Molecular systematics and global phylogeography of angel sharks (genus Squatina)
Björn Stelbrink, Thomas von Rintelen, Geremy Cliff, Jürgen Kriwet

Molecular Phylogenetics and Evolution 54 (2010) 395–404

Contents lists available at ScienceDirect
Molecular Phylogenetics and Evolution
journal homepage: www.elsevier.com/locate/ympev

ARTICLE INFO
Article history:
Received 20 April 2009
Accepted 20 July 2009
Available online 30 July 2009

Keywords:
Squatinae
Biogeography
Vicariance
Dispersal
Molecular clock

ABSTRACT
Angel sharks of the genus Squatina represent a group comprising 22 extant benthic species inhabiting continental shelves and upper slopes. In the present study, a comprehensive phylogenetic reconstruction of 17 Squatina species based on two mitochondrial markers (COI and 16s rRNA) is provided. The phylogenetic reconstructions are used to test biogeographic patterns. In addition, a molecular clock analysis is conducted to estimate divergence times of the emerged clades. All analyses show Squatina to be monophyletic. Four geographic clades are recognized, of which the Europe–North Africa–Asia clade is probably a result of the Tethys Sea closure. A second sister group relationship emerged in the analyses, including S. californica (eastern North Pacific) and S. dumeril (western North Atlantic), probably related to the rise of the Panamanian isthmus. The molecular clock analysis show that both lineage divergences coincide with the estimated time of these two geological events.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

The angel shark genus Squatina (Chondrichthyes: Elasmobranchii: Neoselachi: Squalea: Squatinidae) comprises 22 extant, morphologically homogenous, benthic species (cf. Vooren and Da Silva, 1992; Compagno et al., 2005; Castro-Aguirre et al., 2006; Last and White, 2008), which inhabit continental shelves and upper slopes down to 500 m (Compagno et al., 2005). They are moderately-sized (total length about 1–2 m) and globally distributed in temperate to tropical seas (Compagno et al., 2005; Last and White, 2008). While some species occur over a wide geographic range, the majority are restricted to a smaller area (Compagno et al., 2005). Restriction in geographic range might be as a result of the behavior of Squatina species, which are ambush predators with a corresponding stationary bottom-dwelling habit (Compagno et al., 2005). Thus, trans-ocean migration is extremely unlikely, even though large-scale coastal migratory patterns have been reported in species such as S. squatina (Wheeler et al., 1975) and S. californica (Kato et al., 1967; Eschmeyer et al., 1983; Natanson and Cailliet, 1986; Compagno et al., 2005).

Based on their distribution, eight distinct putative zoogeographic species groups can be distinguished (distribution areas based on Compagno et al., 2005; Last and White, 2008): (1) Eastern North Atlantic–Mediterranean–North Africa (S. aculeata, sympatric with S. oculata and S. squatina in the Mediterranean), (2) southwest Indian Ocean, South Africa (S. africana), (3) western North Pacific, i.e. Asian species (S. formosa, S. japonica, S. nebulosa and S. tergocellatoidea), (4) western South Pacific and eastern Indian Ocean, i.e. Australian species (S. australis) and Squaliformes (dogfish sharks), which is congruent with morphological characters is still widely debated (cf. Compagno, 1973; Maisey, 1984; Thies and Reif, 1985; Seret, 1986; Shirai, 1992; de Carvalho, 1996). Recent mtDNA and nDNA studies by Dosady et al. (2003) and Winchell et al. (2004) show closer relationships between Squatiniformes (angel sharks), Pristiorhiformes (saw sharks) and Squaliformes (dogfish sharks), which is congruent with morphological data.

In the present paper we present the first comprehensive phylogeography of the Squatiniid sharks based on two mitochondrial

* Corresponding author. Fax: +49 30 2093 8565.
E-mail addresses: bjorn.stelbrink@mfn-berlin.de (B. Stelbrink), thomasvonrintelen@mfn-berlin.de (T. von Rintelen), cliff@shark.co.za (G. Cliff), kriwet.smns@ naturkundemuseum-bw.de (J. Kriwet).

