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Molecular Phylogeny and Historical Biogeography of Pacific Island Boas (*Candoia*)

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The enigmatic biogeography of Pacific island boas of the genus *Candoia* is examined using DNA sequence variation from a portion of the mitochondrial cytochrome *b* gene. Estimates of the phylogenetic relationships and genetic distances between *Candoia* and the Old World (*Sanzinia*) and New World boids (*Boa, Corallus,* and *Epicrates*) suggest that a recent dispersal event from the Americas is not responsible for *Candoia*'s Papuan distribution. Multiple populations from the three *Candoia* species are sampled to distinguish whether substantial evolutionary partitions exist within species as a result of colonization patterns or geographical barriers and to assess whether genetic partitions are concordant with species boundaries. In all analyses, *Candoia* and the Madagascan *Sanzinia* are sister taxa, and *C. bibroni* is basal to *C. aspera* and *C. carinata*. Mean sequence divergences between *Candoia* and the other boid genera *Sanzinia, Corallus, Epicrates,* and *Boa* are 0.19, 0.23, 0.24, and 0.25, respectively. Within *Candoia,* mean interspecific sequence divergence ranges from 0.13, between *C. aspera* and *C. bibroni,* to 0.16 for both *C. aspera*/*C. carinata* and *C. bibroni*/*C. carinata* pairwise comparisons. Large intraspecific sequence divergence (up to 0.13 within *C. carinata*) exists within *Candoia* species demonstrating deep separations corresponding to patterns of island colonization and geographic barriers in New Guinea.

THE Superfamily Booidea (Serpentes) is composed of two families, the egg-laying pythons (Pythonidae) and the live-bearing boas (Boidae; McDowell, 1987). These families have disjunct distributions with pythons being primarily restricted to the Old World and boas primarily restricted to the New World. There are, however, a few striking exceptions to this distribution most notably the boid genera *Acrantophis* and *Sanzinia* found in Madagascar and *Candoia* distributed across the Papuan-Pacific region (McDowell, 1987; Cadle, 1987).

The enigmatic distribution of *Candoia* makes this genus of particular interest for understanding the biogeographic origins of Papuan and Pacific faunas. Based on morphological and biochemical data, *Candoia* is a monophyletic boid genus (Schwaner and Dessauer, 1981; Cadle, 1987; Kluge 1991). All three species of *Candoia* are defined by synapomorphies, including a distinct flat rostrum that gives a unique angular profile to the snout (McDowell, 1979). The phylogenetic relationships between *Candoia* and other boid snakes, however, have been contentious (Underwood, 1976; McDowell, 1987; Kluge, 1991). *Candoia* is thought by some authors to have close phylogenetic affinities to the Madagascan boids (*Sanzinia* and *Acrantophis*; Underwood, 1976; Branch, 1981; Gibbons, 1985), whereas other authors have suggested a close affinity between *Candoia* and Neotropical boids (*Corallus*, *Epicrates*, and *Eunectes*; Mertens, 1972; McDowell, 1979; Harlow and Shine, 1992). Morphological work by Kluge (1991), however, places *Candoia* as basal to all other boids.

The basal placement of *Candoia* has been used to argue that the distribution of *Candoia* is relictual (Kluge, 1991). In contrast, the authors who propose a sister relationship between *Candoia* and the American boids argue for dispersal from the Americas as the best explanation for the distribution of *Candoia*. Inference of biogeographic process from strictly distributional data is problematical. Even when the phylogenetic relationships of taxa are well supported, it may not be possible to precisely infer the biogeographic process that produced current distributions. Dispersal and vicariance hypotheses, therefore, are not easily distinguishable given

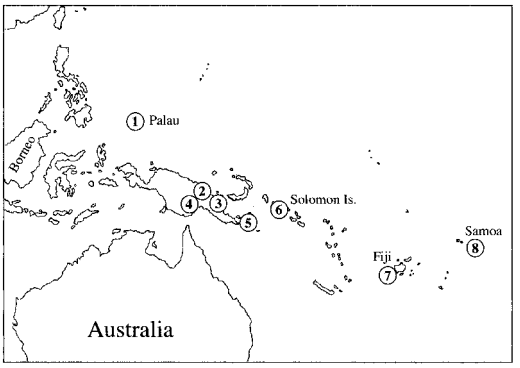

Fig. 1. Map of localities for the *Candoia* used in this study. Numbers 1, 2, 5, and 6 designate *Candoia carinata* populations. Numbers 3 and 4 represent *Candoia aspera* populations. *Candoia bibroni* samples are from localities 7 and 8.

that all the areas involved are fragments of Gondwana. A phylogenetic hypothesis combined with genetic distance data may, however, greatly improve our abilities to infer biogeographic patterns. Relative genetic distance information, even without a precise calibration, would allow us to distinguish between recent dispersal from the Americas and an ancient vicariant/dispersal event.

