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**Phylogeny and phylogeography of *Mantophryne* (Anura: Microhylidae) reveals cryptic  
diversity in New Guinea**

by

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Undergraduate honors thesis under the direction of

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## Abstract

The island of New Guinea is one of only five high-biodiversity wilderness areas in the world and frog diversity is exceptionally large, with more than 400 species known to date and many new species described annually. The microhylid frog genus *Mantophryne* is endemic to New Guinea and presently contains four terrestrial species, three of which have narrow geographic distributions. In contrast, the fourth species, *Mantophryne lateralis*, has a broad distribution that extends throughout the lowland forests on the eastern half of the island. This broad range is concordant with many other amphibian groups and thus makes *M. lateralis* a good candidate for examining patterns of genetic diversity in eastern New Guinea. Here, we sequence 104 ingroup samples for 3 mitochondrial and 3 nuclear loci to reconstruct the first phylogeny of the genus and to examine spatial patterns of diversity. Biogeographic analysis suggests the genus evolved on the eastern Papuan peninsula before dispersing west along the north and south coasts and east to the D'Entrecasteaux and Louisiade archipelagos. Phylogenetic results show that *M. infulata* is more closely related to the sister genus *Hylophorbus* and that the monotypic genus *Pherohapsis* is nested within *Mantophryne*. The wide-ranging *M. lateralis* is composed of at least nine geographically separated and well-supported lineages that represent putative species.

## **Introduction**

New Guinea is the world's largest and highest tropical island and is one of the most biodiverse regions on the planet (Beehler 1993; Dinerstein & Wikramanayake 1993; Myers et al. 2000). The complex geological history of New Guinea has played a major role in the high level of biodiversity (Heads 2002). Relatively recent tectonic activity, such as island accretion, montane uplift, and volcanism, have created a complex landscape with extreme habitat heterogeneity and topographic relief along a steep elevational gradient from sea-level to over 5,000 meters. Some of the major habitat types include lowland tropical rainforests, sago palm swamps, eucalypt savannas, cloud forests, montane moss forests and alpine grasslands. Accretion of the Inner and Outer Melanesian Island Arcs onto the Australian plate (southern portion of New Guinea) as the plate migrated north resulted in the formation of the central mountain range approximately 5-10 million years ago; the Outer Melanesian Arc now makes up the northern edge of the island (Abbott et al. 1994; Allison 1996; Hall 1997; Heads 2002; Tregoning et al. 1999). Lowland rainforest currently exists both north and south of the central mountain range.

The frog diversity on New Guinea represents approximately 8% of global diversity despite accounting for only about 0.6% of global land area. However, frog biodiversity is extremely underestimated; species accumulation curves demonstrate that the total number of described frog species is still increasing exponentially over time, and implying that the true number is likely double the current 410 described species (Allison 1996; Allison et al. 2010; Austin et al. 2008; Günther 2006). If this prediction is correct, it would mean that New Guinea would represent an astonishing biogeographic focal center of frog diversity.



The frog family Microhylidae is the most diverse group of frogs in New Guinea with over 218 described species representing 53% of New Guinea amphibian diversity, almost all of which are endemic (Zweifel and Tyler 1982, Allison et al. 2010). Globally, microhylids are one of the most diverse frog families, with over 487 described species, representing 8% of global amphibian diversity (Frost 2011; Meijden et al. 2007). New Guinean microhylid frogs occupy a wide diversity of ecological niches and include aquatic, fossorial, terrestrial, and arboreal forms (Köhler & Günther 2008; Meijden et al. 2007). All New Guinean microhylid species have direct development, in which the embryo bypasses the aquatic stage and hatches directly into a small froglet, and there have been repeated origins of paternal parental care (Bickford 2004; Günther 2006; Köhler & Günther 2008).

Based predominantly on call structure and subtle differences in morphology, Günther recently described six new species from the previously monotypic microhylid genus *Hylophorbus*, showing that the widespread “*H. rufescens*” is actually composed of multiple geographically separated species (Günther 2001). However, only the westernmost portion of the range was examined and *Hylophorbus* extends into the eastern part of New Guinea as well. Günther’s results suggest that other widespread species in lowland New Guinea might also be composite. Another microhylid frog with a similarly broad geographic range is *Mantophryne lateralis*, which occurs throughout the lowlands of eastern New Guinea (Boulenger 1897; Burton 1986; Menzies 2006; Zweifel 1972). This broad range is concordant with *Hylophorbus* and with many other amphibian species and makes *M. lateralis* a good candidate for examining patterns of genetic diversity in eastern New Guinea. *Mantophryne* currently includes only three other species, all with restricted geographic ranges (Figure 1): *M. axanthogaster*, restricted to Sudest Island (Kraus & Allison 2009); *M. infulata*, restricted to Arau and the Huon Peninsula (Burton

1986; Zweifel 1972); and *Mantophryne lousiadensis*, restricted to Rossel Island (Burton 1986; Parker 1934; Zweifel 1972).

Here we use data from multiple loci to reconstruct the phylogeny of the genus *Mantophryne* in order to examine spatial and temporal patterns of diversity. In addition, we use Bayesian methods of species tree inference to inquire if the widespread *M. lateralis* represents one or more independent evolutionary lineages (i.e. putative species). Finally, we conduct a biogeographic analysis using ancestral reconstructions to decipher areas of origin and patterns of dispersal. Our analyses, while confined to a single genus, may provide a template for examining cryptic diversity in other frogs in New Guinea.

## **Materials and Methods**

### *Sampling*

A total of 104 ingroup samples were examined, representing all four described species of *Mantophryne*: 94 samples spanning the distribution of *M. lateralis*, four *M. lousiadensis*, five *M. axanthogaster*, and one *M. infulata* (Fig. 1). To test the monophyly of *Mantophryne*, we also included 24 samples representing four described and multiple undescribed species of *Hylophorbus*, one sample of the monotypic genus *Pherohapsis* (*P. menziesi*) and eight samples of a putative new species of *Pherohapsis*. Based on higher-level asterophryine phylogenetics (Köhler & Günther 2008), we also included outgroup samples of the genera *Austrochaperina*, *Callulops*, *Choerophryne*, and *Sphenophryne*. Voucher numbers and collection localities are provided in Appendix 1.

### *DNA isolation, amplification, and sequencing*

Whole genomic DNA was extracted from liver tissue using the Qiagen DNeasy Blood and Tissue Kit (Valencia, California, USA). All tissues were digested overnight and eluted in 300µL of AE Elution Buffer. Three mitochondrial gene regions (12S, 16S, and cytochrome oxidase b) and three nuclear loci (Tyrosinase, c-myc exon 2, and c-myc exon 3) were selected based on their utility in previous studies that included microhylid frogs primarily as outgroups (e.g, (Richards & Moore 1996, Bossuyt & Milinkovitch 2000, Wiens et al. 2005, Köhler & Günther 2008), and sequenced using previously published primers (Table 1). Target gene regions were amplified via the polymerase chain reaction following Austin et al. (2010) and were purified using Exonuclease I and Antarctic Phosphatase (New England Biolabs, Ipswich, MA, USA) as in Austin et al. (2010). Purified amplicons were then cycle sequenced in both directions with BigDye v. 3.1 Terminator Sequencing Kit (Applied Biosystems, Foster City, CA, USA) using previously published protocols and sequenced on an ABI 3100 automated capillary sequencer (Austin et al. 2010).

