Molecular phylogeny and historical biogeography of the land snail genus *Solatopupa* (Pulmonata) in the peri-Tyrrhenian area

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Abstract

The land snail genus *Solatopupa* consists of six species and has a peri-Tyrrhenian distribution; most of the species have a very narrow range and all of them except one (*Solatopupa cianensis*, which inhabits porphyritic rocks) are strictly bound to calcareous substrates. One species (*Solatopupa guidoni*) is limited to Sardinia, Corsica, and Elba Island. Because the potential for dispersal of these snails is low, the insular range of this species has been traditionally related to the Oligocene detachment of the Sardinia–Corsica microplate from the Iberian plate and its subsequent rotation towards the Italian peninsula. In this study, we used sequences of three mitochondrial and one nuclear gene to reconstruct the evolutionary history of the genus. Our phylogenetic results are consistent with the genetic relationships found using allozymes, but contrast with the phylogenetic hypotheses based on karyology and morphology. Molecular clock estimates indicate that the main cladogenetic events in the genus occurred between the middle Miocene and the middle-late Pliocene. Patterns of phylogenetic relationships and geological considerations suggest that the cladogenesis of the genus can be explained by vicariant (tectonic) processes. Our datings do not support a causal relation between the split of *S. guidoni* from its continental sister taxon and the initial phases of the detachment of the Corsica–Sardinia microplate from the mainland. On the contrary, time estimates coincide with the very last phase of detachment of the microplate (from 5 to 3 Myrs ago). Overall, our molecular clock estimates are in good agreement with the latest geological views on the tectonic evolution of the peri-Tyrrhenian area.

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1. Introduction

The peri-Tyrrhenian area is of central interest for biogeographers because its geological evolution is well known and many tectonic events are placed in a well-defined temporal framework. Amongst others, the disjunction and the rotation of the Corsica–Sardinia microplate from the Iberian plate has been traditionally considered a very important event responsible for the similar pattern of disjunct (vicariant) distribution currently observed in a variety of taxonomically unrelated organisms with limited dispersal abilities. Corsica and Sardinia split most probably as a single microplate around 29 Myrs ago, while the rotation of the microplate and the disjunction of the two islands started 15 Myrs ago and was completed by 9 Myrs ago (Alvarez, 1972, 1974; Bellon et al., 1977; Bonin et al., 1979; Esu and Kotsakis, 1983; Lanza, 1984). Recently, new geological data challenged this classic scenario. Robertson and Grasso (1995) and Carmignani et al. (1995) dated the beginning of the split of the
microplate at 24–20 Myrs. The maps presented in Meulenkamp and Sissingh (2003) support these views but also suggest that the microplate remained connected with the border of the Paleo-Europe during its anti-clock wise rotation through a land bridge that will constitute the future Maritime Alps and the Ligurian Apennines. The final detachment of the microplate from the mainland was contemporary with the onset of the uplift of Tuscany in continental Italy, and occurred in the Pliocene (around 5 Myrs ago). A schematic representation of these two alternative scenarios is presented in Fig. 1.

Several molecular studies have been carried out on a number of different groups, which show a disjunct distribution in the area; these include earthworms (Cobolli Sbordoni et al., 1992), stone flies (Fochetti, 1994; Fochetti et al., 2004), subterranean aquatic isopods (Ketmaier et al., 1999, 2000, 2003), cave beetles, and newts (Caccone et al., 1994, 1997; Caccone and Sbordoni, 2001). Most of these studies demonstrated a clear link between cladogenetic events and the initial phases of the split of the microplate from the Iberian plate, supporting the vicariance hypothesis over the dispersal one.

Here, we present another study case in the peri-Tyrrhenian area by using the land snail genus Solatopupa as a model organism. This genus of pulmonate gastropod belongs to the family Chondrinidae and has a suite of features that makes it a reliable model for biogeographic studies. It shows a peri-Tyrrhenian distribution, ranging from Northern Spain to Central Italy, including Sardinia, Corsica and Elba Island. The genus comprises only six species (Solatopupa similis, Solatopupa juliana, Solatopupa psarolena, Solatopupa guidoni, Solatopupa pallida, and Solatopupa cianensis) thus allowing testing of alternative biogeographic hypotheses within a well-defined phylogenetic framework. S. cianensis, S. psarolena, and S. pallida are restricted to limited areas between Southern France and Liguria while S. guidoni is confined to Corsica, Sardinia, and Elba Island. The S. similis range spans from Northern Spain to Eastern Liguria and S. juliana is distributed from Western Liguria to Northern Latium. Species ranges are shown in Fig. 2. These species have a very low potential for long distance dispersal, as it is the case in the vast majority of terrestrial mollusks. They are all xero-

Fig. 1. The peri-Tyrrhenian area (A) from the Early Oligocene to the Middle Pliocene (B and C). Maps in (B) show the movement of Corsica and Sardinia from the Iberian plate according to traditional views. Maps in (C) illustrate the alternative hypothesis on the movement of Corsica and Sardinia from the Iberian plate and the connection between the microplate and the mainland (see Section 1 for appropriate references). Triangles indicate the main areas of subduction. The maps were drawn on the basis of present geography. (C) Redrawn from Meulenkamp and Sissingh (2003).
phile, rock dwelling, and strictly bound to limestone, except *S. cianensis*, which inhabits porphyritic rocks. Therefore, the presence of *S. guidoni* in Corsica, Sardinia, and Elba Island is particularly intriguing from a biogeographic point of view. This species might have split from its continental ancestor sometime in the past between 24 and 5 Myrs ago, following the beginning of the detachment of the Corsica–Sardinia microplate or when the connection between the microplate and the mainland was definitively lost. Alternatively, the species might have reached these islands by dispersal.

*Boato* (1986) demonstrated an allopatric distribution of populations of *S. similis* with different chromosome numbers (2N = 60 in populations from Spain to Western Liguria, 2N = 58 in populations from Eastern Liguria to Northern Latium). Subsequently, the same author found more than a 40% of genetic divergence at 28 allozyme loci and slight morphological differences between the two chromosomal forms (*Boato*, 1988, 1991). On these bases, Eastern populations of *S. similis* with 2N = 58 were attributed to *S. juliana*. In addition, previous phylogenetic hypotheses are available for the whole genus, based on morphology, allozymes, and karyology (*Boato*, 1986, 1988, 1991; Gittenberger, 1984).

