Short Communication

Taxonomic status of a newly described island population of the smooth newt *Lissotriton vulgaris* (Linnaeus, 1758) from Bozcaada (Çanakkale, Turkey)

Nurşen ÇÖRDÜK[1], Çiğdem GÜL[1], Murat TOSUNOĞLU[1], Konstantinos SOTIROPOULOS[2],*
[1]Department of Biology, Faculty of Arts and Sciences, Çanakkale Onsekiz Mart University, Çanakkale, Turkey
[2]Department of Biological Applications and Technologies, School of Health Sciences, University of Ioannina, Ioannina, Greece

Abstract: The taxonomic status and phylogenetic position of the recently recorded smooth newt (*Lissotriton vulgaris*) population from the island of Bozcaada (Çanakkale, Turkey) is clarified on the basis of morphological and molecular phylogenetic analyses. The *L. vulgaris* population from Bozcaada presents body proportions and morphological features of subsp. *schmidtlerorum*, such as small body length, absence of the tail filament, and dorsal crest with pointed free margin. Similarly, phylogenetic analyses of mitochondrial DNA sequences (ND4, 16S rRNA) place the Bozcaada population within Clade E of the recent *L. vulgaris* phylogeny, which consists of *L. v. schmidtlerorum* populations.

Key words: *Lissotriton vulgaris schmidtlerorum*, mitochondrial DNA, 16S rRNA, ND4, taxonomy, Bozcaada (Tenedos), Turkey

The smooth newt, *Lissotriton vulgaris* L., is a polytypic amphibian species with a wide range across Eurasia, extending from Western Europe, excluding Iberia, further east to Western Siberia and Western Anatolia. The species breeds in aquatic habitats ranging from sea level up to 1400 m a.s.l. in the southern limits of its distribution (Griffiths, 1996).

Subspeciation in the smooth newt is considered to have evolved in allopatry during the various Pleistocene cycles in different refugia, and is more prominent in southern Europe and Western Anatolia (Raxworthy 1989, 1990; Krizmanić et al., 1997; Babik et al., 2005). All 7 valid subspecies have been described on the basis of male external phenotypic characters (Raxworthy, 1990; Griffiths, 1996; Schmidtler and Franzen, 2004). However, these morphologically designated subspecies do not form monophyletic groups on the basis of mitochondrial gene genealogies (Babik et al., 2005; Pabijan et al., 2015).

Lissotriton vulgaris has been reported from only 3 islands in the Aegean Sea: Evvoia (Buresch and Zonkov, 1941), Samothraki (Mettouris and Kornilios, 2015), and Bozcaada (Tenedos) (Gül and Tosunoğlu, 2013; Tok and Çiçek, 2014). An older record from the island of Tinos (Bird, 1935; Werner, 1938) has never been confirmed and is not considered valid (Sotiropoulos et al., 1995). The recently reported population of the island of Samothraki has been classified as subsp. *schmidtlerorum* (Raxworthy 1988) or *schmidtleri* (according to Dubois, 2007; Dubois and

Raffaëlli, 2009), on the basis of mitochondrial sequences (Mettouris and Kornilios, 2015). This geographically restricted subspecies is distributed along the Marmara coast and in Western Anatolia, reaching further west the area of Thrace (Pabijan et al., 2015; Wielstra et al., 2015). Given the geographic proximity of Bozcaada Island to the Anatolian coast, the corresponding smooth newt population should also belong to subsp. *schmidtlerorum*.

In the present study, we used morphological and molecular analyses to investigate the taxonomic status of the *L. vulgaris* population from Bozcaada, in comparison to nearby subsp. *schmidtlerorum* populations from Kavakkoy (Gallipoli, Thrace) and Çanakkale, as well as to clarify its phylogenetic position within smooth newt phylogeny.

Bozcaada (Tenedos) is a northeastern Aegean island, located in the southwest of the Çanakkale Strait (the Dardanelles). The island covers an area of 37.51 km²; its coastline is 36.41 km long. With the exception of a small permanent lake, Bozcaada lacks any kind of permanent wetlands (Tosunoğlu et al., 2009). Seventeen smooth newts (9 male; 8 female) were collected from several seasonal rain puddles (39°50′N, 26°01′E; sea level) in March and April 2013.