The three species of *Candoia* (*C. aspera, C. bibroni,* and *C. carinata*) range from American Samoa in the east, through Melanesia, to Sulawesi in the west (Gibbons, 1985; McDowell, 1979). *Candoia bibroni* (Fig. 1) is found from American Samoa in the east, the Loyalty Islands in the southwest, and the eastern part of the Solomon Islands group in the west (McDowell, 1979; Harlow and Shine, 1992; Gibbons, 1985). *Candoia carinata* is found in virtually all the islands of the Solomon Island archipelago, including Santa Cruz, Rennell, and Bellona, and occurs westward, through New Guinea to Sulawesi, and as far north as the Palau archipelago (McDowell, 1979; McCoy, 1980; Harlow and Shine, 1992). *Candoia aspera* is found throughout New Guinea below 1500 m and the Bismarck Archipelago and Manus Island (McDowell, 1979; McCoy, 1980; Harlow and Shine, 1992). All species except *C. bibroni* occur in sympatry with at least one species of python.

Candoia species vary in both body-size and shape (Harlow and Shine, 1992). *Candoia aspera* is a short heavy-bodied species with a large head and is strictly terrestrial. *Candoia bibroni* is a long and gracile species with a relatively small head and is primarily arboreal. *Candoia carinata* is intermediate in body size and shape and head shape and is semiarboreal (McCoy, 1980; Harlow and Shine, 1992). Despite significant differences in morphology, there appear to be no differences in diet between the three species (Harlow and Shine, 1992). In contrast to the ecological information, little is known concerning the interrelationships of *Candoia* (Kluge, 1991).

In this paper, the phylogenetic relationships within *Candoia* as well as the relationships between *Candoia* and the Old World (*Sanzinia*) and New World boids (*Boa, Corallus,* and *Epicrates*) are estimated using DNA sequence data from a portion of the mitochondrial cytochrome *b* gene. Several populations from the three *Candoia* species are included to ascertain whether gene genealogies from cytochrome *b* are concordant with presently recognized species boundaries. Quantitative estimates of molecular divergence and phylogenetic relationships are used to reevaluate the biogeography of boids and the history of *Candoia,* and alternate hypothesis of relationships, based on morphology, are tested statistically.

Materials and Methods

Specimens and tissue samples.—Six *C. aspera* from three localities, three *C. bibroni* from two localities, and six *C. carinata* from four localities were used for this study (Appendix 1, Fig. 1). *Boa constrictor, Sanzinia madagascariensis, Epicrates striatus,* and *Corallus caninus* were included in the analysis to determine the phylogenetic affinities of *Candoia* (Appendix 1). Sequence data for *Epicrates* and *Corallus* were obtained from the literature (Henderson and Hedges, 1995). *Python reticulatus* was used as an outgroup (McDowell, 1987; Kluge, 1991). Tissues from *Eunectes* (South America) and *Acrantophis* (Madagascar) were not available.

DNA isolation, amplification, and sequencing.—DNA was isolated from either muscle or liver tissues following the protocols of Hillis et al. (1990). Tissue samples, however, were digested with 20 μl of 10 mg/ml proteinase K for three hours.

The protocols of Hillis et al. (1990) were followed to amplify double-stranded products of a portion of the mitochondrial cytochrome *b* gene. The primers used were L14841 5′AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA3′ and H15149 5′AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A3′ (Kocher et al., 1989). Cytochrome *b* was chosen because of its general utility for resolving divergences among vertebrates (Graybeal, 1994). Double-stranded PCR products were amplified using a Corbett FTS 320 Thermal cycler. The

specific thermal cycle used is as follows: (1) one cycle at 94 C × 3 min, 47 C × 1 min, and 72 C × 1 min; (2) 34 cycles at 94 C × 45 sec, 47 C × 45 sec, and 72 C × 1 min; (3) one cycle at 72 C × 6 min. PCR products were purified 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 obtained with an ABI 377 DNA automated sequencer.

Phylogenetic analysis.—Twenty-one sequences were unambiguously aligned (no insertions or deletions) using Clustal V (Higgins et al., 1991). Both parsimony and likelihood phylogenetic reconstruction methods were used because they have been shown to be two of the most consistent and accurate methods available (Edwards, 1972; Felsenstein, 1981; Huelsenbeck and Hillis, 1993).

The presence of a bias in the type of base substitutions has been well documented (Brown et al., 1982; Knight and Mindell, 1993; Vigilant et al., 1989). Transitions generally occur at a higher frequency than transversions (Vigilant et al., 1989). Estimation of the transition:transversion bias from the data may underestimate the ratio due to multiple substitutions (Wakeley, 1996; Purvis and Bromham, 1997). Maximum likelihood was used to estimate the transition: transversion (TI:TV) ratio using PAUP* test version 4.0, written by D. L. Swofford.

All phylogenetic estimation was done using PAUP*. The two-parameter HKY'85 model was implemented, which uses nucleotide frequencies estimated from the data and corrects for unequal base frequencies, for all likelihood analyses (Hasegawa et al., 1985). Because of the large number of taxa, all searches were done using the heuristic search options in PAUP* with 100 random addition sequences. The tree bisection-reconstruction (TBR) branch-swapping method was used.

The hypothesis of boid relationships based on morphology (Kluge, 1991) was tested statistically against the relationships derived from this molecular analysis using the Templeton (1983), winning-sites, and Kishino-Hasegawa tests (Kishino and Hasegawa, 1989). These tests examine the probability of significant differences in pairwise comparisons of trees based on the null model of no difference between the two trees.

