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Genetic and reproductive evidence for two species of ornate wobbegong shark *Orectolobus* spp. on the Australian east coast

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This study reports on evidence for reproductive isolation among *Orectolobus ornatus* and *Orectolobus halei*, two previously cryptic and recently redescribed species of wobbegong shark (Orectolobiformes: Orectolobidae) from the east coast of Australia. The evidence is based on disparity in size at sexual maturity, diagnostic nuclear and mitochondrial DNA variants, and marked phylogenetic divergence. Plots of total length (L_T) and maturity for the two species were non-overlapping and illustrative of statistically significant size dimorphism. Genetic analyses and phylogenetic reconstruction did not provide indication of hybridization between *O. ornatus* and *O. halei*. In fact, sequence divergence between them was higher than in comparisons with another congeneric and largely co-distributed wobbegong species (*Orectolobus maculatus*). The assumption of a molecular clock revealed that the two species have evolved in isolation for *c.* 3·9 million years. These results challenge a paradigm often mentioned in the biodiversity literature that most cryptic species are the product of recent speciation events and will contribute to the development of effective management strategies for wobbegong sharks.

Key words: conservation; elasmobranch; phylogeography; reproductive isolation; taxonomy.

INTRODUCTION

The identification of unrecognized or cryptic species is an important component of biological and evolutionary research (Claridge *et al.*, 1997). There is widespread disagreement, however, on the criteria used to define species (Hey, 2001), which may impede the identification of taxonomic boundaries. Divergence in morphological traits has formed the basis of traditional systematics, but this approach has been criticized due to its susceptibility to trait homoplasy (Baker & Gatesy, 2002), and the potential for morphologically cryptic species (Mayr & Ashlock, 1991). Thus, accurately interpreting morphological characteristics in a taxonomic sense requires extremely close evaluation (Knowlton, 1993). The advent of molecular techniques, however, has resulted in some movement away from purely morphological systematics (Hey, 2001).

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For sexually reproducing organisms at least, the need to demonstrate reproductive isolation among putative species has become central to defining taxonomic boundaries (Lee, 2003; Wiens, 2004).

Information from mitochondrial and nuclear DNA divergence and variation in reproductive traits can identify reproductively isolated species, elucidate taxonomic confusion or strengthen morphology-based taxonomy (Avise & Walker, 1999). Molecular data are used increasingly to identify marine invertebrate and teleost species (Knowlton, 1993; deVargas *et al.*, 1999; Beheregaray & Sunnucks, 2001). In comparison, molecular approaches have been used to distinguish cryptic species for few elasmobranch groups: namely, angel sharks *Squatina* spp. (Sole-Cava & Levy, 1987), thresher sharks *Alopias* spp. (Eitner, 1995), hound sharks *Mustelus* spp. (Heemstra, 1997; Gardner & Ward, 2002), shovelnose guitarfish *Rhinobatos* spp. (Sandoval-Castillo *et al.*, 2004), hammerhead sharks *Sphyrna* spp. (Quattro *et al.*, 2006) and catsharks *Galeus* spp. (Castilho *et al.*, 2007).

Wobbegong sharks are medium to large demersal sharks that are endemic to coastal waters from Australia to Japan (Compagno *et al.*, 2005). They inhabit a variety of substrata from topographically complex rocky and coral reefs to open sandy flats (Carraro & Gladstone, 2006). They are highly sedentary ambush predators, often concealed by their ornamented skin pattern and colouration (Compagno *et al.*, 2005). Two species, *Orectolobus maculatus* (Bonnaterre) (the spotted wobbegong) and *Orectolobus ornatus* (De Vis) (the ornate wobbegong), have historically been recognized off eastern Australia. Observations of substantial size dimorphism in sexually mature *O. ornatus*, however, suggested the existence of a third species in this region. The cryptic morphotype has the appearance of a dwarf variant of *O. ornatus* (Fig. 1). Whitley (1940) distinguished two sub-species of ornate wobbegong shark, *O. ornatus halei* (the large morphotype) from southern Australia and *O. ornatus ornatus* (the dwarf morphotype) from the north-east. Later, lack of research material resulted in synonymy of the two sub-species as *O. ornatus* (Last & Stevens, 1994). Although taxonomic uncertainty regarding

FIG. 1. Dimorphism in sexually mature male *Orectolobus halei* (left, total length L_T = 1796 mm) and *Orectolobus ornatus* (right, L_T = 895 mm) from the east coast of Australia.

the conspecific status of the dwarf and large ornate morphotypes is mentioned in the literature (Compagno *et al.*, 2005), their inclusion as a single taxon persisted until recently. Huveneers (2006) redescribed the large and dwarf ornate morphotypes based on morphomeristic characters. The two morphotypes differ in several traits, such as maximum length, number of precaudal vertebrae, number of spiral valve whorls, number of dermal lobes, presence of supraorbital knobs and position of the pelvic fin. The study by Huveneers (2006) culminated in the redescription of the dwarf morphotype as *O. ornatus* with elevation of the large ornate morph to species level as *O. halei*.

