Molecular systematics of gadid fishes: implications for the biogeographic origins of Pacific species

Steven M. Carr, David S. Kivlichan, Pierre Pepin, and Dorothy C. Crutcher

Abstract: Phylogenetic relationships among 14 species of gadid fishes were investigated with portions of two mitochondrial DNA (mtDNA) genes, a 401 base pair (bp) segment of the cytochrome *b* gene, and a 495 bp segment of the cytochrome oxidase I gene. The molecular data indicate that the three species of gadids endemic to the Pacific Basin represent simultaneous invasions by separate phylogenetic lineages. The Alaskan or walleye pollock (*Theragra chalcogramma*) is about as closely related to the Atlantic cod (*Gadus morhua*) as is the Pacific cod (*Gadus macrocephalus*), which suggests that *T. chalcogramma* and *G. macrocephalus* represent separate invasions of the Pacific Basin. The Pacific tomcod (*Microgadus proximus*) is more closely related to the Barents Sea navaga (*Eleginus navaga*) than to the congeneric Atlantic tomcod (*Microgadus tomcod*), which suggests that the Pacific species is derived from the *Eleginus* lineage and that *Eleginus* should be synonymized with *Microgadus*. Molecular divergences between each of the three endemic Pacific species and their respective closest relatives are similar and consistent with contemporaneous speciation events following the reopening of the Bering Strait ca. 3.0–3.5 million years BP. In contrast, the Greenland cod (*Gadus ogac*) and the Pacific cod have essentially identical mtDNA sequences; differences between them are less than those found within *G. morhua*. The Greenland cod appears to represent a contemporary northward and eastward range extension of the Pacific cod, and should be synonymized with it as *G. macrocephalus.*

Résumé : Les relations phylogénétiques ont été étudiées chez 14 espèces de gadidés à l'aide de segments de deux gènes d'ADN mitochondrial (ADNmt), un segment de 401 paires de base du gène cytochrome *b* et un segment de 495 paires de base du gène cytochrome oxydase I. Les données moléculaires indiquent que les trois espèces de gadidés endémiques dans le bassin du Pacifique représentent des invasions simultanées de lignées phylogénétiques indépendantes. La Goberge de l'Alaska (*Theragra chalcogramma*) est à peu près aussi apparentée à la Morue franche (*Gadus morhua*) que la Morue du Pacifique (*Gadus macrocephalus*), ce qui semble indiquer que *T. chalcogramma* et *G. macrocephalus* ont envahi le bassin du Pacifique séparément. Le Poulamon du Pacifique (*Microgadus proximus*) est plus apparenté au Navaga de la mer de Barents (*Eleginus navaga*) qu'à son congénère le Poulamon atlantique (*Microgadus tomcod*), ce qui semble indiquer que le Poulamon du Pacifique est dérivé de la lignée d'*Eleginus* et le nom *Eleginus* devrait être considéré comme synonyme de *Microgadus*. Les divergences moléculaires entre les trois espèces endémiques du Pacifique et les taxons qui leur sont le plus apparentés sont semblables et rappellent les cas de spéciation qui se sont produits après la réouverture du détroit de Bering il y a ca. 3,0–3,5 millions d'années. Par ailleurs, l'Ogac (*Gadus ogac*) et *G. macrocephalus* ont des séquences d'ADNmt essentiellement identiques et les différences entre ces taxons sont moins importantes que celles qui prévalent chez *G. morhua*. Il semble donc que l'Ogac représente une expansion d'aire contemporaine vers le nord et vers l'est de la Morhue de la Pacifique et devrait donc être considéré comme un synonyme de *G. macrocephalus*.

[Traduit par la Rédaction]

Introduction

The family Gadidae is a group of benthopelagic fishes that inhabit coastal zones, continental shelves, and slopes, primarily in the northern oceans at depths up to 1300 m (Scott and Scott 1988). The family is currently classified into three subfamilies: Phycinae (27 species in 6 genera), Lotinae (4 species in 3 genera), and Gadinae (22 species in 12 genera) (Cohen et al. 1990). Phycines and lotines resemble the ancestral form of the gadids, characterized by one or two dorsal fins and one caudal fin and an elongate laterally compressed body. Gadines are the more morphologically derived members of the family, with greater differentiation of the dorsal and caudal fins; there is considerable diversity among species. The gadines are an important group of commercially exploited fish, representing more than one-tenth of the total fish caught worldwide (Cohen et al. 1990).

The greatest diversity of gadines occurs in the Atlantic Ocean, where species such as *Gadus morhua* (Atlantic cod), *Pollachius virens* (saithe or black pollock), *Pollachius pollachius* (yellow pollock), and *Melanogrammus aeglefinus*

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Table 1. Scientific names, with references, for the 14 gadine species in eight genera used in this study (after Cohen et al. 1990).

