


Article

Recognition and Distribution of Two North Atlantic *Gadiculus* Species, *G. argenteus* and *G. thori* (Gadidae), Based on Otolith Morphology, Larval Pigmentation, Molecular Evidence, Morphometrics and Meristics

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Academic Editor: Maria Angeles Esteban

Received: 28 July 2017; Accepted: 3 August 2017; Published: 29 August 2017

Abstract: The silvery pout genus *Gadiculus* consists of small aberrant codfishes with several extinct and currently only one recognized extant species. The oldest representatives of a *Gadiculus* lineage known from otoliths are Early Miocene in age. Fossil evidence has showed *Gadiculus* to originate from older genera diverging early from other true cods of the family Gadidae. As adult specimens of different species have been found to be highly similar and difficult to distinguish based on meristic and morphometric data, the number of species in this gadid genus has been controversial since different larval morphotypes were first discovered some 100 years ago. For almost 70 years, *Gadiculus thori* and *Gadiculus argenteus* have been considered subspecies only, with a distribution in the Northeast Atlantic Ocean including the Mediterranean. In this study, we resolve the long-standing issue of extant *Gadiculus* not being monotypic. New results in the form of distinct adult otoliths and molecular data unambiguously show two species of *Gadiculus* present—in agreement with larval morphotypes. Morphometric, meristic and molecular characters, as well as larval pigmentation are discussed in addition to present and past geographic distributions of the two taxa from distributions of fossil otoliths. At present, the cold-water species *Gadiculus thori* (northern silvery pout) is distributed in cold-temperate and subarctic latitudes in the Northeast Atlantic, including a new range extension off Southeast Greenland. *Gadiculus argenteus* (southern silvery pout) occurs in warmer waters and is distributed in the warm-temperate East Atlantic and Mediterranean. Fossil otoliths show that both species often co-existed in the Mediterranean from the Late Pliocene to the Middle Pleistocene.

Keywords: taxonomic revision; otolith; *Cox1* barcode; larval melanophore pattern; morphometrics; meristics; (palaeo)geographic distribution; Gadidae classification

1. Introduction

Silvery pouts of the genus *Gadiculus* are the smallest extant cods in the family Gadidae. Only one species, *Gadiculus argenteus* (Guichenot 1850), is currently recognized with a second species described *Gadiculus thori* (Schmidt 1913) currently considered a synonym [1]. Most gadid species are economically important, although silvery pouts have limited commercial value due to their small size and predominantly artisanal use in some Mediterranean countries. This becomes evident considering the relatively limited number of studies present on these fishes compared to other, larger gadids. However, *Gadiculus* have high regional abundances and have been identified as important forage

prey in trophic ecosystem dynamics [2]. Juveniles and adults of *Gadiculus* feed almost exclusively on various groups of pelagic crustaceans, but also eat small fish [3–6].

Gadiculus fishes are meso- to bathypelagic and show gregariousness, forming large schools in the deeper parts of the shelf and above the continental slope—usually at depths between 100 and 1000 m. However, some differences have been reported concerning depth distributions between northern and southern populations, with the former mainly between 125 and 400 m [6] and the latter mainly between 200 and 400–500 m [7]. Their occurrence in the colder, deeper waters in the more southern Atlantic waters and in the Mediterranean is likely an overlooked factor concerning depth distributions. The younger stages of *Gadiculus* fishes are known to inhabit relatively shallow depths. *Gadiculus* fishes have currently been observed in the northeastern Atlantic from off the North Cape of Norway and the western part of the Barents Sea [8] southwards to Morocco, including the western and central Mediterranean [9]. Observations are also present from the Adriatic Sea [10], the entire Aegean Sea, Thracian Sea [11], off the Turkish coast [12], off the Syrian coast [13], and off the coast of Israel [14]. However, no records from the tropical or north-western Atlantic have ever been reported.

Guichenot [15] (1850) described *Gadiculus argenteus* from the Mediterranean coast of Algiers erecting the new genus *Gadiculus*. The establishment of the genus was justified due to its large eye/body ratio compared to other gadids, i.e., the eye diameter is longer than the snout, although less than 40% head length. In addition, *Gadiculus* shows a unique oblique mouth that is angled steeply upwards. Other distinguishing characteristics of *Gadiculus* are the large deciduous scales and the sensory canals with seven large open pits (mucous cavities) on the dorsal margin of the head (Svetovidov) [16]. Subsequently, Schmidt [17] described *Gadiculus thori* from the North Atlantic Ocean based on the following characteristics:

- Different melanophore patterns in post-larvae of the two species. The post-larvae of *G. argenteus* show three transverse pigmented bars, whereas *G. thori* only exhibit one (Figure 1).
- At the same stage of development, the post-larvae of *G. thori* are, in general, larger than those of *G. argenteus* (Figure 1).
- At the same stage of development, the post-larvae of *G. thori* are slender compared with *G. argenteus*, which are stouter and shorter (Figure 1).
- Different number of vertebrae in the two species. Schmidt found that *G. thori* has 41–43 (usually 42), whereas *G. argenteus* has 39–41 (usually 40).
- Geographic distribution. The number of *G. thori* specimens declines drastically from Ireland in the North to the French Atlantic coast. Conversely, *G. argenteus* occurs in increasing numbers going south from the mouth of the river Gironde along the Atlantic east coast.



Figure 1. Illustrations of *Gadiculus* post-larvae at the same stage of development by Schmidt [17]. (A). *Gadiculus argenteus*; (B). *Gadiculus thori*. The post-larvae were originally used to distinguish two different species although largely neglected in subsequent works until this study. Scale bars: 1 mm.

Subsequently, Svetovidov [18] reclassified the two taxa as subspecies, a classification that subsequently became common usage. Mercader and Vinyoles [1] went even further and synonymized the two subspecies as one indistinguishable eastern North Atlantic species based primarily on external morphometric and meristic characters of adult specimens.

The objective of the present study was to resolve the taxonomic status of extant *Gadiculus* by carefully comparing all characteristics available, including old and new data that were not taken into consideration by the revision of Mercader and Vinyoles [1].

2. Materials and Methods

2.1. Otoliths

Gadiculus thori: 213 specimens, Standard Length (SL) 101–147 mm or Total Length (TL) 111–164 mm, June 26th 1977, Kvinnherad Fjord, Norway (59°58' N, 5°59' E), catch-depth ca. 170 m, collected and identified by (Coll./ID) T. Bakke, otoliths extracted by P. Gaemers; three specimens Trondheim Fjord, Norway, Coll./ID G. Van der Velde.

Gadiculus argenteus: 10 specimens, SL 59–104 mm, Barcelona, Spain, Mediterranean Sea, catch-depths 220 and 385 m, sagittae extracted, Coll./ID C. Allué; two right sagittae, off Casablanca, Morocco, southeast Atlantic Ocean, catch-depth 350 m, Coll./ID D. Nolf; one specimen off Mallorca, Spain, Mediterranean Sea, Coll./ID P. Gaemers; one pair of sagittae, off Agadir, Morocco, Coll./ID P. Gaemers; one pair of sagittae, Mediterranean Sea (fish market, Leiden, The Netherlands), stomach content of *Conger conger*, Coll./ID P. Gaemers.

The aforementioned otoliths were deposited in the collection of P. Gaemers.

Furthermore, sagittae of 46 specimens of *Gadiculus argenteus* (TL 82–145) from off the Portuguese coast, catch-depth 140–401 m, mainly 370–401 m, Coll./ID C.A. Assis, 40 specimens in the collection of C.A. Assis and 6 specimens in the collection of P. Gaemers.

Otoliths of *Gadiculus* are easy to dissect due to their large size, with otolith length (OL) approximately 5% of the TL. There are two possible reasons for this: the smallest species in a genus or family tend to have the largest otoliths, and the size of the eyes and the otoliths are correlated (P. Gaemers, data not shown). The otoliths were dissected by approaching the fishes from the ventral side. The uncovered neurocranium is cut at the sagittal plane with a sharp knife. After the removal of the brain the otoliths could easily be taken out of the two halves of the skull. This method is more time-consuming than a transversal cut on the dorsal part of the head of the fish, which is the usual procedure in collecting otoliths in fisheries surveys, but provides the best chance to retrieve the otoliths intact. The otoliths were stored dry. In contrast to fossil otoliths, it is difficult to take photographs of recent otoliths that show the superficial morphology in sufficient detail, because of their white colour, their gloss and, frequently, their transparency. Therefore, the otoliths were drawn with the aid of a drawing mirror on a Wild M5 binocular microscope (Joint Stock Company, Heerbrugg, Switzerland) with a low angle of incidence of the light.

Otolith terminology and nomenclature presented in the current study follows Chaine and Duvergier [19], Schwarzhans [20], Gaemers [21] and Nolf [22].

2.2. New Record of *Gadiculus thori* off Greenland

One specimen of *Gadiculus thori* was caught by *R/V Pâmiut*, Greenland Institute of Natural Resources (GINR), leg 4, haul 72, August 11, 2012, in Denmark Strait Southeast Greenland at 64°19' N, 36°45' W and deposited at the Zoological Museum University of Bergen as ZMUB 16483 (tissue JYP#952) (Figure 2). Coll./ID J.Y. Poulsen. The specimen was caught during a routine survey with a non-closing Alfredo III trawl probing Greenland halibut abundances; therefore, the catch depth is uncertain. The bottom and fishing depth was 419–424 m with bottom temperature of 3.81 °C. This fish specimen from the Denmark Strait, Southeast Greenland, was digitally X-rayed at the Australian Museum using the industrial X-Ray model EXR 150-23 BW (Seifert Systems, Sydney, Australia),

and examined under a stereomicroscope (Zeiss, model 475052-9901, West Germany). Morphological measurements were taken with a digital calliper to the nearest 0.1 mm, listed in Table 1 and compared to data by Raitt [23].