Wobbegongs are commercially harvested in the New South Wales trap and line fishery for their marketable flesh. Approximately 122 000 t of wobbegong was recorded commercially landed in 1990–1991. By 1999–2000, commercial landings had declined by >60% (New South Wales Department of Primary Industries, unpubl. data). It is unclear whether changes in fishing effort have contributed to this decline, as catch composition and effort records are inconsistent and not species specific. As wobbegongs exhibit K-selected life-history traits, they have limited capacity to rebound from anthropogenically generated population declines (Holden, 1974; Smith *et al.*, 1998; Walker, 1998) and this has prompted conservation concern for these fishes. The IUCN classified the spotted and ornate wobbegongs as 'Vulnerable' in New South Wales and 'Near-Threatened' globally (Pollard *et al.*, 2003). Despite the recognized threat, species-specific management strategies for the commercial harvest of wobbegongs are currently non-existent. The development of such strategies is dependent on comprehensively addressing the possibility of unidentified species in eastern Australia.

Congruence between morphological and molecular data provides sound evidence that a species boundary has been accurately identified (Baker & Gatesy, 2002). In practice, however, a strictly morphological approach is often adopted and reproductive isolation among divergent taxa is assumed but not empirically tested (Turner, 1999). This study comprises a comparative survey of wobbegong sharks from eastern Australia based on mitochondrial and nuclear DNA divergence, and reproductive data. The data are used to explore the extent of reproductive isolation among two redescribed species of wobbegongs and to assess the recently revised taxonomy.

MATERIALS AND METHODS

REPRODUCTIVE SAMPLE COLLECTION

Total length (L_T) and state of maturity were recorded for 561 fishes (Table I) from three locations in New South Wales on the east coast of Australia: Nambucca Heads

TABLE I. Sample size and sex of individuals used for reproductive analyses of the three wobbegong species. Fishes were sampled from NA, PS and SY (see Fig. 2)

	Orectolobus ornatus	*Orectolobus halei*	*Orectolobus maculatus*	Total
Male	101	81	81	263
Female	125	104	69	298
Total	226	185	150	561

(NA), Port Stephens (PS) and Sydney (SY) (Fig. 2). All samples were obtained opportunistically from commercial fishermen and included male and female representatives of the three species (*O. ornatus*, *O. halei* and *O. maculatus*; Table I). The L_T was measured from the snout to the tip of the stretched caudal fin. Males were determined to be immature, maturing or mature by assessing clasper calcification (Walker, 2005). Females were considered immature (state of maturity 1) when uteri were indistinguishable from the isthmus with no discernable follicles in the ovaries, maturing (state of maturity 2) when uteri were thin but distinguishable from the oviducts and had macroscopically visible white follicles 1–8 mm diameter within the ovary and mature (state of maturity 3) when the uteri wall had thickened along its entire length and when follicles were macroscopically visible within the ovaries (modified from Walker, 2005).

GENETIC SAMPLE COLLECTION

Seven locations were sampled for genetic analyses: Stradbroke Island (SI) in Queensland, Nambucca Heads (NA), Port Stephens (PS), Sydney (SY) and Eden (ED) in New South Wales, Gulf St Vincent (SA) in South Australia and Augusta (WA) in Western Australia. Muscle tissue samples were taken from two specimens of each species at each site (Fig. 2), with the exception of SA where only one individual was available. One sample of the brown-banded bamboo shark, *Chiloscyllium punctatum* Müller & Henle, was obtained for use as the outgroup in the phylogenetic analysis. Tissue was preserved in either 95% ethanol or a salt-saturated solution of 20% dimethyl sulphoxide (DMSO).

