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Another Blood Feeder? Experimental Feeding of a Fruit-Piercing Moth Species on Human Blood in the Primorye Territory of Far Eastern Russia (Lepidoptera: Noctuidae: Calpinae)

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Introduction

The genus *Calyptra Ochsenheimer* (Lepidoptera: Noctuidae: Calpini) includes what are commonly known as vampire moths, so named because of their ability to pierce mammalian flesh and feed on blood. These are medium sized moths, with wingspans ranging from 35 to 72 mm in size (Bänziger 1983; Table 1; Figs. 1, 2 and 3). Species in this genus occur in S. Europe, eastern Africa, sub-Himalayan regions of S. Asia, the Manchurian subregion, and are broadly distributed throughout S. E. Asia. *Calyptra* species have enjoyed popularity among members of the entomological community due to their modified proboscides equipped with strongly sclerotized barbed hooks used for piercing through both thick and hard skinned fruits such as peaches, plums, and citrus as well as mammals (Bänziger 1982; Zaspel, personal observation; Fig. 4).

Of the 17–19 described *Calyptra* species (Bänziger 1983; Poole 1989), *C. eustrigata* (Hampson), *C. minuticornis minuticornis* (Guenée), *C. orthograpta*

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Table 1 Summary of Feeding Behaviors for Moth Specimens Collected in Primorye Territory of Far Eastern Russia from 07/14/2006 to 07/15/2006 and from 07/17/2006 to 07/20/2006

Species	Site	Sex	Date collected	Vial number	Feeding trial	Behavior	Time (min)
C. thalictri	Subsite 1a	M	7-14-06	1	1	PBF	2
					2	PBF	6
C. thalictri	Subsite 1a	M	7-14-06	2	1	LM	10
					2	LM	10
C. thalictri	Subsite 1a	M	7-14-06	3	1	LM	10
					2	LM	10
C. thalictri	Primary 1	M	7-15-06	4	1	PBF	11
C. thalictri	Subsite 1b	M	7-15-06	5	1	LM	10
					2	PBF	4.5
C. thalictri	Subsite 1b	M	7-15-06	6	1	LM	10
					2	LM	10
C. thalictri	Subsite 1b	M	7-15-06	7	1	LM	10
					2	LM	10
C. thalictri	Subsite 1b	M	7-15-06	8	1	LM	10
					2	LM	10
C. thalictri	Subsite 1b	M	7-15-06	9	1	LM	10
					2	LM	10
C. lata	Subsite 1b	M	7-15-06	10	1	NA	10
					2	NA	10
C. lata	Subsite 1b	M	7-15-06	11	1	NA	10
					2	NA	10
C. lata	Subsite 1b	M	7-15-06	12	1	NA	10
					2	NA	10
C. lata	Subsite 2a	M	7-17-06	13	1	LM	10
					2	PA	10
C. thalictri	Subsite 2b	M	7-18-06	14	1	LM	10
					2	LM	10
C. thalictri	Subsite 2b	M	7-18-06	15	1	LM	10
					2	LM	10
C. thalictri	Subsite 2b	M	7-18-06	16	1	LM	10
					2	LM	10
C. thalictri	Subsite 2b	M	7-18-06	17	1	LM	10
					2	LM	10
C. thalictri	Subsite 2b	M	7-18-06	18	1	NA	10
					2	NA	10
C. thalictri	Subsite 2b	M	7-20-06	19	1	LM	10
					2	DT	n/a
C. thalictri	Subsite 2b	M	7-20-06	20	1	LM	10
					2	NA	10
C. lata	Subsite 2b	M	7-20-06	21	1	NA	10
					2	NA	10
C. lata	Subsite 2c	M	7-21-06	22	1	NA	10
					2	NA	10
C. lata	Subsite 2c	M	7-21-06	23	1	NA	10
					2	NA	10
C. lata	Subsite 2c	M	7-21-06	24	1	NA	10
					2	NA	10
C. lata	Subsite 2c	M	7-21-06	25	1	NA	10
					2	NA	10
C. lata	Subsite 2c	M	7-21-06	26	1	NA	10
					2	NA	10

LM Licking moisture off skin with proboscis, *PA* piercing attempted, *PBF* piercing and blood-feeding, *NA* no attempt to lick moisture, pierce skin, or feed on blood, *DT* dead at time of trial

Fig. 1 Adult habitus, *Calyptra thalictri*, male.

