



Molecular phylogeny of Asian pipesnakes, genus *Cylindrophis* Wagler, 1828 (Squamata: Cylindrophidae), with the description of a new species from Myanmar

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Abstract

Cylindrophis is a genus of secretive, semi-fossorial, non-venomous snakes comprising 14 species, characterized by a generally cylindrical body, uniform scales (with barely enlarged ventrals), and vestiges of pelvic and limb bones, the latter terminating in a claw lateral to the vent. We reconstructed a concatenated molecular phylogeny of seven taxa of *Cylindrophis* taxa based on one nuclear (R35) and two mitochondrial (16S, ND2) genes. Analyses recovered the Sri Lankan endemic *C. maculatus* as sister to all other sampled *Cylindrophis*. The mainland Southeast Asian species *C. burmanus* and *C. jodiae* form successive sister lineages to a monophyletic Wallacean island group containing *C. boulengeri*, *C. isolepis*, and *C. yamdena*. We also describe a new species of *Cylindrophis*, morphologically similar to *C. burmanus*, from Kachin State in northern Myanmar. *Cylindrophis slowinskii* **sp. nov.** is distinguished from all congeners by the following combination of characters: 19 dorsal scale rows at midbody, 216–220 ventrals, eight subcaudals, a dark venter with > 60 very narrow diffuse pale blotches, and a pale bar running along the posterior border of the prefrontals. In our phylogeny, the new species is strongly supported as the sister species of *C. burmanus*. It is the 15th currently recognised species in the genus, and the fourth from mainland Southeast Asia.

Key words: Chindwin River, Irrawaddy River, phylogenetics, Reptilia, Serpentes, species complex, systematics, taxonomy

Introduction

Asian pipesnakes comprise a single genus (*Cylindrophis* Wagler, 1828) in the monotypic family Cylindrophidae. There are 14 currently recognized species distributed in Sri Lanka and Southeast Asia (Kieckbusch *et al.* 2016, 2018; Uetz *et al.* 2020). These snakes are generally secretive but can be encountered in forests, wetlands, canals, flooded rice fields, and even suburban gardens and inside houses (Wall 1925; Smith 1943; Das 2015; Mader & Mecke 2018). Species in the genus *Cylindrophis* are moderately-sized snakes, with a maximum total length of 87 cm (SM & MK, pers. obs.). They have a cylindrical body with a short tail, smooth scales, narrow ventrals (barely wider than those on the dorsum), vestigial pelvic and hindlimb elements terminating in cloacal spurs, and a conspicuous colour pattern of pale (or bright red) and dark blotches on the venter (with the exception of adults in *C. yamdena*

Smith & Sidik, 1998). The short, blunt tail of these gape-limited burrowers is often reddish in coloration (especially ventrally), and, together with the posterior part of the body, can be flattened, inverted, and displayed when the snake is threatened (Flower 1899; Greene 1973, 1983; Kieckbusch *et al.* 2016, 2018). Viviparity is also a characteristic feature of this group (Amarasinghe *et al.* 2015; Kieckbusch *et al.* 2016, 2018).

Cylindrophis is characterized by significant endemism, with 11 of the 14 species only found on single islands or island groups. Island-endemic pipesnake species include *C. maculatus* (Linnaeus, 1758) from Sri Lanka, *C. engkariensis* Stuebing, 1994 and *C. lineatus* Blanford, 1881 from Borneo, *C. subocularis* Kieckbusch, Mecke, Hartmann, Ehrmantraut, O’Shea & Kaiser, 2016 from Java, *C. melanotus* Wagler, 1828 from Sulawesi and some northern Moluccan islands (see Kieckbusch *et al.* 2018), *C. isolepis* Boulenger, 1896 from Jampea, *C. osheai* Kieckbusch, Mader, Kaiser, & Mecke, 2018 from Boano, *C. opisthorhodus* Boulenger, 1897 from Lombok, Sumbawa, Komodo, and Flores (see Auffenberg 1980), *C. boulengeri* Roux, 1911 from Timor and Wetar (see Lang 2011, O’Shea *et al.* 2015), *C. yamdena* from Yamdena, and *C. aruensis* Boulenger, 1920 from Damar and/or Aru (see Lang 2013) (Fig. 1).

The phylogenetic position of the genus *Cylindrophis* has long been disputed, and little is known about the intrageneric relationships of the included species. Romer (1956) tentatively grouped *Cylindrophis* and *Anomochilus* Lidth de Jeude, 1890 (dwarf pipesnakes) together with *Uropeltis* Cuvier, 1829 (earth snakes) and its allies into the subfamily Uropeltinae Müller, 1831 (shieldtails; currently treated as a family-level taxon) in the family Aniliidae Stejneger, 1907 (false coral snakes; a family that today only includes the neotropical *Anilius scytale* (Linnaeus, 1758)). Since then, most researchers have recognized a close relationship between *Cylindrophis* and uropeltids (e.g., McDowell 1975, 1987; Rieppel 1977, 1979; Cadle *et al.* 1990; Lee & Scanlon 2002; Slowinski & Lawson 2002; Gower 2003; Burbrink & Crother 2011; Burbrink *et al.* 2020). *Cylindrophis* has also often been hypothesized to be most closely related to *Anomochilus*, and in some studies both genera were included in a single subfamily, the Cyliindrophinae Fitzinger, 1843 (Fitzinger’s taxon “Cyliindrophes”) within the Uropeltidae (McDowell 1975, 1987; Rieppel 1977, 1979; Groombridge 1979). However, in-depth comparisons of skeletal, muscular, and visceral characters in several major snake clades resulted in a phylogeny that showed uropeltids to be paraphyletic, leading to a taxonomic change, that is, the allocation of *Anomochilus* to its own family, Anomochilidae Cundall, Wallach, & Rossman, 1993, whereas *Cylindrophis* was grouped into its own family, the Cyliindrophidae (Cundall *et al.* 1993).

The inclusion of cyliindrophiid and anomochilid species in molecular analyses has led to several phylogenies that either hypothesized a monophyletic Cyliindrophidae (e.g., Pyron *et al.* 2013a, 2013b; Reeder *et al.* 2015) or a relationship in which *Anomochilus* rendered *Cylindrophis* paraphyletic (Gower *et al.* 2005; Figueroa *et al.* 2016; Zheng & Wiens 2016). The recovered relationship in the paraphyletic scenario suggests that “*C. ruffus*”¹ (Laurenti, 1768) is the sister species of *C. maculatus* + *A. leonardi* Smith, 1940. This relationship has been recovered with low to moderate support across studies, likely due to the availability of only short, partial gene sequences of 12S and 16S, the use of only one sample per species, and/or the lack of inclusion of multiple species of *Cylindrophis* and/or *Anomochilus*. Irrespective of this, and despite major morphological differences between the two genera, some have included *Anomochilus* in a broader concept of *Cylindrophis* (e.g., Vitt & Caldwell 2014). Recently, without a more complete species-level sampling but using genomic-scale data, *Cylindrophis* was supported as the sister taxon to uropeltids, which as a group are distantly related to a clade containing aniliid and tropidophiid snakes (dwarf boas) (Burbrink *et al.* 2020). However, *Anomochilus* was not included in the latter study, thus the phylogenetic placement of Anomo-chilidae is still uncertain. Inclusion of additional specimens of *Cylindrophis* and *Anomochilus*

1 The sequence data obtained from GenBank by Gower *et al.* (2005) originated from a specimen (MNHN 1999.9021) listed in Vidal & Hedges (2002). This specimen, purchased from a pet shop that regularly imported amphibians and reptiles from Java (Nicolas Vidal, in litt.), was identified as *Cylindrophis subocularis* by SM & MK (see “Appendix 2”). The sequence data obtained from GenBank by Figueroa *et al.* (2016, see their S1 Table) originated from various sources, including Townsend *et al.* (2004), Dong & Kumazawa (2005), Noonan & Chippindale (2006), and Wiens *et al.* (2008). Specimens sampled by Townsend *et al.* (2004) are identical to vouchers used herein (CAS 210518, 206622 = *C. burmanus*). Dong & Kumazawa (2005) provided an accession number for their sampled voucher: NUM-Az0376, a specimen in the Nagoya University Museum (Japan). However, no locality data or further information were provided that would immediately help to identify their specimen. The other publications lack any voucher information. Kieckbusch *et al.* (2016) restricted *C. ruffus*, a taxon for which tissue samples appear unavailable, to parts of Maritime Southeast Asia (mainland Malaysia, Singapore, and the Greater Sunda Islands). This indicates that the sequence data for “*C. ruffus*” used by Figueroa *et al.* (2016) likely stem from mainland taxa that were previously included in *C. ruffus* (i.e., *C. burmanus* and/or *C. jodiae*). The same may apply to Zheng & Wiens (2016), who provide an alignment for “*C. ruffus*” in their supplemental information, but no further data.

for subsequent studies remains problematic due to the secretive nature and potential rarity of many of the relevant species, some of which are known from only very few (and sometimes historical) specimens with genetic samples unavailable (e.g., McDowell 1975; Stuebing & Goh 1993; Stuebing 1994; Gower *et al.* 2005; Kieckbusch *et al.* 2016, 2018).

As a result, intrageneric relationships for *Cylindrophis* have recently been based on morphological characters. Amarasinghe *et al.* (2015) surmised four groups based on the number of dorsal scale rows at midbody in the 13 then-known species of *Cylindrophis*, including *C. mirzae* Amarasinghe, Ineich, Campbell & Hallermann, 2015, a taxon synonymized with *C. ruffus* by Kieckbusch *et al.* (2016). These four groups included, respectively, species with 23 dorsal scale rows (*C. aruensis* and *C. opisthorhodus*), 21 rows (*C. isolepis*, *C. jodiae* Amarasinghe, Ineich, Campbell, & Hallermann, 2015, *C. lineatus*, *C. maculatus*, and *C. yamdena*), 19 rows (*C. boulengeri*, *C. burmanus* Smith, 1943, *C. melanotus*, and Javan *C. ruffus*), and 17 rows (*C. engkariensis*). However, these groups do not accurately divide *Cylindrophis*, as *C. maculatus* is well known to have a variable number of 19 or 21 dorsal scale rows at midbody (Boulenger 1893; Mahendra 1936; SM & MK, pers. obs.), and the same applies to *C. ruffus* (Kieckbusch *et al.* 2016). The monophyly of these groups has not yet been tested using other characters or molecular data.

Here, we use a multilocus genetic dataset and thorough sampling to infer phylogenetic relationships amongst a selection of currently described *Cylindrophis*, and describe a new species of the genus from Myanmar.

