# A new Diplolepis Geoffroy (Hymenoptera, Cynipidae, Diplolepidini) species from China: a rare example of a rose gall-inducer of economic significance 

Juli Pujade-Villar', Yiping Wang², Wenli Zhang ${ }^{2,3}$, Noel Mata-Casanova', Irene Lobato-Vila ${ }^{1}$, Avar-Lehel Dénes ${ }^{4}$, Zoltán László ${ }^{4}$<br>I Department of Animal Biology, University of Barcelona, Barcelona 08028, Catalonia, Spain 2 College of Forest and Biotechnology, Zhejiang Agricultural and Forestry University, Lin'an 311300, China $\mathbf{3}$ Lanzhou Agro-technical research and Popularization Center, Lanzhou, 730010, China 4 Hungarian Department of Biology and Ecology, Babes,-Bolyai University, Cluj-Napoca 400006, Romania<br>Corresponding author: Zoltán László (laszlozoltan@gmail.com)

Academic editor: A. Köhler \| Received 13 September 2019 | Accepted 2 December 2019 | Published 17 January 2020
http://zoobank.org/49036F44-5497-44C4-A0E7-77BC87099A44
Citation: Pujade-Villar J, Wang Y, Zhang W, Mata-Casanova N, Lobato-Vila I, Dénes A-L, László Z (2020) A new Diplolepis Geoffroy (Hymenoptera, Cynipidae, Diplolepidini) species from China: a rare example of a rose gall-inducer of economic significance. ZooKeys 904: 131-146. https://doi.org/10.3897/zookeys.904.46547


#### Abstract

A new species of the genus Diplolepis Geoffroy, Diplolepis abei Pujade-Villar \& Wang sp. nov. is described on host plant Rosa sertata Rolfe $\times R$. rugosa Thunb. from China with an integrative approach based on molecular and morphological data. Diagnosis, distribution and biology of the new species are included and illustrated. This species is the first known rose gall-inducer of economic importance. A review of Eastern Palearctic species of Diplolepis is given and a key to the Chinese fauna is presented.


## Keywords

gall wasp, Kushui rose, new species, phytophagous, taxonomy

## Introduction

The family Cynipidae (Hymenoptera, Cynipoidea) includes around 1400 species, all of them exclusively phytophagous and divided into 12 tribes (Ronquist 1999; Ronquist et al. 2015). The tribe Diplolepidini induces galls exclusively on Rosa spp. L. and

[^0]is distributed in the Holarctic Region. Diplolepidini is a monophyletic group characterized by a unique autapomorphy within Cynipidae: the presence of a longitudinal furrow on the mesopleuron of the mesosoma. Only two genera, Diplolepis Geoffroy, 1762 and Liebelia Kieffer, 1903, are currently included in this tribe, together comprising 58-62 species (Melika 2006; Ronquist et al. 2015). Diplolepis and Liebelia can be easily distinguished on the basis of the following diagnostic morphological characters (Melika 2006): in Diplolepis, antennae of females and males are $12-15$-segmented, the radial cell of the forewing is closed along the wing margin and the hypopygium is plough-shaped, while in Liebelia antennae are 16-segmented, the radial cell is open and the hypopygium is not plough-shaped.

The genus Diplolepis includes six European species (Pujade-Villar 1993; PujadeVillar and Plantard 2002), some of them also occurring in Western Asia and North Africa (Morocco) (Mimeur 1949; Melika 2006); eight Eastern Palaearctic species (Abe et al. 2007; Wang et al. 2013) and 31 North American species (Burks 1979; Shorthouse and Ritchie 1984). The relatively low number of species recorded from the Eastern Palaearctic could be due to a lack of sampling efforts; herein we describe a new species for the Eastern Palaearctic fauna.

Guo et al. (2013) and Wang et al. (2013) published the first record of D. rosae in China, which later has been proved to be a misidentification. The eastern boundary of $D$. rosae's distribution range may be the Ural, Altay, Tianshan, Pamir, Hindukush Mountains (Belizin 1957). However, a single mention of a more eastern location: India, appears in the key of Belizin (1957: 11), a record that has not been confirmed since then. Therefore, we presume that $D$. rosae does not occur in China.

Diplolepis is morphologically characterized by having both the head and the mesosoma black or reddish brown; antennae 14-15 segmented (except D. hunanensis with only 12) in both sexes and with relatively large, cylindrical flagellomeres; pronotum dorsomedially short; pronotal plate not pronounced; scutellar foveae faint or absent; mesopleuron with a broad, crenulate mesopleural furrow; propodeum rugose and lateral propodeal carinae usually indistinct; metanotal trough broad, apically truncate; forewings moderately or strongly uniformly or partially infuscate, margins with short but distinct cilia; radial cell closed along the wing margin; 2 r of forewings usually with a prominent median vein stump anterolaterally projected; nucha dorsally short; hypopygium plough-shaped and hypopygial spine slightly longer than broad, with some sparse short setae (Melika 2006; Ronquist et al. 2015).