Complementary strands were assembled and visually edited in Sequencher v4.7 (Gene Codes Corp., Ann Arbor, MI, USA) and aligned in ClustalX2 (Larkin et al. 2007) using default parameters (gap-opening penalty=15, gap extension penalty=6.66). Protein-coding regions were translated to amino-acid sequences using Mesquite v2.73 (Maddison & Maddison. 2010) to visually verify that sequences contained no premature stop codons. The number of variable sites for each locus was computed using Mega 5.0 (Koichiro et al. 2011).

### *Phylogenetic Analysis*

To estimate phylogenetic relationships among samples, Bayesian inference, maximum likelihood (ML), and maximum parsimony (MP) were implemented on the concatenated dataset of all six loci. Maximum parsimony analyses were conducted in PAUP\* ver.4.0b10 (Swofford 2003) using PAUPrat (Sikes & Lewis 2001) to implement the parsimony ratchet (Nixon 1999). Fifty replicates of 5,000 ratchet iterations and 20% character perturbation were conducted using Tree bisection-reconstruction (TBR) branch swapping to find the most parsimonious trees using the CIPRES portal (Warnow 2008). Branch support was assessed with 1,000 maximum parsimony bootstrap (MPBS) replicates, each with 25 random addition sequences and TBR branch swapping. For Bayesian and ML analyses, a variety of partitioning strategies was tested, including partitioning by locus and by codon position (for the protein-coding cytochrome oxidase b gene). The best-fit model of sequence evolution for each partition was selected in jModeltest v0.1.1 (Posada 2008) using the corrected Akaike Information Criterion (AICc; Table 2). ML analyses were implemented in Garli v2.0 (Zwickl 2006) using default parameters and five search replicates per partitioning strategy. The best-fit partitioning strategy was then selected using the Akaike Information Criterion (AIC) and 50 total search replicates were conducted on the best-fit partitioning strategy to ensure the maximum likelihood phylogeny was found. Branch support was assessed with 1,000 maximum likelihood bootstrap (MLBS) replicates with five search replicates per bootstrap replicate. Bayesian analyses were implemented in MrBayes v.3.1.2 (Huelsenbeck & Ronquist. 2001; Ronquist & Huelsenbeck. 2003). As in ML analyses, a variety of partitioning strategies was tested and the best-fit strategy was selected by the AIC. For each partitioning strategy, Bayesian analyses consisted of two independent runs, each with four chains, run for 25 million generations sampling every 1,000

generations. Substitution model parameters were unlinked among partitions and rate priors were set to “variable” to allow differing substitution rates among partitions. The branch length prior was set to an unconstrained exponential distribution with a mean of 50; all other priors were left at default settings. Convergence was assessed by examining the potential scale reduction factors in MrBayes, as well as by examining the traces of all parameters and the effective sample sizes in Tracer v1.5 (Rambaut & Drummond 2007) and by comparing the Bayesian posterior probabilities (BPP) of all splits in Are We There Yet (AWTY) (Nylander et al. 2008).

These analyses assume that all loci have the same underlying topology; however, due to processes such as incomplete lineage sorting, heterogeneity among gene genealogies may be substantial (Degnan & Rosenberg 2006; Degnan & Rosenberg 2009; Edwards et al. 2007). Therefore, in addition to concatenated phylogenies, we estimated the underlying species tree using \*BEAST (Heled & Drummond 2010) which accounts for heterogeneity among gene trees due to incomplete lineage sorting. Species tree analyses, such as \*BEAST, require samples to be assigned *a priori* to species that accurately represent independently evolving evolutionary lineages. In this case, we suspected *Mantophryne lateralis* represents a cryptic complex of multiple species. Therefore, we generated species trees assuming well-supported (>0.95 BPP, >75 MLBS, >75 MPBS) clades with 3, 4, 5, and 6% divergence in the concatenated phylogenetic analyses represented distinct lineages. Putative species groupings generated using 3 and 4% divergence were identical, and all analyses resulted in identical topologies with similar support; thus, we present the results from the 4% divergence analysis. Analyses were conducted using an uncorrelated lognormal molecular clock for 250 million generations, sampling every 1,000 generations, the first 50,000 of which were discarded as burn-in. Mitochondrial loci were partitioned as in the concatenated analysis, but were constrained to a single gene genealogy due

to the rarity of recombination within the mitochondrial genome. Each nuclear locus was allowed to evolve independently under its own best-fit model. Convergence and stationarity were assessed by examining traces and effective sample sizes of all parameters in Tracer 1.5 (Rambaut & Drummond 2007). Defining species using divergence thresholds is clearly problematic; however, these divergent lineages represent putatively independently evolving species and valid species will be determined integrating molecular data with morphological and mating call data. Since the number of variable sites between mitochondrial and nuclear loci are drastically different, suggesting that they are evolving at different rates, it is not appropriate to use species delimitation methods that do not take into account phylogenetic uncertainty. Therefore, concatenation methods and identification of geographically isolated, strongly supported and divergent clades remains the best option.

### *Biogeographic Reconstruction*

To examine the biogeographic history of *Mantophryne*, we used maximum likelihood ancestral state reconstruction (MLASR) (Schluter et al. 1997), Statistical-Dispersal Vicariance Analysis (S-DIVA) (Yu et al. 2010), and Dispersal-Extinction Cladogenesis (DEC) (Ree et al. 2005) analyses. These methods require discrete regions of occurrence, which we defined by geologic history (Fig. 2): 0) North Coast (including accreted portions of Outer Melanesian Island Arc), 1) Papuan Peninsula (East Papuan Composite Terrane), 2) D'Entrecasteaux Islands, 3) Louisiade Islands (an extension of the Owen Stanley Terrane on the Papuan Peninsula), and 4) Southern and Western New Guinea (Australian Plate). MLASR was conducted in Mesquite v. 2.74 (Maddison & Maddison. 2010) using the maximum clade credibility species tree estimated in \*BEAST. However, this method assumes each species (extant and ancestral) occurred in

single regions, whereas, in reality, it is possible that some ancestral species spanned multiple regions. Thus, we also investigated biogeographic history using S-DIVA and DEC analyses. Both S-DIVA and DEC allow for more realistic, though more complex, models by allowing distributions to span multiple regions and by limiting dispersal between distant regions (e.g., it is unlikely for frogs to disperse from the D'Entrecasteaux Islands (region 2) to southern or western New Guinea (region 4) without dispersing through the Papuan Peninsula). S-DIVA was implemented in RASP ver.1.107 (Yu et al. 2011; Yu et al. 2010) using a posterior distribution of 20,000 species trees from \*BEAST analysis (thinned to one per 10,000 iterations) to integrate reconstructions over phylogenetic uncertainty. DEC was conducted using lagrange ver.2.0.1 (Ree & Smith 2008) in Python ver.2.72, using the maximum clade credibility species tree estimated in \*BEAST. We constrained ancestral distributions to adjacent areas, i.e. 0-1, 1-2, 1-3, 1-2-3, or 1-4. Unsuitable montane habitat occurs between regions 0 and 4, making dispersal directly between these regions (i.e. without passing through region 1) highly unlikely. Therefore, we did not allow for a 0-4 distribution, or dispersal directly between 0 and 4. Similarly, we also constrained dispersal between island systems (i.e., D'Entrecasteaux and Louisiade Islands) and either North coast or southern/western populations, since these dispersals are unlikely without traversing mainland Papuan peninsula. All other dispersals were allowed, i.e., 0-1, 1-2, 1-3, 2-3, 1-4.

