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First evidence of Sette Fratelli cave salamander *Speleomantes sarrabusensis* (Urodela: Plethodontidae) consumption by the Sardinian grass snake *Natrix helvetica cetti* (Squamata: Natricidae)

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Abstract – European cave salamanders, classified under the genus *Speleomantes*, are primarily endemic to Italy, with one sub-endemic species also occurring in south-eastern France. These terrestrial salamanders seek refuge in subterranean habitats with stable, humid, and cool microclimates, especially during periods of high heat or dryness. Above-ground sightings mostly occur during cooler, damp periods, either at night or on very humid days. Many studies have explored their diet, but information on predators is limited. This work presents the first documented evidence of predation on adult Sette Fratelli cave salamander *S. sarrabusensis* by the Sardinian grass snake *Natrix helvetica cetti*. During a field survey in the Sette Fratelli forest complex, South Sardinia, three adult cave salamanders were regurgitated by a captured grass snake, indicating predation. Notably, the observation suggests nocturnal activity of the grass snake in early spring despite low temperatures. This finding underscores significant gaps in our understanding of the Sardinian grass snake's ecology and the predation risks faced by European cave salamanders, highlighting the need for further field research.

Key words: *Hydromantes*, Ophidia, Plethodontid, Predation, Sardinia

Riassunto – Prima evidenza di consumo di geotritoni del Sarrabus *Speleomantes sarrabusensis* da parte della natrice di Cetti *Natrix helvetica cetti*.

I geotritoni, ascritti al genere *Speleomantes*, sono urodeli endemici dell'Italia, eccetto una specie, *S. strinatii*, presente anche nel sud-est della Francia. Queste salamandre terrestri trovano rifugio in ambienti sotterranei con microclimi stabili, umidi e freschi, specialmente durante periodi caldi e siccitosi. Gli avvistamenti epigei avvengono principalmente durante periodi freschi e umidi, di notte o in giornate particolarmente umide. Numerosi studi hanno esplorato la loro dieta, ma le informazioni sui predatori sono limitate. Questo lavoro presenta la prima evidenza documentata di predazione su adulti di geotritone del Sarrabus (*S. sarrabusensis*) da parte della natrice di Cetti (*Natrix helvetica cetti*). Durante un'attività di campo nel complesso forestale dei Sette Fratelli, Sud Sardegna, tre geotritoni adulti sono stati rigurgitati da una natrice catturata, indicando un'avvenuta predazione. L'osservazione suggerisce l'attività notturna della natrice di Cetti a inizio primavera nonostante le temperature relativamente basse. Questo ritrovamento evidenzia significative lacune nelle conoscenze dell'ecologia di questa natrice e dei rischi di predazione affrontati dai geotritoni, sottolineando la necessità di ulteriori ricerche di campo.

Parole chiave: *Hydromantes*, Ophidia, plethodontide, Predazione, Sardegna

Eight species of lungless salamanders (Urodela: Plethodontidae) are currently recognised in Europe, all classified under the genus *Speleomantes* Dubois 1984 (although sometimes identified as *Hydromantes* – see Costa et al., 2023) and commonly known as the European cave salamanders. In particular, they are endemic to Italy, with the exception of *S. strinatii* that reaches a limited area of southeastern France (Renet et al., 2012; Speybroeck et al., 2020; Di Nicola et al., 2021). Three of these species are continental: *Speleomantes strinatii* (Aellen 1958), which ranges from the northern Apennines to Provence in France (including the Principality of Monaco), *S. ambrosii* (Lanza 1955) and *S. italicus* (Dunn 1923), which inhabit the Apennine Peninsula (the latter also occurring in the Republic of San Marino). The remaining five species – *S. flavus* (Stefani 1969), *S. genei* (Temminck & Schlegel 1838), *S. imperialis* (Stefani 1969), *S. sarrabusensis* Lanza, Leo, Forti, Cimmaruta, Caputo & Nascetti 2001, and *S. supramontis* (Lanza, Nascetti & Bullini 1986) – are all endemic to Sardinia [see Di Nicola et al. (2021) and supplementary material in Corti et al. (2022) for distribution maps].

