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PHYLOGENETIC RELATIONSHIPS OF *LIPINIA* (SCINCIDAE) FROM NEW GUINEA BASED ON DNA SEQUENCE VARIATION FROM THE MITOCHONDRIAL 12S rRNA AND NUCLEAR *C-MOS* GENES

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(with two text-figures)

ABSTRACT.- A molecular phylogenetic analysis of four species of *Lipinia* from New Guinea, and one species from the Palau archipelago, is presented based on DNA sequence variation from seven hundred and eleven aligned sites from the mitochondrial 12S rRNA and nuclear *c-mos* genes. There is strong support for the basal placement of *Lipinia leptosoma* from Palau, resulting in a monophyletic New Guinean clade. Previous hypotheses of relationships suggested a New Guinean origin of the genus *Lipinia*. These results, however, suggest that *Lipinia* may have biogeographic origins in the Philippines or south-east Asia rather than New Guinea.

KEY WORDS.- Biogeography, data-partitions, likelihood, Lygosominae, multiple datasets, Pacific, Papua, Scincidae.

INTRODUCTION

The genus *Lipinia* includes 21 species with two centres of species abundance. Eight described species occur in the Philippines (Greer, 1974; Brown and Alcala, 1980) and seven described species occur in New Guinea (Greer, 1974; Zweifel, 1979; Greer and Mys, 1987; Austin, 1995). One species, *Lipinia noctua*, has a large range that extends from the Papuan region throughout Oceania to the Hawaiian Islands in the north-east and Easter Island and Pitcairn Island in the south-east. The remaining species occur in the Indonesian region with only one species reaching mainland south-east Asia (*L. vittigera*). The phylogenetic affinities of *Lipinia* with other lygosomine scincid genera is unclear, but Greer (1974) suggests a close relationship between *Lipinia* and three other genera from New Guinea (*Lobulia*, *Papuascincus*, and *Prasinohaema*). This phylogenetic relationship would suggest a Papuan origin for *Lipinia* with a subsequent invasion into island south-east Asia and radiation in the Philippines. A recent description of the monotypic genus *Paralipinia* from Vietnam, however, provides some evidence that *Paralipinia* is the sister taxa to *Lipinia* and suggests that *Lipinia* has origins in south-east Asia, and quite possibly mainland Vietnam (Darevsky and Orlov, 1997). In addition to *Lipinia*, *Lobulia*, *Papuascincus*, and *Prasinohaema* Greer's 'group I' includes *Scincella* and *Ablepharus*. The last two genera have broad distributions including south-east Asia, and *Lipinia* may have closer affinities to these taxa than to the New Guinea genera.

In this paper I examine the phylogenetic relationships of certain *Lipinia* species from the Papuan region to address questions concerning intrageneric relationships and biogeography. A hypothesis of phylogenetic relationships is based on DNA sequence variation from two genes: the mitochondrial ribosomal RNA 12S gene and the single-copy nuclear proto-oncogene *c-mos*. Mitochondrial genes, and the 12S rRNA gene in particular, have been widely used because of their ability to recover phylogenetic relationships. Nuclear genes suitable for phylogenetic use, however, have been

far more difficult to identify. Graybeal (1994), however, distinguished several potential nuclear candidate genes that might prove useful for vertebrate phylogenetic reconstruction. The single copy nuclear proto-oncogene *c-mos*, one of the genes identified by Graybeal (1994), has recently been shown to be a useful phylogenetic marker for resolving squamate relationships (Saint et al., in press).

MATERIALS AND METHODS

Specimens and tissue samples.- Muscle and liver tissue samples were dissected from freshly sacrificed specimens and either stored at - 80 °C or in 70% ethanol. Specimens and tissue samples were collected for the following five species (Figure 1): *Sphenomorphus leptofasciatus* (Texas Natural History Collection [TNHC 51918]), from Madang Province, Papua New Guinea. *Lipinia rouxi* (TNHC 51436), from the Lelet Plateau, New Ireland Island/Province, Papua New Guinea. *Lipinia longiceps* (TNHC 51284), from Garaina Valley, Morobe Province, Papua New Guinea. *Lipinia pulchra* (TNHC51290), from Garaina Valley, Morobe Province, Papua New Guinea. *Lipinia leptosoma* (presently being catalogued at the California Academy of Sciences; field number RNF 415), from Babeldaob Island Palau.

