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LIFE HISTORY AND MORPHOLOGY OF THE BLACK CUPID BUTTERFLY, *TONGEIA KALA* (DE NICÉVILLE)(LYCAENIDAE), FROM MYANMAR

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ABSTRACT. A rare lycaenid butterfly, *Tongeia kala*, is distributed from NE India to eastern Myanmar. In appearance there are no closely related species in the genus, and therefore it is of much interest to study this species from an evolutionary point of view. In order to extract phylogenetic information of *T. kala*, the immature stages and biology were studied in a high mountain area of Chin State, Myanmar. We describe egg, larval, pupal stages, adult wing pattern and male genitalia of the species, and compare with its related species. We also record *Sedum multicaule*, as its hostplant.

Additional key words: Chin State, Everes section, Polyommatini, *Sedum multicaule, Sedum emarginatum*.

The Black Cupid butterfly, *Tongeia kala* (de Nicéville, 1890), is known as a rare lycaenid species, occurring only from Naga Hills of NE India to southern Shan State of eastern Myanmar (Bingham 1907; Evans 1932; Wynter-Blyth 1957). About 15 *Tongeia* species are distributed in the East and SE Asia (D'Abrera 1986, 1993; Bridges 1988; Huang & Chen 2006), but the genetic status of *T. kala*, *T. potanini* (Alphéraky, 1889) and *T. arcana* (Leech, 1890) seems to be doubtful because of their unique wing markings (Kawazoé & Wakabayashi 1976). Therefore, it is of much interest to study the speciation process of *T. kala* and the monophyly of *Tongeia*. However, there are few available data to shed light on phylogenetic aspects of this species. Only short morphological and biological notes have been published (de Nicéville 1890; Bingham 1907; Seitz 1927; Evans 1932; Wynter-Blyth 1957; Cantlie 1964; Huang & Chen 2006).

In November 2009, we conducted a butterfly research project in the northern part of Chin State, Myanmar, in cooperation with the Department of Hotel and Tourism of Myanmar and the Myanmar Japan Relations Center. During the survey, the third author discovered adults, eggs and larvae of *T. kala* at Mt. Kennedy of the Letha Mountains. Although during his 23 research trips over the past 12 years in Myanmar he had found two congeners, *T. potanini* and *T. ion* (Leech, 1891), *T. kala* had never been recorded anywhere. For the purpose of clarifying the immature stages of this species, we reared some larvae of the species at Mandalay, Mandalay Division.

Here we describe the morphological and biological characteristics of *T. kala* based on the morphology of immature and adult stages. Moreover, we discuss the similarities to and differences from other *Tongeia* species, and provide some preliminary data to elucidate the evolutionary processes relating to this species.

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MATERIALS AND METHODS

The adults and larvae of *T. kala* were found at a high altitude (2,000 m) of Mt. Kennedy, in the Letha Mountains (Figs 1–2) on 3–12 November 2009. Mt. Kennedy (2,704 m) is located 30 miles west of Kalaymyo and 16 miles southeast of Tiddim in Chin State, Myanmar. Although the climatic condition of this area is characterized by a tropical monsoon with comparatively distinct rainy (May–October) and dry (November–April) seasons, rather high altitudes of the mountains are characterized by a consistently cool mountainous climate with chilly nighttime temperatures.

The third author collected some eggs, two second instar and one third instar larvae of *T. kala* in the field. We reared them individually in plastic cases (90 mm diameter, 30 mm height), placed in a room (22±1°C; 14L–10D) at Mandalay, which is the second largest city in Myanmar and the last royal capital of Burma. Until they reached the pupal stage, the larvae were fed on two hostplants, and the details are discussed in the results of this paper. Although the eggs did not hatch, the growth of the larvae and pupae was monitored every day until they reached the adult stage. The individuals were recorded using a digital camera Nikon D70 with a micro lens (Nikon AF Micro Nikkos 60 mm) and a combined electronic flash (Nikon Wireless Speedlight Commander SU-800 and Nikon Wireless Remote Speedlight SB-R200), and with a Kenko Extension Tube 12/20/36 mm as necessary. The body lengths of all the larval instars were measured just before diapause.

