

Taxonomy, biology and natural history of the Milos viper *Macrovipera schweizeri* (Werner, 1935): literature review and observations on autumnal activity and importance of residual pools

(Serpentes, Viperidae)

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Di Nicola, M. R., Pozzi, A. V., Avella, I., Mezzadri, S. & Paolino, G. 2022. Taxonomy, biology and natural history of the Milos viper *Macrovipera schweizeri* (Werner, 1935): literature review and observations on autumnal activity and importance of residual pools (Serpentes, Viperidae). *Spixiana* 45(1): 111–120.

The Milos viper (*Macrovipera schweizeri*) is an endangered species restricted to the western side of the Cyclades archipelago, endemic to the islands of Kimolos, Milos, Polýaigos and Sifnos. This threatened viper has been at the centre of several studies aimed to better understand its peculiar biology and ecology. Through the extensive analysis of the available literature, here we provide a detailed summary of the current knowledge regarding taxonomic status, biogeography, morphology, reproductive biology, ecology, toxinology, and conservation of *M. schweizeri*. The major goal of this literature review is to condensate the outcomes of various studies and short notes, and to highlight differences and similarities between them. Furthermore, here we report some observational records regarding the daily activity pattern of *M. schweizeri* in early autumn and some considerations on the ecological importance of residual water bodies for this species.

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Introduction

The Milos viper (*Macrovipera schweizeri*) is a viperine snake endemic to the western Cyclades islands of Kimolos, Milos, Polýaigos and Sifnos (Greece, Fig. 1) (Adamopoulou et al. 1997, Sindaco et al. 2013, Nilson 2019). The species can be found from sea level up to 400 m a. s. l. (Nilson 2005, Kreiner 2007).

The largest *M. schweizeri* population lives on Milos, where Nilson et al. (1999) estimated a presence of less than 3000 individuals, mainly located in the western part of the island. In the past, several authors reported the presence of this viper also for Antimilos and/or assumed it to be present in Kithnos, too (see De Smedt (2006) for a summary). Nevertheless, records of *M. schweizeri* from these two islands remain unconfirmed. The overall limited distribution area, comprising just over 100 km², together with the illegal collection of individuals, direct persecution, roadkill and habitat loss has led to the classification of *M. schweizeri* as 'Endangered' by the IUCN Red List of Threatened Species (Böhme et al. 2009).

The natural history of the Milos viper has been investigated, directly or indirectly, in several studies over the past years. This has generated a considerable amount of information that we enclose in the present work, in order to provide a comprehensive summary of the information currently available and, in some cases, also to bring out differences between data presented by different authors. Furthermore, here we report some new ecological observations of the autumnal activity of *M. schweizeri* in the proximity of residual water bodies.

Material and methods

All available literature on the Milos viper (i.e., scientific articles, thematic books, guides) was gathered by consulting the PubMed and Scopus database, the social network ResearchGate, and by using the Google Scholar web search engine.

The data considered most relevant were critically analysed, and reported in a discursive, non-systematic way, in order to provide a homogeneous account of the species' biology.

The personal observations were made on Milos Island by two of the authors (MRDN and GP) on an occasional, unscheduled, and transect-free basis, during a 3-day field trip at the end of September 2021. The authors searched different spots with known presence of the Milos viper (choosing only areas along freely passable paths), in the different environmental types suitable for the species in shrublands and cultivated lands (e.g., dry stone walls, bushes, banks of seasonal streams, ponds). In order to investigate the presence of active individuals at different times of the day, surveys took place three times daily (i.e., during the morning, in the

afternoon, and in the early night). Surveys were entirely non-invasive, as the authors exclusively aimed to confirm the presence of active vipers. The search was performed by examining both the ground and suitable vegetation (e.g., shrubs).

Literature review

Taxonomic status

In ancient times, Plinius the Elder (AD 23–79) reported that the Greek island of Kimolos was infested by venomous snakes (Zwinnenberg 1979, De Smedt 2006). Plinius likely referred to the Milos viper, the only venomous snake currently present on the island. Bedriaga (1882) and Schweizer (1932, 1935) produced some of the earliest herpetological manuscripts clearly mentioning the presence of vipers in the Milos Archipelago, although not yet described as a distinct taxon from the Blunt-nosed viper *Macrovipera lebetinus* (Linnaeus, 1758). The new taxon was then described by Werner (1935) based on a specimen from Milos and two specimens from Sifnos, as a subspecies of the *M. lebetinus* (at that time *Vipera lebetina schweizeri*), without defining a type specimen. Curiously, Werner (1938) himself later referred to the vipers of the Milos archipelago as *Vipera lebetina lebetina*. More than a decade later, Mertens (1951, 1955) indicated Milos as the type locality for the taxon *schweizeri* and elected a lectotype (not according to Buchholz (1955), who proposed one of the two specimens from the island of Sifnos described by Werner in 1935 as a lectotype, and Sifnos as the type locality).

At the time of the original description, the Milos viper was considered as belonging to the genus *Vipera* Laurenti, 1768. Reuss (1927) described the genus *Macrovipera* with the type species *M. lebetina* and used it also for the original description of *M. lebetina cypriensis* (Reuss 1933), now considered *M. lebetinus lebetinus*. Schwarz (1936) treated *Macrovipera* as a synonym of *Vipera*. Obst (1983) considered the *Vipera* group too heterogeneous and separated the larger species by reconsidering the genus *Daboia* Gray, 1842 (that originally included *D. elegans*, *D. russelii* and *D. pulchella* and was later synonymised with *Vipera* by Strauch in 1869), in which he included the *lebetina* group. Following biochemical studies, Herrmann et al. (1992) revalidated the genus *Macrovipera*, indicating the following taxa: *M. lebetina*, *M. schweizeri*, *M. mauritanica* and *M. deserti*. Currently, the genus includes three species: *M. lebetinus*, *M. razii*, and *M. schweizeri* (Speybroeck et al. 2020, Uetz et al. 2022).

The taxonomic status of the Milos viper is still debated. Nilson & Andrén (1988) elevated the taxon to species rank on morphological basis, as later

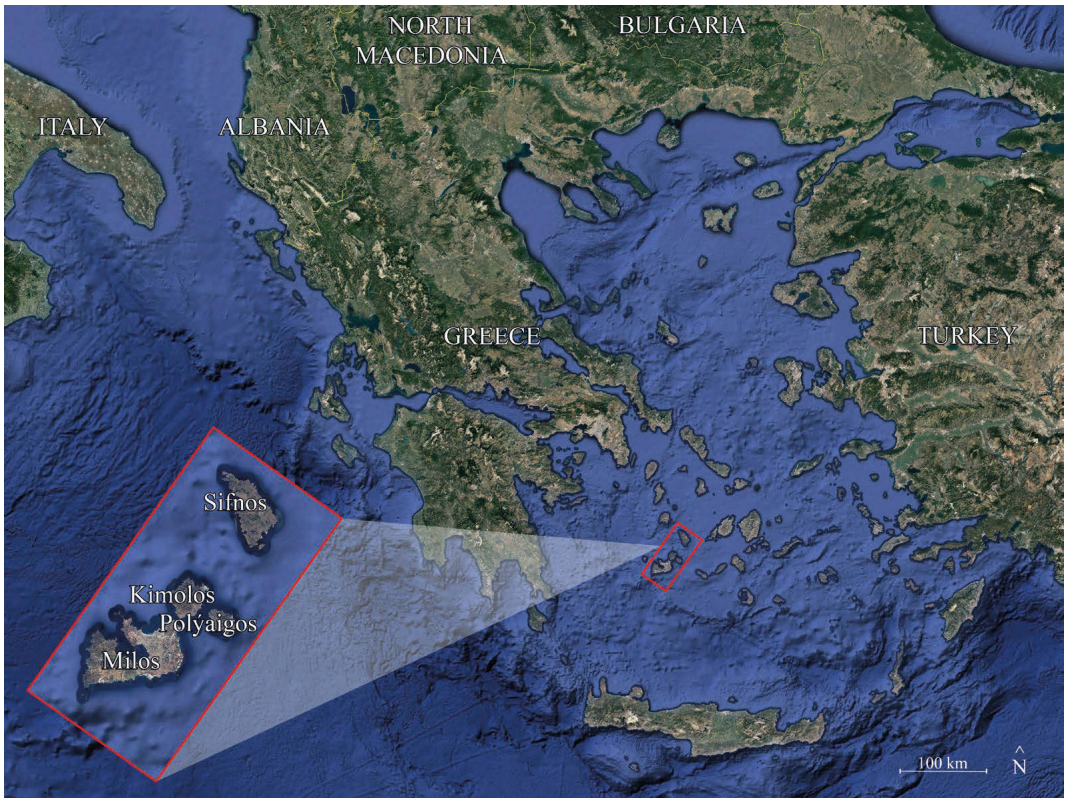


Fig. 1. Map of Greece. The red rectangle includes the islands of Kimolos, Milos, Polyaigos and Sifnos, where *Macrovipera schweizeri* lives. Map credits: Google Earth (modified).

