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Mitochondrial phylogeography of the Dalmatian wall lizard, *Podarcis melisellensis* (Lacertidae)

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Abstract

A 903 bp section of the mitochondrial cytochrome b gene was sequenced from 73 specimens of *Podarcis melisellensis* collected at 52 localities distributed over the major part of the species' range. In addition, parts of the 12S (about 470 bp) and 16S rRNA (about 500 bp) genes were analysed for 11 representative samples leading to a congruent phylogeny. Our study includes representatives of all 20 subspecies recognized today. The phylogenetic analysis of the sequence data revealed three main clades: mainland with nearby islands, Vis archipelago, and Lastovo archipelago. The degree of mitochondrial DNA divergence among these clades suggests a separation of the respective population groups during the earliest Pleistocene. The phylogenetic pattern observed within the species is in sharp contrast to the actual taxonomic division into subspecies. A correlation between genetic diversity of *P. melisellensis* populations and paleogeography of the regions they inhabit is discussed.

Keywords: *Podarcis melisellensis*; Phylogeography; Subspecies; Mitochondrial DNA

See also **Electronic Supplement** at http://www.senckenberg.de/odes/04-08.

Introduction

Podarcis melisellensis (Braun, 1877) is thought to be an autochthonous species of the eastern coastal Adriatic regions, inhabiting the coastal mainland and most of the islands, ranging from the area of Monfalcone (Italy) in the north-west to Tuzi and Shkoder (Albania) in the south-east. It is one of the most abundant members of the herpetofauna of the Croatian coast and islands, and therefore the biogeography of this species can be regarded as a paradigm for understanding the biogeography of the eastern Adriatic region.

The eastern part of the Adriatic Sea contains more than 1000 islands, islets and cliffs that have been isolated for less than 18,000 years. During periods of low sea levels today's northern islands were not more than hills elevated from the dry sea bottom of the northern Adriatic basin, and in central and southern Dalmatia the offshore islands were connected to the mainland. Nevertheless, the island populations exhibit considerable geographic variability in size, colour, pattern, and in particular in pholidosis values (Gorman et al. 1975; Clover 1979). For example, there are populations of *P. melisellensis* comprising large (adult male weight >10 g)

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jet-black lizards, others with small (adult males about 5 g) brown lizards with a solid green mid-dorsal area, and still others in which pigmentation is highly patterned (Gorman et al. 1975).

The great eagerness for describing subspecies, especially concerning insular isolates, that was very common from the end of 19th to the middle of the 20th century led to the description of 20 subspecies of *P. melisellensis*, of which 19 represent insular isolates and 17 are even restricted to a single islet each (see Tiedemann and Henle 1986). These subspecies were described on the basis of morphometric characters by means of a highly subjective approach, the so-called "conventional" procedure for describing subspecies (Thorpe 1987). Although the mainland populations inhabit the majority of the distribution range of the species, these were never included in comparative studies (Gorman et al. 1975; Clover 1979; Thorpe 1980). Radovanović (1956) described new subspecies of *P. melisellensis* on the basis of very small samples not likely to include all colour variants occurring in local populations (Clover 1979). Moreover, according to our experience, the diagnostic features given for some subspecies are incorrect. Therefore, it is not suprising that several former investigations based on morphometric and allozyme data (Gorman et al. 1975; Clover 1979; Thorpe 1980) revealed relationships between populations that clearly differ from current taxonomy.

At present, we are dealing with a paradoxical taxonomic situation: on the one hand, most subspecies inhabit relatively small geographic areas, and on the other, all populations from islands close to the mainland that are not ascribed to a distinct subspecies are by default considered as belonging to *P. m. fiumana*. A similar situation is found with *P. m. lissana* concerning the populations on the islets around Vis.

Since the large number of doubtful subspecies does not contribute to but rather obscures the understanding of the phylogeography of the species (Thorpe 1980), one goal of the present investigation was to test whether mitochondrial haplotypes of *P. melisellensis* populations match the existing subspecies-level taxonomy. Another aim was to reveal the phylogeographic pattern of *P. melisellensis* populations, and to explain it as a result of the geological history and geographical features of the regions they inhabit.

Material and methods

We investigated 73 specimens of *P. melisellensis* from the mainland (eight specimens, eight localities) and from 40 islands (65 specimens, 45 localities), including all presently recognized subspecies (see Organisms Diversity and Evolution Electronic Supplement 04-08, Part 1) and covering almost the entire distribution range of the species (Fig. 1). Sequences of *P. muralis muralis* (Austria, Baden), *P. sicula sicula* (Italy, Sicily, Mti. Peloritani), and *P. sicula campestris* (Croatia, Split) were included in the analyses as outgroup taxa (Electr. Suppl. 04-08, Pt. 1).

DNA was extracted from deep-frozen or ethanol-preserved soft tissues and tail tips by standard phenol-chloroform protocols (Sambrook et al. 1989). In order to discriminate the authentic mitochondrial sequences from possibly present nuclear pseudogenes, mitochondrial DNA was purified from nine samples (SVE1, BRU3, VIS2, JAB2, ZEZ, KAM1, LAS1, BIS3, and SAN2) by the method described by Beckman et al. (1993).