Diplolepis are known to cause galls mainly on feral roses. Accidental infections on cultivated Rosa rugosa and its hybrids were reported from North America, but neither of the Diplolepis species became serious pests of rose cultivars (Shorthouse 2001). There are no published data on the large-scale economic importance of Diplolepis.

## Material and methods

The terminology for the morphology of cynipid gall wasps used in this work follows Liljeblad and Ronquist (1998) and Melika (2006). Abbreviations for the forewing
venation are taken from Ronquist and Nordlander (1989), and the cuticular surface terminology, from Harris (1979). Measurements and abbreviations used in this work are: F1-F12, first and subsequent flagellomeres; post-ocellar distance (POL), the distance between the inner margins of the posterior ocelli; ocellar-ocular distance (OOL), the distance from the outer margin of the posterior ocellus to the inner margin of the compound eye; LOL, 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.

Scanning electron microscope (SEM) images of the described species were taken with a FEI Quanta 200 ESEM at high voltage ( 15 kV ) without gold coating in the "Serveis de Microscopia Electrònica" of the University of Barcelona.

Specimens of the new species were collected in Lanzhou City of Gansu Province and they are deposited in the Hymenoptera Collection of Zhejiang Agricultural and Forest University (ZAFU) and in the University of Barcelona (UB, Col. JP-V).

Genomic DNA was extracted from two individuals using an ISOLATE II Genomic DNA Kit (Bioline, Germany), following the protocol provided by the manufacturer. The mitochondrial cytochrome c oxidase subunit I (COI) sequences were amplified using the standard LCO1490 and HCO2198 primer pair (Folmer et al. 1994) in a 50 $\mu$ reaction volume at a $42{ }^{\circ} \mathrm{C}$ annealing temperature. PCR products were purified with the Wizard SV Gel and PCR Clean-Up System (Promega, USA) and sent for sequencing to Macrogen Inc. (Europe).

Sequences were downloaded and verified with the BLAST (Johnson et al. 2008). Further, sequences for all available Diplolepis species were also downloaded from the NCBI database and the BOLD System (see Fig. 5 for reference numbers). The sequences were aligned using a Clustal W algorithm (Thompson et al. 1994) in BioEdit (Hall 1999). A Bayesian inference (BI) tree was generated in MrBayes (Ronquist et al. 2012) for $1,000,000$ generations, sampled every $1000^{\text {th }}$ step, until the average standard deviation of split frequencies fell below 0.01 . The first $25 \%$ of the sampled trees were discarded as burn-in. The GTR $+\mathrm{G}+\mathrm{I}$ model of molecular evolution was selected as most suitable for this analysis, based on the Bayesian information criterion in jModelTest2 (Darriba et al. 2012). Interspecific $p$-distances were calculated in MEGA X (Kumar et al. 2018).

## Results

## Diplolepis abei Pujade-Villar \& Wang sp. nov.

http://zoobank.org/E35C7053-8145-4CAC-B453-C6A657A11330
Figs 1, 2

Type material. Holotype: $Q$ deposited in UB with the following labels: 'Lanzhou (Gansu Province), ex Rosa sertata $\times$ R. rugosa, (03.ii.2011) 15.iii.2011, col. Sheng Maoling' (white label); 'Diplolepis abei Pujade-Villar \& Wang, desig. JP-V-2017' (red label). Paratypes: $11 q q$ with the same labels as the holotype: $8 q+$ in ZAFU, $3 q q$ in UB.

Diagnosis. This species is characterized by having the following morphological characters: head smooth to alutaceous, mesoscutum alutaceous with piliferous punctures, scutellum rugose with a more delicate sculpture in the centre of the disk; legs, including coxae, reddish; forewings hyaline but slightly smoky in both the radial and the $3^{\text {rd }}$ cubital cells, never with a dusky cloud around veins; second metasomal tergite short. It differs from the rest of species known from China because veins of its forewings are not infuscate. In addition, the deciduous galls have numerous long stout sharp-pointed spines unlike other known species. Molecular results: the two sequenced individuals represent one haplotype (GeneBank accession number: MN434062). Based on the BI tree the species is part of a polytomous clade with a group consisting of D. fructuum, D. mayri and D. rosae, and with D. spinosissimae (Fig. 5). The average $p$-distance compared to the other species is $9.73 \%$ (Table 1 ), with the lowest values shown when compared to $D$. fructuum ( $6.38 \%$ ) and D. spinosissimae (6.39\%).

