PHYLOGENETIC SYSTEMATICS OF DISPHOLIDINE COLUBRIDS (SERPENTES: COLUBRIDAE)

by

THOMAS GERNOT EIMERMACHER

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

THE UNIVERSITY OF TEXAS AT ARLINGTON

August 2012

ACKNOWLEDGEMENTS

First and foremost, I'd like to thank my collaborators Don Broadley, Beryl Wilson, Zoltan Nagy, Axel Barlow, Wolfgang Wüster, and Eli Greenbaum were instrumental in facilitating the research involved in this project. I also wish to thank my Master's advisor Brian Crother, my parents, and my wife and best friend, Claudia Marquez, for providing the at times extraordinary amount of moral support needed for me to complete this dissertation. In addition, Coleman Sheehy, Heath Blackmon, Jeff Streicher, Christian Cox, Paul Pasychnik, Walter Schargel, Emily Deese Cooper, Amanda Thomas, Martin Smit, Thea Litschka-Koen, Clifton Koen, Donald Schultz, Johan Marais, Bill Branch, Wulf Haacke, Elizabeth Scott, Joe Switalski, Steffen Stjerne, Joe Mathis, Nikki Gibbs, and Pg Du Plessis repeatedly provided assistance of various kinds.

Of course, I'd also like to thank my advisors and members of my PhD dissertation committee for their willingness to serve in that function. Eric Smith, Jonathan Campbell, James Robinson, Paul Chippindale, and Andre Pires da Silva all had a profound impact on my journey through the program. I would like to emphasize my appreciation and hope that I will one day be able to reciprocate their contributions. Field Research for the collection of specimens and/or tissues in Africa has been possible through funding from the University of Texas at Arlington, a grant to Eric Smith from Instituto Bioclon (Mexico), three UTA-Phi Sigma Biological Sciences Honors Society research grants, two UTA-Graduate Student Senate grants, a UTA-William L. and Martha Hughes Award for the Study of Biology, and personal funds. Laboratory work has been possible through funding provided by Eric Smith, from UTA start-up funds and a grant from Instituto Bioclon, as well as laboratory surplus and computational resources by Ellen Pritham and Cedric Feschotte. Other institutional support was received from the McGregor Museum and the South African National Biodiversity Institute,

July 29, 2012

ABSTRACT

PHYLOGENETIC SYSTEMATICS OF DISPHOLIDINE COLUBRIDS (SERPENTES: COLUBRIDAE)

Thomas Gernot Eimermacher, PhD

The University of Texas at Arlington, 2012

Supervising Professors: Eric Smith and Jonathan Campbell

Dispholidine colubrids are a group of arboreal African snakes that are distributed throughout sub-Saharan Africa. Despite their vast distribution and medical significance, the systematic relationships of this group remain poorly understood. I used molecular and morphological data in both a phylogenetic and a multivariate framework to study the evolutionary relationships and external morphology of these snakes.

The results of the phylogeographic investigation based on two mitochondrial markers indicated the presence of two distinct evolutionary lineages of *Dispholidus* sp. in southern Africa that are largely geographically separated by the Great Escarpment and associated habitat. A study of the molecular systematics of dispholidine snakes using both mitochondrial and nuclear markers further corroborated those results, and suggest the presence of multiple distinct evolutionary lineages within the genus *Dispholidus*. It also provides strong support for the paraphyly of *Thelotornis* to the exclusion of *Xyelodontophis uluguruensis*.

Lastly, a multivariate analysis of the external morphological characters commonly utilized in taxonomic keys and species accounts indicated that those characters are only partially able to distinguish taxa within this group. Snout-vent length, tail length, subcaudal and mid-dorsal scales were able to differentiate within the genera *Thrasops* and *Rhamnophis*, as well as specimens of *Dispholidus* sp. from Pemba Island.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

Figure Page

LIST OF TABLES

CHAPTER 1

PHYLOGEOGRAPHY OF BOOMSLANGS (*DISPHOLIDUS TYPUS*) IN SOUTHERN AFRICA

1.1 Introduction

1.1.1 Introduction to Dispholidus typus

The African colubrid genus *Dispholidus* includes the boomslang, *Dispholidus typus* (A. Smith 1828), one of the most widely-distributed colubrine snakes throughout sub-Saharan Africa. Boomslangs are found from Senegal in West Africa through Ethiopia and Somalia in East Africa, and south to coastal South Africa. An impressive range of regional color forms are known to occur in adults, including black, brown, green, black and green, black and yellow, and grey, among others. They are also sexually dichromatic, with adult females typically being brown, whereas adult males may attain any of the aforementioned colors. Regardless of geographical origin or gender, juvenile specimens are usually marked with grey and brown stipples, often with small blue marks anteriorly (Spawls and Branch 1995). Despite the substantial morphological diversity, the genus is currently considered to be monotypic, containing only a single species, *Dispholidus typus*. It is the single most wide-ranging taxon among dispholidine colubrids, ranging throughout most of sub-Saharan Africa.

The genus has had a volatile taxonomic history, with multiple species and subspecies ranking among the junior synonyms of *D. typus*. Andrew Smith (1828, 1829, 1838, and 1841) described five synonyms of *Dispholidus*, including *Bucephalus typus* A. Smith 1828, *B. bellii* A. Smith 1828, *B. gutturalis* A. Smith 1828, *B. jardineii* A. Smith 1828, *B. viridis* A. Smith 1838, and *B. capensis* A. Smith 1841. In addition, *Dispholidus Lalandii* Duvernoy 1832, *Dendrophis colubrine* Schlegel 1837, and *Dendrophis pseudodipsas* Bianconi 1848 were described.

Since the genus name *Bucephalus* was found to be occupied by a group of trematode flatworms (Baer 1827), the group was moved to the genus *Dispholidus* Duvernoy 1832 (Schmidt 1923). Finally, Laurent (1955) described the subspecies *D. t. kivuensis* and *D. t. punctatus*, while also resurrecting *D. t. viridis*. However, only *D. typus* was subsequently recognized, and the genus remains monotypic today.

1.1.2 Phylogeography of Southern Africa

Southern Africa includes the countries of South Africa, Lesotho, Swaziland, Namibia, Botswana, Zimbabwe, Mozambique, Angola and Zambia. This subcontinent is known for its tremendous levels of biodiversity, with its reptile diversity being one of the richest in the world and the richest on the African continent (Bauer 1993, Branch 1999), including over 520 known species (Branch 1998). In addition, its reptile fauna displays high levels of endemicity (Branch 2006). Despite these high levels of diversity and endemicity, a recent review of the literature found that only 15% of phylogeographic studies focused on systems from the southern hemisphere, and on a continental scale merely 8% focused on African systems (Beheregaray 2008). Among reptiles alone, almost 80% of species of southern African reptiles are considered to be taxonomically problematic, and many are thought to contain additional cryptic species (Branch 2006). This indicates a pressing need for broad systematic reviews of many of the reptile genera that are distributed in this region, using modern techniques that will help to elucidate the evolutionary relationships and the underlying phylogeographic patterns that generate these.

In South Africa, *D. typus* occurs throughout most of the country, with the exception of parts of the southern Kalahari, the western and central portions of the Nama Karoo, the western coastal belt, the Namaqua highlands, and the Orange River gorge. It is also absent from most of Lesotho, the central Highveld, and parts of the south eastern uplands (Broadley 1983, Branch 1988, Marais 2004).

Many species display some degree of population structure that can be analyzed and interpreted in both spatial and temporal contexts. The study and inference of such diversifications form an integral part of molecular phylogeographic studies, and as such have provided significant insights to speciation (Avise et al. 1998, Kohn 2005), historical biogeography (Avise 2000, Riddle and Hafner 2006), as well as biodiversity research and taxonomy (Avise and Ball 1990, Beheregaray and Caccone 2007). Molecular phylogeography can reveal places, processes and time-periods associated with diversification by comparative analyses of phylogeographic patterns across multiple lineages, which in turn enables us to identify historical processes with far-reaching effects which impacted multiple organismal groups in southern Africa.

Southern African species of reptiles that have been investigated in a phylogeographic context include the angulate tortoise (*Chersina angulata*) (Lesia et al. 2003, Daniels et al. 2007), the speckled padloper (*Homopus signatus*) (Daniels et al. 2010), cordylid lizards (Daniels et al. 2004), dwarf chameleons (*Bradypodion*) (Tolley et al. 2004, Tolley et al. 2006, Tolley et al. 2008), the rock agama (*Agama atra*) (Matthee and Flemming 2002, Swart et al. 2009), sand lizards of the genus *Pedioplanis* (Makokha 2006, Makokha et al. 2007, Tolley et al. 2009), and rock skinks of the genus *Trachylepis* (Portik 2009, Portik et al. 2010, Portik et al. 2011). However, no southern African species of snakes have been studied in that context, despite their local abundance and potential medical significance.

In this study, I investigated the phylogeography of the genus *Dispholidus* within South Africa.

1.2 Materials and Methods

1.2.1 Sampling Methodology

A total of 69 samples were obtained from localities throughout South Africa and Swaziland (figure 1.1) by field collecting and by requesting tissues from other workers with

access to relevant areas. In the field, specimens were located by driving along roads that penetrate suitable habitat. Samples of integument, liver or muscle tissue were collected for molecular analyses, and stored in lysis buffer (1M TrisBase, 0.5M NaCl, 0.5M EDTA, 10% SDS), 95-100% ethanol, or an RNA stabilization reagent. The whole specimens were then fixed in 10% formalin (3.7% formaldehyde, 0.6-1.5% methanol), and maintained in 70% ethyl alcohol. The collected specimens were deposited at the McGregor Museum in Kimberley, South Africa, where a subset of them is currently waiting to be exported UT Arlington, Texas, USA. Tissue samples were deposited at the South African National Biodiversity Institute (SANBI), in accordance with the provincial collecting permits. All tissue samples used in this work were obtained from dead specimens from museum collections. No animals were sacrificed as a result of this research by researcher(s) from UT Arlington.

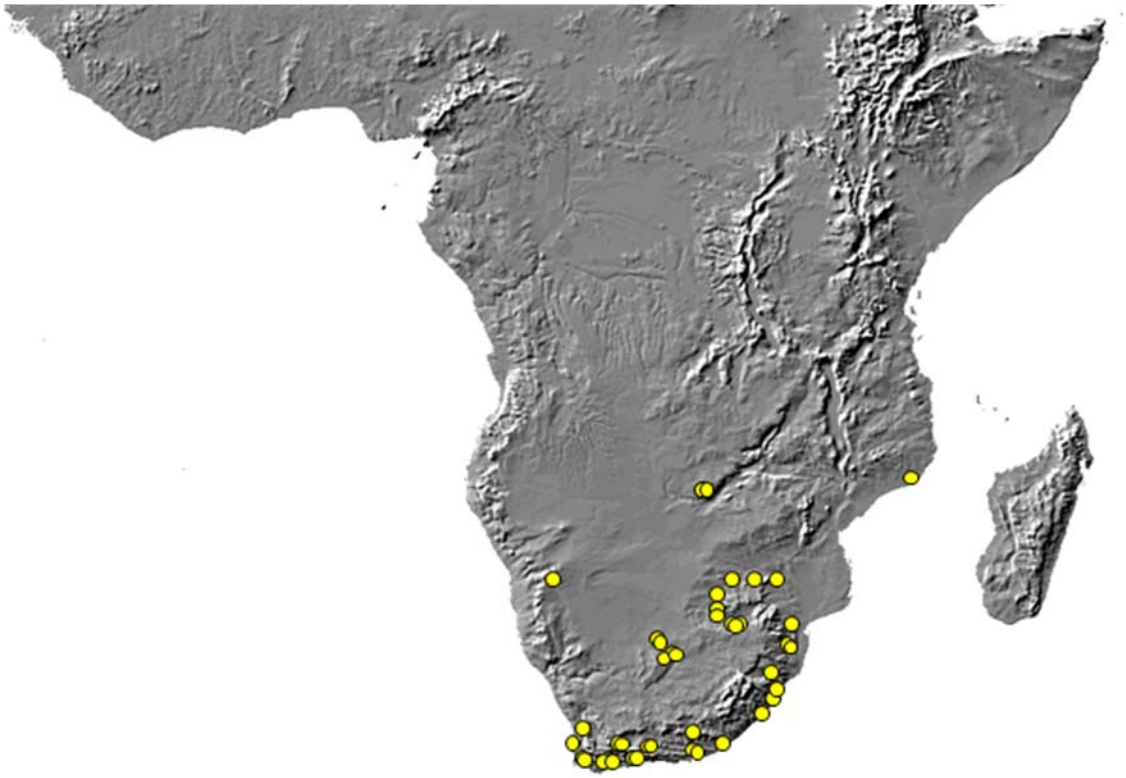

Figure 1.1. Distribution of samples of *D. typus* used in this study.

1.2.2 Molecular Data Generation

Whole genomic DNA was extracted from the tissue samples and stored following the protocols of Burbrink et al. (2000) or the Qiagen DNeasy Blood & Tissue Kit, and PCR amplifications were carried out in 25 µL volumes in an Eppendorf Mastercycler EP Gradient thermocycler, using the Promega GoTaq® Green Master Mix. Samples amplified using the Gludg/AtrCB3 (cyt-b) primer set were subjected to an initial denaturation at 94 °C for 180 s, 2 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 60 s, and extension at 72 °C for 45 s, and 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 45 s, and extension at 72 °C for 45 s. This was followed by a final extension at 72 °C for 300 s. Samples amplified using the H14910/THRSN2 (cyt-b) primer set were subjected to an initial denaturation at 95 °C for 150 s, 2 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 60 s, and extension at 68 °C for 90 s, and 40 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 45 s. This was followed by a final extension at 72 °C for 900 s. Samples amplified using the ND4/LEU (ND4) primer set were subjected to a single cycle of denaturation at 94 °C for 210 s, annealing at 42 °C for 60 s, and extension at 68 °C for 90 s, and 39 cycles of denaturation at 94 °C for 30s, annealing at 48 °C for 30 s, and extension at 72 °C for 60 s. This was followed by a final extension at 72 °C for 900 s.

Table 1.1. List of Samples utilized in this study. Sample IDs represent field, specimen, or GenBank accession numbers, and the locality data include the nearest town, province, and country of origin in standard two-letter code. Legend: TGE = T. Eimermacher; CMRK = C. Kelly; ELI & EBG = E. Greenbaum; BILL = B. Branch; WW = W. Wüster; all other acronyms are of unknown meaning.

Sample ID	Taxon	Locality Data	ND4	Cyt-b
A1-45	*Dispholidus typus*	Hluhluwe, KwaZulu-Natal, ZA	JX301489	JX316980
A1-57	*Dispholidus typus*	Ladysmith, KwaZulu-Natal, ZA	JX301448	JX316942
A1-58	*Dispholidus typus*	Ladysmith, KwaZulu-Natal, ZA	JX301449	JX316943
BILL 729	*Dispholidus typus*	Elandela Reserve, Limpopo, ZA	JX301469	JX316960

Table 1.1 - *Continued*

CMRK 250	*Dispholidus typus*	Kazungula, BW	JX301490	JX316981
DPS 23	*Dispholidus typus*	2km N of Mogoditshane, North-West, ZA	N/A	JX317043
HB016	*Dispholidus typus*	Prince Albert, Western Cape, ZA	JX301443	JX316937
HLMD RA-2974	*Dispholidus typus*	Cape Town, Western Cape, ZA	N/A	AY188012
I28674	*Dispholidus typus*	Cape Town, Western Cape, ZA	JX301445	JX316939
I29417	*Dispholidus typus*	~25km SW of Regone, MZ	JX301488	JX316979
I29420	*Dispholidus typus*	~14km SE of Kurland, Western Cape, ZA	JX301447	JX316941
JM 1857	*Dispholidus typus*	Kalumbila, ZM	N/A	JX317034
KTH0708	*Dispholidus typus*	Cape Town, Western Cape, ZA	JX301444	JX316938
KTH09294	*Dispholidus typus*	~23km N of Windhoek, NA	JX301484	JX316975
MB21431	*Dispholidus typus*	~70km NE of Kuruman, Northern Cape, ZA	JX301466	N/A
MBUR00351	*Dispholidus typus*	~11km NW of Bochum, Limpopo, ZA	JX301460	N/A
MWD070041	*Dispholidus typus*	SE of Vredenburg, Western Cape, ZA	JX301450	N/A
TGE T10-13	*Dispholidus typus*	Magersfontein, Northern Cape, ZA	N/A	JX317037
TGE T1-12	*Dispholidus typus*	Pinetown, KwaZulu-Natal, ZA	JX301434	JX316928
TGE T11-30	*Dispholidus typus*	W of Hans Merensky NR, Limpopo, ZA	N/A	JX317041
TGE T11-60	*Dispholidus typus*	NW of Masebe NR, Limpopo, ZA	JX301471	JX316962
TGE T11-67	*Dispholidus typus*	Leeupoort Vakansiedorp, Limpopo, ZA	N/A	JX317042
TGE T1-17	*Dispholidus typus*	Kathu, Northern Cape, ZA	JX301451	JX316944
TGE T11-71	*Dispholidus typus*	E of Kimberley, Northern Cape, ZA	JX301470	JX316961
TGE T1-18	*Dispholidus typus*	Kathu, Northern Cape, ZA	JX301452	JX316945
TGE T1-19	*Dispholidus typus*	Fourways Johannesburg, Gauteng, ZA	JX301453	JX316946

Table 1.1 - *Continued*

TGE T1-20	*Dispholidus typus*	Lephalale, Limpopo, ZA	JX301454	JX316947
TGE T12-13	*Dispholidus typus*	~5km E of McGregor, Western Cape, ZA	N/A	JX317044
TGE T1-50	*Dispholidus typus*	Hoedspruit, Limpopo, ZA	JX301455	JX316948
TGE T1-51	*Dispholidus typus*	Hoedspruit, Limpopo, ZA	JX301456	JX316949
TGE T1-52	*Dispholidus typus*	Kapama River Lodge, Limpopo, ZA	JX301458	JX316951
TGE T2-42	*Dispholidus typus*	Hluhluwe, KwaZulu-Natal, ZA	JX301464	JX316956
TGE T3-17	*Dispholidus typus*	~37km W of Kimberley, Northern Cape, ZA	JX301465	JX316957
TGE T3-39	*Dispholidus typus*	E of Kimberley, Northern Cape, ZA	N/A	JX317038
TGE T3-9	*Dispholidus typus*	~8km S of Lingelihle, Eastern Cape, ZA	JX301437	JX316931
TGE T4-12	*Dispholidus typus*	~16km W of Campbell, Northern Cape, ZA	N/A	JX317040
TGE T4-16	*Dispholidus typus*	~0.5km SE of NC-FS border, ZA	N/A	JX317039
TGE T4-19	*Dispholidus typus*	Twin Pines Farm, Free State, ZA	JX301467	JX316958
TGE T4-20	*Dispholidus typus*	Twin Pines Farm, Free State, ZA	JX301468	JX316959
Tm 83452	*Dispholidus typus*	Farm Buffelsdrift, Pretoria dist., Gauteng, ZA	N/A	JX317035
Tm 85298	*Dispholidus typus*	no locality data available	N/A	JX317036
WW 1293	*Dispholidus typus*	Hoedspruit, Limpopo, ZA	JX301457	JX316950
WW 1755	*Dispholidus typus*	Grootkraal, Western Cape, ZA	JX301428	JX316922
WW 1764	*Dispholidus typus*	Prince Albert, Western Cape, ZA	JX301430	JX316924
WW 1839	*Dispholidus typus*	Pringle Bay, Western Cape, ZA	JX301438	JX316932
WW 1863	*Dispholidus typus*	Minwater, Western Cape, ZA	JX301439	JX316933
WW 1869	*Dispholidus typus*	Grootvadersbosch, Western Cape, ZA	JX301440	JX316934
WW 1909	*Dispholidus typus*	Cederberg, Western Cape, ZA	JX301441	JX316935

Table 1.1 - *Continued*

WW 2074	*Dispholidus typus*	Vleesbaai, Western Cape, ZA	JX301427	JX316921
WW 2093	*Dispholidus typus*	Springerbaai, Western Cape, ZA	JX301442	JX316936
WW 2097	*Dispholidus typus*	Kasane, BW	JX301485	JX316976
WW 2108	*Dispholidus typus*	Pilanesberg National Park, Northwest, ZA	JX301463	JX316955
WW 2239	*Dispholidus typus*	De Hoop, Western Cape, ZA	JX301435	JX316929
WW 2271	*Dispholidus typus*	10km E of Mkhaya GR, SZ	JX301459	JX316952
WW 2298	*Dispholidus typus*	Niseal Nature Reserve, SZ	JX301462	JX316954
WW 2317	*Dispholidus typus*	Tjaneni, SZ	JX301486	JX316977
WW 2381	*Dispholidus typus*	Oudtshoorn, Western Cape, ZA	JX301431	JX316925
WW 2404	*Dispholidus typus*	Hartenbos, Western Cape, ZA	JX301432	JX316926
WW 2417	*Dispholidus typus*	Grabouw, Western Cape, ZA	JX301446	JX316940
WW 2439	*Dispholidus typus*	8km N of Brits, North-West, ZA	JX301487	JX316978
WW 2579	*Dispholidus typus*	Oudtshoorn, Western Cape, ZA	JX301429	JX316923
WW 2588	*Dispholidus typus*	Port Elizabeth, Eastern Cape, ZA	JX301426	JX316920
WW 2636	*Dispholidus typus*	Margate, KwaZulu-Natal, ZA	JX301424	JX316918
WW 2655	*Dispholidus typus*	East London, Eastern Cape, ZA	JX301422	JX316916
WW 2672	*Dispholidus typus*	Ballito, KwaZulu-Natal, ZA	JX301433	JX316927
WW 2780	*Dispholidus typus*	De Hoop NR, Western Cape, ZA	JX301425	JX316919
WW 2894	*Dispholidus typus*	Fransmanshoek, Western Cape, ZA	JX301436	JX316930
WW 2939	*Dispholidus typus*	Mabula Game Reserve, Limpopo, ZA	JX301461	JX316953
A1-54	*Philothamnus punctatus*	Ngezi, Pemba Island, TZ	JX301272	JX317025

For an overview of the primer sequences used, see table 2. All amplification runs included negative controls to check for contamination, and were quantified on a 1% TAE-agarose gel. The subsequent clean-up was conducted using Agencourt AMPure XP magnetic beads or the USB ExoSAP-IT reagent to remove excess primers and unincorporated dNTPs. These amplifications were then sequenced using an Applied Biosystems 3130xl Genetic Analyzer at the Genomics Core Facility at the University of Texas at Arlington to create the mitochondrial and nuclear data. The final data set contains sequences from two mtDNA gene fragments, including NADH dehydrogenase subunit 4 (681 bp) and cytochrome b (1092 bp). The tRNAs that are typically amplified by the ND4 primer LEU were not included in this study, as amplification of that part of the sequence in this group was relatively poor. Additional sequences of both ingroup and outgroup taxa were imported from Genbank to supplement the dataset.

Table 1.1. Primers used for generating sequence data.

Primer Name	Locus	Direction	Primer Sequence	Reference
Gludg	Cyt-b	Forward	5' TGA CTT GAA RAA CCA YCG TTG 3'	Parkinson et al. (2002)
AtrCB3	Cyt-b	Reverse	5' TGA GAA GTT TTC YGG GTC RTT 3'	Parkinson et al. (2002)
H14910	Cyt-b	Forward	5’ GAC CTG TGA TNT GAA AAA CCA YCG TT 3’	Burbrink et al. (2000)
THRSN2	Cyt-b	Reverse	5’ CTT TGG TTT ACA AGA ACA ATG CTT TA 3’	Burbrink et al. (2000)
ND4	ND4	Forward	5’ CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC 3’	Arevalo et al. (1994)
LEU	ND4	Reverse	5’ CAT TAC TTT TAC TTG GAT TTG CAC CA 3’	Arevalo et al. (1994)

1.2.3 Phylogenetic Analyses

Sequence editing and assembly of contigs was conducted using Sequencher ver. 4.5, aligned using MEGA ver. 5.05, and manually adjusted with MacClade ver. 4.08. Phylogenetic analyses were conducted using maximum likelihood and maximum and weighted parsimony, using RAxML ver. 7.0.4 and TNT ver. 1.1, respectively. The data were partitioned *a priori* on the basis of gene identity (ND4, Cyt-b).

A maximum likelihood analysis was conducted using the program RAxML ver. 7.0.4. (Stamatakis et al. 2008) as implemented on the Cipres portal ver. 2.0, was used for the tree search and the bootstrap analysis on the two concatenated gene fragments.

The data were further analyzed by using the maximum parsimony optimality criterion. In order to reduce computational time, the software program TNT ver. 1.1 (Goloboff et al. 2008) was used. Other authors have found significant differences in speed between TNT and other programs, such as PAUP* and NONA/Pee-Wee (Goloboff 1994a, 1994b), and it has been effectively utilized to infer phylogenetic relationships in similar studies (e.g., Hedin and Bond 2006, Monaghan et al. 2007, Benjamin et al. 2008). For example, TNT was able to find optimum trees for a 228-taxa dataset by McMahon and Sanderson (2006) in an average time of 30 minutes, which took PAUP* 1700 hours of computational time using the ratchet (Goloboff et al. 2008). TNT is made highly efficient by incorporating multiple approaches to finding global optima, including the ratchet (Nixon 1999), tree-drifting (Goloboff 1999), tree-fusing (Goloboff 1999), and sectorial searches (Goloboff 1999). The two mitochondrial gene fragments were concatenated and analyzed both separately and combined. In each of these analyses, 1000 random addition sequence replicates employing all four algorithms with default parameters were first used to find global optima, and were subsequently driven with a score bound in an attempt to find more parsimonious trees. In order to estimate clade support, non-parametric bootstrap values (Felsenstein 1985) were obtained from 1000 pseudo-replicates, in which optimal trees were found

using the new technology search under the same parameters as above. In addition, analyses using implied weighting were conducted in order to construct trees using differential character weighting (Goloboff 1993a). This approach is considered to be superior to successive weighting, because it implements an optimality criterion (maximum total fit, calculated as a function of homoplasy) to constructing trees and weighting characters (Goloboff 1993a). Implied weighting analyses were conducted for *K*-values =3 (default) for 500 replicates.

Average sequences divergence rates were estimated using the maximum composite likelihood model (Tamura et al. 2004) in MEGA5 (Tamura et al. 2011), with groups defined *a priori*, as determined by the results of the maximum likelihood and parsimony analyses. In order to maximize accuracy, samples for which only a single fragment was available and positions with less than 95% site coverage were omitted in the combined analysis of both gene fragments.

1.3 Results

1.3.1 Maximum Parsimony

For the combined data set, TNT recovered five most parsimonious trees, each with a total length of 588 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. Both the strict consensus tree and the 50% majority rule consensus tree (not shown here) indicate the presence of two monophyletic lineages within the ingroup. This was strongly supported by a high proportion of bootstrap replicates (figure 1.2). When implied weighting was incorporated in the analysis, eight most parsimonious trees were recovered, each with a best score of 21.49. The resulting consensus (figures 1.2) was congruent with that of the equally weighted analyses, but support from bootstrap proportions was marginally higher. When the fragment of cyt-b was analyzed by itself, TNT recovered eight most parsimonious trees, each with a length of 369 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. Both the strict consensus and the 50% majority rule consensus (not shown here) indicate the presence of two monophyletic lineages within the ingroup. This was strongly

supported by a high proportion of bootstrap replicates (figure 1.2). When implied weighting was employed in the analysis, six most parsimonious trees were recovered, each with a best score of 15.85. The resulting consensus was congruent with that of the equally weighted analyses, but support from bootstrap proportions was marginally higher.

When the fragment of ND4 was analyzed individually, TNT recovered two most parsimonious trees, each with a length of 208 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. Both the strict consensus and the 50% majority rule consensus indicate the presence of two monophyletic lineages within the ingroup. This was strongly supported by a high proportion of bootstrap replicates. When implied weighting was employed, two most parsimonious trees were recovered, each with a best score of 5.15. The resulting consensus was congruent with that of the equally weighted analyses, but support from bootstrap proportions was marginally higher.

1.3.2 Maximum Likelihood

For the combined data set, the analysis involved 68 nucleotide sequences, with a total of 1774 positions. The tree with the highest log likelihood value (-5858.77) corroborates the presence of two distinct phylogeographic lineages (figure 1.2), which is further supported by high bootstrap proportions.

The separate analysis of the fragment of cyt-b included 65 nucleotide sequences, with a total of 1093 positions. The tree with the highest log likelihood value (-3652.69) supports the presence of two distinct phylogeographic lineages. Support from bootstrap proportions is strong for the monophyly of the southern clade, whereas the bootstrap consensus shows low support for the monophyly of the northern clade. The analysis of the fragment of ND4 included 61 nucleotide sequences, with a total of 681 positions. The tree with the highest log likelihood value (-2004.21) supports the presence of the two distinct phylogeographic lineages recovered in the previous

analyses. Support from bootstrap proportions is strong for the monophyly of the northern clade, but limited for that of the southern clade.

1.3.3 Sequence Divergence

After the omission of incomplete sequences and positions with less than 95% site coverage, the final data set included 54 sequences, with a total of 1226 positions. The divergence rates indicate a significant average divergence between the sequences of samples in the southern clade and those in the northern clade (7.2%; see table 1.3). Average divergences between the southern clade and its KwaZulu-Natal subclade were relatively small (1.1%), as were those between the northern clade and its Botswana and Mozambique subclades (2.9% and 3.0%, respectively).

Table 1.2. Estimates of net evolutionary divergence. Values represent the numbers of base substitutions per site from estimation of net average between groups.

