Dermaphis coccidiformis sp. nov. (Hemiptera), an aphid species with asymmetrically sclerotized apterae and “winter alates”

Shigeyuki AOKI1, Utako KUROSU2, Keigo UEMATSU3,4, Takema FUKATSU3 and Mayako KUTSUKE3

1Faculty of Economics, Rissho University, Tokyo, Japan, 2Faculty of Economics, Chuo University, Hachioji, Japan, 3Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan and 4Department of General Systems Studies, University of Tokyo, Tokyo, Japan

Abstract

Dermaphis coccidiformis sp. nov. (Hormaphidinae: Nipponaphidini) is described from Japan. Apterous adults of the species were found between winter buds (or between a winter bud and a leaf petiole) of the evergreen oaks Quercus glauca, Q. myrsinifolia and Q. salicina. Their morphology is peculiar in that their tergites are heavily sclerotized only in the part that seems to have been exposed to sunlight. The new species is also peculiar in that nymphs to be alates (sexuparae) were found on the upper surfaces of leaves of the host oak only during winter, from December to March or early April, before the bud break of the oak. Our molecular phylogenetic analysis indicated that the new species is closely related to Dermaphis spp., therefore it was placed in the genus. The analysis incidentally indicated that “Dinipponaphis” autumna, a monoecious species forming galls on Distylium racemosum, was included in the clade of the genus Dermaphis, and therefore it was transferred to this genus.

Key words: Dinipponaphis autumna, life cycle, molecular phylogeny, Nipponaphidini, Quercus.

INTRODUCTION

The aphid tribe Nipponaphidini (Hormaphidinae) contains approximately 80–100 species (Remaudière & Remaudière 1997; Favret 2017). They basically have host-alternating life cycles. So far as is known, their primary hosts are evergreen trees of the genus Distylium (Takahashi 1962; Sorin 1987; Noordam 1991; Blackman & Eastop 1994; Chen et al. 2011) and its allied genera, Distyliopsis and Syropsis (Yeh 2009). Their secondary hosts are mainly evergreen trees of Fagaceae, Lauraceae and Moraceae (Takahashi 1958; Blackman & Eastop 1994; Yeh et al. 2008; Aoki et al. 2015). Apterous adults produced on the secondary host are sessile, and covered with hard exoskeletons. In the course of studying life cycles of Nipponaphidini, we found tiny but bizarre apterous adults, whose exoskeletons were irregularly and in most cases asymmetrically sclerotized, between or under winter buds of the evergreen oak Quercus glauca in Japan (Fig. 1a). To our surprise, this aphid species produced alate sexuparae only during winter. In this paper, by comparing its mitochondrial DNA sequence with those of other nipponaphidines, we settle its taxonomic position and describe it as a new species, and report its life cycle on the host oak.

MATERIALS AND METHODS

Sampling the focal aphids

Apterous adults and nymphs of the focal species, Dermaphis coccidiformis sp. nov., were mainly sampled from two trees (Trees A and B) of Q. glauca in Tama, Tokyo, Japan, in 2015–2017. Trees A and B were 22 and 21 cm in diameter, respectively, at breast height. Buds to which apterus adults were seen attached were cut off the trees. Later, aphids were carefully detached from the plant tissue under a dissecting microscope and preserved in 80% ethanol. Some were deposited in 99% ethanol for extracting DNA. To
confirm that the aphids live on *Q. glauca* all year round, sampling was carried out at least once every month (except November).

We noticed that *D. coccidiformis* nymphs to be developed into alates appeared on the upper surfaces of host leaves with good sunlight and fed there. To know when these nymphs are produced, leaves of Tree A were searched for the nymphs six times (approximately once every 12 days) from 1 February to 4 April 2015, and leaves of Tree B 16 times (approximately once every 9 days) from 28 November 2016 to 12 April 2017. When nymphs to be alates were found on leaves, the leaves were cut off and kept in plastic containers with a sheet of tissue paper slightly soaked with water. Early-instar nymphs and some (wingpadded) fourth-instar nymphs were deposited in 80% ethanol (usually within the day of collection), but a number of fourth-instar nymphs on the leaves were kept in the laboratory at room temperature to obtain alates. Even when the leaf was cut off the tree, most of the fourth-instar nymphs became alates. A total of 47 (including one that was collected in the field) and 45 alates were obtained in 2015 and 2017, respectively. Thirty-six alates, which had emerged from 10 February to 15 March 2015, were confined, together with a piece of paper, in 5 mL cotton-plugged glass vials to force their larviposition there. Two to nine days later, after confirming first-instar nymphs walking in the vials, 80% ethanol was poured into them. These nymphs were slide-mounted (see the next section), and it was determined whether they were sexuals or virginoparae (i.e. whether their mothers were sexuparae or secondary migrants). To supplement this, 81 alates (including those that did not give birth to all their embryos in the vials) were slide-mounted, and it was determined whether the embryos in their bodies were the same in morphology as the first-instar nymphs born in the glass vial.

