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The first molecular insight into the genus *Turanium* Baeckmann, 1922 (Coleoptera: Cerambycidae: Callidiini) with a description of a new species from Middle Asia

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http://zoobank.org/CEE85B14-2314-4581-AE0D-EB1E2D5587CD

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Abstract

This paper sheds the first light on the phylogeny of the Central Asian genus *Turanium* Baeckmann, 1922. By applying an integrative taxonomy approach, we revealed and described a new species from Kyrgyzstan—*Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** Distinguishing characters from closely related *Turanium pilosum* (Reitter, 1891) are presented and their ecological associations are discussed. The key characters, including the male terminalia, were examined by means of scanning electron microscopy. High-quality stacked photographs of the habitus of the specimens are presented for both species and their geographical distributions are mapped. While the new species shows stable morphological characters that allow its differentiation from *T. pilosum* and the COI genetic distance between them is approx. 3%, the different species delimitation methods gave discordant results. Although the new species remained unrecognized for so long, it seems that these cerambycids are common in the region and both can be considered potentially invasive as they are apparently highly polyphagous. It has also been documented that they occur sympatrically in Kyrgyzstan. Both the Bayesian and maximum likelihood analyses of COI sequences confirmed the monophyly of the genus *Turanium* with strong support (PP 1 and BS 90, respectively). Moreover, the recently revealed polyphyly of the tribe Callidiini was supported by our analyses and, consequently, the discussion on the establishment of a new tribe Ropalopini is raised. This study further corroborates the effectiveness of DNA barcoding as a tool in detecting new species and provides some of the first sequences for Central Asian cerambycids, which remain almost completely unknown in terms of molecular studies.

Key words

BOLD, DNA barcoding, Central Asia, longhorned beetles, SEM, taxonomy

1. Introduction

The genus *Turanium* Baeckmann, 1922 is a small group of the family Cerambycidae, subfamily Cerambycinae that is distributed in Central Asia—from northeastern Iran (Bojnord) to central and southeastern Kazakhstan (Dzungarian Alatau) and western part of Kyrgyzstan, including almost the entire territory of Tajikistan (Danilevsky 2001; Sama et al. 2008). This so far relatively poorly studied group comprises seven hitherto described species that were classified into two subgenera: *Chalcoturanium* Jankowski, 1934 and *Turanium* (Tavakilian and Chevillotte 2021). Five species (i.e. *Turanium hladili* Kratochvíl, 1985, *Turanium pilosum* (Reitter, 1891), *Turanium rauschorum* Holzschuh, 1998, *Turanium scabrum* (Kraatz, 1882), and *Turanium tekeorum* Danilevsky, 2001) were placed in the nominative subgenus, while *Turanium badenkoi* Danilevsky, 2001 and *Turanium johannis* Baeckmann, 1922 belong to *Chalcoturanium*. The latter species was hitherto commonly divided into two subspecies: *T. j. johannis* and *Turanium johannis juglandis* Jankowski, 1934. Interestingly, despite the latter taxon has been synonymised with *T. johannis* (Danilevsky 2001), it was later still listed as a valid subspecies by the same author (Danilevsky 2020).

Plavilstshikov (1940) was the first who summarised information about this genus and proposed a key for four species that were known at the time. However, since he considered *T. johannis* and *T. juglandis* as valid species, according to the current taxonomy, his key includes in fact only three of the seven (now eight) described species. The most recent and very informative study of this genus, together with the description of two new species, was presented two decades ago by Danilevsky (2001). However, although all so far known species have been presented, some key elements of a revision are missing, such as the presentation of male genitalia or photographs of the type material for all species. Therefore, a new and more comprehensive revision containing all these elements and a reconstruction of a possible phylogeny based on morphological and/or molecular data is needed.

DNA barcoding is a technique that involves sequencing of 658 bp from the 5' end of the mitochondrial cytochrome oxidase subunit I gene (Hebert et al. 2003). Although there are various situations when the use of this method or mitochondrial markers in general can be seriously limited (e.g. hybridising species, maternally-inherited endosymbionts) (Raupach et al. 2020), numerous studies across a broad range of taxa have already proven that DNA barcoding provides a useful tool to speed up taxonomic procedures, such as automatic species identification by matching new sequences to existing taxa in reference libraries (e.g. Schmidt et al. 2015; Grebennikov et al. 2017; Coral Şahin et al. 2019; Kelnarova et al. 2019; Schmid-Egger et al. 2019; Çakmak et al. 2020), associating dimorphic sexes (e.g. Zhai et al. 2017; Zhang et al. 2019) or different life stages (e.g. Miller et al. 2005; Zhou et al. 2007; Stur and Ekrem 2011; Karpiński et al. 2021), as well as differentiating of closely related or sibling species (e.g. Hebert et al. 2004; Vieites et al. 2009). This method, however, should always be supported by other evidence (particularly morphology). International Barcode of Life Data Systems (BOLD; www.boldsystems.org) is a public and freely available database that can serve as a great tool for storage, acquisition, analysis and publication of DNA barcode records. Among various analytical tools implemented in the BOLD workbench, one of the key elements is Barcode Index Number (BIN) that allows barcodes to be analysed using the system that clusters them to produce operational taxonomic units (Ratnasingham and Hebert 2007).

During the routine barcoding of longhorned beetles distributed in Central Asia, which remain almost completely unknown regarding their molecular sequences, we noticed that the specimens of '*Turanium pilosum*' that were sampled in Tajikistan and Kyrgyzstan formed two well-defined clades. Hence, this study aimed at investigating (morphology and species delimitation methods) the existence of a possible new taxon and at better understanding the taxonomic position of this group by revealing results of the first molecular-based (COI) phylogeny of the genus *Turanium* and providing the sequences for further studies. Taking advantage of the opportunity, we also decided to examine the phylogenetic position of the morphologically closest genera of the tribe in trying to test the recently questioned (Lee and Lee 2020) monophyly of Callidiini.

2. Material and Methods

2.1. Material examined

This study is based on the examination of more than 80 specimens of the genus *Turanium* from the territory of Kyrgyzstan, Tajikistan, Kazakhstan and Uzbekistan. The following acronyms for institutional and private collections are used in the text:

CJH Collection of Jacek Hilszczański, Sękocin Stary, Poland
CKL Collection of Krzysztof Łoś, Łomianki, Poland
CLK Collection of Lech Kruszelnicki, Siemianowice Śląskie, Poland
CRP Collection of Radosław Plewa, Sękocin Stary, Poland
HNHM Hungarian Natural History Museum, Budapest, Hungary
MIZ Museum and Institute of Zoology, Polish Academy of Sciences, Poland

Other abbreviations used: **BI**, Bayesian inference; **BL**, body length; **BS**, maximum likelihood bootstrap values; **HT**, holotype; **ML**, maximum likelihood inference; **PP**, Bayesian posterior probability; **PT**, paratype.

The individuals that were used for the detailed morphological and molecular analyses were collected by the authors during the entomological expeditions to Tajikistan (2014), Kazakhstan (2017, 2018) and Kyrgyzstan (2018, 2019). Some of the localities that were posted on professional websites (www.cerambyx.uochb.cz; www.zin.ru/animalia/coleoptera) were taken into consideration and presented only when combined with photographs, enabling accurate identification. After publication, the holotype will be deposited in the collection of MIZ.

2.2. Morphological analyses

The beetles were examined using an Olympus SZH10 Stereo Microscope at 7–140× magnification, a PROLAB MSZ Stereo Microscope at 7–90× magnifications, and a Hitachi S-3400N Scanning Electron Microscope. To examine the sclerotized parts of the male terminalia, the specimens were relaxed in distilled water for 12–24 h at room temperature. Then, the genitalia and last abdominal segment were separated from the other abdominal structures using pins or forceps, without removing the rest of the abdomen. Separated genitalia were put into 15% KOH solution at room temperature for some 24 h.

2.3. Photography and preparation of the figures

The scanning electron microscope (SEM) images were taken using a Hitachi S-3400N SEM at MIZ. Photographs of the habitus were taken with a Canon EOS 50D digital camera equipped with a Canon 100 mm f/2.8 USM Macro lens. The images that were produced were stacked, aligned, and combined using Helicon Focus (www.heliconsoft.com) and Zerene Stacker (www.zerenesystems.com) software. Photographs of the habitats were taken with a Canon EOS 600D and a Nikon Coolpix AW110 cameras. All plates were prepared using Adobe Photoshop CS5 and GIMP v2.10.14. The distribution of the species was illustrated in Quantum GIS (QGIS) v3.6.0 'Noosa' (QGIS Development Team 2021) using National Geographic World Map (National Geographic et al. 2021) as the raster layer. Geographical coordinates of the localities are given in decimal format and WGS84 projection.

2.4. Molecular analyses

DNA barcoding, the analysis of a standardised segment from the 5' end of the mitochondrial cytochrome c oxidase subunit I (COI) gene, was performed on a representative selection of species (four of eight including members of both subgenera; six specimens) of the targeted group: *T. pilosum* (Kyrgyzstan), *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.**, *T. scabrum* (Kazakhstan), and *T. johannis* (Kyrgyzstan) (Table S1). Additionally, we barcoded specimens of two extremely rare Central Asian species from the most closely related genus *Ropalopus* Mulsant, 1839: *Ropalopus nadari* Pic, 1894 and *Ropalopus mali* Holzschuh, 1993, which belong to the same tribe (Callidiini) and occur in the same habitats. Sequencing of some additional specimens that were collected, killed and preserved under the same conditions was unsuccessful, however, each species tested has obtained at least one sequence of a satisfactory length. We chose this gene because it has proven very informative in our previous studies (e.g. Plewa et al. 2018; Karpiński et al. 2021), and COI sequences of many species representing the most closely related genera (Cerambycini, Clytini, Hylotrupini) already exist in BOLD (www.boldsystems.org) and GenBank (www.ncbi.nlm.nih.gov/Genbank).

The individuals that were reared from the inhabited material collected in the field (*T. losi* Karpiński, Plewa & Hilszczański **sp. nov.**, Kyrgyzstan) were preserved in 96% ethanol, which was subsequently replaced in order to avoid diluting the alcohol. For the remaining species, only dried specimens (collected between 2014 and 2019; killed with ethyl acetate) were utilised. The specimens were processed for DNA barcoding in 2020. The right mid femur was cut open on both ends to expose the muscle tissue and then partly crushed with forceps and placed in a sealed well that contained two drops of 95% ethanol on a standard 96-well microplate, which was used for the tissue submission.

All of the laboratory work for extracting, purifying, amplifying and sequencing the DNA was performed at the 'Canadian Centre for DNA Barcode' (CCDB, http://www.ccdb.ca), University of Guelph, Ontario, Canada, following the standard protocol (Ivanova et al. 2006).