Phylogenetic confidence.—Confidence in the phylogenetic signal for this molecular dataset was assessed in four 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; Hillis and Bull, 1993; Swofford and Olsen, 1990). Third, because of the relatively large degree of divergence observed and thus possible saturation of third positions, maximum-parsimony and maximum-likelihood analyses were done for transversions only. The degree of congruence between all analyses is used as an assessment of topological confidence. Finally, presence of a significant phylogenetic signal was assessed using the g_1 statistic estimated from 100,000 random trees (Hillis and Huelsenbeck, 1992) and the permutation-tailed-probability (PTP) test implemented in PAUP*.

RESULTS

Two hundred seventy aligned sites for 21 taxa were used in the phylogenetic analysis (Appendix 2). No insertions or deletions were present. Of these, 112 sites were variable, and 89 sites were parsimony informative. All new sequences used in this study are available from GenBank (accession numbers AF153065–AF153008). For the entire data matrix, a TI:TV ratio of 2.5 was estimated using maximum likelihood. This TI: TV ratio was used as a weighting scheme in all respective analyses except for the transversions-only analysis.

The matrix for inter- and intrageneric amino acid differences and pairwise HKY'85 corrected genetic distances for all nucleotide sites is presented in Table 1. Cytochrome *b* is a protein-encoding gene, and, as expected, most of the variation was at third position sites (82/112), with fewer (30/112) changes at first and second positions. Mean intergeneric pairwise corrected distances were relatively large and ranged from 0.180 (between *Python* and *Corallus*) to 0.249 (between *Epicrates* and *Boa* and between *Python* and *Epicrates*; Table 1). Mean intergeneric absolute amino acid differences ranged from eight differences (between *Corallus* and *Boa* and between *Python* and *Epicrates*) to 11.3 differences (between *Candoia* and *Epicrates*). Within *Candoia,* mean sequence divergence ranged from 0.137 between *C. aspera* and *C. bibroni* and 0.163 between *C. aspera* and *C. carinata* (Table 1). Mean intergeneric amino acid differences within *Candoia* ranged from 2.1 (between *C. aspera* and *C. bibroni*) to 2.4 (between *C. bibroni* and *C. carinata*; Table 1).

The single maximum-parsimony (MP) and maximum-likelihood (ML) trees are presented in Figure 2. Both reconstruction methods esti-

TABLE 1. SUMMARY MEAN AMINO ACID DIFFERENCES (ABOVE THE DIAGONAL) AND HKY'85 CORRECTED GENETIC DISTANCES (BELOW THE LINE; HASEGAWA ET AL., 1985).

		1	2	3	4	5	6	7	8
1	*Python1*	—	0	11	8	9	9	10	10
2	*Python2*	0.00372	—	11	8	9	9	10	10
3	*Sanzinia*	0.20101	0.20622	—	10	10	9	9	9
4	*Epicrates*	0.25223	0.24639	0.24674	—	11	10	12	12
5	*Corallus*	0.18253	0.17747	0.21698	0.20655	—	8	9	9
6	*Boa*	0.22622	0.22087	0.24698	0.24863	0.20016	—	10	10
7	*aspera1*	0.19176	0.19694	0.19092	0.21877	0.20665	0.23297	—	0
8	*aspera2*	0.18666	0.19177	0.18088	0.21872	0.20141	0.22745	0.01507	—
9	*aspera3*	0.19693	0.20217	0.19092	0.21877	0.21195	0.23297	0.01507	0.00747
10	*aspera4*	0.19693	0.20217	0.19092	0.21877	0.21195	0.23297	0.01507	0.00747
11	*aspera5*	0.21701	0.22242	0.20112	0.22841	0.23245	0.27167	0.05052	0.05882
12	*aspera6*	0.21709	0.22251	0.20118	0.22850	0.23253	0.26587	0.04643	0.05466
13	*bibroni1*	0.20040	0.19531	0.15823	0.23937	0.19613	0.23552	0.12807	0.12334
14	*bibroni2*	0.20040	0.19531	0.15823	0.23937	0.19613	0.23552	0.12807	0.12334
15	*bibroni3*	0.20054	0.19544	0.17323	0.26140	0.18611	0.24158	0.12735	0.11803
16	*carinata1*	0.21678	0.21147	0.20283	0.29720	0.26632	0.24941	0.16757	0.17276
17	*carinata2*	0.21677	0.21145	0.20283	0.29728	0.26632	0.24942	0.16759	0.17278
18	*carinata3*	0.20062	0.19551	0.17251	0.26151	0.23832	0.25595	0.16831	0.17353
19	*carinata4*	0.21663	0.22201	0.22304	0.22676	0.23878	0.28298	0.12789	0.13263
20	*carinata5*	0.20217	0.20746	0.21806	0.25093	0.23246	0.24977	0.16280	0.16794
21	*carinata6*	0.19701	0.20225	0.21274	0.25685	0.22706	0.23854	0.16287	0.15775

mate the same intra- and intergeneric relationships. The two trees differ only in the intraspecific relationships within *C. aspera*. Monophyly of each *Candoia* species is supported by bootstrap analysis (5000 and 100 pseudoreplicates for MP and ML, respectively): *C. aspera* (94/82), *C. bibroni* (73/76), and *C. carinata* (79/78). The sister taxon to *Candoia* is the Madagascan *Sanzinia* is based on moderate levels of bootstrap support (61/72). The g_1 (estimated from 100,000 randomly generated trees) was −0.709, indicating significant phylogenetic signal ($P <$ 0.01; Hillis and Huelsenbeck, 1992). The PTP test resulted in a significant ($P = 0.01$) difference between the most parsimonious tree and trees generated from random permutations of the data matrix, demonstrating presence of significant phylogenetic signal.

