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## Comparative brain anatomy of lamniform sharks (Elasmobranchii: Lamniformes) and its implications to function, behavioral ecology, and evolution

Francesco Guzzo

DePaul University, francescoguzzo12101989@yahoo.com

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**Comparative brain anatomy of lamniform sharks (Elasmobranchii: Lamniformes) and its  
implications to function, behavioral ecology, and evolution**

A Thesis Presented in  
Partial Fulfillment of the  
Requirements for the Degree of  
Master of Science

Summer 2019

By

Francesco Guzzo

Department of Biological Sciences  
College of Science and Health  
DePaul University  
Chicago, Illinois

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## Abstract

Understanding the diversity of brain morphology is important to understand the evolution of cognitive ability and how ecology and phylogeny have influenced the variation in brain complexity. I examined the morphological variation of the brain in the shark order Lamniformes based on museum specimens and literature. Where I illustrate a wide range of morphological diversity in lamniform brains, my study shows that there is a strong positive correlation between brain size and body size, and that sharks with a larger brain tend to have a more foliated cerebellum, whereas the body weight over brain weight did not correlate with cerebellar complexity. In addition, the brain size is found to be affected by ontogeny where younger individuals tend to have larger brains than older conspecific individuals. I also demonstrate that different sizes of different parts of the brain with different functions reflect different lifestyles. Some ecological specializations are reflected in the brain anatomy of certain lamniforms, such as adaptations to deep-water (*Mitsukurina*), filter feeding (*Megachasma*), tail-based prey hunting (*Alopias*), and thunniform swimming (Lamnidae). My study also shows that more derived lamniform taxa (e.g., Alopiidae, Cetorhinidae, and Lamnidae) have highly foliated cerebellum, where limited foliation is regarded as plesiomorphic. Lamnids have relatively small brain, whereas alopiids have a large brain, where a mid-sized brain can be interpreted as plesiomorphic. My study represents the first investigation into the morphological variation and diversity of the brain focusing on lamniforms and demonstrates how ecological factors such as habitat, diet, and behavior drive brain evolution.

## I. INTRODUCTION

The complex workings of the brain has fascinated and baffled scientists for centuries where Santiago Ramón y Cajal, a pioneer neurobiologist, was the first to conduct a comparative study on different regions of the human brain (Cajal, 1899). Exemplified by Cajal's work, investigations into how the brain works are important because they help understanding how sensations are made, how movement is coordinated, how memories are made and stored, and how the organization of the brain shapes behavior (Gebauer et al., 2014; Russo et al., 2014; Hu et al., 2016). However, a mere understanding of how the brain works does not explain the origin of the brain as a functional organ, why do different animals have different types of brains, or what effects different aspects of behavior, such as feeding, migration, or reproduction, may impact the morphology of the brain (Jerison, 1973; Gittleman, 1986; Striedter, 2005). An effective approach to begin answering these questions is by studying the evolution of brains and how differences in ecology and behavior can shape the brain anatomy.

Sharks are a large group of cartilaginous fishes comprising over 500 extant species (Weigmann, 2016) under two major groups (superorders) Squalomorphi and Galeomorphi (Naylor et al., 2012) and representing one of the most well-studied vertebrate lineages. Sharks have several unique adaptations, such as having jaws detached from their cranium (Compagno, 1999), undergoing continuous tooth replacement throughout their life (i.e., polyphyodont dentition), and possessing large brains in relations to other vertebrates (Bauchot et al., 1976; Northcutt, 1977, 1978; Striedter, 2005). Originating nearly 400 million years ago and having survived several mass extinction (Compagno, 1977), the brains of sharks (Fig. 1) have become as diverse as the sharks themselves (Masai, 1969; Okada et al., 1969; Northcutt, 1977, 1978;



Kruska, 1988; Ito et al., 1999; Yopak et al., 2007, 2019, Yopak and Montgomery, 2008; Yopak and Frank, 2009). Understanding such diversity is important in helping to understand the cognitive ability of sharks and how ecology and phylogeny have led to the variation in their brain complexity. For example, one of the five basic regions in vertebrate brains, the mesencephalon (Fig. 1B), is responsible for coordination of the eye as well as visual and auditory processing (Kurkcuoglu, 2017), and a previous study has found a correlation between mesencephalon size and the water depth that sharks are found in (Yopak and Lisney, 2012). The folding (foliation) of another region of the brain, the metencephalon (specifically the cerebellum), ranges greatly from none to having numerous deep groves in sharks (Fig. 1C: Yopak et al., 2007; Yopak and Montgomery, 2008). The folds in the cerebellum increase the volume and surface area and allow for greater fine motor control, faster reaction time, and better coordination of the body (Haier et al., 2004; Montgomery and Perks, 2019)—thus, the higher the foliation, the higher the cerebellar complexity and more derived the brain.

Yet, many aspects of the diversity and evolution of brain morphology of sharks remain limited, unlike well-studied teleosts (Bauchot et al., 1977; Huber and Rylander, 1992; Kotrschal and Palzenberger, 1992; Huber et al., 1997; Kotrschal et al., 1998). Previous studies have found that sharks possess large brains relative to other vertebrates (e.g., Bauchot et al., 1976; Northcutt, 1977, 1978; Striedter, 2005). Within sharks, those in the Galeomorphi are known to have larger brains than those of Squalomorphi, where their brain mass increases positively with body mass (Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov and Hirnforsch, 1991). Whereas the influence of phylogeny on their brains had not been investigated until recently (Yopak et al., 2007; Yopak and Montgomery, 2008; Yopak et al., 2019), many basic questions still remain

unanswered, including how ecology has played a role in the evolution of brain anatomy in sharks through their phylogeny.

In this study, I examine the morphological variation of the brain across 14 of the 15 known extant species of a monophyletic shark order, Lamniformes (Fig. 2). Lamniformes is an ideal group of sharks to conduct comparative studies because its members exhibit remarkable ecological specializations (Ebert et al., 2013; Kim et al., 2013). For example, the goblin shark, *Mitsukurina owstoni*, has a highly protrusive jaw apparatus evolved to quickly snap up prey, whereas thresher sharks, *Alopias* spp., use their exceptionally elongate tail to stun prey (Compagno, 1984, 2002; Oliver et al., 2013). Behaviorally, lamniform sharks are also diverse with some species having geographically very large migration routes, such as the basking shark, *Cetorhinus maximus*, whereas other species, such as the megamouth shark, *Megachasma pelagios*, are known to show vertical diel migration (Nelson et al., 1997; Skomal et al., 2009; Nasby-Lucas et al., 2019). Lamniforms also have a wide variety of prey species from small krill consumed by *C. maximus* to large mammals fed by the white shark, *Carcharodon carcharias* (Hallacher, 1977; Dudley et al., 2000; Tucker et al., 2019).

Here, I specifically examine the relative development of the brain size (encephalization), the relative size differences among the five major brain regions (telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon: Fig. 1B), and the variation in cerebellar folding within the metencephalon using the 'foliation index' developed by Yopak et al. (2007) (Fig. 1C). Based on my results, I discuss the variation in brain attributes among samples and taxa as well as functional implications of proportional size differences among the five brain regions observed across different lamniform taxa. I also examine any relationship of my brain data with published habitat (e.g., preferred depth), behavioral (e.g., feeding strategies and presence or

absence of migratory patterns), and physiological (ectothermic vs. endothermic) data to discuss the ecological implications of observed variation of brain morphology in lamniform sharks. In addition, I discuss the evolutionary implications of the observed variation by mapping my quantitative brain data onto published phylogenetic trees to test whether or not more derived species of lamniform sharks will have more complex brains.

## **II. MATERIALS AND METHODS**

### **A. SPECIMEN EXAMINED**

I directly observed preserved specimens of 13 lamniform species (Fig. 2) and one carcharhiniform species, *Scyliorhinus retifer* (Carcharhiniformes: Scyliorhinidae), housed in the following institution: Bernice P. Bishop Museum (BPBM) in Honolulu, Hawaii; Cornell University Museum of Vertebrates (CUMV) in Ithaca, New York; Field Museum of Natural History (FMNH) in Chicago, Illinois; Natural History Museum of Los Angeles County (LACM) in Los Angeles, California; National Museum of Nature and Science (NSMT) in Tsukuba, Ibaraki Prefecture, Japan; Royal Ontario Museum (ROM) in Toronto, Ontario; Scripps Institution of Oceanography (SIO) in La Jolla, California; and University of Florida's Florida Museum of Natural History (UF) in Gainesville, Florida. Except for *Megachasma pelagios*, the brain in each shark was extracted from each specimen that was originally fixed in formaldehyde and was subsequently placed in 70% alcohol for long-term preservation. The brain of *M. pelagios* is the same specimen described by Ito et al. (1999), that was also originally fixed in formaldehyde and is now preserved in alcohol at NSMT where no dissection was involved;

rather, besides simple external observations, digital data generated from magnetic resonance imaging (MRI) were used to obtain additional data (see below). Although attempts were made to obtain specimens of *Cetorhinus maximus* and *Odontaspis noronhai* for the purpose of this study, they were not available due to their rarity in museum collections. However, I was able to obtain a comparable set of brain data of *C. maximus* from literature (i.e., Kruska, 1988). Therefore, my primary data set encompasses 14 of the 15 known extant lamniform species and all 10 extant lamniform genera ('ingroup': Fig. 2), along with the brains of *S. retifer* ( $n = 2$ ) for outgroup comparison (see Fig. 1A for one of the two specimens). In addition, eight specimens of *Squalus acanthias* (Squaliformes: Squalidae), that were caught off the coast of New Jersey, USA (western Atlantic Ocean), were examined for a pilot study (see below) and are now deposited in FMNH.

## **B. EXAMINED VARIABLES**

### **1. Brain Mass**

Except for *Megachasma pelagios* and *Cetorhinus maximus*, each brain (e.g., Fig. 1A, C) was detached through dissection from the spinal cord caudal to the terminal end of the myelencephalon marked by the tip of the fossa rhomboidea. Blood vessels, choroid plexa, olfactory bulbs, peduncle, and connective tissue (e.g., meninges) were removed, whereas cranial and sensory nerves were detached 3 mm from their base in order to preserve their positions for future research. Each brain was then cut into the five major brain regions (i.e., telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon: Fig. 1B) for the purpose of examining the brain organization and measuring a wet mass of each brain region. To remove

excess alcohol, I gave a dab with paper towel. Afterwards, each of the five brain regions was weighed using a U.S. Solid 0.1 mg  $120 \times 0.0001$  g laboratory analytical balance digital precision scale based on the criteria used by Northcutt (1978), and all five weight values were subsequently added together to find the total brain mass. Brain mass data for *M. pelagios* and *C. maximus* were obtained from Ito et al. (1999) and Kruska's (1988) work, respectively.

## **2. Brain Organization**

Except for *Megachasma pelagios* and *Cetorhinus maximus*, the measured weight of each of the five major brain regions (see above) was divided by the total brain weight, giving the proportion of each brain region in percentage. Comparable brain data for *C. maximus* were obtained from Kruska's (1988) work. The brain specimen of *M. pelagios* that was originally described by Ito et al. (1999) was not dissected into the five brain regions. Instead, MRI scanning was conducted to generate three-dimensional (3D) digital data that were then utilized to create a life-size plastic mold of the brain using a 3D injection mold printer. I then manually cut the mold into the five brain regions using a heated razor blade and subsequently calculated the proportion for each brain region represented by a sectioned mold by dividing its mold weight by the total mold weight. Whereas the density of the plastic is uniform throughout the mold, the density of the entire shark brain was also assumed to be uniform for the purpose of obtaining the needed brain mass data for *M. pelagios*.

## **3. Cerebellar Foliation Index**

Yopak et al. (2007) developed a five-tiered scale to describe the degree of foliation observed externally on the cerebellum of the myelencephalon in sharks. I used the same scale,

the foliation index (FI) that ranges from 1 through 5, to describe the degree of folding in each species of examined lamniforms (Fig. 2C). The FI is based on the length, depth, and number of folds in the cerebellum. A FI of 1 is characterized by a cerebellum with no foliation resulting in a smooth cerebellar surface. A cerebellum with a FI of 2 shows limited foliation where a few shallow transverse grooves run parallel to one another without branching. A FI of 3 describes a cerebellum with moderate foliation characterized by shallow to moderately deep grooves that show slight branching. A cerebellum with significant foliation formed by rather symmetrical, moderately deep and branched grooves characterizes a FI of 4. A FI of 5 is assigned to an extremely foliated cerebellum with deep branching grooves and distinctive multiple sections.