For the purpose of male genitalia examination, apical parts of the abdomen were placed in 10 % KOH solution at about 100°C for 10 minutes. After this treatment, they were washed with distilled water and placed in 80 % ethanol for dissection and examination. The genitalia were examined and illustrated using a Leica L2 and stereoscopic microscope with magnifications of up to 40X. Terminology of the male genitalia followed Shirôzu (1960), except for the substitution of 'falx' for 'brachium'.

RESULTS

Description. *Egg* (Figs 3–4). Approximately 0.7 mm (n = 5) in diameter, and 0.3 mm (n = 5) in height. Exochorion whitish, disc-liked, rounded in dorsal view, turban-shaped laterally, with upper surface almost flattened but forming gentle slope from its shoulder toward micropylar area, bottom surface flattened. Micropylar area greenish, smooth, rather small, enclosed by weak elevation but slightly depressed in central axis. Remaining surface composed of concave chorionic cells and thick prominent ridges without spines. Chorionic cells depressed, circular and usually surrounded by four intersected nodular processes and four-sided chorionic ridges. Cells largest at lateral side, becoming smaller (about one-tenth) toward micropylar region. In dorsal view chorionic cells radiate in spiral shape from central axis, but in lateral view forming checker-pattern with their ridges.

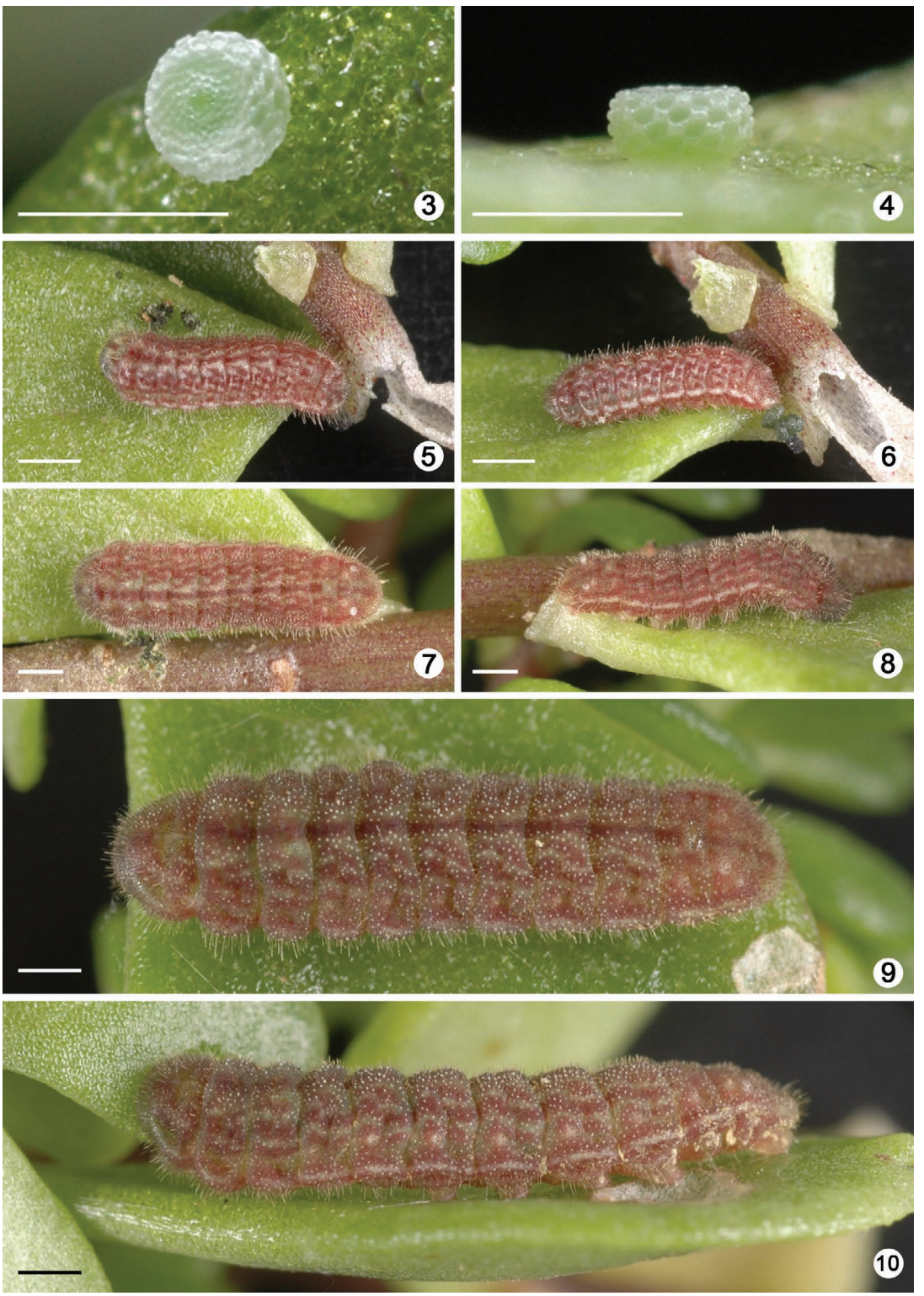
The eggs were laid singly at the base of flowers of the hostplant, on stems near the flowers, and on a bifurcation between stems of the flowers.