Due to the quality of the material, only eight specimens were successfully sequenced for a 619–658 bp long DNA barcoding fragment. The sequences were submitted to GenBank under the accession numbers OK073067–OK073074 (Table S1).

The obtained sequences and additional relevant information such as the specimen images, primers, gel images and trace files were uploaded to the 'Barcode of Life Database' (=BOLD, http://www.boldsystems.org) in the public online dataset '*Turanium Central Asia LK*' (DS-LKCCAT; DOI: dx.doi.org/10.5883/DS-LKCCAT). All of the voucher specimens reported herein are part of the BOLD project 'LECHK Cerambycidae Central Asia L. Karpinski' and were deposited in the LK's Cerambycid DNA-grade specimen bank at MIZ and in the collection of Forest Research Institute, Poland.

2.5. Phylogenetic analysis

Phylogenetic trees were reconstructed using both Bayesian inference (BI) and maximum likelihood (ML) methods. The obtained trees were also used to infer possible species delimitations. In addition to the barcoded specimens, sequences of the following species of Callidiini (11 species of 6 genera) and the most closely related tribes (16 species of 6 genera in 3 tribes) were obtained from GenBank and BOLD as closest related outgroup: Callidiini—

Callidium aeneum [KM286341.1], *Callidium coriaceum* [KU918865.1], *Callidium violaceum* [KU914026.1], *Oupyrrhidium cinnabarinum* [KY683721.1], *Phymatodes pusillus* [KM285884.1], *Phymatodes rufipes* [HQ953905.1], *Phymatodes testaceus* [HQ954560.1], *Pyrrhidium sanguineum* [KU918701.1], *Ropalopus femoratus* [KM446501.1; KJ964459.1; KM286267], *Ropalopus sanguinicollis* [COLAT043-08; CERLF390-08], *Semanotus japonicus* [LC492880.1]; Cerambycini—*Cerambyx cerdo* [KM285966.1], *Cerambyx scopoli* [KJ962934.1], *Cerambyx miles* [KM286032.1]; Clytini—*Chlorophorus annularis* [MK689190.1], *Chlorophorus diadema* [KC135923.1], *Chlorophorus figuratus* [JF889542.1], *Chlorophorus sartor* [KM449257.1], *Chlorophorus signaticollis* [FJ559042.1], *Chlorophorus varius* [KM286012.1], *Clytus arietis* [JF889522.1], *Clytus lama* [KU918981.1], *Plagionotus arcuatus* [JF889541.1], *Plagionotus detritus* [KM442177.1], *Xylotrechus rusticus* [KM286086.1], *Xylotrechus stebbingi* [MN182963.1], and Hylotrupini—*Hylotrupes bajulus* [MH020456.1]. *Prionus coriarius* [MH020283.1], belonging to the subfamily Prioninae was chosen as the most distantly related outgroup. The resulting topology was visualized in FigTree v1.4 (Rambaut 2014). Most of the selected species represent a wide range in the Palaearctic—from Europe (e.g. *R. femoratus*) to the Russian Far East, Korean Peninsula and China (*O. cinnabarinum*), and Japan (*S. japonicus*), although a Nearctic species (*R. sanguinicollis* from Canada) was also considered for broader taxon sampling.

Sequences of COI were aligned in Geneious v9.1.7 (Biomatters Ltd, Auckland, New Zealand, 2005) using the MAFFT plugin v1.3.6, based on MAFFT (Katoh et al. 2002). The sequence alignment is provided in fasta format (Supplementary File 3). The matrix contained molecular data (657 bp) for the total number of taxa under study (39). The alignment was initially partitioned by codon position in PartitionFinder v2 (Lanfear et al. 2016) using the Bayesian Information Criterion (BIC) and the 'greedy' algorithm (Lanfear et al. 2012).

Bayesian analysis was performed using MrBayes v3.2.7a (Ronquist et al. 2012): four chains (one cold and three heated) and two runs of 10 million generations with default prior settings were conducted. The stationarity and convergence of MCMC were assessed in Tracer v1.7.1 (Rambaut et al. 2018), as well as by the examination of Potential Scale Reduction Factor (PSRF) values and Average Standard Deviation of Split Frequencies in the MrBayes output.

Maximum likelihood (ML) analysis was run with RAxML v8.2.12 (Stamatakis 2014) on CIPRES Science Gateway v3.3 (Miller et al. 2010) using the same set of partitions. Node support was evaluated with 1000 bootstrap replicates (BS) (Guindon et al. 2010).

We considered maximum likelihood bootstrap values (BS) 90–100 as strong, 75–89 as moderate, and 50–74 as weak; Bayesian posterior probability from MrBayes (PP) 0.95–1 as strong, 0.85–0.94 as moderate, and 0.70–0.84 as weak support. Nodes with BS < 50 and PP < 0.70 were considered to be unsupported.

2.6. Species delimitation

Distance-based and tree-based species delimitation methods were performed to investigate species boundaries of *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** In order to receive a robust estimate of its entities, three approaches were used: Assemble Species by Automatic Partitioning (ASAP; Puillandre et al. 2021), Poisson Tree Process (PTP; Zhang et al. 2013) and its modification—Bayesian implementation of the Poisson tree processes (bPTP).

The ASAP distance-based analyses were run on the 'ASAP web' server (https://bioinfo.mnhn.fr/abi/public/asap) using Kimura 2-Parameter as a substitution model for matrices of genetic distances. Only partitions showing the lowest asap-score were considered.

The best-score ML tree from RAxML was used as input for PTP, with the most distantly related outgroup removed. ML inference was performed using the single-rate method and was run with mPTP v0.2.4 (Kapli et al. 2016). MCMC analysis was performed for 100 million generations, sampling parameters every 10 thousand generations and the first 10% generations were used as burn-in. The default value for the -minbr parameter was used, and analyses started from the ML random delimitation estimate.

The tree from MrBayes was used for bPTP analysis which was run on the 'bPTP server' (species.h-its.org). Similarly to the previous analyses, the most distantly related outgroup was removed. MCMC chains were run for 500 thousand generations, and all other settings were left as default.

3. Results

3.1. Taxonomy

Initiated by the conspicuous divergence in COI sequences, detailed morphological studies of the specimens representing both sequenced populations from Tajikistan and Kyrgyzstan confirmed the existence of two closely related species. Subsequently, additional individuals representing populations from other localities in the region were investigated. The results of this study are presented as both SEM and stacked colour plates. The general habitus of the beetles is presented dorsally (Fig. 1) and ventrally (Fig. 2). Particular body parts illustrating the key characters are presented in Figs 3–6. Additionally, male terminalia (lateral lobes of tegmen) are shown in Fig. 7. The habitus of the holotype of *Turanium pilosum* (Reitter, 1891) is depicted in Figs 8 and 9. The comparison of our material with the Reitter's type (HNHM) revealed that the barcoded specimens that represent the population from Tajikistan (Takob) belong to the existing species, and the sequenced specimens from Kyrgyzstan (vicinity of Arkit village) represent a new species of the genus *Turanium*. However, subsequent examination of additional individuals and Internet resources not only revealed the pres-

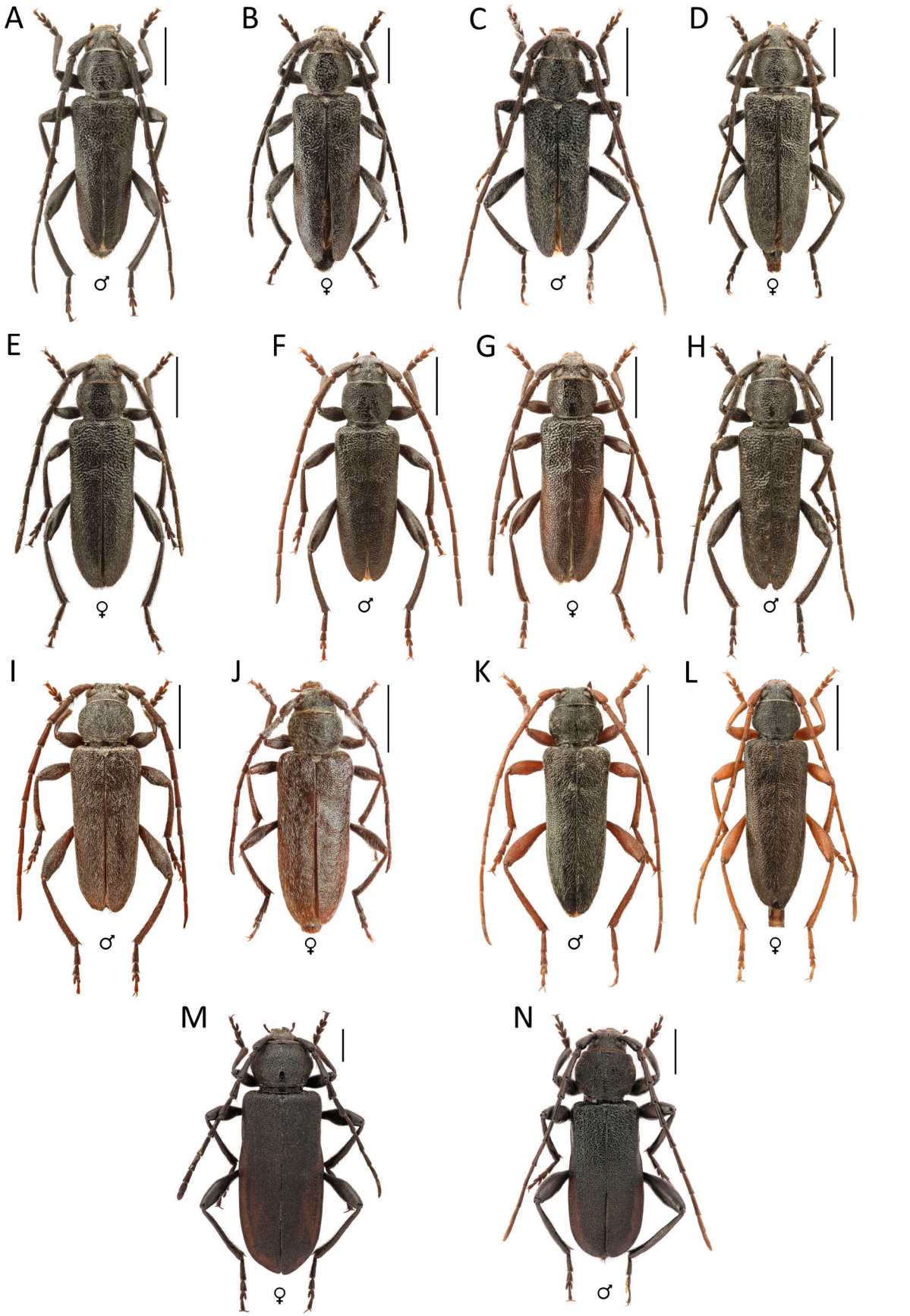

Figure 1. Habitus (dorsal view) of some representatives of the genera *Turanium* and *Ropalopus*. **A**–**E** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**: male holotype (4 km N of Arkit, Kyrgyzstan), female (ibid), male (8 km N of Arkit, Kyrgyzstan), female (Kara-Alma, Kyrgyzstan), female (Torkent, Kyrgyzstan), respectively **F**–**H** *Turanium pilosum*: male (Takob, Tajikistan), female (ibid), male (Kara-Alma, Kyrgyzstan), respectively **I**, **J** *Turanium scabrum*: male (Kara-Alma, Kyrgyzstan), female (Karashota, Kazakhstan), respectively **K**, **L** *Turanium johannis*: male (Urumbash, Kyrgyzstan), female (ibid), respectively **M** *Ropalopus nadari* female (Takob, Tajikistan) **N** *Ropalopus mali* male (Karakul, Kyrgyzstan). Scale bar: 3 mm.