Scientific name	Genus authority
Boreogadus saida (Lepechin, 1774)	*Boreogadus* Gűnther, 1862
Eleginus navaga (Pallas, 1811)	*Eleginus* Fischer, 1812
Gadus macrocephalus Tilesius, 1810	*Gadus* L., 1758
Gadus morhua L., 1758	*Gadus* L., 1758
Gadus ogac Richardson, 1836	*Gadus* L., 1758
Melanogrammus aeglefinus (L., 1758)	*Melanogrammus* Gill, 1862
Merlangius merlangus (L., 1758)	*Merlangius* Geoffroy, 1767
Microgadus proximus (Girard, 1854)	*Microgadus* Gill, 1865
Microgadus tomcod (Walbaum, 1792)	*Microgadus* Gill, 1865
Pollachius virens (L., 1758)	*Pollachius* Nilsson, 1832
Theragra chalcogramma (Pallas, 1811)	*Theragra* Lucas, 1899
Trisopterus esmarkii (Nilsson, 1855)	*Trisopterus* Rafinesque, 1814
Trisopterus luscus (L., 1758)	*Trisopterus* Rafinesque, 1814
Trisopterus minutus (L., 1758)	*Trisopterus* Rafinesque, 1814

(haddock) are found in both eastern and western regions. *Gadus ogac* (Greenland cod) and *Microgadus tomcod* (Atlantic tomcod) are found primarily in coastal areas of the western Atlantic and extend into the Arctic Ocean. *Merlangius merlangus* (whiting) and the three species of the genus *Trisopterus* (*T. esmarkii* (Norway pout), *T. luscus* (pouting), and *T. minutus* (poor cod)) occur exclusively in the eastern Atlantic and into the Mediterranean Sea. *Boreogadus saida* is found in the northern Atlantic and has a circumpolar distribution in the Arctic Ocean. Species of *Eleginus* are found primarily in Arctic regions: *Eleginus navaga* is restricted to coastal areas of the Barents, White, and Karas seas, and *Eleginus gracilis* is found in the Bering Sea and the North Pacific. Three species of gadines are endemic to Pacific waters: *Theragra chalcogramma* (walleye or Alaska pollock) and *Gadus macrocephalus* (Pacific cod) are found on both the eastern and western continental shelves of the Pacific Ocean, and *Microgadus proximus* is found from the southern Bering Sea to central California and inhabits coastal areas much like the Atlantic species of the genus.

Although the gadids are a commercially important family, their evolutionary relationships are relatively poorly understood. Many of the species were known to Linnaeus, and all of those described above were originally placed in the genus *Gadus*; there has since been little agreement on their classification (reviews by Svetovidov 1948; Fahay and Markle 1984; Cohen 1989; Cohen et al. 1990) (Table 1). Previous systematic work has relied on comparisons of morphology (Svetovidov 1948) or adult and juvenile osteological characters (Dunn 1989), and has emphasized elucidation of higher order relationships among families and subfamilies. Several recent studies have examined genetic relationships within and among closely related species. Protein electrophoretic comparisons have been made within *T. chalcogramma* (Grant and Utter 1980), for *G. morhua* and *G. macrocephalus* (Grant et al. 1987; Grant and Ståhl 1988), and for *G. morhua, G. macrocephalus, G. ogac, B. saida, T. chalcogramma, E. gracilis*, and *M. tomcod* (Renaud et al. 1986; Renaud 1989). Direct DNA sequencing of mitochondrial DNA (mtDNA) has been used to study within-species variation in *G. morhua* (Carr and Marshall 1991*a*, 1991*b*; Pepin and Carr 1993; Carr et al. 1995; Crutcher 1996[2]; Carr and Crutcher 1998). Interspecies sequence comparisons have been made between *G. morhua* and *G. ogac* (Carr and Marshall 1991*b*) and many other fish species (reviewed by Lydeard and Roe 1997).

In this study, we extend the examination of mtDNA sequence variation to include 14 species in nine genera from the subfamily Gadinae in order to clarify their evolutionary relationships and understand their biogeographic origins and distributions. We are particularly interested in the relationships of the three endemic Pacific species.