Supplementary materials of *D. coccidiformis* were collected from *Q. glauca* in Kagoshima (Kagoshima Prefecture), from *Q. myrsinifolia* in Hachioji (Tokyo), Tsukuba (Ibaraki Prefecture), and Kashihara (Nara Prefecture), and from *Q. salicina* in Tanegashima Is. (Kagoshima Prefecture) (Table 1). Their morphology and mitochondrial DNA sequences were examined to confirm whether they were conspecific with the samples collected from *Q. glauca* in Tama.

**Examination of aphid morphology**

For slide preparation, aphids preserved in 80% ethanol were cleared in heated 10% KOH solution. These aphids were stained with either Evans' blue or acid fuchsin, dehydrated in a mixture of glacial acetic acid
and methyl salicylate for 1 day, and mounted in balsam via a mixture of xylol–phenol and pure xylol. Many slide-mounted specimens (including alates, apterous adults, first-instar nymphs produced by the alates, nymphs to be apterae and nymphs to be alates) were examined under a light microscope. Measurements for description were made using a digital camera (FX630; Olympus, Tokyo, Japan) equipped with image analysis software (FlvFs; Flovel, Tachikawa, Japan).

For the description of the alate, first-instar nymph to be apterae, first-instar nymph to be male and first-instar nymph to be (sexual) female, ten specimens of each morph were used. Because it was difficult to examine all the characters used for the description from each aptera owing to the strongly sclerotized tergites, the description of the aptera was based on observable characters of 20 individuals, including a few “teneral” individuals with less strongly sclerotized tergites (e.g. aptera of Fig. 2a).

Slide-mounted specimens including the holotype and the paratypes of D. coccidiformis, and the voucher specimens of this and other species for the DNA sequences (Table 1) are deposited in the collection of Systematic Entomology, Hokkaido University (Sapporo, Japan), as voucher specimens. Pref., prefecture; –, no accession number.

