Polar Biol
DOI 10.1007/s00300-011-0974-5

ORIGINAL PAPER

The Antarctic fish genus *Artedidraco* is paraphyletic (Teleostei, Notothenioidei, Artedidraconidae)

Guillaume Lecointre · Cyril Gallut · Céline Bonillo · Arnaud Couloux · Catherine Ozouf-Costaz · Agnès Dettaï

Received: 16 August 2010 / Revised: 12 January 2011 / Accepted: 13 January 2011

Abstract Artedidraconids (Plunderfishes) are small benthic notothenioid fishes of the Antarctic and South Georgia shelf and slope. The family Artedidraconidae is monophyletic; however, the relationships within the family have remained poorly explored until now, and based on a small sample of the genus *Artedidraco*. The present study focuses on the interrelationships among the artedidraconid genera and the phylogeny of the genus *Artedidraco*. 2,353 base pairs from 77 specimens were sequenced from the partial mitochondrial cytochrome oxidase I gene and cytochrome b gene, the partial mitochondrial control region and the partial nuclear rhodopsin retrogene. The genus *Artedidraco* is not monophyletic, confirming the preliminary relationships found by Derome et al. (Mol Phylogenet Evol 24:139–152, 2002): *Pogonophryne*, *Dolloidraco* and *Histiodraco* are well embedded within the genus *Artedidraco*. From *Artedidraco skottsbergi* and *A. loennbergi* to *A. orianae* and *A. mirus*, the tree shows that there is an increasing number of upper lateral line tubular scales and decreasing number of disc-shaped scales. There is also a trend toward a decrease in the number of epipleural ribs and an increase in number of pleural ribs along the tree.

Keywords Artedidraconidae · Notothenioidei · Systematics

G. Lecointre (✉)
UMR 7138 UPMC-CNRS-MNHN-IRD «systématique, Adaptation, Evolution», département «Systématique and Evolution», Muséum National d'Histoire Naturelle, CP 39, 57 rue Cuvier, 75005 Paris, France
e-mail: lecointr@mnhn.fr

C. Gallut · C. Ozouf-Costaz · A. Dettaï
UMR 7138 UPMC-CNRS-MNHN-IRD «systématique, Adaptation, Evolution», département «Systématique and Evolution», Muséum National d'Histoire Naturelle, CP 26, 57 rue Cuvier, 75005 Paris, France

C. Bonillo
UMS 2700 CNRS, département «Systématique and Evolution», Muséum National d'Histoire Naturelle, CP 26, 57 rue Cuvier, 75005 Paris, France

A. Couloux
Genoscope, Centre National de Séquençage, 2, rue Gaston Crémieux, CP5706, 91057 Evry, France

Introduction

Artedidraconids (Plunderfishes) are small notothenioid fishes of 10-34 cm total length living at the bottom of the Antarctic shelf and slope and around South Georgia within the depth range 18–2,542 m (Eastman 1993). They have a sedentary life style and are sit-and-wait predators of actively moving organisms such as amphipods, isopods, polychaetes and mysids. The family is currently composed of 27 valid species (Fishbase.org 2010; Eakin et al. 2009) grouped into four genera: the monotypic *Histiodraco* and *Dolloidraco*, and the more species rich *Artedidraco* (6 species) and *Pogonophryne* (19 species). More than one-fourth of the species have been described in the last 12 years (Balushkin and Eakin 1998; Eakin and Balushkin 1998; Eakin and Eastman 1998; Balushkin 1999; Eastman and Eakin 1999; Eakin and Balushkin 2000; Eakin et al. 2008, 2009). Artedidraconids are easily distinguished from other notothenioids by the presence of a mental barbel. The shape of the barbel has been considered to be species-specific, but a recent study casts doubt on its specificity (Eakin et al. 2006). Artedidraconids also share hooked-shaped operculars and a number of other anatomical features (Eakin 1981). Artedidraconinae were initially included into Harpagiferidae (Eakin 1981). They now form

Published online: 06 March 2011

a separate family (Eakin 1990) and the new Harpagiferidae contains only the genus *Harpagifer*.

Artedidraconids are monophyletic and do form the sister-group of the Harpagiferidae in every phylogenetic scheme, opinion or molecular phylogeny published to this day (Eastman 1993; Ritchie et al. 1997; Balushkin 2000; Bargelloni et al. 2000; Derome et al. 2002; Near et al. 2004; Near and Cheng 2008). However, in spite of the remark by Eastman and Eakin (1999) more than 10 years ago that "*a cladogram of relationships within the family is not yet available*", no strictly cladistic analysis of anatomical characters within the family has been performed since. Moreover, the artedidraconid taxonomic sampling of most of the molecular studies remained poor: Ritchie et al. (1997) and Bargelloni et al. (2000) only sampled *Histiodraco* and *Pogonophryne* while Near et al. (2004) and Near and Cheng (2008) only included *Dolloidraco* and *Pogonophryne* in their trees (Fig. 1). In all these papers, no *Artedidraco* species were included and very few of the *Pogonophryne* species were present. Only Derome et al. (2002) sampled the four genera and found the paraphyly of the genus *Artedidraco* with high support. The authors did not comment further on that result (Derome et al. 2002: 147) because only two species of *Artedidraco* were sampled (Fig. 1) and the study was focused on the phylogeny of the Bathydraconidae. The present study includes nine species sampled across all four genera, among which 5 species of the genus *Artedidraco.* It also includes several specimens for all the included species thanks to the barcoding sampling (Dettaï et al. 2010) performed during the CEAMARC cruise (2007–2008) in the Eastern Antarctic seas (http://www.caml.aq/voyages/aurora-australis-200708/index.html). This sampling design allows a more rigorous test of the possible paraphyly of the genus *Artedidraco.* The discrepancy in topology between the molecular study of Derome et al. (2002) and the comparative anatomy-based study of Balushkin (2000) (*Histiodraco* closer to *Pogonophryne;* a topology also proposed by Eakin 1981, Fig. 1) will also be reassessed with more markers. Four markers (2,353 base pairs) were sequenced for 77 specimens: the partial mitochondrial cytochrome oxidase I gene and cytochrome b gene, the mitochondrial control region and the partial nuclear rhodopsin retrogene. Our phylogenetic analysis corroborates the relationships found by Derome et al. (2002) and shows that the genus *Artedidraco* is not monophyletic.

