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# Taxonomic Affinities within Australian and New Zealand *Mustelus* Sharks (Chondrichthyes: Triakidae) Inferred from Allozymes, Mitochondrial DNA and Precaudal Vertebrae Counts

M. G. Gardner and R. D. Ward

**The relationships of *Mustelus* species in Australian and New Zealand waters were investigated by analysis of allozymes (28 loci), polymorphisms in the length of restriction fragments from mitochondrial DNA (mtDNA; whole genome, 10 restriction enzymes), and precaudal vertebrae counts. We confirm the existence of four *Mustelus* species in this region: a nonspotted *Mustelus* sp. A (a single specimen genetically examined from the North-West Shelf off Western Australia), and three spotted species—*M.* sp. B (from Western Australia, the North-West Shelf south to Perth—extending its known range); *Mustelus antarcticus* (from Bunbury in Western Australia around the southern Australian coast and as far north as Townsville, Queensland), and *M. lenticulatus* from New Zealand. *Mustelus* sp. A was the most divergent of these species, with fixed differences at six allozyme loci and a unique mtDNA profile in digests with six of the 10 restriction enzymes. The other species were much more similar to each other, with no completely fixed differences detected at any of the allozyme loci assayed. *Mustelus* sp. B differed very significantly from *M. antarcticus* in allele frequencies for three allozyme loci, and one restriction enzyme gave a unique mtDNA profile. *Mustelus lenticulatus* was distinguished from *M. antarcticus* by significant frequency differences at nine allozyme loci, and two restriction enzymes were nearly diagnostic. MtDNA nucleotide diversity (average 0.0323) and nucleotide divergence (average 0.0315) between species was low. Allozyme variation in *M. antarcticus* was high ($H = 0.100$) but low for the other three species (*M.* sp. A = 0.000; *M.* sp. B = 0.025; *M. lenticulatus* = 0.019). *Mustelus antarcticus* also had the highest mtDNA haplotype diversity of 0.534 (± 0.022); *M.* sp. B had 0.385 (± 0.149), and *M. lenticulatus* had 0.000 (this parameter could not be estimated for *M.* sp. A because only a single specimen was genetically examined). The within-species nucleotide diversities showed a higher value for *M.* sp. B (0.0021) than for *M. antarcticus* (0.0014). Significant differences between species were also observed for precaudal vertebrae counts. These are lowest in *M.* sp. B (77.39 ± 2.79), followed by *M. antarcticus* (85.61 ± 4.03), *M.* sp. A (90.50 ± 1.05), and *M. lenticulatus* (94.88 ± 1.46).**

*MUSTELUS* comprise 25 species of small (less than 2 m in total length) demersal sharks inhabiting temperate and tropical waters (Heemstra, 1997). Where abundant, they are important in commercial fisheries (Heemstra, 1997). Three Australian *Mustelus* species are identified in Last and Stevens (1994): *Mustelus antarcticus* Günther (the southern gummy shark), in temperate waters from Geraldton (Western Australia) to Port Stephens (New South Wales), and possibly off southern Queensland; *Mustelus* sp. A (the grey gummy shark) identified from off northwestern Australia (Dampier to Darwin), and possibly also off Queensland (Townsville); and *Mustelus* sp. B (the white-spotted gummy shark) from tropical Australia—from both Western Australia (Dampier) and Queensland (Cairns to Bowen). *Mustelus lenticulatus* Phillipps (the rig or spotted estuary smooth-hound shark) occurs in New Zealand waters. *Mustelus antarcticus* and *M. lenticulatus* are heavily targeted in shark fisheries in southern Australia and New Zealand, respectively.

*Mustelus lenticulatus* is morphologically very similar to *M. antarcticus*, and there is some conjecture over their distictivness (MacDonald, 1988; Last and Stevens, 1994). It is primarily distinguishable from *M. antarcticus* by a higher number of precaudal vertebrae, 87–95, (Heemstra, 1973; Francis and Mace, 1980) as opposed to 79–86 for *M. antarcticus* (Last and Stevens, 1994).

