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Variation in Brain Organization and Cerebellar Foliation in Chondrichthyans: Sharks and Holocephalans

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Key Words
Allometry · Ecomorphology · Nervous system · Cerebellum · Morphometrics · Neuromorphology · Comparative brain morphology · Chondrichthyan

Abstract
The widespread variation in brain size and complexity that is evident in sharks and holocephalans is related to both phylogeny and ecology. Relative brain size (expressed as encephalization quotients) and the relative development of the five major brain areas (the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla) was assessed for over 40 species from 20 families that represent a range of different lifestyles and occupy a number of habitats. In addition, an index (1–5) quantifying structural complexity of the cerebellum was created based on length, number, and depth of folds. Although the variation in brain size, morphology, and complexity is due in part to phylogeny, as basal groups have smaller brains, less structural hypertrophy, and lower foliation indices, there is also substantial variation within and across clades that does not reflect phylogenetic relationships. Ecological correlations, with the relative development of different brain areas as well as the complexity of the cerebellar corpus, are supported by cluster analysis and are suggestive of a range of 'cerebrotypes'. These correlations suggest that relative brain development reflects the dimensionality of the environment and/or agile prey capture in addition to phylogeny.

K.E.Y. and T.J.L. contributed equally to this work.

Introduction

Structural diversity can be used as a tool to understand brain function and evolution. Despite the basal position of chondrichthyans, or cartilaginous fishes, they have received less attention in this regard than other vertebrate classes. The class Chondrichthyes is comprised of approximately 1,100 extant species worldwide [Compagno, 1999] and represents a very successful vertebrate lineage. They are divided into two unequal subclasses: the Elasmobranchii, i.e. the modern sharks and batoids (skates and rays), representing 96% of described species, and the Holocephalii, i.e. chimaeras, elephant fishes, ratfishes, and spookfishes, that make up the remaining 4% [Compagno, 1999]. The Elasmobranchii and Holocephalii are thought to have diverged approximately 350 million years ago [Compagno, 1977], but aspects of the evolutionary relationships between taxa within these subclasses remain unresolved [Compagno, 1973, 1999; Maisey, 1984;

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Shirai, 1992b; Dunn and Morrissey, 1995; de Carvalho, 1996; McEachran et al., 1996; Shirai, 1996; Maisey et al., 2004].

Chondrichthyans, especially sharks and batoids, are known to possess relatively large brains, especially in comparison to other ectothermic vertebrates [Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov, 1991]. However, although detailed and descriptive illustrations of brain morphology from a number of species have provided evidence of substantial interspecific variation of component parts [Garman, 1913; Kappers et al., 1936; Masai, 1969; Okada et al., 1969; Northcutt, 1977, 1978; Smeets et al., 1983; Kruska, 1988; Demski and Northcutt, 1996; Smeets, 1998; Hofmann, 1999; Ito et al., 1999], there is a lack of quantitative information on brain organization and the relative development of major brain areas across this group, specifically the way in which this variation correlates with phylogeny and ecology [Northcutt, 1977, 1978; Kruska, 1988; Narendra, 1990; Demski and Northcutt, 1996]. In contrast, there are large quantitative data sets on brain organization of other vertebrate groups such as teleost fishes, birds, and mammals. Strong correlations have been found between brain patterns and various ecological factors, such as diet and feeding habits in teleosts [Bauchot et al., 1977; Huber and Rylander, 1992; Kotrschal and Palzenberger, 1992; Schellart and Prins, 1993; Huber et al., 1997; Kotrschal et al., 1998] and mammals [Eisenberg and Wilson, 1978; Pirlot and Jolicoeur, 1982; Harvey and Krebs, 1990; Hutcheon et al., 2002], habitat complexity in teleosts [Huber et al., 1997], birds [Riddell and Corl, 1977], and mammals [Barton et al., 1995], and increased sociality and/or cognitive skills in birds [Lefebvre et al., 1998, 2002] and mammals [Kudo and Dunbar, 2001]. A recent conclusion based on these studies is the recognition of groups of species that share certain common characteristics in the relative development of brain areas; these commonalities are termed 'cerebrotypes' [Clark et al., 2001; Iwaniuk and Hurd, 2005]. Although the extent to which cerebrotypes relate to phylogeny or ecology varies among taxa [Iwaniuk and Hurd, 2005], there are a number of cases where species that possess the same cerebrotype are also linked as a group by shared lifestyle similarities, such as habitat, feeding strategy, or cognitive capability.

In this paper, we have concentrated on sharks and, to a much lesser extent, the holocephalans. Northcutt [1977, 1978] proposed an initial broad classification of the brains of sharks into two major categories, using the taxonomic schema proposed by Compagno [1973, 1977]. Accordingly, the more ancestral squalomorph and squatinomorph sharks (which includes the orders Hexanchiformes, Squaliformes, Pristioformes, and Squatinoformes), possess a smooth, undifferentiated cerebellar corpus and smaller telencephalon, whereas the advanced galeomorph sharks (the Heterodontiformes, Orectolobiformes, Lamniformes, and Carcharhiniformes) have a foliated cerebellum and hypertrophied telencephalon. The aim of the present study was to extend this initial phylogenetic classification across a wider range of species and to investigate both relative brain size (encephalization) and brain organization in sharks (and to a lesser degree, holocephalans) in relation to ecological factors and the cerebrotype concept, with the aid of multivariate statistics. The relative development of five major brain areas (the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla), was assessed using two sectioning techniques in a broad selection of shark species and one species of holocephalan from a number of habitats representing a range of different lifestyles. Additionally, particular attention was paid to variation in the morphology of the corpus cerebellum, which was assessed using a novel visual grading method. This brain area first appeared in early chondrichthyans [Butler, 2003] and is clearly related to the cerebellar-like structures of the adjacent hindbrain. There is substantial variation in both the degree of foliation and symmetry exhibited by this structure in sharks (and indeed other chondrichthyans) [Kappers et al., 1936; Northcutt, 1977, 1978; Smeets et al., 1983], but as the functional role of this brain area is still controversial, the adaptive significance of this variation is unclear [Northcutt, 1989; New, 2001]. Although much cerebellar research has been conducted on mammals [Bard and Macht, 1958; Marr, 1969; Albus, 1971; Ito, 1984; Gordon et al., 1993; Lackner and Dizio, 1994; Shadmehr and Musso-Ivaldi, 1994; Bastian et al., 1996; Gao et al., 1996; Lang and Bastian, 1999; Earhart and Bastian, 2000, 2001], the earliest vertebrates to have evolved a cerebellum have received comparatively little scrutiny. Early studies of elasmobranchs did not find strong ecological correlations for cerebellar hypertrophy and convolution outside the observation that elasmobranchs that move more rapidly seem to have a more complicated cerebellum [Northcutt, 1989]. Behavioral research suggests that the cerebellum modulates motor tasks [Paul and Roberts, 1979; New, 2001] and error correction [Gluck et al., 2001; Montgomery et al., 2002]. However, other evidence points to the cerebellum as involved in coordination of target tracking and the analysis of the consequences of an organisms' own movements, rather than control of these movements themselves [Paulin, 1993].

Materials and Methods

Specimen Collection

Individuals from 43 species of shark and one species of holocephalan were obtained from various localities in Australasia and Hawaii (according to the ethical guidelines of the National Health and Medical Research Council of Australia and/or the University of Auckland), using a range of fishing methods. Adult individuals were used wherever possible to limit allometric bias [Brandstätter and Kotrschal, 1990].

Each animal was deeply anaesthetized in either 0.4 g/l seawater of MS222 (m-aminobenzoic acid ethyl ester, methansulfate salt), or 10 ml/l of 2-phenoxyethanol 99% (ethylene glycol-monophenylether), or euthanized by severing the spinal cord. The brain was excised from each specimen and preserved in a range of aldehyde-based fixatives (10% formalin in 0.1 M phosphate buffer, 4% paraformaldehyde in 0.1 M phosphate buffer, and Karnovsky's; 2% paraformaldehyde and 2.5% glutaldehyde in 0.1 M cacodylate buffer). In most cases, the brains were immersion fixed, but some animals were either transcardially perfused with fixative or were donated frozen by other researchers and were therefore thawed while immersed in fixative [Demski and Northcutt, 1996; Ito et al., 1999]. All brains were post fixed for at least four months.

Brain Mass

Each brain was detached from the spinal cord caudal to the southern tip of the fossa rhomboidea in the region of the first complete cervical spinal nerve. The meninges, blood vessels, choroid plexa, olfactory bulbs and peduncles, and connective tissue were dissected away and the cranial and sensory nerves were transected to within 3 mm from their base. Each brain was blotted and weighed to the nearest 0.01 g. The sampling error of using this method was estimated to be less than 1.3%, based on ten repeated measurements of brains from six different species. Brain masses were not corrected for shrinkage due to fixation. Body mass information was recorded on fresh, unfixed samples.

Brain Organization

The relative size of five brain areas, the telencephalon, diencephalon, mesencephalon, cerebellum and medulla (fig. 1), was assessed in each species. Two separate sets of data were used, each of which employed a different sectioning method (see table 1). For the first method (termed 'Process 1' or P1), the five brain areas were identified using the criteria of Northcutt [1977, 1978], dissected, and weighed to give the relative size of each brain area as a proportion of total brain mass. The sampling error of using this method was estimated to be ±1.06%, based on ten repeated measurements of each major brain area from the shark *Sphyrna mokarran*.

In the second method (P2), each brain was embedded in a cube of agar (concentration 12 g/200 ml H_2O) post-fixation and sectioned transversely into 1-mm slices using a Vibrotome (Campden Instruments Ltd, Loughborough, England). Each section was photographed (Nikon E990) microscopically and analyzed using the SigmaScan® image analysis program (Systat Software Inc., Richmond, Calif., USA). The five major brain areas were delineated digitally. The area of each structure within a 1-mm-thick section was determined and digitally 'restacked' to reconstruct individual volumes of each brain area. The volumes of each brain area were then estimated by multiplying its area on each image by the depth of each cross section and summing the individual volumes to create a whole, to give the relative size of each brain area as a proportion of total brain volume. This method was only appropriate for specimens that had a maximum post-mortem time of three hours prior to fixation.

The relative size of each brain area was independently assessed using both methods in individuals of four species. The maximum variation found from using both methods was less than 3.2%, which is within the range of the intraspecific variation in the relative size of the five brain areas in similarly-sized individuals for which more than three specimens were analyzed. Therefore the two data sets were combined for further analysis.

Cerebellar Foliation Index

A visual grading method was developed in order to assess the degree of foliation exhibited by the cerebellar corpus of each species. Using photographs of the dorsal, lateral and ventral aspects of each brain along with direct microscopic examination of each specimen, a visual foliation index was created, grading the foliation of the cerebellar corpus from 1–5 (fig. 2). This visual grading method was then validated using the SigmaScan® image analysis program, which quantified length, depth, and number of folds in the corpus. These values corresponded well with the grouping described by the foliation index.