1055-7903/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2009.07.029
markers, cytochrome oxidase subunit I (COI) and 16S rRNA, using different tree reconstruction methods. In addition, fossil and geological calibration points are used to estimate divergence times among the Squatina species and to test biogeographic patterns of inferred species groups.

2. Materials and methods

2.1. Material examined

In this study, 17 of the 22 described Squatina species from different localities of all geographic species groups (see Section 1) were analyzed for molecular purposes (Table 1, Fig. 1). Tissue samples could not be obtained from Squatina argentina, S. heteroptera, S. mexicana, S. nebulosa or S. punctata.

In addition, 22 cytochrome oxidase subunit I (COI) sequences of four Australian and two Asian species of Squatina from Ward et al. (2005, 2008) were used as reference data for molecular analyses (Table 1). Two sequences of Squalus were included as an outgroup in the molecular analyses (Table 1).

2.2. Molecular methods

Genomic DNA was isolated from about 1 to 3 mm³ muscle tissue or a corresponding amount of blood using a CTAB extraction protocol (Winnepenninckx et al., 1993). Muscle tissue was dried, cut into small pieces and macerated in CTAB buffer containing proteinase K. Fragments of the mitochondrial 16S rRNA (~570 bp) and COI (658 bp) genes were amplified and sequenced by polymerase chain reaction (PCR) using universal and modified primers, respectively (Table 2). Amplifications were conducted in 25 μL volumes containing 50–100 ng DNA, 1× PCR buffer, 200 mM of each dNTP, 0.5 mM of each primer, 2 mM MgCl₂ and 1 U of Taq polymerase. After an initial denaturation step of 3 min at 94 °C, 35 cycles of 30 s at 94 °C, 60 s at 40–45 °C (COI) or 50 °C (16S rRNA) and 60 s for COI) s at 72 °C were performed, followed by a final extension step of 5 min at 72 °C. PCR products were purified using NucleoSpin Extract II Kits (Macherey–Nagel). Both strands of the amplified gene fragments were cycle-sequenced using the primers employed in PCR with Big Dye Terminator chemistry version 1.1 (Applied Biosystems Inc.). Sequences were visualized on an Applied Biosystems 3130xl Genetic Analyser.

2.3. Alignment and phylogenetic analyses

Forward and reverse strands were assembled with CodonCode Aligner v. 2.0.6 (CodonCode Corporation, Dedham, MA, USA). 16S rRNA sequences were aligned using ClustalX v. 2.0.3 (Thompson et al., 1997; default settings) and corrected by eye. Substitution models for maximum likelihood (ML) and Bayesian inference (BI) analyses were estimated using MrModeltest v. 2.3 (Nylander, 2004) (Table 3). Two of four hierarchical likelihood ratio tests (hLRTs; e.g. Posada and Crandall, 1998) implemented in MrModeltest v. 2.3 selected HKY+Γ as the best-fit model. This model was also selected by the Akaike information criterion (AIC). For 16S rRNA, the best-fit model of all hLRTs and the AIC was GTR+I+Γ. Haplotypes were identified using DAMBE (Xia and Xie, 2001), uncorrected genetic p-distances were calculated using MEGA v. 4.1 (Tamura et al., 2007). Phylogenetic analyses were performed using maximum parsimony (MP) as implemented in PAUP* v. 4.0b10 for Windows (Swofford, 2002; MP parameters: heuristic search with 10 random addition cycles (maximum number of saved trees), tree bisection and reconstruction (TBR) branch swapping and 100 bootstrap replicates), ML using TREEFINDER v. June, 2008 (Jobb et al., 2004; ML parameters: search depth = 2, No. of bootstrap replicates = 1000) and BI using MrBayes v. 3.1.2 (Ronquist and Huelsenbeck, 2003; BI parameters: 5,000,000 generations, sample frequency = 1000, No. of chains = 4, burnin value = 2500).