To clarify the taxonomic relationships of Australasian *Mustelus* species, we made a genetic comparison of these species, using both allozymes and polymorphisms in the lengths of restriction fragments (RFLP) from mitochondrial DNA. We also compared precaudal vertebrae counts to provide an additional, meristic, char-

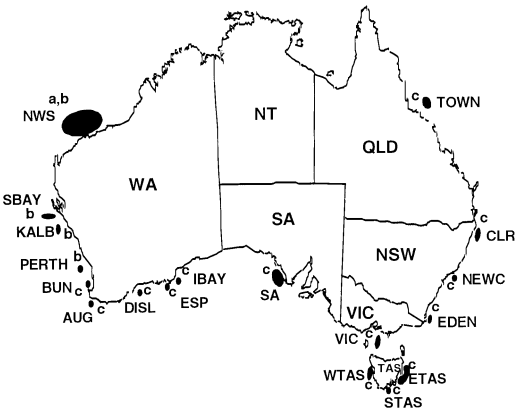


Fig. 1. Australian *Mustelus* sampling locations: a = *Mustelus* sp. A; b = *Mustelus* sp. B; c = *Mustelus antarcticus*. See text for an explanation of sample localities and for the location of the New Zealand sample.

acter for species comparisons, because differences in vertebrae counts were suggested by Last and Stevens (1994) and also Heemstra (1997) to be useful in distinguishing *Mustelus* species.

## Materials and Methods

*Specimen acquisition.*—Eighteen collections of sharks from Australia (Fig. 1) and one from New Zealand were obtained. These include 14 collections, totaling 550 individuals, of *M. antarcticus* from Bunbury (code = BUN; $n = 23$); Augusta (AUG; $n = 21$); Doubtful Islands (DISL; $n = 16$); Esperance (ESP; $n = 16$); Israelite Bay (IBAY; $n = 33$); South Australia (SA; $n = 123$); Victoria (VIC; $n = 100$); west Tasmania (WTAS; $n = 15$); south Tasmania (STAS; $n = 33$); east Tasmania (ETAS; $n = 85$); Eden, New South Wales (EDEN; $n = 14$); Newcastle, New South Wales (NEWC; $n = 22$); Clarence River, New South Wales (CLR; $n = 45$); and Townsville, Queensland (TOWN; $n = 4$). Collection details for all *M. antarcticus* samples can be found in Gardner and Ward (1998). Unfortunately, it proved extremely difficult to collect the tropical Australian *Mustelus*; thus, collection sizes from these regions were very small (one to six per collection). A single specimen of *Mustelus* sp. A was collected from the North-West Shelf (NWS). Fourteen specimens of *Mustelus* sp. B were collected from Western Australia: North-West Shelf (NWS; $n = 6$); Shark Bay (SBAY; $n = 3$); Kalbarri (KALB; $n = 2$); and Perth (PERTH; $n = 3$). A total of 110 *M. lenticulatus* samples were collected from a single location in-between the North and South Islands of New Zealand (41°S, 174°E).

Most specimens were tissue-sampled soon after capture, with samples of liver and muscle being taken and frozen immediately. Some collections were snap-frozen in liquid nitrogen, others were maintained at −20 C. A minority were sent frozen, intact, to the CSIRO laboratory. In the laboratory, these sharks were thawed, labeled, sexed, and samples of muscle and liver taken. They were refrozen at −20 C for later measurement, possible radiographing for vertebrae counts, or for fixing and adding to the CSIRO ISR MUNRO Ichthyological Collection. All tissue samples in the laboratory were maintained at −70 C.

Specimens of fixed (preserved) *Mustelus* sharks from the CSIRO Fish Collection were used to provide additional specimens for x-ray (vertebrae count) analysis. The museum accession numbers of these specimens are available from the authors.

*Allozyme electrophoresis.*—Allozyme variation was examined on Helena Titan III cellulose acetate plates with a tris-glycine [0.02 M tris, 0.192 M glycine; for further details, see Hebert and Beaton (1993)] or a 75-mM tris-citrate (pH 7.0) buffer system [for further details, see Richardson et al. (1986)]. Liver and muscle homogenates were assessed for 21 enzymes encoded by 28 loci (see Gardner and Ward [1998] for enzymes and buffers used). Tris-glycine gels were run at 200 V at room temperature, typically for 30 min. Tris-citrate gels were run at 100 V at 4 C, typically for 60 min. Staining procedures follow those of Hebert and Beaton (1993) and Richardson et al. (1986). Phenylalanine leucine was used in the peptidase stain, and general proteins were stained with Coomassie Blue.

