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Population structure, genetics and conservation of the Maltese wall lizard, *Podarcis filfolensis*, on Linosa Island (Reptilia, Lacertidae)

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ABSTRACT

Podarcis filfolensis is a lacertid lizard endemic to the Maltese and Pelagian archipelagos (Channel of Sicily). In Italy, this species occurs on Linosa and Lampione islands only, where the populations are referred to the endemic ssp. *laurentiimuelleri*. The Linosa population was studied using capture/recapture methods during two sampling seasons (1993, 2001), in order to analyse various ecological parameters and to assess habitat distribution and overall conservation status. A clear preference to xeric Mediterranean habitats dominated by *Pistacia lentiscus* was seen. The lizard density of the whole Linosa population, estimated by various methods, is extremely high. Molecular analyses (partial sequencing of mitochondrial $tRNA^{Phe}$ and 12S rDNA genes) and electrophoretic analysis of 26 presumptive gene loci were also carried out on samples representing the three *P. filfolensis* populations from Malta, Filfola and Linosa islands. Both molecular and allozyme data indicate that the populations of the Maltese Archipelago (Malta and Filfola) are closely related to each other, and that these populations are genetically relatively differentiated from the Linosa population. High levels of genetic variability characterise the latter population. Recent observations of the species on Lampione Islet indicate that it is locally widespread and abundant. Even though *P. filfolensis* does not seem to be threatened on either Linosa or Lampione, the populations occurring on these islands need to be regularly monitored as island populations are known to be more susceptible to change and extinction than mainland ones.

KEY WORDS: *Podarcis filfolensis* - Lacertidae - Ecology - Genetics - Pelagie Islands.

ACKNOWLEDGEMENTS

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INTRODUCTION

Podarcis filfolensis (Bedriaga, 1876) (Fig. 1) is a lacertid lizard endemic to the Maltese Archipelago and to Linosa and Lampione islands (Pelagian Islands) (Lanza, 1973; Turrisi & Vaccaro, 1998; Corti & Lo Cascio, 1999). Five subspecies were described: *P. f. filfolensis* (Bedriaga, 1876), occurring on Filfola Islet (Maltese Archipelago); *P. f. maltensis* Mertens, 1921, on Malta, Gozo and Comino islands (Maltese Archipelago, cfr. Despott, 1915); *P. f. generalensis* (Gulia, 1914), on the Fungus Rock Islet (Maltese Archipelago); *P. f. kieselbachi* (Fejérváry, 1924), on Saint Paul Islet (Maltese Archipelago); *P. f. laurentiimuelleri* (Fejérváry, 1924), on Linosa and Lampione islands (Pelagian Archipelago) (Fejérváry, 1924; Mertens, 1926; Lanza & Bruzzone, 1960; Bischoff, 1986; Capula, 1994; Bischoff, 1997; Turrisi & Vaccaro, 1998; Corti & Lo Cascio, 1999).

From a phylogenetic point of view, Oliverio *et al.* (1998, 2000), using molecular data (12S rDNA), stressed a great affinity between *P. filfolensis* and *P. wagleriana* Gistel, 1868, a species endemic to Sicily and the Egadi Islands. This result confirms immunological studies carried out by Lanza & Cei (1977) and geological data, suggesting the occurrence of some geological connections between southern Sicily and the Maltese Archipelago during the Quaternary. According to Oliverio *et al.* (2000) *P. filfolensis* and *P. wagleriana* can be considered sister species and represent an ancient clade closely related to *P. muralis* (Laurenti, 1768), a species widely distributed in southern Europe. On the other hand, Capula (1994), using allozyme data, found that *P. filfolensis* was genetically more similar to *P. sicula* (Rafinesque, 1810) (average Nei's $D = 0.306$) than to *P. wagleriana* (average Nei's $D = 0.526$), thus testifing to a closer relationship between *P. filfolensis* and *P. sicula* than between the former and *P. wagleriana*.

