# Natural history of the social millipede *Brachycybe lecontii* (Wood, 1864)

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## Scientific Abstract

The millipede *Brachycybe lecontii* Wood, 1864 is a social millipede known for forming pinwheel-shaped groups and for paternal care of eggs. *Brachycybe lecontii* is endemic to the eastern U.S., and its distribution overlaps with another species within the genus, *Brachycybe petasata*, from the Southern Appalachian Mountains. Molecular data, however, show that the closest relative of *B. lecontii* is *Brachycybe nodulosa* from East Asia. Here, I investigated various aspects of the life history, paternal care, defense, feeding, and social behavior of *B. lecontii*, and provided morphological and anatomical descriptions using light and scanning electron microscopy. Based on detailed observations of millipedes from 14 localities in the distribution of *B. lecontii*, I found the following natural history aspects. The oviposition period of *B. lecontii* was from mid-April to late June and the incubation period lasted 3–4 weeks. Males exclusively cared for eggs, but care of juveniles was not observed. In one case, the clutches of two males became combined and they were later cared for by only one of the males. The defensive compound of *B. lecontii* consisted of two isomers of the alkaloid deoxybuzonamine. Defense glands were large, occupying up to a third of the paranotal volume, and were present on all but the first four body rings. Stadia I juveniles do not have defensive secretions and stadia II juveniles have defensive pores but do not secrete. Secretions were observed only in stadia III millipedes and older. I observed *Brachycybe lecontii* feeding on liquids from fungi of the order Polyporales, and describe a cuticular structure on the tip of the labrum that may relate to fungivory. I found that pinwheel-shaped aggregations do not form in the absence of fungus and suggested the aggregation is associated with feeding. I describe and illustrate a previously undescribed comb-like structure on the tibia and tarsi of the six foremost leg-pairs and measure and analyze the spectral reflectance of *B. lecontii* exoskeleton.

**General Audience Abstract**

Millipedes are important members of the ecosystem as decomposers. By eating dead vegetation such as wood, leaves and other detritus, millipedes fragment the material thereby allowing further breakdown by fungi, bacteria, and other microbes. Microbial decomposition further reduces the detritus into its chemical constituents (e.g., carbon, nitrogen, and simple sugars) thereby releasing these materials into the ecosystem for future generations of life to use. In addition to the millipedes' critical role in the ecosystem as decomposers, they are fascinating and yet understudied. For example, millipedes of the species *Brachycybe lecontii* are pink, have males that exclusively care for the young (a rarity amongst arthropods), form star-shaped aggregations of individuals, and emit a novel alkaloid-based chemical secretion. By understanding the natural history of this local Appalachian species, I provided a fundamental basis for future studies of its sociality, chemical defense, and evolution.

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## 1. Introduction

The genus *Brachycybe* (Platydesmida: Andrognathidae) consists of severn nominal species with a fragmentary distribution in southeastern and south-central U.S., western U.S. along the Pacific Coast, Japan, South Korea, east central China, and Taiwan (Decker, 2014; Shelley et al., 2005). Based on a molecular phylogenetic dating analysis, the genus diverged from related andrognathid taxa approximately 50 MYA; the divergence between Asian, eastern North American, and western North American species of *Brachycybe* occurred 17–20 MYA; and the minimum divergence between all U.S. species is 13 MYA (Brewer et al., 2012).

Across its distribution, *Brachycybe* can be found in mesic temperate deciduous forests on decaying logs in groups of up to a hundred individuals. Field notes by Cope (1870), Hoffman (1950) and Shelley et al. (2005) on *Brachycybe* habitat describe encountering them in the following microhabitats “under bark of fallen logs”; “on a dry hillside in oak woods”; “riparian hardwood forest”; “under rock”; “side of a tree”; “in incision in beech tree”; “litter in cultivated area near falls”; and “under dead pigs”. They are frequently found in close proximity to fungus and are assumed to feed on the fungal tissue (Gardner, 1974). One species, *B. producta,* was observed eating corticioid Basidiomycete fungi in the genus *Peniophora* (Gardner, 1974). The identification of the fungal food sources of the other species of *Brachycybe* remain unknown.

Despite their small body size and outwardly uniform appearance, *Brachycybe* individuals are clothed with a variety of microscopic phaneres including previously-unnoticed adornments of the cuticle when viewed under magnification. *Brachycybe* individuals are small, only reaching 4–5 cm in length and up to 4 mm in width (Gardner, 1974). Like all platydesmidan millipedes, they are lightly pigmented with coloration varying from pale pink to salmon and red (Gardner, 1974; Shelley et al., 2005). The flat, keel-shaped paranota are large, comprising up to 60% of an individual's body width, and contain defense glands described as the shape of “slender, lengthy tubes” (Gardner, 1974; Wood, 1864; Alsop, unpublished observations). Heads of *Brachycybe* are small, roughly one-third their body width, and exhibit reduced

mandibles and chewing musculature, including the apodemes (Gardner, 1974; Manton, 1961). Eyes are absent and the anterior portion of the head is covered by the facial shield, a large plate (Gardner, 1974; Shelley et al., 2005). The small head, which when viewed from above, is mostly or entirely obscured by the relatively large collum (the first body ring), thereby providing the basis for the genus name *Brachycybe*, Greek for "short head" (Gardner, 1974). Their dorsoventrally flattened body and closely spaced paranota impart a feather-like appearance, providing the colloquial name the "feather millipedes".

Species-level morphological identification does not include features of the gonopods as they are largely uniform due to their primitive leg-like appearance (Brewer, 2012; Gardner, 1974). Distinct gonopodal features between species have been observed, but insufficient data have been collected on gonopod shape variation of *Brachycybe* species to determine if these traits are indicative if species-level differences. The small size of the gonopods made them impractical for identification purposes without the use of high magnification optics like scanning microscopy (Brewer, 2012; Gardner, 1974). Somatic features, for example the appearance of the paranota and collum, have been traditionally used for species identification (Brewer, 2012; Gardner, 1974). For example, the two eastern species—*B. lecontii* and *B. petasata*—can be confidently differentiated in the field based on the presence of a deep anterior incision on the collum of the latter.

A notable aspect of *Brachycybe* millipedes is that they display social behaviors, and occur in perennial colonies of individuals displaying overlapping generations (Gardner, 1974). Sociality meaning that the organisms have one or more of the following characteristics: (1) division of labor with a caste system composed of reproductive and non-reproductive members, (2) cooperation in caring for the young, and (3) overlapping generations (Gullan and Cranston, 2014). A biological rarity in in arthropods, this trait has evolved independently in the Platydesmida since all other millipedes are solitary. One exception, are a few species of the Polydesmida that have been found in masses of hundreds of individuals that move synchronously taking on the appearance of a single larger animal (Bellairs et al., 1983). Several platydesmidan taxa, including *Brachycybe*, are known for their aggregative behavior where multiple individuals arrange themselves radially, with their heads placed together and their bodies radiating outward like spokes of a wheel (Lewis, 1984; Shear, 2015). These aggregations are stable and persistent, and individuals in the formation do not appear to move when viewed with the unaided eye. Henceforth called

"pinwheels", these aggregations in platydesmidan millipedes have been hypothesized to be related to defense or feeding (Dury et al., 2014; Lewis, 1984; Shear, 2015).

Platydesmidan millipedes display subsocial behavior and not eusociality since they apparently lack a caste system and all individuals are likely able to reproduce. Members of Platydesmida show cooperation in care for the young. Paternal care of eggs is displayed by the colobognath genera *Brachycybe*, *Platydesmus*, *Pseudodesmus*, and *Yamasinaium* (Kudo et al., 2009; Kudo et al., 2010; Lewis, 1984). In *Brachycybe*, paternal care has been observed in five species, and likely occurs in the other three species as well: *B. lecontii* and *B. petasata* in the eastern U.S., *B. producta* and *B. rosea* in the western U.S., and *B. nodulosa* in Japan and South Korea (Gardner, 1974; Kudo et al., 2009; Kudo et al., 2010; Murakami, 1963; Tallamy, 2001). Research on paternal care has focused primarily on *B. nodulosa*, the sister species to *B. lecontii*. In *B. nodulosa*, females lay clutches of 23–78 eggs directly into care of the male that receives the eggs in a basket formed by the legs. The eggs are then brooded by the male until hatching. Paternal care has been experimentally shown to be required for the survival of the eggs (Kudo et al., 2010; Murakami, 1962a; Murakami, 1963).

### 1.1 Study Taxon - *Brachycybe lecontii*

*Brachycybe lecontii* (Wood, 1864) was the first androgнathid millipede described from the U.S. based on collections from "Georgia", with no further locality details provided. Wood (1864) first placed *B. lecontii* in the family Siphonophoridae. However, in 1869, the family Androgнathidae was created by Edward Drinker Cope to contain the new genus *Androgнathus* (type species *Androgнathus corticarius*), and a year later, Cope included *Brachycybe* to this family (Cope, 1869; Cope, 1870). Androgнathids were

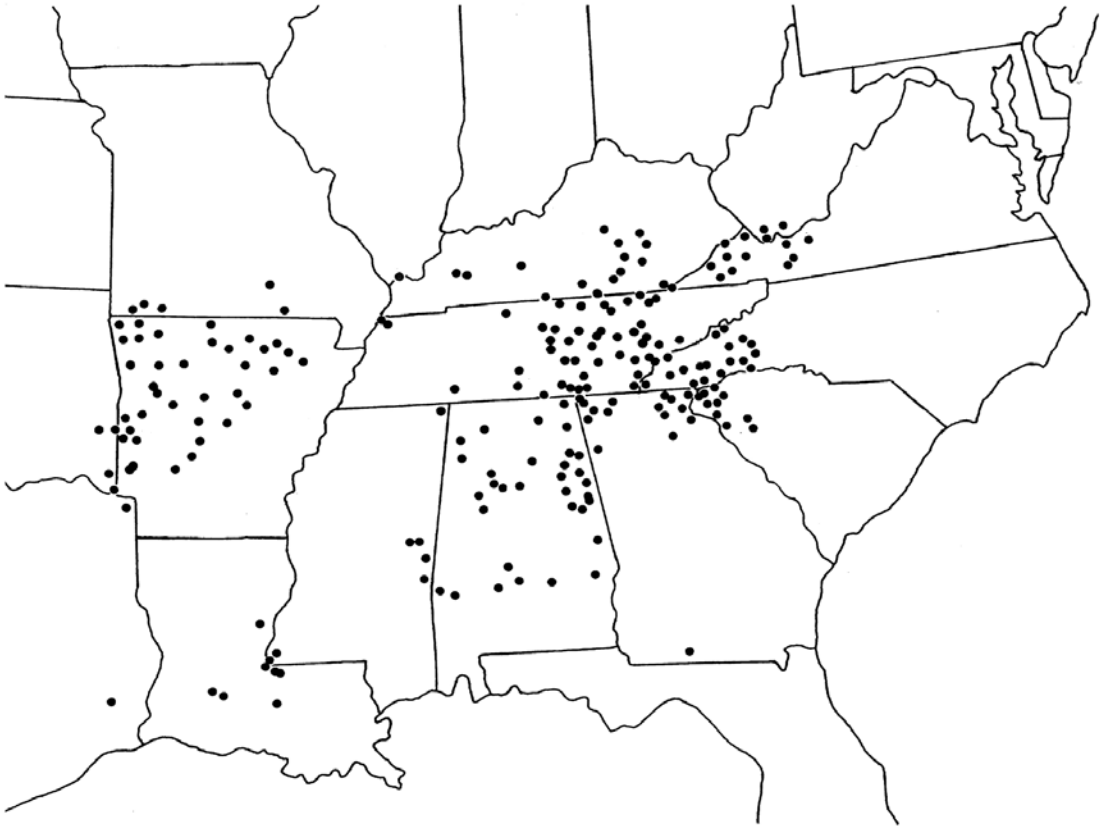

**Figure 1.1. *Brachycybe lecontii* has a large, fragmented range, extending from southern Missouri and West Virginia to eastern Texas and Georgia. Map adapted from Shelley et al. (2005).**

subsequently divided up and placed in the families Polyzoniidae and Platydesmidae until 1928, when Cook and Loomis resurrected the family Androgнathidae and placed it in the order Platydesmida (Bollman, 1983; Brolemann, 1900; Gardner, 1974; McNeill, 1888).

*Brachycybe lecontii* shares its genus with eight species: *Brachycybe petasata* (Southern Appalachians), *Brachycybe picta* (coastal California), *Brachycybe producta* (California and Oregon), *Brachycybe rosea* (Sierra Nevada Range, California), *Brachycybe nodulosa* (Japan, South Korea),

*Brachycybe disticha* (Taiwan), and *Brachycybe cooki* (China) (Shelley et al., 2005). The species are differentiated by whether the collum conceals the head (*B. picta* and *B. petasata*), and the tubercle pattern on the tergum. Brewer et al. (2012) reconstructed a species phylogeny of the genus (excepting *B. cooki*) using two mitochondrial genes and a nuclear gene, and found that *B. nodulosa* is phylogenetically nested within a clade containing the U.S. species, and is the sister species of *B. lecontii*. The molecular phylogeny indicated that the genus *Brachycybe* may be under-split taxonomically and contain additional unnamed species in the U.S. The clade composing *B. lecontii* is made up of four phylogenetically and geographically distinct (parapatric) clades; however, the groups are not distinguishable by morphological features (Brewer et al., 2012). Shelley et al. (2005) synthesized available locality information on *B. lecontii* and showed that the species has a large range across the eastern U.S. [Figure 1.1]. The authors indicated that that the widespread eastern species *B. lecontii* is composed of five geographically delineated relictual populations in the Ozark-Appalachian Region (Shelley et al., 2005) suggested. Brewer et al. (2012) refrained from naming the clades discovered in their analysis as species, as they indicated they were relatively recent in origin and lacked support from morphological characters.