Analysis

The new data were combined with data from Northcutt [1977, 1978], resulting in an overall data set of 46 shark species from 20 families and 2 species of holocephalan. For species where data for more than one individual were available, means were used, but not all data for all 48 species were used in all analyses.

The brain mass and body mass data for 41 species of shark and one species of holocephalan were analyzed using both the raw species data (where species are treated as independent data points) and phylogenetically independent contrasts [Felsenstein, 1985]. This was done because statistical methods that treat species values as statistically independent points are not valid; closely related species share many characters through common descent rather than through independent evolution [Harvey and Pagel, 1991]. Independent contrasts were calculated using the CAIC software package [Purvis and Rambaut, 1995a, b] and Shirai's [1992a, 1996] phylogeny, with additional information for Orectolobiformes [Goto, 2001], Lamniformes [Martin et al., 1992], Carcharhiniformes [Compagno, 1988], and carcharhinids and sphyrnids [Naylor, 1992] (fig. 3). Because the branch lengths for many taxa are unknown, it was assumed that all branch lengths were equal [Purvis and Rambaut, 1995a, b].

The raw species data were plotted on logarithmic coordinates and the regression line describing the allometric relationship was calculated using least squares (LS) or Model I regression using the equation

$$y = ax^b$$

where y = brain mass, x = body mass, a is the allometric coefficient, and b is the allometric component. Encephalization quotients (EQs), the ratio of actual brain size to expected brain size for an animal of a given mass [Jerison, 1973], were calculated using the formula:

$$EQ = E_a/E_e$$

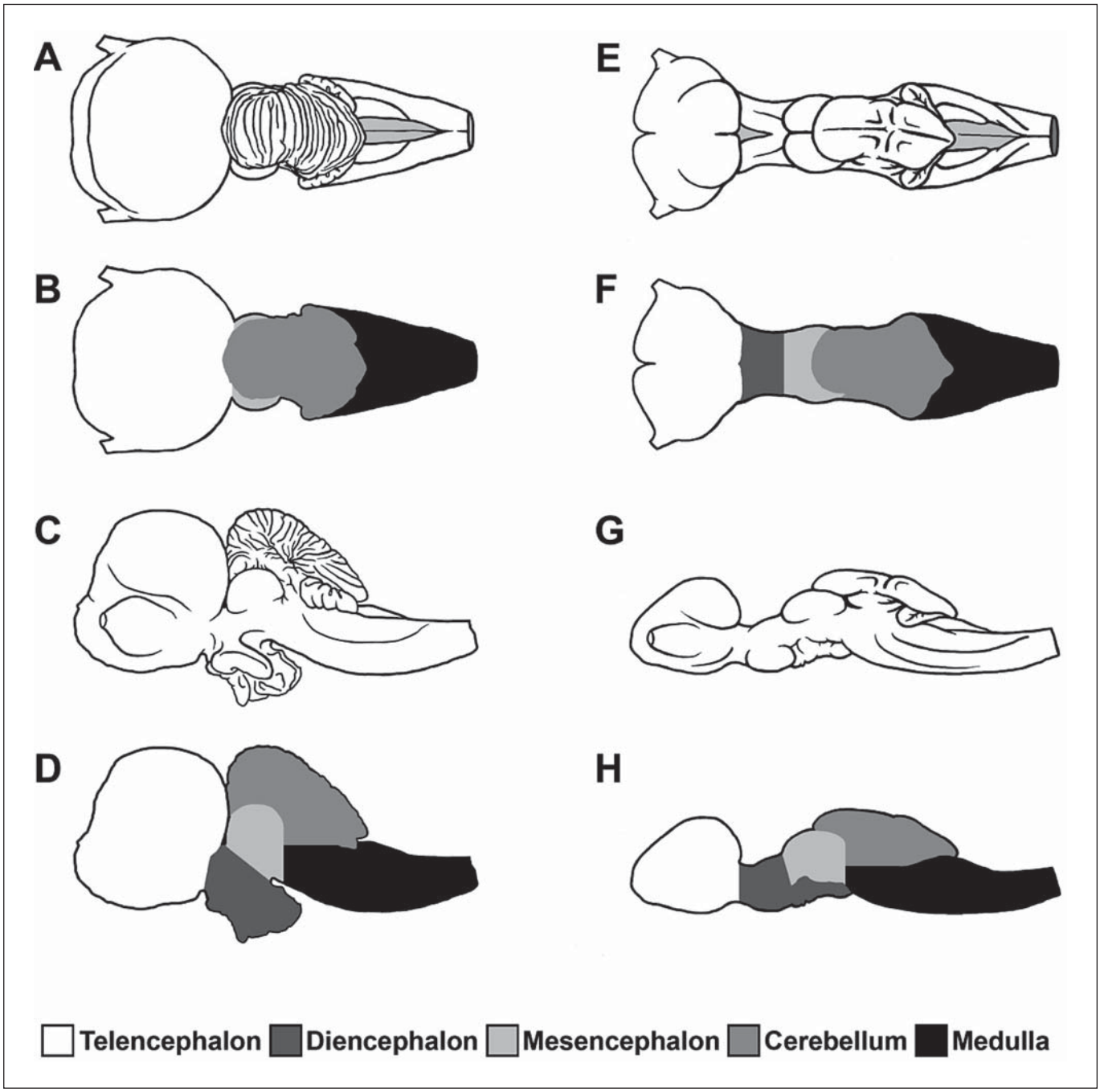


Fig. 1. Dorsal and lateral views of the brains from the sharks *Carcharhinus leucas* (**A–D**) and *Orectolobus ornatus* (**E–G**), illustrating the five major areas of the brain identified in this study. Brains are not to scale.

where E_a = actual brain mass and E_e = expected brain mass. The expected brain mass for a species was calculated using the allometric equation for the brain mass to body mass relationship. EQs of >1.0, 1.0, and <1.0 indicate that the species of interest has a relative brain mass that is greater than, average, or less than expected for its body mass, respectively.

Independent contrasts were obtained by $\log_{10}$ transforming the data and analyzing brain mass and body mass together using the CRUNCH algorithm within CAIC, with body mass as the independent variable. The dependent variable (brain mass contrasts) was then regressed on the control variable (body mass contrasts) using LS regression forced through the origin [Garland et

Table 1. Brain mass, body mass, encephalization quotient (EQ), and residual values for 46 species of shark and 2 holocephalans

Species abbrev.	Family	Genus	Species	n	Structural delineation method	Body weight, kg ± SD	Brain weight, g ± SD	EQ	Residual
NC	Hexanchidae	*Notorhynchus*	*cepedianus*	3	P2	22.17 ± 5.9	5.70 ± 0.95	0.43	0.24
SSp	Squalidae	*Squalus*	*sp.*	3	P2	3.98 ± 0.46	4.96 ± 0.09	0.94	0.47
SA		*Squalus*	*acanthias*	3	P2	0.98 ± 0.43	2.36 ± 0.43	0.95	0.38
SM		*Squalus*	*megalops*	1	P2	0.42	2.24	1.43	0.49
CB		*Cirrhigaleus*	*barbifer*	2	M	–	–	–	–
DC	Centrophoridae	*Deania*	*calcea*	1	M	–	–	–	–
EH[N]	Etmopteridae	*Etmopterus*	*hillianus*	1	P1	–	–	–	–
DL	Dalatiidae	*Dalatias*	*licha*	1	P1	24.00	3.37	0.24	0.00
PC	Pristiophoridae	*Pristiophorus*	*cirratus*	3	P2	1.08 ± 1.10	1.66 ± 0.79	0.64	0.21
BW	Brachaeluridae	*Brachaelurus*	*waddi*	1[b]	P2	0.69	1.39	0.68	0.21
OM	Orectolobidae	*Orectolobus*	*maculatus*	1	P2	12.30	3.23	0.33	0.10
OO		*Orectolobus*	*ornatus*	5	P1; P2	3.41 ± 1.16	2.11 ± 0.34	0.43	0.12
CP	Hemiscyllidae	*Chiloscyllium*	*punctatum*	2	P1	0.7	2.1	1.05	0.39
HO		*Hemiscyllium*	*ocellatum*	3	P1	0.6 ± 0.10	1.96 ± 0.17	1.04	0.38
NF	Ginglymostomatidae	*Nebrius*	*ferrugineus*	1	P1	32.2	15.16	0.92	0.61
CT	Odontaspidae	*Carcharias*	*taurus*	1	P1	152.4	14.25	0.37	0.32
PK	Pseudochariidae	*Pseudocarcharias*	*kamoharai*	1	P1	3.9	4.8	0.92	0.46
AS	Alopiidae	*Alopias*	*superciliosus*	1	P1	62.73[a]	30.2	1.28	0.80
AV		*Alopias*	*vulpinus*	1[c]	P2	5.83	11.13	1.71	0.76
CC[a]	Lamnidae	*Carcharodon*	*carcharias*	3[b]	P1; P2	727.27	29.53	0.41	0.47
IO		*Isurus*	*oxyrinchus*	3	P2	186.53 ± 8.24	25.59 ± 3.91	0.60	0.54
AA		*Asymbolus*	*analis*	1	P1	0.32	0.94	0.70	0.16
AR		*Asymbolus*	*rubiginosus*	1	P1	0.26	0.80	0.66	0.13
CI	Scyliorhinidae	*Cephaloscyllium*	*isabellum*	3	P2	1.25 ± 1.35	1.38 ± 0.39	0.49	0.10
CL		*Cephaloscyllium*	*laticeps*	3[c]	P2	0.18 ± 0.06	0.57 ± 0.12	0.58	0.04
GB		*Galeus*	*boardmani*	3	P1	0.2 ± 0.04	0.87 ± 0.09	0.83	0.21
SR[N]		*Scyliorhinus*	*retifer*	1	P1	–	–	–	–
GA	Pseudotriakidae	*Gollum*	*attenuatus*	1	M	–	–	–	–
ML	Triakidae	*Mustelus*	*lenticulatus*	3	P2	2.29 ± 0.76	5.97 ± 1.17	1.53	0.64
MA[N]		*Mustelus*	*antarcticus*	1[c]	P2	0.58	3.84	2.06	0.67
MC		*Mustelus*	*canis*	1	P1	6.50	7.15	1.04	0.55
GG		*Galeorhinus*	*galeus*	3	P2	12.18 ± 8.8	10.38 ± 3.98	1.07	0.60
HM	Hemigaleidae	*Hemigaleus*	*microstoma*	2	P1	2.33	5.17	0.87	0.40
CA	Carcharhinidae	*Carcharhinus*	*amblyrhynchos*	5	P1	25.54 ± 16.5	35.28 ± 4.45	2.44	1.01
CB		*Carcharhinus*	*brachyurus*	1[c]	P2	2.19	9.14	2.40	0.83
CF		*Carcharhinus*	*falciformes*	1	P1	97.96[a]	51.48	1.72	0.95
CLe		*Carcharhinus*	*leucas*	1	P1	72.85[a]	42.31	1.66	0.92
CMe		*Carcharhinus*	*melanopterus*	1	P1	7.65	17.93	2.38	0.92
CPl		*Carcharhinus*	*plumbeus*	1[b]	P1	16.4	21.86	1.92	0.88
GC		*Galeocerdo*	*cuvier*	1	P1	148.6[a]	19.85	0.53	0.47
NA		*Negaprion*	*acutidens*	1[c]	P1	1.74[a]	10.07	2.99	0.91
PG		*Prionace*	*glauca*	7	P1; P2	75.37 ± 3.86	18.83 ± 4.30	0.72	0.56
TO		*Triaenodon*	*obesus*	2	P1	16.30	15.36	1.35	0.73
SL	Sphyrnidae	*Sphyrna*	*lewini*	1	P1	25.00	47.03	3.29	1.14
SMo		*Sphyrna*	*mokarran*	1	P1	148.50	99.14	2.64	1.17
SZ		*Sphyrna*	*zygaena*	2[b]	P1	63.50	63.63	2.68	1.12
HC[N]	Chimaeridae	*Hydrolagus*	*colliei*	1	P1	–	–	–	–
CM	Callorhinchidae	*Callorhinchus*	*milii*	3	P2	2.85 ± 1.71	3.12 ± 0.72	0.71	0.32

