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LIFE CYCLE OF *DISHOLCASPIS QUERCUSVIRENS* (HYMENOPTERA: CYNIPIDAE) WITH A DESCRIPTION OF THE SEXUAL GENERATION

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ABSTRACT

The life cycle of *Disholcaspis quercusvirens* Ashmead (Hymenoptera: Cynipidae), a species previously known only from its asexual generation, is closed with the association of generations confirmed using molecular data. The description and diagnosis of the sexual generation of *D. quercusvirens* is given. Morphological and molecular data are used to determine that *Disholcaspis quercussuccinipes* Ashmead is a **new synonym** of *D. quercusvirens*.

Key Words: Cynipini, life cycle, alternation of generations, *Disholcaspis*

RESUMEN

El ciclo biológico de *Disholcaspis quercusvirens* Ashmead (Hymenoptera: Cynipidae), una especie conocida previamente sólo de su generación asexual, ha sido cerrado gracias a la asociación de las generaciones a partir de datos moleculares. Se describe y se dan los caracteres diagnósticos de la generación sexual de *D. quercusvirens*. Se utilizan los datos morfológicos y moleculares para determinar que *Disholcaspis quercussuccinipes* es una **nueva sinónimo** de *D. quercusvirens*.

Palabras Clave: Cynipini, ciclo biológico, generaciones alternantes, *Disholcaspis*

For most of its history the genus *Disholcaspis* Dalla Torre & Kieffer has been known only from the Nearctic, with 38 species in America north of Mexico (Burks 1979), and 12 from Mexico (Beutenmüller 1911; Kinsey 1920, 1937, 1938; Pujade-Villar et al. 2009). Two species, *Disholcaspis weldi* (Beutenmüller) and *D. lapiei* Kieffer have been synonymized and transferred to the genus *Kinseyella* Pujade-Villar & Melika (Pujade-Villar et al. 2010). Recently, 2 species, *Disholcaspis bettyannae* Medianero & Nieves-Aldrey and *D. bisethiae* Medianero & Nieves-Aldrey, were described from Panama (Medianero & Nieves-Aldrey 2011) and one species, *D. costaricensis* Melika & Pujade-Villar, from Costa Rica (Melika et al. 2011). Thus, there are currently 51 species of *Disholcaspis* known from the Nearctic and Neotropics. All species of *Disholcaspis* induce detachable stem galls on white oaks (section *Quercus* of the genus *Quercus* L.; Fagales: Fagaceae), with the exception of the Californian species, *D. chrysolepidis* (Beutenmüller), which is associated with *Quercus chrysolepis* Liebm. from the golden-cup oak section (*Quercus* section *Protobalanus*). All but one species are known only from their asexual generations; the exception, *D. eldoradensis* (Beutenmüller), distributed in California, Oregon and Washington (USA), is also known from a sexual

generation that induces small inconspicuous bud galls (Evans 1972).

In this paper we describe the sexual generation of another known species, *Disholcaspis quercusvirens* (Ashmead). The identity of the sexual generation was demonstrated by biological experiments (results will be published elsewhere), and with the use of molecular data, presented here.

Materials and Methods

Morphological Methods

The current terminology for morphological structures and abbreviations for forewing venation follows Melika et al. (2006); cuticular surface terminology follows that used by Harris (1979). Measurements and abbreviations used here include: F1-F15, 1st and subsequent flagellomeres; POL (post-ocellar distance) is the distance between the inner margins of the posterior ocelli; OOL (ocellar-ocular distance) is the distance from the outer edge of a 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 is measured from the margin of the wing to the Rs vein.

Digital images of wasp anatomy were produced with a digital Nikon Coolpix 4500 camera attached to a Leica DMLB compound microscope, followed by processing in CombineZP (Alan Hadley) and Adobe Photoshop 6.0 by G. Melika.

Specimens involved in morphological and molecular analyses, including the voucher specimens, are deposited in the collection of the Plant Health and Molecular Biology Laboratory, National Food Chain Safety Office, Budapest, Hungary (curator G. Melika).

Molecular Methods

DNA was extracted using a chelex method (see Nicholls et al. 2010) from a single leg from 6 asexual females that had emerged from galls fitting the description of those induced by *D. quercussuccinipes* Ashmead, 8 asexual females that had emerged from *D. quercusvirens* galls, and 4 sexual males from the putative sexual generation galls of *D. quercusvirens*. Collection details for these specimens are provided in Table 1. Different subsets of these were sequenced for 2 genes that show levels of variation in gall wasps that allow differentiation even of sister species (see Nicholls et al. 2012): a fragment of the mitochondrial cytochrome *b* gene was sequenced using the primers CB1/CB2 (Jermiin & Crozier 1994) following methods described in Nicholls et al. (2010), and the ITS2 gene was sequenced using the primers ITS2f/ITS2r and methodology described in Campbell et al. (1993). PCR products were sequenced using BigDye v3.1 terminator chemistry and run on an ABI3730 capillary sequencer. Sequences were checked and aligned using the software Sequencher v4.9.

Results

Diagnosis for the Asexual Females of *Disholcaspis* (Fig. 1; Suppl. Fig. 1)

Fully winged robust specimens, with dense setae and coriaceous or rugose surface sculpture on the head and mesosoma; metasoma densely pubescent; the head is usually narrower than the mesosoma, transverse in anterior view, with genae strongly broadened behind eyes, the malar sulcus always absent; notauli usually are incomplete, impressed in the posterior ⅔ of the mesoscutum; scutellar foveae indistinctly delimited, confluent; all tarsal claws with strong basal lobe. The prominent part of the ventral spine of the hypopygium is short, 2.0-4.0 times as long as broad or shorter, subapical setae are long, dense, reaching far beyond the apex, never form a dense truncate tuft (Melika & Abrahamson 2002).

