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DNA-based identification of Alaska skates (*Amblyraja*, *Bathyraja* and *Raja*: Rajidae) using cytochrome *c* oxidase subunit I (coI) variation

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Variation at the mitochondrial cytochrome *c* oxidase subunit I (mt-COI) gene was examined in 15 species of North Pacific skates. Thirteen species had unique sequences, indicating that a DNA-based barcoding approach may be useful for species identification.

Key words: coI; cytochrome *c* oxidase subunit I; DNA barcode; mitochondrial DNA; species identification; skates.

The skates of the North Pacific Ocean and Bering Sea (family Rajidae) are represented by a complex of 15 species in three genera, *Raja* L., *Amblyraja* Malm, 1877 and *Bathyraja* Ishiyama, 1958 (Mecklenburg *et al.*, 2002; Stevenson *et al.*, 2004; Stevenson & Orr, 2005). The genus *Raja*, known as 'stiff-snout' skates due to a robust rostral cartilage, is represented by two species (*Raja rhina* Jordan and Gilbert, 1880 and *Raja binoculata* Girard, 1855). Twelve species are presently recognized in *Bathyraja*, the genus of 'soft-snout' skates possessing a flexible rostral cartilage (Table I). A third genus represented by the deepwater species *Amblyraja badia* (Garman, 1899) was recently discovered in Alaska (Stevenson & Orr, 2005).

Skate abundance and diversity vary considerably across the eastern North Pacific Ocean and the Bering Sea (Stevenson, 2004). The Alaska skate, *Bathyraja parmifera* (Bean, 1881) dominates the skate species complex on the eastern Bering Sea shelf, while the Aleutian skate, *Bathyraja aleutica* (Gilbert, 1896) and whiteblotched skate, *Bathyraja maculata* Ishiyama & Ishihara, 1977, are more dominant in the eastern Bering Sea slope and Aleutian Islands regions, respectively (Gaichas *et al.*, 2005).

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TABLE I. Skate species, number of individuals sequenced (*n*), GenBank accession numbers and voucher status (UW = University of Washington Fish Collection)

Common name	Scientific name	*n*	GenBank accession number	Voucher
Longnose skate	*Raja rhina* Jordan & Gilbert, 1880	4	DQ104880–DQ104883	Photographs on file
Big skate	*Raja binoculata* Girard, 1855	4	DQ104884–DQ104887	Photographs on file
Deep-sea skate	*Bathyraja abyssicola* (Gilbert, 1896)	2	DQ104888–DQ104889	Photographs on file
Aleutian skate	*Bathyraja aleutica* (Gilbert, 1896)	6	DQ104890–DQ104895	Photographs on file
Sandpaper skate	*Bathyraja interrupta* (Gill & Townsend, 1897)	9	DQ104896–DQ104904	UW 49400, 111883
Commander skate	*Bathyraja lindbergi* Ishiyama & Ishihara, 1977	5	DQ104905–DQ104909	UW 45829
Whiteblotched skate	*Bathyraja maculata* Ishiyama & Ishihara, 1977	2	DQ104910–DQ104911	UW 49537
Butterfly skate	*Bathyraja mariposa* Stevenson *et al.*, 2004	5	DQ104912–DQ104916	UW 47197-9, 47201, 47205
Whitebrow skate	*Bathyraja minispinosa* Ishiyama & Ishihara, 1977	3	DQ104917–DQ104919	Photographs on file
Alaska skate	*Bathyraja parmifera* (Bean, 1881)	4	DQ104920–DQ104923	UW 47245, 48083
Golden skate	*Bathyraja smirnovi* (Soldatov & Pavlenko, 1915)	1	DQ665297	UW 114999
Mud skate	*Bathyraja taranetzi* (Dolganov, 1983)	7	DQ104924–DQ104930	UW 47204
Roughtail skate	*Bathyraja trachura* (Gilbert, 1892)	3	DQ104931–DQ104933	UW 46456
Okhotsk skate	*Bathyraja violacea* (Suvorov, 1935)	1	DQ665298	UW 113468
Broad skate	*Amblyraja badia* (Garman, 1899)	1	DQ385444	UW 115021