We designed this study to obtain a molecular phylogeny of the genus *Solatopupa* by using sequences of the three most commonly used mtDNA genes (cytochrome oxidase I, COI; small ribosomal DNA subunit, 12s rRNA, and large ribosomal DNA subunit, 16s rRNA) and of one nuclear gene (histone H3). Our aims are first, to evaluate whether the relationships among species parallel the paleo Geography of the peri-Tyrrhenian area and, second to provide an approximate temporal framework for the evolution of the genus. We will then use our time estimates and phylogenetic hypotheses to test the vicariance vs. dispersal scenarios for the *S. guidoni* species occurring in Sardinia, Corsica, and the Elba Island (Fig. 2). Should we accept vicariance as the most likely scenario, we will address the timing of the *S. guidoni* divergence from its continental closest relative. Finally, we want to compare our hypotheses with relationships based on morphology, karyology, and allozymes (*Boato*, 1986, 1988, 1991).

### 2. Materials and methods

#### 2.1. Sampling

Table 1 reports population and species of *Solatopupa* sampled for this study as well as information on the collecting sites and dates of collection. All the species of the

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**Table 1**

<table>
<thead>
<tr>
<th>Species (2N)</th>
<th>Locality</th>
<th>UTM coordinates</th>
<th>Sampling date</th>
<th>N</th>
<th>Code</th>
<th>Accession numbersb</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. similis</em> (60)</td>
<td>Montpellier, France</td>
<td>31TEJ63</td>
<td>6-05-1986</td>
<td>5</td>
<td>MN</td>
<td>DQ305042/DQ305057/DQ305084/DQ305095</td>
</tr>
<tr>
<td></td>
<td>Var, France</td>
<td>32TLP32</td>
<td>21-06-1984</td>
<td>3</td>
<td>VA</td>
<td>DQ305043/DQ305058/DQ305073/DQ305088</td>
</tr>
<tr>
<td></td>
<td>Mentone, France</td>
<td>32TLP74</td>
<td>4-06-1982</td>
<td>3</td>
<td>ME</td>
<td>DQ305044/DQ305059/DQ305085/DQ305096</td>
</tr>
<tr>
<td></td>
<td>Finalborgo, Liguria, Italy</td>
<td>32TMP4691</td>
<td>24-10-1981</td>
<td>4</td>
<td>FI</td>
<td>DQ305045/DQ305060/DQ305078/DQ305092</td>
</tr>
<tr>
<td><em>S. juliana</em> (58)</td>
<td>Isola del Tino, Liguria, Italy</td>
<td>32TNP6875</td>
<td>19-05-1996</td>
<td>3</td>
<td>TI</td>
<td>DQ305052/DQ305067/DQ305074/DQ305089</td>
</tr>
<tr>
<td></td>
<td>Monte Cetona, Tuscany, Italy</td>
<td>32TQN3457</td>
<td>2-06-2003</td>
<td>3</td>
<td>MC</td>
<td>DQ305050/DQ305065/DQ305082/DQ305097</td>
</tr>
<tr>
<td></td>
<td>Stigliano, Tuscany, Italy</td>
<td>32TPN88</td>
<td>2-06-2003</td>
<td>3</td>
<td>ST</td>
<td>DQ305051/DQ305066/DQ305075/DQ305090</td>
</tr>
<tr>
<td><em>S. guidoni</em> (58)</td>
<td>Monte Grosso, Elba Is., Tuscany, Italy</td>
<td>32TPN1546</td>
<td>4-03-1975</td>
<td>2</td>
<td>GR</td>
<td>DQ305046/DQ305061/DQ305076/DQ305100</td>
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<td></td>
<td>Sabara, Corsica, France</td>
<td>32TNN1196</td>
<td>22-07-1979</td>
<td>4</td>
<td>SB</td>
<td>DQ305047/DQ305062/DQ305080/DQ305091</td>
</tr>
<tr>
<td></td>
<td>Francardo, Corsica, France</td>
<td>32TNN1594</td>
<td>2-12-1983</td>
<td>3</td>
<td>FR</td>
<td>DQ305048/DQ305063/DQ305077/DQ305098</td>
</tr>
<tr>
<td></td>
<td>Capo Caccia, Sardinia, Italy</td>
<td>32TMK29</td>
<td>29-03-1977</td>
<td>3</td>
<td>CC</td>
<td>DQ305049/DQ305064/DQ305079/DQ305101</td>
</tr>
<tr>
<td><em>S. psarolena</em> (56)</td>
<td>Imperia, Liguria, Italy</td>
<td>32TLP9371</td>
<td>24-10-1981</td>
<td>4</td>
<td>FI</td>
<td>DQ305045/DQ305060/DQ305078/DQ305092</td>
</tr>
<tr>
<td><em>S. pallida</em> (58)</td>
<td>Porto Spinone, Liguria, Italy</td>
<td>32TQN88</td>
<td>2-06-2003</td>
<td>3</td>
<td>ST</td>
<td>DQ305051/DQ305066/DQ305075/DQ305090</td>
</tr>
<tr>
<td><em>S. cianensis</em> (56)</td>
<td>Gorges of Cians, France</td>
<td>32TLP37</td>
<td>25-04-1993</td>
<td>1</td>
<td>GO</td>
<td>DQ305055/DQ305070/DQ305072/DQ305087</td>
</tr>
</tbody>
</table>

For each species the chromosome number (2N) as reported in *Boato* (1986) is also given. Numbers in the sixth column (Map) correspond to collecting sites in Fig. 2. The last four columns report GenBank accession numbers.

a Populations/species used for comparisons to previous morphological, karyological, and allozyme data.

b Accession numbers for the outgroup species *C. avenacea* are: DQ305056/DQ305071/DQ305086/DQ305093 for 12s, 16s, COI, and H3, respectively.
2.2. DNA extraction, PCR amplification, and sequencing