GENETIC METHODS

Genomic DNA was extracted using a modified salting-out protocol (Sunnucks & Hales, 1996). Data were obtained from three mtDNA genes *via* amplification and sequencing of the control region (CR) and the adenosine triphosphatase subunits six

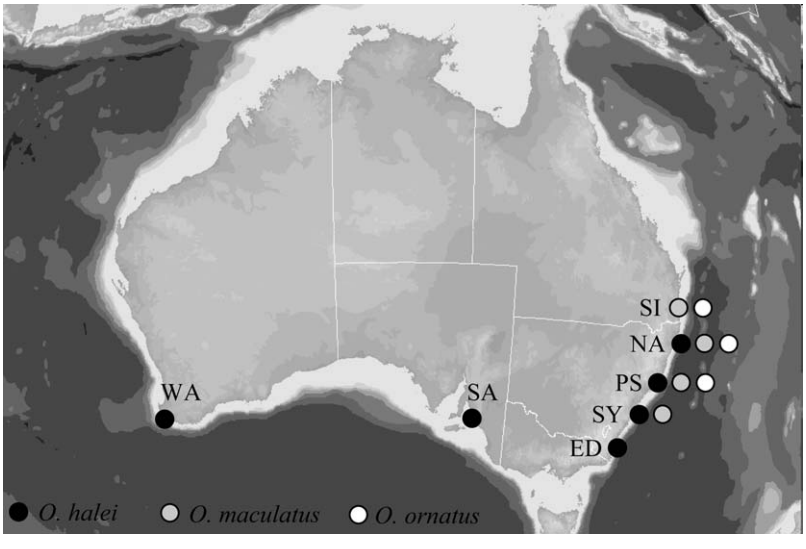

FIG. 2. Location of sampling sites and the species sampled at each site: Stradbroke Island (SI), Nambucca Heads (NA), Port Stephens (PS), Sydney (SY) and Eden (ED) in New South Wales, Gulf St Vincent in South Australia (SA) and Augusta in Western Australia (WA). Two specimens of each morphotype from each site were used for genetic analyses. Reproductive data were collected from a larger sample ($n = 561$) from NA, PS and SY.

and eight (ATPase). Control region fragments were amplified using primers MtGN-F and MtGN-R (Stow *et al.*, 2006). Each 40 μl reaction contained 0·5 μM primers, 2·5 mM $MgCl_2$, 200 μM each dNTP, 10× buffer (10 mM Tris–HCl pH 8·3, 50 mM KCl, 0·1% Triton X-100), 1·5 U Taq (Qiagen, Valencia, CA, U.S.A.) and 50–100 ng template DNA. The polymerase chain reaction (PCR) cycling programme consisted of initial denaturation at 94° C for 5 min followed by a 'touchdown' process of 94° C for 30 s, annealing temperature for 30 s, and 72° C for 60 s. Annealing temperatures of the touchdowns decreased from 59 to 51° C with -2° C cycle^{-1} before stabilizing at 51° C for 31 cycles. This was followed by a final extension step of 72° C for 5 min. ATPase fragments were amplified using primers ATP8.2 and CO3.2 (Bermingham & Martin, 1998). Each 40 μl reaction contained 1 μM primers, 2·5 mM $MgCl_2$, 200 μM each dNTP, 10× buffer (10 mM Tris–HCl pH 8·3, 50 mM KCl, 0·1% Triton X-100), 1·5 U Taq (Qiagen) and 50–100 ng template DNA. A 61–53° C touchdown cycling programme with -2° C cycle^{-1} was used here, consisting of denaturation at 94° C for 5 min followed by 94° C for 30 s, annealing temperature for 30 s, and 72° C for 60 s. Twenty-eight cycles were performed at 53° C, followed by a final extension step of 72° C for 5 min. All PCR products were separated using 2% TAE agarose gel electrophoresis, excised from the gel and purified using an ULTRA CLEAN 15 DNA purification kit (Mo Bio Laboratories, West Carlsbad, CA, U.S.A.). Purified DNA was sequenced in both directions using Big Dye Terminator chemistry and electrophoresed on an ABI 377 genetic analyser (Applied Biosystems, Foster City, CA, U.S.A.).

