

## Molecular confirmation of the rhino rat snake *Rhynchophis boulengeri* in southern Vietnam

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In 2019, we made the first record of a rhino rat snake *Rhynchophis boulengeri* (Mocquard, 1897) in southern Vietnam (Nguyen et al., 2020), a single male specimen (ITBCZ 4506) found dead on a road in Song Chinh Protected Forest, Phu Yen Province. This amounted to a 600 km range extension. The morphology of the specimen was broadly congruent with *R. boulengeri*.

In 2021, *R. boulengeri* was split so that populations in Hainan Province, China are now assigned to *Rhynchophis hainanensis* (Peng et al., 2021). Given that there is a substantial distance between southern Vietnam and all other locations where *R. boulengeri* is found and that there are now two species within the genus, there is clearly interest in checking for potential cryptic species. Consequently, we sequenced the cytochrome b (cytb) gene for the specimen collected in Phu Yen Province and calculated uncorrected p-distances between this sequence and other available *Rhynchophis* sequences on GenBank (see Table 1 & Table 2) following methods described in Kane et al. (2023).

Uncorrected p-distances between the sequences obtained from the specimen we collected in Phu Yen

**Table 1.** List of taxa of *Rhynchophis* specimens and cytb sequences used in this study

Species	Collection site	Voucher	GenBank	Reference
<i>Rhynchophis boulengeri</i>	Phu Yen, Vietnam	ITBCZ 4506	PV588734	This study
<i>Rhynchophis boulengeri</i>	Lao Cai, Vietnam	ANU2012083	MW495262.1	Peng et al., 2024
<i>Rhynchophis boulengeri</i>	Not reported	MVZ Herp 218698	LC640447.1	Kambayashi et al., 2022
<i>Rhynchophis boulengeri</i>	Not reported	NA	AF471053.1	Lawson et al., 2005
<i>Rhynchophis boulengeri</i>	Guangdong, China	ANU2018176	MW495261.1	Peng et al., 2024
<i>Rhynchophis hainanensis</i>	Hainan, China	ANU20190002	MW495258.1	Peng et al., 2021
<i>Rhynchophis hainanensis</i>	Hainan, China	HNU20190001	MW495259.1	Peng et al., 2022
<i>Rhynchophis hainanensis</i>	Hainan, China	ANU20190003	MW495260.1	Peng et al., 2023

**Table 2.** Uncorrected p-distances between species of *Rhynchophis* sequences used in this study based on cytb sequences.

	1	2	3	4	5	6	7	8
1. PV588734 <i>R. boulengeri</i>								
2. MW495262.1 <i>R. boulengeri</i>	0.79%							
3. LC640447.1 <i>R. boulengeri</i>	1.36%	0.56%						
4. AF471053.1 <i>R. boulengeri</i>	1.36%	0.56%	0.00%					
5. MW495261.1 <i>R. boulengeri</i>	0.91%	0.11%	0.68%	0.68%				
6. MW495258.1 <i>R. hainanensis</i>	3.51%	2.66%	2.78%	2.78%	2.78%			
7. MW495259.1 <i>R. hainanensis</i>	3.02%	2.42%	2.54%	2.54%	2.54%	0.45%		
8. MW495260.1 <i>R. hainanensis</i>	3.38%	2.54%	2.65%	2.65%	2.66%	0.34%	0.34%	

Province and *R. boulengeri* from Lao Cai Province more than 1,000 km to the north was 0.79% and up to 1.36% from sequences from specimens with no associated locality data (Table 2). This is typical of within species variation for this gene (e.g. Laopichienpong et al., 2016). Uncorrected p-distances between *R. boulengeri* from Phu Yen Province and *R. hainanensis* ranged from 3.02–3.51%. Furthermore, the specimen from Phu Yen Province has a single loreal scale and black orbital stripe, clearly distinguishing it from *R. hainanensis* (Peng et al., 2021). We conclude that the specimen from Phu Yen Province is indeed *R. boulengeri*.

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