Total cellular DNA was extracted from the whole body after removal of the shell. After homogenization, tissues were digested overnight at 55°C with Proteinase K followed by a standard phenol/chloroform extraction. The primer pairs 12sa/12sb and 16sa/16sb (Palumbi et al., 1991) were used to amplify about 450bp of 12s gene and about 550bp of 16s gene. Primer pair LCO1490/HCO2198 (Folmer et al., 1994) was used to amplify about 660bp of the Cytochrome Oxidase I gene (COI); the primer pair H3F/H3R (Colgan et al., 2000) was used to amplify 250bp of the Histone 3 (H3) gene. Double stranded PCRs were performed on a Touchdown thermal cycler (Hybaid). PCR conditions were 2 min at 94°C, followed by 35 cycles of 1 min at 95°C, 30 s at 50°C (for 12s, 16s and H3)/45°C (for COI) and 1 min at 72°C with a final elongation of 2 min at 72°C. These conditions allowed full-length PCR amplifications of the recently collected samples (S. juliana). For the older samples (collections time reported in Table 1), we were unable to amplify directly the whole PCR fragment due to DNA degradation. For these samples, we used a nested PCR approach where we first amplified the native DNA with the above mentioned primer pairs. This first round of PCR amplification was followed by a second round of PCR cycling, using as template the undiluted PCR products from the first PCR amplification. PCR primers (Table 2) used for this second round were designed on the S. juliana DNA sequences aligned with orthologues sequences of published gastropods. Nested PCR conditions were 2 min at 94°C, followed by 45 cycles of 1 min at 94°C, 1 min at 50°C and 1 min 30 s at 72°C with a final elongation of 6 min at 72°C. PCR products were purified using a GeneClean Kit (Bio 101, Carlsbad, CA). Automated sequencing was carried out using an ABI Prism 3100 DNA Sequencer and the Big Dye terminator chemistry (Perkin-Elmer Cetus), following manufacturer’s protocols. Amplified DNA was sequenced in both directions. GenBank accession Nos. are reported in Table 1.

2.3. Phylogenetic analyses

Sequences were edited using Sequencher 3.1.1 (Gene Code Corporation, Ann Arbor, MI) and easily aligned by eye. Alignment was also checked with Clustal X (Thompson et al., 1997). Genes were analyzed separately and combined in all possible combinations. We used PAUP* 4.0b10 (Swofford, 2003) to calculate variability estimates, the number of transitions (Ti) and transversions (Tv), and to perform \( \chi^2 \) tests for homogeneity of base frequencies. Saturation of sequences was investigated by plotting the absolute number of Ti and Tv against the percentage sequence divergence. This was done for each gene separately and for all genes combined. For the COI and H3 fragments, this analysis was performed at all codon positions and at 3rd codon positions.

Aligned sequences were analysed by maximum parsimony (MP; heuristic searches, ACCTRAN character-state optimisation, 100 random stepwise additions, TBR branch-swapping algorithm) (Farris, 1970), maximum likelihood (ML; heuristic searches, 100 random stepwise additions, TBR branch swapping algorithm) (Felsenstein, 1981), neighbor-joining (NJ) (Saitou and Nei, 1987), and Bayesian methods (Huelsenbeck, 2000; Larget and Simon, 1999; Mau and Newton, 1997; Mau et al., 1999; Rannala and Yang, 1996). MP, ML, and NJ analyses were performed using PAUP* 4.0b10; Bayesian analysis was carried out using MRBAYES (Huelsenbeck, 2000). MP searches were run giving equal weight to all substitutions and down weighting Ti three times Tv (Tv3 \times Ti). We ran the ML analyses on PAUP* 4.0b10 after having determined the best model of DNA substitutions for the different gene combinations using MODELTEST (Posada and Crandall, 1998). NJ analyses were carried out on ML distances calculated with the same parameters used for ML analyses. For the Bayesian approach, we employed the same models of sequence evolution allowing site-specific rate variation partitioned by gene and, for COI and H3, by codon positions. MRBAYES was run for 2 million generations with a sampling frequency of 100 generations. We ran one cold and three heated Markov chains. To establish if the Markov chains had reached stationarity, we plotted the likelihood scores of sampled trees against

Table 2
Forward (F) and reverse (R) primers used in nested PCRs

<table>
<thead>
<tr>
<th>Gene/prime name</th>
<th>Sequence 5’-3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>12s</td>
<td>TTTAAGTCCAGCTCATGAAAA</td>
</tr>
<tr>
<td>12s-329 (R)</td>
<td></td>
</tr>
<tr>
<td>16s</td>
<td>CTTGACTGTTGCAAAAGGTAGC</td>
</tr>
<tr>
<td>16s-109 (F)</td>
<td></td>
</tr>
<tr>
<td>16s-421 (R)</td>
<td>TAGGCCCTAATCCCAACATCG</td>
</tr>
<tr>
<td>COI</td>
<td>TGGTAACTGCTCATGCTTTTG</td>
</tr>
<tr>
<td>COI-140 (F)</td>
<td></td>
</tr>
<tr>
<td>COI-463 (R)</td>
<td>AGCTTATTCAGGTGCTCGTA</td>
</tr>
<tr>
<td>H3</td>
<td>AGACGGGCCGAAAATCTACTG</td>
</tr>
<tr>
<td>H3-7 (F)</td>
<td></td>
</tr>
<tr>
<td>H3-183 (R)</td>
<td>AATCCTGTGGAATGGCAGTTT</td>
</tr>
</tbody>
</table>

Primer names indicate the different genes, numbers represent the position of the 3’ base of the oligonucleotide in the S. juliana sequences.

a This primer was used in conjunction with the universal primer 12sa (Palumbi et al., 1991).
generation time. Trees generated before the stationarity phase were discarded as “burn-in” (first 10% of the sampled trees), and posterior probability values for each node were calculated based on the remaining 90% of sampled trees. These trees were used to construct a 50% majority rule consensus tree using PAUP* 4.0b10. The robustness of the phylogenetic hypotheses was tested by bootstrap replicates (1000 replicates for MP and NJ and 100 replicates for ML) (Felsenstein, 1985).

Competing phylogenetic hypotheses were tested using the approximately unbiased tree selection test (AU; Shimodaira, 2002) in the software package CONSEL (Shimodaira and Hasegawa, 2001). We always compared tree topologies simultaneously (Shimodaira and Hasegawa, 1999). For comparison, we also performed the Shimodaira and Hasegawa (SH) test (1999) as implemented in PAUP* 4.0b10, with the resampling estimated log-likelihood (RELL) technique. SH and AU tests are appropriate in comparing both a priori and a posteriori hypothesis. However, the SH test is conservative, as the number of trees included in the confidence set increases as the number of trees being considered increases, while the AU test uses a multiscale bootstrap approach to remove this bias (Strimmer and Rambaut, 2002).

