ABSTRACT

RINCHHUANAWMA, RINCHHUANAWMA. Ensign Wasps (Hymenoptera: Evaniidae) of Madagascar. (Under the direction of Dr. Andrew R. Deans.)

This research focuses on the taxonomy of the ensign wasps (Hymenoptera: Evaniidae) of Madagascar. This project involves taxonomic descriptions, specimen processing, databasing, and DNA barcoding for the purpose of better understanding the ensign wasp diversity of Madagascar. Seventeen new species are described and twelve species are redescribed on this project. Fifty three DNA barcodes are obtained by sequencing a 680-bp portion of the mitochondrial cytochrome oxidase 1 (CO1) gene.
BIOGRAPHY

Awma Rinchhuanawma, a native of Mizoram, was born in India. He attended elementary school at English Congregation School in Mizoram. He joined Woodstock School, an international boarding school, at Grade 6. Awma completed both middle school and high school at Woodstock School. After finishing high school, he attended Messiah College, PA, to study biology and chemistry. During his sophomore year, he transferred to Messiah College's satellite campus in Philadelphia. In Philadelphia, Awma took classes at Temple University and interned at The Academy of National Sciences for two semesters. Awma graduated from Messiah College with a Bachelor of Science degree majoring in Biology and minoring in Chemistry. Awma then joined the Deans Lab at North Carolina State University for a Master of Science degree.
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INTRODUCTION

Ensign wasps (Hymenoptera: Evaniidae) are a family of solitary wasps that can easily be recognized by their laterally compressed flag-like metasomas. They are distinct from other Hymenoptera in having a tubular petiole that is highly attached on the propodeum. These wasps are often colorful and generally have short stout bodies and relatively long legs. Rearing records indicate that evaniid larvae develop as solitary egg predators within the oothecae of cockroaches (Deans 2005). Evaniidae is currently comprised of over 450 extant species in 21 genera (Deans & Huben 2003; Deans & Kawada 2008). In addition, 19 fossil species and 11 fossil genera are documented and recognized as valid (Deans 2005).

There is only one previously published paper on the taxonomy of Evaniidae of Madagascar. Four genera and 13 species are recognized on this paper (Benoit 1952). Benoit (1952) placed 7 species under the genus *Evania* Fabricius, 2 species under *Zeuxevania* Kieffer, 2 species under *Parevania* Kieffer, and 2 species under *Micrevania* Benoit. Deans (2005) transferred 6 of the 7 *Evania* species to *Mircrevenia* based on molecular data.

There is high likelihood that almost all of Madagascar's Evaniidae species that are not recognized on Benoit's (1952) publication are undescribed in taxonomic literature. The island of Madagascar has been isolated from other landmasses for over 80 million years (more than 150 million years from Africa and less than 90 million years from India) and is well known for its exceptionally high levels of endemism for many biological groups (Wilmé et al. 2006; Myers et al. 2000; Storey et al. 1995). For example, 100% of primate species, 99% of
amphibian species, 95% of reptile species, and 96% of ant species found in Madagascar are endemic (Yoder et al. 2005; Fisher & Girman 2000).

This project focuses on the taxonomic revision of the ensign wasps of Madagascar. Morphological and molecular methods are used in redescribing previously described species and describing new species.

MATERIALS AND METHODS

Specimen Collection

The specimens used for this research were collected as part of a biodiversity project, Terrestrial Arthropod Inventory of Madagascar, funded by the United States National Science Foundation. The Evaniidae specimens were collected by Malaise traps and stored in glass vials containing 70% ethanol. The Terrestrial Arthropod Inventory of Madagascar is an ongoing project and newly collected Evaniidae specimens from this project continue to arrive at the Deans Lab at North Carolina State University. Over 4,000 Evaniidae specimens from Madagascar have been collected and sent to the Deans Lab thus far. Holotype specimens were borrowed from Muséum national d'histoire naturelle (MNHN), the National Museum of Natural History in Paris.
Specimen Processing

Over 2,000 specimens were critical point dried using a SPI-DRY Critical Point Dryer (#13200-AB Manual CPD). Specimens collected from different parts of Madagascar, which were stored in 70% ethanol by collecting event, were systematically critical point dried. Five to 50 specimens from two to four different collecting events were critical point dried in each cycle.

