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The skate fauna in the northern Northeast (NE) Atlantic is poorly investigated, and misidentifications are common. Here, 'DNA barcoding' was used to analyse 105 specimens of 15 species previously reported from the area to investigate the occurrence of species. Of these 15 species, three were new to the region and confirmed with voucher specimens. Three previously reported taxa were not obtained from the study area, providing a total number of 12 skate species for the northern NE Atlantic. Only one specimen of the critically endangered Dipturus batis complex was found. It occurs frequently in the literature and commercial fisheries catch records, and we argue that the vast majority of these are misidentifications. Due to striking differences in Amblyraja radiata life history parameters across the North Atlantic, cryptic species diversity has previously been suspected. A total of 80 A. radiata cytochrome c oxidase subunit I sequences from across the North Atlantic were sampled, and the highest fixation index (F_{ST}) was found when maximising geographical distance ($F_{ST} = 0.133$). A lower index was found when grouped according to life history ($F_{ST} = 0.067$). These results are not strongly supportive for the occurrence of cryptic species.

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Introduction

Skates (Chondrichthyes: Rajiformes) represent a specious group comprising 361 species worldwide, with 65 new species described in the last 10 years (Eschmeyer & Fong 2013). They are bottom dwelling and distributed in most of the world's oceans from the shoreline to at least 4156 m (Orlov et al. 2006; Ebert & Compagno 2007). Skates have low resilience to industrial fisheries due to slow growth, late sexual maturity and low fecundity (Walker & Hislop 1998; Stevens et al. 2000). Despite the high species number, their inter-specific variation in morphology is limited (McEachran & Dunn 1998) and this has caused problems distinguishing between species (Stevens et al. 2000; Tinti et al. 2003; ICES 2007; Stevenson et al. 2007, 2008). Sexual dimorphism and ontogenetic changes add to the confusion, meaning that distribution maps and conservation assessments may be error-prone (Lynghammar et al. 2013). However, accurate species identification remains vital for credible assessments, and where commercial landings of

skate are simply grouped as ‘Skates and rays’ (Stevens et al. 2000; Stevenson et al. 2008; Igl esias et al. 2010), declines in vulnerable species might have taken place unnoticed (Casey & Myers 1998; Iglesias et al. 2010).

Use of genetic markers overcomes many of these issues surrounding species identification from morphology alone. Molecular approaches also have a significant logistic advantage; very large specimens may be difficult to store in freezers at sea, and the cost bringing them from vessels to laboratory can be high. A small tissue sample for genetic analysis, however, is easily shipped. The mitochondrial gene cytochrome c oxidase subunit I (COI) has proven valuable in species identification (Hebert et al. 2003; Ward et al. 2009). The method allows non-taxonomists to identify specimens with a high degree of certainty if a similar ‘barcode’ already exists in a database. This approach has been linked to the idea of a ‘barcode gap’ where the genetic variation between species is larger than within species (although this term should be treated with caution, see e.g. Moritz & Cicero 2004; Meyer & Paulay 2005). Besides identification, it is a tool for species discovery, as a genetic approach may reveal hidden diversity as well as pinpointing potential cryptic species that could be further investigated using additional tools (Hebert et al. 2004a; Hajibabaei et al. 2007; Smith et al. 2008; Cannas et al. 2010).

The starry ray Amblyraja radiata is an abundant skate in the North Atlantic and thrives within wide temperature and depth ranges. Although not targeted by industrial fisheries or landed to any extent in the Barents Sea (Dolgov et al. 2005a), it is the most numerous skate here, comprising 96% of skates caught (Dolgov et al. 2005b). In the Canadian Atlantic, A. radiata is commercially targeted and comprises about 90% of all skates caught (Anonymous 2007). It is also the most common skate in the North Sea (Walker & Heessen 1996; Skjæraasen & Bergstad 2000). In the Red List of the International Union for Conservation of Nature and Natural Resources (IUCN, http://www.iucnredlist.org), the starry ray is classified as ‘Vulnerable’ in the Canadian Atlantic and ‘Critically Endangered’ in USA waters due to its decline. In the northern Northeast (NE) Atlantic, however, it is classified as ‘Least Concern’ and is stable or increasing (Kulka et al. 2009).

Templeman (1984) found a cline in the number of median dorsal thorns and tooth rows from north to south in starry rays from the NW Atlantic. Specimens obtained from Iceland expressed intermediate values for both characters, and factors such as temperature and genetics were discussed as potential explanations for these features. Further investigations carried out by Templeman (1987) revealed a geographical difference in size at maturity and maximum size. Early maturation occurred off West Greenland and northern Iceland, Baffin Island, Labrador, on the NE Newfoundland Shelf and in the Gulf of St. Lawrence, where males matured at 400–500 mm total length (TL) and females at 400–470 mm TL. Maximum TL was 720 and 630 mm for the males and females, respectively. Late maturation was observed in the Grand Bank, St. Pierre Bank and vicinity, where males matured at 760–830 mm TL and females at 610–740 mm TL. Maximum TL there was 1040 mm for the males and 940 mm for the females. In the North Sea and the Skagerrak (northern NE Atlantic), both sexes of A. radiata mature at 300 mm TL and they have a maximum TL of 720 mm (Skjæraasen & Bergstad 2000). Along with Templeman’s observations, the pronounced variation in body size could be

indicative of a cryptic species. The aim of this paper was to (i) demonstrate the use of DNA barcoding as a tool for discrimination between the northern NE Atlantic skate species, (ii) provide an updated checklist of skate species in the region and (iii) investigate whether DNA barcoding can distinguish between potential populations in A. radiata across the North Atlantic Ocean.

Materials and methods

Sample collection

A total of 105 skates were collected from 15 putative species (Table 1, Fig. 1A) across the northern NE Atlantic, here defined as the zones 200 nautical miles around the Norwegian mainland (the exclusive economic zone), Svalbard Archipelago (the fishery protection zone) and around Jan Mayen Island (the fishery zone). The Norwegian Institute of Marine Research, who is carrying out most of the scientific trawling in this area, was asked to look for less common species of skate since 2007, when the study was initiated. In cases where few or no specimens of a previously reported species were obtained, supplementary samples were collected from the British Isles to ensure unambiguous genetic identification. Note that the two species in the Dipturus batis complex follow the nomenclature suggested by Iglesias et al. (2010), namely Dipturus cf. intermedia and Dipturus cf. flossada. An additional 70 specimens of A. radiata from the northern NE Atlantic, NW Atlantic and East (E) Greenland were sampled for population genetic structure analyses (Fig. 1B). Specimens from the NW Atlantic required internal examination to be categorised as early or late maturing, using maturity status vs. TL (C. Miri, Fisheries and Oceans Canada, pers. comm.). Northern NE Atlantic and E Greenland specimens were assumed to mature at small sizes based on the lack of larger specimens in samples, but maturity status has not been assessed in these regions. Skates were caught by bottom trawling or by recreational anglers, and tentatively identified to species on site (ID guide for skates in Norwegian waters, A. Lynghammar, unpubl.). Tissue samples were collected by removing a small piece of skin and muscle from the pectoral or dorsal fins and stored in 96% ethanol at 20°C for subsequent DNA barcoding. Sampling and specimen information, including catalogue number for the specimens kept in natural history museums, are available in the project 'DNA barcoding of the northern NE Atlantic skates' at the Barcode of Life Datasystems (http://www.boldsystems.org; Ratnasingham & Hebert 2007). GenBank accession numbers for all specimens are KF604118–KF604292, and among these are all the A. radiata specimens (n = 80) with numbers between KF604128–KF604207.