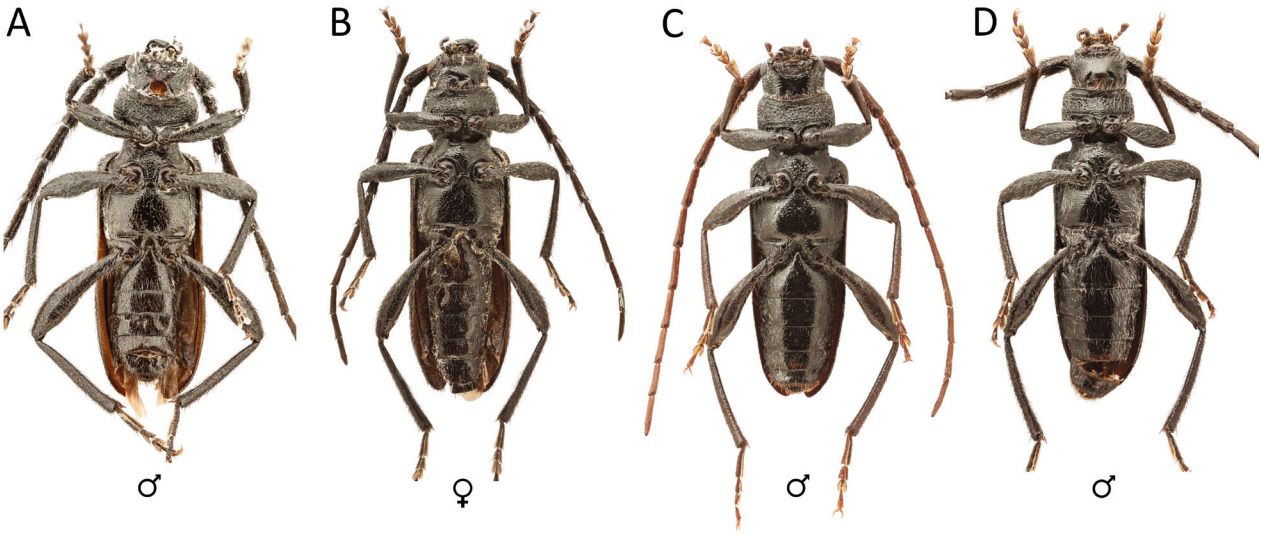

Figure 2. Habitus (ventral view) of two *Turanium* species. **A**, **B** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**: male (type locality, Kyrgyzstan), female (ibid), respectively **C**, **D** *Turanium pilosum*: male (Takob, Tajikistan), male (Arkit, Kyrgyzstan), respectively.

ence of *T. pilosum* in both discussed countries and *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** in Uzbekistan, which suggests their apparently wider distribution in the region, but also that both species can occur in the same localities and habitats. The geographical distribution of all taxa is mapped and presented in Fig. 10.

Turanium losi Karpiński, Plewa & Hilszczański sp. nov.

Figs 1A–E, 2A, B, 3A–C, G–I, M, 4A, B, F–H, K–M, 5A, B, E, G, K, M, 6B, 7A, B, G

http://zoobank.org/71E5EB8B-A67A-430B-84F9-247C1B-4126AB

Type material examined. 8 ♂♂ and 13 ♀♀. — **Holotype:** male (Figs 1A, 3A, B, G, H, 7A): KYRGYZSTAN, Jalal-Abad Region [Жалалабат облусу]: 4 km N of Arkit [Аркит] (41.836; 71.954), 1480 m a.s.l., 17.06.2019, *Prunus* sp., ex larva: 14.01.2020, R. Plewa leg. (MIZ). — **Paratypes.** KYRGYZSTAN, Jalal-Abad Region [Жалалабат облусу]: 4 km N of Arkit [Аркит] (41.836; 71.954), 1480 m a.s.l., 5 ♂♂ and 9 ♀♀, 17.06.2019, *Prunus* sp. and *Malus* sp., ex larva: 14.11–24.12.2019 and 15.12.2020–02.02.2021 (probably younger larvae from originally inhabited material), *Carpinus betulus*, ex ovo (II generation): 07.07.2020, R. Plewa leg. (CRP, CJH, MIZ); 8 km N of Arkit [Аркит] (41.871; 71.973), 1880 m a.s.l., 1 ♂ and 1 ♀, 12.06.2017, ex larva: 12.06.2017 (sic!), P. Hubeny leg. (CLK); 5 km E of Torkent [Торкент] (41.849; 73.203), 990 m a.s.l., 1 ♀, 06.05.2018, K. Łoś leg. (CKL); 17 km E of Kara-Alma [Кара-Алма] (41.253; 73.545), 1810 m a.s.l., 1 ♂, 20.06.2019, K. Łoś leg. (CKL); 5 km E of Kara-Alma [Кара-Алма] (41.193; 73.392), 1620 m a.s.l., 2 ♀♀, 20.06.2019, J. Hilszczański leg. (CJH).

Description. ***Morphology.*** Body relatively slender, strongly flattened dorsoventrally; BL in males: 10.5–14.0 mm (HT 12.0 mm), in females: 11.0–15.0 mm. Humeral width in males: 3.0–3.9 mm (HT 3.5 mm), in females: 3.0–4.2 mm. Integument black; legs and antennae black; elytra black (sometimes with slightly brownish areas on sides behind middle) with slight metallic luster. Pubescence of whole body made by sparse but distinct, long and usually erect whitish hairs and short black setae, being the longest on pronotum and anterior part of elytra, in its posterior part with many shorter and semi-decumbent hairs especially along epipleura; on ventral side distinct long whitish hairs, varying in intensity among specimens but usually denser on abdominal sternites; on femora semi-decumbent, relatively long and sparse whitish hairs; on tibiae and tarsi mostly replaced by denser blackish setae forming brush; on antennae very dense, relatively long, erect setae, especially rich on first five antennomeres and gradually disappearing towards last joint, on last antennomeres in form of single spike-like setae. Head broad, with coarse sculpture; frons with longitudinal furrow of variable depth between antennal tubercles; clypeus and labrum broad and well-pronounced; mandibles and palpi stout; eyes large, surrounding antennal tubercles; genae narrow, approx. 0.15–0.2 of eye width. Antennae thick and strong, relatively long, exceeding elytral apex by 1.5–2 last antennomeres in males, and clearly shorter, never reaching elytral apex in females; average length ratio of antennomeres in males: 1:0.25:1.25:1.0:1.08:1.08:1.13:1.0:0.93:0.83:1.13, in females: 1:0.25:1.2:0.95:1.0:0.95:0.95:0.86:0.8:0.7:0.9; antennomeres 3–10 with tooth of variable depth on outer side; in males antennomere 11 always clearly divided in approx. 2/3 of length, forming almost completely separated antennomere 12. Prothorax very wide and pronounced, distinctly narrower at the base, gradually widening towards upper edge, strongly flattened, with clearly rounded outer edges, in females less pronounced and slightly more oblong, also with clearly rounded outer edges; approx. 1.32 (HT 1.37) in males and 1.4 in females times as wide as long, approx. 3.65 (HT 3.6) in males and 4.15 in females times

Figure 3. Comparison of particular body parts of two *Turanium* species. **A**–**C**, **G**–**I**, **M** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**: **A** head of male holotype **B** pronotum of male holotype **C** pronotum of female (Kara-Alma, Kyrgyzstan) **G** elytra (apex) of male holotype **H** elytra (basal part) of male holotype **I** elytra (basal part) of female (Kara-Alma, Kyrgyzstan) **M** prosternal process of male (type locality) **D**–**F**, **J**–**L**, **N**, **O** *Turanium pilosum*: **D** head of male (Takob, Tajikistan) **E** pronotum of male (ibid) **F** pronotum of female (ibid) **J** elytra (apex) of male (ibid) **K** elytra (basal part) of male (ibid) **L** elytra (basal part) of female (ibid) **N** prosternal process of male (ibid) **O** prosternal process of female (ibid).

shorter than elytral length and 1.2 (HT 1.2) in males and 1.26 in females times narrower than elytra at humeri. Pronotum rather regularly (less regularly in males) and entirely punctate, in males usually with smooth area at middle near base; punctation rather uniform (more uniform in females), relatively sparse but clearly finer and denser at

