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Predation of the Japanese keelback (*Hebius vibakari* Boie, 1826) by the Slender racer (*Orientocoluber spinalis* Peters, 1866)

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Abstract

Background: The Slender racer (*Orientocoluber spinalis* Peters, 1866) has recently been reclassified to the new genus *Orientocoluber* from *Hierophis*. Ecological knowledge of this species is limited due to its highly mobile behavior. On 17 July 2020, we captured a female *O. spinalis* on Oeyeon Island, Boryeong-si, Republic of Korea, and collected its feces for a diet analysis. We observed snake scales from the collected feces and subsequently determined the prey species through morphological and molecular methods.

Results: We initially hypothesized that the extracted fecal sample scales belonged to *H. vibakari*, due to their thin keel and rhombus shape. We also amplified *H. vibakari* DNA from the extracted fecal sample using Illumina sequencing methods. Our morphological and molecular results suggest that *O. spinalis* predated *H. vibakari* on Oeyeon Island.

Conclusion: This is the first report of *O. spinalis* predating another snake species, ophiophagy, and implies that *H. vibakari* may be a crucial prey item for *O. spinalis* on Oeyeon Island.

Keywords: *Hebius vibakari*, Island, Ophiophagy, *Orientocoluber spinalis*, Predation

Background

The Slender racer (*Orientocoluber spinalis* Peters, 1866), which is a cylindrical and slender bodied non-venomous oviparous snake with a medium to moderately long tail, has recently been classified to a new genus *Orientocoluber* from *Hierophis* (Kharin 2011; Park et al. 2020). Although *O. spinalis* has a wide geographic range which spans across northeast Asia including China, Kazakstan, North and South Korea, Russia, and Mongolia (Kharin and Akulenko 2008), there are few studies examining its general ecology due to its highly mobile behavior (Kim and Han 2009). In Korea, they are mainly found on hill-sides or in high, dry valleys, where they can easily escape into grass or low shrubs (Shannon 1956). *Orientocoluber spinalis* is likely a diurnal species and may prey upon

lizards, frogs, and rodents (Won 1971; Kim and Han 2009), although there have been no official publications on the diet of this species. Understanding the diet of *O. spinalis* can provide valuable ecological knowledge and allow for the proactive conservation of local populations (Sih et al. 1998; Werhahn et al. 2019). In this study, we are the first to identify scales and DNA of the Japanese keelback (*Hebius vibakari* Boie, 1826) in the feces of *O. spinalis*.

Materials and methods

The initial capture and observations were made on Oeyeon Island (36.228851° N, 126.084288° E; 143 m above sea level), Boryeong-si, Chungnam, Republic of Korea. Oeyeon Island is approximately 2.18 km² in size and is located 30 km from the west coast of the Korean Peninsula. To date, five reptile species have been reported on the island: four snakes (*O. spinalis*, *Elaphe dione*, *H. vibakari*, and *Gloydius brevicaudus*) and one

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lizard (*Scincella vandenburghi*; NIE 2016). On 17 July 2020, at 9:40 am, one *O. spinalis* was observed basking on stone stairs near the entrance of a hiking trail and was subsequently captured utilizing a snake hook. Morphometric measurements including snout-vent length (SVL) and tail length (TAL) were recorded using a tape measure. The individual's body weight (BW) and sex were recorded using a spring balance and probe, respectively. Fecal samples were collected by gently pressing the snake's abdomen and directing the cloaca into a 2 ml conical tube, containing 99.5% ethanol (Beja-Pereira et al. 2009). The snake was immediately released at the initial capture site after all morphometric data and the fecal sample were collected. The fecal sample was initially analyzed under a stereoscopic microscope (Swift S7 7×-45×; Swift Optical Instruments, USA) and an optical microscope (DW-BMS1000; Dongwon, Republic of Korea). From our microscopy work, we discovered snake scales within the fecal sample.

