Rediscovery of Ludlow's Bhutan Glory, *Bhutanitis ludlowi* Gabriel (Lepidoptera: Papilionidae): morphology and biology

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Abstract

The papilionid butterfly, *Bhutanitis ludlowi* Gabriel, was rediscovered after a lapse of 76 years since it was first collected in Trashiyangtse Valley of Trashi Yangtse, eastern Bhutan. The immature stages of this butterfly are recorded and illustrated for the first time. The morphology and biology of adult, egg and larval stages are compared with those of the other *Bhutanitis* species, in particular *Bhutanitis lidderkalii* Atkinson. *Aristolochia griffithii* (Aristolochiaceae) is recorded as the larval hostplant of *B. ludlowi*. The habitat, conservation needs and biogeography of the butterfly are also briefly discussed.

Keywords

Conservation, early stages, endemic species, hostplant, larva, national butterfly, ovum, Trashiyangtse Valley.

Introduction

Ludlow's Bhutan Glory, *Bhutanitis ludlowi* Gabriel, 1942, was discovered by two English botanical collectors, Frank Ludlow (1885 - 1872) and George Sherriff (1898 - 1967), from "Trashiyangtsi Valley" (the north of the present "Trashiyangtse" along Kulong Chu) located in northeastern Bhutan (Stearn, 1976). They collected in August 1933 and 1934 three males and two females, which Alfred G. Gabriel (1942) subsequently described as a new species. The original description states that the type specimens were captured at alt. 7,500 - 8,000 ft. (2,250 - 2,400 m). The types are deposited in the Natural History Museum, London (BMNH). According to the data labels, the holotype male was collected on "Aug. 12, 1933" and the allotype female on "Aug. 1934" (Iwase, 1969). The collection locality was written as "Tobrang, Makang". Since its discovery many researchers and collectors have attempted to rediscover this mysterious swallowtail but without success. Although Chou (1994, 1999) illustrated one male of this species from Yunnan, this record has been questioned by subsequent researchers because this male specimen is identical to the holotype stored in BMNH.

In July 2009, one of the authors (Karma Wangdi) working at the Ugyen Wangchuck Institute for Conservation and Environment in Bumthang (central Bhutan) had the opportunity to see and talk with Italian researchers, Mr. Gian C. Bozano and his colleague. They provided him scant information known at the time about the mysterious *B. ludlowi*. After the Italian researchers left Bhutan, he continued to search for the butterfly and finally succeeded in capturing two females and obtained photographs of the living adults at Tobrang, Trashiyangtse Valley. This news was immediately transmitted to Europe and then to Japan. In autumn 2010, a Bhutanese delegation led by the Honorable Lyonpo Dr. Pema Gyamtsho (Minister of the Ministry of Agriculture and Forests [MoAF], Royal Government of Bhutan) participated in CBD - COP 10 (the Convention on Biological Diversity - the 10th meeting of the Conference of the Parties). On that occasion some members of the Butterfly Society of Japan (BSJ)
submitted a research proposal to study B. ludlowi. This request was supported by Dr. Takashi Tannowa (Chairman of the Research Institute of Evolutionary Biology, Tokyo) who is affiliated with the Royal Government of Bhutan. After six months negotiation, the government gave special permission to undertake investigations of the Bhutanese butterfly fauna by a joint research team between MoAF and BSJ.

Between Aug. 11-18 2011, the joint research team surveyed the butterflies in Tobrang and adjacent areas, where Karma Wangdi had observed adults of B. ludlowi in 2009. As a result of this survey, Ludlow’s Bhutan Glory was observed and five specimens (three males and two females) were collected within the permitted limit. Various aspects of its morphology and biology, such as larval hostplant, mating, oviposition behavior and the immature stages, were also recorded. However, a detailed comparative study of adult morphology was not possible then because specimens were not allowed to be exported out of Bhutan.

In November 2011, His Majesty the King of Bhutan, Jigme Khesar Namgyel Wangchuck, and Her Majesty the Queen, Jetsun Pema Wangchuck, were invited to Japan as state guests. On that great occasion two specimens of B. ludlowi mounted in boxes with specially curved wood decorations were gifted to institutes to which three Japanese members of the joint research team belong, as part of ongoing friendly relations between Bhutan and Japan. One specimen was deposited in The University Museum, The University of Tokyo, and the other in the Research Institute of Evolutionary Biology. These invaluable specimens enabled the authors to undertake a comparative study of the adult morphology of all four Bhutanitis species.