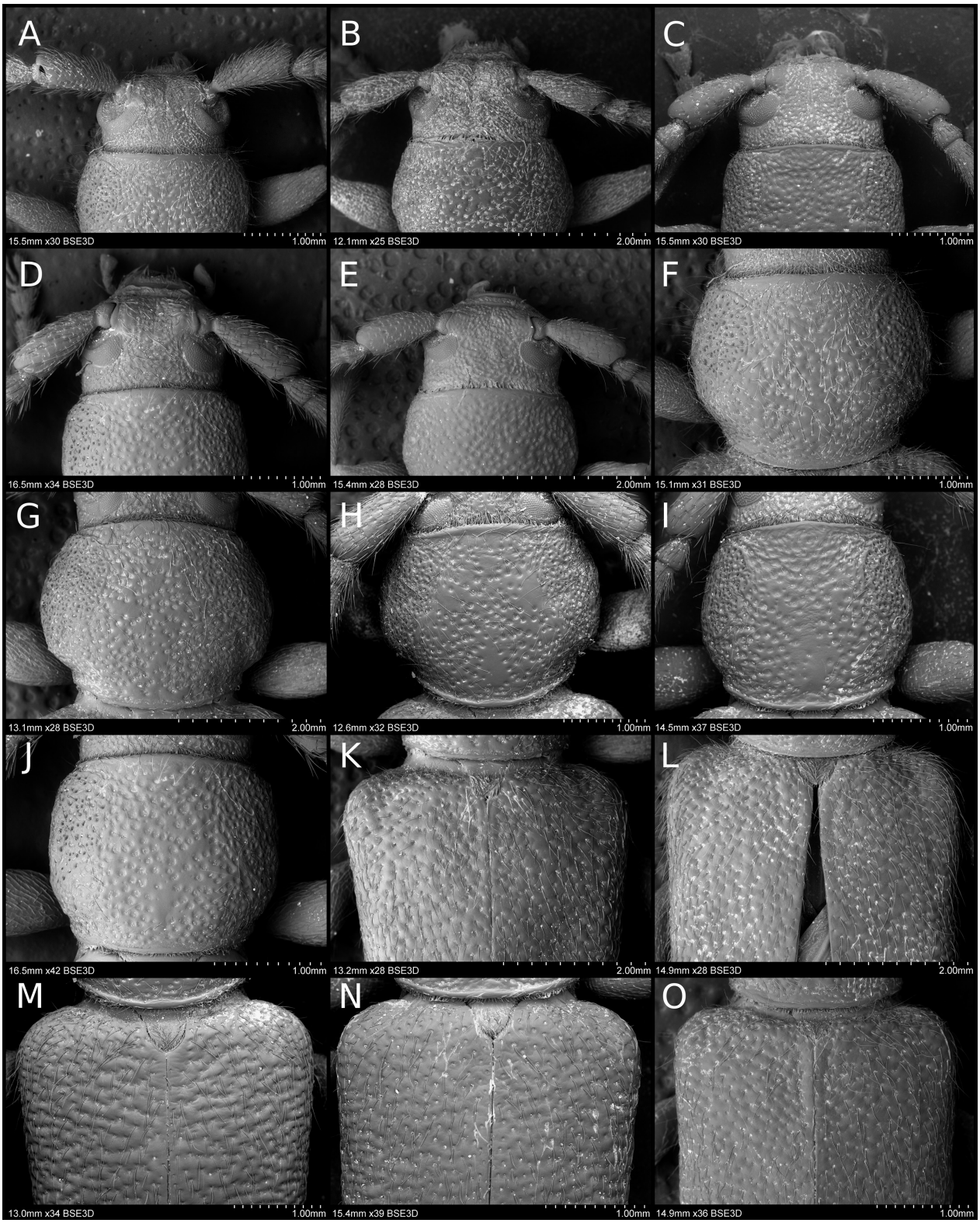

Figure 4. Variability of particular body parts in two *Turanium* species. **A**, **B**, **F**–**H**, **K**–**M** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**: **A** head of male (type locality) **B** head of female (Kara-Alma, Kyrgyzstan) **F** pronotum of male (type locality) **G** pronotum of male (ibid) **H** pronotum of male (Kara-Alma, Kyrgyzstan) **K** elytra (basal part) of male (type locality) **L** elytra (basal part) of male (ibid) **M** elytra (basal part) of male (Kara-Alma, Kyrgyzstan) **C**–**E**, **I**, **J**, **N**, **O** *Turanium pilosum*: **C** head of male (Takob, Tajikistan) **D** head of male (Arkit, Kyrgyzstan) **E** head of female (Takob, Tajikistan) **I** pronotum of male (ibid) **J** pronotum of male (Arkit, Kyrgyzstan) **N** elytra (basal part) of male (Takob, Tajikistan) **O** elytra (basal part) of female (ibid).

sides. Prosternum finely and sparsely punctate, distinctly creased longitudinally. Prosternal process short and very thin, barely exceeding middle of procoxae, slightly curved dorsally. Elytra moderately long, approx. 2.25 (HT 2.22) in males and 2.33 in females times as long as humeral width, gradually tapering towards apex in males

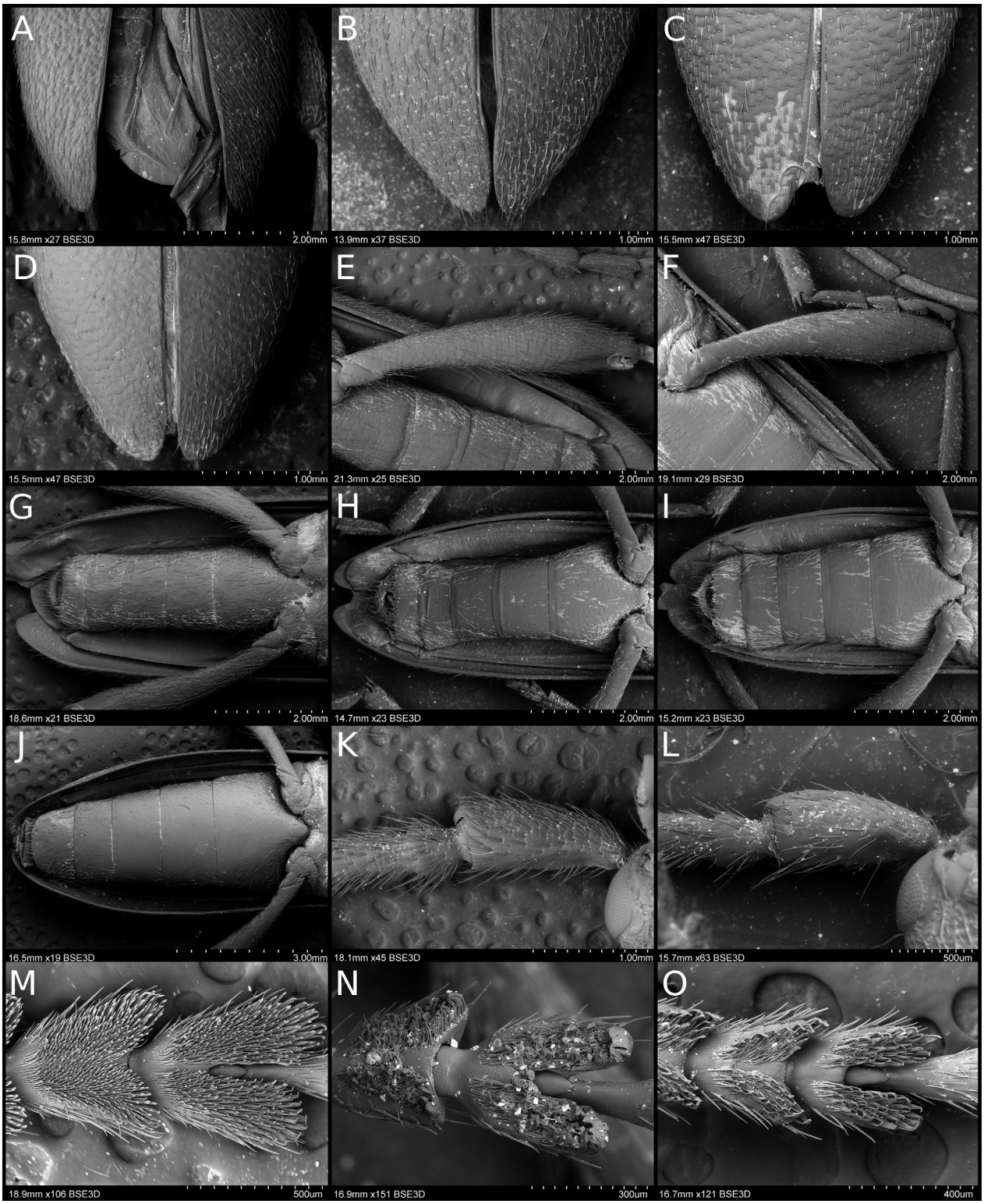

Figure 5. Variability of particular body parts in two *Turanium* species. **A**, **B**, **E**, **G**, **K**, **M** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**: **A** elytra (apex) of male (type locality) **B** elytra (apex) of female (Kara-Alma, Kyrgyzstan) **E** metafemur (ventral view) of male (type locality) **G** abdomen (ventral view) of male (ibid) **K** first two antennomeres (ventral view) of male (ibid) **M** tarsal pads of protarsomere 2 and 3 (ventral view) of male (ibid); **C**, **D**, **F**, **H**–**J**, **L**, **N**, **O** *Turanium pilosum*: **C** elytra (apex) of male (Takob, Tajikistan) **D** elytra (apex) of female (ibid) **F** metafemur (ventral view) of male (ibid) **H** abdomen (ventral view) of male (ibid) **I** abdomen (ventral view) of male (ibid) **J** abdomen (ventral view) of female (ibid) **L** first two antennomeres (ventral view) of male (ibid) **N** tarsal pads of protarsomere 2 and 3 (ventral view) of male (ibid) **O** tarsal pads of protarsomere 2 and 3 (ventral view) of female (ibid).

and usually almost parallel-sided in females, in both sexes with single indentation on each elytron about 1/3 of anterior length; elytral sculpture composed mainly by regular, sparse, fine, shallow punctures, with coarse surface between them (Fig. 4K–M), rather uniform at entire length; scutellum of variable shape, rather triangular but

Figure 6. Detailed view of the last male antennomere of two *Turanium* species. **A** *Turanium pilosum* **B** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** with clearly visible, almost completely developed antennomere 12.

usually with rounded edges, covered with whitish hairs. Tarsomeres similar in both sexes; protarsomeres pronounced, lobes of protarsomere 3 elongate and cordate, protarsomere 5 slightly longer than 1; metatarsomere 1 about as long as 2 and 3 combined and slightly longer than 5; tarsal pads broad, with numerous compact and very dense setae (Fig. 5M). Femora flattened similarly in both sexes. Metatibiae in males clearly exceeding elytral apex, slightly shorter in females. — ***Male terminalia.*** Lateral lobes of tegmen (Fig. 7A, B) short and thin, generally slightly tapering towards apex but sometimes more or less variable in shape, with rather long and thick setae apically; manubrium elongated, with broad edges. Median lobe of variable shape, usually robust and relatively short, gradually tapering towards apex.

DNA barcoding. COI sequences of two individuals of the new species were uploaded to BOLD and GenBank under the accessions: LK0101/LK0114 and OK073067/OK073074, respectively. The new taxon was registered under Barcode Index Number (BIN): BOLD:AEF9068.