The scattered distribution of *P. filfolensis* on the Pelagian Islands needs some explanation. The absence of lacertid lizards on Lampedusa Island is probably due to the presence of snake predators such as *Malpolon monspessulanus* (Hermann, 1804) and *Macropotodon cucullatus* (Geoffroy, 1827). This hypothesis is supported by the presence of a lizard [*Psammodromus algirus* (Linnaues, 1758)] on the Conigli Islet, a very small fringing island, separated from Lampedusa Island by a very short linear distance, where snakes are absent. On the other hand, *P. filfolensis* occurs on Lampione, a calcare-

ous island located about 18 km W NW of Lampedusa, and on Linosa, a volcanic island located between Malta and Lampedusa. *Podarcis filfolensis* and *P wagleriana* probably originated from a common ancestor after the separation of Sicily and the Maltese Archipelago (Oliverio *et al.*, 2000). It may well be that *P filfolensis* reached the Pelagian Islands by dispersal events, probably anthropocorous (Capula, 1994).

The main threats for the species are represented by the presence of natural predators such as kestrels, sparrows (Fornasari & Zava, 2001) and possibly other migratory birds. Moreover, it should be highlighted that the local people systematically kill these lizards, in order to prevent damage to grapes and other crops, which are normally eaten by *P. filfolensis*.

Calcara (1851) was the first to report information on the presence of lizards on Linosa Later, lizards were observed by Giglioli (1879), Escherich (1893), Sommier (1906-1908), Fejérváry (1924) (who described the ssp. *laurentiimulleri*), Mertens (1926) Lanza & Bruzzone (1960), Zavattari (1960), Corti *et al* (1997); Corti & Lo Cascio (1999).

In the last decade, only two ecological investigations were carried out on *P. f. laurentiimuelleri*, respectively on trophic ecology (Sorci, 1990) and on population structure (di Palma, 1991), but both were characterised by a low number of records. In the present paper, the results of a large study focusing on population structure, genetics and conservation of the Linosa population are reported. The trophic niche of *P filfolensis*, compared with that of *Chalcides ocellatus*, is also being analysed and the results will be reported in a separate contribution (Bombi *et al.*, in preparation).

MATERIALS AND METHODS

Study area

Ecological and genetic research was carried out on the Linosa population, while on Lampione only faunistic observations were performed.

Linosa (between 35°51'07"N and 35 52'34' N, and 12 50 43"E and 12°52'34"E) (Fig. 2) and Lampione (35 33 00 'N 12 19'11 'E) Islands (Agrigento Province) are situated in the Mediterranean Sea, in the Channel of Sicily, about 150 km and 200 km respectively SW of Sicily, and 140 km and 120 respectively NE of Tunisia

From a geological point of view, Linosa is a volcanic island with a basaltic belt and inner tufaceous formations On the con trary the other two Pelagian Islands, i e Lampione and Lampe dusa, are calcareous and belong to the African continental plate (Agnesi & Federico, 1995)

The climate of Linosa is semi arid (Fantoli 1960 Vittorini, 1973 Brullo & Piccione, 1985· Agnesi & Federico, 1995). The vegetation is characterised by xerophilic Mediterranean maquis (Brullo & Piccione, 1985) the main crops includes grapes, prickly pears capers, cereals and lentils

Population structure

Field research was carried out in July 1993 and in June 2000.

In 1993, the whole island was subdivided into 28 grid squares, each side being 500 m (Fig 1), using the kilometric UTM grid as reference. In each square two capture sessions were carried out using five pitfall traps filled with pieces of ripe fruit, placed at a distance of 3 metres from each other

During the same year, two capture sessions using a square grid (40 m x 40 m) of 100 pitfall traps were carried out in a single prickly pear cultivation The captured lizards were sexed, measured for total length, weighed, marked with nail polish and released The classical proportion $m M \quad n.N$ (M· number of captured specimens in the first ession m· number of recaptured specimens in the second session n number of captured individuals in the second session; N· number of specimens of the population) was used to determine the numeric size of the population

Fig. 1 *Podarcis filfolensis laurentiimuelleri* from Linosa Island (photo M Capula).

