Herpetology Notes, volume 10: 205-208 (2017) (published online on 25 April 2017)

# A glitch in the *Natrix*: cryptic presence of alien grass snakes in Switzerland

Sylvain Dubey[1,2,*], Sylvain Ursenbacher[3,4], Johan Schuerch[1], Joaquim Golay[1], Pascale Aubert[5] and Christophe Dufresnes[2]

**Abstract.** We report the occurrence of alien grass snakes (*Natrix natrix* ssp.) in Western Switzerland (Lausanne), at a locality where striped grass snakes, presumably from Croatia (*N. n. persa*), escaped from outdoor enclosures of a reptile park in the 1970s, within the natural range of the threatened barred grass snake (*N. n. helvetica*). Three gravid females were captured at the introduction site in 2016: two exhibited colour patterns typical of native *N. n. helvetica* and the third had an intermediate phenotype. Yet, their neonates featured diverse colour patterns, several typical to *N. n. persa*. Genetic analyses identified two distinct mitochondrial alien lineages: one specific to *N. n. persa*/*cypriaca* and one specific to south-Alpine *N. n. helvetica* (genetically unrelated to native Western Swiss *N. n. helvetica*). These results suggest that alien grass snakes of multiple origins have established sustainable populations at the introduction site for the past 40 years, and potentially hybridized and introgressed with the local taxa. Hence, it alarms on the captive breeding practices of potentially invasive reptiles in animal parks, and adds to a long list of uncontrolled herps' introductions, a major threat to biodiversity in Western Europe.

**Keywords:** Colubridae, Europe, Invasive species, mitochondrial DNA, Reptiles

## Introduction

The difficulty to detect invasive species depends of numerous factors, such as morphological similarities with local taxa (Dubey et al., 2014), low densities (Fitzpatrick et al., 2009), cryptic behaviours (Mazzotti et al., 2015), or remote distribution ranges (Lass et al., 2002). In some circumstances, alien taxa hybridize with local ones resulting in individuals with intermediate phenotypes. Their challenging detection requires sophisticated tools such as genetic analyses (e.g. Dufresnes et al., 2017). Even large size alien taxa can thus remain unnoticed for decades, which might have important ecological consequences and prevents their efficient eradication in early stages (e.g. Dufresnes et al., 2016).

In this respect, about 20-30 alien striped grass snakes [*Natrix n. persa* (Pallas, 1814)] from Western Croatia (Rijeka) escaped from the outdoor enclosures of a reptile park in the 1970s in Lausanne, Western Switzerland (J. Garzoni, pers. comm.), but were never detected by local authorities or specialists the following years. In the region, only the barred grass snake [*N. n. helvetica* (Lacépède, 1798)] is naturally present (Fig. 1), where this species is considered threatened (Status "Vulnerable" in the Swiss red List of reptiles; Monney and Meyer, 2005). These two large colubrids (>1.20m) differs in colour patterns: *N. n persa* is characterized by two white or yellow stripes running down the back (additional dark spots can also be present) with highly variable lateral patterns, whereas *N. n helvetica* presents dorsal dark spots only (stripes are absent) with lateral patterns of one row or more of dark bars, which can be fragmented in spots (Kreiner, 2007). In order to assess the presence of alien grass snakes in this area,

[1] Hintermann & Weber SA, Rue de l'Eglise-Catholique 9b, 1820, Montreux, Switzerland
[2] Department of Ecology & Evolution, Biophore Building, University of Lausanne, 1015, Lausanne, Switzerland
[3] KARCH, Passage Maximilien-de-Meuron 6, 2000, Neuchâtel, Switzerland
[4] Department of Environmental Sciences, Section of Conservation Biology, University of Basel, St. Johanns-Vorstadt 10, CH-4056 BASEL, Switzerland
[5] Ville de Lausanne, Service des parcs et domaines, Avenue du Chablais 46, CP 80, 1000 Lausanne, Switzerland
* Corresponding author e-mail: dubey@hintermannweber.ch