Differential diagnosis. *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** differs from its closest relative, *T. pilosum* (Figs 1F–H, 2C, D, 3D–F, J–L, N, O, 4A, B, F–H, K–M, 5C, D, F, H–J, L, N, O, 6A, 7C–F, 8, 9), principally by the different outer edges of the pronotum that are clearly rounded and not parallel-sided (Fig. 3B vs. E), the coarse punctation of the elytra (Fig. 3H vs. K), the different color of the pubescence (especially on antennae and elytra) that is made by whitish hairs and black setae (not brownish and russet as in *T. pilosum*) and its much stronger abundance on the ventral side of the body (Fig. 5G vs. H–J; although Kyrgyz individuals of *T. pilosum* seem to be slightly more densely pubescent on the ventral side: Fig. 2D), as well as the different color of the integument, which is black in the new species and usually brown or brownish in *T. pilosum*. Tarsal pads are broader in the new species, with pronounced, clearly more compact and abundant, longer setae, with a narrower separating line along middle, which sometimes may not be even visible on the protarsomere 2 (Fig. 5M). Moreover, regardless of body size, all studied male specimens of *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** have clearly visible and almost completely developed antennomere 12 (Fig. 6B), while its origin can be barely detectable even in the utmost males of *T. pilosum* (Fig. 6A). Female antennae are distinctly shorter in the new species, clearly not reaching the elytral apex. Generally, the body of the new species is more corpulent and of a bigger size, with the smallest male measuring 10.5 mm (avg. 13 mm) and the biggest females reaching 15 mm (avg. 14 mm), while all studied specimens of *T. pilosum* are within the range of 10–12 mm including females. Although this character appears to be variable, it is worth to mention that most specimens of *T. pilosum* lack the longitudinal furrow on the frons, or it is only slightly marked, while all studied individuals of the new species have this furrow present and it is strongly marked in the majority of specimens. Similarly with the prosternal process; although it is usually wider in the new species (Fig. 3M vs. N, O), this difference appears to result from individual variability and it requires more caution. Regarding male genitalia, while many individuals of both species show sufficiently strong differences in the shape and pubescence of the lateral lobes (Fig. 7A, B vs. D–F), the structures of some individuals differ significantly from the pattern (Fig. 7G), objectively making this character too variable for use in species separation. Similar situation was confirmed also in the individuals of the closely related genus *Ropalopus* (Karpiński et al. 2020) and in the genus *Anoplistes* Audinet-Serville, 1833 (Cerambycinae: Trachyderini) (Karpiński et al. 2021)—some individuals of closely related but clearly independent (morphologically, ecologically, and genetically) species may share almost identical parameres, not to mention the highly variable shape of the median lobe. The molecular distance in COI sequences of *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** and *T. pilosum* was also compared and it reaches almost 3%, with almost no intraspecific variability (Table 1).

Table 1. Summary of the intraspecific and mean interspecific COI genetic distances between the representatives of the genera *Turanium* and *Ropalopus*, estimated using maximum composite likelihood model. See Supplementary Table (S2) for the genetic distances between all analysed species and individuals. 'n/a' indicates species in which only a single specimen was sequenced.

Intraspecific variability [%]	Species	Mean interspecific distances [%]							
			1	**2**	**3**	**4**	**5**	**6**	**7**
0.2	*Turanium pilosum*	**1**							
0.0	*Turanium losi* sp. nov.	**2**	2.7						
n/a	*Turanium scabrum*	**3**	12.1	11.2					
n/a	*Turanium johannis*	**4**	12.3	11.5	13.6				
n/a	*Ropalopus nadari*	**5**	13.6	13.0	13.8	14.9			
n/a	*Ropalopus mali*	**6**	17.3	15.6	17.2	18.4	11.6		
0.0	*Ropalopus sanguinicollis*	**7**	14.7	14.6	17.7	15.5	16.0	17.2	
0.0–0.6	*Ropalopus femoratus*	**8**	16.4	16.5	17.7	16.8	15.3	14.2	16.1

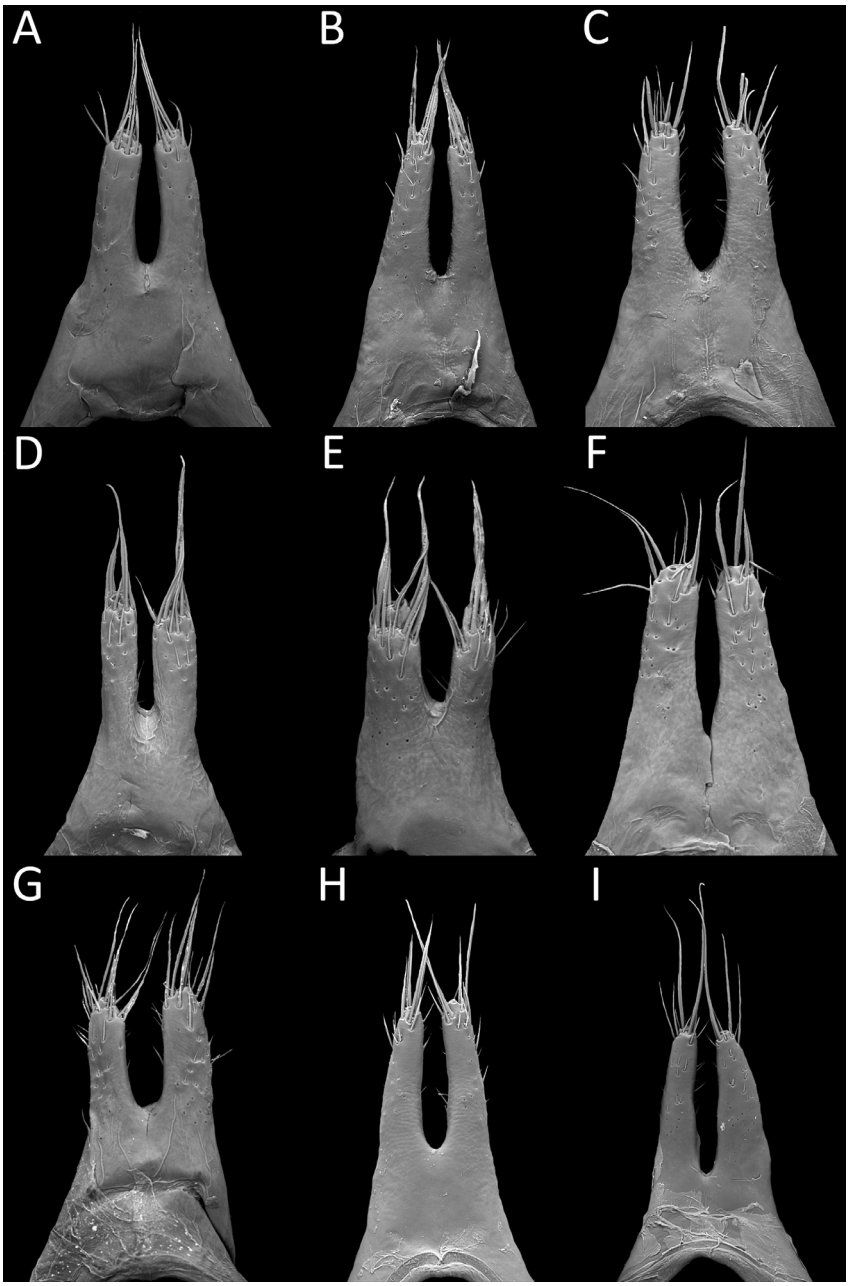

Figure 7. Lateral lobes of *Turanium* species. **A**, **B**, **G** *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**: male holotype, male (type locality), male (ibid), respectively **C**–**F** *Turanium pilosum*: male (Kara-Alma, Kyrgyzstan), male (Takob, Tajikistan), male (ibid), male (Arkit, Kyrgyzstan), respectively **H** *Turanium scabrum* male (Kara-Alma, Kyrgyzstan) **I** *Turanium johannis* male (Urumbash, Kyrgyzstan).

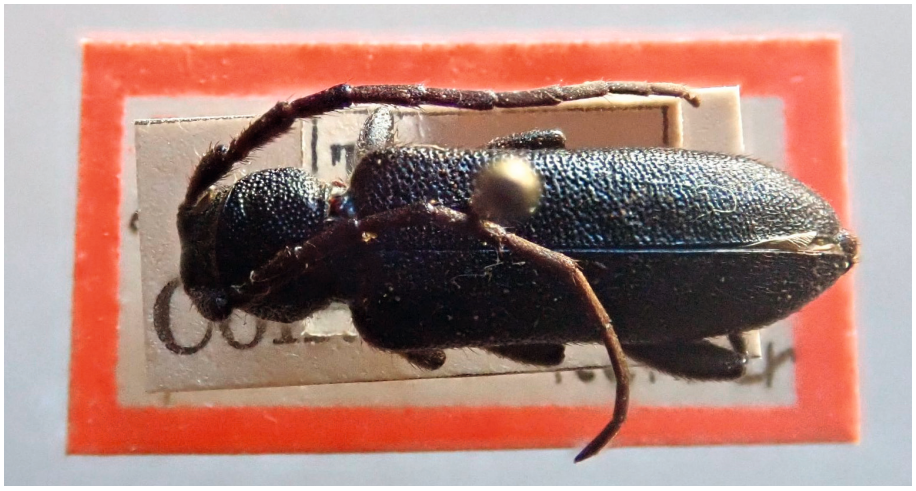

Figure 8. Habitus (dorsal view) of the holotype of *Turanium pilosum* (Reitter, 1891) (**HNHM**).

Figure 9. Habitus (latero-dorsal view) of the holotype of *Turanium pilosum* (Reitter, 1891) (**HNHM**) with the original labels ('Turkestan' = locality in present-day Uzbekistan).

The new species can also easily be separated from *T. rauschorum* (habitus of male paratype presented in Danilevsky (2001)), another 'blackish' species of the discussed group that is distributed in the region, by the much longer antennae in both sexes (in males of *T. rauschorum* about as long as body and reaching posterior elytral fourth in females), by the different shape of pronotum in males ('always without smooth areas' in *T. rauschorum*), and by considerably bigger body dimensions, which in huge series (75 specimens) of *T. rauschorum* vary in males between 7.9–12.4 mm, and in females between 11.0–12.0 mm (10.5–14.0 mm and 11.0–15.0 mm, respectively in *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.**). Furthermore, *T. rauschorum* is considered a monophage restricted to *Atraphaxis* (Polygonaceae) and it is only known so far from southern Kazakhstan and one locality in northernmost Kyrgyzstan (Danilevsky 2001).

Other species in the genus clearly differ in body proportions and colouration, the punctation of the pronotum and elytra, and other characters. The habitus of *T. scabrum* and *T. johannis* is presented in Fig. 1 (I, J and K, L, respectively), and their lateral lobes of tegmen in Fig. 7. Their COI sequences have also been compared and the molecular distance is remarkable (Table 1), even despite rather poor divergence in their parameres (Fig. 7H vs. I).

