Temporal speciation pattern in the western Mediterranean genus *Tudorella* P. Fischer, 1885 (Gastropoda, Pomatiidae) supports the Tyrrhenian vicariance hypothesis

Markus Pfenninger a,⁎, Errol Vélab, Ruth Jesse c, Miren Arantzazu Elejalde d, Fabio Libertob, Frédéric Magninb, Alberto Martínez-Ortí eb

a LabCentre, Research Centre Biodiversity & Climate, Siemesstrasse 70, 60323 Frankfurt, Germany
b Institut Méditerranéen d’Écologie et de Paléoécologie, Université Paul Cézanne, Européé méditerranéen de l’Arbois, Domaine du Petit Arbois, bat. Villenin. BP 80, 13545 Aix-en-Provence, cedex 04, France
c Institute of Ecology, Evolution and Diversity, Goethe University, Siemesstrasse 70 (Building A), 60054 Frankfurt, Germany
d Departamento de Zoología y Biología Celular Animal, Facultad de Farmacia, Universidad del País Vasco, Paseo de la Universidad 7, 01006 Vitoria, Spain
e Departamento de Zoología, Facultad de Ciencias Biológicas, Universitat de València & Museu Valencià d’Història Natural, Passeig de la Petxina 15, E-46008 Valencia, Spain

Article history:
Received 25 April 2009
Revised 14 September 2009
Accepted 16 September 2009
Available online 20 September 2009

Keywords:
Western Mediterranean palaeogeography
Species delimitation
Tyrrhenian vicariance hypothesis

1. Introduction

It is a major challenge of biodiversity research to understand the processes responsible for the current distribution of species. Particularly taxon ranges of organisms with low dispersal capacities, but disjunct distributions, like terrestrial or freshwater organisms separated by the sea, require dispersal hypotheses that are compatible with the taxon’s biology (Jesse et al., 2009). In these cases the processes of vicariance and dispersal as dominant forces underlying biogeographical patterns are often controversially discussed (Austin et al., 2003; Givnish et al., 2004; Yoder and Nowak, 2006). Dispersal theory’s main principle is that of “centres of origin”, from which taxa spread, became isolated and speciated (Dobzhansky, 1937; Mayr, 1954). In contrast, the central assumption of vicariance theory is that ancestral taxa were widespread and speciation was caused by subdivision of the ancestral range (Yoder and Nowak, 2006; Sparks and Smith, 2005).

The land snail genus *Tudorella* P. Fischer, 1885 has a distribution that raises the question whether dispersal or vicariance were the prevailing processes, as the genus range comprises areas currently separated by the Mediterranean Sea. It is thus an example for the debated biogeographical dilemma that Northern Africa is faunistically and botanically more related to southern–western Europe than to the rest of Africa, to which it is geographically connected (Jeannel, 1952). The genus occurs along the western Mediterranean coasts from the Iberian peninsula to Malta in Southern Europe and from Morocco to Libya in Northern Africa (Sacchi, 1958; Giusti and Manganelli, 1984) This disjunct distribution, accompanied by a high level of phenotypic variation and sexual dimorphism (Martínez-Ortí and Robles, 2005), has long since encouraged authors to describe new taxa, mostly on the subspecific level, within the genus (*Potiez and Michaud, 1838; Sowerby, 1847; Pallary, 1898*). Apart from *Tudorella ferruginea*, all populations are therefore generally attributed to *Tudorella sulcata* (Draparnaud, 1805) with the number of subspecific entities varying in dependence of the author from one (Giusti et al., 1995) to five (Sacchi, 1958). *Tudorella* has a quite covert life style. The snails are most of the time deeply bored in soil and debris under calcareous rocks. They are active only under suitable (i.e. moist) weather conditions. These behavioural characteristics render the rather large snails to unlikely can-
candidates for the suggested long range over-sea passive dispersal processes of snails (wind, bird migration, rafting on logs), apart from anthropogenic dispersal.

