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Extensive paraphylies within sharks of the order Carcharhiniformes inferred from nuclear and mitochondrial genes

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Abstract

Using nuclear coding and mitochondrial ribosomal genes we try to clarify relationships within Carcharhiniformes with special focus on the two most problematic groups: scyliorhinids and triakids. The mitochondrial aligned sequences are 1542 bp long, and include principally portion of 16S rRNA gene. They are obtained for two outgroup species and 43 Carcharhiniformes species, covering 5 of the 8 families and 15 of the 48 genera of the order. The nuclear RAG1 sequences are 1454 bp long, and are obtained for 17 species representative of the diversity of all species sampled. We used Maximum Parsimony and Maximum Likelihood criteria for tree reconstruction. Paraphylies within the family Scyliorhinidae was proposed for the first time by Maisey [Zool. J. Linn. Soc. 82, 33, 1984] in a morphological cladistic analysis. This result has never been proposed again until recently from molecular phylogenies [Mol. Phylogenet. Evol. 31, 214, 2004]. Here, independent and simultaneous analyses of nuclear and mitochondrial data are congruent in supporting the paraphyly of scyliorhinids. Two groups of scyliorhinids are obtained, thoroughly in line with discrimination proposed by previous authors, based on presence/absence of supraorbital crests on the chondrocranium. The first group (*Scyliorhinus* + *Cephaloscyllium*) is basal within carcharhiniforms and the second group (*Apristurus* + *Asymbolus* + *Cephalurus* + *Galeus* + *Parmaturus*) is sister group of all the other families investigated (Carcharhinidae, Proscyllidae, Pseudotriakidae, and Triakidae). The paraphyly of triakids appeared probable but more investigations are needed. In conclusion several independent morphological and molecular phylogenetic studies support paraphyly within scyliorhinids. So we propose a new classification for the group, with the redefinition of the family Scyliorhinidae sensu stricto and the resurrection of the family Pentanchidae with a new definition.

Keywords: Carcharhiniformes; Scyliorhinidae; Triakidae; Pentanchidae; Molecular phylogeny; Paraphyletic group; mtDNA; RAG1; Sharks

1. Introduction

Based on the oldest known fossil, the order Carcharhiniformes (ground sharks) is a lineage that at least originated from 144 to 151 million years ago, in the Lower Tithonian, Upper Jurassic (Cappetta, 1987). With 228 extant recognized species, it is the largest of the 8 shark orders, representing more than half living sharks. The monophyly of Carcharhiniformes (Compagno, 1973) is largely accepted and is supported by three synapomorphies: (1) suborbitalis with two divided heads; (2) nictitating lower eyelid present; (3) accessory terminal cartilage of the pelvic fin not spinous or modified into the external mesorhipidion (Shirai, 1996). The monophyly of the order is also strongly supported by molecular investigations (Douady et al., 2003; Winchell et al., 2004); however relationships within the order are still largely unresolved by morphology due to many convergences and reversals (Shirai, 1996), and

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under-investigated by molecular approach. Seventeen Carcharhiniformes have been used in previous molecular phylogenetic analyses using DNA sequences (Douady et al., 2003; Martin, 1993, 1995; Martin and Palumbi, 1993; Martin et al., 1992; Winchell et al., 2004), representing 7.5% of the order's diversity, and leaving questions about the phylogeny of the group unanswered. Within carcharhiniform sharks, sphyrnids were the most represented with seven species included in molecular phylogenies: *Eusphyrna blochii*, *Sphyrna corona*, *S. lewini*, *S. media*, *S. mokarran*, *S. tiburo*, and *S. tudes*. Other families have been poorly explored despite the large number of species they included. Only five carcharhinids were included in molecular phylogenies: *Carcharhinus plumbeus*, *C. porosus*, *Galeocerdo cuvier*, *Negaprion brevirostris*, and *Prionace glauca*, only three species among scyliorhinids: *Scyliorhinus canicula*, *S. torazame*, and *Apristurus profundorum*, and only two species among triakids *Mustelus manazo* and *Triakis semifasciata*. Finally, no representatives of hemigaleids, leptochariids, proscyllids, and pseudotriakids were included in molecular phylogenies.

Forty-eight genera from eight families are currently recognized within Carcharhiniformes: Carcharhinidae or requiem sharks (12 genera; 50 species); Hemigaleidae or weasel sharks (4 genera; 7 species); Leptochariidae or barbeled hound sharks (1 species); Proscyllidae or finback cat sharks (4 genera; 7 species); Pseudotriakidae or false cat sharks (1 species); Scyliorhinidae or cat sharks (15 genera; 115 species); Sphyrnidae or hammerhead sharks (2 genera; 8 species); and Triakidae or hound sharks (9 genera; 38 species) (updated from Compagno, 1999). The order Lamniformes (mackerel sharks) is commonly considered as the sister group for Carcharhiniformes but recently using molecular data (Winchell et al., 2004) proposed an alternative hypothesis where Lamniformes + Orectolobiformes (carpet sharks) is the sister group of Carcharhiniformes.

Scyliorhinids are the largest shark family, representing about a quarter of living shark species. Description of new species is still frequent within cat sharks (13 were described between 1993 and 2003, and several ones are still undescribed). Before Maisey (1984) and as noted by himself, scyliorhinids have commonly been considered as the most primitive group within Carcharhiniformes (Bigelow and Schroeder, 1948; Compagno, 1970, 1973; Garman, 1913; Nakaya, 1975; Regan, 1906; Springer, 1979; White, 1937), and have invariably been diagnosed phenetically by the absence of "higher carcharhinoid" characters. These non-cladistic visions have constrained scyliorhinids as a grade. Some authors (Compagno, 1973; Nakaya, 1975; White, 1936, 1937) had noted transitional series between "lower" and "higher" Carcharhiniformes, suggesting by this comment that scyliorhinids might be paraphyletic, but the idea has not been pursued elsewhere. In his analysis mainly based on vertebral calcification patterns and development of supraorbital crests and palatoquadrate levator muscles on the chondrocranium, Nakaya (1975) considered that scyliorhinids and carcharhinids lie "on the same phyletic line," but concluded that similarities shared between these groups had arisen independently in each group. However in his intuitive tree, Nakaya (1975, Fig. 43, p. 87) had proposed two alternative hypotheses for scyliorhinids evolution; one where the family appear monophyletic (Fig. 1A1) and the other where it appear paraphyletic (Fig. 1A2), with *Scyliorhinus* sister group of *Proscyllidae* + *Triakidae* + *Carcharhinidae* + *Apristurus* + *Galeus* + *Parmaturus*. Later on, Maisey (1984) reused the data of Nakaya (1975) in a cladistic analysis, and for the first time, provided evidence for paraphyly of scyliorhinids, with *Scyliorhinus* as sister group of triakids + carcharhinids + *Galeus* (Fig. 1C). Despite cladistic methods revealed scyliorhinid paraphyly in a truly explicit manner, none of the morphological studies posterior to Maisey (1984) hypothesized that paraphyly, because no strict cladistic analysis was used (Compagno, 1988), because the group was still analysed phenetically (Herman et al., 1990) (Fig. 1E), because the group was under-sampled (Douady et al., 2003; Shirai, 1996) (Figs. 1H and K), or because authors have been influenced by the long "tradition" of the use of scyliorhinids (Sato, 2000) (Fig. 1J). In his strict cladistic analysis of scyliorhinid interrelationships, Sato (2000) followed the hypothesis of Compagno (1988) (Figs. 1D1 and D3) and did not check other Carcharhiniformes, so he implicitly did the hypothesis of monophyly for scyliorhinids. Recently two scyliorhinid species (*A. profundorum* and *S. torazame*) were involved for the first time in a molecular cladistic analysis of elasmobranchs (Winchell et al., 2004) and the family was found paraphyletic (Fig. 1L) along with the hypothesis of Maisey (1984).

Whatever the hypothesis of monophyly or paraphyly for scyliorhinids, the genera *Cephaloscyllium* and *Scyliorhinus* always appeared basal among scyliorhinids. Compagno (1988) as Sato (2000) have found *Asymbolus* and *Galeus* sister groups of *Apristurus* + *Parmaturus*. Compagno (1988) was the first to attempt subdivisions within *Apristurus*, recognizing 10 phenetic species groups in the genus. But later Nakaya and Sato (1999) revisited completely the *Apristurus* interrelationships and just three species groups were recognized based on snout length, spiral valves counts and labial furrows length (Fig. 1I). Later on, in his cladistic analysis Sato (2000) more accurately proposed five morphotypes for *Apristurus*: the morphotypes 1 and 2 were a division of the *longicephalus* group of Nakaya and Sato (1999); the morphotype 3 was synonymous of the *spongiceps* group, and the morphotypes 4 and 5 were a division of the *brunneus* group (Fig. 1J).