Polymerase chain reactions (PCR) were performed on an Eppendorf 'Mastercycler personal' thermocycler, using the primers listed in Electr. Suppl. 04-08, Pt. 2. Twenty-five-µl reactions were used, containing PCR buffer with 1.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.4 µM of each primer, 0.5 u of Taq polymerase (Pharmacia), and approximately 100 ng of genomic DNA. When purified mitochondrial DNA served as a PCR template, 2% of total DNA obtained from approximately 10 mg of tissue was used. Amplification conditions involved an initial denaturation step of 2 min at 94 °C; 35 cycles of 10 s at 95 °C, 20 s at 50 °C, 90 s at 72 °C, and a final extension step of 7 min at 72 °C. PCR products were purified with the PCR products purification kit (Roche). Sequencing was carried out by MWG-BIOTECH (Ebersberg, Germany) with the primers listed in Electr. Suppl. 04-08, Pt. 2.

Sequence data and phylogenetic analyses

A 903 bp section of the cytochrome b (cyt b) gene was sequenced from all 73 samples included in the study, while ribosomal sequences (about 470 bp of 12S, 500 bp of 16S rRNA) were obtained from 11 samples. Sequence alignments were made using ClustalX (Thompson et al. 1997) and corrected by eye. The basic sequence statistics, pairwise comparison of uncorrected sequence divergence (*p*-distance), corrected sequence divergences (Kimura two-parameter; Kimura 1980), and nucleotide composition, were analysed with MEGA version 2.1 (Kumar et al. 2001). Correlation plots of sequence divergences (*p*-distances) among the three gene fragments were made in order to reveal differences in evolutionary rates.

Analyses were conducted on combined and individual data sets separately, using the neighbor-joining method (NJ; Saitou and Nei 1987) on Kimura-2-parameter distances as implemented in MEGA, and Maximum Parsimony (MP; Camin and Sokal 1965; Swofford et al. 1996) as implemented in PAUP.