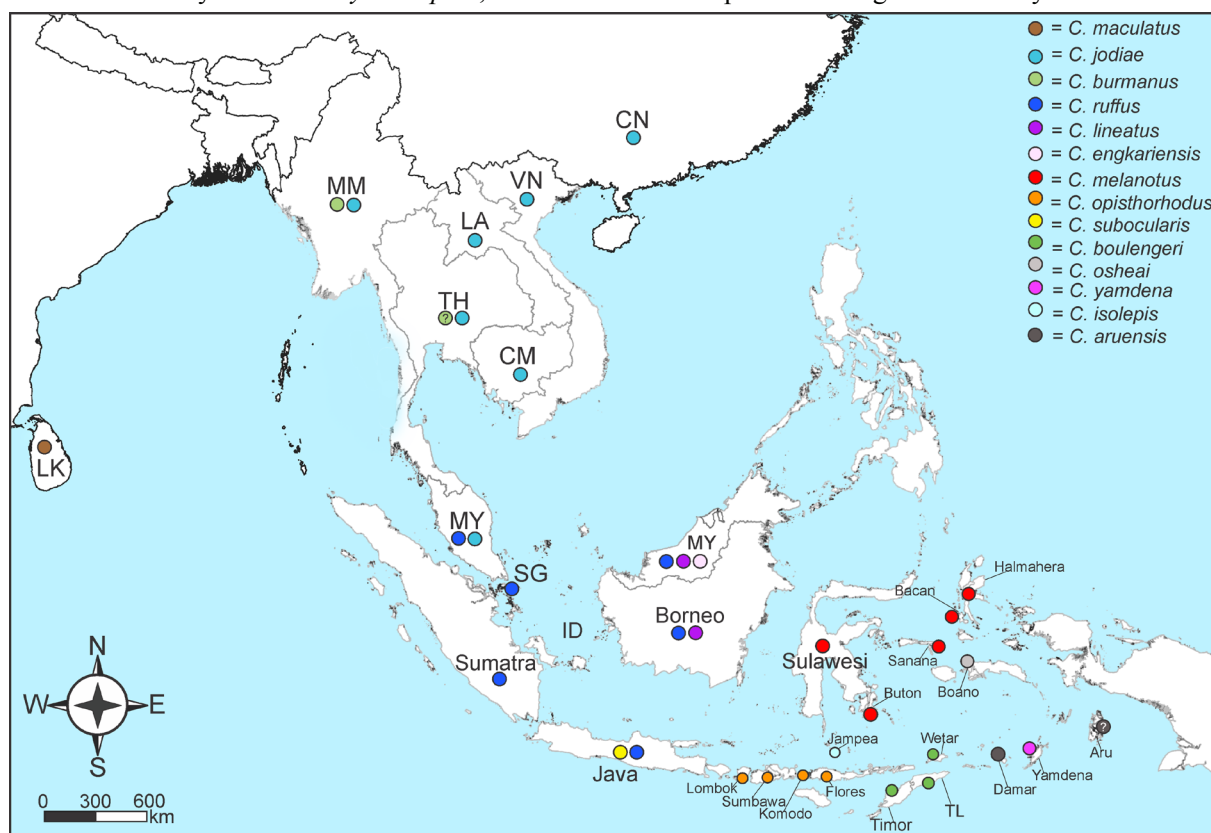


FIGURE 1. Map of South and Southeast Asia showing country and island records for the heretofore recognized species of *Cylindrophis* (colored circles). Abbreviations used for countries are as follows: CM (Cambodia), CN (China), ID (Indonesia), LA (Laos), LK (Sri Lanka), MM (Myanmar), MY (Malaysia), Singapore (SG), TH (Thailand), TL (Timor-Leste), VT (Vietnam). A question mark within a circle refers to an uncertain country or island record. Note that the circles only indicate the presence of a taxon within a political or geographical entity. The map does not depict the actual distribution of *Cylindrophis* species. Map prepared by Justin Bernstein.

Materials and methods

Genetics

Sample collection. Muscle and liver tissues of all snakes used for genetic analyses in this study were obtained from collections at the following institutions, abbreviated according to Sabaj (2016): CAS, LSUMZ, MVZ, MCZ, ROM, USNM, and WAM.

Molecular methods. Tissues were incubated and lysed at 55°C in a solution of Proteinase K, 10% sodium dodecyl sulphate, and extraction buffer (1 M Tris, 5 M NaCl, and 0.25 M EDTA in distilled water). Total genomic DNA was extracted using a 5 M NaCl solution or Qiagen DNAeasy blood and tissue kits. Two mitochondrial genes (16S rRNA and NADH dehydrogenase subunit 2 [ND2]) along with WANCY tRNAs and one nuclear gene (R35) were amplified using polymerase chain reaction (PCR) with the primer pairs listed in Table 1. For ND2, amplification was performed using a combination of Metf1 and CO1R1 or CO1R8, and sequencing was conducted using Metf1 and CO1R1, CO1R8, or L5002. Successful gene amplification was verified using 1.5% agarose gels and subsequent PCR products were cleaned using magnetic bead methods. All amplicons were sequenced using an ABI 3730xl DNA analyser at GENEWIZ, Inc. (South Plainfield, New Jersey, USA).

TABLE 1. List of primers used for gene amplification and sequencing in this study. PCR program temperatures indicate annealing temperatures. All PCR primers were also used for subsequent DNA sequencing.

Primer	Gene—Direction	Primer Sequence (5' – 3')	PCR Program	Fragment Length (bp)	Source
16SA	16S—Forward	CGCCTGTTTATCAAAAACAT	34 cycles /50°C	~ 500	Palumbi <i>et al.</i> , 1991
16SB	16S—Reverse	CCGGTCTGAACTCAGATCACGT	34 cycles /50°C	~ 500	Palumbi <i>et al.</i> , 1991
Metf1	ND2—Forward	AAGCTTTCGGGCCCATACC	34 cycles /50°C	~ 1400	Macey <i>et al.</i> , 1997
CO1R1	ND2—Reverse	AGRGTGCCAATGTCTTTGTGRTT	34 cycles /50°C	~ 1400	Macey <i>et al.</i> , 1997
CO1R8	ND2—Reverse	GCTATGTCTGGGGCTCCAATTAT	32 cycles /52°C	~ 1400	Weisrock <i>et al.</i> , 2001
L5002	ND2—Sequencing	AACCAAACCAACTACGAAAAAT	34 cycles /50°C	~ 1400	Macey <i>et al.</i> , 1997
R35F	R35—Forward	TCTAAGTGTGGATGATYTGAT	34 cycles /50°C	~ 700	Fry <i>et al.</i> , 2006
R35R	R35—Reverse	CATCATTGGRAGCCAAAGAA	34 cycles /50°C	~ 700	Fry <i>et al.</i> , 2006

Phylogenetic analyses. Sequences and tissues of *Anomochilus* were not available. Hence, *Python molurus* (Linnaeus, 1758), *Rhinophis philippinus* (Cuvier, 1829), and *R. phillipsi* (Nicholls, 1929) were chosen as outgroups. Particularly, species of *Rhinophis* Hemprich, 1820 were used as outgroups because previous comprehensive phylogenies recovered uropeltids as closely related to or as the sister taxon of cylindrophiiids, with *Python* positioned outside of the uropeltids and cylindrophiiids (Pyron *et al.* 2013a; Burbrink *et al.* 2020). Sequences of *P. molurus* were obtained from other studies through Genbank (Dubey *et al.* 2012; Wiens *et al.*, 2012; see “Appendix 1”). All DNA sequences were aligned using the MUSCLE alignment function with default settings in Geneious v7.1.8 (<https://www.geneious.com>). We found no heterozygotes in the R35 (nuclear) data. To create contiguous sequences from any low-quality reads, we used high-quality reads as reference sequences. Alignments were then manually edited by eye to ensure correct alignment and to check for sequencing errors. Because there were no cases of conflicting relationships among different gene trees, we ran one set of analyses using concatenated data.

The final alignments were 526, 1403, 680, and 2608 base pairs (bp) long for 16S, ND2, R35, and the concatenated (all three genes) alignments, respectively. We constructed gene trees using maximum likelihood (ML) and Bayesian inference (BI) methods on the CIPRES Science Gateway v3.3 (Miller *et al.* 2010). Bootstrap support for ML analyses were considered to be moderately or strongly supported for values of 70–100. Internal branches in BI trees were considered to be well supported if posterior probabilities were ≥ 0.95 . We produced ML trees for each alignment using RAxML v8.2.10 (Stamatakis 2014); analyses were run for 1000 bootstrap iterations using a GTRGAMMA nucleotide substitution model. Partitioning schemes for DNA alignments were chosen based on results using PartitionFinder2 under the Akaike information criterion (AICc; Lanfear *et al.* 2016). Bayesian inference trees were run for 100 million generations in MrBayes v3.2.6 (Ronquist *et al.* 2012). The GTR+I+G and HKY+I

models of evolution were used for the 16S and R35 BI analyses, respectively. The GTR+G and HKY+G models were used for the ND2 BI analysis, and GTR+I+G, HKY+G, GTR+G, and HKY+I for the concatenated BI analysis. For species tree analysis, all gene alignments were analysed simultaneously using BEAST2 (Bouckaert *et al.* 2014). Because this program uses a Bayesian framework to reconstruct species trees, we implemented the package bModelTest v1.2.1 in BEAST2 for evolutionary model selection and averaging (Lanfear *et al.* 2016; Bouckaert & Drummond 2017). The BEAST analysis was run for 100 million generations with linked mitochondrial gene (16S and ND2) trees and sampled every 10000 generations under a Yule model of speciation and an uncorrelated lognormal relaxed clock to allow for branch rate heterogeneity. The analysis was checked for convergence (10% burn-in) and effective sample size (ESS) values were checked in Tracer v1.6 (Rambaut *et al.* 2018). All trees from the analysis were combined using TreeAnnotator.

Morphology

Metrics. The following measurements were taken with digital callipers (to the nearest 0.1 mm, except for SVL and TL, which were measured to the nearest 1 mm; see Kieckbusch *et al.* 2016, 2018): snout–vent length (SVL; from tip of snout to cloaca); tail length (TL; from cloacal opening to tip of tail); body diameter (BD; calculated as the mean of body height and body width at midbody); head length (HL; from rostral to end of mouth gap); head width (HW; at level of anterior margin of the parietals); snout length (SL; from rostral to anterior margin of orbit); snout width (SW; at level of the nares); eye diameter (ED; measured horizontally); interorbital distance (IOD; measured as shortest distance between the eyes across head); internarial distance (IND; between interior margins of the nares). The following head scale characters were measured, with dimensions expressed as the maximum length, height, or width: rostral height and width; nasal length and width; prefrontal length and width; frontal length and width; parietal length and width; supraocular length and width; postocular length and height; anterior temporal length and height; upper posterior temporal length and height; mental height and width; anterior chin shield length and width; posterior chin shield length and width; mental groove length. Characters occurring bilaterally were measured on the right side of the body.

Scale counts and qualitative scalation characteristics. We counted the following scale characters: number of dorsal scale rows, counted in an inverse “V”-shape (to include all dorsal scales developmentally associated with a single pair of ribs) at (A) one head length behind the head, (B) at midbody, and (C) one head length anterior to the cloacal opening, displayed in a formula as “A-B-C”; ventrals, beginning with the gular scale bordered by the posterior chin shields (see Kieckbusch *et al.*, 2016 for more details); subcaudals, counted from the cloacal opening to the end of the tail, excluding the terminal spine; temporals, including (a) the number of anterior temporals and (b) the number of posterior temporals, expressed in a formula as a + b; and the number of supralabials and infralabials. We also recorded the following qualitative scale characteristics: the specific supralabials contacting the orbit and the condition of the cloacal scute (divided or entire). Scale counts and external observations of morphology were made with the use of a dissection microscope. Characters occurring bilaterally were counted on both sides of the body; if counts differed on either side, this is indicated by “R|L”.

