Description of the Asian chili pod gall midge, Asphondylia capsicicola sp. n., with comparative notes on Asphondylia gennadii (Diptera: Cecidomyiidae) that induces the same sort of pod gall on the same host plant species in the Mediterranean region

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Abstract:	A new species of the genus Asphondylia (Diptera: Cecidomyiidae) that infests pods of chili, Capsicum annuum Linnaeus and C. frutescens Linnaeus (Solanaceae), is described as A. capsicicola sp. n. based on specimens collected from Indonesia and Viet Nam. The new species is similar to Asphondylia gennadii (Marchal) (= A. capsici) that induces chili pod galls in the Mediterranean region but is distinguishable from it by the morphological features of pupa such as nonlinear arrangement of lower frontal horns, narrower longitudinal band of transverse wrinkles on the tergite of mesothorax. Differences between the two species in the DNA sequencing data were 69 bp (16 %) to 77 bp (19 %) among 413 bp of the partial COI region examined, supporting the morphological identification. This is one of the examples in which two congeners induce the same sort of gall on the same host plant organ and species, which means that the two species cannot be distinguishable based solely on gall shape and host plant information unlike many other gall midges
Response to Reviewers:	Authors' response to the review's comments

We thank reviewers for careful reading our manuscript and for giving us useful comments.
We have revised the manuscript following the Reviewers' comments. We look forward to seeing our paper published in Applied Entomology and Zoology.

Our responses to the #1 Reviewer's comments are as follows:
line 221: frutescence >> frutescens
lines 291-305 should be the first paragraph of Discussion
=> this paragraph was moved to lines 316 – 330.
line 335: please specify the polyphagy of A. gennadii viz. (Ceratonia/Fabaceae, Capparis/Capparaceae, Capsicum/Solanaceae; Urginea/Asparagaceae, former Liliaceae)
=> host plants of A. gennadii were described in lines 335-337.
line 434: Sarothamni scoparius (L.) Wimmer. >> Sarothamnus scoparius (L.) Wimmer (correct name and delete '.')
line 442 Stelter von >> Stelter (delete 'von')
line 460: Galls >> galls;
Caption Fig. 6: add '.' after 'gennadii'
Table 2, column 'host plant' furtescens >> frutescens (3 X)

In addition, we added minor changes, mainly according to the 'Instructions for authors';
line 15: A. capsicicola => Asphondylia capsicicola
line 150: Pseudasphondylia matatabi (Yuasa and Kumazawa 1938) => Pseudasphondylia matatabi (Yuasa and Kumazawa); in the References, Yuasa and Kumazawa (1938) was deleted.
line 215: …on third through ninth segments. => … on first through eighth segments.
line 274: … Asphondylia yushimai Yukawa and Uechi and Asphondylia aucubae Yukawa and Ohsaki, … => A. yushimai and A. aucubae
line 293: … Asphondylia capsicicola is …=> … A. capsicicola is …
line 320: A. itoi Uechi and Yukawa => Asphondylia itoi Uechi and Yukawa
line 325: A. pilosa Kieffer => Asphondylia pilosa Kieffer
line 325: (= A. mayeri Liebel) => (= Asphondylia mayeri Liebel)
line 327: A. punica Marchal, A. conglomerata De Stefani => Asphondylia punica Marchal, Asphondylia conglomerata De Stefani
line 362 (in the Acknowledgements): Mr. Ayman K. Elsayed (Saga University) => (Kagoshima University)
Table 3: A. mayeri => A. punica

Cited references in the text, were placed in alphabetical order. Misspellings of authors and pages were corrected.
Periods at the end of figure captions were deleted.

Running title: The Asian chili pod gall midge

Description of the Asian chili pod gall midge, *Asphondylia capsicicola* sp. n., with comparative notes on *Asphondylia gennadii* (Diptera: Cecidomyiidae) that induces the same sort of pod gall on the same host plant species in the Mediterranean region

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Abstract A new species of the genus *Asphondylia* (Diptera: Cecidomyiidae) that infests pods of chili, *Capsicum annuum* Linnaeus and *C. frutescens* Linnaeus (Solanaceae), is described as *Asphondylia capsicicola* sp. n. based on specimens collected from Indonesia and Viet Nam. The new species is similar to *Asphondylia gennadii* (Marchal) (= *A. capsici*) that induces chili pod galls in the Mediterranean region but is distinguishable from it by the morphological features of pupa such as nonlinear arrangement of lower frontal horns, narrower longitudinal band of transverse wrinkles on the tergite of mesothorax. Differences between the two species in the DNA sequencing data were 69 bp (16 %) to 77 bp (19 %) among 413 bp of the partial COI region examined, supporting the morphological identification. This is one of the examples in which two congeners induce the same sort of gall on the same host plant organ and species, which means that the two species cannot be distinguishable based

solely on gall shape and host plant information unlike many other gall midges.

Keywords *Capsicum annuum* · *Capsicum frutescens* · lower frontal horns · pupal morphology · new species · genetic analysis

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Introduction

The genus *Asphondylia* (Diptera: Cecidomyiidae) contains 320 nominal species worldwide (Gagné and Jaschhof, 2014). Some species of *Asphondylia* induce galls on beneficial plants. For example, *Asphondylia beguni* Mani induces galls on flowers of eggplant (Mani 1973), *Asphondylia gennadii* (Marchal) on chili pods (Gagné and Orphanides 1992), *Asphondylia ilicicola* Foote on ornamental holly berries of *Ilex* spp. (Aquifoliaceae) (e.g. Vasvary 1990), *Asphondylia morivorella* (Naito) on mulberry buds (Sunose 1983) and *Asphondylia yushimai* Yukawa and Uechi on soybean pods (Yukawa et al. 2003). The alfalfa gall midge, *Asphondylia websteri* Felt, attacks immature fruiting bodies of native (Gagné and Woods 1988) and exotic crop plants in the southwestern USA (Barnes 1946, Gagné and Wuensche 1986). Species identification of pest gall midges is essential to establish control measures against those of economic importance.

Most *Asphondylia* species are monophagous or oligophagous, inducing galls on specific organs, such as leaf bud, flower bud, flower, and fruit of their host plants. Their galls are frequently specific in shape and structure to each gall-inducing species (e.g. Gagné 1989, 1994; Skuhravá 1986; Yukawa and Masuda 1996). Therefore, in many cases, *Asphondylia* species, as well as other gall-inducing cecidomyiids, can be identified to the species level based on the shape and structure of gall and host plant information even though morphological differences of adults of some species of *Asphondylia* are obscure. However, our recent molecular analyses of gall midges have demonstrated that, in some cases, gall midges cannot be identified based solely on gall and host plant information when they exhibit polyphagy (Tokuda et al. 2005; Uechi et al. 2003), seasonal alternation of host plant organ or species (e.g. Uechi and Yukawa 2006a), and polymorphism in gall shape (Ganaha et al. 2007). Polyphagy across different plant families, which is not so common in *Asphondylia*, has been recognized in *A. websteri* (Gagné and Wuensche 1986). Seasonal alternation of host plants has been confirmed by means of molecular analysis for the following four species: *A. yushimai*

(Uechi et al. 2005; Yukawa et al. 2003), *A. gennadii* (Uechi et al. 2004), *Asphondylia baca* Monzen (Uechi et al. 2004), and *Asphondylia sphaera* Monzen (Uechi and Yukawa 2006b). Host organ alternation has been known for *Asphondylia sarothamni* H. Loew, which induces galls on leaf buds of *Cytisus* (=*Sarothamnus*) *scoparius* (Linnaeus) Link (Fabaceae) in winter and pods of the same plant species in summer (Parnell 1964). Gall size polymorphism occurs in *Asphondylia aucubae* Yukawa and Ohsaki, whose fruit galls on *Aucuba japonica* Thunberg (Garryaceae) are smaller than normal fruit in southern localities, while distinctly larger on *A. japonica* var. *borealis* Miyabe et Kudo in northern localities (Yukawa and Ohsaki 1988).