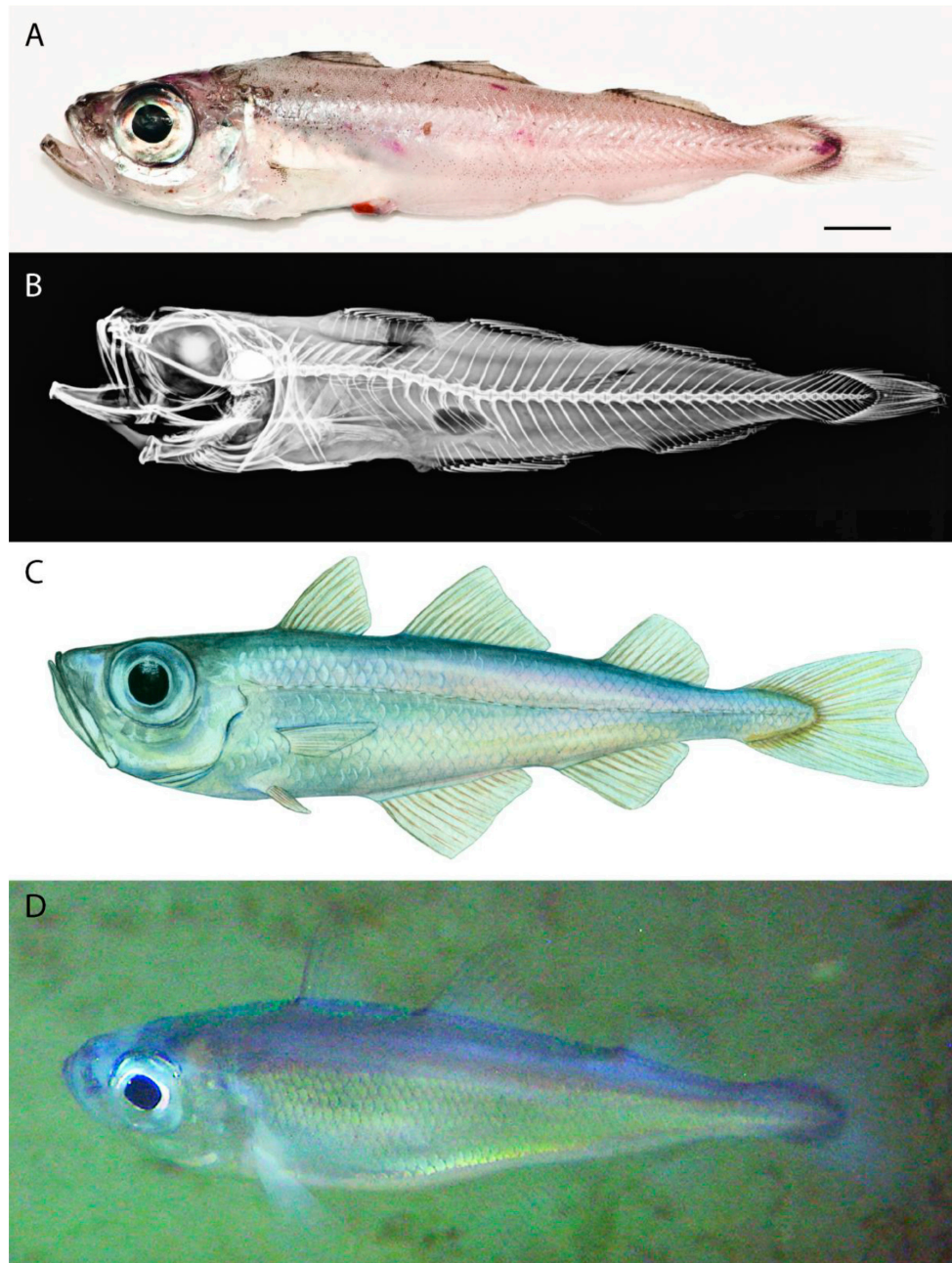


Figure 2. *Gadiculus thori*. (A). ZMUB 14683 (registration number of Museum of Zoology, University of Bergen, Norway). Newly caught off Southeast Greenland in 2012, providing a range expansion into the northwestern Atlantic (Photo: Greenland Institute of Natural Resources, Greenland). Scale bar: 10 mm; (B). X-ray of ZMUB 14683. Meristics and morphometrics presented in Table 2; (C). *Gadiculus thori* newly illustrated for this study; (D). Live specimen *G. thori* (67°48' N, 10°54' E) at 227 m depth filmed during the MAREANO expedition off Arctic Norway in 2011.

Table 1. Meristics and morphometrics of *Gadiculus thori* (ZMUB 14683) caught off Southeast Greenland in 2012, providing a new range extension into the Western North Atlantic (Figure 2). The specimen was compared to *G. thori* “northern” specimens presented by Raitt [23] from off the west coast of Scotland (ranges shown in parentheses).

Characteristics	<i>Gadiculus thori</i>
TL	126.2
SL	115.9 (65.0–135.0)
% SL	
Head length	30.6 (26.8–35.7)
Pre-dorsal dist.	31.7 (28.0–40.0)
Pre-anal dist.	44.6
Pre-pelvic dist.	25.5
Pre-orbital dist.	9.2
Orbit	11.2 (8.8–12.6)
Inter-orbital dist.	7.5
1. Dorsal base	11.2
1st–2nd Dorsal dist.	3.3 (0.9–3.7)
2. Dorsal base	12.1
2nd–3rd Dorsal dist.	5.4 (2.2–6.1)
3. Dorsal base	12.3
1. Anal base	14.4
1st–2nd Anal dist.	5.0 (2–6.9)
2. Anal base	15.1
Body depth	16.1 (13.8–25.8)
Caudal depth	5.5
Premaxillary length	12.0
1. Dorsal fin rays	10 (9–13)
2. Dorsal fin rays	11 (10–16)
3. Dorsal fin rays	17 (15–17)
1. Anal fin rays	17 (15–18)
2. Anal fin rays	17 (16–17)
Vertebrae	42 (39–43)

Total length (TL) and Standard length (SL) are in mm.

2.3. Molecular Analyses

The new record off Greenland of *Gadiculus thori* (ZMUB 14683) was Cytochrome Oxidase 1 (*Cox1*) barcoded as part of the Greenland Fishes (GLF) barcoding project [24] and the sequence deposited in the BOLD repository [25] as GLF136. Additional materials included for molecular comparisons were either newly generated (20 specimens) as part of the GLF project (see [24] for laboratory works) or downloaded (76 specimens) from BOLD. We calculated uncorrected distances for the two taxa including the smallest interspecific distance (barcoding gap) that potentially indicates species delimitation based on *Cox1* DNA sequences (Meier and Paulay [26] and Meier et al., 2008 [27]). In addition, the mitogenome (complete mitochondrial genome) was determined for the specimen according to mitogenomic laboratory work [28]. Newly generated sequences are available at DDBJ (DNA Data Bank of Japan), EMBL (European Molecular Biology Laboratory, Heidelberg, Germany) or GenBank as LC146692–711 (Table 2), and *G. thori* mitogenome as AP018148. The *Cox1* sequences are also available from BOLD, either individually (GLF) or as a single dataset including all 96 barcoded specimens used in this study DS-PGJP (data set P. Gaemers & J. Poulsen, Table 2). Catch localities of all *Gadiculus* specimens used for molecular comparisons are depicted in Figure 3, corresponding also with Table 2, including metadata found in the BOLD repository. Attempts at obtaining samples from the greater regions of the Bay of Biscay were not successful.

Table 2. 96 Specimens used for molecular comparisons of the *Cox1* barcode in this study. Numbers for *Gadiculus* spp. correspond to Figure 8. The dataset is available as (DS-PGJP) from BOLD.

Specimens	Record ID BOLD	NCBI	Museum	Region, Country and Year of Sampling	Position	Study
Lotidae						
<i>Brosme brosme</i>	GLF058	LC146711	ZMUB 21890	SE Greenland 2013	64.25° N, 36.51° E	This study
<i>Brosme brosme</i>	SCFAC287-06	KC015253	ARC 25650	SE Canada 2006	41.93° N, 65.81° E	[29]
<i>Lota lota</i>	ANGBF9234	GU126680	-	Idaho, USA 2009	-	Unpubl.
<i>Lota lota</i>	IFCZE0693	HQ961085	-	Ohre, Czech Republic 2010	50.11° N, 12.40° E	Unpubl.
<i>Molva dipterygia</i>	GLF056	LC146709	ZMUB 21948	SE Greenland 2013	64.18° N, 36.50° E	This study
<i>Molva dipterygia</i>	SCFAC413	KC015694	ARC 25589	Unknown, Canada	-	[29]
<i>Molva molva</i>	GLF071	LC146695	No voucher	SE Greenland 2013	66.50° N, 30.28° E	This study
<i>Molva molva</i>	GLF176	LC146701	ZMUB 22720	SE Greenland 2014	64.27° N, 37.20° E	This study
Gaidropsaridae						
<i>Ciliata mustela</i>	BNSFI129	KJ204805	MT05378	NW Germany 2010	54.14° N, 07.90° E	[30]
<i>Ciliata mustela</i>	BNSFI128	KJ204804	MT05377	NW Germany 2010	54.14° N, 07.90° E	[30]
<i>Enchelyopus cimbrius</i>	SCAFB093	KC015336	ARC 24883	SE Canada 2005	44.94° N, 66.09° E	[29]
<i>Enchelyopus cimbrius</i>	BNSFI132	KJ204840	MT05365	NW Germany 2010	54.14° N, 07.90° E	[30]
<i>Gaidropsarus mediterraneus</i>	FCFPS166	JQ774626	MB85-005350	S Portugal	-	[31]
<i>Gaidropsarus mediterraneus</i>	GBGCA10850	KP136735	J1Bsex-80	Turkey	-	Unpubl.
<i>Gaidropsarus vulgaris</i>	SFM036	-	AF0036	NW Spain 2013	-	Unpubl.
<i>Gaidropsarus vulgaris</i>	GBGCA8490	KJ128491	NRM46985	SW Sweden 2001	57.88° N, 11.58° E	Unpubl.
<i>Onogadus argentatus</i>	GLF114	LC146708	ZMUB 21814	SE Greenland 2013	61.57° N, 40.58° E	This study
<i>Onogadus argentatus</i>	SCAFB229	KC015387	ARC 26385	E Canada 2006	69.83° N, 65.28° E	[29]
<i>Onogadus ensis</i>	SCAFB1182	KC015394	ARC 28289	SE Canada 2007	44.02° N, 59.01° E	[29]
<i>Onogadus ensis</i>	GLF117	LC146696	ZMUC P376048	W Greenland 2013	63.31° N, 56.31° E	This study
Phycidae						
<i>Phycis blennoides</i>	GLF151	LC146700	ZMUB 22773	SE Greenland 2014	61.42° N, 41.04° E	This study
<i>Phycis blennoides</i>	BIM338	-	P. 15193	W Israel 2013	32.27° N, 34.36° E	Unpubl.
<i>Phycis chesteri</i>	GLF017	LC146703	ZMUC P375728	SE Greenland 2009	62.12° N, 40.29° E	This study
<i>Phycis chesteri</i>	SCFAC747	KC015799	ARC 25896	SE Canada 2002	42.80° N, 63.19° E	[29]
<i>Urophycis chuss</i>	SCFAC720	KC016017	ARC 25893	SE Canada 2006	43.03° N, 61.61° E	[29]
<i>Urophycis chuss</i>	SCFAC714	KC016018	ARC 25697	SE Canada 2006	41.39° N, 66.12° E	[29]
<i>Urophycis tenuis</i>	SCFAC522	KC016033	ARC 25942	SE Canada	48.55° N, 63.07° E	[29]
<i>Urophycis tenuis</i>	SCFACB855	KC016030	ARC 26827	SE Canada 2007	44.36° N, 66.50° E	[29]
Gadidae						
<i>Eleginus gracilis</i>	WXYZ007	-	UW150495	Alaska, USA 2010	60.99° N, 167.34° E	Unpubl.
<i>Eleginus gracilis</i>	WXYZ005	-	UW150494	Alaska, USA 2010	60.99° N, 167.34° E	Unpubl.
1. <i>Gadiculus argenteus</i>	CSFOM036	KJ709531	CSFOM-044	Sicily, Italy	-	[32]
2. <i>Gadiculus argenteus</i>	FCFPS164	JQ774620	MB85-005348	S Portugal	-	[31]
3. <i>Gadiculus argenteus</i>	FCFPS133	JQ774622	MB85-005315	S Portugal	-	[31]
4. <i>Gadiculus argenteus</i>	FCFPS130	JQ774618	MB85-005317	S Portugal	-	[31]
5. <i>Gadiculus argenteus</i>	FCFPS154	JQ774619	MB85-005338	S Portugal	-	[31]

