Molecular Phylogenetics and Evolution 54 (2010) 395–404

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

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

Molecular systematics and global phylogeography of angel sharks (genus *Squatina*)

Björn Stelbrink [a,*], Thomas von Rintelen [a], Geremy Cliff [b], Jürgen Kriwet [c]

[a] Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity at the Humboldt University Berlin, Invalidenstr. 43, 10115 Berlin, Germany
[b] KwaZulu-Natal Sharks Board, Private Bag 2, Umhlanga 4320, South Africa and Biomedical Resource Unit, University of KwaZulu-Natal, South Africa
[c] State Museum of Natural History Stuttgart, Rosenstein 1, 70191 Stuttgart, Germany

ARTICLE INFO

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

Keywords:
Squatinidae
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.

1. Introduction

The angel shark genus *Squatina* (Chondrichthyes: Elasmobranchii: Neoselachii: 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. tergocellatoides*), (4) western South Pacific and eastern Indian Ocean, i.e. Australian species (*S. australis* along the south coast of Australia, partly sympatric with *S. albipunctata* along south-eastern Australia and with *S. tergocellata* along south-western Australia; *S. pseudocellata* occurs along north-western Australia) and *S. legnota*, which is recorded from Indonesia, (5) eastern North Pacific (*S. californica*), (6) eastern South Pacific (*S. armata*), (7) western North Atlantic (*S. dumeril*, sympatric with *S. heteroptera* and *S. mexicana* in the Gulf of Mexico) and (8) western South Atlantic (*S. argentina*, sympatric with *S. guggenheim*, *S. occulta* and *S. punctata*). These species groupings can be used to formulate and test biogeographic hypotheses, such as the influence of the rise of the Panamanian isthmus and the Tethys Sea closure on speciation.

Interspecific relationships of angel sharks have not been investigated except for a study by Furtado-Neto and Carr (2002) on three Brazilian species. The position of the squatinids within the elasmobranchs (sharks, skates and rays) based on 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 Douady et al. (2003) and Winchell et al. (2004) show closer relationships between Squatiniformes (angel sharks), Pristiophoriformes (saw sharks) and Squaliformes (dogfish sharks), which is congruent with morphological data.

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

* Corresponding author. Fax: +49 30 2093 8565.
E-mail addresses: bjoern.stelbrink@mfn-berlin.de (B. Stelbrink), thomas.rintelen@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 mm^3 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 $MgCl_2$ 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 (90 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 with 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+I+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. 4.0b010 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 $\delta = 2\ (\ln L_1 - \ln L_0)$ (e.g. Huelsenbeck et al., 1996; Huelsenbeck and Crandall, 1997), where L_1 is the alternative hypothesis (log likelihood of the run with no clock-enforced) and

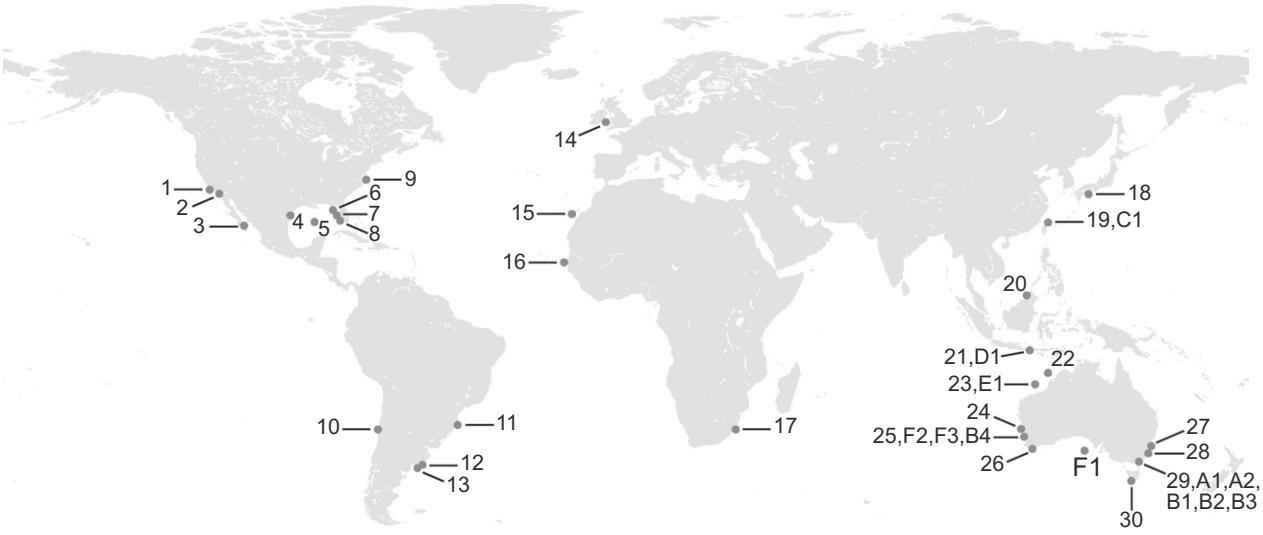

Fig. 1. Distribution of sample sites (see Table 1 for sample site and specimen information).

Table 1
Material examined in this study (for sample site numbers see Fig. 1.) See Supplement material for specimen and locality information.

