Naked dragonfishes *Gymnodraco acuticeps* and *G. victori* (Bathydraconidae, Notothenioidei) off Terre Adélie are a single species

by

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ABSTRACT. - Taxonomists have generally considered the two bathydraconid species *Gymnodraco acuticeps* Boulenger, 1902 and *Gymnodraco victori* Hureau, 1963 as synonymous based on field experience or from personal communications. However, no study has been devoted to that question and here we address it using three lines of evidence: comparison of variable DNA sequences, morphology and cytogenetics. We sequenced four molecular markers for a large sampling to test the delimitation between the two species from individuals caught off Terre Adélie (type locality of *G. victori*); among them is the cytochrome oxidase I (COI) gene, for which a large reference database is available in the Barcode of Life project for many taxa. However it contains only two *Gymnodraco acuticeps* sequences and none from *G. victori*. The karyotype of *G. victori* was obtained and compared with the one from *G. acuticeps*. From specimens from the MNHN collections, we recorded the two external characters most commonly used in the field to assign individuals to these species. The maximum divergence recorded in the COI sequences between two *Gymnodraco* specimens is 0.005% (K2P,) or 0.03% (p-distance), and there are no distinct clusters separating the two morphological entities with any of the four markers: all sequences were identical or nearly identical. No difference was found between the karyotypes of the two species. When a large number of specimens are studied, the pattern of the anterior canine teeth of the lower jaw and the other most visible external features used to distinguish the species by Hureau (1963) are actually more variable than originally described. We place *G. victori* and *G. acuticeps* into synonymy, the former being a junior synonym of *G. acuticeps*.

RÉSUMÉ. - Les dragons nus *Gymnodraco acuticeps* et *G. victori* (Bathydraconidae, Notothenioidei) de Terre Adélie sont une seule espèce.

Les taxinomistes ont généralement considéré les deux espèces de bathydraconidés *Gymnodraco acuticeps* Boulenger, 1902 et *Gymnodraco victori* Hureau, 1963 comme synonymes sur la base de leur expérience du terrain ou de communications personnelles. Cependant aucune étude n'a été dévolue à cette question ; et celle-ci est étudiée ici grâce à la conjonction de trois types de données : la comparaison de séquences d'ADN de gènes variables, la morphologie et la cytogénétique. Nous avons séquencé quatre marqueurs moléculaires pour un large échantillonnage de spécimens afin d'évaluer la délimitation entre les deux espèces à partir d'exemplaires capturés près de la Terre Adélie, la localité type de *G. victori*. Parmi ces marqueurs figure le gène de la cytochrome oxidase I (COI) pour lequel une large base de données de référence est disponible pour de nombreux taxons dans le cadre du projet Barcode of Life. Cependant elle ne contient que deux séquences de *Gymnodraco acuticeps* et aucune de *G. victori*. De plus, le caryotype de *G. victori* a été obtenu afin de le comparer à celui de *G. acuticeps*. Enfin, nous avons étudié les états des deux caractères les plus utilisés sur le terrain pour assigner les individus aux espèces, à partir des spécimens disponibles dans les collections du MNHN. La divergence maximale trouvée entre séquences de la COI de deux individus de *Gymnodraco* est de 0,005% (K2P,) ou 0,03% (p-distance), et les analyses de similitude globale des séquences ne montrent aucun regroupement des individus de chacune des deux espèces, et ceci quel que soit le marqueur. En fait, pour tous les marqueurs étudiés, les séquences sont toutes identiques ou quasi-identiques. Il n'y a pas de différences non plus dans les caryotypes des deux espèces. Les caractéristiques externes, notamment le patron des dents antérieures de la mandibule, sont plus variables que ce qui a été décrit originalement (Hureau, 1963). Nous confirmons que *G. victori* est un synonyme plus récent de *G. acuticeps*.

Key words. - Bathydraconidae - *Gymnodraco* - East Antarctic - Terre Adélie.