Databasing Specimens

Critical point dried specimens were pinned, labeled, and databased. Each specimen was given a unique identification number. The web-based content management system, “mx” (Yoder et al. 2006-present), was used to database all available specimen information including collecting event information, mophological data, and molecular data.

Sorting

A stereo microscope (Olympus SZX16) was used to study the morphology of both critical point dried specimens and specimens in ethanol. The specimens were first sorted to genera using the key provided by Deans and Huben (2003). Specimens were then sorted to morphospecies based on morphological characters used in previous Evaniidae taxonomic treatments and new morphological characters. Specimens sorted in ethanol were place in new vials containing 95% ethanol and given temporary labels. The specimens were continuously resorted as more specimens and characters were studied.
Imaging

A digital camera (JVC KY-F75U) was used for imaging. Archimed Pro® (GT Vision) was used for image processing.

Morphological Terminology

Terminology of wing venation follows Deans and Huben (2003). All other morphological terms follow Hymenoptera Anatomy Ontology (Yoder et al. 2010).

DNA Extraction

DNA was extracted from 129 specimens. The majority of the specimens chosen for DNA extraction were specimens stored in ethanol at -20°C (collected in 70% ethanol and transferred to 95% ethanol after sorting). Forty critical point dried specimens were also used. DNA extracts were prepared using QIAGEN’s “DNeasy Blood & Tissue Kit” (July 2006 edition). The protocol, “Purification of Total DNA from Animal Tissues”, was used with the following changes: (1) on the first step of the procedure, an entire hind leg of a specimen was ground with a plastic pestle and the amount of Buffer ATL used was reduced from 180 µl to 170 µl; (2) on the second step of the procedure, the amount of proteinase K used was increased from 20 µl to 40 µl.
DNA Amplification and Gel Electrophoresis

DNA from the extracts was amplified using the polymerase chain reaction (PCR). For PCR, a 25 µl solution was prepared using the following reagents for each sample:

1.50 µl Template DNA
1.00 µl CO1-LCO1490-F forward primer (5’ to 3’: GGTCACAATCTCAAAAGATATTGG)
1.00 µl CO1-R-DAmod reverse primer (5’ to 3’: TTCTGGRTGACCAAAAAATC)
0.25 µl Taq polymerase
0.50 µl dNTP
2.00 µl MgSO₄
2.50 µl Buffer
16.25 µl PCR water

Thermalcycler settings for PCR were as follows: one cycle of 5 minutes at 94°C; thirty cycles of 45 seconds at 94°C, 45 seconds at 45°C, 1 minute 10 seconds at 72°C; and a final cycle of 7 minutes at 72°C. After the final cycle, the thermalcycler was held at 4°C until the samples were removed.

Gel electrophoresis was used to test whether DNA amplification was successful. 5 µl of each post-PCR sample was run on regular 1% agarose gel with DNA ladder and then observed under ultraviolet light.
**DNA Purification and Sequencing Reaction**

ExoSAP-IT was used to purify successfully amplified DNA (PCR product) for sequencing. The given protocol for ExoSAP-IT (2010) was followed with one change: 0.5 µl of ExoSAP-IT (instead of 2 µl) was used for each 5 µl of PCR product.

Purified PCR products were prepared for sequencing reaction using BigDye® ‘Terminator v3.1 Cycle Sequencing Kit’. For each sample, a 10 µl solution was prepared using the following reagents:

1.00 µl Template DNA

0.50 µl Primer (Forward or Reverse)

1.50 µl 5X Buffer

6.00 µl PCR Water

1.00 µl Big Dye

Thermalcycler settings for the sequencing reaction were as follows: one cycle of 1 minute at 96°C; twenty-five cycles of 10 seconds at 96°C, 5 seconds at 50°C, 4 minutes at 60°C; and a final cycle of 30 seconds at 4°C. After the final cycle, the thermalcycler was held at 4°C until the samples were removed. The samples from the sequencing reaction were sent to the Genomic Sciences Laboratory (GSL) at North Carolina State University for sequencing.