[N] Data obtained from Northcutt [1978].
[a] Brain weight and body weight values for CC are from the sub-adult only. Relative volumes include 2 juvenile specimens.
[b] Sub-adult, [c] juvenile. Otherwise specimen was a mature adult.
[d] Indicates that body mass was calculated using a length-to-mass relationship;

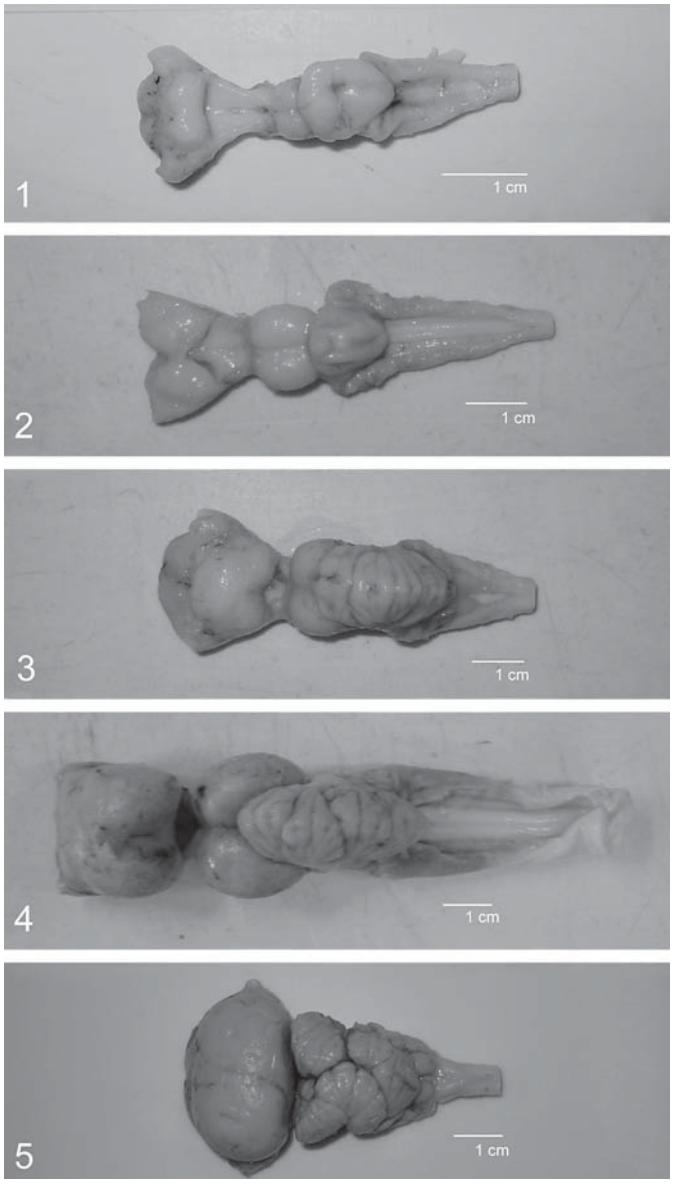


Fig. 2. Dorsal views of the brains of five species of shark, illustrating the cerebellar foliation index devised as part of this study, which involves assigning a quantitative score (1–5) to the length, depth, and number of folds in the cerebellum. Delineations were as follows: 1: No foliation, smooth cerebellar surface, cerebellar symmetry; 2: Minimal foliation, shallow grooves running parallel to one another without branching; 3: Moderate foliation, shallow to moderate grooves, slight branching; 4: Very foliated, moderate to deep, branched grooves with cerebellar symmetry; 5: Extremely foliated, deep, branched grooves, distinctive cerebellar sections; cerebellar asymmetry.

al., 1992]. The resultant regression equation, which described the allometric brain mass to body mass relationship independent of phylogenetic constraints, was then fitted to the raw species data (which were $\log_{10}$ transformed) and used to calculate residuals (the difference between the actual y value and that 'expected' by the regression equation and which are comparable to the EQs calculated using the raw species data) for each species [Purvis and Rambaut, 1995a]. Contrasts at the phylogenetic root were not included in the analysis in order to exclude 'grade' effects [Purvis and Rambaut, 1995b].

The relative sizes of each of the brain areas were expressed as percentages, which were then used for further analysis. The relative size of each brain area was compared among species using a weighted factor (θ) analysis, where the relative volume of each brain area was divided by the average for all the species [Wagner, 2001a, b]. Multivariate hierarchical cluster analysis (CLA), using Euclidean distances, was also used to determine the connectivity between individuals. As noted by Wagner [2001a, b], groupings produced by Euclidean distances strengthen the results and mitigate the limitations of a calculated deviation from the relative average (θ). The data were arcsine-transformed and analyzed using PRIMER 6 software (PRIMER-E Ltd, Plymouth, UK).

In order to relate the findings to ecology, animals were grouped according to their primary lifestyle and habitat, based on discussed [C. Duffy, personal communication] and published [Compagno, 1984a, b, 1998, 2001; Compagno and Niem, 1998a, b; Carrier et al., 2004] information. Three habitat/lifestyle categories were identified: benthic (living on the bottom), benthopelagic (living near the bottom), and pelagic (living in the water column); within these categories, five microhabitats were delineated: bathyal, demersal, reef-associated, coastal-oceanic, and oceanic.

Results

Allometric Relationships

The 41 species of sharks and one species of holocephalan studied exhibited wide variation in both body mass and brain mass (table 1). Using species as independent data points, brain mass (y) increased with body mass (x) according to the allometric relationship $y = 2.4979x^{0.5421}$ ($r = 0.872$, $n = 42$; fig. 4a). The removal of the one holocephalan species did not significantly alter the allometric relationship ($y = 2.5269x^{0.5405}$; $r = 0.872$, $n = 41$) and so the former equation was used to calculate encephalization quotients (EQs) for each species (table 1). EQs were found to range from 3.29 in *Sphyrna lewini* to 0.24 in *Dalatias licha*. The three sphyrnid species, along with *Negaprion acutidens*, had the highest EQs (>2.5), followed by three species of carcharhinid, *Carcharhinus amblyrhynchos*, *C. brachyurus*, and *C. melanopterus*. A range of species had the lowest EQs (<0.5); the two orectolobids, two lamniformes (*Carcharodon carcharias* and *Carcharias taurus*), and three species from different orders, *Cephaloscyllium isabellum*, *Notorhynchus cepedianus*

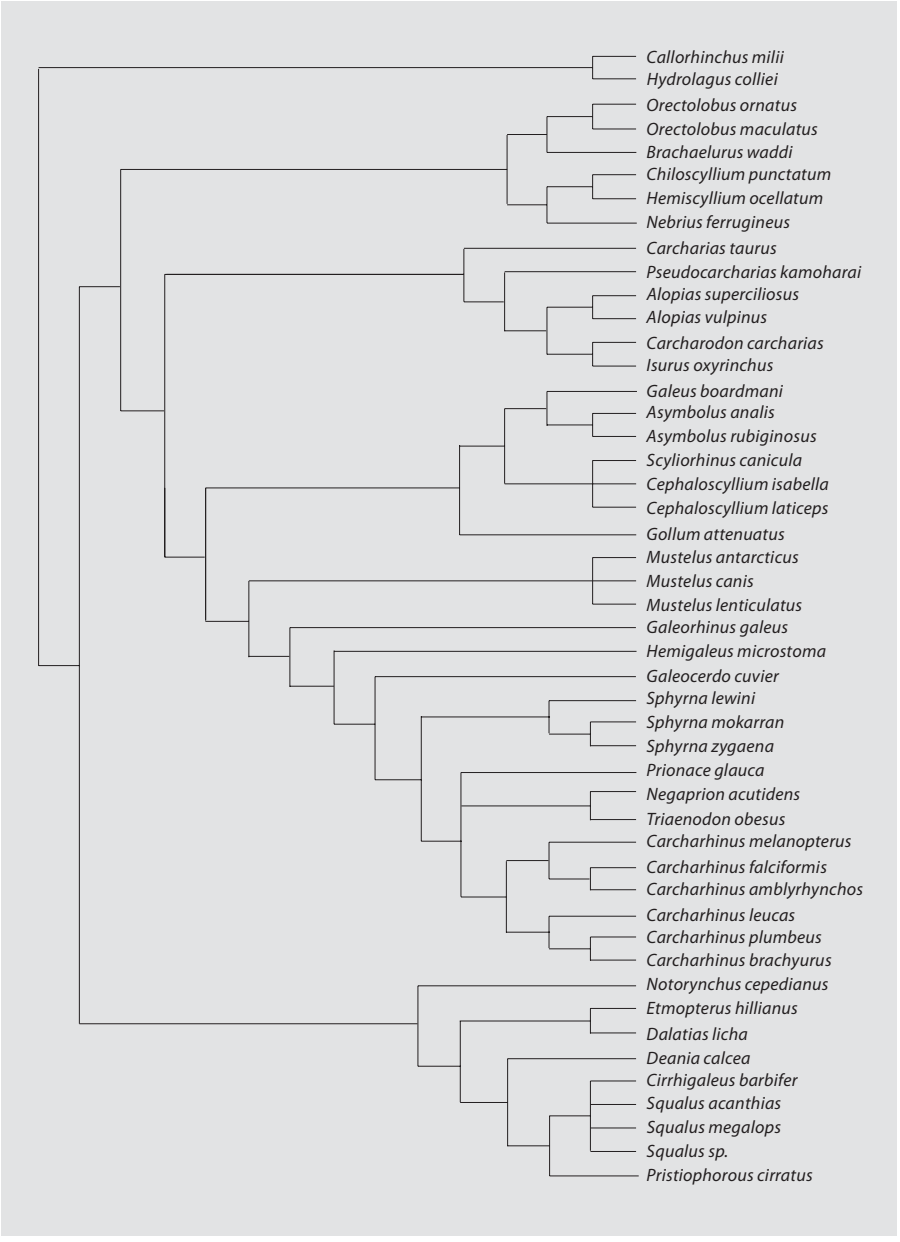


Fig. 3. A phylogenetic tree of the 48 species used in this study. The relationships between species are primarily based on Shirai's [1992b, 1996] phylogeny, with additional information from Compagno [1988], Martin et al. [1992], Naylor [1992] and Goto [2001].

and *Dalatias licha*. The brain of the holocephalan *Callorhinchus milii* was below average relative to body size (EQ = 0.71).

