Purdue University Department of Entomology Undergraduate Capstone Project Summary

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# **Project Title:**

Reassessment of the *Calyptra minuticornis* (Guenée) Species Complex (Insecta: Lepidoptera: Erebidae)

### Introduction:

*Calyptra* is the only genus within all of Lepidoptera that contains both fruit piercers and blood feeders, with 10 out of the 17 species exhibiting the blood feeding behavior (*sensu* Bänziger 1983). Blood feeding is thought to be a way for some male species to sequester salts to help with their mating success (Adler 1982; Smedley and Eisner 1995), but has not been tested experimentally.

Within the genus, three species show feeding behavior based on geographical location. These are: *Calyptra thalictri* (Borkhausen), *C. fletcheri* (Berio), and *C. minuticornis* (Guenée). *Calyptra thalictri* is known to feed on fruits in southern Europe and Scandinavia but feeds on blood in the Russian Far East (Zaspel et al. 2007). It is still not clear whether or not *C. fletcheri*, and *C. minuticornis* have distinct populations that exhibit blood-feeding behavior.

*Calyptra minuticornis* is distributed throughout the Indomalayan and Australasian ecozones (Figure 1). It is thought that the species spread from South East Asia to Australia via Wallacea, which is hypothesized to be an area that facilitated faunal exchanges between Asia and Australia (Bänziger 1983). The populations within SE Asia have been observed piercing fruits, such as mandarins, peaches, plums and figs (Bänziger 1986, 2007); and feeding on blood in the wild (Bänziger 1986, 1989). While individuals occurring in the Australasian region have only been seen piercing on fruit in the lab and never on vertebrate hosts (Bänziger 1986).

*Calyptra minuticornis* was considered two subspecies, *C. minuticornis novaepommeraniae* (Strand), by Bänziger (1983) based on minor morphological differences within the male genitalia, wing venation, and larvae. There has been disagreement among authors regarding the status of the subspecies (Strand 1917, Berio 1956, Common 1990) and the characters have never been examined within an empirical framework.

The goal of this study was re-examine Banziger's characters and survey morphology of both males and females for possible additional characters and determine the taxonomic status of *C. minuticornis*.

### **Materials and Methods:**

#### Specimens Examined

Specimens were obtained from Australian National Insect Collection (ANIC), Taiwan Forestry Research Institute (TRFI), Bernice Pauahi Bishop Museum (BPBM), and the United

States Museum of Natural History (USMNH). Twenty-nine specimens were examined and 19 were dissected. 3 male and female pairs were selected from the ANIC and TRFI loans.

### Specimen Preparation

One fore wing and one hind wing were removed from the thorax. Abdomens were removed from the specimens and placed into 10% KOH solution. The abdomens were heated in a water bath for 25 minutes or until abdomen was soft and cleared. Once cleared, the abdomen was placed into glacial acetic acid to halt the clearing process. The abdomen was laterally cut along the ride side spiracles using scissors. Genitalia was removed from the pelt and both were cleaned and stained with Chlorazole black E (Sigma). Images of the stained genitalia where taken with a Leica M165C Stereomicroscope. These images as well as pinned specimens were used for coding characters and morphological terminology followed Zaspel et al. (2008), Bänziger (1983), Scoble (1995) and Goater (2003).

The wings of all individuals were cleared in a 10% bleach solution, stained and slide mounted. When the wings had cleared (about 2- 5 minutes) the procedures for staining with aqueous Eosin Y (1% in distilled water; Fisher Scientific, Pittsburgh, PA) were followed. Permanent slide mounts were made with Euparol (Bioquip, Garden City, CA). Slides were placed on to a warmer to cure for 2 days or until the air bubbles were removed.

*Material Examined:* Shanping, Kaohsiung Co, Taiwan, Y.B. Fan, 2/18/-; Female, Wulai Taipei Co, Taiwan, Y.B. Fan, 4/28/1987, Dissection Number JS031; Male, Neihu, Taipei City, Taiwan, Y. J. Chang, 2/7/1985, Dissection Number JS030; Lianhuachi Yuchi, Chinese Taipei, Taiwan, Y.B. Fan, 5/2/1996; Female, Chaofeng, Chinese Taipei, Taiwan, Y.B. Fan, 2/17/1994, Dissection Number JS029; Female, Peipu, Chinese Taipei, Taiwan, S. S. Lu, 3/16/1994, Dissection Number JS028, Male; Shanping, Chinese Taipei, Taiwan, Y.B. Fan, 11/5/1991, Dissection Number JS032; Female, Shanping, Chinese Taipei, Taiwan, Y.B. Fan, 2/21/1989, Dissection Number JS027, all from the TFRI