To determine the prey snake species collected from the fecal sample, we compared its general morphological features (shape, presence of keels, etc.) with those of the scales of other snake species found on Oeyeon Island (Koo et al. 2017). We also molecularly determined the species of snake the fecal sample scales may have belonged to. DNA was extracted from the fecal sample using the DNeasy Mericon Food kit (Qiagen, Germany). The extracted DNA was amplified with the vertebrate universal primer (12S-V5) for mitochondrial 12S ribosomal RNA (12S rRNA) gene using the PCR protocols reported (Riaz et al. 2011). The amplified PCR products were sent to Macrogen (Republic of Korea) for next-generation (Illumina) sequencing. The fecal sample DNA library was sequenced using the Illumina Miseq platform (Illumina, USA) with a 2 × 300 bp paired end run using Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2 (Illumina, USA). The sequenced DNA data was trimmed using Cutadapt (Martin 2011) to remove any Illumina adaptor and primer sequences. Sequence reads were quality-filtered, denoised, merged, and any chimeric reads were removed using DADA2 bioinformatics package (Callahan et al. 2016) in RStudio v3.5.3 (Team RStudio 2015). The resulting amplicon sequence variant (ASV) FASTA file from fecal DNA of *O. spinalis* was queried against the NCBI database using the Basic Local Alignment Search Tool (BLAST).

Results and discussion

The captured *O. spinalis* was measured, weighed, and sexed (SVL, 54.6 cm; TAL, 18.1 cm; BW, 35.6 g, female). Some of the dorsal scales extracted from the fecal sample had a thin keel running down the center, were rhomboidal in shape, and were morphologically similar to both *H. vibakari* and *G. brevicaudus*, which are known

inhabitants of Oeyeon Island (Koo et al. 2017). However, the thick keels observed on the extracted scales, more closely resembled scales from *H. vibakari* (Fig. 1). *Orientalocoluber spinalis* lacks any keel morphology on its dorsal scales (Kim and Han 2009; Kharin 2011). Consequently, we could exclude *O. spinalis* as the potential prey species. The width and length of the 10 ventral scales, extracted from the fecal sample, were 6.3 ± 0.3 mm and 1.2 ± 0.1 mm, respectively, and the rounded edges of the dorsal scales both corroborated our hypothesis that the prey snake may have been a juvenile *Hebius* spp. (Fig. 1). Juvenile *Hebius* spp. have rounded dorsal scales which gradually become more notched as the animal matures (Tsai et al. 2018). In addition to our microscopic observations, we also applied Illumina sequencing methods to identify potential prey items from the collected fecal sample.

Our molecular analysis amplified two reptile species, *H. vibakari* and *O. spinalis*. Given that *O. spinalis* was the predatory species, we concluded that the extracted fecal sample scales were likely from *H. vibakari*, although this does not completely rule out the possibility that *O. spinalis* may be cannibalistic. The 12S rRNA sequence from fecal DNA assay yielded 249,148 total sequence reads, and 91,170 preprocessed reads. The highest proportion (40.0%) of these reads corresponded to *O. spinalis*, followed by *H. vibakari* (17.1%).

The results of our morphological and molecular analysis support our hypothesis that the prey snake scales, extracted from the fecal sample, are likely from *H. vibakari*. In this study, we did not collect the snakes gut contents through regurgitation because this method is likely more invasive and stressful on the animal and would have likely yielded similar results to our fecal collection protocol. Currently, the prey breath of *O. spinalis* is only anecdotally known and includes lizards, frogs, and rodents (Won 1971; Kim and Han 2009). This is the first report of *O. spinalis* consuming another snake species, ophiophagy, and potentially cannibalizing its own species. The phylogenetically related but smaller *Eirenis* spp. (mean SVL ~30 cm, Nagy et al. 2004; Das et al. 2019) forages on lizards and arthropods but has not been reported preying other snakes (Çiçek and Mermer 2007; Mahlow et al. 2013). On the other hand, the larger *Hierophis viridiflavus* and *H. gemonensis* (mean SVL ~130 cm) may consume other snakes including *Zamenis longissimus* and *Vipera aspis* (Lisičić et al. 2011; Lelièvre et al. 2012). Additional studies should be conducted to identify the prey breadth of *O. spinalis* and to verify if *H. vibakari* is commonly consumed by *O. spinalis*.

Conclusion

We report the predation of *H. vibakari* by *O. spinalis* on Oeyeon Island, Korea. The predation of *H. vibakari* by