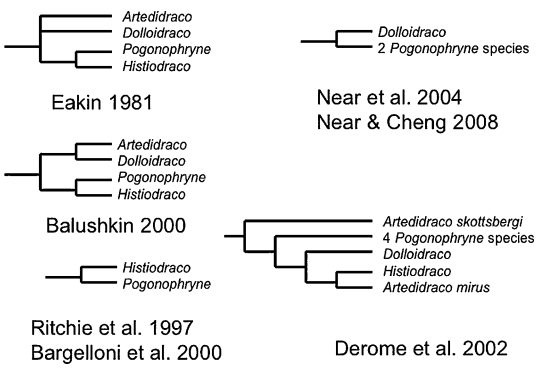

Fig. 1 Various hypotheses about relationships among artedidraconid genera over the last 30 years (Eakin 1981 and Balushkin 2000 from morpho-anatomical data, others based on molecular data)

Materials and methods

The present taxonomic sampling (Table 1) is focused on the genus *Artedidraco* (the only missing species is the recently described *Artedidraco glareobarbatus* Eastman and Eakin 1999) and the interrelationships among artedidraconid genera. The monotypic genera *Histiodraco* and *Dolloidraco* are included. A few species from the genus *Pogonophryne* are included. Many species of that genus are very rare, and they are most probably very closely related to one other (Lombarte et al. 2003; Eakin et al. 2009) as also suggested by their homogeneous chromosomal formulae (Ozouf-Costaz et al. 1991) and the present molecular data. Eakin et al. (2009) sequenced 11 of the 19 *Pogonophryne* species for the ND2 mitochondrial gene, confirming that the *Pogonophryne* species are very closely related, though inter-specific relationships are far from fully resolved. The relationships among the 19 known *Pogonophryne* species would, therefore, require a specific study with a complete taxonomic sampling and multiple specimens for each species, as well as a combined molecular and morphological overview of the species delimitations with ultravariable markers, as suggested by Dettaï et al. (2010). However, this is not within the possibilities and scope of the present study. Hereafter, the genus *Pogonophryne* will be taken as a single taxonomic unit (Balushkin and Eakin 1998; Lombarte et al. 2003). Trees were rooted using sequences of the sister-family, the Harpagiferidae (genus *Harpagifer*), and species from another family from the "High Antarctic Clade" (Near and Cheng 2008), the Bathydraconidae (genus *Gymnodraco*).

For each sample (Table 1), a small piece of muscle tissue was stored at −24°C or fixed in 70% ethanol and stored at 3°C. All DNA extractions followed either a classical CTAB protocol with a chloroform isoamylalcohol step (Winnepenninckx et al. 1993), or were performed on an ABI Prism 6100 Nucleic Acid Prepstation with a Nuc Prep kit (ABI Prism) following the instructions of the manufacturer. DNA amplification was performed by PCR in a final 25 µL volume containing 5% DMSO, 1 µL of dNTP 6.6 mM, 0.15 µL of Taq DNA polymerase (MP Biomedicals or Qiagen), using 2.5 µL of the buffer provided by the manufacturer, 100 u. µL-1 and 0.4 µL of each

Table 1 Sampling data. Sequence accession numbers provided in columns 5–8 are those of BOLD (Barcode of Life Data Systems); in most of the cases the same accession number is given for the four genes of the project EATF (Cytochrome oxidase gene sequence, Cytochrome b gene sequence, d-loop sequence and Rhodopsin nuclear retrogene sequence) for a single specimen

Species name	Specimen tag	Voucher reference	Catch	COI	CYTB	DLOOP	Rhodo	Matrix 27	Matrix 77
Artedidraco loennbergi	si105n653	MNHN 2009–0935	2008	EATF104-10	EATF104-10	EATF104-10	EATF104-10		X
Artedidraco loennbergi	si10n90	MNHN 2009–0937	2008	EATF010-10	EATF010-10	EATF010-10	EATF010-10		X
Artedidraco loennbergi	si12n92	MNHN 2009–0940	2008	EATF012-10	EATF012-10	EATF012-10	EATF012-10		X
Artedidraco loennbergi	si13n93	MNHN 2009–0946	2008	EATF013-10	EATF013-10	EATF013-10	EATF013-10		X
Artedidraco loennbergi	si29n235	MNHN 2009–0968	2008	EATF029-10	EATF029-10	EATF029-10	EATF029-10		X
Artedidraco loennbergi	si358n2567	MNHN 2009–0989	2008	EATF353-10	EATF353-10	EATF353-10	EATF353-10		X
Artedidraco loennbergi	si359n2568	MNHN 2009–0990	2008	EATF354-10	EATF354-10	EATF354-10	EATF354-10		X
Artedidraco loennbergi	si422n2882	MNHN 2009–0999	2008	EATF417-10	EATF417-10	EATF417-10	EATF417-10		X
Artedidraco loennbergi	si7n87	MNHN 2009–1028	2008	EATF007-10	EATF007-10	EATF007-10	EATF007-10	X	X
Artedidraco loennbergi	si8n88	MNHN 2009–1032	2008	EATF008-10	EATF008-10	EATF008-10	EATF008-10	X	X
Artedidraco loennbergi	si9n89	MNHN 2009–1034	2008	EATF009-10	EATF009-10	EATF009-10	EATF009-10	X	X
Artedidraco mirus	3	b	1998	EATF606-10	EATF606-10	EATF606-10	0	X	X
Artedidraco mirus	SG	SAIAB 74945	2004	EATF609-10	EATF609-10	0	EATF609-10	X	X
Artedidraco orianae	1301	d	d	EATF608-10	EATF608-10	EATF608-10	EATF608-10		X
Artedidraco orianae	si519n3637	MNHN 2009–1004	2008	EATF510-10	EATF510-10	EATF510-10	EATF510-10	X	X
Artedidraco orianae	si520n3638	MNHN 2009–1005	2008	EATF511-10	EATF511-10	EATF511-10	EATF511-10		X
Artedidraco orianae	si522n3640	MNHN 2009–1007	2008	EATF513-10	EATF513-10	EATF513-10	EATF513-10	X	X
Artedidraco orianae	si523n3641	MNHN 2009–1008	2008	EATF514-10	EATF514-10	EATF514-10	EATF514-10		X
Artedidraco orianae	si526n3644	MNHN 2009–1011	2008	EATF517-10	EATF517-10	EATF517-10	EATF517-10	X	X
Artedidraco shackletoni	si103n619	MNHN 2009–0933	2008	EATF102-10	EATF102-10	EATF102-10	EATF102-10		X
Artedidraco shackletoni	si157n723	MNHN 2009–0948	2008	EATF156-10	EATF156-10	EATF156-10	EATF156-10		X
Artedidraco shackletoni	si162n1062	MNHN 2009–0952	2008	EATF161-10	EATF161-10	EATF161-10	EATF161-10		X
Artedidraco shackletoni	si353n2562	MNHN 2009–0984	2008	EATF348-10	EATF348-10	EATF348-10	EATF348-10		X
Artedidraco shackletoni	si354n2563	MNHN 2009–0985	2008	EATF349-10	EATF349-10	EATF349-10	EATF349-10		X
Artedidraco shackletoni	si355n2564	MNHN 2009–0986	2008	EATF350-10	EATF350-10	EATF350-10	EATF350-10		X
Artedidraco shackletoni	si369n2680	MNHN 2009–0991	2008	EATF364-10	EATF364-10	EATF364-10	EATF364-10		X
Artedidraco shackletoni	si370n2681	MNHN 2009–0992	2008	EATF365-10	EATF365-10	EATF365-10	EATF365-10		X
Artedidraco shackletoni	si371n2682	MNHN 2009–0993	2008	EATF366-10	EATF366-10	EATF366-10	EATF366-10	X	X
Artedidraco shackletoni	si372n2683	MNHN 2009–0994	2008	EATF367-10	EATF367-10	EATF367-10	EATF367-10		X
Artedidraco shackletoni	si374n2685	MNHN 2009–0996	2008	EATF369-10	EATF369-10	EATF369-10	EATF369-10		X
Artedidraco shackletoni	si378n2491	MNHN 2009–0997	2008	EATF373-10	EATF373-10	EATF373-10	EATF373-10	X	X
Artedidraco shackletoni	si544n2607	MNHN 2009–1020	2008	EATF535-10	EATF535-10	EATF535-10	EATF535-10		X
Artedidraco shackletoni	si545n2606	MNHN 2009–1021	2008	EATF536-10	EATF536-10	EATF536-10	EATF536-10	X	X
Artedidraco shackletoni	si546n2605	MNHN 2009–1022	2008	EATF537-10	EATF537-10	EATF537-10	EATF537-10		X
Artedidraco shackletoni	si90n368	MNHN 2009–1033	2008	EATF090-10	EATF090-10	EATF090-10	EATF090-10		X