The two bathydraconid species *Gymnodraco acuticeps* Boulenger, 1902 and *Gymnodraco victori* Hureau, 1963 were first distinguished in 1963 in the description of *G. victori* by J.C. Hureau, from specimens caught close to Terre Adélie (East Antarctic). The two species were separated on the basis of the arrangement of the anterior canine teeth of the lower jaw, the otolith morphology, the vertebral count, the lengths of the upper jaw, snout and dorsal fin base, the predorsal distance, the position of the nostril and more questionably, the colour patterns. The relative size of the lower canine teeth and the length of the upper jaw were probably the characters that have been used the most in the field, as they are exter-
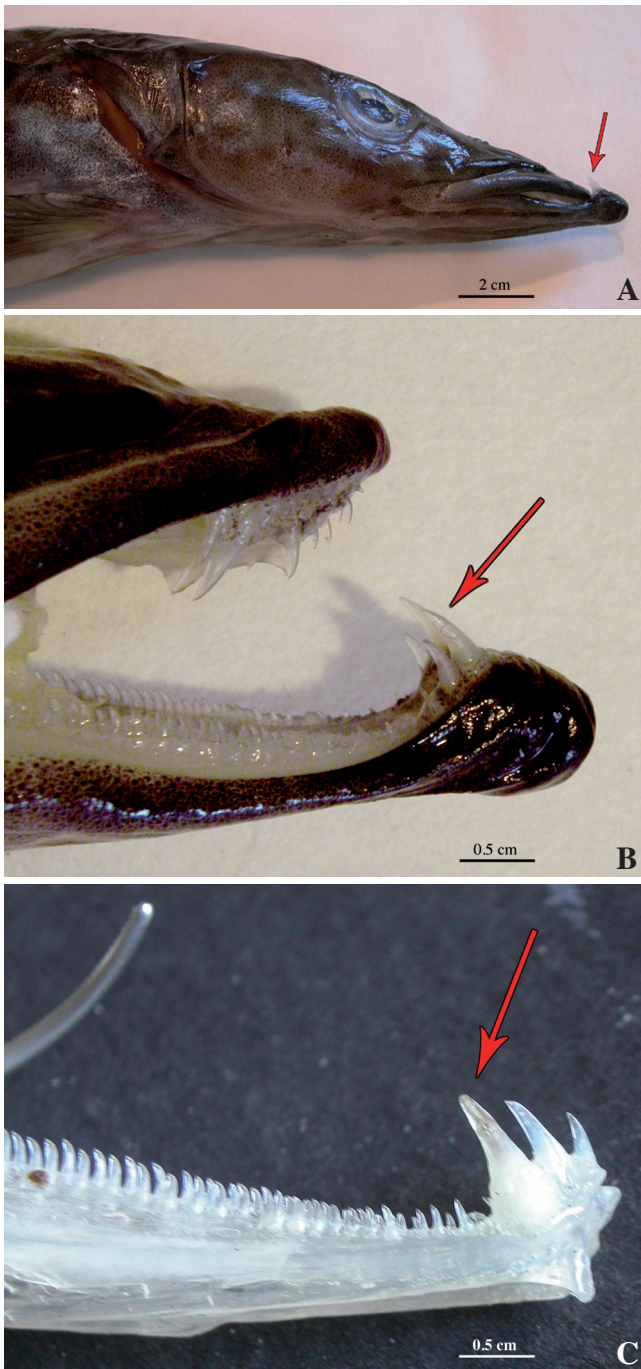
Figure 1. - **A**: Typical head of *Gymnodraco victori* as described by Hureau (1963), with the posterior part of the maxillae ending in front of the eye and the most anterior canine of the lower jaw, which is the strongest (arrow). **B**: Teeth of the canine group of the lower jaw as exhibited by *G. victori*, according to Hureau (1963). There are two large canines and the most anterior is the strongest (arrow). **C**: Teeth of the canine group of the lower jaw as exhibited by *G. acuticeps*, according to Hureau (1963). There are more than two canines and the canine of the rear is the strongest (arrow). Photos G. Lecointre.

nally visible features (Hureau, 1987: 259; Fig. 1), and are less difficult to estimate than the relative position of the nostril or the colour pattern that both show only slight differences. *G. victori* was described as having two canine teeth on the anterior end of the dentary, the strongest being the front one (Hureau, 1963: 23; Fig. 1B). In this species, the maxillary bone – actually the upper jaw – was described as ending posteriorly under the anterior half of the eye (Hureau 1963). *G. acuticeps,* on the other hand, was characterized as having three canine teeth on the anterior end of the dentary (four in Hureau, 1987), the strongest being the rear one (Hureau, 1963: 23; Fig. 1C), and the upper jaw posteriorly ending under the posterior half of the eye. However Gon (1990: 373) suspected *G. victori* to be a junior synonym of *G. acuticeps*: he found "*no consistency in the arrangement of the canines of the lower jaw. The largest tooth may be either in the front or the rear of the canine group and the arrangement can differ from one side of the jaw to the other of the same fish. The vertebral count and morphometric characters of* G. victori *were within the range found in* G. acuticeps". Gon (1990) also mentioned greater similarities in the otolith morphology between species than within species. As a result *G. victori* was frequently not recorded as a distinct species in inventories of notothenioid diversity [Kock, 1992; Eastman, 1993; Eastman and Eakin, 2000; Fishbase (http://www.fishbase.org)], and was never recorded from other parts of the Southern Ocean. However, none of these inventories provide a study designed to justify this position. Only Miller (1993: 383) mentioned Gon (1990) and a personal communication from DeWitt about the potential synonymy, while provisionally maintaining a page for *G. victori* "*until this can be determined from a study of a more adequate series*".

The aim of the present work is to investigate the species delineation between the two taxonomic entities by using molecular markers. Cytochrome oxidase I (COI) gene has been described as a suitable marker for species delimitation in most of the fish species sampled with several specimens per species (Ward *et al*., 2005, 2009), and especially within notothenioids (Dettaï *et al*., 2011). However, only two sequences are currently available in the Barcode of Life Database (BOLD, Ratnasingham and Hebert, 2007). Several other markers were also used to complete this molecular taxonomy study. Moreover, the karyotype of *G. victori* was obtained in order to check for differences between the two species. As a result, it appears that the two taxonomic entities cannot be distinguished: all genetic markers, including cytochrome oxidase I and the more rapidly evolving d-loop, exhibited identical or almost identical sequences between specimens of *G. acuticeps* and *G. victori*. Karyotypes are identical as well. In all kind of data, including canine teeth patterns, the slight differences found are more in line with intraspecific variability than with interspecific differences.

