

Description of *Synergus castaneus* n. sp. (Hymenoptera: Cynipidae: Synergini) Associated With an Unknown Gall on *Castanea* spp. (Fagaceae) in China

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ABSTRACT A species of the tribe Synergini (Hymenoptera: Cynipidae) was reared from a gall collected in China on *Castanea mollissima* Blume and *Castanea seguinii* Dode. The morphological features of this gall wasp indicate that it is an undescribed species belonging to the genus *Synergus* and is herein described as a new species, *Synergus castaneus* Pujade-Villar, Bernardo et Viggiani sp. nov. (Hymenoptera Cynipidae: Synergini). This is the first known instance of a species of *Synergus* emerging from *Castanea* galls (Fagaceae). Data on the diagnosis, distribution, and biology of the new species are given. Morphological features of this species are discussed and illustrated. No other known gall-inducing species emerged from the collected galls, whereas tens of specimens of parasitoids and adults of the inquiline micromoth *Andrioplecta pulverula* Meyrick (Lepidoptera: Tortricidae) did. 28S-D2 and COI sequences confirmed that *S. castaneus* is a distinct species belonging to the genus *Synergus*.

KEY WORDS *Andrioplecta pulverula*, Cynipidae, *Dryocosmus kuriphilus*, gall wasp, new species

During a survey of parasitoids of *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) in *Castanea* fruit orchards in China, several woody galls were collected on *Castanea mollissima* Blume and *Castanea seguinii* Dode (Fagaceae). From them emerged many specimens of an undescribed cynipid species belonging to the tribe Synergini (Hymenoptera: Cynipidae). This tribe includes all of the cynipids developing as inquilines within the galls induced by gall wasps belonging to the tribes Cynipini (on Fagaceae in Holarctic and Neotropical areas and on sumac plants in South Africa), Diplolepidini (on *Rosa* spp. in the Holarctic area), and Aylacini (on *Rubus* spp. in the Nearctic area and in Japan) (Ronquist and Liljeblad 2001, Melika et al. 2006).

The genus *Synergus* is present in the Holarctic and Neotropical regions. According to Melika (2006) and Abe et al. (2007), it includes 43 species in the Palae-

arctic and Oriental areas, and species are mainly inquilines in oak galls (*Quercus*). Four species of *Synergus* have been reported from the Eastern Palaearctic area (Abe et al. 2007): *Synergus chinensis* (Melika, Ács, and Bechtold, 2004), *Synergus gifuensis* (Ashmead, 1904), *Synergus japonicus* (Walker, 1874), and *Synergus xialongmeni* (Melika, Ács, and Bechtold, 2004); and five species of *Synergus* described from Japan are considered of uncertain status (Abe et al. 2007).

To date, *D. kuriphilus* (Asian chestnut gall wasp) is the only known gall-maker wasp native of the Palaearctic region affecting *Castanea* spp. Despite Asian chestnut gall wasp being a widely collected species, only a single female of the inquiline *Synergus* sp. was obtained in Japan from its galls (Ôtake et al. 1982). This record was recompiled in Aebi et al. (2006), but it was omitted in Abe et al. (2007), probably because the authors considered the record unreliable.

The aim of this study is to describe a new species of *Synergus* collected on *Castanea* spp., together with the galls from which it emerges, and to provide preliminary data on its life cycle.

Materials and Methods

In September 2011 and late March 2012, several galls were collected from *C. mollissima* and *C. seguinii* in Jingzhai, in the Anhui Province of China. Further observations were carried out at the same locality during the spring of 2012 to check for the development of new galls. Managed chestnut trees (predominantly

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Table 1. Specimens sequenced in this study

Code	Species	Plant	Accessions	
			28S-D2	COI
SynCast1	<i>Synergus castaneus</i>	<i>Castanea mollissima</i>	KC533839	KC533845
SynCast2	<i>S. castaneus</i>	<i>C. mollissima</i>	KC533840	KC533846
SynCast3	<i>S. castaneus</i>	<i>C. mollissima</i>	KC533841	KC533847
SynCast4	<i>S. castaneus</i>	<i>C. mollissima</i>	KC533842	KC533848
SynCast5	<i>S. castaneus</i>	<i>Castanea seguinii</i>	KC533843	KC533849
SynCast6	<i>S. castaneus</i>	<i>C. seguinii</i>	KC533844	KC533850

C. mollissima) were located on a hill in close association with tea plants and bamboos, and the field was surrounded by several species of oaks.

Galls were collected by hand from branches, separated from any nongall plant material and then stored in isolators with net at room temperature. Samples collected on the two different species of chestnut were kept separate. On the very same trees, galls of *D. kuriphilus* were also collected and stored in distinct isolators. ≈ 700 unknown galls were collected from *C. mollissima* and 19 from *C. seguinii*. Isolators were daily checked to collect the emerged insects until the following summer. At the end of September 2012, the isolators were emptied to collect any dead specimens remaining in the net.

