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Ecomorph Convergence in Stick Insects (Phasmatodea) with Emphasis on the Lonchodinae of Papua New Guinea

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ABSTRACT

Ecomorph Convergence in Stick Insects (Phasmatodea) with Emphasis on the Lonchodinae of Papua New Guinea

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Phasmatodea exhibit a variety of cryptic ecomorphs associated with various microhabitats. Multiple ecomorphs are present in the stick insect fauna from Papua New Guinea, including the tree lobster, spiny, and long slender forms. While ecomorphs have long been recognized in phasmids, there has yet to be an attempt to objectively define and study the evolution of these ecomorphs. Using principal component analysis, PERMANOVA, ANOVA, and phylogenetic reconstructions, we examined the evolution of ecomorphs in the Lonchodinae stick insects of Papua New Guinea. Phylogenetic reconstructions were performed via maximum likelihood and Bayesian methods and ecomorphs were mapped onto recovered topologies to assess patterns of ecomorph evolution. Statistical test supported a general tree lobster ecomorph grouping with overlap of the slender and spiny ecomorph groups. Phylogenetic reconstructions recovered predominantly congruent topologies, with indications of ecomorph convergence across Phasmatodea. Three independent origins of the tree lobster ecomorph were recovered within the subfamily Lonchodinae. When ecomorph evolution was examined across Phasmatodea, multiple origins of the slender, spiny, tree lobster, and large winged ecomorphs were also recovered.

Keywords: Phasmatodea, ecomorph, convergence, phylogeny

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INTRODUCTION

Phasmatodea, an insect order well known for its camouflage and crypsis, is considered a mesodiverse (~3200 spp.) insect order that exhibits a variety of ecomorphs. Phasmid ecomorphs include long slender, large winged, short wingless, leaf imitating, small spiny, and stout-ground dwelling forms (Buckley et al., 2009) (Figure 1). An ecomorph is a morphological form associated with similar ecological occupancy (Garland and Losos, 1994; Losos, 1994; Losos, 2010; Williams, 1972). Ecomorphs are observed in other animal groups, including mantids, katydids, lice, spiders, and *Anolis* lizards, with several instances of convergence of different forms (Blackledge and Gillespie, 2004; Langerhans et al. 2006; Velasco and Herrel; 2007, Svenson and Whiting, 2009; Yamagishi et al., 2014; Mugleston et al., 2016; Rodríguez et al., 2016; Grisales-Martínex et al. 2017). Many phasmid ecomorphs can be easily observed and identified. However; there are also species whose body forms would be considered an intermediate between two given ecomorphs. The inability to distinguish the ecomorphs of these intermediate forms partially results from the lack of formal description of each phasmid ecomorph. Finding a way to objectively define and determine phasmids ecomorphs would prove helpful in dealing with newly described species and intermediate forms, as well as understanding the evolution of these ecomorphs. Before phasmid ecomorphs can be described we must determine if the ecomorphs can be distinguished from one another using an objective framework. In this study we will use various morphometric methods to test the possibility of objective delimitation of ecomorphs.

In addition to testing for distinct ecomorph groupings, this study aims to determine the evolutionary history of various phasmids ecomorphs. The stout-ground dwelling ecomorph, commonly known as the tree lobster, is well known for the Lorde Howe Island stick insect,

which was once thought to be extinct (Priddel et al., 2003). Tree lobsters are typically found at the base of trees and are characterized by their robust body size and enlarged hind femora (Buckley et al., 2009; Buckley, 2010). Ecomorphs appear to have evolved multiple times across Phasmatodea; and the tree lobster form is hypothesized to have evolved at least three times across the order. These three origins include 1) close phylogenetic relationship of 1. *T. guentheri* and *Eurycantha*, 2) the species *Dryococelus australis*, and 3) the genus *Canachus*. (Buckley et al., 2009).

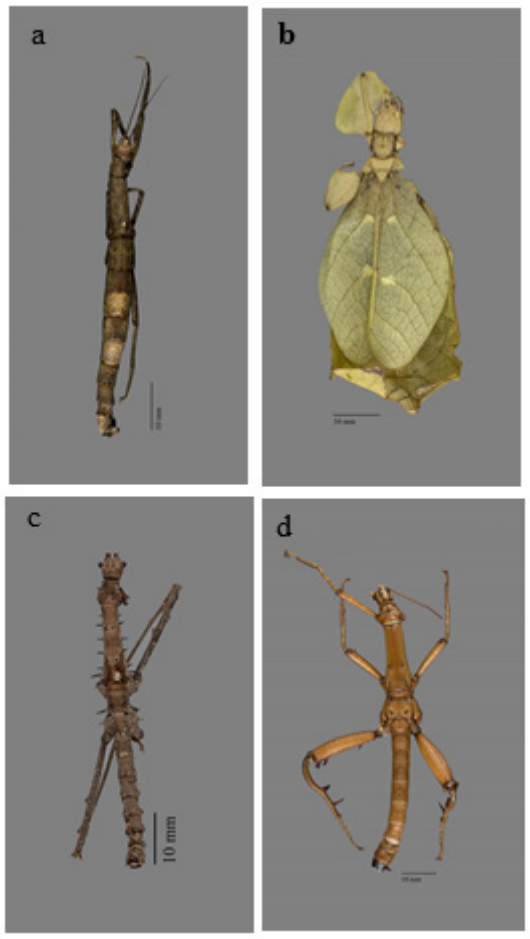

Figure 1
Examples of four different phasmid ecomorphs a. long slender ecomorph (*Oxyartes sp.*), b. leaf mimic ecomorph (*Phyllium sp.*), c. small spiny ecomorph (*Erinaceophasma vepres vepres*), and d. tree lobster ecomorph (*Thaumatobactron guentheri*)

Recent studies have helped further elaborate the evolution of species within Phasmatodea (Bradler et al. 2014; Robertson et al, in press). The present study focuses on specific lineages of Phasmatodea within the subfamily Lonchodinae to investigate evolution of the tree lobster and other ecomorphs more extensively. Lonchodinae is a subfamily of stick insects that exhibits a variety of ecomorphs, including tree lobsters, the spiny ecomorph, and long slender ecomorph. The subfamily consists of 52 genera and 390 described species. The tree lobsters of Lonchodinae are traditionally proposed to have one origin within the subfamily (Bradler, 2009; Buckley et al., 2009). However, previous studies were based on a limited taxon sampling. Buckley et al. (2009) incorporated only four species from the subfamily, while Bradler (2009) included just eight of the Papua New Guinea Lonchodinae. To more extensively examine the relationships in Lonchodinae, a total of 52 Lonchodinae species were used in this analysis. This increased taxon sampling includes six new undescribed Lonchodinae species. Species descriptions will not be presented in this paper. Species will be described after further systematic clarification within the group.