Table I. - Sampling data. All samples of *Gymnodraco* used for the molecular study were caught near the coast of the French station Dumont d'Urville or off Terre Adélie except TNB. The place and date of catch are associated with the voucher specimen in the collection databases (for MNHN: http://coldb.mnhn.fr/colweb/form.do?model=GICIM. wwwichtyo.wwwichtyo.wwwichtyo), except for: b: provided by Luca Bargelloni, d: provided by Rafael Zardoya, same place as "d" in the table I of Papetti *et al.* (2007). For each of the four genes sequenced, the amplicon tag and the BOLD accession number of the corresponding sequence are given. For each specimen, the number of canine teeth is given, if necessary with precision regarding the left (L) and the right (R) sides of the lower jaw. The strongest canine tooth is given (according to Hureau (1963) the typical formula for *G. acuticeps* is then 3-3 and the one for *G. victori* is 2-1). The position of the posterior end of the maxillary is indicated (either in front of the eye (A) or in the posterior part of the eye (P) as drawn in Hureau, 1987).

Genus	Species	Field tag	Voucher number	COI PCR tag and accession nb	Rh PCR tag and accession nb	dloop PCR tag and accession nb	Cytb PCR tag and accession nb	Fang nb	Strongest	Mx end Ant/Post	Chromosome preparation	Sex
Gymnodraco	victori	TA341GYVI1		613/EATF611-11	260/EATF611-11	754/EATF611-11	634/EATF611-11					
Gymnodraco	victori	TA341GYVI2			288/EATF612-11	755/EATF612-11	708/EATF612-11					
Gymnodraco	victori	TA341GYVI1		615/-	262/-	756/-	635/-					
Gymnodraco	victori	TA341GYVI2		832/EATF612-11	289/-	757/-	707/-					
Gymnodraco	victori	TA382GYVI1	MNHN 2009-0658	617/EATF613-11	293/EATF613-11	758/EATF613-11	710/EATF613-11	broken				
Gymnodraco	victori	TA382GYVI2	MNHN 2009-0659	618/EATF614-11	265/EATF614-11	759/EATF614-11	636/EATF614-11	1 L 3 R	3	A		
Gymnodraco	victori	TA381GYVI1	MNHN 2009-0657	619/EATF615-11	266/EATF615-11	760/EATF615-11	637/EATF615-11	3	3	A		
Gymnodraco	victori	TA384GYVI1	MNHN 2009-0660	406/EATF616-11	482/EATF616-11	725/EATF616-11	642/EATF616-11	2	1	A		
Gymnodraco	victori	TA388GYVI1	MNHN 2009-0661	424/EATF617-11	470/EATF617-11	742/EATF617-11	679/EATF617-11	2	1 L 2 R	A		
Gymnodraco	victori	TA388GYVI2	MNHN 2009-0662	620/EATF618-11	267/EATF618-11	768/EATF618-11	761/EATF618-11	3	3	A		
Gymnodraco	victori	TA388GYVI3	MNHN 2009-0663	415/EATF619-11	606/EATF619-11	734/EATF619-11	671/EATF619-11	2	1 L 2 R	A		
Gymnodraco	victori	TA390GYVI1	MNHN 2009-0664	436/EATF620-11	451/EATF620-11	723/EATF620-11	667/EATF620-11	2	2	A		
Gymnodraco	victori	TA390GYVI2	MNHN 2009-0665	416/EATF621-11	607/EATF621-11	735/EATF621-11	672/EATF621-11	2	2	A	yes	f
Gymnodraco	victori	TA391GYVI1	MNHN 2009-0667	806/EATF622-11	460/EATF622-11	732/EATF622-11	669/EATF622-11	1	1	A	yes	f
Gymnodraco	victori	TA391GYVI2	MNHN 2009-0666	428/EATF623-11	515/EATF623-11	746/EATF623-11	683/EATF623-11	2	equal L 2 R	A	yes	m
Gymnodraco	victori	TA396GYVI1	MNHN 2009-0668	405/EATF624-11	604/452/EATF624-11	724/EATF624-11	641/EATF624-11	1	1	A		
Gymnodraco	victori	TA409GYVI1	MNHN 2009-0669	410/EATF625-11	457/EATF625-11	729/EATF625-11	646/EATF625-11	2	1	A	yes	f
Gymnodraco	victori	TA255GYVI1	MNHN 2002-1709	809/EATF626-11	610/EATF626-11	741/EATF626-11	678/EATF626-11				yes	f
Gymnodraco	victori	TA03GYVI1		408/EATF627-11	784/EATF627-11	727/EATF627-11	644/EATF627-11					
Gymnodraco	victori	TA63GYVI1	MNHN 2001-1137	418/EATF628-11	465/EATF628-11	737/EATF628-11	674/EATF628-11					
Gymnodraco	victori	TA49GYVI1	MNHN 2001-1136	414/EATF629-11	484/EATF629-11	733/EATF629-11	670/EATF629-11					
Gymnodraco	victori	TA479GYVI1	MNHN 2009-0672	446/EATF630-10	517/EATF630-10	750/EATF630-10	687/EATF610-10				yes	f
Gymnodraco	victori	TA473GYVI1		447/EATF630-11	518/EATF630-11	751/EATF630-11	688/EATF630-11				yes	f
Gymnodraco	victori	TA484GYVI1		429/EATF631-11	475/516/EATF631-11	747/EATF631-11						
Gymnodraco	victori	TA504GYVI1	MNHN 2009-0865	434/EATF601-10	480/EATF601-10	752/EATF601-10	689/EATF601-10				yes	f
Gymnodraco	victori	TA500GYVI1	MNHN 2009-0867	445/EATF600-10	477/EATF600-10	749/EATF600-10	686/EATF600-10				yes	f
Gymnodraco	victori	TA481GYVI1			476/EATF632-11	748/EATF632-11	685/EATF632-11					
Gymnodraco	victori	TA461GYVI1	MNHN 2006-0131		519/EATF633-11	753/EATF633-11	690/EATF633-11	2	1	A		
Gymnodraco	victori	TA582GYVI1		contigTA582/EATF634-11	contigTA582/EATF634-11	contigTA582/EATF634-11	contigTA582/EATF634-11					
Gymnodraco	victori	TA658GYVI1	MNHN 2009-1085	contigTA658/EATF635-11	contigTA658/EATF635-11	contigTA658/EATF635-11	contigTA658/EATF635-11	1	1	A	yes	f
Gymnodraco	acuticeps	TA288GYAC1	MNHN 2009-0655	822/EATF598-10	483/EATF598-10	726/EATF598-10	643/EATF598-10	3	3	A	yes	f
Gymnodraco	acuticeps	TA10GYAC1	MNHN 2001-1135	412/EATF636-11	459/EATF636-11	731/EATF636-11	648/EATF636-11					
Gymnodraco	acuticeps	TNB	MNHN 1999-0373	440/EATF637-11	608/EATF637-11	738/EATF637-11	675/EATF637-11					
Gymnodraco	acuticeps	TA10GYAC1	MNHN 2001-1135	808/-	467/-	739/-	676/-					
Gymnodraco	acuticeps	TA10GYAC1	MNHN 2001-1135	421/-	609/-	740/-	677/-					
Gymnodraco	acuticeps	TA10GYAC1	MNHN 2001-1135	425/-	611/-	743/-	680/663/-					
Gymnodraco	acuticeps	TA109	MNHN 2007-0255	626/824/EATF597-10	268/294/605/EATF597-10	728/769/EATF597-10	645/711/762/EATF597-10				yes	f
Outgroups:												
Artedidraco	shackelloni	TA64ARSH1	MNHN 2001-1133	825/EATF602-10	473/EATF602-10	745/EATF602-10	875/EATF602-10					
Artedidraco	skottsbergi	TA653ARSP	0	866/EATF603-10	860/EATF603-10	864/EATF603-10	868/EATF603-10					
Artedidraco	skottsbergi	TA654ARSK1	0	867/EATF604-10	861/EATF604-10	865/EATF604-10	869/EATF604-10					
Artedidraco	skottsbergi	TNB230	MNHN 1999-0367		886/EATF638-11	744/EATF638-11	881/EATF638-11					
Artedidraco	skottsbergi	1205	d	827/EATF607-10	291/EATF607-10	770/EATF607-10	873/EATF607-10					
Artedidraco	mirus	SG241	SAIAB 74945	830/EATF609-10	290/273/EATF609-10	834/EATF609-10	843/EATF609-10					
Artedidraco	mirus	3	b	823/EATF606-10		736/878/EATF606-10	880/EATF606-10					