Speleomantes are terrestrial oviparous salamanders that exhibit direct development (Lanza et al., 2006; Lunghi et al., 2018b,c). Their peculiar physiological traits make them highly sensitive to their environmental conditions (Lanza et al., 2006; Lunghi et al., 2018b). In response to excessive heat or dryness, they typically seek refuge in subterranean habitats where a stable microclimate characterised by high humidity and cool temperatures can be found throughout the year. Consequently, sightings of European cave salamanders in above-ground settings are generally confined to the cooler and more humid periods of the year, especially at night or on rainy or foggy days (Lanza et al., 2007; Lunghi et al., 2015; Lunghi et al., 2018b). The subterranean habitats they utilise vary widely, ranging from temporary shelters such as under rocks, logs, or in the leaf litter, to larger cavities like burrows in the soil, karstic caves, and artificial structures including mines and cellars (Lanza et al., 2007; Di Nicola et al., 2021).

Several studies have extensively explored the dietary spectrum of these amphibians (e.g. Salvidio, 1992; Vignoli et al., 2006; Salvidio et al., 2012; Lunghi et al., 2018a,b; Lunghi et al., 2022; Casali et al., 2023; Cianferoni & Lunghi, 2023), while little documented information is available on their predators. Notably, predation on juvenile continental *Speleomantes* by the spiders *Meta menardi* (Latreille 1804) (Pastorelli & Laghi, 2006) e *M. bourneti* Simon 1922 (Lunghi & Corti, 2021) has been confirmed. Additionally, Gene (1839) reported the finding of a *S. genei* in the stomach of a small Sardinian grass snake *Natrix helvetica cetti* Gene 1839 near Iglesias, Sardinia, Lanza (1946, 1948) found a *S. ambrosii* in the stomach of a slow worm *Anguis veronensis* Pollini 1818 on the Apuan Alps, Tuscany, and Gerlach & Biggi (2022) documented a Barred grass snake *N. helvetica sicula* (Cuvier 1829) swallowing a *S. strinatii* near Genova, Liguria. The hypothesis of cannibalism is discussed in section 3.5 of Cianferoni and Lunghi (2023). Lastly, there are no documented reports of predation by birds or mammals (Salvidio et al., 2017).

In this study, we present the first evidence of predation on adult Sette Fratelli cave salamanders *Speleomantes sarrabusensis* by a Sardinian grass snake.

The Sardinian grass snake is a subspecies endemic to Sardinia, where it has a rather fragmented distribution, primarily in the highlands of the island's eastern and southern areas (Schultze et al., 2020; Di Nicola et al., 2021). A recent study by Di Nicola and colleagues (2023) has reviewed the taxonomy, morphology, and ecology of this taxon, provided updated distribution data, and modelled its potential range. Information on the distribution and ecology of *N. helvetica cetti* remains relatively insubstantial, possibly due to its elusive nature, low population density, and insufficient research effort. This has led to a limited knowledge of this snake habits. The diet of the Sardinian grass snake is among the least studied aspects, with information derived from a sample of only twelve food items found in nine snakes from a single area (i.e. Supramonte of Oliena, Nuoro Province). These included six adults and five tadpoles of *Hyla sarda* (de Betta 1853) and one *Podarcis tiliguerta* (Gmelin 1789) (Capula et al., 1994; Di Nicola et al., 2023).

On April 3rd, 2024, in the Sette Fratelli forest complex, South Sardinia (39°18' N, 9°24' E, WGS 84), the authors conducted a field survey aimed at screening for *Ophidiomyces ophidiicola* (Guarro,

Deanna A. Sutton, Wickes & Rajeev) Sigler, Hambl. & Paré, 2013, a pathogenic fungus affecting snakes that has not yet been detected in the Sardinia (see Di Nicola et al., 2022; Marini et al., 2023). The survey followed a transect along a dirt path through a *Quercus ilex* L. forest on a northwest-facing slope, covering a distance of 2.2 km round trip, with an altitudinal range between 420 and 730 m a.s.l.

During the survey, which lasted approximately 3 hours from 11 am to 2 pm, twelve snakes were observed, all basking along the path's edge, near shelters formed by rocks and plant material located at the edge of the forest on a sloping hillside. These included nine adult *N. helvetica cetti* (all temporarily captured to collect skin swabs, photo documentation, and routine measurements and then released; see Fig. 1 and Table 1) and three adult *Hierophis viridiflavus viridiflavus* (Lacépède 1789). When two or more snakes were found close together and captured simultaneously, they were placed in individual cloth bags to avoid possible cross-contamination and were temporarily kept there pending completion of health screening procedures on the other snakes. In one instance, upon removing a Sardinian grass snake from the bag, the individual regurgitated three prey items. The preys were three adult male individuals of *Speleomantes sarrabusensis*, one of which still complete (total length: 98 mm) and the other two missing their posterior parts, likely due to ongoing digestive processes. The sex was recorded by checking for the presence of the male mental gland (Lanza et al., 2006; Di Nicola et al., 2021). The condition of the cave salamanders indicated that regurgitation started from the head; hence, the consumption began from the tail. Given the circumstances, we believe the three individuals have been predated. The Sardinian grass snake responsible for the predation was an adult female showing melanistic colouration (total length: 512 mm; snout to vent length: 413 mm; tail length: 99 mm; No. of ventral scales: 169; No. of subcaudal scales: 55, plus the terminal scale) (Fig. 2).

