Biological Journal of the Linnean Society (2001), *72:* 203–229. With 14 figures
doi: 10.1006/bijl.2000.0487, available online at http://www.idealibrary.com on IDEAL®

Phylogenetic systematics of Southeast Asian flying lizards (Iguania: Agamidae: *Draco*) as inferred from mitochondrial DNA sequence data

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Received 12 December 1999; accepted for publication 15 August 2000

Phylogenetic analysis of mitochondrial DNA sequence data using maximum parsimony, minimum evolution (of log-determinant distances), and maximum-likelihood optimality criteria provided a robust estimate of *Draco* phylogenetic relationships. Although the analyses based on alternative optimality criteria were not entirely congruent, non-parametric bootstrap analyses identified many well-supported clades that were common to the analyses under the three altrenative criteria. Relationships within the major clades are generally well resolved and strongly supported, although this is not the case for the Philippine *volans* subclade. The hypothesis that a clade composed primarily of Philippine species represents a rapid radiation could not be rejected. A revised taxonomy for *Draco* is provided.

ADDITIONAL KEYWORDS: DNA sequences – likelihood-ratio test – LogDet – maximum likelihood – maximum parsimony – rapid radiation.

INTRODUCTION

The flying lizards (genus *Draco*) of Southeast Asia are well-known for their ability to glide large distances, using wing-like patagial membranes supported by elongate thoracic ribs to generate lift forces (Herre, 1958; Klingel, 1965; Colbert, 1967). Nevertheless, it is generally unappreciated that these lizards represent a remarkable radiation composed of 40 or more species, with at least six, and possibly as many as eight, species found in sympatry on the Sunda Shelf (Inger, 1983; Musters, 1983; McGuire, pers. observ.). Consequently, flying lizards have much potential as a model system for the study of diverse evolutionary phenomena including such topics as the evolution of gliding performance, the evolution of display structures and behaviour, the evolution of niche partitioning and community assembly, and the evolution of sexual size dimorphism and dichromatism (McGuire, 1998). Such studies will be undertaken most fruitfully in the context of a robust estimate of phylogenetic relationships. The goal of the present study is to provide a comprehensive species-level phylogenetic estimate for *Draco* that will serve as a framework for future comparative investigations.

In order to generate a meaningful species-level phylogenetic estimate for any taxon, it is necessary to have a reasonable understanding of the species diversity within that group. Although the alpha taxonomy of *Draco* has received several recent revisions (Inger, 1983; Musters, 1983; Ross & Lazell, 1991; McGuire & Alcala, 2000) and additions (Lazell, 1987,

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0024–4066/01/020203+27 $35.00/0

1992; McGuire & Alcala, 2000), there remain several instances in which we disagree with the present taxonomy. Therefore, several taxonomic modifications are proposed herein, although a number of additional taxonomic changes that are beyond the scope of this paper (McGuire, unpublished data) are also discussed.

MATERIAL AND METHODS

TAXONOMIC SAMPLING

Mitochondrial DNA sequence data were obtained for 53 *Draco* species and/or populations and four outgroup taxa (Appendix 1). Taxonomic representation includes all currently recognized species of *Draco* except *D. dussumieri*, *D. jareckii*, and the questionable species *D. affinis*. We also lack sequence data for a currently recognized subspecies (*D. lineatus modiglianii*) that we here elevate to species status. As alluded to above, the *Draco* taxonomy followed here differs in many respects from the taxonomies presented in the most recent systematic treatments of the entire genus (Inger, 1983; Musters, 1983). Much of this disagreement is associated with Philippine taxa; therefore, the taxonomy provided for the Philippine assemblage by McGuire & Alcala (2000) will be followed here. However, there remain several additional cases of taxonomic disagreement, including our recognition of the following species: *D. boschmai*, *D. indochinensis*, *D. formosus*, *D. beccarii*, *D. bourouniensis*, *D. modiglianii*, *D. rhytisma*, *D. spilonotus*, *D. sumatranus*, and *D. timoriensis*. Several undescribed species are also discussed in the present paper. These are denoted by locality names in quotation marks such as *D.* 'Luwuk' and *D.* 'Tagulandang'. Justification for the recognition of these species is provided below. A taxonomy for the genus *Draco* is provided in Table 1.

TAXONOMIC RECOMMENDATIONS

The following taxonomic recommendations are proposed in the context of the general lineage concept of species (de Queiroz, 1998, 1999) and represent a logical extension of the lineage-based taxonomy recently recommended for the Philippine *Draco* assemblage by McGuire and Alcala (2000). Although we are not opposed to the recognition of nondiagnosable lineages as distinct species (in the true spirit of lineage-based species concepts), the taxonomic recommendations that we propose below should not be controversial. In this section, we elevate to species status several diagnosable and allopatrically distributed taxa that were either described as species and later reduced to subspecies or originally described as subspecies. For each taxon that we elevate, Musters (1983) published morphological descriptions, synonymies, and detailed distribution maps, as well as data indicating diagnosability and allopatry. Where necessary, additional character data are presented herein as evidence of diagnosability. All character state differences listed below were verified by the senior author in alcohol-preserved and osteological specimens (see Appendix 2 for specimens examined).

Inger (1983) considered *Draco indochinensis* to be a synonym of *D. blanfordii*, whereas Musters (1983) recognized it as a subspecies of *D. blanfordii*. We treat *D. indochinensis* as a distinct species because it is both allopatrically distributed and clearly diagnosable from *D. blanfordii*. The most compelling character state differences are associated with the dewlap of males. The dewlap of *D. indochinensis* is widest at its base, decreases in width over its entire length, and terminates in a sharp point. In contrast, the dewlap of *D. blanfordii* is distally expanded with a basal constriction, and terminates in a rounded distal edge. The latter type of dewlap is characteristic not only of *D. blanfordii*, but also of *D. formosus*, *D. obscurus*, and *D. taeniopterus* and suggests that *D. blanfordii* and *D. indochinensis* may not even be sister taxa, let alone conspecifics. *Draco indochinensis* also differs from *D. blanfordii* in the presence (in both sexes) of a thick, black transverse band that extends across the posterior gular region from one throat lappet to the other, and in the presence of dark radial bands on the dorsal surfaces of the patagia in both sexes rather than in females only.

Inger (1983) considered *Draco formosus* to be a synonym of *D. obscurus*, and Musters (1983) recognized *D. formosus* as a subspecies of *D. obscurus*. Because these taxa are diagnosable and allopatrically distributed (*D. formosus* occurs on the Malay peninsula, *D. obscurus* on Borneo and Sumatra), we treat them as distinct species. *Draco formosus* and *D. obscurus* differ in the degree of distal expansion of the dewlap in males (greatly expanded in *D. formosus*, unexpanded or only slightly expanded in *D. obscurus*), in maximum body size (*D. formosus* reaches 114 mm SVL [$n=62$], whereas *D. obscurus* reaches only 100 mm SVL [$n=25$]), and in several colour pattern features (*D. obscurus* males lack the dark radial bands on the dorsal patagium that are present in *D. formosus* males, and have a peach-coloured eye ring that is lacking in *D. formosus*). We have not examined specimens of *D. obscurus* from Sumatra, and our recognition of Sumatran populations as *D. obscurus* follows Musters (1983).

Draco beccarii, *D. bourouniensis*, and *D. spilonotus* were considered to be synonyms of *D. lineatus* by Inger (1983), whereas Musters (1983) recognized each as subspecies of *D. lineatus*. Musters (1983) also described an additional subspecies, *D. l. rhytisma*. We recognize each (including *D. l. rhytisma*) as full species on the

Table 1. Listing of *Draco* species recognized in the present study. 'Authority' refers to one or both of the two most recent monographic revisions of the genus (Inger, 1983; Musters, 1983) when the taxonomic status of the species has remained static, or to a more recent publication recommending that the taxon be recognized as a distinct species. No decisions regarding taxonomic status are offered for the following taxa: *D. affinis*, *D. blanfordii norvilli*, *D. lineatus lineatus*, *D. lineatus ochropterus*, *D. maculatus divergens*, *D. m. haasei*, *D. m. whiteheadi*, and *D. obscurus laetipictus* (see text)

Species	Authority
1. *Draco beccarii*	This study
2. *Draco biaro*	Lazell, 1987
3. *Draco bimaculatus*	Inger, 1983
4. *Draco blanfordii*	Inger, 1983; Musters, 1983
5. *Draco boschmai*	This study
6. *Draco bourouniensis*	This study
7. *Draco caerhulians*	Lazell, 1992
8. *Draco cornutus*	Honda *et al.*, 1999a
9. *Draco cristatellus*	Inger, 1983
10. *Draco cyanopterus*	McGuire & Alcala, 2000
11. *Draco dussumieri*	Inger, 1983; Musters, 1983
12. *Draco fimbriatus*	Inger, 1983; Musters, 1983
13. *Draco formosus*	This study
14. *Draco guentheri*	McGuire & Alcala, 2000
15. *Draco haematopogon*	Inger, 1983; Musters, 1983
16. *Draco indochinensis*	This study
17. *Draco jareckii*	Lazell, 1992
18. *Draco maculatus*	Inger, 1983; Musters, 1983
19. *Draco maximus*	Inger, 1983; Musters, 1983
20. *Draco melanopogon*	Inger, 1983; Musters, 1983
21. *Draco mindanensis*	Inger, 1983; Musters, 1983
22. *Draco modiglianii*	This study
23. *Draco obscurus*	This study
24. *Draco ornatus*	Ross & Lazell, 1991
25. *Draco palawanensis*	McGuire & Alcala, 2000
26. *Draco quadrasi*	McGuire & Alcala, 2000
27. *Draco quinquefasciatus*	Inger, 1983; Musters, 1983
28. *Draco reticulatus*	McGuire & Alcala, 2000
29. *Draco rhytisma*	This study
30. *Draco spilonotus*	This study
31. *Draco spilopterus*	Musters, 1983; McGuire & Alcala, 2000
32. *Draco sumatranus*	This study
33. *Draco taeniopterus*	Inger, 1983; Musters, 1983
34. *Draco timoriensis*	This study
35. *Draco volans*	This study
36. *Draco* sp. 'Luwuk'	This study
37. *Draco* sp. 'Tagulandang'	This study
38. *Draco* sp. 'Camiguin Norte'	Lazell, 1989; McGuire & Alcala, 2000

basis of their allopatric distributions and because they are clearly diagnosable on the basis of external morphology (see Musters, 1983). Although it is unrepresented in this analysis, we recognize *D. modiglianii* on the same basis (see Musters, 1983). 'Tagulandang' represents an undescribed species (McGuire, unpublished data) from Tagulandang island in the Sangir–Talaud island group that appears closely related to the other Sangir–Talaud endemics, *D. biaro* and *D. caerulhians* (Lazell, 1987, 1992).

Draco sumatranus, *D. boschmai*, and *D. timoriensis* were considered to be synonyms of *D. volans* by Inger (1983), whereas each was recognized as a subspecies of *D. volans* by Musters (1983). We recognize *D. sumatranus* and *D. volans*, which occur on the Sunda Shelf, as distinct species because they are allopatrically

distributed and diagnosable (see below). We also recognize *D. boschmai* and *D. timoriensis* of the Lesser Sunda Islands as species, as they are clearly distinct from one another as well as from *D. volans* and *D. sumatranus*. However, we emphasize that *D. boschmai* and *D. timoriensis* are each composed of several diagnosable, allopatric lineages and further taxonomic modification of this group will be necessary (McGuire, unpublished data).

Draco sumatranus and *D. volans* have distinct patagial colour patterns that exhibit little geographic variation, whereas *D. boschmai* and *D. timoriensis* exhibit substantial intraspecific variation between populations that occur on islands separated by deep-water channels (which themselves probably represent distinct species). Populations of *D. sumatranus* from the Malay Peninsula, Borneo, and Sumatra share the same colour pattern. The dorsal patagium of both sexes is characterized by large, rounded, white, pale yellow, or pale orange spots over most of its surface, with the base colour of the distal half of the patagium black. *Draco volans* (from both Java and Bali) are sexually dichromatic with respect to the patagial colour pattern. The patagium of males is characterized by a pale tan to pale orange base coloration overlain with several thick, black, concentrically arranged radial bands. Females lack the discrete black radial bands, instead having irregular, black sinuous blotches that are small and relatively diffuse proximally, grading to large and distinct distally.

Draco boschmai and *D. timoriensis* can be distinguished from both *D. sumatranus* and *D. volans* based on the presence of an enlarged series of keeled paravertebral scales and very different colour patterns. Like *D. volans*, *D. timoriensis* is sexually dichromatic. The dorsal patagium of males is bright yellow, overlain with a diffuse series of gray radial bands, the ventral patagium lacking melanic pigments. In *D. timoriensis* females from Timor, Roti, and Semau, the dorsal patagium is black or dark brown with white horizontally oriented striations and the entire ventral patagial surface is saturated with melanic pigments. In *D. timoriensis* females from Alor and Wetar, the ventral patagium either lacks melanic pigments entirely or has a few scattered dark spots. *Draco boschmai* exhibits substantial inter-island variation in the dorsal colour pattern, but none of the populations for which I have examined specimens approach the colour patterns present in *D. timoriensis*, *D. sumatranus*, or *D. volans*. Like *D. volans* and *D. timoriensis*, *D. boschmai* are sexually dichromatic. In some *D. boschmai* populations, both the dorsal and ventral surfaces of the patagia of males are entirely suffused in melanic pigments. Females from these populations have patagia characterized by large pale spots on a dark base and lack melanic pigments on the ventral surface of the patagium. In other populations of *D. boschmai*, neither males nor females have extensive melanic pigments on either the dorsal or ventral surfaces of the patagium. We should emphasize that the colour pattern differences listed here are not intended to be exhaustive and a thorough evaluation of the status of these taxa is beyond the scope of this paper. A taxonomic revision *D. boschmai* and *D. timoriensis* will be published elsewhere.

Musters (1983) described *Draco fimbriatus hennigi* from the island of Java. Musters offered no diagnostic character states distinguishing this subspecies from *D. f. fimbriatus* and we were also unable to find diagnostic differences during our own examination of specimens. Therefore, we treat *D. f. hennigi* as a junior synonym of *D. fimbriatus*.

Musters (1983) recognized several additional taxa for which we make no taxonomic recommendations. These include *Draco affinis*, *D. blanfordii norvillii*, *D. lineatus lineatus*, *D. l. ochropterus*, *D. maculatus divergens*, *D. m. haasei*, *D. m. whiteheadi*, and *D. obscurus laetepictus*. Based on the type description provided by Bartlett (1894), *D. affinis* probably represents a junior synonym of *D. cornutus*. However, we have not examined the type specimen in the Sarawak Museum and therefore do not offer a taxonomic recommendation at this time. Likewise, we have been unable to examine specimens of *Draco blanfordii norvillii* or *D. obscurus laetepictus*. We have examined specimens *D. maculatus divergens*, *D. m. haasei*, and *D. m. whiteheadi*, and it appears likely that each will eventually be synonymized with *D. maculatus*. However, without having seen live specimens representing the three subspecies in question, particularly from contact zones in their geographic distributions, we are unwilling to formally synonymize these taxa. We suggest that a phylogeography study or a fine-scaled morphometric analysis will be required to resolve this problem. Finally, we make no formal taxonomic recommendation regarding *D. l. ochropterus* of the Kai Islands of eastern Maluku Province, Indonesia because the specimens presently available are insufficient to determine their appropriate taxonomic status. Of the four specimens that formed Werner's (1910) original type series (two males and two females), only the two females survived World War II. We have examined the two females and determined that they cannot be distinguished with confidence from *D. bourouniensis*. Furthermore, the senior author visited Kai Kecil island but could not locate any additional specimens, despite searching in forest habitat that appeared excellent for *Draco*. Therefore, we have not ruled out the possibility that *Draco ochropterus* is a synonym of *D. bourouniensis* described on the basis of specimens with incorrect locality data, but only additional field work in the Kai Islands is likely to resolve this issue.

