PENSOFT.

SENCKENBERG
world of biodiversity

Leibniz
Leibniz
Association

Torymus sinensis and its close relatives in Europe: a multilocus phylogeny, detailed morphological analysis, and identification key

Ionela-Mădălina Viciriuc[1], Mircea-Dan Mitroiu[2], Richard Robinson Askew[3], Nicolas Ris[4], Lucian Fusu[2], Nicolas Borowiec[4]

1 *Laboratory of Interdisciplinary Research on the Marine Environment and Marine Terrestrial Atmosphere, Al. I. Cuza University of Iasi, Prof. Dr. Ioan Borcea Marine Biological Station, Nicolae Titulescu str. no. 163, Agigea, Constanţa, Romania*

2 *Research Group in Invertebrate Diversity and Phylogenetics, Faculty of Biology, Al. I. Cuza University of Iasi, Carol I BD. no. 11, 700506 Iaşi, Romania*

3 *Le Bourg, St Marcel du Périgord, 24510, France*

4 *Sophia Agrobiotech Institute, UMR INRAE-CNRS-Université Côte d'Azur, 400 Route des Chappes BP 167, 06903 Sophia Antipolis Cedex, France*

https://zoobank.org/A42A883F-FE28-4EC2-8C3B-F877D52EDB5B

Corresponding author: Lucian Fusu (lucfusu@hotmail.com)

Abstract

The introduction of the biological control agent *Torymus sinensis* Kamijo (Hymenoptera, Chalcidoidea, Torymidae) to control the populations of the chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae) is considered one of the successful programs in biological control. The species was involved in interspecific hybridisation in Japan and the specimens imported into Europe were derived from this hybrid lineage, showing signs of introgression. The discovery of mitochondrial haplotypes or possible *Enolase* haplotypes from *T. beneficus* or of specimens with shorter ovipositor does not necessarily imply that *T. beneficus* is present in Europe, only that the European specimens are of hybrid origin. Of the native European *Torymus* species associated with *D. kuriphilus*, the molecular and morphometric results indicate *Torymus notatus* (Walker) as the closest species to *T. sinensis*. The two are part of the same species-group (*cyaneus* group), are nested together in the multivariate ratio analysis and are the closest genetically based on all three nuclear markers: *Enolase* (1.5% divergence), *Wingless* (2%) and *ITS2* (13%). However, on the mitochondrial marker *COI* the closest species is *Torymus rubi* (Schrank) at 9.9% divergence. As such, *T. notatus* is the most likely candidate for accidental interspecific hybridisation if this is to happen in Europe. We provide an illustrated identification key for the European species of *Torymus* associated with *D. kuriphilus*, an important but lacking tool for biological control programs.

Key words

Biological control, chestnut gall wasp, DNA barcoding, hybridisation, identification key, morphometrics, multilocus phylogeny

1. Introduction

Within Chalcidoidea, the family Torymidae comprises six subfamilies including around 1000 described species (Janšta et al. 2018; Noyes 2019). Torymidae species have different feeding strategies: most of them are ectoparasitoids of gall wasps (Cynipidae) and gall midges (Cecidomyiidae), some are endoparasitoids of eggs or pupae of Lepidoptera and Symphyta, and even fewer species are phytophagous (Grissell 1995; Janšta et al. 2011, 2013, 2018). Since family Megastigmidae, previously a subfamily of Torymidae and a group containing numerous phytophagous species, was recently elevated to family rank (Janšta et al. 2018), the torymids as currently understood contain many fewer phytophagous species.

The genus *Torymus* Dalman (Toryminae) is a diverse group with about 400 species, many of them being ectoparasitoids of gall makers (Cynipidae and Cecidomyiidae) (Grissell 1995; Graham and Gijswijt 1998; Noyes 2019). One of the best known and economically important species, *Torymus sinensis* Kamijo, has been used in the last 50 years to control the invasive chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), native to China (Rieske 2007; Quacchia et al. 2008; Matošević et al. 2015; Borowiec et al. 2018). *Dryocosmus kuriphilus* was accidentally imported to Japan in 1941 where it became a major pest of chestnut trees (Murakami 1997), being afterwards detected in many other countries. The chestnut gall wasp has a highly negative impact on the architecture of the tree and the production of chestnuts with losses of approximately 50–80% (Payne 1975; Battisti et al. 2014; Kenis et al. 2017), a loss in basal area increment in chestnut coppices of 40–79% (Marcolin et al. 2021), and it can even kill the trees, especially concurrently with other stress factors (Moriya et al. 2003; Gehring et al. 2020). The introduction of *T. sinensis* has positive effects improving the health of chestnuts (Gehring et al. 2018), the first results being visible even from the first year after the introduction (Colombari and Battisti 2016; Matošević et al. 2017; Borowiec et al. 2018; Ferracini et al. 2018). An effective control is achieved when parasitism rates reach about 80% (Gehring et al. 2020), usually with significant recovery of tree growth after 2–3 years (Marcolin et al. 2021) and nut production recovery of about 50% in 3–5 years (Colombari and Battisti 2017). Even if the efficiency is not the same in all affected regions (Gil-Tapetado et al. 2023), this can be considered a successful example of biological control (Kenis et al. 2019).

In 1975 *T. sinensis* was first introduced to Japan (Murakami et al. 1977; Ôtake et al. 1984) and during research on the native species attacking *D. kuriphilus* in this country, another species was identified, i.e. *Torymus beneficus* Yasumatsu et Kamijo, which is very close morphologically, ecologically and genetically to *T. sinensis* (Moriya et al. 1988; Yara 2004; Yara 2006). *Torymus beneficus* was further subdivided into two strains with different emergence periods (early-spring, TbE and late-spring, TbL). Females of both strains can allegedly be differentiated from *T. sinensis* using the ratio between ovipositor sheath length and the length of the thorax (i.e. mesosoma) in lateral view (O/T ratio), but males can only be separated using the emergence period (Ôtake 1987; Murakami 1988; Yara et al. 2000). A variation of the above ratio is the ovipositor index, i.e. the ratio between lengths of ovipositor sheath and hind tibia, used by Matsuo (2020) to separate the two species. However, recent studies (Montagna et al. 2019; Viciriuc et al. 2021) showed that the TbL strain of *T. beneficus* and *T. sinensis* are conspecific and that only TbE should be considered as *T. beneficus*. The use of the O/T ratio to discriminate these species (Yara 2004) is the starting point for this confusion since values of this ratio overlap between the two species (Viciriuc et al. 2021). Morphological differences between the two taxa do exist but are very subtle and concern the antenna length (Kamijo 1982; Viciriuc et al. 2021). The antennal flagellum is comparatively longer in *T. beneficus* (Kamijo 1982), but likely because accurate measurements of the antenna are difficult to achieve on air dried specimens, it has been disregarded by all subsequent authors. The separation between the two species was made almost exclusively for female individuals using the O/T ratio (Ôtake 1987; Yara et al. 2000; Yara 2006) even if the length of the ovipositor can vary drastically in the same species of *Torymus*, including between distinct generations (Askew 1965). Because *T. sinensis* and *T. beneficus* are so close, Viciriuc et al. (2021) discuss the taxonomic status of *T. sinensis* relative to *T. beneficus*, but for the stability of name usage, they recommend treating them as distinct species.

First signs of hybridisation between *T. sinensis* and *T. beneficus* were observed under laboratory conditions where fertile hybrid females could be produced (Moriya et al. 1992, 2003). A few years later, hybrids between these two species were detected at low frequency in the field using malic enzyme as a molecular marker (Yara et al. 2000). Whereas intraspecific hybridisation may be intentionally used to increase efficiency of biological control agents (BCA) (Benvenuto et al. 2012), for example by altering their host preference and increasing fitness (Mathenge et al. 2010), unintentional interspecific hybridisation involving an introduced species as one of the parents could affect native communities and/or reduce the efficiency of the BCA (Fischer et al. 2015). For this phenomenon to happen there are several requirements: to be close species, to share the same habitat or have the same reproduction period and the same host (Gibbs et al. 2011). *Torymus sinensis* and *T. beneficus* fulfil most of these prerequisites.

In the case of *T. sinensis* and *T. beneficus* the shorter ovipositor of *T. beneficus*, as well as its lower fecundity, may have represented a reproductive disadvantage (Moriya et al. 1992, Viciriuc et al. 2021) and because of both competition and introgression, *T. beneficus* was displaced by *T. sinensis* in the areas where *D. kurphilus* was present (Yara et al. 2007). Nowadays, *T. beneficus* is considered as a rare species in Japan and only two individuals were recently reported from *Dryocosmus murakamii* Ide and Abe (Matsuo et al. 2021). For a review of the hybridisa-

tion history, see Viciriuc et al. (2021). Besides this case, there are only a few documented examples of interspecific hybridisation between biological control agents and native species (Naka et al. 2005, 2006; Davies et al. 2009; Havill et al. 2012).

To the best of our knowledge, all the European biological control programs have been implemented using the Japanese stock of *T. sinensis*. The history of introduction began in 2002 with the import of over 2000 specimens to Italy from Japan (Quacchia et al. 2008) followed by natural dispersal and by intentional releases of "italian *T. sinensis*" (Borowiec et al. 2014) from Italy to France. Using molecular markers (*COI* and *ITS2*) Montagna et al. (2019) established that the Italian populations of *T. sinensis* showed no signs of hybridisation, but later several *T. sinensis* specimens proving mitochondrial capture from *T. beneficus* were found in France, Italy and Spain (Viciriuc et al. 2021, Gil-Tapetado et al. 2023). The morphology of the Japanese and European *T. sinensis* is likewise intermediate between *T. sinensis* and *T. beneficus* (Viciriuc et al. 2021). However, this interspecific hybridisation seems to have had no impact on *T. sinensis* efficiency since populations of *D. kuriphilus* are currently under control (Borowiec et al. 2018; Muru et al. 2021).

In Europe, nine native species of *Torymus* have been reported to be associated with galls of *D. kuriphilus*: *Torymus auratus* (Müller) (Speranza et al. 2009; Kos et al. 2015; Muru et al. 2021), *Torymus erucarum* (Schrank) (Speranza et al. 2009), *Torymus fastuosus* Boheman (Kos et al. 2021), *Torymus flavipes* (Walker) (Aebi et al. 2007; Speranza et al. 2009; Kos et al. 2015), *Torymus nobilis* Boheman (Kos et al. 2021), *Torymus notatus* (Walker) (Gil-Tapetado et al. 2021), *Torymus formosus* (Walker) (Kos et al. 2015), *Torymus geranii* (Walker) (Kos et al. 2015) and *Torymus scutellaris* (Walker) (Quacchia et al. 2012). No reproductive compatibility has however been observed in laboratory crosses between *T. sinensis* and the tested native species such as *T. notatus* and *Torymus cyaneus* Walker (phylogenetically close to *T. sinensis*), or *T. auratus*, *Torymus affinis* (Fonscolombe), *T. flavipes* and *T. geranii* (phylogenetically distant from *T. sinensis*) (Quacchia et al. 2014; Ferracini et al. 2017, Gil-Tapetado et al. 2023). It is noteworthy that *T. sinensis* can also attack nontarget oak galls, like those of *Andricus aries* (Giraud), *Andricus curvator* Hartig, *Andricus cydoniae* Giraud, *Andricus inflator* Hartig, *Andricus kollari* (Hartig), *Andricus lignicolus* (Hartig), *Andricus polycerus* (Giraud) and *Biorhiza pallida* (Olivier), but so far with a low parasitism rate of 0.1–9.1% (Pogolotti et al. 2019).

Since *T. sinensis* hybridized with *T. beneficus* in Japan it was very important to confidently establish which European species, if any, could be potential candidates for interspecific hybridisation. So far, in interspecific laboratory crosses no attempted mating with European species were reported (Quacchia et al. 2014; Ferracini et al. 2017; Gil-Tapetado et al. 2023), while the use of the *COI* and *ITS2* molecular markers showed ambiguous results as to which is the closest species to *T. sinensis* (Montagna et al. 2019) or identified *T. notatus* as the closest species (Pogolotti et al. 2019; Gil-Tapetado et al. 2022). Even if

no signature of hybridisation between *T. sinensis* and any European *Torymus* has been observed to date in field-collected individuals, monitoring should be continued. By using two additional single copy nuclear genes for a multilocus phylogenetic reconstructions and a morphological and morphometric analysis, the present study aims to identify (i) the closest indigenous species that are potential candidates for interspecific hybridisation and (ii) the native species of *Torymus* developing on *D. kuriphilus* in France. Finally, to provide technical help for field surveys, we also propose an illustrated identification key for the species of *Torymus* that are known to develop in *D. kuriphilus* galls in Europe.

2. Materials and methods

2.1. Sampling

Native parasitoids were obtained from 2011 to 2016 by sampling galls of *D. kuriphilus* on *Castanea sativa* in France. The galls were reared in bulk in outdoor cages, under the meteorological conditions of Sophia Antipolis (France) (Viciriuc et al. 2021). In this study, we used the individuals of *T. sinensis* obtained previously (Viciriuc et al. 2021). We also sampled in 2021 in Fozières, but with very low success since *D. kuriphilus* was at extremely low density. Also, to complete the molecular and morphological datasets, galls of other Cynipinae species were collected in France and Romania, as follows: sexual generation of *B. pallida* and asexual generation of *Cynips longiventris* Hartig on *Quercus* spp. and *Diplolepis rosae* (Linnaeus) on *Rosa canina* (Table S1). The parasitoids were stored in 80% ethanol at –22°C to ensure a good conservation of their DNA. All emerged *Torymus* adults were identified using an unpublished key by Askew and Thuroczy, as well as the keys of Graham and Gijswijt (1998).

2.2. Molecular markers and workflow

Several individuals per species were chosen for the molecular analysis. DNA was isolated individually by incubating whole specimens at 75°C for 3h and 95°C for 5 min using the prepGEM Insect kit (ZyGEM Hamilton, New Zealand). This ensures that enough DNA leaches into the solution, while the specimens remain in good shape for the subsequent morphological identification and morphometric analyses. After extraction the insects were rinsed in water and stored in 80% ethanol for mounting and morphological analysis.

The molecular analyses were based on four molecular markers: one mitochondrial (Cytochrome c oxidase I, *COI*) and three nuclear (the protein-coding genes Wingless, *Wg* and Enolase, *Eno*, and the ribosomal internal transcribed spacer 2, *ITS2*). The amplified region for *Eno* includes both variable regions (introns) and conserved re-

gions (exons). *COI* was amplified using two pairs of primers: the pair LCO1490 / HCO2198 developed by Folmer et al. (1994) that amplifies the standard barcode region (Herbert et al. 2003) and in case of failure the primers COI pF2 / COI 2437d developed by Simon et al. (1994) and modified by Kaartinen et al. (2010), which amplify a fragment largely overlapping with the barcode region. The resulted dataset was not trimmed even if the length of *COI* sequences differed in the phylogenetic analyses since most sequences were the standard barcode and trimming would result in a much shorter alignment. For the other markers we used: LepWg1 and LepWg2 (Brower and DeSalle 1998) for *Wg*; ITS2-F and ITS2-R2 (Yara 2006) for *ITS2*; ENOf3 and ENOr4 (Viciriuc et al. 2021) for *Eno*. The conditions for amplification were the same as in Viciriuc et al. (2021).

