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The *Mesocallis* Matsumura (Hemiptera: Aphididae) of the Korean Peninsula with Descriptions of Two New Species

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Yerim Lee, Mariusz Kanturski, and Seunghwan Lee (2018) This study reviewed the aphid genus *Mesocallis* from the Korean peninsula. A total of five species are recognized using morphological and molecular evidence, including two new species, *Mesocallis (Mesocallis) carpinicola* sp. nov. and *M. (Paratinocallis) occulta* sp. nov., one newly recorded species, *M. (M.) pteleae* Matsumura, 1919, and the two species *M. (M.) sawashibae* (Matsumura, 1917) and *M. (P.) corylicola* (Higuchi, 1972). Species description, illustrations and distributional and biological data were provided for all Korean *Mesocallis* species along with a pictorial key. Pairwise distances, a neighbor-joining tree and a median-joining network are also given.

Key words: Aphids, Biodiversity, Calaphidinae, COI, New record, Panaphidini.

BACKGROUND

The genus *Mesocallis* Matsumura, 1919 (Aphididae: Calaphidinae: Panaphidini) is one of the least known aphid genera in the world. It is comprised of eight East Asian species in the two subgenera: *Mesocallis* Matsumura, 1919 and *Paratinocallis* Higuchi, 1972 (Favret 2017, Remaudière and Remaudière 1997). Most of the *Mesocallis* species occur on host plants belonging to the family Betulaceae, except for one species, *M. (M.) fagicola* Matsumura, 1919, from the family Fagaceae (Blackman and Eastop 2017, Holman 2009, Quednau 2003). This genus differs from the closely related genus *Pterocallis* Passerini, 1860 by its narrow elliptical-formed secondary rhinaria on the 3rd antennal segment and different spinal and marginal setae patterns on the abdomen tergites

(Quednau 2003).

Only two species have been recorded in the Korean peninsula thus far (Quednau and Lee 2001): Paik (1972) first recorded *M. (M.) sawashibae* (Matsumura, 1917) from South Korea and Quednau (1979) added *M. (P.) corylicola* (Higuchi, 1972), which was collected in North Korea. Since then, a DNA barcoding study on this group revealed that five distinct groups are distributed in South Korea (Lee et al. 2017). Between 2001 and 2016, a large number of *Mesocallis* samples were collected in South Korea and examined them together with the museum specimens of *Mesocallis* collected in North and South Korea between 1985 and 2001. For the freshly collected specimens, molecular analyses with the partial mitochondrial cytochrome oxidase subunit I (COI) were also conducted.

The results of the morphological examination

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and molecular analyses identified a total of five valid species in the Korean peninsula. In the present study, we proposed two new species, *M. (M.) carpinicola* sp. nov. and *M. (P.) occulta* sp. nov., and a new faunistic record of *M. (M.) pteleae* Matsumura, 1919. All five species were illustrated and keyed along with their morphological descriptions. A pictorial key to species of *Mesocallis* in the Korean Peninsula was provided. Pairwise distance analyses, neighbor-joining tree and median-joining network based on the partial *COI* were also given.

MATERIALS AND METHODS

Morphological identification: All specimens are deposited in the National Academy of Agricultural Science (NAAS), Jeonju-si, South Korea and the College of Agriculture and Life Sciences, Seoul National University, South Korea (CAL SNU). North Korean specimens were collected by Jan Havelka, Institute of Entomological Academy of Science of the Czech Republic, during scientific expeditions between 1985 and 2001. Aphid samples for this study were collected in South Korea between 2001 and 2015. All samples were preserved in 90% ethanol for over one month and then mounted in Canada balsam following Blackman and Eastop (2000) and Martin (1983). Illustrations for each species were taken by a digital camera attached to a microscope (Leica 400B, Leica Microsystems, Germany) at a resolution of 600 dpi. Measurements for each specimen were taken from the digital images using the analysis software Active measure ver. 3.0.3 (Mitani Co. Ltd, Japan).

Aphid samples were identified using keys to *Mesocallis* species from Ghosh and Quednau (1990) and Quednau (2003). For further confirmation, DNA barcoding results were also applied.

The abbreviations used in this study were listed as follows. CB: Chungcheongbuk-do, South Korea; CN: Chungcheongnam-do, South Korea; GG: Gyeonggi-do, South Korea; GN: Gyeongsangnam-do, South Korea; GW: Gangwon-do, South Korea; JN: Jeollanam-do, South Korea; PB: Pyeonganbuk-do, North Korea; YG: Yanggang-do, North Korea; BL: body length, ANT: antennae, ANT I-VI: antennal segments I-VI, BASE: basal part of last antennal segment, PT: processus terminalis of last antennal segment, Ls ANT III: longest setae on ANT III, BD III: basal

diameter of ANT III, URS: ultimate rostral segment, Cu: cubitus, Rs: radial sector, FEMUR III: hind femur, TIBIAE III: hind tibiae, HT I: first segment of hind tarsus, HT II: second segment of hind tarsus, SIPH: siphunculus, ABD I-VIII: abdominal tergite I-VIII.

Main morphological characters like measurements (in mm), number of setae on antennal segments, number of rhinaria and body parts ratios of Korean *Mesocallis* are given in table 1.

Holotypes and paratypes of the new species are deposited in CALS SNU. Paratypes will be also deposited in the Hemiptera collection of the Department of Zoology, the University of Silesia in Katowice (DZUS).

DNA extraction and Molecular identification: Whole genomic DNA was extracted from each sample using the DNeasy Blood and Tissue Kit (Qiagen, Dusseldorf, Germany) according to the modified manufacturer's protocols. We conducted the nondestructive method to confirm morphological features (Lee et al. 2017).

A 658bp of the *COI* gene region was amplified using a universal primer set: LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAATCA-3'. Polymerase chain reaction (PCR) was performed with AccuPower PCR PreMix (Bioneer, Daejeon, Korea) in 20 ml reaction mixtures under the following conditions: initial denaturation at 94°C for 3 min; followed by 35 cycles at 94°C for 30s, an annealing temperature of 45.2°C for 30s, an extension at 72°C for 1 min; and the final extension at 72°C for 5 min. Successfully amplified samples were purified using a QIAquick PCR purification kit (Qiagen, Inc.), and then sequenced directly using an automatic sequencer (ABI PrismH 3730 XL DNA Analyzer) at Bionics Inc. (Seoul, Korea).

A total of 45 *COI* sequences of five *Mesocallis* species were used for the analyses (Appendix 1). Sequences were aligned using MEGA 7 (Kumar et al. 2016). Intra- and inter-specific distances were calculated using a pairwise distance method based on the Kimura-2-Parameter (K2P) model (Kimura 1980). Neighbor-joining analysis (NJ) based on K2P for the final data set of 658bp was also conducted. The *COI* haplotypes for the final dataset were analyzed using DnaSP ver.5.1. (Librado and Rozas 2009). Median-joining network (MJ) was constructed by Network ver. 5.0.0.1. (Polzin and Daneshmand 2003).

RESULTS

TAXONOMY

Genus *Mesocallis* Matsumura, 1919

Mesocallis Matsumura, 1919: 103.

Neocallis Matsumura, 1919: 104.

Nippochaitophorus Takahashi, 1961: 247.

Type species: *Myzocallis sawashibae* (Matsumura, 1917), by original designation.

Generic Diagnosis: According to Quednau (2003), *Mesocallis* belongs to the *Pterocallis* group of genera. This genus is similar to *Pterocallis* Passerini but can be distinguished by having two setae on ABD VIII, black-bordered fore tibiae and Cu1b of forewing distinctly dark bordered. Representatives of *Mesocallis* can be also distinguished from *Pterocallis* by oval to narrow elliptical secondary rhinaria on ANT III, epicranial suture weakly developed but present on the head of apterous viviparous females.

Host plant: Species belonging to this genus

are known to feed on *Alnus* spp., *Carpinus* spp., *Corylus* spp., *Ostrya* spp. (Betulaceae) and *Fagus* sp. (Fagaceae).

Distribution: This genus is known from South-East Asian countries (China, India, Japan, Korea, Nepal, and Siberia).

Key to the subgenera and species of *Mesocallis*

1. ABD margin with a single pointed seta on ABD I-IV (subgenus *Mesocallis*) 2
- ABD margin with 2-3 setae on ABD I-IV (subgenus *Paratinocallis*) 7
2. Head vertex and ANT I-III pale 3
- Head vertex including ANT I-III blackish 5
3. PT 0.80-1.25 × BASE, URS with 4-5 accessory setae *M. (M.) sawashibae*
- PT 0.60-0.70 × BASE, URS with 2-3 accessory setae 4
4. ANT 0.59-0.61 × BL, ANT III with 9-11 secondary rhinaria, URS 0.80-0.90 × HT II (Gosh and Quednau 1990) *M. (M.) alnicola*
- ANT 0.61-0.75 × BL, ANT III with 4-7 secondary rhinaria, URS 0.50-0.55 × HT II (Gosh and Quednau 1990) *M. (M.) obtusirostris*
5. ANT III with 6-9 secondary rhinaria, URS 0.06-0.07 mm with 2 accessory setae (Quednau 2003) *M. (M.) taoi*

Table 1. Biometric data of alate viviparous females of *Mesocallis* spp. in the Korean peninsula

	<i>M. (M.) carpinicola</i> sp. nov.	<i>M. (M.) pteleae</i>	<i>M. (M.) sawashibae</i>	<i>M. (P.) corylicola</i>	<i>M. (P.) occulta</i> sp. nov.	
Body parts	(n = 20)	(n = 20)	(n = 20)	(n = 20)	(n = 20)	
Length of	ANT I	0.05-0.06	0.05-0.07	0.05-0.07	0.05-0.06	0.04-0.06
	ANT II	0.04-0.05	0.05-0.06	0.05-0.06	0.05-0.06	0.04-0.06
	ANT III	0.29-0.36	0.33-0.43	0.30-0.39	0.28-0.40	0.21-0.33
	ANT IV	0.21-0.23	0.23-0.27	0.19-0.26	0.23-0.32	0.12-0.22
	ANT V	0.17-0.20	0.18-0.22	0.15-0.19	0.19-0.25	0.13-0.18
	BASE	0.09-0.11	0.09-0.14	0.08-0.11	0.10-0.13	0.08-0.12
	PT	0.06-0.08	0.08-0.10	0.08-0.11	0.11-0.13	0.08-0.11
	FEMUR III	0.31-0.37	0.37-0.45	0.30-0.42	0.30-0.39	0.23-0.32
	TIBIAE III	0.51-0.59	0.65-0.80	0.45-0.64	0.55-0.70	0.41-0.63
	Ls ANT III	0.01	0.01	0.01	0.01	0.01
No. of setae on	ANT I	3	3-4	3	3-4	3-5
	ANT II	3-4	2-4	2-3	2-3	2-3
	ANT III	4-5	5-9	6-11	4-6	3-4
	BASE	1	1	1	1	1
	SIPH	0	0	0	0	0
	ABD VIII	2	2-3	2	2	2
	Ratio of	PT / BASE	0.60-0.80	0.71-0.91	0.80-1.25	0.92-1.10
PT / ANT III		0.19-0.24	0.21-0.29	0.21-0.32	0.30-0.39	0.33-0.38
SIPH / BL		0.03-0.04	0.02-0.03	0.03-0.04	0.03-0.06	0.03-0.07
SIPH / ANT III		0.14-0.18	0.09-0.14	0.12-0.15	0.13-0.22	0.15-0.33
SIPH / FEMUR III		0.14-0.18	0.08-0.13	0.12-0.16	0.13-0.20	0.16-0.30
SIPH / Cauda	0.56-0.75	0.33-0.45	0.44-0.60	0.50-0.78	0.56-0.86	

Abbreviations used in this table are listed in list of abbreviations.