Table 1 continued

Species name	Specimen tag	Voucher reference	Catch	COI	CYTB	DLOOP	Rhodo	Matrix 27	Matrix 77
Artedidraco shackletoni	TA64ARSH1	MNHN 2001–1133	2008	EATF602-10	EATF602-10	EATF602-10	EATF602-10		X
Artedidraco skottsbergi	1205	d	d	EATF607-10	EATF607-10	EATF607-10	EATF607-10		X
Artedidraco skottsbergi	TA654ARSK1	0	2007	EATF604-10	EATF604-10	EATF604-10	EATF604-10		X
Artedidraco skottsbergi	si104n652	MNHN 2009–0934	2008	EATF103-10	EATF103-10	EATF103-10	EATF103-10		X
Artedidraco skottsbergi	si106n654	MNHN 2009–0936	2008	EATF105-10	EATF105-10	EATF105-10	EATF105-10		X
Artedidraco skottsbergi	si314n2410	MNHN 2009–0969	2008	EATF309-10	EATF309-10	EATF309-10	EATF309-10	X	X
Artedidraco skottsbergi	si315n2411	MNHN 2009–0970	2008	EATF310-10	EATF310-10	EATF310-10	EATF310-10		X
Artedidraco skottsbergi	si316n2412	MNHN 2009–0971	2008	EATF311-10	EATF311-10	EATF311-10	EATF311-10	X	X
Artedidraco skottsbergi	si318n2414	MNHN 2009–0973	2008	EATF313-10	EATF313-10	EATF313-10	EATF313-10		X
Artedidraco skottsbergi	si319n2415	MNHN 2009–0974	2008	EATF314-10	EATF314-10	EATF314-10	EATF314-10		X
*Artedidraco skottsbergi*i	si320n2416	MNHN 2009–0975	2008	EATF315-10	EATF315-10	EATF315-10	EATF315-10		X
Artedidraco skottsbergi	si322n2418	MNHN 2009–0976	2008	EATF317-10	EATF317-10	EATF317-10	EATF317-10	X	X
Artedidraco skottsbergi	si323n2419	MNHN 2009–0977	2008	EATF318-10	EATF318-10	EATF318-10	EATF318-10		X
Artedidraco skottsbergi	TA653ARSP	0	2007	EATF603-10	EATF603-10	EATF603-10	EATF603-10		X
Dolloidraco longedorsalis	si131n834	MNHN 2009–0941	2008	EATF130-10	EATF130-10	EATF130-10	EATF130-10	X	X
Dolloidraco longedorsalis	si132n838	MNHN 2009–0942	2008	EATF131-10	EATF131-10	EATF131-10	EATF131-10		X
Dolloidraco longedorsalis	si133n839	MNHN 2009–0943	2008	EATF132-10	EATF132-10	EATF132-10	EATF132-10		X
Dolloidraco longedorsalis	si134n840	MNHN 2009–0944	2008	EATF133-10	EATF133-10	EATF133-10	EATF133-10		X
Dolloidraco longedorsalis	si135n841	MNHN 2009–0945	2008	EATF134-10	EATF134-10	EATF134-10	EATF134-10		X
Dolloidraco longedorsalis	si181n1369	MNHN 2009–0954	2008	EATF178-10	EATF178-10	EATF178-10	EATF178-10		X
Dolloidraco longedorsalis	si186n1401	MNHN 2009–0956	2008	EATF183-10	EATF183-10	EATF183-10	EATF183-10		X
Dolloidraco longedorsalis	si251n1963	MNHN 2009–0963	2008	EATF246-10	EATF246-10	EATF246-10	EATF246-10	X	X
Dolloidraco longedorsalis	si254n1988	MNHN 2009–0964	2008	EATF249-10	EATF249-10	EATF249-10	EATF249-10	X	X
Gymnodraco acuticeps	TA109	MNHN 2007–0255	1998	EATF597-10	EATF597-10	EATF597-10	EATF597-10		X
Gymnodraco acuticeps	TA500GYVI1	MNHN 2009–0867	2005	EATF600-10	EATF600-10	EATF600-10	EATF600-10	X	X
Gymnodraco victori	TA288GYAC1	MNHN 2009–0655	2001	EATF598-10	EATF598-10	EATF598-10	EATF598-10		X
Gymnodraco victori	TA479GYVI1	MNHN 2009–0672	2005	EATF610-10	EATF610-10	EATF610-10	EATF610-10	X	X
Gymnodraco victori	TA504GYVI1	MNHN 2009–0865	2005	EATF601-10	EATF601-10	EATF601-10	EATF601-10	X	X
Harpagifer				EATF605-10	GU214222	AF490646	EATF605-10	X	X
Harpagifer antarcticus		d	d	0	GU214222	0	0		
Harpagifer georgianus		a	a	0	0	AF490646	0		
Harpagifer kerguelensis	412	c (MNHN 2000–0269)	1999	EATF605-10	0	0	EATF605-10		
Histiodraco velifer	si125n732	MNHN 2009–0939	2008	EATF124-10	EATF124-10	EATF124-10	EATF124-10	X	X
Histiodraco velifer	si241n977	MNHN 2009–0961	2008	EATF236-10	EATF236-10	EATF236-10	EATF236-10	X	X
Histiodraco velifer	si493n3191	MNHN 2009–1677	2008	EATF485-10	EATF485-10	EATF485-10	EATF485-10	X	X
Histiodraco velifer	TA49	MNHN 2001–1131	2008	EATF599-10	EATF599-10	EATF599-10	EATF599-10		X

Table 1 continued

Species name	Specimen tag	Voucher reference	Catch	COI	CYTB	DLOOP	Rhodo	Matrix 27	Matrix 77
Pogonophryne scotti	si282n103	MNHN 2009–0966	2008	EATF277-10	EATF277-10	EATF277-10	EATF277-10	X	X
Pogonophryne scotti	si425n2912	MNHN 2009–1398	2008	EATF420-10	EATF420-10	EATF420-10	EATF420-10	X	X
Pogonophryne scotti	si528n3628	MNHN 2009–1014	2008	EATF519-10	EATF519-10	EATF519-10	EATF519-10		X
Pogonophryne sp1	si234n1707	MNHN 2009–0960	2008	EATF229-10	EATF229-10	EATF229-10	EATF229-10		X
Pogonophryne sp1	si496n3339	0	2008	EATFR001-10	EATFR001-10	EATFR001-10	EATFR001-10		X
Pogonophryne sp1	si497n3340	MNHN 2009–1001	2008	EATF488-10	EATF488-10	EATF488-10	EATF488-10	X	X
Pogonophryne sp1	si529n3652	MNHN 2009–1015	2008	EATF520-10	EATF520-10	EATF520-10	EATF520-10		X
Pogonophryne sp1	si530n3653	MNHN 2009–1016	2008	EATF521-10	EATF521-10	EATF521-10	EATF521-10		X
Pogonophryne sp2	si6n60	MNHN 2009–1025	2008	EATF006-10	EATF006-10	EATF006-10	EATF006-10		X

