Phylogenetic Relationships of the Flying Lizards, Genus Draco (Reptilia, Agamidae)

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ABSTRACT—Phylogenetic relationships among 12 species of the genus *Draco* were inferred from 779 base pairs of mitochondrial 12S and 16S rRNA genes and allozymes for 20 presumptive loci. Results indicated the presence of at least four distinct lineages within the genus. The first lineage consists of *D. volans* and *D. cornutus*, whereas the second only of *D. lineatus*, which exhibits a great genetic divergence between two subspecies. The third is monotypic with *D. dussumieri*, the only species distributed in southern India. The fourth included all the remaining species. The third and fourth lineages are supposed to exclusively share a common ancestor. It is likely that the common ancestor of whole *Draco* originally diverged into three groups, the ancestors of the first, second, and third and fourth lineages, by vicariance. In the fourth lineage, *D. blanfordii*, *D. haematopogon*, *D. melanopogon*, *D. obscurus* and *D. taeniopterus* are likely to be exclusively close to each other. The resultant phylogenetic tree contradicts the dichotomous relationships previously hypothesized on the basis of morphological characters.

INTRODUCTION

The genus *Draco* Linnaeus, 1758, consisting of some 21 species, is one of the most prominent genera of the family Agamidae, characterized by the presence of patagium (a wing-like skin-extension, supported by elongated ribs, along the flank). This genus is distributed in southern India and throughout Southeast Asia (Fig. 1). Based on morphological characters, Hennig (1936), Inger (1983) and Musters (1983) revised the taxonomy of *Draco*. However, phylogenetic relationships among its species are still poorly understood. Hennig (1936) briefly discussed diversification of *Draco* by assuming two major groups on the basis of the number of ribs supporting patagium — 5 or 6. However, this view was negated because of the presence of variation in the patagium rib number among obviously closely related populations (Musters, 1983). Musters (1983) also recognized two major lineages within the genus, that are, however, characterized by the direction of nostrils — outward or upward. His phylogenetic hypothesis was

derived from the clustering of the distance matrix from morphological characters without an outgroup. It is argued that such a clustering suffers fundamental problems in constructing a phylogenetic tree (e.g., Wiley, 1981).

Karyological studies sometimes give fruitful information to the classification and phylogenetic inference of agamid lizards (e.g., Kupriyanova, 1984; Moody and Hutterer, 1978; Ota, 1988; Ota *et al.*, 1992; Sokolovsky, 1974). However, recent karyological surveys indicated that the karyotype of *Draco* is conservative, and that the divergence within the genus has been accompanied by little chromosomal differentiation (Ota and Hikida, 1989).

Analysis of sequence variations in some mitochondrial DNA genes, such as 12S and 16S ribosomal RNA (rRNA), is effective for estimating phylogenetic relationships among closely related species (e.g., Hedges and Bezy, 1993; Hedges *et al.*, 1991; Reeder, 1995). Allozyme electrophoresis is also useful to infer phylogenies of closely related species and populations (e.g., Hillis, 1985). In the present study, we analyze data for mitochondrial DNA sequence and frequency of allozyme alleles in *Draco* species. Our purposes are: (1) to assess the infrageneric phylogenetic hypothesis on *Draco*

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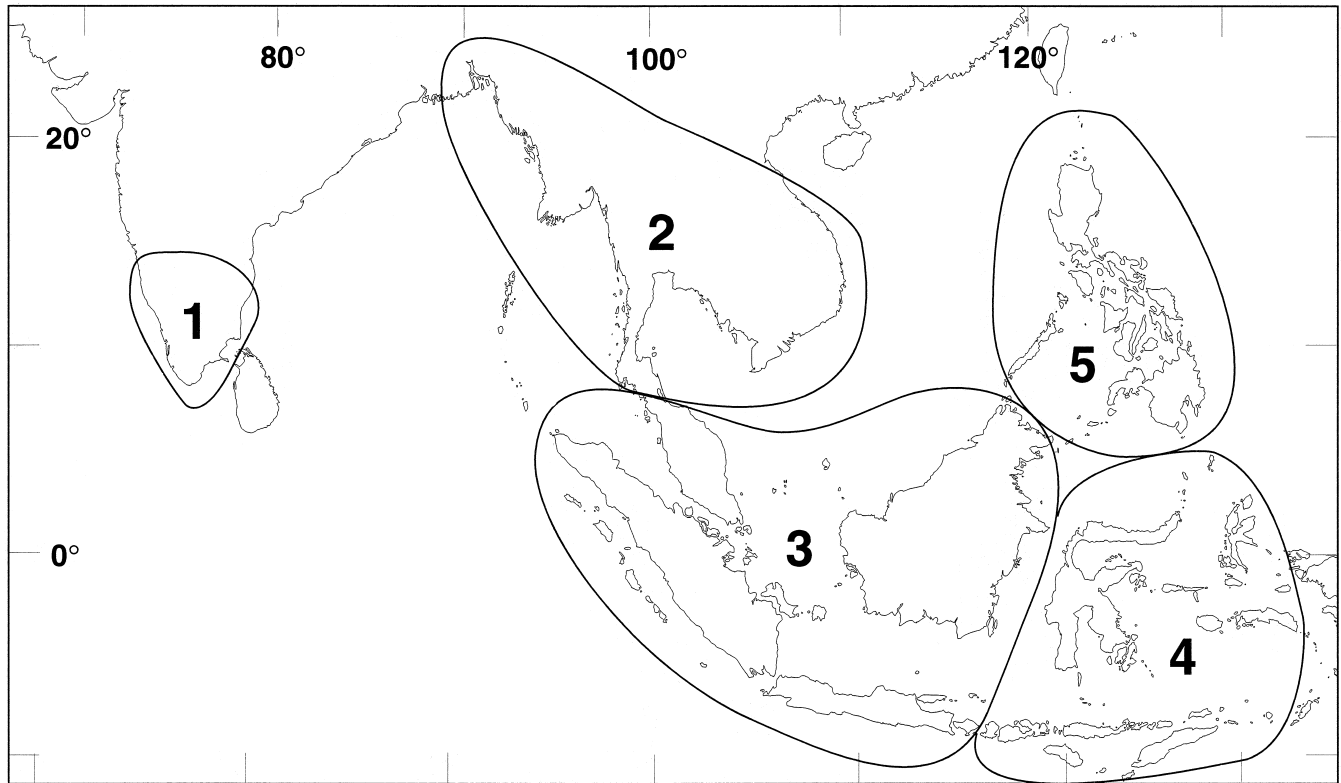   FAX. +81-75-753-4114.

Fig. 1. A map of Southeast and South Asia showing distribution of *Draco*. Following five areas are recognized on the basis of distributional patterns in *Draco* and other taxonomic groups: 1, Southern India; 2, Indo-China Peninsula; 3, Malay Peninsula and Greater Sunda Islands exclusive of Sulawesi; 4, Lesser Sunda Islands, Sulwaesi and Maluk Islands; 5, Philippines.

proposed by Musters (1983) and, when necessary, to submit an alternative hypothesis; and (2) to discuss the historical biogeography of the genus on the basis of the best fitting hypothesis determined in the above process.

Taxonomy of *Draco*

Linnaeus (1758) described *Draco* with *volans* from Java. Although some authors (e.g., Fitzinger, 1843) once split *Draco* into two genera, no questions have recently been posed on the monophyly of this genus, because of the exclusive possession of the highly specialized patagia for gliding by all of its members (e.g., Colbert, 1967).

Within the genus, however, taxonomy is much confused even at present, largely because of the presence of extensive intraspecific variation in morphological characters (Inger, 1983; Schlegel, 1844). Günther (1864), Boulenger (1885) and de Rooij (1915) recognized 14, 21 and 24 species, respectively, although the last author listed only species of Indo-Australian Archipelago. Hennig (1936) revised the genus and lumped 62 of the 64 nominal taxa described to that date into 14 valid species and 19 valid subspecies.

Recently two authors simultaneously revised the species taxonomy of *Draco* on the basis of morphological features (Inger, 1983; Musters, 1983). Despite their concurrency and methodological similarity, accounts of these authors substantially differed from each other. Musters (1983) recognized fol-

lowing 15 species (and 19 subspecies) for the genus: *D. blanfordii* (*D. b. blanfordii*, *D. b. indochinensis* and *D. b. norvillii*), *D. cornutus*, *D. dussumieri*, *D. fimbriatus* (*D. f. fimbriatus* and *D. f. hennigi*), *D. haematopogon*, *D. lineatus* (*D. l. lineatus*, *D. l. beccarii*, *D. l. bimaculatus*, *D. l, bourouniensis*, *D. l. modiglianii*, *D. l. ochropterus*, *D. l. rhytisma* and *D. l. spilonotus*), *D. maculatus* (*D. m. maculatus*, *D. m. divergens*, *D. m. haasei* and *D. m. whiteheadi*), *D. maximus*, *D. melanopogon*, *D. mindanensis*, *D. obscurus* (*D. o. obscurus*, *D. o. formosus* and *D. o. laepitecus*), *D. quinquefasciatus*, *D. spilopterus*, *D. taeniopterus*, and *D. volans* (*D. v. volans*, *D. v. boschmai*, *D. v. reticulatus*, *D. v. sumatranus* and *D. v. timorensis*). He also listed *D. affinis* as a valid species tentatively because no specimens of this species were available and the description by previous author (Bartlett, 1894) did not provide any useful information regarding its validity. However, he did not include *D. affinis* in his discussions because he thought this species might be a synonym of *D. v. sumatranus*. We thus did not include *D. affinis* in the following discussions, either. On the other hand, Inger (1983), while recognizing 15 species and no subspecies, synonymized *D. cornutus* and *D. spilopterus* with *D. volans*, and revalidated *D. bimaculatus* (a subspecies of *D. lineatus* in Musters, 1983) and *D. cristatellus* (a synonym of *D. fimbriatus* in Musters, 1983) as full species. Since then, three additional species, *D. biaro* and *D. caerulhians* from the Sangihe Archipelago, and *D. jareckii from*

the Batan and Babuyan Islands, have been described (Lazell, 1987, 1992), and three species from the Philippines, *D. bimaculatus* (see above), *D. everetti* (a synonym of *D. v. reticulatus* in Musters, 1983) and *D. ornatus* (a synonym of D. spilopterus in Musters, 1983) have been revalidated (Ross and Lazell, 1990).