The natural history of *Brachycybe* is limited to taxonomic studies and the detailed work of Murakami on the development of the East Asian species *B. nodulosa* (Murakami, 1962a, 1962b, 1963). Youngsteadt and McAllister (2014) recorded notes on *B. lecontii* from Arkansas, and suggested that the species feeds on microorganisms that live on rotting wood; however, the authors did not directly observe the millipedes feeding on microorganisms or describe how this was inferred. Youngsteadt and McAllister observed molting, a process that occurred over 10 days, and found that the millipedes did not construct a molting chamber or eat their shed exoskeletons as in other millipedes (Youngsteadt and McAllister, 2014). They observed a single adult male with a clutch of 24 eggs, which hatched in 21 days (Youngsteadt and McAllister, 2014).

Like many species of millipede, *B. lecontii* produces a chemical defense compound that is stored within cuticle-lined glands located inside their paranota (Eisner, 1978; Shear, 2015). The defense glands line both sides of the body, and begin on ring four. The chemical compound is structurally similar to the alkaloid buzonamine produced by the millipede *Buzonium crassipes* (Shear, 2015; Shelley et al., 2005). Buzonamine was found to repel the ant *Formica obscuripes* in an experimental antipredator bioassay

(Wood, 2000). Alkaloids such as buzonamine appear to be an evolutionarily derived defense chemical, and the primitive chemicals in millipedes are lower mass phenols and cresols (Shear, 2015).

### 1.2 Objectives

Despite its cryptic diversity and fascinating biological aspects, little is known about millipedes in the genus *Brachycybe*. Here, I synthesize available published data for the species, *B. lecontii*, and provide new observations on the life history of this species that include descriptions of anatomy, morphology, development, observations of social behavior, and aspects of parental care. My natural history study of *B. lecontii* combines field and laboratory observations, and focuses on feeding, pinwheel aggregations, and chemical defenses.

## 2. Materials and Methods

I collected *Brachycybe lecontii* specimens from 14 localities in six states in the eastern U.S. during the summers of 2015–2017: Alabama, Arkansas, North Carolina, Missouri, Tennessee, and Virginia [Figure 1.2, Table 1.1]. Millipedes were located by visiting deciduous forests and examining the undersides of logs, or by raking leaf litter and collecting specimens by hand according to Means et al. (2015). Specimens were stored in vials or small plastic containers. To maintain a humid and habitable environment for the live specimens, the containers were filled with moistened pieces of decaying wood, fungus, and moss. Specimens were retained in their original containers and kept alive in the laboratory at room temperature in dark cabinets for observation and the experimentation. Containers were monitored and if the substrate in the containers was almost dry to the touch, deionized water was added to maintain a mesic environment.

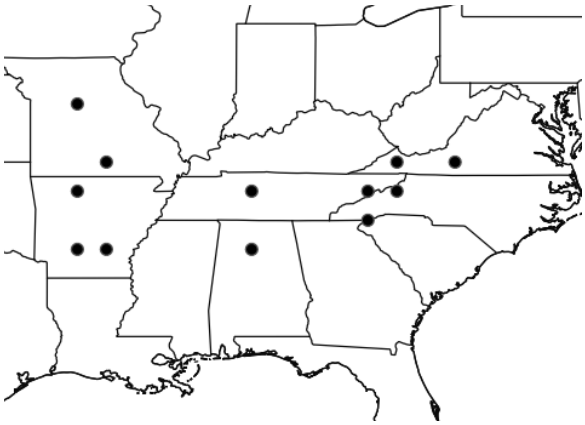

**Figure 1.2. A map of localities where I collected *B. lecontii* specimens. Several points represent multiple localities in close proximity to one another.**

I recorded natural history observations in the field during Summer 2017, noting the arrangement of individuals in pinwheels using drawings and photographs. Specimens were observed for a minimum of 10 minutes, and I recorded their arrangements, any behaviors, and the interactions of the specimens during this period. Notes and drawings were recorded on acid-free collection cards. Collection cards were printed with pre-determined empty fields, including information on localities and the specimens collected at the localities; specifically, the fields were: collection code, state or district, county or subdistrict, locality description, global positioning system (GPS) latitude, GPS longitude, barometric elevation or GPS elevation, number of GPS satellites, GPS accuracy, mountain or mountain range, collecting method, GPS waypoint name, date, time, habitat, and collectors. The collection card also included fields for the sex, abundance, and developmental stage of specimens and—if present—pinwheel diameter, number of individuals within the pinwheel, and the sex and developmental stage of individuals composing a pinwheel. The sex and developmental stage (stadium, number of rings) of individuals were recorded in the laboratory

[Table 1.2]. For this study, the term “ring” is used to refer to diplosegments. Counts of the body rings include the telson. The systematic observation of natural history described above was recorded from specimens collected by VLW in 2017. Other specimens, including those collected earlier than 2017 and where VLW was not listed as a collector were not part of the natural history observations presented in this study [Table 1.1].

Each specimen was assigned a unique identification number. Individuals collected were given an identifier beginning with “MPE-” or “BLIV-” and followed by a unique five-digit string. Pinwheel aggregations of individuals were given an identification number of their own: “BLCV-” or “BLC-” followed by a four-digit string [Table 1.1].

I used scanning electron microscopy to examine the adult morphology and document the development from egg to adult. Specimens for imaging were fixed in 70% isopropyl alcohol, then air-dried at room temperature and humidity before mounting on 12.7 mm or 25.4 mm diameter aluminum SEM specimen mounts (“stubs”). We used 12 mm and 25 mm adhesive PELCO Tabs (Ted Pella, Inc.) or graphite conductive adhesive #112 (Electron Microscopy Sciences) to attach specimens to the stubs. Specimens were sputter coated with a 20-nm thick layer of a mixture of platinum and palladium metals in a Leica EM ACE600 High Vacuum Coater. Images were acquired using a FEI Quanta 600 FEG environmental scanning electron microscope at the Virginia Tech Institute for Critical Technology and Applied Science.

**Table 1.1. The localities of *B. lecontii* specimens, with notes on the habitat in which they were found.**

| Date | Locality code | State | Coordinates | Collectors | Habitat | Specimens |
|---|---|---|---|---|---|---|
| **29.xii.2015** | VLW-2015-001 | Virginia | N37.02528 W-80.77522 | VL Wong, P Marek, J Means, P Shorter | Under tulip poplar log | MPE00811 - MPE00813 |
| **22.iv.2016** | MK-2016-010 | Arkansas | N34.611944 W-93.165556 | M Kasson | - | BLC10 |
| **23.v.2016** | JCM-2016-033 | Alabama | N34.099670 W-87.31973 | J Means, DA Hennen | On big leaf magnolia log; pine, maple, beech, oak, big leaf magnolia | MPE02306 - MPE02321 |
| **25.v.2016** | JCM-2016-042 | Tennessee | N36.26516 W-82.23001 | J Means, DA Hennen | Moist litter with dark, loamy soil; hemlock, maple, oak, tulip poplar, rhododendron | MPE02064, MPE02066, MPE02071, MPE02076, MPE02080 |
| **9.x.2016** | JCM-2016-111 | Tennessee | N36.04861 W-83.74870 | J Means, DA Hennen | Dry deciduous litter, some dead branches | MPE02302 - MPE02305 |
| **12.v.2017** | DAH-2017-0512-02 | Tennessee | N35.899060 W-83.948364 | DA Hennen, J Means, VL Wong | On hardwood log with fungus, in leaf litter; moist (raining); oak, hickory, beech, maple | BLIV0001 - BLIV0007 |
| **13.v.2017** | DAH-2017-0513-02 | Tennessee | N36.10172 W-87.28539 | DA Hennen, J Means, VL Wong | On hardwood logs, branches with lichen and fungi; in moist deciduous leaf litter, particularly beech | BLCV0001-001 - BLCV0001-015, BLCV0002-001 - BLCV0002-011, BLCV0003-001 - BLCV0003-011, BLIV0008 - BLIV0020 |
| **16.v.2017** | DAH-2017-0516-01 | Arkansas | N34.7005 W-92.2606 | DA Hennen, J Means, VL Wong | On hardwood log with fungi on underside; ironwood, hickory, maple, pawpaw, oak | BLCV0004-001 - BLCV0004-029, BLCV0005-001 - BLCV0005-007, BLIV0021 - BLIV0045 |

| Date | Locality code | State | Coordinates | Collectors | Habitat | Specimens |
|---|---|---|---|---|---|---|
| **16.v.2017** | DAH-2017-0516-02 | Arkansas | N36.03763 W-93.34127 | DA Hennen, J Means, VL Wong | On hardwood branch, log with fungus, in leaf litter; beech, ironwood, maple, musclewood, oak | BLIV0046 - BLIV0065 |
| **17.v.2017** | DAH-2017-0517-01 | Arkansas | N36.43071 W-93.75764 | DA Hennen, J Means, VL Wong | On bark on underside of fallen branch; maple, sycamore, oak, pawpaw, cedar | BLIV0066 |
| **17.v.2017** | DAH-2017-0517-02 | Missouri | N37.13226 W-92.32411 | DA Hennen, J Means, VL Wong | On hardwood branch with fungus, in dry litter; oak, hickory, black cherry, juniper | BLCV0006-001 - BLCV0006-005, BLIV0067 - BLIV0070 |
| **21.v.2017** | DAH-2017-0521-02 | Virginia | N37.29701 W-82.30064 | DA Hennen, J Means, VL Wong | On hardwood branch, log, under bark; moist leaf litter; oak, hemlock, rhododendron, maple, tulip poplar | BLIV0071 - BLIV0076 |
| **9.viii.2017** | JCM-2017-047 | Tennessee | N36.502222 W-82.4825 | J Means, DA Hennen | Dry oak, maple, hickory forest | BLIV0077 - BLIV0079 |

**Table 1.2. Localities, sexes, and developmental stages of *B. lecontii* specimens observed in the field during Summer 2017. Indet. = indeterminate, i.e. specimens too young to be sexed by the presence or absence of gonopods.**

| Date | Locality code | Specimen identifier | Sex | Maturity | No. rings |
|---|---|---|---|---|---|
| **12.v.2017** | DAH-2017-0512-02 | BLIV0001 | Male | Adult | 44 |
| | | BLIV0002 | Female | Adult | 51 |
| | | BLIV0003 | Female | Adult | 45 |
| | | BLIV0004 | Female | Adult | 50 |
| | | BLIV0005 | Female | Adult | 52 |
| | | BLIV0006 | Female | Adult | 44 |
| | | BLIV0007 | Male | Adult | 45 |
| **13.v.2017** | DAH-2017-0513-02 | BLCV0001-001 | Male | Adult | 39 |
| | | BLCV0001-002 | Female | Juvenile | 30 |
| | | BLCV0001-003 | Female | Juvenile | 32 |
| | | BLCV0001-004 | Female | Juvenile | 24 |
| | | BLCV0001-005 | Female | Juvenile | 25 |
| | | BLCV0001-006 | Indet. | Juvenile | 22 |
| | | BLCV0001-007 | Male | Adult | 40 |
| | | BLCV0001-008 | Indet. | Juvenile | 23 |
| | | BLCV0001-009 | Male | Juvenile | 30 |
| | | BLCV0001-010 | Male | Adult | 41 |
| | | BLCV0001-011 | Male | Adult | 44 |
| | | BLCV0001-012 | Female | Adult | 39 |
| | | BLCV0001-013 | Male | Adult | 45 |
| | | BLCV0001-014 | Female | Juvenile | 24 |
| | | BLCV0001-015 | Indet. | Juvenile | 19 |
| **13.v.2017** | DAH-2017-0513-02 | BLCV0002-001 | Male | Adult | 38 |
| | | BLCV0002-002 | Female | Juvenile | 34 |
| | | BLCV0002-003 | Female | Adult | 56 |
| | | BLCV0002-004 | Male | Adult | 42 |
| | | BLCV0002-005 | Female | Adult | 48 |
| | | BLCV0002-006 | Female | Adult | 52 |
| | | BLCV0002-007 | Female | Adult | 56 |
| | | BLCV0002-008 | Male | Adult | 48 |
| | | BLCV0002-009 | Female | Adult | 41 |
| | | BLCV0002-010 | Male | Adult | 49 |
| | | BLCV0002-011 | Female | Adult | 52 |
| **13.v.2017** | DAH-2017-0513-02 | BLCV0003-001 | Female | Adult | 52 |
| | | BLCV0003-002 | Male | Adult | 48 |
| | | BLCV0003-003 | Female | Adult | 46 |
| | | BLCV0003-004 | Female | Adult | 42 |
| | | BLCV0003-005 | Female | Adult | 51 |
| | | BLCV0003-006 | Female | Adult | 47 |
| | | BLCV0003-007 | Female | Adult | 41 |
| | | BLCV0003-008 | Male | Adult | 45 |
| | | BLCV0003-009 | Male | Adult | 39 |
| | | BLCV0003-010 | Female | Adult | 50 |
| | | BLCV0003-011 | Male | Adult | 40 |
| **13.v.2017** | DAH-2017-0513-02 | BLIV0008 | Female | Adult | 48 |
| | | BLIV0009 | Female | Adult | 44 |
| | | BLIV0010 | Male | Adult | 45 |
| | | BLIV0011 | Female | Adult | 43 |

