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INSECTA MATSUMURANA

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REDESCRIPTION OF THE PHYTOPHAGOUS LADYBIRD BEETLE HENOSEPILACHNA DIEKEI AND DESCRIPTIONS OF TWO RELATED SPECIES FROM INDONESIA (COCCINELLIDAE, COCCINELLINAE, EPILACHNINI)

By KEI W. MATSUBAYASHI, SIH KAHONO, SRI HARTINI, NAOYUKI FUJIYAMA and HARUO KATAKURA

Abstract

MATSUBAYASHI, K. W., KAHONO, S., HARTINI, S., FUJIYAMA, N. and KATAKURA, H. 2016. Redescription of the phytophagous ladybird beetle *Henosepilachna diekei* and descriptions of two related species from Indonesia (Coccinellidae, Coccinellinae, Epilachnini). *Ins. matsum. n. s.* 72: 1–16, 7 figs, 1 table.

The phytophagous ladybird beetle *Henosepilachna diekei* Jadwiszczak & Węgrzynowicz was redescribed on the basis of specimens from Indonesia and other south and southeastern Asian countries, and two new species related to it were described as *H. nakanoi* Matsubayashi & Katakura sp. nov. and *H. uenoi* Matsubayashi & Katakura sp. nov. *Henosepilachna nakanoi* is easily discernible from congeners by the unique structure of male and female genitalia. *Henosepilachna uenoi* has genitalia being nearly identical with *H. diekei*, but the two species sympatric in Bali are separable by the habitus shape, elytral maculation pattern, and host plants. A phylogenetic analysis based on mitochondrial NADH dehydrogenase subunit 2 (ND2) gene sequences also indicated that *H. uenoi* and *H. diekei* are genetically distinct, though very close to each other.

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Introduction

The phytophagous ladybird beetle *Henosepilachna diekei* Jadwiszczak & Węgrzynowicz, 2003, originally described from the Philippines under the name *Epilachna emarginata* by Dieke (1947), is widely distributed in Indonesia, covering Sumatra, Java, Kalimantan, Sulawesi, Bali, and Lombok (Katakura *et al*. 2001; Matsubayashi *et al*. 2016; S. Nakano, personal communication). This species also occurs in the southern part of the Malay Peninsula and Sri Lanka (Matsubayashi *et al*. 2016). In the course of our faunistic and evolutionary studies of Epilachnini beetles in Indonesia, we noticed a large geographic variation in external appearance and genitalia of *H. diekei* along with its divergent host plant use (Matsubayashi *et al*. 2011, 2013, 2016; Fujiyama *et al*. 2013). Multivariate analyses of morphological characters unveiled the presence of two morphologically divergent entities in *H. diekei* in Indonesia; one occurring in western islands (Java, Kalimantan, Bali), and another in eastern ones (Sulawesi, Lombok) (Matsubayashi *et al*. 2016).

In this study, we first provide a brief redescription of Indonesian *H. diekei*, with notes on the above mentioned two groups of populations that are discernible by the subtle but stable difference in male genitalic structure, body shape and elytral maculation pattern. We then describe two new species related to *H. diekei* from Indonesia. The phylogenetic relationship of these three species is also provided based on mitochondrial NADH dehydrogenase subunit 2 (ND2) gene sequences.

Material and Methods

Terminology followed Ślipiński & Tomaszewska (2010) except some terms concerning male genitalia (i.e., basal knife edge, apical thorn), for which we followed Dieke (1947). The naming of the elytral spots followed Dieke (1947). The following body parts were measured: TL, total body length from apical margin of clypeus to apex of elytra; PL, pronotum length from the middle of anterior margin to margin of basal foramen, PW, pronotum width; EL, elytral length along suture including scutellum; EW, elytral width at the widest part; EH, elytral height in lateral view. Measurements were done using a Nikon SMZ1500 microscope (Nikon Instech, Co., Ltd.) by recording images with Digital Sight camera system (DS-L1, Nikon Instech, Co., Ltd.). Image stacking for the dorsal and lateral view of habitus was performed with CombineZP (Alan Hadley, 2010; http://www.hadleyweb.pwp.blueyonder.co.uk/). Then, beetle specimens were dissected and genitalia were eviscerated. These genitalia were cleaned with 0.1% KOH solution for 12 hours, washed in 0.1% lactic acid, and then observed under the microscope.

Half of the specimens including the holotypes and some paratypes are deposited in Museum Zoologi Bogoriense, Indonesia (MZB.COLE. 84288–84395, 84641–84653, 84778–84780), and other specimens, including some paratypes, are deposited in the Hokkaido University Museum (SEHU. 60601–60720, 62414–62429).

Taxonomy

Recently, Szawaryn *et al*. (2015) made an extensive revision of the genera of the phytophagous ladybird beetles (Epilachnini) on the basis of phylogenetic relationships

inferred by morphological and molecular data. All the three species treated in this paper belong to their revised concept of the genus *Henosepilachna* Li in Li et Cook, 1961, which contains the Asian species of former *Henosepilachna* along with *Subafissa* Bielawski, 1963 (for the phylogenetic relationships with other species of *Henosepilachna*, also see Katoh *et al*. 2014). According to Szawaryn *et al.* (2015), the synapomorphic characters of their new concept of *Henosepilachna* are (1) in males, parameres of the tegmen with apical thorns (= small internal teeth in the terminology of Szawaryn *et al*. 2015), and (2) in females, sperm duct originated ventrally from the bursa copulatorix. As mentioned subsequently, parameres are equipped with apical thorns in all the three species treated here, and in two species, the sperm ducts are originated laterally from the bursa; no information is available for the position of the sperm duct in the remaining one species.

Henosepilachna diekei Jadwiszczak & Węgrzynowicz
(Figs 1–3)

Henosepilachna diekei: Kobayashi *et al*. 2009; Matsubayashi *et al*. 2016.
Henosepilachna emarginata diekei Jadwiszczak & Węgrzynowicz, 2003: 133.
Epilachna emarginata Dieke, 1947: 49–50 [the Philippines].

Distribution: Asian tropic region (Matsubayashi *et al*. 2016): the Philippines, Indonesia (Sumatra, Java, Bali, Kalimantan, Sulawesi, Lombok), Malaysia (Borneo, Malay Peninsula), Thailand (Malay Peninsula), Sri Lanka.

Host plants: Asteraceae: *Mikania micrantha* Kunth, *Mikania scandens* B. L. Rob. Lamiaceae: *Leucas lavandulifolia* Sm., *Leucas* sp.; *Plectranthus* spp. Acanthaceae: *Dicliptera* sp.

