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RESEARCH

New Gall Wasp Species Attacking Chestnut Trees: *Dryocosmus zhuili* n. sp. (Hymenoptera: Cynipidae) on *Castanea henryi* from Southeastern China

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ABSTRACT. A new gall wasp species, *Dryocosmus zhuili* Liu et Zhu, is herein described from the southeastern Fujian province of China. The new species induces galls on trees of Henry's chestnut, *Castanea henryi*, which is also a native host for the notorious Oriental chestnut gall wasp (OCGW, *Dryocosmus kuriphilus* Yasumatsu). *D. zhuili* overlaps with OCGW in emergence time and induces galls morphologically similar to that of OCGW on similar plant parts. In a previous study, we reported considerable divergence between mtDNA CO1 (mitochondrial DNA Cytochrome c oxidase subunit I) sequences of these wasps and the true OCGW wasps and suggested the existence of a cryptic species. Herein, we confirm the identity of the new species based on morphological and biological differences and provide a formal description. Although the new species is relatively easily separated from OCGW on basis of morphology, field identification involving the two species can still be problematic because of their small body size, highly similar gall morphology, and other life history traits. We further discussed the potential of the new species to be a pest for the chestnut industry and the consequences of accidental introduction of this species into nonnative areas, especially with regard to the bisexual reproduction mode of the new species in contrast to the parthenogenetic reproduction mode of OCGW.

Key Words: *Dryocosmus zhuili*, new species, gall wasp, chestnut, southeastern China

Oriental chestnut gall wasp (OCGW), *Dryocosmus kuriphilus* Yasumatsu, 1951 (Insecta, Hymenoptera, Cynipoidea, Cynipidae, Cynipini) is the only confirmed gall wasp species known to induce galls on chestnut trees in the genus *Castanea* Mill. (Fagaceae) (Buffington and Morita 2009, Melika et al. 2011). A new inquiline species, *Synergus castaneus* Pujade-Villar, Bernardo et Viggiani, was recently described, and specimens of the species were reported to be reared from galls on chestnut that apparently did not belong to OCGW, suggesting the existence of a different gall inducer species (Bernardo et al. 2013). As a notorious pest species of global importance, OCGW attacks various chestnut species in its native China and in almost every other place where chestnut trees are grown (Rieske 2007, Murakami 2009, Zhang 2009, Bosio et al. 2010, Rieske and Cooper 2011, Borowiec et al. 2014). In a recent study on gall wasps reared from apparent OCGW galls collected on three *Castanea* species, *i.e.*, *Castanea mollissima* Blume, *Castanea henryi* (Skan), and *Castanea seguinii* Dode, we reported the identification of a distinct COI haplotype among chestnut gall wasps reared from galls on *C. henryi* collected from Fujian province in southeastern China and considered it to represent a cryptic species (Lu et al. 2012). Subsequent evaluation of morphological and biological differences supported the conclusion based on molecular data, and we herein provide a formal description of the new gall wasp species *Dryocosmus zhuili* (DZ) Liu et Zhu, sp. nov. The new species belong to the same genus as OCGW and induces galls on chestnut trees (*Castanea* spp.). Furthermore, it also induces galls that are very similar to that of OCGW on similar plant structures and overlaps with the latter in emergence time. A detailed comparison between the life history traits of the new species and OCGW suggests that the new species may be a potentially dangerous pest for the global chestnut industry like its congener OCGW.

