapical seta, 26 and 17 μm, respectively (Fig 2H and 2I). Accessory setae on sternites setiform, shorter than principal setae, seta $Pc$ on sternite VII shorter than $P1a$ setae (Fig 2M). Sternite I without pores, sternites II – V with single $spm$ pore posterior to Ac (Fig 2H and 2I). Sternite VI and VII with two pairs of $spm$ and $psl$ pores and with a group of three sternal anteromedial pores ($sam$) anterior to Ac and above cuticular lines (Fig 2M). Sternites II – III anteriorly with a cuticular line (Fig 2H and 2I), sternites IV – VII with two parallel cuticular lines (Fig 2M).

Abdominal segment VIII with distinct striate band and with a regular row of small, scattered denticles anteriorly (Fig 2D and 2M). Comb VIII composed of 14–16 slender, teeth of varying lengths (Fig 1H). Pore $psm$ with several surrounding teeth, other pores absent (Fig 2D). Laterotergites VIII with row of granules in anterior part and with 8–10 small teeth on posterior margin (Fig 2L). Pore $psm$ with 1–2 accompanying teeth. Setae 1a present on sternite VIII. Pores absent (Fig 2M).

Hind margin of tergites and sternites IX – XI smooth. Dorsal lobe of segment XII with simple median pore, hind margin smooth. Ventral lobe of segment XII with about 10 teeth on hind margin (Fig 2E) and with 1+1 sternal anterolateral pores.

Female squama genitalis with pointed acrostyli (Fig 1G). Male squama genitalis with 6+6 $setae$, additional setae absent (Fig 2N).

**Body measurements (based on 19 adults, in μm):** Maximum body length 1780, head 180–195, pseudoculus 11–12, posterior part of maxillary gland 17–19; hind cephalic seta $d7$ 20–21, seta $sd7$ 25–26, pronotal seta 1 56–60, pronotal seta 2 26–30, mesonotal setae $P1$ 44–48, $P1a$ 50–58, $P2$ 70–73, $P2a$ 15–16, foretarsus 120–125, claw 40–42, empodial appendage 5–7.

**Chaetal variability:** Single specimens varied as follows: sternite III with 5 $A$-setae — symmetrical absence of seta $AI$ (on 2 specimens), sternite III with duplication of seta $Ac$ (1 specimen), sternite III with asymmetrical absence of seta $AI$ (3 specimens), sternite VII with $Pc$ absent (1 specimen).

**Type material and deposition:** Holotype female (slide no. 77.1) from sample collected in litter and steep southwest slope with *Quercus pubescens* over platy marl, Leopoldsberg, 48°16′40″ N, 16°20′37″ E, Vienna, Austria, 13.March.2012, coll. N. Szucsich and C. Resch.

Paratypes: 16 female and 2 male paratypes (no. 77.2 – 77.7, no. 6619 – 6625, HP 100–HP 103, HP 106, HP 107), and other material: 12 preimagos, 1 maturus junior (no. 6626 – 6638), collected together with the holotype. The holotype, 5 female and 1 male paratypes (slides no. 77.1 – 77.7) are deposited in the collection of the State Museum of Natural History of the National Academy of Sciences of Ukraine, L’viv (SMNH). Five female paratypes are deposited in the Museum of Natural History Vienna, Austria. Six females and one male paratypes (slides no. 6619 – 6625) and other material (12 preimagos and 1 maturus junior) are deposited in the collection of the Institute of Systematics and Evolution of Animals of the Polish Academy of Sciences, Kraków (ISEA).

**Distribution:** Austria, known so far only from the type locality.

**Etymology:** We have the honour of dedicating the new species to Dr. Erhard Christian, Vienna, in appreciation of his merits in studying the apterygote fauna of Austria.

