

MOLECULAR SYSTEMATICS AND PHYLOGENY OF OLD AND NEW WORLD RATSNAKES, *Elaphe* AUCT., AND RELATED GENERA (REPTILIA, SQUAMATA, COLUBRIDAE)

Urs Utiger,^{1,5} Notker Helfenberger,² Beat Schätti,³
Catherine Schmidt,¹ Markus Ruf,⁴ and Vincent Ziswiler¹

Submitted January 30, 2002.

The phylogenetic relationships of the Holarctic ratsnakes (*Elaphe* auct.) are inferred from portions of two mitochondrial genes, 12S rRNA and COI. *Elaphe* Fitzinger is made up of ten Palaeartic species. *Natrix longissima* Laurenti (type species) and four western Palaeartic species (*hohenackeri*, *lineatus*, *persicus*, and *situla*) are assigned to *Zamenis* Wagler. Its phylogenetic affinities with closely related genera, *Coronella* and *Oocatochus*, remain unclear. The East Asian *Coluber porphyraceus* Cantor is referred to a new genus. This taxon and the western European *Rhinechis scalaris* have an isolated position among Old World ratsnakes. Another new genus is described for four Oriental species (*cantoris*, *hodgsonii*, *moellendorffi*, and *taeniurus*). New World ratsnakes and allied genera are monophyletic. *Coluber flavirufus* Cope is referred to *Pseudelaphe* Mertens and Rosenberg. *Pantherophis* Fitzinger is revalidated for *Coluber guttatus* L. (type species) and further Nearctic species (*bairdi*, *obsoletus*, and *vulpinus*). *Senticolis triaspis* is the sister taxon of New World ratsnakes including the genera *Arizona*, *Bogertophis*, *Lampropeltis*, *Pituophis*, and *Rhinocheilus*. The East Asian *Coluber conspicillatus* Boie and *Coluber mandarinus* Cantor form a monophyletic outgroup with respect to other Holarctic ratsnake genera and are referred to *Euprepiophis* Fitzinger. Three Old World species, viz. *Elaphe* (sensu lato) *bella*, *E.* (s.l.) *frenata*, and *E.* (s.l.) *prasina* remain unassigned. The various groups of ratsnakes (tribe Lampropeltini) show characteristic hemipenis features.

Key words: Lampropeltini, new genera, *Arizona*, *Bogertophis*, *Coronella*, *Elaphe*, *Euprepiophis*, *Lampropeltis*, *Oocatochus*, *Pantherophis*, *Pituophis*, *Pseudelaphe*, *Rhinechis*, *Rhinocheilus*, *Senticolis*, *Zamenis*, mtDNA, COI, 12S rDNA, phylogeny, Nearctic, Oriental, Palaeartic.

INTRODUCTION

Until quite recently, the systematic concept of the Old and New World ratsnakes (*Elaphe* auct.) dated back to Boulenger (1894, as *Coluber* L.). Nowadays, and without taking account of six Oriental species recently assigned to *Coelognathus* Fitzinger, 33 nominal species of ratsnakes including *E. lineata* (Came-

rano) are commonly referred to the genus *Elaphe* (sensu Schulz, 1996 and Helfenberger, 2001). These species inhabit the Palaeartic, Oriental, Nearctic, as well as the extreme northern part of the Neotropical region. The type species, *Elaphe sauromates* Pallas, is a Palaeartic taxon.

Based on scutellation, hemipenis, and osteological features of American ratsnakes and several Old World taxa, Dowling (1958) referred two Oriental species, *Coluber oxycephalus* Boie, 1827 and *Gonyosoma janseni* Bleeker, 1858, to *Gonyosoma* Wagler, 1828. Using immunological and comparative protein studies, Minton (1976), Lawson and Dessauer (1981), Dowling et al. (1983), Dessauer et al. (1987), and other authors concluded that *Elaphe* auct. as

¹ Zoologisches Museum der Universität Zürich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.

² Käseereistrasse 4, CH-9305 Berg, Switzerland.

³ Apartado postal 383, San Miguel de Allende, Gto. 37700, República Mexicana.

⁴ Kirchplatz 2, CH-9246 Niederbüren, Switzerland.

⁵ Address correspondence and reprint requests to Urs Utiger, Fax: +41 (1) 635-6818; E-mail: utiger@zoolmus.unizh.ch.

presently understood represents a large polyphyletic group.

Based on scale features, osteology, and hemipenis morphology, Dowling and Fries (1987) assigned *Coluber triaspis* Cope to the monotypic genus *Senticolis*. Dowling and Price (1988) referred *Coluber rosaliae* Mocquard and *Coluber subocularis* Brown to another new genus, *Bogertophis*, due to microdermatoglyphic pattern, karyotype, and immunological data. Schulz (1996) recognized twelve groups of ratsnakes under the generic name *Elaphe*, including *Gonyosoma* and *Bogertophis* spp. as well as *Senticolis triaspis*, and further stressed the heterogeneous character of this taxon.

Utiger (1996) performed a RFLP analysis of eleven Palaearctic and one Nearctic (*guttata*) ratsnake species. Small genetic distances were found between the Japan endemics *Elaphe climacophora* and *E. quadrivirgata* as well as Asian mainland species (*E. dione*, *E. carinata*) as compared to the Japanese *E. conspicillata* and the remaining taxa under consideration.

The molecular analysis of Rodriguez-Robles and De Jesus-Escobar (1999) contributed to a better understanding of the relationships among the New World ratsnakes or Lampropeltini (see, e.g., Dowling et al., 1983), viz. the genera *Arizona* Kennicott in Baird, 1859, *Bogertophis* Dowling and Price, 1988, *Cemophora* Cope, 1860, *Elaphe* (auct.), *Lampropeltis* Fitzinger, 1843, *Pituophis* Holbrook, 1842, *Rhinocheilus* Baird in Baird and Girard, 1853, *Senticolis* Dowling and Fries, 1987, and *Stilosoma* Brown, 1890. Their phylogenetic reconstruction from a portion of the mitochondrial (mt) ND4 gene as well as three different tRNA genes suggests monophyly of the genera *Cemophora*, *Lampropeltis* (including *Stilosoma*), *Pituophis*, and the Nearctic species *Elaphe* (auct.) *bairdi*, *guttata*, *obsoleta*, and *vulpina*.

Based on anatomy, osteology, and electrophoresis data of virtually all Old World ratsnakes, Helfenberger (2001) described a new genus, *Oocatochus*, for the semiaquatic and viviparous *Tropidonotus rufodorsatus* Cantor. Furthermore, this author revalidated the genus *Coelognathus* Fitzinger, 1843 for six Oriental species (*erythrurus*, *flavolineatus*, *helena*, *philippinus*, *radiatus*, and *subradiatus*) formerly parading under *Elaphe* (auct.) and confirmed the validity of the monotypic western Mediterranean *Rhinechis* Michahelles, 1833 (*R. scalaris*).

Today, the comparison of portions of mtDNA is one of the most frequently used and effective tools in modern systematics. Especially for snakes with their highly reduced, functional, and conservative anatomy, this technique has proved useful to reveal the phylogenetic relationships of formerly inextricable species assemblages (e.g., Knight and Mindell, 1993; Burbrink et al., 2000). The COI gene which is slowly evolving compared to other protein-coding mitochondrial genes has been widely used for estimating molecular phylogenies (Russo et al., 1996) and is a good performer in recovering an expected tree (Zardoya and Meyer, 1996). Ribosomal RNA genes (e.g., 12S rRNA) are evolving more slowly than protein-coding genes and are particularly important for the phylogenetic analysis of distantly related species (Moritz et al., 1987).

The combination of COI and 12S rRNA is appropriate to distinguish the taxa of interest at different taxonomic levels. For the purpose of this study, new primers were developed for these fragments to guarantee a high reliability and specificity of the PCR, in particular for the coding COI gene with its highly variable third position of the amino acid codon, and a maximal reading length of the amplified product.

The hemipenis is a diagnostic feature often used for supraspecific systematic studies of snakes (e.g., McDowell, 1961; Branch, 1986). Its morphology is conspicuously different among certain ratsnake groups (see Schulz, 1996: Figs. 17–36; Schmidt, 2000). In this study, the overall shape of the hemipenis and its basal structures are compared within and between closely related ratsnake groups.

The main purpose of this investigation is to use mtDNA sequences to infer phylogenetic relationships among *Elaphe* auct., elucidate the evolutionary history within the various lineages, discuss the implication of our findings in a biogeographical context, and to determine the phylogenetic relationships of ratsnakes (*Elaphe* auct.) with presumably closely related Palaearctic and New World genera, i.e., *Arizona*, *Bogertophis*, *Coronella*, *Lampropeltis*, *Oocatochus*, *Pituophis*, *Rhinechis*, *Rhinocheilus*, and *Senticolis*. Analyzing samples from virtually all taxa, this study intends to contribute to a better understanding of the groups under consideration and a systematic revision of all ratsnakes and related genera (Lampropeltini).

MATERIAL AND METHODS

Initially, sequences from 93 individuals belonging to 52 species including *Rhinocheilus lecontei* and ten outgroup taxa (except *E. (sensu lato) frenata* and *E. (s.l.) prasina*, see below) were considered. Most sequences were obtained from fresh liver tissue (mtDNA). For three specimens (*Coluber constrictor*, *Masticophis flagellum*, *Rhinocheilus lecontei*, see **Appendix A**), genomic DNA (gDNA) was extracted from frozen muscle.

Whenever possible, at least two individuals of each species were sequenced (not so in *Bogertophis rosaliae*, *Coronella austriaca*, *C. girondica*, *Elaphe anomala*, *E. davidi*, *E. (sensu lato) frenata*, *E. (s.l.) prasina*, *E. schrenckii*, *Lampropeltis ruthveni*, *Ptyas mucosus*, *Rhinocheilus lecontei*, as well as *Elaphe (auct.) cantoris*, *hodgsonii*, and *lineata*). Data for five outgroup species (viz., *Eirenis modestus*, *Hemerophis socotrae*, *Hemorrhoids hippocrepsis*, *Hierophis caspius*, and *Platyceps rhodorachis*) are from an earlier study (Schätti and Utiger, 2001).

The origin of the tissue samples as well as the scientific names of the investigated taxa with their author and year of description are compiled in **Appendix A**. Complete citations for taxa not listed there are usually given upon their first mention in this paper. Acronyms used in the text (i.e., **Taxonomic Changes**, **Appendix A**) are BMNH for The Natural History Museum [British Museum (Natural History)], London and MHNG for Muséum d'Histoire naturelle, Geneva.

Virtually all species of Old World ratsnakes (sensu Helfenberger, 2001) except *Elaphe (s.l.) bella* (Stanley) and *E. (s.l.) perlacea* Stejneger (see **Discussion**) were analyzed. Apart from the monotypic genera *Cemophora* and *Stilosoma*, most New World lampropeltine taxa were investigated. Because *Cemophora coccinea* Cope, 1860, *Lampropeltis* and *Pituophis* spp., and *Stilosoma extenuatum* Brown, 1890 are monophyletic (Rodríguez-Robles and De Jesus-Escobar, 1999, 2000), only two species of each *Lampropeltis* and *Pituophis* were examined.

Several species of Old and New World racer genera (*Coluber*, *Eirenis*, *Hemerophis*, *Hemorrhoids*, *Hierophis*, *Platyceps*, and *Masticophis*) as well as *Coelognathus*, *Ptyas*, and *Salvadora* spp. were tested for their suitability as comparative groups for phylogenetic reconstructions.

DNA extraction. MtDNA was isolated from fresh liver tissue (Spolsky and Uzzell, 1986) and purified by phenol/chloroform extraction and ethanol precipitation (Sambrook et al., 1989). Pure mtDNA

was resuspended in TE buffer (10 mM Tris-HCl, pH 8, 1 mM Na₂EDTA) and stored at -70°C. Genomic DNA was extracted from frozen muscle using the Dneasy Tissue Kit from Qiagen.

Primer synthesis. Fragments of two mitochondrial (mt) genes, cytochrome oxidase subunit I (COI) and the small 12S rRNA subunit, were amplified with the Polymerase Chain Reaction (PCR, Saiki et al., 1988). Primers for COI were obtained by comparing a fragment of mtDNA of seven different species of ratsnakes, for 12S rDNA by comparing the mtDNA sequences of *Homo sapiens* with *Dinodon semicarinatus*. The distance between the primer pair was optimized to a length of about 600 base pairs (bp) to ensure long but qualitatively sufficient sequencing runs. Primers for the COI fragment were developed as follows.