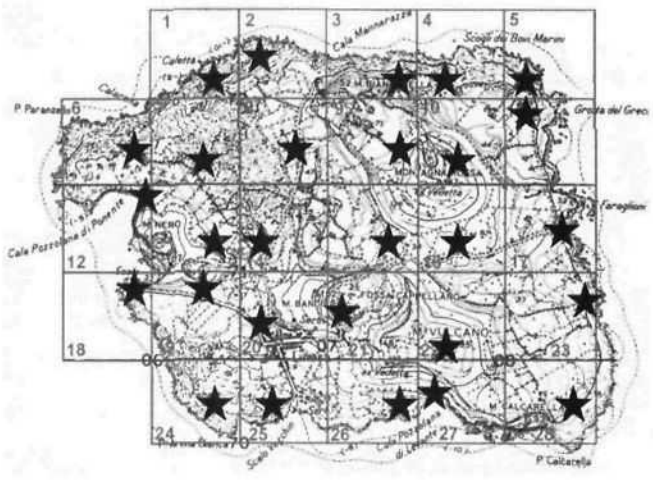

Fig 2 Linosa Island with the network of reference (parallel lines are separated by 500 m) and the pitfall traps positions (black stars).

Data collected on these sample were also used to analyse habitat preference, population structure density, and biometry.

In 2000, the field research was focused primarily on studying the species biometrics and trophic ecology. Captures were made in a sampling area of about half an hectare, near the shoreline where specimens were captured by hand, using a little noose or by pitfall traps filled with a small quantity of water. Each captured specimen was sexed and measured for total length· the cloacal temperature of each lizard was also taken After the measurements were taken, the animals were marked with a colour pen and then released Moreover, the faecal pellets and the stomach contents (obtained through stomach flushing· see: Legler & Sullivan, 1979, and Bombi & Bologna, 2002, for the methodology) were collected and the air and soil temperatures were recorded. Data collected from these samplings were only used here for the analysis of the sex ratio and biometrics

Genetics

Molecular and genetic analyses were carried out respectively by partial sequencing of mitochondrial $tRNA^{Phe}$ and 12S rDNA genes and by multilocus electrophoresis of gene enzyme systems on samples representing the three populations from Malta, Filfola and Linosa islands. Specimens used for DNA analysis are listed in Table I. The material for these analyses was obtained by collecting muscle tissue samples from the tail of the captured specimens or by drawing blood samples directly from the heart of the lizards. Permits for collecting specimens or part of their body, were obtained from the Italian and the Maltese Ministries of the Environment

Molecular analyses Total DNA extraction, designed primers, PCR and cloning conditions were described in Oliverio *et al.* (1998, 2000) Plasmid DNA from positive clones was sequenced using an ABI model 373A automated DNA sequencer using Dye Terminator Ready Reaction Kit (Perkin Elmer) according to the manufacturer protocol

Nucleotide sequences were aligned by eye also taking account of the secondary structure of the 12S rRNA domains I and II (De Rijk *et al.*, 1992), which is very useful in improving the alignment, no ambiguous alignment positions were scored (Oliverio *et al.*, 2000) The divergence indices (uncorrected "p") between the sequences were calculated. To test whether multiple substitutions had a saturation effect on the analysed sites, pairwise transition and transversion proportions were plotted against the corresponding divergence indices

The aligned lacertid sequences were then analysed by the neighbour joining (NJ· Saitou & Nei, 1987) method. Node support in the resulting tree was estimated by 1000 bootstrap replicates; the Ts/Tv ratio was then estimated along the trees. Sequences were also analysed using the Maximum Parsimony (MP: Farris, 1970) method with a heuristic search and node support analysed with a search on 1000 bootstrap replicates. Indels (positions including insertions deletions, aligned by gaps) were included in a first analysis, then excluded to score the influence of gaps on the topologies but preference was given to results from the analyses on the gap-excluding dataset Equal weight was initially given to transitions and transversions; all analyses were then replicated by imposing a weight to transversions 2, 2.5, 3, 5 and 10 times that of transitions.

