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The snake family Psammophiidae (Reptilia: Serpentes): Phylogenetics and species delimitation in the African sand snakes (*Psammophis* Boie, 1825) and allied genera

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ABSTRACT

This study constitutes the first evolutionary investigation of the snake family Psammophiidae—the most widespread, most clearly defined, yet perhaps the taxonomically most problematic of Africa's family-level snake lineages. Little is known of psammophiid evolutionary relationships, and the type genus *Psammophis* is one of the largest and taxonomically most complex of the African snake genera. Our aims were to reconstruct psammophiid phylogenetic relationships and to improve characterisation of species boundaries in problematic *Psammophis* species complexes. We used approximately 2500 bases of DNA sequence from the mitochondrial and nuclear genomes, and 114 terminals covering all psammophiid genera and incorporating approximately 75% of recognised species and subspecies. Phylogenetic reconstructions were conducted primarily in a Bayesian framework and we used the Wiens/Penkrot protocol to aid species delimitation. *Rhamphiophis* is diphyletic, with *Rhamphiophis acutus* emerging sister to *Psammophylax*. Consequently we transfer the three subspecies of *Rhamphiophis acutus* to the genus *Psammophylax*. The monotypic genus *Dipsina* is sister to *Psammophis*. The two species of *Dromophis* occupy divergent positions deeply nested within *Psammophis*, and we therefore relegate *Dromophis* to the synonymy of *Psammophis*. Our results allow division of the taxonomically problematic *Psammophis* '*sibilans*' species complex into two monophyletic entities, provisionally named the '*phillipsii*' and '*subtaeniatus*' complexes. Within these two clades we found support for the status of many existing species, but not for a distinction between *P.p. phillipsii* and *P. mossambicus*. Additionally, *P. cf. phillipsii occidentalis* deserves species status as the sister taxon of *P. brevirostris*.

1. Introduction

Psammophiidae Bourgeois, 1968 occurs throughout Africa, the Middle East, Madagascar, southern Europe and south-central Asia, and currently includes eight genera and about 50 extant species (Table 1), of which around 30 belong to the type genus *Psammophis* Boie, 1825. Other genera include *Dipsina* Jan, 1863 (one species), *Dromophis* Peters, 1869 (two species), *Hemirhagerrhis* Boettger, 1893 (four species), *Malpolon* Fitzinger, 1826 (three species), *Mimophis* Günther, 1868 (one species), *Psammophylax* Fitzinger, 1843 (four species), and *Rhamphiophis* Peters, 1854 (five species). Monophyly of the family has been demonstrated in numerous phylogenetic studies based on immunological data (Cadle, 1994) and mitochondrial DNA sequences (Gravlund, 2001; Vidal and Hedges, 2002; Kelly et al., 2003; Nagy et al., 2003). Additionally, Psammophiidae is defined by several morphological synapomorphies; primarily the vestigial nature of the male genitalia (hemipenes), which are short, thin, and devoid of the ornamentation characteristic of the hemipenes of other snakes (Bogert, 1940; Broadley, 1983). Consequently, almost all morphological studies since the influential work of Bogert (1940) have recognised this assemblage as a natural group (Bourgeois, 1968; Dowling and Duellman, 1978; Zaher, 2000).

No study, morphological or molecular, has analysed phylogenetic relationships within the Psammophiidae, and taxon sampling has been highly restricted in those studies with psammophiid representatives. Furthermore, whilst generic delimitations and the species boundaries within many genera have been reasonably stable (e.g., Broadley, 1977b; Chirio and Ineich, 1991; Broadley and Hughes, 2000), taxonomic opinion on species delimitation within *Psammophis* has varied widely since systematic work on the genus began in the late 1800s (Boulenger, 1896; Loveridge, 1940; Broadley, 1966, 1977a; Hughes, 1999). The most difficult group taxonomically has become known as the *Psammophis* '*sibilans*' species complex, a name coined for a group of taxa that have at some stage been considered synonyms or subspecies of *Psammophis sibilans* Linnaeus, 1758. The prevailing uncertainty in *Psammophis* species boundaries is widely viewed as one of the most protracted and challenging problems in African snake taxonomy, though substan-

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Table 1
Generic composition of the Psammophiidae, indicating the type species of each genus, the total number of species, and the number of species included in this study

Genus	Type species	Total no. species	No. species sampled
Dipsina Jan 1863	*multimaculata* (A. Smith, 1847)	1	1
Dromophis Peters, 1869	*praeornatus* (Schlegel, 1837)	2	2
Hemirhagerrhis Boettger, 1893	*kelleri* Boettger, 1893	4	3
Malpolon Fitzinger, 1826	*Natrix lacertina* Wagler, 1824 (=*Coluber monspessulanus* Herrmann, 1804 by original designation)	3	2
Mimophis Günther, 1868	*madagascariensis* Günther, 1868	1	1
Psammophis Boie, 1825	*sibilans* (Linnaeus, 1758)	~30	23
Psammophylax Fitzinger, 1843	*rhombeatus* (Linnaeus, 1758)	4	4
Rhamphiophis Peters, 1854	*rostratus* Peters, 1854	5	3

tial progress has been made in the identification and morphological delineation of southern African species (Broadley, 1966, 1975, 1977a, 1983, 2002). A revision of the entire genus was conducted by Brandstätter (1995), but this was generally not considered to be authoritative (Hughes, 1999; Broadley, 2002) and will not be discussed further.

Our study seeks to address the poor phylogenetic understanding of Psammophiidae and the taxonomic disarray of *Psammophis*, and our objectives are: (1) to reconstruct the phylogeny of the Psammophiidae using an extensive taxon sample spanning the entire family; (2) to provide a provisional DNA-based hypothesis of species limits in the problematic *Psammophis* '*sibilans*' complex, which can in future be tested against and integrated with other forms of data; and (3) to use these results to propose taxonomic alterations where the evidence for such change is deemed sufficient.

2. Methods

2.1. Taxon sampling and laboratory protocols

Our taxon set comprised 114 terminals (individual samples), including 39 of the approximately 50 recognised psammophiid species from all eight genera, five outgroup taxa from other major African snake lineages, and one outgroup from the Elapidae (Appendix A, and Table 1). Most (but not all) specimens used in this study have been seen and identified by CMRK. No recent authoritative revision of the genus *Psammophis* exists, and the taxonomy applied in this study follows the most recent literature dealing with subsets of the genus. Note that some *Psammophis* species and subspecies are very poorly delineated, and identification of certain specimens is tentative. The specimen from Senegal (haplotype PEM1) has not been seen by any of the authors and was not conclusively identified by its collectors. On the basis of our phylogenetic results this specimen is provisionally treated as *Psammophis cf. rukwae*. Hughes and Wade (2004) described *Psammophis phillipsii occidentalis* largely in recognition of a colour variant and no DNA data are available for the types; the specimens treated here as *P. cf. phillipsii occidentalis* were provisionally identified (by CMRK) on the basis of potentially labile characters associated with pattern and colouration. Similarly, the Ethiopian specimens we refer to as *P. cf. sibilans* are so-called to reflect their close morphological similarity to the true *P. sibilans* described from Egypt; they resemble the latter in colouration and head scalation (five infralabials are typically in contact with the anterior sublinguals). There is some speculation on the validity of *Psammophis sudanensis*; the specimen on which the name is based (the type of *Psammophis subtaeniatus* var. *sudanensis* Werner, 1919) was formerly allocated to *P. sibilans* (Broadley, 1977a), but reinspection of the specimen (by DGB) indicates that this name is probably valid.

Our mitochondrial data set included sequences from the cytochrome b (Cytb, 1100 bases) and NADH dehydrogenase subunit four genes (ND4, 693 bases), and about 169 bases from three transfer RNA (tRNA) genes flanking the 3′ end of the ND4 gene ($tRNA^{His}$, $tRNA^{Ser}$, $tRNA^{Leu}$). Our nuclear data set comprised 570 bases from the c-mos proto-oncogene. Appendix A lists all sequences used in this study. Novel DNA sequences were generated for 105 specimens (Cytb), 106 specimens (ND4 and tRNA), and 22 specimens (c-mos). Using primers published elsewhere (Cytb: L14910, de Queiroz et al., 2002; L14919, H16064, Burbrink et al., 2000. ND4: ND4, Leu, Arévalo et al., 1994. c-mos: S77, S78, Slowinski and Lawson, 2002). These new sequences were deposited in the GenBank, with accession numbers as in Appendix A. Tissue samples were in the form of liver, muscle or blood preserved in 80–96% ethanol or in 20% dimethyl sulfoxide (DMSO) and saturated sodium chloride. Whole genomic DNA was extracted and stored following the protocols of Sambrook et al. (1989) and Burbrink et al. (2000), and PCR amplifications were carried out in 25 μl volumes using Ready-To-Go PCR Beads (Amersham Biosciences). Thermocycling conditions for mtDNA amplification included an initial denaturation step at of 5 min at 94 °C, followed by 40 cycles of (denaturation, 40 sec, 94 °C; annealing, 40 sec, 47 °C; extension, 1 min, 72 °C), and a final 5 min extension phase at 72 °C. Conditions for c-mos amplification comprised an initial denaturation of 1 min at 95 °C, 5 cycles of (30 s, 95 °C; 45 s, 48 °C; 1 min, 72 °C), 30 cycles of (30 s, 95 °C; 45 s, 54 °C; 1 min, 72 °C), and a terminal 5-min extension step at 72 °C. Fragments were isolated by electrophoresis on a 2% agarose gel and purified using a GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences) according to the manufacturer's protocol. Forward and reverse sequencing of the double stranded purified fragments was carried out in 10-μl volumes using the BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, ABI) and the following thermocycling conditions: 25 cycles of (10 s, 94 °C; 5 s, 50 °C; 4 min, 60 °C). Products were analysed on ABI3700 or ABI3730-automated DNA sequencers.