Materials and Methods

Galls collected in the field were cage reared at room temperature in the laboratory. Emerged wasps were killed and preserved in 100% alcohol. Specimens were examined under an Olympus SZX12 stereo dissecting microscope. For SEM examination, specimens were either transferred to 75% alcohol for at least overnight, dissected in 75% alcohol, cleansed in 5% ammonia, rinsed in distilled water, and dehydrated gradually through 50%, 75%, 90%, and finally stored in 100% alcohol. Dehydrated specimen parts were air-dried before being mounted onto aluminum stubs (Ted Pella, Inc. Redding, CA) with Carbon Conductive tape (Ted Pella, Inc. Redding, CA). Gold-coated specimens were examined with LEO EVO-60 SEM (Carl Zeiss, Germany) at the Field Museum of Natural History (FMNH) with 20 KV voltage or Hitachi S-3500N SEM (Hitachi, Japan) at School of Technology, Eastern Illinois University (EIUST) with 5–15 KV voltage, and selected frames were saved as digitized high-resolution images. Wings were mounted on slides in Euparol mounting medium and photographed using a Konica-Minolta Maxxum 7D camera (Konica-Minolta, Japan) attached Olympus SZX12 stereo dissecting microscope through an adaptor (Olympus, Japan). Type specimens are deposited in the National Zoological Museum of China, Beijing, China (NZMC), Field Museum of Natural History, Chicago, USA (FMNH), Central South University of Forestry and Technology Insectorium, Changsha, Hunan, China (CSUFT), US National Museum of Natural History, Washington, DC (USNM), American Museum of Natural History, NYC, NY (AMNH),

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and Illinois Natural History Survey, Champaign, IL (INHS). In addition, several paratypes were also placed in ZL's personal Cynipoidea collection (ZLCC).

We follow Ronquist and Nordlander (1989) for structural terminology, Melika (2006) for measurement definitions, and Harris (1979) for sculpture descriptions.

Nomenclature

This paper and the nomenclatural act it contains have been registered in Zoobank (www.zoobank.com), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:30F6E8A1-5260-415F-AA86-A71C515B6D1F.

Results

Dryocosmus zhuili Liu et Zhu, New Species (Figs. 1–13)

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Diagnosis. The new species is very similar to *D. kuriphilus* but can be distinguished from the latter by a combination of several morphological features (Table 1). This comparison is based on examination of several hundreds of *D. kuriphilus* specimens collected from a broad range of locations from Fujian and Guangxi provinces in the south and Henan and Shandong provinces in the north of China and high-resolution SEM images from the online database of Morphbank by Liljablad and Ronquist (available at http://www.morphbank.net/Browse/ByImage/?tsn=705660). Among these features listed in Table 1, features 1–3 are relatively reliable and can be used for diagnostic purpose, while features 4–7 are less reliable for distinguishing between the two species but can be provide additional information when combined with data on features 1–3. In addition, the species is also different from *D. kuriphilus* in having sexual reproduction and distinct COI haplotype (Lu et al. 2012).

Description. Adult female (Fig. 1). Color (alive or freshly killed): head dark brown, mesosoma pitch black, and metasoma mostly black and ventrally and posteriorly brownish black; legs and base of antenna creamy yellow; distal half of antenna darkish yellow.

Head (Figs. 2, 7, 8, and 12). Frons rugulose, sparsely setose; lower face covered by dense appressed setae; short striae radiating from clypeo-pleurostomal line dorsally towards ventral margin of eye and to lower margin of antennal sockets, reaching only to middle of lower face; malar sulcus absent; clypeus protruding, marked by prominent epistomal sulcus, smoothly curved anteriorly, and pinched laterally by clypeo-pleurostomal line, densely covered with uniform appressed setae somewhat longer than setae on lower face. Eyes long with sparse minute setae; height of eye 3.4 times as long as breadth of malar space. Gena broadly rounded and distinctly expanded behind eyes; gena and vertex gently shagreened, covered in sparse, short appressed setae. Toruli impressed into face with distinct trench present (Figs. 2 and 7). Occiput and postgena coriaceous, mostly covered with appressed setae; area around occipital foramen distinctly impressed, devoid of setae, and with week horizontal striation immediately above occipital foramen; postgena broadly impressed with distinct vertical striation from level of foramen and below; gula moderately narrowed in lower half, shortest distance between gular sulci about 0.4 times the height of gula (Fig. 8). Antenna nonclavate, with 12 flagellomeres; F1 is 4.6 times as long as median width in dorsal view, relative length of F1-12: 2.2/2.0/1.8/1.7/1.4/1.4/1.1/1.1/1.1/1/1/1.8; F12 with a depressed ring in the middle; short appressed setae on all flagellomeres (Fig. 12).