| Date | Locality code | Specimen identifier | Sex | Maturity | No. rings |
|---|---|---|---|---|---|
| | | BLIV0012 | Male | Adult | 52 |
| | | BLIV0013 | Female | Adult | 49 |
| | | BLIV0014 | Female | Adult | 53 |
| | | BLIV0015 | Male | Adult | 40 |
| | | BLIV0016 | Male | Adult | 44 |
| | | BLIV0017 | Female | Adult | 46 |
| | | BLIV0018 | Female | Adult | 53 |
| | | BLIV0019 | Male | Adult | 39 |
| | | BLIV0020 | Female | Adult | 45 |
| **16.v.2017** | DAH-2017-0516-01 | BLCV0004-001 | Female | Juvenile | 32 |
| | | BLCV0004-002 | Female | Juvenile | 32 |
| | | BLCV0004-003 | Male | Juvenile | 35 |
| | | BLCV0004-004 | Female | Juvenile | 32 |
| | | BLCV0004-005 | Female | Adult | 46 |
| | | BLCV0004-006 | Male | Juvenile | 31 |
| | | BLCV0004-007 | Male | Juvenile | 29 |
| | | BLCV0004-008 | Male | Juvenile | 35 |
| | | BLCV0004-009 | Female | Juvenile | 31 |
| | | BLCV0004-010 | Male | Juvenile | 35 |
| | | BLCV0004-011 | Male | Juvenile | 32 |
| | | BLCV0004-012 | Female | Juvenile | 35 |
| | | BLCV0004-013 | Female | Adult | 40 |
| | | BLCV0004-014 | Female | Juvenile | 33 |
| | | BLCV0004-015 | Male | Juvenile | 29 |
| | | BLCV0004-016 | Female | Juvenile | 32 |
| | | BLCV0004-017 | Indet. | Juvenile | 23 |
| | | BLCV0004-018 | Indet. | Juvenile | 23 |
| | | BLCV0004-019 | Female | Juvenile | 27 |
| | | BLCV0004-020 | Female | Juvenile | 26 |
| | | BLCV0004-021 | Indet. | Juvenile | 23 |
| | | BLCV0004-022 | Male | Juvenile | 25 |
| | | BLCV0004-023 | Female | Juvenile | 24 |
| | | BLCV0004-024 | Female | Juvenile | 33 |
| | | BLCV0004-025 | Male | Adult | 35 |
| | | BLCV0004-026 | Female | Juvenile | 34 |
| | | BLCV0004-027 | Female | Juvenile | 33 |
| | | BLCV0004-028 | Indet. | Juvenile | 19 |
| | | BLCV0004-029 | Indet. | Juvenile | 17 |
| | | BLCV0005-001 | Female | Adult | 39 |
| | | BLCV0005-002 | Female | Juvenile | 28 |
| | | BLCV0005-003 | Indet. | Juvenile | 23 |
| | | BLCV0005-004 | Female | Juvenile | 27 |
| | | BLCV0005-005 | Female | Juvenile | 34 |
| | | BLCV0005-006 | Male | Juvenile | 24 |
| | | BLCV0005-007 | Female | Juvenile | 34 |
| | | BLIV0021 | Indet. | Juvenile | 18 |
| | | BLIV0022 | Female | Juvenile | 28 |
| | | BLIV0023 | Indet. | Juvenile | 23 |
| | | BLIV0024 | Indet. | Juvenile | 24 |
| | | BLIV0025 | Female | Juvenile | 31 |
| | | BLIV0026 | Female | Juvenile | 32 |
| | | BLIV0027 | Male | Adult | 38 |
| | | BLIV0028 | Male | Adult | 46 |
| | | BLIV0029 | Male | Adult | 37 |

| Date | Locality code | Specimen identifier | Sex | Maturity | No. rings |
|---|---|---|---|---|---|
| | | BLIV0030 | Male | Adult | 39 |
| | | BLIV0031 | Male | Adult | 36 |
| | | BLIV0032 | Male | Juvenile | 29 |
| | | BLIV0033 | Male | Adult | 41 |
| | | BLIV0034 | Male | Juvenile | 32 |
| | | BLIV0035 | Female | Juvenile | 31 |
| | | BLIV0036 | Male | Juvenile | 30 |
| | | BLIV0037 | Male | Adult | |
| | | BLIV0038 | Male | Adult | 38 |
| | | BLIV0039 | Female | Adult | 48 |
| | | BLIV0040 | Female | Adult | 47 |
| | | BLIV0041 | Female | Adult | 48 |
| | | BLIV0042 | Male | Adult | 39 |
| | | BLIV0043 | Male | Adult | 40 |
| | | BLIV0044 | Female | Adult | 41 |
| | | BLIV0045 | Female | Juvenile | 33 |
| **16.v.2017** | DAH-2017-0516-02 | BLIV0046 | Female | Adult | 50 |
| | | BLIV0047 | Female | Adult | 47 |
| | | BLIV0048 | Female | Juvenile | 30 |
| | | BLIV0049 | Male | Adult | 44 |
| | | BLIV0050 | Male | Adult | 41 |
| | | BLIV0051 | Male | Adult | 48 |
| | | BLIV0052 | Male | Adult | 42 |
| | | BLIV0053 | Female | Adult | 50 |
| | | BLIV0054 | Female | Adult | 51 |
| | | BLIV0055 | Female | Adult | 47 |
| | | BLIV0056 | Female | Adult | 47 |
| | | BLIV0057 | Female | Adult | 42 |
| | | BLIV0058 | Female | Adult | 40 |
| | | BLIV0059 | Female | Adult | 46 |
| | | BLIV0060 | Female | Adult | 53 |
| | | BLIV0061 | Female | Adult | 39 |
| | | BLIV0062 | Female | Adult | 48 |
| | | BLIV0063 | Female | Adult | 45 |
| | | BLIV0064 | Female | Adult | 41 |
| | | BLIV0065 | Female | Adult | 49 |
| **17.v.2017** | DAH-2017-0517-01 | BLIV0066 | Female | Adult | |
| **17.v.2017** | DAH-2017-0517-02 | BLCV0006-001 | Female | Adult | 50 |
| | | BLCV0006-002 | Female | Juvenile | 28 |
| | | BLCV0006-003 | Female | Juvenile | 29 |
| | | BLCV0006-004 | Indet. | Juvenile | 20 |
| | | BLCV0006-005 | Indet. | Juvenile | 18 |
| **21.v.2017** | DAH-2017-0521-02 | BLIV0067 | Male | Adult | 49 |
| | | BLIV0068 | Female | Juvenile | 36 |
| | | BLIV0069 | Female | Adult | 47 |
| | | BLIV0070 | Female | Juvenile | 49 |
| | | BLIV0071 | Female | Adult | 51 |
| | | BLIV0072 | Female | Adult | 50 |
| | | BLIV0073 | Female | Adult | 52 |
| | | BLIV0074 | Female | Adult | 42 |
| | | BLIV0075 | Female | Adult | 58 |
| | | BLIV0076 | Female | Adult | 51 |

The cuticle of *B. lecontii* is transparent thereby allowing examination of internal anatomy such as defense glands with light microscopy and photography, and without needing to enzymatically clear tissues. I used a Canon 6D digital camera with 65 mm and 50 mm lenses attached to a Visionary Digital Passport II system (Dunn Inc., Charlottesville, VA) to photograph live specimens. A Leica M125 stereomicroscope and Zeiss Axio Imager 2 light microscope was used to examine defense glands, internal organs, and gut contents of dissected specimens. Images were taken on the Zeiss microscope with a Zeiss Axiocam ERc 5s camera and Axiovision imaging software (AxioVs40 V 4.8.2.0 Carl Zeiss MicroImaging, Germany). Microscope specimens were killed by freezing for 5–10 minutes before examination. To observe gut contents, specimens that were observed feeding were selected prior to being frozen and placed in alcohol for examination. I used Adobe Illustrator CS6 and Adobe Photoshop CS6 for illustrating morphological features.

Chemical analysis of the defensive secretion of *B. lecontii* was done in collaboration with Dr. Tappey Jones of the Virginia Military Institute (VMI) to confirm the previous identifications of Shear (2015). Ten adult millipedes from a single locality were placed in a vial with 1 ml of 100% methanol (HPLC grade). The vials were sealed with Teflon-lined lids and mailed to VMI, where they were analyzed utilizing gas chromatography–mass spectrometry techniques. Specimens were selected from two Tennessee collection sites (i.e. DAH-2017-0512-02, DAH-2017-0513-02), three Arkansas collection sites (i.e. DAH-2017-0516-01, DAH-2017-0516-02, DAH-2017-0517-01), and one Virginia collection site (i.e. DAH-2017-0521-02).

To quantify the color of *B. lecontii*, I measured reflectance spectra from 18 live specimens (11 females, seven males) from seven localities in Arkansas, Missouri, Tennessee, and Virginia. I used an Ocean Optics USB4000 spectrometer calibrated with a Labsphere white reflectance standard (AS-01158-060 USRS-99-010 BZ37A), DH-2000-BAL UV-VIS light source, and Ocean Optics SpectraSuite software. Specimens were held 6 mm from the end of the spectrometer probe and oriented so the widest section of the dorsal side of specimens were at a 90-degree angle to the spectrometer probe to ensure standardized color readings. The relationship between the percent reflectance by wavelength was graphed using the R package pavo (Maia et al., 2013).

## 3. Results

### 3.1 Life History

On May 13 and 16, 2017, stadium I juveniles and males brooding eggs were observed in the field at the Tennessee and Arkansas localities (DAH-2017-0513-02 and DAH-2017-0516-01). Eggs were 6–7 mm in diameter. I examined them using light and scanning electron microscopy and found that the eggs were light orange in color, with no apparent surface sculpturing [Figure 1.3]. The eggs from these localities hatched on May 24, 11 and 8 days after they were observed in the field. A colony of live adult *B. lecontii* from the Arkansas locality (DAH-2017-0516-02) were maintained in the lab and laid separate clutches of eggs on May 24 and June 2. The May 24 clutch hatched on June 16, 24 days after they were laid, and the June 2 clutch hatched on June 27, 26 days after they were laid.

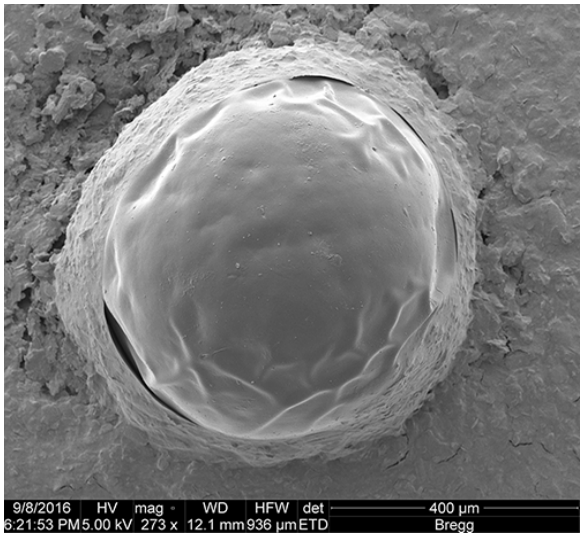


**Figure 1.3. Scanning electron micrograph of a *B. lecontii* egg. Aside from some shallow indentations due to desiccation, the egg surface is smooth.**

I observed the hatchling of these clutches from Arkansas and found the eggshells were not consumed by the newly-hatched stadium I, nor were they consumed by any mature *B. lecontii* individuals. Of 50 stadium I individuals examined, all were 1.5–2 mm in length and had seven rings and five pairs of walking legs [Figure 1.4, Figure 1.5]. The number of rings and leg pairs varied from stadium II onwards [Figure 1.6]. The transition of leg pairs 9–10 from walking legs to gonopods in males could be discerned at a minimum of 24 rings (BLCV0005-006). The gonopods were

**Figure 1.4. A hatching *B. lecontii*. Five leg pairs and seven body rings are visible.**

observed to be fully sexually developed at a minimum of 35 rings (BLCV0004-025). Dissection of females to determine the presence of mature eggs in the ovaries indicated that sexual maturity occurred at a minimum of 39 rings (BLCV0005-001).

Molting was observed in 10 individuals. These individuals were selected because of their characteristic pre-molting appearance where their bodies appeared swollen, particularly in the posterior rings. These molting individuals were observed for 3–4 days. The individuals moved either deeper to the bottom of their containers or into a crevice in the substrate where they curled up for a period of 3–5 days. The process of molting lasted 1–2 days and was only observed in eight individuals since two were hidden in the substrate and were concealed from view. The dorsal side of the exuvium split horizontally, roughly halfway down each individual's body; maintaining a curled position, individuals then wriggled from the old exuvium. Freshly molted millipedes were soft and fragile but when prodded with a soft paintbrush were capable of locomotion within a day of emerging. New rings were visibly whiter in color and the defensive glands (observed through the translucent cuticle) contained no visible secretion. Color darkened and gland contents were present within two weeks. I did not observe *B. lecontii* constructing molting chambers or consuming shed exuvia.