MtDNA of eighteen different ratsnake species was treated in a restriction digest assay with *Hind III* (Amersham Pharmacia Biotech) following the manufacturer's conditions and using an overdose of restriction enzyme and an elongated incubating time of 12 h. The digested mtDNA was separated via gel electrophoresis on a 0.7% agarose gel supplemented with ethidium bromide and submersed in 1×TBE buffer. An identical restriction fragment of about 2.1 kilo base pairs was observed for all species and cut out with a sterile scalpel. The fragment of seven species was extracted from the gel slice using the QIAquick Gel Extraction Kit (Qiagen) and eluted in a total volume of 30 µl ddH₂O. 10 µl of the purified fragment and 2 µl of pBluescript KS(-) vector [40 ng/µl], previously linearized with *Hind III* and dephosphorylated with *Shrimp alkaline phosphatase* (Amersham Pharmacia Biotech), were ligated using *T4 DNA ligase* (Amersham Pharmacia Biotech). 10 µl of the ligation mix was added to 200 µl of competent XL1-Blue cells, mixed and incubated on ice for 30 min. Then the samples were heat-shocked at 42°C for 90 sec and incubated on ice for another 5 min. 1 ml LB medium was added, the samples incubated at 37°C with moderate agitation and then plated on LB agar supplemented with the necessary antibiotics. Plates were grown overnight at 37°C in an inverted position.

A selection of three transformed bacteria colonies for each individual were inoculated in 2 ml LB medium supplemented with the appropriate antibiotics and grown overnight at 37°C with moderate agitation. The cultures were transferred to microcentrifuge tubes and the cells were pelleted at 13,000 rpm for 30 sec. The supernatant was discarded and the pellet

was resuspended in 100 µl resuspension buffer provided by the supplier of the Wizard Plus SV Minipreps DNA Purification System (Promega). The following steps were performed according to the protocol provided by the supplier. Usually the plasmid DNA was eluted from the column with 50 µl ddH₂O and 5 µl was used for restriction analysis to find the recombinant constructs. Fourteen from a total of twenty four clones from seven different species were found to have recombinant plasmids. Purified recombinant plasmids were sequenced with standard primers (T7, T3) following the ABI Prism[®] BigDye[™] Terminator Cycle Sequencing Ready Reaction Kit protocol, version 2.0, using the ABI 377 automated system (PE Biosystems). The ends of the fragment comprised portions of the mt cytochrome oxidase genes (COI and COII), identified with a BLASTN search performed using the NCBI WWW server (<http://www.ncbi.nlm.nih.gov/BLAST/>).

The ratsnake sequences were aligned with the corresponding sequence of *Dinodon semicarinatus* (GenBank entry NC 001945) using Clustal X (Thompson et al., 1994) and suitable PCR primers were designed for a portion of the COI gene, that is COI(+)*deg1*, 5'-AAGCTTCTGACTNCTACCACC-NGC-3', and COI(-)*bdeg*, 5'-ATTATTGTTGCGCTGTRAARTAGGCTCG-3'. During the analysis, one primer was modified to increase the annealing specificity: COI(+)*b*, 5'-TAAATAATATAAGCTTC-TGACTGCTACCACC-3'. The PCR primers for the sequence 12S rDNA fragment were developed by comparison of *Dinodon semicarinatus* with *Homo sapiens* (accession No. NC 001807) and screened for conserved sequence regions. The developed primers are 12S268(+), 5'-GTGCCAGCGACCGCGGTTA-CACG-3', and 12S916(-), 5'-GTACGCTTACCAT-GTTACGACTTGCCCTG-3'.

PCR and sequencing. PCR was performed with a PTC-100[™] thermocycler (MJ Research, Inc.) using the following thermal profile: 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 56.5°C, 1 min at 72°C, and a final step of 10 min at 72°C. Double-stranded PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and both strands were sequenced using an ABI 377 automated system or the MegaBACE 1000 DNA Analysis System, Molecular Dynamics Inc (Amersham Pharmacia Biotech). The 12S rDNA fragment comprised 677 bp and COI 632 (642 with primer COI(+)*b*) including primers. The fragments correspond to the positions 303–980 and 6519(6518)–7151, respectively, of *Dinodon semicarinatus*. The

DNA sequences of all individuals examined within the scope of this study are deposited in the GenBank (AY122683-850).

Alignment. DNA sequences were edited with EditView (Perkin Elmer). A total of 617 positions including insertions or deletions (indels) of the 12S rDNA and 513 bp of COI sequences were aligned with Clustal X (Thompson et al., 1994). The initial alignment of the 12S rDNA fragment was improved in a second step with the *SeqLab* program of the Wisconsin Package (Genetics Computer Group, 1999) using the secondary structure information of *Homo sapiens* (Gutell et al., in prep.). The final alignment includes a total of 33 indels at 20 different regions. Twelve of them consist of one position, six of two, and three three sites. The alignment data file is available from www.unizh.ch/zoolmus/eHerpetologie.html.

Measurement of phylogenetic signal. Before performing basic statistics and phylogenetic reconstructions, RASA 3.0 (Lyons-Weiler, 2001) was used to measure the tree-independent phylogenetic signal (tRASA) for each gene separately and for the combined genes with gaps coded as fifth character (data not shown). Because of computational limits, only different haplotypes from the ingroup and only one sequenced specimen from each outgroup species was analysed. After the phylogenetic signal was measured, taxon variance ratios were calculated and examined to screen for potential long-branch taxa (Fig. 1, A). Taxon-variance outliers to inconsistent trees when included in phylogenetic analyses (Lyons-Weiler and Hoelzer, 1997). The taxon-variance outliers from the initial ingroup, viz. *Elaphe* (s.l.) *frenata* and *prasina*, were shifted to the outgroup (Fig. 1, A).

The outgroup taxa with the smallest taxon variance values were added in different combinations to 68 individuals of 39 species of the ingroup and phylogenetic signal was measured (Fig. 1, B). The combination with the highest phylogenetic signal was selected as the most suitable outgroup for the phylogenetic reconstructions. For the total evidence approach, *Ptyas mucosus* (see **Appendix A**) had the highest signal. Therefore, this species was chosen as the only outgroup for all subsequent analyses, supposing that it stands closest to the ingroup and has the least synapomorphic changes in its sequence (Lyons-Weiler et al., 1998).

Basic statistics and phylogenetic analysis. All phylogenetic analyses and descriptive statistics were performed with PAUP* version 4.0b5 for Unix and version 4.0b8 for Mac (Swofford, 1998). For the 12S

rDNA fragment, 235 (221 with gaps treated as missing characters) of 272 (254) variable characters including gaps as a fifth character state are parsimony-informative. Mean base frequencies of the L-strand are 39.5% A, 24.1% C, 16.2% G, and 20.2% T. For the COI fragment, 194 of 200 variable characters are parsimony-informative. Base composition for the coding L-strand (513 aligned sites) is 28.7% A, 25.9% C, 15.6% G, and 29.7% T. Uncorrected (p) and corrected pairwise sequence divergence of COI was plotted against the same measure of 12S rDNA (Fig. 2). In contrast to the substitution values of 12S rDNA, which increase without an apparent upper limit, p values of COI show considerable saturation tendency by losing the linear correlation with 12S rDNA at approximately 10% and tapering to a value of 18%. The third position of the codon is responsible for most of the overall variation (83.5%).

Whereas only five out of 171 (2.9%) variable sites were observed at the second position and 28 (16.4%) at the first position, 167 variable sites (97.7%) were located at the third position of the amino acid codon. The effect of corrected distance values of COI calculated with MODELTEST (Posada and Crandall, 1998) is illustrated in Fig. 2. The linear correlation with 12S rDNA extends above the 10% level, but the variance of the corrected distance values seems to be rather high.

The model of DNA evolution which best fits the data under the maximum likelihood criterion was estimated with the program MODELTEST. The parameter settings selected for COI and 12S rDNA, i.e., TrN+I+G (see Tamura and Nei, 1993), are less complex than in the general time-reversible model (GTR, Hasegawa et al., 1985). The latter was selected for the combined data set with substitution rates following a γ distribution and a shape parameter α (G), and with a proportion of invariable sites (I), estimated via maximum likelihood (GTR+G+I).

Neighbor joining (NJ, Saitou and Nei, 1987) and minimum evolution (ME) analyses were executed under the suitable substitution model and the latter with heuristic searches and tree-bisection-reconnection (TBR) branch swapping. Weighted maximum parsimony (MP) analysis was performed with heuristic search and TBR branch swapping treating gaps as a fifth character state. Character weights were calculated from the rescaled consistency index (Farris, 1989) of a preceding unweighted parsimony calculation. Nonparametric bootstrap values (Felsenstein, 1985) from NJ and weighted MP analyses were calculated including 1000 replicates.

A partition homogeneity test implemented in PAUP* was performed with ten random stepwise additions using TBR branch swapping and 1000 randomizations. A P value of 0.342 indicates that the two data sets, COI and 12S rDNA, are not significantly different in their phylogenetic content under the assumption of parsimony. This result contrasts with significant test results of previous analyses using the same gene fragments but different taxa (Schätti and Utiger, 2001). A high phylogenetic signal (Fig. 1, B) also supports a combined calculation of the two data sets. Therefore, a total evidence approach was pursued in the subsequent phylogenetic reconstructions.

Hemipenis preparation. The hemipenis shape of 33 species was drawn from everted organs. Four organs were redrawn after published illustrations, viz. *Coronella girondica* (Domergue, 1962), *Arizona elegans*, *Lampropeltis getula*, and *Pituophis melanoleucus* (Dowling and Fries, 1987). Specimens used for hemipenis preparation are listed in **Appendix B**.

Hemipenes were everted with 4% formaldehyde and tied off with thread. To conserve a maximal swelling, the organ was filled with 2% agar or vaseline (Ziegler and Böhme, 1997), cut off, and stored in 70% ethanol. The sulcate view of the right organ was illustrated with emphasize on the presence or absence of basal hooks and the overall shape of the organ (subcylindrical, bulbous or bilobed, Dowling and Savage, 1960). The shape from base to apex changes continuously (i.e., unarticulate) or abruptly (articulate). For a more detailed analysis and descriptions see Schmidt (2000).

RESULTS

Two most parsimonious trees from the total evidence approach were calculated with the weighted MP method. They are identical except for a small intraspecific difference within *Elaphe* (sensu stricto) *climacophora*. The tree (Fig. 3) has a length of 490.52 and a rescaled consistency index (RC) of 0.33. 429 out of 472 variable characters from a total of 1130 are parsimoniously informative and 373 characters have a weight different from 1. The RC lowers to 0.32 when gaps are excluded from the analysis, a clear indication of the usefulness to include indels in phylogenetic analyses.

Except in two out of 27 cases, bootstrap support increases in a combined analysis (Fig. 3). One of them confirms the basal position of *Euprepiophis conspicillatus* and *E. mandarinus* (see below). The value lowered considerably, probably due to satura-

tion effects in COI and, thus, this partition does not support the outgroup position of these species. The other confirms the sister group position of *Oocatochus* Helfenberger, 2001 and *Coronella* Laurenti, 1768. This value lowered only slightly from 65 to 58%. For all other bifurcations, the COI partition contributes with its phylogenetic tracks and an appropriate substitution model to increased bootstrap values in the total evidence approach compared with the analysis of the 12S rDNA partition only.

To illustrate the influence of different reconstruction methods on the general branching pattern, a strict consensus tree (Fig. 4) was derived from the trees resulting from the weighted MP and the ME analyses of the total evidence approach. It indicates that all bifurcations with bootstrap values over 50% from the weighed MP analysis (Fig. 3) were retained, as were four nodes with values between 39 and 47%, viz. *Senticolis triaspis* vs. all other New World taxa (see footnote 6), *Elaphe carinata* — {*E. quadrivirgata* [*anomala*, (*schrencki*)]}, *Zamenis hohenackeri* — {*Z. lineatus* [*longissimus*, (*situla*)]}, and *Euprepiophis* vs. all other ingroup species.

The rough overall pattern of the phylogenetic relationships between the investigated taxa shows eight different lineages and can be summarized as follows.

Elaphe Fitzinger, 1833 is a Palaearctic genus made up of *E. sauromates* (type species), *E. anomala*, *E. bimaculata*, *E. carinata*, *E. climacophora*, *E. davidi*, *E. dione*, *E. quadrivirgata*, *E. quatuorlineata*, and *E. schrenckii*.