Skates are particularly vulnerable to exploitation due to their relatively low fecundity, late maturity and large maternal investment per offspring. Like many elasmobranch fishes, skates appear to be 'equilibrium' life-history strategists (Winemiller & Rose, 1992), exhibiting very low intrinsic rates of growth, and sustainable yields in fisheries can be achieved only at very low-to-moderate fishing mortality rates (King & McFarlane, 2003). Large species exhibiting late maturation appear to be most vulnerable to overfishing (Dulvy *et al.*, 2000), and large-scale extirpations of several eastern North Atlantic species across their historic ranges has been documented (Casey & Meyers, 1998; Walker & Hislop, 1998; Dulvy *et al.*, 2000, and references therein). Little is known regarding life-history parameters of skate species in the eastern North Pacific Ocean and the Bering Sea, where they constitute a substantial fraction of by-catch in other directed fisheries, particularly in the hook-and-line fishery for Pacific cod, *Gadus macrocephalus* Tilesius, 1810 (Gaichas *et al.*, 2005). In addition, a large-mesh trawl fishery for skates was developed in the Gulf of Alaska during 2003, primarily targeting the large *Raja* species, although the fishery takes unknown numbers of *Bathyraja* species.

Uncertainty regarding species composition as by-catch or in directed fisheries has become a management issue for skates. Until recently, North Pacific groundfish observers were unable to identify skates to the species level (Stevenson, 2004). Although observers began consistent species-level identification of skates in 2004, some identification problems persist, particularly on longline vessels where observers typically only identify skates to genus (Gaichas *et al.*, 2005). Observers stationed at processing plants may be presented with only the 'wings' (*i.e.* pectoral fins) of skates, making identification even more difficult.

The utility of DNA 'barcoding' as a robust method for determining species identity using mitochondrial cytochrome *c* oxidase subunit I (COI) has been established for a number of vertebrate and invertebrate taxa (Hebert *et al.*, 2003, 2004*a*, *b*), and has prompted international efforts to standardize screening of species diversity and to accelerate the discovery of new species. This paper describes a DNA barcoding approach to identifying North Pacific skates and its potential applications to fishery management and conservation.

Skates were collected during research surveys conducted by the Resource Assessment and Conservation Engineering Division of the Alaska Fisheries Science Center. A plug of muscle tissue was removed from each specimen for genetic analysis and preserved in 95% ethanol; whole specimens preserved as vouchers were fixed in formalin and preserved in 70% ethanol. Voucher specimens and tissues were archived in the University of Washington Fish Collection. Several large individuals were photographed for voucher purposes and then discarded at sea, a method proven reliable for North Pacific skates (Stevenson *et al.*, in press). The geographic range of samples was broad (Fig. 1) but did not encompass the entire species' range for any given species.

Skate genomic DNA (Table I) was extracted from muscle tissue using DNeasy tissue kits (Qiagen Inc., Valencia, CA, U.S.A.; reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA). Primers HCO2198 and LCO2190 (Folmer *et al.*, 1994) were initially used to amplify a 710 bp fragment of COI *via* the polymerase chain reaction (PCR). Amplifications were conducted in 10 μl volumes containing *c.* 100 ng