The Lonchodinae of Papua New Guinea is recovered as a monophyletic group in a previous study (Robertson et al. in press) providing us a system to study the evolution of ecomorphs within this lineage. Using both mitochondrial and nuclear loci, we present a phylogenetic reconstruction of the lineage which consists of the Papua New Guinea phasmids, in order to assess the evolution of the tree lobster ecomorph from Papua New Guinea.

METHODS

Taxon sampling

Taxa were sampled to incorporate the range of ecomorphs present in the Papua New Guinea Lonchodinae (see index Table 4). These included the diminutive spiny form represented by the genera *Neopromachus* (Giglio & Tso 1912) and *Erinaceophasma* (Zompro 2001), the long slender forms represented by the genera *Hyrtacus* (Stål 1915) and *Eupromachus* (Brunner Von Wattenwyl 1902), and the tree lobsters represented by *Eurycantha* (Boisduval 1835) and *Thaumatobactron* (Hennemann and Conle 1997). Ingroup sampling consisted of 15 assumed *Neopromachus* spp., two *Erinaceophasma* subspecies, seven *Hyrtacus* spp., two *Eupromachus* spp., seven *Eurycantha* species, and one *Thumatobactron* species (*Thuamatobactron guentheri*). An additional eleven Lonchodinae species were included, as well. Additional ingroup taxa were selected from Diapheromerinae, Clitumninae, Gratidiini, Agathemeridae, Pseudophasmatinae, Heteropteryginae, Pharnaciini, Cladomorphinae, Stephanacridini, Lanceocercata, Phylliinae, and Necrosciinae. The sister group to Euphasmatodea, *Timema*, was used as an outgroup (Kristensen,1975; Tilgner, 2002; Whiting et al., 2003; Terry & Whiting, 2005; Bradler, 2009; Klug & Bradler, 2006; Tomita et al., 2011; Friedemann et al., 2012; Gottardo et al., 2012; Wan et al., 2012).

Morphometrics

Body measurements were taken for eight morphological structures (Figure 2) from 135 specimens. Only adult specimens were measured and multiple representatives of each species including both male and female, up to seven specimens per sex, were measured. Both ethanol preserved and pinned specimens were measured. Males and females were analyzed separately in

each morphometric analysis. Measurements included: head width (HW), head length (HL), mean mesonotal width (MnW), mean mesonotal length (MnL), mean mesotibial length (MtL), mean abdominal width (AW), mean abdominal length (AL), and total body length (BL) (Fig. 1). Mean mesonotal width and mean abdominal width were calculated by averaging the width of the anterior margin and the posterior margin of each structure. Mean mesonotal length and mean abdominal length were calculated by averaging three length measurements: the length of the right side of the specimen, the left side of the specimen, and the length down the midline of the specimen. Abdominal measurements were measured from the second abdominal segment to the ninth segment because the first abdominal segment in Phasmatodea is fused to the thorax and genitalia vary across separate species. A total body length measurement was taken from the most distal point of the head to the end of the genitalia. Measurements were then converted into three ratios, including head width to head length (H), mesonotal width to mesonotal length (Mn), and abdominal width to abdominal length (A).

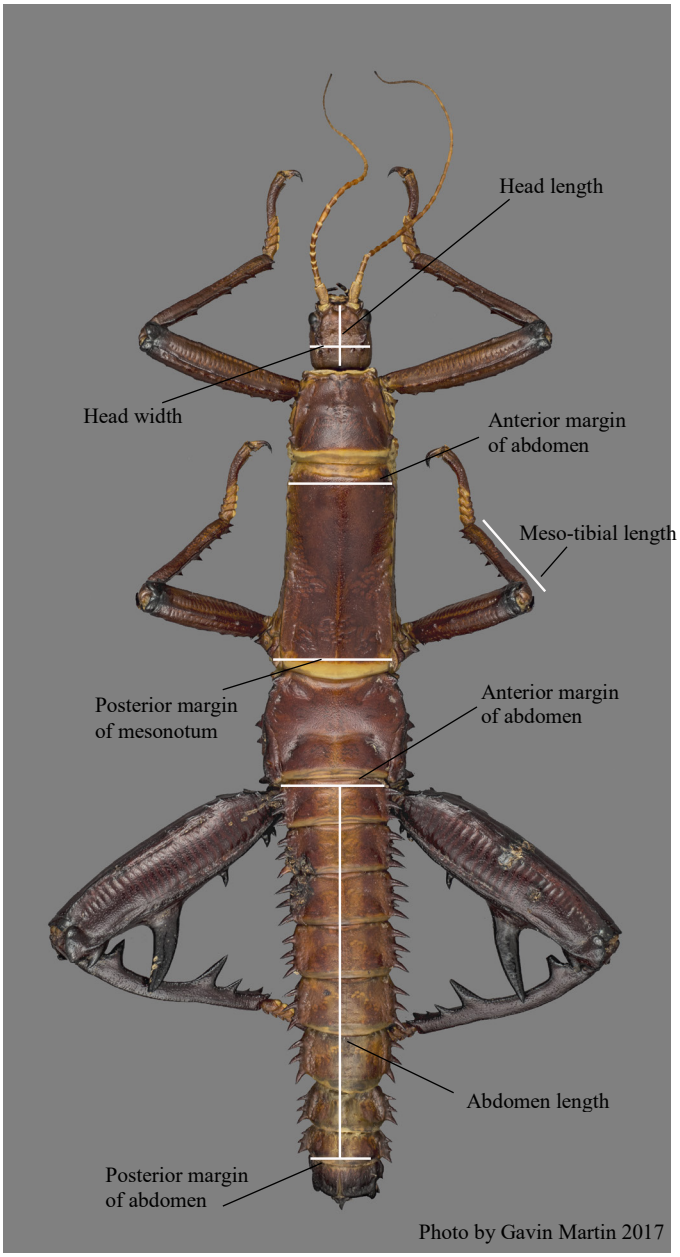

Figure 2
Morphometric characters that were measured for statistical analyses

Statistical methods

Two separate principal components analyses (PCA)were performed. The first analysis included only the average linear measurements along with total body length and the second analysis included the three ratios (H, Mn, and A), mean tibial length, and total body length. PCAs were performed in R (R Core Team, 2013). Principal components one (PC1) and principal components two (PC2) were plotted against each other, for each analysis and each sex, to assess for the presence of ecomorph groupings. The factor loadings of each measured and calculated character were estimated to determine which, if any, element had a significant influence on either PC variable.