CHOICE OF OUTGROUP TAXA

A recent molecular phylogenetic analysis of agamid relationships (Macey *et al*., 2000) found strong support for a clade composed primarily of Southeast Asian taxa. Their Southeast Asian clade included *Draco*, *Acanthosaura*, *Aphaniotis*, *Bronchocela*, *Calotes*, *Ceratophora*, *Gonocephalus*, and *Japalura*. In their study, a clade including *J. tricarinata* and *J. variegata* was placed as the sister group of *Draco* with strong support (bootstrap proportion of 99, decay value of 14). However, *Japalura* was found to be paraphyletic, with a clade composed of *J. fasciatus*, *J. flaviceps*, and *J. splendida* relatively distantly related to *Draco*. In this analysis, we have relatively broad sampling from within the Southeast Asian clade. Representative outgroup taxa include *J. tricarinata*, *J. splendida*, *A. fusca*, and *B. cristatella*. *Aphaniotis fusca* and *B. cristatella* also were suggested to be closely related to *Draco* in the unpublished dissertation of Moody (1980).

DNA SEQUENCING

DNA was obtained using phenol/chloroform (Maniatis, Frisch & Sambrook, 1982) or Chelex (Walsh, Metzger & Higuchi, 1991) extraction. Amplification of the entire *ND2* protein coding gene, together with portions of three flanking tRNAs, was performed using the polymerase chain reaction (Saiki *et al*., 1988) following the protocol of Palumbi (1996). The external primers employed in this analysis inclue METf.1: 5′-AAGCAGTTGGGCCCATRCC-3′ and ALAr.2m: 5′-AAAGTGTCTGAGTTGCATTCRG-3′ and the internal primers used included ND2f.5: 5′-AACCAAACCCAACTACGAAAAAT-3′ and ND2r.6: 5′-ATTTTTCGTAGTTGGGTTTGRTT-3′ (Macey *et al*., 1997). The external primers amplify a fragment that corresponds to positions 4437–5617b in the human genome (Anderson *et al*., 1981). Single-stranded PCR products were purified using Promega Wizard PCR Prep kits, sequenced using ABI Prism terminator cycle sequencing kits, purified again using Princeton Separations centri-sep spin columns, and visualized on an ABI 377 automated sequencer following standard protocols.

Alignment of the *ND2* sequences was performed by eye, although MacClade 3.04 (Maddison & Maddison, 1992) was used to verify that the sequence remained in frame throughout its length. Gaps in the *ND2* gene were detected in five sequences, two of which are three bases in length and represent autapomorphies for their respective taxa. The remaining three gaps are six bases in length and occur in the same position in the *ND2* gene, suggesting that they represent a single deletion event. The tRNAs were aligned according to secondary structural models (Kumazawa & Nishida, 1993; Macey & Verma, 1997). Those regions that could not be aligned with confidence were excluded from the analysis. The regions excluded due to alignment difficulties represent 20 nucleotide positions, including portions of the D- and T-loops of $tRNA^{Trp}$ and a short spacer region between $tRNA^{Trp}$ and $tRNA^{Ala}$. One species, *Draco mindanensis*, has a $\sim$258 bp insertion between the *ND2* and $tRNA^{Trp}$ genes. Most of the insertion sequence is identical to a segment of the *ND2* sequence.

Because it is well documented in the literature that portions of the mitochondrial genome transpose to the nucleus, it is important to evaluate whether the recovered sequences are in fact authentic, orthologous mitochondrial gene fragments. It is diffficult to verify that sequences are of mitochondrial origin without using purified mitochondrial DNA as the template for PCR amplification. Nevertheless, there are several indicators that might suggest that ones sequences are paralogous (Zhang & Hewitt, 1996): (1) multiple bands appear persistently during PCR amplification, (2) indels have occurred resulting in frameshifts or stop codons, (3) nucleotide base frequencies differ substantially from those of other putatively authentic mitochondrial sequences, or (4) the nucleotide sequences themselves, or the phylogenetic estimate derived from the sequences, differ dramatically from prior expectations. With respect to the first three criteria, all of the sequences presented here appear to satisfy the expectations of authentic, orthologous mitochondrial sequences. However, one taxon (*D. dussumieri*) was not incuded in the present study precisely because its sequence was of dubious mitochondrial origin. Clues suggesting that the sequence might be paralogous included that the gene fragment was difficult to amplify, was not the correct size, and the portion of the sequence corresponding to the *ND2* gene was followed by sequence that could not be matched to anything in the mitochondrial genome. Notably, the recovered sequences did not include stop codons, frameshifts, or an unexpected nucleotide composition. We should emphasize, however, that we are by no means certain at this time that the sequence we obtained for *D. dussumieri* is a nuclear insertion. Finally, with respect to the fourth criterion, the readers will have to determine for themselves whether the recovered phylogenetic estimate differs sufficiently from prior expectations to suggest that we have sequenced paralogous versus orthologous gene fragments.

DATA ANALYSES

Phylogenetic analyses were performed using PAUP* 4.0b2 (Swofford, 1999). Parsimony analyses employed the heuristic search option with tree bisection-reconnection (TBR) branch swapping, MULPARS, and random addition of taxa (100 replicates). Parsimony analyses were performed under a variety of character

weighting protocols to assess the effect that differential character weighting has on the phylogenetic estimate. In addition to applying equal weight to all nucleotide substitutions, we also estimated the transition-transversion bias using maximum-likelihood (under the HKY + Γ + I model) and reweighted transversions proportionally using step matrices. A bias of five transversions per transition was estimated from the data, and transversions were therefore weighted five times greater than transitions in these analyses. The frequency of nucleotide substitutions at each codon position were also estimated from the data and used as a basis for differential weighting. Finally, analyses were conducted in which differential weighting was based on both transition/transversion bias and codon position bias. Single-site gaps in the coding portion of the sequence (within the *ND2* gene) were treated as a fifth base in all analyses and were weighted equivalently with transitions. Phylogenetic signal for each treatment of the data set was evaluated using the g_1 statistic (Fitch, 1979, 1984; Hillis, 1991; Huelsenbeck, 1991; Hillis & Huelsenbeck, 1992), which measures the skewness of the distribution of random trees (10 000 random trees were used for each analysis). Tree support was assessed using the nonparametric bootstrap (Felsenstein, 1985; 1000 replicates). Bootstrap analyses utilized random addition of taxa, but with only one addition-sequence replicate per bootstrap replicate. For the analyses that employed differential character weighting, only the 50% majority-rule bootstrap consensus trees are presented, although these trees differ in each case from the strict consensus in the resolution of one or a few nodes that receive weak support in the bootstrap analysis. This is done primarily for space considerations, but also because, where they differ, we have more confidence in nodes recovered on the 50% majority-rule consensus tree than those recovered on the strict consensus tree. Nevertheless, because there is a community of systematists that is only interested in the most parsimonious tree under equal character weighting, we do provide both the strict consensus and bootstrap consensus trees for the analysis under this weighting scheme.

The sequence data also were analyzed under a maximum-likelihood optimality criterion. The protocol of Huelsenbeck & Crandall (1997) was followed such that less parameter-rich models of sequence evolution were employed initially and more complex models were applied thereafter, unless a likelihood-ratio test could not detect a significant increase in the likelihood scores of the phylogenetic estimates. However, it is possible that adding particular parameters will have a greater effect on some substitution models than on others (for example, adding a parameter describing the proportion of sites assumed to be invariant may significantly improve the likelihood score under the F81 model but may not significantly improve the likelihood score under the HKY model). Therefore, although we followed the Huelsenbeck & Crandall (1997) protocol to its logical conclusion, we then verified that the optimal model provided a significantly better fit to the data than did all less parameterized models that could be evaluated with PAUP*. The likelihood-ratio tests were evaluated using the χ^2 distribution (Goldman, 1993; Yang, Goldman & Friday, 1995; Yang, 1996). Whelan & Goldman (1999) tested the assumption that the true distribution of likelihood-ratio test statistics can be approximated by the χ^2 distribution for five model parameters including κ (transition/transversion rate ratio), α (a parameter describing among-site rate heterogeneity), and the three parameters (π) required to allow base frequencies to vary. They found that the χ^2 distribution was appropriate for the κ and π parameters, but deviated significantly from the true distribution for parameter α. Nevertheless, Whelan & Goldman (1999) argued that the deviation from the χ^2 distribution will have a limited affect when the differences in the likelihood scores are large, which was the case in all of the comparsions made in this analysis.

Maximum-likelihood is computationally intensive and, following standard procedures, can require extensive CPU time to complete analyses of 57 taxa using all but the simplest models of evolution. To reduce the amount of time required to complete the likelihood analyses, we used a successive approximations approach (terminology of Voelker & Edwards, 1998) as follows. First, a starting tree was obtained by performing a weighted parsimony analysis (with transversions weighted five times greater than transitions). Maximum likelihood model parameters were then optimized on this parsimony tree. By fixing these model parameters and swapping off of this starting tree, we obtained an optimal topology for this particular set of model parameters. Once an optimal topology was recovered, the process was undertaken again by reoptimizing model parameters on the new likelihood tree. This procedure was repeated until PAUP* could no longer find an alternative tree with a higher likelihood score (in other words, PAUP* did not find improved estimates when reoptimizing model parameters on the tree recovered during the swapping phase of the procedure). This approach substantially reduces computation time because PAUP is never required to simultaneously optimize model parameters and tree topologies.

A nonparametric bootstrap analysis was conducted under the maximum likelihood criterion. Because of the extreme computational intensiveness of this analysis, two compromises were required. First, the optimized model parameters under the GTR + Γ + I model were fixed for the entire bootstrap analysis, rather than allowing parameters to be reoptimized for each

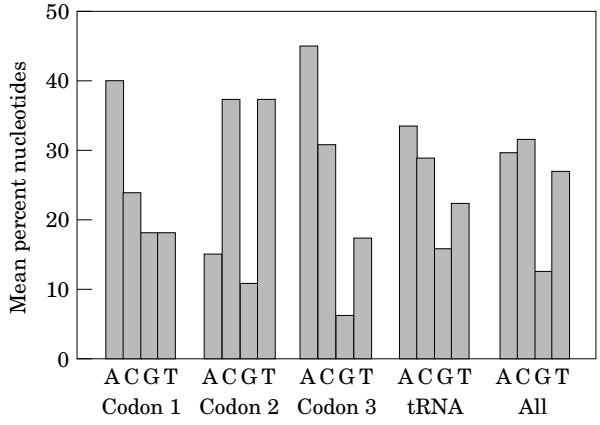

Figure 1. Base frequencies for the three codon positions of the *ND2* gene as well as for the tRNA sites. Bias C of Irwin (1991) is reported for each data partition. Codon 1 = 0.200, 2 = 0.325, 3 = 0.351, tRNA = 0.163, All = 0.167.

resampled data set. Second, only 100 replicates were possible, rather than the 1000 that were performed under the parsimony optimality criterion.

RESULTS

SEQUENCE VARIATION

After ambiguously aligned gap regions and the primer sequences were excluded from consideration, the mitochondrial DNA data set was comprised of 1120 base positions. Of the 1120 included sites, 773 were variable with 671 of those representing parsimony-informative characters. Variation was observed in 229 (68.4%) of the first codon positions, 145 (43.3%) of the second codon positions, 335 (99.7%) of the third codon positions, and 64 (56.1%) of the tRNA sites. Consequently, in the analyses in which the codon positions and tRNA sites were weighted proportionally to the number of observed substitutions, the first position, second position, and tRNA substitutions were weighted 1.5, 2.3, and 2.0 times greater than third position substitutions, respectively. Within the *ND2* protein-coding gene, 241 of 343 amino acid sites (70.3%) were variable. The percentage of variable sites remains high (207 of 343, 60.3%) even when the outgroup taxa are excluded from consideration. Observed pairwise sequence divergence values (uncorrected) between *Draco* terminal taxa ranged between 1.5 and 26.2%. Excluding divergence values observed within species, the minimum observed value was either 1.9% (if *D. biaro* and *D.* 'Tagulandang' are distinct species) or 5.0% (between *D. biaro* and *D. caerulhians*). Divergence values between ingroup and outgroup species ranged between 24.1 and 36.1%.

Base compositional bias (Bias C of Irwin, Kocher & Wilson, 1991) is evident in our DNA sequence data, particularly in the second and third codon positions (Fig. 1). However, the nature of the bias differs substantially between the four data partitions (the three codon positions plus the tRNA sites). The third codon position exhibits the greatest bias (C = 0.351), which is derived primarily from a high frequency of A (45.3%) and a low frequency of G (6.2%). The large second position bias (C = 0.325) results primarily from high frequencies of C (37.1%) and T (37.3%), together with a low frequency of G (10.7%). In contrast to the third position sites, which exhibit a high frequency of A (45.3%), the frequency of A at the second position sites is only 15.0%. First positions and tRNA sites exhibit moderate base compositional bias (0.200 and 0.163, respectively), with similar individual base frequencies.

Observed differences in nucleotide base composition among taxa were not significant when the three codon positions plus the tRNA sequences are considered together ($\chi^2 = 164.9$, $P = 0.55$, df = 168). Base compositional differences also were not significant when considering the first codon position ($\chi^2 = 64.9$, $P = 1.0$, df = 168), second codon position ($\chi^2 = 18.8$, $P = 1.0$, df = 168), and tRNA sites ($\chi^2 = 32.3$, $P = 1.0$, df = 168) independently. However, base compositional differences between taxa were highly significant when considering third codon position sites alone ($\chi^2 = 303.6$, $P < 0.0001$, df = 168). Base compositional differences between taxa are a potential source of systematic error when using phylogenetic methods that assume stationary models (Lockhart *et al.*, 1994; Swofford *et al.*, 1996). Lockhart *et al.* (1994) showed using theoretical and biological data sets that sequences with similar base compositions tend to be grouped together regardless of their evolutionary history. Given that the likelihood models employed here make this explicit assumption (and parsimony analysis makes the assumption implicitly), our phylogenetic results could be affected. The LogDet is a transformation that is generally robust to base compositional differences (Lockhart *et al.*, 1994). Therefore, to test whether sets of taxa appeared to be grouped on the basis of base compositional biases alone in the parsimony and maximum likelihood analyses, a minimum evolution analysis was conducted using the LogDet (see below). In our LogDet analyses, we incorporated the proportion of invariant sites estimated in the GTR + Γ + I maximum likelihood analysis, as Waddell (1995) and Swofford *et al.* (1996) suggested that this could ameliorate problems induced by the occurrence of rate heterogeneity.