Although Inger (1983) examined three species groups of this genus (*D. volans* including *D. cornutus* and *D. spilopterus*, *D. cristatellus* and *D. fimbriatus*, and *D. blanfordii*, *D. obscurus* and *D. taeniopterus*) in detail, there still exist some problems in their classification (e.g., on the validity of *D. cornutus*: Honda, *et al.*, 1999). Because the work by Musters (1983), deriving from examination of samples much greater than those of Inger (1983), seems to be more comprehensive and is accompanied by a hypothesis on the infrageneric phylogeny, we chiefly regarded his classification as the most recent working hypothesis, from which our study should start. Of the six species described or revalidated more recently (see above), *D. biaro*, *D. bimaculatus* and *D. caerulhians* were tentatively assumed to be closest to *D. lineatus*, *D. ornatus* to *D. spilopterus*, and *D. everetti* and *D. jareckii* to *D. volans* in the phylogenetic and biogeographical discussions. We have had no chances to examine those six species, and these *a priori* assumptions are based on their morphological similarities that are seemingly obvious from descriptions in Musters (1983), Lazell (1987, 1992) and Ross and Lazell (1990).

MATERIALS AND METHODS

Samples analyzed

A total of 25 specimens belonging to 16 species/subspecies of *Draco* and 176 to 15 species/subspecies of the genus were, respectively, subjected to DNA and allozyme analyses (Table 1). Voucher specimens were deposited in the herpetological collection of the Department of Zoology, Kyoto University (KUZ) (Appendix 1).

Livers, removed from anesthetized specimens, were stocked at $-80°C$ until experiments. Conspecific and consubspecific samples from Malay Peninsula and Borneo were designated as separate operational taxonomic units (OTUs), because in a few species (e.g., *D. haematopogon* and *D. quinquefasciatus*: Hennig, 1936; Taylor, 1963) morphological differentiations have been reported for populations from these two regions. In his phylogenetic analysis of Agamidae based on the weighted Wagner tree algorithm, Moody (1980) hypothesized the sister group relationship of *Draco* with *Ptyctolaemus* and placed *Aphaniotis* as one of the closely related genera to this clade. Thus, we selected two species, *Aphaniotis fusca* and *Ptyctolaemus phuwuanensis*, as outgroups among non-*Draco* agamids for which tissues were available to us. In the allozyme analysis, *D. haematopogon* from Malay Peninsula, *D. quinquefasciatus* from Borneo, *D. volans volans* and *Aphaniotis fusca* were excluded, because only one specimen was available for each. Of the species recognized by Musters (1983), *D. fimbriatus*, *D. mindanensis* and *D. spilopterus* could not be examined in the present study.

DNA amplification and sequencing

Samples for DNA sequencing were prepared following the method by Wada *et al.* (1992). The frozen tissues were powdered, and were lysed in TE buffer (1 mM Tris-HCl 0.1 M EDTA, pH 8.0) containing 0.5% sodium dodecyl sulfate. After digesting samples with proteinase K (100 μg/ml) at 50°C for three hours, DNA was extracted with phenol, and was precipitated in ethanol with an equal volume of 5.0 M ammonium acetate. Samples resuspended in TE buffer were further purified by RNase digestion (20 μg/ml) at 37°C for one hour, followed by ethanol precipitation.

A part of mitochondrial 12S and 16S rRNA genes were amplified using the polymerase chain reaction (PCR) in a thermal cycler (Perkin-Elmer Corp., Norwalk, USA) using primers L1091 (5'-AAACTG-GGATTAGATACCCCACTAT-3') and H1478 (5'-GAGGGTGAC-GGGCGGTGTGT-3'), and L2606 (5'-CTGACCGTGCAAAGGT-AGCGTAATCACT-3') and H3056 (5'-CTCCGGTCTGAACTCAG-ATCACGTAGG-3'), respectively (Kocher *et al.*, 1989). The numbering system followed the human sequence (Anderson *et al.*, 1981).

Table 1. Localities and sample sizes for samples used for DNA and allozyme analyses.

Sample	Locality	Allozyme (N)	DNA (N)	Abbreviation
Draco blanfordii blanfordii	Peninsular Malaysia, Thailand	10	2	BL
D. cornutus	Borneo	3	1	CR
D. dussumieri	India	3	1	DS
D. haematopogon	Peninsular Malaysia	–	1	HMM
	Borneo	7	1	HMB
D. lineatus beccarii	Sulawesi	3	1	LNB
D. l. spilonotus	Sulawesi	11	1	LNS
D. maculatus maculatus	Thailand	8	1	MCM
D. m. haasei	Thailand	21	3	MCH
D. maximus	Borneo	2	1	MX
D. melanopogon	Peninsular Malaysia, Thailand	18	1	MLM
	Borneo	3	1	MLB
D. obscurus obscurus	Borneo	7	1	OBO
D. o. formosus	Peninsular Malaysia	20	1	OBF
D. quinquefasciatus	Peninsular Malaysia	–	1	QNM
	Borneo	5	1	QNB
D. taeniopterus	Thailand	20	2	TN
D. volans volans	Java	–	1	VV
D. v. sumatranus	Peninsular Malaysia	15	2	VSM
	Borneo	20	1	VMB
Ptyctolaemus phuwuanensis	Thailand	10	1	PP
Aphaniotis fusca	Peninsular Malaysia	–	1	AF