Çanakkale Province is located in western Turkey (Figure 1), expanding both in Asia (Biga Peninsula) and Europe (Gallipoli Peninsula). Twenty smooth newts (12 male; 8 female) were captured in a water canal in the city

* Correspondence: ksotirop@cc.uoi.gr

center of Biga Peninsula (40°06′N, 26°24′E; 42 m a.s.l.) in March 2013.

Kavakköy is located near the Kavak Delta wetland (Saros Gulf), in the Thrace region. Five smooth newts (2 male; 3 female), captured in April 2014 from seasonal rain puddles around Kavakköy (40°36′N, 26°50′E; 4 m a.s.l.), along with 5 museum specimens (3 male; 2 female) (Zoology Department, Çanakkale Onsekiz Mart University, Collection Number: 64/2009) were analyzed.

All samples were collected following the guidelines of the local ethics committee (Çanakkale Onsekiz Mart University, 2011/09–03) as a part of a project (Scientific and Technological Research Council of Turkey) in Turkey.

Total body length (TBL), snout–vent length (SVL), tail length (TL), head length (HL), and head width (HW) were measured with a Mitutoyo digital caliper with 0.01 mm precision. The following ratios of body measurements were calculated: SVL/TBL, SVL/TL, and HL/HW. The body measurements, as well as color and pattern properties, were determined according to Başoğlu et al. (1994) and Olgun et al. (1999). The descriptive statistics of measurements and ratios were calculated using SPSS (version 10.0; IBM, Chicago, IL, USA). The nonparametric Mann–Whitney U test was used in comparing measurements and proportions among the three studied populations.

Muscle samples or tail tips were ground to a fine powder in liquid nitrogen using a micropestle. Total genomic DNA was extracted using a Geneaid Genomic DNA Mini Kit (Kat. No. GT050) following the manufacturer's instructions. Total DNA concentrations were determined on a 1% agarose gel and estimated spectrophotometrically with Spectroquant Pharo 300 spectrophotometer. For the molecular phylogenetic analysis, partial sequences of the mitochondrial genes encoding 16S rRNA (16S) and NADH dehydrogenase subunit 4 (ND4) were selected. We amplified an approximately 500-bp fragment from the mitochondrial 16S rRNA gene using the primer pair 16Sar-L and 16Sbr-H (Palumbi et al., 1991), following the PCR conditions described in Sotiropoulos et al. (2007). The primer pair 'ND4' and 'Leu' (Arévalo et al., 1994) was used to amplify an approximately 920-bp fragment, which covers 727 bp of the ND4 gene, the complete tRNA-His and tRNA-Ser genes, and 15 bp from the 5′ end of the tRNA-Leu gene (further referred to as 'ND4'), following Babik et al. (2005). Successfully amplified products were sequenced by BM Lab Systems (Ankara, Turkey) using the same primers as in PCR.

Additionally, and in order to better classify the newly reported population within the smooth newt's phylogeny, we used 16S and ND4 sequences from 25 other populations corresponding to all previously recognized clades and subclades (Babik et al., 2005; Pabijan et al., 2015) distributed in southeastern Europe and Turkey (Table 1; Figure 1).

The following taxa were used as outgroups: *Ichthyosaura alpestris* (16S: DQ481506/DQ481493 – Sotiropoulos et al., 2007; ND4: KT933184/KT933185 – Sotiropoulos unpublished), *Lissotriton italicus* (16S: DQ092269 – Carranza and Amat, 2005; ND4: AY951653 – Babik et al., 2005), *L. helveticus* (16S: DQ092267 – Carranza and Amat, 2005; ND4: AY951648 – Babik et al., 2005) and *L. boscai* (16S: GQ380401 – Garcia-Gonzalez and Garcia-Vazquez, 2011; ND4: AY951650 – Babik et al., 2005).