After visualisation of the amplified PCR products on a QIA-excel Advanced System (Qiagen), they were sent to Beckman Coulter Genomics (Stansted, U.K) or Genewiz (Radolfzell, Germany) for purification and sequencing in both directions.

2.3.   Molecular data analysis

The sequences were assembled, and a consensus produced using the Staden package (Bonfield et al. 1995); to exclude possible contaminants they were also compared with sequences deposited on GenBank. Protein-coding loci (*COI, Wg* and *Eno* exons) were aligned in MEGA v.6.06 (Tamura et al. 2013) using the ClustalW algorithm (Thompson et al. 1994) and were translated to amino acid sequences to check for stop codons to detect possible pseudogenes. For *Eno* we delimited the regions corresponding to exons and introns as described in Viciriuc et al. (2021). The *ITS2* sequences (that showed numerous indels in interspecific comparisons) were aligned using the E-INS-i algorithm in MAFFT v.7.475 (Katoh and Standley 2013) followed by minor manual adjustments.

For all four markers we first obtained individual phylogenetic trees using RAxML-NG (Kozlov et al. 2019) by performing 20 distinct maximum likelyhood searches starting from 10 random and 10 parsimony-based starting trees and selecting the best-scoring tree; confidence levels of the observed clades were assessed with 1000 standard bootstrap iterations. For the phylogenetic analyses data blocks were first defined based on assumed characteristics of sites (Kainer and Lanfear 2015) as follows: three data blocks each for *COI* and *Wg* (corresponding to the first, second and third codon positions); four blocks for *Eno* (three for the exon regions, with a distinct one for each codon position, and the fourth for the introns); *ITS2* was treated as a single data block. The best partitioning scheme was selected using PartitionFinder2 on R (v.4.2.2) (Lanfear et al. 2017) using the greedy algorithm (Lanfear et al. 2012) and branch lengths set to proportionally linked.

After concatenation and model selection as described above, the dataset was analysed with both a maximum likelihood (ML) and a Bayesian inference (BI) method. For maximum likelihood we used RAxML-NG as above. Bayesian reconstruction was done using MrBayes v.3.2.7 (Ronquist et al. 2012). We ran two parallel analyses, each with four Monte Carlo Markov chains, for a total of 2×10^6 generations, with trees and lnLs sampled every 100 generations; all the estimated parameters were unlinked across partitions except for branch lengths. The trace files were examining in Tracer v.1.7.1 (Rambaut et al. 2018) to ensure that all parameters and sample size values were above 200. The first 25% from the cold chains were treated as burn-in and discarded. The phylogenetic trees were edited with Fig Tree v.1.4.2 and Adobe Illustrator. *P*-distances between taxa were calculated using MEGA v.6.06 with uniform rates among sites and pairwise deletion of missing data.

Besides the newly generated sequences, we used those of *T. sinensis*, *T. auratus* and *T. geranii* previously published by Viciriuc et al. (2021) and the *ITS2* sequences for *T. beneficus* with accession numbers AB200270 and AB200271 from Yara (2006) as well as LC579763 from Matsuo et al. (2021). For the specimens of *T. sinensis* with heterozygous SNPs (one specimens for *Wg* and four for *Eno*), these were coded with N. For a specimen heterozygous for a two nucleotides indel in the *ITS2* sequence, we kept the longest sequence. We used as outgroup sequences of *Glyphomerus stigma* (Fabricius) from the subfamily Glyphomerinae obtained in this study plus sequences of two species from the subfamily Toryminae in the genera *Pseudotorymus* Masi and *Senegalella* Risbec [available from Janšta et al. (2018) under GenBank accession numbers MF956204 and MF956379 for *COI*; MF955908 and MF955957 for *Wg*].

2.4. Morphological characterisation

To ensure that specimens do not collapse, as frequently happens in specimens with a thin exoskeleton especially after DNA extraction, they were dried using a hexamethyldisalizane (HMDS) protocol (Cowan 1995; Heraty and Hawks 1998). Measurements for species identification and morphometric analyses were made either using a Keyence VHX-2000 microscope or a Leica M205C stereomicroscope equipped with a DFC 500 digital camera and illuminated with a LED5000 HDI light source. For the antennae and the head in dorsal view the measurements were made from photographs using Image J (Schindelin et al. 2015). The images for the identification key made with the Leica M205C microscope were processed as described in Fusu and Polaszek (2017), and those made with the Keyence VHX-5000 were not processed. The same characters as in Viciriuc et al. (2021) were used and consisted of 18 measurements for females (Table S2) and 23 for the males (Table S3), to complete the previous dataset. We mainly used specimens of *Torymus* species collected on *D. kuriphilus* in France (*T. auratus*, *T. flavipes*, *T. geranii*, *T. notatus*, *T. sinensis*), but also *T. affinis* and *T. cyaneus* phylogenetically related to *T. sinensis*.

Table 1. Collection years for native *Torymu*s species reared from fresh (GV) and dry (GS) galls of *Dryocosmus kuriphilus*.

	2011	**2012**	**2013**	**2014**	**2015**	**2016**
T. auratus	GV GS	GV GS	GV GS	GS	GS	GV GS
T. flavipes	GV	GV	GV	—	—	GV
T. geranii	—	—	GV	—	—	—
T. notatus	—	GS	GS	—	GS	GV
T. rubi	—	—	—	—	—	GS

To have a broader view and to complete the identification key of the European *Torymus* species attacking *D. kuriphilus*, we added the species that have not been identified in France but are reported from this host elsewhere in Europe. They come from the personal collection of RRA as follows: *T. fastuosus*, *T. nobilis*, *T. formosus*, *T. erucarum* and *T. scutellaris* (Table S4) and were used only for the key. We also used four female and four male paratypes of *T. beneficus* as detailed in Viciriuc et al. (2021). The terms for describing the type of sculpture follow Gibson (2009) and Gibson and Fusu (2016), while morphological terms, the method of measurement and orientation of the anatomical parts follow Graham and Giswijt (1998). To ensure correct interpretation, observations of sculpture were made using diffuse light as explained in Fusu et al. (2018) and Talamas et al. (2017).

2.5. Morphometric analysis

Since principal component analysis (PCA) in its commonly used form is known to be affected by size-related statistical artefacts (Berner 2011), we used instead the multivariate ratio analysis (MRA) of Baur and Leuenberger (2011). This method allows both the detection of the effect of size and the direct interpretation of the results from PCA and linear discriminant analysis (LDA) in terms of body ratios. These tools have been recently used to disentangle cryptic species complexes or very similar species in several groups of Hymenoptera (László and Tóthmérész 2013; Baur et al. 2014; Fusu 2017; Gebiola et al. 2017; Borowiec et al. 2019; Triapitsin et al. 2020; László et al. 2021; Prebus 2021). The steps of the analysis were as described in Viciriuc et al. (2021) and the analyses were conducted using R v.4.1.2 (R Core Team 2021) using the R-scripts from Baur and Leuenberger (2011).

2.6. Abbreviations of morphological terms

fu1–fu3 – first to third funicular segments; **O/T** – the ratio between the length of ovipositor sheath and mesosoma ('thorax' in Otake 1987); **OI** – ovipositor index, the ratio between the length of ovipositor sheath and hind tibia; **OOL** – ocellocular line.

3. Results

3.1. Native *Torymus* parasitoids

We have identified five species of *Torymus* attacking *D. kuriphilus* in France: *T. auratus*, *T. flavipes*, *T. geranii*, *T. notatus* and *T. rubi* (Schrank), the latter species being reported for the first time from *D. kuriphilus* (a single female obtained in 2016 from dry galls). Moreover, during the additional collecting of indigenous gall wasps, one male specimen of *T. sinensis* was reared from the sexual generation of *B. pallida*. *Torymus auratus* and *T. flavipes* were identified since the first year of the survey (2011), the first species being recovered from both dry and fresh galls, while the second only from fresh galls. *Torymus geranii* was recovered from fresh galls in 2013, while *T. notatus* was recovered from dry galls starting from 2012 but only in 2015 from dry galls (Table 1). The most recent survey in 2021 in Fozières (France) did not yield any native *Torymus*. Because the frequency of *D. kuriphilus* galls in this region is currently very low, only four monocular galls were found during a 12 hours search. This is very low compared to some regions such as Dordogne when in 2021 it was still plentiful of galls (R.R. Askew, unpublished data).

3.2. Molecular discrimination between *Torymus* species

Six taxa have been successfully amplified and sequenced for the four different molecular markers (*COI*, *Wg*, *Eno* and *ITS2*): *T. notatus*, *T. cyaneus*, *T. rubi*, *T. flavipes*, *T. bedeguaris* and *Glyphomerus stigma*. For *T. sinensis*, *T. auratus* and *T. geranii* we used the sequences from our previous study (Viciriuc et al. 2021). For all phylogenetic reconstructions the best models and partitioning schemes are presented in Table 2.

3.2.1. Cytochrome c oxidase I

This molecular marker allows the discrimination of all *Torymus* species previously identified using morphological characters, with all conspecific specimens being grouped with bootstrap values above 90%. As expected, *T. sinensis* is divided in two groups, one with most indi-

Table 2. The best substitution models and partitioning schemes for the phylogenetic analyses. The substitution models used for each partition in maximum likelihood and Bayesian inference: F81 (Felsenstein 1981); GTR (Rodriguez et al. 1990); HKY (Hasegawa et al. 1985); K80 (Kimura 1980); K81uf (Kimura 1981); SYM (Zharkikh 1994); TRN, TRNef (Tamura and Nei 1993); TIM (Posada 2003).

Gene	Data block	Substitution models and partition number					
		Single locus		Multilocus phylogeny			
		RAxML-NG		RAxML-NG	MrBayes	
COI	1st codon position	TRN+G	#1	TRN+G	GTR+G	#1
	2nd codon position	F81+I	#2	F81+I	F81+I	#2
	3rd codon position	TIM+G	#3	TIM+G	GTR+G	#3
Wg	1st codon position	TRNef+I	#1	HKY+I	HKY+I	#4
	2nd codon position	TRNef+I	#1	HKY+I	HKY+I	#4
	3rd codon position	K81uf+G	#2	K81uf+G	GTR+G	#5
Eno	Exon, 1st codon position	HKY+I	#1	HKY+I	HKY+I	#4
	Exon, 2nd codon position	K80+G	#2	F81+I	F81+I	#6
	Exon, 3rd codon position	K80+G	#2	HKY+G	HKY+G	#7
	Intron	HKY+I	#3	HKY+I	HKY+I	#8
ITS2	Entire length	F81+G	#1	K80+G	K80+G	#9

viduals of *T. sinensis* and the other with the few *T. sinensis* that harbour *T. beneficus* (TbE) haplotypes, the two being sister groups with a bootstrap of 99%. The *sinensis-beneficus* cluster is sister (bootstrap value 73%) to one that contains all other species of *Torymus*. The genetic *p*-distance between the sequences of *T. sinensis* and those derived from *T. beneficus* are the smallest, of only 5.8%. The native species that are closest to *T. sinensis* are *T. rubi* (9.9%), *T. geranii* (10.3%) and *T. auratus* (10.4%) (Table S5A), with *T. notatus* at 11.5%. The *p*-distances between the analysed taxa vary from 8.8% (*T. auratus – T. geranii*) to 15% (*T. bedeguaris – T. cyaneus*).

3.2.2. Wingless

The phylogenetic reconstruction (Figure S1) shows some notable differences compared to the mitochondrial *COI*: the cluster *T. sinensis* is not subdivided anymore and it is part of a larger polytomy that include the other *Torymus* species. This genetic marker cannot discriminate between very close species such as *T. auratus* and *T. geranii*, as they are placed in the same molecular clade without clear separation, though the two species do not share any haplotypes. With a few exceptions, both internal and apical nodes are well supported on this phylogeny compared to *COI*, most nodes with values above 60%. The interspecific *p*-distance varies from 0.5% (*T. auratus – T. geranii*) to 5.8% (*T. auratus – T. cyaneus*) (Table S5B). With this nuclear marker, *T. notatus* (2%) and *T. cyaneus* (3.7%) are the native species closest to *T. sinensis*.

3.2.3. Enolase

This phylogeny differs from the two previous ones (Figure S3). Due to the introns (variable regions) the *T. sinensis* cluster is more subdivided and it is sister to *T. notatus* (86% bootstrap); the two species group with *T. cyaneus*

(83% bootstrap). *Torymus geranii* is distinct from *T. auratus*. The values of the *p*-distance vary from 1.1% (*T. auratus – T. geranii*) to 7.6% (*T. cyaneus – T. flavipes*). Native species closest to *T. sinensis* are *T. notatus* with a *p*-value of 1.5% followed by *T. geranii* and *T. auratus* with 3.3% and 3.7%, respectively (Table S5C). Notably, specimens 22158 and 22191 of *T. sinensis* are 1.3% divergent from 21943 and several intraspecific pairwise comparisons on *Eno* are 1.1%. This divergence is larger or the same as the one between *T. auratus* and *T. geranii* and close to that between *T. sinensis* and *T. notatus* (Table S5C). These two divergent specimens have *T. sinensis* mitochondrial haplotypes while 21943 has *T. beneficus* mitochondrial sequences (Figure S2).