In this paper, we describe morphological and biological features of B. ludlowi based on observations of the adult and immature stages. We show the affinities to and differences from the other three Bhutanitis species, especially the closely related B. lidderdalii. We also briefly discuss its habitat, conservation needs and biogeography.

Materials & Methods
1. Abbreviations for institutes

2. Members of joint research team
The first joint research team comprised a total of about 30 persons: Japanese research members from BSJ (Japanese team leader M. Harada, subleader M. Yago, Y. Igarashi, Y. Aoki, S. Yamaguchi and Y. Watanabe), NHK crew staff (reporter M. Saito, director T. Uchiyama and cameraman Y. Moriyama) and staff of the Ministry of Agriculture and Forests (Bhutanese team leader Sonam Wangdi, subleader Sherub, Karma Wangdi, Rinchen Wangdi and Sangay Drukpa), Bhutanese guides (Chencho Durkpa, Tshagay Dorji and Tandin Tshering), a few drivers, cooks and several porters with horses.

The second joint research team consisted of two Bhutanese research members (Karma Wangdi and Sangay Drukpa), three NHK crews (M. Saito, T. Uchiyama and Y. Moriyama), three guides and two drivers. Since then, several continuous researches were conducted by Karma Wangdi, Rinchen Wangdi and Sangay Drukpa.

3. Research schedule and sites
On Aug. 1, 2011, the Bhutanese and Japanese research teams joined forces at Thimpu, the capital of Bhutan. The first joint research team left for eastern Bhutan by cars on Aug. 4, and visited UWICE in Bumthang, central Bhutan, on Aug. 6 to have the preliminary discussion and to gather additional members. They arrived at Trashiyangtse (alt. 1,700 m) on Aug. 8, and then left for Tobrang on Aug. 10. The team reached Tobrang on Aug. 11 where a base camp was established, and then spent the next 8 days at Tobrang and its surroundings (2,200 - 2,500 m) (Figs 1-3,
33). Tobrang (27°44′27″N, 91°25′34″E) is located 20 km north-northwest of Trashiyangtse in Trashi Yangtse pref., Bhutan. Although the climate of this area is characterized by a tropical monsoon with comparatively distinct rainy (June - September) and dry (October - May) seasons, the rather high elevations are characterized by a consistently cool montane climate with low nighttime temperatures. The second joint research team started on Sep. 24 when three Japanese members arrived at Thimphu. This team conducted fieldwork at Tobrang from Sep. 28 to Oct. 2. After these two field expeditions, the Bhutanese members have continued to observe the immature stages of the butterfly in the field once or twice a month.

4. Observation, rearing and collection

Observations of B. ludlowi and its immature stages were recorded from August - October 2011. Eggs and larvae were reared at the field site in an outdoor cage (35 cm diameter, 60 cm height) made of a nylon net (Fig. 34). The cage was hung about 250 cm above the ground and the bottom was attached to a living hostplant, covering its apical part. Also, one cluster of eggs was reared by Sherub and Karma Wangdi in a plastic box (21 cm diameter, 8 cm height) at the UWICE.

The individuals were recorded using a digital camera Nikon D7000 with a micro lens (Tamron SP AF 90 mm F/2.8 Di Macro 1:1), and digital cameras Nikon D70 and D90 with lenses (Nikon AF - S Nikkor 28 - 300 mm, Nikon AF DX Fisheye-Nikkor 10.5 mm and Sigma DC Macro 17 - 70 mm) and a combined electronic flash (Sigma EF-500 DC Super).

Some butterflies were collected at the field sites, using standard construction nets (50 cm diameter) with sliding rods (maximum lengths 150, 560, 600 and 700 cm). Specimens were immobilized by manually squeezing the thorax. Depositories for the specimens collected are mentioned in the results.