For the tranversion-only analysis, 82 positions were parsimony informative, and there were three equally parsomonious trees (not shown) that only differ from the analysis where the TI: TV was estimated by maximum likelihood in that the *Candoia* intraspecific relationships are not fully resolved. The resulting bootstrap support for the transversion-only analysis is incorporated in Figure 2. The g_1 (estimated from 100,000 randomly generated trees) for the transversion-only analysis was −0.839 ($P <$ 0.01), and the PTP test was also significant ($P = 0.01$), indicating phylogenetic signal.

When the morphological topology was statistically compared with the molecular results from this study, the Templeton test was nonsignificant ($P = 0.0582$), but the Kishino-Hasegawa and winning-sites tests were both significant ($P = 0.0438$ and $P = 0.0309$, respectively). Results from all phylogenetic analyses are congruent with respect to the generic level topology (Fig. 2) and thus appear to be in conflict with the most recent work of boid relationships based on morphology (Kluge, 1991).

DISCUSSION

The enormous diversity of snakes, combined with their relative uniform body-plan, poor fossil record, and apparent long evolutionary history have impeded a comprehensive resolution of phylogenetic relationships (Cadle, 1987; McDowell, 1987; Kluge, 1991). The higher level phylogenetic relationships of snakes, and in particular the relationships within and between the pythons and boas, has been problematical (Rieppel, 1979; Underwood and Stimson, 1990; Kluge, 1993). In particular, the recent paper by Heise et al. (1995) provides evidence that relationships in the Boidae are complex and require reexamination.

The well-supported monophyly of *Candoia* species is not surprising because all three species are defined by numerous synapomorphies

TABLE 1. CONTINUED.

		9	10	11	12	13	14	15	16
1	*Python1*	10	10	11	11	8	8	10	11
2	*Python2*	10	10	11	11	8	8	10	11
3	*Sanzinia*	9	9	10	10	7	7	9	9
4	*Epicrates*	12	12	13	13	10	10	12	10
5	*Corallus*	9	9	10	10	9	9	11	12
6	*Boa*	10	10	11	11	8	8	10	10
7	*aspera1*	0	0	1	1	2	2	2	4
8	*aspera2*	0	0	1	1	2	2	2	4
9	*aspera3*	—	0	1	1	2	2	2	4
10	*aspera4*	0.00000	—	1	1	2	2	2	4
11	*aspera5*	0.05882	0.05882	—	0	3	3	1	5
12	*aspera6*	0.05466	0.05466	0.00372	—	3	3	1	5
13	*bibroni1*	0.12334	0.12334	0.16770	0.16263	—	0	2	4
14	*bibroni2*	0.12334	0.12334	0.16770	0.16263	0.00000	—	2	4
15	*bibroni3*	0.12267	0.12267	0.16153	0.15655	0.06303	0.06303	—	4
16	*carinata1*	0.17278	0.17278	0.15857	0.15356	0.14341	0.14341	0.14739	—
17	*carinata2*	0.17280	0.17280	0.15859	0.15358	0.14341	0.14341	0.14740	0.00747
18	*carinata3*	0.17355	0.17355	0.16449	0.16983	0.12935	0.12935	0.14304	0.05897
19	*carinata4*	0.13264	0.13264	0.13260	0.13745	0.15592	0.15592	0.17012	0.12965
20	*carinata5*	0.17844	0.17844	0.16787	0.17316	0.18357	0.18357	0.19308	0.10652
21	*carinata6*	0.16803	0.16803	0.17843	0.17325	0.18368	0.18368	0.18244	0.10655

based on internal and external morphology (McDowell, 1979; Kluge, 1991). Indeed McDowell (1979:11) states that ''the characters separating the species of *Candoia* are perhaps more clear-cut than those separating the genera of Pythoninae.'' The sister taxon relationship between *C. aspera* and *C. carinata* is well supported based on this molecular dataset and morphology (Kluge, 1991).

Kluge's (1991) work is the most recent attempt to resolve the relationships within boids. Kluge's (1991) morphological analysis supports

TABLE 1. CONTINUED.