Lacerta bilineata was chosen as a direct outgroup, and the corresponding mtDNA sequence was analysed also from this species (see Oliverio *et al*, 2000). All analyses were performed using the licensed package PAUP 4* (Swofford, 1999)

Electrophoretic analyses: Specimens used for the electrophoretic analysis were obtained from Malta Island (N = 19), Linosa Island (N = 19) and Filfola Islet (N = 3). Standard horizontal starch gel electrophoresis was performed on tail-muscle tissue which was crushed in distilled water. Gene products for the following 23 presumptive enzyme loci were analysed: *αGpd, Ldh 1 Ldh-2, Mdh-1, Mdh 2, Me 1, Me-2, Idh 1 Idh-2, 6Pgd, Gapd, Sod-1, Np, Got-1, Got 2, Ck Ak, Pgm 1, Pgm 2, Ada, Ca-2, Mpi* and *Gpi* In addition, three unidentified non-enzymatic proteins (*Gp-1, Gp-2, Gp-4*) were studied. The buffer systems electrophoretic procedures and staining techniques were those described by Capula (1994). Genotypic proportions expected on the basis of Hardy-Weinberg equilibrium were calculated by Levene's (1949) formula for small samples. The statistical significance of departures from Hardy-Weinberg equilibrium was estimated using a test for calculating exact significance probabilities, analogous to Fisher's exact test (Haldane, 1954). The genetic variability of populations was estimated using the following parameters: mean number of alleles per locus (*A*), proportion of polymorphic loci at the 99% level (*P*); observed mean heterozygosity per locus (H_O); expected mean heterozygosity per locus (H_e, unbiased estimate, Nei, 1978) The genetic relationships among the studied populations were evaluated using Nei's (1978) unbiased genetic identity (I) and genetic distance (*D*). All genetic-variability and genetic-distance measures were calculated using the computer program BIOSYS-1 (Swofford & Selander 1989).

TABLE I - *Specimens of* Podarcis filfolensis *used for DNA analysis* (MZUR: *Zoological Museum of "La Sapienza" Roma University; MZRT: Zoological Museum of "Roma Tre ' University).*

Podarcis filfolensis laurentimuelleri (Fejérváry, 1924):
Italy, Sicily, Agrigento Prov., Linosa Island. 2.IV.1990, M. Bologna leg (Pfl#5) (MZUR R830)

Podarcis filfolensis maltensis Mertens, 1921:
Malta, Gozo Island, Ramla, 21 I.1997, P. Schembri leg. (Pfm#1) (MZRT); Malta, Malta Island, Zeytun, 2 II.1997, P Schembri leg. (Pfm#2) (MZRT).

Podarcis wagleriana Gistel, 1868
Italy, Sicily, Palermo Prov , Godrano, 690 m asl, 31.III.1973, G Carpaneto leg. (Pwa#1) (MZUR R-902); Italy, Sicily, Trapani Prov., Egadi Islands, Marettimo Island, 4.XII.1992, M. Mei leg. (Pwa#2) (MZUR R-878).

Lacerta bilineata Daudin 1802:
Italy, Latium, Rome Prov. Castel di Decima, 2 XI.1996, M. Bologna leg. (Lbi#1) (MZRT).

RESULTS

This research allowed us to confirm the existence of a large *P. filfolensis* population on Linosa Island Only a

few brief surveys were performed on Lampione Island: in particular in 1997 (M. Capula pers. comm.), in 2001 (25/04/2001) and in 2002 (3/3/2002) (S. Pasta, pers. comm.). During each survey, several lizards were observed and the species appeared to be common and widespread.

Population estimation

Seven hundred and thirty two individuals were caught during the 1993 sampling activity in all of the 28 grid squares of the island, 351 during the first trapping sessions and 381 during the second trapping sessions; 64 marked lizards were checked. These results allowed us to estimate a total of 2090 individuals for the areas surrounding the trapping transects, but of course they represent only a small subset of the entire island population. For each sampling site, a mean number of 74.6 lizards were assessed. The mean distance covered by the lizards trapped in both sessions was assessed at 2.4 m for males and 2.6 for females.

