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Short Communication

Molecular identification of *Eremias stummeri* (Squamata: Lacertidae) as a prey for *Gloydius halys* complex (Serpentes: Viperidae) from Kyrgyzstan

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The genus *Gloydius* comprises 13 species of venomous Asian pit vipers (Wagner *et al.* 2016) that range from east of the Ural Mountains to Japan and the Ryukyu Islands (McDiarmid *et al.* 1999). The *Gloydius halys* (Pallas, 1776) complex occurs from Azerbaijan, Iran, through Central Asia to eastern Siberia, Mongolia, and China. Traditionally, *Gloydius halys* from Kyrgyzstan has been considered a subspecies—i.e., *G. halys caraganus* (Eichwald, 1831). However, given recent descriptions of cryptic taxa from Kyrgyzstan and unresolved phylogenetic relationships, they are called a species complex (Wagner *et al.* 2016). These snakes usually prey on small vertebrates, including rodents and lizards, but several kinds of invertebrates also were found in the digestive tract of this taxon (reviewed by Joger and Stümpel 2005).

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Racerunners (Lacertidae: *Eremias*) are widely distributed lizards occurring from southeastern Europe throughout most of the Asian continent (Ananjeva *et al.* 2006). Their systematic status is not yet fully resolved owing to their morphological resemblance to one another and the syntopic occurrence of several species (Pouyani *et al.* 2012, Liu *et al.* 2014, Poyarkov Jr. *et al.* 2014). These lizards often occur in the same localities as pit vipers (cf. Sindaco and Jeremcenko 2008) and are eaten by snakes of the *G. halys* complex (e.g., Yakovleva 1964).

On 13 May 2016 we found a dead, freshly killed subadult male of the *Gloydius halys* complex near Jeti-Ögüz village (42.403° N, 78.221° E; 1888 m a.s.l.), Issyk-Kul Region in northeastern Kyrgyzstan. The snake was 285 mm long with a head that was 12 mm long and 9 mm wide. We did not observe any superficial wounds on the snake's body. The site features a slight rocky slope that has sparse vegetation and no trees or shrubs. The pit viper was found in an area frequently used for grazing; thus, the

individual might have been killed by local farmers. We cannot exclude the possibility of predation by a larger vertebrate, possibly a bird of prey as they are known to mob crotaline snakes (Sazima 2015); however, the body was not damaged. On inspecting the cadaver, we noticed that the abdomen contained an incompletely digested lizard (~100 mm long) of the genus *Eremias* (Figure 1). The lizard was sufficiently decomposed to preclude species determination. However, based on distribution (Sindaco and Jeremcenko 2008), the slightly visible color pattern back of the body, and our observation of several individuals of *E. stummeri* Wettstein, 1940 at the locality, we assume that the prey item may have been this species of racerunner.

Comparative DNA sequence analysis confirmed our supposition. A tissue sample (a piece of the tail and digit) was collected at the locality, preserved in 96% ethanol, and deposited in the private tissue collection of the first author at the Department of Zoology, Comenius University in Bratislava under the number 3981. Total genomic DNA, obtained from the tissue sample of the specimen was extracted using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) following the protocols of the respective manufactures. DNA was amplified by widely used *16S* ribosomal RNA marker (*16S*) with primers 16Sar-L and 16Sbr-H (Palumbi *et al.* 2002) and sequenced by Macrogene Inc. (Amsterdam, Netherlands). The *16S* fragment (528 bp) was aligned using the Clustal W algorithm (Thompson *et al*. 1994) as implemented in BioEdit (Hall 1999) and compared via the BLAST algorithm (Altschul *et al.* 1990). Our resulting sequence alignment corresponded to 99% with the *16S* part of the mitochondrion of *E. stummeri* originating from Kazakhstan (GenBank accession number KT372881; Zhou *et al.* 2015). Sequence divergence between our sample from Kyrgyzstan and that published from Kazachstan is 0.4% (uncorrected *p*-distances using MEGA 7.0; Kumar *et al.* 2016). This documents the genetic affiliation of our sample to *E. stummeri*; the sequence is deposited in the GenBank sequence database under accession number KY486773.

There are several recent taxonomic and distributional studies of *Gloydius* from various parts of Asia (Orlov *et al.* 2014, Kropachev *et al.* 2016, Wagner *et al.* 2016). We can supplement these reports by providing some ecological information about these pit vipers. The food spectrum of *G. halys* depends on the type of habitat in which they occur and the age of the snake (Simonov 2009). The diet of adult snakes mainly consists of warm-blooded prey, such as small mammals, but observations of individuals

Figure 1. Dissected specimen of *Gloydius halys* complex with part of the body of *Eremias stummeri*. (**A**) Ventral view (dorsal view of the lizard's remains). (**B**) Dorsal view (ventral view of the lizard's remains).

with snout–vent lengths less than 400 mm revealed that smaller snakes prefer invertebrates, especially Orthopterans (from 35–61%) and small lizards (Lesnyak 1964, Yakovleva 1964, Bogdanov 1970). This supports our observation. *Eremias arguta* (Pallas, 1773), *E. multiocellata* Günther, 1872, and *E. velox* (Pallas, 1771) have been reported in the diet of *G. halys* from Kyrgyzstan (Yakovleva 1964), but this is the first record of the racerunner *E. stummeri.* Given the abundance of *E. stummeri* at the locality, we can assume that these lizards are one of the main prey items of subadult pit vipers. Similarly, species of *Eremias* also have been recorded as a prey item of *Eryx miliaris* (Pallas, 1773), *E. tataricus* (Lichtenstein, 1823) or *Vipera renardi* (Christoph, 1861) (Yakovleva 1964, Rodríguez-Robles *et al.* 1999).

The body of the racerunner was incomplete, which suggests that the lizard might have been dead before it was eaten. Similarly, Yakovleva (1964) documented part of a limb of *Eremias arguta* found in male *Gloydius halys* with a snout–vent length of 205 mm. Therefore, it seems likely that *G. halys* consumes lizard cadavers, which might indicate occasional necrophagy. This is a rare behavior in Viperidae (Sazima and Strussman 1990).

Direct observation of feeding events is impractical and invasive approaches are inappropriate for protected or rare species. Contents of the digestion tract of freshly killed individuals (e.g., on the road or killed by man) may provide direct information about food preference. Molecular techniques are useful to identify prey items that are quickly destroyed by digestive processes. Dietary composition can provide valuable knowledge about the interaction of these pit vipers with other reptile species, as well as elucidate the composition of local herpetofauna.

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