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Multilocus Phylogeny of *Lycodon* and the Taxonomic Revision of *Oligodon multizonatum*

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Abstract Classification of the Asian snake genera *Lycodon* and *Oligodon* has proven challenging. We conducted a molecular phylogenetic analysis to estimate the phylogenetic relationships in the genus of *Lycodon* and clarify the taxonomic status of *Oligodon multizonatum* using mitochondrial (cyt *b*, ND4) and nuclear (c-mos) genes. Phylogenetic trees estimated using Maximum Likelihood and Bayesian Inference indicated that *O. multizonatum* is actually a species of *Lycodon*. Comparing morphological data from *O. multizonatum* and its closest relatives also supported this conclusion. Our results imply that a thorough review of the evolutionary relationships in the genus of *Lycodon* is strong suggested.

Keywords Bayesian inference, China, classification, c-mos, cyt *b*, *Lycodon*, maximum likelihood, ND4, *Oligodon*

1. Introduction

The genus *Oligodon* Fitzinger, 1826 is widespread throughout central and tropical Asia, containing approximately 70 species (Green *et al*., 2010). Among them, 15 are known to occur in southern China (Zhao *et al*., 1998). Previous studies aimed at classifying the genus have been based on morphological data and yielded conflicting results (Wall, 1923; Pope, 1935; Smith, 1943; Leviton, 1963; Campden, 1969; Wallach and Bauer, 1996; David *et al*., 2008; Tillack and Günther, 2009). However, all of these studies were limited to a species group within this complex or a limited geographic area, and no study constructed a phylogenetic tree. Green *et al*. (2010) produced an updated checklist and key to the entire genus together with a phylogentic tree. The key and checklist were given in his thesis, and the phylogenetic data were later published (Green *et al*., 2010) and concluded that several uncertainties about the classification still exist. However, no study has included molecular data from *Oligodon multizonatum*.

Oligodon multizonatum was described by Zhao and Jiang (1981) from Luding County, Sichuan Province, southwest China. The species was classified as a member of the genus *Oligodon* on the basis of morphological characteristics including a short head that is not distinct from the neck, a large rostral scale that appears protruding when viewed from above, a cylindrical body with paired subcaudals and smooth dorsal scales (Zhao *et al*., 1998). There have been no published attempts to explore the taxonomic position of the species since it was first described, and no new specimens have been reported. Currently, *O. multizonatum* is considered an endemic

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species of China, only occurring in Sichuan and Gansu Provinces (Zhao, 2006). A snake specimen (specimen number KIZ01623, Figure 1) was collected in Luding County (29°55′12.58″ N, 102°13′31.07″ E) during a herpetological survey on July in 2009. A detailed comparison with the species description and the holotype specimen (CIB9964, Figure 2) suggested that it was conspecific with *O. multizonatum*.

Recent studies of snakes (Burbrink and Castoe, 2009; Huang *et al*., 2009), have shown that molecular data are powerful tools for identifying and understanding snake diversity. In view of this, the purpose of the present study was to use molecular methods to clairfy the systematic affinities of *O. multizonatum*. A prior study by us based on molecular analysis with more than three genes and 89 species of Colubridae showed that *O. multizonatum* clustered within *Lycodon*. Recently, the genus *Lycodon* was suggested to include species of the old genus *Dinodon* (Siler *et al*., 2013; Guo *et al*., 2013), suggesting that many relationship within the genus of *Lycodon* still need to be resolved. For example, Siler *et al*. (2013) suggested that currently recognized subspecies may

Figure 1 Photographs of a new *Oligodon multizonatum* specimen (specimen number KIZ01623) collected in Luding province. A–C: Whole body; D–F: Head in dorsal, ventral and right lateral views; G: Cloacal region in ventral view. Photo by Mian HOU.