**Remarks:** *Acerentomon christiani* sp. nov. is similar to the group of species characterized by a short foretarsal sensillum $b$, the apex of which does not reach the base of $γ3$, and a broadened
sensillum \(a\) (\(A.\) gallicum, \(A.\) tenuisetosum, \(A.\) brevisetosum, \(A.\) italicum, \(A.\) fagetica) and \(A.\) nemorale). Furthermore, \(A.\) christiani differs from all \(Acerentomon\) spp. of the “\(doderoi\)” group in possessing a very long foretarsal sensillum \(c\), which is longer than sensillum \(a\) and three times longer than \(b\). In the remaining species sensillum \(c\) is shorter than \(a\) and about 1.5 times longer than sensillum \(b\). The chaetotaxic pattern of the new species is most similar to \(A.\) gallicum, \(A.\) tenuisetosum and \(A.\) brevisetosum. However, \(Acerentomon italicum\), \(A.\) fagetica and \(A.\) nemorale differ in seta \(Pc\) being absent from tergite VII, in the shape of the comb with lower number of teeth and in the length of the foretarsi (see the identification key). \(Acerentomon\) christiani sp. nov. is closest to \(A.\) gallicum in foretarsus and body lengths, and in the shape of laterotergal lines (lines on tergites II–V smooth, line of tergite VII with about 15 teeth and line on tergite VII with one or two strong teeth). These two species clearly differ in the length and position of sensillum \(e\) on the foretarsus (in the new species \(e\) is shorter and located at half the length between the bases of \(d\) and \(f\), in \(A.\) gallicum this sensillum is long and very close to the base of sensillum \(d\)), in the shape of the comb (which possesses 12–14 long teeth in \(A.\) gallicum), and in the relative length ratio of mesonotal setae \(P1\) and \(P1a\) (in \(A.\) christiani sp. nov. the seta \(P1a\) is longer than seta \(P1\), whereas in \(A.\) gallicum \(P1a\) is shorter). The position of the foretarsal sensillum \(e\) of the new species is similar to \(A.\) tenuisetosum, but the new species differs in possessing longer foretarsus and rostral setae and in the shape of the comb (see identification key below). \(Acerentomon\) christiani sp. nov. is closest to \(A.\) brevisetosum in the relative length ratio of mesonotal setae \((P1a\) longer than \(P1))\), but differs in the length of the foretarsus and dorsal setae, in the shape of the anterolateral lines on tergites VI and VII, and in the shape of the comb \((A.\) brevisetosum\) is characterized by shorter foretarsal length, very short dorsal setae of approximately 20 μm, smooth anterolateral lines on tergites VI and VII and possession of about 13 teeth on the comb).

**Molecular description.** The DNA barcode of \(Acerentomon\) christiani sp. nov. is clearly delimited from all other \(Acerentomon\) species sequenced so far. The new species clusters with either \(Acerentomon maius\) (Fig 3, COI), or \(Acerentomon\) dispar (Fig 3, 28S).

**Discussion**

The monophyly of the \(Acerentomon\) “\(doderoi\)” group is supported by the presence of supplementary seta \(x\) on tergite VII. Two other characters were briefly discussed by [15] as additional support: a distinctly protruded rostrum (LR 2.8 to 4.5) and the presence of 6 setae on tergite XI. Within the \(doderoi\) group only \(A.\) novaki has a short rostrum (LR 14). The presence of seta \(P1a\) on sternite VIII mentioned by [7] and [10] as an important character for distinguishing their \(doderoi\) group, was not confirmed by [15], who added species with only four setae on sternite VIII (\(A.\) franzi and \(A.\) noseki) into the group. Within the group only \(A.\) franzi is mentioned as having just four setae on tergite XI in the original description of [16]. However, in two male specimens from Vienna tergite XI has 6 setae. Two species (\(A.\) skuhravyi and \(A.\) granulatum) form a small subgroup, characterized by 6 setae on sternite IX and by an anterior position of setae \(P1a\) on sternite VIII ([17], [18]). All other chaetotaxic characters are uniform among all species of the group, with the exception of the chaetotaxy of segment VII. The presence or absence of seta \(Pc\) on tergite VII and sternite VII, and the absence of seta \(A1\) and presence of setae \(P3a\) on tergite VII, are of limited phylogenetic value, since [9] noted interspecific variability in these characters. The number of anterior setae on sternites I and III varies from 5 to 7 in different species, but this variation can be intraspecific, as observed both in the new species and mentioned by [9]. Species of the \(Acerentomon\) doderoi group clearly differ in length of sensillum \(b\), shape of sensilla \(a\) and \(b\), position of sensillum \(e\) on the foretarsus, shape of comb and in foretarsal length. The new species differs from all others by a very long foretarsal sensillum \(c\),
which reaches the base of the sensillum t3 and is about three times longer than sensillum b (see identification key).

Both the tree based on the COI barcoding fragment and the tree based on the 28S rDNA fragment are fully congruent with morphological systematics. Since *A. christiani* sp. nov. is
known only from a single locality little can be said with respect to its intraspecific variation. Distances are large among populations of Acerentomon dispar, sampled from four different locations in Austria (Fig 3, COI), a result congruent with [2]. However, intraspecific distances in the same species are nearly lacking in 28S rDNA sequences (Fig 3, 28S). A more conclusive contribution of molecular data to relationships among Acerentomon species awaits a denser sampling at both population- and species-level.