Coloration and pattern. We recorded the number of transverse ventral blotches present along the body, from the first blotch behind the head to the last blotch anterior to the cloacal opening, as well as the pattern and coloration of head, dorsum, and tail. Coloration of the examined museum vouchers referred herein as “pale” may be strikingly yellowish or reddish in living specimens. Descriptions of pattern and coloration follow Köhler (2012). Numbers in parentheses behind the respective capitalized color name refer to the coding therein.

Comparisons. Comparisons were made with museum material representing all but one *Cylindrophis* species (*C. aruensis*), for which we relied on literature sources alone. A list of specimens examined is provided in Appendix 2. Specimens used as comparative material are housed in the following collections (abbreviations follow Sabaj 2016): AMNH, BMNH, CAS, MCZ, MHNG, MNHN, MTKD, NMBA, NMBE, NMW, RMNH, SMF, USNM, WAM, ZMA (now at RMNH), ZMB, ZMH, and ZRC. The type specimens of the new species are housed at the CAS.

Results

Genetics

ML and BI trees were reconstructed for 16S, ND2, R35, and the concatenated datasets (Fig. 2; see “Appendix 3” for BI trees). The 16S genealogies recovered the same topology, with the Sri Lankan endemic *Cylindrophis maculatus* as the sister species to all other *Cylindrophis* included in this study (Fig. 2A–D). In the 16S ML tree, *C. burmanus* from Myanmar appears as sister to a clade containing the recently described *C. jodiae* from Vietnam and species from islands in the Wallacea region (Jampea, Timor and Wetar, and Yamdena). These island species form their own clade, in which *C. isolepis* from Jampea, South Sulawesi, is recovered as the sister species of *C. boulengeri* from the Lesser Sunda islands of Timor and Wetar + *C. yamdena* from Yamdena in the Tanimbar Archipelago. Species pairwise distances for ND2 for the Wallacean clade range from 6.80–9.50%, whereas they are 11.97–14.49% for the entire genus. At the species level, the ML 16S and ND2 trees have strongly supported clades and identical topologies (Fig. 2B, C). The BI 16S and ND2 trees have strong support with Bayesian posterior probabilities (PP) ≥ 0.95 for all clades, with the exception of one within *C. burmanus*. ML 16S and ND2 gene trees also recovered clades representing species that were moderately supported by bootstrap values (≥ 70), but have poor to moderate bootstrap support at deeper internal branches. The *C. burmanus* samples were recovered with strong support in the ML concatenated analysis as comprising two clades, with the exception of one specimen (CAS 241554; see below). All specimens of *C. burmanus* west of the Irrawaddy River cluster together in one clade, which is sister to a clade that contains specimens from the Irrawaddy Delta and east of the Irrawaddy River. Although *C. burmanus* and *C. maculatus* represent distinct taxa, most relationships are unresolved in both R35 trees (Fig. 2D; see “Appendix 3”). The ML and BI phylogenies for the concatenated datasets are similar to the separate 16S and ND2 trees. Similarly, the BEAST analysis recovered the same topology as the concatenated dataset with strong support values (Fig. 3). Almost all ESS values were above 1000 and only one below the recommended value of 100, which was a base frequency statistic for the R35 dataset.

We note that a specimen from Kachin State, northern Myanmar (CAS 241554), collected from a region that is enclosed by the Chindwin and Irrawaddy Rivers, and originally identified as *C. burmanus*, was recovered as the sister taxon to the rest of the *C. burmanus* specimens in the ND2, concatenated, and species trees with strong support (Figs. 2, 3). The 16S and ND2 uncorrected pairwise distances between this specimen and *C. burmanus* are 2.43–3.04% and 5.52–6.18%, respectively. A second specimen from Kachin State (USNM 590030) was included in our dataset. Although we were only able to obtain ND2 data, when included in the genealogy, this specimen is genetically identical to CAS 241554 (100% sequence identity; Fig. 2A, C). Hence, given the genetic distinctiveness of these two specimens from the rest of *C. burmanus*, from which they also differ in morphology, we describe the lineage from Kachin State as a new species, below.

Species description

Cylindrophis slowinskii sp. nov.

(Fig. 4)

Holotype. CAS 241554 (field number: CAS-MHS 26113), an adult specimen from Indawgyi Wildlife Sanctuary in the vicinity of Lwe Mon Village, west of Indawgyi Lake, Mohnyin Township, Kachin State, Myanmar (ca. 25.1787° N, 96.2917° E, elevation 245 m), collected by J.A. Wilkinson, K.T. Kyaw, and J.V. Vindum on 26 July 2008 (Fig. 4).

Paratype. USNM 590030 (field number: CAS-MHS 26114), an adult specimen, collection data as for the holotype.

Diagnosis. A species of the genus *Cylindrophis*, identified by a combination of the following characteristics: (1) small size: 297–333 mm SVL; (2) 17-19-17 dorsal scale rows; (3) 216–220 ventrals; (4) eight subcaudals; (5) 65–69 very narrow diffuse pale ventral blotches that are branching, creating the impression of a diffuse reticulum; and (6) a pale bar running along the posterior border of the prefrontals (Fig. 4). These morphological characters are sufficient to distinguish *C. slowinskii* from all other species of Asian pipesnakes.

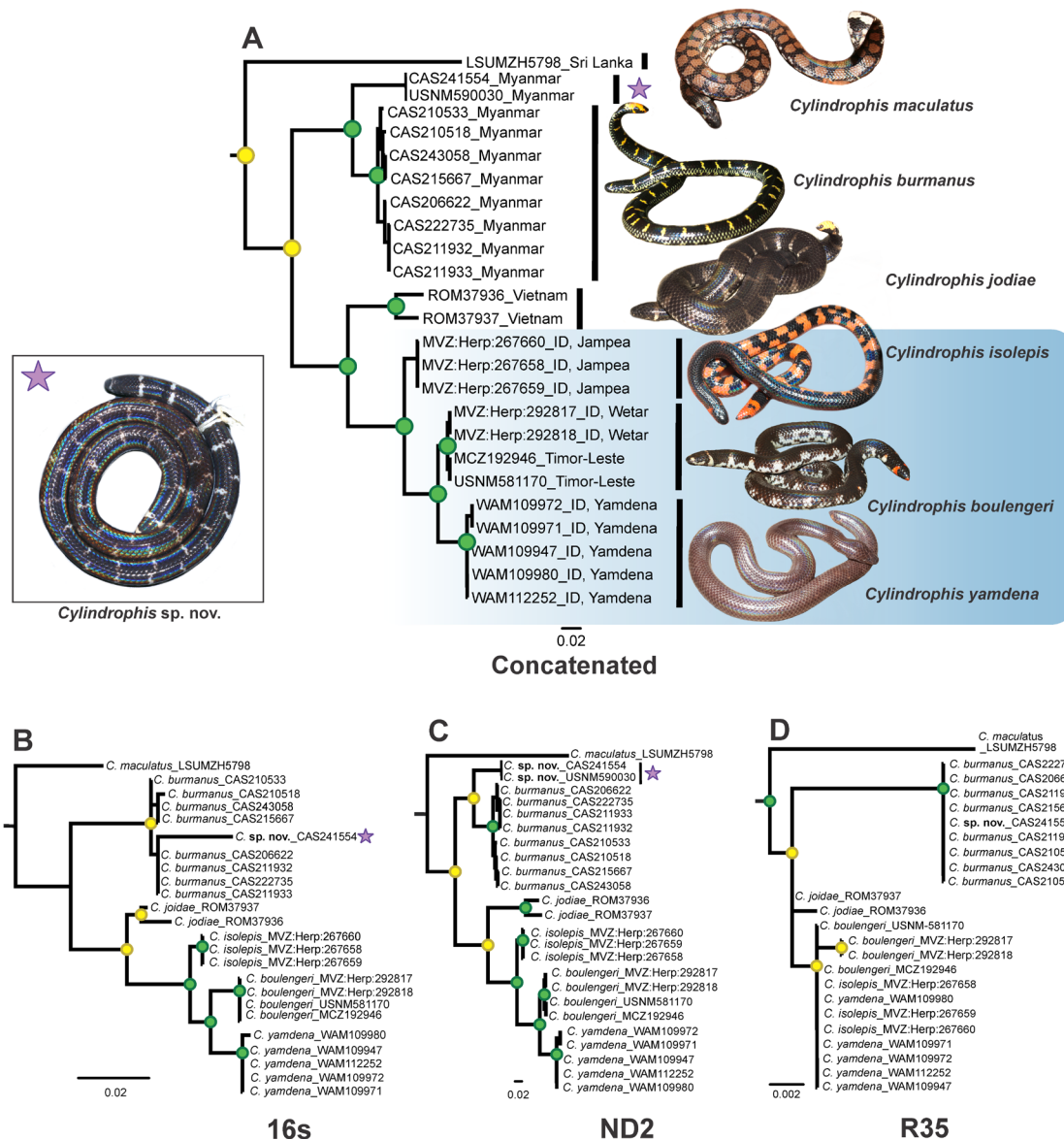


FIGURE 2. Maximum likelihood (ML) trees of *Cyliodrophis*. Yellow and green circles represent bootstrap support values of 70–94 (moderate support) and 95–100 (strong support), respectively. Clades without circles represent bootstrap values of 0–69 (poor support). Scale bars indicate the estimation of nucleotide substitutions per site. For each terminal, a voucher number and political unit (country) are given. For specimens from Indonesia (ID), island localities are additionally provided. A purple star (★) represents specimens of the new species described herein. **A)** Concatenated ML phylogeny of *Cyliodrophis* using 16S, ND2, and R35 loci. The shaded clade highlights a Wallacean group containing *C. isolepis*, *C. boulengeri*, and *C. yamdena*. **B–D)** ML genealogies using 16S (**B**), ND2 (**C**), and R35 (**D**). Photo credits: *Cyliodrophis maculatus*: Ruchira Somaweera (The Commonwealth Scientific and Industrial Research Organisation/CSIRO); *Cyliodrophis sp. nov.* (holotype designated herein, CAS 241554): Justin M. Bernstein (Rutgers University-Newark); *C. burmanus*: Hla Tun (photograph registered and archived at CAS: ID: MHS_209); *C. jodiae*: Teo Eng Wah (University of Malaya); *C. isolepis*: Jimmy A. McGuire (MVZ); *C. boulengeri*: Sven Mecke (Naturkundemuseum Paderborn); *C. yamdena*: Ron E. Johnstone (WAM).