At the completion of adult emergence, the diameter of 100 randomly chosen galls was measured. The volume of each gall was calculated by using the formula for a sphere ($4/3\pi r^3$). Because the galls were not perfectly spherical, a mean value between the maximum and the minimum diameter was used to improve the estimate. Thirty galls were randomly sampled from the initial 100 to be dissected, allowing us to count the number of chambers per gall.

The emerged insects (included cynipids and moths) were separated into morphospecies and sexed. All hymenopterans were preserved in 95% ethanol at -20°C . Successively, they were treated with xylol-ethanol (1:1) for 24 h and amyl acetate for 24 h according to a modified version of Vockeroth's (1966) protocol and card mounted. The material was deposited at the Pujade-Villar collection, Universitat de Barcelona (UB), Barcelona, Spain; at the insect collection of the Istituto per la Protezione delle Piante (IPP)-UOS Portici, Italy; at the Museo di Entomologia F. Silvestri (MES), Università degli Studi di Napoli "Federico II," Portici, Italy; at the Natural History Museum (NHM), London, United Kingdom; at the Melika collection, Pest Diagnostic Laboratory (PDL), Budapest, Hungary; at the National Museum of Natural History (NMNH), Smithsonian Institution, Washington; and at the National Zoological Museum of China (NZMC)/Institute of Zoology, Chinese Academy of Sciences.

The specimens were studied by using a Leica MSY226 stereomicroscope and with environmental scanning electron microscopy (SEM). To perform SEM imaging, a female was dissected and coated with gold by using a sputter coater (Jel JFC-1100) to a thickness of ≈ 30 – 40 nm. SEM pictures were taken with the Stereoscan Leica-360 (Cambridge Instru-

ments) operated with a voltage of 10 kV. The male antenna was imaged by using the FEI Quanta 200 Environmental SEM at 15 KV without any coating. The forewings were photographed by using a Zeiss Discovery V8 compound microscope with an Infinity-21C digital camera attached; the computer program DeltaPix View-Pro AZ was used to obtain a single in-focus image starting from a series of 20 focal planes. Specimens were also photographed at multiple focal planes by using a Leica DFC450 digital camera, and pictures were subsequently stacked by using the freely available software CombineZM (Image Stacking Software by Alan Hadley).

Terminology used to describe the gall wasp morphology follows recent cynipid studies (Melika 2006, Liljeblad et al. 2008). Abbreviations for forewing venation follow Ronquist and Nordlander (1989), and cuticular surface terminology follows Harris (1979). The following abbreviations were used for morphological traits and measurements: F1–F12 for the first and subsequent flagellomeres; POL (postocellar distance) for the distance between the inner margins of the posterior ocelli; OOL (ocellar-ocular distance) for the distance from the outer edge of a posterior ocellus to the inner margin of the compound eye; and LOL (lateral-frontal ocelli distance) for the distance between lateral and frontal ocelli. The width of the forewing radial cell was measured from the margin of the wing to the Rs vein. All means are presented with their standard deviations (mean \pm SD).

Molecular Characterization. Total genomic DNA was extracted from six individuals by using a nondestructive Chelex and proteinase-K-based method (Walsh et al. 1991), as modified by Gebiola et al. (2009). The standard COI barcoding fragment was amplified for all specimens by using primers LCO1490 and HCO2198 (Folmer et al. 1994). 28S-D2 was amplified by using primers D2 F and D2R (Campbell et al. 1993). Polymerase chain reaction cycles for COI and 28S-D2 were as described by Gebiola et al. (2009). Amplicons were directly sequenced by using Sanger technology at the Beijing ZhongKeXiLin Biotechnology Co., Ltd., Beijing, China. Newly generated sequences were deposited in GenBank with accession numbers KC533839–KC533850 (Table 1).

Chromatograms were assembled with Bioedit 7.0 (Hall 1999). COI sequences were verified for protein coding frameshifts and nonsense codons by using DnaSP5 (Librado and Rozas 2009) and aligned manually. All available COI and 28S-D2 sequences of *Synergini* were retrieved from GenBank for phylogenetic



Fig. 1. Female of *Synergus castaneus* n. sp. (Online figure in color.)

analyses. The alignment of COI sequences was straightforward, whereas 28S-D2 sequences were aligned by using the G-INS-I algorithm in MAFFT 6 (online version) (Kato and Toh 2008). Maximum likelihood (ML) trees were obtained by using RAxML 7.0.4 (Stamatakis 2006) after 1,000 multiple inferences on the original alignments by using the GTRGAMMAI nucleotide model as inferred by jModeltest2 (Darriba et al. 2012), starting from a random parsimonious tree, and default initial rearrangement settings and number of rate categories. ML was performed on single genes and on a concatenated data set that was partitioned by gene, and within COI, by first + second codon position, and third codon position. Based on the availability of sequences in GenBank, the COI data set included 216 taxa, the 28S-D2 data set 150 taxa, and for the combined dataset, 117 taxa were retrieved for which both genes were available. Branch support was based on 1,000 bootstrap pseudoreplicates.



Fig. 2. Male of *S. castaneus* n. sp. (Online figure in color.)