Species	Sample site and specimen no.	EMBL Accession No. (COI)	EMBL Accession No. (16S rRNA)	Source
Squatina aculeata (n = 2)	**16** (acu_Pi 56, 57)	FN431671, FN431672	FN431790, FN431791	this study
Squatina oculata (n = 1)	**16** (ocu_Pi 58)	FN431754	FN431873	this study
Squatina squatina (n = 3)	**14** (squ_Pi 36, 37), **15** (squ_Pi 102)	FN431761, FN431762, FN431760	FN431880, FN431881, FN431879	this study
Squatina africana (n = 16)	**17** (afr_Pi 80 - 98)	FN431674 - FN431688	FN431792 - FN431808	this study
Squatina formosa (n = 5)	**18** (for_Pi 134 - 136), **19** (for_Pi 38, 47)	FN431741 - FN431743, FN431744, FN431745	FN431860 - FN431862, FN431863, FN431864	this study
Squatina formosa (n = 2)	**C1** (EU399040, EU399041)	EU399040, EU399041		Ward et al. (2008)
Squatina japonica (n = 1)	**18** (jap_Pi 101)	FN431750	FN431869	this study
Squatina tergocellatoides (n = 1)	**20** (tgs_Pi 48)	FN431766	FN431885	this study
Squatina albipunctata (n = 5)	**28** (alb_Pi 53, 54, 70, 71), **29** (alb_Pi 72)	FN431689, FN431690, FN431691, FN431692, FN431693	FN431809, FN431810, FN431811, FN431812, FN431813	this study
Squatina albipunctata (n = 2)	**A1** (EU399043), **A2** (EU399044)	EU399043, EU399044		Ward et al. (2008)
Squatina australis (n = 3)	**25** (aus_Pi 68), **28** (aus_Pi 67), **30** (aus_Pi 69)	FN431698, FN431697, FN431699	FN431818, FN431817, FN431819	this study
Squatina australis (n = 8)	**B1** (DQ108193, DQ108203), **B2** (DQ108200, DQ108201), **B3** (DQ108202), **B4** (EU399036, EU399037, EU399038)	DQ108193, DQ108203, DQ108200, DQ108201, DQ108202, EU399036, EU399037, EU399038		Ward et al. (2005, 2008)
Squatina legnota (n = 1)	**21** (leg_Pi 76)	FN431751	FN431870	this study
Squatina legnota (n = 1)	**D1** (EU399042)	EU399042		Ward et al. (2008)
Squatina pseudocellata (n = 3)	**22** (pse_Pi 75), **23** (pse_Pi 73, 74)	FN431757, FN431755, FN431756	FN431876, FN431874, FN431875	this study
Squatina pseudocellata (n = 2)	**E1** (EU399045, EU399046)	EU399045; EU399046		Ward et al. (2008)
Squatina tergocellata (n = 3)	**24** (tga_Pi 77), **25** (tga_Pi 78, 79)	FN431763, FN431764, FN431765	FN431882, FN431883, FN431884	this study
Squatina tergocellata (n = 5)	**F1** (DQ108194, DQ108195, DQ108196), **F2** (EU399047), **F3** (EU399048)	DQ108194, DQ108195, DQ108196, EU399047, EU399048		Ward et al. (2005, 2008)
Squatina sp. (n = 2)	**27** (spX_Pi 24, 26)	FN431758, FN431759	FN431877, FN431878	this study
Squatina californica (n = 5; n = 29 from the Sea of Cortez)	**1** (cal_Pi 49, 50), **2** (cal_Pi 27, 28, 29), **3** (cal_Pi 105 - 133)	FN431732, FN431733, FN431729, FN431730, FN431731, FN431700 - FN431728	N/A, FN431852, FN431849, FN431850, FN431851, FN431820 - FN431848	this study
Squatina armata (n = 3)	**10** (arm_Pi 21 - 23)	FN431694 - FN431696	FN431814 - FN431816	this study
Squatina dumeril (n = 7)	**4** (dum_Pi 32), **5** (dum_Pi 51, 52), **6** (dum_Pi 18), **7** (dum_Pi 19), **9** (dum_Pi 30, 31)	FN431738, FN431739, FN431740, FN431734, FN431735, FN431736, FN431737	FN431857, FN431858, FN431859, FN431853, FN431854, FN431855, FN431856	this study
Squatina guggenheim (n = 4)	**11** (gug_Pi 33), **12** (gug_Pi 60), **13** (gug_Pi 61, 62)	FN431746, FN431747, FN431748, FN431749	FN431865, FN431866, FN431867, FN431868	this study
Squatina occulta (n = 2)	**11** (occ_Pi 34, 35)	FN431752, FN431753	FN431871, FN431872	this study
Squalus acanthias (n = 1)	no geographic data available	NC_002012	NC_002012	Rasmussen and Arnason (1999)
Squalus cubensis (n = 1)	**8** (Scub_Pi 20)	FN431670	FN431789	this study

Table 2
Mitochondrial primers used in this study. M = A or C, Y = C or T and R = A or G.