Although there is support for the close relationship among *Lipinia*, *Lobulia*, *Prasinohaema* and Papuascincus, there is some doubt as to the monophyly of these genera. *Sphenomorphus leptofasciatus*, therefore, was chosen as a conservative outgroup (Greer, 1974).

DNA isolation, amplification, and sequencing.- Isolation of DNA from either muscle or liver tissue was conducted following the protocols of Hillis et al. (1990) with one exception. Tissue samples were not ground in a mortar and pestle with liquid nitrogen. Rather, ~50 mg of tissue was digested with 20 (1 of 10 mg/ml proteinase K for three hours.

Double-stranded DNA products were amplified following the protocols of Palumbi et al. (1991). For the 12S gene two oligonucleotide primers synthesized at the South Australian Museum were used with the polymerase chain reaction (PCR) to amplify and sequence both complementary strands. The 12S primers used were: forward SAM(M1): 5'-TGA CTG CAG AGG GTG ACG GGC GGT GTG T-3' and reverse SAM(M2): 5'-AAA AAG CTT CAA ACT GGG ATT AGA TAC CCC ACT AT-3'. The nuclear c-mos primers (Saint et al., in press) used were: forward SAM(G73) 5'- GCG GTA AAG CAG GTG AAG AAA-3' and reverse SAM(G74) 5'-TGA GCA TCC AAA GTC TCC AAT C-3'.

Double-stranded PCR products were amplified using a Corbett FTS 320 Thermal cycler. The specific thermal cycle used is as follows: (i) one cycle at 94 °C X 3 min, 47 °C X 1 min, and 72 °C X1 min; (ii) thirty four cycles at 94 °C X 45 seconds, 47 °C X 45 seconds, and 72 °C X 1 min; (iii) one cycle at 72 °C X 6 min. PCR products were cleaned using BresaClean (Bresatec Ltd.) and then cycle sequenced on Corbett FTS1 Thermal cycler using ABI Prism dye-terminators (ABI) and protocols specified by the manufacturer. Sequences were determined on an ABI 377 DNA automated sequencer.

Phylogenetic analysis.- *Lipinia* belongs to the *Sphenomorphus* group of lygosomine skinks and trees were rooted using *Sphenomorphus leptofasciatus* as an outgroup (Greer, 1974).

Sequences from the 12S rRNA and *c-mos* genes were aligned using Clustal V (Higgins et al., 1991). Both parsimony and likelihood phylogenetic reconstruction methods were used as they are two of the most robust and accurate methods available (Felsenstein, 1981; Huelsenbeck and Hillis, 1993). The presence of a transition/transversion bias has been well documented with transitions occurring at a higher frequency than transversions (Brown et al., 1982; Vigilant et al., 1989; Knight and Mindell, 1993). Maximum likelihood was used to estimate the transition/transversion (TI/TV) ratio because estimation of the transition/transversion bias from the data themselves may underestimate the ratio due to multiple substitutions (Wakeley, 1996; Purvis and Bromham, 1997).

All phylogenetic analyses were done using PAUP* test version 4.0d64, written by D.L. Swofford. The two parameter HKY'85 model was implemented, which uses nucleotide fre-