We used the AU and SH tests also to compare our phylogenetic hypotheses with those generated by Boato (1991) using morphological characters and allozymes (28 loci). Morphological data include eight characters of the shell and 5 of the male genital ducts. Boato (1991) deduced them from classical comparative analysis of shell and genital features. To make the different data sets comparable, we reduced our data to a single population per species. Populations included in these new partitions were chosen on the basis of their geographical proximity to populations analyzed by Boato (1991) (Table 1). Given the lack of an outgroup in the morphological and allozyme data sets, we did not include C. avenacea in these analyses and we treated trees as unrooted. We re-determined the appropriate models of DNA evolution that best fitted the trimmed DNA data sets with MODELTEST (Posada and Crandall, 1998).

We used the Trace Character History facility in Mesquite 1.05 (Maddison and Maddison, 2003) and the Parsimony Reconstruction method to plot the morphological data of Boato (1991) onto our molecular phylogeny. In addition, we also plotted diploid chromosome numbers (2N) and the presence/absence of dependence on limestone on our tree.

2.4. Molecular clock and divergence times

The molecular clock hypothesis was tested with the likelihood ratio test (LRT; Goldman, 1993), which compares the log-likelihood of the ML trees with and without assuming a molecular clock. The LRT test was carried out on the following partitions: (I) 12s; (II) 16s; (III) COI-1st codon positions; (IV) COI-2nd codon positions; (V) COI-3rd codon positions; (VI) COI-1st+2nd codon positions; (VII) COI-all codon positions; (VIII) H3-all codon positions; (IX) all genes combined excluding COI 3rd codon positions; (X) all genes combined. We used MODELTEST (Posada and Crandall, 1998) to infer all substitution model parameters for each partition before calculation of the LRT tests.

Time estimates were calculated according to the formula $T = D_{ML}/2r$, where $D_{ML}$ is the maximum likelihood genetic distance and $r$ is the mutation rate. $D_{ML}$ distances were calculated using the substitution model parameters selected for each data partition with MODELTEST (Posada and Crandall, 1998). We used several different previous published rates for mollusks. Rates for mitochondrial ribosomal genes vary between 0.5 and 0.6% per million years for gastropods (Ozawa and Okamoto, 1993; Rumbak et al., 1994). However, Chiba (1999) and Thomaz et al. (1996) reported accelerated rates (10–12.9% per million year) in the mitochondrial ribosomal genes of the land snail genera Mandarina and Cepaea. Marko (2002) calibrated rates for COI and H3 genes for six gaiemate pairs of bivalve mollusks (family Arcidae) taking advantage of the geological datings available for the formation of the Isthmus of Panama. COI rates vary between 0.03 and 6.84% per million years, depending on the codon position considered. H3 rates range from 0.02 to 0.20% per million years.

3. Results

3.1. Sequence variation

By using nested primers we were able to sequence 421 bp from 12s rRNA gene, 330 bp from 16s rRNA gene, 346 bp from COI gene and 191 bp from H3 gene, totaling about 1288 bp for each individual. Different individuals of the same population always had identical sequences; hence, we used a single sequence per population for our analysis. We found few indels in the alignment of ribosomal genes and a single 3-bp deletion in the COI gene in the outgroup species (C. avenacea). No indels were found in the H3 alignment. No stop codons were observed in COI and H3 sequences. As expected mitochondrial genes show an excess of A’s and are biased against G, especially in COI 3rd codon positions. The latter partition is by far the most variable one; the first and 2nd codon positions of H3 are the least variable partitions. Details on sequence variability are given in Appendix A. Inspection of the saturation plots (not shown; available upon request from the first author) suggests that 12s and H3 and the combined data set have not reached saturation; saturation was only beginning to be apparent in outgroup comparisons for 16s and COI (all positions), while the COI 3rd codon positions have reached saturation. Removal or inclusion of indels in the phylogenetic analyses (indels were counted as one single mutation each, regardless of size) did not result in significant differences in tree topologies.

3.2. Phylogenetic analyses

We conducted our analyses on all genes combined (data partition I; DPI). According to the COI saturation curves,
we analyzed data excluding COI 3rd codon positions (data partition II; DPII) and considering COI only (data partition III; DPIII). We also analyzed separately ribosomal genes, ribosomal genes+H3 and COI+H3. Results obtained on these additional three partitions are not shown because they are almost identical to results obtained on DPI, DPII and DPIII.

Fig. 3 shows the ML tree obtained on the complete data set (DPII) using the TVM+I+G model of evolution ($\alpha = 0.528$; model chosen with MODELTEST) and summarizes the results of the other phylogenetic methods employed in the study. Phylogenetic analyses strongly support a sister taxa relationship for *S. similis/S. guidoni* and *S. pallida/S. juliana* while the position of *S. psarolena* and *S. cianensis* is not supported. At the intraspecific level multiple populations of *S. similis*, *S. guidoni*, and *S. juliana* always form strongly supported monophyletic clades. Within *S. guidoni*, the population from the Elba Is. (GR) is placed as the closest relative to the Sardinian population (CC). The sister taxa relationships between *S. similis* and *S. guidoni* is not supported when COI 3rd codon positions are excluded from the analysis, while the clade grouping *S. psarolena*, *S. pallida*, and *S. juliana* is still present in the tree but its topology is less supported. Also in this case species represented by more than one population are clearly monophyletic. Analyses on the COI gene fragments alone produced quite a different topology; *S. similis* and *S. guidoni* are placed as sister taxa but *S. psarolena* is embedded within *S. similis* while *S. cianensis* is placed basal to a lineage including *S. guidoni*, *S. similis*, and *S. psarolena*. COI gene also provided a strong support for a sister species relationship between *S. pallida* and *S. juliana*. With the only exception of the placement of *S. psarolena* within *S. similis*, analyses of the different data sets always produced identical topologies at the intraspecific level.

