

Where are you from? Origin determination of the introduced green whip snake, *Hierophis viridiflavus* (Squamata: Colubridae), in Switzerland

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Abstract. The negative impacts of invasive alien species on native biota are common due to ecological interactions, such as competition, predation, hybridisation, or the introduction of new pathogens. Among them, some snake species are considered to have a dramatic impact on local biodiversity. Here, we focus on a large-sized colubrid species, the Green Whip Snake, *Hierophis viridiflavus* (Lacépède, 1789) with established populations outside of its natural distribution in western Switzerland as a result of multiple introductions during the second half of the 20th century. The aim of this study was to determine the subspecies and geographical origin of the populations at three locations using molecular methods. The subspecies assessment was based on morphological features and mitochondrial DNA (*Cyt-b* gene) sequencing. Specimens from the two introduced populations in the area of Onnens and Chamblon (North of the canton of Vaud) could be assigned to the subspecies *H. v. viridiflavus*, while those from a population near the river La Gryonne (southeast of the canton of Vaud) belong to the subspecies *H. v. carbonarius*. However, due to the low diversity in the mitochondrial gene (*Cyt-b*) in both subspecies, it was not possible to assess the geographical origin of the introduced populations. Nevertheless, based on historical faunistic data there is strong evidence the three examined populations were not the result of a natural colonisation process. To propose adequate management actions, future changes in the distribution of the species and its ecological impact on native species at the introduction sites need to be investigated.

Keywords. Snakes, *Cyt-b*, haplotype-network, alien species management

Introduction

In the present time of globalisation, organisms are moved all over the planet for numerous reasons, such as ornamental purposes, consumption, pet trade, or

unintended transport (Hulme, 2009; Amano et al., 2016). Some of these organisms exhibit life-history traits allowing them to potentially thrive and become invasive when released into the wild out of their native range (Kowarik, 1995). The negative impacts of invasive alien species on native biota are common due to ecological interactions, such as competition, predation (Kats and Ferrer, 2003; Bonnaud et al., 2011), hybridisation (Dufresnes et al., 2017), or the introduction of new pathogens (Alderman, 1996; Sainsbury et al., 2020).

Many snake species exhibit, thanks to their high mobility, fast reproduction, and opportunistic behaviour, the potential to establish populations when introduced to suitable regions outside of their natural distribution. Multiple examples have shown that snakes can even become invasive, such as the Brown Tree Snake, *Boiga irregularis* (Bechstein, 1802) that was moved unintentionally by troop transports to the island of Guam during the World War II (Rodda et al., 1992; IUCN, 2017). Today, this species occurs in Guam with an enormous density which not only led to the extinction of most of the native bird and small mammal species but also effectuated a tremendous decline

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in the abundance of lizard species (Savidge, 1988; Rodda and Fritts, 1992). Even though the presence of invasive snake species is more likely to be documented in (sub-)tropical regions as snakes show the highest diversity there, plenty of introductions of snakes in more temperate regions, such as the Western Palearctic, are reported. Extensive genetic analyses by Silva-Rocha et al. (2015) showed that several snake species introduced to the Balearic Islands, like the Horseshoe Whip Snake *Hemorrhois hippocrepis* (Linnaeus, 1758), the Montpellier Snake *Malpolon monspessulanus* (Hermann, 1804), the Algerian False Smooth Snake *Macroprotodon mauritanicus* Guichenot, 1850, and the Ladder Snake *Zamenis scalaris* (Schinz, 1822), were originating from the Iberian Peninsula and have most likely been introduced unknowingly by the transport of olive trees (Pinya and Carretero, 2011; Silva-Rocha et al., 2015, 2018; Febrer-Serra et al., 2021). However, for other species such as the Californian Kingsnake *Lampropeltis californiae* (Blainville, 1835), a species native to the United States and Mexico introduced on the Canary Islands before 1998, the introduction pathway via the pet trade is the most likely. This species is threatening endemic lizards from the genus *Gallotia* and birds by preying on them (Bowler, 2018; Gallo and Mateo, 2020). Also in Switzerland, several snake species have been introduced to various allochthonous locations. The most famous example is the Dice Snake, *Natrix tessellata* (Laurenti, 1768), a species native to Switzerland south of the Alps, which has been introduced to various other locations north of the Alps, including the lakes of Zurich, Alpnach, Brienz, and Geneva (Hofer et al., 2001; Gautschi et al., 2002). While there have been no observed ecological impacts of the introduced snakes at most of the introduction sites, indications of competitive interactions were observed with the closely related Viperine snake, *Natrix maura* (Linnaeus, 1758) at Lake Geneva (Dubey et al., 2015). The latter species, which is considered as critically endangered in Switzerland (Monney and Meyer, 2005), natively occurs along the Rhône river in Switzerland and its populations are distinctly declining in correlation with the spread of *N. tessellata* around Lake Geneva (see Lenz et al., 2009).

