TAXONOMIC STUDIES OF NITIDULIDAE (COLEOPTERA: CUCUJOIDEA) IN NORTH AMERICA

by

COURTNEY LYNN BRISSEY

(Under the Direction of Joseph V. McHugh)

ABSTRACT

The sap beetle family Nitidulidae is one of the most diverse families of beetles containing 4,500 species in 350 genera worldwide (Ślipiński, Leschen, and Lawrence, 2011). The subfamilies Carpophilinae and Epureainae contain some of the most economically and ecologically significant species which can be difficult to identify using current literature. I provide an overview of features and technical specifications used to build an interactive web-based key for identification of Carpophilinae (Coleoptera: Nitidulidae) in eastern North America. The terminal taxa list used in the key represents the most current account of carpophiline diversity known to occur in the area. I also provide redescription of the larvae and first description of the pupae of *Epuraea ocularis* Fairmaire (Nitidulidae), and diagnosis for the adult. Habitus and character images used for the descriptions are provided. New state records for this non-native sap beetle are reported for Georgia and California, illustrating a large range expansion across North America.

INDEX WORDS: Morphology, Sap beetle, Characters, Taxonomy, Determinations, Interactive key, Identification key, Multi-entry key, Matrix key

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by

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DEDICATION

To my parents, Johnny and Lisa Brissey, for providing continuous support and always making me feel like I could do anything I set my mind to.

And to my boyfriend Aaron Di'Lorenzo, who went above and beyond in facilitating my pursuit of this degree.

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This work was partially supported by grants from the H.H. Ross Fund and from the Georgia Peach Council. We thank J. Grant and B. Gochnour for collecting many of the Georgia samples from outside of Clarke and Oconee County. We also thank F.W. Shockley of the Smithsonian Institution National Museum of Natural History (NMNH), T.C. McElrath of the Illinois Natural History Survey Insect Collection (INHS-INHSIC), and P.E. Skelley and K. Schnepp of the Florida State Collection of Arthropods (FSCA) for loans of authoritatively determined specimens.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

The sap beetle family (Nitidulidae: Coleoptera) is one of the most diverse groups in the superfamily Cucujoidea, comprising 4,500 species in 350 genera worldwide (Slipinski, Leschen, and Lawrence 2011). The North American sap beetle fauna is represented by 165 species in 35 genera (Habeck 2002). While most sap beetles are either saprophagous or mycetophagous, other feeding strategies exhibited include feeding on flowers, pollen, over ripened fruit, fermenting juices, stored products, decaying plant matter, carrion, honey comb, and other beetles (Habeck 2002). Due to their associations with fruits and stored products, many species have been transported globally through trade routes and are now considered cosmopolitan. Two of the most prevalent, widespread, and destructive subfamilies within Nitidulidae are Carpophilinae and Epuraeinae. Many members of these subfamilies can become pests in agricultural settings, food processing facilities, and stored food products.

Carpophilinae is represented by 3 genera and 21 species in eastern North America. Some carpophilines, especially those in the genus *Nitops*, are anthophagous, feeding on decaying flowers and pollen, and therefore have been studied as potential pollinators (Nadel and Pena 1994, Higuchi et al. 2014). Carpophilines also have been associated with the spread of pathogens, such as *Ceratocystis fagacearum* (Bretz) Hunt which causes oak wilt disease (Cease and Juzwik 2001). Carpophilines are most often associated with over ripened, rotting, or dried fruits and vegetables; however, many

species have the ability to damage healthy fruit and transmit bacterial pathogens, making them pests of fruit industries (Leschen and Marris 2005). They are able to transmit some microorganisms that produce mycotoxins (Dowd 1991, Dowd 1995) and also transport *Monilinia* spp., which results in brown rot of stone fruits (Kable 1969, Williams and Salles 1986).

When carpophilines become pests in crops or food storage facilities, one of the most effective management techniques utilizes trap and kill bait stations (Hossain et al. 2006, Bartelt and Hossain 2006). Effective pesticides are available, however, since sap beetles do not usually arrive until crops are ripening, residues from pesticide use can remain on harvested produce. Initially trap and kill bait stations relied solely on fermenting, rotting fruit, but in the last few decades, species-specific pheromones have been incorporated. The use of fermenting fruit volatiles along with species-specific sap beetle pheromones has resulted in trap and kill bait stations becoming as effective as conventional pesticides in controlling carpophiline populations (Bartelt and Hossain 2010).

Since trap and kill bait stations rely on lures containing species-specific pheromones, it is important for the user to identify the sap beetle species involved so the correct pheromone can be deployed. Correct species identification is also important for port and border inspectors, enabling them to prevent the entry of non-established species at ports of entry. In Chapter 2, I use the key building tool Lucid^{TM} to create a matrix-based interactive key to the Carpophilinae of Eastern North America. The key utilizes user friendly terminology and a wide variety of images to allow for easy species identifications by specialist and non-specialists. I also discuss the ways in which matrix

based keys are superior to traditional dichotomous keys, emphasizing how this relatively new format is able to resolve long standing issues faced by taxonomists creating and using identification keys.

The subfamily Epuraeinae contains 13 genera, one of which is the genus *Epuraea* Erichson, 1843 (Kirejtshuk 2008). *Epuraea* is one of the largest genera in the sap beetle family, with more than 300 known species. Considered one of the most taxonomically problematic genera of Nitidulidae, *Epuraea* sensu lato includes more than 15 subgenera, for which no identification key exists. There are more than 30 described species of *Epuraea* in the Nearctic Region (Cline and Audisio 2011) with many species still awaiting description. Creating a key to the species of *Epuraea* in North America is beyond the scope of this paper due to unresolved nomenclature issues, difficulty in obtaining authoritatively determined specimens, and the fact that several species collected in the region are still awaiting full description.

To address the issue of lacking descriptions of *Epuraea*, in Chapter 3 I provide the first detailed descriptions of the larvae and pupae of the non-native species *Epuraea* (*Haptoncus*) *ocularis* Fairmaire. I also provide diagnostic characters for all life stages, images of diagnostic characters, and habitus images. Further, I discuss the range expansion of *E. ocularis* in North America since its first discovery in the region by Cline and Audisio (2011). This will enable the identification of *E. ocularis*, in any life stage, as it continues to spread across the continent.

CHAPTER 2

CARPOPHILINE-ID: AN INTERACTIVE MATRIX-BASED KEY TO THE CARPOPHILINAE (COLEOPTERA, NITIDULIDAE) OF EASTERN NORTH AMERICA[1]

[1]Brissey C.L., G.S. Powell, A.R. Cline, and J.V. McHugh. To be submitted to *ZooKeys*.

2.1 Abstract: We provide an overview of features and technical specifications used to build an interactive web-based key for the identification of Carpophilinae (Coleoptera: Nitidulidae), a beetle subfamily of agricultural and ecological significance, in North America east of the Mississippi River. The list of the terminal taxa used in the key represents the most current account of carpophiline taxa occurring in the area. We also discuss the importance of utilizing matrix-based, free access keys to facilitate identifications and decrease the frequency of misidentifications of difficult taxa.

Keywords: Matrix key, morphology, interactive key, sap beetle, characters, taxonomy, determinations, identification key, multi-entry key

2.2 Introduction:

Matrix-based keys, such as Lucid™ keys, are superior to traditional dichotomous keys. They allow users to follow many paths to determine species, use subsets of characters, non-traditional characters (e.g., biological, geographical, phenological, and genetic data), multi-state characters, and allow creators to incorporate extensive supporting graphics to aid in identification (Penev et al. 2009, 2012, Cerretti 2012). Lucid™ keys are built around a data matrix of all available diagnostic characters scored for each taxon in the key. Identifications proceed by users selecting any character in the key and indicating the state observed in the specimen. The software then eliminates taxa that do not match. This format makes the key "undirected", allowing users to take multiple paths, skipping difficult, missing, or inapplicable characters to identify specimens. Since undirected keys work by eliminating taxa that do not match the character states observed on the subject, users may choose more than one state option if uncertain. Lucid™ provides a web hosting service, making these keys widely accessible

for free to the scientific community and the general public. These many advantages make Lucid™ keys superior to traditional dichotomous keys, especially when dealing with specimens that are difficult to identify.