Table 2. Cont.

Specimens	Record ID BOLD	NCBI	Museum	Region, Country and Year of Sampling	Position	Study
6. <i>Gadiculus argenteus</i>	FCFPW097	JQ775028	MB85-010501	W Portugal 2005	40.28° N, 09.59° W	[31]
7. <i>Gadiculus argenteus</i>	FCFPW079	JQ775027	MB85-010519	W Portugal 2005	40.18° N, 09.59° W	[31]
8. <i>Gadiculus argenteus</i>	FCFPW078	JQ775024	FCFOPB064-03	W Portugal 2005	40.18° N, 09.59° W	[31]
9. <i>Gadiculus argenteus</i>	FCFPW076	JQ775025	MB85-010520	W Portugal 2005	40.18° N, 09.59° W	[31]
10. <i>Gadiculus argenteus</i>	FCFPW077	JQ775026	MB85-010496	W Portugal 2005	40.18° N, 09.59° W	[31]
11. <i>Gadiculus argenteus</i>	FCFP065	JQ774831	MB85-004995	W Portugal 2005	39.08° N, 10.00° W	[31]
12. <i>Gadiculus argenteus</i>	FCFP067	JQ774828	MB85-004994	W Portugal 2005	39.08° N, 10.00° W	[31]
13. <i>Gadiculus argenteus</i>	FCFP066	JQ774829	MB85-004998	W Portugal 2005	39.08° N, 10.00° W	[31]
14. <i>Gadiculus argenteus</i>	FCFP069	JQ774830	MB85-004996	W Portugal 2005	39.08° N, 10.00° W	[31]
15. <i>Gadiculus argenteus</i>	FCFP068	JQ774832	MB85-004997	W Portugal 2005	39.08° N, 10.00° W	[31]
16. <i>Gadiculus argenteus</i>	FCFPS065	JQ774623	MB85-005249	S Portugal	-	[31]
17. <i>Gadiculus argenteus</i>	FCFPS132	JQ774624	MB85-005314	S Portugal	-	[31]
18. <i>Gadiculus argenteus</i>	FCFPS131	JQ774621	MB85-005318	S Portugal	-	[31]
19. <i>Gadiculus argenteus</i>	FCFPS134	JQ774625	MB85-005316	S Portugal	-	[31]
20. <i>Gadiculus argenteus</i>	CSFOM091	KJ709532	CSFOM-117	Sicily, Italy	-	[32]
1. <i>Gadiculus thori</i>	GLF136	LC146704	ZMUB 21452	SE Greenland 2012	64.19° N, 36.45° W	This study
2. <i>Gadiculus thori</i>	NAF001	LC146706	ZMUB 21333	SW Norway 2012	62.04° N, 05.02° E	This study
3. <i>Gadiculus thori</i>	BNSFI055	KJ204873	MT04119	N United Kingdom 2012	59.71° N, 00.56° W	[30]
4. <i>Gadiculus thori</i>	BNSFI030	KJ204872	MT04118	SW Norway 2012	58.22° N, 04.38° E	[30]
5. <i>Gadiculus thori</i>	BNSFI056	KJ204867	MT04120	N United Kingdom 2012	59.71° N, 00.56° W	[30]
6. <i>Gadiculus thori</i>	BNSFI029	KJ204865	MT04117	SW Norway 2012	58.22° N, 04.38° E	[30]
7. <i>Gadiculus thori</i>	BNSFI028	KJ204864	MT04116	SW Norway 2012	58.22° N, 04.38° E	[30]
8. <i>Gadiculus thori</i>	BNSF269	KJ204869	MT02313	SW Norway 2012	59.14° N, 03.13° E	[30]
9. <i>Gadiculus thori</i>	GBGCA6718	KJ128488	NRM476	SE Norway 2000	58.07° N, 10.02° E	Unpubl.
10. <i>Gadiculus thori</i>	BNSFI057	KJ204871	MT04121	N United Kingdom 2012	59.71° N, 00.56° W	[30]
<i>Arctogadus glacialis</i>	GLF145	LC146697	ZMUB 22974	W Greenland 2014	68.36° N, 55.10° W	This study
<i>Arctogadus glacialis</i>	DSFNG010	-	ZMUB 21027	NE Greenland 2010	72.00° N, 21.02° W	Unpubl.
<i>Boreogadus saida</i>	GLF148	LC146698	ZMUB 22936	W Greenland 2014	69.31° N, 51.53° W	This study
<i>Boreogadus saida</i>	GLF105	LC146694	ZMUB 21932	SE Greenland 2013	65.38° N, 30.19° W	This study
<i>Gadus ogac</i>	GLF065	LC146707	ZMUB 21811	SW Greenland 2013	60.43° N, 46.02° W	This study
<i>Gadus ogac</i>	SCAFB565	KC015369	ARC 26244	SE Canada 2006	50.05° N, 57.88° W	[29]
<i>Gadus macrocephalus</i>	FMV221	JQ354100	UW110223	NE Pacific 2004	-	Unpubl.
<i>Gadus macrocephalus</i>	UKFBI444	KF929903	KU 28473	NE Pacific 1999	55.16° N, 133.99° W	Unpubl.
<i>Gadus morhua</i>	GLF052	LC146693	No voucher	SE Greenland 2013	65.28° N, 33.45° W	This study
<i>Gadus morhua</i>	NOFIS088	-	NHMO-f-541	S Norway 2009	58.11° N, 08.13° E	Unpubl.
<i>Gadus chalcogrammus</i>	FMV536	JQ354517	UW150214	NE Pacific 2008	33.87° N, 118.43° W	Unpubl.
<i>Gadus chalcogrammus</i>	ABFJ129	JF952737	-	NE Japan 2005	-	[33]
<i>Merlangius merlangus</i>	ANGBF9794	FN689176	-	Iceland 2003	-	[34]
<i>Merlangius merlangus</i>	ANGBF9862	FN689040	-	Black Sea, Turkey 2003	-	[30]
<i>Melanogrammus aeglefinus</i>	GLF171	LC146702	ZMUB 22913	SE Greenland 2014	66.35° N, 29.15° W	This study
<i>Melanogrammus aeglefinus</i>	GLF057	LC146710	ZMUB 21891	SE Greenland 2014	64.25° N, 36.51° W	This study
<i>Microgadus proximus</i>	FMV009	JQ354228	UW047300	NW USA 2003	-	Unpubl.
<i>Microgadus proximus</i>	WXYZ011	-	UW 150512	NW USA 2010	47.13° S, 122.69° W	Unpubl.
<i>Microgadus tomcod</i>	BCF621	EU524129	ROM-T03570	SE Canada 2006	47.06° S, 70.42° W	[35]

Table 2. Cont.

