Molecular Phylogenetics and Evolution xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Molecular phylogeny and biogeographic history of the European *Maja* spider crabs (Decapoda, Majidae)

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ARTICLE INFO

Article history:
Received 25 March 2009
Revised 11 May 2009
Accepted 13 May 2009
Available online xxxx

Keywords:
Maja crabs
Eastern Atlantic/Mediterranean
Mitochondrial phylogeny
Biogeography
Indo-West Pacific origin

ABSTRACT

We have assessed for the first time the phylogenetic relationships and biogeographic history of the crabs of the genus *Maja* that inhabit European coasts: *M. brachydactyla*, *M. crispata*, *M. goltziana* and *M. squinado*. Using mitochondrial markers, we have recovered a well-resolved phylogenetic tree that supports a single origin for the European species, most likely from an Indo-West Pacific ancestor during the Early Miocene. In this phylogeny, *M. goltziana* appears as the basal European species, with a sister lineage bifurcating into an Eastern Atlantic (*M. brachydactyla*) and a Mediterranean (*M. crispata* and *M. squinado*) clade. We propose the Tethyan Seaway as the initial colonization route, although an entrance through South Africa cannot be discounted. The Eastern Atlantic/Mediterranean split seems to predate the Messinian salinity crisis, which, in turn, could have promoted the recent divergence within the Mediterranean. In addition, Pleistocene glaciations could explain the current diversity in the Eastern Atlantic Ocean, where a unique mitochondrial lineage is found. According to this, the genetic profile of South African crabs appears to belong to *M. brachydactyla*, questioning the validity of the putative species *M. capensis*.

1. Introduction

Among the true crabs (Decapoda, Brachyura), the spider crabs (Majoidea) are one of the most diverse groups, with over 800 species (see Ng et al., 2008). The monophyly of this group, currently classified as superfamily, is broadly accepted, but its internal relationships are not well-understood (see Hultgren and Stachowicz, 2008; Mahon and Neigel, 2008; Ng et al., 2008). Majoid classification has been largely based on larval and adult morphology, which may be subject of widespread convergent evolution due to the adaptive radiation of different lineages into similar ecological niches (Hultgren and Stachowicz, 2008). According to the paleontological record, majoids probably arose during the last wave of the brachyuran radiation in the Early Eocene, about 50 million years ago (mya) (Spears et al., 1992), although molecular dating suggests a rather older origin in the Late Permian about 250 mya (Porter et al., 2005).

The type genus of spider crabs is *Maja* Lamarck, 1801 (Stevcic, 2005), included in the family Majidae and comprising around 19 extant species (Ng et al., 2008). This genus is mainly distributed in the Indo-West Pacific region (Griffin and Tranter, 1986; Neumann, 1996), considered the center of brachyuran diversity (Ng et al., 2008). Outside this region, 4 species have been described along the European coasts (Neumann, 1998): *M. brachydactyla* Balss, 1922; *M. crispata* Risso, 1827; *M. goltziana* D'Oliveira, 1888; and *M. squinado* (Herbst, 1788). Within the European group, the wide morphological variability has also complicated the identification and classification. *M. brachydactyla* and *M. squinado* are the most similar species. They reach the largest body sizes (exceeding 20 cm in carapace length) and are found from subtidal areas to about 50 m of depth. *M. brachydactyla* is restricted to the Eastern Atlantic, from the British Islands to Senegal, while *M. squinado* inhabits the Mediterranean (Neumann, 1998; Sotelo et al., 2008b). *M. crispata* and *M. goltziana* have been recorded in the Atlantic, from Portugal to the Gulf of Guinea, and across the Mediterranean (Neumann, 1996); however, *M. crispata* is seldom found outside the Mediterranean (Carmona-Suárez, 2003), while *M. goltziana* is rare in both basins (Soppelsa et al., 2005). *M. goltziana* attains intermediate sizes and is found in deeper habitats (to 300 m) (Lelli et al., 2007). *M. crispata* is the smallest species and lives in shallow littoral waters (less than 15 m). An open taxonomic question is the identity of the purported *Maja* taxa from Senegal down to South Africa. Although the scarce specimens analyzed from this region showed some character overlap with *M. brachydactyla*, they were provisionally assigned to a different (putative) species, *M. capensis* Ortmann, 1894, while waiting for additional material to contrast this decision (Neumann, 1998).