The origins of the genus *Tudorella* can be traced in the fossil record to the beginning of the Miocene (Véla et al., 2008). Unfortunately, it is not possible to attribute these fossilised shells to extant lineages, which precludes a fossil calibration of a molecular clock. This time frame includes several potential, mutually not exclusive distribution scenarios that do not require transoceanic dispersal. These start with the substantial tectonic processes, including the split, dislocation and connection of continental micro-plates, shaping the western Mediterranean Basin between the Strait of Gibraltar and Malta in the last 30 my (Gueguen et al., 1998; Rosenbaum et al., 2002a). Though debated in detail, the scenario that during the Oligocene terranes now found in Calabria, Sicily, Corsica, Sardinia, Kabylies (Algeria), Balearic Islands and Rif range in Morocco were part of the Tyrrenhian plate situated on the Southern European and Iberian margin is now widely accepted. From the late Oligocene on (~30 million years ago) this plate split from mainland Europe. By 22 million years ago latest, the component parts of the plate were separated from each other, except for the Betis/Rif region that stuck together until 12 million years ago. About 10 million years ago, the joining of the Rif region with Northern Africa brought them into secondary contact with the Kabylies region, having attached to this continent some 5 millions years earlier. All other terranes reached their current positions approximately in the late Pliocene (Rosenbaum et al., 2002a). Intriguingly, these terranes coincide largely with the current distribution of the genus *Tudorella*. Another major event, connecting most of the current distribution by land bridges and thus providing the opportunity of land based dispersal, was the Messinian Salinity Crisis in the Pliocene (Duggen et al., 2003; Hsi, 1972). However, during this short time interval between 5.9 and 5.3 million years ago (Krijgsman et al., 1999), the Mediterranean Basin may not have completely dried out (Cipollari et al., 1999; Manzi et al., 2005) and at least the lower parts of the area may not have supported much life (Quézel, 1995). Also the major Pleistocene sea level changes (Rohling et al., 1998) might have constituted opportunities to link several but not all current distribution areas. Lastly, as mentioned above, a relatively recent anthropogenic introduction from a single source area is also a possible scenario.

Our goals for the present study were thus twofold: First, we aimed to delimit comprehensively the western Mediterranean taxa of the genus *Tudorella*, applying the unified species concept of De Queiroz (2007). We have chosen this species concept, because it comprises the biological species concept without the need to prove actual reproductive isolation. In short, the unified species concept equates species with separately evolving metapopulation lineages, which is appropriate in a case of allopatric relations. Second, we evaluated the relative support of several hypotheses on the temporal and thus biogeographical splitting pattern of the identified evolutionary lineages, using a Bayesian phylogenetic model selection approach.

2. Materials and methods

2.1. Sampling

The present study covered all putative taxonomical entities and all described occurrences of the genus *Tudorella* in the western Mediterranean region (Fig. 1). Forty-two sequences were made specifically for this study. Data on Algerian, French, Iberian, Moroccan and Maltese localities were integrated from previous studies (Martínez-Ortí et al., 2008; Véla et al., 2008). For each sampled population, several individuals were preserved in 70–90% alcohol and with few exceptions, two to five specimen per locality were analysed. As potential outgroup species in Bayesian analyses *Pomatias rivulare* (Eichwald, 1829), *P. elegans* (O.F. Müller, 1774), *Leonia mammillaris* (Lamarck, 1822) and two species of the genus *Cochlosoma* Jan, 1830 were included.

2.2. Molecular analyses

The operculum was forced open without damaging the shell and a small part of the foot muscle was taken. The shell, operculum

Fig. 1. Sampling sites and clade distributions of *Tudorella* across the western Mediterranean. This represents also the current knowledge on the distribution of the genus, since most recently and securely confirmed localities were sampled. The names of the regions discussed are indicated in italics. The insert shows the presumed Oligocene distribution of the current distribution areas (modified from Rosenbaum et al., 2002a,b).
adopted from the study of Pfenninger et al. (2005). PCR products were purified using Pure Link™ PCR Purification Kit (Invitrogen, USA). For 57 individuals, a 670 bp segment of the mitochondrial ribosomal gene was PCRamplified. A mostly overlapping set of 46 individuals was also characterised for a section of the nuclear ITS-1 locus (368 bp). For 24 selected individuals representing the major evolutionary lineages, a 473 bp fragment of the mitochondrial ribosomal gene was additionally sequenced. PCR conditions and primers were adopted from the study of Pfenninger et al. (2005). PCR products were sequenced on an ABI 377 automated DNA sequencer. Sequences were deposited in GenBank (see Table 1).