confirmed by biochemical studies of Herrmann et al. (1992). However, based on the analysis of mtDNA, Stümpel & Joger (2009) and Stümpel (2012, PhD thesis) suggested to consider *M. schweizeri* as a subspecies of *M. lebetinus* (i. e., *M. l. schweizeri*). Due to these genetic data having yet to be published in a peer-reviewed journal, Speybroeck et al. (2020) suggested keeping the taxon at the species level, as done by several authors who recently cited the Milos viper in their works (e.g., Lymberakis et al. 2018, Thanou & Kornilios 2018, Yanenko et al. 2018, García-Arredondo et al. 2019, Nilson 2019, Šmíd & Tolley 2019, Zollinger et al. 2019, Cattaneo 2020, Pizzigalli et al. 2020, Chowdhury et al. 2021a, Degen & Brock 2021, Chowdhury et al. 2022, Di Nicola et al. 2022, Kontsiotis et al. 2022, Tzoras et al. 2022). Nevertheless, Freitas et al. (2020) suggested again to consider *M. schweizeri* as a subspecies of *M. lebetinus*, having found a genetic distance of only 2% (cyt-b) between the two taxa in the phylogenetic analyses they performed. Moreover, some authors proposed to separate the populations of Sifnos from the others.

Wettstein (1952) described *Vipera lebetina siphnensis*, mainly based on a different number of dorsal scale rows of four specimens from bibliographic data (25 rows instead of the 23 normally present), but the considered characteristic was not reliable (see Schweizer 1957, Cattaneo 2020); Cattaneo (1989, 2020) and Mallow et al. (2003) suggested the re-consideration of the subspecies *siphnensis* based on chromatic, dimensional and pholidotic characters. Nilson (2005) hypothesised a possible specific rank consideration for the populations of Sifnos, on the basis of a 1.6 million year separation between this island and Milos. Sindaco et al. (2013) indicated the presence of the nominal and *siphnensis* subspecies. Nilson (2005, 2019) deferred to any future taxonomic decisions: "...Wettstein separated the Siphnos population as a separate subspecies, *Vipera lebetina siphnensis*, which if accepted should be *Macrovipera schweizeri siphnensis*".

Anyhow, in the absence of proven, consistent differences (e.g., genetics-based) between the populations of Sifnos and those of the other islands,

the validity of a potential *M. s. siphnensis* is highly questionable.

The complete mitochondrial genome of *M. schweizeri* (total length: 17 152 base pairs) has been assembled through next-generation sequencing by Thanou & Kornilios (2018), and could provide clearer insight into the taxonomic status of this taxon.

Hypothesis on the origins

Viperids are relatively abundant in fossil records, although mainly confined to the Neogene (23.03–2.58 mya) and often consisting of nothing more than isolated vertebrae. Because of this, viper fossils are associated with extant taxa from derived lineages, thus not helping to understand the beginning of the group's evolutionary history (Szyndlar & Rage 1999, Zaher et al. 2019). To date, the oldest fossil record traced back to a viperid is a fang dating back to the early Miocene (21 to 23 mya) found in Germany, attributed to *Proviper* *boettgeri* Kinkelin, 1892 (Szyndlar & Rage 2002, Zaher et al. 2019). Considering the type of fossil, it is not possible to safely attribute it to a viperine or a crotaline (Szyndlar & Rage 2002). Other fossils from more or less the same period, attributable with greater certainty to viperine snakes, belong to *Vipera antiqua* Szyndlar, 1987a (from Germany and the Czech Republic) and to another possible species of the same genus from France (Szyndlar & Rage 2002).

Regarding the “oriental vipers” (i.e., genera *Montivipera* and *Macrovipera*), there are different fossil records dating back to the early Miocene (see Appendix II in Szyndlar & Rage (2002) and Supplementary Table S3 in Šmíd & Tolley (2019) for a summary).

In particular, an extinct taxon believed to be an ancestor of the “oriental vipers” is *Vipera platyspondyla* Szyndlar, 1987b, with findings from Germany and Czech Republic (17–18 mya). Other extinct taxa attributable to *Macrovipera* spp. are known, like *M. burgenlandica* (Bachmayer & Szyndlar, 1987) from Austria (8.5–8.9 mya), *M. gedulyi* (Bolkay, 1913) from Hungary (5.3–6.2 mya), *M. kuchurganica* (Zerova, 1987) from Ukraine (4.8–5.2 mya), and an unidentified *Macrovipera* sp. (Venczel & Stiuca 2008) from Romania (11.8–12 mya) (Šmíd & Tolley 2019). Also interesting is the discovery of a *Vipera* sp. from Spain (5.0–5.3 mya) attributable to the *Macrovipera*/*Montivipera* clade (Bailon et al. 2010, Šmíd & Tolley 2019).

No fossils attributable to the genus *Macrovipera* have been found in Greek territory (Georgalis & Delfino 2022).

The viperine (Viperinae Oppel, 1811a,b) also known as ‘true vipers’, ‘pitless vipers’ or ‘Old World vipers’, are a subfamily of viperids found only in Africa, Asia, and Europe (Phelps 2010, Alencar et

al. 2016), comprising about 100 species belonging to 12 genera (Uetz et al. 2022), with a long and often complex taxonomic history. Many authors have applied different approaches to try to frame them at taxonomic level (see Freitas et al. 2020) and reconstruct their evolutionary history. Currently, discrepancies exist between the results obtained by the application of the two main approaches, i.e., paleontological and molecular (Szyndlar & Rage 2002). According to Šmíd & Tolley (2019), who used the modern fossilised birth-death model (Heath et al. 2014) to calibrate the tree of modern viperine snakes, diversification within this subfamily started at the Eocene/Oligocene boundary (about 34 mya), and its biogeographic origin is still unclear.

Eurasian vipers constitute a monophyletic group within the subfamily Viperinae, comprising species distributed mainly in the Palaearctic region, and currently divided into four genera: *Daboia*, *Macrovipera*, *Montivipera*, and *Vipera* (Šmíd & Tolley 2019, Freitas et al. 2020). The diversification within Eurasian vipers started more than 20 mya (see Šmíd & Tolley 2019 and Freitas et al. 2020). Among them, large-bodied species belonging to the genera *Daboia*, *Macrovipera* and *Montivipera* have been identified as a separated group, and generally named “Oriental vipers” (Szyndlar 1987b, 1988). This group cannot be defined as a clade, due to lack of morphological, biochemical, and/or phylogenetic evidence (Šmíd & Tolley 2019), and currently does not include the genus *Daboia* (see Szyndlar & Rage 1999, 2002, Rage & Bailon 2005, Rage & Danilov 2008).

Depending on the authors, different divisions are applied within Eurasian vipers. For example, Šmíd & Tolley (2019) divide the group into two clades: one including the genera *Macrovipera* and *Montivipera*, and one including the genera *Daboia* and *Vipera*. Freitas et al. (2020) instead indicate a clade including *Vipera*, *Macrovipera*, and *Montivipera*, and another comprising only *Daboia*.

