



## A recent shark radiation: Molecular phylogeny, biogeography and speciation of wobbegong sharks (family: Orectolobidae)

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### ARTICLE INFO

#### Article history:

Received 16 October 2008

Revised 2 March 2009

Accepted 11 March 2009

Available online 19 March 2009

#### Keywords:

Elasmobranch

Taxonomy

Ribosomal protein S7

Mitochondrial DNA

Phylogeography

### ABSTRACT

The elasmobranch fish are an ancient, evolutionarily successful, but under-researched vertebrate group, particularly in regard to their recent evolutionary history. Their lineage has survived four mass extinction events and most present day taxa are thought to be derived from Mesozoic forms. Here we present a molecular phylogenetic analysis of the family Orectolobidae that provides evidence for recent events of diversification in this shark group. Species interrelationships in Orectolobidae were reconstructed based on four mitochondrial and nuclear genes. In line with previous morphological work, our results do not support current taxonomic arrangements in Orectolobidae and indicate that a taxonomic revision of the family is warranted. We propose that the onset of diversification of orectolobid sharks is of Miocene age and occurred within the Indo-Australian region. Surprisingly, we also find evidence for a recent (~last 2 million years) and rapid radiation of wobbegong sharks. Allopatric speciation followed by range expansion seems like the general most likely explanation to account for wobbegong relationships and distributions. We suggest that the evolution of this shark group was mostly influenced by two temporal scenarios of diversification. The oldest relates to major geological changes in the Indo-West Pacific associated with the Miocene collision of the Indo-Australian and Eurasian plates. The most recent scenario was influenced by changes in oceanography and the emergence of biogeographic barriers related to Pleistocene glacial cycles in Australian waters.

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### 1. Introduction

The elasmobranch fish are considered one of the most ancient and successful vertebrate lineages. According to fossil evidence, they were present from the lower Devonian (possibly Silurian) and radiated during the “Age of Fishes” to become widely distributed, represented by diverse morphological and ecological forms (Grogan and Lund, 2004). Their lineage has survived four mass extinction events (Raup and Sepkoski, 1982) and most present day taxa are derivable from Mesozoic forms (Grogan and Lund, 2004). Despite a highly successful evolutionary trajectory, the elasmobranchs are a relatively under-researched group of fish, probably owing to their historically low economic value (Walker, 1998). In recent years however, improved recognition of the key role that apex predators play in ecosystem functioning, together with emerging conservation concern for many commercially harvested species, has stimulated a surge in elasmobranch research (Last, 2007). A large proportion of this research has been classically systematic and descriptive in approach, with about one third of the world’s chondrichthyan (elasmobranchs and holocephalans) fauna described (almost exclusively on the basis of morphological char-

acters) during the last 30 years (Last, 2007). Although there has been a concerted effort to address taxonomic uncertainty within elasmobranchs, seldom have these data been used to infer relationships between species within a phylogenetic or evolutionary framework. Also particularly lacking are studies that adopt a molecular approach to studying these fish. The majority of molecular phylogenetic appraisals of elasmobranchs are limited to higher taxonomic levels and mostly concerned with the origin and relative phylogenetic placement of study taxa (Bernardi and Powers, 1992; Douady et al., 2003; Dunn and Morrissey, 1995; Iglesias et al., 2005; Martin, 1993, 2001, 1995; Naylor, 1992; Naylor et al., 1997; Winchell et al., 2004). Relationships at or below the family level have rarely been reconstructed (Cavalcanti, 2007; Eitner, 1995; López et al., 2006) and studies that investigate recent biogeographic history (i.e. phylogeographic patterns) within elasmobranchs are extremely limited (Beheregaray, 2008). For instance, between 1987 and 2006 elasmobranchs accounted for only 1.5% of all articles published in the fish phylogeography literature (Beheregaray, 2008).

One group of sharks that reflect these research patterns are the wobbegong sharks (family: Orectolobidae, Gill, 1896). Wobbegongs are dorso-ventrally flattened, demersal sharks that can be distinguished from other elasmobranch families by the presence of dermal lobes on the lateral sides of the head (Compagno, 2001).

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These sharks are powerful ambush predators that occur over varied bottom topography from complex rocky and coral reefs to open sandy flats (Carraro and Gladstone, 2006; Compagno, 2001), camouflaged by their variegated colouration and dermal lobes (Compagno, 2001). Wobbegongs are endemic to the temperate and tropical continental waters of the western Pacific and eastern Indian oceans, but are most diverse in Australian waters (Fig. 1). They

are harvested as a food source at several locations throughout their distribution including Australia, China, Japan and Malaysia (Compagno, 2001). In Australia, they are commercially targeted in New South Wales and Western Australia where there is evidence suggesting that populations are declining (New South Wales Department of Primary Industries, unpublished data and R. McAuley, personal communication).

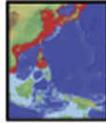
Species name	Vernacular	Voucher reference	Reference	Distribution
<i>Eucrossorhinus dasyopogon</i>	Tasselled wobbegong	Specimen alive at Omaha Henry Doorly Zoo	Compagno, 2001	
<i>Sutorectus tentaculatus</i>	Cobbler wobbegong	Specimen identified by Justin Chidlow	Compagno, 2001	
<i>Orectolobus japonicus</i>	Japanese wobbegong	AMS I 43794-002	Compagno, 2001	
<i>Orectolobus wardi</i>	Northern wobbegong	Specimen identified by Charlie Huveneers and Justin Chidlow, not retained.	Compagno, 2001	
<i>Orectolobus maculatus</i>	Spotted wobbegong	Specimen collected and identified by Charlie Huveneers, not retained.	Compagno, 2001	
<i>Orectolobus ornatus</i>	Dwarf ornate wobbegong	AMS I 43621-001	Huveneers, 2006	
<i>Orectolobus halei</i>	Gulf wobbegong	AMS I 43628-002	Huveneers, 2006	
<i>Orectolobus hutchinsi</i>	Western wobbegong	Specimen identified by Justin Chidlow, not retained	Last et al., 2006	
<i>Orectolobus flavidus</i>	Floral banded wobbegong	CSIRO H5936-01	Last and Chidlow, 2008	
<i>Orectolobus parvimaclulatus</i>	Dwarf spotted wobbegong	CSIRO H5633-01	Last and Chidlow, 2008	

Fig. 1. Geographic distributions of species, species and common names and details of voucher specimens.

The current taxonomic arrangement of the family follows Compagno et al. (2005) with the amendments of Huveneers (2006) and Goto (2008) as well as recent additions by Last et al. (2006), Last and Chidlow (2008) and Last et al. (2008). Orectolobidae comprises three genera and eleven species. *Eucrossorhinus* Regan, 1908 includes a single species *Eucrossorhinus dasyopogon* Regan, 1908 which is diagnosed by the presence of dermal lobes on the lower jaw (Compagno, 2001). *Sutorectus* Whitley, 1939 is also monotypic. *Sutorectus tentaculatus* (Peters, 1864) is most readily identified by the presence of warty tubercles on the dorsal surface and short dorsal fins relative to the height of the body of the shark (Compagno, 2001). The type genus *Orectolobus* Bonaparte, 1834, is the most diverse consisting of nine species (Fig. 1) that can be distinguished from other genera by the absence of dorsal warty tubercles and dermal lobes from the lower jaw, and tall dorsal fins relative to body height (Compagno, 2001). In addition to these, it is thought that undescribed taxa may exist in Northern Australia (Last et al., 2008), Indonesia (White et al., 2006), Borneo (Manjaji, 2002) and the Philippines (Compagno et al., 2005).