Specimen examined (113♂♂106♀♀; MZB.COLE.84307–84395; 84641–84653, 84778–84780; SEHU. 60622–60720; 62414–62429). *Indonesia*: 8♂♂6♀♀, Mt. Rinjani, Lombok, 25 Jan. 2013 (on *Leucas* sp.) (K. Matsubayashi *et al*.); 6♂♂2♀♀, Kebun Raya Bali, Bali, 5 Dec. 2005 (on *Mikania* sp.) (H. Katakura *et al*.); 1♂7♀♀, Kebun Raya Bali, Bali, 9 Nov. 2007 (on *Mikania* sp.) (N. Fujiyama *et al*.); 4♂♂5♀♀, Kebun Raya Bali, 17 Jun. 2008 (on *Mikania* sp.) (H. Katakura *et al*.); 5♂♂1♀, Rurukan, North Sulawesi, 27 Nov. 1999 (on Labiatae) (H. Katakura *et al*.); 10♂♂9♀♀, Rantepao, Sulawesi, 30 Nov. 2011 (on *Plectranthus* sp.) (K. Matsubayashi *et al*.); 10♂♂8♀♀, Tinokari, Sulawesi, 29 Nov. 2011 (on *Mikania* sp.) (K. Matsubayashi *et al*.); 10♂♂10♀♀, Bogor, West Java, 17 Nov. 2011, (on *Leucas lavandulifolia*) (K. Matsubayashi *et al*.); 10♂♂10♀♀, Bogor, West Java, 19 Nov. 2011 (on *Mikania micrantha*) (K. Matsubayashi *et al*.); 1♀, Cibinong, West Java, 11–14 Nov. 2002 (on *Mikania* sp.) (Fujiyama); 1♀, Cibodas, West Java, 1 Nov. 1990 (on *Mikania* sp.) (Katakura & Kahono); 3♂♂2♀♀, Pagilaran, Bogor, West Java, 19 Oct. 1990 (on *Mikania* sp.) (Katakura *et al*.); 10♂♂10♀♀, Patuha, West Java, 17 Feb. 2009 (on *Dicliptera* sp.) (K. Matsubayashi *et al.*); 1♀, Gunung Medan near Sitiung, West Sumatra (on *Mikania* sp.) (H. Katakura); 1♂1♀, near Simpang Empat, Padang, West Sumatra, 5 Mar. 1989 (on *Mikania* sp.) (S. Nakano); 2♂♂, near Simpang Empat, Padang, West Sumatra, 5 Mar. 1989 (on *Mikania* sp.) (Zaldi, Asril, Nagamasu, S. Nakano); 4♂♂4♀♀, Padang, West Sumatra, 22 Jan. 2013 (on *Mikania* sp.) (H. Katakura *et al*.); 13♂♂10♀♀, Balikpapan, Kalimantan, 28 Nov. 2011 (on *Mikania micrantha*) (K. Matsubayashi *et al*.). *Malaysia*: 8♂♂10♀♀, Poring, Kinabalu, Borneo, 12 Mar. 2008 (on *Mikania* sp.) (H. Katakura & Y. Ohta); 3♂♂2♀♀, Kuala Lumpur, 7 Dec. 2013 (on *Mikania* sp.) (Katakura & Fujiyama); 1♂4♀♀, Kuala Lumpur, 8 Dec. 2013 (on *Mikania* sp.) (Katakura & Fujiyama).

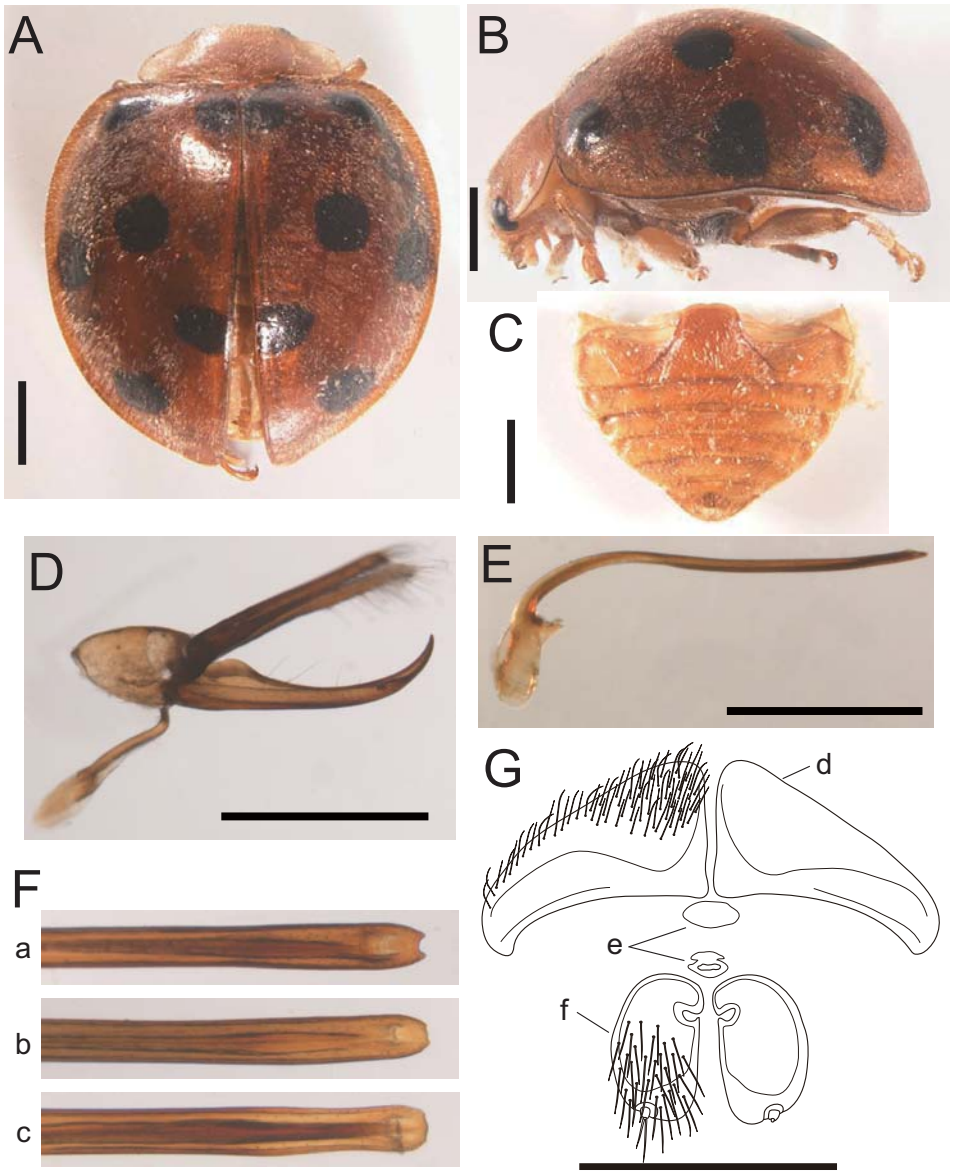

Fig. 1. *Henosepilachna diekei* Jadwiszczak & Węgrzynowicz. (A) dorsal view; (B) lateral view; (C) ventral view of abdomen; (D) lateral view of tegmen; (E) lateral view of penis; (F) dorsal view of apical edge of penis (a, emarginate; b, truncate; c, convex); (G) Female genitalia (d, ventrite 6 (=sternite VIII); e, sternite IX; f, coxite). Scale bar = 1mm.

