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A new water mite species of the genus *Teutonia* Koenike, 1889 from Corsica, France, based on morphological data and DNA barcodes (Acari, Hydrachnidia, Teutoniidae)

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Original research

ABSTRACT

We describe a new species, *Teutonia corsicana* **sp. nov.** from Corsica, France using morphological data and DNA barcodes. Morphologically the new species can be separated on the basis of the female pregenital sclerite partially covered by genital flaps, and in both sexes a broad-based, triangular ventral projection of P-2 and slender P-3 (L/H ratio 1.6-1.7). The average K2P-distance between DNA-barcode sequences of *T. corsicana* **sp. nov.** and widely distributed *T. cometes* (Koch, 1837) was estimated at 18.7±1.9 %. An updated key for the European species of *Teutonia* is provided.

Keywords water mites; new species; taxonomy; species delimitation; France
Zoobank http://zoobank.org/F83B5FED-0C63-41BC-A2F5-A331E03CD95E

Introduction

The family Teutoniidae Koenike, 1910 comprises of two genera, the monotypic *Limnolegeria* Motaş, 1928, which was for a long time considered as a representative of the Anisitsiellidae (see Cook 1974), and the widely distributed *Teutonia* Koenike, 1889. The latter genus, recorded from the Holarctic, comprises of two subgenera, the nominate subgenus and *Subteutonia* Habeeb, 1958 known only from Canada (Smit 2020).

Currently, two species of the genus *Teutonia* are known from the Western Palaearctic (Di Sabatino *et al.* 2010), i.e., the widely distributed *T. cometes* (Koch, 1837), inhabiting cold montane lakes and pools of shaded streams (Di Sabatino *et al.* 2010), and *T. subalpina* Thor, 1897. The latter, little known species is restricted to the far north of Europe (alpine and subalpine coniferous forests of Norway and Sweden) (Di Sabatino *et al.* 2010).

In this paper we used morphological data and DNA barcode of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene sequence to describe one new species of the genus *Teutonia* from Corsica.

Material and methods

Water mites were collected by hand netting by the junior author, sorted live in the field, and immediately preserved in 96% ethanol for the purpose of the molecular analyses. A part of the material was also preserved in Koenike-fluid. After DNA extraction, two voucher specimens were dissected and slide mounted in Faure's medium. Holotype and paratypes of the new

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species are deposited in Naturalis Biodiversity Center in Leiden (RMNH). The DNA sequences prepared in the course of this study were deposited in the Barcode of Life Data System (BOLD) and GenBank (Table 1).

All measurements are in µm. Morphological nomenclature follows Gerecke *et al.* (2016). The length of genital flaps and acetabula were measured on both sides, and therefore their dimensions are given as a range. The photograph of ejaculatory complex was made using a camera on a Samsung Galaxy smartphone. The following abbreviations are used: Ac – acetabula; a.s.l. – above sea level; Cx-I-IV – first-fourth coxae; dL – dorsal length; H – height; I-IV -L-1-6 – first-sixth segments of first- fourth legs; L – length; P-1-P-5 – palp segment 1-5; RMNH – Naturalis Biodiversity Center, Leiden; vL – ventral length; W – width.

Molecular analyses were conducted at the Canadian Centre for DNA Barcoding (Guelph, Ontario, Canada (CCDB; http://ccdb.ca/)), where the specimens were sequenced for the barcode region of COI using standard invertebrate DNA extraction, amplification and sequencing protocols (Ivanova *et al.* 2007; Ivanova and Grainger 2007a, b). In CCDB the chromatograms were assembled into consensus sequences for each specimen and uploaded to the barcode of life database (BOLD; https://www.boldsystems.org/). The sample identifiers in BOLD are given for each barcoded specimen.

The final dataset for the phylogenetic analysis includes 17 COI sequences representing *Teutonia cometes* (n=7), and *T. corsicana* **sp. nov**. (n=10). *Limnolegeria longiseta* Motaş, 1928 (BOLD/GenBank SEPTA083-21/OM321062) was used as outgroup (Table 1).

Sequence comparisons were performed using MUSCLE alignment (Edgar 2004). Intra- and interspecific genetic distances were calculated based on the Kimura 2-parameter model (K2P; Kimura 1980), using MEGA-X (Kumar *et al.* 2018). MEGA-X software was used to calculate Neighbour-Joining (NJ) trees based on K2P distances (standard for barcoding studies) and pairwise deletion of missing data. The support for tree branches was calculated by the non-parametric bootstrap method (Felsenstein 1985) with 1000 replicates. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair.