Alleles were identified by the anodal electrophoretic mobility of their product relative to that of the most common allele (= 100) in the east Tasmanian (ETAS) collection of *M. antarticus*, rounded to the nearest 5%. Locus notation follows that of Shaklee et al. (1990). When an enzyme was encoded by two loci, the more anodally migrating enzyme was suffixed as 1.

Loci were defined as polymorphic if the most common allele had a frequency of 0.95 or less. Polymorphic loci were tested for goodness-of-fit to Hardy-Weinberg expectations, and chi-squared comparisons of allele frequencies were performed using the pseudo-probability approach of Zaykin and Pudovkin (1993). Chi-squared comparisons of allele frequencies were performed using the pseudo-probability approach of Zaykin and Pudovkin (1993). Nei's (1972, 1978) distance measures were calculated

between collections and species to facilitate comparisons with other studies.

*Mitochondrial DNA procedures.*—Total DNA was extracted from ~100 mg of white muscle tissue, using a modified CTAB (hexadecyltrimethylammoniumbromide) protocol described by Grewe et al. (1993). Individuals were screened using 10 restriction enzymes (*Apa*I, *Ava*I, *Bcl*I, *Eco*RI, *Hind*III, *Nco*I, *Pst*I, *Pvu*II, *Sac*II, and *Xba*I).

Restriction fragments were separated in horizontal 1.0% agarose gels submerged in a trisborate-EDTA (TBE) buffer system (Sambrook et al., 1989). DNA was transferred to a nylon membrane filter (Hybond N+, Amersham Ltd.) by southern transfer (Sambrook et al., 1989). The nylon membrane filters were probed with blue eye trevalla (*Hyperoglyphe antarctica*, Teleostei: Stromateoidei) mtDNA (50 ng used per 10 20-cm × 20-cm blots) purified by caesium chloride ultra centrifugation. The trevalla probe was labeled with [$^{32}$P] dCTP (Bresatec Pty Ltd.) by a GIGAprime DNA labeling kit (Bresatec Pty Ltd). The membrane filters were then exposed to Kodak x-ray film for 12–48 h, routinely at −20 C without intensifying screens.

Restriction profiles for each enzyme were given letter designations in order of discovery, with the first pattern being designated ''A,'' the second ''B,'' and so on. The scoring of restriction patterns from *Mustelus* samples was performed concurrently with a study on *Galeorhinus galeus*, and hence patterns presented here may have missing letter designations. This scoring is also consistant with our previously published analysis of stock structure in *Mustelus antarcticus* (Gardner and Ward, 1998). Haplotypes of each fish were then identified by the combination of letters representing the restriction profiles for each restriction enzyme used. Restriction fragments were sized with the assistance of the program DNAGEL (Kieser, 1984; modified by P. Grewe in QuickBasic) run on an IBM.

Genetic distances between mtDNA haplotypes were compared by converting site information (inferred from digests of single restriction enzymes) to a nucleotide substitution matrix of d-values with the program REAP (McElroy et al., 1992), using the equations of Nei and Tajima (1981) and Nei and Miller (1990). Site information was used for input to phylogenetic analysis rather than fragment data, as the latter violates the assumption of independence among characters (see Swofford et al., 1996).

Haplotype (nucleon) diversity of mitochondrial DNA (using the formulations of Nei, 1987), nucleotide diversity (using the formulations of Nei and Tajima, 1981), and sequence divergence (from site information, using the formulations of Nei, 1987) were computed with the REAP package (McElroy et al., 1992). A matrix of sequence divergences between collections and species was converted to a phylogram with a neighbor-joining algorithm in PHYLIP (unpubl.).

*X-ray counts.*—Precaudal vertebrae were counted from x-ray films of 25 individuals used in the genetic analysis and 69 other sharks from the CSIRO Fish Collection. The precaudal vertebrae were delineated from the anterior caudal vertebrae by inserting a pin perpendicularly at the upper origin of the caudal fin and counting vertebrae only to this pin. Counts of the four *Mustelus* species were compared by analyses of variance.

*Significance levels for statistical analysis.*—In all analyses involving multiple tests the predetermined experimentwise significance level, $\alpha$, of 0.05 was adjusted by Bonferroni procedures (Miller, 1980). The $\alpha$ level was divided by the number of tests to derive a new $\alpha$ level, and *P*-values had to be less than this corrected $\alpha$ value to be deemed significant.