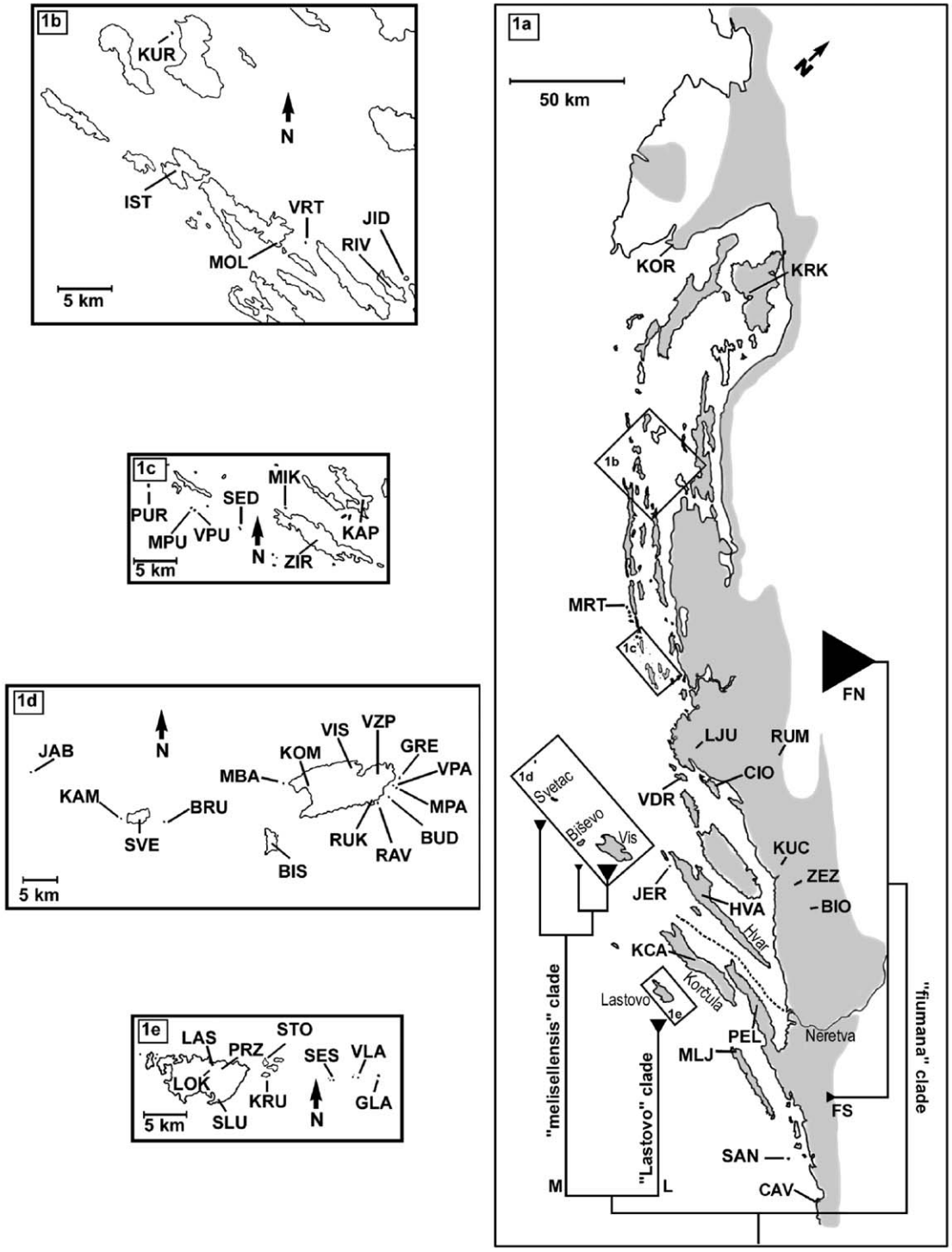

Fig. 1. Maps of the eastern Adriatic (a: overview, b–e: details) showing sampling localities for specimens used in molecular analyses; sample codes as in Electronic Supplement, Part 1. Grey shading = distribution range of *P. melisellensis*; dashed line = ancient course of Neretva river. Fig. 1a also shows correlation between geographic distribution of clades and main topology of phylogenetic tree (NJ/MP) based on cytochrome b sequences; FN = "northern fiumana" clade, FS = "southern fiumana", M = "melisellensis", L = "Lastovo" clade.

The congruence of 12S and 16S rRNA sequence data was tested with a partition homogeneity test (PAUP Version 4.0b10; Swofford 2002). Since the test revealed no significant conflict between fragments ($P = 0.2$), the sequences of 12S and 16S rRNA were concatenated for all further analyses. The sequences of the cyt b gene,

Table 1. Percentages of uncorrected pairwise sequence divergence (*p*-distances): (A) within and between main clades, (B) within and between subclades of the "fiumana" clade

	Cytochrome b			12S + 16S rRNA		
(A)	Fiumana	Melisellensis	Lastovo	Fiumana	Melisellensis	Lastovo
Fiumana	0.1–1.3			0.1–0.7		
Melisellensis	5.5–6.5	0.1–1.3		1.6–2.2	0.1–0.7	
Lastovo	5.9–6.4	4.5–5.4	0.1–0.4	1.8–2.1	1.8–2.3	0.1
(B)	Northern fiumana		Southern fiumana	Northern fiumana		Southern fiumana
Northern fiumana	0.1–0.4			0.1		
Southern fiumana	0.8–1.3		0.1–0.3	0.5–0.7		0.1

which were obtained from a much larger sample, were analysed separately. NJ and MP analyses of combined data sets for all three partitions were also performed. For NJ analysis, gaps in the alignments of 12S and 16S rRNA sequences were handled by means of pairwise deletion. Statistical support for nodes was estimated by bootstrapping (2000 replicates). For MP analysis, all sites and nucleotide substitutions were weighted equally, and gaps in ribosomal sequences were treated as fifth character state. MP analyses of all data sets were conducted using the heuristic search mode with 100 repeats, randomized input orders of taxa, and tree bisection-reconnection (TBR) branch swapping. Non-parametric bootstrapping (100 pseudoreplicates, 10 addition-sequence replicates for MP) was used to assess the stability of internal branches in cladograms.

In order to examine the shallow population structure and to infer recent population history, a cyt b 95% parsimony network (Templeton et al. 1992) was constructed using TCS (v1.13:2; Clement et al. 2000) software.

Results

Sequence data

A total of 41 different cytochrome b haplotypes were identified. Out of the 109 variable characters, 20% were at the first, 3% at the second, and 77% at the third codon position; 85 variable characters were parsimony informative. The mean transition to transversion ratio was 13.0. Uncorrected (*p*) sequence divergence values between haplotypes ranged from 0.1% to 6.5% (Table 1). Translation of DNA data into protein sequences revealed 16 (out of 301) variable amino acid sites. In the alignments of ribosomal sequences 35 characters were variable, 29 of these were informative under parsimony. The mean ratio of transitions to transversions was 3.5. Uncorrected (*p*) sequence divergence values between samples reached up to 2.3% (Table 1). Linear regression of the *p*-distances of analysed genes (not shown) revealed similar evolutionary rates for 12S and 16S rRNA, and a rate approximately 3 times faster for cyt b.

The cyt b sequences obtained from the purified mitochondrial DNA were identical to those obtained from total genomic DNA, stop codons were absent, and tree topologies obtained independently with each of the three investigated genes matched perfectly. In combination, this provides sufficient evidence that the analysed DNA sequences are not nuclear pseudogenes, but coding mitochondrial sequences.

Phylogenetic analyses

Phylogenetic analyses revealed a strong phylogeographic structure within *P. melisellensis*, which was consistent across different methods. Three main mtDNA clades were found designated in the following as the "fiumana" (two subclades: FN and FS), "melisellensis" (M), and "Lastovo" (L) clade, respectively (see Figs. 1 and 2).