PHYLOGENETIC SIGNAL

The results of the g_1 analyses indicate that under each of the four weighting procedures employed in the parsimony analyses, the data set contains substantial phylogenetic structure. For each treatment, the g_1 values were significantly left-skewed at $P < 0.01$ (Hillis & Huelsenbeck, 1992).

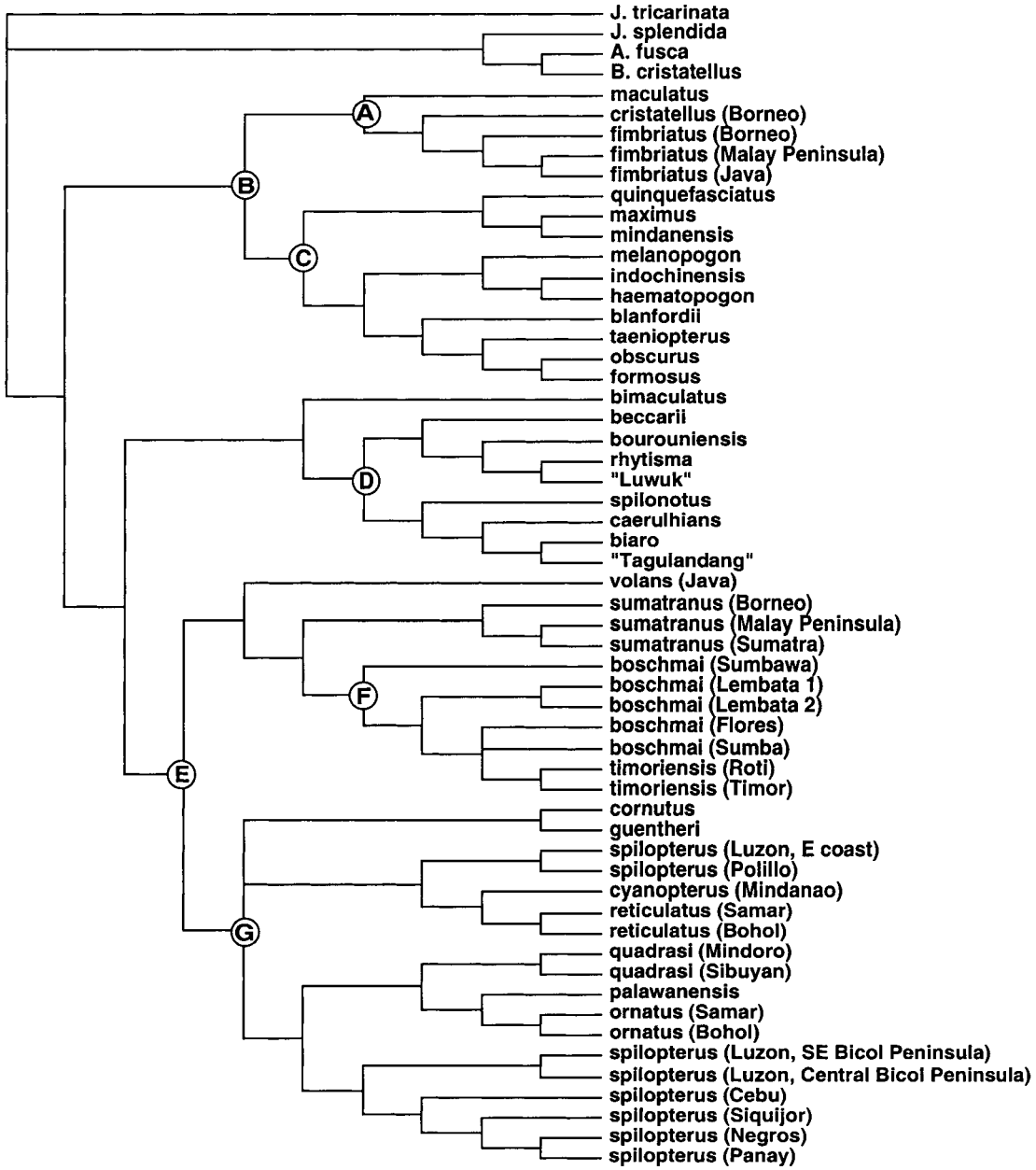

Figure 2. Strict consensus of six equally most parsimonious trees obtained in the analysis with all sites equally weighted. Tree length = 5005; CI (excluding uninformative characters) = 0.270; RI = 0.560; RC = 0.161. The nodes identified by letters refer to the following informally recognized clade names: A = *fimbriatus* group, B = lacrimal bone group, C = dorsal nostril group, D = *lineatus* group, E = *volans* group, F = Lesser Sunda group, G = Philippine *volans* group.

PARSIMONY ANALYSES

Parsimony analysis of the data set with all base positions and gap characters equally weighted resulted in six equally parsimonious trees of length 5005, the strict consensus of which is presented in Figure 2. The 50% majority-rule bootstrap consensus tree, which differs from each of the six equally most parsimonious trees in the placement of *D. bimaculatus*, is presented in Figure 3.

Analysis of the data set with transversions weighted five times greater than transitions resulted in four equally parsimonious trees of length 6445. The 50% majority-rule bootstrap consensus tree, which differs from the four equally most parsimonious trees in the

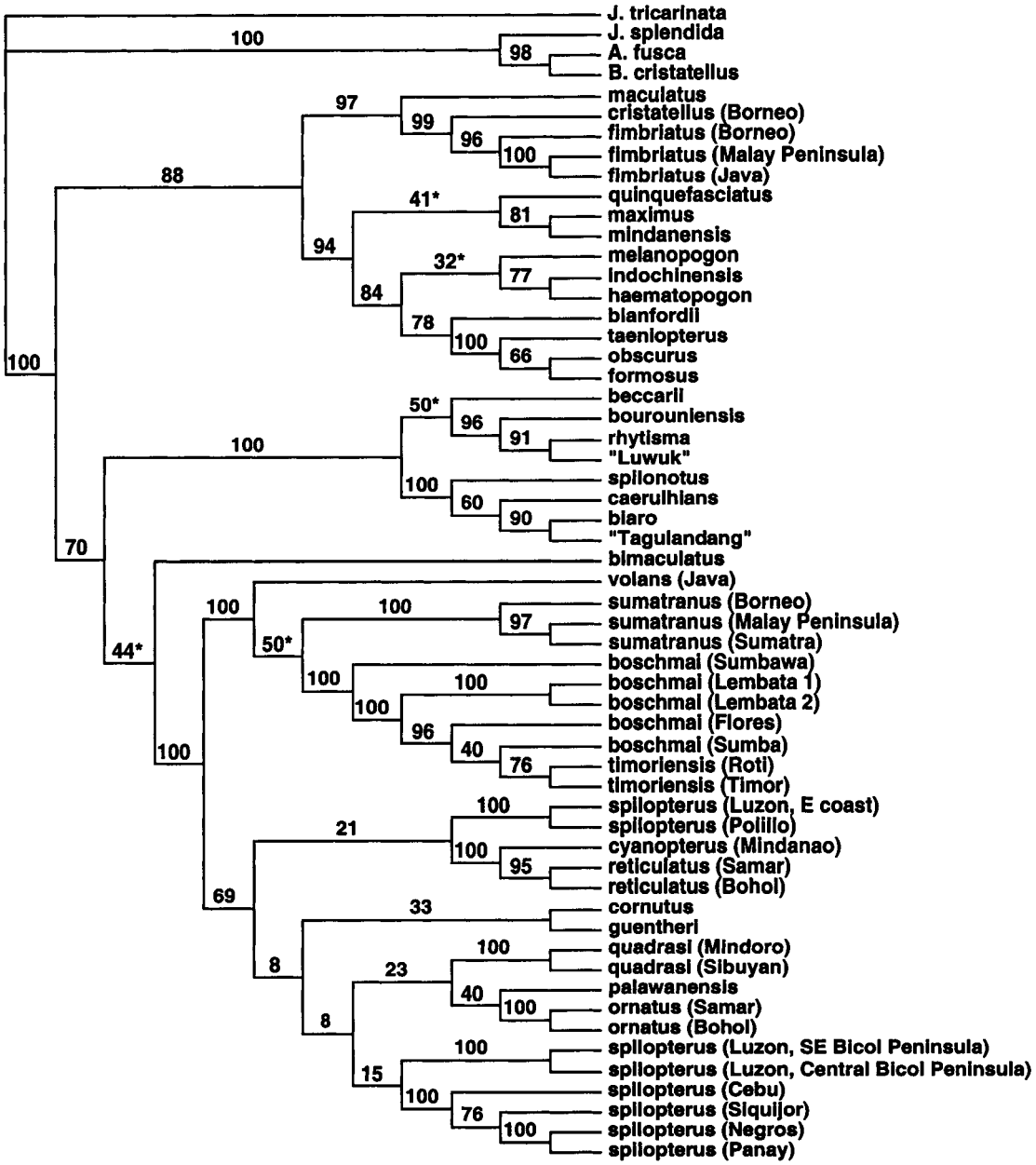

Figure 3. The 50% majority rule consensus tree obtained in the nonparametric bootstrap analysis with all characters equally weighted (1000 pseudoreplicates). The five nodes common to the parsimony bootstrap trees under all four weighting criteria, but interpreted as weakly supported, are identified with asterisks.

placement of *D. melanopogon* and in the resolution of much of the Philippine *volans* group, is presented in Figure 4.

Analysis of the data set with substitutions at first codons positions weighted 1.5 times greater than those at third positions, substitutions at second positions weighted 2.3 times greater than those at third positions, and substitutions at tRNA sites weighted 2.0 times greater than those at third positions resulted in a single most parsimonious tree of length 39 065. The 50% majority-rule bootstrap consensus tree, which differs from the single most parsimonious tree in the placement of *D. bimaculatus* and in the relative positioning of three subclades in the Philippine *volans* group, is presented in Figure 5.

Analysis of the data set with transversions weighted five times greater than transitions and the three codon positions plus tRNA sites differentially weighted as described in the paragraph above resulted in a single most parsimonious tree with a length 49 753. The 50%

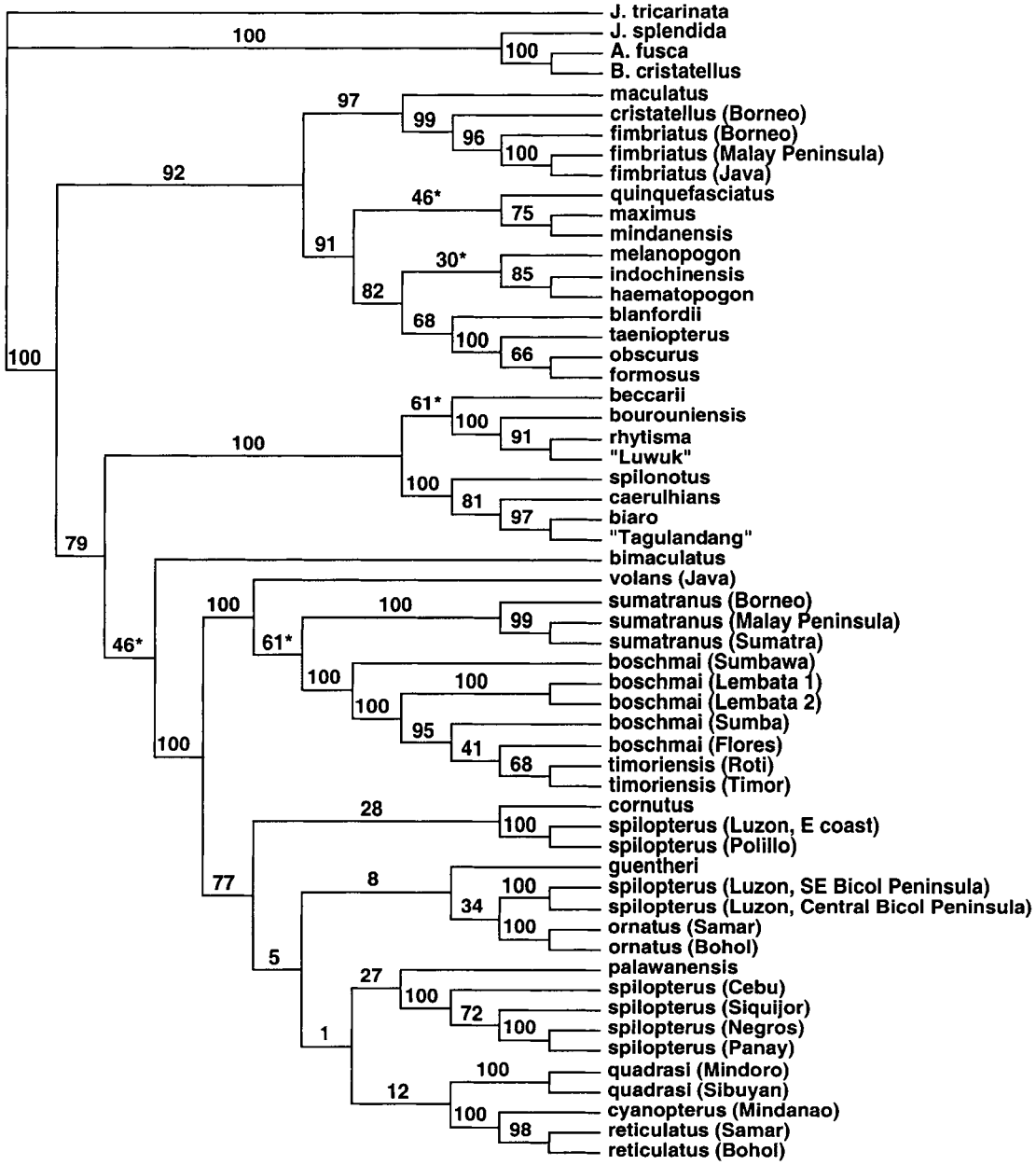

Figure 4. The 50% majority rule consensus tree obtained in the nonparametric bootstrap analysis with transversions weighted five times greater than transitions (1000 pseudoreplicates). The four equally parsimonious trees recovered under this weighting criterion had the following characteristics: tree length = 6445; CI (excluding uninformative characters) = 0.283; RI = 0.601; RC = 0.180.

majority-rule bootstrap consensus tree is presented in Figure 6.

