Report on the Emergence Time of a Species of *Thitarodes* Ghost Moth (Lepidoptera: Hepialidae), Host of the Caterpillar Fungus *Ophiocordyceps sinensis* (Ascomycota: Ophiocordycipitaceae) in Uttarakhand, India

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Abstract

Ghost moths in the genus *Thitarodes* Viette, 1968, are hosts of the economically important caterpillar fungus, which is harvested in high mountain meadows across the Hengduan mountains and the Himalayas. In northwestern India, although caterpillar fungus has been collected in the state of Uttarakhand, no adults of *Thitarodes* species have been recorded. We report the sighting of a female pupa of *Thitarodes* sp. and its last-instar molt at Munsiyari, Uttarakhand, India, on 5 July 2019 and estimate that the adult emergence time in this habitat should be late July or early August. Although the habitats of caterpillar fungus in Uttarakhand are geographically closer to those in Nepal, they are climatically more similar to habitats of caterpillar fungus in Qinghai province in China. Among records at the same elevation, climatic variables are highly predictive of the emergence date of *Thitarodes* adults (adjusted- R^2 : 0.7925, F = 6.27, P = 0.03). Our result highlights the role of the Himalayan mountains as both a north–south climatic barrier and an east–west climatic gradient. We encourage local stakeholders and scientists in Uttarakhand to survey adult emergences of *Thitarodes* from July to mid-August.

Key words: Ophiocordyceps sinensis, Thitarodes, Uttarakhand, climatic gradient

Ghost moths in the genus Thitarodes Viette, 1968, are hosts of the entomophagous fungus, Ophiocordyceps sinensis (Berk.) Sung et al. (2007) (see Wang and Yao 2011 for review on known host distribution; Wang et al. 2019 for review on current phylogenetic status of the group). The fungal sclerotium encased in the exoskeleton of an infected Thitarodes larva is known as 'caterpillar fungus'. It is avidly collected in high mountain meadows across the Hengduan mountains and the Himalayas for their reputed medicinal benefits. Caterpillar fungus collection accounts for as much as 40% of the rural cash income in the Tibet Autonomous Region of China (Winkler 2008, 2010; Sulek 2019), whereas in the western Himalayas, the collection contributes to over 70% of many households' local cash income (Shrestha and Bawa 2014, Laha et al. 2018, Shrestha et al. 2019, Yadav et al. 2019). Known ranges where communities rely on caterpillar fungus collection stretch from the Hengduan mountains, Qianghai-Tibetan plateau to the Himalayan ranges of Bhutan, Nepal, and India (Hopping et al. 2018).

Caterpillar fungus trade in the Indian Himalayas was first discussed by Sharma (2004). In the Indian state of Uttarakhand, caterpillar fungus collection has become an integral part of the economic activities of some local communities (Laha et al. 2018, Yadav et al. 2019), while the risk of overexploiting this profitable natural resource remains high (Negi et al. 2016). The state of Uttarakhand borders Darchula District of Nepal, where local conflicts related to caterpillar fungus collection (Pant et al. 2017) and illicit cross-border trades (Wallrapp et al. 2019) are known to occur. Despite ongoing investigation in the social economics of caterpillar fungus collection, no *Thitarodes* moth—the obligate host of caterpillar fungus—has been recorded in India. In reviewing the ghost moths of India, Grehan and Ismavel (2017) pointed out that *Thitarodes* 'has not been documented from within India although its presence is almost certain because it is known from adjacent Bhutan, China, and Nepal'.

Identifying the emergence time of *Thitarodes* adults in the state of Uttarakhand is crucial to developing local captive cultivation

projects that could maintain the sustainable harvest of caterpillar fungus. Since *Thitarodes* larvae live under the soil, they are difficult to collect without significantly damaging alpine meadow vegetation. Any project that aims at sustainably rearing *Thitarodes* moths would need to start by collecting them during the short time period of their emergence (and mating) in natural habitat. Moreover, in captive breeding, it is often necessary to replenish and hybrid multiple stocks of *Thitarodes* to avoid inbred pressure, which requires the breeder to know the emergence time of several species of *Thitarodes* to obtain the adults (Zhou et al. 2014; Li et al. 2016, 2019). The emergence time of *Thitarodes* is also an important parameter to control in captive breeding: by adjusting the environmental temperature species with different emergence dates in the wild can be brought to emerge within the same week (Tao et al. 2015).

Here we report our observations of a *Thitarodes* sp. pupa in Munsiyari, Uttarakhand, India, and from this, we estimate the adult emergence time in this habitat. We compare our estimated emergence time of *Thitarodes* sp. in Uttarakhand with that of other known *Thitarodes* moths at the same elevation to show that delay in emergence date of *Thitarodes* sp. in Uttarakhand is likely driven by the colder climate in the western Himalayas.