The two morphology-based studies available for the extant Orectolobidae indicate that the systematics of the family is problematic (Dingerkus, 1986; Goto, 2001). Dingerkus (1986) suggests that *Eucrossorhinus* is the most derived genus and sister to *Orectolobus*, while *Sutorectus* was placed in a separate and ancestral clade (Dingerkus, 1986). The work of Goto (2001) which considers a more diverse representation of the family, similarly suggests *Sutorectus* as a possible ancestral taxon and *Eucrossorhinus* more derived. However, in that study *Orectolobus* is paraphyletic, with some derived species clustering with *Eucrossorhinus* (Goto, 2001). Determining the polarity of morphological character states is inherently difficult in elasmobranchs and trait homoplasy is common (Fechhelm and McEachran, 1984; Maisey, 1984; Martin, 1995). These factors complicate inferences of relationships based on morphology, such as those of Dingerkus (1986) and Goto (2001). In addition, the recent descriptions of several species, some that are morphologically cryptic or that appear to have characteristics intermediate between genera (Compagno, 2001; Corrigan et al., 2008; Huveneers, 2006; Last and Chidlow, 2008; Last et al., 2006) further complicate interpretation of their results.

Phylogenetic reconstruction on the basis of character state variation at gene loci (Avice, 2004) provides a promising alternative approach to estimating relationships among wobbegong taxa and for addressing taxonomic uncertainties. A molecular approach is also timely given the recent surge in de novo species descriptions for these sharks. Furthermore, adopting such a framework will elucidate aspects of orectolobid evolution and biogeography that have not previously been considered. Vicariant events associated with plate tectonics may have influenced wobbegong speciation events in the Indo-Pacific (Hall, 1998), while various coastal and oceanographic features may have promoted wobbegong diversification in the more tectonically stable Australian waters (White, 2006). Orectolobids are endemic to these two regions and thus provide an excellent elasmobranch model for studying biogeographic history in the Indo-Pacific and Australia as well as oceanographic processes that might have shaped species distributions in elasmobranchs.

The aim of this study was to conduct a molecular phylogenetic analysis of the family Orectolobidae, the wobbegong sharks. Our sample set is more taxonomically diverse than that used by previous studies and includes both recently described species and taxa that are new to science. We believe that this represents one of the most comprehensive family-level molecular phylogenetic studies presented for any elasmobranch to date. We reconstruct species-interrelationships based on both mitochondrial and nuclear DNA data and discuss the several systematic implications of our results. We also propose a biogeographic scenario of diversification for the

group that includes both relatively old events of vicariance and dispersal in the Indo-Australian Archipelago (IAA) and rapid speciation within temperate Australian waters.

## 2. Materials and methods

### 2.1. Sample collection

Tissue samples were obtained from ten orectolobid species (Fig. 1) either by opportunistic sampling of commercially harvested animals or by sampling from museum and scientific collections and aquaria. Our sample set includes all described species of orectolobid shark excluding one very recently described species, *Orectolobus reticulatus* (Last et al., 2008) which is known only from museum specimens and from which tissue could not be obtained. Also included are samples that represent an undescribed wobbegong shark known from Indonesia, referred to herein as *Orectolobus cf ornatus* (sensu White et al., 2006). Samples of the blind shark, *Brachaelurus waddi* (Bloch and Schneider, 1801; voucher reference AMS I 41268-005) and the leopard shark, *Stegostoma fasciatum* (Hermann, 1783) were obtained for use as outgroups in the phylogenetic analysis. Tissue was preserved in either 95% ethanol or a salt-saturated solution of 20% DMSO.

### 2.2. Genetic methods

Genomic DNA was extracted using a modified salting out protocol (Sunnucks and Hales, 1996) and data obtained from both the mitochondrial and nuclear genomes. Three mitochondrial DNA (mtDNA) genes were amplified and sequenced: the control region (CR) and the adenosine triphosphatase subunits 6 and 8 (ATPase 6 and ATPase 8). From the nuclear genome we obtained data from the second intron of the ribosomal protein S7 (S72). Details of the reaction and the polymerase chain reaction (PCR) cycling conditions for each locus can be found in Corrigan et al. (2008). Despite substantial optimisation effort, S72 reactions consistently produced multi-banded products such that it was not possible to isolate pure amplification of the second intron. Consequently, the PCR product for each species was cloned using TOPO TA Cloning vectors (Invitrogen), transformed into chemically competent *Escherichia coli* cells and plated on Luria–Bertani agar. Multiple colonies from each cloning reaction (each species) were sequenced until a colony containing the second intron insert could be identified. Purified DNA was sequenced in both directions on an ABI 3730xl genetic analyser (Applied Biosystems) using Big Dye Terminator chemistry according to the manufacturer's directions.

### 2.3. Phylogenetic analyses

Sequence data were aligned in SEQUENCHER v4.1 (Gene Codes Corporation, Ann Arbor, MI). Sequence saturation and biases in base composition or substitution type can obscure phylogenetic signal (Swofford et al., 1996). To assess saturation, independent plots of the number of transitions and transversions versus uncorrected 'p' genetic distance were obtained for each of the mitochondrial genes (CR, ATPase 6 and ATPase 8) and the nuclear intron. A partition-homogeneity test (Farris et al., 1994) implemented in PAUP\* v4.0b10 (Swofford, 1998) was also applied to the mitochondrial data to evaluate whether the three gene regions contained similar phylogenetic signals. There was no significant incongruence between CR and ATPase data ( $p = 0.09$ ) indicating it was appropriate to combine them in a concatenated sequence for phylogenetic analysis.

We employed character-based, distance-based and model-based (Maximum Parsimony, MP; Neighbour-joining, NJ; Maxi-