2.4. Molecular clock analyses

Both data sets (COI and 16S rRNA) were tested for nucleotide substitution saturation using the test by Xia and Xie (2001) implemented in DAMBE. The test revealed no significant saturation for COI. 16S rRNA was excluded from the following steps, because substantial saturation for an (unlikely) extreme asymmetrical tree was found. Alignments were modified by deleting identical haplotypes in order to reduce the number of degrees of freedom (df), which are crucial for the following likelihood ratio tests (LRTs) (see Wilke et al., 2009). Two BI runs for COI were conducted using MrBayes (BI parameters: see Section 2.3.) with and without a molecular clock constraint (strict clock, simple clock model, default settings). Maximum log likelihoods of both runs were subjected to a LRT using the equation δ = 2 (ln L₁ − ln L₀) (e.g. Huelsenbeck et al., 1996; Huelsenbeck and Crandall, 1997), where L₁ is the alternative hypothesis (log likelihood of the run with no clock-enforced) and
Table 1
Material examined in this study (for sample site numbers see Fig. 1.) See Supplement material for specimen and locality information.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample site and specimen no.</th>
<th>EMBL Accession No. (COI)</th>
<th>EMBL Accession No. (16S rRNA)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squatina aculeata (n = 2)</td>
<td>16 (acu Pi 56, 57)</td>
<td>FN431671, FN431672</td>
<td>FN431790, FN431791</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina aculeata (n = 1)</td>
<td>16 (ocu Pi 58)</td>
<td>FN431754</td>
<td>FN431873</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina sp. (n = 3)</td>
<td>14 (squ_Pi 36, 37), 15 (squ_Pi 102)</td>
<td>FN431761, FN431762, FN431760</td>
<td>FN431880, FN431881, FN431879</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina africana (n = 16)</td>
<td>17 (afri Pi 80 - 98)</td>
<td>FN431674 - FN431688</td>
<td>FN431792 - FN431808</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina formosa (n = 5)</td>
<td>18 (for_Pi 134 - 136), 19 (for_Pi 38, 47)</td>
<td>FN431741 - FN431743, FN431744, FN431745</td>
<td>FN431860 - FN431862, FN431861, FN431864</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina formosa (n = 2)</td>
<td>C1 (EU399040, EU399041)</td>
<td>EU399040, EU399041</td>
<td>EU399040, EU399041</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina japonica (n = 1)</td>
<td>18 (jap_Pi 101)</td>
<td>FN431750</td>
<td>FN431869</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina kerguelenoides (n = 1)</td>
<td>20 (tga Pi 48)</td>
<td>FN431766</td>
<td>FN431885</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina albipunctata (n = 5)</td>
<td>28 (alb_Pi 53, 54, 70, 71), 29 (alb_Pi 72)</td>
<td>FN431689, FN431690, FN431691, FN431692, FN431693</td>
<td>FN431809, FN431810, FN431811, FN431812, FN431813</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina kerguelenoides (n = 2)</td>
<td>A1 (EU399044)</td>
<td>EU399044</td>
<td>EU399044</td>
<td>this study</td>
</tr>
<tr>
<td>Squatina australis (n = 3)</td>
<td>25 (aus_Pi 68), 28 (aus_Pi 67), 30 (aus_Pi 69)</td>
<td>FN431698, FN431697, FN431699</td>
<td>FN431818, FN431817, FN431819</td>
<td>this study</td>
</tr>
</tbody>
</table>
| Squatina australis (n = 8)   | B1 (DQ108193, DQ108203), B2 (DQ108200, DQ108201), B3 (DQ108202), B4 (EU399036, EU399037, EU399038) | FN431732, FN431733, FN431729, FN431730, FN431731, FN431700 - 1.4.1 (Rambaut and Drummond, 2007) and δ was subjected to a two-sided χ²-test. LRT parameters: L₁ = -3061.12; L₀ = -3075.78; δ = 29.32; df = s - 2 (s is the number of taxa used in the analysis) = 32; χ² upper critical value (α/2 = 0.025) = 49.48; χ² lower critical value (1 - α/2 = 0.975) = 18.29. As the estimated value (δ) does not exceed both upper and lower critical values the null hypothesis (all branches have a homogeneous nucleotide substitution, i.e. a strict clock) could not be rejected for COI.