Despite the fact that *S. canicula* (the small-spotted cat shark) is the most important model for understanding

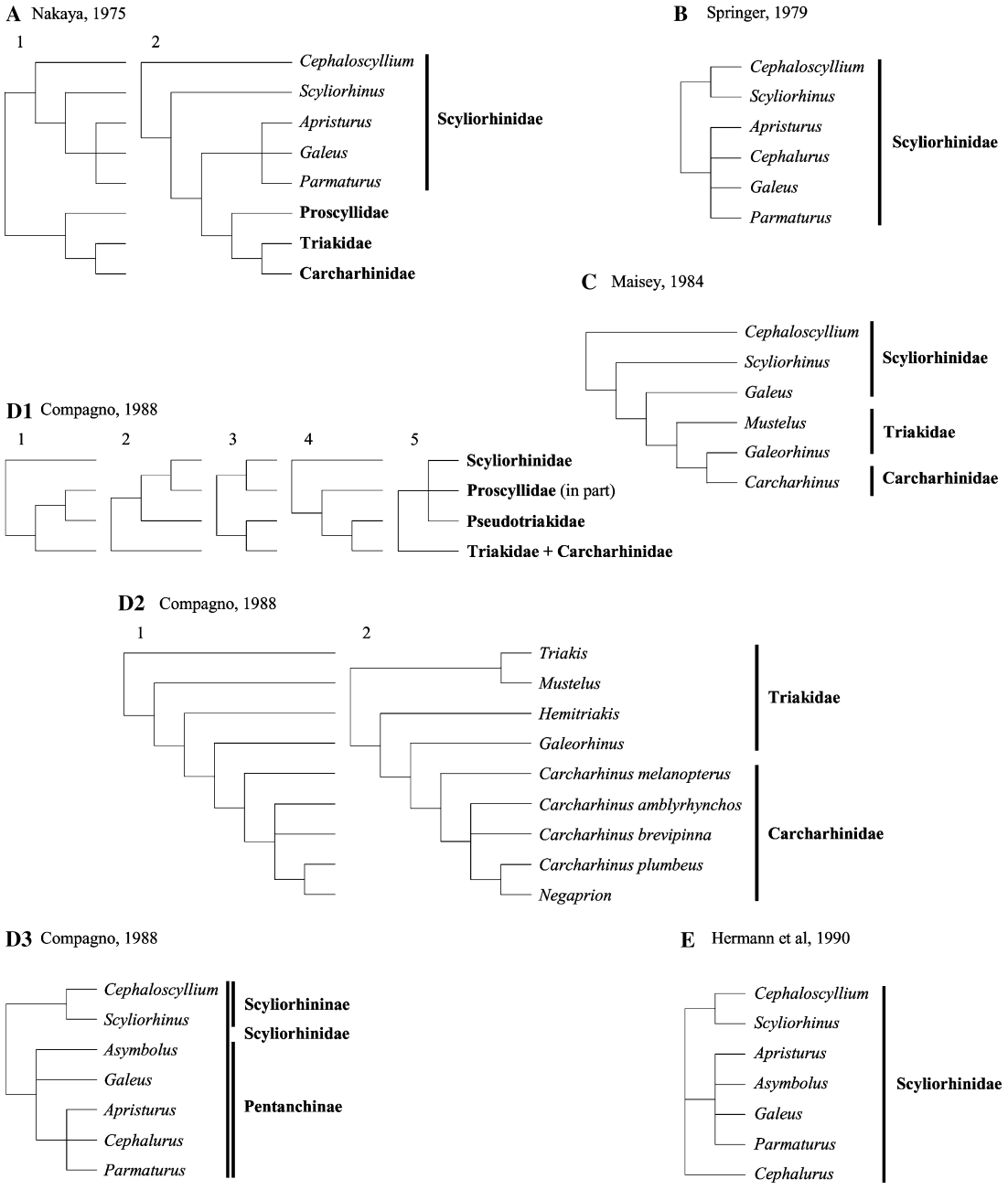

Fig. 1. Previous hypotheses for carcharhiniform interrelationships. Taxa shown in the trees are restricted to those also present in our analysis. Species names and rank names (family) are updated when required, using the classification of Compagno (1999) except for the subfamily names Scyliorhininae and Pentanchinae of Compagno (1988). Family and subfamily names are in bold. (A) Hypothesis of Nakaya (1975) for scyliorhinid and related taxa interrelationships, based on morphology, with 2 alternative trees. (B) Hypothesis of Springer (1979) for scyliorhinid interrelationships, based on morphology. (C) Hypothesis of Maisey (1984) for carcharhiniform interrelationships, based on a cladistic analysis of the morphological characters proposed by Nakaya (1975). (D) Hypothesis of Compagno (1988) based on a non-strictly cladistic analysis of morphological characters; (D1) for carcharhiniform families interrelationships, with five alternative trees; (D2) for triakid and carcharhinid interrelationships, with 2 alternative trees; (D3) for scyliorhinid interrelationships. (E) Hypothesis of Herman et al. (1990) for scyliorhinid interrelationships, based on teeth anatomy. (F) Hypothesis of Naylor (1992) for carcharhinid interrelationships, based on the cladistic analysis of allozyme electrophoresis, with four alternative trees. (G) Hypothesis of Lavery (1992) for carcharhinid interrelationships, based on the cladistic analysis of allozyme electrophoresis, with two alternative trees. (H) Hypothesis of Shirai (1996) for carcharhiniform interrelationships, based on a cladistic analysis of morphological characters. (I) Hypothesis of Nakaya and Sato (1999) for *Apristurus* interrelationships, based on morphology. (1) Added from Nakaya and Séret (1999); (2) added from Sato et al. (1999); (3) added from Iglésias et al. (2004). (J) Hypothesis of Sato (2000) for scyliorhinid interrelationships, based on a cladistic analysis of morphological characters. (K) Hypothesis of Douady et al. (2003) for carcharhiniform interrelationships, based on a cladistic analysis of DNA sequences. (L) Hypothesis of Winchell et al. (2004) for carcharhiniform interrelationships, based on a cladistic analysis of DNA sequences.

