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***Thiasophila szujeckii* sp. n. (Coleoptera, Staphylinidae, Aleocharinae)—a cryptic species associated with *Formica truncorum* in Poland**

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Abstract

The article describes a new rove beetle species, *Thiasophila szujeckii* sp. n., in southeastern Poland. This new species is associated exclusively with *Formica truncorum*. The authors describe its sexual dimorphism of habitus, structure of antennae, eighth abdominal tergite and eighth sternite. *T. szujeckii* sp. n. shares most morphological features with *T. angulata* and *T. lohsei* known in Europe. Characters of adults which differentiate the new species from the above-mentioned ones include body size, coloration, structure of ligula, aedeagus, parameres and spermatheca. In order to confirm morphological distinctiveness of *T. angulata* and *T. szujeckii*, mitochondrial cytochrome oxidase II gene (COII) partial sequences of both taxa was analyzed.

Key words: Rove beetle, myrmecophile, new species, entomology, taxonomy, cytochrome oxidase II gene, COII, Poland

Introduction

Genus *Thiasophila* Kraatz, 1856 (Staphylinidae, Aleocharinae, Oxypodini), includes 11 species (†‡*T. angulata* (Erichson, 1837), *T. aymumosir* Maruyama & Zerche, 2014, †*T. bercionis* Berhauer, 1926, †‡*T. canaliculata* Mulsant & Rey, 1875, †‡*T. inquilina* (Märkel, 1845), *T. kaszabi* Zerche, 1987, †*T. lohsei* Zerche, 1987, *T. nipponica* Maruyama & Zerche, 2014, *T. pexa* Motschulsky, 1860, *T. shinanonis* Maruyama & Zerche, 2014, and †*T. wockii* (G. Schneider, 1862)) distributed in the Palearctic region, among them 6 species were recorded in Europe (†) and 3 are known in Poland (‡) (Zerche 1987; Smetana 2004; Maruyama & Zerche 2014). Members of this genus (body length of the Palearctic species: 1.8–4.3 mm) are myrmecophilous rove beetles associated with ants of the genera *Formica*, *Lasius* and *Camponotus*. However, most *Thiasophila* species are associated exclusively with single host species thus their distribution ranges are usually fairly limited (Zerche 1987; Maruyama & Zerche 2014; Smetana 2004). Among the European species, only *T. angulata*, is distributed throughout Europe and in some Asian regions, and is associated with ten ant host species (*Formica aquilonia*, *F. lugubris*, *F. polycrena*, *F. pratensis*, *F. rufa*, *F. sanguinea*, *F. truncorum*, *F. uralensis*, *Lasius brunneus* and *L. fuliginosus*) (Zerche 1987; Päivinen *et al.* 2002, 2003; Smetana 2004; Staniec & Zagaja 2008). Faunistic studies on Staphylinidae associated with ant colonies, conducted in south-eastern Poland, revealed that *T. angulata* is a dominant species in the assemblages of those beetles. It constitutes approximately 22% of all rove beetles captured in ant colonies (Staniec & Zagaja 2008).

Recently, detailed morphology of its immature developmental stages was described (Zagaja *et al.* 2014). In this study the authors try to determine whether there are any differences in the structure of the preimaginal stages of *T. angulata* depending on the host species. Previously, host-dependent interspecific variability of adult structure of this species was recorded several times by Lohse (1974), Burakowski *et al.* (1981) and Koch (1989). The study revealed that this variability occurs in larvae. Mature larvae of *T. angulata* inhabiting *F. truncorum* nest mounds are larger and have more slender antennae than larvae associated with other ant hosts (*F. rufa* and *F. polycrena*) (Zagaja *et al.* 2014). Host-dependent variability of *T. angulata* adults was also revealed by the conducted studies (authors'

unpublished data). The differences include phenology and seasonal abundance. In the vegetation season, three generations of *T. angulata* inhabiting *F. rufa* and *F. polyctena* colonies, and one generation of the beetle inhabiting *F. truncorum* colonies were observed. The information obtained inspired us to formulate the following presumption: in the explored area most specimens of *Thiasophila* associated with *Formica truncorum*, defined before as members of *T. angulata*, belong to a yet undescribed species.

In the present study, we describe this new species and analyze its morphological characteristics in comparison to its most similar congeners. Additionally, we provide genetic differences between *T. angulata* and the new species based on mitochondrial cytochrome *c* oxidase subunit II gene (COII) sequences.

Material and methods

Examined material. Adults of *Thiasophila* were collected by sifting the nest material of *Formica polyctena*, *F. rufa*, *F. truncorum* and *F. pratensis* (only for molecular study), from 1 May to 10 October 2010 and 29 July 2014 in Leżajsk Forest Division, located in the central part of the Sandomierska Basin ($50^{\circ}18'12.72''N$, $22^{\circ}16'14.74''E$, UTM–EA87) of SE Poland (Fig. 31A). Number of specimens examined: for morphometric and statistical analysis – 60 of *T. angulata* associated with *F. polyctena* (30) and *F. rufa* (30), and 30 of the new species associated with *F. truncorum*, within them 20 of *T. angulata* associated with *F. polyctena* (10) and *F. rufa* (10), and 10 specimens of the new species associated with *F. truncorum* were dissected for detailed morphological studies; for DNA sequence analysis – 15 of *T. angulata* associated with *F. polyctena* (6), *F. rufa* (6) and *F. pratensis* (3), and 8 of the new species associated with *F. truncorum* were homogenized.