A strict molecular clock analysis was performed using BEAST v. 1.4.8 (Drummond and Rambaut, 2007; substitution model: HKY+Γ; tree prior: constant size; 5,000,000 generations; log parameters every 200; burnin value = 1000). The fossil taxon Squatina cranei (96.55 ± 3.05 mya; from the Cenomanian, zone b of Holaster subglobosus, Clayton, Brighton, Sussex, UK; JK, pers. obs.) was subjected to a χ²-test. LRT parameters: L₁ = -3061.12; L₀ = -3075.78; δ = 29.32; df = s - 2 (s is the number of taxa used in the analysis) = 32; χ² upper critical value (α/2 = 0.025) = 49.48; χ² lower critical value (1 - α/2 = 0.975) = 18.29. As the estimated value (δ) does not exceed both upper and lower critical values the null hypothesis (all branches have a homogeneous nucleotide substitution, i.e. a strict clock) could not be rejected for COI.

A strict molecular clock analysis was performed using BEAST v. 1.4.8 (Drummond and Rambaut, 2007; substitution model: HKY+Γ; tree prior: constant size; 5,000,000 generations; log parameters every 200; burnin value = 1000). The fossil taxon Squatina cranei (96.55 ± 3.05 mya; from the Cenomanian, zone b of Holaster subglobosus, Clayton, Brighton, Sussex, UK; JK, pers. obs.) was subjected to a χ²-test. LRT parameters: L₁ = -3061.12; L₀ = -3075.78; δ = 29.32; df = s - 2 (s is the number of taxa used in the analysis) = 32; χ² upper critical value (α/2 = 0.025) = 49.48; χ² lower critical value (1 - α/2 = 0.975) = 18.29. As the estimated value (δ) does not exceed both upper and lower critical values the null hypothesis (all branches have a homogeneous nucleotide substitution, i.e. a strict clock) could not be rejected for COI.

A strict molecular clock analysis was performed using BEAST v. 1.4.8 (Drummond and Rambaut, 2007; substitution model: HKY+Γ; tree prior: constant size; 5,000,000 generations; log parameters every 200; burnin value = 1000). The fossil taxon Squatina cranei (96.55 ± 3.05 mya; from the Cenomanian, zone b of Holaster subglobosus, Clayton, Brighton, Sussex, UK; JK, pers. obs.) was subjected to a χ²-test. LRT parameters: L₁ = -3061.12; L₀ = -3075.78; δ = 29.32; df = s - 2 (s is the number of taxa used in the analysis) = 32; χ² upper critical value (α/2 = 0.025) = 49.48; χ² lower critical value (1 - α/2 = 0.975) = 18.29. As the estimated value (δ) does not exceed both upper and lower critical values the null hypothesis (all branches have a homogeneous nucleotide substitution, i.e. a strict clock) could not be rejected for COI.
was used to set a calibration point for the root height, i.e. the time of the most recent common ancestor (tmrca; BEAST setting: normal distribution) of *Squalus* and *Squatina* (Table 6).

### 3. Results

#### 3.1. Molecular phylogeny and geographic patterns

All three phylogenetic methods showed *Squatina* to be monophyletic and yielded essentially the same topology (Fig. 2). Four major clades were found in all analyses (COI, 16S rRNA and concatenated sequences). These clades correspond to geographic regions and comprise (1) the European and North African species *S. aculeata*, *S. oculata* and *S. squatina*, together with the Asian species *S. formosa*, *S. japonica*, *S. legnota* and *S. tergocellatoides* (clade 1, blue), (2) the South African species *S. africana* (clade 2, yellow), (3) the four Australian species *S. albipunctata*, *S. australis*, *S. pseudocellata* and *S. tergocellatoides* (clade 3, green) and (4) all North and South American species *S. armata*, *S. californica*, *S. dumeril*, *S. guggenheim* and *S. oculata* (clade 4, red). Each of the four clades is well supported, but the relationship between these clades remains unresolved (Fig. 2). The intergeneric genetic distance between *Squalus* and *Squatina* ranges from 14.8% to 18.3%.