Table 1 List of sequenced specimens used in this study.

Locality (country, name)	Lat/Long	BOLD/GenBank Acc. Nos	Voucher code
***Teutonia cometes* (Koch, 1837)**			
Norway, Trondheim, Bymarka, Lauglovatnet, st. 2	63.3617° N, 10.2410° E	MMHYD003-19	NTNU-VM 228621
Norway, Trondheim, Bymarka, Store Leirsjoe, st. 3	63.3862° N, 10.2872° E	MMHYD012-19	NTNU-VM 228623
Norway, Stjoerdal, Stjoerdalsvassdraget, Fugla, st. 1	63.4430° N, 10.9235° E	MMHYD060-19	NTNU-VM 228625
Norway, Orkdal, Orklavassdraget, Gjoesjoeen, st. 1	63.2349° N, 9.6626° E	MMHYD069-19	NTNU-VM 228627
Montenegro, Podgorica, Mareza canal	42.4790° N, 19.1813° E	DNAEC068-20/ OL870217	33. M19_20_3_F11
Montenegro, Danilovgrad, spring Svinjška vrela	42.6384° N, 19.0074° E	DNAEC079-20/ OL870180	Hyd_MN_VP5
Germany, Baden-Württemberg, Schönbuch, Goldersbach near Teufelsbrücke	-	JN018321	MNHN-JAC69
***Teutonia corsicana* sp. nov.**			
France, Corsica, Porto, Ruisseau d`Enova at crossing with road D124	42.2659° N, 8.7141° E	NLACA1050-17	RMNH.5070808
France, Corsica, Ruisseau de Tuara, crossing road D81	42.3496° N, 8.6650° E	NLACA1051-17	RMNH.5070809
France, Corsica, Ruisseau de Tuara, crossing road D81	42.3496° N, 8.6650° E	NOVMB001-21/ OM952168	CCDB 38559 A01
France, Corsica, Ruisseau de Tuara, crossing road D81	42.3496° N, 8.6650° E	NOVMB002-21/ OM952167	CCDB 38559 A02
France, Corsica, Ruisseau de Sattu	41.6907° N, 9.1525° E	NOVMB033-21/ OM952166	CCDB 38559 C09
France, Corsica, Riviere La Solenzara	41.8383° N, 9.3306° E	NOVMB048-21/ OM952165	CCDB 38559 D12
France, Corsica, Rivière La Solenzara	41.8383° N, 9.3306° E	NOVMB049-21/ OM952164	CCDB 38559 E01
France, Corsica, Ruisseau de Battesta at crossing with road D50	41.5449° N, 9.0229° E	NOVMB068-21/ OM952163	CCDB 38559 F08
France, Corsica, Ruisseau de Battesta at crossing with road D50	41.5449° N, 9.0229° E	NOVMB069-21/ OM952162	CCDB 38559 F09
France, Corsica, Ruisseau d' Enova, at crossing with road D124	42.2659° N, 8.7141° E	NOVMB076-21/ OM952161	CCDB 38559 G04
***Limnolegeria longiseta* Motaş, 1928**			
Turkey, Burdur, Bucak at Kizilli Village	37.3425° N, 30.9256° E	SEPTA083-21/ OM321062	CCDB 38363 G11