Using independent contrasts, brain mass was found to increase with body mass according to the relationship y = 0.3801x (r = 0.610, n = 37; fig. 4b). Residuals were calculated for each species to give an indication of relative brain size. The pattern of results was very similar to the EQs (table 1) and there was a highly significant correlation between the rank position of each species, ranked from 1 to 42 on the basis of the size of its corresponding EQ or residual, as calculated using each of the regression equations ($r_s = 0.8728$, n = 42, $p < 0.0001$; Spearman rank, two-tailed).

In both analyses, the largest-brained species tend to be benthopelagic or pelagic and are largely found in reef or coastal-oceanic subhabitats. In comparison, the species with the smallest brains are benthic or benthopelagic and are found in bathyal, demersal, or reef subhabitats.

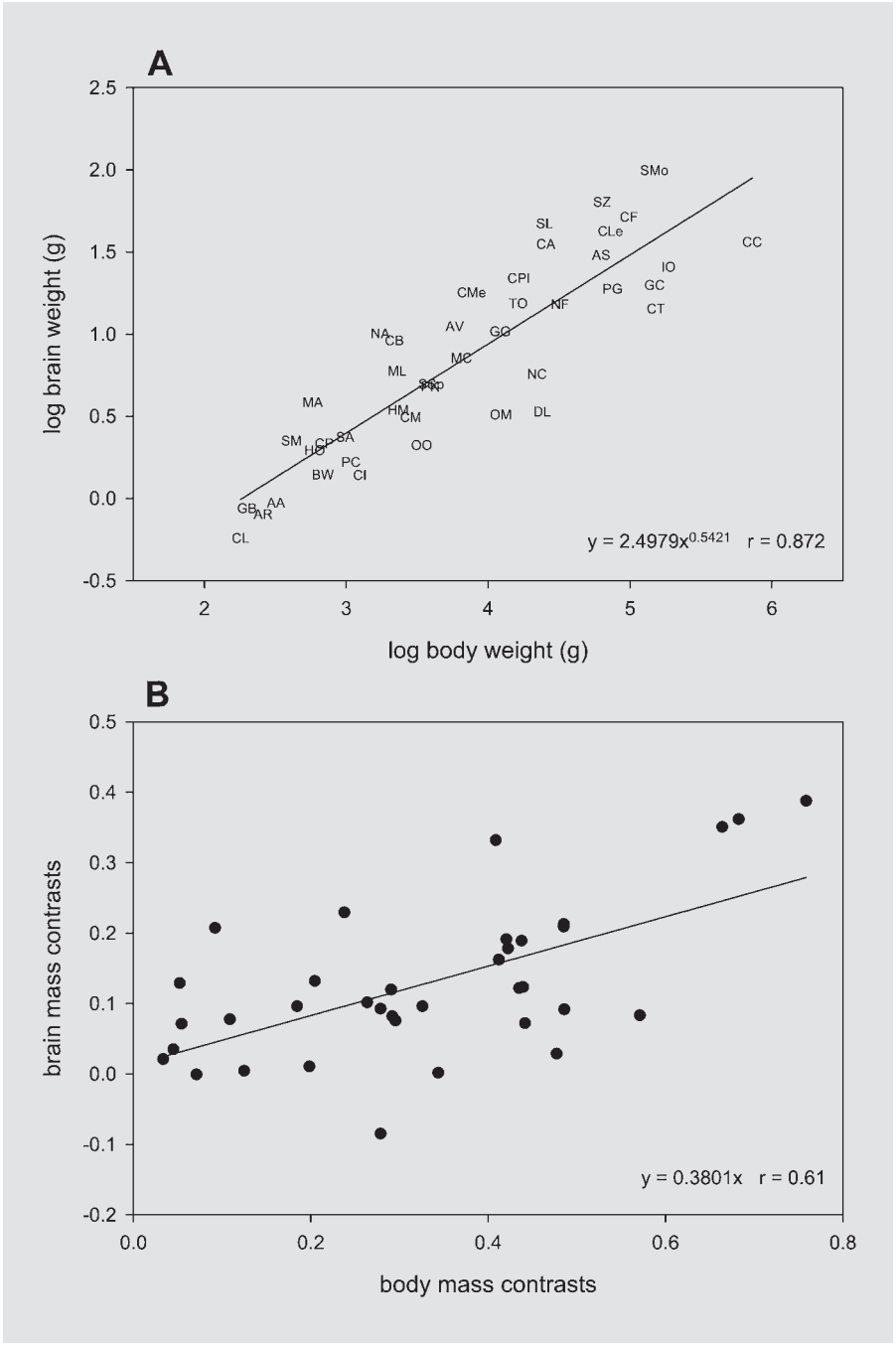


Fig. 4. Scaling of brain mass with body mass in sharks and one species of holocephalan using (**A**) species as independent data points and (**B**) phylogenetically independent contrasts.

Brain Organization

Substantial variation in the relative size of the five major brain areas (the telencephalon, diencephalon, mesencephalon, cerebellum, and medulla) was found (table 2; fig. 5). There is a general phylogenetic trend towards neural advancement from early squalomorphs, such as members of Squalidae and Hexanchidae, to modern galeomorphs, such as Carcharhinidae and Lamnidae. Squalomorph sharks generally have small telencephalons and average-sized cerebellums, although they have well-developed medullas and mesencephalons, whereas galeomorph sharks exhibit increasingly large telencephalons. The sharks with the highest level of neural development, the sphyrnid sharks, clearly show greatly hypertrophied

Table 2. The relative sizes (as a proportion of the total brain) and weighted factors (θ) for the five major brain areas (telencephalon, mesencephalon, diencephalon, cerebellum and medulla) in 43 species of shark and two species of holocephalan

Species[a]	Relative structure weight (%) ± SD									
	Telencephalon		Mesencephalon		Diencephalon		Cerebellum		Medulla	
	weight, %	θ	weight, %	θ	weight, %	θ	weight, %	θ	weight, %	θ
NC	31.31 ± 2.84	–0.27	17.20 ± 2.13	0.40	5.44 ± 1.71	–0.13	13.78 ± 2.33	–0.23	32.27 ± 1.46	0.56
SSp	30.53 ± 1.55	–0.29	18.05 ± 1.14	0.47	5.92 ± 0.42	–0.05	15.36 ± 0.69	–0.15	30.14 ± 0.73	0.46
SA	31.26 ± 7.99	–0.27	15.02 ± 4.54	0.22	6.65 ± 0.44	0.07	17.66 ± 2.90	–0.02	29.43 ± 1.96	0.42
SM	27.33	–0.36	18.06	0.47	7.17	0.15	19.44	0.08	27.99	0.35
EH[N]	31.87	–0.25	10.99	–0.11	9.89	0.59	17.58	–0.02	29.67	0.43
DL	21.37	–0.50	14.68	0.19	23.24	2.73	17.59	–0.02	23.12	0.12
PC	20.09 ± 4.9	–0.53	18.83 ± 3.37	0.53	5.25 ± 1.27	–0.16	19.81 ± 1.29	0.10	36.01 ± 1.67	0.74
BW	41.67	–0.03	8.01	–0.35	4.39	–0.29	19.25	0.07	26.67	0.29
OM	42.40	–0.01	10.34	–0.16	6.15	–0.01	14.60	–0.19	26.50	0.28
OO	37.90 ± 6.04	–0.11	11.64 ± 3.61	–0.05	4.86 ± 1.13	–0.22	16.89 ± 1.90	–0.06	28.82 ± 2.00	0.39
CP	49.07	0.15	8.80	–0.29	5.56	–0.11	21.76	0.21	14.81	–0.28
HO	52.04 ± 2.70	0.22	6.12 ± 0.30	–0.50	4.59 ± 0.74	–0.26	20.92 ± 2.79	0.16	16.32 ± 0.67	–0.21
NF	58.30	0.36	4.39	–0.64	5.24	–0.16	21.92	0.22	10.16	–0.51
CT	30.57	–0.29	9.47	–0.23	11.42	0.83	25.28	0.41	23.26	0.12
PK	33.13	–0.23	20.42	0.66	6.88	0.10	16.04	–0.11	23.54	0.14
AS	27.19	–0.36	16.19	0.31	3.15	–0.50	32.09	0.78	21.39	0.03
AV	26.79	–0.37	15.60	0.27	1.66	–0.73	30.60	0.70	33.02	0.22
CC	38.86 ± 2.59	–0.09	14.28 ± 0.71	0.16	5.57 ± 0.98	–0.11	17.66 ± 0.42	–0.02	23.63 ± 2.38	0.14
IO	37.70 ± 2.83	–0.12	18.18 ± 2.45	0.48	3.35 ± 1.67	–0.46	17.03 ± 0.65	–0.05	23.74 ± 3.70	0.15
AA	42.55	0.00	12.77	0.04	6.38	0.02	17.02	–0.05	21.28	0.03
AR	40.00	–0.06	15.00	0.22	6.25	0.00	18.75	0.04	20.00	–0.03
CI	41.93 ± 6.28	–0.02	12.72 ± 1.39	0.03	5.91 ± 0.43	–0.05	14.81 ± 1.77	–0.18	24.64 ± 5.95	0.19
CL	46.62 ± 4.12	0.09	14.12 ± 1.37	0.15	5.29 ± 2.31	–0.15	12.13 ± 1.62	–0.33	21.83 ± 1.77	0.05
GB	37.93 ± 1.79	–0.11	14.94 ± 0.41	0.21	6.90 ± 0.85	0.11	17.24 ± 1.06	–0.04	22.99 ± 1.31	0.11
SR[N]	40.70	–0.05	13.95	0.13	8.14	0.31	17.44	–0.03	19.77	–0.05
ML	41.04 ± 3.50	–0.04	12.47 ± 0.82	0.01	4.34 ± 0.53	–0.30	17.47 ± 1.39	–0.03	24.68 ± 1.82	0.19
MA[N]	44.99	0.05	14.23	0.16	5.36	–0.14	17.77	–0.01	17.65	–0.15
MC	37.21	–0.13	12.79	0.04	8.14	0.31	18.60	0.03	23.26	0.12
GG	38.19 ± 2.53	–0.11	17.14 ± 1.40	0.39	6.07 ± 1.21	–0.02	17.19 ± 2.34	–0.04	21.41 ± 0.49	0.03
HM	48.07	0.12	12.96	0.05	5.71	–0.08	16.15	–0.10	17.12	–0.17
CA	64.23 ± 2.02	0.50	8.3 ± 2.14	–0.33	4.73 ± 1.72	–0.24	13.07 ± 1.84	–0.27	9.67 ± 1.39	–0.53
CB	54.90	0.28	12.75	0.04	4.90	–0.21	13.11	–0.27	14.34	–0.31
CF	63.48	0.48	8.31	–0.32	4.99	–0.20	12.53	–0.30	10.68	–0.48
Cle	60.69	0.42	6.07	–0.51	5.86	–0.06	14.04	–0.22	13.33	–0.36
CMe	58.34	0.36	7.86	–0.36	6.19	–0.01	15.84	–0.12	11.77	–0.43
CPl	54.16	0.27	8.46	–0.31	7.23	0.16	14.23	–0.21	15.92	–0.231
GC	50.03	0.17	11.79	–0.04	7.00	0.12	16.88	–0.06	14.30	–0.31
NA	50.35	0.18	9.63	–0.22	5.56	–0.11	13.51	–0.25	20.95	0.01
PG	49.65 ± 0.61	0.16	15.43 ± 1.5	0.25	5.96 ± 1.66	–0.04	10.97 ± 0.87	–0.39	17.98 ± 1.24	–0.13
TO	56.84	0.33	8.98	–0.27	5.34	–0.14	15.89	–0.12	12.96	–0.37
SL	53.75	0.26	6.53	–0.64	4.44	0.05	23.71	0.32	11.57	–0.44
SMo	66.52	0.56	3.75	–0.70	3.39	–0.46	18.41	0.02	7.93	–0.62
SZ	58.10	0.36	6.08	–0.51	3.93	–0.37	22.25	0.24	9.63	–0.53
HC[N]	33.33	–0.22	15.05	0.22	6.45	0.04	22.58	0.26	22.58	0.09
CM	21.46 ± 1.07	–0.50	17.93 ± 1.45	0.46	7.39 ± 1.49	0.19	22.32 ± 2.67	0.24	30.90 ± 1.47	0.49