Within clade 1 (blue), two major subclades (even though not well supported) can be distinguished (Fig. 2): the European and North African subclade comprising the strongly supported sister group *S. aculeata* (Senegal) and both populations of *S. squatina* (British Isles and Canary Islands), and the second subclade consisting of the populations of *S. formosa*, *S. japonica*, *S. legnota* and *S. tergocellatoides*. *Squatina formosa* (populations from Taiwan, Borneo and Japan) and *S. legnota* (Lombok, Indonesia) form a strongly supported sister group. The latter form a group with the remaining Asian species within clade 1 (*S. japonica* and *S. tergocellatoides*), but this node and also the most basal node, which suggests a sister group relationship of *S. oculata* (Senegal) to all remaining species of clade 1, are only strongly supported by BI posterior probabilities. The same is true for the sister group relationship between the European and North African species on the one hand and the Asian species on the other hand. The interspecific genetic distances in the European and North African species Asian species clade 1 (*S. africana*), was defined using the fossil taxon *Squatina cranei* with an approximate age of 96.55 ± 3.05 my (JK, pers. obser.), the estimated ages for the single nodes and clades (identified by numbers in Fig. 5) are given in Table 6. The mean clock rate for this topology is 2.38 ± 0.71 × 10⁻⁹ substitutions site⁻¹ year⁻¹ with an ESS (effective sample size) value of 1028.

### 4. Discussion

#### 4.1. Reliability of divergence time estimates

Estimation of divergence times using fossil taxa is, with good reason, hotly debated and frequently criticized (e.g. Heads, 2005;
Pulquerio and Nichols, 2007). Squatina cranei was used, as it is the oldest known, verified fossil squatinid, to determine the tmrca of Squalus and Squatina. However, it cannot be excluded that the squatinid lineage is even older depending on the exact position of the fossil within Squatina (see e.g. Magallon, 2004), resulting in generally older mean node ages and thus a slower mean clock rate. While this is considered unlikely, a re-assignment of Squatina cranei to one of the four clades found in the present study would increase mean node ages at least twofold. Hence, the lineage divergence would considerably precede the geological events discussed below (see Section 4.2.).

The estimated mean clock rate of the present study is $2.38 \pm 0.71 \times 10^{-9}$ substitutions \textit{site}^{-1} \textit{year}^{-1}. This is slightly faster compared with the substitution rate of the mitochondrial genes ND4 and ND5 in carcharhinoids and lamnoids (Martin et al., 1992). Although the possibility of a comparison between
these genes and shark groups, respectively, might be rather debatable, it nevertheless indicates a general reliability of the temporal data. In addition, this study supports the results by Martin et al. (1992) that evolutionary rates in sharks are generally slower in comparison with warm-blooded vertebrates (e.g. Pesole et al., 1992) that evolutionary rates in sharks are generally slower in comparison with warm-blooded vertebrates (e.g. Pesole et al., 1992) than those in cold-blooded vertebrates. This is expected because the cold-blooded vertebrates are not as active as the warm-blooded ones.

Irrespective of the remaining phylogenetic ambiguities, several highly interesting patterns emerged, particularly on a smaller geographic scale, and are discussed here for each clade.

4.2. Molecular analyses and biogeographic patterns

A comprehensive discussion of relationships among the four geographic clades remains speculative, because they remain essentially unresolved in this study (except in the BEAST analysis; see Fig. 5). However, all four clades corresponding to biogeographic groups are well supported by the phylogenetic analyses, only the European–North African–Asian clade has somewhat less support. No morphological apomorphies are as yet attributable to each clade: a comprehensive comparative morphological study of all recent species as initiated by Walsh and Ebert (2007) for the western North Pacific species would be very helpful to test the congruence between molecular and morphological data.

