Can mitochondrial DNA draw the phylogenetic picture of Central Mediterranean island *Podarcis*?

Five species of wall lizards (genus *Podarcis*) occur on the Central Mediterranean Islands of Corsica, Sardinia, Sicily, the Malta Archipelago and surrounding islets: *P. sicula* (RAFINESQUE-SCHMALTZ, 1810) (Corsica, Sardinia and Sicily), *P. tiliguerta* (GMELIN, 1789) (Corsica and Sardinia), *P. filfolensis* (BEDRIAGA, 1876) (Malta Archipelago), *P. wagleriana* (GISTEL, 1868) (Sicily) and *P. raffonei* (MERTENS, 1952) (some islets in the Aeolian Archipelago). The specific status of the latter species was recently established on the basis of allozyme electrophoretic results comprising former subspecies of *P. sicula* and *P. wagleriana* (CAPULA 1994). *Podarcis raffonei* is obviously the sister species of *P. wagleriana*. Samples of all five species have been included in a phylogenetic analysis of the genus by OLIVERIO et al. (2000) based on mitochondrial *12SrRNA* sequences. HARRIS & ARNOLD (1999) also published *12SrRNA* sequences of *Podarcis* species occurring on Central Mediterranean islands (*P. tiliguerta*, *P. filfolensis*, *P. sicula*) but used a different part of the gene. Unfortunately, these two *12SrRNA* segments overlap only about 70 bp. Hence, the results cannot be compared directly. In a paper on mitochondrial DNA data of *P. tiliguerta* accepted for publication and kindly provided by the authors, HARRIS et al. (2005) demonstrated high genetic divergence between the populations from Sardinia and Corsica and suspected that this species would represent a species complex. In the present paper sequences from a DNA segment covering both parts of the *12SrRNA* gene (here refered to as 5'-section vs. 3'-section) of samples of all above species except *P. raffonei* are presented and compared separately with the data of both OLIVERIO et al. (2000) (data set I) and HARRIS & ARNOLD (1999) (data set II).

We sequenced approximately 920 bp of the mitochondrial *12S rRNA* gene from four samples of *P. tiliguerta*, and one sample each from *P. wagleriana*, *P. filfolensis* and *P. sicula*.

The conditions for the amplification of the 3'- and 5'-sections, as well as the amplification and sequencing primers are described in PODNAR et al. (2005). Sequencing was carried out by MWG-BIOTECH® (Ebersberg, Germany). Sequences were aligned using ClustalX® (THOMPSON et al. 1997) and corrected by eye. In order to compare our data with previously published sequences (HARRIS & ARNOLD 1999; OLIVERIO et al. 2000) this alignment was partitioned into two data sets: data set I comprises the sequences of the 5'-section (about 555 bp) representing the section published by OLIVERIO et al. (2000), data set II those of the 3'-section (about 350 bp) representing the segment published by HARRIS & ARNOLD (1999). Calculation of *p*-distances was performed using MEGA® version 2.1 (KUMAR et al. 2001). Phylogenetic analyses were conducted on both data sets separately using Maximum Parsimony (MP) and Maximum Likelihood (ML) as implemented in PAUP® (PAUP Version 4.0b10, SWOFFORD 2002). *Podarcis sicula* (sample sic-P) was selected as the outgroup since it appears to be basal to all other species from the Central Mediterranean islands (OLIVERIO et al. 2000). For ML analyses the optimal model of sequence evolution was determined by means of ModelTest® software (Version 3.06, POSADA & CRANDALL 1998), the HKY+Γ model (HASEGAWA et al. 1985; YANG 1993) for data set I, and TrN+Γ model (TAMURA & NEI 1993) for data set II. Bootstrap (BS) analysis was performed with 1000 replicates for MP trees and with 100 bootstrap replicates for ML trees. All sequences are deposited in Genbank (see appendix).