Study techniques. Morphometry and morphological observation: specimens were measured, examined and dissected using Olympus SZX16 stereomicroscope. Measurements were made by Stream Motion 1.7 software, and given in millimeters. Analysis of variance (ANOVA) was carried out on the measurements of the seven morphometric characteristics (body length, head and pronotum width and length, elytral and abdominal width) to determine whether there was a difference in the *Thiasophila* specimens coming from nests of various *Formica* species (*F. polyctena*, *F. rufa* and *F. truncorum*). Post hoc Tukey Honest Significant Difference (HSD) multiple comparison tests were performed to determine which mean values differed significantly. A statistical analysis was conducted using STATISTICA 6.0 (StatSoft. Inc.). For illustrating the morphometric variability between individuals of *T. angulata* (coming from *F. rufa* and *F. polyctena*) and the new species (coming from *F. truncorum* nests) the data obtained were analyzed using statistical procedure PCA (Principal Component Analysis) by means of the MVSP 3.1 program (Kovach 2005).

For the purpose of preparing detailed morphological analyses, the heads and terminal segments (VIII–X) including genitalia were treated with a 10% KOH solution for approximately 2–3 days, rinsed in distilled water, and placed in lactic acid for subsequent preparation on temporary microscope slides. The genitalia, tergites VIII, sternites VIII and antennae of both sexes of the new species were examined. For distinguishing of the new species from the closely related *T. angulata* (Erichson, 1837) the ligulae, parameres and genitalia were compared. Photographs were taken by a digital camera (Olympus DP72) mounted to an Olympus SZX16 compound microscope (Figs 1, 2, 2A–E) or Olympus BX61 compound microscope (Figs 3–28), and corrected by means of graphic software CorelDRAW Graphics Suite X5.

DNA extraction, PCR and sequencing analyses: for 23 specimens of *T. angulata* and the new species (preserved in 96% ethanol) total genomic DNA was extracted according to the manufacturer's protocol for the Genomic mini (A&A Biotechnology, Gdańsk, Poland). The COII region examined in this study was amplified by a set of primers TL2-J-3046 and C2-N-3661 (Simon *et al.* 1994; Elven *et al.* 2010). PCR was performed in 20 µL with 3 µL of the genomic DNA with 10 µL 2xPCR Master Mix (A&A Biotechnology, Gdańsk, Poland) and 0.4 µL of each primer. The amplification involved 3 min of denaturation at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s of primer annealing at 48–53°C, and 1 min of extension at 72°C, followed by a final 3-min extension at 72°C. Amplified DNA templates were purified and sequenced by a commercial company, Genomed, using the same primers. Chromatograms were checked by eye using ChromasPro 1.45 (McCarthy 1998) and the alignment was performed using BioEdit v.7.0.5.2 (Hall 1999). A final alignment was analyzed, where the selected outgroups included *Oxypoda praecox* and *Atheta hampshirensis* (Table 1).

The final data set was then analyzed using maximum likelihood (ML) method. The best-fit model of nucleotide substitution evolution under corrected Akaike Information Criterion was estimated using JModelTest 2.1.4 (Darriba *et al.* 2012). Model GTR+I+G was chosen and used in the phylogenetic analyses. ML trees were built in PhyML 3.0 (Guindon *et al.* 2010) running 1,000 bootstrap replicates and searching for the best-scoring ML tree. Pairwise distances were obtained using MEGA 6 software based on the Kimura two-parameter model (Tamura *et al.* 2013).

Chosen fragment of the COII gene, has been successfully used for the identification of species (Jeon & Ahn 2007, 2009) and of phylogenetic analyses at taxonomically higher levels (Maekawa *et al.* 2000; Normark 2000; von Dohlen *et al.* 2002, 2006).

Partial COII sequences (658 bp) of *T. angulata* and *T. szujeckii* sp. n. have been deposited in GenBank under accession numbers KP677504–KP677507 (Table 1).

Voucher specimens have been deposited in the collection of the Department of Zoology of the Mariae Curie-Sklodowska University of Lublin. Morphological terminology and a formula description mainly follow that used by Maruyama and Zerche (2014).

TABLE 1. Taxa included in the phylogenetic analyses and GenBank accession numbers for sequences.

Species	GenBank accession numbers	Reference
<i>Thiasophila angulata</i> H1	KP677504	this study
<i>Thiasophila angulata</i> H2	KP677505	this study
<i>Thiasophila angulata</i> H3	KP677506	this study
<i>Thiasophila szujeckii</i> sp. n. H4 (= <i>Thiasophila angulata</i> H4)	KP677507	this study
<i>Oxypoda praecox</i>	GQ980882	Elven <i>et al.</i> (2010)
<i>Atheta hampshirensis</i>	GQ980915	Elven <i>et al.</i> (2010)

