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## Phylogenetic position of the cryopelagic codfish genus *Arctogadus* Drjagin, 1932 based on partial mitochondrial cytochrome b sequences

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**Abstract** In order to elucidate the phylogenetic position of the cryopelagic cod genus *Arctogadus*, gadine phylogenetic relationships were examined using the mitochondrial DNA cytochrome b gene. A segment of 401 base pairs was sequenced from 6 gadine species [*Arctogadus borisovi* Drjagin, *A. glacialis* (Peters), *Gadiculus argenteus* Guichenot, *Micromesistius poutassou* (Risso), *Pollachius pollachius* (L.), *Pollachius virens* (L.)] and from 4 gadiform outgroup species. With additional data from GenBank, a dataset of all 12 gadine genera (19 species) was analysed using parsimony and neighbour-joining. *Arctogadus* appeared in a terminal clade as sistergenus to *Boreogadus* and closely related to *Gadus* and *Theragra*. The relatively small genetic difference between these four genera indicates a need for taxonomic revision, and possibly that *Arctogadus* should be synonymised with *Boreogadus* or *Gadus*. A difference of only 0–2 base pairs between specimens of *A. borisovi* and *A. glacialis* indicates that they are conspecific, with *A. borisovi* as the junior synonym.

### Introduction

Codfishes in the subfamily Gadinae are probably the most studied of all fishes owing to their great importance to commercial fisheries (Cohen et al. 1990). However,

only two phylogenetic analyses of these fishes have been published. Dunn (1989) presented an analysis based on 28 morphological characters of 11 genera (all gadine genera except *Arctogadus* Drjagin, 1932), represented by 11 species. Carr et al. (1999) provided an analysis based on cytochrome b and cytochrome oxidase I genes (401 + 495 bp) including 14 species in 9 genera (all genera except *Arctogadus*, *Gadiculus* Guichenot, 1850 and *Micromesistius* Gill, 1863). Both studies concluded that more work is needed.

There is general agreement that the 12 genera in Gadinae (sensu Svetovidov 1948) form a monophyletic group, representing the most derived subfamily of codfishes. This group has been raised to family rank by several authors (e.g. Cohen 1984; Dunn 1989; Markle 1989). The assumed monophyly of the group is based on several apomorphic character states such as number of dorsal and anal fins, loss of oil globules in eggs, loss of X/Y bones, number of hypural and suborbital bones, and adpression of first neural spine to the skull (Svetovidov 1948; Dunn 1989). The relationship of the group to other subfamilies and families, and its classification are still a matter of debate (Cohen et al. 1990; Howes 1991; Nelson 1994). In the present study, we follow the classification provided by Nelson (1994): Gadinae and Lotinae are regarded as subfamilies of Gadidae and the phycid and merlucciid genera are placed in separate families. Phylogeny at the subfamily/family level is of great importance to the cladistic analysis of gadine phylogeny, as outgroups should be chosen among the most closely related gadid taxa.

The codfish genus *Arctogadus*, endemic to the Arctic Ocean, has never before been included in phylogenetic analyses of gadiform fishes and seldom appears in comparative studies. It is probably the least-studied genus within the Gadinae, although ongoing studies aim to reveal more information on the physiology and general biology of the genus (Jordan et al. 2001). *Arctogadus* differs from other codfishes in having dentigerous palatine bones and elliptical and imbricate scales (Nielsen and Jensen 1967). It is a cryopelagic genus that spends at

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least a part of its life-cycle in close association with sea ice (Andriashev et al. 1980). Studies of the ecology of *Arctogadus* reveal an opportunistic feeding behaviour, with pelagic crustaceans as the predominant food items (Andriashev et al. 1980; Süfke et al. 1998). Reports of mass occurrences of *Arctogadus* (Walters 1961; Andriashev et al. 1980) indicate that these fishes play an important role in the Arctic ecosystem.

*Arctogadus* has a relatively confusing taxonomic and nomenclatorial history, summarised by Nielsen and Jensen (1967). They considered *Arctogadus glacialis* (Peters, 1872) and *A. borisovi* Drjagin, 1932 as valid species, and concluded that *A. borisovi* differs from *A. glacialis* in the presence of a well-developed barbel, number of gill rakers (31–35 vs 27–34), wider, soft interorbital length [5.1–7.0% standard length (SL) vs 4.4–6.8% SL], smaller diameter of orbit (6.2–8.5% SL vs 8.0–11.0% SL) and larger maximum size (up to 502 mm SL vs up to 325 mm SL). Nielsen and Jensen (1967) have been followed by all recent authors (e.g. Dunn 1989; Cohen et al. 1990; Coad et al. 1995). Nielsen and Jensen (1967), however, reported a typical specimen of *A. borisovi* caught off South Greenland, but without a barbel, and with no indication of an accidental loss, thus weakening the only distinct diagnostic character for the separation of the two species. Recently, several “barbel-less” *A. borisovi* have been caught in Greenland waters, indicating the need for a new morphological revision of the genus, which is currently being carried out at the Zoological Museum, University of Copenhagen (A.D. Jordan, unpublished data).

