Assessing the phylogenetic relationships of softnose skates (*Bathyraja*) based on RADseq and mtDNA data

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A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Marine Affairs

University of Washington

2024

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School of Marine and Environmental Affairs

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Abstract

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Despite their high species diversity, skates (Rajidae) exhibit morphological conservatism which may hamper identification and assessment efforts. *Bathyraja*, the most species-rich genus of skate, is known to have particularly unclear systematics and several ambiguous species boundaries. To elucidate these phylogenetic relationships, I assessed RADseq data from 139 specimens across 17 species of *Bathyraja* available in the University of Washington Fish Collection. I compared these results to a phylogenetic tree generated from COI sequences available on GenBank to evaluate whether genome-wide sequencing can provide greater resolution than a single gene approach. Both trees demonstrated possible instances of incomplete lineage sorting, resolving *B. panthera* as paraphyletic in the RADseq tree and *B. parmifera* as paraphyletic in the COI tree. Incomplete lineage sorting was also possible for *B. mariposa* and *B.*

violacea, sister species that exhibited reciprocal monophyly within the *B. interrupta* clade. The COI tree failed to separate *B. maculata* and *B. lindbergi* into genetically isolated groups, as has been observed previously. Conversely, the RADseq tree resolved *B. maculata* and *B. lindbergi* into distinct lineages. Neither phylogenetic tree showed clear genetic divergence between *B. kincaidii* and *B. interrupta*, a species pair with particularly uncertain boundaries. The species pair did exhibit population structure in the Salish Sea, a pattern that has not yet been observed for either species. Morphological and genetic assessments of samples throughout the entire *B. interrupta/B. kincaidii* range is needed to resolve whether *B. kincaidii* is a junior synonym of *B. interrupta*.

1. Introduction

Skates (Rajidae) are dorsally flattened, cartilaginous fish that spend most of their lives on the seafloor. They are 'equilibrium' life history strategists, with low fecundity, high juvenile survivorship, long life spans, and late maturity (Frisk 2010). Alaska skates (*Bathyraja parmifera*), a species that represents 90% of all skate abundance in the North Pacific (Markowitz et al. 2023), reach maturity at 9 and 10 years, for males and females respectively (Matta and Gunderson 2009). Some skate species do not reach sexual maturity until more than 20 years of age (Fry Maurer 2009, Ainsley et al. 2011b). Like other equilibrium strategists, skates can be particularly vulnerable to overfishing because their slow population growth hampers their ability to quickly rebound from reductions in population size (King and McFarlane 2003, Dulvy et al. 2000).

A targeted skate fishery does not currently exist in the North Pacific; however, they are incidentally caught in commercial fisheries (Cronin-Fine 2023). As of 2023, skates are not

subjected to overfishing and maintain a stock status of "no concern" in Alaska (Cronin-Fine 2023, Tribuzio et al. 2023). Species-specific management has been suggested for skates, but this system has not been implemented in federally managed fisheries in the United States; all *Bathyraja* species, except for *B. parmifera* in the Bering Sea and Aleutian Islands, are managed in aggregate (Cronin-Fine 2023, Tribuzio et al. 2023). This system emerged in the absence of species-specific data and the relatively low economic importance of skates in commercial fisheries, as well as logistical difficulties of identifying and considering each species separately (Ormseth 2021). Data sources began to improve in 2004, when fisheries observers were required to identify skates to species (Stevenson 2004). Four years later, observers identified over 95% of skates to genus and over 50% to species (Stevenson and Lewis 2010). While this is a positive advancement, accurate identification hinges on accurate systematics, which have been brought under scrutiny for *Bathyraja*.

Bathyraja systematics have been described as problematic, poorly resolved, and in need of descriptive work (Ebert 2004, Stevenson et al. 2004, Naylor et al. 2012). Several factors contribute to this confusion. First, skates are highly morphologically conserved despite having high species diversity (McEachran and Dunn 1998), which can pose a significant challenge when identifying species from morphology. Second, many *Bathyraja* species were described from a single holotype, some of which have since disintegrated or been lost (Fricke et al. 2024). Unique holotypes are problematic because they do not provide insight on intraspecific variation and are inherently risky; if the holotype is lost, it can no longer be referenced to resolve taxonomic uncertainties that emerge later. Third, *Bathyraja* research has progressed considerably in recent years, putting forth new species descriptions (Orr et al. 2011, Misawa et al. 2020, Ebert et al. 2022), taxonomic revisions (Stehmann et al. 2021), and identifying potential cryptic speciation

(Spies et al. 2021). While these studies enhance our knowledge of *Bathyraja*, a genus-wide genetic assessment can provide an overview of current skate systematics and may illuminate intraspecific cryptic diversity.