Accession numbers that do not start with EATF are Genbank accession numbers. The place and date of catch are associated with the voucher specimen collection databases (for MNHN: http://coldb.mnhn.fr/colweb/form.do?model=GICIM.wwwichtyo.wwwichtyo.wwwichtyo) and in BOLD, except for: a: specimen caught at King Gorge Island, provided by Luca Bargelloni, used in Derome et al. (2002), b: provided by Luca Bargelloni, c: no voucher specimen, however the specimen was caught at the same place and date as MNHN 2000–0269, d: provided by Rafael Zardoya, same place as "d" in Table 1 from Papetti et al. (2007)

of the two primers at 10 pM (Table 2); 1 μL of DNA extract was added. After denaturation for 2 min, the PCR was run for 40–50 cycles of 20 S, 94°C; 20 S, hybridisation temperature (see Table 2); 50 S to 1 min 10 S, 72°C using a Biometra trioblock cycler (T3000). The result was visualized on ethidium bromide-stained agarose gels. Sequencing was performed by the National Center for Sequencing (Genoscope) using the same primers.

All sequences were obtained in both directions and checked manually against their chromatogram using Sequencher 4.8 (Gene Codes Corporation). They were controlled for mix-ups and contaminations by pairwise sequence comparison. This yielded four datasets: partial COI gene, partial rhodopsin retrogene, partial cytochrome b gene and partial control region. All new COI sequences were deposited in the BOL database with their accompanying information when vouchers were available. The other nuclear sequences and the mitochondrial sequences without vouchers were deposited in GenBank (http://www.ncbi.nlm.nih.gov) (accession numbers listed in Table 1).

Alignments were performed using Clustal X (Thompson et al. 1997) using BioEdit (Hall 1999) and were checked by eye and adjusted when necessary (they are available upon request). Quick neighbor-joining analyses of similarity based on p-distances were performed under MEGA4 (Tamura et al. 2007). Phylogenetic analyses using parsimony were performed using PAUP4 (Swofford 2003). A neighbor-joining approach based on p-distances was also performed using PAUP4 to detect differences between clustering by global similarity and inference of clades (data not shown). In parallel, a bayesian phylogenetic approach was also used. MrBayes 3. 2 (Ronquist and Huelsenbeck 2003) was used with GTR as model, with the default settings for the priors for the proportion of invariable sites and for the gamma shape parameter. The analyses were run on all datasets (complete and reduced sampling, and all markers separately as well as concatenation), using the 1st, 2nd and 3rd codon positions as different partitions for the coding datasets, and with a single partition for Dloop. Four analyses were run for each dataset with the following parameters: four chains, 5 million generations, sampling of every 200th tree and discarding of the first 10% trees after checking the burn-in zone was included in this interval. After checking convergence had been reached, the trees and parameters resulting from the five analyses of a dataset were pooled and combined in a consensus.

Separate and simultaneous analyses (Nixon and Carpenter 1996; Lecointre and Deleporte 2005) have been performed using maximum parsimony (heuristic searches) in order to check for possible bias among individual markers (separate analyses: phylogenetic analyses performed on each genetic marker taken separately), to evaluate the overall robustness of the clades (simultaneous

Table 2 Primers used

Gene	Name	HT (°C)	Sequence (5′–3′)	References
Cytochrome Oxidase I	COIF1	52	TTCTCCAACCACAAAGACATTGGCAC	Ward et al. (2005)
	COIR1	52	ACGTGGGAGATAATTCCAAATCCTG	Ward et al. (2005)
Rhodopsin retrogene	Rhd193F	53	CNTATGAATAYCCTCAGTACTACC	Chen et al. (2003)
	Rhd1039R	53	TGCTTGTTCATGCAGATGTAGA	Chen et al. (2003)
Control region	L15926	52	TCAAAGCTTACACCAGTCTTGTAAACC	(this study)
	LPR02	52	AACTCCCACCACTAACTCCCAAAGC	Sanchez et al. (2007)
	H16498	52	CTTGAAGTAGGAACCAGATG	Derome et al. (2002)
	HDL2	52	AAGTAGGAACCAGATGCCAGNAAT	Sanchez et al. (2007)
Cytochrome b	CytbL650	50–60	AYAARTCTCNTTCCACCC	(this study)
	CytbL15026	50–60	CCGAGGVCTDTACGGCTC	Sanchez et al. (2007)
	S-CytL	50–60	TTTTGRGGYGCAACTGTAATTAC	Sanchez et al. (2007)
	CytbH1052	50–60	GAMGCRAYTTGGCCGATG	(this study)
	CytbH15915	50–60	TCTCCATTTCTGGTTTACAAGAC	(this study)
	Cytb-HNoto	50–60	CTCAGCTTMTTCCCCTYGCAG	(this study)

HT is the hybridization temperature

analysis: a single phylogenetic analysis of all the available data), and to better assess their reliability (Chen et al. 2003; Li and Lecointre 2009). Robustness was estimated both by a resampling method (bootstrap) and a non-resampling method (Bremer index). A first simultaneous analysis was performed on 77 individuals using a heuristic search (TBR search, 500 random addition sequences, characters unordered and unweighted). Most of the terminals are specimens of the same species that clearly group together. For resampling approaches such as bootstrap, uncertainties in relationships among closely related individuals of the same species having almost identical sequences add considerably to the calculation time. As resolution of intraspecific relationships was not our goal, and all included species were monophyletic in the analyses including all terminal taxa (see also Dettaï et al. 2010), a subset of three individuals per species has been chosen to calculate node robustness, especially for inter-specific nodes, in order to decrease the calculation time required for robustness analyses. We selected individual specimens in order to keep the maximum sequence divergence within species, calculated from the concatenated sequences. Such a secure decrease of sampling also allowed a branch and bound search for the final simultaneous analysis using parsimony. Table 3 summarizes information about the size of the data sets, their informative contents, as well as information about trees.