PERMANOVA analyses were performed in order to determine if measured characters differed significantly across ecomorphs. Analyses were performed in RStudio (RStudio Team, 2016) using the package vegan (Oksanen et al., 2017) with 1000 permutations. The multivariate analysis uses distance methods to compare the presented elements across the three ecomorphs. The results will be used to determine if the measured elements, as a whole, differ significantly between ecomorphs.

ANOVA analyses were performed to determine if each measured character differed significantly between ecomorphs. Analyses were executed in RStudio (RStudio Team, 2016) with the package vegan (Oksanen et al., 2017). This was done by comparing the means in each ecomorph grouping of individual characters, to determine if the means from each ecomorph grouping differ significantly.

DNA extraction and PCR amplification

Specimens vouchers are preserved in 99% ethanol, stored at -80ºC, and deposited in the Insect Genomics Collection at Brigham Young University. Tissue samples were extracted by removing muscle from the hind leg and coxa of specimens. DNA was extracted from tissue samples with a Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Seven loci were targeted and amplified via polymerase chain reaction (PCR): nuclear 18S rRNA (18S), 28S rRNA (28S), histone 3 (H3); mitochondrial 12S rRNA (12S), 16S rRNA (16S), cytochrome c oxidase subunit I (COI), and cytochrome c oxidase subunit II (COII). PCR amplification used standard insect primers and previously described protocols (Svenson and Whiting, 2009; Robertson et al., 2013). PCR products were cleaned and sequencing reactions were completed using Big Dye terminator sequencing. Sequences were prepared for gel electrophoresis and complementary strands were sequenced at the Brigham Young University DNA Sequencing Center (Provo, UT).

Multiple sequence alignment

Sequence fragments were assembled into contigs, ends were trimmed, and sequences were BLAST searched for contamination in Geneious version 10.1.3 (Kearse et al., 2012). Each locus was aligned individually on the MAFFT server using MAFFT version 7 (Katoh and Standley, 2013) with default settings. Aligned sequences were then concatenated in Geneious (Kearse et al., 2012).

Phylogenetic reconstruction

Both maximum likelihood (ML) and Bayesian methods were used to infer the topology of the Papua New Guinea Lonchodinae. Partitions were implemented for the ML reconstructions for each of the 7 loci. Partitions were then analyzed in ModelFinder (Kalyaanamoorthy et al., 2017) in IQ-Tree (Trifinopoulos et al. 2016) to determine the best fit model for each partition and establish if any partitions should be merged, to reduce overparameterization. Tree reconstruction was performed in IQ-Tree (Trifinopoulos et al. 2016) with 1000 bootstrap replicates (Hoang et al., in press).

Bayesian analyses were performed in Mr. Bayes version 3.2.6 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) using the BYU supercomputer (https://marylou.byu.edu/). The same seven partitions used in the ML analysis were implemented to the multiple sequence alignment. Runs were checked every 100,000 generations for a total of 50,000,000 generations with a 25% burn in. Bayesian analysis was run under the GTR+I+G model and resulting posterior probabilities were used to assess nodal support.

Ancestral state reconstructions were performed in a parsimony framework in Mesquite (Maddison and Maddison, 2017), for both likelihood and Bayesian topologies. Each ecomorph was coded as an unordered multi-state character (0: spiny, 1: slender, 2: tree lobster, 3: leaf, 4: large winged, and 5: intermediate leaf/spiny 6: intermediate tree lobster).

RESULTS

Morphometrics and Statistical analyses

The first and second axis of the PCA were plotted against each other (Figure 3); general ecomorphs groupings were recovered with some overlap among groups.

In analyses of male specimens, PC1 and PC2 account for 91.08% of the variation. PC1 accounted for 79.87% of the variation and PC2 accounted for 11.2% of the variation. The was no one specific dimension that influenced PC1 significantly. Mean tibia length had the greatest influence on PC2 with a factor loading of 0.78 (Table 1).

For females, PC1 and PC2 account for 84.65% of the variation. PC1 accounted for 71.49% of the variation and PC2 accounted for 13.16% of the variation. There was no one specific dimension that influenced PC1 significantly. Mean tibia length had the greatest influence on mean tibia length in PC2, with a factor loading of 0.60.