The sister group relationship of both subclades (European–North African and Asian) in clade 1 is one of the most interesting results of this study. According to paleogeographical reconstructions, a connection between the Mediterranean and the Tethys Sea in the Miocene existed until it was closed with the break-up of the Red Sea Land Bridge ca. 18–15 mya, separating the Atlantic from the Indian Ocean (Steininger and Rögl, 1984; reviewed e.g. in Barber and Bellwood, 2005). We suggest that the pattern of relationship in Squatina has been caused by the Tethys Sea closure, thus constituting an example of vicariance (i.e. the separation of the geographical range of a species into two or more parts through the development of a barrier (or barriers) sensu de Queiroz, 2005). In this vicariant scenario it has to be assumed that Squatina populations have occurred and maintained gene flow along the continental shelves of the Tethys Sea. Geographically separated populations, in the Mediterranean and in the Indian Ocean, could then have evolved into (incipient) species when the connection between these populations was disrupted ca. 18–15 mya during the Tethys closure. The age of the Tethys closure coincides with the estimated age for the split of both subclades (node 3 in Fig. 5: 22.22 ± 7.23 mya), providing further support for this hypothesis. Similar sister group relationships, for which an involvement of the Tethys Sea closure might be suspected, were also observed in molecular studies on other fish groups, e.g. in killifishes (Hrbek and Meyer, 2003), needlefishes (Banford et al., 2004), and are also found but not discussed in triakid (Iglesias et al., 2005; Human et al., 2006; Mustelus spp.) and scyliorhinid sharks (Iglesias et al., 2005; Apristurus spp.). For Squatina, the analysis of specimens reported from the Bay of Bengal (Nishida and Sivasubramaniam, 1986; merely identified as squatiniids) and from Mediterranean populations of S. aculeata, S. oculata and S. squatina, which were unfortunately not available for this study, will be crucial for testing the Tethys Sea closure hypothesis. The sister relationship of S. oculata from Senegal to all remaining species of clade 1 (Fig. 2) does not contradict the vicariant hypothesis suggested here, as two haplotype lineages may already have existed before the Tethys Sea closure.

Interestingly, the BEAST analysis revealed, in contrast to the non-clock analyses, a well supported sister group relationship between S. africana and clade 3 (Australian species) (Fig. 5). Divergence was estimated to have occurred in the Miocene and Oligocene (node 2 in Fig. 5: 24.86 ± 8.68 mya) when both land masses were already separated about 100 mya (Gondwanan break-up; see recent review by Upchurch, 2008). Therefore, a dispersal scenario of a post-Gondwanan population along the pre-existing coastal shelves seems to be the only possible explanation. Grant and Bowen (1998) studied the interrelationship of globally distributed species of sardines (Sardinia and Sardinops). In this molecular study, species from South Africa (Sardinops ocellatus) and Australia (Sardinops neopilchardus) were found to be sister groups possessing the same haplotype for the mitochondrial cytochrome b. The authors conclude that this pattern might indicate a probable dispersal along the Indian Ocean rim. The same scenario might be applied to S. africana and the Australian species. However, dispersal by early ontogenetic stages probably plays a more important role in teleost fishes than in sharks due to different reproductive strategies (i.e. small teleost eggs and larvae are more prone to dispersal than far bigger shark neonates).

In clade 3 (Australian species), Squatina australis is sister to the other three species. All S. australis specimens in this study share the same haplotype, although sampling was conducted along the en-
tire south Australian coast (Fig. 1). This suggests either a recent spread of *S. australis* from a smaller ancestral range or the capability to maintain gene flow across large distances in this species. In the Miocene (node 4 in Fig. 5; 13.02 ± 5.38 mya), an ancestral Australian lineage diverged into the *S. australis* lineage and a second lineage, which might have occurred either along the northern or the southern Australian coast. Possible subsequent geological events might have resulted in additional lineage divergences separating the eastern *S. pseudocellata* from a western lineage, which then diverged into *S. albipunctata* and *S. tergocellata*. The southern Australian coast consists of three different biogeographic provinces (see Waters and Roy, 2003; Waters et al., 2004), which are a result of temperature gradients, historical glacial cycles, and/or oceanographic patterns (reviewed in Möller et al., 2008). According to these provinces, an east–west distribution along South Australia was shown for both invertebrates (Waters et al., 2004, 2005) and vertebrates (Möller et al., 2008). The observed distribution pattern in these examples is thought to arise from both Plio-Pleistocene glacial cycles and ocean currents (Western Australian Leeuwin Current and East Australian Current), respectively, supporting dispersal. It might be possible that these factors also gave rise to the lineage separation scenario for the Australian Squatina species mentioned above. Hence, the present (partly sympatric) distribution pattern of the Australian species might be a result of secondary range extension. However, the reliability of this scenario depends on the accuracy of the species distributions shown in Fig. 4. It remains to be assessed whether the distribution gaps between species are real, particularly if *Squatina* is actually absent in the North of Australia, or if these gaps are rather sampling artifacts.