Potential biases that may arise through exclusive reliance on the mtDNA genome were eliminated by comparing results from mtDNA data with data obtained from two nuclear microsatellite markers and sequence data from the second intron of the ribosomal protein S7 (S72). Microsatellite loci originated from a library that is currently under development for *Orectolobus*. The library was constructed using a modified enrichment method that selectively amplifies microsatellite repeats annealed to biotinylated probes using a magnetic field (Beheregaray *et al.*, 2004). Samples from each wobbegong species (10 *O. ornatus*, 10 *O. halei* and seven *O. maculatus*) were genotyped at the loci *O65* and *O78* (primer sequences available from corresponding author). Microsatellites were amplified in 10 μl radiolabelled PCR reactions containing 1 μM primers, 1 mM $MgCl_2$, 200 μM dCTP, dGTP, dTTP and 20 μM dATP, 0·05–0·08 μl [α-^{33}P] dATP at 1000 Ci mmol^{-1}, 10× buffer (10 mM Tris–HCl pH 8·3, 50 mM KCl, 0·1% Triton X-100), 1·5 U Taq (Qiagen) and 50–100 ng template DNA. PCR cycling conditions consisted of a 3 min denaturation at 94° C, followed by 30 cycles of 94° C for 30 s, 30 s annealing at 60 and 65° C for *O65* and *O78*, respectively, and 72° C for 60 s. Final extension occurred at 72° C for 4 min. PCR products were separated using 6% polyacrylamide gel electrophoresis and visualized autoradiographically. Alleles were identified by comparison with a size standard marker.

The S72 intron was amplified for one representative of each species in a 40 μl reaction containing 1·2 μM primers (Chow & Hazama, 1998), 2·5 mM $MgCl_2$, 200 μM each dNTP, 10× buffer (10 mM Tris–HCl pH 8·3, 50 mM KCl, 0·1% Triton X-100), 1·5 U Taq (Qiagen) and 50–100 ng template DNA. The PCR cycling programme consisted of initial denaturation at 94° C for 5 min followed by 94° C for 30 s, 48° C for 30 s and 72° C for 45 s. This was followed by a final extension step of 72° C for 4 min. As this reaction produced a multi-banded PCR product, it was not possible to isolate pure amplification of the second intron. Thus, the PCR product was cloned using TOPO TA Cloning vectors (Invitrogen, Carlsbad, CA, U.S.A.), transformed into chemically competent *Escherichia coli* cells and plated on Luria-Bertani agar. Five colonies from each cloning reaction were sequenced to identify a colony that contained the second intron insert.

DATA ANALYSIS

Morphometric data were highly skewed and failed to meet assumptions of homogeneity of variance and normality despite multiple transformations. Therefore, comparisons of size at sexual maturity between the three species were made using non-parametric tests (Siegel, 1956).

MtDNA sequences were aligned in SEQUENCHER 4.1 (Gene Codes Corporation, Ann Arbor, MI, U.S.A.). MODELTEST 3.06 (Posada & Crandall, 1998) was used to estimate the most likely model of sequence evolution for the mtDNA data set. Maximum likelihood values for different models were obtained and assessed using the Akaike information criterion (AIC). Corrected genetic distances and the number of base-pair differences were calculated between wobbegong haplotypes using PAUP* 4B10 (Swofford, 1998). Phylogenetic analysis based on maximum parsimony (MP; Farris, 1970) and neighbour-joining (NJ; Saitou & Nei, 1987) methods was also implemented in PAUP*. Analyses were conducted using branch-and-bound searches using *C. punctatum* as an outgroup. Bootstrap resampling (Felsenstein, 1985) based on 1000 replicates was used to test the support of the resolved relationships. The genealogical relationships among wobbegong haplotypes were depicted by a network based on the statistical parsimony method of Templeton *et al.* (1992) as implemented in TCS 1.21 (Clement *et al.*, 2000). TCS collapses sequence data into haplotypes and calculates the maximum number of substitutions required to link all haplotypes parsimoniously with 95% confidence.

RESULTS

VARIATION IN REPRODUCTIVE CHARACTERS AMONG *ORECTOLOBUS* SPP.

Marked and non-overlapping differences in morphology of reproductive characters can be observed among the three species by plotting L_T and clasper length (L_C) for each maturity class in males (Fig. 3) and against state of maturity for females (Fig. 4). Statistically significant dimorphism in size at sexual maturity was detected among the three species (Kruskall–Wallis, d.f. = 2, $P < 0{\cdot}001$). *Orectolobus ornatus* was sexually mature at the smallest size, followed by *O. maculatus* and then *O. halei*. Female *O. ornatus* mature between 795 and 864 mm, males between 796 and 830 mm. Female *O. halei* mature between 1605 and 1871 mm, and males between 1600 and 1684 mm. *Orectolobus maculatus* females mature between 1206 and 1380 mm, and males between 1194 and 1308 mm (Figs 3 and 4 and Table II).