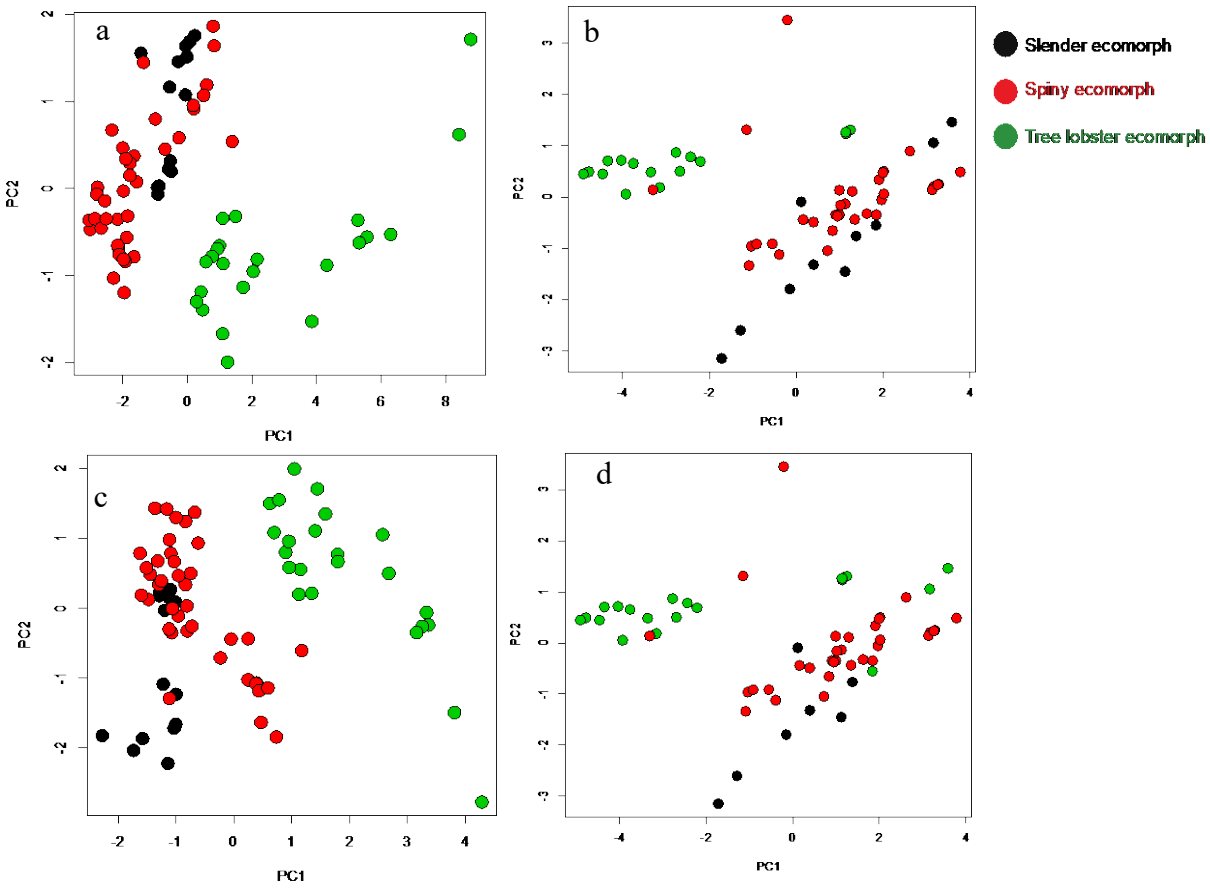

Figure 3
PCA plots of PC1 v. PC2 for a. male linear measurements, b. female linear measurements, c. male ratios, and d. female ratios.

Principal components analyses were also performed on ratios in order to adjust for differences in diet. PC1 was plotted against PC2 (Fig. 3a-b) in both males and females and similar results to the linear measurement analyses were recovered, with no distinct ecomorph groupings.

In males, PC1 and PC2 accounted for 69.58% of the variation. PC1 accounted for 46.83% of the variation, and Mn had the greatest influence on PC1, with a factor loading of 0.61. PC2 accounted for 22.75% of the variation and mean tibial length had the highest influence on PC2, with a factor loading of -0.79.

In females, PC1 and PC2 accounted for 76.28% of the variation. PC1 accounted for 46.38% of the variation, while no one dimension influences had a significant influence on the on PC1. PC2 accounted for 29.9% of the variation, while no one dimension influences had a significant influence on the on PC2.

The multivariate analysis (PERMANOVA) of male linear measurements suggests a significant difference between the three ecomorphs ($F_{2,132}$=45.82, $p < 0.001$*). Additionally, the multivariate analysis of female linear measurements suggests a significant difference between the three ecomorphs ($F_{2,132}$=17.06, $p < 0.001$*).

When ratios were examined via PERMANOVA the analysis of ratios for male only specimens ($F_{2,132}$= 41.74, $p < 0.001$*) and female only specimens ($F_{2,132}$=19.90, $p < 0.001$*) also suggests a significant difference between ecomorphs.

Analysis of variance test were performed on each individual linear measurement character. Across males, the means of all linear characters differing significantly between ecomorphs, except for tibial length ($F_{2,132}$= 0.036, $p = 0.96$). The analysis of female specimens

recovered a significant difference in the means of all linear characters, except tibial length ($F_{2,132}$=0.41, p = 0.67). (See Table 2 for a full summary of ANOVA results).

The result of the ANOVA test for the three ratios examined, mean tibial length, and total body length across males recovered a non-significant difference in H across ecomorphs ($F_{2,73}$ = 0.50, p= 0.61). Additionally, the mean tibial length, in male specimens, was found to not differ significantly between ecomorphs ($F_{2,73}$ = 2.73, p = 0.96), while all other characters' means differed significantly. The analysis of female specimens recovered similar results, with all characters' means differing significantly between ecomorphs, except tibial length ($F_{2,73}$ =32.48, p = 0.67). (See table 3 for a full summary of ANOVA results).

Overall, PCA results recovered broad ecomorph groupings while PERMANOVA and ANOVA analyses do indicate significant differences among ecomorphs, with some overlap of ecomorph groups.

Phylogenetic reconstruction

The best fitting models for each locus were: TVM+I+G4 for 12S and H3, GTR+I+G4 for COII and 28S, K3Pu+I+G4 for 16S, TVM+G4 for COI, and SYM+I+G4 for 18S. ML reconstruction recovered *Neopromachus* (spiny morph) as non-monophyletic, consistent with previous morphological and molecular studies (Figure 4) (Bradler 2009, Robertson et al. in press). *Hyrtacu*s, *Eupromachus*, and *Eurycantha* were also recovered as non-monophyletic. *Eurycantha* and *T. guentheri* were recovered as polyphyletic, contrary to previous studies (Bradler, 2009; Buckley et al., 2009). The non-monophyly of *Eurycantha* is due to the placement of a single taxon, *Eurycantha sp.* 1. Further morphological work and observations should be completed to verify if this taxon is indeed a *Eurycantha* species, or a possible new

genus. An unidentified Eurycanthini sp. (Eurycanthini sp. 1) was recovered as sister to *T. guentheri* (bootstrap value = 100). The Lonchodinae subfamily was recovered as sister to the subfamily Necrosciinae (bootstrap support = 83, posterior probability = 1), congruent with results of Robertson et al. (in prep.).

Mapping of ecomorphs indicated three separate origins of the tree lobster ecomorph within Lonchodinae and a total of five independent origins of the tree lobster ecomorph across all Phasmatodea. Ancestral state reconstruction recovered the spiny form as the ancestral state of the majority *Eurycantha* assemblage and the single *Eurycantha sp.* 1 taxon. The slender, tree lobster, and intermediate tree lobster forms were recovered as equally parsimonious ancestral states of the *T. guentheri* lineage. The ancestral states of the two other tree lobster lineages, *Dyococelus australis* and *Canachus* were recovered as the large winged ecomorph and the tree lobster, slender, or large winged ecomorph respectively. Within Lonchodinae, one independent origin of the spiny ecomorph was recovered, with a total of five independent origins of this ecomorph across Phasmatodea. The slender ecomorph was recovered as the ancestral state of the Lonchodinae lineage, with at four separate origins of the slender ecomorph within the subfamily. Across Euphasmatodea five origins of the spiny ecomorph were recovered, six origins of the slender ecomorph, two origins of the large winged ecomorph, and one origin of the leaf ecomorph. The intermediate short stalky form and slender ecomorph were recovered as equally parsimonious ancestral states of Euphasmatodea.