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         1                                  50                                 100
AF  GCCTAGCTAT–AATCACCACACGCCAAAAAAGTTACAAGCGCAA––GCTTAAAACTTAAAAGACCTGGCGGTACTCTATATCACCCTAGAGGGGCCTGTC
PP  ....TA.CG.T..A..AA.A.T......G....–..G...C..CTCA...T......T.G.................C.....................
BL  ....GA..G.A...T..T.––.............–...........CGC––C...C.....C.....T.G.................A...T...........C.
CR  ...CGA.C..A..C.C.–..––...........–..GG..C.––C..C.....T..G...........G....C.................C.
DS  ....GA..G.A..C.T.T.––.............–.....TA.C–C...C...........................G.............C...
HMM ....GA..G.A...CT..T.––.............–..G...C.––C.....T..G.................C...A...........C.
HMB ..CGA..G.G...T..T.––.............–..G...C.––C.....T..G.................A...........C.
LNB ....GA..G.A...T..A.––.............–..G...CA.C––....G.................C...GA.........T.....
LNS ....A..G.A...T..T.––.............–..TG...C.GC–CA...G...G.................A...........T....
MCM ....G...G.A...T..TT.––.............–.......CA.––C...G.....G.................G.................CT
MCH ....G...G.A...T..T.––.............–.......CA.––C...G.....G.................G.................CT
MX  ....GA..G.A...CT–.T.––.............–.......C..––C...C.....T.G.................A.................C.
MLM ....GA..G.A...CT..T.––.............–.......C..––C...C.....T.G.................A.................C.
MLB ....GA..G.A...CT..T.––.............–.......C.C––C...C.....T.G.................A.................C.
OBO ....GA..G.A..CT.AT.––.............–..G...C.––T...G....C.T..G.................G.....G.........CT
OBF ....GA..G.A..CT.AT.––.............–.......C.––C...G.....T.G.................C...A...G.........C.
QNM ....GA..G.A..CT.TT.––.............–..TG..C.C––C...C.....T.G.................C...A...........T...C.
QNB ....GA..G.A..CT..T.––.............–..TG..C..––C...C.....T.G.................A.................C.
TN  ....A..G.A..CT.AT.––.............–..G...CT––C...G.....T..G.................C...A...G.........C.
VV  ...C.A..G.A...TTTT.––.............–..G...C.––C.....T.G.................C.C..A...........T.C.C.
VSM ...C.A..G.A...TTT–.––.............–..G...T.––C.....T.G.................C...GT.........T.C.C.
VMB ...C.A..G.A...TTT–.––.............–..G...C.––C.....T.G.................C...AT.........T.C.C.
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         101                                150                                200
AF  TAGTAATCGATACTACACGAAAAAACCTAACCTCTTCTAGCCACT–TCAGCCTATATACCGCCGTCGAAAGCTTATCTCACAAGAAATAGAAGCTAAAAAG
PP  CCA.........A.C.C..CTCT....T...AT....G...C.CTC......G.............CC...ACG.T...........G–.........C..
BL  CT....C.....A.C.....G.....C...ATC...T...T–––.................TCC...AC..T......A....G–G...T..C..
CR  CT....C.....ACC...........T...ATC.A.T....––C.................TCC...AC..T......A...G–.....G...T..
DS  C.A...C.....A.C....T.....CT...G.C..C.......–................TCC...AC..T.TT..A...G.....CT..T..
HMM CT....C.....A.C.....G.....C..A.....G..C–––C–...............TTC...AC..T......A...G–.....C..T..
HMB CTA...C.....ACC......G.....C....T.........C–––C...............TTC...AC..T......A...G–.....CG.T..
LNB CTA.......C.A.C.G.........C...ATC...T....T–C................TCC...AC..T.TC.......G–.....A...C..
LNS CTA...C.....A.C.......T...ATC...T....––C................TTC...AC..T......A...G–.....C...C..
MCM C...........A.C......G.....C..AT...T....––................TTC...AC..T......A...G–.........C..
MCH C...........A.C.......C...AT...T....––................TTC...AC..T......A...G–.........C..
MX  C.A...C.....A.C.......T...ATC......T––................TTC...AC..T......A...G–.....CC..C..
MLM C.A...C.....A.C.......C...A.....C..C––...............TCC...AC..T......A...G–.....CC..C..
MLB C.A.........A.C.......C...AT....C..C––...............TTC...AC..T......A...G–......C..C..
OBO C.A...C.....A.C......G.....T...ATC.......––C................TTC...AC..T......A...G–.....CC..C..
OBF C.A...C.....A.C......G.....T..G.C..G....––...C................TCC...AC..T......A...G–.....CC..C..
QNM CT....C.....ACC......G.....C...ATCC..C...T––................TTC...AC..T......A...G–.....CC..T..
QNB CT....C.....ACC......G.....C...A.CC..C...T––...............TTC...AC..T......A...G–.....CCG.T..
TN  C.A...C.....A.C......G.....T...A.C.......T––C................TTC...AC..T......A...G–.....CC.GT..
VV  CTA...C.....GA.C...G.......T...A.CC..T....––................TCC...AC..T......A...G–.....AG..C..
VSM CT....C.....GA.C.C..G.......T...A.CC..T....––................TCC...AC..T......A...G–.....A...C..
VMB CT....C.....GA.C.C..G.......T...A.CC..T....––C...............TCC...AC..T......A...G–.....A...C..
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         201                                250                                300
AF  TATAATTAGACTAACACGTCAGGTCAAGGTGTAGCATATGAAAAGGA––TAAGATTGGCCACAGTAC–––––AACACATACGAAAAACACCGCGAAATACGT
PP  CCC.––––CC........................TC......T..GG–CG............T...CTC..GT.C..AG...CTGTAAT....CCACA
BL  ...T–AC.C...G....................TC....G.C..ATA.GCGCG.A.....C...AAC...–.C.....C.C.ATT....C...G
CR  CCC..–GC.C––..GT..........G.........CC....G.T..GT–.G..G.G.A.....A..TCA–..–..C..A.....C..T.CT....CG.A.
DS  ...T–.ATC....GT..................T......GC...––..GCGCGA......A..ATAT...–GC..TG...C.CTGTT......TAG
HMM ..CC–ACGC.....T...................TC.....GT..GA–A.GCG.G.......T..TAAC...–.C..AG...C.C.GTT....C..AG
HMB ..CC–.CGC.....T...................TC.....GA–A.GCG.G.......T..TAAC...–.C..AG...C.C.GTT.......AG
LNB .CCC–AC.C...G..................CC...G.T...––AG..GCG.A....A.G.CGC...–.C..A....C...ATT....C.T..
LNS .T.C–ACGC...G....................T.....GGT....––AGC..CG.A......A...CAC...–CC..AG...C...ACT....CGT.A
MCM .C..–AC.C....................CC.......T..G–TA–GCGCG.A......A...CAC...–GC..A....T.TTATT....C.TAA
MCH .C..–AC.C....................TC.......T..GGTA–GCGCG.A......A...CAC...–GC..A....T.TTATT....C.TAA
MX  .TC–––CA.C...G...............CA...G.T..A–A.GCGCG.A.....T..AGAT...–.C..AG...C.CTGCT....C..AG
MLM ..C–––CC.C...................CC.......T..GATG.GCG.G.......C.GTAAT...–.C..AG...C.C.TCT....C.A.G
MLB ..CT–CC.C...................CC.......T..ATA.GCG.G.......T.G.–––..–...C..AG...C.C.ACT....C...G
OBO ..C.–AC.C...G................TC....GGT...G–A.GCGCG.A.....T.GAAAT..T–.C..A....CC.CTGCT....CG.AG
OBF ...C–AC.C...G................TC.C.GGC....A–A.GCGCA.......T.GAAAT...–.C..AGG.GC.CTGCT....CG.AG
QNM ..CC–.CGC.....T.............CA....G.T..A–A..AGCG.A.....C.GTAAT...–.C..AG...C.C.ATT....C.T.G
QNB ..CC–.CGC.....T.............CA...GGT..A–A..AGCG.A.....C.TAAT...–.C..AG...C.CTATT....C.TAG
TN  ..CC–.C.C...................TC...GGT...A–A.GCGCGA......T..AAAT.C..–...AG...C.CTGTT....C..AA
VV  CTC.CACCC––..G............G...........CA....GGT..GC–.G..G.G.A.....A...CA–..A–..C..AG...C....CT...C..A.
VSM CCC.CAC.C––..G............G...........CA....GGT..GT–.G..G.G.A.....A...CA–..A–..C..AG...C....CT...CG.A.
VMB CCC.CAC.C....G............G.........TA....GGT..GT–.G..G.G.A.....A...CA–..A––.C..AG...C....CT...CG.A.
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        301                             350                 *              400
AF      AAT---TAATAAGAATTTAGTAGTAAGAAAACCAACGAATAACTTTCTGAAAC-TTTCCCGCCCTAGAGTATGCTGTCCTCCAAATAAGGACCAGTATGAA
PP      C.GTTG..G.T.G.....C......TG.GGA..T...C.C.ACA.....C.----.GG.....T.....C...............................
BL      GG.-GA..G.C.......C--GGAGC.....GC.CCG.....C-AAA-.A...........C.........T....G.......C.....
CR      GG.-AA..G.C.......A....T--TGG-.....C.CAA.....C--ACTA......C.C............G...............
DS      G.CAGA..G.........C......G--.GGGTT....GC.C.C.....C--C.C.A.....C.........T....G.......C.....
HMM     G..-AA..G.C.......C......C--GGAGC.....GC.CCA.....C.AAAA.A...........C.........T....G.......C.....
HMB     G..-GA..G.C.......C......C--.GAGC.....GC.CCA.....C.AAAA.A...........C.........T....G.......C.---
LNB     GGA-GG..G.........C......--GGGGC.....GC.CC.....C------..T......C.........T....G.......
LNS     GGA-AA..G.C.......C......C--GGAGCT....GC.CCC.....CA--GC............C.........T....G.......
MCM     G..-GG..G.C.......C......C--GGA-TA.....C.CCG.....C.AAA-.A...........C.........T....G.......C.....
MCH     G..-GG..G.C.......C......C--GAA-TA.....C.CCG.....C.AAA-.A...........C.........T....G.......C.....
MX      GG.-AA..G.C.......C......C--GGACT......C.CCC.....CTAAGA.A...........C.........T....G.......C....
MLM     GG.-GG..G.C.......C......C--GGA.T......C.CCA.....CTAAAATA...........C.........T.............C.C.....
MLB     G..-GG..G.T.......C......--GGAGC.....GC.CCA.....CTAAAA.A...........C.........T.............C.....
OBO     G..-GA..G.C...C...C......T--GGA.T......GC.CCA.....C.GAAA.AT.........C.........T.............C.....
OBF     ...-GA..G.C...C....C......C--GGGGTT....GC.CCA.....C.AAAA.AT.........C.........T....G.......C.....
QNM     GG.-GA..G.C.......C......C--GAGGC.....GC..CA.....C.AAAG.............C.........T...............
QNB     GG.-GA..G.C.......C......--.GGGC.....GC.CCA.....C.AAA-.............C.........T.............C.....
TN      GG.-GA..G.C...C...C......C--GGA.TT....GCTCCG.....T.AAAA.AT.........C.............G.......C.....
VV      GG.-GA..G.C.......A....T--.GGG.....GC.C.A.....C--CCA...........C.............G.......C.....
VSM     GG.-GA..G.C.......A....T--.GG.......GC.C.A.....C--AC...........C.............G.......C.....
VMB     GG.-GA..G.C.......A....T--.GG.......GC.C.A.....C--AC...........C.............G.......C.....


        401                             450                                500
AF      TGGCAACATGAGAAAGAAACTGTCTCTCTAGGCTAGCCAATGAACCTGATCTGCCCGTACAAACGCAGGCATACAAAAACAAGACAAAAAGACCCTGTGA
PP      ...................TA....C...................TT..A...A...A.....TT.......................
BL      C.............TGC.......T.........T..G...A.........T.......A.........A.....................
CR      A.............GGC.......T.....A..T..G.........A..T...G..A........A.....................
DS      ...............GGC.......TA....C.......A........T..T.......A.........A.....................
HMM     ..............G.......T.T.A..C.T..G....T.........T.......A........TA.....................
HMB     ..............G.......T.T..TCGCT..G....A.........T.......A........A....G.....................
LNB     C.............GGC.......T..A...G.T.GG.........T.......A........GT.....................
LNS     ..............GC.......T..A...G.T..G.........T.......A.........T.....................
MCM     ..............ACCC.......TC.A....T..G...A.........T..TA...A.................T.....................
MCH     ..............A.CC.......TC.A....T..G...A.........T..TA...A.........G...T.....................
MX      C.............C.........T.....T..G...A.........T.......A........G.....................
MLM     C.............C.........T.T.....T..G...A.........T.......A.........A.....................
MLB     C.............C.........T.T.....T..G...A.........T.......A...A...GA.....................
OBO     C....T.........C.........TC.....T..G...A.........T.......A.........A.....................
OBF     ..............C.........T.......T..G...A.........A..A...A.....................
QNM     ..............C.........T.C...C.T..G...A.........T.......A........GT.....................
QNB     ..............C.........T.......T..G...A.........T.......A........GT.....................
TN      C.............GC.......T.........T..G...A.........T.......A.......A.G..G.....................
VV      A.............GGC.......T.....A..T..G.........A..T.......A.....T..A.....................
VSM     A...........G..GGC.......TA...A..T..G.........A..T.......A.........A.....................
VMB     A...........G..GGC.......TA.....T..G.........T.......A.........A.....................


