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SHORT COMMUNICATION

Development and characterization of 79 nuclear markers amplifying in viviparous and oviparous clades of the European common lizard

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Abstract

The European common lizard (*Zootoca vivipara*) is a widely distributed species across Europe and Asia exhibiting two reproductive modes (oviparity/viviparity), six major lineages and several sublineages. It has been used to tackle a large variety of research questions, nevertheless, few nuclear DNA sequence markers have been developed for this species. Here we developed 79 new nuclear DNA sequence markers using a clonation protocol. These markers were amplified in several oviparous and viviparous specimens including samples of all extant clades, to test the amplification success and their diversity. 49.4% of the markers were polymorphic and of those, 51.3% amplified in all and 94.9% amplified in 5–7 of the extant *Z. vivipara* clades. These new markers will be very useful for the study of the population structure, population dynamics, and micro/macro evolution of *Z. vivipara*. Cross-species amplification in four lizard species (*Psammodromus edwardsianus, Podarcis muralis, Lacerta bilineata*, and *Takydromus sexlineatus*) was positive in several of the markers, and six makers amplified in all five species. The large genetic distance between *P. edwardsianus* and *Z. vivipara* further suggests that these markers may as well be employed in many other species.

Keywords *Zootoca vivipara* · Lacertidae · Nuclear DNA · Clonation · Reptile · Representative genes

Introduction

The European common lizard (*Zootoca vivipara*) is a widely distributed species in Europe and Northern Asia exhibiting several genetic lineages (Surget-Groba et al. 2006) and two reproductive modes: oviparous (lineages: A, B1 and B2) and viviparous reproduction (C, D, E and F). Despite the existence of species-specific mitochondrial markers (e.g. Surget-Groba et al. 2006), only around 40 microsatellites loci (nuclear markers) have been developed (e.g. Boudjemadi et al. 1999; Horreo et al. 2017) and no species-specific makers amplifying nuclear sequences exist. The existing nuclear markers have been mainly used to measure sexual selection (e.g. Breedvled and Fitze 2016; Fitze et al. 2010; Fitze and Le Galiard 2011, 2008; Le Galiard et al. 2008; Richard et al. 2005, 2009; San-Jose et al. 2014) and the few studies investigating population structure used unspecific AFLPs (Mila et al. 2013), or well conserved nuclear gene fragments amplifying in a multitude of species (e.g. Cornetti et al. 2014). Consequently, there is a great lack of information regarding nuclear DNA in this species.

For these reasons, new nuclear sequence markers were developed for *Z. vivipara* using a clonation protocol and they were amplified in all extant *Z. vivipara* lineages to determine the amplification success, their diversity, and the applicability to different *Z. vivipara* lineages. In addition, we tested cross-species amplification of the newly developed genes in four lizard species with different phylogenetic relationships, in order to test their applicability.

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Table 1 79 nuclear markers newly developed in *Zootoca vivipara*