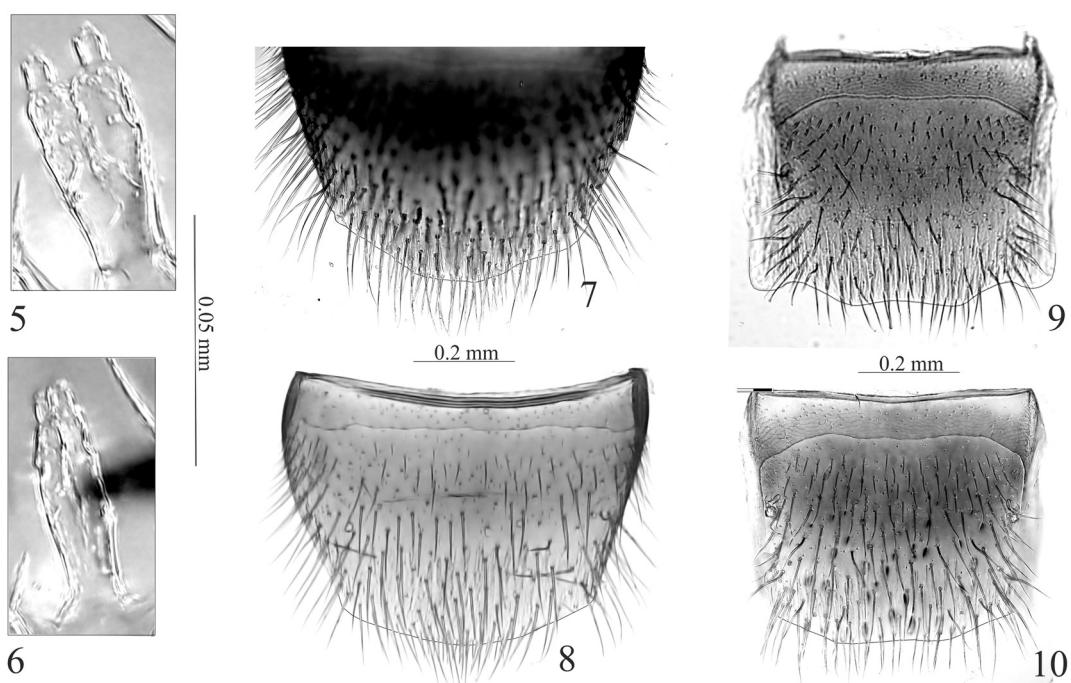
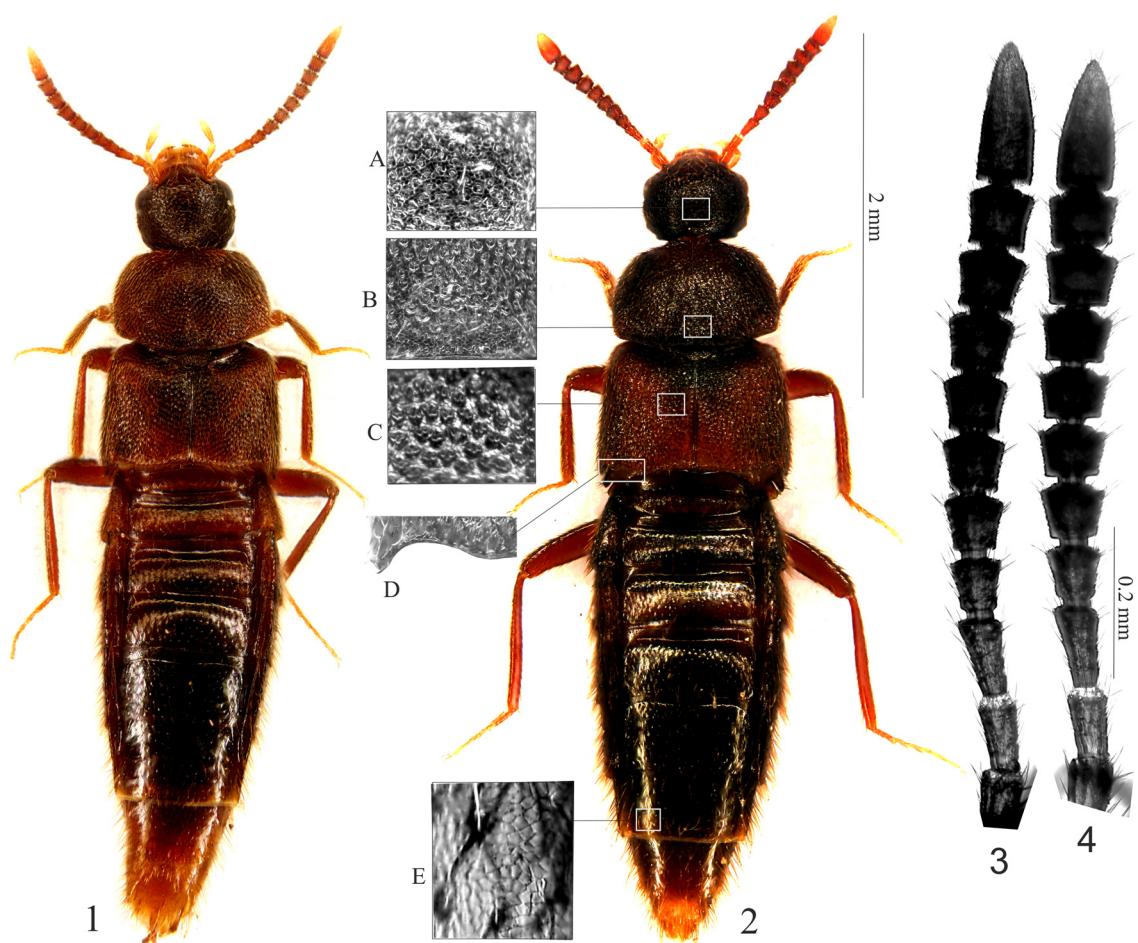
Taxonomy

Thiasophila szujeckii Zagaja & Staniec, sp. n.