Sequence analysis revealed extremely low differences between species on the one hand, and unusually high divergences within some species on the other hand. In data set I, the *p*-distances between species were from 0.2% to 7.4%. Distances within species were up to 6.6% for *P. tiliguerta*, up to 0.9% for *P. filfolensis*, and up to 4.8% for *P. wagleriana* samples.

ML (data set I: single tree, -ln L = 5524.15262, data set II: single tree, -ln L = 821.20514) and MP (data set I: three equally parsimonious trees; length = 116, CI = 0.767, RI = 0.827, RC = 0.634, data set II: fourteen equally parsimonious trees; length = 63, CI = 0.746, RI = 0.709, RC = 0.529) analyses of data set I revealed similar over-

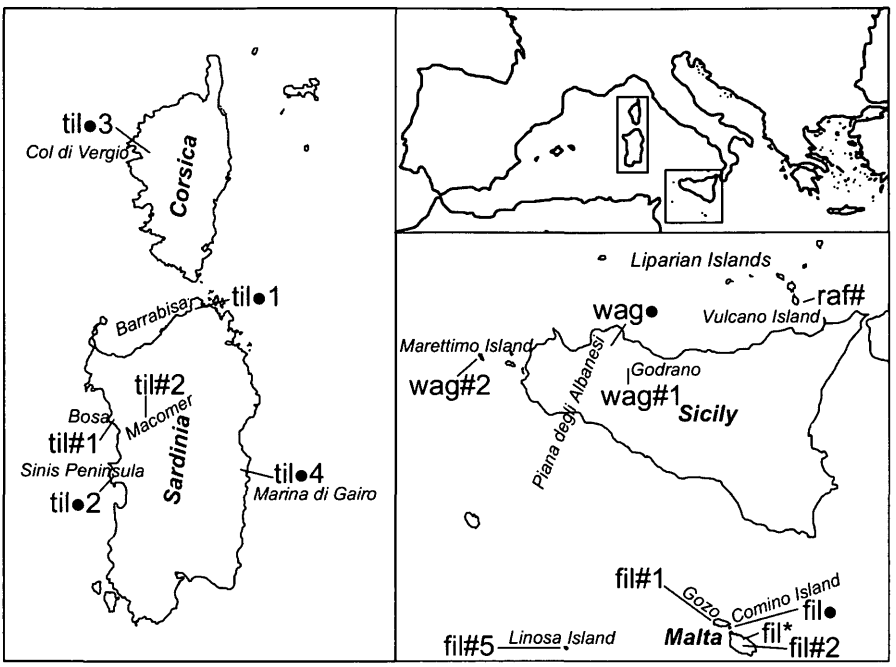

Fig. 1: The Central Mediterranean islands of Sardinia, Corsica, Sicily and the Malta and Aeolian Archipelagos. Localities of samples analyzed in this study. Abbreviations: see Appendix.

all topologies of phylogenetic trees with three clades with low bootstrap support: The "*filfolensis-wagleriana*" clade encompasses our and OLIVERIO's samples of *P. filfolensis* as well as both *P. wagleriana* samples of OLIVERIO et al. (2000). The "*raffonei-wagleriana*" clade, contains our *P. wagleriana* and OLIVERIO's *P. raffonei* sample. The third one comprises our western and northern Sardinian samples of *P. tiliguerta* and OLIVERIO's samples of that species. Our *P. tiliguerta* samples from Corsica and southeastern Sardinia appear as separate lineages in the MP phylogenetic trees. In ML analysis they cluster with the "*filfolensis-wagleriana*" clade, however, with poor BS support. For both data sets, the Shimodaira-Hasegawa test (full optimization with 1000 bootstrap replicates, SHIMODAIRA & HASEGAWA 1999; GOLDMAN et al. 2000) was used to compare the ML topology to a constrained topology that enforced monophyly of *P. tiliguerta* lineages. Tests resulted in no significant differences in likelihood between the ML tree and constrained topology ($p > 0.05$ for both data sets), hence, monophyly of *P. tiliguerta* cannot be rejected.