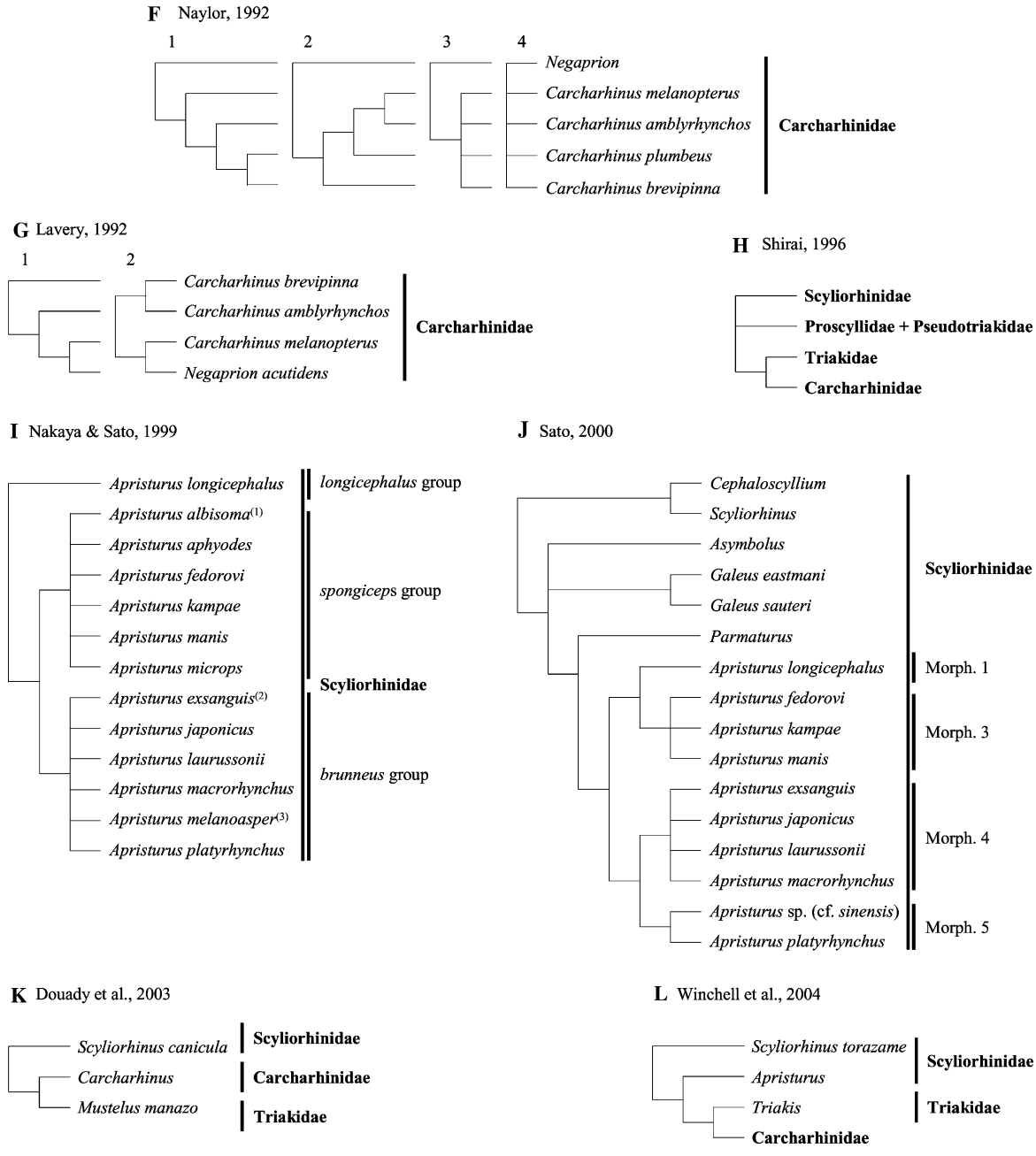

Fig. 1. (*continued*)

the chondrichthyan pattern of development, physiology, neurology or molecular biology; little is known about the evolutionary history of the family Scyliorhinidae and the position of this species in a phylogenetic tree with high taxonomic density. The complete mtDNA sequence for *S. canicula* was the first one obtained for a chondrichthyan species (Delarbre et al., 1998) and a hundred of nucleotide sequences from that species are now available online in GenBank, with several ones useful for phylogeny, as mitochondrial genes and nuclear ribosomic RNA genes. Despite these facts it was not included in a molecular phylogeny of sharks until very recently (Douady et al., 2003) (Fig. 1K).

Triakids are always considered by authors to be the sister group of carcharhinids (Fig. 1) and concomitant species (sphyrnids, not show on Fig. 1). Triakids are commonly considered as a para- or polyphyletic group in modern works, and *Galeorhinus* is supposed to be the sister group of *Carcharhinus* (e.g., Compagno, 1988; Maisey, 1984) (Figs. 1C and D2). Interrelationships of this group are unresolved and no consensus emerges among authors.

Carcharhinids are generally better known than scyliorhinids or triakids because they include large size species, they are occasionally involved in shark attacks and they have a commercial importance for fisheries. However,

the marked morphological uniformity among most carcharhinids did not permit authors to resolve intra-family relationships. For first time both Lavery (1992) and Naylor (1992) have investigated carcharhinids interrelationships by using allozyme electrophoresis (Figs. 1F and G). Because most species sampled are not common to the two studies, comparison of the phylogenetic hypotheses obtained is not possible. All subsequent molecular phylogenies based on the more higher informative DNA sequences had used very few carcharhinids, so as for scyliorhinids and triakids, interrelationships of the group is still largely unresolved.

The objectives of this study are: (1) Discriminate among the previous hypotheses about monophyly or paraphyly of scyliorhinids and triakids using new independent markers (mitochondrial ribosomal and nuclear coding sequences). (2) Enlarge knowledge of phylogeny of carcharhiniforms by using better taxonomic sampling. (3) Start to investigate intra-genus relationships, especially within *Apristurus*, the second largest genus among extant sharks. Forty of the 45 species used in this study have never been included in any molecular phylogenetic analysis and 60 of the 62 sequences used are new. Separate and simultaneous analyses have been conducted to evaluate reliability and robustness, respectively (Lecointre and Deleporte, 2005).

2. Materials and methods

2.1. Taxonomic sampling and DNA extraction

Specimens were collected during cruises on commercial and research vessels, in fish markets or provided directly by professional fishermen. They come from EN Atlantic, New Caledonia, New Zealand, Japan, Taiwan, and Peru. All the voucher specimens newly studied except *Cetorhinus maximus* have been catalogued in the collections of the Muséum National d'Histoire Naturelle (MNHN), and those of the Laboratory of Marine Zoology, Faculty of Fisheries, Hokkaido University (HUMZ) (Table 1). Catalogue numbers of tissue samples (BPS) are from the collection of S.P. Iglésias. Forty-five species of elasmobranchs were analysed: 2 from the order Lamniformes as outgroup and 43 Carcharhiniformes, representing 5 of the 8 families and 15 of the 48 genera of the order. Most of the sampled species are scyliorhinids (30 species). A complete list of species examined and related data is given in Table 1. Complementary data concerning the specimens deposited in the MNHN collections are available online at: http://www.mnhn.fr/collections/gicim.

Species were identified by the first author with help from specialists of the groups when presenting difficulties. Because many specimens have been collected in poorly studied areas, especially in deep-waters, several of them are new species for Science and still undescribed. Twelve species are unnamed in the present work (Table 1): *Apristurus* sp. 1 is apparently an undescribed species (Nakaya, com. pers.); *Apristurus* sp. 2 is possibly *Apristurus macrorhynchus*; *Apristurus* sp. 3 is the *Apristurus* sp. B described by Last and Stevens (1994); *Apristurus* sp. 4 is an unidentified or undescribed species; *Apristurus* sp. 5 is possibly *Apristurus platyrhynchus*; *Apristurus* sp. 6 is apparently an undescribed species, very similar to *A. melanoasper* recently described (Iglésias et al., 2004); *Apristurus* sp. 7 is an unidentified or undescribed species; *Cephalurus* sp. 1 is an undescribed species (Takahashi and Nakaya, com. pers.); *Asymbolus* sp. 1, *Galeus* sp. 1; *Hemitriakis* sp. 1 and *Parmaturus* sp. 1 are undescribed (Séret, com. pers.). Unidentified or yet undescribed species make no problem here as long as the corresponding sequences are referenced with the voucher specimen deposited in museum collections, allowing future updates of the taxonomy of both specimens and sequences.

Muscle tissue was taken on fresh specimens and stored frozen in 80% ethanol. Total DNA was extracted following the standard CTAB method modified from Jones (1953).

2.2. Loci selection and PCR amplification

Two loci were sequenced because different genes are often informative at different hierarchical levels and above all, independent loci allow discussing the reliability of the groups obtained. We sequenced a mitochondrial sequence including partial 12S rRNA (24 positions), complete Valine tRNA (72 positions) and 16S rRNA (1446 positions) genes for 45 species and a nuclear sequence of 1454 bp of partial RAG1 (from 1629 to 3082 when aligned with the sequence U62645 of *Carcharhinus leucas*) for 17 species representing the diversity of all sampled species to test scyliorhinid paraphyly from an independent marker. The loci were amplified by polymerase chain reaction (PCR) using the puReTaq Ready-To-Go, PCR Beads (Amersham Biosciences), using the primers Chon-Mito-S005 and Chon-Mito-R017 for the mitochondrial sequence (Table 2). To amplify the nuclear sequence nested PCR were performed because faint quantities of products were obtained from single PCRs. The PCR fragments obtained with the first PCR was purified using the QIAquick Gel Extraction Kit (Qiagen) and was used for a second PCR, with a couple of internal primers. For the two successive PCRs, various primer combinations were used depending of species, including the three forward primers Chon-Rag1-S018, Chon-Rag1-S024, and Chon-Rag1-S026 and the two reverse primers Chon-Rag1-R021 and Chon-Rag1-R029 (Table 2). PCR was carried out using a TGradient thermocycler (Biometra). Thermal cycling for mitochondrial sequence consisted of an initial step at 94 °C denaturing for 1 min; 10 cycles

Table 1
List of the 45 species analysed and related data on specimens and sequences