Marker	Size	Fordward primer	Reverse primer	Clades amplified	Pe	Pm	Lb	Ts
nZV1	437	CCCCTGGACTCACATGGTAA	ACCCCTGTCAGGAAGACTCA	All	–	–	–	–
nZV2	611	ACCTGCCAATCATCAAGTCC	AAGTGCAGCTATCCCATTGC	All	+	–	+	–
nZV3	462	TTTTCACTGCAGTACGCATCTT	GACCTGTTCATCCTGTAATCAGTG	All	–	–	+	–
nZV4	432	TCTTAAATGTATTCAGCAGTCTTGG	GTGTGTATGGGAGCGTGTTC	B2, C, D, F	–	–	–	–
nZV5	212	TTTTGCCACAAATTGCTTCA	TTGATGGTGGAACAGTGCAT	B1, B2, C, D, E, F	+	+	+	+
nZV6	391	TTGGTAAGTTATTTTTGCCAAGG	TATTAAAGTTCTAGGGGTTGAGAAGT	A, B1, B2, C	–	–	–	–
nZV7	365	ACTCGGCATGCCAGGTATT	AATTCCCCCACACCCTTAAC	All	–	–	–	–
nZV8	189	TTGCAATCATTCAAATGCAAG	AGATTCCAGACAAGGCCAAG	B1, B2, C, D, E, F	–	+	+	+
nZV9	499	GCAGCAGATTCCTACAGTGG	TTCACAAAATCCCACAATGC	B1, B2, C, D, E, F	–	–	–	–
nZV10	411	GCCAGCAGTCCTCTTCCTTA	AGAACGATGGGGGAGGAG	All	+	+	+	+
nZV11	229	TTTCATGGATGTGATTGATGC	CCCACCTCACTGTGTATTGGT	B2, C, D, E, F	–	+	+	–
nZV12	262	GCCGCTGCTTAGAATGTCTG	ACTGCAGTCAGGCCAGGA	A, B2, C, D, E, F	–	+	–	+
nZV13	255	GAAAACAAAAAGCAACCATTCC	TTCTGTGAGTTGGATTGTCCAT	B1, B2, C, D, E, F	–	+	–	–
nZV14	497	ATTGAGAGGAAAGGGGAACC	CCCCAAGTATAGTATTATGGGAGGT	A, B2, D, E, F	–	–	–	–
nZV15	478	GGGTGGTTTTATTTGCAATTCT	CCACATGCATTTGAAGCTGA	All	+	+	+	+
nZV16	238	CCATGCCATAACATCACTGC	GGTTGGCAGCTGCTACAAGT	B1, B2, C, D, F	+	+	+	+
nZV17	535	CCAACCAGAAGCTGTGATGA	AAAGCCAACATGAAGCCAAC	All	–	–	+	–
nZV18	578	AGAGTGACACAGGCCAAGGT	TGAGGCAGACCAAACAAATG	All	–	+	–	–
nZV19	410	AAGGATTCTGAAGGGCAACA	ACAACCCACCCTCAGAGATG	All	–	–	–	–
nZV20	425	TCCGTCTAGCCCAACATACC	CCACCTTTTCAGAACTTGGAA	All	–	–	–	–
nZV21	610	TTAACGGCTGCTTCCAGTCT	TCAGGTTTGACTGCTCATGC	A, B1, B2, C, D, E	–	–	+	+
nZV22	570	TGAAGGAATATTCATAGAAATAGATGG	CCCCAATCAGTATGGTTAGGAA	All	–	–	–	–
nZV23	333	GGACCTGGGAGACCAGAGTT	GACACCAGAGAGCATTGCAG	A, B1, B2, D, E, F	–	+	+	+
nZV24	507	TGCATACTTATTGCAGTAGTCAGTC	TTTCGCCCACATCTAGGTTC	All	–	+	+	–