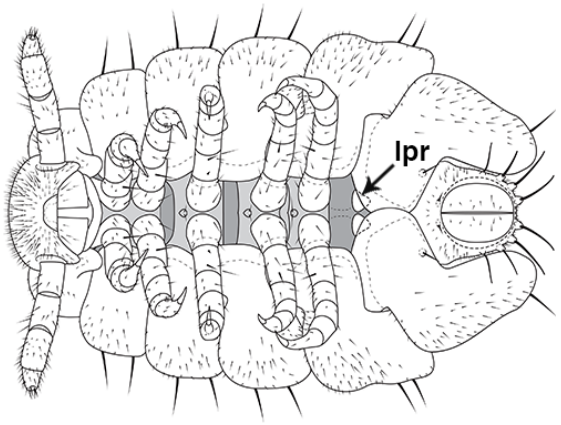


**Figure 1.5. Illustration of a stadium I *B. lecontii*. Coxal sacs have yet to develop on leg pairs. Leg pair rudiments (lpr) may be visible but are frequently hidden by overlapping pleurotergites.**

*Brachycybe lecontii* has coxal sacs that first develop in stadium II on leg pairs 3–5 [Figure 1.7]. Frequently referred to as eversible glands, coxal sacs are found in many species of colobognath millipedes and function to absorb water from their surroundings. I found that during development coxal sacs were frequently not visible on newly added leg pairs. Of 24 specimens examined, 23 had legs without coxal sacs, with the exception of one adult male that had coxal sacs on all walking legs [Figure 1.8]. In one specimen, asymmetric development of coxal sacs was observed, and the left leg developed a coxal sac prior to the right one [Figure 1.9].

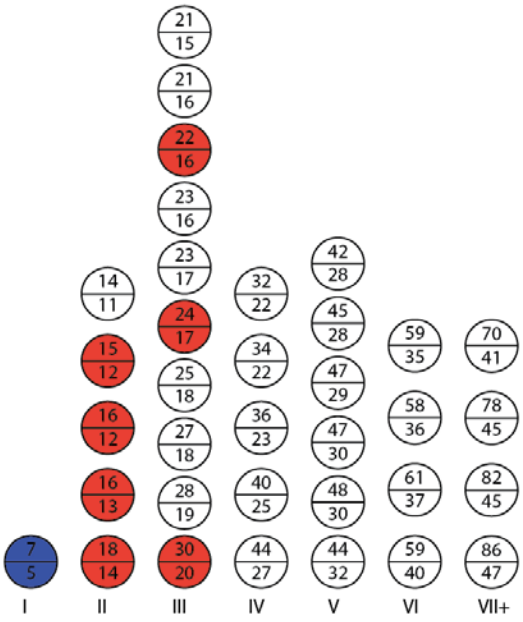


**Figure 1.6. Number of body rings (top) and leg pairs (bottom) in *B. lecontii* specimens for which stadium number could be established, not encompassing the total possible range of rings present in each stadium. White circles = 1 individual, red = 2-5, blue = 50.**

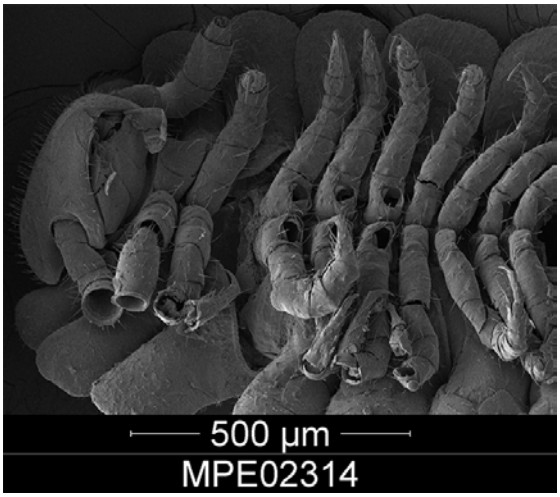


**Figure 1.7. A stadium II *B. lecontii* with coxal sacs visible on leg pairs 3–5.**

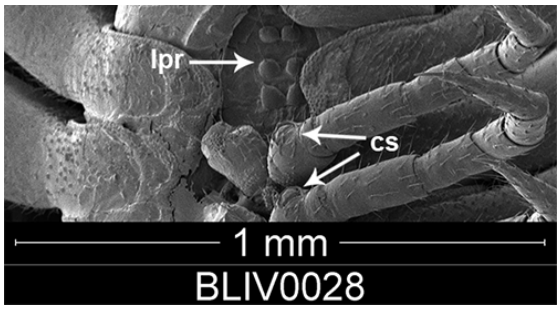


**Figure 1.8. Scanning electron micrograph showing coxal sacs (cs) on the posterior fully-developed walking legs of an adult male *B. lecontii*. Three leg pair rudiments (lpr) are also visible.**

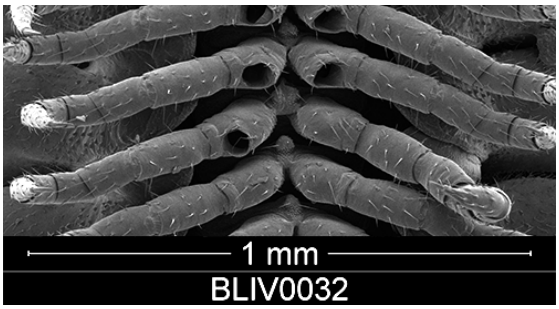


**Figure 1.9. A juvenile male *B. lecontii* (BLIV0032) displaying asymmetrical coxal sac development.**

### 3.2 Paternal Care

Male *B. lecontii* with eggs were observed in the field at the Tennessee and Arkansas localities: DAH-2017-0513-02 (BLIV0010) and DAH-2017-0516-01 (BLIV0037). Subsequently in the lab, three additional males from the Arkansas locality possessed clutches of eggs (individuals BLIV0050-0052 from

DAH-2017-0516-02). The males curled around the clutches of eggs with the entire length of their body trunks but with some posterior legs anchoring the brooding male to the substrate [Figure 1.10]. When lightly prodded with a paintbrush, the males would curl tighter around their eggs but would not abandon the clutch. When exposed to bright light from the fiber optic light source of a microscope, the males would crawl towards the shade, with the anterior portion of the body being used to walk and the posterior half remaining wrapped around the eggs.

When artificially separated from their eggs, the males would seek them out and collect them. Males did not move far from the original location of brooding when seeking out the eggs, and would frequently fail to relocate and collect eggs if relocated further than 2 cm away from the millipede. Non-brooded eggs consistently did not hatch, while the majority of brooded eggs successfully hatched, with 0–2 nonviable eggs observed per clutch. In the field, no adults were seen brooding juvenile millipedes. In the lab, adult males with clutches were observed to stop brooding and move away from the natal site once eggs in the clutch began to hatch.

**Figure 1.10. Male *B. lecontii* (BLIV0037) with his clutch of eggs, and an aggregation of newly-hatched stadia I juveniles departing from their natal site. Photograph by Derek Hennen.**

Males did not appear to discriminate between eggs from their own clutch and eggs from other males' clutches. In a laboratory colony of individuals, one male (BLIV0051) abandoned his clutch; subsequently, a second male—also with a clutch (BLIV0052)—collected BLIV0051's eggs to increase its own clutch size to 115 eggs. Observed clutch sizes, excluding the aforementioned combined clutch, ranged from 54 to 70 eggs, and average clutch size—including the aforementioned combined clutch—was 59 eggs (n = 5).

### 3.3 Defense

Like other platydesmidan millipedes, *Brachycybe* individuals have a type 2 defensive gland architecture, which comprise a single chamber containing the liquid defensive secretions, and a duct leading from the sac to an ozopore, the opening of the gland [Figure 1.11] (Eisner et al., 1978). I found the defensive glands to be large, and occupied up to a third of the paranotal volume [Figure 1.12]. The defensive chemical was composed of two isomers of the alkaloid deoxybuzonamine (J. Tappey, pers. comm.), which is liquid, and has a bubbled appearance when the glands are viewed from outside of the cuticle [Figure 1.13]. When millipedes were disturbed by handling, the defensive chemical was secreted from the ozopores in discrete droplets. After oozing from the ozopore the droplets from adjoining paranota coalesced together to form a single clear, colorless droplet. The millipedes appeared to be able to control the location where defense secretions emanated and individual ozopores were triggered based on the location of disturbance along the length of the trunk and from left to right sides.

First stadia *B. lecontii* possessed visible ozopores [Figure 1.14] but defensive compounds

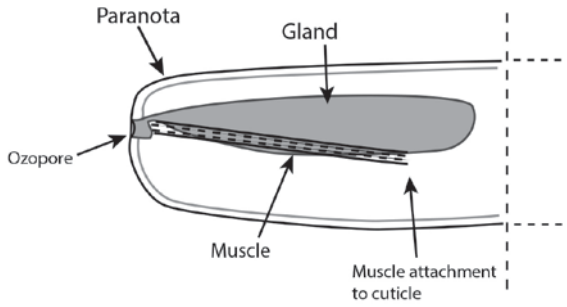


**Figure 1.11. An illustration of a *B. lecontii* repugnatorial gland. The exact position of the gland and ozopore vary: anterior paranota have glands and paranota positioned anteriorly, medial paranota have the structures positioned medially, and posterior paranota have the structures arranged more towards the posterior.**

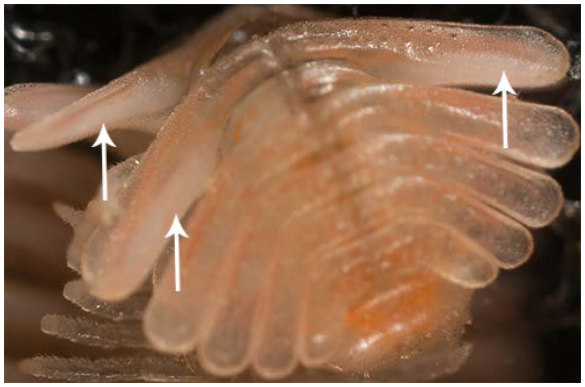

**Figure 1.12. Shown from several angles are the large repugnatorial glands of a live *B. lecontii* (BLIV0080), visible in the figure as white in color.**

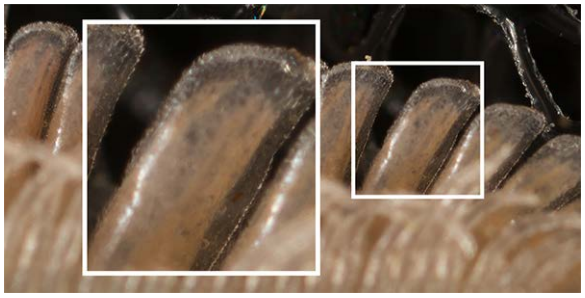

**Figure 1.13. A photograph of a live *B. lecontii* (BLIV0080) with the inset a magnified view showing the bubbles of defensive secretion visible within its defense gland.**

were apparently not present (both visible within the glands and via secretion). In second stadia millipedes, defensive compounds were visible within the paranota, but disturbance of individuals with forceps failed to produce visible secretions. Obvious secretions (both visible within the glands and through secretion) were observed from third stadia onwards.

When I disturbed individuals in pinwheels with a soft-tipped forcep or a paintbrush, defensive secretions were not readily produced. Probing with a paintbrush would make the subjects recoil and flee, but only occasionally would they produce defensive compounds. While disturbing individuals in pinwheels, I did not observe any reaction in non-disturbed individuals.

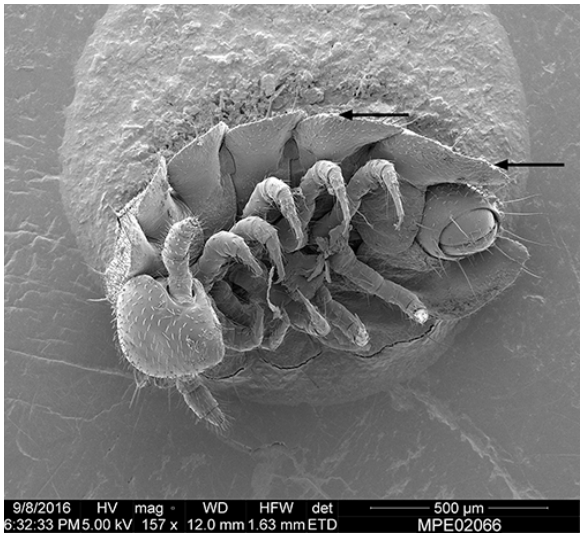


**Figure 1.14. Scanning electron micrograph of a stadium I *B. lecontii* (MPE02066). Arrows point to ozopores, present on body segments 5–6.**

In 2016, during field observations, I observed that when the adults were removed from a pinwheel containing both adults and early-stadia juveniles, an ant (*Camponotus* sp.) grabbed a juvenile and absconded. It is unknown whether the juvenile millipede was subsequently killed and consumed by the ants. In 2017, I observed no predation on *B. lecontii* or interactions between *B. lecontii* and other organisms. While at a locality in Arkansas (DAH-2017-0516-01), I observed a *B. lecontii* individual (BLIV0027) walking in the leaf litter and encountering several ants. The ants neither attacked or investigated the millipede, even though it was contacted by the ants several times. Both pinwheel aggregations and individuals were found on logs inhabited by termites at several localities in Tennessee and Arkansas (TN: DAH-2017-0512-02, DAH-2017-0513-02; AR: DAH-2017-0516-01).