imum Likelihood, ML and Bayesian inference, BI) methods of phylogenetic analysis. All analyses were conducted on the concatenated mtDNA and nuclear intron data sets independently. A second data set, with in-dels removed was also analysed for the intron data. Analysis based on MP (Farris, 1970) was conducted in PAUP\*. All characters were treated as unordered and unweighted and in-dels were coded as a fifth base. A heuristic search for the most parsimonious trees was conducted with random addition sequence (100 replicates) and tree bisection–reconnection (TBR) branch-swapping. A majority-rule consensus tree was constructed and bootstrap resampling (Felsenstein, 1985) based on 1000 replicates was used to assess support of relationships. For the NJ and ML analyses we used MODELTEST v3.06 (Posada and Crandall, 1998) to estimate the most likely model of sequence evolution for each of the data sets (mtDNA and nuclear intron). Based on maximum likelihood values and the Akaike Information Criterion (AIC, Posada and Buckley, 2004), the TIM+I+G model was selected as the most likely model of sequence evolution for the mtDNA data set ( $-\ln l = 5290.5591$ ,  $AIC = 10597.1182$ ) while the HKY+G ( $-\ln l = 761.4131$ ,  $AIC = 1532.8262$ ) and K80+G ( $-\ln l = 626.5803$ ,  $AIC = 1257.1605$ ) models were selected to best fit the nuclear data sets with and without in-dels included, respectively. Corrected genetic distances and the number of base pair differences between wobbegong species were calculated using PAUP\*. NJ trees were also constructed using distances corrected according to the above model parameters and assessed using 1000 bootstrap replicates. A tree based on ML criteria was also obtained using PAUP\*. Here, we employed heuristic searches again assuming the appropriate models of sequence evolution. Nucleotide frequencies were estimated from both the mtDNA and nuclear data sets. For the mtDNA data, substitution rates were estimated to follow a gamma distribution with shape parameter  $\alpha = 0.64$ . For the nuclear data  $\alpha = 0.63$ . The proportions of invariable sites were set to 0.29 and 0.00 for the mtDNA and nuclear data sets respectively. In both cases, starting branch lengths were obtained using the Rogers–Swofford approximation method and starting trees were obtained via step-wise addition. TBR branch swapping and 100 replicates of random addition of taxa were performed to estimate a tree. Non-parametric bootstrap support for each node was estimated using 100 heuristic bootstrap replicates. Bayesian inference was implemented in MR BAYES v3.1.2 (Huelsenbeck and Ronquist, 2001). This method has advantages over the ML approach as it allows the application of mixed-models to concatenated sequences that are partitioned into separate gene fragments. The best-fit models of sequence evolution were selected for each of the mtDNA fragments (CR and ATPase) and the S72 data independently using MR MODELTEST v2 (Nylander, 2004) based on AIC. The nucleotide substitution models selected were GTR+G for CR and HKY+G for both ATPase and S72. For the mitochondrial analysis the data were divided into two partitions, one representing each gene fragment. The appropriate parameters, derived from MR MODELTEST, were applied to each partition. The S72 was treated separately in an independent analysis. For all analyses, the prior probability of a flat Dirichlet distribution for the substitution rates and stationary nucleotide frequencies was used under the assumption of no prior knowledge. The Bayesian analysis was run using the Metropolis-coupled Markov Chain Monte Carlo (MCMC) algorithm from randomly generated starting trees for 5 million generations with sampling every 1000 generations. Two runs were performed simultaneously in which there were three heated chains and one cold chain, each with a temperature parameter of 0.2. The standard deviation of splits frequencies and the potential scale reduction factor were used as convergence diagnostics to determine that stationarity of the posterior probability distribution had been achieved. The burn-in was set to discard the initial 25% of samples.

#### 2.4. Molecular dating of speciation events

Speciation events were dated using a MCMC approach implemented in the program BEAST v1.4.6. (Drummond and Rambaut, 2007). The fossil record for true wobbegongs is poor (Kemp, 1991; Pledge, 1985; Siverson, 1995) such that a reliable fossil calibration point for our tree could not be obtained. Similarly, a reasonable biogeographic calibration point is not apparent thus, it was not possible to develop a wobbegong specific molecular clock. In light of this, we have based our dating analyses solely on the mitochondrial control region (CR) data and have assumed the available elasmobranch CR molecular clock of Duncan et al. (2006). This clock used divergences between taxa across the Isthmus of Panama to estimate a rate of CR evolution of 0.8% per million years for the hammerhead shark (*Sphyrna lewini*). The S72 data was excluded from dating analyses as it failed to resolve the phylogenetic structure of the group due to possible paralogous sequences in the data set. It was therefore deemed uninformative to include this data in further analyses (see Section 3.3 for further details). The validity of the molecular clock assumption was first assessed by performing a likelihood ratio test (Felsenstein, 1981) using DAMBE (Xia and Xie, 2001). The null hypothesis of a constant evolutionary rate across lineages was rejected by the data ( $p = 0.0008$ ). Therefore, we estimated time to most recent common ancestor ( $T_{mrc}$ ) statistics for the major clades in our tree using a relaxed clock method that allows branch-specific rate variation, drawn from a lognormal distribution (Drummond et al., 2006), while assuming the average mutation rate of Duncan et al. (2006). Tree priors were modelled according to a Yule speciation process and all other priors were set to their default values. The MCMC analyses were run for 60 million generations, and sampled every 100th generation with the first 10% of samples discarded as burn-in. Inspection of the results using TRACER v1.4 (Rambaut and Drummond, 2006) confirmed that stationarity and adequate effective sample sizes had been obtained for all parameters.

### 3. Results

#### 3.1. Sequence characteristics

The concatenated mtDNA data set was comprised of 1412 base pairs per species (587 bp of CR, GenBank Accession Nos. DQ885482, DQ885485, DQ885486, EU888892–EU888899; and 825 bp of ATPase, GenBank Accession Nos. DQ886665, DQ886667, DQ886668, EU910196–EU910205). These haplotypes were defined by 542 variable characters (CR 271/587; ATPase 6 214/669; ATPase 8 57/156), of which 195 were parsimony informative (CR 66/587; ATPase 6 105/669; ATPase 8 24/156). The average transition to transversion ratio (Ti/Tv) was 2.5 and base frequencies were 31.8% T, 23.4% C, 32.5% A and 12.2% G.

The nuclear data set consisted of 149 base pairs per species (GenBank Accession Nos. EU379563–EU379565 and EU910206–EU910214), of which 79 characters were variable and 50 were parsimony informative. The average Ti/Tv was 1.3 and base frequencies were 21.8% T, 26.1% C, 31.5% A and 20.6% G.

No evidence of sequence saturation was observed in the mtDNA data (graphs available from corresponding author upon request). For the mtDNA data, TIM+I+G corrected pairwise sequence divergence among wobbegong species ranged from 18.9% between *O. wardi* and *S. tentaculatus*, to 0.3% between *O. japonicus* and *O. cf ornatus* (Table 1). The average sequence divergence in the ingroup was 6.7%. For the nuclear data, HKY85+G corrected distance ranged from 51.8% between *S. tentaculatus* and *O. parvimaculatus* and 0% between *O. maculatus* and *O. cf ornatus* with average sequence divergence in the ingroup of 26.0% (Table 1).

**Table 1**

Distance matrix of pairwise genetic distance among wobbegong species. Bottom diagonal is (TIM+I+G) corrected genetic distance based on 1412 bp of the mtDNA control region and ATPase 6, 8. Top diagonal is (HKY85+G) corrected genetic distance based on 149 bp of the second intron of the ribosomal protein S7 (S72).

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>Stegostoma fasciatum</i>	–												
2 <i>Brachaelurus waddi</i>	0.713	–											
3 <i>Orectolobus wardi</i>	0.635	0.336	–										
4 <i>Eucrossorhinus dasyopogon</i>	0.549	0.257	0.185	–									
5 <i>Orectolobus hutchinsi</i>	0.540	0.284	0.149	0.072	–								
6 <i>Sutorectus tentaculatus</i>	0.547	0.279	0.189	0.085	0.046	–							
7 <i>Orectolobus parvimaculatus</i>	0.551	0.304	0.167	0.079	0.029	0.055	–						
8 <i>Orectolobus floridus</i>	0.549	0.266	0.184	0.081	0.047	0.013	0.055	–					
9 <i>Orectolobus ornatus</i>	0.648	0.291	0.186	0.083	0.051	0.056	0.057	0.057	–				
10 <i>Orectolobus halei</i>	0.569	0.284	0.163	0.074	0.022	0.045	0.027	0.044	0.045	–			
11 <i>Orectolobus maculatus</i>	0.572	0.285	0.154	0.076	0.018	0.049	0.032	0.047	0.052	0.017	–		
12 <i>Orectolobus japonicus</i>	0.534	0.276	0.147	0.077	0.021	0.046	0.034	0.045	0.050	0.018	0.013	–	
13 <i>Orectolobus cf ornatus</i>	0.534	0.275	0.146	0.073	0.019	0.044	0.032	0.044	0.048	0.014	0.012	0.003	–

### 3.2. Phylogenetic analyses based on mtDNA: systematic implications

All different methods of analyses of the mitochondrial DNA data resulted in largely concordant tree topologies that strongly supported the monophyly of Orectolobidae and several other key relationships. Five major clades were consistently resolved (Fig. 2). These correspond to *O. wardi*, *E. dasyopogon*, *O. ornatus* and a clade consisting of a sister relationship between *O. floridus* and *S. tentaculatus*. The fifth relationship consists of a recently derived clade that includes all remaining wobbegong species. As shown in the fully resolved ML tree (Fig. 2, tree score of 5288.74), *O. ornatus* appears ancestral to the *S. tentaculatus* and *O. floridus* clade and *O. hutchinsi* is sister to *O. parvimaculatus*. *Orectolobus halei*, on the other hand, appears ancestral to a recently derived clade consisting of *O. maculatus*, *O. japonicus* and *O. cf ornatus*.