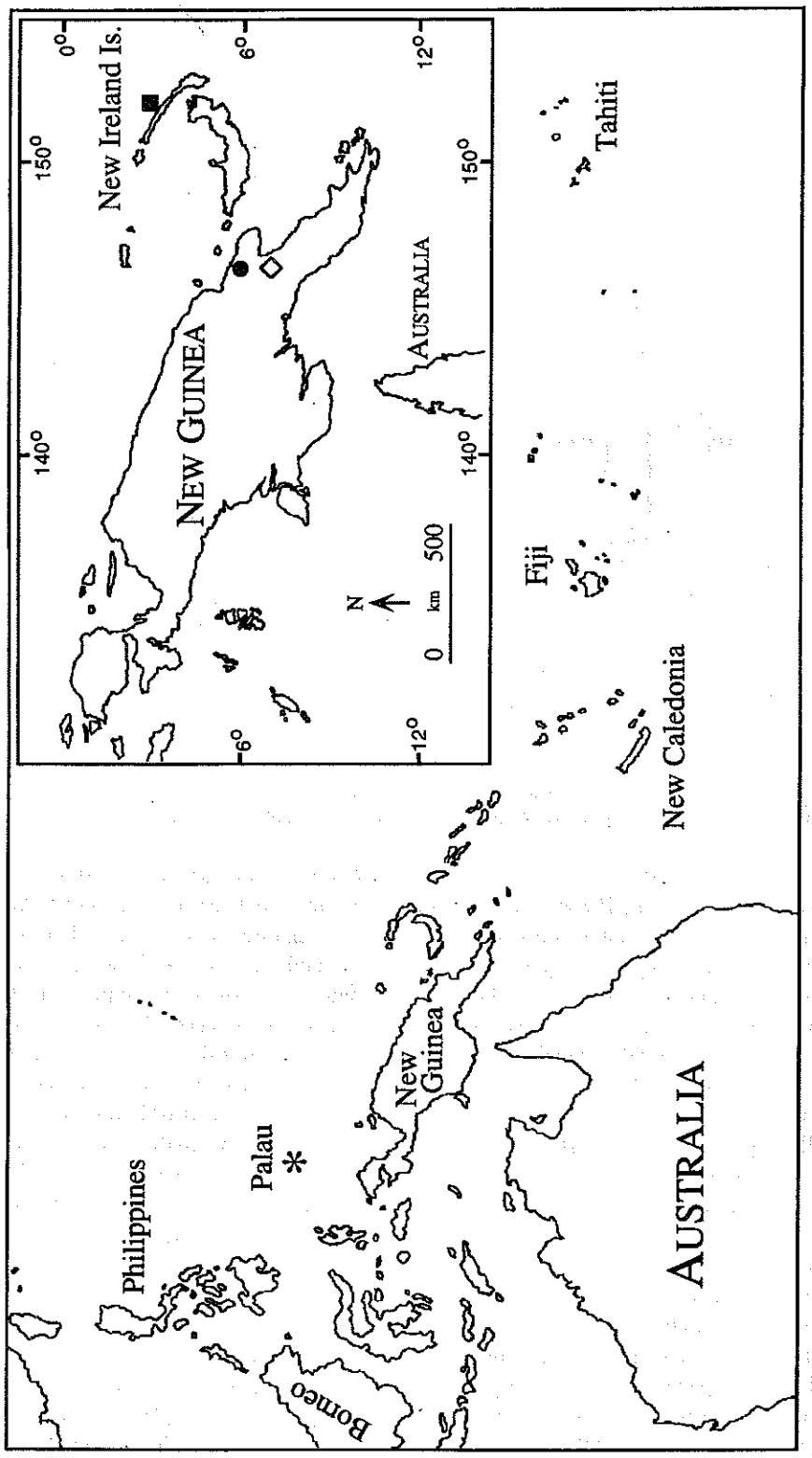

FIGURE 1: Map of sample localities for specimens used in this study: *Lipinia leptosoma*, denoted by asterisk; *Lipinia rouxi*, denoted by solid square; *Lipinia pulchra* and *Lipinia longiceps*, denoted by open diamond; *Lipinia nocuta* and *Sphenomorphus leptofasciatus*, denoted by closed circle.