MAXIMUM-LIKELIHOOD ANALYSES

Maximum-likelihood analyses were conducted under four primary models of sequence evolution: the Jukes-Cantor model (JC; Jukes & Cantor, 1969), the Felsenstein model (F81; Felsenstein, 1981), the Hasegawa–Kishino–Yano model (HKY; Hasegawa, Kishino & Yano, 1985), and the general time-reversible model (GTR; Lanave *et al.*, 1984). The JC model is the least complex in that equal nucleotide frequencies are enforced and transitions and transversions are assumed equally likely. The F81 model adds three additional parameters in that the nucleotide frequencies are allowed to vary. The HKY85 model incorporates one additional parameter relative to the F81 model because

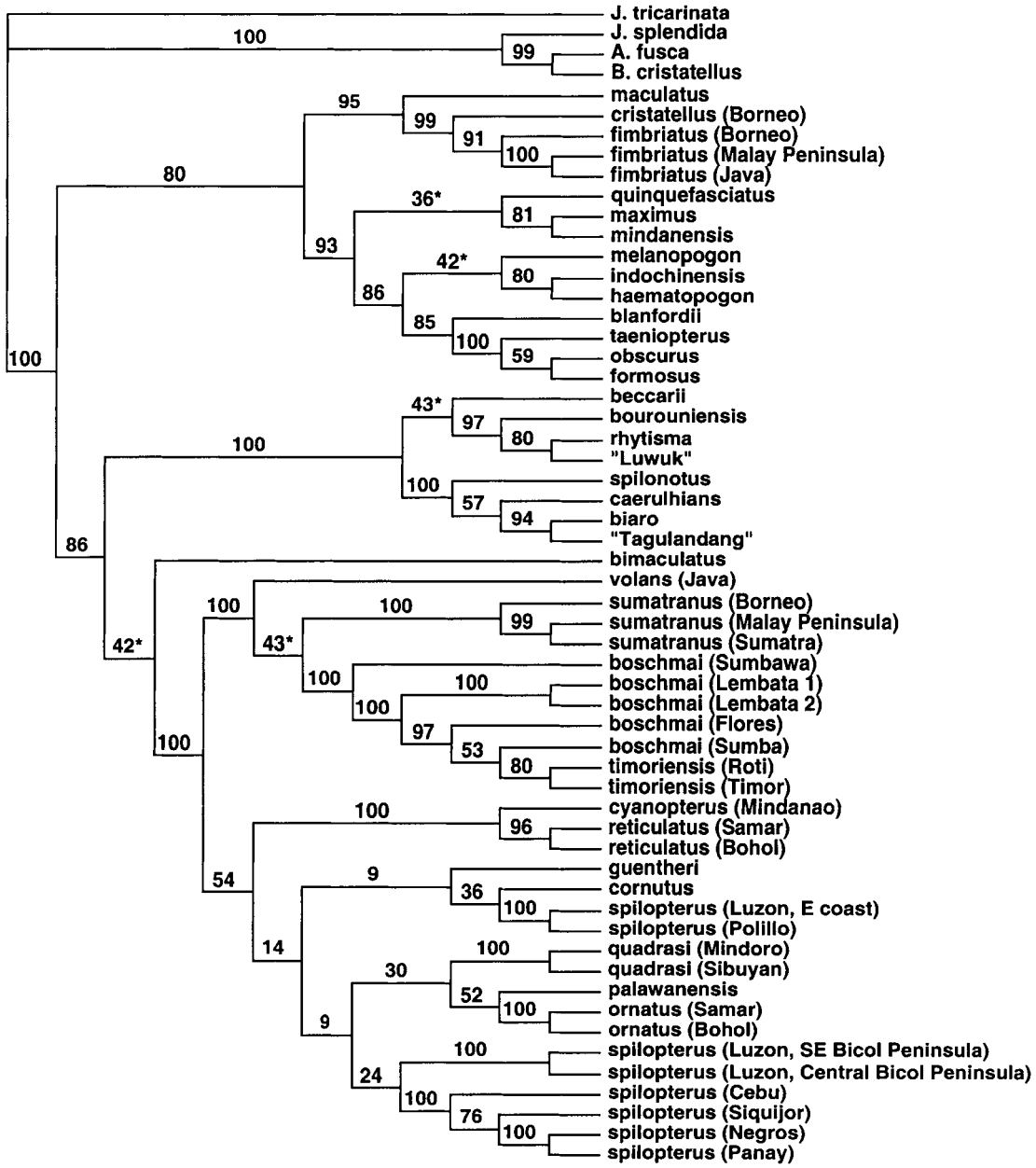

Figure 5. The 50% majority rule consensus tree obtained in the nonparametric bootstrap analysis with first codon positions weighted 1.5 times greater than those at third positions, substitutions at second positions weighted 2.3 times greater than those at third positions, and substitutions at tRNA sites weighted 2.0 times greater than those at third positions (1000 pseudoreplicates). The single most parsimonious tree obtained under this weighting regime had the following characteristics: tree length = 39 065; CI (excluding uninformative characters) = 0.282; RI = 0.578; RC = 0.177.

transitions and transversions are allowed to occur at unequal rates. Finally, the GTR model adds four more parameters relative to the HKY model by allowing six different types of character state changes, rather than the two types of changes (transitions and transversions) allowed by the HKY85 model. In all analyses in which nucleotide frequencies were allowed to vary, the frequencies were estimated via maximum likelihood rather than using empirical frequencies. Whelan & Goldman (1999) found that empirical frequencies can be poor approximations of the maximum likelihood estimates and therefore the use of the empirical values can substantially reduce recovered likelihood scores.

Our initial analysis employed the JC model. This

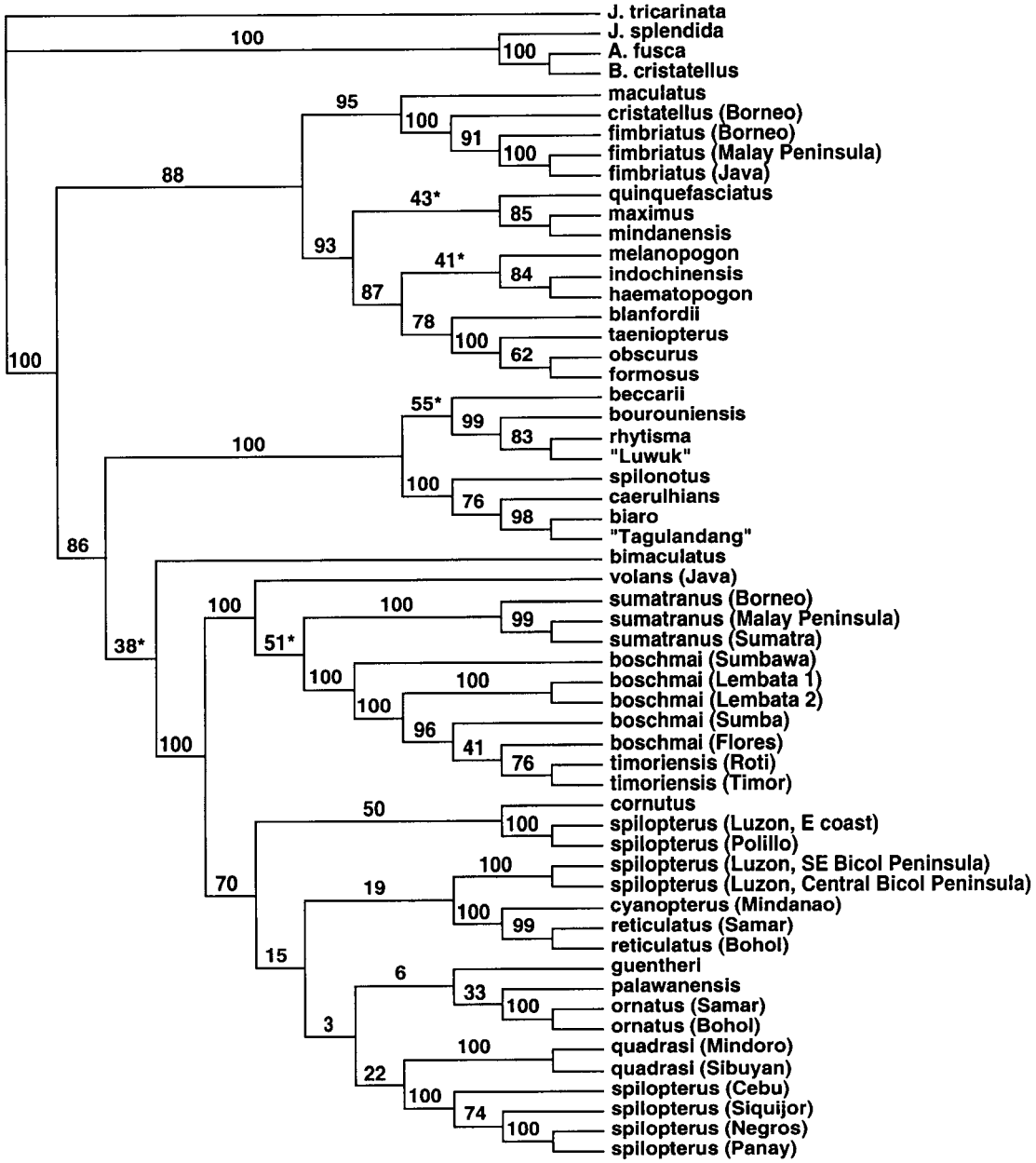

Figure 6. The 50% majority rule consensus tree obtained in the nonparametric bootstrap analysis with tranversions weighted five times greater than transitions and the three codon positions and tRNA sites weighted as described in Figure 5 (1000 pseudoreplicates). The single most parsimonious tree obtained under this weighting regime had the following characteristics: tree length = 49 753; CI (excluding uninformative characters) = 0.297; RI = 0.615; RC = 0.197.

was followed by an analysis that incorporated a rate heterogeneity parameter (JC + Γ see Yang, 1996) and a third analysis that also incoporated a parameter allowing some sites to be invariant (JC + Γ + I; see Gu, Fu & Li, 1995, Waddell & Penny, 1996). Likelihood-ratio tests indicated that the inclusion of the rate heterogeneity parameter significantly improved the estimated likelihood score over that obtained with the JC model alone. Similarly, the addition of the invariant sites parameter (JC + Γ + I) significantly improved the likelihood score relative to that obtained with the JC + Γ model. Therefore, subsequent analyses using the more parameterized models of sequence evolution (i.e. F81, HKY, and GTR) also included rate heterogeneity and invariant sites parameters. The results of the maximum likelihood analyses and the corresponding likelihood-ratio tests are presented in Table 2. These analyses show that the incorporation of in-

Table 2. Results of the likelihood ratio tests. JC = Jukes–Cantor model, F81 = Felsenstein model, HKY = Hasegawa–Kishino–Yano model, GTR = general time-reversible model, Γ = a rate-heterogeneity parameter modeled with a discrete gamma distribution, I = a parameter describing the proportion of sites estimated via maximum likelihood to be invariant

Models compared	$\log L_0$	$\log L_1$	$-2 \log \Lambda$	df	P
H0: JC H1: JC + Γ	26 024.85	24 144.55	3760.60	1	<0.0001
H0: JC + Γ H1: JC + Γ + I	24 144.55	24 093.78	101.54	1	<0.0001
H0: JC + Γ + I H1: F81 + Γ + I	24 093.78	23 939.78	308.00	3	<0.0001
H0: F81 + Γ + I H1: HKY + Γ + I	23 939.78	21 890.55	4098.46	3	<0.0001
H0: HKY + Γ + I H1: GTR + Γ + I	21 890.55	21 855.15	70.80	4	<0.0001

creased model complexity significantly increases the likelihood scores and, based on these results, the tree estimated under the GTR + Γ + I model (Fig. 7) is here considered to be the preferred tree.

Figure 8 illustrates that two model parameters have the greatest affect on the likelihood analyses. Incorporation of a rate heterogeneity parameter (Γ) substantially improves the fit of all four primary models (i.e. JC, F81, HKY, GTR) to the data with the addition of this one parameter increasing the likelihood scores by an average of ~2127 (an improvement of ~7 would be significant at $P = 0.0001$). The addition of the parameter allowing transitions and transversions to have different substitution rates (the switch from JC and/or F81 to HKY) also has a substantial effect on the likelihood score with an average improvement relative to the F81, F81 + Γ, and F81 + Γ + I models of ~1870.

The results of the nonparametric bootstrap analysis under the maximum likelihood criterion are similar to those obtained in the parsimony and LogDet bootstrap analyses (Fig. 7). In order to illustrate branch lengths, bootstrap proportion values are indicated on the optimal maximum likelihood tree. However, two clades that are reasonably well-supported in the parsimony analyses and present as well on the preferred maximum likelihood tree illustrated in Figure 7 were not best-supported according to the likelihood bootstrap analysis, which indicated better support for clades composed of *Draco formosus* + *D. taeniopterus* (BP = 49), and *D. spilopterus* (Cebu) + *D. spilopterus* (Siquijor) (BP = 59).

LogDet

The minimum evolution analysis employing Log Determinant (LogDet) distances resulted in the phylogenetic estimate presented in Figure 9. Nonparametric bootstrap proportion values are indicated on the optimal neighbor-joining tree.

DISCUSSION

LogDet

Because the LogDet results (Fig. 9) were very similar to those obtained under the parsimony and maximum likelihood criteria (Figs 2–7), we conclude that the phylogenetic estimates obtained under those criteria probably are not significantly affected by nonstationarity resulting from the interspecific differences in base composition.

Parsimony analyses

Parsimony analyses undertaken with four alternative sets of weighting procedures resulted in phylogenetic estimates that were largely, but not completely, congruent with one another. Forty-four of 52 nodes present on the 50% majority-rule bootstrap consensus tree obtained with all characters weighted equally were also recovered in the three analyses that employed differential character weighting. Not surprisingly, most of the nodes that were common to all four analyses (39 of 44 common nodes with mean bootstrap values ≥ 63) were relatively strongly supported $\bar{x} = 91.4$). The five nodes common to the four parsimony bootstrap trees but interpreted as weakly supported are identified by asterisks in Figures 3–6.

Several major clades were strongly supported under all four weighting procedures. For ease of discussion, we will refer to these clades informally as the *'fimbriatus'*, 'lacrimal bone' (because the constituent species have lacrimal bones that are absent in the remaining species of *Draco*), 'dorsal nostril' (all taxa

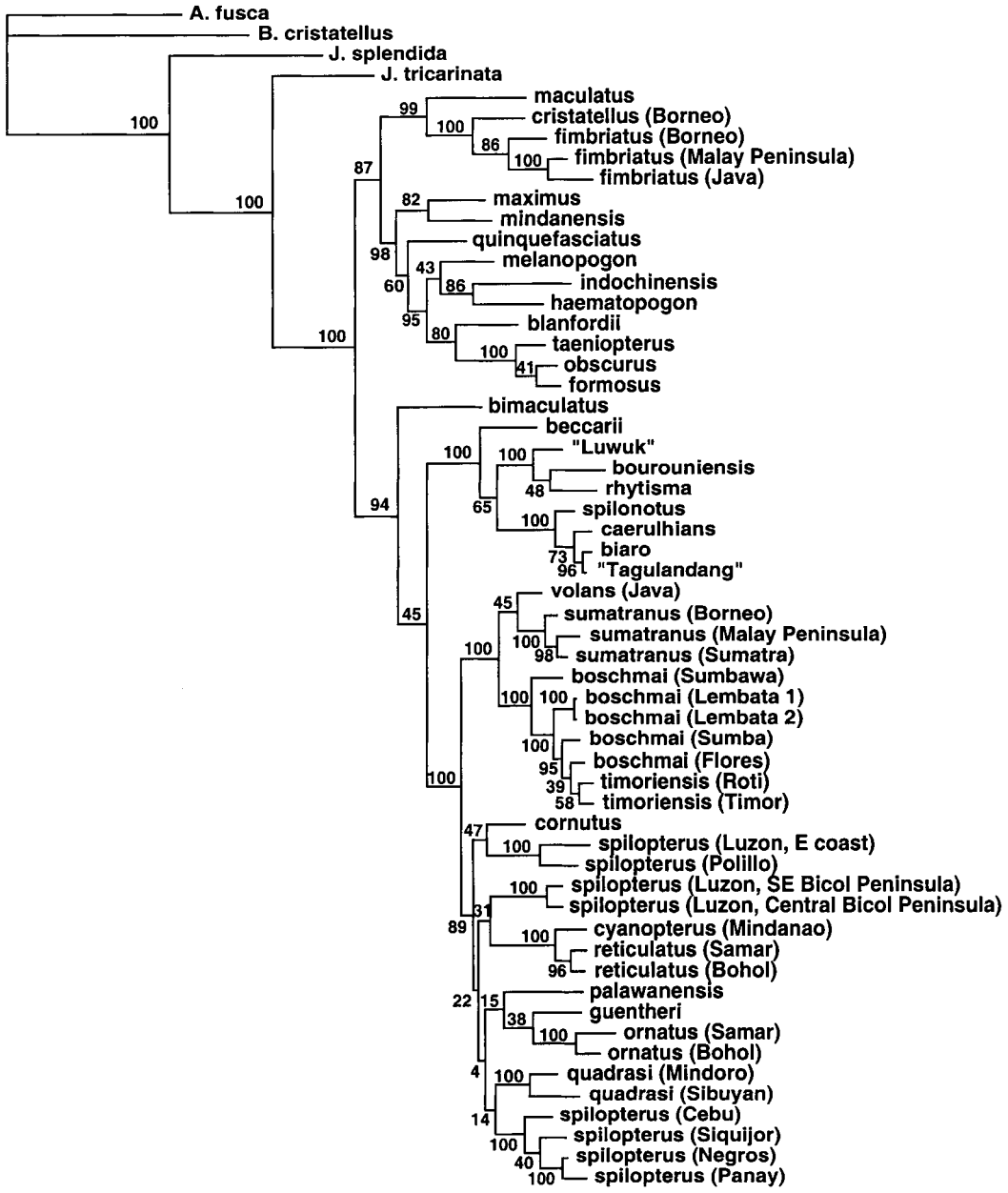

Figure 7. Results of the maximum-likelihood analyses under the GTR + Γ + I model. The log-likelihood score obtained in this analysis was 21 855.15; estimated base frequencies were A = 0.375, C = 0.332, G = 0.069, T = 0.223; proportion of sites estimated to be invariant = 0.242; estimated value of gamma shape parameter = 0.762. The numbers at each node represent nonparametric bootstrap proportion values (100 replicates, see text for details).