2.3. Phylogenetic reconstruction

The orthologous DNA sequences were initially aligned using the default settings of CLUSTAL X (Larkin et al., 2007) and optimised by eye. In an initial analysis, we used the COI and ITS-1 data sets to identify evolutionary lineages. For the former marker, a 99.9% credible set of phylogenetic trees was estimated with the program MrBayes vers. 3.1.2 (Ronquist and Huelsenbeck, 2003) by sampling the tree space using a Metropolis coupled Monte Carlo Markov chain, implementing a GTR + $\Gamma + I$ model of sequence evolution (where $\Gamma$ denotes General Time Reversible, $I$ is the shape parameter of the gamma distribution and $I$ the proportion of invariant sites), because the most parameter-rich model makes most use of the Bayesian approach. The parameter space was not constrained by a priori expectations, but estimated during the runs. Initial runs as well as a posterior inspection of the likelihoods in the final run showed that a burn-in phase of 10,000 generations

| Table 1 |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Sampling site               | Code                        | Latitude                    | Longitude                   |
| Orihuela, Cañada de la Estaca, Alicante, Spain | ALI 38°05'01"N 0°56'37"W | EF052335                    | EF052350                    |
| Alghero, La Nurra, Sardinia, Italy | ALN1,2,3 40°33'11"N 08°19'15"E | GQ370434 GQ370435 GQ370422 | GQ370413                    |
| Alghero, Via Oristano, Sardinia, Italy | ALO1,2 40°34'31"N 08°19'39"E | GQ370436 GQ370446 GQ370447 | GQ370414 GQ370415            |
| Bonnico, Bouches-du-Rhône, France | BON1,2,3 43°20'42"N 05°01'32"E | GQ370442 GQ370456 GQ370397 | GQ370390                    |
| Cabo de los Caballeros, Menorca, Spain | CAB1,2,3,4 40°05'14"N 04°05'27"E | GQ370423                     |                         |
| Capo Caccia, Sardinia, Italy | CAC1 40°48'56"N 08°48'53"E | GQ370449                    | GQ370420 GQ370411           |
| Caltanissetta, Sicily, Italy | CAL1,2 37°29'19"N 14°03'49"E | GQ370437 GQ370430            | GQ370410                    |
| Rass el Hamra «Caentarea», Annaba, Algeria | CEN1,2,4,5,6 36°57'51"N 07°46'38"E | GQ370428 GQ370429            | GQ370410                    |
| Collinass, Medioi Campidano, Sardinia, Italy | CO1 39°38'29"N 08°50'24"E | GQ370405                    | GQ370403                    |
| Capo Gallo, Palermo, Sicily, Italy | GAI1,2,3,4 36°13'15"N 13°19'00"E | GQ370424 GQ370425            | GQ370403                    |
| Gorges de Ben Harun, Jijel/Mila, Algeria | GBH1,4,5 36°36'07"N 06°16'58"E | GQ370405                    | GQ370403                    |
| El Tajo del Escalate, Motril, Granada, Spain | GRA 36°44'40"N 03°31'01"E | EF052334                    | EF052349                    |
| Rass el Hamra «Argiles», Annaba, Algeria | HAM1,2,3,4 36°57'42"N 07°46'25"E | GQ370409                    | EF052352 GEF052357         |
| El Khbous, Constantine, Algeria | KHR1 36°16'17"N 06°54'22"E | GQ370433                    | EF052352 GEF052357         |
| Lago di Baratz, Sardinia, Italy | LAB1 40°48'54"N 08°48'07"E | GQ370440 GQ370391            | GQ370408                    |
| Citra, Malta Island, Malta | MAL1,2 35°59'14"N 14°19'42"E | GQ370450                    | EF052363EF052357         |
| Tanout, Kif, Morocco | MOR 32°41'57"N 09°04'29"W | EF215453                    | EF215452                    |
| Cape Rama, Palermo, Sicily, Italy | RAM1,2 36°08'00"N 13°03'39"E | GQ370426 GQ370427            | GQ370406 GQ370398          |
| Resquidou, Bouches-du-Rhône, France | RES1,2,3 43°21'15"N 05°16'33"E | GQ370447                    | GQ370398                    |
| Roucas-Blanc, Bouches-du-Rhône, France | ROU1,2,3 43°16'28"N 05°22'33"E | GQ370448                    | GQ370399 GQ370400          |
| Monte Albo, Nuoro, Sardinia, Italy | MA 40°27'21"N 09°31'24"E | EF052345                    | EF052360                    |
| Porto Conte, Sardinia, Italy | POC1,2 40°48'00"N 08°48'00"E | GQ370451                    | EF052360                    |
| Rass el Hamra «Seseli», Annaba, Algeria | SES1,2,4 36°57'43"N 07°46'35"E | GQ370431 GQ370432            | GQ370408                    |
| Monte Gallo, Palermo, Sicily, Italy | SIC1,2,3,4 38°10'50"N 13°16'22"E | GQ370439                    | EF052352 GEF052357         |
| Sugiton, Bouches-du-Rhône, France | SIG1,2,3,4 43°12'55"N 05°26'45"E | GQ370444 GQ370445            | EF052352 GEF052357         |
| Trapani, San Vito Lo Capo, Sicily, Italy | TNA1,2 37°07'28"N 12°44'41"E | GQ370438 GQ370439            | EF052352 GEF052357         |
| Yemira, Sfax, Tunisia | TRA1 37°10'31"N 10°11'03"E | GQ370441 GQ370443            | GQ370412                    |
| Yemima Couraya, Bejaia, Algeria | YEM1,2,3,4 36°46'10"N 05°05'22"E | GQ370441 GQ370442            | GQ370412                    |
| Cape Zafferano, Palermo, Sicily, Italy | ZAF3 38°06'30"N 13°32'00"E | GQ370441 GQ370443            | GQ370412                    |
| Outgroups |                      |                              |                              |
| Cochlostoma punctatum, Luberon, Vaucluse, France | 43°48'55"N 05°13'50"E | GQ370416                    |                      |
| Cochlostoma septempunctata, Luberon, Vaucluse, France | 43°48'44"N 05°13'49"E | GQ370417                    |                      |
| Pomatias rivulare, Marmaris, Turkey | 36°51'16"N 28°16'10"E | GQ370418                    |                      |
| Pomatias elegans: Roucas-Blanc, Bouches-du-Rhône, France | 43°16'28"N 05°22'33"E | GQ370419                    |                      |
| Leontia mammalliana: Pilar de la Horadada, Alicante, Spain | 37°51'53"N 00°48'35"E | GQ370421 GQ370425            |                      |

