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Reinstatement of *Carposina ottawana* Kearfott, 1907 (Lepidoptera: Carposinidae) as a valid species

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Abstract. *Carposina ottawana* Kearfott, 1907 (Lepidoptera: Carposinidae), **revised status**, formerly considered a synonym of *C. sasakii* Matsumura, 1900, is returned to species status. Morphological features that separate the Asian species *C. sasakii* and *C. niponensis* Walsingham, 1900 from the North American *C. ottawana* are described and illustrated. A heuristic maximum likelihood (ML) analysis based on the mitochondrial gene cytochrome oxidase I (DNA barcode) further supports *C. ottawana* and *C. sasakii* as distinct taxa.

Key words. *Carposina sasakii, Carposina niponensis,* Asia, North America, pest species.

Introduction

The genus *Carposina* Herrich-Schäffer occurs throughout North America, Europe, Asia, and Oceania. Most species are borers in various plant parts, and the majority are internal fruit-feeders (Scoble 1992). The larvae of Old-World members, in particular, are known to bore into fruit, twigs, stems, leaves, or bark of their hosts (Ponomarenko 1999). A small number of *Carposina* species are of economic importance (Davis 1969; Diakonoff 1989), including *Carposina sasakii* Matsumura, a well-known pest of pome and stone fruits in China and Japan (Davis 1969; CABI 2020). Adults of *Carposina* are small, drab colored, and unremarkable; consequently, they are often overlooked by collectors. In many cases, the lack of specimens, poor preservation, and non-descript wing patterns have resulted in the incorrect lumping of specimens under established species names.

Carposina sasakii was described in 1900 by S. Matsumura. However, three years earlier, in a government report on the pests of Japan, Sasaki reported this species as "*Carpocapsa persicana*" (Matsumura 1897). The latter reports were not circulated, and the name has gone largely ignored. Nasu et al. (2010) recommended to continue the use of *C. sasakii* for stability since *Carpocapsa persicana* has only been used in four publications and all uses occurred prior to 1906. For purposes of stability, we use the widely accepted name *Carposina sasakii* Matsumura, in lieu of *Carpocapsa persicana* Sasaki, 1897.

Carposina ottawana was described by Kearfott in 1907 from specimens collected by C.H. Young in Ottawa, Canada during the summer of 1906. In his revision of North American Carposinidae, Davis (1969) treated *C. ottawana* as a subspecies of *C. niponensis* Walsingham, 1900. Unfortunately, this decision was based on a limited number of specimens from a rearing facility in Yokohama, Japan, and those specimens are in actuality *C. sasakii*. Diakonoff (1989) synonymized *C. n. ottawana* with *C. sasakii*, but it is unclear whether his decision was based on the examination of specimens or merely a review of the published literature. A decade later, *C. niponensis* was also synonymized with *C. sasakii* by Ponomarenko (1999), who argued that the type was most likely an anomalous specimen of *C. sasakii*

because the species had not been seen or collected in Japan for more than 100 years. This was reversed when Nasu et al. (2010) dissected unknown *Carposina* specimens in pheromone traps from Japan that matched the type of *C. niponensis*.

In our study of North American *Carposina*, we found both morphological and molecular support that *Carposina ottawana* is distinct from its Asian congeners, and herein we restore it to species status. We provide descriptions and illustrations of the males of *C. sasakii*, *C. ottawana*, and *C. niponensis*, along with descriptions and illustrations of the larvae and female genitalia of *C. ottawana* and *C. sasakii* for comparison.

Materials and Methods

Depositories. Specimens were examined at or borrowed from the following institutions: NMNH, U.S. National Museum of Natural History, Washington, DC, USA; MEM, Mississippi Entomological Museum Collection, Mississippi State University, MS, USA (2018); FSCA, Division of Plant Industry, Florida State Collection of Arthropods, Gainesville, FL, USA (2018); MGCL, McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA (2019); and UMRM, Enns Entomological Museum, University of Missouri, Columbia, MO, USA (2019).