The resultant topologies of different analytical approaches (NJ, MP, ML and BI) differed only in the level of resolution at two particular points in this tree. Firstly, there was discordance in the placement of *O. ornatus* relative to *O. floridus* and *S. tentaculatus*. Similarly, the relative placements of *O. hutchinsi*, *O. parvimaculatus* and *O. halei* varied slightly across the trees. The discrepancies mentioned above are reflected in the bootstrap values and posterior probabilities displayed in Fig. 2. The MP analysis resulted in three equally parsimonious trees that were 821 steps in length, CI = 0.78, HI = 0.22 and RI = 0.53. Computation of a majority-rule consensus tree resulted in the general tree topology described above. The same applies to results from NJ and BI.

The phylogenetic placements of the three wobbegong genera (*Eucrossorhinus*, *Sutorectus* and *Orectolobus*) are of systematic interest. All methods of analysis indicate that *Orectolobus* is polyphyletic. The monotypic genus *Sutorectus* forms a very well-supported sister relationship with *O. floridus* in a recently derived clade. *Eucrossorhinus*, on the other hand, is placed at a basal position in the radiation of the family. Also of systematic interest is the close relationship (only 0.29% corrected sequence divergence; Table 1) between *O. japonicus* and *O. cf ornatus*, the Indonesian undescribed taxon.

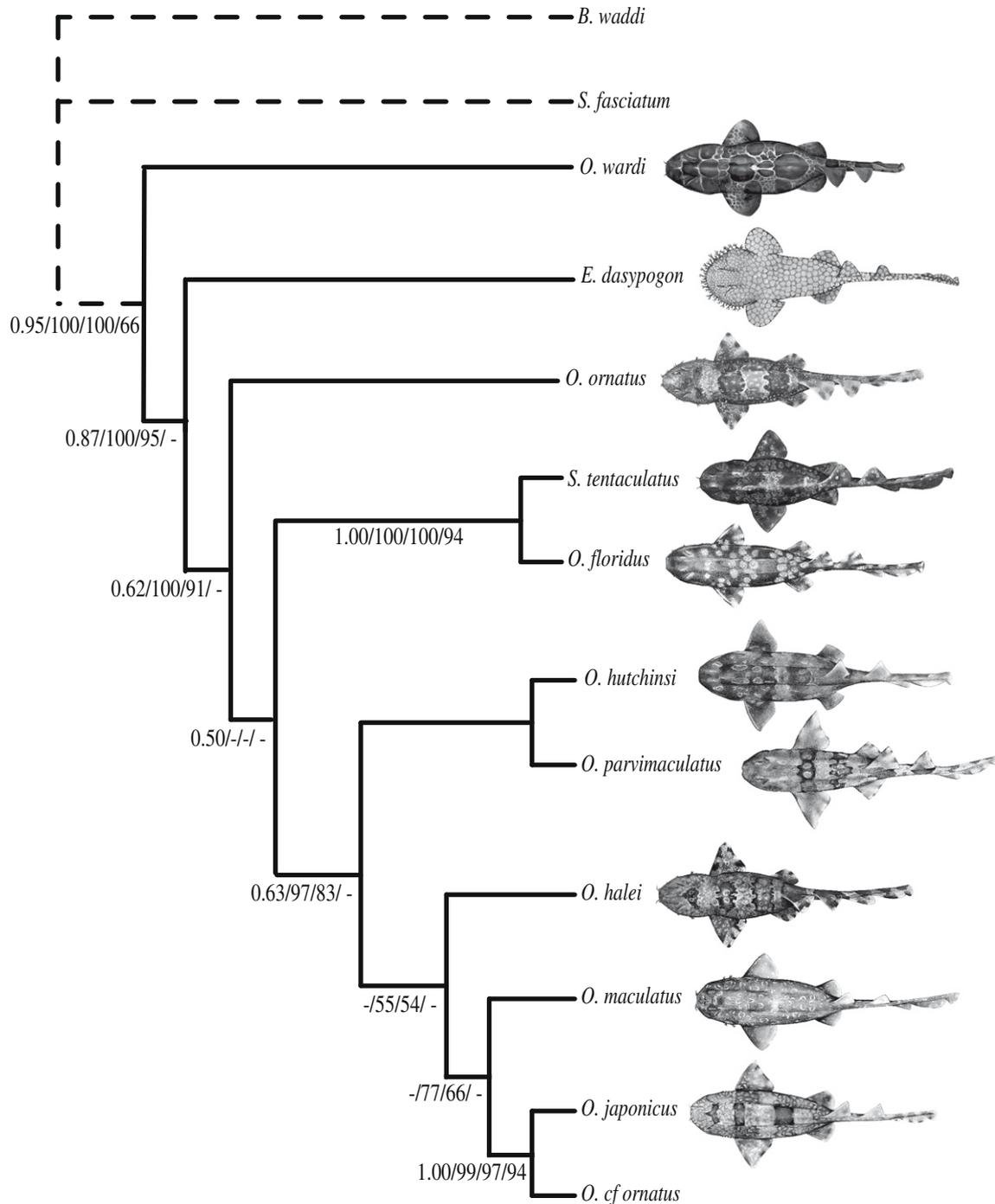
### 3.3. Phylogenetic analyses based on nuclear DNA: problematic signal with S72

