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

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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 Palaearctic species. *Natrix* longissima Laurenti (type species) and four western Palaearctic 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-
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. quadriovirgata as well as Asian mainland species (E. dione, E. carinata) 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, Rhinechis Baird in Baird and Girard, 1853, Senticolis Dowling and Fries, 1887, 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, Oocatophus, for the semiaquatic and viviparous Tropidonotus rufodorsatus Cantor. Furthermore, this author revalidated the genus Coelognathus Fitzinger, 1843 for six Oriental species (erythrus, flavolineatus, helenae, philippinensis, 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, Oocatophus, Pituophis, Rhinechis, Rhinechelis, 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 Rhinoceros lecontee 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, Rhinoceros lecontee, 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 austrica, C. gironica, Elaphe anomala, E. davidi, E. (sensu lato) frenata, E. (s.l.) prasina, E. schrenckii, Lampropeltis rathveni, Pityas mucosus, Rhinoceros lecontee, as well as Elaphe (auct.) cantoris, hodgsonii, and lineata). Data for five outgroup species (viz., Eirenis modestus, Hemerophis socotrae, Hemorrhois hippocrepis, 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 (Rodriguez-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, Eiremis, Hemerophis, Hemorrhois, Hierophis, Platyceps, and Masticophis) as well as Coelognathus, Pityas, 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'-AAGCTTCTGACTNCTACCACCNGC-3', and COI(-)-deg, 5'-ATTATTGTGCYGCTGTRAARTAGGCTCG-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'-GTGCCAGCGACCGGTTA CTGACCTGACC-3', and 12S916(-), 5'-GTACGCTTACCATTGTACGACTTGCCCTG-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 (Amer sham Pharmacia Biotech). The 12S rDNA fragment comprised 677 bp and COI 632 (642 with primer deg) 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 (rRASA) for each gene seperately 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 invariant 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 gironica* (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 vasline (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-
Four Oriental ratsnake species, i.e., *Coluber cantoris* Boulenger, *Cynophis moellendorffi* Boettger, *Elaphe taeniura* Cope, and *Spilotes h 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 *orthos* (ορθός), meaning dawn, and *ophis* (οphis), i.e., snake; the gender is masculine.

**Diagnosis.** Subocular usually present (often absent in *hodgsonii*, sometimes also in *cantoris* and *taeniurus*); one or several presuboculars common for *cantoris*, *moellendorffi*, and *taeniurus*; elevated number of ventral scales (212 in *cantoris* 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* (ορός), meaning mountain, and *ophis* (οphis), 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-
**Molecular Systematics and Phylogeny of Ratsnakes**

**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.

**Outgroup taxa**

- *Hemorophis socotrae*
- *Platycps rhodorachis*
- *Hemorrhois hippocrepis*
- *Hierophis caspius*
- *Eirenis modestus*
- *Coelognathus erythrus*
- *Elaphe (s.l.) frenata*
- *Elaphe (s.l.) prasina*
- *Ptyas mucosus*
- *Salvadora grahamiae*
- *Coluber constrictor*
- *Masticophis flagellum*
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*.

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 (Colubrini). 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 (Rodriguez-Robles and De Jesus-Escobar, 1999).

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

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 (Rodriguez-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. linearus 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.

Orthriophis porphyraceus exhibits moderate genetic distances vis-à-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.

Orthriophis porphyraceus differs from Orthriophis spp. in a number of external morphological, osteological, and anatomical characters such as, for instance,
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. davidii 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. davidii, 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. erythrurus, 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 (Colubridae).

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 withOld 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) hemipenis 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.) prasina (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 anomalus 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.
Fig. 5. Slightly adapted consensus tree of Fig. 4 (Pantherophis obsoletus as paraphyletic species with two branches). Color defines monophyletic groups, based on the branching pattern at three levels, viz. the outgroup species, Euprepiophis, and the remaining taxa. Species branches without hemipenis grayish. Asterisks mark redrawn organs (see Material and Methods). See comment in Fig. 3 concerning Elaphe climacophora, E. davidi, and New World genera.
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, Rhinocelus, and Senticolis, are monophyletic and related next to several Palaearctic 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 Palaearctic. 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 Palaearctic 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 Palaearctic 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 Palaearctic 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 porphyreus* Cantor.

*Orthriophis porphyreus* (Cantor)  
*Coluber porphyreus* 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. Boulen-ger (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: Tsonomirri, 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)  
*Elaepe taeniurus* Cope, 1861:655. Type locality: Ningpo [Ningbo], Zhejiang Province, China.

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

*Pantherophis bairdi* (Yarow)  
*Coluber bairdi* Yarow, 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 vulgaris* (Baird and Girard)  
*Scotophis vulgaris* 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 flavirufa* 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 lineatus* (Camerano)  
*Callopeltis 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).