(Butler), *C. bicolor* (Moore), *C. fasciata* (Moore), *C. ophideroides* (Guenée), *C. parva* Bänziger and *C. pseudobicolor* Bänziger have been reported to pierce mammalian skin, the latter five also of man, under natural conditions while *C. fletcheri* (Berio) has done so in experiments (Bänziger 1968, 1989). These species are considered facultative or opportunistic blood-feeders primarily in subtropical areas in southern Asia and tropical Southeast Asian countries: their hosts are typically ungulates such as cattle, tapirs, zebu, and occasionally elephants and humans; female *Calyptra* adults have not been documented feeding on blood (Bänziger 1989). At least four additional closely related genera (*Eudocima*, *Gonodonta*, *Oraesia*, and *Plusiodonta*) have apparently homologous proboscides modifications used for fruit-piercing, but the occurrence of blood-feeding in those species has not been observed (Bänziger 1979 and personal communication, Zaspel, unpublished data). It is known that some fruit-piercing moths are of great economic importance, e.g., *Eudocima fullonia* and to a lesser degree even some *Calyptra* spp. (Fay 2002; Fay and Halfpaff 2006; Sands et al. 1993; Todd 1959; Yoon and Lee 1974); their potential as vectors of human or animal disease remains a possibility, however, whether a real danger of vectoring disease exists is unknown (Bänziger 1980, 1989). The purpose of this paper is to document the first case of *Calyptra*

Fig. 2 Adult habitus, *Calyptra lata*, male.

Fig. 3 Adult habitus, *Calyptra hokkaida*, male.

feeding on human blood in Far Eastern Russia and the novel finding of blood feeding by the species *C. thalictri* under experimental conditions.

Materials and Methods

Description of Observation Sites

During an expedition in the Primorye Territory of Far Eastern Russia (Fig. 5) in July, 2006, we sought to observe feeding behaviors of the three *Calyptra* species (*C.*

Fig. 4 Proboscis of *Calyptra thalictri*: *TH*, tearing hooks.

Fig. 5 Map of Primorye region of Far Eastern Russia.

hokkaida Wileman, *C. lata* Butler, and *C. thalictri* Borkhausen) in this region (Kononenko 1990; Remm 1980). None of these species had been recorded feeding on mammalian blood and have been considered exclusive fruit piercers. Since the complete geographic occurrence—and precise phylogenetic origins—of blood feeding *Calyptra* remain unknown, an objective of this work was to determine whether *Calyptra* species that occupy the northern extent of their range were hematophagous.

The southern part of the Russian Far East (Primorye territory) lies in a zone of Manchurian coniferous and mixed coniferous and broad leaved forests with very rich and diverse vegetation. The main forest formations in the region are *Abies-Picea taiga* in the upper and mid mountain belts, *Pinus koraiensis* mixed forest in the mid mountain belt, *Abies nephrolepsis* mixed forest in the south of the region, deciduous broad leaved forest and forest dominated by *Quercus mongolica* (Kurentzov 1965; Richter 1961). The mixed and deciduous forests of the south Far East contain many east Asian trees and shrubs that are absent from Siberia, such as *Quercus*, *Fraxinus*, *Acer*, *Tilia*, *Ulmus*, *Carpinus*, *Phellodendron*, *Maakia*, *Aralia*, *Calopanax*, *Actinidia*, *Schisandra* (Kurentzov 1965; Richter 1961). The sub-alpine, and mountain tundra belts are fragmentary, and occur in the Sikhote-Alin mountain range above 1,500 m in the central and northern part of the region. The forest-steppe zone occurs mainly in the southwestern portions of the region (Kurentzov 1965; Richter 1961).