Larva (Figs 5–10). All larval instars except the first instar were examined (n = 3). From second to final instar larvae, larval shape unisciform as in other lycaenids, but somewhat slender and flattened. Larval body color reddish in general with pale creamy white in pattern. Prothoracic shield pale creamy white with green tint. In dorsal view, mid dorsal line distinguished by darker color running from mesothorax to last abdominal segment, and bordered by pale creamy white subdorsal line, which is distinct in early instar larvae but



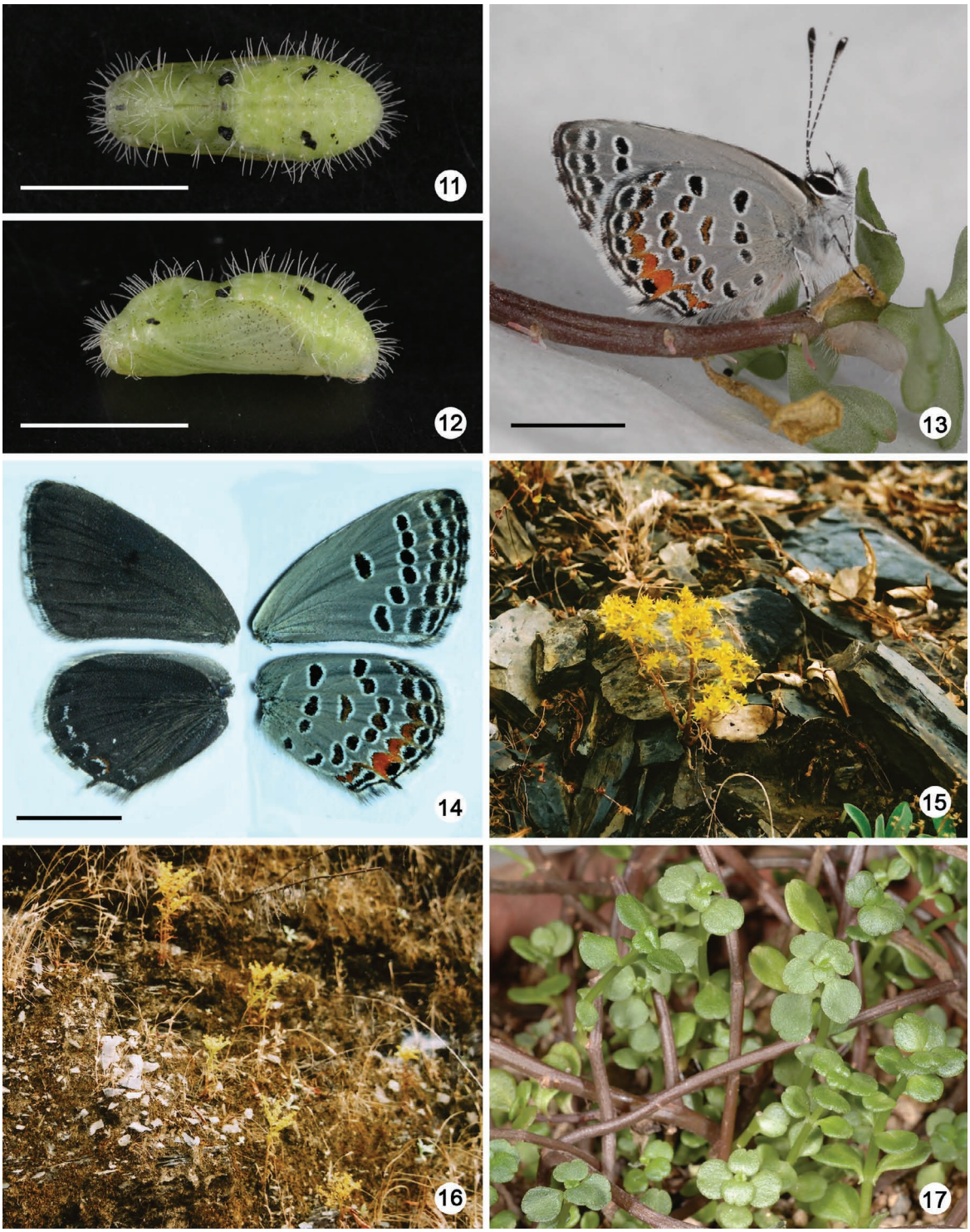
FIGS 1–2. Habitat of *Tongeia kala*. **1**) A view of Mt. Kennedy and its surroundings in Chin State, Myanmar. **2**) A sunny cliff (2,000 m alt.) along a mountain track running sparse shrubs. The butterfly adults fly actively on the cliff, where the hostplant also sparsely grows.