The sap beetles in the family Nitidulidae are represented by 4,500 species in 350 genera worldwide (Ślipiński, Leschen, and Lawrence 2011). Of those, approximately 165 species in 30 genera are known to occur in North America (Habeck 2002). East of the Mississippi River, the subfamily Carpophilinae is represented by 3 genera and 21 species, most of which are cosmopolitan. Carpophilinae are distinguished from other Nitidulidae in the Nearctic Region by the following combination of characters: Elytra short and truncate apically, not covering pygidium and 1-2 preceding tergites; terminal segment of the labial palpi somewhat enlarged, shorter to slightly longer than wide, widely truncate at apex; antennal grooves often very long, almost confluent posteriorly; elytra lacking sutural striae, long marginal hairs laterally, longitudinal carinae, and rows of longitudinal setae, hairs, or punctures (Habeck 2002).

Several species of carpophilines, especially those in the genus *Nitops*, feed on pollen as adults; therefore, research has been ongoing to determine their usefulness as pollinators, especially for plants in the genus Annona (George et al. 1989, Tsukada Tanaka and Higuchi 2008, Higuchi et al. 2014). Carpophilines are most often associated with ripe, rotting, or dried fruits and vegetables; however, many species have the ability to damage healthy fruit and transmit bacterial pathogens, making them pests of food commodity production (Leschen and Marris 2005). Currently, the best management techniques for Carpophilinae in stone fruits are field sanitation, harvesting fruits before they fully ripen, and using trap and kill bait stations to keep populations reduced (Hossain

et al. 2006, Bartelt and Hossain 2006). Trap and kill bait stations rely on lures containing species-specific pheromones to capture beetles. In order to successfully utilize these traps, it is important to correctly identify the species involved so the correct pheromones can be deployed. Due to their association with ripe and stored food crops, many species have become cosmopolitan due to international food trades. The ability for port and border inspectors to correctly identify detected carpophilines is paramount in preventing the entry of non-established species at ports of entry.

Though traditional dichotomous keys to the Carpophilinae of the USA are available (Parsons 1943, Connell 1977, Connell 1991), they exclude several eastern species and rely on some difficult characters, limiting their usefulness. In addition, high quality graphics illustrating many species and difficult characters are lacking from those works. To aid in correct species identification of this difficult and important group, we developed a web-based Lucid^{TM} key to the Carpophilinae genera and species east of the Mississippi River in the USA and east of 90° longitude in Canada.

2.3 Project description:

Taxonomic coverage

This key covers all identified Carpophilinae east of the Mississippi River in the USA and east of 90° longitude in Canada, including all 3 genera and 21 of the 35 species currently known to occur in America north of Mexico.

List of the terminal taxa included in the current version of the identification key

(last update July, 2018)

Carpophilus antiquus Melsheimer, 1844; ***Carpophilus brachypterus*** (Say, 1825); ***Carpophilus corticinus*** Erichson, 1843; ***Carpophilus dimidiatus*** (Fabricius, 1792);

Carpophilus discoideus LeConte, 1858; ***Carpophilus freemani*** Dobson, 1956; ***Carpophilus fumatus*** Boheman, 1851; ***Carpophilus hemipterus*** (Linnaeus, 1758); ***Carpophilus lugubris*** Murray, 1864; ***Carpophilus marginatus*** Erichson, 1843; ***Carpophilus marginellus*** Motschulsky, 1858; ***Carpophilus melanopterus*** Erichson, 1843; ***Carpophilus mutilatus*** Erichson, 1853; ***Carpophilus pilosellus*** Motschulsky, 1858; ***Carpophilus sayi*** Parsons, 1943; ***Carpophilus tempestivus*** Erichson, 1843; ***Nitops craigheadi*** (Dobson, 1972); ***Nitops floralis*** Erichson, 1843; ***Nitops ophthalmicus*** Murray, 1864; ***Nitops pallipennis*** (Say, 1823); ***Urophorus humeralis*** (Fabricius, 1798)

Images of terminal taxa

For each species represented in the key, there is a minimum of one dorsal and one ventral habitus photograph. For species with variable morphology, multiple figures are provided to illustrate the range in color and/or size. All specimens imaged were determined by the second and third authors (GSP & ARC). Figures illustrating each character and their various states are provided in the key. Species-specific character images are included within the Species Fact Sheets section of the website, along with larger dorsal and ventral figures. These Species Fact Sheets can be accessed by hyperlinks provided for each species in the Entities section of the key.

Characters used in the key

General features

Characters used as diagnostic features in the key were derived from existing literature (Parsons 1943, Connell 1977, Connell 1991) and from museum specimen data. Published attributes of species were confirmed using specimens in the Smithsonian Institution National Museum of Natural History (NMNH), Illinois Natural History

Survey Insect Collection (INHS-INHSIC), Florida State Collection of Arthropods (FSCA), and University of Georgia Collection of Arthropods (UGCA). Anatomical terminology follows that used by Parsons (1943) and Connell (1977). An anatomical atlas is included in the Features section to aid non-specialists in interpreting the characters used in the key.

The data matrix for the key includes 37 anatomical, distributional, and ecological characters. These characters appear in the key in the Features section, each one with two to five possible character states from which to choose. All features refer to either external anatomical structures of adults that can be easily seen with a stereomicroscope or locality information about where the specimen was collected. For the length and ratio features, ranges provided were derived from the literature (Parsons 1943, Connell 1977, Connell 1991) and from measurements taken using museum specimens to ensure more accuracy. Morphological features are grouped by the following structures/regions: antenna, eye, pronotum, prothorax, mesothorax, elytra, metathorax, pygidium, ventrites. This allows the user to quickly find characters of interest. Characters based on the distribution, ecology, and overall specimen appearance are grouped under the heading "general features." Since some morphological features are only present in either the male or female, characters not relevant for a particular specimen can be excluded quickly from consideration by indicating the sex of the specimen in the key using the helpful images as a guide.

List of characters used in the key

GENERAL: sex (male/female); length (mm); host association; geographic distribution; body convexity (lateral view); body surface overall appearance (glossy/dull)

ANTENNA: antenna club shape (round/oval); antennomere coloration (abruptly darker at club/gradually darker towards club/unicolorous throughout)

EYE: ratio of eye width at widest point: intraocular distance at narrowest point (1:3 or less/between 1:4 and 1:9/1:10 or more)

PRONOTUM: pronotal disk setation length (long/not distinctly long); pronotal disk punctation density (dense/sparse/not conspicuously dense or sparse); pronotum coloration (black/dark brown/medium brown, light brown, or orange); pronotum posterior angles (broadly rounded/truncated/not broadly rounded or truncated)

ELYTRA: elytra coloration with pattern (conspicuous yellowish humeral and apical patches/light humeral patches only/darker coloration near scutellum and apex/darker coloration near scutellum only/dark coloration near apex only); elytra coloration unicolorous (unicolorous and distinctly darker than body/unicolorous and distinctly lighter than body/unicolorus similar to body color); elytra apical shape (straight/squarely truncated or rounded/arching posteriorly)

MESOTHORAX: posterior rim of mesocoxal cavities (crenulate, not forming axillary space/smooth, not forming axillary space/smooth, forming small axillary space extending approximately ¼ down metepisternal suture/smooth, forming large axillary space extending halfway down metepisternal suture); mesosternum median longitudinal ridge (present/absent); mesosternum anterior impunctate edge along with median longitudinal ridge (present/absent); mesosternum impunctate area near center (present/absent)

METATHORAX: male metathoracic tibia shape (abruptly dilated apically/gradually dilated apically); male metathoracic femur (bearing small toothlike

projection on inner margin near trochanter/lacking a tooth-like projection near trochanter); metathoracic preapical tibial spurs along posterior margin (present, distinct/absent)