## Results

*Allozyme data.*—Quaternary structures of all variable loci accorded with known structures (Ward et al., 1992), and genotype numbers at all loci accorded with Hardy-Weinberg expectations after Bonferroni adjustments were made. Allele-frequency data for 28 loci (available from the authors) were estimated from genotype data, with *M. antarcticus* collections pooled into their three previously determined stocks (BUN-EDEN, NEWC, and CLR, and TOWN, see Gardner and Ward, 1998). Not all collections could be examined for all loci (contact the authors for numbers of specimens genotyped from each locality). Three collections in particular were poorly preserved on arrival in Hobart: PERTH (*M.* sp. B), DISLE (*M. antarcticus* BUN-EDEN stock), and IBAY (*M. antarcticus* BUN-EDEN stock).

Only 11 of 19 collections could be examined for all 28 loci: NWS (*M.* sp. B), ESP, STAS, WTAS, ETAS, VIC, EDEN, NEWC, CLR, TOWN (*M. antarcticus*), and NZ (*M. lenticulatus*). The two *PROT** loci were not scored from *M.* sp. A, but the remaining 26 loci were scored. Thus, 12 collections were scored for 26 loci, and 11 collections were scored for 28 loci. Because there were no significant allelic differences between

TABLE 1. LEVELS OF ALLOZYME AND MTDNA DIVERSITY IN *Mustelus* SPECIES (*Mustelus antarcticus* STOCKS COMBINED). H = heterozygosity; P = polymorphism (0.95 criteria); na = this value is not applicable. Values presented for allozyme analyses are from samples scored for all loci (i.e., 28 or 26 only for *M.* sp A).

| | Allozymes | | | | mtDNA (10 restriction enzymes) | | |
|---|---|---|---|---|---|---|---|
| Species | Number of fish | Number of loci | Mean *H* | Mean *P* | Number of fish | Haplotype diversity (± SE) | Nucleotide sequence diversity |
| *M.* sp A | 1 | 26 | 0.000 | 0.000 | 1 | na | na |
| *M.* sp. B | 6–14 | 28 | 0.025 | 0.064 | 14 | 0.385 ± 0.149 | 0.0021 |
| *M. antarcticus* | 134–503 | 28 | 0.100 | 0.242 | 162 | 0.534 ± 0.022 | 0.0014 |
| *M. lenticulatus* | 22–96 | 28 | 0.019 | 0.071 | 94 | 0.000 ± 0.000 | 0.0000 |

the collections of *M.* sp. B, the data were pooled for further analysis.

Of the four species of *Mustelus* collected, *M.* sp. A was, not surprisingly, the least variable (Table 1), with only a single specimen being genotyped. However, the lack of variability in *M.* sp. A was pronounced, because this specimen was homozygous at all 26 loci examined. *Mustelus* sp. B and *M. lenticulatus* showed a few polymorphic loci out of the 28 examined, with percentage polymorphism levels of 6.4 and 7.1, respectively, and average heterozygosities per locus of 0.025 and 0.019, respectively. *Mustelus antarcticus* was considerably more variable, with percentage polymorphism per collection (for those scored for all 28 loci) ranging from 21.43 to 32.14 (species mean 24.20), and average heterozygosities per locus ranging from 0.079 to 0.151 (species mean 0.100; see Gardner and Ward, 1998).

*Mustelus* sp. A, although represented by only a single specimen, was well differentiated from all other sharks. Six of the 26 loci examined (23%) were diagnostic (*ADA-1**, allele 105; *CK-A**, allele 400; *MPI**, allele 130; *ODH**, allele 85; *PEP-1**, allele 125; *sSOD**, allele 95). As this specimen exhibited unique alleles at six loci, it is therefore well differentiated from the other species examined, especially considering of the lack of variation in *M.* sp. B and especially in *M. lenticulatus*.

None of the other species showed absolutely diagnostic loci, although *Mustelus* sp. B differed ($P < 0.001$) from *M. antarcticus* in having a very high frequency of allele *ESTD-2**150 (*M. antarcticus* has *ESTD-2**100 as its common allele), and alleles *LDH-2**-60 and *PEP-1**120 fixed (these alleles are uncommon in southern Australian populations of *M. antarcticus*). *Mustelus lenticulatus*, like *M.* sp. B, was fixed for *LDH-2**-60, monomorphic for several loci that are highly polymorphic in *M. antarcticus* (*ACP, CK-A**, *ESTD-2**, *G6PDH**, *PEP-1**; all $P < 0.001$) and nearly monomorphic for *MPI** ($P < 0.001$). Additionally, *M. lenticulatus* was variable for two loci that were monomorphic in *M. antarcticus* (*AAT-2**, *ME-1**; both $P < 0.001$).