Two Nearctic genera are revalidated, viz. *Pantherophis* Fitzinger, 1843 (*P. bairdi*, *P. guttatus*, *P. obsoletus*, and *P. vulpinus*) and *Pseudelaphe* Mertens and Rosenberg, 1943 (*P. flavirufa*). The monophyly of *Bogertophis rosaliae* and *B. subocularis* is confirmed. New World Lampropeltini share a common ancestor.

The western Palaearctic genus *Zamenis* Wagler, 1830 includes *Natrix longissima* Laurenti (type species), *Coluber hohenackeri* Strauch, *Callopetalis longissimus* var. *lineata* Camerano, *Coluber longissima* var. *persica* Werner, and *Coluber situla* Linnaeus (see **Taxonomic Changes**).

The validity of the monotypic genus *Rhinechis* Michahelles, 1833 is confirmed. *Coronella austriaca* and *C. girondica* are monophyletic and closely related to *Oocatochus rufodorsatus* (monotypic). *Coluber conspicillatus* Boie and *Coluber mandarinus* Cantor are referred to *Euprepiophis* Fitzinger, 1843 (see **Discussion**).

Four Oriental ratsnake species, i.e., *Coluber cantoris* Boulenger, *Cynophis moellendorffi* Boettger, *Elaphe taeniura* Cope, and *Spilotes hodgsonii* Günther make up a distinct genus within Old World ratsnakes and related genera (see **Taxonomic Changes**).

***Orthriophis* gen. nov.**

Etymology. This genus is named for the early morning activity pattern of its species. *Orthriophis* stems from the Greek words *orthros* (ορθηρος), meaning dawn, and *ophis* (οφις), i.e., snake; the gender is masculine.

Diagnosis. Subocular usually present (often absent in *hodgsonii*, sometimes also in *cantis* and *taeniurus*), one or several presuboculars common for *cantis*, *moellendorffi*, and *taeniurus*; elevated number of ventral scales (212 in *cantis* to 305 in *taeniurus*) and precaudal vertebrae (222–272); longitudinal dorsal scale rows with an increase between 13 and 26% of the total number of ventral scales (sometimes absent in *taeniurus*), 21 or more rows on forebody usually followed by a reduction around midbody; 19–27 dorsal rows at midbody (50% ventrals), at least one reduction involving sixth row or situated more paravertebral. Head long and slender; medium to large adult size (up to 230 cm total length in *taeniurus*). Hemipenis bilobed (unknown for *hodgsonii*), basal hooks absent. Eggs with comparatively strong shells.

Coluber porphyraceus Cantor has an isolated position among the Lampropeltini and is apparently without close affinities to the remaining Old World ratsnake genera (see **Discussion**).

***Oreophis* gen. nov.**

Etymology. This genus is named for its occurrence in mountainous areas. *Oreophis* stems from the Greek words *oros* (ορος), meaning mountain, and *ophis* (οφις), i.e., snake; the gender is masculine.

Diagnosis. Subocular and presuboculars absent; ventrals 174–218, 193 precaudal vertebrae; usually 19–19–17 (sometimes 21–19–17, rarely 18 or 15 prior to anal scale) longitudinal dorsal scale rows on anterior part of trunk, at midbody, and prior to the vent; reduction on posterior part of body involving rows 3–5 between 51 and 72% of the total number of ventrals. Hemipenis more or less cylindrical, completely covered with spines (apical ones very fine and numerous), and with a basal hook.

Probably, *Oreophis* is polytypic. Some of the seven currently recognized subspecies of *O. porphy-*

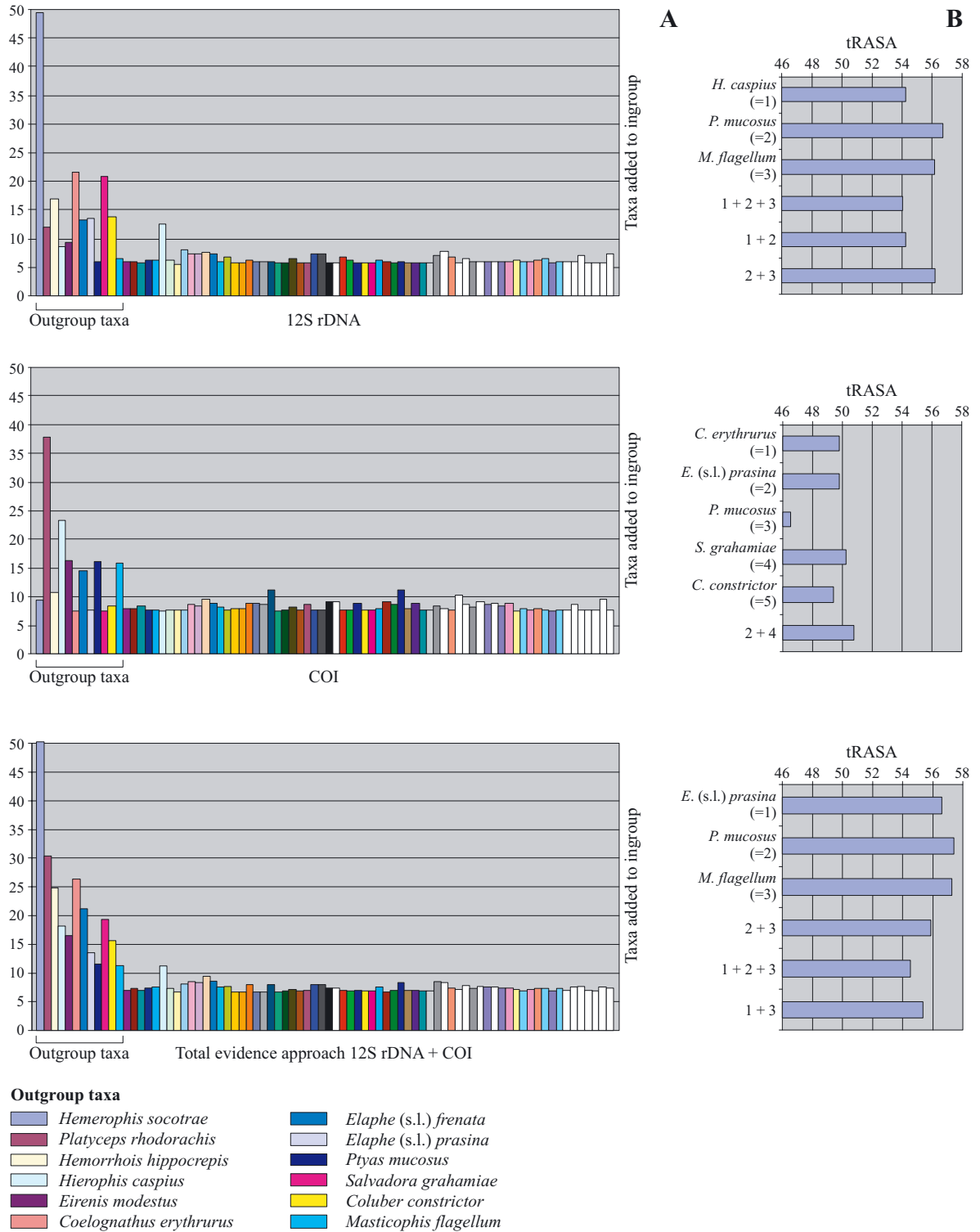


Fig. 1. A: Taxon-variance ratio for 12S rDNA, COI, and the combined sequences including twelve outgroup taxa. Most of these species including *Elaphe* (sensu lato) *frenata* and *E. (s.l.) prasina* are outliers relative to ratsnake species. B: Phylogenetic signal using several outgroup combinations. The strongest shows the total evidence approach and the weakest signal indicates the COI sequence.

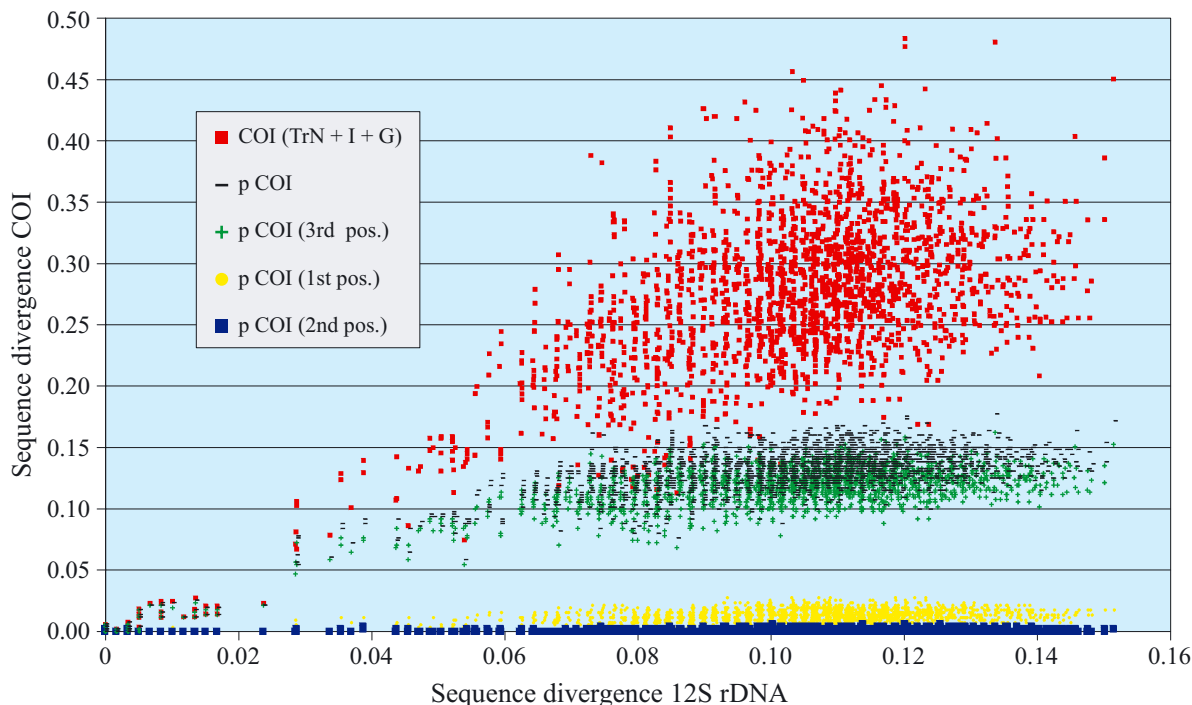


Fig. 2. Uncorrected (p) and corrected pairwise sequence divergences of the coding COI sequence vs. *p* of 12S rDNA.

raceus (e.g., *O. p. vaillanti*, see Schulz and Helfenberger, 1998) are likely to be valid species.

DISCUSSION

Elaphe Fitzinger is made up of ten species ranging from southern Europe to the Far East and including two Japanese endemics, *E. climacophora* and *E. quadrivirgata* (see **Biogeographical Aspects**). The systematic reality of this Palaearctic genus is well supported by molecular data (bootstrap values 97%).

Contrary to the branching pattern in the phylogenetic trees (Figs. 3–4), a final analysis performed after the completion and submission of this paper generated a slightly different arrangement revealing that *Elaphe climacophora* is the sister taxon of all other *Elaphe* spp., i.e., *climacophora* and *davidi* change their position (see footnote 6).

Our data suggest Nearctic ratsnakes constitute a monophyletic group that comprises all New World taxa examined within the scope of this study, i.e., species of the genera *Arizona*, *Bogertophis*, *Lampropeltis*, *Pantherophis*, *Pituophis*, *Pseudelaphe*, *Rhinocheilus*, and *Senticolis*⁶.

This result is in contradiction with the findings of Keogh (1996). Based on the absence of an intrapulmonary bronchus in *Senticolis*, this author removed *S. triaspis* from the Lampropeltini and placed the genus in the racers (Colubri). Following these results, Wallach (1998) concluded that Old World *Elaphe* (auct.) and *Coronella* have to be assigned to this tribe.

Senticolis triaspis is the most basal species within New World ratsnakes. Its outgroup position is well established by enzyme electrophoresis (Lawson and Dessauer, 1981), pulmonary features (Keogh, 1996), and, with certain reservations, molecular data (Rodríguez-Robles and De Jesus-Escobar, 1999).