- ANT III with 12-16 secondary rhinaria, URS 0.10-0.14 mm with 4 or more accessory setae 6
- 6. PT 0.06-0.08 mm long, URS 0.10-0.11 mm long with 4-6 accessory setae, cauda with 10-15 setae
..... *M. (M.) carpinicola* Lee Y. sp. nov.
- PT 0.08-0.10 mm long, URS 0.11-0.14 mm long with 8-9 accessory setae, cauda with 7-11 setae *M. (M.) pteleae*
- 7. PT 0.6-0.7 × BASE, ABD dorsum with spinulose capitate spinal setae (Quednau 2003) *M. (P.) yunnanensis*
- PT 0.83-1.10 × BASE, ABD dorsum without spinulose capitate spinal setae 8
- 8. ANT 0.75-0.92 × BL and URS 0.80-1.00 × HT II
..... *M. (P.) corylicola*
- ANT 0.60-0.77 × BL and URS 1.00-1.14 × HT II
..... *M. (P.) occulta* Lee Y. sp. nov.

***Mesocallis (Mesocallis) carpinicola* Lee Y., sp. nov.**

(Figs. 1, 2, 3a-b, 5, 10; Table 1)

urn:lsid:zoobank.org:act:966B034F-6ABD-40AA-972B-4FBDADB1ED2D

Material examined: *Holotype*: 1 alate viviparous female, Jangsan-forest, Jangsan-ri, Maam-myeon, Goseong-gun, GN, South Korea, 35°02'48"N, 128°24'44"E, on *Carpinus laxiflora*, Yerim Lee and Hyoseok Lee leg., 3.v.2015, no. 150503YR-11.

Paratypes: 19 alate viviparous females, same data as the holotype, including DNA voucher no. Mes1-2, Mes1-3, Mes1-4, Mes1-5.

Etymology: The species name, *carpinicola*, is derived from the genus name of the host plant (*Carpinus*) and the Latin suffix '-cola' (dweller, inhabitant).

Diagnosis: This species is close to *M. (M.) pteleae* in having similar coloration and Cu1b of forewing dark bordered. However, the new species can be distinguished from *M. (M.) pteleae* by its shorter PT, 0.06-0.08 mm (0.08-0.10 mm in *M. pteleae*) and shorter URS length, 0.10-0.11 mm (0.11-0.14 mm in *M. pteleae*), ANT III with 4-5 setae (5-9 in *M. pteleae*), URS with 4-6 accessory setae (8-9 in *M. pteleae*), cauda with 10-15 setae (7-11 in *M. pteleae*).

Description (Alate viviparous female): **Color in life**: Head pale yellow, head vertex and ANT I-III fuscous, distal 1/3 of ANT IV and ANT V darken, BASE and distal 1/2 of PT also darken. Thorax and abdomen pale yellow. Legs pale, fore and hind tibiae including 1/9 of femur and tarsus black, 1/9 of middle tibiae and tarsi dark. Wing vein Cu1b of forewing bordered with black pigment. SIPH pale. Cauda and anal plate pale.

Morphology: Body oval, BL, 1.30-1.55 mm. Head smooth with a flat median protrusion on

frons, head vertex with 0.01 mm pointed setae, epicranial suture weakly developed, head dorsum without tubercles. ANT 6-segmented, 0.92-1.05 mm long, ANT 0.66-0.78 × BL, ANT III longest with 12-16 narrow transversely elliptical secondary rhinaria in a row on the whole segment, with 4-5 short and pointed setae, ANT III-VI imbricated with very short and inconspicuous setae, Ls ANT III × 0.50 BD III, ANT IV without secondary rhinaria, ANT V shorter than ANT IV, PT 0.60-0.80 × BASE. Rostrum not reaching to middle coxae, URS 0.10-0.11 mm long with 4-6 accessory setae, URS 1.00-1.22 × BASE, 1.22-1.38 × HT II. Thorax smooth, without tubercles. Fore coxae weakly enlarged, longest setae on TIBIAE III 0.67-1.00 × middle width of TIBIAE III, HT I with 7 chaetotaxy, HT II 0.08-0.09 mm long. Wing vein Rs of forewing weakly developed. Dorsal spinal setae sometimes on small elevations, conspicuous tubercles not developed, ABD VIII with a pair of spinal setae, ABD margin I-IV with a single seta on short cone-shaped marginal tubercles, 4th marginal tubercle 0.03 mm. SIPH cylindrical, truncated, 0.05-0.06 mm long. Cauda knobbed, 0.07-0.09 mm long, bearing 10-15 setae. Anal plate bilobed, each lobe with 6-8 setae.

Distribution: So far, the species is known from Goseong-gun in the southern part of South Korea.

Host plant: The species feed on the underside of leaves of *Carpinus laxiflora* (Betulaceae).

Remarks: This species was first referred to as *Mesocallis* sp. in Lee et al. (2017).

***Mesocallis (Mesocallis) pteleae* Matsumura, 1919**
(Figs. 1, 2, 3c-d, 6; Table 1)

Mesocallis pteleae Matsumura, 1919: 103.

Agrioaphis corylicola Shinji, 1935: 1148.

Agrioaphis hashibamii Shinji, 1935: 287.

Material examined: 3 alate viviparous females, Mt. Beagunsan, Gwangyang-si, JN, South Korea, 35°06'26"N, 127°37'19"E, on *Carpinus* sp., Yerim Lee leg., 25.vii.2013, no. 130725YR-23, including DNA voucher no. Ca2; 33 alate viviparous females, Mt. Chilgapsan, Cheongyang-gun, CN, South Korea, 36°24'48"N, 126°53'03"E, on *Corylus* sp., Yerim Lee and Hyoseok Lee leg., 10.v.2014, no. 140510YR-1, including DNA voucher no. Co10-1, Co10-2, Co10-3, Co10-4, Co20; 2 alate viviparous females, Mt. Chilgapsan, Cheongyang-gun, CN, South Korea, 36°24'48"N, 126°53'03"E, on *Corylus* sp., Yerim Lee and Hyoseok Lee leg., 6.v.2016, no. 160506YR-2.

Diagnosis: This species is similar to *M.*

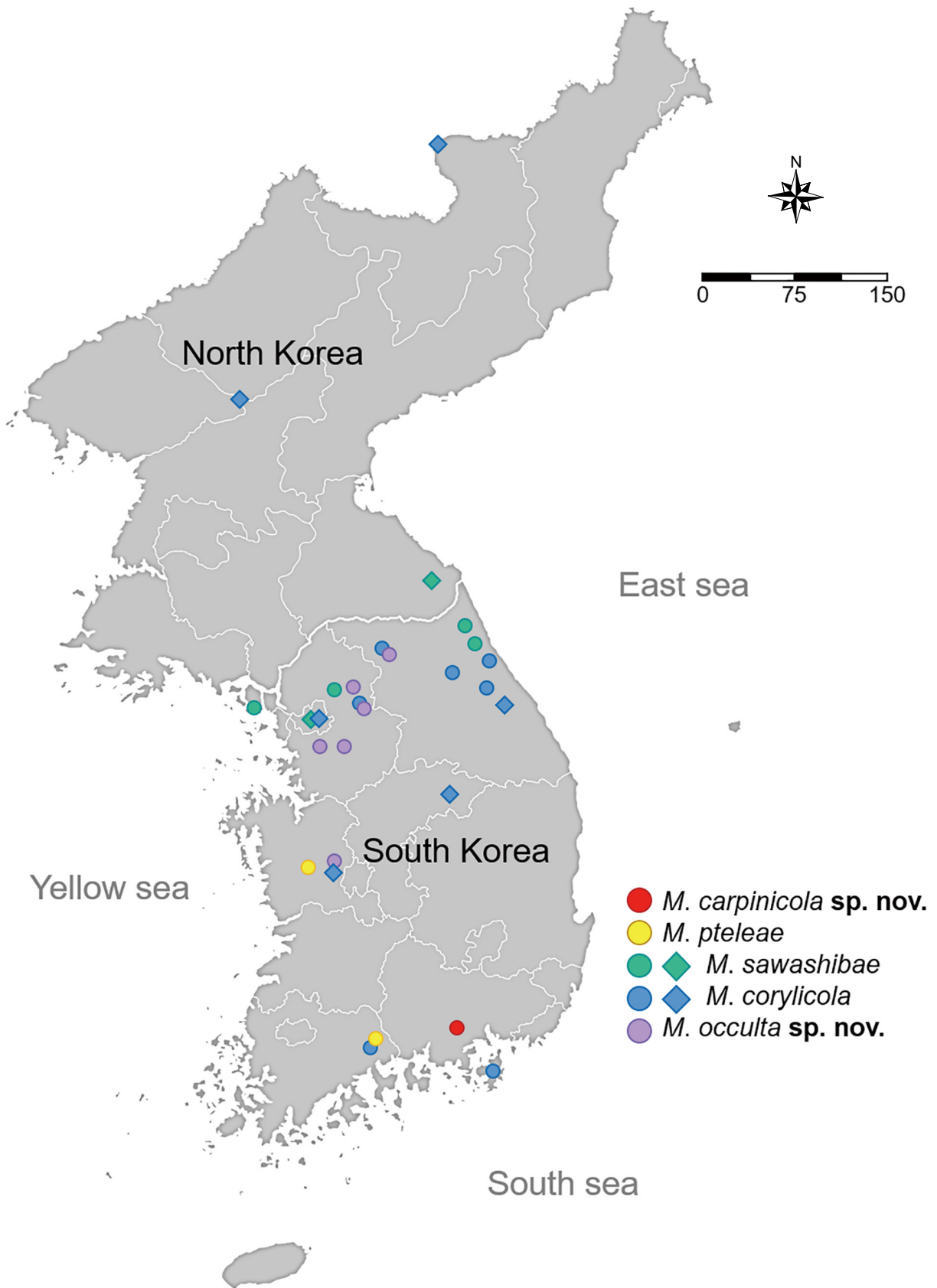


Fig. 1. Collection sites of *Mesocallis* spp. specimens collected in this study (●) and museum specimens (◆).

(*M.*) *taoi* Quednau by the color of ANT and black pigmented frons. However, it can be distinguished by its longer ANT III length, 0.33-0.43 mm (0.14-0.20 mm in *M. tatoi*) and long and slender URS, 0.11-0.14 mm (0.06-0.07 mm in *M. tatoi*).

Redescription (Alate viviparous female):

Color in life: Head pale, head vertex and ANT I-III fuscous, compound eye red, distal joint of ANT IV-V, BASE and distal 1/2 of PT also dark. Thorax and abdomen pale. Legs pale, fore tibiae including 1/9 of femur black, distal margin of FEMUR III dark, tarsus dark. Wing vein Cu1b of forewing dark bordered. SIPH and cauda pale.