Mesosoma (Figs. 2, 9, 10, and 13). Pronotum narrow medially, length in median dorsal line 1/7.5 times as long as the greatest length on outer lateral margin. Lateral surface of pronotum areolate-rugose on dorsal 1/3 along dorsal margin, smooth coriaceous on anteroventrally, and with several broadly spaced longitudinal to oblique ridges posteriorly; ventral margin with a deep, transversely ridged trench; sparsely setose except in anteroventral smooth area; lateral pronotal carina lacking, submedial pronotal depressions deep, open laterally (Fig. 9). Mesopleuron mostly smooth, glabrous below mesopleural triangle, with distinct diagonal striae in middle, with sparse appressed setae ventrally; mesopleural triangle deeply impressed, with sparse short setae, ventral edge well defined anteriorly (Fig. 9). Mesoscutum completely smooth, glabrous, with sparse setae along notauli and lateral edges (Figs. 9 and 10); anteroadmedian signum indistinct; median mesoscutal impression lacking; notauli complete, gradually slightly widened posteriorly; parascutal signa indistinct (Fig. 8). Scutellum sloped posteriorly (Figs. 2, 9, and 10), disk rugulose, covered with appressed setae (Fig. 9); scutellar fovea broad, interspaced by multiple parallel longitudinal ridges; axillar area rugulose and setose (Fig. 9).

Metapleural-propodeal complex (Figs. 2, 10, and 13). Metapleuron sparsely setose anteriorly and posteriorly covered with long, white setae; metapleural sulcus reaching posterior margin of mesopleuron slightly above middle; semilunar upper metapleural area, rectangular upper metapleural area, and metepisternum distinctly excavated, and smooth in semilunar upper metapleural area irregularly uneven elsewhere (Fig. 10). Setal pits at ventral margin of metapleuron distinct; posterior aspects of propodeum flat (Fig.10); propodeal carinae distinct in anterior 2/3, being broadly curved in the middle; posterior 1/3 becoming indistinct or lacking posteriorly; area between propodeal carinae heavily, longitudinally rugulose (Figs. 9 and 10); area laterad to propodeal carina covered with long white setae; nucha very short, dorsally heavily rugulose with a few sparse long setae (Figs. 9 and 13).

Wings (Fig. 3). radial cell elongate, open along anterior margin, length 3.75 times as long as wide; 2nd abscissa curved distally; areolet present and triangular; Rs + M, M, and Cu1a veins represented by trace veins; Rs + M meeting basalis at posterior 2/3; short setae present on wing surface and margins ciliate.