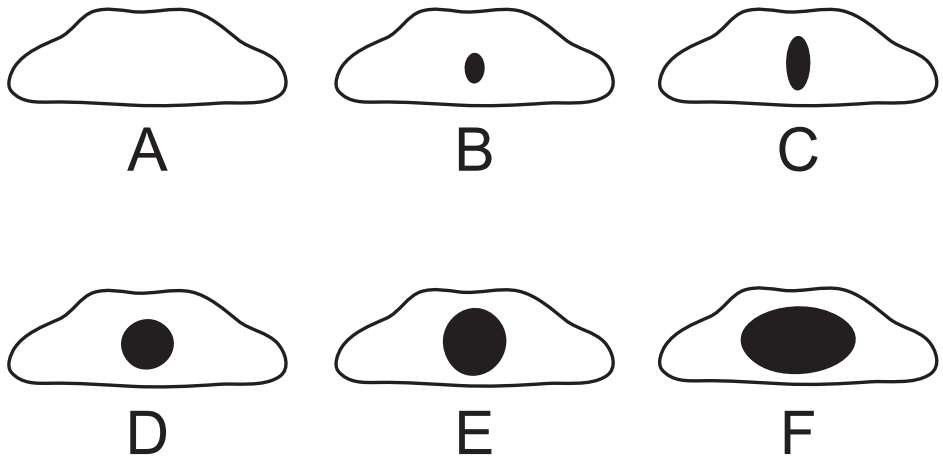

Fig.2. Spot patterns of pronotum in *Henosepilachna diekei*.

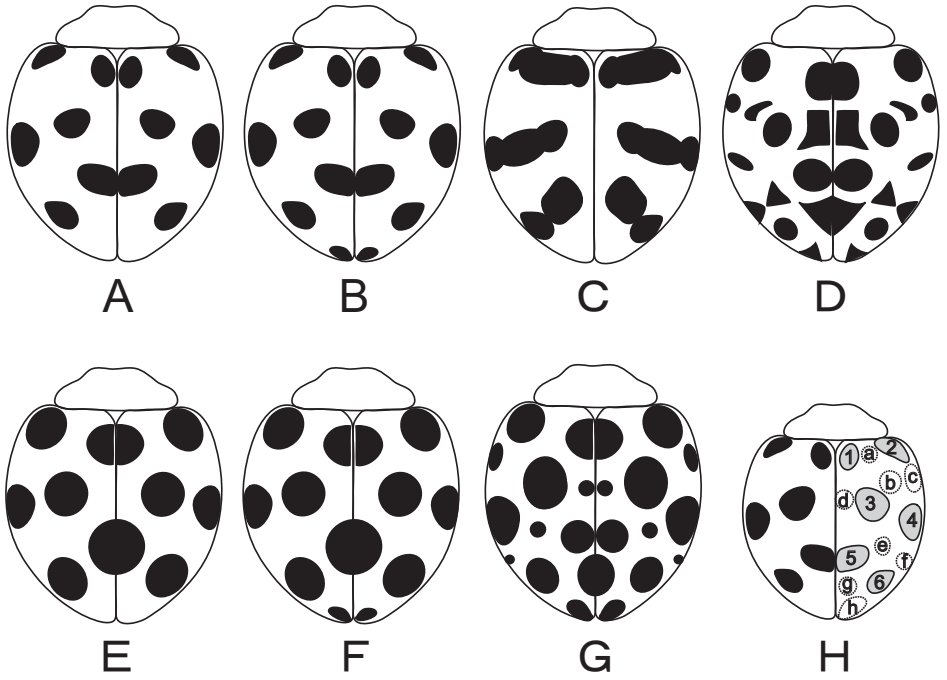

Fig. 3. Variation of the elytral spot pattern in *Henosepilachna diekei*. Persistent spots (1 - 6) and non-persistent spots (*a* - *h*) of Asian species of *Henosepilachna* (after Dieke 1947 plate 1.7) are shown in H for reference. In *H. diekei* examined in this study, spot *a* was always lacking.

Thailand: 1♂, Ban Han, South Thailand, 5 Dec. 2013 (H. Katakura & N. Fujiyama). *Sri Lanka*: 1♂1♀, Gannoruwa, 8 Oct. 2003 (on *Mikania scandens*) (S. Basnagala); 2♂♂1♀, Gannoruwa, 20 Oct. 2003 (on *Mikania scandens*) (S. Basnagala).

Redescription: Body round oval, convex above (Fig. 1). Head exposed, transverse, spotless. Pronotum transverse, spotless or with a black spot of various sizes (Fig. 2). Scutellum small, triangular, light in color. Elytron with six persistent spots and up to seven non-persistent spots (spot *a* is always lacking; Fig. 3). Spot 1, very close to suture (Fig. 3A–C) and often partly of entirely fused with the counterpart on suture (Fig. 3D–G); spot 5 often partly or entirely fused on suture, and spot *g* (if present) partly or entirely fused on suture; spot 5 not entirely fused on suture when there is spot *g* (Fig. 3D, G). Elytron basically with 6 or 7 spots (persistent spots plus spot *h*) in the areas other than Bali and Lombok though some additional spots were occasionally found; basically 6 or seven spots but some individuals possessed 26 spots (Fig. 3D) in Bali; 22 to 26 spots in Lombok (Fig. 3G). Spots 1 and 2, spots 3 and 4, and spots 5 and 6 sometimes fused to form fasciae, especially in the Patuha population in Java (Fig. 3C). Abdomen and legs with various degrees of melanization according to localities. Tarsal claw bifid, with subtriangular basal tooth. Hind margin of ventrite 5 truncate in male, sinuate in female. Ventrite 6, male, entire; female, split. Hind margin of female tergite VIII nearly truncate or weakly emarginate medially.