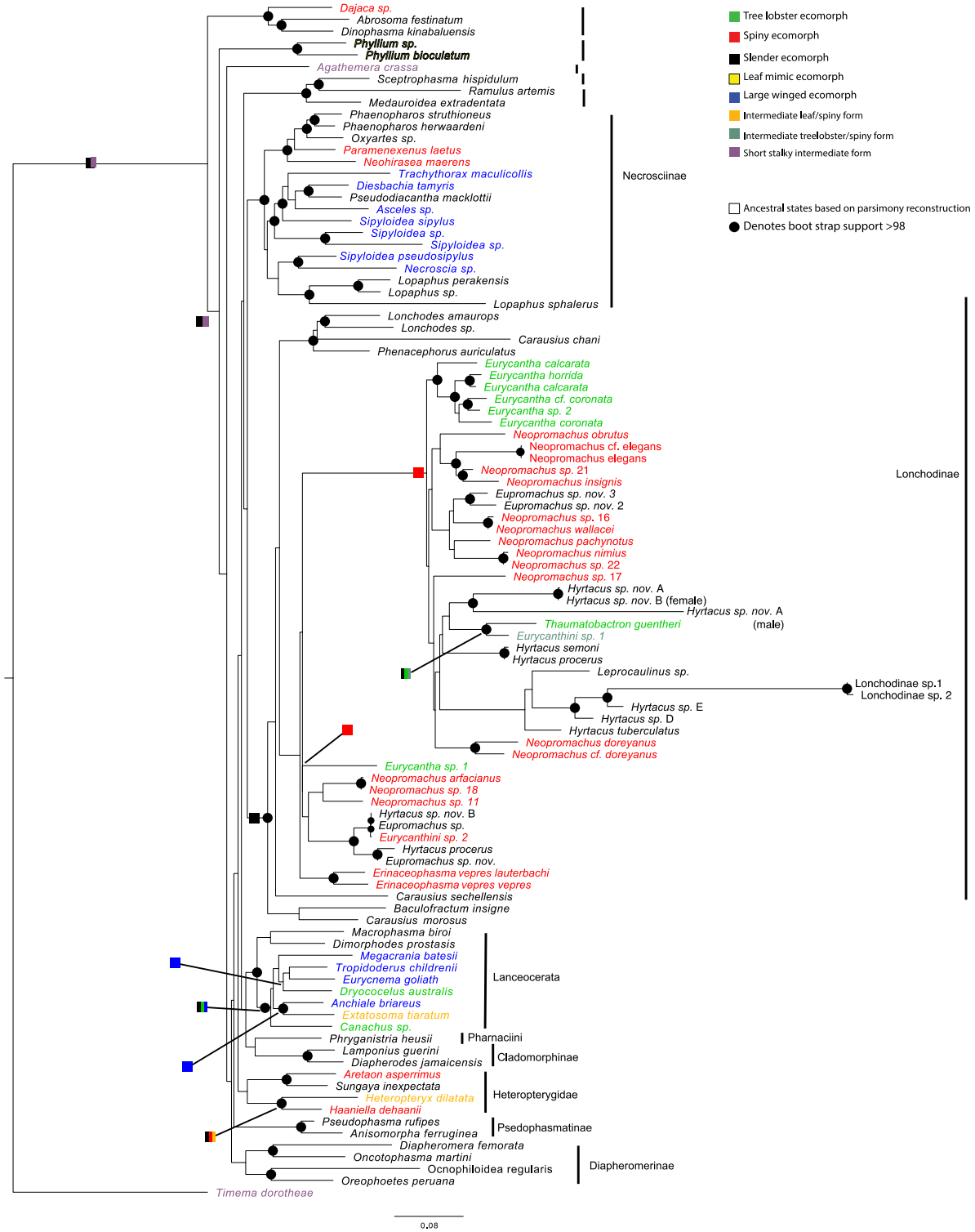

Figure 4
Maximum likelihood topology with ancestral state reconstruction indicated at nodes of interest.

Bayesian and ML topologies shared many similarities, particularly in the sister species relationships of the Lonchodinae. Both topologies recovered a monophyletic Lonchodinae and Necrosciinae as the sister group to Lonchodinae, consistent with previous studies (Robertson et al, in prep.). Within the subfamily, three origins of the tree lobster form were recovered, consistent with the ML results. The ancestral state of the *T. guentheri,* was recovered as slender, tree lobster, or intermediate tree lobster form as equally parsimonious. The tree lobster ecomorph was recovered as the ancestral state for the larger *Eurycantha* group and the spiny ecomorph was recovered as the ancestral state for *Eurycantha sp.* 1. The ancestral states for the remaining tree lobster lineages within Phasmatoadea, *Canachus* and *D. australis*, were both recovered as the slender ecomorph and ambiguous, respecitivley. Three independent origins of the spiny ecomorph were recovered within the subfamily, along with three origins of the slender ecomorph. The ancestral state to the Lonchodinae was recovered as the slender ecomorph. Across all of Euphasmatodea, five independent origins of the tree lobster ecomorph were recovered, along with seven origins of the spiny ecomorph, two origins of the large winged ecomorph, six origins of the slender ecomorph, and a single origin of the leaf ecomorph were recovered. The slender form was recovered as the ancestral state to Euphasmatodea.

While some conflicts occur between analyses, both topologies indicated five independent origins of the tree lobster ecomorph, two origins of the large winged ecomorph and a single origin of the leaf mimic ecomorph, and multiple origins of the spiny and slender ecomorphs. Significant topology differences include the placement of Eurycanthini sp. 2 and *N. sp.* 17 and *N. sp.* 11, resulting in one origin of the spiny ecomorph within Lonchodinae via the ML reconstruction and three origins of the spiny ecomorph via the Bayesian reconstruction. Additionally, the placement of Eurycanthini sp. 2 as sister to *H. sp. nov.* B + *Eupromachus sp.* in

the ML reconstruction versus the polytomy of Eurycanthini sp. 2 + *H. sp. nov.* B + *Eupromachus sp.* in the Bayesian reconstruction accounts for the difference of four origins of the slender ecomorph as opposed to three origins respectively.

Other topology differences include Necrosciinae + Lonchodinae as sister to (*Sceptrophasma hispidulum* + *Ramulus artemis*) + *Medauroidea extradentata* in ML topology compared to Necrosciinae + Lonchodinae as sister to ((*S. hispidulum* + *R. artemis*) + *M. extradentata*) + Heteropyterygidae in the Bayesian topology. Additionally, in the ML topology *Phyllium* and *Agathemera* are arise earlier on the topology than Diaphermoderidae. However, the low nodal support for both topologies suggests the topology recovered in Robertson et al. (in prep.) more likely, with high nodal support for *Phyllium* arising earlier than Diaphermoderidae, and Diaphermoderidae as more arising before *Agathermera*. The placement of *Agathermera* in the ML reconstruction also contributes to the differing ancestral states of Euphasmatodea.

