Original Paper

The Comparative Morphology of the Cerebellum in Caprimulgiform Birds: Evolutionary and Functional Implications

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Abstract

Interspecific variation in the structure of the avian cerebellum is poorly understood. We present the first comparison of cerebellar morphology within the avian order Caprimulgiformes. Using a range of qualitative descriptions and quantitative measurements of cerebellar morphology we compared caprimulgiform birds with hummingbirds and swifts (Apodiformes) and owls (Strigiformes), two groups that are putative sister taxa to the Caprimulgiformes. Our results demonstrate that the owlet-nightjars (Aegothelidae), nightjars (Caprimulgidae) and potoos (Nyctibiidae) are more similar to apodiforms than they are to other taxa. All of these species have a reduced anterior lobe characterized by particularly small folia II and III and a relatively large posterior lobe. The frogmouths (Podargidae) possess a markedly different cerebellum that is more similar to that of owls than any of the caprimulgiform or apodiform birds. The monotypic oilbird (*Steatornis caripensis*, Steatornithidae) possesses a cerebellum with some nightjar-like features and some owl-like features, but overall it too resembles an owl more than a nightjar. This cerebellar diversity within the order Caprimulgiformes has significant implications for understanding the evolutionary relationships within the order, how the avian cerebellum has evolved and whether interspecific differences in cerebellar morphology reflect behavior.

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Introduction

The cerebellum varies tremendously in relative size and morphology among vertebrate classes from the relative simple commissure-like structure in cyclostomes to the multi-lobed structure of birds and mammals. There is also a large amount of variation in cerebellar size and morphology within vertebrate classes. This is especially true for birds and mammals where the cerebellum is complexly foliated. Within both of these classes, there is considerable variation in the number and size of folia. In mammals, the degree of cerebellar foliation and the size of individual regions within the cerebellum have often been linked to the evolution of novel behaviors and/or complex behaviors. For example, the platypus *(Ornithorhynchus anatinus)* possesses a large, heavily fissured cerebellum that is involved in electroreception [Scheich et al., 1986]. Bats have relatively small cerebella, but the parafloccular lobes and lobule VIII of the vermis are

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greatly enlarged [Henson, 1970; Larsell, 1970] and are involved in auditory processing [Sun et al., 1990; Kamada and Jen, 1990]. Lastly, it is suggested that the presence of large hemispheres of the anterior lobe and simplex and ansiform lobules of primates is due to their dexterous forelimbs [Ito, 1984]. Thus, in mammals at least some of the interspecific variation in cerebellar morphology is associated with distinct behavioral differences.

In two seminal publications, Larsell [1967] and Senglaub [1963] documented the diversity of cerebellar morphology across a range of birds. Both authors linked some of this morphological diversity with behavioral differences. For example, the absence of folium III and expansion of folia IV, V and VI in hummingbirds was attributed to their relatively weak hindlimbs and strong wings, respectively. The large folium VII in eagles, on the other hand, is thought to reflect their 'visual power' [p. 251, Larsell, 1967]. Variation in other folia and taxa, however, was restricted to qualitative descriptions of cerebellar morphology, with little quantitative data, thus precluding any statistical analyses. The development of a data set that is amenable to statistical analysis would allow for a more systematic treatment of cerebellar evolution in birds. This is important for at least two reasons. First, it will provide a quantitative evaluation of species differences rather than simply qualitative observations. Second, it may yield insight into the functional organization of the avian cerebellum, particularly those folia whose function is poorly understood, if at all (e.g., I, II and IXab).