## **Results**

### *Phylogenetic analyses*

The final aligned length of the combined mitochondrial and nuclear dataset was 1,621 basepairs. The length of each partition, number of variable and parsimony informative sites, and models for nucleotide substitution (as determined by the AICc) are provided in Table 2. The

same best-fit partitioning scheme was selected (using the AIC) for both ML and Bayesian inference: 12S; 16S; cyt b 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> codon positions; Tyrosinase; c-myc exon 2; c-myc exon 3. Tracer plots of all parameters, posterior probabilities, and likelihood were stationary at similar values for both Bayesian inference analyses after removal of burn-in samples, and all ESSs were substantially greater than 200 at run completion. Additionally, AWTY plots comparing the posterior probabilities of all splits were linear, suggesting that Bayesian inference analyses had reached convergence.

The phylogenetic reconstruction (Figure 3) suggests that *M. lateralis* is monophyletic and split into nine divergent clades: (A) Mt. Shungol, Morobe Province, (B) North Coast New Guinea – Huon Peninsula (Outer Melanesian Arc), (C) Northern Milne Bay Province, (D) Central and Oro Province, (E) Southwestern New Guinea and Southern Highlands Province, (F) Haia, Gulf Province (G) Sobo and Haia, Gulf Province, (H) Southern Milne Bay Province, (I) Normanby Island of the D’Entrecasteaux islands. Each *M. lateralis* clade is strongly supported with 100 MPBS, >89 MLBS and 1.0 BPP, and is geographically isolated, with one exception: samples from Haia, Gulf Province were recovered in both clades F and G. *Pherohapsis* and a putative new species from Amau (*P. Amau* sp.) are sister taxa and were recovered as embedded within *Mantophryne*, sister to the *M. lateralis* complex. *Mantophryne lousiadensis* + *M. axanthogaster* were recovered as sister taxa and sister to *M. lateralis* + *Pherohapsis*. Furthermore, *M. infulata* was recovered within the sister genus *Hylophorbus*, rather than within *Mantophryne*.

The species tree estimates (Figure 4) largely corroborate the concatenated phylogeny. The only exception is the relationships among *M. lateralis* clades E, F, and G. In the concatenated phylogeny, E is sister to F+G. However, in the species tree, F is sister to E+G. This



clade is strongly supported in both the species tree (BPP=0.99) and the concatenated phylogeny (MPBS=90, MLBS=92, BPP=1.0). Although the sister relationship of clades F+G is strongly supported in the concatenated phylogeny (MPBS=100, MLBS=89.7, BPP=1.0), the sister relationship of clades E+G is not strongly supported in the species tree (BPP=0.81). Lower-level relationships are often discordant between these two methods due to heterogeneity among gene trees (Degnan & Rosenberg 2006, 2009; Edwards et al. 2007).

### *Biogeography*

MLSR, S-DIVA, and DEC produced similar results (Table 3). MLSR suggests that *Mantophryne* originated in the Papuan Peninsula (p=0.54) over the Louisiade Islands (p=0.19). DEC (p=0.73) and S-DIVA (p=0.99) support an origin between both the Papuan peninsula and the Louisiade Islands. MLSR (p=0.75, 0.81), S-DIVA (p=0.99, 0.99), and DEC (p=0.79, 0.55) all suggest that mainland *Mantophryne* and the *M. lateralis* complex originated in the Papuan peninsula. Furthermore, all analyses suggest that the North Coast and South Coast populations each originated from single dispersal events out of the Papuan Peninsula region.

### **Discussion**

This is the first phylogeographic study of any New Guinean frog. Our molecular data for the widespread *M. lateralis* demonstrate that there are at least nine independently evolving lineages from across its range in eastern New Guinea. These data, combined with the recent work by Günther and others on the genus *Hylophorbus* in central and western New Guinea, suggest that some, possibly many, of the currently widespread frog species in New Guinea may in fact represent cryptic species complexes (Günther 2001; Kraus & Allison 2009; Richards &

Oliver 2007). While Günther (2001) primarily focused his species analyses to *Hylophorbus* on the westernmost tip of New Guinea, our phylogenetic analyses included several populations of *Hylophorbus* from the central and eastern part of the island. These samples were deeply divergent, and may represent independent evolutionary lineages. These taxa are also obvious based on morphology and call characteristics in the field. *Hylophorbus* exhibits similar ecology to *Mantophryne* and could, therefore, display similar topological and spatial patterns. In addition, we included two disparate populations of the widespread *Sphenophryne cornuta*. These populations, sampled from north and south of the central cordillera, similarly show a large degree of sequence divergence, and may represent distinct species.

Recent work on New Guinean reptiles has also found cryptic diversity in wide ranging species including two skinks (*Sphenomorphus jobiensis* and *Carlia fusca*) and three snakes (2 *Aspidomorphus* species and *Morelia viridis*) (Austin et al. 2011; Donnellan & Aplin 1989; Metzger et al. 2010; Rawlings & Donnellan 2003; Zug 2004). Identical results are being found in work done by others and us involving additional lizard “species.” These data, in concert with our findings, suggest that cryptic species might be more common in New Guinea than previously thought. Current understanding has been based in large part on studies of museum specimens, in which details of color and pattern are often difficult to discern. Research involving living animals clearly supports the fact that many New Guinean taxa currently treated as single species in fact represent complexes of multiple species (F. Kraus, unpubl. data).

Biogeographic reconstructions indicate that *Mantophryne* originated on the Papuan Peninsula while the Louisiade Islands were still connected to the mainland (strongly supported by DEC, and S-DIVA analyses). MLASR has split probabilities between the Papuan Peninsula and the Louisiade Islands. Since MLASR fails to take into account distributions across multiple

biogeographic regions, this is likely a result of the two regions being connected historically. Interestingly, the genus *Hylophorbus* also occurs in both the Louisiade and D'Entrecasteaux archipelagos off the eastern tip of New Guinea (as do other frog genera: *Cophixalus*, *Austrochaperina*, *Litoria*, *Nyctimystes*, *Hylarana*, *Copiula*, *Oreophryne*, and *Callulops*), suggesting that the similar distribution of these two genera may mirror common historical and ecological processes.

Our results based on an improved understanding of phylogenetic relationships highlight some taxonomic confusion associated with the closely related genera *Mantophryne*, *Hylophorbus* and *Pherohapsis*. The monotypic *Pherohapsis* is embedded within *Mantophryne* and thus should be synonymized. Our results also show that *M. infulata* is nested within *Hylophorbus* and should be included in that genus. We are currently analyzing call and morphological data to assess whether nine clades of the *lateralis* complex, as well as the putative new species of “*Pherohapsis*” from Amau, are conspecific lineages or distinct species. We are currently in the process of directing efforts towards a comprehensive species delimitation analyses in *Mantophryne*.