		17	18	19	20	21
1	*Python1*	11	9	9	10	10
2	*Python2*	11	9	9	10	10
3	*Sanzinia*	8	6	8	9	9
4	*Epicrates*	10	9	11	12	12
5	*Corallus*	12	10	10	9	9
6	*Boa*	10	9	9	10	10
7	*aspera1*	3	3	2	0	0
8	*aspera2*	3	3	2	0	0
9	*aspera3*	3	3	2	0	0
10	*aspera4*	3	3	2	0	0
11	*aspera5*	4	4	3	1	1
12	*aspera6*	4	4	3	1	1
13	*bibroni1*	3	1	2	2	2
14	*bibroni2*	3	1	2	2	2
15	*bibroni3*	3	3	2	2	2
16	*carinata1*	1	3	4	4	4
17	*carinata2*	—	2	3	3	3
18	*carinata3*	0.05066	—	3	3	3
19	*carinata4*	0.12011	0.12436	—	2	2
20	*carinata5*	0.09741	0.09703	0.11021	—	0
21	*carinata6*	0.09743	0.10613	0.11952	0.00747	—

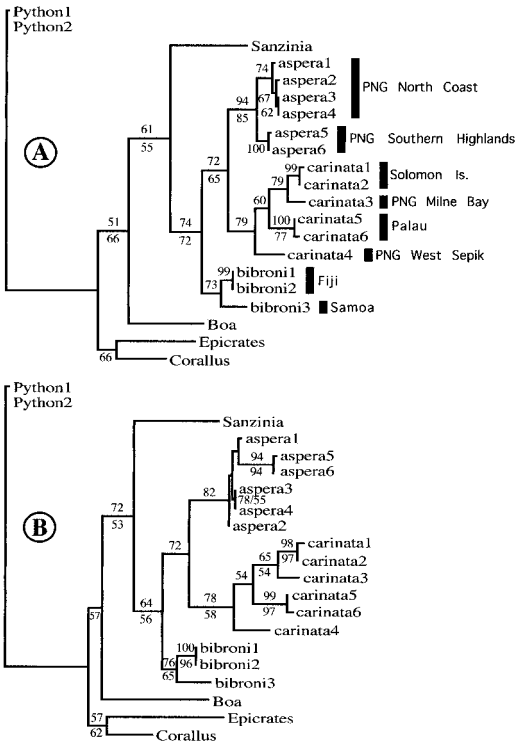

Fig. 2. Phylogram of the maximum parsimony tree (A) and maximum likelihood tree (B) obtained from PAUP* searches using the *Python reticulatus* sequences as the outgroups. Numbers at nodes represent bootstrap proportions for 5000 and 100 pseudoreplicates for parsimony and likelihood analyses, respectively. Bootstrap proportions above the lines correspond to the TI/TV weighted analysis and numbers below the lines correspond to the bootstrap proportions to the transversion only analysis. Bootstrap proportions less than 50% are not shown. The maximum likelihood tree (B) differs from the single most parsimonious tree only in the arrangement of *Candoia aspera* populations. For both analyses *Sanzinia* is the sister taxon to *Candoia*.

the relationships (*Candoia* (*Corallus* ((*Epicrates, Eunectes*) (*Boa* (*Sanzinia, Acrantophis*))))). He argues for a relictual distribution for *Candoia* as a result of its basal position. He also synonymizes the Madagascan boids *Sanzinia* and *Acrantophis* with *Boa* from the Americas. The results from this study dispute both the basal placement of *Candoia* and the synonymization of the Madagascan boids with *Boa*. Phylogenetic analyses do not suggest a close relationship between *Boa* and *Sanzinia* to the exclusion of *Candoia*. Rather *Sanzinia* and *Candoia* consistently group together, and *Boa* only marginally groups with the *Sanzinia/Candoia* clade (Fig. 2). The Kishino-Hasegawa and winning-sites tests both demonstrated that the morphology tree (Kluge, 1991) was significantly different given the molecular dataset. The Templeton, however, was not significant ($P = 0.0582$). Clearly more data are necessary to fully resolve relationships among the boid genera.

Intraspecific divergence.—The limited samples in this study suggest that substantial evolutionary partitions exist within *Candoia* species. The largest levels of intraspecific sequence divergence within *C. carinata* (up to 13%; Table 1), are similar to species level divergence recorded from lizards (Hedges et al., 1991; Thorpe et al., 1994; González et al., 1996) and snakes (Henderson and Hedges, 1995). Indeed McDowell (1979) discusses morphological differences between ''long-tailed'' and ''short-tailed'' forms of *C. carinata*. Although the samples from this study do not directly test McDowell's hypothesis of two species of *C. carinata*, these data are compatible with species level divergence (González et al., 1996). The main east-west running cordillera dividing New Guinea appears to be a geographic barrier to *C. carinata* populations. The Milne Bay Province (southeastern New Guinea) *C. carinata* (n = 1) shows 12% sequence divergence from samples from northern New Guinea (n = 1). Further, the Milne Bay Province sample groups with the *C. carinata* from the Solomon Islands (n = 2; MP and ML bootstrap proportions of 79 and 65, respectively). This suggests that the colonization of the Solomon Islands was from *C. carinata* populations from southern New Guinea via the D'Entrecasteaux islands of the Louisidae archipelago rather than from northern populations via the Bismarck archipelago. The population of *C. carinata* from Palau (n = 2) also shows a relatively large degree of sequence divergence (12%) from the New Guinea and Solomon Islands populations sampled.