Distribution. *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** is known to occur in northwestern Kyrgyzstan and easternmost Uzbekistan (Fig. 10), however, this species is most likely widely distributed in the

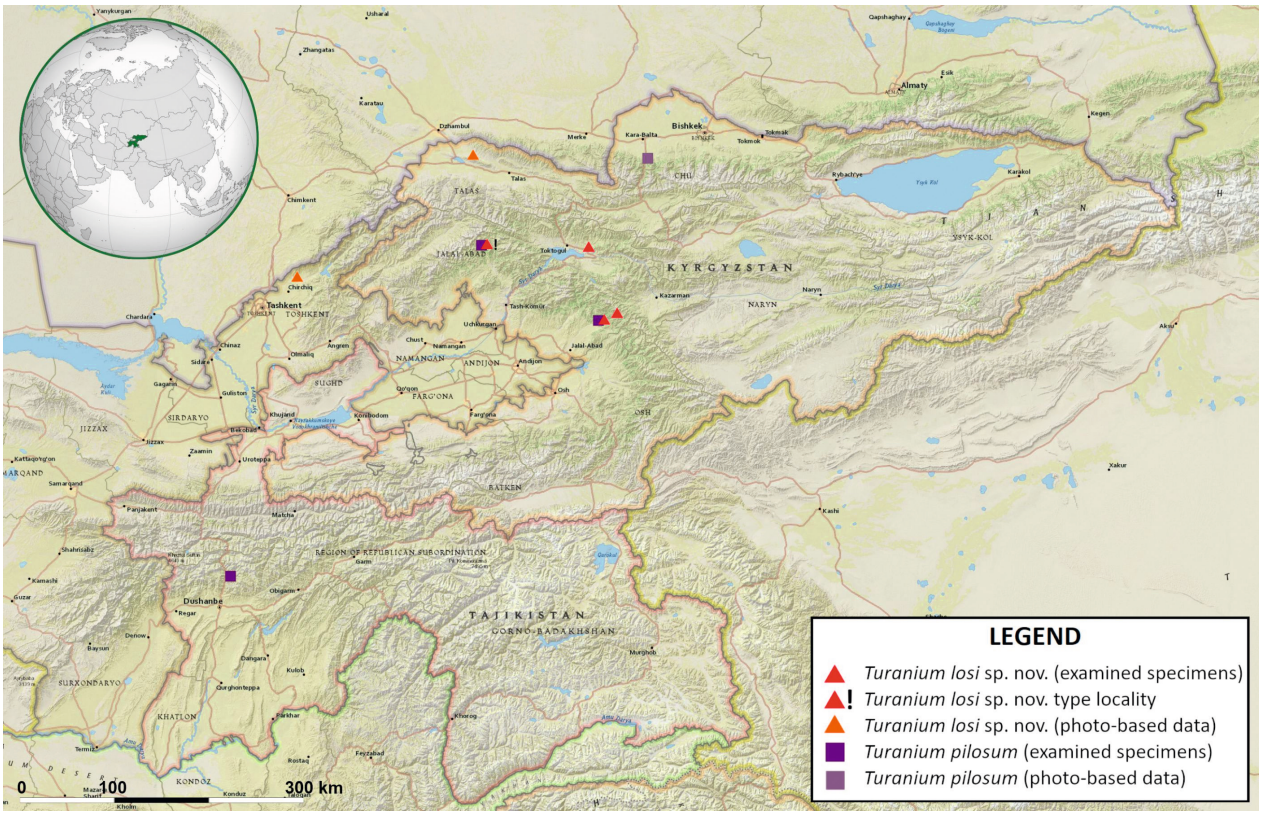

Figure 10. Distribution of *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** and *Turanium pilosum* in the eastern part of Middle Asia. Raster layer by National Geographic et al. (2021).

region, including southern Kyrgyzstan and northern Tajikistan since the high mountains of Tian Shan (Turkestan Range) seems not to be the sufficient distributional barrier for ecologically very close *T. pilosum*.

Bionomics. Adults of the new species were collected from the first days of May to the third quarter of June, at altitudes between 1000 and 1900 m a.s.l. The earliest observation was done at the lowest altitude (May 6, at 994 m a.s.l.). The holotype and the main series of paratypes have been reared from larvae collected in mid-June at an altitude of 1500 m a.s.l. At the time, imagoes of *T. pilosum* (exclusively males) were collected in the same locality and despite careful investigation no adults of the new species were observed at this plot. At the higher altitude (1620 m a.s.l.), females of the new species were found on June 20, together with a single male of *T. pilosum*. In type locality in Kyrgyzstan the species is related to mountain deciduous open forests with a substantial share of *Fraxinus* L. (Oleaceae), *Prunus* L., and *Malus* L. trees (Rosaceae) (Fig. 11A, B). In Tajikistan (at 1850 m a.s.l.), adults of *T. pilosum* were mating in the mid-July in a mountain valley with *Juglans* L. (Juglandaceae) and *Malus* trees (Fig. 11C, D) (Kadyrov et al. 2016). Considering the above, it seems reasonable to conclude that *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** is a phenologically relatively early species emerging in the first decade of May at lower elevations, and surviving in nature to the end of June at higher altitudes, while *T. pilosum* occurs later—from the mid-June to the end of July or even to the first decade of August in higher located sites.

The new species seems to be common in the region and its larvae are most likely wide polyphages as the adults that were reared from Kyrgyz *Prunus* L. and *Malus* L. were able to continue breeding on the wood of European hornbeam *Carpinus betulus* L. (Betulaceae). Moreover, Danilevsky (2001) listed numerous host plants (both deciduous and coniferous) for '*T. pilosum*' that certainly include some records of the new species (see more in Remarks). The depicted male specimen was collected on June 2 and the female was reared from *Picea* A. Dietr. (Pinaceae).

Based on own observations, the life cycle usually lasts two years, however in the laboratory rearing, a single male emerged from the wood material that was added already in Poland (II generation) after only eight months, which means that the cycle can be shortened in nature to one year under optimal conditions.

Remarks. As *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** is rather common in Middle Asia, there were already some specimens imaged in the Internet or in scientific papers that present the new species but were clearly misidentified. One example could be a male of '*T. johannis*' from Uzbekistan (www.cerambyx.uochb.cz; accessed on: 10.01.2021, the image has been replaced) and another a female of '*Turanium rauschorum*' from Kyrgyzstan, close to the Kazakh border (www.zin.ru). Regarding the revision of the genus (Danilevsky 2001), although the quality of the images in the distributed version of this paper does not allow the reliable identification of the depicted specimens, it seems that both presented '*T. pilosum*' (fig.

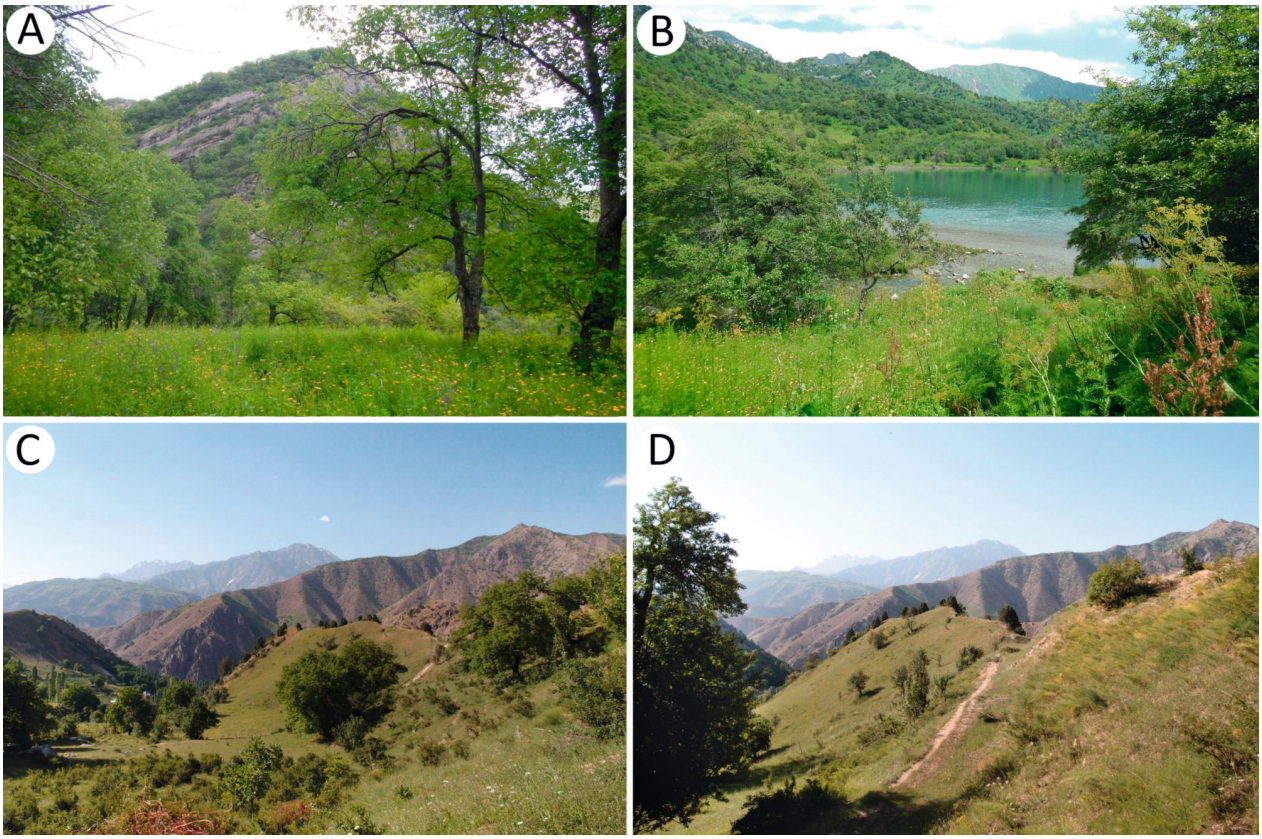

Figure 11. Habitats of *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** and *Turanium pilosum*. **A**, **B** vicinity of Arkit village (Kyrgyzstan) **C**, **D** vicinity of Takob village (Tajikistan).

3a,b; p. 583), which were collected in close vicinity of the type locality of the new species, represent in fact *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** Besides certain morphological characters (pronotal shape, integument colouration), the collecting date of the male (02.06.1978) clearly links to the new species.

Etymology. We are pleased to dedicate this species to a Polish entomologist, Krzysztof Łoś, our close friend and one of the main organizers of the 2017–2019 trips to Kazakhstan and Kyrgyzstan.