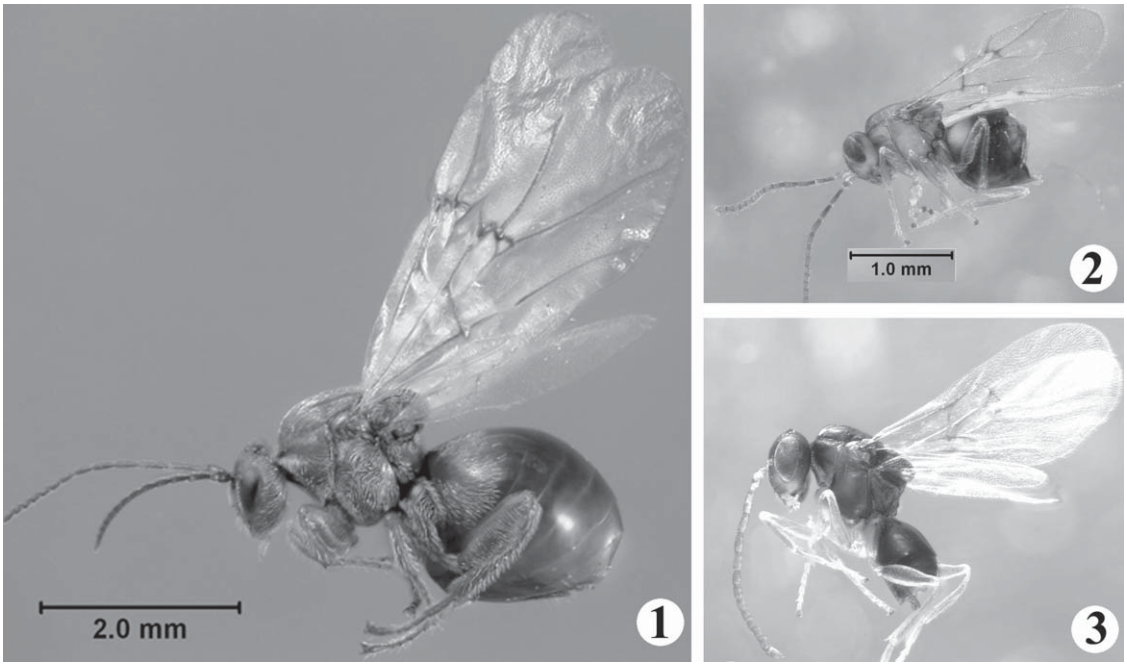
Diagnosis for the Sexual Females of *Disholcaspis* (Figs. 2-3, 12-20; Suppl. Figs. 2-3, 12-20)

Fully winged small specimens, 2.1-3.5 mm, with brown body. The head is equal or slightly narrower than the mesosoma, rounded in anterior view. Female antennae with 12 flagellomeres, male with 13. The mesosoma with setae only on the pronotum laterally; the mesoscutum anteriorly microreticulate or delicately coriaceous, smooth and shiny posteriorly; notauli incomplete, absent or very indistinct in the anterior ¼ of the mesoscutum; the mesoscutellum only slightly elongated, nearly quadrangular, uniformly rugose; scutellar foveae indistinctly delimited, with a shiny smooth bottom. Forewings longer than the body, with distinct brown veins and long cilia on margins. Tarsal claws with a distinct basal lobe. The metasoma with sparse short white setae laterally on the 2nd metasomal tergite, subsequent tergites smooth, bare. The prominent part of the ventral spine of the hypopygium is short, with subapical setae reaching behind the apex of the spine.

The sexual generation of *Disholcaspis* is similar to those of some other Cynipini genera. It differs from sexual generations of *Dryocosmus* Giraud and *Biorhiza* Westwood by the presence of the basal lobe on the tarsal claws. Three other genera, *Andricus* Hartig, *Acraspis* Mayr and *Cynips* L., possess a basal lobe on their tarsal claws, like *Disholcaspis*. In *Andricus*, however, scutellar foveae are distinctly delimited, the mesoscutum with stronger surface sculpture, the mesoscutellum is rounded, as long as broad and only very slightly overhanging the metas-

TABLE 1. DETAILS OF SPECIMENS USED IN MOLECULAR ANALYSES INCLUDING GENBANK ACCESSION NUMBERS.

Species	Sex	Generation	Collection Locality	Collection date	Host oak species	cytb sequence	ITS sequence
Disholcaspis quercussuccinipes	Female	Asexual	Archbold Biological Station, FL	21-Oct-2007	*Q. geminata*	KF039986	KF040003
Disholcaspis quercussuccinipes	Female	Asexual	Archbold Biological Station, FL	21-Oct-2007	*Q. geminata*	KF039987	
Disholcaspis quercussuccinipes	Female	Asexual	Archbold Biological Station, FL	21-Oct-2007	*Q. geminata*	KF039988	KF040004
Disholcaspis quercussuccinipes	Female	Asexual	Archbold Biological Station, FL	21-Oct-2007	*Q. geminata*	KF039989	KF040005
Disholcaspis quercussuccinipes	Female	Asexual	Archbold Biological Station, FL	21-Oct-2007	*Q. geminata*	KF039990	
Disholcaspis quercussuccinipes	Female	Asexual	Archbold Biological Station, FL	1-Dec-1994	*Q. geminata*		KF040006
Disholcaspis quercusvirens	Female	Asexual	Archbold Biological Station, FL	1-Nov-1994	*Q. geminata*	KF039991	
Disholcaspis quercusvirens	Female	Asexual	Archbold Biological Station, FL	18-Oct-2007	*Q. geminata*	KF039992	KF040007
Disholcaspis quercusvirens	Female	Asexual	Archbold Biological Station, FL	18-Oct-2007	*Q. geminata*	KF039993	
Disholcaspis quercusvirens	Female	Asexual	Penney Farms, FL	18-Dec-2007	*Q. virginiana*	KF039994	
Disholcaspis quercusvirens	Female	Asexual	Penney Farms, FL	18-Dec-2007	*Q. virginiana*	KF039995	
Disholcaspis quercusvirens	Female	Asexual	Penney Farms, FL	18-Dec-2007	*Q. virginiana*	KF039996	
Disholcaspis quercusvirens	Female	Asexual	Jacksonville, FL	19-Oct-2007	*Q. virginiana*	KF039997	KF040008
Disholcaspis quercusvirens	Female	Asexual	Jacksonville, FL	19-Oct-2007	*Q. virginiana*	KF039998	
Disholcaspis quercusvirens	Male	Sexual	Penney Farms, FL	4-Apr-2008	*Q. virginiana*	KF039999	
Disholcaspis quercusvirens	Male	Sexual	Penney Farms, FL	4-Apr-2008	*Q. virginiana*	KF040000	
Disholcaspis quercusvirens	Male	Sexual	Penney Farms, FL	4-Apr-2008	*Q. virginiana*	KF040001	KF040009
Disholcaspis quercusvirens	Male	Sexual	Lake Manatee SRA, FL	16-Apr-2008	*Q. geminata*	KF040002	