Male genitalia: Fig. 1D–F. Coloration of tegmen and penis normally amber or light brown, but rather dark in the Eastern form (Sulawesi and Lombok) and also in Patuha population on Java island. Tegminal basal piece with a pair of small protrusions (spines) on inner margin near base of tegminal strut. Penis guide gently curved upward, with weakly developed basal knife edge. Number of hairs on penis guide highly variable among and within populations, from 0 up to 13 (median = 3). Paramere subequal to penis guide, with apical thorn. Apical edge of penis variable among populations, from emarginate, via truncate, and to convex (Fig. 1F).

Female genitalia: Fig. 1G. Coxite, usually round beanlike in shape, but sometimes slender; inner edge with distinct incision medio-basally. Sternite IX rudimentary and often disappeared. Sperm duct originated laterally from the bursa copulatorix (Katakura *et al*. 1994).

Measurements: Males (n = 69): body length = 5.1–7.2 mm, pronotum length = 1.1–2.0 mm, pronotum width = 2.1–3.0 mm, elytra length = 4.1–5.9 mm, elytra width = 2.0–2.8 mm, elytra height = 2.0–3.0 mm. Females (n = 69): body length = 5.6–7.7 mm, pronotum length = 1.5–2.6 mm, pronotum width = 2.3–3.3 mm, elytra length = 4.6–6.2 mm, elytra width = 2.1–3.5 mm, elytra height = 1.9–3.4 mm.

Remarks: This species was referred to as *Epilachna* sp. 3 in Katakura *et al.* (1994, 2001). A more detailed account of its distribution and variations in some metric characters and penial apex structure is given in Matsubayashi *et al*. (2016). It is distinguishable by the combination of the habitus, pronotum and elytral spot patterns, the food plant, and the shape of male genitalia from other Southeast Asian species of *Henosepilachna* except for *Henosepilachna altera* (Dieke, 1947) (*sensu* Jadwiszczak & Węgrzynowicz 2003), *H. reducta* (Dieke, 1947), *H. shulzei* Bielawski, 1965, and *H. vigintioctopunctata sumbana* (Bielawski, 1959).

Adoption of the name *H. diekei* in this paper is based on the assumption that *E*. sp. 3 is identical with *Epilachna emarginata* Dieke described from the Philippines (Dieke 1947). A detailed note for this taxonomic treatment was given in Kobayashi *et al*. (2009)

Table 1. Synopsis of some diagnostic characters in *Henosepilachna diekei*, *H. uenoi* and four related taxa.

	H. diekei[1)]	*H. uenoi*[1)]	*H. altera*[2)]	*H. reducta*[2, 3)]	*H. schultzei*[3)]	*H. vigintioctopunctata sumbana*[4)]
Pronotum	Spotless or with a black median spot	3–5 black spots	No black spot	Almost black	No black spot	4 black spots
Elytral spots	12–26	26	26	26	12	28
Basal knife edge of penis guide	Present	Present	Present	Present	Absent or very weak	Weak?
Hairs on penis guide	0–13	0–2	Present	Absent	Absent	Present
Apex of penis	Emarginate, truncate, or weakly convex	Emarginate	Emarginate	Weakly emarginate	Deeply emarginate	Rounded to slightly emarginate

Source: 1) Present study; 2) Dieke (1947); 3) Bielawski (1965); 4) Bielawski (1959).

and is reproduced here with slight modification: "We referred to a 12-spotted species as *Henosepilachna* sp. 3 in our previous study, which is distributed in various parts of Indonesia. This time we identified this species with the taxon originally described from the Philippines as *Epilachna emarginata* Dieke, 1947, or more strictly its 12-spotted subspecies *Epilachna emarginata emarginata* described from Samar Island. Since the name *Epilachna emarginata* was already in use for another species, Jadwiszczak and Węgrzynowicz (2003) proposed the use of the name *Epilachna altera* Dieke, 1947, originally given to a multipunctate (26-spotted) subspecies of *Epilachna emarginata* occurring on Luzon and Mindanao. Since *E. emarginata emarginata* needs a name by this treatment, Jadwiszczak and Węgrzynowicz (2003) further proposed a replacement name *Henosepilachna emarginata diekei* for this subspecies. However, it should be *H. altera diekei* so the situation is somewhat confusing. Moreover, in the light of the current knowledge of the intraspecific variation of spot patterns in Asian epilachnines, it is not clear whether Dieke's two subspecies really represent two locally differentiated intraspecific variations or not. They might represent a mere individual variation, or represent two distinct species. We here adopt the name *Henosepilachna diekei* for our 12-spotted taxon, tentatively regarding it as specifically different from *Henosepilachna altera altera* (Dieke, 1947) (*sensu* Jadwiszczak & Węgrzynowicz 2003)."

Recognition of the two taxa (irrespective of subspecies or species) on the basis of the difference in the number of elytral spots alone is now not justifiable, because *H. diekei* in our sense is not necessarily 12 spotted. The number of elytral spots in the specimens of *H. diekei* was variable, ranging from 12 to 26 (Fig. 3). However, since little is known about the morphological variation and biology, especially host plant use, of *H. altera*, further comparison to clarify the detailed relationship of the two "subspecies" is difficult at present (Table 1).

There are two other *Henosepilachna* species apparently related to *H. diekei*, namely, *H. reducta* and *H. schultzei*, both described from the Philippines (Table 1). Dieke (1947) mentioned that the genitalia of *Epilachna reducta* and *E. emarginata* (= *H. reducta* and

H. diekei, respectively, in this paper) were practically identical in structure, but the two species differed considerably in external appearance (i.e., more spots on the elytra and more slender body shape in *reducta*). According to Bielawski (1965), *H. reducta* is very similar to *H. emarginata altera* (= *H. altera*, *sensu* Jadwiszczak & Węgrzynowicz) but is distinguished from the latter in having almost black pronotum and glabrous penis guide. With regard to *H. schultzei*, Bielawski (1965) notes that it is discernible from *H. emarginata emarginata* (= the 12 spotted form of *H. diekei* in the present paper) by the lack of basal knife edge and hairs on the penis guide. However, the degree of melanization and the number of spots on the pronotum and elytra are known to be variable within a species of Epilachnini beetles including *H. diekei*, and the number of hairs on the penis guide is variable in our *H. diekei*.