The alignment was performed on the concatenated 16S and ND4 sequences in MEGA6 (Tamura et al., 2013). Phylogenetic relationships were estimated using the Bayesian inference (BI) method in MrBayes v3.2 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2011), using *Ichthyosaura alpestris* as the outgroup. We used the Bayesian information criterion (BIC), implemented in MrModeltest v2.3 (Nylander, 2004), to estimate the best-fit model of nucleotide substitution for each gene partition; 16S: HKY + I + G; ND4: GTR + G. We run four chains of 10^7 generations, saving to file one tree every 100 generations, which resulted in 4×10^5 trees. The first 10^4 trees of each run were discarded (10% burn-in).

Body measurements and ratios of the three studied populations are given in Table 2. Significant differences were detected between males from Çanakkale and Kavakköy (Gallipoli) populations (TBL: P = 0.04; z = –2.049), as well as between males from Bozcaada and Kavakköy (Gallipoli) populations (TBL: P = 0.003; z = –2.928; SVL: P = 0.003; z = –2.928). Significant differences in TBL (P = 0.009; z = –2.626) and SVL (P = 0.006; z = –2.731) were also detected between males from Çanakkale and Bozcaada populations. Significant differences in TBL (P = 0.002; z = –3.057) and SVL (P = 0.006; z = –2.741) were detected between females from Çanakkale and Kavakköy populations, and between females from Bozcaada and Kavakköy (Gallipoli) populations (TBL: P = 0.004; z = –2.867; SVL: P = 0.006; z = –2.733). Comparisons of ratios revealed no significant differences in the SVL/TBL (P = 0.558; z = –0.586), SVL/TL (P = 0.884; z = –0.146), or HL/HW (P = 0.464; z = –0.732) either between males or between females (SVL/TBL: P = 0.058; z = –1.897; SVL/TL: P = 0.206; z = –1.265; HL/HW: P = 0.833; z = –0.211) from Çanakkale and Kavakköy (Gallipoli) populations.

Similarly, no significant differences in the SVL/TBL (P = 0.462; z = –0.735), SVL/TL (P = 0.141; z = –1.470), or HL/HW (P = 0.529; z = –0.630) were detected either between males or between females (SVL/TBL: P = 0.055; z = –1.919; SVL/T: P = 0.356; z = –0.924; HL/HW: P = 0.570; z = –0.569) from Çanakkale and Bozcaada populations.

Finally, no significant differences in the SVL/TBL (P = 0.079; z = –1.757), SVL/TL (P = 0.143; z = –1.464), or HL/HW (P = 0.057; z = –1.903) were detected between males or between females (SVL/TBL: P = 0.386; z = –0.867; SVL/

Table 1. Sample localities of *Lissotriton vulgaris* used in the study.

