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THE EVOLUTION OF FEMALE PARENTAL CARE IN POISON FROGS OF THE GENUS *DENDROBATES*: EVIDENCE FROM MITOCHONDRIAL DNA SEQUENCES

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ABSTRACT: We used nucleotide sequences from portions of the mitochondrial cytochrome b, cytochrome oxidase I and 16s rRNA gene regions to evaluate phylogenetic relationships within the genus *Dendrobates*, a group of neotropical poison frogs with complex parental behaviors. Mapping of parental care behaviors on the phylogenetic tree derived from the molecular analysis suggests that female-only care has evolved once within *Dendrobates*, after passing through a biparental stage involving male egg attendance and female tadpole transport and feeding. Phylogenetic analysis also suggests that female provisioning behaviors observed in some Amazonian species of poison frogs may have arisen independently from male care in this genus. Low levels of divergence between members of previously delimited groups within *Dendrobates* suggest that the members of these groups from Central and South America may have speciated relatively recently (after the formation of the current Panamanian land bridge in the Pliocene).

Key words: Poison frogs; *Dendrobates*; Parental care; Evolution; mtDNA

THE bright coloration, extreme toxicity, and complex behaviors of the poison frogs (*Dendrobatidae*) have made them the sub-

ject of considerable interest (e.g., Myers and Daly, 1983; Wells, 1978, 1981; Weygoldt, 1980, 1987; Zimmermann and Zimmermann, 1984, 1988). Nevertheless, the evolutionary relationships of the poison

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frogs are not well resolved, which impedes our understanding of the evolution of their complex behavioral strategies (Caldwell, 1997).

Controversy still surrounds the relationships of the dendrobatids to other anurans; phylogenetic research utilizing morphological characters suggests that the family is of ranoid ancestry (Ford, 1993), but recent analysis of mitochondrial DNA sequences groups dendrobatids with bufonids (Ruvinsky and Maxson, 1996). Members of the family Dendrobatidae are believed to form a monophyletic group (Ford and Cannatella, 1993), including at least six genera (*Aromobates*, *Colostethus*, *Epipedobates*, *Minyobates*, *Dendrobates*, and *Phyllobates*). Zimmermann and Zimmermann (1988) proposed two new genera of poison frogs, *Phobobates* and *Allobates*, from species previously placed within *Epipedobates*. However, these new genera have not been recognized as legitimate by all members of the scientific community (e.g., Myers et al., 1991). Two new genera of dendrobatids have also been proposed for some species previously placed in *Colostethus*: *Mannophryne* (La Marca, 1992a) and *Nephelobates* (La Marca, 1992b). The toxic dendrobatids are thought to form a monophyletic group exclusive of *Aromobates* and *Colostethus* (Myers et al., 1978, 1991; Silverstone, 1975, 1976), although recent molecular evidence suggests that *Phobobates trivittatus* and some members of *Colostethus* may be sister taxa (Ruvinsky and Maxson, 1996).

Dendrobates and *Phyllobates* were diagnosed as a monophyletic clade based on the presence of lipophilic alkaloids (Myers et al., 1978, 1991). *Dendrobates* was subsequently divided into several genera on the basis of morphology, acoustic parameters of the mating call, and the chemical structure of skin toxins (Myers, 1987): *Epipedobates* (22 species), *Minyobates* (eight species), *Phyllobates* (five species), and *Dendrobates* (21 species). Myers (1987) posited *Dendrobates* and *Phyllobates* as sister taxa, with *Minyobates* as their sister taxon, then *Epipedobates*.

The systematics of the poison frogs are of special importance to those interested

in the evolution of their complex parental and social behaviors. Members of the genus *Dendrobates* exhibit the most elaborate parental behaviors observed in the family (Weygoldt, 1987), including three parental care types—male, female, and biparental.

In *D. auratus*, *D. leucomelas*, *D. azureus*, *D. tinctorius*, and *D. truncatus*, small (2–8 eggs) clutches are laid in the leaf litter and are attended by the male (Summers, 1989, 1990; Wells, 1978; Weygoldt, 1987). Once the eggs have developed into mature tadpoles (10–14 days), they are typically carried by the male to small pools of water that form in treeholes (Dunn, 1941; Eaton, 1941; Wells, 1981).

In *D. histrionicus*, *D. speciosus*, *D. granuliferus*, and *D. pumilio*, eggs are also deposited in the leaf litter, but females carry the young and deposit them in small accumulations of water that form in the leaf or stem axils of plants (e.g., bromeliads). The female then returns to the pools periodically and lays infertile eggs that are eaten by the tadpole (Brust, 1993; Weygoldt, 1980; Zimmermann and Zimmermann, 1981). This form of feeding is probably obligatory in *D. pumilio*; the tadpole will neither grow nor survive if not provisioned with trophic eggs (Brust, 1993). The male performs egg attendance in some of these species [i.e., *D. pumilio* (Weygoldt, 1980) and *D. granuliferus* (Meyer, 1992)], but the female is the main care provider (Weygoldt, 1987). In *D. histrionicus* and *D. speciosus*, females carry out all aspects of parental care (Jungfer, 1985; Zimmermann and Zimmermann, 1981). The form of parental care in *D. arboreus* is not known, but attempts to keep it in captivity suggest that nutritive eggs are required for tadpole growth, implying that females feed their offspring (Walls, 1994). This species is thought to be closely related to *D. pumilio* on the basis of morphology and calling parameters (Myers et al., 1984).

In captivity, several species of a wide-ranging Amazonian group of poison frogs exhibit what appears to be biparental care. For example, in a study of captive *D. reticulatus*, Zimmermann and Zimmermann

(1984) observed that both parents attend the clutch, which is oviposited above a pool in the leaf axil of a bromeliad. The male carries the tadpoles to a different pool and returns periodically to call, thus attracting his mate who lays eggs at the surface of the pool, which the tadpoles consume. Evidence of biparental care has also been observed in other dendrobatids from the Peruvian Amazon, including *D. ventrimaculatus* [referred to as *D. quinquevittatus* by Zimmermann and Zimmermann (1988), but see Caldwell and Myers (1990)], *D. variabilis*, *D. imitator*, and *D. fantasticus* (Zimmermann and Zimmermann, 1988), although these behaviors are not well documented in captivity (Walls, 1994; Weygoldt, 1987) and have not been studied in the field.

Recently, biparental care has been observed in a field study of *D. vanzolinii*, another Amazonian poison frog (Caldwell, 1997). In this species, males carry tadpoles to small pools of water in treeholes, and females feed tadpoles with unfertile nutritive eggs. Males and females maintain an intimate association, or "pair bond" throughout the period of parental care (Caldwell, 1997).

Recent field research on a population of *D. ventrimaculatus* in Amazonian Ecuador suggests that this population (or species) has male care, rather than biparental care. *Dendrobates ventrimaculatus* is probably a complex of closely related species (Caldwell and Myers, 1990). Briefly, in the Ecuadorian population, the mating system is promiscuous, unrelated individuals oviposit in the same axil, and females do not return to pools to feed tadpoles (Summers and Amos, 1997; Summers, unpublished data).