        501                             550                                600
AF      AGCTTTA-AATGTGAGTCAACCTAATGACCAACACATTTT-TGGTTGGGGCAACCAAAGAGAAATATAAACCTCGAACCA-TCACACGGCCAACTAGCCA
PP      .A...C.-...A.TC.....AA...T....A.T....A.T....-.A...........TC.G...T..-.CC..T.CTCTC.ATCAAC..A.......A.....
BL      .A.....-..G..CCTT.....A.T.-TGA.TGGG..C..TC...............T...-..C.......AT.AA.-....A...AC..A....T
CR      .A.....-....CCCTC.....A.C.-TGGGGGA.......TC.................-..-.C.......TT.AA.-....CA...AG..A....T
DS      .A.....-....CCCTC.....A..-TGGA.GGG......TC.................-..-.C.......CT.T.-.....A.A..A...A.....
HMM     .A.....T.G..CCCT......GT.-TGA..GGG......TC.................T...-..-.C.......AT.AA.-.....A...AT..A.....
HMB     .A.....T.G.ACCCC....T.AT.-TGA..GGG..C...TC...............T...-..-.C.......AT.AA.-.....A.A.AT..A...G
LNB     .A...A.-....CCCC.....TAG.-TGAG.GGGT.....T..................-..-.C.......AT.ATT-..G..A.......A...T
LNS     .A.....-....CCCC.....A.G-TGAGG.AG......T..................-..-.C.......AT.A..GC..TGA.......A...T
MCM     .A...C.-.G...CTT.......T.-TGA...G...C...TC................T...-..-.......AT.AA.-...T.A...AC..G...G
MCH     .A...C.-.G...CTT.......T.-TGA...G...C...TC................T...-..-.......AT.AA.-...T.A...AC..A...G
MX      .A...C.-.G..CCTT......AC.-TGAA.GAG......TC................T...-..-GC......AT.AA.-....A.A.AC..A.....
MLM     .A...A.-....ACCCT.....C..-TGA..GGG......TC................T...-..-.C.......AT.AA.-...GA.A.AC..A....T
MLB     .A.....-....A.CCT......C..-TGA..GGG......TC................T...-..-.C.......AT.AA.-....A.A.AC..A.....
OBO     .A.....-....ACCCT......AC.-TGATGGGC......TC................T...-..-GC.......AGGA..-.....A...T..A.....
OBF     .A.....-....ACCCT...G..AC.-TGAT.GGT......TC................T...-..-GC.......AG.A..-.....A...T..AG....
QNM     .A...C.-....ACCCT......AC.-TGAT.GGG......TC................T...-..-G.......AT.AA.-...A.A....CG.A.....
QNB     .A.....-....CCCT.....AT.-TGA..GGG......TC................T...-..-.C.......AT.AA.-...TGA...AC..A.....
TN      .A.....-....ACTCT.....AC.-TGAT.GACT.....-.C................T...-..-GC.......AT.A..-...T.A...T..A.....
VV      .A.....-....CCCTC.....AC.CTGGA..GG......TC................-..-...G.....AT..A.-.TG..A.A.AT..A...T.T
VSM     .A.....-....CCCTC.....AC.CTGGA..GG......TC................-..-GC.........T.AG.-.T...A.A.AC..A..T.T
VMB     .A.....-....CCCTC.....AC.CTGGA..GG......TC................-..-GC.G.....AT.AG.-.T...A.A.AC..A..T.T
```