**Sequence Editing and Alignment**

The files containing the DNA sequence information were downloaded from the Genomic Sciences Laboratory’s website. The sequences were manually edited using the
software, Sequencher 4.8® (Gene Codes Corporation). Sequence editing was clear-cut as most of the sequences were high-quality. Low-quality sequences (unclear chromatograms) were not edited. The edited sequences were manually aligned using BioEdit Sequence Alignment Editor 7.0.5.3 (Copyright© 1997-2005 Tom Hall).

**Tree Construction**

Neighbor-Joining and Maximum Parsimony trees were constructed using MEGA 4.0.2 (Copyright© 1993-2008).

**Mini-barcoding for degraded DNA**

Since the majority of DNA extracts could not be amplified, mini-barcoding (Meusnier et al. 2008) was attempted. The purpose of mini-barcoding is to amplify shorter fragments within the barcode region for specimens from which the entire barcode region cannot be amplified (e.g. when the genomic DNA is highly degraded).

All available Malagasy Evaniidae CO1 sequences from online databases were downloaded and aligned using BioEdit Sequence Alignment Editor. The sequences were studied for primer designing. After the CO1 sequences were carefully examined and all important primer design guidelines were taken into consideration, the following internal primers were designed for mini-barcoding of Malagasy Evaniidae:

1.) CO1_Awma_F1 (5’ to 3’): TTGGRGGATTYGGNAAYTG (used with CO1-R-DAmod)
2.) CO1_Awma_F2 (5’ to 3’): GGAACRGGWGTAACNRTWTA (used with CO1-R-DAmod)

3.) CO1_Awma_R (5’ to 3’): TTRTTTAWNCGNGGRAANGC (used with CO1-LCO1490-F)

These primer combinations were first tested on 10 DNA extracts that had been successfully amplified previously using CO1-LCO1490-F forward primer and CO1-R-DAmod reverse primer. They were then tested on 20 DNA extracts that previously failed to amplify.
RESULTS

(The 17 new species described and 12 species redescribed on this project are not all included on this thesis document. All 29 species descriptions from this project will be submitted to Zootaxa [http://www.mapress.com/zootaxa/] by July 2011 and likely be available for access shortly after. The holotype and paratype specimens are currently under negotiation and their repositories and identifiers will also be included on this submission to Zootaxa. A completed dichotomous “Key to the Ensign Wasp species of Madagascar” will also be included.)

Taxonomic Descriptions

Zeuxevania Kieffer of Madagascar

Diagnosis: Zeuxevania species of Madagascar differ from species of other Malagasy genera by a combination of the following character states: fore wing with 6 complete cells; fore wing 1 RS absent; sculpture of lower face not costate; nitid-smooth patch on propodeal-metapctal complex immediately posterior to mesopleuron absent.

Zeuxevania 01 sp. nov. Rinchhuanawma

Diagnosis:

Zeuxevania 01 sp. nov. differs from all other Zeuxevania species by a combination of the following character states: head and mesosoma dark brown to black; pronotum in dorsal view
enlarged (often reticulate) and significantly exceeding tegula laterally; mesoscutum highly convex medially.

**Male Description**

Body length: 2.5 – 3.5 mm. Fore wing length: 2.5 – 3.5 mm.

**Color:**


**Head:**

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: densely setose. Sculpture of lower face: smooth. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending to margin of eye. Malar space length: 0.7 – 0.8 times basal width of mandible.

widest point. Number of mandibular teeth: four. Scape length: 5 times as long as wide. Pedicel length: as long as wide. Longest flagellomere: II. Shortest flagellomere: IX, X.

**Mesosoma:**

Metasoma:


Wings:


Female: Unknown.
Figure 1. *Zeuxevania 01 sp. nov.* Rinchhuwahuwma in lateral view.
Figure 2. *Zeuxevania 01* sp. nov. Rinchhuanawma in lateral view.
Figure 3. *Zeuxevania 01 sp. nov.* Rinchhuanawma in frontal view.
Figure 4. *Zeuxevania 01 sp. nov.* Rinchhuawma in dorsal view.
Zeuxevania 02 sp. nov. Rinchhuanawma

Diagnosis:

Zeuxevania 02 sp. nov. differs from all other Zeuxevania species by a combination of the following character states: head and mesosoma bright yellow; pronotum in dorsal view enlarged, not reticulate, and slightly exceeding tegula laterally; mesoscutellum strongly depressed posterolaterally.