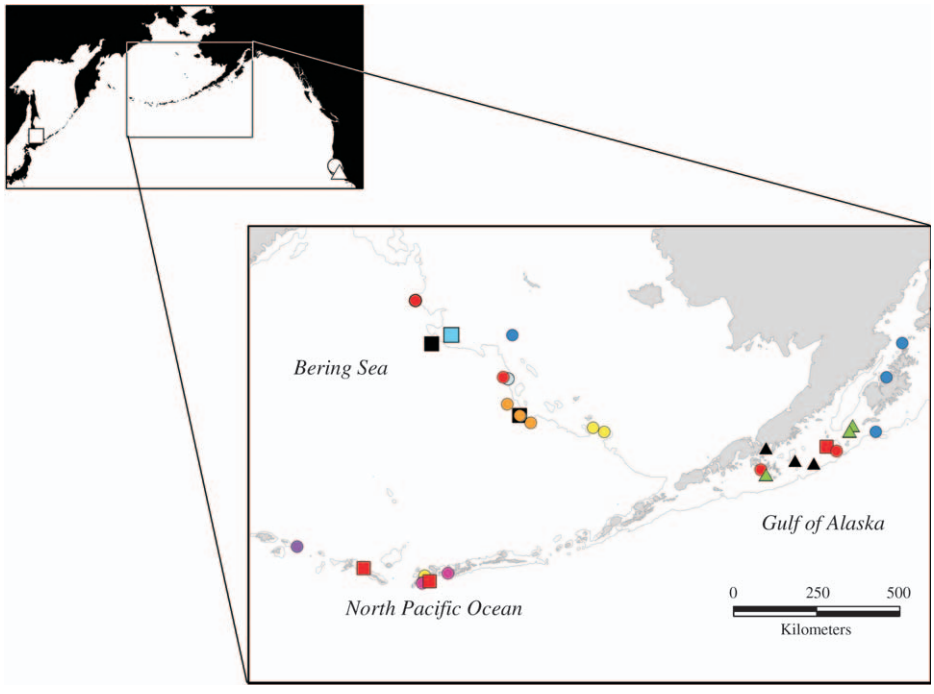

FIG. 1. Map showing skate sample collection locations. △, *A. badia*; □, *B. smirnovi*; ○, *B. abyssicola*; ▲, *R. rhina*; ▲, *R. binoculata*; ●, *B. aleutica*; ●, *B. interrupta*; ■, *B. parmifera*; ●, *B. minispinosa*; ●, *B. lindbergi*; ■, *B. maculata*; ●, *B. mariposa*; ■, *B. trachura*; ●, *B. taranetzi*; ●, *B. violacea*.

template DNA, 10 mM Tris-HCl (pH 8·3), 50 μM KCl, 3·0 mM $MgCl_2$, 1·5 mM dNTPs, 0·5 pM of each primer and 0·5 U Bioline *Taq* polymerase (Bioline USA, Inc., Boston, MA, U.S.A.). The thermal cycle profile, following Hebert *et al.* (2002), was conducted in an MJ PTC-200 DNA Engine thermocycler (MJ Research, Inc., Waltham, MA, U.S.A.).

Although the primers successfully amplified targeted co1 fragments in all species of *Bathyraja*, they did not work in either species of *Raja*. New primers were designed using Primer3 software (Rozen & Skaletsky, 2000) and the complete mtDNA sequence from *Raja radiata* Donovan, 1808 (Genbank accession number NC000893). These new primers, COI_RajaF (5′-CCGCTTAACTCTCAGCCATC-3′) and COI_RajaR (5′-TCAGGGTGACCAAAGAATCA-3′) were subsequently used to amplify a 750 bp fragment of co1 in all three genera using the same PCR and thermal cycle protocols described above, except for an annealing temperature of 57° C. PCR amplicons were treated with ExoSAP-IT^R (USB Corp., Cleveland, OH, U.S.A.) to degrade unincorporated primers and deoxynucleotides. About 35 fmoles of PCR product were used to seed a cycle sequencing reaction using ThermoSequenase II cycle sequencing chemistry (GE Healthcare/Amersham Biosciences, Buckinghamshire, U.K.). Primers used for cycle sequencing reaction were the same as those used for initial PCR amplification except that forward and reverse primers were 5′ labelled with IRD700 and IRD800, respectively (LI-COR Biosciences, Lincoln, NE,

U.S.A.). Automated sequencing of both strands was performed on a LI-COR 4300S (LI-COR Inc., Lincoln, NE, U.S.A.) and analysed using LI-COR eSeq v2.0 software.

58 individuals from three genera and 15 species were sequenced (Table I). Sequence contigs were assembled using Sequencher v4.2 (Gene Codes Corp., Ann Arbor, MI, U.S.A.). A 498 bp fragment was chosen for analyses based upon sequence overlap and quality and aligned consensus sequences were created using BioEdit 7.0.4.1 (Hall, 1999). The number of polymorphic sites, sequence diversity and pair-wise differences between species were determined using DnaSP ver. 3 (Rozas & Rozas, 1999) and sequence divergence was calculated with BioEdit. A total of 170 mutations were found in 142 segregating sites and 16 sites (14 transitions and two transversions) were polymorphic within species (Table II). The majority (88%) of substitutions occurred in the third nucleotide position within codons, while the remaining 12% were found in the first position. The GC% of codon positions 1 and 2 for the three North Pacific genera, calculated using CODONTREE (Pesole *et al.*, 1988), were similar to those reported for co1 sequences from 61 species of Australian sharks and rays (Ward *et al.*, 2005). However, the GC% for codon position 3 was *c.* 10% higher for the two *Raja* species and *A. badia* (39·2 and 39·2%, respectively) and *c.* 8% lower (21·8%) in *Bathyraja* than those reported for sharks and rays by Ward *et al.* (2005). Intraspecific nucleotide diversity (π) was low, a result consistent with studies of other elasmobranch fishes. For example, π estimated for the largest sample [*Bathyraja interrupta* (Gill & Townsend, 1897; $n = 10$] was 0·13%, somewhat lower than the range of 0·31–0·72% reported for the more variable control regions of *Raja* spp. (Valsecchi *et al.*, 2005) and blacktip shark, *Carcharhinus limbatus* (Müller & Henle, 1839) ($\pi =$ 0·21%; Keeney *et al.*, 2005).