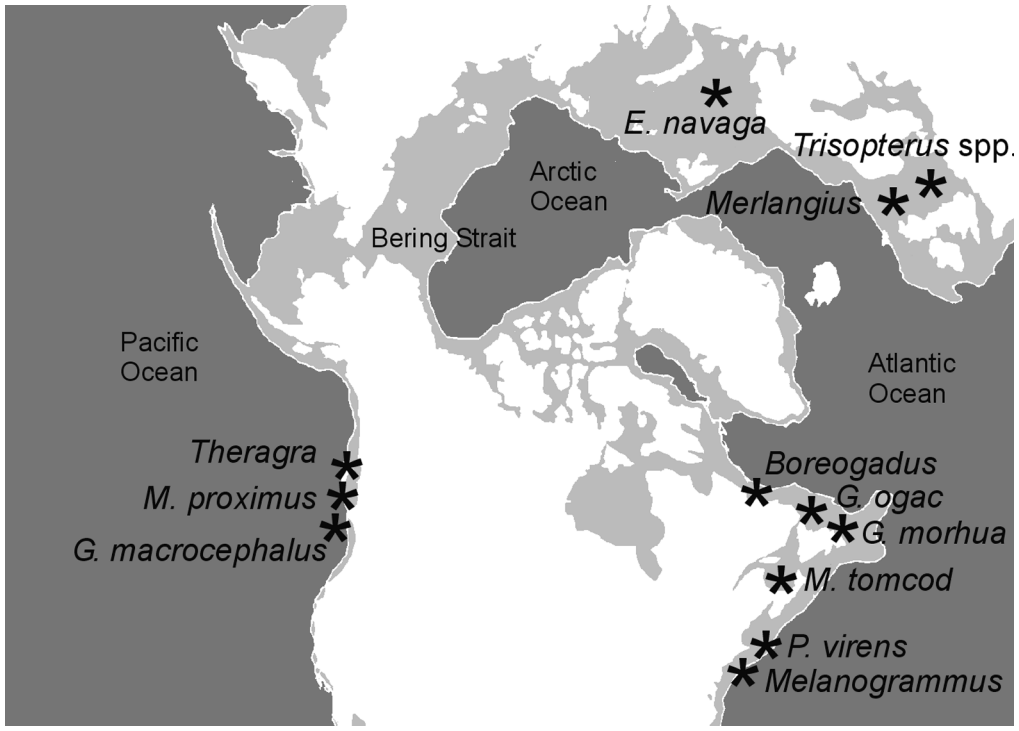
Materials and methods

Specimens of *B. saida*, *G. ogac*, and *G. morhua* were collected on the Newfoundland shelf between 1991 and 1993 (Department of Fisheries and Oceans, St. John's, Nfld.). *Theragra chalcogramma*, *M. proximus*, and *G. macrocephalus* were collected from the Hecate Strait off the west coast of British Columbia in 1993 (Department of Fisheries and Oceans, Nanaimo, B.C.). *Eleginus navaga* was collected in the Barents Sea in 1994 (Norsk Institutt for fiskeri og havbruksforskning AS, Tromsö, Norway). *Melanogrammus aeglefinus* and *P. virens* were collected from George's Bank off the east coast of New England in 1991 (National Marine Fisheries Service, Woods Hole, Mass., U.S.A.). *Merlangius merlangus* and the three species of *Trisopterus* (*T. esmarkii*, *T. luscus*, and *T. minutus*) were collected from the North Sea in 1991 (Ministry of Agriculture, Food and Fisheries, Lowestoft, U.K.). *Microgadus tomcod* was collected from the Gulf of St. Lawrence (Université Laval, Québec, Que.). Collecting localities for all samples are shown in Fig. 1.

DNA was extracted from frozen muscle tissue with the acid guanidium thiocyanate – phenol – chloroform DNA extraction method (Bartlett and Davidson 1992). Two portions of the mitochondrial genome were amplified by the polymerase chain reaction (PCR). A region including a 401 base pair (bp) segment at the 5′ end of the cytochrome *b* sequence was amplified with oligonucleotide primers 5′-cgaagcttgatatgaaaaaccatcgttg-3′ (L14724 of

[2] D.C. Crutcher. 1996. Population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic as determined by mitochondrial DNA sequence data. B.Sc. (Hons.) thesis, Memorial University of Newfoundland, St. John's.

Fig. 1. Polar projection map showing locations of sample collections.



Irwin et al. 1991) and 5′-gcccctcagaatgatatttgtcctca-3′ (H15149 of Kocher et al. 1989). A region including a 495 bp segment of the cytochrome oxidase I gene was amplified with oligonucleotide primers 5′-cctgctggaggaggtgatcc-3′ (Co1fcod-L, modified from Kessing et al. 1989) and 5′-ccagagattagagggaatcagtg-3′ (Co1e-H of Kessing et al. 1989). PCR reactions were carried out as described by Carr and Marshall (1991*a*, 1991*b*), Crutcher (1996, see footnote 2), and Kivlichan (1997).[3] DNA sequencing was accomplished with a fluorescent dye terminator chemistry and an Applied Biosystems 373A automated DNA sequencer, and the same primer pairs that were used for amplification (Carr and Marshall 1991*b*). At least two individual fish from each species were analyzed. The DNA sequences reported here are a consensus of the two complementary strands; all sequences are given as their coding-strand equivalents.