Morphology. Abdomens of adults were prepared by soaking in an 8% solution of sodium hydroxide overnight at room temperature. Gentle heating was applied to specimens that required further maceration. Specimens were dissected in distilled water and later transferred to ethanol. Terminology for the structures of the adult genitalia follows Klots (1956) and larval characters and their abbreviations follow Stehr (1987).

One valva was removed from each genital capsule of male specimens to allow closer inspection of the inner surface. This was necessary because the valvae in their natural state are partially fused to the transtilla, vinculum, and the adjoining valva. Female specimens were processed using conventional methods, and genitalia were separated from the abdominal integument to allow for closer inspection. For both sexes, the abdominal integument and genitalia were transferred to glycerin for permanent storage. Slide-mounting was avoided to prevent distortion of three-dimensional structures and to prevent obscuring diagnostically important features. A total of 105 specimens of *Carposina* from the U.S. and Asia was examined, 37 of which were either *C. ottawana* or *C. sasakii*. The larvae used in this study were reared from eggs of identified adults or were from rearing colonies.

DNA barcodes. Cytochrome oxidase subunit I (COI) sequences of *Carposina* species were downloaded from the Barcode of Life Data (BOLD) Systems database (http://www.barcodinglife.com/) for downstream analyses. Alignment of sequences was based on amino acid reading frame facilitated with Mesquite 3.51 (Maddison and Maddison 2011) resulting in 659 aligned base pairs. Nineteen North American and Asian *Carposina* taxa, including *C. fernaldana* Busck, 1907*, C. sasakii*, and *C. ottawana* from multiple locales, were included in the analysis. PartitionFinder 2.1.1 (Lanfear et al. 2016) was used to simultaneously select the best-fit partitioning scheme and corresponding nucleotide substitution models for the COI data. The analysis was run with the data initially partitioned by codon position, implementing a greedy search scheme with all models considered using the corrected Akaike information criterion (AICc). Phylogenetic inference was performed using heuristic maximum likelihood (ML) analyses as implemented in RAxML (Stamatakis et al. 2005). We performed 750 rapid bootstrap replicates with a subsequent search for the optimal ML tree implementing the partition schemes and models suggested by PartitionFinder. FigTree 1.4.3 (Rambaut 2009) was used to visualize the phylogenetic results, and trees were rooted to *Carposina fernaldana*.

A haplotype network was constructed using Population and Evolutionary Genetics Analysis System (Paradis 2010). A total of 37 sequences representing 30 *Carposina* species from around the world were selected from publicly available data in BOLD. The network generated was modified using Photoshop Elements 12.0 to organize and group taxa from similar biogeographic regions.

Results

Morphological assessment

Carposina sasakii, *C. niponensis*, and *C. ottawana* cannot be separated reliably by external morphology and/or wing markings. Inspection of the genitalia is required for positive identification of adult specimens.

Carposina niponensis Walsingham, 1900

Material examined. Published material only. Based on images of the type and specimens collected by Nasu et al. (2010).

Male genitalia (Fig. 1a). The costal margin of the valve lacks sclerotization. The terminal spines (gn.s) of the gnathos (gn) are uniform in diameter and length, with all spines projecting posteriorly. The harpe (hrp) is approximately 2 times as long as broad and terminates in several spines giving it a distinctly "toothed" appearance. The juxta (jx) is well developed with 2 long posterior projecting arms. The uncus (un) has the upper edge unsclerotized and covered with long thin setae (removed in illustration) that are half the length of the arms of the gnathos. The basal process (bp) of the valva is approximately half the length of the arms of the gnathos. The transtilla is absent or obscured in the published publications, and the saccus (sa) is subequal to the length of the valva.

Female. Unknown.

Larva. Unknown.

Carposina ottawana Kearfott, 1907, revised status

Material examined. The type specimens are lost, and the lectotype designated by Klots (1942) could not be found (AMNH). Specimens collected by C.H. Young in Ottawa, Canada the two days following the collection of the type and co-types designated by Kearfott were examined (NMNH), along with the type of *C. nicholsana* Forbes, 1923, which was designated a synonym of *C. n. ottawana* by Davis (1969). In total, 25 specimens of *C. ottawana* **stat. rev.** were examined (UMRM, FSCA, MEM, NMNH).