In Europe, all these species are important members of the coastal fauna from an ecological perspective and also as economic resources, especially *M. brachydactyla* and *M. squinado*, which are extensively exploited by European fisheries. While morphology (Guerao et al., 2008; Neumann, 1996, 1998), reproduction and

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doi:10.1016/j.ympev.2009.05.009

some behavior traits are fairly well described for most of these species (e.g., Carmona-Suárez, 2003; Corgos and Freire, 2006; Fürböck and Patzner, 2005; García-Flórez and Fernández-Rueda, 2000), genetic data are limited to the identification and population structure description of *M. brachydactyla* (Sotelo et al., 2008a,b). Despite that "It is only through a full appreciation of morphological and genetic diversity, and why this has come about, that we can hope to successfully manage, maintain and conserve healthy ecosystems" (Ng et al., 2008), nothing is known so far about the historical biogeography of the genus *Maja*, neither in the Eastern Atlantic-Mediterranean nor in the Indo-West Pacific regions. The few *Maja* sequences available have been included in phylogenetic studies of higher taxa such as decapods (Porter et al., 2005) or majoids in general (Hultgren and Stachowicz, 2008), but a phylogenetic study of this genus has never been attempted before.

Here, we focus on the Eastern Atlantic and Mediterranean species with the aim of reconstruct their phylogenetic relationships and temporal diversification. To do so, we use two mitochondrial fragments (partial COI and 16S genes) and incorporate three congeners from the Indian Ocean: *M. japonica* Rathbun, 1932, *M. kominatoensis* Kubo, 1936 and *M. spinigera* (De Haan, 1837). The main questions we are interested to solve are: Do European species have a single origin? Are they a basal lineage within *Maja* or are instead derived from an Indo-West Pacific ancestor? When and how did they reach and spread into the Eastern Atlantic and Mediterranean basins?

2. Materials and methods

The analysis included seven *Maja* species (Table 1): the four European, *M. brachydactyla*, *M. crispata*, *M. goltziana* and *M. squinado*; and three from the Indian Ocean, *M. japonica*, *M. kominatoensis* and *M. spinigera*; plus two dubious specimens from South Africa putatively identified as *M. squinado* and *M. capensis* (hereafter referred to as *M.* sp. SA1 and *M.* sp. SA2, respectively). *Schizophrys aspera*, another spider crab of the family Majidae widespread across the whole Indo-West Pacific, was used as outgroup. Several samples of *M. brachydactyla*, *M. crispata* and *M. squinado* were available, but for the remaining species we only could obtain one individual, as we were largely limited by suitable museum material (Table 1). For *M. brachydactyla*, *M. crispata* and *M. squinado*, we selected one representative individual carrying the most common COI-16S haplotype for each species (Sotelo et al., 2008b).

Genomic DNA was extracted from muscle tissue preserved in pure ethanol, using the NucleoSpin Tissue kit (Macherey-Nagel) according to the manufacturer's instructions. We amplified two mitochondrial fragments: 709 bp of the COI gene using LCO1490 and HCO2198 primers (Folmer et al., 1994); and 668–674 bp of the 16S gene using 16L29 and 16HLeu (Schubart, 2009). PCRs were carried out in a final volume of 20 μl, containing 1 μl of DNA extraction, 2 μl of 10× PCR buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl, pH 8.8, 0.1% Tween 20), 1 μl of 50 mM $MgCl_2$, 1 μl of 0.1% BSA (Amersham Life Science), 1 μl of 10 mM dNTP Mix (Applied Biosystems), 0.5 μl of each primer (20 μM), 0.2 μl BIOTAQ polymerase (5 U/μl, Bioline) and 13 μl of sterile bidistilled water. PCR profiles were as follows: 5 min at 95 °C, 35 cycles of 20 s at 95 °C, 30 s at 42 °C, 30 s at 72 °C, and 7 min at 72 °C for COI; and 5 min at 95 °C, 35 cycles of 20 s at 95 °C, 30 s at 50–55 °C, 30 s at 72 °C and 7 min at 72 °C for 16S. For verification, PCR products were run in 2% agarose gels stained with ethidium bromide. Both fragments were always sequenced using the forward and reverse primers to ensure consistency. PCR products were purified with the NucleoSpin Extract II kit (Macherey-Nagel). Sequences were performed with BigDye v1.1 chemistry (Applied Biosystems), precipitated with ethanol and run in an ABI PRISM 310 (Applied Biosystems). Electropherograms were visualized and manually checked using BioEdit (Hall, 1999).