Genetic methods are commonly used to complement morphological species descriptions and interrogate species boundaries. Perhaps the most common genetic approach involves cytochrome c oxidase subunit I (COI), a mitochondrial gene that has become a standard 'barcode' for marking species biodiversity (Hebert et al. 2003a). The COI gene is particularly useful because it is represented in mitochondrial genomes across all animal phyla and possesses a strong phylogenetic signal (Hebert et al. 2003a). For recently separated species, however, phylogenetic relationships derived from COI data may not align with species boundaries (Neigel and Avise 1986). This appears to be the case for some closely related *Bathyraja* species pairs, whose COI sequences diverge by less than 1% (Spies et al. 2006). Of these species pairs, the commander skate (*Bathyraja lindbergi*) and the whiteblotched skate (*Bathyraja maculata*) show no fixed differences between their COI, despite having clear morphological differences (Spies et al. 2006). In these instances of high COI conservation, a genome-wide approach may be better suited to discern minute genetic differences of slowly-evolving species (Martin et al. 1992, Ball et al. 2016).

One cost-effective form of genome-wide sequencing is restriction-site-associated DNA sequencing (RADseq), a high-throughput technique that provides gigabases of DNA across the genome of non-model organisms (Davey and Blaxter 2010). With RADseq, restriction enzymes cut DNA at specific sites across the genome then the adjacent regions are sequenced. When those regions are compared across samples, single nucleotide polymorphisms (SNPs) can be detected and used to determine genetic relationships (Davey and Blaxter 2010). This technique has

become increasingly useful for delimiting species of fishes, including plunder fishes (Parker et al. 2022), red snappers (Pedraza-Marrón et al. 2019), and Lake Victoria cichlids (Wagner et al. 2013). It has also been used to resolve genus and family-wide phylogenies of snailfishes (Orr et al. 2019), tunas (Díaz-Arce et al. 2016), and surfperches (Longo and Bernardi 2015). Within *Bathyraja*, RADseq has been used to study population genetics and genetic diversity within skate egg 'nurseries' (Spies et al. 2021a, Spies et al. 2021b), but not to clarify taxonomic relationships across all *Bathyraja* species that occur in the North Pacific.

In this study, I used RADseq and COI data to assess phylogenetic relationships of skates within the genus *Bathyraja* and to investigate whether genome-wide sequencing is better suited to discern genetic variations between slowly evolving species. To investigate these questions, I examined 139 *Bathyraja* specimens from the University of Washington Fish Collection, spanning 17 species that occur in the North Pacific. Using SNPs generated from RADseq data, I produced a phylogenetic tree to visualize the relationships between species. I then described problematic species groups, assessed the validity of *B. kincaidii*, and commented on potential sources of unclear boundaries.

2. Materials and Methods

2.1. Taxon sampling

I included a total of 139 specimens across 17 *Bathyraja* species from the University of Washington Fish Collection in my analysis (Table 1 & Table S1). To allow for morphological comparison, only vouchered specimens were selected and an emphasis was placed on post-egg specimens (>200 mm in total length; Hoff 2008). I increased sample sizes of *B. parmifera*, *B.*

panthera, and *B. interrupta* (n=56, n=20, and n=40, respectively) relative to the other species to detect suspected cryptic speciation.

2.2. DNA extraction, RADseq library assembly, and sequencing

DNA was extracted from tissue samples using the Qiagen DNeasy blood and tissue kit according to manufacturers' instructions (Qiagen, Inc., Valencia, CA), then sent to the NGS Facility at University of Wisconsin-Madison for library assembly and RAD sequencing. Samples were sequenced using ddRADseq, a form of RADseq that introduces two restriction enzymes to 'double digest' the DNA. The additional digestion creates a subset of DNA fragments that meet specific size-selection criteria, which improves the resolution and coverage of reads across the genome and (Peterson et al. 2012). It can also accommodate small starting amounts of DNA material, which is advantageous when working with tissue samples that are poor in quality (Peterson et al. 2012).