The results of nuclear analyses were in conflict with those obtained from mtDNA. The phylogeny was poorly resolved by the nuclear marker and the monophyly of Orectolobidae was not supported (see Supplementary data). In light of this we do not base any of our interpretations on the information obtained from S72. Instead, we discuss the limitations of this marker here in order to caution other researchers against its use for phylogenetic infer-

ence. Development of a nuclear sequence marker for orectolobid sharks was problematic. We selected eight potential nuclear markers from the literature, namely Trop, LDHb, RP40 (Friesen et al., 1999), S71, S72 (Chow and Hazama, 1998), LDHa6 (Stoner et al., 2003), ITS1 and ITS2 (Pank et al., 2001) which include markers that have been used in other elasmobranch fishes, and screened them for their utility in wobbegongs. Despite extensive optimisation effort, the majority of these markers failed to amplify or was monomorphic among species. S72 was the single marker from which we could obtain successful amplification and polymorphic sequence data. We cloned the S72 PCR product in wobbegongs, sequenced colonies, and confirmed its sequence homology with exons 2 and 3 of the ribosomal protein S7 in teleost fish (Benson et al., 2008). Surprisingly though, sequencing of multiple colonies per species revealed several alleles (more than could be accounted for by heterozygosity at this locus) with apparent homology to S72. This was unexpected given that S72 is known to be single copy in teleosts (Chow and Hazama, 1998; Cooke and Beheregaray, 2007). Possibly, pseudo-genes of the ribosomal protein S7 may exist in this group of sharks; alternatively the ribosomal protein is encoded for by multiple loci in elasmobranchs (to the best of our knowledge this marker has not been previously used for elasmobranchs). Evidence of pseudo-genes of ribosomal protein genes is documented for mammals (Annilo et al., 1998). In light of this and despite obtaining some regions of homology across species, we believe that the discordance observed between our mitochondrial and nuclear data sets is due to comparison of paralogous nuclear fragments across species. Determining the true explanation for the observed pattern in our intron data will require further exploration that is not within the scope of this study. Nonetheless, our results are important since they suggest that researchers should be cautious when using S72 (a popular intron in teleosts) to reconstruct relationships in elasmobranch groups. Given that our mitochondrial results lend themselves to a logical biogeographical interpretation and are largely consistent with those obtained from previous morphological studies (discussed below), we base all further interpretations on the mtDNA data.

### 3.4. Estimates of $T_{mrca}$ 's: rapid diversification in wobbegong sharks

The estimated values of  $T_{mrca}$  and their 95% highest posterior densities can be found in Table 2 and are displayed in Fig. 3. The  $T_{mrca}$  of orectolobid sharks suggests that diversification began during the late Miocene, approximately 10.7 million years ago (95% HPD 6.4–15.8 Ma; Table 2, node 1 in Fig. 3). *Orectolobus wardi* emerges as the most basal taxon within the group. This is followed by another basal split during the late Miocene–early Pliocene involving the ancestral lineages of *E. dasyopogon* and that of a clade



**Fig. 2.** Maximum likelihood phylogeny depicting genetic relationships among wobbegongs inferred from the mtDNA ATPase 6, 8 and CR genes. Numbers below branches are posterior probability and bootstrap support values based on BI/NJ/MP/ML trees (5,000,000 generations/1000/1000/100 replicates), respectively. The branches leading to the outgroups, *Stegostoma fasciatum* and *Brachaelurus waddi*, have been shortened for the purposes of illustration (indicated by the dashed line).

that includes *O. ornatus* and a group of recently radiated species. Speciation of all other wobbegong species seems very recent, dated between the very end of the Pliocene and beginning of the Pleistocene. The split between the *O. hutchinsi* and *O. parvimaculatus* clade from that encompassing *O. halei*, *O. maculatus*, *O. japonicus* and *O. cf ornatus* began during the late Pliocene, approximately 2.7 Ma (95% HPD 1.4–4.5 Ma; Table 2, node 5 in Fig. 3). This was followed by a Pliocene (~2 Ma, 95% HPD 0.9–3.3 Ma; Table 2) split involving the ancestral lineage of *O. halei* and other taxa from a recently derived clade (node 6 in Fig. 3). The two most recent splits, one involving *S. tentaculatus* and *O. floridus* and another involving *O. japonicus* and

*O. cf ornatus* were dated at approximately 1 Ma (95% HPD 0.2–2.1 Ma; Table 2, node 9 in Fig. 3) and 400 ka (95% HPD 0.03–1.0 Ma; Table 2, node 8 in Fig. 3), respectively.

#### 4. Discussion

Here we propose a hypothesis of evolutionary relationships for the wobbegong sharks (family Orectolobidae) based on a taxonomically diverse sampling that includes both recently described species and taxa that are new to science. Below we discuss the systematic implications of our findings and propose a biogeo-

**Table 2**

$T_{mrc}$  statistics and 95% lower and upper highest probability densities as calculated in BEAST v1.4.6 using a relaxed clock method that allows branch-specific rate variation, drawn from a lognormal distribution while assuming an average mutation rate of 0.8% per million years after Duncan et al. (2006).

Node number	$T_{mrc}$	95% HPD lower	95% HPD upper
1	10.692	6.406	15.833
2	6.379	3.486	10.524
3	3.852	1.971	6.496
4	2.985	1.790	4.442
5	2.711	1.381	4.529
6	2.038	0.945	3.296
7	1.391	0.556	2.408
8	0.445	3.085E-2	1.022
9	1.008	0.194	2.096

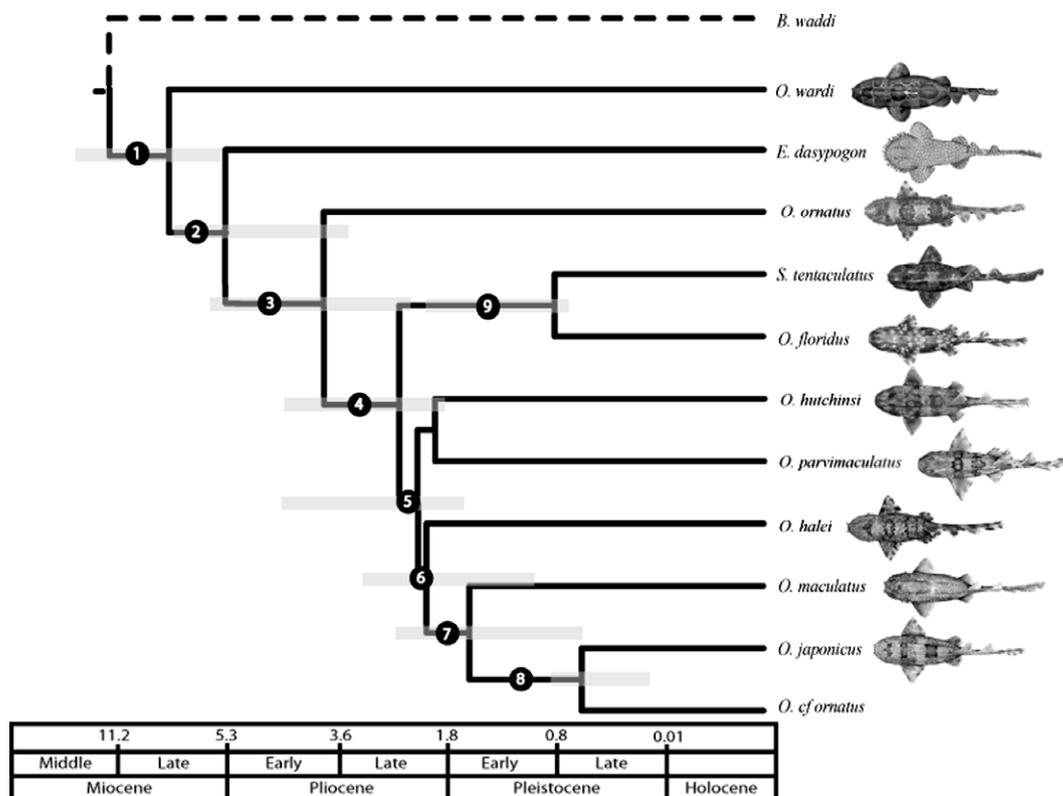
graphic scenario to account for orectolobid diversification in the coastal waters of the Indo-Pacific and Australia. We also comment on strong evidence of a surprisingly recent and rapid radiation detected for wobbegong sharks.