Results

All four markers analyzed separately provided parsimony or Bayesian (BA) trees where each species of *Artedidraco*, *Histiodraco* and *Dolloidraco* was monophyletic (except for the rhodopsin data set where the BA tree does not provide enough resolution). The molecular markers used here all agree on species delimitations. Species of the genus *Pogonophryne* are out of the scope of the present paper, but the genus appears monophyletic in all analyses with the present sampling whether with maximum parsimony or Bayesian analyses. The discrepancies among trees based on different genetic markers were not significant, i.e. provoked by non robust deeper nodes with very short branch length (data not shown, however, information about trees is given Table 3). Note that some data sets seem poorly informative such as the rhodopsin retrogene data set. However, that data set contains 18 informative positions with a C.I. of 0.87 and a R.I. of 0.98 (Table 3), which indicates that it does provide phylogenetic information. These numbers are the same in the 76 specimens tree and the 26 specimens tree (three specimens per species), which indicates that this phylogenetic information concerns interspecific relationships. Moreover, the three mitochondrial data sets belong to the same unit of inheritance whereas the rhodopsin retrogene is inherited independently since it is nuclear. The rhodopsin data set is therefore, important to take into account. The tree based on the combined data (Fig. 2) also shows that each species sampled here is monophyletic. Genera *Histiodraco*, *Dolloidraco* and *Pogonophryne* are well embedded within the genus *Artedidraco*. The topology is the same in BA analysis (data not shown). The tree based on the subsample of 27 specimens (Fig. 3) obtained from a branch and bound search allows estimation of the robustness of the deepest nodes of the tree. The grouping of *Histiodraco*, *Dolloidraco* with *A. shackletoni*, *A. mirus* and *A. orianae* is supported by a bootstrap proportion of 85% and a Bremer

Table 3 Information about the data sets and trees. "*n* terminals" stands for number of taxa, "*n* characters" stands for number of characters, "*n* constant" stands for number of constant characters, "*n* informative" stands for number of characters informative for parsimony, *n* trees stands for number of equiparsimonious trees, "tree length stands for the number of steps, "CI" stands for consistency index, "RI" stands for retention index

	n terminals	*n* characters	*n* constant	*n* informative	*n* trees	Tree length	CI	RI
Analyses of the complete taxonomic sampling								
COI	77	655	550	69	8	147	0.7619	0.9488
Cytb	77	577	443	91	2,872	224	0.6875	0.9364
Dloop	76	420	207	161	10,000	375	0.7493	0.9431
Rhodo	76	701	674	18	2	33	0.8788	0.9807
COI + Cytb + Dloop + Rhodo	77	2,553	1,874	339	10,000	796	0.7236	0.9396
	n terminals	*n* characters	*n* constant	*n* informative	*n* trees	Tree length	CI	RI
Analyses of the taxonomic sampling reduced to three specimen per species								
COI	27	655	558	67	8	129	0.7984	0.8903
Cytb	27	577	461	88	8	183	0.7268	0.8670
Dloop	26	420	209	149	20	334	0.8144	0.8901
Rhodo	26	701	676	18	1	31	0.8710	0.9481
COI + Cytb + Dloop + Rhodo	27	2,553	1,904	322	12	688	0.7776	0.8780

index of 5. The species *A. skottsbergi* is the first to diverge, then *A. loennbergi*, then *Pogonophryne*. *A. mirus* is the sister-species of *A. shackletoni* (BP of 97%, Bremer index of 7), however, the interrelationships of those two species with *Histiodraco* and *Dolloidraco* are not resolved. *A. orianae* is the sister-species of those four. The robustness of most of the clades in Fig. 3 is high. The topology is also found by BA trees (data not shown).

Discussion

Artedidraco as a stem group

As in Derome et al. (2002), the genus *Artedidraco* is paraphyletic in the present study. Two markers are shared between the two studies but two other markers and more specimens are added here. Neither the diagnosis by Balushkin (1992) nor the two characters given by Eakin (1981: 139) for the diagnosis of the genus *Artedidraco* actually were conceived as synapomorphies for the genus. Considering our topology, the "first dorsal fin above base of pectoral fin" and "more than three spines in the first dorsal fin" are to be considered as primitive conditions with regard to what is found in the three other genera. In the same way, the characters given for *Artedidraco* by Balushkin (2000) appear to be subject to parallelisms; and they are even polymorphic according to this author. These are "rows in interneuralia of the first dorsal fin and the second dorsal fin separated", "elongated rays on first dorsal fins", and "sharply elongated caudal fin rays". It is not surprizing to find the genus *Artedidraco* as a stem group: the genus has never been defined in terms of unambiguous synapomorphies like the family itself was. Actually, none of the four genera were cladistically defined. However, it is interesting to note that Lombarte et al. (2003) using morphological characters associated with the sensory organs and the size of the mouth, found it more difficult to characterize *Artedidraco* than any other genus of the family. Through an ecomorphological approach to the four genera, *Artedidraco* appears to be "*the least morphologically differentiated group*" with "*a less specialized diet, poorly developed barbels and medium-size eyes*", which is considered by most to be the characteristics of stem groups.

Homoplastic characters

According to Balushkin (2000), non homoplastic characters linking *Pogonophryne* and *Histiodraco* (Fig. 1) are "the head wide and flattened", the "presence of a post-temporal crest" and "two or three rows of teeth on the premaxillae". According to the same study there is a single non homoplastic character uniting *Dolloidraco* and *Artedidraco* which is "the reduced number of rays in pectoral fins to 13–18". All those characters appear to be convergences according to the tree presented (Fig. 3), however, further investigations should be carried out taking into account all species including the more recently described.

Trends within Artedidraco

The present tree leads to a new interpretation of the evolutionary trends of some features discussed by Eakin (1981) and Eastman and Eakin (1999). The number of