Laboratory analysis

DNA extraction was performed with E.Z.N.A. Tissue DNA kit (Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. To amplify the 5^0 end of the mitochondrial gene COI, the following reagents were used: 2.0 lL DNA template, 2.5 lL 10 9 PCR buffer (Qiagen, Hilden, Germany), 2.0 lL dNTP (Thermo Scientific, Hudson, NH, USA), 1.0 lL MgCl, 1.0 lL primer, 0.2 lL Hot Star Taq (Qiagen) and 16.3 lL dH_2O (to a total volume of 25.0 lL). PCR cycles started at 95°C for 15 min and 94°C

for 60 s. Then 35 cycles at 94°C for 30 s, 51°C for 30 s and 72°C for 60 s followed, with a final extension at 72°C for 5 min. Samples of Bathyraja spinicauda were ampli

fied with the primers HCO2198 and LCO1490 (Folmer et al. 1994), whereas samples from the remaining species were amplified with the M13-tailed COI-3 primer cocktail (Ward et al. 2005; Ivanova et al. 2007).

All samples were sequenced forward and reverse with an ABI PRISM 3130 xl Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The sequences were edited in SEQUENCHER 4.8 (Gene Codes Corp., Ann Arbor, MI, USA) or MEGA 5.05 (Tamura et al. 2011), and aligned in the latter.

Statistical analysis

Haplotype and nucleotide diversities were calculated with DnaSP (Librado & Rozas 2009). Maximum intra

and inter- specific genetic distance (using the Kimura-2-Parameter distance model, K2P) was calculated using the Barcode Gap Analyses tool in BOLD (http://www.boldsystems.org, Ratnasingham & Hebert 2007). The Bayesian tree was made in MRBAYES 3.2 (Ronquist et al. 2012) using the model HKY+I+G, selected by AIC in MRMODELTEST 2.3 (Nylander 2004) and run for 2,000,000 MCMC with a 10% burn-in. Four chains were used, with heating parameter 0.1. The neighbour joining (NJ) and maximum likelihood (ML) trees were made in MEGA 5.05 (Tamura et al. 2011). Both trees were re-sampled by bootstrap with 1000 replicates. The former tree was built with the K2P substitution model, while the latter was built with the HKY+G model (five discrete gamma categories). The haplotype network was made with TCS 1.21 (Clement et al. 2000) and manually edited in Illustrator CS6 (Adobe Systems Inc., San Jose, CA, USA). Pairwise fixation indices (F_{ST}), analysis of molecular variance (AMOVA) and neutrality tests were computed in ARLEQUIN 3.5. (Excoffier et al. 2005).

Results

A total of 105 specimens representing 15 species in six genera and two families were analysed (Table 1). The genetic results support the morphological species identifications. From a total of 651 base pairs, 208 showed variation and all mutations were found in the third codon. There were ten amino acid substitutions, and all specimens clustered with their conspecifics in all trees. In the Bayesian tree (Fig. 2), all species and genera are well supported, with the exception of the Dipturus and Rajella genera that do not form a monophyletic group. Further phylogenetic analyses with NJ and ML trees (Data S1A and B, respectively) also demonstrate good support for the species and genera included. However, the ML tree has the same issues as the Bayesian tree, with Dipturus nidarosiensis being paraphyletic with Raja. Dipturus was monophyletic in the NJ tree, whereas Rajella species failed to form a monophyletic taxon.

'Barcode gaps' were present between all species pairs in the data set (Table 2). Genetic distances (K2P) ranged from 1.87% for Dipturus oxyrinchus vs. D. cf. intermedia (12

fixed nucleotide differences) to 18.38% for B. spinicauda vs. Rajella fyllae (102 fixed nucleotide differences). Distances to nearest neighbour for most species were 2.3~6%, with an overall average of 5.20%. The average for the family Rajidae without B. spinicauda was 3.97%. The intra-specific variation (number of haplotypes) varied considerably across species (Table 1). With the proviso that for some species only three specimens were sequenced, six species showed no variation and seven species comprised only two haplotypes. Rajella fyllae and A. radiata were more variable with four and eight haplotypes, respectively.

Given the high haplotypic variation in A. radiata, 70 additional specimens were analysed (Fig 1B), increasing the sample size to a total of 80. Bringing in samples from the NW Atlantic and E Greenland resulted in 22 additional haplotypes and an extraordinary total of 30 haplotypes for this species. There were 33 variable sites, and all mutations occurred in the third codon with one exception.

Groups were assigned in different ways to test for genetic structure, taking geography and early or late maturation into account. Four groups were generated (Tables 3 and 4): (i) NW Atlantic late-maturing type, (ii) NW Atlantic early-maturing type, (iii) E Greenland and (iv) northern NE Atlantic. The highest fixation index was found between the northern NE Atlantic and the NW Atlantic late-maturing type ($F_{ST} = 0.133$). A lower index was found for the northern NE Atlantic vs. the NW Atlantic early-maturing type, with $F_{ST} = 0.066$ – both values were significant ($P < 0.001$ and $P = 0.009$, respectively). Compared to the E Greenland samples, no significant F_{ST} values were found between any of the other three groups. The AMOVA results showed that 93% of the variation was found within the groups and the global F_{ST} was 0.070 ($P = 0.002$). Merging the two types in the NW Atlantic into one resulted in an F_{ST} value of 0.085 ($P < 0.001$) between the NW and the northern NE Atlantic, with E Greenland samples still being not significant. Finally, specimens were assigned to size at maturation and illustrated by a haplotype network (Fig. 3), giving a pairwise F_{ST} of 0.067 ($P < 0.001$) between the early- and late-maturing type. About half of the specimens were distributed in five haplotypes, and the remaining half was distributed in 25 haplotypes. The Tajima's D neutrality test was approximately 1.00 for all three groups regardless of assignment, and none were significant.