In what concerns the genus *Bogertophis* (see below), the present results confirm the monophyly of *B.*

⁶ A weighted MP analysis performed after the submission of this paper and including *Rhinocheilus lecontei* produced reversed positions for two Palaearctic *Elaphe* spp. (viz. *E. climacophora* and *E. davidi*) and a different branching pattern within New World Lampropeltini, i.e., [(*Arizona*, *Rhinocheilus*: 52%), *Lampropeltis* spp.: <50%], (*Bogertophis*, *Pseudelaphe*), [*Pantherophis*, *Pituophis* spp.: 87%], *Senticolis*: 76%. The new positioning of *climacophora* and *davidi* makes sense in terms of zoogeographical reasons, and we consider this topography to be better than the one shown in Figs. 3–5.

rosaliae and *B. subocularis* concluded from morphological features, karyotype, and immunological data (Dowling and Price, 1988).

Pantherophis and *Pituophis* are sister taxa (bootstrap support 92%, Figs. 3–4) and the monophyly of the former is well supported by a bootstrap value of 74% (see footnote 6). This relationship is congruent with previous results from a mtDNA study (Rodríguez-Robles and De Jesus-Escobar, 1999). A cladistic analysis using morphological characters including visceral anatomy and scutellation (Keogh, 1996) confirms close relationship of *Pantherophis* spp.

Dowling and Maxson (1990) remarked that the semifossorial genus *Pituophis* appears to be derived from the generalized terrestrial and/or arboreal ancestors of *Pantherophis vulpinus* or *P. guttatus*. This scenario agrees with the branching pattern of the ME tree from the total evidence analysis (not shown) where *Pituophis* spp. form the sister group of *P. vulpinus*. This would render the more generalized genus *Pantherophis* paraphyletic, an inevitable result in the process of evolution⁷.

Based on enzyme electrophoretic data, Lawson and Dessauer (1981) noted that the monotypic genus *Arizona* (*A. elegans*) has a smaller genetic distance to *Pituophis melanoleucus* and other New World ratsnakes (i.e., *Pantherophis* spp. and *Bogertophis subocularis*) than to *Lampropeltis*. A similar conclusion was drawn from morphological and immunological investigations (Dowling and Maxson, 1990) whereas a molecular analysis (Rodríguez-Robles and De Jesus-Escobar, 1999) revealed close affinities of *Arizona* with *Bogertophis* and *Lampropeltis*. In the latter study, *Rhinocheilus lecontei* appeared as the outgroup vis-à-vis all other examined Nearctic Lampropeltini. Our results (see footnote 6) indicate that *A. elegans* is phylogenetically closest to *R. lecontei* (bootstrap support 52%). Compared with other New World ratsnakes, *Arizona* and *Rhinocheilus* have single (1–3 in *R. lecontei*) instead of paired apical pits. However, the sister group relationship of *Arizona* and *Rhinocheilus* with *Lampropeltis* requires further investigations including *Cemophora coccinea* and *Stilosoma extenuatum*.

Pseudelaphe flavirufa has no consistently supported relationship with any of the New World Lampropeltini (Fig. 4). A similar result was reported from an earlier analysis based on enzyme electrophoretic

data (Lawson and Dessauer, 1981). Uncorrected pairwise distances of *P. flavirufa* to *Bogertophis* are in the same range (10.3–12%) as to *Arizona* (11.0–11.1%), *Lampropeltis* (10.5–11.0%), *Pantherophis* (10.2–11.7%), and *Pituophis* spp. (10.2–10.8%). *P. flavirufa* differs from other Nearctic ratsnake genera except *Lampropeltis* (*L. getula*, Schmidt, 2000) in an unarticulate and bilobed hemipenis (unarticulate and clavate in *Arizona elegans*, *Bogertophis* spp., and *Pituophis melanoleucus*, unarticulate and subcylindrical in *Senticolis triaspis*, articulate and bilobed in *Pantherophis* spp., see Fig. 5).

Similarities of *Pseudelaphe flavirufa* and *Bogertophis* spp. in morphology (slender body, large eyes) and behavior (nocturnal activity) were noted by Keogh (1996), and the present molecular data (see footnote 6) argue for close affinities of these species.

Quite surprisingly, the western Palaearctic genus *Zamenis* belongs to a radiation group different from *Elaphe* (sensu stricto). A maximum likelihood topology based on mt cytochrome b sequences (Lenk et al., 2001) produced the same branching pattern for *Zamenis* spp. as shown in the weighed MP analysis (Fig. 3). Morphologically, these species are best characterized by the presence of a distinct basal hook of the hemipenis instead of more or less uniform spines as in *Elaphe* Fitzinger. Interestingly, *Z. lineatus* and *Z. longissimus* have a bulbous apex of the hemipenis whereas it is subcylindrical in the remaining species (Fig. 5). For the rest, a phenogram based on 19 enzyme electrophoresis loci (Helfenberger, 2001) confirms the monophyly of *Zamenis*.

Although the western Mediterranean *Rhinechis scalaris* appears as a basal member of *Zamenis* in the ME topology (data not shown), this monotypic genus represents a basal lineage within the evolution of the Old World ratsnakes as proposed by Minton (1976) and Lenk et al. (2001).

Lenk et al. (2001) stated that “the East Asian *E. [laphe (auct.)] porphyracea* clusters with the *E. longissima* group,” i.e., the genus *Zamenis*. Certainly, this result is due to the restricted sample size with a single East Asia species examined.

Oreophis porphyraceus exhibits moderate genetic distances vis-a-vis other ratsnakes lineages. To conclude from the taxon-variance ratio plot (no taxon-variance outliers), a definite statement about the phylogenetic relationships of *O. porphyraceus* is not possible for the time being.

Oreophis porphyraceus differs from *Orthriophis* spp. in a number of external morphological, osteological, and anatomical characters such as, for instance,

⁷ This is also evident, for example, in the sister group position of *Stilosoma extenuatum* and *Lampropeltis getula* within the otherwise monophyletic genus *Lampropeltis* (Rodríguez-Robles and De Jesus-Escobar, 1999).

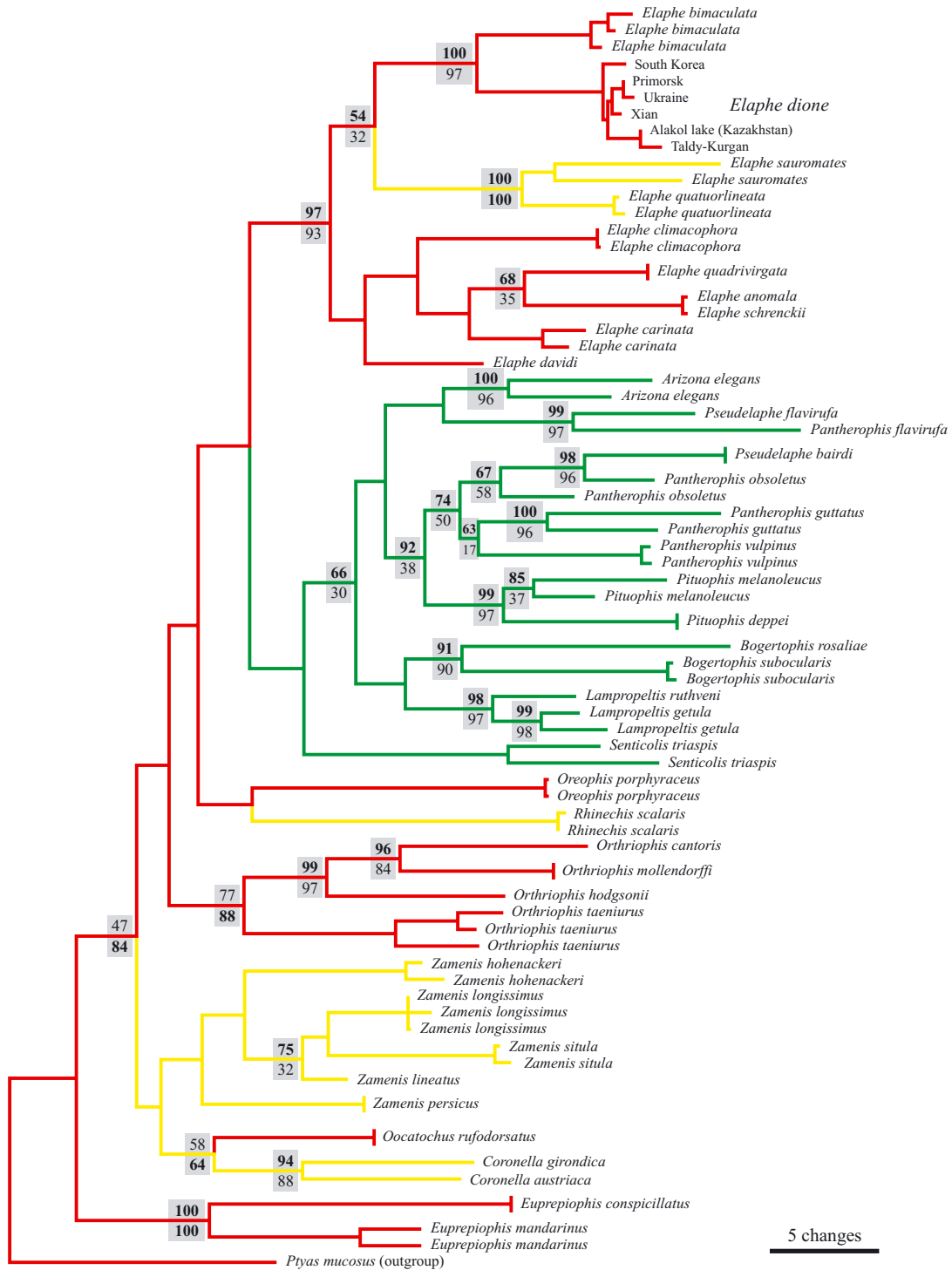


Fig. 3. Weighted MP tree from the total evidence approach. Bootstrap values above branches are from the total evidence approach, below from the 12S rDNA sequence. Higher value at each node bold-faced; bootstraps under 50% from both analyses omitted. Red: mainly eastern Palaearctic or Oriental distribution; yellow: mainly western Palaearctic distribution; green: mainly New World distribution. See text and footnote 6 with regard to the position of *Elaphe climacophora* and *E. davidi* as well as the branching pattern within Nearctic ratsnakes and the position of *Rhinocheilus lecontei*.

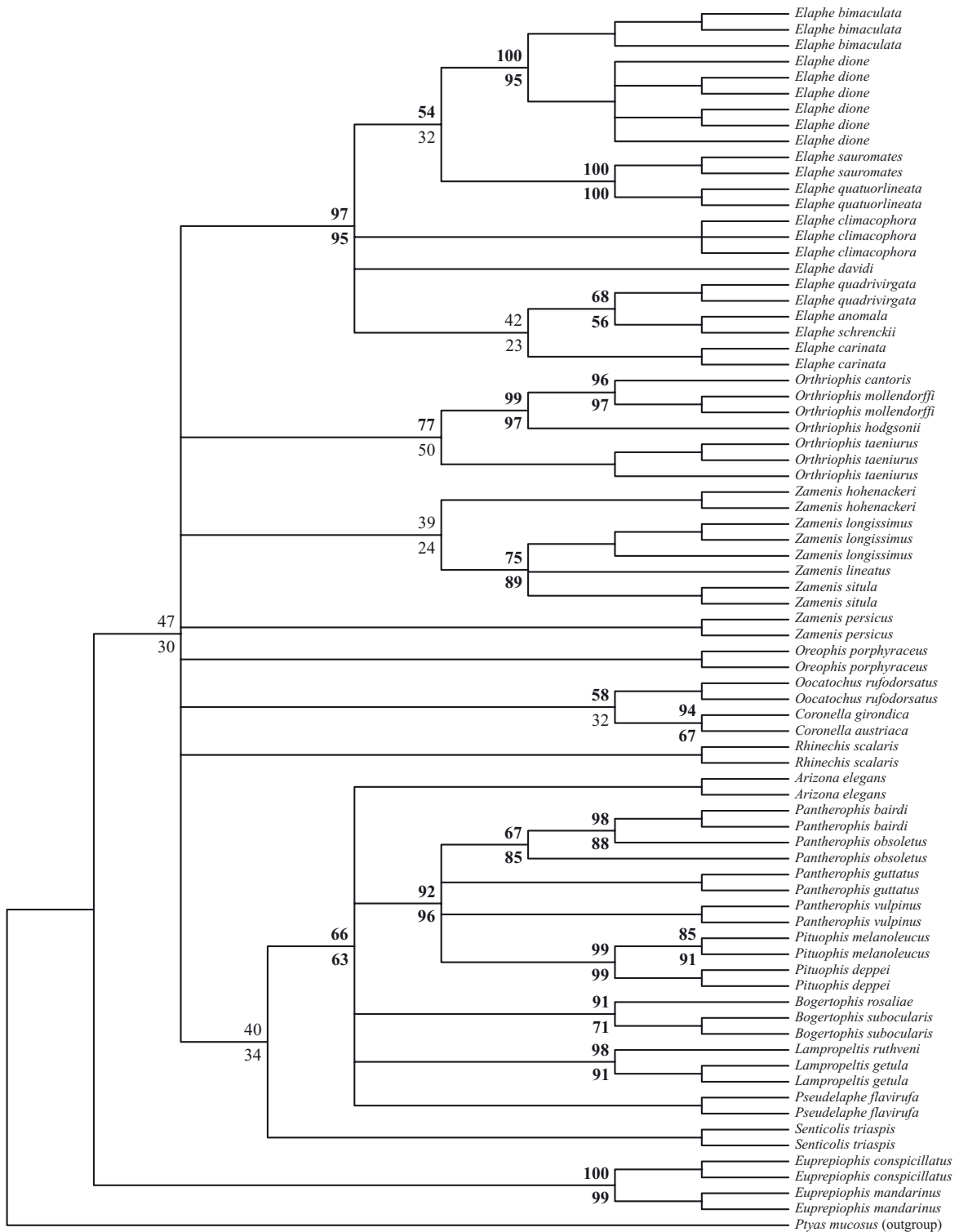


Fig. 4. Strict consensus tree from the best MP and ME analyses (total evidence). Bootstrap values are from 1000 replicates of the MP (above) and the NJ (below) analysis (values over 50% bold-faced). See comment in Fig. 3 concerning *Elaphe climacophora*, *E. davidi*, and New World genera.