The Green Whip snake, *Hierophis viridiflavus* (Lacépède, 1789) has been the subject of several systematic studies in recent decades. Even though Schätti and Vanni (1986) declared the species as monotypic based on pholidosis, more recent studies found evidence for the existence of two distinct lineages corresponding to these two subspecies based on mtDNA, karyotypes,

and morphometry analyses (Nagy et al., 2002; Rato et al., 2009; Mezzasalma et al., 2015). However, the suggestion to lift the subspecies *H. v. carbonarius* to the species level (Mezzasalma et al., 2015) was refused by the taxonomic committee of the SEH due to insufficient examinations of gene flow in the contact zone between these two lineages (Speybroeck et al., 2020). While the nominated subspecies *H. v. viridiflavus* occurs from eastern Spain, southern France, southwestern Switzerland to western Italy, the mainly melanotic subspecies *H. v. carbonarius* (Bonaparte, 1833) occupies habitats in south-eastern Switzerland, eastern Italy, Slovenia, and Croatia. In Switzerland, the subspecies *H. v. viridiflavus* naturally inhabits the Canton of Geneva, and *H. v. carbonarius* inhabits the Canton of Ticino, some valleys in the south of Canton of Graubünden and south of the Simplon Mountain in the Canton Valais (Fig. 1). It is presumed that, similar to *N. tessellata*, *H. viridiflavus* has also been introduced at various allochthonous sites in Switzerland in the Canton of Vaud (Hofer, 2001; Fig. 1). *H. viridiflavus* has been recorded in the Canton of Vaud in three different locations since the 1950's, and recently (2018) a few observations were recorded in the Canton of Valais (Fig. 1). Hofer (2001) suggested that the introduced population in one of the sites in the Canton of Vaud (near the river la Gryonne) originated from specimens from the Canton of Ticino. However, the origin of these populations has never been examined genetically. Empirical data indicates that the presence of *H. viridiflavus* has caused population declines in native Asp Viper *Vipera aspis* (Linnaeus, 1758) due to competition and predation (pers. obs.). Since 2016, specimens of *H. viridiflavus* have been periodically caught at the introduced sites, euthanised, and preserved at the Musée de Zoologie in Lausanne.

The aim of this study was to determine with genetic analyses the geographical origin and associated subspecific level of several introduced *H. viridiflavus* populations in the Canton of Vaud. Assessing the origin(s) of these *H. viridiflavus* populations does not only improve our knowledge about possible introduction pathways but could also serve as a basis for future management.

Materials and Methods

The two subspecies *viridiflavus* and *carbonarius* were shown to be differentiated by 4% genetic divergence (and 1% within each subspecies) on the mitochondrial genes *ND4* and *Cyt-b* (Rato et al., 2009; Mezzasalma et al., 2015). While the genetic structure seems to be quite