2.2. Phylogenetic methods

2.2.1. Alignment and genetic distances

Sequences were aligned using ClustalX 1.64 (Thompson et al., 1997) and manually adjusted with BioEdit 5.0.9 (Hall, 1999). DAMBE (Xia and Xie, 2001) was used to screen the aligned sequences for frame-shift mutations and premature stop codons, which may indicate the presence of pseudogenes (e.g., transpositions of mtDNA to the nuclear genome: Sorenson and Fleischer, 1996; Bensasson et al., 2001). Calculations of genetic distances within and between clades were based on Cytb and ND4 sequences. Regression of ND4 against Cytb for pairwise K2P distances (not shown) revealed a slope of 0.94, suggesting that these genes evolve at very similar rates, so data from the two genes were combined for distance calculations. We used MEGA2 (Kumar et al., 2001) with the K2P model of distance correction to calculate mean and maximum within-group divergences, and minimum between-group divergences.

2.2.2. Data combination and splitting of the data set

The ILD test of data homogeneity Farris et al. (1995) has been shown to be problematic (Barker and Lutzoni, 2002), so we used PAUP* 4.0b10 (Swofford, 2002) to conduct non-parametric bootstrap analyses under the MP criterion for each gene. No significant bootstrap support for conflicting nodes was evident (exceeding 70%; Hillis and Bull, 1993), so we combined data from different genes for subsequent phylogenetic analyses. Since nuclear sequences were only generated for a fraction of the terminals, two overlapping data sets were created for independent analysis. The

'mitochondrial' data set comprised mitochondrial sequences from all 114 terminals, whilst the pruned 'mixed' data set included both mitochondrial and nuclear sequences from 21 ingroup and all outgroup taxa (see Appendix A).

2.2.3. Phylogenetic inference and clade support

Phylogenetic analyses were conducted using parametric (maximum likelihood, ML and Bayesian inference, BI) and non-parametric (maximum parsimony, MP) approaches. For DNA sequence data, MP can be inconsistent even under simple models of cladogenesis (Huelsenbeck and Lander, 2003), so parametric methods are generally favoured. Notwithstanding recent methodological advances in the use of ML to reconstruct phylogenies (e.g. Stamatakis, 2006; Zwickl, 2006), Bayesian approaches are still able to implement more complex (and hopefully more realistic) models of sequence evolution. Consequently, we relied primarily on Bayesian methods for phylogenetic reconstruction, whilst ML and MP approaches were used to perform non-parametric bootstrap analyses (Felsenstein, 1985). In assessing the reliability of phylogenetic hypotheses, we follow the majority of recent literature in considering Bayesian posterior probability values $\geqslant$95% and ML and MP bootstrap proportions $\geqslant$70% to indicate significant support.

We used MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) for phylogeny reconstruction by BI and employed the rigorous model selection procedure described by Castoe et al. (2004) to identify the model of DNA substitution and data partitioning that best fits each of our data sets. An outline of this procedure is provided below, but readers are referred to Castoe et al. (2004) for full methodological details. Initially, the optimal overall DNA substitution model and parameters for each data set were selected using hierarchical likelihood ratio tests (hLRTs) and the Akaike Information Criterion (AIC), implemented in ModelTest 3.7 (Posada and Crandall, 1998). This 'global' optimal model of DNA substitution was applied in all subsequent analyses. For the mitochondrial data set (Cytb, ND4, tRNA) the tRNA genes were pooled and treated as a single tRNA partition, and three models of data partitioning were defined based on sequence properties of potential biological relevance: (A) the unpartitioned data set, (B) protein-coding versus tRNA genes, and (C) Cytb, ND4 and tRNA partitioned independently. Within models B and C, a further two models (SSR, site-specific rates) were defined based on the degree to which evolution of the partitions is assumed to be independent. First, partitions have the same underlying parameter values and only among-site rates (overall rates, not α rate-heterogeneity values) are allowed to vary across-partitions (SSR model). Second, all of the parameters are unlinked across partitions and are free to achieve unique optimal values in the different partitions (SSR-unlinked model). Overall, five different models were defined: A, B-SSR, B-SSR-unlinked, C-SSR, and C-SSR-unlinked. These five models were analysed independently in MrBayes, applying the global optimal model of DNA substitution. For the mixed data set (Cytb, ND4, tRNA, c-mos), four models of data partitioning were defined: (A) as above, (B) mitochondrial versus nuclear genes, (C) mitochondrial protein-coding versus mitochondrial tRNA versus nuclear genes, and (D) all genes partitioned independently. SSR and SSR-unlinked models were defined within models B–D, to give a final total of seven different models: A, B-SSR, B-SSR-unlinked, C-SSR, C-SSR-unlinked, D-SSR, D-SSR-unlinked. As above, these models were analysed independently in MrBayes.

Bayesian analyses were conducted with flat priors as per the MrBayes defaults, and with model parameters estimated as part of the procedure. Each analysis comprised two independent runs (starting from different random trees) of one cold and three incrementally heated MCMC chains, sampling the chains every 100 generations. We used the built-in convergence diagnostic (average standard deviation of split frequencies) to monitor the mixing of chains and convergence of independent runs, and we discarded as burn-in all generations prior to this diagnostic reaching a value of 0.01. Initial analyses providing data for comparison of the different models were run for 1.4 million generations, and analyses applying the final best-fit model were run for 5 million generations.

MP bootstrap analyses (1000 pseudoreplicates) were implemented using PAUP* 4.0b10, with uninformative characters excluded, alignment gaps coded as a fifth state, simple sequence addition, and tree bisection–reconnection branch swapping. ML bootstrap analyses (1000 pseudoreplicates) were performed with RAxML version 7.0.0 (Stamatakis, 2006), using the closest available equivalent to the best-fit model of DNA substitution and data partitioning selected for Bayesian analyses.

2.3. Detailed investigation of the Psammophis 'sibilans' species complex

Since c-mos sequences were only generated for a minority of *Psammophis* terminals, the following analyses were based on mitochondrial data only.

2.3.1. Phylogenetic networks

We used phylogenetic networks to visualise relationships in cases where the underlying evolutionary history of the data may not be purely hierarchical (intraspecific relationships), and also as a tool to visualise ambiguity in the data (conflicting phylogenetic signal). For network construction we used SplitsTree 4 (Huson and Bryant, 2004) to implement the Neighbor-Net procedure (Bryant and Moulton, 2004), which is a distance method based on the NJ algorithm. The K2P model of distance correction was used to account for multiple substitutions per site.

2.3.2. Provisional species delimitation

The strongest support for species hypotheses is the concordance of conclusions from multiple operational criteria and independent data sets (e.g., Wiens and Penkrot, 2002; Morando et al., 2003; Dayrat, 2005). However, our study was limited to use of mtDNA data alone, and the species boundaries delineated here must be treated as highly provisional. Wiens and Penkrot (2002) provide an explicit tree-based species delimitation protocol (henceforth termed the WP strategy) designed for application to phylogenies of non-recombining DNA haplotypes. This approach informs species-level decisions by applying a dichotomous key to terminals currently classified as a single species (the 'focal species'). Wiens and Penkrot suggest that their method be used in conjunction with nested clade analysis (NCA: Templeton et al., 1987, 1995; Templeton and Sing, 1993). This combined approach uses the WP strategy to delimit species at the deepest levels of divergence, then subsequent application of NCA to well-sampled clades to see whether any further splitting may be statistically justified. However, our sampling of individuals and localities within most 'focal species' is limited and likely to be insufficient for useful application of NCA. In addition, serious concerns have been raised about the high incidence of false positive results returned by NCA (e.g. Panchal and Beaumont, 2007; Petit, 2008), and we concur with Petit (2008) that use of this method should be deferred pending thorough critical evaluation. Following Wiens and Penkrot (2002), we use the term "exclusive" in place of "monophyletic" in the context of species delimitation since true monophyly may not occur at the intraspecific level. The concept of monophyly incorporates the closely related properties of "exclusivity" and "common ancestry", and above the species level these two properties coincide. However, reticulating relationships lead to a decoupling of these properties, and entities of exclusive common ancestry do not exist where reticulation predominates (de Queiroz and Donoghue, 1990). All species may at some stage in their history be nonexclusive for any given gene, due to incomplete lineage sorting of ancestral polymorphisms (Neigel and Avise, 1986). This may be especially common in situations where a widespread species

gives rise to a much more restricted species, which thus develops exclusivity more rapidly than the other (Wiens and Penkrot, 2002). The WP strategy permits discovery of non-exclusive species.

3. Results

3.1. Phylogenetic analyses

3.1.1. Alignment and genetic distances

Alignment was unambiguous for all protein-coding genes, and no frame-shift mutations or premature stop codons were detected. For tRNA, eight gaps were required for preservation of homology, ranging from one to six bases in length. The mitochondrial data set comprised 1962 characters (including outgroups—1077 variable, 953 potentially parsimony-informative; excluding outgroups—979 variable, 893 potentially parsimony-informative). The mixed data set comprised 1957 mtDNA characters (including outgroups—1023 variable, 825 potentially parsimony-informative; excluding outgroups—879 variable, 685 potentially parsimony-informative) and 570 nuclear characters (including outgroups—111 variable, 35 potentially parsimony-informative; excluding outgroups—74 variable, 23 potentially parsimony-informative).

Genetic distances within and between selected *Psammophis* taxa, including those in the *Psammophis* '*sibilans*' complex, are presented in Table 2.

3.1.2. Model selection and Bayesian MCMC

For the mitochondrial data set, TVM + I + Γ was selected as the global optimal model of DNA substitution. However, this model is not implemented in MrBayes so the closely related GTR + I + Γ model was used instead. B-SSR-unlinked was tentatively selected as the best-fit model, but inspection of preliminary analyses revealed that certain parameters were not unique. Unlinking was thus reduced to allow only among-site rates and state frequencies to vary between partitions, and further testing resulted in choice of this refined model (B-SSR-unlink-statefreq) as the final model of best fit. For the mixed data set, GTR + I + Γ was selected as the global optimal model of DNA substitution, and B-SSR as the model of best fit. In all analyses of both data sets, independent runs converged on identical consensus topologies and very similar parameter estimates, and all runs reached stationarity rapidly (usually within 50,000 generations).