Discussion

In the present study, DNA barcoding successfully separated the commonly captured and previously considered valid skate species in the northern NE Atlantic, and characteristic barcode gaps were present between all species pairs. The general intraspecific variance was low, with only one haplotype identified in six of the species. Among these was Raja clavata, and this is in direct contrast to Serra-Pereira et al. (2011), where this species was more variable. Most R. clavata specimens in the present study were obtained from fjords in the central part of Norway (~64°N^0). This is at its northern distributional limit, and the low diversity gives support to the 'leading edge' hypothesis, where low diversity is expected due to bottleneck events (Hewitt 1993; but see also Chevolot et al. 2006).

In BOLD (http://www.boldsystems.org, Ratnasingham & Hebert 2007), species pairs

with genetic distances of <2% are highlighted as potential problematic taxa, and this occurred in D. oxyrinchus vs. D. cf. intermedia (1.87% distance). However, morphological characters such as the number of median dorsal spines and snout length vs. distance between eyes clearly support two valid species. Some authors suggest a universal gap value of 3.5% for fishes (Ward et al. 2009) or 10 9 the mean intraspecific variance as suggested for birds (Hebert et al. 2004b). A fixed gap value would imply a non-evolving and fixed feature of the species, which is highly unlikely (Wiemers & Fiedler 2007; Trewick 2008). In other words, DNA barcoding does not solve all of the issues surrounding species demarcation, and other aspects such as ecology and morphology still need to be taken into account. Regardless of thresholds, the lowest distance to the nearest neighbour species could be a standard measure to maintain conservative estimates, rather than using mean distances (Meier et al. 2008). Bathyraja spinicauda differs from the nearest neighbour by more than 18%, indicating a distant relationship to the remaining species. This is consistent with higher taxonomic levels, as B. spinicauda belong to family Arhynchobatidae and not to Rajidae. Success in species identification using COI barcoding may be dependent of the geographical scale. Increasing proportions of non-monophyly, higher levels of intraspecific distance and lower distance to nearest neighbour are expected with increasing geographical sampling range (Bergsten et al. 2012). Previous regional studies have reported difficulties in separating sibling species of skates using the COI gene, such as Raja clavata and R. maderensis (Serra-Pereira et al. 2011), Amblyraja hyperborea, A. jenseni and A. badia (Coulson et al. 2011), and Raja montagui and R. asterias (Lago et al. 2012). In the Bering Sea, Spies et al. (2006) found minor differences between Bathyraja lindbergi and B. maculata, although adult morphology and egg case characters clearly suggest they are separate species.

The recent morphology-based reallocation of Dipturus linteus to the genus Rajella (Stehmann 2012) was well supported in the phylogenetic analysis, although it is important to note that members of Rajella remained poorly resolved in the trees. The grouping of D. nidarosiensis with Raja in the Bayesian and ML trees was not expected, but we acknowledge that DNA barcoding is not a strong phylogenetic tool to explore evolutionary histories and more genes should be included for such use (Hajibabaei et al. 2006). A similar issue was reported by Serra-Pereira et al. (2011), where D. oxyrinchus clustered with Raja in a Maximum Parsimony tree. Finally, DNA barcodes successfully separated the two species previously combined in D. batis (Griffiths et al. 2010; Igl esias et al. 2010), providing the first published reference COI barcodes for D. cf. intermedia and D. cf. flossada. The division into these two species continues to be problematic for conservation and management as their distribution and abundance still remain poorly established, although additional molecular genetic evidence presented here supports the taxonomic revision of the group. More research into this topic is urgently needed to address this issue.

Skates in the northern NE Atlantic suffer from the lack of accurate identification. A total of 15 skate species (including the two formerly known as D. batis) are reported from the region (e.g. Pethon 2005; Williams et al. 2008), but many records are dubious and voucher specimens rarely exist. An example of this problem is found in statistics received

from the Norwegian Directorate of Fisheries (A. Østreim, pers. comm.): a total of ~640 metric tonnes of skates were landed in the years from 2011 to 2013. Of these, 96% were registered as 'Raja sp.', whereas the remaining ~3% were denoted as D. batis. Seven species comprise the last ~1%. The collective group Raja sp. gives no information about species composition, and the latter is highly unlikely because only one specimen of this species complex (D. cf. intermedia) was obtained in this study despite sampling since 2007. Most skate catches in Norway are bycatch, and considerable discards can be assumed because of low commercial value. It is notable that the Norwegian Institute of Marine Research stopped recording species such as the D. batis complex, Leucoraja fullonica, Raja brachyura and Raja montagui from northern Norway and the Barents Sea as a result of improved species identification in the last ~5 years. Records of the above-mentioned species in, for example, Williams et al. (2008) and Dolgov et al. (2005a) should generally be regarded as misidentifications as no voucher specimens are available for re-examination. Another question arising is whether both, or only one, of the two species in the D. batis complex are present in Norwegian waters. This is particularly important in the case of D. cf. intermedia, because it is considered to be much more vulnerable to fisheries pressures due to its very large size (Walker & Hislop 1998), but it has been frequently recorded in the seas around Scotland (Griffiths et al. 2010). A survey of voucher specimens in Icelandic, Swedish and Danish natural history museums revealed that records of both species are from the south in the Skagerrak region (E. Viinamäki unpubl.). Several specimens of D. cf. flossada were collected in Icelandic waters as late as 2010, leaving the possibility that both species could be present in the northern NE Atlantic.

Three species new to Norwegian waters were identified and preserved in natural history collections. Raja montagui (Data S2A) was caught by a commercial vessel on 3rd September 2009 in the Skagerrak (57.486°N, 07.596°E). The specimen is deposited in the Göteborg Natural History Museum, Sweden (cat. nr. GNM2009-22.103:2). This is a single observation, but the species has previously been reported from central Norway (Pethon 2005). One of these is deposited in the Natural History Museum in Oslo, Norway, but was re-identified as R. clavata (A. Lynghammar, 2008, pers. obs.). Leucoraja circularis (Data S2B) was caught by a commercial vessel on 6th July 2009 in Frohavet (63.785°N, 09.056°E) and is deposited in the NTNU University Museum, Norway (cat. nr. NTNU-VM4100). Two more L. circularis specimens were collected from the same area, where it seems to have a restricted distribution (Williams et al. 2008). A third species, Leucoraja naevus (Data S2C), was found 28th July 2010 in the North Sea (61.314°N, 2.097°E). DNA extraction failed (probably due to repeated freezing and thawing), but species identity was confirmed using morphology. The specimen will be deposited in a museum collection and should be regarded as present in Norwegian parts of the northern NE Atlantic. On the other hand, L. fullonica, a species reported to be relatively abundant in the late 1800s (e.g. Storm 1880), was not encountered, despite sampling since 2007.