Table I. - Continued.

Genus	Species	Field tag	Voucher number	COI PCR tag and accession nb	Rh PCR tag and accession nb	dloop PCR tag and accession nb	Cytb PCR tag and accession nb	Fang nb	Strongest	Mx end Ant/Post	Chromosome preparation	Sex
Artedidraco	*orianae*	1301	d	828/EAITF608-10	876/EAITF608-10	771/EAITF608-10	840/EAITF608-10					
Complement:												
Gymnodraco	*acuticeps*		MNHN 1963-0064					3	3	A		
Gymnodraco	*victori*		MNHN 1963-0065					2 L 1 R	1	A		
Gymnodraco	*victori*		MNHN 1963-0066					2	1	A		
Gymnodraco	*acuticeps*		MNHN 1963-0067					3	3	A		
Gymnodraco	*acuticeps*		MNHN 1963-0068					3	3	A		
Gymnodraco	*victori*		MNHN 1962-0792					2	1	A		
Gymnodraco	*acuticeps*		MNHN 1965-0490					2	1	A		
Gymnodraco	*acuticeps*		MNHN 1965-0491					2	1	A		
Gymnodraco	*acuticeps*		MNHN 1965-0492					2	2	A		
Gymnodraco	*acuticeps*		MNHN 1982-1269					2	1	A		
Gymnodraco	*acuticeps*	178-EPOS3	MNHN 1990-1195					3 L 2 R	1	A		
Gymnodraco	*acuticeps*	178-EPOS3	MNHN 1990-1196					2 L 3 R	2 L 3 R	A		
Gymnodraco	*acuticeps*	178-EPOS3	MNHN 1990-1197						1	A		
Gymnodraco	*acuticeps*	188-ICEFISH	MNHN 1999-0373					1		A		
Gymnodraco	*acuticeps*	188-ICEFISH	MNHN 1999-0374					2		A		
Gymnodraco	*acuticeps*	188-ICEFISH	MNHN 1999-0375					1	1	A		
Gymnodraco	*acuticeps*	2401	MNHN 2009-1083					2	1	A		

All these elements collectively confirm that *G. victori* should indeed be considered a junior synonym of *G. acuticeps*.