[a] For species abbreviations see table 1.
[N] Data obtained from Northcutt [1978].

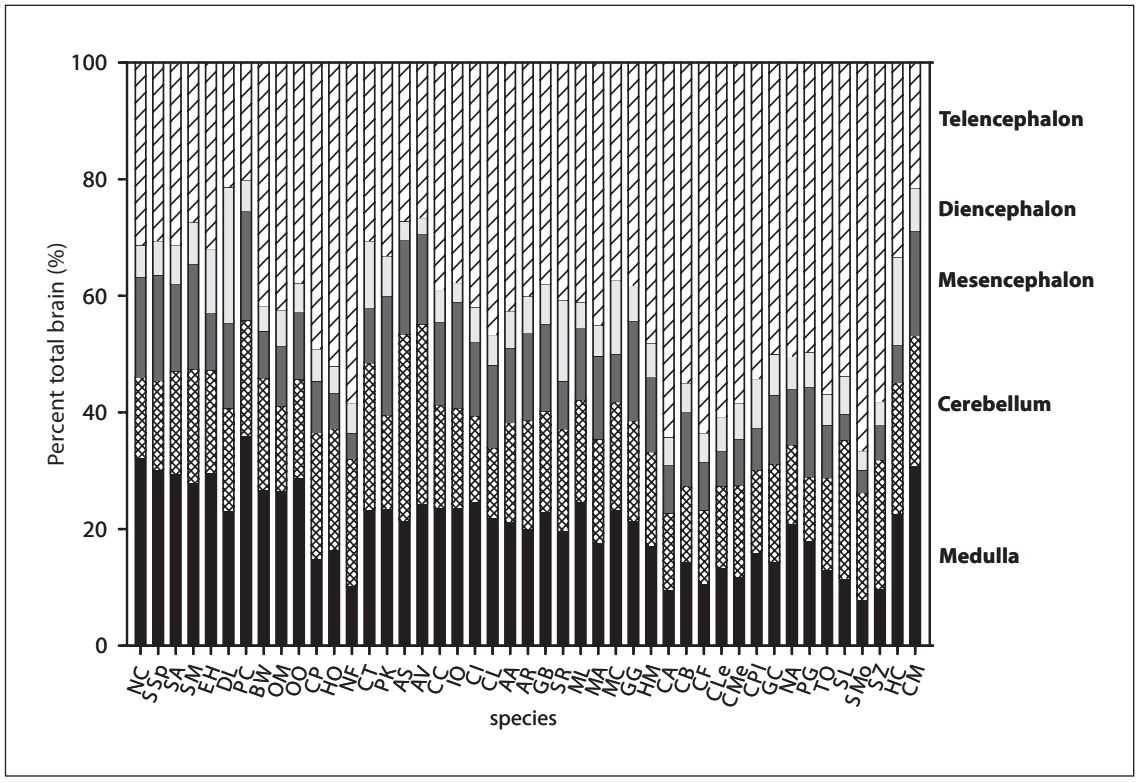
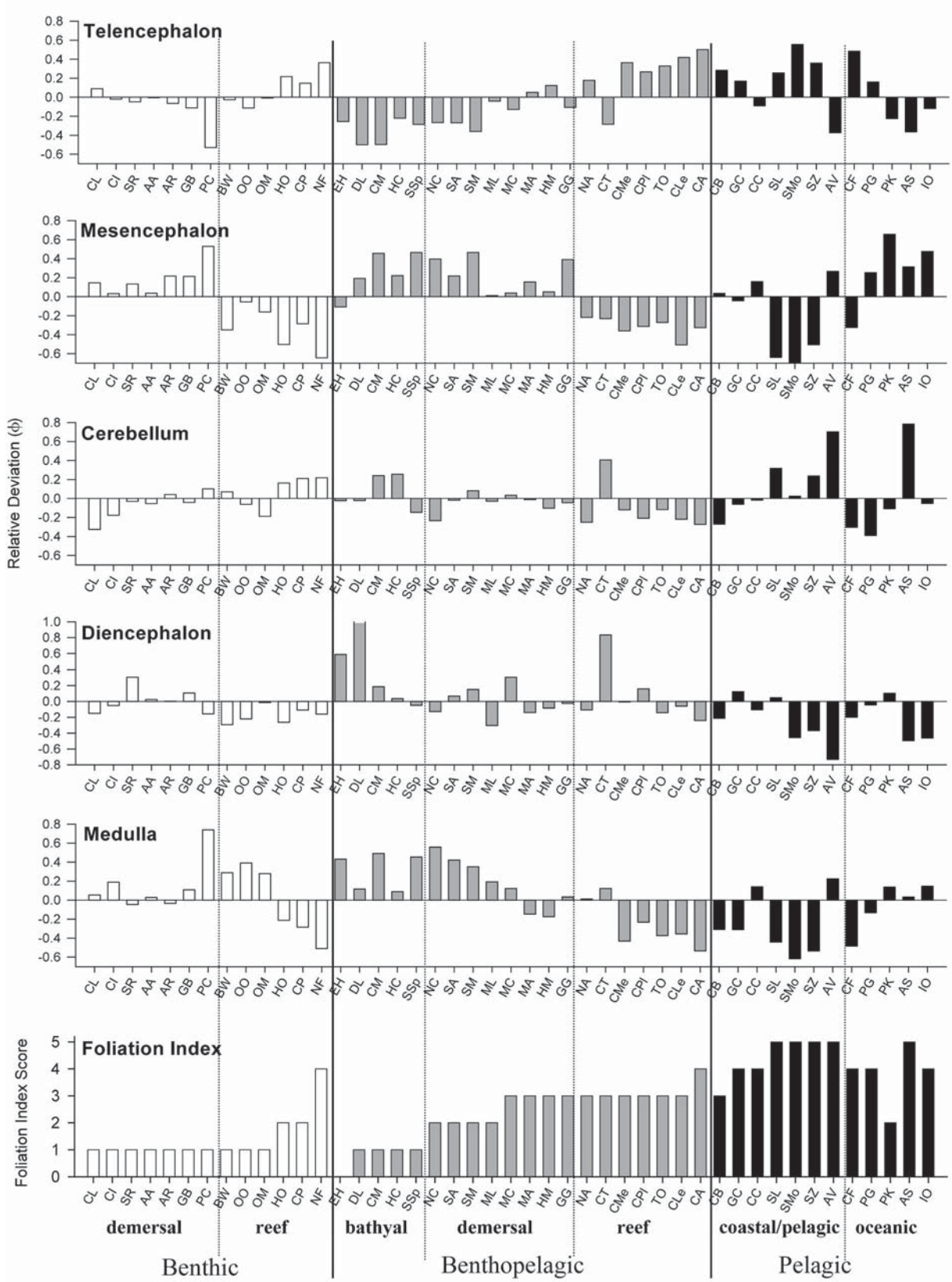


Fig. 5. Bar graph showing interspecific variation in brain area proportions in 43 species of sharks and 2 holocephalans. Standard error bars have been omitted for clarity. For standard error values and species abbreviations see table 1.

telencephalons and enlarged cerebellums, with these areas occupying at least 50 and 20% of their total brain, respectively. *Sphyrna mokarran*, in particular, has evolved a particularly large telencephalon that accounts for almost 67% of its brain. Members of the genus *Alopias* also have extremely enlarged cerebellums comprising, on average, 31% of their total brain. Unlike *Sphyrna*, however, the telencephalon of these animals comprises only 26% of the total brain size. Rather, *Alopias* also shows a larger than average mesencephalon (accounting for, on average, 16% of the total brain). Both holocephalan species have an enlarged medulla and these species also have very large cerebellums.