3.2.4. ITS2

The bootstrap values show good support for the apical nodes (> 93%; with the exception of *T. sinensis* with 68%), but generally weak support (37–90%) for the internal nodes (Figure S4). The clade that contains *T. sinensis* and *T. beneficus* is uniform and sister to *T. notatus* but the support is low. With this marker, *T. cyaneus* is not the closest to them, instead being basal to the clade that includes *T. auratus*, *T. geranii* (the two being distinct), *T. rubi*, *T. bedeguaris* and *T. flavipes*. For this marker, the *p*-distance varies considerably: from only 3.4% between *T. auratus* and *T. geranii* to 25% between *T. bedeguaris* and *T. cyaneus*. *Torymus notatus* and *T. cyaneus* are the closest species to *T. sinensis*, with a *p*-distances of 13% and 16.1%, respectively (Table S5D).
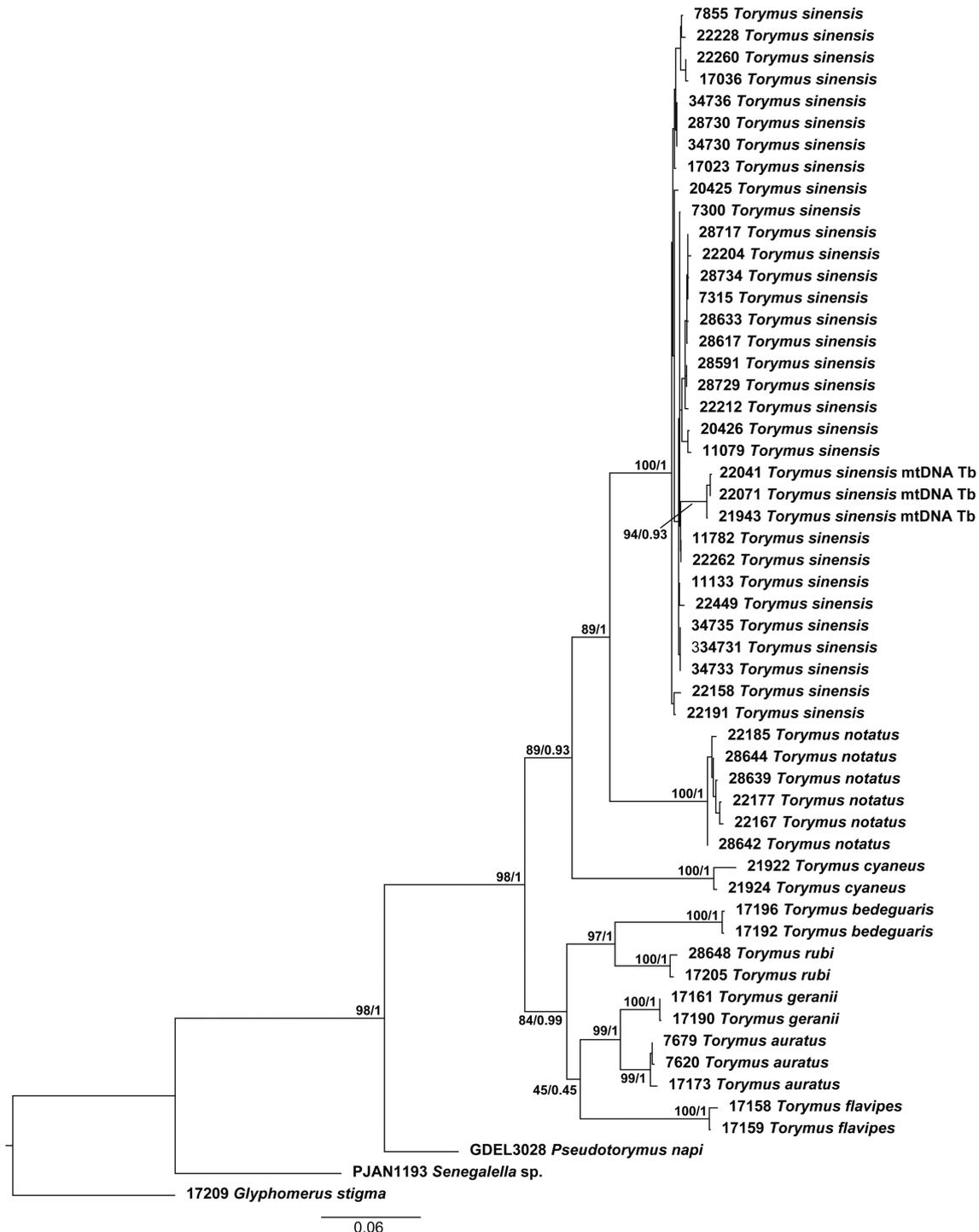
3.3. Multi-locus phylogeny

The topologies observed for each marker are consistent enough to reconstruct a multi-locus phylogeny. As shown in Fig. 1 the phylogenetic analysis using both methods al-

lows unambiguous discrimination (bootstraps >99% and posterior probability of 1) of each species of European *Torymus*, providing a reliable basis for species delimitation, morphological characterisation and identification of diagnostic characters. Both phylogenies have the same topology, except for some differences at intraspecific level. *Torymus sinensis* and *T. notatus* are sister species, the two being grouped with *T. cyaneus*, while all the other *Torymus* species form a second clade.

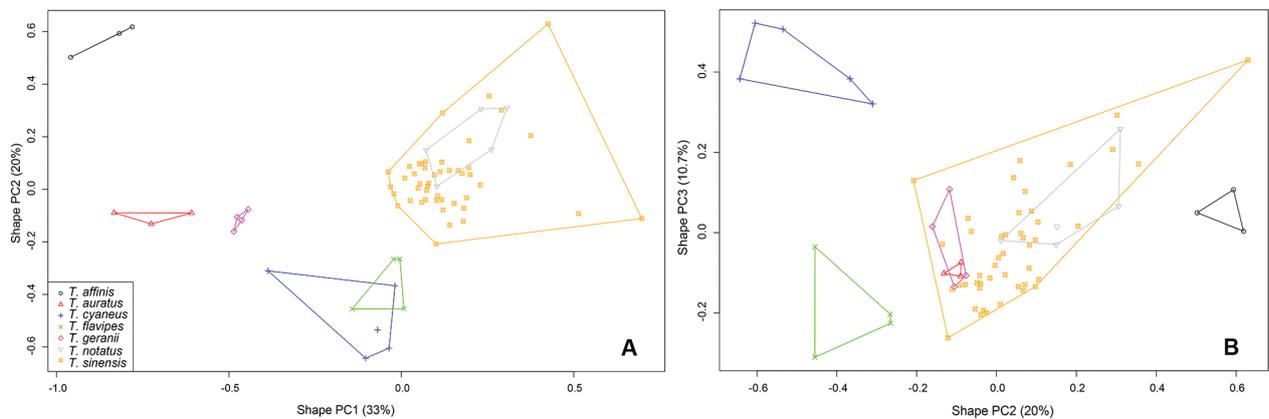## 3.4.   Multivariate Ratio Analysis

For the females, we used the first three components that accounted for 63.8% of the total variance of the dataset (33.0%, 20.0% and 10.7% for the first, second and third component, respectively), to perform the shape PCA. The first component is the most discriminant, with four native species of *Torymus* (*T. affinis*, *T. auratus*, *T. geranii* and *T. cyaneus*) that can be separated from each


Figure 1. Phylogenetic tree inferred using RAxML-NG based on a partitioned multilocus dataset. The reconstruction is based on the sequences of individuals in this study with coverage for at least 3 markers. Bootstrap values and posterior probabilities from an analysis in MrBayes are shown next to the nodes. mtDNA Tb – individuals with mitochondrial DNA derived from *T. beneficus*.

other and from *T. sinensis* (Fig. 2A). A slight overlap is nevertheless observed between females of *T. affinis* and *T. auratus*, and between those of *T. cyaneus* and *T. flavipes*. Along the second component, only *T. affinis* was clearly separated from the other native species, as well as some *T. cyaneus* and some *T. flavipes* (Fig. 2B). The two latter species are also the only ones that can be separated from *T. sinensis*. Along the third component, only some *T. cyaneus* and some *T. flavipes* are discriminated from other species of *Torymus* (Fig. 2B). On all three first components, females of *T. notatus* are nested within those of *T. sinensis*.

For the males, the three first components accounted for 65.2% of the total variance (36.3%, 20.2% and 8.7% for the first, second and third component, respectively). Along the first component, all native species (only partially for *T. notatus*) can be discriminated from *T. sinensis* but with a strong overlap between each other (Fig. 3). Only *T. flavipes* and some *T. auratus* and *T. geranii* can be separated from *T. sinensis* along the second component. By using the LDA ratio extractor we obtained the ratios listed in Table S6 that can be used to discriminate *T. sinensis* from the native species associated with *D. kuriphilus* in Europe.

Figure 2. Results of the multivariate ratio analysis (MRA) for females of several *Torymus* species. **A** Shape PCA of the first component versus second component. **B** Shape PCA of the second component versus third component.
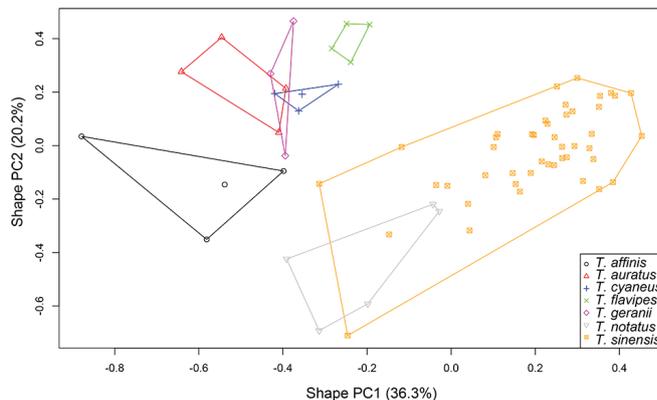
Figure 3. Results of the multivariate ratio analysis (MRA) for males of several *Torymus* species. Shape PCA of the first component versus second component.

3.5.   Key to the species of *Torymus* parasitizing *D. kuriphilus* in Europe

1   Mesoscutellum with frenum distinct, with or without frenal line, i.e. frenal area differently sculptured from the rest of mesoscutellum and frequently glabrous (Fig. 4A, C–E) **2**

–   Mesoscutellum without frenal line and frenum indistinct, mesoscutellum uniformly sculptured and setose (Fig. 4B) **7**

2(1)   Surface of frenum entirely smooth, exceptionally with some weak striae posterolaterally (Fig. 4A, D, E).......**3**

–   Frenum entirely yet finely sculptured (Fig. 4C) **5**

3(2)   Mesoscutellum almost smooth except for piliferous punctures (Fig. 4A); body blue-green to blue, sometimes with violet iridescence (Fig. 5E, F); ocelli large, OOL less than maximum diameter of posterior ocellus (Fig. 6I)..***T. cyaneus* (Walker)**
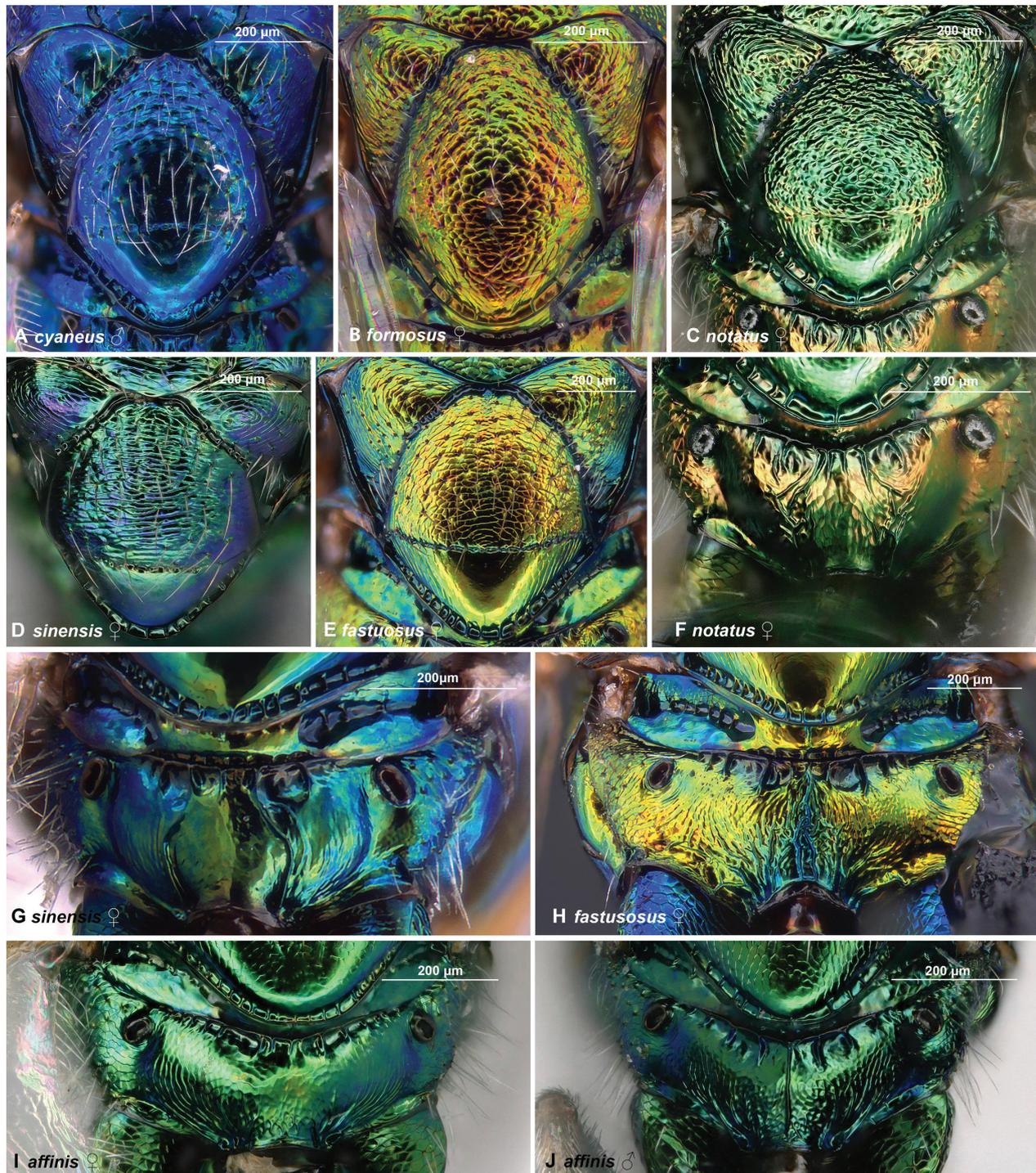

Figure 4. Mesoscutellum (**A–E**) and propodeum (**F–J**) in different species of *Torymus*.