the absence of a subocular scale and presuboculars, a low number of ventrals and precaudal vertebrae (174–218 and 193 vs. 212–305 and 222–272, respectively), dorsal scale rows (e.g., 19 vs. 21 or more at midbody) including the reduction pattern thereof (see above) and a lack of anterior additions (present in *Orthriophis* spp.), as well as a cylindrical (vs. articulate and bilobed) hemipenis with a basal hook in *O. porphyraceus* (absent in *Orthriophis* spp.).

With regard to some morphological characters, *Orthriophis* spp. are similar to Oriental colubrids formerly referred to *Elaphe* (auct.), i.e., *Coelognathus* spp. (see Helfenberger, 2001). These features include, for instance, high ventral scale and precaudal vertebra counts (198–278 and 217–240, respectively), a long and slender head, and adult maximum lengths exceeding 200 cm in *C. radiatus* and *C. subradiatus*. Apart from *C. radiatus* (range extending to Indo-China), these species including *C. erythrus*, *C. flavolineatus*, *C. helena*, and *C. philippinus* are distributed in the Indo-Malayan region, viz. from Kashmir and Nepal (*helena*, *radiatus*) to Timor (*subradiatus*) and the Nicobar and Andaman islands (*flavolineatus*). They differ from *Orthriophis* spp., for instance, in the absence of a subocular (sometimes present in *C. subradiatus*) and presubocular scales, three (instead of two) supralabials, usually fourth to sixth, entering the orbit (except in *helena*, sometimes only two in *flavolineatus* and *subradiatus*), the dorsal scale row reduction pattern including the absence of additions (sometimes in *C. helena*), a single anal plate (vs. divided), and hemipenis features, i.e., a long and slender shape and conspicuous differences in overall ornamentation. For the rest, molecular analyses (in preparation) indicate that *Coelognathus* is no ratsnake genus but belongs to the Old World racers (Colubriini).

Although the branching pattern between various Old World groups of ratsnakes is not well supported by our data, there can be hardly any doubt that the genus *Euprepiophis* is the outgroup of the Holarctic and Oriental ratsnakes and their allies (84% from the 12S rDNA, 47% from the total evidence approach, Fig. 3). Sister group status for *E. conspicillatus* and *E. mandarinus* vis-à-vis all other examined ratsnakes and related genera is justified on the basis of moderate genetic distances (11.2–14.6% from the total evidence approach) compared with Old and New World ratsnakes and the taxon-variance ratio (Fig. 1). A very small (sometimes lacking) loreal, smooth or slightly keeled dorsal scales, and a subcylindrical (*conspicillatus*) or weakly bilobed (*mandarinus*) he-

mipenis with basal hooks characterize *Euprepiophis* spp. However, further investigations are necessary to clarify the validity of this genus (see below). *Elaphe perlacea* Stejneger, 1929 is either a junior synonym of *E. mandarinus* or a closely related taxon.

With the present study, 30 out of 33 nominal species of Old and New World ratsnakes (*Elaphe* sensu Schulz, 1996 and Helfenberger, 2001) are referred to a total of nine genera, viz. *Bogertophis*, *Elaphe* (sensu stricto), *Euprepiophis*, *Oreophis*, *Orthriophis*, *Pantherophis*, *Pseudelaphe*, *Senticolis*, and *Zamenis*. *Coronella bella* Stanley, 1917 is a senior synonym of *Coluber leonardi* Wall, 1921 (Schulz et al., 2000). This Indo-Chinese species has 19–19–17 longitudinal rows along the trunk and a single paired reduction involving rows 4+5. Also, *Elaphe* (s.l.) *bella* has few supralabials (7–8, third and fourth in contact with eye) but is peculiar for lacking a loreal scale. *Elaphe* (s.l.) *bella* (Stanley) requires comparison with *Euprepiophis* spp. To judge from morphological characters, *Elaphe* (s.l.) *frenata* (Gray, 1853) and *E.* (s.l.) *prasinana* (Blyth, 1854) are most closely related to *Gonyosoma* Wagler, 1828 (in preparation).

Speciation Events

Based on external morphological characters, *Elaphe bimaculata* cannot be distinguished from *E. dione* and these taxa were considered to be conspecific (Brunner, 1995). However, Schulz (1996) treated them as valid species due to slight differences in color pattern. In fact, genetic distances among *dione* from distant regions within its large range are much smaller than those found between Chinese populations of *dione* and *bimaculata* (Fig. 3), and species status for *bimaculata* is beyond any doubt (see Helfenberger, 2001). Most probably, the separation of these taxa occurred long before the geographic expansion of *dione*.

Genetic distances found among individuals of *Elaphe sauromates* are of the magnitude as otherwise observed between closely related species (e.g., *E. dione* and *E. bimaculata*). Our data confirm its specific distinctness from *E. quatuorlineata* as suggested by Helfenberger (2001) and supported by sequences of a portion of the mt cytochrome b gene (Lenk et al., 2001). However, considerable morphological and biochemical differences between *E. schrenckii* and *anomala* reported by Helfenberger (2001) cannot be confirmed with the present data. Although a recent speciation process cannot be ruled out, the systematic status of *Coluber anomalus* Boulenger, 1916 is open to question.

Zamenis lineatus was specifically separated from *Z. longissimus* only recently (Lenk and Wüster, 1999). Based on the present data, specific status for *lineatus* is fully confirmed; the genetic distance *longissimus* is much larger than intraspecific variation among various populations of the latter (see **Appendix A**).

Zamenis persicus differs from *Z. longissimus* in color pattern and development of the ventral keels (Nilson and Andrén, 1984). Specific status can be confirmed by slightly higher genetic distances of these taxa (7.6–7.8%, total evidence approach) than between *Z. longissimus* and *Z. situla* (7.0–7.4%). A similar result was obtained by Lenk et al. (2001).

Biogeographical Aspects

New World ratsnakes and their allies, viz. the genera *Arizona*, *Bogertophis*, *Lampropeltis*, *Pantherophis*, *Pituophis*, *Pseudelaphe*, *Rhinocheilus*, and *Senticolis*, are monophyletic and related next to several Palaeartic and Oriental ratsnake lineages. Therefore, we conclude that the Nearctic and, later, the northern Neotropical region were probably invaded by an Old World stock only once in the evolutionary history of ratsnakes and their relatives (Lampropeltini).

During the early Miocene, a great wave of migrants including 16 genera of mammals as well as Esocoid fishes arrived the Nearctic region from Asia (Sychevskaya, 1986; Briggs, 1987). The first modern Nearctic colubrid genus so far reported, i.e., *Salvadora* Baird in Baird and Girard, 1853, is recorded from the Hemingfordian, 20–16 million years ago (m.y.a., Holman, 2000). *Elaphe kanensis*†, the oldest known New World ratsnake, appeared in the Early Barstovian (16–14.5 m.y.a.).

Based on immunological distances, Dowling et al. (1983) estimated the beginning of Nearctic ratsnake evolution 14–15 m.y.a., and Dowling and Maxson (1990) dated the separation of their main radiation groups within New World ratsnakes (i.e., the “Lampropeltini” and “Elaphini”), as a late Miocene event.

A major Neogene thermal optimum occurred at the end of the early Miocene 17.2–16.5 m.y.a. The Beringia was covered with mixed forests and the appearance of tropical bats, for instance, indicate that a warm and humid climate dominated over the northern hemisphere (Zubakov and Borzenkova, 1990) facilitating a northward migration of ratsnakes in the eastern Palaeartic. It is likely that during this period a single ancestor invaded the Nearctic region over the Bering land bridge. This event resulted in a fast and

extensive radiation leading to the recent diversity with more than twenty species and a plethora of subspecies, ecological and morphological adaptation such as burrowing habits (*Pituophis*, *Cemophora*, *Stilosoma*), and an evolutionary divergence producing the highest density of ratsnakes species in the world.

A minor radiation event took place in the eastern Palaeartic giving rise to *Elaphe* (sensu stricto). The ancestor of *E. sauromates* and *E. quatuorlineata* reached the Mediterranean subregion. Spreading westward, *quatuorlineata* specifically separated from the eastern *sauromates*.

The western Palaeartic was invaded by ratsnakes at least twice (Fig. 3) including an ancestral stock leading to the modern genera *Coronella*, *Rhinechis*, and *Zamenis*. In what concerns the latter genus, the basal position of eastern species (*hohenackeri*, *persicus*) compared with western Mediterranean species (*lineatus*, *longissimus*, Fig. 3) clearly argue for an east-west invasion route.

Another evolutionary lineage gave rise to the western Palaeartic *Coronella* with an uncorrected genetic distance of 9.6% from the total evidence approach between *C. austriaca* and *C. girondica*. The clustering of *Coronella* spp. with the monotypic Chinese genus *Oocatochus* may be surprising at first glance. However, *O. rufodorsatus* and *C. austriaca* are the only viviparous taxa among several dozen closely and more distantly related colubrids. Viviparity certainly represents a derived state characterizing members of this group. A parallel distribution pattern can be found in the racer genus *Hierophis* Fitzinger, 1843 with only one species, *H. spinalis* (Peters), from Kazakhstan to Korea is a mainly western Palaeartic taxon (Schätti, 1988).

Interestingly, the three Japanese and Kuriles species, *Euprepiophis conspicillatus*, *Elaphe climacophora*, and *E. quadrivirgata* inhabit island groups where two East Asian ratsnakes, viz. *Elaphe carinata* and *Orthriophis taeniurus* (Senkaku and Ryukyu islands; see Schulz, 1996), are absent (Utiger, 1996). This high degree of ratsnake endemism in Japan is most probably the result of a long-term isolation, and it is likely that a (presumably one-time) land bridge between Korea and southwest Japan during the Pleistocene, approx. 160,000 years ago (Fujii, 1990), was not practicable for ratsnakes.

SUMMARY OF TAXONOMIC CHANGES

Euprepiophis Fitzinger, 1843:26. Type species: *Coluber conspicillatus* Boie (by monotypy).

Euprepiophis conspicillatus (Boie)

Coluber conspicillatus Boie, 1826:211. Type locality: "Japan."

Euprepiophis mandarinus (Cantor)

Coluber mandarinus Cantor, 1842:483. Type locality: Chusan [Zhoushan] Island, Zhejiang Province, China.

Oreophis Utiger, Helfenberger, and Schätti [hoc loco]. Type species: *Coluber porphyraceus* Cantor.

Oreophis porphyraceus (Cantor)

Coluber porphyraceus Cantor, 1839:51. Type locality: Mishmee [Mishmi] Hills, Assam [Arunchal Pradesh], India.

Orthriophis hoc loco. Type species: *Cynophis moellendorffi* Boettger.

Orthriophis cantoris (Boulenger)

Coluber reticularis Cantor, 1839:5 (non *Coluber reticularis* Daudin, 1803).

Coluber cantoris Boulenger, 1894:[26, 29] 35. Type locality: not specified.

