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43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641035, India
Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: Whale Shark *Rhincodon typus* and Reef - made with poster colours. © P. Kritika.

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COMMUNICATION

Biology of *Bhutanitis ludlowi* Gabriel, 1942 (Lepidoptera: Papilionidae) Bumdeling Wildlife Sanctuary, Bhutan

Tshering Dendup [1], Namgay Shacha [2], Karma Tempa [3] & Tez Bdr Ghalley [4]

[1] Pemagatshel Divisional Forest Office, Department of Forests and Park Services, Ministry of Energy and Natural Resources, Royal Government of Bhutan.

[2,4] Bumdeling Wildlife Sanctuary, Trashiyangtse, Department of Forests and Park Services, Ministry of Energy and Natural Resources, Royal Government of Bhutan.

[3] Mongar Territorial Division, Mongar, Department of Forests and Park Services, Ministry of Energy and Natural Resources, Royal Government of Bhutan.

[1] tsheringdendup@moaf.gov.bt, [2] nshacha@moaf.gov.bt (corresponding author), [3] ktempa@moaf.gov.bt, [4] tbghalley@moaf.gov.bt

Abstract: It was in 1933–34 that the first discovery of *Bhutanitis ludlowi* was made by plant explorer Frank Ludlow and George Sheriff at Tobrang, upper part of Trashiyangtse valley, Bhutan. It was rediscovered after a lapse of 76 years in 2009 by Bhutanese forester Karma Wangdi. *Bhutanitis ludlowi* is native to Trashiyangtse and occurs in few pockets of areas in northeastern part of Bumdeling Wildlife Sanctuary (BWS). There has been no report which described complete life cycle of *Bhutanitis ludlowi* till date. Listed in CITES appendix II, the endemic species is also the first butterfly species to be included under Schedule I of FNCRR of Bhutan, making it a totally protected species in Bhutan. For the first time a complete life cycle stages of *Bhutanitis ludlowi* was recorded at BWS on the host plant *Aristolochia griffithii* Hook.f. & Thomson ex Duch in ex situ environment. A greenhouse with dimensions of 12 X 5 X 3 m was constructed and a thriving habitat was created artificially through establishment of *Aristolochia griffithii* nursery inside the greenhouse. The eggs were brought down to the new environment at an elevation of 1,752 m from their natural habitat (elevation of 2,200 m). The eggs were incubated in the greenhouse with host plants in ex-situ conditions. Eggs incubated inside the greenhouse were examined at eight hour intervals daily (twice a day: 0900 h and 1700 h) to record physical changes in eggs such as colours. *Bhutanitis ludlowi* is univoltine brood where the life cycle completes in 365–367 days with egg hatching (Average = 16 days; SD = 2), larva (Avg. of 174 days SD 3), pupa (174 days, SD 1.53)) and adults emerged after 366 days. Predators of *Bhutanitis ludlowi* include spider, wasps, praying mantis, snails, and ants. *Bhutanitis ludlowi* protects themselves by avoiding predators through mechanisms like odour, camouflaged colorations, and excretion of poisons. Advocacy programs on its conservation importance and formation of community conservation support groups to protect its habitats is seen as one of the priority activities in the identified range of *Bhutanitis ludlowi.*

Keywords: Chrysalis, eggs, host plants, instars, life history, metamorphosis, molts.

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Author details: Mr. Tshering Dendup, Deputy Chief Forestry Officer currently working under Pemgatshel Divisional Forest Office, Department of Forests and Park Services (DoFPS), Bhutan. Mr. Namgay Shacha, Forestry Officer working under Bumdeling Wildlife Sanctuary, DoFPS, Bhutan. Karma Tempa, Chief Forestry Officer currently working under Mongar Territorial Division, DoFPS, Bhutan. Tez Bdr Ghalley, Forestry Officer working under Bumdeling Wildlife Sanctuary, DoFPS,Bhutan