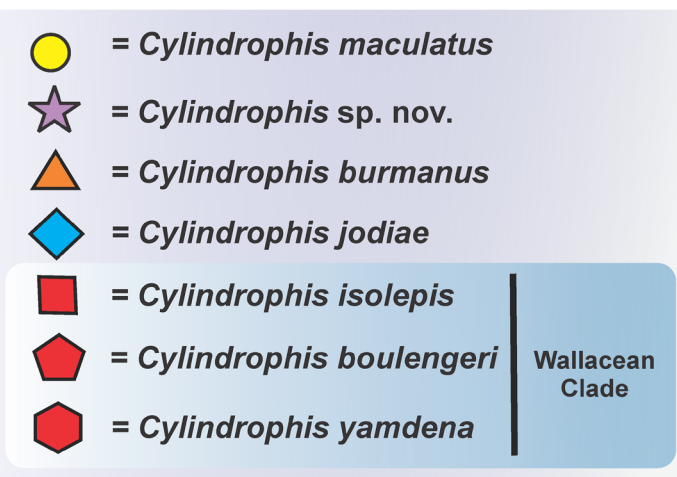
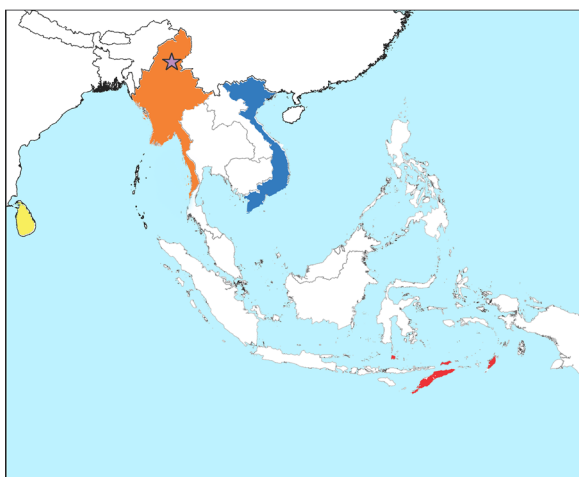
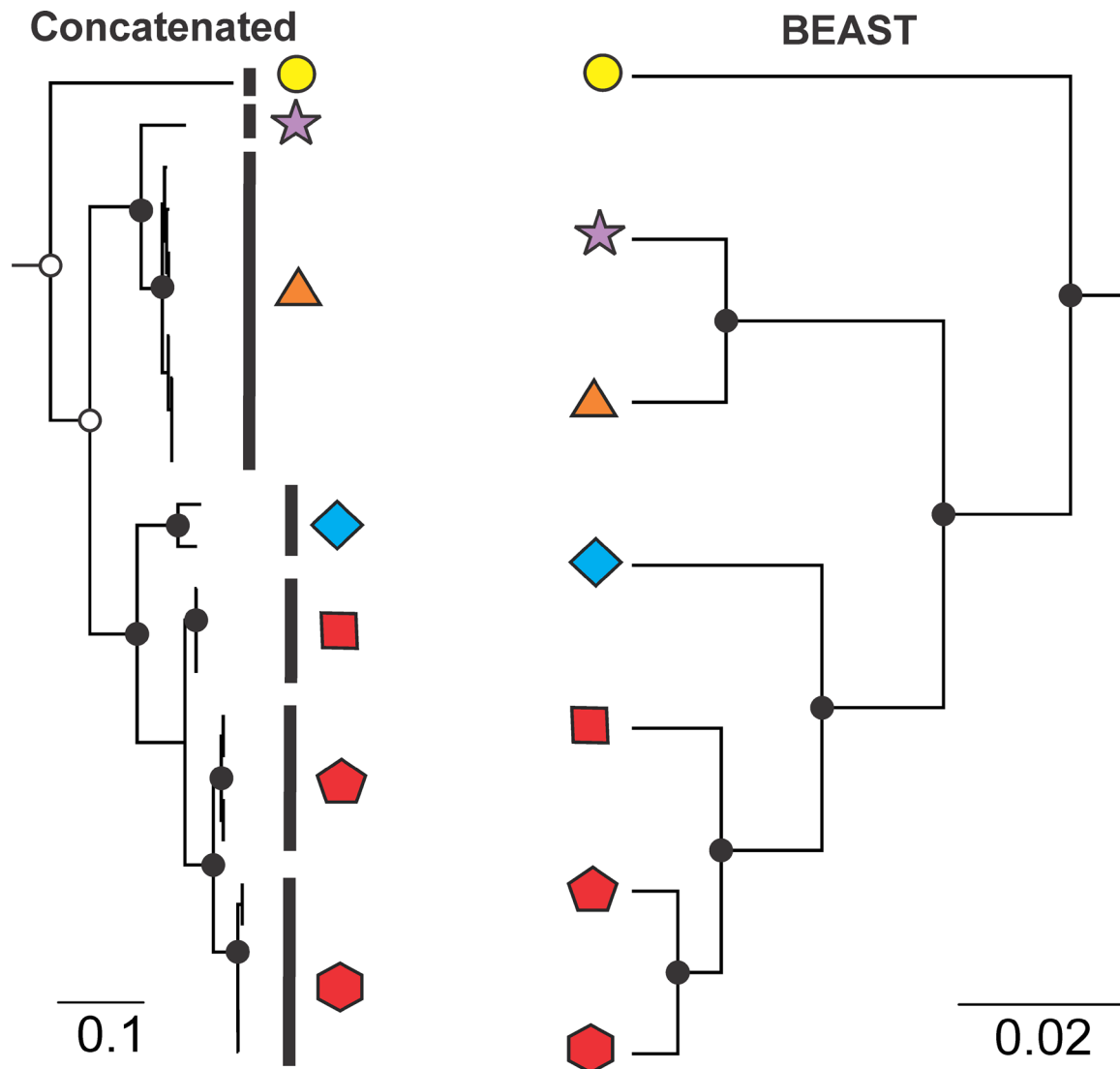


FIGURE 3. Concatenated (left) and species (right) trees of *Cylindrophis*. Open circles (○) indicate ML bootstrap values of 70–94; filled circles (●) represent ML bootstrap values of 95–100 (concatenated) or PP of 0.95–1 (BEAST). Symbols behind each clade refer to taxa as defined in inset, with symbols for species of the Wallacean clade consistently colored red. In the map, the origins, as political units (countries) or islands, of the specimens used for the phylogenies are shaded, and do not represent the total geographic range of the respective taxon. A purple star (★) indicates the type locality of the new species described herein. For country records of all species of *Cylindrophis*, see Fig. 1.

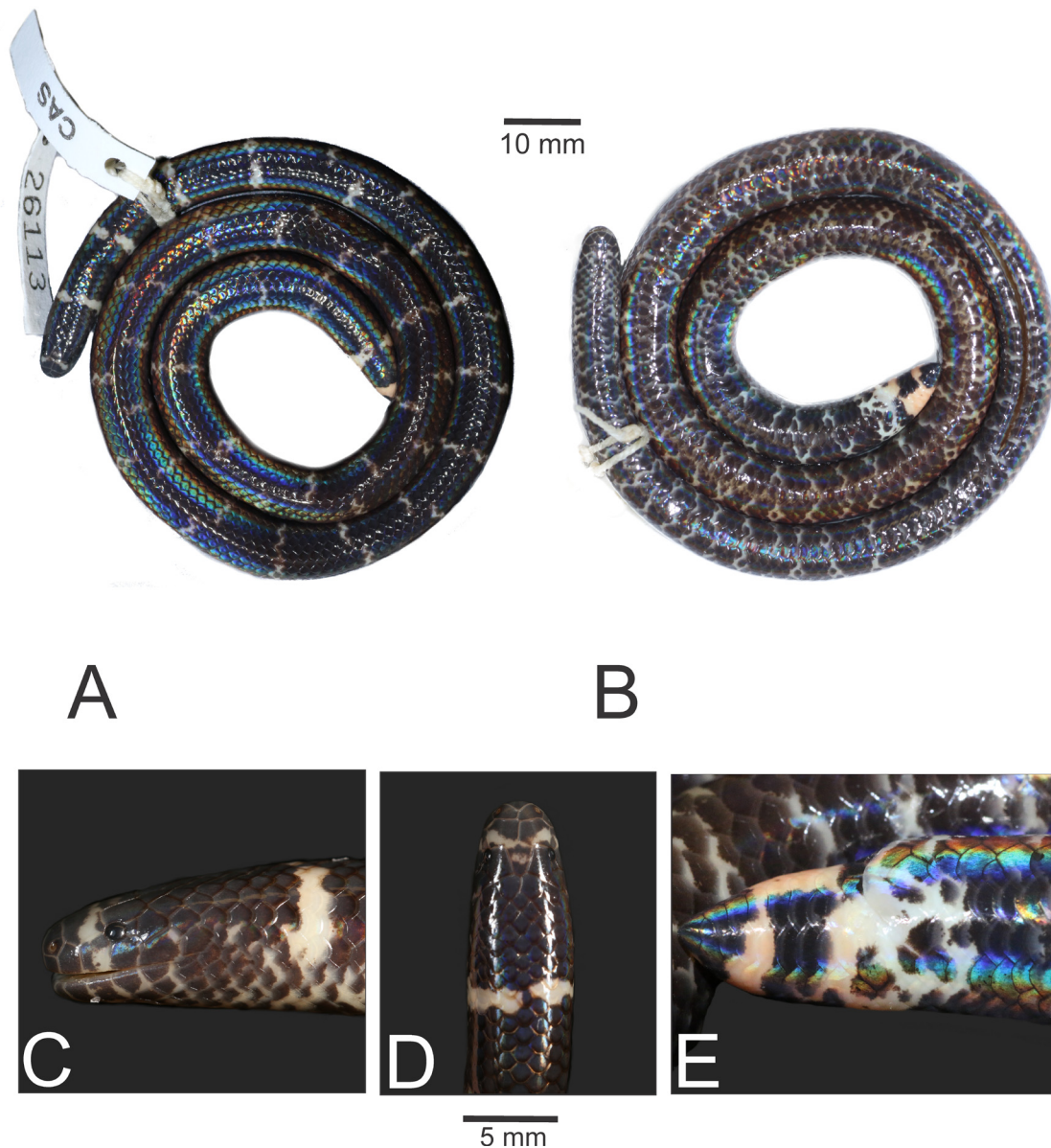


FIGURE 4. Holotype of *Cylindrophis slowinskii* sp. nov. (CAS 241554) from Kachin State, Myanmar in dorsal (A) and ventral (B) view. The head is shown in lateral (C) and dorsal (D) view, the tail in ventral view (E). The 10 mm and 5 mm scale bars are for panels A+B and C+D+E, respectively. Photos by Justin M. Bernstein.

Description of the holotype. *Metrics.* A relatively short body, 333 mm SVL + 7 mm TL = 340 mm TTL (336 mm in life), tail blunt and very short (2% of SVL); BD 7.4 mm (2.2% of SVL); head not wider than body, HL 7.4 mm (2.2% of SVL), HW 4.9 mm (66.2% of BD); snout rounded in dorsal and lateral views, relatively long, 2.7 times as long as diameter of the eye, SL 3.4 mm (45.9% of HL), SW 2.8 mm; a small eye, ED 0.9 mm (12.1% of HL); IOD 3.3 mm; IND 2.0 mm; rostral height 1.2 mm, width 1.6 mm; paired nasals, length of a single nasal 1.1 mm, width 1.8 mm; paired prefrontals, length of a single prefrontal 1.6 mm, width 1.9 mm; frontal length 2.0 mm, width 1.6 mm; paired parietals, length of a single parietal 1.7 mm, width 1.5 mm; paired supraoculars, length of a single supraocular 1.9 mm, width 1.5 mm; postocular length 0.7 mm, height 0.8 mm; anterior temporal length 1.2 mm, height 1.6 mm, upper posterior temporal length 1.5 mm, height 0.8 mm; mental height 0.7 mm, width 1.3 mm; two pairs of chin shields, anterior chin shield length 1.8 mm, width 1.6 mm, posterior chin shield length 1.5 mm, width 1.0 mm; mental groove length 3.1 mm. The holotype weighed 12.5 g in life.