Primer	Sequence (5′–3′)	Source
COI		
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al. (1994)
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al. (1994)
LCO Squatina	TCTACMAAYCACAAAGATATCGG	This study
HCO Squatina	TAAACTTCTGGGTGRCCRAAGAATCA	This study
16S rRNA		
L2510	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
H3080	CCGGTCTGAACTCAGATCACGT	Palumbi et al. (1991)

L_0 represents the null hypothesis (log likelihood of the clock-enforced run). Log likelihoods were visualized using TRACER v. 1.4.1 (Rambaut and Drummond, 2007) and δ was subjected to a two-sided χ^2-test. LRT parameters: $L_1 = -3061.12$; $L_0 = -3075.78$; $\delta = 29.32$; $df = s - 2$ (s is the number of taxa used in the analysis) = 32; χ^2 upper critical value ($\alpha/2 = 0.025$) = 49.48; χ^2 lower critical value ($1 - \alpha/2 = 0.975$) = 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+I+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. obser.)

Table 3
Substitution models selected by MrModeltest v. 2.3. I, proportion of invariable sites; Γ, gamma distribution shape parameter. I and Γ values refer to the AIC.

Gene	Substitution model	Among-site rate variation		Base frequencies			
		I	Γ	A	C	G	T
COI	HKY	0.6152	3.2267	0.2773	0.2510	0.1370	0.3347
16S rRNA	GTR	0.7071	0.7133	0.3153	0.1935	0.1905	0.3006

Table 4
Inter- and intraspecific genetic p-distance range (COI) in % within the European–North African–Asian clade.

	European and North African species				Asian species			
	aculeata	*oculata*	*squatina* (Canary Islands)	*squatina* (Britsh Islands)	*formosa*	*japonica*	*legnota*	*tergocellatoides*
aculeata	–							
oculata	7.2	–						
squatina (Canary Islands)	6.1	8.4	–					
squatina (Britsh Islands)	6.4–6.5	8.5–8.7	0.3–0.5	–				
formosa	6.7–6.8	6.2–6.4	7.3–7.5	7.3–7.6	–			
japonica	9.3	7.9	9.0	9.3–9.4	6.2–6.4	–		
legnota	5.9	5.3	7.0	7.2–7.3	2.3–2.4	6.5	–	
tergocellatoides	7.5	7.2	8.8	9.0–9.1	5.6–5.8	7.5	4.9	–

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. tergocellata* (clade 3, green), and (4) all North and South American species *S. armata*, *S. californica*, *S. dumeril*, *S. guggenheim* and *S. occulta* (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 Asian group are generally smaller in comparison to the European and North African species and specimens (Table 4). The smallest genetic distance is observed within the sister group *S. formosa* and *S. legnota*, which are also geographically close. In the other subclade the smallest genetic interspecific distance is observed between *S. aculeata* (Senegal) and *S. squatina* from the Canary Islands (Table 4).

All studied *S. africana* specimens (clade 2, yellow) share the same haplotype for COI and 16S rRNA. These specimens were all sampled along the South African east coast, in a geographic range of ca. 150 km, extending from Durban south to Trafalgar.

Most topologies for the Australian species (clade 3, green) show a well supported relationship (*S. australis* (*S. albipunctata* (*S. pseudocellata* + *S. tergocellata*))). *Squatina pseudocellata* and *S. tergocellata* both occur along the western coast, *S. albipunctata* along the eastern Australian coast and *S. australis* is recorded from southern Australian waters (Fig. 4). Two previously unidentified specimens (spX_Pi24 and spX_Pi26) from New South Wales cluster within the *S. albipunctata* subclade (see Supplementary material for inter- and intraspecific genetic distances).

The phylogenetic reconstructions for the North and South American species (clade 4, red) show *S. armata* from Chile (eastern South Pacific) to be sister group to the remaining species. A close relationship exists for the Brazilian specimens of *S. guggenheim* and *S. occulta* (western South Atlantic; however, the support for this grouping differs among the reconstruction methods; see also Supplementary material) and the strongly supported adelphotaxon *S. californica* and *S. dumeril*. These two species are geographically separated with the former, found on the Californian coast (also including the population from the Sea of Cortez; eastern North Pacific) and the latter occurring in the Gulf of Mexico north to Massachusetts (USA) and possibly also in the Caribbean (western North Atlantic; Compagno et al., 2005). Five COI haplotypes were found in *S. californica*, of which four were exclusively found in specimens sampled in the Sea of Cortez, a fifth haplotype is only found in the specimens off the Californian coast (Fig. 3); the sister taxon *S. dumeril* is represented by six different haplotypes. Inter- and intraspecific genetic distances are shown in Table 5.

3.2. Molecular clock analysis and divergence times

The BEAST analysis resulted in a slightly better resolved topology than the one formed in the non-clock analyses (Fig. 5), with the European and North African clade 1 (blue) being sister group to the remaining clades. Conspicuously, the *S. africana* clade 2 (yellow) is shown to be the sister group of the Australian species (green) with a moderately high posterior probability (88; Fig. 5). The four major clades are well supported in the BEAST analysis, however, the posterior probability for the clade node comprising clade 2, clade 3 and clade 4 is below the 50% limit (Fig. 5).