Note: Locality codes, geographical coordinates and GenBank accession numbers of Tudorrellia specimens and outgroups haplotypes (Missing COI accession numbers of outgroups will be provided at proof stage).
was largely sufficient for both analyses to allow the likelihood values to reach convergence. The chain was run for 10,000,000 generations and sampled every 100th generation. A rooted majority consensus tree was computed from the sampled trees, excluding the trees sampled in the burn-in phase. For the ITS-1 data set, we applied a Maximum Parsimony search with 1000 bootstrap replicates in MEGA4 (Tamura et al., 2007), because mainly insertions and deletions appeared to be informative, while the remaining sequence variation was rather negligible. As both coding each insertion/deletion position as new character and each insertion/deletion as single character yielded the same results, we present here only the first option to illustrate the sequence divergence.

The concatenated COI and 16S datasets, including five outgroups, was also subjected to a Bayesian analysis. The parameters used were identical to the previous analyses with the exception that the Markov-chain was run with a separate instance of the GTR + I model for each marker. Outgroup status was assigned to Cochlostoma patulum (Draparnaud, 1801).

2.4. Phylogenetic model selection

We used hypotheses on the temporal splitting pattern of the Tudorella lineages in conjunction with the knowledge on the geological history of the western Mediterranean (Rosenbaum et al., 2002a,b) for a Bayesian model comparison approach.

Given the posterior probabilities of the inferred clades, we assumed the existence of the following most recent common ancestors (mrca) and their successive branching: The ancestor of all Tudorella lineages in the dataset (mrca Tudorella, split 1 in Fig. 2), the mrca of the sister taxon to T. ferruginea (Lamarck, 1822) (mrca FerrSister, split 2 in Fig. 2), the mrca of the sister taxon to T. mauretanica (Pallary, 1898) (mrca MauSister split 3 in Fig. 2) and the mrca of the three T. sulcata s.l. lineages (SL, split 4 in Fig. 2).