Clade	**Southern Clade**	**KwaZulu-Natal Subclade**	**Northern Clade**	**Botswana Subclade**	**Mozambique Subclade**
Southern Clade					
KwaZulu-Natal Subclade	0.011				
Northern Clade	0.072	0.071			
Botswana Subclade	0.070	0.067	0.029		
Mozambique Subclade	0.063	0.065	0.030	0.029	
Outgroup (*Philothamnus*)	0.223	0.226	0.231	0.231	0.224

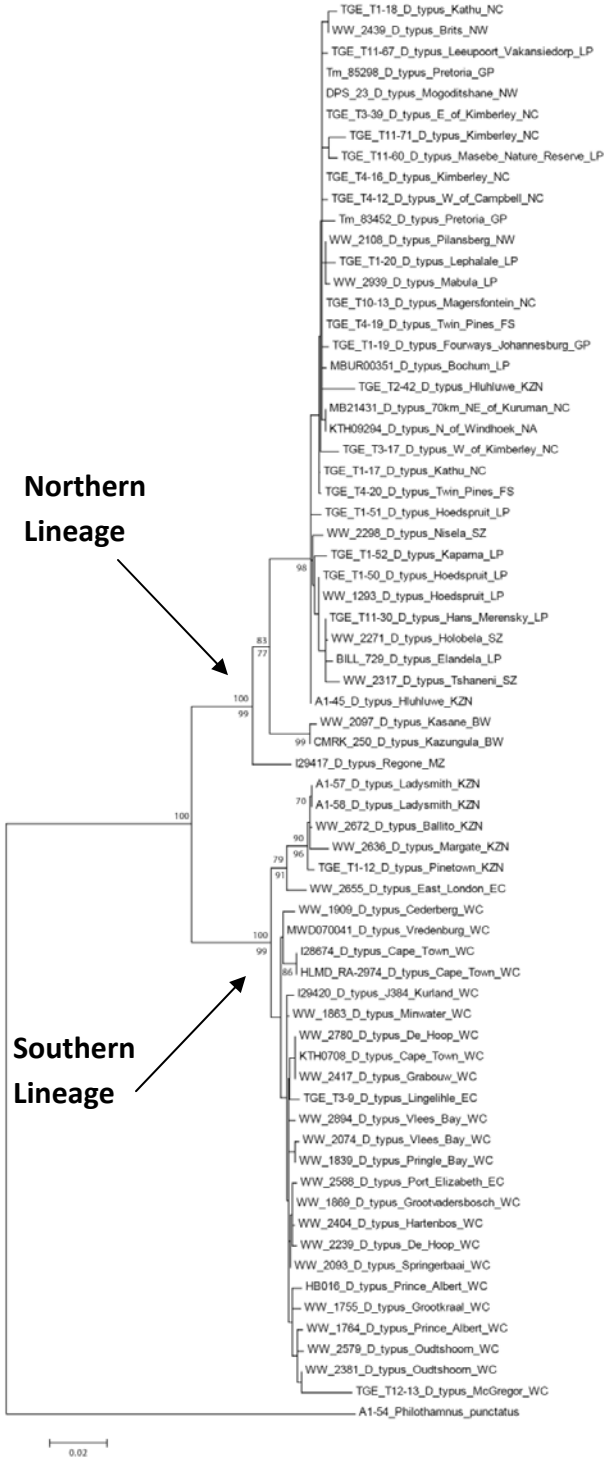

Figure 1.2. Maximum likelihood tree with the highest log likelihood (-5858.76) of the combined data set. Numbers represent bootstrap proportions from 1000 bootstrap pseudo-replicates from the parsimony (above nodes) and likelihood (below nodes) analyses.

1.4 Discussion

1.4.1 Biogeographic Lineages

Based on these results, there are two distinct maternal clades present within Southern Africa, a southern clade and a northern clade. The southern clade includes samples from Vredenburg and Cape Town in the Western Cape of South Africa (MWD070041, HLMD RA-2974, I28674, and KTH0708), and east to Ballito and Ladysmith in KwaZulu-Natal, South Africa (WW 2672, and A1-57 and A1-58, respectively). Within the southern clade, specimens from East London, Eastern Cape (WW 2655), and those from KwaZulu-Natal (TGE T1-12, WW 2672, WW 2636, A1-57, A1-58) are contained within a subclade that is sister to a clade containing all other samples, including those from throughout the Western Cape and east to Cradock (approximately 225 km northwest of East London, Eastern Cape). The northern most samples are those from central KwaZulu-Natal (A1-57, A1-58). Support from bootstrap proportions is strong in the combined analysis (figure 1.2), but inconsistent in the analyses of the individual gene fragments (not shown here).

The northern clade includes samples from Kathu in the Northern Cape of South Africa (TGE T1-17, TGE T1-18), east to the Hoedspruit region of Limpopo, South Africa (BILL 729, TGE T1-50, TGE T1-51, TGE T1-52, and WW 1293). It further includes samples from southeastern South Africa through Swaziland (WW 2271, WW 2298, and WW 2317) and south into Hluhluwe, KwaZulu-Natal (A1-45 and TGE T2-42). The northern most samples are those from north of Windhoek, Namibia (KTH 09294) and from southwest of Regone, Mozambique (I29417). Specimens from northern Botswana (CMRK 250, WW 2097) form a subclade that is sister to a clade that contains all other northern samples. Support from bootstrap proportions for this subclade is strong in all analyses (figure 1.2).

1.4.2 Geographic Isolation

The northern and southern lineages are largely geographically separated by the several major biogeographic barriers, including the Great Escarpment, a 3000-km long mountain range

that is located 50-300 km inland from the coast (Brink 1992), and extends from Angola and Namibia south to South Africa, where it runs eastward, roughly parallel to the coast, to Swaziland, Mozambique, and north to Zimbabwe. The mountains of the Great Escarpment range from 1500 to 3500 m in elevation (Ollier and Marker 1985), and is bordered by the Highveld in the north, an area of subtropical and temperate grasslands at elevations between 1500 and 2100 m. The history of the uplift of the Great Escarpment is complex and difficult to reconstruct (Brink 1992), but there is some evidence that it is an Upper Paleozoic feature (Erlank et a. 1984) that underwent subsequent erosion toward the coast in the Early to Late Cretaceous (Brink 1992). Historical records and distribution maps show that *D. typus* appear to be largely absent in the region immediately northwest of the Great Escarpment (Broadley 1983, Branch 1988, 1998, Marais 2004), an area known as the Great Karoo. That area is one of marked aridity, and parts of this may represent an ecological barrier to *D. typus*.

In the eastern part of South Africa, the northern and southern clades approach one another in central KwaZulu-Natal. Samples from as far east as Ballito (WW 2672) and Ladysmith (A1-57, A1-58) are part of the southern clade, whereas samples from Hluhluwe (A1-45, TGE T2-42), only approximately 250 km east of Ladysmith, fall into the northern clade. Repeated attempts were made to collect samples from within the potential area of contact in central KwaZulu-Natal, but additional samples from that area of interest were unable to be obtained, despite extensive collecting attempts. There are no obvious topographic or ecological barriers present in those areas that would maintain the separation of the two distinct lineages that we detected in this study (figure 1.3).

The Cape Floristic Region in the western part of South Africa is known for its high levels of diversity and endemism (Goldblatt 1997, Linder 2003, 2005, Cowling et al. 2009), in particular for reptiles (Mittermeier et al. 2004, Turner et al. 2007). A number of lizard clades are known to converge geographically in the western part of that region (e.g., Daniels et al. 2004, Swart et al. 2009), and there is some evidence that similar patterns occur in snakes (Wuster, pers. comm.). These patterns of diversification are thought to be a result of Plio-Pleistocene climatic shifts

(Tolley et al. 2009), which resulted in the regression of the forest biome in the area (Linder et al. 1992, Scott 1995, Linder 2003). However, the results of this study are incongruent with those phylogeographic patterns. Instead, samples from the Cape Floristic Region are nested within the southern clade, which also samples from as far east as Cradock, Eastern Cape (TGE T3-9).

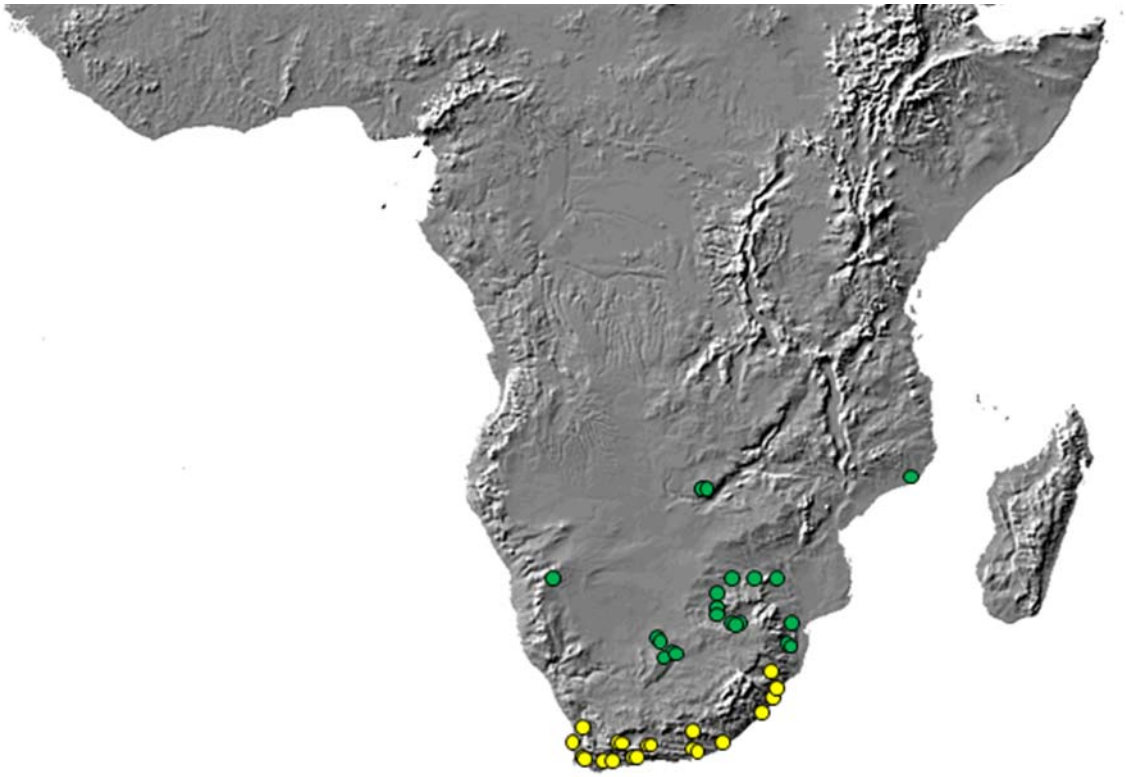

Figure 1.3. Geographic distribution of clades. Legend: yellow circles = southern clade; green circles = northern clade.

1.4.3 Patterns Observed in Other Studies

Previous studies have identified several factors that may have acted as drivers of the contemporary diversity of southern Africa. Based on mitochondrial sequence data, Tolley et al. (2008) concluded that the dwarf chameleon diversity within the South African diversity hotspots appears to be primarily the result of a Late Pliocene radiation, associated with shifts in habitat types that in turn correspond with shifts from C_3 to C_4 environments, and later with the development of the winter-rainfall regime. Similar results of diversification being driven by climatic changes in the Pliocene have been reported in a number of vertebrates and invertebrates (e.g., Matthee and Flemming 2002, Daniels et al. 2004). Previous investigations into the phylogeographical structure of southern African taxa have identified at least three major monophyletic clades (Matthee and Flemming 2002, Smit et al. 2007, Swart et al. 2009), based on mitochondrial markers. The distribution of those clades corresponds broadly to patterns observed in other vertebrates, with vicariance being the likely driving force behind the observed patterns (Lamb and Bauer 2000, Matthee and Flemming 2002). However, the patterns observed in the results of this paper are largely incongruent with the previously mentioned studies, which may simply be an artifact of the different types of habitat preferences by *D. typus*, as compared to the taxa investigated in the aforementioned studies.

1.5 Conclusions

The results indicate the presence of two distinct evolutionary lineages that are present in southern Africa. The southern clade is confined to the southern parts of South Africa, including the Western and Eastern Cape, and east to central KwaZulu-Natal. The northern clade is largely geographically separated from the southern clade by the mountains of the Great Escarpment and the associated habitat, and is distributed throughout the northern parts of South Africa, as well as Namibia, Swaziland, and Mozambique. The two clades approximate one another in KwaZulu-Natal, where they are found within 250 km of one another, without any obvious biogeographic barriers. The sequence divergence rates indicate that the two lineages may constitute two

different taxa, and further emphasize the need for a complete systematic revision of the genus using both mitochondrial and nuclear data. Future efforts should involve additional sampling in the area of potential contact between the two clades, as well as those areas north of the localities sampled in this study.

CHAPTER 2

MOLECULAR SYSTEMATICS OF DISPHOLIDINE SNAKES (SERPENTES: COLUBRIDAE)

2.1 Introduction

2.1.1 Introduction to Dispholidine Colubrids

Dispholidine snakes form a hypothesized clade of arboreal African colubrines that includes the genera *Dispholidus* Duvernoy 1832, *Thelotornis* A. Smith 1849, *Thrasops* Hallowell 1852, *Rhamnophis* Guenther 1862, and *Xyelodontophis* Broadley & Wallach 2002. Bourgeois (1968) first hypothesized the monophyly of this group, and concluded that it formed the subfamily Dispholidinae Bourgeois 1968 that was closely related to the subfamilies Philothamninae Bourgeois 1968 (genera *Hapsidophrys* Fischer 1856 and *Philothamnus* A. Smith 1840) and Boiginae Stejneger 1907 (genera *Boiga* Fitzinger 1826, *Crotaphopeltis* Fitzinger 1843, *Dipsadoboa* Günther 1858, and *Telescopus* Wagler 1830). However, with some exceptions (e.g., Welch 1982), that classification was not subsequently accepted until they were reinstated the group as a colubrine tribe, Dispholidini Broadley and Wallach 2002. The latter authors included the aforementioned genera in this tribe, in which twelve species are currently recognized. The monotypic genus *Dispholidus* includes only *D. typus*, which has the single largest known distribution range of any dispholidine snake, ranging through much of sub-Saharan Africa. *Rhamnophis* includes two species, *R. aethiopissa* Günther 1862 and *R. batesii* Boulenger 1908, which are primarily found in West and Central Africa. The genus *Thelotornis* contains four species, *The. capensis* A. Smith 1849, *The. kirtlandii* Hallowell 1844*, The. mossambicanus*

Bocage 1895, and *The. usambaricus* Broadley 2001. *Thelotornis kirtlandii* is a West African species, *The. capensis* occurs in Southern Africa, and *The. mossambicanus* and *The. usambaricus* are found in East Africa. The genus *Thrasops* also contains four species, including *Thr. flavigularis* Hallowell 1852, *Thr. jacksonii* Günther 1895, *Thr. occidentalis* Parker 1940, and *Thr. schmidti* Loveridge 1936. *Thrasops flavigularis* and *Thr. jacksonii* are mostly found in Central Africa, *Thr. occidentalis* occurs only in West Africa, and *Thr. schmidti* is restricted to a small area in Kenya. The monotypic genus *Xyelodontophis* contains the recently described *X. uluguruensis* Broadley and Wallach 2002, which is known only from Tanzania.

Proposed synapomorphies for this group sensu Bourgeois (1968) include a forked ectopterygoid with a resulting ectopterygoid-maxillary hole, a large optic fenestra, an interorbital vacuity, the presence of three large posterior maxillary teeth that are separated from 18 or fewer small anterior teeth by a diastema, a single loreal scale, at least one preocular scale, and the presence of narrow dorsal scales with small apical cavities (Bourgeois 1968).

2.1.2 Taxonomic History and Systematics

Bourgeois (1968) also hypothesized that the genus *Rhamnophis* represents the ancestral lineage within this group, based on the retention of a number of primitive morphological characters. The same author considered the genus *Thrasops* to be more derived, as made evident by the marked shortening of the palatomaxillary. The genera *Dispholidus* and *Thelotornis* were considered to be the most derived members of this group (Bourgeois 1968), based on dentition and pupil structure. In contrast, Eimermacher (2007) found that the genus *Thrasops* represents the basal lineage, with the genus *Rhamnophis* being the sister taxon to the genus *Dispholidus*.

The validity of the genus *Rhamnophis* has also been controversial. Some authors (e.g. Loveridge 1957, Leston and Hughes 1968, Hughes and Barry 1969, Pitman 1974, Spawls 1978, Hughes 1983, Obst et al. 1984, 1988, Coborn 1991, Fischer and Hinkel 1992, Frank and Ramus

1995, Trape and Roux-Estève 1995, Chippaux 1999, 2001, 2006, Pauwels et al. 2002a, 2002b) have treated *Rhamnophis* as a synonym of *Thrasops*, while others (e.g., Dowling and Duellman 1978, Ferrarezzi 1994) have considered both of these genera to be members of the tribe Philothamnini Dowling and Duellman 1978. Perret (1961) considered both *Thrasops* and *Rhamnophis* to be two distinct colubrine genera (along with *Philothamnus* and *Hapsidophrys*), whereas both *Dispholidus* and *Thelotornis* were contained within the subfamily Boiginae. In a review of dispholidine snakes, Broadley and Wallach (2003) concluded that *Thrasops* and *Rhamnophis* were basal taxa within the tribe Dispholidini, which was subsequently rejected (Eimermacher 2007) based on their differential placement within that group. Broadley and Wallach (2003) also described a new species, *Xyelodontophis uluguruensis* from the Uluguru Mountains in Tanzania, which was thought to be transitional between the genera *Rhamnophis* and *Thelotornis* (Broadley and Wallach 2003). Eimermacher (2007) concluded that the genera *Thelotornis* and *Xyelodontophis* were sister taxa that were in turn sister to the *Dispholidus*-*Rhamnophis* clade.

The genus *Dispholidus* has a volatile taxonomic history, and has been treated as polytypic in the past (e.g., Laurent 1955, 1956; Witte 1962; Golay et al. 1993). It was first described as *Bucephalus typus* by Andrew Smith (1828) from the eastern districts of South Africa. In the same paper, Smith (1828) also described *Bucephalus jardineii* from Cape Town, *B. gutturalis* from the forests of the eastern districts of South Africa, and *B. bellii* from the eastern districts of South Africa. Smith (1828) considered those taxa to be distinct species, based on their external coloration. While *B. typus* was described as being “uniform lightish brown” dorsally and “silvery gray speckled with brown” ventrally (Smith 1828), *B. jardineii* was described as being blackish green dorsally and laterally, and yellow ventrally “with a black line extending along the posterior margin of each abdominal plate” (Smith 1828). *Bucephalus gutturalis* was described as being greenish brown dorsally and laterally, with most scales containing a greenish white mark. Ventrally, *B. gutturalis* is colored “light grayish brown mottled with dark greenish brown” (Smith

1828). This taxon also possesses a transverse orange-colored throat band, and is referred to as the yellow throated boom-slang (Smith 1828). *B. bellii* is blackish green dorsally, with a greenish white dot on most scales, and yellowish green ventrally, with the posterior edge of each scale being marked by a blackish line (Smith 1828). Duvernoy (1832) described *Dispholidus lalandii* from the Cape of Good Hope, and Schlegel (1837) described *Dendrophis colubrina* from Rondesbosch, South Africa. In 1838, Smith described *B. viridis* from Old Latakoo, South Africa, and later *B. capensis* (A. Smith 1842) from the Cape Province of South Africa. Bianconi (1948) described *Dendrophis pseudodipsas* from Inhambane, Mozambique, and Mertens (1937) described *Thrasops jacksonii mossambicanus* from the same locality. Laurent (1955) described two new subspecies, including *D. t. kivuensis* from Uvira, Democratic Republic of Congo, and *D. t. punctatus* from Dundo, Angola, both of which were distinguished by adult coloration and subcaudal scale counts. Finally, Perret (1961) described *D. t. occidentalis* from Cameroon. In addition, *D. typus* from Pemba Island, Tanzania was hypothesized by Hughes to represent a distinct species within that genus (cited by Broadley and Wallach 2003). Nonetheless, *Dispholidus* is currently considered to be a monotypic genus, despite some anecdotal evidence suggesting otherwise (Broadley and Wallach 2003). The systematic relationships of this genus are further complicated by its widespread distribution and extensive morphological polymorphism.

Consequently, the evolutionary relationships of this group remain largely unknown, and have never been investigated using a molecular approach. Therefore, the objective of this study was to elucidate the evolutionary relationships of the Dispholidini, and to address several associated questions:

1. Do dispholidine colubrids form a monophyletic group?
2. Which group is basal within dispholidines?
3. Is *Thrasops* paraphyletic to the exclusion of *Rhamnophis*?
4. Does *Dispholidus* contain multiple evolutionary lineages?

2.2 Materials and Methods

2.2.1 Taxonomic Sampling

I used a total of 155 samples of dispholidine snakes, including 9 of the 12 species that are currently contained within this group. Tissue samples of *Thr. flavigularis*, *Thr. occidentalis*, and *Thr. schmidti* were unavailable, despite numerous attempts made to obtain samples from areas near the corresponding type localities. However, multiple samples of *Thr. jacksonii* were included in this study as representatives for that genus. Table 2.1 provides a complete list of the specimens utilized in this study, including the associated sample ID, taxon, and locality.

In order to test the monophyly of Dispholidini, a substantial amount of outgroups (Table 4) were utilized, many of which have been identified as potential sister taxa to dispholidine snakes. The genera *Philothamnus* and *Hapsidophrys* have long been considered close relatives of dispholidine snakes (e.g., Bourgeois 1968, Lawson et al. 2005), and are included in this study with 21 samples, representing 10 of the 22 currently accepted species within that group. In a study by Kelly et al. (2009), *D. typus* was the lone dispholidine taxon used, and was found to be sister to a clade consisting of *Boiga dendrophila*, *Lycodon* sp. and *Dinodon* sp. In another study, *D. typus*, *The. capensis*, and *Thr. jacksonii* formed a clade that was sister to a large group that also included *Boiga pulverulenta*, *Dasypeltis scabra*, *Dasypeltis atra*, *Dipsadoboa unicolor*, *Crotaphopeltis tornieri*, *Telescopus fallax*, and *Dinodon semicarinatum* (Pyron et al. 2011). In that same study, representatives of the genera *Philothamnus* and *Hapsidophrys* were found to be rather distantly related to dispholidine snakes, being sister to representatives of the genus *Coelognathus*.

Consequently, I included samples of *Boiga dendrophila*, *Boiga* (*Toxicodryas*) *pulverulenta*, *Crotaphopeltis hotamboeia*, *Crotaphopeltis tornieri*, *Dasypeltis atra*, *Dasypeltis medici*, *Dasypeltis scabra*, *Dinodon rufozonatum, Dipsadoboa unicolor, Lycodon zawi*, *Lycodon capucinus*, *Telescopus semiannulatus*, *Telescopus fallax*, and *Toxicodryas blandingii* and in this

data set. A total of 46 outgroup samples were included in this study, for a combined total of 176 samples in the final data set (table 2.1).

Table 2.1. Specimens used in molecular analysis. Sample IDs represent field, specimen, or GenBank accession numbers, and the locality data include the nearest town, province, and country of origin in standard two-letter code. Legend: TGE = T. Eimermacher; CMRK = C. Kelly; ELI & EBG = E. Greenbaum; BILL = B. Branch; WW = W. Wüster; all other acronyms are of unknown meaning.

Sample ID	Taxon	Locality Data	ND4	Cyt-b	C-MOS	NT3
U49393	*Boiga dendrophila*	no locality data available	U49393	N/A	N/A	N/A
NV	*Boiga dendrophila*	no locality data available	N/A	AF471089	AF471128	N/A
AF428022	*Crotaphopeltis hotamboeia*	Mount Rungwe, Rungwe Mission, TZ	N/A	AF428022	N/A	N/A
AF428036	*Crotaphopeltis tornieri*	no locality data available	N/A	AF428036	N/A	N/A
CAS 168957	*Crotaphopeltis tornieri*	no locality data available	N/A	AF471093	AF471112	N/A
MBUR01603	*Dasypeltis* sp.	no locality data available	JX301537	N/A	N/A	N/A
CAS 201641	*Dasypeltis atra*	no locality data available	N/A	AF471065	AF471136	N/A
I29445	*Dasypeltis medici*	Tupito camp, Moma, MZ	JX301540	JX317028	JX301275	JX301407
I18469	*Dasypeltis scabra*	Satara Camp, Kruger NP, Mpumalanga, ZA	JX301538	JX317026	JX301273	JX301405
I29431	*Dasypeltis scabra*	no locality data available	JX301539	JX317027	JX301274	JX301406
AY235729	*Dasypeltis scabra*	no locality data available	N/A	AY235729	N/A	N/A
CIB 098274	*Dinodon rufozonatum*	no locality data available	JF827649	JF827672	JF827695	N/A
CAS 201660	*Dipsadoboa unicolor*	no locality data available	N/A	AF471062	AF471139	N/A
AF428037	*Dipsadoboa unicolor*	no locality data available	N/A	AF428037	N/A	N/A
EBG 2673	*Dispholidus typus*	North of Uvira, South Kivu, CD	JX301474	JX316965	JX301211	JX301347
ELI 79	*Dispholidus typus*	ca. 5km West of Kabongo, Katanga, CD	JX301479	JX316970	JX301216	JX301351
CMRK 103	*Dispholidus typus*	30km North of Butare, BW	JX301475	JX316966	JX301212	JX301348
CMRK 250	*Dispholidus typus*	Kazungula, BW	JX301490	JX316981	JX301227	JX301362
CMRK 286	*Dispholidus typus*	20km Southwest of Morogoro, TZ	JX301491	JX316982	JX301228	JX301363

Table 2.1 – *Continued*

WW 1438	*Dispholidus typus*	Watamu, KE	JX301481	JX316972	JX301218	JX301353
WW 1439	*Dispholidus typus*	Watamu, KE	JX301482	JX316973	JX301219	JX301354
WW 1293	*Dispholidus typus*	Hoedspruit, Limpopo, ZA	JX301457	JX316950	JX301196	JX301334
DPS 23	*Dispholidus typus*	2km North of Mogoditshane, Northwest, ZA	N/A	JX317043	JX301290	JX301416
A1-45	*Dispholidus typus*	Hluhluwe, KwaZulu-Natal, ZA	JX301489	JX316980	JX301226	JX301361
A1-57	*Dispholidus typus*	Ladysmith, KwaZulu-Natal, ZA	JX301448	JX316942	JX301188	JX301326
A1-58	*Dispholidus typus*	Ladysmith, KwaZulu-Natal, ZA	JX301449	JX316943	JX301189	JX301327
FMNH 250444	*Dispholidus typus*	8km NNW of Armani, Muheza Distr., TZ	U49302	N/A	N/A	N/A
HLMD RA-2974	*Dispholidus typus*	Cape Town, Western Cape, ZA	N/A	AY188012	AY187973	N/A
WW 1755	*Dispholidus typus*	Grootkraal, Western Cape, ZA	JX301428	JX316922	JX301169	JX301309
WW 1764	*Dispholidus typus*	Prince Albert, Western Cape, ZA	JX301430	JX316924	N/A	JX301311
WW 1839	*Dispholidus typus*	Pringle Bay, Western Cape, ZA	JX301438	JX316932	JX301178	JX301318
WW 1863	*Dispholidus typus*	Minwater, Western Cape, ZA	JX301439	JX316933	JX301179	JX301319
WW 1869	*Dispholidus typus*	Grootvadersbosch, Western Cape, ZA	JX301440	JX316934	JX301180	JX301320
WW 1909	*Dispholidus typus*	Cederberg, Western Cape, ZA	JX301441	JX316935	JX301181	JX301321
WW 2074	*Dispholidus typus*	Vleesbaai, Western Cape, ZA	JX301427	JX316921	JX301168	JX301308
WW 2093	*Dispholidus typus*	Springerbaai, Western Cape, ZA	JX301442	JX316936	JX301182	JX301322
WW 2097	*Dispholidus typus*	Kasane, BW	JX301485	JX316976	JX301222	JX301357
WW 2108	*Dispholidus typus*	Pilanesberg National Park, Northwest, ZA	JX301463	JX316955	JX301201	JX301339
WW 2239	*Dispholidus typus*	De Hoop, Western Cape, ZA	JX301435	JX316929	JX301175	JX301315
WW 2271	*Dispholidus typus*	10km East of Mkhaya Game Reserve, SZ	JX301459	JX316952	JX301198	JX301336
WW 2298	*Dispholidus typus*	Niseal Nature Reserve, SZ	JX301462	JX316954	JX301200	JX301338
WW 2317	*Dispholidus typus*	Tjaneni, SZ	JX301486	JX316977	JX301223	JX301358
WW 2381	*Dispholidus typus*	Oudtshoorn, Western Cape, ZA	JX301431	JX316925	JX301171	JX301312
WW 2404	*Dispholidus typus*	Hartenbos, Western Cape, ZA	JX301432	JX316926	JX301172	N/A
WW 2417	*Dispholidus typus*	Grabouw, Western Cape, ZA	JX301446	JX316940	JX301186	JX301324

Table 2.1 – *Continued*

WW 2439	*Dispholidus typus*	8km North of Brits, North-West, ZA	JX301487	JX316978	JX301224	JX301359
TGE T1-12	*Dispholidus typus*	Pinetown, KwaZulu-Natal, ZA	JX301434	JX316928	JX301174	JX301314
TGE T1-17	*Dispholidus typus*	Kathu, Northern Cape, ZA	JX301451	JX316944	JX301190	JX301328
TGE T1-18	*Dispholidus typus*	Kathu, Northern Cape, ZA	JX301452	JX316945	JX301191	JX301329
TGE T1-19	*Dispholidus typus*	Fourways Johannesburg, Gauteng, ZA	JX301453	JX316946	JX301192	JX301330
TGE T1-20	*Dispholidus typus*	Lephalale, Limpopo, ZA	JX301454	JX316947	JX301193	JX301331
TGE T1-50	*Dispholidus typus*	Raptor's View Estate, Hoedspruit, Limpopo, ZA	JX301455	JX316948	JX301194	JX301332
TGE T1-51	*Dispholidus typus*	Raptor's View Estate, Hoedspruit, Limpopo, ZA	JX301456	JX316949	JX301195	JX301333
TGE T1-52	*Dispholidus typus*	Kapama River Lodge, Limpopo, ZA	JX301458	JX316951	JX301197	JX301335
TGE T2-42	*Dispholidus typus*	Hluhluwe, KwaZulu-Natal, ZA	JX301464	JX316956	JX301202	JX301340
TGE T3-9	*Dispholidus typus*	~8km South of Lingelihle, Eastern Cape, ZA	JX301437	JX316931	JX301177	JX301317
TGE T3-17	*Dispholidus typus*	~37km West of Kimberley, Northern Cape, ZA	JX301465	JX316957	JX301203	JX301341
TGE T3-39	*Dispholidus typus*	East of Kimberley, Northern Cape, ZA	N/A	JX317038	JX301285	N/A
TGE T4-12	*Dispholidus typus*	~16km West of Campbell, Northern Cape, ZA	N/A	JX317040	JX301287	N/A
TGE T4-19	*Dispholidus typus*	Twin Pines Farm, Free State, ZA	JX301467	JX316958	JX301204	N/A
TGE T4-20	*Dispholidus typus*	Twin Pines Farm, Free State, ZA	JX301468	JX316959	JX301205	JX301342
WW 2579	*Dispholidus typus*	Oudtshoorn, Western Cape, ZA	JX301429	JX316923	JX301170	JX301310
WW 2588	*Dispholidus typus*	Port Elizabeth, Eastern Cape, ZA	JX301426	JX316920	JX301167	JX301307
WW 2636	*Dispholidus typus*	Margate, KwaZulu-Natal, ZA	JX301424	JX316918	JX301165	JX301305
WW 2655	*Dispholidus typus*	East London, Eastern Cape, ZA	JX301422	JX316916	JX301163	JX301304
WW 2672	*Dispholidus typus*	Ballito, KwaZulu-Natal, ZA	JX301433	JX316927	JX301173	JX301313
WW 2780	*Dispholidus typus*	De Hoop Nature Reserve, Western Cape, ZA	JX301425	JX316919	JX301166	JX301306

