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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

[^0]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 16 s r RNA of $\sim 700$ nucleotides were amplified. Double-stranded (DS) PCR amplifications were performed in a final volume of $50 \mu$ l containing $0.4 \mu 1$ of each primer, $1.0 \mu \mathrm{l}$ of each dNTP, $3.0 \mu \mathrm{l}$ of 25 mM MgCl , and 1.25 units of Taq (Thermus aquaticus) DNA polymerase; the reaction was overlaid with $50 \mu 1$ of mineral oil. PCR conditions were as follows: $94^{\circ} \mathrm{C}$ for $60 \mathrm{~s}, 57^{\circ} \mathrm{C}$ for 60 s , and $72^{\circ} \mathrm{C}$ for 60 s , with 25 cycles for the 12 S amplification and 30 cycles for the 16 S amplification. Amplified product was purified using Wizard® PCR Preps Kit (Promega). Of the purified DS fragment, $0.5 \mu$ were mixed with $1.5 \mu 1$ of a single IRD-labeled primer, $7.2 \mu 1$ of Sequencing Buffer, $1.0 \mu 1$ of Sequitherm Excel ${ }^{\text {TM }}$ II (Epicentre Technologies Co.) DNA polymerase, and $6.8 \mu \mathrm{l}$ of $\mathrm{dH}_{2} \mathrm{O}$. Subsequently, $4.0 \mu \mathrm{l}$ of this mix was added to each of 4 tubes containing $2 \mu 1$ of each nucleotide respectively. PCR conditions were as follows ( 30 cycles): $92^{\circ} \mathrm{C}$ for $30 \mathrm{~s}, 55^{\circ} \mathrm{C}$ for 30 s , and $70^{\circ} \mathrm{C}$ 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 83912 S and 648 16 S 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 12 S rDNA gene and 647 bp to the 16 S 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, 12 S , and 16 S data sets) as well as combined analyses (i.e., $12 \mathrm{~S}+16 \mathrm{~S}$ 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 downweighting transitions to transversions 5:1.

## RESULTS

There is modest variation in the $12 \mathrm{~S}, 16 \mathrm{~S}$, and $12 \mathrm{~S}+16 \mathrm{~S}$ data sets (Tabs.1-3). The maximum sequence divergences between pairs of taxa are $21 \%$ for the 12 S data, $16 \%$ for the 16 S data, and $18 \%$ for the $12 \mathrm{~S}+16 \mathrm{~S}$ 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 16 S 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 12 S data (Tab.1), but not at all clear in the 16 S 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 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 $12 \mathrm{~S} \& 16 \mathrm{~S}$ 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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APPENDIX 2

continuation

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## snxpəт̣๔ Riveroi Silvani Bufoniu Fuscuss Chaquen Insular Myloide Pentada Vanzoli Adenhya Lithody Lithody

ACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGCATCCTAGT ACAGGGAAATCCACTTTAAGGGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTACCCCAGT ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTGTCCCAGT ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCTAGT ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCTAGT ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCCAGT ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTTCGACCTCGATGTTGG-ATCAGGGTATCCCAGT ACAGCGCAATCCATTTCAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTACCCCAGT ACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGCCCTCGATGTTGG-ATCAGGGNNCCN-AGT ACAGCGCAATCCACTTCAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCTAGT ACAGCGCAATCCACTTTAAGAGCTCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTTCCCTAGT ACAGCGCAATCCACTTTAAGAGCTCTTATCGACAAGTGGGCTTACGACCTCGATGTTGG-ATCAGGGTACCCCAGT ACAGCGCAATCCACTTCAAGAGCCCCTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCCAGT ACAGCGCAATCCACTTCAAGAGCTCTTATCGACAAGTGGGTTTACGACCTCGATGTTGG-ATCAGGGTATCCCAGT
GGTGTAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTGCTTACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTGATGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTAATGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTAATGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTGCTAACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGCAATCC GGTGCAGCCGCTACTAACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTGCTAACGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGCAATCC GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC GGTGCAGCCGCTACTAAAGGTTCGTTTGTTCAACGATTAAAACCCTACGTGATCTGAGTTCAGACCGGAGTAATCC

Diedrus
Riveroi
Silvani
Bufoniu
Fuscuss
Chaquen
Insular
Tyloide
Melanon
Pentada
Vanzoli
Adenhya
Lithody
Physala


AGGTCAGTTTCTATCTATAAAGAGTTTTCTCCAGTACGAAAGGACCGAAAAAACATGGCCAATGCCCCCAGTAAGCC AGGTCAGTTTCTATCTATAAAGAGCTTTTTCTAGTACGAAAGGACCGGAAAAGCATGGCCCATGCTAACTGCAAGCC
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ATAACAACCTATTTATG-ACACAAT ATAACAATTA-TTTATG-ATACAAC
GTAGCAACCAATTTATG-ACACAGC
GTAACAACCAACTTATG-ACATAGT
ataAchatatait tatg-acacanc ATAACAACTAATTTATG-ACACAAC ATAGCAACTTATTTATG-ACTTAAC ATAACGCTCAATTTATG-ACTAAAC ATAACAGCCAATTTATG-ACATAAC ATACCATTC-ATTTATG-AATTTAT
АТТСТААТТААСТТТTG-АСТТААС
Diedrus
Riveroi
Silvani
Bufoniu
Fuscuss
Chaquen
Insular
Tyloide
Melanon
Pentada
Vanzoli
Adenhya
Lithody
Physala


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