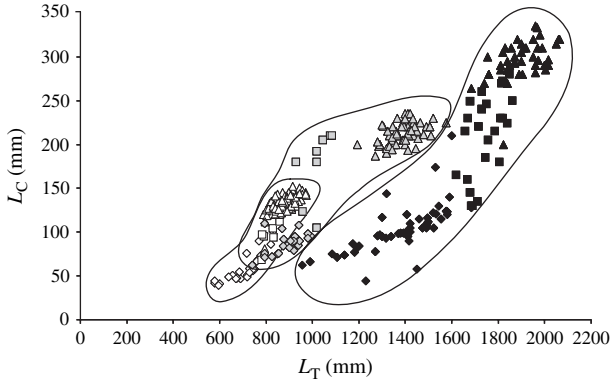

FIG. 3. The relationship between total length (L_T) and clasper length (L_C) of immature (◇, ◈, ◆), maturing (□, ▩, ■) and mature (△, ◬, ▲) male wobbegong sharks (sample sizes as in Table I): *Orectolobus ornatus* (◇, □, △), *Orectolobus maculatus* (◈, ▩, ◬) and *Orectolobus halei* (◆, ■, ▲).

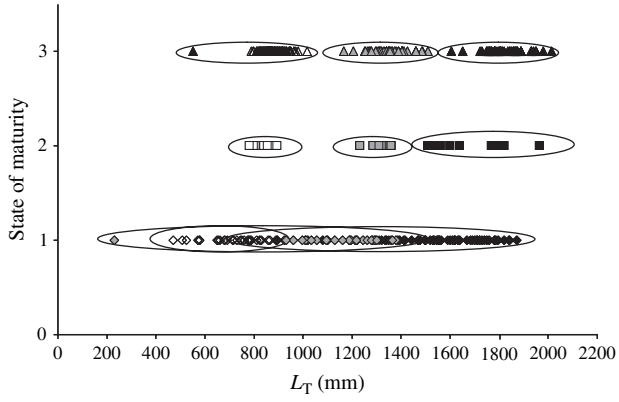

FIG. 4. The relationship between total length (L_T) and state of maturity for immature (◇, ◈, ◆) maturing (□, ▩, ■) and mature (△, ◬, ▲) female wobbegong sharks (sample sizes as in Table I): *Orectolobus ornatus* (◇, □, △), *Orectolobus maculatus* (◈, ▩, ◬) and *Orectolobus halei* (◆, ■, ▲).

GENETIC VARIATION AMONG *ORECTOLOBUS* SPP.

Mitochondrial ATPase and CR haplotypes were resolved for six *O. ornatus*, 12 *O. halei* and eight *O. maculatus* individuals as well as one representative of *C. punctatum*. The mtDNA data set is represented by 1398 bp (574 bp of CR and 824 bp of ATPase). Seven CR (GenBank accession # DQ885480 – DQ885486) and five ATPase (GenBank accession # DQ886665 – DQ886669) haplotypes were identified within *Orectolobus*, corresponding to eight haplotypes in the combined mtDNA data set. These haplotypes were defined by 72 polymorphic sites (CR 27/574; ATPase 45/824), of which 71 were parsimony informative (CR 26/574; ATPase 45/824). The AIC used by MODELTEST selected the TrN + I model as the most likely model of sequence evolution for the mtDNA data set ($-\ln l = 2870{\cdot}6030$, AIC = 5751·2061). This model assumes variable base and transition frequencies and equal transversion frequencies. TrN + I pair-wise sequence divergence between species ranged from 1·7% between *O. maculatus* and *O. halei* to 4·8% between *O. ornatus* and *O. halei* (Table III). Sequence divergence was highest between the two cryptic species, *O. ornatus* and *O. halei* (4·2–4·8%; Table III).