We aligned the sequences for each fragment with MAFFT version 6 (Katoh and Toh, 2008) using the L-INS-i algorithm and default parameters. The alignments were concatenated into a single dataset and a Bayesian phylogenetic analysis was performed with MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003). To do so, the dataset was partitioned by gene and by codon position in the COI region. This was selected as the best partitioning strategy according to Bayes factors (see Brandley et al., 2005) (data not shown). Substitution parameters were unlinked between partitions; for each partition, we set the number of distinct relative substitution rates and the kind of rate variation among sites (gamma distribution and/or proportion of invariable sites) that best adjusted to the nucleotide substitution model previously calculated under the AICc criterion in jModelTest (Posada, 2008). Topology and branch lengths remained linked between partitions without any prior constrain, while the prior on the substitution rate was set to variable to accommodate rate differences across partitions. We performed a MCMC search with two simultaneous runs starting with random trees. Each run consisted of four chains, with default heating parameters, and 10^7 generations sampled every 10^3 steps. We used the standard deviation of split frequencies as a convergence index (<0.001). Also, to visually check for convergence we used the online version of AWTY (Wilgenbusch et al., 2004). After discarding the first 10^3 samples for burn-in, 9000 sampled trees were summarized with a majority rule (50%) consensus tree. Branch length information was retained and nodal support expressed as posterior probabilities (*bpp*).

We used BEAST v1.4.8 (Drummond and Rambaut, 2007) to infer the time to the most recent common ancestor (TMRCA) for nodes of interest in the phylogeny (see below). This software also implements a Bayesian MCMC analysis, and is able to co-estimate trees and divergence times. We analyzed the concatenated dataset with the same partition scheme (the input file was properly formatted with the BEAUti utility included in the same program package). We unlinked substitution model parameters for each partition and they were estimated under a HKY+I+G model. We specified a relaxed clock with an uncorrelated lognormal distribution (Drummond et al., 2006) and a speciation Yule process as tree prior. Taking into account published substitution rates for COI and 16S in other crabs (Ketmaier et al., 2003; Schubart et al., 2000; Stillman and Reeb, 2001), we set the *meanRate* prior as a uniform distribution between 0.005 and 0.015 substitutions per site per lineage per mya, and the midpoint of this interval (0.01) as the starting value of the *ucld.mean* prior. To estimate the TMRCA for the European *Maja* species as well as for other nodes of interest, we defined the appropriate groups but without constraining them to be monophyletic. The analysis was run for 3×10^7 generations sampled every 3×10^3 steps and the first 10^3 samples were discarded as

Table 1
Taxon sampling. Species names and site of collection, according to the indicated references.

Species	Location	Reference
Maja brachydactyla	Cádiz (Southern Iberian Peninsula)	Sotelo et al. (2008b)[a]
Maja sp. SA1	Port Elizabeth (South Africa)	ZRC 2005.0014[b]
Maja sp. SA2	Southern South Africa	This study
Maja squinado	Capraia Island	Sotelo et al. (2008b)
Maja crispata	Greece	Sotelo et al. (2008b)
Maja goltziana	Sicily	This study
Maja japonica	Singapore	ZRC 1995.311
Maja spinigera	Taiwan	ZRC 2001.0057
Maja kominatoensis	Philippines	AURORA 2007 CC2743
Schizophrys aspera	Taiwan	ZRC 2001.0068

[a] JCB 28(1): 76–81, 2008.
[b] Zoological Reference Collection, Raffles Museum, Singapore (catalog number).

burn-in. To check for convergence and to visualize the results we used the program Tracer v1.4 (Rambaut and Drummond, 2007).