Table 2.1 – *Continued*

WW 2894	*Dispholidus typus*	Fransmanshoek, Western Cape, ZA	JX301436	JX316930	JX301176	JX301316
WW 2939	*Dispholidus typus*	Mabula Game Reserve, Limpopo, ZA	JX301461	JX316953	JX301199	JX301337
WW 3101	*Dispholidus typus*	Gede, KE	JX301423	JX316917	JX301164	JX301461
WW 3104	*Dispholidus typus*	Gede, KE	JX301483	JX316974	JX301220	JX301355
I29420	*Dispholidus typus*	~14km Southeast of Kurland, Western Cape, ZA	JX301447	JX316941	JX301187	JX301325
I28674	*Dispholidus typus*	Cape Town, Western Cape, ZA	JX301445	JX316939	JX301185	JX301323
I29417	*Dispholidus typus*	~25km Southwest of Regone, MZ	JX301488	JX316979	JX301225	JX301360
TGE T10-13	*Dispholidus typus*	Magersfontein, Northern Cape, ZA	N/A	JX317037	JX301284	N/A
TGE T10-75	*Dispholidus typus*	St. Lucia, KwaZulu-Natal, ZA	N/A	N/A	JX301302	N/A
TGE T11-60	*Dispholidus typus*	Northwest of Masebe NR, Limpopo, ZA	JX301471	JX316962	JX301208	JX301344
TGE T11-67	*Dispholidus typus*	Leeupoort Vakansiedorp, Limpopo, ZA	N/A	JX317042	JX301289	N/A
TGE T11-71	*Dispholidus typus*	East of Kimberley, Northern Cape, ZA	JX301470	JX316961	JX301207	JX301343
TGE T12-13	*Dispholidus typus*	~5km East of McGregor, Western Cape, ZA	N/A	JX317044	JX301291	JX301417
TGE T11-30	*Dispholidus typus*	West of Hans Merensky NR, Limpopo, ZA	N/A	JX317041	JX301288	N/A
MWD070041	*Dispholidus typus*	Southeast of Vredenburg, Western Cape, ZA	JX301450	N/A	N/A	N/A
TB39	*Dispholidus typus*	~30km North of Suarimo, AO	JX301476	JX316967	JX301213	N/A
MB21431	*Dispholidus typus*	~70km Northeast of Kuruman, Northern Cape, ZA	JX301466	N/A	N/A	N/A
MBUR00351	*Dispholidus typus*	~11km Northwest of Bochum, Limpopo, ZA	JX301460	N/A	N/A	N/A
HB016	*Dispholidus typus*	Prince Albert, Western Cape, ZA	JX301443	JX316937	JX301183	N/A
KTH0708	*Dispholidus typus*	Cape Town, Western Cape, ZA	JX301444	JX316938	JX301184	N/A
KTH09294	*Dispholidus typus*	~23km North of Windhoek, NA	JX301484	JX316975	JX301221	JX301356

Table 2.1 – *Continued*

BILL 634	*Dispholidus typus*	Klein's Camp, Mara, TZ	N/A	JX317033	JX301280	JX301413
BILL 635	*Dispholidus typus*	Port Elizabeth, Eastern Cape, ZA	N/A	N/A	JX301294	JX301419
BILL 636	*Dispholidus typus*	Mount Mulanje, MW	N/A	N/A	JX301295	JX301420
BILL 729	*Dispholidus typus*	Elandela Reserve, Limpopo, ZA	JX301469	JX316960	JX301206	N/A
BILL 690	*Dispholidus typus*	Camp Chiri, AO	JX301477	JX316968	JX301214	JX301349
Tm 85298	*Dispholidus typus*	Pretoria, Gauteng, ZA	N/A	JX317036	JX301283	N/A
ssT	*Dispholidus typus*	no locality data available	N/A	N/A	JX301296	N/A
Tm 83452	*Dispholidus typus*	Plot 54 Farm Buffelsdrift, Pretoria dist., Gauteng, ZA	N/A	JX317035	JX301282	N/A
Tm 83455	*Dispholidus typus*	Plot 54 Farm Buffelsdrift, Pretoria dist., Gauteng, ZA	N/A	N/A	JX301300	N/A
RSP 219	*Dispholidus typus*	Kammanasie, Western Cape, ZA	N/A	N/A	JX301303	N/A
JM 1857	*Dispholidus typus*	Kalumbila, ZM	N/A	JX317034	JX301281	JX301414
JM 1858	*Dispholidus typus*	Kalumbila, ZM	JX301478	JX316969	JX301215	JX301350
ELI 1316	*Dispholidus typus*	road between Fizi and Mokanga, South Kivu, CD	JX301473	JX316964	JX301210	JX301346
ELI 1434	*Dispholidus typus*	Kihungwe village, South Kivu, CD	JX301480	JX316971	JX301217	JX301352
ELI 1280	*Dispholidus typus*	5 km West of Rutegama, BI	JX301472	JX316963	JX301209	JX301345
TGE T4-16	*Dispholidus typus*	~0.5km SE of Northern Cape-Free State border, ZA	N/A	JX317039	JX301286	JX301415
FJ434104	*Hapsidophrys smaragdina*	no locality data available	N/A	N/A	N/A	FJ434104
CAS 219171	*Hapsidophrys smaragdina*	ST	N/A	DQ112075	DQ112078	N/A
AF544691	*Hapsidophrys smaragdina*	no locality data available	N/A	N/A	AF544691	N/A
FJ434104	*Hapsidophrys smaragdina*	no locality data available	N/A	N/A	N/A	FJ434104
USNM 340053	*Lycodon capucinus*	Iloilo, Panay Province, PH	U49317	N/A	N/A	N/A
CAS 210323	*Lycodon zawi*	no locality data available	N/A	AF471040	AF471111	N/A

Table 2.1 – *Continued*

EGB 1921	*Philothamnus angolensis*	Kamango village, North Kivu, CD	JX301262	JX317016	JX301394	JX301525
CMRK 374	*Philothamnus battersbyi*	40km North of Addis Ababa, ET	JX301267	JX317021	JX301399	JX301531
EGB 1363	*Philothamnus carinatus*	Irangi, South Kivu, CD	JX301269	JX317022	JX301401	JX301533
PEM R5938	*Philothamnus carinatus*	Rabi complex, GA	N/A	FJ913498	N/A	N/A
CAS 201619	*Philothamnus heterodermus*	Bwindi Impenetrable National Park, Kabale Dist., UG	N/A	AF471055	AF471149	N/A
CMRK 228	*Philothamnus hoplogaster*	8km West of Sodwana Bay, KwaZulu-Natal, ZA	JX301263	JX317017	JX301395	JX301526
TGE T2-53	*Philothamnus hoplogaster*	~6km South of Ekuseni, KwaZulu-Natal, ZA	JX301266	JX317020	JX301398	JX301530
TGE T3-3	*Philothamnus natalensis*	Mhlume, SZ	JX301264	JX317018	JX301396	JX301528
CMRK M02	*Philothamnus n. occidentalis*	Salem, Eastern Cape Province, ZA	JX301268	N/A	JX301400	JX301532
A1-32	*Philothamnus punctatus*	Ngezi, Pemba Island, TZ	JX301271	JX317024	JX301403	JX301535
A1-54	*Philothamnus punctatus*	Ngezi, Pemba Island, TZ	JX301272	JX317025	JX301404	JX301536
EGB 1285	*Philothamnus ruandae*	Mugaba, South Kivu, CD	JX301261	JX317015	JX301393	JX301524
CER 983	*Philothamnus ruandae*	RW	JX301270	JX317023	JX301402	JX301534
CMRK 006	*Philothamnus semivariegatus*	Kewke, ZW	JX301260	JX317014	JX301392	JX301523
PEM R13214	*Philothamnus semivariegatus*	Moebase Camp, Zambezia Province, MZ	N/A	FJ913497	N/A	N/A
TGE T10-79	*Philothamnus sp.*	Road to Hluhluwe-Imfolozi GR, KwaZulu-Natal, ZA	N/A	N/A	JX301301	N/A

Table 2.1 – *Continued*

TGE T3-4	*Philothamnus sp.*	~3.5km S of RZA-Swaziland, ~1.5km N of MR24, SZ	JX301529	JX317019	JX301265	JX301397
EGB 1888	*Rhamnophis aethiopissa*	Muhobo village, North Kivu, CD	JX301254	JX317008	JX301386	JX301517
I29383	*Rhamnophis aethiopissa*	Rabi, GA	JX301520	JX317011	JX301257	JX301389
I29375	*Rhamnophis aethiopissa*	Rabi, GA	JX301518	JX317009	JX301255	JX301387
I29377	*Rhamnophis aethiopissa*	Rabi, GA	JX301519	JX317010	JX301256	JX301388
CRT 4257	*Rhamnophis aethiopissa*	CD	JX301516	JX317007	JX301253	JX301385
I29394	*Rhamnophis batesii*	Rabi, GA	JX301515	JX317006	JX301252	JX301384
I29322	*Telescopus fallax*	ca. 45 km East of Estehard, IR	JX301276	JX317029	JX301409	JX301542
HLMD S90	*Telescopus fallax*	Tasan, JO	N/A	AY188039	AY188000	N/A
LSUMZ 37967	*Telescopus fallax*	no locality data available	N/A	AF471043	AF471108	N/A
I29446	*Telescopus semiannulatus*	Tenbani Village, Moma, MZ	JX301277	JX317030	JX301410	JX301543
I28563	*Telescopus sp.*	JO	JX301408	N/A	N/A	JX301541
CMRK 32B	*Thelotornis capensis*	Mtunzini, Kwazulu-Natal, ZA	JX301505	JX316996	JX301242	JX301376
CMRK 337	*Thelotornis mossambicanus*	Bustoni Village, Mnt Kilimanjaro, KE/TZ border	JX301510	JX317001	JX301247	JX301379
MTSN 5470	*Thelotornis mossambicanus*	Mkalazi, Uzungwa Scarp F.R., Udzungwa Mtns, TZ	JX301509	JX317000	JX301246	N/A
LSUMZ 22073	*Thelotornis capensis*	no locality data available	N/A	AF471042	AF471109	N/A
TGE T1-32	*Thelotornis capensis*	~2.5km West of Hoedspruit, Limpopo, ZA	JX301498	JX316989	JX301235	JX301369

Table 2.1 – *Continued*

TGE T2-40	*Thelotornis capensis*	Hluhluwe, KwaZulu-Natal, ZA	JX301499	JX316990	JX301236	JX301370
TGE T2-41	*Thelotornis capensis*	Hluhluwe, KwaZulu-Natal, ZA	JX301500	JX316991	JX301237	JX301371
TGE T2-54	*Thelotornis capensis*	~2.5km Northeast of Ekuseni, KwaZulu-Natal, ZA	JX301501	JX316992	JX301238	JX301372
TGE T2-58	*Thelotornis capensis*	~2km S of Ngomane, SZ	JX301502	JX316993	JX301239	JX301373
WW 2656	*Thelotornis capensis*	Cape Vidal, St. Lucia, KwaZulu-Natal, ZA	JX301497	JX316988	JX301234	JX301368
TGE T11-03	*Thelotornis capensis*	Zululand Rhino Reserve, KwaZulu-Natal, ZA	JX301503	JX317041	JX301240	JX301374
BILL 749	*Thelotornis capensis oatesi*	Kaulmbila, ZM	JX301504	JX316995	JX301241	JX301375
I29389	*Thelotornis kirtlandii*	Rabi, GA	JX301495	JX316986	JX301232	JX301366
CRT 4053	*Thelotornis kirtlandii*	CD	JX301496	JX316987	JX301233	JX301367
CRT 4255	*Thelotornis kirtlandii*	CD	JX301494	JX316985	JX301231	JX301365
ELI 373	*Thelotornis kirtlandii*	road between Nyunzu and Kalemie, Katanga, CD	JX301492	JX316983	JX301229	JX301364
ELI 388	*Thelotornis kirtlandii*	road from Bukavu to Uvira, South Kivu, CD	JX301493	JX316984	JX301230	N/A
MTSN 5272	*Thelotornis 'kirtlandii'*	Masisiwe Village, Kilolo dist., Udzungwa Mtns, TZ	JX301506	JX316997	JX301243	N/A
MTSN 5236	*Thelotornis 'kirtlandii'*	Kihanga, Udzungwa Mtns, TZ	N/A	N/A	JX301298	N/A
BILL 73	*Thelotornis mossambicanus*	3km West of Moebase Village, MZ	N/A	N/A	JX301293	N/A
BILL 79	*Thelotornis mossambicanus*	Moebase village, MZ	N/A	N/A	JX301297	N/A

Table 2.1 – *Continued*

I29422	*Thelotornis mossambicanus*	MZ	N/A	JX317045	JX301292	JX301418
JS02	*Thelotornis sp.*	Watamu, KE	JX301512	JX317003	JX301249	JX301381
Xu	*Thelotornis sp.*	TZ	JX301511	JX317002	JX301248	JX301380
JS01	*Thelotornis sp.*	Watamu, KE	JX301513	JX317004	JX301250	JX301382
T_usam	*Thelotornis usambaricus*	TZ	JX301514	JX317005	JX301251	JX301383
ELI 509	*Thrasops jacksonii*	Byonga village near Kitutu, South Kivu, CD	JX301258	JX317012	JX301390	JX301521
CRT 4258	*Thrasops jacksonii*	CD	JX301259	JX317013	JX301391	JX301522
Tjack	*Thrasops jacksonii*	no locality data available	N/A	N/A	JX301299	JX301421
LSUMZ 37488	*Thrasops jacksonii*	no locality data available	N/A	AF471044	DQ112084	N/A
LSUMZ H-6819	*Thrasops jacksonii*	no locality data available	N/A	AF471044	N/A	N/A
I28732	*Toxicodryas blandingii*	Somoria, GN	JX301278	JX317031	JX301411	JX301544
I29380	*Toxicodryas pulverulenta*	Cavally, CI	JX301279	JX317032	JX301412	JX301545
Xyelo	*Xyelodontophis uluguruensis*	Summit of Nguru Mountains, TZ	JX301507	JX316998	JX301244	JX301377
BILL 661	*Xyledontophis uluguruensis*	Nguru Mts, TZ	JX301508	JX316999	JX301245	JX301378

2.2.2 Sampling Methodology

Samples were accumulated by field collecting and by requesting tissues from other workers with access to relevant taxa (see figures 2.1–2.3). In the field, roadkill specimens were located by driving along roads that penetrate suitable habitat. Specimens were stored in appropriately-sized cloth bags and plastic containers. Samples of integument, liver or muscle tissue were collected for molecular analyses, and stored in lysis buffer (1M TrisBase, 0.5M NaCl, 0.5M EDTA,10% SDS), 95–100% ethanol, or an RNA stabilization reagent. Whole specimens were then fixed in 10% formalin (3.7% formaldehyde, 0.6–1.5% methanol), and maintained in 70% ethyl alcohol. The collected specimens were deposited at the McGregor Museum in Kimberley, South Africa, where a subset of them is currently waiting to be exported to the Reptiles and Amphibian Diversity Research Center at the University of Texas at Arlington, Texas, USA. Tissue samples were deposited at the South African National Biodiversity Institute (SANBI), in accordance with the provincial collecting permits. All tissue samples used in this work were obtained from dead specimens from museum collections. No animals were sacrificed as a result of this research by researcher(s) from UT Arlington.

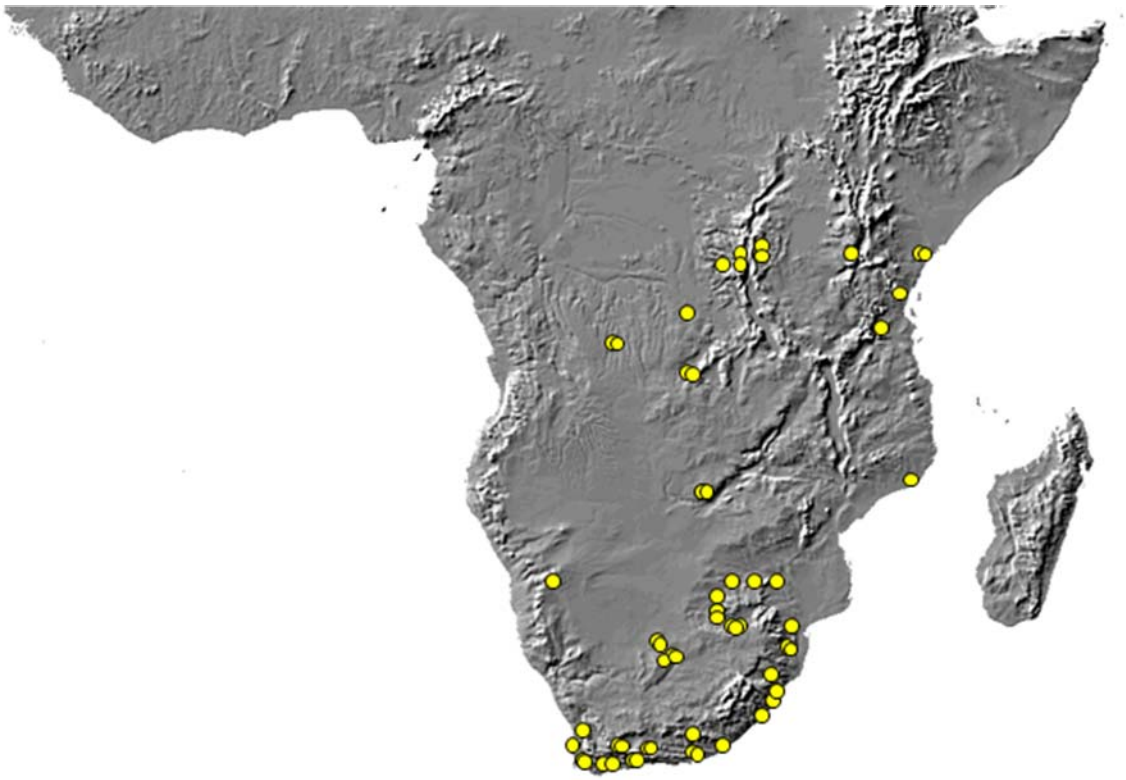

Figure 2.1. Distribution of samples of *D. typus* included in the data set.

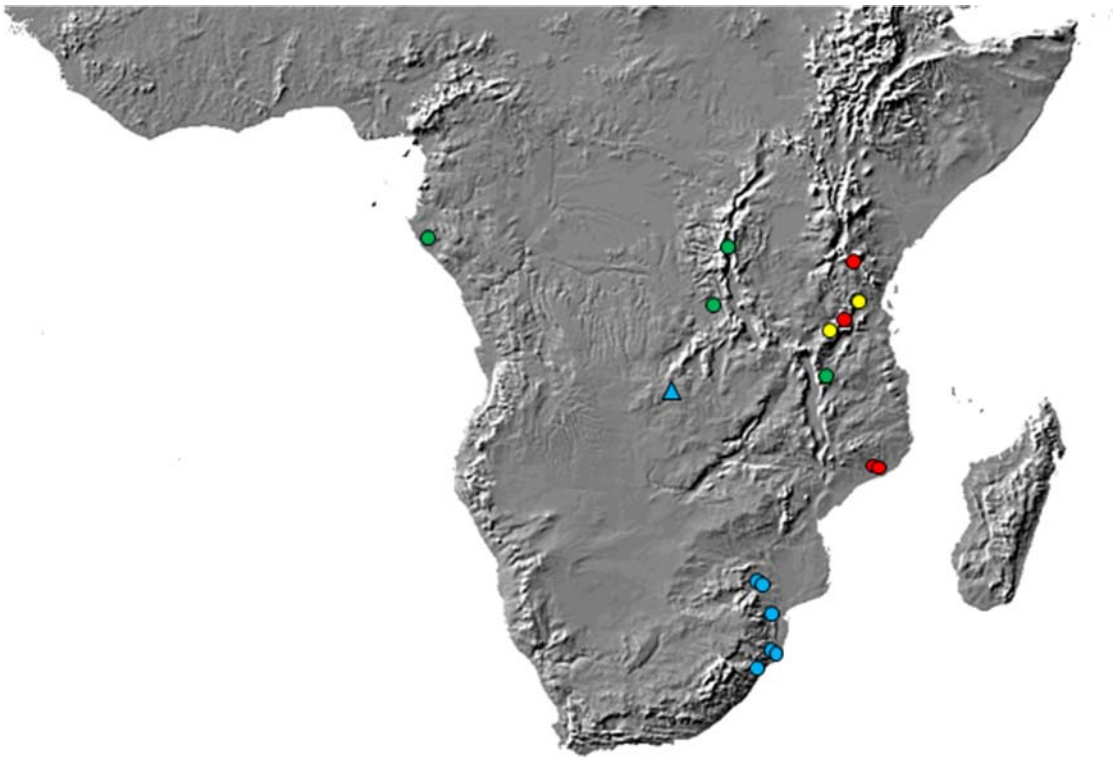

Figure 2.2. Distribution of samples of *Thelotornis* and *Xyelodontophis* included in the data set. Legend: *The. kirtlandii* = green; *The. mossambicanus* = red; *The. c. oatesii* = blue triangle; *The. c. capensis* = blue circle; *X. uluguruensis* = yellow.

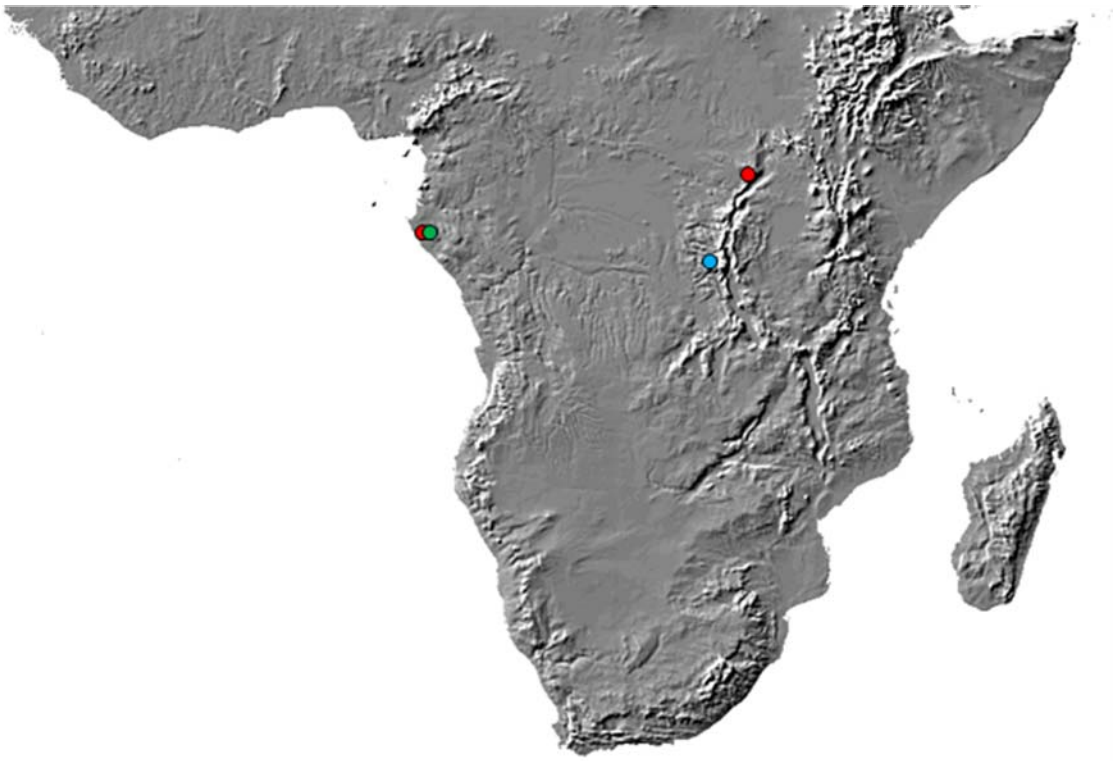

Figure 2.3. Distribution of samples of *Rhamnophis* and *Thrasops* included in the data set. Legend: *R. aethiopissa* = red; *R. batesii* = green; *Thr. jacksonii* = blue.

2.2.3 Molecular Data Generation

Whole genomic DNA was extracted from the tissue samples and stored following the protocols of Burbrink et al. (2000) or the Qiagen DNeasy Blood & Tissue Kit, and PCR amplifications were carried out in 25 µL volumes in an Eppendorf Mastercycler Gradient thermocycler, using the Promega GoTaq® Green Master Mix and the appropriate set of primers (table 2.2). The mitochondrial data set included fragments of cytochrome b (cyt-b) and NADH dehydrogenase subunit 4 (ND4), and the nuclear data set was comprised of fragments of the c-mos proto-oncogene (c-mos) and neurotrophin-3 (NT3). Samples amplified using the Gludg/AtrCB3 (cyt-b) primer set were subjected to an initial denaturation at 94 °C for 180 s, 2 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 60 s, and extension at 72 °C for 45

s, and 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 45 s, and extension at 72 °C for 45 s. This was followed by a final extension at 72 °C for 300 s. Samples amplified using the H14910/THRSN2 (cyt-b) primer set were subjected to an initial denaturation at 95 °C for 150 s, 2 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 60 s, and extension at 68 °C for 90 s, and 40 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 45 s. This was followed by a final extension at 72 °C for 900 s. Samples amplified using the ND4-1/LEU-1 (ND4) primer set were subjected to a single cycle of denaturation at 94 °C for 210 s, annealing at 42 °C for 60 s, and extension at 68 °C for 90 s, and 39 cycles of denaturation at 94 °C for 30s, annealing at 48 °C for 30 s, and extension at 72 °C for 60 s. This was followed by a final extension at 72 °C for 900 s. Samples amplified using the S77/S78 (c-mos) primer set were subjected to an initial denaturation at 94 °C for 90 s, 5 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, extension at 72°C for 90 s, 5 cycles of denaturation 94 °C for 30 s, annealing at 49 °C for 30 s, extension at 72 °C for 90 s, and 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 60 s, extension at 72 °C for 90 s. This was followed by a final extension at 72 °C for 420 s. Samples amplified using the NT3-F3/NT3-R4 (NT3) primer set were subjected to an initial denaturation at 94 °C for 90 s. 5 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, extension at 72 °C for 90 s, 5 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 90 s, 10 cycles of denaturation 94 °C for 30 s, annealing at 49 °C for 30 s, extension at 72 °C for 90 s, and 30 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 30 s, extension at 72 °C for 90 s. This was followed by a final extension at 72 °C for 420 s. All amplification runs included negative controls to check for contamination, and were quantified on a 1% TAE-agarose gel. The subsequent clean-up was conducted using Agencourt AMPure XP magnetic beads or the USB ExoSAP-IT reagent to remove excess primers and unincorporated dNTPs. These amplifications were then sequenced using an Applied Biosystems 3130xl Genetic Analyzer at the Genomics Core Facility at the University of Texas at Arlington to create the mitochondrial and nuclear data. The mitochondrial data set contains sequences from two mtDNA gene fragments, including

NADH dehydrogenase subunit 4 (681 bp) and cytochrome b (1092 bp). The nuclear data set is comprised of two nuDNA gene fragments, including Neurotrophin-3 (503 bp) and C-mos (562 bp). This combination of using two mitochondrial and two nuclear gene fragments has frequently been used to investigate questions of phylogenetic inference of a group of closely-related organisms in the past (e.g., Bossuyt and Milinkovitch 2000, Che et al. 2007). Additional sequences of both ingroup and outgroup taxa were imported from Genbank to supplement the dataset.

Table 2.2. Primers used in generating nucleotide sequence data.

Primer Name	Locus	Direction	Primer Sequence	Reference
Gludg	Cyt-b	Forward	5' TGA CTT GAA RAA CCA YCG TTG 3'	Parkinson et al. (2002)
AtrCB3	Cyt-b	Reverse	5' TGA GAA GTT TTC YGG GTC RTT 3'	Parkinson et al. (2002)
H14910	Cyt-b	Forward	5’ GAC CTG TGA TNT GAA AAA CCA YCG TT 3’	Burbrink et al. (2000)
THRSN2	Cyt-b	Reverse	5’ CTT TGG TTT ACA AGA ACA ATG CTT TA 3’	Burbrink et al. (2000)
ND4	ND4	Forward	5’ CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC 3’	Arevalo et al. (1994)
LEU	ND4	Reverse	5’ CAT TAC TTT TAC TTG GAT TTG CAC CA 3’	Arevalo et al. (1994)
S77	C-mos	Forward	5' CAT GGA CTG GGA TCA GTT ATG 3'	Lawson et al. (2005)
S78	C-mos	Reverse	5' CCT TGG GTG TGA TTT TCT CAC CT 3'	Lawson et al. (2005)
NT3-F3	NT3	Forward	5’ ATA TTT CTG GCT TTT CTC TGT GGC 3’	Noonan and Chippindale (2006)
NT3-R4	NT3	Reverse	5’ GCG TTT CAT AAA AAT ATT GTT TGA CC 3’	Noonan and Chippindale (2006)

2.2.4 Phylogenetic Analyses

Sequence editing and assembly of contigs was conducted using Sequencher ver. 4.5, aligned using MEGA ver. 5.05, and manually adjusted with MacClade ver. 4.08. Phylogenetic analyses were conducted using maximum likelihood and maximum and weighted parsimony,

using RAxML ver. 7.0.4 and TNT ver. 1.1, respectively. The data were partitioned *a priori* on the basis of gene identity (ND4, Cyt-b, NT3, C-mos) and gene location (mitochondria, nucleus).

A maximum likelihood analysis was conducted using the program RAxML ver. 7.0.4. (Stamatakis et al. 2008) as implemented on the Cipres portal ver. 2.0, was used for the tree search and the bootstrap analysis on all four concatenated gene fragments, as well as on the two mitochondrial and the two nuclear fragments as separate partitions. The only substitution model implemented in RAxML was GTR (Stamatakis 2006), and thus the GTR GAMMA model was used across all partitions, as recommended in the program documentation and implemented by others (Grazziotin et al. 2012). In order to quantify clade support, one thousand pseudo-replications of non-parametric bootstrap were performed using the cluster hosted at the Pritham-Feschotte laboratories at the University of Texas at Arlington.

The data were further analyzed by using parsimony optimality criteria. In order to reduce computational time, the software program TNT ver. 1.1 (Goloboff et al. 2008) was used. Other authors have found significant differences in speed between TNT and other programs, such as PAUP* and NONA/Pee-Wee (Goloboff 1994a, 1994b), and it has been effectively utilized to infer phylogenetic relationships in similar studies (e.g., Hedin and Bond 2006, Monaghan et al. 2007, Benjamin et al. 2008). For example, TNT was able to find optimum trees for a 228-taxa dataset by McMahon and Sanderson (2006) in an average time of 30 minutes, which took PAUP* 1700 hours of computational time using the ratchet (Goloboff et al. 2008). TNT is made highly efficient by incorporating multiple approaches to finding global optima, including the ratchet (Nixon 1999), tree-drifting (Goloboff 1999), tree-fusing (Goloboff 1999), and sectorial searches (Goloboff 1999).