The climate of the Primorye territory is dominated in part by monsoon features. The annual temperature in Primorye, is +4°C, temperature in January: 10–15°C, in July it is over +20°C (Kurentzov 1965; Richter 1961). The annual precipitation is 800–1,200 mm, roughly 70–75% of which falls from July to September. North-westerly winds dominate in winter, while south-east winds prevail in the summer (Kurentzov 1965; Richter 1961).

We collected Calyptra specimens at two primary sites (designated sites 1 and 2; Figs. 6, 7, 8 and 9) and three subsites (designated subsites 1a and 1b; subsites 2a, 2b,

Fig. 6 Primary collecting site 1.

and 2c, respectively). Collecting site 1 (including subsites 1a and 1b) was in the vicinity of the Kraunouka Village at the Borisovskoe Hunting Area, roughly 20 km west of Ussuriisk, Russia (Figs. 6 and 8: N 43° 44.577, E 131° 38.218; 287 ft). This is a popular hunting site for wild boar (*Sus scrofa* sp.), two species of deer (*Cervus nippon* and *Capreolus capreolus*), and bear (*Ursus thibetanus*), situated in the easternmost spurs of the East Manchurian montane system (Fig. 6). Although it has not yet been observed in this region, these mammals could potentially serve as hosts of adult *Calyptra* species. This area contains low elevation mountains (100–300 m), hills and cliffs in the upper reaches of the Kraunouka River. The vegetation around the collecting sites consists primarily of broad leaf forests dominated by oak (*Quercus mongolica*; Kurentzov 1965; Richter 1961). The vegetation along the river valley is considerably more diverse broad-leafed forest with variously dominant species such as *Juglans mandshurica*, *Ulmus japonica*, and, *Vitis amurensis*. Mixed broad-leaf coniferous forest with coniferous trees such as: *Abies holophila*, *A. nephrolepsis*, *Pinus koraiensis*, and *P. funebris*, cover the upper reach of Kraunouka

Fig. 7 Primary collecting site 2.

Fig. 8 Map of primary collecting site 1.

River (Kurentzov 1965; Richter 1961). Most relevant, are the presence of larval food plants of the local species of *Calyptra*: *Menispermum dahuricum*, *Thalictrum contortum*, *T. simplex*, and *T. amurensis* are abundant in meadows (Kurentzov 1965; Richter 1961).

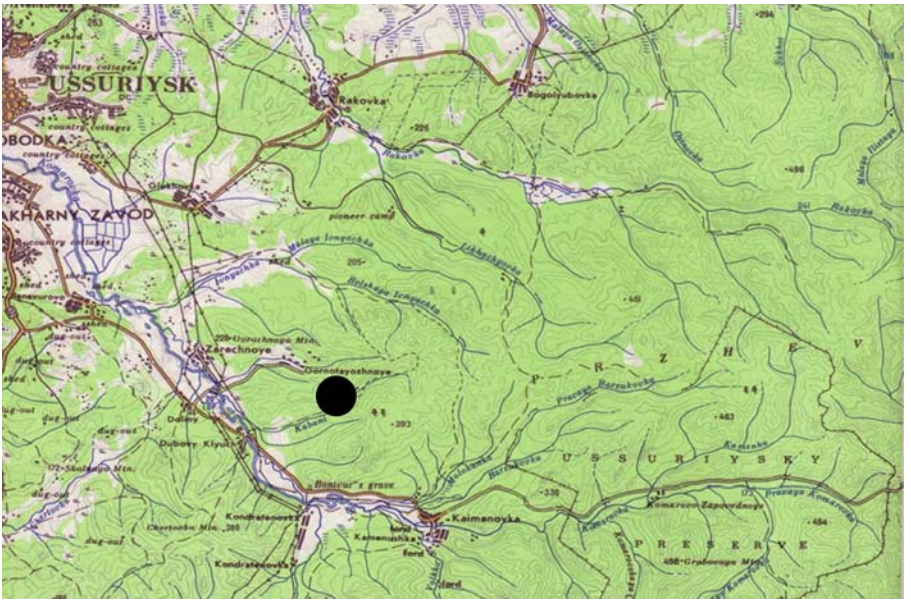

Fig. 9 Map of primary collecting site 2.