Figs. 1-3. *Disholcaspis quercusvirens*: 1, asexual female, 2, sexual female, 3, male (photos by J. Platt Bird).

cutellum; the mesopleuron sculptured, the head usually slightly transverse in anterior view; the prominent part of the ventral spine of the hypopygium 4.0 times or more longer than broad. Sexual *Disholcaspis* most closely resembles the sexual *Acraspis* and *Cynips*. In *Disholcaspis* the head is rounded in anterior view (always transverse or trapezoid in *Acraspis* and *Cynips*, Figs. 21 and 24; Suppl. Figs. 21 and 24); notauli incomplete (always complete in *Acraspis* and *Cynips*, Figs. 24 and 29; Suppl. Figs. 24 and 29), the mesoscutum at least in the anterior half coriaceous (mesoscutum entirely smooth and shiny in *Acraspis* and *Cynips*, Figs. 22 and 25; Suppl. Figs. 22 and 25); the mesoscutellum is subquadrangular, only slightly longer than broad, scutellar foveae more or less well-delimited, with smooth shiny bottom, the mesoscutellum only slightly overhanging the metascutellum (mesoscutellum distinctly longer than broad, scutellar foveae absent, the mesoscutellum strongly overhanging the metascutellum in *Acraspis* and *Cynips*, Figs. 23 and 26; Suppl. Figs. 23 and 26). The sexual generation of *Philonix* Fitch differs from all the mentioned genera by the entirely smooth and polished mesoscutum and mesoscutellum.

Disholcaspis Quercusvirens (Ashmead, 1881)

Cynips quercusvirens Ashmead, 1881 (female and gall).

Cynips quercussuccinipes Ashmead, 1881 (female and gall), **syn. nov**.

Andricus (*Andricus*) *virens* Ashmead, 1885.

Andricus (*Andricus*) *succinipes* Ashmead, 1885.

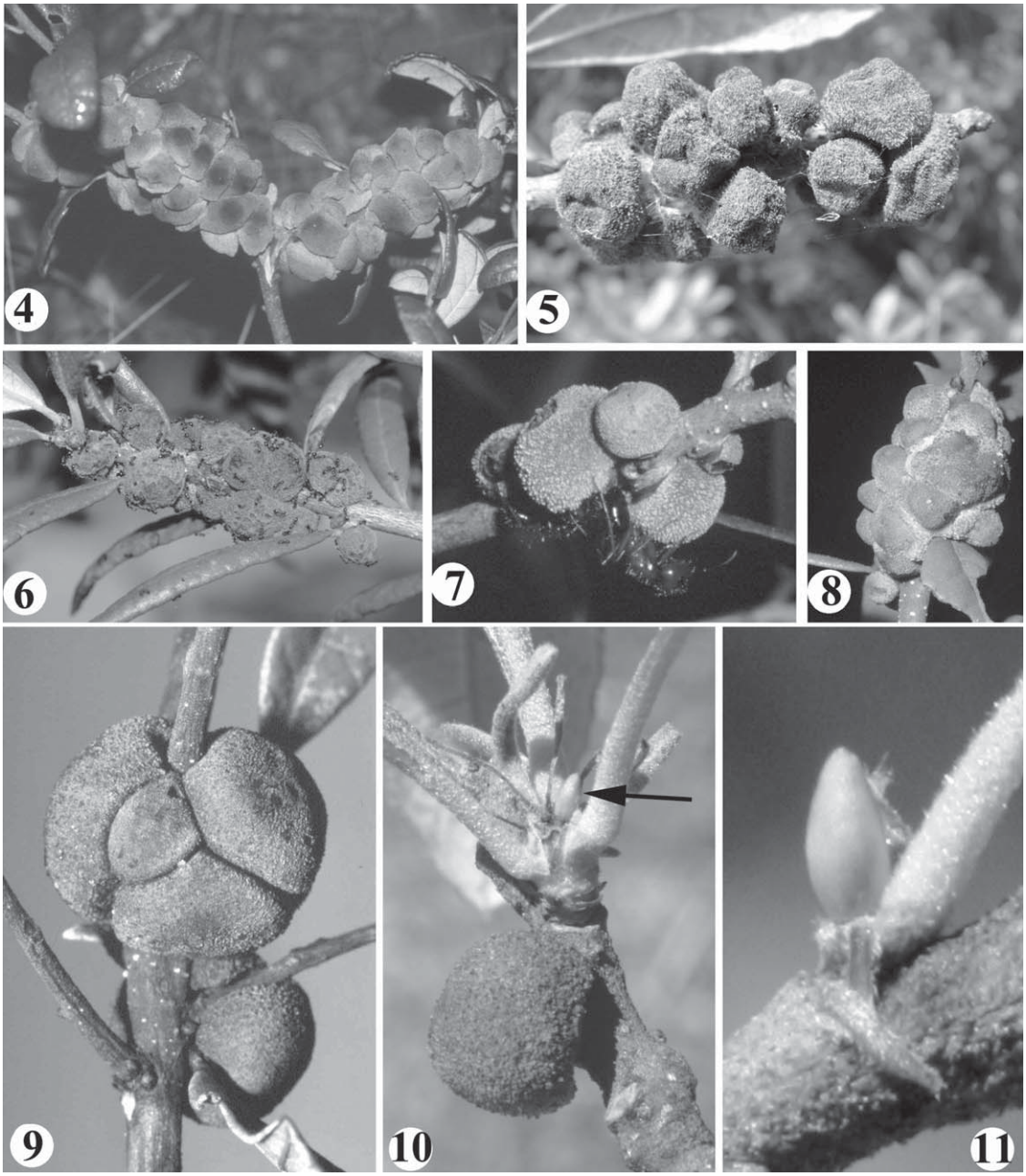
Cynips quercusficigera Ashmead 1885.