Eight samples to be optimized were chosen at random to generate RADseq libraries with each enzyme offered by the facility. The enzyme combinations used were ApeKI, PstI/MspI, PstI/BfaI, NsiI/MspI, and NsiI/BfaI. The libraries were run on an Agilent Tapestation 4200 (Agilent Technologies, Inc., Santa Clara, CA) to observe the distribution of fragment sizes. PstI/BfaI was chosen based on the smooth profile and concise fragment size range.

RADseq libraries were prepared as in Elshire et al (2011) with minimal modification. In short, 150 ng of DNA was digested using PstI and BfaI (New England Biolabs, Ipswich, MA) after which barcoded adapters amenable to Illumina sequencing were added by ligation with T4 ligase (New England Biolabs, Ipswich, MA). Ninety-six adapter-ligated samples were pooled and amplified to provide library quantities amenable for sequencing, and adapter dimers were

removed by SPRI bead purification. Quality and quantity of the finished libraries were assessed using the Agilent Tapestation and Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY), respectively. Libraries were sequenced on Illumina NovaSeq X Plus using paired end 150 bp reads.

2.3. Sequence processing and locus assembly

Sequences were processed using the Stacks 2.4.1 pipeline (Catchen et al. 2013) on the University of Washington's High Performance Computer Cluster. First, I ran the *process_radtags* module to filter and demultiplex RAD sequences into their respective samples. In this module, sequences were trimmed to 105 base pairs in length, a length chosen based on the MultiQC report provided by University of Wisconsin–Madison. Next, the *clone_filter* module was used to identify and remove PCR duplicates.

Because a reference genome for *Bathyraja* was not available, the trimmed and filtered reads were aligned de novo using the module *denovo_map.pl*. Within *denovo_map.pl*, the sub-module *ustacks* aligns short-read sequences into putative alleles using a maximum likelihood framework (Hohenlohe et al. 2010). *Ustacks* accepts three parameters: '-M' (maximum nucleotide mismatches allowed *between* stacks to be considered a loci) '-m' {minimum depth of read coverage required to form a stack), and '-N' (maximum nucleotide mismatches allowed to align secondary reads to primary stacks). These parameters can be adjusted to optimize RAD loci demarcations based on genetic distances between individuals. Because my analysis spanned 17 species, I increased '-M' from 2 to 4 allowable mismatches to account for greater genetic distances. Parameters '-m' and '-N' were kept at default values of 3 and M+2, respectively.

Within *denovo_map.pl*, the sub-program *populations* was used to generate files in PLINK format, which could then be filtered with PLINK software v.1.90b7.2. Specifically, PLINK argument '-geno' was set to 0.50 to remove loci present in less than 50% of individuals, ensuring adequate loci coverage for my phylogenetic analysis (Table 2). The '-mind' argument, which sets a minimum threshold for individual genotypic rates, was not applied so that all 139 individuals were retained for phylogenetic analysis. To remove noise generated by sequencing errors, minor alleles present in less than 5% of the population were discarded using the '-maf' argument. The 24,055 loci that passed these filters were then compiled into a whitelist. *Populations* was re-run with this whitelist to generate align sequences and generate a PHYLIP file (Felsenstein 1993) containing all variable sites (36,500 base pairs) from these loci.

2.4. Phylogenetic analyses

All available COI sequences on GenBank from *Bathyraja* species included in RADseq analysis (N = 156) were retrieved (GenBank accession numbers available in Table S2). I aligned COI sequences in Geneious and analyzed them using Bayesian phylogenetic inference in the program MrBayes ver. 3.2 (Ronquist et al. 2012). I used a GTR+G substitution model for the alignment of 36,500 bp. The analysis consisted of two parallel Markov Chain Monte Carlo runs, each with four chains, run for 20,000,000 generations. Convergence of parallel runs was assessed by comparing plots of log-likelihood vs. generation in Tracer ver. 1.5 (Rambaut and Drummond 2007), evaluating the standard deviation of split frequency statistic and the Potential Scale Reduction Factor statistic in MrBayes, and by visually inspecting the resulting topologies of consensus trees from each run. Taxa with large amounts of missing data and poor quality sequences were removed, which lowered the total number of specimens and species represented

on the RADseq tree to 114 and 16, respectively. I also verified that estimated sample sizes for each parameter from each run were greater than 200 using Tracer.