```
           601                                  650                                                 700
AF   ACTGACCAAATACT--AAGACCCAGTATTACTGAGCAAAGGACCAAGTTACTCCAGGGATAACAGCGCTACATTCTTGAAGAGTCCACATCAACAAGAAT
PP   .AGT.G.C..C..CACC...T......AA.....C.....A.....C.......................C.......T........T...........
BL   .A-----TG..C...-A....T......C......T.......A...C.......................C.......T........T...........
CR   .G-----TG..C..GATT-..T...........T.....A..A...C.......................C.......A.......TT...........
DS   .G-----TG..C.AAGA....T.....CC......T...T.A..A...C.....................C.......A.......TT...........
HMM  .G-----TG..C.T.-A....T......C......T...A..A...C.......................C.......A........T...........
HMB  .G-----TG..ACT.-G...T......C......T.....A..A...C.......................C.......A........T...........
LNB  .A-----....CCAA-A....T......C......T...A..G...C.......................C.......A.......TT...........
LNS  .A-----TG..CTAA-A....T.....CC......T...A..A...C.......................C.......A.......TT...........
MCM  .A-----.G.GC..C-A.T..T......CC......T...A..A...C.......................C.......A........T...........
MCH  .A-----.G.GC..C-A.T..T......CC......T...A..A...C.......................C.......A........T...........
MX   .G-----TG..C.TA-G...T.....CC......T.......A...C.......................C.......A........T...........
MLM  .A-----TG..CTA.-A....T......C......T.T.....A...C.......................C.......A........T...........
MLB  .G-----TG..CTAA-A....T......C......T.....A...C.......................C.......A........T...........
OBO  .A-----.C.T.-A.C..T......C......T...........C.......................C.......TT.....T...........
OBF  .A-----TG..CCT.-G.C..T......C......T...........C.......................C.......TT.....T...........
QNM  .T-----TG..CG.A-G...T......C......T.T.....A...C.......................C.......A........T...........
QNB  .T-----TG..C..A-A....T......C......T.T.....A...C.......................C.......A........T...........
TN   .A-----TG..C.T.-A.C..T......C......T...........C.......................C.......TT.....T...G.T......
VV   .G-----...GC..AAA.T..T......AA.....T...A..A...C.......................C.......AG......TT...........
VSM  .G-----T...C..AAA.-..T......AA.....T...A..A...C.......................C.......AG......TT...........
VMB  .G-----TG.GC..AAA.-..T......AA.....T...A..A...C...C.......................C.......AG......TT...........
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           701                                  750
AF   ACCTACGACCTCGATGTTGGATTAGGGTACCCAGATGGAGTAAAAATTATCAATGGTTCGTTTGTTCAACGATTAAAAC
PP   ....................C..A..T..TA....C.C.G..GC...T..G...................C...C.T
BL   ....................C....C....C.....C.C.G..GC...TG.A.......................G.
CR   ....................C........CA....C.C....GC...T..A.........................
DS   ....................C.........A....C.C....GC...T..A.........................
HMM  ....................C....C....CAG...C.C....GC...T..A.......................G.
HMB  ....................C....AC....C.C....GC...T..A.......................GT
LNB  ....................C.....T..TA....C.C....GC...T..A.........................
LNS  ....................C........CA....C.C....GC...T..A.........................
MCM  ....................C....C.....A....C.C.G..GC...T..A.......................GT
MCH  ....................C....C.....A....C.C.G..GC...T..A.......................GT
MX   G...................C....C.....A....C.C....GC...T..A..C................G.
MLM  ....................C........CA....C.C....GC...T..A.......................G.
MLB  ....................C....C....CA....C.C....GC...T..A.......................G.
OBO  ....................C....C....CA....C.C....GC...T..A.......................G.
OBF  ....................C....C....CA....C.C....GC...T..A.......................G.
QNM  ....................C....C.....A....C.C....GC...T..A.......................G.
QNB  .T..................C....C.....A....C.C....GC...T..A.......................G.
TN   ....................C....CC...CA....C.C....GC...T..A.......................G.
VV   ....................C.....T..TA....C.C....GC...T..A.........................
VSM  ....................C.....T..TA....C.C....GC...T..A.........................
VMB  ....................C.....T..TA....C.C....GC...T..A.........................
```

Fig. 2. Aligned sequences of a 779 bp segment of the 12S and 16S rRNA gene sequence. The 16S rRNA gene sequence begins at the asterisk. Dot indicates an identity with the first sequence; dash denotes gaps. See Table 1 for abbreviations.

Amplification proceeded in 50 μl of 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, with 0.2 mM each dNTP, 50 pmol primer, template DNA (5–50 μg) and 1 U Taq polymerase (Toyobo Co. Ltd., Osaka, Japan). The temperature regimen of 30 cycles was one minute at 94°C, two minutes at 55°C, and three minutes at 72°C. Amplified DNA was purified by electrophoresis in 0.8% agarose gel.

Nucleotide sequences were determined for both strands with a dye terminator cycle sequencing FS Ready Reactions Kit and ABI PRISM 377 DNA Sequencer (Perkin-Elmer Corp., Norwalk, USA), using the primers described above.

Allozyme electrophoresis

A total of 20 loci encoding 14 enzyme systems were scored (Appendix 2). Enzyme nomenclature and enzyme commission numbers followed the recommendations of the Nomenclature Committee of the International Union of Biochemistry (IUBNC, 1984). The frozen tissues were homogenized in equal volumes of 0.01 M Tris-HCl pH 7.1 buffer containing 0.001 M EDTA. Extracts of homogenates were absorbed into paper wicks (Whatman No. 3), and were subjected to the horizontal starch gel electrophoresis. Starch gels were prepared using both Hydrolysed Potato Starch (Starch Art Corp., Smithville, USA) and Starch-Hydrolysed (Connaught Lab., Ontario, Canada) mixed in a 4:1 ratio at a starch concentration of 13%. The staining procedure for specific enzymes followed those outlined by Shaw and Prasad (1970), Harris and Hopkinson (1976) and Allendorf *et al.* (1977). Genetic interpretations of allozyme data were based on criteria developed by Selander *et al.* (1971). Allozyme designations followed Murphy and Crabtree (1985).

Phylogenetic analyses

For DNA sequences, alignments were determined based on maximum nucleotide similarity. Using the aligned sequences, we eliminated gap sites and prepared a pairwise matrix of distance by Kimura's (1980) two-parameters model for transition/transversion bias. The neighbor-joining (NJ) method (Saitou and Nei, 1987) was applied to infer genetic relationships among OTUs on the basis of the distance matrix. Degrees of supports for internal branches of each tree were assessed by 1,000 bootstrap pseudoreplications (Felsenstein, 1985). These analyses were performed by use of Clustal W (Thompson *et al.*, 1994)

For allozyme data, Nei's genetic distance coefficients (D) (Nei, 1978) were computed from observed electromorph frequencies. The NJ method (Saitou and Nei, 1987) was applied to the computed distances. The degrees of support for branches were assessed by 1,000 times bootstrapping (Felsenstein, 1985). These analyses were performed by use of PHYLIP 3.54c (Felsenstein, 1993).

The interpretation of bootstrap proportions (BPs) is still in a state of uncertainty (see Felsenstein and Kishino, 1993; Hillis and Bull, 1993). We tentatively followed Shaffer *et al.* (1997), and considered BPs≥90% as highly significant, 70≤BPs<90% as marginally significant, and BPs<70% as constituting limited evidence of monophyly.

RESULTS

DNA analysis

Aligned sequences from two mitochondrial genes are presented in Fig. 2. The 12S rRNA fragment consisted of 373 total aligned sites, 189 of which (50.7%) were variable. For the 16S rRNA fragment, there were 406 total sites, 159 of which (39.2%) were variable. Interspecific nucleotide replacements within *Draco* varied from 48 base pairs (bp) (*D. haematopogon* from Malay Peninsula versus *D. melanopogon* from Borneo) to 120 bp (*D. taeniopterus* versus *D. volans volans*). Nucleotide replacements between subspecies of *D. maculatus* (*D. m. maculatus* versus *D. m. haasei*), *D. volans* (*D. v. volans* versus *D. v. sumatranus*) and of *D. lineatus* (*D. l. beccarii* and *D. l. spilonotus*) involved seven, 27 and 67 bp, respectively. So, inter-subspecific nucleotide replacements within *D. lineatus* were larger than those between a few combinations of different species. On the other hand, nucleotide replacements between samples from Malay Peninsula and Borneo were observed in 10, 29, 30 and 40 bp in *D. v. sumatranus*, *D. quinquefasciatus*, *D. melanopogon* and *D. haematopogon*, respectively. The values for the latter three species were larger than those between subspecies of *D. maculatus* or of *D. volans* (see above).

The NJ dendrogram derived from mitochondrial DNA dis-
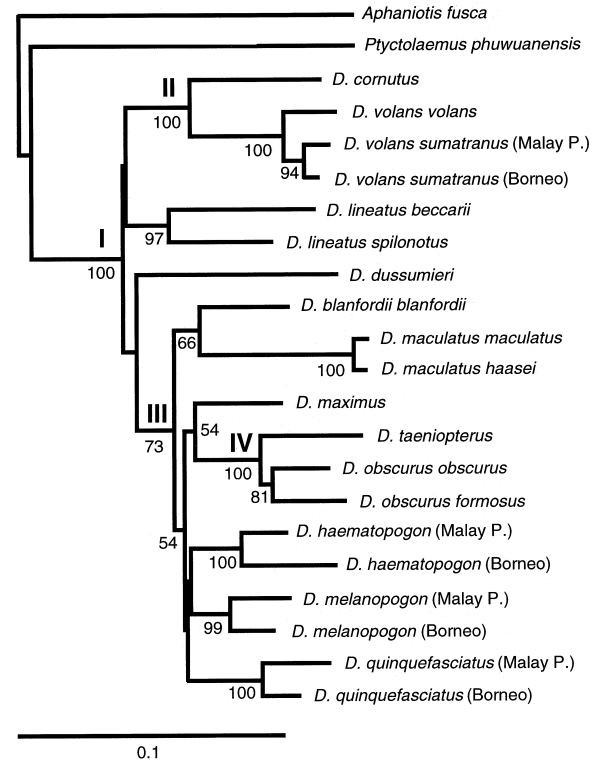
Fig. 3. NJ dendrogram deriving from distance matrix from 12S and 16S rRNA sequence data. Numbers at branch indicate bootstrap proportions (BPs) in 1,000 bootstrap pseudoreplications. Branches without BP values were not supported in ≥50% of the replicates. Bar equals 0.1 Kimura two-parameter distance.

tance matrix is shown in Fig. 3. The monophyly of *Draco* was supported in all of the 1,000 bootstrappings (node I: BP=100%). The ingroup formed a nearly trichotomous cluster, which consists of *D. cornutus–D. volans* cluster (node II: BP=100%), *D. lineatus* cluster (BP=97%) and a cluster accommodating all the remaining species (BP=42%). The last