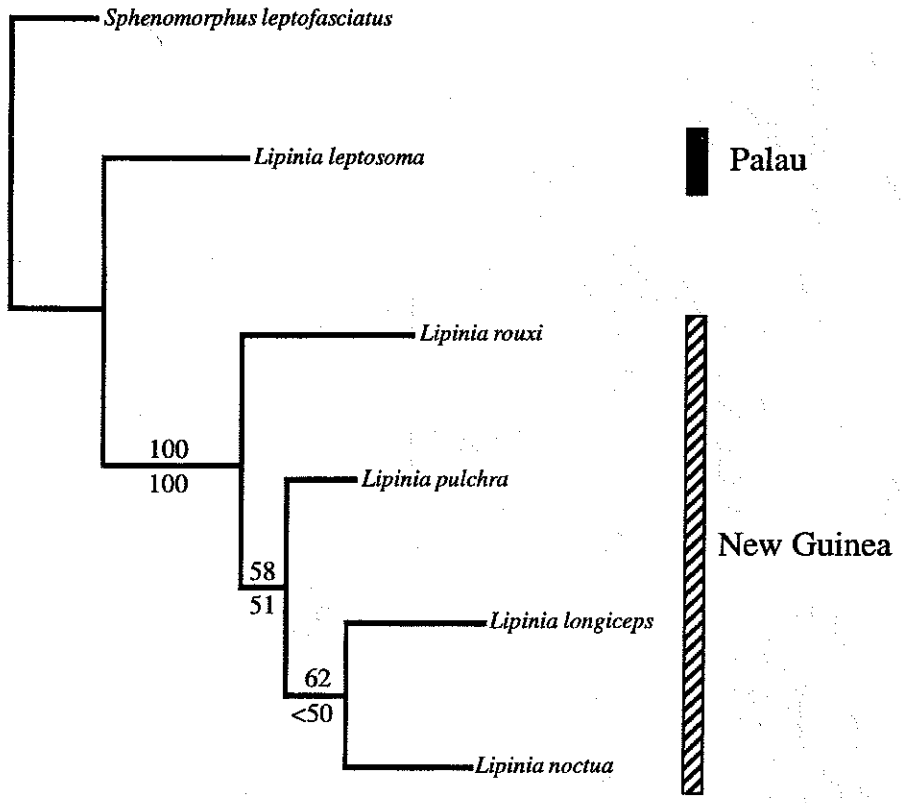

FIGURE 2: Phylogram, showing relative branch lengths, of the single maximum parsimony tree obtained from branch and bound PAUP* searches using *Sphenomorphus leptofasciatus* as the outgroup. Numbers at nodes represent bootstrap proportions for 1,000 and 100 pseudoreplicates for parsimony (above the line) and likelihood analyses (below the line).

quencies estimated from the data, for all likelihood analyses (Hasegawa et al., 1985). Branch and bound searches, which guarantee to find the shortest tree, were used for all parsimony analyses including the bootstrap replicates. The branch and bound method was also used to find the optimal likelihood tree. In order to facilitate a reasonable number of bootstrap pseudoreplicates for likelihood, which is computationally expensive, the heuristic search option was implemented. The tree bisection-reconstruction (TBR) branch swapping method was used.

The partition-homogeneity test was used to asses if both genes should be combined in a single analysis. The test examines heterogeneity by calculating the sum of the tree lengths from the original dataset and comparing that with the tree length distribution from pseudopartitions (of original partition size)[Huelsenbeck et al., 1996].

Phylogenetic Confidence.- Confidence in the phylogenetic signal for this molecular data set was assessed in three ways. First, both maximum parsimony and maximum likelihood were used to estimate a phylogenetic hypothesis (Kim, 1993). Second, both maximum parsimony and maximum likelihood analyses were bootstrapped to assess confidence for each node (Felsenstein, 1985; Swofford and Olsen, 1990; Hillis and Bull, 1993). Finally, presence of a significant phylogenetic signal was assessed using the g1 statistic estimated from 100,000 random trees (Hillis and Huelsenbeck, 1992).

RESULTS

A total of seven hundred and eleven aligned sites, three hundred and fifty aligned sites for 12S and three hundred and sixty one aligned sites for *c-mos*, were used in the phylogenetic analysis (Appendix I). Of these, eighty two sites were in-

formative under the parsimony criterion. For the entire data matrix a TI/TV ratio of 1.96 was estimated using maximum likelihood. This TI/TV ratios was used as a weighting scheme in all phylogenetic analyses. The partition homogeneity test was non-significant (P = 1.0), indicating datasets from the two genes should be combined.