Our *P. wagleriana* sample (wag●) and those published by OLIVERIO et al. (2000) represent two highly divergent mitochondrial lineages. The *p*-distances between them are about 5%, a value usually found between well separated *Podarcis* species. In contrast, the *p*-distance between OLIVERIO's *P. wagleriana* (samples wag#1 and wag#2) and our *P. filfolensis* sample (fil●) is surprisingly low for distinct species (0.2%, corresponding to one substitution only). In addition, the *p*-distances between the subspecies *P. filfolensis maltensis* MERTENS, 1921 (fil#1, fil#2 and fil●) and *P. f. laurentimuelleri* (FEJÉRVÁRY, 1924) (fil#5) are higher than the distances between *P. f. maltensis* and OLIVERIO's samples of *P. wagleriana* (wag#1, wag#2).

The observed phenomena can be explained by introgression of *P. filfolensis* mitochondrial DNA into that of *P. wagleriana*. The connection of the Malta archipelago with Sicily during the last glacial period (Würm) enabled a contact between both

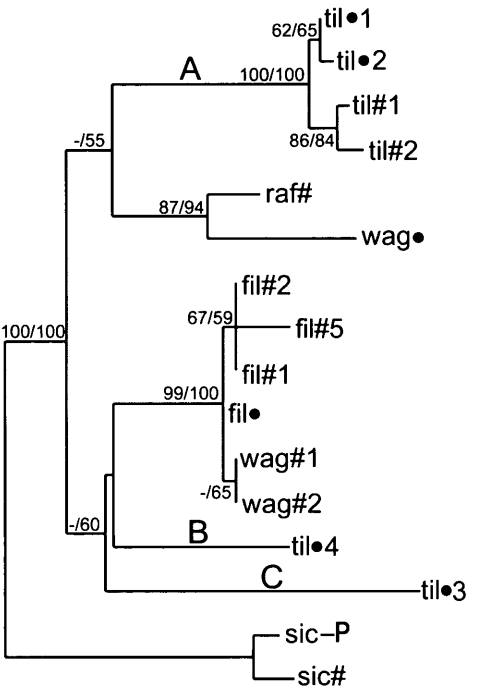

Fig. 2A: Phylogram from ML analyses of data set I. Numbers at the branches indicate ML and MP bootstrap values respectively. BS values smaller than 50 are indicated by a dash. Capital letters at the branches designate the different mitochondrial lineages of *Podarcis tiliguerta*.

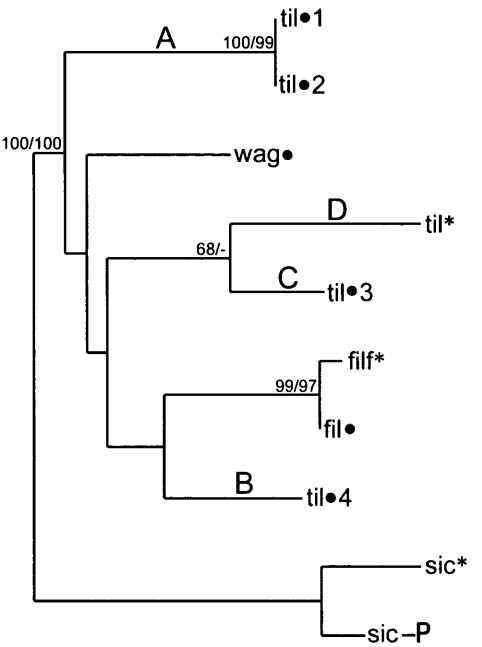

Fig. 2B: Phylogram from ML analyses of data set II. Numbers at the branches indicate ML and MP bootstrap values respectively. BS values smaller than 50 are indicated by a dash. Capital letters at the branches designate the different mitochondrial lineages of *Podarcis tiliguerta*.

species possibly followed by a limited episode of hybridization and mitochondrial DNA introgression. The *p*-distance of 2.6% found between the sequence of *P. wagleriana* (wag●) and *P. raffonei antoninoi* (raf#) is relatively small for *Podarcis* species. This confirms the hypothesis of CAPULA (1994) that *P. raffonei* originated from a colonisation of *wagleriana*-like lizards from Sicily. Thus, we assume that the autochthonous haplotype of *P. wagleriana* is the *raffonei*-like and not the *filfolensis*-like haplotype.