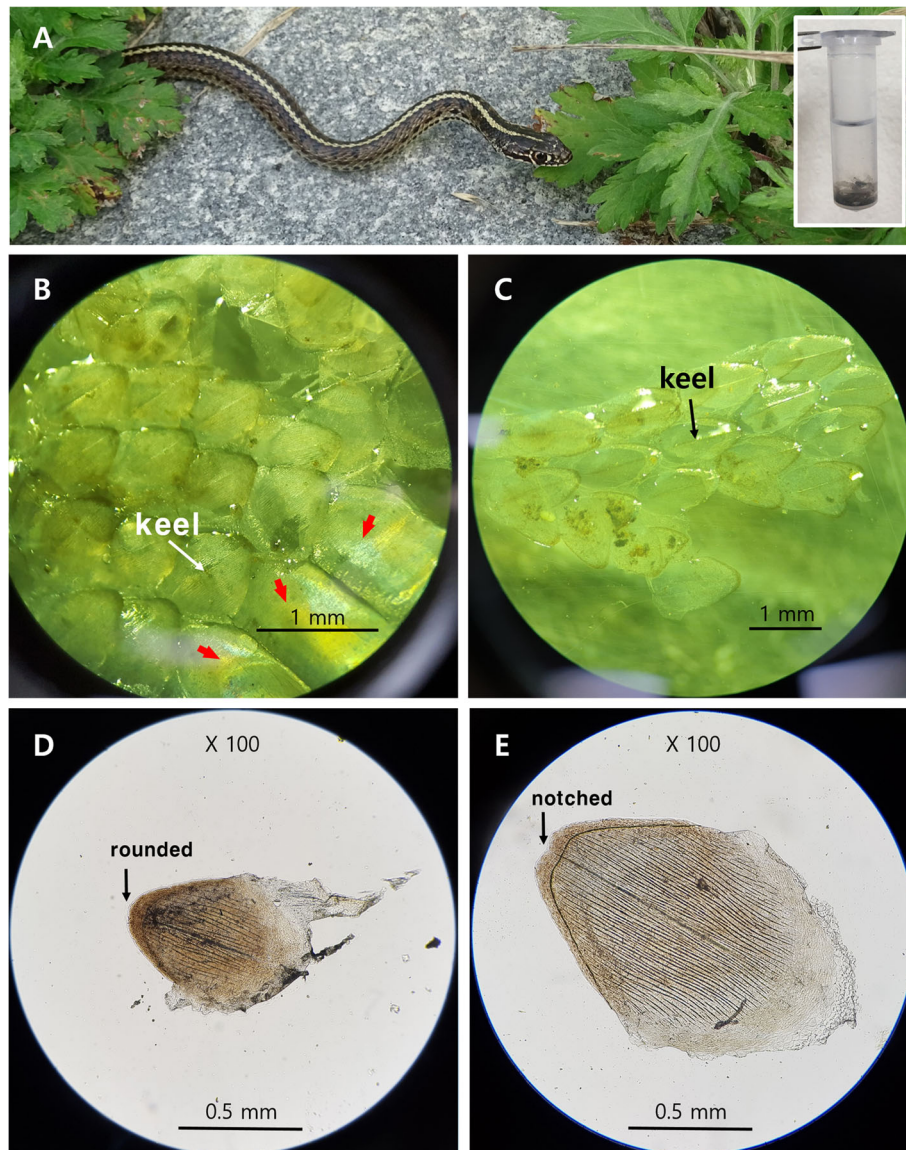


Fig. 1 (A) The captured and sampled female *Orientocoluber spinalis*, from Oeyeon Island, Republic of Korea, with dorsal and ventral scales placed within a conical vial. (B) Microscopic images of the dorsal and ventral scales (designated by red arrows) collected from the fecal sample. (C) Microscopic images of the extracted dorsal scales to exemplify the keel morphology. (D) Magnified photographs of the extracted dorsal scales and of a sub-adult *H. vibakari* (E) captured on the same island, for comparison

G. ussuriensis and *Sibynophis chinensis* has been previously reported in Jeju Island, Republic of Korea (Chang et al. 2010; Kim and Oh 2014). Considering the relatively simple food web on island ecosystem (Boback 2003; Roslin et al. 2014), *H. vibakari* may be a crucial prey item for *O. spinalis* on Oeyeon Island. Thus, the reduction of *H. vibakari* on Oeyeon Island may have negative population-level effects on *O. spinalis*. This diet information has the potential to better refine future conservation efforts of *O. spinalis* on Oeyeon Island and beyond.

Abbreviations

SVL: Snout-vent length; PCR: Polymerase chain reaction; NCBI: National Center for Biotechnology Information; BLAST: Basic local alignment search tool

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Not applicable.

Authors' contributions

IP, JJP, JHP, and SM carried out the field study and performed the analysis. IP, JJP, AG, and DP wrote and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data involved in this study is available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee in Kangwon National University (KW-200707-3). The study, capture, handling, and release of the Slender racer on Oeyeon Island was conducted with permission from the Chungnam Provincial Government (2020-1).

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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