The aim of the present study is to provide the first gadine phylogeny including all 12 gadine genera, and thereby to elucidate the phylogenetic position of the high-Arctic genus *Arctogadus*. The addition of the genera *Arctogadus*, *Gadiculus*, *Micromesistius* and three outgroup genera to the Carr et al. (1999) cytochrome b dataset is expected to enhance understanding of the phylogeny of the subfamily. Furthermore, the analysis will provide a measure of the genetic distance between the nominal species *A. glacialis* and *A. borisovi*, which can be used to evaluate the existence of two species.

## Materials and methods

### Samples

Samples of blood, muscle tissue or gill-arch tissue from 12 gadine specimens [*A. borisovi* ( $n=3$ ), *A. glacialis* ( $n=1$ ), *Gadiculus argenteus* Guichenot, 1850 ( $n=1$ ), *Micromesistius poutassou* (Risso, 1827) ( $n=1$ ), *Pollachius pollachius* (L., 1758) ( $n=1$ ), *P. virens* (L., 1758) ( $n=1$ )] and 4 outgroup species [*Merluccius merluccius* (L., 1758), family Merlucciidae; *Enchelyopus cimbrus* (L., 1766), family Phycidae; *Molva dipterygia* (Pennant, 1784), *Molva molva* (L., 1758), family Gadidae, subfamily Lotidae] were obtained from the tissue collection at the Zoological Museum, University of Copenhagen (ZMUC). The tissue samples were stored at  $-40^{\circ}\text{C}$  in 25% DMSO in a saturated solution of NaCl (Amos and Hoelzel 1991) or in 95% ethanol. Vouchers were fixed in 4% formaldehyde and stored in 70% ethanol at ZMUC. Sequences of 14 taxa sequenced by Carr et al. (1999) were downloaded from GenBank. Thus, the

present study includes all 12 genera of Gadinae, and 19 of the 22 valid species (sensu Cohen et al. 1990). Species not included are *Eleginus gracilis*, Tilesius, 1810, *Micromesistius australis*, Norman, 1937 and *Theragra finnmarchica* Koefoed, 1956, due to lack of samples. A list of all sequenced specimens is presented in Table 1.

The four specimens of *Arctogadus* were identified following Nielsen and Jensen (1967). A 140-mm total length (TL) specimen, caught on the upper continental slope off Baffin Island, was identified as *A. glacialis*, based on the lack of a barbel. Three large specimens (260, 380 and 385 mm TL), caught inshore at Uummannaq Fjord, West Greenland, were identified as *A. borisovi*, on the basis of the well-developed barbel (3.9 and 3.7% SL) on two of the specimens and the large body size. The lack of a barbel in the third specimen (and other similar specimens) could be because: (1) the barbel had been lost accidentally; (2) the specimen is an extremely large *A. glacialis*; (3) the presence or absence of a barbel is variable in *A. borisovi* (which might then be a junior synonym of *A. glacialis*).

### DNA extraction, PCR amplification and sequencing

DNA was extracted from blood, muscle tissue or gill-arch fragments using QIAmp Tissue Kit from Qiagen following the supplier's protocol. A section of the mitochondrial gene cytochrome b was amplified using 1  $\mu\text{l}$  of the extracted DNA as template. The PCR reaction was a standard 50- $\mu\text{l}$  reaction (Saiki et al. 1988) using the following parameters: 1 initial cycle of denaturation ( $94^{\circ}\text{C}$ , 2 min), followed by 30 cycles ( $94^{\circ}\text{C}$  for 30 s,  $49^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 40 s), and finally 1 cycle ( $94^{\circ}\text{C}$  for 30 s,  $49^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 10 min). Primers used for amplification of the cytochrome b fragment were: GLU-(L)-TGA CTT GAA GAA CCA C/TCG TTG-3' (Palumbi 1996) and CB2-(H)-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3' (Kocher et al. 1989). The resulting PCR product was purified using QIAquick PCR Purification Kit (Qiagen, Valencia, Calif.) and 1  $\mu\text{l}$  used as template in the following 10- $\mu\text{l}$  cyclic sequence (cs) reaction using ABI prism DNA Sequencing Kit (dRhodamine Terminator Cycle Sequencing Ready Reactions). The conditions for the cyclic sequence reaction were: 1 initial cycle ( $96^{\circ}\text{C}$ , 2 min), followed by 34 cycles ( $96^{\circ}\text{C}$ , 20 s,  $49^{\circ}\text{C}$ , 10 s,  $60^{\circ}\text{C}$ , 2 min), and finally 1 cycle ( $96^{\circ}\text{C}$ , 20 s,  $49^{\circ}\text{C}$ , 10 s,  $60^{\circ}\text{C}$ , 10 min). The product of the cs reaction was purified using ethanol precipitation and run on a Perkin Elmer ABI Prism 377 DNA Sequencer in a 5% polyacryl amid gel. The resulting chromatographs were aligned manually using the computer program Sequencher (Perkin Elmer).