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INTRODUCTION

Discovery of *Bhutanitis ludlowi* (Lepidoptera: Papilionidae) was first made in 1933–34 by Frank Ludlow (1885–1972) and George Sheriff (1898–1967) during their botanical expedition at Tobrang Makang, upper part of Trashiyangtse valley. *Bhutanitis ludlowi* was sporadically distributed between altitude ranges of 2,200–2,500 m (Harada et al. 2012). *Bhutanitis ludlowi* was named after the foreign botanist Frank Ludlow and it was only after eight years of discovery that it was described as new species in 1942 (Gabriel 1942). Except species description (Gabriel 1942), there has been no reports which described complete life cycle of *Bhutanitis ludlowi.* The only reference to life cycle of the concern species was made by a joint research team from Ministry of Agriculture and Forests (MoAF), Bhutan and a group of Japanese lepidopterists (Harada et al. 2012). In their paper they compared *Bhutanitis ludlowi* morphological characteristics with *Bhutanitis lidderdalii* Atkinson (Bhutan Glory) till second instar (Harada et al. 2012).

Bhutanitis ludlowi was accorded increased protection status in the provisions of Forest and Nature Conservation Act 1995 and Forest and Nature Conservation Rules and Regulation (FNCRR) 2017. Listed in CITES appendix II, it was also the first butterfly species to be included under Schedule I of FNCRR making it a totally protected species (Singh & Chib 2015). Endorsed during 123rd sitting of Cabinet, *Bhutanitis ludlowi* was officially declared as Bhutan's National Butterfly in 2012 (Singh & Chib 2015). The paper was written after nine years of rediscovery of the concern species, adding invaluable memorandum of complete life history report to other existing three congeneric species: *Bhutanitis lidderdalii* Atkinson 1873, *Bhutanitis thaidina* Blanchard 1871, and *Bhutanitis mansfieldi* Riley 1939. The present study, which is the first of such kind in Bhutan & the world, demystifies and elucidates the complete life cycle of *Bhutanitis ludlowi* on its only novel larval host plant *Aristolochia griffithii.*