Female, Southport, Queensland, Australia, 5/30/1940, Dissection Number JMZ 524;
Bucasia, North Queensland, Australia, Ken J. Sandery, 4/27/2003; Female, Mt. Keira, New
South Wales, Australia, V.J. Robinson, 2/22/1965, Dissection Number 525; Cooks Hut, Iron
Range, Queensland, Australia, J.C. Cardale, 7/10/1998; Smith Point, Cobourg Pen., Northern
Territory, Australia, E.D. Edwards, 2/3/1977; Male, Smith Point, Cobourg Pen., Northern
Territory, Australia, E.D. Edwards, 1/23/1977, Dissection Number 527;
Male, Keatings Gap 3km SW of Cooktown, Queensland, Australia, I.F.B. Common and E.D.
Edwards, 5/16/1977, Dissection Number JS034; Male, Cooks Hut, Iron Range, Queensland,
Australia, J.C. Cardale, 7/7/1998, Dissection Number 526; Male, 11-12 Combined St Windham,
New South Wales, Australia, J. Stockard, 5/23/1990, Dissection Number JS033; Cambridge
Plateau, Richmond Range, New South Wales, Australia, J.C. Keast, 10/16/1993, all from the

Male, Wau, Morobe Distr., New Guinea, 1200m, 3/30/1965, Dissection Number JS035 and Female, J. Sedlacek, Dissection Number JS036 from the BPBM

Matale Dist. Bandarapola, Sri Lanka, Gans and Prasanna, 5/12/1974; Male, Assam, India, Genitalia slide by SAB USNM 42.319, JS043; Male, Kan. Dist. Udawattakele, Sri Lanka, S. and

P. B. Karunaratne, March 26-30, 1975, Dissection Number JS037; Sun Moon Lake, NanTow Co., Taiwan, D. R. Davis, June 20-25, 1980; Ceylon, kan. Dist. Kandy 1800ft. Peak View Motel, Sri Lanka, Davis and Rowe, January 7-14, 1970; Mt. Makiling Luzon, Philippines, Baker, Genitalia slide by SAB USNM 42.317, JS042; Sun Moon Lake, NanTow Co., Taiwan, all from the USNMNH

Male, Mae Sa, Mae Rim, 400m, Chiang Mai Prov., Thailand, Hans Banziger, 6/20/1982, Dissection Number JS041; Female, Wang Ta Khrai, 200-500m, Nakhorn Nayok Prov., Thailand, Hans Banziger, 4/11/1975, Dissection Number JS040, from the Banziger material.

Female, Kathmandu, 1300m, Nepal, M. Fibiger, October 4-6, 1980, Dissection Number JS038; Madinna Springs Farm, Pa Pae Village, 1500m, Chiang Mai Prov., Thailand, Zaspel, Cummins, Konoenko, June 8-20, 2013, Dissection Number JS006 and JS039, all from the PERC.

# Morphological Characters

Male genitalia characters were coded in a matrix (Table 1), specifically the juxta and the aedeagus. The aedeagus is the reproduction organ that delivers sperm to the female and the juxta is made up of two processi that support the aedeagus. Characters used to describe the aedeagus include the presence or absence of small teeth on the inner or outer sides of the curvature. When looking at the juxta there was a difference in the structures over all and how the processi were shaped. The overall form of the processi could be considered higher than wide or wider than high. When looking at each process, the posterior edge was either more rounded or rectangular in shape.

### **Results:**

The male genitalic characters relate to geographical distribution (figure 2). When looking at the juxta of the male genitalia you can see a distinct separation of the subspecies by geographical location. *C. minuticornis minuticornis* in South Eastern Asia and *C. minuticornis novaepommeraniae* in Papua New Guinea and Australia (AU), but as you examine and code more characters the distribution is not as clear. Three specimens in northern AU (JMZ526, JMZ527, and JS034) exhibit characters of both.

-	Processi higher than wide (0/1)	Juxta rounded (0/1)	Juxta square/ rectangluar (0/1)		0	Aedeagus with outer row of teeth (0/1)
JS030	1	1	0	0	1	1
JS032	1	1	0	0	1	1
JS034	0	0	1	0	1	1
JS033	0	0	1	0	1	0
JS037	1	1	0	0	1	1
JS039	1	1	0	0	1	1
<b>JMZ527</b>	0	0	1	0	1	1
JS035	0	0	1	1	1	0
JS006	1	1	0	0	1	1
JMZ526	0	0	1	1	1	1
JS041	1	1	0	0	1	1
JS042	1	1	0	0	1	1
JS043	1	1	0	0	1	1

Table 1: Character Matrix of male genitalia characteristics of Calyptra minuticornis (Guenée).

Figure 1: Distribution map of the full extent of *Calyptra minuticornis* (Guenée) range.