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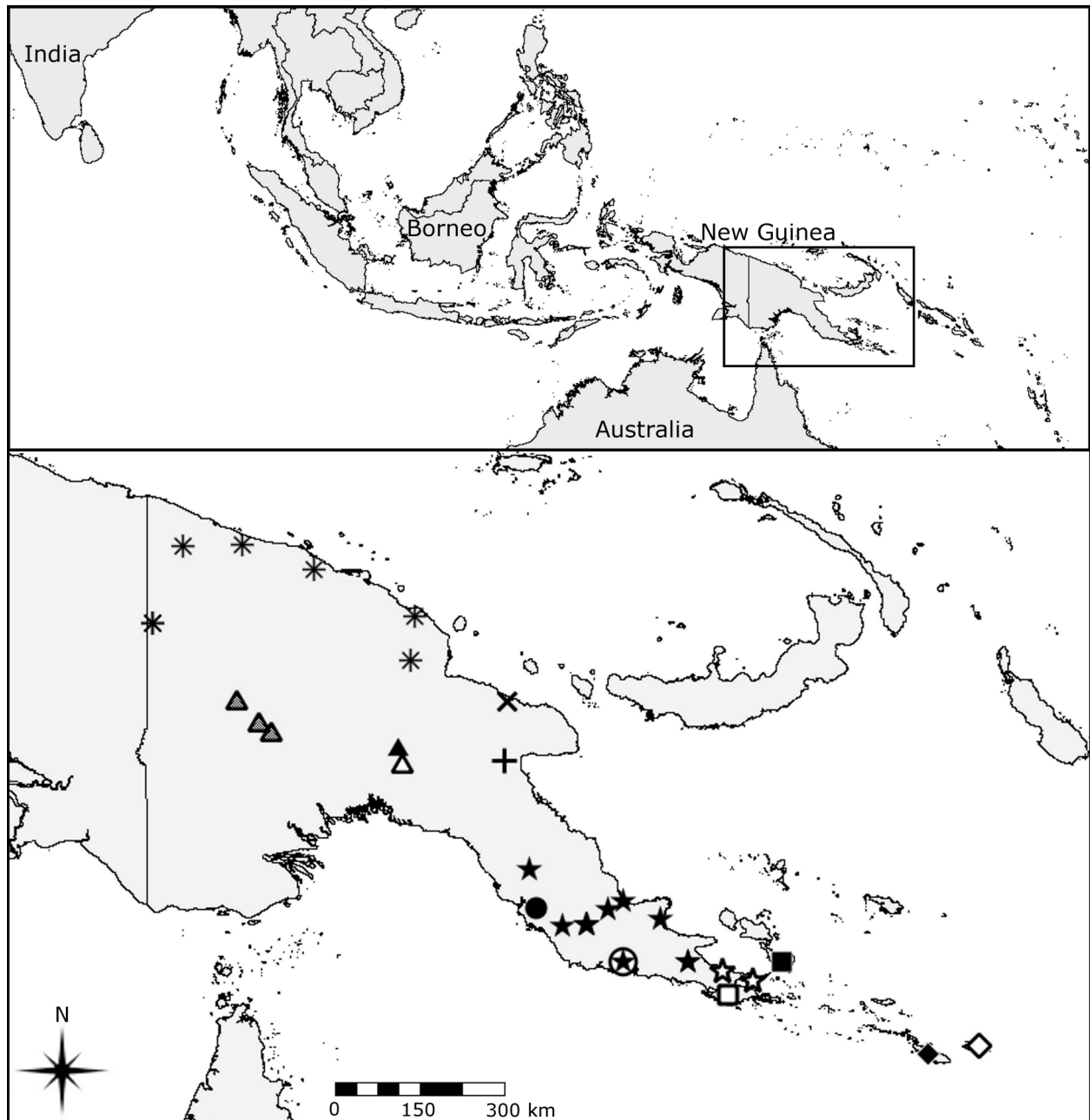
## Figure Legends

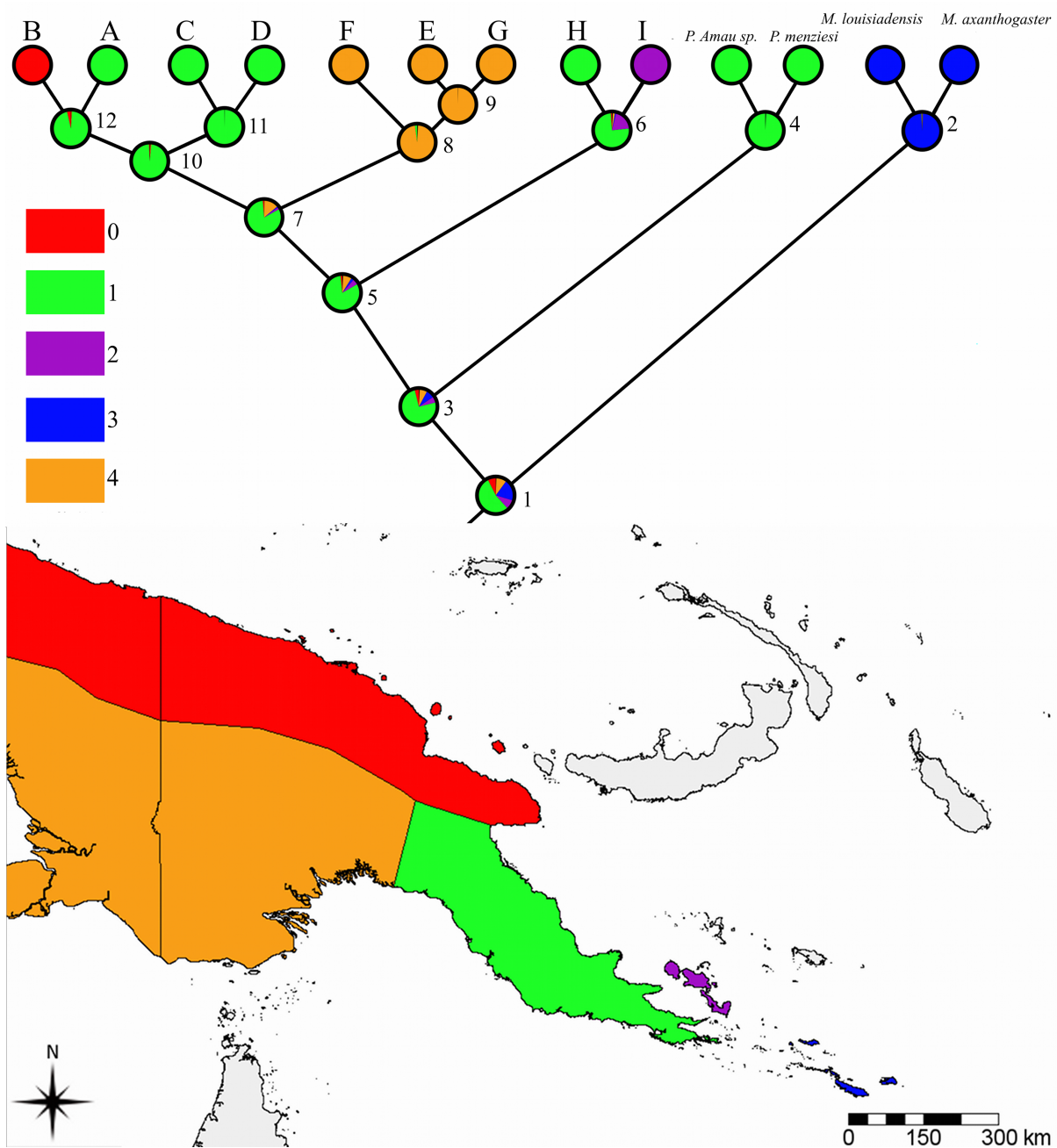
**Figure 1.** Distribution map of all putative *Mantophryne* putative species. Symbols correspond to those in Figure 3. Two species occur in Amau, indicated by an open circle and a closed star.