This paper deals with an additional case in which gall and host plant information is useless to identify gall midges because two different species of *Asphondylia* induce the same sort of pod gall on the same host plant species, *Capsicum annuum* Linnaeus (Solanaceae) in the Mediterranean and Southeast Asian countries. On the basis of gall shape (Fig. 1) and adult morphological similarity, the chili pod gall midge has been regarded in Indonesia as the Mediterranean species, *Asphondylia capsici* Barnes (e.g. Busniah 2014) but *A. capsici* was synonymized under *A. gennadii* (Marchal) by Gagné and Orphanides (1992). However, we noted that pupal morphological features of the Asian *Asphondylia* species are different from those of *A. gennadii* and our preliminary genetic analysis for several *Asphondylia* species demonstrated that the chili pod gall midge in tropical Asia should not be included with the clade of *A. gennadii*.

In this paper, we describe the Asian chili pod gall midge as a new species of *Asphondylia* together with DNA sequencing data and demonstrate an example that the two congeners induce the same sort of galls on the same plant organ and species.

Materials and methods

Collection and preservation of specimens, morphological observation, and type depository

We collected chili pod galls induced by a species of *Asphondylia* from Indonesia and Viet Nam (Table 1). Most of the galls collected were dissected soon after collecting under a binocular microscope to obtain full-grown larvae and pupae, and the remaining galls were kept in plastic bags for several days to rear adults. Specimens obtained were kept either in 75 % ethanol for morphological observation or in 99 % ethanol for DNA analysis.

Body contents of one male (later designated as holotype), six females and three mature larvae (as paratypes) were extracted with the DNeasy Blood and Tissue Kit (Qiagen, Japan), following the manufacturer's instructions. The cleared adults and larvae were kept in 99 % ethanol, transferred to clove oil and then mounted on slides with Canada balsam for morphological observation. The extracted body contents of these specimens, together with those of other specimens (Tables 2, 3), were subjected to genetic analysis (see below under Genetic analysis). It should be emphasized here that this method enables us to connect morphological features of type specimens with genetic sequencing data. Four pupal cases (as paratypes) were mounted on slides with Canada balsam through 75 and 99 % ethanol and clove oil. In addition, two males and two females were prepared for SEM photographs to show morphological features.

Morphological terminology of pupae follows Gagné (1994) and Yukawa et al. (2003). These specimens were examined with bright–field and phase–contrast microscopy, using a Nikon ECLIPSE E400 microscope. Special attention was paid to pupal morphological features because the number and arrangement of upper- and lower frontal horns of *Asphondylia* species frequently exhibit species–specific features (e.g. Yukawa et al. 2003).

The holotype and some paratypes of the newly described species, and other slide-mounted and ethanol-stored specimens of gall midges used in this study, are deposited in the collection of the Entomological Laboratory, Faculty of Agriculture, Kyushu University, Japan. Some paratypes are deposited in the collection of Zoological Museum (LIPI), Cibinong, Indonesia.

Genetic analysis

Genetic analyses were performed in Kyushu University (Fukuoka, Japan) and Institute of Fruit Tree and Tea Science, NARO (Tsukuba, Japan). A region of the cytochrome oxidase subunit I (COI) gene of mitochondrial DNA was amplified, purified, sequenced and electrophoresed following the methods described by Yukawa et al. (2003). The primers used in the analysis were: forward; COIS 5'-GGA TCA CCT GAT ATA GCA TTC CC-3' or LCO1490 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3' (Folmer et al. 1994) and reverse; COIA 5'-CCC GGT AAA ATT AAA ATA TAA ACT TC-3' (Funk et al. 1995) or HCO2198 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al. 1994). COIS has been used to detect intra- and interspecific variations in Cecidomyiidae (e.g. Tokuda et al. 2004, 2008; Uechi et al. 2003; Yukawa et al. 2003) in combination with COIA (Funk et al. 1995). Another primer set, LCO1490 and HCO2198, has been adopted for the DNA barcoding project of animals (Hebert et al.

2003). In this paper, homologous 413 bp were used in the analysis to compare DNA sequences with those of the Asian chili pod gall midge and *A. gennadii* (Accession No. AB115569), which has been registered on DNA databases. In addition, 12 Palaearctic species of *Asphondylia* that were registered on the DNA database were included in the analysis together with an outgroup taxon, *Pseudasphondylia matatabi* (Yuasa and Kumazawa) (Diptera: Cecidomyiidae) (Accession No. AB085873).

Based on these sequence data, a neighbor-joining (NJ) tree was constructed and bootstrap analysis was conducted with 1,000 pseudoreplications and evolutionary distances were computed by Kimura's two-parameter distances (Kimura 1980) using MEGA6 (Tamura et al. 2013).

Results

Description

***Asphondylia capsicicola* Uechi, Yukawa and Tokuda sp. n.** (Figs. 2–5)

[English name: The Asian chili pod gall midge]

[Japanese name: Ajia tougarashi saya tamabae]

Generic synopsis of *Asphondylia*: see Yukawa (1971).

Male (Fig. 2). Eyes connate, 6 to 7 facets long at vertex (Fig. 2a); facets all closely adjacent. Frontoclypeal setae 24 to 36 in number. Palpus 2 segmented, bearing scattered setae and no scales; first palpal segment 2.0 to 2.4 times as long as wide; second 1.3 to 1.4 times as long as first. Flagellomeres consisting of 12 segments; first and second flagellomeres not connate; node of fifth flagellomere about 180 µm, 2.3 to 3.0 times as long as wide (Fig. 2b). Wing unmarked, length 2.5–3.5 mm, about 2.6 times as long as wide; R_5 almost straight, joining costa a little beyond wing apex. Mesepimeral setae 14 to 16 in number. Fore leg with femur about 1.05 mm, tibia about 1.03 mm, second tarsomere about 1.00 mm, third about 0.48 mm, fourth about 0.28 mm and fifth about 0.15 mm; middle leg with femur about 0.80 mm, tibia about 0.88 mm, second tarsomere about 0.70 mm, third about 0.40 mm, fourth about 0.25 mm and fifth about 0.15 mm; hind leg with femur about 1.13 mm, tibia about 1.05 mm, second tarsomere about 0.85 mm, third about 0.48 mm, fourth about 0.33 mm and fifth about 0.18 mm; tarsal claws bent beyond mid length (Fig. 2c); empodia nearly as long as or slightly shorter than claws. First through seventh abdominal tergites rectangular, with single, sparse row of posterior setae, several lateral setae that are more numerous in posterior tergites, and dense scales; no discernible trichoid sensillum; eighth tergite without setae. Terminalia (Fig. 2d) showing typical shape for *Asphondylia* (Fig. 2d); cerci caudo-laterally developed into 2 setose elliptical lobes; tegmen rather deeply emarginated dorsally, rather shallowly emarginated ventrally; gonostylus subglobular, with solid, sclerotized bidentate tooth; aedeagus tapering; apodemes divided.

Female (Fig. 3). Node of fifth flagellomere about 130 µm, 2.2 to 2.4 times as long as wide (Fig. 3a). Frontoclypeal setae 20 to 34 in number. Wing length 3.8 to 4.0 mm, about 2.6 to 2.8 times as long as wide (Fig. 3b). Mesepimeral setae 18 to 33 in number (Fig. 3c, in a circle). Abdominal tergites without discernible trichoid sensillae (Fig. 3d). Ovipositor showing the typical shape for *Asphondylia*; needle part of ovipositor 1.3 to 1.4 mm, 2.6 to 2.8 times as long as the length of seventh sternite (Fig. 3e). Otherwise practically as in male.

Full-grown larva (Fig. 4). Second antennal segment short, conical, 11.2 μm in length, 5.0 μm in basal width; 2 ventral and 2 lateral cervical papillae each with a seta. Spiracles present on prothorax and first through eighth abdominal segments; 4 dorsal papillae distinct, each with a seta on all abdominal segments except eighth segment (Fig. 4a, in circles) (most outer papillae are included in pleural papillae in some cases when they are close to stigmatal protuberance: see Möhn (1955) and Yukawa (1971); 2 pleural papillae present on each side, each with a seta; 2 dorsal papillae of eighth abdominal segment each with a seta (Fig. 4b, in circles); 2 of 6 terminal papillae somewhat cone-shaped, the remaining 4 each with a short seta. Sternal spatula strongly sclerotized, about 150 μm in length, 2.6 to 2.8 times as long as maximum width, distally with 4 lobes, which are usually pointed apically (Fig. 4c); outer lobes longer than inner lobes; width between tips of 2 outer lobes about 45 μm; sternal and inner pleural papillae each with a seta on all thoracic segments; 3 inner and 2 outer lateral papillae each with a seta on all thoracic segments; 2 anterior ventral papillae and 2 posterior ventral papillae each with a seta (Fig. 4d, in circles); 2 ventral papillae of eighth abdominal segment each with a seta; anal papillae without setae.