In addition to the apparent trends seemingly associated with phylogeny, there is evidence that much of the interspecific variation might be independent of phylogenetic position, as the relative development of the five major brain areas is similar in distantly-related species that occupy similar habitats or lifestyles (fig. 6). With the exception of *Pristiophorus*, which is a transitional species that different authorities have classified as either a ray [Maisey, 1984; Shirai, 1992b; Winchell et al., 2004] or a shark [Last and Stevens, 1994; Compagno, 1999], demersal benthic sharks have average-sized telencephalons, cerebellums, and medullas. They do, however, have slightly enlarged mesencephalons, averaging approximately 13% of their total brain. More reef-associated benthic species, such as *Orectolobus* and *Brachaelurus waddi*, have reduced mesencephalons and enlarged medulla, constituting on average 9 and 27%, respectively, of the brain. Others, such as members of Hemiscyllidae, have enlarged telencephalons and cerebellums and reduced medullas (the medulla accounts for only approximately 16% of their brains).

Bathyal and demersal benthopelagic species show similar trends in brain development. These sharks generally have well developed mesencephalons and medullas, with these structures comprising on average 14 and 25%, respectively, of their brain, but have reduced telencephalons and have fairly average-sized cerebellums. An exception to these patterns is *Mustelus lenticulatus*. In this species, the relative deviation is average to negative in all


Telencephalon
Mesencephalon
Cerebellum
Diencephalon
Medulla
Foliation Index
Relative Deviation (ϕ)
Foliation Index Score
CL CI SR AA AR GB PC BW OO OM HO CP NF EH DL CM HC SSp NC SA SM ML MC MA HM GG NA CT CMe CPl TO CLe CA CB GC CC SL SMo SZ AV CF PG PK AS IO
demersal
reef
bathyal
demersal
reef
coastal/pelagic
oceanic
Benthic
Benthopelagic
Pelagic

brain areas except the medulla. In contrast, reef-associated benthopelagic species, such as members of *Carcharhinus*, show extremely enlarged telencephalons, accounting for over 50% of their total brain.

The brains of pelagic species tend to show specialization rather than generalization in their structural development. In the lamniform species *(Alopias, Carcharodon, Isurus,* and *Pseudocarcharias)*, the mesencephalons and medulla are hypertrophied, accounting for, on average, 17 and 25% of the total brain, respectively. *Alopias* and *Pseudocarcharias* also show extreme hypertrophy of the cerebellum and diencephalon, respectively. Pelagic carcharhiniform sharks *(Carcharhinus brachyurus, C. falciformes, Galeocerdo, Prionace,* and *Sphyrna)* show hypertrophy of the telencephalon, which comprises at least 54% of their total brain, whereas the mesencephalons, medulla, and diencephalons are generally below average. The majority of truly oceanic species from either order have an enlarged mesencephalon that comprises, on average, more than 17% of their total brain.

Cerebellar Foliation Index

Sharks exhibit widespread variation in cerebellar foliation, which was quantified on a scale of 1–5 using a cerebellar foliation index. When assigned a foliation index grading, a progression of development of the length, depth, and number of folds was observed that correlates with phylogeny (fig. 6). The more basal species *(Squalus, Notorhynchus, Pristiophorus)* have low foliation, whereas some of the more recently evolved sharks *(Alopias*, lamnids, *Sphyrna)* represent the group with the highest foliation (fig. 6). However, members of the same ecological group, although not necessarily the same family group, also tend to exhibit similar levels of foliation. Benthic animals that hide in crevices along the reef and are often sedentary on the sea floor, such as *Cephaloscyllium, Asymbolus,* and *Galeus boardmani*, have low foliation gradings from 1–2. The benthopelagic shark species that also have a foliation grading of 1–2 are those that live in bathyal habitats and are more demersal, such as *Mustelus lenticulatus* and *Gollum attenuatus*. The two bathydemersal holocephalans fall into this category as well, both with a foliation grading of 1. The upper range of the benthopelagic sharks (with an average grade of 3) belong to more fast-swimming, reef-associated species, such as *Carcharhinus* and *Triaenodon obesus*. The most complex cerebellums, with index gradings of 4–5 on average, are found in those species that occupy pelagic habitats and hunt agile prey, such *Sphyrna, Alopias,* and *Isurus oxyrhinchus*.

As species of the Carcharhiniformes and Lamniformes groups cover the full spectrum of foliation gradings, relationships between the level of cerebellar foliation and both phylogeny and ecology were explored further using the data from these families. When ordered phylogenetically (fig. 7a), there is again a visible pattern of increasing foliation through evolutionary time. When the Carcharhiniformes and Lamniformes are grouped on the basis of habitat and lifestyle rather than phylogeny (fig. 7b), there is a tendency for those animals that occupy similar ecological habitats to have similar levels of cerebellar foliation.

Although some sharks, such as *Alopias* and *Sphyrna*, possess both relatively large and heavily foliated cerebellums, whereas others, such as the two orectolobids, have relatively small, largely unfoliated cerebellums, there is no overall correlation between foliation index score and the relative size of the cerebellum ($r_s = 0.107$, $n = 42$, $p = 0.500$; Spearman's rank).

Multivariate Analysis

Interspecific differences in the relative proportions of the five major areas in 43 species of shark and 2 species of holocephalans were assessed statistically using cluster analysis. The analysis yielded a dendrogram that is shown in figure 8, which produced six clusters.

The first two clusters each contained members of a single genus. The first consists of a single species, *Dalatias licha*, which has an extraordinarily large diencephalon, compromising 23% of its total brain, as well as an enlarged mesencephalon and medulla. The two alopiid sharks were grouped together in the second cluster on the basis of their large cerebellums and mesencephalons.

The third cluster includes all members of the genus *Sphyrna* along with *Nebrius ferrugineus*. These species all have large telencephalons, accounting for at least 50% of their total brain size, and large cerebellums. These species also exhibit some of the highest foliation gradings of 4–5. All species in the fourth cluster also share the common trait of an enlarged telencephalon, which on average comprises 54% of the brain. This cluster can further be

Fig. 6. Weighted factors (θ) for the telencephalon, mesencephalon, diencephalon, cerebellum, and medulla for 43 species of elasmobranch and 2 holocephalans, showing the deviation [Wagner, 2001a, b] from the average relative volume for each brain structure. In addition, foliation index scores for each species, grouped according to primary habitat, are presented. For species abbreviations see table 1.

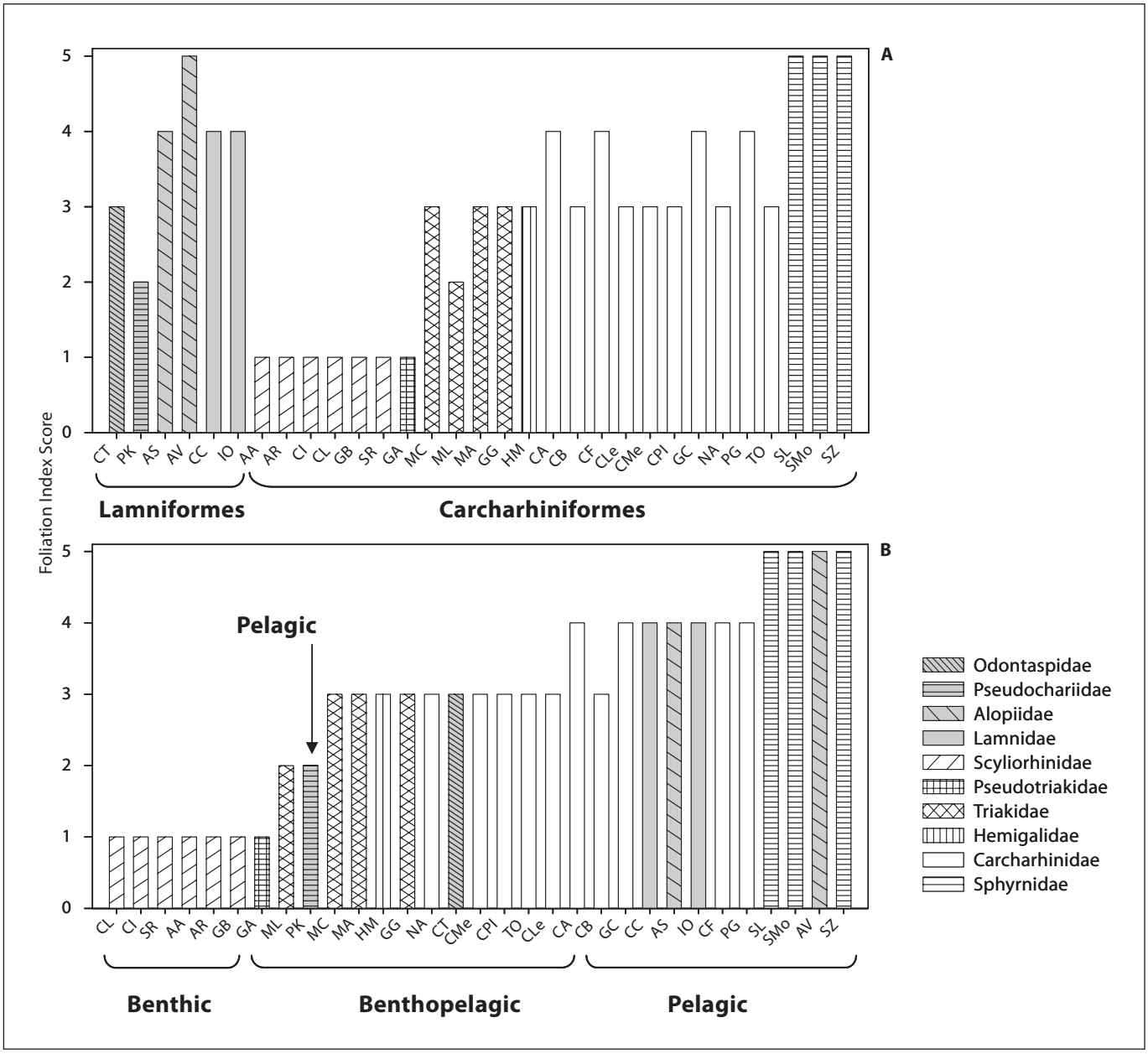
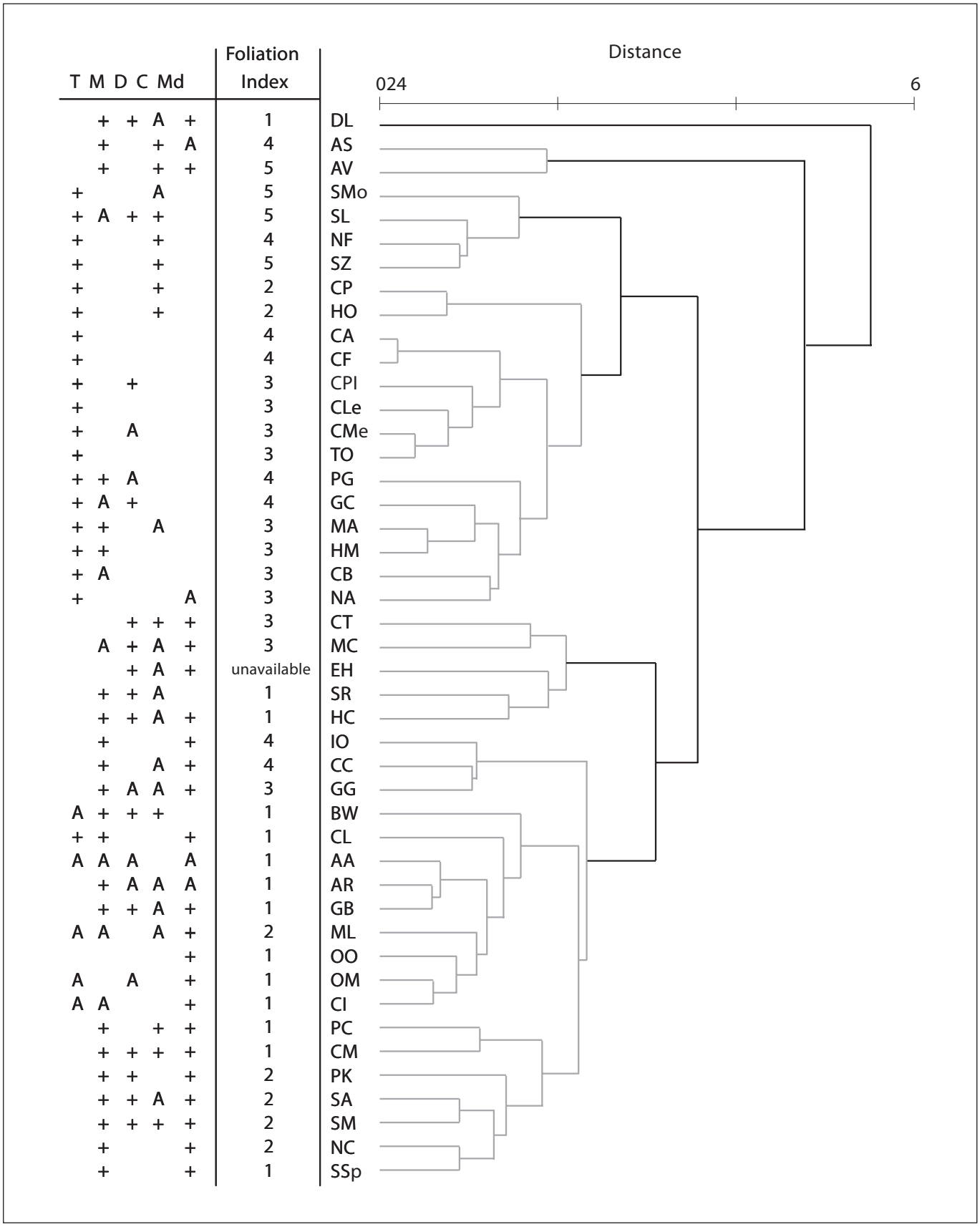