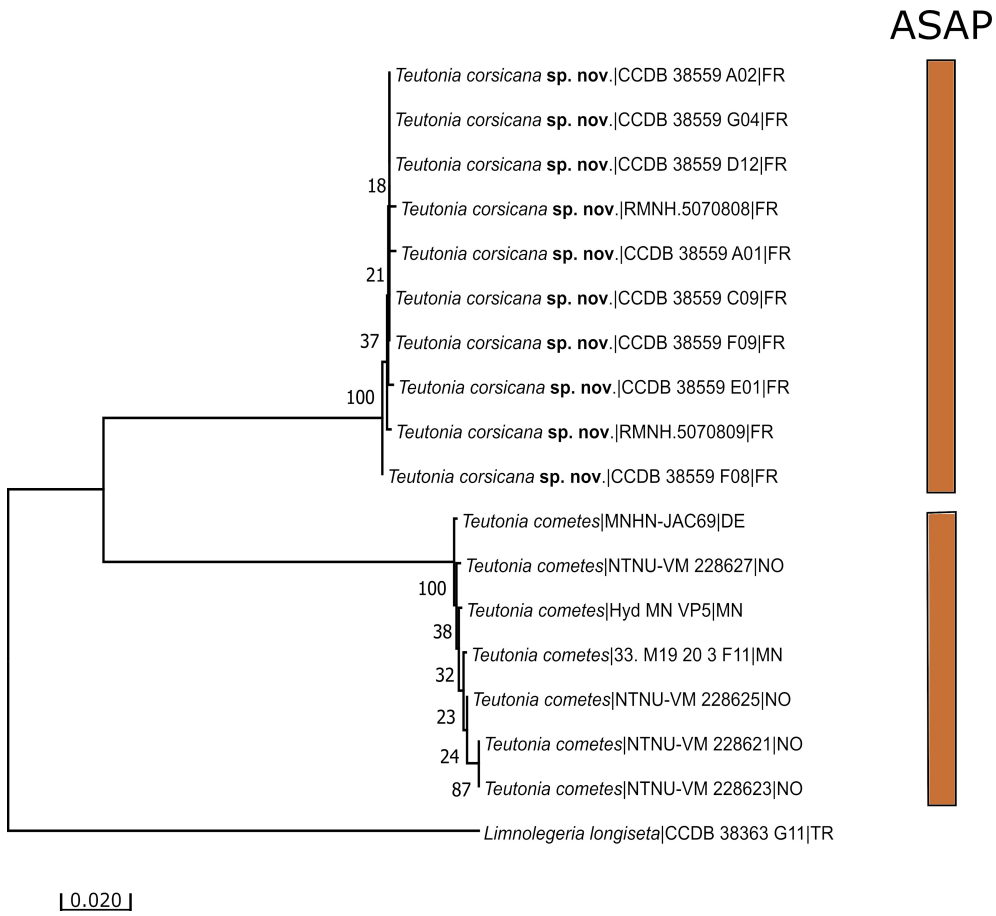

Figure 1 Neighbour-Joining tree of the genus *Teutonia*, obtained from 17 nucleotide COI sequences.and the results of species delimitation analyses. Values near branches show bootstrap support (BS). The results of species delimitation by ASAP procedure are indicated by vertical bars. Country codes (alpha-2 code): DE – Germany, FR – France, MN – Montenegro, NO – Norway, TR – Turkey.

In order to assess the genetic differentiation of species we used the online ASAP (assemble species by automatic partitioning) version (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) with default settings and the K2P distance model. The latter procedure was designated to a list of partitions of species hypotheses using genetic distances, calculated between DNA sequences and ranked by their ASAP-scores: the lower the score, the better the partition (Puillandre *et al.* 2021).

Results and discussion

Species delimitation using DNA-barcodes

The final alignment for species delimitation using COI sequence data included 17 *Teutonia* specimens listed in Table 1 and one outgroup, *Limnolegeria longiseta* from Turkey. The final alignment consisted of 837 nucleotide positions. The NJ tree is presented in Figure 1.

The sequences retrieved from the *Teutonia* specimens from Corsica appeared as a sister clade of the clade that encompasses specimens of *T. cometes* from Norway, Germany and Montenegro (Figure 1). The average K2P genetic distance between *T. corsicana* **sp. nov**. from Corsica and *T. cometes* was estimated 18.7±1.9%. Both species possesses a low intraspecific distance, 0.15±0.07% K2P for *T. corsicana* **sp. nov**. and 0.36±0.15% K2P for *T. cometes*.