Holcaspis succinipes Ashmead, 1887.

Holcaspis ficigera Ashmead, 1887.

Ashmead (1887) did not mention his previously described *Cynips quercusvirens*. Dalla Torre (1893) mentioned *Holcaspis succinipes* and *H. ficigera*, however, *Andricus virens* (Ashmead) was not included into his catalogue under any name. Beutenmüller (1909), in his revision of the genus *Holcaspis* Mayr, mentioned only *H. succinipes* and *H. ficigera*, without naming *H. virens*.

Dalla Torre & Kieffer (1910) moved *Holcaspis quercus-ficigera* (Ashmead) and *H. succinipes* into the newly established *Disholcaspis*; however, *Andricus virens* was left in *Andricus*, re-named as *Andricus quercusvirens* (Ashmead). Weld (1921) synonymized *Cynips quercusficigera* to *Disholcaspis virens*. Later, Weld (1951) listed 2 species: *Disholcaspis succinipes* (Ashmead) and *D. virens* (Ashmead). The names *Disholcaspis quercussuccinipes* and *D. quercusvirens* were used by Weld (1959) in his "Cynipid galls of the Eastern United States".



Figs. 4-11. Galls of *Disholcaspis quercusvirens*. 4-9, asexual galls, 10, an old asexual and young growing sexual gall; 11, sexual bud gall (photos by J. Nicholls and G. Melika).

Synonymy of *Disholcaspis quercusvirens* (Ashmead) and *D. quercussuccinipes* (Ashmead) and Matching of *D. quercusvirens* Generations

Types of *Cynips virens* Ashmead and *Cynips quercussuccinipes* Ashmead, deposited at the USNM, were examined. We were unable to locate the type of *Cynips quercusficigera* Ashmead. Large series of asexual females of *D. quercusvirens* and *D. quercusuccinipes*, deposited in the general collection of USNM and also a large number of specimens reared by the authors were examined. The original descriptions of *D. quercusvirens* and *D. quercussuccinipes* (Ashmead 1881) are identical with no apparent differences. Only minor differences in the gall shape can be detected, with galls

induced by *D. quercussuccinipes* typically being smaller, more rounded in latitudinal cross-section with a more pointed top. However, such differences may well be caused by the influence of different host oak individuals (Figs. 4-10; Suppl. Figs. 4-10). Since no appreciable characters were found for the separation of adults of *D. quercusvirens* and *D. quercussuccinipes*, the 2 species are synonymized herein: *D. quercussuccinipes* is a **syn. nov.** of *D. quercusvirens*.

DNA sequence data confirmed the synonymisation of *D. quercussuccinipes* with *D. quercusvirens*, and also confirmed the matching of sexual and asexual generations in *D. quercusvirens* that was proposed by Bird et al. (2013). In total 17 cytochrome *b* sequences were obtained (5 from asexual females emerged from *quercussuccinipes*-type galls, 8 from asexual females emerged from *quercusvirens*-type galls and 4 sexual *D. quercusvirens* males; see Table 1 for GenBank accessions). Variation among this set was limited, with a maximum of 2 bases out of 433 (0.46%) different between any 2 sequences, well within the variation levels found within other species (e.g., Nicholls et al. 2012). In the majority of cases sequences were identical between individuals from *quercussuccinipes*-type galls and *quercusvirens*-type galls, and between sexual and asexual *D. quercusvirens*. A similar pattern was found in the ITS2 data (4 from asexual females from *quercussuccinipes*-type galls, 2 from asexual females from *quercusvirens*-type galls and one sexual *D. quercusvirens* male; Table 1), with identical or virtually identical sequences among the 3 categories of specimen.

Sexual Generation of *Disholcaspis quercusvirens* (Ashmead)

Material Examined

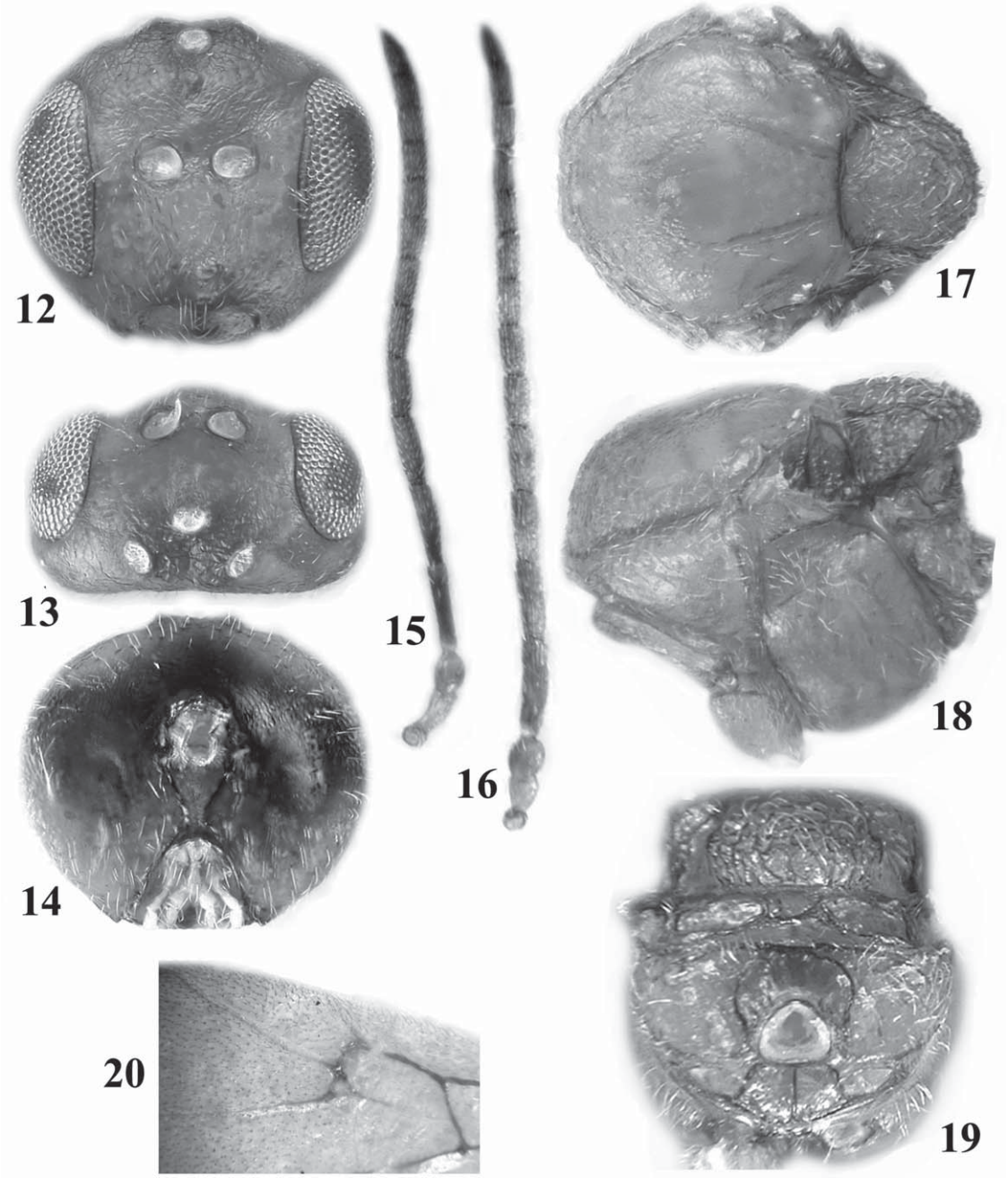
Four females and 2 males "USA, FL, Clay Co (USA), Penny Farms, Shadownlawn Nursery, *Q. virginiana*, 4-IV-08 J.Platt & J.Cash"; 2 females and one male: "USA, FL, Lake Manatee State Recreation Area, coll. 16 Apr 2008, on *Q. geminata*, leg. J.A. Nicholls". Two of the males from Penney Farms and one male from Lake Manatee SRA were included in the molecular analyses.