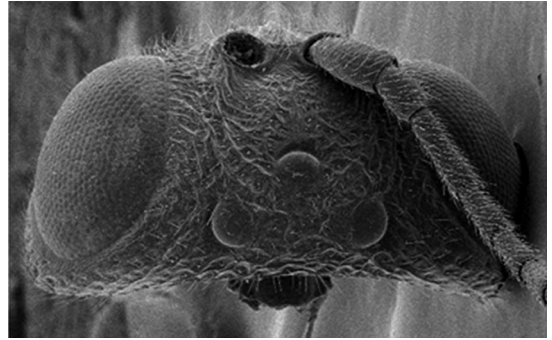


Fig. 3. *S. castaneus* n. sp. (♀): head in dorsal view.

Results

Synergus castaneus Pujade-Villar, Bernardo et Viggiani sp. nov. (Figs. 1–16).

Etymology. The species is named after its host, *Castanea*.

Type Material. HOLOTYPE (♀): deposited at UB, with the following labels: “CHINA, Jinzhai, Lo’an, Anhui, 149 m. asl, (30–iii–2012) iv–v–2012” (white label); “ex *Castanea mollissima* Blume and *C. seguinii* Dode, col. U. Bernardo” (white label); “Holotype ♀ of *Synergus castaneus* n. sp. desig. J.P-V” (red label); *Synergus castaneus* ♀ Pujade-Villar, Bernardo et Viggiani, det 2012.” PARATYPES (29♂ and 28♀) with the same data as holotype are stored as follows: 10♂ and 9♀ at UB, 2♂ and 2♀ at PDL, 2♂ and 2♀ at NMHM, 5♂ and 5♀ at IPP, 5♂ and 5♀ at MES, 5♂ and 5♀ at NHM, ♂ and 5♀ at NZMC.

Additional Material. Eight ♂ and 7 ♀ at UB, 20 ♂ and 20 ♀ at IPP, with the same data as of holotype.

Diagnosis. *S. castaneus* n. sp. differs from all other *Synergus* species by having the radial cell opened (basally and apically), and from the known species of the closely related genus *Saphonecrus* by the following combination of characters: female antenna with 11

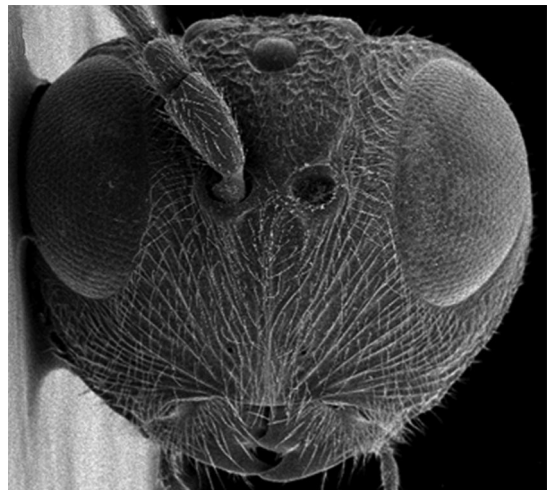


Fig. 4. *S. castaneus* n. sp. (♀): head in frontal view.

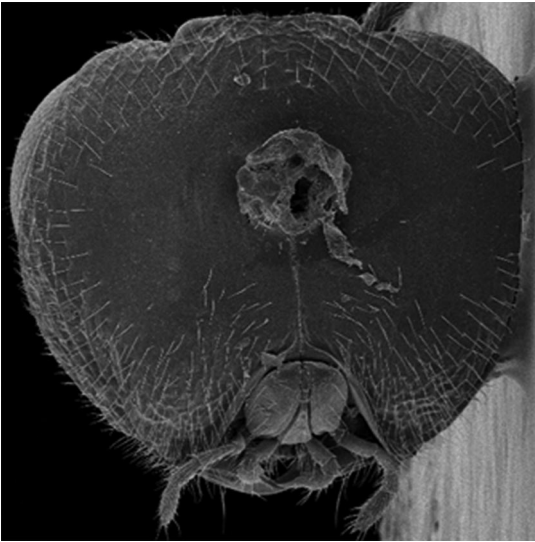


Fig. 5. *S. castaneus* n. sp. (♀): head in posterior view.



Fig. 7. *S. castaneus* n. sp. (♀): mesosoma in lateral view.

flagellomeres (the last long and sometimes incompletely divided), lateral frontal carinae absent or very weak, pronotal lateral carinae absent, mesoscutum median sulcus present, and male antenna with 13 flagellomeres. Only *Synergus plagiotrochi* (Nieves-Aldrey and Pujade-Villar, 1987), *Synergus variabilis* (Mayr, 1872), *Synergus flavipes* (Hartig, 1843), *Synergus acsi* (Melika and Pujade-Villar, 2006), and *Synergus palmirae* (Melika and Pujade-Villar, 2006) do not have pronotal lateral carinae, but in *S. variabilis*, *S. flavipes*, *S. acsi*, and *S. palmirae*, the radial cell is closed. *S. plagiotrochi* has a partially closed radial cell, but its frons is coriaceous (with some delicate rugae in *S. castaneus*), mesosoma with weak sculpture, and median sulcus very short (with interrupted sharp and widely spaced transverse rugae and long medial sulcus in *S. castaneus*). Morphologically, *S. castaneus* is closely related to *Synergus apicalis* and *Synergus tibialis*. Nevertheless, in *S. castaneus*, the radial cell is open, the notauli percurrent, the medial sulcus very long, and the pronotal carina absent; male also differs from *S. tibialis* because the head is yellowish in frontal view and F1 curved and swollen basally and apically.