Order Family Species	Specimen catalog No.	Locality	Tissue sample No.	Sequence	Sequence length (bp)	GenBank Accession No.
Lamniformes						
Cetorhinidae						
Cetorhinus maximus	Uncatalogued	Western Europe	BPS-0197	12S-16S	1500	AY462146[d]
	Uncatalogued	Western Europe	BPS-0197	RAG1	1454	AY462147[d]
Odontaspididae						
Odontaspis ferox	MNHN-2003-1991[*]	New Caledonia	BPS-0195	12S-16S	1500	AY462144[d]
	MNHN-2003-1991[*]	New Caledonia	BPS-0195	RAG1	1454	AY462145[d]
Carcharhiniformes						
Carcharhinidae						
Carcharhinus amblyrhynchos	MNHN 2002-1183[*]	New Caledonia	BPS-0198	12S-16S	1506	AY462148[d]
Carcharhinus brevipinna	MNHN 2003-1992[*,a]	New Caledonia	BPS-0196	12S-16S	1506	AY462149[d]
Carcharhinus melanopterus	MNHN 2002-1203[*]	New Caledonia	BPS-0199	12S-16S	1505	AY462150[d]
Carcharhinus plumbeus	MNHN 2002-1175[*]	New Caledonia	BPS-0200	12S-16S	1509	AY462151[d]
	MNHN 2002-1175[*]	New Caledonia	BPS-0200	RAG1	1454	AY462152[d]
Negaprion acutidens	MNHN 2002-1201[*]	New Caledonia	BPS-0201	12S-16S	1502	AY462153[d]
Proscyllidae						
Proscyllium habereri	HUMZ 171536[*]	Taiwan	BPS-0084	12S-16S	1504	AY462183[d]
	HUMZ 171536[*]	Taiwan	BPS-0084	RAG1	1454	AY462184[d]
Pseudotriakidae						
Pseudotriakis microdon	MNHN 2002-2852[*]	Western Europe	BPS-0024	12S-16S	1504	AY049049[d]
	MNHN 2002-2852[*]	Western Europe	BPS-0024	RAG1	1454	AY462185[d]
Scyliorhinidae						
Apristurus albisoma	MNHN 2003-1930[*]	New Zealand	BPS-0166	12S-16S	1507	AY462154[d]
Apristurus aphyodes	MNHN 2000-1744	Western Europe	BPS-0009	12S-16S	1505	AF358916[d]
Apristurus exsanguis	MNHN 2003-0551[*]	New Zealand	BPS-0030	12S-16S	1504	AY049048[d]
Apristurus fedorovi	MNHN 2003-1046[*]	Japan	BPS-0069	12S-16S	1504	AY462155[d]
Apristurus japonicus	MNHN 2003-1045[*]	Japan	BPS-0068	12S-16S	1501	AY462156[d]
Apristurus kampae	HUMZ 174359	Peru	BPS-0076	12S-16S	1505	AY462157[d]
Apristurus laurussonii	MNHN 2000-1745[c]	Western Europe	BPS-0006	12S-16S	1500	AF329376[e]
Apristurus longicephalus	MNHN-2002-3077[*,b]	New Caledonia	BPS-0165	12S-16S	1505	AY462158[d]
	MNHN-2002-3077[*,b]	New Caledonia	BPS-0165	RAG1	1454	AY462159[d]
Apristurus manis	MNHN 2000-1769[*]	Western Europe	BPS-0007	12S-16S	1506	AF329375[d]
	MNHN 2000-1769[*]	Western Europe	BPS-0007	RAG1	1454	AY462160[d]
Apristurus melanoasper	MNHN 2000-1755[c]	Western Europe	BPS-0005	12S-16S	1500	AF329374[e]
	MNHN 2000-1755[c]	Western Europe	BPS-0005	RAG1	1454	AY462161[d]
Apristurus microps	MNHN 1999-0779[*]	Western Europe	BPS-0008	12S-16S	1507	AF382947[d]
Apristurus sp. 1	HUMZ 171435[*]	Taiwan	BPS-0085	12S-16S	1503	AY462162[d]
Apristurus sp. 2	MNHN 2003-1983[*]	New Caledonia	BPS-0187	12S-16S	1501	AY462163[d]
Apristurus sp. 3	MNHN 2003-0553	New Zealand	BPS-0032	12S-16S	1500	AY049051[d]
Apristurus sp. 4	MNHN 2003-1964[*]	New Caledonia	BPS-0178	12S-16S	1500	AY462164[d]
Apristurus sp. 5	MNHN 2003-1967[*]	New Caledonia	BPS-0180	12S-16S	1500	AY462165[d]
Apristurus sp. 6	MNHN 2003-1981[*]	New Caledonia	BPS-0185	12S-16S	1500	AY462166[d]
Apristurus sp. 7	HUMZ 173310	Peru	BPS-0073	12S-16S	1506	AY462167[d]
Asymbolus sp. 1	MNHN 2003-1989[*]	New Caledonia	BPS-0193	12S-16S	1505	AY462168[d]
	MNHN 2003-1989[*]	New Caledonia	BPS-0193	RAG1	1454	AY462169[d]
Cephaloscyllium umbratile	HUMZ 170373[*]	Taiwan	BPS-0081	12S-16S	1508	AY462170[d]
Cephalurus sp. 1	HUMZ 180178[*]	Peru	BPS-0082	12S-16S	1502	AY462171[d]
	HUMZ 180178[*]	Peru	BPS-0082	RAG1	1454	AY462172[d]
Galeus eastmani	HUMZ 175851	Japan	BPS-0079	12S-16S	1504	AY462173[d]
Galeus melastomus	MNHN 2000-1729	Western Europe	BPS-0003	12S-16S	1504	AF329372[d]
Galeus murinus	MNHN 2000-1733	Western Europe	BPS-0004	12S-16S	1503	AF329373[d]
	MNHN 2000-1733	Western Europe	BPS-0004	RAG1	1454	AY462174[d]
Galeus sauteri	HUMZ 170379[*]	Taiwan	BPS-0083	12S-16S	1502	AY462175[d]
Galeus sp. 1	MNHN 2003-1985[*]	New Caledonia	BPS-0189	12S-16S	1504	AY462176[d]
Parmaturus sp. 1	MNHN 2003-1987[*]	New Caledonia	BPS-0191	12S-16S	1502	AY462177[d]
	MNHN 2003-1987[*]	New Caledonia	BPS-0191	RAG1	1454	AY462178[d]
Scyliorhinus canicula	Uncatalogued	Western Europe	Uncatalogued	12S-16S	1509	Y16067[f]
	MNHN 2000-1728[*]	Western Europe	BPS-0002	RAG1	1454	AY462179[d]

Table 1 (*continued*)

Order Family Species	Specimen catalog No.	Locality	Tissue sample No.	Sequence	Sequence length (bp)	GenBank Accession No.
Scyliorhinus stellaris	MNHN 2000-1727*	Western Europe	BPS-0001	12S-16S	1508	AF327706[d]
	MNHN 2000-1727*	Western Europe	BPS-0001	RAG1	1454	AY462180[d]
Scyliorhinus torazame	MNHN 2003-1040*	Japan	BPS-0066	12S-16S	1505	AY462181[d]
	MNHN 2003-1040*	Japan	BPS-0066	RAG1	1454	AY462182[d]
Triakidae						
Galeorhinus galeus	MNHN 2003-0533*	Western Europe	BPS-0057	12S-16S	1503	AY462186[d]
Hemitriakis sp. 1	MNHN 2002-1202*	New Caledonia	BPS-0202	12S-16S	1506	AY462187[d]
Mustelus asterias	MNHN 2001-1112*	Western Europe	BPS-0049	12S-16S	1507	AY049050[d]
	MNHN 2001-1112*	Western Europe	BPS-0049	RAG1	1454	AY462188[d]
Mustelus manazo	Uncatalogued	Japan	Uncatalogued	12S-16S	1506	AB015962[g]
Mustelus mustelus	MNHN 2003-1050*	Mediterranean sea	BPS-0087	12S-16S	1505	AY462189[d]
Triakis scyllium	MNHN 2003-1038*	Japan	BPS-0064	12S-16S	1505	AY462190[d]
	MNHN 2003-1038*	Japan	BPS-0064	RAG1	1454	AY462191[d]

Note. *Specimen which photography appears in the tree Fig. 2.

[a] Complementary data on the specimen provided by Caraguel and Iglésias (2004).

[b] Complementary data on the specimen provided by Iglésias et al. (2005).

[c] Complementary data on the specimen provided by Iglésias et al. (2004).

[d] Sequences new to this study.

[e] Iglésias et al. (2004).

[f] Delarbre et al. (1998).

[g] Cao et al. (1998).

at 94 °C denaturing for 1 min, 60 °C annealing for 1 min and 72 °C extension for 3 min; 10 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 4 min and 20 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 5 min. Thermal cycling for nuclear sequence consisted of an initial step at 94 °C denaturing for 1 min; 10 cycles at 94 °C denaturing for 1 min, 52 °C annealing for 1 min and 72 °C extension for 3 min; 10 cycles at 94 °C for 1 min, 50 °C for 1 min and 72 °C for 4 min and 25 cycles at 94 °C for 1 min, 50 °C for 1 min; and 72 °C for 5 min. The PCR products are deposited on an agarose gel and the PCR fragments were purified as above.

