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A PHYLOGENETIC ANALYSIS OF *VANZOLINIUS* HEYER, 1974 (AMPHIBIA, ANURA, LEPTODACTYLIDAE): TAXONOMIC AND LIFE HISTORY IMPLICATIONS [1]

(With 1 figure)

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ABSTRACT: The validity of the monotypic leptodactylid frog genus *Vanzolinius* Heyer, 1974 has been questioned recently. We explore the relationships of *Vanzolinius discodactylus* within the cluster of closely related genera *Adenomera, Leptodactylus,* and *Lithodytes* with both morphological and molecular data sets. Morphological and combined morphological and molecular data were analyzed using maximum parsimony; molecular data sets were analyzed with maximum likelihood methods. The resultant relationships are unambiguous in *Vanzolinius* being imbedded within *Leptodactylus.* In order to maintain *Leptodactylus* as a monophyletic genus, *Vanzolinius* is placed in the synonymy of *Leptodactylus* Fitzinger, 1826. The implications of relationships analyzed in this study are discussed in terms of both nomenclature and life-history evolution.

Key words: *Leptodactylus. Vanzolinius.* Phylogenetic relationships. Life history evolution.

RESUMO: Análise filogenética de *Vanzolinius* Heyer, 1974 (Amphibia, Anura, Leptodactylidae): implicações taxonômicas e sobre a história de vida.

A validade do gênero monotípico de leptodactilídeo *Vanzolinius* Heyer, 1974, tem sido questionada recentemente. Neste estudo exploramos as relações de *Vanzolinius discodactylus* dentro do agrupamento de gêneros proximamente relacionados *Adenomera, Leptodactylus* e *Lithodytes* por meio de dados morfológicos e moleculares. Dados morfológicos e dados morfológicos e moleculares combinados foram analizados por parcimônia máxima, dados moleculares foram analisados por máxima verossimilhança. As relações resultantes são inequívocas em *Vanzolinius* ter que ser incluído em *Leptodactylus.* Para manter *Leptodactylus* como um gênero monofilético, *Vanzolinius* Heyer 1974, é colocado na sinonímia de *Leptodactylus* Fitzinger, 1826. As implicações dos relacionamentos analisados neste estudo são discutidas em termos de nomenclatura e evolução dos modos reprodutivos.

Palavras-chave: *Leptodactylus. Vanzolinius.* Relações filogenéticas. Evolução da história de vida.

INTRODUCTION

The frog genera *Adenomera* Fitzinger, 1867*, Lithodytes* Fitzinger, 1843*, and Vanzolinius* Heyer, 1974 have, at one time or another, been included in the genus *Leptodactylus.* BOULENGER (1883) described the currently recognized monotypic *Vanzolinius* as *Leptodactylus discodactylus.* HEYER (1970) associated this taxon with the *Leptodactylus melanonotus* species group. Later, HEYER (1974a) placed the taxon within *Lithodytes* commenting on its possible distinctiveness and subsequently created the genus *Vanzolinius* to accommodate this species (HEYER, 1974b). The most recent morphological analysis indicated that *Vanzolinius* shared distinctive characteristics with *Leptodactylus diedrus* (HEYER, 1998). Previous analyses of relationships agreed that within the subfamily Leptodactylinae the genera *Adenomera, Leptodactylus, Lithodytes,* and *Vanzolinius* formed a monophyletic clade and that the genus *Physalaemus* Fitzinger, 1826, was more distantly related to this clade (HEYER, 1974a, 1975; LYNCH, 1971).

It is necessary to establish convincingly whether the genus *Leptodactylus* as currently understood is monophyletic, if we wish to understand the

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evolution of life history variation in *Leptodactylus*. In this paper, we are particularly interested in determining the phylogenetic relationships of *Vanzolinius*. Preliminary findings on relationships of previously proposed monophyletic clades in the *Leptodactylus* cluster (*Adenomera, Leptodactylus, Lithodytes, Vanzolinius*) are also presented in this paper, and we discuss the implications of our results for understanding aspects of life history evolution in this cluster.

MATERIAL AND METHODS

Taxon sampling – Species groups within *Leptodactylus* were previously recognized on the basis of morphological and life history characters (HEYER, 1969). We included samples from each of the four species groups to sample the morphological diversity within *Leptodactylus*. *Leptodactylus riveroi* Heyer & Pyburn, 1983, a species of uncertain species group affinity, and *L. silvanimbus* McCranie *et al.*, 1980, a species recently suggested as basal within the genus (HEYER, DE SÁ & MULLER, 2005), were also included. *Physalaemus* has been shown to function well as an outgroup for *Leptodactylus* using both morphological and molecular data (HEYER, 1998; HEYER, DE SÁ & MULLER, 2005); herein *Physalaemus gracilis* (Boulenger, 1883) was the outgroup taxon.

The taxa analyzed in this study are: *Leptodactylus bufonius* Boulenger, 1894, *L. fuscus* (Schneider, 1799) (*fuscus* species group); *L. leptodactyloides* (Andersson, 1945), *L. melanonotus* (Hallowell, 1861) (*melanonotus* species group); *L. chaquensis* Cei, 1950, *L. insularum* Barbour, 1906 (*ocellatus* species group); *L. pentadactylus* (Laurenti, 1768) (*pentadactylus* species group); *L. diedrus* Heyer, 1994, *L. riveroi, L. silvanimbus* (*Leptodactylus* of unclear species group affinity); *Adenomera hylaedactyla* (Cope, 1868), *Lithodytes lineatus* (Schneider, 1799), *Vanzolinius discodactylus* (Boulenger, 1883); and *Physalaemus gracilis* (as the outgroup). For both the morphological and molecular data, the data for *L. pentadactylus* are from Middle American specimens. See Tissue Voucher Specimens section at the end of this paper for specimen data used for molecular analyses. Museum abbreviations follow LEVITON *et al.* (1985).

Morphological data set – The morphological matrix is provided in Appendix 1. The character state descriptions and ordering information are the same as those published in HEYER (1998) with the following exceptions. We had no tissue samples for *Adenomera marmorata* and *Physalaemus pustulosus*, two of the taxa used in HEYER (1998), so we used morphological data for *Adenomera hylaedactyla* and *Physalaemus gracilis,* for which we do have molecular data. Data taken for *A. hylaedactyla* and *P. gracilis* were taken from HEYER (1974a), HEYER, DE SÁ & MULLER (2005), USNM 292477 (cleared-and-stained *A. hylaedactyla*) and RdS 511 (larval *P. gracilis* from Uruguay, Canelones, Balneario Atlantida, Rafael de Sá field number). These two species have a few states that differ from their congeners, and require recoding of states and/or redefinition of states as follows.

Character 7, toe webbing. *Physalaemus pustulosus* was coded as having a unique state in the data set of HEYER (1998), toes with weak basal fringes and webbing. *Physalaemus gracilis* has toes without web or fringes, a condition found in other taxa in the data set. The new definitions are: State 0 – toes without web or fringes; State 1 – toes with fringes extending length of toes except for tips; State 2 – females with weakly developed lateral toe fringes and males either with ridges or weakly developed fringes. The state ordering is 0-1-2.

Character 15, depressor mandibulae muscles. The depressor mandibulae may have one to three slips of origin, from the dorsal fascia (df), the zygomatic and/or otic ramus of the squamosal (sq), and the tympanic annulus (at) (following the terminology defined by STARRETT, 1968). Lower case indicates small slips of the muscle, upper case indicates large slips. *Physalaemus pustulosus* has the dfSQat condition, whereas *P. gracilis* has DFSQat. The DFSQat condition is state 0 in our data matrix.

Character 18, anterior petrohyoideus muscle. *Adenomera hylaedactyla* has a state not found in the data set of HEYER (1998). The new definitions are: State 0 – the anterior petrohyoideus muscle inserts entirely on the edge of the hyoid apparatus; State 1 – the muscle inserts on the edge of the hyoid and on the ventral body of the hyoid in part; State 2 – the muscle inserts entirely on the ventral surface of the hyoid body. The state ordering is 0-1-2.

Character 24, sartorius muscle. The condition in *P. gracilis* does not differ from some other taxa in the data set, in contrast to the condition found in *P. pustulosus*. The new definitions are: State 0 – muscle moderate; State 1 – intermediate condition between States 0 and 2; State 2 – muscle broad. The state ordering is 0-1-2.

Character 32, sacral diapophyses. *Physalaemus gracilis* does not differ in this character from other taxa. Thus characters 32-37 in our data set equal characters 33-38 in the HEYER (1998) data set.

Molecular methodology – DNA extraction followed HILLIS *et al.* (1996). Two segments of the mitochondrial genome were amplified using the polymerase chain reaction (PCR). A segment of the 12S r RNA of ~ 900 nucleotides and a segment of the 16s r RNA of ~ 700 nucleotides were amplified. Double-stranded (DS) PCR amplifications were performed in a final volume of 50µl containing 0.4µl of each primer, 1.0µl of each dNTP, 3.0µl of 25mM MgCl, and 1.25 units of *Taq* (*Thermus aquaticus*) DNA polymerase; the reaction was overlaid with 50µl of mineral oil. PCR conditions were as follows: 94°C for 60s, 57°C for 60s, and 72°C for 60s, with 25 cycles for the 12S amplification and 30 cycles for the 16S amplification. Amplified product was purified using Wizard® PCR Preps Kit (Promega). Of the purified DS fragment, 0.5µl were mixed with 1.5µl of a single IRD-labeled primer, 7.2µl of Sequencing Buffer, 1.0µl of Sequitherm Excel™II (Epicentre Technologies Co.) DNA polymerase, and 6.8µl of dH_20. Subsequently, 4.0µl of this mix was added to each of 4 tubes containing 2µl of each nucleotide respectively. PCR conditions were as follows (30 cycles): 92°C for 30s, 55°C for 30s, and 70°C for 30s. SS amplified and IR labeled fragments were sequenced in a LI-COR 4200 IR DNA Sequencer on 6% acrylamide gels. A total of 839 12S and 648 16S nucleotide positions were aligned unambigously using Clustal X and positions of ambiguous alignments were not used in the phylogenetic analyses. GenBank accession numbers for the sequence data are AY943217—242. The alignment matrix is provided in Appendix 2.