Insertion/deletion (indels) events are present in the alignment for both genes. *c-mos* is a protein-encoding gene and an open reading frame was observed for all taxa. As expected, indels present in *c-mos* are in multiples of three nucleotides (corresponding to a single codon), thus preserving the reading frame. For *c-mos* a two codon (six nucleotide) deletion was observed for *Lipinia pulchra*, and a single codon (three nucleotide) deletion was observed for *L. noctua* (Appendix I). As the ribosomal 12S gene is not a protein-encoding gene, indels need not be in multiples of three nucleotides.

The single maximum parsimony (MP) tree is presented with bootstrap support from both the MP and maximum likelihood (ML) analyses (Fig. 2). Bootstrap proportions in Fig. 2 are for 1000 and 100 pseudoreplicates, for MP and ML respectively. There is strong bootstrap support (100) for the basal placement of *Lipinia leptosoma* from Palau rendering the New Guinea species monophyletic. The matrix for both uncorrected and HKY'85 corrected genetic distances, is presented in Table 1. The g1 (estimated from 100,000 randomly generated trees) was -1.41, indicating significant phylogenetic signal (P 0.01) [Hillis and Huelsenbeck, 1992]. For the parsimony analysis, the tree length was 258.4 with a consistency index (CI) of 0.854. For the likelihood analysis the likelihood value was -1963.6. Fractional tree length and likelihood values for the parsimony and likelihood analyses result from a fractional estimate of the transition/transversion ratio.

DISCUSSION

Zweifel (1979) synonymized *Lipinia rouxi* with *L. nocuta*, but non-traditional morphological data by Greer and Mys (1987) and allozyme data by Austin (1995) showed that *L. rouxi* is clearly a distinct species. The results of both Greer and Mys (1987) and Austin (1995), however, were equivocal as to whether *L. rouxi* was the sister to *L. noctua*. The results from this study further demonstrate the specific status of *L. rouxi* from New Ireland Island as well as provide support that *L. rouxi* is basal within the New Guinea clade.

Based on the molecular data presented in this paper, the New Guinean *Lipinia* are monophyletic. Three additional species of *Lipinia* are recorded from New Guinea, however, these species either have isolated ranges or are known from just a few specimens and were not included in the analysis. *Lipinia cheesmanae* is known from only four specimens, the holotype from the Cyclops Mountains of Irian Jaya collected in 1938, and three additional specimens collected by the 1938-39 Archbold Expedition 190 km south-west of the type locality (Parker, 1940; Zweifel, 1979). Based on colour pattern variation and morphology, *L. cheesmanae* appears to be closely related to *L. longiceps* (Parker, 1940). *Lipinia venemai*, described by Brongersma (1953), is known only from two

TABLE 1: Summary of genetic distance values. Uncorrected genetic distances above the diagonal, HKY'85 corrected distances below the diagonal (Hasegawa et al., 1985).

		1	2	3	4	5	6
1	*Sphenomorphus leptofasciatus*	-	0.10110	0.07802	0.09070	0.10677	0.09480
2	*Lipinia rouxi*	0.10939	-	0.10519	0.06913	0.09091	0.07305
3	*L. leptosoma*	0.08309	0.11424	-	0.08903	0.10805	0.09890
4	*L. pulchra*	0.09746	0.07303	0.09544	-	0.06035	0.06665
5	*L. longiceps*	0.11611	0.09783	0.11818	0.06329	-	0.07310
6	*L. noctua*	0.10218	0.07734	0.10716	0.07041	0.07779	-

specimens, the male holotype from Ajamaroe, and female paratype from Djidmaoe, both from the Vogelkop Peninsula of Irian Jaya. Although the specific status of *L. venemai* was upheld by Zweifel (1979) the relationship of this species with the other New Guinean *Lipinia* is undetermined (Zweifel, 1979). *Lipinia miota* was described by Boulenger (1895) from Fergusson Island off the east coast of New Guinea, but Zweifel (1979) synonomized it with *Lipinia* noctua. Given the specific status of *L. rouxi*, which was similarly synonomized with *L. noctua*, and strong morphological conservatism in this group (Austin, 1995), it remains unclear as to whether *L. miota* should be recognized as a distinct species; further molecular work should clarify this issue.