The two mitochondrial and the two nuclear gene fragments were concatenated and analyzed both separately and combined. In each of these analyses, 1000 random addition sequence replicates employing all four algorithms with default parameters were first used to find global optima, and were subsequently driven with a score bound in an attempt to find more

parsimonious trees. In order to estimate clade support, non-parametric bootstrap values (Felsenstein 1985) were obtained from 1000 pseudo-replicates, in which optimal trees were found using the new technology search under the same parameters as above. In addition, analyses using implied weighting were conducted in order to construct trees using differential character weighting (Goloboff 1993a). This approach is considered to be superior to successive weighting, because it implements an optimality criterion (maximum total fit, calculated as a function of homoplasy) to constructing trees and weighting characters (Goloboff 1993a). Implied weighting analyses were conducted for *K*-values = 3 (default) for 500 replicates.

Divergence estimates were calculated using the maximum composite likelihood model (Tamura et al. 2004) in MEGA ver. 5.05 with the concatenated sequences, consisting of all four gene fragments (ND4, cyt-b, c-mos, and NT3). All positions with less than 95% site coverage were eliminated from the analysis. Estimates of standard error were obtained by a bootstrap procedure with 1000 pseudo-replicates.

2.3 Results

2.3.1 Combined Analyses

In the analysis of the complete dataset with all four gene fragments, TNT recovered six most parsimonious trees, each with a total length of 5930 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. Both the strict consensus and the majority consensus support the monophyly of dispholidine snakes relative to the outgroups, with *Philothamnus-Hapsidophrys* being the sister taxon. *Thrasops* is the basal lineage within that clade, while *Rhamnophis* is sister to a clade consisting of *Dispholidus* and *Thelotornis-Xyelodontophis*. *Xyelodontophis* is nested within *Thelotornis*, and there are multiple clades contained within *Dispholidus*. When implied weighting was incorporated in the analysis, three most parsimonious trees were recovered, each with a best score of 458.27. The results of the maximum likelihood analysis (figures 2.4–2.5) were congruent with those recovered using

parsimony with the best tree having a highest log likelihood value of -30184.95. Outgroup samples that contained substantial amounts of missing data were omitted the final analyses in order to obtain a more accurate assessment of support values.

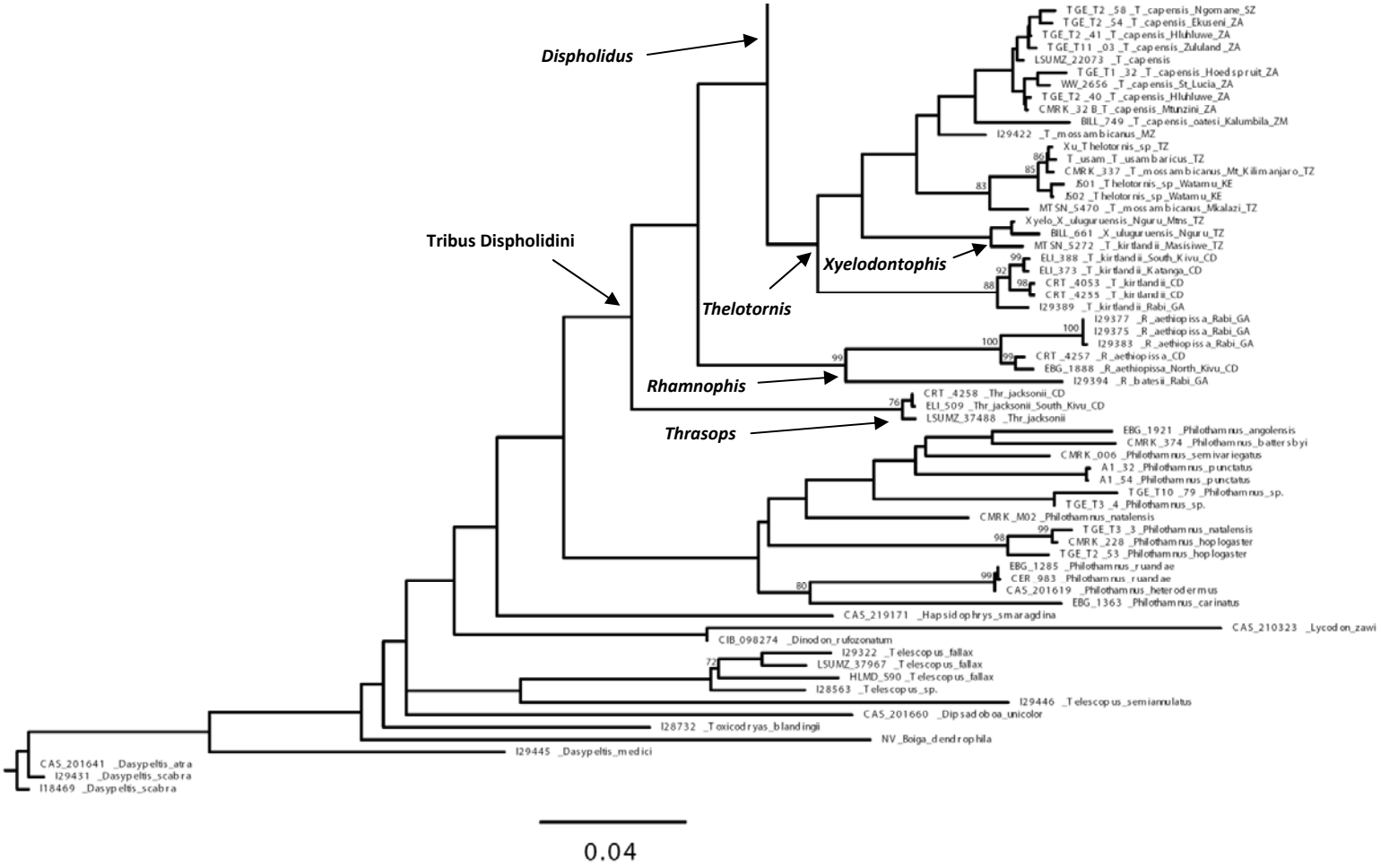

Figure 2.4. Maximum likelihood tree with the highest log likelihood value (-30184.95) of the combined data set, including all gene fragments. Numbers indicate weighted parsimony bootstrap proportions from 1000 pseudo-replicates.

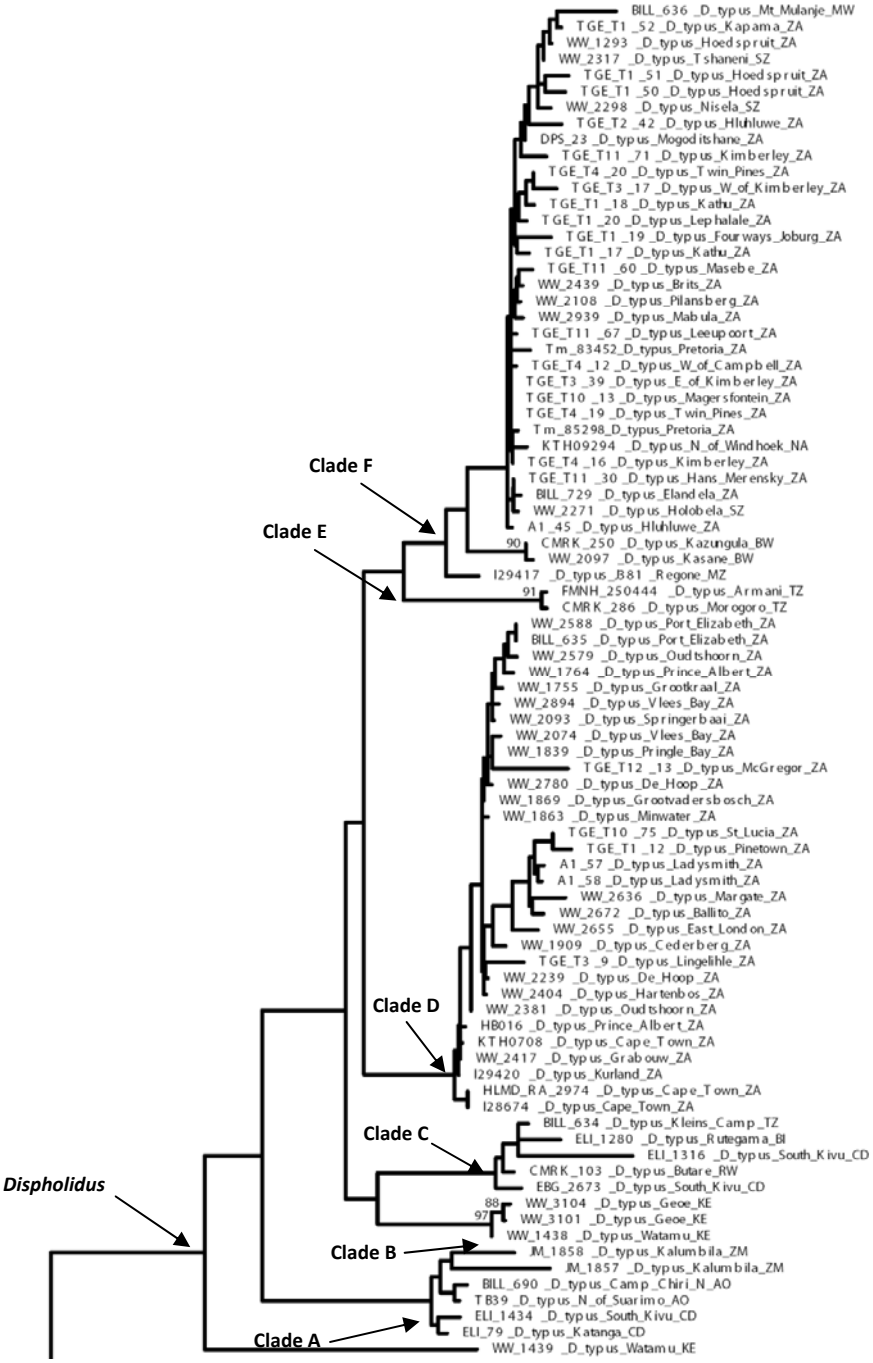

Figure 2.5. *Dispholidus* clade of the maximum likelihood tree with the highest log likelihood value (-30184.95) of the combined data set, including all gene fragments. Numbers indicate weighted parsimony bootstrap proportions from 1000 pseudo-replicates.

Table 2.3. Estimates of net evolutionary divergence between groups of concatenated sequences, including 2838 bp of ND4, cyt-b, c-mos, and NT3. Numbers below the diagonal represent the number of base substitutions per site from estimation of net average between groups of sequences. Numbers above the diagonal represent the standard error estimate(s).

Taxa	1	2	3	4	5	6	7	8	9	19	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. *Dispholidus typus* (KZN)		0.011	0.003	0.011	0.010	0.015	0.010	0.010	0.013	0.023	0.022	0.022	0.021	0.033	0.028	0.029	0.033	0.037	0.035	0.032	0.037	0.037	0.037	0.032	0.032	0.033	0.036	0.033	0.045	0.033	0.034	0.050
2. *Dispholidus* sp. (Kenya)	0.053		0.010	0.012	0.012	0.017	0.012	0.012	0.015	0.023	0.023	0.024	0.022	0.031	0.029	0.030	0.032	0.035	0.036	0.031	0.040	0.038	0.036	0.030	0.032	0.033	0.036	0.033	0.043	0.033	0.035	0.050
3. *Dispholidus typus*	0.009	0.053		0.010	0.010	0.015	0.010	0.009	0.012	0.021	0.022	0.022	0.021	0.033	0.029	0.030	0.033	0.038	0.035	0.033	0.038	0.037	0.036	0.032	0.032	0.034	0.036	0.033	0.046	0.032	0.035	0.050
4. *Dispholidus viridis*	0.052	0.056	0.052		0.011	0.016	0.006	0.006	0.012	0.022	0.022	0.022	0.021	0.030	0.029	0.032	0.032	0.039	0.036	0.034	0.040	0.038	0.038	0.033	0.033	0.034	0.034	0.033	0.046	0.035	0.037	0.051
5. *Dispholidus kivuensis*	0.053	0.055	0.053	0.053		0.015	0.012	0.011	0.013	0.022	0.022	0.023	0.021	0.033	0.028	0.030	0.030	0.037	0.035	0.034	0.038	0.037	0.038	0.032	0.031	0.036	0.037	0.033	0.045	0.034	0.036	0.058
6. *Dispholidus punctatus*	0.073	0.078	0.073	0.078	0.077		0.015	0.014	0.015	0.021	0.021	0.023	0.022	0.030	0.029	0.029	0.032	0.037	0.036	0.033	0.038	0.035	0.035	0.030	0.032	0.031	0.036	0.032	0.046	0.030	0.035	0.050
7. *Dispholidus viridis* (Botswana)	0.049	0.057	0.050	0.022	0.059	0.077		0.006	0.011	0.023	0.022	0.021	0.021	0.032	0.029	0.031	0.032	0.036	0.036	0.033	0.039	0.037	0.037	0.030	0.032	0.035	0.036	0.034	0.048	0.035	0.034	0.051
8. *Dispholidus viridis* (Mozambique)	0.048	0.056	0.045	0.022	0.054	0.072	0.022		0.011	0.022	0.021	0.020	0.020	0.031	0.029	0.030	0.033	0.037	0.035	0.033	0.039	0.036	0.036	0.031	0.031	0.035	0.035	0.032	0.047	0.035	0.036	0.051
9. *Dispholidus* sp. (Tanzania)	0.054	0.071	0.052	0.053	0.067	0.077	0.053	0.047		0.022	0.022	0.024	0.022	0.032	0.031	0.032	0.034	0.037	0.037	0.034	0.038	0.036	0.037	0.032	0.033	0.034	0.038	0.035	0.048	0.034	0.036	0.055
10. *Thelotornis kirtlandii*	0.111	0.115	0.108	0.113	0.119	0.105	0.118	0.110	0.112		0.016	0.016	0.018	0.029	0.023	0.026	0.031	0.033	0.032	0.029	0.037	0.034	0.033	0.032	0.032	0.035	0.035	0.033	0.046	0.030	0.032	0.050
11. *Thelotornis c. capensis*	0.108	0.116	0.108	0.110	0.119	0.106	0.111	0.104	0.111	0.077		0.013	0.009	0.028	0.025	0.027	0.030	0.030	0.033	0.027	0.037	0.032	0.032	0.030	0.029	0.032	0.034	0.031	0.042	0.031	0.035	0.051
12. *Xyelodontophis uluguruensis*	0.108	0.114	0.109	0.109	0.118	0.111	0.110	0.103	0.118	0.080	0.069		0.013	0.026	0.024	0.025	0.029	0.029	0.034	0.027	0.037	0.030	0.033	0.031	0.031	0.032	0.032	0.033	0.043	0.033	0.032	0.046
13. *Thelotornis mossambicanus-usambaricus*	0.108	0.110	0.106	0.105	0.112	0.111	0.107	0.102	0.111	0.085	0.049	0.068		0.028	0.026	0.028	0.033	0.032	0.034	0.029	0.039	0.036	0.033	0.031	0.031	0.035	0.034	0.035	0.043	0.033	0.033	0.051
14. *Rhamnophis batesii*	0.155	0.146	0.155	0.145	0.161	0.147	0.153	0.148	0.152	0.138	0.143	0.127	0.134		0.023	0.030	0.033	0.038	0.036	0.032	0.037	0.037	0.038	0.035	0.032	0.030	0.036	0.034	0.042	0.032	0.034	0.055
15. *Rhamnophis aethiopissa*	0.136	0.141	0.138	0.139	0.136	0.136	0.137	0.136	0.145	0.116	0.129	0.121	0.129	0.110		0.028	0.028	0.028	0.028	0.029	0.037	0.030	0.031	0.031	0.030	0.028	0.033	0.033	0.039	0.029	0.030	0.050
16. *Thrasops jacksonii*	0.140	0.146	0.141	0.152	0.151	0.138	0.152	0.144	0.150	0.130	0.133	0.129	0.138	0.145	0.139		0.034	0.032	0.032	0.029	0.033	0.035	0.031	0.035	0.036	0.029	0.034	0.032	0.039	0.030	0.034	0.050
17. *Philothamnus semivariegatus*	0.160	0.156	0.160	0.154	0.152	0.155	0.154	0.158	0.158	0.145	0.141	0.142	0.151	0.158	0.144	0.157		0.025	0.016	0.025	0.036	0.019	0.016	0.023	0.017	0.030	0.035	0.033	0.041	0.029	0.032	0.054
18. *Philothamnus ruandae*	0.175	0.168	0.175	0.181	0.174	0.171	0.173	0.172	0.170	0.148	0.144	0.141	0.152	0.174	0.141	0.147	0.119		0.026	0.023	0.039	0.025	0.027	0.022	0.026	0.031	0.033	0.032	0.038	0.032	0.031	0.051
19. *Philothamnus angolensis*	0.176	0.171	0.176	0.175	0.177	0.170	0.177	0.171	0.176	0.159	0.161	0.162	0.160	0.170	0.151	0.152	0.079	0.125		0.025	0.033	0.021	0.013	0.025	0.017	0.030	0.034	0.036	0.037	0.031	0.030	0.054
20. *Philothamnus hoplogaster-natalensis*	0.169	0.165	0.171	0.170	0.175	0.170	0.169	0.171	0.170	0.146	0.139	0.139	0.148	0.161	0.143	0.148	0.124	0.113	0.131		0.036	0.023	0.028	0.023	0.025	0.027	0.030	0.029	0.038	0.028	0.028	0.052
21. *Crotaphopeltis hotamboeia*	0.186	0.200	0.187	0.195	0.192	0.185	0.190	0.191	0.188	0.178	0.175	0.177	0.187	0.193	0.184	0.166	0.173	0.180	0.164	0.183		0.037	0.038	0.037	0.035	0.032	0.042	0.035	0.041	0.032	0.037	0.057
22. *Philothamnus* sp. (Swaziland)	0.184	0.181	0.182	0.179	0.179	0.162	0.181	0.175	0.175	0.157	0.151	0.150	0.170	0.176	0.149	0.168	0.090	0.120	0.098	0.120	0.180		0.020	0.025	0.022	0.032	0.038	0.036	0.041	0.031	0.030	0.052
23. *Philothamnus battersbyi*	0.182	0.176	0.177	0.181	0.183	0.168	0.178	0.174	0.181	0.164	0.161	0.158	0.159	0.177	0.161	0.154	0.083	0.135	0.064	0.141	0.178	0.098		0.027	0.018	0.033	0.035	0.036	0.044	0.030	0.032	0.054
24. *Philothamnus carinatus*	0.157	0.154	0.161	0.159	0.163	0.148	0.151	0.157	0.153	0.146	0.147	0.155	0.151	0.166	0.155	0.166	0.119	0.106	0.126	0.121	0.181	0.128	0.138		0.024	0.031	0.036	0.034	0.042	0.030	0.032	0.051
25. *Philothamnus punctatus*	0.156	0.155	0.154	0.160	0.155	0.152	0.159	0.154	0.158	0.149	0.141	0.149	0.149	0.154	0.152	0.163	0.079	0.126	0.091	0.132	0.171	0.104	0.099	0.121		0.031	0.035	0.033	0.038	0.030	0.031	0.050
26. *Dasypeltis scabra*	0.164	0.161	0.167	0.163	0.173	0.152	0.169	0.170	0.168	0.166	0.161	0.159	0.166	0.150	0.147	0.144	0.146	0.145	0.146	0.142	0.162	0.154	0.158	0.149	0.145		0.022	0.030	0.033	0.023	0.024	0.047
27. *Dasypeltis medici*	0.181	0.180	0.181	0.170	0.180	0.172	0.174	0.171	0.183	0.168	0.166	0.162	0.164	0.179	0.164	0.167	0.168	0.154	0.165	0.156	0.198	0.176	0.165	0.167	0.173	0.115		0.032	0.037	0.029	0.029	0.053
28. *Telescopus fallax eberus*	0.177	0.176	0.178	0.171	0.175	0.165	0.177	0.172	0.182	0.167	0.161	0.166	0.170	0.168	0.170	0.154	0.160	0.159	0.174	0.154	0.169	0.175	0.174	0.174	0.164	0.153	0.165		0.035	0.029	0.034	0.052
29. *Telescopus semiannulatus*	0.215	0.210	0.219	0.218	0.214	0.217	0.226	0.221	0.222	0.204	0.198	0.204	0.197	0.197	0.193	0.185	0.191	0.187	0.180	0.173	0.198	0.198	0.209	0.193	0.180	0.160	0.182	0.173		0.034	0.034	0.061
30. *Toxicodryas blandingii*	0.168	0.169	0.167	0.170	0.175	0.155	0.174	0.175	0.166	0.152	0.152	0.164	0.162	0.168	0.149	0.144	0.144	0.154	0.155	0.142	0.154	0.152	0.157	0.145	0.149	0.114	0.147	0.143	0.163		0.021	0.042
31. *Toxicodryas pulverulenta*	0.173	0.171	0.175	0.183	0.182	0.171	0.174	0.177	0.177	0.153	0.168	0.159	0.152	0.155	0.145	0.167	0.156	0.151	0.149	0.144	0.179	0.153	0.152	0.154	0.156	0.122	0.145	0.166	0.167	0.117		0.044
32. *Dinodon rufozonatum*	0.214	0.212	0.218	0.216	0.239	0.204	0.217	0.218	0.226	0.209	0.213	0.193	0.210	0.232	0.213	0.212	0.224	0.210	0.227	0.226	0.240	0.219	0.235	0.213	0.210	0.209	0.225	0.223	0.253	0.187	0.199	

2.3.2 Sequence Divergence Estimates

The results of the sequence divergence estimates show an average divergence of 5.3% between *Dispholidus* from southern South Africa (*D. typus*) and those from the northern parts of southern Africa (*D. viridis*; see table 2.3.). In Central Africa, *D. kivuensis* and D. punctatus showed a 7.7% divergence from each other, and a 5.3% and 7.3% divergence from *D. typus*, respectively. The southern

subclades of *D. typus* from KwaZulu-Natal and the two northern subclades of *D. viridis* from Mozambique and Botswana showed only moderate levels of sequence divergence from their respective main clades. The two East African clades (*Dispholidus* sp. Kenya and *Dispholidus* sp. Tanzania) showed a sequence divergence of 5.3% and 5.2% from *Dispholidus typus* proper, respectively. Taxa within the *Thelotornis-Xyelodontophis* clade showed divergence estimates between 4.9% (between *T. c. capensis* and *T. mossambicanus-usambaricus*) and 8.5% (between *T. kirtlandii* and *T. mossambicanus-usambaricus*). The divergence between the two taxa within the genus *Rhamnophis* were particularly strong at 11.1%.

2.3.3 Mitochondrial Data

The analysis of the two mitochondrial fragments (ND4 and cyt-b) in an equally weighted manner yielded seven most parsimonious trees, each with a length of 5338 steps. A driven search with a score bound was unable to retrieve any trees of shorter length. When implied weighting was incorporated into the analysis, eight most parsimonious trees were recovered, each with a best score of 422.51. Both the strict consensus and the majority rule consensus support the monophyly of dispholidine snakes, with *Philothamnus* being the sister taxon. In all seven most parsimonious trees, *Thrasops* is basal within the ingroup, and *Rhamnophis* is sister to a clade consisting of *Dispholidus* and *Thelotornis-Xyelodontophis*. There are several distinct clades contained within *Dispholidus*. The maximum likelihood analysis yielded a best tree with a log likelihood value of -26088.84, which was largely congruent with the topology recovered using parsimony (figure 2.6). Outgroup samples that contained substantial amounts of missing data were omitted the final analyses in order to obtain a more accurate assessment of support values.

The analysis of the ND4 fragment individually yielded three most parsimonious trees, each with a length of 2104 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. When implied weighting was incorporated into the analysis, three most parsimonious trees were recovered, each with a best score of 164.46. All most parsimonious

trees support the monophyly of dispholidine snakes, with *Philothamnus* being the sister taxon. *Thrasops* is the basal lineage within the ingroup, and *Rhamnophis* is sister to a clade consisting of *Dispholidus* and *Thelotornis-Xyelodontophis*, with the latter being nested within *Thelotornis*. Support from bootstrap proportions was relatively low for many of the clades. The maximum likelihood analysis yielded a best tree with a log likelihood of -11276.58. The topography of the major relationships was largely congruent with the results of the parsimony analysis.

The separate analysis of the cytochrome b fragment recovered two most parsimonious trees, each with a length of 3195 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. All six most parsimonious trees support the monophyly of dispholidine snakes, with most supporting a sister relationship with *Philothamnus*. *Thrasops* is basal within the ingroup, and *Rhamnophis* is sister to a clade that consists of *Dispholidus* and *Thelotornis-Xyelodontophis*. When implied weighting was incorporated into the analysis, five most parsimonious trees were recovered, each with a best score of 256.59. The major relationships recovered are largely congruent with those from the unweighted analysis. However, while *Xyelodontophis* is nested within *Thelotornis* in the unweighted analysis, it is sister to a clade that consists of *Dispholidus* and *Thelotornis* under implied weighting. Clade support from bootstrap proportions is slightly higher under implied weighting. The results of the maximum likelihood analysis yielded a log likelihood score of -131295.67. The topography of the major relationship was largely congruent with the results of the parsimony analysis.

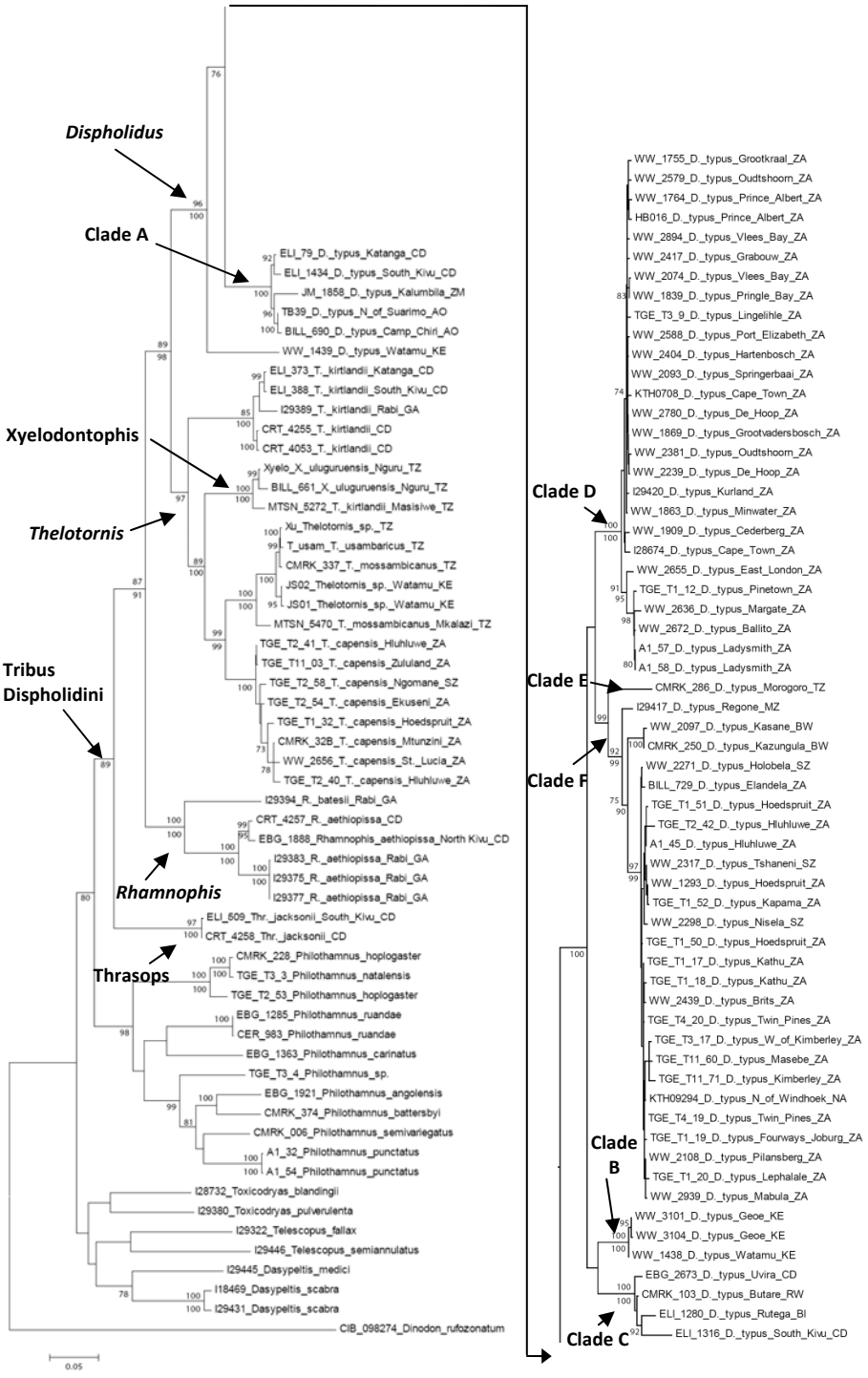

Figure 2.6. Maximum likelihood tree with the highest log likelihood value (-26088.84), inferred using both mitochondrial fragments. Numbers represent bootstrap proportions from 1000 bootstrap pseudo-replicates from the parsimony (above nodes) and likelihood (below nodes) analyses.

2.3.4 Nuclear Data

When the two nuclear gene fragments (c-mos and NT3) were analyzed, six most parsimonious trees were recovered, each with a total length of 518 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. When implied weighting was incorporated into the analysis, seven most parsimonious trees were recovered, each with a best score of 28.48. The most parsimonious trees do not support the monophyly of dispholidine snakes to the exclusion of *Philothamnus* and *Hapsidophrys*. The major relationships were poorly resolved, with *Thrasops*, *Philothamnus-Hapsidophrys*, and a clade containing all other dispholidine snakes producing a large polytomy. Within the latter clade, *Rhamnophis* is sister to a clade containing *Dispholidus* and *Thelotornis-Xyelodontophis*. Support from bootstrap replicates was higher under implied weighting, but overall suffered from relatively low proportions. The maximum likelihood results were not congruent with the most parsimonious trees, in that the tree with the highest likelihood (-5193.40, figure 2.7) did support the monophyly of dispholidine snakes. However, samples that had significant amounts of missing characters, such as those for which only a single fragment was available, were omitted from the analysis, and subsequently showed congruence with the mitochondrial data.