However, there appears to be consensus on the phylogenetic relationships within the *Macrovipera*/*Montivipera* clade: the diversification between the two genera would have occurred about 13 mya, less than 9 mya within the genus *Macrovipera*, and about 3 mya between *M. lebetinus* and *M. schweizeri* (see Šmíd & Tolley 2019 and Freitas et al. 2020).

Considering that the western Cyclades islands have been separated from mainland Greece since the Pliocene (Dermitzakis 1989), it might be that vipers of the Milos archipelago underwent geographical isolation for 4–5 mya, thus differentiating themselves from the mainland populations (Nilson 2019). Another possible scenario considers that, during the Pliocene, the Aegean region was sectioned by wide sea channels, with the Cyclades probably still con-

nected to mainland Greece by several land bridges, which disappeared when the rise in sea level reduced the Cyclades to islands. About 300 000 years ago, during the Mindel-Riss interstadial period, the connection with Asia Minor was also finally interrupted, completely separating *M. schweizeri* from the surrounding populations of *M. lebetinus*, thus leading to the diversification of these two taxa (De Smedt 2006).

Morphology

As all members of the genus *Macrovipera*, *M. schweizeri* is a robust, thickset viper. The species presents a large head, clearly distinct from the neck, and a characteristically rounded snout; the eyes are relatively small, with the vertical elliptical pupil typical of Viperidae (De Smedt 2006, Kreiner 2007, Valakos et al. 2008, Cattaneo 2020) (Fig. 2A,B).

The average and maximum total lengths reported in the literature for the Milos viper vary slightly between different publications. According to Schweizer (1957), Arnold & Burton (1978), Adamopoulou et al. (1997), and De Smedt (2006), adults of this species reach up to 80 cm of total length (TL). De Smedt (2006) also indicates that adult individuals measure on average 65–75 cm, and that captive individuals can exceed one metre (as in the case of an individual reported by Schweizer (1949), measuring 120 cm). Nilson (2005, 2019) reports a maximum TL of 98.5 cm on Milos, and of 107 cm on Sifnos; Kreiner (2007) reports the maximum total lengths indicated by Nilson (2005), and comments that wild specimens rarely exceed 85 cm of TL, as also indicated by Kwet (2016). Valakos et al. (2008) report a TL of 1.5 m (without specific references); Speybroeck et al. (2016), in the *Macrovipera lebetinus* ssp. sheet, report total lengths of up to 70 cm and, occasionally, 100 cm for European individuals. Finally, Cattaneo (2020) provides more in-depth data: the total lengths of 21 wild males from Milos ranged between 64.5 and 83.2 cm, while the TL of 17 wild females of the same origin went from 50 cm to 68.4 cm. From Milos, the author reports a 97 cm TL for a 684 g male with incomplete tail, and a 73 cm TL for a 252 g female. From Sifnos, the TL records in the wild are 107 cm for a male (660 g) and 88 cm for a female (720 g), both with an incomplete tail. For captive individuals from Milos, the record is 127.8 cm for a male weighing 1485 g with an incomplete tail, and 89 cm for a 502 g female. For captive specimens from Sifnos, the record TLs are 145.3 cm for a male (1820 g) and 112.4 cm for a female (995 g). Cattaneo (2020) also provides some data about the total length of newborn Milos vipers: the TL of 18 newborn captive individuals from Milos ranged between 18.7 and 21.1 cm; 3 individuals from Sifnos had TLs of 21, 22, and 22.5 cm.

The Milos viper has keeled scales on the dorsum and head, with the exception of the temporal scales, which are mainly smooth. The head shields are completely fragmented in small scales, including the supraoculars (Arnold & Ovenden 2002, Nilson 2005, De Smedt 2006, Kreiner 2007). Both in the populations of Milos and Sifnos, there are usually 23 rows of dorsal scales at midbody, more rarely 19–25 (Nilson 2005, Kreiner 2007, Cattaneo 2020). De Smedt (2006), based on Zwijnenberg (1979) and Nilson & Andr n (1988), compiled a table with the following mean pholidotic values ($n=14$): 8.3 interoculars; 29.5 first row circumoculars; 30.5 second row circumoculars; 14.6 loreals; 25.8 sublabials (for the last 4 parameters, the sum of the values of both sides of the head is considered). Also, he reports a range of 142–160 ventrals and 40–45 pairs of subcaudals for males, and a range of 148–157 ventrals and 33–41 pairs of subcaudals for females.

Kreiner (2007), based on Nilson (2005), reports the following pholidotic values: 2–3 subocular shields between orbit and supralabials; 10 (rarely 11) supralabials; 140–164 ventrals; 40–47 pairs of subcaudals for males and 38–44 for females; anal scute undivided. According to Nilson (2019), the ventrals are 142–163 in males and 148–164 in females. Finally, the following data are presented by Cattaneo (2020): 33–39 intersupraoculars; 13–19 intercanthals; 2 canthals per side; 2 apicals; 2–3 supraoculars per side; 10–11 supralabials per side; 12–14 sublabials per side; 14–16 circumoculars per side; 7–12 longitudinal series of scales between the eyes. For individuals from Milos: 144–158 ventrals in males ($n=22$), 151–155 in females ($n=8$); 41–45 pairs of subcaudals for males ($n=12$), 40–43 for females ($n=6$). For individuals from Sifnos: 154–163 ventrals in males ($n=14$), 155–158 in females ($n=5$); 37–44 pairs of subcaudals for males ($n=8$), 37–42 for females ($n=4$).

The dorsal colouration of the Milos viper is quite variable, and generally dull (Arnold & Ovenden 2002). The background colour is usually light grey (Kreiner 2007, Nilson 2019) (Fig. 2C), but it can present different tonalities (e.g., bluish-grey, beige, sandy, rosy, brownish, reddish-brown, dark brown, blackish; De Smedt 2006, Kreiner 2007, Nilson 2019). The dorsal-lateral pattern is usually made up of 4 rows of subretangular crossbars, with the two central ones joined on the dorsal midline and irregularly alternating (Fig. 2C). These can be grey, yellowish, olive, reddish, or brown/dark brown (Arnold & Ovenden 2002, Kreiner 2007, Nilson 2019). Some individuals have a finely dappled pattern along the whole body (De Smedt 2006). Reddish, orangish, and brownish individuals with reduced or non-visible dorsal pattern (Fig. 2D) and even melanistic

individuals have been recorded (Dimitropoulos 1992, De Smedt 2006, Kreiner 2007, Nilson 2019), the latter probably rarer than “monochromatic” ones (De Smedt 2006). The belly has a whitish/light grey background colour, irregularly speckled with black to reddish (Arnold & Ovenden 2002, Kreiner 2007) (Fig. 2E,F). The terminal part of the tail is often orangish/yellowish (De Smedt 2006, Kreiner 2007, Cattaneo 2020) (Fig. 2G,H). Males are usually darker than females, and their pattern (when present) is typically more contrasted (Arnold & Ovenden 2002, Kreiner 2007, Cattaneo 2020).

According to Kreiner (2007), juveniles seem to be a little darker than adults, but they do not differ in pattern. Arnold & Ovenden (2002) describe the juveniles as usually blue-grey with dark olive cross-bars, the two central ones usually not meeting across the back. For juveniles from Milos born in captivity, Cattaneo (2020) reports a grey background colouration, with a very conspicuous dark brown pattern appearing just after the postnatal moult.

Cattaneo (2020) also indicates chromatic differences between the vipers from Sifnos and individuals from other populations. Specifically, the author argues that the individuals of Sifnos ultimately display two main background colourations, namely an ochre one and a more greyish one. Furthermore, Cattaneo comments that in Milos, in May, he only found brownish-grey individuals, which became greyish in July right after moulting while kept in captivity. At last, the author found Sifnos vipers to be generally paler and duller in colour, to the point that the dorsolateral crossbars often turned into shaded blotches with undefinable outlines.