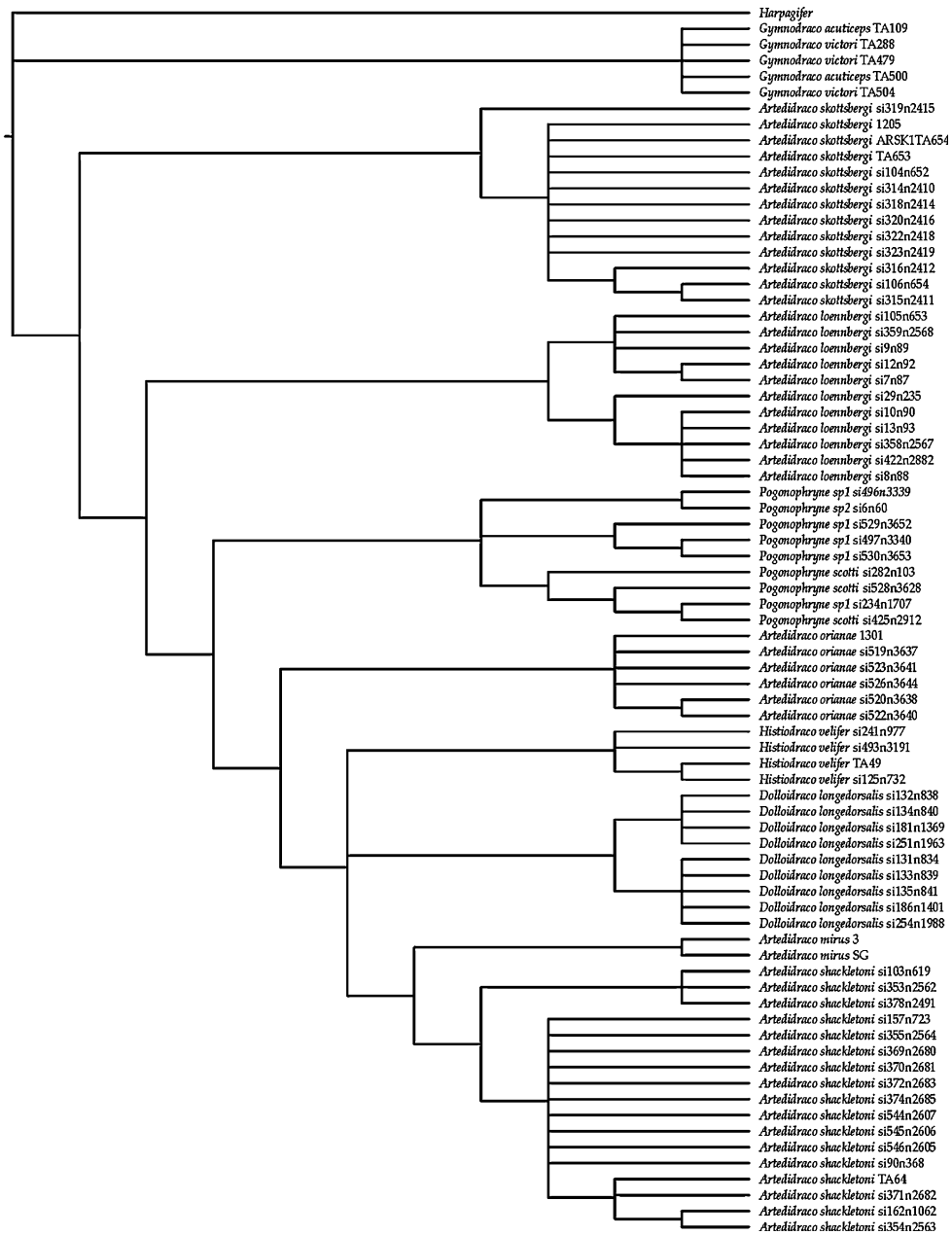

Fig. 2 Strict consensus tree of 10,000 most parsimonious trees of 796 steps (heuristic search with 500 random addition sequences) based on concatenated sequences (four markers). C.I. and R.I. are given in Table 3

upper lateral line tubular scales as sampled by Eastman and Eakin (1999) increases as species exhibit a more derived position in our trees (Fig. 3) from *Artedidraco skottsbergi* and *A. loennbergi* to *A. orianae* and *A. mirus*: they are 3–9 in the sample of Eastman and Eakin (1999) of *A. skottsbergi* with a mean of 4.55, 2–6 in *A. loennbergi* with a mean of 4.75, 11–17 in *A. shackletoni* with a mean of 13.38 and 18–22 in *A. orianae* with a mean of 20.8. The number of disc-shaped scales parallels our tree (Fig. 3) in decreasing from *A. skottsbergi* and *A. loennbergi* to *A. mirus* and *A. orianae*. The parhypural and the lower hypural plate are fused with the pleurostyle in *A. skottsbergi* and *A. loennbergi* and they are autogenous in *A. orianae* and *A. mirus*. The direction of the character transformations is reversed compared to the interpretation of Eakin (1981). Last, there is a trend toward the decrease of the number of epipleural ribs along the tree (from 15 in *A. skottsbergi* and 13–15 in *A. loennbergi* to 12 in *H. velifer* and *D. longedorsalis*), along with the increase of number of pleural ribs from none in *A. skottsbergi* to 8 in *A. orianae* and *A. mirus*. However, these last trends are difficult to interpret as there is considerable variation

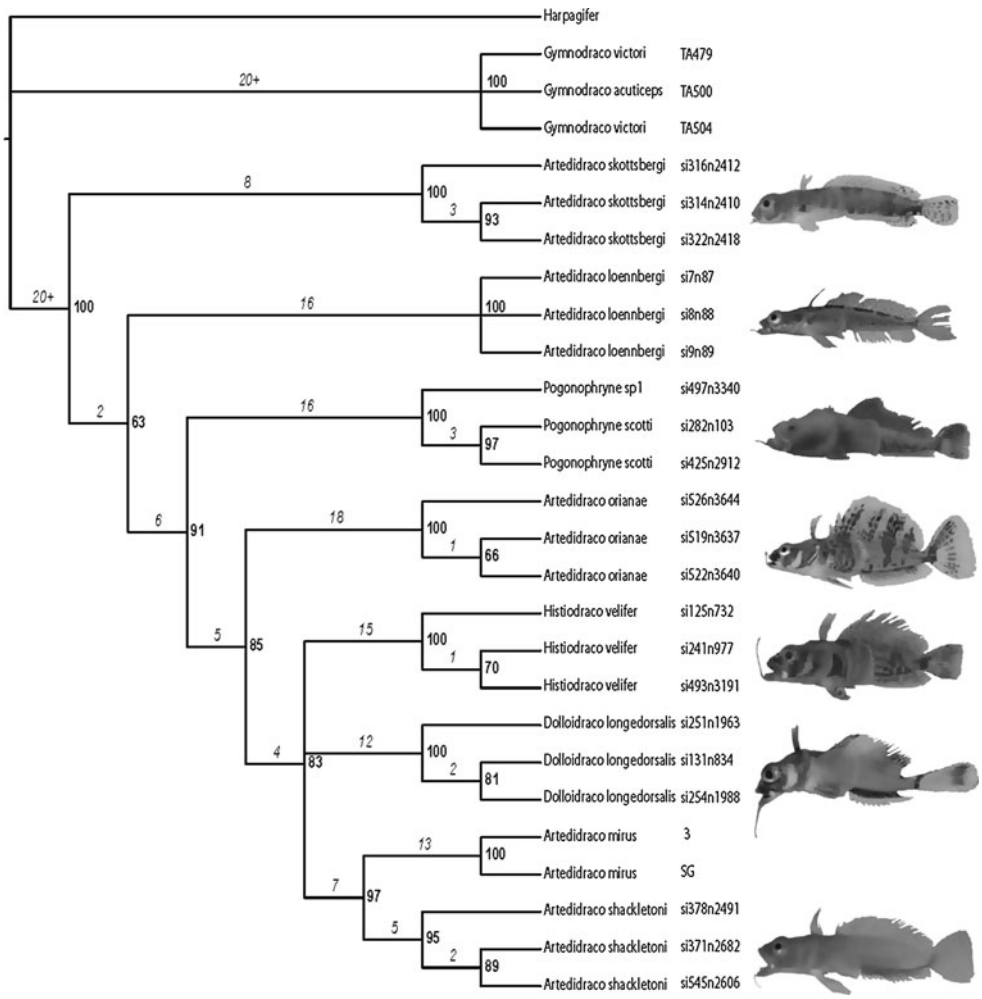

Fig. 3 Strict consensus of the 12 most parsimonious trees of length 688 steps (branch and bound search performed on a subset of 27 taxa, three individuals per species sampled for bootstrap proportions calculations). To the right of each node, in *bold*: bootstrap proportions in the maximum parsimony approach, above the node, in *italics*: Bremer index for the node. CI and RI are given Table 3. 20+ means that the Bremer index is higher than 20 steps. Photos credits: S. Iglésias/F. Busson/CEAMARC/MNHN

within the genus *Pogonophryne* (13–16 epipleural ribs and 0–8 pleural ribs, but see Eakin 1981; Eakin et al. 2009). In summary, additional studies are necessary in a phylogenetic framework to find morpho-anatomical synapomorphies supporting the nodes of the present tree. An answer cannot be directly found in Eakin (1981) or Eastman and Eakin (1999), unless by arbitrarily claiming, for instance, that the clade grouping *A. orianae* and *A. shackletoni* is supported by having a mean number of tubular upper lateral line scales of more than 10.