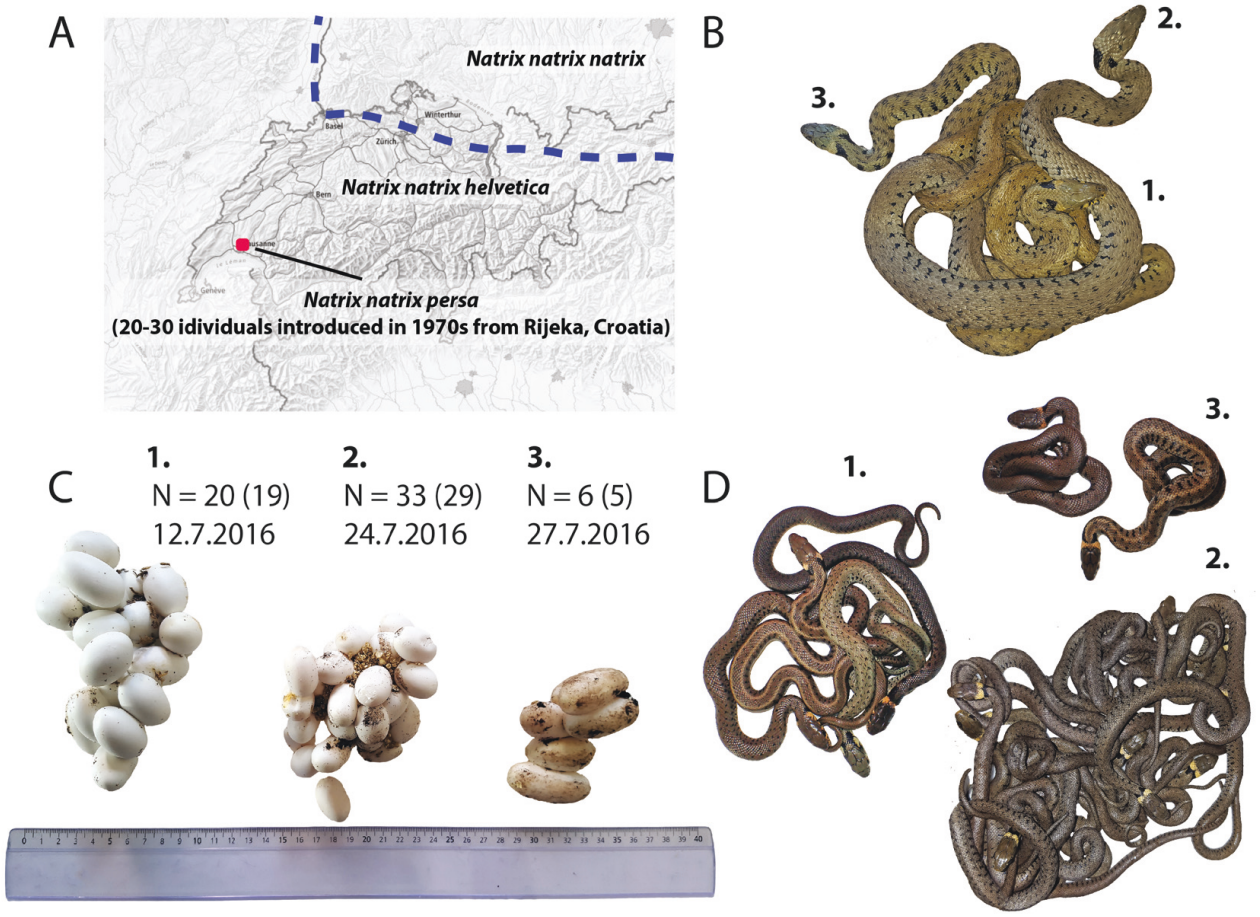


**Figure 1.** (A) Localisation of introduced *N. n. persa* presumably from Rijeka (Croatia) in Switzerland in 1970s (red dot) and distribution of autochthonous subspecies (*helvetica* and *natrix*; (B) adult gravid females captured in 2016 at the introduction site; (C) their corresponding clutches (with number of eggs and hatchling [between brackets]) and egg laying date; (D) Colour variation among some of their juveniles.

we conducted a field survey and genotyped captured individuals at a mitochondrial marker.

## Methods

### *Sampling*

The introduction site (Lausanne's former vivarium, 46.5427° 6.6423°) was surveyed over spring and summer 2016. Snakes were caught by hand and tissue samples for genetic analyses were taken with buccal swabs. Gravid females were kept in captivity till they laid their eggs and clutches were incubated at 29°C.

### *Genetic Analyses*

Total genomic DNA was obtained from small pieces of one ventral scale. DNA extraction was performed with the DNeasy Blood & Tissue Kit (Qiagen). We amplified and sequenced ~840 bp of the NADH dehydrogenase subunit 4 gene with the adjacent region coding for tRNA-His, tRNA-Ser and tRNA-Leu (ND4 + tRNAs) with primers used in previous phylogenetic studies of grass snakes and close relatives (Guicking et al., 2006; Fritz et al., 2012; Kindler et al., 2013). PCR thermal conditions consisted of 40 cycles of 45" at 94°C (denaturation), 45" at 55°C (annealing), and 60" at 72°C (elongation), followed by 10' at 72°C (final elongation). The PCR was performed with a Mastercycler Pro (eppendorf) using the *Taq* PCR Core Kit (Quiagen) in 25 µl reaction volumes with 3 µl DNA template, 0.5-1 U *Taq* polymerase, 1×buffer, 2 M $MgCl_2$, 0.2 mM dNTP, 2mg/ml Q solution and 0.5 µl of each primers. Sequencing was outsourced to Macrogen Europe (Amsterdam, the Netherlands).