*Mitochondrial DNA data.*—A subset of specimens (1 of *M.* sp. A; 14 *M.* sp. B; 162 *M. antarcticus*; 94 *M. lenticulatus*) were examined with 10 restriction enzymes and haplotype frequencies were calculated (Table 2). Fragment sizes are available from request from the authors. See Table 2 for a locality breakdown of specimens examined within each species. Additionally, see Gardner and Ward (1998) for details of BUNEDEN specimens examined for the 10 restriction enzymes. Considering within-population measures (Table 1), haplotype diversity was highest for *M. antarcticus* at 0.534 ± 0.022, followed by 0.385 ± 0.149 for *M.* sp. B and 0.000 for *M. lenticulatus*. No valid within-species haplotype diversity value (or nucleotide diversity) can be calculated for *M.* sp. A because only a single specimen was examined genetically. *Mustelus* sp. B had the most variable mtDNA genome when nucleotide diversities are considered, with a value of 0.0021 compared with 0.0014 for *M. antarcticus* and 0.0000 for *M. lenticulatus*.

The single specimen of *M.* sp. A showed the most divergence (haplotype i in Table 2) with estimates of evolutionary distance between it and all others ranging from 4.4% (i and x) to 6.1% (i and vi). It had unique fragment patterns for six enzymes: *Eco*RI (restriction profile E), *Hind*III (I), *Nco*I (D), *Pst*I (D), *Pvu*II (D), and *Xba*I (C).

In *M.* sp. B, one restriction enzyme, *Nco*I, gave a unique fragment pattern (C). *Xba*I gave three restriction profiles, B (common in *M. antarcticus*), D (found in a very few *M. antarcticus*), and E (rare, but so far unique to *M.* sp. B).

In *M. lenticulatus*, two of the 10 restriction enzymes gave nearly unique fragment patterns. These were *Apa*I (restriction profile E) and *Ava*I (C). All 94 specimens had the same 10 enzyme

TABLE 2. *Mustelus*—ALL SPECIES (*Mustelus* sp A, *Mustelus* sp. B, *Mustelus antarcticus, M. lenticulatus*). Composite mitochondrial DNA haplotype frequencies from the 10 restriction enzymes (in order) *Apa*I, *Ava*I, *Bcl*I, *Eco*RI, *Hind*III, *Nco*I, *Pst*I, *Pvu*II, *Sac*II, and *Xba*I.

| Composite restriction profiles | Combined haplotype | *M.* sp. A | *Mustelus* sp. B | | | | *Mustelus antarcticus* | | | *M. lenticulatus* |
|---|---|---|---|---|---|---|---|---|---|---|
| | | NWS | NWS | SBAY | KALB | PERTH | BUN–EDEN | NEWC/CLR | TOWN | NZ |
| EBCEIDDDAC | i | 1.00 | — | — | — | — | — | — | — | — |
| BBBBEABBAB | ii | — | — | — | — | — | 0.63 | 0.51 | 0.25 | — |
| BBBBEABBAD | iii | — | — | — | — | — | — | 0.04 | — | — |
| CBBBEABBAB | iv | — | — | — | — | — | 0.30 | 0.46 | 0.25 | — |
| CBBDEABBAB | v | — | — | — | — | — | 0.05 | — | — | — |
| CBBCEABBAB | vi | — | — | — | — | — | — | — | 0.25 | — |
| BBCBEABBAB | vii | — | — | — | — | — | — | — | 0.25 | — |
| ECBBEABBAB | viii | — | — | — | — | — | 0.01 | — | — | 1.00 |
| BBDBEABBAB | ix | — | — | — | — | — | 0.01 | — | — | — |
| BBCBECBBAB | x | — | 0.67 | 0.67 | 1.00 | 1.00 | — | — | — | — |
| BBCBECBBAD | xi | — | 0.17 | 0.33 | — | — | — | — | — | — |
| BBCBECBBAE | xii | — | 0.17 | — | — | — | — | — | — | — |
| *n* | | 1 | 6 | 3 | 2 | 3 | 99 | 59 | 4 | 94 |

composite haplotype. This haplotype (viii) is the same as two of the 162 *M. antarcticus* examined and quite similar to other *M. antarcticus* haplotypes (range 0.49–0.97%).