ID	Locality	Coordinates	Country	Clade*	Haplotype	GenBank Acc. Nr. (16S/ND4)	Reference
1	Zevgolatio	37°15′N, 21°57′E	Greece	M	M1	EF089303/KT933169	Sotiropoulos et al., 2007/this study
2	Nestani	37°37′N, 22°27′E	Greece	M	M1	KT933142/JF681230.1	this study/Ivanovic et al., 2012
3	Milia	37°36′N, 22°24′E	Greece	M	M1	KT933143/KT933170	this study
4	Kalavryta	38° 03′N, 22°02′E	Greece	M	M1	KT933144/KT933171	this study
5a	Kalogria forest	38°07′N, 21°22′E	Greece	C	C3	KT933140/KJ852607	this study/Sotiropoulos & Karli unpbl.
5b	Kalogria forest	38°07′N, 21°22′E	Greece	C	C4	KT933141/KJ852608	this study/Sotiropoulos & Karli unpbl.
6	Arhontochori	38°45′N, 21°01′E	Greece	C	C5	KT933145/KJ852609	this study/Sotiropoulos & Karli unpbl.
7	Lefkada Isl.	38°38′N, 20°37′E	Greece	C	C6	KT933148/KJ852610	this study/Sotiropoulos & Karli unpbl.
8a	Paliambela	38°55′N, 20°57′E	Greece	C	C7	KT933146/KJ852611	this study/Sotiropoulos & Karli unpbl.
8b	Paliambela	38°55′N, 20°57′E	Greece	C	C8	KT933147/KJ852612	this study/Sotiropoulos & Karli unpbl.
9a	Kerkyra Isl.	39°41′N, 19°49′E	Greece	C	C2	KT933149/KJ852606	this study/Sotiropoulos & Karli unpbl.
9b	Kerkyra Isl.	39°41′N, 19°49′E	Greece	C	C1	KT933150/KT933172	this study
10	Kato Pedina	39°54′N, 20°40′E	Greece	K	K1	KT933151/KT933173	this study
11	Ano Kalliniki	40°52′N, 21°27′E	Greece	K	K3	KT933154/KT933175	this study
12a	Lake Prespa	40°50′N, 21°06′E	Greece	K	K4	KT933152/KT933174	this study
12b	Lake Prespa	40°50′N, 21°06′E	Greece	K	K2	KT933153/KJ852615	this study/Sotiropoulos & Karli unpbl.
13	Seli	40°34′N, 21°57′E	Greece	K	K3	KT933155/KT933176	this study
14	Bergina	40°29′N, 22°19′E	Greece	K	K1	KT933156/KT933177	this study
15	Kentriko	41°10′N, 22°54′E	Greece	K	K5	KT933157/KT933178	this study
16	Dojran	41°13′N, 22°42′E	FYROM	K	K3	KT933158/JF681231.1	this study/Ivanovic et al. 2012
17	Suvo jezero	42° 08′N, 22° 01′E	FYROM	L	L72	KT933168/KT933183	this study
18	Valjevska podgorina	44°16′N, 19°53′E	Serbia	L	L5	KT933167/KT933182	this study
19	Romanija	43°54′N, 18°45′E	Bosnia & Herzegovina	L	L16	KT933166/KT933181	this study
20	Svitava	43°00′N, 17°48′E	Bosnia & Herzegovina	D	D2	KT933165/KT933180	this study
21	Nova voda, Dolovi	42°19′N, 18°47′E	Montenegro	D	D7	KT933163/KT933179	this study
22	Sinj	43°42′N, 16°38′E	Croatia	L	L73	KT933164/KJ852626	this study/Sotiropoulos & Karli unpbl.
23	Zarkadia	41°02′N, 24°39′E	Greece	E	E15	KT933159/KJ852618	this study/Sotiropoulos & Karli unpbl.
24	Aghios Kosmas	41°06′N, 24°40′E	Greece	E	E15	KT933160/KJ852618	this study/Sotiropoulos & Karli unpbl.
25a	Thyrea	41°25′N, 26°32′E	Greece	E	E16	KT933161/KJ852619	this study/Sotiropoulos & Karli unpbl.
25b	Thyrea	41°25′N, 26°32′E	Greece	E	E17	KT933162/KJ852620	this study/Sotiropoulos & Karli unpbl.
26	Kavakkoy	40°36′N, 26°50′E	Turkey	E	E3	KT961697/KT989039	this study
27	Çanakkale	40°06′N, 26°24′E	Turkey	E	E5	KT961695/KT989037	this study
28	Bozcaada	39°50′N, 26°01′E	Turkey	E	E5	KT961696/KT989038	this study

*after Babik et al., 2005; Pabijan et al., 2015

Figure 1. Geographic positions of the smooth newt populations used. Locality information is given in Table 1. Colors correspond to the various major clades (named after Babik et al., 2005; Pabijan et al., 2015). The three studied populations are given with a different symbol (star).

TL: P = 0.947; z = −0.067; HL/HW: P = 0.739; z = −0.333), from Kavakköy (Gallipoli) and Bozcaada populations.

Bozcaada females exhibit a light yellowish green dorsal ground color, while the back has a brown stripe extending from the back of the head until the end of the tail on both sides. In males, the back ground color is a dark green tone with rounded dark markings on the body and tail. The dorsal crest continues uninterrupted until the tail end. There is no filament on the tail end. Color and pattern features of Çanakkale and Kavakköy populations are almost the same as those of the Bozcaada population.