3.1.3. Evolutionary relationships

Figs. 1 and 2 illustrate the 50% majority-rule Bayesian consensus topologies for the mitochondrial and mixed data sets, respectively, and both data sets led to identical psammophiid phylogenetic relationships. The approximate geographic distributions of clades A–D and 1–9 are presented in Fig. 3 (Maps a–1). Figs. 1 and 2 include clade support values in the form of Bayesian posterior probabilities, ML bootstrap proportions, and MP bootstrap proportions. Support is generally high, with significant support for 81% (BI), 90% (ML) and 74% (MP) of ingroup nodes in the mitochondrial topology, and 80% (BI), 85% (ML) and 40% (MP) of ingroup nodes in the mixed topology. There is no consistent difference between the two topologies in the support values for equivalent nodes, but two important basal nodes (marked with black circles in Figs. 1 and 2) were more strongly supported by the mixed data set than by the mitochondrial data alone.

No detailed topological descriptions will be attempted here, and readers are referred to Figs. 1 and 2 for details of tree topology. At a higher level, four major lineages were defined for ease of discussion, all with strong support in the mixed topology (A–D; Figs. 1 and 2). Surprisingly, *Rhamphiophis acutus* is placed in clade B as sister to *Psammophylax*, instead of grouping with its supposed congeners in clade A. In clade D the two species of *Dromophis* occupy divergent positions deeply nested within the genus *Psammophis*.

Within clade D, nine distinct lineages were defined for ease of discussion, based on analysis of the mitochondrial data set (1–9; Fig. 1). Results from the mixed data set (Fig. 2) are congruent with those from the mitochondrial data alone. The West and Central African *Dromophis praeornatus* is placed in clade 5 with *Psammophis elegans*, *Psammophis schokari* and *Psammophis punctulatus*, rather than with *Dromophis lineatus* in clade 7. Clades 8 and 9 have usually been collectively referred to as the *Psammophis* '*sibilans*' complex (e.g.,

Table 2
Within-taxon and between-taxon distances for selected *Psammophis* taxa, including those in the *Psammophis* '*sibilans*' complex (calculated using pooled Cytb and ND4 data and applying the K2P model of distance correction)

Taxon	Mean within-taxon distance (%)	Max. within-taxon distance[a] (%)	Min. between-taxon distance[b] (%)
Clade 3			
namibensis	–	–	0.39 (PEM13 ↔ *leightoni*: TM83620)
leightoni	–	–	0.39 (TM83620 ↔ *namibensis*: PEM13)
Clade 6			
biseriatus	0.23	0.23 (169 ↔ BK6)	11.38 (BK6 ↔ *tanganicus*: 90)
tanganicus	1.42	2.74 (90 ↔ 88)	11.38 (90 ↔ *biseriatus*: BK6)
Clade 8 ('*phillipsii*' complex)			
leopardinus	0.17	0.17 (PEM14 ↔ PEM3)	8.02 (PEM3 ↔ *brevirostris*: 277)
brevirostris	0.76	1.07 (186 ↔ 237)	3.75 (277 ↔ *cf. phillipsii occidentalis*: 71) 7.02 (277 ↔ *mossambicus*: 376)[c]
cf. phillipsii occidentalis	0.87	1.30 (71 ↔ 381)	3.75 (71 ↔ *brevirostris*: 277) 6.62 (381 ↔ *mossambicus*: TM83688)[d]
phillipsii phillipsii	–	–	1.59 (PEM12 ↔ *mossambicus*: Bills)
mossambicus	2.82	4.29 (PEM15 ↔ 231)	1.59 (Bills ↔ *phillipsii phillipsii*: PEM12) 6.62 (TM83688 ↔ *cf. phillipsii occidentalis*: 381)[e]
Clade 9 ('*subtaeniatus*' complex)			
cf. sibilans	0.10	0.17 (352 ↔ 358)	9.69 (358 ↔ *orientalis*: 161)
rukwae and *cf. rukwae*	5.32	7.80 (PEM1 ↔ BK4)	8.93 (PEM1 ↔ *orientalis*: 172)
subtaeniatus	0.61	0.73 (4 ↔ 249)	8.65 (282 ↔ *orientalis*: 161)
orientalis	3.15	6.59 (177 ↔ 187)	8.11 (161 ↔ *sudanensis*: 91)
sudanensis	2.26	4.04 (386 ↔ BK2)	8.11 (91 ↔ *orientalis*: 161)

Clade numbers follow Fig. 1, and haplotype labels follow Appendix A and Figs. 1, 2 and 4.
[a] Haplotypes between which the quoted distance was observed are enclosed in parentheses.
[b] Haplotypes between which the quoted distance was observed are enclosed in parentheses, and the taxon membership of the external haplotype is also identified.
[c] Min. distance to a taxon other than *cf. phillipsii occidentalis*.
[d] Min. distance to a taxon other than *brevirostris*.
[e] Min. distance to a taxon other than *phillipsii phillipsii*.

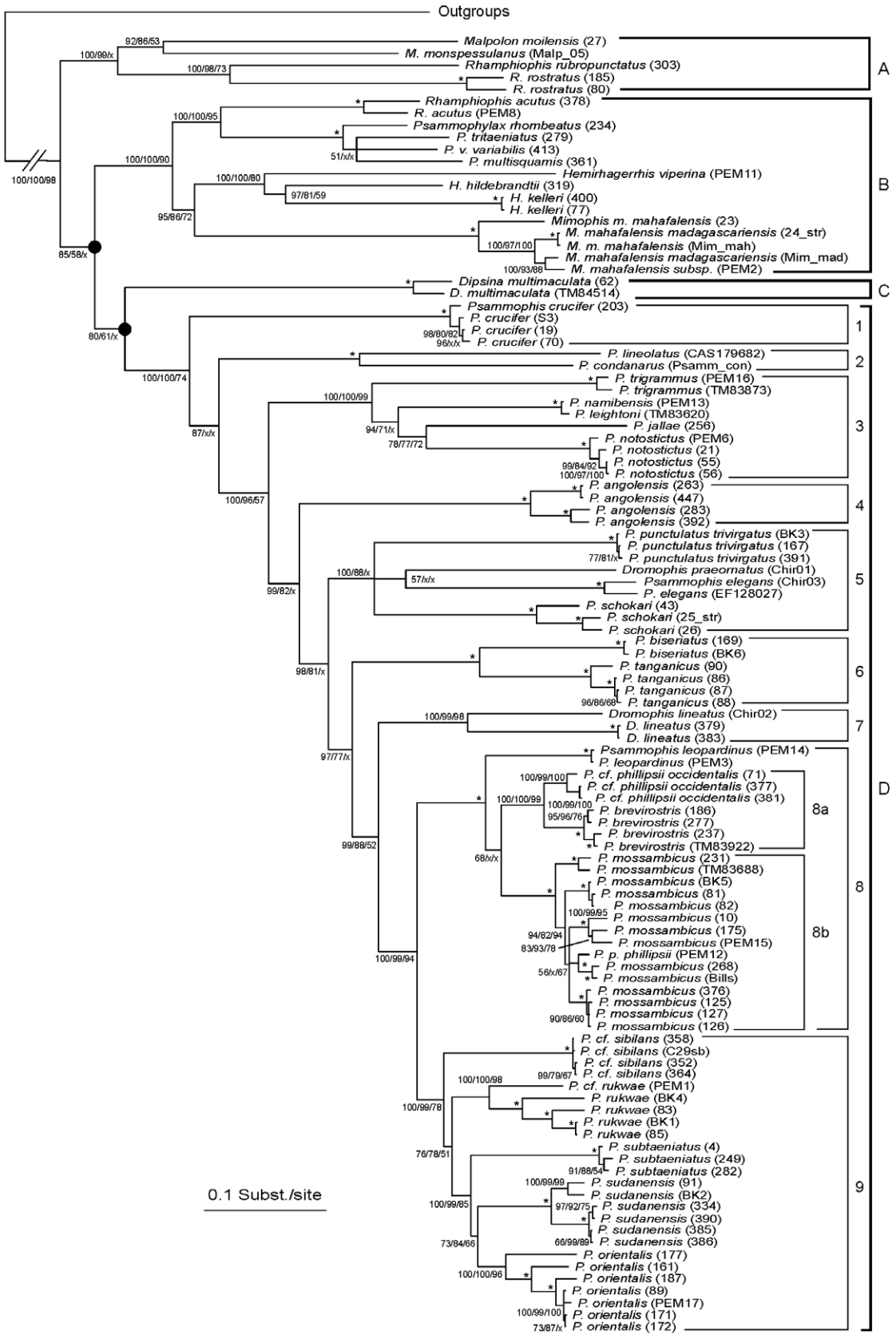

Fig. 1. Bayesian Inference 50% majority-rule consensus phylogram from the mitochondrial data set (Cytb, ND4, tRNA), under the B-SSR-unlink-statefreq and GTR + I + Γ models of data partitioning and nucleotide substitution. 45,000 trees from two runs (22,500 each) were sampled from the posterior distribution at stationarity, and branch lengths are averaged over all input trees. Node support (ingroup only) is in the format: Bayesian posterior probability/ML bootstrap proportion/MP bootstrap proportion. Support values not exceeding 50% are denoted by a cross (×), and asterisks identify nodes that received 100% support from all three indices. Analysis of the mixed data set resulted in substantially higher support values for the two nodes marked with black circles (see Fig. 2). Each terminal is labelled with its corresponding haplotype code (in parentheses) matching Appendix A, and clade labels are as in the text. Outgroups are listed in Appendix A.