### Phylogenetic analyses

The dataset resulting from the sequenced specimens and the GenBank sequences was analysed in PAUP\*4 (Swofford 1998). Representatives of the gadiform families Merlucciidae (*Merluccius merluccius*), Phycidae (*Enchelyopus cimbrus*) and the gadid subfamily Lotinae (*Molva dipterygia* and *Molva molva*) were designated as outgroup taxa. For the resulting topologies, the Consistency Index (CI) was calculated to estimate the levels of homoplasy (Kluge and Farris 1969). In parsimony analysis, only minimal-length trees were retained and zero-length branches were collapsed. The heuristic search algorithm was used and employed 1,000 random input orders of taxa and tree bisection-reconstruction (TBR) branch swapping. The robustness of the clades recovered in the phylogenetic hypotheses was evaluated using the Bremer Support (Bremer 1994).

A neighbour-joining heuristic analysis (Saitou and Nei 1987) was carried out deploying a random input order of the taxa, and basing the distances on mean character evolution and minimum evolution. Nodal support was estimated by bootstrap resampling analysis (400 replicates) (Felsenstein 1985).

## Results

In the initial parsimony analysis of all 26 taxa, 401 bp were included, of which 168 bp (42%) were variable and

**Table 1.** List of species, museum numbers, GenBank numbers, geographic positions and collection dates for specimens and GenBank data used in this study. Classification follows Nelson (1994). Specimens marked with \* are taken from Carr et al. (1999)

Species	Museum number	GenBank Accession no.	Collection locality	Date (yymmdd)
<b>Merlucciidae</b>				
<i>Merluccius merluccius</i>	ZMUC P375154	AF469623	North Sea, NE Atlantic	001113
<b>Phycidae</b>				
<i>Enchelyopus cimbricus</i>	ZMUC P374380	AF469624	Skagerrak, NE Atlantic	990429
<b>Gadidae</b>				
<b>Lotinae</b>				
<i>Molva dipterygia</i>	No voucher	AF469625	Denmark Strait, NE Atlantic	980629
<i>Molva molva</i>	ZMUC P374175	AF469626	North Sea, NE Atlantic	961010
<b>Gadinae</b>				
<i>Arctogadus borisovi</i> (barbel 0.0% SL)	ZMUC P375151	AF469627	Uummannaq Fjord, NW Atlantic	000912
<i>Arctogadus borisovi</i> (barbel 3.9% SL)	ZMUC P375152	AF469628	Uummannaq Fjord, NW Atlantic	000912
<i>Arctogadus borisovi</i> (barbel 3.7% SL)	ZMUC P375153	AF469630	Uummannaq Fjord, NW Atlantic	000912
<i>Arctogadus glacialis</i> (barbel 0.0% SL)	ZMUC P375091	AF469629	Baffin Bay, NW Atlantic	991009
<i>Boreogadus saida</i> *	No voucher	AF081686	Newfoundland Shelf, NW Atlantic	91-93
<i>Eleginus navaga</i> *	No voucher	AF081690	Barents Sea, NE Atlantic	94
<i>Gadiculus argenteus</i>	ZMUC P374208	AF469631	North Sea, NE Atlantic	961011
<i>Gadus macrocephalus</i> *	No voucher	AF081683	Hecate Strait, NE Pacific	93
<i>Gadus morhua</i> *	No voucher	AF081682	Newfoundland Shelf, NW Atlantic	91-93
<i>Gadus ogac</i> *	No voucher	AF081684	Newfoundland Shelf, NW Atlantic	91-93
<i>Melanogrammus aeglefinus</i> *	No voucher	AF081687	George's Bank, NW Atlantic	91
<i>Merlangius merlangus</i> *	No voucher	AF081688	North Sea, NE Atlantic	91
<i>Microgadus proximus</i> *	No voucher	AF081691	Hecate Strait, NE Pacific	93
<i>Microgadus tomcod</i> *	No voucher	AF081692	Gulf of St. Lawrence, NW Atlantic	?
<i>Micromesistius poutassou</i>	ZMUC P374240	AF469632	North Sea, NE Atlantic	961010
<i>Pollachius pollachius</i>	ZMUC P375156	AF469633	North Sea, NE Atlantic	010705
<i>Pollachius virens</i>	ZMUC P374176	AF469634	North Sea, NE Atlantic	960806
<i>Pollachius virens</i> *	No voucher	AF081689	George's Bank, NW Atlantic	91
<i>Theragra chalcogramma</i> *	No voucher	AF081685	Hecate Strait, NE Pacific	93
<i>Trisopterus esmarkii</i> *	No voucher	AF081695	North Sea, NE Atlantic	91
<i>Trisopterus luscus</i> *	No voucher	AF081694	North Sea, NE Atlantic	91
<i>Trisopterus minutus</i> *	No voucher	AF081693	North Sea, NE Atlantic	91

125 bp (31%) were phylogenetically informative. A matrix of the observed uncorrected paired nucleotide differences is shown in Table 2. Similar sequences were found between *Gadus macrocephalus* Tilesius, 1810 and *G. ogac* Richardson, 1836 (Carr et al. 1999), and between *A. glacialis* and *A. borisovi* (barbel 3.7% SL). These specimens were therefore merged in the following analyses. The least similar pair of in-group taxa was *Melanogrammus aeglefinus* (L., 1758) and *Trisopterus esmarkii* (Nilsson, 1855), which differed by 79 substitutions (19.7%). In the second parsimony analysis rooted with *Gadiculus*, 142 bp (35%) were variable and 107 bp (27%) were phylogenetically informative.