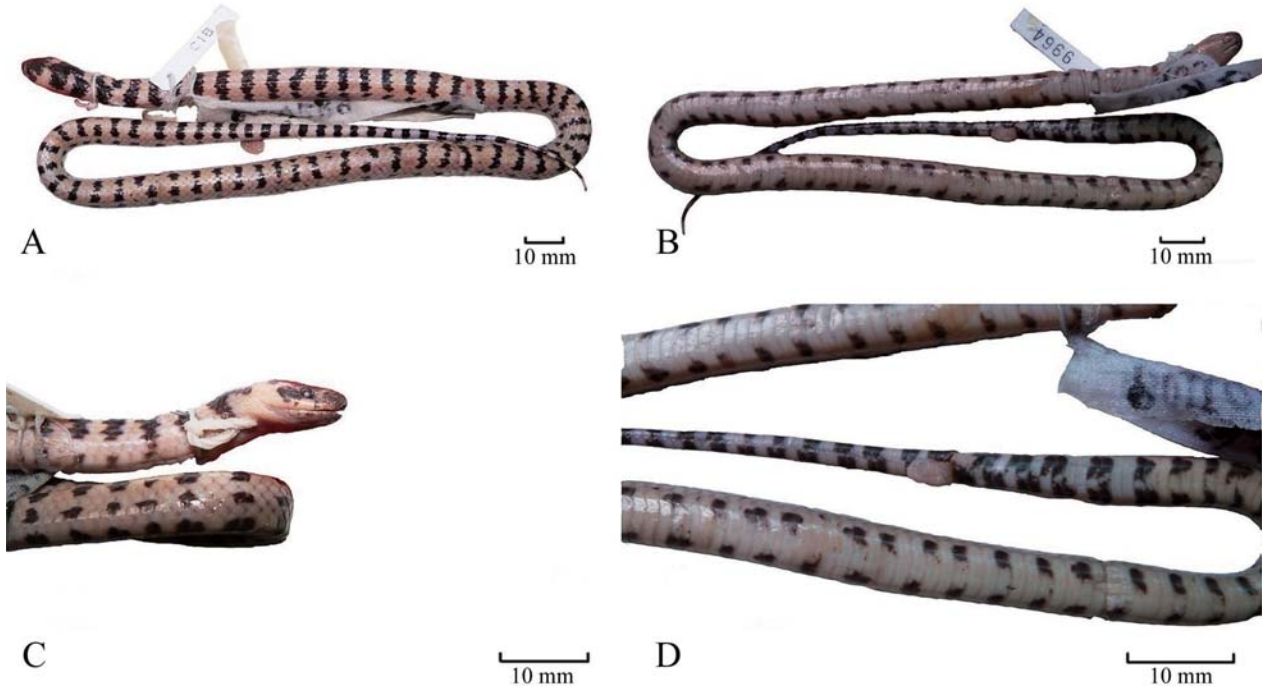

Figure 2 Photographs of the holotype specimen (CIB9964) of *Oligodon multizonatum*. A and B: Whole body; C: Ventral views; D: Cloacal region in ventral view and hemipenis. Photo by Juan LEI.

need to be elevated to species in further studies. Hence, in this study, we sampled species from both *Lycodon* and *Dinodon* in order to resolve these issues. For the convenience of our discussion, the historic taxonomic genera *Lycodon* and *Dinodon* continue to be used. Additionally, we also compared the morphological data of *O. multizonatum* with its closest relative as identified by molecular data analysis to verify this conclusion.

2. Materials and methods

2.1 Morphology Measurements, except body and tail lengths, were taken with a slide-caliper to the nearest 0.1 mm; all body lengths were made to the nearest millimeter using a tape measure. The number of ventral scales was counted according to Dowling (1951). Divided ventrals were counted as one. The first scale posterior to the cloaca was regarded as the first subcaudal, the terminal scute was not included in the number of subcaudals. The dorsal scale rows were counted at one head length behind the head, at midbody (i.e., at the level of the ventral plate corresponding to half the total number of ventrals), and at one head length before the vent. We considered sublabials being those shields that were completely below a supralabial. Values for paired head characters are given in left/right order.

The hemipenes of *O. multizonatum* and *L. liuchengchaoi* were compared. The method for preparing the hemipenes of preserved specimens followed Jiang (2010) and Pesantes (1994). Hemipenial descriptive terminology followed Dowling and Savage (1960), Branch (1986) and Zhang *et al*. (1984). Drawings were made with the aid of a stereomicroscope.

2.2 Taxon sampling Previous studies indicated that the systematics of the genera *Oligodon*, *Lycodon* and *Dinodon* are complex and possibly intertwined (Pope, 1935; Smith, 1943; Vogel and Brachtel, 2008; Green *et al*., 2010; Guo *et al*., 2013). Therefore, data from seven species in *Oligodon*, 16 species in *Lycodon* and one species in *Dinodon* from GenBank were used along with new data generated during the present study from *O. multizonatum*, *O. formosanus*, *O. chinensis*, *L. ruhstrati*, *L. liuchengchaoi*, *D. rufozonatum* and *D. flavozonatum* (Table 1). We also selected 10 taxa representing 10 genera of Colubrinae from GenBank. The choice of outgroup taxa (*Boa constrictor* and *Cylindrophis ruffus*) was based on Huang *et al*. (2009). Accession numbers from the Chengdu Institute of Biology (CIB), Kunming Institution of Biology (KIZ) and the laboratory of Ding Li (DL) for all these specimens are provided in Table 1.