The root height, i.e. the tmrca of *Squalus* and *Squatina*, 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 $2.38 \pm 0.71 * 10^{-9}$ 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;

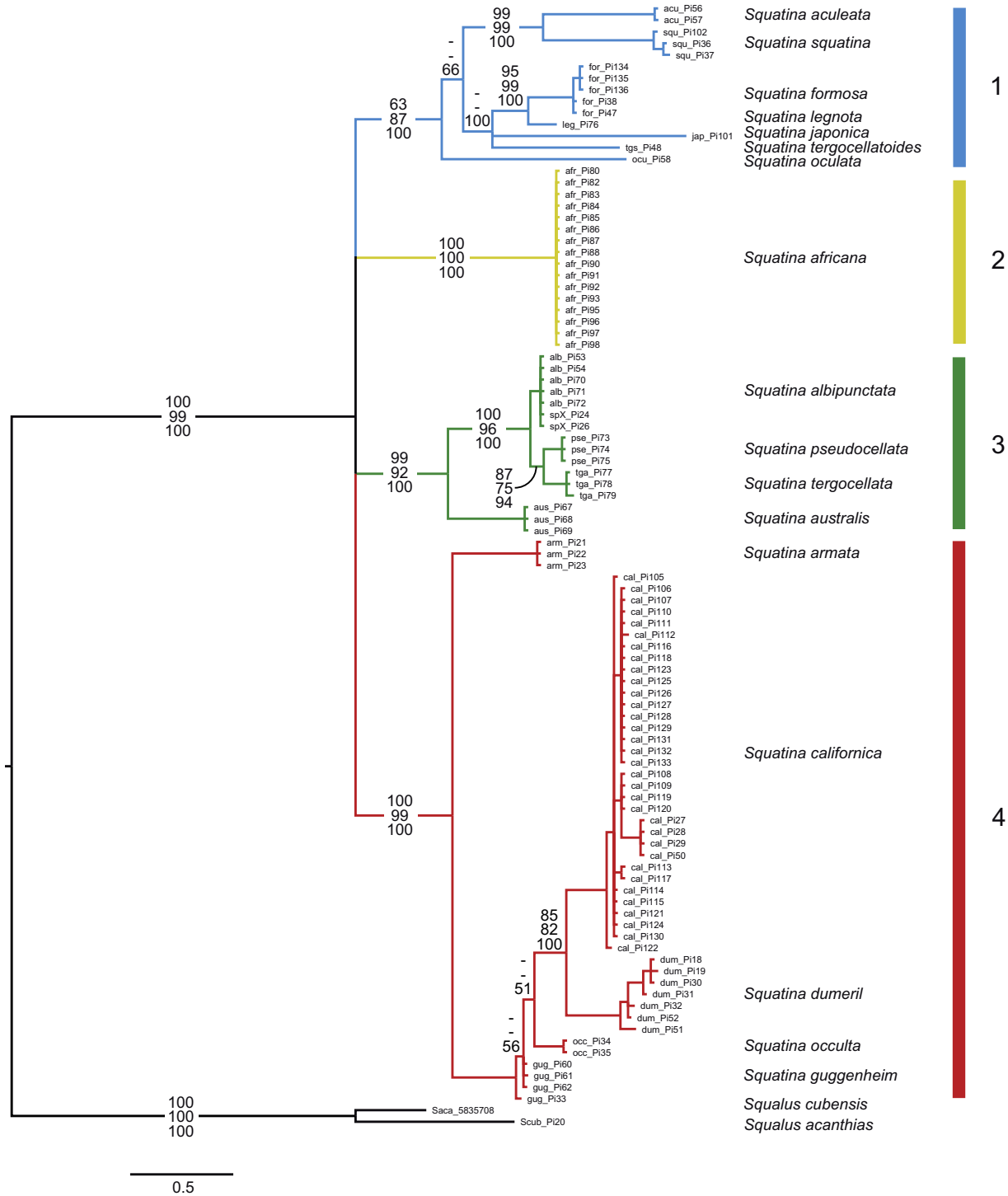

Fig. 2. BI phylogram of the concatenated data set (COI and 16S rRNA). Clades have been color-coded: 1 (Europe–North Africa–Asia) – blue; 2 (North and South America) – red; 3 (Australia) – green; 4 (South Africa) – yellow. The numbers on branches are MP and ML bootstrap values and posterior probabilities of BI, respectively (from top to bottom). (For interpretation of color mentioned in this figure legend, the reader is referred to the web version of the article.)

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 $2.38 \pm 0.71 * 10^{-9}$ substitutions $*$ site^{-1} $*$ 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

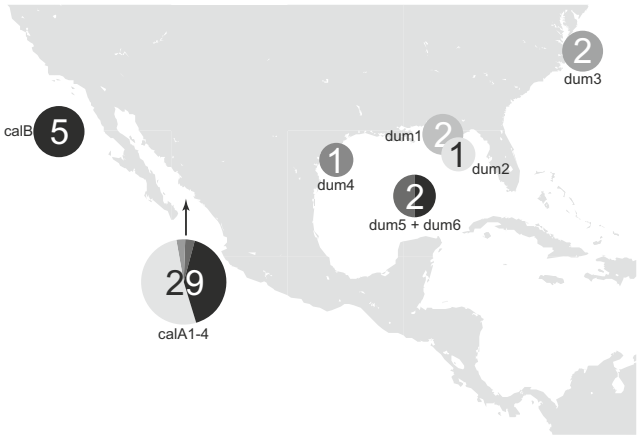

Fig. 3. COI haplotype diversity and sample provenience of the sister species *S. californica* and *S. dumeril*. Pie diagrams represent haplotype diversity, the numbers show the total number of specimens within the population or haplotype. The localities of haplotypes dum5 and dum6 could only be determined as Gulf of Mexico.