3.2. Phylogeny

Two following partitions were found: COI1 + COI2 and COI3. For MrBayes SYM+I+G model was selected as the best supported for the first one and HKY+G for the second. The maximum likelihood (Fig. 12) and Bayesian (Fig. 13) inference trees showed similar topologies, however, most likely due to insufficient data (COI only), some clades in the Bayesian tree were not resolved. Both the ML and BI analyses of the molecular sequences revealed the monophyly of the genus *Turanium* with strong support (BS 90 and PP 1, respectively). *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.** from Kyrgyzstan and *T. pilosum* from Tajikistan formed a well-defined clade with almost maximal support in both trees. The resemblance of these two taxa is strongly supported morphologically, which clearly indicates close sister relationships between them. *Turanium scabrum* (Kazakhstan) of the same subgenus was revealed as more closely related compared to early-branching *T. johannis* (Kyrgyzstan) of the subgenus *Chalcoturanium*, which was placed as a sister group to all remaining *Turanium* species. Interestingly, the genus *Ropalopus* was revealed as paraphyletic in both analyses, although the nodes were moderately or weakly supported and the representation of this genus might not be comprehensive enough to draw far-reaching conclusions (see more in the Discussion). The analyses further indicated that both Central Asian *Ropalopus*: *R. nadari* and *R. mali* were clustered together with strong support (BS 91, PP 1), in the position of a sister group to the *Turanium* clade (BS 59, PP 0.79). It also indicated *R. sanguinicollis* as sister to the clade of all remaining *Ropalopus* and *Turanium* in both trees (BS 66, PP 0.90).

Callidiini was recovered as polyphyletic, separated in a few clades, although generally with weak support value of each subgroup: *Phymatodes* + *Pyrrhidium* as a part of Clytini, and *Callidium* and *Ropalopus* + *Turanium* as separate (ML) or unresolved (BI) clades.

3.3. Species delimitation

For additional verification, we also tested the putative new species using a few species delimitation methods. Different methods gave, however, discordant results, either confirming the species status of *T. pilosum* and *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.**, accepting

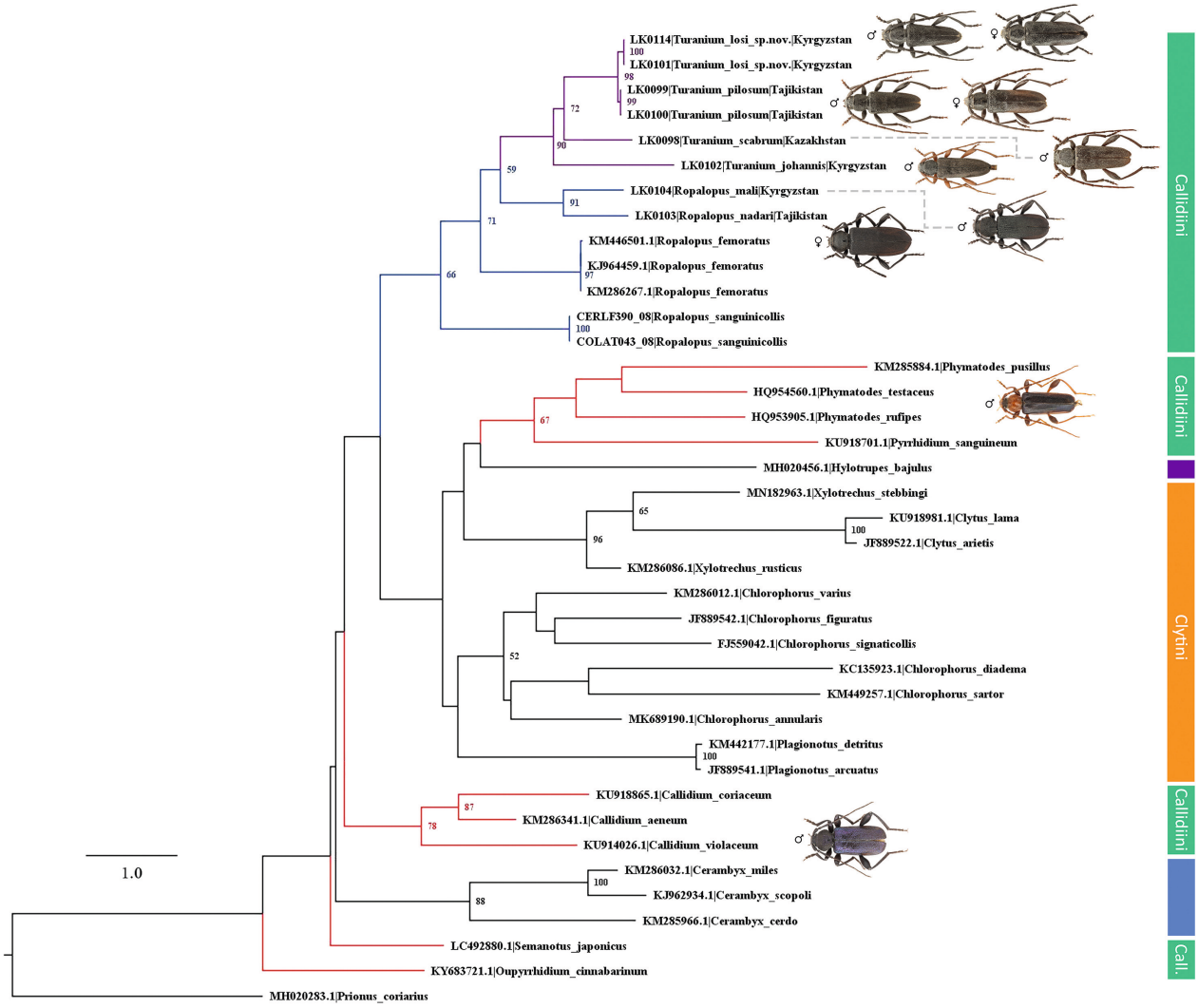

Figure 12. Maximum likelihood phylogenetic tree based on the COI sequences of the representatives of *Turanium* and *Ropalopus* and some closely related genera of Callidiini and other Cerambycinae tribes. Maximum likelihood bootstrap values >50 are shown. Clades within Callidiini were marked by coloured branches as follows: purple—*Turanium*, blue—*Ropalopus*, red—remaining genera. Additional interpretation for the trib labels on the right side: purple—Hylotrupini, blue—Cerambycini.

them partly, or rejecting them as distinct taxa. *Turanium losi* Karpiński, Plewa & Hilszczański **sp. nov.**, besides strong morphological support, was confirmed as a separate species in the ASAP method. On the contrary, the PTP method recognised both *Turanium* as a single taxon, while the bPTP method gave inconclusive results, supporting their divergence in 72% of analyzes (while the author of the method suggests treating results as sufficiently accurate with the support of approx. 0.90). It is worth mentioning, however, that two species of the genus *Plagionotus* Mulsant, 1842: *Plagionotus arcuatus* (Linnaeus, 1758) and *Plagionotus detritus* (Linnaeus, 1758) have also been revealed as a single species by this method, with very similar support (0.78) as for *Turanium* taxa. Similarly, in the PTP method, both *Plagionotus* species were revealed as a single taxon. There are no justified reasons not to consider *P. arcuatus* and *P. detritus* as two separate species. The COI genetic distance between them is approx. 3%, nearly the same as in case of *T. pilosum* and *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** (Table S2). We also investigated that the authors of the original paper (Hendrich et al. 2014) have identified those species correctly (their sequences were submitted also to BOLD, where images of exact specimens are available). A genetic distance threshold may exist below which the delimitation methods are not able to resolve relationships based on single specimens.

The results from each method are presented on the Bayesian inference tree (Fig. 13) for the entire *Ropalopus* + *Turanium* clade and for both *Plagionotus* species.

4. Discussion

4.1. Plausible separation of the tribe Ropalopini

Callidiini is a cosmopolitan tribe comprising 30 genera (41 including subgenera) (Tavakilian and Chevillotte 2021). After exclusion of *Callidium* Fabricius, 1775 and

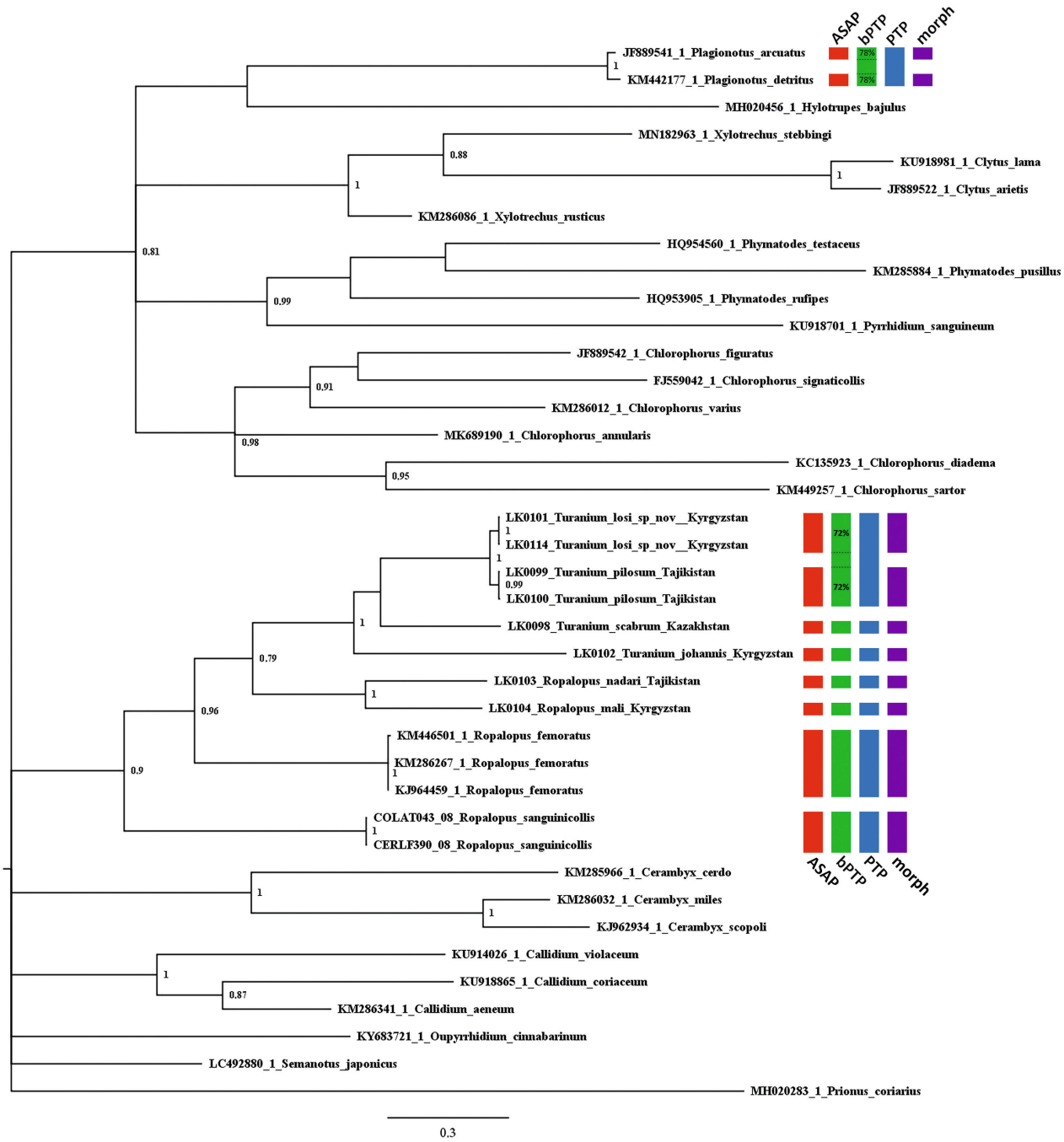

Figure 13. Bayesian phylogenetic tree based on the COI sequences reporting the results of the species delimitation analyses (*Turanium* + *Ropalopus* clade). Bayesian posterior probabilities >0.70 are shown. Vertical bars correspond to morphology (purple) and to the species delimitation results obtained by ASAP (red), bPTP (green), and PTP (blue) methods.