**Figure 1.15. An adult female (BLIV0066) *B. lecontii* collected at locality DAH-2017-0517-01. The only individual uncovered at this locality was found on the underside of a log feeding on fungus.**

### 3.4 Feeding

*Brachycybe lecontii* were often found associated with fungi. Based on my field collections in 2016, I collected fungi that was later identified by mycologist Matthew Kasson (West Virginia University) as species in the order Polyporales, genera *Irpex* and *Trametopsis*, (M. Kasson, pers. comm.). In the field and laboratory, I observed *B. lecontii* with their heads held above or embedded within fungal tissue [Figure 1.15]. Pits or indentations in the surface of the fungus, henceforth called "feeding bowls", were frequently observed when millipedes were removed from the fungal mats [Figure 1.16]. Feeding behavior was determined by the noticeable presence of fungus and the head of the millipede in close proximity to the fungal tissue. Feeding was observed at five of the seven locations in the field [Table 1.3].

I did not observe any visible trace of plant or fungal matter in the gut during dissections. I did not observe *B. lecontii* defecating solid feces though I twice observed *B. lecontii* defecating liquid. The liquid was an approximately 1 mm droplet of clear, colorless fluid. I also observed millipedes regurgitating clear liquid after submergence in 70% isopropyl alcohol.

**Figure 1.16. A pinwheel (BLCV0002) of *B. lecontii* millipedes encircling a fungal growth. In the center of the pinwheel is a depression in the fungus, a sign of fungal feeding (indicated by the arrow). Photograph by Derek Hennen at locality DAH-2017-0513-02.**

**Table 1.3. Fungal proximity of *B. lecontii* specimens observed during Summer 2017. Millipedes were engaged in feeding on fungal mats at all, spare two localities. The two exceptions (DAH-2017-0517-02, DAH-2017-0521-02) had fungus present on the log that the millipedes inhabited, but the millipedes were not directly observed on the fungal mat.**

| Locality | On fungus | Fungus nearby* | Fungus not nearby† |
|---|---|---|---|
| **DAH-2017-0512-02** | 0 | 7<br>(BLIV001 - BLIV007) | 0 |
| **DAH-2017-0513-02** | 39<br>(BLCV0001 - BLCV0003, BLIV0019 - BLIV0020) | 3<br>(BLIV0008, BLIV0009 - BLIV0011) | 7<br>(BLIV0010, BLIV0013 - BLIV0018,) |
| **DAH-2017-0516-01** | 41<br>(BLCV0004, BLCV0005, BLCV0007, BLCV0008, BLIV0041 - BLIV0045) | 7<br>(BLIV0021-BLIV0026, BLIV0037) | 11<br>(BLIV0027 - BLIV0036, BLIV0038) |
| **DAH-2017-0516-02** | 6<br>(BLCV0009, BLIV0048 - BLIV0048) | 17<br>(BLIV0049-0065) | 0 |
| **DAH-2017-0517-01** | 1<br>(BLIV0066) | 0 | 0 |
| **DAH-2017-0517-02** | 0 | 5<br>(BLCV0006) | 4<br>(BLIV0067 - BLIV0070) |
| **DAH-2017-0521-02** | 6<br>(BLIV0071 - BLIV0076) | 0 | 0 |

* Fungus present on the log or branch from where the individual or pinwheel was encountered, or within 10 cm of the individual or pinwheel.

† No fungus present on the log or branch from where the individual or pinwheel was encountered, or not within 10 cm of the individual or pinwheel. The majority of individuals that were not found on or near fungus were found in leaf litter.

### 3.5 Feeding Structures

The gnathochilarium and labrum of *B. lecontii* were visibly appressed. The tip of the labrum was composed of a ramifying, fibrous cuticle with scattered sensilla and sensory pits on the surface [Figure 1.17]. This structure was roughly semicircular in shape with a radius of approximately 20 µm in a stadium I juvenile and 50 µm in an adult female [Figures 1.18, Figure 1.19]. The fibrous structure was concave and therefore not continuous with the overall convex head capsule. The outer semicircular ridge of the fibrous structure was wrinkled in appearance with fibrous strands emanating ventrally towards the gnathochilarium. At the tip of the structure was a ridge, 15 µm in length in an adult, and vertically oriented. In several older individuals, this fibrous labral structure and surrounding setae appeared frayed and worn,

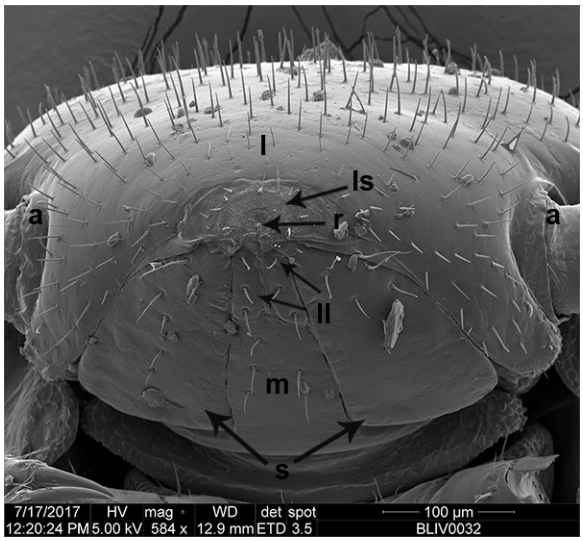


**Figure 1.17. Scanning electron micrograph showing the general shape and location of the cuticular labral structure (ls) and its ridge (r) on a *B. lecontii* head. The labrum (l), antennae (a), and components of the millipede gnathochilarium: the lamina linguales (ll), mentum (m), and stipes (s) are also visible (Gardner, 1974).**

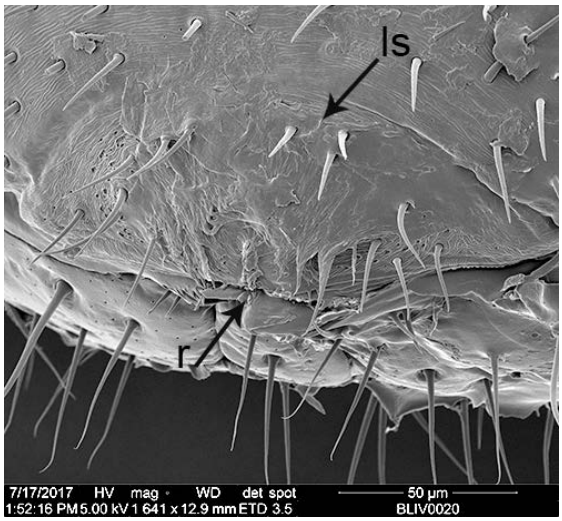


**Figure 1.18. Scanning electron micrograph of the cuticular labral structure (ls) on the tip of the labrum of an adult *B. lecontii* (BLIV0020).**

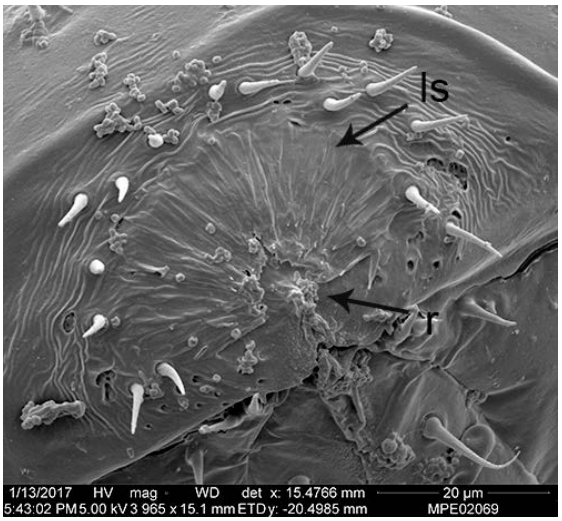


**Figure 1.19. Scanning electron micrograph of the cuticular labral structure (ls) on the labrum of a stadium I *B. lecontii* (MPE02069).**

with setae on the structure broken or missing. In stadium I individuals, the structure showed less damage.

### 3.6 Pinwheeling

The formation of pinwheels was associated with the presence of fungus. In the laboratory, on occasions when no fungus was present, millipedes would aggregate but would not assemble into pinwheels. The centers of pinwheels were frequently atop fungus. Of the 9 pinwheels observed in the field, six of them (BLCV0001, BLCV0002, BLCV0003, BLCV0007, BLCV0008, BLCV0009) included millipedes with their heads visibly embedded in fungus. Three pinwheels (BLCV0004, BLCV0005, BLCV0006) had fungus present elsewhere on the branch or limb upon which the millipedes were aggregated [Figure 1.20].

**Figure 1.20. Two small pinwheel aggregations on fungal mats at collection site DAH-2017-0516-01 in Arkansas. Photograph by Derek Hennen.**

Pinwheels persisted for several weeks. In the laboratory, a *B. lecontii* colony collected in the field from Arkansas site MK-2016-010 maintained a pinwheel for 27 days, from May 23 to June 18. Individuals entered and exited the pinwheel, and the overall formation, particularly the location of the central hub, remained in a constant location.

Individuals in pinwheels varied in developmental stage and sex [Figure 1.21]. Although pinwheels often consisted of numerous juveniles that could not be sexed, among those individuals whose sex could be determined, the pinwheels had a significant difference in sex proportions with consistently more females than males [Figure 1.22]. A chi-squared test of goodness of fit using the totals of males and females in all observed pinwheels found a significant difference from the null hypothesis of a 1:1 sex ratio ($\chi^2 = 6.21$, df = 1, p = 0.013).

At one locality (DAH-2017-0516-01), over a dozen pinwheels of stadia I and stadia II *B. lecontii* were observed [Figure 1.23]. These were not collected as sex could not be determined from early-stadia individuals. Stadia I–II juveniles were also observed pinwheeling with mature female *B. lecontii* on fungus, and when the adults were collected, the juveniles dispersed (locality BLIV0039-0040).

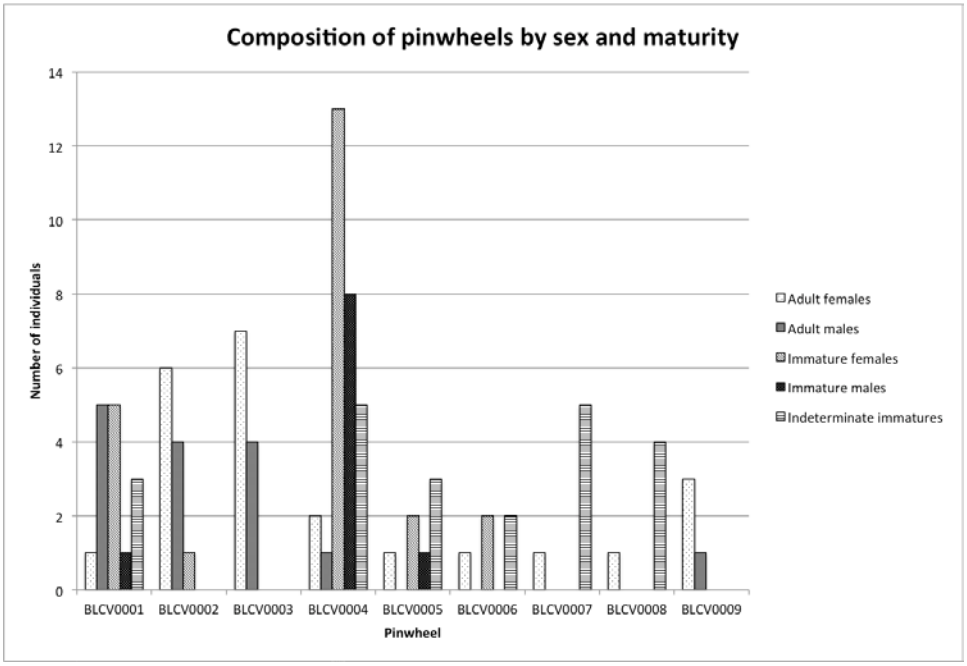


**Figure 1.21. The composition of pinwheels observed during summer 2017, by sex and maturity.**

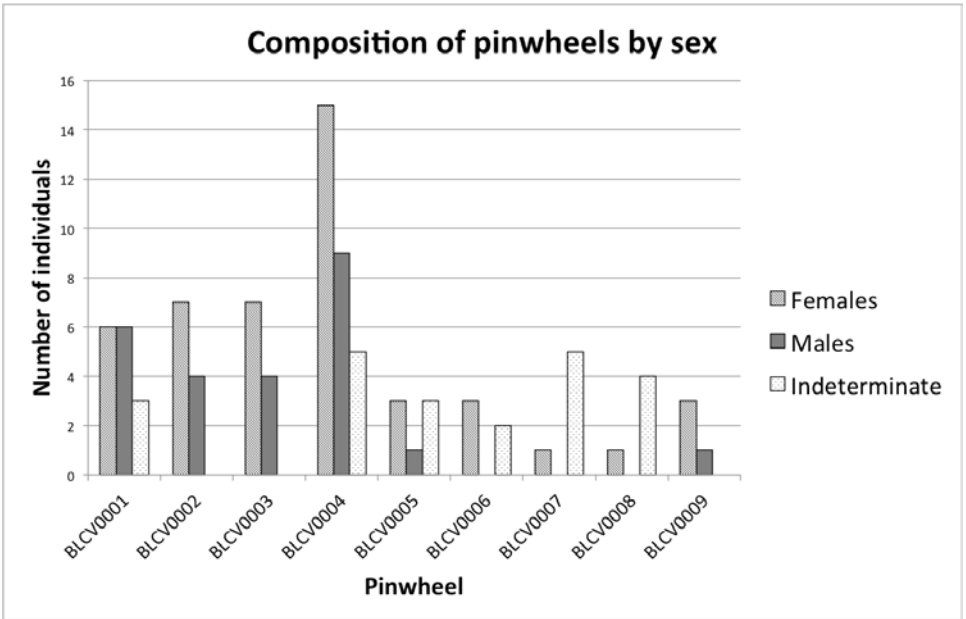


**Figure 1.22. The composition of pinwheels by sex.**

**Figure 1.23. Several clusters of stadium I *B. lecontii* at locality DAH-2017-0516-01 in Arkansas.**

### 3.7 Leg Morphology

A feature consisting of a single row of comb-like setae present on the tarsus was found on the six anterior-most leg pairs of both male and female *B. lecontii*, referred to as "comb teeth" [Figure 1.24]. On adults, the modified setae had short, thick shafts which flatten and terminate in blunt ends [Figure 1.25]. Comb teeth were visible as early as the first stadium. Although the undeveloped comb teeth of juveniles lacked the flattened shaft and blunt tips present on the adults, they were recognized by their alignment in a single-row and stoutness relative to other unmodified setae [Figures 1.26, Figure 1.27]. Larger, older millipedes possessed a greater number of comb teeth and more developed setae than younger millipedes [Table 1.4].