nZV25	635	TTGGGGACCCCTAATCTAGC	CTGGGCTTTCCTCATCATTC	B2, C, D, E, F	–	–	–	–
nZV26	567	CTCCGCACCTTTTTAATGGA	GTTTGGAAGAGGCTCAGGAA	A, B1, B2, D, E, F	–	+	+	–
nZV27	643	TCTGCCCTCCTTACAGCAAT	GCTGGAGGTCCTTTGGTACA	All	–	–	+	–
nZV28	500	GGGTGCATGGAAAGAGAACT	GGGCAGCTTCCAACAAAATA	B1, B2, C, D, F	–	–	–	–
nZV29	462	GGCCATCAGGAGAGCATTAT	TGCTAGAGCTTCTCATTCAGACA	All	–	+	+	+
nZV30	363	ATTGCAGTTTCCGAGTCACC	CATGGGACATGGCTCACC	All	–	–	–	–
nZV31	361	GGGAAGTGACAGGCATCAAT	CAGTTTGCGACAAAGCAGAG	B1, B2, C, D, E	–	–	+	–
nZV32	544	CTTGCAAATCCCATTGTGAA	GGCAATTGAATCCCATTTTG	A, B2, C, D, E, F	–	–	–	–
nZV33	481	TTTCACCCAAAGCCTTATGC	TAGAGCATGCACCACTCTGG	All	–	–	–	–
nZV34	371	AAATTGGTCACACCCCAGAG	AGGGAGCCAACAAGGAGAAT	A, B1, B2, C, D, F	–	+	–	+
nZV35	345	CTCAAACGAGGAGGCAAGC	TGCCATTTGTACCCTCCGAA	A, B1, B2, D, E, F	–	–	–	–
nZV36	238	AAGGGTTGCACATGGATTGC	AGTGGATCTCAGTAAACACCAGA	All	–	+	+	–
nZV37	292	TGAAACCATGAACCATAAGCAGG	AAGGGTTGCACATGGATTGC	All	+	+	+	+
nZV38	615	TCTTCACCCAGCCCAATTCT	TTGGACAGTGTGGCATCAGT	All	–	–	+	–
nZV39	420	AAAGCACAACACATAATCTCTCCA	GGTCCCTTTGGTGCTTGTAA	All	–	–	–	–
nZV40	249	AATGCAAGAACCGACATTTTG	TGACGCAGAAATTGAGCAAG	B2, C, D, E, F	–	–	–	–
nZV41	684	AATTGTCATTCCTGGGCTGT	CGCAACAGGAATGTTCTCAG	All	–	–	–	–
nZV42	596	TGGCTGAGTCGAGGAAGAGT	CTGAGGGGAGAGATGCGTAG	All	–	–	–	–
nZV43	312	TGGATCCTGAAGAGAAGCAAA	TGAAGGCCTGTAAAAATTTGG	All	–	+	–	–
nZV44	527	CGGCTTCGAAAGTACGACAT	TGTCATATTTGTTTCCCCACA	B1, B2, C, E, F	–	+	+	–
nZV45	642	GGCAGCACAAGGAAAAAGAG	GGGGCAAAGGAAGGAAAC	All	–	+	+	+
nZV46	490	GGGAAGGGGCTTTTTAGAGA	GGGGAGAAAAGGAAGAAAGC	A, B1, B2, C, D, F	–	+	+	–
nZV47	783	GCTTGCACGCAAGTAGGAAT	CAGGAAGCACATCTGGAGAA	All	–	–	–	–
nZV48	499	ACTCTGCCCCTCACTCCAC	AGCAATGGCCAGCTGAGT	All	–	–	–	+
nZV49	630	TCCTAAAATGGAGGGCACTG	TTTGCTGTGGTGCCATAGTG	B1, B2, C, D	–	–	–	–
nZV50	594	TGATTGGAATGAGACCCAGA	AGTTTGCCTATGCCTGCTGT	B1, B2, C, E	–	–	–	–