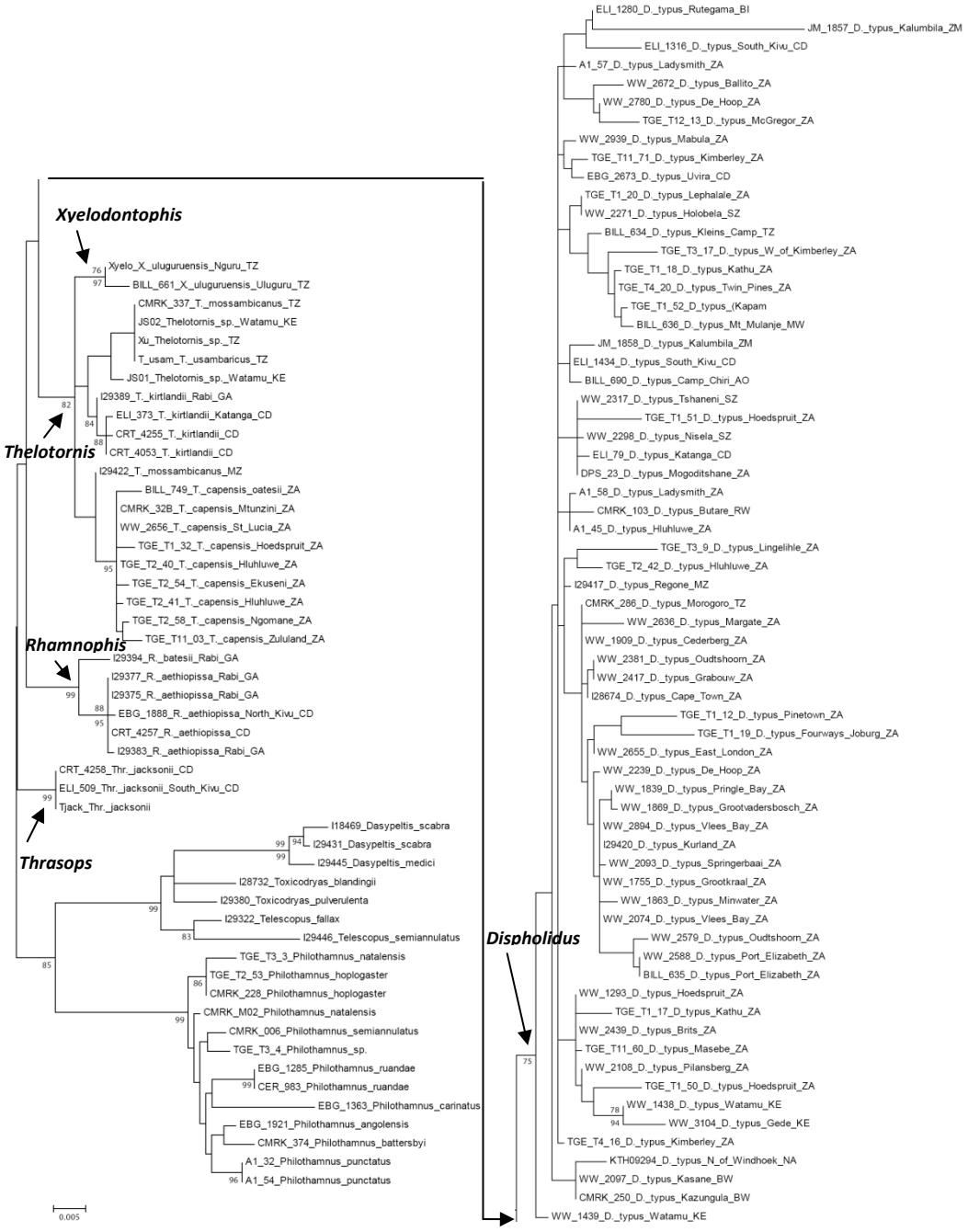

Figure 2.7. Maximum likelihood tree with the highest log likelihood value (-5193.40), inferred using both nuclear fragments. Numbers represent bootstrap proportions from 1000 bootstrap pseudo-replicates from the parsimony (above nodes) and likelihood (below nodes) analyses.

The analysis of the c-mos fragment individually produced five most parsimonious trees, each with a length of 137 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. When implied weighting was incorporated into the analysis, five most parsimonious trees were recovered, each with a best score of 3.70. The most parsimonious trees do not support the monophyly of dispholidine snakes to the exclusion of the outgroups with whom they form a large polytomy. Major relationships within the ingroup are poorly resolved, with *Dispholidus* and *Thelotornis* contained in a large polytomy. Support from bootstrap replicates was higher under implied weighting, but overall suffered from relatively low proportions. Results from the maximum likelihood analysis were congruent with those inferred using parsimony. The tree with the highest log likelihood value (-1764.84) did not support the monophyly of dispholidines, relative to the outgroups. While many of the individual taxa did group together, the overall topology was poorly resolved and suffered from low bootstrap support.

The analysis of the NT3 fragment individually yielded five most parsimonious trees, each with a length of 362 steps. A driven search with a score bound was unable to retrieve any more parsimonious trees. When implied weighting was incorporated into the analysis, seven most parsimonious trees were recovered, each with a best score of 22.10. All seven most parsimonious trees support the monophyly of dispholidine snakes to the exclusion of *Philothamnus-Hapsidophrys*. *Thrasops* is basal within the ingroup, and *Rhamnophis* is sister to *Thelotornis-Xyelodontophis*, which in turn is sister to *Dispholidus*. Support from bootstrap proportions was high for the monophyly of dispholidine snakes, but relatively low within the ingroup. The tree with the highest log likelihood value (-3171.50) corroborated the results from the parsimony analysis. Support from bootstrap proportions was inconsistent within the ingroup, but did support the monophyly of the ingroup taxa at the genus level.

2.4 Discussion

2.4.1 Monophyly of Dispholidine Snakes

Most of the analyses provide strong support for the monophyly of dispholidine snakes, as hypothesized by Broadley and Wallach (2002). The sister taxon are philothamnine snakes, including of the genera *Philothamnus* and *Hapsidophrys*. These results support the hypothesis of Bourgeois (1968) that dispholidine snakes form a clade that is closely related to members of the genera *Hapsidophrys*, *Philothamnus*, *Boiga*, *Crotaphopeltis*, *Dipsadoboa*, and *Telescopus,* and is thus more distantly related to other colubrine snakes, such as *Dasypeltis*, *Lycodon*, or *Dinodon*. I therefore recommend treating dispholidine snakes as a colubrine tribe *sensu* Broadley and Wallach (2002), and I reject earlier hypotheses by Dowling and Duellman (1978) and Ferrarezzi (1994), who considered *Rhamnophis* and *Thrasops* to be members of the tribe Philothamnini, and *Dispholidus* and *Thelotornis* members of the tribe Boigini.

2.4.2 Basal Lineage within Dispholidini

Based on these results, the hypothesis that *Rhamnophis* diverged precociously from the ancestral lineage (Bourgeois 1968) is rejected. Instead, these data show that *Thrasops* diverged early in the evolution of this clade, as hypothesized by Eimermacher (2007). All of the samples of the genus *Thrasops* were of the same species, *Thr. jacksonii*, and hence the results do not allow for further inference regarding the systematics within that genus.

2.4.3 Status of Rhamnophis and Thrasops

The results support the monophyly of *Rhamnophis* to the exclusion of *Thrasops*, thereby rejecting the synonymy of the two (e.g. Loveridge 1957, Leston and Hughes 1968, Hughes and Barry 1969, Pitman 1974, Spawls 1978, Hughes 1983, Obst et al. 1984, 1988, Coborn 1991, Fischer and Hinkel 1992, Frank and Ramus 1995, Trape and Roux-Estève 1995, Chippaux 1999,

2001, 2006, Pauwels et al. 2002a-b), while corroborating Broadley and Wallach (2002) and Eimermacher (2007).

Within the genus *Rhamnophis*, *R. batesii* was represented by just a single sample, which is clearly distinct from and sister to *R. aethiopissa*. The two species within this genus are particularly poorly understood, and I was unable to obtain a thorough sampling for either taxon. *R. aethiopissa* has a wide distribution, ranging from Ghana to Kenya, and south to Angola and Zambia. Three subspecies have been described, including the nominate form, *R*. *a*. *aethiopissa* (Günther 1862) from West Africa, *R. a. ituriensis* (Schmidt 1923) from Niapu in the Democratic Republic of Congo, and *R. a. elgonensis* (Loveridge 1929) from the Lukosa River in Kenya, at the foot of Mount Elgon. While the majority of the samples of *R. aethiopissa* used in this study were from Gabon, one sample (EBG 1888) was collected near the town of Owicha in the Democratic Republic of Congo, which is approximately 400 km southeast of the type locality of *R*. *a*. *ituriensis*, and approximately 500 km west of the type locality of *R*. *a*. *elgonensis*. The collected specimen was unavailable at the time of this writing, so none of the specimens used in this study were designated subspecific status. Therefore, no further inferences regarding the status of any of the aforementioned taxa are possible at this point.

2.4.4 Systematic Relationships of Thelotornis and Xyelodontophis

The *Thelotornis* clade includes multiple distinct lineages. *T. kirtlandii* is sister to a clade consisting of *T. capensis* and *T. mossambicanus*-*T. usambaricus*. None of the samples of *T. kirtlandii* that were included in this study were collected near the type locality of this species (Liberia), but the vast majority of the specimens grouped together in the analyses, as expected. The two exceptions that were common to most analyses were two particular samples (MTSN 5236 and MTSN 5272) from the Udzungwa Mountains of Tanzania, which consistently came out sister to *Xyelodontophis*. Efforts are currently underway to obtain those specimens, as there is

strong support to the effect that these may be misidentified specimens of *X. uluguruensis*, or perhaps even a new species.

Sister to the *T. kirtlandii* clade is a clade that consists of three distinct lineages. First, it contains a clade with *T. capensis* of southern Africa. All the specimens from South Africa and Swaziland fall into that clade, as well as one *T. c. oatesii* (BILL 749) from Zambia and one specimen that was identified as *T. mossambicanus* from Mozambique (I29422). Since BILL 749 was the only representative of the subspecies *T. c. oatesii*, which was collected rather distantly (at least 800 km) from the type locality of that taxon (Matebeland, Zimbabwe), no further inferences can be made about the validity of that taxon as a distinct lineage. The same goes for sample I29422, *T. mossambicanus* from Mozambique, which may be another case of misidentification in the field. *T. c. schilsi* (Derleyn 1978) from the Ruzizi plain in Burundi was described on the basis of possessing a shorter tail and consequently lower subcaudal scale counts (Broadley 2001), and was subsequently not accepted (Broadley and Wallach 2002, Eimermacher 2007). Samples from near the type locality of that taxon were unavailable, and consequently no inference can be made regarding its status.

Sister to the Southern African *T. capensis* is a clade of East African *Thelotornis*, containing samples of both *T. mossambicanus* and *T. usambaricus*, neither of which are monophyletic to the exclusion of the other. *T. mossambicanus* was described by Bocage (1895), with the type locality of Manica, Mozambique. The closest locality of the samples of *T. mossambicanus* used in this study (BILL 73; vicinity of Moebase Village, ~190 km east of Mocuba, Mozambique) is approximately 650 km northeast of the type locality. According to the distribution maps by Broadley (2001) and Broadley and Wallach (2002), no other species of *Thelotornis* is found in that area. However, those samples showed poor amplification in the lab, with only the sequence of the c-mos fragment being included in the analyses. That particular sample is nested within a clade along with MTSN 5236, a sample of *T. kirtlandii* from the Udzungwa Mountains of Tanzania that has shown to have close affinity with *Xyelodontophis*, to

which both of these are sister. Other samples of *T. mossambicanus* are in a clade with *T. usambaricus*, the other East African member of that genus. Due to the inconsistent phylogenetic placement of this taxon, no further conclusions are possible at this point, until a sample from the type locality can be obtained.

T. usambaricus (Broadley 2001) was represented in this study by only a single sample (T_usam), a captive specimen that was imported from Tanzania, and lacks detailed locality information. That sample is nested within a clade that also contains a sample of *T. mossambicanus* (CMRK 337) from Mount Kilimanjaro at the Kenya-Tanzania border, as well as several samples of *Thelotornis* sp. from Watamu, Kenya (JS01, JS02). More samples including the type locality (Amani Nature Reserve, Tanzania) are needed before conclusions regarding its phylogenetic placement and taxonomic validity can be made.

Perhaps the most interesting phylogenetic placement associated with *Thelotornis* is that of *Xyelodontophis*, which is nested deep within the *Thelotornis* clade, rendering the latter paraphyletic. Both samples (Xyelo and BILL 661) of *X. uluguruensis* were consistently placed as the sister taxa to MTSN 5272, a sample of *T. kirtlandii* from the Udzungwa Mountains of Tanzania. As previously mentioned, that sample is thought to be a misidentified *Xyelodontophis* sp. The same may also be the case for other samples that are associated with this clade in some analyses, such as MTSN 5236, another *T. kirtlandii* from the Udzungwa Mountains of Tanzania, and BILL 73, *T. mossambicanus* from Moebase Village, Mozambique. Since the placement of *Xyelodontophis* is highly consistent among the different analyses, and is strongly supported by high bootstrap proportions, I recommend that *X. uluguruensis* is moved to the genus *Thelotornis*.

2.4.5 Systematic Relationships of Dispholidus

The genus *Dispholidus* has long been suspected to contain multiple lineages, but a wide distribution range combined with a complex pattern of polymorphism has complicated the elucidation of evolutionary patterns in this clade. Many species and subspecies have been

proposed in this genus (e.g., Smith 1828, Smith 1841, Laurent 1955, Perret 1961), but none have subsequently been accepted. Based on the results of this study, there are at least four distinct clades of *Dispholidus*. Clade A contains samples of the eastern and southeastern parts of the Democratic Republic of Congo (ELI 1434 and ELI 79, respectively), northwestern Zambia (JM 1857 and JM 1858), and northeastern Angola (BILL 690 and TB39). That clade is sister to a large clade that contains all other lineages of *Dispholidus*. Clade B contains samples from coastal southeastern Kenya (WW 1438, WW 3101 and WW 3104), clade C contains samples from eastern and far eastern Democratic Republic of Congo (ELI 1316 and EBG 2673, respectively), southern Rwanda (CMRK 337), far northern Tanzania (BILL 634), and central Burundi (ELI 1280). Clade D contains samples from the southern parts of South Africa, ranging from Cape Town (KTH 0708) in the West to St. Lucia (TGE T10-75) in the East, and north to Lingelihle (TGE T3-09). Clade E contains two samples from eastern and northeastern Tanzania (CMRK 286 and FMNH 250444, respectively), and is sister to Clade F, which in turn contains samples from the northern parts of South Africa (e.g., TGE T1-17, TGE T4-19), Namibia (KTH 09294), Botswana (CMRK 250, WW 2097), Swaziland (WW 2298, WW 2317) and Mozambique (I29417). That topology is largely consistent throughout all analyses, including the nuclear data.

These results provide strong evidence for the presence of multiple distinct lineages within the genus *Dispholidus*, and in turn raise the need for a reevaluation of the morphological systematics of that group, and a potential formal description of the lineages that were discovered in this study (figure 2.7), which are currently in preparation. The type locality of *D. typus* is the 'Eastern districts of South Africa' (Smith 1828), which refers to what is today known as the Eastern Cape province of South Africa. All of the samples from that area (TGE T3-9, WW 2588, WW 2655, BILL 635, I29420) fall into clade D, thus the name *D. typus* would be retained for that clade only. Clade E contains only two samples (CMRK 286, FMNH 250444), which are nested within clade F in some analyses. More samples are needed to better assess the phylogenetic position of those specimens. Clade F is well-defined, occurring north of the Great Escarpment of

South Africa. The name *D. viridis* (Smith 1838) is available for that lineage with the type locality of 'Old Latakoo' (Smith 1838), which is now known as Dithakong (Lye 1975), a small village in the Northern Cape of South Africa. The closest samples to that locality are MB21431 (approximately 5 km southeast of the type locality), DPS 23 (approximately 100 km East of the type locality), TGE T1-17 and TGE T1-18 (both approximately 115 km West of the type locality), all of which consistently fall within clade F. There is no apparent overlap between clades D and F, as they are largely separated by the Great Escarpment of South Africa. Clade C contains a sample from Uvira, Democratic Republic of Congo (EBG 2673), the type locality of *D. t. kivuensis* (Laurent 1955), which should be resurrected and elevated to species status for that clade. For clade A, the name *D. t. punctatus* (Laurent 1955) should be resurrected and elevated to species status. The closest samples to the type locality of Dundo, Angola are BILL 690 and TB 39 (both approximately 225 km south of the type locality).

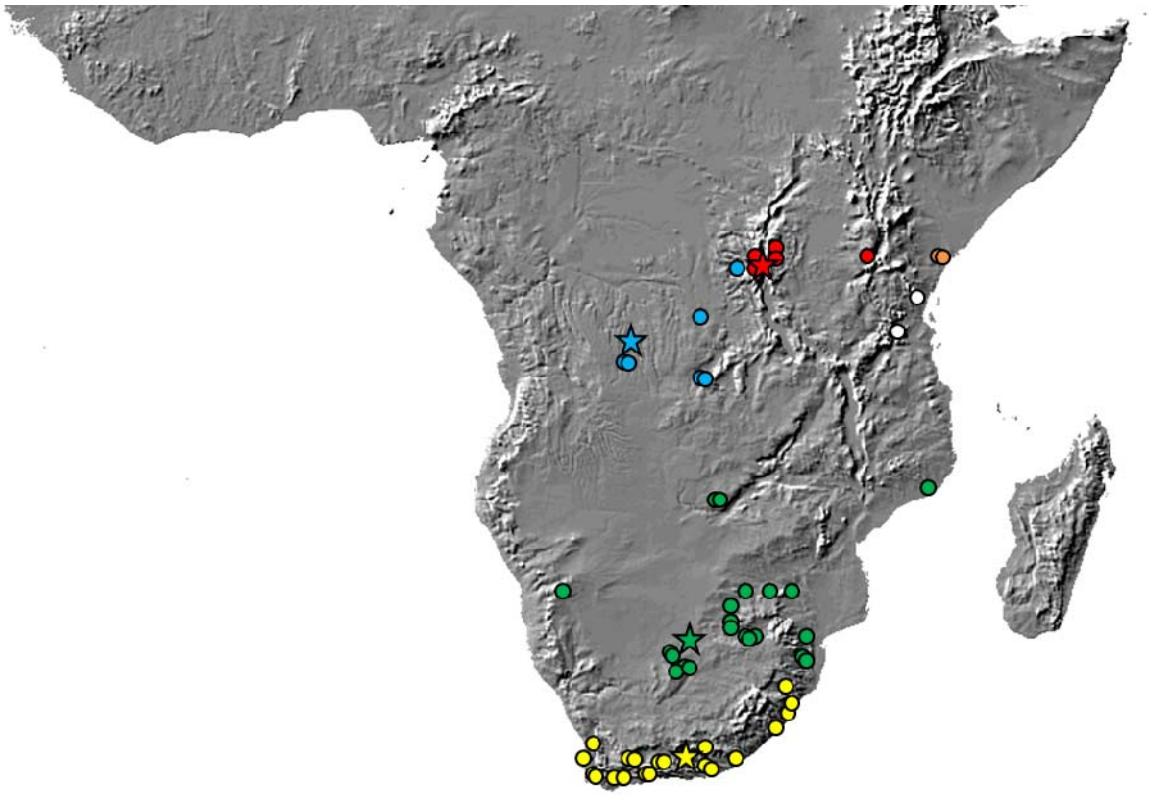

Figure 2.8. Distribution of *Dispholidus clades*. Legend: *Dispholidus* typus A. Smith 1828 = yellow circles; *Dispholidus viridis* A. Smith 1838 = green circles; *Dispholidus punctatus* Laurent 1955 = blue circles; *Dispholidus kivuensis* Laurent 1955 = red circles; *Dispholidus* sp. (Tanzania) = white circles; *Dispholidus* sp. (Kenya) = orange circles. Stars indicate the corresponding type locality, when applicable.

2.4.6 Support from Bootstrap Proportions

Bootstrapping is a tool that is commonly used to estimate sampling error in phylogenetic inference (Page and Holmes 1998). It works by repeatedly resampling the data set and comparing the various individual estimates made, based on a preset number of pseudoreplicates. Under specific conditions (equal rates of change, symmetric phylogeny and internodal change of 20% or less of the characters), bootstrap proportions that are greater than or equal to 70% are thought to correspond to a probability of 95% that the clade in question is real, which indicates a positive relationship between high bootstrap proportions and phylogenetic accuracy (Hillis and Bull 1993). Given those guidelines, many of the bootstrap proportions that are associated with the

clades recovered in this study are relatively low. However, there have been several distinct interpretations of the role of bootstrap values in phylogenetic inference (Berry and Gascuel 1996, Soltis and Soltis 2003), each with emphasis on different aspects of that analysis. For instance, Efron (1979) and Felsenstein (1985) interpreted bootstrap proportions as a measure of repeatability (rather than accuracy). Given the same method of tree construction, high proportions from bootstrap pseudoreplicates would indicate a high probability of inferring the same topology if a different sample was available. Using that interpretation, low bootstrap proportions thus indicate low repeatability, low phylogenetic signal, or erroneous clades. A different interpretation is that of viewing bootstrap proportions as a conservative measure of accuracy or probability of it representing a true clade, given certain conditions (e.g., Sanderson 1989; Zharkikh and Li 1992a, 1992b; Hillis and Bull 1993). Low bootstrap proportions would thus indicate a clade that has a low probability of representing true relationships. Finally, bootstrap proportions can be interpreted as confidence intervals in the sense of a statistical hypothesis test (Felsenstein and Kishino 1993; Efron et al. 1996; Zharkikh and Li 1995). In this case, low bootstrap proportions would indicate that the observed relationships are incorrect, given the null hypothesis.

Despite the variety of interpretations, it tends to be common practice to disregard clades that have low associated bootstrap proportions. In this study, low proportions from bootstrap replicates by themselves are not considered grounds to disregard those clades that are supported by multiple lines of evidence (i.e., both mitochondrial and nuclear markers) and multiple types of phylogenetic inference (i.e., maximum likelihood and maximum parsimony). Instead, low bootstrap proportions are interpreted here as a symptom of variability in the data, which in turn may be an indication of cryptic diversity that is concealed by the small sample size of some of those clades. It may also be an artifact of a large number of compatible but uninformative characters (Faith and Cranston 1991), invariant characters (Kluge and Wolf 1993), and autapomorphies (Carpenter 1992). In addition, there is also evidence that increased taxon sampling size may decrease bootstrap proportions (Sanderson and Wojciechowski 2000). One

also stands to reason that the assumptions underlying the interpretation made by Hillis and Bull (1993) are not realistic for phylogenies (Soltis and Soltis 2003), and that a violation of all of them may lead to an overestimation of accuracy for bootstrap proportions over 50% (Hillis and Bull 1993). For the time being, this author follows the suggested approach of Felsenstein and Kishino (1993), which is that given the bootstrap proportion *P* for a given clade, 1 – *P* is considered the probability of erroneously accepting a clade that is not real (type I error).

2.4.7 Abbreviated Synonomies

Based on these data, a number of taxonomic changes are recommended in order to incorporate these results into the current classification of these snakes. In this section I provide a list of abbreviated synonomies of dispholidine taxa.

Dispholidus kivuensis Laurent

Dispholidus typus kivuensis Laurent, 1955, Revue Zool. Bot. Afr. 51: 127. Type locality: Uvira, Kivu. Congo Belge [= Democratic Republic of Congo]. Holotype: MRAC 17505.

Dispholidus punctatus Laurent

Dispholidus typus punctatus Laurent, 1955, Revue Zool. Bot. Afr. 51: 129. Type locality: Dundo, Angola. Holotype: MRAC 17395.

Dispholidus typus A. Smith

Bucephalus typus A. Smith, 1828, South African Commercial Advertiser 3 (144): 2, col. 4. Type locality: Eastern districts of South Africa; 1829, Zool. Journ., 4: 441 (*B. typicus*).

Bucephalus Jardineii A. Smith, 1828, South African Commercial Advertiser 3 (144): 2, col. 4. Type locality: Cape Town, South Africa; 1829, Zool. Journ. 4: 442.

Bucephalus gutturalis A. Smith, 1828, South African Commercial Advertiser 3 (144): 2, col. 4. Type locality: Forests of the eastern districts of South Africa; 1829, Zool. Journ., 4: 442.

Bucephalus Bellii A. Smith, 1828, South African Commercial Advertiser 3 (144): 2, col. 4. Type locality: Eastern districts of South Africa; 1829, Zool. Journ., 4: 442.

Dispholidus Lalandii Duvernoy, 1832, Ann. Sci. Nat. (Paris) 26: 150. Type locality: Cape of Good Hope.

Dendrophis colubrina Schlegel, 1837, Essai Phys. Serp. 2: 238, Pl. ix, fig. 14-16. Type locality: Rondesbosch, [Western] Cape Province, South Africa.

Bucephalus capensis A. Smith, 1841, Illus. Zool. S. Africa, Rept.: Pl. x-xiii. Type locality: Cape Province, South Africa; Bocage, 1895: 121.

Dispholidus viridis A. Smith

Bucephalus viridis A. Smith, 1838, Illus. Zool. S. Africa, Rept.: Pl. iii. Type locality: Old Latakoo [Northern Cape Province], South Africa.

Dendrophis pseudodipsas Bianconi, 1848, Nuovi Ann. Sci. Nat. (2) 10: 108, Pl. iv, fig. 2 & 1850. Spec. Zool. Mosamb. 40, Pl. iv, fig. 2. Type locality: [Inhambane] Mozambique. Holotype: Bologna 100296.

Thrasops jacksonii mossambicus Mertens, 1937. Abhand. Senckenberg. Naturf. Ges., No. 435: 13. Type locality: Cheringoma Farm, Inhaminga, Mozambique. Holotype: SMF 22246.

Rhamnophis aethiopissa Günther

Rhamnophis aethiopissa Günther, 1862, Ann. Mag. nat. Hist. (3) 9: 129, Pl. x. Type locality: West Africa; Roux-Esteve, 1965: 65, fig. 16: Chippaux, 1999: 97.

Thrasops splendens Andersson, 1901, Bihang Till K. Svenska Vet.-Akad. Handl. 27(5): 11, Pl. 1, fig. 8. Type localities: Bibundi & Mapanja, Cameroon.

Rhamnophis ituriensis Schmidt, 1923, Bull. Amer. Mus. nat. Hist. 49: 81, fig. 4. Type locality: Niapu, Belgian Congo [= Democratic Republic of Congo]; Witte, 1941: 202.

Rhamnophis aethiopissa elgonensis Loveridge, 1929, Bull. U. S. nat. Mus. 151: 24. Type locality: Yala [= Lukosa] River at the foot of Mount Elgon, Kenya; 1944: 129.

Rhamnophis aethiopissa aethiopissa Loveridge, 1944: 126; Perret, 1961: 136; Villiers, 1966: 1739; Stucki-Stirn, 1979: 335.

Rhamnophis aethiopissa ituriensis Loveridge, 1944: 128; Laurent, 1956: 189, 355; 1960: 47 & 1964: 108; Bourgeois, 1968: 109, fig. 43-46; Broadley, 1991: 532.

Thrasops aethiopissa elgonensis Loveridge, 1957: 264; Pitman, 1974: 101, Pl. T, fig. 3; Spawls, 1978: 5.

Thrasops aethiopissa aethiopissa Hughes & Barry, 1969: 1018; Trape & Roux-Esteve, 1995: 40.

Thrasops (*Rhamnophis*) *aethiopissa* Hinkel, 1992: 144, Pl. 130.

Rhamnophis batesii Boulenger

Thrasops batesii Boulenger, 1908, Ann. Mag. nat. Hist. (8) 2: 93. Type localities: Akok and Efulen, Cameroon; Trape & Roux-Esteve, 1995: 40; Chippaux, 1999: 99.

Rhamnophis batesii Schmidt, 1923: 83, fig. 5; Loveridge, 1944: 125; Laurent, 1956: 355, Pl. xx, fig. 1; Perret, 1961: 136; Villiers, 1966: 1739; Stucki-Stirn, 1979: 339.

Thelotornis capensis capensis A. Smith

Thelotornis capensis A. Smith, 1849, Ill. Zool. S. Africa, Rept. App.: 19. Type locality: 'Kaffirland and the country towards Port Natal', i.e. Durban (type lost).

Thelotornis kirtlandii capensis Loveridge, 1944: 154 (part).

Thelotornis capensis capensis Broadley, 1979: 126.

Thelotornis capensis oatesii Günther

Oxybelis Lecomtei (not Dumeril & Bibron) Peters, 1854: 623 (part, Tete).

Dryiophis oatesii Günther, 1881. In Oates' Matabeleland and the Victoria Falls, App.: 330, Col. Pl. D. Type locality: Matabeleland [= western Zimbabwe]. Type: BMNH 1946.1.9.76

Thelotornis Kirtlandii (not Hallowell) Peters, 1882: 131 (part).

Thelotornis kirtlandii capensis Loveridge, 1944: 154 (part).

Thelotornis capensis (not A. Smith) Witte, 1953: 249, fig. 82.

Thelotornis kirtlandii oatesii Loveridge, 1953: 277.

Thelotornis capensis oatesii Laurent, 1956: 231, fig. 35.

Thelotornis kirtlandii (Hallowell)

Leptophis Kirtlandii Hallowell, 1844, Proc. Acad. nat. Sci. Philadelphia: 62. Type locality: Liberia, type ANSP 5271.

Oxybelis Lecomtei Dumeril & Bibron, 1854. Erpet. Gen., 7: 821. Type locality: Gabon.

Tragophis rufulus Dumeril & Bibron, 1854, Erpet. Gen., 7: 827. Type locality: Senegal.

Oxybelis violacea Fischer, 1856, Abhand. Nat. Ver. Hamburg, 3: 91, Pl. ii, fig. 7. Type locality: Edina, Grand Bassa County, Liberia.

Dryiophis Kirtlandii Bocage, 1895: 119 (part).

Thelotornis kirtlandii Schmidt, 1923: 112, Pl. xiv; Bogert, 1940: 69; Witte, 1953: 247, fig. 82; Laurent, 1964: 116.

Thelotornis kirtlandii kirtlandii Loveridge, 1944: 149 (part).

Thelotornis mossambicanus (Bocage)

Oxybelis Lecomtei (not Dumeril & Bibron) Peters, 1854: 623 (part).

Thelotornis Kirtlandii (not Hallowell) peters, 1882: 131 (part), Pl. xix, fig. 2.

Dryiophis Kirtlandii var. *mossambicana* Bocage, 1895, Herp. Angola & Congo: 119. Type locality: Manica, Mozambique. Lectotype: MBL 1843 (destroyed).

Thelotornis kirtlandii capensis (not A. Smith) Mertens, 1937: 14.

Thelotornis capensis (not A. Smith) Bogert, 1940: 70 (part), fig. 11.

Thelotornis capensis capensis (not A. Smith) Laurent, 1956: 230 & 378.

Thelotornis capensis mossambicanus Broadley, 1979: 129.

Thelotornis mossambicanus Broadley, 2001: 60.

Thelotornis uluguruensis Broadley and Wallach

Xyelodontophis uluguruensis Broadley and Wallach 2002, Bull. nat. Hist. Mus. Lond. (Zool.) 68(2): 66. Type locality: Lupanga Peak, Uluguru Mountains, Tanzania. Holotype: KMH 2636; Eimermacher, 2007, Master's Thesis, Southeastern Louisiana University: 15.