Zimmermann and Zimmermann (1988) analyzed relationships among 32 species in six genera of poison frogs using overall similarity in behavior (based on 62 behavioral characters, including some behaviors involved in parental care). They resolved three "species groups" within *Dendrobates*, each exhibiting a specific type of parental care: (1) male parental care (*D. auratus*, *D. leucomelas*, *D. tinctorius*, *D. azureus*, *D. truncatus*), (2) female (or pre-

dominantly female) parental care (*D. pumilio*, *D. granuliferus*, *D. histrionicus*, *D. lehmanni*, and *D. speciosus*), and (3) biparental care (*D. ventrimaculatus*, *D. reticulatus*, *D. fantasticus*, *D. imitator*, and *D. variabilis*). Zimmermann and Zimmermann (1988) hypothesized that these behavioral groups corresponded to monophyletic evolutionary groups.

Weygoldt (1987) and Zimmermann and Zimmermann (1984, 1988) proposed that male parental care is the primitive behavioral condition within dendrobatids, and that male care gave rise to biparental care of the form observed in some Amazonian species, which later gave rise to female care, as seen in *D. pumilio*, *D. granuliferus*, *D. speciosus*, and *D. histrionicus*.

Male parental care was hypothesized as the ancestral state in *Dendrobates* based on studies of parental care in species from *Phyllobates*, *Minyobates*, and *Epipedobates* (Weygoldt, 1987). Four out of five species of *Phyllobates* have been studied, and all exhibit male parental care (Weygoldt, 1987; Zimmermann and Zimmermann, 1988). Fourteen out of 22 species of *Epipedobates* have been studied, and all exhibit male parental care (Weygoldt, 1987; Zimmermann and Zimmermann, 1988). A recent study of *Minyobates minutus* demonstrated that this species also exhibits male parental care (Summers, unpublished data). In most species of *Colostethus*, the males provide care for the tadpoles (Weygoldt, 1987), but females provide care (tadpole transport) in some species of *Colostethus* (Wells, 1981). However, tadpoles are transported to streams (not axil pools) and tadpole feeding does not occur in these species (Weygoldt, 1987). The type of parental care (if any) in the most basal taxon, *Aromobates*, is unknown (Myers et al., 1991). The most parsimonious interpretation of the information currently available is that male parental care is the primitive state within *Dendrobates* (Weygoldt, 1987).

Here we present an analysis of DNA sequence data from the mitochondrial cytochrome b, cytochrome oxidase I, and 16S rRNA genes that helps to resolve the systematic relationships of species in the ge-

TABLE 1.—Forms of parental care observed in the poison frogs included in this analysis. References for these observations are as follows: Jungfer, 1985; Meyer, 1993; Summers, 1989, 1992, unpublished data; Walls, 1994; Wells, 1978; Weygoldt, 1987; Zimmermann and Zimmermann, 1981, 1988.

Species	Egg attendance	Larval transport	Larval feeding	Source
<i>D. auratus</i>	Male	Male	No	Field and captive
<i>D. leucomelas</i>	Male	Male	No	Field and captive
<i>D. fantasticus</i>	Male	Male	Yes	Captive
<i>D. ventrimaculatus</i>	Male	Male	No	Field
<i>D. granuliferus</i>	Male	Female	Yes	Field and captive
<i>D. pumilio</i>	Male	Female	Yes	Field and captive
<i>D. arboreus</i>	Unknown	Female?	Yes?	Captive
<i>D. speciosus</i>	Female	Female	Yes	Field and captive
<i>D. histrionicus</i>	Female	Female	Yes	Field and captive

nus *Dendrobates*, and allows an assessment of the evolution of parental care in this group. In particular, we focus on the species with female or predominantly female care, to test the hypothesis that these species form a monophyletic group. The type of parental care found in each species is listed in Table 1. Phylogenetic analyses of these data contribute to a more complete understanding of the evolutionary relationships of these frogs, and they permit inferences concerning the evolution of parental care and other behaviors that are independent of the behavioral characters of interest.

MATERIAL AND METHODS

We analyzed five out of the seven species in the female care group of *Dendrobates* (see above). We also included four other species of *Dendrobates* (*D. auratus*, *D. leucomelas*, *D. ventrimaculatus*, *D. fantasticus*), and two outgroup species, *Pho-*

bobates trivittatus and *Colostethus talamancae*. These species, the areas where they were collected, the region of mitochondrial DNA sequenced, and the number of individuals sequenced are listed in Table 2. Overall, we sequenced 292 base pairs of the cytochrome b gene, 521 base pairs of the cytochrome oxidase I gene, and 536 base pairs of the 16s rRNA gene (GenBank accession numbers for COI: AF097496-506; for cyt b: AF120008-017; for 16s: AF098740-750). Whole frogs were preserved in liquid nitrogen and stored at -70°C , or in DNA preservation buffer (20% DMSO, 0.25 M EDTA, NaCl to saturate) until analyzed. Tissue dissections were extensive; as a result the remaining carcasses could not be usefully maintained as vouchers. Two species (*D. fantasticus* and *P. trivittatus*) were obtained from the U.S. National Aquarium in Baltimore, Maryland. The specimen of *D. fantasticus* was a second generation offspring from

TABLE 2.—Collection localities and gene regions sequenced for each species in this study. CyB = cytochrome b, COI = cytochrome oxidase I, 16s = 16s rRNA. Numbers in parentheses indicate the number of individuals sequenced for that gene region.

Country	Location	Species	Sequences
Panama	Bocas del Toro	<i>D. pumilio</i>	CyB(2), COI(2), 16s(1)
Panama	Nusagandi	<i>C. talamancae</i>	CyB(1), COI(1), 16s(1)
		<i>D. auratus</i>	CyB(2), COI(2)
Panama	Fortuna	<i>D. speciosus</i>	CyB(1), COI(2), 16s(2)
		<i>D. arboreus</i>	CyB(1), COI(1), 16s(1)
Venezuela	Tabogan	<i>D. leucomelas</i>	CyB(1), COI(2), 16s(2)
Ecuador	Santo Domingo	<i>D. histrionicus</i>	CyB(1), COI(1), 16s(1)
Ecuador	Limoncocha	<i>D. ventrimaculatus</i>	CyB(1), COI(2), 16s(1)
Costa Rica	Corcovado	<i>D. granuliferus</i>	CyB(1), COI(1), 16s(1)
Peru	Chumilla	<i>P. trivittatus</i>	CyB(1), COI(1), 16s(1)
Peru	Yurimaguas (F1)	<i>D. fantasticus</i>	CyB(1), COI(1), 16s(1)

wild-caught animals, whereas the specimen of *P. trivittatus* was a wild-caught individual. The sample of DNA for the 16s sequence of *D. auratus* was obtained from a captive bred animal.

Extraction of DNA

We homogenized approximately 0.5 g of muscle tissue in 300 μ l of lysis buffer (100 mM EDTA, 100 mM Tris pH 7.5, 1% SDS). Samples were homogenized and incubated overnight with 25 μ l of proteinase K solution (20 mg proteinase K/ml in 50% glycerol) at 37 C. The homogenate was centrifuged for 3 min at 14,000 rpm. The supernatant was transferred to a new tube and extracted once with equal volumes of equilibrated phenol, once with phenol-chloroform-isoamyl alcohol (25:24:1), and once with chloroform-isoamyl alcohol (24:1). DNA was precipitated for 30 min at -20 C with ethanol and 3 M sodium acetate, and centrifuged for 20 min at 14,000 rpm. The resulting pellet was rinsed once with 70% ethanol, vacuum dried, resuspended in 100 μ l of dH₂O, and stored at -20 C.