Pupa (Fig. 5). Base of antennae long, strongly sclerotized, finely denticulate on inner margin; cephalic pair of setae 40–41 μm in length; upper frontal horn simple, strongly sclerotized, pointed apically; lower frontal horn consisting of 3 pointed lobes, of which outer 2 are a little longer and situated more anteriorly than central lobe (Fig. 5a); usually a pair of lower facial papillae each with a short seta. Prothoracic spiracle 93–97 μm in length. Scutum medially with a narrow longitudinal band of many transverse wrinkles (Fig. 5b). Abdominal spiracles very short, present on first through eighth segments. One or two anterior rows of short spines and a posterior row of short spines present on second through seventh abdominal tergite. Arrangement of spines on dorsal surface as in Fig. 5c.

Etymology. The specific name, *capsicicola*, means the inhabitant of *Capsicum*.

Host plant. *Capsicum annuum* Linnaeus (Solanaceae), 'chili' and *Capsicum frutescens* Linnaeus (Solanaceae), 'Cabe Rawit' in Indonesian.

Gall. Chili pods do not grow normally but are swollen, twisted, tapered, whirled, sharply bent, and transformed into various shapes (Fig. 1). Surface color remains green in most cultivars of green chili, sometimes partly with a reddish tinge in cultivars of red chili. Usually each gall contains one gall midge larva, occasionally two or more.

Distribution. Indonesia (Java, Sumatra, and Bali), and Viet Nam (Baria Vung Tau Province), and possibly China (see discussion).

Holotype. ♂ (on slide, Cecid. No. CPS001; referable to the accession number LC167140 for DNA sequencing data of this specimen), a galled pod of *Capsicum annuum* was collected by N. Maryana from Cisarua, Bogor, Indonesia (Alt.1000m) on 16 March 2004, an adult emerged in late March 2004, reared by N. Maryana.

Paratypes. 2 ♀♀, 1 pupa and 2 pupal cases (on slides, Cecid. Nos. CPS002–CPS006; accession nos. LC167141, LC167142 and LC164726; DNA was not analyzed for the pupal cases), galled pods of *C. annuum* were collected by N. Maryana, J. Yukawa and N. Uechi from Cisarua (*ditto*) on 16 March 2004, adults emerged in late March 2004, reared by N. Maryana. 1 pupal case (on slide, Cecid. No. CPS007; DNA was not analyzed), collected by N. Maryana on 29 December 2015 from a galled pod of *C. frutescens* in Cisarua (*ditto*). 2 ♀♀ and 2 larvae (on slide, Cecid. Nos. CPS008–CPS011; accession nos. for a female LC167145, and for a larva LC167146, DNA for two others were not analyzed), galled pods of *C. frutescens* were collected by N.

Maryana from Cisarua (*ditto*), on 14 January 2016, adults emerged in late January 2016, reared by N. Maryana. 1 ♂ and 1 ♀ (on slides, Cecid. Nos. CPS012–013), galled pods of *C. annuum* were collected by N. Uechi on 1 October 2001 from Desa Kubang, Kec Guguk, Kab. 50 kota, Dropingi, W. Sumatra, Indonesia and adults emerged on 2 October 2001, reared by N. Uechi. 2 ♀♀ (on slide, Cecid. Nos. CPS014–CPS015; accession nos. LC167143 and LC167144), galled pods of *C. annuum* were collected by P.O. Ngakan, J. Yukawa, N. Uechi and T. Ganaha-Kikumura from Gianyar, Bali, Indonesia on 18 March 2004 and adults emerged in late March 2004, reared by P.O. Ngakan.

Biology. *Asphondylia capsicicola* is multivoltine, requiring 25 days on chili for one generation. Each female has 124 ovarian eggs on average (Busniah 2014, as *A. capsici*).

Pest status. There has been no report on severe infestation by *A. capsicicola* in Indonesia, but the level of infestation sometimes reaches 40 % of chili plants (Anastasia and Maryana 2005). Thus this gall midge seems to have the potential to cause severe damage to cultivated chili plants.

Remarks. We found a clear morphological difference, particularly in pupal characteristics, between *A. capsicicola* and *A. gennadii*. In *A. capsicicola*, the upper frontal horn of the pupal face is shorter than that of *A. gennadii*, the lower frontal horn consists of 3 pointed lobes, of which the outer 2 are distinctly longer and situated more anteriorly than the central lobe (Fig. 5a). In *A. gennadii* the 3 pointed lobes are sub-equal in length and arranged horizontally almost linearly (Fig. 5d, see also Fig. 1 in Gagné and Orphanides 1992), and the tergite of pupal mesothorax medially with a longitudinal band of transverse wrinkles, which are distinctly narrower than the band of *A. gennadii* (Fig. 5b, e, see also Fig. 2 in Gagné and Orphanides 1992). Such difference indicates that they are different species and DNA analysis supported the conclusion based on the morphological differences.

From South America, the origin of solanaceous plants, *Asphondylia fructicola* Maia that induces fruit galls on *Solanum* sp. (Solanaceae) has been described (Maia et al. 2009). The pupa of this species has 1 upper and 3 lower facial horns that are arranged similarly to those of *A. capsicicola*, but these two species are distinguishable in the number of adult frontal spines, wing length, the length of the needle part of ovipositor, and the lengths of cephalic setae and prothoracic spiracle in pupae (Table 4).

Some Japanese species, such as *A. yushimai* and *A. aucubae* are morphologically similar to *A. capsicicola* but can be distinguished from it by the combination of slight differences in the setal counts and the measurements of some body parts (Table 4). In spite of such slight differences, DNA sequencing data indicated that *A. capsicicola* was different from the Japanese species as will be shown in the next section.

Genetic analysis

Nucleotide sequences of *A. capsicicola* that were collected from *C. annuum* and *C. frutescence* in Indonesia (Java, Sumatra and Bali) and Viet Nam were included in the same clade, and their monophyly was supported by a 100 % bootstrap value (Fig. 6). Eleven haplotypes were recognized among individuals of *A. capsicicola*. Intraspecific sequence divergences of *A. capsicicola* were 0 to 13 bp (3 %). Sequence divergences between *A. capsicicola* and *A. gennadii* were 69 bp (16 %) to 77 bp (19 %) (Fig. 6). *A. gennadii* was included in the group consisting of Japanese species except *A.*

morivorella and the monophyly of the group was supported by 68 % bootstrap value (Fig. 6).

Discussion

The current study revealed that *A. capsicicola* is morphologically different from *A. gennadii*. Genetic distance between *A. gennadii* and *A. capsicicola* was 69 bp (16 %) to 77 bp (19 %), which are distinctly higher than the 2 % divergence proposed by Hebert et al. (2003) as an acceptable distance to consider two closely related entities as distinct species. Thus, genetic analysis supported the identification.

Frequently, gall midges cannot be identified based solely on gall and host plant information although various phylogenetic and evolutionary studies have demonstrated that gall shape and structure are 'extended phenotypes' of gall-inducing insects (e.g. Fukatsu et al. 1994; Nyman et al. 2000; Stern 1995; Stone and Cook 1998). The case of *A capsicicola* and *A. genndaii* is a good example to show that the two congeners induce the same kind of galls on the same plant organ and species. Such an example has been noted for the African rice gall midge, *Orseolia oryzivora* Harris and Gagné and the Asian rice gall midge, *Orseolia oryzae* (Wood-Mason) (Harris and Gagné 1982) though these two *Orseolia* species have distinctive morphological differences.

Both morphological observation and genetic analysis indicate that *A. capsicicola* and *A. gennadii* are not closely related to each other and have separately appeared in the Oriental and Palaearctic Region, respectively. They might have been introduced directly into their respective regions from South America together with their solanaceous host plants. Otherwise their ancestors might have expanded their host range to introduced

Capsicum from the original host plants. Polyphagy and host alternation by *A. gennadii* suggest the latter case. We also need to confirm the host range of *A. capsicicola* in the future.