2.3 DNA extraction, amplification, and sequencing Tissue samples were either skeletal muscle or liver preserved in 95% ethanol at the time of collection and

Table 1 The information of sequences retrieved from GenBank and sequenced in this study. New sequences from this study are in bold.

Family	Genus and species	Accession No.		
Subfamily		Cyt *b*	ND4	c-mos
Colubridae				
Colubrinae				
	Dinodon flavozonatum (DL12612)	**KF732927**	**KF732920**	**KF732934**
	Dinodon rufozonatum (DL12611)	**KF732924**	**KF732917**	**KF732931**
	Dinodon semicarinatus	AB008539	AB008539	
	Lycodon alcalai	KC010345		KC010304
	Lycodon aulicus	HQ735416		HQ735418
	Lycodon bibonius	KC010351		KC010309
	Lycodon butleri	KC010359		KC010312
	Lycodon capucinus	KC010354	U49317	KC010313
	Lycodon chrysoprateros	KC010360		KC010318
	Lycodon dumerilii	KC010363		KC010320
	Lycodon effraenis	KC010376		KC010328
	Lycodon fasciatus	KC010366		
	Lycodon jara	KC010367		KC010322
	Lycodon laoensis	KC010370		KC010325
	Lycodon liuchengchaoi (DL14315)	**KF732928**	**KF732921**	**KF732935**
	Lycodon muelleri	KC010375		
	Lycodon osmanhilli		KC347524	KC347403
	Lycodon ruhstrati (DL12678)	**KF732925**	**KF732918**	**KF732932**
	Lycodon stormi	KC010380		KC010331
	Lycodon subcinctus	KC010385		KC010335
	Lycodon zawi	AF471040		AF471111
	Oligodon arnensis	KC347464	KC347504	KC347404
	Oligodon calamarius	KC347478	KC347511	KC347405
	Oligodon chinensis (DL12672)	**KF732930**	**KF732923**	**KF732937**
	Oligodon cinereus	AF471033		AF471101
	Oligodon formosanus (DL12643)	**KF732929**	**KF732922**	**KF732936**
	Oligodon maculatus	KC010387		
	Oligodon multizonatum (KIZ01623)	**KF732926**	**KF732919**	**KF732933**
	Oligodon octolineatus		U49316	
	Oligodon sublineatus	KC347465	KC347521	KC347406
	Oligodon taeniolatus	KC347483	KC347505	KC347407
	Boiga dendrophila	AF471089	U49303	AF471128
	Cemophora coccinea	AF471091	DQ902282	AF471132
	Crotaphopeltis tornieri	AF471093	AF428011	AF471112
	Dasypeltis atra	AF471065		AF471136
	Dipsadoboa unicolor	AF471062	AF428017	AF471139
	Elaphe carinata	DQ902133	DQ902284	DQ902063
	Lytorhynchus diadema	DQ112076		AY187986
	Pituophis melanoleucus	DQ902130	DQ902312	FJ627797
	Senticolis triaspis	DQ902127	AF138775	
	Telescopus fallax	AF471043		AF471108
Outgroups:				
Boidae	*Boa constrictor*	AB177354	AB177354	AF471115
Cylindrophiidae	*Cylindrophis ruffus*	AB179619	AB179619	AF471113

subsequently stored in either ethanol or frozen at –80°C. All specimens sampled are preserved in the collections of CIB. All tissues were treated by the standard method of proteinase K digestion in lysis buffer followed by a high salt DNA extraction procedure (Sambrook *et al*., 1989). The mitochondrial cytochrome b (cyt *b*) gene and the NADH dehydrogenase subunit 4 (ND4) gene, and the nuclear oocyte maturation factor Mos (c-mos) gene were amplified from total DNA extracts using polymerase chain reaction (PCR) with the following primer pairs for cyt *b*: L14910/H16064 (Burbrink *et al.*, 2000), ND4: ND4/Leu (Arévalo *et al*., 1994), and c-mos: S77/S78 (Lawson *et al*., 2005). Amplification was performed in a 20 µl volume reaction with the following settings: initial denaturation step with 4 min at 94°C, 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 46°C for cyt *b* primers and 56°C for ND4 and c-mos, extension for 1 min at 72°C. A final extension at 72°C was conducted for 7 min. Purified PCR products were sequenced in both directions with an ABI automated DNA sequencer (ABI 3700). We conducted a BLAST search of acquired sequences by using the GenBank database to verify that generated sequences were not of pseudogenes. All novel sequences have been deposited in GenBank (Table 1).