One group of particular interest is the avian order Caprimulgiformes. Traditionally, this order is comprised of: potoos (Nyctibiidae), owlet-nightjars (Aegothelidae), frogmouths (Podargidae), nightjars (Caprimulgidae) and the oilbird (Steatornithidae). Although these families share some morphological features, such as weak hindlimbs and soft, cryptic plumage, there is considerable debate concerning how they are related to one another and to other avian lineages [see reviews in Sibley and Ahlquist, 1990; Mayr, 2002; Cracraft et al., 2004]. With respect to the cerebellum, the only published information is that nightjars purportedly possess a markedly reduced anterior cerebellar lobe [Portmann and Stingelin, 1961]. Unfortunately, no details are provided as to what parts of the anterior lobe are reduced or whether this marked reduction is similar to that observed in apodiforms (i.e., hummingbirds and swifts, order Apodiformes). In both swifts and hummingbirds most of the reduction in the anterior lobe appears to be due to a virtual absence of folia II and III [Larsell, 1967]. Larsell [1967] suggested that

this is correlated with the relatively weak hindlimb musculature of both swifts and hummingbirds. Given that caprimulgiform birds also possess weak hindlimbs [Cleere, 1998] and are thought to be closely related to hummingbirds and swifts based upon molecular [Sibley and Ahlquist, 1990] morphological [Livezey and Zusi, 2001; Mayr, 2002] and combined traits [Mayr et al., 2003; Cracraft et al., 2004], we expected that caprimulgiforms would also possess a markedly reduced anterior lobe. Furthermore, some studies have suggested that a clade of apodiform and caprimulgiform birds is closely related to owls [Sibley and Ahlquist, 1990; Livezey and Zusi, 2001], which do not possess a reduced anterior lobe [Senglaub, 1963; Larsell, 1967]. We therefore provide the first qualitative and quantitative study of the cerebellum of caprimulgiform birds and compare them with apodiforms and owls. Using a variety of multivariate statistics, we aimed to assess how much variation in cerebellar morphology is present among caprimulgiform birds. In doing so, we yield insight into how the cerebellum evolved within this order and the functional organization of the avian cerebellum.

Materials and Methods

Specimens

The brains of several caprimulgiform species were obtained from wildlife sanctuaries in Australia and loaned to us from the Bishop Museum (Honolulu, HI) and the National Museum of Natural History (Washington, DC; table 1). Additional species obtained from wildlife sanctuaries were also included for the primary purpose of determining how similar apodiform and caprimulgiform cerebella are to putative sister-groups (apodiforms and owls) and out-groups (galliforms, waterfowl and parrots). For all species, the brains were extracted from the skull and the meninges removed. All birds that we collected were submersion fixed in 10% buffered formalin or 4% buffered paraformaldehyde. The museum specimens were also immersion fixed in 10% buffered formalin, but following adequate fixation, they were kept in 70% ethanol that was replaced on a regular basis. The specimens that were loaned to us were stored in 70% ethanol for between 2 and 45 years. We placed them in 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) for several days prior to processing.

The brains were bisected in the sagittal plane and the cerebellum from one half of the brain was removed by cutting through the cerebellar peduncle. This enabled us to examine the entire lateral aspect of the cerebellum prior to sectioning and use Larsell's [1967] cerebellar taxonomy appropriately (see below). The brains were then placed in 30% sucrose in 0.1 M phosphate buffer until they sank. The brains were subsequently gelatin embedded and sectioned in the sagittal plane on a freezing stage microtome. Sections 40 μm thick were collected in 0.1 M phosphate buffered saline and mounted onto gelatinized slides. After drying, the slides were stained with thionin and coverslipped with Permount.

Table 1. A list of the species examined, sample sizes (n), body mass (g) and the sources of the material. 'Measurements' refers to whether midsagittal or both midsagittal and volume measurements were obtained