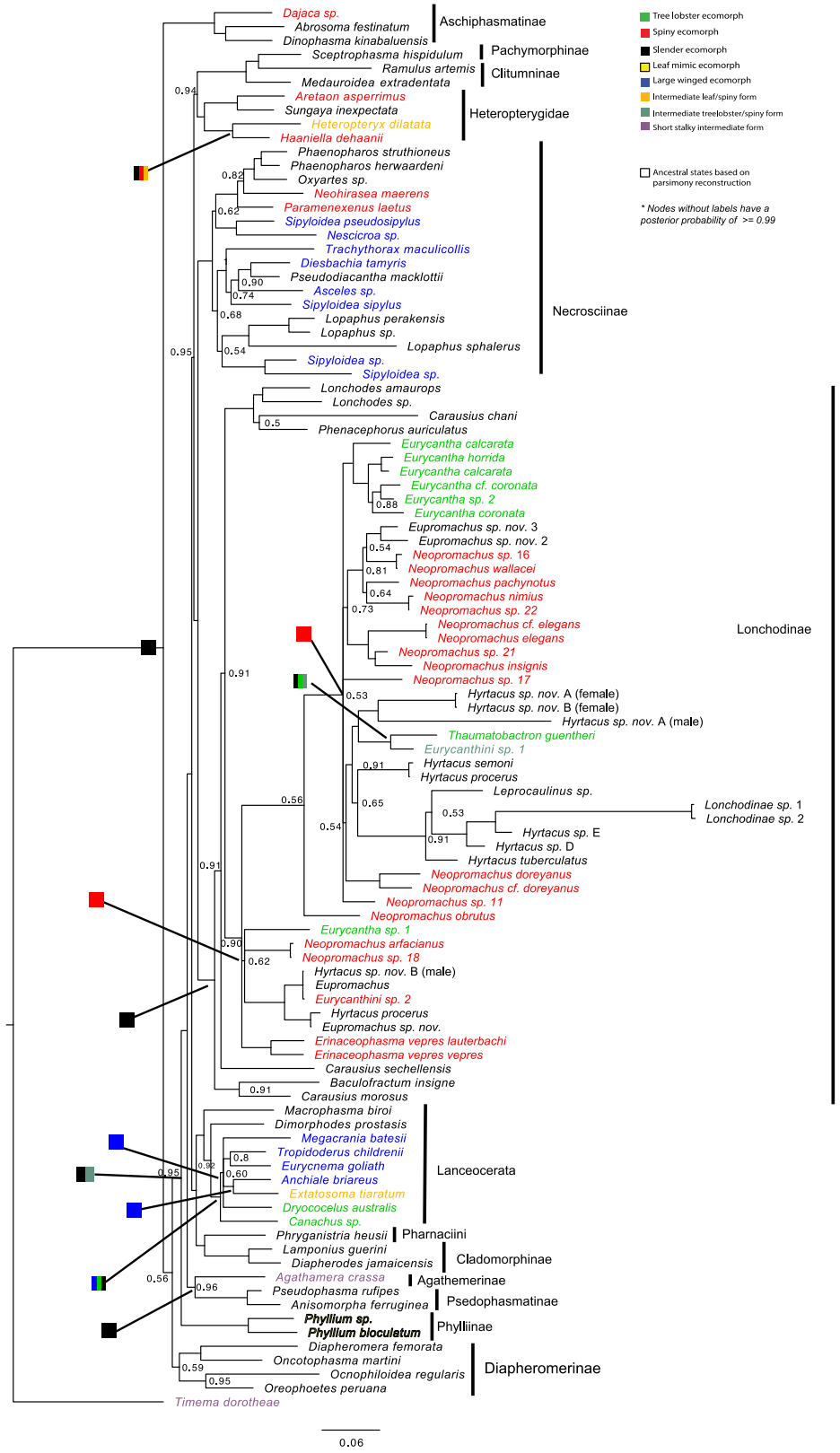

Figure 5
Bayesian topology with ancestral state reconstruction indicated at nodes of interest.

DISCUSSION

Morphometrics and statistical analyses

Morphometrics and principal components analysis were performed in an attempt to objectively categorize phasmid ecomorphs. Ecomorph groupings from both PCAs recovered broad ecomorph groupings with overlap of groups. While discrete groupings were not recovered in PCA analyses, PERMANOVA and ANOVA results further support the presence of statistically different ecomorphs based on measured characters. Similar instances of ecomorph overlap occurs in a variety of organisms including crabs and *Anolis* lizards (Marochi and Masunari, 2016; Irschick et al., 1997; Losos, 1997).

Phylogenetic reconstruction

The recovery of *Eurycantha* as non-monophyletic suggests a possibility of an additional tree lobster genus. Past studies have recovered a monophyletic *Eurycantha*. However, the increased taxon sampling in this study reveals that *Eurycantha* is likely paraphyletic (Bradler, 2009; Buckley et al., 2009; Robertson et al. in prep.). While different topologies were recovered in ML and Bayesian analyses it is apparent that multiple origins of various ecomorphs are present across Phasmatodea. Phylogenetic analysis, from both ML and Bayesian methods, indicate five independent origins of the tree lobster form. Within the Papua New Guinea Lonchodinae, the tree lobster form was originally hypothesized to evolve once (Bradler, 2009; Buckley et al., 2009). However, our analysis indicates three independent origins of the tree lobster ecomorph within the subfamily. Convergence of the tree lobster form is hypothesized across Phasmatodea (Buckley et al., 2009) and demonstrated within the Lonchodinae of Papua

New Guinea. Convergence of the spiny, slender, and large winged ecomorphs are also present in the recovered phylogenies.

The ancestral state of both *Eurycantha* lineages was recovered as the spiny ecomorph and the ancestral state of *D. australis* was recovered as the full winged ecomorph in both topologies. However, the ancestral state of *T. guentheri* is undetermined based on the equally parsimonious ancestral states of slender, tree lobster, and the intermediate stout form. Additionally, the ancestral state of *Canachus* is undetermined. The ancestral state of Lonchodinae was recovered as the slender ecomorph in both analyses, while the ancestral state of Euphasmatodea was recovered as the slender ecomorph in the Bayesian analysis (ancestral state reconstruction was ambiguous in the ML analysis). Multiple origins of the spiny and large winged ecomorph also occur, however; they all evolved from the slender ecomorph.