### 3.3. Mapping of morphological, karyological, and ecological characters

Fig. 4 lists the 8 shell and 5 male genital characters analyzed by Boato (1991) and illustrates their reconstruction along with chromosome numbers and gain/loss of dependence on limestone on the molecular tree based on all genes. In general, when morphological and karyological characters are superimposed on the molecular phylogeny there is a considerable increase in homoplasy. Consistency index (CI) is 0.816 for the tree based on all genes; this value drops to 0.550 when morphological characters are simultaneously considered with the molecular ones. In particular, when we look at the shell morphology characters we find that none of them is free from reversal when mapped onto the DNA-based tree. Given our phylogenetic tree and the observed distribution of character states in the terminal taxa, we need to invoke one/two reversal events for each shell character to fit them in the molecular data. The situation improves when we look at the genital suite of traits. In this case, only two characters (junction between epiphallum and flagellum and proximal/distal epiphallum length) show reversal events. We were also interested in understanding whether chromosome number could be a reliable source of phylogenetic information in *Solatopupa*. Chromosomal rearrangements appear to be only randomly associated with speciation and not particularly informative on phylogenetic relations. Finally, we found that dependence on limestone was acquired only once in the past when all loci are simultaneously considered.

### 3.4. Topology tests

The MP (unweighted and with Tv’s weighted three times more than Ti’s), NJ and Bayesian trees are statistically undistinguishable from the ML trees based on different data partitions with the AU and SH tests (Table 3). The same table also reports the results of AU and SH tests conducted on a variety of independent alternate phylogenetic hypotheses. Because of the biogeographic interest in finding the closest extant relative of *S. guidoni*, we forced each species of the genus to be its sister taxon. AU and SH tests reject a sister relationship between *S. guidoni* and *S. juliana* and between *S. guidoni* and *S. pallida* for most of the partitions analyzed. The AU test also rejects a sister species relationship between *S. guidoni* and *S. psarolena*, regardless of the data partition considered. It is to note that this hypothesis is never rejected by the conservative SH test. Only DPIII rejects the hypothesis of *S. cianensis* being sister to *S. guidoni*. Within *S. guidoni*, phylogenetic searches forcing populations from Corsica and Elba Islands as each other’s closest relative produced trees statistically worst than the unconstrained ones. ML topolo-
Topological tests between the pruned DNA data set and the topology based on morphological or allozymic data indicate that the phylogenetic hypotheses based on all partitions of the DNA sequence data are not statistically distinguishable from the topologies obtained using allozymes, but are statistically different from the one based on morphological data (Table 3).

### 3.5. Divergence times

The results of the LRT tests comparing trees in which the molecular clock was both relaxed and enforced are presented in Table 4. The molecular clock hypothesis can be
Table 4
Summary of the likelihood ratio tests (LRT) (Goldman, 1993) of the molecular clock conducted on ten different data partitions

<table>
<thead>
<tr>
<th>Data partition</th>
<th>Substitution model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$-\ln L$</th>
<th>$-\ln L$ (clock)</th>
<th>$2\Delta L$</th>
<th>df</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>12s</td>
<td>K81uf+Γ</td>
<td>1696.07</td>
<td>1705.95</td>
<td>19.76</td>
<td>13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>16s</td>
<td>TVM+Γ</td>
<td>1284.22</td>
<td>1308.18</td>
<td>47.92</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>COI-1st pos.</td>
<td>TrNeF+Γ</td>
<td>282.99</td>
<td>290.95</td>
<td>15.92</td>
<td>13</td>
<td>&gt;0.250</td>
</tr>
<tr>
<td>COI-2nd pos.</td>
<td>F8I+I</td>
<td>166.22</td>
<td>166.59</td>
<td>0.74</td>
<td>13</td>
<td>&gt;0.975</td>
</tr>
<tr>
<td>COI-3rd pos.</td>
<td>HKY+I</td>
<td>727.11</td>
<td>742.85</td>
<td>31.48</td>
<td>13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>COI-1st + 2nd pos.</td>
<td>HKY+Γ</td>
<td>475.42</td>
<td>495.45</td>
<td>20.06</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>H3-all pos.</td>
<td>HKY</td>
<td>358.93</td>
<td>363.85</td>
<td>9.84</td>
<td>13</td>
<td>&gt;0.500</td>
</tr>
<tr>
<td>DPI (all genes)</td>
<td>TVM+1+Γ</td>
<td>4941.01</td>
<td>5093.95</td>
<td>305.88</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DPII (all genes excl. COI-1st pos.)</td>
<td>TVM+Γ</td>
<td>4048.27</td>
<td>4236.19</td>
<td>375.84</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DPIII (COI only)</td>
<td>TVM+Γ</td>
<td>1328.74</td>
<td>1436.34</td>
<td>215.2</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Models selected with MODELTEST (Posada and Crandall, 1998).

rejected for DPI, DPII, DPIII and for 16s and COI, when analyzed separately. For COI, we conducted LRT tests considering all positions together, all positions separately and pooling 1st and 2nd codon positions. The LRT tests suggest that for COI the molecular clock hypothesis can only be accepted when 1st and 2nd codon positions are considered separately. A clock cannot also be rejected for 12s, and H3 sequences (all positions). Time estimates based on 12s place the split between *S. similis* and *S. guidoni* from 0.25 to 4.83 Myr, depending on the rate adopted (0.6–10%/Myr). COI 1st codon positions yielded estimates that range from 1.2 to 3.66 Myr. Rates for this partition vary between 0.15 and 0.46%/Myr. COI 2nd codon positions and H3 (all positions) are invariant for this split. The divergence time for the *juliana*–*pallida*–*psarolena* clade ranges in age from 0.89 to 14.83 Myr based on 12s; from 10.54 to 32.33 Myr based on COI 1st codon positions (rates for this partition are comprised between 0.03 and 0.19%/Myr) and from 5.75 to 37.5 Myr based on H3. Rates for this gene are 0.02–0.20%/Myr. Within *S. guidoni*, divergence times range between 0.19 and 3.16 Myr (12s estimates) and 2.5–7.66 Myr (COI 1st codon positions). Matrices of corrected pairwise genetic distances based on 12s, H3, and COI (1st and 2nd codon positions) are given in Appendix B.