Within the 1362 sites examined, 29 sites for the 16S and 93 sites for the ND4 were variable. Among the 33 ingroup sequences, a total of 26 different haplotypes were identified (Table 1).

The Bozcaada haplotype (E5), which was found also in Çanakkale (Table 1), showed an uncorrected sequence divergence between 0.6 (to Thyrea) and 4.3% (to Milia,

Nestani, Zevgolatio, and Kalavryta) for the ND4, and between 0.4 (to Thyrea) and 1.9% (to Milia, Nestani, Zevgolatio, Kalavryta, and Arhondochori) for 16S rRNA.

Identical topologies for each of the 4 runs were produced under the Bayesian inference method. However, for some nodes, posterior probabilities differed slightly between each run (Figure 2). A 50% majority-rule consensus tree (mean −ln L = −5357.99; 16S: G-shape parameter = 0.533; proportion of invariable sites = 0.624; nucleotide frequencies: A = 0.37, C = 0.20, G = 0.17, T = 0.26; ND4: G-shape parameter = 0. 275; nucleotide frequencies: A = 0.33, C = 0.25, G = 0.13, T = 0.29) was constructed combining the (remaining after burn-in) 9 × 10^4 trees.

The resulting tree topologies and phylogenetic relations were found to be highly concordant with the already published smooth newt phylogeny (Babik et al., 2005; Pabijan et al., 2015).

Table 2. Body measurements and ratios for the 3 studied smooth newt populations from Turkey. N: Number of specimens, ±SE: Standard error, Min: Minimum value, Max: Maximum value. Abbreviations of measurements and ratios are given in the Materials and methods section.

	BOZCAADA (♂)					BOZCAADA (♀)				
Characters	N	Min	Max	Mean	±SE	N	Min	Max	Mean	±SE
TBL	9	60.86	69.93	64.18	0.984	8	57.11	68.40	63.14	1.316
SVL	9	30.26	34.55	31.78	0.530	8	30.16	34.75	32.83	0.611
SVL/TBL	9	0.48	0.52	0.50	0.004	8	0.51	0.54	0.52	0.004
SVL/TL	9	0.93	1.04	0.97	0.013	8	1.05	1.21	1.10	0.018
HL/HW	9	1.37	1.54	1.45	0.016	8	1.30	1.41	1.36	0.016
	ÇANAKKALE (♂)					ÇANAKKALE (♀)				
Characters	N	Min	Max	Mean	±SE	N	Min	Max	Mean	±SE
TBL	12	62.83	69.94	66.34	0.566	8	64.95	75.66	69.08	1.222
SVL	12	29.87	35.70	31.96	0.507	8	33.24	37.89	35.57	0.495
SVL/TBL	12	0.45	0.54	0.48	0.007	8	0.50	0.54	0.52	0.004
SVL/TL	12	0.82	1.12	0.94	0.027	8	1.00	1.16	1.07	0.020
HL/HW	12	1.31	1.54	1.46	0.018	8	1.31	1.48	1.39	0.019
	KAVAKKÖY (♂)					KAVAKKÖY (♀)				
Characters	N	Min	Max	Mean	±SE	N	Min	Max	Mean	±SE
TBL	5	69.74	76.12	72.37	1.218	5	68.93	78.81	73.86	1.635
SVL	5	33.45	40.10	36.50	1.239	5	35.65	37.98	37.02	0.437
SVL/TBL	5	0.48	0.53	0.50	0.009	5	0.48	0.52	0.50	0.008
SVL/TL	5	0.92	1.17	1.02	0.056	5	1.03	1.11	1.0607	0.016
HL/HW	5	1.30	1.54	1.44	0.041	5	1.34	1.45	1.4064	0.018

The Bozcaada population is grouped together with the Çanakkale, Kavakköy, and northeastern Greek populations (Zarkadia, Aghios Kosmas, Thyrea) within Clade E (Figure 2).