Phylogenetic Analysis – Maximum Parsimony (MP) analysis using PAUP* 4.0 (SWOFFORD, 2002) was used for both the morphological data set and the combined morphological and molecular data set. Molecular data sets were analyzed with maximum likelihood (ML) in PAUP* under the GTR+I+G model recommended by both the Hierarchical Likelihood Ratio Test and the Akaike Information Criterion used by Modeltest 3.04 (POSADA & CRANDALL, 1998).

We obtained a total of 37 morphological characters and 1486 base pairs (bp) for each taxon (839 bp corresponding to the 12S rDNA gene and 647 bp to the 16S rDNA gene). Sequences were aligned using Clustal X (THOMPSON, HIGGINS & GIBSON, 1994). We ran individual analyses for each of the data sets (i.e., morphology, 12S, and 16S data sets) as well as combined analyses (i.e., 12S+16S matrix, morphology+12S+16S matrix). In combined analyses gaps were alternatively considered as missing or as 5th characters; we also evaluated the effect of the substitution bias in the analysis of the combined data matrix using MP by down-weighting transitions to transversions 5:1.

RESULTS

There is modest variation in the 12S, 16S, and 12S+16S data sets (Tabs.1-3). The maximum sequence divergences between pairs of taxa are 21% for the 12S data, 16% for the 16S data, and 18% for the 12S+16S data.

The results of all cladistic analyses are almost identical; consequently we present the maximum parsimony combined data set results and point out where the analyses differ (Fig.1). The parsimony analysis of the combined data matrix results in a single tree (length=1430, consistency index=0.56) in which *Vanzolinius* exhibits a sister taxa relationship with *L. diedrus*. This relationship is also recovered in the analyses of the combined molecular data partitions as well as in all analyses of the 12S data partition. The analyses of the 16S data partition position *Vanzolinius* in the following clade (*L. diedrus* (*L. leptodactyloides*+ *Vanzolinius*)). The distance data matrices show that the close relationship of *L. diedrus* with *Vanzolinius* is unambiguous in the 12S data (Tab.1), but not at all clear in the 16S data, where *L. diedrus* and *Vanzolinius* have lower sequence distance values with *L. silvanimbus* and several members of the *L. fuscus*, *L. melanonotus*, and *L. ocellatus* group members than with each other (Tab.2). The morphological data set demonstrates strong support for a *L. diedrus*–*V. discodactylus* sister species relationship with 100% bootstrap support.

DISCUSSION

Phylogenetic conclusions – The following conclusions are supported by the analyses performed on our data.

First, *Vanzolinius* always clusters within *Leptodactylus*. The data are very clear and convincing for this conclusion. There are two nomenclatural options to resolve the phylogenetic conclusion that *Vanzolinius* is imbedded within *Leptodactylus*: *Vanzolinius* could be synonymized with *Leptodactylus*; or one or more clades within

Leptodactylus could be raised to generic status. Current (unpublished) data are inconclusive regarding the phylogenetic relationships among *Leptodactylus* species, and rule out elevating certain clades within *Leptodactylus* to generic status at this time. However, we think there are compelling arguments for placing *Vanzolinius* in the synonymy of *Leptodactylus*. The previous actions on generic placement of the species *discodactylus* were all based on morphological and karyotype data. The strongest support for generic recognition of *Vanzolinius* as a genus distinct from *Leptodactylus* involved two morphological features of the toes: the toe tips of *V. discodactylus* are expanded into small disks with longitudinal grooves on the dorsal surface and the terminal phalanges are T-shaped (HEYER, 1974b). With the discovery of *Leptodactylus diedrus*, the morphological distinctiveness between *Leptodactylus* and *Vanzolinius* was bridged to a large extent (HEYER, 1998). Thus, the morphological data used to define *Vanzolinius* as a genus distinct from *Leptodactylus* are seriously compromised by inclusion of the data for *L. diedrus* and the molecular data strongly support synonymizing *Vanzolinius* with *Leptodactylus*. Consequently, we hereby synonymize the genus *Vanzolinius* Heyer, 1974 with the genus *Leptodactylus* Fitzinger, 1826.

Second, the genera *Adenomera* and *Lithodytes* may share a sister-group relationship and our data provide support that both are evolutionarily distinct from *Leptodactylus* (including *Vanzolinius*).

Third, the previously recognized "traditional" species groups may not all be monophyletic, although the two members of the *L. fuscus* group form a well-supported clade in this study.

Fourth, a sister-group relationship between *L. discodactylus* and *L. diedrus,* previously suggested by HEYER (1998), is reasonably well supported by the morphological and combined molecular data sets.

Finally, *Leptodactylus riveroi*, a taxon of uncertain relationships, exhibits suggestive affinities to the *L. melanonotus* species group.

Life history implications – All members of the subfamily Leptodactylinae (except *Limnomedusa*), place their eggs in foam nests (LANGONE, 1995). Within the *Leptodactylus* cluster, however, there is variation regarding where the foam nests are deposited and considerable variation occurs in other life history aspects. Two examples illustrate how an understanding of phylogenetic relationships in this group is critical to deciphering life history evolution in the genus *Leptodactylus*.

Table 1. 12S sequence differences between taxon pairs included in study using General Time Reversible (GTR) parameter values.

TAXA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *L. diedrus*	-													
2 *L. riveroi*	0.148	-												
3 *L. silvanimbus*	0.126	0.137	-											
4 *L. bufonius*	0.139	0.144	0.115	-										
5 *L. fuscus*	0.136	0.165	0.135	0.077	-									
6 *L. chaquensis*	0.128	0.119	0.078	0.095	0.097	-								
7 *L. insularum*	0.123	0.133	0.094	0.094	0.096	0.065	-							
8 *L. leptodactyloides*	0.131	0.135	0.086	0.107	0.116	0.042	0.087	-						
9 *L. melanonotus*	0.137	0.146	0.101	0.105	0.117	0.087	0.088	0.097	-					
10 *L. pentadactylus*	0.144	0.160	0.116	0.118	0.118	0.107	0.113	0.115	0.131	-				
11 *V. discodactylus*	0.113	0.166	0.141	0.136	0.129	0.126	0.116	0.130	0.136	0.134	-			
12 *A. hylaedactyla*	0.177	0.197	0.177	0.156	0.157	0.145	0.151	0.156	0.174	0.161	0.168	-		
13 *Lith. lineatus*	0.207	0.203	0.175	0.168	0.173	0.175	0.187	0.178	0.182	0.165	0.190	0.161	-	
14 *P. gracilis*	0.185	0.212	0.167	0.151	0.156	0.161	0.164	0.162	0.164	0.171	0.182	0.160	0.174	-

Table 2. 16S sequence differences between taxon pairs included in study using General Time Reversible (GTR) parameter values.

TAXA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *L. diedrus*	-													
2 *L. riveroi*	0.129	-												
3 *L. silvanimbus*	0.099	0.111	-											
4 *L. bufonius*	0.118	0.136	0.101	-										
5 *L. fuscus*	0.096	0.132	0.099	0.053	-									
6 *L. chaquensis*	0.085	0.105	0.072	0.093	0.079	-								
7 *L. insularum*	0.088	0.098	0.060	0.088	0.072	0.039	-							
8 *L. leptodactyloides*	0.092	0.124	0.109	0.117	0.116	0.085	0.092	-						
9 *L. melanonotus*	0.083	0.100	0.079	0.097	0.091	0.060	0.063	0.084	-					
10 *L. pentadactylus*	0.108	0.128	0.099	0.096	0.082	0.086	0.074	0.117	0.080	-				
11 *V. discodactylus*	0.104	0.118	0.125	0.132	0.114	0.099	0.094	0.087	0.100	0.114	-			
12 *A. hylaedactyla*	0.135	0.136	0.128	0.144	0.133	0.128	0.124	0.131	0.111	0.128	0.147	-		
13 *Lith. lineatus*	0.155	0.146	0.116	0.138	0.125	0.125	0.120	0.156	0.121	0.131	0.160	0.105	-	
14 *P. gracilis*	0.160	0.163	0.126	0.150	0.139	0.126	0.124	0.148	0.145	0.145	0.165	0.143	0.133	-

Table 3. Combined 12S & 16S sequence differences between taxon pairs included in study using General Time Reversible (GTR) parameter values.