Description. Female. Length. Body length 3.3-3.6 mm ( $N=4$ ).
Color (Fig. 2g). Head and mesosoma uniformly black. Antenna black; pedicel, and sometimes also the scapus, lighter. Tegulae brown. Mandibles reddish, with black tips, maxillary and labial palpi brown. Legs reddish, including coxae; last tarsi (and sometimes the $4^{\text {th }}$ tarsomere) darker. Metasoma reddish; basal and posterior parts and hypopygium, brown. Wings hyaline but slightly smoky in both the radial and the $3^{\text {rd }}$ cubital cells; wing veins distinct, dark brown, never with infuscate clouds.

Head (Fig. 1a, b). Head trapezoidal in frontal view, transverse, as wide as the mesosoma, shiny, with short sparse white setae, 1.3 times as broad as high in frontal view and 2.1 times as broad as long seen from above. Lower face smooth to alutaceous, with distinct piliferous punctures; median elevated area alutaceous. Clypeus quadrangular, broader than high, smooth to alutaceous, flattened; anterior tentorial pits, epistomal sulcus and clypeo-pleurostomal line, distinct, ventral margin straight. Gena smooth to alutaceous, with piliferous punctures and basally with some weak carinae, not broadened behind the compound eye (not visible in frontal view) and 2.0 times as broad as the cross diameter of the compound eye in lateral view. Malar space smooth to coriaceous, around 0.5 times as long as height of compound eye. Transfacial distance 1.5 times as long as height of compound eye; diameter of antennal toruli 1.4 times as long as the distance between them, and distance between torulus and eye margin 1.2 times longer than torulus diameter. Inner margins of compound eyes divergent. Frons and vertex shiny, alutaceous; occiput dull, coriaceous. POL 0.75 times as long as OOL; OOL 2.0 times longer than the diameter of the lateral ocelli and 6.6 times longer than LOL.

Antenna (Fig. 1c). 14-segmented, 1.5 times longer than head plus mesosoma; pedicel slightly longer than broad; F1 very long, 4.0 times longer than pedicel and nearly 1.8 times longer than F2; F2 as long as F3; F12 slightly longer than F11; placodeal sensilla present in all the funicular segments, but only apically in F1 and in the anterior half in F2. Antennal formula: 6: 4(x3): 16: 9: 9: 8: 8: 8:7:7:7:7:7:9.

Mesosoma (Fig. 1d-g). Mesosoma curved and slightly longer than high in lateral view, with short white setae. Pronotum very narrow, coarsely punctured, sparsely


Figure I. Diplolepis abei Pujade-Villar \& Wang $q$ sp. nov. a head in frontal view $\mathbf{b}$ genae in dorsal view $\mathbf{c}$ antenna $\mathbf{d}$ mesosoma in lateral view $\mathbf{e}$ mesosoma in dorsal view $\mathbf{f}$ mesosoma in dorso-lateral view $\mathbf{g}$ propodeum $\mathbf{h}$ metasoma in lateral view.
haired in the middle and coriaceous with some carinae in the basal part. Scutum wider than long and at least 1.7 times longer than the scutellum, alutaceous, with distinct punctures. Notauli complete, convergent posteriorly; median mesoscutal line very shallow, reaching at least the level of tegulae; parapsidal lines visible but poorly impressed, narrow, shining, reaching tegulae level; anterior parallel lines distinct,


Figure 2. Diplolepis abei Pujade-Villar \& Wang $q$ sp. nov. a-b galls c dissected gall with last instar larva $\mathbf{d}$ forewing $\mathbf{e}$ lateral and ventral view of the $3^{\text {rd }}$ instar larva $\mathbf{f}$ lateral and ventral view of the last instar larva $\mathbf{g}$ lateral habitus.
smooth, extending to half the length of the scutum. Scutellum as long as wide, with parallel lateral margins, rounded posteriorly, dull, rugose, with a more delicate sculpture in the centre of the disk. Scutellar foveae short, transversal, inconspicuous, rugose,
not delimited posteriorly. Mesopleuron smooth and shiny, with a strong transverse dull rugose furrow; mesopleural triangle with numerous delicate wrinkles. Metapleural sulcus reaching the mesopleuron slightly above half of its height; axillula ovate, smooth, distally with some short rugae, without setae; subaxillular bar coriaceous, with very delicate carinae. Dorsellum smooth with some carinae, inferiorly convex. Metanotal trough smooth, shining, with some longitudinal parallel weak wrinkles and without setae; ventral impressed area alutaceous, without delicate longitudinal wrinkles, shining. Propodeum laterally rugose, medially smooth; lateral propodeal carinae anteriorly with three straight carinae and strongly curved outwards in posterior 2/3, delimiting a closed area.