Scale counts and qualitative pholidosis. 17-19-17 dorsal scale rows; 220 ventrals; eight subcaudals + terminal spine; postnasal, loreal, preocular, and subocular absent; temporal formula 1 + 2; six supralabials, 3rd and 4th contacting the eye; six infralabials; cloacal scute divided; scales on head, trunk, and tail smooth; apical pits absent.

Coloration and pattern in preservative (after 11 years in ethanol). Dorsal surface Jet Black (300), with 31 transverse Pale Buff (1) to Chamois (84) blotches from nape to tail; blotches may be complete or medially interrupted, if interrupted both parts may alternate; first four blotches approximately one scale wide, blotches covering medial part of the body narrowest, covering scale margins only, last dorsal blotch broad, dorsal blotch on the tail Pale Pinkish Buff (3), merging onto the subcaudal region, tail tip dark; a pale bar runs from the border of the prefrontals with the frontal towards the upper margin of the 3rd supralabials, leaving the margin of the prefrontals with the supraoculars dark; posterior half of 3rd supralabial with a pale blotch, slightly connected with the prefrontal bar on the right side only; margin of 5th labial pale; frontal with a pale median blotch; scales on ventral surface of head almost always with pale margins (except for the mental and 1st labials); ground color of venter as for dorsum; 69 pale and very narrow diffuse ventral blotches present, branching, and therefore creating the impression of a diffuse reticulum.

Variation. The paratype (USNM 590030) differs from the holotype (CAS 241554) as follows (metrics of head scales excluded): 297 mm SVL + 8 mm TL = 305 mm TTL; 216 ventrals; 65 pale ventral blotches. In terms of color pattern, the paratype is very similar to the holotype. The bar on the prefrontals is not in contact with the pale color of the supralabials on both sides. All supralabials (except for the 1st and 2nd on the right side) have pale markings.

Comparisons with other species. *Cylindrophis slowinskii* ($n = 2$; characters listed in parentheses below), yet alone differs from all congeners by a combination of unique scalation characters, including 17-19-17 dorsal scale rows, a high number of ventrals (216–220), and eight subcaudals. *Cylindrophis aruensis* possesses 23 (19) dorsal scale rows at midbody, 173–182 (216–220) ventrals, six or seven (eight) subcaudals, 44 (65–69) pale ventral blotches, and pale stripes on the head, running from the nasals onto the posterior temporals (a pale bar on the prefrontals, no pale markings posterior to the eyes) (Boulenger 1920; McDowell 1975; Lang 2013; Amarasinghe *et al.* 2015). *Cylindrophis boulengeri* ($n = 4$) possesses 195–204 (216–220) ventrals, five or six (eight) subcaudals, 48–54 (65–69) pale ventral blotches, and pale wavelike markings along the labials that may run onto the prefrontals but are never connected to form a bar (no wavelike markings on the labials, a pale bar on the prefrontals). *Cylindrophis burmanus* ($n = 20$) possesses 190–220² [median = 209] (216–220; median = 218) ventrals, four to seven (eight) subcaudals, and 29–61 (65–69) pale ventral blotches. *Cylindrophis engkariensis* ($n = 1$) possesses 17 (19) dorsal scale rows at midbody, 230 (216–220) ventrals, five (eight) subcaudals, rugose (smooth) dorsals on the tail, 45 (65–69) pale ventral blotches, a dorsal pattern of two paravertebral rows of dark spots (transverse pale dorsolateral blotches), a pale postocular streak (no postocular streak), and dark prefrontals (a pale bar on the prefrontals). *Cylindrophis isolepis* ($n = 2$) is a relatively large species with a maximum SVL of 500 mm (333 mm; see Amarasinghe *et al.* 2015) and possesses 21 (19) dorsal scale rows at midbody, five or six (eight) subcaudals, nasals that are separated by the rostral (nasals in contact), 27–35 (65–69) pale ventral blotches that are reddish in living specimens, and no markings on the dorsal surface of the head (a pale bar on the prefrontals). *Cylindrophis jodiae* ($n = 90$) is a relatively large species with a maximum SVL of 786 mm (333 mm) and possesses 21 (19) dorsal scale rows at midbody, 177–201 (216–220) ventrals, five to seven (eight) subcaudals, 30–55 (65–69) pale ventral blotches, pale wavelike markings along the labials or heavily blotched labials (labials without prominent pattern), and dark prefrontals (a pale bar on the prefrontals). *Cylindrophis lineatus* ($n = 1$) is a relatively large species with a maximum SVL of 713 mm (333 mm) and possesses 21 (19) dorsal scale rows at midbody, 210–218 (216–220) ventrals, 31 (65–69) pale ventral blotches, a dorsal pattern of stripes that are red and black in living specimens (transverse pale dorsolateral blotches), a pale dorsal surface of head and tail, both being red in living specimens (no extensively colored dorsal surface of head and tail), and dark markings on the temporals and parietals (head uniformly dark with a pale bar on the prefrontals) (see also Amarasinghe *et al.* 2015). *Cylindrophis maculatus* ($n = 36$) possesses 27–49 (65–69) pale ventral blotches, a dorsal pattern of large and roundish, paired pale blotches separated by a narrow dark network, with the blotches being reddish-brown in living specimens (very narrow and widely separated transverse pale blotches on a dark dorsum), and prefrontals that lack a pale bar (a pale bar on the prefrontals). *Cylindrophis melanotus* ($n = 39$) is a relatively large species with a maximum SVL of 678 mm (333 mm) and possesses 228–268 (216–220) ventrals and 38–63 dark blotches on a pale-colored venter that is reddish or white in living specimens (65–69 very narrow diffuse pale blotches on a dark-colored venter). *Cylindrophis opisthorhodus* ($n = 6$) possesses 23 (19) dorsal scale rows at midbody, 185–210 (216–220) ventrals, four to seven (eight) subcaudals, 46–61 (65–69) pale ventral blotches, a pale dorsum with dark speckles forming two paravertebral rows and occasionally a discontinuous vertebral line

2 Among the 20 specimens of *Cylindrophis burmanus* we have examined, only a single specimen (BMNH 1908.6.23.3; paralectotype) has a ventral count (= 220) that falls within the range of *C. slowinskii*. The second highest count in our specimens was 214 ventrals. BMNH 1925.12.22.4 (paralectotype) was stated to possess 225 ventrals by Amarasinghe *et al.* (2015), but has only 214 ventrals (see also Wall (1926), who provided a count of 212 ventrals for this specimen).

(very narrow transverse pale blotches on a dark dorsum), and dark speckles on the pale dorsal surface of the head (head uniformly dark with a pale bar on the prefrontals) (see also Amarasinghe *et al.* 2015). *Cylindrophis osheai* ($n = 3$) possesses 224–226 (216–220) ventrals, 25–28 dark ventral blotches, aligned to form a broad wavy stripe along most of the otherwise pale underside (venter almost entirely black with 65–69 diffuse pale blotches), and no pale bar on the prefrontals (a pale bar on the prefrontals). *Cylindrophis ruffus* ($n = 244$), as defined by Kieckbusch *et al.* (2016), is a relatively large species with a maximum SVL of 857 mm (333 mm) and possesses five to seven (eight) subcaudals and 33–66 (65–69) pale ventral blotches. *Cylindrophis subocularis* ($n = 9$) possesses a subocular scale (no subocular scale), 190–196 (216–220) ventrals, six or seven (eight) subcaudals, and 40–48 pale ventral blotches that are usually as broad as the dark blotches (65–69 very narrow diffuse pale blotches that are widely separated by dark color). *Cylindrophis yamdena* ($n = 5$) is a relatively large species with a maximum SVL of 671 mm (333 mm) and possesses 21 (19) dorsal scale rows at midbody, 183–195 (216–220) ventrals, and no pattern on the dorsal surface of the body and no ventral blotches in adults (a pale bar on the prefrontals, pale blotches on the dorsum and venter).

Geographic distribution, habitat, and natural history. *Cylindrophis slowinskii* might have a more isolated distribution with regard to the other species of the genus and is only known from its type locality in northern Myanmar near Indawgyi Lake (Fig. 5). As the type specimens were found under logs in forests, *C. slowinskii* is assumed to be a secretive forest-dweller with a semi-fossorial lifestyle, as typical for most species of Asian pipesnakes. Due to the limited number of available specimens, the complete geographic distribution and biogeographic history of *C. slowinskii* is uncertain. However, the physiography of northern Myanmar, especially Kachin State, is characterized by scattered stretches of mountains that may act as dispersal and gene flow barriers for species of *Cylindrophis*. Additionally, major rivers such as the Irrawaddy River, and its largest tributary, the Chindwin River, may also largely act as barriers to gene flow. These biogeographic hypotheses, however, can only be tested using more complex analyses, divergence dating estimates, and a more complete sampling of *C. slowinskii* and the closely related *C. burmanus* (see “Remarks on the type series”, below).

Etymology. The specific epithet is a patronym to honour the American herpetologist Joseph Bruno “Joe” Slowinski (1962–2001) and his pioneering work on herpetofauna, especially in Myanmar. In 2001, Joe led a multidisciplinary expedition to Kachin State, Myanmar, the type locality of the new species described herein. For biographical details see Donnelly & Crother (2003) and James (2008).

Suggested common names. Slowinski’s pipesnake (English), Slowinski-Walzenschlange (German),

Remarks on the type series of the closely allied *Cylindrophis burmanus*. The Asian pipesnake *Cylindrophis burmanus* was originally described as a subspecies of “*C. ruffus*” by Malcolm Arthur Smith (1875–1958) based on an unspecified number of vouchers. The distributional range of this new taxon was given as “Tenasserim and Burma as far North as Myitkyina” (today’s Myanmar) without the restriction of a precise type locality. Amarasinghe *et al.* (2015) designated a lectotype (BMNH 1940.3.3.1) from “the presumed type series” (i.e., the syntypes) and listed five additional specimens housed in the collection of the BMNH that they designated as paralectotypes (see also Kieckbusch *et al.*, 2016).

Although Smith (1943; probably based on Wall (1925)) listed Tenasserim, today’s Tanintharyi Region, as the southern limit for the distribution of *Cylindrophis burmanus*, we were not able to confirm the presence of this species south of the Irrawaddy Delta based on voucher specimens. A single, more recent record (JMB & SM, pers. obs.) for an Asian pipesnake from the Tanintharyi Region can be identified as *C. jodiae*.

The lectotype (definitive type) of *Cylindrophis burmanus* was collected from Yangon (today’s Rangoon; see Fig. 5), the most famous port in the Irrawaddy Delta, and capital of all Myanmar (Burma) from 1886 to 2005. Based on its collection locality, this specimen likely belongs to the population that is found in the Irrawaddy Delta and east of the Irrawaddy River and that we identified using molecular genetic methods (Fig. 5). Among all the vouchers of *C. burmanus* we have examined ($n = 20$; see “Appendix 2”), only a single specimen (BMNH 1925.12.22.4; paralectotype) was collected from the temperate hilly zone in northern Myanmar that is enclosed by the Chindwin and Irrawaddy Rivers (see Wall (1926)), and only ~50 km away from the type locality of *C. slowinskii*, the latter located at the western side of Indawgyi Lake. Indawgyi Lake is enclosed by hilly country as well, with the Loipyet Range acting as a potential barrier to the east. The collection locality of BMNH 1925.12.22.4 (Sahmaw; part of the Myitkyina District when the specimen was collected) is located in the lowlands east of this mountain range. Based on morphology (214 ventrals, seven subcaudals, 52 pale ventral blotches) this paralectotype identifies as *C. burmanus*.