Type material: Apparently, Cantor's (1839) specimen from "Chirra Punji" [Cherrypunji] in the Khasi Hills (Meghalaya, India) is not deposited neither in the British Museum nor in another scientific collection. Boulenger (1894) had at hand eleven specimens from "Nepal," "Sikkim," and northern India, i.e., Darjeeling and the Khasi Hills (Arunchal Pradesh). We herewith designate BMNH 58.6.24.3, an adult male specimen from "Nepal" (coll. B. H. Hodgson), as the lectotype of *Orthriophis cantoris*.

Orthriophis hodgsonii (Günther)

Spilotes hodgsonii Günther, 1860:156, pl. 27. Type locality: Tsomoriri, Ladak, Tibet (lectotype, Kramer, 1977).

Orthriophis moellendorffi (Boettger)

Cynophis moellendorffi Boettger, 1886:520. Type locality: Nanning, Yong Jiang River, Guangxi Province, China.

Orthriophis taeniurus (Cope)

Elaphe taeniurus Cope, 1861:565. Type locality: Ningpo [Ningbo], Zhejiang Province, China.

Pantherophis Fitzinger, 1843:25. Type species: *Coluber guttatus* Linnaeus (by monotypy).

Pantherophis bairdi (Yarrow)

Coluber bairdi Yarrow, 1880:492. Type locality: Fort Davis, Apache Mountains, Jeff Davis County, Texas.

Pantherophis guttatus (Linnaeus)

Coluber guttatus Linnaeus, 1766:385. Type locality: "Carolina" (restricted to the vicinity of Charleston, South Carolina, Dowling, 1951).

Pantherophis obsoletus (Say)

Coluber obsoletus Say, 1823:140. Type locality: "On the Missouri River from the vicinity of Isle au Vache (Cow Island) to Council Bluff" (restricted to the vicinity of Cow Island, near Leavenworth, Kansas, Dowling, 1951).

Pantherophis vulpinus (Baird and Girard)

Scotophis vulpinus Baird and Girard, 1853:75. Type locality: Grosse Isle (Detroit River), Michigan.

Pseudelaphe Mertens and Rosenberg, 1943:61. Type species: *Coluber flavirufus* Cope (by monotypy).

Pseudelaphe flavirufa (Cope)

Coluber flavirufus Cope, 1867:319. Type locality: "Yucatan" (see remarks in Schulz, 1996).

Zamenis Wagler, 1830:188. Type species: *Coluber Aesculapii* Lacépède = *Natrix longissima* Laurenti (by subsequent designation, Fitzinger, 1843).

Zamenis hohenackeri (Strauch)

Coluber hohenackeri Strauch, 1873:69, pl. 2. Type locality: description based on five specimens from different localities. Bischoff (1993) restricted the type locality to Chanlar ("Kolonie Helenendorf"), Azerbaijan.

Zamenis lineatus (Camerano)

Callopetalis longissimus var. *lineata* Camerano, 1891:458. Type locality: Naples, Italy.

Zamenis longissimus (Laurenti)

Natrix longissima Laurenti, 1768:74. Type locality: "Austria" (restricted to Vienna, Mertens and Müller, 1928).

Zamenis persicus (Werner)

Coluber longissima var. *persica* Werner, 1913:23. Type locality: Barferush, Mazandaran, Iran.

Zamenis situla (Linnaeus)

Coluber situla Linnaeus, 1758:223. Type locality: "Egypt" (corrected to Izmir, Turkey, Obst et al., 1993).

Acknowledgments. Thanks to Michael Buchert (Melbourne, Australia) for his help with the cloning of the restriction fragments. Migg Angehrn (Lenzerheide, Switzerland), Eugen Deplazes (Mauren, Principality of Liechtenstein), Goran Dusej (Rottenschwil, Switzerland), Jean-Claude Eichmann (Zurich, Switzerland), Rainer Fesser (Leutschach, Austria), Andreas Gumprecht (Cologne, Germany), Helmut Hansen (Baden, Switzerland), Henri Kratzer (Zurich, Switzerland), Sergej Kudryavtsev (Moscow, Russia), Stefan Lingenhölle (Romanshorn, Switzerland), Sergej Mamet (Moscow, Russia), Christian Meienberger (St. Gall, Switzerland), Song Ming-tao (Xian, China), Sergej Ryabov (Tula, Russia), Klaus-Dieter Schulz (Alsdorf, Germany), Michihisa Toriba (Nitta-Gun, Japan), Kamuran Tepedelen (Boulder, USA), and Boris Tunijev (Sochi, Russia) donated or provided specimens. Leo Borkin (St. Petersburg, Russia), Valery Eremchenko (Bishkek, Kyrgyzstan), Nikolaj Orlov (St. Petersburg), Ram Prasad Panday (Katmandu, Nepal), Alexander Panfilov (Bishkek, Kyrgyzstan), and Karan Shah (Katmandu, Nepal) assisted the second author during field work. Thanks to Monika Brunner, Yves Choffat, Gaston Guex, Hansjürg Hotz, Stefan Keller, Michael Spörri, and Giancarlo Tomio (all Zurich, Switzerland) for laboratory assistance. Gerhard Bächli, Jocelyne Leclerc, Hubert Krättli, and Jürg Stauffer (all Zurich, Switzerland) provided technical assistance. We are

grateful to Melissa Knight (Melbourne, Australia) and Andrea Stutz (Winterthur, Switzerland) for reading earlier drafts and the final version of the manuscript, respectively. Peter Beerli (Seattle, USA), Patrick Deese (San Miguel de Allende, Mexico), Jeannette Fanti (Winterthur, Switzerland), Andrew G. McArthur and Monica Medina (Woods Hole, USA), Clemens Müller-Glauser (St. Gall, Switzerland), Michel Guillod (Grandvaux, Switzerland), Markus Ruch, and Marcel Zefferer (both Zurich, Switzerland) contributed in various ways to the present work. This study was supported by a grant of the FAN (Fonds des Zürcher Universitäts-Vereins zur Förderung des akademischen Nachwuchses). This publication was prepared by the senior author as partial fulfillment of the requirements for the Ph.D. in Zoology at the University of Zurich, Switzerland.

REFERENCES

- Baird S. F.** (1859), "Reptiles of the boundary, with notes by the naturalists of the survey," in: *Report on the United States and Mexican Boundary Survey. Part II. Zoology of the boundary*, W. H. Emory (ed.), Govt. Print. Off., Washington, pp. 1–35.
- Baird S. F. and Girard C.** (1853), *Catalogue of North American reptiles in the Museum of the Smithsonian Institution. Part I. Serpents*, Smithsonian Inst., Washington.
- Bischoff W.** (1993), "*Elaphe hohenackeri* (Strauch, 1873) Transkaukasische Kletternatter," in: *Handbuch der Reptilien und Amphibien Europas*, Band 3/1, W. Böhme (ed.), Aula-Verlag, Wiesbaden, pp. 317–329.
- Blanchard F. N.** (1920), "Three new snakes of the genus *Lampropeltis*," *Occ. Pap. Mus. Zool. Univ. Mich.*, **81**, 1–10.
- Bleeker P.** (1858), "*Gonyosoma janseni* Blkr., eene nieuwe slang van Manado," *Nat. Tijdschr. Nederl. Ind.*, **16**, 242.
- Blyth E.** (1854), "Notices and descriptions of various reptiles, new or little known," *J. Asiat. Soc. Bengal*, **23**(3), 287–302.
- Boie F.** (1826), "Generalübersicht der Familien und Gattungen der Ophidier," *J. Asiat. Soc. Bengal*, **19**(10), 981–982.
- Boie F.** (1827), "Bemerkungen über Merrem's Versuch eines Systems der Amphibien. Iste Lieferung. Ophidier," *J. Asiat. Soc. Bengal*, **20**(9), 508–566.
- Boettger O.** (1886), "Die Reptilien und Amphibien des Talysch-Gebietes," in: *Die Fauna und Flora des südwestlichen Caspi-Gebietes. Wissenschaftliche Beiträge zu den Reisen an der Persisch-Russischen Grenze*, G. Radde (ed.), F. A. Brockhaus, Leipzig, pp. 30–82.
- Boulenger G. A.** (1894), *Catalogue of the Snakes in the British Museum (Natural History), Containing the Conclusion of the Colubridae Aglyphae. Vol. II*, Trustees Brit. Mus., London.
- Boulenger G. A.** (1916), "Description of a new snake of the genus *Coluber* from northern China," *Ann. Mag. Nat. Hist.*, **8**(17), 243–244.
- Branch W. R.** (1986), "Hemipenial morphology of African snakes: a taxonomic review. Part 1. Scolecophidae and Boidae," *J. Herpetol.*, **20**(3), 285–299.
- Briggs J. C.** (1987), *Biogeography and Plate Tectonics*, Elsevier, Amsterdam.
- Brown A. E.** (1890), "On a new genus of Colubridae from Florida," *Proc. Acad. Nat. Sci. Philad.*, **42**, 199–200.
- Brown A. E.** (1901), "A new species of *Coluber* from western Texas," *Proc. Acad. Nat. Sci. Philad.*, **53**, 492–495.
- Brunner M.** (1995), *Morphologische Analyse der paläarktischen Schlangengruppe Elaphe dione (Reptilia, Serpentes)*. Diploma Thesis, University of Zurich.
- Burbrink F. T., Lawson R., and Slowinski J. B.** (2000), "Mitochondrial DNA phylogeography of the polytypic North American Rat Snake (*Elaphe obsoleta*): a critique of the subspecies concept," *Evolution*, **54**(6), 2107–2118.
- Camerano L.** (1891), "Monografia degli ofidi Italiani. Parte seconda. Colubridi e monografia dei cheloni Italiani," *Mem. R. Accad. Sci. Fis. Mat. Torino. Ser. 2*, **41**, 403–481.
- Cantor T.** (1839), "Spicilegium serpentium indicorum (II)," *Proc. Zool. Soc. Lond.*, **1839**(7), 49–55.
- Cantor T.** (1842), "General features of Chusan, with remarks on the flora and fauna of that island (III)," *Ann. Mag. Nat. Hist.*, **9**, 481–493.
- Cope E. D.** (1860), "Catalogue of the Colubridae in the Museum of the Academy of Natural Sciences of Philadelphia, with notes and descriptions of new species. Part 2," *Proc. Acad. Nat. Sci. Philad.*, **12**, 241–266.
- Cope E. D.** (1861), "Catalogue of the Colubridae in the Museum of the Academy of Natural Sciences of Philadelphia. Part 3," *Proc. Acad. Nat. Sci. Philad.*, **12**, 553–566.
- Cope E. D.** (1866), "Fourth contribution to the herpetology of Tropical America," *Proc. Acad. Nat. Sci. Philad.*, **17**(2), 123–132.
- Cope E. D.** (1867), "Fifth contribution to the herpetology of Tropical America," *Proc. Acad. Nat. Sci. Philad.*, **18**, 317–323.
- Daudin F. M.** (1803), *Histoire Naturelle Générale et Particulière des Reptiles. Tome 6*, F. Dufart, Paris.
- Dessauer H. C., Cadle J. E., and Lawson R.** (1987), "Pattern of snake evolution suggested by their proteins," *Field. Zool. New Ser.*, **34**, 1–34.
- Domergue A.** (1962), "Observation sur les pénis des ophiidiens (2ème note)," *Bull. Soc. Sci. Nat. Phys. Maroc*, **42**(1/2), 87–105.
- Dowling H. G.** (1951), "A taxonomic study of the Rat-snakes, genus *Elaphe* Fitzinger. 1. The status of the name *Scotophis laetus* Baird and Girard (1853)," *Copeia*, **1951**(1), 39–44.