**Figure 2.** Biogeographic reconstruction of *Mantophryne*. Biogeographic regions: 0-North Coast (includes accreted portions of Outer Melanesia Island Arc), 1-Papuan Peninsula (East Papuan Composite Terrane), 2-D'Entrecasteaux Islands, 3-Louisiade Islands, and 4-Southern and Western New Guinea (Australian Plate). Support values for each node are listed in Table 3.

**Figure 3.** Concatenated phylogeny. Branch support values are listed as maximum parsimony bootstrap/maximum likelihood bootstrap/Bayesian posterior probability (BPP). 100 bootstrap or 1.0 BPP are indicated by an \*. Bootstraps less than 50, and BPP less than 0.75, are not listed. Symbols correspond to those in Figure 1.

**Figure 4.** Species tree from \*BEAST. Node supports are listed as Bayesian posterior probabilities. Support values <0.75 are not listed and values of 1.0 are listed as \*.



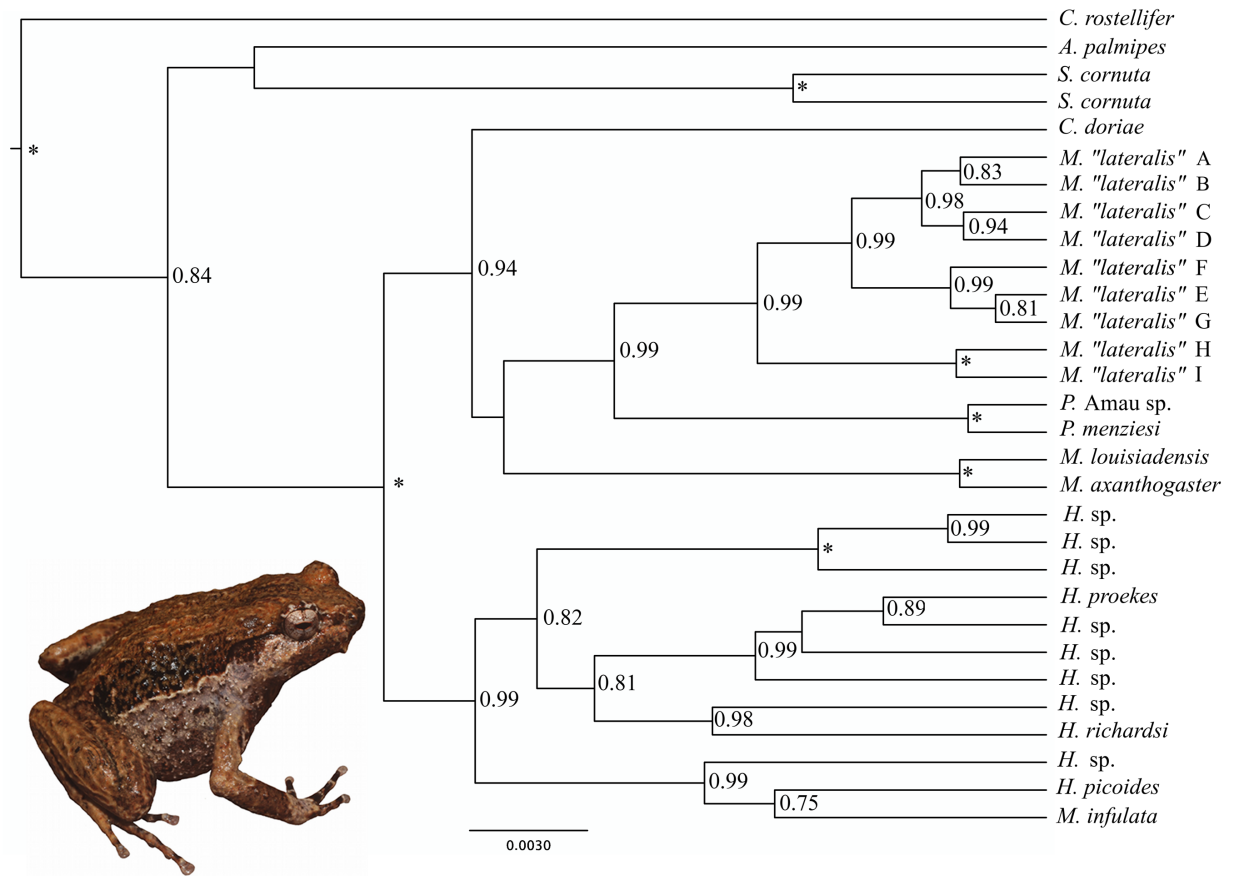


Phylogenetic tree of the genus *Acanthopneuste* based on 18S rDNA sequences. The tree shows relationships between various species, with bootstrap values and posterior probabilities indicated at the nodes. The tree is rooted with *A. palmipes*.

Species and their corresponding symbols:

- S. cornuta* (filled circle)
- H. rufescens* (filled circle)
- H. richardsi* (filled circle)
- H. proekes* (filled circle)
- H. spp.* (filled circle)
- H. rufescens* (filled circle)
- H. spp.* (filled circle)
- M. infulata* (cross)
- H. picoides* (filled circle)
- H. spp.* (filled circle)
- M. "lateralis" Clade I* (filled square)
- M. "lateralis" Clade H* (open square)
- M. "lateralis" Clade G* (open triangle)
- M. "lateralis" Clade F* (filled triangle)
- M. "lateralis" Clade E* (filled triangle)
- M. "lateralis" Clade D* (star)
- M. "lateralis" Clade C* (star)
- M. "lateralis" Clade B* (asterisk)
- M. "lateralis" Clade A* (plus)
- P. Amau sp.* (circle)
- P. menziesi* (filled circle)
- M. louisiadensis* (open diamond)
- M. axanthogaster* (filled diamond)
- C. doriae* (filled circle)
- C. rostellifer* (filled circle)