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., 1999) as well as other fish groups (e.g. Doiron et al., 2002).

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

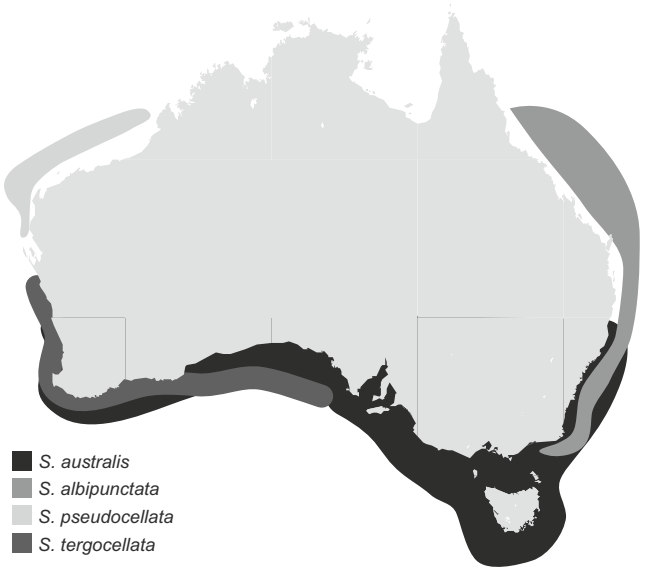

Fig. 4. Geographic distribution of the four Australian species *S. australis*, *S. albipunctata*, *S. pseudocellata* and *S. tergocellata*. Species-specific distribution redrawn from Last and Stevens (1994) and Compagno et al. (2005).

North Pacific species would be very helpful to test the congruence between molecular and morphological data.

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

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 squatinids) 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 (*Sardina* 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-

Table 5
Inter- and intraspecific genetic p-distance (COI) range in % between the *S. californica* and *S. dumeril* haplotypes. calA1–4, *S. californica* haplotypes (Sea of Cortez); calB, *S. californica* haplotypes (Californian coast); dum1–dum6, *S. dumeril* haplotypes.

	calA1	calA2	calA3	calA4	calB	dum1	dum2	dum3	dum4	dum5	dum6
calA1	–										
calA2	0.2	–									
calA3	0.3	0.2	–								
calA4	0.2	0.3	0.5	–							
calB	0.5	0.6	0.8	0.6	–						
dum1	3.2	3.3	3.5	3.0	3.3	–					
dum2	3.3	3.5	3.7	3.2	3.5	0.2	–				
dum3	3.0	3.2	3.3	2.9	3.2	0.2	0.3	–			
dum4	2.9	3.0	3.2	2.7	3.0	0.6	0.8	0.5	–		
dum5	3.0	3.2	3.3	2.9	3.2	0.8	0.9	0.6	0.5	–	
dum6	2.7	2.9	3.0	2.6	2.9	0.5	0.6	0.3	0.2	0.3	–

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 within the last decades (reviewed in Kirby et al., 2008) revealed that the rise of the Panamanian isthmus was a successive process commencing 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 form 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 6
Estimated mean node ages from the strict clock analysis for COI (see Fig. 5). Node 1, *Squatina* ingroup; node 2, *S. africana* 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 *S. californica* and *S. dumeril*. Calibration point: *Squatina cranei* (96.55 ± 3.05 my; correlates with the root height in Fig. 5).

Mean node age ± 95% highest posterior density (HPG) in my						Mean clock rate (substitutions $*$ site^{-1} $*$ year^{-1})/ESS
Node 1	Node 2	Node 3	Node 4	Node 5	Node 6	
33.17 ± 9.85	24.86 ± 8.68	22.22 ± 7.23	13.02 ± 5.38	12.18 ± 4.79	6.11 ± 2.53	2.38 ± 0.71 $*$ 10^{-9}/1028