#### **4. Encephalization Quotient**

Whereas intelligence of animals is difficult to quantify, encephalization quotient (EQ) allows for better interspecific comparisons of different levels of cognitive ability associated with behavioral complexity than the traditional method of simply measuring raw brain weight or brain weight to body weight (Cairo, 2011). An EQ value was calculated for each lamniform shark using the ratio of the actual brain size to its expected brain size for a given mass, using the formula  $EQ = E_a/E_e$ , where  $E_a$  is the actual brain mass and  $E_e$  the expected brain mass (Jerison, 1973; in my study,  $E_a$  and  $E_e$  are referred to aBrW and eBrW, respectively). To calculate an expected brain weight, I used Snell's (1892) equation for simple allometry (Williams, 2002), with equation  $E = ax^b$ , where  $E$  is the expected brain mass,  $x$  the body mass,  $a$  an allometric coefficient, and  $b$  the allometric component. To calculate EQ values, the body weight in fresh state for each species was needed. The body mass data for specimens of *Pseudocarcharias kamoharai* and *Scyliorhinus retifer* when captured were available from each respective museum

of origin. Data from the brain of *Megachasma pelagios* and *Cetorhinus maximus* in fresh state were taken from Ito et al. (1999) and Kruska's (1988) work, respectively. For other lamniform taxa, I estimated the original body weight of each examined specimen using conversion equations that show the relationship between the body weight (BoW) and total length (TL) in literature:  $BoW = 2.02 \times 10^{-8} TL^{3.906}$  for *Mitsukurina owstoni* (Yano et al., 2007);  $BoW = 1.3 \times 10^{-4} TL^{2.4}$  for *Carcharias taurus* (Goldman et al., 2006);  $BoW = 2.166 \times 10^{-6} TL^{3.189}$  for *Odontaspis ferox* (Fergusson et al., 2007);  $BoW = 4.61 \times 10^{-5} TL^{2.494}$  for *Alopias pelagicus* (Liu et al., 1999);  $BoW = 9.1069 \times 10^{-6} TL^{3.0802}$  for *A. superciliosus* (Kohler et al., 1996);  $BoW = 1.8821 \times 10^{-4} TL^{2.5188}$  for *A. vulpinus*;  $BoW = 7.5763 \times 10^{-6} TL^{3.0848}$  for *Carcharodon carcharias* (Kohler et al., 1996);  $BoW = 5.2432 \times 10^{-6} TL^{3.1407}$  for *Isurus oxyrinchus* (Kohler et al., 1996);  $BoW = 4.4 \times 10^{-5} TL^{2.875}$  for *Lamna ditropis* (Goldman and Musick, 2006); and  $BoW = 1.4823 \times 10^{-5} TL^{2.9641}$  for *L. nasus* (Kohler et al., 1996). Because such a conversion equation is not available for *I. paucus* to my knowledge, I generated my own conversion equation,  $BoW = 4.00 \times 10^{-5} TL^{2.7024}$ , ( $r^2 = 0.952$ ) based a total of eight individuals of the species with reported TL (range: 92–372.8 cm) and BoW (range: 5.2–351 kg) data, including two full-term embryos (Guitart-Manday, 1966, 1975; Gilmore, 1983; Queiroz et al., 2007; Bustamante et al., 2009; Wakida-Kusunoki and Ande-Fuente, 2012).

### C. PILOT STUDY

A possible concern for this study is brain shrinkage that could have occurred because of fixation in preserved specimens. However, Kruska (1988) found that the brain of the basking

shark (*Cetorhinus maximus*) shrunk uniformly across the five brain regions (Fig. 1B) after alcohol fixation. To confirm Kruska's (1988) observation and to take the possible shrinkage factor into consideration for my study, I conducted an experiment as a pilot study on eight 'fresh' specimens of *Squalus acanthias* and treated them through typical fixation and preservation processes for long-term storage of fish specimens in museums collections (Emmanuel et al., 2012). First I extracted all eight brains with the same procedure described above and measured each of their body mass (BoW), total length (TL), and brain mass in fresh state (FrBrW) (note that the eight individuals were organized from the smallest TL to the largest TL in sequence and were assigned consecutive catalogue numbers). Second, I submerged the brains completely in 10% formaldehyde for 10 days and measured the formaldehyde-treated brain mass (FoBrW) of each specimen in order to measure any change as a result of specimen fixation. I then discarded the formaldehyde and placed the brains in 70% ethanol for 10 days to measure the ethanol-treated brain mass (EtBrW) in order to examine any effects of alcohol preservation on the shark brains.

My experiment reveals that all the eight brain samples of *Squalus acanthias* (now cataloged as FMNH 141885-1 through 141885-8) increased in mass by an average of 10.6% through formaldehyde fixation and subsequently decreased in mass by an average of 35.5% after ethanol preservation (Table 1). Because of the significantly large changes in brain weights that warrant consideration for the preserved specimens I examined, I made an assumption that all the preserved specimens I examined had also gone through an average of 35.5% decrease in brain mass through their formaldehyde fixation and alcohol preservation processes and factored the changes into their brain weight data. Once the putative amount of shrinkage was determined, I then examined whether or not the brains of *S. acanthias* had shrunk uniformly across all brain



regions. For this examination, I cut four of the eight brain samples (FMNH 141885-2, 141885-4, 141885-6, and 141885-8) into the five brain regions and measured what proportion each brain region comprised the total brain (Table 2; note that the other four brain samples were chosen to be kept intact). I then compared my data to the comparable data for *S. acanthias* presented by Yopak et al. (2007), who examined three brain samples in fresh state for the species. Because my data and Yopak et al.'s (2007) data are very close (Table 2), I determined Kruska's (1988) observation that each brain shrunk uniformly across the five brain regions (Fig. 1B) to be valid, allowing me to assume that it is also the case for the preserved specimens I examined.

#### **D. REGRESSION-BASED COMPARATIVE ANALYSES**

Based on my compiled data for the 14 lamniform species examined, I conducted three separate regression analyses. For the first analysis, I obtained independent contrasts by  $\log_{10}$  transformation of my brain mass and body mass data to allow regression with the brain mass in terms of weight as the dependent variable, where positive scaling has been noted previously in some other taxonomic orders of sharks (Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov and Hirnforsch, 1991; Yopak et al., 2007, 2019; Yopak and Montgomery, 2008). My second analysis entails a regression analysis between the cerebellar foliation index (FI) and 'brain size' in the form of encephalization quotient (EQ) with FI as the dependent variable. My third regression analysis also examines the relationship of FI with the 'brain size' but in terms of the effects of body weight and brain weight where FI is the dependent variable like the second analysis. On my second and third analyses is to examine if brain size increases as foliation increases and if raw brain weights over raw body weights are a predictor of cerebellar foliation, respectively.

## E. CHARACTER MAPPING

I employed character mapping (Harvey and Pagel, 1991) to examine the evolutionary pattern of the brain through lamniform phylogeny using the scyliorhinid carcharhiniform, *Scyliorhinus retifer*, as an outgroup. The EQ and FI for each of the ingroup (14 of the 15 lamniforms species examined) and outgroup species were mapped onto two separate published phylogenetic trees. One of them is Compagno's (1990) morphology-based tree that represents the first proposed phylogenetic interrelationships of all extant lamniform species. Although Compagno's (1990) tree has some shortcomings (Shimada, 2005), subsequent morphology-based phylogenetic studies (Shirai, 1996; Shimada, 2005; Stone and Shimada, in press) have shown little conflict with Compagno's (1990) tree topology. Another phylogenetic tree used for my character mapping is a molecular-based tree presented by Martin et al. (2002). Martin et al.'s (2002) tree includes all the lamniform genera with the most resolved depiction of their interrelationships that are largely consistent with other molecular studies (e.g., Martin and Naylor, 1997; Naylor et al., 1997, 2012; Heinicke et al., 2009). Morphology-based trees typically differ significantly from molecular-based trees in the position of *Carcharias*, *Odontaspis*, *Pseudocarcharias*, *Megachasma*, and *Alopias*, but both types of trees generally place *Mitsukurina* as phylogenetically the most basal taxon among extant lamniforms and that *Cetorhinus* and Lamnidae (*Lamna*, *Isurus*, and *Carcharodon*) form a derived monophyletic clade (Stone and Shimada, in press). In my study, *Scyliorhinus retifer* is depicted as a sister to a clade that includes all the lamniform taxa, where the sister relationship between Carcharhiniformes and Lamniformes is well supported by a variety of phylogenetic studies of elasmobranchs (e.g.,

Shirai, 1996; Maisey et al., 2004; Human et al., 2006; Heinicke et al., 2009; Naylor et al., 2012; da Cunha et al., 2017; Amaral et al., 2018).

### **III. RESULTS**

#### **A. DESCRIPTION OF BRAINS**

##### **1. General Observations**

Figure 3 shows the brains of all 14 lamniform species examined (Fig. 2) in dorsal and left lateral views, whereas the brain of *Scyliorhinus retifer* is illustrated in Figure 1A. Within Lamniformes, the brain shape shows considerable variations, although interspecific similarities within each family are also present. One noticeable difference is with Alopiidae in which all *Alopias* spp. have a short, dorsoventrally thick myelencephalon, whereas the myelencephalon of other lamniform taxa are long and thin. The myelencephalon of *Megachasma pelagios* is also unique in that it is dorsoventrally thin but laterally broad compared to all other lamniform taxa. Another noteworthy difference is with the brains of *Odontaspis ferox* and *Pseudocarcharias kamoharai* in which they are anteroposteriorly elongated and laterally compressed compared to all other lamniforms. When comparing the shape of the brains of Lamniformes (Fig. 3) with the brain of *Scyliorhinus retifer* (Fig. 1A), one major difference is with the telencephalon where it is triangular in *S. retifer* with several gyri towards its anterior end that are absent in all lamniforms.

Table 3 shows my primary quantitative data compared with the brain data presented by Yopak et al. (2007) where the foliation index (FI) and encephalization quotient (EQ) are of particular interests in my study. One notable difference between my study and Yopak et al.'s

(2007) report is with the FI of *Alopias superciliosus* in which Yopak et al. (2007) scored 4 for the species, whereas I scored 5. However, it is noteworthy that all other assignments of FI values are identical for species that were represented in both studies, including *Scyliorhinus retifer*. Another difference is with some variation in EQ values. For example, my EQ values for *Carcharias taurus* and *Isurus oxyrinchus* represented by juvenile specimens are substantially larger than Yopak et al.'s (2007) data that were based on adult specimens. On the other hand, my EQ values for *Pseudocarcharias kamoharai* and *A. superciliosus* are substantially smaller than Yopak et al.'s (2007) data. Nevertheless, the overall relative quantitative relationships among species in my study are similar to those of Yopak et al.'s (2007) study except for *I. oxyrinchus*. For example, the EQ values for *C. taurus* and *Carcharodon carcharias* are the smallest (<0.75), those for *Alopias* spp. the highest (>1.00), and *P. kamoharai* in between those two extremes (0.75–1.00) in both studies. It is particularly noteworthy that *A. vulpinus* has largest EQ value relative to all other lamniform taxa examined in both studies.

Table 4 shows the percent size of each of the five brain regions relative to the total brain size of examined species compared with the percent values of comparable taxa reported by Yopak et al. (2007). There are some minor differences between my data and Yopak et al.'s (2007) data. For example, in *Pseudocarcharias kamoharai*, the mesencephalon is larger than the myelencephalon in my study, whereas the former is smaller than the latter in Yopak et al.'s study. In *Alopias vulpinus* and *Scyliorhinus retifer*, the metencephalon is larger than the myelencephalon in my study, whereas the former is smaller than the latter in Yopak et al.'s (2007) study. Nevertheless, the proportional relationships among the five brain regions (or 'brain organization') as well as their quantitative values for each species in my study are overall very similar to those of Yopak et al. (2007).

## 2. Brain Anatomy by Taxa

**Mitsukurinidae**—The specimen of *Mitsukurina owstoni* (FMNH 117742: 126.5 cm TL) was calculated to have a body weight (BoW) of 3.28 kg. Its actual brain weight (aBrW) was 1.94 g with a FI of 3. Using the 35.5% weight reduction based on my pilot study (see above), the expected brain weight (eBrW) was 2.63 g. Using the eBrW and Snell's (1892) equation for simple allometry, EQ was found to be 0.55 (Table 3). The brain organization of *M. owstoni* was found to be 34.55% telencephalon, 8.76% diencephalon, 9.79% mesencephalon, 21.13% metencephalon, and 25.77% myelencephalon (Table 4). The brain of *M. owstoni* was illustrated by Garman (1913, plate 40) and Masai et al. (1973, figs. 1–8) in which its morphology conforms to the specimen I examined.

**Odontaspidae**—The specimen of *Carcharias taurus* (FMNH 16136: 106 cm TL, was calculated to have a BoW of 9.43 kg, whereas the BoW in *Odontaspis ferox* (BPBM 9335: 297 cm TL) was calculated to be 166.83 kg. The aBrW of *C. taurus* was measured at 3.58 g with a FI of 3 (same FI value as Yopak et al., 2007: Table 3), whereas the aBrW of *O. ferox* was found to be 6.81 g with a FI of 3. Using the 35.5% weight reduction from my pilot study (see above), the eBrW for *C. taurus* was determined to be 4.85 g, whereas that for *O. ferox* was calculated to be 9.26 g. Using the eBrW and Snell's (1892) equation for simple allometry, the EQ of *C. taurus* and *O. ferox* was 0.57 (vs. 0.37 by Yopak et al., 2007) and 0.23, respectively (Table 3). The brain organization of *C. taurus* was found to be 33.00% telencephalon, 10.70% diencephalon, 9.98% mesencephalon, 23.90% metencephalon, and 22.42% myelencephalon (Table 4). The brain organization of *O. ferox* was found to be 22.80% telencephalon, 7.42% diencephalon, 14.54% mesencephalon, 18.90% metencephalon, and 36.34% myelencephalon (see Table 4 that also included brain organization data of Yopak et al., 2007). The brain of *C. taurus* was

illustrated by Garman (1913, plate 41) in which its morphology conforms to the specimen I examined. To my knowledge, the specimen of *O. ferox* depicted in Figure 3 represents the first illustration of the brain of that species. The brain morphology of another species of *Odontaspis*, *O. noronhai*, remains unknown.