3. Results

The final concatenated dataset was 1294 bp long, with 654 bp from the COI gene and 640 bp from the 16S (excluding only primers positions). The best fit model of nucleotide substitution for each partition was: TrNef+I, F81, TIM2+G for 1st, 2nd and 3rd codon positions of COI, respectively; and TIM3+G for 16S (see Posada, 2008). The COI alignment (GenBank accession numbers: GQ153550–GQ153559) rendered 214 variable positions. Eleven of the 218 codons showed replacement changes, one of them between the European species (the codon 148 codified for Leu in *M. crispata* and *M. squinado* and for Met in the remaining species). The 16S alignment (GenBank accession numbers: GQ153560–GQ153569) presented 176 variable positions. Moreover, the length of this fragment varied between species, resulting in 22 sites with gaps (3 between the European samples). Remarkably, the 2 specimens from South Africa (*M.* sp. SA1 and *M.* sp. SA2) carried the same 16S sequence, which was also identical to that of *M. brachydactyla*. For the partial COI gene, they differed in a single position and possessed 8 substitutions respect to *M. brachydactyla*.

MrBayes runs clearly reached convergence, with a final standard deviation of split frequencies smaller than 0.001 and stable likelihood values. The resulting phylogeny was well resolved and highly supported, nodal posterior probabilities were often 1.00 or close to 1.00 (Fig. 1). After rooting the tree with the outgroup (*S. aspera*), the Asian *M. kominatoensis* appeared as the basal ingroup lineage. The remaining species formed two well defined clades: one including *M. japonica* and *M. spinigera* (both from the Indian Ocean) and the other comprising all the Eastern Atlantic and Mediterranean specimens. Within the monophyletic European group, *M. goltziana* was the basal species, while a more recent split separates the "Mediterranean" (*M. crispata* and *M. squinado*) an "Eastern Atlantic" (*M. brachydactyla*, *M.* sp. SA1 and *M.* sp. SA2) lineages.

BEAST parameters had very large effective sample sizes. The inferred mean substitution rate was 1.08% per my, and was close to a strict clock model (*ucld.stdev* = 0.259). The estimated TMRCA for the "European" *Maja* crabs (Fig. 1, node A) was 22.00 mya (Early Miocene), and the split between the "Mediterranean" and the "Eastern Atlantic" lineages (Fig. 1, node B), 9.59 mya (Late Miocene). The *M. squinado*/*M. crispata* divergence (Fig. 1, node C) dates back to 3.95 mya (Pliocene), and to 0.60 mya (Middle Pleistocene) the divergence within the "Eastern Atlantic" clade (Fig. 1, node D). As well, the time estimate of the root of the tree (Fig. 1, node R) was of 86.14 mya. However, all confidence intervals were quite wide, as indicated in Fig. 1.

4. Discussion

The inferred molecular phylogeny clearly shows that the Eastern Atlantic and Mediterranean *Maja* species conform to a single mitochondrial lineage that most likely diverged from an Indo-West Pacific ancestor during the Early Miocene. Within *Maja*, *M. kominatoensis* seems to be the basal species, a result that would be congruent with its morphological data, as this crab can be easily differentiated from the other congeners due to the absence of spines in the median line and in the margins of the carapace. To check the influence of the outgroup selection in this result, we repeated the analysis incorporating other majoid species from different families: *Chionoecetes opilio* (Oregoniidae) and *Pugettia quadridens* (Epialtidae) (these sequences were available at GenBank under the following accession numbers: AB211154 and