The monophyly of the *C. aspera* samples from south of the New Guinean cordillera (n = 2) is supported based on bootstrap proportions from both maximum parsimony (100) and maximum likelihood analyses (94). Based on the maximum parsimony results, *C. aspera* also shows reciprocal monophyly between populations north and south of the main cordillera of New Guinea (n = 4 and 2, respectively). The mean sequence divergence, however, between these small samples is only 5%, which roughly corresponds to subspecies level divergence (Thorpe et al., 1994; Henderson and Hedges, 1995; González et al., 1996).

McDowell (1979:21) recognized two distinct forms of the postorbital bone in *C. bibroni* with one form primarily from the east (Loyalty Is-

lands, Fiji, and Samoa) and another from the western part of the range (Vanuatu, and the south-eastern Solomon Islands). Both samples of *C. bibroni* in this study (n = 3) come from the eastern portion of the range (Fiji and Samoa) and thus presumably represent only one form of McDowell's postorbital bone division. Nevertheless, these samples show over 6% sequence divergence. Sampling populations from the western portion of the range in the future may show levels of divergence indicative of specific differentiation within *C. bibroni*.

Biogeography.—Distinguishing between ancient vicariance and ancient dispersal can be problematical. If the distribution of boids is strictly a result of the breakup of Gondwana, then one might expect a sister relationship, but with a large degree of divergence, between the American boids and the Australia-Papuan boids because South America and Australia-Papua shared a more recent land connection than either with Africa (Smith et al., 1994). If boids originated before the breakup of Gondwana, they may have been much more widespread at one point in time, and the small distributions in Madagascar and Papua may, therefore, represent a relictual distribution (vicariance). Reciprocal monophyly of the Old World and New World boids would support a relictual hypothesis for boids in Madagascar and Papua. However, ancient dispersal from the Americas by a basal lineage, or subsequent extinction of the boid basal lineage in the Americas, could also explain such a distribution. At present, the poor bootstrap support grouping *Boa* with *Sanzinia* plus *Candoia* provides insufficient information concerning potential reciprocal monophyly.

The breakup of Gondwana, however, began around 150 million years ago, and it is unlikely that boids were differentiated by then. If the boid radiation, therefore, occurred sometime after the breakup of Gondwana, then some level of dispersal must account for the present distribution of the group. If both dispersal and vicariance are partly responsible for current patterns, then teasing them apart is even more problematical. Cracraft (1975) considered the sister relationship between Boinae and Pythoninae to be evidence of a late Cretaceous vicariant connection. Underwood and Stimson (1990) also concluded that the distribution of boids was Gondwanan in origin. Kluge (1991) who synonomized the Madagascan boids with *Boa* states that ''boines have had a long and continuous presence in the New World, at least since the divergence of *Corallus* and (*Boa* (*Epicrates, Eunectes*)).'' Kluge (1991), however, suggests that *Candoia*'s enigmatic distribution in the Papuan region was the result of vicariance. Therefore, vicariance appears to be the primary force used to explain boine distribution patterns, as opposed to ancient dispersal.

The Old World versus New World disjunct distribution is also present in other taxa: Iguaniod (Iguanines and Opulurines) lizards, pelomedusid turtles, and chelid turtles. Iguanians (Opulurines) occur in Madagascar but their relationship with other iguaniods groups is unclear (Frost and Etheridge, 1989; Macey et al., 1997; Petren and Case, 1997). The pelomedusid turtle genus *Podocnemis* has a disjunct distribution between Madagascar and South America and chelid turtles show a disjunct distribution between Australia and South America (Underwood, 1976; Kluge, 1991; Legler and Georges, 1993). A relictual hypothesis for the Gondwanan distribution of these taxa, and in particular a close area relationship between Madagascar and South America, has been used to explain the similarity of these biogeographic distributions (Cracraft, 1975).

Other authors have suggested recent dispersal from North America as an explanation for the distribution of *Candoia* and cite a similar distribution for the iguanian *Brachylophus* in Fiji and Tonga. *Brachylophus* is an iguanine iguanian, with its closest relatives apparently being from the Americas (Sites et al., 1996; Petren and Case, 1997). The presence of *Brachylophus* and similar relationships between other Pacific island animal and plant groups with South America suggest that such trans-Pacific dispersal is possible (Croizat, 1958; Cogger, 1974; Gibbons, 1981). *Candoia*, however, does not exactly share the distributional pattern of *Brachylophus*. *Brachylophus* is (currently) restricted to the Pacific, whereas *Candoia* has a distinct Melanesian (Papuan) distribution with only one species (*C. bibroni*) extending into the central Pacific region (McDowell, 1979; Gibbons, 1985). In fact *Candoia* shares a distributional pattern virtually identical with many other Melanesian taxa such as skinks (Adler et al., 1995; Austin, 1999; Zug, 1991), geckos (Fisher, 1997; Case et al., 1994), frogs (Burt and Burt, 1932), and other herpetofauna (De Rooij, 1915; Greer, 1974; Brown, 1991). The basal placement of *C. bibroni*, however, is somewhat in conflict with this idea. The distribution of *C. bibroni* (the only species of *Candoia* not found in sympatry with pythons) may have been pushed eastward because of competition with pythons. Alternatively, it could support ancient invasion into the Pacific with more recent spread of derived species to Papua.