MATERIALS AND METHODS

Table I presents the sampling for the molecular study, primarily based on specimens of *G. acuticeps* and *G. victori* collected off Terre Adélie or near the coast of the French station Dumont d'Urville (type-locality of *G. victori*). One sample was collected in Terra Nova Bay in the Ross Sea. Precise locations of catches are available in the database of the ichthyological collection of the Muséum national d'Histoire naturelle (MNHN), from which numbers are provided.

For each sample, 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 a classical CTAB protocol with a chloroform isoamylalcohol step (Winnepenninckx *et al*., 1993). 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 of the two primers at 10 pM (Tab. II); 1 μL of DNA extract was added. After denaturation for 2 min, the PCR was run for 40 to 50 cycles of (20 s, 94°C; 20 s, hybridisation temperature, see table II; 50 s to 1 min 10 s, 72°C) using a Biometra trioblock cycler (T3000). The result was visualised 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 chromatograms 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 BOLD database with their accompanying information when vouchers were available. The other nuclear sequences and the mitochondrial sequences without vouchers were deposited first in BOLD and then in GenBank (http://www.ncbi.nlm.nih.gov) (accession numbers listed in table I).

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). Terminal regions of aligned sequence data blocks were removed to avoid any impact of experimental differences in sequence lengths. As a result there is a difference between the sequence length really sequenced and the sequence length actually used for similarity analyses (Tab. III).

Table II. - Primers used. HT stands for hybridization temperature.

Gene	Name	HT (°C)	Sequence (5' to 3')	Reference
Cytochrome Oxidase I	FishF1	52	TTCTCCAACCACAAAGACATTGGCAC	Ward *et al.*, 2005
	FishR1	52	TAGACTTCTGGGTGGCCAAAGAATCA	Ward *et al.*, 2005
	LCO1490	50	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.*, 1994
	HCO2198	50	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994
Rhodopsin Retrogene	Rhd193F	53	CNTATGAATAYCCTCAGTACTACC	Chen *et al.*, 2003
	Rhd1039R	53	TGCTTGTTCATGCAGATGTAGA	Chen *et al.*, 2003
Control Region	LPR02	52	AACTCCCACCACTAACTCCCAAAGC	Sanchez *et al.*, 2007
	HDL2	52	AAGTAGGAACCAGATGCCAGNAAT	Sanchez *et al.*, 2007
Cytochrome b	S-CytbL	50-60	TTTTGRGGYGCAACTGTAATTAC	Sanchez *et al.*, 2007
	CytbL399	50-60	GTYCTHCCHTGAGGACA	This study
	CytbH1052	50-60	GAMGCRAYTTGGCCGATG	This study

Analysis of sequence similarities was calculated using pairwise p-distances and pairwise K2P distances from each of the four sequence datasets taken separately (Tab. III). A neighbour-joining approach was used to summarize the degrees of global similarity among sequences using p-distances in MEGA4 (Tamura *et al.*, 2007) with clusters (Figs 2, 3).

Chromosome preparations were obtained on the specimens highlighted in table I, as described in Pisano *et al.* (2001).

RESULTS

The maximum divergence found between the sequences of two specimens of *Gymnodraco* in each dataset is shown in table III. All markers exhibit a maximal pairwise divergence far below 1%. For each marker, there are at least a few specimens of *G. acuticeps* that exhibit no difference with *G. victori* sequences. The absence or quasi-absence of clustering among *Gymnodraco* specimens in figures 2 and 3 is therefore explained by the fact that many sequences are actually identical.

The karyotype of *Gymnodraco acuticeps* was described (Pisano *et al.*, 2001) from three female specimens caught at Terra Nova Bay (Ross Sea). The chromosome diploid number and formula were 2n = 48 (2 metacentrics+ 2 submetacentrics + 44 acrocentrics). The 28S RNA ribosomal genes displayed a very particular localization in the long arm of a submetacentric pair, which was more or less homeomorphic from one specimen to another: in one chromosome, the 28S rDNA clusters almost entirely covered the long arm, whilst in the homologue chromosome, the 28S clusters were intercalated in the long arm.

In Terre Adélie, 12 specimens (11 females and 1 male) of *Gymnodraco* have been karyotyped. Some animals exhibited an external morphology closer to *G. acuticeps*, while others were similar to the original description of *G. victori*. Chromosome preparations could be obtained from eleven of the twelve specimens (Tab. I). In all of them, the chromosome numbers and formulae were found to be perfectly identical to those obtained for the *Gymnodraco acuticeps* specimens of Terra Nova Bay. The individuals caught off Terre Adélie had a typical, marked heteromorphism in the pair bearing the rRNA gene clusters (see an example figure 4). We could not detect any chromosomal character supporting the separation of *Gymnodraco* into two distinct species.