TAXA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *L. diedrus*	-													
2 *L. riveroi*	0.140	-												
3 *L. silvanimbus*	0.114	0.125	-											
4 *L. bufonius*	0.130	0.140	0.109	-										
5 *L. fuscus*	0.118	0.150	0.118	0.066	-									
6 *L. chaquensis*	0.109	0.113	0.075	0.094	0.089	-								
7 *L. insularum*	0.108	0.118	0.079	0.092	0.085	0.054	-							
8 *L. leptodactyloides*	0.114	0.130	0.096	0.111	0.116	0.060	0.089	-						
9 *L. melanonotus*	0.113	0.126	0.092	0.101	0.106	0.075	0.077	0.091	-					
10 *L. pentadactylus*	0.128	0.146	0.109	0.108	0.102	0.098	0.096	0.116	0.108	-				
11 *V. discodactylus*	0.109	0.144	0.134	0.134	0.123	0.114	0.106	0.111	0.120	0.125	-			
12 *A. hylaedactyla*	0.158	0.170	0.155	0.151	0.147	0.138	0.139	0.145	0.146	0.146	0.159	-		
13 *Lith. lineatus*	0.184	0.178	0.148	0.155	0.152	0.153	0.157	0.168	0.154	0.150	0.177	0.136	-	
14 *P. gracilis*	0.174	0.190	0.149	0.150	0.149	0.145	0.147	0.156	0.155	0.160	0.175	0.152	0.156	-

First, two clades (*Adenomera* and the *L. fuscus* species group) within Leptodactylinae share the same pattern of males constructing a terrestrial subsurface chamber, attracting females to the chamber acoustically, and depositing the foam nest in the chamber where at least embryonic and early larval development take place (see KOKUBUM & GIARETTA, 2005 and references cited therein). Our data indicate that this complex life history pattern was independently derived in both clades and is not the result of shared ancestral adaptations. Also, at least some members of the *L. pentadactylus* group use pre-existing terrestrial burrows in which they deposit their foam nest (see GIBSON & BULEY, 2004 and references cited therein). Additional taxon sampling is required to determine whether this pattern served as a precursor to the actual construction of terrestrial incubating chambers in the *L. fuscus* group. Our preliminary data suggest support for this scenario. Second, there is considerable variation in female attendance of foam nests and larvae, whether attending females communicate with their larvae, and how females communicate with their larvae (VAZ-FERREIRA & GEHRAU, 1975; WELLS & BARD, 1988). As far as is known, parental care does not occur in any species of the *L. fuscus* group. Our preliminary data indicate that intensive taxon sampling with additional data is required to resolve relationships among the *Leptodactylus* species that demonstrate female attendance and communication with their offspring in order to understand the evolution of parental care in *Leptodactylus*.

More intensive taxon sampling and the sequencing of nuclear and more slowly evolving genes should provide a well-supported phylogeny for *Leptodactylus* at the species level that will allow a better understanding of the evolution of life history variation in the *Leptodactylus* cluster.

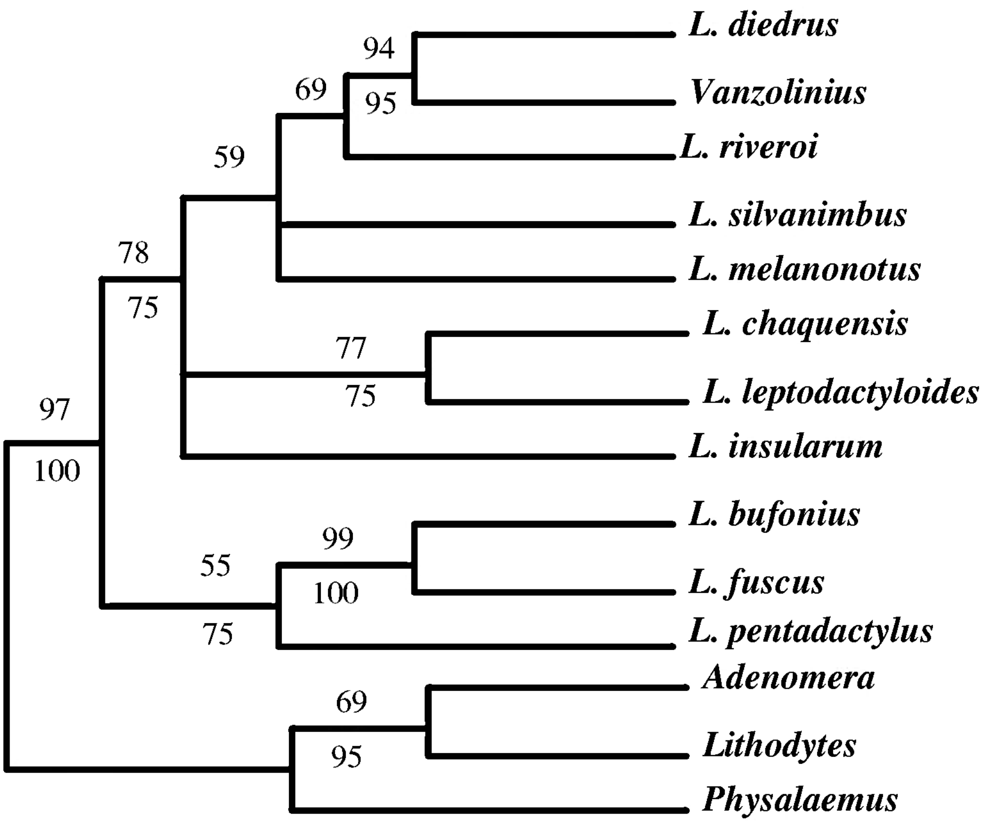

Fig.1- Maximum Parsimony Tree of combined (morphological and molecular) data sets. Gaps were considered as a fifth character. Numbers above branches correspond to bootstrap support in parsimony analysis; numbers below branches are bootstrap support values from Maximum Likelihood analysis of the combined molecular data set.

TISSUE VOUCHER SPECIMENS

Adenomera hylaedactyla – BRAZIL: PARÁ: Alter do Chão (MZUSP 70958)

Leptodactylus bufonius – ARGENTINA: SALTA: 54 km NE of Joaquín V. González on provincial route 41 (USNM field number 175816, deposited in FML).

Leptodactylus chaquensis – ARGENTINA: TUCUMÁN: ca 40 km SE San Miguel de Tucumán at km post 1253 on International Route 9 (USNM 319708).

Leptodactylus diedrus – VENEZUELA: AMAZONAS: Río Negro, near Neblina base camp on left bank of Río Baria (= Río Mawarinuma) (USNM 30715).

Leptodactylus discodactylus – ECUADOR (QCAZ 16788).

Leptodactylus fuscus – BRAZIL: RORAIMA: Caracaranã, near Normandia (MZUSP 67073).

Leptodactylus insularum – PANAMA: PANAMA: Río Indio, camino hacia Las Minas (CH 4956).

Leptodactylus leptodactyloides – BRAZIL: PARÁ: Serra de Kokoinhokren (MZUSP 70969).

Leptodactylus melanonotus – BELIZE: CAYO: between San Jacinto and Spanish Lookout road on Webster Highway, Caesar's Hotel (USNM 535964).

Leptodactylus ocellatus – BRAZIL: SANTA CATARINA: Campeche (MZUSP 68993).

Leptodactylus "pentadactylus" – PANAMA: BOCAS DEL TORO: Isla Popa (USNM 347153).

Leptodactylus riveroi – VENEZUELA: AMAZONAS: Río Negro, Neblina base camp on left bank of Río Baria (= Río Mawarinuma) (USNM 562029).

Leptodactylus silvanimbus – HONDURAS: OCOTEPEQUE; Belén Gualcho (USNM 348631).

Lithodytes lineatus – BRAZIL: MATO GROSSO: Apiacás (MZUSP 80874).

Physalaemus gracilis – URUGUAY: SALTO: Espinillar (RdS 788 field number).

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LITERATURE CITED

BOULENGER, G.A., 1883. On a collection of frogs from Yurimaguas, Huallaga River, northern Peru. **Proceedings of the Zoological Society of London**, London, **1883**:635-638.

GIBSON, R.C. & BULEY, K.R., 2004. Maternal care and obligatory oophagy in *Leptodactylus fallax:* a new reproductive mode in frogs. **Copeia**, Lawrence, **2004**(1):128-135.

HEYER, W.R., 1969. The adaptive ecology of the species groups of the frog genus *Leptodactylus* (Amphibia, Leptodactylidae). **Evolution**, Lawrence, **23**(3):421-428.

HEYER, W.R., 1970. Studies on the frogs of the genus *Leptodactylus* (Amphibia: Leptodactylidae). VI. Biosystematics of the *melanonotus* group. **Contributions in Science, Los Angeles County Museum**, Los Angeles, **253**:1-46

HEYER, W.R., 1974a. Relationships of the *marmoratus* species group (Amphibia, Leptodactylidae) within the subfamily Leptodactylinae. **Contributions in Science, Los Angeles County Museum**, Los Angeles, **191**:1-48.

HEYER, W.R., 1974b. *Vanzolinius*, a new genus proposed for *Leptodactylus discodactylus* (Amphibia, Leptodactylidae). **Proceedings of the Biological Society of Washington**, Washington, **87**(11):81-90.

HEYER, W.R., 1975. A preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. **Smithsonian Contributions to Zoology**, Washington, **199**:1-55.

HEYER, W.R., 1998. The relationships of *Leptodactylus diedrus* (Anura, Leptodactylidae). **Alytes**, Paris, **16**(1-2):1-24.

HEYER, W.R.; DE SÁ, R.O. & MULLER, S., 2005. On the enigmatic distribution of the Honduran endemic *Leptodactylus silvanimbus* (Amphibia: Anura: Leptodactylidae). In: DONNELLY, M.A.; CROTHER, B.I.; GUYER, C.; WAKE, M.H. & WHITE, M.E. (Eds.) **Ecology and evolution in the tropics: a historical perspective**. Chicago: University of Chicago Press. p.81-101.

HILLIS, D.M.; MABLE, B.K.; LARSON, A.; DAVIS, S.K. & ZIMMER, E.A., 1996. Nucleic acids IV: Sequencing and cloning. In: HILLIS, D.M.; MORITZ, C. & MABLE, B.K. (Eds.) **Molecular systematics**. Second edition. Sunderland, Massachusetts: Sinauer Associates, Inc. p.321-381.