Morphology: Body oval, BL 1.51-1.76 mm long. Head smooth with a flat median protrusion on frons, head vertex with 0.01 mm pointed setae, epicranial suture developed, head dorsum

without tubercles. ANT 6-segmented, ANT 0.63-0.74 × BL, ANT III longest with 13-16 transversely elliptical secondary rhinaria in a row on the whole segment, ANT III with 8-10 short pointed setae, ANT III-VI imbricated, ANT IV without secondary rhinaria, ANT V shorter than ANT IV, BASE with a single inconspicuous seta, PT 0.71-0.91 × BASE. Rostrum not reaching to middle coxae, URS 0.11-0.14 mm long with 8-9 accessory setae, URS 0.86-1.27 × BASE, 1.22-1.50 × HT II. Thorax smooth, without tubercles. Fore coxae weakly enlarged, longest setae on TIBIAE III 1.00-1.05 × middle width of TIBIAE III, HT I with 7 chaetotaxy, HT II 0.08-0.10 mm long. Wing vein Rs of forewing weakly developed. Dorsal spinal setae sometimes on small elevations, conspicuous tubercles not developed, ABD VIII with a pair of spinal setae,

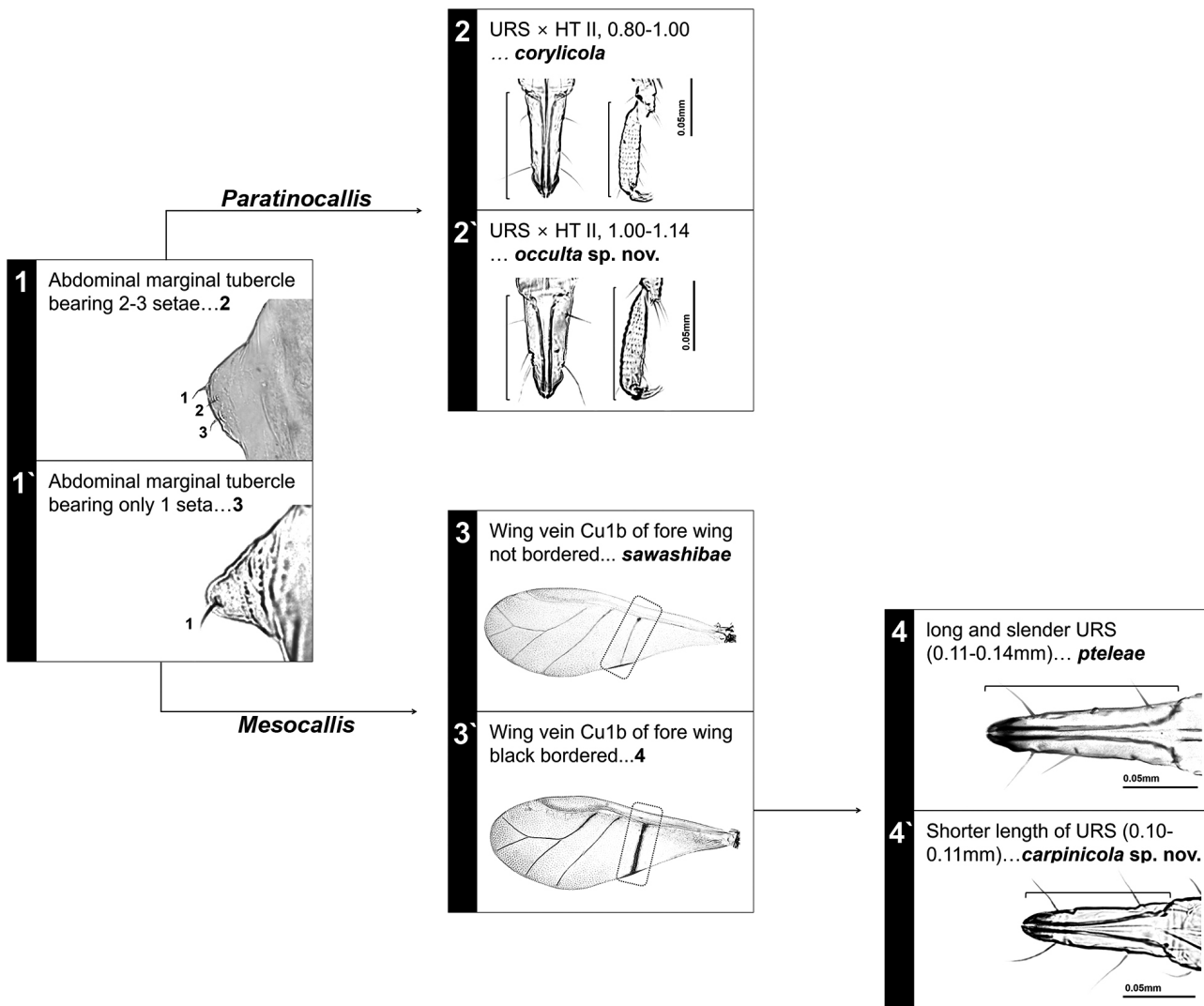


Fig. 2. A pictorial key to the species of *Mesocallis* in the Korean peninsula.

rarely with 3 setae, ABD margin I-IV with a single seta on short cone-shaped tubercles, 4th marginal tubercle 0.02 mm long. SIPH cylindrical, truncated, 0.03-0.06 mm long. Cauda knobbed, 0.09-0.11 mm long, bearing 8-11 setae. Anal plate bilobed, each lobe with 7-10 setae.

Distribution: The species is known from Chungcheongnam-do in western Korea. (First record), China (Zhang and Zhong 1990), Japan

(Higuchi 1972)

Host plant: This species feeds on *Alnus* spp., *Betula* spp., *Carpinus* spp., *Ostrya* spp. (Holman 2009). In Korea, it was only found on *Corylus* spp. (Betulaceae). Matsumura (1919) gave *Ptelea trifoliata* (Rutaceae) as a host plant, however, it is unlikely to be a true host.

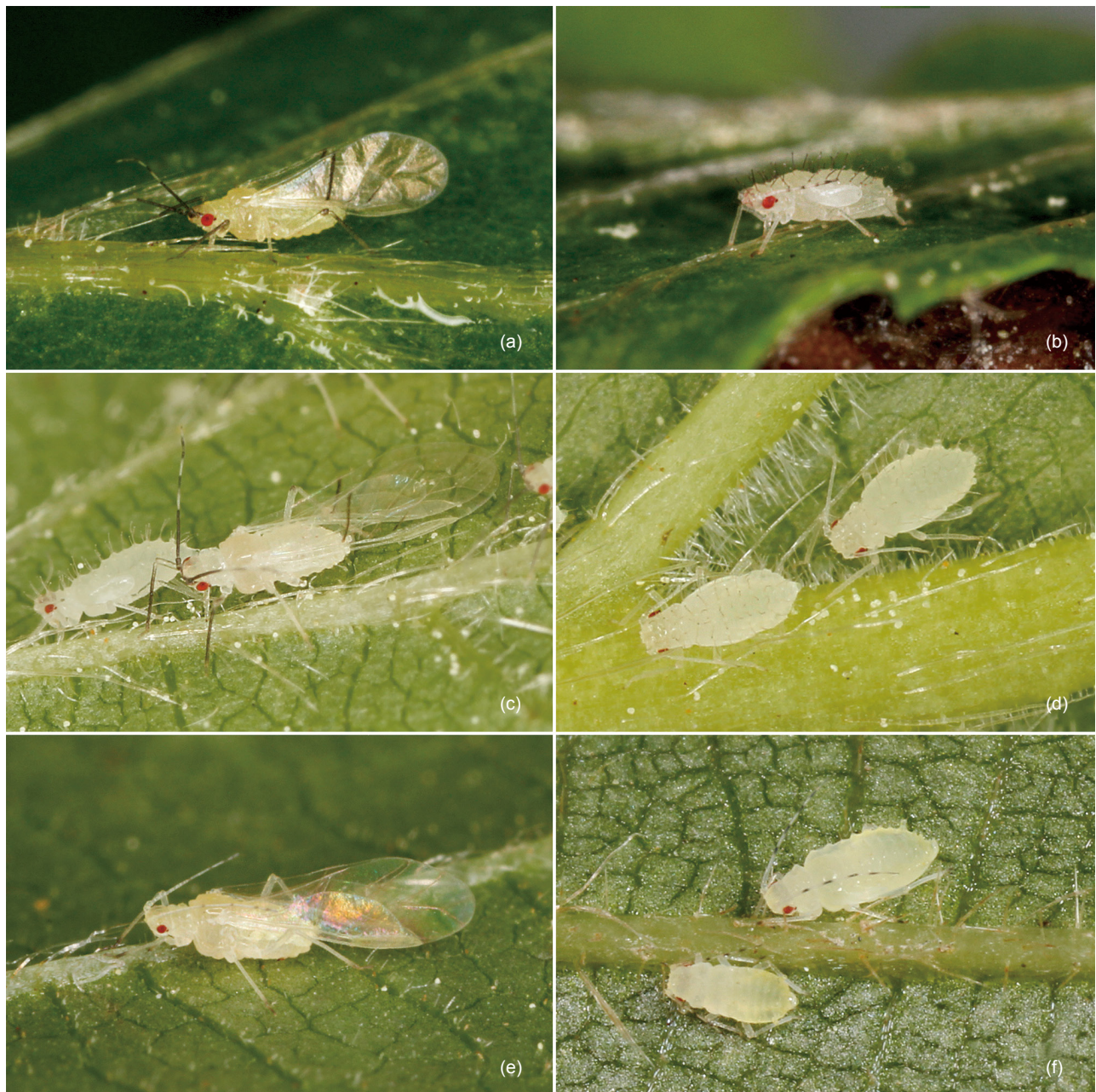


Fig. 3. Representatives of *Mesocallis* (*Mesocallis*) of Korea in life: (a) alate viviparous female of *M. (M.) carpinicola* sp. nov.; (b) alate nymph of *M. (M.) carpinicola* sp. nov.; (c) alate viviparous female of *M. (M.) pteleae*; (d) alate nymph of *M. (M.) pteleae*; (e) alate viviparous female of *M. (M.) sawashibae*; (f) alate nymph of *M. (M.) sawashibae*.

Mesocallis (Mesocallis) sawashibae
(Matsumura, 1917)

(Figs. 1, 2, 3e-f, 7; Table 1)

Myzocallis sawashibae Matsumura, 1917: 374.

Neocallis carpinicola Matsumura, 1919: 105.

Nippochaitophorus moriokaensis Takahashi, 1961: 187.

Material examined: 4 alate viviparous females, Seoul, South Korea, 37°33'59.53"N, 126°58'40.69"E, on *Carpinus erosa*, Woon-Ha Paik leg., 3.xi.1970, no. 6146; 1 alate viviparous female, Seoul, South Korea, 37°33'59.53"N, 126°58'40.69"E, on *Carpinus koreana*, Woon-Ha Paik leg., 6.v.1971, no. 6241; 1 alate viviparous female, Seoul, South Korea, 37°33'59.53"N, 126°58'40.69"E, on *Carpinus erosa*, Woon-Ha Paik leg., 6.v.1971, no. 6247; 1 alate viviparous female, Seoul, South Korea, 37°33'59.53"N, 126°58'40.69"E, on *Carpinus cordata*, Woon-Ha Paik leg., 21.x.1971, no. 6837; 2 alate viviparous females, Manmulsang-area, Mt. Geumgangsán, North Korea, 38°39'24.10"N, 128°6'17.85"E, on *Carpinus cordata*, Jan Havelka, 24.v.1988, no. 88HA2587; 1 alate viviparous female, Sohol-eub, Pocheon-si, GG, South Korea, 37°49'24"N, 127°08'18"E, on *Carpinus laxiflora*, Yerim Lee leg., 2.viii.2013, no. 130802YR-3, DNA voucher no. Ca4; 4 alate viviparous females, Girin-myeon, Inje-gun, GW, South Korea, 37°57'19"N, 128°19'09"E, on *Carpinus* sp., Duwal Keshari Ram leg., 26.ix.2013, no.130926Ram-6, including DNA voucher no. Ca5-2, Ca5-3, Ca5-4; 10 alate viviparous females, Mt. Manisan, Is. Ganghwado, Incheon-si, GG, South Korea, 37°36'41"N, 126°26'05"E, on *Carpinus turczaninowii*, Yerim Lee and Hyoseok Lee leg., 17.v.2014, no. 140517YR-1; 33 alate viviparous females, Yongdae-ri, Inje-gun, GW, South Korea, 38°11'02"N, 128°21'43"E, on *Carpinus* sp., Yerim Lee leg., 4.vi.2014, no. 140604YR-42, including DNA voucher no. Ca10; 13 alate viviparous females, Mt. Manisan, Is. Ganghwad, Incheon-si, GG, South Korea, 37°36'41"N, 126°26'05"E, on *Carpinus turczaninowii*, Yerim Lee and Hyoseok Lee leg., 21.vi.2014, no. 140621YR-3, including DNA voucher no. Ca9.