The second collecting site (subsites 2a, 2b, and 2c) was at the Gornotayeznaya Biological Station Far Eastern Branch of the Russian Academy of Sciences (20 km east of Ussurisk). This collecting site was in the vicinity of Gornotayeznoe village situated in southwestern spurs of Sikhote-Alin montane range (Figs. 7 and 9: N 43° 41.917 E 132° 09.131). The area is in the vicinity of low elevation mountains (200–400 m). The vegetation around the collecting site is similar to first collecting locality and consists of broad-leaf forest in a creek valley. Wild rosaceous plants are less well represented here than in the former site; however, apple, pear, cherry and apricot trees are cultivated in gardens. The fruits of Rosaceae are commonly attacked by many fruit-piercing moths, including some *Calyptra* spp. (Bänziger 1971, 1982). The southern slopes of the hills are covered by secondary forest dominated by *Quercus mongolica* and *Corylus mandschurica* in the understory; the northern slopes are covered by mixed broad leaf—coniferous forest with native and planted trees and pure primary forest dominated *Pinus koraiensis* situated in the upper reaches of the Krivoi kljuch creek (Kurentzov 1965; Richter 1961), roughly 10 km from the collecting site.

Experimental Methods

Moths were collected using standard techniques: suspended white sheets illuminated by a 60 W mercury vapor lamp (HgVpr, Winter 2000). Live specimens were collected into separate ten dram plastic vials. Upon return to the field station at primary collecting sites 1 and 2, male specimens were retained alive overnight. Females were transferred into 95% EtOH and males were placed in a small rearing tent with small pieces of wet cotton for about 4 h. Each specimen was carefully removed from the tent and placed in a numbered live vial (see Table 1). Feeding trials were conducted to determine which, if any, of the additional specimens collected would penetrate human skin and feed on blood. The moths were presented with two separate feeding opportunities conducted over a 24-h period. The first trial was always the morning after the moths were collected, and started between 0800 and 1000 h second trial began in the evening between 1900 and 2100 h. The trials were conducted by inserting a thumb into the vial in such as way so that the moth could not escape, and each moth's behavior was recorded for 10 min. If the moth began to feed, or made an attempt to feed beyond the 10 min time period it was not interrupted. If a moth did take a blood meal, it was eliminated from the experiment and recorded as a positive blood-feeder. This was done to minimize potential injury and/or allergic reactions on the part of the subjects. If no feeding activity was observed after 10 min, or if previous feeding behavior(s) ceased, the thumb was removed from the vial and the moth was given another opportunity to feed during trial 2. All species identifications were confirmed based on external morphology and genitalic dissections.

Results

On July 14th 2006 a male specimen of *Calyptra thalictri* (Fig. 1) was collected in Kraunouka about 2 km from the field station in the hunting area (subsite 1a) at

approximately 2300 h. This specimen was placed in a plastic live vial (designated vial #1, Table 1) and the senior author's thumb inserted into the vial to observe potential piercing behavior. After 3 min, the moth inserted its proboscis into the thumb just below the nail and began to uptake blood for approximately 2 min. Three additional specimens of this species (two males, and one female) were also collected the same night at subsite 1a. The following morning (7–15–06: 1047 h), Specimen in vial #1 was given another opportunity to feed on human blood (Table 1). During this time, the moth grazed its proboscis across the subject's thumb, lapping moisture off of the skin. After approximately 5 min, the moth inserted the tip of its proboscis into a crease in the skin on the joint of the thumb and pierced through the skin (Fig. 10). The moth sucked blood briefly (less than 30 s) and without removing the tip of the proboscis pierced further into the wound and then continued sucking blood. This behavior (intermittent piercing followed by feeding) continued for 6 min before the moth was removed, and the wound examined (Fig. 11). The same methodology was used to determine whether the additional male specimens would feed. However, the specimens only licked the skin, presumably unsuccessful attempts at piercing. All specimens were ultimately placed in 95% EtOH for future molecular study.