Average intergeneric sequence divergence was 17·0% between *Raja* and *Bathyraja* species, and 12·5 and 17·6% between *A. badia v. Raja* and *Bathyraja* species, respectively (Table III). Intrageneric levels of divergence were 10% between the two *Raja* species and averaged 3·6% across congeneric pairs within *Bathyraja*. This latter value is low relative to those reported in other chordate species, where divergence in a 710 bp fragment of COI ranged from 4 to 32% in 94% of congeneric pairs (Hebert *et al.*, 2003). The large percentage of substitutions observed at third nucleotide positions in COI sequences inferred that multiple hits at these sites could have contributed some degree of downward bias in estimated divergence. Alternatively, rates of COI evolution in skates, as in sharks (Martin *et al.*, 1992), may be much lower than those in mammals. Comparisons with other mitogenomic regions may help to elucidate which process is more likely responsible for the low levels of observed intrageneric variation.

Unique COI sequences were obtained from 13 of the 15 skate species examined. Two species, *Bathyraja lindbergi* Ishiyama & Ishihara 1977, and *B. maculata*, displayed 0·5% sequence divergence, but displayed no fixed differences, and shared two nucleotide polymorphisms at positions 138 and 474 (Table II). This was a surprising result, given that species validity is strongly supported by adult morphology (Ishiyama & Ishihara, 1977; Ishihara & Ishiyama, 1985; Mecklenburg *et al.*, 2002) and egg case characters (G. R. Hoff, pers. comm.).

TABLE II. Variable nucleotide sites in 498 bp consensus sequences of cytochrome *c* oxidase subunit I in 15 species of skates. Dots indicate nucleotide identity with the *Raja rhina* sequence. Shaded sites indicate polymorphisms within species; K = G or T, Y = C or T, R = A or G