Concerning *P. tiliguerta*, three clearly separated lineages, two from Sardinia and one from Corsica, were found (branches A, B, C in fig. 2A). Although monophyly of the different lineages could not be rejected, the sequence differences between them are within the range usually found between distinct *Podarcis* species.

Podarcis tiliguerta is a polytypic species. LANZA & POGGESI (1980) reported about strong inter-island geographical variation in morphology and coloration. Morphological variety of lizards is often severely constrained by local environment (LOSOS 1997). The resulting morphological heterogeneity of the whole species is not necessarily accompanied by large genetic divergence. SCHNEIDER (1973) found high morphological variability within and between *P. tiliguerta* of Sardinia and Corsica along a north-south cline; hence, separate taxonomic units cannot be delimited. Furthermore, several studies of allozyme polymorphism (MAYER 1981; CAPULA 1996) showed remarkably high genetic differences between populations, however, without fixed alleles.

Unfortunately, we cannot compare our results with the sequences of HARRIS et

al. (2005) because they are not yet available in Genbank. Nevertheless, according to the localities given in their submitted paper we assume that the sequences are similar to our sequences "til●1", "til●2" from Sardinia and "til●3" from Corsica, respectively. In the draft version of the above paper we miss the reference and discussion of the very different *P. tiliguerta* "til*" published by HARRIS & ARNOLD (1999). This sequence is well separated from all other sequences. In the MP analysis it clusters with Corsican sample til●3, however with poor BS support (data set II, Fig. 2B). Moreover, the sequence divergence between til* and til●3 is rather high (3.2 %), and, therefore, it represents a forth lineage within *P. tiliguerta*, i.e., a third one from Sardinia (branch D in fig. 2B). Mitochondrial DNA divergences between all four haplotypes are in an order of magnitude which is usual among *Podarcis* species isolated from one another for millions of years. Based on allozyme electrophoretic data (*F*-statistics and genetic distances), CAPULA (1996) found three clades within this species corresponding to populations from (1) Corsica, (2) Corsican offshore islands, and (3) Sardinia, but no fixed alleles differentiating Corsican from Sardinian populations. Gene introgression is a quite unlikely explanation for the high mitochondrial sequence divergence among *P. tiliguerta*, because of the distinctiveness of all mitochondrial types compared to all *Podarcis* sequences published up to now. Nevertheless, it is also rather improbable that three haplotype lineages survived on the moderate-sized (only 24.000 km²) island of Sardinia, over a period of millions of years. In conclusion we do not agree with the opinion of HARRIS et al. (2005) that *P. tiliguerta* could represent a species complex, at least not concerning the different clades in Sardinia.

The high intraspecific divergences in both *P. wagleriana* and *P. tiliguerta* have to be investigated more thoroughly, with more samples from more localities comparing nuclear genes and mitochondrial genes to obtain a satisfying explanation of the surprising mitochondrial data.

APPENDIX: Data of samples (see fig. 1), Genbank accession numbers are given in parentheses; sequences from this study are indicated by "●", "#" indicates sequences of OLIVERIO et al. (2000), and "*" sequences of HARRIS & ARNOLD (1999).

Podarcis filfolensis: Malta Archipelago: Comino Island: fil● (DQ017660); Gozo Island fil#1 (AJ001415); Malta Island fil#2 (AJ001463) and fil* (AF133442), Linosa Island fil#5 (AJ250156).