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FIGS 3-10. Egg and larvae of *Tongeia kala*. **3**) Egg, dorsal view. **4**) Egg, lateral view. **5**) Second instar larva, dorsal view. **6**) Second instar larva, lateral view. **7**) Third instar larva, dorsal view. **8**) Third instar larva, lateral view. **9**) Final (fourth) instar larva, dorsal view. **10**) Final (fourth) instar larva, lateral view. Scale bar 1 mm.

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FIGS 11-17. Pupa, adults and hostplants of *Tongeia kala*. **11**) Pupa, dorsal view. **12**) Pupa, lateral view. **13**) Male adult just after emergence on substitute hostplant, lateral view. **14**) Male wings, upperside (left) and underside (light). **15–16**) Hostplant, *Sedum multicaule* (Crassulaceae). **17**) Substitute hostplant, *Sedum emarginatum* (Crassulaceae). Scale bar 5 mm.

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reduced in late instars. On dorsolateral portion, three rows of faint, slanted, pale white lines waved and extending posterolaterally in each segment. In lateral view, spiracles white in color and located parallel to lateral line in each abdominal segment. Lateral line white and running along body edge. All instar larvae with short transparent prominent setae throughout body, especially in lateral side, as in larvae of many lycaenid butterflies. Stellate based setae distinctly visible, represented as small white and brown dots, and dispersed throughout dorsal surface of thorax and abdomen, especially in final instar larvae. Second to final instar larvae with three types of chemical signaling myrmecophilous organs (ant-association organs): dorsal nectary organ (DNO) on dorsum of seventh abdominal segment, paired eversible tentacle organs (TOs) on dorsolateral portion of eighth abdominal segment posterior to spiracle, and pore cupola organs (PCOs) scattered throughout body surface, concentrated near DNO and spiracles. Body length 4.0 mm (n = 2) in second instar, 6.5–7.0 mm (n = 2) in third instar, and 10.5–11 mm (n = 3) in final instar.

The larvae usually eat into thick leaves or stalks of their hostplants and eat from the inside, especially in early instars. They remained four days in the third instar, five days in the final instar, and two days in the prepupal stage.

Pupa (Figs 11–12). Typical lycaenid shaped, slightly long and slender, gourd-like dorsally but rather flattened ventrally, and with abdomen somewhat swollen and rounded, body surface weakly wrinkled and covered with minute long setae except for ventral portion and wings. Ground color light green, more yellowish on abdominal segments, tinged by paired prominent dark brown dots on base of wings and dorsolateral portion of first and fourth abdominal segments, and by small dark brown dots covering body surface, especially on wings. Body length 8.0–8.5 mm (n = 2).