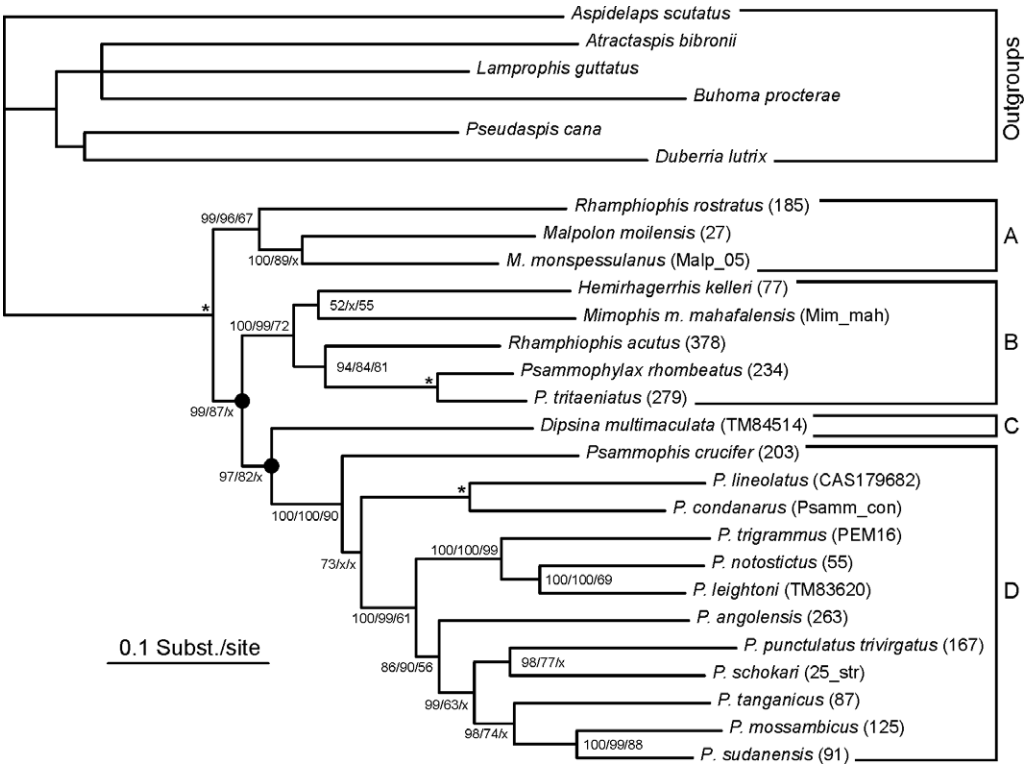

Fig. 2. Bayesian Inference 50% majority-rule consensus phylogram from the mixed data set (Cytb, ND4, tRNA, and c-mos), under the B-SSR and GTR + I + Γ models of data partitioning and nucleotide substitution. 40,000 trees were sampled from the posterior distribution at stationarity, and branch lengths are averaged over all input trees. Node support (ingroup only) is in the format: Bayesian posterior probability/ML bootstrap proportion/MP bootstrap proportion. Support values not exceeding 50% are denoted by a cross (×) and asterisks identify nodes that received 100% support from all three indices. The two nodes marked with black circles received substantially higher support here than in analyses of the mitochondrial data alone (see Fig. 1). Each ingroup terminal is labelled with its corresponding haplotype code (in parentheses) matching Appendix A, and clade labels are as in the text.

Broadley, 1983). Our results suggest that further subdivision of this complex is possible (Fig. 1), and we refer to these clades as the '*phillipsii*' complex (clade 8) and the '*subtaeniatus*' complex (clade 9).

3.2. Detailed investigation of the Psammophis 'sibilans' species complex

3.2.1. Evolutionary relationships

Relationships within the '*sibilans*' complex were investigated using phylogenetic networks in addition to the standard hierarchical phylogenetic methods. Fig. 4 illustrates the network diagram obtained using the Neighbor-Net procedure and applying the K2P model of distance correction. This is mostly congruent with the Bayesian analysis, and also supports subdivision of the '*sibilans*' complex into clades 8 and 9. The '*phillipsii*' complex (clade 8) contains five currently recognised taxa: *P. leopardinus*, *P. brevirostris*, *P. p. phillipsii*, *P. cf. phillipsii occidentalis*, and *P. mossambicus*. The primarily Central African *P. cf. phillipsii occidentalis* groups with the southern African *P. brevirostris* to form clade 8a (Figs. 1 and 4) rather than associating with typical *P. p. phillipsii* of West Africa, and our single representative of this latter taxon is deeply nested within the more extensive sample of *P. mossambicus* (clade 8b; Figs. 1 and 4).

The '*subtaeniatus*' complex (clade 9) also contains five currently recognised taxa (assuming that our *P. cf. sibilans* specimens from Ethiopia are correctly identified): *P. cf. sibilans*, *P. rukwae*, *P. subtaeniatus*, *P. sudanensis*, and *P. orientalis*. A specimen of uncertain identity from Senegal (haplotype PEM1) groups with *P. rukwae* with very strong support (BI = 100%, ML = 100%, MP = 98; Fig. 1), and we provisionally treat this specimen as *P. cf. rukwae*.

3.2.2. Provisional species delimitation

Selection of 'focal species' for assessment with the WP strategy was largely according to current taxonomy, and the following nine species-level hypotheses were tested (see Figs. 1 and 4). Clade 8: *P. leopardinus*, *P. brevirostris*, *P. phillipsii* (incorporating the two taxa *phillipsii* and *cf. occidentalis*), and *P. mossambicus*. Clade 9: *P. cf. sibilans*, *P. rukwae*, *P. subtaeniatus*, *P. orientalis*, and *P. sudanensis*. Given the current data, all but two of these 'focal species' are exclusive and without strongly supported early-diverging lineages concordant with geographic distribution. The exceptions are *P. phillipsii* and *P. mossambicus*, and three points can be made regarding these taxa. First, haplotypes of *P. phillipsii* are non-exclusive with respect to the exclusive *P. brevirostris*, and there is no evidence of gene flow between the earliest-diverging lineages of these two groups. Second, the lone *P. p. phillipsii* haplotype is nested among *P. mossambicus* samples, rendering the latter also non-exclusive. Unfortunately only one sample of *P. p. phillipsii* was available, so no decisions were possible regarding gene flow between the two focal species. Third, within *P. mossambicus* there is good support for a disjunction between the South African haplotypes (231 and TM83688) and the remaining samples (BI = 94%, ML = 82%, MP = 94%; Fig. 1), and these two earliest-diverging lineages are concordant with geographic distribution.

4. Discussion

4.1. Phylogenetic relationships and implications for taxonomy

4.1.1. The family Psammophiidae (clades A–D)

The pattern of relationships retrieved at the generic level is in agreement with that obtained by Gravlund (2001), using 12S rRNA and 16S rRNA gene sequences from single representatives of five psammophiid genera. A more comprehensive psammophiid taxon sample was included in the immunological study of Cadle (1994)—five genera, and seven species of *Psammophis*—but the only resolved relationship at the generic level was a clade uniting *Rham-*

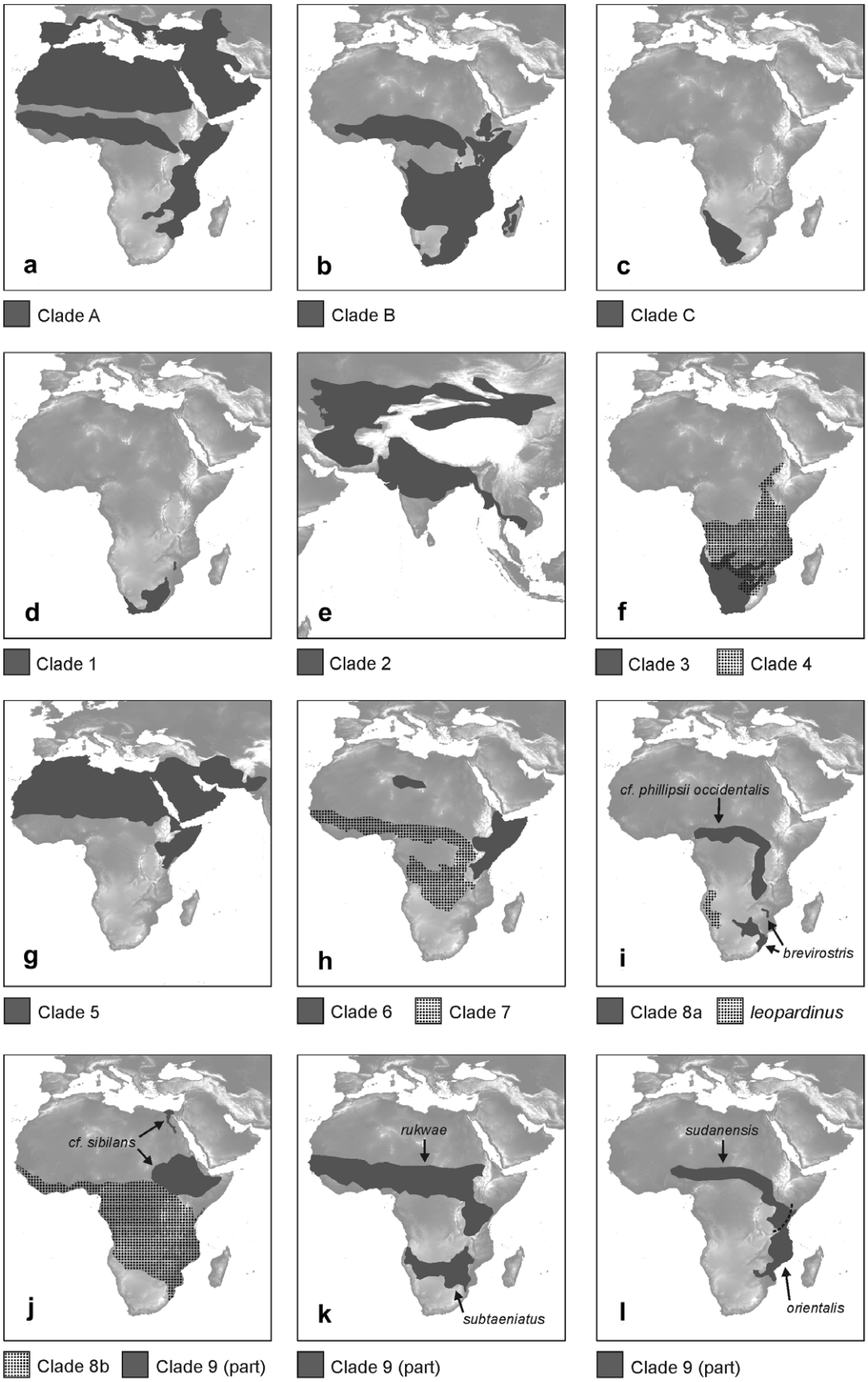

Fig. 3. Maps a–1 show tha approximate distributions of the major clades identified in Fig. 1, compiled from the following sources: Multiple genera—http://www.reptile-database.org/; Broadley, 1983; Broadley and Howell, 1991; Largen and Rasmussen, 1993; Schleich et al., 1996; Chippaux, 2001; Spawls et al., 2002. *Hemirhagerrhis*—Broadley and Hughes, 2000. *Malpolon*—Gasc et al., 1997; Carranza et al., 2006. *Mimophis*—Glaw and Vences, 1994. *Psammophis* and *Dromophis*—Loveridge, 1940; Broadley, 1966, 1975, 1977a, 2002; Brandstätter, 1995; Hughes, 1999; Hughes and Wade, 2002, 2004; Hughes, 2004; Rato et al., 2007. *Psammophylax*—Broadley, 1977b. *Rhamphiophis*—Broadley, 1971; Chirio and Ineich, 1991.