PYGIDIUM: male pygidium lateral margin shape (constricted/not constricted); male supplementary segment (visible in dorsal view/not visible in dorsal view); female pygidium with large oval depression with vague anterior margin at apex (present/absent); female pygidium apical flexion (deflexed ventrally/upturned medially/not flexed upward or downward); female pygidium weak median longitudinal ridge (present/absent); female pygidium grooves along lateral margins (present/absent); female pygidium lateral margin shape (constricted/not constricted); female pygidium apical margin shape (pointed/broadly rounded or truncated); female pygidial disc setation length (very short, short/ medium, long); female pygidial disc setal density (dense/sparse); female abdominal apex setation length (distinctly longer than nearby setae/not distinctly longer than nearby setae)

VENTRITES: male setation on 4th ventrite distinctly longer medially at posterior margin (present/absent); male 5th ventrite setation density, anterior to supplementary segment (less dense or absent/not distinctly different); male 5th ventrite depression (shape, location); male setation on supplementary segment bearing 2 distinctly longer setae (present/absent)

Software technical specifications

Application: Lucid^{TM} Builder 3.5 (www.lucidcentral.org, website provides technical specifications and features list)

Key Version: 1.0

Requirements for use: Java-enabled browser and internet connection

License for use of the key: Creative Commons Attribution License 4.0 (CC-BY), which permits unrestricted use, distribution, reproduction, and editing, provided the original author and source are credited

Web location: https://site.caes.uga.edu/carpophiline-id/

Website features

Species fact sheets (https://site.caes.uga.edu/carpophiline-id/taxon-fact-sheets/)

All 21 species represented as entities in the key are figured with dorsal habitus, ventral habitus, and diagnostic character images. Each species fact sheet includes a diagnosis and summaries of the known biology and distribution, as well as references. Within the interactive key, these pages can be accessed through hyperlinks provided within each species entity entry.

Resources (https://site.caes.uga.edu/carpophiline-id/resources/)

This section provides an anatomical atlas (also available within the key), a glossary of terminology, and diagnoses for the beetle family Nitidulidae and the subfamily Carpophilinae. The anatomical atlas shows all of the structures mentioned in the key indicated on a dorsal and/or ventral habitus image of a male specimen of *Carpophilus marginellus*. The glossary provides definitions of the terms used in the key. Definitions were derived from Torre-Bueno (1937), Parsons (1943), and Connell (1977). The diagnostic pages provide lists of anatomical characters used to recognize beetles belonging to the family Nitidulidae and the subfamily Carpophilinae.

References (https://site.caes.uga.edu/carpophiline-id/references/)

This section provides a list of useful references about Carpophilinae, building interactive keys, and making species fact sheets.

2.4 Conclusions and future work:

Since multi-access keys allow users to skip sex-specific, hard-to-view, and rarely available characters, additional, more difficult diagnostic features (e.g., male genitalic anatomy, features on immature stages, genetic markers, etc.) will be added as they become available. A full taxonomic revision of the Carpophilinae of North America is currently being conducted (by GSP). Upon completion, newly published information could easily be incorporated into the data matrix and species fact sheets of the interactive key.

This key provides a user friendly tool which will make species-level identifications of Carpophilinae beetles possible for specialists and non-specialists. It also allows for additions and updates as new characters become available and as taxonomic changes are made to the group. It could also be expanded to include newly discovered species, or to extend the geographic range of coverage to create a more inclusive tool.

2.5 Acknowledgements:

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CHAPTER 3

DESCRIPTION OF *EPURAEA* (*HAPTONCUS*) *OCULARIS* FAIRMAIRE (COLEOPTERA: NITIDULIDAE) LARVAE AND PUPAE, WITH A NOTE ON THEIR RANGE EXPANSION IN NORTH AMERICA[2]

[2]Brissey C.L., G.S. Powell, A.R. Cline, and J.V. McHugh. To be submitted to *The Coleopterists Society*.

3.1 Abstract: A detailed redescription of the larvae and first description of the pupae of *Epuraea ocularis* Fairmaire (Nitidulidae) are provided, along with a diagnosis for the adult. Habitus images and images of characters used for the descriptions are also provided. New state records of this non-native sap beetle for Georgia and California are reported, showing a recent expansion in the known range of this species in North America.

3.2 Introduction:

Epuraea Erichson, 1843 is one of the largest genera in the sap beetle family Nitidulidae with more than 300 known species. This genus is one of 13 genera in the subfamily Epuraeinae (Kirejtshuk 2008). Species of *Epuraea* are present worldwide and are considered one of the most taxonomically problematic genera of Nitidulidae, due to nomenclatural issues, incomplete descriptions, lacking illustrations, and decades of misidentifications (Cline and Audisio 2011). *Epuraea* sensu lato includes more than 15 subgenera, for which no identification key exists. Well-illustrated, comprehensive, modern identification keys to the species in each subgenus are lacking as well. There are more than 30 described species of *Epuraea* in the Nearctic Region, with many new species awaiting description (Cline and Audisio 2011). A revision of the New World species of *Epuraea* is currently in progress (by ARC).

Haptoncus Murray, 1864 was initially considered to have generic status until Kirejtshuk (1989) designated it as a subgenus of *Epuraea*. There is some uncertainty regarding the monophyly of *Epuraea* (*Haptoncus*) as currently delimited (Jelinek et al. 2016, ARC pers. obs.). Members of the subgenus *Haptoncus* are described as tropical

Epuraea having a short, truncate terminal labial palpomere (Kurochkin and Kirejtshuk 2003).

Epuraea (*Haptoncus*) *ocularis* Fairmaire, 1849 was first detected in North America near Houston, Texas, in 2010 (Cline and Audisio 2011). In 2014 it was recorded in Louisiana (Ferro, 2014). This paper reports new state records of *E. ocularis* from Georgia and California. These new detections suggest that *E. ocularis* is now well established in North America and is spreading, likely through agricultural commerce (Cline and Audisio 2011).

The adult stage of *E. ocularis* has been illustrated (Ewing and Cline 2005) and possesses a distinctive elytral coloration pattern that the native, Nearctic *Epuraea* (*Haptoncus*) species lack. The larvae were partially characterized by Hayashi (1978), but only with respect to *Epuraea harmandi*. Further, only the urogomphi and dorsolateral aspect of the 8th and 9th abdominal segments were illustrated. A resource providing more detailed descriptions and images will aid in the correct identification of this species.

In this paper, we report on newly collected and museum specimens that extend the known distribution for *Epuraea ocularis*. We provide the first detailed descriptions of the larvae and pupae, diagnostic characters for all life stages, images of diagnostic characters, and habitus images.

3.3 Materials and methods:

Specimen acquisition and deposition

For the distributional records, specimens were newly collected or obtained from museum holdings. For the anatomical studies and descriptions, a lab colony was established to provide fresh material. The stock for the lab colony was started with

specimens collected at the University of Georgia's Horticulture Research Farm, in Watkinsville, GA, using green and yellow UNI-traps. The traps were baited with apples and whole wheat bread dough. Some adults were captured and kept alive for colony establishment in the lab while others were placed in 70% ethanol for preservation.

Lab colonies were maintained in 1-quart plastic containers with fine mesh screens on top. Paper towels were placed in the bottom and kept moist. Apples or pineapples were used as a food source and oviposition site. Third instar larvae were removed and placed in 40 ml vials with pineapple for food and moist tissue paper for pupation (Tsukada et al. 2005). Development from egg to adult took between 21 to 35 days, depending on temperature and diet. Tsukada et al. (2005) provide details about the impact of various temperatures on developmental rates in *E. ocularis*.

Anatomical terminology for larvae follows that of Böving and Rozen (1962), Hayashi (1978), Lawrence (1991) and Cline et al. (2013). Pupal terminology follows that of Rozen (1963), Kurochkin and Kirejtshuk (2003), and Cline et al. (2013).

Dissection and imaging protocols.

Habitus images of adults, larvae, and pupae were taken with a Canon EOS-1 digital camera and a Canon Macro Photo MP-E 65mm lens attached to an ML-1000 Digital Imaging System (Microptics, Inc., Ashland, VA). Images were shot with an ISO of 100, f-stop of 3.5, and a shutter speed of 350. Lighting consisted of two Yongnuo Digital Speedlite YN560 III speed flashes pointed directly at a white styrofoam cup for diffusion. For each image, sequential images were made at different focal depths, then combined to create a deep focus image using Helicon Focus 6.4.2. Pro software. Within Helicon Focus, images were stacked using Method B, radius 2, and smoothing 4.