Podarcis tiliguerta: Sardinia: Barrabisa til●1 (DQ017655); Sinis Peninsula til●2 (DQ017656); Marina Gairo til●4 (DQ017658); Bosa til#1 (AJ001478); S. Maria de Sauccu til#2 (AJ001479); "Sardinia" til* (AF133456); Corsica: Col de Vergio til●3 (DQ017657).

Podarcis raffonei: Aeolian Archipelago: Vulcano Island raf# (AJ250156).

Podarcis sicula: Sicily: Mti. Peloritani sic-P (AY770867); Sardinia: Oristano sic# (AJ001476); "Italy" sic* (AF133454).

P*odarcis wagleriana*: Sicily: Piana d. Albanese wag● (DQ017659); Godrano wag#1 (AJ001466); Marettimo Island wag#2 (AJ001467).

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The diet of *Bothrops asper* (GARMAN, 1884) in the Pacific lowlands of Ecuador

The Terciopelo, *Bothrops asper* (GARMAN, 1884), is a large pitviper species ranging from Mexico to northwestern South America (CAMPBELL & LAMAR 2004). It is an important causer of snakebite casualties wherever it occurs (WARRELL 2004). In Ecuador, where *B. asper* is usually referred to as "equis", this species is distributed throughout the Pacific lowlands and adjacent western versant of the Cordillera Occidental of the Andes up to at least 1,700 m a.s.l. (FREIRE & KUCH 1994; CISNEROS-HEREDIA & TOUZET 2004). It has also been collected in two dry inter-Andean valleys in southern Ecuador which are connected by rivers to the Pacific lowlands (CISNEROS-HEREDIA & TOUZET 2004; U. KUCH & F. P. AYALA-V., unpublished). *Bothrops asper* is by far the most commonly encountered and medically most important venomous snake in western Ecuador. It is found in a variety of natural habitats, from dry coastal scrub to cloud forests (CISNEROS-HEREDIA & TOUZET 2004), as well as in agricultural lands and around human habitations. While aspects of the natural history of certain populations of *B. asper* were comprehensively studied (SOLÓRZANO & CERDAS 1989), little has been published about Ecuadorian populations (CAMPBELL & LAMAR 2004; KUCH et al. 2004).

Here we report the results of an analysis of stomach and intestine contents of preserved specimens of *B. asper* in the collection of the Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito, Ecuador (QCAZ).

We dissected 21 specimens of *B. asper*, and found evidence of prey in 14. Of these, only one contained undigested or only partly digested prey. Thirteen specimens were found to contain remains of digested prey (see Appendix for locality information and catalogue numbers). The specimens containing prey (seven males, seven females) were assigned to three classes based on the study by SOLÓRZANO & CERDAS (1989) and personal observations on sexual size dimorphism and size at maturity of Ecuadorian *B. asper*: juveniles (29-38 cm snout-vent-length [SVL], four males; 40-49 cm SVL, four females); subadults (58 cm SVL, one male; 85 and 90 cm SVL, two females); and adults (115 and 95.2 cm SVL, two males; > 83 cm SVL, one female [head missing]). Among the 13 snakes containing only remains of digested prey, evidence that rodents had been eaten (hair of members of the family Muridae, also rodent bones and incisors) was found in six, insect remains in nine (from the orders Coleoptera [1], Diptera [2], Hemiptera [3], Hymenoptera [2], Orthoptera [1], unidentified [3]) and anuran remains arms, legs and/or skull of *Eleutherodactylus achatinus* [BOULENGER, 1898], Leptodactylidae) in three. One of these snakes contained lizard bones, and another plant matter (leaves), apparently ingested along with insect prey.

Eight of the 14 snakes with prey contained at least two different prey species (bird and centipede [1], insect and rodent [3], insect and lizard [1], insect and frog [2], and coleopterans and hemipterans [1]).