Materials and Methods

On 5 July 2019, we visited a caterpillar fungus habitat south of Panchchuli basecamp, north of Munsiyari hill station (30°8′54.14″N, 80°18′074″E, 3,801 m, Fig. 1A). The Munsiyari

region has an average elevation of 2.200 m, with several peaks above 7.000 m. At the habitat, annual mean temperature is around 3°C, annual precipitation is around 1.600 mm, concentrated in July, August, and September. The alpine flora in this habitat consists predominantly of Anemone, Primula, Potentilla, Polygonum, Corydalis, Caltha, Rhododendron, and Tricholepidium, among which the stroma of caterpillar fungus could still be found above ground (Fig. 1F). Upon our accidental encounter of the Thitarodes pupa, we remained at the habitat for two evenings to look for emerging adults and search for pupal molts to check whether any adults have emerged before our arrival in early July. In accordance with local regulations, no sample were taken and no further excavation were conducted to investigate Thitarodes pupae in the soil.

To compare our estimated emergence date of *Thitarodes* sp. in Uttarakhand with the emergence date of other *Thitarodes* moths (that are O. *sinensis* hosts) at the same elevation, we searched through relevant literature with both recorded emergence dates and recorded elevation in the 3,800- to 3,900-m range (Wang and Yao 2011, Ueda 2000). When obtaining climatic data of the selected regions, rather than obtaining direct point climate estimates from WorldClim database (Hijmans et al. 2005), we obtained temperature estimates for these habitats by applying a moist adiabatic lapse rate of 6°C/km to each habitat's average bioclimatic variable obtained in Hopping et al. (2018) to avoid variance caused by inaccurate spatial measurements. The moist adiabatic lapse rate refers to the rate at which saturated air warms or cools at different altitudes (Jacobson

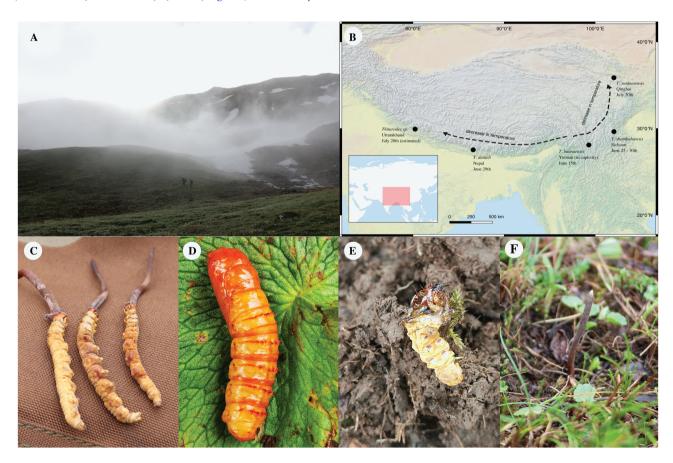


Fig. 1. (A) The habitat of caterpillar fungus in Munsiyari, Uttarakhand, India, 5 June 2019. (B) Map showing emergence date of five species of *Thitarodes*, at the same elevation, along the Himalayas and Hengduan mountains. (C) Caterpillar fungus collected by local stakeholders in Uttarakhand habitat. (D) *Thitarodes* moth pupa in Uttarakhand habitat. (E) Molt of last-instar *Thitarodes* larva in Uttarakhand habitat. (F) Stroma of caterpillar fungus appearing above the soil in Uttarakhand habitat (photograph: Darong Yang).

2005). Regression analysis were conducted in R (version 3.6, R Core Team 2019).

Results and Discussion

Upon setting up our tents, we uncovered a recently pupated female *Thitarodes* pupa and its last-instar molt 35 cm under the soil (Fig. 1D and E). No *Thitarodes* adults or pupal molts were found despite searching for two additional evenings so we concluded that no adults have emerged before early July. We were unable to identify the collected individual to species since it did not emerge during our field trip. Nevertheless, the pupal stage of *Thitarodes* typically lasts more than 30 d (Tao et al. 2015), with no difference in emergence time between male and female; we were therefore able to estimate the adult emergence time of *Thitarodes* in this habitat to be late July or early August.

Among *Thitarodes*, only four species have recorded adult emergence dates in the 3,800- to 3,900-m elevational range. These records span across the distribution range of *Thitarodes* from the Hengduan mountains to the Himalayas and can be compared with our estimated *Thitarodes* adult emergence time in Uttarakhand (Table 1): adults of *T. baimaensis* (Liang et al. 1988) in Yunnan are reared at 3,600- to 3,800-m elevation for captive breeding by the authors; individuals of *T. shambalaensis* (Wang et al., 2019) have recently been described at 3,892-m elevation on Mt. Gongga; *T. danieli* (Viette, 1968) moths were collected at 3,800 m in east Khumjung, eastern Nepal (as reported in Ueda 2000) and adults of *T. xunhuaensis* (Yang et al., 1995) have been reported to occur in Qinghai province at 3,800 m.