Phylogenetic data with fossil data to calibrate

branching points combined with tectonic data offer some of the most convincing evidence for biogeographic scenarios. Fossils are the links that tie phylogenetic relationships with specific timing of plate movements. Unfortunately there are no fossil remains in either Africa or Australia that can be definitively identified as boids. Until adequate fossils are discovered, or new methodologies developed to distinguish vertebrae, the precise dating of branching points is not possible.

A great deal more molecular work combined with fossil and morphological work will be necessary to get an estimate of time of divergence among the major lineages. The molecular results do, however, provide a very rough clock that does allow us to say with relative confidence that the *Candoia-Sanzinia* clade is not the result of a recent dispersal event. Rather, these molecular data suggest a divergence of greater than 40 million years between the Old World and New World boids (Thorpe et al., 1994; Johns and Avise, 1998). Tissues from the boid genus *Acranthophis* from Madagascar were not available, and thus at present, it is unclear whether the Madagascan boids are monophyletic.

Congruent biogeographic patterns between boines and other taxa do not necessarily mean that the same historical/geologic event is the causal agent. The hypothesis of recent waif overwater dispersal from the Americas as an explanation for the distribution of *Candoia* is discounted based on phylogenetic and quantitative measures of genetic distance. In fact, the genetic distance data suggest that the time of divergence between *Candoia* and the New World boids is quite large ($>$ 40 million years). Unfortunately, the lack of understanding of higher level boinae and pythoninae relationships combined with the paucity of fossil data as well as the polemical issue of not being able to distinguish between boinae and pythoninae fossil vertebrae (the vast majority of fossil snakes are known primarily from vertebrae) has proved to hinder the understanding of time of origins of the major clades (Kluge, 1991). Thus although Underwood and Stimpson (1990) viewed the African-Asian-American pythoninae radiation/distribution as being heavily influenced by Laurasian vicariance, whereas the African-Papuan-American boinae radiation/distribution as having its origins in the Gondwanan supercontinent, neither the fossil record, nor robust estimates of phylogeny calibrated by fossil dates, support these hypotheses.

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Appendix 1.

Species, museum identification numbers, and localities for specimens used in this study are listed below. Numbers in parentheses following species names correspond to individual DNA sequences in Appendix 2. Data for *Epicrates striatus* and *Corallus canninus* were taken from Henderson and Hedges (1995). All institutional acronyms follow Leviton et al. (1985).

Python reticulatus (1,2) D687 and D688 (SAMA Australian Biological Tissue Collection), Sumatra. *Boa constrictor* L425 (SAMA Australian Biological Tissue Collection), unknown locality. *Epicrates striatus* SBH No. 103120, Dominican Republic. *Corallus caninus* Audubon Zoo 5902, unknown locality. *Sanzinia madagascariensis* USNM 495770, Madagascar. *Candoia aspera* (1,2) AMS R124100-1, Madang Province, Papua New Guinea. *Candoia aspera* (3,4) AMS R135507-8, West Sepik Province, Papua New Guinea. *Candoia aspera* (5,6) AMS R122352-3, Southern Highlands Province, Papua New Guinea. *Candoia bibroni* (1,2) AMS R135212-3, Kadavu Island, Fiji. *Candoia bibroni* (3) USNM 322766, Savai'i island, Samoa. *Candoia carinata* (1,2) AMS R136298 and 136370, Choiseul Island, Solomon Islands. *Candoia carinata* (3) AMS R129740, Milne Bay Province, Papua New Guinea. *Candoia carinata* (4) AMS R135578, West Sepik Province, Papua New Guinea. *Candoia carinata* (5,6) SAMA R48035 and USNM 220415, Palau.