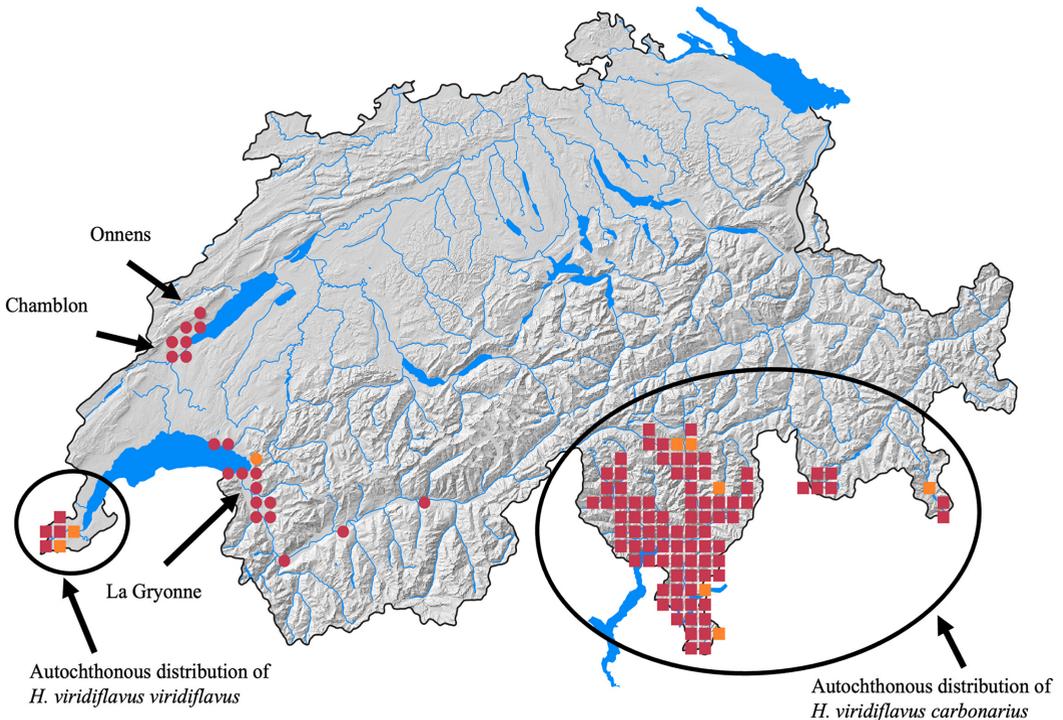


Figure 1. Distribution map of *Hierophis viridiflavus* in Switzerland. Squares represent autochthonous locations, and circles represent introduced sites where the species was observed in a 5 x 5 km area before the year 2000 in orange and red marks after 2000. © info fauna - CCO/KOF, basal map ©Swisstopo

uniform in the subspecies *viridiflavus*, populations from different regions of the subspecies *carbonarius* can be differentiated genetically using mtDNA sequencing. To determine the origin of the putatively introduced populations of *H. viridiflavus*, we sequenced part of the mtDNA (*Cyt-b*) and compared them with reference sequences from Mezzasalma et al. (2015). From a morphological point of view, the subspecies *viridiflavus* has a black or brownish colouration with yellow or whitish dots that tend to form transverse rows in the anterior part of the body and longitudinal rows on the posterior parts. The ventral scales of the subspecies *viridiflavus* usually lack any punctuation. The subspecies *carbonarius* is darker, usually without or with fewer yellow dots. The ventral scales of *carbonarius* are often blotched with dark pigmentation (Heimes, 1993; Di Nicola et al., 2019).

Samples. For morphological differentiation, we only observed the captured individuals and determined visually if they corresponded to the subspecies *viridiflavus* or *carbonarius* based on the distinctive traits described above.

For genetic analyses, liver tissue samples were collected from 33 *H. viridiflavus* specimens that were previously collected at putatively introduced sites in the Canton of Vaud (villages of Onnens, Chamblon, and along the river La Gryonne, between Ollon and Bex). These specimens were prepared and preserved at the Museum of Zoology in Lausanne (Table 1). As references, *H. viridiflavus* samples originating from the native populations in Geneva and Ticino were collected at the corresponding museums: one liver tissue sample of *H. v. viridiflavus* from the Natural History Museum of Geneva (MHNG) and 3 liver tissue samples of *H. v. carbonarius* from the Museo Cantonale di Storia Naturale in Lugano (MCSN).

In addition, 82 reference sequences for the mitochondrial gene *Cyt-b* were downloaded from GenBank (LN551963–LN552044). These sequences correspond to specimens of *H. viridiflavus* ($n = 51$) and *Hierophis gemonensis* (Laurenti, 1768) ($n = 31$) that originated from all over the distribution area of the two species and were published by Mezzasalma et al. (2015). Sequences from *H. gemonensis*, the closest

related species to *H. viridiflavus*, were used to compare genetic distances.

Laboratory protocol. Before DNA extraction, liver samples (ca. 2 x 2 x 2 mm) were rehydrated 2× for 3 h in 1 ml H₂O. Tissue digestion and DNA extraction were then performed following the spin-column protocol (Quiagen, 2020). Part of the mitochondrial gene *Cyt-b* was amplified using adapted PCR protocols from Mezzasalma et al. (2015). The following primers were used: CytbHvF (5'-ATGCCCAACCATCATATAC-3') and CytbHvR (5'-GAGCCTTCAGTATGGAGA-3') (Mezzasalma et al., 2015). PCR parameters were as follows: initial denaturation at 94°C for 5 min; 37 cycles of 94°C for 30 s, 54°C for 45 s, 72°C for 45 s; and final elongation at 72°C for 7 min. A final concentration of 3.5 mM MgCl₂ was applied for the PCR reaction. Amplification success was controlled using agarose gel electrophoresis. PCR products were sent to Macrogen (Amsterdam, the Netherlands) for PCR clean-up and standard Sanger sequencing.