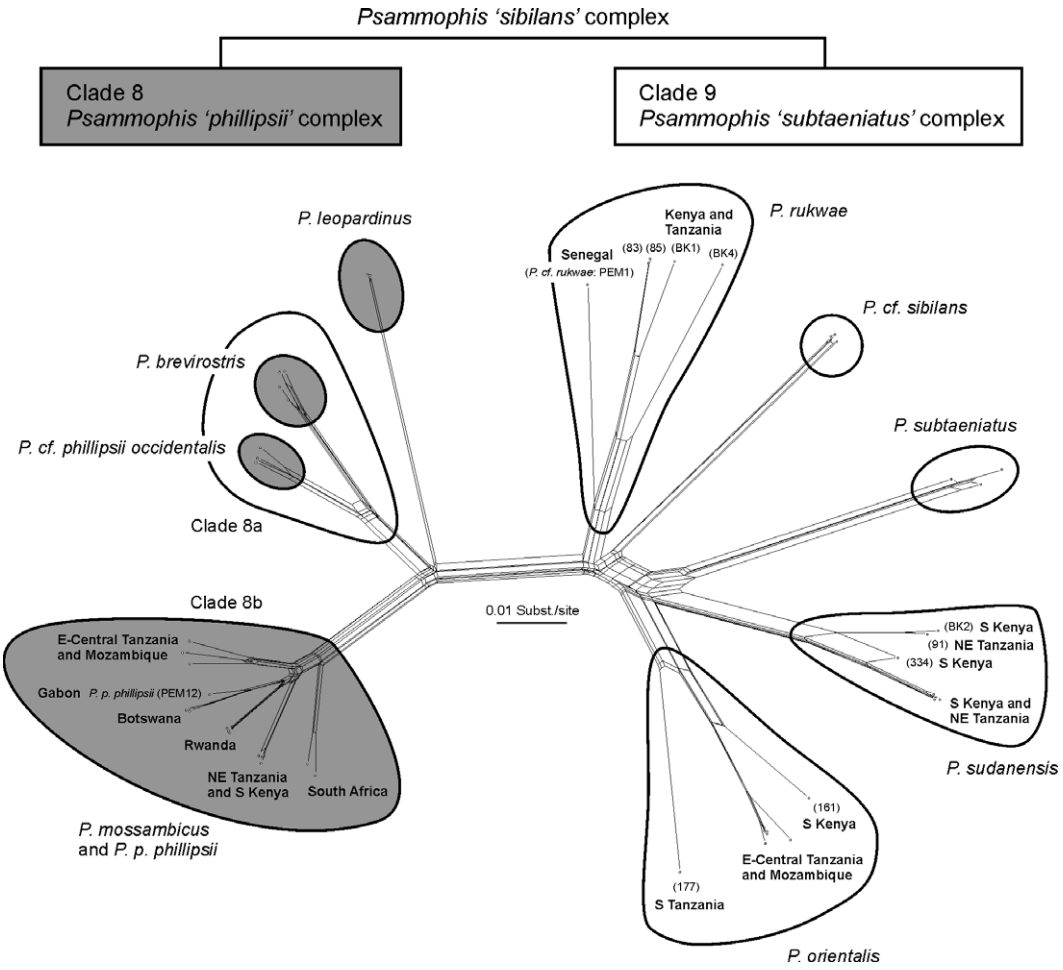

Fig. 4. Phylogenetic network diagram for the *Psammophis* '*sibilans*' complex, constructed using the Neighbor-Net procedure and applying the K2P model of distance correction. The '*phillipsii*' complex (grey) and '*subtaeniatus*' complex are clearly marked and the major constituent clades of each complex are circled and labelled. In clades containing substantial phylogenetic structure, the approximate distributions of groups of terminals are given, and where necessary, terminals are identified with their haplotype codes in parentheses (see Appendix A).

phiophis oxyrhynchus and the monotypic genus *Dipsina*. This conflicts with our results, in which *Rhamphiophis rostratus* (at one time a subspecies of *R. oxyrhynchus*) and *R. rubropunctatus* group with the North African and Eurasian genus *Malpolon* to form clade A. Recent taxonomic work on *Malpolon* has been undertaken by Carranza et al. (2006) and Geniez et al. (2006). *Dipsina multimaculata* resembles *Rhamphiophis* morphologically and was for a long time classified as a species of the latter genus, but on the basis of differing cranial osteology, Broadley (1983) resurrected the genus *Dipsina* Jan, 1863. He considered both *Rhamphiophis* and *Dipsina* to be derived from "ancestral *Psammophylax* stock" (Broadley, 1983), but our study instead provides good support for a sister relationship between *Dipsina* (clade C) and *Psammophis* (clade D).

In clade B, the strong support for a sister relationship between *Psammophylax* and *R. acutus* renders the genus *Rhamphiophis* diphyletic. Broadley (1971) provides a review and taxonomic history of *R. acutus*, noting that in several respects (dentition and colouration) this taxon forms a link between *Rhamphiophis* and *Psammophylax*. The most distinctive synapomorphy of *Rhamphiophis* is its shortened and reinforced skull (adaptations for digging), a characteristic that is less pronounced in *R. acutus* (Broadley, 1971; Chirio and Ineich, 1991). It is clear that this character is homoplasious, and we transfer the three subspecies of *R. acutus* to the genus *Psammophylax*, i.e. *Psammophylax acutus acutus* (Günther, 1888) (comb. nov.), *Psammophylax acutus jappi* (Broadley, 1971) (comb. nov.) and *Psammophylax acutus togoensis* (Matschie, 1893) (comb. nov.). This change increases the dental variation in *Psammophylax* and necessitates the following modification to the diagnosis provided by Broadley (1977b): 'The maxilla bears 9–13 subequal teeth, separated by a diastema from two enlarged grooved fangs on the posterior end of the bone; palatine teeth 7–11; pterygoid 9–23; dentary 15–24.' In *Hemirhagerrhis*, the southwestern African semi-desert species *H. viperina* is sister to an East African clade comprising *H. hildebrandtii* and *H. kelleri*. Broadley and Hughes (2000) undertook a morphological revision of the genus and proposed an evolutionary hypothesis that implied a closer relationship between *H. viperina* and *H. hildebrandtii* than between the latter and *H. kelleri*. Our results contradict this hypothesis, but a complete picture of *Hemirhagerrhis* relationships awaits inclusion of the widespread *H. nototaenia*. There is substantial well-supported phylogenetic structure in *Mimophis*, suggesting the presence of taxonomically unrecognised genetic diversity, and further work is necessary to investigate this.

Dromophis praeornatus and *Dromophis lineatus* occupy divergent and well-supported positions nested deeply within *Psammophis*, in clades 5 and 7 respectively (Fig. 1). We therefore relegate the genus *Dromophis* to the synonymy of *Psammophis*, forming *Psammophis praeornatus* (Schlegel, 1837) (comb. nov) and *Psammophis lineatus* (Dumèril & Bibron, 1854) (comb. nov.). Loveridge (1940) provides a taxonomic revision of both of these genera stating, "*Dromophis* has been included in this paper with the purpose of inviting attention to its close relationship to *Psammophis sibilans* as evidenced by

its synonymy, and the references to *P. sibilans* which should properly be referred to as *D. lineatus*." More recently, Hughes (2004) has also noted the high incidence of misidentification of *D. lineatus* as one of several taxa from the *Psammophis* '*sibilans*' species complex. Indeed, our results show a sister relationship between *D. lineatus* (clade 7; Fig. 1) and the '*sibilans*' complex (clades 8 and 9; Fig. 1). The main character formerly used to separate *Dromophis* and *Psammophis* involves maxillary dentition - in *Psammophis* (but not in *Dromophis*) a pair of enlarged fang-like teeth is present anterior to the fangs, separated from other maxillary dentition by an interspace on each side (Loveridge, 1940; Broadley, 1983). The generic diagnosis provided for *Psammophis* by Broadley (1983) should thus be modified as follows: 'Maxillary teeth usually 10–16, 3–5 small teeth anteriorly, followed after a distinct interspace by two much enlarged recurved and fang-like teeth (below anterior border of eye), which are followed, after a second interspace, by 3–7 small teeth and then two strongly enlarged grooved fangs (below the posterior border of the eye). In *P. praeornatus* and *P. lineatus* (formerly assigned to the genus *Dromophis*) there is a continuous series of 10–16 small teeth (median longest) preceding the large grooved fangs below the posterior border of the eye. Anterior mandibular teeth strongly enlarged.'

4.1.2. The genus Psammophis (clades 1–9)

The only phylogenetic analysis incorporating several *Psammophis* taxa is the immunological study of Cadle (1994), which included the species *biseriatus*, *condanarus*, *elegans*, *phillipsii* (subspecies unknown), *punctulatus* (subspecies unknown), *rukwae*, and *subtaeniatus*. The resultant topology (Cadle's Fig. 3) showed *P. condanarus* sister to a polychotomy containing *biseriatus*, *elegans*, *punctulatus*, and a *phillipsii* + *rukwae* + *subtaeniatus* clade. This is congruent with our results. We defined nine lineages in the genus (Fig. 1) and a high proportion of nodes had significant support. The following points are noteworthy.

(1) The southern African *Psammophis crucifer* (clade 1) and the two exclusively Eurasian taxa (clade 2: *P. condanarus* and *P. lineolatus*) are the earliest-diverging lineages. The juxtaposition of these geographically distantly separated taxa raises interesting biogeographic questions.