Data from cytogenetics

Data from artedidraconid cytogenetics do not include a sufficient number of taxa to allow an interpretation about the general evolutionary trend followed by chromosome formulae from the present tree (Fig. 3). The family is characterized by 46 chromosomes (while there are 48 chromosomes in harpagiferid species and channichthyid species, and a variable number in bathydraconid species, but never 46). The chromosomal formulae are variable within *Artedidraco* species and very stable within *Pogonophryne* species karyotyped to date (suggesting the very close relatedness of the *Pogonophryne* species). Unfortunately chromosome formulae are only known from species of the crown group of our tree, not from the species that were the first to diverge. Known formulae are those of *A. mirus* (2n = 46, 2 m + 2sm + 42a) (Prirodina 1995), *A. shackletoni* (2n = 46, 2 m + 6sm + 38a) (Ozouf-Costaz et al. 1991), *A. orianae* (2n = 46, 2 m + 6sm + 38a) (Ozouf-Costaz et al. 1991), *Pogonophryne* (2n = 46, 2 m + 4sm + 40a) (*P. barsokovi*, *P. marmorata*, *P. scotti*, *P. mentella* in Ozouf-Costaz et al. 1991), and *Histiodraco velifer* (2n = 46, 6sm + 40a) (Caputo et al. 2003). Without knowing the formulae in *A. skottsbergi* and *A. loennbergi*, it is difficult to infer the plesiomorphic condition in the family and to draw any evolutionary trend. In the present trees neither sister-species nor sister-groups share the same formulae, suggesting that each formula in each species was independently acquired, therefore made of non-homologous chromosomal segments (except maybe within *Pogonophryne*). This suggests an interesting venue

for further studies using chromosomal paints. Chromosomal paints use marked chromosomes from one species as probes for marking karyotypes of target species. This allows to identify correspondences in chromosomal segments in different species that are not visible through classical karyotype colorations.

Artedidraconids as a species flock?

According to the present tree, no characteristics are historically shared among species in terms of geographic distribution or depth range as recorded by La Mesa et al. (2006) or by Eakin (1990). In their barcoding approach, Rock et al. (2008) had no species-specific clusters among the artedidraconid species they sampled. From a different sampling Dettaï et al. (2010) found unique molecular clusters for each artedidraconid species with the same marker, except for some unclear cases in the *Pogonophryne* species. Both studies pointed out very low interspecific divergences within the family, even if most intraspecific distances were lower than the interspecific distances in Dettaï et al. (2010). The interspecific divergence were considerably smaller than the divergence found by Steinke et al. (2009) and they were far from the tenfold difference between intra and interspecific divergences suggested by Ward et al. (2009). Interestingly, the other markers used here provided the same groupings of individuals as the COI and the morphologically identified species, suggesting that the currently valid artedidraconid species (at least the ones included in the present study) are indeed separate genetic units. Eakin et al. (2009) also noticed from ND2 mitochondrial gene sequences that *Pogonophryne* species formed separate genetic units with very low divergences among them (maximum of 1.4%). The low genetic divergence among artedidraconid species can now be considered as a confirmed fact and could then be charted as a lack of variability of the COI gene and the other markers due either to a very recent divergence time or a slowdown in DNA rate of change. The later option is less likely because it would imply an overall slowdown of the four markers (mitochondrial and nuclear). The most recent estimate for divergence time is also in favor of the recent divergence. Near (2004, 2009) placed the divergence of the family from harpagiferids at 12.7 Mya and the divergence *Histiodraco/Pogonophryne* around 2.5 Mya. These tentative estimates of the divergence times are compatible with the estimates of Bargelloni et al. (2000). Lombarte et al. (2003) stressed that the ecomorphological diversification of artedidraconids should have taken place through a rapid adaptive evolution, by a process of sympatric or microvicariant speciation as suggested by Bargelloni et al. (2000) who noticed the high degree of sympatry in each of the recent taxonomic components of the benthic species of Artedidraconidae, Trematominae and Channichthyidae. Pliocene (4.8–2.5 Mya) episodes of partial deglaciation could have played a role in modifying benthic coastal habitats, promoting micro-vicariant speciations followed by dispersions. This hypothesis could explain, within artedidraconids, the absence of interspecific large-scale historically organized extrinsic traits such as depth ranges, geographic distributions or ecological niches. This partly corresponds to the definition of a species-flock, and some components of the family—at least the crown group *Histiodraco/Dolloidraco/Artedidraco*, as well as the *Pogonophryne* species studied to date (Lombarte et al. 2003; Eakin et al. 2009)- could be small species "sub-flocks" within the giant notothenioid flock proposed by Eastman and McCune (2000). But to be able to consider the artedidraconids as a flock, the ecological parameter in the definition of a flock would need to be relaxed: the benthic artedidraconids seem less ecologically diverse (Eastman 1993; Lombarte et al. 2003) than the other coastal taxonomic components of roughly the same age, such as trematomines or channichthyids.

Acknowledgments We thank Lukas Rüber, Rafael Zardoya and Luca Bargelloni for sample exchanges. We thank the crew and participants of the cruises involved in the capture of the samples, and especially the chief scientists of the CAML-CEAMARC cruises: G. Hosie, T. Ishimaru, M. Riddle, M. Stoddart. The CAML-CEAMARC cruises of R/V "Aurora Australis" and R/V "Umitaka maru" (IPY project no 53) were supported by the Australian Antarctic Division, the Japanese Science Foundation, the French polar institute "Institut Paul-Emile Victor" (and the IPEV program 281 "ICOTA" co-directed by Catherine Ozouf-Costaz and Philippe Koubbi). Programs associated were supported by the Centre National pour la Recherche Scientifique, the Muséum national d'Histoire naturelle and the Agence Nationale pour la Recherche (Projet Blanc "ANTFLOCKS" USAR no 07-BLAN-0213-01 directed by Guillaume Lecointre). This project is a contribution to the EBA-SCAR program. The present work was also partly based on samples collected during the ICEFISH 2004 cruise (supported by National Science Foundation grant OPP 01-32032 to H. William Detrich (Northestern University)) and the EPOS cruise (1989) for which we are grateful to the Alfred Wegener Institute and the European Science Foundation. This work was also supported by the «Service de Systématique Moléculaire» of the Muséum National d'Histoire Naturelle (UMS 2700 CNRS) and the «Consortium National de Recherche en Génomique» : it is part of the agreement number 2005/67 between the Genoscope and the Muséum National d'Histoire Naturelle on the project 'Macrophylogeny of life' directed by Guillaume Lecointre. We thank Joseph T. Eastman, Craig Marshall and an anonymous reviewer for constructive remarks.