4. Discussion

4.1. Phylogeny

According to DPI and DPII *S. cianensis* is basal in the phylogeny of the genus. On the contrary, its position is noticeably different according to DPIII. Previous allozymic studies (Boato, 1986, 1988, 1991) suggested exactly the same placement as the one produced by DPI and DPIII. It must be emphasized that *S. cianensis* shows a suite of plesiomorphic characters in the shell morphology and genital anatomy. In addition, this species is the only representative of the genus that is not obligatorily bound to calcareous substrates. These observations, taken together, might suggest a possible evolutionary scenario placing the species as the earliest branch in the phylogeny of the group. Gastropod shell morphology has a clear adaptive significance, and thus is not always suitable for historical reconstructions (Giusti and Manganelli, 1988). On the other hand, even characters associated with genital anatomy, traditionally considered useful taxonomic indicators being free from adaptive constraints, are also plesiomorphic (Boato, 1991). The character “dependence on limestone” also supports a basal placement of *S. cianensis*. Such a position implies that this adaptation was acquired only once over the evolutionary history of the genus. Less parsimoniously, one gain and one loss of dependence on limestone are needed to support the non-basal position of the species depicted by DPIII. It is important to note that shifting from a limestone to a no-limestone substrate is not cost free involving profound physiological changes. The substrate has deep effects on the development of the shell, that tends to become thinner and smaller in lime absence (Goodfriend, 1986). This could lead over evolutionary time to pedomorphosis (i.e., truncation of ontogeny by precocious sexual maturation).

In all our phylogenetic searches, *S. juliana* and *S. similis* are never placed as sister taxa, regardless of the data partition considered. This result is in agreement with previous allozyme data that did not place *S. juliana* and *S. similis* as each other’s closest relatives (Boato, 1986, 1988). It is noteworthy that the recognition of *S. juliana* as a separate species from *S. similis* is relatively recent and based, at least in a first instance, only on allozyme and karyological grounds (Boato, 1986, 1988; Boato and Rodinò, 1986). In addition, according to Boato (1991) there are four morphological characters of the male genital ducts discriminating *S. juliana* from its sibling *S. similis*.

We were particularly interested in finding the closest living relative to the insular *S. guidoni*, because of the obvious important implications in the reconstruction of the biogeographic history of the genus. Phylogenetic searches conducted on DPI (all loci) place *S. similis* sister to *S. guidoni*.
with a remarkable support; the same result is obtained when searches are based on DPIII (COI only), but in this case the node linking the two species lacks of statistical support. When all loci are considered simultaneously, but COI 3rd codon positions are excluded (DPII), *S. guidoni* is basal to all the other species except *S. cianensis*. AU and SH tests do not statistically reject the hypothesis of *S. guidoni* sister to *S. similis* for this partition.

According to the results of the topology tests (Table 3), *S. juliana*, *S. pallida*, and *S. psarolena* are not good candidates to be sisters to *S. guidoni*, regardless of the data partition considered. It is noteworthy that the placement of *S. psarolena* as sister to *S. guidoni* is always rejected by the AU tests but never by the SH tests. SH is a conservative test (Strimmer and Rambaut, 2002), thus the failure to reject a particular topology does not imply that the constituent clades have compelling support. Neither the AU test nor the SH test rejected the hypothesis of *S. cianensis* being sister to *S. guidoni* except the AU test conducted on DPIII. The failure of the AU and SH tests to reject this hypothesis could be related to the fact that no data partition is informative enough to provide a robust support for the position of *S. cianensis* in the phylogeny of the group. It is, however, very unlikely that *S. cianensis*, a species with a very narrow range and not dependent upon limestone, might be the closest relative to *S. guidoni*, a species with a wider insular range and dependent upon limestone. Furthermore, the genital anatomy is quite different in the two species. On the contrary, *S. similis*, the most widespread species of the genus, and *S. guidoni* share a more similar genital morphology and are both strictly associated to limestone. Thus, combining the outcomes of phylogenetic searches (see in particular the remarkably robust support for the node placing *S. guidoni* and *S. similis* as sister species in the tree of Fig. 3) with the results of the topology tests and with the considerations discussed above, all suggest that *S. similis* might be the closest relative to *S. guidoni*. The biogeographic implications of this conclusion will be discussed in the last part of Section 4.

At the intra-specific level, we always obtained the same pattern of relationships among genotypes with a remarkable statistical support, regardless of the data partition considered. While most of the relations within *S. similis* and *S. juliana* make biogeographical sense, phylogenetic searches separated the populations of *S. guidoni* in two distinct clusters, one grouping the Corsican populations (SB and FR) and the other placing the populations from Sardinia (CC) and Elba Island (GR) as each others’ closest relatives. The latter result was unexpected, because Elba Island is geographically closer to Corsica than to Sardinia (Fig. 1). Forcing GR to cluster with SB and FR produced a topology significantly worst than the ML topologies based on DPI, DPII, and DPIII (Table 3). Such a relationship might be explained by taking advantage of the results of Boato (1988) based on allozymes. That paper included the same populations we used in our study plus two other populations from coastal areas of Corsica. No samples from the Elba Island have been analyzed with allozymes. These markers suggested the presence in Corsica of two different lineages of *S. guidoni*. The first lineage comprised populations sampled in the Corsican mainland (SB and FR, same locality in both studies), while the second included populations from coastal areas of the island (not analyzed here). Allozymes revealed a close genetic affinity between the latter populations and the Sardinian one (CC, same locality in both studies). It is important to emphasize that the Sardinian locality is placed along the Northern–Western coast of the island. We can therefore hypothesize that the population from the Elba Island (GR, not analyzed with allozymes) belongs to the lineage distributed along the coasts of these Tyrrhenian islands. Evidently, to properly address this point and to arrive to a firm conclusion on the phylogeny of the species we need to sequence a higher number of populations (both from coastal areas and from the mainland) throughout the species’ range.