The TOWN collection of *M. antarcticus* was very small ($n = 4$), but two individuals had haplotypes not seen in any other *M. antarcticus*. However, these two haplotypes (vi and vii) were closely related to the two common *M. antarcticus* haplotypes (range 0.22–0.46%). Haplotype vii was related to the most common haplotype ii by the loss of a single cut site for *Bcl*I, and haplotype vi was related to the second most common haplotype iv by the gain of a cut site for *Eco*RI. No TOWN individuals had the unique fragment pattern (restriction profile C) that digestion with *Nco*I gave for *M.* sp. B, confirming that the TOWN collection was *M. antarcticus* and not *M.* sp. B.

The specific relationship shown by the allozyme analysis is also generally supported by the estimates of mtDNA nucleotide diversity and nucleotide divergence (Nei and Tajima, 1981; Nei, 1987) between species (with *M. antarcticus* split into its three stocks). The nucleotide divergence relationship among species and stocks, which is corrected for within-population polymorphism, is shown in Figure 2. The highest between-species nucleotide divergence of 5.7% (nucleotide diversity = 5.7%) was between *M.* sp. A and *M. lenticulatus*. The smallest between-species divergence was 0.51% (nucleotide diversity = 0.58%) between *M. antarcticus* and *M. lenticulatus*, which is more than twice the largest nucleotide divergence calculated among *M. antarcticus* stocks (range 0.01–0.23%). *Mustelus* sp. A is again seen as the most divergent species.

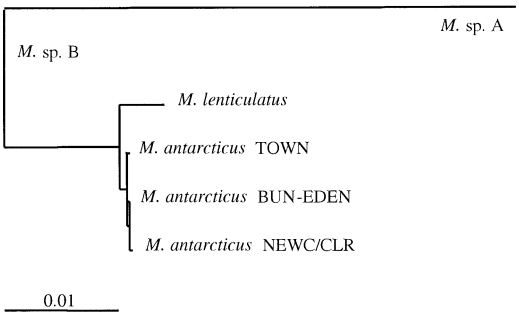


Fig. 2. Phenogram of sequence divergence of mtDNA nucleotides among *Mustelus* species/stocks (Neighbor-joining tree) from 10 restriction enzymes.

*X-ray counts.*—Analyses of variances of precaudal vertebrae counts (Table 3) show that means of this character vary significantly ($P < 0.001$) for the fishes in the 10 collections counted. Mean precaudal vertebrae counts for each of the four species were then pooled for collections within species. The count is lowest in *M.* sp. B (77.39 ± 2.79), followed by *M. antarcticus* (85.61 ± 4.03), *M.* sp. A (90.50 ± 1.05), and *M. lenticulatus* (94.88 ± 1.46). These differences are highly significant ($P < 0.001$). Unfortunately two specimens of *M. antarcticus* from the TOWN collection were unable to be radiographed. Given the wide variation in vertebrae counts in the TOWN sample, it is possible that not all specimens were *M. antarcticus* but that *M.* sp. B may have been present in the sampled. The two ge-

TABLE 3. PRECAUDAL VERTEBRAE COUNTS.

| Species range | *M.* sp A 89–92 NWS | *Mustelus* sp. B 69–82 NWS | SBAY | PERTH | *Mustelus antarcticus* 75–94 ETAS | EDEN | NEWC | CLR | TOWN | *M. lenticulatus* 93–97 NZ |
|---|---|---|---|---|---|---|---|---|---|---|
| *n* | 6 | 10 | 5 | 3 | 16 | 6 | 13 | 14 | 13 | 8 |
| Range | 89–92 | 76–82 | 69–79 | 77–78 | 78–86 | 75–87 | 80–89 | 85–91 | 79–94 | 93–97 |
| Mean | 90.50 | 78.20 | 75.80 | 77.33 | 82.69 | 82.00 | 85.69 | 87.93 | 88.31 | 94.88 |
| ± SD | ±1.05 | ±2.25 | ±4.09 | ±0.58 | ±2.63 | ±4.65 | ±2.75 | ±1.54 | ±4.77 | ±1.46 |

notyped individuals had precaudal vertebrae counts of 87 and 89.