2.4 Phylogenetic analyses The initial alignments of cyt *b*, ND4, c-mos were aligned using ClustalX (Thompson *et al*., 1997) with default parameters, and subsequently verified manually, and translated into amino acid sequences to check for the presence of stop codons. We tested the saturation of 3rd codon positions of the mitochondrial protein-coding genes. These were highly saturated, therefore, we deleted the 3rd codon positions of mitochondrial genes (cyt *b* and ND4). In addition, we also analyzed the phylogeny for each gene independently in order to explore the congruence between different gene data by using likelihood and Bayesian analyses. There was no moderate to highly supported incongruence between cyt *b*, ND4 and c-mos gene and therefore we used the concatenated and combined data for phylogenetic analyses in this study. Because some of taxa were missing data for cyt *b*, ND4 and c-mos, we did exploratory analyses of the combined data set of 41 ingroup and two outgroup taxa and found no missing data exhibited identical relationships. Therefore, we chose to use all data (41 taxa) for subsequent analyses of the combined data set.

Phylogenetic analyses were performed using Bayesian Inference (BI) and Maximum Likelihood (ML) methodology. Partitioned Bayesian Inference (BI) approaches were used to reconstruct phylogeny with combined data set of three partial gene sequences, using MrBayes v 3.1 (Huelsenbeck and Ronquist, 2001). Both mitochondrial and nuclear data sets were partitioned by codon position. The best-fit substitution model (see Table 2) was assigned to each partition using AIC in Modeltest 3.7 (Posada and Crandall, 1998) and PAUP* v4b10 (Swofford, 2003). Two separate runs were performed with four Markov chains. Each run was conducted with 15 000 000 generations and sampled every 1000 generations. When the scores were found to stabilize, a consensus tree was calculated after omitting the first 25% of the trees as burn-in. Node support for the Bayesian consensus tree was determined using posterior probabilities (Erixon *et al*., 2003). Maximum Likelihood (ML) with the non-partitioned strategy with the combined data set was used to infer trees and assess nodal support by using RaxML (Stamatakis *et al*., 2005). The complex model (GTR + Γ) was used for each partition. Support for ML trees was derived from 100 nonparametric bootstrap replicates using RaxML. Each inference was started with a random starting tree, and 100 nonparametric bootstrap pseudoreplicates (Stamatakis *et al*., 2008) was used to assessed the nodal support. Because of less availability of ND4 gene and the fact that the c-mos gene was highly conserved in this study, average divergence estimation between species was calculated from the two mitochondrial genes using Mega 4.0 (Tamura *et al*., 2008).

2.5 Topological test The Bayesian analysis and Maximum Likelihood produced BI and ML trees. The topological structure of phylogenetic trees were slightly different. Results from the Shimodaira-Hasegawa (SH test; Shimodaira and Hasegawa, 1999) and Kishino-Hasegawa tests (KH test; Kishinino and Hasegawa, 1989) indicated that the BI tree was the best fit. Therefore, the conclusion of our analysis are based mainly on the BI tree topological structure.

3. Results

3.1 Morphology The newly collected specimen (KIZ01623) and the specimen of the type series of *O. multizonatum* Zhao and Jiang, 1981 (CIB9964) were used in this study. A Comparison of the main morphological characters between *O. multizonatum* (type specimens, CIB9964–9967), *O. multizonatum* (new specimen, KIZ01623), *L. liuchengchaoi* (description from Zhang *et al*. [2011], CWNU867001, CWNU84002, and FMNH15148), *L. liuchengchaoi* (new specimen, DL14315), and *O. joynsoni* (description from Jiang *et al*.

Table 2 Models of evolution selected by AIC and partitions of mitochondrial (cyt *b*, ND4) and nuclear (c-mos) data applied in model-based analyses.

Partitions	ACI models	Number of characters
cyt *b*, 1st codon position	GTR + G	361
cyt *b*, 2nd codon position	GTR + I + G	361
ND4, 1st codon position	K81uf + G	216
ND4, 2nd codon position	TrN + G	216
c-mos, 1st codon position	K80	188
c-mos, 2nd codon position	T-VM	188
c-mos, 3rd codon position	HKY + Γ	188

Table 3 A Comparison of the main morphological characters between *O. multizonatum* (type specimens, CIB9964–9967), *O. multizonatum* (new specimen, KIZ01623), *L. liuchengchaoi* (description from Zhang *et al*. [2011], CWNU867001, CWNU84002, and FMNH15148), *L. liuchengchaoi* (new specimen, DL14315), and *O. joynsoni* (description from Jiang *et al*. [2012], BMNH1946.1.4.23, BMNH 1969.1809, BMNH 1938.8.7.40, BMNH 1969.1808, MNHN 1896.0633, and KIZ09128). Dimensions in mm.