In *Asphondylia*, there are several different patterns of combination in the number of upper (0, 1, 2) and lower facial horns (0, 1, 2, 3) and the arrangement of lower frontal horns (linear or nonlinear). Most Japanese species have 1 upper and 3 nonlinear lower facial spines (Yukawa, 1971; Yukawa et al. 2003), except *A. morivorella* (Naito) that has 2 upper and 3 nonlinear lower facial spines (Sunose 1983) and *Asphondylia itoi* Uechi and Yukawa that has 1 upper and 3 linear lower facial horns (Uechi and Yukawa 2004). It is remarkable that the arrangement of pupal facial horns of *A. gennadii* is similar to that of *A. itoi*, and these two species are included in the same subgroup in the Japanese *Asphondylia* group (Fig. 6). In European species, *Asphondylia pilosa* Kieffer has 1 upper and 1 lower horns (Gagne and Waring 1990) while *A. sarothamni* (= *Asphondylia mayeri* Liebel) (Stelter, 1957) has the combination similar to that of *A. morivorella*. Interestingly, *Asphondylia punica* Marchal (= *Asphondylia conglomerata* De Stefani) has the combination similar to that of most Japanese species (Dorchin et al. 2014). Thus, the morphological features of pupal facial horns only partly coincided the topography of NJ-tree but did not as a whole.

Chili has been recognized as a host plant of *A. capsicicola* in Indonesia and Viet Nam but there may be interruption between the cultivation seasons of chili plant due to rotation of other crops. Therefore, *A. capsicicola* is presumably polyphagous, utilizing other plant species as hosts simultaneously or alternatively, as has been noted for *A. gennadii*, which has a wide host range including *C. annuum* (Solanaceae), *Ceratonia siliqua* Linnaeus (Fabaceae), *Capparis spinosa* Linnaeus (Capparidaceae), and *Urginea* *maritime* (Linnaeus) Baker (Asparagaceae, formaly Liliaceae), in the Mediterranean region (Orphanides 1975; Uechi et al. 2004; Yukawa et al. 2003). Further intensive field surveys are needed to find alternative wild or cultivated host plants to clarify the annual life cycle of *A. capsicicola*. This is essential to establish control measures against the pest gall midge.

The chili pod gall midge in the Oriental Region has been recorded as *A. capsici* from Turkey (Skuhravá, 1986), India (e.g.; Prasad and Ranganath 2001; Tomar et al. 1997) and Indonesia (e.g., Busniah 2014), and as *A. gennadii* from China (Jiao and Bu 2014). The gall midge in China is possibly identical to *A. capsicicola* as in Indonesia, but that should be reconfirmed in the light of our pupal morphology and DNA sequence data. Because Turkey and India are located far away from Southeast Asia, we are very much interested in the taxonomic status of the chili pod gall midge distributed in Turkey and India.

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References

Anastasia D, Maryana N (2005) Chili pod gall midge, *Asphondylia* sp. (Diptera: Cecidomyiidae) a new pest of chili (*Capsicum annuum*) in Bogor. Program & Abstract the first International Conference of Crop Security 2005. Brawijaya University, Malang Indonesia September 20th – 22th, 2005

Barnes HF (1946) Gall midges of economic importance. Volume II: Gall midges of fodder crops. Crosby Lockwood & Son Ltd, London, UK

Busniah M (2014) Life cycle of gall midge on red chilli in West Sumatra. Int J Adv Sci Engin Inf Tech (IJASEIT) 4: 245–248

Dorchin N, Mifsud D, Askew R (2014) Saltbush-associated *Asphondylia* species (Diptera: Cecidomyiidae) in the Mediterranean Basin and their chalcidoid parasitoids (Hymenoptera: Chalcidoidea). Zootaxa 3869: 383–396

Folmer O, Black M, Hoew H, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3: 294–299

Fukatsu T, Aoki S, Kurosu U, Ishikawa H (1994) Phylogeny of Cerataphidini aphids revealed their symbiotic microorganisms and basic structure of their galls:

implications for host–symbiont coevolution and evolution of sterile soldier castes. Zoolog Sci 11: 613–623

Funk DJ, Futuyma DJ, Orti G, Meyer A (1995) Mitochondrial DNA sequences and multiple data sets: a phylogenetic study of phytophagous beetles (Chrysomelidae: *Opharaella*). Mol Biol Evol 12: 627–640

Gagné RJ (1989) The Plant–Feeding Gall Midges of North America. Cornell University Press, Ithaca, New York

Gagné RJ (1994) The Gall Midges of the Neotropical Region. Cornell University Press, Ithaca, New York

Gagné RJ, Jaschhof M (2014) A catalog of the Cecidomyiidae (Diptera) of the world. 3rd Edition. Digital version 2. [Cited 3 August 2016] Available from URL: http://afrsweb.usda.gov/SP2UserFiles/Place/12454900/Gagne_2014_World_Cecidomyiidae_Catalog_3rd_Edition.pdf

Gagné RJ, Orphanides GM (1992) The pupa and larva of *Asphondylia gennadii* (Diptera: Cecidomyiidae) and taxonomic implications. Bull Entomol Res 82: 313–316

Gagné RJ, Waring GL (1990) The *Asphondylia* (Cecidomyiidae: Diptera) of creosote bush (*Larrea tridentata*) in North America. Proc Entomol Soc Wash 92: 649–671

Gagné RJ, Woods WM (1988) Native American plant hosts of *Asphondylia websteri* (Diptera: Cecidomyiidae). Ann Entomol Soc Am 81: 447–448

Gagné RJ, Wuensche AL (1986) Identity of the *Asphondylia* (Diptera: Cecidomyiidae) on guar, *Cyamopsis tetragonoloba* (Fabaceae), in the southwestern United States. Ann Entomol Soc Am 79: 246–250

Ganaha T, Nohara M, Sato S, Uechi N, Yamagishi K, Yamauchi S, Yukawa J (2007)

Polymorphism of axillary bud galls induced by *Rhopalomyia longitubifex* (Diptera: Cecidomyiidae) on *Artemisia princeps* and *A. montana* (Asteraceae) in Japan and Korea, with the designation of new synonyms. Entomol Sci 10: 209–212

Harris KM, Gagné RJ (1982) Description of the African rice gall midge, *Orseolia oryzivora* sp. n., with comparative notes on the Asian rice gall midge, *O. oryzae* (Wood-Mason) (Diptera: Cecidomyiidae). Bull Entomol Res 72: 467–472, pl. VI

Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proc Roy Soc Lond B Biol Sci 270: 313–321

Jiao K, Bu W (2014) Gall midges (Diptera: Cecidomyiidae) recorded from China during the period from 1900 to 2012, with faunistic comparison between China and Japan. Jpn J Syst Entomol 20: 201–215

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. J Mol Evol 16: 111–120

Maia VC, Santos JC, Fernandes GW (2009) *Asphondylia fructicola*, a new species of Cecidomyiidae (Diptera) associated with *Solanum* sp. (Solanaceae) from Brazil. Rev Bras entomol 53: 166–170

Mani MS (1973) Plant galls of India. Macmillan Company of India, Delhi

Möhn E (1955) Beiträge zur Systematik der Larven der Itonididae (=Cecidomyiidae, Diptera). 1. Teil: Porricondylinae und Itonidinae Mitteleuropas. Zoologica 105: 1–247

Nyman T, Widmer A, Roininen H (2000) Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera: Tenthredinidae). Evolution 54: 526–533

Orphanides GM (1975) Biology of the carob midge complex, *Asphondylia* spp. (Diptera, Cecidomyiidae), in Cyprus. Bull Entomol Res 65: 381–390

Parnell JR (1964) Investigation on the biology and larval morphology of the insects associated with the galls of *Asphondylia sarothamni* H. Loew (Diptera: Cecidomyiidae) on broom (*Sarothamnus scoparius* (L.) Wimmer). Trans Roy Entomol Soc Lond 116: 255–273

Prasad GS, Ranganath HR (2001) Occurrence of chilli gall midge, *Asphondylia capsici* Barnes (Cecidomyiidae: Diptera) in south Andamans, Andaman Islands. J Bombay Nat Hist Soc 98: 468–469

Skuhravá M (1986) Family Cecidomyiidae, pp. 272–297 in Soós A, Papp L (eds) Catalogue of Palaearctic Diptera Vol. 4, Sciaridae-Anisopodidae. Elsevier, Amsterdam

Stelter H (1957) Untersuchngen über Gallmücken. III Die Artberechtigung von *Asphondylia mayeri* Liebel. Arch Nat Meckl 3: 152–160

Stern DL (1995) Phylogenetic evidence that aphids, rather than plants, determine gall morphology. Proc Roy Soc Lond B Biol Sci 260: 85–89

Stone GN, Cook JM (1998) The structure of cynipid oak galls: patterns in the evolution of an extended phenotype. Proc Roy Soc Lond B Biol Sci 265: 979–988

Sunose T (1983) Redescription of *Asphondylia morivorella* (Naito), comb. n. (Diptera: Cecidomyiidae), with notes on its bionomics. Appl Entomol Zool 18: 22–29

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30: 2725–2729

Tokuda M, Harris KM, Yukawa J (2005) Morphological features and molecular phylogeny of *Placochela* Rübsaamen (Diptera: Cecidomyiidae) with implications for taxonomy and host specificity. Entomol Sci 8: 419–427.