S. castaneus is easily distinguishable from the seven species included in the genus *Synophrus* (Pérez et al., 2009) for the presence of lateral frontal carina, the trapezoid head, the metasomal quadrangular shape, and scutellar foveae indistinctly delimited with lon-

gitudinal wrinkles, and by the presence of sexual dichromatism.

Female. Length. 2.0–2.2 mm (2.02 ± 0.154 mm, $n = 10$) (Fig. 1).

Color. Head black, except for lower face, which is chestnut around mouthparts; mandibles yellow with dark brown tips; maxillae and labium yellow; antenna yellow to light brown, with darker areas on scapus and pedicel. Mesosoma black; legs brown or light brown, with coxae black and dorsal femora darker. Metasoma shining black with a very narrow chestnut-brown collar around the distal part of the petiole; hypopygium light brown. Wing veins pale to light brown. Body with sparse white setae.

Head. Slightly wider than long in frontal view, 1.2–1.3 times as broad as high, as wide as metasoma; 2.1–2.2 times as broad as long (Figs. 3–5). Gena with strongly converging sculpture. Malar space ≈ 0.6 times as long as height of eye. POL 1.6–1.7 times as long OOL; OOL only slightly longer than diameter of lateral ocellus and equal LOL; temple length slightly shorter than OOL. Transfacial distance as long as height of eye; diameter of antennal torulus 0.5 times distance between them and slightly shorter than distance between torulus and eye margin. Lower face and elevated median area with uniform delicate striae, radiating from ventral margin of clypeus and reaching eye and antennal toruli. Clypeus striate, indistinctly delimited by anterior tentorial pits, epistomal sulcus and clypeo-pleurostomal line absent, ventrally nearly straight. Frons coriaceous with some delicate rugae,

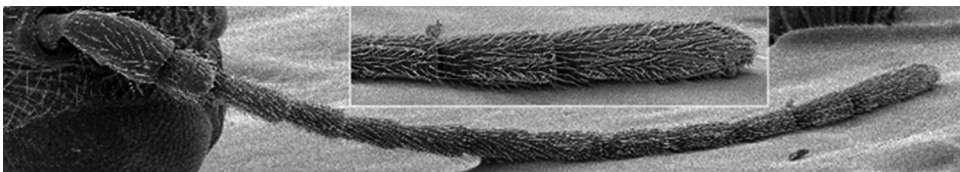


Fig. 6. *S. castaneus* n. sp. (♀): antenna and detail of last antennomeres.

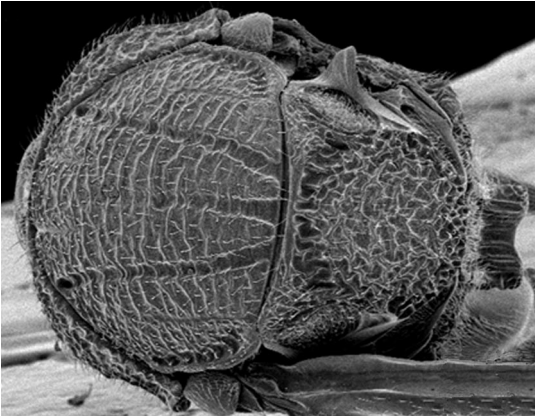


Fig. 8. *S. castaneus* n. sp. (♀): mesosoma in dorsal view.

and without punctures; lateral frontal carinae absent or very weak, indistinct, sometimes reaching lateral ocellus. Vertex, interocellar area, and occiput coriaceous with some delicate rugae, and without punctures. Right mandible with three teeth and left mandible with two; maxillary palp five-segmented; labial palp three-segmented.

Antennae. Shorter than body (1.45 ± 0.074 mm, $n = 10$), 13 segmented, shorter than head + mesosoma; scape 1.8 times as long as pedicel and slightly wider; pedicel 1.6 as long as broad; F1 around 1.7 times as long as F2; F2 to F10 subequal in length, distal flagellomeres slightly broadened; F11 around 1.7 times as long as F10; last flagellar segment clearly derives from the partial or total fusion of two distal segments; placodeal sensilla on F6–F11 (Fig. 6).