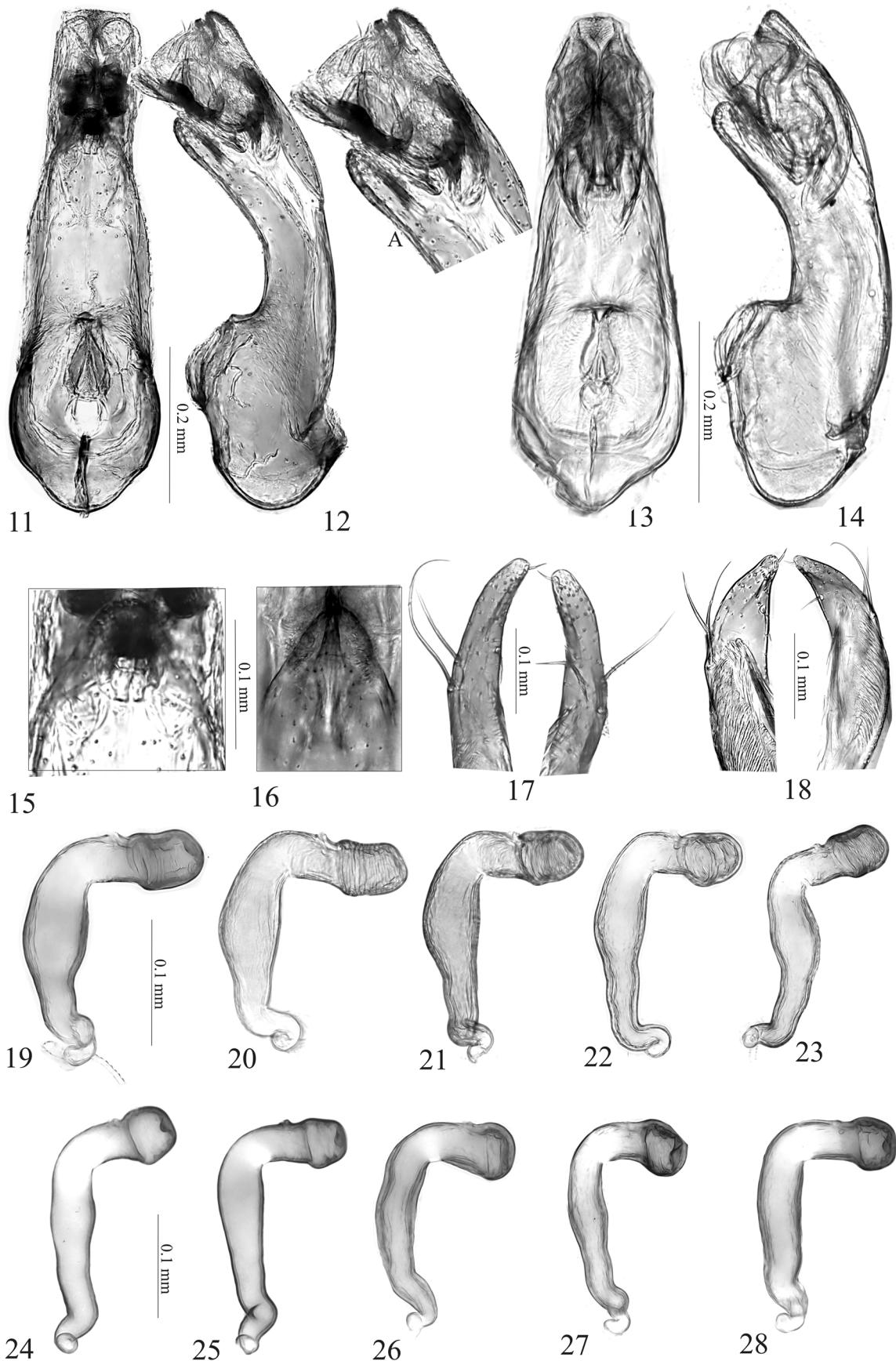
Type locality. South-eastern Poland, the Sandomierska Basin, Wola Zarczycka near Leżajsk, pine forest ($50^{\circ}18'12.72''N$, $22^{\circ}16'14.74''E$, UTM–EA87) (Fig. 31, 31A).

Type series. Holotype (male): Poland, Wola Zarczycka, in the area of the Leżajsk Forest Division, the Sandomierska Basin (SE Poland) ($50^{\circ}18'12.72''N$, $22^{\circ}16'14.74''E$, UTM–EA87), 29 VII 2014, pine forest, in the nest mound of *Formica truncorum*, by sifting the nest material (the collection of the Department of Zoology of the Mariae Curie-Sklodowska University of Lublin). Paratypes (7 females, 4 males): same data as holotype (2 females), same data as holotype but collected from 1 V to 10 X 2010 (5 females, 4 males) by sifting a mound of nest, all collected by M. Zagaja (the collection of the Department of Zoology of the Mariae Curie-Sklodowska University of Lublin).

Description. Body (Figs 1, 2) large, length 3.90–4.21 mm. Dorsal surface of head, pronotum and elytra mostly shagreened but slightly glossy. Head black, 1.0–1.2 as wide as long; surface finely, densely punctured, reticulated (Fig. 2A), but clypeus and frons weakly punctured, clypeus mildly rounded apically. Ligula robust widening gradually to apical part (Fig. 5). Antennae rather slender as long as head and pronotum combined, reddish brown, segments I–III and XI distinctly lighter, segment I slightly widened apically, segment II and III almost equal in length, segment II slightly and segment III distinctly widened apically, both almost twice as long as wide, length to width relation segments IV–XI slightly different depending on sex (Figs. 3, 4; see description below). Pronotum slightly convex, widest near middle, 1.6 times as wide as long, dark brown, but paler around posterolateral corners and lateral margins; anterior margin truncate; lateral margin gently rounded, subparallel-sided in posterior part; posterolateral corners visible, slightly rounded, posterior margin rounded; surface shagreened, reticulated (Fig. 2B). Elytra slightly widened posteriorly, posterior margin deeply notched near posterolateral corners (Fig. 2D); reddish brown, near scutellum, anterior and lateral margin to posterolateral corners dark brown; surface shagreened, reticulated (Fig. 2C). Legs reddish brown, tarsi somewhat paler. Abdominal segments III–IV or III–V reddish brown, segments VI–VII (sometimes V) dark brown with lighter posterior margin, apical segments yellowish brown; tergites glossy but reticulated and densely with setiferous punctures that become sparser towards apical segments (Fig. 2E).



FIGURES 1–10. *T. szujeckii* sp. n. (1–5, 7–10), *T. angulata* (6). Dorsal habitus of holotype, male (1) and paratype, female (2) with microstructure (2A–C, E) and posterior margin near posterolateral corner (2D); right antenna of male (3) and female (4); ligula (5, 6); eighth sternite of male (7) and female (8); eighth tergite of male (9) and female (10).