While multiple shifts to the tree lobster form occur, they evolved from different ancestral states. One explanation for this occurrence may correlate with egg oviposition methods. The majority of tree lobster species deposit their eggs into the ground (records are unknown for *Thaumatobactron*); this method is also utilized by other ecomorphs. As various lineages explored new habitats and shifted to the egg burying method an increased size and coloration similar to the forest floor would be beneficial for defense against predations. The occupancy of similar habitats isolated from each other support the hypothesis of ecomorph convergence in Phasmatodea.

Multiple ecomorph shifts are present in Phasmatodea for all forms except the leaf mimic ecomorph. While studies have not been conducted on the effectiveness of each ecomorph in cryptic predator defense, the tree lobster and spiny ecomorphs possess additional morphological features that help them evade predation. Many tree lobster species possess large spines on their

hind femora that can be used to defend against predators. Additionally, the sharp spines of the spiny ecomorph serve as a physical defense from predation. Alternatively, the complex morphological structure of the leaf mimic may also be the cause behind the single origin of this ecomorph. Perhaps it is more biologically difficulty to shift from a long slender form to a laterally broad yet flat form, rather than increasing in general robustness.

CONCLUSION

The observation of ecomorph groupings and grouping overlap is supported by statistical methods (PERMANOVA and ANOVA). The overlap of ecomorphs in PCA also supports the hypothesis of intermediate ecomorphs. While sperate analyses were preformed based on gender, similar groupings were recovered despite sexual dimorphism. While phasmid ecomorph groupings may be broad, they were found to be statistically significant based on PERMANOVA and ANOVA results of morphometric data. Future studies should investigate additional morphological characters that may contribute to formal descriptions of phasmids ecomorphs. Additionally, it may be relevant to investigate, possible lineage groupings within each ecomorph. This could be beneficial, especially when studying intermediate forms.

The phylogenetic analyses support the need for taxonomic revision within the Lonchodinae of Papua New Guinea, particularly within in the genera *Neopromachus, Hyrtacus,* and *Eupromachus.* Furthermore, recovered topologies suggest the possibility of an additional tree lobster genus within the subfamily. While ML and Bayesian analyses resulted in differing topologies both highly supported three independent origins of the tree lobster form, a single origin of the leaf mimic ecomorph, and two origins of the large winged ecomorph. Multiple origins were also recovered for the spiny and slender ecomorphs. However, the ancestral state of

Euphasmatodea was unclear based on conflicting topologies. Additional work should be done to formally describe phasmids ecomorphs and investigate their evolution and statistical significance on a larger scale.

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APPENDIX

Table 1 Summary of factor loadings for each dimension

Element	PC1	PC2
Head width (male)	0.37	-0.20
Head length (male)	0.38	-0.21
mean mesonotum width (male)	0.37	-0.30
Mean mesonotum length (male)	0.34	0.33
Mean tibia (male)	0.20	0.78
Mean abdomen length (male)	0.37	0.14
Mean abdomen width (male)	0.37	-0.27
Total body length (male)	0.38	0.10
Head width (female)	-0.38	0.24
Head length (female)	-0.39	0.21
mean mesonotum width (female)	-0.37	0.33
Mean mesonotum length (female)	-0.38	-0.25
Mean tibia (female)	-0.27	-0.60
Mean abdomen length (female)	-0.39	-0.25
Mean abdomen width (female)	-0.16	0.55
Total body length (female)	-0.41	-0.08
Head (male)	0.07	-0.12
Mesonotum (male)	0.61	0.27
Mean tibia (male)	0.27	-0.79
Abdomen (male)	-0.52	-0.44
Total body Length (male)	0.53	-0.32
Head (female)	-0.16	0.52
Mesonotum (female)	-0.59	0.10
Mean tibia (female)	-0.30	-0.56
Abdomen (female)	0.49	-0.49
Total body length (female)	-0.54	-0.40

Table 2 Statistical summary of PERMANOVA tests.

Data set	F-statistic	DF	P-value
Linear measurements (Male)	45.824	2, 132	0.001***
Linear Measurements (Female)	17.061	2, 132	0.001***
Ratios (Males)	46.066	2, 132	0.001***
Ratios (Female	19.897	2, 132	0.001***

Table 3 Statistical summary of ANOVA test for linear measurements.

Character	F-Statistic	Df	P-value
Head width (Male)	74.05	2, 132	0.001***
Head length (Male)	60.69	2, 132	0.001***
Mean mesonotum width (Male)	91.89	2, 132	0.001***
Mean mesonotum length (Male)	32.64	2, 132	0.001***
Mean tibial length (Male)	0.036	2, 132	0.96
Mean abdomen length (Male)	46.7	2, 132	0.001***
Mean abdomen width (Male)	58.63	2, 132	0.001***
Total body length (Male)	47.5	2, 132	0.001***
Head width (Female)	46.58	2, 132	0.001***
Head length (Female)	57.55	2, 132	0.001***
Mean mesonotum width (Female)	74.34	2, 132	0.001***
Mean mesonotum length (Female)	11.8	2, 132	0.001***
Mean tibial length (Female)	0.41	2, 132	0.67
Mean abdomen length (Female)	14.82	2, 132	0.001***
Mean abdomen width (Female)	2.93	2, 132	0.001***
Total body length (Female)	20.46	2, 132	0.001***

Table 4 Statistical summary for ANOVA of ratio matrix.

Character	F-Statistic	Df	P-Value
Head width:Head length (Male)	0.5	2, 73	0.61
Mesonotum width:Mesonotum length (Male)	241.65	2, 73	0.001***
Mean tibial length (Male)	0.036	2, 73	0.96
Abdomen width:Abdomen length (Male)	95.51	2, 73	0.001***
Total body length (Male)	47.5	2, 73	0.001***
Head width:Head length (Female)	8.43	2, 73	0.001***
Mesonotum width:Mesonotum length (Female)	76.76	2, 73	0.001***
Mean tibial length (Female)	0.41	2, 73	0.67
Abdomen width:Abdomen length (Female)	32.48	2, 73	0.001***
Total body length (Female)	20.46	2, 73	0.001***

Table 5 Taxon sampling for molecular analysis.