Mesosoma. Slightly longer than high in lateral view (Figs. 7–12). Pronotum delicately coriaceous with some delicate wrinkles; lateral pronotal carinae absent; anterior corners of pronotum rounded. Scutum flattened posteriorly, convex anteriorly; slightly broader than long, with interrupted sharp and widely spaced transverse rugae; interspaces coriaceous. Notauli complete; median mesoscutal line long, reaching at minimum one-third of scutum length; parapsidal

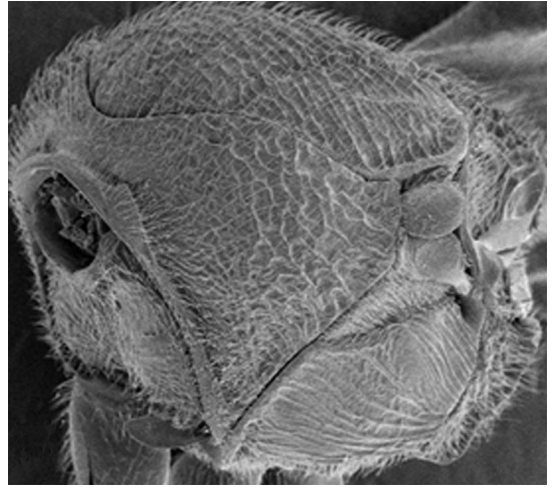


Fig. 10. *S. castaneus* n. sp. (♀): mesosoma in dorso-lateral view.

lines present, extending one-third of scutum length. Mesoscutellum 0.5 times shorter than scutum, as long as broad; dull rugose, interspaces coriaceous; scutellar foveae transversely ovate, indistinctly delimited, with longitudinal wrinkles. Mesopleuron completely striate. Mesopleural triangle with dense pubescence. Metapleural sulcus reaching mesopleuron in upper one-third of its height; axillula square, coriaceous, without piliferous points and with some weak rugae; subaxillular bar smooth and shining posteriorly, and shorter than height of metanotal trough. Metascutellum very short medially; metanotal trough very delicately coriaceous to alutaceous, with few white setae next to dorsellum and some longitudinal carinae; ventral impressed area shining and glabrous; dorsal impressed area shining but setose. Lateral propodeal carinae nearly straight, with setae, and equal in width; central propodeal area delicately coriaceous with a short medial carina, pubescent; lateral propodeal area delicately coriaceous to alutaceous, with relatively dense white setae and very weak longitudinal carinae. Nucha entirely sulcate and with white setae laterally.



Fig. 9. *S. castaneus* n. sp. (♀): mesosoma in anterior view.



Fig. 11. *S. castaneus* n. sp. (♀): propodeum.

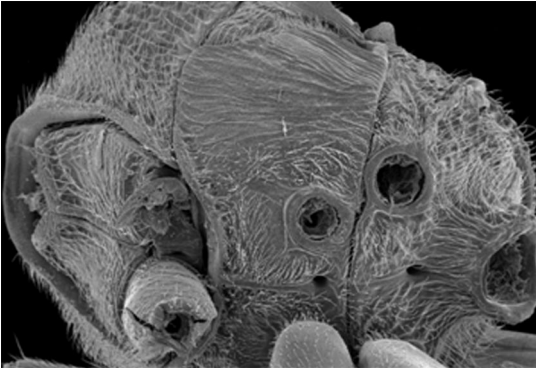


Fig. 12. *S. castaneus* n. sp. (♀): mesosoma in ventro-lateral view.

Forewing. Longer than body (2.37 ± 0.216 mm; $n = 10$), margin with short cilia ($\approx 1/17$ of the wing width); radial cell opened marginally and basally, around 2.6 times as long as broad (2.63 ± 0.106 mm; $n = 10$), Rs very slightly curved; areolet spectral; Rs + M hardly traceable, extending to half distance between areolet and basalis veins, projecting into the middle of the basal vein (Fig. 13).

Legs. Tarsal claws with basal lobe (Fig. 14).

Metasoma. As long as head + mesosoma or slightly shorter, as long as high in lateral view; metasomal tergites 3 and 4 fused, posteriorly with punctures (or very small patch of indistinct punctures present dorso-posteriorly only), with a very few sparse, short white setae antero-laterally. Hypopygium alutaceous without punctures; prominent part of ventral spine very short (Fig. 15).

Male. Length 1.8–2.0 mm. (1.84 ± 0.149 mm; $n = 10$) Similar to female but with lower face and gena yellowish; antenna 15-segmented, shorter than body (1.50 ± 0.070 mm; $n = 10$), F1 (Fig. 16) slightly curved, swollen basally and apically, around 1.8 times as long as F2; subsequent flagellomeres nearly of equal length, as long as pedicel; distal flagellomeres very slightly broadened; placodeal sensilla on F6–F13 (Fig. 2).

Distribution. Anhui province (eastern China).