Male genitalia (Fig. 1b). The terminal spines of the gnathos have 2–3 spines that are significantly larger than other spines in both length and diameter. The spines spiral anteriorly (towards the tegumen) and project laterally. The harpe is 3 times as long as broad and gradually tapering to a point with no apparent teeth or striations present. The harpe continues anterior-ventrally with a strong sclerotized structure that reaches the anterior margin of the valva (hrp.ex). The juxta is reduced to a short, broad, well sclerotized plate. The uncus has the upper edge with a strongly sclerotized margin and covered with long thin setae (removed in illustration) that are half the length of the arms of the gnathos. The basal process of the valva is strait and shorter than the harpe. The transtilla (tra) is broad where it attaches to the pedunculi and narrows at the midpoint. The saccus is short (~0.75× length of the harpe) but well developed and larger than that of *C. sasakii*.

Female genitalia (Fig. 2a, 3a). The ductus bursae has a large area that lacks sclerotization in the posterior third of its length and is without a bend. The corpus bursae, *in situ*, has a pair of forked signa (sig) positioned dorso-ventrally (1 top, 1 bottom). The posterior margin of A8 (pm-A8) strongly projects posteriorly with the opening of the ostium bursae at the tip of the projecting lobe (Fig. 3a). The ventral surface of sternite 8 has sclerotized folds (sf) that produce a valley extending anteriorly from the ostium.

Larva (Fig. 4a). The pinaculum of the lateral setal group (L group) on the prothorax is small, round, and located anterior to and below the spiracle. The thoracic shield has a narrow band of integument lacking granulations along lateral and posterior margins. The pinacula of the meso- and metathorax are small (1 to 1.5 times the diameter of spiracle on prothorax), with the dorsal setae (D1 and D2) on a common pinaculum, and the subdorsal setae (SD1 and SD2) on a common pinaculum. On the abdomen the lateral seta L3 is widely separated from L1 and L2, and it is positioned close to the subventral setae

(SV group). The subventral pinacula of segments A3–A6 are absent, and there is no granulation of the integument in the area between SD1 and the spiracle on segment A7.

Carposina sasakii Matsumura, 1900

Material examined. The deposition of the type is unknown. Eight specimens of *C. sasakii* from a rearing colony in Yokohama, Japan and four wild-caught specimens from Hokkaido, Ishikari, Japan were examined (NMNH).

Male genitalia (Fig. 1c). The terminal spines of the gnathos are uniform in diameter and length, and all project posteriorly. The harpe is long and tapering to an abrupt upturned point, with a narrow, sclerotized structure that continues anterior-ventrally to the margin of the valva. The juxta is reduced to a small brace-like structure that is narrowly sclerotized on the posterior margin. The uncus has the posterior margin strongly sclerotized, covered with long thin setae (removed in illustration) that are half the length of the arms of the gnathos. The basal process of the valva is straight and slightly longer than the harpe in length. The transtilla is very narrow, and the saccus is reduced to a small knob.

Female genitalia (Fig. 2b, 3b). In the ductus bursae the basal third of its length has a large area that lacks sclerotization, and there is a distinct bend half way between the sinus vaginalis and corpus bursae. *In situ*, the corpus bursae has two-forked signa positioned laterally (one left, one right). The posterior margin of A8 barely projects posteriorly, with the opening of the ostium bursae located on the posterior margin (Fig. 3b). The ventral surface of sternite 8 has sclerotized folds that produce a valley extending anteriorly from the ostium.

Larva (Fig. 4b). The prothoracic pinaculum of the L group is large (four times the diameter of the spiracle along its longest axis) and extends below the spiracle. The spiracle is positioned on its own well defined pinaculum. The thoracic shield has granulated integument extending to the edge of the sclerotized plate. The pinacula of the meso- and metathorax are large and conspicuous (three times the diameter of spiracle on the prothorax), and all D and SD setae are on separate pinacula. On the abdomen, the pinacula of SD1 on segments A1–6 are noticeably larger than those of *C. ottawana*. Lateral seta L3 is widely separated from L1 and L2 and positioned close to the SV setae. The SV pinacula of segments A3–A6 are small but present. All abdominal spiracles are surrounded by a concolorous, unpigmented, unsclerotized ring that is free of granulation.