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Molecular Systematics and Phylogeny of Ratsnakes


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), SR 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 austriae (Laurenti, 1768): SH 1103 (Villeneuve-d’Arc, France); C. giron dica (Daudin, 1803): SH 1067 (Camporosso, E of Ventimiglia, Italy). Eirenus 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 (Kurile, Russia), 1173 (Gifu Prefecture, Honshu); E. davi di (Sauvage, 1884): SH 576 (Shaanxi, China); E. dione (Pallas, 1773): SH 545 (Ukraine), 952 (Shaanxi, China), 1152 (Wonju, Korea), 1155 (Lazo, Primorye, Russia), 1389 (Kuriles, Russia), 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). *Hemorrhoides hippocrepis* (Linnaeus, 1758): MHNG 2415.100 (Rabat area, Morocco), GenBank No. AY039126 and AY039176. *Orthriophis cantoris* (Cantor, 1839): SH 1416, 1444 (pet trade). *Oreophis porphyracea* (Cantor, 1839): SH 1091–1092 (K. Tepedelen). *Orthis cantoris* (Boulenger, 1894): SH 2462 (Nepal); *O. hodgsonii* (Günther, 1894): SH 2462 (Nepal); *O. taeniurus* (Cope, 1886): SH 960 (Shaanxi, China), 1169–1170 (K.-D. Schulz). *Pantherophis bairdi* (Yarrow, 1880): SH 446–447 (Hwy 277 North, Val Verde Co., Texas, USA); *P. guttatus* (Boulenger, 1890): SH 581 (origin unknown). *Masticophis flagellum* (Shaw, 1802): SR 492 (5 miles south of Boulder City, Clark Co., Nevada, USA), *Otocatochus 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, 1894): SH 812 (Katmandu, Nepal); *O. moellendorffi* (Boettger, 1886): SH 532, 1096 (pet trade); *O. taeniurus* (Cope, 1886): 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): SH 1148 (Selçuk, Turkey), GenBank No. AY039126 and AY039176. *Rhinechis scalaris* (Schinz, 1822): SH 1091 (Timor Island); *O. tae- niurus* (Cantor, 1839): SH 572 (origin unknown); *E. bimaculata*; SH 1426 (“China”); *E. carinata*; SH 869 (origin unknown); *E. climacophora*; SH 527 (origin unknown); *E. daviidi*; SH 576 (“China”); *E. dione*; SH 1552 (“China”); *E. quadrivirgata*; SH 522 (“Japan”); *E. quadrivirgata*; SH 1112 (Rome, Italy); *E. saurumata*; SH 965 (Selçuk, Turkey); *E. schrencki*; SH 549 (origin unknown). *Euprepiophis conspicillatus*; SH 572 (“Japan”); *E. mandarinus*; SH 968 (“China”). *Otocatochus rufodorsatus*; SH 1443 (“China”). *Oreophis porphyraceus*; SH 930 (origin unknown). *Orthriophis cantoris*; SH 2462 (Ilam, Nepal); *O. moellendorffi*; SH 1101 (Timor Island); *O. tae- niurus*; SH 581 (origin unknown). *Pantherophis bairdi*; SR 447 (Texas, USA); *P. guttatus*; SR 448 (Florida, USA); *P. obsoletus*; SR 400 (Steel City, Florida, USA); *P. vulpinus*; SR 657 (origin unknown). *Pseudepalea flavirufa*; SR 600 (Cancun, Mexico). *Rhinechis scalaris*; SH 863 (origin unknown). *Senticolis triaspis*; SR 616 (Puebla, Mexico). *Zamenis hohenackeri*; SH 554 (Ilgidir, Mt. Ararat, Turkey); *Z. lineatus*; SH 1543 (K.-D. Schulz); *Z. longissimus*; SH 1538 (Croatia), 1550 (Bulgaria).

**APPENDIX B**

Specimens used for hemipenis illustrations. *Bogertophis rosaliae*: SR 351 (Baja California, Mexico); *B. subocularis*: SR 539 (Brewer Co., Texas, USA); *Coronella australica*: 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. daviidi*: SH 576 (“China”); *E. dione*: SH 1552 (“China”); *E. quadrivirgata*: SH 522 (“Japan”); *E. quadrivirgata*: SH 1112 (Rome, Italy); *E. saurumata*: SH 965 (Selçuk, Turkey); *E. schrencki*: SH 549 (origin unknown). *Euprepiophis conspicillatus*: SH 572 (“Japan”); *E. mandarinus*: SH 968 (“China”). *Otocatochus 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*; SR 400 (Steel City, Florida, USA); *P. vulpinus*: SR 657 (origin unknown). *Pseudepalea flavirufa*: SR 600 (Cancun, Mexico). *Rhinechis scalaris*: SH 863 (origin unknown). *Senticolis triaspis*: SH 616 (Puebla, Mexico). *Zamenis hohenackeri*: SH 554 (Ilgidir, Mt. Ararat, Turkey); *Z. lineatus*: SH 1543 (K.-D. Schulz); *Z. longissimus*: SH 1213 (Trabzon, Turkey); *Z. persicus*: coll. Schmidt (Lenkoran area, Azerbaijan); *Z. situla*: SH 1538 (Croatia).