As argued above, it is reasonable not to take the possibility of pre-anthropogenic over-sea dispersal in these land snails into account. Therefore, we assumed that the colonisation of unoccupied areas required the existence of direct land connections. We furthermore assumed that the minimal date of a split between lineages is given by the geological separation of the respective harbouring land masses. If a new lineage occupied a newly connected land mass, we assumed that the split from the ancestral lineage occurred after the connection in the course of the dispersal, thus given us a maximum age for the respective split.

Fig. 3 gives an overview of the temporal and spatial pattern of land connections or isolation of areas that currently harbour Tudorella lineages, according to Rosenbaum et al. (2002a,b). We then fitted the inferred phylogeny according to the assumptions detailed above on this area connection matrix. This resulted in four distinct hypotheses concerning the temporal splitting pattern:

1. Messinian Radiation. The mrca of Tudorella lived in southwest Europe. When the Tyrrhenian plate broke off about 25 mya, T. ferruginea was isolated on the Balearics (split 1). The sister lineage on the continent spread and diverged over North Africa to Sicily, Malta and Sardinia during the Messinian Salinity Crisis (Krijgsman et al., 1999, MSC 5,330,000–5,960,000, splits 2–4), when all areas in question were connected by land bridges.

2. Rif-Rafting. The extant Tudorella lineage was completely on the Tyrrhenian plate that broke off mainland Europe. First, T. ferruginea split off on the Balearics 25 mya the latest (split 1). The mrca of FerrSister rafted on the Betis/Rif terrane. When this terrane attached to Northern Africa about 10 mya, the lineage had from there on the possibility to spread to the East and diverge from T. mauretanica (splits 2 and 3). The earliest possibility to reach Sardinia was the onset of the MSC, whose end also marks the minimum age for split 4.

3. Kabylies Crossing. The extant Tudorella lineage was completely on the Tyrrhenian plate that broke off mainland Europe. First, T. ferruginea split off on the Balearics 25 mya the latest (split 1). The remaining plate split the latest 21 mya, isolating T. mauretanica on the Rif/Betis terrane (split 2). The mrca of MauSister crossed the Mediterranean on one of the Kabylies-fragments and had the possibility to spread and diverge to the East (Tunisia, Sicily, Malta) after this area attached to Northern Africa 15 mya (split 3). The colonisation of Sardinia was achieved during the MSC.

4. Sardinia Sailing. The extant Tudorella lineage was completely on the Tyrrhenian plate that split/broke off mainland Europe. First, T. ferruginea split off on the Balearics 25 mya the latest (split 1). The remaining plate split the latest 21 mya, isolating T. mauretanica on the Rif/Betis terrane (split 2). The mrca MauSister sailed on Sardinia. When this island was connected to Sicily during the MSC, the lineage dispersed and diverged to there, Malta and Northern Africa (splits 3 and 4).

These hypotheses are visualised in Fig. 3. We compared the fit of these temporal diversification hypotheses on the molecular dataset under the assumption of a relaxed molecular clock model of DNA sequence evolution using Bayesian factors. With the software BEAST vers. 1.4.8 (Drummond et al., 2006), we constrained the divergence time of the splits according to the above scenarios, respectively. We applied uniform priors on the Time to the Most Recent Common Ancestor (mrca) of the respective clades, since we had no information or plausible assumption when during a possible period the actual lineage split occurred. The data was partitioned into 16s and COI. For each partition, we used a separate GTR + Γ + I model with four rate categories. We used an uncorrelated lognormal relaxed clock model. As tree prior, a Yule speciation model was chosen. We ran the MCMC chain for 2 × 10^7 generations and sampled every 1000th tree, discarding the first 2000 as burn-in. This assured an effective sampling size (ESS) of at least 1500 for all relevant parameters. Both chain convergence and ESS were monitored using the software Tracer vers. 1.4.1 (Rambaut and Drummond, 2007). From the tree-file output, we calculated the harmonic mean of the sampled tree log likelihoods, excluding the burn-in. This harmonic mean is an estimator of the marginal log likelihood of the model with respect to the prior. The difference of two marginal log likelihoods is the Bayes factor (BF) between them. A BF larger than 3 indicates substantial, over 10 strong and above 30 very strong support for the respective hypothesis. Additionally, a model without temporal restrictions on the clades was estimated for comparison.