Enzymatic Amplifications

Initial polymerase chain reaction amplifications were performed in 50 μ l reactions containing 1 μ l of genomic DNA, 5 μ l of 10 \times buffer, 2.5 μ l each of 10 mM stock solutions of the 16s primers (16sar-L and 16sbr-H; Palumbi et al., 1991), cytochrome oxidase I primers (COIa and COIf; Palumbi et al., 1991), or cytochrome b primers (H14841 and L15182; Kocher et al., 1989), 5 μ l of 10 mM dNTP mix, and 0.25 μ l (1.25 units) of Taq Polymerase. The samples were overlaid with a drop of mineral oil and cycled 30-35 times on a Perkin-Elmer thermal cycler using standard conditions: 94 C for 45 s (denaturing step), 50 C for 45 s (primer annealing step), and 72 C for 60 s (primer extension step). Following amplification, the PCR products were run in 1.5% agarose gels in 1X TBE (89 mM Tris, 89 mM Boric Acid, 2 mM EDTA) and stained with ethidium bromide.

Sequencing was carried out with radioactive labeling for some samples and with

fluorescently labeled dNTPs on an automated sequencer for others. For radioactively labeled sequencing, a single band was visualized and this fragment was cut from the gel and diluted in 400 μ l of dH₂O. For cytochrome oxidase I, 1 μ l of this sample was amplified a second time using the same conditions described above except that we replaced one of the two COI primers with a phosphorylated COI primer and reduced the number of amplification cycles to 20. This second reaction was carried out two times for each sample; one reaction used the phosphorylated COIa primer and the other used the phosphorylated COIf primer.

For the cytochrome b primers, 1 μ l of the sample was used in an asymmetric amplification in which one primer (the limiting primer) was present at 0.01 of the original concentration in the polymerase chain reaction.

Sequencing Template

For radioactive labeling, following the second amplification with COI primers, the DNA strand initiated with the phosphorylated primer was digested with lambda-exonuclease yielding the single-stranded DNA products used as sequencing templates (Higuchi and Ochman, 1989). To the double-stranded DNA product (45 μ l), we added 5 μ l of 10 \times lambda-exonuclease supplement (775 mM glycine, 278 mM KOH, 5.8 mM MgCl₂, 5.8 mg/ml bovine serum albumin) and incubated for 30 min at 37 C with 2.5 units of lambda-exonuclease. The lambda-exonuclease was then heat denatured at 94 C for 5 min. Next the samples were desalted and concentrated over Centricon 30 columns. After the asymmetric amplification using cytochrome b primers, the samples were desalted and concentrated over Centricon 30 columns, as for cytochrome oxidase I.

DNA Sequencing

We carried out dideoxy sequencing reactions using the Sequenase 2.0 kit (United States Biochemical Co.) and following the vendor's protocol. The sequencing reactions were resolved in 6.0% polyacrylamide gels which were run for 2, 4, 7, and

9 h. The gels were dried and exposed to autoradiograph film for 12–48 h.

Automated Sequencing

All 16s and some COI and cytochrome b sequences were obtained via ABI 373 and 377 automated sequencers. A single amplification was carried out as for the other species, then the product was purified using Microcon 100 filters and sequenced in both directions using the ABI Prism Sequencing Reactions Kit and following the protocols therein.

Sequence Alignments and Phylogenetic Analyses

We sequenced DNA from each individual in both directions to check for sequence accuracy. DNA sequences were read from autoradiographs into the MacVector sequence alignment program (IBI, 1990) using the IBI gel reader. Sequence fragments for the same individual from different gels were aligned with the MacVector sequence alignment algorithm, and consensus sequences were constructed for each individual sequenced.

Electropherograms from the automated sequencers were aligned and consensus sequences for each individual were constructed using the ABI Sequence Assembler software (Applied Biosystems). Cytochrome oxidase I and cytochrome b gene region sequences were aligned with the Gene Jockey Sequence Analysis Software (Taylor, 1990), and 16s RNA sequences were aligned with the Clustal Sequence Alignment Program (Thompson et al., 1994). Minor adjustments to the alignments were made by eye after initial alignments were carried out with the programs. There were no gaps in the COI and cytochrome b alignments, and few gaps in the 16s alignments. In the phylogenetic analysis, single gaps (of any size) were coded as single characters if they were informative. The number of such informative gaps was small: gaps accounted for only three informative characters in the analysis, and removing them from the analysis did not change the topology of the most parsimonious tree. The aligned sequences are presented in Appendix I.

In some cases, the nucleotide base at a particular position could not be determined or was ambiguous. In these cases, the base is represented with a question mark, for missing data. Base pair mismatches between different individuals of the same species were extremely rare. Hence consensus sequences were constructed for each species, and mismatches were represented as unknown base pairs. Genetic distances between species were calculated with MEGA (Kumar et al., 1993). Phylogenetic analyses were carried out with PAUP 3.1 (Swofford, 1993). Phylogenetic analysis was carried out using sequence data from all three gene regions combined, consistent with the total evidence approach (Kluge, 1989; Kluge and Wolf, 1993). Character weighting was carried out with a dynamic weighting method that utilizes the negative natural log of transition and transversion frequencies (based on an initial tree derived from an unweighted parsimony analysis) to construct a stepmatrix of transition and transversion costs (Williams and Fitch, 1990). Support for clades within the most parsimonious phylogenetic hypothesis was assessed with bootstrap analysis (Felsenstein, 1985). Character mapping (using parsimony) was carried out with MacClade (Maddison and Maddison, 1992).

Based on morphological, toxicological, and behavioral characteristics, members of the genus *Colostethus* are considered to be outside of a clade formed by the toxic dendrobatids (Myers et al., 1991). There is some question as to whether the genus *Colostethus* is monophyletic or whether it should be broken into two groups (Myers et al., 1991; Rivero, 1984), but the placement of members of this genus as basal to all the toxic dendrobatids has not been questioned until recently. Recent analysis of 16s rRNA sequences suggests that *P. trivittatus* may be more closely related to *Colostethus* than to other toxic dendrobatids (Ruvinsky and Maxson, 1996). We used both *C. talamancae* and *P. trivittatus* as outgroup species in the analysis.

RESULTS

Genetic distances among taxa, based on the Kimura two-parameter model (Kimu-

TABLE 3.—Genetic distances calculated between all pairs of taxa, based on all nucleotide sequence data, using the Kimura 2-parameter model with transversions weighted 2:1 to transitions.

OTU's	1	2	3	4	5	6	7	8	9	10
1) <i>C. talamancae</i>										
2) <i>D. fantasticus</i>	0.2489									
3) <i>D. histrionicus</i>	0.2173	0.2347								
4) <i>D. leucomelas</i>	0.2121	0.2110	0.1756							
5) <i>D. pumilio</i>	0.2237	0.2292	0.0509	0.1714						
6) <i>D. auratus</i>	0.2013	0.2129	0.1662	0.1071	0.1510					
7) <i>D. ventrimaculatus</i>	0.2456	0.1455	0.1938	0.1970	0.1908	0.1958				
8) <i>D. speciosus</i>	0.2275	0.2386	0.0458	0.1735	0.0493	0.1610	0.1985			
9) <i>D. arboreus</i>	0.2195	0.2247	0.0391	0.1674	0.0257	0.1530	0.1941	0.0399		
10) <i>D. granuliferus</i>	0.2318	0.2352	0.0888	0.1631	0.0824	0.1652	0.2155	0.0789	0.0761	
11) <i>P. trivittatus</i>	0.2170	0.2527	0.2069	0.2115	0.2014	0.1946	0.2306	0.2156	0.2004	0.2121

ra, 1980), varied from 4–25% divergence (Table 3). Transition to transversion ratios for species that had high levels of sequence similarity, and were unambiguously closely related in preliminary phylogenetic analyses, ranged from 4–14 transitions per transversion, but declined to <2:1 for the more highly divergent sequence pairs, suggesting the possibility of saturation for the more distantly related taxa (Brown, 1985; Moritz et al., 1987). Dynamic weighting of transitions and transversions (Williams and Fitch, 1990) should ameliorate any noise associated with such saturation, but character weighting had little effect on the topology of the phylogenetic tree produced by parsimony analysis (see below).