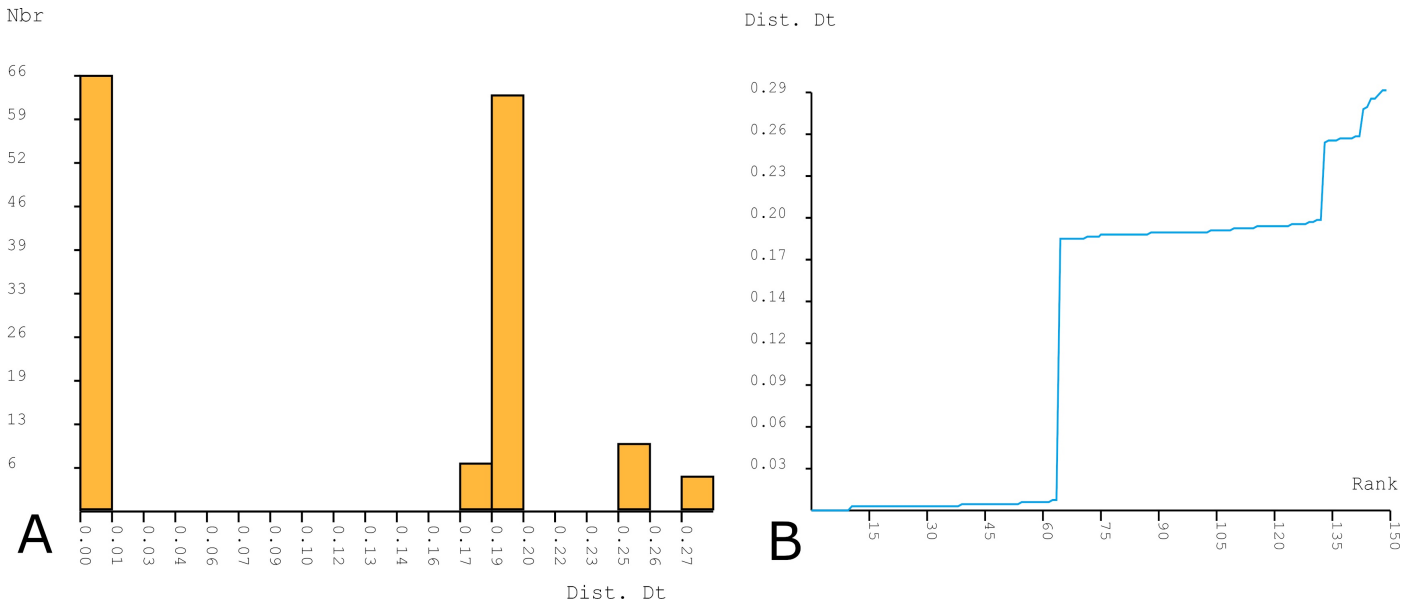

Figure 2 Results of ASAP analysis for COI sequences. (A) Distribution of pairwise differences, (B) Ranked pairwise differences.

The ASAP method observed a barcode gap of about 1-18% K2P distances (Figure 2A-B). The applied ASAP procedure identified two MOTUs (hypothetical species) corresponding to *T. cometes* and *T. corsicana* **sp. nov**. at the threshold distance of 9.51% (K2P) which has the best ASAP-score (1.00) within the available molecular data.

Systematics

Family Teutoniidae Koenike, 1910

Diagnosis — Smit 2020: 268.

Genus *Teutonia* Koenike, 1889

Diagnosis — Smit 2020: 269.

Teutonia corsicana sp. nov.

Zoobank: 5877B3D9-E264-4102-A442-3356BCE46C10

Figures 3-4

Material examined

Holotype ♀, France, Corsica, Ruisseau de Tuara, crossing road D81, 42.3496° N, 8.66497° E, 238 m a.s.l., 14 Apr. 2015, leg. Smit (sequenced [voucher code: CCDB 38559 A01; BOLD/GenBank Nos.: NOVMB001-21/ OM952168]), dissected and slide mounted. Paratype: 1 ♀, same place and data as holotype, sequenced (Table 1); 1♂, 1♀, Ruisseau de Sattu, Corsica, 41.6907° N, 9.1525° E, 370 m a.s.l., 10 Apr. 2015, leg. Smit, 1♂ sequenced (Table 1); 1♂, 1♀, Rivière La Solenzara, Corsica, 41.8383° N, 9.33055° E, 90 m a.s.l., 7 Apr. 2015, leg. Smit, 1♂, 1♀ sequenced (Table 1); 2♂, Ruisseau de Battesta at crossing with road D50, Corsica, 41.5449° N, 9.02292° E, 220 m a.s.l., 8 Apr. 2015, leg. Smit, 2♂ sequenced (Table 1), 1♂ (voucher code: CCDB 38559 F09) dissected and slide mounted; 2♀, Ruisseau de Enova, at crossing with road D124, Corsica, 42.2659° N, 8.71405° E, 105 m a.s.l., 13 Apr. 2015, leg. Smit, 1♀ sequenced (Table 1).