Sequences were analyzed with the SeqEd™ 675 DNA Sequence Editor (Applied Biosystems, Inc.) and the ESEE program (Cabot and Beckenbach 1989). Maximum-likelihood (ML), neighbour-joining (NJ), and maximum-parsimony (MP) analyses were performed with the PAUP (Phylogenetic Analysis Using Parsimony) program (version 4, release d63) (Swofford 1998). ML analyses were made with empirical estimates of the transition/transversion ratio (Ts/Tv) as 8.4 and the gamma parameter, *a*, as 0.158, a heuristic search with five random taxon additions, and the nearest-neighbour branch-swapping option, with and without enforcement of the molecular clock constraint. Bootstrap analyses were performed by a heuristic search with a taxon-addition order determined by NJ and a heuristic search with a single nearest-neighbour-interchange branch-swapping for each of 1000 replicates. NJ analysis was performed on ML and Tamura–Nei (T–N) distances (Tamura and Nei 1993) (Ts/Tv and gamma parameters as above, or a gamma parameter of 0.5); bootstrap analysis was done with 1000 replicates. MP analysis was done by heuristic search (transversion to transition weightings of 1:1, 3:1, 8.4:1, and transversion only, and 10 random taxon additions with the tree-bisection-and-reconnection branch-swapping option); bootstrap analysis of parsimony trees was done with 1000 replicates, each with 5 random taxon additions and the nearest-neighbor-interchange branch-swapping option.

Results

Among 14 gadid species, 273 of the 896 nucleotide positions examined from both gene segments were variable; 217 of these were phylogenetically informative (sensu Nei 1987). Within the 495 bp segment of the cytochrome oxidase I gene, there were 141 variable nucleotide positions of which 122 were phylogenetically informative. Within the 401 bp segment of the cytochrome *b* gene, there were 132 variable nucleotide positions of which 95 were phylogenetically informative. The sequences were submitted to GenBank and have been assigned the accession numbers AF081682–AF081695 (cytochrome *b* sequences for *G. morhua*, *G. macrocephalus*, *G. ogac*, *T. chalcogramma*, *B. saida*, *M. aeglefinus*, *P. virens*, *E. navaga*, *M. proximus*, *M. tomcod*, *T. minutus*, *T. luscus*, and *T. esmarkii*, respectively) and AF091696–AF081709 (cytochrome oxidase I sequences for these species in the same order as above).

Table 2 shows the genetic distances calculated for the combined data set of 896 bp as uncorrected pairwise nucleotide differences and T–N distances. *Gadus macrocephalus* and *G. ogac* have identical sequences for the two gene segments. Identical sequences for *G. macrocephalus* have been obtained from four individuals from two separate sources; identical sequences for *G. ogac* have been obtained from

[3] D.S. Kivlichan. 1997. Biogeographic origins of gadid fishes as inferred from mitochondrial cytochrome oxidase I and cytochrome *b* sequences. B.Sc. (Hons.) thesis, Memorial University of Newfoundland, St. John's.

Table 2. Pairwise numbers of nucleotide differences (lower half of the matrix) and Tamura–Nei distances (upper half of the matrix) calculated from 896 bp of mitochondrial cytochrome *b* and cytochrome oxidase I genes from 14 gadid species.

	G. morhua	*G. macrocephalus*	*G. ogac*	*T. chalcogramma*	*B. saida*	*M. aeglefinus*
G. morhua	0	0.054	0.054	0.043	0.061	0.130
G. macrocephalus	44	0	0	0.045	0.064	0.129
G. ogac	44	0	0	0.045	0.064	0.129
T. chalcogramma	35	37	37	0	0.052	0.130
B. saida	49	51	51	42	0	0.151
M. aeglefinus	95	94	94	94	104	0
M. merlangus	76	77	77	77	82	83
P. virens	82	86	86	80	86	106
E. navaga	97	90	90	95	105	122
M. proximus	104	107	107	105	110	121
M. tomcod	112	109	109	113	110	139
T. minutus	124	119	119	119	127	134
T. luscus	129	120	120	127	133	145
T. esmarkii	127	128	128	127	135	149

multiple individuals (P. Pepin and S. Carr, unpublished observation in Pepin and Carr 1993), with only one nucleotide variant, which is not shared with other taxa (not shown). *Gadus ogac* was therefore removed from subsequent analyses, which were thus done on 13 taxa. Otherwise, the most similar pair is *G. morhua* and *T. chalcogramma*, which differ by 35 substitutions and a distance of 0.043. The least similar pairs are *M. aeglefinus* and *T. esmarkii*, which differ by 149 substitutions and a T–N distance of 0.234, or *M. tomcod* and *T. minutus*, which differ by 146 substitutions and a T–N distance of 0.242.