Furthermore, although the emarginate apical edge of penis was treated as an important diagnostic character of this species in Dieke (1947), there is a large geographic variation in the condition of penial apex (Fig. 1F). By the condition of penial apex, *H. diekei*, especially its multi-spotted form in Lombok, very resembles *H. vigintioctopunctata sumbana*, a 28 spotted form recorded from Sumba and Flores, in detailed morphology of both male and female genitalia (Bielawski 1959) (Table 1). However, the host plant of *H. vigintioctopunctata sumbana* is unknown. *H. diekei* is also similar to *H. cucurbitae* (Richards, 1983) (= *H. vigintioctoctopunctata, sensu* Dieke 1947), a 28 spotted species known as feeding exclusively on cucurbits, in the general appearance of male and female genitalia (Dieke 1947; Richards 1983). (*Additional notes*: Jadwiszczak and Węgrzynowicz 2003, upgraded *H. vigintioctopunctata sumbana* to the species-rank and synonymized *H. cucurbitae* with *H. sumbana*. However, it is premature to justify this action because the information on *H. sumbana* is at present very poor, known only by the original description. We here use the names of the two taxa as originally used.)

Thus, not only the relationship of *H. diekei* to *H. altera* mentioned earlier, but also relationships of *H. diekei* to *H. reducta*, *H. schultzei* and *H. vigintioctopunctata sumbana* are difficult to resolve because of the lack of information about the morphological variations of "diagnostic characters" and host plants in the latter three species. The relationship with *H. cucurbiate* should also be clarified. Moreover, there are some other species that are different in external appearance but have similar genitalia with *H. diekei*, such as *Henosepilachna signatipennis* (Boisduval, 1835) and *H. undecimvariolata* (Biosduvali, 1835) in New Guinea, and *H. solomonensis* (Dieke, 1947) in the Solomon Islands (*see*, Bielawski 1963). For the time being, therefore, we maintain our earlier position and regard the specimens treated here as a distinct species *H. diekei*, unless a reliable taxonomic revision is made for these species incorporating morphological variations, molecular data and other sorts of information.

We here modify descriptions for some characters made by Dieke (1947) as follows: Number of hairs on penis guide highly variable; pronotum spotless or with a black spot of various sizes (Fig. 2); elytra basically 12-spotted, sometimes up to 26 spots with additional non-persistent spots (Fig. 3); penial apex emarginate to convex dependent on populations (Fig. 1F).

This species, recorded from various elevations, exhibits large variations not only in morphological characters, but also in host plant use. We recognize two morphologically differentiated groups in this species, i.e. Western form and Eastern form, which exhibit rather stable difference in the elytral height and the frequencies in the type of apical edge

of penis, and occupied the western and eastern regions separated by the Wallace's line (Matsubayashi *et al*. 2016):

Western form: Populations from Sundaland-originated localities (Java, Kalimantan, Bali, Sumatra, Malay Peninsula) and Sri Lanka exhibiting smaller elytral height; apical edge of penis mostly emarginate (88.1%, n = 42) and sometimes truncate (11.9%); pronotum generally spotless; elytron each usually with six persistent spots, sometimes with several non-persistent spots (especially in Java and Bali).

Eastern form: Populations from a part of Wallacea (Sulawesi and Lombok) exhibiting larger elytral height; apical edge of penis convex (53.6%, n = 28) or truncate (46.4%); pronotum often spotless but frequently with various sizes of spots; elytron each with well developed six persistent spots and often with small additional non-persistent spots.

An analysis of mitochondrial ND2 sequences with some sample materials showed that the two forms are slightly differentiated genetically, too (see PHYLOGENETIC ANALYSIS).

Henosepilachna uenoi Matsubayashi & Katakura sp. nov.
(Figs 4, 5)

Description. Body oval, convex above (Fig. 4). Head exposed, transverse, spotless. Pronotum transverse, with one median and one or two pairs of lateral black markings (Fig. 5); but with a very faint medial spot in the holotype. Scutellum small, triangular, light in color. Elytron each with 13 spots; size of non-persistent spots variable, sometimes small and irregular in shape (Fig. 4A). Abdominal ventrites 1–4 black except for sides. Hind margin of ventrite 5 truncate in male, sinuate in female. Ventrite 6, male, entire; female, split. Hind margin of female tergite VIII mildly rounded. Leg spotless. Tarsal claw bifid, with subtriangular basal tooth.

Male genitalia: Fig. 4D–F. Very similar to those of *H. diekei*. Tegmen and penis dark brown. Tegminal basal piece with a pair of small protrusions on inner margin near base of tegminal strut. Penis guide gently curved upward, with weakly developed basal knife edge. Number of hairs on the penis guide from 0 up to 2 (median = 1; 2 in holotype). Paramere subequal to penis guide, with apical thorn. Apex of penis emarginate.

Female genitalia: Fig. 4G. Coxite, slender beanlike shape, inner edge with distinct incision medio-basally; sternite IX rudimentary and often disappeared. No information available for the position of sperm duct relative to bursa copulatrix.

Measurements: Males (n = 8): body length = 4.8–5.9 mm, pronotum length = 1.6–2.1 mm, pronotum width = 2.2–2.6 mm, elytra length = 4.3–5.1 mm, elytra width = 1.8–2.3 mm, elytra height = 1.9–2.6 mm. Female (n = 12): body length = 5.1–6.4 mm, pronotum length = 1.7–2.0 mm, pronotum width = 2.3–2.8 mm, elytra length = 4.4–5.3 mm, elytra width = 1.8–2.5 mm, elytra height = 2.1–2.9 mm.

Distribution: Highland in Bali, Indonesia.

Host plants: Asteraceae: *Synedrella* sp., *Tithonia* sp.; an unidentified species of Acanthaceae.

Etymology: Dedicated to Dr. Hideki Ueno, a distinguished researcher of ladybird quantitative genetics, who discovered this species for the first time with NF.

Specimen examined: Holotype (♂; MZB.COLE. 84298), Kebun Raya Bali, Bali, Indonesia, 27 Jan. 2013 (on *Tithonia* sp.) (K. Matsubayashi). Paratypes (8♂♂11 ♀♀; MZB.COLE. 84299–