Dissection of larval mouthparts were performed using customized minuten pin tools under a Leica MZ8 stereomicroscope. Larvae and pupae were cleared using 2 KOH pellets dissolved in 1 dram of deionized water and heated using a hot plate. Specimens were checked every hour for 4-6 hours and removed from heat and the KOH solution once fully cleared. Dissected parts and cleared specimens were then temporarily mounted on slides with glycerin for observation and imaging. Images of dissected larval structures and cleared larvae and pupae were produced using a Sony DKC-5000 camera attached to a Leica Leitz DMRB compound microscope. Differential interference contrast microscopy (DIC) was used to interpret membranous structures. When sequential images were necessary to capture a wider focal depth, images were combined using Helicon Focus 6.4.2 Pro software.

A series of 60 voucher specimens were deposited in both the University of Georgia Collection of Arthropods (UGCA) and the California State Collection of Arthropods (CSCA). Yellow voucher labels were placed on the specimens below the data labels, which read "VOUCHER SPECIMEN; Brissey et al., 2018; *E.* (*H.*) *ocularis* Fairmaire; det. A.R. Cline 2017".

3.4 Results and discussion:

New State Records: Collection data from labels is directly quoted, with ";" indicating line breaks.

Georgia. "33.887367, -83.4166799; USA: GA: Oconee; UGA Horticulture Research Farm; 19.JUL.2017, CL Brissey; ex. UNI-trap with WWBD and apples" [UGCA]. "33.936366, -83.371331; USA: GA: Clarke; 8.AUG.2016, CL Brissey; Coll. in SWD trap baited with WWBD" [UGCA]. "31.75317, -82.44336; USA: GA: Appling; 30.JUL.2016,

JA Grant; yeast, sugar, water trap" [UGCA]. "31.51020, -82.45230; USA: GA: Bacon; 15.JUL.2016, JA Grant; yeast, sugar, water trap" [UGCA]. "31.45169, -82.17831; USA: GA: Pierce; 22.JUL.2016, JA Grant; yeast, sugar, water trap" [UGCA]. "31.18022, -82.00486; USA: GA: Brantley; 2.JUL.2016, JA Grant; yeast, sugar, water trap" [UGCA]. "31.15472, -82.599180; USA; GA: Ware; 9.JUL.2016, JA Grant; yeast, sugar, water trap" [UGCA]. "32.1296, -81.1426; USA: GA: Chatham; Garden City: Port of Savannah; 27.JUN.2015, B Gochnour; ex. leaf litter" [UGCA]. Data labels for the CSCA specimens are indicated in the same manner. **California:** "USA: CA: San Bernardino Co.; Montclair, 2-SEP-2010; Ex: found under apple tree; PDR# 1638302. **Louisiana:** "USA: LA: East Baton Rouge Parish; LSU Campus, 28-SEP-2013 – 10-OCT-2013; ML Ferro and BH Reily; Ex: on hedge apple" [LSAM].

Life stage descriptions

Epuraea (*Haptoncus*) *ocularis* Fairmaire, 1849

Material examined. 33.887367, -83.4166799; USA: GA: Oconee; UGA Horticulture Research Farm; 19.JUL.2017, CL Brissey; ex. UNI-trap with WWBD and apples.

Adult diagnosis. Adult *E.* (*H.*) *ocularis* can be readily differentiated from all other New World *Epuraea* species by the characteristic markings on the elytra (Fig. 1A, 1B; see Ewing and Cline, 2005, for illustration). To date, no identification key exists for all members of the subgenus *Haptoncus* and there is no comprehensive systematic revision of the group. There is a small possibility that cryptic species exist in the Old World within the current broadly defined *E.* (*H.*) *ocularis* species concept. A modern combined morphological/molecular revision of this subgenus, and related genera, is critically needed.

Adult description. A redescription of the adult form is unnecessary. Observations of the type for *E.* (*H.*) *ocularis* and other members of the subgenus would be needed to adequately describe this species in a modern sense with well-defined species-level characteristics and diagnostic features. This is beyond the scope of this work.

Variation. Elytral and pronotal markings can be inconspicuous on teneral specimens.

Geographic distribution. The global distribution of *E. ocularis* was documented by Cline and Audisio (2011). This species is found throughout Southeast Asia, China, Japan, Korea, Europe, East Africa, and Australia (Grouvelle, 1913; Kirejtshuk, 1998; Ewing and Cline, 2005; Jelinek et al., 2016; Jelinek and Lason, 2018). In North America *E. ocularis* has been reported from Houston, TX, Mexico, and East Baton Rouge Parish in Louisiana (Cline and Audisio, 2011; Torres, 2013; Ferro, 2014). Our collection efforts have now yielded new records from eight counties in Georgia (Appling, Bacon, Brantley, Chatham, Clarke, Oconee, Pierce, Ware), and San Bernardino County in California.

Third instar larval description. Body elongate, fusiform, widest around A2-A3, average length 3.9 mm (n=10). Coloration white to cream colored in preserved specimens but paler and translucent in fresh specimens (Figs. 1A, 1B). Setation sparse, short, simple, mostly occurring on dorsal setiferous tubercles (Figs. 1C, 2H). Body surface microsculpture finely granulate (Fig. 2H) with coarser granulations on head (Fig. 1D) and pronotum (Fig. 2B). All thoracic sclerites and abdominal segments 1-8 bearing 2 lightly sclerotized tergal plates, each plate bearing setiferous tubercles (Figs. 1A, 2B, 2C, 2D). Abdominal segments 1-7 with sharply angulate lateral margins along posterior half with weakly developed apical tubercles, each tubercle bearing a single seta (Figs. 1A). Spiracular openings located posteriorly on A1-8 on short tubes (laterad to tergal plate)

(Figs. 1A, 2D). Urogomphi and pregomphi present on anal shield of A9 tergite (Figs. 2F, 2G).

Head prognathous, average width at widest point .5 mm (n=10), slightly narrower than T1, surface microsculpture coarsely granulate with several short setae arranged roughly into 4 transverse rows, row 1 between antennal bases bearing 2 setiferous granules, rows 2 to 4 bearing 6-8 setiferous granules, setae projecting anteriad (Figs. 1A, 1D), 1 longitudinal row of 3 frontal median setae present (Fig. 1D).1 prominent, erect seta appearing along lateral margin near widest part of head (Figs. 1A, 1B, 1D). Clypeus bearing 4 elongate setae and a few minute setae (Fig. 1E). Posterior dorsal margin broadly emarginate. Frontal sutures present, weakly lyriform, epicranial stem absent (Fig. 1D). Eye with 4 ocelli, located posterior to antenna, ocelli arranged in 2 longitudinal pairs, the posterior pair greatly reduced with a seta between them (Fig. 1C). Antenna with 3 segments; relative lengths from 1st to 3rd antennomere are 7:14:11; segment 1 slightly wider than segment 2 (Fig. 1F); segment 2 twice as wide as segment 3, with sensorium present at apex (Fig. 1F), posteroventrad to articulation of segment 3; sensorium about half the length of segment 3, with vague transverse annulation near midlength (Fig. 1F); segment 3 with 1 prominent apical seta, 1 shorter apical seta, and 4 shorter preapical setae(Fig. 1F); segment 3 bearing placoid sensillum on dorsal surface near midlength (Fig. 1F). Labrum with anterior margin broad, weakly arcuate, bearing 4 prominent setae, lateral setae twice as long as medial pair (Fig. 1E). Mandibles broad, slightly asymmetrical (differing in number of dorsal teeth), generally pale, appearing darker along incisor plane, prosthecal processes, mola, and condyles (Fig. 2A); with 2 prominent setae visible along lateral margin. Mandibular apex bidentate, left mandible with 3 subapical

dorsal teeth, right mandible with 2; subapical ventral teeth absent. Prosthecal area bearing distal membranous lobe with complex multi-fringed and spinose processes (Fig. 2A) and basal prosthecal fringe with 5 rows of fine setae (Fig. 2A). Mola prominent, possessing transverse bands of asperities, asperities largest distally along mesal surface (Fig. 2A). Maxilla with 3-segmented palp, appearing 4-segmented due to large palpiger (Fig. 2E); segment 3 slightly longer than 2 and about 1.5x length of 1; palpiger irregular, incompletely sclerotized, partially embedded anteriorly (Fig. 2E). Mala with complex uncus (Fig. 2E), bearing 3 lobes each with conical process at apex (Fig. 2E), dorsal lobe about .5x length of others (Fig. 2E).