Host Gall. Sampled *C. mollissima* and *C. seguinii* trees carried two different galls, often on the same branch: galls caused by *D. kuriphilus* and galls from

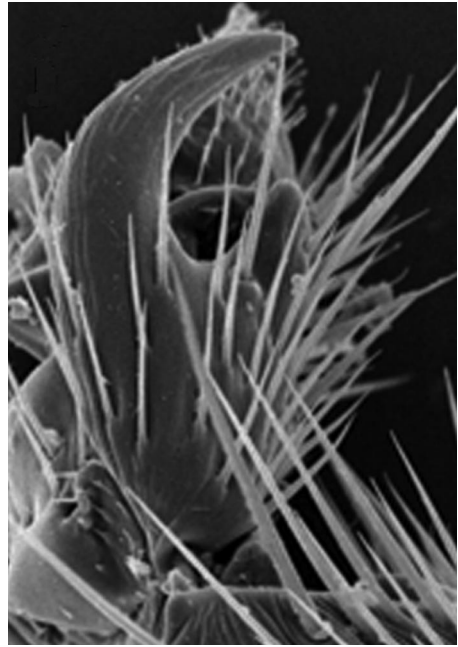


Fig. 14. *S. castaneus* n. sp. (♀): tarsal claws.

which *S. castaneus* emerged. Galls associated with *S. castaneus* have a roughly spherical shape, with a diameter of 0.6–2.3 cm (Figs. 17 and 18). They are multilocular, inserted on twigs at bud sites, variable in exact position and number, in some cases conglomerate, but never on leaves. Spines are present at the tops of young galls, although these are lost as the gall ages. At maturity, galls become woody. The mean gall volume is 1.69 ± 0.920 cm³ (0.2 – 4.6 cm³), $n = 100$, and the mean number of chambers per gall is 43.95 ± 34.405 (6–126), $n = 20$. Ten of the 30 dissected galls had chambers disrupted by tunnels mined by moth larvae.

Life Cycle. *S. castaneus* is a biparental univoltine species. The adult emergence started a few days after the collection (in April) and lasted about 4 wk. The new galls became evident in May–June, and the species overwinters inside the galls. In total, 593 specimens (369 females and 224 males) that emerged from

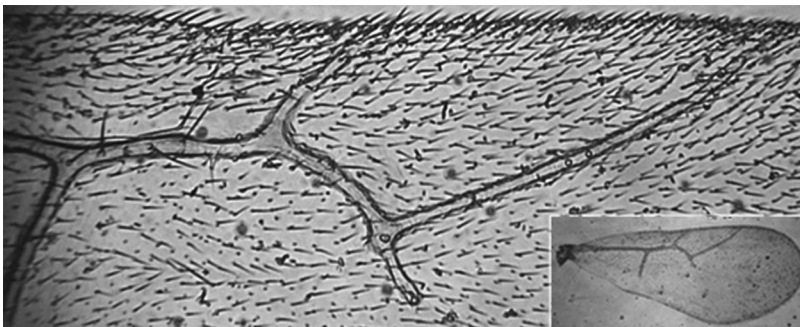


Fig. 13. *S. castaneus* n. sp. (♀): forewings and detail of radial cell.

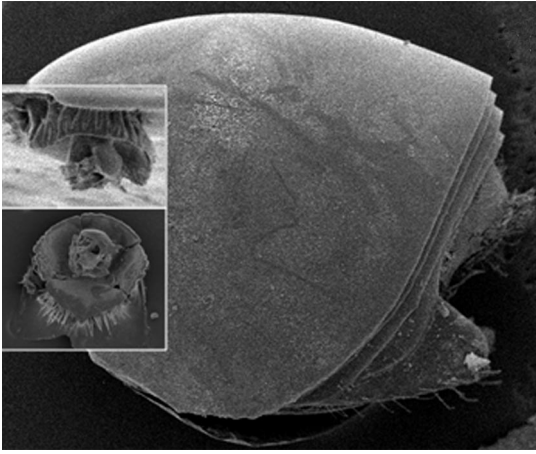


Fig. 15. *S. castaneus* n. sp. (♀): metasomal with details of petiole and first metasomal segment.

galls were collected. The sex ratio was 0.6 with a female bias. A large percentage of galls contained dead specimens (30.26%). The main differences between *D. kuriphilus* and *S. castaneus* life cycles and galls are summarized in Table 2.

Gall Associates. No other cynipids or other species known as gall-inducing emerged from the collected galls. However, several parasitoids belonging to the families Eupelmidae, Eurytomidae, Ormyridae, and Torymidae emerged, together with several specimens of the moth *A. pulverula* (Lepidoptera: Tortricidae).

Molecular Characterization. Trimmed COI sequences of *S. castaneus* resulted in a 642-nt fragment. Two haplotypes were recovered, differing for a single polymorphic A/C site at position 405. 28S-D2 sequences did not show any intraspecific variation. ML phylogenetic analyses (Fig. 19; Supp. Figs. 1 and 2 [online only]) showed that *S. castaneus* represents a genetically distinct species that clusters within the monophyletic genus *Synergus* (we believe that the single *Synergus* species that falls outside of this monophyletic assemblage represents a misidentification because of the difficulty in separating some species of *Synergus* and *Saphonecrus*). The combined analysis

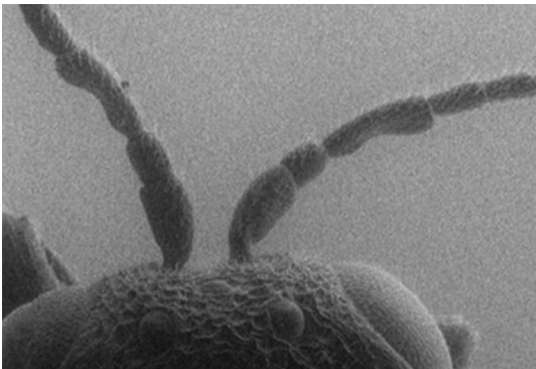


Fig. 16. *S. castaneus* n. sp. (♂): first antennomeres.



