Scientific Note: A new aquatic host plant of *Junonia atlites atlites* (Lepidoptera: Nymphalidae) from India

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Abstract: We recorded *Limnophila heterophylla* (Plantaginaceae) as a new host of the butterfly *Junonia atlites atlites* (Nymphalidae) in India, and we describe our observations of the unique feeding behavior of a *J. atlites atlites* caterpillar on this aquatic plant.

Key words: Aquatic, heterophylly, host plant, iridoid glycoside, Nymphalidae, spiracle

Butterflies exhibit stenophagy, and most species of butterfly thus show host specificity to one or a few families of plants (Robinson *et al.*, 2010). Despite this specificity, butterflies are also involved in continuous co-evolution with their typical host plants, and as a result they may occasionally use novel host plants, likely in response to the kairomonal cues (Berenbaum & Feeny, 2008). Exploiting new or unusual host plants may benefit phytophagous species in terms of opportunities for niche expansion, habitat generalisation and reduction of intra- and inter-specific competition for food sources (McClure & Elias, 2016). Using new host plants also provides scope for adaptive speciation and diversification of insect herbivores, including butterflies (Ehrlich & Raven, 1964). Understanding the range of host plants exploited by herbivorous insects is therefore relevant to broader studies of insect ecology and evolution.

Junonia atlites atlites, (Linnaeus, 1763) [Lepidoptera: Nymphalidae], Oriental Grey Pansy, is a tropical butterfly from India whose larval host plants are generally in the family Acanthaceae, including *Hygrophila auriculata* (Wynter-Blyth, 1957; Kunte, 2000; Robinson *et al.,* 2010; Karmakar *et al.*, 2018), *Hygrophila costata* (Robinson *et al.*, 2010), and *Barleria* sp. (Wynter-Blyth, 1957; Kunte, 2000; Robinson *et al.*, 2010). Here we report a new host plant for *Junonia atlites atlites* from India. Interestingly, this plant is an aquatic angiosperm belonging to a different family to known host plants. Thus, this note also highlights the usage of aquatic vegetation by a butterfly as a larval food source, which is evidently a rare behavioral event for these terrestrial insects.

During a butterfly survey on 23 November 2014 at Rajabhatkhawa, West Bengal, India, we encountered a 3rd instar caterpillar of *Junonia atlites atlites* feeding on the submerged leaves of the aquatic annual herb *Limnophila heterophylla* (Roxb.) Benth (Plantaginaceae) within a shallow water body (Fig. 1a; accompanying video with DOI: 10.5281/zenodo.5519414). This is a freshwater plant which is generally seen in shallow water. The leaves of this plant show distinctive environmental heterophylly: the submerged leaves are highly branched into filamentous projections, each of which is approximately 4 cm long (Fig. 2), whereas the aerial leaves are elliptical with serrated edges, verticillate, and on average 20 mm long.

Intriguingly, during locomotion or feeding, the caterpillar was observed using the network of the submerged leaves as the substratum for its attachment (Fig. 1b). This apparently enabled the caterpillar to keep the maximum part of its body out of water, which possibly led to uninterrupted respiration through the spiracles (Fig. 3). On encountering this new host plant, we documented by video the feeding behaviour of the caterpillar with a Canon EOS 7D camera (DOI: 10.5281/zenodo.5519414), and also recorded the elevation and latitude and longitude of the location with a Garmin etrex 10 GPS (76 m, 26.60951º N, 89.53099º E). We did not find any known host plant of *Junonia atlites* in the vicinity of the *L. heterophylla* plant, suggesting that the caterpillar may have fed only on this host plant from the beginning of its larval stage. If so, then it would obviously be of great interest to observe how the early instars survived in that aquatic habitat and what portion of the plant they fed on (submersed or emersed leaves). Clearly, we also lack information about where the egg was laid.