Legs. Tarsal claws simple, without a basal lobe.
Forewing (Fig. 2d). Radial cell partially closed and margin pigmented, 2.3 times longer than wide, first abscissa of radius nearly straight, 2 r with an additional median prolongation into the radial cell. Areolet distinct, large. Rs+M well-marked and reaching basalis in the lower third.

Metasoma (Fig. 1h). Slightly longer than head plus mesosoma length (1.1×); in lateral view, 1.4 times longer than high. Second metasomal tergite short, reaching 1/3 if the metasoma; metasomal tergites 2 to 5 without punctures, subsequent tergites alutaceous. Hypopygium plough-shaped, shiny, smooth and large; prominent part of the ventral spine of the hypopygium very thin, 3.0 times longer than broad, with sparse white setae, apical setae short, not extending behind apex of the spine.

Male: unknown.
Gall (Fig. 2a-c). Resembles the North American gall Diplolepis bicolor (Harris, 1841), but the new species has more abundant spines and a different coloration. It also resembles D. japonica (Walker, 1874), but the shape and the length of the spines are very different. The galls of the new species are spherical-shaped, appearing as monothalamous or one-celled swellings bearing numerous long, stout and sharp-pointed spines that are longer than the diameter of the galls. Their surface is smooth and glabrous. Galls arise on branches, buds or leaf veins of Rosa sertata Rolfe $\times R$. rugosa Thunb., usually in groups. Young galls are pea green or reddish green and soft, gradually turning greyish green and harder when maturing. The inner cell is large, and the delimiting wall of parenchymatous cells is thick, usually 1.5 mm thick. Mature galls are deciduous.

Host. The new species was collected on the Chinese Kushui rose, a hybrid of Rosa sertata Rolfe $\times R$. rugosa Thunb. which is cultivated mainly in Gansu Province (China) for its oil. Rosa rugosa also occurs at the collection site (a Kushui rose plantation) but no galls were found on them despite growing only a few meters from Kushui roses supporting large numbers of galls. To the best of our knowledge this may be the first known Diplolepis species that causes significant agricultural loss. In Gansu Province (China) the $R$. sertata $\times R$. rugosa hybrid is commonly planted for its high yields of flowers and oil. The infected shrubs may suffer up to $70 \%$ yield loss according to rose oil farmers (We et al. 2014). In infected plantations, $D$. abei is considered a significant pest which reduces rose flower numbers and subsequent rose oil yields.
Table I. P-distance values for sequences of Diplolepis abei sp. nov. and all available Diplolepis species. Abbreviations: dradi: Diplolepis radicum, drosfol: D. rosaefolii, dfusif: D. fusiformans, dtrif: D. triforma, dspinsa: D. spinosa, degla: D. eglanteriae, dbic: D. bicolor, dbass: D. bassetti, doreg: D. oregonensis, dignot: D. ignota, dvari: D. variabilis, dgrac: D. gracilis, dpoli: D. polita, dnodu: D. nodulosa, dnebu: D. nebulosa, dfruc: D. fructuum, dcalif. D. californica, dmayr: D. mayri, drosa: D. rosae, dnerv: D. nervosa, dspinsi: $D$. spinosissimae, dspnov: $D$. abei sp. nov.