Hopping et al. (2018) identified a series of highly correlated bioclimatic variables (such as 'mean temperature of coldest quarter' and 'annual mean temperature') that are predictive of caterpillar fungus growth across the species' distribution range. We found that at 3,800 m, the same variables implemented in Hopping et al. (2018) are also highly predictive of the emergence date of Thitarodes adults (adjusted- R^2 : 0.7925, $F_{DF3} = 6.27$, P = 0.03). For example, although the habitats of caterpillar fungus in Uttarakhand are geographically closer to those in Nepal, they are climatically more similar to the caterpillar fungus habitats in Qinghai. At the northern and western limits of the caterpillar fungus' distribution range, mean temperatures of the coldest quarter in habitats in Uttarakhand and Qinghai are below -9°C (Hopping et al. 2018). After adjusting for elevational differences among habitats (with a moist adiabatic lapse rate of 6°C/ km), at 3,800-m mean temperatures of the coldest quarter are below -7°C in Uttarakhand and Qinghai, colder than any other locality of the same elevation in eastern Himalayas and Hengduan mountains. Our estimation and records of adult Thitarodes emergence in Qinghai and Uttarakhand, which are delayed until late July and August, likely reflects the cold weather in both these regions.

By contrast, at the same elevation, adults of species of *Thitarodes* in Yunnan, whose habitat has the highest annual temperature, emerge as early as mid-June. Two other *Thitarodes* species in Yunnan also have early emerging adults, but were not included in this study as their recorded elevational ranges are either different from or broader than the 3,800- to 3,900-m range: adults of *T. jianchuanensis* (Yang, 1994) were recorded in late May at 3,000–3,500 m; those of *T. yunnanensis* (Yang, Li and Shen, 1992) were recorded in mid-June at 3,600–4,180 m. Current temperature data from habitats in eastern Nepal (similar to the climate in Yunnan) predict an adult emergence of *T. danieli* similar to that of Yunnan in mid-early June. However, the 1964 collection records for *T. danieli* are in late June, potentially reflecting an emergence timing when local temperatures were lower (all other emergence records were collected within the past 30 yr).

Our estimated adult emergence time of *Thitarodes* in Uttarakhand, which could be as late as August, could explain the lack of recorded sightings of this genus in northwestern India. Caterpillar fungus collection in Uttarakhand starts in the spring but ends in early July when the monsoon brings precipitation—local collectors are generally no longer present in the habitat to find species of *Thitarodes* in August. In contrast, the region where different species of *Thitarodes* moths are most thoroughly documented is Yunnan, where the early emergence of the adults overlaps with local collection periods in early June.

Based on these observations, we recommend that local stakeholders and scientists in Uttarakhand survey the habitat in July and August to look for emerging adults of *Thitarodes*. *Thitarodes* found will likely be a new species, considering the high level of endemism observed in this genus (Wang and Yao 2011, Wang et al. 2019). A better understanding of the lifecycle of the local *Thitarodes* sp., particularly its adult emergence time, can help broaden current social economic-driven conservation research to include strategies beneficial to preserving the host of caterpillar fungus. These efforts will also enable the rearing of *Thitarodes* in captivity to increase caterpillar fungus yield, as has been implemented in China (Zhou et al. 2014; Li et al. 2016, 2019; Tao et al. 2015), but not across the Himalayas.

We also note that the role of the Himalayan mountains in determining the phenology of *Thitarodes* warrants further research. The presence of this geographical feature creates a north-south climatic barrier: west of the 81° E latitude, caterpillar fungus does not occur in the arid, northern Himalayas, but can be found in the southern Himalayas, extending west to Himachal Pradesh. The Himalayas also create an east–west climatic gradient, making the western Himalaya climatically similar to northern Qinghai, as reflected by similar emergence dates of *Thitarodes* in both locations. The biological and evolutionary implications of this east–west climatic gradient have recently been discussed in the context of species

Table 1. Adult emergence records of all known *Thitarodes* species in the 3,800-m elevational range and the mean coldest quarter temperature of their habitat as shown in Hopping et al. (2018), with elevational adjustment

| Species | adult emergence | Mean temperature of coldest quarter (°C) | Habitat in Hopping et al. (2018) | Elevation (m) |
|-------------------------------|-------------------------|--|-------------------------------------|---------------|
| Thitarodes baimaensis | Mid-June (in captivity) | -0.60 | Yunnan | 3,600-3,800 |
| Thitarodes danieli | 29 June | -1.60 | Eastern Nepal | 3,800 |
| Thitarodes shambalaensis | 25-30 June | -4.53 | Sichuan | 3,892 |
| Thitarodes xunhuaensis | 20 July | -8.60 | Golog (Qinghai) | 3,800 |
| Thitarodes sp. in Uttarakhand | Late July | -7.55 | Uttarakhand | 3,801 |

[&]quot;Thitarodes baimaensis has been successfully reared in captivity for multiple generations at this elevation by the author (YD). Its natural habitat in Yunnan is at 4,400–4,780 m, with emergence date recorded between 17 and 27 July.

diversity (Rana et al. 2019), but not in the context of plant and animal phenology.

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