Phylogenetic analysis produced a single most parsimonious tree (Fig. 1). Unweighted analysis produced the same topology, with the exception that the *D. arboreus*-*D. pumilio* clade collapsed into a polytomy, and *C. talamancae* collapsed into a polytomy with *P. trivittatus*. Members of the female care group appeared as a monophyletic group in our analysis, supporting previous claims that these species form a monophyletic group. The species with biparental (but predominantly female) care (*D. granuliferus*, *D. pumilio*) fell out as basal within the female care group, whereas the two species with female-only care (*D. histrionicus* and *D. speciosus*) appear to be relatively derived. The position of *D. arboreus* within this clade is consistent with previous suggestions that it is closely related to *D. pumilio*.

Dendrobates auratus and *D. leucomelas* also came out as sister taxa, which agrees with the results of Zimmermann and Zimmermann (1988). Both of these species have male parental care: *D. auratus* is widely distributed in Central America and Colombia, and *D. leucomelas* is found in the Guyana highlands and the Orinoco River basin in Venezuela.

Dendrobates ventrimaculatus and *D. fantasticus* also appeared as sister taxa in our analysis. These species are both members of a large complex of Amazonian species that are morphologically similar and believed to be closely related (Caldwell and Myers, 1990). Our results support that interpretation.

In contrast to the hypothesis proposed by Zimmermann and Zimmermann (1984, 1988) and Weygoldt (1987), our results suggest that the Amazonian species are not the sister group of the *D. granuliferus*, *D. pumilio*, *D. arboreus*, *D. speciosus*, and *D. histrionicus* clade. Instead, our analysis places the *D. granuliferus* clade as the sister taxon to the northern species with male parental care, *D. auratus* and *D. leucomelas*.

DISCUSSION

The phylogenetic hypothesis derived from our mitochondrial DNA data has implications with respect to the evolution of parental care in the genus *Dendrobates*. Figure 2 shows the most parsimonious reconstruction of the evolution of different facets of parental care on the hypothesis

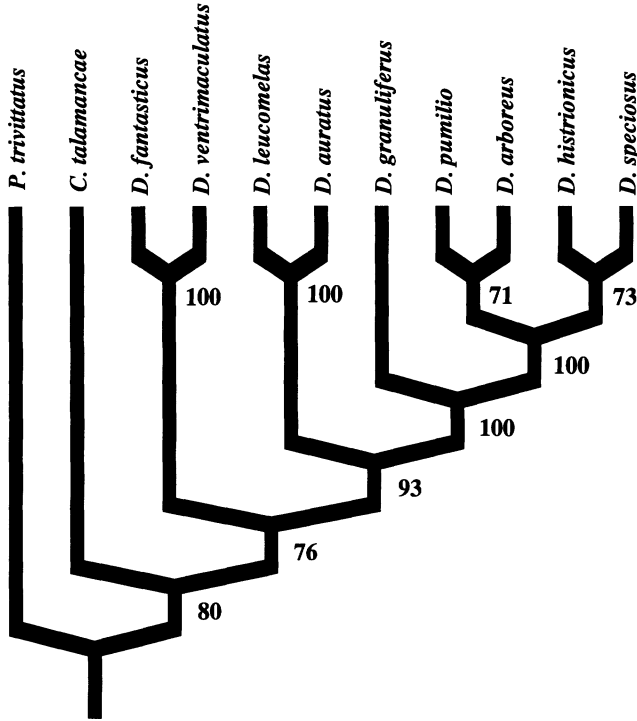


FIG. 1.—Phylogenetic tree derived from a weighted parsimony analysis of mitochondrial DNA substitutions. Substitutions were weighted according to their frequency using a dynamic weighting method (see text). The treelength for the weighted analysis was 2442 (a consistency index was not calculated for the stepmatrix characters). Numbers at the base of each node refer to the percent of trees in which that clade appeared in 1000 bootstrap replicates. An unweighted parsimony analysis produced the same topology, with the exception that *C. talamancae* and *P. trivittatus* formed a basal polytomy, and the *D. arboreus*-*D. pumilio* clade collapsed to form a polytomy with the *D. speciosus*-*D. histrionicus* clade. The treelength was 1061 and the Consistency Index was 0.59 for the unweighted analysis.

of phylogenetic relationships derived from the mitochondrial DNA sequence data. This reconstruction suggests that female parental care in the *D. granuliferus*, *D. pumilio*, *D. arboreus*, *D. speciosus*, and *D. histrionicus* clade evolved from male parental care, passing through a stage in which males cared for eggs and females carried tadpoles.

Our analysis does not support the hypothesis that female parental care evolved from biparental care of the kind seen in certain Amazonian species, as proposed by Zimmermann and Zimmermann (1984, 1988) and Weygoldt (1987). Instead, it suggests that larval feeding behavior by females has evolved independently in two lineages, once in certain Amazonian species (e.g., *D. fantasticus* and *D. vanzolinii*) and once in a Central

American-northern South America clade represented by *D. pumilio*, *D. histrionicus*, *D. arboreus*, *D. granuliferus*, and *D. speciosus*.

This inference is further supported by the placement of *D. granuliferus* as basal to the female care group. This suggests that the female care group originated in Central America (*D. granuliferus* is restricted to southwestern Costa Rica). If this group had a South American origin, then *D. histrionicus* (from Colombia) should appear as the basal species in the female care lineage. The significance of this for parental care is that all the biparental members of the Amazonian species group are restricted to the Amazonian regions of South America, suggesting that it was unlikely that an ancestral member of this clade gave rise to the *D. pumilio*, *D.*

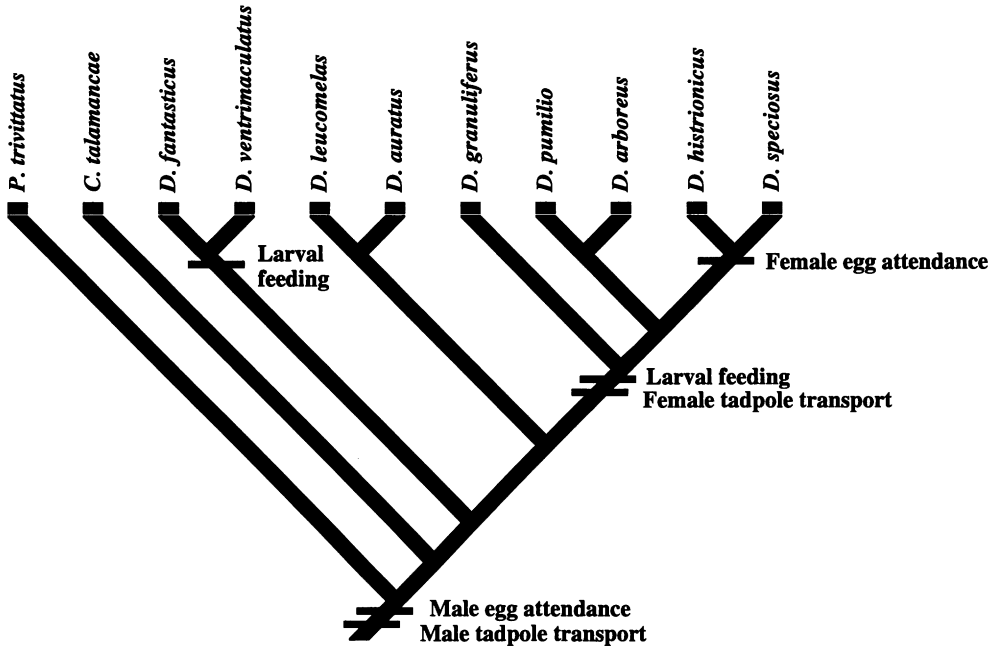


FIG. 2.—The most parsimonious reconstruction of the evolution of female parental care in the genus *Dendrobates*, based the phylogenetic tree derived from the mitochondrial DNA sequence data.

histrionicus, *D. arboreus*, *D. granuliferus*, and *D. speciosus* clade.