Scale bar: 0.08



**Table 1:** List of loci, PCR primers, and annealing temperatures.

| Locus        | Primer      | Primer Sequence 5'–3'      | Temp (°C) | Reference                     |
|--------------|-------------|----------------------------|-----------|-------------------------------|
| 12S          | L2519       | AAACTGGGATTAGATACCCCACTAT  | 55        | (Richards & Moore 1996)       |
|              | H3296       | GCTAGACCATKATGCAAAAGGTA    |           | (Richards & Moore 1996)       |
| 16S          | 16S-L       | TCGAACTTAGAGATAGCTGGTT     | 55        | (Köhler & Günther 2008)       |
|              | 16S-H       | GCGAATGTTTTTGGTAAACA       |           | (Köhler & Günther 2008)       |
| Cytb         | CytbA       | CCATGAGGACAAATATCATTYTGRGG | 46        | (Bossuyt & Milinkovitch 2000) |
|              | CytbB       | CTTCTACTGGTTGTCCTCCGATTCA  |           | (Bossuyt & Milinkovitch 2000) |
| Tyrosinase   | Tyr1A       | AGGTCCTCTTRAGCAAGGAATG     | 55        | (Bossuyt & Milinkovitch 2000) |
|              | Tyr1F       | TCATCTCCCGYCACTTCTGGAT     |           | (Bossuyt & Milinkovitch 2000) |
| C-myc exon 2 | cmcy2F      | ACVGARTTCCTGGGAGGGGACATGG  | 55        | (Wiens et al. 2005)           |
|              | cmcy-ex2d R | TCATTCAATGGGTAAGGGAAGACC   |           | (Wiens et al. 2005)           |
| C-myc exon 3 | c-myc-ex3F  | CCCACCAGTCCAGACCTCACCACAG  | 48        | (Wiens et al. 2005)           |
|              | c-myc-ex3R  | GTTCTCTTTTGAGTTTAACTGTTC   |           | (Wiens et al. 2005)           |

**Table 2:** List of the length, number of variable sites, and partitioning scheme for each locus.

| Partition    | Length | All Samples  | Ingroup Samples                                      | Best-fit Model |
|--------------|--------|--|--|----------------|
|              |        | No. Variable Sites (No. Parsimony Informative Sites) | No. Variable Sites (No. Parsimony Informative Sites) |                |
| 12S          | 687    | 347 (301)  | 218 (188)  | TIM3+G         |
| 16S          | 445    | 298 (271)  | 212 (184)  | HKY+G          |
| Cyt b pos. 1 | 163    | 65 (60)  | 36 (32)  | TrN+G          |
| Cyt b pos. 2 | 163    | 28 (24)  | 12 (11)  | TrN+G          |
| Cyt b pos. 3 | 163    | 160 (159)  | 139 (132)  | TIM1+G         |
| Tyrosinase   | 503    | 155 (95)   | 76 (45)  | HKY+G          |
| c-myc exon 2 | 330    | 52 (28)  | 20 (12)  |                |
| c-myc exon 3 | 356    | 70 (43)  | 32 (16)  |                |



**Table 3:** Biogeographic reconstruction probabilities. 1-Papuan Peninsula (East Papuan Composite Terrane), 2-D’Entrecasteaux Islands, 3-Louisiade Islands and 4-Southern and Western New Guinea (Australian Plate).

| Node | Reconstructed Distribution | MLASR Support | Reconstructed Distribution | S-DIVA Support | Reconstructed Distribution | DEC Support |
|------|----------------------------|---------------|----------------------------|----------------|----------------------------|-------------|
| 1    | [1]                        | 0.542         | [13]                       | 0.999          | [3 1]                      | 0.739       |
|      | [3]                        | 0.198         |                            |                | [3 12]                     | 0.131       |
|      | [4]                        | 0.097         |                            |                |                            |             |
|      | [2]                        | 0.087         |                            |                |                            |             |
|      | [0]                        | 0.075         |                            |                |                            |             |
| 2    | [3]                        | 0.985         | [3]                        | 1.000          | [3 3]                      | 0.992       |
| 3    | [1]                        | 0.752         | [1]                        | 0.996          | [1 1]                      | 0.792       |
|      | [3]                        | 0.079         |                            |                | [12 1]                     | 0.143       |
|      | [4]                        | 0.072         |                            |                |                            |             |
|      | [2]                        | 0.058         |                            |                |                            |             |
| 4    | [1]                        | 0.994         | [1]                        | 1.000          | [1 1]                      | 0.996       |
| 5    | [1]                        | 0.817         | [1]                        | 0.998          | [1 1]                      | 0.559       |
|      | [4]                        | 0.083         |                            |                | [1 12]                     | 0.217       |
|      | [2]                        | 0.055         |                            |                | [14 1]                     | 0.192       |
| 6    | [1]                        | 0.758         | [12]                       | 1.000          | [1 2]                      | 0.968       |
|      | [2]                        | 0.196         |                            |                |                            |             |
| 7    | [1]                        | 0.817         | [14]                       | 1.000          | [4 1]                      | 0.942       |
|      | [4]                        | 0.137         |                            |                |                            |             |
| 8    | [4]                        | 0.972         | [4]                        | 1.000          | [4 4]                      | 0.984       |
| 9    | [4]                        | 0.999         | [4]                        | 1.000          | [4 4]                      | 0.998       |
| 10   | [1]                        | 0.977         | [01]                       | 0.518          | [1 1]                      | 0.757       |
|      |                            |               | [1]                        | 0.482          | [01 1]                     | 0.238       |
| 11   | [1]                        | 0.997         | [1]                        | 1.000          | [1 1]                      | 0.999       |
| 12   | [1]                        | 0.953         | [01]                       | 1.000          | [1 0]                      | 0.941       |

| Genus       | Species   | Catalog No. | Locality                | Latitude | Longitude |
|-------------|-----------|-------------|-------------------------|----------|-----------|
| Mantophryne | lateralis | LSUMZ 94149 | Oro Province: Wanigela  | -9.34    | 149.16    |
| Mantophryne | lateralis | LSUMZ 92102 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92224 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92103 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92104 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92228 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92225 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92105 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92106 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92226 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92227 | Saundaun Province: Utai | -3.40    | 141.58    |
| Mantophryne | lateralis | LSUMZ 92107 | Milne Bay Province      | -10.34   | 150.57    |
| Mantophryne | lateralis | LSUMZ 92108 | Milne Bay Province      | -10.28   | 150.60    |
| Mantophryne | lateralis | LSUMZ 92109 | Milne Bay Province      | -10.28   | 150.60    |
| Mantophryne | lateralis | LSUMZ 92110 | Milne Bay Province      | -10.31   | 150.62    |
| Mantophryne | lateralis | LSUMZ 94796 | Milne Bay Province      | -10.34   | 150.62    |
| Mantophryne | lateralis | LSUMZ 94797 | Milne Bay Province      | -10.34   | 150.57    |
| Mantophryne | lateralis | LSUMZ 94798 | Milne Bay Province      | -10.34   | 150.57    |
| Mantophryne | lateralis | LSUMZ 94799 | Central Province: Amau  | -10.04   | 148.56    |
| Mantophryne | lateralis | LSUMZ 94800 | Central Province: Amau  | -10.04   | 148.56    |
| Mantophryne | lateralis | LSUMZ 94801 | Central Province: Amau  | -10.04   | 148.56    |
| Mantophryne | lateralis | LSUMZ 94802 | Central Province: Amau  | -10.04   | 148.56    |