characterized by dorso-posteriorly oriented nostrils), *'lineatus'*, *'volans'*, 'Lesser Sunda' (endemic to the Lesser Sunda island group of Indonesia), and 'Philippine *volans*' groups (see Fig. 2). With the exception of the Philippine *volans* group, all of these clades were strongly supported in each of the four analyses (minimum bootstrap value of 80, $\bar{x} = 96.0$). Relationships within these major clades generally were well-resolved and strongly supported (Figs 2–6). However, relationships within the Philippines *volans* group proved particularly difficult to recover. Indeed, interspecific relationships within this clade remain essentially unresolved, with only the *D. reticulatus* + *D. cyanopterus* clade strongly supported. Furthermore, monophyly of *D. spilopterus*, which is readily diagnosed morphologically, was unsupported in each of the four

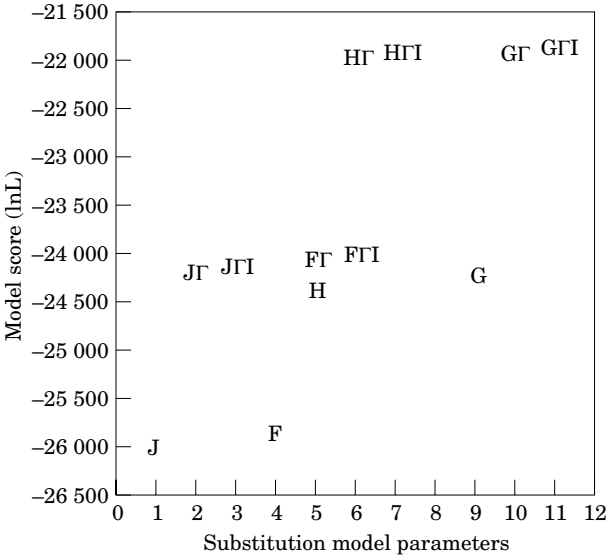

Figure 8. The likelihood scores for the 12 models of sequence evolution evaluated within a maximum likelihood framework. Substitution models are represented by single characters: J = Jukes–Cantor, F = Felsenstein 81, H = Hasegawa–Kishino–Yano, G = general time-reversible. Characters following the substitution models represent among-site rate heterogeneity parameters: I = proportion of invariant sites, Γ = gamma.

analyses, with three strongly supported *D. spilopterus* subclades variably placed throughout the Philippines *volans* clade depending on the weighting procedure employed.

MAXIMUM-LIKELIHOOD ANALYSES

The maximum-likelihood analyses and likelihood-ratio tests indicate that an analysis employing the parameter-rich general-time-reversible model with rate heterogeneity and the proportion of invariant sites estimated via maximum likelihood explains the data significantly better than do analyses based on all of the less parameterized models that were considered (Fig. 8; Table 2). The GTR + Γ + I phylogenetic estimate is largely congruent with the parsimony estimates, sharing 39 of the 44 nodes common to the four parsimony analyses and all but one of the parsimony nodes interpreted as strongly supported. The one node that was strongly supported in the parsimony analyses but not recovered on the GTR + Γ + I tree is the one placing *D. rhytisma* as the sister taxon of *D.* 'Luwuk'. This is a particularly interesting case because *D. rhytisma* and *D. bourouniensis* are very similar morphologically, but *D.* 'Luwuk' occurs on the northeast arm of Sulawesi immediately adjacent to Peleng Island, the home of *D. rhytisma*.

The GTR + Γ + I tree is most similar to the parsimony tree based on differential weighting of transitions and transversions together with differential weighting of each of the three codon positions and the tRNA sites. Indeed, the extent of the similarity between the GTR + Γ + I tree and this parsimony tree is surprising—the only difference in the respective Philippine *volans* group topologies being that maximum likelihood places *D. palawanensis* as the sister taxon of a clade composed of *D. guentheri* and *D. ornatus*, whereas the parsimony tree places *D. guentheri* as the sister taxon of a clade composed of *D. palawanensis* and *D. ornatus*. Despite the high degree of congruence between the GTR + Γ + I tree and this weighted parsimony tree, the weak bootstrap support for these internal nodes indicate that maximum likelihood has difficulty resolving relationships within this group as did the parsimony and LogDet analyses (see Fig. 7).

Cunningham, Zhu & Hillis (1998) investigated the performance of likelihood and distance methods under alternative branch length conditions and found that maximum-likelihood under the GTR model substantially outperformed likelihood under simpler models of evolution (as well as distance methods under simple or complex models) when the true underlying tree had short internal branches and long terminal branches. Their findings suggest that the GTR + Γ + I likelihood results are more likely to reflect the evolutionary history of the Philippine *volans* group than are the parsimony results or the likelihood results based on less complex models of evolution. Furthermore, simulation studies have shown that maximum likelihood outperforms maximum parsimony and distance methods under a variety of conditions (Hillis, Huelsenbeck & Swofford, 1994; Kuhner & Felsenstein, 1994; Tateno, Takezaki & Nei, 1994; Yang, 1994; Huelsenbeck, 1995). For these reasons, we refer to the GTR + Γ + I tree as our preferred tree. All further considerations of the evolutionary biology of *Draco*, including comparisons of our phylogenetic findings with those of other authors (see below) are based on this tree.

COMPARISON WITH PREVIOUS PHYLOGENETIC HYPOTHESES

The only explicit phylogenetic hypotheses for *Draco* thus far presented in the literature are those of Honda *et al.* (1999b; Fig. 10) and Musters (1983; Fig. 11). Honda *et al.* (1999b) estimated phylogenetic relationships based on mitochondrial 12S and 16S rRNA sequence (15 ingroup taxa, 20 OTUs) and allozymic (13 ingroup taxa, 16 OTUs) data sets. Both data sets were converted to distance matrices and analyzed under the neighbor-joining algorithm. Although both the rRNA sequence and allozyme trees were well-resolved, much of this resolution was not strongly

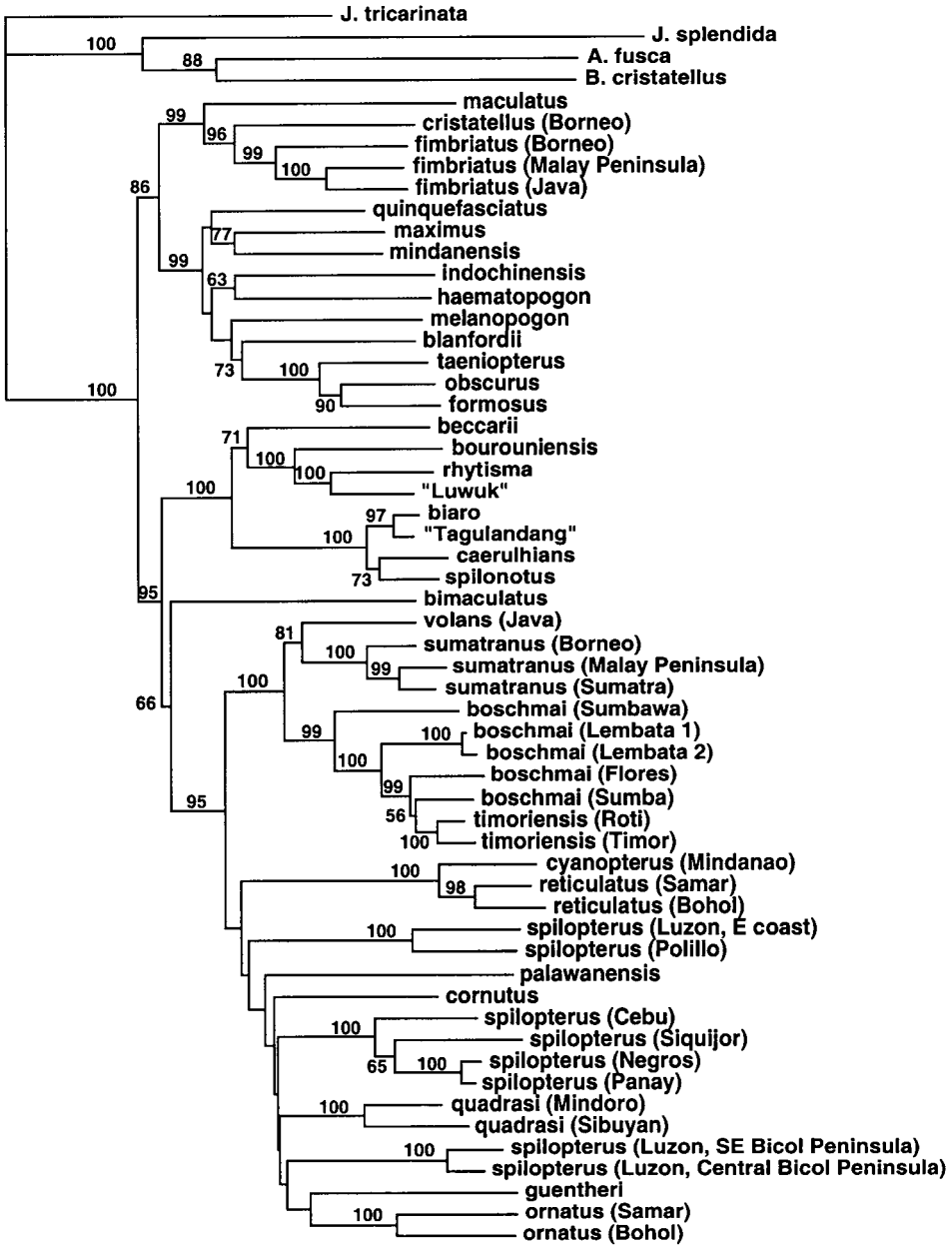

Figure 9. Results of the minimum evolution analysis employing Log Determinant (LogDet) distances. The numbers at each node represent nonparametric bootstrap proportion values (1000 replicates).

supported according to their nonparametric bootstrap analyses. Those interspecific relationships that were reasonably well-supported in one or both analyses (bootstrap proportion values ≥ 70, $n = 8$) were entirely congruent with the results obtained in this study (see Honda *et al.*, 1999b, fig. 5). There was some disagreement between their rRNA and allozyme trees such that one node that was strongly supported by the allozymic data (a clade composed of *Draco blanfordii*, *D. formosus*, *D. haematopogon*, *D. melanopogon*, *D. obscurus*, and *D. taeniopterus*; bootstrap support = 70) was contradicted by the sequence data, which placed *D. blanfordii* as the sister taxon to *D. maculatus* (bootstrap support = 66). With respect to these competing hypotheses, the present study agrees with the allozymic data rather than the rRNA sequence data, as the six taxa together with *D. indochinensis* (not represented in the Honda *et al.* analyses) are strongly supported as a monophyletic grouping.

Musters' (1983) hypothesis of relationships was not

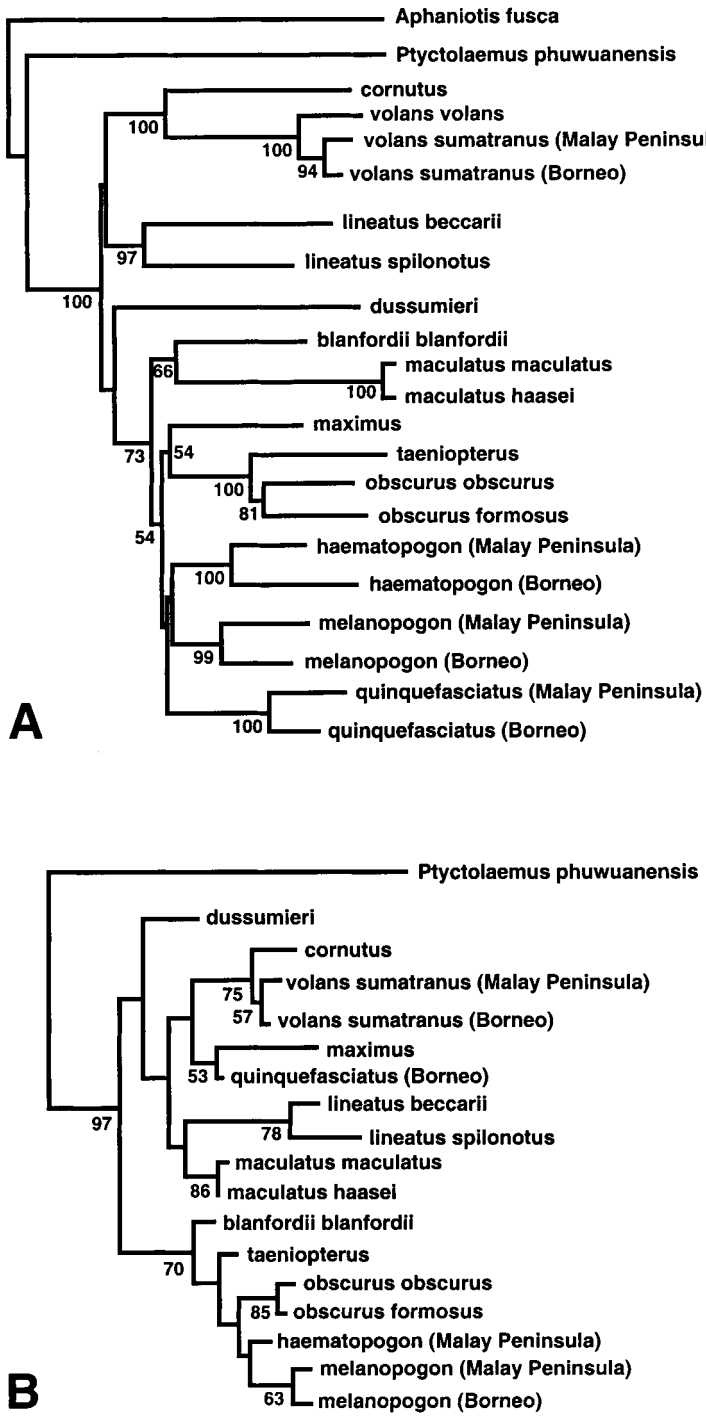

Figure 10. Neighbor-joining dendrograms of Honda *et al.* (1999b) based on 12S and 16S rRNA sequence (A) and allozymic (B) data. The numbers at the nodes represent nonparametric bootstrap proportion values (1000 replicates).

based on an empirical analysis of data, but rather on a thoughtful consideration of a small number of conspicuous and unpolarized morphological characters. In his defense, Musters made it clear from the outset that he considered his hypothesis to be highly speculative and at best a starting point for a more rigorous phylogenetic study. That said, it is not too surprising that the results of the present analysis disagree in many respects with those of Musters (1983). Indeed, only two of the 12 nodes on Musters' tree (Fig.