Molecular analysis. DNA sequences were checked with the CodonCode Aligner (v. 9.0.1.3) (CodonCode Corp., Dedham, MA) for ambiguities in the chromatogram. Clear sequences were then aligned using the Muscle algorithm and cleaned using the trim end function in CodonCode Aligner. Aligned sequences were then imported to POPART (v. 1.7), and a haplotype network analysis was made using the Minimum Spanning Network (epsilon = 0) (Leigh and Bryant, 2015). The haplotype nomenclature prioritises more prevalent haplotypes with lower numbers. However, the haplotype codes are not identical with the ones used in Mezzasalma et al. (2015), because we did only consider *Cyt-b*, but not *16S* and *ND4*.

Results

Clade assignment. Morphologically, the pattern was clear for all observed individuals, allowing unambiguous assignment for each individual to either of the subspecies. Similarly, an unambiguous sequence of *Cyt-b* was obtained for all analysed individuals (sequences uploaded to GenBank: OK668324–OK668362), and a clear assignment to one or the other subspecies was made. Both morphological and molecular analyses revealed that all individuals in Onnens and Chamblon could be assigned to the subspecies of the western clade *H. v. viridiflavus* and in la Gryonne to the subspecies of the eastern clade *H. v. carbonarius* (Table 1). Molecular analyses of the reference samples from Ticino and Geneva confirmed the expected distribution

of *H. v. viridiflavus* in Geneva and *H. v. carbonarius* in Ticino. The two subspecies showed a genetic divergence of 3.1% (16 mutations) on the examined fragment of the mitochondrial gene *Cyt-b* (512 bp). In comparison to that, the genetic divergence between the two subspecies of *H. viridiflavus* and *H. gemonensis* is 8.4% (42 mutations) on the examined fragment of the mitochondrial gene *Cyt-b*.

Haplotypes. The Minimum Spanning Network (Fig. 2) demonstrated the existence of two predominant haplotypes for the examined sequence of the *Cyt-b* gene, which corresponds to the two subspecies (E1 = *carbonarius*, W1 = *viridiflavus*). For both subspecies, specimens differed from these two major haplotypes by one to three mutations (E2–E8; W2–W5). The haplotype E1, which was found in all specimens from la Gryonne, was shared by specimens from Ticino but also by specimens from many other locations, e.g., some from Krk (Croatia), Pescara (Italy), and Ancona (Italy). All specimens from the population in Onnens and most of the specimens from the population in Chamblon were identified with the haplotype W1, which is the most common haplotype for the western subspecies *viridiflavus* and occurs, e.g., in Alessandria (IT), Corse (FR) and Rome (IT). The haplotype assignment for reference sequences is given in Appendix S1. While the populations in la Gryonne (E1) and Onnens (W1) were each composed of only one single haplotype, two haplotypes were found in Chamblon (W1, W2). However, the haplotype W2 was only found in a single specimen in Chamblon and could not be found in any of the reference sequences. Furthermore, the haplotype found in the reference specimen from Geneva (W3) was not shared either with any of the specimens from the introduced populations or any of the reference sequences.

Discussion

The selected genetic marker *Cyt-b* proved to be sufficiently informative to separate the two subspecies of *H. viridiflavus*. Sequence analysis allowed a clear assignment of the populations in Onnens and Chamblon to *viridiflavus*, while the population in la Gryonne was assigned to *carbonarius*. However, *Cyt-b* showed little variation within the two subspecies. Within our sample and the 51 reference sequences, we have found in total 6 haplotypes for the subspecies *viridiflavus* and 8 haplotypes for *carbonarius*. Even though this sounds like reasonable genetic variation, most of the examined specimens were sharing the same haplotype within