Table 2. Nei's genetic distances between samples of *Draco*. See Table 1 for the abbreviations.

	BL	CR	DS	HMM	LNB	MCM	MCH	MX	MLM	MLB	OBO	OBF	QNB	TN	VSM	VMB	PP
BL	–																
CR	1.13	–															
DS	0.83	0.43	–														
HMM	0.25	0.98	0.60	–													
LNB	1.02	0.68	0.66	1.03	–												
LNS	1.37	0.84	0.65	0.96	0.29	–											
MCM	0.67	0.62	0.67	0.82	0.66	0.54	–										
MCH	0.65	0.63	0.67	0.85	0.68	0.56	0.02	–									
MX	0.95	0.57	0.77	1.30	0.96	1.11	0.58	0.53	–								
MLM	0.30	0.91	0.66	0.27	0.93	0.95	0.66	0.66	1.00	–							
MLB	0.35	0.84	0.64	0.32	0.80	0.83	0.59	0.56	1.11	0.11	–						
OBO	0.36	1.24	0.95	0.33	1.09	1.38	0.85	0.81	1.08	0.43	0.54	–					
OBF	0.35	1.19	0.82	0.26	1.07	1.22	0.75	0.71	1.07	0.30	0.37	0.07	–				
QNB	0.51	0.44	0.48	0.85	0.62	0.89	0.29	0.30	0.33	0.74	0.70	0.79	0.82				
TE	0.17	0.98	0.72	0.31	1.05	1.40	0.79	0.74	0.73	0.45	0.49	0.32	0.37	0.64	–		
VSM	1.05	0.13	0.65	1.04	0.67	0.93	0.65	0.60	0.64	0.86	0.79	1.06	1.02	0.46	1.10	–	
VSB	0.91	0.16	0.58	0.85	0.62	0.91	0.69	0.66	0.71	0.76	0.76	1.08	1.03	0.47	1.02	0.07	–
PP	1.77	2.00	1.59	1.86	2.45	2.42	1.76	1.60	2.28	2.26	2.45	1.76	1.80	1.71	1.53	1.66	1.64

cluster further split into *D. dussumieri* and the other (node III: BP=73%). Within node III, *D. blanfordii* and *D. maculatus*, and all the remaining species formed clusters (66%, 54%, respectively), of which the latter further accommodated *D. maximus* –*D. obscurus*–*D. taeniopterus* cluster (BP=54%), and *D. obscurus*–*D. taeniopterus* cluster (node IV: BP=100%). All conspecific samples exclusively constituted lowest clusters with highly or marginally significant BPs.

Allozyme analysis

All of the 20 presumptive loci examined were polymorphic in the present 18 samples (Appendix 3). Table 2 presents pairwise comparisons of the Nei's D values. Nei's D between two species of *Draco* varied from 0.13 (*D. cornutus* versus *D. v. sumatranus* from Malay Peninsula) to 1.40 (*D. lineatus spilonotus* versus *D. taeniopterus*). Intraspecific D in *D. lineatus* showed higher value (0.29) than interspecific D between *D. cornutus* and *D. volans* (0.13–0.16) or between *D. blanfordii* and *D. taeniopterus* (0.17).

The NJ dendrogram constructed on the basis of D matrix is shown in Fig. 4. As in the DNA analysis, the monophyly of *Draco* was supported in highly significant BP value (node I: BP=97%). The ingroup showed a large dichotomy into clusters consisting of *D. blanfordii*, *D. haematopogon*, *D. melanopogon D. obscurus* and *D. taeniopterus* (node V: BP=70%), and of all the remaining species. In the latter, *D.*

Fig. 4. NJ dendrogram deriving from Nei's (1978) distance (D) matrix from allozyme data. Numbers at branch indicate BPs in 1,000 bootstrap replicates. Branches without BP values were not supported in ≥50% of the replicates. Bar equals 0.1 D.

dussumieri was first split to constitute a cluster by itself, whereas the remainder was further divided into four lower clusters that consist of *D. volans* and *D. cornutus* (node II: BP=75%), *D. maximus* and *D. quinquefasciatus* (BP=53%), two subspecies of *D. lineatus* (BP=78%), and of two subspecies of *D. maculatus* (BP=86%).

DISCUSSION

Phylogenetic relationships of *Draco*

Our result indicates that the nucleotide replacements between two subspecies of *D. lineatus* are larger than those between a few combinations of different species. In the allozyme analysis, genetic distance (D) also showed a high value between the two subspecies. We also observed larger nucleotide replacements between peninsular and Bornean samples of *D. haematopogon*, *D. melanopogon* and *D. quinquefasciatus* than between subspecies of *D. maculatus* and *D. volans*. These suggest that those subspecies of *D. lineatus*, and those populations of *D. haematopogon*, *D. melanopogon* and *D. quinquefasciatus* are genetically differentiated to the species and subspecies levels, respectively.

In both DNA (Fig. 3) and allozyme dendrograms (Fig. 4), all conspecific samples were exclusively clustered into lowest single nodes, most of which were supported in marginally to highly significant BPs. At the level of BPs≥50%, the two dendrograms showed only three conflicts in terms of branching topology: (1) *Draco blanfordii* was exclusively clustered with *D. maculatus* in the DNA dendrogram, whereas it composed node V together with *D. haematopogon*, *D. melanopogon*, *D. obscurus* and *D. taeniopterus* in the allozyme dendrogram; (2) *Draco maximus* formed a cluster with node IV (*D. obscurus* and *D. taeniopterus*) in the DNA dendrogram, whereas it was exclusively closest to *D. quinquefasciatus* in the allozyme dendrogram; (3) *Draco haematopogon*, *D. maximus*, *D. melanopogon*, *D. obscurus*, *D. quinquefasciatus* and *D. taeniopterus* formed an exclusive cluster in the DNA dendrogram, whereas the *D. maximus*–*D. quinquefasciatus* cluster was distantly located from the cluster consisting of the other species in the allozyme dendrogram. However, these dendrograms were not in substantial conflict with each other in nodes II–V that were defined in at least one of the dendrograms with significant BP values (BPs≥70%). Figure 5 shows the combined tree sensu Hillis (1987) which incorporates both DNA (as depicted in Fig. 3) and allozyme information (as depicted in Fig. 4). The monophyly of *D. volans* and *D. cornutus* that constitute node II seems to deserve no doubt because this is supported by both dendrograms with significant BPs. As to other relationships, we recognized three clades (clades α and β, and the *D. obscurus*–*taeniopterus* clade, corresponding to nodes III, V and IV, respectively) to maximize phylogenetic resolution, although no single data set fully resolves the relationships among OTUs. We advocate Hillis' (1987) claim that a greater portion of phylogeny can be ascertained by combining two data sets than by analyzing a single data set.

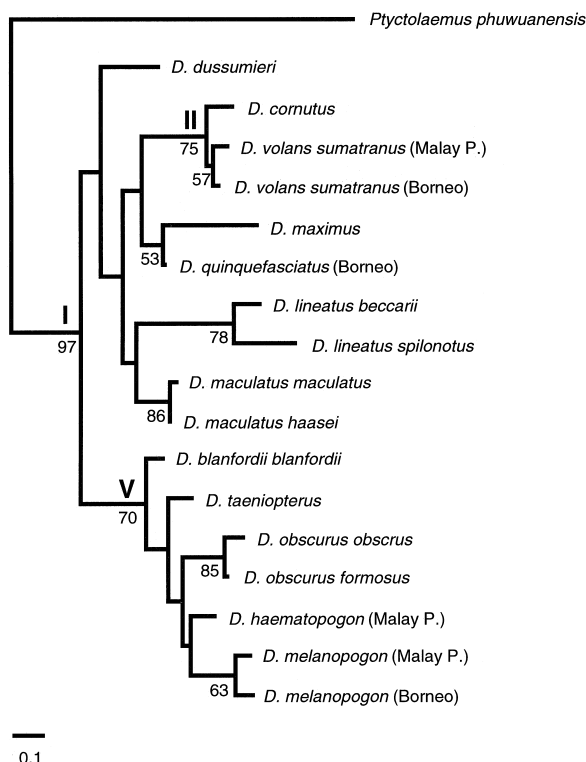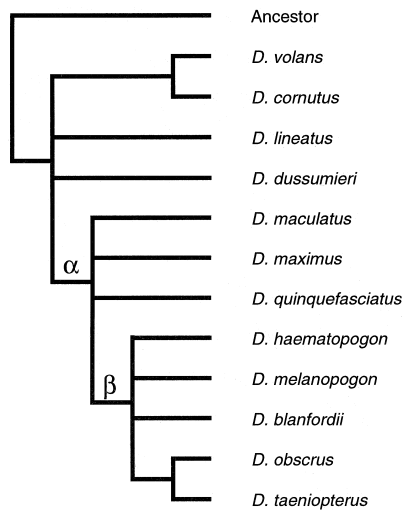As is mentioned above, Musters (1983) hypothesized the

Fig. 5. Phylogenetic tree of the genus *Draco* inferred by combination approach (Hillis, 1987) to consent and complement both DNA (Fig. 3) and allozyme dendrograms (Fig. 4). Nodes are supported in at least one of these dendrograms with BPs ≥70%. Clades α and β correspond to nodes III and V, respectively.
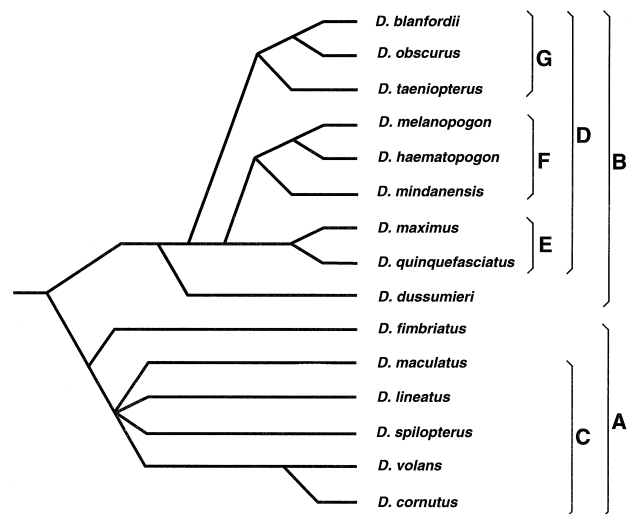

Fig. 6. Phylogenetic tree of the genus *Draco*, proposed by Musters (1983) on the basis of morphological characters and geographic ranges. A, group of species characterized by outward-directed nostrils and more or less developed nuchal crests; B, group of species characterized by upward-directed nostrils and absence of distinct nuchal crests; C, group of species especially closely resembling each other in A; D, Southeast Asian species in B; E, group of species characterized by the presence of nuchal fold and six patagium ribs; F, group of species characterized by the absence of nuchal fold and presence of five patagium ribs; G, group of species characterized by the presence of a nuchal fold and five patagium ribs.

primary dichotomy in *Draco* on the basis of morphological characters. One of the two major clades in his phylogenetic tree (Fig. 6) was characterized by outward-directed nostrils and more or less developed nuchal crests (henceforth referred to as Group A). In this group, *D. fimbriatus* was first diverged from the remaining species (Group C). Although Group C consisted of *D. maculatus*, *D. lineatus*, *D. spilopterus* and the *D. volans–D. cornutus* cluster, their detailed relationships remained uncertain.

The other major clade (Group B) was characterized by upward-directed nostrils and the absence of distinct nuchal crests. *Draco dussumieri*, the only species distributed in southern India, was the first to diverge from the remaining species (Group D). In Group D, a clade consisting of species with a nuchal fold and six ribs in the patagium (Group E), and another clade of those without a nuchal fold and with five patagium ribs (Group F) collectively constituted a sister group to the other clade (Group G), which was characterized by the presence of a nuchal fold and five ribs in the patagium.

Phylogenetic relationships of *Draco* hypothesized on the basis of DNA and allozyme analyses (Fig. 5) do not support the monophyly of the Group A in Musters (1983) at all. Both outward-directed nostrils and more or less developed nuchal crests that characterize Musters' Group A are considered to be primitive, because these characters are common to outgroups (*Aphaniotis* and *Ptyctolaemus*) as well. Thus, monophyly of the Group A is not actually well supported on the morphological ground, either. On the other hand, species of Group B exclusive of *D. dussumieri* (i.e., Group D) are relatively close to each other in clade α of our phylogram. Presences of two apparently synapomorphic characters in Group B (i.e., upward-directed nostril and the absence of nuchal crests) also seem to support the monophyly of those species,

although the possession of these characters by *D. dussumieri* may represent convergence (Fig. 5: see below).