Silverstone (1975) suggested that members of the genus *Dendrobates* colonized Central America from South America during the Pliocene, after the establishment of the present Panamanian land bridge, 3–5 million years ago. The relatively low levels of genetic divergence between the members of the female care clade represented by *D. pumilio*, *D. speciosus*, *D. arboreus*, and *D. granuliferus* from Central America and *D. histrionicus* from South America, are roughly consistent with Silverstone's (1975) argument, whereas those between *D. auratus* from Panama and *D. leucomelas* from Venezuela are somewhat higher than might be expected.

A phylogenetic analysis of mtDNA sequences by Ruvinsky and Maxson (1996) indicated a sister group relationship between *P. trivittatus* and *C. talamancae*. In contrast, in our analysis *C. talamancae* was placed closer to *Dendrobates* than to *P. trivittatus*. This distinction was not, however, upheld in an unweighted parsimony analysis, in which *C. talamancae* and *P. trivit-*

tatus collapsed into a basal polytomy. Resolution of these conflicting results awaits further analysis.

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APPENDIX I

Aligned DNA sequences for each gene region. Abbreviations are as follows: DPUMIL = *D. pumilio*, DAURAT = *D. auratus*, DSPECI = *D. speciosus*, DHISTR = *D. histrionicus*, DLEUCO = *D. leucomelas*, DVENTR = *D. ventrimaculatus*, CTALAM = *C. talamancae*, DFANTA = *D. fantasticus*, DARBOR = *D. arboreus*, PTRIVI = *P. trivittatus*. These sequences have been submitted to GenBank.

Cytochrome Oxidase I

CTALAM ATCCTCCCTGGCT?TGGTATTATCTCTCACGTAGTAACCTACTACTCTAGCAAAAAAGAA
 DFANTA ATGCTTCCGGGTTTTGGTATCATCTCCACGTGGTAACATTTTACTCCA?C?AAAAAAA
 DHISTR ATCCTCCCAGGCTTCGGAATCATCTCCCATGTAGTCACGTTTTACTCAAACAAAAAGAG
 DLEUCO ATCC??CCCGTTTTGGGATTATCTCTCATATAGTAACATTCTATTCAAGCAAAAAAGAA
 DPUMIL ?TCCCTCCAGGCTTCGGAATAATCTCCCATGTAGTCACGTTTTACTCAAGCAAAAAAGAA
 DAURAT ATCCTCCCTGGCTTTGGGAATTATTTCCACGTAGTAACATTTTATTCAAGCAAAAAAGAA
 DVENTR A?TCTTCCAGGTTTTCGGTATCATTTCCACGTAGTTACATTCTATTCCGGCAAAAAAGAG
 DSPECI ATCCTCCCAGGCTTCGGAATCATCTCCCATGTAGTCACATTTTACTCAAGCAAAAAAGAG
 DARBOR ATCCTCCCAGGCTTCGGAATCATCTCCCATGTAGTCACGTTTTACTCAAGCAAAAA?AA
 DGRANU ATCCTCCCAGGTTTTCGGAATCATCTCTCATGTGGTCACATTTTACTCAAGCAAAAA??A
 PTRIVI ATTCTTCCAGGATTTGGGATTATTTCCCATGTCTCACATTTACTCTAGCAAAAAAGGAG

CTALAM CCTTTTGGGTACATAGGCATAGTCTGAGCTATAATATCCATTGGCCTCCTTGGTTTTATT
 DFANTA CCCTTTGGCTATATAGGCATGGTTTTGAGCGATAATATCTATTGGTCTTCTAGGCTTTTATC
 DHISTR CCATTTGGTTACATAGGAATAGTCTGAGCCATAATATCTATTGGCCTCTTAGGTTTCATT
 DLEUCO CCTTTTGGGTACATAGGCATGGTCTGAGCCATAATATCCATCGGCTTACTAGGCTTTTATC
 DPUMIL CCATTTGGTTACATGGGAATAGTCTGAGCCATAATATCTATTGGCCTATTAGGTTTCATT
 DAURAT CCTTTCGGGTATATAGGCATGGTTTTGAGCCATAATATCTATTGGCTTGCTAGGCTTTATT
 DVENTR CCATTCGCGTACATAGGCATAGTTTTGAGCTATAATATCTATTGGACTTTTAGGCTTTTATC
 DSPECI CCATTTGGTTACGTAGGAATAGTCTGAGCCATAATATCTATTGGCCTCTTAGGTTTCATT
 DARBOR CCATTTGGCTACATAGGAATAGTCTGAGCCATAATATCTATTGGCCTCTTAGGTTTCATT
 DGRANU CCATTTGGCTATATGGGGATAGTCTGAGCCATAATATCTATCGGTCTCTTAGGTTTCATT
 PTRIVI CCCTTCGGCTATATGGGGATAGTTTTGAGCTATAATATCAATCGGCTCCTAGGATTCATT

CTALAM GTTTGAGCCCACCACATATTCACCCTGACCTAAATGTAGATACTCGAGCCTATTTTACC
 DFANTA GTCTGACCCCACCATATATTCACTACTGACCTAAATGTAAACACCCGAGCCTACTTTACC
 DHISTR GTGTGAGCTCATCATATATTTACTACAGACTTAAATGTGGATACACGAGCCTACTTTACC
 DLEUCO GTCTGAGCCCACCATATGTTTACTACAGATCTCAACGTGGACACTCGAGCCTATTTTACC
 DPUMIL GTATGAGCTCATCATATATTTACTACAGACTTAAACGTAGACACACGAGCCTACTTTACC
 DAURAT GTCTGGGCCCACCATATATTCACTACAGACTTAAACGTAGACACTCGAGCCTATTTTACC
 DVENTR GTCTGGGCCCACCATATGTTTACTACTGACCTTAAATGTGGACACCCGAGCCTACTTTACC
 DSPECI GTATGAGCCCACATATATTTACTACAGACTTAAACGTGGACACACGAGCCTACTTTACC
 DARBOR GTTTGAGCTCATCATATATTTACTACAGACTTAAACGTAGACACACGAGCCTACTTTACC
 DGRANU GTATGAGCCCACATATGTTTACTACAGACTTAAACGTGGACACAC?AGCCTACTTTACT
 PTRIVI GTTTGAGCCCACCACATGTTTACAACCTGACCTTAAATGTAGATACTCGAGCCTACTTTACC