Semanotus Mulsant, 1839, which in our study were separated from *Turanium* Baeckmann, 1922 and *Ropalopus* Mulsant, 1839 and clustered in Lee and Lee (2020) together with Oemini as a sister group to Phoracanthini + Dichophyiini, and after further exclusion of *Phymatodes* Mulsant, 1839, which was positioned in our study within Clytini and as a sister clade to Compsocerini + Hylotrupini in Lee and Lee (2020), *Ropalopus* is the oldest established genus of Callidiini. It was described in the same year as *Phymatodes* and *Semanotus*. In our study, the genera *Ropalopus* and *Turanium* formed a separate clade, which, however, was not resolved by Bayesian inference. Therefore, although we do not propose herein any changes to the tribal classification, if the distinctiveness of the discussed clade (and perhaps some other yet untested genera) is confirmed in more densely sampled multilocus molecular analysis or well-designed morphological analysis, it will be reasonable to establish a new tribe Ropalopini. The external morphology of adults seems to confirm this affinity, and according to Švácha and Danilevsky (1988), *Turanium* larvae are similar to those of *Ropalopus macropus*. Some representatives of molecularly unstudied genera: *Callidiellum* Linsley, 1940, *Leioderes* L. Redtenbacher, 1845, *Pronocera* Motschulsky, 1859, as well as additional species of *Ropalopus* (especially the type species—*R. clavipes* and some

taxa of the '*ungaricus*/*insubricus*' group) and *Semanotus* definitely need to be sequenced and compared to obtain a relatively objective phylogeny reconstruction.

4.2. Phylogenetic positions of *Turanium* and Callidiini

Besides the monophyly of the genus *Turanium* our analyses revealed the polyphyly of the tribe Callidiini. Although a COI-based phylogeny should not be overinterpreted, we decided to present and discuss some parts of our trees as very similar results were obtained by Lee and Lee (2020) in the analyses based on six molecular markers (mitochondrial protein-coding COI, mitochondrial ribosomal 16S rRNA, nuclear ribosomal 18S rRNA and 28S rRNA, and nuclear protein-coding wingless and CAD), who proposed the most sophisticated to-date tribal and generic relationships within Cerambycinae. In their study, most of the sampled cerambycine tribes were rendered as monophyletic except for Callidiini, Callidiopini, Cerambycini, Cleomenini, and Phoracanthini. Callidiini was recovered as polyphyletic and divided into two distinctly separated and distant clades with high support value of each subgroup: (I) *Phymatodes* and (II) *Callidium* + *Oupyrrhidium* + *Semanotus*. According to those authors, it will be reasonable to treat the Phymatodes-group as an independent tribe after finding morphological characters supporting the presumed monophyly of each group. In our analyses, Callidiini was separated into three clades: (I) *Phymatodes* + *Pyrrhidium* (the latter not tested by Lee and Lee), (II) *Callidium*, and (III) *Ropalopus* + *Turanium* (both genera not tested by Lee and Lee). To support these results, it is worth to note that, in total, six species representing five of six subgenera of the genus *Phymatodes* were analysed and only one (*P. testaceus*, type species of the genus) was common to both discussed projects. We also utilised the sequences of European *P. pusillus* (subg. *Phymatoderus*) and *P. rufipes* (subg. *Phymatodellus*), while Asian *P. maaki* and *P. ermolenkoi* (subg. *Poecilium*), and *P. mediofasciatus* (subg. *Paraphymatodes*) were processed in Lee and Lee (2020). Similarly, in the genus *Callidium*, only one (*C. aeneum*) of four species tested, representing all three subgenera, was utilised in both projects, however, the type species of this genus is *C. violaceum*, and not *C. aeneum* (subg. *Callidostola*) as it was erroneously stated by Lee and Lee (2020). We additionally processed sequences of the nominative *C. violaceum* and *C. coriaceum* (subg. *Palaeocallidium*).

Formation of analogous clades despite the use of different markers and different species sampling seems suggestive. As it was mentioned above, neither *Ropalopus* nor *Turanium* were analysed in Lee and Lee (2020), and the inclusion of the representatives of these two genera in the combined tree would be particularly interesting, especially as *Turanium* is an exclusive Central Asian genus, representation of which region is absent in slightly biased towards Western and Eastern Palaearctic and Oriental regions study of Lee and Lee (2020). Moreover, the morphological traits that appear to merge the genera of Callidiini could have likely arisen as a result of convergence, as the flattened body seems very beneficial for adults when getting beneath the bark to hide from predators or unfavorable weather conditions, as well as wide, clavate femora may be useful for maintaining better adhesion when moving rapidly along branches.

Since we present here the first sequences of *Turanium* and, next to one Palaearctic and one Nearctic species available in GenBank/BOLD, the only sequences of *Ropalopus* (sole for Asian species), these results seem quite novel and except herein widely referenced paper of Lee and Lee (2020) there are unfortunately very few articles that investigate this issue. In the most recent study dealing with cerambycid phylogeny (Nie et al. 2020) the only presented Callidiini species are *Semanotus bifasciatus* and *Pyrrhidium sanguineum* (both were tested in the above discussed analyses). Moreover, as this paper aimed to solve higher-level phylogeny, the total number of taxa representing the subfamily Cerambycinae is too low to conclude on a tribal system. On the other hand, the study attempting to reconstruct a tribal level phylogeny of Lamiinae (Souza et al. 2020) revealed that a relatively high number of described tribes in this subfamily do not comprise monophyletic groups. Therefore, future studies with strong evidence in both morphological and molecular phylogenetic aspects are essential to resolve the taxonomic system of Cerambycinae, and particularly of Callidiini.

4.3. Invasive potential of *Turanium*

Turanium is an exclusive Central Asian genus that includes generally highly polyphagous species. For instance, according to Švácha and Danilevsky (1988), *T. scabrum* is a polyphage of deciduous trees recorded from *Elaeagnus* L., *Prunus*, *Rosa* L., *Halimodendron* Fisch. ex DC., *Tamarix* L., and others; *T. pilosum* is also polyphagous on deciduous trees and it was recorded from *Juglans*, *Amygdalus* L., *Cydonia* Mill., *Malus*, *Armeniaca* (Scop.) Koch, *Cerasus* Mill., *Acer* L., *Salix* L., *Populus* L., *Ulmus* L., *Morus* L., *Sorbus* L., and others; *T. juglandis* (=*johannis*) is polyphagous on both deciduous and coniferous trees: *Picea*, *Abies* Mill., *Juglans*, and 'apparently others'. Danilevsky (2001) provided some additional host plants for most of the species, however, the most important in this regard is *T. pilosum*, as *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** appears to be confused especially with this species and some locality and host plant records clearly refer to the newly described species. In the revisionary paper, *T. pilosum* is considered as polyphagous on both deciduous and coniferous trees, and *Picea* was added to its host plants list. Although species of this genus develop in branches and rather thin stems and, consequently, it is difficult to expect large economic importance, *T. scabrum* has been reported as a serious pest of *Elaeagnus angustifolia* and *Populus diversifolia* in tugai forests (Sinadskiy 1963). The imagoes of *T. scabrum* were observed in Kazakhstan mating on primarily oleaster old branches that formed a flood barrier (a com-

mon solution in the region) (Karpiński et al. 2018), which in case of massive occurrence of beetles may also weaken the durability of this protection. It seems that also *T. losi* Karpiński, Plewa & Hilszczański **sp. nov.** and *T. pilosum* may have some economic significance, but mainly in young plantations of fruit trees.

Another issue is the invasive potential of *Turanium*. At least half of the species are common beetles in Central Asia and their adults, considering individual populations, are present in nature almost throughout the entire growing season. Moreover, it was documented that larvae are also able to develop in dry wood (*T. scabrum*; Karpiński et al. 2018). The life cycle can be shortened to one year under optimal conditions (*T. losi* Karpiński, Plewa & Hilszczański **sp. nov.**, unpublished own observation). Despite the fact that the range of *Turanium* is restricted to the area of Middle Asia, an individual of *T. johannis* was reported from Ukraine (Kerch, Crimea) already in 2009 (www.insecte.org; accessed on: 24.02.2021). According to the International Monetary Fund (World Economic Outlook 2018), all countries in which representatives of this genus occur are still considered developing economies, thus, the invasive potential of these beetles may have not been fully disclosed yet, and as international trade develops in these countries, species of this genus are likely to spread. One example could be velvet longhorned beetle, *Trichoferus campestris*—a relatively closely related (Cerambycinae: Hesperophanini) species with a similar bionomy, native to this region but, unlike *Turanium*, also to highly industrialized China, that is considered a serious wood-boring pest and one of the most rapidly spreading cerambycids (e.g. Grebennikov et al. 2010; Dascălu et al. 2013; Keszthelyi et al. 2019).

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Supplementary material 1

Table S1

Authors: Karpiński L, Szczepański WT, Plewa R, Kruszelnicki L, Koszela K, Hilszczański J (2021)
Data type: .pdf
Explanation note: List of barcoded *Turanium* and *Ropalopus* specimens, their GenBank accessions/BOLD sample IDs, with the collecting data.

Link: https://doi.org/10.3897/asp.79.e65325.suppl1

Supplementary material 2

Table S2

Authors: Karpiński L, Szczepański WT, Plewa R, Kruszelnicki L, Koszela K, Hilszczański J (2021)
Data type: .pdf
Explanation note: Genetic divergence between the COI sequences (in %) estimated using the maximum composite likelihood model implemented in MEGA7.

Link: https://doi.org/10.3897/asp.79.e65325.suppl2

Supplementary material 3

Sequence alignment

Authors: Karpiński L, Szczepański WT, Plewa R, Kruszelnicki L, Koszela K, Hilszczański J (2021)
Data type: .fas
Explanation note: Data file used for phylogenetic analyses.

Link: https://doi.org/10.3897/asp.79.e65325.suppl3