DISCUSSION

Ninety-nine percent of the 294 fish species sampled with multiple specimens in BOLD (as it was in 2008) were successfully distinguished by the COI barcode analysis. That analysis also showed that 93% of the fish intraspecific divergences were less than 1% (Ward *et al.*, 2009). The maximal divergence in the COI sequences between the two *Gymnodraco* species is extremely low (0.005% in K2P, and 0.03% in p-distance). With such low

Table III. - Dataset sizes and the greatest genetic distances recorded in the sample between two *Gymnodraco* specimens, as measured using pairwise p-distances or pairwise K2P distances.

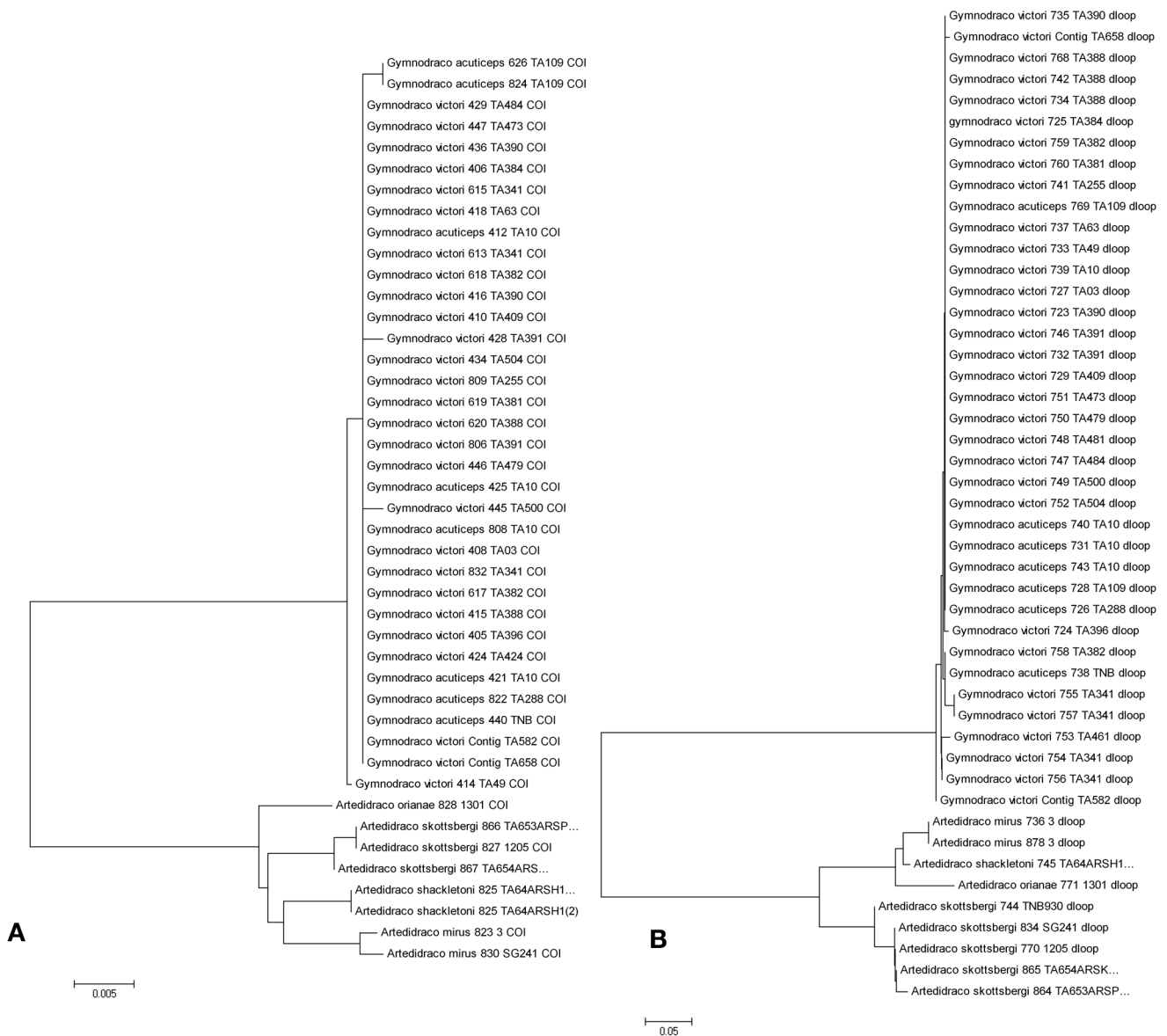| Gene	Number of terminals	Size sequenced	Data set size	Maximal divergence within *Gymnodraco* in %	
				p	K2P
Cytochrome Oxidase I	44	700	562	0.03	0.005
Cytochrome b	47	680	506	0.002	0.002
Rhodopsin retrogene	50	800	646	0.002	0.002
dloop	47	450	349	0.019	0.012

Figure 2. - Global similarity among DNA sequences of *Gymnodraco acuticeps* and *Gymnodraco victori* as measured by p-distances and illustrated using a neighbour-joining method. **A**: Cytochrome oxidase I gene; **B**: Mitochondrial d-loop.