Order	Family	Species	n	Body mass	Measurements	Source
Anseriformes	Anatidae	*Anas platyrhynchos*	1	2,900	Both	A.N. Iwaniuk
Apodiformes	Apodidae	*Apus apus*		38	Midsagittal only	Larsell [1967][a]
		Collocalia esculenta	1	5	Both	USNM 20281
	Trochilidae	*Glaucis hirsuta*	1	7	Both	USNM 616825
		Lampornis sp.		–	Midsagittal only	Larsell [1967]
Caprimulgiformes	Aegothelidae	*Aegotheles insignis*	1	–	Both	BBM-NG 101365
	Caprimulgidae	*Eurostopodus argus*	1	72	Both	A.N. Iwaniuk
		Nyctidromus albicollis	1	57	Both	USNM 504211
	Nyctibiidae	*Nyctibius griseus*	2	257	Both	USNM 504184
						USNM 504185
	Podargidae	*Podargus strigoides*	3	387	Both	A.N. Iwaniuk
	Steatornithidae	*Steatornis caripensis*	1	414	Both	USNM 431365
Columbiformes	Columbidae	*Columba livia*	2	355	Both	D.R.W. Wylie
Galliformes	Phasianidae	*Bonasa umbellus*	1	650	Both	A.N. Iwaniuk
Psittaciformes	Cacatuidae	*Nymphicus hollandicus*	1	83	Both	A.N. Iwaniuk
Strigiformes	Strigidae	*Aegolius acadicus*	1	86	Both	B.J. Frost
		Asio flammeus		5,300	Midsagittal only	Larsell [1967]
		Asio otus		5,310	Midsagittal only	Larsell [1967]
		Bubo virginianus		14,730	Midsagittal only	Larsell [1967]
		Ninox boobook	1	231	Both	A.N. Iwaniuk
	Tytonidae	*Tyto alba*	1	450	Both	A.N. Iwaniuk

[a] Note that sample sizes are not included in Larsell [1967].

Measurements

Prior to measuring the relative sizes of individual folia, we numbered them following Larsell's [1967] cerebellar taxonomy. As shown in a representative drawing of a pigeon *(Columba livia)* cerebellum (fig. 1), each folium is numbered in ascending order from rostral (I) to caudal (X). Primary folia are individually numbered and secondary folia alphanumerically numbered. In the pigeon, for example, folia I, II and III are distinct primary folia, whereas Va and Vb are the two subfolia belonging to V (fig. 1). According to Larsell [1967], primary folia are determined by the presence of fissures on the exterior surface of the cerebellum. The primary fissure separates folia V and VI, and the secondary fissure separates VIII and IXab. Folia I–V and VI–IX, comprise the anterior and posterior lobes, respectively [Larsell, 1967]. Folia IXcd and X comprise the vestibulocerebellum [VbC; Schwarz and Schwarz, 1986]. Larsell [1967] defined individual folia and their subdivisions based upon cerebellar development in chickens *(Gallus domesticus)* and ducks *(Anas platyrhynchos)* and extrapolated this to other species. This is problematic, however, because we do not know whether there are species differences in how the cerebellum develops. Instead of developmental extrapolations, we based our divisions on branching patterns observed throughout the medio-lateral extent of the cerebellum and fissure depth. For example, moving from midsagittal to lateral pole, folia VIa, b and c coalesce into a single folium (VI), which retains a deep fissure between it and folia V and VII. In doing so, the cerebellar taxonomy reflects the branching pattern of the cerebellum more accurately than shape-based or other criteria.

Measurements were taken of the cerebella of each specimen using Scion ImageJ v.1.3. First, we measured the length of the Purkinje cell layer of each folium from a midsagittal section for each specimen ('midsagittal measures'; see fig. 1). We also measured the length of the Purkinje cell layer of midsagittal sections of other owls and apodiforms provided in Larsell [1967] to increase our sample size. Second, we measured the length of the Purkinje cell layer of each folium from serial sagittal sections from the lateral pole of the cerebellum to the midsagittal section. This second measurement yielded an estimate of the relative volume of each folium ('volume measures'). Although these methods provide a quantitative analysis of the relative size of all folia, it should be noted that they do not include any information regarding sub-foliation patterns or the relative positions and orientation of the folia. We therefore also make use of qualitative descriptions of morphology to address such similarities and differences.