**Pseudocarchariidae**—The two specimen of *Pseudocarcharias kamoharai*, BPBM 37113 (111 cm TL; 4.02 kg BoW) and FMNH 117474 (98.1 cm TL; 3.80 kg BoW), were examined and their measurements were averaged where the average TL and BoW were 104.55 cm and 3.91 kg, respectively. The aBrW of BPBM 37113 was found to be 3.05 g and that of FMNH 117474 2.76 g, averaging to an aBrW of 2.90 g with a FI of 2 (same FI value as Yopak et al., 2007: Table 3). Using the 35.5% weight reduction from my pilot study (see above), the eBrW for BPBM 37113 was calculated to be 4.13 g and that for FMNH 117474 3.74 g, giving an average eBrW of 3.93 g. Using the average eBrW and Snell's (1892) equation for simple allometry, the EQ was found to be 0.75 (vs. 0.92 by Yopak et al., 2007: Table 3). The brain organization of *P. kamoharai* (averages) was found to be 33.86% telencephalon (32.22% in BPBM 37113 and 35.50% in FMNH 117474), 7.00% diencephalon (7.30% in BPBM 37113 and 6.70% in FMNH 117474), 21.37% mesencephalon (20.28% in BPBM 37113 and 22.46% in FMNH 117474), 17.68% metencephalon (18.11% in BPBM 37113 and 17.25% in FMNH 117474), and 19.91% myelencephalon (21.73% in BPBM 37113 and 18.09% in FMNH 117474) (see Table 4 that also included brain organization data of Yopak et al., 2007). Lisney and Collins (2006, fig. 2b) illustrated a brain of *P. kamoharai* in which its morphology conforms to the specimen I examined.

**Megachasmidae**—The brain of *Megachasma pelagios* (NSMT-P 134785) comes from a 544-cm-TL individual originally illustrated and described by Ito et al. (1999). It came from an

individual that had a BoW of 1,040 kg, where Ito et al. (1999) also reported the aBrW of 19.80 g. Whereas it has a FI of 2, its eBrW was not needed because the brain mass was taken when the specimen was fresh. Using the aBrW and Snell's (1892) equation for simple allometry, the EQ was found to be 0.18 (Table 3), that marked the smallest brain among all the lamniforms examined in my present study. The brain organization of *M. pelagios* was found to be 22.98% telencephalon, 10.90% diencephalon, 13.75% mesencephalon, 12.22% metencephalon, and 40.15% myelencephalon (Table 4).

**Alopiidae**—The BoW for the specimen of *Alopias pelagicus* (FMNH 117473, 169.9 cm TL), *A. superciliosus* (UF 178509, 200.7 cm TL), and *A. vulpinus* (SIO 64-804A, 144.80 cm TL) was calculated to have 16.59, 55.36, and 14.06 kg, respectively. The aBrW of *A. pelagicus* was 8.03g with a FI of 5. The aBrW of *A. superciliosus* was found to be 13.41g with a FI of 5, that differs from the findings by Yopak et al. (2007) who reported a FI of 4 (Table 3). My decision of assigning a FI of 5 to *A. superciliosus* stems from the fact that the cerebellum of *A. superciliosus* is 1) more foliated than that of any of the taxa in the family Lamnidae (see below) with a FI of 4, and 2) more similar to that of *A. pelagicus* and *A. vulpinus* that exhibit a longitudinal fissure and distinctive cerebellar sections. The aBrW of *A. vulpinus* was 4.64 g with a FI of 5 (same FI value as Yopak et al., 2007: Table 3). Using the 35.5% weight reduction from my pilot study (see above), the eBrW for *A. pelagicus*, *A. superciliosus*, and *A. vulpinus* was calculated to be 10.88, 18.17, and 6.30 g, respectively. Using the eBrW and Snell's (1892) equation for simple allometry, the EQ of *A. pelagicus*, *A. superciliosus*, and *A. vulpinus* was calculated to be 1.45, 1.03, and 1.66 (vs. 1.28 for *A. superciliosus* and 1.71 for *A. vulpinus* by Yopak et al., 2007: Table 3). The brain organization of *A. pelagicus* was found to be 30.83% telencephalon, 2.20% diencephalon, 17.00% mesencephalon, 30.42% metencephalon, and 19.55% myelencephalon

(Table 4). The brain organization of *A. superciliosus* was found to be 24.64% telencephalon, 3.41% diencephalon, 16.40% mesencephalon, 30.01% metencephalon, and 25.54% myelencephalon (see Table 4 that also included brain organization data of Yopak et al., 2007). The brain organization of *A. vulpinus* was found to be 26.40% telencephalon, 1.30% diencephalon, 14.50% mesencephalon, 31.40% metencephalon, and 26.40% myelencephalon (see Table 4 that also included brain organization data of Yopak et al., 2007). The brain of *A. superciliosus* was illustrated by Lisney and Collins (2006, fig. 2a) and that of *A. vulpinus* by Kajiura et al. (2010, fig. 2.9C [sic]) and Yopak and Lisney (2012, fig. 2a) in which each of their morphology conforms to the brain of respective species I examined. I am not aware of any published illustration of the brain of *A. pelagicus*, and if so, Figure 3 would represent the first depiction of its morphology.

**Cetorhinidae**—Kruska (1988) examined and illustrated a brain of *Cetorhinus maximus* that measured 375 cm TL and 385 kg BoW, and my brain data largely come from Kruska's (1988) study. The aBrW measured by Kruska (1988) was 20.70 g, whereas I determined its FI to be 4 based on Kruska's (1988) dorsal and lateral images of the brain (reproduced in Fig. 3). The eBrW was not needed because the brain mass was taken in fresh state. Using the aBrW and Snell's (1892) equation for simple allometry, the EQ was found to be 0.33 (Table 3). The brain organization of *C. maximus* was found to be 34.00% telencephalon, 5.00% diencephalon, 9.00% mesencephalon, 30.00% metencephalon, and 22.00% myelencephalon (Table 4).

**Lamnidae**—The specimen of *Carcharodon carcharias* (LACM 56960-1, 209.25 cm TL), *Isurus oxyrinchus* (SIO 55-85, 82.80 cm TL), *I. paucus* (FMNH 135411, 237.7 cm TL), *Lamna ditropis* (FMNH 117475, 151.3 cm TL), and *L. nasus* (ROM 22751, 84.7 cm TL) was calculated to have a BoW of 84.82, 4.36, 105.45, 81.37, and 5.89 kg, respectively, and an aBrW



of 10.16, 4.42, 12.37, 4.89, and 3.56 g, respectively. All species in this family had a FI of 4 (same FI value as Yopak et al., 2007, who included data from *C. carcharias* and *I. oxyrinchus*: Table 3). Using the 35.5% weight reduction from my pilot study, the eBrW for *C. carcharias*, *I. oxyrinchus*, *I. paucus*, *L. ditropis*, and *L. nasus* was calculated to be 13.77, 5.98, 16.76, 6.63, and 4.83 g, respectively, with the EQ of 0.49, 1.08, 0.56, 0.24, and 0.74, respectively (vs. 0.41 for *C. carcharias* and 0.60 for *I. oxyrinchus* by Yopak et al., 2007: Table 3). The brain organization of *C. carcharias*, *I. oxyrinchus*, *I. paucus*, *L. ditropis*, and *L. nasus* was found to be, respectively: 38.48, 37.57, 38.40, 25.20, and 28.15% telencephalon; 4.85, 4.03, 4.90, 4.10, and 3.27% diencephalon; 16.58, 16.50, 18.40, 21.15, and 19.38% mesencephalon; 17.89, 20.50, 17.90, 26.85, and 27.15% metencephalon; and 22.20, 21.40, 20.40, 22.70, and 22.05% myelencephalon (see Table 4 that also included brain organization data of *C. carcharias* and *I. oxyrinchus* by Yopak et al., 2007). Brains of *C. carcharias*, *I. oxyrinchus*, and *L. ditropis* have been illustrated in literature, including papers by Gilbert (1963, fig. 7), Demski and Northcutt (1996, figs. 1, 2), Kajiura et al. (2010, fig. 2.9D [sic]), and Schaffer et al. (2013, fig. 1.1), where each of their overall morphology conforms to the specimen of respective species I examined. I am not aware of any published illustration of the brain of *I. paucus* or *L. nasus*, and if so, Figure 3 would represent the first depiction of its morphology.

**Scyliorhinidae (outgroup)**—Two specimen of *Scyliorhinus retifer* (CUMV 45864-A, 33.6 cm TL; and CUMV 45864-B, 37.8 cm TL) were examined that had an average TL of 35.70 cm. According to the museum catalog, CUMV 45864-A and CUMV 45864-B had a BoW of 0.15 and 0.18kg, respectively, with an average BoW of 0.16 kg. The aBrW for CUMV 45864-A and CUMV 45864-B was found to be 0.49 and 0.58 g, respectively, with an average aBrW of 0.53 g. Both specimens exhibited a FI of 1 (same FI value as Yopak et al., 2007: Table 3). Using

the 35.5% weight reduction from my pilot study (see above), the average eBrW was calculated to be 0.72 g. Using the average eBrW and Snell's (1892) equation for simple allometry, the average EQ was found to be 0.78 (Table 3; note that EQ of this species was not reported by Yopak et al., 2007). The brain organization of *S. retifer* (averages) was 48.67% telencephalon (49.07% in CUMV 45864-A and 48.27% in CUMV45864-B), 8.30% diencephalon (7.33% in CUMV 45864-A and 9.27% in CUMV 45864-B), 10.28% mesencephalon (9.61% in CUMV 45864-A and 10.95% in CUMV 45864-B), 16.69% metencephalon (16.58% in CUMV 45864-A and 16.80% in CUMV 45864-B), and 16.06% myelencephalon (17.41% in CUMV 45864-A and 14.71% in CUMV 45864-B) (see Table 4 that also shows Yopak et al.'s, 2007, data). The brain of *Scyliorhinus* has been illustrated in literature, including the work on *S. canicula* by Ridet et al. (1973, figs. 3, 4), showing nearly identical morphology observed in the specimens I examined.

## REGRESSION-BASED COMPARATIVE ANALYSES

Figure 4 shows my regression analysis that investigates whether or not the brain mass has any relationship with the body mass in lamniforms. The  $\log_{10}$  transformed regression line with an equation of  $y = 0.323x + 0.478$  and  $r^2$  of 0.797 was found to be statistically significant ( $p < 0.0001$ ), suggesting the presence of a strong positive correlation between brain size and body size. The regression line shows that *Mitsukurina owstoni*, *Carcharias taurus*, *Odontaspis ferox*, *Pseudocarcharias kamoharai*, *Megachasma pelagios*, *Lamna ditropis*, and *L. nasus* have a brain smaller than expected for a shark of that respective size, whereas the overall brain size of all three *Alopias* spp. as well as *Carcharodon carcharias*, *Isurus oxyrinchus*, and *I. paucus* is found to be larger than expected for a shark of that respective size. On the other hand, my analysis shows that *Cetorhinus maximus* has a brain mass expected for a shark of that size.

Figure 5 shows two regression analyses examining whether or not the FI-based cerebellar complexity in lamniform sharks is depended by the brain size in terms of EQ (Fig. 5A) and aBrW/BoW (Fig. 5B). The regression line between the EQ and FI with an equation of  $y = 1.490x + 2.644$  and  $r^2$  of 0.434 (Fig. 5A) has a statistically significant ( $p = 0.0125$ ) positive correlation, meaning that sharks with a larger brain tend to have a more foliated cerebellum. On the other hand, the regression line between the aBrW/BoW and FI (Fig. 5B) that has an equation of  $y = 0.265x + 3.617$  and  $r^2$  of 0.007 shows no significance ( $p = 0.7803$ ), suggesting that the body weight over brain weight has no effect on the cerebellar complexity.

## CHARACTER MAPPING

Figure 6 shows mapping of my FI and EQ data onto a morphology-based phylogenetic tree (Fig. 6A) and a molecular-based phylogenetic tree of the order Lamniformes, both with *Scyliorhinus retifer* as an outgroup (Fig. 6B) (note: explanation about ‘oEQ’ in Figure 6 provided in Discussion below). *Scyliorhinus retifer* has an FI of 1, whereas all the lamniform taxa have FI values of 2 or higher. On the other hand, the relative brain size in terms of EQ within Lamniformes shows no apparent trends in both trees. The three species of *Alopias* have higher EQ values relative to most other lamniforms, but sister taxa in many other clades (e.g., *Isurus* and *Lamna* clades in both trees as well as the *Pseudocarcharias-Megachasma* clade in the molecular-based tree), are represented by both low and high EQ values.

