

## Research article

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# A new species of *Mesopolobus* (Hymenoptera, Pteromalidae) from black locust crops

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**Abstract.** A new species of the genus *Mesopolobus* Westwood, 1833, *Mesopolobus robiniae* Lakatos & László sp. nov., is described and illustrated from east-central Europe (Romania and Hungary). The species was reared from black locust (*Robinia pseudoacacia*) seedpod samples, where it most likely parasitizes the black locust's seed predator *Bruchophagus robiniae* Zerova, 1970. Here we present the new species and report on its ecological relationships within the European seed predator community of black locust. We also give details regarding type material and type locality, a detailed description with images, a differential diagnosis of the new species, and a modification to the identification key published by Graham (1969), that distinguishes this new species from closely related species. In addition, we provide information on the distribution, biology and results of barcoding analysis. We also provide the DNA sequence data to complement the morphological taxonomy.

**Keywords.** New species description, black locust seed predation, seed predator parasitoid, morphometry, multivariate ratio analysis.

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## Introduction

In the last century, the black locust (*Robinia pseudoacacia* L.) became a characteristic component feature of the Central and Eastern European landscape (Vítková *et al.* 2017). Its positive economic, but negative environmental impacts led to conflicts between nature conservationists, forestry workers, urban planning experts, beekeepers and the public (Benesperi *et al.* 2012; Dickie *et al.* 2014; Sádlo *et al.*

2017). As current legislation will determine the future distribution of black locust, we need detailed knowledge, not only from the viewpoint of the forestry and economy, but also from the viewpoint of the species' potential associates, like herbivorous insects and their community (Kleinbauer *et al.* 2010).

The invasive history of black locust follows the characteristic pathway of introduced crops with an initial phase when presumably several independent introductions occurred from North America, which ceased for a long period, then were followed by frequent plantings and a rapid invasion in the wild, resulting in its widespread distribution of today (DAISIE 2009). The invasion of black locust in Central and Eastern Europe was facilitated by extensive plantings, due to the wood's long-term quality, resistance to insects and fungi, rapid growth, easy propagation, and ability to stabilize soils (Vítková *et al.* 2017).

When replacing native vegetation, the black locust reduces local biodiversity (Hanzelka & Reif 2015). Endangered light-demanding plants and invertebrates are threatened by its appearance through reducing light to plants growing beneath the canopy and above the forest floor, and changing the microclimate and soil quality (Lazzaro *et al.* 2018). These impacts can have effects throughout the food chain, by depriving birds of their insect prey, which depend on the plants that have been wiped out by the black locust (Hanzelka & Reif 2015). One of the central problems regarding black locust colonization is its capacity to rapidly increase soil nutrient concentration and to alter soil chemical properties which conditions then facilitate invasion by other non-native nitrophilous plant species (Enescu & Dănescu 2013).

Because of the wide distribution and negative environmental impacts of black locust, an important management tool for invasive populations is limiting their propagation (Redei *et al.* 2001). The crops of black locust are attacked by several pests, among which the seed predators can have a major impact (Zerova 1970; Perju 1998). *Bruchophagus robiniae* Zerova, 1970 (Hymenoptera: Chalcidoidea: Eurytomidae) is a pre-dispersal seed predator, monophagous on black locust seeds (Perju 1998). As with other *Bruchophagus* Ashmead, 1888 species, each *B. robiniae* individual feeds and develops inside one infested seed, so each seed wasp consumes only one seed and each seed houses only one seed wasp (Lakatos *et al.* 2018). This seed predator has a component community comprising several species, including parasitoid wasps (Lakatos *et al.* 2016, 2018).

One member of the *Bruchophagus robiniae* – black locust seed predator community belongs to the genus *Mesopolobus* Westwood, 1833, a group of parasitoid wasps in Pteromalidae Dalman, 1820 (Hymenoptera: Chalcidoidea) containing more than 120 described species (Noyes 2020), over 60 of which are present in Europe (<http://www.fauna-eu.org/>). Species of this genus have a wide host range, although several species are known to be host-specific on galls (Diptera: Cecidomyiidae, Hymenoptera: Cynipidae), bark beetles (Scolytinae), seed predators (Lepidoptera; Coleoptera: Curculionidae, Bruchinae; Hymenoptera: Eurytomidae), etc. (Bouček & Rasplus 1991; Noyes 2020). Several Eurytomidae Walker, 1832 species, such as *Bruchophagus gibbus* (Boheman, 1836) has *Mesopolobus* sp. parasitoids (Noyes 2020), and several *Mesopolobus* species are parasitoids of pests on oilseed rape (*Brassica napus* L.), on alfalfa (*Medicago sativa* L.), or on Norway spruce (*Picea abies* L.) (Noyes 2020).

*Mesopolobus* is a taxonomically complex genus, considering the high number of species belonging to this genus. European *Mesopolobus* were revised by Hans von Rosen (von Rosen 1958, 1959, 1960, 1961), who synonymized species from multiple genera (*Amblymerus* Walker, 1834; *Eutelus* Walker, 1834; *Platyterma* Walker, 1834) under *Mesopolobus*. The last revision of the *Mesopolobus* genus for the Western Palearctic was written by Graham (1969). Later studies dealing with *Mesopolobus* parasitoids of certain host groups have provided further clarifications of species synonymies, notably parasitoids of gall inducing Cynipidae Latreille, 1802 on *Quercus* sp. (Askew 1961; Nieves-Aldrey 1983; Pujade-Villar 1993) and of seed weevils associated with Brassicaceae (Baur *et al.* 2007). Since the latest generic

revision (Graham 1969) several new species have been described from Asia (e.g., Narendran *et al.* 2011; Xiao *et al.* 2016) and North-America (e.g., Doganlar 1979).

The identification of *Mesopolobus* species emerged from black locust crops was based on the most detailed identification key up to date provided by Graham (1969). Using Graham's keys a number of characters (fore wing marginal vein length ratio to stigmal vein length, number of anelli and funicular segments, position of toruli to anterior margin of clypeus and to median ocellus, pilosity of the basal cell of fore wing, position of hypopygium tip along the metasoma) led us to key couplet 16 (Graham 1969: 643), where based on two character combinations, namely metasoma length ratio to head plus mesosoma length and metasoma breadth, which did not match our specimens. This suggested the specimens reared from the black locust pods were not represented in the keys, and were likely undescribed. We thus studied several *Mesopolobus* species represented in the keys and compared them morphometrically to the *Mesopolobus* females emerged from black locust seed pods. This approach provides robust insight into *Mesopolobus* morphology, which may play a major role in resolving the species delimitations in biocontrol studies. Complementing the morphometric study, we also analyzed *mtCOI* sequences of the emerged *Mesopolobus* females from black locust pods, and compared them to the available *mtCOI* sequences from the BOLD System and NCBI databases.

Our objectives were the following: i) to identify those morphometric characters that give the best discrimination of the females emerged from black locust seedpods from other *Mesopolobus* species. ii) to calculate the genetic distance values between the *mtCOI* sequence of the females emerged from black locust and the other *Mesopolobus* species. iii) to describe the females and the males of the species that emerged from black locust seedpods.