The maximum parsimony analysis of the cyt b data resulted in 630 most parsimonious trees (length = 363, CI = 0.771, RI = 0.932), that of the 12S + 16S rRNA data in three most parsimonious trees (length = 159, CI = 0.874, RI = 0.873). The MP strict consensus tree derived from the cyt b sequences was largely congruent with the 12S + 16S rRNA tree obtained by the same method (Fig. 2A), as well as with a neighbour-joining tree calculated from combined sequences of all three genes (Fig. 2B). The three main clades are well supported by high bootstrap values and long branches. The "fiumana" clade contains two subclades comprising southern (FS) and northern (FN) populations, respectively. While in the most parsimonious network of the cyt b haplotypes the "fiumana" (Fig. 3A) and "Lastovo" (Fig. 3B) clades exhibit a simple starburst pattern, the presence of a loop in the "melisellensis" clade (Fig. 3C) indicates that there was more than one most

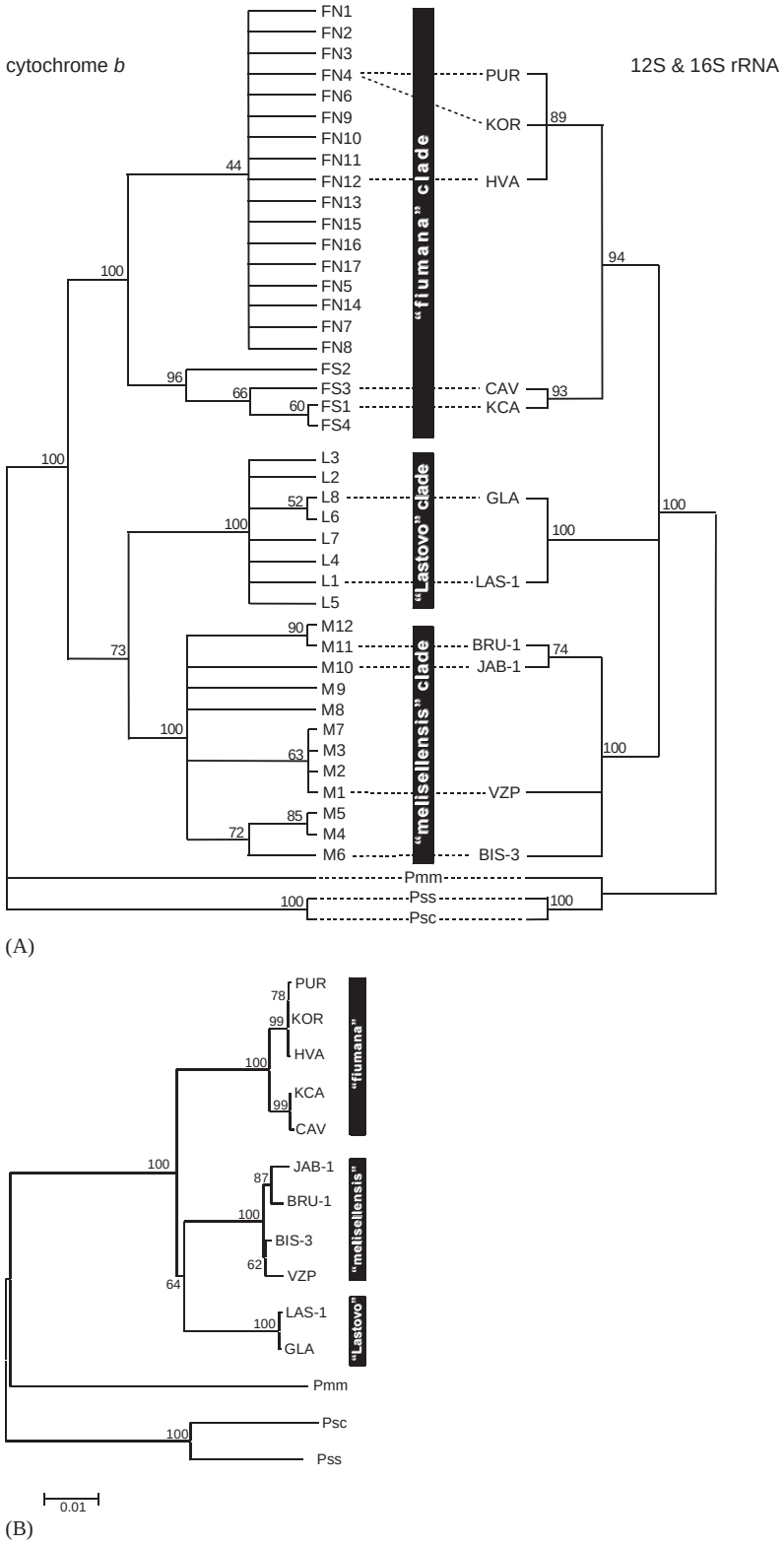

Fig. 2. (A) Strict consensus cladogram based on maximum parsimony analyses of partial cytochrome b (left) and combined partial 12S and 16S rRNA (right) sequences. Numbers at branches are bootstrap percentages (100 pseudoreplicates). (B) Neighbour-joining tree of Kimura two-parameter genetic distances based on combined data sets (cytochrome b, 12S and 16S rRNA). Numbers at branches are bootstrap percentages (2000 replicates).