–   Mesoscutellum sculptured, with interspaces between piliferous punctures mostly alutaceous (Fig. 4D, E); body entirely green, with limited blue or coppery iridescence; ocelli moderate in size, OOL more than maximum diameter of posterior ocellus (Fig. 6J)...**4**

4(3)   Both sexes: central area of propodeum with a pair of curved, submedian carinae delimiting a large and circular median area (Fig. 4G); frenum usually smooth, rarely with weak striae posterolaterally (Fig. 4D). Female: only one row of multiporous plate sensilla on each funicular segment; anellus only slightly transverse (Fig. 6A). Male: at least the first five funicular segments longer than wide, with multiporous plate sensilla disposed in 2–3 rows on each segment (Fig. 6E) ....***T. sinensis* Kamijo** (Fig. 7A, B)

–   Both sexes: central area of propodeum with 2–3 irregular longitudinal carinae on each side of the midline (Fig. 4H); frenum medially smooth, but with superficial and fine reticulation posterolaterally (Fig. 4E). Female:

Figure 5. Habitus for *Torymus affinis* (**A**, **B**), *T. auratus* (**C**, **D**) and *T. cyaneus* (**E**, **F**). The two *T. auratus* are not typical, the species usually having the body more extensively green and yellow legs. The dark legs in the male reared from dry galls are characteristic for the spring generation (Askew 1965: 222); the female was reared in the summer from green galls and has yellow legs.

funicular segments with multiporous plate sensilla disposed in 2–3 rows; anellus strongly transverse (Fig. 6C). Male: funicular segments quadrate to weakly transverse, multiporous plate sensilla hidden by numerous, dense setae (Fig. 6B) .... *T. fastuosus* **Boheman** (Fig. 8C, D)

5(2)   Both sexes: vertex with a sulcus connecting each lateral ocellus with the adjacent eye (better visible in males) (Fig. 6K, L, arrows); mesoscutellum anterior of frenal line uniformly coriaceous-alutaceous. Female: ovipositor sheath usually about twice as long as body; propodeum without or with only weakly visible carinae (Fig. 4I). Male: propodeum with or without median carina (Fig. 4J) .... *T. affinis* **(Fonscolombe)** (Fig. 5A, B)
         [Rarely the ovipositor does not exceed the length of the body; to prevent confusion/misidentification with females of *T. sinensis*, the propodeum should be checked – see couplet 4]

–   Both sexes: vertex without a sulcus connecting each lateral ocellus with the adjacent eye, though sometimes with a slight depression in that area (Fig. 6M); mesoscutellum anterior of frenal line coriaceous-alutaceous to partly rugose. Female: ovipositor sheath shorter than body, but longer than gaster; propodeum with 1–3 longitudinal carinae (more or less interrupted and oblique) (Fig. 4F) Male: propodeum with 1–3 longitudinal carinae **6**

6(5)   Both sexes: fore wing with dark brown venation, evidently pigmented around stigmal vein and with some diffuse infuscation mesally (better seen in females) (Fig. 9G); mesoscutellum anterior of frenal line coriaceous-alutaceous, gradually becoming rugose towards mesoscutum, piliferous punctures partly obliterated (Fig. 4C). Female: anellus slightly transverse (Fig. 6F). Male: at least the first five funicular segments strongly transverse, with multiporous plate sensilla disposed in 2–3 rows on each segment (Fig. 6G) ....
         .... *T. notatus* **(Walker)** (Fig. 10A, B)
         [*T. sinensis* may occasionally key out here since a minority of individuals have weak sculpture on the frenum. They are nevertheless easily differentiated by the characteristic propodeum of *T. sinensis* – see couplet 4]

–   Both sexes: fore wing with light brown venation, at most with very limited pigmentation adjacent to stigmal vein (Fig. 9H); mesoscutellum anterior of frenal line entirely coriaceous, with piliferous punctures distinct (Fig. 4E). Female: anellus twice as wide as long (Fig. 6C). Male: funicular segments quadrate, multiporous plate sensilla not visible due to numerous setae disposed in several rows (Fig. 6B) ....
         .... *T. fastuosus* **Boheman** (Fig. 8C, D)
         [This species has been introduced twice in the key because the interpretation of the sculpture on frenum can vary depending on the magnification used]
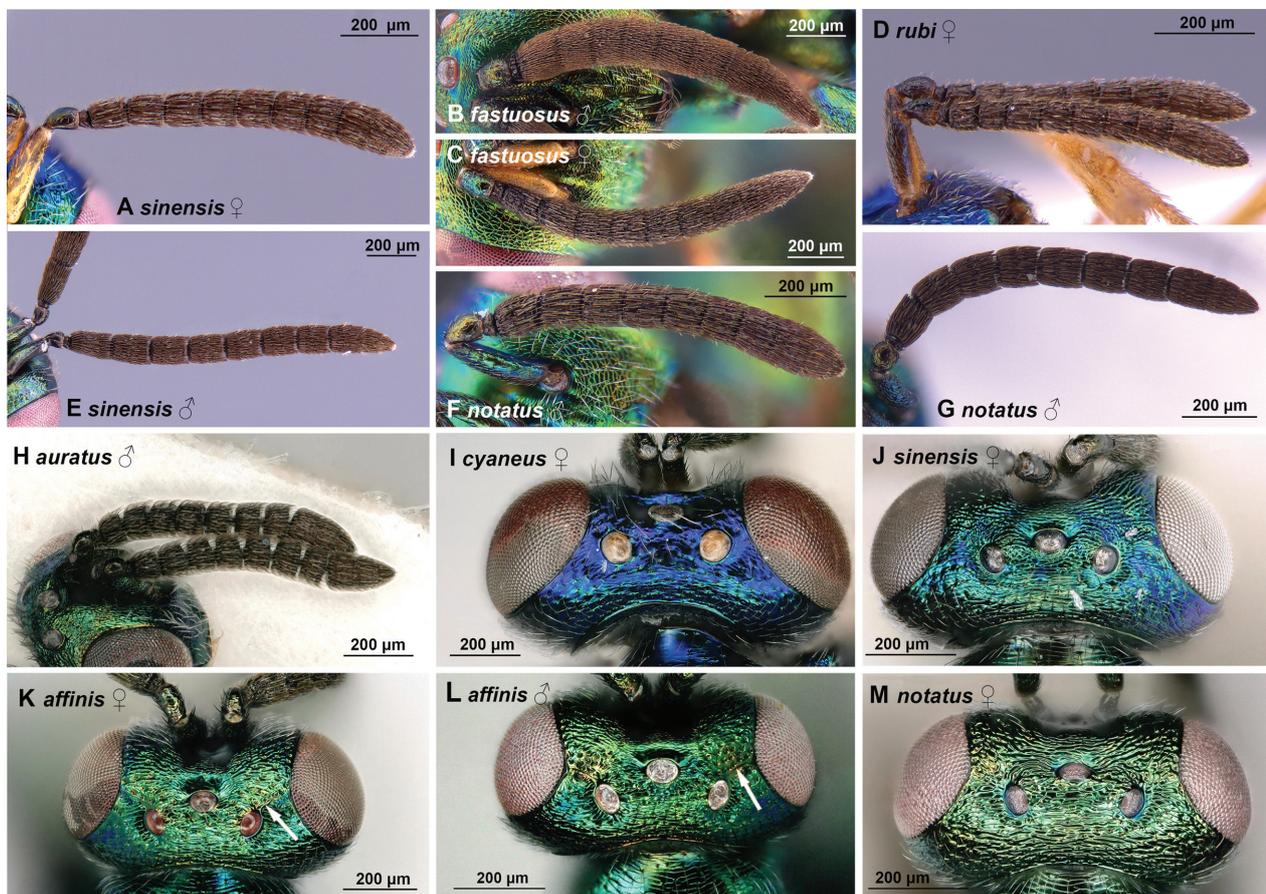

Figure 6. Antenna (**A–H**) and head in dorsal view (**I–M**) in different species of *Torymus*. The arrows point to the sulcus connecting the lateral ocellus with the eye orbit.

7(1) Dorsal edge of hind coxa bare in basal half (Fig. 9B, D)...**8**
–   Dorsal edge of hind coxa with short setae in basal half (Fig. 9E)..**12**
8(6) Gaster with a large, pale, rusty to testaceous subbasal area, especially visible in lateral view (Fig. 11F).........**9**
–   Gaster without a pale subbasal area (Fig. 8F) **10**
9(8) Both sexes: head and mesosoma mainly to entirely dark blue or violet (Fig. 11F). *Female*: ovipositor shorter than body, OI 1.8–2.4 (Fig. 11E) .... ***T. nobilis* Boheman**
–   Both sexes: head and mesosoma greenish, often with extensive coppery and purple iridescence (Fig. 8B). Female: ovipositor longer than body, OI 3.8–4.1 (Fig. 8A) .... ***T. erucarum* (Schrank)**
10(8) Longer spur of hind tibia more than half the length of basitarsus (0.6–0.63×) (Fig. 9C); body entirely bright green to green-blue, with reduced golden iridescence (Fig. 8E, F).....................................***T. flavipes* (Walker)**
–   Longer spur of hind tibia at most half the length of basitarsus (0.3–0.5×) (Fig. 9F); body usually less extensively green, with bronze, copper, purple or violet iridescence (Figs 10F, 11B)...**11**
11(10) Both sexes: Lower mesepimeron large, anterior margin strongly convex (Fig. 9A arrow) and greatest diameter about as long as mid coxa; gaster extensively purplish, with green iridescence confined to basal tergite (Fig. 11A, B). Female: OI 1.8–2.0 ....***T. formosus* (Walker)**
–   Both sexes: Lower mesepimeron smaller, anterior margin almost straight (Fig. 9D arrow) and greatest diameter shorter than mid coxa; gaster mainly bronze-purple, green iridescence sometimes more extensive (Fig. 10E, F). Female: OI 2.7–3 .... ***T. scutellaris* (Walker)**
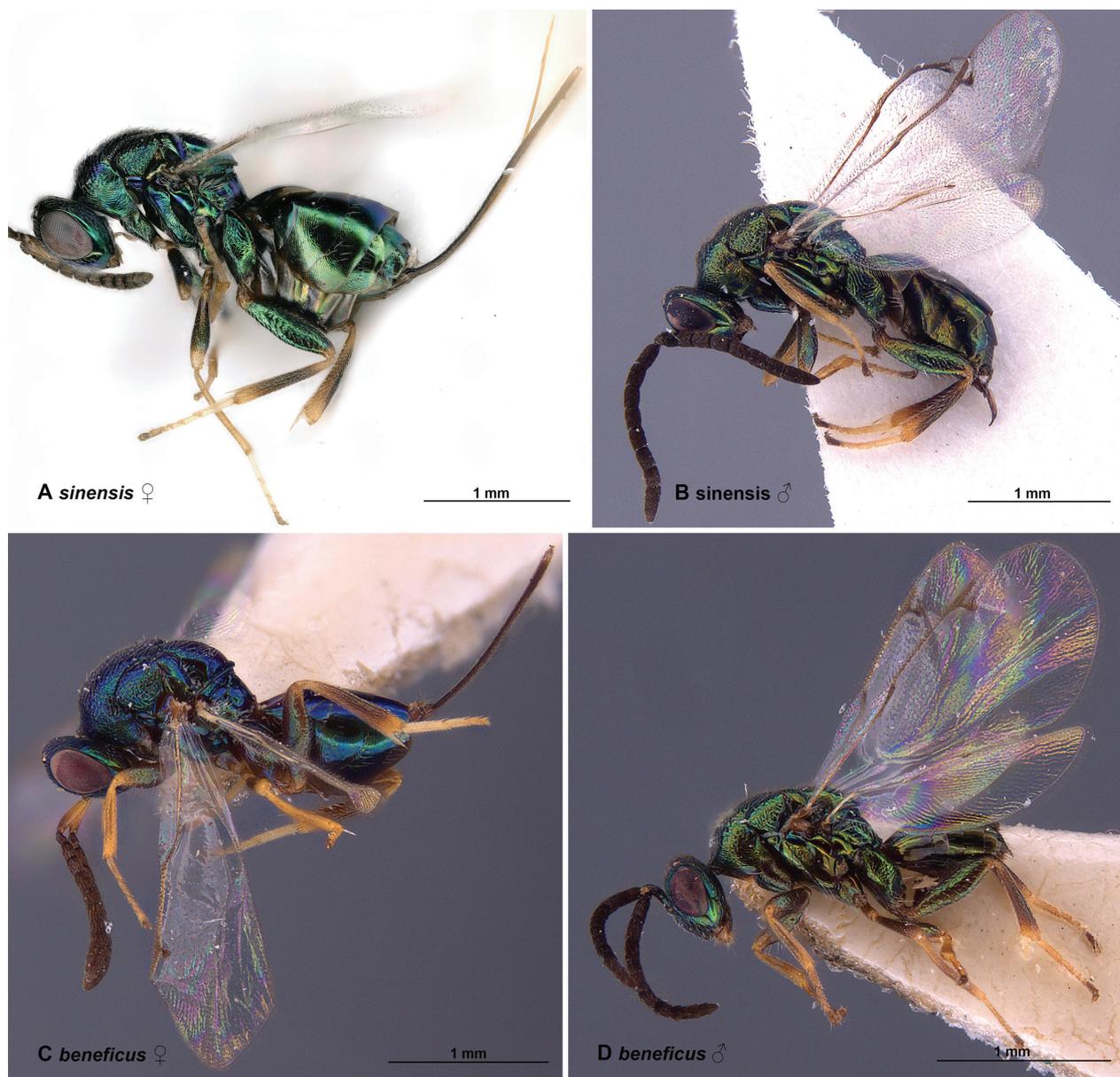

Figure 7. Habitus for *Torymus sinensis* (**A**, **B**) and *T. beneficus* paratypes (**C**, **D**).

12(7) Gaster greenish with violet reflections and pale reddish-yellow subbasal area, mesosoma coppery green (Fig. 11C, D)... ***T. geranii* (Walker)**
– Gaster without pale sub basal area, mesosoma green to bluish-green **13**
13(12) Female: OI 2.1–2.4 (Fig. 10C). Male: at least fu1–fu3 quadrate (Fig. 6D)....... ***T. rubi* (Schrank)** (Fig. 10C, D)
– Female: OI 3.4–4.3 (Fig. 5C). Male: only fu1 quadrate, following funiculars very slightly transverse (Fig. 6H) .... ***T. auratus* (Müller)** (Fig. 5D)
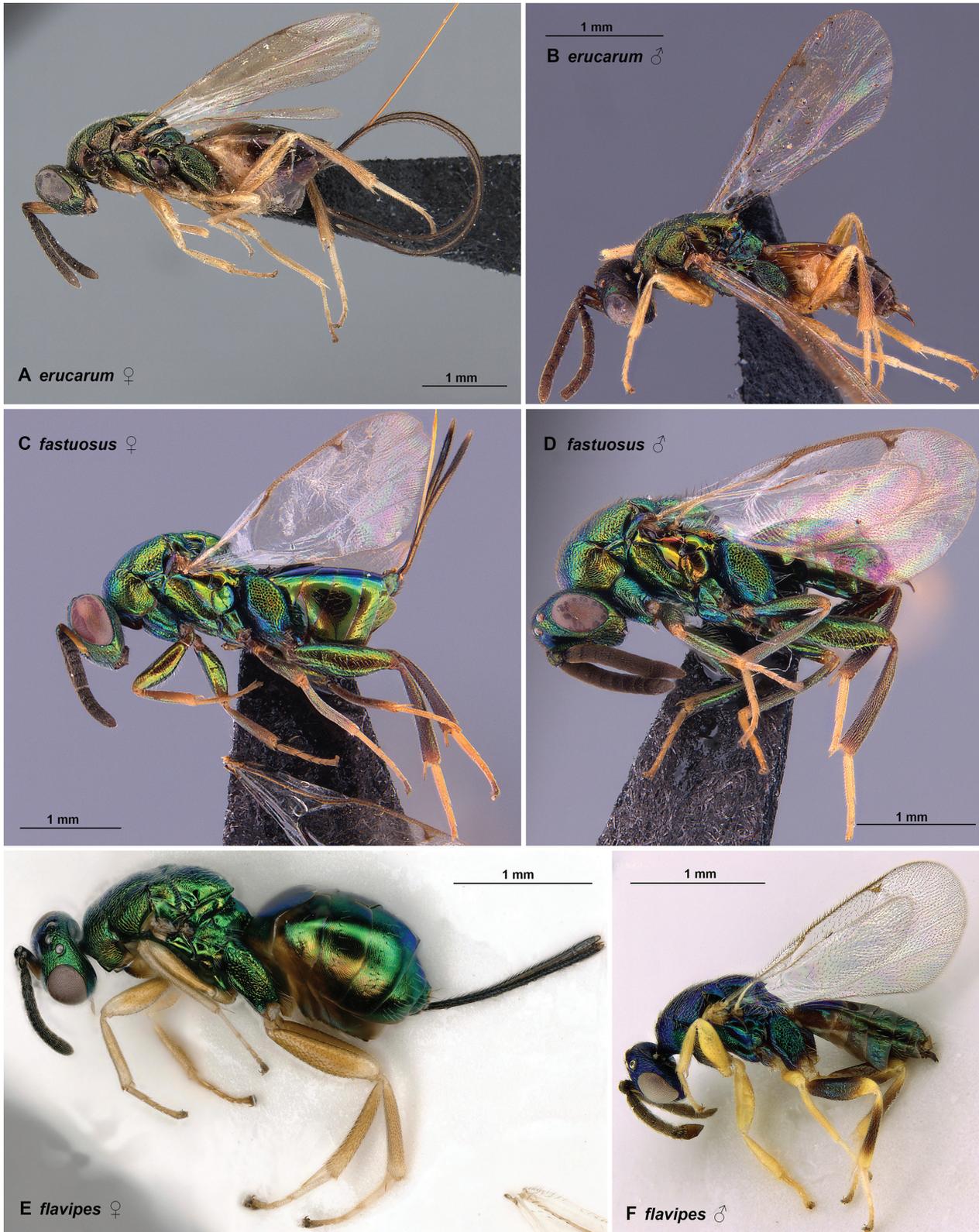
Figure 8. Habitus for *Torymus erucarum* (**A**, **B**), *T. fastuosus* (**C**, **D**) and *T. flavipes* (**E**, **F**).