Species	Nucleotide position																																																	
	1	3	6	9	12	16	18	21	24	27	30	31	33	36	37	39	42	51	54	63	64	75	84	87	90	96	105	108	111	114	120	123	126	129	132	138	141	142	147	153	156	159	162	168	174	177	180	192	195	198
R. rhina	T	A	T	A	C	C	A	T	A	C	G	A	C	C	C	A	T	G	T	C	A	C	T	A	A	T	T	A	A	T	C	A	G	T	C	A	C	G	C	A	C	C	C	C	C	C	A	T	A	T
R. binoculata	·	·	C	G	·	·	G	·	·	T	K	·	A	·	·	G	C	A	·	·	·	·	A	G	G	·	·	·	·	·	·	G	A	·	T	T	·	·	T	R	T	·	·	·	·	T	C	·	·	·
A. badia	·	·	·	·	·	·	·	·	G	·	·	T	A	T	·	·	·	·	·	·	C	·	C	·	·	·	C	·	·	·	·	·	A	C	·	·	·	·	T	G	·	·	T	T	·	T	·	C	·	C
B. abyssicola	C	G	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	G	C	C	·	·	T	C	T	G	T	·	T	G	T	T	T	·	T	T	·	·	·	C
B. aleutica	C	G	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	G	C	C	T	·	T	C	T	G	T	·	T	G	T	T	T	·	T	T	·	·	·	C
B. interrupta	C	G	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	·	T	C	T	·	T	·	T	G	T	·	T	G	·	T	T	·	T	T	·	·	·	Y
B. lindbergi	C	·	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	·	·	C	T	T	T	C	T	R	T	·	T	G	·	T	T	·	T	T	·	·	·	C
B. maculata	C	·	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	·	·	C	T	T	T	C	T	R	T	·	T	G	·	T	T	·	T	T	·	·	·	C
B. mariposa	C	·	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	·	T	C	T	·	T	·	T	G	T	·	T	G	·	T	T	·	T	T	·	·	·	C
B. minispinosa	C	·	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	C	T	·	·	·	·	·	C	·	G	·	C	T	·	T	·	T	G	T	·	T	·	T	T	T	·	T	T	·	·	·	C
B. parmifera	C	G	C	·	T	T	·	C	·	T	A	·	·	T	T	·	·	A	C	T	·	·	·	·	·	C	·	·	·	C	T	·	T	·	T	G	T	·	T	G	T	T	T	·	T	T	·	·	G	C
B. taranetzi	C	·	C	·	T	T	·	·	·	T	A	·	C	T	T	·	·	·	·	T	·	·	·	·	·	C	·	G	·	C	T	·	T	C	T	G	T	R	T	G	T	T	T	·	T	T	·	·	·	C
B. trachura	C	G	C	·	T	T	·	C	·	T	A	·	·	T	T	·	A	·	·	T	·	T	·	·	·	C	·	·	·	C	T	·	T	C	T	G	T	·	T	G	T	T	·	·	T	T	·	·	·	·
B. smirnovi	C	G	C	·	T	T	·	C	·	T	A	·	·	T	T	·	·	A	C	T	·	·	·	·	·	C	·	·	·	C	T	·	T	·	T	·	T	·	T	G	T	T	T	·	T	T	·	·	G	C
B. violacea	C	·	C	·	T	T	·	·	·	T	A	·	·	T	T	·	·	·	·	T	·	·	·	·	·	C	·	·	T	C	T	·	T	·	T	G	T	·	T	G	·	T	T	·	T	T	·	·	·	C

Species	204	201	208	210	213	216	219	222	225	228	231	232	234	237	240	243	246	249	252	255	258	261	264	267	270	279	282	285	288	289	291	294	297	300	301	303	309	312	315	318	324	327	328	339	342
R. rhina	A	T	T	G	C	C	T	C	C	C	C	C	G	C	C	T	G	T	A	T	G	C	A	A	T	T	C	A	T	T	G	A	T	T	C	A	T	A	A	C	A	C	T	C	T
R. binoculata	·	·	C	A	T	·	·	·	·	·	·	T	·	·	T	C	·	A	·	C	·	·	G	·	·	A	·	·	C	·	·	·	G	·	A	·	C	G	G	·	G	·	·	·	·
A. badia	·	·	·	A	·	T	A	T	T	T	·	·	·	·	·	·	·	·	·	C	A	T	·	·	·	C	·	C	·	C	·	T	A	C	·	·	C	G	G	·	·	·	·	·	·
B. abyssicola	·	C	C	A	T	A	·	T	T	G	T	·	A	·	T	C	T	·	·	·	A	A	G	T	C	·	T	C	A	C	A	·	G	·	·	T	·	·	·	T	T	T	·	·	·
B. aleutica	·	C	C	A		A	·	T	T	G	T	·	A	·	T	C	T	·	·	·	A	A	G	T	C	·	T	C	A	C	A	·	A	·	·	T	·	·	·	T	T	T	·	·	·
B. interrupta	·	C	C	A	T	A	·	T	T	G	T	·	A	T	T	C	T	·	·	·	A	A	·	T	C	·	T	C	A	C	A	·	A	·	·	T	·	·	R	T	T	T	·	·	·
B. lindbergi	·	C	C	·	·	A	·	T	T	G	T	·	A	·	T	C	T	·	·	·	A	A	G	T	C	·	T	C	A	C	A	G	G	·	·	T	C	·	·	T	T	T	·	T	Y
B. maculata	·	C	C	·	·	A	·	T	T	G	T	·	A	·	T	C	T	·	·	·	A	A	G	T	C	·	T	C	A	C	A	G	G	·	·	T	C	·	·	T	T	T	·	T	·
B. mariposa	·	C	C	A	T	A	·	T	T	G	T	·	A	T	T	C	T	·	·	·	A	A	·	T	C	·	T	C	A	C	A	·	A	·	·	T	·	·	·	T	T	T	Y	·	·
B. minispinosa	·	C	C	·	T	A	·	T	T	G	T	·	A	T	T	C	T	·	·	C	A	A	·	T	C	·	T	T	A	C	A	·	A	·	·	T	·	·	·	T	T	T	·	·	·
B. parmifera	·	C	C	·	T	A	·	T	T	G	T	·	A	T	T	C	T	·	·	·	A	A	·	T	C	·	T	T	A	C	A	·	A	·	·	T	·	·	·	T	T	T	·	·	·
B. taranetzi	·	C	C	·	T	A	·	T	T	G	T	·	A	·	T	C	T	·	·	·	A	A	G	T	C	·	T	C	A	·	A	·	·	·	·	T	·	·	·	T	T	T	·	·	·
B. trachura	·	C	C	A	T	A	·	T	T	G	T	·	A	·	T	C	T	C	G	·	A	A	C	G	C	·	T	C	A	C	A	·	A	·	·	T	·	·	·	T	T	T	·	·	·
B. smirnovi	·	C	C	·	T	A	·	T	T	G	T	·	A	T	T	C	T	·	·	C	A	A	·	T	C	·	T	T	A	C	A	·	A	·	·	T	·	·	·	T	T	T	·	·	·
B. violacea	G	C	C	A	T	A	·	T	T	G	T	·	A	T	T	C	T	·	·	·	A	A	·	T	C	·	T	C	A	C	A	·	A	·	·	T	·	·	·	T	T	T	·	·	·