On July 15th at approximately 2030 h (dusk) a male *C. thalictri* was captured outside the perimeter of the field station in the Borisovskoe Hunting Area. This specimen was resting on the trunk of a cherry shrub when it was hand collected. The specimen slowly crawled outside the slightly compressed hand through the opening between the forefinger and thumb, where it rested. The moth uncoiled its proboscis several times, touching the skin below the thumbnail. The moth made several attempts to pierce the skin with its proboscis, finally settling on a crease in the skin area 7–8 mm above the border of the thumbnail (the same location as the previous blood-feeding specimen). The moth's proboscis slowly penetrated the skin. As with the first *Calyptra thalictri* specimen collected (specimen in vial #1, Table 1), this moth (vial #4, Table 1) pulled its proboscis slightly out of the wound, and then pierced further into the wound. When the moth pulled its proboscis out of from the wound, it was saturated with blood (Figs. 12, 13 and 14). This feeding continued for an additional 7 min. This incident of blood feeding should be considered "semi-natural," as the moth was not enclosed in a vial, yet did not actively seek out the host and could have been disoriented given the time of day was earlier than its typical

Fig. 10 Image of *Calyptra thalictri* feeding on human thumb (JMZ); Lateral view (Vial 1).

Fig. 11 Image of subject's wound (JMZ) after piercing and feeding by *C. thalictri*.

flight time. The subject observed slight swelling in the area around the wound, and described a feeling of slight stinging pain for 2–3 h following the attack. After feeding ceased, the specimen was placed in a vial and kept alive overnight. At 0130 h the live vial containing this moth had several large drops of blood at the bottom indicating excretion of the blood meal (Fig. 15). Later that morning all blood droplets were absent, suggesting re-uptake of the blood meal, and when the moth was removed from the vial and transferred into EtOH, additional blood droplets were excreted from the tip of the abdomen.

Between the hours of 2230 and 0100 h on 15 July, 13 additional Calyptra specimens were collected at a third site about 10 km from the hunting area (subsite 1b) using the same methods. Of these specimens, eight were *C. thalictri* (Fig. 1; three females and five males), and five were *C. lata* (Fig. 2; one female and four males). All specimens were collected into separate live vials and were transported to the Gornotayeznaya Biological Station. At the Gornotayeznaya Biological Station, the females were given the opportunity to feed, but made no attempts to do so. The lack of attempts to feed or even lick moisture from the subject's thumb corroborates

Fig. 12 Image of *Calyptra thalictri* feeding on human thumb (VK); frontal view (Vial 4).

Fig. 13 Image of *Calyptra thalictri* feeding on human thumb (VK); frontal view (Vial 4).

previously published work that states female *Calyptra* species do not feed on blood (Bänziger 1989). Females were not included in future feeding trials.

In addition to the two blood feeders mentioned above, one of the male *C. thalictri* specimen collected from subsite 1b (vial #5, Table 1) pierced the senior author's thumb and fed on blood for 4 min and 30 s. The remaining *C. thalictri* specimens only attempted to pierce but uptook moisture from the skin. The *C. lata* specimens made no attempt to feed.

During the course of these feeding experiments, seven additional *Calyptra thalictri* specimens, and six specimens of *C. lata* were collected in the vicinity of the Gornotayeznaya Biological Station and subjected to feeding trials. These specimens were presented the same feeding opportunities those specimens taken from the Borisovskoe Hunting Area. Of the *C. thalictri* collected at various sites surrounding the Gornotayeznaya Biological Station, none fed on blood and only one specimen made a weak attempt to pierce the skin (but did not draw blood); all specimens, however, did graze the skin with the proboscis, lapping moisture off of the thumb. No attempts to pierce or lap moisture from skin were observed by any of the *C. lata*

Fig. 14 Image of *Calyptra thalictri* feeding on human thumb (VK); lateral view (Vial 4).