Clade α includes one Group A species (*D. maculatus*) and all Group D species examined. Since only *D. maculatus* has plesiomorphic characters (outward-directed nostrils and developed nuchal crests), it is likely that the presumptive common ancestor of clade α resembled *D. maculatus*, and that the first dichotomy within the clade α occurred between *D. maculatus* and the remaining species.

In the present study, we could not infer detailed relationships among the *D. volans–cornutus* clade, *D. lineatus*, *D. dussumieri* and clade α with significant BPs. Such unresolved nodes may have diverged through a rapid radiation. Considering that *D. dussumieri* and the clade α species formed a cluster in the DNA dendrogram (BP=42%), it is probable that *D. dussumieri* has diverged from a common ancestor with clade α. This idea is circumstantially supported by the fact that the geographical range of *D. dussumieri* (southern India) is nearest, among ranges of the other congeneric species, to Indo-China Peninsula where *D. maculatus*, the possible ancestral stock of the clade α (see above) occurs. We thus suspect that the common ancestor of *Draco* had first diverged into three groups which lead to the *D. volans–cornutus* clade, *D. lineatus*, and a clade consisting of *D. dussumieri* and clade α. The ancestors of these three clades might have been characterized by the symplesiomorphies mentioned above.

Musters (1983) assumed a close relationship among the species without enlarged scales on male gular pouches

(Groups E and F). However, our results, while supporting the monophyly of species with five ribs in the patagium (Groups F and G: as clade β), negated the monophyly of Groups E and F (see further discussion in Biogeography). Musters (1983) also assumed the monophyly of Group G (*D. blanfordii*, *D. obscurus* and *D. taeniopterus*) and Inger (1983) also noted the morphological similarity among those species. Our results support the monophyly of the latter two species, but not of the whole Group G.

We could not examine *D. fimbriatus*, *D. mindanensis* and *D. spilopterus*. Musters (1983) hypothesized close relationships of *D. mindanensis* with *D. haematopogon* and *D. melanopogon* (Group F). Inger (1983) pointed out the morphological similarity of *D. spilopterus* with *D. cornutus* and *D. volans*, and synonymized the former two with *D. volans*. These suggest close affinities of *D. mindanensis* and *D. spilopterus* with clade α and the *D. volans–cornutus* clade, respectively.

Biogeography

Based on the geographic patterns of endemisms, the range of *Draco* can be divided into five areas: Area 1, southern India; Area 2, Indo-China Peninsula; Area 3, Malay Peninsula and Greater Sunda Islands exclusive of Sulawesi; Area 4, Lesser Sunda Islands, Sulawesi and Maluk Islands; and Area 5, Philippines (Fig. 1) (Lazell, 1987, 1992; Musters, 1983; Ross and Lazell, 1990). Area 1 is isolated from the others with a broad geographical gap. Areas 2 and 3 are also assigned to different provinces in zoogeographic classification of Southeast Asia on the basis of other taxonomic groups (e.g., Lepidoptera: Holloway, 1987). In Area 3, Sumatra, Java and Borneo are considered to have been parts of a peninsula projecting southward from Asian continent during the glacial periods of the Pleistocene (e.g., Dunn and Dunn, 1977; Heaney, 1991). Many mammalian species in this region are widely distributed, demonstrating dispersals across the Sunda Shelf during those periods (Heaney, 1984). Between Areas 3 and 5, and Area 4 (between the islands of Bali, Borneo and Mindanao in the west and north, and Lombok, Sulawesi and Sangihe Islands in the east and south), there exist major borders of mammalian, butterfly and bird faunas (Wallace 1860, but see Musser, 1987; Vane-Wright, 1991). This demarcation is known as Wallace's Line. Area 5 has a different geographic history (Hall, 1996) and a high degree of herpetological endemicity (Alcala, 1986). Thus, our setting of five areas can be tied in with geographic pattern illustrated by other faunas. We discuss the zoogeography of *Draco* on the basis of recognitions of these five areas.

Draco dussumieri is the only species distributed in Area 1, *D. blanfordii*, *D. maculatus* and *D. taeniopterus* in Area 2, *D. cornutus*, *D. fimbriatus*, *D. haematopogon*, *D. maximus*, *D. melanopogon*, *D. obscurus* and *D. quinquefasciatus* in Area 3, and *D. mindanensis* and *D. spilopterus* in Area 5. Of these, *D. blanfordii*, *D. maculatus*, and *D. taeniopterus* also occur in the northern part of Area 3, whereas *D. obscurus* is distributed in the southern part of Area 2 as well across the boundary in Malay Peninsula. *Draco lineatus* is mainly distributed in Area 4, but also occurs in the southeastern part of Area 3 and the southern part of Area 5 as well. *Draco volans* has a widest distribution which, while seemingly centering in Area 3, also partially ranges in Areas 2, 4 and 5.

According to the phylogenetic relationships inferred above, the presumptive ancestor of *Draco* seems to have first diverged into three groups, the ancestors of the clade consisting of *D. dussumieri* and clade α, the *D. volans–cornutus* clade, and of the monotypic *D. lineatus* clade. The common ancestor of *D. dussumieri* and clade α, supposedly resembling *D. maculatus* (see above), should have originally been distributed in Area 2, where *D. maculatus* almost exclusively occurs. Accepting this, we can extend the assumption that the primary divergence of *Draco* took place as a series of vicariances among Areas 2, 3, and 4. The common ancestor of *D. dussumieri* and clade α should have invaded to Area 1 from Area 2 to be isolated and diverged into *D. dussumieri* subsequently.

After these primary diversifications, the common ancestor of clade α, originally distributed in Area 2, should have invaded into Area 3 where it further diverged into several species. Musters (1983) assumed that within Group D, Groups E and F from Area 3, and Group G from Area 2 first differentiated through vicariance, followed by the separation between the Groups E and F within Area 3. However, our analyses strongly suggested the monophyly of clade β (Groups F and G), and further of the *D. obscurus–taeniopterus* clade. Therefore, it seems more appropriate to consider that the common ancestor of clade β had split from the other components of clade α within Area 3, and that resultant ancestors of *D. blanfordii* and *D. taeniopterus* independently dispersed into Area 2. Invasion of the *D. blanfordii* lineage might have occurred earlier than that of the *D. taeniopterus* lineage, because the former has diverged into several subspecies within Area 2, whereas the latter is rather monomorphic (Musters, 1983). The sister species relationship of the latter and *D. obscurus* shown in our conbined tree (Fig. 5) lends a further support to this view.

These range extensions seem to have been affected by habitat preferences of lineages involved. During surveys from 1979 to 1997, we directly observed habitats of *Draco* in Areas 2 (Thailand), 3 (Peninsula Malaysia, Borneo and Sumatra) and 4 (Sulawesi) (Honda *et al.*, unpubl. data). Results indicate that those species that are characterized by plesiomorphic characters (outward-directed nostril and developed nuchal crest, referred to as Group A in Musters, 1983: see above), such as *D. maculatus* in Area 2, *D. volans* and *D. cornutus* in Area 3, and *D. lineatus* in Area 4, inhabit open lowlands including secondary forests, cultivated areas and even urban sites. These species did not occur syntopically at all. On the other hand, the species group with the apomorphic characters (Group B in Musters, 1983), such as *D. blanfordii*, *D. haematopogon*, *D. maximus*, *D. melanopogon*, *D. obscurus*, *D. quinquefasciatus* and *D. taeniopterus* in Area 2 and/or Area 3, were found only in deep, largely primary forests with frequent syntopy (e.g., *D. haematopogon*, *D. maximus* and *D.*

quinquefasciatus in Sabah; *D. blanfordii* and *D. taeniopterus* in the central Malay Peninsula). *Draco dussumieri* also occurs in deep forest in Area 1 (Sengoku, pers. comm.). Although species belonging to these two groups were occasionally observed together around the forest boarder, they seem to segregate their major habitats in each area. Based on the fact that only species with the plesiomorphic characters occur in open environments, we assume that the common ancestor of *Draco* emerged in such habitats. It is probable that the forest dwelling members emerged after the dispersal of clade α ancestor from Areas 2 to 3 and then dispersed back to Area 2 where only open habitat dweller had occurred.

Interestingly, clade α are confined to Areas 2 and 3, whereas the *D. volans–cornutus* clade and *D. lineatus* have been spread in both Areas 3 and 4 across Wallace's Line. Considering these, one may argue that the separation of Areas 3 and 4 by sea occurred after the range extensions of the latter two clades, but before the divergence of clade α. However, it would be also possible to attribute such differential distributions to the differences in dispersal ability between these two groups, because inhabitants of open habitats (such as *D. volans*, *D. cornutus* and *D. lineatus*) would more easily extend their ranges across the straits and/or temporary landbriges than the deep forest dwellers (such as most species of clade α). Detailed analyses of genetic variation among populations of *D. volans*, *D. cornutus* and *D. lineatus* are needed to assess these alternative hypotheses.