2.3. DNA sequencing

Sequencing reactions were done using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and carried out on a TGradient thermocycler (Biometra). Thermal cycling consisted of 40 cycles at 94 °C denaturing for 30 s, 60 °C annealing for 30 s, and 72 °C extension for 4 min. The primers used are presented in Table 2 and they vary depending on the species analysed for the RAG1 fragment, whereas the primers used for the mitochondrial fragment are universal for sharks. Amplified DNA was directly sequenced on an ABI Prism 310 automated sequencer (Applied Biosystems). Each fragment used was sequenced in both directions to maximize the accuracy of the sequence. No missing data exist in all the sequences obtained.

2.4. Alignment of sequences

Sequence alignment for mitochondrial locus was performed by eye using ED of the software Must 2000 (Philippe, 1993) and BioEdit version 4.8.6 (Hall, 1999) with no ambiguity and no region was excluded for the phylogenetic analysis. The nuclear sequences do not present difficulty for alignment because all sequences have the same length. Four data sets were used for tree reconstruction (Table 3). Alignments are available upon request from SPI (iglesias@mnhn.fr). The analyses were performed firstly considering gap as missing data and secondly as a fifth character, and very few differences were observed in the results. Some of the sequences obtained for the RAG1 gene presented polymorphic positions. These were coded as ambiguous.

Before combination of matrices the ILD test (Farris et al., 1994) was performed with 1000 iterations on parsimony-informative positions to check for significant character incongruence, using the "Partition Homogeneity Test" of PAUP* version 4.0b8 (Swofford, 2001).

2.5. Phylogenetic reconstruction

The selection of an optimal outgroup is crucial for reliability of phylogenetic inferences. We used here two species of Lamniformes (*C. maximus* and *Odontaspis ferox*) for rooting trees. Phylogenetic analyses under the parsimony criterion were carried out using PAUP* version 4.0b8. Heuristic search option with 1000 iterations of random addition sequence was used for mitochon-

Table 2
List of primers used to amplify and sequence the mitochondrial sequence (portion of 12S and 16S rRNA and complete Valine tRNA genes) and the nuclear sequence (portion of RAG1 gene)

Primer	Sequence 5′–3′	Length (bp)	Forward/ reverse	PCR	Sequencing	Site of fixation[a]
Mitochondrial						
Chon-Mito-S003	TCTCTGTGGCAAAAGAGTGG	20	F	–	x	15260-15279
Chon-Mito-R004	GTTTAATTATCAGTGATTTAATTC	24	R	–	x	15694-15717
Chon-Mito-S005	AGGCAAGTCGTAACATGGTAAG	22	F	x	x	14832-14855
Chon-Mito-S007	CACTGA(CT)AATTAAAC(AG)A(ACT)CCCA	22	F	–	x	15703-15724
Chon-Mito-R008	CCACTCTTTTGCCACAGAGA	20	R	–	x	15260-15279
Chon-Mito-S009	CACGAGAGTTTAACTGTCTCT	21	F	–	x	15993-16013
Chon-Mito-R010	TAGAGACAGTTAAACTCTCGT	21	R	–	x	15992-16014
Chon-Mito-S014	AGTGGGCCTAAAAGCAGCCA	20	F	–	x	15504-15523
Chon-Mito-R017	ATCCAACATCGAGGTCGTAAACC	23	R	x	x	16363-16385
Nuclear						
Chon-Rag1-S018	ACAGTCAAAGCTACTAC(AG)GGGA	22	F	x	–	1576-1597
Chon-Rag1-S019	TGGCAGATGAATCTGACCATGA	22	F	–	x	2096-2117
Chon-Rag1-S020	TGTGAACTGAT(CT)CCATCTGAAG	22	F	–	x	2719-2740
Chon-Rag1-R021	AATATTTTGAAGTGTACAGCCA	22	R	x	x	3094-3115
Chon-Rag1-R022	CTGAAACCCCTTTCACTCTATC	22	R	–	x	2440-2461
Chon-Rag1-R023	CCCATTCCATCACAAGATTCTT	22	R	–	x	1904-1925
Chon-Rag1-S024	CAGATCTTCCAGCCTTTGCATGC	23	F	x	x	1600-1622
Chon-Rag1-R025	TGATG(CT)TTCAAAATG(CT)CTTCCAA	23	R	x	–	3070-3092
Chon-Rag1-S026	TTCC(TA)GCCTTTGCA(CT)GCACTCCG	23	F	x	x	1606-1628
Chon-Rag1-S027	GAGA(CT)TCTCAGAGAGTTAATGCA	23	F	–	x	2749-2771
Chon-Rag1-R028	GT(CT)TCATGGTCAGATTCATC(CT)GC	23	R	–	x	2098-2120
Chon-Rag1-R029	AGTGTACAGCCA(AG)TGATG(CT)TTCA	23	R	x	x	3083-3105
Chon-Rag1-S030	GTGAG(AG)TATTCCTT(CT)AC(AC)ATCATG	24	F	–	x	1975-1998
Chon-Rag1-S031	GA(AG)CGCTATGAAAT(CT)TGGCGTTCA	24	F	–	x	2383-2406

[a] The position of the primers refers to the 5′–3′ position in the complete mitochondrial genome sequence of *Scyliorhinus canicula* (GenBank Accession No. Y16067) and in the RAG1 sequence of *Carcharhinus leucas* (GenBank Accession No. U62645).

drial 45 taxa and 17 taxa data sets. Branch and bound search option was used for RAG1 17 taxa and combined 17 taxa data sets (Table 3). Mutational saturation (i.e., multiple substitutions at a single site) was explored by plotting the pairwise number of observed nucleotide differences against the pairwise number of inferred substitutions. For this, the COMP-MAT program of MUST was used, the pairwise number of observed differences being computed by MUST and the pairwise number of inferred substitutions being computed using PAUP* as the number of steps met in the path joining the two species in the most parsimonious tree. All subtitutions were equally weighted to stay in the logical foundations of parsimony. If weights have to be given, a weighting scheme has to be proposed and in that case we preferred to use a model under the maximum likelihood criterion. Phylogenetic analyses under that criterion have been performed using PAUP* version 4.0b8. The model used

Table 3
Properties of matrices and MP trees

	mtDNA, 45 taxa	mtDNA, 17 taxa	RAG1, 17 taxa	Combined, 17 taxa
No. of taxa	45	17	17	17
No. of homologous sites	1542	1530	1454	2984
No. of parsimony-informative sites	445	354	294	648
No. of variable sites	587	494	410	904
No. of conserved sites	955	1036	1044	2080
No. of sites containing gaps	59	42	0	42
A %	36.3	36.4	32.0	35.2
C %	20.1	21.0	17.7	19.4
G %	16.6	16.5	24.9	18.8
T %	27.0	27.1	25.4	26.6
Base frequence homogeneity (p)	1 ($\chi^2 = 24.55$)	1 ($\chi^2 = 10.21$)	0.99 ($\chi^2 = 17.76$)	0.99 ($\chi^2 = 15.98$)
MP tree length	2059	1173	604	1785
No. of MP trees	12	5	2	2
C.I.	0.41	0.57	0.77	0.63
R.I.	0.66	0.56	0.83	0.67
Analysis option for MP	Heuristic × 1000	Heuristic × 10000	B & B	B & B

is GTR + Γ + I. Model parameters were all estimated through an iterative process of cycling between parameter estimation and optimal tree searching as described in Swofford et al. (1996) and Chen et al. (2003). Robustness of clades (i.e., monophyletic groups) was estimated for the four data sets using 1000 iterations of bootstrapping and using decay index calculated with PAUP*.

3. Results

3.1. Characteristics of the mitochondrial and nuclear gene fragments

Both mitochondrial and nuclear sequences were obtained using forward and reverse internal primers, consequently the obtained fragments were read entirely between the two PCR primers. The mitochondrial fragments sequenced are 1500–1509 bp long depending on species (Table 1). As different primer pairs were used to amplify the RAG1 sequences from the different species, the computational analyses were conducted on the smallest resulting fragment homologous to all taxa, i.e., 1454 bp which was the Chon-Rag1-S026/Chon-Rag1-R029 region. Gaps are not direct observations from nature but are created by primary homologies established during the alignment. As primary homology is a hypothesis of common ancestry based on shared similarities, we did the initial hypothesis that these gaps must come from events of insertion and/or deletion in common ancestors, and for this reason we considered gap as a fifth character in the final analysis. The 62 sequences used here represent an amount of 92,396 bp prior to alignment and 94,108 bp after alignment.