Additionally, at the present time specimens from the large Philippine radiation were not included. Unfortunately many of the Philippine species are also known from a small number of specimens or have very limited ranges (Brown and Alcala, 1980). Inclusion of these taxa along with other potentially closely related taxa such as *Paralipinia* in future analyses will provide the final samples necessary to answer the question of the biogeographic origins of *Lipinia*. Unfortunately, *Paralipinia* is known only from a single specimen and the habitat of the type locality in Vietnam has recently been severely degraded (I. S. Darevsky, pers. comm.). The secretive arboreal behaviour and patchy distribution of most species of *Lipinia*, however, probably make them appear to be less common than they actually are. *Lipinia macrotympanum*, known only from the holotype described by Stoliczka (1873), was recently rediscovered in the Nicobar Islands over one century after its original description (Das, 1997). *Lipinia rouxi* was only known from fifteen specimens until Austin (1995) collected an additional twenty specimens. Given the remote and densely forested areas involved, it is therefore quite possible that additional populations of previously described species will be discovered along with species new to science for future phylogenetic analyses.

The biogeography of the herpetofauna of south-east Asia and Papua is complex, but recent systematic work based on morphology and molecules, and a improved understanding of the heterogeneous tectonic history of this region, has provided greater insight into the evolution of this highly diverse reptile and amphibian fauna. The origins of the genus *Lipinia* are unclear as this genus has two centres of abundance, with one area (Philippines) being closely associated with south-east Asia, and the other (New Guinea) having a mixture of several different faunal histories. The variegated Papuan herpetofauna has a high degree of endemics and includes some members that have a south-east Asia origin, others with an Australian origin, and other faunal elements that appear to be of ancient Gondwanan origin. The biogeographic origin and phylogenetic affinities of *Lipinia* are unclear, but the data presented in this study, although somewhat limited, provide some suggestion that the Papuan *Lipinia* are monophyletic and that either island or mainland south-east Asia, rather than New Guinea, may host *Lipinia*'s closest relatives. Indeed, Paralipinia from Vietnam, may be the only surviving relative to *Lipinia*. Further morphological and molecular work is clearly necessary to fully understand the biogeographic processes that have been responsible for the current distributions and phylogenetic relationships of *Lipinia*.

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APPENDIX 1: Three hundred and fifty base-pair sequence from the mitochondrial 12S rRNA gene and three hundred and sixty one base-pair sequence for the nuclear *c-mos* gene for six taxa. Dots indicate a match with the first taxon (outgroup), dashes indicate gaps.

12S:

S. leptofasciatus	ATAGTACTAACACAACACCATCCGCCAGAGAACTACAAGCGAAAAGCTTG
L. rouxi	...-.T.....T.AT.....A.
L. leptosoma	...A.....TT.....A.
L. pulchra	...-.T....T...C..AT.....A.
L. longiceps	...-.TT...T....T..T.....A.
L. noctua	...-.TT...T.....-T.....G.....A.

S. leptofasciatus	AAACTCCAAGGACTTGGCGGTGCTTCAAACCAACCTAGAGGAGCCTGTCC
L. rouxi	.....
L. leptosoma	......A.....T.....
L. pulchra	.....
L. longiceps	.....T.....
L. noctua	.....T.....

S. leptofasciatus	TATAATCGATACTCCACGTTTTACCTCACCGCTCCTTGAAATTCAGCCTA
L. rouxi	.....AA.....T.CTT...CT.AC.....
L. leptosoma	.....A.....A..C....T...A.CA..A.CC.AC.....
L. pulchra	.....C.....C.....T...C.C.T.A.CCC-C.....
L. longiceps	.....T...C.CG..A.CC.AC.....
L. noctua	.....C.....CGCA..A.TC.AC.....

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S. leptofasciatus   TATACCGCCGTCGCCAGCCTACCTTGTGAAAGAAACAAAGTGAGCAAAAT
L. rouxi            .............T...........A........GA.....AG.......
L. leptosoma        ..................TC...C.A................G.......
L. pulchra          .............T...........A................AGA.....C
L. longiceps        .............T.........C.....G...........A.......C
L. noctua           .............T...........A........G......A........