(2) Five southern African taxa are included in clade 3 and, with the exception of *P. trigrammus*, all were considered by Broadley (1977a) to be related and were later referred to as the "*Psammophis notostictus* complex" (Broadley, 2002). Broadley (1977a, 2002) treated *P. trigrammus* as a member of the "Afro-Asian *P. schokari* group", but our results indicate that it is the earliest-diverging lineage in the *Psammophis notostictus* group. Within this clade, three subspecies of *Psammophis leightoni* were recognised by Broadley (1975)—*leightoni*, *trinasalis* and *namibensis*—all of which were subsequently elevated to species status (Broadley, 2002). We were only able to include single representatives of *P. leightoni* and *P. namibensis*, and their low K2P distance (0.39%; Table 2) would suggest intra- rather than interspecific divergence. Further molecular work on the *P. leightoni* complex is necessary on a broad geographic scale to investigate the existence and limits of putative species boundaries in the group.

(3) *Psammophis angolensis* (clade 4) is a widespread species with several peculiar autapomorphies, including small size, reduced number of teeth, and a substantial reduction in the number of dorsal scale rows. It was originally described under the generic name *Amphiophis* Bocage, 1872, and Brandstätter (1995) suggested that this be revived as a monotypic subgenus. However our phylogenies place it squarely in the genus *Psammophis*.

(4) Clade 5 includes *Psammophis schokari* (subspecies uncertain, probably *schokari*), *P. punctulatus trivirgatus*, *P. elegans*, and *Dromophis* (now *Psammophis*) *praeornatus*. It is likely that *P. schokari aegyptius* and *P. punctulatus trivirgatus* both represent valid species (*aegyptius*: Schleich et al., 1996; Rato et al., 2007. *trivirgatus*: Lanza, 1990; Hughes, 1999), but our data were insufficient to address these issues. Broadley (1977a, 2002) included the species *schokari*, *aegyptius*, *punctulatus*, *elegans* and *trigrammus* in the "*Psammophis schokari* group". With the exception of *P. trigrammus* (placed in clade 3), and with the addition of *P. praeornatus*, our results agree with Broadley's morphological assessment.

(5) The distinction between *Psammophis biseriatus* and *Psammophis tanganicus* (clade 6) was first appreciated by Loveridge (1940), who described *tanganicus* as a subspecies of *P. biseriatus* on the basis of scalation differences. Bezy and Drewes (1985) confirmed and reinforced these distinctions and discovered that the two forms were sympatric at several sites, so elevated them to species level. Subsequently Largen and Rasmussen (1993) analysed a large sample of specimens from Ethiopia and northern Somalia and found higher degrees of morphological overlap than in the earlier studies. The minimum mitochondrial K2P distance between these taxa was 11.38% (Table 2) and our phylogenetic results support their reciprocal monophyly, providing further evidence for their status as separate species.

The remainder of *Psammophis* diversity resides in the '*sibilans*' complex. The following discussion is centred on the two subdivisions of this problematic clade: the '*phillipsii*' complex (clade 8) and the '*subtaeniatus*' complex (clade 9).

4.1.3. Species delimitation in the Psammophis 'sibilans' complex (clades 8–9)

The use of mitochondrial DNA data in delineation of species boundaries is common (e.g., Puorto et al., 2001; Wiens and Penkrot, 2002; Hebert et al., 2004; Roe and Sperling, 2007), and mtDNA has an important advantage for species delimitation compared with nuclear markers (e.g., nuclear DNA and morphology): its smaller effective population size will cause alleles to coalesce approximately four times more recently than in the latter. Thus, species should become distinct in their mtDNA haplotype phylogenies well before this is evident in phylogenies from nuclear data. Mitochondrial gene trees are able to span the interface between intraspecific and interspecific evolution, and are consequently powerful tools that can be used to investigate and delimit the boundaries between species (Templeton, 2001). However, there are also several limitations of mtDNA for inference of species boundaries. It is becoming increasingly evident that ecological selection can produce a reduction in gene flow across habitat boundaries, potentially leading to reproductive isolation (ecotonal speciation: Smith et al., 1997; Thorpe and Richard, 2001; Ogden and Thorpe, 2002). In some cases, ecological selection may be more important than historical vicariance in causing reproductive isolation (e.g., Thorpe and Richard, 2001), and in these cases real species boundaries may be very different from patterns of historical vicariance inferred using mtDNA. Other limitations of mtDNA for species delimitation include: (1) retention of ancestral polymorphism; (2) sensitivity to sexual biases in dispersal and gene flow; (3) inheritance of the entire mitochondrial genome as a single linkage unit; (4) introgression; and (5) paralogy resulting from transpositions of mtDNA to the nuclear genome (e.g., Moritz and Cicero, 2004).

There is insufficient research on the Psammophiidae to determine whether sexually biased dispersal may be an issue. Hybridization appears to be rare, and every effort was made in this study to avoid comparison of paralogous sequences. We have based our investigation of species limits on mtDNA data alone but it is clear that species hypotheses proposed on this basis should be tested against independent data sets, so the species boundaries delineated here must be viewed as provisional. A further caveat concerns the small sample sizes for most of the focal species tested. Identification of species boundaries requires accurate characterisa-

tion of intraspecific diversity, and this can only be based on small samples if these samples extend across population subdivisions as indicated by geography and phenotypic variation (Moritz and Cicero, 2004; Roe and Sperling, 2007). Our sample of *P. mossambicus* was fairly comprehensive, and geographic coverage was reasonable for *P. orientalis* and *P. rukwae*. However for the other taxa, data were unavailable from large sectors of their geographic ranges. Noting these limitations, our results allow the following provisional conclusions.

The '*phillipsii*' complex (clade 8) contains four focal species: *P. leopardinus*, *P. brevirostris*, *P. phillipsii* (including the subspecies *phillipsii* and *occidentalis*), and *P. mossambicus*, all of which have complex taxonomic histories. The first two taxa were formerly regarded as subspecies of *P. sibilans* (e.g., Broadley, 1977a), but both were elevated to species status by Broadley (2002). The original description of *P. phillipsii* (Hallowell, 1844) was based on specimens from Liberia, and Broadley (1977a, 1983) treated this as a widespread species ranging from Senegal to South Africa. Subsequently, Brandstätter (1995) and Hughes (1999) considered *P. phillipsii* to be restricted to West Africa, and Branch (1998) was the first to use the name *P. mossambicus* Peters, 1882 to refer to the former southern and East African populations of *P. phillipsii*. This name is now in common usage. *Psammophis phillipsii occidentalis* (Werner, 1919) was initially described as a colour variant of *P. sibilans*, and Hughes and Wade (2004) resurrected it as a subspecies of *P. phillipsii*.

Our analyses provide support for the species status of *P. leopardinus* and P. *brevirostris*, but results for the other two taxa require a different interpretation. The three samples of *P. cf. phillipsii occidentalis* form a clade sister to *P. brevirostris*, and application of the WP strategy indicates that this clade represents a species-level taxon. As already noted, the identification of our *P. cf. p. occidentalis* specimens was based on potentially labile characters associated with pattern and colouration, and is thus somewhat uncertain. Further, all of these specimens were collected outside the known distribution range of *P. p. occidentalis*, so we refer to these specimens as *P. cf. occidentalis*. On the basis of morphological similarities, we predict that *Psammophis zambiensis* probably belongs in clade 8a with *P. brevirostris* and *P. cf. occidentalis*. The single *P. p. phillipsii* sample is nested among haplotypes of *P. mossambicus* in clade 8b, but since only one sample of the former was available, no decisions were possible regarding gene flow between the two focal species. A primary feature supposedly distinguishing *P. p. phillipsii* from *P. mossambicus* (and from all other West African *Psammophis* species) is the state of the cloacal scale: entire (CSE) in *P. p. phillipsii* and divided (CSD) in the others. However, Hughes and Wade (2004) note a marked east–west cline in this character in West Africa (high proportion of CSD in the east and high CSE in the west), and the two forms are often found in sympatry. Luiselli et al. (2004) could find no ecological differences between sympatric CSE and CSD specimens in southern Nigeria. We tentatively interpret the balance of evidence as indicating that *P. p. phillipsii* and *P. mossambicus* are conspecific, and hereafter we refer to clade 8b as *P. phillipsii*. Note that within this clade there is good support for a disjunction between the South African haplotypes (231 and TM83688) and the remaining samples, and these earliest-diverging lineages are concordant with geographic distribution. It is thus possible that future work could show these South African populations to represent a distinct species.

The '*subtaeniatus*' complex (clade 9) contains five focal species: *P. cf. sibilans* (Ethiopian), *P. rukwae*, *P. subtaeniatus*, *P. sudanensis*, and *P. orientalis*. Largen and Rasmussen (1993) examined a large sample of Ethiopian *Psammophis cf. sibilans* and found the vast majority to agree with typical Egyptian *P. sibilans* in their infralabial arrangement. Broadley (1966) described *Psammophis rukwae* (from southwest Tanzania) as a subspecies of *P. sibilans*, and subsequently elevated it to species status (Broadley, 1977a). *Psammophis subtaeniatus* Peters, 1882 was described from Mozambique, and *P. sudanensis* and *P. orientalis* have at various times both been considered subspecies of this taxon. *Psammophis subtaeniatus* var. *sudanensis* Werner, 1919 was described from the southern Sudan and treated as a subspecies of *P. subtaeniatus* by Loveridge (1940) and Broadley (1966). Later Broadley (1977a) considered the type to be referable to *P. sibilans*, but reinspection of the specimen (by DGB) indicates that *P. sudanensis* is probably a valid name. *Psammophis orientalis* was described as an eastern subspecies of *P. subtaeniatus* by Broadley (1977a), and later elevated to species status on the basis of sympatry with *P. s. subtaeniatus* in southern Zimbabwe.