TABLE II. Size dimorphism among eastern Australian wobbegongs. Mean ± S.D. total length (L_T) is given for samples of immature, maturing and mature sharks of each of the three species

		L_T (mm)		
Morphotype	*n*	Immature	Maturing	Mature
Orectolobus ornatus	226	762 ± 11	830 ± 14	875 ± 03
Orectolobus halei	185	1508 ± 20	1674 ± 30	1851 ± 11
Orectolobus maculatus	150	1142 ± 16	1300 ± 12	1370 ± 08

TABLE III. Distance matrix of pair-wise corrected (TrN) genetic distance (below diagonal) and total character difference (above diagonal) among eastern Australian *Orectolobus* spp. haplotypes. Differences are based on 1398 bp of the mtDNA ATPase 6,8 and control region. Population codes are as in Fig. 2. Haplotype numbers correspond to Fig. 6

		1	2	3	4	5	6	7	8
1	*Orectolobus ornatus* – NA, PS	—	2	54	53	55	56	57	61
2	*O. ornatus* – SI	0·001	—	52	51	53	54	55	59
3	*Orectolobus halei* – PS, SY	0·045	0·043	—	1	1	4	5	23
4	*O. halei* – NA	0·044	0·042	0·001	—	2	5	6	22
5	*O. halei* – NA, ED	0·046	0·044	0·001	0·001	—	3	4	22
6	*O. halei* – SA, WA	0·047	0·045	0·003	0·004	0·002	—	1	23
7	*O. halei* – WA	0·048	0·046	0·004	0·004	0·003	0·001	—	24
8	*Orectolobus maculatus* – SI, NA, PS, SY	0·051	0·049	0·017	0·017	0·017	0·017	0·018	—

Both methods of phylogenetic reconstruction resulted in identical tree topologies. Interestingly, these reconstructions place *O. ornatus* in a distinct clade to the sister relationship between *O. halei* and *O. maculatus* (Fig. 5). This topology was resolved with high bootstrap support (>95%) and is strongly supported by the network analysis conducted with the statistical parsimony approach. The mtDNA haplotype network reveals three groups of haplotypes, corresponding to each of the three species. Sequence differences between *O. ornatus* and either *O. halei* or *O. maculatus* are around twice the values observed between *O. halei* and *O. maculatus* (Fig. 6).

The results of nuclear DNA analyses corroborated those obtained from mtDNA data. Diagnostic alleles were identified for both *O65* and *O78* microsatellite loci. In fact, each of the three species was fixed for a unique microsatellite allele at each locus. At *O65*, *O. halei* was fixed for the smallest allele (96 bp), followed by *O. ornatus* (98 bp) and then *O. maculatus* (100 bp). At *O78*, the smallest allele was again present in *O. halei* (146 bp), followed by *O. maculatus* (148 bp) and then *O. ornatus* (150 bp). There was no overlap in the range of allele sizes across the three species and no evidence for hybridization between them.

Isolation of the second intron of the ribosomal protein S7 was confirmed by sequence homology with exons two and three. Similar to the case of microsatellites, a unique and divergent S72 variant was obtained for each species (GenBank accession # EU379563 – EU379565). Sequence divergence in the corresponding 141 bp S72 fragment was considerably higher among the three species at this nuclear gene (13–15%) than at mtDNA loci. Uncorrected sequence divergences of 13·8 and 14·5% were obtained for pair-wise comparisons of *O. ornatus* with *O. halei* and *O. maculatus*, respectively. Similarly, uncorrected sequence divergence between *O. halei* and *O. maculatus* was 15·2%.

DISCUSSION

Inaccurately defined species boundaries compromise the ability to set conservation priorities (Avise, 2004). A multidisciplinary framework that employs