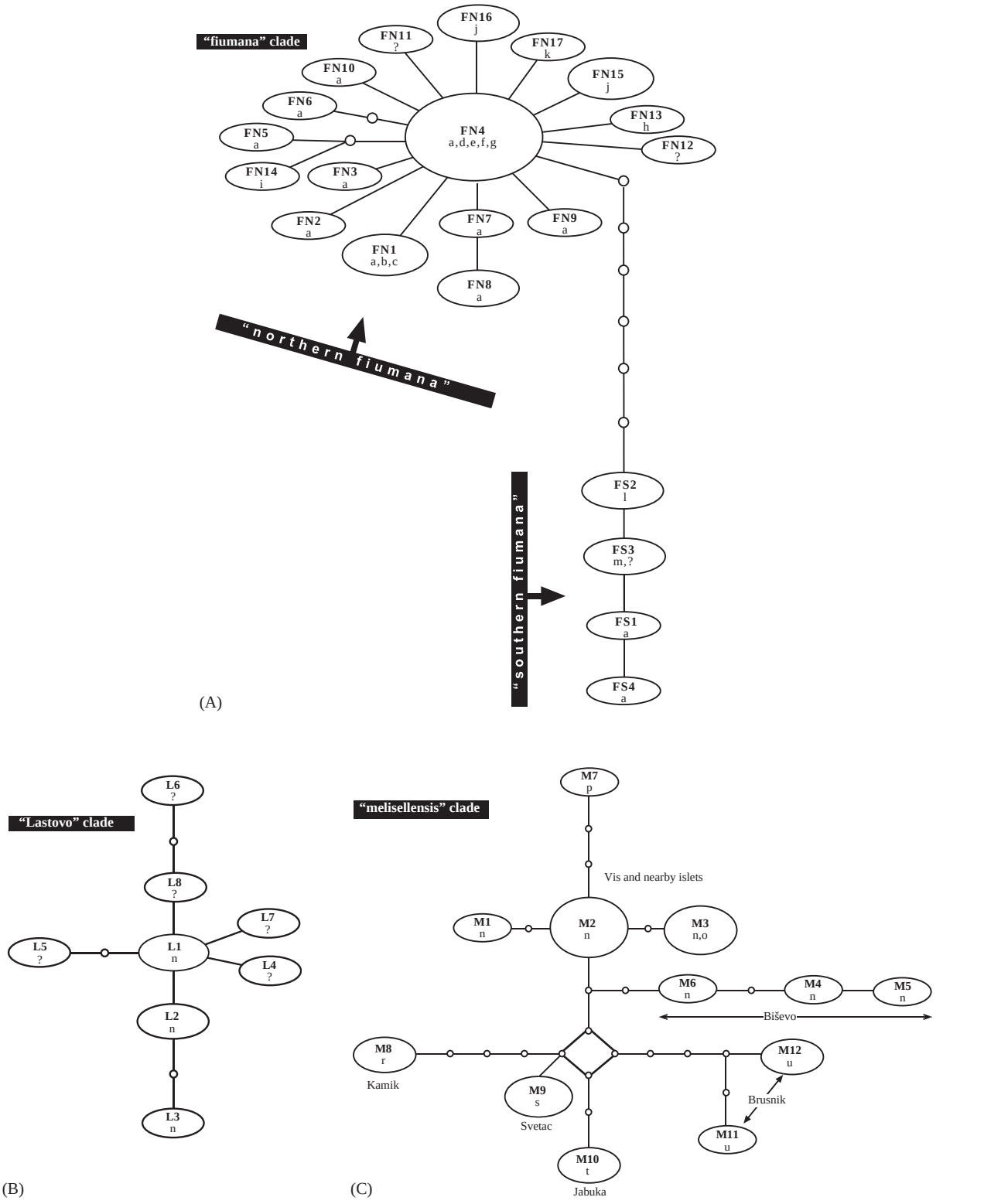

Fig. 3. Ninety-five per cent parsimony networks of cytochrome b haplotypes in *P. melisellensis*: (A) ''fiumana'', (B) ''Lastovo'', (C) ''melisellensis'' clade. Size of ovals corresponds to haplotype frequencies. Capital letters refer to haplotypes, small letters to subspecies codes: (a) *fiumana*, (b) *bokicae*, (c) *aeoli*, (d) *kornatica*, (e) *thetidis*, (f) *jidulae*, (g) *caprina*, (h) *plutonis*, (i) *traguriana*, (j) *mikavicae*, (k) *lupa*, (l) *gigantea*, (m) *curzolensis*, (n) *lissana*, (o) *gigas*, (p) *kammereri*, (r) *galvagnii*, (s) *digenea*, (t) *pomoensis*, (u) melisellensis, (?) undescribed.

parsimonious solution. Since the 95% connection limit was established at 13 steps, the three main clades could not be parsimoniously connected.

The ranges of genetic distance values obtained between and within main clades are shown in Table 1. All three main clades, "fiumana", "melisellensis" and "Lastovo", as well as the two subclades of the "fiumana" clade, are monophyletic. Concerning cyt b, the lowest interclade distance is 3.5 times higher than the highest intraclade distance, and the lowest distance between "southern" and "northern fiumana" haplotypes is twice as high as the highest distance observed within each of the subclades (Table 1).

Discussion

Phylogenetic relationships

Our investigation of *P. melisellensis* revealed three main monophyletic mitochondrial clades (Fig. 2A, B) which must have been separated for a long period of time: (1) the "fiumana" clade, (2) the "Lastovo" clade, and (3) the "melisellensis" clade (Fig. 1).