Fig. 17. *S. castaneus* n. sp. gall associated with *Castanea* spp. (Online figure in color.)

showed the higher statistical support for most clades (Supp. Fig. 2 [online only]), whereas in the COI tree (Fig. 19), which included the greater number of species, *S. castaneus* occupies a basal position within the monophyletic genus *Synergus*.

Discussion

S. castaneus is morphologically intermediate between the genera *Synergus* and *Saphonecrus* and have some characters coincident with *Synophrus*. The current morphological limits between these genera are not satisfactory. The genus *Saphonecrus* was erected by Dalla Torre and Kieffer (1910) to include oak inquiline species differing from *Synergus* by having an open radial cell. Nevertheless, *S. castaneus* and two other *Synergus* (*S. plagiotrochi* and *S. tibialis*) have the



Fig. 18. Section of the *Synergus castaneus* n. sp. gall associated with *Castanea* spp. (Online figure in color.)

Table 2. Principal differences between life cycle and gall morphology of the two cynipids collected on *Castanea* spp. in China

Species	<i>Dryocosmus kuriphilus</i> ^a	<i>Synergus castaneus</i> sp. nov.
Type of reproduction	Thelytokous; male unknown	Biparental
Overwintering site	Inside buds	Inside galls
Emergence of adults	June–July	April–beginning of May
Shape of galls	Very irregular	Roughly spherical
Consistence of galls	Leathery and fragile when dried	Woody
Position of galls	On several vegetable parts, enclosing leaves, stems, and sprouts of new branches	On branches of 1 yr or older, not enclosing other vegetable parts
Type of galls	Uni- or multilocular	Multilocular

^a Bernardo et al. (2013).

radial cell open. The validity of the *Saphonecrus* genus has been questioned by an increasing number of authors over the years (Eady and Quinlan, 1963; Ritchie, 1984; Kierych, 1988; Pujade-Villar and Nieves-Aldrey, 1990; Melika 2006, among others). Further doubts have arisen when phylogenetic reconstructions showed the genus *Saphonecrus* to be paraphyletic with respect to *Synophrus* (Melika et al. 2006, Péntzes et al. 2009, Ács et al. 2010), a finding confirmed by this study.

In *Saphonecrus*, the number of female flagellomeres is 11, as in *Synophrus*, whereas it is 12 in *Synergus*; although *S. castaneus* has 11 flagellomeres, but the last one is very long with at least two whirls of placodeal sensilla (suggesting it is the result of the fusion of two flagellomeres). In addition, some specimens have an incomplete suture in F11, and the scutum and the frons sculptures are strongly impressed as in *Synergus*. For these reasons, we place this new species in the genus *Synergus*.

According to Melika (2006) and Abe et al. (2007), 43 species of *Synergus* have been described from the Palaearctic and Oriental regions, but five of them, described from Japan, are doubtfully placed within the genus. All 38 valid species have the radial cell closed, except *S. plagiostrochi*, *S. castaneus*, and several Iberian specimens of *S. tibialis* Hartig, 1840 (= *Synergus rotundiventris* Mayr, 1872). Nevertheless, *S. castaneus* differs from *S. tibialis* in having no lateral pronotal carinae and from *S. plagiostrochi* in having the sculpture of the head and scutum with spaced transverse rugae (without transverse rugae and with weak strigose sculpture narrowly spaced in *S. plagiostrochi*). All *Synergus* species have lateral pronotal carinae, except *S. plagiostrochi*, *S. variabilis*, *S. flavipes*, *S. acsi*, *S. palmirae*, and *S. castaneus*. The genus *Saphonecrus* includes 21 species (Wang et al. 2010, Wachi et al. 2011, Liu et al. 2012), nine of which are found in the eastern Palaearctic region. Among these species, *Saphonecrus diversus*, *Saphonecrus excisus*, *Saphonecrus flavotibialis*, *Saphonecrus naiquanlini*, and *Saphonecrus yukawai* have the female antenna with 11 flagellomeres. Three of these species (*Sa. diversus*, *Sa. naiquanlini*, and *Sa. yukawai*) have pronotal carinae (lacking in *S. castaneus*), whereas *Sa. excisus* has simple tarsal claws (Kieffer 1904) in contrast with all other *Saphonecrus* species, which have a basal lobe. *S. castaneus* seems closely related to *Sa. flavotibialis*, although it can be easily distinguished by the presence of the mesoscu-

tum median line, the shorter radial cell, and the body color.