In terms of numbers, A. radiata is dominating the northern NE Atlantic skate catches (~96%), indicating that at least eleven species are very rare and vulnerable to local extinction. Whether the species spawn or are only foraging in the area could be of

importance to conservation, and egg cases from B. spinicauda, A. hyperborea, A. radiata, D. nidarosiensis, D. oxyrinchus, R. clavata and possibly R. fyllae have been observed in the study area (A. Lynghammar, pers. obs.). Bycatch rates of skates are considered relatively low in the Barents Sea (Dolgov et al. 2005a), but may have an impact when species are rare. Basic life history parameters such as age at maturation, growth coefficient and longevity are largely unknown, resulting in an uncertain future for skates in heavily fished regions such as the North Sea, the Norwegian Sea and the Barents Sea.

Cryptic speciation has been suspected for the widely distributed A. radiata due to variation in morphology and ecology (Templeman 1984, 1987), in addition to genetic evidence (Coulson et al. 2011). We expected the late-maturing, larger type in the NW Atlantic to differ from the remaining samples due to a significantly different life history, which, however, was only partly supported by our data. The highest significant pairwise F_{ST} value was actually found between the late-maturing type in the NW Atlantic and the northern NE Atlantic specimens. An F_{ST} value of 0.133 indicates some differentiation, but considering the haplotype network (Fig. 3), it does not seem to have biological significance because the haplotypes do not mirror their geographical distribution. The lower but still significant F_{ST} value (=0.067) between the early- and late-maturing type indicates that size at maturation is of less importance than distance in relation to genetic structure. According to Bergsten et al. (2012), 70 samples are sufficient to recover 95% of the genetic variation if the geographic distance is maximised between the new sample and the closest previous sample. Although samples from southern parts of the N Atlantic are missing in the present study, geographic distance was maximised in the north. Considering 30 haplotypes distributed across 80 specimens, one would expect to find a new haplotype for every third specimen sequenced, and it is thus very probable that a large part of the species' variation is still unsampled. Each of the 30 haplotypes was separated by one or two mutations, except for one specimen that required seven mutational steps to be connected to its nearest neighbour. Interestingly, this specimen (BOLD accession no. RNEZ126-13, NW Atlantic larger type) was the only A. radiata with a mutation on the second site in the codon. Coulson et al. (2011) reported a similar case, where the specimen had 3–4% sequence divergence from its congeners. It was discussed whether this could represent a cryptic species, but compared to our 'outlier', there was a ~3.5% sequence divergence between them, implying no closer relationship.

Chevolot et al. (2007) investigated the genetic structure of A. radiata using another mitochondrial gene, the cytochrome b. Some NW Atlantic specimens were included, but the majority were from Iceland, southern Norway, the Kattegat and the North Sea. Skates have no pelagic larval stage, and displacement between areas is a result of active swimming. Deep waters, largely unsampled, separate the NW Atlantic, E Greenland and northern NE Atlantic. However, the ridges in the Denmark Strait from Greenland to Iceland and eastwards via the Faroe Islands to the North Sea could be a possible migration route for A. radiata, avoiding the deepest part of the North Atlantic. This is supported by Chevolot et al. (2007), as Icelandic waters seem to constitute an admixture zone with a high number of haplotypes. However, no clear genetic structure could be detected in the central parts of the N Atlantic.

Conclusions

DNA barcoding is a well-suited method for identifying skate specimens to species level in the northern NE Atlantic, with all species being successfully distinguished. A total of 12 skate species were found in the Norwegian parts of the northern NE Atlantic (Table 1), and three of these were new to the region. Leucoraja fullonica has probably disappeared from the region, and R. brachyura and D. cf. flossada were probably never present. The relatively numerous records in the literature of the critically endangered D. batis complex are most likely all misidentifications. However, whilst DNA barcoding remains a valuable tool in verifying skate identification, it becomes impractical at sea where identification keys still serve as the best tool. The importance of discrimination between species must be communicated to all parts in commercial fisheries and management, to avoid overlooked local or even global extinction of skate species.

The method applied did not support any subdivision between the late- and early-maturing type of A. radiata from the NW Atlantic, but there was some significant genetic subdivisions across the sampling locations. Compared to other skate species in the northern NE Atlantic, this species has a combination of wide geographical range, wide depth range, and is the most abundant skate species in the Barents Sea, the North Sea and the NW Atlantic. All these factors combined may facilitate a large effective population size and maintain high genetic diversity. Markers with higher resolution, such as microsatellites, are currently being applied to further investigate the population structure of A. radiata ((A. Lynghammar, K. Præbel, S.-E. Fevolden, and J. S. Christiansen, in prep.)).