Diagnosis for the Sexual Generation

The only known sexual *Disholcaspis* is *D. eldoradensis* (Beutenmüller), known from the states of California, Oregon and Washington within the USA. In *D. quercusvirens* the body is light brown, F1 of female antenna longer than F2, the mesoscutum coriaceous in the anterior half, notauli incomplete; 2nd metasomal tergite smooth, shiny, while in *D. eldoradensis* the body is dark brown to black, F1 of female antenna nearly equal to F2; the mesoscutum smooth, shiny, notauli are complete; 2nd metasomal tergite punctuate posteriorly. In males of *D. quercusvirens* F1 modified, slightly excavated and swollen apically, while in *D. eldoradensis* F1 is straight, not modified, not excavated and swollen apically.

Descriptions of Sexual Females and Males

Sexual Female (Figs. 2, 12-15, 17-20; Suppl. Figs. 2, 12-15, 17-20). Body, antennae and legs light brown, with dark brown interocellar area and darker central propodeal area; metasomal tergites dark brown to black dorsally. Head delicately coriaceous, rounded in anterior view, with sparse short white setae, especially on lower face; 2.0 times as broad as long from above, 1.2 times as broad as high, as broad as width of mesosoma in anterior view. Gena very delicately coriaceous, not broadened behind eye in front view, 3.0 times narrower than cross diameter of eye from lateral view; malar space very delicately coriaceous, without radiating striae, 0.2 times as long as height of eye. POL 1.3 times as broad as OOL, OOL 1.7 times as long as length of lateral ocellus and 1.4 times as long as LOL. Inner margins of eyes parallel. Transfacial distance nearly as long as height of eye and 1.6 times as long as height of lower face (distance between antennal rim and tip of clypeus); diameter of torulus only very slightly longer than distance between them, distance between torulus and inner margin of eye 1.2 times as large as the diameter of torulus; lower face delicately coriaceous, with relatively dense white short setae, median elevated area delicately coriaceous. Clypeus rectangular, nearly 2.0 times as broad as high, delicately coriaceous, with slightly elevated central area, ventrally not emarginate; anterior tentorial pits and clypeo-pleurostomal line indistinct, shallow; epistomal sulcus broad, deep and shiny, distinctly delimiting clypeus from lower face. Frons very delicately coriaceous to alutaceous, with very few short white setae, in some specimens darker than the rest of head, especially towards the frontal ocellus; central area slightly impressed right before the frontal ocellus. Vertex and occiput uniformly delicately coriaceous; interocellar area more dull rugose, medially impressed. Occiput rounded, gradually, without carina, continuing into postocciput which is delicately coriaceous, impressed around occipital foramen. Labial palpus 3-segmented, maxillary palpus 4-segmented. Antenna 14-segmented, longer than mesosoma; pedicel 1.4 times as long as broad, F1 slightly longer than F2, 2.5 times as long as pedicel; F3-F4 equal in length; subsequent flagellomeres shorter; F12 1.2 times as long as F11; placoid sensillae on F3-F12, absent on F1-F2, long, in one row. Mesosoma 1.2 times as long as high. Pronotum alutaceous, with few



Figs. 12-20. Sexual *Disholcaspis quercusvirens*: 12-14, head, female: 12, anterior view, 13, dorsal view, 14, posterior view. 15-16, antenna: 15, female, 16, male. 17, mesosoma, dorsal view, female, 18, mesosoma, lateral view, female, 19, metascutellum and propodeum, dorsoposterior view, 20, forewing, part, female.