S. castaneus is easily distinguishable from the seven species included in the genus *Synophrus* for the presence of lateral frontal carina (absent in *Synophrus*), the metasomal quadrangular shape (longer than head + mesosoma and longer than high in lateral view in *Synophrus*), and by the presence of sexual dichromatism (absent in *Synophrus*). Almost all species of *Synergus* and *Saphonecrus* are associated with *Quercus* galls caused by other cynipids, but *Saphonecrus hupingshanensis* (Liu et al. 2012) emerged from galls on *Castanopsis carlesii* (Hemsl.) (Fagaceae) and *Sa. yukawai* (Wachi et al. 2011) has been reared from galls of the midge *Ametrodiplosis acutissima* Monzen (Cecidomyiidae) on *Quercus* (sect. *Cerris*) *acutissima* Carruthers.

Recently, the supposed inquiline nature of all *Synergini* has been questioned by the discovery of two new species. The first is *Synergus itoensis* Abe, Ide and Wachi, recently recorded in Japan on *Quercus glauca* (Thunb.), which has been demonstrated to be a gall inducer (Abe et al. 2011). The second is *S. hupingshanensis* (Liu et al. 2012), which was inferred to be a gall maker because no gall maker was reared from the same galls. Similarly, for *S. castaneus*, we were unable to find any other cynipid among the several hundreds of specimens collected, hence we cannot rule out the possibility that this species is a gall inducer.

Phylogenetic analyses confirmed our morphological observations, placing *S. castaneus* within the genus *Synergus*. Furthermore, the COI tree (Fig. 13) suggested that *S. castaneus* is an ancestral species of the genus *Synergus*. Recent DNA sequence data indicate that inquilines have evolved from gall-inducing ancestors several times (Nylander et al. 2004). The phylogenetic placement of *S. castaneus* as an early branching species within *Synergus* based on COI data seems to support this hypothesis in case further studies will unequivocally prove that this species is in fact a gall inducer.

Several species of parasitoids emerged from the collected galls, many of them shared with those reared from galls of Asian chestnut gall wasp collected in China (Bernardo et al. 2013). The sharing of parasitoids in the same habitat and the different life cycles of *S. castaneus* and Asian chestnut gall wasp could increase the parasitism rate on the latter pest. In fact,

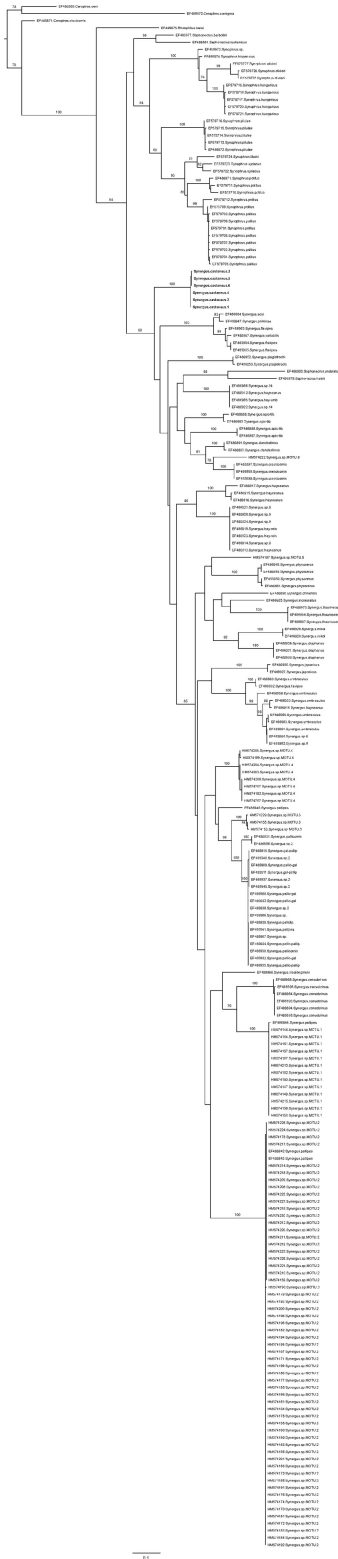


Fig. 19. Maximum likelihood tree based on the COI data set. Bootstrap values higher than 70% are indicated above branches.

as Asian chestnut gall wasp overwinters inside the buds as first instar (a stage too small and unsuitable for several parasitoids), parasitoids could use *S. castaneus* as host and the galls from which emerges as protection to overwinter. Several parasitoids are, indeed, bivoltine (Quacchia et al. 2013) and need another host after Asian chestnut gall wasp before overwintering, which they might find on the very same trees. Furthermore, about one-third of galls examined were damaged by the larvae of *A. pulverula*. This species has never been recorded as gall inducer, and is distributed in Northern India, China, Korea, and Japan (Komai 1992). Recorded hosts are galls of cynipids on *Quercus* and of *D. kuriphilus* galls on *Castanea* spp. Larvae of this species also feed on larvae of gall wasps (Komai 1992).

The tribe Synergini is in need of a thorough revision as numerous important taxonomic, phylogenetic, and biological issues remain unresolved, including the placement of species within genera, the relationships among genera (Pénzes et al. 2009; Liu et al. 2012), and the inquiline and/or gall-inducing nature of its members. The discovery of *S. castaneus* in China confirms the widely held belief among entomologists working on cynipid gall wasps that the species diversity of Cynipidae in Asia is largely underestimated (Abe et al. 2007; Liu et al. 2012).

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