## DISCUSSION

Both genetic analyses and counts of precaudal vertebrae support the existence of four species of *Mustelus* in the Australasian region: the nonspotted *Mustelus* sp. A (a single specimen examined from the North-West Shelf), and three spotted species—*M.* sp. B (from Western Australia, the North-West Shelf south to Perth); *M. antarcticus* (from Bunbury in Western Australia around the southern Australian coast and as far north as Townsville); and *M. lenticulatus* (from New Zealand). Descriptions of these are in Last and Stevens (1994). The identification of these taxa as valid species was also supported by morphometric analysis (unpubl. data). There was no genetic evidence for species other than *M. antarcticus* in the Townsville region. It should be noted that only four specimens were examined genetically there, and the possibility remains that, given the wide range in vertebrae count for that region (79–94, $n = 13$), other species such as *M.* sp. B may coexist there. However, given that *M.* sp. B and *M. antarcticus* are sympatric in at least part of their respective ranges (as is revealed by our samples from off Perth, WA, being *M.* sp. B, and from the range of *M. antarcticus* reported in Last and Stevens [1994]), the allozyme and mtDNA differences between *M.* sp. A, *M.* sp. B from Western Australia, *M. antarcticus*, and *M. lenticulatus* are consistent with these four taxa representing four distinct species. *Mustelus* sp. B, *M. antarcticus*, and *M. lenticulatus* are genetically very similar to one another, with *M.* sp. A being the most divergent species. Estimates of unbiased genetic distance (Nei, 1978) among species confirm their close relationships, with D ranging from 0.057 to 0.318. The two most genetically distant species were *M. antarcticus* (combined stocks) and *M.* sp. A, (0.318) with *M. antarcticus* and *M.* sp. B being the most similar (0.057).

*Mustelus antarcticus* was the most genetically variable species in the study (Table 1). Although the low levels of genetic variation of *M.* sp. A and *M.* sp. B could be caused by limited sample sizes in these two species, this explanation cannot, however, account for the limited variation in *M. lenticulatus*.

*Mustelus sp. A.*—Examination of this nonspotted species, albeit of a single specimen, confirmed that it was genetically (for both allozymes and mitochondrial DNA) quite distinct from the other, spotted, *Mustelus* species. The single genetically examined fish, and some stored formalin fixed specimens with similar color pattern, were radiographed for vertebrae counts: they had a mean precaudal vertebrae count of 90.5, which was higher than *M.* sp. B (mean = 77.4) or *M. antarcticus* (mean = 85.6) but lower than *M. lenticulatus* (mean = 94.9). The specimens of *M.* sp. A examined also had distinct lower/upper labial furrow proportions compared with the other species in the study (unpubl. data), a field character identified by Last and Stevens (1994). All specimens examined came from the North-West Shelf; we were unable to confirm its possible existence off Townsville, Queensland (Last and Stevens, 1994). Molecular and meristic data support this as a valid species.

*Mustelus sp. B.*—The white-spotted gummy shark, *M.* sp. B, was genetically quite similar to the similarly spotted common gummy shark, *M. antarcticus*, although there were large allele-frequency differences at several allozyme loci and unique mtDNA fragment patterns for two of the 10 restriction enzymes tested. These genetic differences, although limited, were significant following a randomization statistical procedure that takes into account the small numbers of samples available. The data therefore support the specific status of *M.* sp. B. The possibility of underestimating the divergence between *M.* sp. B and *M. antarcticus* resulting from reduced sampling and incomplete lineage sorting is lessened by the geographic spread of samples taken for both species. Three small collections—from the North-West Shelf, Shark Bay, and Perth—

were confirmed genetically as *M.* sp. B. These findings verify their previously unconfirmed (Last and Stevens, 1994) existence in Shark Bay and extend its range much further south; its only previously confirmed locality was off Dampier (Last and Stevens, 1994). Precaudal vertebrae counts of *M.* sp. B had a mean of 77.4, a little less than the southern collections (east Tasmania and Eden) of *M. antarcticus* (82.5), and substantially less than the more northern collections (Newcastle and Townsville; 87.3).