Ecology

The vast majority of the ecological aspects of *M. schweizeri*, such as habitat use, diet, foraging ecology and movement patterns, are strongly influenced by seasonal changes (Cattaneo 1989, Nilson et al. 1999).

Milos vipers inhabit a wide variety of different habitats, spanning from highly vegetated areas with rocky outcrops, to cultivated fields, to well-exposed hills and riverbeds (Stubbs 1985, Arnold & Ovenden 2002, Valakos et al. 2008). This viper is mostly found in open shrublands characterised by the presence of *Pistacia lentiscus*, *Genista acanthoclada*, *Juniper phoenicea*, *Nerium oleander*, *Myrtis communis* and *Olea europaea*, surrounded by flowering plants such as *Cistus x incanus* and *Thymus capitatus* (Stubbs 1985, Nilson et al. 1999, Nilson 2019, Cattaneo 2020). A long-term study led by Nilson et al. (1999) on Milos highlighted a strong relationship between the presence of *M. schweizeri* and large bushes (over 10 m²).

Specifically, 77% of the 125 observed vipers were found by the author within such large shrubs, or in immediate proximity of their edges.

Furthermore, during hot days, individuals were observed seeking shelter underneath rock piles located within these large bushes (Nilson et al. 1999). The authors also documented the almost total absence of vipers in areas devoid of these bushes, further highlighting the importance of this kind of vegetation for *M. schweizeri*. At the same time, vipers were also not present in areas with excessive vegetation, probably because lacking microhabitats suitable for thermoregulation (Nilson et al. 1999, Nilson 2019). Interestingly, specimens on the island of Sifnos were observed using different habitat types from those exploited by the vipers on Milos. In fact, Cattaneo (1989, 2020) reported that, on Sifnos, vipers were almost always encountered in the proximity of dry stone walls surrounded by low vegetation and by bushes of *Pistacia lentiscus*. According to the author, these man-made structures would provide the reptiles with shelter and food resources. The recent observation of an adult *M. schweizeri* foraging along one of these constructions in proximity of the coast of Sifnos might provide support to this hypothesis (Degen & Brock 2021).

During spring and autumn, due to alterations in prey availability and composition dictated by seasonal changes, the habitat use of *M. schweizeri* changes significantly (Nilson et al. 1999, Nilson 2019). The vipers are in fact known to converge along water sources (e.g., rivers and rock pools) in spring and subsequently along dry streambeds neighboured by large shrubs of *Nerium oleander*, *Pistacia lentiscus*, *Myrtus communis* and small trees later in autumn (Stubbs 1985, Nilson et al. 1999, Valakos et al. 2008, Nilson 2019). The association between water sources and vipers of the genus *Macrovipera* was also investigated in *M. l. lebetinus* on Cyprus island, but with negative results (Jestrzowski & Kuzyakova 2019).

The Milos viper, as the majority of the members of the subfamily Viperinae, is considered to be mainly a diurnal species (Cattaneo 1989, Valakos et al. 2008, Phelps 2010). Nevertheless, seasonality deeply affects the species activity patterns (Arnold & Ovenden 2002, Valakos et al. 2008, Nilson 2019). During early spring and autumn, individuals from both Milos and Sifnos were observed thermoregulating during the day, between 9:00 and 12:00 am, with the peak of activity at 10:00 am (Cattaneo 1989). Despite this, only a small proportion (less than 10%) of the 7 specimens radio-tracked by Nilson et al. (1999) on Milos was actually observed exposed basking in open grounds. The authors stated that vipers were in fact mainly encountered while “bush basking” within or at the edges of large shrubs. “Bush basking” appears



Fig. 2. *Macrovipera schweizeri* details: **A.** Head in dorsal view; **B.** head profile; **C.** dorsal colouration in a standard individual; **D.** dorsal colouration in a reddish individual; **E.** ventral colouration in a standard individual; **F.** ventral colouration in a reddish individual; **G.** tail colouration (ventral) in a standard individual; **H.** tail colouration (dorsal) in a reddish individual. Photo credits: Matteo R. Di Nicola.

to be a very common behaviour among Milos vipers in spring and autumn, and it's been recorded in 23.5% of 166 encounters (Nilson et al. 1999). With the increase of the mean day temperatures, during late spring, summer, and early autumn, *M. schweizeri* shifts to an almost completely crepuscular and nocturnal activity (Even & Pijnappel 1989, Arnold & Ovenden 2002, Nilson 2019, Cattaneo 2020). During daytime, with temperatures often exceeding 30°C, an almost total lack of viper activity was recorded. Vipers were instead found active both at dawn and at night when the average temperature reached 20°C, mainly along roads (Cattaneo 1989, Nilson 2019). Exceptions to this general trend were recorded. Between the 6th and the 15th of May 1983, Cattaneo (1989) observed multiple times on Milos, specimens of *M. schweizeri* thermoregulating in the sun on open grounds. Similarly, an adult individual was found foraging along a dry stone wall on Sifnos island on the 16th of May 2019, at 15:52 circa (Degen & Brock 2021). These observations might be correlated with the beginning of the mating season for *M. schweizeri* (see below the paragraph on the species' reproductive biology).

Even some phenotypic traits of the Milos viper seem to be affected by seasonal changes. During spring, individuals of this species (especially males), seem to reach the peak of a colour-change process starting in autumn, where the overall body colouration becomes darker and reddish. Cattaneo (1989) reported that this "spring colour phase" disappears in summer with shedding. The author hypothesised that such seasonal colour change might allow the vipers to better thermoregulate during early spring and autumn. Similar seasonal colour patterns have been described in other snake species (e.g., Banks 1981, Johnston 1994, Boback & Siefferman 2010). In winter, *M. schweizeri* hibernates underground in hibernacula located on the sides of south-facing hills. Nevertheless, on the 6th and the 7th of February 1995 three males were observed basking at the entrance of their respective hibernacula, with air temperature between 12.5 and 14°C (Nilson et al. 1999).

Another major aspect of the Milos viper's ecology highly impacted by seasonality is its foraging ecology and behaviour (Arnold & Ovenden 2002, Degen & Brock 2021). *Macrovipera schweizeri* seems to be characterised by two distinct feeding cycles: one in which resident birds are the main prey and one linked to the arrival of migratory birds (Nilson et al. 1999, Nilson 2019). The first feeding cycle takes place in late spring/early summer, when vipers disperse along the valley slopes and spend the majority of their time within large bushes. Here, they appear to mainly feed on local resident birds inhabiting these bushes, such as *Sylvia melanocephala*,

Phylloscopus trochilus, *Phoenicurus phoenicurus*, and *Muscicapa striata* (Nilson et al. 1999). The finding of an adult specimen of *S. melanocephala* within the gut's contentment of a 60 cm long *M. schweizeri* on the 16th of May 1983 seems to confirm this behaviour (Cattaneo 1989). The second feeding cycle is divided into two different "sub-cycles", both correlated with the arrival of migrating birds: one taking place in early spring, and the other taking place in autumn (Nilson 2019, Degen & Brock 2021). During spring, Milos vipers can be found in high densities along rivers and other water sources, where migratory birds gather (Nilson et al. 1999, Nilson 2019). Here, the vipers often lie in ambush with their head on top of rocks and the rest of the body kept in a serpentine position (Nilson 2019). Interestingly, while surveying for *Podarcis erhardii* on Sifnos, Degen & Brock (2021) observed an adult *M. schweizeri* actively foraging along a dry stonewall in a hot and windy afternoon in late spring (16th of May). The viper was observed poking its head in the cracks between the stones making up the wall, likely in search of prey. This episode, which happened on a dry trail distant by sources of freshwater, seems to be in contrast with the above-mentioned spring foraging behaviour observed on Milos Island. A completely different foraging behaviour is shown by *M. schweizeri* during the second "sub-cycle" in autumn, when the vipers come back to the water sources previously exploited during the spring, which usually dry out with the summer heat (Nilson et al. 1999). In the absence of water bodies, the vipers have been observed climbing up small trees located within the proximities of the dry water sources, in order to prey on passerine birds (Nilson et al. 1999, Arnold & Ovenden 2008, Degen & Brock 2021). While on the trees, vipers usually position themselves on the lower branches (1.5–2 metres high), with the head pointing downward and the rest of the body kept in a serpentine position along the branch, with the tail pressing on the tree trunk (Nilson et al. 1999, Nilson 2019). Once a bird has been caught, the viper holds it tightly in its jaws, to reduce any potential chance of escape (Nilson et al. 1999, Cattaneo 2020). This peculiar arboreal foraging behaviour usually takes place between 19:00 and 23:00 circa and almost exclusively in autumn (Nilson et al. 1999). In fact, Nilson et al. (1999) reported that no vipers were located in ambush position on trees during spring, and only one specimen was observed on a tree in July, while 50% of the vipers found in September 1994 were spotted in ambush position on trees.