Results

1. Adults (Figs 4, 5, 8, 9, 12-16)

Male. Differs from the most closely allied species, B. lidderdalii, as follows. Almost the same size as or slightly larger. Forewing length 58.2 - 60.3 mm (n = 3). Wing shape broader, more rounded, and less strongly toothed in hindwing. Hindwing tails wider, particularly the longest tail at tip of vein 4 tongue-shaped. Yellowish lines on fore- and hindwings wider and brighter than those of B. lidderdalii from Bhutan. Postdiscal line of forewing almost straight in cell 1a-3. Postdiscal line of hindwing prominent in cell 5-6 and shifted inwardly in cell 5. Submarginal lunules on hindwing greyish-yellow. Female. Similar to male, but differs as follows. Slightly larger, with more rounded wings. Forewing length 61.0 - 63.0 mm (n = 2). Upperside and undersides a little paler. Yellow hairs on distal portion of abdomen remarkably less. Sphragis (Figs 8, 9) pale yellow, covering flatly lamella antevaginalis and lamella postvaginalis at base, and produced ventrally into needle-like process extending from ostium bursae. Needle-like process about 2 mm (n = 2) in length.

Specimens examined. 13. viii. 2011 (Figs 4, 5; UMUT), 1♂, 1♂, 1♀. 13. viii. 2011 (UWICE), 1♂, 1♀. 16. viii. 2011 (RIEB), 1♂, 1♀, 1♀, 1♀. 13. viii. 2011 (DoFPS), 1♂, 1♀, 1♀. 16. viii. 2011 (DoFPS), Tobrang, Trashiyangtse, Trashi Yangtse pref., Bhutan.

Adults of this species are more active on fine sunny days, but they also fly during cloudy weather when the temperatures are high. Their flight is usually from 8 a.m. to 4 p.m. on fine weather. Both males and females fly slowly above the canopy, among high trees or occasionally on paths. The butterflies are often attracted to clusters of whitish flowers such as Viburnum crinatum (Adoxaceae) (Fig. 14). The peak male emergence period had already passed on our arrival on Aug. 12. Mating behavior and copulation were observed on several occasions on Aug. 14 - 18 (Fig. 15). The male first straddled the female on the wing, and they fall together from the canopy to the ground. Once on the ground the male then attempted to copulate with the female. Only one female was observed laying eggs, which were deposited on the underside of a leaf of the hostplant 2.2 m above the ground. About 30 seconds were spent laying each egg (Fig. 16).

2. Eggs (Figs 17, 18)

Spherical and yellow with red tint. 1.14 - 1.20 mm in diameter and 1.00 mm in height (n = 5). The eggs were laid on the underside of leaves of the hostplant in a cluster comprising a mound. Four clusters of eggs were found at about 2 m above the ground. Each cluster was composed of about 65 - 180 eggs. The egg stage lasted for about 14 days in captivity at UWICE, Bumthang (alt. 2,900 m).

3. First and 2nd instar larvae (Figs 19-21)

Head glossy blackish brown with white setae. Osmeterium short and yellowish orange. Thorax and abdomen cylindrical, whitish-grey, and with low wart-like, yellow processes and whitish setae. Subdorsal and lateral lines represented by two rows of wart-like yellow processes.
Prothoracic shield glossy blackish-brown. Thoracic legs glossy dark brown, and prolegs and anal legs whitish-grey. Anal shield glossy blackish-brown. First instar about 6 mm; 2nd instar about 7 mm in length. The larvae were gregarious and processionary in habit.

4. Parasitoids (Figs 25-27)
In one particular cluster of eggs, about 84% (150 of 178 eggs) were parasitized by Telenomus sp. (Scelionidae, Hymenoptera). The wasps were also observed laying their eggs into the eggs of B. ludlowi while the female butterfly was ovipositing.

5. Hostplant (Figs 28-32)
Aristolochia griffithii (Aristolochiaceae). This plant is distributed in Nepal, Bhutan, N. India, Myanmar and China (Tibet, Xi’an and Yunnan) (Fletcher, 1975; Matsuka, 2001). In Bhutan, it generally occurs in montane regions (alt. 1,800 - 3,000 m) clothed with cool broad-leaved forest (evergreen oak and mixed fir forest). The hostplant is an evergreen woody climber that does not shed its leaves during winter. Leaves are ovate, acute or shortly acuminate (6-15 cm length, 5-9 cm width). The base is strongly auriculate or cordate. Flowers are pale purple. Fruits are oblong (12-18 cm length, 1.5-2.5 cm width) and six-ribbed, and dehiscence into six longitudinal valves (Fletcher, 1975; Grierson & Long, 1984).