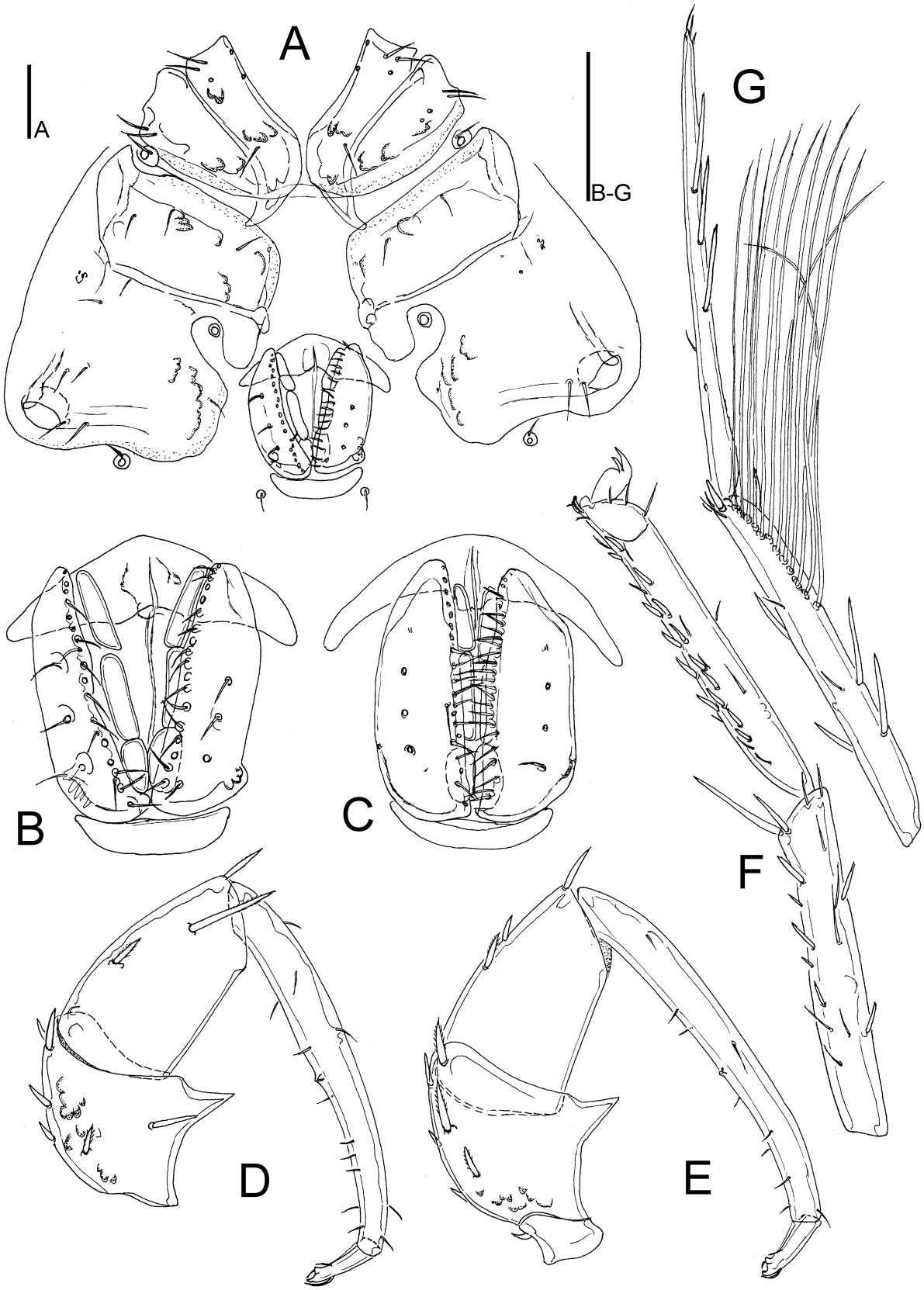

Figure 3 *Teutonia corsicana* **sp. nov**. (A-B, D-G – holotype ♀, Ruisseau de Tuara, France; C – ♀ [CCDB 38559 D12], preserved specimen, Riviere La Solenzara, France): A – coxal and genital field; B, C – genital field; D – palp, medial view (P-1 lacking); E – palp, lateral view; F – I-L-5 and -6; G – IV-L-5 and -6. Scale bars = 100 µm.