## Material and methods

To gather information about black locust seedpod insect inhabitants we collected seedpod samples in black locust plantations and patches for four years, in the early spring of 2009 in Romania and between 2013–2015 in Romania and Hungary (Table 1). Samples were placed in plastic cups, containing 20–100 seedpods and covered with punched plastic wrap. Samples were kept in a covered balcony with a temperature and humidity close to outdoors at Babeş-Bolyai University (Cluj-Napoca, Romania) and at University of Debrecen (Debrecen, Hungary). Emerged individuals were monitored and collected monthly from seedpod samples for a year, and stored in 70% ethanol. The dominant emerging species were the seed predator of black locust seeds, *Bruchophagus robiniae*, and its parasitoid, the undescribed *Mesopolobus* species (Lakatos *et al.* 2018).

Identification and description of the emerged *Mesopolobus* species have been made under an Olympus SZ51 binocular microscope, with an 80× magnification and LED lighting. Images were produced by a Canon EOS 600D and a Canon EF 100 mm f/2.8 USM Macro Lens. Morphological nomenclature follows Graham (1969). The provided identification key is modified from the keys to genus *Mesopolobus* of Graham (1969). Type material is deposited in the Museum of Zoology, Babeş-Bolyai University, Cluj-Napoca (MZBBU). Specimen identification codes: holotype–MZBBU HYM000011; 14 paratypes–MZBBU HYM000012 to HYM000025, measured female specimens: HYM000026 to HYM000039.

For morphological comparison several specimens were loaned from different museums. The specimens of *Mesopolobus amaenus* (Walker, 1834), *M. apicalis* (syn. *thomsonii*) (Thompson, 1878), *M. aspilus* (syn. *elongates*) (Walker, 1835), *M. diffinis* (Walker, 1834), *M. dubius* (Walker, 1834), *M. fasciiventris* Westwood, 1833, *M. semiclavatus* (Ratzeburg, 1848) and *M. typographi* (Ruschka, 1924) were loaned from the Hungarian Natural History Museum, Budapest, Hungary (HNHM). Specimens of *M. verditer* (Norton, 1869), *M. mediterraneus* (Mayr, 1903), *M. tibialis* (Westwood, 1833) and *M. xanthocerus* (Thomson, 1878) were loaned from the Natural History Museum, London, United Kingdom (NHM).

**Table 1.** Collection dates, location and number of *M. robiniae* Lakatos & László sp. nov. individuals. Abbreviations: BH = Bihor County, Romania; CJ = Cluj County, Romania; HB = Hajdú-Bihar County, Hungary.

Collection date	Location	Northing	Easting	N	N <sup>♂</sup>	N <sup>♀</sup>
2009	BH	47.098202	21.975355	9	7	2
2009	CJ	46.827366	23.629258	44	16	28
2013	BH	47.098202	21.975355	4	0	4
2013	CJ	46.801433	23.611995	26	12	14
2014	BH	47.098202	21.975355	69	35	34
2014	CJ	46.801433	23.611995	104	62	42
2014	HB	47.554773	21.591610	58	48	10
2015	BH	47.098202	21.975355	16	8	8
2015	CJ	46.801433	23.611995	23	13	10
2015	HB	47.554773	21.591610	30	14	16

One female specimen of *M. longicollis* Graham, 1969 was measured using ImageJ from photographs of the type provided by Oxford University Museum of Natural History.

We measured 19 morphometric variables, corresponding to those used in the taxonomy of Pteromalidae for calculating typically used ratios (e.g., Graham, 1969) (Table 2), on a total of 55 dry-mounted *Mesopolobus* females belonging to the new species (Supp. file 1). Measurements were made with an Olympus SZ51 stereo microscope (objective: 110AL2X; eyepiece: WHSZ10X) under 60× and 80× magnification using a calibrated eye-piece micrometer (2.5 mm subdivided into 100 units). For all measurements we ensured that the points of reference were equidistant from the objective of the microscope.

Body ratios of *Mesopolobus* female specimens were analysed using the Multivariate Ratio Analysis (MRA) tool (Baur & Leuenberger 2011). Variation structure of *Mesopolobus* specimens was analysed by PCA in shape space to identify the principal components accounting for the variation. For the visualization of each character's contribution we used PCA ratio spectrum. Body ratios with best discriminant power were determined using the LDA ratio extractor (Baur & Leuenberger 2011). Analyses were made with R statistical software ver. 3.6.3 (R Core Team 2020).

Genomic DNA was extracted from three individuals using DNeasy Blood and Tissue kits (Qiagen Inc., Valencia, CA), following the protocol provided by the manufacturer. Mitochondrial cytochrome c oxidase subunit I (COI) sequences were amplified using the standard LCO1490 and HCO2198 primer pair (Folmer *et al.* 1994) in a 50 µl reaction volume at a 45°C annealing temperature. PCR products were purified with the Wizard SV Gel and PCR Clean-Up System (Promega, USA) and sent for sequencing to Macrogen Inc. (Korea).

Sequences were downloaded and verified with the Basic Local Alignment Search Tool (BLAST) (Johnson *et al.* 2008). Further, sequences for all available *Mesopolobus* species were also downloaded from the NCBI database and the BOLD System (for reference numbers see Fig. 2). The sequences were aligned using a Clustal W algorithm (Thompson *et al.* 1994) in BioEdit (Hall 1999). A phylogenetic tree was inferred in MrBayes (Ronquist *et al.* 2012), assuming a GTR+G+I model. Interspecific *p*-distances were calculated in MEGA X (Kumar *et al.* 2018).

**Table 2.** The selected morphometric characters of female *Mesopolobus* Westwood, 1833 specimens, abbreviations used, and their description.

Abbreviation	Character name	Definition	Magnification
clv.b	Clava breadth	Greatest breadth of antennal clava, lateral view	80 ×
clv.l	Clava length	Length of antennal clava, lateral view	80 ×
hea.b	Head breadth	Greatest breadth of head, dorsal view	60 ×
hea.l	Head length	Head length in dorsal view (Graham 1969), distance between anterior and posterior margin of the head, measured laterally	60 ×
ltg.b	Seventh gastral tergite breadth	Greatest breadth of the seventh gastral tergite, greatest distance between the outermost lateral edges of the seventh gastral tergite	80 ×
ltg.l	Seventh gastral tergite length	Greatest length of the seventh gastral tergite, greatest distance between the anterior and posterior edges of the seventh gastral tergite	80 ×
mv.l	Marginal vein	Length of marginal vein, distance between the point at which the submarginal vein touches the leading edge of the wing and the point at which stigmal vein and postmarginal vein unite (Graham 1969)	80 ×
msc.b	Mesoscutum breadth	Greatest breadth of mesoscutum just in front of level of tegula, dorsal view	80 ×
msc.l	Mesoscutum length	Length of mesoscutum along median line from posterior edge of pronotum to posterior edge of mesoscutum, dorsal view	80 ×
mss.l	Mesosoma length	Length of mesosoma along median line from anterior edge of pronotum collar to posterior edge of nucha, dorsal view	60 ×
ool.l	OOL	Shortest distance between posterior ocellus and eye margin, dorsal view (Graham 1969)	80 ×
pcl.l	Pronotal collar length	Length of pronotal collar along the median line from the edge between neck and pronotal collar to anterior edge of mesoscutum, dorsal view	80 ×
ped.b	Pedicellus breadth	Greatest breadth of pedicel, dorsal view	80 ×
ped.l	Pedicellus length	Greatest length of pedicel, dorsal view	80 ×
pol.l	POL	Shortest distance between posterior ocelli, dorsal view (Graham 1969)	80 ×
ppd.l	Propodeum length	Length of propodeum measured along median line from anterior edge to posterior edge of nucha, dorsal view	80 ×
sct.b	Scutellum breadth	Greatest breadth of the scutellum, greatest distance between the outermost lateral edges of the scutellum	80 ×
sct.l	Scutellum length	Length of scutellum along median line from posterior edge of mesoscutum to posterior edge of scutellum, dorsal view	80 ×
stv.l	Stigmal vein	Length of stigmal vein, distance between the point at which stigmal vein and postmarginal vein unite apically, and the distal end of the stigma (Graham 1969)	80 ×