## IV. DISCUSSION

### A. SOURCES OF VARIATION

#### 1. Sample-Based Variation

The comparison of FI data between my study and Yopak et al.'s (2007) study reveals that FI values are remarkably consistent intraspecifically (Table 3) where the only minor difference observed was for *Alopias superciliosus* (FI of 4 vs. 5: see above). On the other hand, whereas the EQ for *A. vulpinus* and *C. carcharias* was similar between the two studies, the EQ for *Carcharias taurus*, *P. kamoharui*, *A. superciliosus* and *I. oxyrinchus* was noticeably different (Table 3). Although calibrating an EQ value is sensitive to the body mass used, such intraspecific differences in brain size could at least in part be attributed to the differences in ontogenetic age among shark individuals examined, where the majority of the brain growth in vertebrates is generally known to occur while an individual is still young (Dekaban and Sadowsky, 1978; Leigh, 2004; Cofran, 2019). Yopak et al.'s (2007) specimens of *C. taurus* (152.4 kg BoW) and *I. oxyrinchus* ( $n = 3$  with an average of 186.53 kg BoW) were mature, whereas the specimens of these two species in my study were very young (4.44 and 4.36 kg BoW, respectively). Using such young specimens could result in larger brain sizes relative to the body sizes as the growth of the body might simply not have caught up to the growth of the brain. Nevertheless, it is noteworthy that taxa with higher EQ values in one data set are generally also higher in the other data set relative to taxa with lower EQ values, suggesting that different EQ values for each species are still collectively characterizing the brain size attribute for that species.

My data also show that similar effects of age differences could also be at play when comparing the data interspecifically (Table 3). For example, my specimen of *Isurus oxyrinchus* is much smaller than that of *I. paucus*, measuring 4.36 and 105.45 kg BoW, respectively, where *I. oxyrinchus* has a substantially larger EQ value (1.08) than *I. paucus* (0.56). Similarly, the specimen of *Lamna nasus* in this study is considerably smaller than that of *L. ditropis*, weighing 5.89 and 81.37 kg BoW, respectively, where *L. nasus* has a larger EQ value (0.74) than *L. ditropis* (0.24). Because the two species are phylogenetically sisters and live in similar environments (Compagno, 1990, 2002; Naylor et al., 2012; Ebert et al., 2013), one would expect the EQ of both species of each genus to be similar. The discrepancies in my data appear to suggest the possible presence of ontogenetic effects on brain size, but more samples are needed to substantiate the degree to which ontogenetic differences impact EQ values.

Another source of variation in EQ values could be the amount of brain shrinkage. Kruska (1988) noted that the brain of *Cetorhinus maximus* shrunk by 51% after alcohol preservation. Such a large level of shrinkage would substantially affect the calibrated EQ. To compensate for this shrinkage effect, I applied the assumption of the 35.5% reduction based on my pilot study (Table 1) for adjustment to the brain mass data for specimens preserved in alcohol. Although the 35.5% reduction is considerably conservative than the 51% reduction observed by Kruska (1988), it should be pointed out that Kruska's (1988) specimen of *C. maximus* represents the largest brain among all the brain specimens I examined where it is likely that the shrinkage rate may be higher than the remaining brain specimens that are smaller. The fact that the brain sizes of specimens I examined in this study are equivalent or smaller than Kruska's (1988) specimen of *C. maximus*, justifies that the 35.5% reduction is a reasonable, conservative proxy to the general shrinkage rate for alcohol-preserved brains of typical lamniforms, if not all the chondrichthyans.

## **2. Regression-Based Interspecific Variation**

The  $\log_{10}$  transformed regression was calculated to examine any correlation between brain weight and body weight. Previous studies found that brain mass scales positively with body mass in sharks (Bauchot et al., 1976; Northcutt, 1977, 1978; Myagkov and Hirnforsch, 1991; Yopak et al., 2007, 2019; Yopak and Montgomery, 2008). The results of my regression analysis (Fig. 4) suggest that such a positive scaling is also present in the order Lamniformes.

My regression analysis examining the relationship between FI and EQ shows a strong positive correlation (Fig. 5A). This result is consistent with previous studies on mammalian brains (Toro et al., 2008; Germanaud et al., 2012), suggesting that, at least for lamniforms, a more foliated brain requires a larger metencephalon (cerebellum), thus increasing the overall brain size in terms of EQ. Conversely, a less foliated brain would not need a larger brain.

My regression analysis showing body weight divided by the actual brain weight and how it correlates with cerebellar foliation shows no correlations (Fig. 5B). This result indicates that body size does not affect the degree of cerebellar foliation. This interpretation, in turn, suggests that the size of the body is independent of the size of its brain at least in Lamniformes.

## **B. FUNCTIONAL IMPLICATIONS**

### **1. General Background**

Different parts of the brain (Fig. 1B) have different functions. For example, the telencephalon, that largely consists of the cerebrum, is responsible for memory formation and storage (Martin, 2003) including recognition of individuals (Gold et al., 2012). The telencephalon is also for somatosensation (Berlucchi and Vallar, 2018) in response to stimuli like

temperature, pain, pressure, and vibration, and processes sensory information such as sound, taste, smell, touch (Angel, 1977), and quite possibly electromagnetism as well. The diencephalon relays sensory information among different brain regions and controls many autonomic functions of the peripheral nervous system (Penfield, 1934). The mesencephalon, which largely consists of the optic lobe, is responsible for coordination of the eye as well as visual and auditory processing (Kurkcuoglu, 2017). The metencephalon that mostly consists of the cerebellum controls and coordinates fine motor control and bodily movement (Haier et al., 2004; Montgomery and Perks, 2019). The myelencephalon that largely consists of medulla integrates afferent information from a variety of peripheral receptors and produces control signals to effector organs for appropriate physiological responses (Ciriello et al., 1986). Based on these region-specific functional differences, it is reasonable to assume that different sharks with different lifestyles would have different types of brain organization.

## **2. Telencephalon**

My brain organization data (Table 4) show that, among lamniforms, *Carcharodon carcharias* and *Isurus* spp. have the largest telencephalon (ca. 38% of the total brain). Their large telencephalon likely reflects their acute sense of smell, where their capacity to swim fast due to regional endothermy (or heterothermy: Katz, 2002; see below for additional discussion) may be a strategy to reach a distant food source detected through sensitive olfaction faster than most other animals with similar diet preferences. On the other hand, *Odontaspis ferox* and *Megachasma pelagios* have the smallest telencephalon (ca. 23% of the total brain) among lamniforms (Table 4). Whereas the possible explanation for the small telencephalon in *M. pelagios* is discussed below, this fact for *O. ferox* is intriguing because the biology of the species is still poorly

understood (e.g., see Fergusson et al., 2007). It may indicate that *O. ferox* does not rely on smell for prey capturing that is known to feed on small fish and invertebrates such as squid (Compagno, 2002; Fergusson et al., 2007).

It is noteworthy that the scyliorhinid carcharhiniform, *Scyliorhinus retifer*, I examined for comparison has even larger telencephalon than species of *Carcharodon* and *Isurus*, taking up as much as almost 49% of the total brain (Table 4). Scyliorhinids are for the most part benthic sharks that regularly feed on benthic fishes and invertebrates and hunt by searching crevices in rocks using its acute sense of smell and ampullae of Lorenzini (Compagno, 1984; Ebert et al., 2013). Their reliance on smell and electromagnetic signals is the likely driving factor for their enlarged telencephalon, where it is worth pointing out that extant members of Lamniformes do not include benthic forms equivalent to scyliorhinids.

### **3. Diencephalon**

My brain organization data (Table 4) indicate that, among lamniforms, *Carcharias taurus* and *Megachasma pelagios*, followed by *Mitsukurina owstoni*, have the largest diencephalon in relation to the rest of the brain, whereas *Alopias pelagicus* and particularly *A. vulpinus* have the smallest diencephalon. *Carcharias*, *Megachasma*, and *Mitsukurina* are all generally characterized as sluggish swimmers (Compagno, 1984, 2002; Ebert et al., 2013). A larger diencephalon observed in these sharks would mean that there would be more distance for sensory information to travel within it, so the large diencephalon present in these sharks makes sense because they do not require rapidly relaying sensory information within the brain or quickly controlling autonomic functions of the peripheral nervous system. Likewise, *A. pelagicus* and *A. vulpinus* exhibiting a small diencephalon makes sense because they are known to engage in rapid



and complex maneuvering of the body and caudal fin to hunt for prey (Aalbers et al., 2010; Oliver et al., 2013: see below for further discussion on *Alopias* spp.). Their small diencephalon is likely to minimize the traveling distance for rapid transmission of sensory information, including enhanced autonomic functions.

#### **4. Mesencephalon**

My brain organization data (Table 4) show, within Lamniformes, that *Pseudocarcharias kamoharai* has the largest mesencephalon relative to the rest of the brain, whereas the following taxa also have a relatively large (>15%) mesencephalon: *Alopias pelagicus*, *A. superciliosus*, *Carcharodon carcharias*, *Isurus* spp., and *Lamna* spp. Although the biology of *P. kamoharai* and *A. superciliosus* is poorly understood, they together with *A. pelagicus* have large eyes relative to their body (Compagno, 1984, 2002; Ebert et al., 2013), and the large mesencephalon that would allow better eye coordination and visual processing in these sharks makes sense. All lamnids (*Carcharodon*, *Isurus*, and *Lamna*) also have relatively large mesencephalon, suggesting that they too have better eye coordination and visual acuity relative to other lamniforms presumably aiding for hunting active prey (for their biology, see Compagno, 1984, 2002).

It is worth noting that, unlike *A. pelagicus* and *A. superciliosus*, *A. vulpinus* has a relatively small mesencephalon. However, where *A. pelagicus* and *A. superciliosus* are ectothermic, *A. vulpinus* is known to be endothermic to maintain its brain and eye muscles above the ambient water temperature like lamnids (Dickson and Graham, 2004; Patterson et al., 2011). Because warming of the brain and eye muscles has shown to improve reaction time (Fritsches et al., 2005; Helfman et al., 2009), its endothermy may help increasing its visual acuity to compensate for its relatively small size of the mesencephalon (see below for further discussion).

All other lamniform sharks (*Mitsukurina*, *Carcharias*, *Odontaspis*, *Megachasma*, and *Cetorhinus*) are ectothermic with a relatively small (<15%) mesencephalon (Table 4), suggesting that their visual reliance is comparably less than the aforementioned taxa.

## 5. Metencephalon

My brain organization data (Table 4) indicate that all *Alopias* spp. and *Cetorhinus maximus*, followed by *Lamna* spp., have a large (>25%) metencephalon among lamniforms. The enlarged metencephalon (or cerebellum) along with an exceptionally high FI value (FI of 5) in *Alopias* spp. is likely associated with their complex hunting behavior to stun prey using their caudal fin, requiring fine motor control of the body (Aalbers et al., 2010; Oliver et al., 2013: see below for further discussion). On the other hand, the relatively large metencephalon in *C. maximus* is rather puzzling because it is generally characterized as a slow-swimming shark. However, its highly migratory behavior to search for food does demand significant body movement (Skomal et al., 2009; Hueter et al., 2013), and it even exhibit breaching behavior occasionally (Hayes et al., 2018), suggesting that *C. maximus* may have more active lifestyle than it is typically perceived. The rather large metencephalon (ca. 27%) for *Lamna* spp. is intriguing given that other lamnids (*Carcharodon* and *Isurus*) with a large telencephalon have a relatively small metencephalon. This difference is also somewhat puzzling. However, because all lamnids have regional endothermy (see above) that likely result in an increase in agility and improved reaction time which in turn has been shown to increase the ability to receive and synchronize information to and from the peripheral nervous system (Garg et al., 2013), it is possible that the relatively small metencephalon in *C. carcharias* and *Isurus* spp. functions as well as *Lamna* spp. and ectothermic taxa with a larger metencephalon (see below for further

discussion). It should be added that, among lamniforms, an exceptionally small (12%) metencephalon is present in *Megachasma pelagios* that agrees well with its exceptionally sluggish lifestyle (see Compagno, 2002, and references therein).

## **6. Myelencephalon**

My data (Table 4) show that, among the lamniforms examined, *Megachasma pelagios* has an exceptionally enlarged myelencephalon (40% of the total brain), followed by *Odontaspis ferox* (36%), whereas all other lamniforms have a myelencephalon that takes up only 20–26% of the total brain. Whereas the biology of both *O. ferox* and *M. pelagios* is still poorly known (Fergusson et al., 2007; Watanabe and Papastamatiou, 2019), the exact significance of their large myelencephalon is unclear where it is plausible that the condition may simply be due to smaller sizes of their other brain regions because the brain organization in this study is measured based on the proportions of the five brain regions relative to the total brain size.

## **C. ECOLOGICAL IMPLICATIONS**

### **1. Deep-water Sharks**

There have been numerous studies on the physiology and sensory specialization of deep-water vertebrate species (e.g., Angel, 1997; Merrett and Haedrich, 1997; Douglas et al., 1998; Wagner et al., 1998; Herring, 2000; Warrant, 2000; Warrant and Locket, 2004). However, the question about exactly how neural characteristics on deep-water sharks have evolved in response to the demands of the deep-water has remained largely unaddressed until recently. Yopak and Montgomery (2008) examined 12 species of deep-water sharks and found that they all exhibit a

metencephalon with a low FI (1 or 2), small encephalization, and small telencephalon. However, their study did not contain any deep-water sharks in the order Lamniformes.