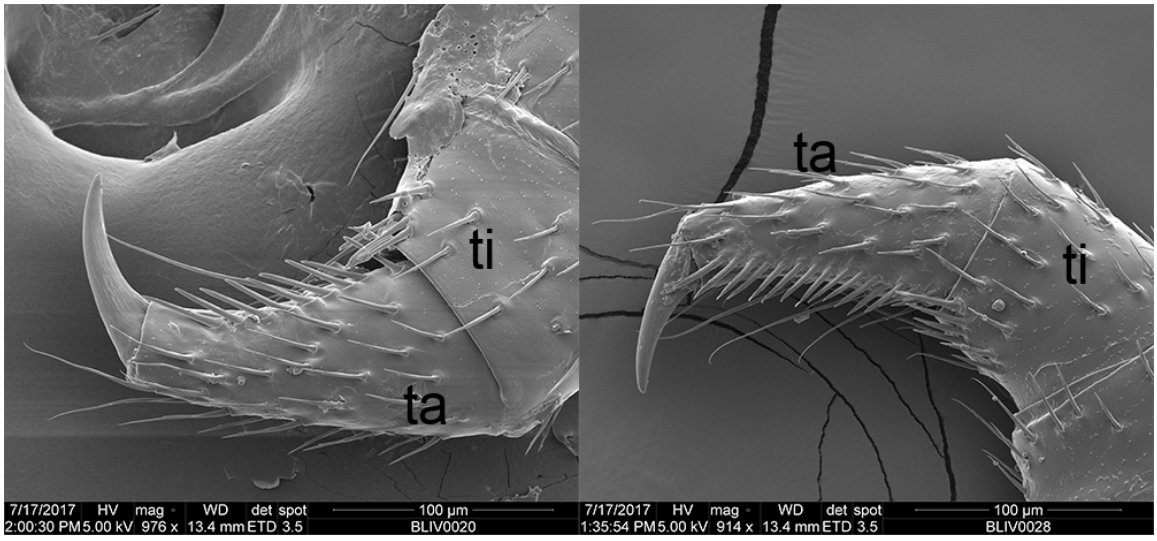


**Figure 1.24. Tibial (ti) and tarsal (ta) combs on leg pair 2 of adult female (left, BLIV0020) and male (right, BLIV0028) *B. lecontii*.**

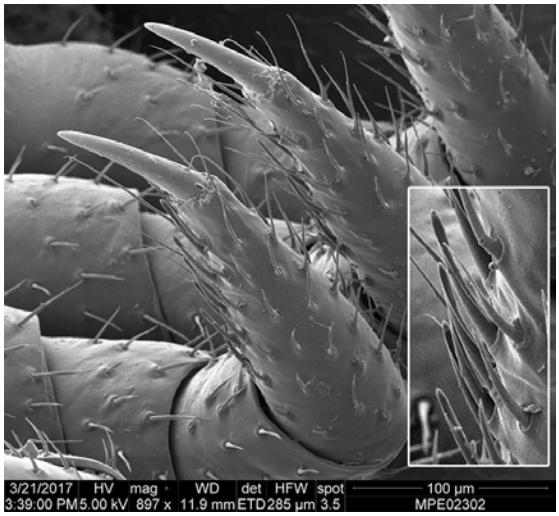


**Figure 1.25. Leg pairs 4-5 of an adult male *B. lecontii* (MPE02302). Inset is a magnified view of the modified setae of the comb.**

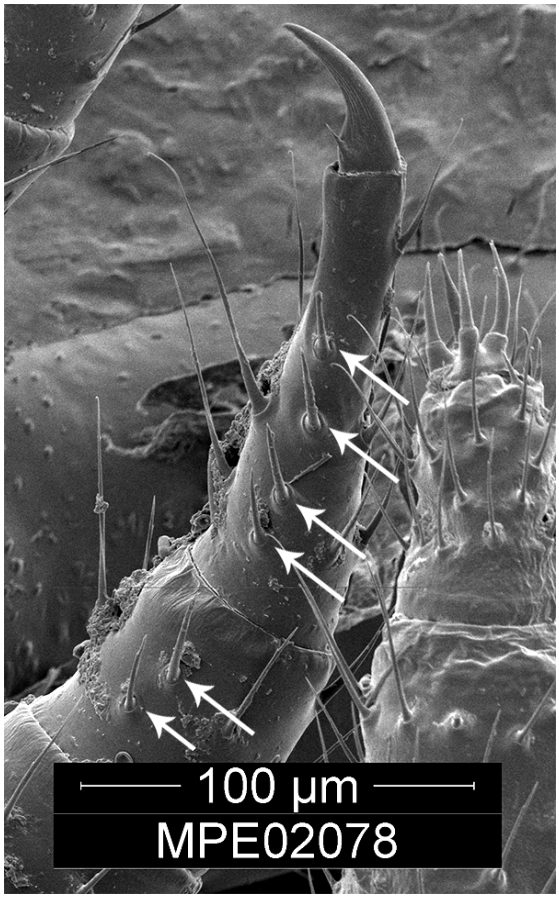


**Figure 1.26. Scanning electron micrograph of the developing combs on the second leg pair of a stadium I *B. lecontii* (MPE02078).**

**Figure 1.27. Illustrations of the comb structures on the first leg pair of a stadium I *B. lecontii* (left) and second leg pair of an adult *B. lecontii* (right). A small seta at the base of the tarsal claw is illustrated.**

**Table 1.4. Counts of comb teeth on the tarsus and tibia (in parentheses) on the six anteriormost leg pairs (LP) of *B. lecontii*. Teeth on the legs of several specimens that were mounted on SEM stubs were obscured and therefore the tooth count could not be obtained. Leg pairs that are missing data are denoted with a "-".**

| Specimen identifier | Sex | No. rings | LP 1 | LP 2 | LP 3 | LP 4 | LP 5 | LP 6 |
|---|---|---|---|---|---|---|---|---|
| **BLIV0020** | Female | 45 | 3 (2) | 8 (3) | 12 (6) | 12 (0) | 10 (0) | 4 (0) |
| **BLIV0028** | Male | 46 | 7 (4) | 15 (4) | 15 (5) | 13 (4) | 4 (0) | 3 (0) |
| **BLIV0029** | Male | 37 | 6 (4) | 9 (4) | 10 (2) | 6 (0) | 3 (0) | 2 (0) |
| **BLIV0031** | Male | 36 | 5 (3) | 9 (3) | 9 (4) | 8 (0) | 4 (0) | 0 (0) |
| **BLIV0032** | Male | 29 | 5 (3) | 7 (3) | 10 (2) | 7 (0) | 3 (0) | 1 (0) |
| **BLIV0077** | Indet. | 16 | 3 (2) | 5 (2) | 5 (2) | - | - | - |
| **BLIV0078** | Indet. | 14 | 3 (1) | 4 (2) | 5 (1) | 1 (0) | - | - |
| **BLIV0079** | Indet. | 20 | 5 (1) | 7 (2) | 6 (0) | 2 (1) | - | - |
| **MPE02315** | Indet. | 22 | - | 6 (3) | 8 (4) | 6 (0) | 4 (0) | - |
| **MPE02316** | Female | 25 | 4 (0) | 9 (2) | 8 (3) | - | 3 (0) | - |
| **MPE02378** | Indet. | 7 | 3 (1) | 4 (2) | 2 (0) | - | - | - |

### 3.8 Color

When viewed alive, recently eclosed stadium I *B. lecontii* were translucent white in color, and as the millipede aged, the coloration became pink. The mature millipedes were generally pink in color with variations in hue from beige to deep salmon. Their coloration was uniform over their body; however, the anteriormost body rings were more strongly pigmented and saturated in color, and the body rings recently added in posterior section of the trunk via anamorphosis were weakly pigmented and less saturated in color. Preserved specimens in 70% isopropyl alcohol faded to a dull beige. Examination of reflectance spectra revealed the least reflectance at short wavelengths, with a median of 4.97% reflectance at 300 nm (minimum 2.10%, maximum 19.53%). The highest percent reflectance occurred at longer wavelengths, with a median of 48.97% at 700 nm (minimum 15.59%, maximum 77.99%) [Figure 1.28]. Inflection points

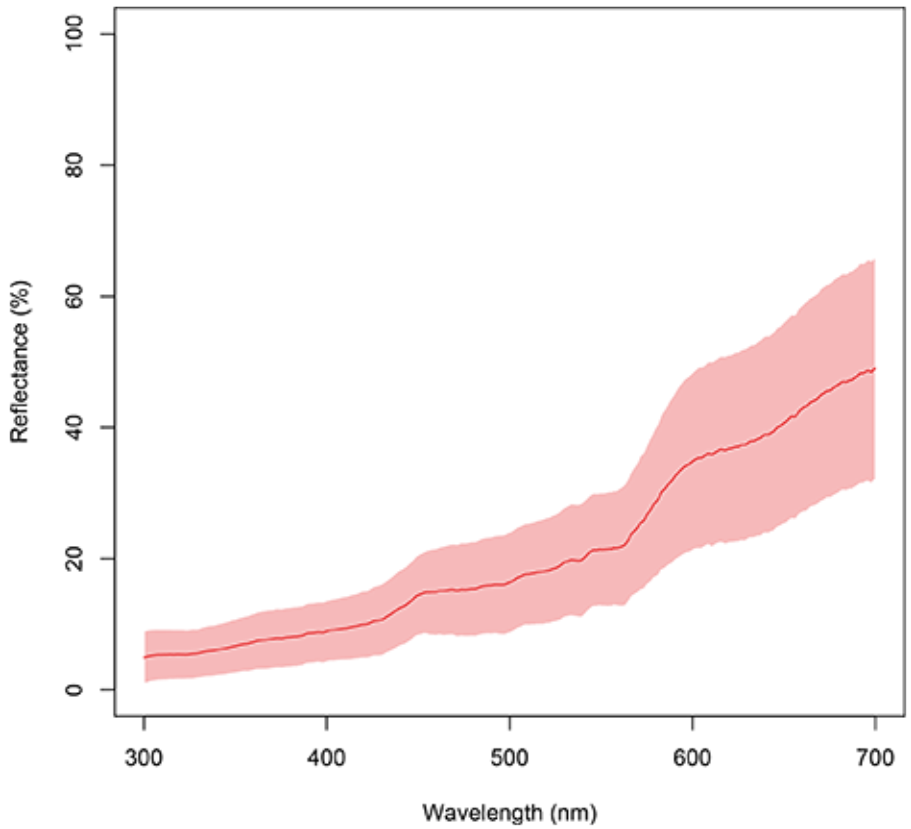


**Figure 1.28. Reflectance spectra (y-axis, percent reflectance; x-axis, wavelength of light), measured in 18 live millipedes and graphed showing median and standard deviation. I observed a sharp increase in reflectance around 450 nm and around 580 nm.**

of the spectrum were present at 450 nm and 580 nm.

## 4. Discussion

### 4.1 Life History

Youngsteadt and McAllister (2014) found that *B. lecontii* millipedes eclosed from the egg in 21 days but did not directly observe the eggs being laid, thereby making conclusions regarding the incubation period inaccurate. From my observations, I found that the oviposition and incubation periods of *B. lecontii* were similar to its sister species *B. nodulosa*. Murakami (1962b) stated that the oviposition period of *B. nodulosa* extended from mid-May until late July, with an incubation period of 3–5 weeks at 13-24º C. Clutches of *B. lecontii* eggs laid in the laboratory were observed hatching 24–26 days later. With my observations of an incubation period of 3–4 weeks at room temperature (20–25º C), and because eggs and stadium I juveniles occur in the field on May 13, the oviposition period for *B. lecontii* begins mid-April and continues until early June. The eclosion from eggs in May by *B. lecontii* would synchronize the development of juvenile millipedes to coincide with warmer temperatures and greater fungal growth.