As its foraging behaviour, also the diet composition of the Milos viper appears to be affected by seasonality. *M. schweizeri* seems to feed almost entirely on passerine birds, both nesting and migrant

(Nilson et al. 1999, Nilson 2019). Within the stomach of adult vipers, Nilson et al. (1999) reported solely the presence of bird feathers, even if the stomachs of a good part of the analysed specimens resulted to be empty. Juvenile specimens, on the other hand, seem to mainly feed on lizards (Arnold & Ovenden 2002). Analysis of the stomach contents of juvenile vipers (length <35 cm) by Nilson et al. (1999) highlighted the presence of different lizard species, such as *Podarcis milensis*, *Ablepharus kitaibelii*, and *Lacerta trilineata hansschwweizeri*'s eggs. The authors thus suggested that the diet of *M. schweizeri* changes ontogenetically, with juveniles feeding on lizards and adults feeding almost exclusively on passerine birds, and seldomly on rodents.

Milos vipers have evolved in an ecosystem lacking native mammals, and rodents such as *Rattus rattus* and *Mus musculus* were introduced by humans in fairly recent times (Nilson et al. 1999). Due to the absence of native mammals, *M. schweizeri* could have thus evolved a strong preference for foraging on birds, as shown in other island vipers (Shine et al. 2002, Marques et al. 2012, Luiselli et al. 2015). Furthermore, the marginal role of mammals in the diet of *M. schweizeri* could be linked to the low density at which these occur. Surveys by Legakis et al. (1997) reported the presence of a single male *M. musculus* found on Milos on the 20th of April 1997, and nine specimens of *R. rattus* found between the 20th of April and the 26th of June 1997. Similarly, 199 night-traps placed on Milos captured only 5 individuals of *M. musculus*, while just a few specimens of *R. rattus* were observed while surveying at night for vipers (Nilson et al. 1999). Nilson et al. (1999) reported that no other mammals were encountered on the island between 1993–1998, except for *Erinaceus roumanicus*. Despite that, the presence of other mammals such as *Oryctolagus cuniculus*, *Lepus europaeus*, and small insectivores such as *Suncus etruscus*, often found in multiple sites, was reported in the past (Legakis et al. 1997). Moreover, according to Nilson et al. (1999), *M. schweizeri* is so dependent on birds as main food source that the body mass index (BMI) of the studied individuals seemed to vary greatly during the years due to the availability of migratory birds, in turn affected by yearly climatic conditions. Indeed, Nilson et al. (1999) reported that in 1993 vipers were found in poor body conditions (BMI=0.141, n=11 vipers), while their situation seemed to have significantly improved in the years from 1994 to 1997 (BMI=0.184–0.257, n=21–36 vipers), just to decline again in 1998 (BMI=0.149, n=8 vipers) (Nilson et al. 1999). The authors suggested that unfavourable climatic conditions affected birds' migratory routes and presence on the island in 1993 and 1998, thus leading vipers to starvation.

Data from different sources just partly support the dietary habits of *M. schweizeri* described by Nilson et al. (1999). The analysis of the gut contents of 12 preserved specimens of *M. schweizeri* collected in spring on Milos revealed that small mammals represented 42.8% of the total prey items, followed by *Podarcis milensis* (21.4%) and unidentified passerine birds (7%) (Adamopoulou et al. 1997). The authors further stated that insects, mainly Coleoptera, made up 28.4% of the total prey items, and that these were exclusively found in small vipers (TL <52 cm). The presence of invertebrates in the stomachs of juvenile Milos vipers was hypothesised to be due to secondary ingestion rather than intentional feeding (Adamopoulou et al. 1997). In accordance with the results of Nilson et al. 1999, the stomach content of 9 specimens of *M. schweizeri* (length circa 65–93 cm) found on Milos during May 1983 revealed the presence of 8 passerine birds, one *Podarcis milensis* (found in the stomach of a juvenile viper), and only 2 rodents (Cattaneo 2020). Despite that, the same analysis highlighted the presence of 2 specimens of *Lacerta trilineata* within the stomach content of two adult vipers. Additionally, examinations of the gut content of 6 specimens of *M. schweizeri* (length 82.2–107 cm) found on Sifnos in 1981 and 1985, revealed the presence of 4 rodents and 2 passerine birds (Cattaneo 2020). On Kimolos, Broggi (2014) observed a specimen of *M. schweizeri* feeding on a mouse. The precise time of this predatory event is unknown, but it likely took place in April. Finally, an adult *M. schweizeri* was seen actively foraging on a dry stone wall with high densities of *Podarcis erhardii* (9 individuals within a wall section of 10 metres, Degen & Brock 2021). In captivity, *M. schweizeri* is known to feed on different prey items, mainly rodents and birds (Cattaneo 1989).

A further aspect of the ecology of *M. schweizeri* influenced by seasonality is its movement ecology (Cattaneo 1989, Nilson et al. 1999, Nilson 2019). Based on data obtained by long-term tracking (14–24 months), Nilson et al. (1999) showed that the home range of six *M. schweizeri* males was characterised by a triangular shape with a hibernaculum in the centre, two of the corners located within a streambed and the third corner positioned at the top of a valley's northern slope. Differences in both movement patterns and home ranges seem to be evident between males and females, with the former having larger home ranges and covering longer distances, especially at the beginning of the reproductive season (Cattaneo 1989, Nilson et al. 1999). The home ranges of male vipers seem to measure between 10 and 20 ha, while females seem to occupy home ranges of about 2 ha. The extension of the females' home ranges was estimated by the analysis of several recapture events of two speci-

mens by Nilson et al. (1999). Mean daily movements, recorded as the distance travelled at night between two consecutive days, was estimated at 28.5 m (178 observations, distance range 0–266 m) (Nilson et al. 1999). Vipers seemed to travel longer distances following the first encounter with the investigator (mean distance 71.86 m), compared to the second consecutive encounter (mean distance 25.39 m) (Nilson et al. 1999). Daily mean movements appear to change considerably during the year. Data from six radio-tracked males highlighted that the vipers moved significantly more in May (63–89 metres/day) and September (40–66 metres/day) compared to the other months of activity (10–40 metres/day) (Nilson et al. 1999).

From the data and observations reported above, it seems possible that differences in habitat use and other ecological aspects occur between the vipers of Milos and Sifnos.

Information about other, less studied aspects of the ecology of *M. schweizeri* are generally provided by sporadic observations. For example, Cattaneo (2020) reported the presence of parasitic nematodes and acari on a few individuals of *M. schweizeri* on Milos. The same author also described the defensive behaviour of Milos viper, capable of raising the front part of its body while hissing and expanding its ribs. This “cobra-like” behaviour was also performed by a male individual tracked by Nilson et al. (1999). Similar defensive postures are assumed also by other large viperids (e. g. genera *Bitis* and *Crotalus*). Data about the potential predators of *M. schweizeri* are currently scarce. The presence of large birds of prey, such as *Aquila chrysaetos* and *Buteo buteo*, known to feed on snakes as part of their diet (Sergio et al. 2002, Collins & Latta 2009) has been reported on Milos (Legakis et al. 1997). Despite that, records of avian predation on *M. schweizeri* are currently absent. Two potential (unspecified) predation events on *M. schweizeri* have been recorded by Nilson et al. (1999) on the only radio-tracked female in 1994, and then on a tracked male in 1995. Two further predation events upon radio-tracked vipers took place in 1997. During these latter events, the recovered transmitters presented evident scratch marks. The Western Cyclades lack any native mid-large size mammals, but a feral colony of cats seems to be established on western Milos. Introduced feral cats may predate on *M. schweizeri* and likely represent a threat especially to the survival of young and sub-adult individuals (Nilson et al. 1999, Nilson 2019).