Identification Key for All Described Species of the Acerentomon “doderoi” Group

1. Foretarsal sensillum b broad, slightly shorter to longer than sensillum c . . . 2
   - Sensillum b slender and short, distinctly shorter than c . . . 10
2. Sensillum b longer than c, sensillum a broadened . . . 3
   - Sensillum b slightly shorter than c . . . 6
3. Sternite VII with seta Pc, rostrum long . . . 4
   - Sternite VII without seta Pc . . . 5
4. Sensillum b distinctly longer than c, its apex reaching base of seta γ4, length of foretarsus about 135 μm . . . A. imadatei Nosek, 1967 (Hungary, Slovakia, Austria)
   - Sensillum b slightly longer than c, its apex not reaching base of seta γ4, length of foretarsus 154–160 μm . . . A. baldense Torti, 1986 (Italy)
5. Rostrum short (LR 14), sternite VIII with 6 setae, length of foretarsus about 105 μm . . . A. novaki Rusek, 1965 (Czech Republic)
   - Rostrum of moderate length (LR 3.8), sternite VIII with 4 setae, length of foretarsus about 135 μm . . . A. franzi Nosek, 1965 (Austria)
6. Sensillum a broadened . . . 7
   - Sensillum a slender . . . 8
7. Tergite VII with 18 posterior setae, comb with about 60 teeth, LR 3.2 . . . A. rostratum Ionesco, 1951 (Romania)
   - Tergite VII with 16 posterior setae, comb with 14–16 teeth, LR 2.6–2.7 . . . A. maius Berlese, 1908 (Italy, Slovakia, Slovenia, Austria)
8. Setae Pla on sternite VIII placed proximally, close to P1 and P2, sternite IX with 6 setae, rostrum long (LR 2.7). . . 9
   - Setae Pla on sternite VIII placed distally, on hind margin of sternite, sternite IX with 4 setae, rostrum of medium size (LR 3.7) . . . A. doderoi Silvestri, 1907 (Italy, Slovenia, other records are questionable)
9. Comb with about 25 teeth, length of foretarsus about 125 μm . . . A. skuhravyi Rusek,1965 (Slovakia, Poland, Ukraine)
   - Comb with about 40 teeth, length of foretarsus about 140 μm . . . A. granulatum Szeptycki, 1993 (Georgia)
10. Foretarsal sensillum b reaching base of seta γ3 . . . 11
    - Foretarsal sensillum b not reaching base of seta γ3. . . 15
11. Sensillum a broadened, sword-shaped . . . 12
    - Sensillum a slender . . . 14
12. Tergite VII and sternite VII with seta Pc Tergite VII with 19 posterior setae, LR 3.3, length of foretarsus about 150 μm . . . A. dispar Stach, 1954 (Poland, Czech Republic, Slovakia, Ukraine, Austria)
    - Tergite VII and sternite VII without seta Pc . . . 13
13. Tergite VII with 14 posterior setae . . . A. kustorae Nosek, 1983 (Slovenia)
- Tergite VII with 16 posterior setae (for additional characters see current redescription [19]) . . . A. italicum Nosek, 1969 (Italy, Austria)

14. Sternite VII with *Pc*, length of foretarsus 156–164 μm, rostrum long (LR 3.1) . . . A. tuxeni Nosek, 1961 (Slovakia, Austria, Czech Republic, Poland)

- Sternite VII without *Pc*, length of foretarsus about 145 μm, rostrum long (LR 2.8) . . . A. giganteum Condé, 1944 (Austria, Czech Republic, France, Germany, Poland, Slovakia, Slovenia, Africa)

15. Tergite VII without seta *Pc* . . . 16

- Tergite VII with seta *Pc* . . . 19

16. Tergite VII with 12 anterior setae (*A1* present) . . . 17

- Tergite VII with 10 anterior setae (*A1* absent) . . . A. nemorale Womersley, 1927 (Great Britain, France, Luxembourg, Germany, Austria, Slovakia, Czech Republic)

17. Sternite VII with seta *Pc*, length of foretarsus about 135 μm . . . 18

- Sternite VII without seta *Pc*, length of foretarsus 105–122 . . . A. fageticola Rusek, 1966 (Czech Republic, Slovakia, Poland, Austria)

18. Sternites VIII and XI with 4 setae, comb with about 32 teeth, rostrum long (LR 3.3) . . . A. noseki Torti, 1981 (Italy)

- Sternites VIII and XI with 6 setae, comb with 10–16 teeth, rostrum of medium size (LR 4.0–4.5) . . . A. omissum Szeptycki, 1980 (Poland, Slovakia, Ukraine)

19. Length of foretarsus about 110 μm . . . 20

- Length of foretarsus about 125 μm . . . 21

20. All dorsal setae very short, comb with about 13 teeth, rostrum long (LR 3.0–3.2) . . . A. brevisetosum Condé, 1945 (France)

- All dorsal setae long, comb with about 8 teeth, rostrum of moderate length (LR 3.8) . . . A. tenuisetosum Nosek, 1973 (Great Britain)

21. Sensillum *a* longer than *c*, comb with 12–14 teeth, mesonotal seta *P1a* shorter than *P1* . . . A. gallicum Ionesco, 1933 (France, Austria, Czech Republic, Germany, Italy, Slovakia, Poland)

- Sensillum *a* shorter than *c*, comb with 18–22 teeth, mesonotal seta *P1a* longer than *P1* . . . A. christiani sp. nov. Shrubovych & Resch (Austria)

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**Author Contributions**

Conceived and designed the experiments: JS DB NUS GP. Performed the experiments: JS DB NUS MCR. Analyzed the data: JS DB NUS. Contributed reagents/materials/analysis tools: JS GP. Wrote the paper: JS DB NUS MCR GP.

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