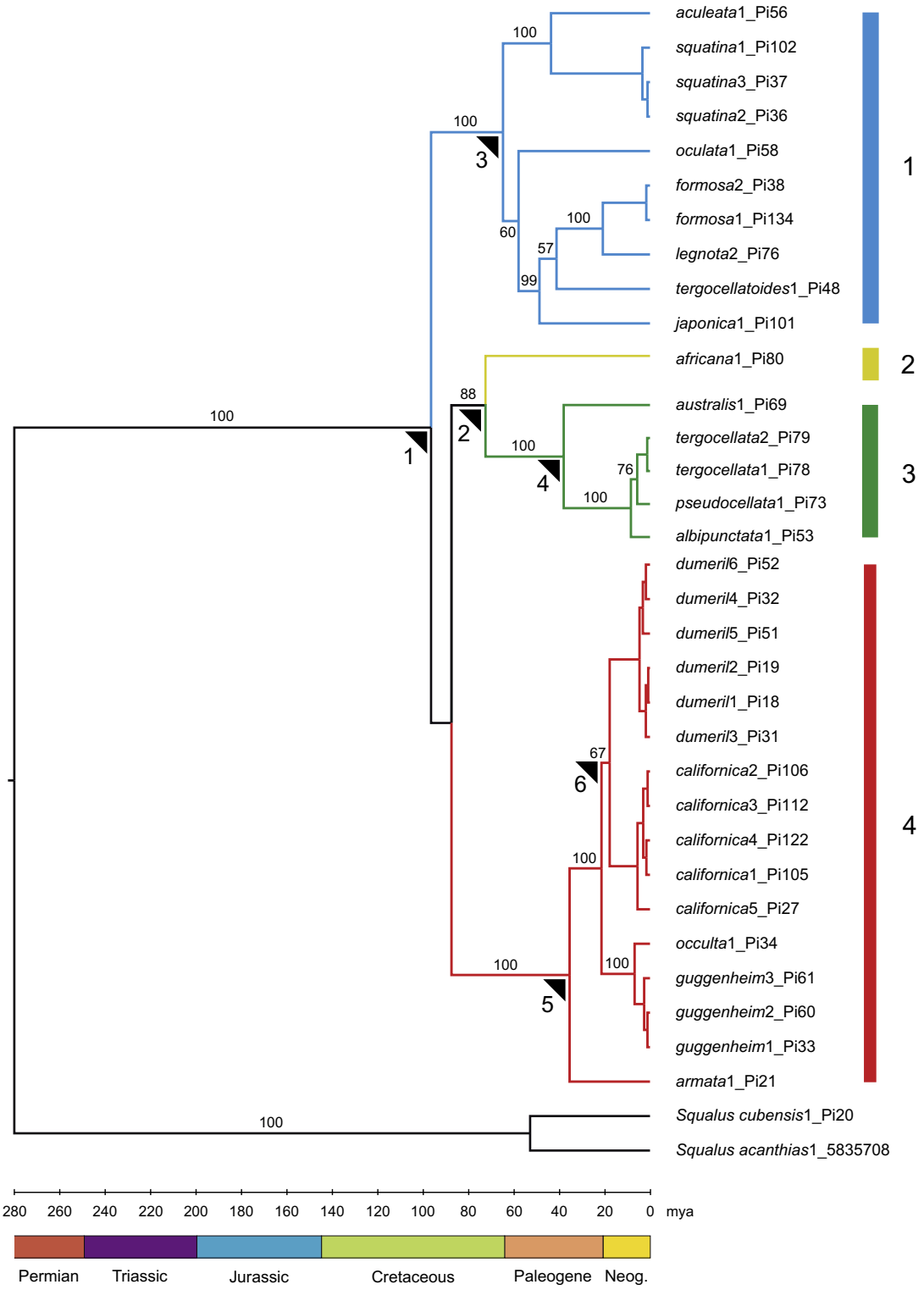

Fig. 5. Strict molecular clock tree for COI. Numbers on branches are posterior probabilities, node numbers with triangles refer to node ages listed in Table 6. Time scale lettering: A, Upper Cretaceous; B, Paleocene; C, Eocene; D, Oligocene; E, Miocene; F, Pliocene; G, Pleistocene; H, Holocene. Numbered grey bars above timescale denote geological events: 1, break-up of the Red Sea Land Bridge ca. 18–15 mya; 2, rise of the Panamanian isthmus ca. 3.5–2.9 mya. (For interpretation of color mentioned in this figure legend, the reader is referred to the web version of the article.)

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-

quences: Julie Carter (NOAA, Charleston, USA), Sebastian Hernandez (Chile) and Nicolas Straube (Berlin/Munich), Mark McGrouther (Australian Museum, Sydney, Australia), Andrew Bentley (Kansas University, Lawrence, USA), Samuel Iglesias (Muséum national d'Histoire naturelle, Paris, France), Gavin Naylor (Florida State University, Tallahassee, USA), Juan M. Diaz de Astarloa (Facultad de Ciencias Exactas y Naturales, Departamento de Ciencias Marinas, Universidad Nacional de Mar del Plata, Argentina), Sue Morrison (Western Australian Museum, Perth, Australia), Alastair Graham (CSIRO Marine and Atmospheric Research, Hobart, Australia), Sabine Wintner (KwaZulu-Natal Sharks Board, Umhlanga, South Africa), Hiromitsu Endo (Kochi University, Japan), Jose Antonio Gonzalez-Perez (Instituto Canario de Ciencias Marinas, Telde, Gran Canaria), Felipe Galvan-Magana (Centro Interdisciplinario de Ciencias Marinas, La Paz, Mexico), and Robert Ward (CSIRO Marine and Atmospheric Research, Hobart, Australia).

This study was financed by two Deutsche Forschungsgemeinschaft (DFG) Grants to JK (KR-2307/1-1; KR-2307/4-1).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.07.029.