Stadia were calculated based on the number of leg pairs lacking coxal (eversible) sacs. As coxal sacs are not present on newly added rings immediately after ecdysis, and the minimum number of rings added during molting is constant, the stadium number can be determined by dividing the number of leg pairs without coxal sacs by those with, and then rounding up. *Brachycybe lecontii* stadium I juveniles possess five leg pairs and seven body rings, which is not found in its sister species or any other millipedes, which typically have three or four leg pairs post eclosion (Enghoff et al., 1993; Murakami, 1962b). From my observations, I found that stadium II individuals had 14–18 leg pairs and 11–14 rings; stadium III had 21–30 leg pairs and 15–20 rings; and stadium IV had 32–44 leg pairs and 22–27 rings [Figure 1.6]. Stadium IV individuals were identified by the presence of gonopods (the gonopods become distinguishable from walking legs at this stage), and the number of rings of stadium IV were determined from these individuals. This mode of development is similar to that found in *B. nodulosa* where males are first discernable at stadium IV due to the presence of the intromittant organs (Murakami, 1963). Assignment of stadium based on leg pair and body ring counts becomes challenging at older stadia, as the variation in the counts can widely overlap, and the number of rings and leg pairs do not appear to follow a rule and are not

tightly correlated as they are in younger millipedes; moreover, an odd or even number of leg pairs may be present, and an odd or even number of leg pairs may be added between molts thereby hampering stadium calculation (Enghoff et al., 1993; Murakami, 1962b). Variability in ring counts among stadia was lower in *B. lecontii* than in *B. nodulosa*, which may be due to the smaller number of specimens that I examined for *B. lecontii*. Murakami (1962a, 1962b, 1963) reared 396 *B. nodulosa* from eggs and observed the molts of these individuals, while approximately 200 *B. lecontii* were examined for this study.

The presence of coxal sacs on all walking legs was observed in a single adult male *B. lecontii*. Because colobognaths are euanamorphic (meaning they add rings and legs for an indeterminate period of time), the full complement of sacs is unlikely to be a feature of a final molt. Millipedes in the order Polydesmida are teloanamorphic, meaning there is an adult stadium where molting, and therefore ring and leg addition, stops. Development of the coxal sacs takes longer than development of the legs between molts, hence the one-molt delay seen in *B. lecontii*. A delay in molting for this individual for reasons such as poor environmental conditions may have allowed the development of the coxal sacs to catch up with the development of the legs. The apparent maturation lag of the coxal sacs, making the posterior-most leg pairs always lacking the structures, may be adaptive to avoid resorption of liquid feces.

Molting in *B. lecontii* occurs according the same four stages observed by Murakami (1963) in *B. nodulosa* they are as follows: prerigidation, rigidation, intermediate, and recovery. In *B. nodulosa*, Murakami wrote that prerigidation, a stage in which the body swells and the pre-anal ring protrudes, lasted up to a week; rigidation, where the millipede curls under the cover of substrate, also lasts up to a week; the intermediate period, when the actual process of ecdysis occurs, may last up to a day; and finally, the recovery period occurs during one to two weeks after the intermediate period. The maximum length of each stage in *B. nodulosa* is slightly longer than what I observed in *B. lecontii*, but may be dependent on environmental conditions and the vigor of the specimens.

### 4.2 Paternal Care

Paternal care in *B. lecontii* was similar to that documented in *B. nodulosa* (Kudo, 2010). Brooding by males appears vital to the survival of eggs, since eggs that I separated from the brooding male always perished; however, I did not examine the cause of death for non-brooded eggs. A number of factors may

lead to the death of eggs without paternal care. However, fungal infection is likely as Kudo (2010) found that eggs of *B. nodulosa* would become covered in fungal hyphae if not tended by a male. Other reasons such as desiccation are also possible. The failure of males to recover all of the eggs from their clutches if they are separated from them suggests an inability to evaluate clutch size or number of eggs. Alternatively, it may be more advantageous to not expend excess time and energy in collecting their whole dispersed clutch: lost eggs may have been prey upon and thus not obtainable; and collection attempts could bring the male to wander away from safety and resources.

Unfortunately, the transfer of eggs from one male to another—whereby a clutch was increased in size to 115 eggs—was not directly observed in the process, so the mechanism is unknown. One male may have accidentally lost his clutch and the eggs were collected by another male; however, this seems unlikely as males wrap tightly around their clutches and are not prone to uncurling. The male may have abandoned his clutch, though there were no apparent differences between eggs in the combined clutch, so it did not make sense that the eggless male discarded an infected brood. Abandonment of clutches by males has been observed by Kudo (2011) in *B. nodulosa* and by Gardner (1974) in *B. rosea* and *B. producta*, as a consequence of disturbance of the males by the researcher. Abandonment was not observed here in *B. lecontii* when males were disturbed when probed with a paintbrush. However, a different outcome may have occurred if a male was repeatedly or constantly handled or disturbed by other millipedes in the colony. Doubling the clutch number could also have been a theft of eggs from one male to be incorporated into the other clutch. Kudo (2011) indicated that females (which are notably blind) are able to assess tergal width and therefore capability as an egg-brooder. Perhaps females are able to assess the fecundity of the males in their ability to care for a large clutch of eggs. If this occurs, then multiple matings would occur, which is consistent with evidence of this type of reproductive strategy in other millipedes (Wojcieszek & Simmons, 2012). Regardless of possible mechanism or cause of brood growth, the singular observation of a clutch redistribution in a laboratory setting may simply be an artifact, reflecting abnormal behavior that would otherwise not be found in the wild.

**4.3 Defense**

I found that the shape of the defense glands, and identity of the chemical secretions were different than previously documented in the literature. Although formerly described as "slender, lengthy tubes " (Eisner, 1978), I found that the glands were long and conical in shape, and more voluminous than previously described, occupying a quarter to a third of the paranotal volume. A possible explanation for this discrepancy in observation may be that previous descriptions examined live specimens that may have expended some or all of their defensive secretions, or dead specimens may have been stored in alcohol, thereby causing the defensive glands to shrink in the preservative. My field observations of the interactions between ants and *B. lecontii*, whereby chemically defended adult *B. lecontii* were avoided, suggests that their defensive chemical, containing the alkaloid deoxybuzonamine, may have similar antipredator properties to buzonamine (Wood, 2000). Because deoxybuzonamine is an alkaloid, which is a relatively more toxic chemical than is typical in the defensive secretions of millipedes, the susceptibility to predation or identity of predators of *B. lecontii* may be different than in other Diplopoda. Other arthropods produce alkaloids such as ladybugs (coccinelline) and monarch butterflies (pyrrolizidine alkaloids); however, their predators are primarily avian (Kelley et al., 1987). Ants are the only known predators of *B. lecontii* and deoxybuzonamine is likely effective as a predator deterrent. A diversity of predators may exist, and field studies of predation with cameras or clay models would help address likely receivers of the chemical. *Brachycybe lecontii* was found to produce two isomers of deoxybuzonamine and examination of the isomers found that *B. lecontii* from different collection sites produced differing proportions of each isomer (T. Jones, pers. comm.). The cause for the production of multiple isomers and differing proportions of each is unknown, with possible causes ranging from (1) energetic cost of producing one isomer over another, (2) production impacted by environmental conditions, (3) overall vigor of the millipedes, and (4) evolutionarily isolated populations that evolved to produce more of one isomer than the other. Chemical isomers may also be an aggregation cue, and the defense chemical of the Indian millipede, *Streptogonopus phipsoni*, containing benzaldehyde acts as an attractant (Bellairs et al., 1983). Additional chemical analyses from a wider range of localities and controlled field predation studies are required before any robust conclusions can be drawn regarding the function of deoxybuzonamine.

### 4.4 Feeding

Fungivory has been observed in several *Brachycybe* species. Gardner (1974) indicated that *B. producta* fed on the corticioid basidiomycete fungus, *Peniophora* sp., and *B. rosea* has been observed on the corticioid fungus *Merulius* sp. (Rockerfeller, 2012). I found that *B. lecontii* fed on the fungal genera *Irpex* and *Trametopsis* of the order Polyporales (M. Kasson, pers. comm.). Evidence of fungal feeding is recorded in the bowl-shaped depressions that *B. lecontii* makes with its head in the fungal tissue. How *B. lecontii* feeds and makes the bowl-shaped depressions is uncertain. The millipedes appear to feed exclusively on liquids from fungi because no solid matter was found in the digestive tracts of dissected millipedes, despite observing the millipedes with their heads embedded in the fungal tissue. Additional support for a liquid-feeding lifestyle was evident in the defecation of liquids and not solids. A common reaction of millipedes when immersed in 70% isopropyl alcohol (for preservation) is the regurgitation of their gut contents. Millipedes in the family Xystodesmidae, which feed on decaying leaves, regurgitate an opaque beige gel (P. Marek, pers. comm.). However, *B. lecontii* preserved in this manner regurgitates a clear liquid, visible in the alcohol by the differential refraction between the two liquids. Blanke and Wesener (2014) used micro-computed tomography and found that *B. lecontii* possesses two large paired gland-like structures extending backwards in the two rings posterior to the head. The glands open to the cephalic region near the buccal cavity. These unknown structures of uncertain homology may be salivary glands related to fungal feeding and secrete a chitinase to liquefy fungal tissue.

### 4.5 Feeding Structures

The mouthparts of *B. lecontii* are like those of other colobognath millipedes, with reduced mandibles (not visible externally), labrum without teeth, distinct gnathochilarial sclerites, and closely appressed labrum and gnathochilarium (Gardner, 1974). The feeding mode may be of a suctorial liquid-feeding nature as Manton (1961) indicated is the mode of some other colobognath millipedes. However, the manner in which they extract liquid from fungus is yet unknown, though Lewis (1974) suggested external digestion as a possibility, and this would be consistent with an association of the spacious cephalic glands found by Blanke and Wesener (2014) with feeding. The semicircular structure on the tip of the labrum is distinctive and not observed in any other group of Diplopoda. The numerous pits and setae set around the

structure suggest that it may be sensory or highly sensitive in nature. The rough texture and raised central ridge of the labrum may be an artifact of wear upon the area and may not have a specialized function. Alternatively, if feeding does not rely on external digestion and instead requires mechanical rasping of fungus to extract liquid, the roughened surface may aid the millipede in tearing and mashing the fungal hyphae. This rough and raised central ridge was found in the mouthparts of the colobognath millipede genus *Illacme* (family Siphonorhinidae) that were suggested to be for rasping plant or fungal tissue (Marek et al., 2012).

**4.6 Pinwheeling**

Pinwheel aggregations have been described in previous studies and authors speculated about their function. Gardner (1974) referred to the aggregations as “star clusters”, but the term had not been adopted in subsequent studies. I chose the term "pinwheeling" for my research as it was descriptive and is itself a more precise behavioral verb, in that "pinwheeling" alludes to the head-in/tail-out pattern of the aggregation, and "star clustering" or "clustering" does not.

The pinwheel assembly may be a feeding behavior associated with the presence of fungus, as millipedes kept in containers without fungus aggregated but did not pinwheel. The aggregation shape may be coincidental, arising as each individual millipede seeks to reach and feed from fungal growths, or may be functional and aid feeding, such as through pooling of digestive secretions for external digestion, suggested by Lewis (1984) in a study of the closely related genus *Pseudodesmus* from Sarawak. The pinwheel arrangement may be evolutionarily co-optimized for feeding and additional functions such as defense. For example, when a pinwheel consisting of a mix of adults and young juveniles was disturbed, an ant (*Camponotus* sp.) carried off one of the juvenile millipedes, while solitary wandering adults were ignored by ants (DAH-2017-0516-01). Pinwheels may be aposematic, signaling to visual predators of their chemical defenses. The pinwheel aggregation may augment the overall aposematic signal, more so than if a solitary individual was viewed by a predator, as by a supernormal stimulus (Joron, 2009). It does not appear that pinwheels serve to enhance chemical defenses via pooling the volume of defensive secretions since when an attack was simulated on an individual millipede in a pinwheel, the other individuals did not

react and also exude chemicals. Gregarious behavior such as pinwheeling may also facilitate finding of mates, be a result of mate-guarding, or function as a lek.

Pinwheeling does not appear to associated with the care of eggs or young. Males with eggs were not observed as members of a pinwheel. Two pinwheels consisting of single adult females with several stadium I-II juveniles were observed. When the females were removed, the juveniles scattered, a behavior also seen when congregations of solely juveniles were disturbed. Parental or brooding behavior was not exhibited by the female adults in these pinwheels.

Communal feeding occurs in other animals, for example the Yellow-bellied Glider (*Petaurus australis*) from Australia. These marsupials occur in family groups of four to six individuals—notably with a greater proportion of females—and in fall and winter feed exclusively on plant exudates such as sap, gum, and even honeydew from hemipteran insects (Smith, 1982). During the remainder of the year, *P. australis* is insectivorous and feeds on beetles and other insects. The gliders exhibit communal feeding where adult individuals scrape the bark with their incisors—of which these teeth often show significant wear—thereby causing sap to flow. Afterwards, other members of the family group lick the bark to ingest the liquid. When a family group is feeding, multiple individuals can be observed in close proximity feeding on a single sap wound on the tree.

**Figure 1.29. One of several *B. lecontii* observed wandering during the day. This individual emerged from the top side of a log, in the afternoon of a sunny day.**

**4.7 Leg Morphology**

Contrary to observations by Manton (1961), the tarsal claw of *B. lecontii* is not simple and instead is armed with a small seta situated at the base of the tarsal claw. Comb teeth counts generally are more numerous in adult individuals, but no pattern is apparent with regards to the predictable increase in number of teeth according to stadium. The comb structure is present on related millipedes of the genera

*Gosodesmus* and *Andrognathus*. As parental care of eggs is not known in either of these genera, the comb structure is likely not for this function, and instead possibly for a more general function such as grooming or burrowing.