3. Results

3.1. Species delimitation with mitochondrial and nuclear markers

The unrooted phylogram derived from Bayesian analysis of the COI data (Fig. 4) showed eight divergent clades within the genus Tudorella, each with a Bayesian posterior probability above 0.87. The most divergent group (Bayesian posterior probability 1.00, clade 1) corresponded to the well known Balearic endemic T. ferruginea. The remaining seven clades are generally attributed to the polytypic T. sulcata sensu lato. A second branch represented the clade from SE-Spain and NE-Morocco (clade 2). This clade was also highly supported (post. prob. 1.00). One strongly distinct
unit was found on Malta (post. prob. 1.00, clade 3), another (post. prob. 0.93, clade 4) originates from northern Sicily (near Palermo). One divergent group comprised individuals sampled from Central-Northern Algeria, South-Eastern France and several localities from Sardinia (post. prob. 1.00, clade 5). The last three closely related units (sequence divergence 0.012–0.021) were well supported clades (post. prob. ranged between 0.87 and 0.94) from Sicily and Tunisia (clade 6), Sardinia (clade 7) and North-Eastern Algeria (clade 8), respectively.

The delineated entities could be partially ascribed to existing taxa, applying the criterion that the describer must have remarked a specific distinctness of the taxon in question in the same area where we located the respective clade:

- Clade 1 corresponded to *T. ferruginea* (Lamarck, 1822), the type species of the genus. It is endemic to the Balearic Islands of Mallorca and Minorca.
- Clade 2 was described as *T. mauretanica* (Pallary, 1898). It is restricted to areas around the Alboran Sea: SE-Spain, NE-Morocco and NW-Algeria, i.e. the Betis and Rif region (Martínez-Ortí et al., 2008) where it was initially described.
- Clade 3 was congruent to *Tudorella melitense* (Sowerby, 1847), endemic to the Maltese Islands of Malta and Gozo (Giusti et al., 1995).

The second phylogram (Fig. 5) was obtained from sequencing nuclear ribosomal DNA from the first internal transcribed spacer (ITS-1) region. All clades previously identified with COI, with the exception of the individuals from SE-Spain and NE-Morocco from which no ITS-1 sequences could be obtained, were also forming well defined units.
Messianian Radiation occurs twice, because of the complicated reticulate area relations. Possible splitting periods for respective indicated nodes (numbers in the bars) of the hypotheses tested:

- Clade 4 could be attributed to Tudorella panormitana (Sacchi, 1954) which is endemic to northern Sicily near Palermo.
- Clade 5 was identified as T. sulcata sensu stricto (Draparnaud, 1805) because this clade includes the Provençal type locality of T. sulcata (Draparnaud, 1805).
- Clade 6 with its distribution restricted to Western Sardinia could not be matched to one of the available names and was thus identified as a new species (Tudorella nov. spec. 1).
- Clade 7 is known from a single region in north-eastern Algeria (Annaba). No available name is corresponding to this local entity. Consequently, it was termed as Tudorella nov. spec. 2.
- For Clade 8, also occurring in Tunisia, Tudorella multisulcata (Potiez and Michaud, 1838) is the oldest available name for localities of Western and Southern Sicily.

### 3.2. Phylogenetic hypothesis

A Bayesian analysis of the concatenated mitochondrial markers (16S and COI), resulting in an alignment of 1143 positions was performed (Fig. 2). The nuclear marker ITS-1 was not included because of the non-tudorella outgroup taxa which required many additional insertion/deletions, rendering a credible overall alignment impossible.

**Tudorella** was distinct from the genera Pomatias Studer, 1789 and Leonia Gray, 1850 that belong to the same family Pomatiidae. The splitting order within **Tudorella** stayed partially unresolved, as evidenced by two polytomies (Fig. 2). The remaining splits, however, showed four well supported unequivocal monophyla. First, the Balearic T. ferruginea split from the remaining Tudorella species. Next, T. mauretanica split from the T. sulcata species complex. First, the latter, T. mellitense seemed to have speciated first. However, the next lower level node had relatively weak support (0.71/0.66, posterior probability/bootstrap proportion) so that this divergence order remains uncertain. The relations between the last phylogenies, T. panormitana, T. sulcata s.str. and the T. sulcata s.l. complex could not be resolved. This was also the case for the three clades within the T. sulcata s.l. complex. A post-hoc power analysis (Walsh et al., 1999; Braun and Kimball, 2001; Walsh and Friesen, 2001) indicated that the combined COI and 16S data set of 1143 bases should have been sufficient to resolve sequential branching that occurred during an interval of at least 6.3% (±1.2%) of the total divergence time between the lineages. This indicated that the radiation of some Tudorella lineages indeed occurred during a relatively short time interval, if not simultaneously, and seemed not due to a poor resolution caused by a lack of data.