KOKUBUM, M.N.C. & GIARETTA, A.A., 2005. Reproductive ecology and behaviour of a species of *Adenomera* (Anura, Leptodactylinae) with endotrophic tadpoles: systematic implications. **Journal of Natural History**, London, **39**(20):1745-1758.

LANGONE, J.A., 1995 ("1994"). Ranas y sapos del Uruguay (reconocimientos y aspectos biológicos). **Museo Damaso Antonio Larrañaga, Serie de Divulgación**, Montevideo, **5**:1-123.

LEVITON, A.E.; GIBBS JR., R.H.; HEAL, E. & DAWSON, C.E., 1985. Standards in herpetology and ichthyology: Part I. Standard symbolic codes for institutional resource collections in herpetology and ichthyology. **Copeia**, Lawrence, **1985**(3):802-832.

LYNCH, J.D., 1971. Evolutionary relationships, osteology, and zoogeography of leptodactyloid frogs. **University of Kansas Museum of Natural History, Miscellaneous Publication,** Lawrence, **53**:1-238.

POSADA, D. & CRANDALL, K.A. 1998. Model-test: testing the model of DNA substitutions. **Bioinformatics**, Oxford, **14**:817-818

STARRETT, P.H., 1968. **The phylogenetic significance of the jaw musculature in anuran amphibians**. Ann Arbor, Michigan. 179p. Dissertation (Ph.D. in Zoology), University of Michigan.

SWOFFORD, D.L., 2002. **PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Version 4.0b10.** Sunderland, Massachusetts: Sinauer Associates.

THOMPSON, J.D.; HIGGINS, D.G. & GIBSON, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. **Nucleic Acids Research**, Oxford**, 22**:4673-4680.

VAZ-FERREIRA, R. & GEHRAU, A., 1975. Comportamiento epimelético de la rana común, *Leptodactylus ocellatus* (L.) (Amphibia, Leptodactylidae) I. Atención de la cría y actividades alimentarias y agresivas relacionadas. **Physis**, Buenos Aires, Sección B, **34**(88):1-14.

WELLS, K.D. & BARD, K.M., 1988. Parental behavior of an aquatic-breeding tropical frog, *Leptodactylus bolivianus*. **Journal of Herpetology**, Athens, **22**(3):361-364.

APPENDIX 1

Morphological (primarily) data matrix used for phylogenetic analysis

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
L. bufonius	3	0	0	0	0	0	0	3	4	0	3	0	1	1	1	0	2	0	0
L. fuscus	3	0	0	3	0	0	0	3	4	0	3	0	1	1	1	0	1	0	1
L. leptodactyloides	1	0	2	1	1	0	1	0	3&5	0	2	0	0	0	1	0	0	0	0
L. melanonotus	1	0	2	0	0	0	1	1	5	0	2	0	0	0	1	0	0	0	0
L. chaquensis	2	0	2	3	0	0	1	2	2	0	2	0	0	0	1	0	0	0	0
L. insularum	1	0	2	2	0	0	1	1	3	0	2	0	0	0	1	0	0	0	1
L. pentadactylus	1	0	1	2	0	0	0	2	4	0	3	1	0	0	1	0	0	0	0
L. diedrus	1	0	2	0	2	0	1	?	?	?	?	?	1	?	1	0	0&1	0	0
L. riveroi	0	0	2	2	0	0	1	0	4	0	2	0	?	?	1	0	0	0	0
L. silvanimbus	1	0	2	0	0	0	2	0	5	0	2	0	0	?	1	0	0	0	0
A. hylaedactyla	1	0	0	0	1	0	0	?	0	0	1	0	1	1	1	1	1	1	0
Lith. lineatus	1	0	0	2	4	0	0	?	1	0	0	0	1	1	1	0	0	0	1
V. discodactylus	1	0	0	0	3	0	1	0	5	0	2	0	1	0	1	0	0	0	1
P. gracilis	4	1	3	0	0	1	0	3	4	1	3	0	1	0	0	1	2	2	0

	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
L. bufonius	0	0	1	0	2	0	0	1	2	1	1	0	0	0	0	2	1	0
L. fuscus	0	0	1	0	0&2	0	0	0	2	1	2	0	0	0	0	2	1	0
L. leptodactyloides	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0&1
L. melanonotus	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0
L. chaquensis	0	0	1	0	0	0	1	0	0	0	2	0	0	0	3	0&3	1&3	0&1
L. insularum	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0&1
L. pentadactylus	0	0	1	0	2	0	1	1	1	1	1	0	0	0	0	2	2	0
L. diedrus	0	0	1	0	0	0	0	1	0	0	1	0	1	0	2	2	1&2	1
L. riveroi	0	0	1	0	0	0	1	1	0	0	1	0	0	1	1	3	3	0
L. silvanimbus	0	0	1	0	1	1	0	0	0	0	1	0	0	0	4	0	1	1
A. hylaedactyla	2	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1
Lith. lineatus	1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1
V. discodactylus	0	0	1	0	0	0	0	1	0	0	1	0	1	0	2	1	1	0
P. gracilis	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	4	3	1

(See text and Heyer, 1998, for character state descriptions).

APPENDIX 2

Molecular data matrix used for phylogenetic analysis. Regions in brackets correspond to ambiguous alignment and were not included in the analyses.

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BEGINS 12S DATA
Diedrus  [AGCGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTAACCCTAAGATCAAC
Riveroi  [-GCGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGCTTGGTCCCGGCCTTAAGATCAAC
Silvani  [-GCGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAGACA]CAAAGGTTTGGTCCTGACCTTAAGATCAAC
Bufoniu  [--CGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTGACCTTAGAATCAAT
Fuscuss  [--CGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAGACA]CAAAGGTTTGGTCCTGACCTTAAAATCAAT
Chaquen  [-GCGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTGACCTTAAAATCAAT
Insular  [AGCGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTGACCTTAAGATCAAT
Tyloide  [----CTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAATA]CAAAGGTTTGGTCCTGACCTTAAAATCAAT
Melanon  [--CGCTGAAAATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTGACCTTAATATCAAC
Pentada  [-GCGCTGAAGATGCTGAGATGGACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTGACCTTAAAATCAAT
Vanzoli  [AGCGCTGAAGATGCTGAGATGGACCTTAAAAAGTCCTTTAGACA]TAAAGGTTTGGTCCTGACCTTAAAATCAGC
Adenhya  [---GCTGAAGATGCTGAGATGAACCCTAAAAAGTCCTTTAAACA]CAAAGGTTTGGTCCTAGCCTTGTAGTCAAT
Lithody  [---GCTGAAGATGCTGAGATGGGCCCTAAAAAGCCCTTTAAACA]CAAAGGTTTGGTCCTAGCCTTGCAATCAAC
Physala  [---GCTGAAGATGCTGAGATGAACCCTAAAAAGTTCTTTAAACA]CAAAGGTTTGGTCCTGGCCTTGAGATCAGT

Diedrus  TCTTACTTAACTTACACATGCAAGTCTCAGCACCCCTGTGAAAACGCCCTTCAACTCCT-ACA-AGGGGCAAGGAG
Riveroi  TCTTACTTAACCTACACATGCAAGTCTCAGCGCCCCGGTGAGAACGCCCTTCAACTCCA-CTA-AGGAACAAGGAG
Silvani  TCTTACTTAACTTACACATGCAAGKCTCAGCACCCCTGTGAAAACGCCCTTCAACTCCC-CC--TGGAGTAAGGAG
Bufoniu  TTTTACTTAATTTACACATGCAAGTCTCCGCACCCCTGTGAAAACGCCCTTAAATTCCCCCTAGCGGGACAAGGAG
Fuscuss  TTTTACTTAATTTACACATGCAAGTCTCCGCACCCCTGTGAGAACGCCCTCAAACCCCT-AAA-AGGGACGAGGAG
Chaquen  TTTTACTTAACTTACACATGCAAGTCTCAGCACCCCTGTGAGAACGCCCTTTAACTCCC-ATT-AGGAACAAGGAG
Insular  TTTTACTTAATTTACACATGCAAGTCTCAGCATCCCTGTGAGAACGCCCTTTAACTCCCCCTA-AGGAGCAAGGAG
Tyloide  TTTTACTTAACTTACACATGCAAGTCTCAGCACCCCTGTGAGAACGCCCTTTAACTCCC-GTT-AGGAACAAGGAG
Melanon  TTTTACTTAATTTACACATGCAAGTCTCAGCATTCCTGTGAAAACGCCCTTTAACTCCT-TTA-CGGAACAAGGAG
Pentada  TGTTACTTAACTTACACATGCAAGTCTCCGCACTCCTGTGAGAACACCCTTTAACCCCT-TTA-AGGGGAAAGGAG
Vanzoli  TCTTACTTAACTTACACATGCAAGTCTCCGCCTTCCTGTGAAAACGCCCTTAGACCCCT-CAA-AGGGGAAAGGAG
Adenhya  TTTTACTTAATTTACACATGCAAGTATCCGCACCCCTGTGAAAACGCCCTTTAATTCCT-TAT-AGGGATAAGGAG
Lithody  TTTTTCTTAACTTACACATGCAAGTATCCGCACCCCCGTGAAAACGCCCTTATATCCCC-GA--TAGGATAAGGAG
Physala  TATTACTTAATATACACATGCAAGTCTCCGCACCCCTGTGAAAACGCCCTTTAATCCCC-TCT-CGGGATAAGGAG
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Diedrus  CCGGTATCAGGCACACCAA--AAGCCCAAGACACCTAGCTATGCCACACCCACAAGGGAACTCAGCAGTGATTAAC
Riveroi  CCGGTATCAGGCACAAGTTTTTAGCCCAAGACACCTAGCCACGCCACACCCACAAGGGAACTCAGCAGTGATTAAC
Silvani  CTGGTATCAGGCGCAAACCT-TAGCCCAAGACACCTAGCTATGCCACACCCACAAGGGAATTCAGCAGTGATTAAC
Bufoniu  CTGGTATCAGGCACAAACAT-TAGCCCAAGACACCTAGCTTTGCCACACCCACACGGGAACTCAGCAGTGATTAAC
Fuscuss  CTGGTATCAGGCACAAACAT-TAGCCCAAGACACCTAGCCATGCCACACCCACAAGGGAAATCAGCAGTGATTAAC
Chaquen  CTGGTATCAGGCACAACCTT-TAGCCCAAGACACCTAGCTACGCCACACCCACAAGGGAATTCAGCAGTGATTAAC
Insular  CTGGTATCAGGCACAAATCT-TAGCCCAAGACACCTAGCCATGCCACACCCACAAGGGAATTCAGCAGTGATTAAC
Tyloide  CTGGTATCAGGCACAACCTT-TAGCCCAAGACACCTAGCTACGCCACACCCACAAGGGAATTCAGCAGTGATTAAC
Melanon  CTGGTATCAGGCACAAATAT-TAGCCCAAGACACCTAGCTACGCCACACCCACAAGGGATCTCAGCAGTGATTAAC
Pentada  TTGGTATCAGGCTCAAACAT-TAGCCCAAGACACCTAGCTAGGCCACACCCACAAGGGAACTCAGCAGTGATTAAC
Vanzoli  CCGGTATCAGGCACATCTCT-TAGCCCAAGACACCTAGCTATGCCACACCCACAAGGGACCTCAGCAGTGATTAAT
Adenhya  CCGGTATCAGGCACATCAATATAGCCCAAAACACCTAGCTATGCCACACCCACAAGGGACCTCAGCAGTGATTAAC
Lithody  CTGGTATCAGGCACAAAATT-TAGCCCAAAACACCTAGCTCAGCCACACCCCCACGGGAACTCAGCAGTGATCAAC
Physala  CTGGTATCAGGCCCAAAATTCT-GCCCAAAACACCTAGCTATGCCACATCCACAAGGAAACTCAGCAGTGATTAAC