References

Banford, H.M., Bermingham, E., Collette, B.B., 2004. Molecular phylogenetics and biogeography of transisthmian and amphi-Atlantic needlefishes (Belonidae: *Strongylura* and *Tylosurus*): perspectives on New World marine speciation. Mol. Phylogenet. Evol. 31, 833–851.

Barber, P.H., Bellwood, D.R., 2005. Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (*Halichoeres*: Labridae) in the Indo-Pacific and new world tropics. Mol. Phylogenet. Evol. 35, 235–253.

Bermingham, E., McCafferty, S.S., Martin, A.P., 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian isthmus. In: Kocher, T.D., Stepien, C. (Eds.), Molecular Systematics of Fishes. Academic Press, New York, pp. 113–128.

Castro-Aguirre, J.L., Espinosa Perez, H., Huidobro Campos, L., 2006. Dos nuevas especies del genero *Squatina* (Chondrichthyes: Squatinidae) del Golfo de Mexico. Rev. Biol. Trop. 54, 1031–1040.

Compagno, L.J.V., 1973. Interrelationships of living elasmobranchs. In: Greenwood, H.P., Miles, R., Patterson, C. (Eds.), Interrelationships of Fishes. Academic Press, London, New York, San Diego, pp. 15–61.

Compagno, L.J.V., Dando, M., Fowler, S., 2005. A Field Guide to the Sharks of the World. Harper Collins Publishing, Ltd., London.

de Carvalho, M.R., 1996. Higher-level elasmobranch phylogeny, basal squaleans and paraphyly. In: Stiassny, M.L.J., Parenti, L.R., Johnson, D.G. (Eds.), Interrelationships of Fishes. Academic Press, London, New York, San Diego, pp. 35–62.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68–73.

Doiron, S., Bernatchez, L., Blier, P.U., 2002. A comparative mitogenomic analysis of the potential adaptive value of arctic charr mtDNA introgression in brook charr populations (*Salvelinus fontinalis* Mitchill). Mol. Biol. Evol. 19, 1902–1909.

Douady, D.J., Dosay, M., Shivji, M.S., Stanhope, M.J., 2003. Molecular phylogenetic evidence refuting the hypothesis of Batoidea (rays and skates) as derived sharks. Mol. Phylogenet. Evol. 26, 215–221.

Drummond, A.J., Rambaut, A., 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7, 214.

Endler, J.A., 1977. Geographic Variation, Speciation and Clines. Princeton Univ. Press, Princeton.

Eschmeyer, W.N., Herald, E.S., Hammann, H., 1983. A Field Guide to Pacific Coast Fishes of North America. Houghton Mifflin Co., Boston, Massachusetts.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.

Furtado-Neto, M., Carr, S., 2002. Molecular genetics of some Brazilian sharks. The IUCN/SSC Shark Specialist Group, Shark News 14, March 2002, unpaginated.

Grant, W.S., Bowen, B.W., 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J. Hered. 89, 415–426.

Heads, M., 2005. Dating nodes on molecular phylogenies: a critique of molecular biogeography. Cladistics 21, 62–78.

Hrbek, T., Meyer, A., 2003. Closing of the Tethys Sea and the phylogeny of the Eurasian killifishes (Cyprinodontiformes: Cyprinodontidae). J. Evol. Biol. 16, 17–36.

Huelsenbeck, J.P., Crandall, K.A., 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu. Rev. Ecol. Syst. 28, 437–466.

Huelsenbeck, J.P., Hillis, D.M., Nielsen, R., 1996. A likelihood-ratio test of monophyly. Syst. Biol. 45, 546–558.

Human, B.A., Owen, E.P., Compagno, L.J.V., Harley, E.H., 2006. Testing morphologically based phylogenetic theories within the cartilaginous fishes with molecular data, with species reference to the catshark family (Chondrichthyes; Scyliorhinidae) and the interrelationships within them. Mol. Phylogenet. Evol. 39, 384–391.

Iglesias, S.P., Lecointre, G., Sellos, D.Y., 2005. Extensive paraphylies within sharks of the order Carcharhiniformes inferred from nuclear and mitochondrial genes. Mol. Phylogenet. Evol. 34, 569–583.

Jobb, G., Haeseler, A., von Strimmer, K., 2004. TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. BMC Evol. Biol. 4, 18 (9 pages).

Jordan, D.S., 1908. The law of geminate species. Am. Nat. 42, 73–80.

Kato, S., Springer, S., Wagner, M.H., 1967. Field guide to eastern Pacific and Hawaiian sharks. US Fish and Wildl. Serv., Circ. 271, 1–47.

Kirby, M.X., Jones, D.S., MacFadden, B.J., 2008. Lower Miocene stratigraphy along the Panama Canal and its bearing on the Central American Peninsula. PLoS ONE 3, 1–14.

Last, P.R., Stevens, J.D., 1994. Sharks and Rays of Australia. CSIRO, Melbourne, Australia.

Last, P.R., White, W.T., 2008. Three new angel sharks (Chondrichthyes: Squatinidae) from the Indo-Australian region. Zootaxa 1734, 1–26.

Magallon, S.A., 2004. Dating lineages: molecular and paleontological approaches to the temporal framework of clades. Int. J. Plant Sci. 165 (4 Suppl.), S7–S21.