Pupation took place on the stalk near a base of the hostplant in rearing. The pupal stage before emergence is 13 to 15 days.

Adult (Figs 13–14). Wing shape very similar to those of congeners, but in female slightly more rounded than that in male. Hindwing with very small, black, white-tipped tail at vein 2. Wing markings on upper- and underside almost the same in both sexes, but wing shape more rounded in female. On upperside, both wings evenly black in ground color. Forewing with rather prominent oval discocellular deep black spot. Hindwing with discocellular deep black spot and submaginal series of oval indistinct black spots, each of which outwardly defined by fine white or pale blue arched line, but somewhat with orange tint in cell 2. Cilia white edged and with black scales at each vein. On underside, both wings somewhat dark grey in ground color. Forewing margined by fine black line, then following with two series of oval dull black spots circled by white line, inner one larger and more quadrate than outer one. Discal spots comprising of series of six black spots circled by fine white line, similar round discocellular spot present. Hindwing with submarginal markings similar to forewing, but inner one arranged by series of wide submarginal orange lunules from cells 1a to 4 or 5 (but orange lunules occasionally narrowed in cells 3–5), then changed to dull fuscous toward cell 7. Discal cell markings represented by eight irregular black spots with orange stain and somewhat parallel with wing margin. Linear discocellular spot and four subbasal spots present as in forewing, but duller in color. Cilia similar to those on upperside, but black scales at each vein more widely expanded. Forewing length 11.5–12 mm (n = 2) in male and 11–12 mm (n = 3) in female.

Male genitalia (Fig. 18). Tegumen short and inclined posterodorsally. Uncus claw-like, bearing thin hairs. Falx slender, shorter than uncus and gently curved inwardly under uncus. Vinculum in lateral view rather broad but slightly concave below. Valva nearly rectangular, hairy on posterior portion and with bifurcate, flattened but sharply pointed apices, of which the lower one (harpe) in ventral view crosses the upper one (ampulla); valvae close to each other at dorsal margin of sacculi. Juxta slender, V-shaped and connected ventrally with sacculi of valvae. Phallus slender, almost straight, as long as height of ring and bearing small hook on posterioventral portion.

Habitat (Figs 1–2, 15–16). The habitat and its surroundings of *T. kala* are covered mostly with mixed evergreen and deciduous broad-leaved forest (Fig. 1), dominanted by members of the Betulaceae (*Alnus*), Fagaceae (*Quercus*) and Ericaceae [*Rhododendron* (*Hymenanthes*)]. This butterfly species was found on a sunny cliff (2,000 m; Fig. 2) facing west along a mountain track with sparse shrubs and with some deforestation for fuel. During fine weather the male and female adults fly actively, close to the cliff between 12:00 and 16:00. Some females are often found on or near the hostplant, and one individual was observed laying an egg on a bifurcation of peduncles (Figs 15–16). The hostplant grows on the cliff where the adults fly (Fig. 2).

Hostplants (Figs 13, 15–17). At the habitat of *T. kala*, all eggs and larvae that the third author found were discovered on or near flowers of *Sedum multicaule* Wallich ex Lindley (Crassulaceae; Figs 15–16). The hostplant is widely distributed from Nepal, India and Myanmar to China (alt. 1,300–3,500 m), along the Himalayas and its surroundings (Ohba 1975; Fu et al. 2001). Rearing at Mandalay, we fed all three larvae on *Sedum emarginatum* Migo (Crassulaceae; Figs 13, 17) as a substitute hostplant. The *Sedum* plant is native to S, SE and E China (alt. 600–1,800 m) (Fu et al. 2001). According to local people, there has been a continuing influx of Chinese immigrants and imports mainly from SW China in the past 20 years, and the substitute hostplant is also likely to have come from Sichuan, China, for ornamental or medicinal purposes.