TABLE II. Continued

Species	Nucleotide position																																													
	345	348	349	351	354	357	358	360	363	366	369	370	372	375	378	384	387	390	393	396	399	402	405	408	411	414	423	426	429	432	435	441	447	450	453	456	457	459	465	471	474	475	477	484	489	492	498
R. rhina	T	C	T	G	A	C	G	T	A	C	T	C	A	C	C	C	C	C	C	C	T	T	T	A	A	A	C	T	G	C	G	C	C	A	G	G	A	T	T	T	C	T	A	A	C	C	T
R. binoculata	·	T	C	A	·	T	·	·	·	T	C	·	G	·	·	·	·	·	·	·	·	·	Y	·	·	G	·	·	A	·	A	T	·	·	A	A	·	·	·	·	·	·	·	·	·	·	C
A badia	·	T	·	·	·	T	A	·	T	·	C	T	·	·	·	·	·	·	·	·	C	C	C	·	·	C	·	·	·	·	·	·	·	G	·	T	·	C	C	C	·	·	G	·	·	·	A
B. abyssicola	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	T	T	T	T	·	·	·	·	·	·	T	A	A	·	A	T	T	·	A	A	G	·	C	·	A	C	·	T	T	·	·
B. aleutica	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	T	T	T	T	·	·	·	·	·	·	T	A	A	·	A	T	T	·	A	A	G	·	C	·	A	C	·	T	T	·	·
B. interrupta	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	·	T	T	T	C	·	·	·	·	·	T	A	A	·	A	T	T	R	A	A	G	·	C	·	A	·	·	T	T	·	·
B. lindbergi	·	·	·	A	C	A	A	·	·	T	C	T	·	A	A	T	T	T	A	T	C	·	·	·	·	·	T	A	A	·	A	T	T	G	A	A	G	·		·	R	Y	·	T	T	·	·
B. maculata	·	·	·	A	C	A	A	·	·	T	C	T	·	A	A	T	T	T	A	T	C	·	·	·	·	·	T	A	A	·	A	T	T	G	A	A	G	·		·	R	C	·	T	T	·	·
B. mariposa	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	·	T	T	T	C	·	·	·	·	·	T	A	A	·	A	T	T	G	A	A	G	·	C	·	A	·	·	T	T	·	·
B. minispinosa	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	T	T	T	C	C	C	·	·	·	·	T	A	A	·	A	T	T	C	A	A	G	·	C	·	A	C	·	T	T	·	·
B. parmifera	·	·	·	A	C	A	A	C	·	K	C	T	·	A	A	T	·	T	T	T	C	·	·	·	·	·	·	A	A	·	A	T	T	C	A	A	G	·	·	·	A	C	·	T	T	T	·
B. taranetzi	C	·	·	A	T	A	A	C	·	T	C	T	·	A	A	T	T	T	T	T	·	·	·	G	G	·	T	A	A	T	A	T	T	R	A	A	G	·	C	·	A	C	·	T	T	·	·
B. trachura	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	·	T	T	T	·	·	·	·	·	·	T	A	A	·	A	T	T	G	A	A	G	·	C	·	A	C	·	T	T	·	C
B. smirnovi	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	·	T	T	T	·	·	·	·	G	·	T	A	A	·	A	T	T	C	A	A	G	·	·	·	A	C	·	T	T	T	·
B. violacea	·	·	·	A	C	A	A	C	·	T	C	T	·	A	A	T	·	T	T	T	C	·	·	·	·	·	T	A	A	·	A	T	T	G	A	A	G	·	C	·	A	·	·	T	T	·	·