All European cave salamander species produce toxic skin secretions, which are thought to act as a defence mechanism against predators (Lanza et al., 2006; Salvidio et al., 2017). The predation on *Speleomantes* provides additional confirmation of the grass snakes' (*N. natrix* complex) aptitude to prey on amphibian taxa capable of secreting relevant toxic substances, such as species from the genera *Bombina* Oken 1816, *Bufo* Garsault 1764, *Bufo* Rafinesque 1815 and *Salamandra* Garsault 1764 (see Luiselli et al., 2005; Rodríguez et al., 2017; Kowalski et al., 2018; Lüddecke et al., 2018; Laciak et al., 2023).

Such evidence of predation on cave salamanders in early April, observed in the Sette Fratelli forest complex, which comprises granite formations (Melis et al., 2017) and thus offers limited subterranean habitats compared to karst environments, suggests nocturnal activity of the Sardinian grass snake even during cool periods. Indeed, the predation event took place despite the relatively low minimum temperatures recorded that week (mean T_{min}: 10.0 °C, mean T_{max}: 19.7 °C; first week of April 2024), as similarly noted by Capula et al. (1994). Their survey in the Supramonte of Oliena (eastern Sardinia) revealed that individuals were active at night in water searching for prey when air temperatures were below 15 °C.

This finding represents the first documented evidence of predation on *Speleomantes sarrabusensis* by *Natrix helvetica cetti*, confirming the early observation by Gené (1839) of predation of *S. genei* and supporting the inclusion of cave salamanders of the genus *Speleomantes* in the diet of the Sardinian grass snake (cf. Lunghi et al., 2018d; Di Nicola et al., 2023), a previously overlooked food item.

The surveyed transect does not include any permanent watercourses in its immediate vicinity. However, for a large part of the year, rivulets and little pools appear along the trail, where the authors frequently observed adults and tadpoles of *Discoglossus sardus* Tschudi in Otth 1837. These frogs could possibly represent another amphibian prey for the Sardinian grass snake.

The present work emphasises the significant gaps that still exist in our understanding of the ecology of the Sardinian grass snake. Further field efforts are crucial not only for this localised snake, but also for more easily detectable species, such as European cave salamanders, for which knowledge about potential predators remains extremely limited.

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Table 1 - Key measurements of the nine observed *Natrix helvetica cetti*. The asterisk indicates the individual that regurgitated the three cave salamanders. / Principali misure delle nove *Natrix Helvetica cetti* osservate. L'asterisco indica l'individuo che ha rigurgitato i tre geotritoni.

Individual	Snout-Vent Length (mm)	Tail Length (mm)	Total Length (mm)	Tail to total Proportion	Subcaudal Scales	Sex
A	420	91	511	0.18	53 + terminal	F
B	329	84	413	0.20	59 + terminal	M
C	399	108	507	0.21	62 + terminal	M
D	459	80	539	0.15	49 + terminal	F
E	507	127	634	0.20	57 + terminal	M
F*	413	99	512	0.19	55 + terminal	M
G	444	111	555	0.20	59 + terminal	M
H	539	102	641	0.16	N/A (injured)	F
I	433	106	539	0.20	59 + terminal	M
Females	473 ± 60.7	91 ± 11.0	564 ± 68.4	0.16 ± 0.015	51 ± 2.8	F
Males	421 ± 58.4	106 ± 14.2	527 ± 72.1	0.20 ± 0.007	58.5 ± 2.3	M

Figures and Figure captions:



Fig. 1 - Dorsal pattern of the nine observed *Natrix helvetica cetti*. / Pattern dorsale delle nove *Natrix Helvetica cetti* osservate.



Fig. 2 – Melanistic female *Natrix helvetica cetti* with three freshly regurgitated *Speleomantes sarrabusensis*. / Femmina melanotica di *Natrix Helvetica cetti* con i tre *Speleomantes sarrabusensis* appena rigurgitati.