Although about half of the 15 extant lamniform species (Fig. 2) are reported from water depth equal to, or greater than, 1,000 m (Weigmann, 2016), *Mitsukurina owstoni* is one taxon generally characterized as a deep-water lamniform (e.g., Ebert et al., 2013) that has been captured at depths of 1,300 m (Weigmann, 2016). Yet, my data (Tables 3, 4) show two strikingly differences from Yopak and Montgomery's (2008) observations on deep-water non-lamniform sharks. First, *M. owstoni* has a metencephalon with a FI of 3 (Table 3). Second, the telencephalon is large, making up about one-third (34%) of the total brain mass (Table 4). One likely explanation for the rather well-foliated metencephalon and large telencephalon in *M. owstoni* is its highly specialized mode of detecting and capturing its prey (i.e., primarily teleosts, but also some invertebrates, such as squids, decapods, and isopods: Yano et al., 2007). *Mitsukurina owstoni* uses its elongated rostrum (Fig. 2) covered in ampullae of Lorenzini to accurately detect the position of prey electromagnetically under the rostrum (Compagno, 1984, 2002). When prey is detected, *M. owstoni* then protracts its highly kinetic jaws with great speed to capture the prey item (Nakaya et al., 2016). In addition, *M. owstoni* has an enlarged nasal apparatus (Masai et al., 1973) that also explains the rather large telencephalon. Therefore, the expansion of telencephalon for enhanced olfaction and electromagnetic sensitivity in *M. owstoni*, coupled with its rather well-foliated cerebellum to enhance motor control (e.g., Haier et al., 2004) for rapid jaw protraction, represents a unique alternative mode of brain-wise deep-water adaptation in sharks. Such an adaptation of *M. owstoni* is considered vital for its survival in the dark waters, particularly considering its sluggish swimming mode (see Nakaya et al., 2016).

## 2. Filter Feeders

There are three extant species of filter-feeding sharks: *Rhincodon typus* (Orectolobiformes: Rhincodontidae), *Megachasma pelagios*, and *Cetorhinus maximus*, all of which grow to immense sizes (Ebert et al., 2013). The brain of each of the three filter feeders has been studied previously (Kruska, 1988; Ito et al., 1999; Yopak and Frank, 2009). However, my present study represents the first to compare all three.

Yopak and Frank (2009) found that *Rhincodon typus* has a small brain, with a large telencephalon, and a highly foliated and large metencephalon. *Rhincodon typus* and *C. maximus* have large migration routes and migrate where food is in high quantities (Skomal et al., 2009; Hueter et al., 2013), and Yopak and Frank (2009) attributed the large telencephalon to the social and migratory behaviors exhibited by both species in which the similarity in their brain organization to be the result of convergent evolution. Because the telencephalon, among its other functions (see above), plays a role in memory formation (Martin, 2003), their need to remember feeding grounds may also explain for their enlarged telencephalon.

In contrast, the brain organization of *Megachasma pelagios* is drastically different from that of *Rhincodon typus* and *Cetorhinus maximus*. Most notably, the telencephalon and metencephalon of *M. pelagios* are the smallest, or one of the smallest, among all the lamniform taxa, whereas its diencephalon and myelencephalon are the largest in proportion among all the lamniforms I examined (Table 4). In fact, the size of its myelencephalon marks the largest proportion observed in any shark to date (e.g., see Northcutt, 1978; Kruska 1988; Yopak et al., 2007, 2019; Yopak and Montgomery, 2008). These differences are striking because they show no sign of convergent evolution of *M. pelagios* with the other two filter-feeding sharks (see Yopak and Frank, 2009). At least based on one acoustic telemetry study, *M. pelagios* is known to

be a vertical migrator that moves between shallow waters at night (12–25 m) and deep waters (120–166 m) during the days (Nelson et al., 1997; Watanabe and Papastamatiou, 2019), and unlike *R. typus* and *C. maximus* (Compagno, 1984, 2002; Ebert et al., 2013), *M. pelagios* has never been found aggregated in groups. In addition, although it has a worldwide geographic distribution, *M. pelagios* is found most frequently caught or sighted along the Asian coasts where they represent some of the biologically richest regions in the world (Grassle and Maciolek, 1992; Fujikura et al., 2010). Taking these pieces of information into account, the small telencephalon and metencephalon in *M. pelagios* may be the reflection of its solitary behavior with limited social behavior and its preferred habitat where food is readily available all year long not requiring the need for memorizing the best feeding grounds. The exact significance of the exceptionally large diencephalon (except for a longer distance for neural signal to travel: see above) and myelencephalon is uncertain, but the condition may simply be the consequence of exceptionally small telencephalon and metencephalon as their sizes in this study are measured based on their proportions to the total brain size.

### **3. ‘Weaponized’ Caudal Fins**

*Alopias* spp. have brains distinct from all other brains in the order Lamniformes. The most notable differences are the large, highly foliated metencephalon (Tables 3, 4; see also Yopak et al., 2007, whose study included *A. superciliosus* and *A. vulpinus*, but not *A. pelagicus*). It is known that the larger metencephalon with the higher cerebellar foliation a brain has, the more volume with more surface area there is in the metencephalon, that in turn indicates a greater capacity for fine, accurate motor control (Haier et al., 2004). One likely explanation for the highly foliated metencephalon is the unique, and exceptionally complex prey hunting

behavior directly observed at least in *A. pelagicus* and *A. vulpinus* in which they use their elongated caudal fin to stun small schooling fish (Aalbers et al., 2010; Oliver et al., 2013). It is worth pointing out that the only other family of sharks that exhibit a FI of 5 is Sphyrnidae (*Sphyrna* spp.: hammerhead sharks) with unique laterally protruded eyes (Yopak et al., 2007). The high cerebellar complexity in sphyrnids may be attributed to their highly unique head morphology that provides high maneuverability with enhanced visual field and increased surface area and density for ampullae of Lorenzini (Kajiura, 2001; Kajiura and Holland, 2002; Kajiura et al., 2003; McComb et al., 2009), and it is also physically used in capturing and handling prey such as batoids (rays) (Chapman and Gruber, 2002).

It is noteworthy that *Alopias vulpinus* is the only member of Alopiidae with the ability to warm its brain and eye muscles above the ambient water temperature (Goldman, 2002; Dickson and Graham, 2004; Patterson et al., 2011). Where higher temperatures have been shown to improve reaction time and increase the ability to receive and synchronize information to and from the peripheral nervous system (Garg et al., 2013), the warming of the eyes and eye muscles has been shown previously to improve the resolution of the image the eyes that capture (Fritsches et al., 2005; Helfman et al., 2009). *Alopias vulpinus* also possesses the largest brain in terms of EQ among all the lamniform sharks examined (1.66–1.71: Table 3). Although sphyrnid sharks with a comparable FI value (see above) have even larger brain (EQ of 2.64–3.29: Yopak et al., 2007) than *A. vulpinus*, unlike ectothermic sphyrnids, the fact that *A. vulpinus* has regional endothermy in the brain makes the brain of *A. vulpinus* quite possibly the most derived and efficient of all sharks, or at least certainly within Lamniformes.

#### **4. Thunniform Swimmers**

The family Lamnidae (*Carcharodon*, *Isurus*, and *Lamna*) comprises five extant species that show regional endothermy capable of keeping the brain warm (Block and Carey, 1985; Wolf et al., 1988; Goldman, 1997; Goldman et al., 2004). Facilitated by their regional endothermy, lamnids employ high-speed thunniform swimming and achieve their propulsive power with their caudal fin (Donley and Shadwick, 2003; Wilga and Lauder, 2004; Watanabe et al., 2015, 2019). These sharks are all wide-ranging, migratory species that hunt very active, agile prey, such as scombrid and salmonid teleosts, and even pinnipeds, and cetaceans for *C. carcharias* (e.g., Compagno, 2002; Watanabe et al., 2019; Tucker et al., 2019, and references therein). My study shows that those five species have a relatively complex brain, all marked by a FI of 4 (Table 3). However, when only the EQ value of the largest examined individual for each lamnid species is used for comparison (i.e., 0.41 for *C. carcharias*; 0.60 for *I. oxyrinchus*; 0.56 for *I. paucus*; 0.24 for *L. ditropis*, and 0.74 for *L. nasus*: Table 3), it becomes evident that the brain of lamnids can be characterized overall as small to mid-size within Lamniformes. Lamnids exhibit regional endothermy which allows them to heat their brain and eyes well above the ambient water temperature compensates their relatively small brain size increasing the ability to receive and synchronize information to and from the peripheral nervous system, which in turn improves reaction time (Garg et al., 2013).

#### **D. EVOLUTIONARY IMPLICATIONS**

The distribution of mapped FI values onto the morphology-based and molecular-based trees (Fig. 6) does not necessarily favor one tree over the other, but it does show some distinct



evolutionary patterns regardless of the trees. For example, more derived lamniform taxa, particularly Alopiidae (*Alopias*), Cetorhinidae (*Cetorhinus*), and Lamnidae (*Carcharodon*, *Isurus*, and *Lamna*), have high (4 or 5) FI values compared to other taxa in each tree. Where all the lamniform taxa have a FI value of 2 or higher, a FI of 1 in *Scyliorhinus retifer* is noteworthy because it can be argued that a FI of 1 is a plesiomorphic condition, where lamniforms evolved at least some degree of cerebellar complexity as an apomorphic condition.

In contrast, there is no apparent trends in the relative brain size in terms of EQ within Lamniformes in both tree, and an EQ of 0.8 for *Scyliorhinus retifer* represents the approximate middle value of the total range of EQ values seen in lamniforms (0.2–1.7) (Fig. 6). However, because EQ values are found to be affected by ontogenetic growth where younger individuals tend to have larger brain than older individuals relative to the body size (see above), I reexamined my data against Yopak et al.'s (2007) data to take an EQ value derived from the largest sample (by BoW) for each species (Table 3). For example my specimen of *Carcharias taurus* was represented by a 9.43-kg individual, whereas Yopak et al.'s sample of the same species was 152.4 kg. This suggests that my sample was substantially younger than Yopak et al.'s sample, so I chose to map the EQ value based on Yopak et al.'s sample for *C. taurus*. Besides *C. taurus*, the reexamination resulted in changes in the EQ value for the following additional species: *Alopias superciliosus*, *Carcharodon carcharias*, and *Isurus oxyrinchus*. Although not perfect, this new data set minimizing inclusions of extremely young individuals is considered to be the most 'optimized encephalization quotient' (oEQ) represented by the largest possible sample for each species in which multiple EQ values are available.

The distribution of mapped oEQ values onto the morphology-based and molecular-based trees (Fig. 6) does not necessarily favor one tree over the other, but the oEQ values do reveal

certain trends that were not evident based only on my EQ values (Fig. 6). For example, all the lamnid species (*Carcharodon carcharias*, *Isurus* spp., and *Lamna* spp.) have an oEQ of  $\leq 0.7$ , whereas all the Alopiids (*Alopias* spp.) have an oEQ of  $\geq 1.3$ . Although a rather large discrepancy between *L. ditropis* and *L. nasus* in oEQ remains from EQ, the oEQ (or EQ) of 0.7 for *L. nasus* is likely an exaggerated value for the species, because the value was taken from a specimen that was substantially smaller (younger) than that of *L. ditropis*. The evolutionary pattern for other taxa in both trees is largely unclear, but because they all have an oEQ of  $\leq 0.8$ , including *Scyliorhinus retifer*, lower (i.e.,  $\leq 0.8$ ) oEQ values are considered plesiomorphic where exceptionally high oEQ values seen in *Alopias* spp. are clearly apomorphic. It is also worth noting that the oEQ of 0.8 for *S. retifer* represents the approximate middle value of the total range of oEQ values (0.2–1.7). If the oEQ of 0.8 is considered to be plesiomorphic, then it is possible to argue that lamniforms developed two evolutionary pathways in terms of oEQ—one group (*Alopias* spp.) attained enlarge the brain (high oEQ) and the other (all other lamniforms) evolved towards having smaller brain (low oEQ)—where both diverging trends can be regarded as two alternative apomorphies in this interpretation.

Both the morphology-based and molecular-based trees (Fig. 6) indicate that regional endothermy in *Alopias vulpinus* evolved independent of that in Lamnidae (see also Ferrón, 2017). Because these sharks can keep the brain and eyes warm above the ambient water temperature (Goldman, 2002; Dickson and Graham, 2004; Patterson et al., 2011), their warm brains and eye muscles allow for improved reaction time. However, whereas *A. vulpinus* has a higher FI (5) than Lamnidae (FI of 4), the fact that *A. vulpinus* has the largest brain (oEQ of 1.7) than any other lamniform shark, that brains of lamnids are relatively small (oEQ of  $\leq 0.7$ ), and that *A. vulpinus* and lamnids are the only extant endothermic sharks, suggests that *A. vulpinus*

arguably has the most derived brain among extant lamniforms, and quite possibly not only among extant elasmobranchs, but even among all the extant chondrichthyans.