Fig. 7. Foliation index scores for Lamniformes and Carcharhiniformes. **A** Foliation index scores grouped phylogenetically. **B** Foliation index scores grouped according to primary habitat.

divided into three subgroups: members of Hemiscyllidae, that enlarged cerebellums which occupy approximately 21% of their brain but low foliation grades (2); reef-associated, benthopelagic, carcharhinid sharks that, despite having below average-sized cerebellums, show average to high foliation grades (3–4) and include four species of *Carcharhinus*, for whom the telencephalon comprises more than 60% of the total brain; and finally a group of

Fig. 8. Cluster analysis dendrogram based on the relative size of each of the five brain areas as a proportion of the total brain, alongside a comparison with both the foliation index score and the brain structures for each species which showed either a positive (+) relative deviation ($\theta > 0.05$) or an average (A) relative deviation ($-0.05 < \theta < 0.05$). The darkened lines indicate those clusters that are significantly different. T = Telencephalon; m = mesencephalon; D = diencephalon; C = cerebellum; Md = medulla.


T M D C Md
Foliation Index
Distance
024
6
+ + A +
1
DL
+ + A
4
AS
+ + A +
5
AV
+ A
5
SMo
+ A + +
5
SL
+ +
4
NF
+ +
5
SZ
+ +
2
CP
+ +
2
HO
+
4
CA
+
4
CF
+ +
3
CPl
+
3
CLe
+ A
3
CMe
+
3
TO
+ + A
4
PG
+ A +
4
GC
+ + A
3
MA
+ +
3
HM
+ A
3
CB
+ A
3
NA
+ + +
3
CT
A + A +
3
MC
+ A +
unavailable
EH
+ + A
1
SR
+ + A +
1
HC
+ +
4
IO
+ A +
4
CC
+ A A +
3
GG
A + + +
1
BW
+ + +
1
CL
A A A A
1
AA
+ A A A
1
AR
+ + A +
1
GB
A A A +
2
ML
+
1
OO
A A +
1
OM
A A +
1
CI
+ + +
1
PC
+ + + +
1
CM
+ + +
2
PK
+ + A +
2
SA
+ + + +
2
SM
+ +
2
NC
+ +
1
SSp

active benthopelagic and pelagic species such as *Prionace glauca* and *Negaprion acutidens*, with again below average cerebellums but average to high foliation grades (3–4), which are grouped due to their average to large mesencephalons and enlarged telencephalons, that comprise, on average, 13 and 54% of their total brain, respectively.

The next major cluster contains just 5 species, which all have a below-average telencephalon and a large diencephalon and medulla, occupying an average of 24 and 11% of their brain, respectively. This small group has the widest range of foliation grades for all clusters (1–3) and includes bathyal, benthopelagic species such as *Etmopterus hillianus*, and reef-associated, benthopelagic species such as *Carcharias taurus*. Despite the variation in cerebellar foliation exhibited by these species of shark, all of them have an average relative cerebellum size.

The sixth and final cluster is a large cluster that comprises 19 species and appears to be further divided into three subgroups. The first subgroup contains just three species: *Isurus oxyrinchus, Carcharodon carcharias*, and *Galeorhinus galeus*. These species have average to high foliation gradings (3–4), and are grouped due to their enlarged mesencephalon, accounting for approximately 23% of their brain, and medulla, which accounts for approximately 17%. The other two subgroups in this cluster all have low foliation gradings of 1–2. The second subgroup contains mostly benthic species, such as *Orectolobus* and *Cephaloscyllium*, which possess an average to large medulla that accounts for at least 20% of their total brain and also contains the largest number of species with average-sized brain structures when compared across all 45 species. The third subgroup consists of species with above-average mesencephalons and medullas (representing, on average, 18 and 29% of the total brain, respectively). These are mainly benthopelagic, demersal, and bathyal species, such as the three squalids, but this subgroup also includes the pelagic, oceanic *Pseudocarcharias kamoharai* and the benthic, demersal *Pristiophorus cirratus*.

Discussion

Variation in brain morphology in 46 species of shark and two species of holocephalan has been investigated by assessing relative brain size (encephalization) and comparing the relative development of five major brain areas (telencephalon, diencephalon, mesencephalon, cerebellum, and medulla) in terms of percentage of total brain size using two sectioning techniques. Variation in cerebellar foliation has also been quantified using a novel visual grading method. The use of such methods is becoming increasingly common as they provide an easy and convenient way of quantifying descriptive data [e.g., Kim et al., 2004; Williams and Babcock, 2004; Gelfand et al., 2005].

The data in this study includes a wide range of shark species and encompasses all of the major clades, 20 of the 36 shark families (representing every order except the Squatiniformes), and two of the three holocephalan families are represented. The species studied also encompass a wide variety of body morphologies, predation strategies, and primary habitats, and is sufficiently representative to explore the issues of comparative brain morphology within these groups.

In a study of this nature, where relative size is the basis for comparison [Kotrschal and Palzenberger, 1992], the following assumptions are made: (1) there are correlations between specific brain areas and functions or behaviors, and (2) although there might be correlations, they might not necessarily represent cause-and-effect relationships, and are most likely the result of a combination of adaptation, phylogeny, and allometric/developmental processes [Harvey and Krebs, 1990; Kotrschal and Palzenberger, 1992; Barton and Harvey, 2000]. This is not a functional analysis, but an attempt to discern ecological patterns within a neuroanatomical framework. The extent to which brain structure size is directly related to, for example, specific behaviors or the specialization of particular sensory modalities, is not addressed in this study and requires further analysis.

Encephalization

The allometric scaling of brain mass with body mass has received some attention in chondrichthyans, and these animals have been found to possess large brains in relation to other vertebrates [Bauchot et al., 1976; Northcutt, 1977, 1978; Striedter, 2005]. Within the sharks, galeomorphs tend to have larger brains than squalomorphs, with the carcharhinid and sphyrnid sharks having the largest brains [Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov, 1991]. Most previous studies, with the exception of that by Myagkov [1991], have suffered from having a small sample size, and none of them have considered the underlying influence of phylogeny. This is in contrast to the current study where a comparatively large number of species have been analyzed, using both raw species data and the phylogenetically independent contrasts method [Felsenstein, 1985].

Brain mass scales positively with body mass in sharks and holocephalans [Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov, 1991]. The coefficient of allometry (b), calculated using species as independent data points (b = 0.5421), is in close accordance with those previously calculated for sharks by Myagkov [1991] (b = 0.54, n = 38) and Demski and Northcutt [1996] (b = 0.543, n = 27). Coefficients of allometry calculated from earlier work [Bauchot et al., 1976 (b = 0.73, n = 10) and Northcutt, 1977, 1978 (b = 0.75, n = 11)] were much higher, but this is probably due to the low number of species they examined.

Encephalization quotients (EQs; raw species data) and residuals (independent contrasts) were calculated in order to assess which species had relatively larger brains. The results from both techniques are similar to each other and the EQs are comparable to those presented previously for sharks by Northcutt [1978]. The species with the largest brains relative to body mass are benthopelagic or pelagic, chiefly found in reef or coastal-oceanic subhabitats, whereas benthic or benthopelagic species found in bathyal, demersal, or reef subhabitats tend to have the smallest brains. In particular, carcharhiniform sharks, especially *Sphyrna* and *Carcharhinus*, have the largest brains. Many of these species live in coastal, often coral-reef-associated habitats, as do the teleosts with the largest brains [Bauchot et al., 1977, 1989], and it has been previously suggested that the requirements for learning the complex spatial organization of the reef habitat and its myriad of inhabitants might have influenced the evolution of brain size in both teleosts and chondrichthyans [Bauchot et al., 1977; Northcutt, 1978, 1989]. Similar relationships between increased relative brain size and habitat complexity have also been reported in mammals [Budeau and Verts, 1986]. However, as noted by Kotrschal et al. [1998], increases in relative brain size are possibly unrelated to habitat complexity per se, but rather to complex social behaviors and intra- and interspecific interactions that are often prevalent in species that live in complex habitats. Such 'social intelligence' has been correlated with brain size in birds and mammals [reviewed by Striedter, 2005], and although the cognitive abilities of cartilaginous fishes are virtually unknown, carcharhinid and sphyrnid sharks are considered to be social animals [Springer, 1967; Myrberg and Gruber, 1974; Klimley, 1985] that aggregate or form true schools that can range in size from less than ten to thousands of individuals. These groupings are often segregated by sex or size and there is some evidence of relatively complex social and reproductive behaviors, such as dominance hierarchies and courtship behavior [Johnson and Nelson, 1973; Gruber and Myrberg, 1977; Klimley, 1985; Ritter and Godknecht, 2000].