Characters	*O. multizonatum* (CIB9964–9967)	*O. multizonatum* (KIZ01623)	*L. liuchengchaoi* (Zhang *et al.* [2011])	*L. liuchengchaoi* (DL14315)	*O. joynsoni* (Jiang *et al.* [2012])
Snout-vent length	173-409	428	595-676	458	568
Tail length	45-90	92	134-152	114	79
Dorsal scale rows	17-17-15	17-17-15	17-17-15	17-17-15	17-17-15
Ventrals	190-195	194	202-206	205	186-200
Subcaudals	68-75	63	68-77	73	40-50
Loreal enters eye	yes	yes	yes	yes	yes
Dorsal bands	55-73	55	40-45	43	no
Tail bands	16-19	11	10-15	12	no
Upper labials	8	8	7-8	7	7-8
Temporals	2 + 3; 1/2 + 2; 2 + 3/2	2 + 3	2 + 2; 2 + 2/1 + 2; 1 + 2	2 + 2	1 + 2
Infralabials	8	8	8	8	7-8
Maxillary teeth	10-11	10	8-9	8	11-12
Anal plate	divided	divided	divided	divided	entire

[2012], BMNH1946.1.4.23, BMNH 1969.1809, BMNH 1938.8.7.40, BMNH 1969.1808, MNHN 1896.0633, and KIZ09128) are shown in Table 3. Based on morphological examination, our results indicated that the new specimen of *O. multizonatum* is same as the type specimen of *O. multizonatum*, but different from the type specimen of *L. liuchengchaoi* and the new specimen of *L. liuchengchaoi*. In addition, Zhao and Jiang (1981) reported *O. joynsoni* is the most similar species to *O. multizonatum*. Our analysis indicates there are many major differences between *O. joynsoni*, *L. liuchengchaoi* and *O. multizonatum*.

The hemipenis of *O. multizonatum* (KIZ01623) (Figure 3) is characterized as follows: smaller base, expanding from middle to tip; relatively short, extending to the eighth subcaudal; unforked, sulcus single and prominent, extending to the tips of the organ; the base to middle of the organ covered with larger hard spines, but changing to tiny spines after middle to the tip; no nick at the tip.

The hemipenis characteristics of *O. multizonatum* (KIZ01623) are similar to the description of the type specimen of *O. multizonatum* (CIB9964) provided by Zhao and Jiang (1981). Based on hemipenis morphology, *O. multizonatum* can be seperated from the species of the genus *Oligodon* that lack a hard spine, such as *O. joynsoni* (Smith, 1917), but is similar to most species of the genera *Oligodon*, *Lycodon* and *Dinodon*, which have a hard spine on the hemipenis (Zhao *et al*., 1998; Zhao, 2008; Green *et al*., 2010). Green *et al*. (2010) reported the hind teeth of snakes of the genus *Oligodon* are broad and strongly recurved. However, our analysis showed that the hind teeth of both new and type specimens of *O. multizonatum* are not strongly recurved. In addition, a striking characteristic of *Oligodon* is the large rostral scale that is clearly visible when viewed from above (Zhao *et al*., 1998; Zhao, 2006). Nevertheless, such observations are to some extent subjective and might even be dependent on the viewing angle (Figures 1–2). Actually the rostral scale of *O. multizonatum* is not as

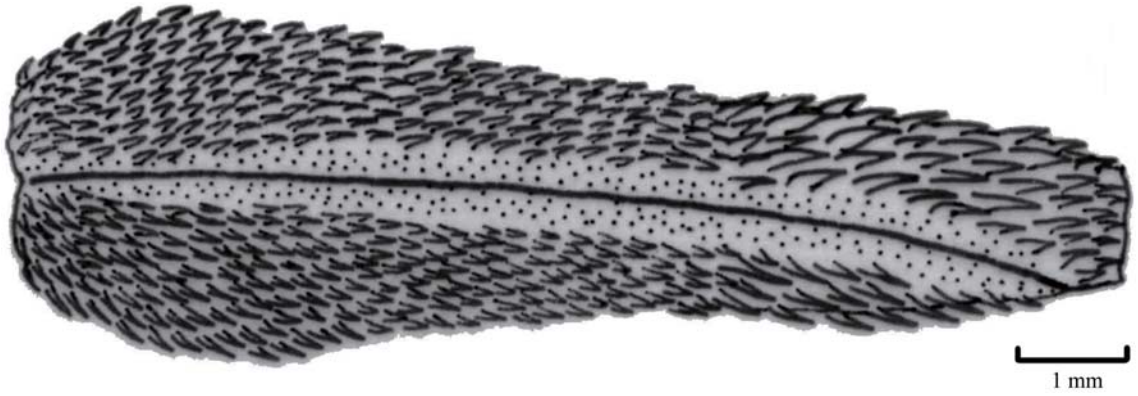

Figure 3 The left hemipenis of *Oligodon multizonatum* (specimen number KIZ01623). Drawing by Ke JIANG..