Tergum of T1 coarsely granulate and slightly darker than other tergal plates (Fig. 2B). Setiferous tubercles on each tergal plate arranged in 6 longitudinal rows of 4 to 6 (Fig. 2B). T2 and T3 with terga bearing setiferous tubercles, arranged in 5 longitudinal rows of 3 setae on each tergal plate (Fig. 3C). A1-A8 terga bearing setiferous tubercles, arranged in 4 longitudinal rows of 3 setae on each tergal plate (Fig. 3D). Medial setiferous tubercles aligning longitudinally with those on other terga (Figs. 2B, 2C, 2D) T2-A8; 3 to 5 transverse rows of asperities along anterior third of each plate, density greatest medially, rows becoming indistinct laterally (Figs. 2C, 2D).

A9 with ratio greatest width to median length is 7:5 (Fig. 2F); tergum bearing 6 setiferous tubercles along lateral margin and with broad band of small granules basally and distally (Fig. 2F); subpregomphi (sensu Hayashi 1978) appearing as a pair of setiferous tubercles, similar in size and shape to those on preceding tergites (Fig. 2F); pregomphi appearing as a pair of longer setiferous tubercles (Fig. 2F); urogomphi lobe-

like projecting posteriad, each bearing conical process apically and setiferous tubercle ventrolaterally (Figs. 2F, 2G).

Thoracic and abdominal ventrites finely granulate (Fig. 3B). Posterior half of A1-A8 bearing 2 prominent setae posteriorly with interspersed sparse smaller, finer setae (Fig. 3B). A10 dorsal surface with single transverse row of fine short setae, and a median pair of more elongate thicker setae, followed by a faint transverse band of asperities extending posteriad to anal opening (Fig. 2F); ventral surface bearing several rows of faint asperities on anterior half, followed by a single transverse row of short fine setae, and 6 stout anal hooks along apex (Figs. 2F, 2G).

Legs with coxae widely separated, ratio of procoxae separation to metacoxae separation is 3:5, coxae on T1 closer than on T2 and T3, coxae slightly converging posteriorly (Fig. 3A). Setae as in Fig. 3A. Tibia approximately .75x length of femur (Fig. 3A); tarsungulus nearly as long as tibia (Fig. 3A). Femur gradually dilating from base to apex, tibia gradually narrowing from base to apex (Fig. 3A). Femur length nearly 2x width, tibia slightly longer than wide, tarsungulus curved and bearing single seta from basal lobe (Fig. 3A).

Variation. Specimens often become distended or contracted depending on the methods used for preservation and storage. If specimens are boiled for preservation, they become opaque and white to creamy in color.

Pupal description. Body length average 2.1 mm long, widest at antennal clubs, average maximum width 1.0 mm (n=10)(Figs. 4A, 4B). Body surface creamy white to yellowish brown in color; tubercles brown; setae sparse, erect, light brown and mostly limited to lateral sides. Dorsal and ventral body surface covered with fine dense asperities (Fig. 4E).

Head appressed to body, concealed in dorsal view (Figs. 4A, 4B). One small semi erect tubercle near posterior medial margin of each eye, arising from mound like base and curving posteriad, bearing 1 setae medially from base (Figs. 4A, 4C). Eyes appearing finely faceted, with inner margins converging for posterior half, nearly parallel for anterior half (Fig. 4A). Frontoclypeal suture complete, clypeus deeply cleft, bearing 1 simple setae on each apical lobe, and two simple setae at the base (Fig. 4D). Mandibles each bearing 2 setae on dorsal surface (Fig. 4F). Maxillary and labial palps appearing as blunt lobes (Fig. 4G). Apical antennomeres with several conical spines present (Fig. 4H).

Pronotum prominent and completely obscuring head in dorsal view (Fig. 4A), anterior margin arcuate, anterolateral angles angulate, posterolateral angles broadly rounded. Armature consisting of 10 tubercles, all weakly curved anteriad; each side of pronotum bearing 1 large tubercle on anterior margin, with a pair of small tubercles posterior to it, smallest 1 directly posterior to it and the other near anterolateral angle; another tubercle arising on each side near posterolateral angle, with larger tubercle mesad of it near midpoint between lateral margin and midline (Fig. 5A). Vestiture sparse with several setae present anterolaterally, several setae near tubercle at posterolateral angle, and many short fine setae throughout the disc, only visible at magnification above 40X; lacking posterior marginal setae (Fig. 5A).

Meso- and metanotum lacking distinctive setae or tubercles, several fine short setae visible at magnification above 40X (Fig. 5B). Metanotum bearing distinctive medial posterior lobe (Fig. 5B). Both pairs of wings curved ventrally beneath body. Mesothoracic wings bearing fine sparse setae, denser toward apex (Figs. 4B, 5C). Mesothoracic wings extending beyond the anterior edge of A3, completely covering

metathoracic wings in dorsal view; metathoracic wings only visible in ventral view (Figs. 4A, 4B). Mesothoracic wing apex truncate, only reaching anterior margin of ventrite 5; metathoracic wing apex rounded, reaching midlength of ventrite 6 (Fig. 4B).

Pro- and mesothoracic legs not obscured by wings; metathoracic leg mostly obscured by wings, only tarsus and femorotibial junction visible. All femora with apex bearing 1 tubercle, and a pair of anterior and pair of posterior setae (Figs. 4B, 5E).

Abdominal tergites lacking medial tubercles and prominent setation, bearing sparse fine setation on A1-A5 becoming denser and longer on A6-A8 (Fig. 5D). A1 lateral margin covered by wings. A2- A8 bearing 1 lateral tubercle with seta arising from base (Figs. 4A, 4E, 5D). A6-A8 bearing additional smaller tubercle mediad of lateral tubercle (Fig. 5D). A9 with 2 large apical tubercles with several short setae at the base (Fig. 6A). Spiracles present near lateral margin of A1-A5 (Fig. 5D).

Abdominal ventrites 1-5 obscured by meso- and metathoracic wings (Fig. 4B). Lateral tubercles on A4-A9 visible in ventral view (Fig. 4B, 6B, 6C). Sparse fine setation present, becoming denser and longer on ventrites 6-8 (Fig. 6B). Ventrite 7 bearing a tubercle mediad of each lateral tubercle (Fig. 6B).

Variation. As pupae age, nearing the pharate adult stage, morphological features become more distinctive, resembling the adult characters. This is true of the mandibles which, in young pupae, appear as blunt lobes and in older pupae develop apical teeth similar to those seen in adult specimens. The legs also appear more defined with age, with preapical spurs of the tibia and individual tarsi becoming apparent.