CTALAM TCAGCTACTATAATCATCGCTATCCCTACCGAGTAAAAGTTTTTCAGCTGATTAGCAACA
 DFANTA TCGGCCACTATAATTATCGCCATCCCTACGGCGTCAAAGTCTTTAGCTGACTAGCCACA
 DHISTR TCAGCCACCATAATCATTTGCTATCCCAACAGGCGTGAAAGTCTTTAGCTGACTTGCCACT
 DLEUCO TCAGCCACTATAATCATCGCTATCCCAACGGGCGTTAAAGTCTTCAGCTGGCTTGCCACA
 DPUMIL TCAGCCACCATAATTTATGCTATCCCAACAGGTGTGAAGTCTTTAGTTGACTTGCTACT
 DAURAT TCAGCCACTATAATTATGCTATCCCAACAGGTGTAAAGTCTTTAGTTGGCTCGCTACA
 DVENTR TCAGCCACCATAATTATCGCCATCCCAACAGGCGTTAAAGTCTTTAGCTGACTAGCCACA
 DSPECI TCAGCCACCATAATCATTTGCTATCCCAACAGGCGTGAAAGTCTTTAGTTGGCTTGCCACT
 DARBOR TCAGCCACCATAATTATGCTATCCCAACAGGCGTGAAAGTCTTTAGTTGACTTGCTACT
 DGRANU TCAGCCACCATAATTATGCTATCCCAACAGGCGTGAAAGTCTTTAGCTGACTTGCCACT
 PTRIVI TCAGCTACAATAATCATTTGCTATCCCAACAGGTGTAAAAGTCTTTAGCTGACTTGCTACC

APPENDIX I.—Continued

CTALAM	ATGCACGGAGGTAATTAATGAGATGCTGCTATGCTCTGAGCCCTGGGATTCATCTTT
DFANTA	ATACATGGGGGGTCAATTAATGAGAAGCTGCTATACTTTGAGCTCTCGGCTTTATTTTC
DHISTR	ATGCATGGGGGGTAAATCAAATGAGAAGCTGCCATGCTTTGGGCCCTAGGGTTTATTTTT
DLEUCO	ATACATGGAGGCATTAATCAAATGAGAAGCCGCAATGCTCTGAGCTCTTGGCTTCATTTTT
DPUMIL	ATACATGGAGGAATTAATCAAATGAGAAGCTGCCATGCTTTGAGCCCTAGGGTTTATTTTT
DAURAT	ATGCATGGAGGCATTAATTAATGAGAAGCCGCAATGCTATGGGCCCTCGGCTTCATTTTT
DVENTR	ATACATGGAGGAGCCATCAAGTGAAGCTGCCATATTATGGGCTCTCGGCTTTATTTTT
DSPECI	ATACATGGAGGGATTATCAAATGAGAAGCTGCTATGCTCTGAGCCCTAGGGTTTATTTTT
DARBOR	ATGCATGGGGGAATTAATCAAATGAGAAGCTGCCATGCTTTGAGCCCTAGGGTTTATTTTT
DGRANU	ATACATGGAGGAATTAATCAAATGAGAAGCTGCTATGCTTTGAGCCCTAGGATTTATTTTT
PTRIVI	ATGCACGGGGAAATCAATTAATGAGATGCCCCATGCTGTGAGCCCTAGGATTCATCTTT
CTALAM	TTGTTACAGTTGGAGGCCAACCGGCATTGTTCTCGCTAACTCCTCTCTAGATATTGTC
DFANTA	CTTTTTACAGTCGGGGGGCTAACTGGAATTGTTCTAGGTAATTTTCT?TAGACATTGTT
DHISTR	TTATTTACTGTGGGTGGCCTAACCAGGAATCGTCTTGGCTAATTCATCTCTTGATATTGTT
DLEUCO	CTTTTCACTGTGGAGGTTAACAGGAATTGCTCTAGCTAATTCCTCCTTAGACATTGTT
DPUMIL	TTATTTACTGTAGGCGGCTTAACGGGATCGTCTTGGCTAATTCATCTCTTGACATCGTT
DAURAT	CTTTTTACCGTGGAGGTTGACCGGAATTGCTCTAGCCAACTCCTCTTAGACATTGTC
DVENTR	CTCTTTACGGTGGGGGGCTGACCGGATGTTCTAGCTAATTCCTCTCTAGATATTGTT
DSPECI	TTATTTACTGTGGGCGGCTGACCGGAATCGTCTTGGCTAACTCATCTCT?GACATCGTC
DARBOR	TTATTTACTGTAGGCGGCTAACTGGAATCGTCTTGGCTAACTCATCTCTTGACATCGTT
DGRANU	TTATTTACTGTAGGGGTCTAACCAGGAATCGTCTTAGCTAATTCATCTCTTGACATTGTT
PTRIVI	TTATTTACAGTTGGAGGACTGACCGGATGTTCTAGCCAATTCCTC?TTAGACATTGTC
CTALAM	CTTCATGACACATATTATGTTGTTGCTCATTTCCACTATGTTCTATCCATGGGAGCTGTA
DFANTA	CTCCACGATACATTATATGTCGTAGCCACTTCCACTACGTCCTAT?TATGGGTGCAGTA
DHISTR	CTTCATGATACATATTACGTAGTCGCACACTTCCATTATGTTTTATCTATAGGAGCAGTC
DLEUCO	CTCCACGACACCTACTATGTTGTTGCCAATTTCCACTATGCTTATCCATGGGTGCGGTC
DPUMIL	CTTCATGACACATATTACGTAGTCGCACACTTCCATTATGTTCTATCTATGGGGGAGTC
DAURAT	CTTCATGATACATATTATGTTGTTGCTCATTTCCACTATGCTTATCCATAGGTGCAGTG
DVENTR	CTTCACGACACTTACTACGTGCTGGCCACTTTCACTACGTTCTGCTATAGGCGCAGTA
DSPECI	CTTCATGACACATATTACGTAGTCGCACACTTCCATTATGTTCTAT?TATAGGAGCAGTC
DARBOR	CTTCATGACACATATTACGTAGTCGCACACTTCCATTATGTTCTATCTATAGGAGCAGTC
DGRANU	TTTCATGACACATATTATGTTAGTCGCACACTTCCATTATGTTCTAT?TATAGGGGAGT?
PTRIVI	CTACACGACACATATTATGTTAGTAGCTCACTTTCACTATGTTCTTCTATAGGAGCAGTA
CTALAM	TTTGCTATTATAGCGGATTTGTTCACTGATTTCCCTCTTTTTTCTGGATATACTCTTCAT
DFANTA	TTTCGCAATTATAGCTGGCTTTGTACACTGATTTCCACTTTTTTACCGG?TACACATTGCAT
DHISTR	TTTGCAATCATAGCCGGCTTTGTCCACTGATTTCCACTTTTTTCTGGGTTCACTCTTCAT
DLEUCO	TTTCGCCAATTATAGCCGGCTTCGTACACTGATTTCCCTTTTTTACCAGGCTTACACTTCAT
DPUMIL	TTTGCAATTATAGCCGGCTTTGTCCACTGATTTCCCGTTTTTTCTGGATATACTCTTCAT
DAURAT	TTTGCCATTATAGCTGGCTTTGTACACTGATTTCCCTTTTTTACGGCTTTACGTTTAC
DVENTR	TTTCGCAATTATAGCTGGCTTTGTACACTGATTTCCGCTTTTTTACTGGCTATACATTGCAC
DSPECI	TTTGCAATCATAGCCGGCTTTGTCCACTGATTTCCGCTTTTTTCTGGATATACTTTTTCAT
DARBOR	TTTGCAATTATAGCCGGCTTTGTCCACTGATTTCCCGTTTTTTCTGGGATATACTCTTCAT
DGRANU	TTTGCAATTATAGCCGGCTTTGTCTCACTGATTTCCCGTTTTTTCTGGATATACTTTTTCAT
PTRIVI	TTTGCTATCATAGCCGGCTTCGTGCACTGATTTCCCTCTTCTCCGGATTTACCCTTCAT
CTALAM	GAAACTTGAACAAAATCCATTTGGGGTGATATTGCGAGG
DFANTA	AGTACCTGAACAAAGATTCACTTTGGTG?GATATTTGCCGG
DHISTR	GAAACATGAACATAAACCCATTTGGTGTAATATTGCCGG
DLEUCO	GAGACCTGAACAAAATTCACTTTGGCGTCATATTGCCGG