### 4.2. DNA sequences versus other characters

Allozymes and morphological data exist for all the *Solatopupa* species (Boato, 1988, 1991), although not for exactly the same populations we sequenced in our study. Consequently, we made the different data sets comparable (see Section 2) to test whether our phylogeny agrees or not with previous hypotheses based on different characters. We are aware that the results of these analyses must be considered cautiously because they are based on a single population per species. We used the AU and SH tests to statistically compare these independent hypotheses. Tree topologies obtained from both genetic data sets are compatible with each other, underscoring our confidence in the inferred phylogenetic hypothesis. When we compare phylogenetic hypotheses based on morphology against the molecular ones, the AU and SH tests indicate that the tree based on morphology is statistically different (worst) from the phylogenies based on allozymes and sequence data. Thus, our results are at odds with the statement of Boato (1991) asserting that for *Solatopupa* “…there is no inconsistency between genetically and morphologically inferred phylogenies, if the morphological data are thoroughly examined.” It is important to note, however, that at the time when this assertion was made, topology tests (especially the likelihood-based ones) were not employed as commonly as they are nowadays; consequently, authors tended to underestimate the differences in the branching patterns of their trees. In addition, Boato (1991) analyzed the morphological data under a parsimony approach assuming constancy of evolutionary rates and absence of homoplasy. We cannot judge from our data whether such constancy holds or not. Certainly, our concurrent analyses of molecular and morphological data demonstrate that homoplasy is not negligible in the morphological data set.
The lack of consensus between morphological and molecular data is not a novelty and this is especially true for gastropods. The systematics and taxonomic arrangement of this group has often relied on dubious morphological characters. Amongst others, the phylogenetic validity of shell morphology has been often called into question (Giusti et al., 1986; Giusti and Manganelli, 1988) because most of its variation can be explained in light of adaptive processes to local conditions (Pfenninger et al., 2003). The reconstruction of the evolution of morphological characters onto our best estimate tree supports this view. Shell characters are the main source of conflict between morphology and molecular data sets, with multiple reversal events needed to accommodate them onto the DNA phylogeny (Fig. 4). Our conclusions are also in agreement with Giokas (2000). According to this author, shell characters must be avoided for phylogenetic purposes in pulmonates, because they often produce misleading hypotheses. One has also to consider that substantial changes took place during the last decade in the field of pulmonate morphological systematics. Pfenninger and Magnin (2001) showed that morphometric analyses of “traditional” shell traits aid in the detection of quantitative vs. qualitative differences among taxa. Quantitative variation is not phylogenetically informative. Our data suggest that a critical re-examination of the morphological traits employed by Boato (1991) in light of recent advance is much needed.

Finally, topology tests generally rejected the hypotheses of a monophyletic origin of those diploid numbers (2N = 56 and 58) shared by more than one species. Thus, our results agree with the general view that changes in chromosome number and morphology are an important isolating mechanism but karyotypic comparisons are poorly informative on phylogenetic relationships (Riesenberg, 2001).

4.3. Biogeography and divergence times

An additional use for phylogenetic studies are as a source of hypotheses of historical biogeography. This especially holds for organisms like land snails with very low, if any, potential for dispersal. Our phylogenetic analyses suggest a sister taxon relationship for the pair S. guidoni–S. similis. However, given the relatively small amount of genetic divergence between the two species (5.4 ± 0.2% DML; all loci), it is hard to conceive that their split coincided with the onset of the separation of the Sardinia–Corsica microplate from the Iberian plate, around 24 Myrs ago. Granted that comparisons among taxonomically unrelated groups are scarcely informative due to the possible occurrence of among-lineage rate variations, sequence divergences between S. similis and S. guidoni are approximately two times and nine times lower for ribosomal and COI genes, respectively, than comparable estimates of divergence for geminate species pairs from a variety of organisms inhabiting the same region, and whose divergence has been associated to the Corsica–Sardinia vs. Iberian plate split (Caccone et al., 1997; Caccone and Sbordoni, 2001; Ketmaier et al., 2003). Furthermore, if we still assume a causal relation between the split of Sardinia–Corsica microplate and the cladogenetic events in Solatopupa and we use 24 Myr as the dating of the divergence between S. guidoni and S. similis, we would obtain crude estimates of the substitution rate equal to 0.131 substitutions per site per million years for 12s and 0.025 substitutions per site per million years for COI 1st codon positions. COI 2nd codon positions and H3 (all positions) are invariant for the S. similis–guidoni split. These rates are remarkably slower (4.6–76.3 times for 12s and 6–18.4 times for COI 1st codon positions) than those previously published for mollusks (Marko, 2002). Though these rates are based on the maximum time of divergence (and thus represent minimum estimates), they still imply that this group of land snail experienced an extreme deceleration in the rate of evolution of the mitochondrial genome.

Low mtDNA rates are not a novelty (Shearer et al., 2002) and a number of mechanisms have been invoked to explain this phenomenon, such as selection, recent bottleneck, introgression, and mismatch repair, though their relative influence is difficult to determine (Govindarajan et al., 2005). There are neither intrinsic (i.e., body size, generation time, and metabolic rate) nor ecological/life history factors (i.e., extreme environmental temperature, and extensive radiation) that could account for such a dramatic deceleration of rate in Solatopupa (Gillooly et al., 2005). Since there are no sufficient evidences to support the hypothesis of a severe deceleration in molecular rates, we argue that the most reasonable explanation for our findings is that the Oligocene events that affected the peri-Tyrrhenian area not the primary forces behind the divergence of the Solatopupa snails.

Given the absence of fossil evidence for this group, which prevents the use of tree based estimates of time divergences (sensu Sanderson, 1997, 2002, 2003), the only possibility to derive time estimates for the main cladogenetic events within the genus is to use published rates for mollusks. We are aware that these estimates might be prone to errors due to lineage-specific heterogeneties. Thus, our datings must be interpreted cautiously. The four data partitions that passed the LRT test (Table 4) gave a broad range of time estimates for the same split. 12s gave a sequence divergence that ranges from 3.8 ± 0.3% within S. guidoni to 17.8 ± 5.4% for the separation of the S. juliana–pallida–psarolena clade from the S. similis–guidoni one. The sequence divergence between S. similis and S. guidoni is 5.8 ± 0.5%. As expected, COI 1st codon positions diverge more slowly than 12s by a factor of about 1.8. COI 2nd codon positions and H3 (all positions) accumulate substitutions at an even slower pace; these data partitions had substitutions only for the deepest split (S. juliana–pallida–psarolena vs. S. similis–guidoni; 0.5 ± 0.1% and 2.3 ± 0.2% of sequence divergence for COI and H3, respectively). The number of substitutions accumulated within these two partitions is too few and clearly not informative over the temporal scale we are considering. Our results are in line with a
previous study on bivalves (Marko, 2002) that demonstrated how substitutions at H3 are very rare and may occur too infrequently to resolve divergence times less than 30 Myr. Thus, we will not use COI 2nd positions and H3 but only 12s and COI 1st positions.  

12s rates vary in gastropods between 0.6% per million years in Littorina to a formally fast 10% per million years in Mandarina (Rumbak et al., 1994; Chiba, 1999). There are, however, no evidences suggesting that accelerate rates of evolution would apply in our case. Based on a divergence rate of 0.6% per million years, we obtained a time estimates of evolution would apply in our case. Based on a divergence rate of 0.6% per million years, we obtained a time estimates of 3.16 ± 0.25 Myrs for the divergence within S. guidoni, 4.83 ± 0.41 Myrs for the S. guidoni–S. similis split and 14.83 ± 4.5 Myrs for the split between the S. juliana– pallida–psarolena and S. similis–guidoni clades.  