#### 4.1. Molecules vs. morphology: systematic implications

The only minor discordance between our molecular phylogeny and the relationships previously proposed for the orectolobid sharks (Dingerkus, 1986; Goto, 2001), involves the relative placements of *Eucrossorhinus* and *Sutorectus*. Based on morphology, *Sutorectus* is placed ancestral to *Eucrossorhinus* (Dingerkus, 1986; Goto, 2001), while our molecular results suggest the converse. Together with the difficulty inherent in determining the polarity of morphological character state change, this discrepancy is probably

largely governed by the lesser taxonomic coverage of the morphological studies. The work of Goto (2001) reveals six equally probable cladograms in which the placements of *Sutorectus* and *Eucrossorhinus* vary, suggesting limited confidence in the proposed relationships. It is likely that the topology of these morphological trees would change further with the addition of newly described taxa, or with the treatment of additional characters. In our molecular phylogeny however, the placements of *Eucrossorhinus* and *Sutorectus* are fixed, with high posterior probability and bootstrap support (Fig. 2), irrespective of analytical approach. Furthermore, considering wobbegong species distributions, these relative placements lend themselves to a plausible biogeographic interpretation (discussed below). Overall, morphological and molecular phylogenies appear fundamentally consistent, providing strong support for the relationships we propose here.

A monophyletic Orectolobidae, with *O. wardi* as the basal taxon (Fig. 2) is consistently resolved with strong support by both morphological (Goto, 2001) and molecular analyses (this study). Also consistent among both data sets is the problematic nature of the phylogenetic placement of genera. Regan (1908) initially proposed the monotypic genus *Eucrossorhinus* for *Crossorhinus dasygogon* Bleeker, 1867 and Whitley (1939) proposed *Sutorectus* for *Crossorhinus tentaculatus* (Peters, 1864). Since then, each genus has received inconsistent recognition in the literature (summarised in Compagno, 2001) and both have previously been considered synonyms of *Orectolobus* (Applegate, 1974; Goto, 2001; Regan, 1909; Stead, 1963). Although Compagno (2001) describes a number of diagnostic characters for *E. dasygogon* and *S. tentaculatus*, these distinctions are autapomorphies that do not provide useful information under the cladistic concept (Hennig, 1966). This point is illustrated most clearly by the highly supported sister relationship



**Fig. 3.** Chronogram of Orectolobidae showing divergence date estimates with 95% lower and upper highest probability densities designated by the grey shading.  $T_{mrc}$  statistics (as in Table 2) were calculated in BEAST v1.4.6 using a relaxed clock method that allows branch-specific rate variation, drawn from a lognormal distribution while assuming an average mutation rate of 0.8% per million years after Duncan et al. (2006). Branch lengths reflect estimated ages of lineages, with respect to the geological time scale. The branch leading to the outgroup, *Brachaelurus waddi*, has been shortened for the purposes of illustration (indicated by the dashed line).

between *S. tentaculatus* and *O. floridus* (corrected genetic distance = 0.013, Table 1 and Figs. 2 and 3).

In the work of Goto (2001), *Sutorectus* and/or *Eucrossorhinus* cluster with *Orectolobus* in all of the six equally parsimonious trees. *Orectolobus* is also paraphyletic with respect to *Eucrossorhinus* and *Sutorectus* according to our molecular data (Fig. 2). Therefore we reach a similar conclusion to Goto (2001) in that the type genus of the family, *Orectolobus*, could only be considered monophyletic with the inclusion of *S. tentaculatus* and *E. dasyopogon*.

In the interest of obtaining taxonomic stability for the group, the logical recommendation would be a taxonomic revision of the family to include all species within a single monophyletic genus – *Orectolobus*. Alternatively, retaining *Eucrossorhinus* and *Sutorectus* as valid genera would require redefinition of the diagnostic traits of genera, particularly that of *Sutorectus*. Furthermore, if both *Eucrossorhinus* and *Sutorectus* are retained then, given the levels of divergence we describe here and the morphological relationships shown by Goto (2001), *Orectolobus wardi* is also worthy of generic status.

Last et al. (2008) describe three colour morphs of the northern wobbegong. One morph represents the true *O. wardi*, the second morph the recently described species, *O. reticulatus*, and the third an undescribed taxon (referred to herein as *O. cf wardi*). Based on morphological similarity, Last et al. (2008) propose that these three taxa represent a ‘wardi complex’ that also potentially includes *S. tentaculatus* and *O. floridus*. The recency of the description and lack of fresh material prevented the inclusion of either *O. reticulatus* or *O. cf wardi* in our study. Therefore, we cannot comment on either the taxonomic status or phylogenetic placement of these taxa from a molecular perspective. However, the *S. tentaculatus*–*O. floridus* clade resolved by our analyses is clearly divergent from all other wobbegongs (corrected sequence divergence ranges 0.044–0.189; Table 1). In fact, corrected sequence divergence is maximal when comparing *O. wardi* with *S. tentaculatus* (0.189; Table 1). Given these results, *S. tentaculatus* and *O. floridus* should not be considered members of a species complex with *O. wardi*.

Finally, White et al. (2006) proposed *O. cf ornatus* as an undescribed taxon although the features that distinguish it from other wobbegong species were not explored. The biology and distribution of *O. cf ornatus* are little known, but it is thought to be endemic to the Philippines and eastern Indonesia (White et al., 2006). A specific status for *O. cf ornatus* is not supported by our molecular analyses. Corrected sequence divergence between *O. cf ornatus* and sister taxon *O. japonicus* (Figs. 2 and 3) is only 0.3% (Table 1), well within the range of intraspecific variation for other species of wobbegong (Corrigan et al., 2008). Therefore, we suggest that *O. cf ornatus* represents an intraspecific variant of the Japanese wobbegong, *O. japonicus*, which also occurs in the Philippines (Goto, 2008). If conspecificity of *O. cf ornatus* and *O. japonicus* is confirmed by further analyses (e.g. population genetics and morphometrics), these findings represent a range extension for *O. japonicus* into Indonesian waters.

#### 4.2. Wobbegong speciation and biogeography: Indo-Australian origins and recent and rapid diversification

The fossil record for true orectolobid sharks is poor (Siverson, 1995), restricted to an early Miocene recording from Victoria (Kemp, 1991) and an early Pliocene occurrence in South Australia (Pledge, 1985). Lack of good fossil recordings has prevented major inferences regarding the evolutionary history of this group except to suggest that they appeared relatively late and have likely been restricted to the western Pacific (Siverson, 1995). Two possible biogeographic scenarios have been proposed to account for present day species distributions, though neither has been empirically investigated. The first assumes that high Australian diversity and

endemism are indicative of an Australian origin for the group. Alternatively, given that wobbegong ancestors are thought to have been widespread along the Tethyan margin (Cappetta et al., 1993; Maisey, 1984; Musick et al., 2004) it is possible that extant orectolobids are relict in Australian waters (Musick et al., 2004). Our molecular phylogeny indicates that the biogeographic history of the orectolobids is in line with the latter scenario, with signatures of both vicariance and dispersal in the Indo-Australian Archipelago (IAA) followed by rapid diversification in temperate Australian waters.