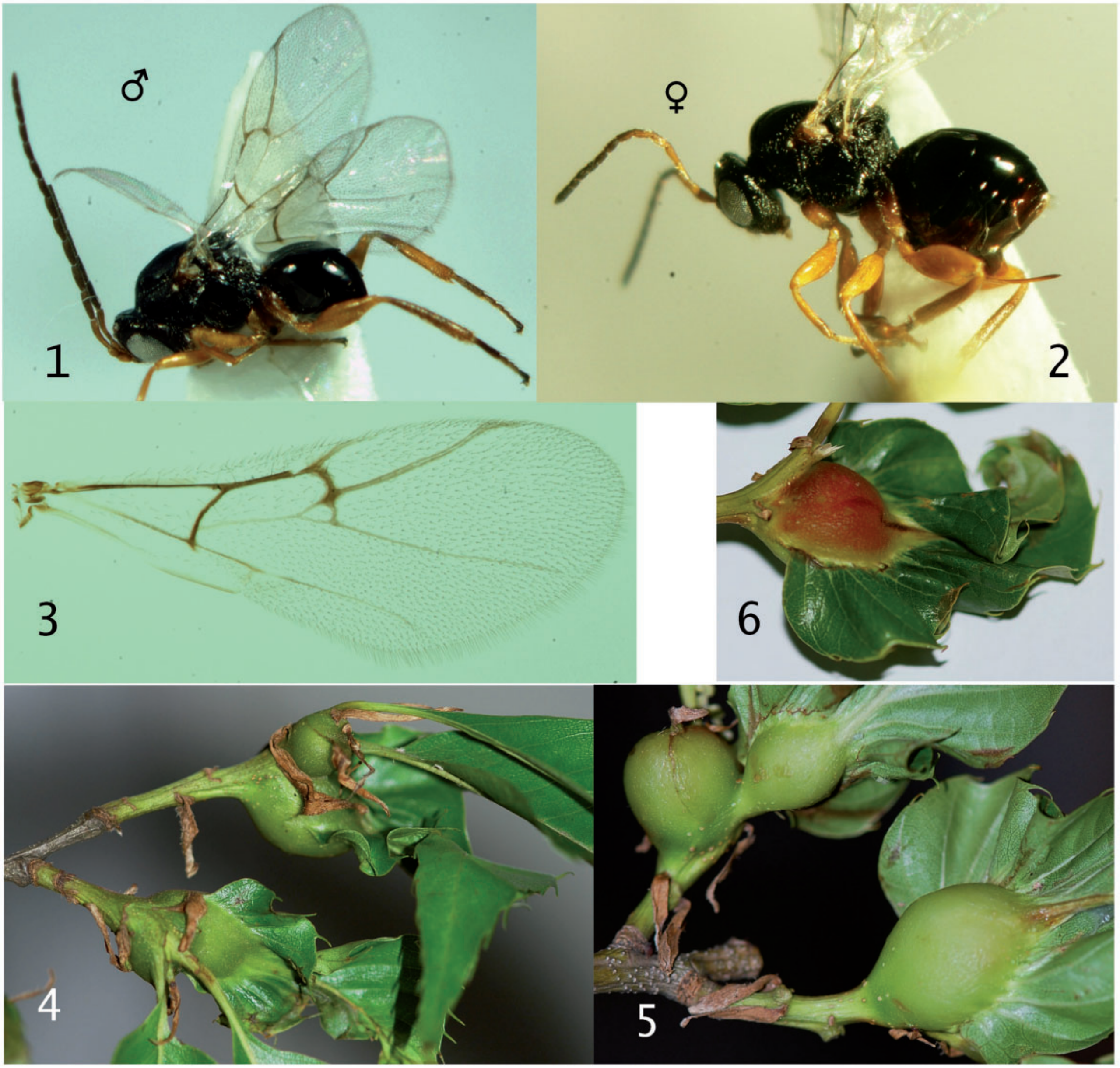
Legs. Coxae mostly covered with sparse to moderately dense setae and glabrous in a central area on the posterior surface and lower part of the anterior surface of metacoxa; appressed setae 7 present sparse to moderately dense on all femorae, dense on front tibiae, and very dense on meso- and meta tibiae. Tarsomeres evenly covered in very dense, appressed setae; length of metatarsomere 4/5 as long as the combined length of tarsomeres 2–5; claw without basal tooth.

Metasoma (Fig. 11). Petiole small, crescent-shaped and surface smooth; all postpetiolar terga free; In nature, relaxed state, relative length of T3-T8: 5.3/2.3/1.0/0.75/0.5/0.5, T9 small, but about as long as T8; posterior margins of T3 and T6 parallel, except that of T6 angled away from lower 2/3 posteriorly, T7 with posterior margin gradually but distinctly angled away from midline posteriorly; T4–T9 with very fine micropores; long setae very sparsely present on T3, sparsely present on T8, moderately densely present on T9 forming moderate tuft; hypopygium (sternite 7) distinctly extended ventrally; prominent part needle-like and short, about 1/5 the entire length of the sternite; sparse, elongate setae present along entire length of extreme ventral margin.

Adult male (Fig. 1). antenna filiform, 1.3 × body length (not including antenna or wings), with 13 flagellomeres, F1 distinctly curved, excavated on dorsal surface, and very slightly twisted laterad; relative length of F1-13 1.9/1.7/1.5/1.5/1.5/1.3/1.2/1.2/1.1/1.0/1.0/1.0/1.1. Gena, ventral half of pronotum, and mesopleuron glabrous. T3 covering about 2/3 of metasoma, 3.3 times as long as T4 measured along median dorsal line; posterior margins of all metasomal terga T4–8 gradually angled away from midline posteriorly. Sparse, long setae present on slightly exposed T9. Otherwise male is very similar to female.