6. Habitats (Figs 2, 3, 33)
The mid-slope, summit and paths (2,237 - 2,475 m) of the mountain ridge along Trashiyangtse Valley, where B. ludlowi and its hostplant were observed, is covered primarily by secondary forest. Evergreen and deciduous broad-leaved trees are dominant at this area, and include members of the Fagaceae (Quercus griffithii), Betulaceae (Alnus nepalensis), Ericaceae (Lyonia sp. and Rhododendron sp.), Lauraceae (Machilus sp.), Adoxaceae (Viburnum cylindricum), Rosaceae (Spiraea sp.) and Diervillaceae (Weigela sp.).

Discussion
In the surveys conducted at Tobrang and its surroundings we found the adults of B. ludlowi and discovered the eggs and early instar larvae. The mating behavior, copulation, oviposition, hostplant and immature stages are also described and illustrated for the first time. Based on this material, we compare the morphology and biology of the adult, egg and larval stages with the three other Bhutanitis species, particularly the most closely allied B. lidderdalii. Although B. ludlowi is sometimes regarded as a subspecies of B. lidderdalii, the following morphological and biological evidences suggest that B. ludlowi should be treated as a distinct species according to the biological species concept.

1. Adults
In the original description of B. ludlowi, Gabriel (1942) noted that B. ludlowi is distinguished from B. lidderdalii by the following three features (Figs 4-7): 1) wings much broader, 2) hindwing less strongly toothed, and 3) submarginal lunules on hindwing upperside greyish. Four additional differences which we observed are as follows (Figs 4-11): 4) hindwing tails broader, 5) postdiscal line of forewing almost straight in cell la-3, 6) postdiscal line of hindwing prominent in cell 5-6 and shifted inwardly in cell 5, and 7) pale yellow sphenagus covering lamella antevagnalis and lamella postvagnalis. In particular, the last feature is unique to B. ludlowi and not observed in the other Bhutanitis species (Figs 8-11).

The behavior of B. ludlowi is quite similar to that of B. lidderdalii (Igarashi, 1989), but B. lidderdalii is more active on cloudy days than on fine days and flies without difficulty even in rainy weather, whereas B. ludlowi is more active on fine days.

2. Eggs
The egg of B. ludlowi is smaller in size than that of B. lidderdalii. The egg diameters are 1.14 - 1.20 mm in B. ludlowi, about 1.37 mm in B. lidderdalii, 1.4 mm in Bhutanitis mansfieldi (Riley, 1939) and 1.05 mm in Bhutanitis thaidina (Blanchard, 1871) (Lee, 1986a, 1986b; Igarashi, 1989).

The most noteworthy feature of B. ludlowi is its oviposition strategy. Although B. lidderdalii, B. mansfieldi and B. thaidina lay eggs in a flat cluster of 7 - 42 eggs, they are not deposited in a mound (Figs 22, 23; Lee, 1986a, 1986b; Igarashi, 1989), whereas B. ludlowi lays its eggs in a mound composed of about 65-180 eggs (Figs 17, 18). As far as we are aware, this type of egg-laying behavior has not been recorded in the Papilionidae (Vane-Wright & Ackery, 1984; Ackery et al., 1999). We hypothesize that mounds are a defense against egg parasitoid wasps. Some ovipositing parasitoid wasps (Telenomus sp.) were observed on all four clusters of eggs at the field site (Fig. 25), and one cluster had a considerably high proportion (about 84%) of parasitism. Almost all eggs distributed at the surface of the cluster were parasitized (Fig. 26), while these eggs that escaped parasitism were located inside the cluster.
3. First and 2nd instar larvae
In appearance, the 1st and 2nd instar larvae of *B. ludlowi* are very similar to those of *B. thaidina* in coloration, but differ from the latter in having whitish setae on the body (blackish setae in *B. thaidina*) (Figs 19-21; Lee, 1986b). Although the larvae of *B. ludlowi* resemble those of *B. lidderdalii*, they are easily distinguished from the latter as follows (Figs 19-21, 24): 1) thorax and abdomen dark purplish grey, and 2) lower wart-like processes reddish orange on mesothorax, 2nd, 3rd, 7th and 8th abdominal segments and greyish-brown on the other segments. In general, the larvae of *B. ludlowi* are gregarious, as are those of all other *Bhutanitis* species (Figs 19, 20, 24).