delicate striae in the most ventro-lateral edge, emarginate and impressed along propleuron; propleuron delicately transversely striate, flat in medio-central part. Mesoscutum delicately uniformly coriaceous to alutaceous, with few short white setae, especially along notauli and lateral edges; very slightly longer than broad in dorsal view (largest width measured on the level of the base of tegulae); from the level of tegula narrowing down towards posterior end. Notauli uniformly broad, incomplete, extending to ⅔ of mesoscutum length, well-impressed only in

posterior half of mesoscutum, slightly converging posteriorly; anterior parallel lines invisible; parapsidal lines distinct, well-impressed, broad, with shiny glabrous surface, extending to ⅓ engthh of mesoscutum; median mesoscutal line absent. Mesoscutellum slightly longer than broad, dull rugose, overhanging metanotum; scutellar foveae indistinctly delimited, with shiny bottom. Mesopleuron smooth with some very delicate indistinct transverse striae, acetabular carina very indistinct delimiting a narrow area laterally; mesopleural triangle uniformly dull coriaceous; dorsoaxillar area smooth, lateral axillar area coriaceous; preaxilla and axilla dull rugose; axillar carina with some longitudinal striae; axillula rugose, without setae; height of subaxillular bar posteriorly more than height of metanotal trough; metapleural sulcus reaching mesopleuron in the upper ⅓ of its height. Metascutellum uniformly delicately coriaceous, very short, slightly higher than height of smooth, shiny ventral impressed area; metanotal trough uniformly smooth, with sparse and short white setae; central propodeal area dark brown, smooth, shiny, very short, only slightly higher than height of ventral impressed area; lateral propodeal carinae strongly bent outwards in the middle; lateral propodeal area coriaceous, with dense white setae. Nucha very short, with some longitudinal delicate carinae. Tarsal claws with basal lobe. Forewing longer than body, hyaline, with long cilia on margins, veins brown, radial cell 2.6-2.8 times as long as broad; R1 invisible, Rs indistinct, nearly straight, nearly reaching margin of wing; areolet small, triangular, closed and indistinct. Metasoma slightly longer than head+mesosoma; metasomal tergite 2 occupies almost half of metasoma length in dorsal view, with few short basal white setae laterally; all tergites smooth, shiny, in some specimens with very sparse delicate, hardly detectable, very superficially impressed micropuntures. Ventral spine of hypopygium short, prominent part 2.0 times as long as broad, with few sparse short white setae, which extend beyond the apex of spine. Body length 2.1-3.4 mm.

Male (Figs. 3, 16; Suppl. Figs. 3, 16). Similar to female; however, head is black, except light brown lower face and clypeus; antennae and legs pale brown to yellow; mesosoma, except light brown pronotum, and metasoma are dark brown. Antenna 15-segmented, F1 slightly excavated and expanded apically, placodeal sensilla on F1-F12.

Gall (Figs. 10-11; Suppl. Figs. 10-11).

Sexual generation. Single galls develop in apical or lateral buds on young shoots, never in clusters. Unilocular. A small, inconspicuous, thin-walled gall, 2-3 mm × 1.5 mm when mature. The gall surface is smooth and light brown, without ribs. A proportion of galls are partially concealed within the bud scales. The apex of the gall is pointed, without a small tuft of hairs.

Biology

Alternate asexual and sexual generations are known, both developing on *Quercus virginiana* Mill., *Q. geminata* Small and *Q. minima* (Sarg.) Small. The sexual generation galls develop through the early spring and mature in late Mar, and the adults emerge immediately from early Apr until May (Bird et al. 2013). The asexual galls form through the summer and mature in autumn. Adult asexual females emerge from late autumn.

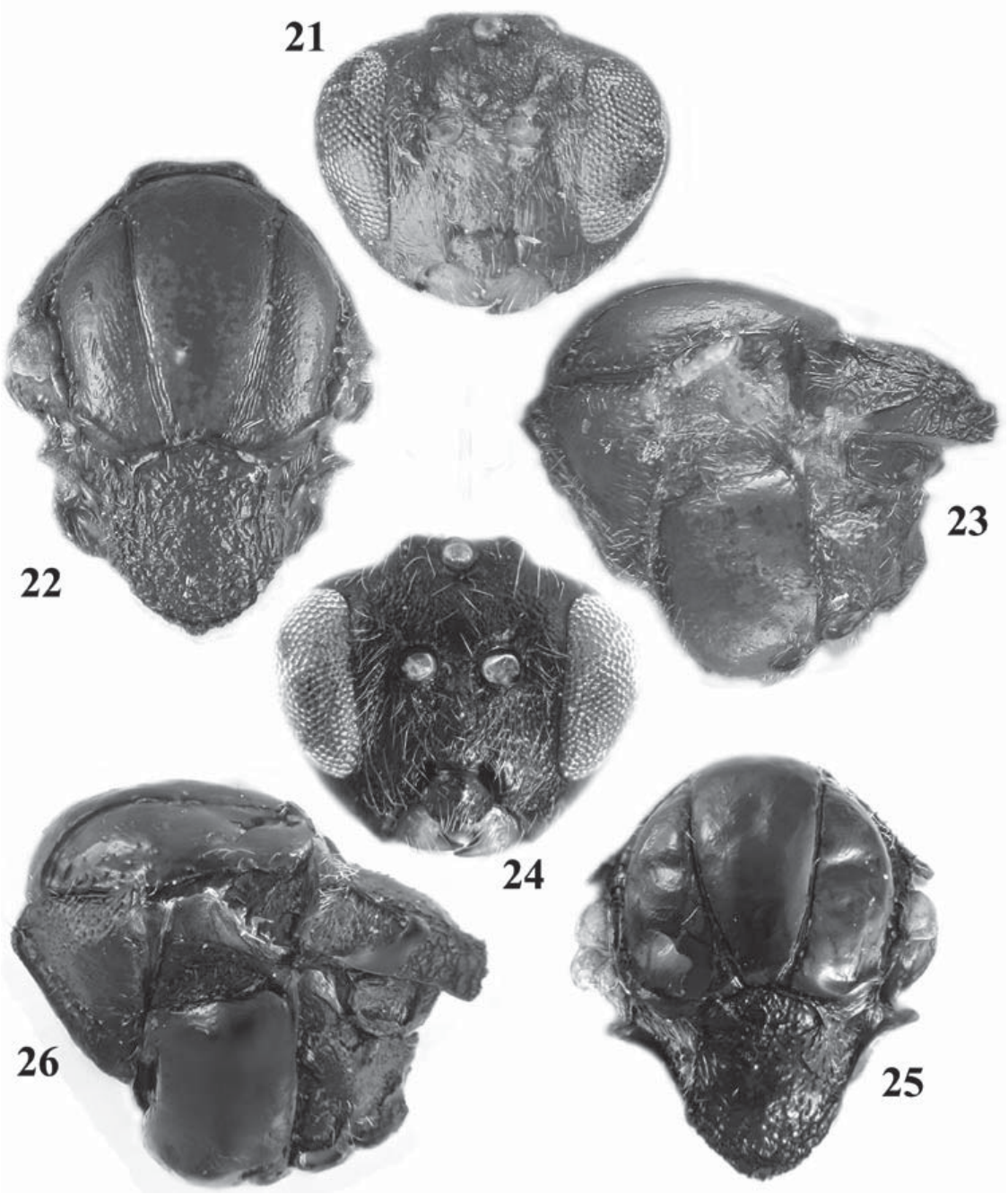
Distribution

USA: Florida, Georgia, South Carolina, Mississippi, Louisiana and Texas (Burks 1979). *Disholcaspis quercussuccinipes* was thought to be restricted to Florida (Burks 1979).