3. Results

3.1. Nuclear and mitochondrial phylogenies

Phylogenetic analyses of RADseq data and COI data resulted in trees with similar topologies (Fig. 1 & Fig. 2). In both trees, interspecific divergences were well-supported with posterior probabilities close to 1 for the following species: *B. abyssicola*, *B. aleutica*, *B. spinosissima*, *B. trachura*, *B. taranetzi*, *B. minispinosa* and *B. smirnovi*. Some intraspecific relationships for *B. parmifera*, *B. panthera*, and *B. interrupta* were less well-supported, with posterior probabilities falling between 0.62 and 1.

3.2. B. interrupta and *B. kincaidii*

One species pair, *B. interrupta* and *B. kincaidii*, did not separate out into distinct clades with either genetic dataset. On the COI tree, *B. kincaidii* was interspersed throughout the *B. interrupta* clade with relationships supported by posterior probabilities between 0.71 and 1. On the RADseq tree, all *B. kincaidii* specimens (n = 4) were represented in one monophyletic group that also contained two *B. interrupta* specimens with high support; each node in the RADseq had a posterior probability of 1. All specimens represented within that monophyletic group were collected from the Salish Sea (Fig. 3).

3.3. B. maculata and *B. lindbergi*

B. maculata and *B. lindbergi* were poorly resolved on the COI tree; they did not sort by species, branch lengths were relatively short, and posterior probabilities were as low as 0.56. On the RADseq tree, these two species were represented as sister species.

3.4. *B. parmifera* and *B. panthera*

Unexpectedly, *B. panthera* did not completely separate from *B. parmifera* on either tree. On the RADseq tree, the *B. panthera* clade was nested within *B. parmifera*, rendering *B. parmifera* paraphyletic. The opposite was true on the COI tree; the *B. parmifera* clade emerged within *B. panthera*. On the RADseq tree, one putative *B. parmifera* individual was grouped within *B. panthera*.

3.5. B. violacea and *B. mariposa*

On the RADseq tree, *B. violacea* and *B. mariposa* separated into reciprocally monophyletic groups within the *B. interrupta* clade. In contrast, on the COI tree, seven *B. violacea* individuals formed a monophyletic clade external to the *B. interrupta* clade, though the other *B. violacea* individual appeared within the *B. interrupta* clade (see Fig. S1 for non-collapsed COI tree). Also on the COI tree, all *B. mariposa* individuals (n = 7) were resolved within the *B. interrupta* clade, contributing to the paraphyly of *B. interrupta*.

4. Discussion

The high morphological conservatism along with subtle intraspecific morphological plasticity in skates have presented challenges for skate identification. These attributes, coupled with the subjectivity in how a species is defined, has mustered concerns about the accuracy of

Bathyraja systematics. Genetic approaches have been used to interrogate morphological species definitions, as well as to provide insight on evolutionary histories. Preliminary studies using mitochondrial markers identified some skate relationships that did not align with morphology-based species delimitations (Naylor et al. 2012, Spies et al. 2006). Here, I demonstrate the utility of RADseq, a genome-wide sequencing approach, in conjunction with COI sequences available on GenBank, to examine a number of questionable taxonomic relationships within *Bathyraja*.

4.1. B. interrupta and *B. kincaidii*

The validity of *B. interrupta* and *B. kincaidii* as separate species has been heavily contested since *B. kincaidii* was first described (Ishihara and Ishiyama 1985, Mecklenburg 2002, Compagno 2005, Last et al. 2016a, Last et al. 2016b, Knuckey and Ebert 2022). Garman (1908) described *B. kincaidii* as a new species from a single holotype collected in Puget Sound. The original species description did not explicitly describe how *B. kincaidii* differed from *B. interrupta*, leaving the distinction between the two species ambiguous (Garman 1908). Years later, Ishihara and Ishiyama (1985) re-examined the *B. kincaidii* holotype and claimed *B. kincaidii* to be a junior synonym of *B. interrupta*. Recent taxonomic work has supported the separation of the two species, citing subtle morphological differences (Knuckey and Ebert 2022), however, this examination only included *B. interrupta* specimens from the Bering Sea, which would not capture any geography-driven morphological variation of *B. interrupta*. Furthermore, characters that are typically used to identify skates, such as mid-dorsal thorns, are inconsistent in the literature; NMFS (2015) suggests that the row of middorsal thorns is *usually* interrupted in *B. interrupta* while Ebert et al. (2017) and Knuckey and Ebert (2022) describe mid-dorsal thorns as discontinuous in *B. interrupta* and continuous in *B. kincaidii*, respectively.