large as that in members of the genus *Oligodon*, and is only clearly visible from above. The placement of *O. multizonatum* in the genus *Oligodon* was based on it having a large rostal scale (ref to original description), but our analysis suggests that the rostal scale of *O. multizonatum* is not particularly prominent.

3.2 Phylogeny The initial aligned data set contained 1085 bp of cyt *b*, 648 bp of ND4 and 564 bp of c-mos for 41 ingroup and two outgroup taxa, whereas the final aligned data set contained 722 bp of cyt *b*, 432 bp of ND4 and 564 bp of c-mos after deleting the 3rd codon positions of mitochondrial genes.

The topologies of trees derived from each dataset and analytical method were nearly identical (see Figures 4–5). BI and ML trees showed strong support (100% PP and 99% BS respectively) for the monophyly of *Oligodon*, adding some novel molecular sequence data of *O. chinensis*, *O. formosanus*, but excluding *O. multizonatum*. Unexpectedly, all analyses demonstrated that *O. multizonatum* is not part of the genus *Oligodon* but is instead nested within the genus *Lycodon*, where it is most closely related to *L. liuchengchaoi* in our sampling. These two species together formed a highly supported clade (100% PP and 99% BS), which are themselves sister to *L. ruhstrati* in the BI tree but to other clades including two species of *L. butleri* and *L. fasciatus* in ML tree. All these five species formed a monophyletic group with support values of 100% PP and 99% BS. However, most of the main nodes were not well solved within *Oligodon* and *Lycodon* in both BI and ML trees. In addition, the genetic distance (uncorrected *P*-distance) between *O. multizonatum* and *L. liuchengchaoi* is 0.066 (cyt *b* gene) and the minimum genetic distance of valid species between *L. aulicus* and *L. capucinus* is 0.047 (cyt *b* gene).

4. Discussion

4.1 Phylogenetic position and morphology of *O. mulizonatum* Although our sampling was incomplete relative to the sampling of Colubridae, multilocus phylogenetic reconstruction has indicated that all representatives from *Oligodon* except *O. multizonatum* formed a strongly supported clade, and those from *Lycodon* with *O. multizonatum* clustered into another highly supported group (Figure 1), in which the species within *Lycodon* and *Dinodon* were shown to be paraphyletic or polyphyletic. Previous studies support the conclusion that *Lycodon* is paraphyletic with respect to *Dinodon* (Siler *et al*., 2013). In addition, Guo *et al*. (2013) concluded that *Lycodon* and *Dinodon* are paraphyletic based on molecular and morphological data, and suggested synonymizing *Dinodon* with *Lycodon*. Moreover, Pyron *et al*. (2013) indicated the genus of *Dryocalamus* which had previously been identified as *Lycodon* nested within the group *Dinodon* and *Lycodon*, suggesting that *Dinodon* and *Lycodon* are not monophyletic. In agreement with our molecular phylogenetic results, previous morphological studies have noted the difficulty of separating *Dinodon* and *Lycodon* (Pope, 1935; Smith, 1943; Vogel and Brachtel, 2008), and thus the validity of these two genera has triggered debate. This is the reason why we sampled widely within these taxa and used different methods of analysis to make our conculsions. Our results support synonymizing the genus *Dinodon* and *Lycodon*.

Unexpectedly, our analysis indicates *O. multizonatum* as the sister species of *L. liuchengchaoi* which is clustered within *Lycodon* based on both mitochondrial and nuclear genes. Based on morphology, Zhao and Jiang (1981) suggested that *O. multizonatum* is closely related to the Indochinese *O. joynsoni*, and then assigned this species to