In the parsimony analysis of all 26 taxa with *Merluccius* as outgroup, a relatively poor resolution was achieved. Monophyly of Gadinae is neither confirmed nor affected, since the outgroup genera *Enchelyopus* Bloch and Schneider, 1801 and *Molva* LeSueur, 1819 appeared in separate clades in a large basal polytomy of eight clades (Fig. 1). The larger of the gadine clades (A) with *Melanogrammus* Gill, 1862, *Merlangius* Geoffroy, 1767, *Gadus* L. 1758, *Theragra* Lucas, 1899, *Boreogadus* Günther, 1862 and *Arctogadus* is similar to a clade provided by Carr et al. (1999), except for the addition of *Arctogadus*. The four specimens of *Arctogadus* occurred in an unresolved polytomy (F), and their relationship to

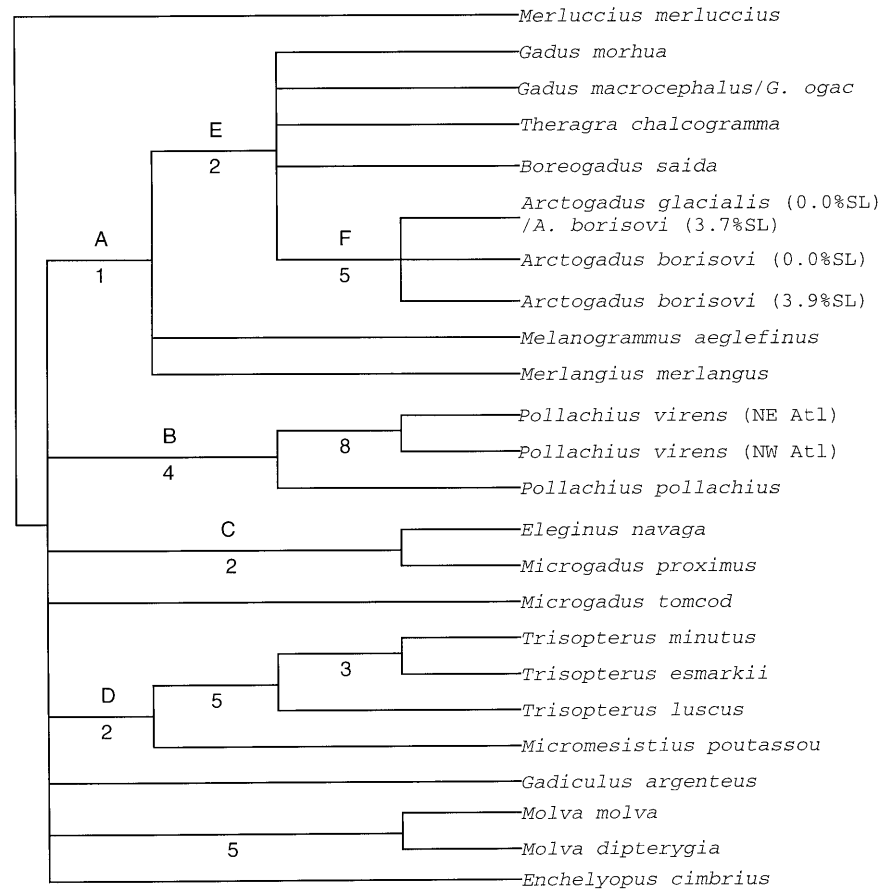
*Boreogadus*, *Theragra* and *Gadus* is not resolved either (E). *P. pollachius* and the two specimens of *P. virens* occurred in a separate clade (B). The topology indicates a close relationship of *Eleginus navaga* (Pallas, 1811) and *Microgadus proximus* (Girard, 1854) (C), but the second species of *Microgadus* Gill, 1865, *Microgadus tomcod* (Walbaum, 1792), is not included in this clade (C). It appeared in the basal polytomy, as did *Gadiculus argenteus*. *Micromesistius* is indicated to be sistergenus to *Trisopterus* Rafinesque, 1814 (D).