Fig. 15 Live vial showing blood droplets excreted by *C. thalictri*; (Vial 4).

specimens. Sixteen additional specimens of *C. lata* and *C. thalictri* were collected on 21 July and were either pinned or transferred into tubes with 95% EtOH for future morphological and molecular study. Of the nine male *C. thalictri* specimens collected at Primary site 1 (and subsites therein), 44% were successful at piercing skin and feeding on blood. *Calyptra lata* specimens from Primary site 1 did not attempt to pierce. One attempt to pierce was made by a *C. lata* specimen from Primary site 2; *C. thalictri* specimens from Primary site 2 made no attempts to pierce. A summary of feeding behaviors for the *Calyptra* specimens subjected to feeding trials during 14–15 July and 17–20 July is provided in Table 1.

Discussion

These are the first recorded experimental observations of blood feeding by a *Calyptra* species in the Primorye Territory of Far Eastern Russia. Although under experimental and semi-natural conditions, they represent the first documented occurrence of blood-feeding by *Calyptra* in a temperate region, and a new blood-feeding species record for the genus. The observed blood feeding behavior of *C. thalictri* specimens in this study consisted of spindle movements, head oscillations, followed by frequent partial withdrawal and re-insertions of the proboscis; thus, the blood feeding behavior of *C. thalictri* under the conditions reported in this paper are identical to those previously described for the blood feeding species *C. eustrigata* (Bänziger 1968, 1980). It was unclear from the feeding experiments whether saliva was released or if blood was regurgitated during feeding.

Although indistinguishable by genitalia and proboscis morphology (including development of armature), *C. thalictri* specimens collected in the Primorye Territory vary phenotypically from those that occur in Palearctic regions: specimens collected in Primorye have dark green forewings as opposed to the red-orange colored Palearctic specimens (compare Figs. 1 and 16). It is possible that the two populations represent two different species, but addressing this question is beyond the scope of this paper. The fact that *C. thalictri* specimens from the second collecting site did not

Fig. 16 *Calyptra thalictri* feeding on raspberry during night observations (under natural conditions) in S. Europe, photo by Hans Bänziger.

pierce skin and feed on blood is also consistent with previous published feeding behavior records for this species (Bänziger 1970, 1971). Similar differential feeding behaviors have also been reported in other *Calyptra* species, e.g., *C. fasciata* (Bänziger 1989).

Whether or not *C. thalictri* are feeding on blood under natural conditions in the Primorye Region is presently under further investigation. It is possible that *C. thalictri* specimens' feeding behavior was different in the enclosed environment as opposed to their behavior under completely natural conditions; however, these preliminary observations indicate that *C. thalictri* can be induced to pierce human skin to suck under experimental conditions, while *C. lata* cannot. These findings are also consistent with previous studies reporting the blood feeding behavior in these moths is restricted to the males. It has been suggested that males may engage in zoophilous feeding behaviors as a result of sugar, salt, or amino acid deficiency (Scoble 1992). When the fruit or nectar hosts supplying these nutrients are unavailable, butterflies and moths will seek alternate substrates on which to feed (puddles, dung, urine, sweat, tears, and blood). Bänziger (1972, 1990) demonstrated that the *eulachryphagous* moth *Lobocraspis griseifusa* Hampson has proteinases with which it can digest protein contents in the tears of mammals while such *hemilachryphagous pyralids* as *Filodes mirificalis Lederer* are, like the very vast majority of adult Lepidoptera, incapable of protein digestion. It is uncertain whether such a mechanism exists in blood feeding *Calyptra* spp; no full analysis of the digestive capabilities of *Calyptra* has yet been published. It should be noted however, that the skin piercing behavior followed by sucking blood from the mammalian host is derived from the fruit piercing habit, as opposed to other zoophilous feeding behaviors, e.g.,

tear feeding. The observations in this study support previous work that suggests blood-feeding moths may engage in this behavior facultatively, depending on regional availability of mammalian versus vegetative hosts.

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