FIGURES 11–28. *T. szujeckii* sp. n. (11, 12, 12A, 15, 17, 19–23), *T. angulata* (13, 14, 16, 18, 24–28). Aedeagus in ventral view (11, 13) and lateral view (12, 14); apex of apical lobe of aedeagus in ventral view (15, 16); apex of apical lobe of parameres in ventral view (17, 18); spermathecae (19–28).

TABLE 2. Morphological differences between *T. szujeckii* sp. n., *T. angulata* and *T. lohsei*; measurements (in mm)—mean, standard error (SE), range; ?—no data, (X)—figure number; X§—figure number and data of measurements by Zerche (1987). Assignment of various superscript letters indicates a highly significant difference (ANOVA, test Tukeya (HSD), $p \leq 0.05$) between means.

Species Character	<i>T. szujeckii</i> sp. n. (N=30)	<i>T. angulata</i> (N=60)	<i>T. lohsei</i> (N=?)
Body length	4.10 ± 0.20^a , 3.90–4.21	3.79 ± 0.028^b , 3.52–4.10 or 2.8–4.3§	2.8–3.7§
Head width	0.57 ± 0.035^a , 0.55–0.61	0.47 ± 0.0044^b , 0.46–0.53	?
Head length	0.44 ± 0.043^a , 0.41–0.48	0.38 ± 0.0044^b , 0.35–0.43	?
Pronotum width	0.90 ± 0.042^a , 0.86–0.93	0.79 ± 0.057^b , 0.75–0.83	?
Pronotum length	0.47 ± 0.039^a , 0.43–0.50	0.43 ± 0.0048^b , 0.39–0.48	?
Pronotum: width to length ratio (mean)	1.91	1.84	1.56
Elytral width	1.01 ± 0.053^a , 0.95–1.10	0.91 ± 0.0080^b , 0.85–0.99	?
Abdomen width	0.97 ± 0.054^a , 0.92–1.00	0.89 ± 0.0076^b , 0.83–0.98	?
Colour	dark	light	dark
Antenna: length ratio of seg. XI and X	male: 2.4:1 female: 2.3:1	male: 2.5:1 female: 2.4:1	male: 2.4:1 (29§) female: 2.8:1
Antenna, ♀: width to length ratio of seg. X	1.3x	1.5x	1.6x
Ligula: shape	long, robust, widened to apical part (5)	long, slim, subparallel-sided (6, 45§)	short, slim, widened to apical part (27§)
Aedeagus: shape	moderately slender (12)	robust (14, 53§)	slender (40§)
Aedeagus: shape of apical lobe	narrow, short, not widened about apex (12, 12A), broadly rounded apically (15)	wide, long, not widened about apex (14, 46§), mildly rounded apically (16)	wide, short, slightly widened about apex (38§), broadly rounded apically (39§)
Aedeagus: shape of median lobe	in the greater part subparallel-sided (11)	distinctly tapering from base to apex (13, 52§)	slightly tapering from base to apex (39§)
Parameres: shape of apical lobe	slightly tapering and widely rounded apically (17)	strongly tapering and moderately rounded apically (18)	strongly tapering and moderately rounded apically (32§)
Spermatheca: shape, size of apical part	cylindrical, large (19–23)	spherical, small (24–28, 54–58§)	cylindrical, large (41–43§)
Spermatheca: basal part	short	short	long
Spermatheca: dilatation on basal part	present (19–23)	lack (24–28, 54–58§)	lack (41–43§)
Host-associated	<i>F. truncorum</i>	<i>F. aquilonia</i> , <i>F. lugubris</i> , <i>F. polyctena</i> , <i>F. pratensis</i> , <i>F. rufa</i> , <i>F. sanguinea</i> , <i>F. truncorum</i> , <i>F. uralensis</i> , <i>Lasius brunneus</i> , <i>L. fuliginosus</i>	<i>F. pratensis</i>
Distribution	south-eastern Poland	Europe, partly Asia	Europe
Sources	present study	present study, Zerche 1987	Zerche 1987

Male (Fig. 1). Antennae more slender than of female (Fig. 3), length to width ratio of segments: IV to V–1.1:1, VI–1:1, VII to X–1:1.1, XI–2.6:1, respectively. Sternite VIII broadly rounded apically (Fig. 7). Tergite VIII with posterior margin slightly emarginated medially, distinctly notched near lateral corners (Fig. 9). Median lobe of aedeagus with sides in the greater part subparallel in ventral view (Fig. 11); apical lobe narrow in lateral view, flagellum short (Figs 12, 12A), widely rounded apically in ventral view (Fig. 15); apical lobe of paramere curved

inside, slightly and gradually tapering to the apex, widely rounded apically, with basal setae about 0.8 times as long as apical lobe (Fig. 17).

Female (Fig. 2). Body somewhat more robust than that of male. Antennae (Fig. 4), length to width ratio of segments: IV–1.1:1, V–1:1.1, VI–1:1.2, VII to X–1:1.3, XI–2.3:1, respectively. Sternite VIII broadly rounded apically (Fig. 8). Tergite VIII with posterior margin truncate medially (Fig. 10). Spermatheca (Figs 19–23): apical part large, cylindrical; basal part with clearly visible dilatation near middle, curved inside about at 90°.

Comparative notes. Morphology. The new species in size, body shaped, structure of antennae is closely similar to *T. angulata*, but in structure of genitalia and coloration to *T. lohsei* (Zerche 1987). The unique characters of *T. szujeckii* sp. n. are the following: spermatheca with dilatation on basal part, apical part of parameres slightly, gradually tapering to apex, median lobe of aedeagus in the greater part subparallel-sided, ligula widening gradually to the apical part. The new species is also distinguished from the other known, from the Palearctic region, *Thiasophila* species by its large size (Zerche 1987; Maruyama & Zerche 2014). In this respect, the distinctiveness of *T. szujeckii* from most similar in size *T. angulata* is illustrated in Fig. 29. The smaller group was formed by the larger specimens of the new species, which were collected exclusively in *F. truncorum* nest mounds. The bigger group consisted of the smaller individuals of *T. angulata* associated with *F. rufa* and *F. polycetena*. Characteristics of adults which differentiate the three above-mentioned species are listed in Table 2.