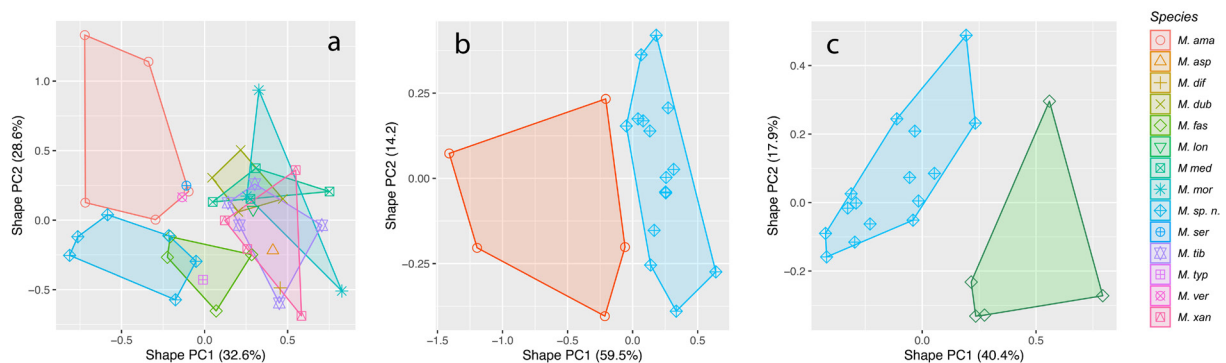
## Results

### Multivariate Ratio Analysis of variation in body size and shape

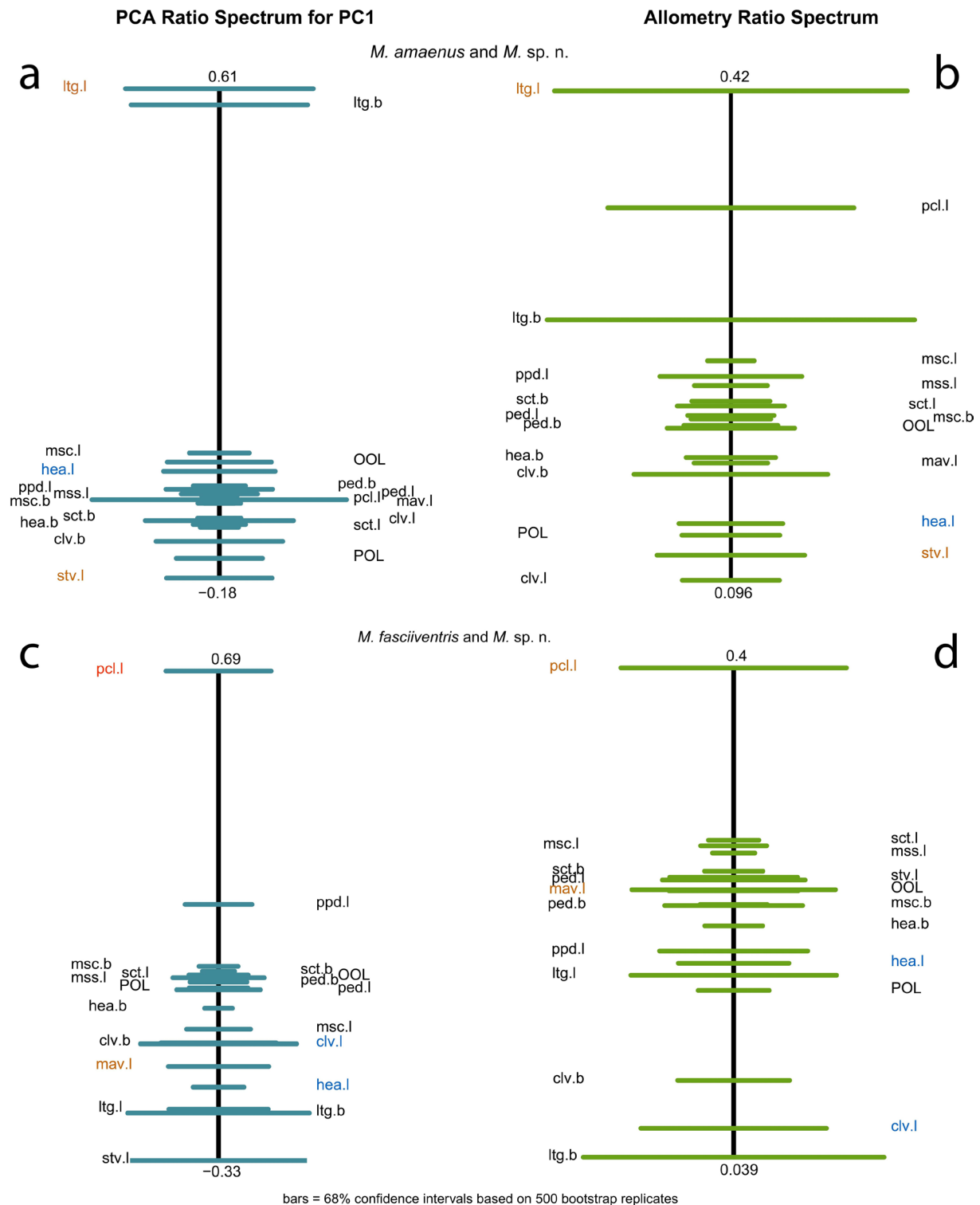
We first performed a series of shape PCAs on all specimens based on 19 morphometric characters. We identified principal components contributing to morphometric variation of all *Mesopolobus* females without prior species-determination by applying the PCA in isometry free shape space function to all specimens as a single group. Then we applied the PCA in isometry free shape space only to the group of females which were closest to those emerged from black locust seedpods. When we included all females in shape space, PC1 and PC2 accounted for 58% of the variation of the entire sampled population. When analysing only those species pairs which were closest to our target group in shape space, PC1 and PC2 accounted for 61% and 74% of the variation respectively. The first principal components are congruent with the separation of species, although a clear cut between the clusters could not be established (Fig. 1). On the first scatterplot (Fig. 1a) only five species (*M. amaenus*, *M. verditer*, *M. sericeus* (Förster, 1770), *M. typographi* and *Mesopolobus robiniae* Lakatos & László sp. nov.) showed a clear separation from the rest, but because of the overplotting with *Mesopolobus robiniae* sp. nov. we also retained *M. fasciiventris* for further analyses. On the other two scatterplots (Fig. 1b–c) the selected species show almost clear separations on PC1, while on PC2 are overlapping.