The nuclear and mitochondrial phylogenies also suggest discordance. On both phylogenetic trees, *B. kincaidii* and *B. interrupta* did not separate into clear monophyletic groupings. The lack of genetic separation indicates that *B. interrupta* and *B. kincaidii* are not distinct, but instead represent a species continuum from the Bering Sea to southern California. Under this conclusion, the purported morphological differences between species may instead be examples of intraspecific phenotypic plasticity. Alternatively, it is plausible that these may be two valid species that display high morphological and genetic conservatism. This theory has been supported by previous studies on the basis of morphology, however, the limited assessment of southern *B. interrupta* specimens undermines this claim. It is also worth noting that as a public database, GenBank may contain sequences that have been mis-identified (Meiklejohn et al. 2019). Given the morphological conservatism and unreliability of dichotomous keys for *B. interrupta* and *B. kincaidii*, it is possible that the COI sequences retrieved from GenBank may not be correctly labeled. To definitively join or separate these two species, an assessment of both morphology and genetics of specimens representing their complete ranges is necessary.

Interestingly, the RADseq tree indicated population structure within the Salish Sea, which has not been previously described for *B. interrupta*. The inclusion of all *B. kincaidii* individuals within one phylogeographic clade may initially seem like evidence of incipient speciation of *B. kincaidii* restricted to the Salish Sea, however, this clade also contains two *B. interrupta* specimens. Additionally, the branch separating this clade from the closest relative, a *B. interrupta* specimen from British Columbia, was relatively short in comparison to the branches separating valid species groups, such as *B. mariposa* and *B. violacea*, from *B. interrupta*.

4.2. Nuclear and mitochondrial phylogenies

In some cases, genome-wide sequencing has been more helpful than mitochondrial markers in clarifying species boundaries (Díaz-Arce et al. 2016, Pedraza-Marrón et al. 2019, Piñeros et al. 2022). In my analysis, the phylogenetic trees derived from COI data and RADseq data resolved species into nearly identical topologies, except for one group: *B. maculata* and *B. lindbergi*. The COI tree was consistent with the findings of Spies et al. (2016), showing little genetic differentiation between *B. lindbergi* and *B. maculata* COI despite their clear morphological differences. While the collective sample size for these two species was limited in the RADseq data set (n = 3), the lineages resolved more clearly than they did on the COI tree, positioning the two as sister species. However, increasing the sample size would capture intraspecific diversity, which may reveal a different phylogenetic relationship between the two species.

For the other closely related species groups (*B. interrupta/B. kincaidii*, *B. mariposa/B. violacea*, *B. parmifera/B. panthera*), the genetic evidence was not as decisive; many species boundary questions persisted across sequencing approaches. It is notable that the COI tree did resolve many species into reciprocally monophyletic groups, indicating that COI *can* capture species boundaries in *Bathyraja*. Presence of pattern suggests that cases of non-reciprocally monophyletic species on the COI tree are evidence of incomplete lineage sorting, where the evolutionary history of a single-gene does not match to the evolutionary history of the organism. It is possible that COI evolves slowly in skates, a phenomenon that has been observed in other elasmobranchs (Martin et al. 1992), which could exacerbate instances of incomplete lineage sorting.

Incomplete lineage sorting was detected for other species in the RADseq tree as well. *B. panthera*, a species described in 2011 (Orr et al. 2011), fell within the *B. parmifera* clade, thereby classifying *B. parmifera* as paraphyletic. Similarly, *B. mariposa* and *B. violacea* were not completely isolated from *B. interrupta*. Rather, their nuclear DNA has not yet diverged sufficiently to place them in reciprocally-monophyletic clades. Because the *B. parmifera/B. panthera* and *B. violacea/B. mariposa* species pairs have been validated as distinct species on the basis of morphology (Stevenson et al. 2004, Orr et al. 2011), this is evidence of incomplete lineage sorting, contributing to the paraphyly of the *B. interrupta* clade.