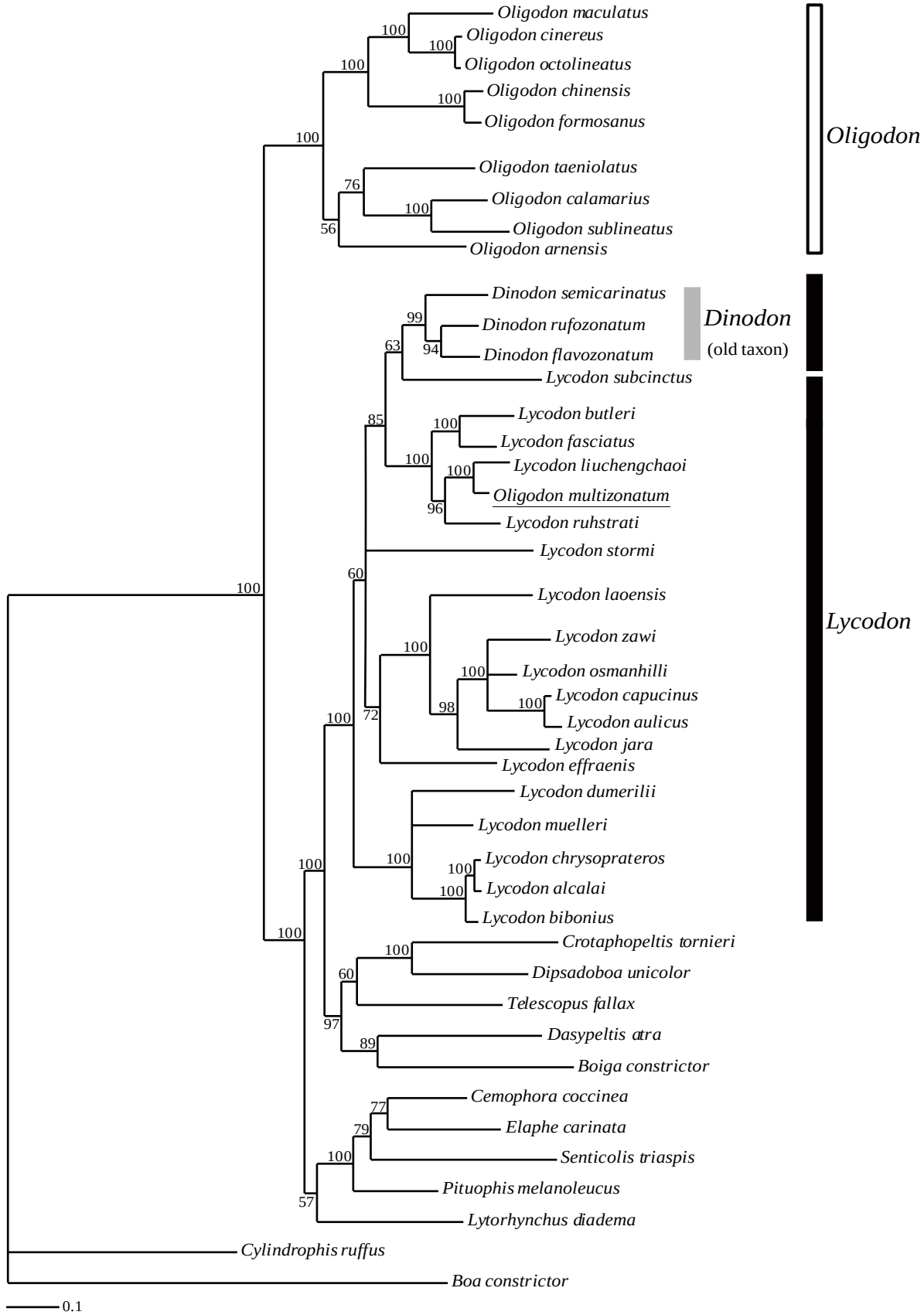

Figure 4 The 50% majority-rule consensus tree from Bayesian analysis based on c-mos, cyt *b* and ND4 combined sequences. Values at nodes are posterior probability support values. Black bar: *Lycodon*; Open bar: *Oligodon*; Gray bar: old *Dinodon*.

Figure 5 Maximum likelihood inferred phylogeny of the c-mos, cyt *b* and ND4 combined data. Bootstrap values are shown at the corresponding nodes. Support values below 50% were not shown in this figure. Black bar: *Lycodon*; Open bar: *Oligodon*; Gray bar: old *Dinodon*.

the genus *Oligodon*. However, there are many differences between these two species. The former species differs from the latter by having: 1) more subcaudal scales, 68–75 pairs *vs* 40–50 pairs; 2) a divided *vs* entire anal plate; 3) eight upper labial scales, the third, fourth and fifth *vs* the fourth and fifth touching the eye; 4) a hemipenis with spines *vs* without spines. The colour pattern and markings of these two species are also quite different (Zhao and Jiang, 1981). The hind teeth are also different being broad and strongly recurved, much like the shape of the kukri knife in *Oligodon* (Green *et al*., 2010), but not recurved in *O. multizonatum.* The recurved shaped teeth of *Oligodon* are used to open reptile eggs (Green *et al*., 2010), upon which they mainly feed. The combined morphological data also indicate that *O. multizonatum* is neither a close relative to *O. joynsoni* nor a member of the genus *Oligodon.*