- Dowling H. G.** (1958), "A taxonomic study of the Ratsnakes. 6. Validation of the genera *Gonyosoma* Wagler and *Elaphe* Fitzinger," *Copeia*, **1958**(1), 38–52.
- Dowling H. G. and Fries I.** (1987), "A taxonomic study of the Ratsnakes. 8. A proposed new genus for *Elaphe triaspis* (Cope)," *Herpetology*, **43**(2), 200–207.
- Dowling H. G., Highton R., Maha C. G., and Maxson L. R.** (1983), "Biochemical evaluation of colubrid snake phylogeny," *J. Zool.*, **201**, 309–329.
- Dowling H. G. and Maxson L. R.** (1990), "Genetic and taxonomic relations of the short-tailed snakes, genus *Stilosoma*," *J. Zool.*, **221**, 77–85.
- Dowling H. G. and Price R. M.** (1988), "A proposed new genus for *Elaphe subocularis* and *Elaphe rosaliae*," *Snake*, **20**(1), 52–63.
- Dowling H. G. and Savage J. M.** (1960), "A guide to the snake hemipenis: a survey of basic structure and systematic characteristics," *Zoologica*, **45**(2), 17–28.
- Duméril A. M. C., Bibron G., and Duméril A.** (1854), *Erpétologie générale ou histoire naturelle complète des reptiles*, Roret, Paris.
- Farris J. S.** (1989), "The retention index and the rescaled consistency index," *Cladistics*, **5**, 417–419.
- Felsenstein J.** (1985), "Confidence limits on phylogenies: an approach using the bootstrap," *Evolution*, **39**, 783–791.
- Fitzinger L. J.** (1833), "*Elaphe parreyssii* — Parreyss's *Elaphe*," in: *Descriptiones et icones amphibiorum*, J. Wagler (ed.), J. G. Cotta, München–Stuttgart–Tübingen.
- Fitzinger L.** (1843), *Systema Reptilium. Amblyglossae*, Braumüller et Seidel, Vindobonae [Vienna].
- Fujii S.** (1990), "Changes of the palaeoenvironment along the Japan Sea since the early Pleistocene," *Daiyonki-Kinkyu*, **29**(3), 173–182.
- Genetics Computer Group** (1999), *Wisconsin Package. Version 10.0*, Madison, WI.
- Gmelin J. F.** (1789), *Caroli a Linné. Systema naturae. 13th Edition. Part I*(3). *Amphibia*, G. E. Beer, Lipsiae [Leipzig].
- Gray J. E.** (1853), "Descriptions of some undescribed species of reptiles collected by Dr. Joseph Hooker, in the Khasia Mountains, East Bengal and Sikkim, Himalaya," *Ann. Mag. Nat. Hist.*, **12**(2), 386–392.
- Günther A.** (1860), "Contribution to a knowledge of the reptiles of the Himalaya Mountains," *Proc. Zool. Soc. Lond.*, **1860**, 148–175.
- Günther A.** (1864), *The Reptiles of British India*, R. Hardwicke, London.
- Günther A.** (1881), "Descriptions of the amphisbaenians and ophidians collected by Prof. I. Bayley Balfour in the Island of Socotra," *Proc. Zool. Soc. Lond.*, **1881**(II), 461–463.
- Gutell R. R., Subashchandran S., Schnare M., et al.** (in preparation), *Comparative Sequence Analysis, Predicting RNA Structure, and the Web*, www.rna.icmb.ntexas.edu.
- Hasegawa M., Kishino H., and Yano T.** (1985), "Dating of the humanape splitting by a molecular clock of mitochondrial DNA," *J. Mol. Evol.*, **21**, 160–174.
- Helfenberger N.** (2001), "Phylogenetic relationships of Old World ratsnakes based on visceral organ topography, osteology, and allozyme variation," *Russ. J. Herpetol.*, **8**(Suppl.), 1–64.
- Holbrook J. E.** (1842), *North American Herpetology, or a Description of the Reptiles Inhabiting the United States. Vol. 4. 2nd Edition*, J. Dobson, Philadelphia.
- Holman A. J.** (2000), *Fossil Snakes of North America*, Indiana Univ. Press, Bloomington.
- Jan G.** (1863), *Elenco sistematico degli ofidi descritti e disegnat per l'Iconografia generale*, Lombardi, Milano.
- Keogh J. S.** (1996), "Evolution of the colubrid snake tribe Lamproptini: a morphological perspective," *Herpetology*, **52**(3), 406–416.
- Knight A. and Mindell D. P.** (1993), "Substitution bias, weighting of DNA sequence evolution, and the phylogenetic position of Fea's Viper," *Syst. Biol.*, **42**(1), 18–31.
- Kramer E.** (1977), "Zur Schlangenfauuna Nepals," *Rev. Suisse Zool.*, **84**(3), 721–761.
- Lacépède B. G. E. Comte de** (1789), *Histoire naturelle des quadrupèdes ovipares et des Serpens. Vol. II*, Hôtel de Thou, Paris.
- Laurenti J.** (1768), *Austriaci Viennensis specimen medicum, exhibens synopsis reptilium [...]*, J. T. Trattner, Wien.
- Lawson R. and Dessauer H. C.** (1981), "Electrophoretic evaluation of the colubrid genus *Elaphe* (Fitzinger)," *Isoz. Bull.*, **14**, 83.
- Lenk P., Joger U., and Wink M.** (2001), "Phylogenetic relationships among European ratsnakes of the genus *Elaphe* Fitzinger based on mitochondrial DNA sequence comparisons," *Amphibia–Reptilia*, **22**, 329–339.
- Lenk P. and Wüster W.** (1999), "A multivariate approach to the systematics of Italian Rat snakes of the *Elaphe longissima* complex (Reptilia, Colubridae): revalidation of Camerano's *Callopettis longissimus* var. *lineata*," *Herpetol. J.*, **9**, 153–162.
- Linnaeus C.** (1758), *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Vol. 1. Editio decima, reformata*, L. Salvii, Holmiae [Stockholm].
- Linnaeus C.** (1766), *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species cum characteribus, differentiis, synonymis, locis. Vol. 1. Part I. Editio duodecima*, L. Salvi, Holmiae [Stockholm].
- Lyons-Weiler J.** (2001), *RASA 3.0 Turbo for the Macintosh*, http://bio.uml.edu/LW/RASA.html.

- Lyons-Weiler J. and Hoelzer G. A.** (1997), "Escaping from the Felsenstein zone by detecting long branches in phylogenetic data," *Mol. Phylogen. Evol.*, **8**(3), 375–384.
- Lyons-Weiler J., Hoelzer G. A., and Tausch R. J.** (1998), "Optimal outgroup analysis," *Biol. J. Linn. Soc.*, **64**, 493–511.
- Martin W. C. L.** (1838), "A collection of snakes procured by the Euphrates expedition," *Proc. Zool. Soc. Lond.*, **1838**[VI], 81–84.
- McDowell S. B.** (1961), "Systematic division and evolution of the colubrid snake genus *Natrix*, with comments on the subfamily Natricinae," *Copeia*, **1961**(4), 502–506.
- Mertens R. and Müller L.** (1928), "Liste der Amphibien und Reptilien Europas," *Abh. Senckb. Naturf. Ges.*, **41**(1), 1–62.
- Mertens R. and Rosenberg H.** (1943), "*Elaphe flavirufa* (Cope), die mexikanische Nachtnatter," *Wochenschr. Aquar. Terrar.*, **37**(3), 60–62.
- Michaëles [Dr.]** (1833), "*Rhinechis* Agassizii — Agassiz's Schnautzen-Natter," in: *Descriptiones et icones amphibiorum*, J. Wagler (ed.), J. G. Cotta'sche Buchhandlung, München–Stuttgart–Tübingen.
- Minton S. A., Jr.** (1976), "Serological relationships among some congeneric North American and Eurasian colubrid snakes," *Copeia*, **1976**(4), 672–678.
- Mocquard M. F.** (1899), "Contribution à la faune herpétologique de la Basse-Californie," *Nouv. Arch. Mus. Hist. Nat.*, **4**(2), 297–343.
- Moritz C., Dowling T. E., and Brown W. M.** (1987), "Evolution of animal mitochondrial DNA — relevance for population biology and systematics," *Ann. Rev. Ecol. Syst.*, **18**, 269–292.
- Nilson G. and André C.** (1984), "A taxonomic account of the Iranian Ratsnakes of the *Elaphe longissima* species-group," *Amphibia–Reptilia*, **5**, 157–171.
- Obst F. J., Shzherbak N. N., and Böhme W.** (1993), "*Elaphe situla* (Linnaeus, 1758) — Leopardnatter," in: *Handbuch der Reptilien und Amphibien Europas, Band 3/I*, W. Böhme (ed.), Aula-Verlag, Wiesbaden, pp. 296–315.
- Pallas P. S.** (1773), "Reise durch verschiedene Theile des Russischen Reichs im 1771sten Jahr," in: *Reise durch verschiedene Provinzen des Russischen Reichs*, Kayserliche Akademie der Wissenschaften, St. Pétersbourg [Reprint 1976, Akad. Druck-und Verlagsanst., Graz], pp. 371–744.
- Pallas P. S.** (1811), *Zoographia Rosso-Asiatica, sistens omnium animalium in extenso imperio rossico et adjacentibus maribus observatorum recensionem, domicilia, mores et descriptiones, anatomem atque icones plurimorum. Animalia monocordia seu Frigidi Sanguinis imperii Rosso-Asiatici. 2, Officina Caes.*, Acad. Sci., Petropoli.
- Posada D. and Crandall K. A.** (1998), "MODELTEST: testing the model of DNA substitution," *Bioinformatics*, **14**(9), 817–818.
- Rodriguez-Robles J. A. and De Jesus-Escobar J. M.** (1999), "Molecular systematics of New World lampropeltine snakes (Colubridae): implications for biogeography and evolution of food habits," *Biol. J. Linn. Soc.*, **68**, 355–385.
- Rodriguez-Robles J. A. and De Jesus-Escobar J. M.** (2000), "Molecular systematics of New World Gopher, Bull, and Pinesnakes (*Pituophis*: Colubridae), a transcontinental species complex," *Mol. Phylogen. Evol.*, **15**(1), 35–50.
- Russo C. A. M., Takezaki N., and Nei M.** (1996), "Efficiencies of different genes and different tree-building methods in recovering a known vertebrate phylogeny," *Mol. Biol. Evol.*, **13**(3), 525–536.
- Saiki R. K., Gelfand D. H., Stoffel S., et al.** (1988), "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase," *Science*, **239**, 487–491.
- Saitou N. and Nei M.** (1987), "The neighbor-joining method: a new method for reconstructing phylogenetic trees," *Mol. Biol. Evol.*, **4**, 406–425.
- Sambrook J., Fritsch E., and Maniatis T.** (1989), *Molecular Cloning: a Laboratory Manual. Second Edition*, Cold Spring Harbor Lab. Press, Cold Spring Harbor.
- Sauvage H. E.** (1884), "Sur quelques reptiles de la collection du Muséum d'Histoire naturelle," *Bull. Soc. Philom. Paris*, **7**(8), 142–147.
- Say T.** (1823), "*Coluber obsoletus*," in: *Account of an Expedition from Pittsburgh to the Rocky Mountains, Performed in the Years 1819 and '20. 1*, E. James (ed.), Longman, Hurst, Rees, Orme and Brown, London, p. 140.
- Schätti B.** (1988), *Systematik und Evolution der Schlangengattung Hierophis Fitzinger, 1843 (Reptilia, Serpentes)*. Ph.D. Thesis, University of Zurich.
- Schätti B. and Utiger U.** (2001), "*Hemerophis*, a new genus for *Zamenis socotrac* Günther, and a contribution to the phylogeny of Old World racers, whip snakes, and related genera (Reptilia: Squamata: Colubrinae)," *Rev. Suisse Zool.*, **108**(4), 919–948.
- Schinz H. R.** (1822), *Das Thierreich eingeteilt nach dem Bau der Thiere als Grundlage ihrer Naturgeschichte und der vergleichenden Anatomie von Ritter von Cuvier. Reptilien und Fische. 2*, F. A. Brockhaus, Leipzig.
- Schmidt C.** (2000), *Morphologie der Hemipenes von Kletternattern (Elaphe Fitzinger, 1833)*. Diploma Thesis, University of Zurich.
- Schmidt K. P.** (1925), "New Chinese amphibians and reptiles," *Am. Mus. Novitates*, **175**, 1–3.
- Schulz K.-D.** (1996), *A Monograph of the Colubrid Snakes of the Genus Elaphe Fitzinger*, Koeltz Scientific Books, Havlicuv Brod.