The two sampling sessions carried out using the grid of 100 pitfall traps allowed further information to be obtained. During the first session, 119 lizards were captured, while 178 during the second session (with 48 lizards checked); a subpopulation of 441 individuals, with a sex-ratio of 1.05 females/males, was assessed in this restricted area (40 × 40 m). According to our estimations, the mean distance covered by each individual was 5.5 m. The surface of "influence" of the grid of traps was consequently estimated to be 2209 m^2, and the relative population density 0.20 lizards/m^2. According to this value, a theoretical population of more than one million of individuals was calculated for Linosa Island. On the other hand, a density of 0.29 lizards/m^2 was found by means of the data obtained in the 28 grid squares. According to this estimate, the theoretical number of individuals occurring on Linosa Island would be even greater than that reported above. However, considering the attractive effect of the traps three times more powerful than that observed (16.5 m instead than 5.5 m), the relative population density estimation would be 0.05 lizards/m^2, and the theoretical population occurring on the island would be ca. 250,000 individuals. The higher value of the influence radius of the pitfall traps is consistent with the maximum distance of 16.9 m covered by a marked lizard. The habitat heterogeneity of Linosa would suggest that the real size of the entire Linosa population could be intermediate between the values reported above.

Biometrics

During the survey carried out in 1993, 37 males and 36 females were analysed for a biometric study. Weight and four linear measurements were calculated (see Table II). The species is sexually dimorphic and males are larger than females: 22.9% of the total length; 21.2% of the forearm; 24.5% of the cranial length; 28.1% of the cranial width; 82.3% of the weight ($P < 0.01$ for each of the t tests).

Other measurements were taken on 41 males, 23 females and 7 young specimens (probably newborn) during the 2000 sampling season. Weight and six linear measurements were recorded (Table III). The earlier period of this second sampling (June vs. July) could explain the differences in the observed weight.

Habitat preference

The habitat preferences of *P. filfolensis* on Linosa Island were defined by its consistency: this could be considered as the ratio between the number of collected specimens and the number of sites referring to each vegetational type (Fig. 3). The highest consistency was observed in the ecosystems with the dominance of *Pistacia lentiscus* Linnaeus in both the xerophilic microvegetation and the typical maquis. A lower density was observed in the xerophilic vegetation without *P. lentiscus*, and in agricultural areas.

Population genetics

Molecular analyses. As far as molecular characteristics are concerned, the DNA sequences were published in a separate paper (Oliverio *et al.* 2000). The secondary structure models adopted for the lacertid 12S rRNA domains I and II was derived by comparison of different taxa (see Oliverio *et al.*, 2000). This comparative analysis of rRNA sequences has greatly improved the reliability of the nucleotide alignment. The folding model of 12S rRNA domain II of Pfm#2 (see Table I) is shown in Fig. 4.

The molecular data confirm the greater affinity between the populations of the Maltese Archipelago than between those and the Linosa population (see Oliverio *et al.*, 2000 for details). Phylogenetic relationships deriving from the NJ method is depicted in Fig. 5.

TABLE II - *Biometrics of* Podarcis filfolensis *specimens collected during the 1993 samplings*

	Total length	Forearm	Cranial length	Cranial width	Weight
Males					
n	37	8	10	7	22
Mean	170.2	7.1	15.5	10.0	7.9
SD	16.4	0.8	1.0	0.8	1.4
Min	140.0	6.1	14.0	8.7	5.0
Max	205.0	8.0	17.2	10.8	10.5
Females					
n	36	13	13	12	20
Mean	138.4	5.8	12.5	7.8	4.4
SD	10.1	0.4	0.6	0.5	0.7
Min	11.0	5.1	11.3	6.9	3.0
Max	155.0	6.5	13.3	8.5	5.5

TABLE III - *Biometrics of* Podarcis filfolensis *specimens collected during the 2000 samplings.*