Diagnosis: This species is morphologically close to *M. (M.) obtusirostris* Gosh by having pale head and body color, and partly pigmented ANT. However, it can be distinguished by having 6-12 secondary rhinaria in a row on basal 2/3 of ANT III (4-7 secondary rhinaria mostly restricted to basal half of ANT III in *M. obtusirostris*).

Redescription (Alate viviparous female): Color

in life: Head and antenna pale yellow, distal joint of Ant.III-VIb dark, compound eye red; Thorax and abdomen pale yellow; Legs pale; cu1b of forewing, slightly dark bordered; SIPH and cauda.

Morphology: Body oval, BL 1.27-1.77 mm. Head smooth with a flat median protrusion on frons, head vertex with 0.01 mm pointed setae, epicranial suture weakly developed, head dorsum without tubercles. ANT 6-segmented, ANT 0.64-0.76 × BL, ANT III longest with 6-12 transversely elliptical secondary rhinaria in a row on 2/3 of segment, bearing 6-11 short pointed setae, ANT IV-VI imbricated with inconspicuous hair-like setae, ANT IV without secondary rhinaria, ANT V shorter than ANT IV, BASE with a single inconspicuous seta, PT 0.80-1.25 × BASE. Rostrum not reaching to middle coxae, URS 0.06-0.08 mm long with 4-5 accessory setae, URS 0.60-0.89 × BASE, 0.67-1.00 × HT II. Fore coxae weakly enlarged, longest setae on TIBIAE III 0.67-1.00 × middle width of TIBIAE III, HT I with 7 chaetotaxy, HT II 0.08-0.10 mm long. Wing vein Rs of forewing weakly developed. Thorax smooth, without tubercles. Dorsal abdominal setae sometimes on small elevations, conspicuous tubercles not developed, ABD VIII with a pair of spinal setae, ABD margin I-IV with a single seta on short cone-shaped tubercles, 4th marginal tubercle 0.02-0.04 mm long. SIPH cylindrical, truncated, 0.04-0.06 mm long. Cauda knobbed, 0.08-0.11 mm long, bearing 11-13 setae. Anal plate bilobed, each lobe with 5-10 setae.

Distribution: This species is widely distributed in Korea (North and South). Also known from China (Zhang and Zhong 1990), Japan (Higuchi 1972), and East Siberia (Quednau and Shaposhnikov 1988).

Host plant: This species was found on different *Carpinus* species (Betulaceae). In Korea also on *Corylus sieboldiana* (Paik 1972).

***Mesocallis (Paratinocallis) corylicola* (Higuchi, 1972)**

(Figs. 1, 2, 4a-b, 8; Table 1)

Paratinocallis corylicola Higuchi, 1972: 30.

Material examined: 1 alate viviparous female, Mt. Gyeryongsán, Gyeryong-si, CN, South Korea, 36°20'7"N, 127°12'3"E, on *Alnus japonica*, Woon-Ha Paik leg., 17.ix.1963; 1 alate viviparous female, Daegwanryeong-myeon, Pyeongchang-gun, GW, South Korea, 37°40'24"N, 128°42'22"E, host plant unknown, Woon-Ha Paik leg., 11-20.viii.1968; 3 alate viviparous females, Seoul, South

Korea, 37°34'1"N, 126°57'5"E, on *Physocarpus insularis*, Woon-Ha Paik leg., 12.v.1971; 1 alate viviparous female, Mt. Myohyangsan, PB, North Korea, 40°1'3"N, 126°19'46"E, on *Corylus heterophylla*, Jan Havelka leg., no. 85HA0789; 3 alate viviparous females, Mt. Baekdusan, Naegok-ri, YG, North Korea, 41°7'52"N, 128°15'42"E, on *Corylus heterophylla*, Jan Havelka leg., 18.vi.1988, no. 88HA3031; 1 alate viviparous female, Danseong-myeon, Danyang-gun, CB, South Korea, 36°56'13"N, 128°19'24"E, on *Corylus* sp., Seunghwan Lee leg., 18.v.2001, no. 011517-SH22; 4 alate viviparous females, Mt. Beagunsan, Gwangyang-si, JN, South Korea, 35°06'26"N, 127°37'19"E, on *Corylus sieboldiana*, Yerim Lee leg., 25.vii.2013, no. 130725YR-22, including DNA voucher no. Co2, Co3; 1 alate viviparous females, Mt. Manwolsan, Yangyang-gun, GW, South Korea, 37°57'11"N, 128°41'37"E on *Corylus sieboldiana*, Yerim Lee leg., 14.viii.2013, no. 130814YR-4, DNA voucher no. Co4; 15 alate viviparous females, Mt. Hwangbyeongsan, Chahang-ri, Pyeongchang-gun, GW, South Korea, 37°42'27"N, 128°41'14"E, on

Corylus sieboldiana, Yerim Lee leg., 15.viii.2013, no. 130815YR-11, including DNA voucher no. Co6-1, Co6-2, Co6-3, Co6-4, Co6-5; 1 alate viviparous females, Silnae-pass, Hwacheon-gun, GW, 38°09'05"N, 127°31'02"E, on *Corylus sieboldiana*, Yerim Lee leg., 1.v.2014, no. 140501YR-15, DNA voucher no. Co8-1; 2 alate viviparous females, Sangdong-ri, Yeongwol-gun, GW, South Korea, 37°47'09"N, 127°17'58"E, on *Corylus sieboldiana*, Yerim Lee leg., 12.vi.2014, no. 140612YR-23, DNA voucher no. Co15-1, Co15-2; 1 alate viviparous female, Is. Geojeodo, GN, South Korea, 34°53'12"N, 128°37'25"E, on *Corylus* sp., Yerim Lee and Hyoseok Lee leg., 14.vii.2014, no. 140714YR-7, DNA voucher no. Co14.

Diagnosis: This species is similar to *M. (P.) yunanensis* (Zhang) but it differs by having pointed setae on the dorsal abdomen, URS 0.80-1.00 × HT II (1.50-1.70 in *M. yunanensis*), and PT 0.90-1.10 × BASE (0.60-0.70 in *M. yunanensis*).

Redescription (Alate viviparous female):
Color in life: Head and antenna pale yellow, distal joint of ANT III-VIb dark. Thorax and abdomen

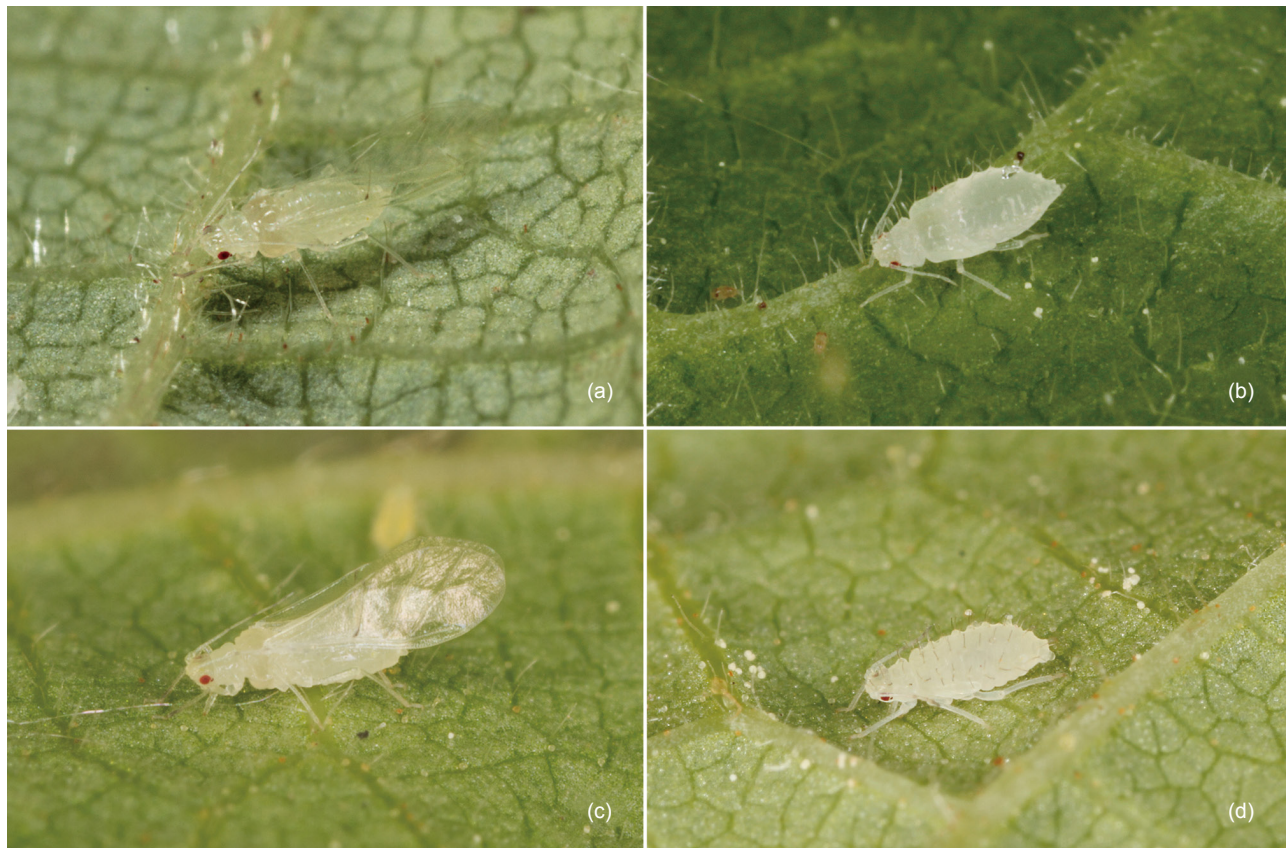


Fig. 4. Representatives of *Mesocallis (Paratinocallis)* of in life: (a) alate viviparous female of *M. (P.) corylicola*; (b) alatoid nymph of *M. (P.) corylicola*; (c) alate viviparous female of *M. (P.) occulta* sp. nov.; (d) alatoid nymph of *M. (P.) occulta* sp. nov.

pale yellow. Legs pale, distal 1/3 of fore tibiae and tarsi fuscous. Wing vein Cu1b of forewing dark bordered. SIPH and cauda.