The PCA ratio spectrum for the species pair *M. amaenus* and *M. robiniae* sp. nov. (Fig. 2a) identified lgt.l at the extreme high end, and stv.l at the extreme low end of the spectrum. These characters were also found to contribute to species discrimination. The allometry ratio spectrum for the first species pair was dominated almost by the same ratio, stv.l and lgt.l (Fig. 2b), which is also the most important ratio concerning the first shape PC which shows to be the most allometric one. The PCA ratio spectrum for the species pair *M. fasciiventris* and *M. robiniae* sp. nov. (Fig. 2c) identified pcl.l at the extreme high end, while stv.l at the extreme low end of the spectrum. These characters, except for stv.l, were found to contribute to species discrimination. The allometry ratio spectrum for the second species pair was dominated almost by the same ratio, pcl.l and lgt.b (Fig. 2d), that is not the most important ratio concerning the first shape PC.

For the species pair *M. amaenus* and *M. robiniae* sp. nov. the LDA ratio extractor identified stv.l/lgt.l and hea.l/stv.l as the first two best discriminating ratios. These two combined ratios successfully separated the two species (Fig. 3a). The calculated ratios for the LDA-suggested characters (*M. amaenus* vs *M. robiniae* sp. nov., range, mean, sd) are: stv.l/lgt.l (1.38–4.25, 2.38, 1.21) vs (0.80–1.15, 0.92, 0.11);



**Fig. 1.** Scatterplot of first against second shape PC based on 19 morphometric variables of females. **a.** All 14 species of *Mesopolobus* Westwood, 1833. **b.** *M. amaenus* (Walker, 1834) and *Mesopolobus robiniae* Lakatos & László sp. nov. emerged from black locust seedpods. **c.** *M. fasciiventris* Westwood, 1833 and *Mesopolobus robiniae* Lakatos & László sp. nov. emerged from black locust seedpods. The variance explained by each shape PC is given in parentheses.



**Fig. 2.** Ratio spectra for the species pairs. **a, c.** PCA ratio spectrum. **b, d.** Allometry ratio spectrum. **a.** The two species pairs. **b.** *Mesopolobus amaeus* (Walker, 1834) and *Mesopolobus robiniae* Lakatos & László sp. nov. **c–d.** *M. fasciventris* Westwood, 1833 and *Mesopolobus robiniae* Lakatos & László sp. nov. Horizontal bars in the ratio spectra represent 68% bootstrap confidence intervals based on 1000 replicates.

hea.l/stv.l (0.11–1.12, 0.93, 0.13) vs (1.11–1.53, 1.26, 0.11). This suggests that the two species can be separated when judgement is based on a series of individuals. Further, the calculated D.shape is much higher than D.size in all of the two best discriminative ratios, indicating that species are mostly separated by differences in shape of characters (Table 3).

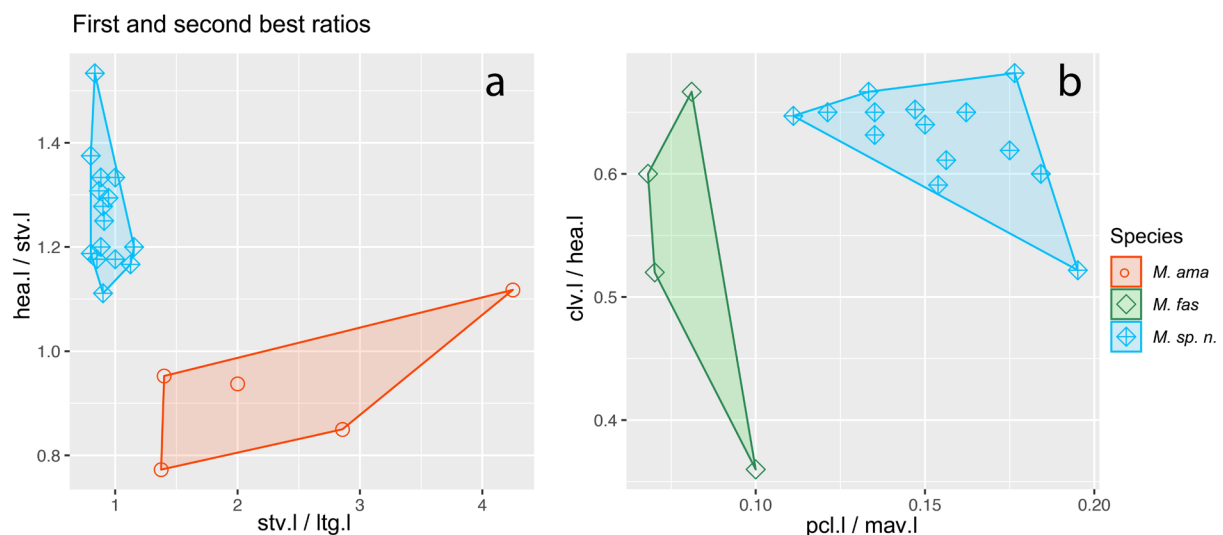
For the species pair *M. fasciiventris* and *M. robiniae* sp. nov. the LDA ratio extractor identified pcl.l/mav.l and clv.l/hea.l as the first two best discriminating ratios. These two combined ratios successfully separated the two species (Fig. 3b). The calculated ratios for the LDA-suggested characters (*M. fasciiventris* vs *M. robiniae* sp. nov., range, mean, sd) are: pcl.l3/mav.l (0.07–0.10, 0.08, 0.02) vs (0.11–0.20, 0.15, 0.05); clv.l/hea.l (0.36–0.66, 0.50, 0.14) vs (0.52–0.68, 0.63, 0.04). This suggests that the two species can be separated when judgement is based on a series of individuals. Further, the calculated D.shape is much higher than D.size in all of the two best discriminative ratios, indicating that species are mostly separated by differences in shape of characters (Table 3).

Because specimens of *M. verditer* and *M. sericeus* were overlapping in the shape PCA with *M. amaeus*, we calculated the best ratios for discriminating *M. amaeus* from *M. robiniae* sp. nov. for these two species as well. *M. typographi* overlapped in the shape PCA with *M. fasciiventris*, so we also calculated the best ratios discriminating *M. fasciiventris* from *M. robiniae* sp. nov. for *M. typographi* (Table 3).

### Molecular species delimitation

Based on molecular analysis of the available samples, *Mesopolobus robiniae* sp. nov. is placed closest to *Mesopolobus verditer* (Norton 1868) (Fig. 4). The three individuals represented only one haplotype (653 bp) that was deposited in GenBank with the MF098549 accession number. The alignment of the downloaded sequences was 468 bp long and consisted of 3 *Pteromalus* species (used as outgroup) and 14 *Mesopolobus* species, including the one described in this paper.