References

Balushkin AV (1992) Classification, phylogenetic relationships, and origins of the families of the suborder notothenioidei (Perciforms). J Ichthyol 32:90–110

Balushkin AV (1999) *Pagonophryne eakini* sp. nova (Artedidraconidae, Notothenioidei, Perciformes): a new species of plunderfish from the Antartica. J Ichthyol 39:799–802

Balushkin AV (2000) Morphology, classification, and evolution of notothenioid fishes of the Southern Ocean (Notothenioidei, Perciformes). J Ichthyol 40:S74–S109

Balushkin AV, Eakin RR (1998) A new toad plunderfish *Pogonophryne fusca* sp. nova (Fam. Artedidraconidae: Notothenioidei) with notes on species composition and species groups in the genus Pogonophryne Regan. J Ichthyol 38:574–579

Bargelloni L, Marcato S, Zane L, Patarnello T (2000) Mitochondrial phylogeny of notothenioids: a molecular approach to antarctic fish evolution and biogeography. Syst Biol 49:114–129

Caputo V, Splendiani A, Nisi Cerioni P, Olmo E (2003) The chromosomal complement of the artedidraconid fish *Histiodraco velifer* (Perciformes: Notothenioidei) from Terra Nova Bay, Ross Sea. Cytogenet Genome Res 101:29–32

Chen WJ, Bonillo C, Lecointre G (2003) Repeatability of clades as a criterion of reliability: a case study for molecular phylogeny of Acanthomorpha (Teleostei) with larger number of taxa. Mol Phylogenet Evol 26:262–288

Derome N, Chen WJ, Dettaï A, Bonillo C, Lecointre G (2002) Phylogeny of Antarctic dragonfishes (Bathydraconidae, Notothenioidei, Teleostei) and related families based on their anatomy and two mitochondrial genes. Mol Phylogenet Evol 24:139–152

Dettaï A, Lautrédou AC, Bonillo C, Goimbault E, Busson F, Causse R, Couloux A, Cruaud C, Duhamel G, Denys G, Hautecoeur M, Iglesias S, Koubbi P, Lecointre G, Moteki M, Pruvost P, Tercerie S, Ozouf-Costaz C (2010) The actinopterygian diversity of the CEAMARC cruises: barcoding and molecular taxonomy as a multi-level tool for new findings. Deep Sea Res II 58:250–263

Eakin RR (1981) Osteology and relationships of the fishes of the antarctic family harpagiferidae (Pisces, Notothenioidei). In: Kornicker LS (ed) Biology of the antarctic seas IX, antarctic research series 31, paper 3. American Geophysical Union, Washington, pp 81–147

Eakin RR (1990) Artedidraconidae. In: Gon O, Heemstra PC (eds) Fishes of the Southern ocean. J.L.B. Smith Institute of Ichthyology, Grahamstown, South Africa, pp 332–356

Eakin RR, Balushkin AV (1998) A new species of toadlike plunderfish *Pogonophryne orangienisis* sp. nova (Artedidraconidae, Notothenioidei) from the Weddell Sea, Antarctica. J Ichthyol 38:800–803

Eakin RR, Eastman JT (1998) New species of *Pogonophryne* (Pisces, Artedidraconidae) from the Ross Sea, Antarctica. Copeia 1998(4):1005–1009

Eakin RR, Eastman JT, Vacchi M (2006) Sexual dimorphism and mental barbel structure in the South Georgia plunderfish *Artedidraco mirus* (Perciformes: Notothenioidei: Artedidraconidae). Polar Biol 30:45–52

Eakin RR, Eastman JT, Matallanas J (2008) New species of *Pogonophryne* (Pisces, Artedidraconidae) from the Bellingshausen Sea, Antarctica. Polar Biol 31:1175–1179

Eakin RR, Eastman JT, Near TJ (2009) A new species and a molecular phylogenetic analysis of the Antarctic fish genus *Pogonophryne* (Notothenioidei: Artedidraconidae). Copeia 4:705–713

Eastman JT (1993) Antarctic fish biology. Academic Press, San Diego

Eastman JT, Eakin RR (1999) Fishes of the genus *Artedidraco* (Pisces, Artedidraconidae) from the Ross Sea, Antarctica, with the description of a new species and colour morph. Antarct Sci 11:13–22

Eastman JT, McCune AR (2000) Fishes on the Antarctic continental shelf: evolution of a marine species flock? J Fish Biol 57:84–102

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–98

La Mesa M, Cattaneo-Vietti R, Vacchi M (2006) Species composition and distribution of the Antarctic plunderfishes (Pisces, Artedidraconidae) from the Ross Sea off Victoria Land. Deep Sea Res II 53:1061–1070

Lecointre G, Deleporte P (2005) Total evidence requires exclusion of phylogenetically misleading data. Zool Scripta 34:101–117

Li B, Lecointre G (2009) Formalizing reliability in the taxonomic congruence approach. Zool Scripta 38:101–112

Lombarte A, Olaso I, Bozzano A (2003) Ecomorphological trends in the Artedidraconidae (Pisces: Perciformes: Notothenioidei) of the Weddell Sea. Antarct Sci 15:211–218

Near TJ (2004) Estimating divergence times of notothenioid fishes using a fossil-calibrated molecular clock. Antactic Sci 16:37–44

Near TJ (2009) Notothenioid fishes (Notothenioidei). In: Hedges SB, Kumar S (eds) The timetree of life. Oxford University Press, New York, pp 339–343

Near TJ, Cheng CC (2008) Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): Inferences from mitochondrial and nuclear gene sequences. Mol Phylogenet Evol 47:832–840

Near TJ, Pesavento JJ, Cheng CC (2004) Phylogenetic investigations of Antarctic notothenioid fishes (Perciformes: Notothenioidei) using complete gene sequences of the mitochondrial encoded 16S rRNA. Mol Phylogenet Evol 32:881–891

Nixon KC, Carpenter JM (1996) On simultaneous analysis. Cladistics 12:221–241

Ozouf-Costaz C, Hureau JC, Beaunier M (1991) Chromosome studies on fish of the suborder Notothenioidei collected in the weddell sea during EPOS 3 cruise. Cybium 15:271–289

Papetti C, Lio P, Rüber L, Patarnello T, Zardoya R (2007) Antarctic fish mitochondrial genomes lack *ND6* Gene. J Mol Evol 65:519–528

Prirodina VP (1995) Chromosomal complement of *Artedidraco mirus* and *Pogonophryne dolichobranchiata* (Artedidraconidae, Notothenioidei) from the Scotia Sea. J Ichthyol 35:295–399

Ritchie PA, Lavoué S, Lecointre G (1997) Molecular phylogenetics and the evolution of Antarctic notothenioid nishes. Comp Biochem Physiol 118A(4):1009–1027

Rock J, Costa FO, Walker DI, North AW, Hutchinson WF, Carvalho GR (2008) DNA barcodes of fish of the Scotia Sea, Antarctica indicate priority groups for taxonomic and systematics focus. Antarct Sci 20:253–262

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12):1572–1574

Sanchez S, Dettaï A, Bonillo C, Ozouf-Costaz C, Detrich III W, Lecointre G (2007) Molecular and morphological phylogenies of the Antarctic teleostean family Nototheniidae, with emphasis on the Trematominae. Polar Biol 30:155–166

Steinke D, Zemlak TS, Boutillier JA, Hebert PD (2009) DNA barcoding of Pacific Canada's fishes. Mar Biol 156:2641–2647

Swofford D (2003) PAUP*, phylogenetic analysis using parsimony (*and other methods), Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Thompson JD, Gibson TJ, Pewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PD (2005) DNA barcoding Australia's fish species. Philos Trans Royal Soc Lond B Biol Sci 360:1847–1857

Ward RD, Hanner R, Hebert PD (2009) The campaign to DNA barcode all fishes. FISH-BOL. J Fish Biol 74:329–356

Winnepenninckx B, Backeljau T, Wachter RD (1993) Extraction of high molecular weight DNA from molluscs. T I G 9:407