Taxon	Subfamily	Location	Voucher
Dajaca sp.	Aschiphasmatinae	West Malaysia	WS316
Abrosoma festinatum	Aschiphasmatinae	West Malaysia	WS140
Dinophasma kinabaluensis	Aschiphasmatinae	Vietnam	WS141
Phyllium sp.	Phylliinae	PNG	WS099
Phyllium bioculatum	Phylliinae	Java	WS012
Agathemera crassa	Agathemerinae	unknown	WS098
Sceptrophasma hispidulum	Pachymorphinae	Thailand	WS027
Ramulus artemis	Clitumninae	Vietnam	WS046
Medauroidea extradentata	Clitumninae	Vietnam	WS033
Phaenopharos struthioneus	Necrosciinae	Malaysia	WS053
Phaenopharos herwaardeni	Necrosciinae	Thailand	WS159
Oxyartes sp.	Necrosciinae	Vietnam	WS077
Paramenexenus laetus	Necrosciinae	Vietnam	WS079
Neohirasea maerens	Necrosciinae	Vietnam	WS028
Trachythorax maculicollis	Necrosciinae	Borneo	WS133
Diesbachia tamyris	Necrosciinae	Sumatra	WS119
Pseudodiacantha macklottii	Necrosciinae	Java	WS004
Asceles sp.	Necrosciinae	Thailand	WS112
Sipyloidea sipylus	Necrosciinae	Madagascar	WS042

Sipyloidea sp.	Necrosciinae	Philippine Islands	WS085
Sipyloidea sp.	Necrosciinae	Australia	WS160
Sipyloidea pseudosipylus	Necrosciinae	PNG	WS084
Paranecroscia sp.	Necrosciinae	PNG	WS074
Lopaphus perakensis	Necrosciinae	Vietnam	WS031
Lopaphus sp.	Necrosciinae	unknown	WS158
Lopaphus sphalerus	Necrosciinae	Vietnam	WS043
Lonchodes amaurops	Lonchodinae	Borneo	WS152
Lonchodes sp.	Lonchodinae	unknown	WS153
Lonchodes chani	Lonchodinae	Borneo	WS150
Lonchodes auriculatus	Lonchodinae	Borneo	WS127
Eurycantha calcarata	Lonchodinae	PNG	WS453
Eurycantha horrida	Lonchodinae	PNG	WS454
Eurycantha calcarata	Lonchodinae	PNG	WS097
Eurycantha cf. coronata	Lonchodinae	PNG	WS460
Eurycantha sp. 2	Lonchodinae	PNG	WS095
Eurycantha coronata	Lonchodinae	PNG	WS063
Neopromachus obrutus	Lonchodinae	PNG	WS072
Neopromachus cf. elegans	Lonchodinae	PNG	WS488
Neopromachus elegans	Lonchodinae	PNG	WS088
Neopromachus sp. 21	Lonchodinae	PNG	WS093
Neopromachus insignis	Lonchodinae	PNG	WS490
Eupromachus sp. nov. 3	Lonchodinae	PNG	WS498
Eupromachus sp. nov. 2	Lonchodinae	PNG	WS497
Neopromachus sp. 16	Lonchodinae	PNG	WS489
Neopromachus wallacei	Lonchodinae	PNG	WS089
Neopromachus pachynotus	Lonchodinae	PNG	WS073
Neopromachus nimius	Lonchodinae	PNG	WS071
Neopromachus sp. 22	Lonchodinae	PNG	WS094
Neopromachus sp. 17	Lonchodinae	PNG	WS090
Hyrtacus sp. nov. A	Lonchodinae	PNG	WS499
Hyrtacus sp. nov. B (female)	Lonchodinae	PNG	WS069
Hyrtacus sp. nov. A (male)	Lonchodinae	PNG	WS096
Thaumatobactron guentheri	Lonchodinae	PNG	WS086
Eurycanthini sp.	Lonchodinae	PNG	WS459
Hyrtacus semoni	Lonchodinae	PNG	WS494
Hyrtacus procerus?	Lonchodinae	PNG	WS068
Leprocaulinus sp.	Lonchodinae	PNG	WS070
Lonchodinae sp.1	Lonchodinae	PNG	WS502

Lonchodinae sp. 2	Lonchodinae	PNG	WS503
Hyrtacus sp. E	Lonchodinae	PNG	WS505
Hyrtacus sp. D	Lonchodinae	PNG	WS504
Hyrtacus tuberculatus	Lonchodinae	Australia	WS156
Neopromachus doreyanus	Lonchodinae	PNG	WS492
Neopromachus cf. doreyanus	Lonchodinae	PNG	WS493
Eurycantha sp. 1	Lonchodinae	PNG	WS064
Neopromachus arfacianus	Lonchodinae	PNG	WS07
Neopromachus sp. 18	Lonchodinae	PNG	WS091
Neopromachus sp. 11	Lonchodinae	PNG	WS487
Hyrtacus sp. nov. B	Lonchodinae	PNG	WS500
Eupromachus sp.	Lonchodinae	PNG	WS508
Eurycanthini sp.	Lonchodinae	PNG	WS501
Hyrtacus procerus	Lonchodinae	PNG	WS496
Eupromachus sp. nov.	Lonchodinae	PNG	WS495
Erinaceophasma vepres lauterbachi	Lonchodinae	PNG	WS087
Erinaceophasma vepres vepres	Lonchodinae	PNG	WS092
Carausius sechellensis	Lonchodinae	Seychelles	WS115
Baculofractum insignis	Lonchodinae	Sumatra	WS157
Carausius morosus	Lonchodinae	India	WS030
Macrophasma biroi	Phasmatinae	PNG	WS067
Dimorphodes prostasis	Xeroderinae	PNG	WS062
Megacrania batesii	Platycraninae	Australia	WS125
Tropidoderus childrenii	Tropidoderinae	Australia	WS035
Eurycnema goliath	Phasmatinae	Australia	WS040
Dryococelus australis	Lanceocercata	Australia	DRA1
Anchiale briareus	Phasmatinae	Australia	WS007
Extatosoma tiaratum	Tropidoderinae	Australia	WS006
Canachus sp.	Lanceocercata	New Caledonia	CAN5
Phobaeticus heusii	Phasmatinae	Philippine Islands	WS057
Lamponius guerini	Bacteriinae	Guadeloupe	WS039
Diapherodes jamaicensis	Bacteriinae	Jamaica	WS165
Aretaon asperrimus	Heteropteryginae	Borneo	WS009
Sungaya inexpectata	Heteropteryginae	Philippine Islands	WS038
Heteropteryx dilatata	Heteropteryginae	West Malaysia	WS008
Haaniella dehaanii	Heteropteryginae	Borneo	WS037
Pseudophasma rufipes	Pseudophasmatinae	Peru	WS011

Anisomorpha ferruginea	Pseudophasmatinae	USA	WS010
Diapheromera femorata	Heteromiinae	USA	WS001
Oncotophasma martini	Heteromiinae	Panama	WS052
Ocnophiloidea regularis	Heteromiinae	Trinidad	WS002
Oreophoetes peruana	Heteromiinae	Peru	WS003
Timema dorotheae	Timematinae	USA	WS105