Phylogenetic analysis. The analysis of the partial COII sequence of *T. angulata* and *T. szujeckii* sp. n. found in four ant species of the *Formica* genus, exhibited the existence of four haplotypes (H1–4). Haplotypes H1, H2, H3 concern *T. angulata*, whereas, H4 is a haplotype of the new species. Genetic distances between obtained haplotypes varied from 0.002 between haplotypes H1, H2, H3 to 0.012 between H3 and H4, indicating a 1.2% difference between the sequence of these haplotypes (Table 3). Minor differences in the COII gene sequence, less than 1%, were also observed between two species of butterflies; *Ostrinia latipennis* and *O. ovalipennis* (Ohno et al. 2006). Cognato (2006) comparing the differences between the sequence of mitochondrial and nuclear DNA markers of sixty two species of insects, determined the interspecies difference in the sequence of these markers in the range between 0.04 and 26.0%. Based on the obtained sequence (alignment) of the COII gene fragment a polygenetic tree was constructed using the ML method (Fig. 30). Two major mtDNA clades were retrieved; first, the H4 haplotype, belonging to the *T. szujeckii* sp. n., the second to the other three haplotypes (H1–3) representing *T. angulata*. The branch grouping of these two evolutionary lines is supported by a high bootstrap ratio of 99. The typology of the polygenetic tree, constructed on the basis of the COII gene sequence, supports the existence of two separate species in the test material.

TABLE 3. Pairwise genetic distances (K2P distances) based on 658 positions of COII sequences between haplotypes.

Haplotype	H1	H2	H3	H4
<i>T. angulata</i> (H1)	-			
<i>T. angulata</i> (H2)	0.002	-		
<i>T. angulata</i> (H3)	0.002	0.003	-	
<i>T. szujeckii</i> sp. n. (H4)	0.011	0.011	0.012	-

Supplement to the key. In order to include *T. szujeckii* sp. n. in the key to the Palearctic *Thiasophila* species except Japan (Zerche 1987)§, the following modifications separately for male and female are proposed at couplet 3.

- 3 (male). Paramere strongly tapering with moderately rounded apex (Figs 18, 32§). Aedeagus slender or robust with wide apical lobe (Figs 14, 38§); median lobe from base at least slightly tapering to apex (Figs 13, 39§, 52§) 3a.
 - Paramere slightly tapering with wide rounded apex (Fig. 17). Aedeagus moderately slender with narrow apical lobe (Fig. 12); median lobe in its greater part subparallel-sided (Fig. 11). Ligula long, widened to apical part (Fig. 5). Coloration: usually dark. Length: 3.90–4.21 mm. Symbiotic host: *Formica truncorum* *Thiasophila szujeckii* sp. n.
 3a. Aedeagus robust (Fig. 14); apical lobe long, not widened about apex (Figs 14, 46§). Ligula long, subparallel-sided (Figs 6, 45§). Coloration: usually light. Length: 3.52–4.10 mm or 2.8–4.3 mm (Zerche 1987). Symbiotic host: *F. aquilonia*, *F. lugubris*, *F. polycetena*, *F. pratensis*, *F. rufa*, *F. sanguinea*, *F. truncorum*, *F. uralensis*, *Lasius brunneus*, *L. fuliginosus*. *T. angulata*
 - Aedeagus slender (Fig. 40§); apical lobe short, slightly widened about apical (Fig. 38§). Ligula short, widened to apical part (Fig. 27§). Coloration: usually dark. Length: 2.80–3.70 mm. Symbiotic host: *F. pratensis*. *T. lohsei*

- 3 (female). Spermatheca: apical part semi-cylindrical or cylindrical (Figs 19–23, 41–43§); basal part short or long. Antenna: segment X 1.3 or 1.6 × as wide as long; segment XI 2.3 × or 2.8 × longer than segment X 3a.
- Spermatheca: apical lobe semi-spherical or spherical (Figs 24–28, 54–58§); basal part short. Antenna: segment X 1.5 × as wide as long; segment XI 2.4 × longer than segment X. Ligula, coloration, length and symbiotic host the same as male *T. angulata*
- 3a. Basal part of spermatheca elongated, 5–5.2 × longer than apical part, without dilatation (Figs 54–58§). Antenna: segment X 1.6 × as wide as long; segment XI 2.8 × longer than segment X. Ligula, coloration, length and symbiotic host the same as male *T. lohsei*
- Basal part of spermatheca short, 3.5–4.2 × longer than apical part, with dilatation at about half of the length (Figs 19–23). Antenna: segment X 1.3 × as wide as long; segment XI 2.3 × longer than segment X. Ligula, coloration, length and symbiotic host the same as male *T. szujeckii* sp. n.