Tokuda M, Tabuchi K, Yukawa J, Amano H (2004) Inter- and intraspecific comparisons between *Asteralobia* gall midges (Diptera: Cecidomyiidae) causing axillary bud galls on *Ilex* species (Aquifoliaceae): species identification, host range, and the mode of speciation. Ann Entomol Soc Am 97: 957–970

Tokuda M, Yang M-M, Yukawa J (2008) Taxonomy and molecular phylogeny of *Daphnephila* gall midges (Diptera: Cecidomyiidae) inducing complex leaf galls on Lauraceae, with descriptions of five new species associated with *Machilus thunbergii* in Taiwan. Zool Sci 25:533–545

Tomar RKS, Yadav HS, Agrawal RK (1997) Parasitoids of chilli gall midge, *Asphondylia capsici* and their role in chilli ecosystem. Ind J Entomol 59: 173–178

Uechi N, Tokuda M, Yukawa J, Kawamura F, Teramoto KK, Harris KM (2003) Confirmation by DNA analysis that *Contarinia maculipennis* (Diptera: Cecidomyiidae) is a polyphagous pest of orchids and other unrelated cultivated crops. Bull Entomol Res 93: 545–551

Uechi N, Yukawa J (2004) Description of *Asphondylia itoi* sp. n. (Diptera: Cecidomyiidae) inducing fruit galls on *Distylium racemosum* (Hamamelidaceae) in Japan. Esakia 44: 27–43

Uechi N, Yukawa J (2006a) Life history patterns and host ranges of the genus *Asphondylia* (Diptera: Cecidomyiidae), pp. 275–285 in Ozaki K, Yukawa J, Ohgushi T, Price PW (eds) Galling Arthropods and Their Associates: Ecology and Evolution. Springer-Verlag, Tokyo

Uechi N, Yukawa J (2006b) Host range and life history of *Asphondylia sphaera* Monzen (Diptera: Cecidomyiidae): use of short-term alternate hosts. Ann Entomol Soc Am

99: 1165–1171

Uechi N, Yukawa J, Usuba S (2005) Discovery of an additional winter host of the soybean pod gall midge, *Asphondylia yushimai* (Diptera: Cecidomyiidae) in Japan. Appl Entomol Zool 40: 597–607

Uechi N, Yukawa J, Yamaguchi D (2004) Host alternation by gall midges of the genus *Asphondylia* (Diptera: Cecidomyiidae). In Contributions to the Systematics and Evolution of Diptera, D. Elmo Hardy Mem Vol, Bishop Mus Bull Entomol 12 (Evenhuis NL, Kaneshiro KY eds.). Bishop Mus Press, Honolulu. pp 53–66

Vasvary LM (1990) The holly berry midge and its control in New Jersey. Holly Soc J 8: 3–4

Yukawa J (1971) A revision of the Japanese gall midges (Diptera: Cecidomyiidae). Mem Fac Agr Kagoshima Univ 8: 1–203

Yukawa J, Masuda H (1996) Insect and mite galls of Japan in colors. Zenkoku Nôson Kyôiku Kyôkai, Tokyo (In Japanese with English explanations for color plates)

Yukawa J, Ohsaki N (1988) Separation of the aucuba fruit midge, *Asphondylia aucubae* sp. nov. from the ampelopsis fruit midge, *Asphondylia baca* Monzen (Diptera, Cecidomyiidae). Kontyû 56: 365–376

Yukawa J, Uechi N, Horikiri M, Tuda M (2003) Description of the soybean pod gall midge, *Asphondylia yushimai* sp. n. (Diptera: Cecidomyiidae), a major pest of soybean and findings of host alternation. Bull Entomol Res 93: 73–86

Figure caption

Fig. 1 Chili pods infested by a gall midge of the genus *Asphondylia* in Southeast Asia

Fig. 2 Male of *Asphondylia capsicicola*. **a** Head (bar: 100μm). **b** Fifth flagellomere (bar: 50μm). **c** Claw and empodium of fore leg (bar: 20μm). **d** Terminalia (bar: 50μm)

Fig. 3 Female of *Asphondylia capsicicola*. **a** Fifth flagellomere (bar: 50μm). **b** Wing (bar: 10mm). **c** Pleural parts of thorax (bar: 300μm). **d** Fifth abdominal tergite (bar: 200μm). **e** Lateral side of eighth abdominal segment and ovipositor (bar: 100μm)

Fig. 4 Full-grown larva of *Asphondylia capsicicola*. **a** Fifth and sixth abdominal tergites (bar: 200μm). **b** Eighth and ninth abdominal tergite (bar: 50μm). **c** Ventral side of head and prothorax, showing sternal spatula (bar: 100μm). **d** Sixth abdominal sternite (bar: 200μm)

Fig. 5 Pupa of *Asphondylia capsicicola*. (**a**–**c**) and *Asphondylia gennadii* (**d**–**f**). **a, d** Frontal area, showing upper and lower frontal horns (bar: 200μm). **b, e** Dorsal surface of mesothorax, showing longitudinal band of many transverse wrinkles (bar: 200μm). **c, f** Abdominal tergites (bar: 200μm)

Fig. 6 Neighbor-joining tree for the Asian chili pod gall midge and some Palaearctic species of *Asphondylia* including the Mediterranean chili pod gall midge, *A. gennadii*