The "fiumana" clade encompasses the mainland populations and populations from islands closely associated with the mainland. Inside the "fiumana" clade, there are two distinct subclades (FN and FS) representing northern and southern populations, respectively. The 95% parsimony network of the "northern fiumana" subclade (Fig. 3A) reveals a starburst phylogeographic pattern which is, in combination with low nucleotide diversity and high haplotype diversity, the expected signature for a recent radiation (Avise 2000). The FN4 haplotype was found in a large number of samples from north of the Neretva river, encompassing a large geographical range: from Koromačno in the north-west to Žeževica in the south-east. All other haplotypes from this area are separated from FN4 by one or two nucleotide substitutions. The haplotypes of the southern populations form a distinct group separated by at least six substitutions from the northern haplotypes (Fig. 3A).

The "Lastovo" clade (L) comprises the populations from Lastovo Island and the islets of the Lastovo archipelago. It represents a homogeneous assemblage of very similar haplotypes (Fig. 3B), exactly as in the case of the "northern fiumana" subclade. Consequently, we can assume that the populations of the whole Lastovo archipelago also are the result of a recent radiation.

The "melisellensis" clade (M) encompasses the populations of three adjacent island groups in southern Dalmatia: the island Vis with nearby islets, the island Biševo, and the Svetac Island group. Although the genetic distances between these populations are at a level similar to those between the subclades "northern fiumana" and "southern fiumana", the phylogenetic relationships within this clade remain unresolved. Only the populations of Vis Island and nearby islets clearly represent a single genetic stock.

Lineage ages and phylogeography

Some previous authors have estimated evolutionary rates of the cyt b and 12S rRNA genes in lacertids. Most recently, Lin et al. (2002) investigated 12S rRNA sequences of the oriental lacertid genus *Takydromus*. According to two very different isolation scenarios, they discussed a fast rate of 10% and a slow rate of 1.25% sequence divergence per million years (myr). In their investigation of *Lacerta schreiberi*, Paulo et al. (2001) used three different rates for the cyt b gene: a slow rate of 1.7% as the most probable rate, a standard rate of 2% that also coincided with their estimations for the *Gallotia* data sets, and a fast rate of 2.85% they considered less plausible. Taking into account that the evolutionary rate of cyt b is three times faster than that of 12S rRNA, the slow rate estimated by Lin et al. (2002) would well coincide with the fast rate proposed by Paulo et al. (2001). Moreover, immunological albumin distances of 10–17 MC′F units between *Podarcis* species have been found (Lutz and Mayer, unpublished). Assuming that 100 MC′F units correspond to a divergence time of 55 myr (Wilson et al. 1977), a separation time of 5.5–8.5 myr can be calculated. Since the sequence differences between the *Podarcis* species *P. sicula*, *P. muralis*, and *P. melisellensis* are in a range of 6–7% for 12S rRNA genes, an average sequence divergence of 0.9–1.6%/myr is calculated, which fits very well with the slow rate of Lin et al. (2002). If we follow this assumption (1.25% divergence/myr), the divergence of 1.5–2.4% found between 12S rRNA haplotypes of the three main clades of *P. melisellensis* would correspond to a divergence in the earliest Pleistocene, 1.2–1.9 myr ago.

Accepting the commonly used estimate of about 120 m for the eustatic sea level rise during the last 18,000 years following the most recent Pleistocene glacial period (Wurm), probably all Croatian islands (with the exception of Jabuka) were connected to the mainland during the last glaciation. The channel depths of 80–100 m that separate the Vis and the Lastovo archipelagos from one another as well as from the mainland with nearby islands are much greater than in all other channels between islands. However, they are still above the boundary of 120 m, indicating that those archipelagos are not older than 18,000 years. Thus, although intraspecific monophyletic groups distinguished by large genealogical gaps usually arise from long-term extrinsic barriers to gene flow (Avise et al.

1987), the distinctiveness of the ''Lastovo'' and ''melisellensis'' clades cannot be simply explained by a long-time sea barrier preventing gene flow between island populations.

The existing geological data concerning the East Adriatic region are rather poor. However, some interesting facts concerning the paleogeographic evolution of the Apulo-Dalmatic Realm, from the Oligocene to the Pleistocene, were reported by De Giuli et al. (1987). The Apulo-Dalmatic Realm represents an area in the Southern Adriatic region formed by structural high blocks, including (among other elements) the Gargano peninsula and the Mid-Adriatic Ridge, the shallow-sea area between the Gargano and the Split-Dubrovnik region, which often emerged but was only discontinuously connected. Particularly interesting for our subject is the paleogeographic history of the area in the time of the late Pliocene and Pleistocene. According to De Giuli et al. (1987), an ingressive phase started during the Pliocene and progressed until the early Pleistocene, causing extensive flooding of the present west Adriatic coast. During the latest Pliocene and the early Pleistocene the sea reached its major extent in the whole Adriatic area. Although the Vis and the Lastovo archipelagos were never cut off from the main landmass during the time from early Miocene to present, it is worth noting that, in the period of 2.2–1.8 myr ago, these two archipelagos became placed very close to the mainland coastline. This time frame quite well matches the presumed age of our three main *P. melisellensis* lineages, and in our opinion it was then that gene flow between groups became restricted. Although the two archipelagos most probably were not completely isolated islands, there is still a possible explanation for the distinctiveness of the three groups: the area surrounding the archipelagos might have been exposed to frequent sea flooding during ingressive sea phases. During regressive phases salt swamps probably existed, which are not a preferable habitat for *P. melisellensis*. It is likely that distribution areas were in fact expanding during dry periods, but the migration fronts that eventually came in contact and were able to exchange genetic material became extinct during periods of flooding.