distance divergences we would be inclined to directly assign individuals of *G. victori* to *G. acuticeps*. However Dettaï *et al*. (2010, see also Meier *et al*., 2008) recommend prudence with such assignments based on the use of a single raw distance value without having evaluated the actual barcoding gap of the species at hand. For instance, a slowing down of the rate of change of the COI sequences is a possible pitfall that can prevent finding sufficient differences to distinguish actual independently evolving units. The same difficulty can emerge from very recently separated evolving units. Such situations do exist: for instance, within another notothenioid family, the Artedidraconidae, most of the interspecific divergences are well below 1%. Hopefully in that case these low

divergences do not prevent the recovering of well delimited species clusters, as shown by the congruence obtained with additional markers, both more variable mitochondrial markers and a nuclear one, leading to conclude that these very low values were really interspecific divergences (Dettai *et al.*, 2011; Lecointre *et al.*, 2011). To avoid the possible pitfall of a mutation rate slowdown, Ward *et al.* (2009) recommended the sequence analysis of a more rapidly evolving mtDNA like the control region, and the use of nuclear markers is also important for an independent assessment of the clusters (Dettai *et al.*, 2011). The more rapidly evolving d-loop also lacks divergence among specimens (Tab. III) and does not provide clusters (Fig. 2B). While it is still possible
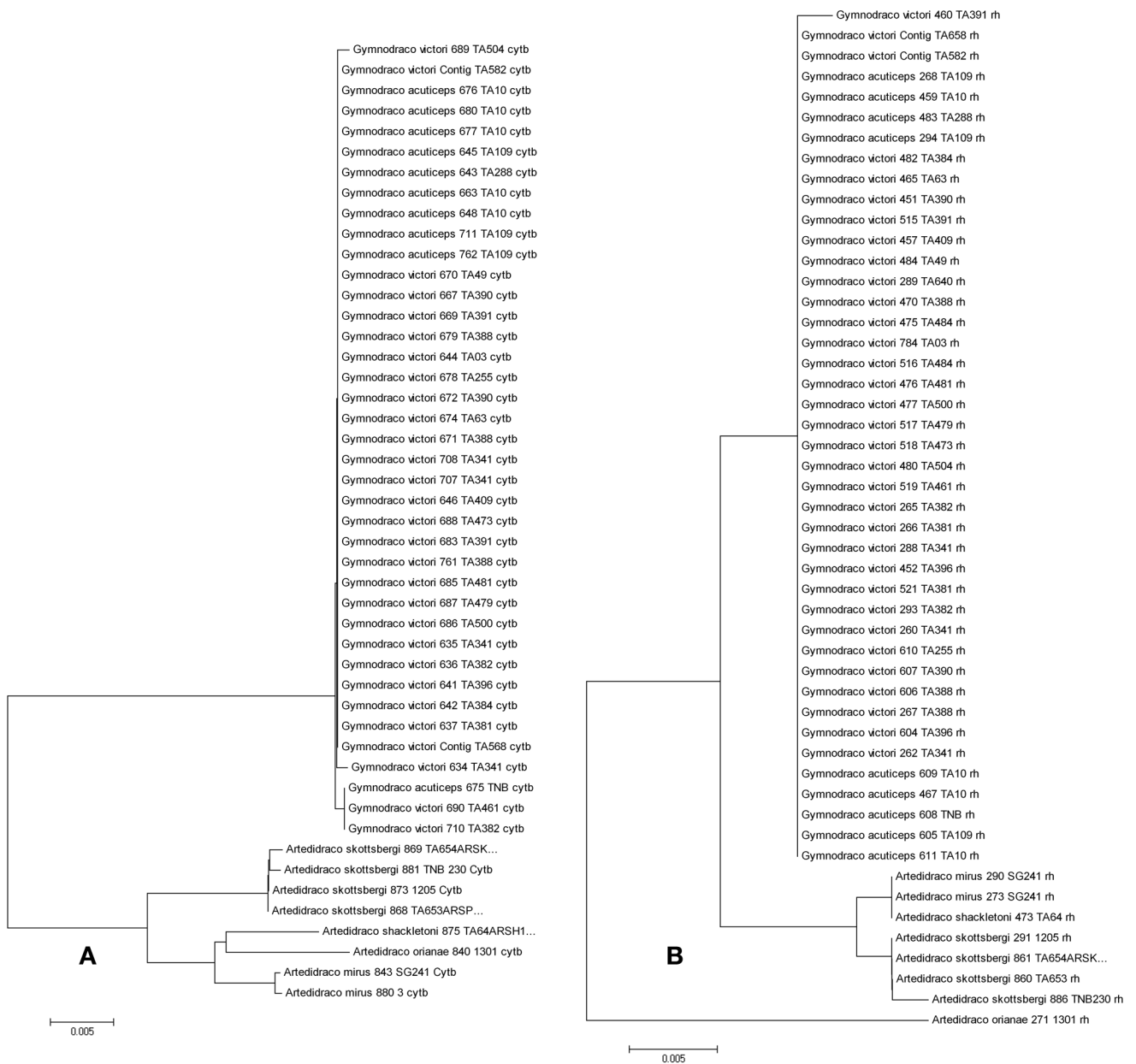
Figure 3. - Global similarity among DNA sequences of *Gymnodraco acuticeps* and *Gymnodraco victori* as measured by p-distances and illustrated using a neighbour-joining method. **A**: Cytochrome b gene; **B**: Rhodopsin nuclear retrogene.

that this is a case of haplotype sharing among very recently diverged species *(Galtier et al.,* 2009; Ward *et al., 2009)*, the present sequence data rather favours the hypothesis of a single *Gymnodraco* species.