Thelotornis usambaricus Broadley

Thelotornis kirtlandii (not Hallowell) Stejneger, 1893: 733.

Thelotornis kirtlandii kirtlandii (not Hallowell) Loveridge, 1944: 149 (part).

Thelotornis capensis mossambicanus (not Bocage) Broadley, 1979: 126 (part); rasmussen, 1997: 138 (part).

Thelotornis usambaricus Broadley, 2001, Afr. J. Herpetol. 50 (2): 58. Type locality: Amani Nature Reserve, (Kwamkoro/Kwemsambia Forest reserve), East Usambara Mountains, Tanzania. Holotype: NMZB 16182.

Thrasops flavigularis (Hallowell)

Dendrophis flavigularis Hallowell, 1852, Proc. Acad. nat. Sci. Philadelphia: 205. Type locality: 'Liberia', later corrected to Gabon.

Hapsidophrys niger Günther, 1872, Ann. Mag. nat. Hist. (4) 9: 25. Type locality: Gabon.

Thrasops pustulatus Buchholz and Peters, 1875, Monatsb. Akad. Wiss. Berlin: 199/ Type locality: Mungo, Cameroon.

Thrasops flavigularis Bocage, 1895: 97; Bogert, 1940: 58; Loveridge, 1944: 132; Trape and Roux-Esteve, 1995: 40; Chippaux, 1999: 95.

Thrasops flavigularis flavigularis Stucki-Stirn, 1979: 319.

Thrasops flavigularis stirnensis Stucki-Stirn, 1979: 632.

Thrasops jacksonii Günther

Thrasops Jacksonii Günther, 1895, Ann. Mag. nat. Hist. (6) 15: 528. Type locality: Kavirondo, Kenya.

Rhamnophis jacksonii Boulenger, 1896: 632.

Thrasops Rothschildi Mocquard, 1905, Bull. Mus. natn. Hist. nat. 11: 287. Type locality: 'Afrique orientale anglaise'.

Thrasops jacksonii jacksonii Loveridge, 1936: 249, 1944: 134 & 1957: 264; Bogert, 1940: 58; Witte, 1953: 200; Laurent, 1956: 187, 354 & 1960: 46; Roux-Esteve, 1965: 66, fig. 17; Villiers, 1966: 1739; Bourgeois, 1968: 124, 278, fig. 51; Pitman, 1974: 99, Pl. G, fig. 4; Spawls, 1978: 5; Broadley, 1991: 532; Hinkel, 1992: 319, Pl. 306; Trape and Roux-Esteve, 1995: 40.

Thrasops schmidti Loveridge

Thrasops jacksonii schmidti Loveridge 1936, proc. Biol. Soc. Washington 49: 63. Type locality: Meru Forest, Mount Kenya, Kenya; 1944: 137 & 1957: 264; Spawls, 1978: 5.

Thrasops occidentalis Parker

Thrasops occidentalis Parker, 1940, Ann. Mag. nat. Hist. (11) 5: 273, fig. 1 & 2a. Type locality: Axim, Gold Coast [= Ghana]; Loveridge, 1944: 131; Cansdale, 1961: 31, Pl. vi, fig. 11 & 12; Hughes and Barry, 1969: 1018, Chippaux, 1999: 100.

2.5 Conclusions

The results provide support for the monophyly of dispholidine snakes, with members of the genera *Philothamnus* and *Hapsidophrys* being sister taxa. Members of the genus *Thrasops* are the most basal taxon within Dispholidini, and are sister to a clade that contains all other taxa. *Rhamnophis* and *Thrasops* are each monophyletic to the exclusion of one another, with *Rhamnophis* being sister to *Dispholidus-Thelotornis*. The relationships within *Thelotornis* are incompletely resolved, and for further investigation with increased sampling from throughout their range. *Xyelodontophis* is consistently nested within *Thelotornis* in the analyses, which provides strong support for moving *X. uluguruensis* to *Thelotornis*. There is also strong evidence for the resurrection of several junior synonyms of *D. typus*, which should further be elevated to species status. This includes *D. viridis* from southern Africa, *D. kivuensis* from the Democratic Republic of Congo, and *D. punctatus* from Angola. *D. typus* proper is reserved for specimens from mostly south of the Great Escarpment South Africa, and west of eastern KwaZulu-Natal. There is also

evidence for additional taxa from East Africa, but more thorough sampling is needed before taxonomic recommendations may be made.

CHAPTER 3

MULTI-VARIATE ANALYSES OF EXTERNAL MORPHOLOGY IN DISPHOLIDINE COLUBRIDS (SERPENTES: COLUBRIDAE)

3.1. Introduction

3.1.1. The Role of External Morphology in Colubrid Systematics

Morphology has played a central role in colubrid systematics, with different authors placing emphasis on different morphological structures, often without an explanation of why a certain class of characters were utilized (Dowling 1967). For instance, Smith (1828) placed emphasis on coloration and size, Dumeril, Bibron, and Dumeril (1854) on dentition, Boulenger (1893) on vertebral structure, Cope (1893, 1895) on hemipenial structure, and Wallach (1991) on viscera. The resulting systematic classification differ not only when a single class of characters is utilized, but also when differential weights are assigned to multiple classes of morphological characters (Dowling 1967). An example of such a case are the classifications inferred by Dunn (1928) and Bogert (1940), who each placed differential weights on vertebral structure and hemipenial characters. Nonetheless, morphological characters play an important role in the study of colubrid relationships, and are widely relied upon throughout the secondary literature.

Like most reptile taxa, dispholidine colubrids have traditionally been described and classified using external morphological characters, such as scale counts and relative body lengths (e.g., Broadley and Wallach 2002, Eimermacher 2007). Scale counts in particular are commonly used in both species descriptions and field guides to delimit and identify taxa (e.g. Broadley 2001, Broadley and Wallach 2002), and have been an important facet of squamate systematics. Some authors (e.g., Wallach 1991, 1998) have emphasized visceral anatomy to

investigate the systematics of snakes, but the utility of visceral characters in the field is often rather limited. Reproductive traits, such as hemipenial characters, have been shown to be useful in systematic studies (e.g., Dowling and Savage 1960, Dowling 1967, Slowinski 1995, Zaher 1999, Schargel and Castoe 2003), but their utility in the field is also limited. On the other hand, external morphological characters are easy to score and can be utilized for proper identification in the field, as they can be easily observed in live specimens with a minimal amount of manipulations.

3.1.2 Quantitative Approaches to Morphological Systematics

Historically, descriptive statistics have extensively been used to investigate the systematic relationships of colubrid snakes (e.g., Malnate 1960), as is the case with the vast majority of literature on dispholidine systematics (Broadley 2001, Broadley and Wallach 2002). However, Eimermacher (2007) investigated the morphological systematics of this group in a phylogenetic frame work, an approach that has also been taken by other authors in colubrid systematics (e.g., Hollis 2006). While that approach allows for the testing of hypotheses, it does require *a priori* knowledge of the natural groups at hand, and is ineffective at the discovery of previously unsuspected clades.

The goal of this study is to evaluate the utility of those external morphological characters that have traditionally been emphasized in dispholidine colubrids, and to see which of them are useful to distinguish between the different taxa within dispholidine colubrids.

3.2 Materials and Methods

3.2.1 Taxonomic Sampling

I utilized a dataset previously used by Eimermacher (2007) that included samples of the sixteen known taxa (table 3.1), as determined by the conclusion of the molecular analysis (see chapter 2). Specimens from West Africa, which were not represented in the molecular analysis, were grouped separately (*Dispholidus* sp. [West Africa]) in order to test for the potential of geographical distinctiveness, as indicated by morphological variation. In addition, specimens from Pemba Island were also treated separately (*Dispholidus* sp. [Pemba Island]) in order to test for

the hypothesis that specimens from that locality represent a distinct species of *Dispholidus* (Hughes, as quoted by Broadley and Wallach 2002).The final data set included 1417 specimens (see Appendix A), representing 18 OTUs. Specimens were housed in the following institutions: Angola - Museu Regional do Dundo (MD), Dundo; Belgium - Institut Royal des Sciences Naturelles de Belgique (IRSNB), Brussels; Musée Royal de l'Afrique Centrale (MRAC), Tervuren; Brazil - Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo; Bulgaria - Asenovgrad Museum (AM), Asenovgrad; Denmark - Universitets København (ZMUC), Copenhagen; France - Museum National d'Histoire Naturelle (MNHN), Paris; Germany - Senckenberg Forschungsinstitut und Naturmuseum (SMF), Frankfurt; Staatliches Museum für Naturkunde (SMNS), Stuttgart; Zoologisches Museum Universität Humboldt (ZMB), Berlin; Zoologisches Museum für Hamburg (ZMH), Hamburg; Italy - Università di Firenze (MZUF), Florence; Kenya – National Museums of Kenya (NMK), Nairobi; Portugal - Museu Bocage (MBL), Lisboa; Senegal - Instituto Fondamental d'Afrique Noire (IFAN), Dakar; South Africa - Albany Museum (AMG), Grahamstown; Durban Museum and Art Gallery (DM), Durban; McGregor Museum (MMK), Kimberley; Natal Museum (NMP), Pietermaritzburg; Port Elizabeth Museum (PEM), Port Elizabeth; South African Museum (SAM), Cape Town; Transvaal Museum (TMP), Pretoria; Spain - Unidad de Zoología Aplicada (UZA), Madrid; Sweden - Naturhistoriska Rijkmuseet (NHRM), Stockholm; Switzerland - Museum d'Histoire Naturelle (MHNG), Genève; United Kingdom - The Natural History Museum (BMNH), London; United States of America - American Museum of Natural History (AMNH), New York; California Academy of Sciences (CAS), San Francisco; Carnegie Museum of Natural History (CM), Pittsburgh; Field Museum of Natural History (FMNH), Chicago; Louisiana Museum of Natural History (LSUMZ), Baton Rouge; Museum of Comparative Zoology (MCZ), Cambridge; San Diego Natural History Museum (SDSNH), San Diego; Florida Museum of Natural History (UF), Gainesville; University of Michigan (UMMZ), Ann Arbor; National Museum of Natural History (USNM), Washington D. C.; Natural History Museum of Zimbabwe (NMZB/NMZB-UM), Bulawayo. A complete list of specimens is included in Appendix A.

Table 3.1. Taxon sampling for Principal Component Analysis.

Taxa	Sample Size (n)
Dispholidus typus	20
Dispholidus kivuensis	100
Dispholidus punctatus	87
Dispholidus viridis	15
Dispholidus sp. (Pemba Island)	3
Dispholidus sp. (West Africa)	3
Thelotornis kirtlandii	118
Thelotornis capensis capensis	160
Thelotornis capensis oatesii	370
Thelotornis mossambicanus	261
Thelotornis usambaricus	58
Xyelodontophis (Thelotornis) uluguruensis	5
Thrasops flavigularis	44
Thrasops jacksonii	57
Thrasops occidentalis	27
Thrasops schmidti	18
Rhamnophis aethiopissa	60
Rhamnophis batesii	24

3.2.2 Morphological Characters

All characters were derived from the external morphology, and included the number of anterior dorsal scale rows, number of mid-dorsal scale rows, number of posterior dorsal scale rows, snout-vent length, tail length, number of subcaudal scales, number of ventral scales, number of upper labial scales, number of lower labial scales, and number of postocular scales. Many of the characters used in this study have previously been utilized for this group (Broadley 2001, Broadley and Wallach 2002, Eimermacher 2007), and have been widely used in other studies of colubrid systematics (e.g., Broadley 1966a, 1966b, 1977, 1992, 1994, 1996; Broadley and Hughes 1993, Hollis 2006, Hughes 1985, Rasmussen 1985, 1993a, 1993b, 1997, Rasmussen and Largen 1992, Rasmussen et al. 1995). Characters were scored from preserved

museum specimens and supplemented with records from the published literature. Lengths were measured to the nearest millimeter using a metric ruler, and the number of ventral scales was scored using the method of Dowling (1951).

3.2.3 Statistical Analyses

We used multivariate statistics to assess whether the external morphological characters that are most commonly relied upon in dispholidine colubrid systematics are able to distinguish the different taxa in that clade. Data sets were partitioned by genus, and in the case of *T. capensis*, additionally by species, to test for distinctiveness at the subspecies level. Morphological differentiation between taxa was assessed using a principal component analysis (PCA) in Systat 11. This provided the advantage of making no *a priori* assumptions about the groupings within the data (McGarial et al. 2000), which was of particular importance, given the uncertainty of the validity of some of the currently accepted taxa, which were poorly represented in the molecular data (see chapter 2). The resulting principal components were then examined using scatter plots, and grouped according to species or subspecies. In order to test the effects of ontogeny and sexual dimorphism, the principal components were also grouped by age (adult vs. juvenile) and gender (male vs. female). Similar approaches using principal component analysis to investigate questions of snake systematics have previously been used by a number of authors (e.g., Thorpe and McCarthy 1978, Wüster et al. 1992a, 1992b, 1997, Lenk and Wüster 1999, Puorto et al. 2001).

3.3 Results

When the principal components were plotted according to age and gender groups, the results showed no distinctiveness in the evaluated characters based on those factors. However, many of the specimens lacked data regarding gender, and nearly all were adult specimens.

3.3.1 Genus Dispholidus

The first two principal components explained the majority of the variance (29.1% and 22.1% variance, respectively; see table 3.2). Plotted against each other, the resulting pattern showed significant amount of overlap in all principal components (Figure 3.1), especially in the

case of *D. kivuensis*, whose principal components overlap with those of every other taxon, except *Dispholidus* sp. from Pemba Island. The latter showed separation in both of the first two principal components, but not in the third principal component. The component loadings and associated factor coefficients indicate the number of subcaudal scales, tail length, and snout-vent length to be the significant factors (Tables 3.3 & 3.4).

Table 3.2. Relative variance in *Dispholidus* explained by principal components.

	PC 1	**PC 2**	**PC 3**
Eigenvalue	2.33	1.77	1.09
Percent of Total Variance	29.15	22.11	13.61

Table 3.3. Component Loadings of principal components of *Dispholidus*.

Character	**PC 1**	**PC 2**	**PC 3**
Tail Length	0.932	0.129	0.009
Snout-Vent Length	0.901	0.288	-0.034
Mid-Dorsal Rows	-0.152	0.762	-0.301
Anterior Dorsal Rows	-0.038	0.680	0.197
Posterior Dorsal Rows	0.326	-0.080	-0.451
Upper Labials	0.423	-0.462	0.399
Ventrals	0.932	0.129	0.009

Table 3.4. Factor Coefficients of principal components of *Dispholidus*.

Character	PC 1	PC 2	PC 3
Tail Length	0.468	0.088	0.009
Snout-Vent Length	0.452	0.196	-0.032
Mid-Dorsal Rows	-0.076	0.517	-0.281
Anterior Dorsal Rows	-0.019	0.462	0.184
Posterior Dorsal Rows	-0.025	0.225	0.710
Upper Labials	0.164	-0.054	-0.420
Ventrals	0.212	-0.314	0.372

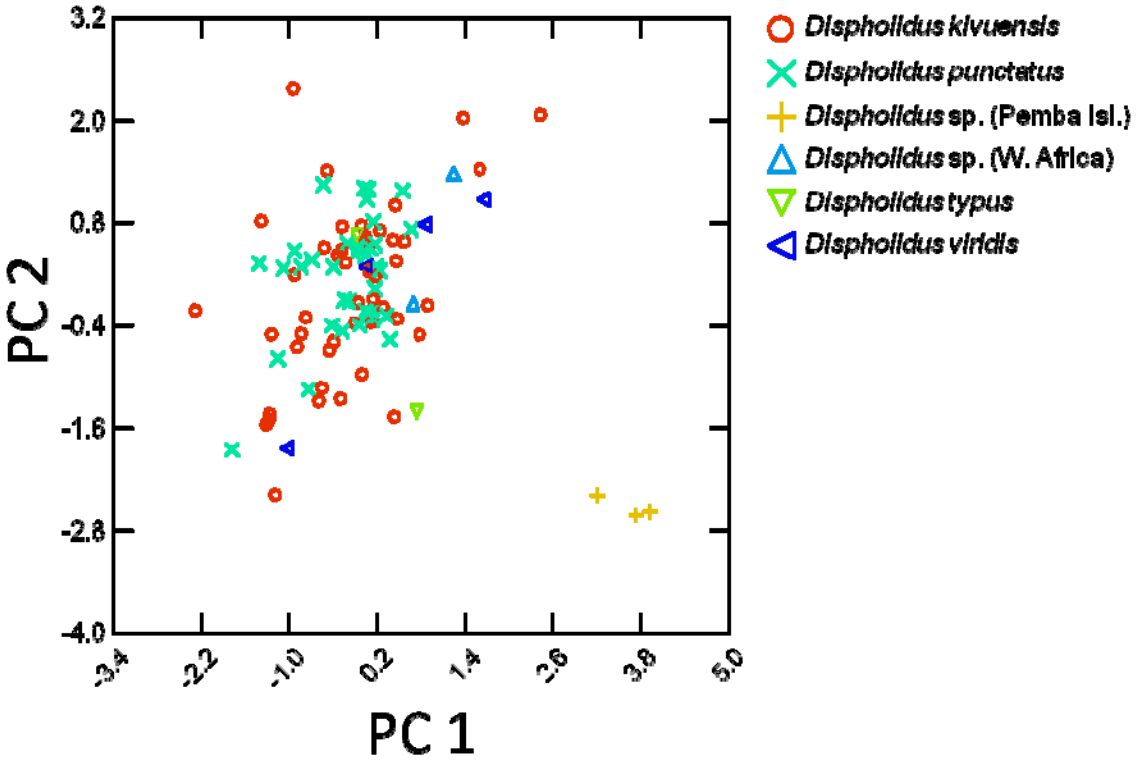

Figure 3.1. Scatter plot of the first two principal components of *Dispholidus*.

3.3.2 Genus Thelotornis

The first two components explain the majority of the variance (22.8% and 16.4%, respectively, see table 3.5). The resulting scatter plot of the first two principal components, grouped by species shows partial separation of *T. kirtlandii*, but lots of overlap in the other taxa (Figure 3.2). *Thelotornis capensis*, *T. mossambicanus*, *T. usambaricus* and *X*. (*Thelotornis*) *uluguruensis* are not distinguishable by the principal components. The second, third, and fourth principal components did not show any separation when grouped by taxa (not shown here).

Table 3.5. Relative variance in *Thelotornis* explained by principal components.

	PC 1	**PC 2**	**PC 3**	**PC 4**
Eigenvalue	2.05	1.48	1.22	1.10
Percent of Total Variance	22.80	16.41	13.50	12.20

Table 3.6. Component Loadings of principal components of *Thelotornis*.

Character	**PC 1**	**PC 2**	**PC 3**	**PC 4**
Tail Length	0.923	0.079	0.222	-0.133
Snout-Vent Length	0.904	0.069	0.037	-0.377
Lower Labials	0.045	0.761	0.192	0.055
Mid-Dorsal Rows	0.212	0.564	-0.239	0.478
Ventrals	0.474	-0.520	-0.200	0.319
Posterior Dorsal Rows	0.161	-0.241	-0.741	0.037
Anterior Dorsal Rows	0.110	0.442	-0.601	0.046
Subcaudals	0.266	-0.173	0.335	0.768
Postoculars	-0.057	0.117	0.087	0.107

Table 3.7. Factor coefficients of principal components of *Thelotornis*.

Character	PC 1	PC 2	PC 3	PC 4
Tail Length	0.450	0.054	0.183	-0.121
Snout-Vent Length	0.440	0.047	0.030	-0.343
Lower Labials	0.022	0.516	0.158	0.050
Mid-Dorsal Rows	0.103	0.382	-0.197	0.435
Ventrals	0.231	-0.352	-0.165	0.291
Posterior Dorsal Rows	0.079	-0.163	-0.610	0.034
Anterior Dorsal Rows	0.053	0.300	-0.495	0.042
Subcaudals	0.129	-0.117	0.276	0.699
Postoculars	-0.028	0.079	0.072	0.097

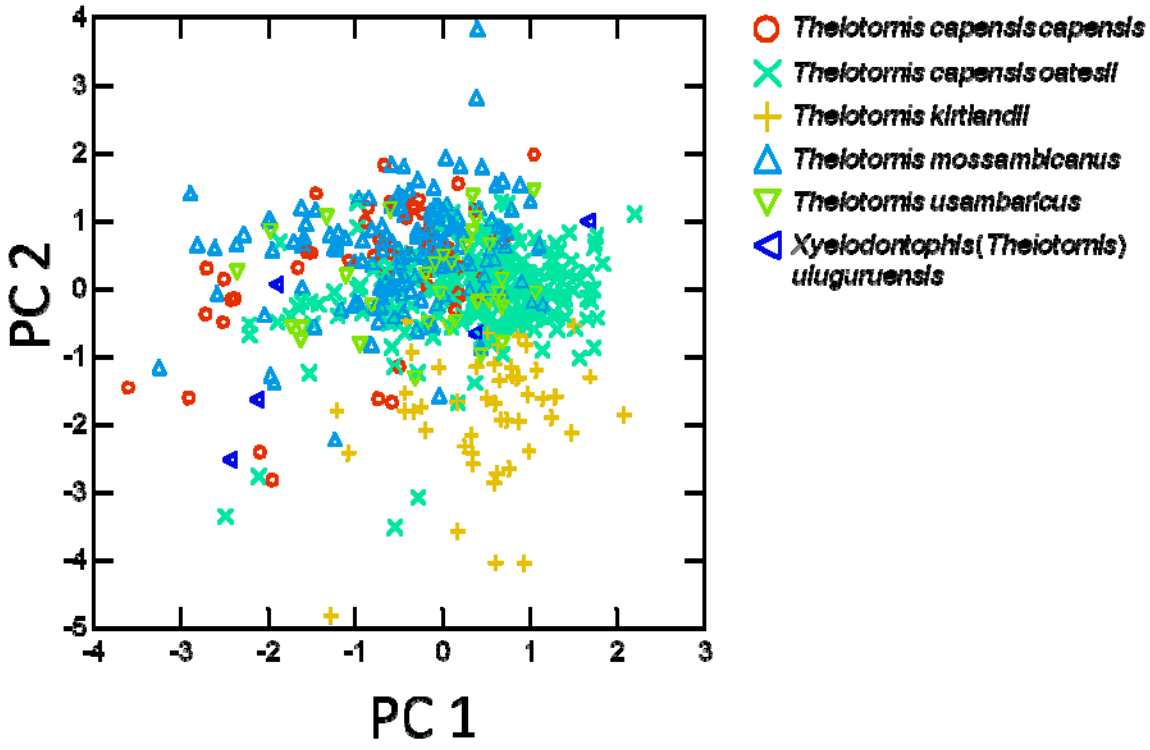

Figure 3.2. Scatter plot of the first two principal components of *Thelotornis*.

3.3.3 Thelotornis capensis

The first two principal components explain the majority of the variance (24.8% and 16.0%, respectively; see table 3.8). The scatter plot of the first two principal components indicates partial distinctiveness between *T. c. capensis* and *T. c. oatesii*, with significant amounts of overlap (figure 3.3). The third and fourth principal components explain only a small amount of the variance (12.9% and 12.3%, respectively; see table 3.8), and the scatter plot shows no separation when graphed with the other principal components (not shown here).

Table 3.8. Relative variance in *Thelotornis capensis* explained by principal components.

	PC 1	**PC 2**	**PC 3**	**PC 4**
Eigenvalue	2.23	1.43	1.17	1.11
Percent of Total Variance	24.83	15.95	12.94	12.32

Table 3.9. Component loadings of principal components of *Thelotornis capensis*.

Character	**PC 1**	**PC 2**	**PC 3**	**PC 4**
Tail Length	0.883	-0.154	-0.224	-0.037
Snout-Vent Length	0.871	-0.123	-0.396	0.12
Anterior Dorsal Rows	0.159	0.651	-0.07	-0.546
Posterior Dorsal Rows	-0.138	0.611	-0.468	0.133
Mid-dorsal Rows	0.486	0.546	0.247	-0.052
Subcaudals	0.285	-0.152	0.628	-0.434
Postoculars	0.031	0.213	0.287	0.68
Lower Labials	0.3	0.409	0.43	0.326
Ventrals	0.495	-0.257	0.107	0.129

Table 3.10. Factor coefficients of principal components of *Thelotornis capensis*.

Character	PC 1	PC 2	PC 3	PC 4
Tail Length	0.395	-0.107	-0.193	-0.033
Snout-Vent Length	0.390	-0.086	-0.340	0.108
Anterior Dorsal Rows	0.071	0.453	-0.060	-0.493
Posterior Dorsal Rows	-0.062	0.426	-0.402	0.120
Mid-dorsal Rows	0.218	0.380	0.212	-0.047
Subcaudals	0.127	-0.106	0.539	-0.392
Postoculars	0.014	0.148	0.246	0.614
Lower Labials	0.134	0.285	0.369	0.294
Ventrals	0.221	-0.179	0.092	0.117

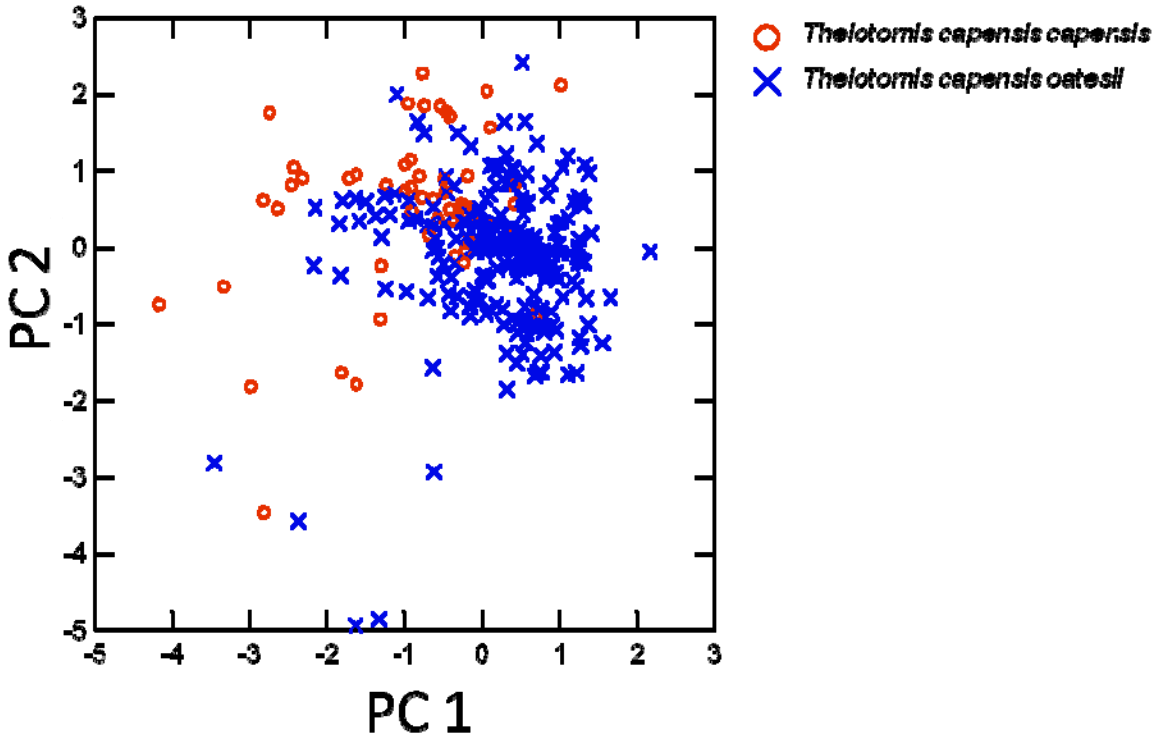

Figure 3.3. Scatter plot of the first two principal components of *Thelotornis capensis*.

3.3.4 Genus Thrasops

The first two principal components explained the majority of the variance (26.6% and 19.1%, respectively; see table 3.11). The scatter plot of the first two principal components showed significant distinctiveness of *T. jacksonii*, *T. flavigularis*, and *T. schmidti*, without any respective overlap (figure 3.4). On the other hand, the principal components of *T. occidentalis* overlapped at least partially with those of all other species within that genus. Snout-vent and tail lengths, as well as mid-dorsal rows were the factors that most prominently loaded the first two principal components (tables 3.12 & 3.13). The third and fourth principal components did not show any significant separation when plotted (not shown here).

Table 3.11. Relative variance in *Thrasops* explained by principal components.

	PC 1	**PC 2**	**PC 3**	**PC 4**
Eigenvalue	2.39	1.72	1.40	1.22
Percent of Total Variance	26.55	19.10	15.53	13.58

Table 3.12. Component loadings of principal components of *Thrasops*.

Character	**PC 1**	**PC 2**	**PC 3**	**PC 4**
Snout-Vent Length	0.913	0.231	0.038	-0.062
Tail Length	0.898	0.254	0.227	-0.098
Ventrals	0.585	-0.504	-0.451	0.030
Subcaudals	0.539	-0.173	0.285	0.275
Mid-dorsal rows	-0.144	0.770	0.049	0.263
Preoculars	-0.072	-0.520	-0.486	0.080
Upper Labials	0.105	-0.518	0.401	0.580
Postoculars	0.270	0.356	-0.790	0.110
Lower Labials	-0.081	0.241	-0.190	0.842

Table 3.13. Factor coefficients of principal components of *Thrasops*.