MATERIALS AND METHODS

Study site

The study was carried out at Bumdeling Wildlife

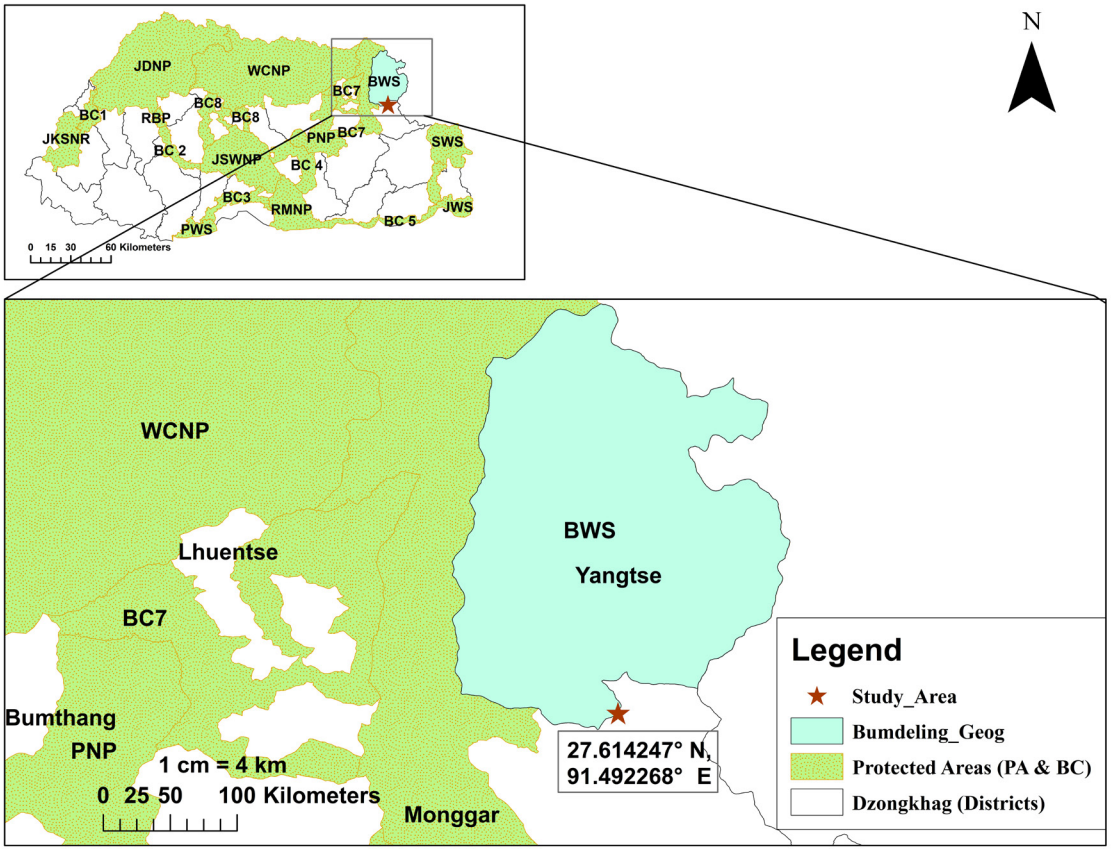

Figure 1. Study area of Bumdeling Wildlife Sanctuary, Yangtse.

Sanctuary (BWS) during 2017. The study site is located at an elevation of 1,752 m (27.614247° N 91.492268° E) in Trashiyangtse (Figure 1). The experimental site experiences a warm temperate climate with an average annual precipitation between 1,000–3,000 mm from June to September and the mean temperatures of 20°C in summer and 10°C in winter. Lighting condition was normal indirect sunlight through high density polyethylene (HDPE) green-agro shade net with mesh size 10 mm. An average hours of sun light received by greenhouse varied from short duration of about seven hours during winter months (November–March: 0800–1500 h) to long duration of about nine hours during summer months (April–October: 0800–1700 h).

Methods

Before eggs were introduced into an ex-situ environment, a green-agro shade net house with dimensions of 12 X 5 X 3 m was constructed (Image 1A,B). A single 2 X 2 m door was opened from one end of the green-agro shade house. Thriving habitat was created artificially through establishment of *Aristolochia griffithii* nursery inside the agro shade house (Image 2AC). The main purpose of agro net is to provide shade to growing host plants and to maintain thriving habitat for *Bhutanitis ludlowi*.

The first cluster of freshly laid *Bhutanitis ludlowi* eggs were collected in mid-August and introduced into ex situ environment of green-agro shade house at the study site with established host plants. Eggs were allowed to hatch in greenhouse in ex situ conditions. Rearing eggs were examined at every eight-hour intervals daily (twice a day: 0900 h and 1700 h) to record eggs physical changes such as colours. The eggs were enumerated as hatched when the larvae came out of them. Fresh young leaves of *Aristolochia griffithii* (Image 2C) were used as food by growing larvae. Molting stages were noted, thus the number of instars and their metamorphosis.

RESULT

Butterflies go through a life cycle known as complete metamorphosis. The stages of their life cycle include egg, larva, pupa, and adult. Female butterflies were very selective about their host plant and laying their eggs. Female *Bhutanitis ludlowi* laid its eggs in clusters on underside of mature host plant leaves in pyramidal

Table 1. Observation on life cycle stages of *Bhutanitis ludlowi*.