The WP strategy provides support for the status of all focal species in the '*subtaeniatus*' complex. The unusually high intraspecific divergences in the *rukwae* and *orientalis* clades (Table 2) were in both cases due largely to single divergent haplotypes (*P. cf. rukwae* (PEM1) in the former and 177 in the latter), though in *P. rukwae* many distances involving other haplotypes were also relatively high (above 5%). The elevated divergence of PEM1 (from Senegal) is perhaps unsurprising given its geographic separation of over 5700 km from its nearest neighbour in our taxon sample. We consider the inclusion of this sample in the *P. rukwae* clade to support claims by Broadley (1977a), subsequently followed by others (Böhme, 1978, 1986; Joger, 1981; Böhme and Schneider, 1987), that this taxon occurs in West Africa. However, the high levels of mitochondrial divergence within *P. rukwae* and *P. orientalis* suggest the presence of taxonomically unrecognised diversity, and additional sampling will be necessary to further investigate species boundaries in these two clades.

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Appendix A

Sequence sources for the analyses presented in this paper

Classification		Geographic origin				Voucher specimen[a]	mtDNA haplotype	GenBank accession number, or author		
Genus	Species (subspecies)	Locality	Country	Lat (decimal degrees)	Long (decimal degrees)			Cytb	ND4 and tRNA	c-mos
Ingroup for phylogenetic reconstruction										
Dipsina	*multimaculata*		Namibia			CMRK62	62	**DQ486370**	**DQ486209**	—
Dipsina[b]	*multimaculata*	Namaqualand	South Africa	−29.02	18.09	TM84514	TM84514	**DQ486357**	**DQ486332**	**DQ486181**
Dromophis	*lineatus*	Bujumbura	Burundi	−3.35	29.29	CMRK379	379	**DQ486426**	**DQ486263**	—
Dromophis	*lineatus*	Bujumbura	Burundi	−3.21	29.29	CMRK383	383	**DQ486428**	**DQ486265**	—
Dromophis	*lineatus*	Bello Tonga	Benin	12.05	3.21		Chir02	—	**EU526861**	—
Dromophis	*praeornatus*	Nienie	Benin	11.36	2.21		Chir01	—	**EU526860**	—
Hemirhagerrhis	*hildebrandtii*	Arusha Region	Tanzania			CMRK319	319	**DQ486418** (short)	**DQ486255** (short)	—
Hemirhagerrhis[b]	*kelleri*	Kingori	Tanzania	−3.28	36.98	CMRK77	77	**DQ486335**	**DQ486311**	**DQ486159**
Hemirhagerrhis	*kelleri*	Kingori	Tanzania	−3.28	36.98	CMRK400	400	**DQ486436**	**DQ486272**	—
Hemirhagerrhis	*viperina*	Okangwati	Namibia	−17.07	13.27	CAS(AMB5989)	PEM11	**DQ486453**	**DQ486289**	—
Malpolon[b]	*moilensis*		Tunisia			HLMD	27	**DQ486333**	**DQ486309**	**DQ486157**
Malpolon[b]	*monspessulanus*						Malp_05	AY058965	AY058989[c]	AY058936
Mimophis	*mahafalensis* (*mahafalensis*)	Mont. des Francais	Madagascar				23	**DQ486363**	**DQ486202**	—
Mimophis	*mahafalensis* (*madagascariensis*)	Mount Ibity	Madagascar				24_str	**DQ486440**	**DQ486276**	—
Mimophis	*mahafalensis* (?)	Tulear District	Madagascar				PEM2	**DQ486461**	**DQ486297**	—
Mimophis	*mahafalensis* (*madagascariensis*)						Mim_mad	AY188031	—	—
Mimophis[b]	*mahafalensis* (*mahafalensis*)						Mim_mah	AY188032	AF544662[c]	AY187993
Psammophis[b]	*angolensis*	Kazungula	Botswana	−17.84	25.23	CMRK263	263	**DQ486410**	**DQ486248**	**DQ486189**
Psammophis	*angolensis*	Kabwe	Zambia	−14.58	28.26	CMRK283	283	**DQ486416**	**DQ486254**	—
Psammophis	*angolensis*	Usa River	Tanzania	−3.37	36.85	CMRK392	392	**DQ486433**	**DQ486270**	—
Psammophis	*angolensis*	Kasane	Botswana	−17.82	24.86	CMRK447	447	**DQ486439**	**DQ486275**	—
Psammophis	*biseriatus*	Arusha	Tanzania	−3.37	36.68	CMRK169	169	**DQ486389**	**DQ486228**	—
Psammophis	*biseriatus*	Watamu	Kenya	−3.35	40.07	BK10724	BK6	**DQ486448**	**DQ486284** (short)	—
Psammophis	*brevirostris*	Marondera	Zimbabwe	−18.18	31.51	CMRK186	186	**DQ486395**	**DQ486234**	—
Psammophis	*brevirostris*	Hole in the Wall	South Africa	−32.03	29.11	CMRK237	237	**DQ486402**	**DQ486241**	—
Psammophis	*brevirostris*	Hole in the Wall	South Africa	−32.03	29.11	CMRK238	237	**DQ486403**	**DQ486242**	—
Psammophis	*brevirostris*	Naboomspruit	South Africa	−24.36	28.83	CMRK277	277	**DQ486412**	**DQ486250**	—

(*continued on next page*)

Appendix A (*continued*)

Classification		Geographic origin				Voucher specimen[a]	mtDNA haplotype	GenBank accession number, or author		
Genus	Species (subspecies)	Locality	Country	Lat (decimal degrees)	Long (decimal degrees)			Cytb	ND4 and tRNA	c-mos
Psammophis	*brevirostris*	Cullinan Mine	South Africa	−25.68	28.52	TM83922	TM83922	**DQ486470**	**DQ486306**	—
Psammophis[b]	*condanarus*						Psamm_con	AF471075	AY058987[c]	AF471104
Psammophis	*crucifer*	Somerset East	South Africa	−32.72	25.58	CMRK19	19	**DQ486360**	**DQ486199**	—
Psammophis	*crucifer*	Jeffrey's Bay	South Africa	−33.94	24.99	CMRK70	70	**DQ486334**	**DQ486310**	—
Psammophis[b]	*crucifer*	Nyanga	Zimbabwe	−18.24	32.77	CMRK203	203	**DQ486397**	**DQ486236**	**DQ486188**
Psammophis	*crucifer*	DeHoop Nat. Res.	South Africa	−34.43	20.48		S3	**DQ486466**	**DQ486302**	—
Psammophis	*elegans*	La Tapoa	Niger	12.05	2.26		Chir03	—	**EU526862**	—
Psammophis	*elegans*						EF128027	—	EF128027	—
Psammophis	*jallae*	Kazungula	Botswana	−17.95	25.23	CMRK256	256	**DQ486409**	**DQ486247**	—
Psammophis	*namibensis*	Port Nolloth	South Africa	−29.25	16.87	PEM R15811	PEM13	**DQ486455**	**DQ486291**	—
Psammophis[b]	*leightoni*	Piketberg	South Africa	−32.90	18.77	TM83620	TM83620	**DQ486467**	**DQ486303**	**DQ486197**
Psammophis	*leopardinus*	Opuwo	Namibia	−18.47	13.80	CAS214763	PEM14	**DQ486456**	**DQ486292**	—
Psammophis	*leopardinus*	Grootberg Pass	Namibia	−19.87	14.07	CAS214727	PEM3	**DQ486462**	**DQ486298**	—
Psammophis[b]	*lineolatus*	Nephtezavodsk	Turkmenistan	39.25	63.18	CAS 179682	CAS179682	**DQ486450**	**DQ486286**	**DQ486195**
Psammophis	*mossambicus*	Tanga	Tanzania	−5.16	38.98	PEM R5679	10	**DQ486359**	**DQ486198**	—
Psammophis	*mossambicus*	Kingori	Tanzania	−3.28	36.98	CMRK81	81	**DQ486373**	**DQ486212**	—
Psammophis	*mossambicus*	Kingori	Tanzania	−3.28	36.98	CMRK82	82	**DQ486374**	**DQ486213**	—
Psammophis[b]	*mossambicus*	Nyagatare	Rwanda	−1.36	30.35	CMRK125	125	**DQ486383**	**DQ486222**	**DQ486185**
Psammophis	*mossambicus*	Nyagatare	Rwanda	−1.29	30.23	CMRK126	126	**DQ486384**	**DQ486223**	—
Psammophis	*mossambicus*	Nyagatare	Rwanda	−1.29	30.23	CMRK127	127	**DQ486385**	**DQ486224**	—
Psammophis	*mossambicus*	Mikumi Nat. Pk.	Tanzania	−7.35	37.08	CMRK175	175	**DQ486392**	**DQ486231**	—
Psammophis	*mossambicus*	Sodwana Bay	South Africa	−27.69	32.37	CMRK231	231	**DQ486400**	**DQ486239**	—
Psammophis	*mossambicus*	Pandamatenga	Botswana	−18.64	25.63	CMRK268	268	**DQ486411**	**DQ486249**	—
Psammophis	*mossambicus*	Butare	Rwanda	−2.69	29.71	CMRK376	376	**DQ486423**	**DQ486260**	—
Psammophis	*mossambicus*	Maun	Botswana	−19.98	23.42		Bills	**DQ486442**	**DQ486278**	—
Psammophis	*mossambicus*	Makuyu	Kenya	−0.90	37.18	BK10357	BK5	**DQ486447**	**DQ486283**	—
Psammophis	*mossambicus*	Moebase	Mozambique	−17.06	38.69	PEM R13258	PEM15	**DQ486457**	**DQ486293**	—
Psammophis	*mossambicus*	Namagure	Mozambique	−17.06	38.69	PEM R13217	PEM15	**DQ486460**	**DQ486296**	—
Psammophis	*mossambicus*	Palaborwa	South Africa	−23.95	31.12	TM83688	TM83688	**DQ486468** (short)	**DQ486304**	—
Psammophis	*notostictus*	Grahamstown	South Africa	−33.30	26.51	PEM R5660	21	**DQ486362**	**DQ486201**	—
Psammophis[b]	*notostictus*	Mtn. Zebra Nat. Pk.	South Africa	−32.17	25.27	PEM R5682	55	**DQ486366**	**DQ486205**	**DQ486182**
Psammophis	*notostictus*	Mtn. Zebra Nat. Pk.	South Africa	−32.17	25.27	PEM R5669	56	**DQ486367**	**DQ486206**	—
Psammophis	*notostictus*	Port Nolloth	South Africa	−29.25	16.87	PEM	PEM6	**DQ486463**	**DQ486299**	—
Psammophis	*orientalis*	Handeni	Tanzania	−5.43	38.02	CMRK89	89	**DQ486380**	**DQ486219**	—
Psammophis	*orientalis*	Gilgil	Kenya	−0.50	36.32	NMK O/3597	161	**DQ486386**	**DQ486225**	—
Psammophis	*orientalis*	Nguru Mtns	Tanzania	−5.43	37.45	CMRK171	171	**DQ486390**	**DQ486229**	—
Psammophis	*orientalis*	Nguru Mtns	Tanzania	−5.43	37.45	CMRK172	172	**DQ486391**	**DQ486230**	—
Psammophis	*orientalis*	Udzungwa Nat. Pk.	Tanzania	−7.58	36.36	CMRK177	177	**DQ486393**	**DQ486232**	—
Psammophis	*orientalis*	Gorongosa	Mozambique	−18.18	34.11	CMRK187	187	**DQ486396**	**DQ486235**	—