Marko (2002) reports a variety of rates (0.15–0.46% per million years) for COI 1st codon positions in bivalves. Here, we use a rate of 0.15–0.22% per million years because such estimates are based on a calibration point of 23–26 Myrs which is appropriated for our study case. COI-based datings place the divergence within S. guidoni at 4.66 ± 2.66–3.18 ± 1.81 Myrs, the divergence between S. guidoni and S. similis at 3.66 ± 1.33–2.50 ± 0.9 while the deepest split in our phylogeny (S. juliana–pallida–psarolena and S. similis–guidoni) is dated at 16.3 ± 2.33–11.2 ± 1.59 Myrs. It is to note that, according to these estimates, the beginning of independent evolution within S. guidoni would have predated the divergence between S. guidoni and S. similis. However, we do not consider this result realistic and we have three explanations to support our opinion. First, both 12s and COI suggest that the splits between S. guidoni and S. similis and within S. guidoni might have occurred within a relatively short time. In such a case, genetic data may not be able to clearly resolve the splits, because of their proximity. Second, COI 1st codon positions diverge slowly and therefore are less precise to date relatively recent separation events. Third, in all our searches multiple populations of S. guidoni are always placed in a strongly supported monophyletic clade.  

Overall, there is a good agreement between time estimates obtained with 12s and COI; both genes suggest cladogetic events from the middle Miocene to the middle-late Pliocene. Interestingly, our results fit almost perfectly with two of the five episodes of major regional change in the paleogeographic and tectonic setting of the peri-Tethys platforms on both sides of the African/Arabian–Eurasian convergent plate boundary (Meulenkamp and Sissingh, 2003, Fig. 1C). These changes had profound effects on the area presently occupied by Solatopupa. Between 18 and 15 Myrs ago, the anti-clockwise rotation of the Corsica–Sardinia block slowed down and came to a halt (Chamot-Rooke et al., 1999; Edel et al., 2001). At that time, the two islands were still connected to the mainland by a land bridge approximately corresponding to the area comprised between Southern France and Liguria (Meulenkamp and Sissingh, 2003). This area was affected by an active oceanic subduction that ultimately led to the formation of the Ligurian Apennines and of the Maritime Alps. The uplift of these mountain systems might have fragmented the range of a continuously distributed ancestor species, curtailling genetic exchanges among populations and, therefore, promoting speciation in this sedentary land snail genus. This hypothesis is supported by the observation that only two of the five continental species (namely, S. similis and S. juliana) have a relatively wide range, while the remaining species (S. pallida, S. cianensis, and S. psarolena) have a much more limited distribution, with S. cianensis and S. psarolena being known only from very restricted area (Fig. 2). Such a pattern of geographical distribution of species may be easily explained on the basis of multiple (possibly coeval) vicariant events. The splitting of S. similis and S. guidoni as well as the differentiation within S. guidoni is placed between 5 and 3 Myrs ago. These estimates coincide with the well-known Pliocene transgressive event that flooded and created the modern Mediterranean Sea (Steininger and Rögl, 1984) and broke off the connection between Corsica–Sardinia and the Alps–Apennines, which were then being formed. Following this event, the two islands became permanently isolated. The changes in the sea level also deeply affected the coastal areas of Tuscany, creating the Tuscan Archipelago, including the Elba Island (Lanza, 1984; Van der Meulen, 1999). Thereby, they promoted speciation in Solatopupa by isolating S. guidoni from its continental sister taxon. Our data also suggest that the differentiation within S. guidoni was coeval with the separation of the latter species from S. similis. This hypothesis makes sense because the same Pliocene transgressive event invoked to explain the S. similis–guidoni split, partially inundated Corsica and Sardinia (Cherchi and Montadert, 1982), producing a powerful barrier for terrestrial organisms and causing the cessation of gene flow among populations separated by the sea.  

5. Conclusions

In this study, we have used mitochondrial and nuclear DNA sequences to test the vicariance vs. dispersal scenarios in the land snail genus Solatopupa. We have also compared our results with previously published data based on morphology, karyology and allozymes. Our phylogenetic analyses revealed a good agreement between sequence and allozyme data, while neither morphology nor karyology proved to be informative to derive phyletic relationships within the group. According to our time estimates differentiation within this genus occurred from the middle Miocene to the middle-late Pliocene (from 18 to 3 Myrs ago). Cladogetic events can be explained on vicariance terms, in light of the area paleogeographic scenarios. Interestingly, our data do not justify a causal relationship between the Oligocene split of the Corsica–Sardinia microphone from the Iberian plate and the cladogenesis of the genus. Rather, all the differentiation events are more recent and occurred when the rotation of the microplate was almost completed. To our knowledge, this is the first study that relates speciation in a peri-Tyrrenian taxon with the ending of the tectonic evolution of the Corsica–Sardinia microphone. Indeed, our
results are at odds with a number of previous studies that revealed a direct link between speciation in an array of taxonomically diverse organisms and the initial phases of the rotation of the microplate (Caccone et al., 1994, 1997; Caccone and Sbordoni, 2001; Cobolli Sbordoni et al., 1992; Fochetti, 1994; Fochetti et al., 2004; Ketmaier et al., 1999, 2000, 2003). It is, however, important to point out that most of these organisms have a distribution strictly limited to Southern-France/Northern Spain, Corsica, and Sardinia, with different species on the three landmasses. On the contrary, the Solatopupa’s range is wider (see Fig. 2) and Corsica, Sardinia and Elba Islands all share the same species (S. guidoni). It is likely that for groups with very poor dispersal ability the faunistic assemblage of this area is the result of a series of vicariance events occurring at different time points. The origin and divergence of the oldest elements is Oligocene, while the diversification of younger lineages might have been achieved during the Miocene and Plio-Pleistocene (Oosterbroek and Arntzen, 1992). This scenario is consistent with the long and continuous tectonic evolution of the area that dates back to the late Rupelian nario is consistent with the long and continuous tectonic evolution of the area that dates back to the late Rupelian (33–29 Myrs ago) (Meulenkamp and Sissingh, 2003).

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Appendix A. Supplementary data


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