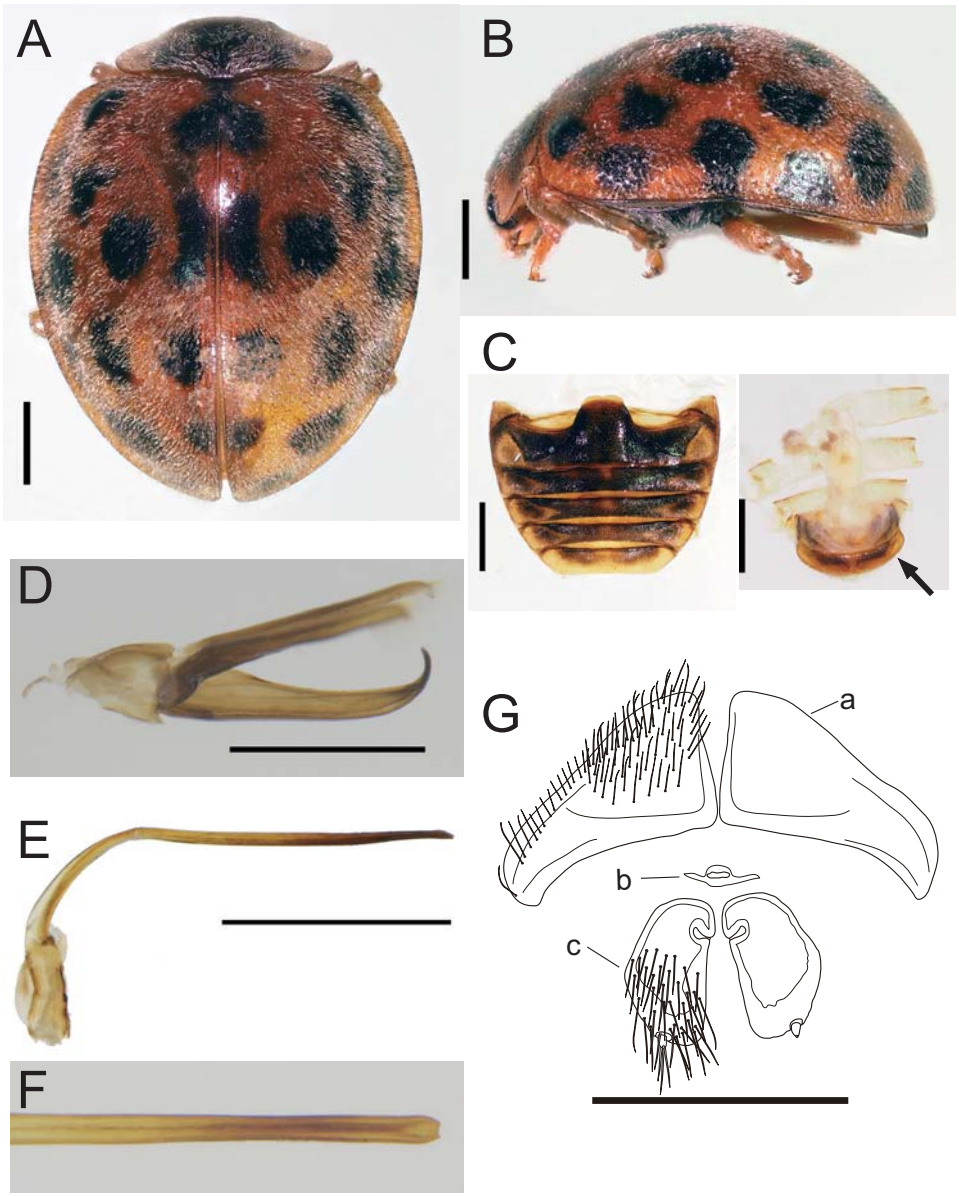

Fig. 4. *Henosepilachna uenoi* Matsubayashi & Katakura sp. nov. (A) dorsal view; (B) lateral view; (C) left: ventral view of abdomen, right: ventrite 6; (D) lateral view of tegmen (tegminal strut not shown; similar to that of *H. diekei*); (E) lateral view of penis (broken at the basal two fifth and so the tip in slightly oblique position); (F) dorsal view of apical edge of penis; (G) Female genitalia (a, ventrite 6; b, sternite IX; c, coxite). A to F, holotype; G, a paratype. Scale bar = 1 mm.

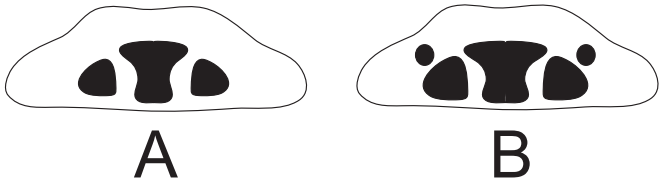

Fig. 5. Spot patterns of pronotum in *Henosepilachna uenoi* sp. nov.

84306; SEHU. 60611–60621): 2♂♂, 3♀♀, data same as the holotype; 1♀, Kebun Raya Bali, Bali, Indonesia, 2 Dec. 2006 (on *Synedrella* sp.) (H. Katakura *et al.*); 2♀♀, Buleleng, Bali, 5 Dec. 1999 (on *Synedrella* sp. and also on unknown plant belonging to Acanthaceae) (N. Fujiyama *et al.*); 5♂♂, 5♀♀, Batur Tengah, near Lake Kintamani, Bali, 2 Dec. 2005 (on *Synedrella* sp.) (H. Katakura *et al.*); 1♂, Kintamani, Bali, Indonesia, 27 Jun. 2013 (on *Synedrella* sp.) (K. Matsubayashi).

Remarks: *Henosepilachna uenoi* was first collected in December 1999 on *Synedrella* sp. (Asteraceae) in Buleleng, Bali, Indonesia. At the same time, the larvae of this species were also collected on *Synedrella* sp. and another unidentified plant belonging to Acanthaceae. Later, additional specimens were collected in December 2005 on the same *Synedrella* sp. at the site near the 1999 collecting site. This species is sympatric with Western form of *H. diekei* and has female and male genitalia being nearly identical with those of the latter. However, the two species in Bali differ in the shape and size of the body (smaller and more slender in *H. uenoi*), maculation patterns on the elytra (Fig. 4) and pronotum (Fig. 5), and the food plants (*Mikania* species in *H. diekei*, *Synedrella* sp. and some others in *H. uenoi*). Comparisons of body length with the general linear model including two main variable factors (Sex and Species) and the interaction (Sex×Species) with *R 2.15.0* software (R Development Core Team, 2012) between these two species collected in the sympatric locality at Bali Island demonstrated significant morphological divergence; *H. uenoi* demonstrated significantly smaller body sizes than that of *H. diekei* (Null model, Resid. Dev. = 15.539; Sex, df = 1, Dev. = 2.046, Resid. Dev. = 13.494, $P < 0.001$; Species, df = 1, Dev. = 7.976, Resid. Dev. = 5.518, $P < 0.001$; Sex×Species, df = 1, Dev. = 1.037, Resid. Dev. = 4.480, $P = 0.003$). This species is also different from *H. diekei* in mitochondrial ND2 sequences as mentioned later. Since co-occurrence of *H. uenoi* and *H. diekei* has been observed on Bali at least from 2005 to 2013, these divergence in multiple characters including morphology (i.e. body size and spot pattern), ecology (i.e. host plant), and mitochondrial haplotypes (see PHYLOGENETIC ANALYSIS) between sympatric populations is stable, suggesting that they represent different biological entities, namely species. *Henosepilachna uenoi* resembles *H. altera* and *H. reducta* in the structure of the genitalia of both sexes and having 26 spots on elytra (Table 1). However, the figures provided by Dieke (1947) and Bielawski (1965) showed some differences in the body shape and position of elytral spots at least between *H. uenoi* and *H. reducta*. *Henosepilachna uenoi* is also somewhat similar to *H. vigintioctopunctata*

sumbana (Table 1). As in the case of *H. diekei* mentioned above, we treat *H. uenoi* as a distinct species until detailed information on the morphological variation and host plants of the putatively related species becomes available (cf. "*Remarks*" for *H. diekei*).