- Schulz K.-D. and Helfenberger N.** (1998), "Eine Revision des Unterarten-Komplexes der Roten Bambusnatter *Elaphe porphyracea* (Cantor, 1839)," *Sauria*, **20**(1), 25–45.
- Schulz K.-D., Helfenberger N., Rao D.-Q., and Cen J.** (2000), "Eine verkannte Colubriden-Art, *Elaphe bella* (Stanley, 1917)," *Sauria*, **22**(1), 11–18.
- Shaw G.** (1802), *General Zoology, or Systematic Natural History. Vol. 3. Part 2. Amphibia*, G. Kearsley, London.
- Spolsky C. and Uzzell T.** (1986), "Evolutionary history of the hybridogenetic hybrid frog *Rana esculenta* as deduced from mtDNA analyses," *Mol. Biol. Evol.*, **3**, 44–56.
- Stanley A.** (1917), "Two new species of Chinese snakes," *J. N.-China Br. Roy. Asiat. Soc.*, **47**, 83–84.
- Stejneger L.** (1929), "A new snake from China," *Proc. Biol. Soc. Wash.*, **42**, 129–130.
- Strauch A.** (1873), "Die Schlangen des Russischen Reichs, in systematischer und zoogeographischer Beziehung," *Mém. Acad. Imp. Sci. St. Pétersb. Ser. 7*, **21**(4), 1–287.
- Swofford D. L.** (1998), *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods). Versions 4.0b5, 4.0b8*, Sinauer, Sunderland.
- Sychevskaya E. K.** (1986), "Role of the Bering land bridge in the distribution of Esocoid fishes," in: *Beringia in the Cenozoic Era*, V. S. Kotheekar (ed.) [Russian Translations Series], A. A. Balkema, Rotterdam, pp. 317–326.
- Tamura K. and Nei M.** (1993), "Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees," *Mol. Biol. Evol.*, **10**, 512–526.
- Thompson J. D., Higgins D. G., and Gibson T. J.** (1994), "Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice," *Nucl. Acids Res.*, **22**, 4673–4680.
- Utiger U.** (1996), *RFLP-Analyse zur Klärung der Verwandtschaftsbeziehungen von japanischen Kletternattern (Elaphe Fitzinger, 1833)*. Diploma Thesis, University of Zurich.
- Wagler J. G.** (1828), *Descriptiones et icones amphibiorum*, J. G. Cotta, München.
- Wagler J. G.** (1830), *Natürliches System der Amphibien, mit vorangehender Classification der Säugethiere und Vögel*, J. G. Cotta, München–Stuttgart–Tübingen.
- Wall F.** (1921), "Notes on some notable additions to the Bombay Natural History Society's snake collection," *J. Bombay Nat. Hist. Soc.*, **28**(1), 43–44.
- Wallach V.** (1998), "The lungs of snakes," in: *Biology of the Reptilia. Vol. 19. Morphology G*, C. Gans and A. S. Gaunt (eds), Soc. Study Amph. Rept., Ithaca, NY, pp. 93–295.
- Werner F.** (1913), "Neue oder seltene Reptilien und Frösche des Naturhistorischen Museums in Hamburg," *Mitt. Nat. Mus. Hamb.*, **30**(2), 1–51.
- Yarrow H. C.** (1880), "*Coluber bairdi* sp. nov.," in: *On the zoological position of Texas*, No. 17, E. D. Cope (ed.), *U.S. Nat. Mus. Bull.*, **41**, 492.
- Zardoya R. and Meyer A.** (1996), "Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates," *Mol. Biol. Evol.*, **13**(7), 933–942.
- Ziegler T. and Böhme W.** (1997), "Genitalstrukturen und Paarungsbiologie bei squamaten Reptilien, speziell den Platynta, mit Bemerkungen zur Systematik," *Mertensiella*, **8**, 1–207.
- Zubakov V. A. and Borzenkova I. I.** (1990), in: *Global Palaeoclimate of the Late Cenozoic [Developments in Palaeontology and Stratigraphy]*, Elsevier, Amsterdam, pp. 335–344.

APPENDIX A

DNA samples used for this study (SH, SR, and SS: tissue collection of N. Helfenberger, M. Ruf, and B. Schätti, respectively; an asterisk (*) means that gDNA extracted from muscle was used). *Arizona elegans* Kennicott in Baird, 1859: SR 526 (El Paso, El Paso Co., Texas, USA), SR 547 (10 miles south of Fort Stockton, Hwy 285, Pecos Co., Texas, USA). *Bogertophis rosaliae* (Mocquard, 1899): SR 351 (Baja California, Mexico); *B. subocularis* (Brown, 1901): SR 358 (Big Bend National Park, Brewster Co., Texas, USA), 359 (north of Del Rio, Hwy 277, Val Verde Co., Texas, USA). *Coelognathus erythrurus* (Duméril, Bibron, and Duméril, 1854): SH 969 (K.-D. Schulz), 1011 (Sibutu, Borneo). *Coluber constrictor* Linnaeus, 1758: SR 649 (Ginger's Lane, Huntsville, Madison Co., Alabama, USA), *MHNG 2415.34 (Woodbine, Dickinson Co., Kansas, USA). *Coronella austriaca* (Laurenti, 1768): SH 1103 (Villeneuve-d'Arc, France); *C. girondica* (Daudin, 1803): SH 1067 (Camporosso, E of Ventimiglia, Italy). *Eirenis modestus* (Martin, 1838): SH 1115 (Konya, Turkey), GenBank No. AY039181 and AY039143. *Elaphe* (sensu stricto) *anomala* (Boulenger, 1916): SH 577 (Shaanxi, China); *E. bimaculata* Schmidt, 1925: SH 1164 (K.-D. Schulz), 1420, 1438 (pet trade); *E. carinata* (Günther, 1864): SH 575, 1177 (pet trade); *E. climacophora* (Boie, 1826): SH 528 (Central Honshu, Japan), 1150 (Kuriles, Russia), 1173 (Gifu Prefecture, Honshu); *E. davidi* (Sauvage, 1884): SH 576 (Shaanxi, China); *E. dione* (Pallas, 1773): SH 545 (Ukraine), 952 (Shaanxi, China), 1152 (Wonju, Korea), 1155 (Lazo, Primorye, Russia), 1389 (Taldy-Kurgan region, Kazakhstan), 2166 (Lake Alakol, Kazakhstan); *E. quadrivirgata* (Boie, 1826): SH 470 (Central Honshu, Japan), 551 (Gifu Prefecture, Honshu); *E. quatuorlineata* (Lacépède, 1789): SH 975 (origin unknown), 1112 (Rome, Italy); *E. sauromates* (Pallas, 1811): SH 556 (Mt. Ararat, Turkey), 972 (Selçuk, Turkey); *E. schrencki* Strauch, 1873: SH 857 (pet trade). *Elaphe*

(sensu lato) *frenata* (Gray, 1853): SH 1010 (K.-D. Schulz); *E. (s.l.) prasina* (Blyth, 1854): SH 2189 (K.-D. Schulz). *Euprepiophis conspicillatus* (Boie, 1826): SH 572–573 (Gifu Prefecture, Honshu, Japan); *E. mandarinus* (Cantor, 1842): SH 578 (Shaanxi, China), 1095 (pet trade). *Hemerophis socotrae* (Günther, 1881): MHNG 2610.88 (Socotra, Yemen), GenBank No. AY039140 and AY039178. *Hemorrhais hippocrepi* (Linnaeus, 1758): MHNG 2415.100 (Rabat area, Morocco), GenBank No. AY039139 and AY039177. *Hierophis caspius* (Gmelin, 1789): SH 1148 (Selçuk, Turkey), GenBank No. AY039126 and AY039164. *Lampropeltis getula* (Linnaeus, 1766): SR 315 (origin unknown), 546 (Hwy 277 North, Val Verde Co., Texas, USA); *L. ruthveni* Blanchard, 1920: SR 405 (origin unknown). *Masticophis flagellum* (Shaw, 1802): SR 492 (5 miles south of Boulder City, Clark Co., Nevada, USA), *MHNG 2414.63 (Houston, Harris Co., Texas, USA). *Oocatochus rufodorsatus* (Cantor, 1842): SH 1416, 1444 (pet trade). *Oreophis porphyracea* (Cantor, 1839): SH 1091–1092 (K. Tepedelen). *Orthriophis cantoris* (Boulenger, 1894): SH 2462 (Nepal); *O. hodgsonii* (Günther, 1860): SH 812 (Katmandu, Nepal); *O. moellendorffi* (Boettger, 1886): SH 532, 1096 (pet trade); *O. taeniurus* (Cope, 1861): SH 960 (Shaanxi, China), 1169–1170 (K.-D. Schulz). *Pantherophis bairdi* (Yarrow, 1880): SR 446–447 (Hwy 277 North, Val Verde Co., Texas, USA); *P. guttatus* (Linnaeus, 1766): SR 444 (Redlands Mesa, east of Currant Creek, Delta Co., Colorado, USA), 448 (near Kinard, Calhoun Co., Florida, USA); *P. obsoletus* (Say, 1823): SR 495 (vicinity of New Braunfels, Comal Co., Texas, USA), 565 (3 miles north of Whispering Pines, Moore Co., North Carolina, USA); *P. vulpinus* (Baird and Girard, 1853): SR 360, 657 (origin unknown). *Pituophis deppei* (Duméril, Bibron, and Duméril, 1854): SR 350, 356 (Tecamac, Edo. Mexico, Mexico); *P. melanoleucus* (Daudin, 1803): SR 394 (Santa Cruz Island, California, USA), 549 (south of Loma Alta on Hwy 277, Val Verde Co., Texas, USA). *Platyiceps rhodorachis* (Jan, 1863): MHNG 2542.47 (Wadi Warazan, Yemen), GenBank No. AY039138 and 039176. *Pseudelaphe flavirufa* (Cope, 1867): SR 600 (vicinity of Cancun, Quintana Roo, Mexico), 634 (Tamaulipas, Mexico). *Ptyas mucosus* (Linnaeus, 1758): SH 2461 (Katmandu, Nepal). *Rhinechis scalaris* (Schinz, 1822): SH 814 (Pont du Gard, France), 1388 (Salamanca, Spain). *Rhi-*

nocheilus lecontei Baird in Baird and Girard, 1853: *SR 646 (Black Gap Road, La Linda, Texas, USA). *Salvadora grahamiae* Baird and Girard, 1853: SR 349 (Hidalgo, Mexico), 494 (~15 km NW Hillsboro, Sierra Co., New Mexico, USA). *Senticolis triaspis* (Cope, 1866): SR 314 (Honduras?), 406 (origin unknown). *Zamenis hohenackeri* (Strauch, 1873): SH 555, 1061 (Mt. Ararat, Turkey); *Z. lineatus* (Camerano, 1891): SH 1543 (K.-D. Schulz); *Z. longissimus* (Laurenti, 1768): SH 1107 (Krasnodar area, Russia), 1111 (Rome, Italy), 1369 (Olivone, Switzerland); *Z. persicus* (Werner, 1913): SH 1140 (K.-D. Schulz), 1156 (Lenkoran area, Azerbaijan); *Z. situla* (Linnaeus, 1758): SH 1538 (Croatia), 1550 (Bulgaria).

APPENDIX B

Specimens used for hemipenis illustrations. *Bogertophis rosaliae*: SR 351 (Baja California, Mexico); *B. subocularis*: SR 539 (Brewster Co., Texas, USA); *Coronella austriaca*: collection of G. Dusej No. 108 (Saluch Aul, Sochi, Russia). *Elaphe anomala*: SH 549 (origin unknown); *E. bimaculata*: SH 1426 (“China”); *E. carinata*: SH 869 (origin unknown); *E. climacophora*: SH 527 (origin unknown); *E. davidi*: SH 576 (“China”); *E. dione*: SH 1552 (“China”); *E. quadrivirgata*: SH 522 (“Japan”); *E. quatuorlineata*: SH 1112 (Rome, Italy); *E. sauromates*: SH 965 (Selçuk, Turkey); *E. schrencki*: SH 549 (origin unknown). *Euprepiophis conspicillatus*: SH 572 (“Japan”); *E. mandarinus*: SH 968 (“China”). *Oocatochus rufodorsatus*: SH 1443 (“China”). *Oreophis porphyraceus*: SH 930 (origin unknown). *Orthriophis cantoris*: SH 2462 (Ilam, Nepal); *O. moellendorffi*: SH 1101 (Timor Island); *O. taeniurus*: SH 581 (origin unknown). *Pantherophis bairdi*: SR 447 (Texas, USA); *P. guttatus*: SR 448 (Florida, USA); *P. obsoletus spiloides*: SR 400 (Steel City, Florida, USA); *P. vulpinus*: SR 657 (origin unknown). *Pseudelaphe flavirufa*: SR 600 (Cancun, Mexico). *Rhinechis scalaris*: SH 863 (origin unknown). *Senticolis triaspis*: SR 616 (Puebla, Mexico). *Zamenis hohenackeri*: SH 554 (İğdir, Mt. Ararat, Turkey); *Z. lineatus*: SH 1543 (K.-D. Schulz); *Z. longissimus*: SH 1213 (Trabzon, Turkey); *Z. persicus*: coll. Schmidt (Lenkoran, Azerbaijan); *Z. situla*: SH 1537 (“Yugoslavia”).