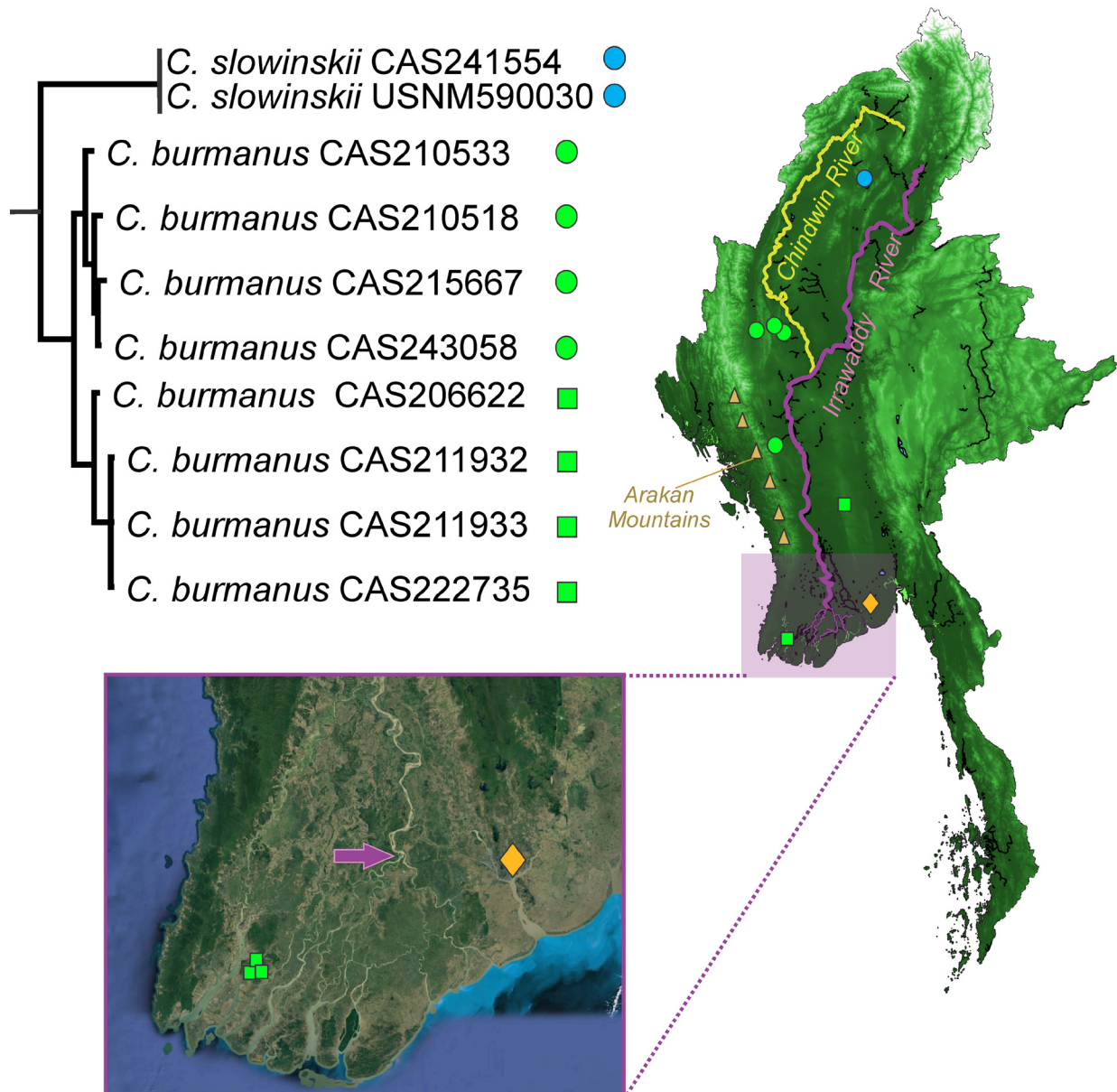


FIGURE 5. Collection localities of the specimens of *Cylindrophis burmanus* (green symbols) and *Cylindrophis slowinskii* (blue circles) used for our molecular analyses. Symbols in the maps correspond to the ones used for the different clades in the accompanying tree. Major clades of *C. burmanus* are distinguished by green circles and squares. The orange diamond represents the collection locality of the lectotype of *C. burmanus* (BMNH 1940.3.3.1 from Yangon (Rangoon); specimen not included in phylogenetic analysis). In the country map of Myanmar, higher elevations appear in brighter and lower elevations in darker shades of green; the Chindwin and Irrawaddy Rivers are marked in yellow and purple, respectively; the Arakan Mountain range is highlighted with yellow triangles. The satellite map is a detailed magnification of the area shaded purple in the country map. An arrow in the inset map indicates where the Irrawaddy splits into its delta. Note that the three green squares in the satellite image represent near-overlapping localities of the southern population of *C. burmanus* that appear as a single symbol in the larger map. Satellite map obtained from Google Earth v7.3.2.5776: Image © 2019 TerraMetrics Data SIO, NOAA, U.S. Navy, NGA, GEBCO. Map prepared by Justin M. Bernstein.

Discussion

In this study we provide the first phylogeny for the genus *Cylindrophis* that includes a sampling of more than two species. Our concatenated and species trees recovered seven species with strong support, including one new taxon. It is worth mentioning that although some species of *Cylindrophis* share the same number of dorsal scale rows at

midbody, a character useful for species identification, the molecular phylogeny does not support the morphological groupings based on this character proposed by Amarasinghe *et al.* (2015). This is evidenced by the sister relationship of *C. boulengeri* (19 dorsals) and *C. yamdena* (21 dorsals) with *C. isolepis* (21 dorsal), which together form the sister group to *C. jodiae* (21 dorsal), a major clade to which *C. burmanus* (19 dorsals) is the sister species (Fig. 2).

Although no formal biogeographic analyses were performed in this study, there is strong support for several mainland Southeast Asian clades, and a Wallacean clade that includes *C. boulengeri*, *C. isolepis*, and *C. yamdena*. Based on our results, *Cylindrophis* originated in South or Mainland Southeast Asia and expanded its range southward into the Indonesian Archipelago. However, the biogeographic implications on the root of our tree are limited, as species of *Anomochilus* were not included in our analysis. Here, we used the South Asian uropeltids (and *Python*) as outgroups. Previous phylogenies have shown *Cylindrophis* to be more closely related to *Anomochilus* than to uropeltids (Pyron *et al.* 2013a; Zheng & Wiens 2016), but fresh tissues of this taxon were not available. We cannot link any particular biogeographic event to the diversification of *Cylindrophis*. Pleistocene sea level fluctuations have occurred at least 50 times within the last 250000 years and allowed for faunal exchanges via land bridges on what is now discontinuous habitat (Voris 2000; Woodruff 2010; Bruyn *et al.* 2014). The age of cylindrophids, based on other studies (Pyron 2016; Harrington & Reeder 2017; Burbrink *et al.* 2020), far precedes these Pleistocene sea-level fluctuations, and it is likely that these fluctuations had an impact in producing the disjunct distribution of cylindrophids we see today. Additionally, *Cylindrophis* that only occur in the Wallacean Archipelago, which remained isolated through its geologic history (Voris 2000; Hall 2013), are hypothesized to have reached these islands by drift dispersal scenarios. Using a molecular clock based on squamate mitochondrial and nuclear genome studies (Zamudio & Green 1997: 0.47–1.32% my⁻¹; Eo & DeWoody 2010: 0.529% my⁻¹), the Wallacean clade of *Cylindrophis* may have diverged between 5.15–7.20 million years ago (mya) or 14.47–20.21 mya using the upper and lower limits of Zamudio & Green's (1997) estimates, respectively. Alternatively, using the estimates from Eo & DeWoody (2010), dates may range between 12.85–17.96 mya, overlapping with the Zamudio & Greene (1997) dating hypotheses. Using these estimates to date all cylindrophids would place a date of divergence for the entire group between 9.06–10.98, 25.47–30.83, or 22.63–27.39 mya for Zamudio & Green's (1977) upper and lower, and Eo & DeWoody's (2010) evolutionary rates, respectively. Assumptions are made when using molecular clocks, many of which are often broken (e.g., rate constancy). Hence, we provide these dates and biogeographic scenarios as hypotheses for future studies, which should include additional analyses, increased sampling of ingroup and outgroups, calibrations for divergence dating, and larger molecular datasets with supplementary geographic and distribution data.

Cylindrophis burmanus shows genetic structure between the eastern side of the Irrawaddy + the Irrawaddy Delta and the western side of this river (Fig. 5). The Irrawaddy River is the largest river in Myanmar, bisecting the country, running from north to south. Although this river can be several miles wide in the plains of central Myanmar during the rainy season, it terminates at the Irrawaddy Delta covering an area of over 1000 square miles, where it splinters into smaller tributaries that may be less than one hundred meters wide. Thus, there is a possibility that populations of *C. burmanus* to the east of the Irrawaddy River and at its mouth may have a more recent history with one another via movement through areas of the Irrawaddy Delta with smaller tributaries (Fig. 5). The wider parts of this river system in central and northern Myanmar might act as barriers to gene flow between the eastern and western populations of *C. burmanus*. Additionally, the north-south oriented Arakan (Rakhine Yoma) Mountains of western Myanmar run parallel to the Irrawaddy River. The lowland area between the middle and southern Arakan Mountains and the Irrawaddy River becomes much narrower (~20–30 km wide) compared to the north, and may also limit gene flow between eastern and western *C. burmanus* (Fig. 5). The rivers of Indochina, including the Irrawaddy River, and their changes in structure through time have resulted in geographically-related signatures in molecular data of other reptiles inhabiting these systems and areas (e.g., homalopsid snakes, Lukoschek *et al.* 2011; *Draco* lizards, Klabacka *et al.* 2020). Similar to our discussion above on the evolutionary origins of the Cylindrophidae, we emphasize that these hypotheses provide starting points for future investigations of Myanmar *Cylindrophis* using more data.

Specimens of *Cylindrophis slowinskii* from Indawgyi Lake, Kachin State, Myanmar (CAS 241554, USNM 590030) were found to be the sister to *C. burmanus* and may be largely geographically isolated from this species by the Irrawaddy River to the east and its largest tributary, the Chindwin River, to the south and west. Additionally, the collection locality of the new species lies within the Kachin Hills in the northern part of the state, which are characterized by multiple mountain chains running in a north-to-south direction, including the Loipyet Hills and adjacent

mountains to the east of Indawgyi Lake. Although more geological data, samples of *Cylindrophis*, and distribution data are required to draw more accurate biogeographic conclusions, the Kachin population may have historically been cut off from the rest of *Cylindrophis* after the orogenic events that created the Kachin Hills and the formation of the Irrawaddy and Chindwin Rivers. It is worth noting that for samples of *C. burmanus* that are separated by 200 km across the Irrawaddy River, or which occur on the same side over 400 km apart, ND2 distances are only 1.16–1.74%. Specimens of *C. burmanus* from the most southern localities in our sampling distribution (CAS 211932, 211933, 222735 from the Irrawaddy Delta) are separated from *C. slowinskii* by just over 1000 km. Despite this, the ND2 distances of the specimens of *C. slowinskii* are, considering our sampling and distances, greater than would be expected in the simplest isolation by distance scenario (expected: 2.85–4.28%; observed: 5.52–6.18%). Thus, the data presented here support the distinctiveness of the Indawgyi Lake population as a species-level lineage of *Cylindrophis*.