Molecular analyses

No sequences of *C. niponensis* were available for the DNA analyses. The best scheme returned by PartitionFinder was partitioning the data by codon position with the following models: TRN+I, F81, and TRN for positions 1, 2, and 3, respectively. Because RAxML allows for only a single model of rate heterogeneity in partitioned analyses, we applied the GTR model to each partition. Mixed model phylogenetic inference in RAxML resulted in a topology strongly supporting the separation of *Carposina ottawana* from *C. sasakii* in branching pattern, nodal support (100% bootstrap) and branch length (Fig. 5). The results of the haplotype network analysis (Fig. 6) support the findings of the phylogenetic analysis that *C. sasakii* and *C. ottawana* have distinct haplotypes.

Discussion

In this study we present morphological features that differentiate *C. ottawana* and *C. sasakii* in both sexes and in last instar larvae. Males of *C. ottawana, C. niponensis,* and *C. sasakii* can be readily distinguished by differences in the arrangement of spines on the gnathos and by the shape and length of the saccus and juxta in the male. *Carposina ottawana* and *C. sasakii* females can be distinguished by the *in situ* orientation of the signa and by the shape of the eighth abdominal sternite; larvae can be distinguished by the size, shape, and position of the pinacula, particularly on the 7th abdominal segment. Moreover, DNA barcodes further support *C. ottawana* and *C. sasakii* as distinct taxa. Additionally, none

of the North American collected material we examined was determined to be *C. sasakii* or *C. niponensis*. We surmise that all previous identifications and mentions of *C. sasakii* or *C. niponensis* as present or established in North America are the result of misidentification or an identification that failed to denote the subspecies as *ottawana*.

Conventional mounting of the female genitalia has historically misrepresented the natural shape of the ductus bursae in *C. sasakii*. The act of forcing this three-dimensional structure flat results in a fold and/or kink (* on Fig. 2b) when slide mounted which is exacerbated by the desire to rotate the corpus bursae to get the signa to lie flat. This morphological artifact of the slide-making process was repeated in illustrations by previous authors, and for consistency, the preparation illustrated in Figure 2b continues this trend; hence, the structures are not shown in their natural orientation. Our phylogenetic and haplotype analyses demonstrate that *C. ottawana* and *C. sasakii* are distinct species; however, it should be noted that their relationship requires further clarification based on increased species sampling. Inclusion of the remaining known North American species, *C. simulator* Davis, 1969, and denser sampling of *C. fernaldana* in a broader study is the next logical step. On a global scale, inclusion of representatives of all *Carposina* species would be necessary to infer whether the North American species represent a monophyletic group or whether multiple invasions of *Carposina* from the Old World into North America occurred. Our haplotype network shows the Asian and North American taxa on separate branches, but a much denser taxonomic and gene sampling is needed to add clarity to the evolutionary and biogeographic history of *Carposina* species. Like other small, drab, nondescript insects, their humble attributes have surely led to the limited scientific attention they have received (e.g., McElrath et al. 2018), and thus it is likely that cryptic diversity within *Carposina* has gone unnoticed.