3.2. Intra-individual polymorphic positions and intra-specific variability

In some cases, two peaks equal in intensity are present at a single position on the electropherogram of the RAG1 sequences, indicating heterozygosity. Six RAG1 sequences have a single intra-individual polymorphic position and one sequence has two polymorphic positions. Six of the eight polymorphic positions are A/G and two are C/T. This polymorphism occurs on the third position of the codon without changing the amino acid in the protein, except for two codons where it occurs on the second position leading to two possible amino acids. Considering all the 17 sequences (24,718 bp) obtained for the RAG1 gene, 0.032% of the nucleotidic positions present intra-individual variations. As the amount of polymorphic positions is extremely low, these positions were coded as ambiguous and it did not affect the cladistic analysis. Comparing the 940 aligned bp between our RAG1 sequence and the RAG1 GenBank sequence AF135476 for *C. maximus*, we observed five different positions and when comparing the 940 aligned bp between our RAG1 sequence and the RAG1 GenBank sequence AF135474 for *O. ferox*, we observed six different positions. Considering the two sequences for the RAG1 gene of these two species, 0.585% of the nucleotidic positions present intra-specific variability. This intra-specific variability observed can be natural or due to lab errors. Anyway it is a low variability, distinctly lower than inter-specific variability and with no noticeable impact on tree reconstruction.

3.3. Data set and trees informations

MP and ML tree-reconstruction algorithms require homogeneity of nucleotide frequencies for optimal performance (Omilian and Taylor, 2001). A test of homogeneity of base frequencies across taxa using χ^2 test was performed using PAUP*. The null hypothesis of homogeneity of base composition across taxa was rejected for none of the four data sets (p value > 5%; see Table 3). No mutational saturation was detected (plots available upon request). The ILD test did not reject the null hypothesis of congruence (p value of 38%). Properties of each data set and data from most parsimonious tree searches are summarized in Table 3. Strict consensus of these MP trees is presented in Figs. 2–4. ML analyses provided the same trees; for that reason we prefer to exhibit results obtained without the burden of models, for the sake of simplicity. Because most of the bootstrap values on tree branches appear >70% and because there is a probability of $\geqslant$95% that a clade is real if the corresponding bootstrap is $\geqslant$70% (Hillis and Bull, 1993; Lecointre et al., 1994), most of the groups obtained in our different trees are considered as strongly supported. Tree of the Fig. 2 includes much more information than trees of the Figs. 3 and 4, because more species are included but the reliability of corresponding nodes is difficult to evaluate because they are based on a unique gene.

Comparison of matrices (Table 3) and trees (Fig. 3) properties obtained for 17 taxa with the equal length mitochondrial and nuclear sequences, led us to consider RAG1 sequences to be slightly more powerful than the mitochondrial sequences for resolving relationship within this group. The phylogenetic analysis of the RAG1 matrix provides higher CI and RI than the analysis of the mitochondrial gene. Also, 11 of the 12 resolved nodes of the RAG1 tree have bootstrap $\geqslant$75% whereas it is only 8 of the 11 resolved nodes for the mitochondrial gene. Only two nodes are unresolved in the RAG1 tree (see polytomy of node "D") whereas it is three in the mitochondrial tree (see polytomies of nodes "D" and "F").

3.4. Phylogenetic relationship

Fig. 2 shows the strict consensus tree obtained from mtDNA sequences for 45 taxa. Fig. 3 shows the two strict consensus trees obtained from mtDNA sequences

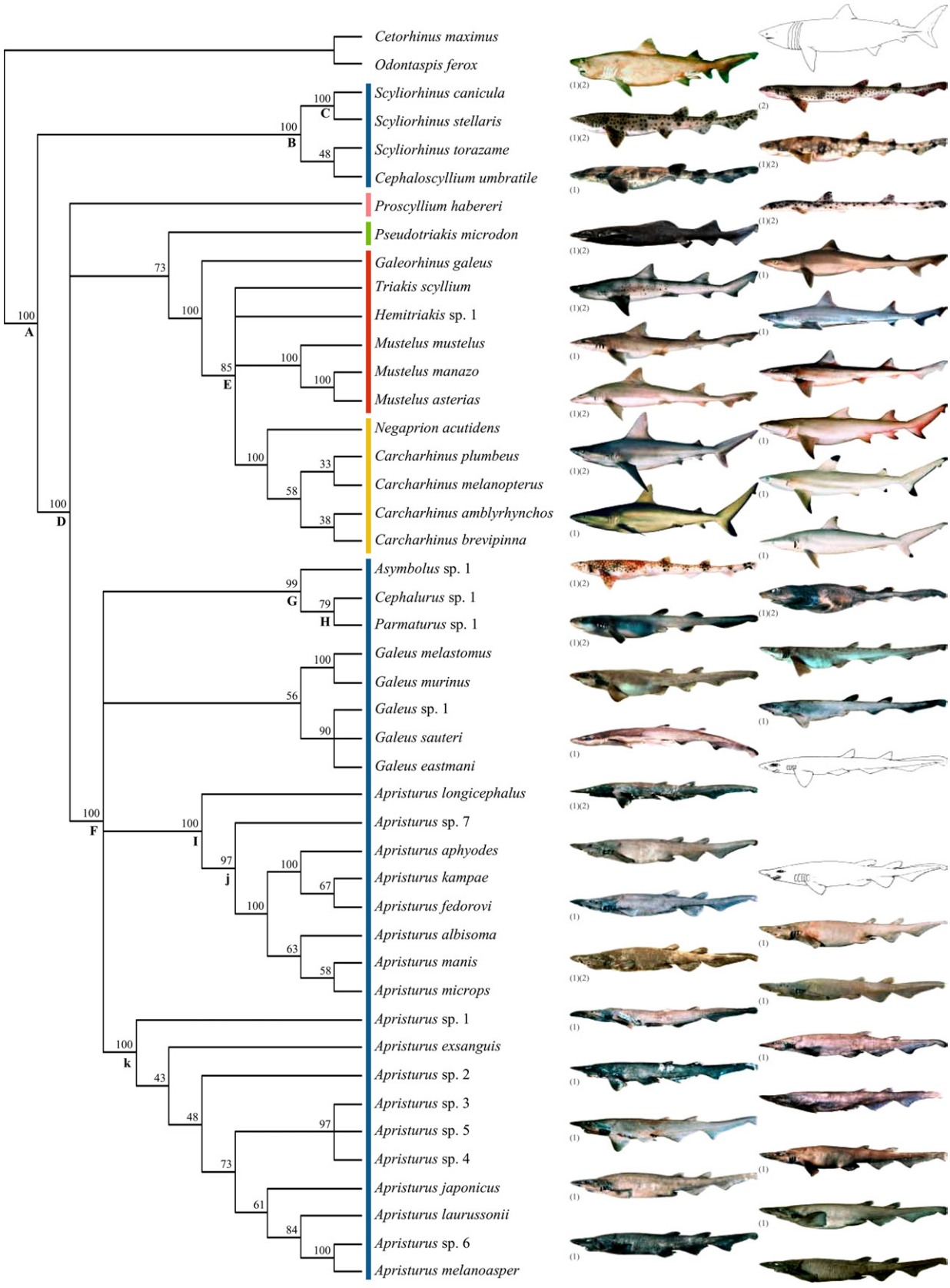

Fig. 2. Strict consensus of 12 MP trees (see values of these trees Table 3) calculated from the mtDNA data set (1542 aligned positions; 45 taxa; length 2059; CI = 0.41; RI = 0.66). MP-bootstrap support values obtained from 1000 replicates appear above the branches. Blue: Scyliorhinidae; pink: Proscyllidae; green: Pseudotriakidae; red: Triakidae; and yellow: Carcharhinidae. (1) Photo from the voucher specimen used for mtDNA sequences; (2) photo from the voucher specimen used for RAG1 sequence. Nodes with capital letters are reliable under repeatability criterion and robust in the analyses of 17 taxa (see Figs. 3 and 4).