Character	PC 1	PC 2	PC 3	PC 4
Snout-Vent Length	0.382	0.135	0.027	-0.051
Tail Length	0.376	0.148	0.162	-0.080
Ventrals	0.245	-0.293	-0.323	0.025
Subcaudals	0.226	-0.101	0.204	0.225
Mid-dorsal rows	-0.060	0.448	0.035	0.215
Preoculars	-0.030	-0.302	-0.348	0.066
Upper Labials	0.044	-0.302	0.287	0.475
Postoculars	0.113	0.207	-0.565	0.090
Lower Labials	-0.034	0.140	-0.136	0.689

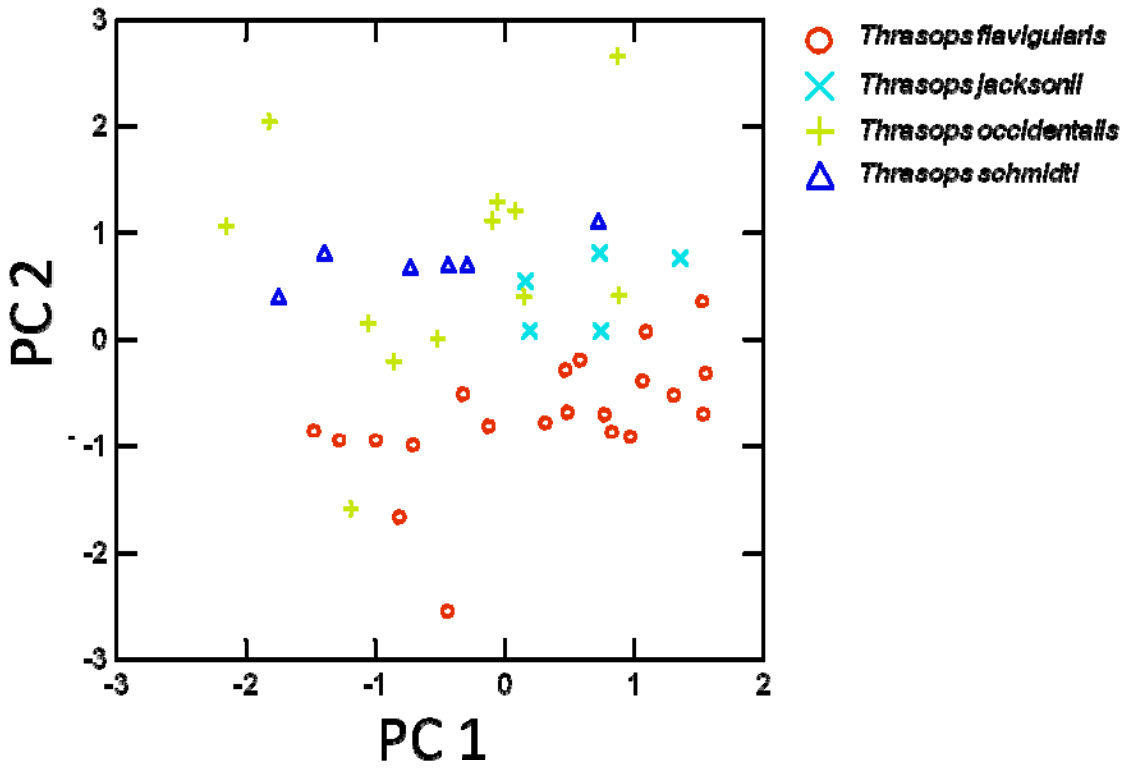

Figure 3.4. Scatter plot of the first two principal components of *Thrasops*.

3.3.5 Genus Rhamnophis

The first two principal components explained the majority of the variance (47.7% and 20.1%, respectively; see table 3.14). The scatter plot of the first two principal components showed significant distinctiveness between the *R. aethiopissa* and *R. batesii* (figure 3.5). Tail-length, the number of mid-dorsal scale rows, and the number of subcaudal and ventral scales were the factors that most significantly loaded the first two principal components (tables 3.15 & 3.16). The third and fourth principal components did not show any separation when plotted (not shown here).

Table 3.14. Relative variance in *Rhamnophis* explained by principal components.

	PC 1	**PC 2**
Eigenvalue	3.34	1.41
Percent of Total Variance	47.74	20.11

Table 3.15. Component loadings of principal components of *Rhamnophis*.

Character	**PC 1**	**PC 2**
Tail Length	0.939	0.202
Mid-dorsal Rows	0.823	0.064
Subcaudals	0.802	0.017
Snout-Vent Length	0.766	0.413
Lower Labials	0.550	-0.549
Ventrals	-0.031	0.806
Upper Labials	0.500	-0.491

Table 3.16. Factor coefficients of principal components of *Rhamnophis*.

Character	PC 1	PC 2
Tail Length	0.281	0.144
Mid-dorsal Rows	0.246	0.045
Subcaudals	0.240	0.012
Snout-Vent Length	0.229	0.293
Lower Labials	0.165	-0.390
Ventrals	-0.009	0.572
Upper Labials	0.149	-0.349

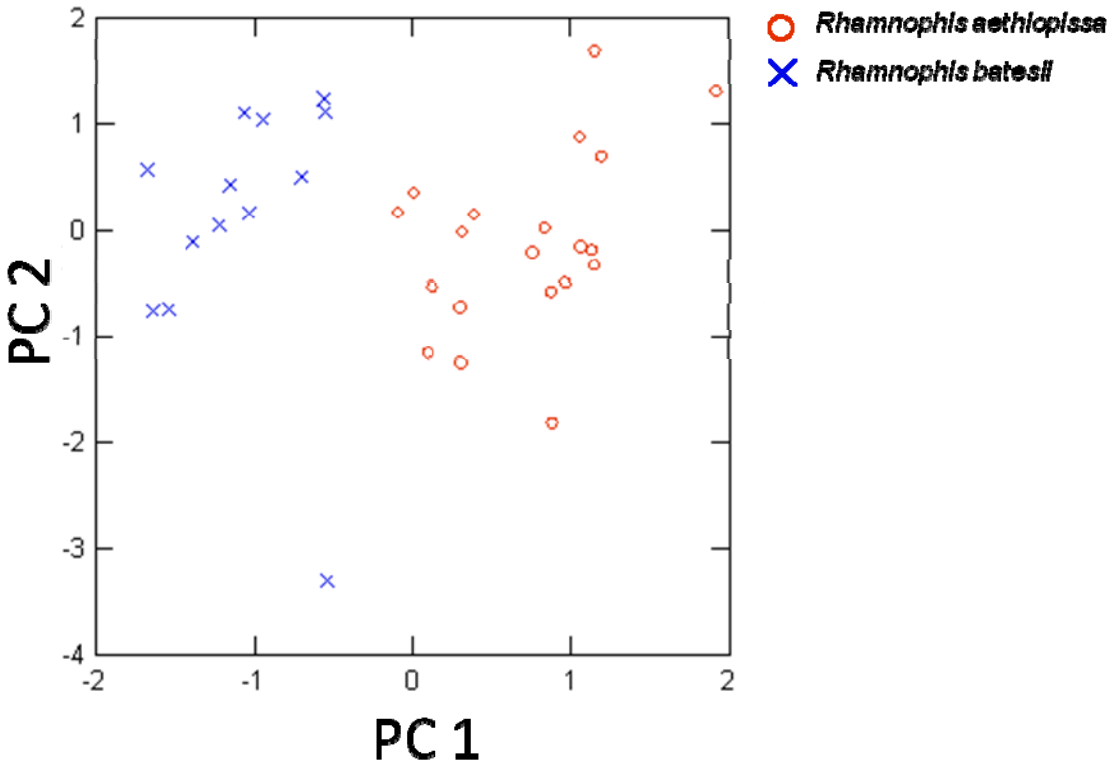

Figure 3.5. Scatter plot of the first two principal components of *Rhamnophis*.

3.4 Discussion

3.4.1 Genus Dispholidus

Within the genus *Dispholidus*, the common external morphological characters were unable to differentiate between the majority of the taxa, with the exception of *Dispholidus* sp. from Pemba Island. According to Broadley and Wallach (2002), Barry Hughes has long held the opinion that specimens from the island that is situated approximately 50 km east of the Tanzanian mainland from a distinct species of *Dispholidus*, and these results provide some support for that hypothesis. Due to the difficulty of obtaining samples from that area, that taxon was not included in the molecular analysis (see chapter 2), and its status remains uncertain. These results support the differentiation of that taxon based on the first two principal components, which are primarily loaded by the number of subcaudal scales, snout-vent length, and tail length. The number of subcaudal scales was 147–77, as opposed to 104–142 in all other species of *Dispholidus* (Broadley and Wallach 2002). Pemba Island specimens also appear to have a longer tail in proportion to the total length of the body (31.88–36.45% in Pemba Island specimens, as opposed to 22.76–29.19% in *D. kivuensis*, 23.29–29.43% in West African specimens, 26.10–27.08% in *D. typus*, 26.10–27.94% in *D. viridis*, and 21.98–28.71% in *D. punctatus*). While these results support the idea of specimens from Pemba Island being distinct from all other specimens, I hesitate to describe these as a new species until samples for a molecular analysis are available.

3.4.2 Genus Thelotornis

In the genus *Thelotornis*, only *T. kirtlandii* is partially distinct in the first two principal components. However, it also shows significant amounts of overlap with the other taxa within that genus, and the third and fourth principal components show complete overlap without any observed distinctiveness. When the two subspecies of *T. capensis*, *T. c. capensis* and *T. c. oatesii*, were analyzed separately, the resulting first and second principal components suffered from significant amounts of overlap, as was the case in the third and fourth principal components. It thus appears that the external morphological characters that were included here are not informative with regard to phylogenetic relationships within that genus.

3.4.3 Genus Thrasops

In the genus *Thrasops*, the first two principal components were able to distinguish between most of the different groups with relatively little overlap. *T. flavigularis* in particular was well distinguished, and both *T. schmidti* and *T. jacksonii* were closely grouped around the centroid. The component loading values indicate that snout-vent length, tail length, and the number of mid-dorsal scale rows were most heavily loading those first two principal components. *T. flavigularis* typically has 13 mid-dorsal scale rows, *T. jacksonii* has 19, and *T. schmidti* has 17 scale rows at midbody. This corroborates the idea that those characters are practical for identification in the field. *T. occidentalis*, which was not distinguished well based on those characters, usually has 15–19 mid-dorsal scale rows. Relative tail (and snout-vent) lengths vary between 21.63–32.28% (*T. flavigularis*), 23.64–31.02% (*T. jacksonii*), and 26.74–34.27% (*T. schmidti*). In *T. occidentalis*, that character varies between 27.08–35.07%.

3.4.4. Genus Rhamnophis

In the genus *Rhamnophis*, the first two principal components clearly distinguished between the two taxa without any significant overlap. The component loadings indicate that tail length, the number of mid-dorsal scale rows, the number of subcaudal scales, snout-vent length, and the number of ventral scales most affected those principal components. *R. aethiopissa* typically has 15–17 midbody scale rows, whereas *R. batesii* usually has 13. In addition, *R. aethiopissa* usually has 117–159 subcaudal scales, while *R. batesii* has 91–114. There is some overlap in the number of ventral scales, with *R. aethiopissa* having 154–179, and *R. batesii* possessing 163–179. With regard to relative body proportions, *R. batesii* tend to have a shorter tail (13.10–30.99%), as compared to *R. aethiopissa* (24.44–37.14%).

3.5 Conclusion

In conclusion, the external morphological characters that are commonly used in dispholidine systematics, field guides, and taxonomic keys are only partially able to distinguish between the different taxa. In the genera *Dispholidus* and *Thelotornis*, only *Dispholidus* sp. from Pemba Island showed distinctiveness based on the number of subcaudal scales, snout-vent

length, and tail length. In the genus *Thrasops*, *T. flavigularis*, *T. jacksonii* and *T. schmidti* showed distinctiveness based on snout-vent length, tail length, and the number of mid-dorsal scale rows. On the other hand, *T. occidentalis* did not show any significant distinctiveness based on those characters. In the genus *Rhamnophis*, both taxa were clearly distinct based on tail length, the number of mid-dorsal scale rows, the number of subcaudal scales, snout-vent length, and the number of ventral scales. These results suggest a need to re-evaluate the type of external morphological characters that are used to investigate the systematics of several of the taxa in this group, especially within the genera *Dispholidus* and *Thelotornis*. The results further indicate a pressing need to evaluate specimens of *Dispholidus* sp. from Pemba Island as a potential undescribed form.

APPENDIX A

SPECIMENS EXAMINED

Dispholidus kivuensis (BMNH 64.11.4.35, 79.11.13.18, 79.11.13.21, 1904.12.23.1, 1940.2.9.24, 1952.1.9.6, 1952.1.9.7, 1952.1.9.8, 1954.1.2.97, 1971.210, 1976.2268, 1978.544; CAS 85988; IRSNB 2141, 2141A, 2141B, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154; MNHN 1904.321, 1990.3955; MRAC 1141, 9808, 11027, 17505, 17506, 17507, 17508, 17509, 17510, 17511, 17512, 17527, 20347, 20399, 20400, 20401, 20818, 20833, 20840, 20841, 20842, 20863, 20924, 20925, 21272, 21320, 21321, 763280, 791523, 791525, 19.938, 19.595, 5 unnumbered; NMZB 1568, 1570, 1571, 1572, 1573, 1574, 1657, 1658, 6924, 6925, 6926; USNM 48591; ZMB 13248, 13321, 17170, 26830, 48154); ***Dispholidus* sp. (Pemba Island)** (BMNH 1947.1.2.41, 1947.1.2.42, 1956.1.6.40); ***Dispholidus* sp. (West Africa)** (MRAC 7317149, MRAC 29636, MNHN 1990.4643); ***Dispholidus typus*** (BMNH 79.11.12.2, 1940.2.22.86; MNHN 5738; MNHN 1990.4643, 1997.6550; MRAC 7317149, 8106200, 29636; ZMB 5879, 23501); ***Dispholidus punctatus*** (BMNH 1905.5.29.32, 1960.1.6.11, 2 unnumbered; CAS 86015; CM 5906; IRSNB 2122, 1 unnumbered; MD 2159, 2179, 2244, 2252, 5018, 5024, 5154, 5187, 5232, 5269, 5275, 5277, 5287, 5450, 5546, 5556, 5559, 5741, 5785, 5833, 5851, 5763A, 5763B; MRAC 263, 607, 1984, 1993, 4354, 5999A, 5999B, 7096, 7942, 9637, 9668, 9853, 10211, 10451, 15810, 16196, 17395, 17396, 17397, 17398, 17399, 17400, 17401, 17402, 17403, 17513, 763282; NMZB 49, 1350, 1616, 2833, 2834, 2990, 3321, 3322, 3323, 3921, 4026, 4317, 4318, 4319, 4320, 4321, 4322, 4461, 4977, 10586, 10661, 10705, 10714, 10715, 10732, 10733, 10760, 10761, 10762; NMZB-UM 687, 4928, 4929; PJ 587; TMP 21203, 21204, 21205; USNM 16258); ***Dispholidus viridis*** (BMNH 1984.886; CAS 85783; MRAC 1738, 3905, 6611, 11497, 17230, 21357, 781727; NMZB 13625, 13871; NMZB-UM 17301, 17302, 17303, 17304, 17305, 17306, 17307, 17308, 17309, 17501); ***Rhamnophis aethiopissa*** (BMNH 62.9.2.28, 1907.4.18.5, 1919.8.16.85, 1946.1.4.99 (61.12.30.61), 1970.2183; IFAN 1014; KMH 4051; MBG 309, 349, 540, 1237, 1263, 1264; MCZ 18198; MD 2308, 5850, 6001, 1 unnumbered; MNHN 8491, 1950.71, 1964.463, 1964.464, 1964.465, 1964.466, 1964.467, 1964.468, 1 unnumbered; MRAC 4728, 11777, 12081, 12257, 16433, 16434, 18120, 18462, 18463, 18464, 18465, 18466, 18467, 18468, 18469; NHRM 1977, 1978, 1979, 1980; NMK I-48; NMZB 10633, 10665, 10706,

10725, 10745, 10793, 16726; NMZB-UM 2548; SMF 3 unnumbered; USNM 49005, 109593; ZMB 10578, 26595; ZMH 11, 135); ***Rhamnophis batesii*** (AMNH 12137, 12503; BMNH 1912.6.27.22, 1933.389, 1934.12.1.25, 1969.1676, 1971.390; IFAN 1004; IRSNB 2813; MBG 342; MCZ 13604, 38393; MNHN 1950.70, 1966.723, 1967.427, 1967.428, 1990.4908; MRAC 826, 2428, 14499, 19070; NMZB 13206; SMNS 9246); ***Thrasops flavigularis*** (AMNH 50573, 50574, 50575; BMNH 1950.1.2.3, 1971.391; IFAN 687, 854, 1246; MHNG 1520.68, 1520.75, 1520.78, 967.2; MNHN 1892.22, 1906.0183, 1992.4593, 1998.0436, 1998.0458, 1998.0459; MRAC 1349, 1403, 28121, 28236, 28284, 28512, 73018.0052; NMZB 16725; SMF 10 unnumbered; ZMB 8335, 20702, 21720, 22796, 24395, 27149; ZMH 146, 186); ***Thrasops jacksonii*** (AMNH 45864, 45865, 50572, 50576; BMNH 2 unnumbered; 1964.47, 1964.469, 1966.719, 1972.72, 1972.73; MRAC 4283, 8662, 11022, 11931, 14607, 14609, 15835, 15836, 15845, 17095, 18453, 18454, 18455, 18456, 18457, 18458, 18459, 18460, 18461, 19064, 19066, 19067, 19068, 20288, 20309, 20325, 20376, 20416, 20649, 20650, 20898, 20951, 20952, 21331, 21332, 21333; NMK I-47; NMZB 10658, 10717, 10749; NMZB-UM 5316, 5392; ZMUC 607-7, 60716, 601199); ***Thrasops occidentalis*** (BMNH 66.1.28.6, 94.3.24.30, 1909.2.23.4, 1911.5.29.9, 1911.5.29.10, 1911.6.30.2, 1912.9.18.3, 1949.1.2.93, 1951.1.6.92, 1963.1045, 1965.781, 1977.1247; IFAN 49-2-9; MNHN 1986.1662, 1986.1797, 1988.157, 1990.4592, 1990.5192; MRAC 29716, 31054, 31056, 80036.0014, 80036.0015; SMNS 9226, 9246; ZMB 11268, 22005); ***Thrasops schmidti*** (MCZ 9276, MNHN 1940.197, 1974.1; NHRM 2 unnumbered, NMK 1222, 1223, 1373, 1374, 1375, 1376, 1609, 1904, 2235, 2750, 3222, 3525); ***Thelotornis capensis capensis*** (AM 1677, 1 unnumbered; AMG 1472, 1520, 4603A, 4603B, 7607; BMNH 97.9.2.5, 1907.4.9.35, 1907.4.17.73, 1914.10.24.3; DM 86/1; FMNH 17676, 191163; JEC 4797; MMK 3 unnumbered; MRAC 76059, 77005.0003, 77005.0008; NKW 2, 76, 153, 217, 293; NMP 369, 371, 379, 401, 425, 426, 427, 1255, 1282; NMZB 472, 877, 920, 1394, 1395, 1398, 1424, 1484, 2316, 3379, 3460, 5735, 6389, 7811, 8412, 8609, 8613, 8614, 8615, 8627, 8678, 8760, 11631, 14484, 17365; NMZB-UM 5382, 17529, 17530, 30685, 30686, 30687, 31588, 31817, 32556; PEM 904, 906, 907, 908, 910, 1440/40; SAM 532, 921, 1737, 1738, 8079, 19271; TMP 5613, 5614, 5615, 5616, 5617, 5619, 5620, 5621,

5622, 5623, 5624, 5625, 5626, 5627, 5631, 5632, 5634, 5636, 5637, 5638, 5639, 5640, 5641, 5642, 5643, 5644, 5645, 10069, 12368, 12387, 12505, 12577, 12723, 13056, 13057, 13059, 13677, 13767, 13902, 13984, 14077, 21819, 24586, 29395, 29462, 29463, 29798, 31228, 33375, 34715, 36682, 41614, 42859, 43988, 44018, 44041, 44223, 44226, 44235, 44245, 44476, 44477, 44478, 44617, 44658, 44735, 45526, 45554, 45639, 45728, 45832, 46033, 47514, 47520, 47991, 48012, 48284; UMMZ 61241; USNM 50936, 142097; ZMB 23526); ***Thelotornis capensis oatesii*** (AMG 512, 6946, 7486; AMNH 51951, 51952, 67776, 67777, 67778, 82414, 82415; BMNH 99.3.20.11, 244, 347, 1915.4.22.15, 1932.5.3.102, 1932.5.3.103, 1932.5.3.104, 1932.9.9.142, 1932.9.9.143, 1932.9.9.144, 1932.12.13.2, 1933.4.3.8, 1946.1.9.76, 1962.47; CAS 147134; CM 6345, 6346, 40514; DM 1330; FMNH 15462, 74252, 74253, 74254, 74255, 134244, 154731; JHVDM 310; JPT 852, 868, 1196, 1202, 1233, 1234, 1246, 1265, 1578, 1826, 3 unnumbered; MCZ 258, 354, 51230, 51231, 51232, 51233, 51234, 51235; MD 2045, 5374, 5391; MMK 2 unnumbered; NMP 2 unnumbered; NMWN 2358A, 2358B, 2358C, 2358D, 2359, 2360, 2361, 2362, 2363, 2592, 4907, 7676; NMZB 77, 217, 218, 231, 232, 263, 273, 275, 277, 278, 279, 317, 348, 351, 359, 362, 363, 366, 391, 404, 406, 462, 585, 612, 613, 799, 873, 875, 876, 878, 880, 972, 1299, 1307, 1561, 1597, 1620, 1627, 1735, 1859, 1923, 1984, 1985, 1986, 1987, 1988, 2563, 2942, 3114, 3115, 3116, 3236, 3242, 3243, 3460, 3448, 3600, 3602, 3775, 3776, 3777, 3828, 3829, 3954, 3975, 4058, 4066, 4094, 4095, 4213, 4218, 4219, 4251, 4252, 4253, 4254, 4255, 4256, 4257, 4258, 4323, 4914, 7624, 8738, 9354, 9361, 9820, 9825, 10172, 10436, 10690, 10691, 10711, 10719, 10720, 11182, 11227, 11229, 11309, 12053, 12488, 12601, 12851, 13577, 13754, 15835, 15897, 16424, 17078; NMZB-UM 88, 339, 557, 689, 993, 1061, 2522, 2565, 2759, 2760, 2761, 2941, 2942, 2943, 2944, 2945, 2946, 2947, 2948, 2974, 3103, 3544, 4518, 4519, 4840, 5374, 5798, 5842, 6461, 7208, 7342, 8520, 9068, 10368, 11026, 11285, 11341, 11524, 11904, 12040, 12555, 12681, 13335, 16057, 16182, 16194, 16195, 16199, 17310, 17311, 17312, 17313, 17314, 17922, 18015, 18029, 18415, 18553, 18560, 18561, 19302, 19803, 20130, 20144, 20783, 20815, 20834, 20843, 20927, 20992, 21279, 21668, 23264, 23378, 23405, 23441, 23576, 23784, 23842, 23933, 24154, 25448, 26581, 26869, 28144, 28163, 29045, 29188,

29386, 29558, 29560, 29599, 29628, 30024, 30253, 30366, 30375, 30681, 30682, 30683, 30684, 30688, 31332, 31450, 31451, 31452, 31923, 32121, 32144, 32145, 32146, 32191, 32196, 32198, 32199, 32207, 32208, 32218, 32250, 32316, 32322, 32479, 32597, 32620, 32826, 32827, 32899, 32954, 33035, 33132, 33154, 33161, 33173, 33297, 33298, 33299, 33300, 33301, 33302, 33303, 33304, 33305, 33306, 33441; NMZB-QVM 30, 76, 552, 581; PSM 41; SAM 17487, 19708; TMP 16055, 16229, 18608, 21666, 21667, 21668, 21669, 22608, 24393, 31017, 38148, 39097, 39929, 44811, 45108, 45167; USNM 132524, 145581, 145582, 164985, 164986, 164993, 200340; UZH 1 unnumbered; ZMB 2386, 27510A, 27510B, 27632A, 27632B; ZMH 1 unnumbered; ZMUC 6393) ***Thelotornis kirtlandii*** (Aarhus 135, 1160; AMNH 5266, 12272, 12273, 12275, 12276, 12277, 12279, 12280, 12281, 12285, 12286, 50531, 65391, 104102, 104103; BMNH 44.1.16.2, 49.3.2.40, 58.2.23.3, 66.1.28.14, 66.1.28.17, 74.10.6.17, 86.10.23.1, 88.8.29.11, 94.8.4.20, 98.11.24.4, 1900.2.17.21, 1901.3.12.102, 1901.6.24.55, 1904.5.2.76, 1906.5.28.16, 1907.5.22.54, 1907.5.22.55, 1910.2.4.3, 1919.8.16.94, 1929.8.7.1, 1936.8.1.718, 1936.8.1.719, 1950.1.2.9, 1952.1.3.48, 1953.1.6.18, 1953.1.6.19, 1953.1.10.88, 1954.1.13.25, 1955.1.14.15, 1955.1.14.16, 1958.1.5.48, 1969.1677, 1970.2174, 1971.403, 1976.2269; CM 6816; FMNH 4019, 4020, 19456, 19835, 52911, 62299, 118996, 121978, 170705, 170706, 178884, 178885, 178933, 205972; IRSNB 5371, 6451, 6454; MCZ 48421, 54736; MD 5192, 6004; MHNG 1463.64, 1463.65, 1520.5, 1520.51, 1520.52; MRAC 11243, 14371, 14372, 18587, 18588, 28055, 28064, 74013.0146, 74013.0063; MZUF 20399, 20400; NMZB 10785; NMZB-UM 3371, 20595, 20596, 32183, 32184, 32185; PEM 1 unnumbered; UMMZ 38839, 65825; USNM 24166, 24225, 62152, 149486, 167090A, 167090B, 167091, 167092, 167093, 167094, 167095; ZFMK 60764, 60767; ZMH 73, 195; ZMUC 631282); ***Thelotornis mossambicanus*** (AMNH 39170, 44303, 44304, 44305, 67757, 67758; BMNH 79.11.13.17, 88.7.14.4, 91.10.15.20, 91.12.17.4, 93.10.26.58, 93.10.26.59, 93.10.26.60, 93.10.26.61, 93.10.26.62, 93.10.26.63, 94.2.13.16, 95.4.17.26, 97.6.9.139, 97.6.9.140, 97.6.9.250, 1928.10.19.71, 1933.4.5.3, 1933.4.5.4, 1937.7.25.9, 1948.1.1.99, 1952.1.3.50, 1970.2173; CM 40442, 40443; Derleyn 2 unnumbered; FMNH 12288, 81122, 81123, 81124, 81125, 81661, 81662, 81663, 81664, 81665, 81666, 81667; IRSNB 5288,

18284; JPT 1490; KMH 19652; MBL 1841, 1842, 1843; MCZ 18476, 23336, 30388, 40677, 40678, 48422, 48423, 48424, 48425, 48426, 51224, 51225, 51226, 51227, 51228, 51229, 51236, 51237, 51238, 51239, 51240, 51241, 51242, 51243, 51244, 51247, 51248, 51249, 51250, 51628, 56922; MHNG 1376.31, 1376.32, 1376.34, 1376.37; MRAC 76.59.R3, 77.5.R3, 77.5.R8; MZUF 2062, 2063, 2064, 2177, 5706, 5719; NMBO 7944; NMZB 202, 1460, 1461, 1562, 1563, 1736, 1921, 1922, 1969, 3896, 3896, 4042, 4154, 4155, 4156, 4157, 6923, 7879, 8337, 8799, 9467, 9651, 9956, 9997, 10068, 10281, 11261, 11262, 11320, 11321, 11390, 11434, 11435, 11622, 11939, 12006, 12204, 14401, 15773, 16346, 16347, 17105; NMZB-UM 3058, 4205, 4249, 7184, 7185, 7187, 7501, 8022, 8023, 8026, 8027, 8028, 8304, 8305, 8350, 8351, 8621, 8653, 8937, 9067, 9168, 9616, 9617, 10554, 10557, 10636, 10897, 10905, 11340, 11974, 12388, 12389, 12421, 12900, 16068, 16393, 16404, 16478, 16938, 17969, 17970, 18412, 19036, 19175, 19305, 20131, 20319, 21669, 22741, 21754, 21766, 21767, 21914, 23086, 23381, 23771, 24060, 24419, 24420, 25403, 25404, 25405, 27426, 27608, 27697, 27832, 28297, 28633, 29367, 29503, 29647, 31101, 31275, 31359, 31454, 31455, 31587, 31654, 32103, 32379, 32339, 32635, 32637, 32803, 32952, 32962, 32963, 32964, 33551, 33650, 32254; PEM 8098, 8099, 8100, 8106, 13201, 13210, 13253, 15472; TMP 13454, 47242; UMMZ 61174, 65706; ZMB 16783, 28001; ZMUC 63862, 63863, 63864; 631197, 631198, 631199, 631200, 631277, 631278, 631279, 631280, 631281, 631283, 631312, 631313); ***Thelotornis usambaricus*** (AMNH 61640, 61641, 61657, BMNH 1971.211, 1971.212, 1974.547, KMH 21306, 21352, 21369, 23214, 23225, 23226, 23405, 23427, 23434; MCZ 23337, 23338, 23339, 23340, 23341, 23342, 23343, 23344, 23345, 23346, 23347, 23348, 23349; NMK 3023, 3148, 3358, 3453, 3459, 3484; NMZB 3347, 6680, 8807, 14103, 14818, 15374, 15375, 15590, 15627, 15628, 15629, 16181, 16182, 16183, 16400; USNM 20097; ZMB 21130, 48245; ZMUC 631190, 631276, 631307, 631308, 631310, 631311); ***Xyelodontophis* (*Thelotornis*) *uluguruensis*** (NMZB 7443, 17088, 17089, 17090; ZMB 48153);

REFERENCES

(AVMA), A. V. M. A. 2007. AVMA Guidelines on Euthanasia. Pp. 36.

Arevalo, E., S. K. Davis, and J. W. Sites. 1994. Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporous grammicus* complex (Phyrynosomatidae) in central Mexico. Systematic Biology 43:387–418.

Avise, J. C. 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA.

Avise, J. C., and R. M. B. Jr. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys in Evolutionary Biology 7:45-67.

Avise, J. C., D. Walker, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proceedings of the Royal Society of London. Series B, Biological Sciences 265:1707–1712.

Bauer, A. M. 1993. African-South American relationships: a perspective from the Reptilia. Pp. 245–288 in P. Goldblatt, ed. Biological relationships between Africa and South America. Yale University Press.

Beheregaray, L. B. 2008. Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. Molecular Ecology 17:3754-3774.

Beheregaray, L. B., and A. Caccone. 2007. Cryptic biodiversity in a changing world. Journal of Biology 6:9.1-9.5.

Benjamin, S. P., D. Dimitrov, R. G. Gillespie, and G. Hormiga. 2008. Family ties: molecular phylogeny of crab spiders (Araneae: Thomisidae). Cladistics 24:708–722.

Berry, V., and O. Gascuel. 1996. On the Interpretation of Bootstrap Trees: Appropriate Threshold of Clade Selection and Induced Gain. Molecular Biology and Evolution 13:999-1011.

Bianconi, J. J. 1848. Alcune Nuove Specie di Rettili del Mozambico. Nuovi Ann. Sci. Nat. Bologna 2:106-109.

Bocage, J. V. B. d. 1895. Herpétologie d'Angola et du Congo. Ministério da Marinha e Colónias.

Bogert, C. M. 1940. Herpetological results of the Vernay Angola expedition. Bulletin of the American Museum of Natural History 77:1-107, figs. 101-118, pl. 101.

Bossuyt, F., and M. C. Milinkovitch. 2000. Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. Proceedings of the National Academy of Sciences of the United States of America 97:6585–6590.

Boulenger, G. A. 1893. Catalogue of the snakes in the British Museum (Natural History).

Bourgeois, M. 1968. Contribution à la morphologie comparée du crâne des ophidiens de l'Afrique Centrale. Publications de l'Université Officielle du Congo à Lubumbashi 18:1-293.