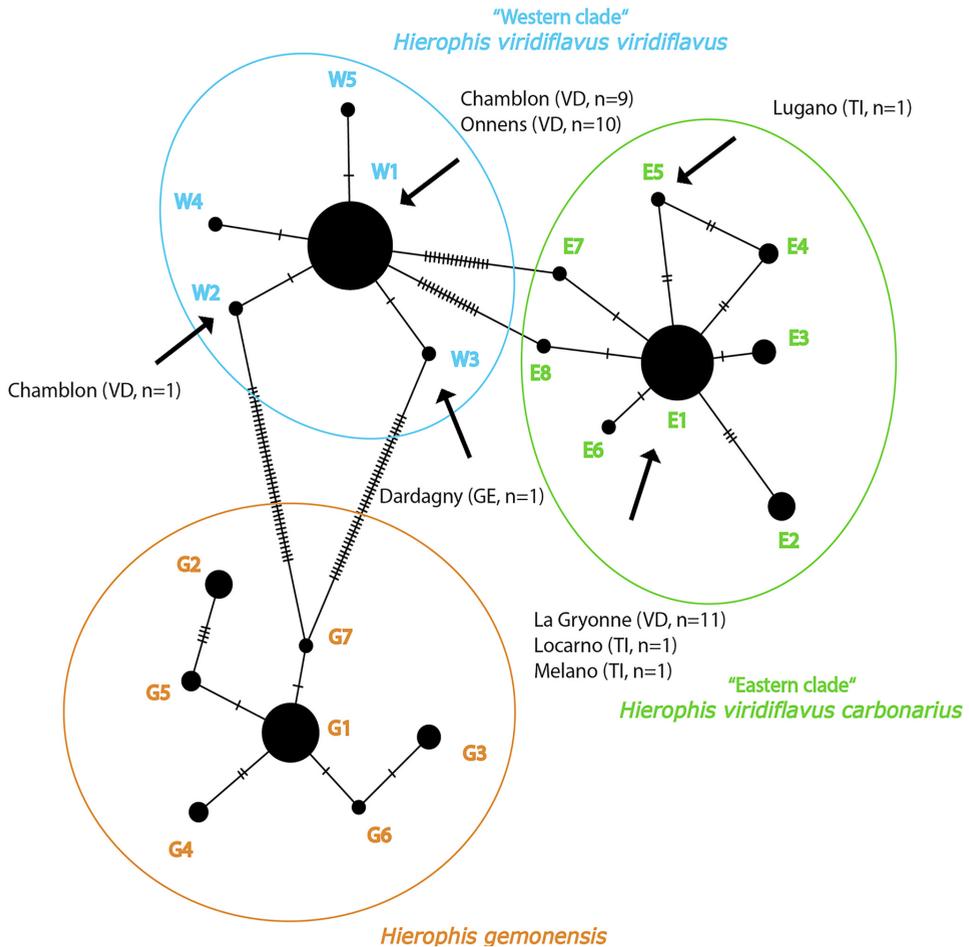


Figure 2. Minimum Spanning Network shows the relatedness between different haplotypes found in the dataset, respectively, the number of mutations by which they are separated (1 hedge = 1 mutation). Haplotypes W1–W5 = *H. v. viridiflavus*, haplotypes E1–E8 = *H. v. carbonarius*, G1–G7 = *H. gemonensis*. The Swiss samples are highlighted with arrows indicating their origins (Swiss cantons and the number of samples in brackets).

each subspecies (W1 and E1; Fig. 2). Furthermore, the other haplotypes differing from W1 and E1 were only separated by usually one, rarely up to three mutations. The haplotypes found in Onnens, Chamblon and la Gryonne matched the most frequent and wide-spread haplotypes W1 and E1, except for a single specimen from Chamblon that differed from W1 by 1 mutation (W2) but was found to be unique in the whole data set. Followingly, based on the genetic data, it was not possible to find evidence either for or against a natural colonisation of *H. viridiflavus* in Canton of Vaud.

Nevertheless, the natural presence of the subspecies *carbonarius* north of the Alps at la Gryonne is very unlikely, as this subspecies is otherwise only present

south of the Alps (Mezzasalma et al., 2015). Currently, this species can exceptionally reach elevations up to 2000 m and is unlikely to cross the Alps (Speybroeck et al., 2016). For the populations of the subspecies *viridiflavus* in Onnens and Chamblon, the lack of records of this species in the Canton of Vaud before the 1950's and the absence of populations around these sites in Switzerland and France provides good evidence against a natural colonisation process. The next populations known are separated by about 70 km along the Jura Mountains and 40 km across, respectively. The Jura Mountains are currently too cold for the species to cross. Consequently, it appears most likely, that all the *H. viridiflavus* populations in the Canton of Vaud were

Table 1. Details of the analysed samples (location and date of capture, voucher ID, museum) with the clade assignment based on a mitochondrial gene (*Cyt-b*) and GenBank numbers. Haplotypes W1–W5 = western clade (*Hierophis viridiflavus viridiflavus*); Haplotypes E1–E8 = eastern clade (*H. v. carbonarius*). Some of the haplotypes are only occurring in the reference sequences (see Appendix S1). Museum acronyms: MZL = Museum of Zoology, Lausanne; MHNG = Natural History Museum of Geneva, MCSN = Museo Cantonale di Storia Naturale, Lugano.