Diedrus  ATTAAACATGAGCGACAGCTTGATTCAGTTAAAGAAAAGAGAGCCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
Riveroi  ATTGTGCATGAGCGCCAGCTCGACTCAATTAAAGTAAAAAGGGCCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
Silvani  ATTGAATATAAGCGACAGCTTGACTCAGTTAAAGTAAAAAGAGCCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
Bufoniu  ATTGAATATAAGCGACAGCTTGACTCAGTTAAAGTAAGAAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Fuscuss  ATTGAATATAAGCGACAGCTTGATTCAGTTAAAGTAAGAAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Chaquen  ATTGAATATAAGCGCCAGCTTGATTCAGTTAAAGTAAAAAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Insular  ATTGAATATAAGCGCCAGCTTGATTCAGTTAAAGTAAAGAGAGCCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
Tyloide  ATTGAATATAAGCGCCAGCTTGATTCAGTTAAAGTAAAAAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Melanon  ATTGGACATAAGCGACAGCTTGATTCAGTTAAAGTAAAAAGAGCCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
Pentada  ATTGAATATAAGCGATAGCTTGATTCAGTTAAAGTAAAAAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Vanzoli  ATTAAACATAAGCGACAGCTTGATTCAGTTAAAGAAAAGAGAGCCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
Adenhya  ATTAAATATCAGCGACAGCTTGATTCAGTTAAAGTAAATAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Lithody  ATTGAACATCAGCGACAGCTGGATTCAGTTAAAGTTTACAGAGCCGGCTAATCTGGTGCCAGCCGCCGCGGTTACA
Physala  ATTGAACATAAGCGACAGCTTGATTCAGTTATGGTAAAAAGAACCGGCAAATCTGGTGCCAGCCGCCGCGGTTACA
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Diedrus   CCACGTGGCTCAAGTTGACCTTGCTCGGCGTAAAGCGTGATTTAAGAAATATGCCCA-TGGTGTCAAAAA-AGTTT
Riveroi   CCACGTGGCTCAAATTGATCTCATCCGGCGTAAAGCGTGATTTAAGAGACAATCCCA-TGGTGCTAAACATGGCAC
Silvani   CCATGAGGCCCTAGTTGACCTTTCTCGGCGTAAAGCGTGATTTAAGAAA-ATATTTA-TGATGTCAAAAA-CTCAC
Bufoniu   CCACGTGGCTCAAATTGATTCTTCTCGGCGTAAAGCGTGATTTAAGGGACATCCTTT-TGGTGTTAAACA-AGCAC
Fuscuss   CCACGTGGCTCTAATTGATTCTGATCGGCGTAAAGCGTGATTTAAGAGATTCCTCCTTTGGTGTCAAAAA-GATAC
Chaquen   CCACGTGGCTCAAATTGATTTTGCTCGGCGTAAAGCGTGATTTAAGAGACCAATTCA-TGGTGTCAAAAA-AGCAC
Insular   CCACGTGGCTCAAATTGATCTTACTCGGCGTAAAGCGTGATTTAAGGGATTAACCAA-TGGTGTCAAAAA-ATTAT
Tyloide   CCACGTGGCTCAAATTGATTTTGCTCGGCGTAAAGCGTGATTTAAGAGATCAATTCA-TGGTGTCAAAAA-AGCAC
Melanon   CCACGTGGCTCTAGTTGATTCTACTCGGCGTAAAGCGTGATTTAAGATA-CTACTCA-TGATGCCAAAAA-AACAT
Pentada   CCACGTGGCTCAAATTGACCTAACTCGGCGTAAAGCGTGATTTAAGGAA-ATACTTT-TGGTGCCAAAAA-TATAC
Vanzoli   CCATGTGGCTCAAGTTGATTTTGTTCGGCGTAAAGCGTGGTTTAAGCGT-TTAATTA-TGGTGTCAAAAA-AGTAC
Adenhya   CCACGTGGCTCAAATTGACCATTTTCGGCGTAAAGAGTGATTTAAGAGT-CCTATAATTGGTGTCAAATT-TTTAC
Lithody   CCACGTGGCTCAAGTTGACCCCCATCGGCGTAAAGCGTGATTTAAGAGACCCAAATT-TGGTACCAAATT-TTTAC
Physala   CCACGTGGTTCAAATTGATTCTTATCGGCGTAAAGCGTGATTTAAGCCATATACGAT-TGAAGTTGAACT-TAAAT

Diedrus   TAAGCTGTGACACGCTTGCTCTTAATAAGACCAAAAACGAAAGTTACACCAACCGCACCTACTTGAACCCACGACA
Riveroi   TAAGCTGTGACACGCTTGTGCCCCCGAAACCCCAAGACGAAAGTTACACCAGCCAAACCAACTTGAACTCACGACA
Silvani   TAAGCTGTGACACGCTTGTGCCCCAGAAGCCCAGAAACGAAAGCTACATCAACC-AACCAACTTGAATTCACGACA
Bufoniu   TAAGCCGTGACACGCTTGTGCTTAAGAAAATCAAAAACGAAAGTTACACCAACTCAACCAACTTGAACTCACGACA
Fuscuss   TAAGCCGTGACACGCTTGTATTCAAGAAGATCAGAAACGAAAGTTACACCAACTTAATCAACTTGAGCTCACGACA
Chaquen   TAAGCTGTGACACGCTTGTGCCTCAGAAGCCCAGAAACGAAAGCTACACCAATATTACCCACTTGAACTCACGACA
Insular   TAAGCCGTGACACGCTTGTGATTTAGAAGCTCAAAAACGAAAGCTACACCAAATATATCAACTTGAACTCACGACA
Tyloide   TAAGCTGTGACACGCTTGTGCCTCAGAAGCCCAGAAACGAAAGCTACACCAATATTACCCACTTGAACTCACGACA
Melanon   TAAGCTGTGACACGCTTATGCTCTAGAAGCTCAAAAACGAAAGTTGCATCAATT-AACCAACTTGAATTCACGACA
Pentada   TAAGCCGTGACACGCTTGTACATTAGAAGACCAAAATCGAAAGCTACACCAACCTAACCAACTTGAACTCACGACA
Vanzoli   TAAGCCGTGACACGCTTGTACATAAGAAGACCTAAAACGAAAGTTACACCAGTCACATAAACTTGAACCCACGACA
Adenhya   TAAGCCGTAACACGCTTGCCTCTTAGAAGCTCTAACACGAAAGTTACCCCAATTTAATCAACTTGAACTCACGACA
Lithody   TAAGCCGTGACACGCTTGTGCAAAAGATGACCTAAAACGAAAGTTGTACCAACTTAGCCAACTTGAACTCACGACA
Physala   TAAGCTGTGACACGCTTGTTTATCAGAAAACCATAAACGAAAGTTACTCCAATTACCTCTACTTGAACTCACGACA
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Diedrus  GCTAGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTAACTTACACCT-CAATCGCCCGGG
Riveroi  GCCGGGGAACAAACTGGGATTAGATACCCCACTATGCCTGGCCATAAACTTTAATTTACAACT-CAATCGCCTGGG
Silvani  GCTTGGGAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTAATTTACA-CTCCAATCGCCAGGG
Bufoniu  GCTAGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTAACTTACAACT-TGATCGCCTGGG
Fuscuss  GTTAGGAAACAAACTGGGATTAGATACCCCACTATGCCTAACCGTAAACTTTAATTTACACCTTTTATCGCCCGGG
Chaquen  GCTTGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTAATTTACACCT-CAATCGCCAGGG
Insular  GCTTGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTAACTTACACCT-CCATCGCCAGGG
Tyloide  GCTTGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACNTTAANTTACACCT-NAATCGCCNGGG
Melanon  GCTTGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTAATTTACA-TTCTTATCGCCAGGG
Pentada  GCTAGGAAACAAACTGGGATTAGATACCCCACTATGCCTAGCCGTAAACTTTTATTTACACCC-ACATCGCCAGGG
Vanzoli  GCTAGGAAACAAACTGGGATTAGATACCCCACTATGCCTAACCGTAAACTTTAACTTACACCC-CGATCGCCAGGG
Adenhya  GCTAAGAAACAAACTGGGATTAGATACCCCACTATGCTTGGCAATAAACCTTAAATTACACCCCCAATCGCCCGGG
Lithody  GTCAAGACACAAACTGGGATTAGATACCCCACTATGCTTGACCGTAAAACTTTATTTACAATATCTATCGCCAGGG
Physala  GTTAAGATACAAACTGGGATTAGATACCCCACTATGCTTAACCGTAAACTTA-ATTTACACCT-TAATCGCCCGGG