The IAA is a large marine biogeographical province with exceptional species richness (Briggs, 1999; Renema et al., 2008). Many of the species that occur there are widely distributed such that Australian zoogeography is heavily influenced by Indo-West Pacific faunal elements (Wilson and Allen, 1987). Northern Australian species are considered part of the Indo-West Pacific faunal region and likely derivatives of Tethyan and their descendant Indo-West Pacific fauna. Temperate Australian waters are also thought to house marine fauna of Indo-West Pacific origin, particularly the Australian west coast. The biogeographic history of wobbegongs largely reflects these patterns. Consistent with the limited fossil evidence available for these sharks (Kemp, 1991), our data indicates that orectolobid diversification began during the Miocene. Between approximately 11 and 6 Ma wobbegong ancestors speciated, giving rise to *O. wardi* and *E. dasyopogon* (Fig. 3 and Table 2). Both of these species occur within the IAA (Fig. 1) suggesting a tropical origin for the orectolobid sharks. As has been proposed for other Indo-West Pacific organisms, wobbegong speciation was likely driven by massive geological restructuring within the region during the Miocene (Renema et al., 2008; Williams and Duda, 2008; Wilson and Allen, 1987). Colliding of the Indo-Australian plate with the Eurasian plate (~25 Ma) and the Philippines–Halmahera–New Guinea arc system, followed by collision of the Ontong Java plateau with the Melanesian arc resulted in volcanic activity and uplift throughout the Miocene. New oceans formed, circulation patterns were altered and new coastline developed, causing a massive increase in the geographical complexity of the region and available shallow-water habitat. This provided ample opportunity for vicariance and speciation in marine taxa (Hall, 1998, 2002; Renema et al., 2008). At the onset of wobbegong diversification (Table 2 and Fig. 3), the Indo-Australian plate was still colliding with Timor causing deformation in this area (White, 2006). Coincidentally, the polar ice volume increased dramatically causing drastic global cooling, glacio-eustatic marine regression (Dawson and Dawson, 2006; Knox, 1980; White, 2006) and the emergence of previously inundated islands and landbridges throughout the IAA. These processes may have instigated vicariance-driven range constriction in wobbegong ancestors promoting speciation. Alternatively, diversification of *E. dasyopogon* may have been ecologically driven. Although the biology of *E. dasyopogon* is little known, it has been suggested that this species could be obligate coral reef dwelling, and its complex dermal lobe arrangement useful for camouflage over the topographically complex substrate (Compagno, 2001). Miocene tectonic events also resulted in an increase in carbonate platforms in the IAA and this has been linked to radiations in reef-dependent organisms (Wilson and Rosen, 1998). Therefore, we speculate that the diversification of *E. dasyopogon* was associated with occupation of newly available coral reef habitat and subsequent ecological specialisation, giving rise to its divergent morphological appearance. Determining this hypothesis awaits the collection of more detailed biological and ecological information for this species.

Following a tropical origin, we detected a Pliocene invasion of temperate Australian waters, occurring at approximately 3.9 Ma (Fig. 3 and Table 2). The distribution of east (*O. ornatus*, *O. maculatus* and *O. halei*) and west (*S. tentaculatus*, *O. hutchinsi*, *O. parvima-*

*culatus* and *O. floridus*) coast species within our tree is intriguing as we do not find discrete eastern and western clades (Fig. 2). We propose two independent colonisation events of temperate Australia: one through the northeast coast and another from the northwest, followed by secondary colonisation of eastern Australian waters via southern Australia. Wobbegongs are demersal and occur to approximately 200 m depth (Compagno, 2001; Kyne et al., 2005). As aplacentally viviparous breeders (Huvneers et al., 2007) they give birth to live young and therefore must actively swim in order to disperse. Consequently, dispersal ability and ultimately the colonisation of Australian waters must have been aided by the availability of suitable shallow-water habitat between northern Australia and Indonesia and likely influenced by the direction of ocean currents.

*Orectolobus ornatus*, despite being regionally sympatric with *O. halei* and *O. maculatus* (Sydney–South Stradbroke Island) is represented by an older and divergent lineage that is restricted to eastern Australian waters (Fig. 1). High sea-level prior to 3 Ma (Lambeck et al., 2002; Lisiecki and Raymo, 2005) combined with tectonic change in the IAA caused an outpouring of warm water via the Indonesian Throughflow (Gordon and Fine, 1996) creating a shallow-water passageway between Indonesia and Australia. We propose that this, together with the poleward flow of the East Australian Current (EAC), a western boundary current that flows along the continental shelf of eastern Australia, penetrating down to 2000 m depth; Ridgway and Dunn, 2003; Tilburg et al., 2001) would have allowed colonisation of northeastern Australia during the Pliocene. Following this colonisation event, the late Pliocene–Pleistocene onset of glacial cycling and associated marine regression would have resulted in the emergence of the Gulf of Carpentaria landbridge, a major biogeographic barrier in northern Australia. This connection between the Australian mainland and Papua New Guinea has been linked to vicariance mediated genetic structuring in a number of taxa (Chenoweth et al., 1998; Gopurenko and Hughes, 2002; Ovenden et al., 2002; Wilson and Allen, 1987). Similarly, the emergence of the landbridge probably constricted the range of wobbegongs allowing evolution in isolation within northeastern Australia and eventually speciation to give rise to the divergent *O. ornatus* lineage. The distribution of *O. ornatus* becomes sparse south of Port Stephens, New South Wales (NSW) where the warm waters of the Coral Sea are replaced by the cooler waters of the Tasman Sea (Tilburg et al., 2001), suggesting that the range of this species is also restricted by a requirement for warmer sea temperatures.

The remaining wobbegong species radiated more recently, beginning in the late Pliocene. We hypothesise that this radiation originated from a separate northwestern colonisation event and occurred predominantly within western Australian waters. The westward movement of the South Equatorial Current through Torres Strait and the Arafura and Timor Seas (Gordon and Fine, 1996), followed by its branching into the poleward flowing Leeuwin Current (the dominant ocean surface current in Western Australia which penetrates to 400 m depth; Cresswell, 1996; Smith et al., 1991) would have allowed colonisation of northwestern Australian and then temperate Australian waters. This hypothesis is consistent with the suggestion by Wilson and Allen (1987) that the southeastward pumping of warm water by the Leeuwin current from the Miocene–Pleistocene is responsible for the maintenance of two biogeographical provinces, comprised mostly of Indo–West Pacific derived fauna, that extend from the northwestern shelf of Australia through to the Great Australian Bight. *Orectolobus halei* and *O. maculatus* are sympatric within the east coast of Australia but are also distributed throughout southern and southwestern Australian waters (Fig. 1). Therefore, both species are also apparently regionally sympatric with *O. hutchinsi* and *O. parvimaclatus*, with which *O. halei* forms a close relationship (Fig. 2). This pattern

suggests that speciation has occurred during the late Pliocene–early Pleistocene (Fig. 3 and Table 2) within the temperate west coast, followed by range expansion of *O. halei* and *O. maculatus* into eastern Australian waters via the Great Australian Bight, crossing the Bassian Isthmus during intermittent periods of high sea-level (Lambeck and Chappell, 2001; Lisiecki and Raymo, 2005). This direction of movement was probably supported by the intensified Leeuwin current during interglacial periods (McGowran et al., 1997; Wilson and Allen, 1987) and flooding of the Bassian Isthmus primarily from the west to form a semi-enclosed bay before complete inundation by encroaching sea-level (Lambeck and Chappell, 2001; Unmack, 2001). Such a scenario accounts for the occurrence and geographic distributions of co-distributed yet phylogenetically divergent lineages of *O. ornatus*, *O. halei* and *O. maculatus*. Furthermore, this hypothesis is supported by a larger comparative phylogeographic data set that shows signal of a north–south colonisation pattern for *O. ornatus* and a western origin for *O. halei* (Corrigan et al., 2008)