APPENDIX I.—Continued

DPUMIL GAAACATGAACAAAAACCCACTTTGGTCTAATATTTGCCGG
 DAURAT GAAACCTGAACAAAAACTCACTTTGGTGTATATTTGCCGG
 DVENTR GACACTGAACAAAAATCCATTTGGTGTAAATATTTGCCGG
 DSPECI GATACATGGGCAAAGACCCACTTTGGTGTAAATATTCGCCGG
 DARBOR GAAACATGAACAAAAACCCACT??GGTCTAATATTTGCCGG
 DGRANU GAATCATGGACAAAAGCACACTTTGGTGTATATTTGTCCG
 PTRIVI GAAGCTGAACAAAAATCAATTTGGCGTCATATTTACAGG

Cytochrome B

CTALAM TTTGGCTCATTGTTAGGTCTTTGCTTAATTGCTCAAATCATTACGGGCTCTTCTTGCT
 DFANTA TTTGGGTCTCTACTCGGTATCTGCTTAGTTATTCAAATCCTAACAGGATTATTC?TGGCA
 DHISTR TTCGGCTCCCTTCTTGGCCTCTGTCTTATTGCCCAAATCATCACTGGCCTTTTTTTAGCA
 DLEUCO TTTGGCTCCCTCCTAGG?CTCTGCCTTATTGCCCAAATCTCACAGGCTTTTCTTGCC
 DPUMIL TTCGGCTCCCTTCTTGGCCTCTGTCTAATTGCCCAAATCATTACTGGCCTTTTTTTAGCA
 DAURAT TTTGGCTCCCTTTTTAGGACTCTGCCTCATTGCCCAGATCCTCACAGGCTTTTCTAGCT
 DVENTR TTCGGCTCCCTCCTCCTCTCTGCCTAATTATACAAATCCTCACCGGGCTCTTCTTGCT
 DSPECI TTCGGCTCCCTTCTTGGCCTCTGTCTTATTGCCCAAATCATCACTGGCCTTTTTTTAGCA
 DARBOR TTCGGCTCC?TTCTTGG?CTCTGTCTTATTGCCCAAATCATCACTGGCCTTTTTTTAGCA
 DGRANU TTTGGCTCC?TTCTTGG?CTCTGTCTTATTGCCCAAATCATCACTGGCCTTTTTTTAGCT
 PTRIVI TTCGGCTCTCTTCTAGGTCTCTGCCTAATTGCCCAGATCGTCACAGGCTCTTCTTAGCC

CTALAM ATACACTACACTGCCGACACATCAATAGCCTTCTCTCCATCGCTCATATCTGCCGAGAT
 DFANTA ATACACTACACATCAGATACAACCACAGCATTTCATCAGTAACACATATCTGCCGAGAC
 DHISTR ATACACTTACTGCAGACACCTCTATAGCTTCTCCTCCATCGCCACATCTGCCGAGAT
 DLEUCO ATACACTCACCAGCAGACACCTCCATAGCCTTCTCCTCCGTAGCCGAGATCTGCCGTGAT
 DPUMIL ATACACTTACTGCAGACACCTCTATAGCCTTCTCCTCCATCGCCACATCTGCCGAGAT
 DAURAT ATACACTTACTGCAGACACCTCTATAGCCTTCTCCTCTGTAGCTCATATCTGCCGTGAT
 DVENTR ATACATTACACCTCGGACATCTCCACAGCCTTCTCCTCCGTAGCCACATCTGCCGAGAT
 DSPECI ATACACTTCTCTGCAGACACCTCAATAGCCTTCTCCTCCATCGCCACATCTGTGAAAT
 DARBOR ATACACTTACTGCAGATACCTCCATAGCCTTCTCCTCCATCGCCACATCTGCCGAGAT
 DGRANU ATACACTTTTCTGCAGACACCTCCATAGCCTTCTCCTCCATCGCTCATATCTGTGAGAT
 PTRIVI ATACACTACAGGCTGACACCTCTATAGCCTTCTCCTCTGTGGCCACATTTGCCGAGAC

CTALAM GTAAATAATGGATGACTTCTTCGTAATGTCCATGCTAATGGCGCATCA?TCTTCTTCATC
 DFANTA GTAAACTACGGCTGATTAATCCGATACATACATGCAAACGGAGCCTCTATATTCTTTATC
 DHISTR GTGAATCATGGATGACTTCTTCGAAATCTTCAGCCAACGGTGCCTCCTTCTTCTTTATC
 DLEUCO GTAAACTACGGATGGCTTTTACGCAACCTTCAG?CTAACGGCGCCTCTTCTTCTTCATC
 DPUMIL GTAAATCACGGATGACTCCTTCGAAACCTACAGCCAACGGCGCCTCCTTCTTCTTTATC
 DAURAT GTAAACTACGGCTGACTTCTACGTAACCTA?????AACGGCGCTTCTTCTTCTTTATC
 DVENTR GTAAACTATGGCTGGCTAATCCGAAATATACAGCCAACGGGGCCTCATTCTTCTTCATC
 DSPECI GTAAATCAGGATGACTTCTTCGAAATCTTCAGCCAACGGCGCCTCCTTCTTCTTTATC
 DARBOR GTTAAATCAGGATGACTTCTTCGAAATCTTCAGCCAACGGCGCCTCCTTCTTCTTTATC
 DGRANU GTAAATCAGGATGACTTCTTCGAAATCTTCAGCCAACGGCGCCTCTTGTCTTTTATC
 PTRIVI GTAAACAACGGCTGACTTCTTCGCAACCTACAGCCAATGGCGCTTCATTTTTCTTCATC

CTALAM TGTATTTACCTTCACATCGGACGAGGCATGTATTATGGCTCATTTTTATTTAAAGAAACA
 DFANTA TGCATATTCTTTCATGTAGGACGAGGCATATATTATGGCTCATATACATTCACAGAAACA
 DHISTR TGCATTTACCTTCACAT?GGCCGCGGGATATACTATGGCTCCTTCTTATTCAAAGAAACC
 DLEUCO TGCATTTACCTTCACAT?GGTCGCGGAATGTACTACGGCTCCTTCTTATTTAAAGAGACC
 DPUMIL TGCATCTACCTTCACATCGGCCGCGGGATATACTACGGCTCCTTCTTATTTAAAGAAACC
 DAURAT TCTATCTACCTTCACATCGGCCGTGGAATATACTACGGCTCCTTCTTATTTAAAGAAACC