Locality	Date	Voucher ID	Museum	Haplotype	Genbank
Chamblon (VD)	21.04.2016	41160	MZL	W1	OK668325
Chamblon (VD)	29.04.2016	41161	MZL	W1	OK668326
Chamblon (VD)	25.05.2016	41162	MZL	W1	OK668327
Chamblon (VD)	22.04.2017	41169	MZL	W1	OK668329
Chamblon (VD)	22.04.2017	41170	MZL	W1	OK668330
Chamblon (VD)	28.04.2018	46517	MZL	W1	OK668331
Chamblon (VD)	25.05.2016	46518	MZL	W2	OK668332
Chamblon (VD)	20.06.2017	46519	MZL	W1	OK668333
Chamblon (VD)	18.05.2018	46534	MZL	W1	OK668334
Chamblon (VD)	18.05.2018	46538	MZL	W1	OK668335
Onnens (VD)	28.05.2016	41163	MZL	W1	OK668353
Onnens (VD)	04.05.2016	41165	MZL	W1	OK668354
Onnens (VD)	04.05.2016	41167	MZL	W1	OK668355
Onnens (VD)	23.06.2016	41168	MZL	W1	OK668356
Onnens (VD)	25.04.2018	41171	MZL	W1	OK668357
Onnens (VD)	16.06.2017	46521	MZL	W1	OK668358
Onnens (VD)	25.04.2018	46523	MZL	W1	OK668359
Onnens (VD)	23.05.2017	46526	MZL	W1	OK668360
Onnens (VD)	20.05.2016	46539	MZL	W1	OK668361
Onnens (VD)	21.05.2018	46544	MZL	W1	OK668362
La Gryonne (VD)	09.06.2016	46429	MZL	E1	OK668337
La Gryonne (VD)	21.04.2016	46431	MZL	E1	OK668338
La Gryonne (VD)	19.04.2016	46450	MZL	E1	OK668340
La Gryonne (VD)	22.05.2018	46528	MZL	E1	OK668341
La Gryonne (VD)	22.05.2018	46529	MZL	E1	OK668342
La Gryonne (VD)	22.05.2018	46533	MZL	E1	OK668343
La Gryonne (VD)	12.06.2018	46540	MZL	E1	OK668345
La Gryonne (VD)	02.06.2017	46546	MZL	E1	OK668346
La Gryonne (VD)	12.04.2017	46550	MZL	E1	OK668347
La Gryonne (VD)	07.04.2017	46557	MZL	E1	OK668348
La Gryonne (VD)	15.05.2017	46558	MZL	E1	OK668349
Dardagny (GE)	08.01.2013	2744.72	MHNG	W3	OK668336
Locarno (TI)	1997	VT 3335	MCSN	E1	OK668350
Lugano (TI)	03.07.2009	VT 5076	MCSN	E5	OK668351
Melano (TI)	25.07.2015	VE 0087	MCSN	E1	OK668352

introduced, as already suggested by Hofer (2001). In order to achieve a better understanding of the origin and colonisation process of *H. viridiflavus* in Canton of Vaud, more extensive genetic analyses would be necessary. As the mitochondrial gene *Cyt-b* is not providing the necessary variation to differentiate populations, and thus allowing to determine the exact origin, genotyping based on fast evolving genetic regions, such as microsatellites or genome-wide analysis (massive parallel sequencing) would be more informative. Furthermore, nuclear DNA sequencing would also allow the detection of putative hybrids between the two subspecies and/or the mixture of individuals from the species but from several geographical origins at one introduction site.

Since an introduction of *H. viridiflavus* in the Canton of Vaud appears to be the most likely scenario based on the available data, several aspects need to be considered for future management strategies. First, further analyses are needed to investigate the colonisation potential of the two subspecies and whether they are both equally likely to colonise new sites and spread throughout the landscape matrix of western Switzerland and what are the possible barriers to their colonisation. If scenarios are in favour of a natural colonisation process, it seems rather short-sighted that a control or an elimination of these introduced populations is requested. Distribution modelling of the western Swiss Alps has shown that the suitable habitat for *Hierophis viridiflavus* is likely to drastically increase in this area through the next decades (Pittet, 2017) due to global warming. Nevertheless, it is crucial to assess the ecological impact of the species at the introduction sites. Negative impacts on prey (birds, lizards, small mammals, other snakes) but also on ophidian competitors, e.g., *Vipera aspis* and *Zamenis longissimus*, are assumed (pers. observation). To prevent the populations from spreading and inhabiting new areas, different management actions should be considered. Currently, the populations are controlled by periodically capturing and euthanising specimens. Similar management actions have been done in Northern Germany, where an introduced population of *H. v. carbonarius* was detected in 2017 at a waste disposal site in Rheinland-Pfalz (Laufer, 2019). Individuals from this introduced population in Rheinland-Pfalz were caught and placed in the hands of experienced reptile keepers. Studies analysing the risks and costs of the management of introduced *Elaphe schrenckii* in the Netherlands showed that population control was much more expensive than local eradication of the species (van de Koppel et al., 2012; Struijk et al., 2020). However, the probability of successful eradication