Several parsimony analyses were tried with all combinations of the four different outgroup taxa (results are not shown), but the resolution was as poor in these analyses as in the one with *Merluccius* as outgroup (Fig. 1). In an attempt to find the most basal gadine genus, a neighbour-joining analysis was conducted (Fig. 2). In this analysis *Molva* (Lotinae) and *Enchelyopus* (Phycidae) form a basal clade (I), as sistergroups to a clade (J) with all gadine genera. The most basal gadine genus appeared to be *Gadiculus*, which was also the result of the morphology-based phylogeny presented by Dunn (1989). The three major clades (A, H, D) are not in conflict with either the parsimony analysis (Fig. 1) or with Carr et al. (1999). *Arctogadus* is again placed in a clade (E) with *Gadus*, *Theragra* and *Boreogadus*. Within *Arctogadus* (F), two specimens of *A. borisovi* (barbel

**Table 2.** Pairwise numbers of nucleotide differences (*lower half* of the matrix) and percentages (*upper half* of the matrix)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
1 <i>Merluccius merluccius</i>	–	23.9	18.7	19.0	21.3	21.4	21.4	20.9	21.9	19.5	20.0	20.7	20.7	20.7	22.7	20.0	18.2	17.7	19.5	20.2	22.7	21.9	19.7	20.7	21.2	22.4	
2 <i>Enchelyopus cimbrius</i>	96	–	17.2	16.0	15.5	16.0	16.2	15.7	17.0	18.7	19.0	16.5	17.5	16.5	19.0	17.5	19.0	19.2	17.7	17.2	16.6	16.7	16.7	19.5	19.2	17.5	
3 <i>Molva dipterygia</i>	75	69	–	7.7	15.7	15.5	14.9	14.7	13.5	14.2	15.4	13.2	13.7	13.2	17.2	13.5	12.2	17.2	14.5	13.7	15.3	14.7	13.5	17.5	15.0	18.0	
4 <i>Molva molva</i>	76	64	31	–	13.9	14.4	14.4	14.5	15.2	14.0	16.5	14.0	15.0	14.0	17.2	15.2	14.5	16.2	14.2	16.7	17.1	16.5	13.2	17.0	15.0	16.7	
5 <i>Arctogadus borisovi</i> (barbel 0.0% SL)	80	58	59	52	–	0.5	0.5	0.5	6.9	10.1	14.0	5.6	5.3	5.6	13.3	7.7	12.5	12.3	11.7	12.8	12.6	12.5	6.7	14.4	15.5	12.0	
6 <i>Arctogadus borisovi</i> (barbel 3.9% SL)	80	60	58	54	2	–	0.3	0.3	6.7	10.4	14.4	5.3	5.1	5.3	13.1	7.5	12.3	12.0	11.5	12.6	12.3	12.3	6.7	14.4	15.5	12.3	
7 <i>Arctogadus borisovi</i> (barbel 3.7% SL)	82	62	57	55	2	1	–	0.0	6.2	9.9	13.8	5.0	4.7	5.0	12.5	7.0	11.7	11.5	11.0	12.0	11.8	11.7	6.5	13.8	14.9	12.0	
8 <i>Arctogadus glacialis</i> (barbel 0.0% SL)	84	63	59	58	2	1	0	–	6.2	10.7	13.7	5.0	5.0	5.0	12.5	7.4	11.7	11.0	11.0	11.5	11.2	11.2	6.5	14.4	14.5	12.5	
9 <i>Boreogadus saida</i> *	88	68	54	61	26	25	24	25	–	12.7	14.9	6.2	6.2	6.2	14.7	9.2	12.2	12.5	10.2	12.0	12.2	12.2	6.2	14.4	14.2	13.7	
10 <i>Eleginus nanaga</i> *	78	75	57	56	38	39	38	43	51	–	14.4	9.0	11.0	9.0	15.0	9.5	6.0	10.7	12.2	13.0	13.5	13.5	10.7	15.5	15.5	15.0	
11 <i>Gadulus argenteus</i>	79	75	61	65	52	53	52	54	59	57	–	14.2	14.7	14.2	17.0	12.6	15.7	14.7	12.4	13.7	15.1	15.2	14.2	17.5	14.9	15.9	
12 <i>Gadus macrocephalus</i> *	83	66	53	56	21	20	19	20	25	36	56	–	4.0	0.0	12.2	8.0	11.7	11.2	11.0	11.0	11.5	11.5	4.5	14.5	13.5	13.5	
13 <i>Gadus morhua</i> *	83	70	55	60	20	19	18	20	25	44	58	16	–	4.0	12.0	7.0	12.5	13.2	11.5	13.0	11.5	11.5	4.0	14.0	15.5	13.7	
14 <i>Gadus ogac</i> *	83	66	53	56	21	20	19	20	25	36	56	0	16	–	12.2	8.0	11.7	11.2	11.0	11.0	11.5	11.5	4.5	14.5	13.5	13.5	
15 <i>Melanogrammus aeglefinus</i> *	91	76	69	69	50	49	48	50	59	60	67	49	48	49	–	11.5	13.7	17.0	16.2	15.5	15.8	15.7	13.0	19.7	18.0	16.0	
16 <i>Merlangius merlangus</i> *	80	70	54	61	29	28	27	30	37	38	50	32	28	32	46	–	9.7	12.2	11.7	10.5	10.0	10.0	8.7	13.2	14.0	11.0	
17 <i>Microgadus proximus</i> *	73	76	49	58	47	46	45	47	49	24	62	47	50	47	55	39	–	9.5	11.0	10.2	11.2	10.7	11.7	15.0	15.5	12.7	
18 <i>Microgadus tomcod</i> *	71	77	69	65	46	45	44	44	50	43	58	45	53	45	68	49	38	–	13.0	10.2	11.0	11.0	12.2	15.7	15.2	15.0	
19 <i>Micromesistius poulassou</i>	78	71	58	57	44	43	42	44	41	49	49	44	46	44	65	47	44	52	–	12.0	11.5	11.5	10.0	12.5	13.0	11.5	
20 <i>Pollachius pollachius</i>	81	69	55	67	48	47	46	46	48	52	54	44	52	44	62	42	41	41	48	–	5.6	5.7	11.2	15.2	15.0	14.2	
21 <i>Pollachius virens</i> (NE Atl.)*	89	65	60	67	46	45	44	44	48	53	59	45	45	45	62	39	44	43	45	22	–	0.3	11.0	14.0	15.3	13.0	
22 <i>Pollachius virens</i> (NW Atl.)*	88	67	59	66	47	46	45	45	49	54	60	46	46	46	63	40	43	44	46	23	1	–	11.0	13.5	15.0	13.0	
23 <i>Theragra chalcogramma</i> *	79	67	54	53	25	25	25	26	25	43	56	18	16	18	52	35	47	49	40	45	43	44	–	14.7	14.0	13.5	
24 <i>Trisopterus esmarkii</i> *	83	78	70	68	54	54	53	58	58	62	69	58	56	58	79	53	60	63	50	61	55	54	59	–	12.4	8.7	
25 <i>Trisopterus luscus</i> *	85	77	60	60	58	58	57	58	57	62	69	54	62	54	72	56	62	61	52	60	60	60	60	56	50	–	11.7
26 <i>Trisopterus minutus</i> *	90	70	72	67	45	46	46	50	55	60	63	54	55	54	64	44	51	60	46	57	51	52	54	35	47	–	