Due largely to tectonically driven vicariant events (Williams and Duda, 2008), the majority of derivatives of Indo–West Pacific shallow-water marine fauna exhibit maximum diversity and geographic distribution in the tropics (Briggs, 1999; Wilson and Allen, 1987). The marine fauna of temperate Australia is typically less diverse and exhibits high species endemism (Wilson and Allen, 1987). It is therefore intriguing that while being highly endemic, wobbegongs are most speciose in the temperate regions of Australia (Wilson and Allen, 1987) and that the radiation began only recently during late Pliocene–Pleistocene (Fig. 3 and Table 2). This pattern was surprising given the general perception that sharks represent a relatively ancient group of fish (Grogan and Lund, 2004) that accumulate mutations slowly in the mtDNA (Martin et al., 1992; Martin, 1995). However, similar Pleistocene radiations in temperate Australian teleost families have been attributed to reduced competition and a greater opportunity for niche specialisation as a result of lower species richness in cooler waters (Wilson and Allen, 1987). Furthermore, the Pleistocene was marked by more than 60 glacial–interglacial epoch (Lambeck and Chappell, 2001; Lisiecki and Raymo, 2005). Regressed sea level (between 100 and 210 m below current levels) during glacial periods resulted in cooler sea temperatures, cold water upwellings over the continental slope, the emergence of the Bassian Isthmus (Gill, 1970; Wells and Okada, 1996), ceasing of the flow of the Leeuwin current (McGowran et al., 1997; Wells and Okada, 1996; Wilson and Allen, 1987) and northward movement of the Sub-tropical convergence zone (Knox, 1980; Wilson and Allen, 1987). During periods of marine transgression however, Bass Strait was flooded and the Leeuwin current recommenced flow, allowing marine organisms to disperse into the Great Australian Bight once more. The pulsing action of the Leeuwin current throughout the Pleistocene, together with the shifting position of the warm-water/cold-water boundary and oscillation in the presence of the Tasmanian land bridge, is thought to have promoted repeated north–south fluctuations in species ranges (Knox, 1980; Wilson and Allen, 1987), causing population subdivision in response to glacial–interglacial cycles and initiating allopatric speciation within temperate Australia (Wilson and Allen, 1987). The geographic distributions of wobbegong species reflect this scenario. Recolonisation of Indo–Pacific waters indicated in our phylogeny by the recently derived *O. japonicus* lineage (Figs. 1–3), was possibly the result of northerly retreat of temperate wobbegong species in response to Pleistocene global-cooling and subsequent isolation through the emergence of the Gulf of Carpentaria landbridge. *Sutorectus tentaculatus*, *O. halei* and *O. maculatus* are distributed throughout southwestern Australia and are regionally sympatric with *O. hutchinsi*, and microendemics *O. floridus* and *O. parvimaclatus* (Fig. 1), indicating that allopatric speciation followed by range expansion is the most likely

explanation for the high diversity and overlapping ranges of closely related wobbegong species on the western Australian coast. However, speciation in the absence of geographical isolation, perhaps driven by ecological adaptation to novel environments or behavioural specialisation is another possibility, particularly in the case of recently diverged *S. tentaculatus* and *O. floridus* (Table 2 and Fig. 3). The range of *O. floridus* is completely overlapping with *S. tentaculatus* throughout the region Geraldton to Augusta, Western Australia. *Sutorectus tentaculatus* is commonly recorded in the catch of commercial gill-net fisherman while *O. floridus* occurs less frequently. Although highly speculative given the recency of the description of *O. floridus* and hence lack of available biological information, this separation may indicate a preference for alternative habitats by these species. In this case, fine-scale niche partitioning could be responsible for maintaining this relationship. Investigating this further is dependent on the collection of biological and ecological information for western Australian species.

Finally, it is surprising that we do not find signal of rapid diversification of orectolobid sharks during the Miocene as shown for other shallow-water marine taxa that originated in the Indo-West Pacific (Williams and Duda, 2008). In addition to the absence of *O. reticulatus* from our study it is possible that other undescribed taxa exist in Northern Australia (Last et al., 2008), Indonesia (White et al., 2006), Borneo (Manjaji, 2002), Japan and the Philippines (Compagno et al., 2005). Considering the tropical locations of these potentially undescribed taxa we can speculate that they would be placed basal within our phylogeny pushing the onset of wobbegong diversification back to the early–middle Miocene. That would also have produced an older radiation in the group (this time of a Miocene age) in response to tectonic activity within the Indo-West Pacific. We should also note here two limitations with our biogeographic interpretation: it relies on a single mitochondrial gene for dating analyses and lacks a wobbegong specific mutation rate. The possibility that introgression or hybridisation may confound the signal in recently derived but sympatric wobbegong species is worthy of consideration. A large scale population-level analysis of both mtDNA and nuclear AFLP (Amplified Fragment Length Polymorphism) markers for *O. halei*, *O. ornatus* and *O. maculatus* (Corrigan et al., 2008) did not show any evidence for hybridisation between these three species. Similar levels of mtDNA divergence were also obtained between other recently derived sister species in the data set (e.g. *S. tentaculatus* and *O. floridus*). Therefore, we are confident that the morphologically distinct species included in our analysis represent true biological units and that mtDNA phylogenetic patterns have not been confounded by introgression or hybridisation events. Adoption of the commonly used mutation rate of 2%/Ma for mtDNA coding regions in bony fishes (Bowen et al., 2001) would translate into an even younger origin (Pliocene age) for the group and an extremely recent radiation. We regard this as unrealistic given the slow mutation rates documented for sharks (Martin et al., 1992) and the fact that the fossil record documents the presence of true orectolobids from at least the Miocene (Kemp, 1991). If anything, observed levels of intraspecific variation suggest that the wobbegong mutation rate is probably slower than the rate we adopt here. Halving the rate to 0.4%/Ma, as was proposed for blacktip sharks (Keeney and Heist, 2006), would effectively double wobbegong divergence times. Even so, this very conservative rate still places wobbegong diversification within the Miocene–Pleistocene timeframe and remains in line with the biogeographic hypothesis we propose here.

## Acknowledgments

We recognise Macquarie University (Research Innovation Fund Grant MQ A006162 to L.B.B.), Birds Eye and the Seaworld Research and Rescue Foundation (Grant SR/9/2004 to L.B.B.) for financial

support. Shannon Corrigan was supported by an Australian Postgraduate Award. We are extremely grateful to Charlie Huvneers, Justin Chidlow, William White, Peter Kyne, Fraser McGregor, Joanna Stead and Christine Dudgeon for provision of tissue samples of various Oreotolobid and outgroup species. Additional samples were also obtained from Omaha Henry Doorly Zoo, Oceanworld Manly, Mark McGrouther and the Australian Museum and CSIRO Hobart (see voucher details within manuscript). We also acknowledge New South Wales and Western Australia commercial fishermen for their cooperation and support during sample collection. We are grateful to Lindsay Marshall of Stickfigurefish for the artwork presented here ([www.stickfigurefish.com.au](http://www.stickfigurefish.com.au)). Tomoaki Goto, Charlie Huvneers and William White provided useful discussion regarding systematic interpretations and we also acknowledge Charlie Huvneers and Anthony Dosseto for their comments on an earlier version of the manuscript.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymp.2009.03.007.

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