Table 1 Insect samples subjected to DNA sequencing

| Insect sample                  | Collection locality       | Collection date | Host plant                          | Accession no. |
|-------------------------------|---------------------------|-----------------|-------------------------------------|---------------|
| *Dermaphis coccidiformis*     | Tama, Tokyo               | 3.i.2015        | *Quercus glauca* (on or near bud)   | LC270791      |
| (#15006)                      |                           |                 |                                     |               |
| *D. coccidiformis* (#16042)  | Tama, Tokyo               | 11.xii.2016     | *Q. glauca* (on leaf)               | –             |
| *D. coccidiformis* (#150322) | Kagoshima, Kagoshima Pref.| 22.iii.2015    | *Q. glauca* (on or near bud)        | LC270795      |
| *D. coccidiformis* (#15163)  | Kashihara, Nara Pref.    | 17.v.2015       | *Quercus myrsinifolia* (on or near bud) | LC270792      |
| *D. coccidiformis* (#170327) | Tsukuba, Ibaraki Pref.   | 27.iii.2017     | *Q. myrsinifolia* (on or near bud)  | LC270793      |
| *D. coccidiformis* (#16002)  | Hachioji, Tokyo           | 28.i.2016       | *Q. myrsinifolia* (on or near bud)  | LC270794      |
| *D. coccidiformis* (#17040)  | Tanegashima Is., Kagoshima Pref. | 19.iii.2017 | *Quercus salicina* (on near bud) | LC270796      |
| *D. coccidiformis* (#17044)  | Tanegashima Is., Kagoshima Pref. | 21.iii.2017 | *Q. salicina* (on or near bud) | LC270797      |
| *Dermaphis japonensis* (#15111) | Tama, Tokyo               | 28.iii.2015     | *Quercus glauca* (on twig)          | LC270798      |
| *D. japonensis* (#15169)     | Orsu, Shiga Pref.         | 8.vi.2015       | *Q. glauca* (on twig)               | –             |
| *D. japonensis* (#15173)     | Orsu, Shiga Pref.         | 8.vi.2015       | *Q. glauca* (on twig)               | LC270800      |
| *D. japonensis* (#15139)     | Kashihara, Nara Pref.    | 18.v.2015       | *Quercus myrsinifolia* (on twig)    | LC270799      |
| *Dermaphis crematogastrii*    | Kitakyushu, Fukuoka Pref. | 29.x.2016       | *Quercus glauca* (on young twig)    | LC270801      |
| (#16017)                      |                           |                 |                                     |               |
| *Dermaphis sp. A* (#15062)   | Ishigaki Is., Okinawa Pref.| 19.iii.2016    | *Quercus miyagii* (on twig)         | LC270802      |
| *D. sp. A* (#15082)          | Ishigaki Is., Okinawa Pref.| 20.iii.2016    | *Q. miyagii* (on twig)              | LC270803      |
| *D. sp. A* (#15093)          | Ishigaki Is., Okinawa Pref.| 20.iii.2016    | *Q. miyagii* (on leaf)              | –             |
| “Dinipponaphis” *autumna*    | Kawanabe, Kagoshima Pref. | 17.vi.1997      | *Distylium racemosum* (in leaf gall) | LC270804      |
| (#2431)                      |                           |                 |                                     |               |
| “D.” *autumna* (#47)         | Shinkiba, Tokyo           | 15.xi.2005      | *D. racemosum* (in leaf gall)       | LC270805      |
| *Allthoracaphis piyananensis* | Ome, Tokyo                | 6.v.2013        | *Quercus glauca* (on leaf)          | LC270806      |
| (#13033)                     |                           |                 |                                     |               |
| *Metathoracaphis isensis*     | Kashihara, Nara Pref.    | 20.v.2015       | *Quercus glauca* (on leaf)          | LC270807      |
| (#15160)                     | Kitakyushu, Fukuoka Pref. | 19.xi.2015      | *Q. glauca* (on leaf)               | LC270808      |
| *Quernaphis tuberculata*     | Kitakyushu, Fukuoka Pref. | 15.xi.2005      | *D. racemosum* (in leaf gall)       | LC270805      |
| (#16020)                     |                           |                 |                                     |               |

1DNA sequences are deposited in the DDBJ/EMBL/GenBank nucleotide sequence database.

2Slide-mounted aphids from the same colony are deposited in the collection of Systematic Entomology, Hokkaido University (Sapporo, Japan), as voucher specimens.

DNA sequencing

Total DNA was extracted from each of fresh or fixed insects using a QIAamp Tissue Kit (Qiagen, Hilden, Germany). From the insect DNA, a 1.6-kb mitochondrial DNA fragment, containing small subunit rRNA,
tRNA-Val and large subunit rRNA genes, was amplified by polymerase chain reaction using two primers, MtrA1 (5'-AAWAAACTAGGATTAGATACCCTA-3') and MtrB1 (5'-CTTTAATYCAACATCGAGGTCGCTA-3'), under the temperature profile of 94°C for 2 min followed by 40 cycles of 94°C for 1 min, 48°C for 1 min, and 65°C for 3 min. The amplified DNA fragment was purified using ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) at 37°C for 15 min followed by 80°C for 15 min, and directly subjected to a sequencing reaction with a BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA, USA). In addition to MtrA1 and MtrB1 primers, the internal primers MtrA2 (5'-ACAAA GTAAARTGTACTGAGAAAGTGT-3'), MtrA3 (5'-ATTTTYATCTGTTTAAACAAACAT-3'), MtrA4 (5'-AGYAATACGTAACAWAGTAGTA-3'), MtrA5 (5'-AATAGCTGCAGTATTTTRACTGTC-3'), MtrB2 (5'-TTAATACGATGTTTTGGTTAACAACGACAG-3'), MtrB3 (5'-AACCTTTC GATACAYTTTACCTTTGCT-3'), MtrB4 (5'-TACTTGTTTTACGACTTTRCTTCT-3') and MtrB5 (5'-ACAGTYAAA ATACTGCGCTATT-3') were used for sequencing under a temperature profile of 94°C for 2 min followed by 30 cycles of 94°C for 1 min, 48°C for 2 min, and 65°C for 3 min. The reaction products were analyzed with a Genetic Analyzer (3130xl; Applied Biosystems). The accession numbers of the DNA sequences determined in this study are listed in Table 1.