DISCUSSION

The descriptions of immature stages of the genus *Tongeia* are still preliminary. Of about 15 described species of the genus, only three have been studied: *T. hainani* (Bethue-Baker, 1914), *T. filicaudis* (Pryer, 1877) from Taiwan (Igarashi & Fukuda 2000) and *T. fischeri* (Eversmann, 1843) from Japan (Fukuda et al. 1984). Here we compare some morphological aspects of *T. kala* to these related species. The eggs of *T. kala* have chorionic cells and chorionic ridges somewhat larger than *T. hainani* and *T. filicaudis* (Igarashi & Fukuda 2000). In appearance, the external morphology of the immature stages in *T. kala* does not differ significantly from those in the three related species. The remarkable difference was found only in coloration. *Tongeia filicaudis* larvae are the most similar to *T. kala*. in that they share several external, morphological characters such as the reddish body color, the series of faint transverse lines and the white lateral line. On the other hand, larvae of *T. hainani* and *T. fischeri* are represented as somewhat plain green in the larval body surface (Igarashi & Fukuda 2000; Fukuda et al. 1984), though they have some body color variations from green to

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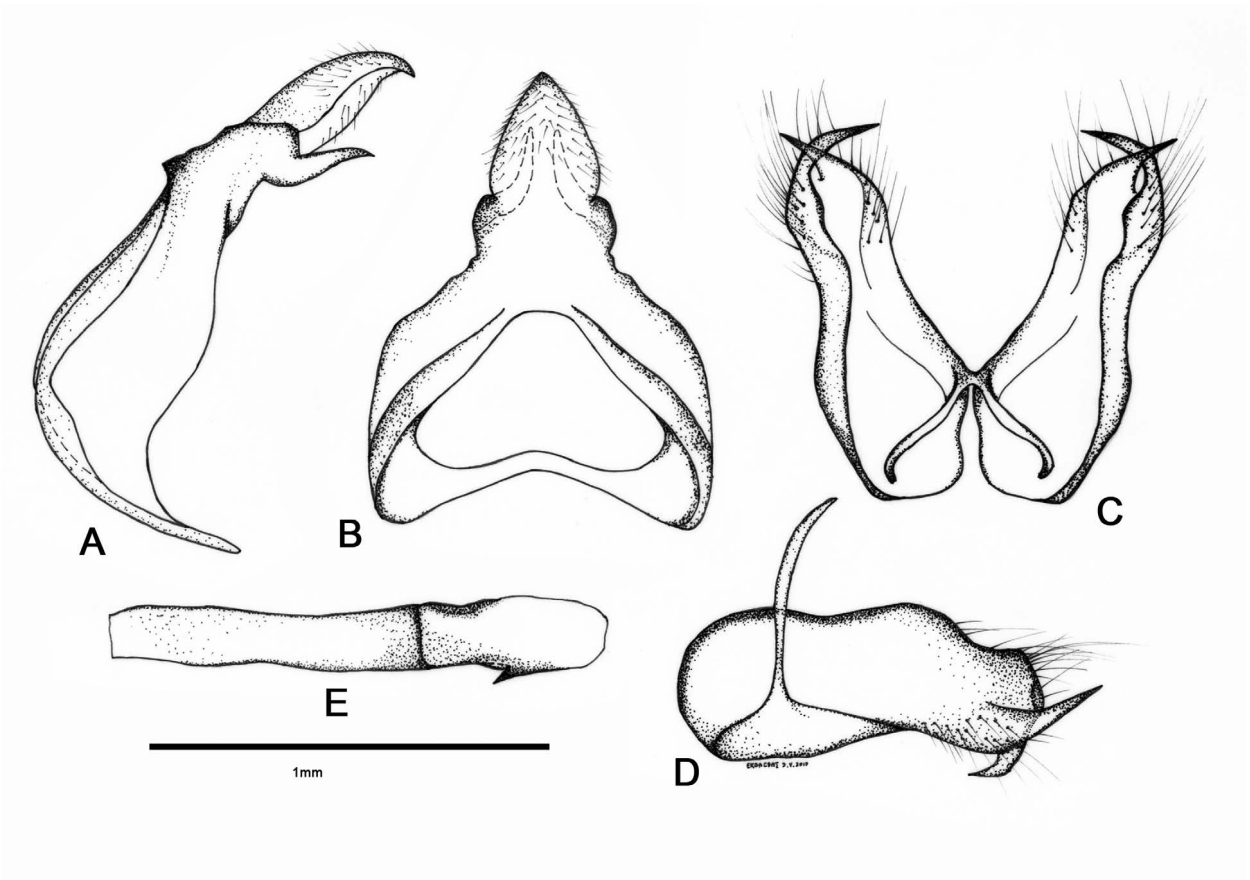