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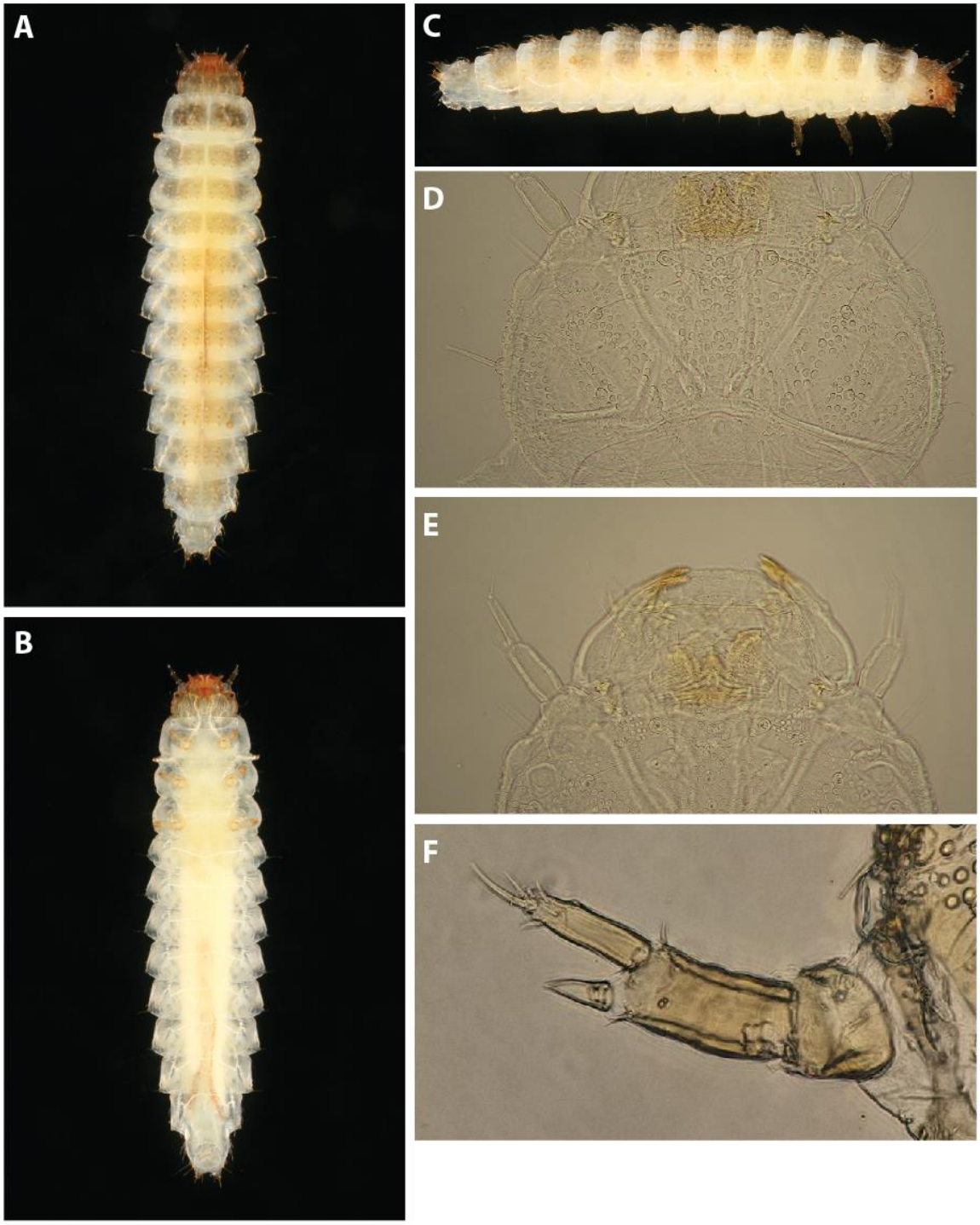

Figure 2.1. Mature 3rd instar larva of *E. ocularis*. A.) Dorsal habitus. B.) Ventral habitus. C.) Lateral habitus. D.) Coarsely granulated head capsule, dorsal view. E.) Clypeus, dorsal view. F.) Left antenna, lateral view.

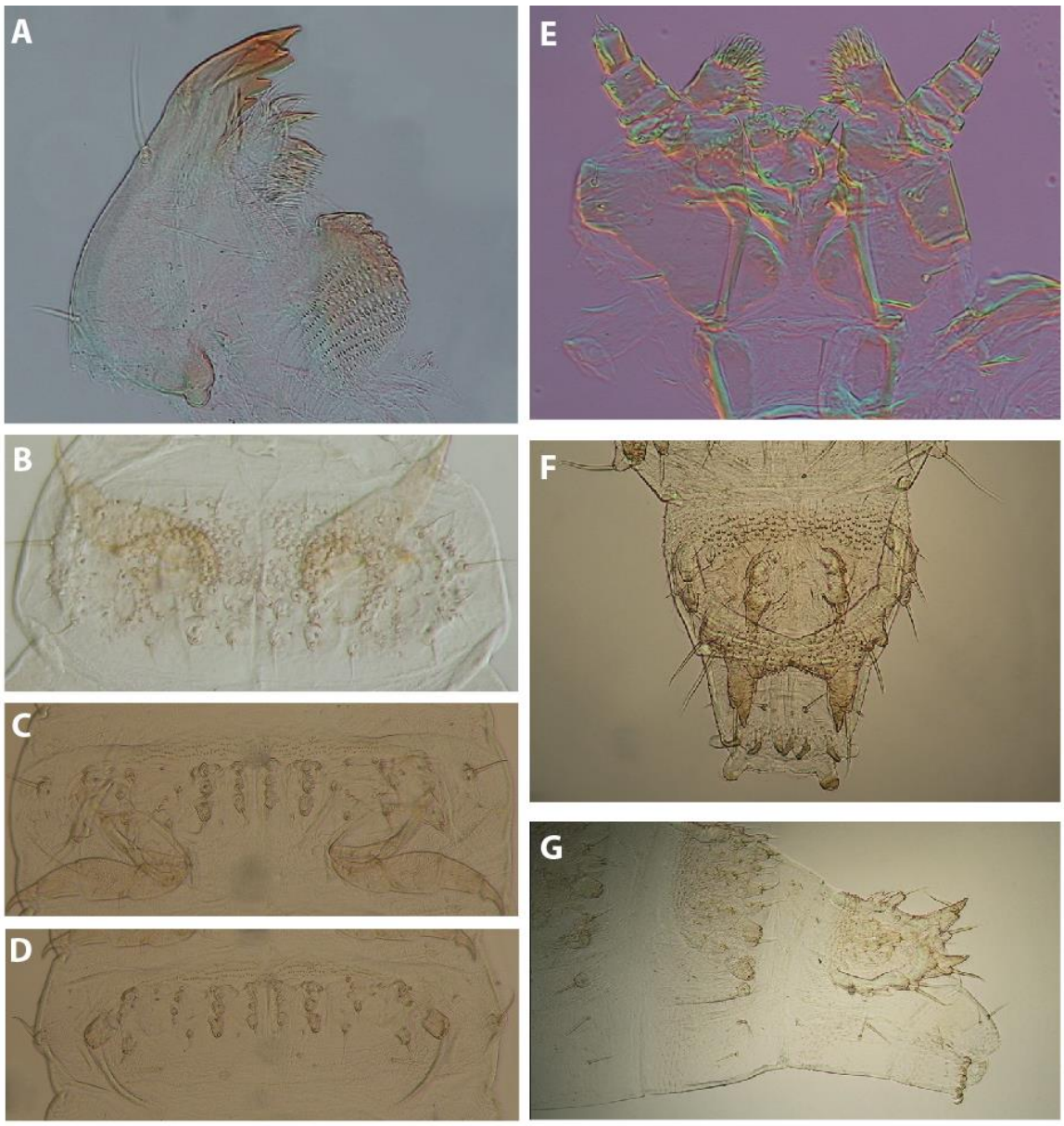

Figure 2.2. Mature 3rd instar larva of *E. ocularis*. A.) Left mandible, dorsal view. B.) T1, dorsal view. C.) T3, dorsal view. D.) A1, dorsal view. E.) Maxillae, maxillary palpi, and labium, ventral view. F.) A9-10, dorsal view. G.) A9-A10, oblique view.