3.6. An updated characterization of *Torymus sinensis*

Torymus sinensis Kamijo

Chresonymy. *Torymus sinensis* Kamijo, 1982: 505–507. Holotype ♀, Museum of the Institute of Zoology, Academia Sinica, Beijing, not examined. Type locality: Taliuchuang, Tsunhua, Hopei, China. — *Torymus sinensis*; Ôtake 1987: 601–609 (compared with *T. beneficus*, illustrated): Izawa et al. 1992: 58–60 (compared with *T. beneficus* using allozymes); Grissel 1995: 287 (catalogued); Yara et al. 2000: 201–206 (compared with *T. beneficus* using morphology and allozymes); Yara 2004: 428–432 (compared with *T. beneficus* using morphology and DNA); Yara 2006: 18–19 (compared with *T. beneficus* using DNA); Yara and Kunimi 2009: 278–279 (compared with *T. beneficus* using DNA); Quacchia et al. 2014: 107, 110 (interspecific crosses); Ferracini et al. 2017: 447, 449–450 (interspecific crosses); Montagna et al. 2019: 334–341 (compared with *T. beneficus* using DNA); Pogolotti et al. 2019: 5–6 (compared with *Torymus* spp. using DNA); Viviani et al. 2019: 5–7 (compared with *Torymus* spp. using DNA); Matsuo 2020: 403, 408, 433–434 (compared with *T. beneficus*, keyed, illustrated); Matsuo et al. 2021: 457–459 (compared with *T. beneficus* using morphology and DNA); Gil Tapetado et al. 2022: 106–110 (compared with *T. cyaneus* and *T. notatus* using DNA); Viciriuc et al. 2021: 5–13 (compared with

Figure 9. Lower mesepimeron (**A**, **D**), hind coxa (**B**, **E**), spurs of the hind tibia and basitarsus (**C**, **F**) and fore wing (**G**, **H**) in different species of *Torymus*. The arrows point to the anterior margin of the lower mesepimeron.

T. beneficus, taxonomy); Gil-Tapetado et al. 2023: 6–9 (compared with *T. beneficus* and *T. notatus* using DNA, interspecific crosses).

Diagnosis. *Both sexes*: Head (Fig. 12A, B) and mesosoma (Fig. 12E, F) from green to blue, occasionally with coppery reflections; gaster usually concolorous with mesosoma (Fig. 7A, B), sometimes mostly brown with green to blue reflections in smaller specimens, where body colour is generally less metallic and darker; scape yellowish brown, darker apically, sometimes completely darkened. Fore wing hyaline with brown venation (Fig. 12C, D). Coxae and femora from dark brown to concolorous with mesosoma; hind tibia less strongly darkened, usually more or less infuscate but not dark brown, occasionally yellow; fore and mid tibiae mostly yellow. In smaller individuals the body colour is less metallic and darker. Scutellum immediately anterior of the frenal line alutaceous, more strongly sculptured or even strigose on the anterior half, frenum smooth and bare, rarely with weak striae posterolaterally; in some small specimens scutellum almost completely smooth. Propodeum with a pair of distinct and curved submedian carinae (Fig. 12G, H), present irrespective of body size; propodeum with or without smaller additional carinae laterad of the central pair. Female: ovipositor index 1.2–2.7.

Remarks. In this study, we analysed several hundred specimens of *T. sinensis* collected from different geographical areas in both Europe and Asia and the most variable character of European individuals was found to be the body colour.
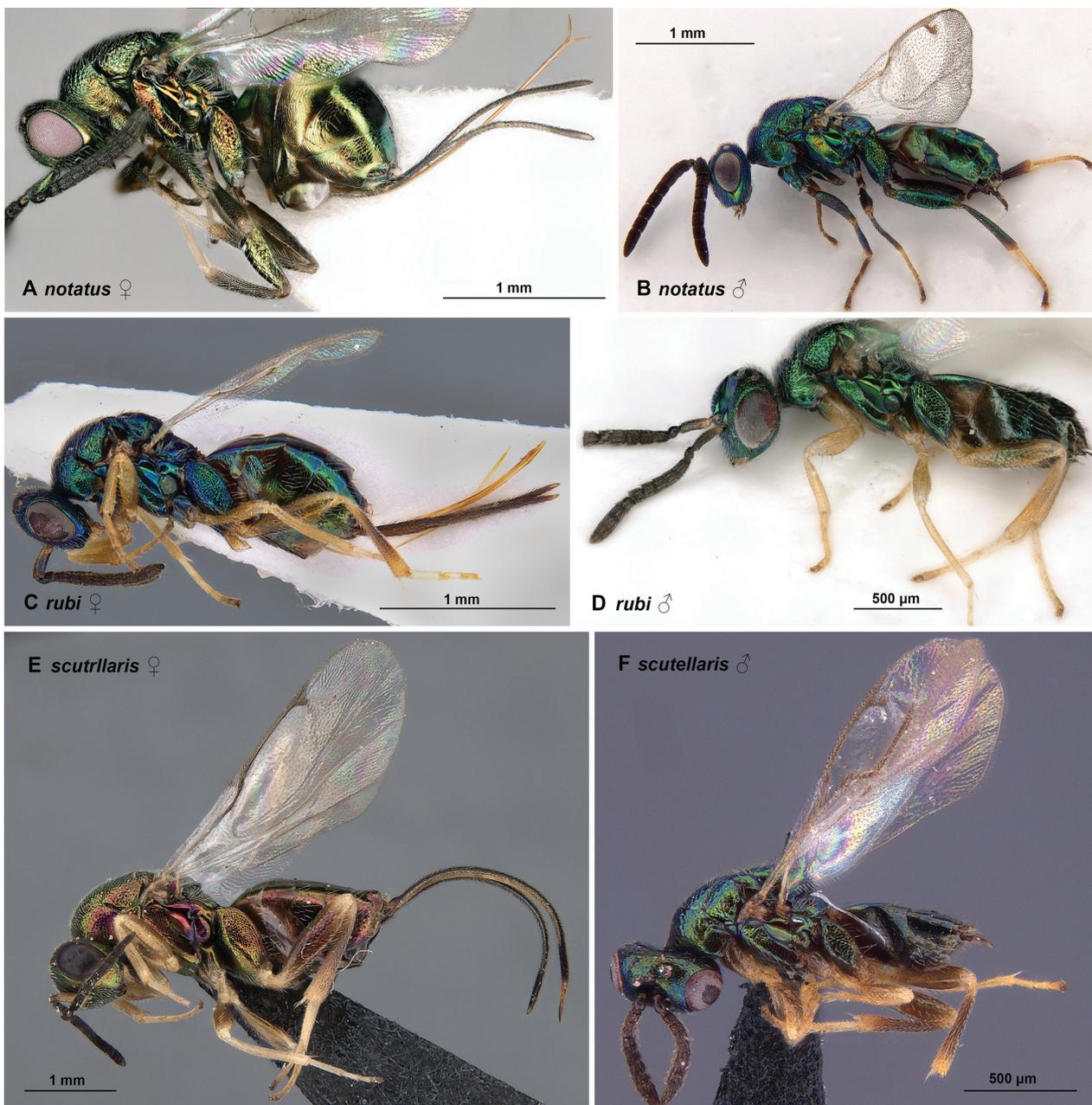
Figure 10. Habitus for *Torymus notatus* (**A**, **B**), *T. rubi* (**C**, **D**) and *T. scutellaris* (**E**, **F**).

Besides the characters given in the key, females of *T. sinensis* differ from those of *T. affinis* that sometimes can have a shorter ovipositor, in having the hind coxa bare in basal half (hind coxa with short setae in basal half in *T. affinis*). From *T. notatus*, besides the more obvious characters used in the key, *T. sinensis* differs in some less obvious ratios obtained in the LDA analysis: width of the first

funicular segment and length of greatest spur for females, and width of the first funicular segment and length of the short spur for males (Table S6).

However, the closest species to *T. sinensis* (Fig. 7A, B) is *T. beneficus* (Fig. 7C, D), which is endemic to Japan. The two can be differentiated using the ratio of antennal flagellum (combined length of anellus, funiculars and cla-
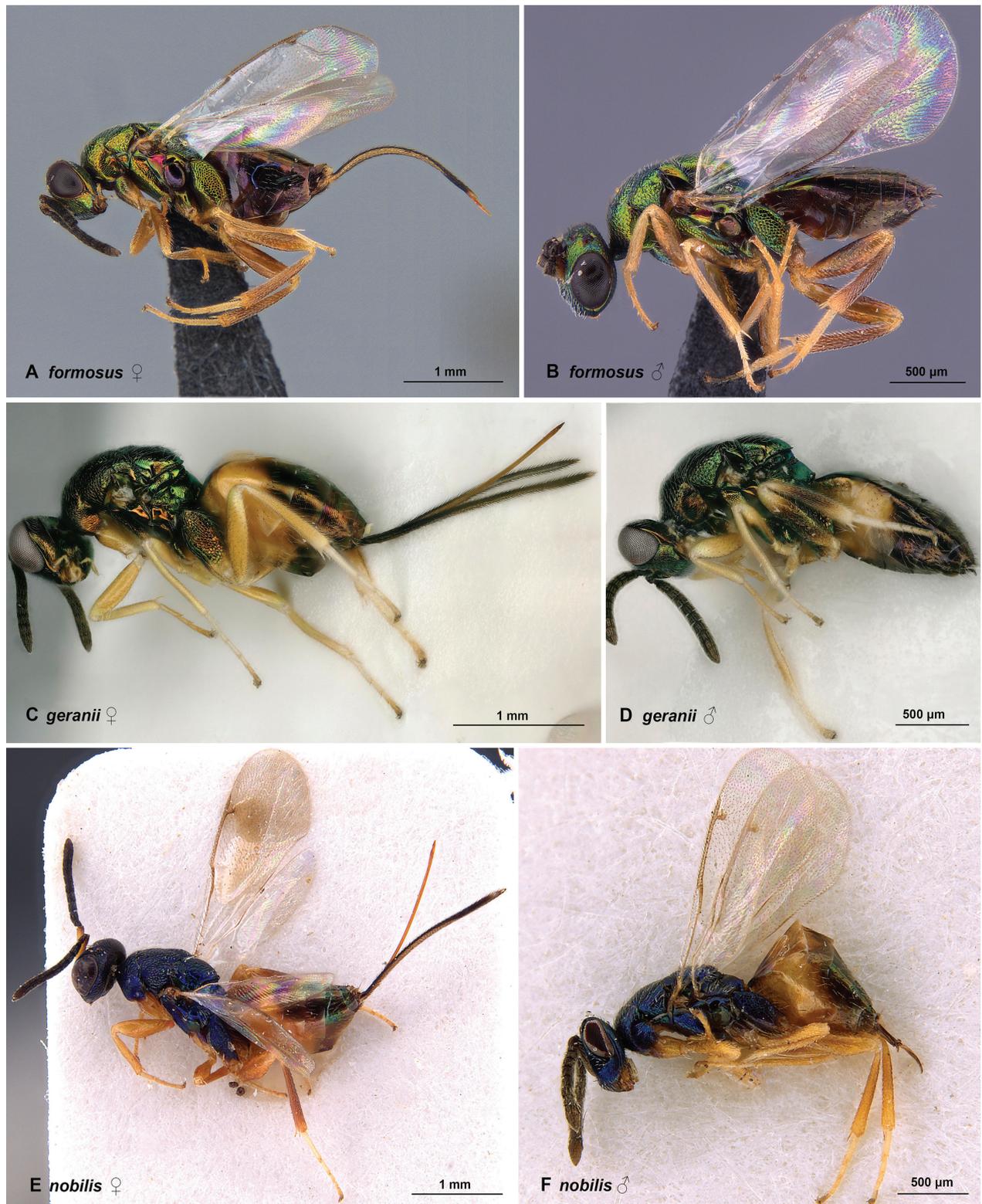
Figure 11. Habitus for *Torymus formosus* (**A**, **B**), *T. geranii* (**C**, **D**) and *T. nobilis* (**E**, **F**).