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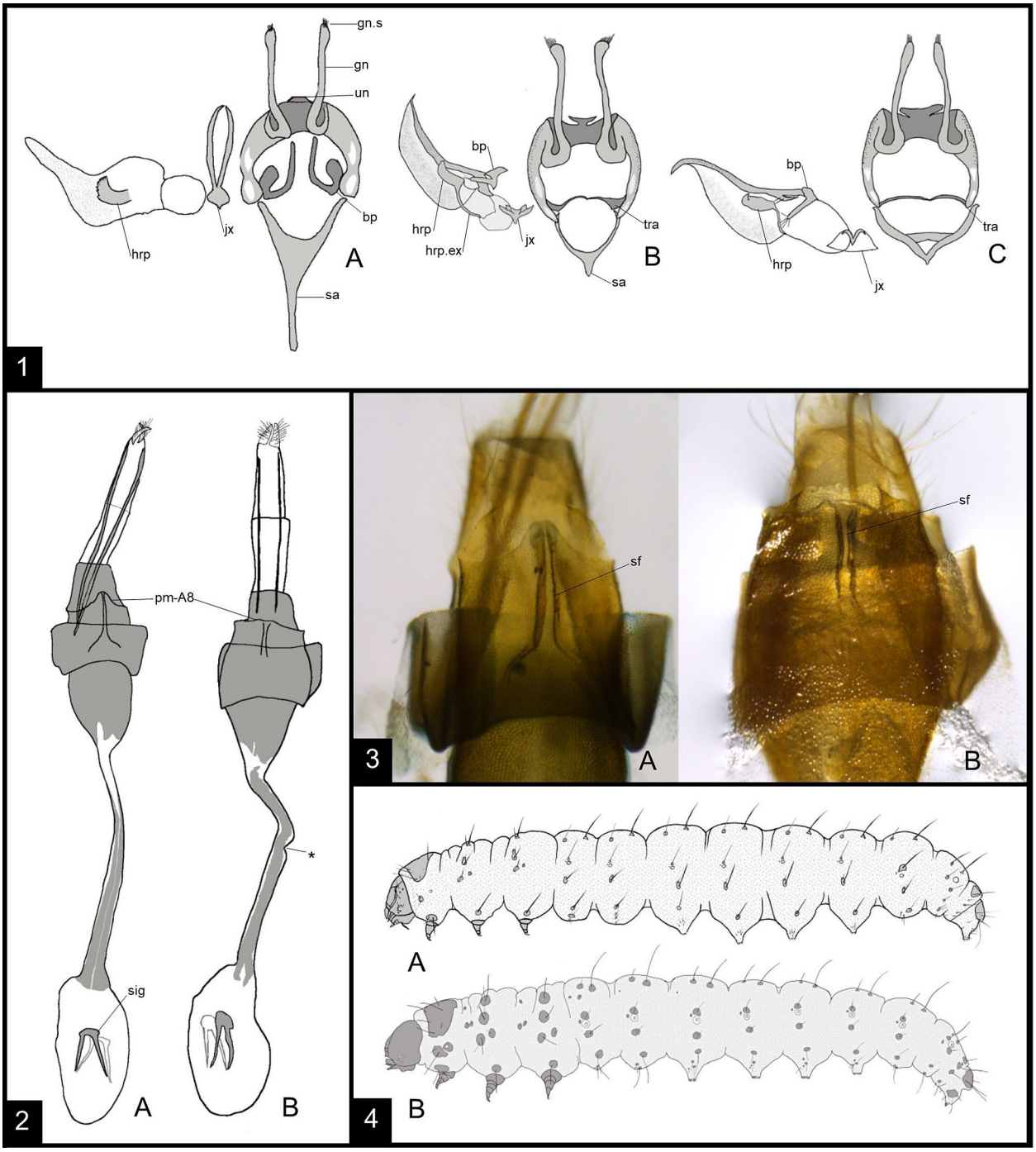
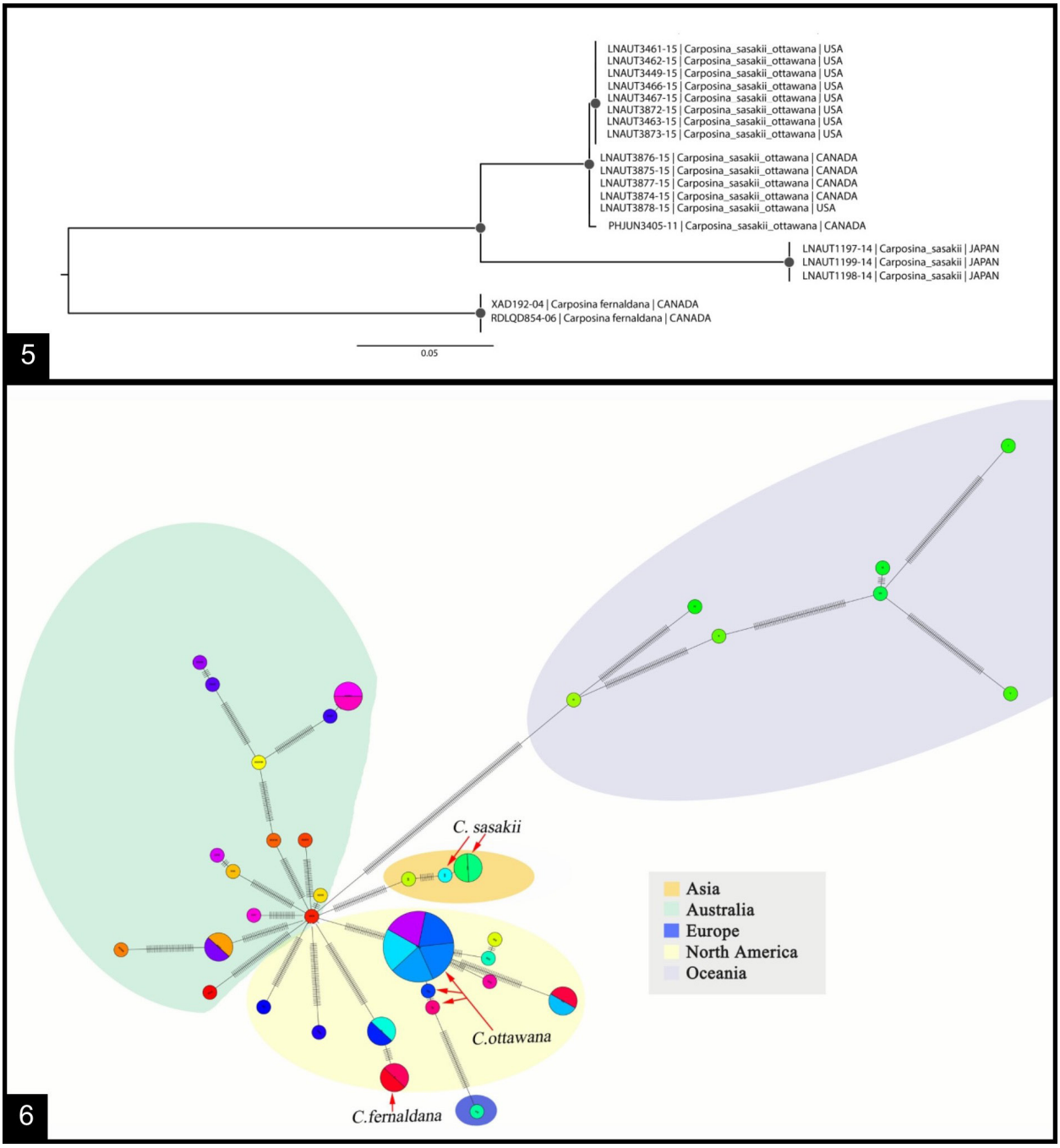