Male Description

Body length: 2.5 – 3.5 mm. Fore wing length: 2.5 – 3.5 mm.

Color:


Head:

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: moderately setose. Sculpture of lower face: smooth. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending past anterior tentorial pit but not reaching margin of eye. Malar space length: 0.6 – 0.7 times basal width of mandible. Position of toruli: slightly ventral to vertical midline of

**Mesosoma:**


**Metasoma:**


**Wings:**


**Female:** Unknown.
Figure 5. Zeuxevania 02 sp. nov. Rinchhuanawma in lateral view.
Figure 6. *Zeuxevania 02 sp. nov.* Rinchhuanawma in lateral view.
Figure 7. *Zeuxevania 02* sp. nov. Rinchhuanawma in frontal view.
Figure 8. *Zeuxevania 02 sp. nov.* Rinchhuhanawma in dorsal view.
**Zeuxevania 03 sp. nov. Rinchhuanawma**

**Diagnosis:**

*Zeuxevania 03 sp. nov.* differs from all other *Zeuxevania* species by a combination of the following character states: head yellow; mesosoma yellow dorsoanteriorly and dark reddish brown ventroposteriorly; mesoscutum sparsely foveolate; mesoscutellum foveolate; upper face and vertex mildly foveolate.

**Male Description**

Body length: 2.5 – 3.5 mm. Fore wing length: 2.5 – 3.5 mm.

**Color:**


**Head:**

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: moderately setose. Sculpture of lower face: punctate. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending to anterior tentorial pit. Malar space length: 0.6 – 0.7 times basal width of

Concavity of medioventral area of upper face: flat, not concave. Presence of vertical carina at the midline of the ventral area of upper face: absent. Sculpture of vertex: mildly foveolate.

Ocelli position: POL > OOL > LOL. Setation of gena: rarely setose. Eye shape: elliptical, 0.4 – 0.5 times as wide as high at widest point. Number of mandibular teeth: three. Scape length: 5 times as long as wide. Pedicel length: 1.2 times as long as wide. Longest flagellomere: II, III. Shortest flagellomere: X.

**Mesosoma:**

smooth posteriorly. Ventral mesopleural area sculpture: foveate. Mesopleural depression
sculpture: smooth dorsally and foveate ventrally. Metanotum sculpture: areolate medially and
rugose laterally. Setation of propodeal-metapectal complex: densely setose. Presence of nitid-
smooth patch on propodeal-metapectal complex immediately posterior to mesopleuron:

**Metasoma:**

Petiole shape: as wide as high. Petiole length: 4 – 5 times as long as wide. Petiole sculpture:
mildly punctate dorsally and smooth ventrally. Gaster shape in lateral view: elliptical.

**Wings:**

Number of complete cells on fore wing: 6. Fore wing 1 RS: absent. Fore wing 2cu-a:
present.

**Female:** Unknown.
Figure 9. *Zeuxevania 03 sp. nov.* Rinchhuanawma in lateral view.
Figure 10. *Zeuxevania* 03 **sp. nov.** Rinchhuanawma in lateral view.
Figure 11. *Zeuxevania 03* sp. nov. Rinchhuanawma in frontal view.
Figure 12. *Zeuxevania 03 sp. nov.* Rinchhuanawma in dorsal view.
Zeuxevania 04 sp. nov. Rinchhuanawma

Diagnosis:

Zeuxevania 04 sp. nov. differs from all other Zeuxevania species by a combination of the following character states: head and mesosoma dark brown to black; mesoscutum strongly depressed anterolaterally; mesoscutum reduced in length, 0.5 – 0.6 times as long as wide; posterolateral corner of mesoscutum highly angulate.

Male Description

Body length: 2.5 – 3.0 mm. Fore wing length: 2.5 – 3.0 mm.