Morphology: Body oval, BL 1.24-1.61 mm. Head smooth with a flat median protrusion on frons, epicranial suture weakly indicated, head vertex with 0.01 mm pointed setae, head dorsum without tubercles. ANT 6-segmented, ANT 1.02-1.29 × BL, ANT III with 7-12 transversely elliptical formed secondary rhinaria in a row on the whole

segment, ANT III-VI imbricated with inconspicuous hair-like setae, ANT IV without secondary rhinaria, ANT V shorter than ANT IV, PT 0.92-1.10 × BASE. Rostrum not reaching to middle coxae, URS 0.08-0.09 mm long with 4-5 accessory setae, 0.80-1.00 × HT II. Thorax smooth, without tubercles. Fore coxae weakly enlarged, longest setae on TIBIAE III 1.00-1.50 × middle width of TIBIAE III, HT I with 7-8 chaetotaxy, HT II 0.08-0.11 mm long. Dorsal spinal setae sometimes on small elevations, conspicuous

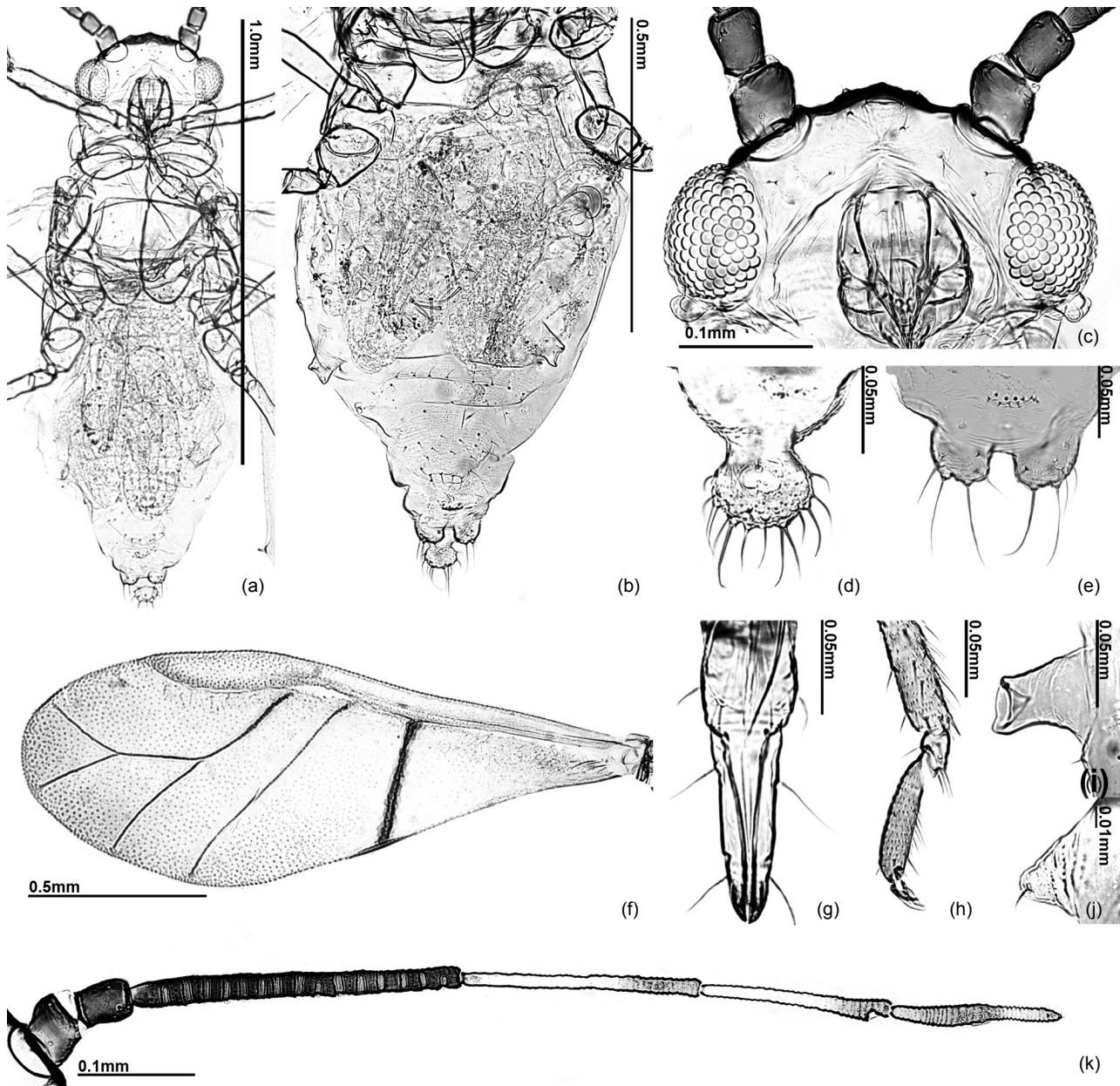


Fig. 5. Alate viviparous female of *M. (M.) carpinicola* sp. nov.: (a) body; (b) abdominal dorsum; (c) head; (d) cauda; (e) anal plate; (f) forewing; (g) URS; (h) HT II; (i) SIPH; (j) marginal tubercle on ABD VI.

tubercles not developed, ABD VIII with a pair of setae, ABD margin I-IV with 2 setae on short cone-shaped tubercles, 4th marginal tubercle 0.02-0.04 mm. SIPH cylindrical, truncated, 0.05-0.07 mm long. Cauda knobbed, 0.08-0.10 mm long, bearing 10-14 setae. Anal plate bilobed, each lobe with 6-11 setae.

Distribution: The species is rather common in the Korean peninsula (both North and South Korea), China (Zhang et al. 1986), and Japan

(Higuchi 1972).

Host plant: The species feeds on several species of *Corylus* (Betulaceae) in almost whole distributional range, but in Russian Far East it was collected from *Quercus dentata* (Holman 2009).

Remarks: In Paik's collection, some specimens were collected on *Alnus japonica* and *Physocarpus insularis*. However, these do not seem to be a true host plants because *M. (P.) corylicola* is typically recorded as *Corylus*.

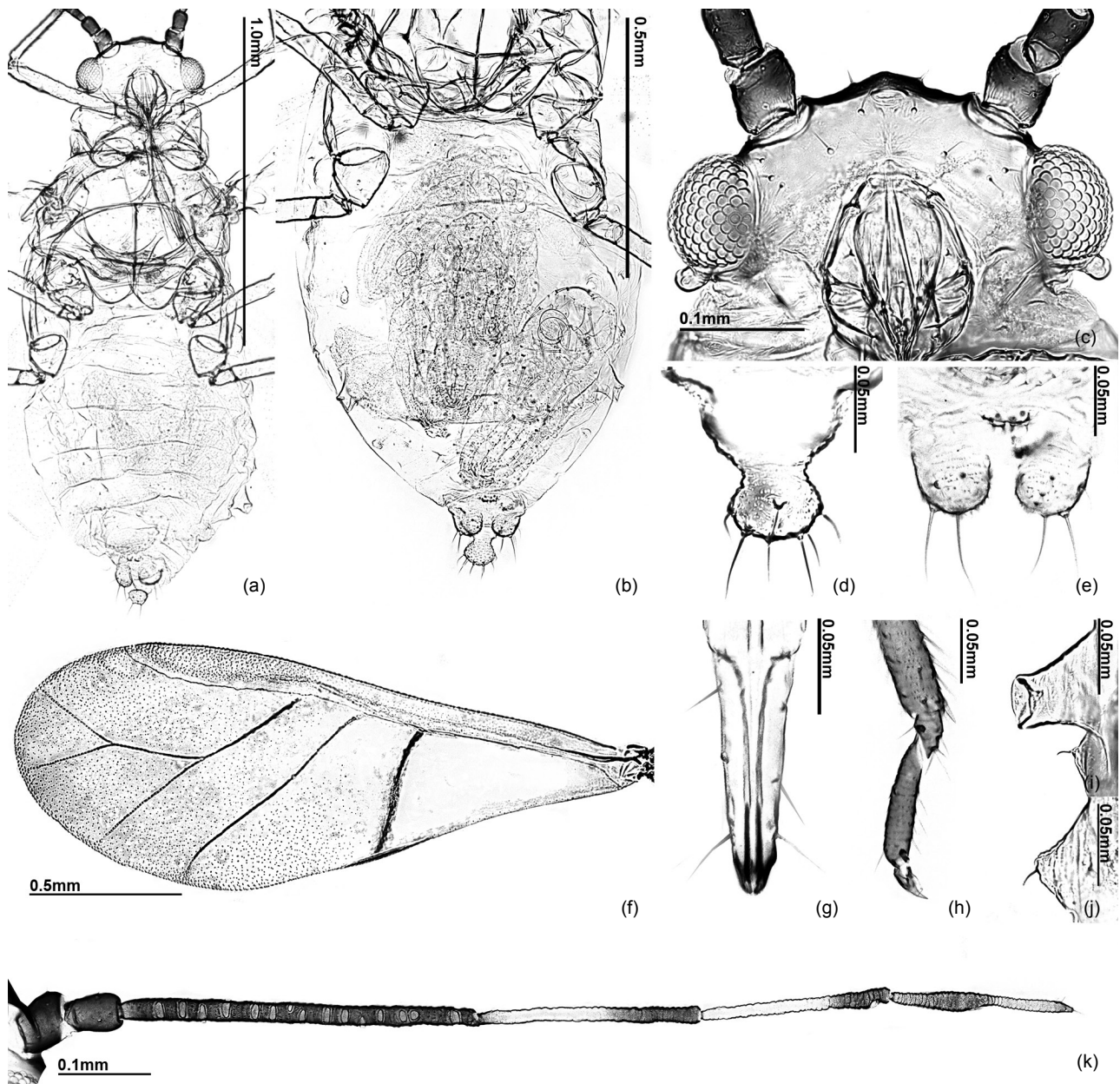


Fig. 6. Alate viviparous female of *M. (M.) pteleae*: (a) body; (b) abdominal dorsum; (c) head; (d) cauda; (e) anal plate; (f) forewing; (g) URS; (h) HT II; (i) SIPH; (j) marginal tubercle on ABD VI.

Mesocallis (Paratinocallis) occulta Lee Y. sp. nov.

(Figs. 1, 2, 4c-d, 9, 11; Table 1)

urn:lsid:zoobank.org:act:21873460-79B3-4ECE-BD7B-BD9100F727A6

Material examined: *Holotype*: 1 alate viviparous female, Sinwon-ri, Yongin-si, GG, South Korea, 37°18'56"N, 127°12'56"E, on *Corylus* sp., Yerim Lee and Hyoseok Lee leg., 16.v.2014, no. 140516YR-12.

Paratypes: 9 alate viviparous females, same

data as holotype, including DNA voucher no. Co12; 1 alate viviparous female, Silnae-pass, Hwacheon-gun, GW, 38°09'05"N, 127°31'02"E, on *Corylus sieboldiana*, Yerim Lee leg., 1.v.2014, no. 140501YR-15, DNA voucher no. Co9; 1 alate viviparous female, Mt. Jugeumsan, Gapyeong-gun, GG, South Korea, 38°08'38"N 126°54'17"E, on *Corylus heterophylla*, Yerim Lee leg., 9.v.2014, no. 140509YR10, DNA voucher no. Co11; 30 alate viviparous females, Mt. Hwayasan, Gapyeong-gun, GG, South Korea, 37°40'17"N 127°25'40"E,