Henosepilachna nakanoi Matsubayashi & Katakura sp. nov.
(Fig. 6)

Description: Body round oval, convex above (Fig. 6). Head exposed, transverse, spotless. Pronotum transverse, spotless. Scutellum small, triangular, light. Elytron with six persistent spots, of which spots 3 and 4 fused (Fig. 6). Ventrites 1–3 dark medially. Hind margin of ventrite 5 truncate in male, weakly sinuate in female. Ventrite 6, male, entire; female, split, narrower and longer than *H. diekei* and *H. uenoi*. Hind margin of female tergite VIII nearly truncate or weakly emarginate. Leg spotless. Tarsal claw bifid, with subtriangular basal tooth.

Male genitalia: Fig. 6D–F. Tegmen and penis black. Tegminal basal piece with a pair of small protrusions on inner margin near base of tegminal strut. Penis guide sharply curved upward, with well developed basal knife edge. Number of hairs on penis guide generally 0, rarely 1 (0 in holotype). Paramere with apical thorn, subequal length with penis guide. Penis pointed slightly upward and distinctly narrowed at apex. Orifice of penis opens at the right side of apical edge seen dorsally (see Fig. 6F).

Female genitalia: Fig. 6G, c. Inner edge of coxite slightly concave at the middle. Sternite 9 well developed (Fig. 6G, b). Sperm duct originated laterally from the bursa copulatrix (Katakura *et al*. 1994).

Measurements: Male (n = 8): body length = 4.4–5.1 mm, pronotum length = 1.4–1.8 mm, pronotum width = 2.0–2.4 mm, elytra length = 3.5–4.3 mm, elytra width = 1.5–2.0 mm, elytra height = 1.6–2.0 mm. Females (n = 11): body length = 4.6–5.8 mm, pronotum length = 1.5–2.3 mm, pronotum width = 2.0–2.6 mm, elytra length = 3.9–5.1 mm, elytra width = 1.4–2.0 mm, elytra height = 1.4–2.3 mm.

Distribution: Highlands in Sumatra and Java, Indonesia.

Host plants: Acanthaceae: *Strobilanthes cernuus* Blume, and an unidentified species.

Etymology: Named after Dr. Susumu Nakano, an expert of epilachnine biology, who collected this species for the first time while he stayed at Andalas University, West Sumatra, as a visiting researcher.

Specimen examined: Holotype (♂; MZB.COLE. 84288), Gn. Salak, Bogor, West Java, Indonesia, 18 Jan. 2013 (on *Strobilanthes cernuus*) (H. Katakura *et al.*). Paratypes (7♂♂12 ♀♀; MZB.COLE. 84289–84297; SEHU. 60601–60610): 2♂♂2♀♀, data same as the holotype; 1♂2♀♀, Mt. Gede, West Java, 29 Jan. 1991 (on *Strobilanthes cernuus*) (S. Kahono); 1♀, Mt. Gede, West Java, 31 Oct. 1991 (on *Strobilanthes cernuus*) (H. Katakura); 3♂♂3♀♀, Mt. Gede, West Java, 29–31 Oct. 1991 (on *Strobilanthes cernuus*) (H. Katakura and S. Kahono); 1♀, Kayu Jao, West Sumatra, 28 Sept 1988 (on Acanthaceae) (S. Nakano); 1♂2♀♀, Gn. Tujuh, Sumatra, 5 Oct. 2004 (on Acanthaceae) (H. Katakura *et al*.); 1♀, Danau Gn. Tujuh, Sumatra, 4 Nov. 2001 (on Acanthaceae) (H. Katakura *et al*.).

Remarks: This new species is similar in the general body shape and elytral spot pattern to some individuals of *H. diekei*, while exhibiting more round body shape and much smaller body size than the latter. This species was referred to as *Epilachna* sp. 5 in Katakura *et al*. (2001). It can be easily recognized by the unique genital morphology both in the male and female, the food plant (e.g. feed on *Strobilanthes cernuus* in Java,