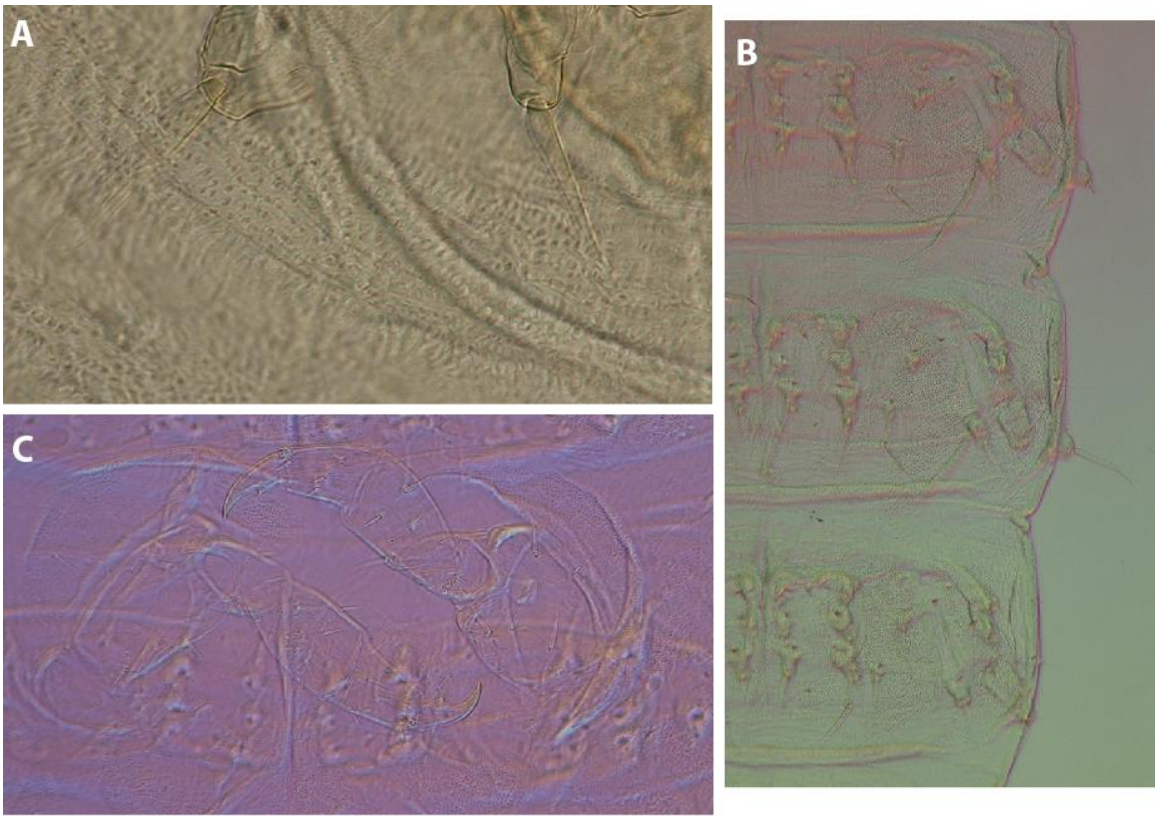

Figure 2.3. Mature 3rd instar larva of *E. ocularis.* A.) Setiferous tubercles. B.) Lateral setiferous tubercles, A6-A8. C.) Prothoracic legs.

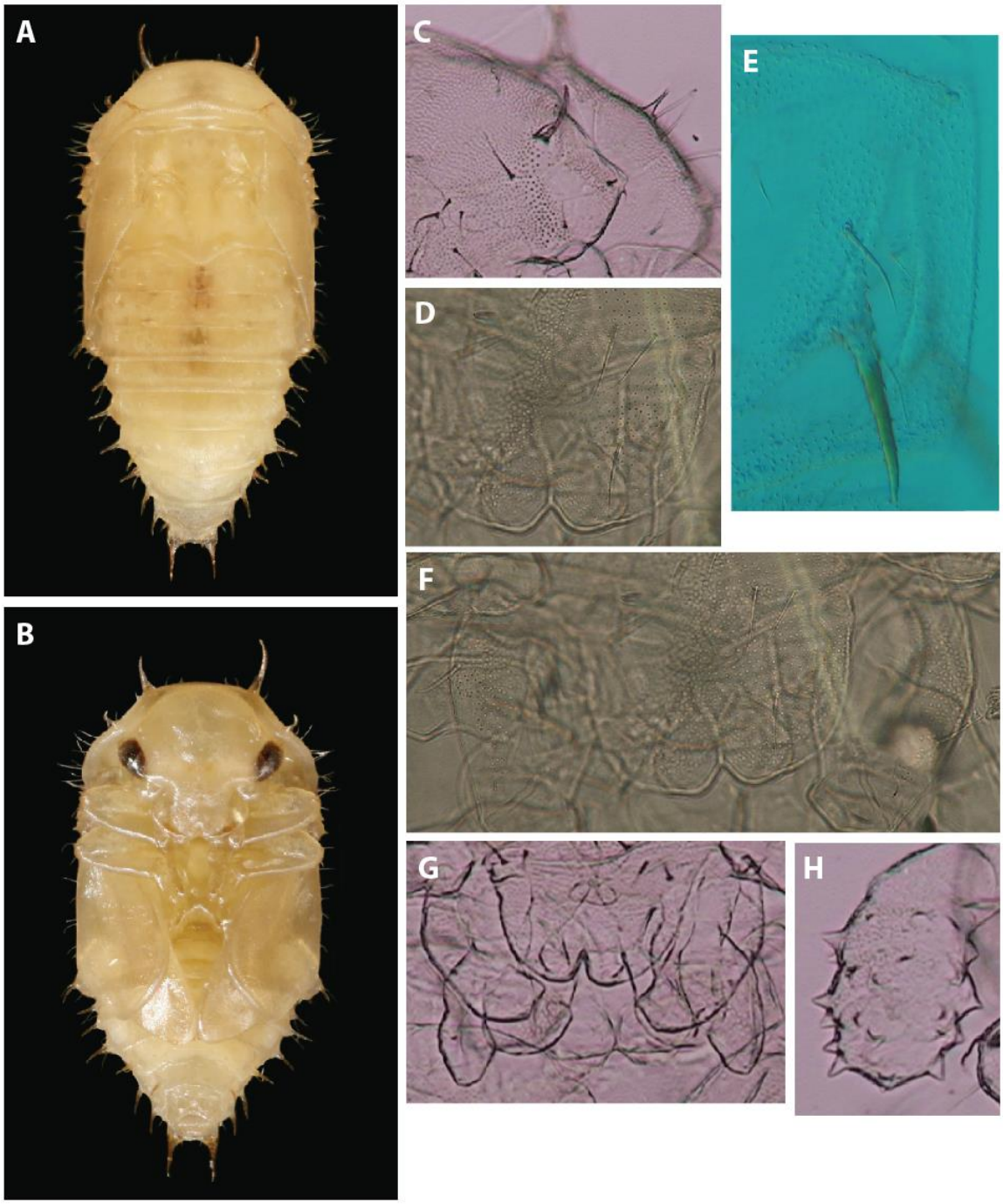

Figure 2.4. *E. ocularis* pupa. A.) Dorsal habitus. B.) Ventral habitus. C.) Tubercle near posterior medial margin of eye. D.) Clypeus. E.) Abdominal tubercle and body surface asperities. F.) Two setae on dorsal surface of mandible. G.) Developing lobes of maxillary and labial palps. H.) Antennal club with conical spines.