ML (Fig. 2A), NJ (Fig. 2B), and MP (Fig. 2C) methods all produced trees with similar topologies. The following groups were identified as clusters (ML and NJ analyses) or clades (MP analysis) in at least 50% of the bootstrap replicates from all three methods: (*i*) all three *Trisopterus* spp., *T. luscus* being the sister to the other two species, and the genus being the sister group to the remaining taxa; (*ii*) the two *Microgadus* spp. plus *E. navaga*, the latter species being more closely related to *M. proximus* than either is to *M. tomcod*; (*iii*) *M. aeglefinus* + *M. merlangus*; and (*iv*) *Gadus* spp. + *B. saida* + *T. chalcogramma*. The relationships of *P. virens* were indeterminate: it falls outside all of the above groups, but its exact relationships are otherwise indeterminate. Groups *iii* and *iv* occurred as sister groups in ML and MP but not NJ analyses.

The lack of consistent bootstrap support for the deeper branches among the different analyses appears to be, in part, a consequence of a high number and unusual pattern of nucleotide substitutions in *M. aeglefinus* and *M. tomcod* (cf. Table 2): there are six unique amino acid substitutions in *M. aeglefinus*, more than in any other taxon in this study. In NJ analyses, T–N distances with the empirically estimated gamma parameter produced extremely long branch lengths in *M. aeglefinus* and *M. tomcod*, whereas ML distances (Fig. 2B) or T–N distances with gamma parameters closer to 0.5 produced results similar to the MP and ML analyses. For relatively short DNA sequences, ML distances seem to be more reasonable estimates of genetic distance than either T–N or Kimura two-parameter models (D. Swofford, personal communication). As well, and in contrast to typical vertebrate patterns (Kocher and Carleton 1997), transversions did not accumulate on deeper branches in these data. In MP analyses with low weightings of transversions to transitions (3:1, or 1:1 as in Fig. 2C), *M. aeglefinus* groups with *M. merlangus*, as it does in the ML and NJ analyses, whereas when transversions are more heavily weighted (empirical 8.4:1), the former species pulls out as a separate lineage.

Separate analyses of the cytochrome oxidase I (Kivlichan 1997, see footnote 3) and cytochrome *b* sequences identified the same four groups described above; however, bootstrap supports were weaker than those in the combined analysis (results not shown).

Discussion

The biogeographic evidence provided by the present-day distribution of gadine species suggests that their original area of endemism was the eastern North Atlantic (Svetovidov 1948). The molecular data confirm this, and further indicate that the three endemic Pacific species of gadids represent distinct phylogenetic lineages that have entered the Pacific Basin independently (Fig. 3). The Atlantic tomcod (*M. proximus*) is more closely related to *E. navaga* from the Barents Sea than to the congeneric Pacific tomcod (*M. tomcod*). *Boreogadus saida* and *T. chalcogramma* are about as closely related to *G. morhua* as is *G. macrocephalus*, which suggests that *T. chalcogramma* and *G. macrocephalus* represent separate invasions of the Pacific Basin.

The genus *Gadus* presently comprises three nominal species, *G. morhua*, *G. macrocephalus*, and *G. ogac* (Cohen et al. 1990). The Greenland cod (*G. ogac*) is usually regarded as more closely related to the partially sympatric Atlantic species *G. morhua* than either is to the allopatric, endemic Pacific species *G. macrocephalus*. Grant and Ståhl (1988) showed that, as measured by protein electrophoresis, *G. macrocephalus* is less genetically variable than *G. morhua*. Low genetic variability and the distribution of allele frequencies in the Pacific species are consistent with the notion that the Pacific species underwent a bottleneck at the time of its origin. Those authors suggest that *G. macrocephalus* is derived

Table 2 (*concluded*).

M. merlangus	*P. virens*	*E. navaga*	*M. proximus*	*M. tomcod*	*T. minutus*	*T. luscus*	*T. esmarkii*
0.100	0.112	0.134	0.146	0.171	0.187	0.191	0.190
0.100	0.117	0.123	0.153	0.165	0.176	0.172	0.188
0.100	0.117	0.123	0.153	0.165	0.176	0.172	0.188
0.102	0.108	0.130	0.149	0.174	0.179	0.186	0.190
0.110	0.120	0.151	0.161	0.170	0.196	0.199	0.207
0.110	0.155	0.179	0.178	0.228	0.204	0.227	0.234
0	0.120	0.136	0.151	0.184	0.177	0.199	0.192
87	0	0.159	0.149	0.159	0.167	0.196	0.178
99	110	0	0.064	0.144	0.214	0.199	0.219
107	105	52	0	0.135	0.199	0.197	0.214
118	105	98	94	0	0.242	0.223	0.237
121	116	141	133	146	0	0.135	0.102
133	131	136	134	144	97	0	0.148
129	122	145	140	145	78	107	0