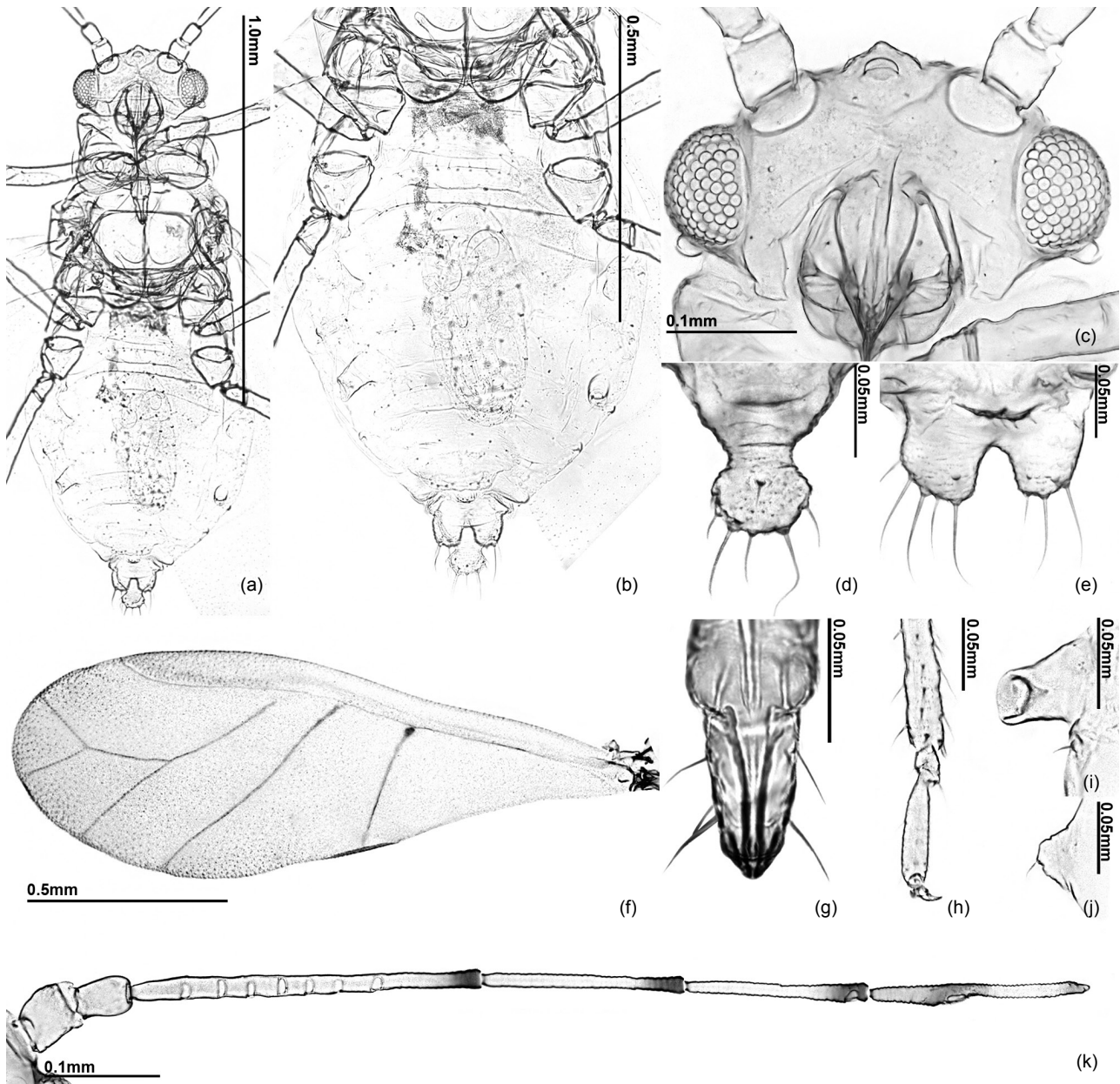


Fig. 7. Alate viviparous female of *M. (M.) sawashibae*: (a) body; (b) abdominal dorsum; (c) head; (d) cauda; (e) anal plate; (f) forewing; (g) URS; (h) HT II; (i) SIPH; (j) marginal tubercle on ABD VI.

on *Corylus heterophylla*, Yerim Lee leg., 26.v.2014, no. 140526YR-1, including DNA voucher no. Co16-1; 2 alate viviparous females, Mt. Gyeryongsan, Gongju-si., CN, South Korea, 36°20'33"N, 127°12'21"E, on *Corylus heterophylla*, Yerim Lee and Hyoseok Lee leg., 24.iv.2015, no. 150424YR-3, DNA voucher no. Mes3-1, Mes3-2; 1 alate viviparous females, Mt. Gwanggyosan, Suwon-si, GG, South Korea, 37°19'40"N, 127°00'54"E, on *Corylus heterophylla*, Yerim Lee and Hyoseok Lee leg., 10.v.2015, no. 150510YR-5, DNA voucher no.

Mes5-1.

Etymology: The species name, *occulta* is derived from Latin adjective, 'occulta' (hidden).

Diagnosis: This species is morphologically very similar to *M. (P.) corylicola* Higuchi in that it has a pale body and URS that is 0.07-0.09 mm in length. However, it can be distinguished by its shorter ANT IV length, 0.12-0.22 mm (0.23-0.32, in *M. corylicola*), shorter ANT V length, 0.13-0.18 mm (0.12-0.22, in *M. corylicola*), ANT 0.60-0.77 × BL (0.75-0.92, in *M. corylicola*) and URS 1.00-1.14 ×

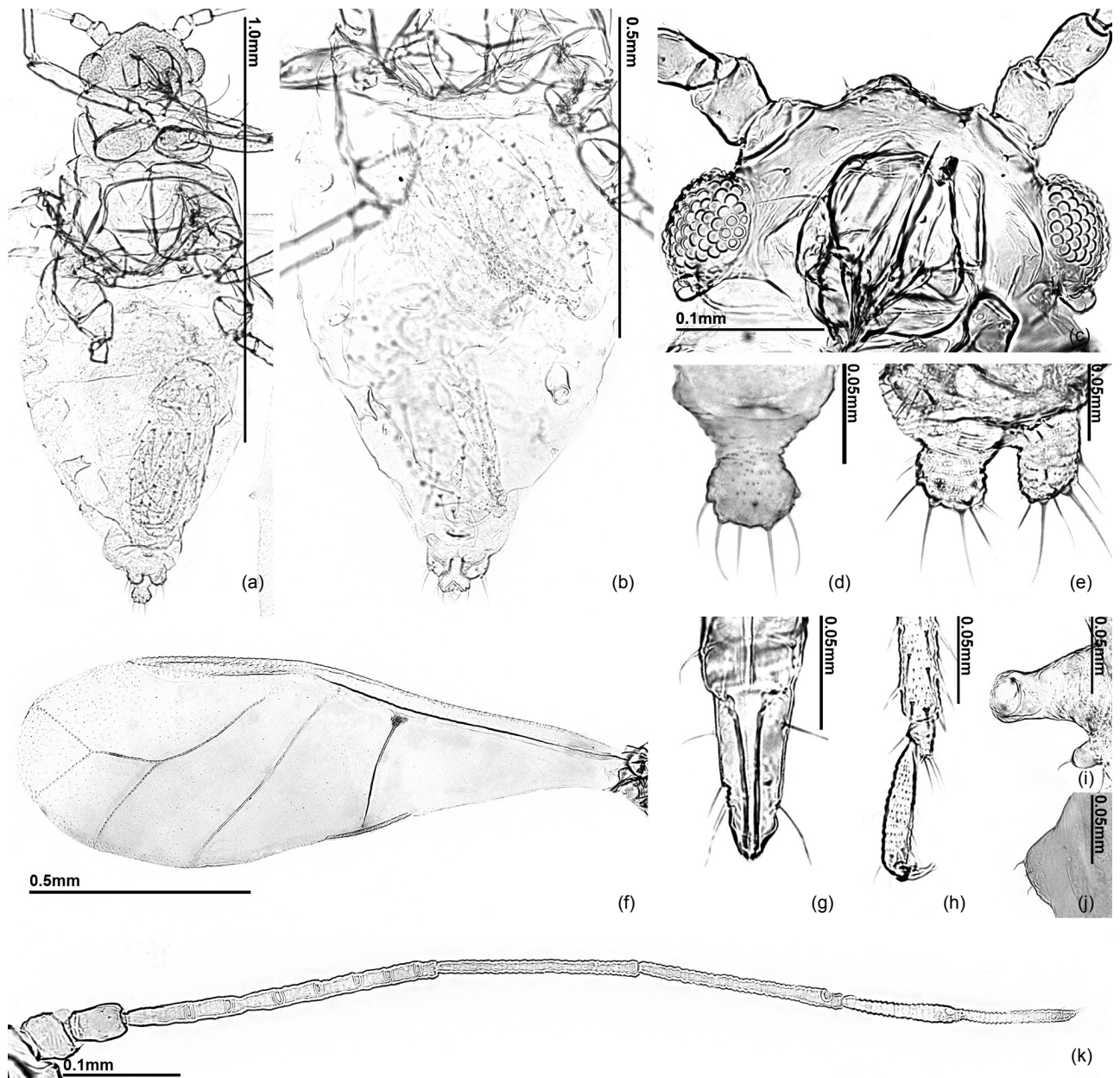


Fig. 8. Alate viviparous female of *M. (P.) corylicola*: (a) body; (b) abdominal dorsum; (c) head; (d) cauda; (e) anal plate; (f) forewing; (g) URS; (h) HT II; (i) SIPH; (j) marginal tubercle on ABD VI.

HT II (0.80-1.00 in *M. corylicola*).

Description (Alate viviparous female): *Color in life:* Head pale yellow, ANT pale with slight dark margin of ANT III and BASE. Thorax and abdomen pale yellow. Legs pale with 1/9 of fore tibiae and tarsi fuscous. Wing vein Cu1b of forewing bordered with brown pigment. SIPH pale. Cauda and anal plate pale.

Morphology: Body oval, BL 0.95-1.44 mm. Head smooth with a flat median protrusion on frons, epicranial suture weakly developed, head

dorsum without tubercles with 8 acuminate setae shorter than 0.01 mm long. ANT 6-segmented, 0.73-1.07 mm long, ANT 0.60-0.77 × BL, ANT III longest with 5-9 narrow elliptical secondary rhinaria in a row on the whole segment, bearing 3-4 short pointed setae, ANT III-VI imbricated with inconspicuous pointed setae, Ls ANT III × 0.50-1.00 BD III, ANT IV without secondary rhinaria, ANT V shorter than ANT IV, BASE with a single inconspicuous seta, PT 0.83-1.10 × BASE. Rostrum not reaching to middle coxae, URS 0.07-