Color:


Head:

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: densely setose. Sculpture of lower face: smooth. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending past anterior tentorial pit but not reaching margin of eye. Malar space length: 0.6 – 0.7 times basal width of mandible. Position of toruli: slightly ventral to vertical midline of

**Mesosoma:**

anteriorly, smooth posteriorly. Ventral mesopleural area sculpture: foveate. Mesopleural
Setation of propodeal-metapetal complex: densely setose. Presence of nitid-smooth patch on
propodeal-metapetal complex immediately posterior to mesopleuron: absent. Propodeal-
metapetal complex sculpture: foveate anteriorly, areolate posteriorly.

Metasoma:
Petiole shape: as wide as high. Petiole length: 4 – 5 times as long as wide. Petiole sculpture:
mildly punctate dorsally and smooth ventrally. Gaster shape in lateral view: elliptical.

Wings:
Number of complete cells on fore wing: 6. Fore wing 1 RS: absent. Fore wing 2cu-a:
present.

Female: Similar to male (species can be diagnosed using male diagnosis) with the following
class differences: antenna shape; gaster shape; setation patterns; color patterns on the
antenna and legs.
Figure 13. *Zeuxevania* 04 **sp. nov.** Rinchuanawma in lateral view.
Figure 14. *Zeuxevania 04 sp. nov.* Rinchhuanawma in lateral view.
Figure 15. *Zeuxevania* 04 sp. nov. Rinchhuanawma in frontal view.
Figure 16. *Zeuxevania* 04 sp. nov. Rinchhuanawma in dorsal view.
Zeuxevania lamellata Benoit

Diagnosis:

Zeuxevania lamellata differs from all other Zeuxevania species by a combination of the following character states: head dark brown to black; mesosoma yellow dorsoanteriorly and dark reddish brown ventroposteriorly; mesoscutellum smooth in sculpture; parapsidal signum present as distinct impression.

Male Description

Body length: 3.0 – 4.0 mm. Fore wing length: 3.0 – 4.0 mm.

Color:


Head:

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: densely setose. Sculpture of lower face: smooth. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending to margin of eye. Malar space length: 0.7 – 0.8 times basal width of mandible. Position of toruli: slightly ventral to vertical midline of eye. Carina extending dorsally from

**Mesosoma:**


**Metasoma:**

**Wings:**

**Female:** Similar to male (species can be diagnosed using male diagnosis) with the following character differences: antenna shape; gaster shape; setation patterns; color patterns on the antenna and legs.
Figure 17. *Zeuxevania lamellata* Benoit in lateral view.
Figure 18. *Zeuxevania lamellata* Benoit in lateral view.
Figure 19. *Zeuxevania lamellata* Benoit in frontal view.
Figure 20. *Zeuxevania lamellata* Benoit in dorsal view.
Zeuxevania variabilis Benoit

Diagnosis:

Zeuxevania variabilis differs from all other Zeuxevania species by a combination of the following character states: head dark brown to black; mesosoma dark brown to black; mesoscutum and mesoscutellum densely punctate; lateral margin of mesoscutum straight; notaulus complete as distinct impression.

Male Description

Body length: 3.0 – 6.0 mm. Fore wing length: 3.0 – 6.0 mm.

Color:


Head:

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: densely setose. Sculpture of lower face: mildly punctate. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending to margin of eye. Malar space length: 0.7 – 0.8 times basal width of mandible. Position of toruli: slightly ventral to vertical midline of eye. Carina extending dorsally from

**Mesosoma:**

**Metasoma:**

Petiole shape: as wide as high. Petiole length: 4.0 – 5.5 times as long as wide. Petiole sculpture: mildly punctate dorsally and smooth ventrally. Gaster shape in lateral view: elliptical.

**Wings:**


**Female:** Similar to male (species can be diagnosed using male diagnosis) with the following character differences: antenna shape; gaster shape; setation patterns; color patterns on the antenna and legs; level of punctures on mesonotum.
Figure 21. *Zeuxevania variabilis* Benoit in lateral view.
Figure 22. *Zeuxevania variabilis* Benoit in lateral view.
Figure 23. Zeuxevania variabilis Benoit in frontal view.
Figure 24. *Zeuxevania variabilis* Benoit in dorsal view.
**Parevania Kieffer of Madagascar**

Diagnosis: *Parevania* species of Madagascar differ from species of other Malagasy genera by a combination of the following character states: fore wing with 7 complete cells; fore wing 1 RS present and mildly or strongly bent toward fore wing Sc + R; sculpture of lower face not costate; nitid-smooth patch on propodeal-metaplectal complex immediately posterior to mesopleuron absent.