Table 1 (continued)

Marker	Size	Fordward primer	Reverse primer	Clades amplified	Pe	Pm	Lb	Ts
nZV51	383	CTTCCCCATTGATGGGATTT	TTCATGGAGAAGAGAGCTGATG	All	–	–	–	–
nZV52	710	TTTGGTATTGAGGAATACCTTTATTT	ATGTTTTGCCTGGAGGTGAG	B1, B2	–	–	–	–
nZV53	166	GTGGCCCTTCTCTCAGTCCT	CTGCCAGTGGAAGGACAAC	All	+	+	+	+
nZV54	737	GGCAGCAAATTCCACAGTTT	CAGCTGGAGCTCAGGAAAAG	B1, B2, C, D, E, F	–	–	–	–
nZV55	784	GCAGCAGTAGATGGGCTTTC	CGCAACCAGAAGCATTCATA	A, B1, B2, C, D, E	–	+	–	–
nZV56	668	AAATATGCCCTGCCCTGTTA	GGATGACTCTTCAGCAGACCA	A, B2, C	–	–	–	–
nZV57	737	TCTGAAATCCGAAGGGAGGT	TTTGACGTGCTTTGGAACTG	B1, B2, C, D	–	–	–	–
nZV58	738	TTCCTCTGTGCCAGAGTCCT	CGAGCCAGCAGTAAATCACA	A, B1, B2, C, D, E	–	–	+	–
nZV59	810	GTTTCCAGGAACTGGTATTCAAA	ACACATGCCAACGTATCTGG	B2, C, D, E	–	–	–	–
nZV60	585	TCTCACAGGAAACAGCATCG	GGGGGATGAACAAGTTGCTA	B1	–	–	–	–
nZV61	702	GGGAAGTTGCTGAATTGGAA	GTGGGGACGCATATTGTTTT	B1, B2	–	–	–	–
nZV62	781	TGTGGCCCATAGTGGTAAAA	CCCAGTGAATCACATTCTCAAA	A, B1, B2	–	+	–	–
nZV63	530	TGTTTTAGGAAGGTTCAATGACT	AGAGATCGCACTGTGTCTGC	B1, B2	–	–	–	–
nZV64	753	TTTTCAGCCCAAAGGTCATC	GCTGCTGTCTAGCCATTGAA	B1, B2	–	+	+	–
nZV65	747	AACGCGCTTACGAATGTCTT	CTGCTAACCAGGTGCACTCA	B1	–	–	–	–
nZV66	591	TCATTATGCCTCACCTTCTGC	AGGTGACTATGGGGTTGCTG	A, B1, B2, D, E, F	–	–	+	–
nZV67	417	ACAAGCATCAAAACTGGACTTTT	GGGACAGCGGTGATTTAGTC	B1, B2, C, D, E, F	–	–	–	–
nZV68	698	TTATGTGGTCTCAGCAGCCA	ACTCCCTGGAAAAGACCCTG	All	–	+	+	+
nZV69	515	GCTTTCTCAGACGCCTTTGG	TGCCTGCTCTAGACAACCC	B1, B2, C, D, E, F	–	–	–	–
nZV70	613	ACATTTACATTCCCCACCCAT	ACCAGTGGTCAGGGAAGATG	B1, B2, D, E, F	–	–	–	–
nZV71	712	TACCACTGTACAGGCCTTCC	GGCTCATCTCTGCACAATGT	B1, B2, E, F	–	–	–	–
nZV72	667	AGCCCTATGGAGACTGAAGTG	TGGAGCACGTTTACTAGGAGA	A, B1, B2, D, F	–	–	–	–
nZV73	560	CCTTTGATGTAGGGCCAAGAG	AAACACACCTACCCACCCAA	All	–	+	–	–
nZV74	572	AACATTGTGGGTTGCCATGG	TGAACCTGAGACCTTCTGCA	All	–	–	–	–
nZV75	574	TTAAGCATGTGTGTCGCAGG	CTGCAGGCTCTAGGTGCTTA	A, B1, B2, C, D	–	–	–	–
nZV76	603	CTTGTCCGTGGCCTTTGTAC	CAGAGAGATCATCGGAGGCA	A, B1, B2, C, D, F	–	–	–	–
nZV77	703	CTCCTGTATCTTGGTGCCCA	CAGGCTCTGGAGTTAGCAGA	All	–	+	+	+
nZV78	712	AGCTTCCAACAGTTCCAAGC	GGAGAATCTAGCTGTCTTGGC	A, B1, B2, C, E, F	–	+	+	–
nZV79	645	GCGGTATCAGCTCACTCAAA	TTTGCCGGATCAAGAGCTAC	All	–	+	+	+

Shown are the sizes of the amplified fragments in basepairs, and the sequences of the forward and reverse primers. Clades with successful amplification are listed. Clade name corresponds to (Surget-Groba et al. 2001) and (Mila et al. 2013) (B1NWcorresponds to B1-NW Spain). Positive (+) or negative (−) cross-species amplification is given for *Psammodromus edwardsianus* (Pe), *Podarcis muralis* (Pm), *Lacerta bilineata* (Lb), and *Takydromus sexlineatus* (Ts)