FIG. 18. Male genitalia of *Tongeia kala*. **A**) Ring (vinculum + tegumen), lateral view. **B**) Ditto, dorsal view. **C**) Valvae, dorsal view. **D**) Right valva, lateral view. **E**) Phallus, lateral view. Scale bar 1 mm.

reddish brown, probably due to the chemical compounds and the color of their hostplants. On the larval body surface, stellate-based or crown-like setae are commonly found in polyommatine butterflies (Ballmer & Pratt 1989; Fiedler 1991), and *T. kala* larvae also possess white or brown stellate-based setae represented as small white and brown dots throughout the body surface. These setae are recognized as white in *T. filicaudis*, but as dark brown in *T. fischeri* and *T. hainani*. (Igarashi & Fukuda 2000; E. Jeratthitikul unpublished). For pupal coloration, *T. kala* has the same color pattern as *T. hainani* and *T. filicaudis* (Igarashi & Fukuda 2000), but differs considerably from *T. fischeri* in having no dark brown smudges over the pupal surface (Fukuda et al. 1984; E. Jeratthitikul unpublished).

The majority of lycaenids are known to have associations with ants. This relationship has exerted strong selection on lycaenid larval morphology (Pierce et al. 2002). *Tongeia kala* also exhibits this trend; for example, the larvae have a thick cuticle and a small head that is retractable under a sclerotized prothoracic plate to defend against ant bites. In addition to more general adaptations, lycaenids possess two highly specialized sets of organs either for chemical or acoustic signaling, used to interact with ants (Pierce et al. 2002; Fiedler 1991). Larvae of *T. kala* possess three types of myrmecophilous organs for chemical signaling: dorsal nectar organ (DNO), tentacle organs (TOs) and pore cupola organs (PCOs), which are similar to those usually found in other lycaenids (Pierce et al. 2002; Fiedler 1991; Kitching 1985) and in reported related species, *T. hainani, T. ion* and *T. fischeri* (Fiedler 1991; E. Jeratthitikul unpublished). The DNO and TOs are easily visible under the stereo microscope or even by the unaided eye in late instar larvae (two small white dots in Fig. 7). Although no acoustic signaling was observed in the larval and pupal stages of *T. kala* according to our preliminary study, Fiedler (1992) suggested that the ability to produce calls may be universal in Lycaenidae, based on a biological study of *Hypolycaena othona* (Hewitson, 1865). In fact, several authors also reported that lycaenid larvae and pupae

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[e.g. *Jalmenus evagoras* (Donovan, 1805)] produce sounds when disturbed or associated with ants (Travassos & Pierce 2000; see review by Pierce et al. 2002).

Despite possessing the myrmecophilous organs, the evidence of ant-association in *T. kala* was not observed during the field study, and the larvae were able to pupate and become adults successfully in the absence of ants under laboratory condition. In the case of other allied species, *T. hainani* and *T. ion* are assigned as moderately myrmecophilous because ant-associations regularly occur at least with part of the larvae (Fiedler 1991). Moreover, it is reported that almost all older larvae of *T. fischeri* are nearly permanently attended by ants (Fukuda et al. 1984; Fiedler 1991). The relationship with ants in *T. fischeri* was also found clearly nonspecific and facultative, since three ant genera in two subfamilies were recorded (E. Jeratthitikul unpublished). Based on the myrmecophily of the closely related species and the presence of myrmecophilous organs in *T. kala*, they must have at least the larval stage of their life history associated with ants, and the association seems to be a facultative relationship because larvae do not necessarily require attendant ants for survival.