Specimens	Record ID BOLD	NCBI	Museum	Region, Country and Year of Sampling	Position	Study
<i>Microgadus tomcod</i>	SCAFB629	KC015691	ARC26844	SE Canada	44.26° S, 64.36° W	[29]
<i>Micromesistius australis</i>	FCHIL259	-	-	S Chile	56.50° S, 68.62° W	Unpubl.
<i>Micromesistius australis</i>	FCHIL239	-	-	W Chile	47.13° S, 75.58° W	Unpubl.
<i>Micromesistius poutassou</i>	GLF149	LC146699	ZMUB 22716	SE Greenland 2014	61.10° N, 41.40° W	This study
<i>Micromesistius poutassou</i>	BNSFI089	KJ205044	MT04159	N United Kingdom 2012	57.85° N, 01.17° E	[30]
<i>Pollachius pollachius</i>	BNSFI033	KJ205137	MT04178	SW Norway 2012	58.22° N, 04.38° E	[30]
<i>Pollachius pollachius</i>	NOFIS084	-	NHMO-f-537	SE Norway 2009	58.11° S, 08.13° E	Unpubl.
<i>Pollachius virens</i>	GLF053	LC146692	No voucher	SE Greenland 2013	65.28° N, 33.45° E	This study
<i>Pollachius virens</i>	SCAFB100	KC015818	ARC 24890	SE Canada 2005	42.91° N, 63.53° E	[29]
<i>Trisopterus capelanus</i>	CSFOM166	KJ709669	CSFOM-246	Sicily, Italy	-	[32]
<i>Trisopterus capelanus</i>	CSFOM165	KJ709671	CSFOM-245	Sicily, Italy	-	[32]
<i>Neocolliolus esmarkii</i>	GLF012	LC146705	ZMUB 21421	SE Greenland 2012	65.53° N, 32.36° W	This study
<i>Neocolliolus esmarkii</i>	GBGCA7771	KJ128652	NRM5415	SW Sweden 2007	57.31° N, 11.47° E	Unpubl.
<i>Trisopterus luscus</i>	FCFP125	JQ774953	MB85-004867	SW Portugal 2005	38.22° N, 08.83° W	[31]
<i>Trisopterus luscus</i>	BNSFI090	KJ205243	MT04227	NW Germany 2011	53.76° N, 06.45° E	[30]
<i>Allotrisopterus minutus</i>	BNSFI133	KJ205252	MT05367	N Germany 2010	54.14° N, 07.90° E	[30]
<i>Allotrisopterus minutus</i>	FCFPW193	JQ775159	FCFOPB086-05	W Portugal 2005	41.62° N, 08.99° W	[31]

NCBI: The National Center for Biotechnology Information, United States of America; Unpublished: BOLD records in the repository without any publication.

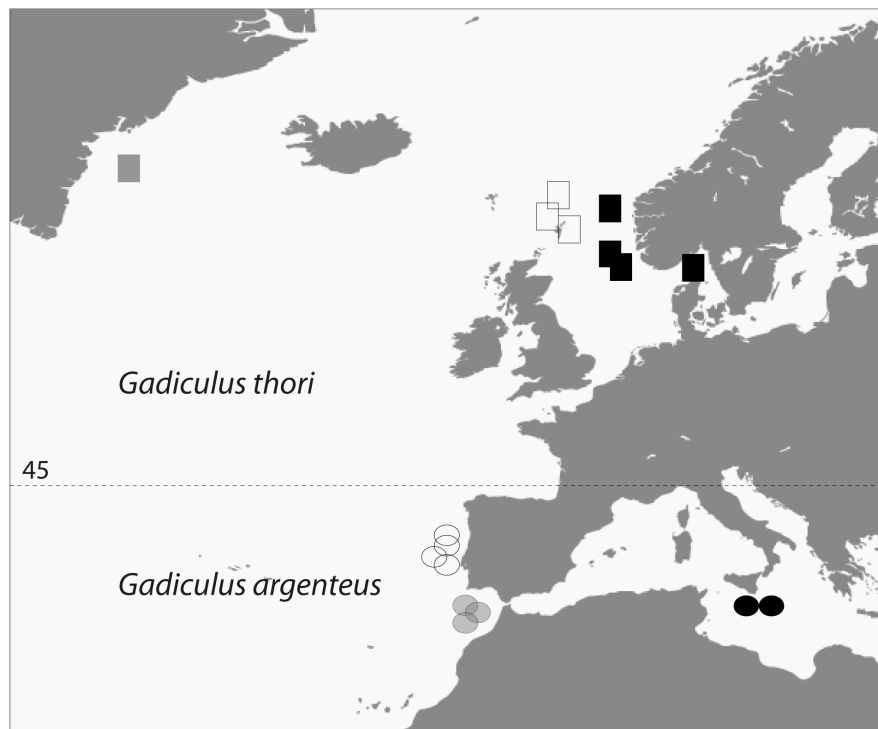


Figure 3. Catch localities of *Gadaculus* spp. used for molecular comparisons in this study (Table 2). Squares depict *G. thori* and circles depict *G. argenteus* with shading corresponding to specimens presented in Figure 8. The dashed line at about 45° N is denoting the approximate boundary separating *G. thori* and *G. argenteus* in the Northeast Atlantic Ocean.

3. Results

3.1. Otolith Characteristics

Gadaculus otoliths are flat (when on their longitudinal side), having a short and high oval outline, with basically a broadly rounded anterior margin and a primarily tapering posterior end. They have a pseudobiostial sulcus opening and the sulcus type is homosulcoid (terminology from Schwarzhans) [20]. The sulcus is wide and rather shallow (Figure 4). The colliculi are short, filling only a small part of the ostium and the cauda. The pseudocolliculum is very long. Clear differences in otolith morphology in *G. thori* and *G. argenteus* have been recognized since Gaemers [36,37], although his inclusion of *G. thori* in the fossil genus *Gadichthys* is currently considered erroneous.

The outline of *G. argenteus* otoliths is stoutly pear-shaped, thus short and high (Figure 5). It can also be described as drop-shaped. The posterior end is bluntly pointed, forming a wide angle, and may be somewhat indented in the largest otoliths. In cases where the posterior end is truncated it is running obliquely to the longitudinal axis of the otolith. The anterior end is regularly rounded with a ventral part that is slightly more prominent than its dorsal part. In full-grown otoliths an indentation often occurs at the anterior end, separating the rostrum from a somewhat shorter antirostrum. In smaller otoliths, this indentation is absent or is only small and shallow. The otoliths rarely show clear dorsal angles—the ones depicted in Figure 5D,G with a distinct postdorsal angle are unusual exceptions. The otoliths show little variation in shape, and variability is mainly limited to the depth of the ornamentation and the length-height ratio in specimens of equal size (Figure 5). Juvenile otoliths tend to be more slender compared to the adult ones and allometry in their length-height ratio during growth is relatively small.

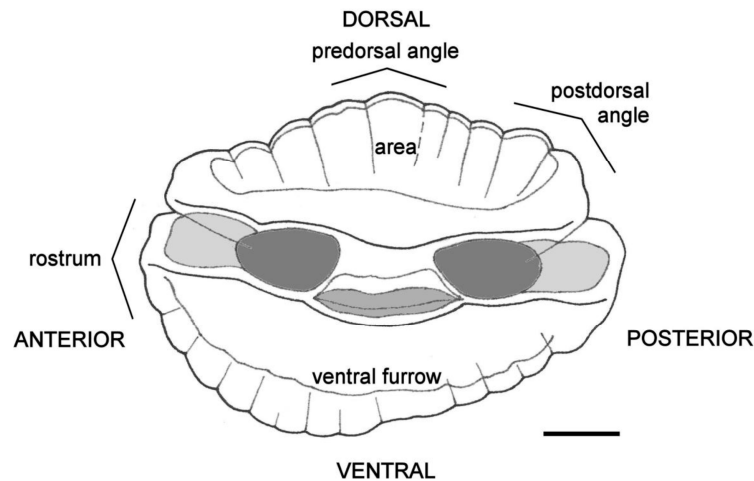


Figure 4. Typical otolith of adult *Gadiculus thori* (Total length (TL) 144 mm, standard length (SL) 135 mm), Kvinnheradford, Hardanger, Norway, showing the medially positioned sulcus acusticus and associated structures. Colliculi dark grey; pseudocolliculum medium grey; very low and indistinct colliculi-like areas light grey. Scale bar: 1 mm.

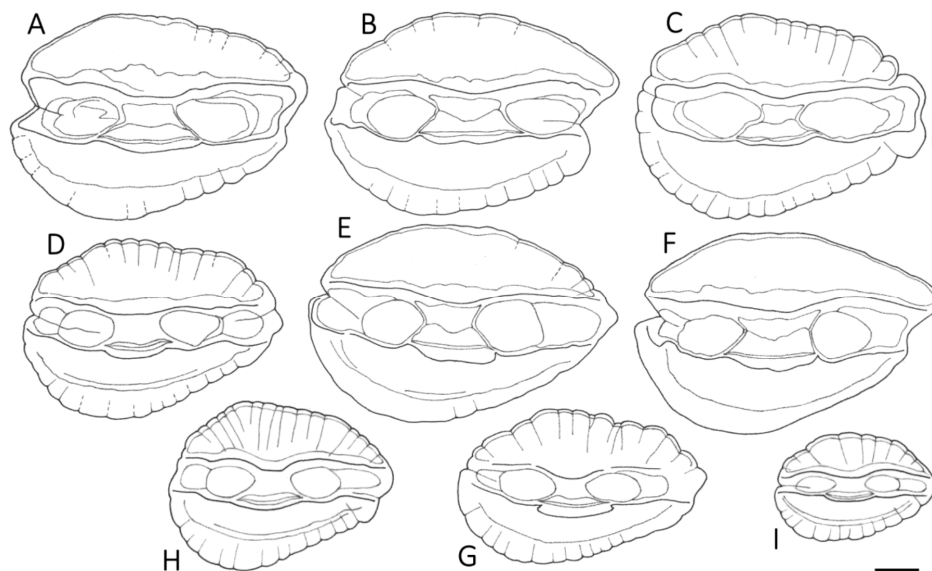


Figure 5. Typical otoliths of *Gadiculus argenteus* showing moderate morphological variabilities. (A–G): Recent otoliths obtained off the Portuguese coast. (A) TL 143, Otolith length (OL) 7.56; (B) TL 139, OL 7.24; (C) TL 145, OL 7.24; (D) SL 104, OL 6.43 (mirror image); (E) TL 133, OL 7.70; (F) TL 133, OL 7.46 (mirror image); (G) SL 94, OL 6.19. The otoliths in E and F are from the same specimen showing asymmetry. (H,I): Fossil otoliths redrawn from Girone et al. [38], Montalbano Jonico section, Basilicata, Italy, early to mid Pleistocene. Scale bar: 1 mm.