va) to head width (Kamijo 1982), and ovipositor sheath and hind tibia (Matsuo 2020). Differences in the length of individual funicular segments were also found by Viciriuc et al. (2021), but they failed to find a difference when using the entire length of the flagellum. Presumably this is because it is difficult to accurately measure the antenna which is usually more or less curved in dry mounted specimens. Our new measurements indicate that the ratio given by Kamijo (1982) is accurate. It is 1.22–1.34 in four female paratypes and 1.75–1.77 in four male para-
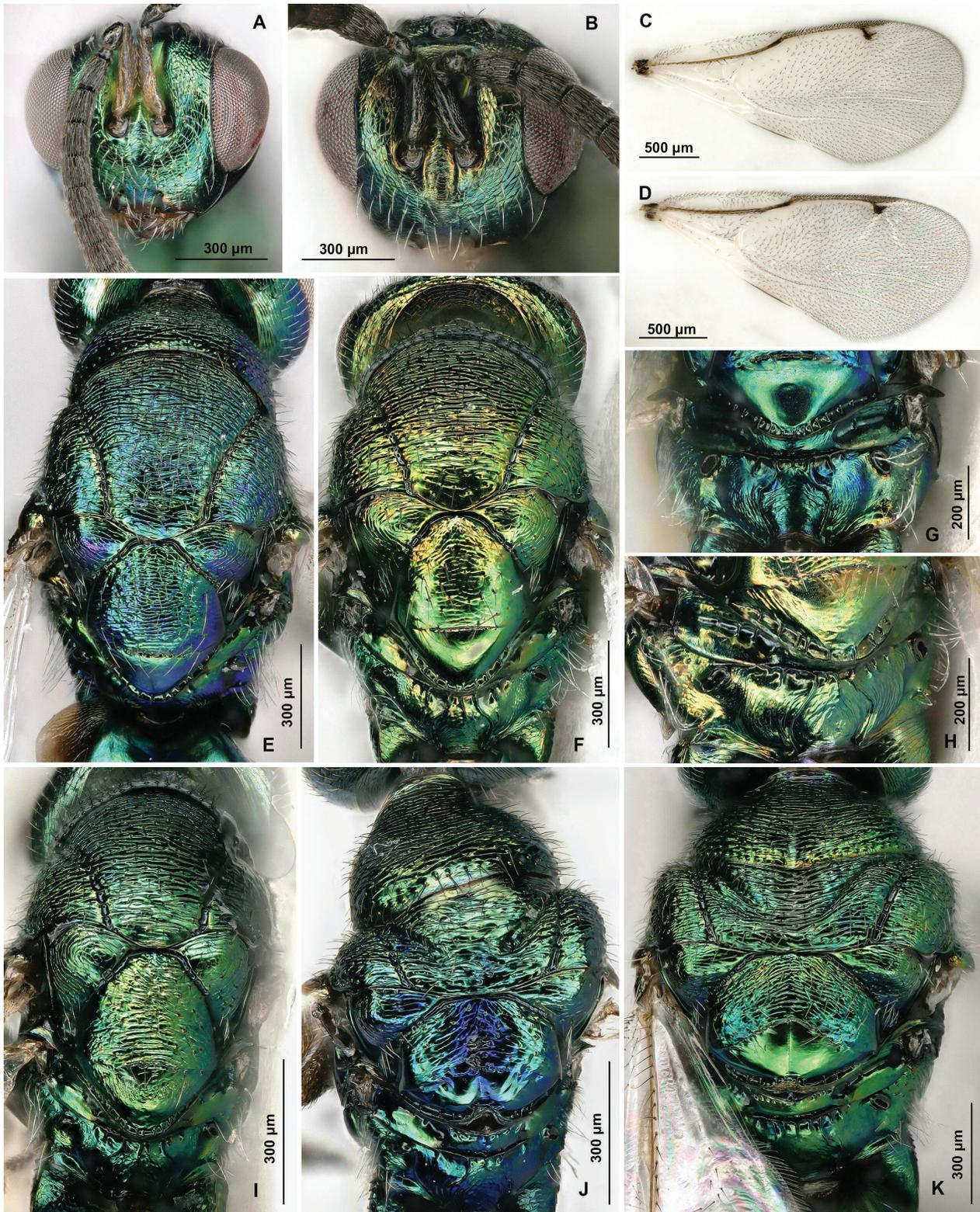
Figure 12. *Torymus sinensis*, head in frontal view: female (**A**), male (**B**); fore wing: female (**C**), male (**D**); mesosoma: female (**E**), male (**F**); propodeum: female (**G**), male (**H**); mesosoma in teratological specimens: male 22204 (**I**), male 28598 (**J**), female 28629 (**K**).

types of *T. beneficus* [repository and label details given in Viciriuc et al. (2021)], and 1.13–1.16 and 1.51–1.69 in eight females and two males of *T. sinensis* from China, respectively. For the European or Japanese specimens this ratio cannot be used because the values for the females correspond to both species (1.17–1.25 in four females from France) and for the males this ratio corresponds to *T. beneficus* (1.64–1.86 in five males). This confirms their intermediate morphology between *T. sinensis* and *T. beneficus* (Viciriuc et al. 2021).

The length of the ovipositor comparatively with the mesosoma ("thorax") cannot be used as originally proposed by Kamijo (1982) when he described *T. sinensis* and used afterwards by many authors following Ôtake (1987). Even if *T. beneficus* has a comparatively shorter ovipositor than *T. sinensis*, the latter species is too variable, including in China, its original distribution area (Yara 2004; Viciriuc et al. 2021). Recently, Matsuo (2020) proposed a separation based on the ovipositor index (the ratio between the length of ovipositor sheath and hind tibia). For the four female paratypes of *T. beneficus* this is 1.61–2.06 and for the seven females of *T. sinensis* from China it is 2.14–2.42. In Europe the interval of the ovipositor index overlaps that of the two species (Viciriuc et al. 2021). Basically, only native *T. sinensis* and *T. beneficus* can be discriminated from each other, but not the specimens used for biological control in Japan or imported from Japan to Europe, due to their intermediate characteristics.

Teratological specimens. During the morphological study of *T. sinensis* collected on *D. kuriphilus* we found a few specimens (two males and a female out of several hundred examined) that have malformations on the mesosoma. If such specimens are found in the future they could be misidentified, especially if the modifications are on the propodeum. The male with the molecular code 22204 (length 2 mm; Fig. 12I), collected in San Lorenzo (France) in 2015, has the central part of the frenum rugose, and the propodeum has two pair of submedian carinae instead of one pair. The male 28598 (2.16 mm; Fig. 12J), collected in St. Georges les Bains (France) in 2016, has teratological modifications in the structure of mesoscutum and mesoscutellum: the mesoscutal median lobe is transverse and has a U-shaped depression; the mesoscutellum has a longitudinal depression along midline and its posterior margin is strongly emarginated. The female 28629 (3.1 mm; Fig. 12K), collected in Prigorieux (France) in 2016, has modifications in the structure of the pronotum, mesoscutum and scutellum: the pronotum has a median groove in its posterior half; the mesoscutal median lobe is transverse and has a U-shaped depression; the frenum has an unusual longitudinal notch; the posterior margin of the mesoscutellum is emarginated.

Biology. *Torymus sinensis* is most frequently reared from galls of *D. kuriphilus* on *Castanea crenata* and *C. molissima* in Asia, *C. dentata* in North America, and *C. sativa* in Europe; with a low incidence it may be reared from other non-target gall wasps (Ferracini et al. 2017; Gil-Tapetado et al. 2023).

The species has one generation per year. In southern France the adults start to emerge in mid-February and emergence continues to early-May, but some individuals emerge from June to August (Borowiec et al. 2018). A diapause as larva of 12 months was identified in Italy in some cases (Picciau et al. 2017).

Distribution. Native to China and introduced to Japan (Murakami et al. 1977), USA (Rieske 2007), Italy (Quacchia et al. 2008), France (Borowiec et al. 2014), Spain (Nieves-Aldrey et al. 2019), Croatia, Hungary, Slovenia (Matošević et al. 2015), Portugal (Amorim et al. 2022) and Turkey (İpekdal et al. 2017).

4.   Discussion

We investigated the phylogenetic relationships between *T. sinensis* and several indigenous species of *Torymus*, that share a similar ecology or morphology, using information from three nuclear and one mitochondrial marker. Our results confirm that *T. notatus*, and to a lesser extent *T. cyaneus*, are the native species that are phylogenetically closest to the "*beneficus-sinensis*" complex, and that the *T. cyaneus* species-group as defined using morphological characters is monophyletic at least concerning the species included in our analysis. The results of our phylogenetic analysis confirm those of Gil-Tapetado et al. (2022), the only notable difference between the two phylogenies being the position of *T. flavipes* and the absence of *T. affinis* from our tree. The same closeness, even overlap, between *T. sinensis* and *T. notatus* is highlighted by the morphometric analyses. Also, the limited available data seem to indicate a vicariant distribution of *T. beneficus* in Japan, *T. sinensis* in China and *T. notatus* in Europe (Graham and Gijswijt 1998). As estimated by Gil-Tapetado et al. (2022) the divergence between *T. sinensis* and *T. notatus* took place 7.9 million years ago (though the 95% highest posterior density credibility interval is rather large).

The large variability within the European *T. sinensis* on *Enolase* (maximum pairwise distance of 1.3%), larger than the divergence between *T. auratus* and *T. geranii* (1.1%) and close to that between *T. sinensis* and *T. notatus* (1.5%) is unexpected. A possible explanation for the presence of such divergent haplotypes is that some of them are the result of introgression, being derived from *T. beneficus*. These most divergent haplotypes were identified in France, with specimens 22158 and 22191 equivalent to haplotype H10, and 21943 to haplotype H7 in Viciriuc et al. (2021). However, because the genetic makeup of *T. beneficus* is unknown, one can only speculate that H10 might originate from this species—H7 is definitely from *T. sinensis* being identified also in China (Viciriuc et al. 2021). Specimen 21943 has a *T. beneficus* mitochondrial haplotype. As in the case of the discovery of mitochondrial haplotypes from *T. beneficus* in France, Italy and Spain (Viciriuc et al. 2021; Gil-Tapetado et al. 2023) or of specimens with shorter ovipositor (Viciriuc et

al. 2021), this does not necessarily imply that *T. beneficus* is present in Europe, only that the European specimens are of hybrid origin, the result of introgression that took place in Japan.

Future interspecific hybridisation in Europe could be favoured by competition for the same host, since this will increase the frequency of interactions between species. In the last years the number of *D. kuriphilus* galls in several areas of France dropped drastically, even where in previous years the pest had a significant impact. This could lead in the first stage to more interspecific competition for the native hosts and, since *T. sinensis* is numerically reduced due to the lack of its specific host, to an increased risk of hybridizing with other close species. The rarity of conspecific individuals increases the frequency of heterospecific crosses (Hubbs 1955, Wirtz 1999). This is not necessarily deleterious for the biological control agent, since it could also increase the fitness of *T. sinensis* (e.g. Lynch 1991). On the other hand, interspecific competition or hybridisation can be a threat for the native populations of *Torymus*.

Five native species parasitizing *D. kuriphilus* have been identified so far in France: *T. auratus*, *T. flavipes*, *T. geranii*, *T. notatus* and *T. rubi*. Of these, *T. rubi* was found to attack *D. kuriphilus* for the first time. Even though the usual host appears to be *Diastrophus rubi* (Bouche) (Cynipidae) this species is not monophagous, being also a parasitoid in galls of other species of Cynipidae (Askew et al. 2006), and also of Cecidomyiidae and Curculionidae (Graham and Gijswijt 1998). Because it is a polyphagous species, and only one individual was identified on *D. kuriphilus*, it is highly likely that this species is not in competition with *T. sinensis* for this host.

Our phylogenetic and morphological data indicate that only *T. notatus*, which can also parasitise *D. kuriphilus*, may have potential for hybridisation with the biological control agent *T. sinensis*. Both species are part of the *cyaneus* group and are also the closest genetically based on all three nuclear markers: *Eno* (1.5%), *Wg* (2%) and *ITS2* (13%), but less so on the mitochondrial marker *COI* (11.5%). However, comparatively, the values of the *p*-distance for the closest species of *Torymus* included in our analyses, *T. auratus* and *T. geranii*, are even smaller: *Eno* (1.1%), *Wg* (0.5%), *ITS2* (3.4%) and *COI* (8.8%). Yet they function as two distinct species after years of coevolution and are not suspected of hybridizing even if they share the same host. The only species that hybridized with *T. sinensis* was *T. beneficus* and this happened in Japan. The genetic distances in this case are known only for *COI* (5.8%) and *ITS2* (0.27%) (Viciriuc et al. 2021) and they are even smaller than for the pair *T. auratus* and *T. geranii*. At the same time, the flight periods for *T. sinensis* and *T. notatus* are almost synchronous, even though the peak of emergence for *T. notatus* is one month earlier than that of *T. sinensis* (Pogolotti et al. 2019).

The other four native species found on *D. kuriphilus* in France cannot be candidates for hybridisation, since they are not part of the same species-group (the *cyaneus* species-group) as *T. sinensis*. On the other hand, *T. fastuosus* was also identified in galls of *D. kuriphilus*; it belongs to the same species-group and is morphologically very close to *T. sinensis* (in the identification key they are placed in the same couplet), but it seems to be a rarer species. If interactions between *T. sinensis* and the indigenous species of *Torymus* were to take place while *T. sinensis* searches to attack a non-target cynipid gall wasp, hybridisation is more likely only in the case of *T. affinis* and *T. cyaneus*, both polyphagous and thus with increased likelihood of encountering *T. sinensis*. However, interaction between these species has been studied (Ferracini et al. 2017, Gil-Tapetado et al. 2023) but only courtship dance with or without antennal contact was observed, without any mating attempts.

Another potential problem could be generated by the numerical reduction of the main host of *T. sinensis*, reported in several European countries. Will in this case *T. sinensis* change its behaviour? Recently at several sites in northern Italy, probably due to a warmer winter, Ferracini et al. (2022), observed a loss of synchronisation between *T. sinensis* and *D. kuriphilus*. This happened because individuals of *T. sinensis* emerged earlier resulting in a lower rate of parasitism than in previous years. This may be the explanation of the different densities of *D. kuriphilus* populations in France, that can be confirmed only after a re-evaluation of the degree of the current impact. On the one hand, this would increase the risk of meeting with indigenous parasitoids, especially with *T. notatus*, and it could also lead to an increase in parasitism of non-target hosts available in the respective period, for example the sexual generations of species of Cynipini.

5. Competing interests

The authors have declared that no competing interests exist.

6. Authors' contributions

Ionela-Mădălina Viciriuc: Conceptualisation, formal analysis, funding acquisition, fieldwork, writing of the original draft. — **Mircea-Dan Mitroiu:** Conceptualisation, writing – review & editing. — **Richard Robinson Askew:** Resources, writing – review & editing. — **Nicolas Ris:** Funding acquisition, fieldwork, writing – review & editing. — **Lucian Fusu:** Conceptualization, formal analysis, writing – original draft. — **Nicolas Borowiec:** Conceptualization, formal analysis, funding acquisition, resources, writing – original draft.

7. Acknowledgements

8. References

Aebi A, Schönenberger N, Melika G, Quacchia A, Alma A, Stone GN (2007) Native introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*. EPPO Bulletin 37(1): 166–171. https://doi.org/10.1111/j.1365-2338.2007.01099.x

Amorim A, Rodrigues R, Nunes LJ, Freitas M, Moura L (2022) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) in Minho (Northern Portugal): Bioecology, Native Parasitoid Communities and Biological Control with *Torymus sinensis* Kamijo (Hymenoptera: Torymidae). Agronomy 12(9): 2184. https://doi.org/10.3390/agronomy12092184

Askew, RR (1965) The biology of the British species of the genus *Torymus* Dalman (Hymenoptera: Torymidae) associated with galls of Cynipidae (Hymenoptera) on oak, with special reference to alternation of forms. Transactions of the Society for British Entomology, 16(9): 217–232.