In previous studies, the nominate subspecies, subsp. *vulgaris*, has been reported to exist in the Thrace region (Freytag, 1957; Raxworthy, 1988; Sotiropoulos et al., 1995). However, Raxworthy (1988, 1990) considered the possibility that *L. v. schmidtlerorum* might extend its range into Europe. Subsequent phylogenetic approaches have confirmed that *schmidtlerorum* expands its range in European Turkey, northeastern Greece, and southeastern Bulgaria (Nadachowska and Babik, 2009; Pabijan et al., 2015), thus updating the distribution of subsp. *schmidtlerorum*, which now covers Western Anatolia, the Marmara coast, and Thrace (Wielstra et al., 2015).

In our molecular phylogenetic approach, all three Anatolian populations (Kavakköy, Çanakkale, and Bozcaada) show increased genetic similarity and are grouped together with the northeastern Greek populations from Thrace, forming a highly supported monophyletic clade (Figure 2). This clade corresponds to "Clade E" of the current smooth newt phylogeny (Babik et al., 2005; Pabijan et al., 2015), which has been proposed to comprise *Lissotriton vulgaris schmidtlerorum* haplotypes.

Similarly, the morphometric measurements of the Bozcaada population (Table 2) were well within the values reported by Olgun et al. (1999) for other *schmidtlerorum* populations (Lapseki (Çanakkale): TBL: 59.9–79.7 mm; Bolayır (Thrace): TBL: 52.6–72.7 mm). Furthermore, the external morphology of Bozcaada smooth newts (small body length, tail filament absent, dorsal crest with pointed free margin) places them in subspecies *L. v. schmidtlerorum* (Raxworthy, 1990; Olgun et al., 1999).

Concluding, in our study, both morphological and molecular approaches clarified the taxonomic status of the
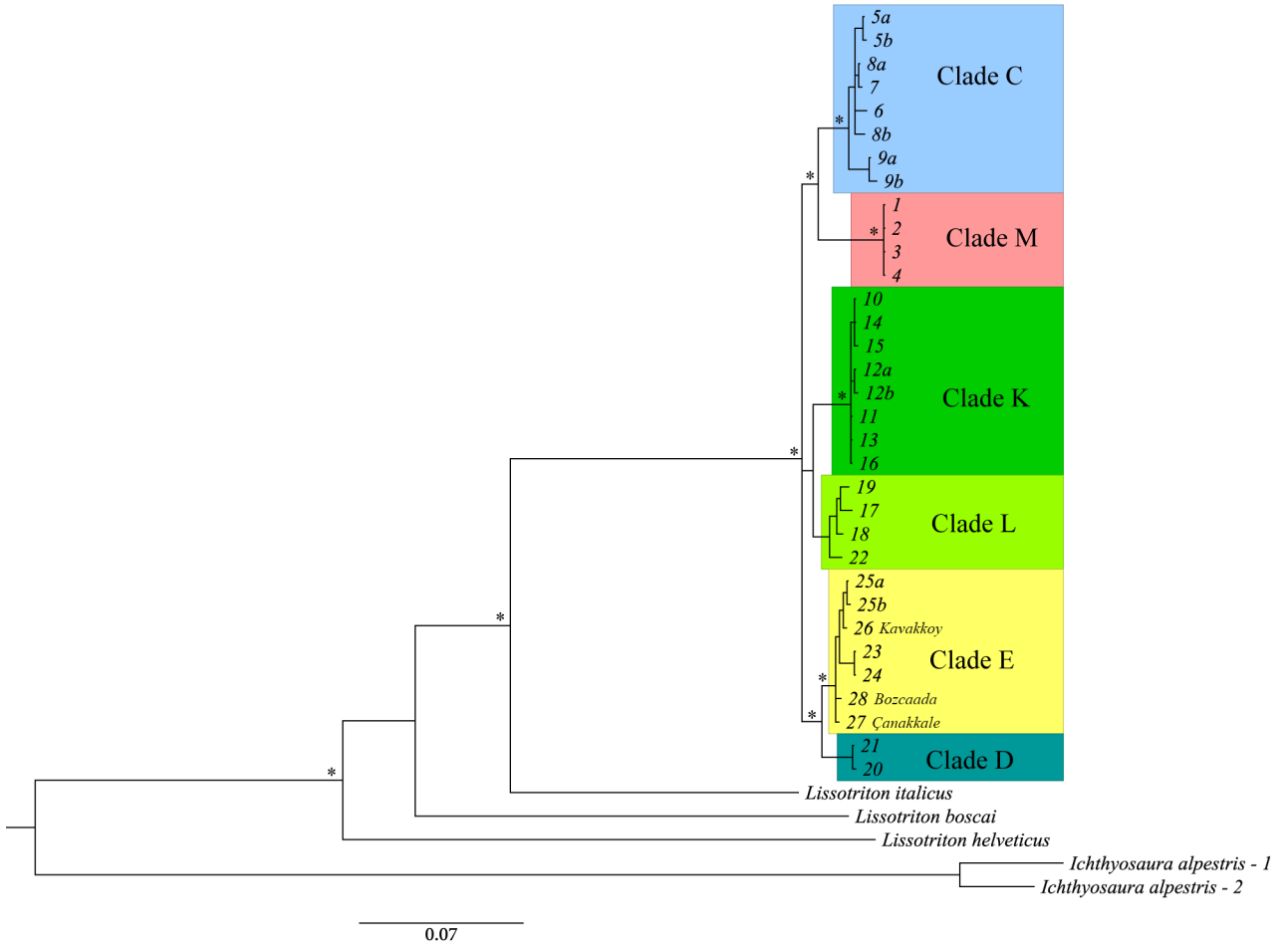
Figure 2. Phylogenetic relationships among studied smooth newt populations based on Bayesian Inference of mtDNA sequences (16S rRNA and ND4). Node posterior probabilities > 0.90 are given with asterisks. Population numbers correspond to Figure 1 and Table 1. The studied populations are given by their full names.