Figure 1

Click here to download Figure Fig_1_Chili_pod_galls.tif

Figure 2

Click here to download Figure Fig_2_Asp_capsicicola_Male.tif

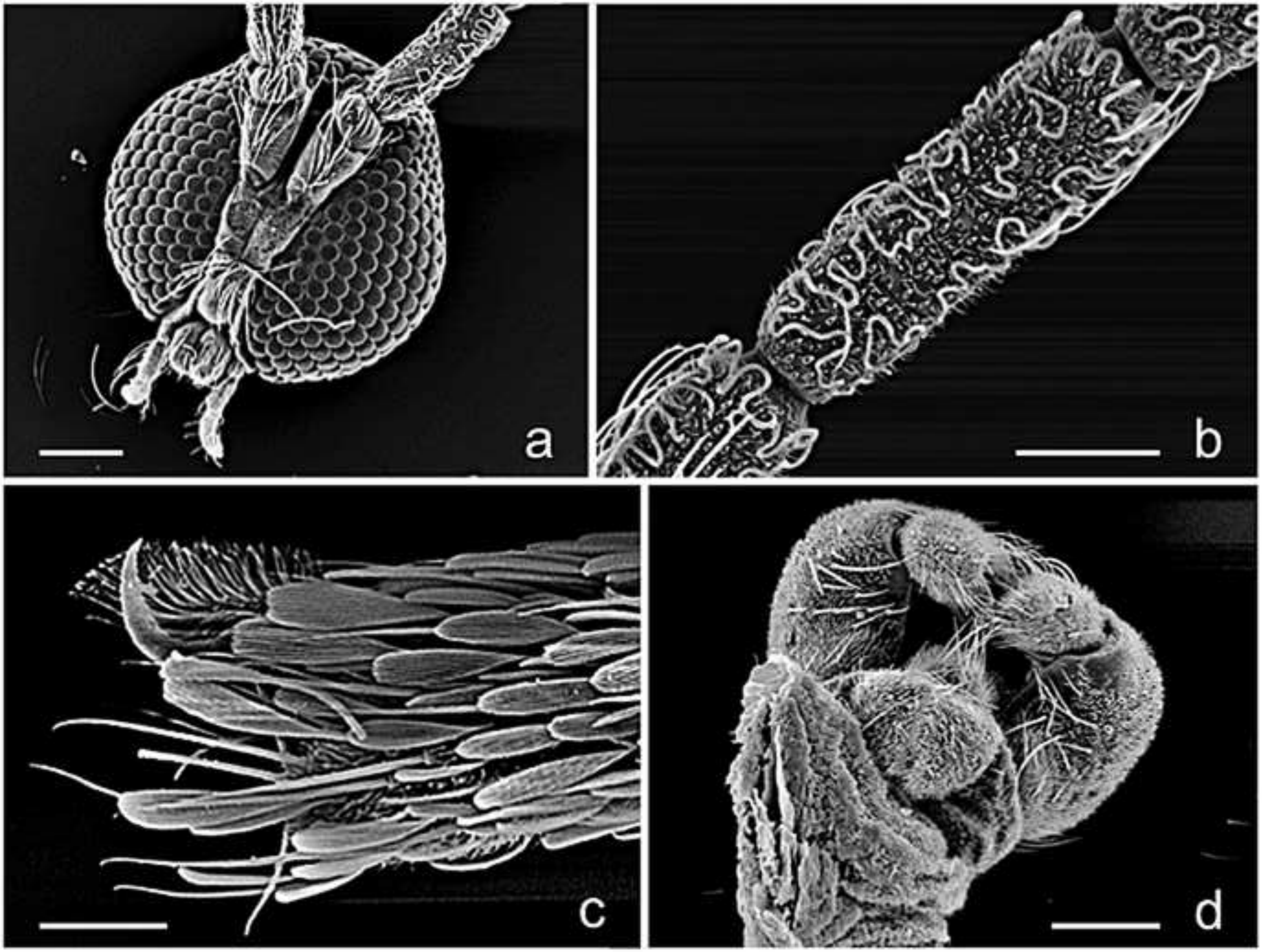

Figure 3

Click here to download Figure Fig_3_Asp_capsicicola_Female.tif

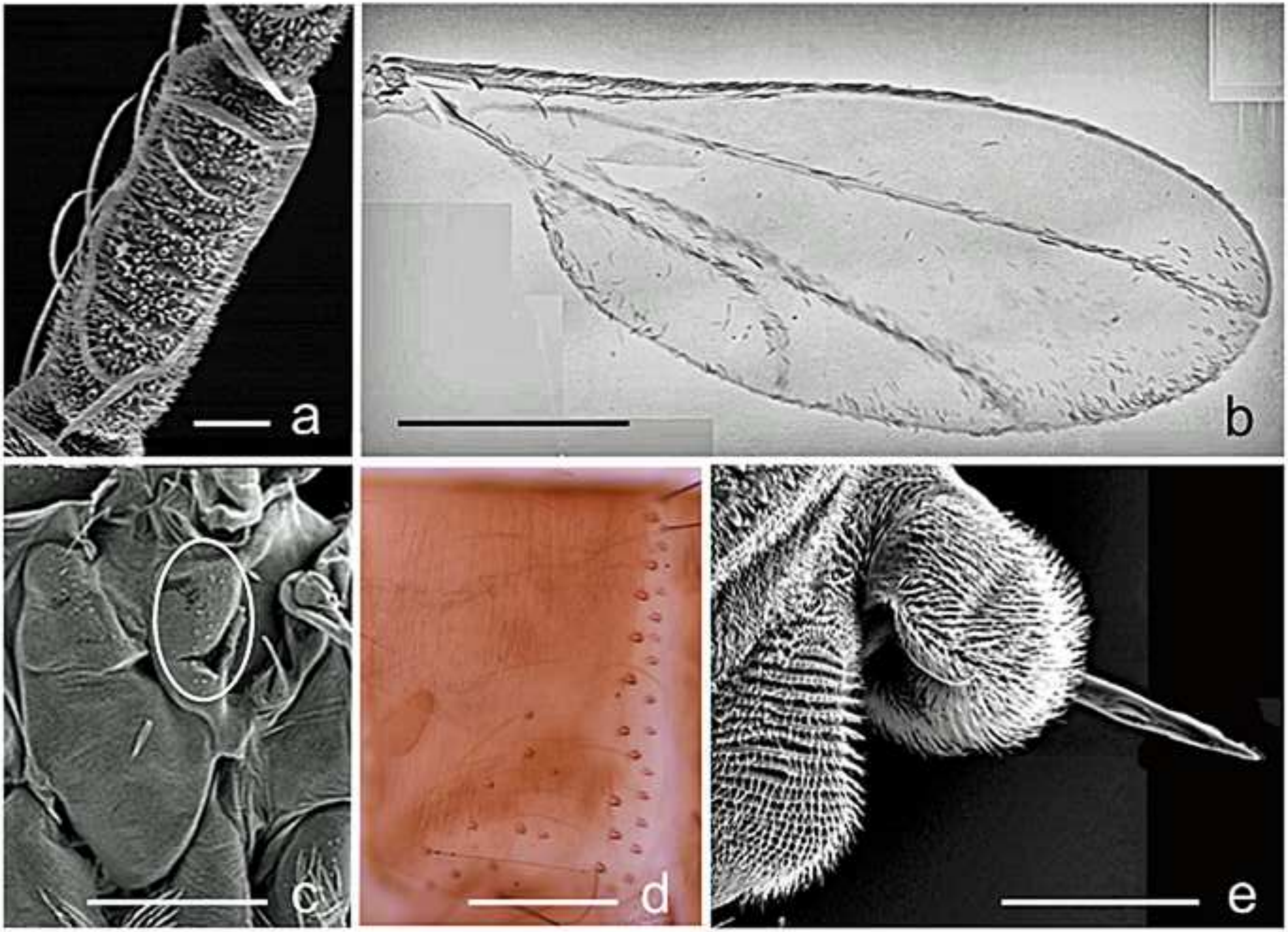

Figure 4 Click here to download Figure Fig_4_Asp_capsicicola_Larva.tif

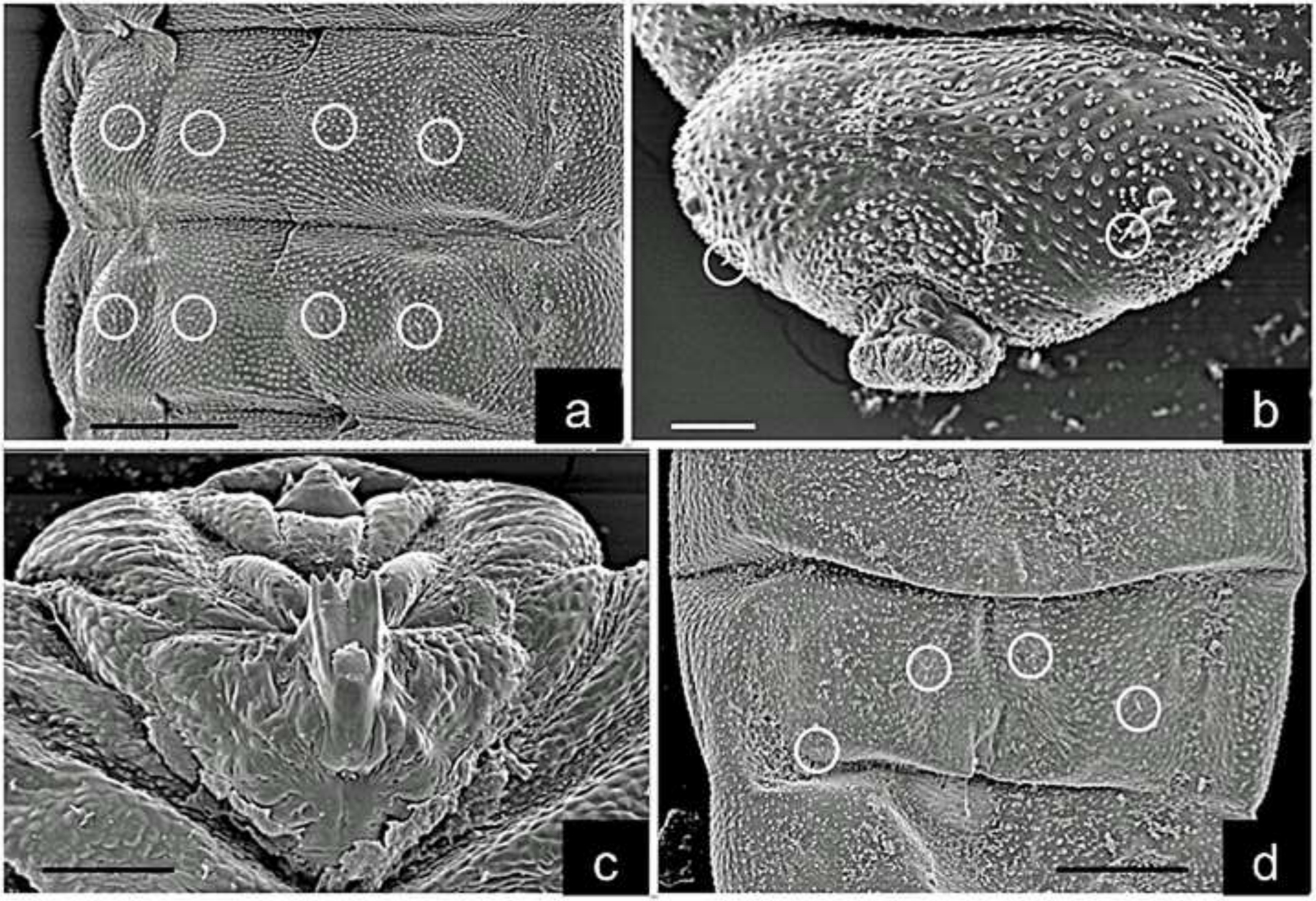

Figure 5

Click here to download Figure Fig_5_Two_Asp_species_Pupae.tif

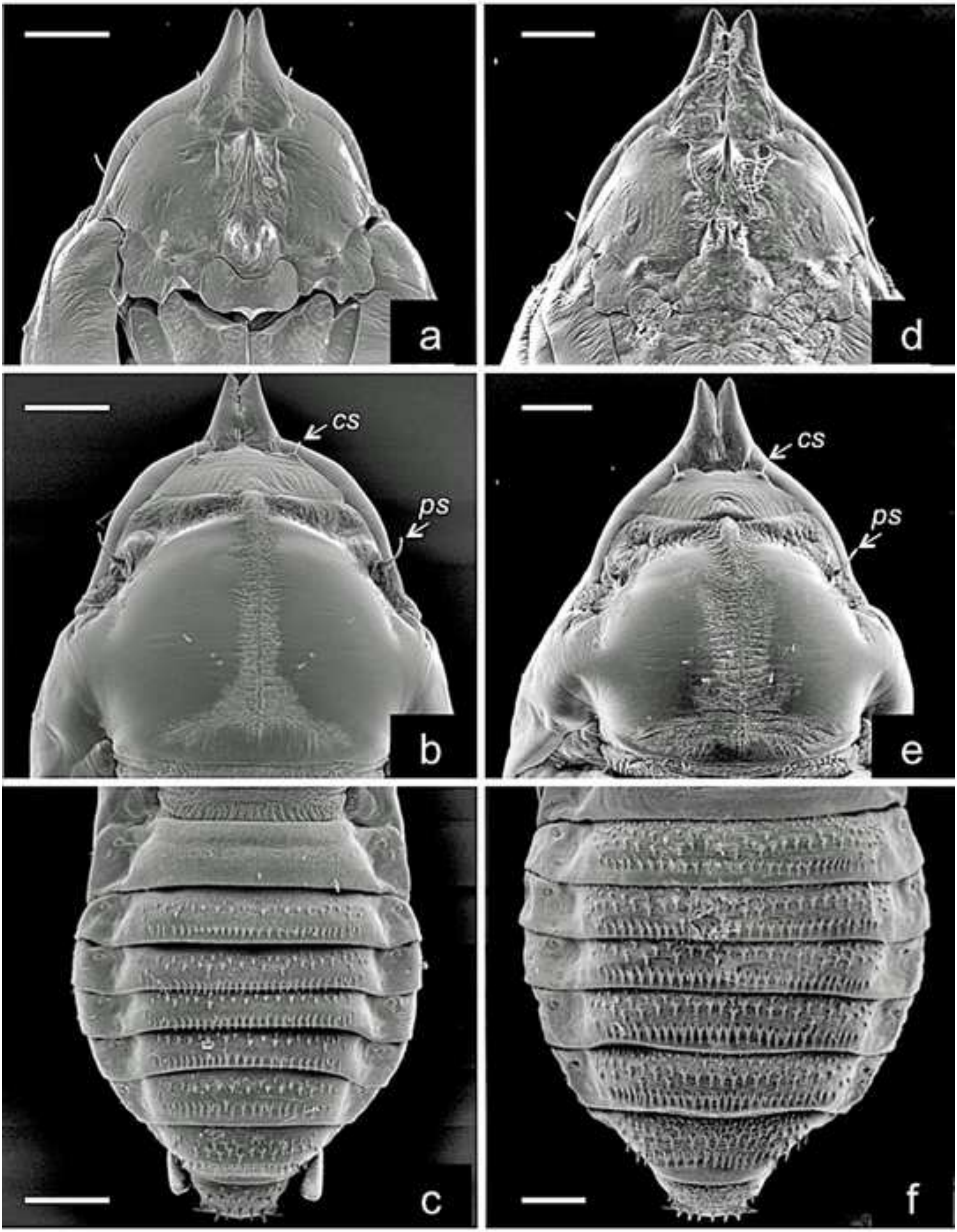

Figure 6

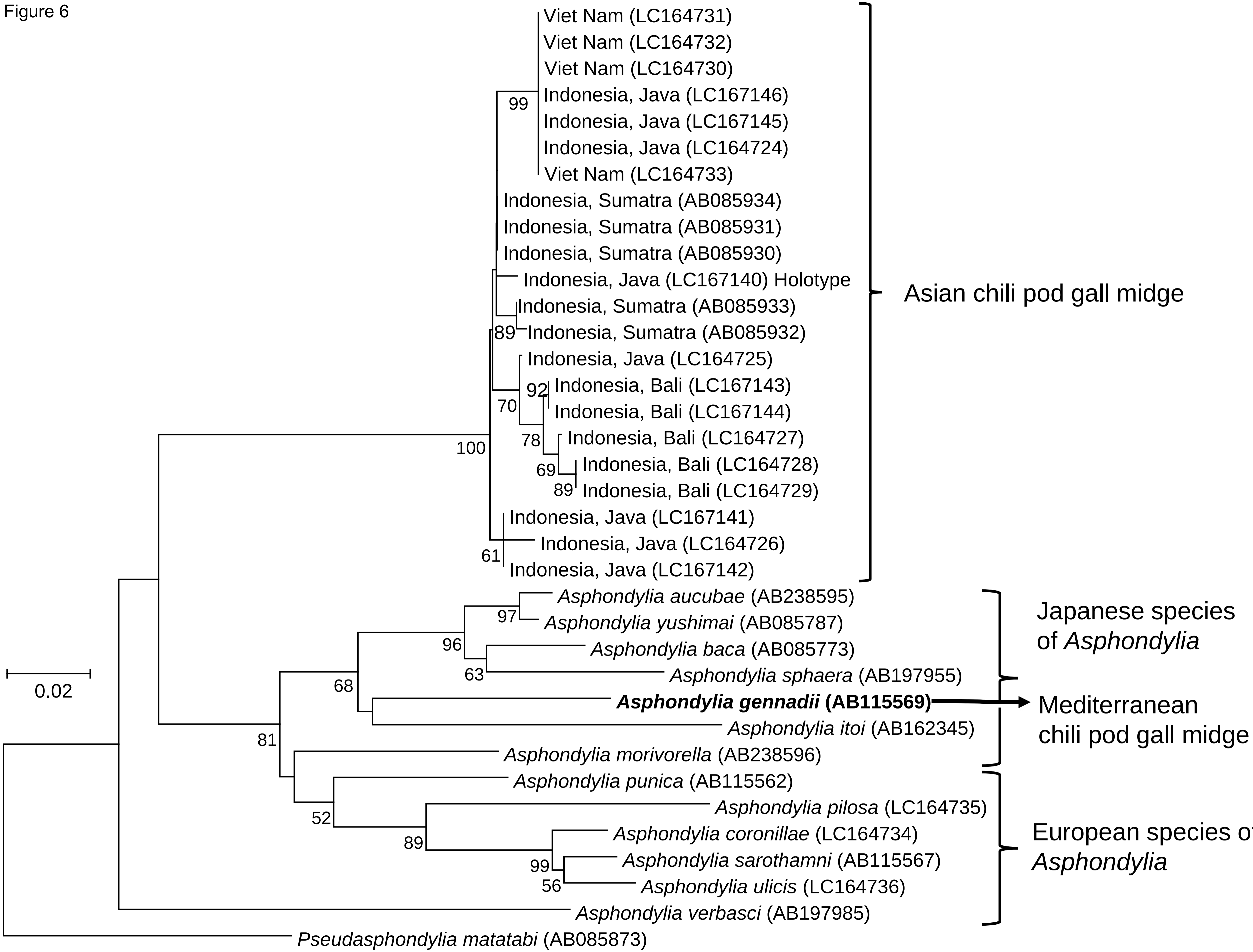
Viet Nam (LC164731)
Viet Nam (LC164732)
Viet Nam (LC164730)
Indonesia, Java (LC167146)
Indonesia, Java (LC167145)
Indonesia, Java (LC164724)
Viet Nam (LC164733)
Indonesia, Sumatra (AB085934)
Indonesia, Sumatra (AB085931)
Indonesia, Sumatra (AB085930)
Indonesia, Java (LC167140) Holotype
Indonesia, Sumatra (AB085933)
Indonesia, Sumatra (AB085932)
Indonesia, Java (LC164725)
Indonesia, Bali (LC167143)
Indonesia, Bali (LC167144)
Indonesia, Bali (LC164727)
Indonesia, Bali (LC164728)
Indonesia, Bali (LC164729)
Indonesia, Java (LC167141)
Indonesia, Java (LC164726)
Indonesia, Java (LC167142)
99
89
92
70
78
100
69
89
61
Asian chili pod gall midge
Asphondylia aucubae (AB238595)
Asphondylia yushimai (AB085787)
Asphondylia baca (AB085773)
Asphondylia sphaera (AB197955)
Asphondylia gennadii (AB115569)
Asphondylia itoi (AB162345)
Asphondylia morivorella (AB238596)
Asphondylia punica (AB115562)
Asphondylia pilosa (LC164735)
Asphondylia coronillae (LC164734)
Asphondylia sarothamni (AB115567)
Asphondylia ulicis (LC164736)
Asphondylia verbasci (AB197985)
Pseudasphondylia matatabi (AB085873)
97
96
63
68
81
52
89
99
56
0.02
Japanese species of *Asphondylia*
Mediterranean chili pod gall midge
European species of *Asphondylia*