Molecular evolutionary analysis

Samples of D. coccidiformis and some related species subjected to our molecular phylogenetic analysis are listed in Table 1. A multiple alignment of the nucleotide sequences (Appendix S1) was generated by using the program package CLUSTALW (Thompson et al. 1994). Aligned nucleotide sites containing gaps were removed from the dataset to generate a reliable alignment. Evolutionary divergence between nucleotide sequences was estimated by MEGA version 7 (Kumar et al. 2016). Molecular phylogenetic analyses were performed by maximum likelihood (ML) and Bayesian methods. The
RESULTS AND DISCUSSION

Description of the focal species

*Dermaphis coccidiformis* Aoki and Kurosu, sp. nov.

**Apterous adult** (Figs 1a,b, 2a)

The following description is based on 20 specimens collected from *Q. glauca* in Tama (Tokyo, Japan) on 11 and 25 December 2016, 3 January 2017, 11 and 23 February 2015, and 21 May 2017.

Body nearly oval in shape, 0.45–0.50 (mean 0.48) mm long. Tergites almost wholly, but weakly, sclerotized; head fused with prothorax, and abdominal tergites I–VIII fused with each other, but dorsal sutures between cephalothorax and mesothorax, between mesothorax and metathorax, and between metathorax and abdomen distinct, without wax plates. Head fused with prothorax, and abdominal segment I fused to form a prosoma. Tergites of prosoma extensively but not wholly sclerotized (Fig. 2a), with the extent of sclerotization varying between individuals; the part that was exposed to sunlight strongly sclerotized with reticulations, whereas the part that was hidden by plant tissues membranous. Head and three thoracic tergites each with two pairs of short marginal setae and a pair of short spinal setae; abdominal tergite I with a pair of short marginal setae and a pair of short spinal setae (setae on membranous part minute and at times lacking). Abdominal tergites II–VII fused to form a strongly sclerotized plate, which is 151–172 (159) μm wide (*n* = 7) with six pairs of marginal setae. Eighth abdominal tergite with two pairs of distinct setae which are stout and longest among setae on the tergites. Antenna usually three-segmented, but in some specimens one antenna reduced, two segmentated, and very short; third segment 24–41 (31) μm long (*n* = 14) with one apical seta, which is up to 11 μm long (*n* = 12). Rostrum reaching near between mid coxae; ultimate rostral segment conical, 51–62 (57) μm long (*n* = 17), without secondary setae (primary setae also invisible). Styles far longer than rostrum. Legs short; femoro-trochanter 52–74 (65) μm long on hind leg (*n* = 6); two tarsal segments united to form a single segment, which is 23–25 (24) μm long on hind leg (*n* = 4), with two small yet distinct claws and a pair of dorsosapical capitete setae, and on foreleg at times with a pair of minute ventral setae at base. Cornicle pore absent.

**First-instar nymph to be aptera** (Fig. 4a)

The following description is based on ten specimens collected from *Q. glauca* in Tama (Tokyo, Japan), on 12 January 2015 and 1 February (fixed on 13 February) 2015.

Body nearly oval in shape, 0.45–0.50 (mean 0.48) mm long. Tergites almost wholly, but weakly, sclerotized; head fused with prothorax, and abdominal tergites I–VIII fused with each other, but dorsal sutures between cephalothorax and mesothorax, between mesothorax and metathorax, and between metathorax and abdomen distinct, without wax plates. Head 0.17–0.20 (0.18) mm wide across eyes. Antenna four-segmented; apical two segments weakly imbricate; segment III 38–46 (42) μm long, without seta; segment IV 40–47 (43) μm long, with four apical setae, two of which are long and up to 36–44 (39) μm. Rostrum reaching mid coxae; ultimate rostral segment conical, 49–55 (52) μm long, without secondary setae; styles very long, far longer than rostrum, singly looped in...
body, possibly in “crumena” as known in adelgids (Ponsen 2006), when not extended from apex of rostrum, with the loop extending near end of abdomen. Hind femorotrochanter 72–81 (77) \(\mu m\) long. Segment I of hind tarsus with a pair of long spatulate setae; segment II 32–35 (34) \(\mu m\) long on hind leg, dorsally with a pair of pointed setae at basal 1/3–1/2 and a pair of long, capitate setae near apex, ventrally with a pair of short pointed setae at apical 1/3–1/2, and laterally with a pair of spatulate setae at apical 1/3; empodial setae spatulate, extending a little beyond apices of the claws. Tergites surrounded by marginal setae: head and three thoracic tergites each with two pairs of marginal setae; abdominal tergites I–VIII each with a pair of marginal setae; these marginal setae pointed, rather thick except for two pairs on segments V and VI which are much shorter and thin. Head, three thoracic tergites and abdominal tergite I each with a pair of minute spinal setae. No pleural setae on tergites. Cauda with two short setae; anal plate with four short setae. Cornicle pore absent. (No cornicles appear throughout the instars in the apterous generation.)