Materials and methods

The development of new nuclear sequences consisted of a cloning-based protocol (Murphy et al. 1996). DNA was extracted following protocols described in detail by Horreo et al. (2015). Thereafter, BamHI and Bg1II restriction enzyme digestions and *Escherichia coli* XL10 strain (Stratagene) transformation with a pBlueScript SK + vector were conducted. After incubation, plasmids from white clones were purified and sequenced with universal M13 primers and BigDye Terminator v3.1 Cycle Sequencing Kit in an ABI PRISM 3700 (Applied Biosystems) automatic sequencer. Primer sets were then designed and tested in all extant *Z. vivipara* clades. PCR were done with up to 100 ng of template DNA in a total reaction volume of 25 µl (5PRIME MasterMix Kit). PCRs included initial denaturation (94 °C, 5 min) followed by 35 cycles at 94 °C for 30 s, annealing at 59 °C for 30 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 5 min. PCR amplification was checked in 1.5% agarose gels. If positive, PCR fragments were purified using standard ethanol precipitation and sequenced. Resulting sequences were edited and aligned using Sequencher v.4.10.1 (Applied Biosystems) and Aliview v.1.17 (Larsson 2014) software and homologs were searched using GenBank.

Zootoca vivipara samples of all clades (A, B1, B2, C, D, E, F; Surget-Groba et al. 2001) were employed to test amplification of the developed primer sets. Used samples originated from Bacher (Slovenia; clade A), Sierra do Xistral (Spain; clade B1), Pinet (France; B2), Brousset (France; clade B2), Moosbrunn (Austria; clade C), Izsak (Hungary; clade D), Pian delle Streghe (Italy; clade E), and Emberger

(Austria; clade F). In the case of positive PCR amplification, the genetic variability of each marker (number of variable sites, number of haplotypes, haplotype diversity and nucleotide diversity) was estimated with DNAsp software v.5.10.1 (Librado and Rozas 2009).

Four lizard species (one specimen per species) with different phylogenetic relatedness with *Z. vivipara* were used for testing cross-species amplification of the developed genes, namely: *Psammodromus edwardsianus* (Valencia, Spain), *Podarcis muralis* (from the Spanish Pyrenees), *Lacerta bilineata* (Burgos, Spain), and *Takydromus sexlineatus sexlineatus* (central Burma). While Takydromus evolved after the split with Zootoca, the lineage including *P. muralis* and *L. bilineata* branched off earlier, and within the *Lacertidae*, the lineage of *Psammodromus* branched off first (Pyron et al. 2013). Thus, Takydromus is closely related with Zotooca, while Psammodromus is the least related taxa used to test cross-amplification. PCR conditions were identical with those used for *Z. vivipara* samples, except that annealing temperature was relaxed to 57 °C, in order to facilitate amplification. Amplification was checked in 1.5% agarose gels. Amplification of each primer was tested using a positive control (one *Z. vivipara* sample), a negative control, and the four test-species.

Results and discussion

In total, 79 clones of *Z. vivipara* DNA were positive and primer sets were designed. All primer sets amplified in at least one clade (Table 1). 64 (81%) sets amplified in 5 or more clades and 39 sets (49.4%) were polymorphic (Table 2). 20 (51.3%) polymorphic markers amplified in all extant *Z. vivipara* clades and of the remaining ones 17 (43.9%) amplified in 5–6 clades (Table 1). Six markers (all monomorphic: nZV52, 61–65) amplified only in specimens belonging to the oviparous clade, while all others amplified in specimens belonging to both reproductive modalities.

Cross-species amplification of the 79 nuclear markers tested in *P. edwardsianus, P. muralis, L. bilineata*, and *T. sexlineatus* (Table 1) showed that 7 (8.9%), 29 (36.7%), 30 (38.0%) and 17 (21.5%) of markers amplified in these species, respectively. From the 79 markers, 41 (51.9%) only amplified in *Z. vivipara*; in contrast, six markers (nZV5, nZV10, nZV15, nZV16, nZV37 and nZV53) of the remaining 38 markers, amplified in all five species. According to BLAST comparisons (Table 3; http://blast.ncbi.nlm.nih.gov/Blast.cgi), 42 of the markers had similarities with GenBank sequences (Table 3) that ranged from 69 to 96%. Interestingly, all matches were with nuclear sequences of reptiles, suggesting the existence of homologues.