## V. CONCLUDING REMARKS

There are over 500 species of extant sharks (Weigmann, 2016), where they have evolved their brains as diverse as the sharks themselves through nearly 400 million years of history (e.g., Yopak et al., 2007, 2019; Yopak and Montgomery, 2008; Yopak and Frank, 2009). Whereas understanding such diversity is critical for understanding the evolution of their cognitive ability and how ecology and phylogeny have influenced the variation of their brain complexity, many aspects of the diversity and evolution of brain morphology remain poorly understood. In this study, I examined the morphological variation of the brain across 14 of the 15 known extant species of a monophyletic shark order, Lamniformes, which exhibit remarkable ecological specializations (e.g., Ebert et al., 2013). I specifically examined the relative brain size (encephalization), the relative size differences among the five major brain regions, and the variation in cerebellar foliation with the goal to investigate the implications to function, behavioral ecology, and evolutionary variations observed in brain morphology.

Based on preserved museum specimens as well as published sources, my primary data set consisted of 14 of the 15 known extant lamniform species represented by all 10 extant lamniform genera. I also examined two brain samples of scyliorhinid carcharhiniform, *Scyliorhinus retifer*, for comparison as an outgroup. In addition, I examined eight fresh specimens of squalid squaliform, *Squalus acanthias*, for a pilot study to examine the possible shrinkage of brain specimens due to preservatives used, specifically formaldehyde and ethanol. My experimental

pilot study revealed that the brain samples of *S. acanthias* increased in mass by an average of 10.6% through formaldehyde fixation, followed by an average of 35.5% mass decreased after ethanol preservation. Therefore, all the preserved specimens I examined in this study were also assumed to have gone through an average of 35.5% decrease in brain mass that was necessary to extrapolate the original brain mass in fresh state. In addition, using the brains of *S. acanthias*, I also determined that they had shrunk uniformly across all brain regions.

A wide range of morphological diversity was found among the 14 lamniform species examined. Based on my quantitative data, I conducted three separate regression analyses that would examine different brain attributes among them. My first analysis was statistically significant ( $p < 0.0001$ ), suggesting that there is a strong positive correlation between brain size and body size. My second analysis was also statistically significant ( $p = 0.0125$ ), suggesting that sharks with a larger brain tend to have a more foliated cerebellum. My third analysis was not statistically significant ( $p = 0.7803$ ), suggesting that the body weight over brain weight has practically no effect on the cerebellar complexity.

The comparisons of my data with a previously published dataset (i.e., Yopak et al., 2007) indicated that EQ values are affected at least in part by ontogeny where younger (smaller) individuals have larger brains in terms of EQ than older (larger) individuals of the same species likely associated with negative allometry of the head relative to the body. Nevertheless, I found that taxa with higher EQ values in one data set are generally also higher in the other data set relative to taxa with lower EQ values, indicating that different EQ values for each species still collectively characterize the brain size of that species. However, my data also showed that similar ontogenetic effects may also be at play when comparing the data interspecifically. In addition, the amount of brain shrinkage among different samples could be another source of

variation in EQ values where the brain mass reduction could have been slightly greater or lesser than my assumed 35.5% reduction.

Different parts of the brain have different functions that may reflect different lifestyles. My data showed that, among lamniforms, *Carcharodon carcharias* and *Isurus* spp. have the largest telencephalon that likely reflects their acute sense of smell, whereas *Odontaspis ferox* and *Megachasma pelagios* have the smallest telencephalon indicating that they likely do not rely on smell for prey capturing. The largest diencephalon in relation to the rest of the brain was found in sluggish swimmers, such as *Mitsukurina owstoni*, *Carcharias taurus*, and *Megachasma pelagios*, that do not require rapid relaying of sensory information within the brain or quick autonomic responses through the brain region. The smallest diencephalon was found in *Alopias pelagicus* and *A. vulpinus* that require rapid neural signal transmission for their unique tail-based hunting behavior. *Pseudocarcharias kamoharai*, *A. pelagicus*, *A. superciliosus*, *Carcharodon carcharias*, *Isurus* spp. and *Lamna* spp. have a large mesencephalon in relation to the rest of the brain, suggesting that these sharks have better eye coordination and visual acuity relative to other lamniforms. *Alopias* spp. have an enlarged metencephalon likely associated with their complex hunting behavior, whereas *M. pelagios* possesses an exceptionally small metencephalon consistent with its sluggish swimming. A large myelencephalon is present in *M. pelagios* and *O. ferox*, but its biological significance is uncertain at the present time.

Some ecological specializations are reflected in the diversity of brain anatomy seen in certain lamniforms, such as adaptations to deep-water, filter feeding, tail-based prey hunting, and thunniform swimming. For example, *Mitsukurina owstoni* has a metencephalon with a moderate cerebellar foliation, and its telencephalon is enlarged, likely related to enhanced olfaction and electromagnetic sensitivity in the dark coupled with enhance motor control for rapid jaw

protraction to compensate its sluggish swimming. Unlike the other two filter-feeding sharks, *Rhincodon typus* and *Cetorhinus maximus*, *Megachasma pelagios* has a small telencephalon and metencephalon likely reflecting its solitary behavior with limited social and migratory behaviors. *Alopias* spp. have a large, highly foliated metencephalon, indicating a greater capacity for fine, accurate motor control required for their complex tail-based prey hunting behavior. The brain of lamnids (*Carcharodon*, *Isurus*, and *Lamna*) are relatively small within Lamniformes, but it is likely that their regional endothermy allows them to heat their brain and eyes well above the ambient water temperature to compensate their relatively small brain size.

I employed character mapping on a morphology-based tree and a molecular-based tree in order to examine the evolutionary pattern of the brain through lamniform phylogeny using the scyliorhinid carcharhiniform, *Scyliorhinus retifer*, as an outgroup. Whereas my data did not favor one tree over the other, it did show some distinct evolutionary patterns regardless of the trees. For example, more derived lamniform taxa, particularly Alopiidae (*Alopias*), Cetorhinidae (*Cetorhinus*), and Lamnidae (*Carcharodon*, *Isurus*, and *Lamna*), were found to have highly foliated cerebellum, where limited foliation is regarded as plesiomorphic. After optimizing my brain size data by incorporating published data, all the lamnid species (*Carcharodon carcharias*, *Isurus* spp., and *Lamna* spp.) were found to have relatively small (or mid-sized) brain, whereas all the alopiids (*Alopias* spp.) were found to have a large brain, where a mid-sized brain can be interpreted as a plesiomorphic condition. Although the functional efficiency of the smaller brains in lamnids is likely compensated by their warming through regional endothermy, convergently evolved regional endothermy in *A. vulpinus* may arguably have the most derived brain of all among all the extant chondrichthyans.

Because fresh samples of lamniforms are generally difficult to come by due to their general rarity or their typically large sizes, one major limitation of the type of investigation I employed in this study is the small sample size for each species. Another limitation with collection-based studies on shark brains that requires further investigation is a better understanding of the effects of their formaldehyde fixation and alcohol preservation. Nevertheless, my present study is significant because it is the first of its kind to examine the morphological variation and diversity of the brain across almost all the known extant species of Lamniformes and relate to its possible function, ecology, and evolution. This study demonstrates how ecological factors such as habitat, diet, and behavior drive brain evolution.

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**Table 1.** Experiment as pilot study demonstrating changes in brain weights in eight samples of *Squalus acanthias* (Squaliformes: Squalidae) after fixation using 10% formaldehyde from fresh condition, followed by preservation in 70% ethanol. Abbreviations in listed sequence: FMNH, FMNH catalog number; BoW, body mass; TL, total length; FrBrW, fresh brain weight; FoBrW, formaldehyde-treated brain weight; Fo%CfFr, percent weight change of formaldehyde-treated brain from fresh brain; EtBrW, ethanol-treated brain weight; Et%CfFr, percent weight change of ethanol-treated brain from fresh brain.

FMNH	BoW (kg)	TL (cm)	FrBrW (g)	FoBrW (g)	Fo%CfFr (%)	EtBrW (g)	Et%CfFr (%)
141885-1	0.87	67.8	2.59	2.98	+15.05	1.47	-43.24
141885-2	0.99	67.2	3.26	3.72	+14.11	2.29	-29.75
141885-3	1.07	70.2	3.53	3.81	+8.11	2.22	-37.11
141885-4	1.07	71.8	3.64	3.97	+9.07	2.29	-37.11
141885-5	1.41	73.6	3.87	4.21	+8.79	2.34	-39.53
141885-6	1.54	74.2	4.03	4.55	+12.90	2.76	-31.51
141885-7	1.61	81.2	4.03	4.35	+7.94	2.72	-31.51
141885-8	1.57	82.7	4.06	4.41	+8.62	2.71	-33.25
Average	1.27	73.6	3.64	4.00	+10.57 ±2.74SD	2.31	-35.50 ±4.54SD

**Table 2.** Pilot study showing percent size of each of five brain regions relative to total brain size (100%) of four samples of squalid squaliform, *Squalus acanthias*, compared with percent values of *S. acanthias* from Yopak et al. (2007, table 2). Abbreviations in listed sequence: Tel, telencephalon; Die, diencephalon; Mes, mesencephalon; Met, metencephalon; Mye, myelencephalon (see Fig. 1B).

Specimen	Tel	Die	Mes	Met	Mye	Total
This study						
FMNH 141885-2	31.29	6.44	16.70	16.45	29.12	100
FMNH 141885-4	31.80	6.10	15.30	17.50	29.30	100
FMNH 141885-6	30.54	6.07	15.20	18.20	29.99	100
FMNH 141885-8	31.56	6.65	14.50	17.47	29.82	100
Average ( $n = 4$ )	31.30	6.32	15.43	17.41	29.56	100.02
Yopak et al.'s (2007) study						
Unspecified ( $n = 3$ )	31.26*	6.65*	15.02*	17.66*	29.43*	100.02*

\* average of three samples



**Table 3.** Examined specimens in this study and their total length (TL), body mass (BoW), actual brain weight (aBrW), estimated brain weight (eBrW), foliation index (FI: see Fig. 2C), and encephalization quotient (EQ) for 14 species of lamniform sharks (Fig. 1) and one species of scyliorhinid carcharhiniform, *Scyliorhinus retifer* (Sr), compared with Yopak et al.'s (2007, table 1, fig. 8) data. For species codes of lamniform species, see Figures 2 and 3. Unless otherwise indicated, sample size ( $n$ ) equals one. Body weight data were calculated using total length to weight conversation equations from following literature: a, Yano et al. (2007); b, Goldman et al. (2006); c, Fergusson et al. (2007); d, Drew et al. (2015); e, Kohler et al. (1996); f, Goldman and Musick (2006). Estimated brain weight is calculated based on a 35.5%-reduction in brain weight due to alcohol preservation as found from pilot study (Table 1; see text).

Species	Specimen	TL (cm)	BoW (kg)	aBrW (g)	eBrW (g)	FI	EQ
This study							
Mo	FMNH 117742	126.50	3.28 <sup>a</sup>	1.94	2.63	3	0.55
Ct	FMNH 16136	106.00	9.43 <sup>b</sup>	3.58	4.85	3	0.57
Of	BPBM 9335	297.00	166.83 <sup>c</sup>	6.91	9.26	3	0.23
Pk ( $n = 2$ )	BPBM-FMNH*	104.55**	3.91**	2.90**	3.93**	2**	0.75**
Mp	NSMT-P 134785	544.00***	1,040.00***	19.80***	-	2	0.18
Ap	FMNH 117473	169.90	16.59 <sup>d</sup>	8.03	10.88	5	1.45
As	UF 178509	200.70	55.36 <sup>e</sup>	13.41	18.17	5	1.03
Av	SIO 64-804A	144.80	14.06 <sup>e</sup>	4.64	6.30	5	1.66
Cm	Kruska's specimen	375.00****	385.00****	20.70****	-	4	0.33
Cc	LACM 56960-1	209.25	84.82 <sup>e</sup>	10.16	13.77	4	0.49
Io	SIO 55-85	82.80	4.36 <sup>e</sup>	4.42	5.98	4	1.08
Ip	FMNH 135411	237.70	105.45	12.37	16.76	4	0.56
Ld	FMNH 117475	151.30	81.37 <sup>f</sup>	4.89	6.63	4	0.24
Ln	ROM 22751	84.70	5.89 <sup>e</sup>	3.56	4.83	4	0.74
Sr ( $n = 2$ )	CUMV 45864-A, B	35.70**	0.16**	0.53**	0.72**	1**	0.78**
Yopak et al.'s (2007) study							
Ct	Unspecified adult	-	152.4	14.25	-	3	0.37
Pk	Unspecified adult	-	3.9	4.8	-	2	0.92
As	Unspecified adult	-	62.73	30.2	-	4	1.28
Av	Unspecified juvenile	-	5.83	11.13	-	5	1.71
Cc	Unspecified subadult	-	727.27	29.53	-	4	0.41
Io ( $n = 3$ )	Unspecified adult	-	186.53**	25.59**	-	4**	0.60**
Sr	Unspecified adult	-	-	-	-	1	-

\* BPBM 37113 and FMNH 117474

\*\* average of multiple samples

\*\*\* based on Ito et al. (1999)

\*\*\*\* based on Kruska (1988)