Discussion

Most oak gall wasps (tribe Cynipini) have 2 generations per year, with alternating sexual and asexual generations. The loss of the sexual generation resulting in a species with only a single asexual generation per year is exceedingly rare, being confirmed from only 3 species within the entire radiation of approximately 1,400 species of Cynipini (Csóka et al. 2005). Hence it is likely that further study of the appropriate host oaks will reveal sexual generations for the other species within the genus *Disholcaspis*. However, discovering these sexual generations is likely to require some effort as the 2 currently known sexual generation galls of *Disholcaspis* are small, often hidden in bud scales and develop very rapidly. To date there is preliminary evidence for 2 further, as yet undescribed, sexual generations within *Disholcaspis*: sexual adults reared from bud galls very similar to those described herein that most likely represent the sexual generation of *D. quercusmamma* (C. McEwen & S. Digweed pers. comm.), and observations by the authors of similar (but as yet unreared) bud galls on *Q. chapmanii*, the host oak species for *D. quercusomnivora*.

Morphological variation suggests that the genus *Disholcaspis* does not form a monophyletic group, a pattern confirmed by preliminary molecular phylogenetic reconstructions (J. A. Nicholls, unpubl. data) and consistent with the confused taxonomic history of this genus. The majority of recognized species form a natural grouping based on both adult morphology and molecular information, with both *D. quercusvirens* and the type species of the genus, *D. quercusglobulus*, falling into this group of "typical"



Figs. 21-26. 21-23, *Acraspis gemmula*, sexual female: 21, head, anterior view, 22, mesosoma, dorsal view, 23, mesosoma, lateral view. 24-26, *Cynips quercusfolii*, sexual female: 24, head, anterior view, 25, mesosoma, dorsal view, 26, mesosoma, lateral view.

Disholcaspis. However, a small group of species from the western United States, comprising *D. chrysolepidis* (Beutenmüller), *D. conalis* Weld, *D. corallina* (Bassett), *D. plumbella* Kinsey, *D. sulcata* (Ashmead), *D. truckeensis* (Ashmead), and *D. washingtonensis* (Gillette) have been provisionally placed into *Disholcaspis*, but are morphologically distinct. In this group the ventral spine of the hypopygium is broad throughout its entire length, the length of the projecting part of

the spine is less than or equal to its width, Rs is slightly curved in the apical one third and slightly expanded, the lateral propodeal carinae are curved and lyre-shaped. In the "typical" *Disholcaspis* species the prominent part of the ventral spine is longer and needle-like, the projecting part at least 2.0-3.5 times as long as broad, Rs is straight, the radial cell is slightly longer and the propodeal carinae are fragmented. Melika & Abrahamson (2002) also erroneously transferred 3 *Andricus* species into this group: *A. lasius* (Ashmead), A. *reniformis* McCracken & Egbert, and *A. spectabilis* Kinsey. Given recent examination of adult morphology and gall structure of all these distinct species, and taking into account the apparent rarity of shifts among host oak section (Stone et al. 2009), it appears that there are 3 major lineages within *Disholcaspis*. One consists of the "typical" *Disholcaspis*, including the species examined in detail in this paper, all of which gall white oaks (*Quercus* section *Quercus*) including the type species of the genus. The second is a morphologically distinct group associated with golden cup oaks (*Quercus* section *Protobalanus*): *D. chrysolepidis*, *D. truckeensis*, *D. lasius* and *D. reniformis*. The third consists of the morphologically-divergent species that are associated with white oaks: *D. conalis*, *D. corallina*, *D. plumbella*, *D. sulcata* and *D. washingtonensis* placed by Burnett (1977) into a new genus *Weldia;* however, this name is not valid since it was not published and the name *Weldia* was preoccupied by Yoshimoto (1962) for species within the Eucoilidae. Finally, the very distinct species *D.* (*Andricus*) *spectabilis*, with a closed radial cell in the forewing, forms a unique entity, divergent from all other *Disholcaspis* species. Further work is still required to provide better resolution of the taxonomy of this group, using both morphological and molecular methods.

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References Cited

Ashmead, W. H. 1881. On the cynipidous galls of Florida. Trans. American Entomol. Soc. 9: ix-xxviii.

Ashmead, W. H. 1885. On the cynipidous galls of Florida with descriptions of new species. Trans. American Entomol. Soc. 12: 5-9.

Ashmead, W. H. 1887. On the Cynipidous galls of Florida, with descriptions of new species and Synopses of the described species of North America. Trans. American Entomol. Soc. 14: 125-149.

Beutenmüller, W. 1909. The species of *Holcapsis* and their galls. Article V. Bull. American Mus. Nat. Hist. 26: 29-45.

Beutenmüller, W. 1911. Two new species of *Holcaspis* from Mexico. Psyche 18: 86-87.

Bird, J. P., Melika, G., Nicholls, J. A., Stone, G. N., and Buss, E. A. 2013. Life history, natural enemies, and management of *Disholcaspis quercusvirens* (Hymenoptera: Cynipidae) on live oak trees. J. Econ. Entomol. 106(4): 1747-1756.

Burks, B. D. 1979. Superfamily Cynipoidea, pp. 1045-1107 *In* K. V. Krombein, P. D. Hurd, Jr., D. R. Smith, and B. D. Burks, [eds.], Catalog of Hymenoptera in America North of Mexico. Volume 1. Symphyta and Apocrita. Smithsonian Institution Press, Washington, D.C.