|  | dradi | drosfol | dfusif | dtrif | dspinsa | degla | dbic | dbass | doreg | dignot | dvari | dgrac | dpoli | dnodu | dnebu | dfruc | dcalif | dmayr | drosa | dnerv | dspinsi |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| dradi |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| drosfol | 13.14 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| dfusif | 11.81 | 4.55 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| dtrif | 6.54 | 11.98 | 10.87 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| dspinsa | 6.60 | 12.59 | 11.87 | 7.15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| degla | 15.25 | 13.90 | 12.81 | 13.81 | 14.31 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| dbic | 14.17 | 11.54 | 9.57 | 13.56 | 14.73 | 12.06 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| dbass | 14.59 | 11.36 | 9.48 | 13.14 | 14.98 | 12.31 | 6.24 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| doreg | 6.99 | 12.63 | 10.62 | 7.82 | 8.90 | 13.56 | 11.81 | 12.48 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| dignot | 11.59 | 10.93 | 9.32 | 10.48 | 11.98 | 11.98 | 10.40 | 11.81 | 10.15 |  |  |  |  |  |  |  |  |  |  |  |  |
| dvari | 11.92 | 10.75 | 9.15 | 10.48 | 11.98 | 11.98 | 10.40 | 11.81 | 10.15 | 0.83 |  |  |  |  |  |  |  |  |  |  |  |
| dgrac | 13.09 | 9.97 | 8.65 | 11.48 | 12.31 | 12.65 | 10.40 | 10.65 | 10.98 | 5.66 | 5.49 |  |  |  |  |  |  |  |  |  |  |
| dpoli | 14.14 | 11.13 | 8.71 | 12.65 | 13.70 | 12.42 | 5.68 | 2.61 | 11.56 | 10.59 | 10.59 | 9.93 |  |  |  |  |  |  |  |  |  |
| dnodu | 7.04 | 11.68 | 10.76 | 5.71 | 7.60 | 15.09 | 13.84 | 13.87 | 8.21 | 10.87 | 10.87 | 11.20 | 12.92 |  |  |  |  |  |  |  |  |
| dnebu | 11.76 | 10.75 | 9.15 | 10.32 | 12.15 | 11.81 | 10.23 | 11.65 | 9.98 | 0.17 | 0.67 | 5.49 | 10.43 | 10.70 |  |  |  |  |  |  |  |
| dfruc | 11.76 | 9.62 | 8.32 | 10.82 | 12.48 | 12.81 | 9.40 | 9.15 | 9.48 | 8.65 | 8.65 | 9.48 | 7.82 | 10.21 | 8.49 |  |  |  |  |  |  |
| dcalif | 5.75 | 12.38 | 11.13 | 6.58 | 6.66 | 14.24 | 14.32 | 14.32 | 7.70 | 11.49 | 11.74 | 11.49 | 13.43 | 6.86 | 11.66 | 10.99 |  |  |  |  |  |
| dmayr | 11.00 | 8.60 | 7.54 | 10.39 | 12.23 | 12.06 | 9.13 | 8.88 | 9.38 | 8.71 | 8.71 | 9.38 | 7.76 | 10.44 | 8.54 | 2.35 | 10.39 |  |  |  |  |
| drosa | 11.33 | 9.21 | 8.21 | 10.80 | 12.81 | 12.40 | 9.72 | 9.72 | 10.18 | 8.71 | 8.71 | 9.55 | 8.71 | 10.78 | 8.54 | 3.02 | 10.90 | 2.09 |  |  |  |
| dnerv | 13.46 | 10.27 | 9.76 | 12.39 | 13.69 | 10.23 | 10.83 | 11.44 | 13.78 | 11.09 | 11.09 | 12.48 | 10.80 | 13.06 | 10.92 | 10.23 | 12.40 | 9.71 | 10.14 |  |  |
| dspinsi | 12.27 | 9.36 | 8.67 | 11.48 | 13.52 | 12.50 | 8.84 | 8.50 | 12.25 | 7.91 | 8.08 | 9.61 | 7.71 | 10.85 | 7.91 | 6.03 | 11.91 | 5.61 | 6.29 | 9.62 |  |
| $d s p n$ | 12.89 | 10.13 | 8.89 | 12.00 | 13.03 | 12.26 | 9.15 | 10.10 | 11.05 | 8.81 | 8.63 | 8.80 | 8.49 | 11.54 | 8.63 | 6.38 | 12.19 | 6.90 | 7.94 | 10.11 | 6.39 |

Biology. Only females are known (Fig. 2g). Galls appear in mid-April and larvae occupy the most part of the larval chamber (Fig. 2c, e, f). Adults emerge in early March of the following year.

Comment. In Wang et al. (2013) and Guo et al. (2013), the material corresponding to this new species is determined as D. rosae. In Wang et al. (2013) seven males and nine females were cited. The reason why there are more females (12) in the present paper than those mentioned in Wang et al. (2013) is that the sexes were confused in Wang et al. (2013): four of the specimens were considered males, although they were females. The other four specimens of the 16 mentioned in Wang et al. (2013) are lost.

Distribution. China (Gansu Province).
Etymology. Named in honour of the Japanese cynipidologist and friend, Prof. Yoshihisa Abe (Biosystematics Laboratory, Graduate School of Social and Cultural Studies, Kyushu University, Fukuoka, Japan).

## Discussion

According to Abe et al. (2007), D. brunneipes (Ashmead, 1904) from Japan has an uncertain status, and D. kunugi Shinji, 1938, also from Japan, is not a Diplolepis. Thus, based on Abe et al. (2007) a total of 11 species occur in the Palaearctic Region of which five are distributed in the Eastern Palearctic (Fig. 3). Wang et al. (2013) subsequently described three new Diplolepis species from China, increasing the number of recorded species present in the Eastern Palaearctic to eight: $D$. japonica (Walker, 1874) from Japan and possibly also from China (see comments below); D. nigriceps Vyrzhikovskaja, 1963, D. nitidus Vyrzhikovskaja, 1963 and D. variegatus Vyrzhikovskaja, 1963 from Kazakhstan; D. radoszkowskii Kieffer, 1904 from Uzbekistan and Tajikistan; and D. flaviabdomenis Wang, Liw \& Chen, 2013, D. hunanensis Wang, Liw \& Chen, 2013 and D. minoriabdomenis Wang, Liw \& Chen, 2013 from China). From Asia Minor and Western Palearctic region six species are known: $D$. fructuum (Rübsaamen, 1895) from Turkmenistan; $D$. mayri (Schlechtendal, 1876) from Siberia, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan and Kyrgyzstan; D. nervosa (Curtis, 1838) (= D. centifoliae (Hartig, 1840)) from Western Kazakhstan; D. rosae (Linnaeus, 1758) from Southern Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan and Kyrgyzstan; D. spinosissimae (Giraud, 1859) from Kazakhstan; and a single species, D. eglanteriae (Hartig, 1840), from Europe and North Africa (Morocco).