### 3.3. Bayesian phylogenetic model selection

The current distribution of the genus **Tudorella** was best explained by geologic processes shaping the western Mediterranean since the Oligocene/Miocene. From the tested hypotheses, *Kabylyes Crossing* received best support in the data (marginal log likelihood = −3779.70). This model had substantial support over *Rif-Rafting* (*BF* = 6.16) and *Sardinia Sailing* (*BF* = 6.33) and very strong support over the Messinian Radiation scenario (*BF* = 90.34). The chosen model was also not much worse than a temporally unconstrained model (marginal log likelihood = −3772.68). All possible scenarios required explaining the presence of *T. sulcata* either in the Provence and Sardinia or Provence and Kabylyes with recent anthropogenic dispersal.

### 4. Discussion

#### 4.1. Species concept and status of lineages in the genus Tudorella

All Tudorella samples appeared as a well supported monophyletic taxon. The relation to the single *Leonia* species and the also well supported genus *Pomatias*, however, could not be resolved, as a trichotomy at the basis of this clade shows (Fig. 2). This result supports the generic distinction of the former “Cyclostoma” into *Tudorella* and *Pomatias* following Picard (1949).

We accomplished species delimitation of the genus *Tudorella* based on a representative sampling across the whole area and analyses of both mitochondrial and nuclear genetic markers. In our analyses, we found eight well supported evolutionary lineages (Figs. 4 and 5). We recognised these lineages as eight different species according to the unified species concept (De Queiroz, 2007), because these entities (i) were reciprocally monophyletic and deeply divergent at two independently inherited marker loci, (ii) represented thus independent coalescence processes and (iii) showed a non-overlapping allopatric distribution, which indicates absence of gene-flow in these poorly dispersing animals (Fig. 1). This finding excludes already the recent anthropogenic dispersal from a single source hypothesis as explanation for the current distribution.

At our knowledge, this species complex is more or less truly cryptic, as it is only partially recognisable by quantitative characters like the mean size of the shell or the shell colour (Véla et al., 2008). Nevertheless, most of these molecularly recognised taxa were previously described at species or subspecies rank based on shell characteristics (Potiez and Michaud, 1838; Sowerby, 1847; Sailing (vertically hatched bars).
Kobelt, 1878–1879; Pallary, 1898; Sacchi, 1954) except for Clades 6 and 7. The first of these new species of *Tudorella* has a restricted distribution in Western Sardinia, the second is an endemic confined to the Edough peninsula near Annaba, a "continental fossil island" (Lanza, 1984) from where also other cryptic animal or plant species are known (Carranza and Wade, 2004; Véla and Benhouhou, 2007).

However, additional studies are necessary to see whether morpho-anatomical characteristics distinguishing the delimited entities can be found. The delineation of these highly divergent cryptic species underlines the importance of the use of molecular markers in the estimation of biodiversity (Pfenninger and Schwenk, 2007).

### 4.2. Biogeography of *Tudorella*

We relied on a Bayesian phylogenetic model selection approach to distinguish among several biologically plausible temporal diversification models by measuring the fit of the data to the models. A better fit of data in the present case means less variation in sequence evolution rate along the branches of the phylogeny, i.e. less introduced rate changes to fit the observed branch lengths to the applied temporal diversification pattern. The advantage of such approaches to hypothesis testing is the possibility to test several models simultaneously, instead of testing a single hypothesis against a null model. However, one has to be aware that all model selection approaches measure solely the relative support of the
models tested by the data — regardless whether the true model was among them or not. Here, we have taken only biogeographic diversification models into account that did not require prehistoric transoceanic dispersal, because this appeared biologically most plausible for the targeted species.