Askew RR, Plantard O, Gomez JF, Nieves MH, Nieves-Aldrey JL (2006) Catalogue of parasitoids and inquilines in galls of Aylacini, Diplolepidini and Pediaspidini (Hym., Cynipidae) in the West Palaearctic. Zootaxa 1301(1): 1–60. https://doi.org/10.11646/zootaxa.1301.1.1

Battisti A, Benvegnù I, Colombari F, Haack RA (2014) Invasion by the chestnut gall wasp in Italy causes significant yield loss in *Castanea sativa* nut production. Agricultural and Forest Entomology 16(1): 75–79. https://doi.org/10.1111/afe.12036

Baur H, Leuenberger C (2011) Analysis of ratios in multivariate morphometry. Systematic Biology 60(6): 813–825. https://doi.org/10.1093/sysbio/syr061

Baur H, Kranz-Baltensperger Y, Cruaud A, Rasplus J-Y, Timokhov AT, Gokhman VE (2014) Morphometric analysis and taxonomic revision of *Anisopteromalus* Ruschka (Hymenoptera: Chalcidoidea: Pteromalidae) – an integrative approach. Systematic Entomology 39(4): 691–709. https://doi.org/10.1111/syen.12081

Benvenuto C, Cheyppe-Buchmann S, Bermond G, Ris N, Fauvergue X (2012) Intraspecific hybridisation, life history strategies and potential invasion success in a parasitoid wasp. Evolutionary Ecology 26(6): 1311–1329. https://doi.org/10.1007/s10682-011-9553-z

Berner D (2011) Size correction in biology: how reliable are approaches based on (common) principal component analysis?. Oecologia 166: 961–971. https://doi.org/10.1007/s00442-011-1934-z

Bonfield JK, Smith KF, Staden R (1995) A new DNA sequence assembly program. Nucleic Acids Research 23(24): 4992–4999. https://doi.org/10.1093/nar/23.24.4992

Borowiec N, Thaon M, Brancaccio L, Warot S, Vercken E, Fauvergue X, Ris N, Malausa JC (2014) Classical biological control against the chestnut gall wasp *Dryocosmus kuriphilus* (Hym. Cynipidae) in France. Plant Protection Quartely 29(1): 7–10.

Borowiec N, Thaon M, Brancaccio L, Cailleret B, Ris N, Vercken E (2018) Early population dynamics in classical biological control: establishment of the exotic parasitoid *Torymus sinensis* and control of its target pest, the chestnut gall wasp *Dryocosmus kuriphilus*, in France. Entomologia Experimentalis et Applicata 166(5): 367–379. https://doi.org/10.1111/eea.12660

Borowiec N, La Salle J, Brancaccio L, Thaon M, Warot S, Branco M, Ris N, Malausa JC, Burks R (2019) *Ophelimus mediterraneus* sp. n. (Hymenoptera, Eulophidae): A new Eucalyptus gall wasp in the Mediterranean region. Bulletin of Entomological Research 109(5): 678–694. https://doi.org/10.1017/S0007485318001037

Brower AV, DeSalle R (1998) Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of *wingless* as a source of characters for phylogenetic inference. Insects Molecular Biology 7(1): 73–82. https://doi.org/10.1046/j.1365-2583.1998.71052.x

Colombari F, Battisti A (2016) Native and introduced parasitoids in the biocontrol of *Dryocosmus kuriphilus* in Veneto (Italy). EPPO Bulletin 46(2): 275–285. https://doi.org/10.1111/epp.12297

Colombari F, Battisti A (2017) A successful case of classical biological control of a gall wasp. In: Mason PG, Gillespie DR, Vincent C (Eds.) Proceedings of the 5th International Symposium on Biological Control of Arthropods, Langkawi (Malaysia), September 11–15, 2017. CAB International, Wallingford UK, 102–104. https://doi.org/10.1079/9781786394118.0102

Cowan DP (1995) Another method of drying chalcids. Chalcid Forum 18: 4–5. https://www.ars.usda.gov/ARSUserFiles/80420580/cf18.pdf

Davies AP, Takashino K, Watanabe M, Miura K (2009) Parental genetic traits in offspring from inter-specific crosses between introduced and indigenous *Diadegma* Foerster (Hymenoptera: Ichneumonidae): possible effects on conservation genetics. Applied Entomology and Zoology 44(4): 535–541. https://doi.org/10.1303/aez.2009.535

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17: 368–376. https://doi.org/10.1007/BF01734359

Ferracini C, Ferrari E, Pontini M, Nova HLK, Saladini MA, Alma A (2017) Post-release evaluation of the non-target effects of *Torymus sinensis*, a biological control agent of *Dryocosmus kuriphilus* in Italy. BioControl 62: 445–456. https://doi.org/10.1007/s10526-017-9803-2

Ferracini C, Bertolino S, Bernardo U, Bonsignore CP, Faccoli M, Ferrari E, Lupi D, Maini S, Mazzon L, Nugnes F, Rocco A, Santi F, Tavella L (2018) Do *Torymus sinensis* (Hymenoptera: Torymidae) and agroforestry system affect native parasitoids associated with the Asian chestnut gall wasp? Biological Control 121: 36–43. https://doi.org/10.1016/j.biocontrol.2018.01.009

Ferracini C, Pogolotti C, Alma A (2022) A mismatch in the emergence of *Torymus sinensis* may affect the effectiveness of this biocontrol agent? Biological Control 174: 105029. https://doi.org/10.1016/j.biocontrol.2022.105029

Fischer MJ, Brewster CC, Havill NP, Salom SM, Kok LT (2015) Assessment of the potential for hybridisation between *Laricobius nigrinus* (Coleoptera: Derodontidae) and *Laricobius osakensis*, predators of the hemlock woolly adelgid (Hemiptera: Adelgidae). Biocontrol Science and Technology 25(12): 1467–1482. https://doi.org/10.1080/09583157.2015.1061099

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294–299.

Fusu L (2017) An integrative taxonomic study of European *Eupelmus* (*Macroneura*) (Hymenoptera: Chalcidoidea: Eupelmidae), with a molecular and cytogenetic analysis of *Eupelmus* (*Macroneura*) ve-

sicularis: several species hiding under one name for 240 years. Zoological Journal of the Linnean Society 181(3): 519–603. https://doi.org/10.1093/zoolinnean/zlw021

Fusu L, Polaszek A (2017) Description, DNA barcoding and phylogenetic placement of a remarkable new species of *Eopelma* (Hymenoptera: Eupelmidae) from Borneo. Zootaxa 4263(3): 557–566. https://doi.org/10.11646/zootaxa.4263.3.7

Fusu L, Askew RR, Ribes A (2018) Rediscovery of *Calymmochilus russoi* Gibson, 1995 (Hymenoptera, Chalcidoidea, Eupelmidae), and revision of European *Calymmochilus Masi*, 1919. Zootaxa 4504(4): 501–523. https://doi.org/10.11646/zootaxa.4504.4.4

Gebiola M, Monti MM, Johnson RC, Woolley JB, Hunter MS, Giorgini M, Pedata PA (2017) A revision of the *Encarsia pergandiella* species complex (Hymenoptera: Aphelinidae) shows cryptic diversity in parasitoids of whitefly pests. Systematic Entomology 42(1): 31–59. https://doi.org/10.1111/syen.12187

Gehring E, Bellosi B, Quacchia A, Conedera M (2018) Assessing the impact of *Dryocosmus kuriphilus* on the chestnut tree: branch architecture matters. Journal of Pest Science 91: 189–202. https://doi.org/10.1007/s10340-017-0857-9

Gehring E, Bellosi B, Reynaud N, Conedera M (2020) Chestnut tree damage evolution due to *Dryocosmus kuriphilus* attacks. Journal of Pest Science, 93: 103–115. https://doi.org/10.1007/s10340-019-01146-0

Gibson GA (2009) Revision of new world Spalangiinae (Hymenoptera: Pteromalidae). Zootaxa 2259(1): 1–159. https://doi.org/10.11646/zootaxa.2259.1

Gibbs M, Schönrogge K, Alma A, Melika G, Quacchia A, Stone GN, Aebi A (2011) *Torymus sinensis*: a viable management option for the biological control of *Dryocosmus kuriphilus* in Europe?. BioControl 56: 527–538. https://doi.org/10.1007/s10526-011-9364-8

Gibson GA, Fusu L (2016) Revision of the Palaearctic species of *Eupelmus* (*Eupelmus*) Dalman (Hymenoptera: Chalcidoidea: Eupelmidae). Zootaxa 4081(1): 1–331. https://doi.org/10.11646/zootaxa.4081.1.1

Gil-Tapetado D, Cabrero-Sañudo FJ, Gómez JF, Askew RR, Nieves-Aldrey JL (2021) Differences in native and introduced chalcid parasitoid communities recruited by the invasive chestnut pest *Dryocosmus kuriphilus* in two Iberian territories. Bulletin of Entomological Research 111(3): 307–322. https://doi.org/10.1017/S000748532000067X

Gil-Tapetado D, Durán-Montes P, García-París M, López-Estrada EK, Sánchez-Vialas A, Jiménez-Ruiz Y, Gómez JF, Nieves-Aldrey JL (2022) Host specialization is ancestral in *Torymus* (Hymenoptera, Chalcidoidea) cynipid gall parasitoids. Zoologica Scripta, 51(1): 91–118. https://doi.org/10.1111/zsc.12515

Gil-Tapetado D, López-Estrada EK, Ruiz YJ, Cabrero-Sañudo FJ, Gómez JF, Montes PD, Durán Montes P, Rey del Castillo C, Rodríguez-Rojo MP, Polidori C, Nieves-Aldrey JL (2023) *Torymus sinensis* against the invasive chestnut gall wasp: Evaluating the physiological host range and hybridization risks of a classical biological control agent. Biological Control 180: 105187. https://doi.org/10.1016/j.biocontrol.2023.105187

Graham MWRV, Gijswijt MJ (1998) Revision of the European species of *Torymus* Dalman (s. lat.) (Hymenoptera: Torymidae). Zoologische Verhandelingen 317: 1–202.

Grissell EE (1995) Toryminae (Hymenoptera: Chalcidoidea:Torymidae): a redefinition, generic classification and annotated world catalogue of species. Memoirs of Entomology International 2: 1–474.

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160–174. https://doi.org/10.1007/BF02101694

Havill NP, Davis G, Mausel DL, Klein J, McDonald R, Jones C, Melissa F, Scott S, Caccone A (2012) Hybridisation between a native and introduced predator of Adelgidae: An unintended result of classical biological control. Biological Control 63(3): 359–369. https://doi.org/10.1016/j.biocontrol.2012.08.001

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. Proceedings. Biological sciences 270(1512): 313–321. https://doi.org/10.1098/rspb.2002.2218

Heraty J, Hawks D (1998) Hexamethyldisilazane: A chemical alternative for drying insects. Entomological news 109: 369–374.

Hubbs CL (1955) Hybridisation between Fish Species in Nature. Systematic Zoology 4(1): 1–20. https://doi.org/10.2307/2411933

Izawa H, Osakabe M, Moriya S (1992) Isozyme discrimination between an imported parasitoid wasp, *Torymus sinensis* Kamijo and its sibling species, *T. beneficus* Yasumatsu et Kamijo (Hymenoptera: Torymidae) attacking *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). Japanese Journal of Applied Entomology and Zoology 36(1): 58–60.

İpekdal K, Emin A, Kuzucu AŞ, Karadağ M, Koçluk M, Açıcı Ö, Şah S, Aksu Y, Colombari F (2017) Rearing and releasing *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), larval parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae). Türkiye Entomoloji Bülteni 7(2): 113–129.

Janšta P, Vilímová J, Hanson P (2011) Description of a new genus, *Boucekinus* (Hymenoptera: Chalcidoidea: Torymidae), with two new species and a discussion of its placement. Zootaxa 2762(1): 49–55. https://doi.org/10.11646/zootaxa.2762.1.4

Janšta P, Křížková B, Vilímová J, Rasplus JY (2013) Description of a new genus, *Chileana* (Hymenoptera: Chalcidoidea: Torymidae), with four new species. Zootaxa 3745(1): 49–63. https://doi.org/10.11646/zootaxa.3745.1.4

Janšta P, Cruaud A, Delvare G, Genson G, Heraty J, Křížková B, Rasplus JY (2018) Torymidae (Hymenoptera, Chalcidoidea) revised: molecular phylogeny, circumscription and reclassification of the family with discussion of its biogeography and evolution of life-history traits. Cladistics 34(6): 627–651. https://doi.org/10.1111/cla.12228

Kainer D, Lanfear R (2015) The effects of partitioning on phylogenetic inference. Molecular Biology and Evolution 32(6): 1611–1627. https://doi.org/10.1093/molbev/msv026

Kaartinen R, Stone GN, Hearn J, Lohse K, Roslin T (2010) Revealing secret liaisons: DNA barcoding changes our understanding of food webs. Ecological Entomology 35(5): 623–638. https://doi.org/10.1111/j.1365-2311.2010.01224.x

Kamijo K (1982) Two new species of *Torymus* (Hymenoptera, Torymidae) reared from *Dryocosmus kuriphilus* (Hymenoptera, Cynipidae) in China and Korea. Kontyû, Tokyo 50(4): 505–510.

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. https://doi.org/10.1093/molbev/mst010

Kenis M, Roques A, Santini A, Liebhold AM (2017) Impact of non-native invertebrates and pathogens on market forest tree resources. In: Vilà M, Hulme PE (Eds) Impact of biological invasions on ecosystem services. Springer, Cham, 103–117.