The origin of two subgroups in the ''fiumana'' clade can probably be ascribed to the existence of two separate Wurm glacial refugia, one placed south, the other north of the Neretva river. Gene flow between island populations was interrupted as a result of the last rising of the see level. Although 17 different haplotypes were found in the populations of the ''northern fiumana'' subclade, genetic differences between them are minimal, ranging from 0.1% to 0.4%. The haplotype with the highest frequency in our sample set, FN4, is present in a large geographical area. On the island Krk as well as on the tiny islet Mikavica, several different haplotypes were found. This suggests a rapid expansion from a smaller area of a single population with multiple haplotypes (Avise 2000), followed by a very recent isolation of the island populations due to rising sea levels. Although our sample size concerning the ''southern fiumana'' subclade is very small, the pattern is similar to that found in the northern populations.

The southernmost population belonging to ''northern fiumana'' was found on the islet Jerolim near Hvar Island. The population of the next island to the south, Korčula, already belongs to ''southern fiumana''. The southernmost mainland population belonging to ''northern fiumana'' was found in the Biokovo mountains, but a ''southern fiumana'' haplotype was found on the Pelješac peninsula. The genetic distances between samples of both subclades indicated that there is no considerable difference between the mainland and nearby islands. In other words, today's distribution of both subclades can be regarded as remnants of subrecent continuous areas. We assume that the border between the two ''fiumana'' subclades is located between the islands Hvar and Korčula. The most plausible explanation for this border is that it represents the extension of the course of the Neretva river. Before the rise of the see level, the Neretva flowed between today's islands Hvar and Korčula (Hydrographic Institute of the Republic of Croatia 1994), and thus could have divided the ranges of two ''fiumana'' groups. Even today the wide, swampy Neretva valley does not present suitable habitat for *P. melisellensis*, and it can be assumed that it still serves as a barrier to gene flow between the northern and southern ''fiumana'' subclades. On the other hand, a limited amount of genetic exchange between the two groups cannot been excluded, particularly because of several records of *P. melisellensis* from the Neretva region (Tiedemann and Henle 1986). However, the latter all came from inland localities in the Neretva valley where ecological characteristics better meet the preferences of *P. melisellensis*. Nevertheless, we presume that this contact occurred only recently, because during the harsh conditions of the Wurm period this area was probably not inhabited by the species.

The pattern of divergence within the ''Lastovo'' clade (Fig. 3B) is similar to that of the ''fiumana'' subclades. Probably, the populations of the small islets of the Lastovo archipelago were separated from the main island only recently. We therefore estimate the separation to have occurred at about the same time as between the island populations of ''northern fiumana'', i.e. as a consequence of the latest rising of the sea level.

The maximum distances between haplotypes observed within the ''melisellensis'' clade (1.3%) lie in the same range as between the two ''fiumana'' subclades, but the phylogenetic pattern is more complicated. The haplotypes found on the Vis archipelago, on Biševo Island as

well as on the islands of the Svetac Island group, stand somewhat separated but not as much as the "fiumana" subclades. The haplotypes of the Svetac archipelago populations are more divergent than those within the other island groups. Although the two small islands of Brusnik (M11, M12) and Kamik (M8) might be considered as fringing islands of Svetac, neither of their lizard populations shows particularly strong affinity with Svetac (M9) (Fig. 3C). The same result was obtained in a previous allozyme study (Gorman et al. 1975). The haplotype from the distant islet Jabuka (M10) is more similar to that of the Svetac population than are the haplotypes of the populations from its fringing islands (M8, M11, M12). That could be an indication for a rather recent over-water colonisation of Jabuka.

Taxonomic implications

In general, the phylogenetic relationships revealed in this study on the basis of mitochondrial DNA sequences are not in accordance with the current division of subspecies in *P. melisellensis*.

Thirteen of the 20 recognized subspecies fall into the "fiumana" clade, ten of them to its northern and two to its southern subclade, while *P. m. fiumana* itself appears as part of both "fiumana" subclades (Fig. 3A), making this subspecies paraphyletic from a mitochondrial DNA point of view. On the other hand, in some cases a certain haplotype was associated with different subspecies. For example, the most widespread haplotype, FN4, was found in populations of five subspecies: *P. m. fiumana*, *kornatica*, *thetidis*, *jidulae* and *caprina*, and the FN1 haplotype was found in *P. m. fiumana*, *bokicae* and *aeoli*.