Reproductive biology

Due to the secretive nature of snakes, their reproductive habits and biology are often hard to investigate, typically requiring long-term monitoring, as well as

insights from captive settings (Slip & Shine 1988, Clark et al. 2014). These data are fundamental for understanding not only the natural history of these reptiles, but also to create and implement successive conservation strategies, both in-situ and ex-situ (Braz et al. 2019).

The information regarding the reproductive biology of *M. schweizeri* has been gathered from multiple long-term studies of wild populations (Cattaneo 1989, 2020, Nilson et al. 1999, Nilson 2019) and captive bred individuals (Schweizer 1949, 1957, Perry & Blody 1986, Cattaneo 1989), as well as from the analysis of museum specimens (Adamopoulou et al. 1997).

Interestingly, *M. schweizeri* is the only European member of the family Viperidae characterised by an oviparous reproductive mode (Adamopoulou et al. 1997, Arnold & Ovenden 2002, Valakos et al. 2008). It is in fact estimated that the vast majority of vipers are live-bearing (about 80% of currently known viperids, Wüster et al. 2008). Oviparity is a trait common to all the members of the genus *Macrovipera*, and strong evidence suggests that it was also shared by the ancestor of the *Macrovipera*–*Montivipera* clade (Fenwick et al. 2012). It is likely that the members of the genus *Montivipera* have independently evolved a viviparous reproductive mode, as happened multiple times along the evolutionary history of Viperidae (Wüster et al. 2008), as a potential result of environmental and climatic pressures (Lynch 2009).

The breeding season of *M. schweizeri* is reported to begin in early May. Between March and April, prior to the beginning of the mating season, males shed their skin, thus potentially triggering the breeding phase and the process of spermatogenesis (Cattaneo 1989, 2020). Three females with developing eggs were observed on Sifnos between the 17th and the 25th of May 1985 by Cattaneo (1989). The same author observed the beginning of the reproductive activity on Milos between the 6th and the 15th of May 1983. This phenomenon was reportedly highlighted by an extreme vagility of male vipers. Analysis of four female specimens of *M. schweizeri* collected in May on Milos, stored and preserved in the herpetological collections of the Museum Koenig in Bonn and the Natural Museum of Vienna, revealed the presence of developing eggs in three of them (Adamopoulou et al. 1997). Further and more direct evidence regarding the temporal setting of the mating season of the Milos viper was obtained from the long-term monitoring project of Nilson and colleagues (Nilson et al. 1999, Nilson 2019). In the years between 1994 and 1997, the authors observed several instances of courting and mating, which took place between the 5th and 18th of May. On the 5th of May 1994, a radio-tracked male was spotted

within a thick bush courting a female. A significant courtship activity was then observed on the 18th of May 1996 when a male was recorded curled on the back of a female while intensively flicking his tongue and tapping the female's body with its head. After that event, on the 12th of May 1995, another pair of vipers was caught mating within the same area of the last described event. The same place was also the setting of another courtship event observed by Nilson and colleagues in May 1997. Interestingly, the abovementioned location where the majority of the courtship observations described by Nilson et al. (1999) took place, was characterised by the presence of a rock pool, which may highlight the importance of natural water reservoirs for the reproductive biology of these vipers.

Macrovipera schweizeri seems to be characterised by a single reproductive season (Nilson et al. 1999), a trait shared with *M. lebetinus*, even if extra-seasonal egg-laying, potentially due to long-term sperm storage or embryonic diapause, has been observed in the latter (Iskenderov 2021). Such post-copulatory mechanisms and extra-seasonal clutches have not been recorded in the Milos viper.

Taking into consideration the timing of male shedding and the unimodal reproductive cycle, Cattaneo (2020) stated that *M. schweizeri* is potentially characterised by a “berus” reproductive cycle (sensu Saint Girons 1976). Within this reproductive cycle, spermatogenesis begins at the end of spring and continues through autumn. Following hibernation, the process of spermiogenesis starts, and thus the sperm cells are produced in time for the mating season (Aldridge et al. 2020).

Based on the fact that about 50 % of the females caught by Nilson and colleagues during the reproductive seasons in the years between 1993 and 1997 appeared to be gravid, it has been suggested that *M. schweizeri* is characterised by a biennial reproductive mode (Nilson et al. 1999, Nilson 2019). A biennial reproductive cycle seems to be very common among European vipers (Nilson & Andr n 1983, Bonnet & Naulleau 1996, Luiselli & Zuffi 2002, Strugariu et al. 2011). Regarding *M. schweizeri*, the implementation of such reproductive mode may be due to its specialised diet and fluctuations in prey availability, which are likely to not provide females with enough fat storage to reproduce during every mating season (Cattaneo 2020) (see ecology paragraph).

Following reproduction, the eggs are initially incubated within the female body, allowing in this way the fast development of the embryos and a relatively low extracorporeal incubation time (Cattaneo 1989). Female Milos vipers have been reported to lay their eggs towards the end of July, after moulting (Cattaneo 2020). Milos vipers typically lay small

clutches consisting of 4–11 eggs (Arnold & Ovenden 2002, Valakos et al. 2008, Nilson 2019). Schweizer (1949, 1957) reported three depositions from three different females, two laying 7 eggs each, and one laying 11 eggs. Adamopoulou et al. (1997) revealed the presence of 11, 6, and 4 eggs in three gravid museum specimens. On Milos and Sifnos, Cattaneo collected four gravid females that later laid between 7 and 10 eggs each (Cattaneo 2020).

At deposition, eggs measure 33–47 mm in length and 24–25 mm in width (Schweizer 1949, 1957, Perry & Blody 1986, Cattaneo 1989). Adamopoulou et al. (1997) recorded egg lengths between 12.9 and 31.5 mm, but the smaller size of the eggs discovered within the preserved females is likely to be attributed to their underdeveloped status. Cattaneo (2020) highlighted differences in size and shell thickness between the eggs laid by Milos and Sifnos individuals, with the latter producing larger eggs with a thicker eggshell.

Captive data show that juveniles hatch about 30–50 days after deposition (Cattaneo 1989). Incubation temperature appears to strongly influence the incubation period, where eggs incubated at 29.5°C took 31 days to hatch, while eggs incubated at a temperature of 26.5°C took up to 50 days (Cattaneo 1989, 2020).

Nilson et al. (1999) did not find any hatchlings during their long-term study but reported that the smallest individual found was 18 cm in length, and probably a year old. Data from the observations of captive specimens from Cattaneo (1989, 2020) report offspring lengths between 18.7 and 20.1 cm for the Milos individuals, and lengths between 21 and 22.5 cm for the specimens from Sifnos. According to the author, juveniles go through their first shed 11–14 days after hatching (Cattaneo 1989).

Hatchlings survival rate under natural circumstances is unknown, but it has been estimated that just 10 % of them reach adulthood (Nilson 2019). Schweizer (1949, 1957) reported that, in captivity, around 50 % of the eggs survived the incubation process. Higher estimates arose from the captive monitoring of Perry & Blody (1986), who reported that 70 % of the eggs were fertilised and subsequently hatched. Similar data came out from the work of Cattaneo (1989), which showed a hatching rate between 40 % and 84 %.

Venom

To date, little is known about the venom of *M. schweizeri*. This apparent neglect is likely attributable to this species' limited distribution, and to its little incidence in terms of snakebite accidents (Cattaneo 2020). Reports of envenomations caused by the Milos viper are, in fact very rare, and do not high-

light any major symptoms or fatal outcomes for the bitten subjects (Nilson 2005). This viper is currently included in the World Health Organization's list of medically important snake species and is considered of secondary medical importance because of the seemingly limited threat it poses to human health (World Health Organization 2020).