5. Conclusion

Genome-wide sequencing and COI sequencing produced a similar level of resolution for *Bathyraja* species. The agreement between the two genetic approaches indicates that unclear genetic delineations between species do not result from lack of sequencing resolution, but rather are evidence of external events, such as incomplete lineage sorting or taxonomic over-splitting. In particular, the debate on the validity of *B. kincaidii* as a distinct species or junior synonym of *B. interrupta* persists. This study provides preliminary genetic insight into the complicated relationship between *B. interrupta* and *B. kincaidii*. In the light of their contentious history, *B. kincaidii* and *B. interrupta* would benefit from a comprehensive genetic analysis that examines the two species throughout the entirety of their ranges.

Tables and Figures

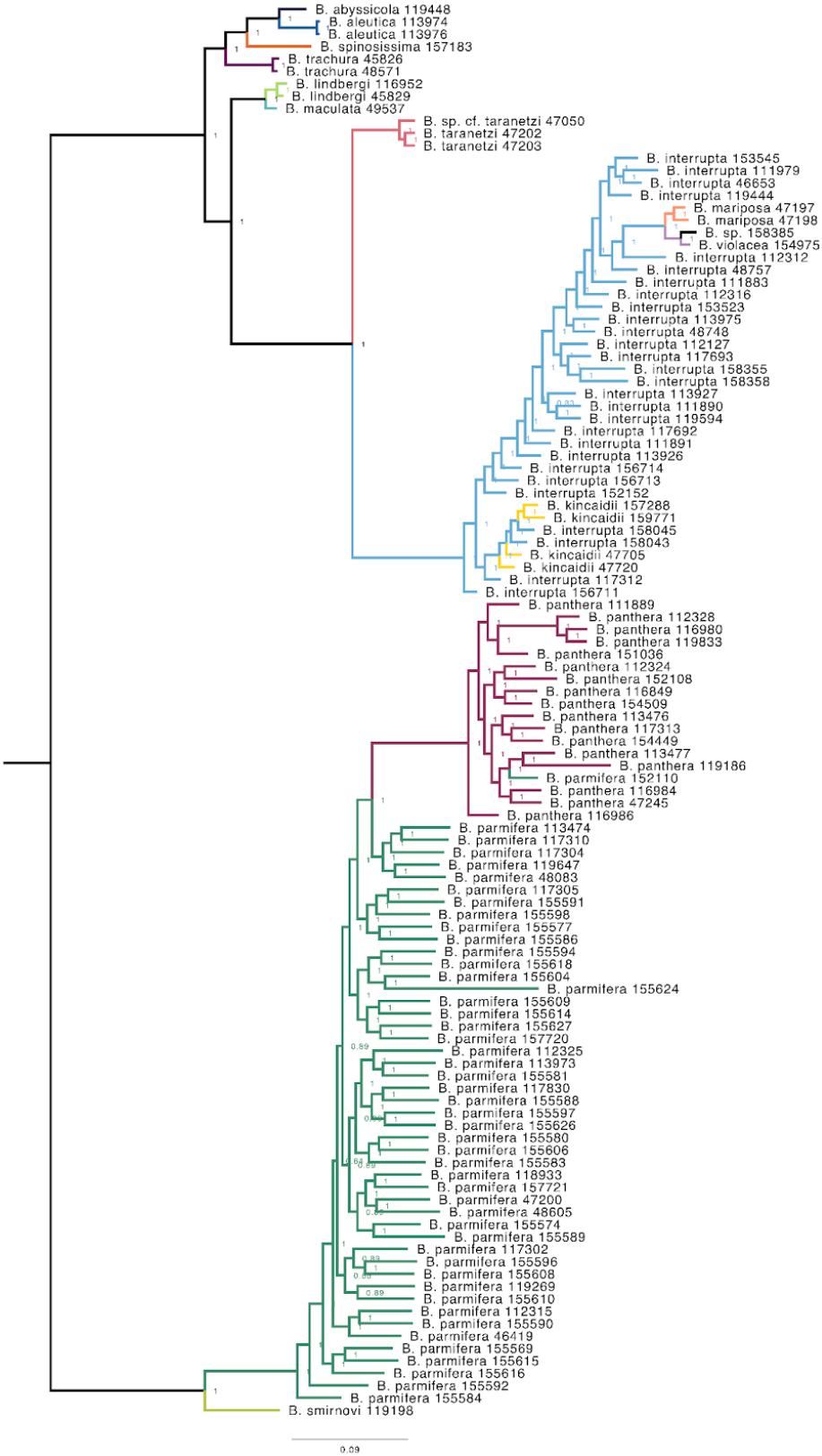

Figure 1. Hypothesis of the phylogenetic relationships of 114 *Bathyraja* specimens representing 16 species based on RADseq data. These relationships were inferred using Bayesian phylogenetic inference with a GTR+G substitution model. Node values represent posterior probabilities from the MCMC chain.