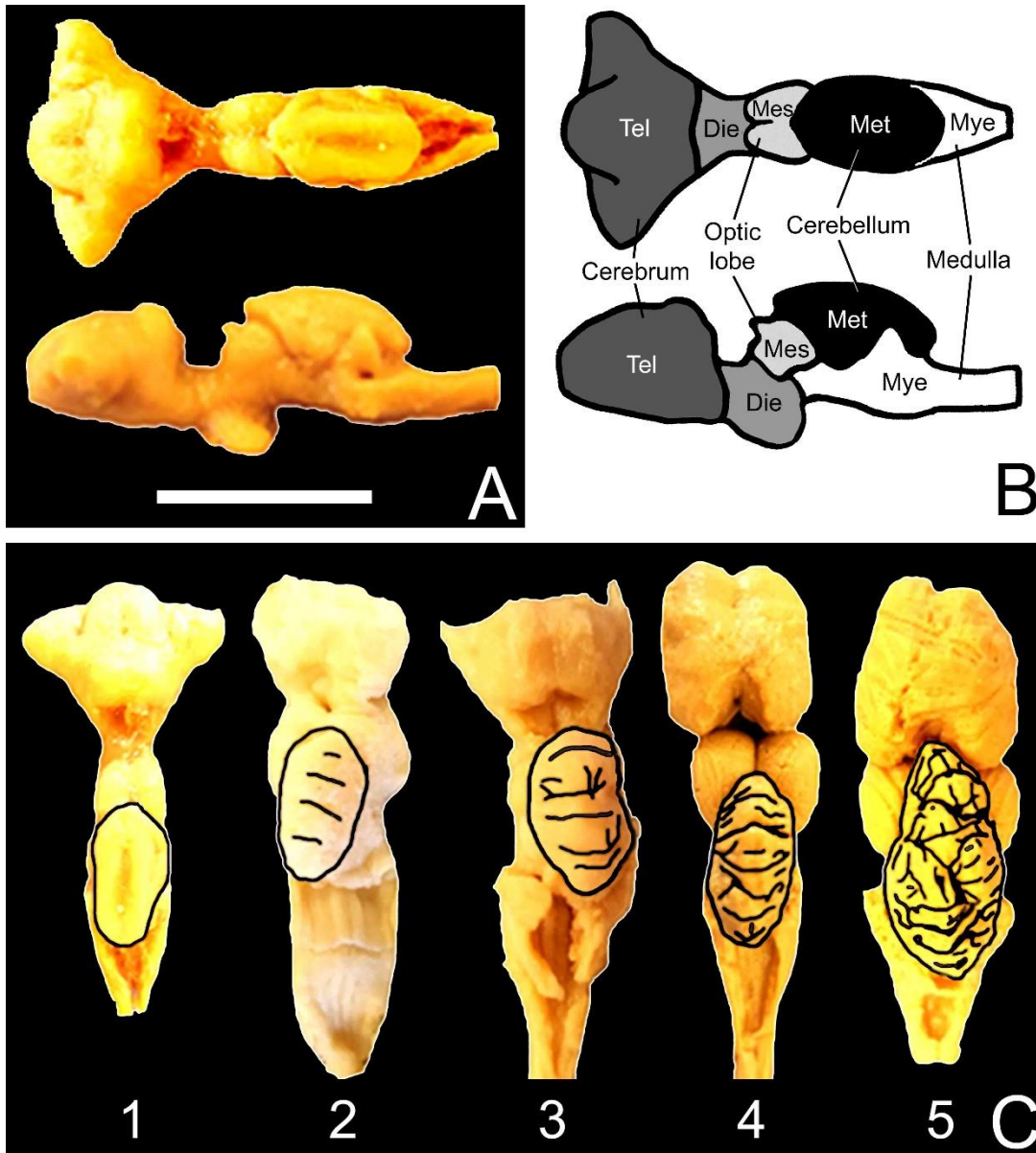
**Table 4.** Percent size of each of five brain regions relative to total brain size (100%) of 14 species of lamniform sharks and one species of scyliorhinid carcharhiniform, *Scyliorhinus retifer* (Sr), compared with percent values of comparable taxa from Yopak et al. (2007, table 2). For species codes of lamniform species, see Figures 1 and 3. Unless otherwise indicated, sample size (*n*) equals one. Abbreviations in listed sequence: Tel, telencephalon; Die, diencephalon; Mes, mesencephalon; Met, metencephalon; Mye, myelencephalon (see Fig. 1B).

Species	Tel	Die	Mes	Met	Mye	Total
This study						
Mo	34.55	8.76	9.79	21.13	25.77	100
Ct	33.00	10.70	9.98	23.90	22.42	100
Of	22.80	7.42	14.54	18.90	36.34	100
Pk ( <i>n</i> = 2)	33.86*	7.00*	21.37*	17.68*	19.94*	99.85*
Mp	22.98	10.90	13.75	12.22	40.15	100
Ap	30.83	2.20	17.00	30.42	19.55	100
As	24.64	3.41	16.40	30.01	25.54	100
Av	26.40	1.30	14.50	31.40	26.40	100
Cm	34.00**	5.00**	9.00**	30.00**	22.00**	100
Cc	38.48	4.85	16.58	17.89	22.20	100
Io	37.57	4.03	16.50	20.50	21.40	100
Ip	38.40	4.90	18.40	17.90	20.40	100
Ld	25.20	4.10	21.15	26.85	22.70	100
Ln	28.15	3.27	19.38	27.15	22.05	100
Sr ( <i>n</i> = 2)	48.67*	8.30*	10.28*	16.69*	16.06*	100*
Yopak et al.'s (2007) study						
Ct	30.57	11.42	9.47	25.28	23.26	100
Pk	33.13	6.88	20.42	16.04	23.54	100.1
As	27.19	3.15	16.19	32.09	21.39	100.1
Av	26.79	1.66	15.60	30.60	33.02	107.67***
Cc ( <i>n</i> = 3)	38.86*	5.57*	14.28*	17.66*	23.63*	100*
Io ( <i>n</i> =3)	37.70*	3.35*	18.18*	17.03*	23.74*	100*
Sr	40.70	8.14	13.95	17.44	19.77	100

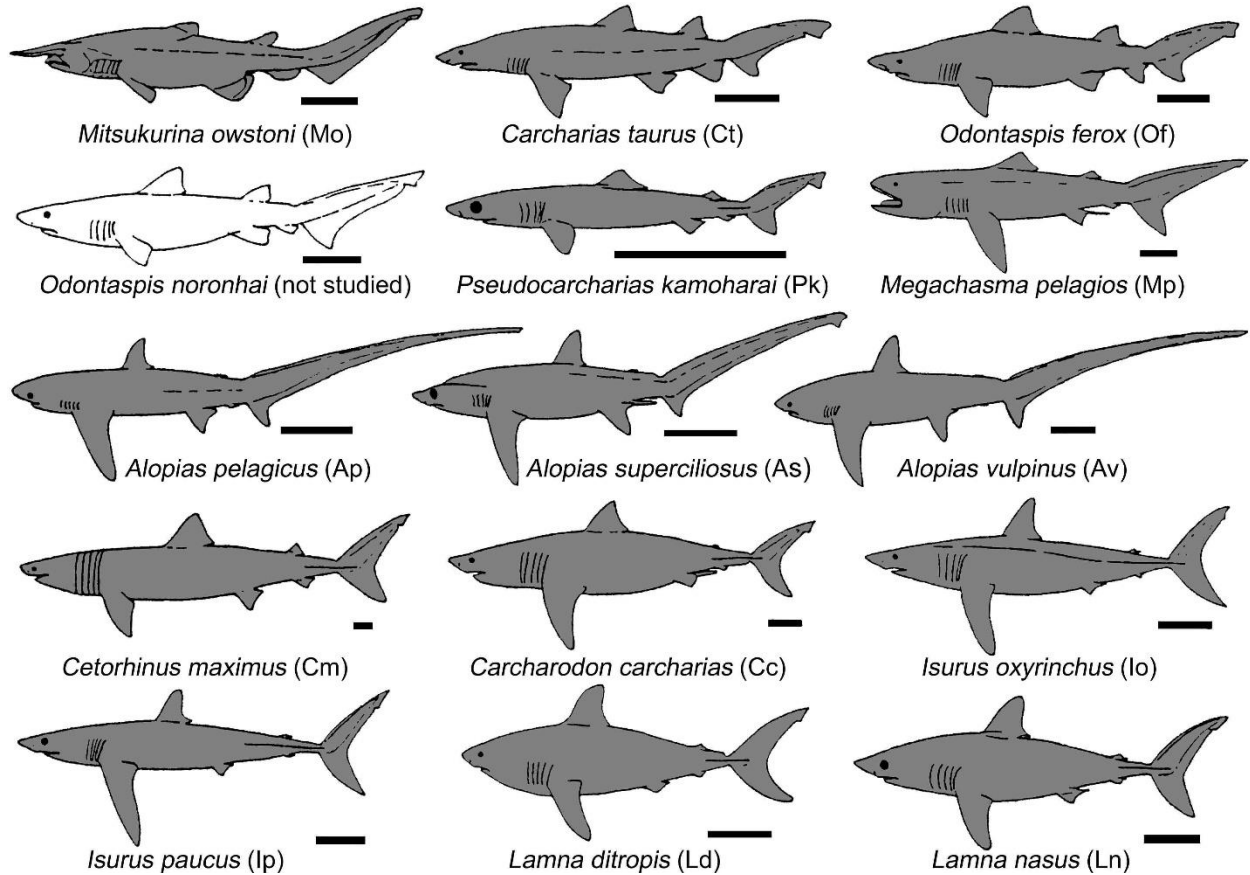
\* average of multiple samples

\*\* based on Kruska (1988)