Old World vipers (subfamily Viperinae) typically possess mainly haemotoxic and cytotoxic venoms, containing high amounts of snake venom metalloproteinases (SVMPs), snake venom serine proteases (SVSPs), phospholipases A₂ (PLA₂s), L-Amino acid oxidases (LAOs), and C-type lectin-like proteins (CTLs), together with many other toxins (e.g. disintegrins, cysteine-rich secretory proteins, etc.) (Damm et al. 2021). The venom composition of the Levantine viper *M. lebetinus*, arguably the most studied species of the genus *Macrovipera*, appears to mirror this same general trend (Bazaa et al. 2005, Sanz et al. 2008, Igci & Demiralp 2012). Considering the very close phylogenetic relationship existing between *M. lebetinus* and *M. schweizeri* (Freitas et al. 2020), the venoms of these two species might be quite similar in composition. Given the lack of detailed data about the composition of the latter's venom, however, this consideration is purely speculative.

Early studies on the venom of *M. schweizeri* indicate that it presents kininogenase, arginine esterase, and trypsin-like activities, which are generally correlated with inflammation, hypotension, and coagulopathy symptoms (Al-Joufi et al. 1991, 1994, Bilbis et al. 1999). These findings are concordant with the description of severe local tissue damage and systemic coagulopathy caused by envenomations by species belonging to the genus *Macrovipera* (Mallow et al. 2003, Göçmen et al. 2006, Valenta et al. 2019). Specifically, recent studies have shown the venoms of four *M. lebetinus* subspecies (i.e. *M. l. cernovi*, *M. l. lebetinus*, *M. l. obtusa*, *M. l. turanica*) and *M. schweizeri* to be potentially procoagulant (Chowdhury et al. 2021a,b), as a consequence of SVMPs activating blood coagulation factor X, and thus inducing systemic haemodynamic disturbances (Siigur et al. 2001, Chowdhury et al. 2021a). Interestingly, the venoms of these taxa presented different factor X activation rates, with *M. schweizeri* having the lowest ones (Chowdhury et al. 2021a). Furthermore, a recent study investigating the taxonomically specific neurotoxicity of several genera of Palearctic vipers found that, while the venoms of three *M. lebetinus* subspecies (i.e. *M. l. cernovi*, *M. l. obtusa*, *M. l. turanica*) strongly targeted amphibian mimotopes, *M. schweizeri* venom presented a higher affinity for the lizard mimotopes (Chowdhury et al. 2022). Although a comprehensive characterisation of the venom of *M. schweizeri* is currently lacking, these

results suggest that it might be quite different from the venoms of other *Macrovipera* species, at least in terms of biochemical activity.

Conservation

Island endemic species, due to their limited geographic ranges and complex evolutionary histories, and to the precarious equilibrium of island environments, are significantly more threatened by extinction compared to mainland taxa (Simberloff 2000, Gerlach 2008, Russell & Kueffer 2019). Despite accounting for less than 6% of the planet's surface, islands represent central hotspots for biodiversity harbouring almost 50% of the species globally classified as threatened (Walker & Bellingham 2011, Spatz et al. 2017). Habitat loss and the introduction of non-native species, followed by climate change and overexploitation, represent the main threats to island endemics (Johnson et al. 2017, Russel & Keuffer 2019).

The distribution of *M. schweizeri* is limited to the western side of the Cyclades archipelago, where the species can be found on the islands of Milos, Sifnos, Kimolos, and Polyaiagos (Phelps 2010). The global number of specimens seems to be estimated around three thousand individuals, with most of them concentrated in the western part of Milos (Böhme et al. 2009). Surveys conducted within an area of 1 km² of optimal habitat in Milos' Chalepa valley between 1994 and 1996 yield 50 adult individuals of *M. schweizeri* (Nilson et al. 1999). Taking into consideration detectability rate and the number of encountered specimens, the authors estimated a density of 0.5 viper per ha of optimal habitat. While on the unexploited islands of Kimolos and Polyaiagos this species seems to not face any relevant external threats (Nilson et al. 1999), the Milos population of *M. schweizeri* has declined dramatically during the past decades (Böhme et al. 2009, Nilson 2019). Habitat loss and alteration have been the major threats for the survival of Milos viper on the homonym island (Buttle 1993, Nilson et al. 1999).

The main cause of habitat loss in Milos must be attributed to the mining activity that has interested the island for almost 10 thousand years (Nilson et al. 1999, Lichrou & O'Malley 2006). The first testimonies of mining on the island date back to the Neolithic, when the Aegean civilization began to extract the Milos obsidian in order to produce rudimentary weapons and tools (Renfrew et al. 1965). Further evidence showed that manufactures made up of Milos obsidian were traded across the eastern side of the Mediterranean Sea and Anatolia, highlighting the presence of a solid commercial network and the economic importance of this igneous rock for the

Aegean civilization (Kuzmin et al. 2020). A significant increase in the exploitation of the mineral resources of Milos took place after the Second World War (Stefanakis 2018). Currently, the mining sector accounts for 30% of the island's domestic product (Tzintzos 2013). Founded in 1939, S&B (Silver & Baryte Ores Mining Co. S.A) established itself as the major player in the local mining industry, making Milos the largest producer of bentonite in the EU (Stefanakis 2018). In its five-years-long study, Nilson et al. (1999) raised concern about the impact of mining activity on the habitat of *M. schweizeri*, especially in the western part of Milos Island. The authors argued that the presence of two large and expanding quarries on this side of the island represent a serious threat to the survival of the species, especially in the absence of subsequent habitat recovery plans. Furthermore, the authors highlighted how the potential expansion of the mining activity on the western side of Milos represents the major threat to the habitat of *M. schweizeri*. The deleterious effects of mining activity on reptiles and amphibians, causing habitat loss/alteration, and increasing individuals' displacement and mortality, have been widely reported in the literature (Pedler 2010, Sasaki et al. 2016, Marrugo-Negrete et al. 2019, Myani-Parás et al. 2019). In order to reduce the long-term impacts of mining on the herpetofauna of Milos, S&B has put at the centre of its activity policy the restoration of disused quarries (Kurbanov & Verzhinia 2018, Stefanakis 2018). A clear example of the S&B's quarries restoration program is represented by the case of the Chivaldolimni site. Comparison of the reptile communities between this restored perlitic mine and an undisturbed area showed that the nine-year reclamation process has led to the comeback of a rich and diverse assemblage of reptiles (including *M. schweizeri*), at a level almost identical to that of the untouched site (Adamopoulou & Legakis 2006). Nilson et al. (1999) also highlighted how, on Milos, vehicular traffic, strongly correlated with the mining activity, constitutes another significant threat to the survival of *M. schweizeri* on the island. Similarly, Broggi (2000) underlined the threat posed by the extensive and busy road network of Milos on the survival of *M. schweizeri*, speculating that this threat might represent a bigger issue than mining-related habitat loss. Snakes and other reptiles often use roads as thermoregulation sites, typically staying motionless on the pavement using it as a source of heating, especially at night. This behaviour very often leads to direct mortality from contact with vehicles (Mccardle & Fontenot 2016). On Milos, *M. schweizeri* seems to highly exploit roads' pavement for thermoregulation during the night, especially during the warmer periods of the year (Nilson et al. 1999). Road

surveys performed from 1993 to 2006 highlighted that between 183 to 537 vipers (more than 10% of the overall population) were killed annually on the roads of Milos (Nilson 2019). Similar data were reported for the period between 1993 and 1997, where around 300 vipers were estimated to die annually on the road network of Milos (Nilson et al. 1999). Considering these findings, in 2005 the local authorities proceeded with the installation of a system of road tunnels and concrete barriers in the proximity of three crossing interest points previously identified (Yannis 2011). Subsequent surveys in 2006 highlighted the absence of viper casualties on the road in the vicinity of the road tunnels, showing that 77% of the specimens that encountered an underpass used it to cross the road with a frequency of 0.8–1.8 passages per day (Yannis 2011, Nilson 2019). Noteworthy, roads can have other negative effects on reptiles, for example by inducing habitat fragmentation and consequential loss of gene flow (Clark et al. 2010).