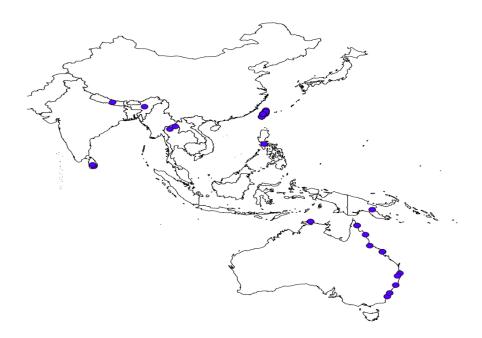
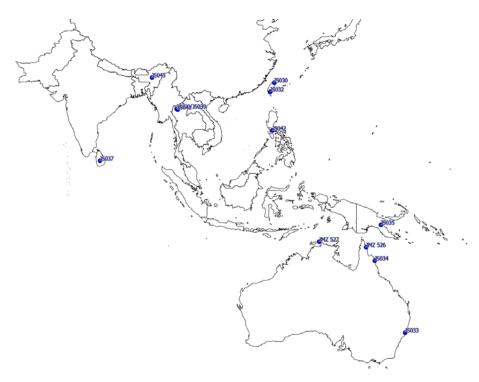


Figure 2: Distribution map of dissected males of Calyptra minuticornis (Guenée).



# **Conclusions:**

Banziger's characters are not all consistent (aedeagus with tiny teeth on the inner side of the curvature), yet some variation does seem to occur, and even perhaps correlate with geographic distributions. Without examining more material from the transition zones in Wallacea, we are unable to make a taxonomic recommendation with confidence at this time. Additional material from Sulawesi, Lombok, Timor and Buru, along with molecular data will allow us to clarify these unresolved issues. Future directions of the study include: travelling to Australia to collect fresh specimens from both northern extents of the subspecies range in Australia this February (Cobourg and Cape York), and visiting the British Natural History Museum to address the sampling gap by examining material from Wallacea.

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# **References:**

Adler, P.H. 1982. Soil- and puddle-visiting habits of moths. *Journal of the Lepidopterists' Society* 36: 161-173.

Bänziger, H. 1975. Skin-piercing blood-sucking moths I: Ecological and ethological studies on *Calpe eustrigata* (Lepidoptera: Noctuidae). *Acta Tropica* (Basel) 32, 125-144

Bänziger, H. 1979. Skin-piercing blood-sucking moths II: Studies on a further 3 adult *Calyptra* [*Calpe*] sp. (Lepidoptera, Noctuidae). *Acta Tropica* 36: 23-37.

Bänziger, H. 1980. Skin-piercing blood-sucking moths III. Feeding act and piercing mechanism of *Calyptra eustrigata* (Hmps.) (Lepidoptera: Noctuidae). *Mittellungen der Schweizerischen Entomologischen Gesellschaft* 53: 127-142.

Bänziger, H. 1983. A taxonomic revision of the fruit-piercing and blood-sucking moth genus Calyptra Ochsenheimer = Calpe Treitschke (Lepidoptera: Noctuidae). *Entomologica Scandinavica*. 14, 467–491.

Bänziger, H. 1986. Skin-piercing blood-sucking moths IV: Biological studies on adults of 4 Calyptra species and 2 subspecies (Lepidoptera, Noctuidae). *Mittellungen der Schweizerischen Entomologischen Gesellschaft* 59: 111-138.

Bänziger, H. 1989. Skin-piercing blood-sucking moths V: Attacks on man by 5 Calyptra spp.(Lepidoptera: Noctuidae) in S. and S.E. Asia. *Mittellungen der Schweizerischen Entomologischen Gesellschaft* 62: 215-233.

Bänziger, H. 2007. Skin-piercing blood-sucking moths VI: Fruit-piercing habits in Calyptra (Noctuidae) and notes on the feeding strategies of zoophilous and frugivorous adult Lepidoptera. *Mittellungen der Schweizerischen Entomologischen Gesellschaft* 80: 271-288.

Berio, E. 1956. Appunti su alcune apecie del genere Calpe Tr. (Lep., Noctuidae). *Memorie della Societa Entomologica Italiana*. 35: 109-119

Common, I.F.B. 1990. Moths of Australia. Melbourne University Press. Carlton, Victoria.

Goater, B., L. Ronkay, and M. Fibiger. 2003. Catocalinae and Plusiinae. Noctuidae Europeae Vol. 10. Entomological Press; Sorø. 452 p.

Scoble MJ (1995) The Lepidoptera: form, function and diversity. New York, USA: Oxford University Press. 404 p.

Smedley, S. R. and T. Eisner. 1995. Sodium uptake by puddling in a moth. *Science* 270: 1816-1818.

Strand, E. 1917. H. Sauter's Formosa Ausbeute: Noctuidae I. Arch. Naturgesch. 83: 129-162

Zaspel, J. M. 2008. Systematics, biology, and behavior of fruit-piercing and blood-feeding moths in the subfamily Calpinae (Lepidoptera: Noctuidae). Ph.D. dissertation, University of Florida, Gainesville.

Zaspel, J.M., C.H. Scott, S.R. Hill, V.S. Kononenko, Ignell, R., & Weller, S.J. 2014. Geographic distribution, phylogeny and genetic diversity of the fruit and blood feeding moth Calyptra thalictri Borkhausen (Insecta: Lepidoptera: Erebidae). *Journal of Parasitology* 100: 583-591.