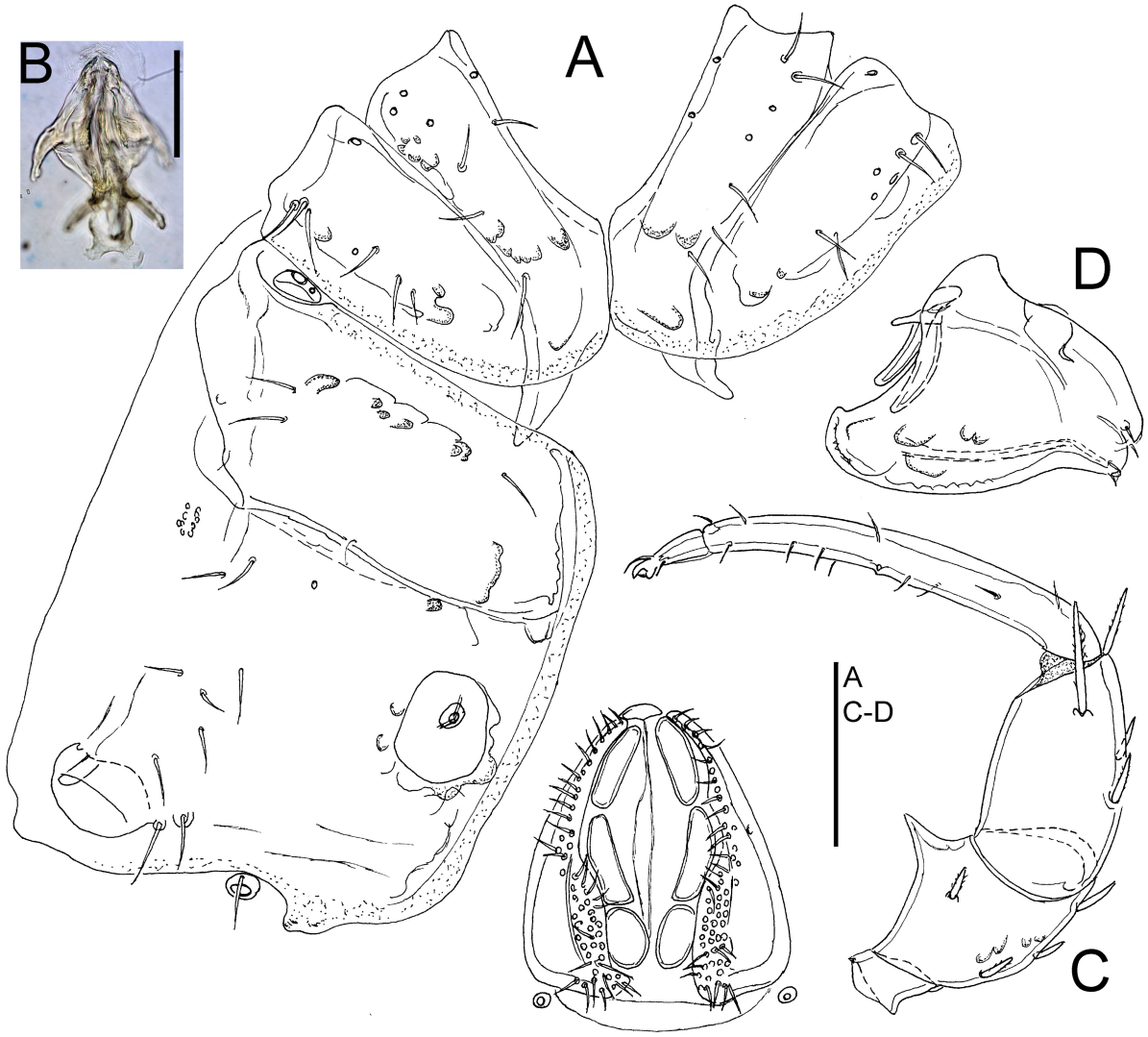

Figure 4 *Teutonia corsicana* **sp. nov.**, ♂ [CCDB 38559 F09], Ruisseau de Battesta, France: A – coxal and genital field, partial view; B – photograph of ejaculatory complex; C – palp, medial view; D – gnathosoma. Scale bars = 100 µm.