Table 1 Collecting data of chili pod galls induced on *Capsicum* spp. by *Asphondylia* sp. in Indonesia and Viet Nam

Locality	Host plant	Collecting date	Collector
Indonesia			
Cisarua, Bogor, Java	*C. frutescens*	16 Mar. 2003	N. Maryana, J. Yukawa & N. Uechi
ditto	*C. annuum* & *C. frutescens*	16 Mar. 2004	N. Maryana, J. Yukawa, N. Uechi & T. Ganaha-Kikumura
ditto	*C. frutescens*	29 Dec. 2015	N. Maryana
ditto	*C. frutescens*	14 Jan. 2016	N. Maryana
ditto	*C. frutescens*	5 Jan. 2016	N. Maryana
Gianyar, Bali	*C. annuum*	18 Mar. 2004	P. O. Ngakan, J. Yukawa, N. Uechi & T. Ganaha-Kikumura
Dropingi, Sumatra	*C. annuum*	1 Oct. 2001	N. Uechi, F. Kodoi & R. Rustam
Viet Nam			
Bana Town, Baria Vung Tau Province	*C. annuum*	11 Dec. 2005	J. Yukawa, N. Uechi & T. Ganaha-Kikumura

Table 2 Specimens of *Asphondylia capsicicola* used for genetic analysis

Collecting site	Collection date	Specimens	Host plant	Accession No.
Indonesia				
Cisarua, Bogor, Java	16 Mar. 2004	male	*Capsicum annuum*	LC167140
		2 females	*C. annuum*	LC167141, LC167142
		pupa	*C. frutescens*	LC164726
	14 Jan 2016	female	*C. frutescens*	LC167145
		larva	*C. frutescens*	LC167146
	14 Jan. 2016	2 pupae	*C. annuum*	LC164724, LC164725
Gianyar, Bali	18 Mar. 2004	2 females	*C. annuum*	LC167143, LC167444
		adult	*C. annuum*	LC164727 – LC164729
Dropingi, Sumatra	1 Oct. 2001	5 adults	*C. annuum*	AB085930 – AB085934
Viet Nam				
Bana Town, Baria Vung Tau Province	11 Dec. 2005	4 adults	*C. annuum*	LC164730 – LC164733

Table 3 Specimens of *Asphondylia* species (other than *A. capsicicola*) and a related taxon used for genetic analysis

Species	Literature cited or collecting site	Accession No.
Asphondylia. aucubae	Uechi & Yukawa (2006a)	AB238595
A. baca	Uechi et al. (2004)	AB085773
A. coronillae	This paper (Callestoafa, Italy, M Solinas leg.)	LC164734