APPENDIX I.—Continued

DVENTR	TGCATCTACCTTCACA?????GAGGCCTATACTACGGCTCCTACCTCTATAAAGAAACA
DSPECI	TGCATTTATCTTCACA?GGCCGCGGGGTATACTACGGCTCCTTCTATTCAAAGAAACC
DARBOR	TGCATCTACCTTCACATCGGCCGCGGGATATACTACGGCTCCTTCTATTAAAGAAACC
DGRANU	TGCATCTATCTCCACATCGGCCGCGGAATCTACTACGGCTCCTTCTATTCAAGGAAACC
PTRIVI	TGCATCTACTTTACATCGGCCGAGGTATATACTACGGCTCATTCATTTAAAGAGACA
CTALAM	TGAAATATTGGCGTGTACTTTTTTTCTTAGTTATAGCCACTGCATTGTGTTG
DFANTA	TGGAATATTGGAATTATACTACTCTTCGCCGTAATAGCATCCGCATTACTAG
DHISTR	TGAAACATTGGGGTAATTTTATTCTTTCTAGTGATAGCTACAGCATTCTGTAG
DLEUCO	TGAAATATCGGCGTCGACTATTTTTTCTTGTATAGCCACAGCATTGTGTTG
DPUMIL	TGAAACATTGGAGTAATTTTACTCTTTCTAGTGATAGCTACAGCATTCTGTAG
DAURAT	TGAAATATTGGAGTCGACTACTTTTTCTAGTTATGGCCACAGCATTGTGTTG
DVENTR	TGAAACATTGGAGTGATCCTACTCCTACTCGTTATAATAACCGCATTCTGTGG
DSPECI	TGAAACATTGGAGTAATTTTACTCTTTCTAGTGATAGCTACAGCATTCTGTAG
DARBOR	TGGAACATTGGAGTAATTTTATTCTTTCTAGTGATAGCTACAGCATTCTGTAG
DGRANU	TGAAACATTGGGGTAATTTTACTATTTTTTAGTTATAGCCACAGCATTCTGTAG
PTRIVI	TGAAACATTGGTGTAGTTCTTTTATTTTTTAGTTATAGCCACTGCCTTCTGTGG
16s RNA	
CTALAM	CGTTGAACAAACGAACCGTTAGTAGCTGCTACACCACTGGGATACCCCTGATC
DFANTA	CGTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
DHISTR	CGTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
DLEUCO	CGTTGAACAAACGAACCTTTAGTAGCGGCTGCACCACTGGGATACCCCTGATC
DPUMIL	????AACAACTATCCATCAGTAGCGGCTGCACCACTAGGATACCCCTGATC
DAURAT	CGTTGAA?AAAC?AACCTTTAGTA?CGGCTGC?CCACCAGGATACCCCGATC
DVENTR	CGTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
DSPECI	CGTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
DARBOR	CGTAGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
DGRANU	?GTTGAACAAACGAACCATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
PTRIVI	CGTTGAACAAACGAAC-ATTAGTAGCGGCTGCACCACTAGGATACCCCTGATC
CTALAM	CAACATCGAGGTCGTAAACCCGCTGTGCGATAAGAGCT-CTTAAG--GCGGATTGCGCTG
DFANTA	CAACATCGAGGTCGTAAACCTACTTGTGCGATATGAGCT-CTTAAA--GTAGATTGCGCTG
DHISTR	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAGAGCT-CTCGAA--GTAGATTGCGCTG
DLEUCO	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAAGAACTTCTCGGAAGGAAGATTGCGCTG
DPUMIL	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAGAGCT-CTCGAA--GTAGATTGCGCTG
DAURAT	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAAGAACT-CTCGAA--GTAGATTGCGCTG
DVENTR	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAGAGCT-CTTAAA--GTAGATTGCGCTG
DSPECI	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAGAGCT-CTCGAA--GTAGATTGCGCTG
DARBOR	CAACATCGAGGTCGTAAACCTACTTGTGCGATAAGAGCT-CTCGAA--GTAGATTGCGCTG
DGRANU	CAACATCGAGGTCGTAAACCCACTTGTGCGATAAGAGCT-CTCGAA--GTAGATTGCGCTG
PTRIVI	CAACATCGAGGTCGTAAACCTACTTGTGCGATATGAGCT-CTTGAA--GTAGATTGCGCTG
CTALAM	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAATAATTGGGTCAATTTAGGTCAATATCT
DFANTA	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAATTATTGGGTCAATGGGAGTCAATGTGT
DHISTR	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAAGTAATTGGGTCAATGGAAGTCAATGTAT
DLEUCO	TTATCCCTAGGGTAACCTGGTTCGTTGGTCAAAAAATTGGGTCAATGGAAGTCAATGTGT
DPUMIL	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAAGTAATTGGGTCAATGGAAGTCAATGTGT
DAURAT	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAATAATTGGGTCAATGGAAGTCAATGTGT
DVENTR	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAATAATTGGGTCAATGGAAGTCAATGTGT
DSPECI	TTATCCCTAGGGTAACCTGGTTCGTTGATCAAAGTAATTGGGTCAATGGAAGTCAATGTAT

APPENDIX I.—Continued

CTALAM	GATTGAATAAAGGAGACAGTATAGCTTTCGTGGGGCCATTACTAGTCCTCATTAAAA
DFANTA	GATTAGAAAAAGGAGACAGTGTAGTCCCTCGTGGGGCCGTTACTAGTCCTCAATTTAAA
DHISTR	GATTAGAAAAAGGAGACAGTGTAGTCTTCGTGAAGCCATTACTAGTCCTCAATTTAAA
DLEUCO	GATTGGAAAAAGGAGACAGTGTAGTCTTCGTGGAGCCGTTACTAGTCCTCAATTTAAA
DPUMIL	GATTAGAAAAAGGAGACAGTGTAGTCTTCGTGAAGCCATTACTAGTCCTCAATTTAAA
DAURAT	GATTAGAAAAAGGAGACAGTGTAGTCTTCGTGAAGCCGTTACTAGTCCTCAATTTAAA
DVENTR	GATTAGAAAAAGGAGACAGTGTAGTCCCTCGTGGGGCCGTTACTAGTCCTCAATTTAAA
DSPECI	GATTAGAAAAAGGAGACAGTGTAGT?TTCGTGAAGCCATTACTAGTCCTCAATTTAAA
DARBOR	GATTAGAAAAAGGAGACAGTGTAGTCTTCGTGAAGCCATTACTAGTCCTCAATTTAAA
DGRANU	GATTAGAAAAAGGAGACAGCGAAGTCTTCGTGAAGCCATTACTAGTCCTCAATTTAAA
PTRIVI	GATTAGAAAAAGGAGACAGTGCAGCCCTCGTGGGGCCATTACTAGTCCTCATTAAAA

CTALAM	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAAT--GTT
DFANTA	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGA--GTC
DHISTR	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGT--GTC
DLEUCO	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAATATGTC
DPUMIL	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGT--GTC
DAURAT	GAACAAGTGATTACGCTAC??TCGCACGGTTAGGATACCGCG?CCGTTGAACA?T--GT?
DVENTR	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGT--GTC
DSPECI	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGT--GT?
DARBOR	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGT--GTC
DGRANU	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAGT--GTC
PTRIVI	GAACAAGTGATTACGCTACCTTCGCACGGTTAGGATACCGCGGCCGTTGAACAAA--GTC

CTALAM	ACTG
DFANTA	ACTG
DHISTR	ACTG
DLEUCO	ACTG
DPUMIL	A?TG
DAURAT	????
DVENTR	ACTG
DSPECI	ACTG
DARBOR	ACTG
DGRANU	ACTG
PTRIVI	ACTG