Diedrus  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTACAATCG
Riveroi  AACTACAAGCCAAGCTTTAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Silvani  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCATCTAGAGGAGCCTGTCCTATAATCG
Bufoniu  AACTACGAGCCAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Fuscuss  AACTACGAGCCAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Chaquen  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCATCTAGAGGAGCCTGTCCTATAATCG
Insular  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCATCTAGAGGAGCCTGTCCTATAATCG
Tyloide  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Melanon  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Pentada  AACTACGAGCCAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCCTCTAGAGGAGCCTGTCCTGTAATCG
Vanzoli  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Adenhya  AACTATGAGCAAAGCTTAAAACCCAARGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Lithody  AACTACGAGCTATGCTTAAAACCCAARGGACTTGACGGTACCCCAAATCCACCTAGAGGAGCCTGTCCTATAATCG
Physala  AACTACGAGCAAAGCTTAAAACCCAAAGGACTTGACGGTACCCCATATCCACCTAGAGGAGCCTGTCCTATAATCG
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Diedrus   ATACTCCCCGCTTAACCTCACCTCTTTTAGTCATTCAGTCTGTATACCTCCGTCGCCAGCTTACCCTATGAGCGTC
Riveroi   ATAACCCCCGCTTAACCTCACCTCTTTTTGTTAATCAGCCTGTATACCTCCGTCGTCAGCTTACCGCGTGAGCGCG
Silvani   ATAACCCCCGTTTAACCTCACCACTTTTAGCCTATCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAGCGTC
Bufoniu   ATAACCCCCGTTCAACCTCACCACTTCTTGTCTTTCAGCCTGTATACCTCCGTCGCCAGCTTACCGCATGAGCGCT
Fuscuss   ATAACCCCCGTTCAACCTCACCACTTCTTGTCTATCAGCCTGTATACCTCCGTCGCCAGCTTACCGCATGAGCGTT
Chaquen   ATAACCCCCGTTTAACCTCACCACTTTTTGCCTATCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAGCGCT
Insular   ATAACCCCCGTTTAACCTCACCACTTATTGTTTGTCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAGCGTT
Tyloide   ATAACCCCCGCTTAACCTCACCTCTTATTGCCCGTCAGCCTGTATACCTCCGTCGTCAGCTTACCACGTGAGCGCC
Melanon   ATAACCCCCGTTAAACCTCACCACTTATTGCCTTTCAGCCTGTATACCTCCGTCGTCAGCTTACCTCGTGAGCGTT
Pentada   ATAACCCCCGCTTAACCTCACCACTTCTAGCAAATCAGCCTGTATACCTCCGTCGTCAGCTTACCTCGTGAGCGCC
Vanzoli   ATAACCCCCGCTTAACCTCACCTCTTGTTGTCCATCAGCCTGTATACCTCCGTCGTCAGCTTACCTCGTGAGCGTC
Adenhya   ATAACCCCCGCTTTACCTCACCACTTCTAGCTAATCAGCCTGTATACCTCCGTCGTCAGCTTACCTCGTGAGCGAA
Lithody   ATAACCCCCGCTTAACCTCACCATTTTTTGAAAATCAGCCTGTATACCTCCGTCGTCAGCTTACCATGTGAACGTC
Physala   ATAACCCCCGCTTAACCTCACCAACTCTTGCTATTCAGCCTGTATACCTCCGTCGTCAGCTTACCTCGTGAGCGAA

Diedrus   ACTAAGTGAGCCAAATGCCCGCACGCCAACACGTCAGGTCAAGGTGCAGCTAATAAAGAGGGAAGAGATGGGCTAC
Riveroi   ACTCAGTGAGCTTAATGCCCGTAAGCCAACACGTCAGGTCAAGGTGCAGCTAATAAAGAGGGAAGAGATGGGCTAC
Silvani   ACTAAGTGAGCTTAATGTCTATACATCAACACGTCAGGTCAAGGTGCAGTCAATGAAGTGGAAAGAGATGGGCTAC
Bufoniu   ATTAAGTGAGCTTAATGACAATACGCCAACACGTCAGGTCAAGGTGCAGCTAATGAAGTGGGAAAAGATGGGCTAC
Fuscuss   CTTAAGTGAGCCCAATGCCCATACGCCAACACGTCAGGTCAAGGTGCAGCTAATGAAGTGGGAAGAGATGGGCTAC
Chaquen   ATTGAGTGAGCTTAATGCCCCTACGCCAACACGTCAGGTCAAGGTGCAGCTAATGAAGTGGGAAGAGATGGGCTAC
Insular   ATTAAGTGAGCTTAATGCCCCTACGCCAACACGTCAGGTCAAGGTGCAGCTAATGAAGTGGGAAGAGATGGGCTAC
Tyloide   ACTAAGTGAGCCCAATGTTTATACATCAACACGTCAGGTCAAGGTGCAGCTAATGAAAAGGGAAGAGATGGGCTAC
Melanon   ACCAAGTGAGCTTAATGCCTGTCCGCCAACACGTCAGGTCAAGGTGCAGCTAATAAAGTGGGAAGAGATGGGCTAC
Pentada   TTTAAGTGAGCCCAATGCCAATACGCCAATACGTCAGGTCAAGGTGCAGCTAATGAAATGGGAAGAGATCGGCTAC
Vanzoli   ATTAAGTGAGCCTAATGCCCCCGCGTCAACACGTCAGGTCAAGGTGCAGCTAATAAAGAGGGAAGAGATGGGCTAC
Adenhya   ATATAGTGAGCTCAATGCCATTTCACCAATACGTCAGGTCAAGGTGCAACTCATGAAGTGGGAAGAGATGGGCTAC
Lithody   TTATAGTGAGCTTAACGCTTATTCACCAGTACGTCAGGTCAAGGTGCAGCTAATGAAATGGACAGAGATGGGCTAC
Physala   TATTAGTGAGCTTAATGTCT-TTCACCAATACGTCAGGTCAAGGTGCAGCACATGAGCTGGAAAGAGATGGGCTAC
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Diedrus   ACTCTCTACT-CTAGAAGAAA-CAAAAGACTA--TATGAAAC-TTAGTCTGAAGGAGGATTTAGTAGTAAAAAGAA
Riveroi   ACTCTCTAAA-ATAGAAGAAA-CGAAAGACT--TTATGAAAC-CTAGTCGAAAGGAGGATTTAGTAGTAAAAAGGG
Silvani   ACTTTCTAGT-ATAGAAGAAA-CGAAAGACTATTTATGAAAC-CTGGTCAGAAGGAGGATTTAGTAGTAAAAAGAA
Bufoniu   ACTTTCTACC-GTAGAAAAAA-CGAAAAACTATTTATGAAAT-CTAGTCGGAAGGAGGATTTAGTAGTAAAAAGAA
Fuscuss   ACTTTCTACC-CTAGAAAAAAACGAAAGACTACCTATGAAAT-CTAGTCAGAAGGAGGATTTAGTAGTAAAAAGAA
Chaquen   ACTTTCTATT-TTAGAAGAAA-CGAAAGACTATATATGAAAT-CTAGTCAGAAGGAGGATTTAGTAGTAAAAAGAA
Insular   ACTTTCTACG-ATAGAAAAAA-CGAAAGACTATATATGAAAC-CTAGTTAGAAGGAGGATTTAGTAGTAAAAAGAA
Tyloide   ACTTTCTAAT-TTAGAAAAAA-CGAAAAGCTATTTATGAAAC-CTAGCCAGAAGGAGAATTTAGTAGTAAAAAGAA
Melanon   ACTTTCTAAT-TTAGAAAAAA-CGAAAGGCTATATATGAAAC-CTAACCAGAAGGAGGATTTAGTAGTAAAAAGAA
Pentada   ACTCTCTATTTATAGAAAAAA-CGAAAGACCACTTATGAAAC-CTGGTCAGAAGGAGGATTTAGCAGTAAAAAGAG
Vanzoli   ACTCTCTACC-TTAGAAAAAA-CAAAAGACTACATATGAAACCCTAGTCAGAAGGCGGATTTAGTAGTAAAAAGAA
Adenhya   ACTCCCTAAA-CTAAGGCACA-CGAAAAACTATCTATGAAAT-CTAGTTTGAAGGCGGATTTAGAAGTAAAAAGAA
Lithody   ACTTTCTAAC-ATAGAATATA-CGAAAGATTACTTATGAAAC-CTAATCTGAAGGCGGATTTAGAAGTAAAAAGAA
Physala   ACTTTCTAAT-CTAGAAAATA-CAAAAGACTACCTATGAAAT-CTAGTCTGAAGGCGGATTTAGAAGTAAAAAGAA