Psammophis	*orientalis*	Moma	Mozambique	−16.76	39.22	PEM R15622	PEM17	**DQ486459**	**DQ486295**	—
Psammophis	*phillipsii* (*phillipsii*)	Loango Nat. Pk.	Gabon	−2.36	9.64	PEM R5451	PEM12	**DQ486454**	**DQ486290**	—
Psammophis	*cf. phillipsii* (*occidentalis*)	Serenje	Zambia	−12.76	30.93	CMRK71	71	**DQ486371**	**DQ486210**	—
Psammophis	*cf. phillipsii* (*occidentalis*)	Kizuka	Burundi	−3.90	29.39	CMRK377	377	**DQ486424**	**DQ486261**	—
Psammophis	*cf. phillipsii* (*occidentalis*)	Bubanza	Burundi	−3.06	29.43	CMRK381	381	**DQ486427**	**DQ486264**	—
Psammophis[b]	*punctulatus* (*trivirgatus*)	Lolkisale	Tanzania	−3.77	36.42	CMRK167	167	**DQ486387**	**DQ486226**	**DQ486186**
Psammophis	*punctulatus* (*trivirgatus*)	Arusha Region	Tanzania			CMRK391	391	**DQ486432**	**DQ486269**	—
Psammophis	*punctulatus* (*trivirgatus*)	Watamu	Kenya	−3.35	40.02	BK10476	BK3	**DQ486445**	**DQ486281**	—
Psammophis	*rukwae*	Kondoa Region	Tanzania	−4.90	35.78	CMRK83	83	**DQ486375**	**DQ486214**	—
Psammophis	*rukwae*	Kondoa Region	Tanzania	−4.90	35.78	CMRK85	85	**DQ486376**	**DQ486215**	—
Psammophis	*rukwae*	Lake Baringo	Kenya	0.47	35.97	BK10358	BK1	**DQ486443**	**DQ486279**	
Psammophis	*rukwae*	Kakuyuni	Kenya	−3.22	40.00	BK10620	BK4	**DQ486446**	**DQ486282**	—
Psammophis	*cf. rukwae*		Senegal	13.98	−14.57	MNHN	PEM1	**DQ486452**	**DQ486288**	—
Psammophis	*schokari* (? *schokari*)	Hazoua	Tunisia			HLMD	26	**DQ486364**	**DQ486203**	—
Psammophis	*schokari* (? *schokari*)	Tantan	Morocco				43	**DQ486365**	**DQ486204**	—
Psammophis[b]	*schokari* (? *schokari*)	Bou Hedma	Tunisia			HLMD	25_str	**DQ486441**	**DQ486277**	**DQ486194**
Psammophis	*cf. sibilans*	Keriyo Hamlet	Ethiopia	9.40	38.65	CMRK352	352	**DQ486419**	**DQ486256**	—
Psammophis	*cf. sibilans*	Keriyo Hamlet	Ethiopia	9.40	38.65	CMRK358	358	**DQ486420**	**DQ486257**	—
Psammophis	*cf. sibilans*	Keriyo Hamlet	Ethiopia	9.40	38.65	CMRK364	364	**DQ486422**	**DQ486259**	—
Psammophis	*cf. sibilans*	Derba	Ethiopia	9.40	38.67		C29sb	**DQ486449**	**DQ486285**	—
Psammophis	*subtaeniatus*	Kwekwe	Zimbabwe	−18.92	29.82	NMZB	4	**DQ486358**	—	—
Psammophis	*subtaeniatus*	Kazungula	Botswana	−17.96	25.23	CMRK249	249	**DQ486408**	—	—
Psammophis	*subtaeniatus*	Kariba	Zimbabwe	−16.52	28.80	CMRK282	282	**DQ486415**	**DQ486253** (short)	—
Psammophis[b]	*sudanensis*	Kingori	Tanzania	−3.28	36.98	CMRK91	91	**DQ486382**	**DQ486221**	**DQ486184**
Psammophis	*sudanensis*	Loitokitok	Kenya	−2.84	37.52	CMRK334	334	—	**DQ486307**	—
Psammophis	*sudanensis*	Athi River	Kenya	−1.45	36.98	CMRK385	385	**DQ486429**	**DQ486266**	—
Psammophis	*sudanensis*	Athi River	Kenya	−1.45	36.98	CMRK386	386	**DQ486430**	**DQ486267**	—
Psammophis	*sudanensis*	Namanga	Tanzania	−2.87	36.72	CMRK390	390	**DQ486431**	**DQ486268**	—
Psammophis	*sudanensis*	Tsavo Nat. Pk.	Kenya	−2.98	38.47	BK10603	BK2	**DQ486444**	**DQ486280**	—
Psammophis	*tanganicus*	Dodoma Region	Tanzania			CMRM86	86	**DQ486377**	**DQ486216**	—
Psammophis[b]	*tanganicus*	Dodoma Region	Tanzania			CMRK87	87	**DQ486378**	**DQ486217**	**DQ486183**
Psammophis	*tanganicus*	Dodoma Region	Tanzania			CMRK88	88	**DQ486379**	**DQ486218**	—
Psammophis	*tanganicus*	Arusha Region	Tanzania			CMRK90	90	**DQ486381**	**DQ486220**	—
Psammophis[b]	*trigrammus*	Sesfontein	Namibia	−19.17	13.57	CAS214751	PEM16	**DQ486458**	**DQ486294**	**DQ486196**
Psammophis	*trigrammus*	Brandberg	Namibia	−21.13	14.58	TM83873	TM83873	**DQ486469**	**DQ486305**	—
Psammophylax	*multisquamis*	Doroba	Ethiopia	9.33	38.92	CMRK361	361	**DQ486421**	**DQ486258**	—
Psammophylax[b]	*rhombeatus*	Grahamstown	South Africa	−33.30	26.51	CMRK234	234	**DQ486342**	**DQ486318**	**DQ486166**
Psammophylax[b]	*tritaeniatus*	Mvuma	Zimbabwe	−19.53	30.73	CMRK279	279	**DQ486414**	**DQ486252**	**DQ486190**

(*continued on next page*)

Appendix A (*continued*)

Classification		Geographic origin				Voucher specimen[a]	mtDNA haplotype	GenBank accession number, or author		
Genus	Species (subspecies)	Locality	Country	Lat (decimal degrees)	Long (decimal degrees)			Cytb	ND4 and tRNA	c-mos
Psammophylax	*variabilis* (*variabilis*)	Udzungwa Mtns	Tanzania	−8.28	35.90	CMRK413	413	**EU526863**	**EU526859**	—
Rhamphiophis[b]	*acutus* (*acutus*)	Gitaba	Burundi	−3.85	29.98	CMRK378	378	**DQ486425**	**DQ486262**	**DQ486192**
Rhamphiophis	*acutus* (*acutus*)	Luzamba	Angola	−9.12	18.06	PEM R13485	PEM8	**DQ486464**	**DQ486300**	—
Rhamphiophis	*rostratus*	Dodoma Region	Tanzania			CMRK80	80	**DQ486336**	**DQ486312**	—
Rhamphiophis[b]	*rostratus*	Bubye River	Zimbabwe	−21.70	30.51	CMRK185	185	**DQ486394**	**DQ486233**	**DQ486187**
Rhamphiophis	*rubropunctatus*	Kilimanjaro Airport	Tanzania	−3.43	37.07	CMRK303	303	**DQ486417**	—	—
Outgroups for phylogenetic reconstruction										
Aspidelaps[b]	*scutatus*							AF217828	AY058969[c]	AY058923
Atractaspis[b]	*bibronii*							AY188008	U49314[c]	AY187969
Buhoma[b]	*procterae*	Udzungwa Nat. Pk.	Tanzania	−8.37	35.97	ZMUC R631315		**DQ486353**	**DQ486328**	**DQ486177**
Duberria[b]	*lutrix*	Limuru	Kenya	−1.11	36.61	NMK O/3578		**DQ486337**	**DQ486313**	**DQ486161**
Lamprophis[b]	*guttatus*	Ngotshe District	South Africa	−27.85	31.33	TM84363		**DQ486355**	**DQ486330**	**DQ486179**
Pseudaspis[b]	*cana*[b]	Nata	Botswana	−19.92	26.15	CMRK246		**DQ486343**	**DQ486319**	**DQ486167**

Original sequences are in bold, and where available, voucher and locality data are given for the associated specimens. Haplotype labels are reflected in the figures, tables and text. Psammophiid taxonomy follows the most recent literature and does not incorporate the changes proposed in this paper.

[a] Institutional codes for voucher specimens: BK, Bio-Ken collection, Watamu, Kenya; CAS, California Academy of Sciences, San Francisco, USA; CMRK, Christopher M. R. Kelly, private collection; HLMD, Hessisches Landesmuseum Darmstadt, Germany; MNHN, Museum National d'Histoire Naturelle, Paris, France; NMK, National Museums of Kenya; NMZB, Natural History Museums of Zimbabwe, Bulawayo; PEM, Port Elizabeth Museum, South Africa; TM, Northern Flagship Institution, Pretoria, South Africa; ZMUC, Zoological Museum, University of Copenhagen, Denmark.

[b] Taxa comprising the pruned 'mixed' data set in which mitochondrial and nuclear sequences were combined.

[c] Sequences lacking tRNA data.

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