|             |           |                |  |        |        |
|-------------|-----------|----------------|--|--------|--------|
| Mantophryne | lateralis | LSUMZ<br>94804 | Central Province: Amau                 | -10.04 | 148.56 |
| Mantophryne | lateralis | LSUMZ<br>94805 | Gulf Province: Sobo                    | -6.92  | 145.07 |
| Mantophryne | lateralis | LSUMZ<br>94808 | Gulf Province: Sobo                    | -6.92  | 145.07 |
| Mantophryne | lateralis | LSUMZ<br>94809 | Gulf Province: Sobo                    | -6.92  | 145.07 |
| Mantophryne | lateralis | ABTC<br>42829  | Haia                                   | -6.70  | 145.07 |
| Mantophryne | lateralis | ABTC<br>42858  | Haia                                   | -6.70  | 145.07 |
| Mantophryne | lateralis | ABTC<br>46121  | Namosado                               | -6.25  | 142.78 |
| Mantophryne | lateralis | ABTC<br>46332  | Namosado                               | -6.25  | 142.78 |
| Mantophryne | lateralis | ABTC<br>46662  | Namosado                               | -6.25  | 142.78 |
| Mantophryne | lateralis | ABTC<br>46708  | Agofia                                 |        |        |
| Mantophryne | lateralis | ABTC<br>49942  | Milne Bay Province:<br>Normanby Island | -10.07 | 151.23 |
| Mantophryne | sp?       | ABTC<br>98682  | Libano                                 | -6.40  | 142.98 |
| Mantophryne | lateralis | ABTC<br>101917 | Juha                                   | -5.90  | 142.43 |
| Mantophryne | lateralis | ABTC<br>111949 | Utai                                   | -3.40  | 141.58 |
| Mantophryne | lateralis | ABTC<br>111950 | Utai                                   | -3.40  | 141.58 |
| Mantophryne | lateralis | ABTC<br>111971 | Imnai Village                          | -4.63  | 141.10 |
| Mantophryne | lateralis | ABTC<br>111972 | Imnai Village                          | -4.63  | 141.10 |
| Mantophryne | lateralis | ABTC<br>111978 | Yapsiei Village                        | -4.63  | 141.10 |
| Mantophryne | lateralis | ABTC<br>111980 | Yapsiei Village                        | -4.63  | 141.10 |
| Mantophryne | lateralis | ABTC<br>112066 | Wamangu                                | -3.79  | 143.65 |
| Mantophryne | lateralis | ABTC<br>112067 | Wamangu                                | -3.79  | 143.65 |
| Mantophryne | lateralis | ABTC<br>112152 | Managalas Plateau                      | -9.06  | 148.57 |
| Mantophryne | lateralis | ABTC<br>112160 | Managalas Plateau                      | -9.20  | 148.33 |

|             |           |                |                                      |        |        |
|-------------|-----------|----------------|--------------------------------------|--------|--------|
| Mantophryne | lateralis | ABTC<br>112192 | Moroarab Village                     | -4.51  | 145.15 |
| Mantophryne | lateralis | ABTC<br>112193 | Moroarab Village                     | -4.51  | 145.15 |
| Mantophryne | lateralis | ABTC<br>112209 | Moroarab Village                     | -4.51  | 145.15 |
| Mantophryne | lateralis | ABTC<br>112224 | Moroarab Village                     | -4.51  | 145.15 |
| Mantophryne | lateralis | ABTC<br>112283 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | ABTC<br>112284 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | ABTC<br>112290 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | ABTC<br>112291 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | ABTC<br>112292 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | ABTC<br>112293 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | ABTC<br>112436 | Wanang, Sogeram                      | -5.23  | 145.20 |
| Mantophryne | lateralis | BPBM<br>15410  | Milne Bay Province:<br>Cloudy Mts    | -10.50 | 150.23 |
| Mantophryne | lateralis | BPBM<br>15411  | Milne Bay Province:<br>Cloudy Mts    | -10.50 | 150.23 |
| Mantophryne | lateralis | BPBM<br>15412  | Milne Bay Province:<br>Cloudy Mts    | -10.50 | 150.23 |
| Mantophryne | lateralis | BPBM<br>15415  | Milne Bay Province:<br>Cloudy Mts    | -10.50 | 150.23 |
| Mantophryne | lateralis | BPBM<br>15416  | Milne Bay Province:<br>Cloudy Mts    | -10.50 | 150.23 |
| Mantophryne | lateralis | BPBM<br>15425  | Milne Bay Province:<br>Duabo         | -10.42 | 150.31 |
| Mantophryne | lateralis | BPBM<br>15426  | Milne Bay Province:<br>Duabo         | -10.42 | 150.31 |
| Mantophryne | lateralis | BPBM<br>15427  | Milne Bay Province:<br>Duabo         | -10.42 | 150.31 |
| Mantophryne | lateralis | BPBM<br>15428  | Milne Bay Province:<br>Duabo         | -10.42 | 150.31 |
| Mantophryne | lateralis | BPBM<br>15429  | Milne Bay Province:<br>Duabo         | -10.42 | 150.31 |
| Mantophryne | lateralis | BPBM<br>15432  | Milne Bay Province:<br>Owen Stanleys | -10.28 | 150.15 |
| Mantophryne | lateralis | BPBM<br>15433  | Milne Bay Province:<br>Owen Stanleys | -10.28 | 150.15 |

|             |           |               |  |        |        |
|-------------|-----------|---------------|--|--------|--------|
| Mantophryne | lateralis | BPBM<br>17023 | Milne Bay Province:<br>Bunisi Village    | -10.02 | 149.60 |
| Mantophryne | lateralis | BPBM<br>17024 | Milne Bay Province:<br>Bunisi Village    | -10.02 | 149.60 |
| Mantophryne | lateralis | BPBM<br>17025 | Milne Bay Province:<br>Bunisi Village    | -10.02 | 149.60 |
| Mantophryne | lateralis | BPBM<br>17026 | Milne Bay Province:<br>Bunisi Village    | -10.02 | 149.60 |
| Mantophryne | lateralis | BPBM<br>18341 | Central Province: Fane                   | -8.55  | 147.08 |
| Mantophryne | lateralis | BPBM<br>18343 | Morobe Province: Mt<br>Shungol           | -6.82  | 146.69 |
| Mantophryne | lateralis | BPBM<br>19265 | Central Province: Laronu                 | -9.44  | 147.98 |
| Mantophryne | lateralis | BPBM<br>19266 | Central Province: Mt<br>Obree            | -9.44  | 148.01 |
| Mantophryne | lateralis | BPBM<br>19267 | Central Province: Mt<br>Obree            | -9.44  | 148.01 |
| Mantophryne | lateralis | BPBM<br>19269 | Central Province: Mt<br>Obree            | -9.45  | 148.00 |
| Mantophryne | lateralis | BPBM<br>19270 | Central Province: Mt<br>Obree            | -9.45  | 148.00 |
| Mantophryne | lateralis | BPBM<br>20396 | Milne Bay Province:<br>Normanby Island   | -10.03 | 150.98 |
| Mantophryne | lateralis | BPBM<br>22506 | Central Province:<br>Iamarere            | -9.45  | 147.61 |
| Mantophryne | lateralis | BPBM<br>22507 | Central Province                         | -9.45  | 147.61 |
| Mantophryne | lateralis | BPBM<br>22508 | Central Province:<br>Iamarere            | -9.45  | 147.61 |
| Mantophryne | lateralis | BPBM<br>22780 | West Sepik Province:<br>Torricelli Mtns  | -3.39  | 142.53 |
| Mantophryne | lateralis | BPBM<br>34728 | East Sepik Province:<br>Mindangua Stream | -3.61  | 143.49 |
| Mantophryne | lateralis | BPBM<br>34729 | East Sepik Province:<br>Mindangua Stream | -3.61  | 143.49 |
| Mantophryne | lateralis | BPBM<br>34730 | Madang:<br>Mandeherimbika                | -4.71  | 145.41 |
| Mantophryne | lateralis | BPBM<br>34731 | Madang:<br>Mandeherimbika                | -4.71  | 145.41 |
| Mantophryne | lateralis | BPBM<br>34732 | Madang: Keki Lodge                       | -4.40  | 145.40 |
| Mantophryne | lateralis | BPBM<br>37266 | Oro Province: Mt.<br>Victory             | -9.22  | 149.11 |
| Mantophryne | lateralis | BPBM<br>37267 | Oro Province: Mt.<br>Victory             | -9.22  | 149.11 |