In Area 5, two endemic species, *D. mindanensis* and *D. spilopterus*, are distributed. Of these, the former may have possibly been derived from the ancestral form of clade α dispersed from Area 3. *Draco spilopterus*, on the other hand, might have originated from the ancestor common with *D. volans* and *D. cornutus*, but direction of dispersal remains uncertain due to the current wide range of *D. volans* (see above).

ACKNOWLEDGMENTS

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Appendix 1. Catalogue number of specimens examined in this study. *: both for allozyme and DNA analyses; **: only for DNA analysis.

Draco blanfordii blanfordii: Kaki Bukit, Peninsular Malaysia, KUZ 22138, 22141, 22147, 22162–63, 27670; Khao Sok, Thailand, 32781; Erawan, Thailand, 35322*; Pa Lao U, Thailand, 35412, 35416; Khao Chang, Thailnad, 37834**.

D. cornutus: Sepilok, Sabah, 9105; Lambir, Sarawak, 12850*; Matang, Sarawak: 16004.

D. dussumieri: Kerala, India, 35183–84, 35185*.

D. haematopogon (Malay Peninsula): Bukit Larut, Peninsular Malaysia, 21250**.

D. haematopogon (Borneo): Niah, Sarawak, 12743, 12744*, 12777, 12790; Lambir, Sarawak, 12815, 12856, 27021.

D. lineatus beccarii: Maros, Sulawesi, 20841*, 20842-43.

D. l. spilonotus: Dumoga, Sulawesi, 18570–71, 21009, 21063, 21082; Maros, Sulawesi, 21064; Monado, Sulawesi, 20967–70, 20971*.

D. maculatus maculatus: Ko Samui, Thailand, 32655, 32659, 32674, 32690*, 32691, 32693, 32711–12.

D. m. haasei: Khao Srabab, Thailand, 35612, 35743; Ko Chang, Thailand, 35452–53, 35456, 35458*, 35459*, 35460, 35475, 35477, 35478*, 35479–80, 35482–85, 35488, 35492–93, 35495, 35498.

D. maximus: Lambir, Sarawak, 12844, 27014*.

D. melanopogon (Malay Peninsula): Gombak, Peninsular Malaysia, 16054, 16056, Penang Island, Peninsular Malaysia, 21183-86; Tempelar Park, Peninsular Malaysia, 21520–23, 22067–68, 22072; Khao Luang, Thailand, 27643–46, 27647*, 27648–49.

D. melanopogon (Borneo): Matang, Sarawak, 16002; Niah, Sarawak, 12755*; Sabah (detailed loc. unknown), 21541.

D. obscurus obscurus: Lundu, Sarawak, 27084*, Matang, Sarawak, 12416, 12879–80, 12883, 27230, 27231.

D. o. formosus: Bukit Larut, Peninsular Malaysia, 21249; Gombak, Peninsular Malaysia, 16010, 16014, 16018, 16038–42, 21485–86, 21504, 21512; Penang Island, Peninsular Malaysia, 21188–21189; Tempelar Park, Peninsular Malaysia, 21515, 22069, 22076; Gunong Jerai, Peninsular Malaysia, 22178, 22180*, 22250.

D. quinquefasciatus (Malay Peninsula): Gombak, Peninsular Malaysia, 22032**.

D. quinquefasciatus (Borneo): Niah, Sarawak, 12757, 12776, 12796; Matang, Sarawak, 16006; Lundu, Sarawak, 27069*.

D. taeniopterus (Malay Peninsula): Kaki Bukit, Peninsular Malaysia, 22161*, 22164, Chanthaburi, Thailand, 32842, 32846–47, 32850–52; Khao Soi Dao, Thailand, 35525, 35527, 35529, 35538, 35563, 35576, 35607, 35610, 35613, 35617, 35618*, 35619.

D. volans volans: Borobudur, Java, 38831**.

D. v. sumatranus (Malay Peninsula): Gombak, Peninsular Malaysia, 16037, 21499*, 21500; Janda Baik, Peninsular Malaysia, 21460; Penang Island, Peninsular Malaysia, 21179–21181, 22288–91, 22293–94, 22296, 22297*, 22298.

D. v. sumatranus (Borneo): Kuching, Sarawak, 26003, 26010; Matang, Sarawak, 12903, 12905, 12907, 12911–13, 12918–19, 27171, 27186, 27217, 27218*, 27222; Lundu, Sarawak: KUZ 27070–71, 27073–74, 27076.

Ptyctolaemus phuwuanensis: Phu Wua, Thailand, 40221–23, 40226–27, 40409, 40353, 40355*, 40356, 40566.

Aphaniotis fusca: Mimaland, Peninsular Malaysia, 22062**.

Appendix 2. Enzyme, loci and buffer systems used in the present study. Mitochondrial and supernatant loci, determined on the basis of anodal/cathodal criterion following Harris and Hopkinson (1976), are denoted by "m" and "s", respectively.

Enzyme	Enzyme commission number	Locus	Buffer Condition*
Aspartate aminotransferase	4. 2. 1. 3	*mAat-A*	TBE
		sAat-A	TBE
Non-specific esterase	—	*Est-1*	TC7
Fumarate hydratase	4. 2. 1. 2	*Fum-A*	TBE
Glycerol-3-phosphate dehydrogenase	1. 1. 1. 8	*G3pdh-A*	TC8
Glucose phosphate isomerase	5. 3. 1. 9	*Gpi-A*	TC7
D-3-hydrooxybutyrate dehydrogenase	1. 1. 1. 30	*Hbdh-A*	TC7
Isocitrate dehydrogenase	1. 1. 1. 42	*mIcdh-A*	CAPM6.1
		sIcdh-A	CAPM6.1
Lactate dehydrogenase	1. 1. 1. 27	*Ldh-A*	CAPM6.1
		Ldh-B	CAPM6.1
Malate dehydrogenase	1. 1. 1. 37	*mMdh-A*	CAPM6.1
		sMdh-A	CAPM6.1
Mannose phosphate isomerase	5. 3. 1. 8	*Mpi-A*	Li-OH
Nucleoside phosphrylase	2. 4. 2. 1	*Np-1*	Li-OH
Peptidase (leu-ala substrate)	3. 4. 11	*Pep-A*	Li-OH
(leu-gly-gly substrate)	3. 4. 11	*Pep-C*	Li-OH
(leu-pro substrate)	3. 4. 11	*Pep-D*	Li-OH
Phosphoglyceromutase	2. 7. 5. 1	*Pgm-A*	TBE
Superoxide dismutase	1. 15. 1. 1	*Sod-1*	TC8

*: TC7 = Tris-citrate pH 7.0; TC8 = Tris-citrate pH 8.0; TBE = Tris-borate-EDTA pH 8.7; CAPM6.1 = Citric acid-Aminopropyl morpholin pH 6.1; Li-OH = Lithium Hydroxide pH 8.1.

Appendix 3. Electromorph freqencies, mean number of alleles per locus (A), percnt loci polymorphic (P, no criterion), and mean heterozygos-abbreviations of samples.

Locus	BL (N=10)	CR (N=3)	DS (N=3)	HMM (N=7)	LNS (N=3)	LNB (N=11)	MCM (N=8)	MCH (N=21)	MX (N=2)	MLM (N=18)	MLB (N=3)	OBO (N=7)	OBF (N=20)
mAat-A	d 1.000	d 1.000	d 1.000	c 0.429 d 0.571	d 1.000	d 1.000	d 1.000	d 1.000	d 1.000	d 1.000	d 1.000	c 1.000	b 0.450 c 0.500 d 0.050
sAat-A	c 1.000	c 0.167 e 0.833	d 1.000	b 0.429 c 0.571	d 1.000	d 1.000	d 1.000	c 0.048 d 0.952	c 1.000	c 0.722 d 0.278	c 1.000	b 0.714 c 0.286	b 0.925 c 0.075
Est-1	b 1.000	a 0.167 b 0.500 e 0.333	b 1.000	b 0.857 c 0.143	d 1.000	d 1.000	a 1.000	a 0.952 b 0.048	e 1.000	b 1.000	b 1.000	c 1.000	b 0.175 c 0.825
Fum-A	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000	a 1.000
G3pdh3-	b 1.000	c 1.000	c 1.000	c 1.000	b 0.500 c 0.500	b 0.045 c 0.955	b 1.000	a 0.238 b 0.667 c 0.095	b 1.000	c 1.000	c 1.000	a 0.857 b 0.143	a 0.875 b 0.125
Gpi-A	c 0.950 d 0.050	b 0.833 c 0.167	c 1.000	c 1.000	c 1.000	c 0.955 d 0.045	b 0.125 c 0.813 d 0.063	b 0.048 c 0.952	c 0.500 d 0.500	c 0.028 d 0.972	d 1.000	c 0.929 e 0.071	b 0.025 c 0.525 d 0.450
Hbdh-A	b 0.950 c 0.050	c 1.000	c 1.000	b 1.000	c 1.000	c 1.000	c 1.000	b 0.119 c 0.881	c 1.000	b 0.056 c 0.944	b 0.667 c 0.333	c 1.000	b 0.088 c 0.912
mIcdh-A	d 1.000	c 1.000	c 1.000	d 1.000	e 1.000	b 1.000	c 1.000	c 1.000	c 1.000	d 1.000	d 1.000	d 1.000	d 1.000
sIcdh-A	f 1.000	e 1.000	e 1.000	f 1.000	c 1.000	b 0.222 d 0.389 f 0.111 g 0.278	f 1.000	d 0.200 f 0.800	e 1.000	f 1.000	f 1.000	f 1.000	b 0.050 d 0.100 f 0.850
Ldh-A	e 0.750 g 0.250	b 1.000	b 0.333 d 0.667	g 1.000	d 1.000	d 1.000	e 1.000	e 1.000	g 1.000	f 1.000	f 1.000	g 1.000	g 1.000
Ldh-B	b 1.000	a 0.833 b 0.167	a 1.000	a 1.000	a 0.333 b 0.667	a 0.864 b 0.136	a 0.375 b 0.625	a 0.048 b 0.952	b 1.000	b 1.000	b 1.000	b 1.000	b 1.000
mMdh-A	c 1.000	a 1.000	a 1.000	c 1.000	a 1.000	b 1.000	c 1.000	c 1.000	a 1.000	c 1.000	c 1.000	c 1.000	c 1.000
sMdh-A	c 0.050 e 0.950	d 0.670 e 0.333	e 1.000	e 1.000	e 1.000	e 1.000	e 1.000	b 0.238 e 0.762	b 1.000	b 0.028 e 0.972	e 1.000	e 1.000	e 1.000
Mpi-A	c 1.000	e 0.500 f 0.500	e 1.000	e 1.000	e 0.333 e 0.667	e 1.000	e 1.000	e 1.000	b 1.000	d 0.028 e 0.972	e 1.000	d 1.000	b 0.025 d 0.100 e 0.875
Np-1	b 1.000	c 1.000	a 1.000	b 1.000	d 1.000	d 1.000	c 1.000	c 1.000	c 1.000	b 0.899 c 0.111	b 0.833 d 0.167	b 1.000	b 1.000
Pep-A	a 1.000	b 1.000	a 1.000	a 1.000	b 1.000	b 1.000	b 1.000	b 1.000	b 1.000	a 0.912 b 0.088	a 0.167 b 0.833	a 1.000	a 1.000
Pep-B	a 0.650 b 0.350	a 0.333 b 0.666	b 1.000	a 1.000	a 1.000	a 0.636 b 0.364	a 0.500 b 0.500	a 0.310 b 0.690	a 0.500 b 0.500	a 0.944 b 0.056	a 0.500 b 0.500	a 0.583 b 0.417	a 0.579 b 0.421
Pep-D	b 1.000	b 1.000	b 1.000	b 0.929 c 0.071	b 1.000	a 1.000	a 1.000	a 1.000	a 1.000	b 0.528 c 0.472	b 1.000	b 1.000	b 1.000
Pgm-A	b 1.000	b 1.000	b 1.000	b 1.000	a 1.000	a 1.000	b 1.000	b 1.000	b 1.000	b 1.000	b 1.000	b 1.000	b 1.000
Sod-1	e 1.000	f 1.000	a 0.167 c 0.833	e 1.000	f 1.000	f 1.000	f 1.000	c 0.042 e 0.048 f 0.929	c 1.000	e 1.000	e 1.000	e 1.000	e 0.950 f 0.050
A	1.25	1.40	1.10	1.20	1.15	1.35	1.20	1.60	1.10	1.45	1.20	1.20	1.70
P	25	35	10	20	15	25	15	50	10	45	20	20	50
H	0.025	0.067	0.017	0.007	0.083	0.041	0.019	0.065	0.050	0.031	0.050	0.061	0.096

ity (H, direct count) in *Draco*. See Table 1 for the

QNB (N=5)	TN (N=20)	VSM (N=15)	VMB (N=20)	PP (N=10)
d 1.000	d 1.000	d 1.000	d 1.000	a 1.000
c 0.800	c 1.000	c 0.133	c 0.100	a 1.000
d 0.200		e 0.867	e 0.900	
a 0.400	a 0.575	b 0.200	b 0.775	a 1.000
b 0.600	b 0.425	c 0.600	c 0.225	
		e 0.200		
a 1.000	a 1.000	a 1.000	a 1.000	b 1.000
b 1.000	b 1.000	c 1.000	c 1.000	d 1.000
c 1.000	b 0.025	a 0.600	a 0.025	c 1.000
	c 0.975	b 0.400	b 0.650	
			c 0.325	
c 1.000	b 1.000	b 0.200	b 0.200	a 1.000
		c 0.800	c 0.800	
c 1.000	d 1.000	c 1.000	c 0.778	a 1.000
			e 0.222	
d 0.200	e 1.000	d 0.733	a 0.075	d 1.000
f 0.800		f 0.267	d 0.725	
			f 0.200	
e 1.000	g 1.000	a 0.167	b 1.000	c 1.000
		b 0.844		
a 0.300	b 1.000	a 0.300	a 0.425	c 1.000
b 0.700		b 0.700	b 0.575	
a 1.000	c 1.000	a 1.000	a 0.975	e 1.000
			d 0.025	
e 1.000	c 0.050	d 0.633	d 0.075	a 1.000
	e 0.950	f 0.367	f 0.925	
b 1.000	b 0.050	e 1.000	e 1.000	a 1.000
	d 0.950			
c 1.000	a 0.200	c 1.000	c 1.000	e 1.000
	b 0.800			
b 1.000	a 1.000	a 0.100	a 0.250	a 1.000
		b 0.900	b 0.750	
a 0.333	a 0.400	a 0.433	a 0.800	b 1.000
b 0.667	b 0.600	b 0.576	b 0.200	
b 1.000	b 1.000	b 1.000	b 1.000	d 1.000
b 1.000	b 1.000	b 1.000	b 1.000	b 1.000
c 0.700	e 1.000	d 0.033	d 0.175	b 1.000
f 0.300		f 0.967	e 0.825	
1.30	1.30	1.60	1.70	1.00
30	30	55	60	0
0.040	0.050	0.113	0.120	0.000