	Stages	Observation
1	Egg: The Beginning Stage	Golden yellow colour of the eggs turned darkish pale after 14–15 days and apical region of the eggs got darkened considerably before hatching (Image 3C). Freshly laid egg hatched after 14–18 days (Image 3CD) in an ex situ environment inside the greenhouse. Soon after emergence from the egg, larva consumed its eggshell before it spread to feed on young tender leaves. A complete life cycle of *Bhutanitis ludlowi* passed through eight larval instar stages including a pupal stage to emerge as an adult butterfly over an incredible period of 367 days.
2	Larval: The Feeding Stage	A young caterpillar that first hatches from its egg is referred to as instar caterpillar (Image 3CE) and the caterpillar's first molt is referred to as second instar. First cluster of eggs were hatched in early September and the young caterpillar or larva that emerged from eggs began their first feast by eating their eggshell along with tender parts of host plant. During the feeding stage all individuals generally fed on individual vine at the same time of the day. Larvae of all instars were generally inactive in nights. Instars caterpillars continued to eat and grow until it became too big for the exoskeleton to support. The caterpillar molts transform into next instar until they pupated to adults which is a dramatic change in ground colour occurred with the molt (sixth, seventh and eighth (final) instars). The dark yellow ground color of the body became ashy grey and processes into darker shades thus appearing more pronounced (Image 4A). The wart-like reddish orange processes on sub-dorsal part became more prominent making body segments more distinct and clearer. During these stages, instars intervals became longer with fewer activities. It could be in response to unfavorable and inhibiting conditions of cold winter months of November, December, January, and February. The remarkable differences in appearance of the instar stages were increased body size and length and more prominent setae on the body (Image 4A). The pattern of tubercle arrangement of the body remains same throughout the rest of larval life, however, change in coloration pattern of chalazae becomes more conspicuous. Ludlow's Bhutan Glory *Bhutanitis ludlowi* butterfly underwent eight larval instars to emerge as an adult.
3	Molting: The exuviating stage	Caterpillar stopped eating and stayed still in one place in group (during early instars) or single (later instars) for around seven–eight days as it prepared to molt. When molted, old head capsule slid forward and dropped off. Old exoskeleton then split just behind the head allowing the caterpillar to walk forward out of its former skin. The larva took one–two hours to inflate its body by drawing in surrounding air to sufficiently toughen to continue normal feeding without injuring itself. At this point, larva often ate its exuviae of ecdysis. *Bhutanitis ludlowi* caterpillar underwent seventh molt to pupate. During this stage, feeding potentiality was found to be very high in contrast to previous stages. At maturation, larva stopped feeding and vigorously moved in search of pupation site.
4	Adult (Imago): The reproductive stage	It is the fourth stage of a butterfly. An adult butterfly (Image 4D) is ready to emerge from a chrysalis after 188 days (six months and seven days). The adult emerged in morning hours after the sun had come up at around 10–12 pm. As the adult butterfly emerged from its chrysalis, its wings were weak, folded against its body and wet. It was not able to take flight instantly. Adult butterfly emerged from pupa in third week of August after 365–367 days from the eggs. The adult butterfly pumped fluids from its abdomen through the veins in its wings, which causes wings to expand to their full size. It dries its wings in the sun by keeping it flat (Image 4F), occasionally flaps to exercise flight muscles before it could fly. It took several hours depending on the weather before it is ready to flutter away. In this reproductive stage, adult butterfly mates to reproduce, searches for the proper host plant to lay her eggs and the cycle continues. Duration of life cycle ranged between 365–367 days (egg-18 days, larva- five months & eight days), pupa- six months & seven days).

Image 1. A—*Aristolochia griffithii* nursery inside greenhouse | B— *Aristolochia griffithii*, the host plant in greenhouse. © Tshering Dendup

Image 2. *Aristolochia griffithii*, novel host plant of *Bhutanitis ludlowi* in greenhouse: A—Flower | B—Fruit | C—Leaves. © Tshering Dendup

mound, covered with very thin and hardly seen shiny sticky substance, which attached them to the leaf surface (Image 3A). Each cluster composed of 60–160 number of eggs. The butterfly eggs underwent colour change (Image 3B) as young larvae developed inside them and the whole observation are recorded (Table 1).