TABLE III. Pair-wise nucleotide differences (below diagonal) and percent sequence divergence (above diagonal) in 498 bp consensus sequences of cytochrome *c* oxidase subunit I from 15 species of skates

	R. rhina	*R. binoculata*	*A. badia*	*B. abyssicola*	*B. aleutica*	*B. interrupta*	*B. lindbergi*	*B. maculata*	*B. mariposa*	*B. minispinosa*	*B. parmifera*	*B. taranetzi*	*B. trachura*	*B. smirnovi*	*B. violacea*
R. rhina	—	10·3	11·7	16·9	17·1	16·7	16·9	16·7	16·5	17·1	17·1	17·3	17·1	17·3	16·5
R. binoculata	51	—	13·3	17·9	18·7	18·5	18·5	18·3	18·5	18·9	18·9	19·1	17·7	18·3	18·3
A. badia	58	66	—	17·5	17·5	17·3	17·1	16·7	16·9	17·5	18·5	18·7	17·7	18·5	16·9
B. abyssicola	84	89	87	—	0·9	2·9	4·1	3·7	2·9	3·3	4·1	2·7	3·3	4·1	2·9
B. aleutica	85	93	87	4	—	2·9	3·7	3·3	2·9	3·3	4·1	2·9	3·3	4·1	2·9
B. interrupta	83	92	86	14	14	—	4·7	4·5	1·1	3·3	3·3	4·3	3·7	3·4	0·6
B. lindbergi	84	92	85	20	18	23	—	0·5	4·1	5·1	5·5	4·9	5·7	5·2	3·5
B. maculata	83	91	83	18	16	22	2	—	3·9	4·7	5·1	4·5	5·3	5·1	3·7
B. mariposa	82	92	84	14	14	5	19	19	—	2·8	2·8	4·0	3·6	3·7	0·5
B. minispinosa	85	94	87	16	16	16	25	23	14	—	2·9	3·9	4·9	2·9	2·9
B. parmifera	85	94	92	20	20	16	27	25	14	14	—	5·1	4·9	1·2	3·2
B. taranetzi	86	95	93	13	14	21	24	22	20	19	25	—	4·7	4·5	3·7
B. trachura	85	88	88	16	16	18	28	26	18	24	24	23	—	4·9	3·7
B. smirnovi	86	91	92	20	20	16	24	24	17	14	5	22	24	—	18
B. violacea	82	91	84	14	14	2	16	17	1	14	15	18	18	18	—

Additional mtDNA sequence data would be needed to investigate this finding. The level of sequence divergence between *Bathyraja abyssicola* (Gilbert, 1896) and *B. aleutica* was also low (0·8%), but included four apparently fixed nucleotide sites.

While additional COI sequencing on specimens taken across species ranges would be necessary to fully resolve fixed and polymorphic differences within and among North Pacific skates, these preliminary results indicate that DNA barcoding can successfully complement traditional field and laboratory methods for species identification. It provides a framework for the development of simple, inexpensive, PCR-based assays (*e.g.* single nucleotide polymorphism or restriction length fragment polymorphism protocols) that can clearly differentiate virtually all skate species known from Alaskan waters. Applications developed from a barcoding approach may be particularly useful in situations where fisheries observer skills or coverage, or both, are incomplete. DNA collections can be obtained quickly from small samples of tissue without damage to processed skate products, preclude the expense and difficulty of preserving large specimens in field surveys, and represent a potential method to identify cryptic species within the North Pacific skate fauna.

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