Diedrus   ATCAGAATGTTCTCTTTAACCCGGCACTGGGGCATGTACACACNGCCCG
Riveroi   ATCAGAGAGCTCTTTTTAACCCGGCACTGGGGTGTGCACACACCGCCCG
Silvani   ACCAGAGTGTTCTTTTTAACCCGGCACTGGGGTGTGTACACACCGCCCG
Bufoniu   ACCAGAGTGTTCTTTTTAACCTGGCACTGGGGTGTGTACACACCGCCCG
Fuscuss   ATCAGAGCGTTCTTTTTAATCTGGCACTGGGGTGTGTACACACCGCCCG
Chaquen   AACAGAGAGTTCTTTTTAACCCGGCACTGGGGTGTGTACACACCGCCCG
Insular   AGCAGAGTGTTCTTTTTAACCCGGCACTGGGGTGTGTACACACCGCCCG
Tyloide   ACTAGAGTGTTCTTTTTAACCCGGCACTGGGGTGCGTACACACCGCCCG
Melanon   AACAGAGTGTTCTTTTAAACCCGGCACTGGGGTGTGTACACACCGCCCG
Pentada   AACATAGTGCTCTTTTTAACCCGGCACTGGGGTGTGTACACACCGCCCG
Vanzoli   AACAGAGTGTTCTTTTTAACTTGGCCCTGGGGTGCGTACACACCGCCCG
Adenhya   ACCAAAGAGTTCTTTTTAACCCGGCACTGGGGTGTGTACACACCGCCCG
Lithody   ACCAGAGTGTTCTTTTTAACCCGGCACTGGGGTGTGTACACACCGCCCG
Physala   ACAAGAGTGTTCTCTTTAAATAGGCACTGGGGTGTGTACACACCGCCCG
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BEGINS 16S DATA
Diedrus  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Riveroi  ATAAGAGGTCCGGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCAAAGGTAGCGTAAT
Silvani  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Bufoniu  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TTT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Fuscuss  ATGAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Chaquen  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TTT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Insular  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Tyloide  ATTAAAGGTCTAGCCTGCCCA-GTGAC-TTT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Melanon  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Pentada  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Vanzoli  ATGAGAGGTCCAGCCTGCCCA-GTGAC-TTT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Adenhya  ATAAGAGGTCTAGCCTGCCCA-GTGAC-ATT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Lithody  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCT--GTTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT
Physala  ATAAGAGGTCCAGCCTGCCCA-GTGAC-TCA--ATTCAACGGCCGCGGTATCCTAACCGTGCGAAGGTAGCGTAAT

Diedrus  CACTTGTTCTCTAAATAAGGACTAGTATGAATGGCACCACGAGGGTTATACTGTCTCCTTCCTCCAATCAGTGAAA
Riveroi  CACTTGTTCTTTAAATGAGGACCGGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTTTTCTAATCAGTGAAA
Silvani  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTTTTTTAATCAGTGAAA
Bufoniu  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTTTTCTAATCAGTGAAA
Fuscuss  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTTTTCTAATCAGTGAAA
Chaquen  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCAGCAAGGTTATACTGTCTCCTTTCTTTAATCAGTGAAA
Insular  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTTCTTTAATCAGTGAAA
Tyloide  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCATCACGAGGGTTTTACTGTCTCCTCCCTCTAATCAGTGAAA
Melanon  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCACGAAGGTTATACTGTCTCCTTCCTCTAATCAGTGAAA
Pentada  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCACCACGAAGGTTATACTGTCTCCTCTCTCTAATCAGTGAAA
Vanzoli  CATTTGTTCTTTAATTGAGGACTAGTATGAACGGCACCACGAAGGTTATACTGTCTCCTCTCTCTAATCAGTGAAA
Adenhya  CACTTGTTCTTTAAATGAGGACTAGTATGAAAGGCATCACGAGAGTCATACTGTCTCCTTTCTCTAATCAGTGAAA
Lithody  CACTTGTTCTTTAAATAAGGACTAGTATGAAAGGCACCACGAGGGTTGCACTGTCTCCTTTCTCTAATCAGTGAAA
Physala  CACTTGTTCTTTAAATGAGGACTAGTATGAATGGCATCACGAGGGTTACACTGTCTCCCTTTTTTAATCAGTGAAA
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Diedrus  CTAATCCCCCCGTGAAGAAGCGAGGATAAACCTATAAGACGAGAAGACCCTATGGAGCTTTAAACAC-AGTAACAA
Riveroi  CTAACCCCCCCGTGAAGAGGCGGGGATGAGCCTATAAGACGAGAAGACCCTATGGAGCTTAAAACTC-AATAACAT
Silvani  CTAATCTTCCCGTGAAGAAGCGGGAATAAATATATAAGACGAGAAGACCCTATGGAGCTTTAAACACAAGTAACAA
Bufoniu  CTAATCTTCCCGTGAAGAAGCGGGAATAAACATATAAGACGAGAAGACCCTATGGAGCTTTAAACA-AACATACAA
Fuscuss  CTAATCTTCCCGTGAAGAAGCGGGAATAAAAATATAAGACGAGAAGACCCTATGGAGCTTTAAACA-AACATACAA
Chaquen  CTAATCTCCCCGTGAAGAAGCGGGGATAAGCCTATAAGACGAGAAGACCCTATGGAGCTTTAAACATAAGTAACAA
Insular  CTAATCTCCCCGTGAAGAAGCGGGGATAAATATATAAGACGAGAAGACCCTATGGAGCTTTAAACA--AGTAACAA
Tyloide  CTAATCTCCCCGTGAAGAAGCGGGGATAAACCTATAAGACGAGAAGACCCTATGGAGCTTTAAACAC--ACAACAA
Melanon  CTAATCTCCCCGTGAAGAAGCGGGGATAAACCTATAAGACGAGAAGACCCTATGGAGCTTTAAACAT-AGTAATAA
Pentada  CTAATCTCCCCGTGAAGAAGCGGGGGTAATTATATAAGACGAGAAGACCCTATGGAGCTTTAAACT-AAGAATCAA
Vanzoli  CTATTCTCCCCGTGAAGAAGCGGGGATGAACTTATAAGACGAGAAGACCCTATGGAGCTTTAAACAT-AACAACAA
Adenhya  CTAATCCCCCCGTGAAGAAGCGGGGATAGAAATATAAGACGAGAAGACCCTATGGAGCTTTAAACAC--ATAATAT
Lithody  CTAATCTTCCCGTGAAGAAGCGGGAATAAAAATATAAGACGAGAAGACCCTATGGAGCTTTAAACT-AAATAATAA
Physala  CTAATCTTCCCGTGAAGAAGCGGGAATACAATTATAAGACGAGAAGACCCTATGGAGCTTTAAACT-AAACAGCAA