**Results and Discussion**

Three gravid grass snakes individuals were captured in July 2016, two exhibiting colour patterns very similar to the native barred grass snake (*N. n. helvetica*, Fig 1B; individual 1 and 2) and one with an abnormal number of dorsal dark spots (Fig 1B; individual 3). However, none of these adult snake featured colouration typical of striped grass snakes (*N. n. persa*). Individuals 1 to 3 laid 20, 33, and 6 eggs, resulting in 19, 29, and 5 neonates respectively (Fig. 1B and C). Interestingly, several neonates did not exhibit maternal morphotypes: some presented dorsal stripes typical of *N. n. persa* (5 from individual 1; 1 from individual 3), or intermediate to both subspecies (abnormal number of dorsal dark spots with an absence of stripes). See fig. 1D for example of colour polymorphism found in hatchlings.

Genetic analyses revealed that individuals 1 and 2 (Genbank KY973647-48) belong to the mitochondrial lineage shared by *N. n. persa* from Eastern Europe/ Anatolia and *N. n. cypria* from Cyprus (Clade 7 in Kindler et al., 2013; 99% identity to the closest sequences). Individual 3 (Genbank KY973649) belongs to a lineage of *N. n. helvetica* restricted to Northern Italy and Southern Switzerland (Ticino) (Clade C in Kindler et al., 2013; 99% identity to the closest sequence). Note that *N. n. helvetica* native to western Switzerland (Clade 3 in Kindler et al., 2013) are genetically unrelated to those from Italy and Swiss Ticino (Clade C). Consequently, at least two alien mitochondrial lineages coexist at the introduction site.

Altogether, these results suggest the following scenario: (1) escaped individuals, originating from at least two southern regions (and not only Western Croatia, as assumed), (2) successfully survived and reproduced in western Switzerland and (3) they likely hybridized and introgressed with local *N. n. helvetica*. Indeed, more than 40 years after the introduction, most of them feature *N. n. helvetica* characteristics but *N. n. persa* phenotypes are still produced, as shown by the morphological characteristics of some of the juveniles (Figure 1).

Documenting the hybridization history and thus the exact taxonomic nature of these snakes will require genetic analyses of nuclear markers. Especially, it will inform on whether they result from crosses between (a) autochthonous *N. n. helvetica* × alien *persa*, (b) alien *N. n. helvetica* × *persa*, or (c) a mix of these three different lineages. In any case, our findings raise serious concerns for the conservation of *N. n. helvetica* in western Switzerland, as well as on the way to manage this population. Given the strong dispersal potential of grass snakes over the Swiss Plateau (large home range: on average 40 ha, Wisler et al., 2008; favourable landscape matrices enabling gene flow, Meister et al., 2010), the distribution of *N. natrix* alien lineages may have expanded outside the introduction site, stressing for a detailed regional survey.

In a more general context, species or subspecies of herps (reptiles and amphibians), from southern Europe (mainly Italy) seem tolerant to current climatic conditions in Western Switzerland, despite their Mediterranean origins. Numerous alien (and often invasive) taxa are present in this area, such as dice snakes (*Natrix tessellata*, Dubey et al., 2015), whip snakes (*Hierophis viridiflavus carbonarius*, www.karch.ch), Italian wall lizards (*Podarcis muralis nigriventris*, www.karch.ch; see Schulte et al., 2013, for similar data concerning Germany), Italian crested newts (*Triturus carnifex*, Dufresnes et al., 2016), meridional smooth newt (*Lissotriton vulgaris meridionalis*, www.karch.ch), Italian tree frogs (*Hyla intermedia,* Dubey et al., 2006; Dufresnes et al., 2015), Italian pool frogs (*Pelophylax bergeri*, Dubey et al., 2014; Dufresnes et al., in press), Levant green frogs (*Pelophylax bedriagae* Dubey et al., 2014), and Balkan water frogs (*Pelophylax kurtmuelleri* Dubey et al., 2014). Most of these species are currently replacing their local counterparts through competition, introgressive hybridization or predation.

As a conclusion, our study highlights the issue of careless captive breeding of exotic reptiles, especially when it involves species susceptible to establish wild populations. It also emphasizes that the origins of these animals may often be misinformed. These two aspects stresses for strict actions by relevant authorities to better control captive breeding in reptile parks. Moreover, this new case of introduction further contributes to the precarious situation of native wildlife in Western Switzerland, which can unfortunately be considered as a hotspot of alien herp diversity.