Kenis M, Hurley BP, Colombari F, Lawson S, Sun J, Wilcken C, Weeks R, Sathyapala S (2019) Guide to the classical biological control of insect pests in planted and natural forests. FAO, Rome, 113pp.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–20. https://doi.org/10.1007/BF01731581

Kimura M (1981) Estimation of evolutionary distances between homologous nucleotide sequences. Proceedings of the National Academy of Sciences 78(1): 454–458. https://doi.org/10.1073/pnas.78.1.454

Kos K, Kriston E, Melika G (2015) Invasive chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), its native parasitoid community and association with oak gall wasps in Slovenia. European Journal of Entomology 112(4): 698–704. https://doi:10.14411/eje.2015.091

Kos K, Lacković N, Melika G, Matošević D (2021) Diversity and surge in abundance of native parasitoid communities prior to the onset of *Torymus sinensis* on the Asian chestnut gall wasp (*Dryocosmus kuriphilus*) in Slovenia, Croatia and Hungary. Journal of Forestry Research 32: 1327–1336. https://doi.org/10.1007/s11676-020-01197-5

Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A (2019) RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics 35: 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Lanfear R, Calcott B, Ho SY, Guindon S (2012) PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29(6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772–773. https://doi.org/10.1093/molbev/msw260

László Z, Tóthmérész B (2013) The enemy hypothesis: Correlates of gall morphology with parasitoid attack rates in two closely related rose cynipid galls. Bulletin of Entomological Research 103(3): 326–335. https://doi.org/10.1017/S0007485312000764

László Z, Lakatos KT, Denes AL (2021) A new species of *Mesopolobus* (Hymenoptera, Pteromalidae) from black locust crops. European Journal of Taxonomy 740: 118–137. https://doi.org/10.5852/ejt.2021.740.1285

Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. Evolution 45(3): 622–629. https://doi.org/10.1111/j.1558-5646.1991.tb04333.x

Mathenge CW, Holford P, Hoffmann JH, Zimmermann HG, Spooner-Hart RN, Beattie GAC (2010) Hybridisation between *Dactylopius tomentosus* (Hemiptera: Dactylopiidae) biotypes and its effects on host specificity. Bulletin of Entomological Research 100(3): 331–338. https://doi.org/10.1017/S0007485309990344

Matošević D, Lacković N, Melika G, Kos K, Franić I, Kriston É, Bozsó M, Seljak G, Rot M (2015) Biological control of invasive *Dryocosmus kuriphilus* with introduced parasitoid *Torymus sinensis* in Croatia, Slovenia and Hungary. Periodicum Biologorum 117(4): 471–477. https://doi.org/10.18054/pb.2015.117.4.3445

Matošević D, Lacković N, Kos K, Kriston E, Melika G, Rot M, Pernek M (2017). Success of classical biocontrol agent *Torymus sinensis* within its expanding range in Europe. Journal of Applied Entomology 141(9): 758–767. https://doi.org/10.1111/jen.12388

Matsuo K (2020) A revision of Japanese *Torymus* Dalman (Hymenoptera: Torymidae). Zootaxa 4758(3): 401–441. https://doi.org/10.11646/zootaxa.4758.3.1

Matsuo K, Ide T, Yara K, Matsunaga K, Gyoutoku N, Higashiura Y, Yukawa J, Abe Y, Moriya S (2021) Discovery of a native host of

Torymus beneficus (Hymenoptera: Torymidae), with description of a new *Dryocosmus* (Hymenoptera: Cynipidae). Applied Entomology and Zoology 56: 451–463. https://doi.org/10.1007/s13355-021-00752-8

Marcolin E, Pividori M, Colombari F, Manetti MC, Pelleri F, Conedera M, Gehring E (2021) Impact of the Asian gall wasp *Dryocosmus kuriphilus* on the radial growth of the European chestnut *Castanea sativa*. Journal of Applied Ecology 58(6): 1212–1224. https://doi.org/10.3389/ffgc.2022.1095185

Montagna M, Gonella E, Mereghetti V, Magoga G, Ferrari E, Pontini M, Ferracini C, Alma A (2019) Molecular species delimitation of the Asian chestnut gall wasp biocontrol agent released in Italy. Insect Systematics & Evolution 50(3): 327–345. https://doi.org/10.1163/1876312X-00002188

Moriya S, Inoue K, Ôtake A, Shiga M, Mabuchi M (1989) Decline of the chestnut gall wasp population, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) after the establishment of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae). Applied Entomology and Zoology 24(2): 231–233. https://doi.org/10.1303/aez.24.231

Moriya S, Inoue K, Shiga M, Mabuchi M (1992) Interspecific relationship between an introduced parasitoid, *Torymus sinensis* Kamijo, as a biological control agent of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, and an endemic parasitoid, *T. beneficus* Yasumatsu et Kamijo. Acta Phytopathologica et Entomologica Hungarica 27: 479–483.

Moriya S, Shiga M, Adachi I (2003) Classical biological control of the chestnut gall wasp in Japan. In: VanDriesche RG (Ed.) Proceedings of the 1st International Symposium on Biological Control of Arthropodes, Honolulu (Hawaii), January 2002. USDA Forest Service, Washington, 407–415.

Murakami Y, Umeya K, Ono N (1977) A preliminary introduction and release of a parasitoid (Chalcidoidea, Torymidae) of the chestnut gall wasp, *Drycosmus kuriphilus* Yasumatsu (Cynipidae) from China. Japanese Journal of Applied Entomology and Zoology 21: 197–203.

Murakami Y (1988) Ecotypes of *Torymus* (*Syntomaspis*) *beneficus* Yasumatsu et Kamijo (Hymenoptera: Torymidae) with different seasonal prevalence of adult emergence. Applied Entomology and Zoology 23(1): 81–87. https://doi.org/10.1303/aez.23.81

Murakami Y (1997) Natural Enemies of the Chestnut Gall Wasp. Approaches to Biological Control. Kyushu University Press, Fukuoka, Japan, 308 pp.

Muru D, Borowiec N, Thaon M, Ris N, Viciriuc IM, Warot S, Vercken E (2021) The open bar is closed: restructuration of a native parasitoid community following successful control of an invasive pest. Peer Community Journal 1: e30. https://peercommunityjournal.org/articles/10.24072/pcjournal.43

Naka H, Mitsunaga T, Mochizuki A (2005) Laboratory hybridisation between the introduced and the indigenous green lacewings (Neuroptera: Chrysopidae: Chrysoperla) in Japan. Environmental Entomology 34(3): 727–731. https://doi.org/10.1603/0046-225X-34.3.727

Naka H, Haruyama N, Ito K, Mitsunaga T, Nomura M, Mochizuki A (2006) Interspecific hybridisation between introduced and indigenous green lacewings (Neurop., Chrysopidae: Chrysoperla) at different adult densities. Journal of Applied Entomology 130(8): 426–428. https://doi.org/10.1111/j.1439-0418.2006.01084.x

Nieves-Aldrey JL, Gil-Tapetado D, Gavira ON, Boyero JR, Polidori C, Blanco D, Rey del Castillo C, Rodríguez-Rojo MP, Wong ME, Vela JM, Lombardero MJ (2019) *Torymus sinensis* Kamijo, a biocontrol agent against the invasive chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu in Spain: its natural dispersal from France and the first

data on establishment after experimental releases. Forest Systems 28(1): e001. https://doi.org/10.5424/fs/2019281-14361

Noyes JS (2019) Universal Chalcidoidea Database. World Wide Web electronic publication. https://www.nhm.ac.uk/our-science/data/chalcidoids/database

Ôtake A (1987) Comparison of some morphological characters among two strains of *Torymus beneficus* Yasumatsu et Kamijo and *T. sinensis* Kamijo (Hymenoptera: Torymidae). Applied Entomology and Zoology 22(4): 600–609. https://doi.org/10.1303/aez.22.600

Ôtake A, Moriya S, Shiga M (1984) Colonization of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae), a parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), introduced from China. Applied Entomology and Zoology 19(1): 111–114. https://doi.org/10.1303/aez.19.111

Payne JA, Menke AS, Schroeder PM (1975) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), an oriental chestnut gall wasp in North America. USDA Cooperative Economic Insect Report 25: 903–905.

Picciau L, Ferracini C, Alma A (2017) Reproductive traits in *Torymus sinensis*, biocontrol agent of the Asian chestnut gall wasp: implications for biological control success. Bulletin of Insectology 70(1): 49–55.

Pogolotti C, Cuesta-Porta V, Pujade-Villar J, Ferracini C (2019) Seasonal flight activity and genetic relatedness of *Torymus* species in Italy. Agricultural and Forest Entomology 21(2): 159–167. https://doi.org/10.1111/afe.12316

Posada D (2003) Using MODELTEST and PAUP* to select a model of nucleotide substitution. Current Protocols in Bioinformatics 1: 6.5.1–6.5.14. https://doi.org/10.1002/0471250953.bi0605s00

Prebus MM (2021) Phylogenomic species delimitation in the ants of the *Temnothorax salvini* group (Hymenoptera: Formicidae): an integrative approach. Systematic Entomology 46(2): 307–326. https://doi.org/10.1111/syen.12463

Quacchia A, Moriya S, Bosio G, Scapin I, Alma A (2008) Rearing, release and settlement prospect in Italy of *Torymus sinensis*, the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*. BioControl 53: 829–839. https://doi.org/10.1007/s10526-007-9139-4

Quacchia A, Ferracini C, Nicholls JA, Piazza E, Saladini MA, Tota F, Melika G, Alma A (2012) Chalcid parasitoid community associated with the invading pest *Dryocosmus kuriphilus* in north-western Italy. Insect Conservation and Diversity 6(2): 114–123. https://doi.org/10.1111/j.1752-4598.2012.00192.x

Quacchia A, Bosio G, Moriya S (2014) Effectiveness of *Torymus sinensis* in the Biological Control of *Dryocosmus kuriphilus* in Italy. Acta Horticulturae 1043: 199–204. https://doi.org/10.17660/ActaHortic.2014.1043.26

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67(5): 901–904. https://doi.org/10.1093/sysbio/syy032

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria: https://www.R-project.org.

Rieske LK (2007) Success of an exotic gall maker, *Dryocosmus kuriphilus*, on chestnut in the USA: a historical account. EPPO Bulletin 37(1): 172–174. https://doi.org/10.1111/j.1365-2338.2007.01100.x

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice

across a large model space. Systematic Biology 61(3): 539–542. https://doi.org/10.1093/sysbio/sys029

Schindelin J, Rueden CT, Hiner MC, Eliceiri KW (2015) The ImageJ ecosystem: An open platform for biomedical image analysis. Molecular Reproduction & Development 82(7–8): 518–529. https://doi.org/10.1002/mrd.22489

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87(6): 651–701. https://doi.org/10.1093/aesa/87.6.651

Speranza S, Stacchiotti M, Paparatti B (2009) Endemic parasitoids of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cinipidae) in Central Italy. Acta Horticulturae 844: 421–424. https://doi.org/10.17660/ActaHortic.2009.844.58

Talamas EJ, Buffington ML, Hoelmer K (2017) Revision of Palearctic *Trissolcus* Ashmead (Hymenoptera, Scelionidae). Journal of Hymenoptera Research 56: 3–185. https://doi.org/10.3897/jhr.56.10158

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10(3): 512–526. https://doi.org/10.1093/oxfordjournals.molbev.a040023

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA 6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22(22): 4673–4680. https://doi.org/10.1093/nar/22.22.4673

Triapitsyn SV, Andreason SA, Power N, Ganjisaffar F, Fusu L, Dominguez C, Perring TM (2020) Two new species of *Ooencyrtus* (Hymenoptera, Encyrtidae), egg parasitoids of the bagrada bug *Bagrada hilaris* (Hemiptera, Pentatomidae), with taxonomic notes on *Ooencyrtus telenomicida*. Journal of Hymenoptera Research 76: 57–98. https://doi.org/10.3897/jhr.76.48004

Viciriuc IM, Thaon M, Moriya S, Warot S, Zhang J, Aebi A, Nicolas R, Fusu L, Borowiec N (2021) Contribution of integrative taxonomy to tracking interspecific hybridisations between the biological control agent *Torymus sinensis* and its related taxa. Systematic Entomology 46(4): 839–855. https://doi.org/10.1111/syen.12493

Viviani A, Bernardi R, Cavallini A, Rossi E (2019) Genotypic Characterization of *Torymus sinensis* (Hymenoptera: Torymidae) after its Introduction in Tuscany (Italy) for the biological control of *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae). Journal of Insect Science 19(4): 1–8. https://doi.org/10.1093/jisesa/iez080

Wirtz P (1999) Mother species-father species: unidirectional hybridisation in animals with female choice. Animal Behaviour 58(1): 1–12. https://doi.org/10.1006/anbe.1999.1144

Yara K (2004) Relationship between the introduced and indigenous parasitoids *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae) as inferred from mt-DNA (COI) sequences. Applied Entomology and Zoology 39(3): 427–433. https://doi.org/10.1303/aez.2004.427

Yara K (2006) Identification of *Torymus sinensis* and *T. beneficus* (Hymenoptera: Torymidae), introduced and indigenous parasitoids of the chestnut gall wasp *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), using the ribosomal ITS2 region. Biological Control 36(1): 15–21. https://doi.org/10.1016/j.biocontrol.2005.08.003

Yara K, Yano E, Sasawaki T, Shiga M (2000) Detection of hybrids between introduced *Torymus sinensis* and native *T. beneficus* (Hymenoptera: Torymidae) in central Japan, using malic enzyme. Applied Entomology and Zoology 35(2): 201–206. https://doi.org/10.1303/aez.2000.201

Yara K, Sasawaki T, Kunimi Y (2007) Displacement of *Torymus beneficus* (Hymenoptera: Torymidae) by *T. sinensis*, an indigenous and introduced parasitoid of the chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae), in Japanese chestnut fields: possible involvement in hybridisation. Biological Control 42(2): 148–154. https://doi.org/10.1016/j.biocontrol.2007.04.017

Yara K, Kunimi Y (2009) Discrimination of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) and *T. beneficus* Yasumatsu et Kamijo and their hybrids by allele-specific PCR. Applied Entomology and Zoology 44(2): 275–280. https://doi.org/10.1303/aez.2009.275

Supplementary Material 1

Table S1

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .xls
Explanation note: Collection information for *Torymus* species used in this study and GenBank accession numbers.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/asp.81.e98141.suppl1

Supplementary Material 2

Table S2

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: The values and abbreviations of the measured characters for females of *Torymus* species.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/asp.81.e98141.suppl2

Supplementary Material 3

Table S3

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: The values and abbreviations of the measured characters for males of *Torymus* species.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/asp.81.e98141.suppl3

Supplementary Material 4

Table S4

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: Collection information for *Torymus erucarum*, *T. fastuosus*, *T. formosus*, *T. nobilis* and *T. scutellaris*.
Link: https://doi.org/10.3897/asp.81.e98141.suppl4

Supplementary Material 5

Table S5A–D

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: Pairwise distance matrix (*p*-distance, %) for *Cytochrome c oxidase I* (**A**), *Wingless* (**B**), *Enolase* (**C**) and *ITS2* (**D**).
Link: https://doi.org/10.3897/asp.81.e98141.suppl5

Supplementary Material 6

Table S6

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: Best ratios found by the LDA ratio extractor for separating females and males of *T. sinensis* and the close species of *Torymus*.
Link: https://doi.org/10.3897/asp.81.e98141.suppl6

Supplementary Material 7

Figure S1

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: *COI* phylogenetic tree inferred in RAxML-NG. mtDNA Tb – individuals with mitochondrial DNA derived from *Torymus beneficus*.
Link: https://doi.org/10.3897/asp.81.e98141.suppl7

Supplementary Material 8

Figure S2

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: *Wingless* phylogenetic tree inferred in RAxML-NG. mtDNA Tb – individuals with mitochondrial DNA derived from *Torymus beneficus*.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/asp.81.e98141.suppl8

Supplementary Material 9

Figure S3

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: *Enolase* phylogenetic tree inferred in RAxML-NG. mtDNA Tb – individuals with mitochondrial DNA derived from *Torymus beneficus*.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/asp.81.e98141.suppl9

Supplementary Material 10

Figure S4

Authors: Viciriuc I-M, Mitroiu M-D, Askew RR, Ris N, Fusu L, Borowiec N (2023)
Data type: .pdf
Explanation note: *ITS2* phylogenetic tree inferred in RAxML-NG. mtDNA Tb – individuals with mitochondrial DNA derived from *T. beneficus*.
Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.
Link: https://doi.org/10.3897/asp.81.e98141.suppl10