**Fig. 1.** Parsimony analysis of 19 species of Gadinae and 4 gadiform outgroup taxa, strict consensus of 64 most parsimonious trees; tree length = 551, consistency index = 0.41. Bremer support values (1–8) provided. Percentages given for *Arctogadus* specimen represent barbel length as percent of standard length. Letters are given to identify various clades



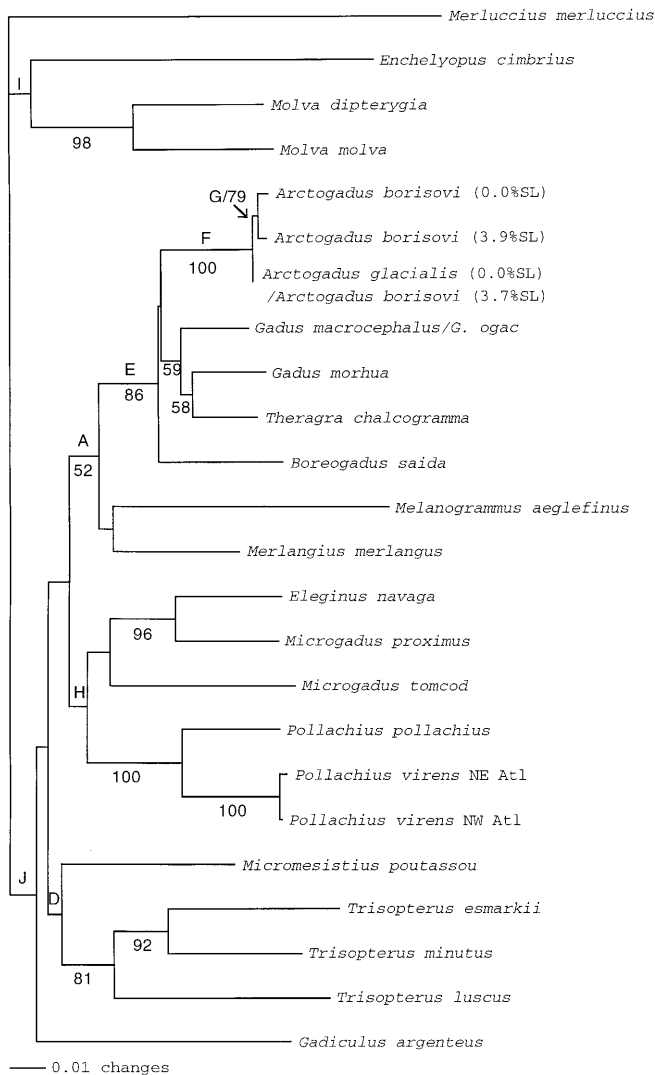
lengths 0.0 and 3.9% SL occur in a separate clade (G) as sister group to the two similar specimens: *A. glacialis* and *A. borisovi*, barbel 3.7% SL.