All models involving some speciation due to vicariance events during the Miocene received significantly better support than a rapid radiation during the comparatively short MSC, the only event potentially connecting the areas in question by land in their more or less current positions. A necessary assumption, of course, is, that all terranes implicated were not completely submerged during any time of the process. This is, however, a quite reasonable assumption, since all areas harbour mountains of at least 1000 m altitude and their appearance is usually attributed to the alpidic orogenesis prior to their detachment from Europe (Schmid et al., 2004).

Among these well supported models, the Kabylies Crossing scenario best fitted the data. This scenario is also supported by the fossil record insofar as *Tudorella*-like shells appeared first in Europe and subsequently in the strata of the respective regions only after the inferred arrival period (Véla et al., 2008). Unfortunately, the fossil remains cannot be reliably associated to extant lineages, which rendered a molecular clock calibration with them impossible.

However, we found current disjunct distributions of *Tudorella* populations clearly belonging to the same coalescence process as evidenced by not reciprocally monophyletic relations and even shared haplotypes (*T. mauretanica* in Spain and Morroco, *T. multisulcata* in Sicily and Tunisia and *T. sulcata* in Algeria, Sardinia and Provence). These disjunct populations belong therefore to the respectively same species according to the applied criteria and their respective most recent common ancestor is presumably younger than the geologic events taken into account. Therefore these distribution patterns require additional studies as both the current sample strategy and sampling size do not allow distin-
guising between relatively recent vicariance events in the late Pliocene or Pleistocene and a postglacial anthropogenic introduction.

The current distribution pattern of the *Tudorella* species in the western Mediterranean is therefore most probably not the result of active or passive dispersal processes, but was caused by vicariance events, followed by subsequent dispersal to areas that can later into contact with the drifting micro-plates. This study thus supports the hypothesis of Giusti and Manganelli (1984) who forwarded these tectonic events shaping the western Mediterranean region as cause for the disjunct distribution of several snail taxa.

In the northern peri-Tyrrhenian area, this sequence of tectonic events is corroborated by molecular clock analyses of the land snail genus *Solotopupa* (Ketmaier et al., 2006). A recent study on cork oaks found a chloroplast haplotype distribution pattern that was also best explained by the above described tectonic vicariance scenario (Magri et al., 2007). Even though additional studies with more taxa are required, the Tyrrhenian vicariance hypothesis seems to be the emerging explanation for the paradoxical taxonomic composition of North African biodiversity and thus provide a partial explanation for the high endemism rate in the western Mediterranean Basin (Verlaque et al., 1997; Médail and Diadéma, 2009).

Acknowledgments

We are grateful to Monia Ben Romdhane for providing samples from Tunisia. Sebastian Klaus provided valuable input on Mediterranean paleogeography. We thank Annette Klussmann-Kolb, Folco Giusti, Barbara Feldmeyer Rebecca Bloch and two reviewers for helpful comments on the manuscript. The study received support by the research funding programme “LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-Ökonomischer Exzellenz” of Hesse’s Ministry of Higher Education, Research, and the Arts.

References


Caranza, S., Wade, E., 2004. Taxonomic revision of Algero-Tunisian Pleurodeles (Ketmaier et al., 2006). A recent study on cork oaks found a chloroplast haplotype distribution pattern that was also best explained by the above described tectonic vicariance scenario (Magri et al., 2007). Even though additional studies with more taxa are required, the Tyrrhenian vicariance hypothesis seems to be the emerging explanation for the paradoxical taxonomic composition of North African biodiversity and thus provide a partial explanation for the high endemism rate in the western Mediterranean Basin (Verlaque et al., 1997; Médail and Diadéma, 2009).

Acknowledgments

We are grateful to Monia Ben Romdhane for providing samples from Tunisia. Sebastian Klaus provided valuable input on Mediterranean paleogeography. We thank Annette Klussmann-Kolb, Folco Giusti, Barbara Feldmeyer Rebecca Bloch and two reviewers for helpful comments on the manuscript. The study received support by the research funding programme “LOEWE – Landes-Offensive zur Entwicklung Wissenschaftlich-Ökonomischer Exzellenz” of Hesse’s Ministry of Higher Education, Research, and the Arts.

References


Yoder, A.D., Nowak, M.D., 2006. Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Annual Reviews in Ecology, Evolution and Systematics 37, 405–431.