Figures 1–4. Genitalia and larvae of *Carposina* species. **1)** Male genitalia. **a)** *Carposina niponensis*, **b)** *C. ottawana*. **c)** *C. sasakii*. **2)** Female genitalia. **a)** *C. ottawana*. **b)** *C. sasakii*. **3)** Female sternite of abdominal segment 8 showing difference in the shape of the posterior margin and cuticular folds along the midline. **a)** *C. ottawana*. **b)** *C. sasakii*. **4)** Late instar larvae. **a)** *C. ottawana*. **b)** *C. sasakii*. bp: basal process of the valva, gn: gnathos, gn.s: spines of the gnathos, hrp: harpe, hrp.ex: anterior extensions of the harpe, jx: juxta, pm-A8: posterior margin of abdominal segment 8, sa: saccus, sig: signa, sf: sclerotized folds, tra: transtilla, un: uncus, *: kink on ductus bursae.



Figures 5–6. Molecular analysis of *Carposina* species. **5)** Mixed model COI maximum likelihood tree. Japanese *Carposina sasakii* are strongly supported as distinct from *C. sasakii ottawana* from North America in both relationship and molecular distance. Nodes with 100% bootstrap support are indicated with gray circles. **6)** Haplotype network of *Carposina* species based on COI data. Each circle or part of a circle represents a different sequence in the dataset. Each branch and each hash mark represent a single nucleotide change. Background shading was added to delineate geographic regions.