**4.8 Color**

The pink coloration of *B. lecontii* is directly related to high reflectance in the blue (450 nm) and red parts (> 600 nm) of the color spectrum. These colors additively mix to appear pink in the human visual system. Because the brightness (area beneath the spectrum) is low, the pink hue appears darker. In addition, the saturation or chroma (slope coefficients of the blue and red spectral peaks) is low, and pink color appears less vivid. For example, and in contrast with *B. lecontii*, a pink orchid has high brightness and chroma. Spectral measurements of their reflectance shows negligible ultraviolet (UV) reflectance, indicating the color is likely not derived from UV-reflecting pigments such as carotenoids that reflect at a peak wavelength of 250 nm. With low UV reflectance, their coloration would lack a radiation-protectant quality in the UV range, an unsurprising finding given my frequent observations of *B. lecontii* occurring beneath decaying logs and in forest understories with very low light conditions (Hoffman, 1950; Shelley, 2005) [Figure 1.29]. The color of *B. lecontii* has been described as varying geographically within the species from east to west with individuals from Arkansas redder than those from Virginia that are light pink (Gardner, 1974; Shelley et al., 2005). The pink color spectrum of *B. lecontii* is similar to human skin lacking melanin. However, the chemical source and function for the pink coloration of *B. lecontii* is unknown. Other millipedes have six types of pigments including quinones, coproporphyrins, ommochromes, melanins, flavins, and tetrapyrroles (Hopkin and Read, 1992). Of these pigments, coproporphyrin is the likeliest because it produces a red hue. This pink coloration may be related to predator defense since chemically defended arthropods have a high apparency to their predators and are aposematic. However, pink is typically not a frequent aposematic color in nature and millipedes that have colors that have been directly tested to be aposematic are long wavelength colors (yellow to red) and, in an unusual case, short wavelength bioluminescence (495 nm) (Marek et al., 2011). To address the hypothesis that pink coloration in *B. lecontii* serves as an aposematic signal, then controlled predator studies to test

association of the millipede's appearance with its toxicity should be conducted either in the laboratory or field where the millipedes occur.

## 5. Annotated Literature Review

### 5.1 Literature pertaining to the family Androgathidae, the family that contains *Brachycybe lecontii*

**Wood, H. C. (1864). Description of new genera and species of North American Myriapoda.** ***Proceedings of the Academy of Natural Sciences of Philadelphia 16*****, 186-187.** This paper includes the generic description of *Brachycybe*, based on examination of the type species *B. lecontii*.

**Cope, E. D. (1869). Synopsis of the extinct Mammalia of the cave formations in the United States, with observations on some Myriapoda found in and near the same, and on some extinct mammals of the caves of Anguilla, W.I., and other localities.** ***Proceedings of the American Philosophical Society*****, 171-192.** While millipedes were not the focus of this paper, notes on the locality and behavior of several millipedes are recorded, including the new genus and species *Androgathus corticarius*, from which the family Androgathidae was based.

**McNeill, J. (1888). A list, with brief descriptions of all the species, including one new to science, of Myriapoda of Franklin County, Indiana.** ***Bulletin of the Brookville Society of Natural History 3*****, l–20.** This list includes species of millipedes and centipedes found in Franklin County, Indiana, including androgathid millipedes which were placed by the author in the family Polyzoniidae.

**Bollman, C. H. (1893). The Myriapoda of North America.** ***Bulletin - United States National Museum, 46*****, 1-210.** This article contained a comprehensive list of myriapods of North America at the time as well as descriptions of new species. The study synonymized the genus *Brachycybe* with the tropical genus *Platydesmus* in family Polyzoniidae (now the Platydesmidae).

**Brolemann, H. W. (1900). Myriapodes d'Amerique.** ***Mémoires de la Société zoologique de France, 13*****, 89-131.** This paper described myriapods of America. In the study, androgathid millipedes are placed in the family Platydesmidae. The genus *Brachycybe* is confirmed as a synonym of the genus *Platydesmus*.

**Cook, O. F., Loomis, H. F. (1928). Millipeds of the order Colobognatha, with descriptions of six new genera and type species, from Arizona and California.** ***Proceedings of the United States National Museum 72*****, 1–26.** This paper provides descriptions and natural history information of

millipede species in the taxon Colobognatha, including Platydesmida and Androgna thidae. Based on morphology of the coxae, *Brachycybe* is removed from synonymy from genus *Platydesmus.*

**Gardner, M.R. (1974). Revision of the millipede family *Androgna thidae* in the Nearctic region. *Memoirs of the Pacific Coast Entomological Society* 5: 1–61.** This article contains a taxonomic revision of the family Androgna thidae and includes descriptions and natural history information of species in the family. The article documented that *Brachycybe producta* individuals consume a basidiomycotan corticioid fungus of the genus *Peniophora*.

**Shelley, R. M., McAllister, C. T., & Tanabe, T. (2005). A synopsis of the milliped genus *Brachycybe* Wood, 1864 (Platydesmida: Androgna thidae). *Fragmenta faunistica 48,* 137-166.** This recent synthesis of the genus provided descriptions of *Brachycybe* species as well as a consideration of the Palearctic species with reference to the Nearctic taxa.

**Brewer, M. S., Spruill, C. L., Rao, N.S., & Bond, J.E. (2012). Phylogenetics of the millipede genus *Brachycybe* Wood, 1864 (Diplopoda: Platydesmida: Androgna thidae): patterns of deep evolutionary history and recent speciation. *Molecular phylogenetics and evolution 64,* 232-242.** This first molecular phylogenetic study of the genus *Brachycybe* indicated *B. lecontii* contains four genetically differentiated, geographically non-overlapping clades indicative of distinct species. However, variation of the male genitalia (gonopods), which is traditionally used for species taxonomy of millipedes, was non-informative and not consistent with species boundaries. The article also indicated that *B. lecontii* is more closely related to the East Asian species *B. nodulosa* than it is to its Appalachian neighbor *B. petasata*.

**Decker, P. (2014). Revision of the family Androgna thidae in Southeast Asia (Diplopoda: Platydesmida) with descriptions of six new species. doi: 10.13140/2.1.4348.6723.** A conference poster from the 16th International Congress of Myriapodology, this study evaluates the relationship of the Southeast Asian androgna thid genus *Pseudodesmus* with the genera *Brachycybe* and *Platydesmus*. In the study, the author proposes six new species.

**5.2 Literature documenting the biology of *Brachycybe lecontii***

**Cope ED (1870) On some new and little known Myriapoda from the southern Alleghenies.** ***Transactions of the American Entomological Society*** **3: 65-67.** In this article, the author provides descriptions and natural history observations of millipedes found in the southern Allegheny Mountains, including a brief discussion of the microhabitat *B. lecontii*.

**Hoffman RL (1950) Records and descriptions of diplopods from the southern Appalachians.** ***Journal of the Elisha Mitchell Scientific Society*** **66: 11-33.** This paper documents new localities of *B. lecontii* from North Carolina and South Carolina.

**Manton SM (1961) The evolution of arthropodan locomotory mechanisms. Part 7. Functional requirements and body design in Colobognatha (Diplopoda), together with a comparative account of diplopod burrowing techniques, trunk musculature, and segmentation.** ***Journal of the Linnean Society of London Zoology*** **44: 383–462.** This study investigates the biomechanics of several colobognath millipedes. Manton showed that *B. lecontii* burrows underground with a wedge-like biomechanical mechanism that allows the millipede to exert pushing forces larger than those of previously recorded relative to millipede body size (body width).

**Eisner T, Alsop D, Hicks K, Meinwald J. (1978) Defensive secretions of millipedes. Arthropod venoms. Springer-Verlag Berlin Heidelberg, New York, 41-72.** This study showed that *B. lecontii* possesses “type 2 defensive glands” that have the anatomical appearance of slender tubes, a shape that that differs from cyanide-generating millipedes that possess spherical bi-compartmental glandular sacs.

**Youngsteadt, N. W., & McAllister, C. T. (2014). Natural history notes and new county records for Ozarkian millipeds (Arthropoda: Diplododa) from Arkansas, Kansas and Missouri.** ***Journal of the Arkansas Academy of Science, 68*****(1), 177-182.** In this article, a new locality record for *B. lecontii* is provided along with some behavioral observations on molting, feeding, and parental care of eggs.

**Shear, WA (2015) The chemical defenses of millipedes (Diplopoda): biochemistry, physiology and ecology.** ***Biochemical Systematics and Ecology*****, 61, 78-117.** In this paper, the gregarious behavior of *Brachycybe* and other platydesmidan millipedes is described, and the pinwheel shape is

described as a rosette. The authors suggest that the alkaloid defensive chemical produced by *Brachycybe* is structurally similar to buzonamine; however, the chemical lacks the element oxygen.

**McAllister CT, Connior MB (2016) A new geographic record for *Brachycybe lecontii* (Diplopoda: Platydesmida: Andrognathidae) from Oklahoma. *Proceedings of the Oklahoma Academy of Science* 99: 46-52**. This study provides documentation of the westward most locality for *B. lecontii* in northeastern Oklahoma that extends the species range further than previously known.

**5.3 Studies investigating the biological aspects of other animals also present in *Brachycybe lecontii***

**Murakami Y (1962) Postembryonic development of the common Myriapoda of Japan. Life history of *Bazillozonium nodulosa* (Colobognatha, Platydesmidae). *Zoological Magazine* 71: 250-255, 291-294.** This paper contains a natural history of *Brachycybe (=Bazillozonium) nodulosa* with the description of the development, eggs, and juveniles of the species. The post-embryonic development of millipedes where legs and rings are added as the individual ages, is referred to as anamorphosis and varies by millipede lineages. This article describes the type of anamorphosis that occurs in *B. nodulosa* based on observations of captive laboratory colony. In *B. nodulosa*, and later found to be present in other colobognath taxa, anamorphosis is indeterminate meaning that development (and therefore segment and leg addition) occurs continuously throughout the life of the millipede. Designated euanamorphosis, this process is responsible for the superlative leg count of 750 in the colobognath species *Illacme plenipes* (order Siphonophorida).

**Murakami Y (1962) Postembryonic development of the common Myriapoda of Japan. Life history of *Bazillozonium nodulosum* (Colobognatha, Platydesmidae). *Zoological Magazine* 72: 40-47.** This article describes the male brooding behavior of *B. nodulosum* (=*Brachycybe nodulosa*).

**Murakami Y (1963) Postembryonic development of the common Myriapoda of Japan. Life history of *Bazillozonium nodulosum* (Colobognatha, Platydesmidae). *Zoological Magazine* 72: 40-47.** This study documents the molting process and body segment number variation during molting of *B. nodulosa*.

**Lewis, J.G.E. (1984) The Myriapoda of the Gunung Mulu National Park. *The Sarawak Museum Journal* 30, 35–51.** A field natural history study of the platydesmid genus *Pseudodesmus* from Gunung Mulu National Park, Malaysia. The author provides natural history observations of an undescribed species of *Pseudodesmus*. These millipedes were found in groups of up to 70 individuals arranged radially around fungus. Lewis provided several hypotheses for the rosette formations, including feeding, aposematism, external communal digestion, and facilitation of mate-finding.

**Enghoff, H., Dohle, W., & Blower, J. G. (1993) Anamorphosis in millipedes (Diplopoda)—the present state of knowledge with some developmental and phylogenetic considerations. *Zoological Journal of the Linnean Society*, 109(2), 103-234.** A systematic overview of postembryonic development pertaining to ring and leg addition, anamorphosis in major orders of Diplopoda.

**Wood, WF, Hanke, FJ, Kubo, I, Carroll, JA, & Crews, P (2000). Buzonamine, a new alkaloid from the defensive secretion of the millipede, *Buzonium crassipes*. *Biochemical systematics and ecology*, 28(4), 305-312.** In this study, the defensive secretion of *Buzonium crassipes* is analyzed and chemically identified as a novel chemical product, buzonamine, using GC-MS and NMR techniques. The authors conducted a behavioral assay and provided experimental evidence of the efficacy of buzonamine as an antifeedant against ants.

**Tallamy, DW (2001) Evolution of exclusive paternal care in arthropods. *Annual review of entomology* 46(1), 139-165.** This article provides a review of parental care in arthropods, and references *B. lecontii* paternal care and an incubation period of three weeks.

**Koshio, C., Tanabe, T. (2009). Male egg‐brooding in the millipede *Yamasinaium noduligerum* (Diplopoda: Andrognathidae). *Entomological science* 12(3), 346-347.** In this study of the Japanese andrognathid species *Yamasinaium noduligerum*, the authors document paternal care behavior and the how males brood eggs.

**Kudo SI, Akagi Y, Hiraoka S, Tanabe T, Morimoto G (2011) Exclusive male egg care and determinants of brooding success in a millipede. *Ethology* 117: 19-27.** This article describes male egg brooding in the Japanese species *Brachycybe nodulosa*, and experimentally demonstrates that hatching is unsuccessful without paternal care. The experiment involved artificially separating

eggs from the brooding male to determine hatching success. Traits associated with brooding success include male body width and egg grooming. Body width is positively correlated with survivorship of eggs; the trunk acts as a protective "roof" to guard the eggs from desiccation and predation, and apparently females are able to assess male body width during courtship. Eggs permanently separated from brooding males quickly became infested with fungus. Male grooming removed pathogenic fungus and other microbes lethal to the developing eggs.

**Dury GJ, Bede JC, Windsor DM (2014) Preemptive circular defense of immature insects: definition and occurrences of cycloalexy revisited. *Psyche: A Journal of Entomology* 2014.** This study defines "cycloalexy" where animals form a defensive circle with their best-defended extremities facing outwards on the periphery of the circle. Juveniles, elderly, or less-protected individuals are situated in the central hub. For example, American bison face outwards with their anterior end including their horns and hooves facing the threat. Examples of cycloalexy are in mammals (musk oxen and American bison), insects (sawflies and leaf beetles), and perhaps in millipedes (*Brachycybe* and other species of Platydesmida).

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