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Supporting Information

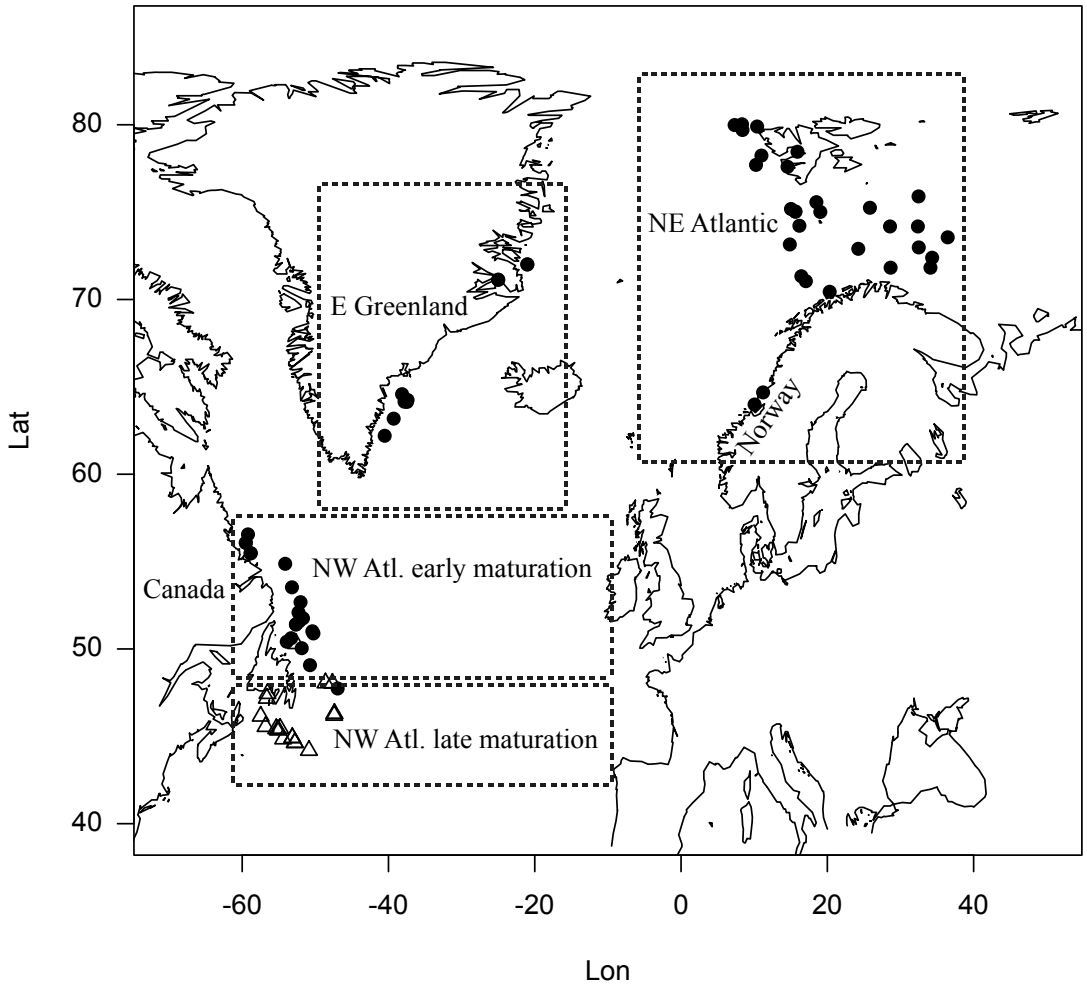
Additional Supporting Information may be found in the online version of this article:

Data S1. Gene trees for 105 skate specimens.

Data S2. Three new skate species from Norwegian waters.

Figures and tables

A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NE Atlantic
Norway
Canada
NW Atl. early maturation
NW Atl. late maturation

B

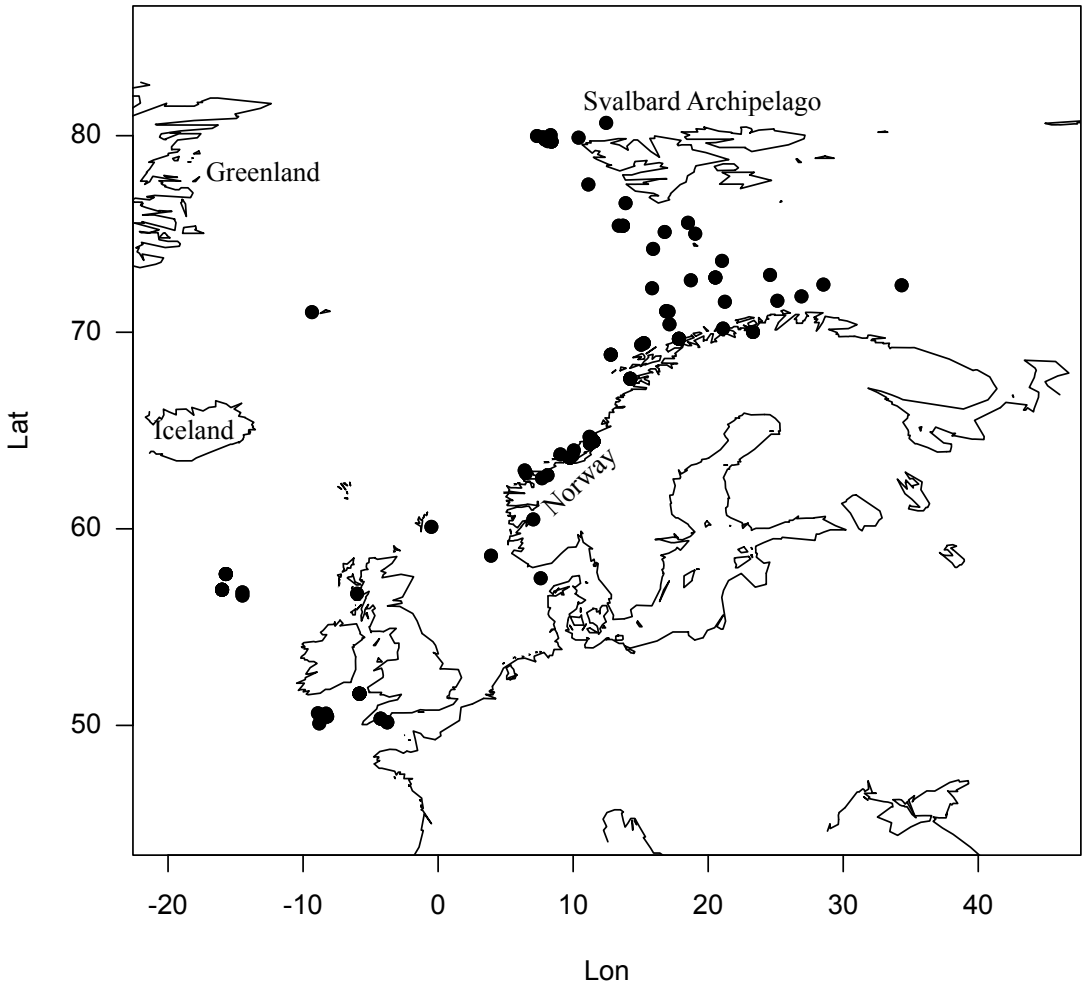


Fig. 1. A. All sampling sites from the northern Northeast Atlantic (n = 105) and B. *Amblyraja radiata* sampling sites, where black dots represents the early maturing type (n = 60) and triangles the late maturing type (n = 20). One data point may represent several specimens. The maps are produced in the software R (R Core Team 2013).