Other material

France, Corsica: Ruisseau de Battesta, at crossing with road D50, 41.54487° N, 9.02292° E, 220 m a.s.l., 8 Apr. 2015, 3♀, 1 deutonymph; Ruisseau de Campaghiu, Porto, 42.24128° N, 8.73240° E, 188 m a.s.l., 11 Apr. 2015, 1♀, 1 deutonymph; Ruisseau d'Aitone, at crossing with road D84, 42.26365° N, 8.84597° E, 1115 m a.s.l., 12 Apr. 2015, 1♀; Ruisseau de Casa Infurcata, at crossing with road D124, 42.25600° N, 8.76513° E, 217 m a.s.l., 13 Apr. 2015, 1♀; Ruisseau d'Enova, at crossing with road D124, 42.26590° N, 8.71405° E, 105 m a.s.l., 13 Apr. 2015, 1♂, 5♀, 2 deutonymphs; Rivière Le Fango, at crossing with road D81, 42.41503° N, 8.68300° E, 6 m a.s.l., 14 Apr. 2015, 1♀, 4 deutonymphs; Ruisseau de Tuara, at crossing with road D81, 42.34958° N, 8.66497° E, 230 m a.s.l., 14 Apr. 2015, 3♀, 4 deutonymphs; tributary of Ruisseau de Canne, at crossing with road D81, 42.38307° N, 8.69903° E, 225 m a.s.l., 14 Apr. 2015, 1♀, 2 deutonymphs; Rivière de Tartagine, at Maison Forestière Tartagine, 42.49410° N, 8.99288° E, 706 m a.s.l., 16 Apr. 2015, 1♀; unnamed stream N of Suarella, at crossing with road D18, 42.39033 N, 9.13762° E, 387 m a.s.l., 17 Apr. 2015, 1♀.

Diagnosis

Female gonopore pregenital sclerite partly covered by genital flaps; in both sexes P-2 with broad-based ventrodistal projection, P-3 slender (L/H ratio 1.6-1.7).

Description

General features — Integument finely striated. Coxae medially touching, but not fused, with a few setae. Ac-1-2 rectangular, Ac-3 roundish. P-2 with a generally broad-based ventrodistal projection, P-3 with nearly straight, or slightly convex ventral margin, P-3 lateral seta long, shifted to medial surface, P-4 slender, bearing several fine hair-like setae, mainly in anteroventral part. Legs: claws with claw blade and ventral clawlets; swimming setae restricted to III-/IV-L-3-4, numbers: III-L-3, 3; III-L-4, 8; III-L-5, 8-9; IV-L-3, 3; IV-L-4, 8-9; IV-L-5, 11-12. *Male* — Glandularium on Cx-IV completely surrounded by coxal sclerotization. Genital field: genital flaps medially with a dense cover of fine setae, pregenital sclerite small, postgenital sclerite as wide as genital field. *Female* — Glandularium on Cx-IV partly surrounded by coxal sclerite, medially in contact with the perigenital membrane. Genital field: genital flaps with a row of regularly arranged medial setae, pregenital sclerite large, crescent shaped, partly covered by genital flaps (but only slightly covered in Koenike-fixed specimens), gonopore shorter than the genital flaps, postgenital sclerite shorter than pregenital sclerite.

Description — **Female** (holotype) – Idiosoma L 1190, W 905. Coxal field: L 581, W 878; Cx-III W 563; L Cx-I+II 228, Cx-III+IV 434. Genital flaps L 177-181; pregenital sclerite W 200, postgenital sclerite W 106; L Ac-1-3: 59, 56-60, 39-43. Eggs maximum diameter (n=2) 173-178.

Palp total L 684, dL/H, dL/H ratio: P-1, 33/44, 0.75; P-2, 142/117, 1.21; P-3, 164/102, 1.61; P-4, 289/41, 7.1; P-5, 56/15, 3.8; L ratio P-2/P-4 0.49. Gnathosoma vL 194; chelicera L 256, basal segment L 219, claw L 44, L ratio chelicera basal segment/claw 5.0. Leg segments dL: I-L-2-6: 122, 166, 228, 244, 253; IV-L-1-6: 156, 156, 200, 253, 264, 331.

Male (paratype, CCDB 38559 F09) – Idiosoma L 750, W 588. Coxal field: L 495, W 703; Cx-III W 459; L Cx-I+II 197, Cx-III+IV 380. Genital field L/W 166/147, genital flaps L 156-163, pregenital sclerite W 119; L Ac-1-3: 50, 52-53, 34-38. Ejaculatory complex (Figure 3B) L 192.

Palp total L 581, dL/H, dL/H ratio: P-1, 28/36, 0.77; P-2, 120/96, 1.25; P-3, 142/84, 1.69; P-4, 244/34, 7.2; P-5, 47/14, 3.3; L ratio P-2/P-4 0.49. Gnathosoma vL 165; chelicera L 230, basal segment L 186, claw L 44, L ratio chelicera basal segment/claw 4.3. Leg segments dL: I-L-1-6: 71, 111, 135, 191, 200, 209; IV-L-1-6: 131, 144, 175, 215, 236, 284.

Etymology

Named after the island where the new species was discovered.