Gall morphology (Figs. 4–6). Subglobular, succulent, and fleshy integral galls are formed on young buds and leaf petiole and mid rib of leaves close to leaf base on new shoots. Galls are usually green colored, occasionally rosy on the sunny side. Gall size: about 8.0 mm in width by 8.0–25.0 mm in length.

Material examined. Holotype ♀ (NZMC). China: Fujian, Zhounin Co., Chunchi Township (27.14°E, 119.19°N), galls collected on 2011-V-21 and wasp reared in early June, Peng-Fei Lu, coll., ex *C. henryi*.



Figs. 1–6. (1–3) *DZ* Liu et Zhu, new species. (1) Lateral habitus of female paratype. (2) Lateral habitus of male paratype. (3) Forewing of female. (4–6) Galls induced by *DZ* Liu et Zhu, new species on *C. henryi*: (4) galls formed on new shoots, upper view, (5) galls formed on petiole and mid rib of leaf, in ventral view, and (6) single gall on petiole and mid rib of leaf, in upper view.

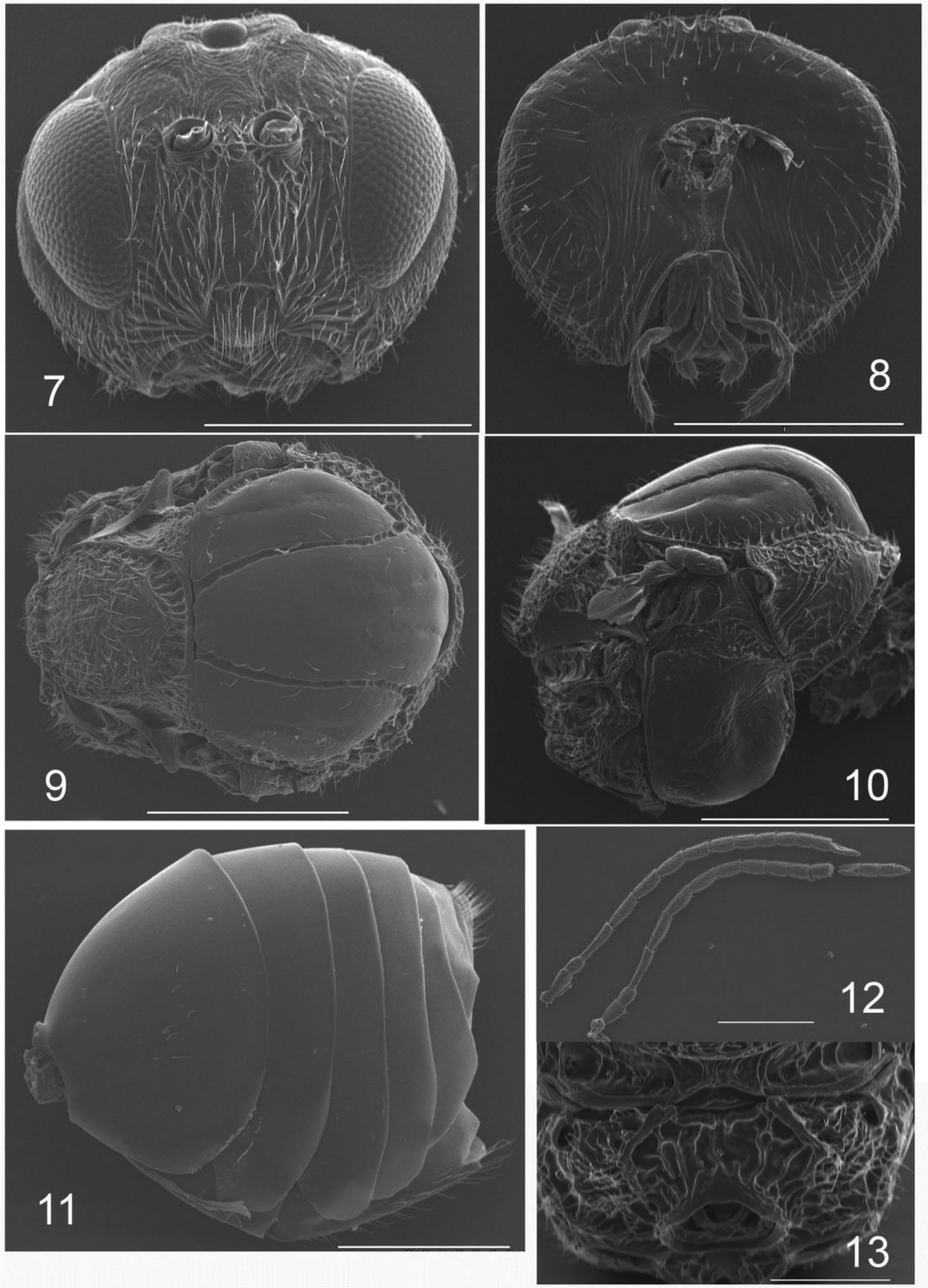
Paratypes. 37 ♀♀, 36 ♂♂, data as Holotype: NZMC (5 ♀♀, 5 ♂♂), FMNH (2 ♀♀, 2 ♂♂), CSUFT (14 ♀♀, 14 ♂♂), AMNH (2 ♀♀, 2 ♂♂), USNM (2 ♀♀, 2 ♂♂), ZLCC (12 ♀♀, 11 ♂♂, including 3 ♀♀, 2 ♂♂ on SEM stubs and wings in slides).