The phylogenetic relationship between the species is unresolved based on the available COI sequence data, but the tree shows a well-supported differentiation (PP = 1) of the new species, with *M. verditer*

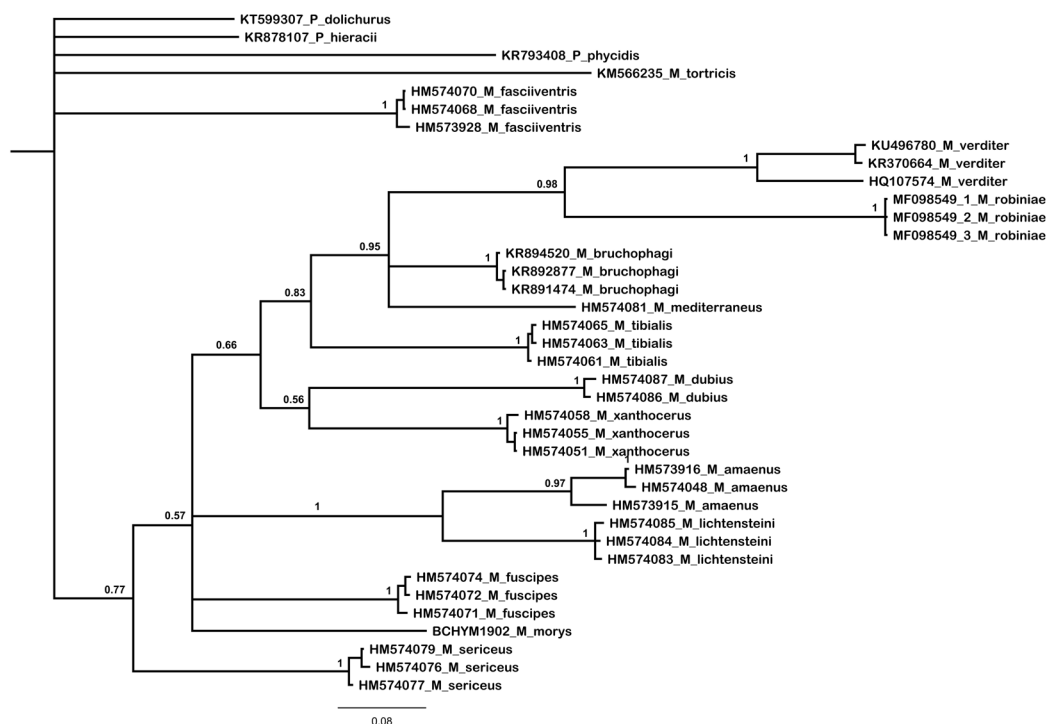


**Fig. 3.** Scatterplots of the two most discriminating ratios for females. **a.** *Mesopolobus amaeus* (Walker, 1834) and *Mesopolobus robiniae* Lakatos & László sp. nov. **b.** *M. fasciiventris* Westwood, 1833 and *Mesopolobus robiniae* Lakatos & László sp. nov. Both plots show first versus second ratio from LDA ratio extractor analysis.



**Table 3.** First and second-best ratios found by the LDA ratio extractor for separating various groups and specimens of *Mesopolobus* Westwood, 1833 females.

Group / species comparison	Best ratios	Range group 1	Range group 2	D.shape	D.size
<i>M. amaenus</i> vs <i>M. robiniae</i> sp. nov.	stv.l/lgt.l	1.38–4.25	0.80–1.15	0.73	0.03
	hea.l/stv.l	0.11–1.12	1.11–1.53	0.72	0.04
<i>M. verditer</i>	stv.l/lgt.l	1.91–2.50			
	hea.l/stv.l	1.14			
<i>M. sericeus</i>	stv.l/lgt.l	1.41			
	hea.l/stv.l	1.15			
<i>M. fasciiventris</i> vs <i>M. robiniae</i> sp. nov.	pcl.l/mav.l	0.07–0.10	0.11–0.20	0.65	0.01
	clv.l/hea.l	0.36–0.66	0.52–0.68	0.63	0.01
<i>M. typographi</i>	pcl.l/mav.l	0.1			
	clv.l/hea.l	0.55			

**Fig. 4.** Bayesian inference (BI) tree of the species of *Mesopolobus* Westwood, 1833 that have available mitochondrial COI sequences. Numbers on the branches represent posterior probabilities (PP).



as the closest species (Fig. 4). The differentiation is also supported by the *p*-distance values with a minimum of 12.5% between *M. robiniae* sp. nov. and *M. verditer*, and a maximum of 16.52% between *M. robiniae* sp. nov. and *M. tibialis* (Table 4).

### Taxonomy

Class Insecta Linnaeus, 1758  
Order Hymenoptera Linnaeus, 1758  
Suborder Apocrita Latreille, 1810  
Superfamily Chalcidoidea Latreille, 1817  
Family Pteromalidae Dalman, 1820  
Subfamily Pteromalinae Dalman, 1820

Genus *Mesopolobus* Westwood, 1833

*Mesopolobus* Westwood, 1833: 443 (type species: *Mesopolobus fasciiventris* Westwood, 1833 by monotypy).

*Mesopolobus robiniae* Lakatos & László sp. nov.

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Fig. 5

### Diagnosis

*Mesopolobus robiniae* sp. nov. is characterized by having the following morphological characters: head with uniform reticulation, antennae inserted at or above ventral edge of compound eyes, with three anelli, head broader than mesoscutum, pronotal collar moderately long, marginal vein of fore wing about twice as long as the stigma vein, metasoma longer than mesosoma, with only slightly projecting ovipositor sheaths, body green, antennae proximally testaceous with funiculus and clava infusate, femora and tibiae testaceous, with hyaline wings. *Mesopolobus robiniae* sp. nov. differs from closely resembling species by the ratios of metasoma to head plus mesosoma length, of temples to eye length, of marginal to stigma vein, of pronotal collar to mesoscutum length and of antennal clava to head length. Molecular results: the three sequenced individuals represented one haplotype (GenBank accession number: MF098549); based on the BI tree the new species, shows a well-supported differentiation from *M. verditer* as the closest species and from other congeners with the maximal differentiation from *M. tibialis*.

### Etymology

The new *Mesopolobus* species is named after the host plant of its seed predator host, the black locust (*Robinia pseudoacacia*).

### Material examined

#### Holotype

ROMANIA • ♀; Bihor County, near Săldăbagiu de Munte; 47.096354° N, 21.984963° E; 11 Mar. 2015; T.K. Lakatos leg.; emerged on 1 Apr. 2015; MZBBU HYM000011.