In the wing markings, all *Tongeia* species including *T. kala* share the blackish upperside in both sexes and no androconia in males (Corbet & Pendlebury 1978, 1992). As described by some researchers (e.g. Kawazoé & Wakabayashi 1976), however, there are many distinctive differences between *T. kala* and the other congeneric species. In particular, *T. kala* has the following unique characters on the wing underside: 1) ground color dark grey, 2) all black dots evenly larger and prominent, 3) submarginal orange lunules on hindwing extremely well developed, 4) black scales of cilia widely expanded. These conditions are also never found in species of allied genera (e.g. *Everes* and *Shijimia*), though submarginal orange lunules are somewhat expanded in some species of Everes. Accordingly, the four character states are regarded as apomorphic within the genus.

The male genitalia of *Tongeia* species have been illustrated in the following literature: Shirôzu (1960) for *T. hainani* and *T. filicaudis*, Kawazoé & Wakabayashi (1976) for *T. fischeri*, Corbet & Pendlebury (1978, 1992) for *T. potanini*, Huang (1998) for *T. ion*, *T. zuthus* (Leech, 1893) and *T. menpae* Huang, 1998, Huang (2001) for *T. bella* Huang, 2001, *T. amplifascia* Huang, 2001 and *T. pseudozuthus* Huang, 2001, Huang (2003) for *T. confusa* Huang, 2003, and Huang & Chen (2006) for *T. dongchuanensis* Huang & Chen, 2006. Although those of *T. kala* can be also found in Cantlie (1964), there has been no detailed work on the comparative morphology of the male genitalia. In this study, we re-examined the male genitalia of *T. kala* and other congeners. *Tongeia* species share the following genital characters: 1) uncus produced into claw-like process and tapered to posterior portion, 2) valva almost rectangular, 3) ampulla and harpe rather flattened, 4) ampulla produced ventrally and pointed at apex. Since these conditions are usually not present in species of allied genera such as *Everes* and *Shijimia*, some of them may be synapomorphies of *Tongeia* species. In addition, *T. kala* differs from other congeners in having the following features: 1) phallus straight (curved ventrally in other congeners), 2) ampulla and harpe of valva sharply pointed and crossed each other (harpe lobed with or without projection in other congeners). However, similar conditions are observed in species of *Everes* and *Shijimia* (Kawazoé & Wakabayashi 1976). Thus, the character states seem to be more plesiomorphic than apomorphic within *Tongeia*.

In the present paper, *Sedum multicaule* is recorded not only as a hostplant of *T. kala* but also for lepidopterans for the first time. Plants in the family Crassulaceae are known as a major hostplant for the genus *Tongeia* (Igarashi & Fukuda 2000; Fiedler 1991; Fukuda et al. 1984). Although one hostplant and one substitute hostplant of *T. kala* larvae are reported here, other related species use various hostplant species as follows. *Tongeia hainani* from Taiwan feeds on *Kalanchoe pinnata, K. daigremontiana, K. garmbiensis, K. spathulala, Sedum alfredii, S. formosanum* etc. of Crassulaceae as well as *Gynura formosana* (Compositae) and *Hoya carnosa* (Asclepiadaceae). *Tongeia filicaudis* from Taiwan feeds on *S. nokoense* and *S. sekiteiense* (Igarashi & Fukuda 2000), and *T. fischeri* from Japan on *Orostachys erubescens, O. iwarenge, O. aggreatus, S. sordidum, S. makinoi, S. tricarpum, S. lineare, S. oryzifolium, S. japonicum* and *Hylotelephium sieboldi* (Crassulaceae) (Fukuda et al. 1984). Moreover, *T. potanini* from Thailand was recorded feeding on *K. integra* (Ek-Amnuay 2006). The data mentioned above indicate the wide host range among *Tongeia* species, so that they usually use more than one hostplant species within one genus, several genera or even different families. Although *T. kala* is perhaps a stenoligophagous or oligophagous species as compared with other related species, the use of crassulaceous plants as common hostplants among *Tongeia* butterflies could be a synapomorphy supporting the monophyly of the genus.

Morphological and ecological characters of *Tongeia* species from previous reports and this study show that *T. kala* should be surely assigned to the genus *Tongeia*, and that it seems to share many characteristics inherited from the common ancestor of the genus. Judging from

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the adult morphology, however, this species may form a unique subgroup within the genus. Other analyses such as using SEM as well as genetic evidence will allow us to discover other features not reported here or previously, that likely will support our hypothesis.

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