**Parevania 01 sp. nov. Rinchhuanawma**

**Diagnosis:**

*Parevania 01 sp. nov. differs from all other Parevania species by a combination of the following character states: body length less than 3.5 mm; head yellow; mesosoma yellow dorsoanteriorly and dark brown to black ventroposteriorly; upper face, gena, and mesonotum smooth-nitid; lateral margin of mesoscutum straight; mesoscutellum not depressed posterolaterally;**

**Male Description**

Body length: 2.5 – 3.3 mm. Fore wing length: 2.5 – 3.0 mm.

**Color:**


**Head:**


**Mesosoma:**

Mesosoma shape in lateral view: 0.8 – 0.9 times as long as high. Pronotum in dorsal view: not enlarged, not exceeding tegula laterally. Mesoscutum shape: 0.6 times as long as wide. Mesoscutum sculpture: smooth-nitid. Convexity of mesoscutum medially: mildly convex. Depression of mesoscutum anterolaterally: not depressed. Lateral margin of mesoscutum: straight. Posterolateral corner of mesoscutum: slightly angulate. Setation of mesoscutum:


Metasoma:


Wings:

Number of complete cells on fore wing: 7. Fore wing 1 RS: present and slightly bent toward fore wing Sc + R. Fore wing 2cu-a: present.

Female: Similar to male (species can be diagnosed using male diagnosis) with the following character differences: antenna shape; gaster shape; setation patterns; color patterns on the antenna.
Figure 25. *Parevania 01 sp. nov.* Rinchhuanawma in lateral view.
Figure 26. *Parevania* 01 *sp. nov.* Rinchhuanawma in lateral view.
Figure 27. *Parevania 01* sp. nov. Rinchhuanawma in frontal view.
Figure 28. *Parevania 01* sp. nov. Rinchhuanawma in dorsal view.
**Parevania 03 sp. nov. Rinchnuanawma**

**Diagnosis:**

_Parevania 03 sp. nov._ differs from all other _Parevania_ species by a combination of the following character states: head and mesosoma bright yellow; mesoscutum strongly depressed anterolaterally; posterolateral corner of mesoscutum highly angulate; mesoscutellum strongly foveolate.

**Male Description**

Body length: 5.5 – 6.5 mm. Fore wing length: 5.0 – 6.0 mm.

**Color:**


**Head:**

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: densely setose. Sculpture of lower face: smooth. Lower face immediately ventral to toruli: flat. Malar carina presence: present. Malar carina length: extending past anterior tentorial pit but not reaching margin of eye. Malar space length: 0.7 – 0.8 times basal width of mandible. Position of toruli: slightly ventral to vertical midline of...

**Mesosoma:**

dorsally and foveolate ventrally. Metanotum sculpture: areolate. Setation of propodeal-
metapetal complex: densely setose. Presence of nitid-smooth patch on propodeal-metapetal
complex immediately posterior to mesopleuron: absent. Propodeal-metapetal complex
sculpture: areolate.

Metasoma:

Petiole shape: as wide as high. Petiole length: 5 – 6 times as long as wide. Petiole sculpture:
mildly punctate dorsally and smooth ventrally. Gaster shape in lateral view: elliptical.

Wings:

Number of complete cells on fore wing: 7. Fore wing 1 RS: present and bent toward fore
wing Sc + R. Fore wing 2cu-a: present.

Female: Similar to male (species can be diagnosed using male diagnosis) with the following
character differences: antenna shape; gaster shape; setation patterns; color patterns on the
antenna.
Figure 29. *Parevania 03* sp. nov. Rinchhuanawma in lateral view.
Figure 30. *Parevania 03* sp. nov. Rinchhuanawma in lateral view.
Figure 31. *Parevania 03 sp. nov.* Rinchhuanawma in frontal view.
Figure 32. *Parevania 03* sp. nov. Rinchhuanawma in dorsal view.
**Parevania 04 sp. nov. Rinchhuanawma**

**Diagnosis**

*Parevania 04* sp. nov. differs from all other *Parevania* species by a combination of the following character states: head mostly black, dorsoposteriorly yellow; mesosoma mostly black, mesonotum yellow; notaulus complete as significantly broad impression; vertex punctate-foveolate; mesocutellum strongly depressed laterally.