#### Paratypes

ROMANIA – Bihor County • 1 ♂; same collection data as for holotype; MZBBU HYM000012 • 1 ♀; near Săldăbagiu de Munte; 47.100895° N, 21.967509° E; 8 Mar. 2014; T.K. Lakatos leg., emerged on 22 Apr. 2014; MZBBU HYM000017 • 1 ♀; near Săldăbagiu de Munte; 47.098182° N, 21.975352° E; 11 Mar. 2014; T.K. Lakatos leg.; emerged on 23 Apr. 2014; MZBBU HYM000018 • 2 ♂♂; near Săldăbagiu

de Munte; 47.098182° N, 21.975352° E; 8 Mar. 2014; T.K. Lakatos leg.; emerged on 22 Apr. 2014; MZBBU HYM000019, HYM000020 • 1 ♂; near Săldăbagiu de Munte; 47.079446° N, 21.970817° E; 14 Mar. 2009; T.K. Lakatos leg.; emerged on May 2009; MZBBU HYM000021 • 1 ♂; near Săldăbagiu de Munte; 47.098519° N, 21.984808° E; 11 Mar. 2015; T.K. Lakatos leg., emerged on Apr. 2015; MZBBU HYM000022. – **Cluj County** • 1 ♀; near Cluj-Napoca; 46.777109° N, 23.674495° E; 17 Mar. 2015; T.K. Lakatos leg.; emerged on 10 Apr 2015; MZBBU HYM000013 • 1 ♀; same collection data as for preceding; 18 Mar. 2014; T.K. Lakatos leg.; emerged in May 2014; MZBBU HYM000014 • 2 ♀♀; near Cluj-Napoca; 46.768086° N, 23.568935° E; 17 Mar. 2009; T.K. Lakatos leg.; emerged in Apr. 2009; MZBBU HYM000015, HYM000016 • 1 ♂; near Cluj-Napoca; 46.834976° N, 23.651004° E; 22 Mar. 2014; T.K. Lakatos leg.; emerged in May 2014; MZBBU HYM000024 • 1 ♂; near Cluj-Napoca; 46.768086° N, 23.568935° E; 17 Mar. 2009; T.K. Lakatos leg.; emerged in May 2009; MZBBU HYM000025.

HUNGARY • 1 ♂; Hajdú-Bihar County, near Debrecen; 47.554773°N 21.591610°E; 2 Mar. 2015; T.K. Lakatos leg.; emerged on Apr. 2015; MZBBU HYM000023

**Other material** (specimens used for the genetic analysis)

ROMANIA • 3 ♀♀; Bihor County, near Săldăbagiu de Munte; 47.0968° N, 21.98525° E; 13 Mar. 2014; T.K. Lakatos leg.; emerged on 17 Apr. 2014; GenBank accession number: MF098549. This specimens were fully processed for the DNA extraction.

**Description**

**Female**

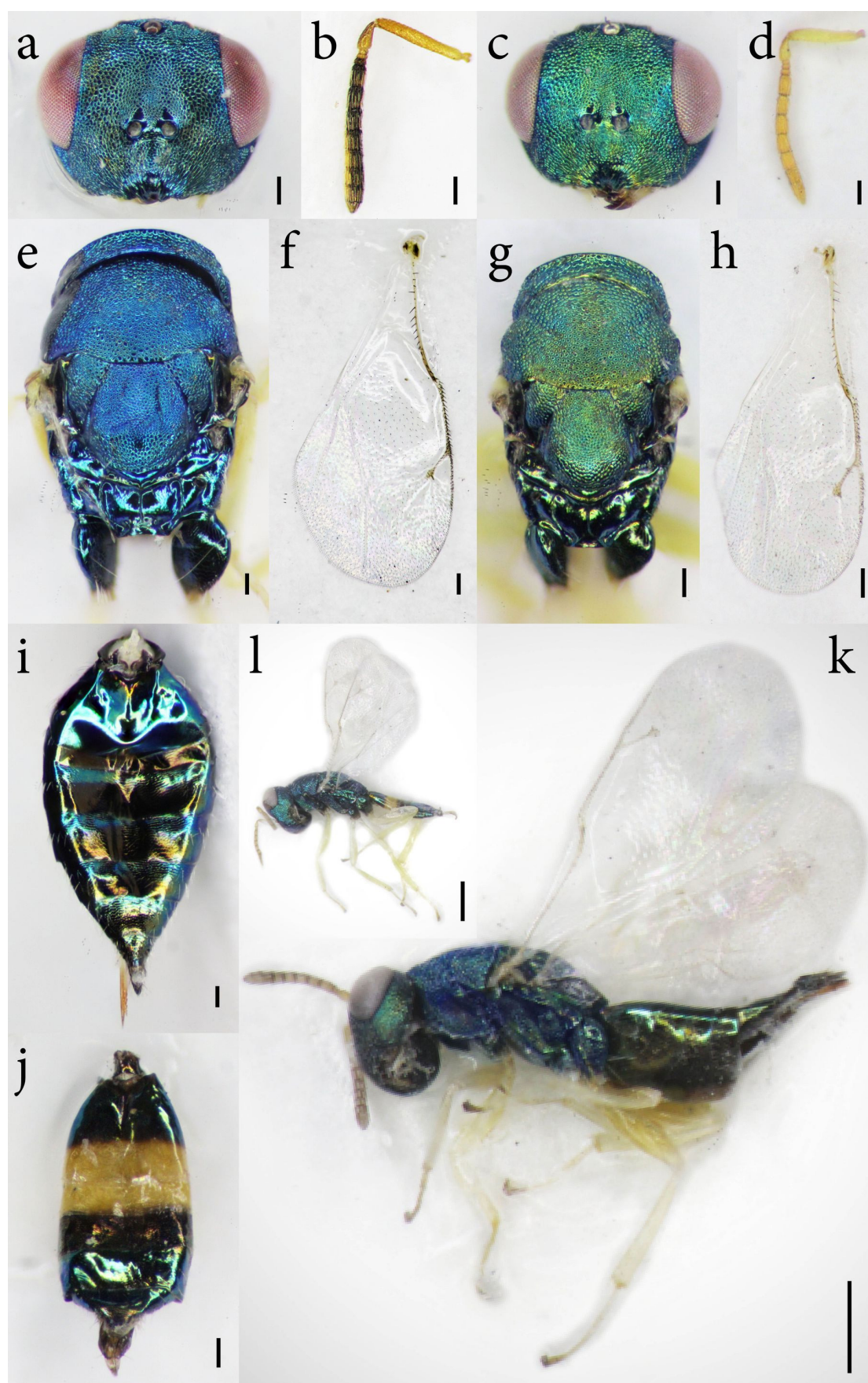
LENGTH. 2.05 to 3.00 mm (N = 15, mean = 2.6, sd = 0.29 mm).

COLORATION. Body green, sometimes with golden reflections; metasoma bronze-black distally, some of the tergites occasionally with blue or violet flecks. Coloration of antennae: scape, pedicellus and anelli testaceous, sometimes last anellus infuscate, all funicular segments and clava infuscate, occasionally brown. Coxae concolorous with the mesosoma, femora and tibiae testaceous, the tips of the fifth tarsi fuscous to black. Tegulae hyaline, usually slightly yellow posteriorly. Wings hyaline; venation pale yellow.