from *G. morhua* and migrated through the Bering Strait when it first opened ca. 3.0–3.5 million years BP (Herman and Hopkins 1980). Although the Isthmus of Panama was also submerged at this time, it is unlikely that gadids reached the Pacific Basin by that route, as no extant species occur that far south. *Gadus ogac* is distinguished from *G. macrocephalus* by the coloration of the peritoneum and by a marked difference in size; *G. ogac* is not usually longer than 70 cm, whereas *G. macrocephalus* can grow to over 100 cm. The smaller size of *G. ogac* may be due to the harsh Arctic environment (Scott and Scott 1988; Brander 1995). Growth of *G. macrocephalus* is much faster in the south and spawning occurs at a younger age than in colder northern waters (Cohen et al. 1990). However, despite these differences, the current data, as well as previous protein electrophoretic comparisons, show that *G. macrocephalus* and *G. ogac* are genetically indistinguishable. Renaud et al. (1986) examined 21 electrophoretic loci in *Gadus*. Of these, 17 loci were fixed for identical alleles between the Pacific *G. macrocephalus* and the Atlantic *G. ogac* and 4 differed only by the presence of minor alleles at low frequencies. In contrast, the sympatric *G. morhua* and *G. ogac* showed fixed allele differences at 7 of these loci. In the same region of the cytochrome *b* gene examined here, more than 50 genotypes have been identified in *G. morhua*, some of which differ by as many as six nucleotide substitutions (Pepin and Carr 1993; Carr et al. 1995; Crutcher 1996, see footnote 2; Carr and Crutcher 1998). Although our population samples are small, *G. ogac* and *G. macrocephalus* seem to be much less genetically differentiated than transatlantic populations of *G. morhua*, or even populations on the Grand Bank and the adjacent Flemish Cap (Crutcher 1996, see footnote 2; Carr and Crutcher 1998). We therefore suggest that *G. ogac* is simply a northward and eastward extension of the range of *G. macrocephalus*, and that gene flow probably continues throughout this region. *Gadus macrocephalus* and *G. ogac* should be synonymized: *Gadus macrocephalus* Tilesius, 1810 has nomenclatorial priority over *Gadus ogac* Richardson, 1836. A lower level of cytoplasmic genetic diversity with respect to *G. morhua* throughout the range of a geographically widespread *G. macrocephalus* (sensu lato, including *G. ogac*) may stem from the same bottleneck that reduced nuclear gene variation (Wilson et al. 1985). Another indication of similarity between *G. macrocephalus* and *G. ogac* is that both produce negatively or neutrally buoyant eggs. This is in contrast to *G. morhua*, *T. chalcogramma*, and most other gadines, which have positively buoyant eggs (*Microgadus* and *Eleginus* also have negatively buoyant demersal eggs, see below).

Relationships among the nominal genera *Gadus*, *Theragra*, and *Boreogadus* are not well resolved; however, pairwise sequence differences (Table 2 and Fig. 2D) suggest the occurrence of more or less simultaneous separations contemporaneous with those within *Gadus*. If *G. macrocephalus* were the outgroup to the remaining taxa (cf. Fig. 2A), or less closely related to *G. morhua* than was *T. chalcogramma* (cf. Fig. 2D), then the nomenclature of *Boreogadus* and *Theragra* might require revision. Pending clarification of the relative branching order of these taxa, we suggest that the third endemic Pacific species, *T. chalcogramma*, is yet another independent invasion of the Pacific Basin that occurred as part of a rapid adaptive radiation at the time of the reopening of the Bering Strait. Grant and Utter (1980) showed that this species, like *G. macrocephalus*, is genetically depauperate compared with *G. morhua* with respect to its protein-electrophoretic alleles and that it, like *G. morhua*, does not occur as multiple genetically distinct local stocks (cf. Pepin and Carr 1993; Carr et al. 1995; Bentzen et al. 1996; Carr and Crutcher 1998). Life-history characteristics that have placed *G. morhua* and *T. chalcogramma* among the world's most successful fishery resources may well be the result of a common evolutionary history.

The molecular data indicate that *M. proximus* is more closely related to *E. navaga* than to the other congeneric tomcod, *M. tomcod*. The genetic distance between *E. navaga* and *M. proximus* (0.064) is approximately equivalent to that between *G. macrocephalus* and *G. morhua* (0.053), whereas the distance between the two *Microgadus* species (0.135) is more than twice as large. If the divergence of the two *Gadus* species corresponds to a separation at the time of the first opening of the Bering Strait, these data would suggest that the separation of the Pacific and Atlantic lineages of

Fig. 2. Relationships among 14 species of gadids suggested by three methods of phylogenetic analysis of 896 bp of mitochondrial cytochrome *b* and cytochrome oxidase I genes. The number below the branch indicates the percent occurrence of that branch among 1000 bootstrap replicates. (A) ML analysis: Ts/Tv = 8.4 and gamma parameter = 0.16. (B) NJ analysis: ML distances, Ts/Tv, and gamma parameter as above. (C) MP analysis: transitions and transverions equally weighted. (D) MP relationships among *Gadus* spp., *B. saida*, and *T. chalcogramma*. The inferred number of nucleotide substitutions along each branch is indicated. None of the branches are supported by bootstrap analysis (cf. C).