The outline of *G. thori* otoliths shows considerable changes throughout ontogeny. Juvenile otoliths are regularly pear-shaped and are often difficult to distinguish from those of *G. argenteus*. Adult otoliths have an irregular oval outline. In larger otoliths, the posterior end usually becomes more truncated, often ending with a clear indentation. The truncation at the posterior end is running perpendicular to the longitudinal axis of the otolith. Sometimes the posterior end differs considerably between the left and right otoliths from the same specimen, resulting in a truncated otolith that is much shorter and higher than the otolith without this truncation (Figure 6A,B). Larger otoliths often develop a clear indentation at the anterior end as well, with a clear rostrum and antirostrum. The most extreme example shows a very deeply and sharply indented anterior margin and a strongly truncated and

indented posterior margin (Figure 7I). Adult otoliths sampled from the same fish population are found to display an extensive variability in the general outline (Figure 7). Often, the dorsal margin before and/or after the predorsal angle is concave, accentuating the dorsal angles. The otoliths of *G. thori* show very strong allometric growth concerning the length-height ratio, with the juvenile otoliths being slender compared with the adult ones.

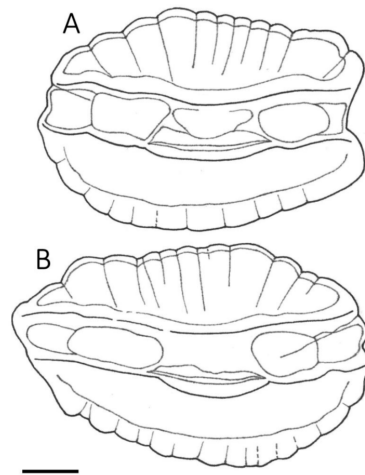


Figure 6. Otoliths of a single *Gadidculus thori* specimen (TL 143, SL 133) showing strong asymmetries between the right (A) and left (B) side otoliths (Kvinnheradford, Hardanger, Norway at approximately 170 m depth). Scale bar: 1 mm.

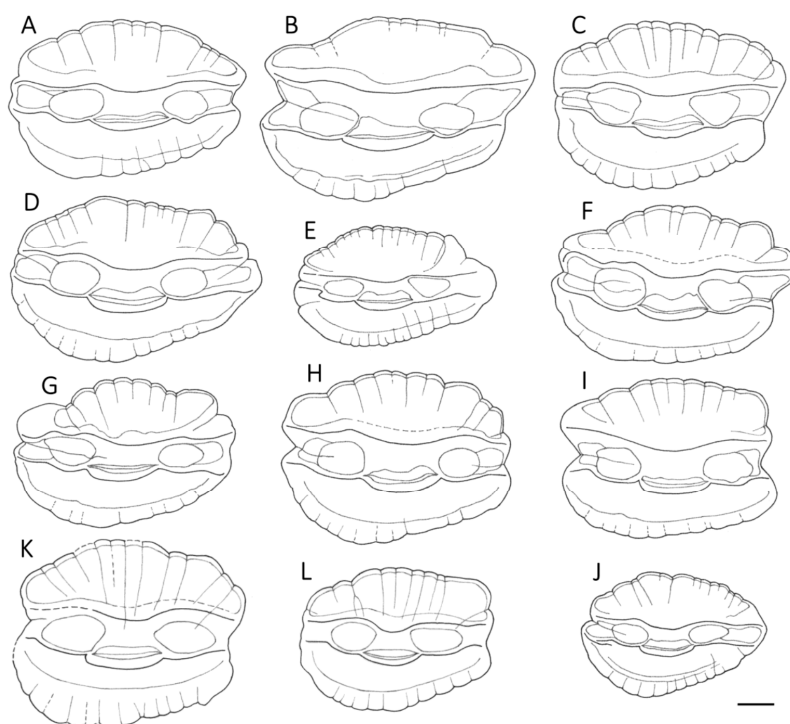


Figure 7. Otoliths of *Gadidculus thori* showing large morphological variability. (A–J): Recent otoliths from the Kvinnheradford, Hardanger, Norway. (A) TL 151, OL 7.12; (B) TL 158, OL 8.37; (C) TL 147, OL 6.88; (D) TL 151, OL 7.50; (E) TL 108, OL 6.07; (F) TL 146, OL 7.40; (G) TL 138, OL 6.65; (H) TL 164, OL 6.90; (I) TL 147, OL 6.42; (J) TL 104, OL 5.62. (K,L): Fossil otoliths redrawn from Girone et al. [38] (K) Furnari section, Sicilia, Italy, early Pleistocene; (L) Vallone Catrica section, Calabria, Italy, mid Pleistocene. Scale bar: 1 mm.

Summary of the most distinct differences of the adult otoliths in *Gadiculus*: *G. argenteus* otoliths are more regularly drop-shaped with normal, less conspicuous dorsal angles, whereas those of *G. thori* are clearly truncated at the posterior end and/or having a variable dorsal margin with usually more prominent and irregular angles. There are no apparent overlaps between the shapes of the adult otoliths in the two *Gadiculus* species despite variation observed within both species.

3.2. Molecular Analysis

A Kimura-2-parameter (K2P) neighbour-joining cladogram is presented in Figure 8, including all recognized Gadidae taxa except *Eleginus nawaga* (Pallas, 1814), and rooted with the Phycidae (for the classification levels of gadiform family groups in this study, see Section 4.8). Two distinctly different groups corresponding to *G. thori* and *G. argenteus* are found with little intraspecific variation between individuals (Figure 8). After unambiguous alignment the following values are observed for 650 base pairs of the *Cox1* DNA sequence; the maximum intraspecific variation between *G. thori* specimens was 0.62%, the maximum intraspecific distance between *G. argenteus* specimens was 0.31% and the barcoding gap (smallest interspecific distance, i.e., substitutions between the two species excluding the few random one-specimen substitutions) is 1.54%. We note that the barcoding gap seems appropriate in the case of *Gadiculus* despite low values observed. However, the clear structure in the variation of the barcodes delimiting the two species is the informative data in this particular case (Figure 8). We note that the random substitutions witnessed in single *G. thori* specimens that results in a variation of 0.62%, could potentially be from sequencing/editing errors that we have no chance of verifying. The two different groups correspond to a geographical separation that is illustrated in Figure 3 by the samples used in the northern and southern Northeast Atlantic Ocean, the latter including the Mediterranean Ocean. The genus *Micromesistius* is found as a sister group to the *Gadiculus* lineage with the longest branch in the tree being *Gadiculus* (note a three-fold shortening of the branch for practical purposes). *Gadiculus* and *Micromesistius* taxa constitute a sister group to *Trisopterus* (see Section 4.4). The group comprising *Gadiculus*, *Micromesistius* and *Trisopterus* is the sister group to the remaining true codfishes in the family Gadidae. The Lotidae family is found to be a sister family to the Gadidae, although the burbot *Lota lota* is rendering this family non-monophyletic (see Section 4.8). Molecular results not directly related to *Gadiculus* and/or classification (see below) will not be discussed further, except for the trisopterine fishes, as the results are highly similar to previous molecular studies on Gadiformes [39]. The mitogenome DNA sequence of *G. thori* consists of 16,713 base pairs and includes the 2 rRNA genes, the 22 tRNA genes and the 13 protein coding genes as observed in vertebrates. In addition, it shows a 258 base pairs intergenic T-P spacer sequence between tRNAs Thr and Pro, the T-P spacer being observed in all gadiform taxa [39].