HEAD. 1.1 (range 1.02–1.18) times as broad as mesoscutum; in dorsal view 2.25 (2.07–2.52) times as broad as long, with temples rounded off and between one third and one fourth as long as eyes; the distance between posterior ocelli (POL) 2.11 (1.75–2.80) times oculo-ocellar distance (OOL). Head in front view suboval with the genae moderately buccate. Eyes separated about 1.59 (1.18–1.74) times their length. Malar space more than half (0.68 (0.55–0.76)) the length of an eye. Breadth of oral fossa 1.93 (1.69–2.36) times malar space. Clypeus strigose, its anterior margin moderately emarginate. Head uniformly and moderately reticulate. Antennae inserted low on head, lower edge of toruli at or hardly above level of ventral edge of eyes; distance between clypeal margin and toruli 0.69 (0.54–0.8) times the distance between median ocellus and toruli. Scape length 1.23 (1.09–1.4) times eye length, scape almost reaching lower edge of median ocellus; combined length of pedicellus and flagellum 0.87 (0.76–0.96) times breadth of head; pedicellus (profile) 2.06 (0.75–2.5) times as long as broad, about as long as anelli plus first funicular segment; flagellum rather weakly clavate, proximally as stout as or slightly

**Fig. 5** (opposite page). *Mesopolobus robiniae* Lakatos & László sp. nov. **a–b, e–f, i.** ♀ (MZBBU HYM000034). **c–d, g–h, j.** ♂ (MZBBU HYM000035). **k.** Paratype, ♀ (MZBBU HYM000014). **l.** ♂ (MZBBU HYM000036). **a.** Head, frontal view. **b.** Antenna. **c.** Head, frontal view. **d.** Antenna. **e.** Mesosoma. **f.** Fore wing. **g.** Mesosoma. **h.** Fore wing. **i.** Gaster, dorsal view. **j.** Gaster, dorsal view. **k.** Habitus. **l.** Habitus. Scale bars: a–j = 0.1 mm; l–k = 0.5 mm.







stouter than pedicellus; first and second anelli short, twice or slightly more than twice as broad as long, third anellus longer than previous anellus and about 1.5 times as broad as long; funicular segments subquadrate, proximal ones sometimes slightly longer than broad, distal ones occasionally very slightly transverse; clava 1.9 (1.5–2.29) times as long as broad, 0.83 (0.66–1.15) as long as three preceding funicular segments together; sensilla in one row on each segment, sparse on funicle, more numerous on the clava.

MESOSOMA. 1.52 (1.38–1.74) times as long as broad. Pronotal collar moderately long medially, 0.21 (0.16–0.26) times (one sixth to one fifth) as long as mesoscutum, and much longer at sides, strongly and coarsely reticulate, clearly margined. Mesoscutum 1.58 (1.28–1.82) times as broad as long, rather coarsely reticulate discally, more finely laterally, without piliferous punctures. Scutellum 0.9 (0.82–0.94) times as broad as long, moderately convex, finely reticulate, frenum slightly more coarsely reticulate. Axillae finely reticulate. Dorsellum a narrow, alutaceous transverse crest separated from scutellum by simple suture. Propodeum medially slightly less than half (0.41 (0.36–0.48)) as long as scutellum; median area 2.39 (2–3) times as broad as long, well-defined laterally, plicae distinct throughout and sharp over at least their distal half; median carina distinct, straight; panels of median area finely, slightly irregularly reticulate; nucha transversely aciculate, separated from median area by impressed line; posterior foveae, at sides of nucha, moderately deep; spiracles oval, longer than broad, separated by nearly half their length from metanotum. Postspiracular sclerite broad, shiny, weakly and irregularly sculptured. Mesepisternum moderately finely reticulate, its upper triangular area smooth; mesepimeron rather more coarsely reticulate than mesepisternum, metapleuron smooth. Legs rather short; femora rather stout; mid tibiae fairly slender, 7.44 (4.88–9) times as long as their maximum breadth. Fore wing rather broad; costal cell fairly broad, its upper surface bare, lower surface with a complete row of hairs and some additional hairs scattered over distal third to half; basal cell bare, open below; basal vein bare or with one to three hairs; speculum open below, on upper surface of wing extending below proximal end of the marginal vein; surface beyond speculum thickly pilose; marginal vein 2.19 (2–2.47) times as long as stigmal vein; postmarginal vein shorter than marginal, 0.73 (0.63–0.81) times as long as marginal.

METASOMA. Ovate, 1.24 (1.16–1.33) times as long as mesosoma, 0.8 (0.66–0.96) times as broad as mesosoma, 2.37 (1.91–2.96) times as long as broad; basal tergite occupying from slightly more than one quarter to nearly one third of total length; last tergite somewhat shorter than basal breadth, its length 1.07 (0.72–1.79) times its breadth; ovipositor sheaths projecting at most very slightly; hypopygium slightly reaching the middle of metasoma, ratio of hypopygium length to metasoma length is 0.44 (0.35–0.54).

### Male

LENGTH. 1.8 to 2.25 mm (N = 15, mean = 2.02, sd = 0.16 mm).

COLORATION. Head and mesosoma bright green; metasoma greenish dorsally with second tergite (T2) and posterior half of first tergite (T1) yellow, third tergite (T3) purplish; antennae bright testaceous; legs except coxae yellow, last tarsal segments grey-brown.

HEAD. Antenna with 3 anelli and 5 funicular segments, length of pedicel plus flagellum 0.97 (0.85–1.04) times as breadth as head; scape 5.33 (4.6–6.25) times as long as broad, without a boss on its anterior surface; flagellum proximally not broader than pedicel, F1–F4 longer than broad, F5 subquadrate. Mouthparts unmodified; no patch of modified sculpture behind malar sulcus.

MESOSOMA. Pronotal collar as long as in females, about 0.21 (0.18–0.27) times mesoscutal length. Middle tibiae unmodified, 7.42 (6.29–8.8) times its breadth, tibial spur 1.47 (1.17–1.8) times breadth of first tarsus.

METASOMA. Oblong, ovate, 0.91 (0.83–1.02) times as long as mesosoma, 2.24 (1.63–2.63) times as long as broad with a yellow ventral plica; T1 with triangular depression at base.

### Morphological comparison

Females of *Mesopolobus robiniae* sp. nov. were not identifiable based on Graham's keys (Graham 1969), but several morphologically and morphometrically related species were found for which the differing characters will be enumerated in the order that the species appear in Graham's key. The species *M. robiniae* sp. nov. has a shorter metasoma compared to head plus mesosoma in *M. maculicornis*. The species *M. jucundus* (Walker, 1834) has a curved stigmal vein compared to *M. robiniae* sp. nov. *Mesopolobus robiniae* sp. nov. differs from *M. fasciventris* by its males having 3 anelli and 5 funicular segments while in the latter there are 2 anelli and 6 segments. Females of *M. robiniae* sp. nov. differ from those of *M. fasciventris* in the ratios of  $pcl.l3/mav.l$  and  $clv.l/hea.l$  (for abbreviations see Table 2, for differences Table 3). The head of *M. apicalis* in dorsal view has temples nearly three quarters as long as the eyes, while *M. robiniae* sp. nov. has its head in dorsal view with temples appearing one quarter to one third as long as the eyes. The metasoma of *M. amaenus* is less than twice as long as broad and almost as long as the mesosoma, while in the case of *M. robiniae* sp. nov. the metasoma is not less than twice as long as broad, but it is as long as the mesosoma. The species *M. longicollis* has the pronotal collar  $1/7$  to  $1/6$  as long as the mesoscutum and its metasoma is less than twice as long as broad compared to *M. robiniae* sp. nov. The species *M. diffinis* and *M. mediterraneus* differ from *M. robiniae* sp. nov. because the latter has longer marginal vein (1.4 to 1.6) than length of the stigmal vein.