Within the secondary causes of habitat loss in Milos, Nilson et al. (1999) highlighted the threats posed by wildfires and intensive grazing. The authors pointed out how uncontrolled wildfires could be an issue for the survival of *M. schweizeri* in Milos, due to the destruction of the vegetation structures representing vital habitat for migrating birds during spring and autumn. As already discussed before, birds represent a major component of the diet of the Milos viper at least during part of the year, and a decrease in their abundance could thus lead to a decline of the vipers (Nilson et al. 1999, Andrén et al. 2007). Following the impact of wildfires, overgrazing could also interfere with the reforestation process needed to restore the habitat of both *M. schweizeri* and its prey items (Nilson et al. 1999). At the same time, moderate grazing could potentially be useful for habitat management, especially within areas with excessive vegetation density (Nilson et al. 1999), although the role of grazing as an effective habitat management tool for reptiles is controversial (Reading & Jofré 2015, 2016, Worthington-Hill & Gill 2019, Mizsei et al. 2020).

Other anthropogenic pressures threatening the Milos viper depend on how humans perceive this species. In the first place, *M. schweizeri* is very popular in the pet trade, and illegal collection has menaced the survival of this species on Milos for various decades (Buttle 1993, Nilson et al. 1999). Fortunately, in recent times the number of poached specimens seems to have declined from almost one thousand to less than 100 individuals per year (Dimitropoulos 1992, Nilson 2019). On the other side, the locals are typically afraid of this venomous snake, a phenomenon that led to the formation of a bounty hunter system that survived until 1977 (Nilson 2019). A similar phenomenon seems to have taken place



Fig. 3. Residual pool where 5 different Milos viper individuals were observed. Photo credits: Matteo R. Di Nicola.

also on Sifnos (Cattano 2020). Intensive hunting represented the major cause of decline and extirpation for other venomous snakes, such as the timber rattlesnake *Crotalus horridus* in North America and Canada (Furman 2007). Following the acquisition of legal protection in 1981 (Nilson 2019), the number of *M. schweizeri* specimens directly killed by humans strongly declined. Specifically, Nilson et al. (1999) report that, over a time frame of four years, they found just less than five specimens intentionally killed by humans. Nevertheless, Nilson will later contradict himself stating that “specimens are still killed in significant numbers”, implicitly declaring that around 100 individuals may be killed by humans yearly on Milos (Nilson 2019). This latter data seems to be supported by the attitude of locals towards *M. schweizeri* and its conservation. In 2003, a written inquiry to the European parliament highlighted the opposition of Milos professionals and stakeholders to the development of a “viper reserve”, as Milos vipers were considered a serious threat for both public health and the island’s economic growth (Trakatellis 2003). Furthermore, the same negative attitude emerged from a study conducted on a demographic and social heterogeneous sample of over one thousand Greek people, where the conservation of the Milos viper, perceived as “ugly” and dangerous, was largely not supported (Liordos et al. 2017).

Currently, *M. schweizeri* is classified as Endangered by the IUCN, even if the available assessment needs to be updated (Böhme et al. 2009). Despite the unique ecology and limited distribution of this species, and its current IUCN status, the Milos viper was not included in the list of viper species for

global conservation priorities compiled by Maritz et al. (2016). In fact, the current *M. schweizeri* population trends seem stable, mostly thanks to the legal protection of habitats on the western side of Milos under the Natura2000 scheme. A major role is played, of course, also by the legal protection of the species itself, currently inserted in annex II of the European directive 92/43/EEC as a priority species, in annex IV as a species of Community interest, and also in annex I and annex II of the Berne Convention (Trakatellis 2003, Böhme et al. 2009, Nilson 2019, Cattaneo 2020).

Observational data

Between 24th and 26th of September 2021, a short field survey aimed at observing individuals of *M. schweizeri* active in the wild was carried out on the island of Milos by authors MRDN and GP.

The three days were characterised by clear weather and variable wind (from 0 to 28 km/h), and followed by a long period of drought (according to the Hellenic National Meteorological Service, Milos suffered a total lack of rainfall in the summer of 2021). The long drought was also confirmed by seasonal streams being completely dry and almost devoid of residual pools. The only exception in the surveyed sites was a small residual pool with a maximum size of about 5 m×1 m, and an average depth of less than 30 cm (Fig. 3).

A total of six active individuals of *M. schweizeri* were observed, with one individual being encountered twice – for a total of seven encounters. Details of each encounter are described below.



Fig. 4. *Macrovipera schweizeri* individuals observed “in situ” near the residual pool during the day. Photo credits: Matteo R. Di Nicola.

The first record is from the 24th of September, at 8:58 h (sunny weather, $T=19^{\circ}\text{C}$, wind = 17 km/h). An individual with uniform, reddish colouration, likely a female (TL = ~50 cm), was observed moving among the stones of a completely dry seasonal stream bed (approx. coordinates: 36.66, 24.40; elev. 67 m a. s. l.) (Fig. 2D). In the stretch of stream visited there was no residual water, and the only other vertebrates encountered in activity were Kotschy’s geckos (*Mediodactylus kotschyi*, $n=2$) and Milos wall lizards (*Podarcis milensis*, $n=5$).

The second, third, and fourth records are also from the 24th of September, and were all registered at 12:05 h (clear weather, $T=22^{\circ}\text{C}$, wind = 17 km/h). Three individuals, one likely a female (TL = ~50 cm) and two males (TL = ~40 cm), all with standard colouration, were observed on oleander (*Nerium oleander*) branches less than a meter from the ground, with the body partly in shade. The three vipers were about 3 meters from each other, all located around the residual pool mentioned above (approx. coordinates: 36.67, 24.34, elev. 44 m a. s. l.) (Fig. 4A, B, C).

The fifth and sixth records are from 24 September, at 22.00 h (clear weather, $T=18^{\circ}\text{C}$, wind = 9 km/h). One of the individuals from the third record (recognized by photo identification) and another one a

couple of meters from the former have been observed in nocturnal activity. They were still on oleander branches about 60 cm from the ground, close to the residual pool mentioned above (the “in situ” photos are not available since the snakes suddenly dropped to the ground due to our presence).

The seventh and last record is from the 26th of September, at 9:05 h (clear weather, $T=22^{\circ}\text{C}$, wind = 15 km/h). An individual, likely a female (TL = ~50 cm) was observed on the ground, almost completely in the shade, near the shore of the already mentioned residual pool (Fig. 4D).

The afternoon of September 24th and the whole day of September 25th were dedicated to surveys in other spots such as beds of other seasonal streams, dry stone walls and banks of large water reservoirs. No active snakes were observed.

The residual pool where the observations of September 24th and 26th were made represented the only trace of water found in the seasonal streams we visited. Several vertebrates were present there for watering: Milos wall lizards and passerine birds such as Sardinian warblers (*Sylvia melanocephala*), spotted flycatchers (*Muscicapa striata*) and red-breasted flycatchers (*Ficedula parva*) were observed near the pond during the day; a rodent (probably *Mus mus-*

culus) on the shore and an unidentified mammal on the branches of a tree adjacent to the pool were observed in the evening.

As reported in the paragraph “Ecology”, *M. schweizeri* prey on lizards, birds, and occasionally mammals. The presence of several Milos vipers on the vegetation at a short height above ground level, both at day and night, around the residual pools, is compatible with ambush predation activity.

Our field observations show how residual pools likely represent important habitats for *M. schweizeri*, as they seem to host considerable concentrations of prey, and might thus constitute favourable feeding grounds for the vipers, likely both at day and night.

Acknowledgements

The authors would like to thank Bobby Bok, Mirko Galuppi and Grégoire Meier for their advice.

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