newly reported smooth newt population from Bozcaada as belonging to subsp. *schmidtlerorum*. Smooth newts might have colonized Bozcaada during the Late Pleistocene's glacial periods, when the sea level dropped approximately 120 m lower than present and the island of Bozcaada was connected to the mainland even until the end of the Last Glacial Maximum (Perissoratis and Conispoliatis, 2003).

References

Arévalo E, Davis SK, Sites JW Jr (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in Central Mexico. Syst Biol 43: 387-418.

Babik W, Branicki W, Crnobrnja-Isailovic J, Cogălniceanu D, Sas I, Olgun K, Poyarkov NA, Garcia-Paris M, Arntzen JW (2005). Phylogeography of two European newt species – discordance between mtDNA and morphology. Mol Ecol 14: 2475-2491.

Başoğlu M, Özeti N, Yılmaz İ (1994). Türkiye Amfibileri. Ege Üniversitesi Fen Fakültesi Kitaplar Serisi No: 151. 1-221 (in Turkish).

Bird CG (1935). The reptiles and amphibians of the Cyclades. Ann Mag Nat Hist London 16: 274-284.

Buresch I, Zonkov J (1941). Untersuchungen über die Verbreitung der Reptilien und Amphibien in Bulgarien und auf der Balkanhalbinscl. III. Schwanzlurche (Amphibia, Caudata). Izvest tsarsk prirod Inst, Sofiya 14: 194-237.

Carranza S, Amat F (2005). Taxonomy, biogeography and evolution of Euproctus (Amphibia: Salamandridae), with the resurrection of the genus Calotriton and the description of a new endemic species from the Iberian Peninsula. Zool J Linn Soc 145: 555-582.

Dermitzakis MD (1990). Paleogeography, geodynamic processes and event stratigraphy during the late cenozoic of the Aegean area. International Symposium on Biogeographical Aspects of Insularity, Rome, 18–22 May 1987, Academia Nazionale Dei Lincei, 85: 263-288.

Griffiths RA (1996). Newts and Salamanders of Europe. 1st ed. London, UK: T & AD Poyser, Natural History.

Gül Ç, Tosunoğlu M (2013). New Herpetofaunal Locality Records on the Bozcaada (Tenedos) and Ecological Observations. Second Scientific Conference on Ecology. (November 1st 2013), Plovdiv Bulgaria.