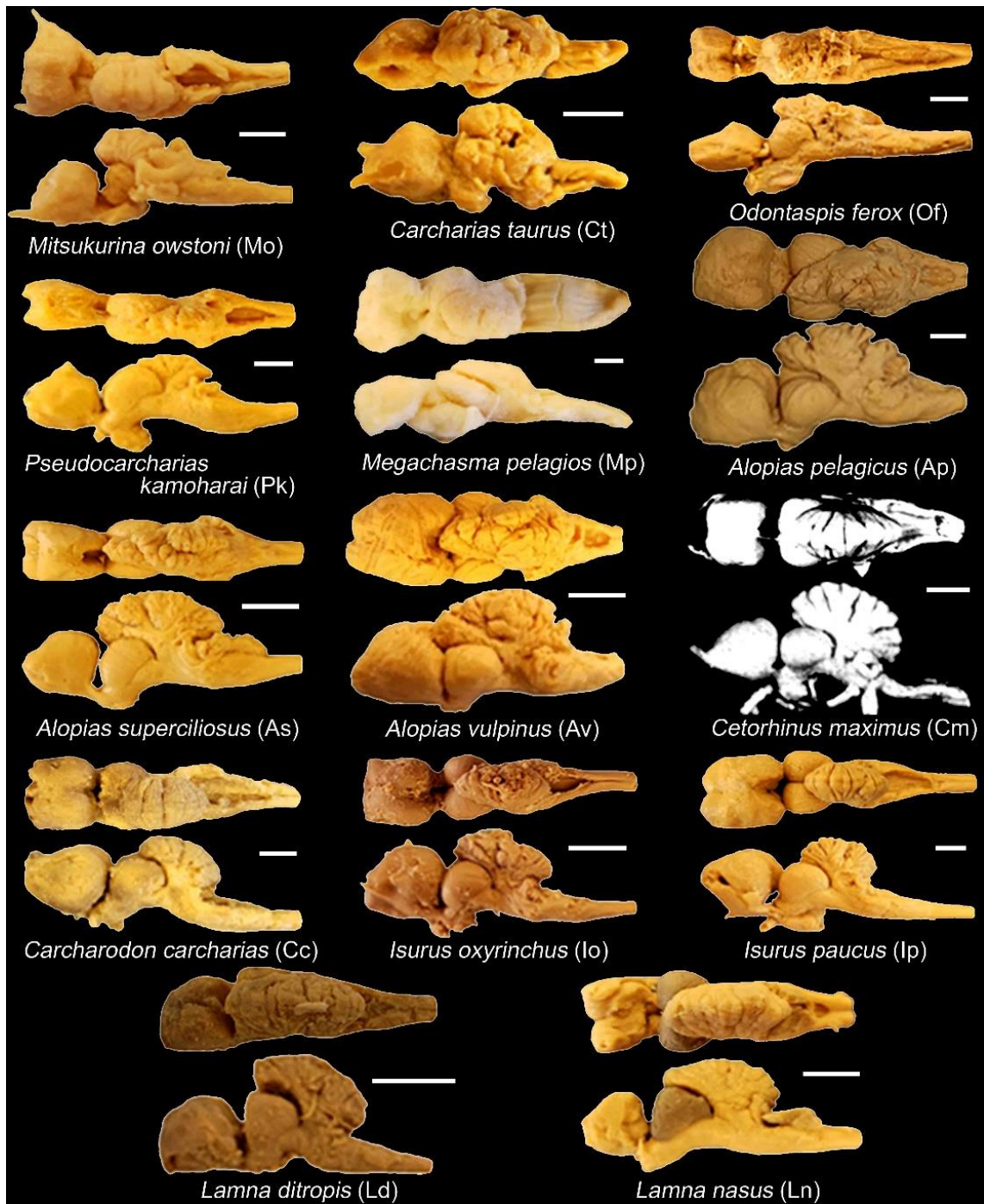
\*\*\* sum based on questionable data



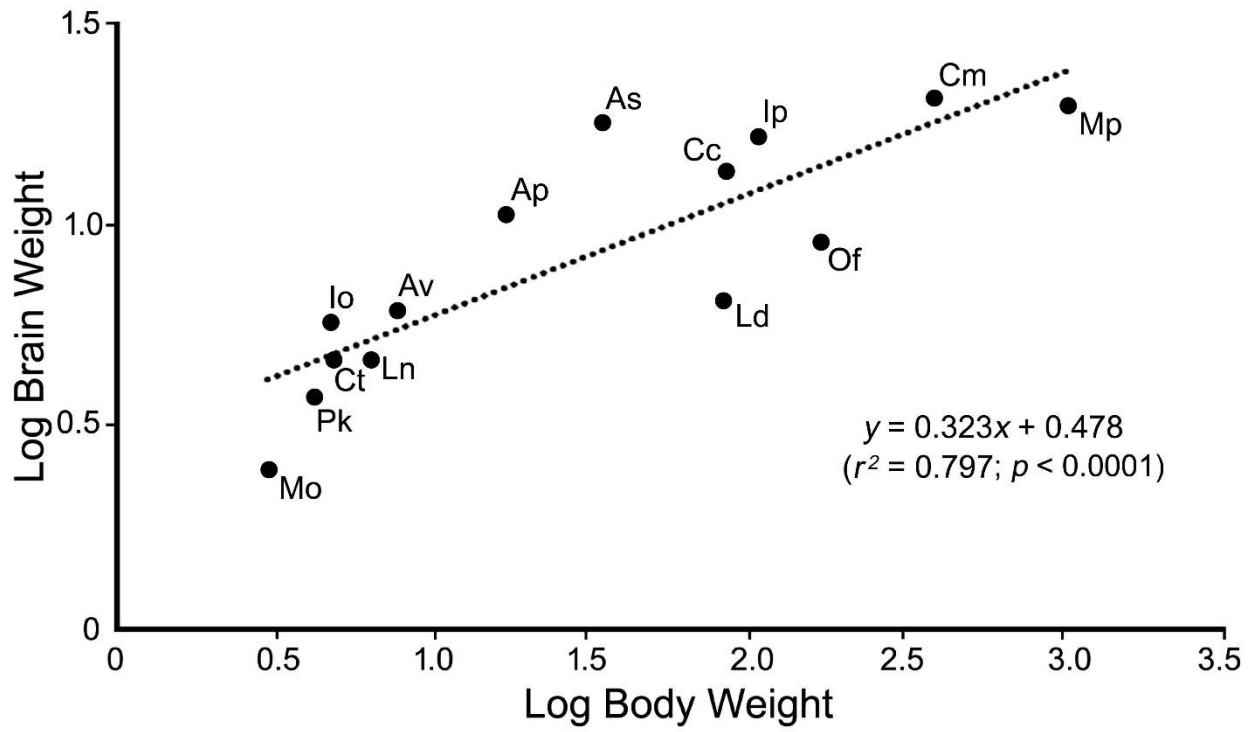
**Figure 1.** Example of shark brain (A), brain anatomy and terminology (B), and cerebellum-based variable examined in this study (C). **A**, brain of scyliorhinid carcharhiniform, *Scyliorhinus retifer* (chain catshark; CUMV 45864; scale = 1 cm) in dorsal (top) and left lateral (bottom) views. **B**, schematic drawings of brain of *S. retifer* (cf. Fig. 2A) illustrating five major regions (telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon in different shades) and specific brain terminology used in this study. **C**, five shark brains in dorsal view (not to scale) illustrating five tiers of cerebellar complexity in terms of 'foliation index' (FI: 1–5) devised by Yopak et al. (2007), where from left to right, *Scyliorhinus retifer* (FI = 1), *Megachasma pelagios* (FI = 2), *Mitsukurina owstoni* (FI = 3), *Isurus paucus* (FI = 4), and *Alopias vulpinus* (FI = 5).



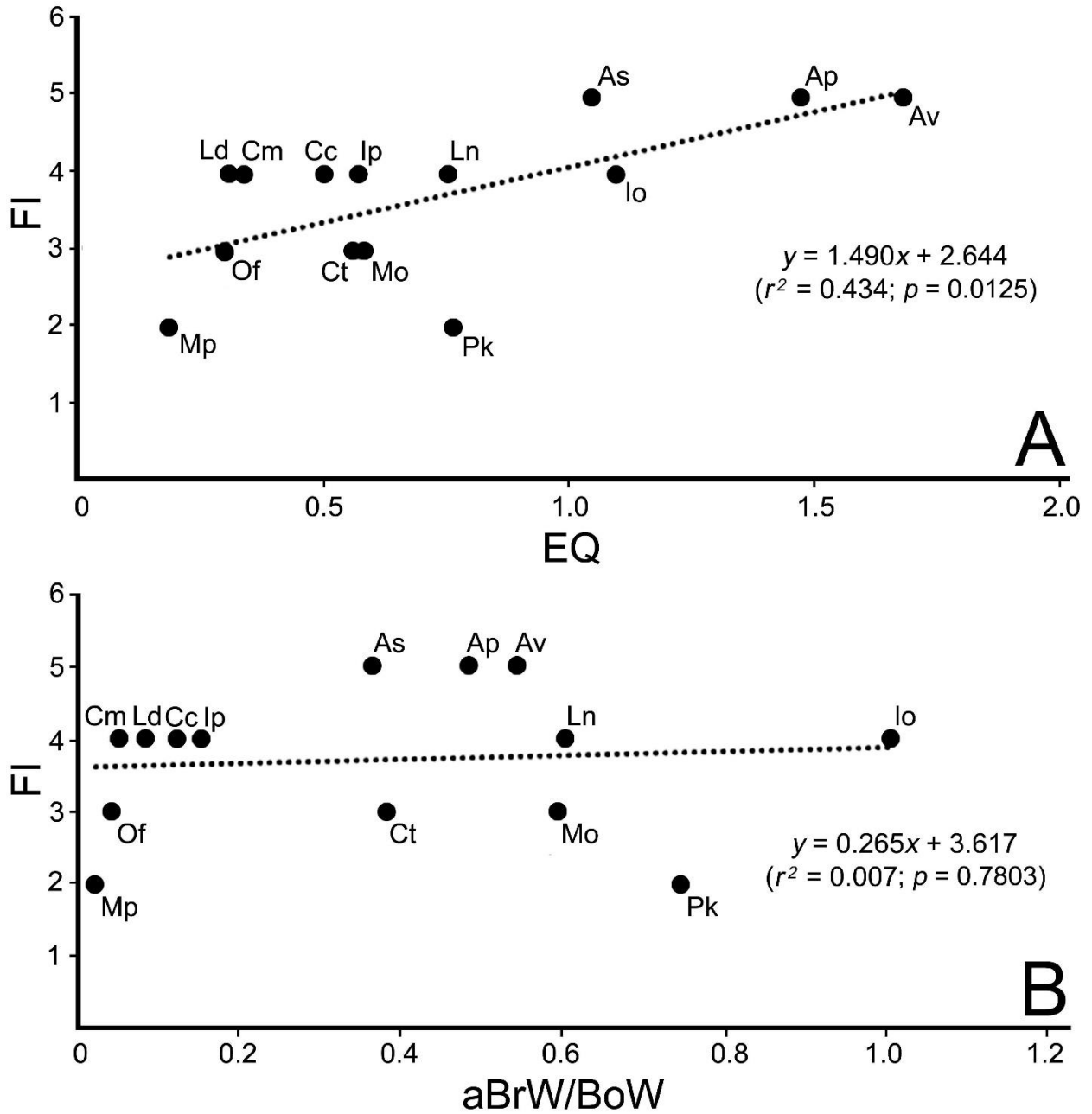
**Figure 2.** Fourteen (shaded in gray) of 15 known extant lamniform species examined in this study, along with their species codes in parentheses (scale = 50 cm: drawings after Compagno, 1984; Shimada, 2005). Common name of each species with its family in parentheses in illustrated sequence: Mo, goblin shark (Mitsukurinidae); Ct, sandtiger shark (Odontaspidae); Of, smalltooth sandtiger (Odontaspidae); *Odontaspis noronhai*, bigeye sandtiger (Odontaspidae); Pk, crocodile shark (Pseudocarchariidae); Mp, megamouth shark (Megachasmidae); Ap, pelagic thresher (Alopiidae); As, bigeye thresher (Alopiidae); Av, common thresher (Alopiidae); Cm, basking shark (Cetorhinidae); Cc, white shark (Lamnidae); Io, shortfin mako (Lamnidae); Ip, longfin mako (Lamnidae); Ld, salmon shark (Lamnidae); Ln, porbeagle (Lamnidae).



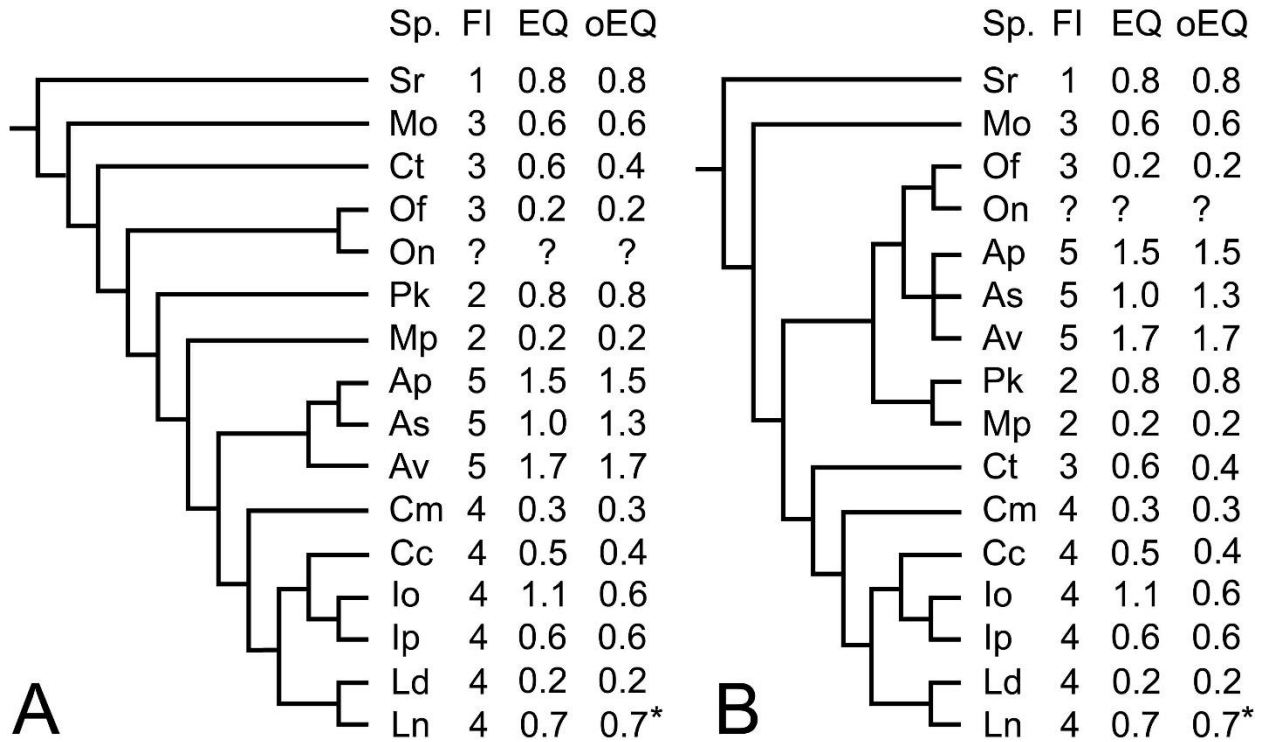
**Figure 3.** Dorsal (top) and lateral (bottom) views of brain in each of 14 extant lamniform species (scale = 1 cm), along with their species codes in parentheses (see caption of Fig. 2 for their common names and families). Illustrated specimens: Mo, FMNH 117742; Ct, FMNH 16136; Of, BPBM 9335; Pk, FMNH 117474; Mp, NSMT-P 134785; Ap, FMNH 117473; As, UF 178509; Av, SIO 64-804A; Cm, images from Kruska (1988, fig. 3: reproduced with permission by Karger Publisher); Cc, LACM 56960-1; Io, SIO 55-85; Ip, FMNH 135411; Ld, FMNH 117475; Ln, ROM 22751.



**Figure 4.** Log<sub>10</sub> regression between body mass and brain mass in terms of weight in 14 species of lamniform shark (for species codes, see Figs. 2, 3).



**Figure 5.** Regression between brain size (EQ) and cerebellar foliation (FI) in 15 species of extant lamniform sharks (**A**), and that between actual brain weight (aBrW) divided by body weight (BoW) of the shark and FI (**B**) (for species codes, see Figs. 2, 3).



**Figure 6.** Foliation index (FI), encephalization quotient (EQ), and ‘optimized’ encephalization quotient (oEQ: see text) mappend onto morphology-based (**A**) and molecular-based (**B**) phylogenetic trees of extant lamniforms (for species [sp.] codes, see Figs. 2, 3) and scyliorhinid carcharhiniform, *Scyliorhinus retifer* (Sr), as outgroup (see text for sources of phylogenetic trees; see Table 3 for raw data where EQ and oEQ values are rounded to one decimal in this figure; asterisk [\*] indicates likely deviation due to young individual).