B. lindbergi DQ104908
B. maculata JN602398
0.8
B. maculata DQ104910
0.56
B. lindbergi DQ104907
B. lindbergi JN602397
0.96
B. lindbergi DQ104909
1
B. lindbergi DQ104905
0.97
B. lindbergi DQ104906
B. maculata DQ104911
B. maculata JN602399
B. taranetzi
B. violacea
B. aleutica JN602385
B. aleutica
0.99
B. abyssicola
0.98
B. spinosissima MF158863
B. trachura
B. interrupta GQ449760
B. interrupta JN602393
B. interrupta DQ104897
B. interrupta GQ449776
B. interrupta DQ104898
B. interrupta GQ449761
B. interrupta GQ449777
B. mariposa GQ449783
0.98
B. violacea DQ665298
0.62
B. mariposa DQ104912
B. mariposa DQ104913
B. mariposa DQ104914
B. mariposa DQ104915
B. interrupta GQ449765
B. interrupta GQ449763
B. interrupta GQ449764
B. interrupta GQ449772
B. mariposa JN602400
B. interrupta JN602394
B. interrupta JQ354010
B. interrupta DQ104902
B. interrupta GQ449768
B. interrupta DQ104896
B. interrupta DQ104899
B. interrupta DQ104900
B. interrupta DQ104901
B. interrupta DQ104903
B. interrupta DQ104904
B. mariposa DQ104916
B. interrupta GQ449762
B. interrupta GQ449766
B. kincaidii KF317726
B. interrupta FJ164382
0.99
B. kincaidii JQ354009
1
B. interrupta FJ164383
B. interrupta FJ164381
B. interrupta JQ354007
B. interrupta JQ354008
1
1
1
B. minispinosa
B. smirnovi
B. parmifera
1
B. panthera DQ104923
B. panthera DQ104921
B. panthera FJ869221
B. panthera FJ869223
B. panthera FJ869224
B. panthera FJ869225
B. panthera FJ869226
B. panthera FJ869227
B. panthera FJ869228
B. panthera HQ712317
0.003

Figure 2. Hypothesis of the phylogenetic relationships of *Bathyraja* species based on COI sequences available on GenBank. Clades representing only one species are collapsed, with triangle length corresponding to genetic diversity within that clade.