|             |               |                |                                      |        |        |
|-------------|---------------|----------------|--------------------------------------|--------|--------|
| Mantophryne | lateralis     | BPBM<br>37268  | Oro Province: Mt.<br>Victory         | -9.24  | 149.14 |
| Mantophryne | lateralis     | BPBM<br>37269  | Oro Province: Mt.<br>Victory         | -9.26  | 149.15 |
| Mantophryne | lateralis     | BPBM<br>37270  | Oro Province: Mt.<br>Victory         | -9.24  | 149.14 |
| Mantophryne | sp            | LSUMZ<br>95094 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95095 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95096 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95098 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95099 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95100 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95101 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | sp            | LSUMZ<br>95102 | Central Province: Amau               | -10.04 | 148.56 |
| Mantophryne | axanthogaster | BPBM<br>20397  | Milne Bay Province:<br>Sudest Island | -11.49 | 153.41 |
| Mantophryne | axanthogaster | BPBM<br>20398  | Milne Bay Province:<br>Sudest Island | -11.49 | 153.41 |
| Mantophryne | axanthogaster | BPBM<br>20399  | Milne Bay Province:<br>Sudest Island | -11.49 | 153.41 |
| Mantophryne | axanthogaster | BPBM<br>20400  | Milne Bay Province:<br>Sudest Island | -11.49 | 153.41 |
| Mantophryne | axanthogaster | BPBM<br>20402  | Milne Bay Province:<br>Sudest Island | -11.49 | 153.41 |
| Mantophryne | lousiadensis  | BPBM<br>20143  | Milne Bay Province:<br>Rossel Island | -11.36 | 154.22 |
| Mantophryne | lousiadensis  | BPBM<br>20427  | Milne Bay Province:<br>Rossel Island | -11.36 | 154.22 |
| Mantophryne | lousiadensis  | BPBM<br>20428  | Milne Bay Province:<br>Rossel Island | -11.36 | 154.22 |
| Mantophryne | lousiadensis  | BPBM<br>20429  | Milne Bay Province:<br>Rossel Island | -11.36 | 154.22 |
| Mantophryne | infulata      | ABTC<br>98542  | Surim: Huon Peninsula                | -5.87  | 146.72 |
| Pherahapsis | menziesi      | LSUMZ<br>94178 |                                      | -9.17  | 147.18 |
| Hylophorbus | cf rufescens  | LSUMZ<br>94942 | Gulf: Sobo                           | -6.92  | 145.07 |

|             |              |                |   |        |        |
|-------------|--------------|----------------|---|--------|--------|
| Hylophorbus | cf rufescens | LSUMZ<br>94943 | Gulf: Sobo                              | -6.92  | 145.07 |
| Hylophorbus | AR sp 2      | LSUMZ<br>95551 | Central: Amau                           | -10.04 | 148.56 |
| Hylophorbus | AR sp 2      | LSUMZ<br>94918 | Central: Amau                           | -10.04 | 148.56 |
| Hylophorbus | rufescens    | LSUMZ<br>94897 | Milne Bay Province                      | -10.30 | 150.44 |
| Hylophorbus | rufescens    | LSUMZ<br>94903 | Milne Bay Province                      | -10.39 | 150.08 |
| Hylophorbus | sp 1         | LSUMZ<br>93040 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 1         | LSUMZ<br>93041 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 1         | LSUMZ<br>93060 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 1         | LSUMZ<br>93070 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 3         | LSUMZ<br>93067 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 3         | LSUMZ<br>93069 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 4         | LSUMZ<br>93073 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp 4         | LSUMZ<br>93072 | Saundaun Province: Utai                 | -3.40  | 141.58 |
| Hylophorbus | sp?          | BPBM<br>20369  | Milne Bay Province:<br>Sudest Island    | -11.45 | 153.43 |
| Hylophorbus | sp?          | BPBM<br>20375  | Milne Bay Province:<br>Sudest Island    | -11.49 | 153.41 |
| Hylophorbus | sp?          | BPBM<br>22737  | West Sepik Province:<br>Torricelli Mtns | -3.39  | 142.53 |
| Hylophorbus | sp?          | BPBM<br>22738  | West Sepik Province:<br>Torricelli Mtns | -3.39  | 142.53 |
| Hylophorbus | picoides     | BPBM<br>34726  | Madang Province:<br>Mongan              | -4.71  | 145.40 |
| Hylophorbus | picoides     | BPBM<br>34727  | Madang Province:<br>Mongan              | -4.70  | 145.40 |
| Hylophorbus | proekes      | BPBM<br>22761  | West Sepik: Torricelli<br>Mtns          | -3.39  | 142.53 |
| Hylophorbus | proekes      | BPBM<br>22762  | West Sepik: Torricelli<br>Mtns          | -3.39  | 142.53 |
| Hylophorbus | richardsi    | BPBM<br>33749  | Southern Highlands<br>Province          | -5.67  | 142.62 |
| Hylophorbus | richardsi    | BPBM<br>33751  | Southern Highlands<br>Province          | -5.67  | 142.62 |

|                     |             |                |                |        |        |
|---------------------|-------------|----------------|----------------|--------|--------|
| Sphenophryne        | cornuta     | LSUMZ<br>92230 | Saundaun: Utai | -3.40  | 141.58 |
| Sphenophryne        | cornuta     | LSUMZ<br>92231 | Saundaun: Utai | -3.40  | 141.58 |
| Sphenophryne        | cornuta     | LSUMZ<br>94793 | Gulf: Sobo     | -6.92  | 145.07 |
| Sphenophryne        | cornuta     | LSUMZ<br>94767 | Gulf: Sobo     | -6.92  | 145.07 |
| Austrochaperi<br>na | palmipes    | LSUMZ<br>95074 | Milne Bay      | -10.31 | 150.62 |
| Austrochaperi<br>na | palmipes    | LSUMZ<br>95075 | Milne Bay      | -10.31 | 150.62 |
| Choerophryne        | rostiffiler | LSUMZ<br>96101 | Saundaun: Utai | -3.40  | 141.58 |
| Choerophryne        | rostiliffer | LSUMZ<br>96098 | Saundaun: Utai | -3.40  | 141.58 |
| Callulops           | doriae      | LSUMZ<br>94845 | Gulf: Sobo     | -6.92  | 145.07 |
| Callulops           | doriae      | LSUMZ<br>94846 | Gulf: Sobo     | -6.92  | 145.07 |