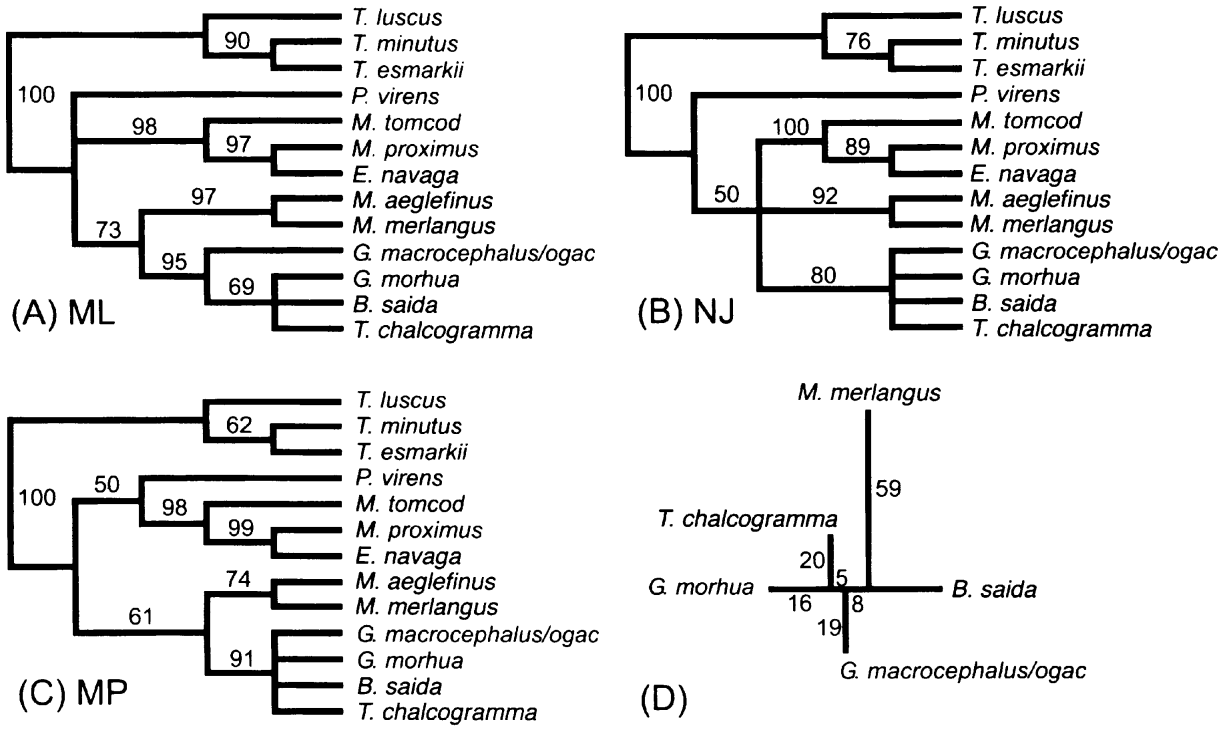
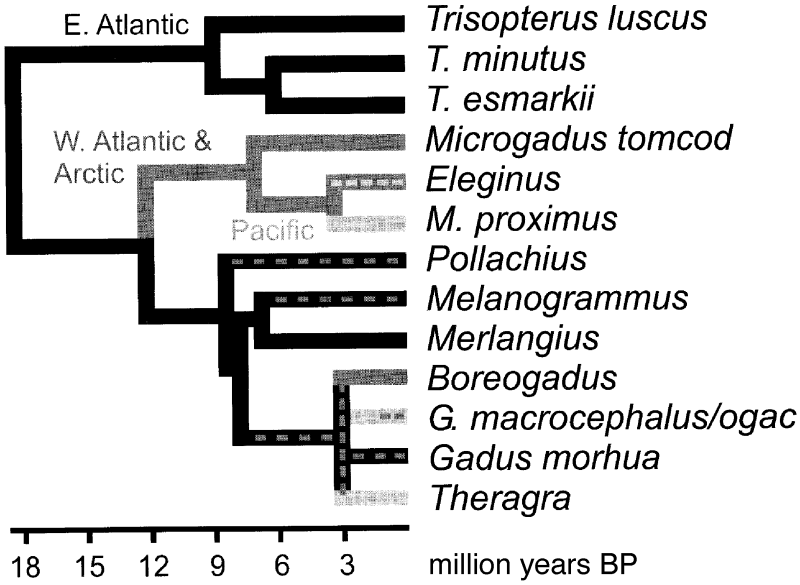


Fig. 3. Hypothetical reconstruction of the biogeographic origins and distributions of gadids as inferred from DNA sequence data. The cladogram is based on a ML analysis (cf. Fig. 2A) with the molecular clock constraint enforced. Relationships among *Gadus* spp., *Theragra*, and *Boreogadus* are unresolved and have been collapsed at the average distance among these taxa. Shaded lines indicate present-day distribution of extant lineages; lines with dashes indicate secondary distributions. Distributions of *Eleginus* and *Pollachius* include those for *E. navaga* and *P. pollachius*, which were not included in this study (see the text). The time scale is based on data in Table 2; the calibration rate is 1.5% per million years per pair of lineages (cf. Bermingham et al. 1997, p. 119).