A. gennadii	Uechi et al. (2004)	AB115569
A. itoi	Uechi & Yukawa (2004)	AB162345
A. morivorella	Uechi & Yukawa (2006a)	AB238596
A. pilosa	This paper (Oregon, USA, GP Markin leg.)	LC164735
A. punica	Uechi et al. (2004)	AB115562
A. sarothamni	Uechi et al. (2004)	AB115567
A. sphaera	Uechi & Yukawa (2006b)	AB197955
A. ulicis	This paper (Bracknell UK, KM Harris leg.)	LC164736
A. verbasci	Uechi & Yukawa (2006b)	AB197985
A. yushimai	Yukawa et al. (2003)	AB085787
Pseudasphondylia matatabi	Yukawa et al. (2003)	AB085873

Table 4

Table 4 Comparison between *Asphondylia capsicicola* and its congeners in the setal counts and the measurements of some body parts in adults and pupae

Body part	*A. capsicicola*		*A. fructicola*[1)]		*A. yushimai*[2)]		*A. aucubae*[3)]	
	N	Min–Max	N	Min–Max	N	Min–Max	N	Min–Max
Number of frontoclypeal setae (male)	2	24 – 36	11	14 – 22	35	18 – 54	49	25 – 37
Number of frontoclypeal setae (female)	4	20 – 34	5	16 – 20	28	25 – 60	54	19 – 38
Wing length (male) (mm)	2	2.5 – 3.5	5	2.0 – 2.4	27	2.5 – 3.6	44	2.5 – 3.5
Wing length (female) (mm)	4	3.8 – 4.0	4	2.7 – 2.9	21	3.1 – 4.2	62	2.5 – 4.0
Needle part of ovipositor (mm)	4	1.3 – 1.4	4	1.6 – 1.8	19	1.0 – 1.4	*5*	*1.3 – 1.5*
Ovipositor / seventh sternite (times)	4	2.6 – 2.8	4	3.0 – 3.3	19	1.8 – 2.4	*5*	*3.8 – 4.4*
Cephalic setae in pupa (µm)	2	40 – 41	6	55 – 60	3	*55 – 70*	*4*	*45 – 50*
Prothoracic spiracle in pupa (µm)	2	93 - 97	4	160 – 170	3	*90 – 100*	*3*	*80 – 100*

Reference for the setal counts and measurements: [1)] Maia et al., 2009, [2)] Yukawa et al. (2003), [3)] Yukawa & Ohsaki (1988). Italic numerals indicate data newly obtained in the current study based on the specimens used in the descriptions. N: the number of specimens examined, Min: minimum value of data, Max: maximum value of data