Most interesting for clade 4 (American species) in regard to paleo- and biogeography is the sister group relationship of *S. californica* and *S. dumeril*, which occur in the eastern North Pacific and in the western North Atlantic/Caribbean Sea, respectively (Compagno et al., 2005) separated by the Panama land bridge. Jordan (1908) was one of the first authors to recognize morphologically similar teleost fish species on either side of the Panamanian isthmus, which were regarded as identical in the past. The author concluded that this similarity between the “geminate species” (twin species) might be a result of an earlier geological event, particularly, the rise of the Isthmus of Panama in the Miocene (Jordan, 1908). The studies of the Central America geology and the paleo- and biogeography is the sister group relationship of *Squatina* (Compagno et al., 2005) separated by the Panama land bridge. The rise of the Panamanian isthmus was a successive process occurring in the Middle Miocene (ca. 20 mya) and terminating in the Late Pliocene with the final rise of the Panamanian isthmus ca. 3.5–2.9 mya (cf. Bermingham et al., 1997; Kirby et al., 2008). During this geological process several narrow straits like the Culebra Strait and Atrato Seaway might have provided the opportunity for gene flow between *Squatina* populations on both sides of the isthmus. Gene flow must have been finally disrupted by the rise of the Panamanian isthmus in the Late Pliocene, followed by allopatric speciation (Endler, 1977; i.e. speciation as a result of geographic isolation between populations) giving rise to the sister species *S. californica* and *S. dumeril*. The BEAST analysis estimated an approximately age of 6.11 ± 2.53 my (Table 6) for this split (node 6 in Fig. 5), suggesting that gene flow between both lineages was disrupted slightly before or during the rise of the Panamanian isthmus.

The molecular data also shed some light on taxonomic problems of clade 4 species (American species). In this study, a *S. californica* population from the Sea of Cortez was examined to confirm the reports that it represents an undescribed species from this area, i.e. to see if there are substantial genetic differences in comparison to *S. californica* populations from the Californian coast. Four different haplotypes were found within the Sea of Cortez population, which differ from the single haplotype of the remaining population (Fig. 3). Additionally, the Sea of Cortez population forms a sister group to the remaining *S. californica* population, at least for ML and BI inferred from COI and for ML inferred from the concatenated sequences (see Supplementary material). However, it remains to be elucidated whether the Sea of Cortez population represents a “good species” (i.e. if the genetic differences are also supported by e.g. morphological, anatomical or ecological traits, thus increasing the probability of reproductive isolation). Moreover, *S. californica* populations from other localities along the Pacific coast should be examined to extend the data base.

Another taxonomically difficult species group occurs in the western South Atlantic (see Section 1), of which *S. guggenheim* and *S. occulta* could be included in the present study. The species validity of *S. occulta*, which has been regarded as being synonymous with *S. guggenheim* by some authors (cf. Vooren and Da Silva, 1992; Soto, 2001; Compagno et al., 2005), is supported by our results, where both species are shown to be clearly distinct. Their relationship remains dubious, though, as *Squatina guggenheim*