DISCUSSION

Aristolochia species is used by most of the Swallowtail butterflies as host plants to lay their eggs to complete life cycle. *Bhutanitis ludlowi* Atkinson laid its eggs on *Aristolochia griffithii.* Similarly, Young (1973) found that food plants acceptance study of swallowtail butterfly *Paridesarcas mylotes* (Papilionidae) on *Aristolochia constricta* Grisebach and *Aristolochia labiata* was readily accepted. Concomitant to the number of eggs per cluster (65–180) of Harada et al. (2012), eggs of the *Bhutanitis ludlowi* were also laid in clusters or batch of 60–160 individuals in pyramidal dome mound, generally in dorsal side of the leaves of *Aristolochia griffithii*. Young (1973) wrote that eggs of *Paridesarcas mylotes* were found on ventral side (occasionally on the crotches of small stems and petioles) of the mature leaves of *Aristolochia constricta* as opposed to *Bhutanitis ludlowi* and *B. lidderdalii* that laid eggs on dorsal side of leaves.

As reported by Igarashi (1989), its closest kin *Bhutanitis lidderdalii* laid 20–40 eggs at a time in flat clusters (not mound) on *Aristolochia griffithii* (Bhutan), *Aristolochia kaempferi* Willdenow (Japan), *Aristolochia mandshuriensis* (Korea), and *Aristolochia shimadai* Hayata (Taiwan). *Bhutanitis Mansfield* Riley and *Bhutanitis thaidina* Blanchard were also found laying eggs in flat cluster of 7–42 eggs (Igarashi 1989; Harada et al. 2012). *Paridesarcas mylotes* lay single but usually in loose clusters of 2–5 eggs on a single mature leaf of *Aristolochia constricta* (Young 1973). The ovi-positioning habit of *Bhutanitis ludlowi* conformed to those of *Bhutanitis lidderdalii*, preferring underside of mature *Aristolochia griffithii* leaves (Suzuki 1987; Igarashi 1989). Ovi-positioning took place between August–September for *Bhutanitis ludlowi* and they do not prefer highly exposed and disturbed areas whereas, *Bhutanitis lidderdalii* took place between late September and early October (Igarashi 1989).

Larval Stage of *Bhutanitis ludlowi*

The eggs of *Parnassius* overwintered and spent approximately 10 months to hatch (Igarashi 1989). A Monarch was an egg for 3–8 days (Howard 2006) *Bhutanitis lidderdalii* took about 30 days in egg stage (Igarashi 1989) and hatched out between mid-October and mid-November while *Bhutanitis ludlowi* took 14–18 days to enter the first instar between first-second weeks of September. The larvae proved to be very hardy and passed through winter months of November, December, and January before they pupated in mid-February. Larvae were gregarious throughout their life. Similar to *Bhutanitis liddarlii* larvae (Igarashi 1989), *Bhutanitis ludlowi* larvae had tendency to disperse into smaller groups as they grow older to the extent that the last mature instar larva lived in solitary. As opposed to the feeding habit of *Bhutanitis liddarlii* that fed on leaves, mature larvae of *Bhutanitis ludlowi* fed on leafstalks and tender shoots agreeing to feeding habit of *Pachliopta* (Swallowtail butterflies) and *Atrophaneura* (Red-bodied Swallowtails) of Papilionidae family. *Bhutanitis ludlowi* underwent eight larval instars as opposed to other swallowtails like *Bhutanitis lidderdalii* (Igarashi 1989) and *Paridesarcas mylotes* (Young 1973) of five instars. It remained in caterpillar stage for 161 days while monarch butterfly caterpillar stage was for only 7–17 days (Howard 2006).