of *H. viridiflavus* at these introduced sites should be considered doubtful because of the limited success rate in juvenile detection and specimen capture (pers. observation) and due to the large dispersal range of these species. In addition to the investigation of these local faunistic aspects, further research on the systematics of *Hierophis viridiflavus* is needed to clarify the specific status of *H. v. carbonarius*.

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Appendix S1. Reference sequences used for the molecular analysis the with haplotype assignment based on *Cyt-b*. Collection Codes: BEV= Biogéographie et Ecologie des Vertébrés (EPHE-CEFE, Montpellier); CD= Collezione Dall'Asta; DBUNF = Dipartimento di Biologia Università di Napoli Federico II;DPC= Dall'Asta personal collection; MCSTF= Museo Civico di Storia Naturale di Ferrara; MSNP= Museo di Storia Naturale dell'Università di Pisa; MCSNT= Museo Civico di Storia Naturale di Trieste; MSNT= Museo Civico di Storia Naturale di Torino; NHMC= Natural History Museum of Crete; ts=tissue sample (Mezzasalma et al., 2015).

GenBank Accession Number	Sequence ID	Haplotype (<i>Cyt-b</i>)	Locality	Voucher	Museum
>LN551963.1	Hv1	E1	Lagonegro (Italy)	Hievir01	DBUNF
>LN551964.1	Hv2	E2	Pietraraja (Italy)	Hievir02	DBUNF
>LN551965.1	Hv3	W1	Arties (Spain)		ts
>LN551966.1	Hv4	W4	Pisa (Italy)	1249	MSNP
>LN551967.1	Hv5	E7	Lentiai (Italy)	1309	MSNP
>LN551968.1	Hv6	W1	Sefro (Italy)	Hievir03	DBUNF
>LN551969.1	Hv7	E2	Avellino (Italy)	Hievir04	DBUNF
>LN551970.1	Hv8	W1	Monterano (Italy)	Hievir05	DBUNF
>LN551971.1	Hv9	E3	Cetraro (Italy)	Hievir06	DBUNF
>LN551972.1	Hv10	E1	Ancona (Italy)	Hievir07	DBUNF
>LN551973.1	Hv11	E1	Cava dei Tirreni (Italy)	Hievir08	DBUNF
>LN551974.1	Hv12	E3	Vallone Argentino (Italy)	1254	MSNP
>LN551975.1	Hv13	E1	Bovino (Italy)	Hievir09	DBUNF
>LN551976.1	Hv14	E1	Ancona (Italy)	Hievir10	DBUNF
>LN551977.1	Hv15	E6	Padriciano (Italy)	2637	MCSNT
>LN551978.1	Hv16	E2	Amalfi (Italy)	Hievir11	DBUNF
>LN551979.1	Hv17	E1	Pavia (Italy)	Hievir12	DBUNF
>LN551980.1	Hv18	E8	Palinuro (Italy)	Hievir13	DBUNF
>LN551981.1	Hv19	W1	Capraia (Italy)	Hievir14	DBUNF
>LN551982.1	Hv20	E4	Iria (Italy)	Hievir15	DBUNF
>LN551983.1	Hv21	E1	Montenovo (Italy)	RE000363	MCSTF
>LN551984.1	Hv22	E1	Mantova (Italy)	Hievir16	DBUNF
>LN551985.1	Hv23	W1	Calanca, Corsica (France)	1163	MSNP
>LN551986.1	Hv24	E1	Lecce (Italy)	1217	MSNP
>LN551987.1	Hv25	W5	Blegiers (France)	11025	BEV
>LN551988.1	Hv26	W1	Montagnac la Crempx (France)	9093	BEV
>LN551989.1	Hv27	E1	Valle Strona (Italy)	3280	MNST
>LN551990.1	Hv28	W1	Asti (Italy)	2114	MNST
>LN551991.1	Hv29	W1	Brusques (France)	8897	BEV
>LN551992.1	Hv30	W1	Campi Bisenzio (Italy)	1292	MSNP
>LN551993.1	Hv31	W1	Lamone (Italy)	1299	MSNP
>LN551994.1	Hv32	E1	Obzova, Krk (Croatia)	2085	MCSNT
>LN551995.1	Hv33	E1	Talponedo (Italy)	2587	MCSNT
>LN551996.1	Hv34	W1	Tombolo (Italy)	1301	MSNP
>LN551997.1	Hv35	W1	Calci (Italy)	1032	MSNP
>LN551998.1	Hv36	W1	Roma (Italy)	Hievir17	DBUNF
>LN551999.1	Hv37	W1	Castelnaud Chalosse (France)	1462	BEV
>LN552000.1	Hv38	W1	Millau (France)	10391	BEV
>LN552001.1	Hv39	W1	Larzac (France)	11525	BEV
>LN552002.1	Hv40	E1	Monzuno (Italy)	RE000194	MCSTF
>LN552003.1	Hv41	W1	Biguglia, Corsica (France)	1164	MSNP