**First-instar nymph to be alate** (Fig. 4b)
The following description is based on ten specimens collected from leaves of *Q. glauca* in Tama (Tokyo, Japan) on 5 December 2016.

A little larger than first-instar nymph to be aptera; body 0.47–0.53 (mean 0.50) mm long. Tergites extensively sclerotized with mosaic-like structures, but narrowly membranous between three thoracic tergites and first two abdominal tergites; head fused with prothorax, and abdominal tergites III–VIII fused with each other, without wax plates. Head 0.18–0.20 (0.19) mm wide across eyes. Antenna four-segmented; apical two segments weakly imbricate; segment III 49–59 (52) \(\mu m\) long, without seta; segment IV 41–52 (46) \(\mu m\) long, with four apical setae, two of which are long and up to

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*Figure 3* Molecular phylogenetic analysis of nipponaphidine species on the basis of their mitochondrial rRNA gene sequences. The maximum likelihood (ML) phylogeny inferred from unambiguously aligned 1,521 nucleotide sites is shown. The Bayesian inference analysis substantially exhibits the same topology. Bootstrap probability (ML) and posterior probability (Bayesian) values in percentage are indicated at the nodes, on the left and on the right, respectively. The minus symbol (−) indicates a support value lower than 50%.
26–35 (32) μm. Rostrum reaching mid coxae; ultimate rostral segment truncated at apex, 35–40 (38) μm long, without secondary setae; styles far longer than rostrum but much shorter than those of first-instar nymph to be aptera, up to 274 μm, extending near the end of abdomen but not beyond it. Hind femorotrochanter 88–102 (95) μm long. Segment II of hind tarsus 35–40 (38) μm long; setal arrangement as in first-instar nymph to be aptera. Arrangement of setae on tergites, cauda and anal plate as in first-instar nymph to be aptera, but marginal setae shorter than those of the latter. Cornicle pore not sclerotized around, located near fifth and sixth marginal setae on abdomen. (Distinct cornicles appear from the second instar onward.)

Alate sexupara (Figs 1d, 2b)

Unless the sample size is indicated in parentheses, the following description is based on ten specimens collected as nymphs from leaves of Q. glauca in Tama (Tokyo, Japan) between 1 February and 3 March 2015, and emerged in the laboratory between 10 February and 11 March 2015.

Body 1.1–1.5 (mean 1.3) mm long. Head 0.31–0.34 (0.33) mm wide across compound eyes, ventrally with 2–4 (2.3) short setae between compound eyes near bases of antennal sockets. Antenna (Fig. 2b) five-segmented; segment III 326–399 (361) μm long, longer than fore tibia, 2.26–2.81 (2.53) times as long as segments IV and V combined; segment IV 80–111 (91) μm long; segment V shorter than IV, 46–60 (52) μm long; processus terminalis very short, with only three short apical setae; secondary rhinaria narrow, often more than half or two-thirds encircling circumference of the segment; segments III, IV and V with 16–21 (18.4), 4–6 (4.4) and 2–3 (2.4) secondary rhinaria, respectively; primary rhinaria on segments IV and V united with the distal secondary rhinaria. Ultimate rostral segment 65–68 (67) μm long (n = 9), without secondary setae. Legs slender; fore tibia 264–326 (293) μm long; hind femorotrochanter 269–316 (294) μm long; tarsi two-segmented; segment I with a pair of long, spatulate setae, and one shorter spine-like seta or two on fore and mid legs; segment II 77–87 (81) μm on hind leg, mid-dorsally with a pair of pointed setae and apically with three pairs of setae, of which dorsoapical and lateroapical pairs are long and widened at apex, and ventroapical pair are short and pointed; empodial setae reaching apices of the claws, spatulate at apex. Forewing with once-branched media, and bases of the two branches usually (but not always) connected. Cornicle ring-like, only slightly elevated, 31–39 (35) μm in outer diameter at apex. Abdominal tergite VII membranous, with only a marginal pair of setae; tergite VIII weakly sclerotized, with a pair of setae mesially and at times with a marginal seta on one or both sides. Cauda constricted at base, 58–66
μm wide, with a pair of long setae and 8–10 (9.4) shorter setae. Anal plate bilobed, with a total of 13–16 (14.3) setae. Genital plate with 12–16 (14.0) setae.