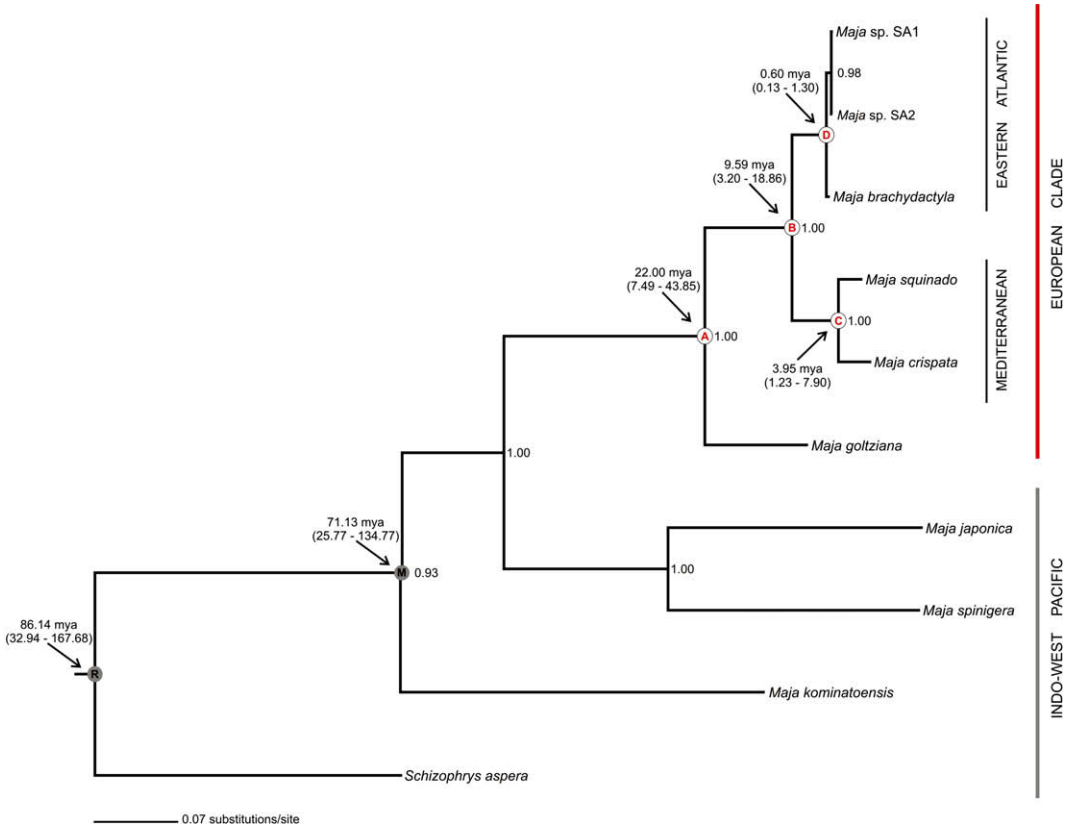

Fig. 1. Bayesian phylogeny of *Maja* species based on partial COI and 16S genes, rooted with *Schizophrys aspera*. Numbers at nodes are posterior probabilities. Arrows point to nodes with estimated TMRCAs, showing mean values in million years (mya) and the 95% confidence intervals (within parentheses).

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AB188684; EU682866 and EU682821). In this case, the relationships within *Maja* did not change (data not shown), although the support of this clade decreased (*bpp* = 0.61) while *S. aspera* appeared as a basal sister lineage of all *Maja* with *bpp* = 1.00. Despite its current classification, *S. aspera* was included within *Maja* for some time (see Griffin and Tranter, 1986; Ng et al., 2008), and our results suggest that its systematic position is not completely clear.

The root of the genus *Maja* is unambiguously placed into the Indo-West Pacific basin, which together with the older age and phylogenetic position of the *M. japonica–M. spinigera* clade, suggests an Indo-West Pacific origin of the European *Maja* crabs. However, it is difficult to be more concrete about the lineages that colonized the Atlantic and Mediterranean oceans, as we only obtained three out of putative 15 species in the Indo-West Pacific (Griffin and Tranter, 1986; Ng et al., 2008). An ancestor of the European lineage inhabiting the current Indo-West Pacific at some point from the Cretaceous to the Early Miocene seems quite logical given the history of this region. The Indo-West Pacific is regarded as a center of origin for many marine organisms, especially for those with a current high concentration of species in this area (e.g., Frey and Vermeij, 2008, and references therein), and it is also a center of biodiversity for decapods in general (Feldmann and Schweitzer, 2006) and brachyurans in particular (Ng et al., 2008). Moreover, from the Cretaceous to the Eocene, several brachyuran radiations took place from the Southern Hemisphere and led to many of the modern families of crabs (Feldmann and Schweitzer, 2006; Schram, 2001). Thus, the first fossil of Majidae in Europe (Italy) effectively dates from the Eocene (Larghi, 2002).