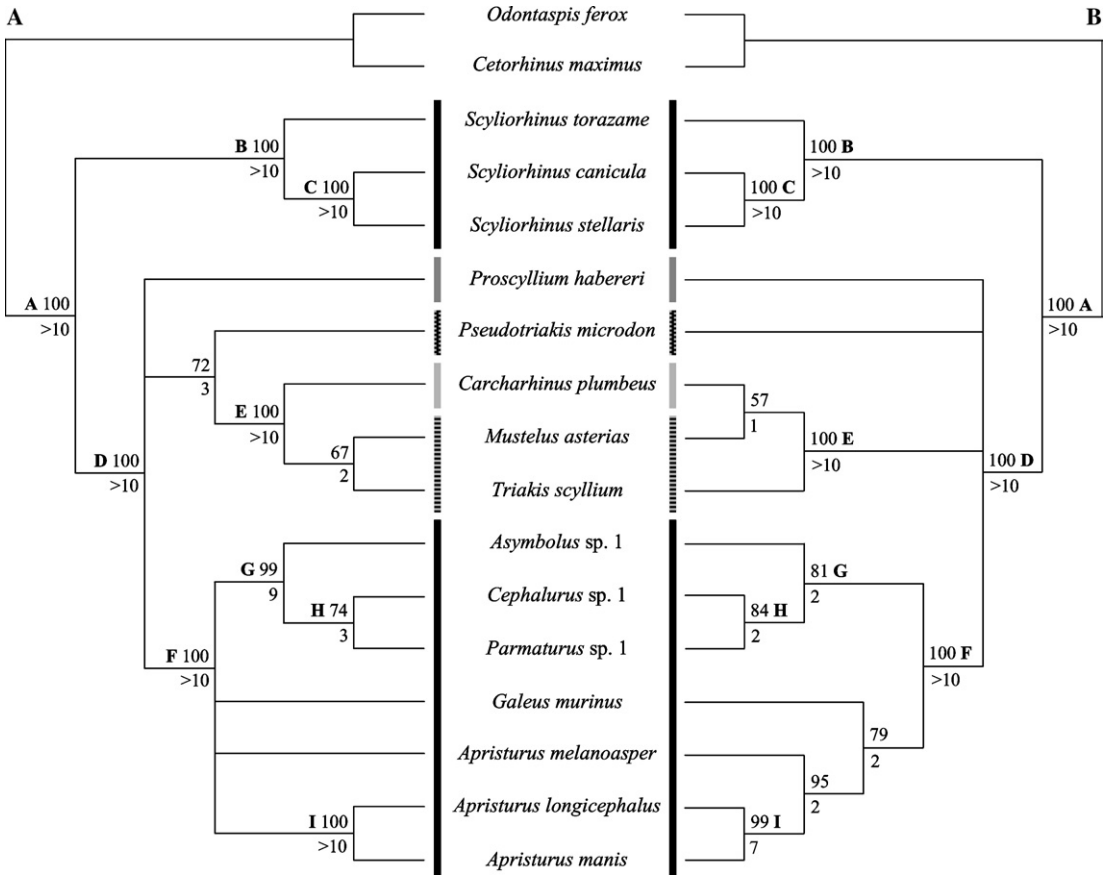

Fig. 3. (A) Strict consensus of five equally-parsimonious trees from mtDNA data set (1530 aligned positions; 17 taxa; length 1173; CI = 0.57; RI = 0.56); (B) Strict consensus of two equally-parsimonious trees from the RAG1 data set (1454 aligned positions, 17 taxa; length 604; CI = 0.77; RI = 0.83). MP-bootstrap support values obtained from 1000 replicates appear above the branches; decay indices appear under the branches. Black bar: Scyliorhinidae; dark grey bar: Proscyllidae; black with white spots bar: Pseudotriakidae; pale grey bar: Carcharhinidae; and grey striped bar: Triakidae. Nodes with capital letters are reliable under the repeatability criterion.

and from RAG1 sequences for the same 17 taxa. Fig. 4 shows the MP tree calculated from combined mtDNA + RAG1 sequences for the 17 taxa. The two trees of the Fig. 3 present 9 common nodes so these nodes are considered as reliable (nodes "A" to "I"). The same nodes "A" to "I" are also obtained within the tree from the simultaneous analysis (Fig. 4) with high robustness and obtained from ML approaches (data not shown).

Node "A" shows the studied carcharhiniforms as monophyletic. Node "B" shows *Scyliorhinus* as a clade (*Cephaloscyllium* is added in this clade for the tree in Fig. 2) corresponding to a first subgroup of scyliorhinids. Node "D" shows that the Proscyllidae, Pseudotriakidae, Carcharhinidae, Triakidae and a second subgroup of Scyliorhinidae (node "F") do form a clade. Node "E" is the clade grouping *Triakis*, *Mustelus*, and *Carcharhinus* (*Hemitriakis* and *Negaprion* are added in this clade in the tree of the Fig. 2). Node "F" groups *Asymbolus*, *Cephalurus*, *Parmaturus*, *Galeus*, and *Apristurus*, corresponding to the second subgroup of scyliorhinids. Node "I" groups *Apristurus longicephalus* and *A. manis* as a clade (Six others *Apristurus* are included in this clade in the tree of the Fig. 2).

An important result observed in these trees is the strongly supported and repeatable paraphyly of scyliorhinids (node "D"). All the other families investigated in the present study (Carcharhinidae, Proscyllidae, Pseudotriakidae, and Triakidae) are sister group to the subgroup of scyliorhinids composed by *Apristurus* + *Asymbolus* + *Cephalurus* + *Galeus* + *Parmaturus* (node "F"). The other subgroup of scyliorhinids composed by *Scyliorhinus* + *Cephaloscyllium* (node "B") has the most basal branching within Carcharhiniformes. This paraphyly is obtained with the two genes and is supported in all trees by bootstrap values of 100 and decay indices higher than 10. A second important result observed is the possible paraphyly of triakids. This family appeared paraphyletic in Fig. 2 (node "E") and Fig. 3B but monophyletic in Fig. 3A and Fig. 4. Paraphyly for triakids in Fig. 2 is supported by a bootstrap value of 85% with the branching of *Galeorhinus galeus*. In Fig. 3 the branching of *Triakis scyllium* supports paraphyly of triakids from RAG1 gene (Fig. 3B) but mono-

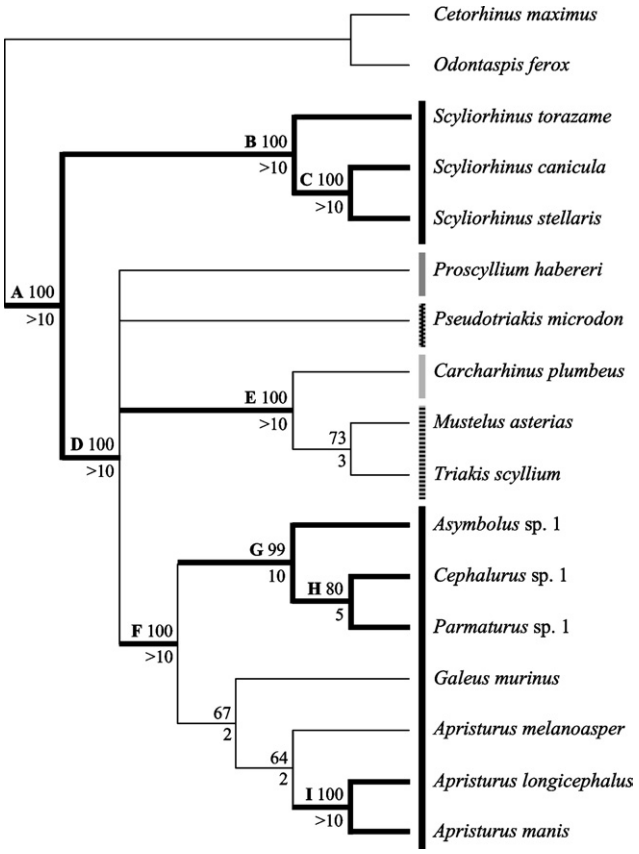

Fig. 4. MP tree from the simultaneous analysis of mtDNA + RAG1 sequences (2984 aligned position, length 1785; CI = 0.63; RI = 0.67). MP-bootstrap support values obtained from 1000 replicates appear above the branches; decay indices appear under the branches. Black bar: Scyliorhinidae; dark grey bar: Proscyllidae; black with white spots bar: Pseudotriakidae; pale grey bar: Carcharhinidae; and grey striped bar: Triakidae. Bold branch in the tree as nodes with capital letters are those reliable and robust (see also Fig. 3).

phyly of triakids from mitochondrial sequences (Fig. 3A). This is the only discrepancy between the trees obtained with nuclear and mitochondrial sequences.