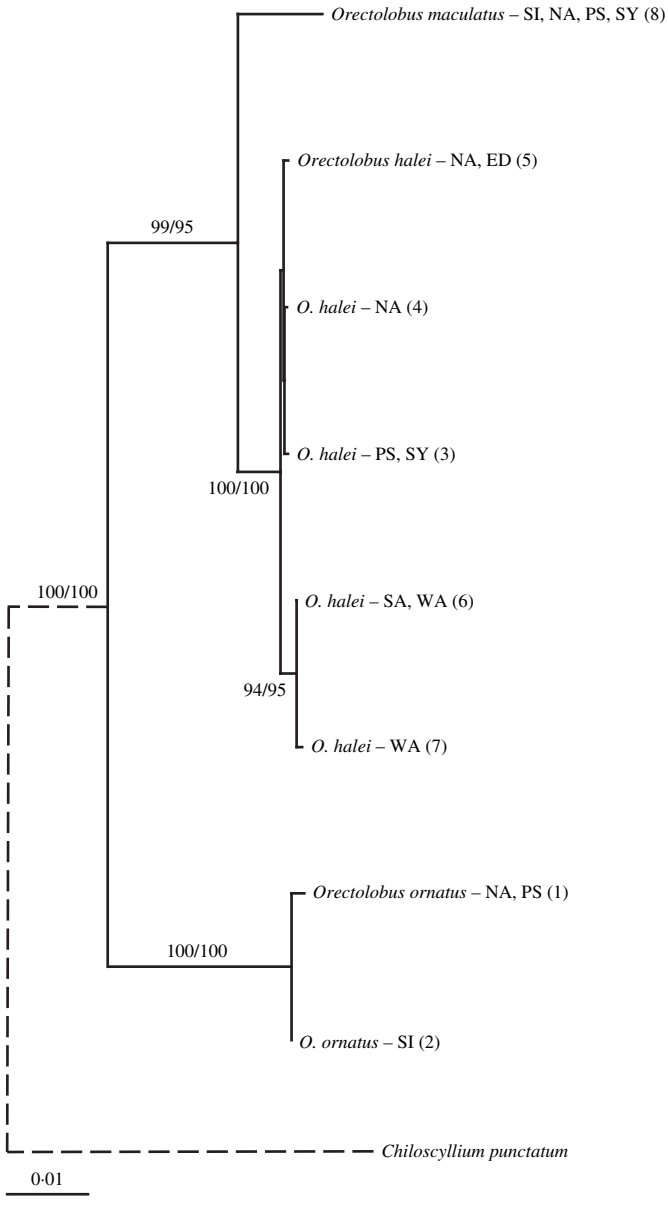

FIG. 5. Neighbour-joining (NJ) tree depicting genetic relationships for eastern Australian *Orectolobus* spp. based on 1398 bp of the mtDNA ATPase 6,8 and control region. Numbers above and below branches are bootstrap support values based on NJ and maximum parsimony (MP) trees (1000 replicates), respectively. Population codes are as in Fig. 2, haplotype numbers are in parentheses. The branch leading to the outgroup, the brown-banded bamboo shark *Chiloscyllium punctatum*, has been shortened for the purposes of illustration (– – – –). Stradbroke Island (SI), Nambucca Heads (NA), Port Stephens (PS), Sydney (SY), Eden (ED), South Australia (SA) and Western Australia (WA).

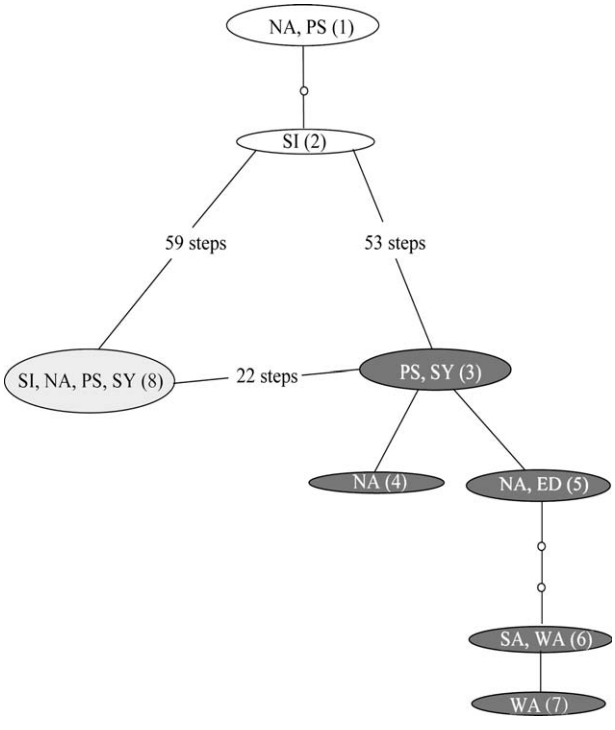

FIG. 6. Haplotype network based on combined mtDNA ATPase 6,8 and control region sequences (○, *Orectolobus ornatus*; ◎; *Orectolobus maculatus*; ●, *Orectolobus halei*). Oval size is proportional to the frequency of the haplotype in the sample. Single lines between haplotypes indicate a single mutational change or 'step'. Small open circles represent extinct or unsampled haplotypes (following Templeton *et al.*, 1992). Haplotype numbers are in parentheses. Stradbroke Island (SI), Nambucca Heads (NA), Port Stephens (PS), Sydney (SY) and South Australia (SA).