One of the most important tectonic events during the Miocene was the collision of the African and Eurasian plates, which caused the closure of the Tethyan Seaway. This corridor connected the Atlantic and Indian Oceans through the Mediterranean Sea, allowing the exchange of fauna among basins. Although there is not consensus in the exact date of the closure, it could have begun in the Early Miocene, around 20 mya, and have completed in the Middle Miocene, around 10 mya (see Teske et al., 2007). This event represented the isolation of the Eastern Atlantic-Mediterranean from the Indo-West Pacific regions, and therefore the independent evolution of the biota on both sides. This episode promoted speciation in several marine organisms; such as the sea urchins *Diadema* (Lessios et al., 2001), the sand gobies Gobiidae (Huyse et al., 2004), the snails *Echinollitorina* (Williams and Reid, 2004) and *Nerita* (Frey and Vermeij, 2008), the seahorses *Hippocampus* (Teske et al., 2007), or the spiny lobsters *Palinurus* (Groeneveld et al., 2007).

According to our data, the estimated age of the European species (22 mya) predates the final Tethyan closure, and so it is possible that their ancestor could have reached the Mediterranean and began to diverge before the complete isolation of both basins. Similar scenarios have been suggested in relation with the uplift of the Isthmus of Panama for species adapted to deeper habitats, which could have become isolated even before the total rising of the Isthmus (Teske et al., 2007; Williams and Reid, 2004). The first split leading to the European *Maja* crabs would fit to this pattern, as its basal lineage, *M. goltziana,* which occurs in both the Mediterranean and the Atlantic, is characteristic of deeper waters (Lelli et al., 2007). The larger genetic divergence of *M. goltziana* is also in agreement with morphology, as it is considered the most differentiated species within the European clade (Neumann, 1996; Soppelsa et al., 2005).

The second split within the European species resulted into a "Mediterranean" (*M. squinado and M. crispata*) and an "Eastern Atlantic" (*M. brachydactyla* and South African crabs) clade, around 9.59 mya. If the dating is correct, this split would have taken place before the Messinian salinity crisis (5.9–5.33 mya, Krijgsman et al., 1999), the major vicariance event between the Mediterranean and the Atlantic (Huyse et al., 2004, and references therein). This incident consisted of a dramatic desiccation of the Mediterranean Sea that isolated it from the Atlantic Ocean (Krijgsman et al., 1999), and that caused a severe extinction of the ancestral Indo-Pacific fauna in the Mediterranean (Huyse et al., 2004). Although *M. goltziana* presumably survived this crisis, we cannot discard the extinction of other *Maja* lineages that had possibly existed in the Mediterranean. *M. goltziana* has been recorded from the Eastern Atlantic as well, but the individual that we obtained for this study was from the Mediterranean Sea (Sicily). Therefore, it would be very interesting to obtain an Atlantic sample in order to confirm whether it effectively belongs to the same mitochondrial lineage, and to understand whether *M. goltziana* spread into the Atlantic once, before or after the Messinian crisis, or perhaps twice.

At the end of the Messinian salinity crisis, the reopening of the Strait of Gibraltar 5.33 mya not only reconnected the two basins because of the re-flooding of the Mediterranean with Atlantic waters but also generated new available habitats and niches within the Mediterranean Sea, i.e., suitable conditions for new species to arise. The divergence between *M. squinado* and *M. crispata*, dated in the Pliocene (3.95 mya), would fit this hypothesis. Indeed, these two species are found at different depth ranges, the first from subtidal areas up to 50 m deep, while the second only occurs in shallow shores. On the contrary, the shared ecological characteristics of *M. squinado* and *M. brachydactyla* could have prevented their co-occurrence in the same basin, as they would compete for the same niche. Furthermore, their large morphological resemblance could be also due to convergent evolution (adaptation to the same habitat), a feature that may be quite common within majoids (Hultgren and Stachowicz, 2008) and in other crustaceans (e.g., Harrison and Crespi, 1999; Reuschel and Schubart, 2006).