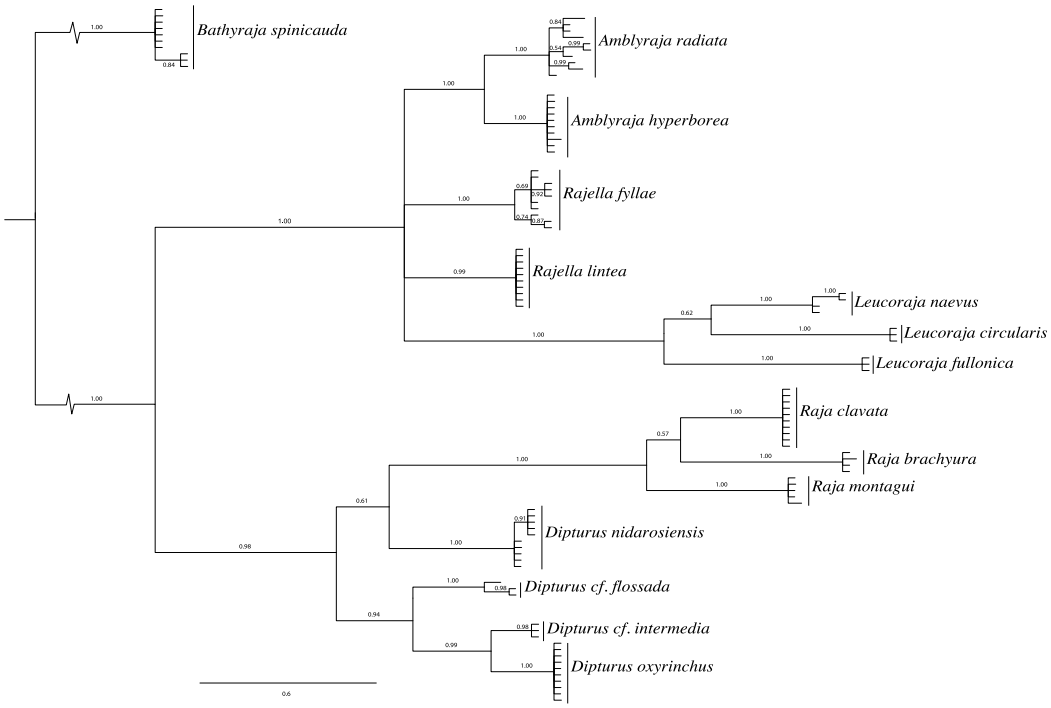


Fig. 2: Bayesian Inference of the 105 northern NE Atlantic skate specimens. Numbers on the branches are posterior probabilities.

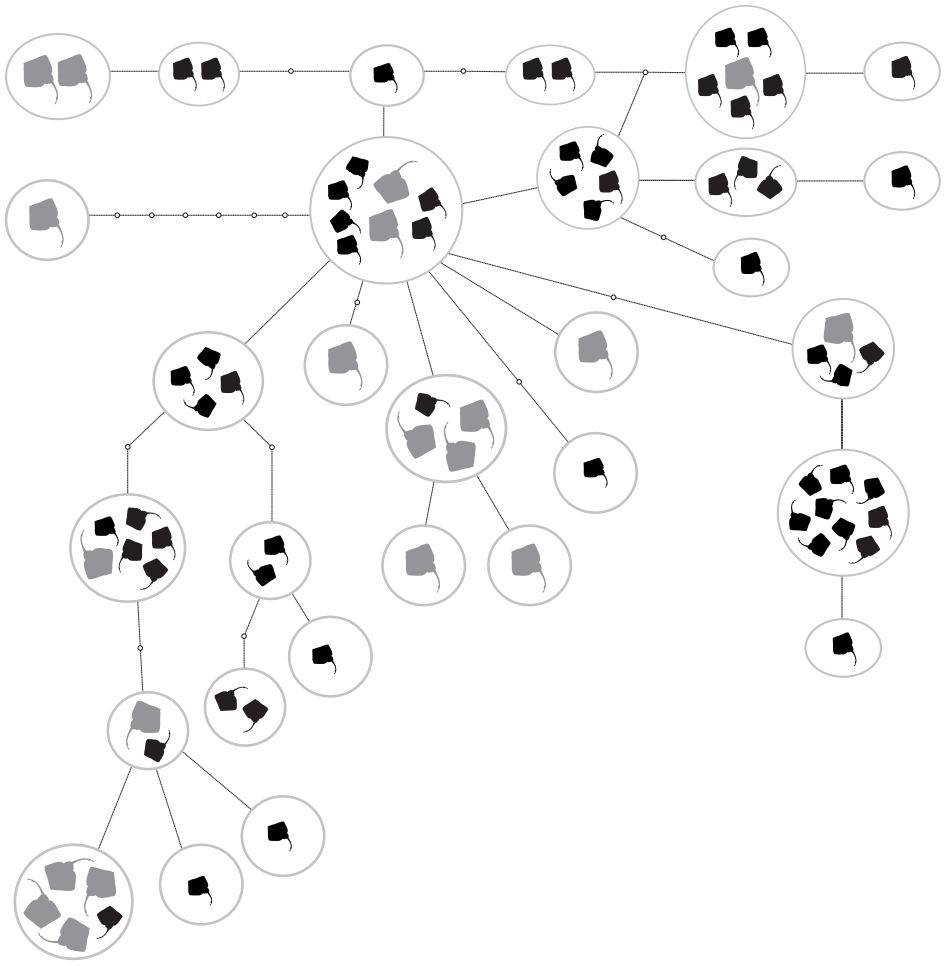


Fig. 3: Haplotype network of *Amblyraja radiata* COI sequences (n = 80). Each circle represents one haplotype, and the number of skate icons in each circle represents the number of specimens in each haplotype. Small, black icons (n = 60) are the early maturing type, while the large, grey icons (n = 20) are the late maturing type. Connecting lines represent one mutation, and small open circles are additional mutations or unsampled haplotypes.

Table 1: List of species analysed and genetic parameters for 105 NE Atlantic skate specimens. The GenBank accession numbers for *Amblyraja radiata* are not limited to ten specimens, but include the 70 additional North Atlantic samples. Scientific name and author follow Catalog of Fishes (Eschmeyer 2013), common name follow Froese & Pauly (2014), except for the *Dipturus batis* complex (*D.* cf. *flossada* and *D.* cf. *intermedia*) which follows nomenclature suggested by Iglésias *et al.* (2010). Abbreviations: n, number of samples; S, number of segregating sites; N_h, number of haplotypes; H_h, haplotype diversity; π, nucleotide diversity and GenBank accession numbers. The rightmost column indicates if the species is found in the study area after 2007.