**Male Description**

Body length: 4.5 – 5.0 mm. Fore wing length: 4.0 – 4.5 mm.

**Color:**


**Head:**

Head shape in lateral view: hemispherical. Head shape in frontal view: circular, as wide as high. Setation of face: densely setose. Sculpture of lower face: punctate medially and foveolate-reticulate laterally. Lower face immediately ventral to toruli: slightly convex.

Malar carina presence: present. Malar carina length: extending past anterior tentorial pit but not reaching margin of eye. Malar space: 0.7 – 0.8 times basal width of mandible. Position of

**Mesosoma:**


**Metasoma:**

**Wings:**
Number of complete cells on fore wing: 7. Fore wing 1 RS: present and slightly bent toward fore wing Sc + R. Fore wing 2cu-a: present.

**Female:** Unknown.
Figure 33. *Parevania 04 sp. nov.* Rinchhuanawma in lateral view.
Figure 34. *Parevana 04 sp. nov.* Rinchhuanaawma in lateral view.
Figure 35. *Parevania 04 sp. nov.* Rinchhuanawma in frontal view.
Figure 36. *Parevania 04 sp. nov.* Rinchhuanawma in dorsal view.
DNA Barcoding Results

DNA Extraction and Amplification

Only 43 out of the 129 DNA extracts could be successfully amplified. This is most likely due to the specimens having highly degraded DNA. It is unlikely that failed amplifications were due to primer issues because several combinations of primers were used in the mini-barcoding process.

There was correlation observed between DNA extracts from specimens of the same collecting event and the likelihood of successful amplification. For example, if DNA extract from a specimen of a particular collecting event failed to amplify, then it was likely that the rest of the specimens from that same collecting event would also not amplify. Due to specimens being carefully chosen by collecting event based on previous DNA amplification results, 43 successful amplifications out of 129 DNA extracts does not indicate that ~33% of specimens used in this project have DNA that can be amplified by the methods used. The percentage of specimens with DNA that can be amplified with the methods used would be much lower than 33% if specimens were chosen at random.

Mini-barcoding

Mini-barcoding was only successful for extracts that were amplified before. The newly designed primers could amplify fragments of the CO1 gene when they were tested on previously amplified extracts. However, amplification with the new primer combinations was
unsuccessful for extracts that previously failed to amplify. No additional sequences were obtained from mini-barcoding.

**DNA Sequences**

A 680-bp portion of the mitochondrial cytochrome oxidase 1 (CO1) gene was successfully obtained from all 43 amplified DNA extracts (each DNA sequence is preceded by the unique identification number of the specimen from which DNA was extracted):

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76
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>CASENT 2032328
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85
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>CASENT_2032254
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>CASENT 2032255
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>CASENT 2032260
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>CASENT 2032266
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>CASENT 2032269
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>CASENT 2032270
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>CASENT 2154657
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> CASENT 2154431
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91
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>CASENT 2154426
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>CASENT 2154763
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>CASENT 2145417
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>CASENT 2145453
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>CASENT 2145454
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>CASENT 2145202
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>CASENT 2145468
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>CASENT 215498
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>CASENT 2032332
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>CASENT 2032333
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>CASENT 2032334
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>CASENT 2032335
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Key to *Zeuxevania* species of Madagascar (Hymenoptera: Evaniidae)

1. Pronotum in dorsal view enlarged and laterally exceeding tegula.................. 2
   - Pronotum in dorsal view not enlarged and not laterally exceeding tegula........ 3
2. Head and mesosoma dark brown to black.................. *Zeuxevania 01* sp. nov.
   - Head and mesosoma bright yellow.................. *Zeuxevania 02* sp. nov.
3. Mesoscutellum foveolate............................... *Zeuxevania 03* sp. nov.
   - Mesoscutellum smooth or punctate (small punctures)............................ 4
4. Mesoscutum strongly depressed anterolaterally.................. *Zeuxevania 04* sp. nov.
   - Mesoscutum not depressed anterolaterally........................................ 5
5. Mesoscutellum yellow and smooth.......................... *Zeuxevania lamellata*
   - Mesoscutellum black to dark brown and punctate............................... *Zeuxevania variabilis*
Figure 37. Neighbor-Joining Tree constructed with MEGA 4.0.2 from nucleotide p-distance.
Figure 38. Maximum Parsimony Tree constructed with MEGA 4.0.2 using the Close-Neighbor-Interchange algorithm at a search level of 1 (Bootstrap - 1000 replications).
DISCUSSION