Field expeditions and studies have contributed to the ongoing descriptions of herpetofaunal species from Myanmar, with particular focus on karst ecosystems and caves (e.g., *Cyrtodactylus*; Bauer 2003; Grismer *et al.* 2018; 2019a). The Irrawaddy Basin and Kachin State in particular may represent areas of significant herpetofaunal biodiversity and/or endemism, as evidenced by the description of *Tylotriton newts* (Grismer *et al.* 2019b), *Cyrtodactylus* geckos, and colubrid (Grismer *et al.* 2019a) and homalopsid snakes (Quah *et al.* 2017). As the number of reptiles and amphibians described from Myanmar increases, as well as the recognition of broad versus narrow-ranged taxa, continued research on the evolutionary histories, population structures, and conservation statuses of these fauna are encouraged.

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APPENDIX 1. Voucher information for phylogeny. Specimens for which molecular data were obtained in this study. For each specimen the institutional/museum voucher ID and collection locality are provided. Names applied for species of *Cylindrophis* are based on a detailed morphological examination of museum vouchers or unambiguous identification of specimens in the field by ourselves. Only for applying the names “*C. jodiae*” to the specimens from Vietnam and “*C. maculatus*” to the voucher from Sri Lanka did we use a geographic criterion, as these species are the only Asian pipesnakes known from the specified political units (see our “Appendix 2”). Museum acronyms follow Sabaj (2016).

Species	Voucher ID	Locality	Genbank Accn. (16S ND2 R35)
<i>Python molurus</i>	NA	NA	HM581978 HM581978 JN703057
<i>Rhinophis philippinus</i>	LSUMZ herpsTissues:5785	Sri Lanka	MT436264 MT434899 MT434900
<i>Rhinophis phillipsi</i>	LSUMZ herpsTissues:5788	Sri Lanka	MT436265 MT434898 MT434901
<i>Cylindrophis boulengeri</i>	MVZ Herp:292817	Indonesia: Maluku Province: Wetar: vicinity of Desa Ilwaki	MT366623 MT366650 MT366672
<i>Cylindrophis boulengeri</i>	MVZ Herp:292818	Indonesia: Maluku Province: Wetar: vicinity of Desa Ilwaki	MT366624 MT366649 MT366673
<i>Cylindrophis boulengeri</i>	MCZ Herp-R:192946	Timor-Leste: Manufahi District: Suco Soibada, Aldeia, 3 km E Soibada, Sahen River	MT366621 MT366652 MT366667
<i>Cylindrophis boulengeri</i>	USNM 581170	Timor-Leste: Aileu District: Suco Fatisi, Laulara, 8 km S Comoro River bridge, confluence Comoro-Bemos Rivers	MT366622 MT366651 MT366671
<i>Cylindrophis burmanus</i>	CAS Herp:206622	Myanmar: Bago Region: Bago Yoma	MT366611 MT366638 MT366659
<i>Cylindrophis burmanus</i>	CAS Herp:210518	Myanmar: Sagaing Region: Kabaing Village	MT366616 MT366640 MT366660
<i>Cylindrophis burmanus</i>	CAS Herp:210533	Myanmar: Magway Region: Minbu District: Padan Village	MT366615 MT366639 MT366661
<i>Cylindrophis burmanus</i>	CAS Herp:211932	Myanmar: Ayeyarwady Region: Mwe Hauk Village	MT366612 MT366643 MT366662
<i>Cylindrophis burmanus</i>	CAS Herp:211933	Myanmar: Ayeyarwady Region: Mwe Hauk Village	MT366613 MT366633 MT366663
<i>Cylindrophis burmanus</i>	CAS Herp:215667	Myanmar: Sagaing Region: Monywa District: A.K. Park, Pya Bon, Mountain Camp	MT366617 MT366641 MT366664

.....continued on the next page

APPENDIX 1. (Continued)

Species	Voucher ID	Locality	Genbank Accn. (16S ND2 R35)
<i>Cylindrophis burmanus</i>	CAS Herp:222735	Myanmar: Ayeyarwady Region: Mwe Hauk Village	MT366614 MT366634 MT366676
<i>Cylindrophis burmanus</i>	CAS Herp:243058	Myanmar: Magway Region: Gangaw District: Gangaw Township, Po Oo Village	MT366618 MT366642 MT366666
<i>Cylindrophis isolepis</i>	MVZ Herp:267658	Indonesia: South Sulawesi Province: Jampea	MT366625 MT366644 MT366668
<i>Cylindrophis isolepis</i>	MVZ Herp:267659	Indonesia: South Sulawesi Province: Jampea, vicinity of Makmanasa	MT366626 MT366645 MT366669
<i>Cylindrophis isolepis</i>	MVZ Herp:267660	Indonesia: South Sulawesi Province: Jampea, Telkom Tower Rd.	MT366627 MT366646 MT366670
<i>Cylindrophis jodiae</i>	ROM 37936	Vietnam: Can Tho Province: Phung Hiep	MT366619 MT366648 MT366674
<i>Cylindrophis jodiae</i>	ROM 37937	Vietnam: Can Tho Province: Phung Hiep	MT366620 MT366647 MT366675
<i>Cylindrophis maculatus</i>	LSUMZ herpsTissue:5798	Sri Lanka	MT366609 MT366635 MT366658
<i>Cylindrophis sp. nov.</i>	CAS Herp:241554 (CAS-MHS 26113), holotype	Myanmar: Kachin State: Myitkyina District: Moenyin Township, Indawgyi Lake Wildlife Sanctuary, vicinity of Lwe Mon Village	MT366610 MT366637 MT366665
<i>Cylindrophis sp. nov.</i>	USNM 590030 (CAS-MHS 26114), paratype	Myanmar: Kachin State: Myitkyina District, Moenyin Township, Indawgyi Lake Wildlife Sanctuary, vicinity of Lwe Mon village	NA MT366636 NA
<i>Cylindrophis yamdena</i>	WAM R:109947, paratype	Indonesia: Maluku Province: Tanimbar Islands, Yamdena, Latdalam	MT366628 MT366654 MT366677
<i>Cylindrophis yamdena</i>	WAM R:109971, paratype	Indonesia: Maluku Province: Tanimbar Islands, Yamdena, Latdalam	MT366629 MT366657 MT366678
<i>Cylindrophis yamdena</i>	WAM R:109972, paratype	Indonesia: Maluku Province: Tanimbar Islands, Yamdena, Latdalam	MT366630 MT366656 MT366679
<i>Cylindrophis yamdena</i>	WAM R:109980, paratype	Indonesia: Maluku Province: Tanimbar Islands, Yamdena, Latdalam	MT366631 MT366655 MT366680
<i>Cylindrophis yamdena</i>	WAM R:112252, holotype	Indonesia: Maluku Province: Tanimbar Islands, Yamdena, Latdalam	MT366632 MT366653 MT366681

APPENDIX 2. Comparative material used for morphological analyses

Museum acronyms follow Sabaj (2016). Prefixes referencing herpetological/reptile collections excluded. Island localities underlined.

- Cylindrophis boulengeri*.—**Indonesia**: Maluku Province: Wetar: RMNH 5529A.168, 5529B.169; Ilwaki: SMF 16996 (holotype). **Timor-Leste**: Timor: Manufahi District: Suco Soibada, Aldeia, 3 km E Soibada, Sahen River: MCZ 192946.
- Cylindrophis burmanus*.—**Myanmar**: without precise locality data: BMNH 1908.6.23.3 (paralectotype). Ayeyarwady Region: Mwe Hauk Village: CAS 211932–211933, 222735. Bago Region: Bago Yoma: CAS 206622. Kachin State: Bhamo: NMBA 479, NMW 21552.3–4, ZMB 11619, ZMH 6256; Myitkyina District, Sahmaw: BMNH 1925.12.22.4 (paralectotype). Magway Region: Minbu District, Padan Village: CAS 210533, Ngape Township, along Padan Village to Ngape Road: CAS 231144; Gangaw District, Gangaw Township, Po Oo Village: CAS 243058. Mandalay Region: Pyn Oo Lwin Distrikt, The Bake Kyin Township, Shwe U Daung Wildlife Sanctuary: CAS 216130, 216155. Rakhine State: “Aracan”: MTKD 14867. Sagaing Region: Kabaing Village: CAS 210518; Monywa District, A.K. Park, Pya Bon, Mountain Camp: CAS 215667. Yangon-Division: Rangoon: BMNH 1940.3.3.1 (lectotype). **Thailand** (questionable locality): NMW 21556.5.
- Cylindrophis engkariensis*.—**Malaysia**: Sarawak: Borneo: Second Division: Lubok Antu District, Lanjak-Entimau, headwaters of the Engkari River, Nanga Segerak: ZRC 2.3398 (holotype).
- Cylindrophis isolepis*.—**Indonesia**: South Sulawesi Province: Jampea: RMNH 11269A.171, 11269B.172.
- Cylindrophis jodiae*.—**China**: Hong Kong: MCZ 5489. **Malaysia**: Kedah State: NMW 39624.2; Penang: NMW 21570.1–2, 21570.4. **Myanmar**: Tanintharyi Region: Kawthaung District, Khamaukyi Township: CAS 247469. **Thailand**: without precise locality data: NMW 21556.4, ZMH 9798–99, 9801–02, MTKD 24126–27, SMF 16987, 16991, ZMB 5261, 30205. “Don Pia Fei Mountains”: NMW 21565.1–6, 21566.1–6. Bangkok Province: Bangkok: MHNG 1335.17, NMW 21561, 21562.1–4, 21563.1–2, 21564.1–11, RMNH 11254.111, 25717, SMF 58675, 58697, 61903, 64838, ZMB 4394, 4545, 58428, ZMH 9794, ZRC 2.4583. Chiang Mai Province: MTKD 39216. Khon Kaen Province: Muang Pou Vieng (Pu Wiang): NMW 21567.1–2. Nakhon Sawan Province: Lat Yao District, 25 km west of Nakhon Sawan: ZMA 16679. Phang Nga Province: Khaolak-Luk National Park: ZMB 55188. Phetchaburi Province: Puek Tian: NMW 21569.1–3. Saraburi Province: Saraburi: MHNG 1471.30, MHNG 1530.9. Ubon Ratchathani Province: Dangrek Mountains: Phu Khi (Pu-Kin, Don-Rek): NMW 21556.2, 21569.1–3. **Vietnam**: without precise locality data: MCZ 5967 (three specimens), NMBE 1015768–69. “South Vietnam”: MHNG 1325.30, 1551.18–20. Ho-Chi-Minh Province: Ho-Chi-Minh City: NMBE 1015764–66, ZMB 31123, 50774. Tay Ninh Province: MCZ 4076 (two specimens). Thua Thien-Hue Province: Hue: MCZ 25158.
- Cylindrophis lineatus*.—**Singapore** (in error for Borneo): AMNH 12872.
- Cylindrophis maculatus*.—**Sri Lanka** (occasionally labeled “Ceylon”): without precise locality data: AMNH 3046, MHNG 762.65, 1199.44, 2745.34, MTKD 14873–76, NMW 21574.1–5, 21575.1–2, RMNH 160–63, SMF 16995, ZMB 1456, 18550, 18551.A–B, 24125, 49460, 77698, ZMH 9785, 9792, 9795–96. Central Province: Kandy District, Peradeniya: ZMB 31506. Sabaragamuwa Province: Kitulgala: MHNG 2156.29; Ratnapura: MHNG 2156.30. Western Province: near Colombo: MHNG 1199.30–32.
- Cylindrophis melanotus*.—**Indonesia**: North Maluku Province: Bacan: SMF 16975; Halmahera: ZMB 34313 (holotype of *Cylindrophis heinrichi* Ahl, 1933). Sula/Sanana: RMNH 5104.17. Southeast Sulawesi Province: Buton: Bau Bau: RMNH 11265.87; Kolaka: RMNH 11276.89. Sulawesi (occasionally labeled “Celebes”): without precise locality data: RMNH 17.83–84, 17.86, ZMA 11459.120, ZMB 1450, 4049 (type ? of *Tortrix rufa* Var. *Celebica* Schlegel, 1844). “North Sulawesi”: RMNH 5461.34–40, ZMA 11451.112–115. Central Sulawesi Province: Poso: ZMA 11453.117–19; Lake Wawontoa: ZMB 62929. North Sulawesi Province: Lake Moat: ZMB 50020; Manado: RMNH 19.82, 173.18B, 174.18A, 5459.41–42. South Sulawesi Province: Lake Tempe: ZMA 11464.116; Makale: RMNH 11274.88; “Patmmang” (possibly Ujung Pandang, today’s Makassar): NMW 21571.1–3. **Philippines** (in error): NMW 21553.1–2.
- Cylindrophis opisthorhodus*.—**Indonesia**: East Nusa Tenggara Province: Flores: SMF 23301, ZMB 33787. West Nusa Tenggara Province: Lombok: SMF 23299, ZMA 12135, 14082. Sumbawa: SMF 23300.
- Cylindrophis osheai*.—**Indonesia**: Maluku Province: Central Maluku Regency, Boano: RMNH 5460 (holotype), 47930 (paralectotype). Sulawesi (in error): RMNH 11262.
- Cylindrophis ruffus*.—**Indonesia**: without precise locality data: ZMB 1454, ZMH 9749, 9786, 9793, 9797. Bangka-Belitung Islands Province: Bangka: ZMA 10487, 23067–8, 23070; Belitung: ZMA 11471.177–79. Borneo: without precise locality data: RMNH 2.20–22, SMF 16989, ZMB 80280. East coast of Borneo: RMNH 3924.15–17. Central Kalimantan Province: Buntok:

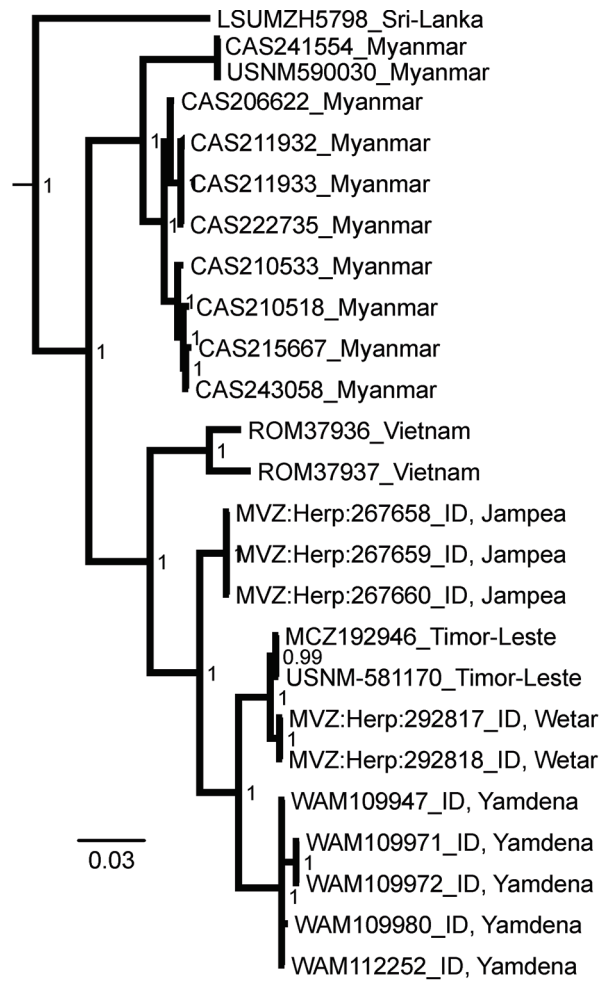
AMNH 71524; Muara Teweh: NMW 21554.3, 21554.6. *West Kalimantan Province*: Badau: NMW 21554.5; Landak: ZMA 10488, 23064; Pasir: MCZ 22651; Pontianak: RMNH 8234.2–3, 8264.5–6, 8264.8–11, 8264.14, SMF 16972. *Java*: without precise locality data: AMNH 3047, AMNH 24673, MHNG 2745.35–38, MTKD D5614–15, D7071, D14868–72, NMW 13835–36, 21551.2, 21558.1, 21558.3, 21558.6, 21558.8, 21559.2–14, NMBE 1015767, RMNH 1.65–68, 46, 47927–28, SMF 16976–78, 16981–86, 16990, ZMA 10495, 11452.145, 11467.151–53, 14460, ZMB 1455, 4908, 13129, 29696; ZMH 9790. *South Java*: ZMB 14443, 58433. *Central Java Province*: Kagok, Tegal: ZMA 11455.155; Pekalongan: ZMA 11468.157; Rembang: RMNH 11252.105; Semarang (Samarang): RMNH 5.60–61, ZMA 11461.158, ZMB 14351, 58429–30. *East Java Province*: without more precise locality data: RMNH 6928.52–55; Kediri: ZMA 11462.159, 11454.146–50; Malang (Malary): NMW 21558.4–5; Mount Arjuno (Ardjoeno): RMNH 11260.108–109, 11261.93–94; Surabaya (Surabaja, Soerabaja): RMNH 5791.49, 5999.58, 11251, 11252.105, 47933, ZMA 11457.154; Tengger Mountains: NMBA 471–73. *Jakarta Province*: Jakarta (occasionally labeled “Batavia”) MTKD D14750, NMBA 20441, RMNH 46. *West Java Province*: Bogor (occasionally labeled “Buitenzorg”): MCZ 172775–76, MCZ 172778, NMBA 462–70, RMNH 11256.110, 11258.92, 11272.98, SMF 16979–80, 16992–94, ZMB 20525; Cirebon (Cheribon): ZMA 11469.129–33; Indramayu (Indramajoe): RMNH 8956.56, 8972.62–64; Itjabe: MHNG 676.67; Sukabumi (Soekaboemi): ZMA 11456.156. *Sumatra*: without precise locality data: NMW 21550.5, ZMB 30847. *Aceh Province* (occasionally labeled “Atje”): NMW 21550.2; Dolok Merangir: MCZ 37717–37718; Lhokseumawe: SMF 22430. *North Sumatra Province*: “Dolokhili”: MHNG 1325.27–28; Langkat: MCZ 37752–37753, RMNH 6349.25–26, ZMB 30899, 30904; Tanah Merah, Bindjey Estate: ZMH 9751–52; “Sultanate of Deli”: NMW 21550.1, 21550.3–4, 21568.1–6, RMNH 6968.27–33, SMF 16973, 16988, ZMA 10490, 11463.125, 11465.127, 11466.124. “Sultanate of Serdang”: ZMA 11460.123. *Riau Province*: Rantau: RMNH 8185.13; Sungai Lala: ZMH 9787. *South Sumatra Province*: Palembang: NMBA 1015770–71; Tanjung Enim: ZMA 11458.126. **Malaysia**: *Johor State*: without precise locality data: AMNH 12873; Johor Bahru: ZRC 2.3009–10. *Penang State*: NMW 21570.3. *Kelantan State*: Kuala Lebir: ZRC 2.3011. *Malacca State*: NMW 21553.3. *Terengganu State*: 25 km west of Marang: ZMB 53293. *Sarawak*: Borneo: without precise locality data: NMBA 1015763; Baram: MHNG 748.28, NMW 21554.1, 21554.4; Kuching: MCZ 15245, SMF 71060; Sungai Tangap, Niah: AMNH 111923. **Singapore**: without precise locality data: MHNG 1325.29, NMW 21555.1–3, ZMH 9788–89, ZRC 2.3017–23, 2.6907, 2.6916; Bukit Timah Road: ZRC 2.3022; Sembawang: Naval Base: ZRC 2.3029; “University of Malaya, Dunearn Hostel”: MCZ 58895.

Cylindrophis subocularis.—No collection data: ZMB 53459. **Indonesia**: *Java*: without precise locality data: MNHN 1999.9021, NMW 21559.1 (paratype). *Central Java Province*: Purworejo Regency, Grabag: RMNH 8785 (holotype), 8958–59 (paratypes), 11257 (paratype), 11263 (paratype), 47929 (paratype).

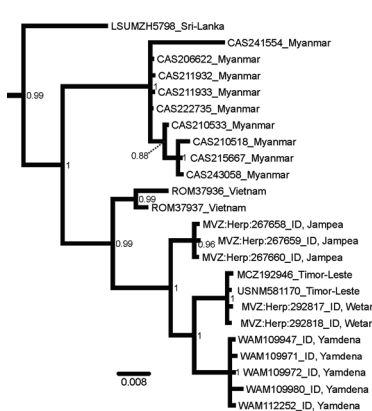
Cylindrophis yamdena.—**Indonesia**: *Maluku Province*: Tanimbar Islands/Yamdena: Latdalam: WAM 109947 (paratype), WAM 109971–72 (paratypes), WAM 109980 (paratype), WAM 12252 (holotype).

APPENDIX 3. Bayesian inference trees

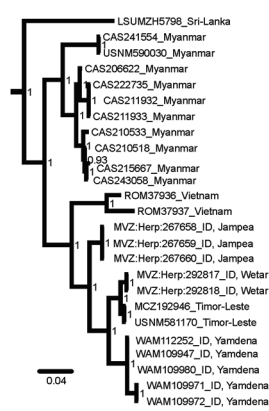
16S, ND2, R35, and concatenated Bayesian inference trees of *Cylindrophis* run for 100 million generations under selected models of molecular evolution (see “Materials and methods: Phylogenetic analyses”). Strongly supported nodes have Bayesian posterior probabilities (PP) of 0.95–1.



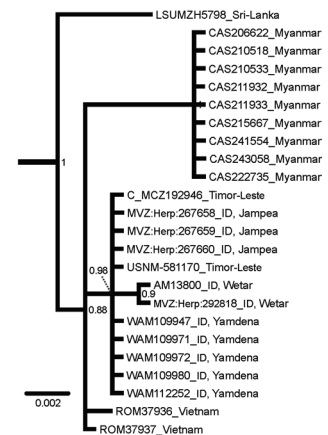
Concatenated



16s



ND2



R35