Diplolepis rosae is also recorded from India (Belizin 1957), which must be confirmed, and erroneously from China (Guo et al. 2013; Wang et al. 2013) - this record was a misidentification of the new species described here. Records of $D$. nervosa and $D$. spinosissimae by Kovalev (1965) must be confirmed too. The only D. japonica specimen mentioned from China (Wang et al. 2013) collected in Malaise trap presents some differences in the sculpture with respect the redescription of D. japonica provided by Yasumatsu and Taketani (1967) (see taxonomic key below); for this reason, we consider


Figure 3. Distribution of the 11 species of Diplolepis of the Palearctic; in yellow, the species distributed exclusively in the Eastern Palearctic. Palaearctic map obtained from https://www.google.com/maps/@57.7 $164944,49.0396796,9792440 \mathrm{~m} /$ data $=!3 \mathrm{~m} 1!1 \mathrm{e} 3$. The inset image pointing out in red the Gansu Province (and thus the collecting location) was obtained from https://en.wikipedia.org/wiki/Gansu.
this specimen here as Diplolepis nr japonica, pending collection of more specimens and its gall. Diplolepis nr japonica could be an undescribed species.

The new species, $D$. abei Pujade-Villar \& Wang induces spherical galls with spines resembling $D$. japonica and, some forms of $D$. nervosa in the Palaearctic Region. However, $D$. abei differs from these species by producing galls with relatively longer, pointed, hard and woody spines. Adults of $D$. nervosa differ from the new species by having POL slightly longer than OOL, the scutum coriaceous, the scutellum strongly elongated (nearly 2.0 times longer than wide) with subparallel margins and slightly constricted basally, the scutellar foveae present (large, transversely ovate and smooth) and forewings are hyaline (neither with infuscate areas nor smoky marks); on the other hand, in D. japonica the radial cell is shorter (around 2.0 times as long as wide), forewings are hardly infuscate around radial cell, the face is coarsely rugose, the mesoscutum is smooth and the $2^{\text {nd }}$ tergite occupies more than half the length of metasoma.

## The species of Diplolepis present in China can be differentiated from each other according to the following key:

1 Radial cell relatively long, longer than 2.5 times as long as broad (Figs 2d, 4a).... 2

- Radial cell shorter, around 2.0 times as long as broad (Fig. 4b-d)

2 Radial cell closed, with infuscate veins and 2 r vein without projection into the radial cell (Fig. 4a). Malar distance long, around 0.75 times as long as compound eye height.
D. flaviabdomenis

- Radial cell partially open in margin, without infuscate veins and 2 r vein with a projection into the radial cell (Fig. 2d). Malar distance shorter, around 0.5 times as long as compound eye height
D. abei sp. nov.

3 Head strongly transverse in frontal view, 1.7 times wider than high (Fig. 4e) and slightly wider than mesosoma. Median mesoscutal line faintly present posteriorly over $1 / 4$ of the entire length of mesoscutum. Occiput sculptured but never with striae. Propodeum sparsely setose 4

- Head trapezoid-shaped in frontal view, around 1.5 times wider than high (Fig. 4g) and distinctly narrower than mesosoma. Median mesoscutal line absent or only present by a very short depression (extending over $1 / 10$ of mesoscutum length). Occiput smooth and shiny with striae. Propodeum densely pubescent (Fig. 4h).... 5
4 Vertex and mesoscutum smooth and shiny. Occiput coarsely punctured. From Japan and Korea.
D. japonica
- Vertex (Fig. 4f) and mesoscutum (Fig. 4i) distinctly alutaceous to coriaceous. Occiput coriaceous. From China
D. japonica

5 Antennae 12-segmented, with scapus and pedicel yellowish-brown. POL around 2.0 times longer than OOL. Parapsidal lines absent, almost invisible. Radial cell closed (Fig. 4b). Third and following metasomal tergites with distinct punctures dorso-laterally
D. bunanensis

- Antennae 14-segmented, with scapus and pedicel black. POL around 3.0 times longer than OOL. Parapsidal lines distinct and extending almost the entirely length of mesoscutum. Radial cell completely open in margin (Fig. 4c). All metasomal tergites without punctures
D. minoriabdomenis

Diplolepis abei is the first Diplolepis associated with a gall from China; D. flaviabdomenis, $D$. hunanensis and $D$. minoriabdomenis were described from material collected by Malaise traps (Wang et al. 2013). This new species occurs on R. sertata $\times$ rugosa, and there are around 100 described species of Rosa in China (Wu et al. 2003) of which at least 65 species are endemic (eFloras 2008); therefore, the richness of Diplolepidini (Diplolepis and Liebelia) is probably greater. As an example of how poorly understood Diplolepis is in Eastern Palaearctic, a species of Periclistus Förster, 1869, which are obligate inquilines of Diplolepis, was recently described from China (Pujade-Villar et al. 2015). Its host remains unidentified, but the gall morphology differs from that of $D$. abei.