Huelsenbeck JP, Ronquist F (2001). MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17: 754-755.

Ivanović A, Sotiropoulos K, Üzüm N, Džukić G, Olgun K, Cogălniceanu D, Kalezić ML (2012). A phylogenetic view on skull size and shape variation in the smooth newt (Lissotriton vulgaris, Caudata, Salamandridae). J Zool Syst Evol Res 50: 116-124.

Krizmanić I, Mesaroš G, Džukić G, Kalezić ML (1997). Morphology of the smooth newt (Triturus vulgaris) in former Yugoslavia: taxonomical implications and distribution patterns. Acta Zoologica Academiae Scientiarum Hungaricae 43: 345-357.

Mettouris O, Kornilios P (2015). A first record of the Smooth newt, Lissotriton vulgaris (Linnaeus, 1758), from Samothraki island, NE Aegean Sea, Greece. Herpetology Notes 8: 483-484.

Nadachowska K, Babik W (2009). Divergence in the face of gene flow: the case of two newts (Amphibia: Salamandridae). Mol Biol Evol 26: 829-841.

Nylander JAA (2004). MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Olgun K, Baran İ, Tok CV (1999). The taxonomic status of Triturus vulgaris (Linnaeus, 1758) populations in Western Anatolia, Turkey. Turk J Zool 23: 133-140.

Pabijan M, Zieliński P, Dudek K, Chloupek M, Sotiropoulos K, Liana M, Babik W (2015). The dissection of a Pleistocene refugium: phylogeography of the smooth newt, Lissotriton vulgaris, in the Balkans. J Biogeogr 42: 671-683.

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (1991). The Simple Fool's Guide to PCR. Version 2. University of Hawaii, Zoology Department, Honolulu, HI.

Perissoratis C, Conispoliatis N (2003). The impacts of sea-level changes during latest Pleistocene and Holocene times on the morphology of the Ionian and Aegean seas (SE Alpine Europe). Marine Geology 196: 145-156.

Raxworthy CJ (1988). A description and study of a new dwarf subspecies of smooth newt, Triturus vulgaris, from Western Anatolia, Turkey. J Zool (Lond) 215: 753-763.

Raxworthy CJ (1990). A review of the smooth newt (Triturus vulgaris) subspecies, including an identification key. Herp J 1: 481-492.

Ronquist F, Huelsenbeck J, Teslenko M (2011). MrBayes version 3.2 Manual: Tutorials and Model Summaries. Online publication. http://mrbayes.sourceforge.net/

Schmidtler JF, Franzen M (2004). Triturus vulgaris (Linnaeus, 1758) – Teichmolch. In: Böhme W, editor. Handbuch der Reptilen und Amphibien Europas. Band 4/IIB, Wiebelsheim. Germany: Aula, pp. 847-967.

Sotiropoulos K, Legakis A, Polymeni RM (1995). A review of the knowledge on the distribution of the genus Triturus in Greece. Herpetozoa 8: 25-34.

Sotiropoulos K, Eleftherakos K, Džukić G, Kalezić ML, Legakis A, Polymeni RM (2007). Phylogeny and biogeography of the alpine newt Mesotriton alpestris (Salamandridae, Caudata), inferred from mtDNA sequences. Mol Phylogenet Evol 45: 211-226.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30: 2725-2729.

Tok CV, Çiçek K (2014). Amphibians and reptiles in the Province of Çanakkale (Marmara Region, Turkey). Herpetozoa 27: 65-76.

Tosunoğlu M, Gül Ç, Uysal İ (2009). The herpetofauna of Tenedos (Bozcaada, Turkey). Herpetozoa 22: 75-78.

Werner F (1938). Die Amphibien und Reptilien Griechenlands.- Zoologica, Stuttgart (Schweizerbart) 94: 1-116.

Wielstra B, Bozkurt E, Olgun K (2015). The distribution and taxonomy of Lissotriton newts in Turkey (Amphibia, Salamandridae). Zookeys 484: 11-23.