First-instar nymph to be (sexual) female (Fig. 5a,c)
The following description is based on ten specimens deposited by alates in vials, which were fixed on 15 March 2015. The alates were collected as nymphs from leaves of *Q. glauca* in Tama (Tokyo, Japan) on 23 February 2015 and 3 March 2015, and emerged in the laboratory.

Body 0.56–0.63 (mean 0.60) mm long. Tergites membranous, without wax plates (Fig. 5a,c). Head 0.19–0.24 (0.21) mm wide across eyes. Antenna four-segmented; apical two segments weakly imbricate; segment III 64–78 (70) μm long, without seta; segment IV 59–78 (70) μm long, with four apical setae (rarely one additional seta at middle), two of which are long and up to 20–30 (27) μm. Rostrum reaching mid coxae; ultimate rostral segment 44–49 (46) μm long, without secondary setae; stylets thin, as long as rostrum, not looped in body. Hind femorotrochanter 110–123 (114) μm long. Hind tibia with no spine-like setae near apex. Segment II of hind tarsus 47–52 (50) μm long; setal arrangement as in first-instar nymph to be aptera. Tergites surrounded by marginal setae: head, three thoracic tergites each with two pairs of marginal setae; abdominal tergites I–VIII each with a pair of marginal setae. Head, three thoracic tergites and abdominal tergites I–VII each with a pair of spinal setae, but one or both often lacking on abdominal segments II–VII. No pleural setae on tergites. A pair of small cornicle pores, which are weakly sclerotized along the anterior margin, appearing near marginal setae on abdominal tergite VI (Fig. 5c). Cauda with two (rarely 3) setae; anal plate with four setae.

First-instar nymph to be male (Fig. 5b,d)
The following description is based on ten specimens deposited by alates in vials, which were fixed on
15 March 2015. The alates were collected as nymphs from leaves of *Q. glauca* in Tama (Tokyo, Japan) on 23 February 2015 and 3 March 2015, and emerged in the laboratory.

Body 0.48–0.51 (mean 0.49) mm long. Tergites extensively sclerotized, with small, round, and distinctly demarcated wax plates (Fig. 5d): a marginal pair of wax plates on three thoracic tergites and abdominal tergites I–III, VI and VII (at times additional one pair or a single wax plate on prothorax, and in one specimen a single plate appearing on abdominal tergite IV); a spinal pair of wax plates on head, three thoracic tergites and abdominal tergites II and III (additional one to three wax plates on head, usually additional one or two on prothorax, and rarely additional one or two on abdominal tergite I or IV). Head 0.18–0.21 (0.20) mm wide across eyes. Antenna four-segmented; apical two segments weakly imbricate; segment III 51–56 (52) μm long, without seta; segment IV 42–52 (48) μm long, with four apical setae, two of which are long and up to 32–39 (37) μm. Rostrum reaching mid coxae; ultimate rostral segment 40–47 (45) μm long, without secondary setae; styles longer than rostrum, looped in the body. Hind femorotrochanter 95–102 (99) μm long. Hind tibia distally with two spine-like setae arranged in an axial row on the inner side. Segment II of hind tarsus 39–42 (41) μm long; setal arrangement as in first-instar nymph to be aptera. Tergites surrounded by marginal setae, which are thicker than those of the first-instar female: head and three thoracic tergites each with two pairs of marginal setae (one of them lacking on two specimens); abdominal tergites I–VIII each with a pair of marginal setae. Head, three thoracic tergites and abdominal tergite I each with a pair of spinal setae. No pleural setae on tergites. Cornicle absent. Cauda with two setae; anal plate with four setae.

**Discrimination from related species**

The apterous adult (on the secondary host) of *D. coccidiformis* can be easily discriminated from those of other *Dermaphis* species by its small size, and also by its partially membranous tergites from *D. japonensis* and *D. crematogastri*. (Apterous adults of *D. sp. A* may have partially membranous tergites. The species will be described in a separate paper when more information is available.) The alate sexupara of *D. coccidiformis* may be discriminated from the alate of *D. japonensis* by the shorter antennal segment III and the smaller ratio of the length of the antennal segments IV + V to that of the segment III: 326–399 μm and 0.36–0.44 in *D. coccidiformis*, while 426 μm and 0.58 in *D. japonensis* (Sorin 2006). Apterous adults of the genus *Neodermaphis* may resemble those of *D. coccidiformis*, but one facet of their trichomatidation is located apart from the remaining two and both of their antennae are reduced to two segmented in *Neodermaphis* (Sorin 2006).