Finally, D. abei Pujade-Villar \& Wang is morphologically closely related to 'rosae' clade according to Pujade-Villar and Plantard (2002). This clade includes four Western Palaearctic species: D. rosae, $D$. mayri, $D$. fructuum and $D$. spinosissimae. It is defined morphologically, according to Pujade-Villar (1993), by the following characters: scutellum rounded, medial sulcus absent or rudimentary (in species with smoky wing


Figure 4. a forewing of $D$. flaviabdomenis $\mathbf{b}$ forewing of $D$. hunanensis $\mathbf{c}$ forewing of $D$. minoriabdomenis, and $\mathbf{d}$ forewing of $D$. nr japonica $\mathbf{e}$ head in frontal view of $D$. nr japonica (reused from Wang et al. 2013) $\mathbf{f}$ head in dorsal view of $D$. nr japonica (reused from Wang et al. 2013) $\mathbf{g}$ head in frontal view of $D$. hunanensis $\mathbf{h}$ lateral mesosoma of $D$. minoriabdomenis $\mathbf{i}$ mesoscutum of $D$. nr japonica.
areas) or present (in species without strongly smoky areas), F1 at least 1.7 times F2, straight in females (curved and shortly expanded in males), head in frontal view oval and galls not detachable from plant tissues. The 'rosae' clade has been also confirmed


Figure 5. Bayesian inference (BI) tree of the Diplolepis species that have available mitochondrial COI sequences. Numbers on the branches represent posterior probabilities (PP).
by Zhang et al. (2019) as the 'Palaearctic multi-chamber subclade'. The closeness of $D$. abei to the 'rosae' clade is also confirmed by the molecular genetic results based on COI sequences. It is the first species of this group present in China.

## Acknowledgments

We are grateful to Yoshihisa Abe (Kyushu University, Fukuoka, Japan) for his comments regarding Diplolepis japonica and the gall of the species described here. The authors are grateful to Chris Looney (Washington State Department of Agriculture, Olympia, United States) for his review, comments and suggestions of the manuscript.

The authors are also thankful for Jessica Awad, Yuanmeng M. Zhang and Evandson J. dos Anjos-Silva for their reviews on the submitted manuscript. The molecular work was done at the Interdisciplinary Research Institute on Bio-Nano-Sciences of BabeșBolyai University, Treboniu Laurian 42, 400271, Cluj-Napoca Romania. The study was supported by the National Natural Science Foundation of China (31472032 and 31071970) and Zhejiang Provincial Natural Science Foundation for Distinguished Young Scholars (LR14C040002).

## References

Abe Y, Melika G, Stone GN (2007) The diversity and phylogeography of cynipid gallwasps (Hymenoptera: Cynipidae) of the Oriental and Eastern Palaearctic Regions, and their associated communities. Oriental Insects 41: 169-212. https://doi.org/10.1080/00305316 .2007.10417504
Belizin VI (1957) Rose gall wasps (Hymenoptera, Cynipidae) in the Fauna of the USSR. Entomologicheskoje Obozrenije 36: 925-934. [in Russian]
Burks BD (1979) Superfamily Cynipoidea. In: Krombein KV, Hurd Jr PD, Smith DR, Burks BD (Eds) Catalog of Hymenoptera in America North of Mexico. Symphyta and Apocrita (Parasitica) (Vol. 1). Smithsonian Institution Press, Washington, DC, 1045-1059.
Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nature Methods 9: 1-772. https://doi.org/10.1038/nmeth. 2109
eFloras (2008) Published on the Internet. http://www.efloras.org [accessed 13 January 2019] Missouri Botanical Garden, St. Louis, MO \& Harvard University Herbaria, Cambridge, MA.
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: 294-299.
Guo R, Wu BM, Zhang WL, Wang YQ, Wang YP (2013) First discovery of an invasive gallformer insect pest, Diplolepis rosae, in China. Chinese Journal of Applied Entomology 50(2): 500-504.
Hall T (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, Nucleic Acids Symposium Series 41: 95-98.
Harris R (1979) A glossary of surface sculpturing. State of California, Department of Food and Agriculture. Occasional Papers of Entomology 28: 1-31.
Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. Nucleic acids research 36: 5-9. https://doi.org/10.1093/ nar/gkn201
Kovalev OV (1965) Gall wasps (Hymenoptera, Cynipidae) from the south of the Soviet Far East. Entomologicheskoye Obozrenije 44(1/2): 46-73. [In Russian] [English translation in Entomological Review 44: 25-38]
Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:15471549. https://doi.org/10.1093/molbev/msy096