Burnett, J. A. 1977. Biosystematics of the new oak-gallwasp genus *Weldia*, of Western North America. Ph.D. Dissertation, University of California, Riverside.

Campbell, B. C., Steffen-Campbell, J. D., and Werren, J. H. 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. Insect Mol. Biol. 2: 225-237.

Csóka, G., Stone, G. N., and Melika, G. 2005. Biology, ecology, and evolution of gall-inducing Cynipidae, pp. 573-642 *In* A. Raman, C. W. Schaefer, and T. M. Withers T. M. [eds.], Biology, ecology, and evolution of gall-inducing arthropods. Science Publishers, Enfield, NH.

Dalla Torre, K. W., von. 1893. Cynipidae. Catalogus Hymenopterorum hucusque descriptorum systematicus et synonymicus. Vol. 2. Lipsiae, Sumptibus Guilelmi Engelmann: VIII + 1-140.

Dalla Torre, K. W., and Kieffer, J. J. 1910. Cynipidae. Tierreich. Berlin: Friedlander & Sohn 24, XXXV, 1-891.

Evans, D. 1972. Alternate generations of gall cynipids (Hymenoptera: Cynipidae) on Garry oak. Can. Entomol. 104: 1805-1818.

Govaerts, R., and Frodin, D. G. 1998. World checklist and bibliography of Fagales. Kew: Royal Botanic Gardens, Kew.

Harris, R. 1979. A glossary of surface sculpturing. State of California, Dept. Food and Agr. Occasional Papers in Entomol. 28: 1-31.

Jermiin, L. S., and Crozier, R. H. 1994. The cytochrome-b region in the mitochondrial DNA of the ant *Tetraponera rufoniger* - sequence divergence in Hymenoptera may be associated with nucleotide content. J. Molec. Evol. 38: 282-294.

Kinsey, A. C. 1920. New Species and Synonymy of American Cynipidae. Bull. American Mus. of Nat. Hist. 42: 293-317.

Kinsey, A. C. 1937. New Mexican Gall Wasps (Hymenoptera, Cynipidae). Rev. Entomol. 7(1): 39-79.

Kinsey, A. C. 1938. New Mexican Gall Wasps (Hymenoptera, Cynipidea). IV. Proc. Indiana Acad. Scienc. 47: 261-280.

Liljeblad, J., and Ronquist, F. 1998. A phylogenetic analysis of higher-level gall wasp relationships (Hymenoptera: Cynipidae). Syst. Entomol. 23: 229-252.

Medianero, E., and Nieves-Aldrey, J. L. 2011. First record of the genus *Disholcaspis* Dalla Torre & Kieffer (Hymenoptera: Cynipidae: Cynipini) in the Neotropics, with description of two new species from Panama. Zootaxa 2802: 23-33.

Melika, G. 2006. Gall Wasps of Ukraine. Cynipidae. Vestnik zoologii, supplement 21(1-2), 1-300, 301-644.

MELIKA, G., AND ABRAHAMSON, W. G. 2002. Review of the world genera of oak cynipid wasps (Hymenoptera: Cynipidae, Cynipini), pp. 150-190 *In* G. Melika and Cs. Thuróczy [eds.], Parasitic Wasps: Evolution, Systematics, Biodiversity and Biological Control. Agroinform, Budapest.

MELIKA, G., HANSON, P., AND PUJADE-VILLAR, J. 2011. A new species of *Disholcaspis* Dalla Torre and Kieffer oak gallwasp from Costa Rica (Hymenoptera: Cynipidae: Cynipini). Dugesiana 18(1): 17-22.

NICHOLLS, J. A., PREUSS, S., HAYWARD, A., MELIKA, G., CSÓKA, G., NIEVES-ALDREY, J. L., ASKEW, R. R., TAVAKOLLI, M., SCHÖNROGGE, K., AND STONE, G. N. 2010. Concordant phylogeography and cryptic speciation in two Western Palaearctic oak gall parasitoid species complexes. Mol. Ecol. 19: 592-609.

NICHOLLS, J. A., CHALLIS, R. J., MUTUN, S., AND STONE, G. N. 2012. Mitochondrial barcodes are diagnostic of shared refugia but not species in hybridising oak gallwasps. Mol. Ecol. 21: 4051-4062.

PUJADE-VILLAR, J., EQUIHUA-MARTÍNEZ, A., ESTRADA-VENEGAS, E. G., AND CHAGOYÁN-GARCÍA. C. 2009. Estado del conocimiento de los Cynipini (Hymenoptera: Cynipidae) en México: perspectivas de estudio. Neotropical Entomology 38(6): 809-821.

PUJADE-VILLAR, J., ROMERO-RANGEL, S., CHAGOYAN-GARCIA, C., EQUIHUA-MARTINEZ, A., ESTRADA-VENEGAS, E. G., AND MELIKA, G. 2010. A new genus of oak gallwasps, *Kinseyella* Pujade-Villar & Melika, with a description of a new species from Mexico (Hymenoptera: Cynipidae: Cynipini). Zootaxa 2335: 16-28.

STONE, G. N., HERNANDEZ-LOPEZ, A., NICHOLLS, J. A., DI PIERRO, E., PUJADE-VILLAR, J., MELIKA, G., AND COOK, J. M. 2009. Extreme host plant conservatism during at least 20 million years of host plant pursuit by oak gallwasps. Evolution 63: 854-869.

WELD, L. H. 1921. American Gallflies of the family Cynipidae producing subterranean galls on oak. Proc. U.S. Nat. Mus. 59: 187-246.

WELD, L. H. 1951. Superfamily Cynipoidea, pp. 594-654 *In* C. F. W Muesebeck, K. V. Krombein, H. K. Townes et al. [eds.], Hymenoptera in America North of Mexico. Synoptic Catalogue. U.S. Department of Agriculture. Agricultural Monograph No. 2: 1420 pp.

WELD, L. H. 1959. Cynipid galls of the Eastern United States. Ann Arbor, Michigan. Privately published: 1-124.

YOSHIMOTO, C. M. 1962. Revision of the Hawaiian Eucoilinae. Pacific Insects 4: 799-845.