To monitor further development of the caterpillar on *L. heterophylla* we collected the caterpillar along with the larval host. We created a similar environment in the bottom half of a rearing box with the host plant leaves submerged in water and raised the caterpillar in it under room temperature and humidity (temp. c. 20ºC, RH c. 55%). As larvae of *Junonia atlites atlites* are typically completely terrestrial, we examined the pattern of usage on the submersed and emersed leaves. We observed that the caterpillar fed on submersed leaves and rested upon the emersed portion of the plant as well as under the lid of the rearing box. However, it underwent a normal larval development feeding only on *L. heterophylla* and successfully pupated on 31 November 2014, 8 days after its discovery. We could not calculate the total larval duration as the caterpillar when collected was in the 3rd instar. As usual, pupation took place distant from the host plant and thus away from the water, reducing the chances of physical damage caused by the water and thereby ensuring a safe, uninterrupted emergence of the adult (Fig. 4). The pupal duration was 7 days and the adult

Figure 1. a. Feeding of *Junonia atlites atlites* caterpillar on submerged leaves of *Limnophila heterophylla*; b. The caterpillar using the same leaves as the substrate of attachment for locomotion.

butterfly emerged on 7 December 2014. After the emergence we released the butterfly into the wild.

Plant-herbivore interactions are shaped by multiple adaptive frameworks. As every life history stage of a butterfly is typically terrestrial, the morphology and behavior of each stage are highly adapted to terrestrial environments. As a result, the plants mostly chosen as larval food sources are also terrestrial. In India, some widely used primary host plants of *Junonia atlites atlites* (such as *Hygrophila auriculata*, *Hygrophila costata*) prefer to grow in damp areas, in the vicinity of water bodies (such as pond margins) as well as in wetlands, but every plant is strictly terrestrial. *Junonia divaricata* from Central America uses the aquatic plant *Utricularia hispida* (Lentibulariaceae) as larval host, but this butterfly has also been reported to feed only on the emersed flower stocks (Brévignon, 2009; Brévignon & Brévignon, 2012). Thus, except for some aquatic moths (mostly belonging to the families Crambidae and Pyralidae) (Herlong, 1979; Dorn *et al.*, 2001; Vallenduuk & Cuppen, 2004; Pabis, 2014; Redekop *et al.*, 2018), feeding on aquatic submersed vegetation has hardly been reported from the order Lepidoptera, especially among the butterflies. In this context, our opportunistic sighting of the larval feeding behavior described here appears to be quite exceptional.

Figure 2. Structure of the submerged leaves of *Limnophila heterophylla.*

Similarities in chemical cues can be an important driving factor behind a phytophagous insect using a unusual host plant of a related or unrelated genus or family (Becerra & Venable, 1999), as well as in shifts to novel hosts. *Junonia* mostly prefer plants from the family Acanthaceae as their larval host (Robinson *et al.*, 2010). But, unlike *Junonia atlites atlites*, a few species of *Junonia* have previously been reported to feed

Figure 3. a. Position of a spiracle in a *Junonia atlites atlites* caterpillar; b. Close-up view of the spiracle.

Figure 4: Pupa of the reared individual of *Junonia atlites atlites*.

on the family Plantaginaceae as well. For example, in India, *Misopates orontium* (Plantaginaceae) is a host plant of *Junonia orithya orithya* (Wynter-Blyth, 1957; Kunte, 2000; Nitin et al. 2018). Similarly, *Junonia almana almana* has been recorded feeding on *Antirrhinum majus* (Plantaginaceae) from the Oriental region (Robinson *et al.*, 2010). In Central America, *Junonia coenia* has been observed to use *Plantago lanceolata* (Plantaginaceae) as a common host plant (Pereyra & Bowers, 1988). Interestingly, plants of Plantaginaceae and Acanthaceae share a special group of secondary plant metabolite, iridoid glycosides, which have been found to provide oviposition stimulation to the females of *Junonia* species (Bowers, 1984). Aucubin and catalpol, two iridoid glycosides isolated from *Plantago lanceolata* (Plantaginaceae), are used by female *Junonia coenia* as oviposition cues (Pereyra & Bowers, 1988). These compounds have also been found to protect *Junonia* larvae from ant predators and to thus increase survival (Dyer & Bowers, 1996). Thus, in our case also, iridoid glycosides may potentially play a crucial role in explaining the usage of these two plant families by the same butterfly species. Hence, we suggest that a thorough phytochemical analysis of *L. heterophylla* and additional attempts to study the life history of *Junonia atlites atlites* on the same plant could provide insights into the chemical cues that resulted in the feeding event we observed, as well as the potential of this plant as more widespread larval host for this species than has been realized to date.

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