APPENDIX 2. TWO HUNDRED AND SEVENTY BASE-PAIR SEQUENCE FOR 21 TAXA FOR THE CYTOCHROME *b* GENE ALIGNED WITH THE OUTGROUP *Python reticulatus*. Sequence data for *Epicrates* and *Corallus* was taken from Henderson and Hedges (1995). Dots indicate a match with the first taxon (*Python1*; see Appendix 1). The first base corresponds to the first position of a codon.

```
Python1     TTCGGCTCAATATTACTAACATGTCTAGCCTTACAAGTACTAACCGGCTTTTTCCTAGCCGTCCATTACACAGCAAACATTAACCTAGCATTTTCATCCATTATCCACATCACCCGAGACGTCCCATACGGCTGAATAATACAAAACCTT
Python2     ..............................................................................................C.............................................
Sanzinia    ..............T.......CTC....C.....A.TA....A..T..C........A..A..C.....................G.....C..C......G.......A..A............C.T..T.................A
Epicrates   .....A..C...C....TG.T..CT..T.TC.....C....T..A..A..C...T....T..A..C...........T..............C.....T..C........T........T........T..A..................
Corallus    .....A..T...C.T..C..C...TC....C.............A.....C..T........A.................CG..T.......C......G.CG....T..T.T......T.....C.....A..................
Boa         .....G......C....G.....CTCCATAA.T...........A..G..C...T.......A.................C........C..C.........CG.A..T.....A.....T..A....GT..G...........G.....C
aspera1     .....A..............C...TC...A.........A....A..G..C...T..T.AA.A..............T...G..........C.....T........T..T..A............C.T..A.................A
aspera2     .....A..............C...TC...A.........A....A..A..C...T..T.AA.A..............T...G..........C.....T........T..T..A............C.T..A.................A
aspera3     .....A..............C...TC...A.........A....A..A..C...T..T.AA.A..............T...G..........C.....T........T..T..A........T...C.T..A.................A
aspera4     .....A..............C...TC...A.........A....A..A..C...T..T.AA.A..............T...G..........C.....T........T..T..A........T...C.T..A.................A
aspera5     .....A......C.......C...TC...A.........A....A..A..C...T..T.AA.A..............T...G....G.....C.....T...........T..A............C.T..A..............T..A
aspera6     .....A......C.......C...TC...A.........A....A..A..C...T..T.AA.A..............T...G....G.....C.....T...........T..A............C.T..A..............T..A
bibroni1    .....A..............C...TC...AC........A....A..A..C......T.A..A..C........G.....C....................C.....T..T..A........T...C....A.....G.............
bibroni2    .....A..............C...TC...AC........A....A..A..C......T.A..A..C........G.....C....................C.....T..T..A........T...C....A.....G.............
bibroni3    .....A..............C...TC...AC........A....A.............T.AA.A..C..T...........C....................C.....T..T..A........G...C....A...................
carinata1   ..T..G......C.......C...TC.A.A.........AC......G......T..T.AA.A.................C..T..G.....C........C...........A........T...C...................T..G
carinata2   ..T..G......C.......C...TC.A.A.........A.......G......T..T.AA.A.................C..T..G.....C........C...........A........T...C...................T..G
carinata3   ..T..A......C.......T...TC...A.........A....A..G.........T.A..A..C..............C..T..G.....C........C...........A........T...C...................T..G
carinata4   ..T..A......C.G..G..C...TC...A.........A.......A......T..T.AA.A..C........G..T..C...........C.................T..A...........CC.....................G
carinata5   ..T..G..G...C.GT....C..CTC...A.........A.......G......T..T.AA.G..C..............CG....G.....C....................A.....T......C.....................G
carinata6   ..T..G..G...C.GT....C..CTC...A.........A.......G......T..T.AA.G..C..............CG....G.....C....................A.....T......C.....................G
```

APPENDIX 2. CONTINUED.

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CACGCTATCGGAGCATCCATATTCTTCATCTGCATCTACATCCACATCGCACGAGGCCTATACTACGGATCATACCTCAACAAAGAAACCTGAATATCAGGCATCACCCTACTCATCACA
.....
..T..A........C..AG.G.....T..T..T........T.....G.....C.................C..T..A...........A...C.......A.CA.....C..A....T.
........T..G..C..AG....A..T..T..T..T..T.....T............T.G..T..T..G..G...T.A..............C.C.....T.C...AT....A....T.
.....A........C..A..............T...........T........C...........T..G.....T..A..............C.........C.T.TT....A....T.
.....C..A.....C..A.............................T.....C..A..T.....T.....C.....T..............GT.G.....G.C...AT....A....T.
.....C...........T.....T..T.....T.......CA.....T........AT..........C......T.A........G..A...C.........C...A.....A....T.
.....C...........T.....T..T.....T.......CA.....T........A...........C..G.....A........G..A...C.........C...A.....A....T.
.....C...........T.....T..T.....T.......CA.....T........A...........C..G...T.A........G..A...C.........C...A.....A....T.
.....C...........T.....T..T.....T.......CA.....T........A...........C..G...T.A........G..A...C.........C...A.....A....T.
.....C...........T..G..T........T.......CA.....T........GT....T.....C......T.A......A....A...C....T....CG..A.....A....T.
.....C...........T..G..T........T.......CA.....T........AT....T.....C......T.A......A....A...C....T....CG..A.....A....T.
.....C........C...........T..T..T.......CA...........C..AT.............C.....A.....G.....A...C.......A.CA..A..T..A..T.T.
.....C........C...........T..T..T.......CA...........C..AT.............C.....A.....G.....A...C.......A.CA..A..T..A..T.T.
.....C..T.....C...........T.....T.......CA.....T.....C..A...........G..T..T..A......A....A...C.......A.CA..A..T..A..T.T.
.............C...G.G..T..T........T....CA...........C..AT.............C..T..A...........A...C....T....CA..A..G..A..T.T.
.............C...G.G..T..T........T....CA...........C..AT.............C..T..A..T........A...C....T....CA..A..G..A..T.T.
..T...........C...G....T..T........T....CA...........C..GT.............C..T..A..T........A...C....C....CA..A.....A....T.
.....A..T..G..C.....G..T..T.....T..T.....A.............GT.............C...T.A..T.....G..A...C....T....C...A.....A....T.
.............C........T..T........T....CA..T...........GT.......T.....C.....A..T............C....G....CA..A.....A....T.
.............C........T..T........T....CA..T...........A........T.....C.....A..T............C....G....CA..A.....A....T.
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