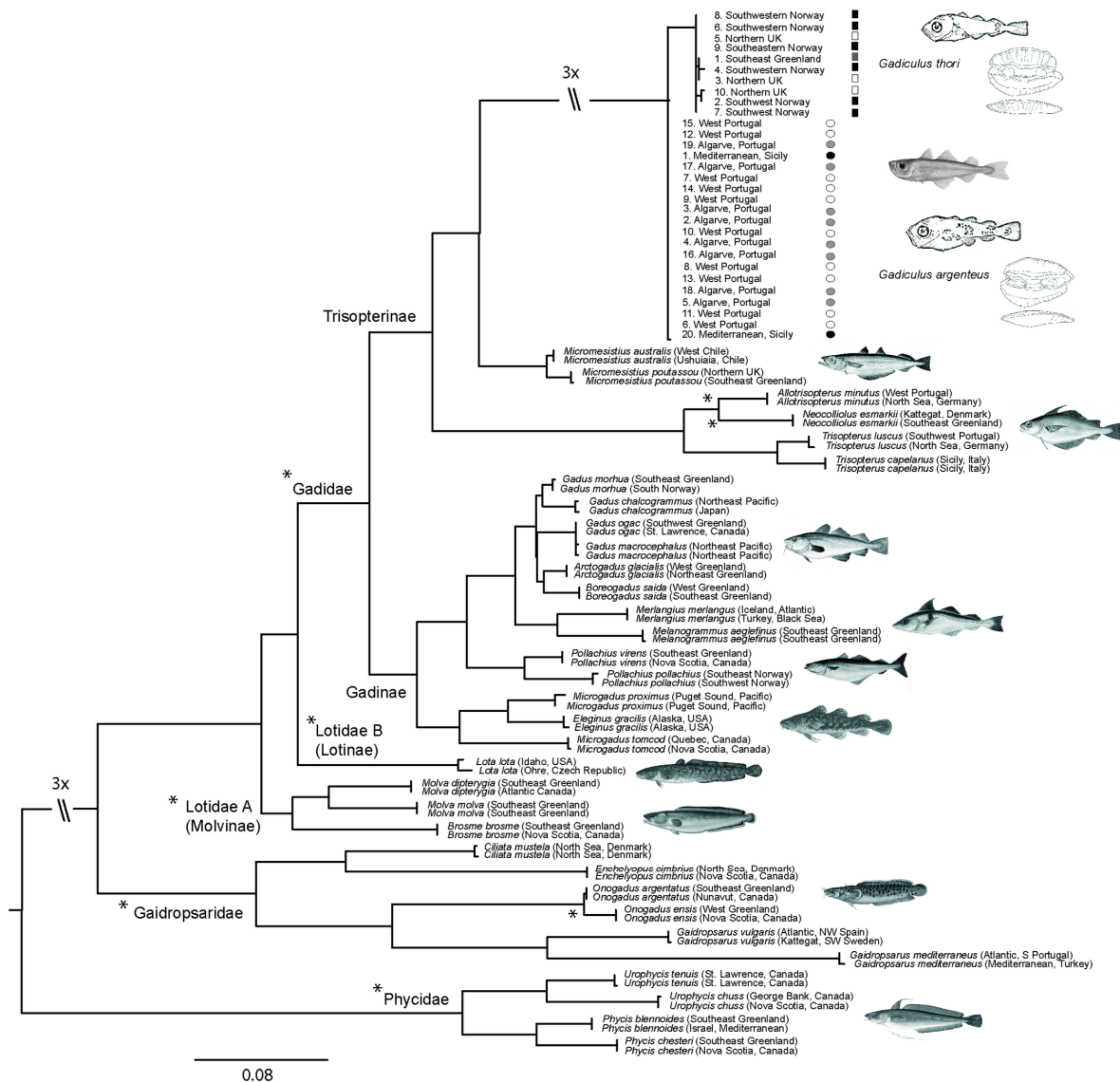


Figure 8. Distance-based K2P neighbour-joining cladogram of the family Gadidae, including taxa of Lotidae, Gaidropsaridae and Phycidae employed as outgroups. We note that the cladogram is intended for molecular taxonomy and not an attempt at resolving Gadidae phylogeny, hence the omission of support values. All currently valid Gadidae taxa are included, except *Eleginus narwaga* (Pallas, 1814) (no *Cox1* available). Catch locations are noted in parentheses. Numbers listed of *Gadiculus* spp. correspond with Figure 3 and Table 2. Double bars (3x) indicate a three-fold shortening of the branches for practical purposes only. Asterisk (*) denotes classificatory names used in this Figure that are supported by otoliths, although currently not employed in morphological and molecular taxonomy (see main text sections). It is of note that the longest branch present in the family Gadidae is representing the *Gadiculus* lineage, having two extant taxa and several extinct taxa. Post-larvae (Schmidt [17]) and general adult otolith morphology (this study) are included for both *Gadiculus* taxa. *Gadiculus thori* otolith: SL 150, OL 7.85; *G. argenteus* otolith: SL 123, OL: 7.56.

4. Discussion

4.1. Size and Age

The difference in size between the two *Gadiculus* species appears to be much larger than previously reported: Heessen et al. [6] show that the TLmax (maximum TL) for *G. thori* (200 mm) is considerably

larger than that for *G. argenteus* (150 mm). Several differences related to morphology between these species can be explained by this difference in size. The relatively larger head and eyes in the smaller species, *G. argenteus*, was statistically validated by Pope in Raitt [23]. The differences observed in several proportions are normal for closely related species of different sizes. The same is true when larger and smaller specimens of a species are compared with one another: during ontogeny allometric changes occur. The most important parts and organs of an animal (like for instance the head and the eyes) need to be relatively larger in smaller specimens, causing allometry. Likewise, the otoliths of *G. argenteus* are relatively larger than those in *G. thori*. Gaemers [40] observed the same relationship between the relative size of the otoliths of *Trisopterus capelanus* (Lacepède, 1800) and the larger *T. luscus* (Linnaeus, 1758). In addition, Schmidt's [17] observations, that the post-larvae of *G. thori* are larger and slenderer compared with post-larvae of *G. argenteus* at the same stage of development, is in accordance with the difference in maximum size.

According to Albert [5], the largest *G. thori* individuals are at least 170 mm TL and Heessen et al. [6] reported 200 mm for northwestern European specimens, results based on a large number of specimens with broad geographic sampling. The TL_{max} of this species in the latter, comprehensive study was firmly based on fish populations with gradually declining length distribution towards the maximum size [41]. Stergiou and Politou [42] reported a maximum TL of 140 mm for *G. argenteus* in Greek Mediterranean waters. Tuset et al. [43] illustrated an otolith from a 145 mm TL specimen caught on the Atlantic coast off the Iberian Peninsula. The maximum TL of this taxon generally agreed upon is usually 150 mm [9], in accordance with a specimen recorded by Vassilopoulou [44] from the northern Euboian Gulf, Greece.

Age determinations using cross sections of whole *G. argenteus* otoliths from the southern Aegean Sea, showed that the oldest fishes were two years of age when ranging between 90 and 121 mm [45]. Therefore, the largest known fishes of this species are at least 3 years old. Age determination of *G. thori* otoliths remains to be fully established, although separation of sex and partitioning into 5 mm length classes revealed three year-classes of northern Atlantic specimens [5]. Therefore, this indicated that this species rarely lives to be older than three years, but the largest known fishes reaching 170–200 mm TL, which were not included in Albert's study are likely to be older, reaching at least 4 years of age.

It could be possible that *G. argenteus* remains smaller due to environmental factors varying between the Mediterranean and the Atlantic Ocean, for example in *Merlangius merlangus* (Linnaeus, 1758) that attains a much smaller maximum TL in the Adriatic Sea compared with specimens from the North Sea and Atlantic Ocean, which was not due to overfishing [46,47]. However, *G. argenteus* has not been observed to grow larger outside the Mediterranean. If the two *Gadiculus* taxa were to be considered a single species, *G. argenteus* would have to attain a larger size northwards in the Atlantic. All available data on the size of *Gadiculus* fishes indicate size difference between the two taxa.

4.2. Morphometric and Meristic Data

The most important studies related to the status of *Gadiculus* fishes concerning morphological characteristics are: Schmidt [17], Raitt [23], and Mercader and Vinyoles [1]. However, the omission of Raitt's thorough investigations in the latter is here noted and is problematic. The most distinctive characteristics found by Raitt (and validated by Pope's statistics in Raitt) are:

1. The relatively larger eye in *G. argenteus* in relation to SL
2. The relatively larger head in *G. argenteus* in relation to SL
3. The number of vertebrae in *G. thori* (39–43)—39 and 40 rarely observed—and *G. argenteus* (37–41)—37 and 41 rarely observed
4. The number of D3 fin rays in *G. thori* (15–17) and *G. argenteus* (11–16)
5. The number of A1 fin rays in *G. thori* (15–18)—18 rarely observed—and *G. argenteus* (11–16)—11 rarely observed

Mercader and Vinyoles [1] included only six specimens of *G. thori*, whereas Raitt's study consisted of 645 specimens of this species and included vertebral counts that are missing in Mercader and Vinyoles [1]. Raitt counted the fin rays of 85 specimens of *G. thori* and of 65 *G. argenteus* specimens. Mercader and Vinyoles studied 69 specimens of *G. argenteus* (48 from the Mediterranean and 21 from the Atlantic) including fin-ray counts. Raitt had only two specimens of *G. argenteus* available from the Atlantic and no fin-ray counts were given.

The variability in the number of A1 fin rays of *G. thori* (12–18) in Mercader and Vinyoles (1, Table 5) is extreme considering the small number of specimens included for this species. Raitt noted 15–18, using a much larger data set. The A1 fin ray counts for *G. thori* in Svetovidov [16], also incorporated into Table 5 in [1], disagree with those listed in Svetovidov [18,47]. The original data of Svetovidov [18,48] are in agreement with Raitt's counts, thereby proving the data listed by Svetovidov [16] to be erroneous as he exchanged the A1 ray counts of *G. thori* for those of *G. argenteus*. The low A1 fin rays counts of *G. thori* given by Mercader and Vinyoles have apparently been copied from Svetovidov [16] and must be disregarded.

The large variability of the *G. argenteus* D3 fin ray counts (12–20) in Mercader and Vinyoles [1] is peculiar and may also be incorrect. None of the five studies they referenced included data similar to these, and nor did Raitt [23] who found numbers greater than 18. Additionally, Mercader and Vinyoles unfortunately did not list each specimen for each discrete character, which is customary in most elaborate taxonomic studies, making the use of their data difficult. Therefore, it is not possible to note which counts are rare and which specimens are within the overlap ranges. The high fin ray counts of the D2 and A2 for *G. argenteus* in Mercader and Vinyoles [1] were similarly not found by other authors and are also questionable.

All dorsal and anal fin ray counts of the two taxa overlap. According to Raitt [23], the most distinctive fins for the separation of taxa are the D3 and A1. The fin ray counts in Mercader and Vinyoles [1] are ambiguous. The interspecific differences of the D3 and A1 were found to be greater by Raitt than noted by the latter study, although these characteristics alone are not sufficient to distinguish the two taxa. These counts are informative only in combination with other characters.

With a few exceptions, individuals of *Gadiculus* can be distinguished to species by the number of vertebrae [23] as observations of specimens showing an overlap concerning this character are rare. The majority of *G. argenteus* specimens show 38–40 vertebrae and *G. thori* shows 41–43 vertebrae. Accordingly, the *G. thori* specimen caught off Greenland shows 42 vertebrae. However, it is currently believed that many species with an extensive north-south distribution often have a gradually decreasing number of vertebrae towards southern warmer waters. Hodges [49] mentions that T.N. Gill in 1863 found that the number of vertebrae in fishes is temperature dependent. This is in agreement with Wheeler and Jones [50] who found that the number of vertebrae is affected by the egg developing temperature, corresponding to Jordan's ecogeographic rule [51]. On that note, it would be interesting to clarify the number of vertebrae for the northernmost *G. argenteus* specimens; however, this is beyond the scope of this study. Mercader and Vinyoles [1] did not include the number of vertebrae in their study—unfortunate as this meristic character is the best discriminator between adults of the two taxa. Raitt [23] had only two *G. argenteus* specimens from southern Portugal at his disposal both showing 41 vertebrae. This indicates that the number of vertebrae in *G. argenteus* is in fact increasing in colder waters although more data are necessary for this to be verified.

4.3. Post-Larval Pigmentation

The different pigmentation observed in the post-larvae of *Gadiculus* (Figure 1), being the most important reason for Schmidt [17] to erect *G. thori* as a new species, is an important, although often overlooked feature for the distinction of *Gadiculus* species.