both morphological and molecular markers is increasingly advocated as a robust approach to species identification (Baker & Gatesy, 2002). This study represents the first assessment of genetic divergence and reproductive isolation in wobbegong sharks. It provides conclusive evidence that the three morphologically defined species are genetically distinct and therefore, apparently reproductively isolated. Given the marked separation in size at sexual maturity (Figs 3 and 4), it seems unlikely that interbreeding would occur between *O. ornatus* and *O. halei*. This is corroborated by the results of nuclear and mitochondrial loci analyses, which indicate genetic isolation between the three eastern Australian species. For instance, diagnostic alleles for each species were identified for the two microsatellite loci examined with no evidence for hybridization. This apparent reproductive isolation among the two initially cryptic ornate species was observed in parapatric as well as in sympatric (NA and PS) conditions. Furthermore, the levels of mtDNA sequence divergence revealed here (*c.* 4·8%) are comparable to those reported in studies of other cryptic elasmobranch species, thus supporting the designation of specific status to *O. ornatus* and *O. halei*. Sandoval-Castillo *et al.* (2004) report 2·5% control region sequence divergence

between cryptic species of the shovelnose guitarfish *Rhinobatos* spp. from the Gulf of California, while Quattro *et al.* (2006) revealed 5·3% control region sequence divergence among cryptic species of hammerhead shark *Sphyrna* spp.

Interestingly, higher levels of mtDNA sequence divergence were detected between the cryptic *O. ornatus* and *O. halei* (4·2–4·8%) than between *O. halei* and *O. maculatus* (1·7%), a wobbegong taxon with uncontested specific status. The results of both phylogenetic and genealogical analyses (Figs 5 and 6) point to a long history of evolutionary separation of *O. ornatus*. By tentatively assuming that the elasmobranch control region evolves at a rate of *c.* 0·8% per million years (Duncan *et al.*, 2006), the data suggest that *O. halei* and *O. ornatus* have evolved in isolation for *c.* 3·9 million years. These results challenge the paradigm that recent speciation would account for the apparent morphological stasis observed in many cryptic species. Although this paradigm is often mentioned in the biodiversity literature, an increasing number of DNA-based studies suggest that morphologically similar species can be the product of ancient speciation events (Beheregaray & Caccone, 2007).

This study provides irrefutable evidence to support the recently revised taxonomy of eastern Australian *Orectolobus* spp., showing that three morphologically, genetically and evolutionarily distinct species are distributed along this coast. Importantly, the patterns reported here have remained consistent over a more extensive sampling regime undertaken for an ongoing phylogeographic study (represented by 450 *Orectolobus* spp. samples; unpubl. data). Research to date suggests that *O. halei* is distributed from south-west Western Australia around the southern Australian coastline to Moreton Bay in south-east Queensland. Sydney, New South Wales probably represents the southern limit of the range of *O. ornatus*, which extends north beyond the limit of *O. halei* into northern Queensland (Huveneers, 2006). All three Australian east coast species (*O. maculatus*, *O. halei* and *O. ornatus*) are apparently sympatric in coastal waters from Sydney, New South Wales to Moreton Bay, Queensland. Given that no strong historical biogeographic barriers are known in this region (Waters *et al.*, 2005), it is possible that reproductive isolation among these taxa evolved in sympatric conditions. Alternatively, overlapping distributions may be the consequence of range expansion of geographically separated populations. Elucidation of reproductive isolating mechanisms among the three species awaits further investigation and should follow from the accumulation of data on life-history traits, migration patterns and population history.

Conservation interest in the potentially severe effects of fishing activity on Australian shark populations is increasing. This point was substantiated by the development of the National Plan of Action for the Conservation and Management of Sharks. The key objectives of this plan include facilitating the collection of species-specific catch, landings, biological and trade data, and improving the species identification capabilities of all resource users (Lack, 2004). Fulfilling these objectives and developing sound management strategies for Australian shark populations is critically dependent on accurate taxonomy. The work presented here contributes to existing literature describing the utility of molecular techniques in recognizing unidentified, reproductively isolated species of sharks and investigating evolutionary relationships among species (Sandoval-Castillo *et al.*, 2004; Quattro *et al.*, 2006). Confirmation of *O. ornatus*

and *O. halei* as distinct species will contribute to the development of effective management strategies for wobbegong sharks on the Australian east coast. Anatomical characteristics that may be used by fisherman to distinguish *O. ornatus* from *O. halei* in their catch have also been identified (Huveneers, 2006). More accurate species-specific catch and landings data can now be collected, allowing independent assessments of response to harvest and ultimately the development of species-specific management plans.

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