In order to get a better resolution of Gadinae, we excluded all non-gadine taxa and used *Gadiculus* as outgroup in a second parsimony analysis (Fig. 3), since it was the basalmost gadine genus in the neighbour-joining analysis. This second parsimony analysis resulted in two equally parsimonious trees, differing only in the topology of the four specimens of *Arctogadus*. One of the trees is similar to the consensus tree (Fig. 3), where *Arctogadus* specimens appear unresolved (F), whereas in the other (tree not shown) *A. borisovi* (barbel lengths 0.0 and 3.9% SL) form a separate clade (G) as in the neighbour-joining analysis (Fig. 2). The topology of the strict consensus tree with three major clades (A, H, D) (Fig. 3) is essentially similar to that of Carr et al. (1999), except for the addition of new taxa. A basal clade (D) consists of *Micromesistius* as sistergroup to *Trisopterus*. The next clade (K), containing the rest of the genera, branches into two lineages (A and H). In clade (H), with *Pollachius* Nilsson, 1832, *Eleginus* Fischer, 1812 and *Microgadus*, the first-mentioned appear to be monophyletic with *P. pollachius* as sistergroup (B) to *P. virens*, represented by both northeast Atlantic and northwest Atlantic specimens (L). The last two differ by only one nucleotide (Table 2). In clade (A), *Melanogrammus* and *Merlangius* form a clade (M) which is sistergroup to a

clade (E) with *Gadus* spp., *Theragra*, *Boreogadus* and *Arctogadus* spp. The latter branches into a clade (P) with *Gadus macrocephalus/ogac* as sistergroup to the sister-species *Gadus morhua* L., 1758 and *Theragra chalcogramma* (Pallas, 1811) (O), and into a clade (N) with *Boreogadus saida* as sistergroup to *Arctogadus* spp.

## Discussion

The suggested close relationship of *Arctogadus* and *Boreogadus* (Fig. 3) is in accordance with the small amount of morphological difference between the two taxa. *Arctogadus* differs from *Boreogadus* by the presence of palatine teeth, overlapping scales, less emarginate caudal fin, fewer gill-rakers (32–34 vs 37–34) and larger eye (Svetovidov 1948; Andriashev 1954). *B. saida* and specimens of *Arctogadus* are often confused at sea and mixed samples are quite common in museum collections. The genetic difference between *Arctogadus* and *Boreogadus* (6.2–6.9%) and between *Arctogadus* and *Gadus* (including *Theragra*) (4.7–6.7%) is less than the difference within the genera *Trisopterus* (8.7–12.4%), *Microgadus* (9.5%) and *Molva* (7.7%), and only slightly larger than the distances found within *Gadus* (including *Theragra*) (4.0–4.5%) and *Pollachius* (5.6–5.7%) (Table 2). This indicates that the taxonomy of *Arctogadus*, *Boreogadus*, *Theragra* and *Gadus* needs a morphological



**Fig. 2.** Neighbour-joining analysis of 19 species of Gadinae and 4 gadiform outgroup taxa. ME score 1.17. Branch lengths shown. Percentages given for *Arctogadus* specimens represent barbel length as percent of standard length. Letters as in Fig. 1

revision, and possibly that *Arctogadus* should be synonymised with *Boreogadus* or even *Gadus*. Additional morphological and molecular studies are needed before nomenclatorial changes can be made.

The small genetic difference observed between the examined specimens of the two nominal species *A. borisovi* and *A. glacialis* (0–2 nucleotides) is comparable to the differences between the two specimens of *P. virens* from each side of the Atlantic, and to the intraspecific variation in cytochrome b sequences previously described for *Gadus morhua* (Carr and Marshall 1991). The results also resemble the lack of genetic variation between *Gadus macrocephalus* and *Gadus ogac* (Carr et al. 1999), which was used as an argument for synonymising of the two, with *Gadus ogac* as junior synonym. In fact, similarity between *Gadus macrocephalus* and *Gadus ogac* was indicated by Schultz and Welander (1935), who were unable to find any morphological difference in

shape of swimbladder, counts of fin rays and vertebrae and numerous measurements of body and fins. Walters (1955) also suggested that *A. glacialis* and *A. borisovi* are identical. In summary, the current data indicate that presence or absence of the barbel in *Arctogadus* is not species-specific and that *A. borisovi* is a junior synonym of *A. glacialis*. A critical study of the hitherto-used specific meristic and morphological characters is currently in progress at the ZMUC.