	SVL	Femur	Tibia	Cranial length	Cranial width	Mouth length	Weight
Males							
n	41	40	41	40	41	41	42
Mean	61.7	10.4	11.4	14.8	9.8	11.8	6.6
SD	3.5	1	0.9	0.8	0.8	1	1.2
Min	53.9	7.9	8	12.6	8	8.8	4
Max	69.1	13.1	13	16	11.2	13.8	9
Females							
n	23	23	23	23	23	22	24
Mean	58.2	8.8	9.6	12.2	8.0	10	4.2
SD	3.4	1	0.5	0.4	0.4	0.8	0.5
Min	52.5	7.3	8.3	11.5	7	8.8	3
Max	68.4	11	10.5	12.9	8.8	12	5
Young							
n	7	7	7	7	7	7	7
Mean	31.5	5.2	5.7	7.9	4.7	5.8	0.5
SD	0.9	0.5	0.3	0.2	0.1	0.6	0
Min	30.6	4.2	5.3	7.6	4.6	4.8	0.5
Max	33.2	5.6	6.2	8.1	4.9	6.5	0.5

Electrophoretic analysis. Of the 26 presumptive gene loci analysed (*αGpd, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Idh-1, Idh-2, Gapd, Sod-1, Np, Got-1, Got-2, Ak, Ada, Ca-2, Mpi, Gp-1, Gp-2*), 18 were found to be monomorphic and fixed for the same allele in all the studied samples. The remaining 8 loci *(Me-1, Me-2, 6Pgd, Ck, Pgm-1, Pgm-2, Gpi, Gp-4)* were polymorphic in at least one sample. No significant deviation from Hardy-Weinberg equilibrium was found in the studied populations. The considered genetic variability parameters are given in Table IV. The highest percentages of polymorphism and heterozygosity were found in the Linosa sample ($P_{99\%}$ = 26.9; H_O = 0.080), while noticeably lower levels of genetic variability ($P_{99\%}$ = 7.7; H_O = 0.038) were pointed out in the Filfola sample. The values of Nei's standard genetic identity and genetic distance for each pairwise comparison are given in Table V. The samples from the Maltese Archipelago (Malta and Filfola) were genetically similar to each other (D = 0.027). On the other hand, the comparison between the Maltese samples and the Linosa population gave relatively higher values of genetic distance (average D = 0.088).

DISCUSSION

On the basis of the present data, *Podarcis filfolensis laurentiimuelleri* can still be considered widespread and quite common both on Linosa and Lampione islands. Moreover, according to the results of the molecu-

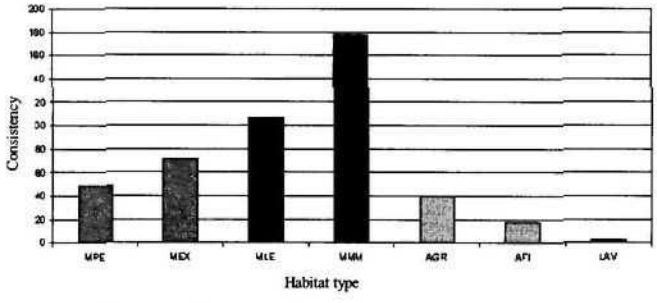

Fig. 3 - Habitat preferences of *Podarcis filfolensis* on Linosa Island. The consistency is defined as number of specimens collected/number of sites referable to each vegetational type. MPE: maquis dominated by *Periploca laevigata*; MEX: xerophilic microvegetation; MLE: maquis dominated by *Pistacia lentiscus*; MMM: xerophilic microvegetation mixed with maquis; AGR: agricultural areas; AFI: prickly pear cultivation; LAV: coastal lavic areas.