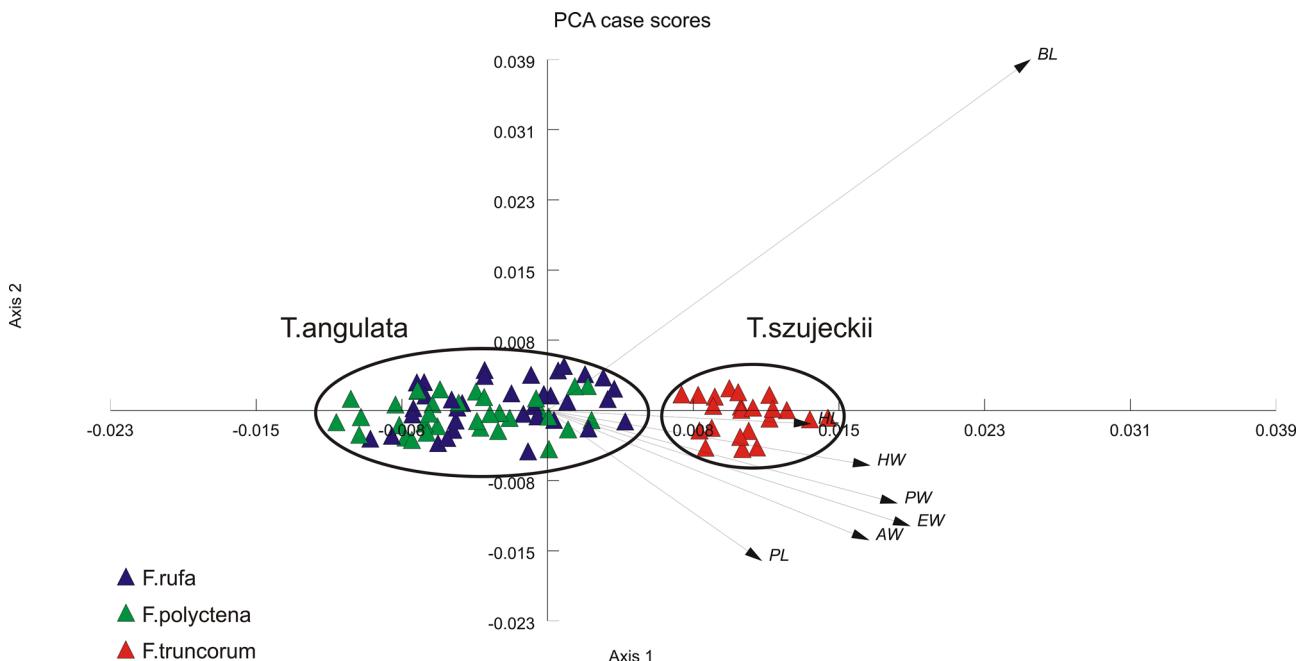


FIGURE 29. Principal Component Analysis (PCA) with some morphometric data of *T. szujeckii* sp. n. (associated with *F. truncorum*) and *T. angulata* (associated with *F. rufa* and *F. polyctena*).

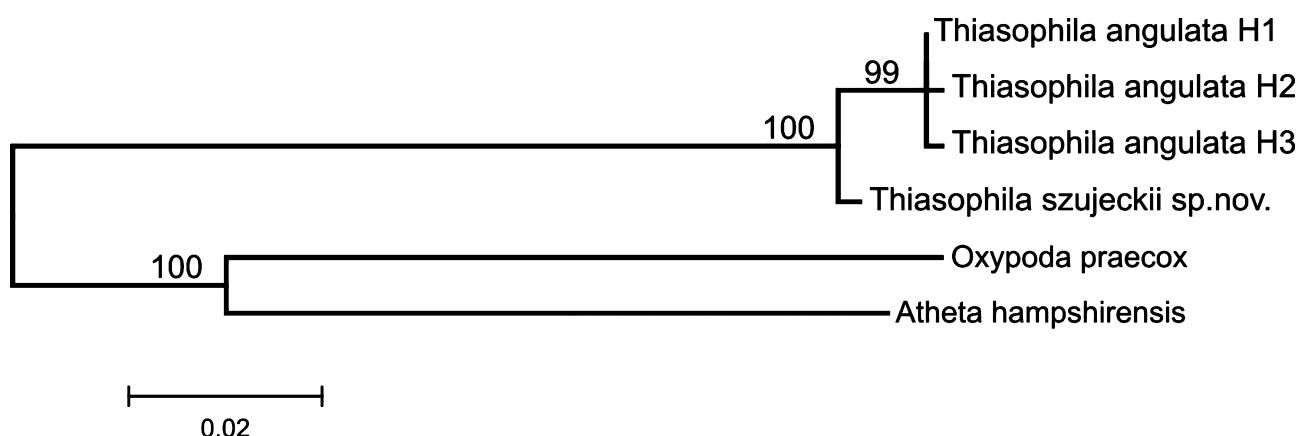


FIGURE 30. Phylogenetic tree (ML) of the *T. angulata* (H1–3) and *T. szujeckii* sp. n. (H4) haplotypes obtained based on COII sequence analysis. Bootstrap values larger than 50% above nodes were pointed.

Etymology. The new species is dedicated to the Polish coleopterologist and forest ecologist prof. Andrzej Szujecki, whose many fundamental researches have contributed much to the knowledge of Polish rove-beetles.

Distribution and natural history. *T. szujeckii* sp. n. discovered in south-eastern Poland (Fig. 31A) is probably broadly distributed in Europe. All specimens of the species were collected exclusively from *Formica*

truncorum nest mounds in pine forest (Fig. 31). Recently, our observations of the natural history (life cycle, phenology, seasonal abundance etc.) of *T. angulata* and *T. szujeckii* sp. n., in natural and laboratory conditions, were completed. These data will be provided in a future article.



FIGURE 31. The nest mound of *Formica truncorum* inhabited by *T. szujeckii* sp. n. with type locality (black circle on UTM map) of this species in Poland (A).