Table S1. Continued.

GenBank Accession Number	Sequence ID	Haplotype (Cyr-b)	Locality	Voucher	Museum
>LN552004.1	Hv42	E1	S. Bartolomeo in Bosco (Italy)	RE000564	MCSTF
>LN552005.1	Hv43	W1	Alessandria (Italy)		ts
>LN552006.1	Hv44	E1	Aosta (Italy)	Hievir18	DBUNF
>LN552007.1	Hv45	W1	Asinara (Italy)	Hievir19	DBUNF
>LN552008.1	Hv46	E3	S. Nicola Arcella (Italy)	Hievir20	DBUNF
>LN552009.1	Hv47	E4	Lago Spartà (Italy)	1193	MSNP
>LN552010.1	Hv48	W1	Gréolières (France)	8143	BEV
>LN552011.1	Hv49	W1	Clumanc (France)	1689	BEV
>LN552012.1	Hv50	E1	Pescara (Italy)		ts
>LN552013.1	Hv51	E2	Soccavo (Italy)	Hievir21	DBUNF
>LN552014.1	Hg1	G1	Pag (Croatia)		ts
>LN552015.1	Hg2	G2	Krk (Croatia)		ts
>LN552016.1	Hg3	G4	Voulikanou (Greece)	8032581	NHMC
>LN552017.1	Hg4	G1	Aoos river, Iperios (Greece)	8032534	NHMC
>LN552018.1	Hg5	G1	Megalo Peristeri (Greece)	8032537	NHMC
>LN552019.1	Hg6	G5	Araxos (Greece)	8032563	NHMC
>LN552020.1	Hg7	G2	Mursini, Crete (Greece)	8032568	NHMC
>LN552021.1	Hg8	G2	Voni, Crete (Greece)	8032567	NHMC
>LN552022.1	Hg9	G1	Tebloni, Corfù (Greece)	8032577	NHMC
>LN552023.1	Hg10	G5	Kalogria (Greece)	8032560	NHMC
>LN552024.1	Hg11	G7	Kalogria (Greece)	8032559	NHMC
>LN552025.1	Hg12	G1	Foloi (Greece)	8032561	NHMC
>LN552026.1	Hg13	G1	Chani Panopoulou (Greece)	8032562	NHMC
>LN552027.1	Hg14	G2	Crete (Greece)	8032549	NHMC
>LN552028.1	Hg15	G1	Michoi (Greece)	8032570	NHMC
>LN552029.1	Hg16	G1	Makrokythira, Kithira (Greece)	8032519	NHMC
>LN552030.1	Hg17	G3	Argos (Greece)	8032534	NHMC
>LN552031.1	Hg18	G6	Mt. Parnasos (Greece)	8032538	NHMC
>LN552032.1	Hg19	G1	Lampaina (Greece)	8032580	NHMC
>LN552033.1	Hg20	G1	Ostros (Montenegro)		ts
>LN552034.1	Hg21	G1	Nafpaktos (Greece)	Hiegem02	DBUNF
>LN552035.1	Hg22	G1	Sibinj (Croatia)	1079	CD
>LN552036.1	Hg23	G1	Zadar (Croatia)		ts
>LN552037.1	Hg24	G1	Lastva (Montenegro)		ts
>LN552038.1	Hg25	G1	Debar (Macedonia)		ts
>LN552039.1	Hg26	G1	Vilusi (Montenegro)		ts
>LN552040.1	Hg27	G1	Tzoumerka (Greece)	8032584	NHMC
>LN552041.1	Hg28	G4	Kalamata (Greece)	Hiegem03	DBUNF
>LN552042.1	Hg29	G3	Argos (Greece)		ts
>LN552043.1	Hg30	G1	Fitiai (Greece)	Hiegem04	DBUNF
>LN552044.1	Hg32	G3	Argos (Greece)		ts