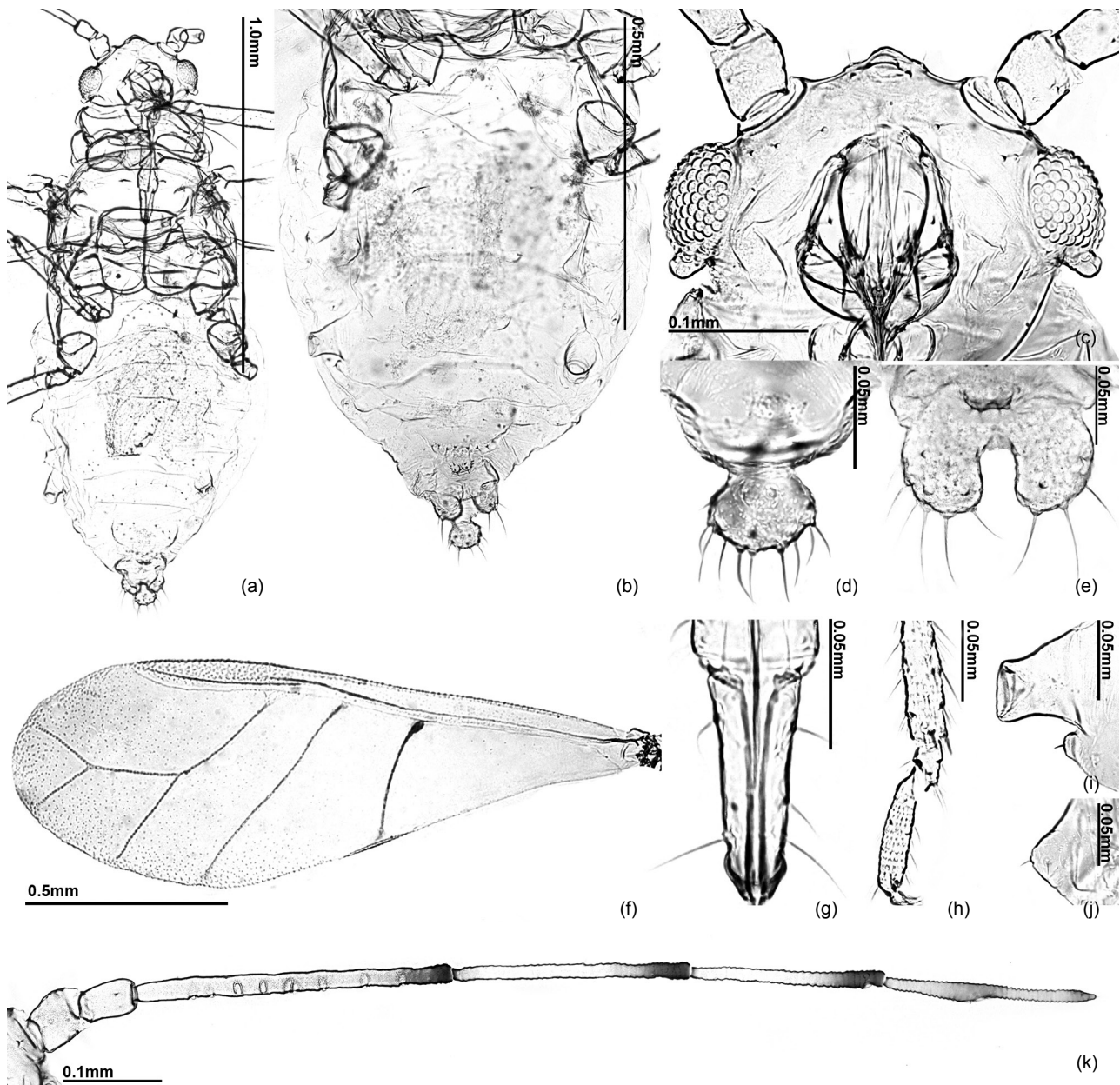


Fig. 9. Alate viviparous female of *M. (P.) occulta* sp. nov.: (a) body; (b) abdominal dorsum; (c) head; (d) cauda; (e) anal plate; (f) forewing; (g) URS; (h) HT II; (i) SIPH; (j) marginal tubercle on ABD VI.

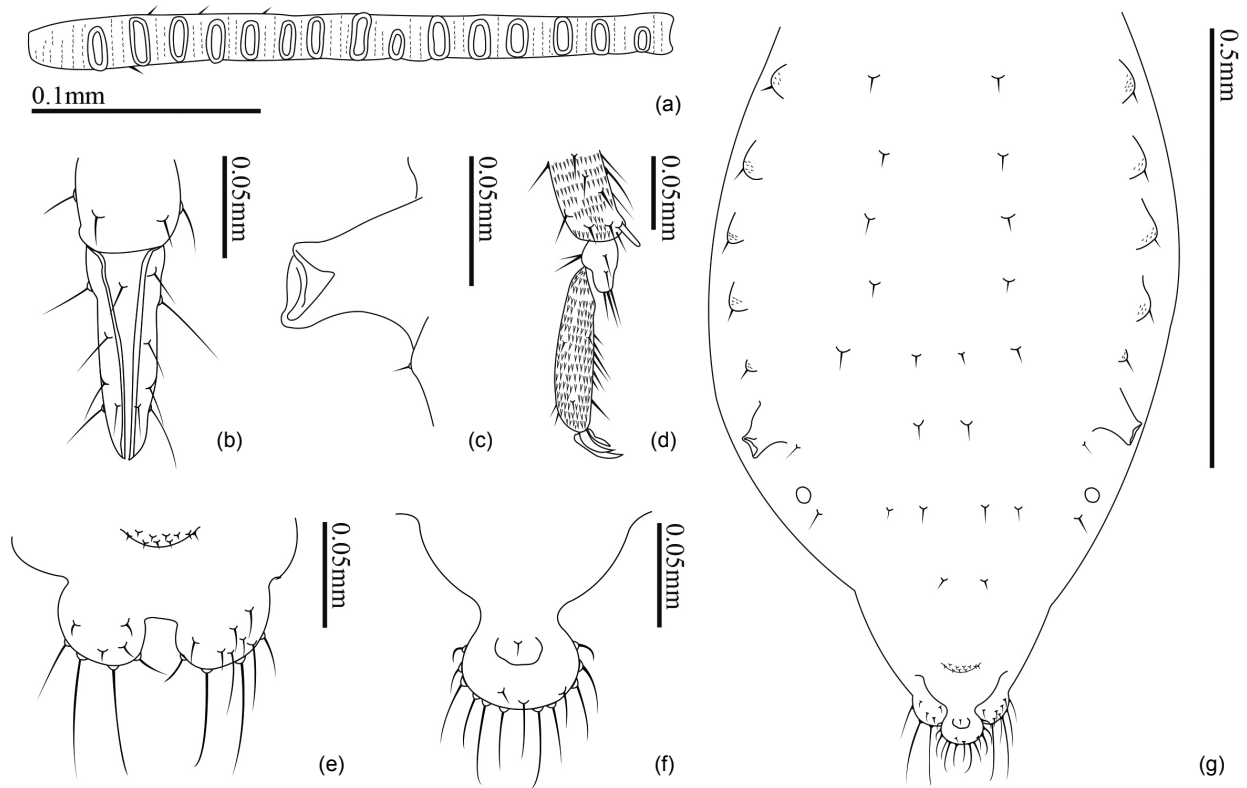


Fig. 10. Line illustration of *M. (M.) carpinicola* sp. nov.: (a) ANT III; (b) URS; (c) SIPH; (d) HT II; (e) anal plate; (f) cauda; (g) body.

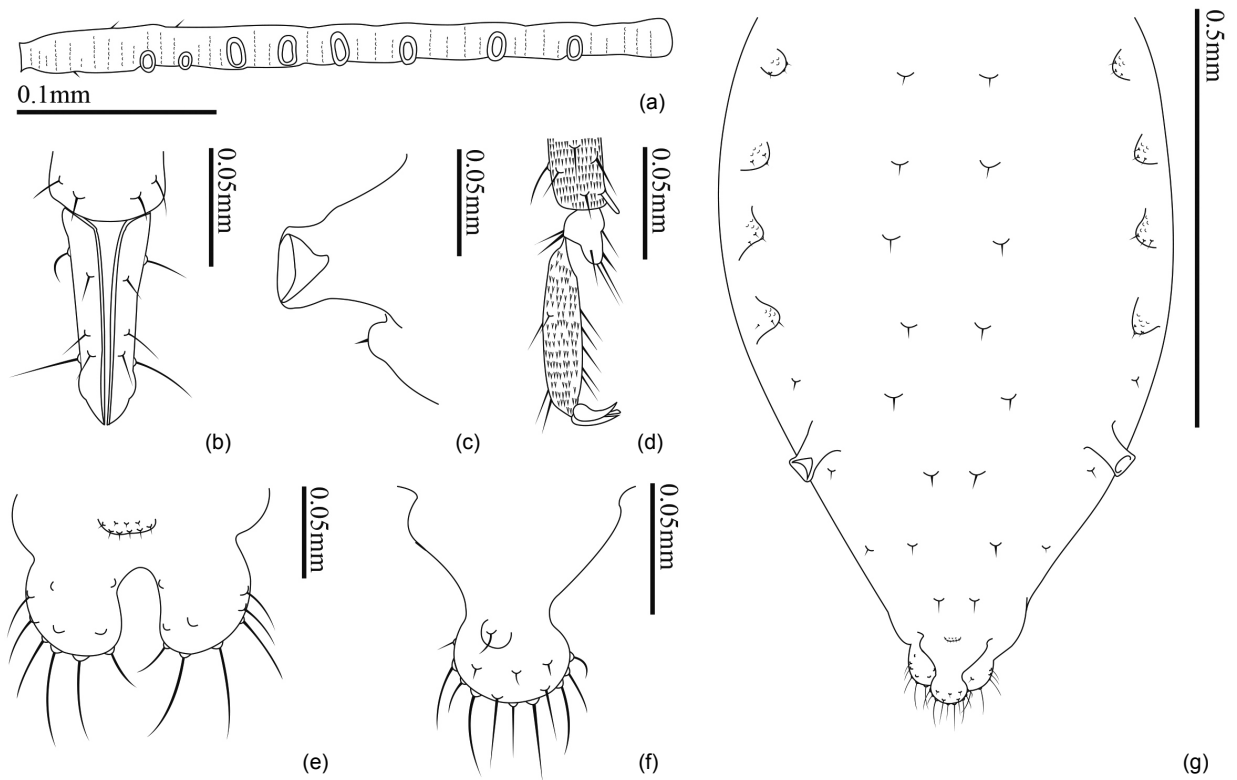


Fig. 11. Line illustration of *M. (P.) occulta* sp. nov.: (a) ANT III; (b) URS; (c) SIPH; (d) HT II; (e) anal plate; (f) cauda; (g) body.

0.09 mm long with 4-6 accessory setae, URS 0.75-1.00 × BASE, 1.00-1.14 × HT II. Thorax smooth without tubercles. Fore coxae slightly enlarged, longest setae on TIBIAE III 1.50 × middle width of TIBIAE III, HT I with 8 chaetotaxy, HT II 0.07-0.09 mm long. Wing vein Rs weakly developed. Dorsal spinal setae sometimes on small elevations, conspicuous tubercles not developed, ABD VIII bearing a pair of short setae, ABD margin I-IV with 0.01-0.02 mm long of 2 setae on short cone-shaped tubercle, 4th marginal tubercle 0.01-0.02 mm. SIPH cylindrical, truncated, 0.04-0.06mm long. Cauda knobbed, 0.07-0.09 mm with 9-13 setae. Anal plate bilobed, each lobe with 7-10 setae.

Distribution: So far, the new species is known from Gangwon-do, Gyeongsangnam-do, and Jeollanam-do in South Korea.

Host plant: Representatives of the new species feed on the underside of leaves of *Corylus* sp. (Betulaceae).

Remarks: This species was first recognized as a cryptic species of *M. (P.) corylicola* in the previous DNA barcoding study (Lee et al. 2017).

Molecular identification

The overall mean genetic distance was 7.33% for the 45 partial *COI* sequences of the 5 *Mesocallis* species. All species showed very low intraspecific distance level, ranging from 0% to 1.07% (Table 2). Among the five species, intraspecific distance was most varied in *M. corylicola*, with ranges of up to 1.07%; in contrast, 0% of intraspecific distance was observed among *M. carpinicola* sp. nov. In the remaining species, intraspecific distances were below 0.61%. Interspecific distance ranged from 6.21% to 12.72%. 11.81% to 12.72% of highest interspecific distances were observed between *M. pteleae* and *M. corylicola* (Table 2). *M. corylicola* and *M.*

occulta sp. nov. showed 6.21% to 7.40% of lowest interspecific distances.

The *COI* sequence NJ tree showed that the 45 sequences are clearly divided into five specific groups (Fig. 12). This result indicates that molecular identification based on *COI* barcoding region is effective for *Mesocallis*.

Haplotype analysis results found 128 variable positions in the 658bp *COI* region. Overall, 19 different haplotypes were observed, consisting of 1 haplotype for *M. carpinicola* sp. nov., 3 for *M. pteleae*, 4 for *M. sawashibae*, 9 for *M. corylicola* and 2 for *M. occulta* sp. nov. (Fig. 13).

DISCUSSION

In this study, five *Mesocallis* species were recognized based on morphological and molecular evidence. Among them, morphological similarities were observed within two different species pairs, *M. carpinicola* sp. nov. and *M. pteleae* (e.g. having a similar coloration and Cu1b of forewing dark bordered), and *M. corylicola* and *M. occulta* sp. nov. (e.g. having a pale body color and length of URS 0.07-0.09 mm). In particular, the latter species pair shows only some length differences. *M. occulta* sp. nov. has recently been disclosed with the comprehensive DNA barcoding of Calaphidinae (Lee et al. 2017). This species could have been overlooked as a result of intraspecific variation in *M. corylicola*, considering its ambiguous morphology and overlapping host-plant range.