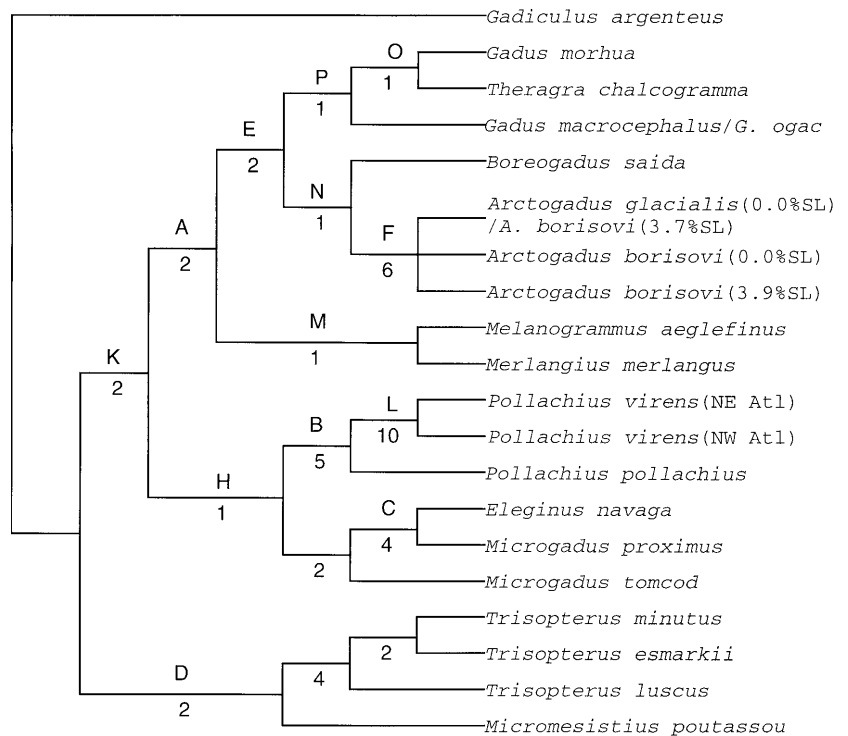
The position of *Gadiculus* as the basal genus in Gadinae agrees with Dunn (1989). *Gadiculus* differs morphologically from all other genera within Gadinae in having a truncated ventral flange of the mesethoid and ontogenetic loss of a bifurcate posterior margin of the maxilla, which Dunn (1989) considered as autapomorphic. Furthermore, *Gadiculus* possess several plesiomorphic character states not shared with most other gadines (moderate length of the posterior process of the quadrate, low number of precaudal vertebrae, and caudal fin rays) (Dunn 1989). The last two characters and the presence of a pointed dorsal flange of the mesethoid are shared with *Trisopterus*. These characters support the relatively basal placement of *Trisopterus* indicated by the present study, which is in contrast to the terminal position advocated by Dunn (1989). Dunn, however, speculated that his placement of *Trisopterus* could be wrong, due to the number of similarities with *Gadiculus*. Carr et al. (1999) did not include non-gadine outgroups in their analysis and apparently rooted their cladograms with *Trisopterus luscus* (L., 1758), without justification. They agreed with Svetovidov (1948), who regarded *Trisopterus* as primitive to all other gadines.

Our basal assignment of *Micromesistius* is not in agreement with Dunn (1989), who interpreted it as a sistergroup to *Boreogadus*, among the derived taxa of Gadinae. This position was based on a few homoplastic character states (high number of precaudal vertebrae and loss of posterior process of basipterygia), and an assumed unique long distance between dorsal fins. The basal position of *Micromesistius* indicated by the present study suggests that the long distance between dorsal fins has evolved convergently in *Micromesistius* and *Arctogadus*/*Boreogadus*. However, the indicated sister-relationship between *Micromesistius* and *Trisopterus* is currently not supported by morphological shared derived characters (Dunn 1989).

### Biogeography

The results of the present analysis with *Gadiculus*, *Micromesistius* and *Trisopterus* as basal branches in the phylogeny support previous molecular and morphological evidence of a northeast Atlantic origin of gadine fishes (Svetovidov 1948, 1959; Carr et al. 1999). Today, three genera (*Arctogadus*, *Boreogadus* and *Eleginus*) occur in high-Arctic regions, and three species (*Theragra chalcogramma*, *Gadus macrocephalus*, *Microgadus proximus*) are endemic to the Pacific (Svetovidov 1948;

**Fig. 3.** Parsimony analysis of 19 species of Gadinae, strict consensus of 2 most parsimonious trees; tree length = 382, consistency index = 0.43. Bremer support values provided. Percentages given for *Arctogadus* specimens represent barbel length as percent of standard length. Letters as in Fig. 1



Andriashev 1954; Cohen et al. 1990). The relatively small genetic distance within the *Gadus* lineage and between *Eleginus navaga* and *Microgadus proximus* was explained by relatively recent speciation events connected to the re-opening of the Bering Strait ca. 3.0–3.5 million years BP (Carr et al. 1999). Subsequent cooling of the Arctic Ocean during the Pleistocene is a vicariant event that has apparently divided populations in both the *Gadus* and *Microgadus* lineages and thereby generated the speciation of Pacific (*Gadus macrocephalus/ogac*, *Microgadus proximus* and *Theragra chalcogramma*) and Arctic/Arctic–Atlantic (*Eleginus navaga*, *Gadus morhua*, *A. borisovi* and *A. glacialis*) species. *B. saida*, *Eleginus gracilis* and possibly *Gadus macrocephalus/ogac* are the only gadine species found in both the North Pacific and the Arctic Ocean (Andriashev 1954; Cohen et al. 1990; Carr et al. 1999). The exact speciation scenario of the closely related species of *Arctogadus* and *Boreogadus* is not easily explained by geographic or thermal events. Possibly, their sympatric, circumarctic distribution might be a result of a secondary invasion of *B. saida* into the Arctic Ocean, as suggested for *Gadus macrocephalus/ogac* by Carr et al. (1999), which might also be the case with *Eleginus gracilis*. A more detailed analysis of the evolutionary history of the Arctic gadines must await more studies on their morphology, ecology, genetics and physiology.

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