Within the "Eastern Atlantic" clade, the low differentiation of the South African specimens formerly identified as separate taxa (*M. squinado* and *M. capensis*), both between themselves and *M. brachydactyla*, suggests that all these taxa are in fact the same species (*M. brachydactyla*), in congruence with the morphological affinities already described between *M. capensis* and *M. brachydactyla* (Neumann, 1998). If we assume a single species in the Eastern Atlantic, from the British Islands to South Africa, *M. brachydactyla* would have diverged from a plausible Mediterranean ancestor during the Late Miocene (9.59 mya), although its current diversity would be explained by a more recent expansion during the Pleistocene (600,000 ya) (Sotelo et al., 2008a). Indeed, it is possible that despite having reached the Atlantic during the Miocene, Pleistocene glaciations caused severe bottlenecks and the retreat of the *M. brachydactyla* populations to southern refuges, as described for most marine taxa in this region (see Chevolot et al., 2006, and references therein). Supporting this idea, a fossil of *Maja* was recorded from the Pliocene in Belgium (van Bakel et al., 2006), which indicates its presence before Pleistocene glaciations. The same pattern has been observed and discussed in the spiny lobster *Palinurus elephas*, with similar speciation and expansion dates in the Eastern Atlantic (Groeneveld et al., 2007; Palero et al., 2008). While the wide distribution of *M. brachydactyla* in the Eastern Atlantic is congruent with the high dispersal capacity of its pelagic larvae (the two zoea stages spend around two weeks in the water column), its eurithermic condition, and the influence of oceanic currents (see Sotelo et al., 2008a), the southernmost expansion seems more difficult to explain, as the main current in southern Africa, the Benguela Current, which flows clock-wise from east to west coasts, already appeared in the Miocene and intensified in the Late Pliocene (Lessios et al., 2001). Alternatively, a putative *M. capensis* taxon could have independently diverged during the Miocene, having hybridized only recently with *M. brachydactyla* in a contact zone, likely around Cape Verde Islands, where Canary and Benguela Cur-

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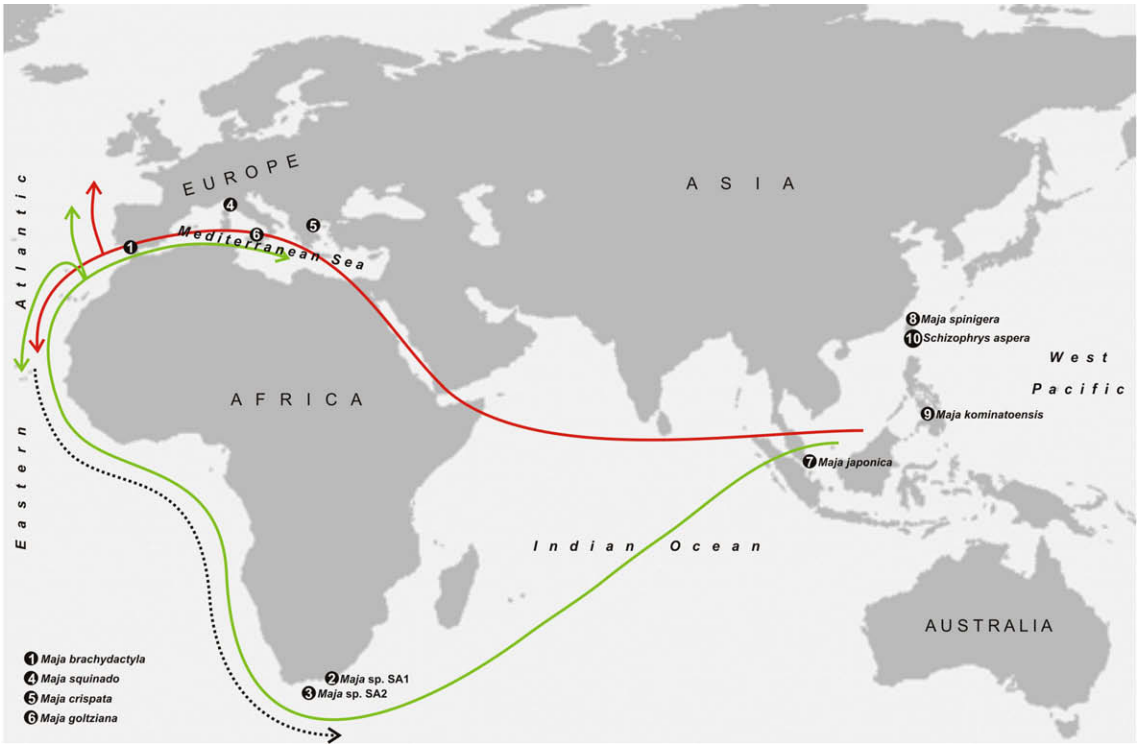