Microgadus must have occurred well before this time, and that the Pacific species was derived from *Eleginus* at about the same time as the two species of *Gadus* separated (Fig. 3). The ancestor of *M. proximus* evidently evolved in the Barents Sea and migrated eastward through the Siberian Sea and Bering Strait to the Pacific Ocean (Messtorff 1973).

To keep *Microgadus* monophyletic (sensu Farris 1974), *E. navaga* should be included in the genus as *Microgadus navaga* (Pallas, 1811). The relationships of *E. gracilis* (which was not included in this investigation) will require clarification. We hypothesize that this species represents a fourth independent invasion of the Pacific Basin and is most closely related to *M. navaga*. The ranges of *E. gracilis* and *M. proximus* overlap extensively in the waters around the Aleutian Islands south of Alaska. *Eleginus* and *Microgadus* are distinct from all other gadines in possessing continuous lateral lines along all or part of their length, which would seem to be a synapomorphy of the group. These taxa also have negatively buoyant eggs, unlike most other gadines except *G. macrocephalus* sensu lato, as noted above. The expanded parapophyses of the precaudal vertebrae in *M. navaga* would appear to be autapomorphic and the partial expansion of these structures in *E. gracilis* an intermediate stage. Alternatively, *E. gracilis* might be more closely related to *M. proximus* and its distribution north of the Bering Strait a reinvasion of this area. This seems less likely, both because secondary northward passage through the Bering Strait has been discouraged by a drop in the temperature of Arctic Ocean waters in the last 3 million years (Emiliani 1961) and because this would require parallel changes in the structure of the parapophyses along two distinct lineages. (The third alternative, a close relationship of *E. gracilis* with *M. tomcod*, is unlikely on biogeographic grounds.)

This analysis may help to clarify the morphological evolution of gadines. The most recent quantitative analysis of gadid relationships is that of Dunn (1989), who performed a cladistic analysis of 28 morphological characters in 11 genera of gadines, including all of those in the present study, with the hake (*Merluccius*) as outgroup. (For his analysis of genera, Dunn used composites of characters for *G. morhua* and *G. macrocephalus*, for both species of *Pollachius*, and for all three species of *Trisopterus*, and used *E. gracilis* to represent *Eleginus*.) Except for a close relationship among *Gadus*, *Theragra*, and *Boreogadus*, there is little or no similarity between Dunn's (1989) cladogram and the evolutionary relationships implied by the molecular data: for example, there are no indications of a close relationship between *Eleginus* and *Microgadus* (Dunn separated these into distinct subfamilies within the Gadidae), and *Trisopterus* appears to be more closely related to *Merlangius* than to any other taxon in the present study. Reanalysis of Dunn's (1989) data for the nine taxa common to our two studies indicates that Dunn's tree would require 30 morphological changes, whereas the trees equivalent to those shown in Fig. 2 would each require 35 changes; these lengths cannot be differentiated by bootstrap analysis. In contrast, the distribution of nucleotide substitutions among the same species in the trees equivalent to those shown in Fig. 2 can be explained with no more than 450 substitutions, whereas the minimum-length morphology tree (equivalent to Fig. 29 in Dunn 1989) would require at least 502 substitutions; the latter can be rejected.

In his classic study of gadiform evolution, Svetovidov (1948) constructed a key to gadid genera that can be interpreted as a phylogenetic hypothesis. As presented in Fig. 1 of Dunn (1989), each successive taxon in this key is the sister to the remaining succeeding taxa. For the nine taxa analyzed by both Dunn (1989) and ourselves, Svetovidov's arrangement requires 33 changes in the morphological data and 517 changes in the molecular data. Svetovidov recognized *Trisopterus* as the outgroup to the remaining gadines, and a close relationship between *Microgadus* and *Eleginus* as successive taxa in his key (though they are therefore not each other's closest relative). The poor fit of Svetovidov's arrangement to the molecular data is due in part to his separation of *Gadus* from *Boreogadus* and *Theragra*.

We conclude that although morphological data are compatible with the molecular tree, the reverse is not true. Dunn (1989) acknowledged that analyses of gadiform morphological data are troubled with a high degree of homoplasy (parallel change) and difficulties in determining character polarity. Many of the internal and external features of these species seem to have arisen independently in different lineages. We concur, and suggest that reevaluation of the evolution of osteological and morphological characters among gadids is required in light of the molecular analysis presented here.

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