The "melisellensis" clade (Fig. 3C) comprises seven currently accepted subspecies. Six different haplotypes were found in populations assigned to *P. m. lissana* from the Vis archipelago, one of them (M3) was found also in *P. m. gigas*. The most remote haplotype found within the Vis island group, M7 (*P. m. kammereri* from Mali Barjak), is still closer to the M2 haplotype than the haplotypes observed within the *P. m. lissana* population from Biševo (M4 and M5). The haplotypes of the remaining subspecies, *P. m. galvagnii* (M8), *digenea* (M9), *pomoensis* (M10), and *melisellensis* (M11 and M12), are unique, but differences between them and the above-mentioned haplotypes are quite small.

The taxonomic situation with the "Lastovo" clade is of particular interest. Boulenger (1920) and Wettstein (1926) ascribed the Lastovo population to *P. m. lissana*, the subspecies originally described from Vis (Werner 1891). However, although it has never been described as a distinctive taxon, several former investigations indicated that this population may be different from the populations from Vis. Wettstein (1926) named the Lastovo population "the green *lissana*", and Radovanović (1956) emphasised that the Lastovo population differs from that from Vis regarding body size and the number of dorsal scales. In a morphometric analysis performed by Clover (1979), the differences between these two populations were greater than between *P. m. lissana* (from Vis) and four other subspecies of *P. melisellensis*. Allozyme investigations performed by Gorman et al. (1975) revealed that *P. m. lissana* from Vis is electrophoretically more similar to at least six other recognized subspecies than to the homonymous population from Lastovo. Our mitochondrial DNA data confirm these results: The haplotype group found in the Lastovo archipelago is separated by a large genetic distance from *P. m. lissana* from Vis, and the subspecies *P. m. lissana* thus appears as a paraphyletic assemblage.

The taxonomy of a group should be consistent with its evolutionary history (Frost and Hillis 1990). It is often the case that the deepest phylogeographic partitions in intraspecific mtDNA gene trees agree with traditional taxonomic partitions (Avise 2000). However, this is not the case for *P. melisellensis*. On the one hand, there is a number of subspecies with minimal or no difference in mtDNA, particularly in the "fiumana" clade; on the other hand, one of the three main phylogeographic units, the population group from the Lastovo archipelago, has not been recognized as a distinct taxon. The incongruence between subspecies-level taxonomy and the results of mtDNA analysis becomes visible also within the "melisellensis" clade: each melanistic population has been described as a distinct subspecies, although the differences between them and the other populations of this clade are of the same order as the differences between northern and southern populations of *P. m. fiumana* that were never classified as different subspecies. By using principal coordinate analysis and a Wagner network, Thorpe (1980) re-evaluated morphological data sets published by Clover (1979) and showed that populations of *P. melisellensis* tend to segregate into a northern and a southern group. However, only 10 presently recognized subspecies and no mainland samples were included in his study. Nevertheless it is worth mentioning that, with the exception of the Lastovo population which fell into Thorpe's "southern group" (the subspecies from Vis archipelago), all his other results are highly consistent with our mtDNA data: his "northern group" (subspecies *P. m. fiumana*, *kornatica*, *thetidis*, *traguriana*, and *mikavicae*) is equivalent to our "fiumana" clade, and his "southern group" (subspecies *P. m. lissana*, *galvagnii*, *digenea*, *pomoensis*, and *melisellensis*) corresponds to our "melisellensis" clade.

It is not our intention to enact major taxonomic consequences only on the basis of mitochondrial DNA data. However, our results agree with the allozyme

elecrophoretic data published by Gorman et al. (1975), as well as with morphological analysis (Thorpe 1980), and contradict the current subspecific division in the species. This is not surprising because: (1) most of the subspecies have been described on the basis of very small samples and considering only few characters, and (2) the characteristics of island subspecies have never been compared with those of the mainland populations (*P. m. fiumana*). Therefore, it is obvious that most of the 20 currently accepted subspecies cannot be regarded as real phylogenetic units. Considering the phylogeographic structure and the fact that most of the presently accepted subspecies seem to represent not more than local varieties, our opinion is that the evolutionary history of *P. melisellensis* would most appropriately be described by recognizing only three taxonomic units corresponding to the three main clades (''fiumana'', ''Lastovo'', ''melisellensis''). Clades on that level of differentiation are often established as species according to an evolutionary species concept. In the present case the population groups are in fact separated (by sea channels), but there is no sign that they are reproductively isolated as well. Therefore, we prefer a more conservative concept of only one species with three subspecies for all taxa assigned to *P. melisellensis* today. According to this concept, all subspecies within the ''fiumana'' group (*P. m. fiumana, bokicae, aeoli, kornatica, thetidis, jiduale, caprina, plutonis, traguriana, mikavicae, lupa, gigantea*, and *curzolensis*) should be placed in synonymy with *P. m. fiumana*. Similarly, all subspecies of the ''melisellensis'' group (*P. m. lissana, kammereri, gigas, galvagnii, digenea, pomoensis*, and *melisellensis*) should be synonymized with *P. m. melisellensis*. Concerning the populations of the Lastovo archipelago there is still a controversy between morphological (Thorpe 1980) and molecular data (Gorman 1975; present study). Therefore, a critical morphometric analysis should be performed prior to the description of a third subspecies for the populations of the ''Lastovo'' group.

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