Biology and Distribution. Bisexual, with sex ratio ≈1: 1 ($n > 300$). The species is only known to induce galls on *C. henryi*, and it is not yet known whether the species also attacks the chestnut species *C. mollissima* found extensively in the same area, where the emergence time of the new species is concurrent with that of DK. The new species is known only from the type locality, Zhounin County and the neighboring Jianou Co. (Shuiyuan Township, 27.09° E, 118.44° N) in northern Fujian Province. Since the host plant, *C. henryi*, has a broad distribution, covering 14 provinces (Anhui, Fujian, Guangdong, Guangxi, Guizhou, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Sichuan, Yunnan, Zhejiang) in the south and east of China (Huang et al. 2013), the natural distribution of the new species might be rather extensive.

Etymology. The species is named after the Chinese pinyin name of the host plant *C. henryi*.

Discussion

The genus *Dryocosmus* currently comprises 28 species widely distributed in the northern hemisphere (Melika et al. 2011), including the recently described species *Dryocosmus rileypokei* (Buffington and Morita 2009) and *DZ* n.sp as described herein. The genus is particularly fascinating with regard to its broad range of host use; species of the genus induce galls on almost all genera of the beech family, including *Quercus* (Burks 1979, and references therein), *Chrysolepis* (Buffington and Morita 2009), *Castanopsis* (Melika et al. 2011), *Lithocarpus* (Melika et al. 2011), and *Castanea*. A recent study of higher level phylogeny of Fagaceae supported a monophyletic relationship of *Quercus, Chrysolepis, Castanopsis*, *Lithocarpus,* and *Castanea,* with *Quercus* being the most derived genus, while the other four genera being at the



Figs. 7–13. *DZ* Liu et Zhu, sp. nov. (7) Face (PT1), (8) occiput (PT2), (9) thorax, dorsal view (PT3), (10) thorax, lateral view (PT2), (11) metasoma, lateral view (PT3), (12) female antennae (PT2), (13) Propodeum (PT3). Scale bars in all SEM images are 500 μm, except 200 μm for Fig. 12.