Liljeblad J, Ronquist F (1998) A phylogenetic analysis of higher-level gall wasp relationships (Hymenoptera: Cynipidae). Systematic Entomology 23: 229-252. https://doi. org/10.1046/j.1365-3113.1998.00053.x
Melika G (2006) Gall Wasps of Ukraine. Cynipidae. Vestnik zoologii, supplement 21(1): 1-300.
Mimeur JM (1949) Contribution a l'etude des zoocecidies du Maroc. Encyclopedie Entomologique 24 P. LeChevalier, Paris, 259 pp.
Pujade-Villar J (1993) Revisió de les espécies del génere Diplolepis de l'Europa centro occidental (Hym., Cynipidae) amb una especial atenció a la Península Ibérica. Historia Animalium 2: 57-76.
Pujade-Villar J, Plantard O (2002) About the validity of Diplolepis fructuum (Rübsaamen) and some new synonyms in Diplolepis nervosa (Curtis). In: Melika G, Thuróczy C (Eds) Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. Agroinform, Budapest, 135-142.
Pujade-Villar J, Wang YP, Guo R, Chen XX (2015) Revision on Palaearctic species of Periclistus Förster with description of a new species and its host plant gall (Hymenoptera, Cynipidae). ZooKeys 596: 65-75. https://doi.org/10.3897/zookeys.596.5945
Ronquist F (1999) Phylogeny, classification and evolution of the Cynipoidea. Zoologica Scripta 28: 139-164. https://doi.org/10.1046/j.1463-6409.1999.00022.x
Ronquist F, Nordlander G (1989) Skeletal morphology of an archaic cynipoid, Ibaliarufipes (Hymenoptera: Ibaliidae). Entomologica Scandinavica, supplement 33: 1-60.
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: 539-542. https://doi.org/10.1093/sysbio/sys029
Ronquist F, Nieves-Aldrey JL, Buffington ML, Liu Z, Liljeblad J, Nylander JAA (2015) Phylogeny, Evolution and Classification of Gall Wasps. The Plot Thickens. PLoS ONE 10(5): e0123301. https://doi.org/10.1371/journal.pone. 0123301
Shorthouse JD (2001) Galls induced by cynipid wasps of the genus Diplolepis (Cynipidae, Hymenoptera) on cultivated shrub roses in Canada. Acta horticulturae 547: 91-92. https:// doi.org/10.17660/ActaHortic.2001.547.10
Shorthouse JD, Ritchie AJ (1984) Description and biology of Diplolepis triforma, new species (Hymenoptera: Cynipidae) inducing galls on the stems of Rosa acicularis. The Canadian Entomologist 116(12): 1623-1636. https://doi.org/10.4039/Ent1161623-12
Thompson JD, Higgins DG, Gibson TJ (1994) ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Research 22: 4673-4680. https://doi. org/10.1093/nar/22.22.4673
Wang YP, Guo R, Liu ZW, Chen XX (2013) Taxonomic study of the genus Diplolepis Geoffroy (Hymenoptera, Cynipidae, Diplolepidini) in China, with descriptions of three new species. Acta Zootaxonomica Sinica 38(2): 317-327.
We BM, Wang YQ, Zhang WL, Xu Q, Ma H, Wang X (2014) Preliminary report of investigation on Torymus bedeguaris. Plant protection 40(5): 148-151. [in Chinese]

Wu C, Li C, Lu L, Jiang S, Alexander V, Bartholomew B, Brach AR, Boufford DE, Ikeda H, Ohba H, Robertson KR, Spongberg SA (2003) Rosaceae AL Jussieu. In: Wu ZY, Raven PH, Hong DY (Eds) Flora of China (Vol. 9). (Pittosporaceae through Connaraceae). Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis.
Yasumatsu K, Taketani A (1967) Some remarks on the commonly known species of the genus Diplolepis in Japan. Esakia 6: 77-86.
Zhang Y, László Z, Looney C, Dénes AL, Hanner R, Shorthouse JD (2019) DNA barcodes reveal inconsistent species boundaries in Diplolepis rose gall wasps and their Periclistus inquilines (Hymenoptera: Cynipidae). The Canadian Entomologist 151(6): 717-727. https://doi.org/10.4039/tce.2019.59


[^0]:    Copyright Juli Pujade-Villar et al. This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