Species	Name	n	S	N_h	H_h	π	GenBank no.	Found in study area
Family: Arhynchobatidae								
Bathyraja spinicauda (Jensen, 1914)	Spinetail ray	10	3	2	0.467	0.00215	KF604208–KF604217	Yes
Family: Rajidae								
Amblyraja hyperborea (Collett, 1879)	Arctic skate	10	1	2	0.200	0.00031	KF604118–KF604127	Yes
Amblyraja radiata (Donovan, 1808)	Starry ray	10	10	8	0.956	0.00498	KF604128–KF604207	Yes
Dipturus cf. *flossada*	'Blue skate'	3	4	2	0.667	0.00409	KF604218–KF604220	No
Dipturus cf. *intermedia*	'Flapper skate'	3	0	1	0.000	0.00000	KF604221–KF604223	Yes
Dipturus nidarosiensis (Storm, 1881)	Norwegian skate	10	1	2	0.556	0.00085	KF604234–KF604243	Yes
Dipturus oxyrinchus (Linnaeus, 1758)	Longnosed skate	10	0	1	0.000	0.00000	KF604244–KF604253	Yes
Leucoraja circularis (Couch, 1838)	Sandy ray	3	0	1	0.000	0.00000	KF604254–KF604256	Yes
Leucoraja fullonica	Shagreen ray	3	0	1	0.000	0.00000	KF604257–	No

(Linnaeus, 1758)							KF604259	
Leucoraja naevus (Müller & Henle, 1841)	Cuckoo ray	4	3	2	0.667	0.00307	KF604260–KF604263	Yes
Raja brachyura Lafont, 1873	Blonde ray	4	1	2	0.500	0.00077	KF604264–KF604267	No
Raja clavata Linnaeus, 1758	Thornback ray	10	0	1	0.000	0.00000	KF604268–KF604277	Yes
Raja montagui Fowler, 1910	Spotted ray	5	1	2	0.400	0.00061	KF604278–KF604282	Yes
Rajella fyllae (Lütken, 1887)	Round ray	10	4	4	0.778	0.00269	KF604283–KF604292	Yes
Rajella lintea (Fries, 1838)	Sailray	10	0	1	0.000	0.00000	KF604224–KF604233	Yes

Table 2: Data generated from the Barcode Gap Analysis tool in the Barcode of Life Data System (BOLD, http://www.boldsystems.org), using the K2P distance model. The maximum intraspecific variation (Max_{intra}) is given with the nearest related species. The rightmost column gives genetic distance (in per cent) and the BOLD accession number to the nearest specimen.

Species	Max_{intra} (%)	Nearest species	Distance (%) to nearest neighbour (accession no.)
Bathyraja spinicauda	0.46	*Rajella fyllae*	18.38 (RNEZ081-10)
Amblyraja hyperborea	0.15	*Amblyraja radiata*	2.35 (RNEZ160-13)
Amblyraja radiata	0.93	*Amblyraja hyperborea*	2.35 (RNEZ014-10)
Dipturus cf. *flossada*	0.62	*Dipturus oxyrinchus*	3.47 (RNEZ042-10)
Dipturus cf. *intermedia*	0	*Dipturus oxyrinchus*	1.87 (RNEZ042-10)
Dipturus nidarosiensis	0.15	*Dipturus* cf. *flossada*	5.47 (RNEZ032-10)
Dipturus oxyrinchus	0	*Dipturus* cf. *intermedia*	1.87 (RNEZ181-13)
Leucoraja circularis	0	*Leucoraja naevus*	5.65 (RNEZ056-10)
Leucoraja fullonica	0	*Leucoraja naevus*	6.27 (RNEZ056-10)
Leucoraja naevus	0.46	*Leucoraja circularis*	5.65 (RNEZ048-10)

Raja brachyura	0.15	*Raja clavata*	5.12 (RNEZ064-10)
Raja clavata	0	*Raja montagui*	5.12 (RNEZ071-10)
Raja montagui	0.15	*Raja clavata*	5.12 (RNEZ064-10)
Rajella fyllae	0.62	*Amblyraja hyperborea*	4.62 (RNEZ098-13)
Rajella lintea	0	*Rajella fyllae*	4.64 (RNEZ082-10)

Table 3: Genetic parameters for four *Amblyraja radiata* groups (total n = 80), divided into groups by geography and size at maturity (NW Atlantic early maturing type, NW Atlantic late maturing type, northern NE Atlantic and East Greenland). Abbreviations: n, number of samples; N_h, number of haplotypes; S, number of segregating sites; H_h, haplotype diversity and Tajima's D with p-values in paranthesis.

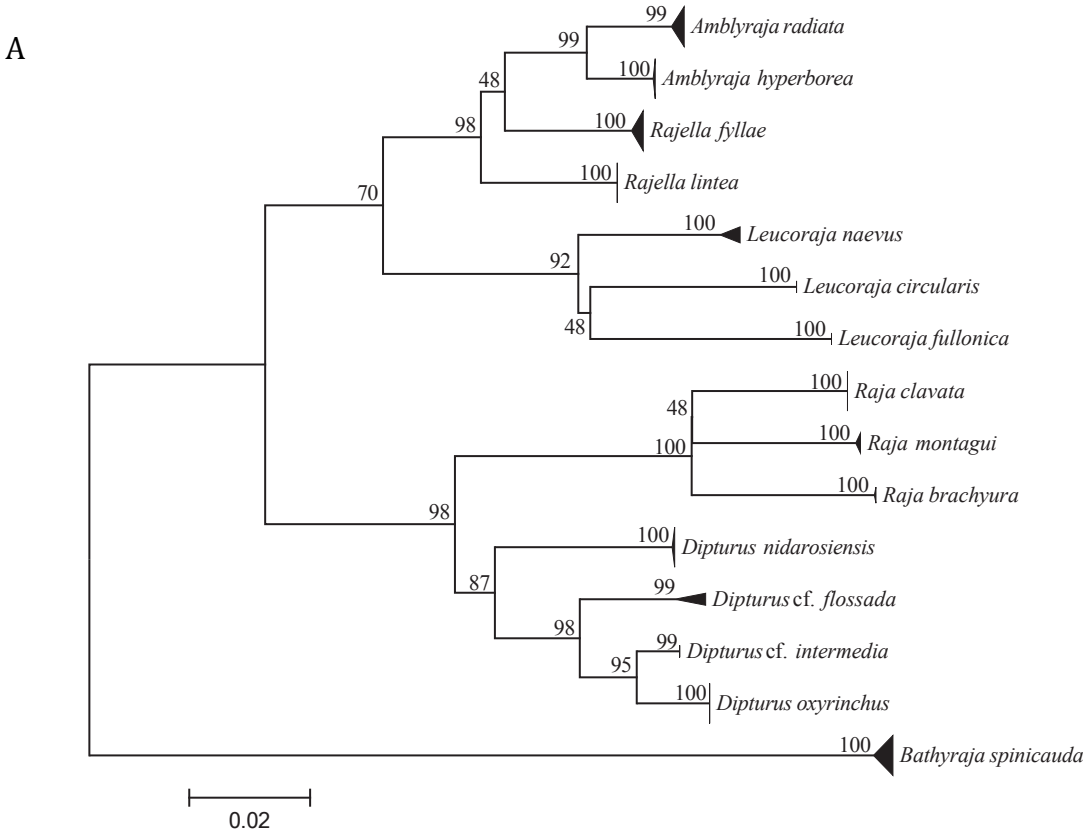
Group	n	N_h	S	H_h	Tajima's D (p-value)
NW Atlantic (early mat.)	20	12	21	0.9368	-0.65713 (> 0.10)
NW Atlantic (late mat.)	20	13	24	0.9421	-1.30677 (> 0.05)
NE Atlantic	30	13	17	0.9103	-0.87732 (> 0.10)
E Greenland	10	8	12	0.9556	-1.15667 (> 0.10)

Table 4. Pairwise F_{ST} differences between four *Amblyraja radiata* groups divided into groups by geography and size at maturity (see Table 3). Significant numbers (p-value < 0.05) are in bold.