Table 1. Morphological differences between DZ from DK

Features	DZ Liu et Zhu, new species	DK (/OCGW)
1. Ratio of height of eye to breadth of malar space	3.40–3.5	2.6–3.1[a]
2. Irradiating striation on lower face	Only distinct in lower half, absent or indistinct on upper half	Usually distinct throughout
3. Diagonal striation on mesopleuron	Distinct in the middle	Faint or completely without central striation.
4. Lateral propodeal carinae	Incomplete posteriorly	Percurrent[b]
5. Body color	Mostly black, except legs and proximal half of antenna	Varies, from brown, yellowish brown, to black
6. Setae in anteroventral area of mesopleuron	Sparse, but distinct	Absent
7. Appressed setae on lower face	Dense	Less dense

An anonymous reviewer reported that [a]up to 3.3 ratio was observed in OCGW and [b]incomplete lateral propodeal carinae were also observed in OCGW.

base of the clade (Nixon and Crepet 1989, Manos and Steele 1997, Manos et al. 2001). All six species of genus known from Western Palaearctic induce galls on white oaks, whereas most of the Nearctic species induce their galls on red oaks (section Lobatae) (Melika et al. 2011). The broad host spectrum of host use observed in the genus is unusual among oak gall wasps, or the tribe Cynipini. As more taxonomic work is done on the poorly studied Cynipini fauna in eastern Asia, where *Castanopsis*, *Lithocarpus,* and *Castanea* are disproportionately represented compared with the other regions of the world (Huang et al. 2013), more species of the genus may be discovered in the future.

Melika et al. (2011) commented that the taxonomy of the genus is currently problematic and badly needs revision. Although species with questionable membership in the genus seem to be those associated with the North American endemic oak section of "red oaks," or Lobate (Nixon 1985, 1997), some of those associated with the red oaks are indeed "true" *Dryocosmus*, such as *Dryocosmus punctata* (Z.L., unpublished data). Existing molecular studies indicated that the genus as currently perceived is polyphyletic (Acs et al. 2007; Stone et al. 2009), and hence, the genus is probably a conglomerate of species that actually belong to several genera (Melika et al. 2011). When revisionary work on genus mentioned by Melika et al. (2011) becomes available and the phylogeny of the genus is properly studied, the evolution of host use in Cynipini may be better understood in the light of the evolution of host use of the species currently included in the genus *Dryocosmus*.

Dryocosmus also contains the potentially devastating and globally important pest species attacking various chestnut tree species (Rieske 2007, Murakami 2009, Zhang 2009, Bosio et al. 2010, Rieske and Cooper 2011, Borowiec et al. 2014). OCGW is originally from China, although it was described for the first time from Japan, where it had become a pest of chestnut trees in a mere 10 yr since an accidental introduction (Yasumatsu 1951, Murakami 2009). In its native China, OCGW occurs in all the 16 chestnut producing provinces and currently causes a 15–30% yield loss each year in the country (Zhang 2009), although no outbreak was initially reported (Moriya et al. 2003). The species had spread rapidly throughout Japan by 1962 and has become one of the most important pests of chestnut trees in the country (Murakami 2009). It was subsequently introduced into Korea (Yasumatsu 1951, Abe et al. 2007), Nepal (Ueno 2006), North America (where it has spread to encompass at least 13 US states) (Payne et al. 1975; Cooper and Rieske 2007, 2010, 2011, Rieske 2007; Rieske and Cooper 2011; Lizotte and Fulbright 2015), and Europe, where it is found in Italy, France, Switzerland, Croatia, and Slovenia (EPPO 2005, 2006, 2007, 2008, 2010; Brussino et al. 2002; Bosio et al. 2010; Borowiec et al. 2014). DZ is the only other species in the genus that attacks chestnut trees and induce galls like OCGW on new shoot terminals that otherwise may flower and bear nuts. Therefore, the new species certainly deserves special attention as a potential candidate of important pest species of chestnuts.

We have provided a list of morphological features that can be used to separate DZ and *D. kuriphilus* (DK; OCGW) (Table 1), including some features that are relatively apparently highly reliable. However, it should be noticed that the morphological data on the new species DZ are based on samples taken from the limited known local populations, and higher degree of variation might be observed within the species as more populations are discovered and surveyed in the future. If that indeed happens, molecular methods should used to assist in the identification of species. Based on our previously published molecular study (Lu et al. 2012), the two species can be reliably separated using the *mt*DNA gene COI—the within species genetic distance for DK was 0.3% as sampled from eight populations from China and one population from Italy, whereas the genetic distance between DK and DZ were 2%. In addition, phylogenetic reconstruction clearly supported the monophyly of each species represented by multiple samples, with 99% bootstrap support for DK and DZ, respectively. Future study should explore the use of two other genes for this purpose, i.e., mtDNA cytb gene and nuclear 28S ribosomal RNA D2, which have also shown potential in species identification of species of Cynipidae (Acs et al. 2010).