Four stages of *G. thori* larvae and post-larvae (in the range 3.8–17.3 mm SL) were presented by Halbeisen and Schöfer [52], and another series by Izeta [53] that showed eight stages of larvae and post-larvae of *G. argenteus* from the southern Bay of Biscay (2.9–19.0 mm SL). Both studies presented the

development of melanophore patterns similar to type material descriptions, thus confirming the results by Schmidt [17]. It is of note that Bay of Biscay materials showed *G. argenteus* present, considering this region to be near the putative boundary of the separation of the two *Gadiculus* species (Figure 3). However, we were unable to obtain new materials from this region, *G. thori* has never been found so far south and the two species have never been found together. The importance of post-larvae melanophore patterns has been demonstrated repeatedly [54,55], and is unambiguous for the separation of *Gadiculus* taxa. The fact that larger individuals of the two *Gadiculus* species are so similar does not alter the importance of post-larval pigmentation. Therefore, Mercader and Vinyoles [1] not fully acknowledging its importance is untenable.

4.4. Otolith Morphology

Otoliths are a powerful tool for distinguishing closely related species, reconstructing phylogenies, and estimating geological events [37,40]. *Gadiculus* otoliths show several plesiomorphic characters within the Gadidae. The presence or absence as well as the size and shape of the pseudocolliculum, a feature described by Schwarzhans [20], and the collum are very important for the identification of species and genera within the Gadidae, including phylogenetic considerations. *Gadiculus* and *Micromesistius* are the only extant gadid genera possessing a pseudocolliculum on the medial surface of their sagittae. This symplesiomorphic character supports the close relationship of *Gadiculus* and *Micromesistius* identified from molecular data (Figure 8).

Many small-sized cod species possessed a well-developed pseudocolliculum and collum in the Oligocene and Miocene (Gaemers [56,57], Schwarzhans [58,59]), but the number of species decreased in the Pliocene, reaching an all-time low number of species in the Gadidae in the present day. The gadicoline fishes were very diverse and abundant during the Oligocene and Miocene. The medially situated pseudocolliculum is apparently an ancestral character that fishes in the family Gadidae have in common with many species of the gadiform rattails, family Macrouridae. Both *Gadiculus* and *Micromesistius* otoliths have deep roots in the past: based on fossil otoliths their common ancestor appears to have lived at the beginning of the Early Oligocene, i.e., about 34 million years ago (Gaemers, in preparation). Considering the distant geological past of this common ancestor, it is, therefore, unsurprising that *Gadiculus* and *Micromesistius* have their own peculiar advanced characteristics.

The oldest common ancestor of species currently attributed to the genus *Trisopterus* must have lived even earlier, in the Late Eocene. It is interesting that the oldest trisopterine lineage known from the geological record also possesses a pseudocolliculum just like *Gadiculus* and *Micromesistius* [40]. This further confirmed the plesiomorphic character of collum and pseudocolliculum in the gadids.

Otolith and adult fish morphology, habitat and food preferences all show that *Trisopterus esmarkii* (Nilsson, 1855) is clearly different from the other *Trisopterus* species [40]. The genus *Neocolliolus* was erected for this taxon by Gaemers [36] based on its different otolith morphology. Unfortunately, this reclassification still remains to be implemented in subsequent works on *Trisopterus*. Establishment of the genus name *Neocolliolus* for *T. esmarkii* is supported by molecular data showing a relatively large molecular distance to its closest relative *Trisopterus minutus* (Linnaeus, 1758) from *Cox1* barcodes (Figure 8). Based on otoliths, meristic and some external characteristics, *Trisopterus minutus* is more closely related to *Neocolliolus esmarkii* than to *Trisopterus luscus* (Linnaeus, 1758) and *T. capelanus* (Lacepède, 1800). A new genus, *Allotrisopterus*, is therefore introduced by Gaemers [40] for *Trisopterus minutus* and is confirmed by the molecular data presented in this study (Figure 8).

Otoliths of adult *Gadiculus* specimens identify the two species well, because they are nearly always very distinctive, at least since the Early Pleistocene. The adult otoliths of *G. argenteus* are essentially regularly drop-shaped, whereas those of *G. thori* show a distinctly truncated posterior end and/or an irregularly shaped and variable dorsal margin. In the Mediterranean, the two species already co-existed in the Late Pliocene [38] and their otoliths apparently could be distinguished in deposits formed during that period. Unfortunately, illustrations of Late Pliocene *G. thori* otoliths from the Mediterranean or elsewhere have not been published yet. In the light of general otolith morphology,

it is remarkable that adult *G. argenteus* shows otoliths that are fairly uniform in shape whereas those of *G. thori* show high variability.

4.5. Molecular Data

Two clearly separate groups based on *Cox1* barcodes within *Gadiculus* unambiguously support two species whether considering the molecular gap or the low intraspecific variation observed within both species (Figure 8). The molecular data are therefore in agreement with vertebral counts [23], pigmentation of post-larvae [17], geographic distribution (Figure 3) and with the differences present in the adult otoliths (Figures 3, 5 and 6). It is of note that the *Cox1* barcodes are showing virtually no intraspecific variation within the Mediterranean/Lusitanian Atlantic and the Boreal Atlantic populations. It is also of note that the edge length of the *Gadiculus* lineage is the longest observed within the Gadidae compared to all other genera (Figure 8). The many substitutions delimiting *Gadiculus* taxa are in agreement with a relatively long history of gadiculine fishes in the Gadidae, and are therefore in accordance with the relatively complicated evolutionary history observed in the fossil otolith records.

The *Cox1* barcodes show a closer relationship between *Gadiculus* and *Micromesistius* than with other Gadidae (Figure 8). This is in agreement with the results found by Teletchea et al. [60] that used two mitochondrial genes. This relationship corresponds with the otoliths of both genera that are separated from all other extant Gadidae taxa by having a pseudocolliculum.

4.6. Present Geographic Distributions

The distributional patterns of the two species are noteworthy as there is strong empirical support for a cold- and a warm-water Northeast Atlantic separation from both morphological and molecular data. This is particularly evident as Mediterranean *G. argenteus* specimens, without exception, cluster with specimens caught off Western Portugal and more south in the East Atlantic Ocean (Figures 3, 4, and 8). Therefore, separation of *G. argenteus* and *G. thori* is observed at approximately the 45° N latitude, although details remain obscure in this region as additional sampling is needed for a more accurate line of separation (Figure 3). However, separation is roughly corresponding to the boundaries of the subtropical gyre currents in the North Atlantic, originating in the warm western tropical Atlantic as the Gulf Stream and diverging to the East into the North going North Atlantic Drift and the South going Canary Current [61,62]. This divergence likely acts as a species barrier for *Gadiculus* spp. from either distinct temperatures in the opposite going currents or the hindrance of mixing in current swept eggs.

It is of note that *G. argenteus* is not tropical as it has never been reported in tropical East Atlantic waters, being confined to the warm-temperate latitudes between 20° N and 45° N. This is in agreement with other gadids that show anti-tropical distributions [9] although two distinct closely related species as observed for *Gadiculus* are not usually the case [63]. It is, therefore, appropriate to deem *G. thori* the cold-water adapted form confined to the cold-temperate latitudes between 72° N and 45° N, and *G. argenteus* the warm-water adapted form distributed south to off northwestern Africa. The distributions of both *Gadiculus* species show similarities to the distribution of *Micromesistius poutassou* (Risso 1827), the most closely related genus and also found throughout the eastern North Atlantic and in the Mediterranean. Similar to *G. argenteus*, *M. poutassou* appears restricted towards the south by the Canary current, an extensive system that is responsible for extensive upwelling off Northwest Africa [64]. Distributional patterns of *G. thori* appear similar to those found in *Neocolliolus esmarkii* [40].

Gadiculus thori and *G. argenteus* are associated with landmasses, as they have been exclusively reported from upper slope and lower shelf habitats [9]. However, we here present one specimen caught in 2012 off southeast Greenland, providing a range extension into the western part of the subarctic Atlantic [65]. The finding of *G. thori* off southeast Greenland means that the species was either transported by the North Atlantic Drift originating in warmer waters, very unlikely as this route is oceanic originating in the western tropical Atlantic, or distributed via the eastern Atlantic route across

Iceland to Norway. The latter explanation is plausible, as *Gadiculus* has been reported in Icelandic waters for some time [66]. However, the route via Iceland means crossing a deep-sea barrier in the northeastern Atlantic, which is not impossible due to the pelagic mode of life of *Gadiculus*. Theoretically, it could also belong to a separate *G. thori* population of Iceland and/or Greenland. However, molecular results presented in this study show no indications of separate populations in *G. thori* (Figure 8). It is difficult, however, to assess whether a true distribution is present in Greenland waters as *G. thori* has not been observed subsequently to 2012 despite extensive yearly surveys conducted in the region by the Greenland Institute of Natural Resources (J.Y. Poulsen, personal observation). The Greenland-Iceland submarine ridge could potentially facilitate distribution of shelf-associated species such as *G. thori* [24]. Distributional changes for pelagic fishes are a complicated matter in subarctic Atlantic waters, as yearly seawater temperature changes are heterogeneously occurring at various depths [67]. In addition, the distinction of coastal versus oceanic habitat is difficult at times, including also a general problematic sampling and taxonomic effort in this region [68,69]. Byrkjedal and Høines [8] found *Gadiculus* in the southwestern part of the Barents Sea. Additionally, the recent discovery of this species in the Russian part of the Barents Sea [70] is a further extension of its area that possibly could be explained by changed temperatures in these Arctic waters.

The case of *Gadiculus* presents a good example of how important taxonomy is for the distribution and monitoring of taxa in relation to ocean temperature and climate changes. If organisms that show a latitudinal cline in their distributions are to be used as biological monitoring markers, for example in relation to temperature affinities, species distributions and temperature tolerance are baseline knowledge. *Gadiculus* has been treated as a single temperature-changing tolerant entity ranging from approximately 20° N to 70° N in the eastern North Atlantic Ocean until this study. The fact that two species were not recognized lately, with all evidence showing different temperature adaptations to have evolved in this genus, speaks volumes concerning the standard use of distributional changes without proper taxonomic assessment. Using distribution of taxa as a tool in climate change research requires a thorough investigation of the individual marker. This evidently begins with proper taxonomic considerations. With this in mind, the finding of *G. thori* off southeast Greenland close to the Arctic Circle is unsurprising.