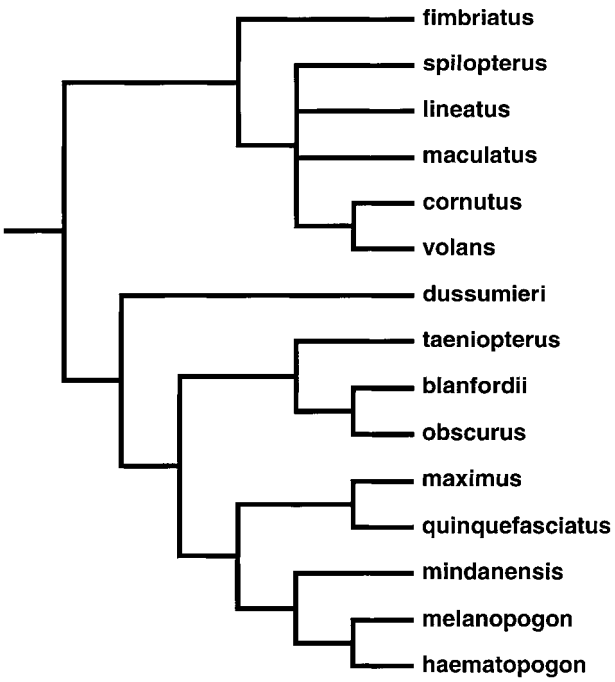

Figure 11. The phylogenetic hypothesis of Musters (1983).

11) were recovered in the present study. The shared nodes represent the two clades that are most easily diagnosed on the basis of external morphology. The first shared clade is the dorsal nostril group, which is characterized by unique turret-like nostrils that are oriented dorsally and posteriorly on the snout. The second clade, composed of *D. blanfordii*, *D. formosus*, *D. obscurus*, and *D. taeniopterus*, is characterized by the presence in males of a distally expanded dewlap that is overlain with greatly enlarged scales. Musters (1983) also suggested that *D. melanopogon* and *D. haematopogon* are sister taxa. If *D. indochinensis* is ignored (Musters considered *D. indochinensis* to be a subspecies of *D. blanfordii* and, consequently, did not include this taxon on his phylogenetic tree), this hypothesis is consistent with the likelihood and parsimony results (but not the LogDet). The remaining nodes on the Musters (1983) phylogenetic tree are not consistent with any of the analyses conducted here.

Although Honda *et al.* (1999b) and Musters (1983) provided the only explicit phylogenetic hypotheses, some authors have implied phylogenetic relationships with their taxonomies. For example, Musters (1983) followed Hennig (1936) in recognizing *Draco bimaculatus* as a subspecies of *D. lineatus*. This taxonomy implies that *D. bimaculatus* and the lineatus group taxa form a clade. Although the equally-weighted parsimony analysis is consistent with this hypothesis, all other analyses, including the preferred GTR + Γ + I maximum-likelihood analysis indicate that *D. bimaculatus* is neither the sister taxon of, nor nested within, the *D. lineatus* group (Figs 2–7, 9).

Inger (1983) treated all of the members of the *volans* group as a single species, *Draco volans*. His conservative taxonomy is consistent with the phylogenetic results presented here in that all of the taxa that he synonymized with *D. volans* form a monophyletic group. Other clades recovered here that Inger (1983) treated as single species include *D. formosus* + *D. obscurus* (recognized as *D. obscurus* by Inger), and the *lineatus* group (recognized as *D. lineatus* by Inger).

The phylogenetic data also provide evidence that *Draco indochinensis* should not be treated as a synonym of *D. blanfordii* (Inger, 1983), or as a subspecies of *D. blanfordii* (Musters, 1983), as it is neither the sister taxon of *D. blanfordii* nor a member of the clade that includes *D. blanfordii*, *D. taeniopterus*, *D. formosus*, and *D. obscurus* (Figs 2–7, 9). The phylogenetic data are consistent with the morphological and distributional data (see above) indicating that *D. indochinensis* is a distinct species.

Rapid radiation of the Philippine *volans* group

All of our analyses support the monophyly of a clade composed primarily of Philippine species that I refer to informally as the Philippine *volans* group. The parsimony and maximum likelihood analyses suggest that this clade is well-supported (see Figs 3–7), although the LogDet analysis (Fig. 9) only weakly supports this grouping. All of the species in the Philippine *volans* group occur in the Philippines except the Bornean species *Draco cornutus*. However, a second species, *D. palawanensis*, might also be considered a 'non-Philippine' species despite its occurrence on the Philippine island of Palawan because this island is generally treated as an extension of the Sunda Shelf (particularly Borneo) and consequently is excluded from the Philippine Biogeographic Province (Everett, 1889; Heaney, 1985, 1986). Based on the phylogenetic conclusions presented here, McGuire and Alcala (2000) suggested that the biogeographical relationship between Palawan and the remainder of the Philippines may be understated in the biogeographical literature.

One unexpected finding was that monophyly of the morphologically similar populations of *Draco spilopterus* was not supported by the data. Our analysis included eight individuals of *D. spilopterus* representing six separate Philippine islands (Fig. 12). In all of our analyses, we obtained strong support for three *D. spilopterus* clades, but little or no support for the monophyly of any combination of the three groups. Monophyly of each of the three strongly supported subsets of *D. spilopterus* is consistent with the geological and geographical history of the region (see Fig. 13). One clade is composed of species on the Western

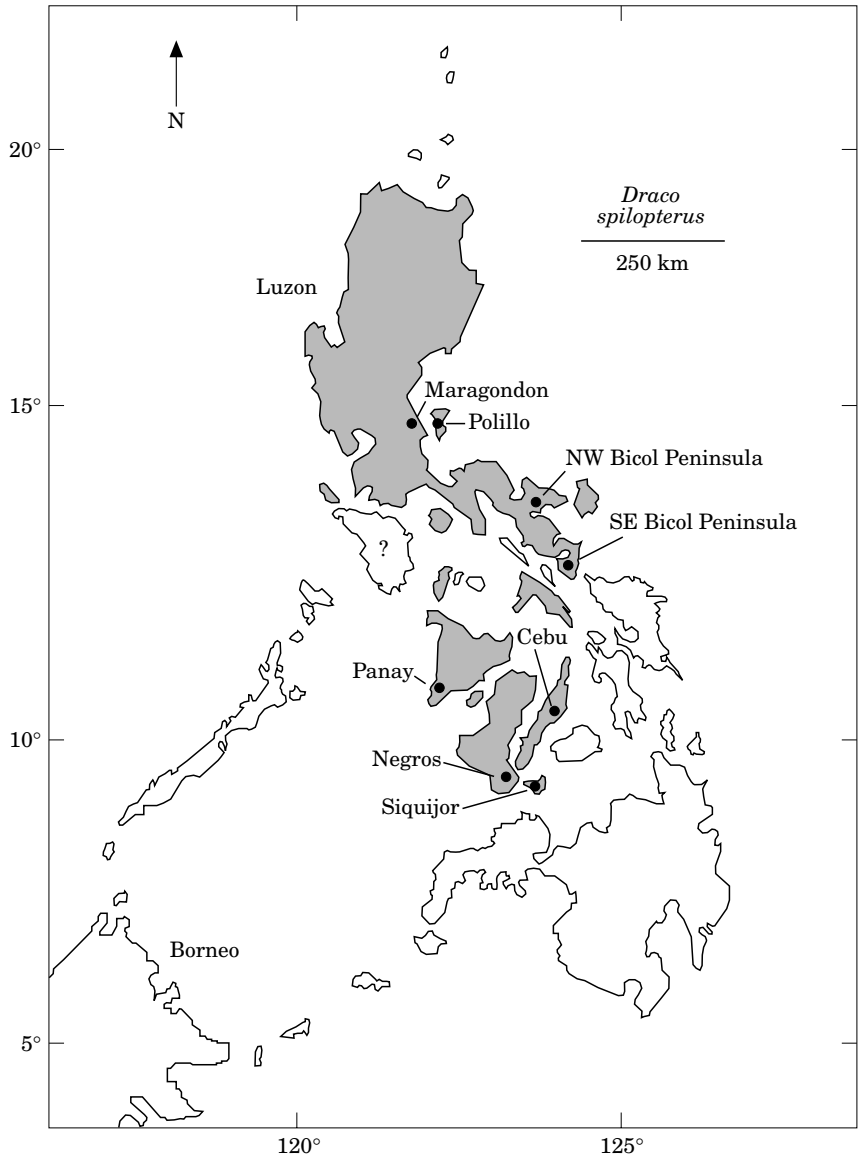

Figure 12. Map of the Philippine Archipelago illustrating the distribution of *Draco spilopterus*. The shaded areas represent islands known to be inhabited by *D. spilopterus*, with the question mark identifying the questionable locality of Mindoro Island (see McGuire and Alcaca, 2000). The black dots denote the eight localities represented in the phylogenetic analysis.

Visayan islands of Cebu, Negros, Panay, and Siquijor. Cebu, Negros, and Panay are separated from one another by shallow marine barriers and therefore represented a single island during the late Pleistocene 10–18 000 years ago. Siquijor, although separated from these islands by a deep marine channel, lies off of the SE coasts of Panay and Cebu and is shielded by those islands from any other islands inhabited by *D. spilopterus*. The other four *D. spilopterus* samples are from islands separated from the Western Visayas by deep marine barriers, indicating the potential for a long period of isolation. A second clade is composed of two individuals from the Bicol Peninsula of Luzon Island, an attenuated arm projecting southeastward from the main body of the island. The Bicol Peninsula is known to have existed as a separate island until about 5–10 Myr ago in the Miocene (Hall, 1998) and it is therefore not surprising that these two individuals are more closely related to one another than either is to any other *D. spilopterus* samples in this analysis. The third *D. spilopterus* clade is composed of one individual from the east coast of Luzon and another from the adjacent island of Polillo. A close relationship between these two individuals is expected because

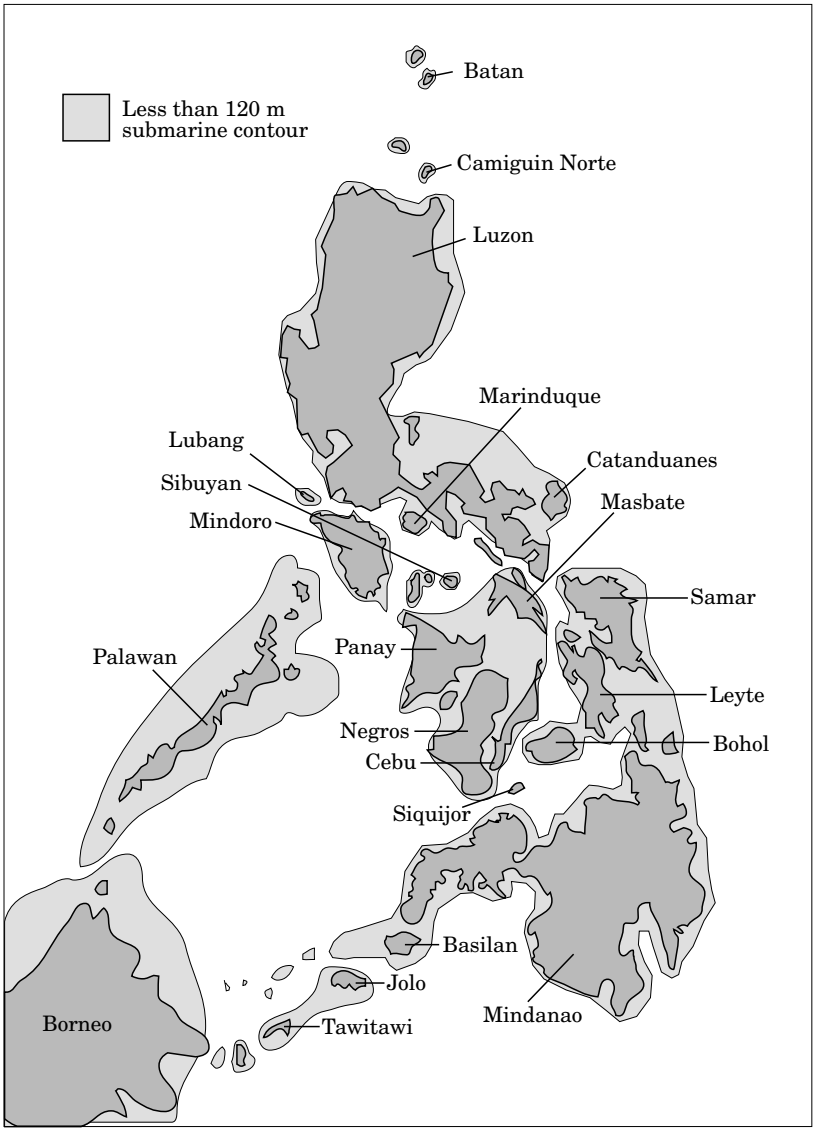

Figure 13. Map illustrating the extent of the Philippine islands during the late Pleistocene (18 000 years before present) when sea-levels were approximately 120 m lower; from Heaney (1986).

Polillo is separated from the east coast of Luzon by a shallow marine channel and was therefore connected to Luzon by dry land in the last 10–18 000 years. However, it is surprising that the second and third *D. spilopterus* clades, both of which are primarily associated with Luzon Island, are evidently not closely related to one another, despite the fact that there is continuous habitat in the intervening area separating these divergent populations. In fact, the pairwise genetic distances (uncorrected) between these taxa are between 18.6% and 20.7%, which represent even greater divergences than those observed between these individuals and other Philippine species that are easily distinguished from *D. spilopterus* on the basis of numerous morphological character state differences (pairwise sequence divergences between Philippine *Draco* species range between 7.4% and 21.2%). These findings suggest a long period of reproductive isolation between the members of the three clades. Although this clearly suggests the existence of multiple species within *D. spilopterus*, taxonomic adjustment would be premature at this time given that our sampling is relatively sparse and that we cannot distinguish some of these populations morphologically (and thus would not know the precise distributional limits of some of the resultant species). Also, this finding could be the result of paralogous sequences and should be verified with independent nuclear markers.