DZ may have a much broader natural distribution than it is currently known for several reasons. First, the galls of the two species are very similar in morphology, and the two species also have almost identical phenology, at least with regard to the known summer generation of DZ. Second, field identification can be difficult and inaccurate even though the species can be easily separated from OCGW by means of molecular sequence typing (Lu et al. 2012) as well as by adult morphology, especially using SEM. On the other hand, even the separation of male and the female *Dryocusmus* wasps might be problematic without proper training, given the fact that the bisexual DZ has not become known until now. Furthermore, the identification of the host species of the two *Dryocosmus* species, *C. mollissima* and *C. henryi*, respectively, can be problematic when acorns and nuts are not available, and certainly hybridization occurs naturally (Liu et al. 2009).

The life history characteristics of OCGW are bewilderingly fascinating. On the one hand, there seems to be little variation in life history throughout the extensive geographic range of OCGW, including the expanded range of the species due to introduction, which may be attributable to its asexual reproduction mode that generate clones. On the other hand, OCGW appears to be very adaptive in host use and readily exploits local *Castanea* species in new habitats it invades (Bosio et al. 2010, Lin et al. 2010, Rieske and Cooper 2011). Recorded hosts include *C. mollissima* Blume*, C. henryi* (Skan)*, C. senguinii* Dode (Sun and Fan 1965, Long and Wang 2011), *Castanea crenata* Siebold & Zuccarini (Otake 1980), *Castanea dentata* (Marshall) (Payne et al. 1975, Dixon et al. 1986), and *Castanea sativa* Miller (Brussino et al. 2002; EPPO 2005; EFSA Panel on Plant Health 2010). Only two wild North American species of chestnut, *Castanea ozarkensis* Ashe and *Castanea pumila* Miller, are not known to have galls caused by OCGW, and caged inoculation experiments on *C. pumila* failed to induce gall formation (Dixon et al. 1986, but see Nixon (1997) for correct nomenclature of North American *Castanea* species). The host spectrum of DZ is not yet known but certainly needs to be clarified given the similarities in life history traits between the two species.

Details of the life history of the DZ are not yet known; the only data came from our field collection and laboratory rearing (Lu et al. 2012). In addition to sharing one of the several host plant species of OCGW, gall morphology, host plant parts utilized, and emergence time of the new species are extremely similar to that of the latter. OCGW is invariably univoltine (Tamura 1960, Sun and Fan 1965, Payne et al. 1975, Payne 1978, Zhang 2009, Long and Wang 2011, Matosevic et al. 2014), with adult females emerging in late May to early July and laying eggs in the buds of chestnut trees (Tamura 1960, Cho and Lee, 1963, Sun and Fan 1965, Long and Wang 2011). Larvae hatch within 30–40 d and early-instar larvae overwinter inside buds (Cho and Lee 1963, Dixon et al. 1986, Long and Wang 2011). The overwintering larvae start to develop the following spring when the buds of chestnut trees burst, inducing more or less globular galls on green chestnut organs including shoot tip, leaf base, and main vein of leaf. Galls are usually multilocular and are green or red in color. Larvae feed for 20–30 d inside galls and then pupate (Dixon et al. 1986, Long and Wang 2011).

The greatest life history difference between DZ and OCGW is that the former reproduces bisexually, while the latter reproduces asexually. Since sexual reproduction generates genetic diversity rapidly through genome recombination, and asexual reproduction generates clones with limited variation, species that reproduce sexually often have increased adaptability than asexual species, rendering pest management measures less effective compared with the latter (e.g., Burdon and Marshall 1981, Marie-Solange et al. 2007, Paynter et al. 2012). Given the known amazing adaptability of the asexual OCGW to new host species in new areas, and the fact that DZ and OCGW cause similar damage to chestnut trees, an accidental introduction of the sexual DZ may potentially create a pest problem even more complicated than that caused by OCGW.

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