Gon (1990) found tooth patterns difficult to interpret in many specimens. It is likely that some of the variability in the number of large anterior canine teeth is attributable to the normal cycle of tooth replacement. Our field experience does confirm that fact. However, the identification of specimens in the field might have been somewhat confused by a mistake in the identification key most commonly used in the

field (Hureau, 1987: 259): the two species names have been swapped, each being accompanied by the description of the other species. Moreover, Hureau (1963) mentioned three canine-like teeth in *G. acuticeps,* while four teeth are mentioned in the 1987 key (Hureau, 1987). We have checked the two external parameters cited in figures 15 and 16 of Hureau (1987: 259, with the right names associated with the right description); i.e., the number and size of lower canine teeth and the length of the upper jaw (Tab. I) for the specimens used for the molecular study that are vouched in the MNHN collections, as well as for additional specimens from the
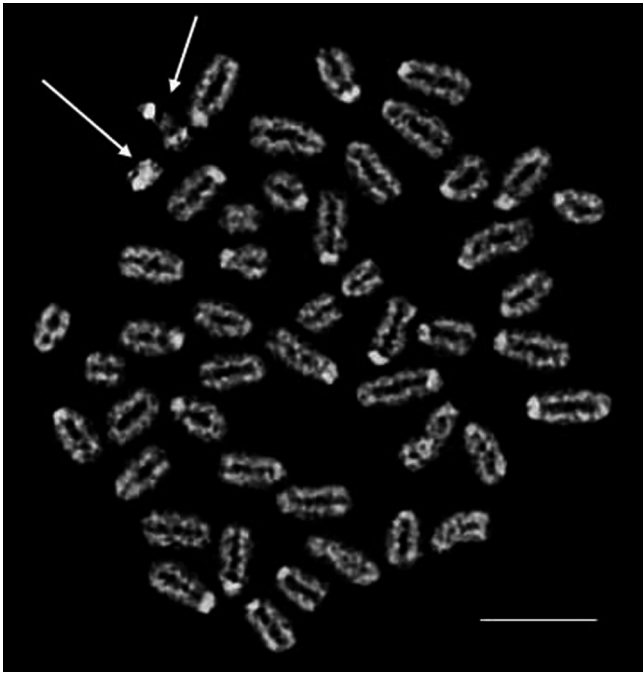
Figure 4. - Black and white photo of a DAPI-stained metaphase plate of the specimen T658GYVI1 (MNHN 2009-1085). 28SrRNA ribosomal gene bearing pair (arrows) shows a strong heteromorphism as described in Pisano *et al.* (2001). Scale bar = 10 μm.

MNHN collections including the syntypes of *G. victori*. All specimens exhibit a posterior end of the maxillar bone under the anterior part of the eye, and never under the posterior part of the eye as drawn in Hureau (1987). Variations in the number of canine teeth from right to left side of the same animal were also observed (MNHN 2009-0658, MNHN 2009-0659, MNHN 1963-0065), but we cannot exclude that these are due to broken fang-like teeth. More interestingly, the position of the largest fang may vary from side to side in the same animal. For example, two individuals labelled *G. victori* (MNHN 2009-0661 and MNHN 2009-0663) presented two canine teeth in each side, with the first being the strongest on the left side and the second on the right side. In another specimen, the two canine teeth are of the same size on the left side only (MNHN 2009-666). We confirmed the identifications of the specimens used by Hureau (1963) either to *G. victori* or to *G. acuticeps*. However, the expanded sampling series allows detection of variations in the tooth pattern, corroborating Gon's (1990) observations. We found a specimen with three fangs, the first one being the strongest. Others exhibit a single fang in the front without obvious signs of a broken second posterior one. This variability could be responsible for the errors in specimen attribution to either species. MNHN 2009-0659, MNHN 2009-0657, MNHN 2009-0662 are labelled *G. victori* but have the typical canine tooth pattern of *G. acuticeps*. MNHN 1965-0490, MNHN

1965-0491, MNHN 1982-1269, MNHN 1999-0374, MNHN 2009-1083 are labelled *G. acuticeps* but have the canine tooth pattern of *G. victori*. There are still no differences in the sequences whatever the marker even taking into account only the specimens *correctly* and unambiguously identifiable as either *G. acuticeps* or *G. victori*. The existence of a great diversity of shapes in the morphological characters used to differentiate the species is also more in line with intraspecific than with interspecific divergences. This is also in agreement with the lack of variability displayed at the level of the karyotypes.

The description of *G. victori* (Hureau, 1963) was based on a small number of specimens of both species due to the limited fishing possibilities of those times (10 specimens, plus three syntypes at the BMNH). The low number of specimens did not reflect accurately the whole range of variability in *Gymnodraco acuticeps*, and the species *G. victori* might have been created on the basis of extreme points in what is actually a continuum of variation in the chosen characters. By expanding the sampling of both species to 24 specimens, Gon (1990) had to reevaluate the evidence supporting their separation based on those morphological characters.

No element in the three lines of evidence investigated here supports the existence of a discontinuity between the specimens identified as *G. victori* and those identified as *G. acuticeps*. Because the former taxon does not appear to be a distinct entity from the latter, we propose to make *Gymnodraco victori* a junior synonym of *Gymnodraco acuticeps*.

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