In terms of pattern, *O. multizonatum* is most similar to *L. liuchengzhaoi* except for the fact that the number and color of bands of the former are greater and deeper than those of the latter (5–73 orange rings spaced along the black body, and 16–19 orange rings spaced along the black tail *vs* 40–45 well-defined yellow rings evenly spaced along the entire length of the black body, and more than 10–15 yellow rings evenly spaced along the black tail), but differs by the following traits: more maxillary teeth (10–11 *vs* 8–9), fewer ventrals (190–195 *vs* 202–206) (Zhao and Jiang, 1981; Zhang *et al*., 2011).

Therefore, we suggest that the species previously assigned to *O. multizonatum* needs to be transferred to *Lycodon*. Zhao (2006) reported that *O. multizonatum* feed on reptile eggs, but no analysis of the stomach content of this species has been reported. Further studies on prey types consumed by species within *Oligodon* and *Lycodon* are needed to confirm or provide some new evidence to support the view that most of *Oligodon* feed on retile eggs whereas snakes and lizards are the major food of *Lycodon*. Considering that the genus *Dinodon* has been merged into *Lycodon*, we suggested that the scientific name of *O. multizonatum* should be renamed as *Lycodon multizonatum*. Consistent with this we propose a new common English name, the Luding wolf snake, referring to the type locality, Luding County, China.

4.2 The validity of *O. multizonatum* and *L. liuchengzhaoi* For many species, selective or developmental constraints either prevent morphological divergence (Colborn *et al*., 2001) or promote convergence (Wake, 1991), complicating our understanding of group composition based on evolutionary relationships inferred from morphology (Guo *et al*., 2013). On the other hand, within species individual variations in morphology can make species identification difficult. A good example is *Lycodon*, one of the most diverse genera of Asiatic colubrids (sensustricto, see Pyron *et al*., 2011). Recently, *L. futsingensis* (Pope, 1928), which was subsequently synonymized with *L. ruhstrati* by Pope himself (Pope, 1935) was revalidated by Vogel *et al*. (2009). In 2010 and 2011, two new endemic species were described from China: *L. synaptor* (Vogel and David, 2010) and *L. gongshan* (Vogel and Luo, 2011). Based on the specimens collected from northern Sichuan Province, China, Zhang *et al*. (2011) described *L. liuchengzhaoi*. They are similar to *L. fasciatus* in shape and were identified as *L. fasciatus* previously. By careful examination of the specimens it was noticed that they could be distinguished from *L. fasciatus* and other species of the *L. fasciatus* group by several morphological characters (Vogel *et al*., 2009). However, *O. multizonatum* was not compared with these specimens. Thus it should be cautioned that *O. multizonatum* is the closest related specie to *L. liuchengchaoi* from our molecular phylogenetic analysis (Figures 4–5). Although they shared very similar morphlogical characters, such as same dorsal scale rows, loreal enters eye, same infralabial, similar temporals and similar subcaudals (Table 3), the genetic distance between these two species reached the level of interspecific differentiation. Our molecular data showed that the genetic distance (uncorrected *P*-distance) between *O. multizonatum* and *L. liuchengchaoi* is 0.066 (cyt *b* gene), which is greater than the minimum genetic distance of the valid species difference between *L. aulicus* and *L. capucinus* which is 0.047 of cyt *b* gene. Therefore, we strongly suggest that *O. multizonatum* and *L. liuchengchaoi* are valid as distinct species.

Currently, these two species of *Lycodon* are known in Sichuan Province. The three sites where *L. liuchengchaoi* was found are from the east of the Hengduan mountains, at the eastern edge of Qinghai-tibet Plateau. *O. multizonatum* is known only from Luding county, Sichuan Province, Tianshui and Kang counties, Gansu Province. These specimen records and published literature suggest that *O. multizonatum* might be distributed in the middle eastern and northern edge of Hengduan mountains which is sympatric with the *L. liuchengchaoi*. However, it is interesting to notice that the specimen of *O. multizonatum* sourced from Gansu province had fewer rings in its body on the photograph (Zhao, 2006) is rather similar to *L. liuchengchaoi*. Therefore, we suggest the distribution of *O. multizonatum* in Gansu province might be questionable.

Unfortunately, the molecular phylogeny presented here did not resolve the relationships among *Lycodon* and *Dinodon*. Considering the morphological and phylogenetic results in this study, we suggest future studies need to add more markers to resolve the relationships among *Lycodon* and *Dinodon*.

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