4.7. Former Geographic Distributions

The present geographic distribution of *G. thori* and *G. argenteus* coupled with catch rates, already gives a clue for the existence of two species as aforementioned. An even stronger argument can be found in the geological past.

Fossil otoliths have proven that both species co-existed in the Mediterranean during parts of the Late Pliocene (younger part of the Gelasian) and Early to Middle Pleistocene (Calabrian to Ionian) [38]. The Pleistocene otoliths of both taxa in these periods are easily distinguishable and very similar to the recent ones (Figures 5 and 7). Unfortunately, illustrations of Mediterranean *G. thori* otoliths from Late Pliocene specimens have not yet been published, but can be distinguished from Late Pliocene *G. argenteus* otoliths according to Girone et al. [38]. This shows that the two taxa are distinct and separated at least from *c.* three million years ago. Subspecies cannot co-exist in the same area without interbreeding. The fossil otoliths show that interbreeding apparently did not occur and corroborate the presence of two taxa present then and now. Hypothetically, if fossil otoliths from *G. argenteus* and *G. thori* were to be subspecies only and not species, overlapping variation would have been observed, resulting in the absence of two clearly different shape types of otoliths showing the same characteristics as observed in the two extant *Gadiculus* species. The size difference between the two *Gadiculus* species indicates that resource partitioning must have occurred in these fishes, resulting in less competition between co-existing fishes. There might also have been some depth segregation with *G. thori* on an average living in deeper somewhat colder water than *G. argenteus*.

The past co-occurrence of *G. argenteus* and *G. thori* in the Mediterranean Sea might be correlated with a much more southern course of the North Atlantic Drift and/or a weaker presence of this drift

during several ice ages when arctic and subarctic environments covered a much larger area on the northern hemisphere. This would have pushed the geographical distribution of *G. thori*, as well as that of many other species, further southwards. The present distribution separating the two species, strongly suggests that they are vicariant species, but the fossil otoliths show that they were co-existing in the same regions during longer geological periods. This observation is important and proves that distribution is a complicated matter on multiple levels, as the fossil record and temperature tolerance are not usually known for most fishes. Otoliths indicate that sister species co-existing in the same area for long time periods is a normal phenomenon in gadids. When the earliest fossil otoliths of such closely related species co-occur in the same sediment samples and represent the beginning of lineages, they provide strong evidence for sympatric speciation. A good example of this is found for *T. luscus* and *T. capelanus* that have their origins in the Early Miocene North Sea region, and have co-existed in this region until the Late Pliocene [40]. The oldest representatives of the *G. argenteus* lineage are known from the late Early Miocene, although otoliths of the *G. thori* lineage are not yet known from before the Late Pliocene (Gaemers, in preparation). Additional data are therefore necessary to resolve whether the two extant *Gadiculus* species have a sympatric origin or not.

It is finally important to mention that Schwarzhans [71] described *Gadiculus (Gadiculus) antipodus* from the Early Miocene of New Zealand. However, this species is not a member of one of the two *Gadiculus* lineages leading to the two recent species, because its collum and pseudocolliculum are shorter than in *Gadiculus*, in addition to very small colliculi. Therefore, it is more related to the extinct genus *Circagadiculus*, known from the Late Oligocene to Early Miocene of the North Sea Basin [56], and should be included within that genus. The maximum size of the otoliths found by Schwarzhans also agrees with that of *Circagadiculus* otoliths that never attain the size of *Gadiculus* otoliths. This means that *Gadiculus* is still unknown from the southern hemisphere, but it is interesting to note that earlier gadiculines managed to cross the equator extending as far as New Zealand. Species of the genus *Circagadiculus* were adapted to much warmer waters than *Gadiculus*, because the Late Oligocene and Early Miocene North Sea was considerably warmer than at present. Temperature differences within tropical seas in that period were likely less pronounced than today. Considering the distribution in New Zealand waters, *Circagadiculus antipodus* probably occupied a pelagic habitat.

4.8. Classification of Codfishes in this Study

The ranking and classification of codfishes is generally not agreed upon when considering different types of evidence. This is especially evident concerning the subfamily and family levels (e.g., Endo [72]; Roa-Varón and Ortí, [73]). Concerning family levels, we have noted the gadids, lotids, gaidropsarids and phycids as distinct families due to their distinct otoliths (see Gaemers [37]), corroborated by distinctive external characteristics of the four groups. Following Howes [74] and Fahay [55], we define the Gadidae family as consisting of only the species with three dorsal fins. We note that the burbot of the family Lotidae are found non-monophyletic in this study (Figure 8), similar to results presented by Roa-Varón and Ortí [73] who used a larger dataset and more molecular markers. We have classified the two lotid clades as subfamilies: Lotinae and Molvinae. However, general morphology of *Lota lota* otoliths is not particularly different from those of the other lotid genera *Molva* and *Brosme*. Therefore, the observed non-monophyly of the Lotidae is unresolved at present (Figure 8). Few molecular studies of gadiform fishes are available, which is surprising considering their large-scale importance economically, and investigations of additional molecular information might reveal new results related to longer DNA sequences. However, it is encouraging that molecular and otolith data of gadiform fishes correspond well in most instances.

The gaidropsarid genus *Onogadus* is used in this study (Figure 8) according to evidence that supports the replacement of *Gaidropsarus* for *Onogadus*, i.e., for *Onogadus argentatus* (Reinhardt, 1837) and *Onogadus ensis* (Reinhardt, 1837) by De Buen [75]. The recognition of *Onogadus* is supported by several morphological characters that were described by Howes [74,76,77]. This is in accordance with otoliths that similarly support *Onogadus* as a distinct genus (see [78] under *Platyonos*).

5. Conclusions

Evidence from post-larval pigmentation, otolith morphology, number of vertebrae, molecular data, maximum size and present/former geographic distribution all support two extant species of *Gadiculus*. We conclude that Schmidt [17] was right in recognizing two *Gadiculus* species. Several morphometric and meristic measurements may introduce some ambiguity, although the number of vertebrae will usually allow for the correct identification of specimens. Table 3 shows an overview of all differences between the two *Gadiculus* species discussed in the present study. The questionable meristic data concerning number of vertebrae and fin rays noted by Mercader and Vinyoles [1] are excluded in Table 3.

Table 3. Evidence supporting *Gadiculus argenteus* and *G. thori* as separate species.

Species	<i>Gadiculus argenteus</i>	<i>Gadiculus thori</i>
Maximum size	ca. 15 cm	ca. 20 cm
Head size	Relatively large	Relatively small
Proportion of the eye	Relatively large	Relatively small
OL/OT ratio	Relatively large	Relatively small
Shape of adult otoliths	Drop-shaped Moderately variable Posterior end usually not truncated, but if so, truncation is oblique to long axis	Oval (irregular) Highly variable Posterior end usually truncated; truncation perpendicular to long axis
Postlarvae at same stage of development	Three transverse pigmented bars Relatively small size Relatively stout	One transverse pigmented bar Relatively large size Relatively slender
Number of vertebrae	37–41, usually 40 (usual range 38–40; 37 and 41 rare)	39–43, usually 42 (usual range 41–43; 39 and 40 rare)
Number of D3 fin rays	11–16	15–17
Number of A1 fin rays	11–16 (11 rare)	15–18 (18 rare)
<i>Cox1</i> barcodes	Little intraspecific variation	Little intraspecific variation

The two *Gadiculus* species are not vicariant species, as their present geographical distributions would suggest. Fossil otoliths indicate that they are sister species and have co-existed during long geological periods. Therefore, it is likely that sympatric speciation occurred and *Gadiculus* thereby originating from a single common ancestor. Gaemers [40] also observed this pattern in other Gadidae lineages witnessed from the fossil otolith record.

For *G. thori* and *G. argenteus* we suggest northern and southern silvery pout as vernacular names, respectively.

Acknowledgments: We would like to thank T. Bakke (Norwegian Institute for Water Research, Oslo, Norway; formerly Marin Biologisk Stasjon Espegrend, Blomsterdalen, Norway) for *G. thori* specimens, and G. van der Velde (Biology Department, Radboud University, Nijmegen, The Netherlands) for *G. thori* otoliths, C. Allué (Instituto de Ciencias del Mar, CSIC, Barcelona, Spain), D. Nolf (formerly at the Royal Belgian Institute of Natural Sciences, Brussels, Belgium) and C.A. Assis (Department of Animal Biology, University of Lisboa, Portugal) for *G. argenteus* otoliths, N. Daan (formerly at the Rijksinstituut voor Visserijonderzoek, R.I.V.O., IJmuiden [now IMARES], the Netherlands) for fish size information, L. Lindblom & S. Thorkildsen (University of Bergen, Norway), M. Miya & T. Sado (Chiba Natural History Museum, Japan) for help with molecular lab-work, K. & E. Hjørne (www.naturporten.dk, private) for help with illustrations, T. Menne (Zoological Museum University of Copenhagen, Denmark) for cataloging, R/V Pamiut crew, J. Nielsen (Greenland Institute of Natural Resources, Greenland) and B.H. Sunset (MAREANO, Institute of Marine Research, Norway) for loan of photos and three anonymous reviewers for valuable comments. We owe a special thanks to I. Byrkjedal & G. Langhelle (Natural History Collections Bergen, Norway) for curating, providing materials and logistics whenever needed and to I. Chemshirova (Zoological Society London, UK) for correcting the manuscript.

Author Contributions: Otolith examinations and review of previous works was conducted by P.G.; New records and molecular works were conducted by J.P. Both author wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

TL	Total fish length in mm
TLmax	Largest known total fish length in mm
SL	Standard length in mm
OL	Otolith length in mm
Coll.	Collector
ID	Person who originally identified
Cox1	Cytochrome Oxidase 1
K2P	Kimura-2-parameter model
T-P spacer	Intergenic non-coding region in all gadiforms between the tRNAs Threonine and Proline
ZMUB	Registration number of the Museum of Zoology, University of Bergen, Norway

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