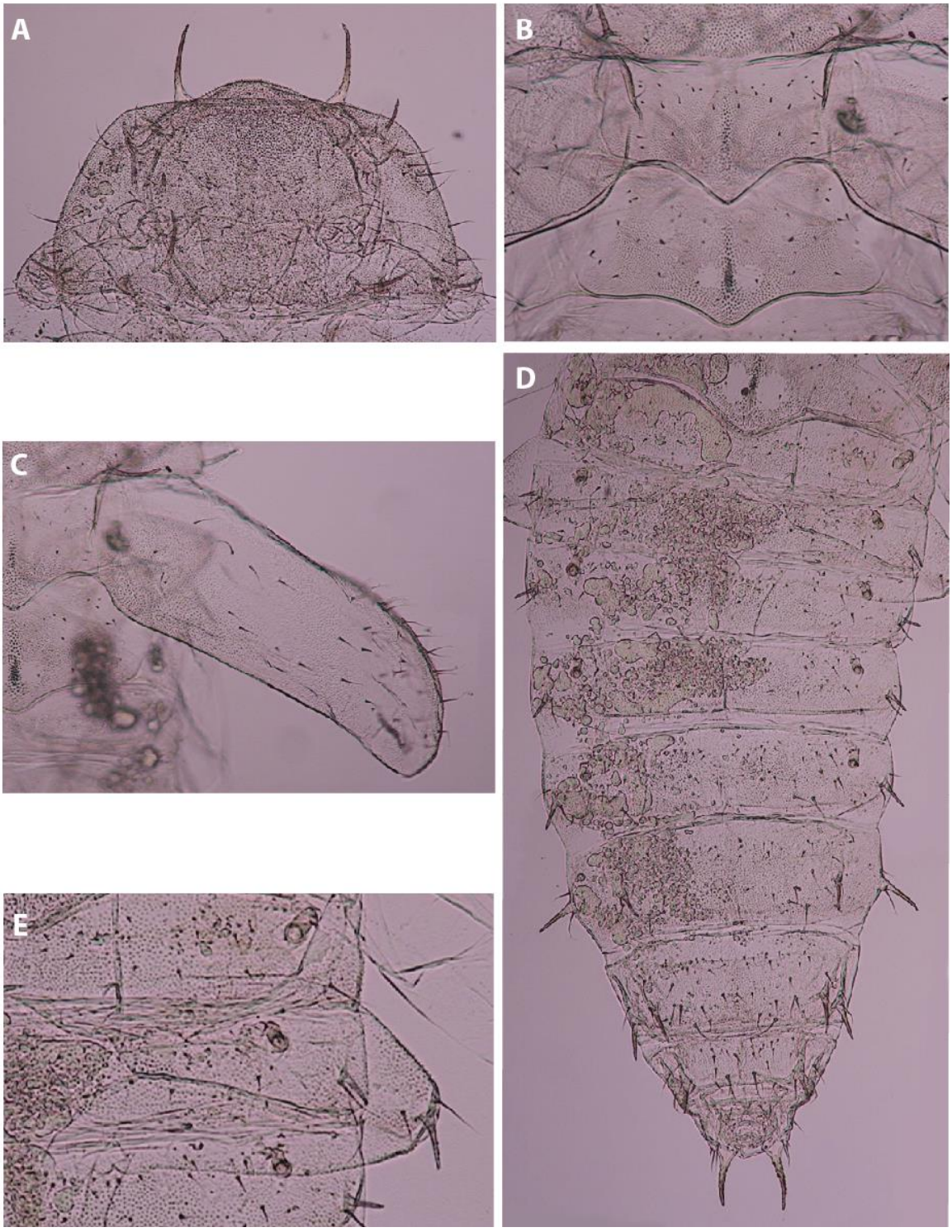

Figure 2.5. *E. ocularis* pupa. A.) Pronotum, dorsal view. B.) Meso- and metathorax, dorsal view. C.) Right elytron, dorsal view. D.) A1-A9, dorsal view. E.) Metathoracic leg tubercle and setae, ventral view.

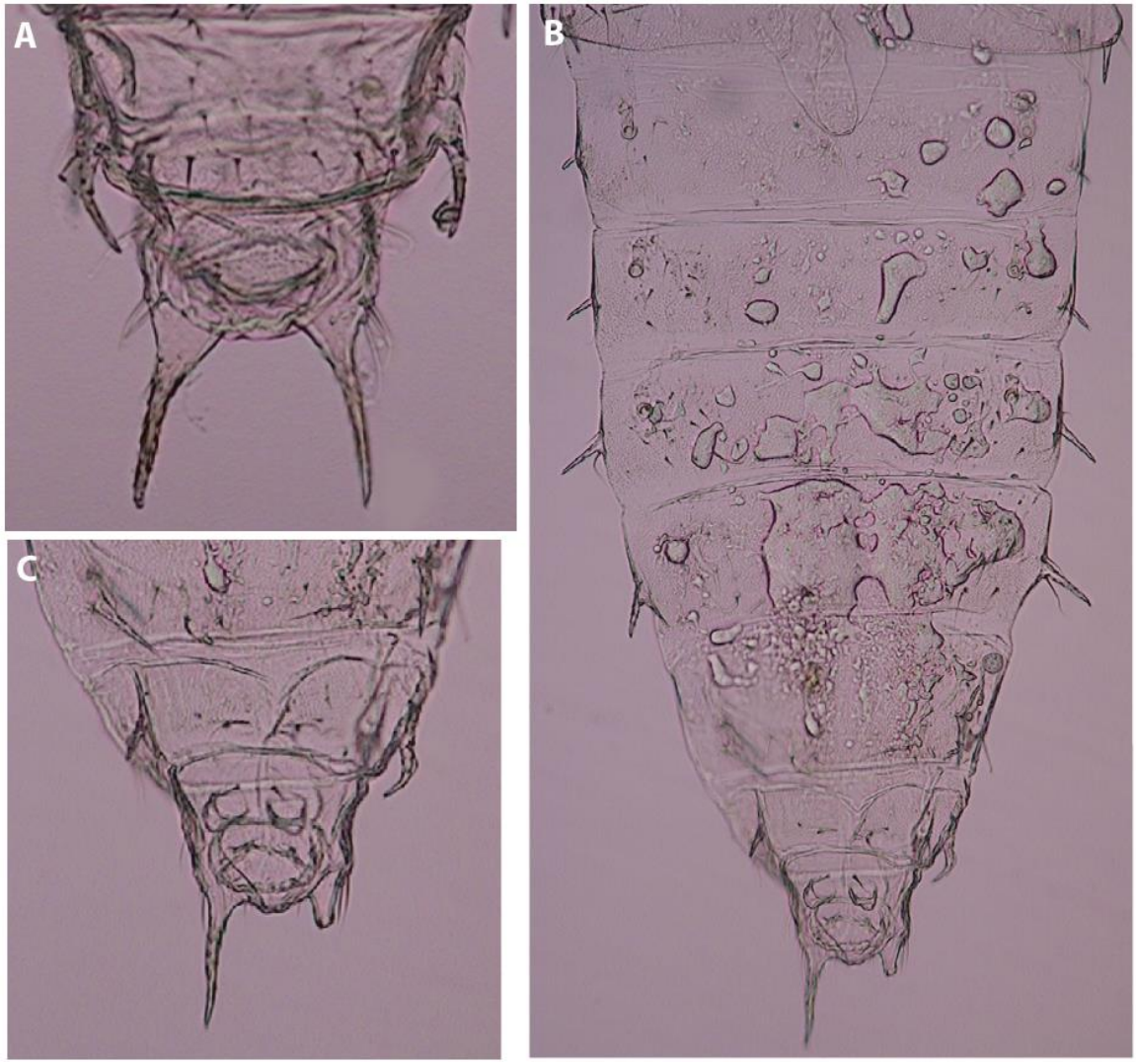

Figure 2.6. *E. ocularis* pupa. A.) A8-A9, dorsal view. B.) A2-A9, ventral view. C.) A7-A9, ventral view.

CHAPTER 4

CONCLUSION

Though Nitidulidae is one of the largest families of cucujoid beetles, species level diagnostic tools contain insufficient coverage of the species present in eastern North America up to this point. Further, many species that have been detected in the Nearctic region have not yet been fully described. This thesis offers a starting point for resolving these long standing issues.

The key to the Carpophilinae of eastern North America provides a much needed resource for port inspectors, growers, extension agents, and entomologists in this region. The key provides a user friendly tool which will make species-level identifications of Carpophilinae beetles possible for specialists and non-specialists. A full taxonomic revision of the Carpophilinae of North America is currently being conducted (by GSP). Since multi-access keys also allow for additions and updates as new characters become available and as taxonomic changes are made to the group, new information will be added as it becomes available. The key could also be expanded to include newly discovered species, or a greater geographic range to create a more inclusive tool.

The new descriptions of the larvae and pupae of *Epuraea ocularis* will enable detection and identification the species in all life stages. The inclusion of detailed character images will also facilitate the correct identifications despite the absence of a key to the genera. This is especially important for species such as *E. ocularis* which are

experiencing an accelerated range expansion aided by human transport. A full revision of the *Epuraea* in the Nearctic region is currently in progress (by ARC).

As technology continues to advance, so will our ability to create better tools for species identification both with interactive, easily accessible keys, and the ability to include high quality images of morphological characters for species descriptions. This paper provides a demonstration of how powerful evolving technology has become in clarifying taxonomic issues which have existed for decades.

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