The polymorphic markers had very different genetic variability (Table 2). The number of variable sites ranged from 1 to 33 (mean 9.03; standard deviation, SD 7.96), the number of haplotypes from 2 to 7 (mean 4.49; SD 1.75), the haplotype diversity from 0.25 to 1 (mean 0.76; SD 0.23), and the nucleotide diversity from 0.001 to 0.026 (mean 0.008; SD 0.007). There existed no significant differences between the markers amplifying in all or in 5–6 clades (Wilcoxon

Table 2 Genetic variability of the polymorphic new developed nuclear markers (nZV1 to nZV39)

Marker	S	H	Hd	Nd
nZV1	3	4	0.750	0.002
nZV2	9	5	0.857	0.005
nZV3	7	4	0.750	0.004
nZV4	1	2	0.600	0.001
nZV5	2	2	0.571	0.005
nZV6	1	2	0.333	0.001
nZV7	4	3	0.607	0.004
nZV8	1	2	0.333	0.002
nZV9	4	3	0.762	0.004
nZV10	1	2	0.250	0.004
nZV11	1	2	0.286	0.001
nZV12	13	6	0.952	0.026
nZV13	3	3	0.524	0.005
nZV14	33	5	0.933	0.024
nZV15	15	4	0.786	0.011
nZV16	10	5	0.933	0.021
nZV17	5	6	0.893	0.002
nZV18	17	7	1.000	0.011
nZV19	10	7	1.000	0.008
nZV20	4	3	0.464	0.002
nZV21	8	6	0.952	0.004
nZV22	10	7	0.964	0.005
nZV23	2	2	0.286	0.002
nZV24	2	3	0.646	0.001
nZV25	20	6	1.000	0.012
nZV26	22	6	0.952	0.016
nZV27	33	7	0.964	0.02
nZV28	9	6	1.000	0.008
nZV29	11	7	0.964	0.007
nZV30	2	3	0.679	0.002
nZV31	18	4	0.800	0.025
nZV32	13	6	0.952	0.011
nZV33	8	4	0.750	0.005
nZV34	5	4	0.714	0.004
nZV35	7	5	0.905	0.009
nZV36	6	5	0.786	0.008
nZV37	7	7	0.964	0.008
nZV38	11	6	0.893	0.008
nZV39	14	4	0.867	0.017

S number of variable sites, *H* number of haplotypes, *Hd* haplotype diversity, *Nd* nucleotide diversity

Table 3 Most similar sequences found when doing BLAST comparisons (http://blast.ncbi.nlm.nih.gov/Blast.cgi) with the newly developed *Z. vivipara* nuclear markers

Marker	BLAST	Long	Identities (%)	Species
nZV4	XM_014070182	84	87	*Thamnophis sirtalis*
nZV5	XM_008103244.2	49	88	*Anolis carolinensis*
nZV8	HQ453278	124	81	*Podarcis lilfordi*
nZV9	XM_015414461	81	95	*Gekko japonicus*
nZV10	AF373382.1	237	93	*Podarcis muralis*
nZV12	KX080857	148	93	*Algyroides moreoticus*
nZV13	KX080857	149	93	*Algyroides moreoticus*
nZV15	DQ023372	268	93	*Podarcis muralis*
nZV16	GU355914	61	85	*Gallotia bravoana*
nZV18	XM_008107868	103	87	*Anolis carolinensis*
nZV19	XM_003222763	284	69	*Anolis carolinensis*
nZV22	DQ023348	70	96	*Podarcis muralis*
nZV23	JQ747339	77	94	*Takydromus stejnegeri*
nZV25	XM_007426839	119	74	*Python bivittatus*
nZV26	HQ453279	118	76	*Podarcis lilfordi*
nZV27	HQ595231	159	87	*Darevskia unisexualis*
nZV28	KX080905	225	83	*Psammodromus hispanicus*
nZV29	XM_007443671	263	90	*Python bivittatus*
nZV30	AF373382	328	92	*Podarcis muralis*
nZV33	AF373382	145	89	*Podarcis muralis*
nZV38	XM_006037131	60	95	*Alligator sinensis*
nZV41	XM_014070182	245	85	*Thamnophis sirtalis*
nZV42	XM_003215205	592	80	*Anolis carolinensis*
nZV43	XM_015415010	197	74	*Gekko japonicus*
nZV50	XM_003221868	41	90	*Anolis carolinensis*
nZV51	XM_015415201	45	87	*Gekko japonicus*
nZV52	BK006913	261	72	*Anolis carolinensis*
nZV53	XR_505673	82	74	*Anolis carolinensis*
nZV54	EU269530	236	75	*Podarcis hispanica*
nZV57	KJ680105	144	90	*Darevskia raddei*
nZV59	FJ587883	88	92	*Psammodromus hispanicus*
nZV61	JQ747142	127	88	*Takydromus formosanus*
nZV62	JQ826682	96	74	*Phyllopezus pollicaris*
nZV66	DQ393697	322	85	*Darevskia raddei*
nZV68	AF373378	277	90	*Podarcis muralis*
nZV69	XM_016994140	44	91	*Anolis carolinensis*
nZV70	KX080857	96	95	*Algyroides moreoticus*
nZV71	GU355917	47	89	*Gallotia bravoana*
nZV72	JN208354	131	89	*Physignathus lesueurii*
nZV76	XM_007439900	152	88	*Python bivittatus*
nZV77	XM_008117162	703	78	*Anolis carolinensis*
nZV78	XM_008117162	723	74	*Anolis carolinensis*