Pupa of *Bhutanitis ludlowi*

Bhutanitis ludlowi pre-pupated into "C" shape for seven–nine days and monarch butterfly 8–15 days (Howard 2006). Pupation took normal girdle position like most of the swallowtails. Unlike *Bhutanitis thaidina* that pupated among the leaves (Igarashi 1989); Bhutanitis ludlowi restlessly roamed around in search of healthy substrata. They pupated in mid-February on strong stems of the host plant and iron rod pillars of greenhouse. Young (1973) reported that Paridesarcas mylotes larva stopped feeding at maturation and moved vigorously in search of pupation sites. The monarch butterfly stayed in pupal stage for 8–15 days (Howard 2006) while *Bhutanitis ludlowi* remained in pupal stage for 188 days, one of the longest of such case for swallowtail butterflies.

Adult of *Bhutanitis ludlowi*

The basic life cycle of a butterfly from egg to adult varied from three weeks to two years (Opler & Krizek 1984). Young (1973) reported a developmental time for *Paridesarcas mylotes* on *Aristolochia species* of about 42 days under similar ex-situ condition. Developmental time of *Bhutanitis ludlowi* on *Aristolochia griffithii* in ex situ greenhouse was 365–367 days which is incredibly lengthier. This long developmental time could be attributed to the aestivating period during

Image 3. Development stages of eggs of *Bhutanitis ludlowi*: A—Freshly laid eggs | B—Maturing eggs | C—Apical region of the eggs get darkened as eggs mature | D—Hatching eggs | E—Newly emerged larvae. © Tshering Dendup

Image 4. A—Mature larva | B—Pre-pupating "C" shape larva | C—Pupating | D—Mature pupa | E &F—Adult *Bhutanitis ludlowi* butterfly. © Tshering Dendup

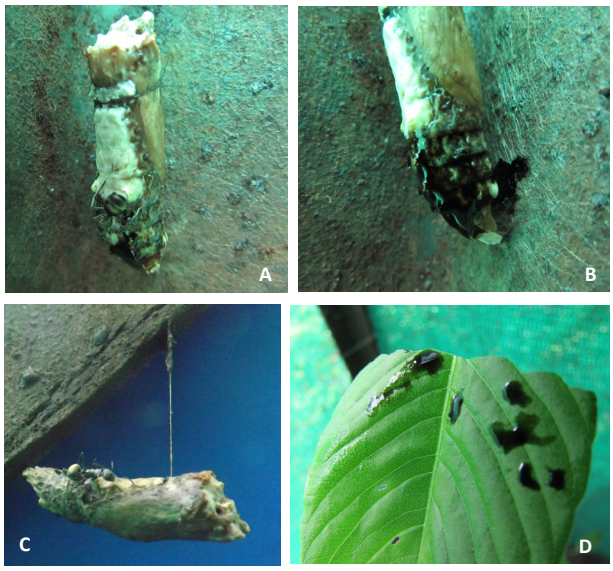

Image 5. Predators: A—Pupa being attacked by an ant | B—Pupa bleeds black fluid due to injury by ants | C—Pupa being attacked by an ant | D—Black fluid oozing out from injured pupa by ants. © Tshering Dendup

over-wintering of chrysalis in diapauses. Straatman (1970) found out that egg-adult time of *Ornithopteraa lexandrae* Rothschild to be 131 days on *Aristolochia schlechteri* Lauterb and 107 days on *Aristolochia tagala* Cham.

Enemies of *Bhutanitis ludlowi* and its conservation measures

Predators varied in each life stages of Bhutanitis ludlowi. Owing to the high mortality rate, very huge majority of them were not able to make it to become a butterfly out of few hundred eggs. There were many predators in all stages of lifecycle, but little information is available about the extent to which each of those predators influenced the population. Invertebrate predators such as snails (terrestrial gastropod mollusks), ants (Formicidae), spiders (Arachnids), wasps (Vespidae), and praying mantis (Mantidae) were among those that was found to prey on immature Bhutanitis ludlowi (eggs, larvae, pupae) on Aristolochia plants (Image 5A–D, 6A–E). This may be because of porous agro net used for captive breeding. Similar results were found by Zhang et al. (2019) while studying lifecycle of Bhutanitis lidderdalii in both natural and captive habitat, where the larva and egg were attacked by parasites and insects (Zhang et al. 2019). Bhutanitis ludlowi has mechanism to protect themselves and avoid from predations such as odour, camouflaged colorations and poisons which might have gain after feeding on host plant. A study carried out in