Diedrus [-CTGCCC-----ACACCCCC---------TTCCTGGGGG-TTAAGTAT---TTGGGCTCC--]TTGATTACAAGTT
Riveroi [-TTGCCCAACACCCACCC---------AATCTCAGGAAACTCGCCCACCACCCGGACATA--]TTGATTACAAGTT
Silvani [-TTGCCCCCTTCCCTATTTTC------AA---CAGAAAATTAATCTATAT-TTAGGCATT--]TTGATTACAAGTT
Bufoniu [-TTGCCTTCA-ACAAAAAAA--------ATTCCAGAAGAAAAACCTTTAT-TTAGGCATC--]CTGTCATGACGTT
Fuscuss [-TTGCCTT-TTCTCATAAAA--------ATTCCAGAAAAAACACTTCT-ATTCAGGCACT--]TTGATATAAAGTT
Chaquen [-CTGCCCTAAATTTTTT----------AATCTCAGGAAATTAATCACGACACTTAGCACT--]TTGATTACAAGTT
Insular [-CTGCCTTATTAATCTCTTA--------ATCTCAGGAAATTTACCCCTTATCCAGGCATT--]TTGATTACAAGTT
Tyloide [-TTGCCCTGCTTCCCCCCCCC------AATCTCCGGAAAGCCACATACTC---GGGCATT--]TTGATTGCAAGTT
Melanon [-ATGCCCCCCCCCCTTTTTTCTATTTAAATCTCCGGAAAACTACT--TTATCTGGGCATC--]CTAATTACAAGTT
Pentada [-CTGCTTTATTCCCCTACA--------AATTTCAGAAGACTAACTTTTAC-CAAAGCACT--]CTGATTTCTAGTT
Vanzoli [-CTGCCCCGCCCCAGTTTTAT-------GTTCCCGAAAAATTATTT---ACCTAAGCATT--]TTGATTGTACGTT
Adenhya [-ATGCCCTTTTAACTTCAAA---------TTCCAGAAAAATCTCTTAT---CTTGGTATA--]ATAACTAATAGTT
Lithody [-TAGCCTACTCATTTACACA--------ACTCCAGATGAATA--CTTTAC-CCTGGCTCG--]ATAATTATTAGTT
Physala [-TTGT-TATATGTTTCCACC---------CTTCAGAGAAATAAATTCTAC-TTTAACATA--]ATGCTCACCAGTT
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Diedrus  TTAGGTTGGGGTGACCACGGAGCAAAAAACAACCTCCGCAGTGAATAGGGCCTTTTTCCCTAAACCCAGGACTAC
Riveroi  TTTGGTTGGGGTGACCGCGGAG-AAAAAACAACCTCCACAATGAATGGGACCCCCC-CCCCTAATTTCAGGGCCAC
Silvani  TTAGGTTGGGGTGACCGCGGAGAAAAAATAA-CCTCCACAATGAACAGGACTTA---TCCTTAAATTTAGGATTAC
Bufoniu  TTAGGTTGGGGTGACCACGGAGTAAAATTTAACCTCCGCAATGAACGGGGCTTT---CCCCTAAGATAAGAGCTAC
Fuscuss  TTAGGTTGGGGTGACCACGGAGTAAAAAACAACCTCCGCAGTGAAAGGGGCTTTT--CCCCTAAGCCAAGGGCTAC
Chaquen  TTAGGTTGGGGTGACCGCGGAGCAAAAAATAACCTCCGCAGTGAATGGAACTTAT--TTCCTAAACCCAGGGCTAC
Insular  TTAGGTTGGGGTGACCGCGGAGCATAAAATAACCTCCACAGTGAACGGACTCAT--TCCCTAAACCAAGGGCTAC
Tyloide  TTAGGTTGGGGTGACCGCGGAGTAAAAATTAGCCTCCACAGCGAATGGGACTTC---TCCCTAAACTCAGGGCTAC
Melanon  TTAGGTTGGGGTGACCCCGGGGTAAAAAATAACCTCCACAGTGAATGGGG-TCTT--CCCCTAAACTCAGGGCCAC
Pentada  TTAGGTTGGGGTGACCACGGAGTAAAAACCAACCTCCGCAATGAACAGGGATCT---TCCCTTAACCAAGGGCCAC
Vanzoli  TTAGGTTGGGGTGACCGCGGAGCAAAAAACAACCTCCACACTGGAAGGGACCTT---TCCCTAAACCCAGGGCCAC
Adenhya  TTTGGTTGGGGTGACCACGGAATAAAAAACAACTTCCACAATGAA-AGAT-TCTCCTTCACTAAGTTAAGGACTAC
Lithody  TTTGGTTGGGGTGACCACGGAGAAAAAAGAAACCTCCGCAATGAAAGACT--CTC--CTTCTTAGTTTAGGACTAC
Physala  TTCGGTTGGGGTGACCACGGAGAATAAAACAACCTCCACGATAAAAGAAACTTAA--TCTCTTAATCCAGAATTAC

Diedrus  AACCCTAAGATTCAACAAAT-TGACACCCATT-GACCCAGTT--TCTGATCAATGAACCAAGTTACCCTAGGGATA
Riveroi  AGCCCTAAAAATCAACAAAT-TGACATATATTTGACCCAATTCTTTTGRGCAACGAACCAAGTTACCCTAGGGATA
Silvani  AATCCCAAAAATCAATAAAT-TGACATCTATT-GACCCAATATTTTTGATCAATGAACCAAGTTACCCTAGGGATA
Bufoniu  GACTCTAATAATCAACAAAT-TGACACCAATT-GACCCAATACACTTGATCAATGAACCAAGTTACCCTAGGGATA
Fuscuss  GACCCTAAGAATCAATAGAT-TGACACTAATT-GACCCAATT-AATTGATCAATGAACCAAGTTACCCTAGGGATA
Chaquen  GACCCTAAGAATCAATAAAT-TGACACTGATT-GACCCAATATTTTTGATCAATGAACCAAGTTACCCTAGGGATA
Insular  AACCCTAAGCATCAATAAAT-TGACACCTATT-GACCCAATA-TTTTGATCAATGAACCAAGTTACCCTAGGGATA
Tyloide  GACCCTAAGAATCAATAAAT-TGACACCCATT-GACCCAATT-TTTTGACCAATGAACCAAGTTACCCTAGGGATA
Melanon  AACCCTAAAAATCAATAAAT-TGACACCCATT-GACCCAATA-TTTTGATCAATGAACCAAGTTACCCTAGGGATA
Pentada  AACCCTAAGAATCAATACAT-TGACATCAATT-GATCCAAAAAATTTGCCCAATGAACCAAGTTACCCTAGGGATA
Vanzoli  AGCCCCCAGAATCAATAAAT-TGACACCTGTT-GACCCAATA-TTTTGACCAATGAACCAAGTTACCCTAGGGATA
Adenhya  AACCCTATACATCAATAAAT-TGACATA-ATT-GACCCAACA-TATTGATCAATGAACCAAGTTACCCTAGGGATA
Lithody  TTTCCTACGCATCAATAAAT-TGACACATATT-GACCCAACAACGTTGATCAATGAACCAAGTTACCCTAGGGATA
Physala  GATTCTAAGTACCAAAAATT-TGATATACATT-GATCCAATT-TATTGATCAACGAACCAAGTTACCCTAGGGATA
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Diedrus   ACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGCATCCTAGT
Riveroi   ACAGGGAAATCCACTTTAAGGGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTACCCCAGT
Silvani   ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTGTCCCAGT
Bufoniu   ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCTAGT
Fuscuss   ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCTAGT
Chaquen   ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCCAGT
Insular   ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTTCGACCTCGATGTTGG-ATCAGGGTATCCCAGT
Tyloide   ACAGCGCAATCCATTTCAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTACCCCAGT
Melanon   ACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGCCCTCGATGTTGG-ATCAGGGNNCCN-AGT
Pentada   ACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCTAGT
Vanzoli   ACAGCGCAATCCACTTTAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTTCCCTAGT
Adenhya   ACAGCGCAATCCACTTTAAGAGCTCTTATCGACAAGTGGGCTTACGACCTCGATGTTGG-ATCAGGGTACCCCAGT
Lithody   ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCCAGT
Physala   ACAGCGCAATCCACTTCAAGAGCTCTTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCCAGT

Diedrus   GGTGTAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Riveroi   GGTGCAGCCGCTGCTTACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Silvani   GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Bufoniu   GGTGCAGCCGCTACTGATGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Fuscuss   GGTGCAGCCGCTACTAATGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Chaquen   GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Insular   GGTGCAGCCGCTACTAATGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Tyloide   GGTGCAGCCGCTGCTAACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGCAATCC
Melanon   GGTGNAGCCGCTNCTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Pentada   GGTGCAGCCGCTACTAACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Vanzoli   GGTGCAGCCGCTGCTAACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGCAATCC
Adenhya   GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Lithody   GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
Physala   GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC
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Diedrus  AGGTCAGTTTCTATCTATAAAGAGTTTTCTCCAGTACGAAAGGACCGAAAAAACATGGCCAATGCCCCCAGTAAGCC
Riveroi  AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGGAAAAGCATGGCCCATGCTAACTGCAAGCC
Silvani  AGGTCAGTTTCTATCTATAAAGAGTTTTTTCCAGTACGAAAGGACCGAAAAAACATGGCCCATACTTCATGCAAGCC
Bufoniu  AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGAAAAAGCACGGCCTATACCTAACGCAAGCC
Fuscuss  AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGAAAAAGCACGGCCTATACTCTAAGCAAGCC
Chaquen  AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGAAAAAGCATGGCCTATACTTCATGCAAGCC
Insular  AGGTCAGTTTCTATCTATAAAGAGCCTTTTCTAGTACGAAAGGACCGAAAAAGCATGGCCTATACTATATGCAAGCC
Tyloide  AGGTCAGTTTCTATCTATAAAGAGTTTCTTCTAGTACGAAAGGACCGAAGAAACATGGCCAATGCCTC-CGTAAGCC
Melanon  AGGTCAGTTTCTATCNATAAAGAGATTTTTCTAGTACGAAAGGACCGAAAAAGCATGGCCCATACTTCATGCAAGCC
Pentada  AGGTCAGTTTCTATCTATAAAAAGCTCTTTCTAGTACGAAAGGACCGAAAAAGCATGGCCTATACTTAATGCAAGCC
Vanzoli  AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGAAAAAACATGGCCAATGCCAA-AGTAAGCC
Adenhya  AGGTCAGTTTCTATCTATAAAGAGCTTTTTTTAGTACGAAAGGACCAAAAAAGCATGGCCAATGCTTC-AATAAGCC
Lithody  AGGTCAGTTTCTATCTATAAAGAGCTTTTTTTAGTACGAAAGGACCAAAAAAGCATGGTCCATGTTTATTACAAACC
Physala  AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGAAAAAGCATGGCCAATATTTTATATAAGCC

Diedrus  ATAACAACCTATTTATG-ACACAAT
Riveroi  ATAACAATTA-TTTATG-ATACAAC
Silvani  ATAACAATCAATTTTATGACACAAC
Bufoniu  GTAGCAACCAATTTATG-ACACAGC
Fuscuss  GTAACAACCAACTTATG-ACATAGT
Chaquen  ATAACAGATAATTTATG-ACACAAC
Insular  ATAACAACTAATTTATG-ACACAAC
Tyloide  ATAGCAACTTATTTATG-ACTTAAC
Melanon  ATAACGCTCAATTTATG-ACTAAAC
Pentada  ATAACAGCCAATTTATG-ACATAAC
Vanzoli  ATAATACCTTATTTATG-ACCAAAT
Adenhya  ATACCATTC-ATTTATG-AATTTAT
Lithody  ATTCTAATTAACTTTTG-ACTTAAC
Physala  ATAGT--CTAATTTATG-TTTATAC
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