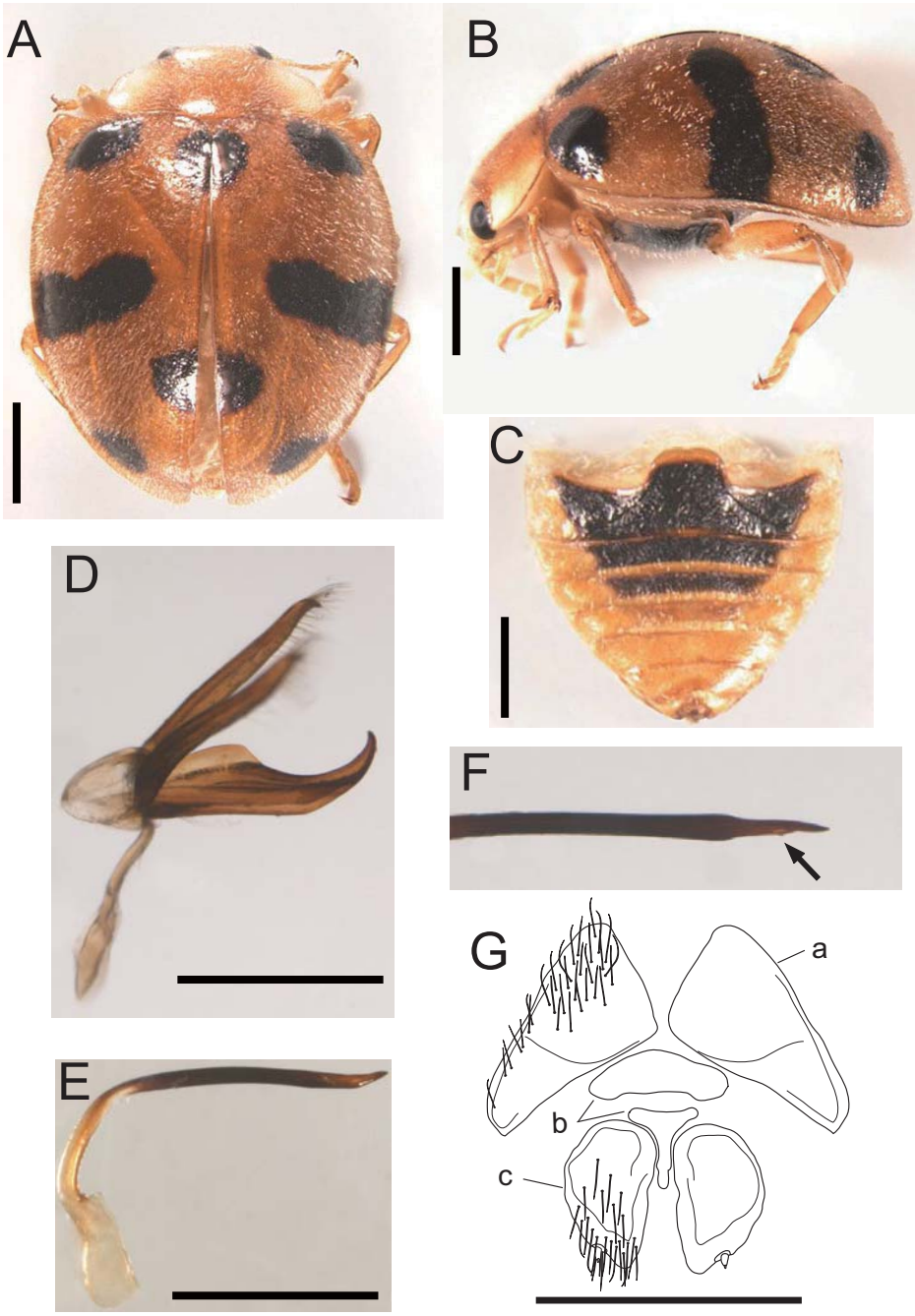

Fig. 6. *Henosepilachna nakanoi* Matsubayashi & Katakura sp. nov. (A) dorsal view; (B) lateral view; (C) ventral view of abdomen; (D) lateral view of tegmen; (E) lateral view of penis; (F) dorsal view of apical edge of penis with an arrow representing sperm orifice; (G) Female genitalia (a, ventrite 6; b, sternite IX; c, coxite). A - F, holotype; G, a paratype. Scale bar = 1mm.

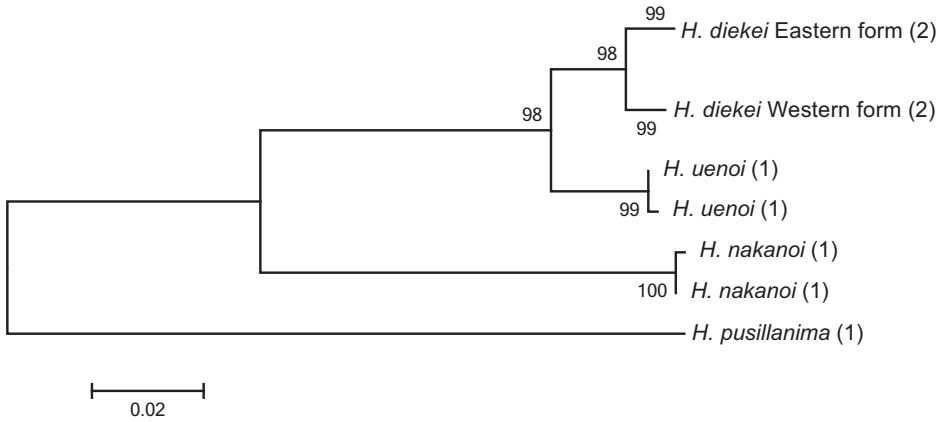

Fig. 7. A maximum likelihood phylogenetic tree based on mitochondrial ND2 sequences of three species, *Henosepilachna diekei*, *H. uenoi* sp. nov., *H. nakanoi* sp. nov., and *H. pusillanima* as the out group. The numerals in the parentheses denote the numbers of specimens.

and on an unidentified Acanthaceae in Sumatra), and mitochondrial sequences from other *Henosepilachna* species.

Phylogenetic Analysis

The phylogenetic relationship among *Henosepilachna diekei*, *H. uenoi* and *H. nakanoi* was analyzed using ND2 gene sequences. For the analysis, a previously published haplotype of *Henosepilachna pusillanima* (Mulsant, 1850) (DDBJ: AB359204) was used as the out-group to root the tree since *H. pusillanima* was shown to be close to *H. diekei* and *H. nakanoi* (Kobayashi *et al*. 2009; Katoh *et al*. 2014). The sequences of two individuals each of *H. uenoi* and *H. nakanoi* were used for the phylogenetic analysis. To investigate the intra-specific genetic variation of the two morphological groups (Western form and Eastern form) of *H. diekei*, two individuals each of Western form (specimens collected on *Mikania* sp. in Bali) and Eastern form (specimens collected on *Plectranthus* sp. in Rantepao, Sulawesi) were used. These eight specimens of the three species were newly analyzed in the present molecular analysis (DDBJ: LC169557, LC169558, LC172183–LC172186). Methods for DNA extraction, PCR amplification, and gene sequence followed Fujiyama *et al*. (2013). A phylogenetic tree was constructed using 639 bp ND2 gene sequences with 129 variable sites based on Maximum Likelihood method (General Time Reversible Model with G +I substitution model) and 1000 times bootstrap test with MEGA 6.05 (Tamura *et al*. 2013). Further, we measured group mean genetic distance (d_A) between *H. uenoi* and *H. diekei*, and between two geographic forms of *H. diekei* based on Maximum Composite Likelihood method with MEGA 6.05.

Henosepilachna uenoi and *H. diekei* formed a cluster, in which the two species were reciprocally monophyletic, and *H. nakanoi* was branched out of the cluster (Fig. 7). Group mean genetic distance between *H. uenoi* and *H. diekei* was 0.0183, while that between two geographic forms of *H. diekei* was 0.0078. The clear genetic divergence between partially sympatric *H. uenoi* and *H. diekei* suggested that the two species are

good biological species being reproductively isolated from each other as suggested by differentiation in some morphological traits and ecology (see above), although their genitalia and general appearance were very similar. Specimens of Eastern form and Western form of *H. diekei* were grouped in reciprocally monophyletic clusters, being consistent with the morphological divergence reported in Matsubayashi *et al.* (2016).

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