One of the more interesting aspects of the Philippine *volans* group is that it appears to have undergone a

rapid radiation. This interpretation is based not only on the general lack of interspecific phylogenetic resolution within this group, but also in the nature of the DNA sequence variation observed within this set of taxa. In the recent phylogenetic literature, the 'rapid radiation' hypothesis is often presented when a general lack of resolution is observed. However, it may be that the unresolved polytomy is obtained due to inadequate or inappropriate data rather than simultaneous divergences within the clade. For example, the phylogenetic marker may be evolving too rapidly relative to the timing of the relevant speciation events within the clade. In such cases, older divergences may be difficult to resolve due to saturation of character state changes (which is likely to present problems for many parsimony analyses of DNA sequence data with equal character weighting). It is also possible that the phylogenetic marker may not be evolving rapidly enough if the divergences were relatively recent. These alternative hypotheses should be considered before resorting to the rapid radiation argument. Jackman *et al*. (1999) suggested a series of analyses that would allow one to test the hypothesis that an unresolved polytomy is actually a 'hard polytomy' (Maddison, 1989)—in other words, a polytomy resulting from simultaneous or near-simultaneous divergences rather than inadequate or inappropriate data. They argued that the first step is to evalate the data to assess whether substitutional saturation is evident. Given that their phylogenetic estimate was derived from a parsimony analysis with equal character weighting, assessment of the degree of substitutional saturation was critical to their study. However, because we have analysed our data in a maximum likelihood framework that compensates for superimposed nucleotide substitutions, this saturation test is less critical for the present study. Nevertheless, the fact that the uncorrected pairwise genetic distances between species in the Philippine *volans* group are no greater than those observed in the well-resolved and well-supported 'dorsal nostril' and 'lacrimal bone' clades, and much greater than those in several other well-resolved and well-supported clades (e.g. within the *'lineatus'* and 'Lesser Sunda' groups) suggests that the degree of substitutional saturation within the Philippine *volans* group cannot explain the lack of phylogenetic resolution obtained in our analyses.

Another series of tests recommended by Jackman *et al*. (1999) more directly evaluates whether the lack of resolution is the result of a hard polytomy versus insufficient data. If a long internal branch was divided by many sequential branching events such that few characters support any particular node, the selective removal of taxa from the analysis should result in increasing character support for specific remaining subsets of taxa (those separated by many branches on the tree). This would suggest that the lack of resolution is the result of too few characters in the analysis rather than simultaneous branching. Alternatively, if the speciation events were effectively simultaneous, removal of taxa from the clade in question should lend no additional support to the remaining subclades. Jackman *et al*. (1999) suggested several statistical approaches that can be used to test the null hypothesis that phylogenetic data are consistent with a hard polytomy. Because the alternative approaches resulted in qualitatively similar findings in their study, we selected one procedure (their permutation test) to evaluate the hard polytomy hypothesis for the Philippine *volans* group. Although we would have preferred to have conducted this analysis within a maximum likelihood framework, the computation time required to complete such an analysis was prohibitive. Therefore, for our permutation test, all phylogenetic analyses employed maximum parsimony with transversions weighted five times greater than transitions and codon positions 1, 2, 3, and the tRNA sites weighted 1.5:2.3:1:2. We followed this approach because our parsimony results under these weighting procedures closely approximated the results of our preferred maximum likelihood analysis under the GTR+Γ+I model. We selected nine individuals from the Philippine *volans* group—one from each of the nine lineages or clades for which inter-relationships were weakly supported in the parsimony and maximum likelihood analyses (Fig. 14). The single individuals selected to represent the six strongly supported subclades were chosen randomly. *Japalura tricarinata* also was retained as a single outgroup representative. To generate random

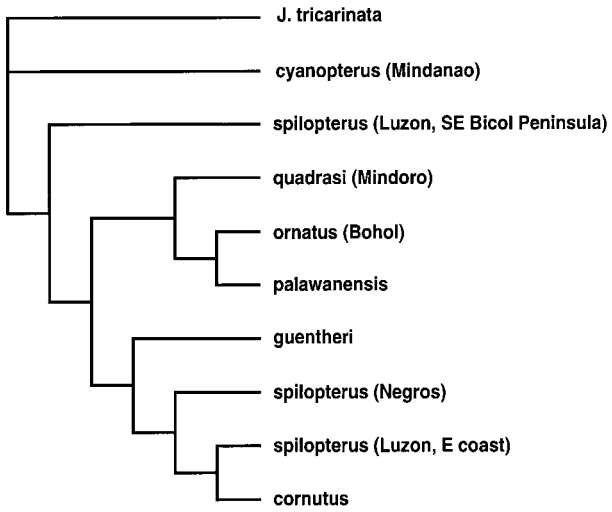

Figure 14. Parsimony estimate for the 10 individuals included in the permutation test. Transversions weighted five times greater than transitions, and codon positions 1, 2, 3, and the tRNA sites weighted 1.5:2.3:1:2.

data similar to those expected if the true history of the group was one of simultaneous branching, this 10-individual data set was shuffled 100 times using MacClade 3.04. The 100 shuffled data sets were used to generate a null distribution composed of 200 bootstrap values (two from each shuffled matrix) obtained following the procedures of Jackman *et al.* (1999). As Jackman *et al.* (1999) indicated, this approach should result in a relatively conservative test such that the null hypothesis of a hard polytomy may be overly difficult to reject. From the randomized data, values corresponding to the 95th percentile were used as the critical value for significance testing. According to this criterion, a bootstrap proportion value of 91 is the critical value for this data set and observed bootstrap proportions equal to or greater than 91 in the subsequent analyses indicate that simultaneous branching can be rejected for the taxon set in question. The 126 possible four-taxon combinations drawn from the unshuffled data were each subjected to a bootstrap analysis and the resulting bootstrap proportion values were compared to this critical value. In the 126 comparisons, a bootstrap proportion greater than or equal to 91 was observed once (mean observed bootstrap proportion value = 56.1, maximum observed value = 91). Thus, according to this rather conservative test, the null hypothesis of a hard polytomy cannot be rejected for the Philippine *volans* group.

CONCLUSIONS

Phylogenetic analysis of mitochondrial DNA sequence data under maximum likelihood, maximum parsimony, and minimum evolution (LogDet) optimality criteria provided a robust estimate of *Draco* phylogenetic relationships. Although this does not represent the first phylogenetic analysis of this group using modern methods, it does provide the first such analysis with extensive taxonomic sampling. The topologies recovered under the three alternative optimality criteria are not entirely congruent, but every node that is well supported under any one of the three criteria was recovered under all three. Relationships within the major clades are generally well-resolved and strongly supported, although this is not the case for the Philippine *volans* group. The hypothesis that lack of well-supported resolution within the Philippine *volans* group is the result of this group having undergone a rapid radiation was evaluated using a permutation test and could not be rejected.

ACKNOWLEDGEMENTS

We are indebted to the following curators for loan of specimens: Darrel Frost (AMNH), Barry Clarke and E. N. Arnold (BMNH), Jens Vindum, Bob Drewes, and Alan Leviton (CAS), Ellen Censky and John Wiens (CM), Robert S. Kennedy and John Ferner (CMNH), Robert Inger, Alan Resetar, and Harold Voris (FMNH), Frank Burbrink and Doug Rossman (LSUMNS), John Cadle and Jose Rosado (MCZ), Marinus Hoogmoed (RMNH), Anna Wong (Saah Museum), Richard Etheridge and Tod Reeder (SDSU), Ron Crombie, Kevin de Queiroz, and Addison Wynn (USNM), Ric How (WAM), and Jakob Hallerman (ZMH). For providing tissue samples, we are grateful to Rafe Brown, Mike Forstner, Ric How, Bob Kennedy, Bob Macey, Bob Murphy, and Steve Goodman. Bob Macey generously provided the DNA sequence for *Japalura tricarinata*. The Smithsonian Institution's Laboratory of Molecular Systematics provided the senior author with supplies and access to lab equipment during the later stages of this study. Thanks also to Dave Swofford at LMS for generously providing computer access. For facilitating collecting and export permits from the government of Indonesia, we thank Dr Jatna Supriatna (University of Indonesia at Depok), LIPI, and the leadership and staff of the Museum Zoologicum Bogor (especially Dr Siti Nuramaliati Prijono and Mumpuni Sancoyo). For facilitating a research pass and collecting and export permits in Malaysia, we are grateful to Munirah Abd. Manan (Unit Perancang Ekonomi, Malaysia), Francis Liew and Maklarin Lakim (Sabah Parks), and Jasmi Bib Abdul and Sivananthan Elagupillay (Department of Wildlife and National Parks, Malaysia). For facilitating collecting permits in the Philippines, we are indebted to Angel Alcala (Silliman University, Philippines) and Roger Sison (Philippine National Museum). For assistance and companionship in the field, JAM would like to thank Chris Austin, Julie Barcelona, Rafe Brown, Lito Bulalacao, Ron Crombie, Robert Dudley, Lee Grismer, Brad Hollingsworth, Ulrich Kuch, Mohamed Izhan Bin Mohamed Ilhan, Humberto Wong, Vicente 'the Terminator' Yngente, and Frank Bambang Yuwono. JAM's field work was supported by grants from the National Geographic Society, The Explorer's Club, The New England Herpetological Society, The University of Texas Department of Zoology, and the Texas Memorial Museum. John Allen, Rafe Brown, David Cannatella, Sharon Messenger, and an anonymous reviewer provided valuable comments on earlier drafts of the manuscript that substantially improved the final product. This work was initiated as part of the senior author's doctoral dissertation at the University of Texas and extended during the course of a postdoctoral fellowship at the Smithsonian Institution.

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APPENDIX 1

TISSUE SAMPLE VOUCHER SPECIMENS AND GENBANK ACCESSION NUMBERS

Aphaniotis fusca (TNHC 57874 – Malaysia: Selangor, 30 km N Kuala Lumpur via Rt. 68, Ulu Gombak Field Studies Centre; AF288228), *Bronchocela cristatella* (TNHC 56517 – Malaysia: Sarawak, junction of Buntal and Santubong roads, 25 km N Kuching; AF288229), *Japalura splendida* (LSUMZ 81212 – locality unknown; AF288230), *Draco beccarii* (LSUMZ 81223 – Indonesia: Sulawesi Island, South Sulawesi Province, 60 km N Palopo; AF288276), *D. biaro* (LSUMZ 81270 – Indonesia: Biaro Island, North Sulawesi Province, Desa Lalinsaheng; AF288277), *D. bimaculatus* (TNHC 57786 – Philippines: Samar Island, Western Samar Province, Hinabangan Municipality, Bagacay, Bagacay Mines;

AF288241), *D. blanfordii* (TNHC 56531 – Malaysia: Perlis, 2 km E Thai border on road between Wang Kelian and border; AF288242), *D. boschmai* – Flores (WAM 104530 – Indonesia: Flores Island, Robo; AF288269), *D. boschmai* – Lembata 1 (WAM 105107 – Indonesia: Lembata Island, Belang; AF288271), *D. boschmai* – Lembata 2 (WAM 105108 – Indonesia: Lembata Island, Belang; AF288272), *D. boschmai* – Sumba (WAM 101714 – Indonesia: Sumba Island, Wailonda; AF288270), *D. boschmai* – Sumbawa (WAM 98623 – Indonesia: Sumbawa Island, Batudulang; AF288273), *D. bourouniensis* (LSUMZ 81297 – Indonesia: Buru Island, Maluku Province, Dusun Labuang [near Namrole]; AF288279), *D. caerulhians* (LSUMZ 81307 – Indonesia: Sangir Besar Island, North Sulawesi Province, Tahuna; AF288281), *D. cornutus* (TNHC 56769 – Malaysia: Sabah, Poring Hot Springs; AF288244), *D. cristatellus* (TNHC 56763 – Malaysia: Sarawak, Gunung Santubang; AF288255), *D. cyanopterus* (TNHC 56842 – Philippines: Mindanao Island, Davao Del Sur Province, Taril Municipality, Barangay Upper Baracatan, Sitio San Roque; AF288245), *D. fimbriatus* – Borneo (TNHC 56764 – Malaysia: Sabah, Poring Hot Springs; AF288254), *D. fimbriatus* – Java (LSUMZ 81441 – Indonesia: Java Island, no specific locality; AF288257), *D. fimbriatus* – Malay Peninsua (TNHC 57954 – Malaysia: Perak, Bukit Larut; AF288256), *D. formosus* (TNHC 56540 – Malaysia: Selangor, 30 km N Kuala Lumpur via Rt. 68, Ulu Gombak Field Studies Centre; AF288263), *D. guentheri* (TNHC 58847 – Philippines: Mindanao, Davao City Province, Calinan Municipality, Malagos Eagle Station; AF288260), *D. haematopogon* (TNHC 56847 – Malaysia: Perak, Bukit Larut; AF288259), *D. indochinensis* (ROM 31987 – Vietnam: Krong Pa; AF288243), *D. maculatus maculatus* (TNHC 56576 – Malaysia: Perlis, 1.5 km W of intersection of Route 7 and westbound road to Kaki Bukit, Forestry Research Institute of Malaysia compound; AF288248), *D. maximus* (TNHC 56803 – Malaysia: Sabah, Poring Hot Springs; AF288231), *D. melanopogon* (TNHC 56584 – Malaysia: Selangor, 30 km N Kuala Lumpur via Rt. 68, Ulu Gombak Field Studies Centre; AF288258), *D. mindanensis* (TNHC 58848 – Philippines: Mindanao, Davao City Province, Calinan Municipality, Malagos Eagle Station; AF288249), *D. obscurus* (TNHC 56814 – Malaysia:, Sabah, Poring Hot Springs; AF288250), *D. ornatus* – Bohol (TNHC 58506 – Philippines: Bohol Island, Bohol Province, Bilar Municipality, Barangay Riverside; AF288253), *D. ornatus* – Samar (TNHC 55072 – Philippines: Samar Island, Western Samar Province, Paranat Municipality, Barangay San Isidro, Sitio Nasarong; AF288252), *D. palawanensis* (TNHC 56719 – Philippines: Palawan Island, Palawan Province, Quezon City, vicinity of National Museum; AF288262), *D. quadrasi* – Mindoro (TNHC 55067 – Philippines: Mindoro Island, Oriental Mindoro Province, San Teodoro Municipality, Barangay Lumang Bayan, 24 km from Calapan on Calapan–Puerta Galera road; AF288261), *D. quadrasi* – Sibuyan (FMNH 236070 – Philippines: Sibuyan Island, Romblon Province, 1.5 km S, 1.25 km E Magdiwang, vicinity of Tampayan; AF288268), *D. quinquefasciatus* (TNHC 56829 – Malaysia: Selangor, 30 km N Kuala Lumpur via Rt. 68, Ulu Gombak Field Studies Centre; AF288232), *D. reticulatus* – Bohol (TNHC 56702 – Philippines: Bohol Island, Bohol Province, Carmen Municipality, Barangay Buena Vista, Chocolate Hills complex; AF288247), *D. reticulatus* – Samar (TNHC 55055 – Philippines: Samar Island, Northern Samar Province, Allen Municipality, Barangay Tasvilla, appx. 3 km N Allen Ferry Landing; AF288246), *D. rhytisma* (LSUMZ 81327 – Indonesia: Peleng Island, Sulawesi Tengah Province Kecematan Liang; AF288280), *D. spilonotus* (LSUMZ 81375 – Indonesia: Sulawesi Island, North Sulawesi Province, Airmadidi, base of Gunung Klabat; AF288282), *D. spilopterus* – Cebu (TNHC 58493 – Philippines: Cebu Island, Cebu Province, Municipality of Cebu City, Barangay Talamban, Sitio Dita; AF288239), *D. spilopterus* – Negros (ROM 774 – Philippines: Negros Island, Dumaguete, Silliman University Marine Laboratory; AF288237), *D. spilopterus* – Panay (TNHC 58484 – Philippines: Panay Island, Antique Province, Barangay Calacja; AF288238), *D. spilopterus* – Siquijor (TNHC 58527 – Philippines: Siquijor Island, Siquijor Province, Barangay Luyang; AF288240), *D. spilopterus* – Polillo (TNHC 55019 – Philippines: Polillo Island, Quezon Province, Polillo Municipality, Barangay Sibucan, Sitio San Francisco; AF288236), *D. spilopterus* – Central Bicol Peninsula of Luzon (CMNH 5903 – Philippines: Luzon Island, Camarines Sur Province, Naga City Municipality, Barangay Binunuaan; AF288234), *D. spilopterus* – SE Bicol Peninsula of Luzon (TNHC 57775 – Philippines: Luzon Island, Sorsogon Province, Irosin Municipality, Barangay Manban, Sitio San Benon, 4 km NNE Irosin Centro at Mateo Hot and Cold Springs Resort, edge of Mt. Gapayao; AF288233), *D. spilopterus* – E coast of Luzon (TNHC 55009 – Philippines: Luzon Island, Quezon Province, Real Municipality, Barangay Maragondon; AF288235), *D. sumatranus* – Borneo (TNHC 56733 – Malaysia: Sarawak, Kuching, Taman Budaya; AF288264), *D. sumatranus* – Malay Peninsula (TNHC 56728 – Malaysia: Selangor, 30 km N Kuala Lumpur via Rt. 68, Ulu Gombak Field Studies Centre; AF288265), *D. sumatranus* – Sumatra (TCWC 73123 – Indonesia: Sumatra Island, Lhoknga, 27 km S Banda Aceh; AF288266), *D. taeniopterus* (TNHC 56685 – Malaysia: Perlis, 2 km E Thai border on road between Wang Kelian and border; AF288251), *D. timoriensis* – Roti (WAM 105619 – Indonesia: Roti Island, Baa; AF288274), *D. timoriensis* – Timor (WAM 107005 – Indonesia: Timor Island, Kupang; AF288275), *D. volans* (LSUMZ 81441 – Indonesia: Java Island, West Java Province, Jakarta; AF288267), *D.* 'Luwuk' (LSUMZ 81258 – Indonesia: Sulawesi Island, Sulawesi Tengah Province, on cross-peninsular road between Luwuk and Kemumu, appx. 10 km N coastline; AF288283), *D.* 'Tagulandang' (LSUMZ 81405 – Indonesia: Tagulandang Island, North Sulawesi Province, Desa Haasi; AF288278).