Table shows their GeneBank accession number (BLAST), the longitude (in base pairs) of the common sequence (long), the percentage of similarity between sequences (identities), and the species of the GeneBank sequence match. Sequences had similarities ≥ 69%

signed-rank test; S: $z = 0.06$, $P = 0.95$; H: $z = -1.10$, $P = 0.27$; Hd: $z = -0.11$, $P = 0.92$; Nd: $z = 1.15$, $P = 0.25$).

79 nuclear markers have been developed and tested in all extant clades of the European common lizard (*Z. vivipara*). 39 markers are clearly polymorphic, while no polymorphism has been detected in the other 40 markers. Given that the markers have been amplified in only eight specimens, it is likely that these markers may as well be polymorphic, but

further analyses are required to demonstrate this hypothesis. Cross-species amplification has been tested in four reptile species *P. edwardsianus, P. muralis, L. bilineata*, and *T. sexlineatus*. Six markers (nZV5, nZV10, nZV15, nZV16, nZV37 and nZV53) amplified in all the five species, suggesting that they amplify regions being more conserved than the other developed markers. This also suggests that they may amplify in more species belonging to Lacertidae than the other markers.

The newly developed markers can be used to investigate the genetic diversity (heterozygosity, F_{ST}, nucleotide diversity, haplotype diversity, genetic distances), microevolution (phylogenies, population structure), population dynamics (population expansion, haplotype networks, hybridization events, gene flow, population demography), and to generate more robust phylogenetic hypotheses. The specific genetic information of each marker (or combination of markers) will also be useful to determine the most representative genes (Horreo 2012) for different study types, different clades/specimens, and different evolutionary and geographic scales. In addition, using a combination between representative nuclear and mitochondrial markers will provide more robust results, that may change the phylogenetic relationships and even the taxonomic hypotheses (Ahmadzadeh et al. 2012; Makokha et al. 2007; Toewls and Brelsford 2012). In fact, mito-nuclear discordance has been described in the Pyrenean *Z. vivipara* populations leading to marker specific patterns of genetic structure and introgression (Mila et al. 2013). This suggests that the here developed markers together with the mitochondrial ones will allow to obtain a more precise understanding of the species' evolutionary history. More broadly, the newly developed tools will provide a considerable amount of new information that can be obtained relatively easy, relatively cheap (compared to a genome analyses), and relatively fast. The analytical easiness and relatively low costs will further allow to run detailed global phylogeographic studies including a large number of specimens, what is especially important for a species with a very large distribution. In conclusion, the newly developed nuclear markers will allow for more robust, more precise, and thus more general studies of micro and macro evolution, biogeography and population dynamics at different geographic scales.

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Compliance with ethical standards

Conflict of interest All the authors declare no conflict of interests.

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