*Mustelus from Townsville.*—The TOWN sample showed an especially wide range of precaudal vertebrae counts. Of the 13 specimens, the lowest had a count of 79, followed by one with a count of 80, up to two specimens with counts of 94. Neither of the two low-count specimens was examined genetically, as both were fixed specimens from the Fish Collection. Two specimens both counted and examined genetically were genetically classified as *M. antarcticus*: they had counts of 87 and 89. We assume that all the TOWN specimens were *M. antarcticus*, although the broad spread of counts suggests that further investigation of the taxonomy of *Mustelus* from this area is warranted: it is conceivable that the two specimens with the lowest counts are a distinct species of *Mustelus*, possibly *M.* sp. B. The specimens with the highest counts are possibly *M.* sp. A, although they had labial furrow proportions (Last and Stevens, 1994) consistent with *M. antarcticus* and not *M.* sp. A. The precaudal vertebrae counts of the two genetically typed specimens from the TOWN collection were very similar (mean of 88) to the Clarence River collection of *M. antarcticus* (87.9), and considerably higher than the mean for *M.* sp. B (77.4), adding support to the genetically typed TOWN specimens being *M. antarcticus* and not *M.* sp. B. This would need to be confirmed genetically for the other specimens and further sampling in this region is warranted.

*Mustelus lenticulatus.*—The New Zealand gummy, *M. lenticulatus*, was genetically distinct from the Australian *M. antarcticus* at several allozyme loci, and two of the ten restriction enzymes gave nearly unique patterns of mtDNA restriction fragments with the New Zealand species fixed for a haplotype that is very rare in *M. antarcticus*. These differences support its identification as a distinct species. Multivariate analysis of morphometric traits further confirmed its distinctiveness from *M. antarcticus* and from *M.* sp. B (unpubl. data). It also had a higher precaudal vertebrae count (94.9) than any of the other *Mustelus* species examined, the next highest was *M.* sp. A with a mean of 90.5.

Smith (1986) also surveyed allozyme variation in *M. lenticulatus*: he identified three out of 38 loci as polymorphic—*G6PDH**, GPI*, and *SOD**. Although allele frequencies were not given, each was specified to have the most common allele at a frequency greater than 0.80, and variation was therefore limited. In our collection, and with our techniques, *G6PDH** and *SOD** were invariant, and *GPI** was not resolved satisfactorily. Two additional loci, *AAT-2** and *MEP-1**, reported as monomorphic by Smith (1986), showed limited variation in our study. Both studies thus found low levels of genetic diversity in *M. lenticulatus*: Smith (1986) reported $P$ of 0.079 and $H$ of $0.001 \pm 0.001$; we report 0.071 and $0.019 \pm 0.011$, respectively.

*Comments on the relationships between Mustelus antarcticus and Mustelus lenticulatus.*—Previously *M. antarcticus* and *M. lenticulatus* were separated on the basis of a difference in precaudal vertebrae counts (Heemstra, 1973), but genetic testing was suggested to evaluate their distinctiveness. The existence of nearly diagnostic mtDNA haplotype differences, and a nearly fixed allele for *LDH-2**, with significant allelic frequency differences at eight other allozyme loci, supports the current taxonomy. However, the small allozyme distances ($D = 0.068$, $I = 0.934$), and mtDNA sequence divergence (0.51%) show that the two species are very closely related. Lavery and Shaklee (1991) similarly found a low allozyme divergence ($D = 0.045$, $I = 0.956$) between the morphologically similar sharks *Carcharhinus limbatus* and *C. tilstoni*. Most congeneric fish species have genetic identities less than 0.8 (Thorpe, 1983) and mtDNA divergences in the range 3.7–13.0% (Billington and Hebert, 1991). The limited geographic and temporal sampling of *M. lenticulatus* could potentially lead to an underestimate of genetic diversity in this species. However, the congruence of the mtDNA, allozymes, and precaudal vertebrae adds support to the specific identities of *M. lenticulatus* and *M. antarcticus*.

Although no tagged *Mustelus* from New Zealand have been caught in Australian waters or vice versa (J. D. Stevens pers. comm.), females of both *M. lenticulatus* and *M. antarcticus* are known to move substantial distances (Francis, 1988). However, *Mustelus* are relatively benthic and are likely to remain in shallow coastal waters rather than move across oceans. It is, thus, unlikely that *Mustelus* do exchange between Australia and New Zealand.

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(MGG, RDW) CSIRO Marine Research, GPO Box 1538 Hobart, Tasmania 7001 Australia. Present address: (MGG) CEH Dorset, Winfrith Technology Centre, Winfrith Newburgh, Dorchester, Dorset DT2 8ZD UK. (MGG) migg@ceh.ac.uk; and (RDW) bob.ward@csiro.au. Send reprint requests to MGG. Submitted: 16 March 2001. Accepted: 19 Nov. 2001. Section editor: J. D. McEachran.