**Acknowledgments.** We thank the city of Lausanne for supporting this project and funding the field survey and J. Garzoni and former collaborators of the vivarium of Lausanne for helpful comments and support. The snakes were collected with the authorization of the 'Direction générale de l'environnement du Canton de Vaud' (No. 2362). We also thank N. Perrin (University of Lausanne) for support.

**References**

Dubey, S., Ursenbacher, S., Fumagalli, L. (2006): Origin of tree frog (*Hyla* spp.) populations in western Switzerland. Revue Suisse de Zoologie **113**: 879–887.

Dubey, S., Leuenberger, J., Perrin, N. (2014): Multiple origins of invasive and "native" water frogs (*Pelophylax* spp.) in Switzerland. Biological Journal of the Linnean Society **112**:442-449.

Dubey, S., Christe, P., Formenti, V., Staub, E., Schuerch, J., Glaizot, O., Ursenbacher, S. (2015): Introduced freshwater blenny influences the diet and body condition of the invasive dice snake in Lake Geneva. Journal of Wildlife Management **79**: 338-343.

Dufresnes, C., Dubey, S., Ghali, K., Canestrelli, D., Perrin, N. (2015): Introgressive hybridization of threatened European tree frogs (*Hyla arborea*) by introduced *H. intermedia* in Western Switzerland. Conservation Genetics **16**: 1507-1513.

Dufresnes, C., Pellet, J., Bettinelli-Riccardi, S., Thiébaud, J., Perrin, N., Fumagalli, L. (2016): Massive genetic introgression in threatened northern crested newts (*Triturus cristatus*) by an invasive congener (*T. carnifex*) in Western Switzerland. Conservation Genetics **17**: 839-8346.

Dufresnes, C., Di Santo, L., Leuenberger, J., Schuerch, J., Mazepa, G., Grandjean, N., Canestrelli, D., Perrin, N., Dubey, S. (2017): Cryptic invasions of Italian pool frogs (*Pelophylax bergeri*) across Western Europe unraveled by multilocus phylogeography. Biological Invasions **19**: 1407–1420.

Fitzpatrick, M.C, Preisser, E.L., Ellison, A.M., Elkinton, J.S. (2009): Observer bias and the detection of low-density populations. Ecological Applications **19**: 1673-1679.

Fritz, U., Corti, C., Päckert, M. (2012): Mitochondrial DNA sequences suggest unexpected phylogenetic position of Corso-Sardinian grass snakes (*Natrix cetti*) and do not support their species status, with notes on phylogeography and subspecies delineation of grass snakes. Organisms Diversity and Evolution **12**: 71-80.

Guicking, D., Lawson, R., Joger, U., Wink, M. (2006): Evolution and phylogeny of the genus *Natrix* (Serpentes: Colubridae). Biological Journal of the Linnean Society **87**: 127-143.

Kindler, C., Boehme, W., Corti, C., Gvoždík, V., Jablonski, D., Jandzik, D., Metallinou, M., Široký, P., Fritz, U. (2013): Mitochondrial phylogeography, contact zones and taxonomy of grass snakes (*Natrix natrix*, *N. megalocephala*). Zoologica Scripta **42**: 458-472.

Kreiner, G. (2007): The snakes of Europe: all species from west of the Caucasus Mountains. Chimaira Edition, Germany.

Lass, L.W., Thil, D., Shafii, B. Prather, T.S. (2002): Detecting spotted knapweed (*Centaurea maculosa*) with hyperscpectral remote sensing technology. Weed Technology **16**: 426-432.

Mazzotti, F.J., McEachern, M., Rochford, M., Reed, R.N., Eckles, J.K., Vinci, J., Edwards, J., Wasilewski, J. (2015): *Tupinambis merianae* as nest predators of crocodilians and turtles in Florida, USA. Biological Invasions **17**: 47-50.

Meister, B., Hofer, U., Ursenbacher, S., Baur, B. (2010): Spatial genetic analysis of the grass snake, *Natrix natrix* (Squamata: Colubridae), in an intensively used agricultural landscape. Biological Journal of the Linnean Society **101**: 51-58.

Monney, J.C., Meyer, A. (2005): Liste Rouge des reptiles menacés en Suisse. In : OFEFP and KARCH (eds), Série OFEFP: L'environnement pratique, OFEFP, Switzerland.

Schulte, U., Veith, M., Mingo, V., Modica, C., Hochkirch, A. (2013): Strong genetic differentiation due to multiple founder events during a recent range expansion of an introduced wall lizard population. Biological Invasions **15**: 2639-2649.

Wisler, C., Hofer, U., Arlettaz, R. (2008): Snakes and monocultures: habitat selection and movements of female grass snakes (*Natrix natrix* L.) in an agricultural landscape. Journal of Herpetology **42**: 337-346.

*Accepted by Spartak Litvinchuk*