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References

- Burakowski, B., Mroczkowski, M. & Stefańska, J. (1981) *Chrząszcze Coleoptera-Staphylinidae*, p. 3. *Katalog Fauny Polski*, p. XXIII. Vol. 8. Polskie Wydawnictwo Naukowe, Warszawa, 330 pp.
- Cognato, A.I. (2006) Standard percent DNA sequence difference for insects does not predict species boundaries. *Journal of Economic Entomology*, 99, 1037–1045.
<http://dx.doi.org/10.1093/jee/99.4.1037>
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9, 772–772.
<http://dx.doi.org/10.1038/nmeth.2109>
- Dohlen, C.D., Kurosu, U. & Aoki, S. (2002) Phylogenetics and evolution of the eastern Asian-eastern North American disjunct aphid tribe, Hormaphidini (Hemiptera: Aphididae). *Molecular Phylogenetics and Evolution*, 23, 257–267.
[http://dx.doi.org/10.1016/S1055-7903\(02\)00025-8](http://dx.doi.org/10.1016/S1055-7903(02)00025-8)

- Dohlen, C.D., Rowe, C.A. & Heie, O.E. (2006) A test of morphological hypotheses for tribal and subtribal relationships of Aphidinae (Insecta: Hemiptera: Aphididae) using DNA sequences. *Molecular Phylogenetics and Evolution*, 38, 316–329.
<http://dx.doi.org/10.1016/j.ympev.2005.04.035>
- Elven, H., Bachmann, L. & Gusarov, V. (2010) Phylogeny of the tribe Athetini (Coleoptera: Staphylinidae) inferred from mitochondrial and nuclear sequence data. *Molecular Phylogenetics and Evolution*, 57, 84–100.
<http://dx.doi.org/10.1016/j.ympev.2010.05.023>
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59, 307–321.
<http://dx.doi.org/10.1093/sysbio/syq010>
- Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium*, Series 41, 95–98.
- Jeon, M.J. & Ahn, K.J. (2007) Descriptions of late instars of three littoral *Cafius* species (Coleoptera: Staphylinidae) by association of life stage with DNA sequences. *Florida Entomologist*, 90, 465–474.
- Jeon, M.J. & Ahn, K.J. (2009) Description of late-instars of *Bryothinusa koreana* Ahn and Jeon (Coleoptera: Staphylinidae: Aleocharinae) by association of life stage based on DNA sequence data. *Florida Entomologist*, 92, 367–373.
<http://dx.doi.org/10.1653/024.092.0224>
- Koch, K. (1989) *Die Käfer Mitteleuropas. Ökologie*, 1. Goecke & Evers Verlag, Krefeld, 440 pp.
- Kovach Computing Services (2005) *Multi-Variate Statistical Package Plus. Version 3.1*. Kovach Computing Services, Pentraeth, Wales, 137 pp. [UK]
- Lohse, G.A. (1974) Staphylinidae II (Hypocyphinae und Aleocharinae). In: Freude, H., Harde, K. & Lohse, G.A. (Eds.), *Die Käfer Mitteleuropas. Vol. 5*. Goecke & Evers, Krefeld, pp. 304.
- Maruyama, M. & Zerche, L. (2014) Japanese species of the myrmecophilous genus *Thiasophila* Kraatz, 1856 (Coleoptera, Staphylinidae, Aleocharinae). *Esakia*, 54, 27–31.
- Maekawa, K. & Matsumoto, T. (2000) Molecular phylogeny of cockroaches (Blattaria) based on mitochondrial COII gene sequences. *Systematic Entomology*, 25, 511–519.
<http://dx.doi.org/10.1046/j.1365-3113.2000.00128.x>
- McCarthy, C. (1998) CHROMAS 1.45. School of Health Science, Griffith University, Queensland, Australia.
- Normark, B.B. (2000) Molecular systematics and evolution of the aphid family Lachnidae. *Molecular Phylogenetics and Evolution*, 14, 131–140.
<http://dx.doi.org/10.1006/mpev.1999.0699>
- Ohno, S., Ishikawa, Y., Tatsuki, S. & Hoshizaki, S. (2006) Variation in mitochondrial COII gene sequences among two species of Japanese knotweed-boring moths, *Ostrinia latipennis* and *O. ovalipennis* (Lepidoptera: Crambidae). *Bulletin of Entomological Research*, 96, 243–249.
- Päivinen, J., Ahlroth, P. & Kaitala, V. (2002) Ant-associated beetles of Fennoscandia and Denmark. *Entomologica Fennica*, 13, 20–40.
- Päivinen, J., Ahlroth, P., Kaitala, V., Kotiaho, J.S., Suhonen, J. & Virola, T. (2003) Species richness and regional distribution of myrmecophilous beetles. *Oecologia*, 134, 587–595.
<http://dx.doi.org/10.1007/s00442-002-1141-z>
- Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved PCR primers. *Annals of the Entomological Society of America*, 87, 651–701.
<http://dx.doi.org/10.1093/aesa/87.6.651>
- Smetana, A. (2004) Aleocharinae. In: Löbl, I. & Smetana, A. (Eds.), *Catalogue of Palearctic Coleoptera. Vol. 2, Hydrophilidea, Histeroidea, Staphyloidea*. Apollo Books, Stenstrup, pp. 353–295.
- Staniec, B. & Zagaja, M. (2008) Rove-beetles (Coleoptera, Staphylinidae) of ant nests of the vicinities of Leżajsk. *Annales UMCS, Biologia*, 63, 111–127.
<http://dx.doi.org/10.2478/v10067-008-0009-y>
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.
<http://dx.doi.org/10.1093/molbev/mst197>
- Zagaja, M., Staniec, B. & Pietrykowska-Tudruj, E. (2014) The first morphological description of the immature stages of *Thiasophila* Kraatz, 1856 (Coleoptera: Staphylinidae) inhabiting ant colonies of the *Formica rufa* group. *Zootaxa*, 3774 (4), 301–323.
<http://dx.doi.org/10.11646/zootaxa.3774.4.1>
- Zerche, L. (1987) Beitrag zur Kenntnis der Gattung *Thiasophila* Kraatz, 1856 (Coleoptera, Staphylinidae, Aleocharinae). *Entomologische Blätter*, 83, 91–114.