This research project is only a beginning step toward a better understanding of the ensign wasp diversity of Madagascar. The species described in this project likely represent only a fraction of Evaniidae found in Madagascar. The vast differences in character states between different species (e.g. length of malar carina, convexity of mesoscutum, various levels depressions on the mesonotum etc.) make many of the species easily distinctive morphologically from other species. However, the diversity of morphological character states also led to challenges in developing robust taxonomic treatments, especially for continuous characters. Attempting to delimit character states from one state to the other was one of the most challenging aspects of this project. For example, it was often difficult to delimit surface sculpturing character states (e.g. foveate vs. foveolate), setation character states (e.g. moderately setose vs. densely setose), and character states that involved the convexity or concavity of a particular body part. The continuousness of character states was an issue even for distinct characters, such as the number of mandibular teeth and the presence or absence of a signum. The mandibular teeth of some specimens are partially fused and the signa (e.g. antero-admedian signum) of many specimens are only visible under relatively high magnification and translucent light from a particular direction.

An interesting finding is the putative conspecificity of morphospecies that vary significantly in color. The majority of previous publications on Evaniidae use color as part of the species diagnosis and there are very few species descriptions of a species that
significantly vary in color patterns. Most of the variance in color patterns within a species is subtle in past publications (Ashmead 1901; Benoit 1952; Benot 1953; Deans & Huben 2003; Deans & Kawada 2008; Elliott 2005; Townes 1949; Townes 1958). Non-color morphological characters and DNA barcoding results suggest the conspecificity of some morphospecies that significantly differ in color patterns *Micrevania seyrigi* is an example (manuscript in preparation).

The most striking aspect of the results from DNA barcoding (680-bp portion of the CO1 gene) is the significant differences observed in nucleotide p-distance between some putative conspecific species. For example, some CO1 sequences of *Zeuxevania variabilis* have p-distances that exceed 0.07 within the species. The unreasonableness of having a universal threshold level for p-distance value for delimiting species based on the barcode region of the CO1 gene has often been highlighted by taxonomists (e.g. Meier et al., Rubinoff et al.). However, CO1 barcoding results in most literature report p-distances of less than 0.05 within species for most species. Due to taxonomic treatments in this project being developed based solely on the 75 external morphological characters studied, it is possible that specimens (e.g. *Zeuxevania variabilis*) treated as conspecific in this project may actually represent two or more species. Further studies on both external and internal morphological characters and character states, in addition to the 75 external characters used in this project, would assist in the process of developing future taxonomic treatments for these specimens with exceptionally high p-distance values within the species.
The significantly low bootstrap support in the Maximum Parsimony tree emphasizes the eminence of acquiring additional molecular data for the inference of a robust phylogenetic tree based on molecular data. Due to the molecular data obtained in this project being significantly limited by highly degraded DNA of the specimens, phylogenetic analysis based on morphological data should be considered in the future. All the characters and character states used in this project should be considered for future phylogenetic studies based on morphology, however, careful attention should be paid towards the use of these characters in terms of consistency and balance. Morphological phylogenies vary significantly in quality and the reasons for inexplicit phylogenies include the number of characters used, indistinct character states, and choice of characters (Arnold 1990; Thiele 1993; Wiens 2001).

There are over 2,000 ensign wasp specimens from Madagascar (available at the Deans Lab) that have not been examined. Additional taxonomic work on these specimens may reveal additional new species and taxonomically significant morphological characters that might have been overlooked in this project. The taxonomy of the ensign wasps of Madagascar is a vital process in the direction towards future phylogenetic and biogeographic studies to enhance our understanding of the ensign wasp diversity of Madagascar.
REFERENCES CITED