Maisey, J.G., 1984. Chondrichthyan phylogeny: a look at the evidence. J. Vertebr. Paleontol. 4, 359–371.

Martin, A.P., Naylor, G.J.P., Palumbi, S.R., 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357, 153–155.

Möller, L.M., Bilgmann, K., Charlton-Robb, K., Beheregaray, L., 2008. Multi-gene evidence for a new bottlenose dolphin species in southern Australia. Mol. Phylogenet. Evol. 49, 674–681.

Natanson, L.J., Cailliet, G.M., 1986. Reproduction and development of the Pacific angel shark, *Squatina californica*, off Santa Barbara, California. Copeia 1986, 987–994.

Nishida, T., Sivasubramaniam, K., 1986. Atlas of deep water demersal fishery resources in the Bay of Bengal. Marine Fishery Resources Management in the Bay of Bengal, Colombo, Sri Lanka.

Nylander, J.A.A., 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Palumbi, S.R., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The Simple Fooĺs Guide to PCR. University of Hawaii Press, Honolulu.

Pesole, G., Gissi, C., De Chirico, A., Saccone, C., 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. J. Mol. Evol. 48, 427–434.

Posada, D., Crandall, K.A., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Pulquerio, M.J.F., Nichols, R.A., 2007. Dates from the molecular clock: how wrong can we be? Trends. Ecol. Evol. 22, 180–184.

Rambaut, A., Drummond, A.J., 2007. Tracer v. 1.4, Available from: <http://beast.bio.ed.ac.uk/Tracer>.

Rasmussen, A.S., Arnason, U., 1999. Phylogenetic studies of complete mitochondrial DNA molecules place cartilaginous fishes within the tree of bony fishes. J. Mol. Evol. 48, 118–123.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

Seret, B., 1986. Classification et phylogenese des chondrichthyens. Oceanis 12, 161–180.

Shirai, S., 1992. Phylogenetic relationships of the angel sharks, with comments on elasmobranch phylogeny (Chondrichthyes, Squatinidae). Copeia 1992, 505–518.

Soto, J.M.R., 2001. Annotated systematic checklist and bibliography of the coastal and oceanic fauna of Brazil. Mare Magnum 1, 51–120.

Steininger, F.F., Rögl, F., 1984. Paleography and palinspastic reconstruction of the Neogene of the Mediterranean and Paratethys. In: Dixon, J.E., Robertson, A.H.F. (Eds.), The Geological Evolution of the Eastern Mediterranean. Blackwell Scientific Publications, Oxford, pp. 659–668.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). Sinauer Associates, Sunderland, MA, USA.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Thies, D., Reif, W.E., 1985. Phylogeny and evolutionary ecology of Mesozoic Neoselachii. Neues Jahrb. Geol. Palaeontol. Abh. 169, 333–361.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25, 4876–4882.

Upchurch, P., 2008. Gondwanan break-up: legacies of a lost world. Trends Ecol. Evol. 23, 229–236.

Vooren, C.M., Da Silva, K.G., 1992. On the taxonomy of the angel sharks from southern Brazil, with the description of *Squatina occulta* sp. N. Rev. Bras. Biol. 51, 589–602.

Walsh, J.H., Ebert, D.A., 2007. A review of the systematics of western North Pacific angel sharks, genus *Squatina*, with redescriptions of *Squatina formosa*, *S. Japonica*, and *S. nebulosa* (Chondrichthyes: Squatiniformes, Squatinidae). Zootaxa 1551, 31–47.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Herbert, P.D., 2005. DNA barcoding Australia's fish species. Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci. 360, 1847–1857.

Ward, R.D., Holmes, B.H., White, W.T., Last, P.R., 2008. DNA barcoding Australian chondrichthyans: results and potential uses in conservation. Mar. Freshwater Res. 59, 57–71.
Waters, J.M., Roy, M.S., 2003. Marine biogeography of southern Australia: phylogeographical structure in a temperate sea-star. J. Biogeogr. 30, 1787–1796.
Waters, J.M., O'Loughlin, P.M., Roy, M.S., 2004. Cladogenesis in a starfish species complex from southern Australia: evidence for vicariant speciation? Mol. Phylogenet. Evol. 32, 236–245.
Waters, J.M., King, T.M., O'Loughlin, P.M., Spencer, H.G., 2005. Phylogeographical disjunction in abundant high-dispersal littoral gastropods. Mol. Ecol. 14, 2789–2802.
Wheeler, A., Blacker, R.W., Pirie, S.F., 1975. Rare and little-known fishes in British seas in 1970 and 1971. J. Fish Biol. 7, 183–201.
Wilke, T., Schultheiß, R., Albrecht, C., 2009. As time goes by: A simple fool's guide for molecular clock approaches in invertebrates. Am. Malacol. Bull. 27, 25–45.
Winchell, C.J., Martin, A.P., Mallatt, J., 2004. Phylogeny of elasmobranchs based on LSU and SSU ribosomal RNA genes. Mol. Phylogenet. Evol. 31, 214–224.
Winnepenninckx, B., Backeljau, T., DeWachter, R., 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet. 9, 407.
Xia, X., Xie, Z., 2001. DAMBE: Data analysis in molecular biology and evolution. J. Hered. 92, 371–373.