TABLE IV - *Genetic variability parameters in* Podarcis filfolensis *popualtions from Malta, Filfola and Linosa islands.* A, *mean number of alleles per locus;* P, *mean proportion of polymorphic loci;* H_0, *observed mean heterozygosity;* H_e, *expected mean heterozygosity. Standard errors are reported in parentheses.*

Population	*A*	*P*	H_O	H_e
Malta	1.2	19.2	0.052 (0.029)	0.049 (0.026)
Filfola	1.1	7.7	0.038 (0.028)	0.028 (0.020)
Linosa	1.3	26.9	0.080 (0.033)	0.078 (0.032)

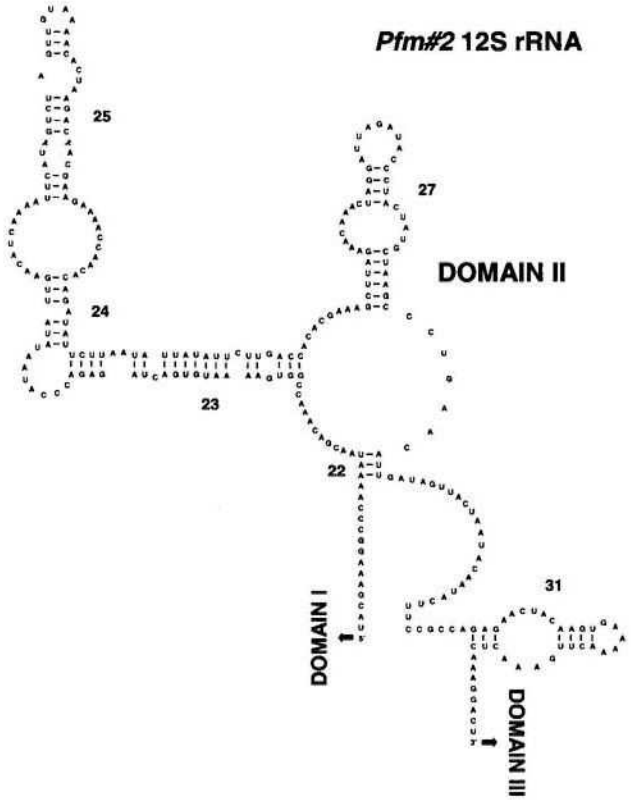

Fig. 4 - Proposed secondary structure model of the mitochondrial 12S rRNA Domain II of *Podarcis filfolensis*. Helix numbering is from Van de Peer et al. (1994).

TABLE V - *Values of Nei's (1978) genetic identity (above the diagonal) and genetic distance (below the diagonal) among* Podarcis filfolensis *popualtions from Malta, Filfola and Linosa islands.*

Population	Malta	Filfola	Linosa
Malta	*****	0.973	0.902
Filfola	0.027	*****	0.921
Linosa	0.098	0.079	*****

lar and electrophoretic analyses, the Linosa population is characterised by high levels of genetic variability and thus is not threatened by the effects of loss of genetic variation pointed out in some phylogenetically related insular species [e.g. *P. raffonei* (Mertens, 1952); Capula, 2004]. However, conservation and correct management of the endemic subspecies *laurentiimuellerii* is particularly important because of the high genetic isolation of the Linosa and Lampione populations and the extremely limited range of this taxon.

Threats due to the accidental introduction of allocthonous predators or competitors, such as e.g. cats, rabbits, snakes or other lizards, must be taken into account

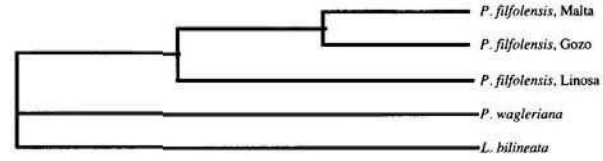

Fig. 5 - Phylogenetic tree using the Neighbour Joining Method, analysing the nucleotidic sequences.

when assessing the potential causes of extinction on the species (Fornasari & Zava, 2001). In addition, it must be noted that the local population of Linosa systematically kill lizards, in order to prevent damage to grape cultivations, regularly eaten by *P. filfolensis*.

In light of these considerations, we suggest changing the conservation status of the lizards from Linosa and Lampione *P. f. laurentiimuelleri*, proposed in the Red Book of Italian Vertebrates (Bulgarini *et al.*, 1998), from "critically endangered" to "at low risk". To prevent future decimation of these lizards, the collection and trade in the species should be prohibited as soon as possible. Moreover, strict control of the presence of alien fauna and prevention of intentional or accidental introductions of predators and competitor species are required.

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