Remarks

The female of the new species from Corsica, is easily distinguished from the two other know species of the genus, *T. cometes* and *T. subalpina* in the shape of genital field having a pregenital sclerite partly covered by the genital flaps. Moreover, in both sexes *T. cometes* differs in P-2 generally with a narrow-based ventrodistal projection (but in larger populations, the latter projection appearing broad-based in some specimens, especially in juveniles) and stouter P-3 (L/H ratio 1.35-1.5 vs. 1.6-1.7 in *T. corsicana* **sp. nov.**). The high genetic distance between the new species and *T. cometes* (COI 18.7±1.9 K2P%) suggests a long independent history of the new species. The former records of *T. cometes* from Corsica (Angelier 1959; Santucci 1971) likely should be assigned to the new species.

The little known *T. subalpina* can be distinguished in the female by the very long gonopore, anteriorly distinctly extending over the margin of the genital flaps (Di Sabatino *et al.* 2010). The taxonomic status of *T. subalpina* is still unclear, and according to Di Sabatino *et al.* (2010),

it is possible that the original description of the latter species was based on freshly hatched, unsclerotized, misshapen adults of *T. cometes*.

Distribution

France (Corsica).

Key to the European species of *Teutonia* Koenike, 1889

1. Female gonopore very long, only the posterior 2/3 flanked by Ac and genital flaps; ventral projection of P-2 in both sexes on a large base, triangular . . . . . *Teutonia subalpina*
— Female gonopore all over it length flanked by Ac and genital flaps; ventral projection of P-2 various 2

2. Female pregenital sclerite not covered by genital flaps (as illustrated in 5A); ventral projection of P-2 in both sexes on a narrow base, peg-like (as illustrated in Figure 5B); P-3 stout, L/H ratio 1.35-1.5 . . . . . *Teutonia cometes*
— Female pregenital sclerite partly covered by genital flaps; ventral projection of P-2 in both sexes on a large base, triangular; P-3 slender, L/H ratio 1.6-1.7 . . . *Teutonia corsicana* **sp. nov**.

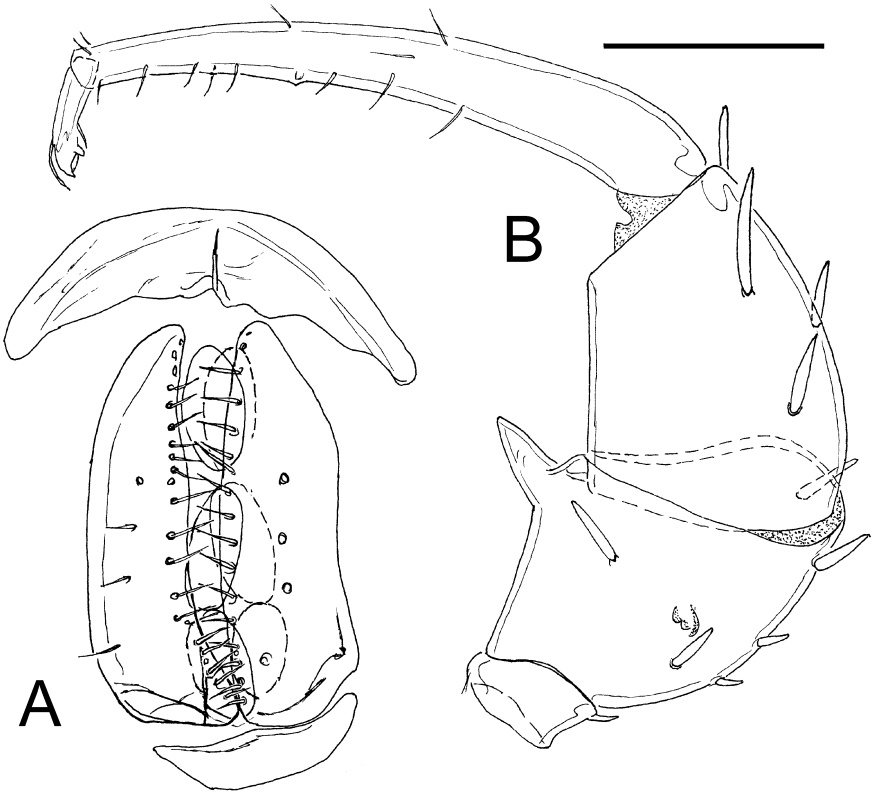

Figure 5 *Teutonia cometes* (Koch, 1837), ♀, Danilovgrad, spring under the bridge over the Zeta river, Montenegro: A – genital field; B – palp, medial view. Scale bar = 100 μm.

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