	NW Atl early mat.	NW Atl late mat.	nNE Atl	E Green
NW Atl early mat. (n = 20)	0.00000			
NW Atl late mat. (n = 20)	0.01692	0.00000		
nNE Atl (n = 30)	**0.06582**	**0.13292**	0.00000	
E Green (n = 10)	-0.00532	0.03943	0.04566	0.00000

Supplementary materials I:

A



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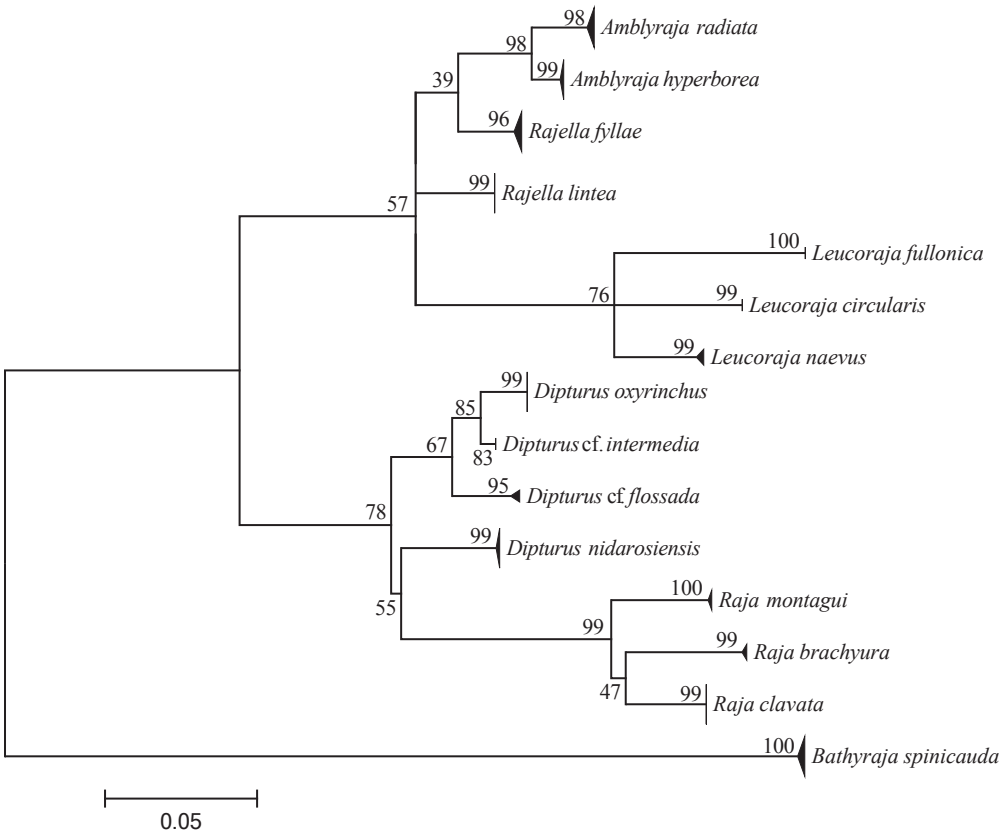
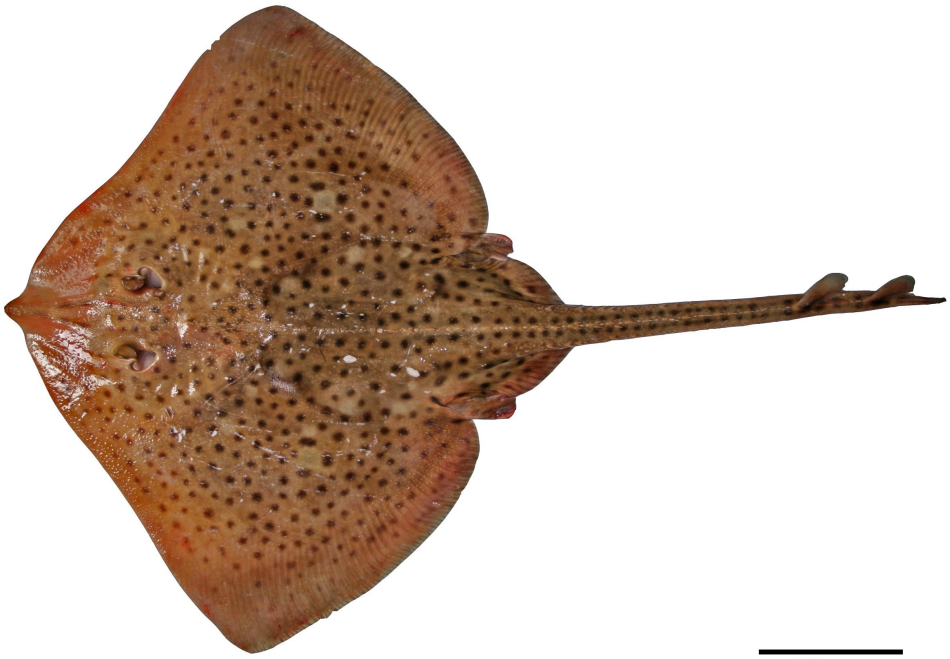


Gene trees for 105 skate specimens. A. Neighbour-Joining (K2P) and B. Maximum Likelihood (HKY+G, 5 discrete gamma categories). Numbers are bootstrap values and specimens are merged into species for improved readability.

Supplementary materials II:


100 mm


100 mm

Three new skate species from Norwegian waters. A. *Raja montagui* (female, 651 mm TL, photo: L. Jonsson, Göteborg Natural History Museum), B. *Leucoraja circularis* (male, 798 mm TL, photo: A. Lynghammar), and C. *Leucoraja naevus* (male, 561 mm TL, photo: A. Lynghammar).