Fig. 2. Biogeographic history of the European *Maja* species, from an Indo-West Pacific ancestor. Two hypotheses are presented: the red line indicates colonization through the Mediterranean Sea, while the green line represents an alternative route through the Atlantic Ocean. The black dotted line remarks the assignment of South African *Maja* crabs to the Atlantic species *M. brachydactyla*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

rents converge. However, to test this hypothesis it would be necessary to include other *Maja* species from the Western Indian Ocean in addition to an extensive sampling in the Eastern Atlantic and the use of nuclear markers. Remarkably, the Benguela Current is considered another biogeographic break between the Eastern Atlantic-Mediterranean and the Indo-West Pacific (e.g., Frey and Vermeij, 2008; Lessios et al., 2001).

So far, we have discussed the evolution of *Maja* crabs in the Eastern Atlantic-Mediterranean region considering the Tethyan Seaway as the initial colonization route; however, we cannot completely discard the entrance through the South African coast instead (Fig. 2). Under this scenario, an ancestral *M. goltziana* would have spread along the Atlantic and Mediterranean coasts during the Miocene, before the establishment of the Benguela Current. In agreement with this hypothesis, *M. goltziana* has been described as a species of subtropical Atlantic origin (Lelli et al., 2007; Soppelsa et al., 2005). Its sister lineage would split later into the Eastern Atlantic *M. brachydactyla* and a "Mediterranean" clade before the Messinian salinity crisis. The Mediterranean ancestor of *M. squinado* and *M. crispata* may have survived the desiccation period and, after that, with the appearance of new available habitats, diverged into species adapted to intermediate depths (*M. squinado*) and to shallow waters (*M. crispata*). These two species could have spread later into the Atlantic, but *M. squinado* could have been displaced due to niche competition with *M. brachydactyla*, while *M. crispata* could manage to successfully colonize this basin.

Indeed, our inferences rely on divergence estimates from mitochondrial data alone and assuming that the mutation rate adjusts to values reported for other crabs, ranging from 0.5% to 1.5% per lineage per my. The inferred rate of 1.08% seems quite reasonable given this, however, rates as low as 0.18–0.36% have also been proposed, for example, for the COI and 16S genes in the spiny lobster genus *Palinurus* (Groeneveld et al., 2007; although see Palero, 2008). Thus, it is clear that the results obtained should be taken as the first step in the reconstruction of the biogeographic history of European spider crabs of the genus *Maja*. Nuclear markers as well as a more comprehensive sampling would be desirable to answer with more precision this question.

Acknowledgments

We are grateful to Jose Cuesta, Paolo Sartor and Costas Triantaphyllidis for providing European samples; and to Tin-Yam Chan, Johan Groeneveld, Joelle Lai and Peter Ng for sending Pacific and Indian Ocean samples as well as for useful comments on the non-European species; and to Gaston Fernandes for helping in the shipment of South African specimens. We especially thank Sara Rocha for constructive discussions on the manuscript; and thanks are also due to Pilar Alvariño and Nieves Santamaría for lab assistance. Graciela Sotelo was supported by a pre-doctoral fellowship from the Xunta de Galicia.

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Please cite this article in press as: Sotelo, G., et al. Molecular phylogeny and biogeographic history of the European *Maja* spider crabs (Decapoda, Majidae). Mol. Phylogenet. Evol. (2009), doi:10.1016/j.ympev.2009.05.009