Monophyly of the genus *Apristurus* is obtained in the tree from simultaneous analysis (Fig. 4) and from the RAG1 data (Fig. 3B) but is unresolved from the mitochondrial data (Figs. 2 and 3A). The monophyly of the genus *Apristurus* is strongly supported with a bootstrap value of 95% from the RAG1 data (Fig. 3B). Two subgroups of *Apristurus* (nodes "I" and "k" in Fig. 2) appeared very well supported from the mitochondrial data, having bootstrap values of 100%.

4. Discussion

4.1. Robustness and reliability

Comparison of trees issued from separate analyses of independent data sets helps in assessing the reliability of clades. Separate and simultaneous analyses must be both conducted to obtain indicators of reliability and robustness, respectively. This is because (1) it is possible that a node repeated among the separate analyses is not recovered in the tree from the simultaneous analysis (Barrett et al., 1991; Dettaï and Lecointre, 2004) and (2) it is possible that a robust node obtained from a tree-reconstruction artefact from a single data set (therefore not repeated) is recovered in the tree from simultaneous analysis (Chen, 2001; Chen et al., 2003), and (3) robustness generally increases in trees based on more data. The repeatability of a clade in several independent trees appears as a more convincing indicator of reliability than bootstrap value or others indices of robustness (Chen et al., 2003). By performing independent trees using mitochondrial and nuclear sequences from the same 17 taxa, the nodes found repeated in the two independent trees through a single method are considered as reliable. In such an approach we do not mix different methods and different data sets, in order (1) not to artificially increase repeatability, and (2) not to mix sensitivity (which is an approach of robustness, in each calculation data being not independent) and repeatability (that must come from independent sources of data).

4.2. Relationships within Carcharhiniformes subgroups

The monophyly of the genus *Apristurus* and the branching of *Galeus* as its sister group is supported by the analysis on the RAG1 gene (Fig. 3B) and by the combined analysis (Fig. 4) but is unresolved from the mtDNA sequence (Figs. 2 and 3A). Polytomy is not contradicting the results from RAG1 data, so we consider the genus as monophyletic and *Galeus* as its sister group. The species groupings within *Apristurus* distinguished by Nakaya and Sato (1999) are the *longicephalus* group, the *brunneus* group and the *spongiceps* group (Fig. 1I). The species clustered with molecular data are in line with the three groups of species according to Nakaya and Sato (1999) but relationships between these groups are different. In our analysis as for Sato (2000) the *longicephalus* group is sister group to the *spongiceps* group (node "j") whereas for Nakaya and Sato (1999) the *brunneus* group (node "k") was supposed to be the sister group of the *spongiceps* group based on the shared character "short snout". Sampling of additional species of the *longicephalus* group will test the monophyly of that group.

In our molecular analysis we found triakids paraphyletic from the 45 taxa mitochondrial matrix and the 17 taxa nuclear matrix but neither from the 17 taxa mitochondrial matrix nor the combined 17 taxa data set. Maisey (1984) as Compagno (1988) found triakids paraphyletic (Figs. 1C and D2), including the carcharhinids, but these authors placed *Mustelus* with a basal branching and *Galeorhinus* in a derived position, whereas in our

analysis this is the opposite. Further studies on the branching of *Galeorhinus* appear important to clarify the probable paraphyly of triakids.

In the tree inferred from the best taxonomic sample (Fig. 2) as for Naylor (1992), *Negaprion* is sister group of *Carcharhinus*, whereas Compagno (1988) and Lavery (1992) found *Negaprion* nested within *Carcharhinus*. Our nodes are poorly supported within Carcharhinids so further analysis are needed to resolve the placement of *Negaprion*. Incomplete taxon sampling within carcharhinids limits discussions about the monophyly of this group.

The monophyly of the subgroup of scyliorhinids composed by the genera *Asymbolus* + *Cephalurus* + *Parmaturus* + *Galeus* + *Apristurus* (node "F") is strongly supported by our molecular analyses and is also always found in morphological analyses (Compagno, 1988; Nakaya, 1975; Sato, 2000). Nevertheless relationships between these genera within the clade "F" are not the same as those from these authors. Our analysis strongly supports *Parmaturus* and *Cephalurus* as sister groups and *Asymbolus* as the sister group of *Cephalurus* + *Parmaturus*.

4.3. Morphological considerations

Presence or absence of supraorbital crests on the chondrocranium is the first character used in dichotomic keys for scyliorhinid genera (Springer, 1979) (Fig. 1B). It separates two groups, a first one with crests on the chondrocranium, composed by the six genera *Atelomycterus*, *Aulohalaelurus*, *Cephaloscyllium*, *Poroderma*, *Schroederichthys*, and *Scyliorhinus*, and a second one without crests on the chondrocranium composed by the nine genera *Apristurus*, *Cephalurus*, *Galeus*, *Halaelurus*, *Haploblepharus*, *Holohalaelurus*, *Parmaturus*, and *Pentanchus*. The paraphyly of scyliorhinids observed in the present study based on seven of the 15 genera of the family proposed two groups, a first one comprising the genera *Scyliorhinius* and *Cephaloscyllium* (Clade "B") and a second one composed by the genera *Apristurus*, *Galeus*, *Parmaturus*, *Asymbolus*, and *Cephalurus* (Clade "F"). These two groups are in line with the two groups of scyliorhinids commonly recognized using the chondrocranium structure. The outgroup (lamniforms) as well as the clade "B," which has the most basal branching within carcharhiniforms, possess supraorbital crests. So the presence of supraorbital crests appears as a primitive character state in our trees. The crests are also present in proscyllids, pseudotriakids and triakids. Absence of crest, which is found in carcharhinids and in the clade "F," appears as a derived character state and can be explained by two putative losses that occurred independently as suggested in Nakaya (1975), once in carcharhinid ancestor and once in the ancestor of the clade "F."

4.4. New classification for scyliorhinids

Three independent cladistic analysis using molecular data (Winchell et al., 2004, present study) and morphology (Maisey, 1984) now support scyliorhinid paraphyly, which must have an impact on the nomenclature of scyliorhinids. The family Scyliorhinidae (Gill, 1862) was defined on the type genus *Scyliorhinus*, so we redefine the Scyliorhinidae sensu stricto as the family including the genera *Atelomycterus*, *Aulohalaelurus*, *Cephaloscyllium*, *Poroderma*, *Schroederichthys*, and *Scyliorhinus*, and characterized by the presence of supraorbital crest on the chondrocranium (node "B" in our trees). The family Pentanchidae (Smith and Radcliffe, in Smith, 1912) was created for the single species *Pentanchus profundicolus* (not sampled in our study) and erroneously placed by these authors within Hexanchiformes based on the absence of first dorsal fin. Regan (1912) correctly noted that *Pentanchus* was a scyliorhinid and synonymized pentanchids with it. *Pentanchus* was regarded by Garman (1913) and subsequent authors as very close to the genus *Apristurus* and was sometimes synonymized with it (e.g., Fowler, 1934). The new ranks Scyliorhininae and Pentanchinae were created by Compagno (1988) for two scyliorhinid subfamilies (Fig. 1D3), and followed the two scyliorhinid groups based on chondrocranium structure, but these subfamilies were abandoned in the next Compagno's studies. As the clade "F" recovers the definition of Pentanchinae in the sense of Compagno (1988), we propose to resurrect here the family name Pentanchidae (Smith and Radcliffe, in Smith, 1912) and we assign it the content of Pentanchinae as in Compagno (1988), i.e., including the genera *Apristurus*, *Asymbolus*, *Cephalurus*, *Galeus*, *Halaelurus*, *Haploblepharus*, *Holohalaelurus*, *Parmaturus*, and *Pentanchus*. That clade is characterized by the absence of supraorbital crest on the chondrocranium (node "F" in our trees).

4.5. Main conclusion and perspectives

Our results from nuclear coding and mitochondrial ribosomal genes are similar to those of Winchell et al. (2004) from nuclear genes (LSU and SSU rRNA) and to those of Maisey (1984) from morphology. Both studies found scyliorhinids as a paraphyletic group including triakids and carcharhinids and also proscyllids and pseudotriakids in the present study. Morphology supports that the non-sampled carcharhiniform families (hemigaleids, leptochariids, and sphyrnids) will be also nested within scyliorhinids. Triakids very probably are paraphyletic, including the family Carcharhinidae, but more studies are needed to clarify it. Molecular phylogenies have logically focused first on clarifying basal nodes within the tree of elasmobranchs and knowledge has made some progress in this field. Works on more terminal nodes with a dense taxonomic sampling will be the new challenge for future molecular phylogenies of sharks.

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