APPENDIX 2

SPECIMENS EXAMINED

Osteological specimens are followed by an 'S'. Museum acronyms follow Leviton *et al.* (1985) except for the following nonstandard abbreviations: CMNH (Cincinnati Museum of Natural History herpetology collection) and RSK (Robert S. Kennedy field numbers).

Draco beccarii – (INDONESIA: Sulawesi: AMNH

63395, CAS 24212, LSUMZ 81214–81256). *D. biaro* – (INDONESIA: Biaro Island: LSUMZ 81259–81270, MCZ 170899–907). *Draco bimaculatus* – (PHILIPPINES: Basilan Island: CAS 60193–94; Leyte Island: CAS 85649–50, USNM 160018, 318426–29; Mindanao Island: TNHC 56770–83, 57787S, 58852, USNM 135978, 229368–70; Samar Island: TNHC 55082–84, 55086–90, 55092–95, 57774–86S). *D. blanfordii* – (BURMA: AMNH 58506–08; MALAYSIA: Pahang: TNHC 57637; Perak: TNHC 56532–39, 57553, 57627–36, 57811–12S, 58582–85; Perlis: TNHC 56521–31, 57554–57, 57638–39, 57806–10S, 58586–94). *D. boschmai* – (INDONESIA: Flores Island: FMNH 154836, TNHC 59289–95, WAM 104530, 104621, 104629, 104647, 105020, 105074, 105385, 105465; Komodo Island: AMNH 32094; Lembata: WAM 105104–08, 105110–12, 105116, 105130–32, 105222–23, 105292–93; Lombok: WAM 99790-91; Sumba: WAM 101714, 101733–44, 101853; Sumbawa: WAM 98608, 98623, 98633). *D. bourouniensis* – (INDONESIA: Ambon Island: CAS 64255, LSUMZ 81271–81; Buru Island: LSUMZ 81294–81304; Seram Island: AMNH 21110, LSUMZ 81282–81293). *D. caerhulians* – (INDONESIA: Sangir Besar Island: LSUMZ 81305–81318, MCZ 173314–18, 173322–26). *D. cornutus* – (INDONESIA: Kalimantan: USNM 51664; MALAYSIA: Sabah: FMNH 239336, 248963, TNHC 56767–69, 56790S). *D. cristatellus* – (MALAYSIA: Sabah: FMNH 63701–02, 76253; Sarawak: FMNH 150621, 150624, TNHC 56763). *D. cyanopterus* – (PHILIPPINES: Camiguin Sur Island: CAS 28200, 28349; Mindanao Island: BMNH 77.10.9.13, 77.12.13.16, CAS 15554–55, TNHC 56838–46, 57801–05S, 58849, USNM 38394, 38885–86, 38998). *D. dussumieri* – (INDIA: CAS 10970, CM 65105–09, 114857, FMNH 95960, LSUMZ 24730, 24732). *D. fimbriatus* – (INDONESIA: Java: CAS 64264, LSUMZ 81445–49; MALAYSIA: Perak: TNHC 57954–56; Perlis: TNH 58565; Sabah: TNHC 56764–66; Sarawak: AMNH 57243; Selangor: TNHC 57591, 57953; Thailand: MCZ 39096S). *D. formosus* – (MALAYSIA: Pahang: TNHC 56543, 56553, 56556–57, 57858–60S; Perak: CAS 10967, TNHC 56560–61, 56564–65; Selangor: TNHC 56540–5642, 56544–52, 56554–55, 56558–59, 56562–63, 56566–67, 57558–66, 57700–16, 57855–57S, 57861–65S, 57866–67; THAILAND: AMNH 8801). *D. guentheri* – ('PHILIPPINES:' BMNH 79.4.16.4; Basilan Island: CAS 60370–72; Jolo Island: CAS 18410–11, 60889–95, USNM 38742, 38744–48; Mindanao Island: CAS 10372, 10972–83, CMNH 5660, 5667, 5671–72, TNHC 56837, 58847, USNM 37407, 34752–55, 229443–44; Siminul Island: CM 1856–57, 1859–65). *D. haematopogon* – (MALAYSIA: Perak: TNHC 56847–61, 57567–75, 57665, 57791–94S, 58561–64; Sarawak: AMNH 111836, CAS 8457–59, USNM 197948–49). *D. indochinensis* – (VIETNAM: ROM 30633, 31987–93). *D. jareckii* – (PHILIPPINES: Batan Island: CMNH 3782–84, 5657, MCZ 44141, PNM 5776–77, RSK 2027, 2063, 2108, USNM 266641S, 266501, 266513). *D. maculatus* – (BURMA: CAS 8427; CHINA: Hainan: AMNH 30904–09, 30945–56; MALAYSIA: Perlis: TNHC 56568–80, 57592–621, 57795–99S; THAILAND: CAS 23590–98, 23600, 23602–23603; VIETNAM: ROM 30721–23, 30729–30, 32001–04). *D. maximus* – (MALAYSIA: Pahang: TNHC 56809; Sabah: TNHC 56795–804, 57831–33S; Sarawak: AMNH 111838; Selangor: TNHC 56794, 56805–08, 56810–11, 57751–52, 58559–60). *D. melanopogon* – (MALAYSIA: Pahang: TNHC 56582, 56592–94, 56627, 56629–33, 57672–75, 57846–47S; Perak: TNHC 57647; Perlis: TNHC 56641; Sabah: TNHC 56595–97; Selangor: TNHC 56581, 56583–89, 56591, 56598–99. 56601–24, 56634–40, 56642, 57640–46, 57648–64, 57666–71, 57676–99, 57840–45S, 57848–54S, 58568–81). *D. mindanensis* – (PHILIPPINES: Leyte Island: CAS 24600, 24639; Mindanao Island: CAS 23561–62, 61974, 133566, 133684, CMNH 5673–75, FMNH 63157, LSUMZ 41679, PNM 5777–78, TNHC 58848). *D. modigliani* – (INDONESIA: Enggano Island: FMNH 97968, MCZ 46912–13, RMNH 19768, 19784, 19803–04, 19815–16, 19818, 19820, USNM 35807–08, 35810–11, ZMH 4906). *D. obscurus* – (MALAYSIA: Sabah: TNHC 56812–20, 57816S; Sarawak: AMNH 111855, CAS 105999, MVZ 111812–13, USNM 197959–61). *D. ornatus* – (PHILIPPINES: Bohol Island: TNHC 58464S, 58505–12, 58851, USNM 228979, 228981, 228983, 228986, 228987–89, 228992, 228994–96, 228998–229004, 229007, 229010–13, 229015–18, 229020, 229022–24, 229030, 229032–33, 229035, 229039–40, 229042–47, 229049–52, 229054, 229057–59, 229061–62; Leyte Island: CAS 24626, 24628; Mindanao Island: CAS 133151, 133254, CM 1919–20, CMNH 5676–77, PNM 5779; Samar Island: FMNH 96498–507, TNHC 55070–81, 57770–73S, USNM 533121–23). *D. palawanensis* – (PHILIPPINES: Palawan Island: CAS 28612, 28614–16, 28649, 157297–98, 157328, 157350, CMNH 5636–42, PNM 5769, 5770, 5771–75, TNHC 56707–25, 57827–30S, 58853, USNM 158260–63, 229494–95, 287406, 287407). *D. quadrasi* – (PHILIPPINES: Mindoro Island: TNHC 55064–69; Romblon Island: USNM 38638; Semirara Island: CAS 127851–52; Sibuyan Island: CAS 62480–81, 73853–57, 139176–78, CM 2238–39, 2240S, 2242–43, 236070, MCZ 20096, USNM 36171–73, 496847–48). *D. quinquefasciatus* – (MALAYSIA: Pahang: TNHC 57817–18S; Sarawak: AMNH 111847–50, USNM 197962–63; Selangor: TNHC 57549–50, 57622–26, 57819S). *D. reticulatus* – ('PHILIPPINES:' BMNH 1946.8.27.28; PHILIPPINES: Bohol Island: FMNH 202747, TNHC 56700–07, 56862–69, 58461S, 58463S, USNM 228978, 228980, 228982, 228984–85, 228990–91, 228993, 228997, 229005–06, 229008–09, 229014, 229019, 229021, 229025–29, 229031, 229034, 229036–38, 229041, 229048, 229053, 229055–56, 229060; Lapinin Chico Island: CAS 27521; Leyte Island: CAS 60922–25, 85640, MCZ 26169, USNM 133720; Samar Island: TNHC 55055–58, 55060–63, 57800S). *D. rhytisma* – (INDONESIA: Peleng Island: LSUMZ 81319–70). *D. spilonotus* – (INDONESIA: Sulawesi: CAS 103623, CM 113414, LSUMZ 81371–99, MCZ 170928–33, 173351–55). *D. spilopterus* – ('Borneo?: ' BMNH 1947.8.27.25, holotype of *D. rostratus*, true locality unknown); PHILIPPINES: Bantayan Island: CAS 124483–84; Boracay Island: CAS 127886, 127916, 127961, 128031; Carabo Island: CAS 128151, 128162, 128169; Catanduanes Island: FMNH 247989, USNM 318700–32; Cebu Island: FMNH 96282–86, 96566–69, TNHC 58462S, 58496–504, 59491–94; Guimaras Island: CAS 125277, 125280–81, 125295, USNM 38990–96; Inampulugan Island: CAS 27966, 28027; Kalotkot Island: CAS 60554–55; Lubang Island: CM 1833S, 1834–36, 1837S, 1838, 1841S, 1842–45, 1847–50, USNM 89140–42; Luzon Island: CAS 61108, CM 1846, 1851–55, PNM

2483, CMNH 4387–89, TNHC 55005–13, 55097–06, 57754–58S, 57766S; USNM 31189, 38635–37, 56677, 58808–09, 163980–84, 180200, 228193–94, 291418–25, 306003–06, 318328–30, 512834–45; Marinduque Island: CM 65111–13, 65114–15S, 65116–18, 65120; Masbate Island: CAS 144231, 144244; Mindoro Island: CAS 20339; Negros Island: AMNH 86604–07, CAS 17962–63, 17967–71, 92865, 92868, 92873, ROM 17455–71, TNHC 58458–60S, 58540–51, 58553–55, 58557–58, USNM 78158, 209380–81, 228314–17, 229588, 305936–54; Panay Island: TNHC 58465–67S, 58471–80, 58482–89, 58850, USNM 38991–92, 496866–67; Polillo Island: TNHC 55014–22, 55098, 55107–11, 57759–65S, 57767–69S, 58453S; Siquijor Island: CAS 26333–36, TNHC 58468–70S, 58513–15, 58516–38, 58854; Tablas Island: CAS 139188, 185499, USNM 496889–90). *D. sumatranus* – (INDONESIA: Sumatra: TCWC 73123; MALAYSIA: Pahang: TNHC 58598–612; Sarawak: TNHC 56729–37, 57820–26S; Selangor: MVZ 81721–24, TNHC 56726–28, 58595–97). *D. taeniopterus* – (MALAYSIA: Perlis: TNHC 56682–99, 57576–90, 57834–39S). *D. timoriensis* – (INDONESIA: Alor Island: WAM 107583–107694–95, 107959, 107962–64, 107969–72, 108020–29, 108031–43; Roti Island: WAM 105618–19, 105664, 105691–92, 105699, 105728, 105733, 105767–71, 105780–86, 105825; Semau Island: WAM 105906; Timor Island: AMNH 102202, CAS 64253, FMNH 154849–50, WAM 101513, 101523, 101528–29, 101553–54, 101560–63, 107005, 107036, 107055–56, 107074–82, 107116, 107118–26, 107129–31, 107171–72, 107272–73, 107279–303, 107360–62; Wetar Island: AMNH 32151, WAM 117579–80, 117626–27, 117640). *D. volans* – (INDONESIA: Java: FMNH 131030–39, LSUMZ 53014, 81429–44). *D.* sp 'Luwuk' – (INDONESIA: Sulawesi: LSUMZ 81257–58). *D.* sp. 'Tagulandang' – (INDONESIA: Tagulandang Island: LSUMZ 81400–28). *D.* sp. 'Camiguin Norte' – (PHILIPPINES: Camiguin Norte Island: USNM field 054876–77).