4. Habitat and conservation
*Bhutanitis ludlowi* probably originally occurred in scattered gaps in primary forests, but its larval hostplant has been sustained by traditional forestry management of the village people in this area (Figs 2, 3, 33). In other words, the butterfly has been conserved by the life style of Bhutanese people. Since Bhutan is one of the greatest countries founded on the principles of environmental protection, its rich ecosystems and biodiversity are preserved by a national policy. In the spring of 2012, *B. ludlowi* was designated as the National Butterfly of Bhutan by the Ministry of Agriculture and Forests with the opportunity of its rediscovery (MoAF, 2012), and it will be protected as a symbol of wildlife conservation in Bhutan. In the near future, poaching by "outsiders" is considered to be the most serious threat facing this species, so it is important that a conservation management plan and captive breeding programme be established as soon as possible.

5. Biogeography
According to Saigusa & Lee (1982), *B. lidderdalii* and *B. ludlowi* are allopatric sister species based on a synapomorphy of the much expanded postdisca of the hindwing containing the enlarged red marking (Figs 4-7). If that is the case then the following selective forces may have contributed to their evolution. At the field site in eastern Bhutan, hot wet air blown north from the Indian Ocean first hits the Khali Hills located to the south of the Himalayas. It dumps considerable amounts of rain on the area, so that hot dry air blows farther north. This wind then meets the cold humid air coming from the higher Himalayas, creating a distinct climate unlike other areas in the southern Himalayas. Furthermore, this area fluctuates quickly from hot-dry to cool-wet climates in a day. Indeed, tropical butterflies, *Junonia orithya* and *Troides aeacus*, coexist with subalpine *Aulocera saraswati* and *Aglais caschmiensis* in this area. Thus, we infer that the ancestral *B. ludlowi* separated from the common ancestor of *B. lidderdalii + B. ludlowi*, perhaps due to a geographical barrier, and then evolved into the extant *B. ludlowi* through adaptation to these unique climatic conditions that presently prevail only in a limited area of Bhutan. This area seems to bear evolutionary and biogeographically interesting phenomena as observed in other species. We hope elucidate the systematic relationships and historical biogeography of this mysterious species with further studies using molecular analyses, as well as to analyze other interesting species in the area.

Acknowledgements
First of all, Japanese members of this research team express their sincere thanks to His Majesty the Druk Gyalpo Jigme Khesar Namgyel Wangchuck, the King of Bhutan, for the presentation of two specimens of *B. ludlowi*. They express their cordial thanks to the Honorable Minister of Agriculture and Forests, Royal Government of Bhutan, Pema Gyamtsho for giving the opportunity to study the butterfly fauna of Bhutan. Special thanks go to the Chairman of the Board of Directors of The Research Institute of Evolutionary Biology, T.Tannowa for access to the Royal Government of Bhutan. They are indebted to Ugyen Tshewang, Dechen Dorji, Karma Dukpa, Sonam Wangchuck, Phub Dorji, Tashi Yangtzone, Nawang Norbu, Pankey Dukpa, Singay Dorji, Vetsop Namgyel, Kinley Tenzin, Chencho Durpa, Tshagay Dorji, Tandin Tshering, Sangay, Sherub, G.C. Bozano, R.I. Vane-Wright, P.R. Ackery, B. Huertas, Y.Hirose, Y.Nishino, K. Yamagishi, T.Nishizaki, O. Yata, Y.Uemura, K. Ueda, S.Ueda and N.Hirai for permissions, agencies, guides, information or literature. In particular, this research was conducted by cooperation with the RGB, namely the NEC, NBC, and DoFPS, UWICE and BWS, MoAF, and by permission of the Department of Immigration, Ministry of Home and Culture Affairs (No. 4487/4493), Royal Bhutan Army (No.126/2011), Nature Conservation Division (No. 0903) and Wildlife Conservation Division (CITES certificate/permit No. WCD-39), DoFPS, MoAF, and Bhutan InfoComm and Media Authority (BICMA/MEDIA/FPF/11/179). We also would like to express our thanks to M.F. Braby, H.Takasaki and
M. Kondo for their cirtilical readings of the manuscript. This study was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 23570111 to MY) from the Japan Society for the Promotion of Science.

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(Submitted April 20, 2012)