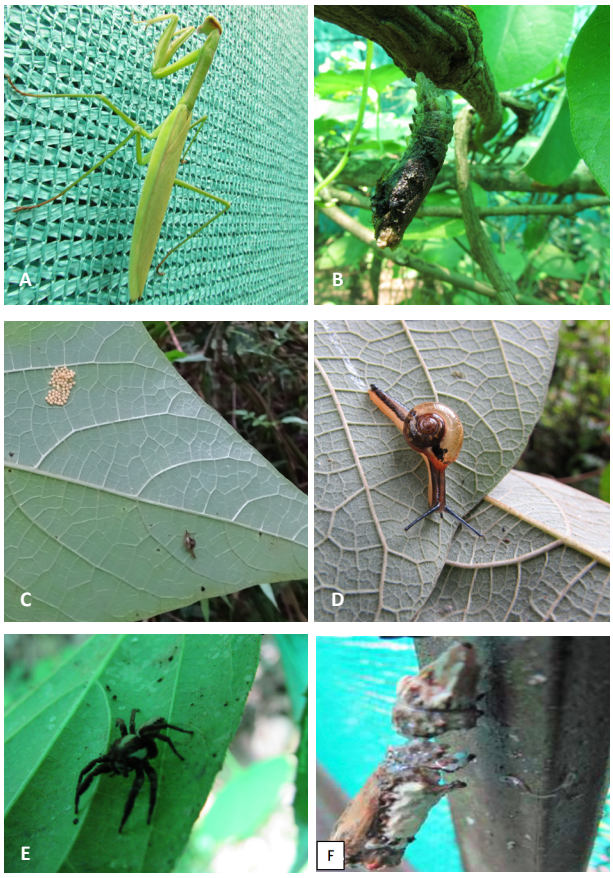

Image 6. Predators: A—Praying mantis | B—Larva attacked by Mantis | C&D—Snail | E—Spider | F—Pupa devoured by unidentified leech like insect. © Tshering Dendup

Chile (2020) on larva and egg of pipevine Swallowtial found that larvae feed exclusively on highly toxic plants of the genus Aristolochia from which they sequester toxins and later used as defense mechanism to protect from predators (Palma-Onetto et al. 2020).

CONCLUSION

Ever since the discovery and rediscovery of *Bhutanitis ludlowi* by plant explorer Frank Ludlow and George Sheriff and Bhutanese researchers respectively, such comprehensive study on its life cycle was not carried out. For the first time, a complete life cycle stages of *Bhutanitis ludlowi* was recorded at Bumdeling Wildlife Sanctuary on the host plant *Aristolochia griffithii* in ex-situ environment. *Bhutanitis ludlowi* is univoltine brood where the life cycle completes in 365–367 days with egg hatching (14–18 days), larva (five months and eight days), pupa (six months and seven days), and adults emerged after eight instars. Predators of *Bhutanitis*

ludlowi include spider, wasps, praying mantis, snails, and ants.

There is need to carry out a comprehensive study of its life history in in-situ habitats. Though *Aristolochia griffithii* is not palatable to cattle disturbances due to presence of 'Tsethar' (lifelong freely released) bulls in *Bhutanitis ludlowi s*core habitats greatly contributed to reduction of natural regeneration of host plant through foot trampling of young shoots. In future it is important to carry out enrichment plantation of host plants in its prime habitats. The current study recommends in-depth studies like its distribution patterns, behaviors, population trends, and conservation threats in future. Advocacy programs on its conservation importance and formation of community conservation support groups to protect its habitats is seen as one of the priority activities in the identified range of *Bhutanitis ludlowi.*

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