In contrast to the similar morphological features, molecular analyses based on the *COI* region strongly supported the separation of five species with high interspecific distance values from 6.21% to 12.72%. Interspecific distances above 6.21% are much higher than the general species delimitation value of 2.5% in the subfamily Calaphidinae (Lee et al. 2017). Such a high genetic

Table 2. Intra- and inter-specific pairwise genetic divergence (%) based on the K2P model for five *Mesocallis* species

	<i>M. carpinicola</i> sp. nov. (n = 4)	<i>M. pteleae</i> (n = 9)	<i>M. sawashibae</i> (n = 6)	<i>M. corylicola</i> (n = 16)	<i>M. occulta</i> sp. nov. (n = 10)
<i>M. carpinicola</i> sp. nov.	0				
<i>M. pteleae</i>	7.71-8.01	0-0.61			
<i>M. sawashibae</i>	9.92-10.10	9.88-10.59	0-0.46		
<i>M. corylicola</i>	10.4-11.1	11.81-12.72	6.87-8.07	0.0-1.07	
<i>M. occulta</i> sp. nov.	11.47-12.01	10.76-12.01	7.86-8.20	6.21-7.40	0-0.46

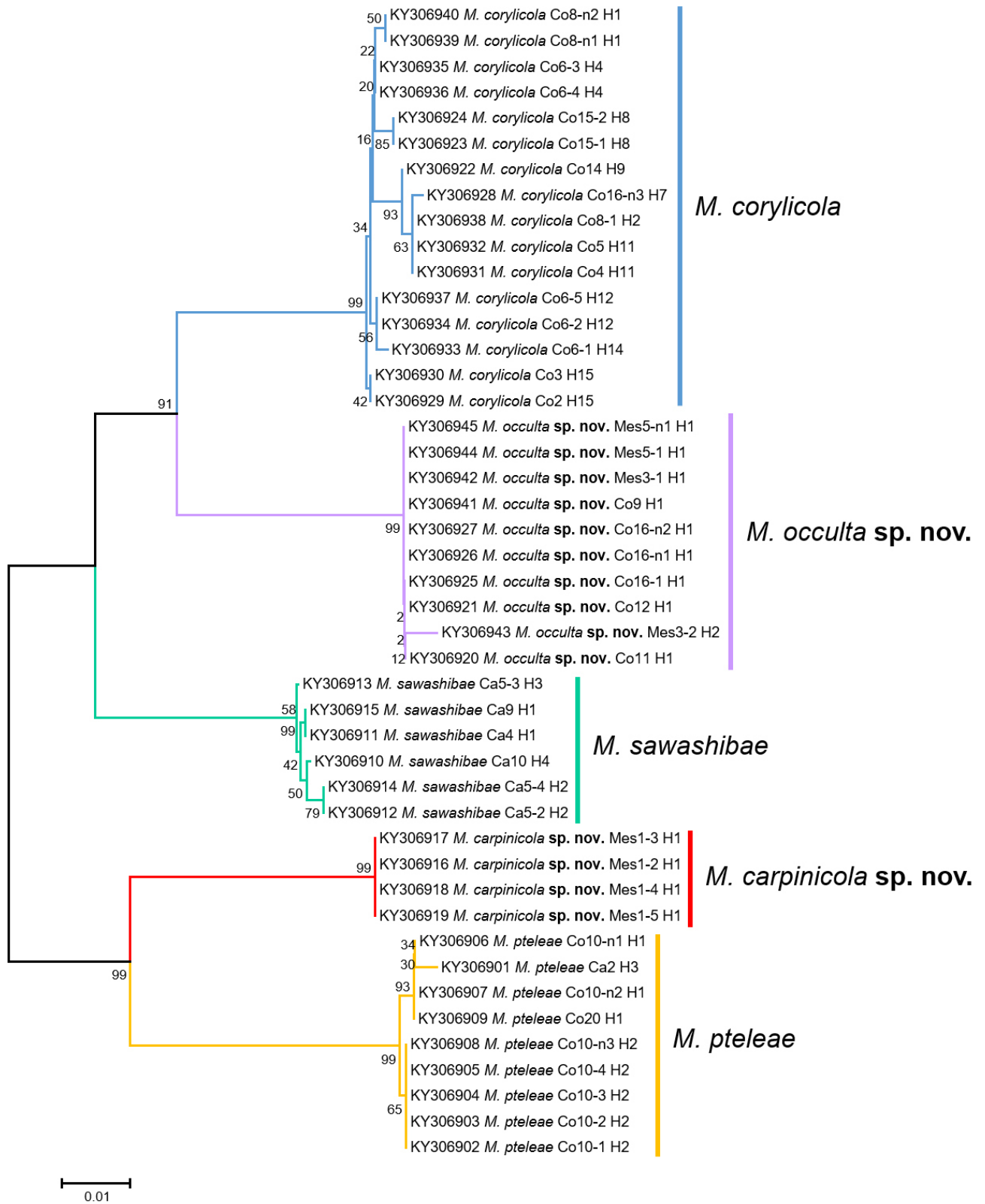


Fig. 12. Neighbor-joining tree of COI partial gene sequences of *Mesocallis* spp. (45 sequences of 5 species)

distance was also supported in the NJ tree and MJ network (Figs. 12-13). Each species group was distinctly separated from other species groups. These results suggest that DNA barcoding can be applied for rapid and reliable species identification in this group.

Haplotype diversity differed greatly depending on species. *M. corylicola* showed highest haplotype diversity with 9 haplotypes (Fig. 13). However, only 1 haplotype was found in *M. carpinicola* sp. nov. These differences may be due to differences in number of collection sites. *M. corylicola* is one of the most common species in the Korean peninsula and thus we could compare various populations from a number of regions of South Korea (Fig. 1). However, we could not make a comparison between various populations of *M. carpinicola* sp. nov. due to its distributional scarcity.

To date, *M. pteleae* has been known to occur on various host plants belonging to the family Betulaceae. However, it is questionable whether this species really has a wide host plant spectrum because polyphagous species are relatively very rare in the subfamily Calaphidinae. In this study,

we found that *M. carpinicola* sp. nov. has similar morphological features to *M. pteleae* and this species has only been collected on *Carpinus laxiflora* thus far. *Carpinus* spp. are some of the best known host plants of *M. pteleae*. Although we collected a single specimen of *M. pteleae* on *Carpinus* sp., a colony of *M. pteleae* (including females and nymphs) was only observed on *Corylus* spp. Among individuals of the above two species, high interspecific distances were detected, ranging from 7.71% to 8.01% (Table 2). Therefore, it will be necessary to reconfirm a true host plant range for *M. pteleae* by performing additional molecular works for different host plants associated groups, considering the *Mesocallis* group share some morphological similarities between its members.

CONCLUSIONS

We reviewed the genus *Mesocallis* in the Korean peninsula. Overall, five species - including two new species and one unrecorded species - are

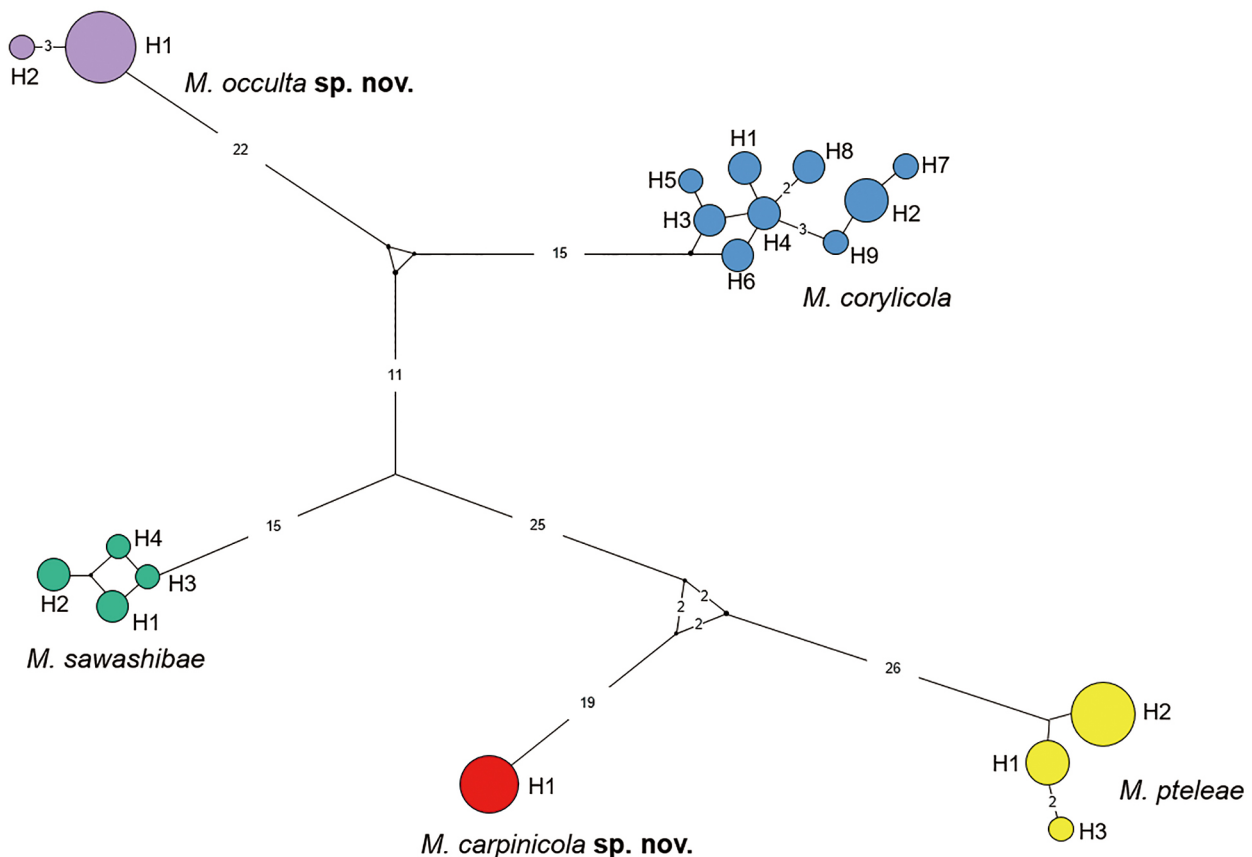


Fig. 13. Median-joining network for COI partial gene sequences of *Mesocallis* spp. (45 sequences of 5 species).

now identified in the Korean peninsula. Our study demonstrated that five *Mesocallis* species are clearly separated with the strong morphological and molecular evidence. We provided a number of morphological diagnostic characters and demonstrated that DNA barcoding is effective enough for species identification in this group. This study only dealt with 50% of all known *Mesocallis* species and thus future studies should compare more species in the genus. Future studies should also conduct molecular phylogenetic studies and investigate *M. pteleae*'s true host plant ranges in order to contribute to our knowledge of the *Mesocallis* group's evolutionary history.

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Authors' contributions: Conceptualization: YL. Data curation: YL. Formal analysis: YL. Funding acquisition: YL, SL. Investigation: YL. Project administration: YL. Specimen collection: YL. Writing - original draft: YL. Writing - review and editing: YL, MK.

Competing interests: YL, MK and SL declare that we have no competing interest.

Availability of data and materials: Our datasets from the manuscript are deposited in Genbank.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Appendix 1. Detailed collection information and Genbank accession numbers. (download)