**Type materials**

**Holotype.** One apterous adult (exule) singly mounted on a slide, labeled “Tama, Tokyo, Japan; 25 xii 2016; ex *Quercus glauca* (on bud); no. 16052; U.K. & S.A. leg.”

**Paratypes.** Six slides containing nine apterous adults (exules), seven alate sexuparae, five first-instar nymphs to be apterae, five first-instar nymphs to be alates and nine first-instar sexuals. They were all collected from *Q. glauca* in Tama except that the first-instar sexuals were borne by alates in a glass vial (and fixed on 15 March 2015). The apterous adults, the first-instar nymphs to be apterae and the first-instar nymphs to be alates were collected on 3 January 2017, 1 February 2015 (fixed on 13 February) and 5 December 2016, respectively. The alates were collected as nymphs on 1 February 2015 (fixed on 15 February) and 3 March 2015 (fixed on 15 March).

**Maximum likelihood and Bayesian trees and the taxonomic position of the focal species**

The results of our molecular phylogenetic analyses based on mitochondrial ribosomal DNA sequences are summarized as the maximum likelihood tree in Figure 3. The Bayesian inference analysis substantially exhibited the same topology. With the bootstrap (ML) and posterior (Bayesian) probabilities of 100%, the seven samples of *D. coccidiformis* formed a monophyletic group. There is no doubt that the aphids on *Q. glauca* and *Q. myrsinifolia* belong to the same species, and that nymphs found on leaves of *Q. glauca* are conspecific with the aphids found on or near buds of the oaks. The genetic distance between aphids from *Q. salicina* (collected in Tanegashima Is., southern Japan) and those from *Q. glauca* and *Q. myrsinifolia* (collected in the main islands Kyushu and Honshu) was 0.7–0.8%, while genetic distances within other *Dermaphis* species, as well as within *D. coccidiformis* populations of Tanegashima Is. and the main islands, were smaller than 0.1% (Table S1). Although the two geographically distant populations of *D. coccidiformis* seem to diverge genetically, at present there is no other evidence to suspect that they might belong to two different species.

The seven samples of *D. coccidiformis*, with the bootstrap and posterior probabilities of 99 and 100%,
respective, formed a clade together with *D. japonensis*, *D. crematogastri*, *D. sp. A*, and “Dinipponaphis” *autumna* (Fig. 3). This offered us a good reason for placing the focal species in the genus *Dermaphis*. In fact, except for its small size and irregular sclerotization, the apterous adult of *D. coccidiformis* is similar to those of other *Dermaphis* species on oaks in the basic structure (cf. Sorin 2006).

The phylogenetic analysis incidentally indicated that “Dinipponaphis” *autumna*, which is monoecious on *Distylium racemosum* (Sorin 1960; Takahashi 1962; Aoki et al. 1999), belongs to this clade. Because the genus *Dinipponaphis* has been monotypic (Takahashi 1962) and because “*D.* *autumna*” has no secondary-host generation, the taxonomic position of the species has hitherto been unclear within the tribe Nipponaphidini. We transfer the species into the genus *Dermaphis*, and treat the genus name *Dinipponaphis* Takahashi, 1962 as a junior synonym of *Dermaphis* Takahashi, 1958.

**Life cycle**

In Tama, Tokyo, live apterous adults (and nymphs to be apterae) of *D. coccidiformis* were found on *Q. glauca* (Trees A and B) all year round. In many cases, apterous adults were hidden between a winter bud and a leaf petiole (Fig. 1a) and were feeding on the petiole. Some were feeding on the bases of winter buds (Fig. 1b). The tergites of apterous adults were irregularly and often asymmetrically sclerotized. The tergites were strongly sclerotized in the part that presumably had been exposed to sunlight, but membranous or only weakly sclerotized in the other part that had been hidden by plant tissue (Fig. 2a). In 2015–2017, new shoots developed from winter buds of Trees A and B in April. In April and May, because the newly formed winter buds were still small on the shoots and there were no crevices that could hide them, apterous adults were not found around the new buds; some were attached near the bases of developed shoots. On 17 June 2015 and 27 July 2015 we found apterous adults and nymphs around new buds.

First-instar nymphs (Fig. 4a) found together with these apterae had very long styles. There is no doubt that they were to be apterae. Because the first-instar nymphs to be apterae have well-sclerotized tergites and well-developed legs and antennae, which are longer than the legs and antennae of the apterous adults, respectively, they are likely to be dispersers between buds of an oak tree, or maybe between oak trees. Apterous adults are thought to reproduce throughout the year on the host oak.