The species *M. verditer* is not present in the keys of Graham (1969) because it has a North American distribution. It differs from *M. robiniae* sp. nov. in the following: antennal funicle segments shorter than their breadth, while in *M. robiniae* sp. nov. they are at least as long as their breadth. The ratio of the stigmal vein to the last gastral tergite length is 1.91–2.50 in *M. verditer*, while in *M. robiniae* sp. nov. is between 0.08–1.15. *Mesopolobus sericeus* differs from *M. robiniae* sp. nov. first by having 2 anelli and 6 funicular segments, but also in having the ratio of the stigmal vein to the last gastral tergite length 1.41, while in the other species this ratio is smaller (0.8–1.15). In *M. typographi* the median area of the propodeum is 1.75–2 times as broad as long (Graham 1969) while in *M. robiniae* sp. nov. is 0.82–0.94 times as broad as long (N = 15).

Based on von Rosen's key (von Rosen 1958), the morphological identification of specimens led us to *M. mediterraneus* as the closest species, from which females of *M. robiniae* sp. nov. differs, apart from the previously mentioned longer marginal than stigmal vein, in also having a longer pronotal collar and a shorter metasoma than the combined length of head and mesosoma.

Gahan (1932: 739) states that *Mesopolobus* (syn. *Amblymerus*) *verditer* (Norton 1868) "...conforms very closely to the characters of the genus *Amblymerus* Walker as represented by *Amblymerus amoenus* Walker..." (syn. *M. amaenus*), when transferring the species to the genus *Amblymerus* Walker, 1834 from the genus *Nasonia* Ashmead, 1904. The hosts of *M. verditer* are usually sawflies (Hymenoptera: Diprionidae) on pines (*Pinus* sp.) (Noyes 2020). *Mesopolobus verditer* is distributed in the Nearctic and Germany (Thompson 1958). Moreover, *M. verditer* differs from *M. robiniae* sp. nov. in having a reticulated middle area of propodeum and oblique wrinkles, as does also from *M. amaenus* and *M. longicollis* (von Rosen 1958).

We propose the following update to the key of *Mesopolobus* species of Graham (1969) for females:

- 16. Either metasoma at least slightly longer than head plus mesosoma, and usually more than twice as long as broad, or metasoma not longer than head plus mesosoma, and at most twice as long as broad ..... 16A
- Metasoma not longer than head plus mesosoma, their ratio is 0.94 (0.88–0.98), metasoma usually more than twice, 2.37 (1.91–2.96) times as long as broad ..... *M. robiniae* Lakatos & László sp. nov.
- 16A. Metasoma at least slightly longer than head plus mesosoma, usually more than twice as long as broad ..... 17
- Metasoma not longer than head plus mesosoma, at most twice as long as broad ..... 27

### Distribution

The type locality for *M. robiniae* sp. nov. is Săldăbagiu de Munte, Bihor County, Romania (47.096354° N, 21.984963° E). The other localities are situated in the neighbouring counties: Cluj County, Romania and Hajdú-Bihar County, Hungary. The species may appear in the Carpathian Basin where its host plant is present, but we expect that it may also be found outside of the Carpathian Basin, in Eastern Europe and maybe throughout Europe.

### Biology

Based on our rearing, *M. robiniae* sp. nov. seems to be an early flying parasitoid species. Individuals of the species emerged during spring consequently in all study years. Our black locust seedpod samples were collected mostly in March, and the emergence peak of *M. robiniae* sp. nov. was in April, with a decrease in May. After May, we rarely encountered any individuals of this parasitoid species.

The host of *M. robiniae* sp. nov. may be *Bruchophagus robiniae* but there is no information regarding the host plant of *B. robiniae* before the introduction of black locust. Another possibility is that *M. robiniae* sp. nov. initially had another host, but switched from it to *B. robiniae*. Either possibility is plausible; before 1970 (Zerova 1970) the species *B. robiniae* was not known, and *M. robiniae* sp. nov. was not described until now. The parasitoid community of black locust is understudied, and the available literature makes no mention of parasitoids in this community (Farkas & Terpó-Pomogyi 1974; Perju 1998), with the exception of our ecological study concerning the seed-predator community of black locust in Eastern Europe (Lakatos *et al.* 2016).

### Discussion

The multivariate ratio analysis (MRA) and the *mtDNA* sequence analysis resulted in the successful separation of the *Mesopolobus* species emerging from black locust seedpods from the other congeneric relatives. The morphometry-based shape PCA helped us identify which species fall closer to the specimens emerged from black locust crops. This delimitation was important since the available specific keys (Graham 1969) did not lead us to a closest relative based on the combination of morphology and morphometry.

In a PCA ratio spectrum, only ratios calculated with variables lying at the opposite ends of the spectrum are relevant for a particular shape PC and the most allometric ratios are also found at the opposite ends of the allometry ratio spectrum (Baur *et al.* 2014) we apply multivariate ratio analysis (MRA). The PCA ratio spectrum and the allometry ratio spectrum plots revealed a large (*M. amaenus* and *M. robiniae* sp. nov.) and moderate (*M. fasciventris* and *M. robiniae* sp. nov.) amount of allometric variation in the identified discriminating morphometric character pairs (Fig. 2). However, this is not of concern in our

case, because on the one hand the species we found to be closely related based on morphometry were clearly separated based on the molecular results, and the combinations of the usually used ratios do not overlap with species in the keys of Graham (1969), since there is no possibility to progress beyond key couplet 16. On the other hand, the ratios found with the LDA ratio extractor tool have small D.size values compared to D.shape values (Table 3) which means that separation was mainly due to shape rather than size. The LDA ratio extractor tool found that the species pairs could be separated without overlapping based on the first ratio pairs. These ratios in combination with morphologic characters gave a confident separation of the closely related species.

The origin of *M. robiniae* sp. nov. is as yet unknown, since it has to be a host shifting species. Black locust was introduced to Europe 300 years ago, and in its native area it has no *Bruchophagus* seed predator, nor the associated parasitoids (Stone 2009). So, the new *Mesopolobus* species may not be monophagous on *B. robiniae*, which is similarly a host-shifting seed predator. Nonetheless, it is befitting of the name *robiniae*, since parasitoids are also affected by the host plant of their herbivorous host. As part of their host finding strategy, parasitoids may search for a specific plant or plant part (as seedpods) housing any potential herbivorous host species (Cronin & Abrahamson 2001).

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**Supp. file 1:** Morphometric measurements of dry-mounted *Mesopolobus* Westwood, 1833 females belonging to the analysed species. <https://doi.org/10.5852/ejt.2021.740.1285.3849>

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