Branch, B. 1988. Bill Branch's Field Guide to the Snakes and other Reptiles of Southern Africa. Ralph Curtis Pub.

Branch, B. 1998. Field guide to the snakes and other reptiles of southern Africa. Ralph Curtis Publishing, Cape Town.

Branch, B. 1999. Reptile systematic studies in southern African: a brief history and overview. Transactions of the Royal Society of South Africa 54:137-156.

Branch, B. 2006. Priorities for systematic studies on southern African reptiles in W. R. Branch, K. A. Tolley, M. Cunningham, A. M. Bauer, G. Alexander, J. A. Harrison, A. A. Turner, and M. F. Bates, eds. A plan for phylogenetic studies of southern African reptiles. South African National Biodiversity Institute, Kirstenbosch, South Africa.

Brink, U. T. 1992. Rift Flank Uplifts and Hinterland Basins: Comparisons of the Transanarctic with the Great Escarpment of Southern Africa Journal of Geophysical Research 97:569-585.

Broadley, D. G. 1977. A revision of the African Snakes of The Genus *Psammophylax* Fitzinger (Colubridae). Occasional papers of the National Museums and Monuments of Rhodesia, Series B, Natural Sciences 6:1-44.

Broadley, D. G. 1983. FitzSimons' Snakes of Southern Africa. Delta.

Broadley, D. G. 1966b. A Review of the African Stripe-bellied Sand-snakes of the Genus *Psammophis*. Arnoldia (Rhodesia), Series of Miscellaneous Publications 2:1-9.

Broadley, D. G. 1966a. A review of the Natal green snake, *Philothamnus natalensis* (A. Smith), with the description of a new subspecies. Annals of the Natal Museum (Pietermaritzburg) 18:417-423.

Broadley, D. G. 1992. The taxonomy and zoogeography of the genus *Lycophidion* (Serpentes: Colubridae). Journal of the Herpetological Association of Africa 40:30-36.

Broadley, D. G. 1994. A revision of the African genus *Scaphiophis* Peters (Serpentes: Colubridae). Herpetological Journal 4:1-10.

Broadley, D. G. 1996. A Revision of the Genus *Lycophidion* Fitzinger (Serpentes: Colubridae) in Africa south of the Equator. Syntarsus 3:1-33.

Broadley, D. G. 2001. A review of the genus *Thelotornis* A. Smith in eastern Africa, with the description of a new species from the Usambara Mountains (Serpentes: Colubridae: Dispholidini). African Journal of Herpetology 50:53-70.

Broadley, D. G., and B. Hughes. 1993. A review of the genus *Lycophidion* (Serpentes: Colubridae) in Northeastern Africa. Herpetological Journal 3:8-18.

Broadley, D. G., and V. Wallach. 2002. Review of the Dispholidini, with the description of a new genus and species from Tanzania (Serpentes, Colubridae). Bulletin of the Natural History Museum (London) 68:57-74.

Burbrink, F. T., R. Lawson, and J. B. Slowinski. 2000. Mitochondrial DNA phylogeography of the polytypic North American ratsnake (*Elaphe obsoleta*): a critique of the subspecies concept. Evolution 54:2107-2118.

Carpenter, J. M. 1992. Random cladistics. Cladistics 8:147-153.

Che, J., J. Pang, H. Zhao, G.-f. Wu, E.-m. Zhao, and Y.-p. Zhang. 2007. Phylogeny of *Raninae* (Anura: Ranidae) inferred from mitochondrial and nuclear sequences. Molecular Phylogenetics and Evolution 43:1-13.

Chippaux, J.-P. 1999. Les serpents d'Afrique occidentale et centrale. IRD, Paris, France.

Chippaux, J.-P. 2001. Les serpents d'Afrique occidentale et centrale. IRD, Paris, France.

Chippaux, J.-P. 2006. Les serpents d'Afrique occidentale et centrale. IRD, Paris, France.

Coborn, J. 1991. The atlas of snakes of the world. T.F.H. Publications, Neptune City, NJ, USA.

Cope, E. D. 1893. Prodromus of a new system of the non-venomous snake. American Naturalist: 477-483.

Cope, E. D. 1895. The classification of the Ophidia. Transactions of the American Philosophical Society XVIII:186-219.

Cowling, R. M., S. Proches, and T. C. Partridge. 2009. Explaining the uniqueness of the Cape flora: Incorporating geomorphic evolution as a factor for explaining its diversification. Molecular Phylogenetics and Evolution 51:64-74.

Daniels, S. R., M. D. Hofmeyr, B. T. Henen, and E. H. W. Baard. 2010. Systematics and phylogeography of a threatened tortoise, the speckled padloper. Animal Conservation 13:237–246.

Daniels, S. R., M. D. Hofmeyr, B. T. Henen, and K. A. Crandall. 2007. Living with the genetic signature of Miocene induced change: Evidence from the phylogeographic structure of the endemic angulate tortoise *Chersina angulata*. Molecular Phylogenetics and Evolution 45:915-926.

Daniels, S. R., P. I. F. N. Mouton, and D. A. Toit. 2004. Molecular data suggest that melanistic ectotherms at the south-western tip of Africa are the products of Miocene climatic events: evidence from cordylid lizards. Journal of Zoology 263:373–383.

Derlyn, P. P. 1978. Notes sur les serpents du Burundi (2me partie). Les genres *Geodipsas* et *Thelotornis*. Revue de Zoologie Africaine 92:208-218

Dowling, H. G. 1951. A proposed standard system of counting ventrals in snakes. British Journal of Herpetology 1:97-99.

Dowling, H. G. 1967. Hemipenes and Other Characters in Colubrid Classification. Herpetologica 23:138-142.

Dowling, H. G., and W. E. Duellman. 1978. Systematic Herpetology: A synopsis of families and higher categories. HISS Publications, New York, USA.

Dowling, H. G., and J. M. Savage. 1960. A guide to snake hemipenes: a survey of basic structure and systematic characteristics. Zoologica 45:17-27.

Dumeril, A. M. C., G. Bibron, and A. H. A. Dumeril. 1854. Erpetologie generale ou histoire naturelle complete des reptiles.

Dunn, E. R. 1928. A tentative key and arrangement of the American genera of Colubridae. Bulletin of the Antivenin Institute of America 2:18-24.

Duvernoy, G. L. 1832. Mémoire sur les charactéres de l'anatomie pour distinguer les Serpents venimeux des serpents non-venimeux. Ann. Sci. Nat. (Paris) 26:113-160.

Efron, B. 1979. Bootstrap methods: another look at the jackknife. Annals of Statistics 7:1-26.

Efron, B., E. Halloran, and S. Holmes. 1996. Bootstrap confidence levels for phylogenetic trees. Proceedings of the National Academy of Sciences of the United States of America 93:13429-13434.

Eimermacher, T. G. 2007. Phylogenetic Relationships of Dispholidini (Serpentes: Colubridae) Based on Morphological Data. Pp. 73. Department of Biology. Southeastern Louisiana University, Hammond, LA, USA.

Erlank, A. J., J. S. Marsh, A. R. Duncan, R. M. Miller, C. J. Hawkesworth, P. J. Betton, and D. C. Rex. 1984. Geochemistry and petrogensis of the Etendeka volcanic rocks from SWA/Namibia Geological Society of South Africa, Special Publication 13:196-245.

Faith, D. P., and P. S. Cranston. 1991. Could a cladogram this short have arisen by chance alone? On permutation tests for cladistic structure. Cladistics 7:1–28.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Felsenstein, J., and H. Kishino. 1993. Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. Systematic Biology 42:193-200.

Ferrarezzi, H. 1994. Uma sinopse dos gêneros e classificação das Serpentes (Squamata): II. Família Colubridae. Herpetologia no Brasil 1:81-91.

Fischer, J.G. 1856. Neue Schlangen des Hamburgischen Naturhistorischen Museums. Abhandlungen des Naturwissenschaftlichen Verein Hamburg 3 (4): 79-116.

Fischer, E., and H. Hinkel. 1992. Natur Ruandas/La nature du Rwanda: spermatophytes & herpetofauna. Ministerium des Innern und fuer Sport, Rheinland-Pfalz (Germany), Mainz, Germany.

Fitzinger LJ. 1826. Neue Classification der Reptilien nach ihren natürlichen Verwandtschaften. Nebst einer Verwandtschafts-tafel und einem Verzeichnisse der Reptilien-sammlung des K. K. zoologischen Museum's zu Wien. J.G. Hübner, Wien. vii, 66 pp.

Fitzinger LJ. 1843. Systema Reptilium. Braumüller et Seidel, Wien: 106 pp.

Frank, N., and E. Ramus. 1995. A complete guide to scientific and common names of reptiles and amphibians of the world. N. G. Publishing, Pottsville, PA, USA.

Golay, P., H. M. Smith, D. G. Broadley, J. R. Dixon, C. McCarthy, J.-C. Rage, B. Schätti, and M. Toriba. 1993. Endoglyphs and Other Major Venomous Snakes of the World. A Checklist. Azemiops, Geneva. Springer-Verlag, New York.

Goldblatt, P. 1997. Floristic diversity in the Cape Flora of South Africa. Biodiversity and Conservation 6:359-377.

Goloboff, P. 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics 15:415–428.

Goloboff, P. 1993a. Estimating character weights during tree search. Cladistics 9:83–91.

Goloboff, P. 1994a. Nona: A Tree-searching Program. Program and documentation, available at http://www.zmuc.dk/public/phylogeny/Nona-PeeWee.

Goloboff, P. 1994b. Pee-Wee: Parsimony and Implied Weights. Program and documentation, available at http://www.zmuc.dk/public/phylogeny/Nona-PeeWee/.

Goloboff, P. 1993b. Pee-Wee, ver. 3.0. Program available at http://www.cladistics.com.

Goloboff, P., J. Farris, and K. Nixon. 2008. TNT, a free program for phylogenetic analysis. Cladistics 24:774-786.

Grazziotin F. G., Zaher H., Murphy R. W., Scrocchi G., Benavides M. A., Ya-Ping Z., Bonatto S. L. 2012. Molecular phylogeny of the New World Dipsadidae (Serpentes: Colubroidea): a reappraisal. Cladistics 1: 1-23.

Günther, A. 1858. Catalogue of Colubrine snakes of the British Museum. London, I - XVI, 1 – 281.

Günther, A. 1862. On new Species of Snakes in the Collection of the British Museum. The Annals and Magazine of Natural History, including Zoology, Botany, and Geology 9:124-132.

Hallowell, E. 1852. On a new Genus and two new species of African Serpents. Proceedings of the Academy of Natural Sciences of Philadelphia:203-205.

Hedin, M., and J. E. Bond. 2006. Molecular phylogenetics of the spider infraorder Mygalomorphae using nuclear rRNA genes (18S and 28S): Conflict and agreement with the current system of classification. Molecular Phylogenetics and Evolution 41:454–471.

Hillis, D. M., and J. J. Bull. 1993. An Empirical Test of Bootstrapping as a Method for Assessing Confidence in Phylogenetic Analysis. Systematic Biology 42:182-192.

Hollis, J. L. 2006. Phylogenetics of the genus *Chironius* Fitzinger, 1826 (Serpentes, Colubridae) based on morphology. Herpetologica 62:435-453.

Hughes, B. 1983. African Snake Faunas. Bonner Zoologische Beiträge 34:311-356.

Hughes, B. 1985. Progress on a Taxonomic Revision of the African Green Tree Snakes (*Philothamnus* spp.). Pp. 511-530 in K.-L. Schuchmann, ed. International Symposium on African Vertebrates. Systematics, phylogeny and evolutionary ecology, Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn, Germany.

Hughes, B., and D. H. Barry. 1969. The snakes of Ghana: a checklist and key. Bull. Inst. Fond. Afr. Noire Ser. A 31:1004-1041.

Kelly, C. M. R., N. P. Barker, M. H. Villet, and D. G. Broadley. 2009. Phylogeny, biogeography and classification of the snake superfamily Elapoidea: a rapid radiation in the late Eocene. Cladistics 25:38-63.

Kluge, A. G., and A. J. Wolf. 1993. Cladistics: What's in a word? Cladistics 9:183–199.

Kohn, L. M. 2005. Mechanisms of fungal speciation. Annual Review of Phytopathology 43:279–308.

Lamb, T., and A. M. Bauer. 2000. Relationship of the *Pachydactylus rugosus* group of geckos (Reptilia: Squamata: Gekkodidae). African Zoology 35:55-67.

Laurent, R. F. 1955. Diagnoses préliminaries de quelques venimeux. Revue de Zoologie et de Botanique Africaines 51:127-139.

Laurent, R. F. 1956. Contribution à l'Herpetologie de la Region des Grands Lacs de l'Afrique Centrale. Annales du Musée Royal du Congo Belge Sér. 8 vo. 48:1-390.

Lawson, R., J. B. Slowinski, B. I. Crother, and F. T. Burbrink. 2005. Phylogeny of the Colubroidea (Serpentes): new evidence from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution 37:581-601.

Lenk, P., and W. Wüster. 1999. A multivariate approach to the systematics of Italian rat snakes of the *Elaphe longissima* complex (Reptilia, Colubridae): revalidation of Camerano's *Callopeltis longissimus* var. *lineata*. Herpetological Journal 9:153-162.

Lesia, M. G. A., M. D. Hofmeyr, and M. E. D'Amato. 2003. Genetic variation in three *Chersina angulata* (angulate tortoise) populations along the west coast of South Africa. African Zoology 38:109-117.

Leston, D., and B. Hughes. 1968. The snakes of Tafo, a forest cocoa-farm locality in Ghana. Bulletin de l'Institute Fondamental d'Afrique Noire Sér. A 30:737-770.

Linder, H. P. 2003. The radiation of the Cape flora, southern Africa. Biological Reviews of the Cambridge Philosophical Society 78:597–638.

Linder, H. P. 2005. Evolution of diversity: the Cape flora. Trends in Plant Science 10:536–541.

Linder, H. P., M. E. Meadows, and R. M. Cowling. 1992. History of the Cape flora. Pp. 113–134 in R. M. Cowling, ed. The Ecology of Fynbos, Nutrients, Fire and Diversity. Oxford University Press, Oxford.

Loveridge, A. 1929. East African reptiles and amphibians in the United States National Museum. Bulletin of the United States National Museum 151:1-135.

Loveridge, A. 1944. Further revisions of African snake genera. Bulletin of the Museum of Comparative Zoology, Harvard 95:121-247.

Loveridge, A. 1957. Check list of the reptiles and amphibians of East Africa (Uganda; Kenya; Tanganyika; Zanzibar). Bulletin of the Museum of Comparative Zoology 117:153-362 + xxxvi.

Lye, W. F. 1975. Andrew Smith's Journal of his expedition into the interior of South Africa / 1834 - 36, an authentic narrative of travels and discoveries, the manners and customs of the Native tribes, and the physical nature of the country, with contemporary illustrations by Charles Davidson Bell. AA Balkema, Cape Town.

Makokha, J. S. 2006. Molecular Phylogenetics and Phylogeography of Sand Lizards, *Pedioplanis* (Sauria: Lacertidae) in Southern Africa. Pp. 86. Department of Botany and Zoology. University of Stellenbosch, Stellenbosch.

Makokha, J. S., A. M. Bauer, W. Mayer, and C. A. Matthee. 2007. Nuclear and mtDNA based phylogeny of southern African sand lizards, *Pedioplanis* (Sauria: Lacertidae). Molecular Phylogenetics and Evolution 44:622-633.

Malnate, E. V. 1960. Systematic Division and Evolution of the Colubrid Snake Genus *Natrix*, with Comments on the Subfamily Natricinae. Proceedings of the Academy of Natural Sciences of Philadelphia 112:41-71.

Marais, J. 2004. A Complete Guide to the Snakes of Southern Africa. Struik Publishers.

Matthee, C. A., and A. F. Flemming. 2002. Population fragmentation in the southern rock agama, *Agama atra*: more evidence for vicariance in Southern Africa. Molecular Ecology 11:465–471.

McGarial, K., S. Cushman, and S. G. Stafford. 2000. Multivariate statistics for wildlife and ecology research. Springer.

McMahon, M., and M. Sanderson. 2006. Phylogenetic supermatrix analysis of GenBank sequences from 2228 papilionoid legumes. Systematic Biology 55:818–836.

Mertens, R. 1937. Reptilien und Amphibien aus dem suedlichen Inner-Afrika. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 435:1-23.

Mittermeier, R. A., P. R. Gil, M. Hoffman, J. Pilgrim, T. Brooks, C. G. Mittermeier, J. Lamoreux, and G. A. B. d. Fonseca. 2004. Hotspots Revisited: Earth's Biological Riches and Most Endangered Terrestrial Ecoregions. Conservation International.

Monaghan, M. T., D. J. G. Inward, T. Hunt, and A. P. Vogler. 2007. A molecular phylogenetic analysis of the Scarabaeinae (dung beetles). Molecular Phylogenetics and Evolution 45:674–692.

Nagy, Z. T., N. Vidal, M. Vences, W. R. Branch, O. S. G. Pauwels, M. Wink, and U. Joger. 2005. Molecular Systematics of African Colubroidea (Squamata: Serpentes). Pp. 221-228 in B. A. Huber, B. J. Sinclair, and K.-H. Lampe, eds. African Biodiversity: Molecules, Organisms, Ecosystems. Springer Verlag, Berlin.

Nixon, K. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15:407–414.

Noonan, B. P., and P. T. Chippindale. 2006. Dispersal and vicariance: The complex evolutionary history of boid snakes. Molecular Phylogenetics and Evolution 40:347–358.

Obst, F. J., K. Richter, and U. Jacob. 1984. Lexikon der Terraristik und Herpetologie. Landbuch-Verlag Hannover, Leipzig.

Obst, F. J., K. Richter, and U. Jacob. 1988. The completely illustrated atlas of reptiles and amphibians for the terrarium. T.F.H. Publications, Neptune City, NJ, USA.

Ollier, C. D., and M. E. Marker. 1985. The Great Escarpment of Southern Africa. Zeitschrift für Geomorphologie Supplement Series 54:37-56.

Page, R. D. M., and E. C. Holmes. 1998. Molecular Evolution, A Phylogenetic Approach. Blackwell Publishing Co.

Parkinson, C. L., J. A. Campbell, and P. T. Chippindale. 2002. Multigene phylogenetic analyses of pitvipers; with comments on the biogeographical history of the group. Pp. 93-110 in G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene, eds. Biology of the Vipers. Eagle Mountain Publishing, Salt Lake City, UT.

Pauwels, O. S. G., A. K. Toham, and C. Chimsunchart. 2002. Recherches sur l'herpétofaune des Monts de Cristal, Gabon. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique (Biol.) 72:59-66.

Pauwels, O. S. G., A. K. Toham, and C. Chimsunchart. 2002. Recherches sur l'herpétofaune du Massif du Chaillu, Gabon. Bulletin de l'Institut Royal des Sciences Naturelles de Belgique (Biol.) 72:47-57.

Perret, J.-L. 1961. Études Herpétologiques Africanes III. Bulletin de la Société Neuchâteloise de Sciences naturelles 84:133-138.

Pitman, C. R. S. 1974. A Guide to the Snakes of Uganda. Wheldon & Wesley Ltd.

Portik, D. M. 2009. Comparative phylogeography of two skink species in southern Africa. Pp. 212. Department of Biology. Villanova University, Villanova.

Portik, D. M., A. M. Bauer, and T. R. Jackman. 2010. The phylogenetic affinities of *Trachylepis sulcata nigra* and the intraspecific evolution of coastal melanism in the Western rock skink. African Zoology 45:1-13.

Portik, D. M., A. M. Bauer, and T. R. Jackman. 2011. Bridging the gap: Western rock skinks (*Trachylepis sulcata*) have a short history in South Africa. Molecular Ecology 20:1744-1758.

Puorto, G., M. G. Salomao, R. D. G. Theakston, R. S. Thorpe, D. A. Warrell, and W. Wüster. 2001. Combining mitochondrial DNA sequences and morphological data to infer species

boundaries: phylogeography of lanceheaded pitvipers in the Brazilian Atlantic forest, and the status of *Bothrops pradoi* (Squamata: Serpentes: Viperidae). Journal of Evolutionary Biology 14:527-538.

Pyron, R. A., F. T. Burbrink, G. R. Colli, A. N. M. d. Oca, L. J. Vitt, C. A. Kuczynski, and J. J. Wiens. 2011. The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. Molecular Phylogenetics and Evolution 58:329-342.

Rasmussen, J. B. 1993a. The current taxonomic status of Tornier's cat-snake (Crotaphopeltis *tornieri*). Amphibia-Reptilia 14:395-409.

Rasmussen, J. B. 1997. On two little known African water snakes *Crotaphopeltis degeni* and C. *barotseensis*. Amphibia-Reptilia 18:191-206.

Rasmussen, J. B. 1985. A Re-evaluation of the Systematics of the African Rear-fanged Snakes of Bogert's Groups XIII – XVI, including a Discussion of some Evolutionary Trends within Caenophidia. Pp. 531-548 in K.-L. Schuchmann, ed. International Symposium on African Vertebrates, Bonn, Germany.

Rasmussen, J. B. 1993b. A taxonomic review of the *Dipsadoboa unicolor* complex, including a phylogenetic analysis of the genus (Serpentes, Dipsadidae, Boiginae). Steenstrupia 19:129-196.

Rasmussen, J. B., K. M. Howell, and M. Andersen. 1995. A review of the Usambara forest snake *Geodipsas vauerocegae* and the Uluguru forest snake *G. procterae*. Amphibia-Reptilia 16:123-136.

Rasmussen, J. B., and M. J. Largen. 1992. A review of *Pseudoboodon* Peracca with the description of a new species from Southwest Ethiopia (Serpentes, Dipsadidae, Lycodontinae, Boadontini). Steenstrupia 18:65-80.

Riddle, B. R., and D. J. Hafner. 2006. Phylogeography in historical biogeography: investigating the biogeographic histories of populations, species, and young biotas. Pp. 161-176

in M. C. Ebach, and R. S. Tangney, eds. Biogeography in a Changing World CRC Press, Boca Raton, FL.

Sanderson, M. J. 1989. Confidence limits on phylogenies: the bootstrap revisited. Cladistics 5:113-129.

Sanderson, M. J., and M. F. Wojciechowski. 2000. Improved bootstrap confidence limits in large-scale phylogenies, with an example from Neo-Astragalus (Leguminosae). Systematic Biology 49:671–685.

Schargel, W. E., and T. A. Castoe. 2003. The Hemipenes of Some Snakes of the Semifossorial Genus *Atractus*, with Comments on Variation in the Genus. Journal of Herpetology 37:718-721.

Schlegel, H. 1837. Essai sur la physionomie des serpens.

Schmidt, K. P. 1923. Contributions to the herpetology of the Belgian Congo based on the collection of the American Museum Congo Expedition, 1909-1915. Part II. Snakes, with field notes by Herbert Lang and James P. Chapin. Bulletin of the American Museum of Natural History 49:1-146.

Scott, L. 1995. Pollen evidence for vegetation and climate change in southern Africa during the Neogene and Quaternary. Pp. 56–76 in E. S. Vrba, G. H. Denton, T. C. Partridge, and L. H. Burckle, eds. Paleoclimate and Evolution with Emphasis on Human Origins. Yale University Press, New Haven.

Slowinski, J. B. 1995. A phylogenetic analysis of the New World coral snakes (Elapidae: *Leptomicrurus*, *Micruroides*, and *Micrurus*) based on allozymic and morphological characters. Journal of Herpetology 29:325-338.

Smit, H. A., T. J. Robinson, and B. J. v. Vuuren. 2007. Coalescence methods reveal the impact of vicariance on the spatial genetic structure of *Elephantulus edwardii* (Afrotheria, Macroscelidae). Molecular Ecology 16:2680-2692.

Smith, A. 1828. Descriptions of new or imperfectly known Objects of the Animal Kingdom, found in the South of Africa. South African Commercial Advertiser 3:2.

Smith, A. 1838. Illustrations of the Zoology of South Africa. Part I, Plate III.

Smith, A. 1840. Illustrations of the Zoology of South Africa, Reptilia. Smith, Elder, and Co., London.

Smith, A. 1841. Illustrations of the Zoology of South Africa: Reptiles.

Smith, A. 1842. Illustrations of the Zoology of South Africa: Reptiles. Smith, Elder and Co., London.

Smith, A. 1849. Illustrations of the Zoology of South Africa: Reptiles.

Soltis, P. S., and D. E. Soltis. 2003. Applying the Bootstrap in Phylogeny Reconstruction. Statistical Science 18:256-267.

Spawls, S. 1978. A checklist of the snakes of Kenya. Journal of the East Africa Natural History Society & National Museum 31:1-18.

Spawls, S., and B. Branch. 1995. Dangerous Snakes of Africa: Natural History – Species Directory – Venoms and Snakebite. Ralph Curtis Pub.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A rapid bootstrap algorithm for the RAxML Web-Servers. Systematic Biology 57:758-771.

Stejneger, L. 1907. Herpetology of Japan and adjacent territory. U. S. National Museum, Bull. 58, 1907. xx, 577 p.

Swart, B. L., K. A. Tolley, and C. A. Matthee. 2009. Climate change drives speciation in the Southern rock Agama (*Agama atra*) in the Cape Floristic Region, South Africa. Journal of Biogeography 36:78-87.

Tamura, K., M. Nei, and S. Kumar. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences of the United States of America 101:11030-11035.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution 28:2731-2739.

Thorpe, R. S., and C. J. McCarthy. 1978. A preliminary study, using multivariate analysis, of a species complex of African house snakes (*Boaedon fuliginosus*). Journal of Zoology 184:489-506.

Tolley, K. A., M. Burger, A. A. Turner, and C. A. Matthee. 2006. Biogeographic patterns and phylogeography of dwarf chameleons (*Bradypodion*) in an African biodiversity hotspot. Molecular Ecology 15:781-793.

Tolley, K. A., B. M. Chase, and F. Forest. 2008. Speciation and radiations track climate transitions since the Miocene Climatic Optimum: a case study of southern African chameleons. Journal of Biogeography 35:1402-1414.

Tolley, K. A., J. S. Makokha, D. T. Houniet, B. L. Swart, and C. A. Matthee. 2009. The potential for predicted climate shifts to impact genetic landscapes of lizards in the South African Cape Floristic Region. Molecular Phylogenetics and Evolution 51:120-130.

Tolley, K. A., C. R. Tilbury, W. R. Branch, and C. A. Matthee. 2004. Phylogenetics of the southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae). Molecular Phylogenetics and Evolution 30:354-365.

Trape, J. F., and R. Roux-Estève. 1995. Les serpents du Congo: liste commentée et clé de détermination. Journal of African Zoology 109:31-50.

Turner, A. A., A. L. d. Villiers, and E. H. W. Baard. 2007. Reptiles. Pp. 56-74 in Anonymous, ed. Western Cape Province State of Biodiversity.

Wagler, J. G. 1830. Natürliches System der Amphibien mit vorangehender Klassifikation der Säugetiere und Vögel. Ein Beitrag zur vergleichenden Zoologie. J. G. Cotta, München, Stuttgart, and Tübingen. 354pp.

Wallach, V. 1991. Comparative Visceral Topography of African Colubrid Snakes of the Subfamilies Aparallactinae and Atractaspidinae. Pp. 980. Louisiana State University and Agricultural and Mechanical College, Baton Rouge.

Wallach, V. 1998. The visceral anatomy of blindsnakes and wormsnakes and its systematic implications (Serpentes: Anomalepididae, Typhlopidae, Leptotyphlopidae). Pp. 611. Northeastern University.

Welch, K. R. G. 1982. Herpetology of Africa: a checklist and bibliography of the orders Amphisbaenia, Sauria and Serpentes. Robert E. Krieger Publishing Company, Malabar, FL, USA.

Witte, G.-F. d. 1962. Genera des serpents du Congo et du Ruanda-Urundi. Musee Royal de L'Afrique Centrale, Tervuren, Belgium.

Wüster, W., S. Otsuka, A. Malhotra, and R. S. Thorpe. 1992a. Population systematics of Russell's viper: a multivariate study. Biological Journal of the Linnean Society 47:97-113.

Wüster, W., S. Otsuka, R. S. Thorpe, and A. Malhotra. 1992b. Morphological variation in Russell's viper in Burma and Thailand. Herpetological Journal 2:99-101.

Wüster, W., M. G. Salomao, R. S. Thorpe, G. Puorto, M. F. D. Furtado, S. A. Hoge, R. D. G. Theakston, and D. A. Warrell. 1997. Systematics of the *Bothrops atrox* species complex: insights from multivariate analysis and mitochondrial DNA sequence information. Pp. 99-130 in R. S. Thorpe, W. Wüster, and A. Malhotra, eds. Venomous Snakes: Ecology, Evolution and Snakebite. Symposia of the Zoological Society of London. Clarendon Press, Oxford.

Zaher, H. 1999. Hemipenial morphology of the South American xenodontine snakes: with a proposal for a monophyletic Xenodontinae and a reappraisal of colubroid hemipenes. Bulletin of the American Museum of Natural History. No. 240: 1-168.

Zharkikh, A., and W. Li. 1995. Estimation of confidence in phylogeny: the complete-and-partial bootstrap technique. Molecular Phylogenetics and Evolution 4:44-63.

Zharkikh, A., and W. Li. 1992a. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences: I. Four taxa with a molecular clock. Molecular Biology and Evolution 9:1119-1147.

Zharkikh, A., and W. Li. 1992b. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences: II. Four taxa without a molecular clock. Journal of Molecular Evolution 35:356-366.

BIOGRAPHICAL INFORMATION

Thomas Eimermacher was born in Muenster, Germany, in 1977, but spent significant amounts of time in his mother's native country of Bolivia. It was there that his interest in reptiles was first awakened, fueled by accounts of snake encounters by members of his family. By the age of thirteen, he was reading every book on snakes that the local city library had in stock. In 1994, Thomas moved to East Texas to finish high school, before moving to New Orleans, Louisiana to attend college. Uncertain about his future path, he majored in management at the University of New Orleans, where he earned a Bachelor of Science degree in 2000. At that time, he became deeply involved with local herpetological organizations, such as the Louisiana Gulf Coast Herpetological Society (LGCHS), for which he would later serve as board member and president. In 2002, he earned an M. B. A. in management information systems at the University of New Orleans, but nonetheless continued to find himself drawn to herpetology. In 2003, he first spent time in Central America, before leading a National Geographic-funded research expedition to East Africa, where he conducted his first research project, studying the systematics of an aquatic elapid snake. In 2004, he returned to school, and earned a Master of Science degree in evolutionary biology from Southeastern Louisiana University (SELU) in 2007, studying under Dr. Brian Crother. That same year, he began working on his Ph. D. in quantitative biology under Drs. Eric Smith and Jonathan Campbell at the University of Texas at Arlington (UTA).

Following the completion of that program at UTA, Thomas intends to focus on teaching, while at the same time continuing his research on African snakes at the McGregor Museum in Kimberley, South Africa, where he was awarded an associate researcher position in 2010.