Nymphs to be alates (Fig. 1c) were noticed for the first time on 1 February 2015 on leaves of *Q. glauca*, and had been seen until 3 March 2015. In the winter season of 2016/2017, these nymphs were seen from 5 December to 5 April (not found on 28 November 2016 nor on 12 April 2017). First-instar nymphs were found from 5 December to 8 February in 2016/2017. There were usually one or a few nymphs on a single leaf, and all of them (all nymphal instars) were on the upper surface. That is, first-instar nymphs produced by apterous adults on or under winter buds appear on the upper surfaces of nearby leaves. These first-instar nymphs to be alates (Fig. 4b) were different from first-instar nymphs to be apterae (Fig. 4a) in morphology; they had much shorter styles and shorter marginal setae on the tergites. Differentiation in the morphology of first-instar nymphs between alate and apterous generations on the secondary host is already known in some nipponaphidines such as *Thoracaphis kashifolia* (Uye, 1924) (Kurosu et al. 2016). Because cast-off skins of (wingpadded) fourth-instar nymphs were also found on the upper surfaces of leaves, the emergence to the alate adult should have taken place there. A teneral alate, which seemed to have emerged on a leaf, was found on the underside of the leaf in the field on 11 February 2015. In the laboratory, too, teneral alates were found on the undersides of leaves (Fig. 1d) in plastic containers. The newly emerged alates therefore move onto the undersides of the leaves before the final takeoff. No ant was observed attending nymphs on leaves, or apterae around buds.

The first-instar nymphs deposited by alates in the vials showed a clear dimorphism. Nymphs of one morph (Fig. 5a,c) had membranous tergites with no demarcated wax plates, thin and short styles, and a pair of cornicle pores, while nymphs of the other morph (Fig. 5b,d) were smaller and had more strongly sclerotized tergites with small, round wax plates but without cornicle pores, and thicker styles which were looped in the body and longer than the rostrum. There is little doubt that the former were of the (sexual) female and the latter of the male, and that the mother alates were sexuparae. The first-instar nymphs of both morphs were distinct from first-instar nymphs to be (parthenogenetic) apterae and alates. All 92 alates we examined were sexuparae, and no secondary migrants as known in *Thoracaphis kashifolia* (Kurosu et al. 2016) were found. The sexuparae would fly back to their primary host, but it is unknown whether the primary host is *Distylium* or other trees.

Two features of the life cycle of *D. coccidiformis* are worth pointing out. First, it is peculiar that the species produces alates during winter, or “winter alates.”
Many nipponaphidines that use evergreen trees as the secondary host, *Nipponaphis monzeni* and *N. distyliicola*, for example, grow and reproduce (though slowly, presumably due to the low temperature) on the host trees during winter in the south-western half of Honshu, Japan (Sorin 1958). The two species, however, produce alates in April and May (Kurosu & Aoki 1998, 2009), when the host oaks (*Q. glauca* and *Q. myrsinifolia*) are producing new shoots and, as in other tree-dwelling aphid species (Dixon 2005), may get enough nutrition to reproduce a lot. In contrast, *D. coccidiformis* ceases producing alates before the host oak trees begin budding. Second, nymphs to be alates feed on the upper, sunlit surfaces of leaves. They remain there until they become alates and then move onto the underside. Although they are tiny, it is rather easy for human eyes (and therefore perhaps also for birds) to detect these nymphs on the upper surface of a leaf. Considering the fact that they are black in color (Fig. 1c), the nymphs to be alates might utilize radiant heat from the sun for their growth.

There may be other nipponaphidine species that produce such “winter alates.” *Dermaphis japonensis* is a candidate. Sorin (2006) found alates of *D. japonensis* from *Q. glauca* in Ise (Mie Prefecture, Japan) on 20 January 1984, and we also found two alates from *Q. glauca* in Niiza (Saitama Prefecture, Japan) on 21 January 1996. It remains to be confirmed whether nymphs to be alates of *D. japonensis* grow on the upper surfaces of leaves as in *D. coccidiformis*.

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SUPPORTING INFORMATION
Additional Supporting Information may be found online in the supporting information tab for this article.
Appendix S1 Multiple sequence alignment used for the molecular evolutionary analyses.
Table S1 Pairwise genetic distances of mitochondrial DNA sequences within and between the aphid species used in the molecular phylogenetic analysis.