S. leptofasciatus   AGTTA-ACAACTAGTACGTCAGGTCAAGGTGTAGCACACGAA-GCGGTAG
L. rouxi            .....-.T.....AC.......................T.GGGAG.-A..
L. leptosoma        .....-.......A.......................A....G-.T.....
L. pulchra          .....-.T.....AA.......................T.GGG.G..C..
L. longiceps        .AC..-.T..T..AA.......................T.CGG.G.-C..
L. noctua           .....C.......AC.......................T.TGTCG.A-..

S. leptofasciatus   AGATGGGCTACATTTTTACAAAGAAAAAACACGAATAGCACGTTGAAATC
L. rouxi            ................C.-.C.ATG..C.........A..T.C.....CA
L. leptosoma        ........................T..C.......C..TGT.C.....CA
L. pulchra          ................C.-.--AC...C.T.......ATGT.......CA
L. longiceps        ................CT-..GATG..C.T..AG.C.AA.T.......CA
L. noctua           ................C.-.-.ACG..C.T.....C.AT.T.......CA

S. leptofasciatus   CCTGCTCGAAGGTGGATTTAGTAGTAAAATAAAAAAGAAAAATTATTTTA
L. rouxi            .A..T.TA....................GAGT.T.....G.CATC..G.
L. leptosoma        .TCA..T.....................G.GT.......C........
L. pulchra          .A.AT.T.....................CG.CC.......G.ATA..G.
L. longiceps        .A.AT.A.....................T...GC.....T.C.AT...G.
L. noctua           .G.AT.T.....C..............C..G..........CATC..G.

C-mos:

S. leptofasciatus   AAGAACCGGTTGGCATCAAGACAAAGCTTCTGGGCAGAACTAAATGTGGT
L. rouxi            .........C........C.............................A.C
L. leptosoma        .................C.............................A.C
L. pulchra          .................CA...C.........................C
L. longiceps        .................C....C.........................C
L. noctua           .......A.........C....C.........................C

S. leptofasciatus   ACGCCTTAGTCATAACAATGTGGTACGTGTAATAGCTGCTAGTGCATGTT
L. rouxi            ....................................A............
L. leptosoma        ............G.A.....................................
L. pulchra          ....................................C............
L. longiceps        ....................................A............
L. noctua           ................................................
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S. leptofasciatus  CTCCTACCAATCAGAACAGTTTGGGTACCATCATAATGGAATATGTAGGT
L. rouxi           .............................................
L. leptosoma       .......T.G...................................
L. pulchra         ............................T................
L. longiceps       ......T.....................T................
L. noctua          ......................A......................

S. leptofasciatus  AACAGCACTTTGCACCATGTTATCTATGGGACAGGATGTACTGTAGCAAA
L. rouxi           ...G.....................................A...A....
L. leptosoma       ........................................TG.......
L. pulchra         ...G.....................................-------..
L. longiceps       ..TG.....................................A..GA....
L. noctua          ...G....A.....................A..........A...A....

S. leptofasciatus  AAGGAAGGATAATGAGCTTGGTTGTGGCTATGAACCTTTGAGTATAATGC
L. rouxi           ........C...........A.....A.............A........
L. leptosoma       ....................A................A.........
L. pulchra         ........C...................................
L. longiceps       ........CA..................................
L. noctua          ........C......---..........................

S. leptofasciatus  AGTCTCTGAGCTACTCATGTGACATTGTGGCAGGCTTGGTCTTTCTCCAT
L. rouxi           .C..............A.G.........................
L. leptosoma       .............................................
L. pulchra         .............................................
L. longiceps       .....T..........C............................
L. noctua          ................C.....................A.....

S. leptofasciatus  TCACAATTAACTGTGCATCTGGATTTAAAACCTGCCAACATATTCATCAC
L. rouxi           .............................................
L. leptosoma       .............................................
L. pulchra         .............................................
L. longiceps       .............................................
L. noctua          .............................................

S. leptofasciatus  TGAACAAA
L. rouxi           ........
L. leptosoma       ........
L. pulchra         ........
L. longiceps       ........
L. noctua          ........
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