Relative brain size also appears to be correlated with mode of reproduction. Chondrichthyans have evolved a number of reproductive strategies that can be broadly divided into two groups: oviparous (egg-laying) and viviparous (live-bearing) [Carrier et al., 2004]. Those with the relatively largest brains (the Carcharhiniformes) are viviparous and have evolved placental analogues or placental viviparity, which greatly increase the energy flow from the mother to the offspring [Wourms, 1977]. Indeed, Martin [1996] has suggested that such an increased energy flow from the mother to the fetus is a prerequisite for the development of the relatively large brains found in mammals.

Brain Organization and Cerebellar Variation

Cluster analysis was used to investigate relationships in the relative size of five brain areas among species. The overall clustering pattern, as shown by the cluster analysis dendrogram (fig. 8), is quite different from the cladogram based on Shirai's [1996] phylogeny for sharks and holocephalans (fig. 3), suggesting that phylogeny is not the sole or dominant force driving variation in the organization of shark and holocephalan brains. However, within each of the six clusters there is a tendency for more closely related species to be grouped together. There is also some evidence for the existence of shark/holocephalan cerebrotypes, but unlike some previous studies on other vertebrate taxa [Huber et al., 1997; de Winter and Oxnard, 2001; Wagner, 2001a, b; Iwaniuk and Hird, 2005] the relationships were not absolute. Additionally, sharks and holocephalans exhibit substantial variation in cerebellar foliation, which appears to be correlated with both locomotor abilities and sensory-motor integration [New, 2001] and prey capture [Paulin, 1993], lending evidence to both arguments regarding the functional role of this structure.

Forty-two of the 45 species are grouped into clusters 3–6. Cluster 1 contained just one species, *Dalatias licha*, whereas cluster 2 comprised the two alopiid species. The relative size of the diencephalon appears to be the main reason for the cluster pattern of this species, given that, like *D. licha*, a number of the other deepwater species investigated also had above average diencephalons, mesencephalons and medullas (e.g., *Squalus acanthias, S. megalops*, and the holocephalan *Hydrolagus colliei*), but a different clustering pattern might arise if additional deepwater species that are more closely related to *D. licha* were

included in the analysis. The unique pattern of brain organization found in *Alopias*, which is characterized by a relatively large mesencephalon and cerebellum (which is also heavily foliated), could be related to the evolution of a novel method of prey capture in these sharks, which involves the use of an extraordinarily elongated upper lobe of the caudal fin to stun and capture prey [Compagno, 1984a; Last and Stevens, 1994; Lisney and Collin, 2006]. It has been proposed that species with larger cerebellums might have the ability to perform more multifaceted motor tasks than their close relatives lacking cerebellar hypertrophy [New, 2001].

The remaining four clusters (3–6) are divided into two separate lineages, based to a large extent on relative telencephalon size. All of the species in clusters 3 and 4 have a larger than average telencephalon and this characteristic also corresponds with a cerebellar foliation grading of 3 or higher in most species. These sharks are all galeomorphs, predominantly carcharhiniforms, although some orectolobiform species are also present and represent the largest-brained species. As mentioned above, many of these species dwell in complex reef environments, such as *Carcharhinus amblyrhynchos, C. melanopterus*, and *Trienodon obesus*, and some coastal-oceanic species such as *Galeocerdo* and *Sphyrna* are also associated with reefs. This provides evidence that increases in the relative size of the brain and the telencephalon are associated with complex environments in sharks, a situation found in many other vertebrates [Riddell and Corl, 1977; Barton et al., 1995; Huber et al., 1997; Striedter, 2005]. Carcharhiniform sharks also tend to be active hunters that live in a 3-dimensional environment and feed on fishes, cephalopods, and other chondrichthyans [Compagno, 1984a, b; Last and Stevens, 1994; Cortés, 1999]. In addition, as mentioned previously, these sharks also show potential for social behavior [Springer, 1967; Myrberg and Gruber, 1974; Klimley, 1985].

The pelagic galeomorph sharks from clusters 3 and 4, along with the two *Alopias* species (cluster 2) and the two lamnids (cluster 6), have the most highly foliated cerebellums (4–5). These are all wide-ranging, migratory species and hunt very active, agile prey, such as scombrid teleosts, other chondrichthyans, pinnipeds and cetaceans [Compagno, 1984a, b; Compagno et al., 1989; Long, 1991; Cortés, 1999]. Pelagic species that achieve high swimming speeds (e.g., *Carcharodon* and *Isurus*) employ the thunniform swimming style, obtaining the majority of their propulsory power from their caudal fins, whereas those pelagic sharks that utilize a subcarangiform swimming mode (e.g., *Galeocerdo, Prionace, Sphryna*, and *Carcharhinus falciformes*) are capable of long-distance swimming with high maneuverability [Donley and Shadwick, 2003; Wilga and Lauder, 2004].

The sharks and two holocephalans grouped in clusters 5 and 6 tended to have smaller than average telencephalons, average cerebellum size, low to average foliation (1–3), and an enlarged mesencephalon and/or medulla. Although these clusters contain a mixture of galeomorph and squalomorph sharks, all of these species tend to be more sluggish benthic or benthopelagic animals that occupy demersal and bathyal habitats, with the exception of the two active pelagic lamniform species, *Carcharodon* and *Isurus*, found in cluster 6. Unlike other pelagic carcharhiniform species, *Carcharodon* and *Isurus* do not have particularly hypertrophied telencephalons, despite living in similar environments and feeding on similar prey items, so these differences might reflect differences in social behavior between these groups of sharks [Demski and Northcutt, 1996].

In contrast to *Carcharodon* and *Isurus*, most of the species in clusters 5 and 6 feed in a more 2-dimensional environment on benthic and demersal teleosts and invertebrates [Compagno, 1984a, b; Last and Stevens, 1994; Cortés, 1999]. They utilize either anguilliform swimming (e.g., *Cephalosyllium* and *Orectolobus*) or a modified slow-moving subcarangiform mode, as in *Galeus boardmani* and *Scyliorhinus retifer* [Webb and Keyes, 1982; Wilga and Lauder, 2004], whereas many benthic species spend significant amounts of time resting on the seafloor. Therefore, there appears to be a relationship between the level of cerebellar foliation and both swimming speed and mode of locomotion in sharks, with slow-moving species that rely on axial undulation of the body having low levels of foliation, and faster-swimming species that employ subcarangiform or thunniform swimming having higher levels of foliation.

Benthic species in particular, with their general lack of structural hypertrophy, might also be more 'ecologically flexible' and better able to adapt to new or altered environments. Research on cyprinids has shown that species with 'basic brains' [Schiemer, 1988], that is, brains with no apparent structural enlargement, might be more ecologically flexible and thus more successful as a species [Brabrand, 1985; Lammens et al., 1987]. The same might be true for opportunistic benthic shark species, whose generalized neural development could be a mechanism to maintain an adaptable lifestyle [Wagner, 2002].

Two exceptions to the general patterns of brain organization identified in this study are the benthic reef-dwelling *Nebrius ferrugineus*, with a foliation score of 4

and an enlarged telencephalon, and *Pseudocarcharias kamoharai*, a pelagic species with a foliation grade of 2 and a reduced cerebellum in relative terms. In *N. ferrugineus*, the organization of the cerebellum might be related to prey capture for, unlike many benthic sharks, this species feeds predominantly on cephalopods [Smale, 1996], which, in contrast to the common prey items of many benthic species, are fast and/or agile. In the case of *P. kamoharai*, although this shark occupies an oceanic habitat, certain characters, such as its very small pectoral and dorsal fins and the presence of high levels of low-density squalene oil in its liver [Last and Stevens, 1994], resemble those of squaliform sharks rather than highly active pelagic species, suggesting that this shark has a very different locomotory (and probably prey capture) strategy compared to other pelagic species.

Holocephalans are generally demersal, deepwater species, with enlarged medullas, mesencephalons, and cerebellums, and they feed on bony fishes, crustaceans, and polychaetes [Armstrong, 1996; Didier, 2004]. Unlike sharks, whose locomotory strategies involve varying degrees of undulation along the axial body, they swim using undulation of the pectoral fins [Wilga and Lauder, 2004]. Although not greatly foliated, the cerebellum is relatively large in these animals, which may be related to dexterity of the pectoral fins and enhanced motor capabilities.

In contrast to previous reports [Larsell, 1967; Hildebrand, 2001], the species with the relatively largest cerebellums did not necessarily also exhibit the highest levels of foliation. For example, the two holocephalans have relatively large cerebellums but low levels of foliation, whereas *Isurus oxyrinchus, Prionace glauca* and *Carcharhinus falciformis* are species with high foliation scores (4) but relatively small cerebellums. It appears that there is a trade-off in most species between relative cerebellar size and foliation. The exceptions appear to be the highly derived *Alopias* and *Sphyrna*. However, it is difficult to determine whether this finding has a functional significance, because although the analysis of brain divisions as percentages of total brain size indicates which brain areas are highly developed, it fails to account for the possibility of independent enlargement or reduction of other brain divisions [Northcutt, 1978]. Previous work on mammalian brains has also shown that the use of volume or mass to assess the relative sizes of different brain areas can also result in an underestimation of the importance of folded brain areas [Sultan, 2002].

In conclusion, sharks and holocephalans exhibit widespread variation in brain size and morphology. This could be due, in part, to phylogenetic constraints, as ancestral groups appear to have smaller brains, relatively smaller telencephalons and lower cerebellar foliation indices. However, there is substantial variation within these clades that does not appear to track phylogenetic relationships. Although it has been previously shown that structural enlargement does not necessarily predict ecological patterns [Kotrschal and Palzenberger, 1992], it is possible that in sharks and holocephalans, brain size and the relative size of each of its component structures is a consequence of phylogenetic grouping, locomotory behavior, habitat, and lifestyle.

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