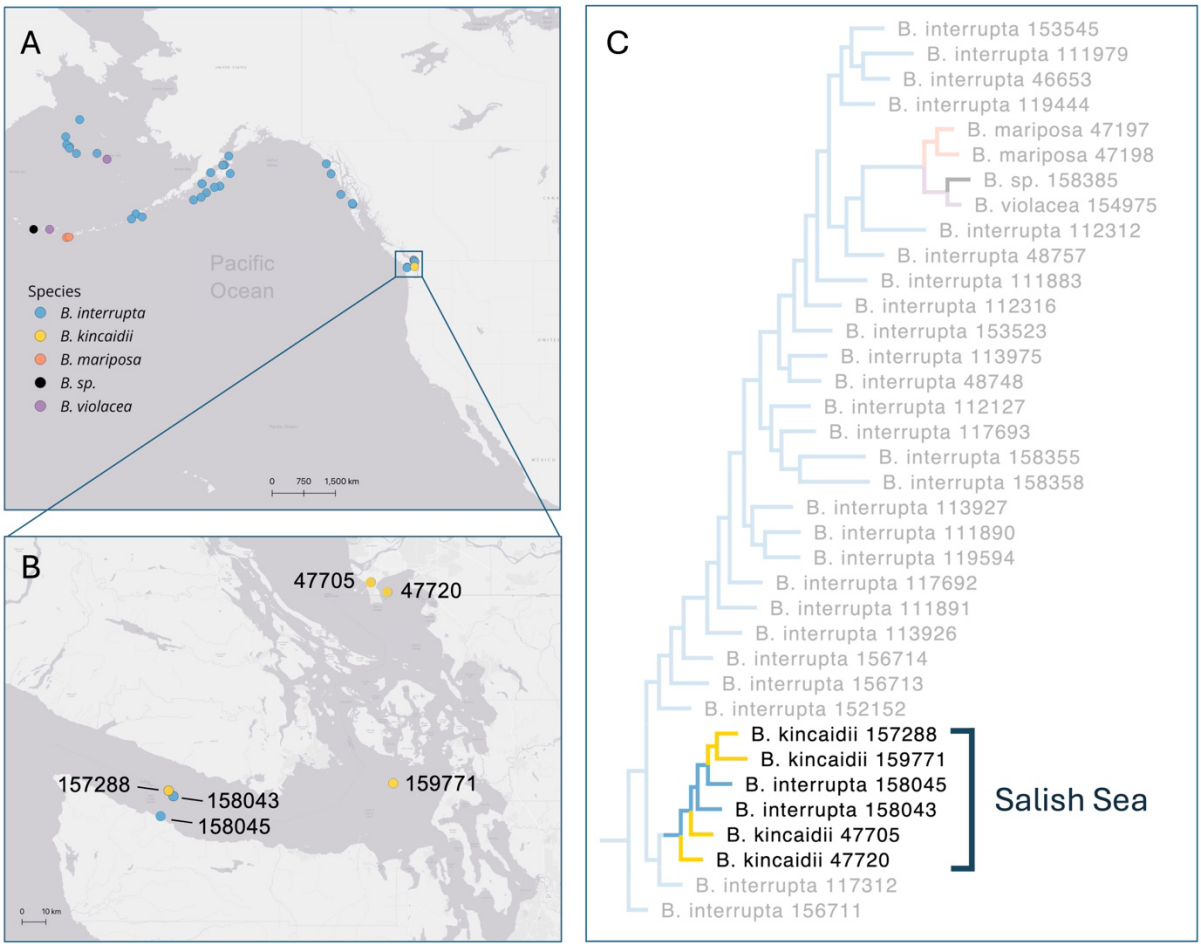

Figure 3. (A) Map showing collection sites of specimens included in the RADseq phylogeny. Specifically, collection sites for species closely related to *B. interrupta* are included: *B. interrupta*, *B. kincaidii*, *B. mariposa*, *B. violacea* and *B. sp.* Collection sites for specimens that did not meet RADseq filtering thresholds are also included. (B) Inset map of the Salish Sea, where six specimens (two *B. interrupta*, four *B. kincaidii*) were collected. (C) Selected clade of RADseq phylogeny that emphasizes the relationships between *B. interrupta* and *B. kincaidii* that were sampled from the Salish Sea.

Species	Common Name	Sample size
B. parmifera	Alaska skate	55
B. interrupta	Bering skate	40
B. panthera	Leopard skate	20
B. lindbergi	Commander skate	2
B. mariposa	Butterfly skate	2
B. minispinosa	Whitebrow skate	2
B. trachura	Roughtail skate	2
B. violacea	Okhotsk skate	1
B. maculata	White-blotched skate	2
B. aleutica	Aleutian skate	2
B. kincaidii	Sandpaper skate	4
B. taranetzi	Mud skate	2
B. sp. cf. taranetzi	N/A	1
B. sp.	N/A	1
B. smirnovi	Golden skate	1
B. abyssicola	Deep-sea skate	1
B. spinosissima	Pacific white skate	1

Table 1. Latin name, common name, and sample size of each species selected from the University of Washington Fish Collection.

-geno	SNPs retained	SNPs removed
0.1	32	918803
0.2	10419	908416
0.3	16490	902345
0.4	37896	880939
0.5	*237163*	*681672*

Table 2. The number of SNPs retained for different filtering parameters within PLINK software. The --geno filter sets a threshold for the percentage of individuals that share a certain loci (–geno 0.1 removes all loci that were genotyped in less than 90% of individuals). Minor allele frequency (--maf) filter was set to 0.05 for all -geno thresholds to remove alleles that occurred at low frequencies, typically resulting from sequencing error. The -geno value (0.5) that was selected for analysis is italicized.

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