### Table 5
<table>
<thead>
<tr>
<th></th>
<th>calA1</th>
<th>calA2</th>
<th>calA3</th>
<th>calA4</th>
<th>calB</th>
<th>dum1</th>
<th>dum2</th>
<th>dum3</th>
<th>dum4</th>
<th>dum5</th>
<th>dum6</th>
</tr>
</thead>
<tbody>
<tr>
<td>calA1</td>
<td>0.2</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calA2</td>
<td>0.3</td>
<td>0.2</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calA3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calB</td>
<td>0.5</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dum1</td>
<td>3.2</td>
<td>3.3</td>
<td>3.5</td>
<td>3.0</td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dum2</td>
<td>3.3</td>
<td>3.5</td>
<td>3.7</td>
<td>3.2</td>
<td>3.5</td>
<td>0.2</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dum3</td>
<td>3.0</td>
<td>3.2</td>
<td>3.3</td>
<td>2.9</td>
<td>3.2</td>
<td>0.2</td>
<td>0.3</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dum4</td>
<td>2.9</td>
<td>3.0</td>
<td>3.2</td>
<td>2.7</td>
<td>3.0</td>
<td>0.6</td>
<td>0.8</td>
<td>0.5</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dum5</td>
<td>3.0</td>
<td>3.2</td>
<td>3.3</td>
<td>2.8</td>
<td>3.2</td>
<td>0.8</td>
<td>0.9</td>
<td>0.6</td>
<td>0.5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>dum6</td>
<td>2.7</td>
<td>2.9</td>
<td>3.0</td>
<td>2.6</td>
<td>2.9</td>
<td>0.5</td>
<td>0.6</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 6
<table>
<thead>
<tr>
<th>Mean node age ± 95% highest posterior density (HPD) in my</th>
<th>Mean clock rate (substitutions × site⁻¹ × year⁻¹)/ESS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node 1, Squatina ingroup; node 2, <em>S. africana</em> and Australian species; node 3, European–North African–Asian species; node 4, Australian species; node 5, North and South American species; node 6, sister group <em>S. californica</em> and <em>S. dumeril</em>. Calibration point: <em>Squatina cranii</em> (96.55 ± 3.05 my; correlates with the root height in Fig. 5).</td>
<td></td>
</tr>
<tr>
<td>33.17 ± 9.85</td>
<td>24.86 ± 8.68</td>
</tr>
</tbody>
</table>
and *S. occulta* form a strongly supported sister group only in the BEAST analysis (Fig. 5), while this is not well supported for the other phylogenetic reconstruction methods (see Fig. 2 and Supplementary material).

**Acknowledgments**

This study would not have been possible without the following colleagues, who provided tissue material and additional se-


Paleontol. 4, 359–371.


population in sharks are slow compared with mammals. Nature 357, 153–155.

evidence for a new bottlenecked species in southern Australia. Mol.


angel shark, Squatina californica, off Santa Barbara, California. Copeia 1986, 587–

592.


resources in the Bay of Bengal. Marine Fishery Resources Management in the

Bay of Bengal, Colombo, Sri Lanka.


Evolutionary Biology Centre, Uppsala University.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G.,

Pesole, G., Gissi, C., De Chirico, A., Saccone, C., 1998. Nucleotide substitution rate of


bio.ubio.univie.ac.at/Tracer/


DNA molecules place cartilaginous fishes within the tree of bony fishes. J. Mol.

Evol. 48, 118–123.


Sert, R., 1986. Classification and phylogenese der chondrichthyes. Oceans 16, 121–

180.

Shirai, S., 1992. Phylogenetic relationships of the angel sharks, with comments on


and oceanic fauna of Brazil. Mare Magnum 1, 51–120.

Steininger, F.F., Rigl, F., 1984. Paleogeography and palynoplastic reconstruction of the

Neogene of the Mediterranean and Parathethys. In: Dixo, J.E., Robertson, A.H.F.
(Eds.), The Geological Evolution of the Eastern Mediterranean. Blackwell


Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary


Thies, D., Reif, W.E., 1985. Phylogeny and evolutionary ecology of Mesozoic


Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The

ClustalX windows interface: flexible strategies for multiple sequence alignment


Evol. 23, 229–236.


angel sharks, genus Squatinus, with redescriptions of Squatinus formosa, S.


Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Herbert, P.D., 2005. DNA barcoding


1857.