Statistical Analysis

Although phylogenetic effects do play a significant role in the evolution of the avian brain [Nealen and Ricklefs, 2001; Iwaniuk, 2003; Iwaniuk and Hurd, 2005], the rationale of this study was to determine how cerebellar morphology varied among caprimulgiform birds irrespective of phylogenetic relationships. In addition, phylogenetic relationships among the species examined are largely uncertain because of the variability in phylogenetic trees generated from different data sets. Some studies have found that the order is paraphyletic or polyphyletic [Johansson et al., 2001; Livezey and Zusi, 2001; Mayr, 2002; Mayr and Clarke, 2003; Mayr et al., 2003;
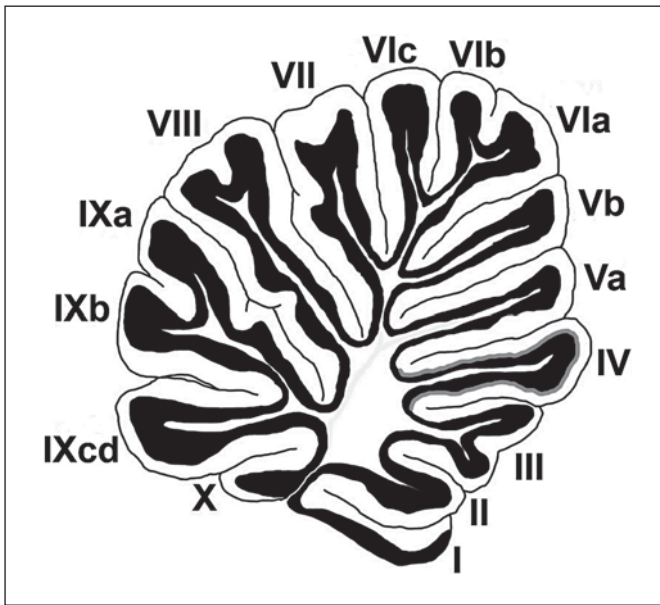
Fig. 1. A parasagittal view of the cerebellum of a pigeon *(Columba livia)*. Each of the folia is labeled from I through X in a rostral-caudal direction using the same terminology as Larsell [1967]. The anterior lobe consists of folia I–V whereas the posterior lobe consists of folia VI–IXab. Folia IXcd and X comprise the vestibulo-cerebellum. The black areas indicate the granule cell layer. The Purkinje cell layer, which is one cell deep, sits atop the granule cell layer. The grey line indicates the Purkinje cell length for folium IV in this section.

Cracraft et al., 2004; Fain and Houde, 2004], which would necessitate including numerous intermediate taxa to accurately trace the evolution of the cerebellum. Furthermore, uncertain phylogenetic relationships can generate significant problems in analyzing and interpreting results from phylogenetically-based statistics [Symonds, 2002; Iwaniuk, 2004]. We therefore present analyses using species as independent data points only, but recognize that the inclusion of phylogenetic information might alter our conclusions.

First, we performed a discriminant function analysis. The discriminant function is a formula that correctly classifies data based upon a training set of pre-assigned classes. Once this function has been calculated from the training set data, it can then be applied to cases whose class membership is not known to calculate the probability that a test case is a member of each of the training set classes. Because of the statistical requirements for training the MDA, this could only be performed with the midsagittal data and not the volumetric measures. The MDA was calculated using the lda function of the MASS library [Venables and Ripley, 2002] under the R statistical package [R Development Core Team, 2004]. The independent variables were the relative lengths (i.e., proportion of total length) of the following folia: I, II, III, IV, V, VI, VII, VIII, IXab, IXcd, and X. Although the inclusion of more variables is possible (e.g., folium VIII is subfoliated in some species) we chose those that best characterized the qualitative differences that we observed among the species sampled (see below). The three dependent vari-

able classes were: apodiform (i.e., hummingbird or swift), owl or nightjar. The training set consisted of four apodiforms *(Apus apus, Collocalia esculenta, Glaucis hirsuta* and *Lampornis* sp.), two 'nightjars' *(Eurostopodus argus* and *Nyctidromus albicollis)* and six owls *(Aegolius acadicus, Asio flammeus, Asio otus, Bubo virginianus, Ninox boobook, Tyto alba)*. Once the coefficients of the linear discriminants were calculated from the training data, the functions were applied to the feline owlet-nightjar *(Aegotheles insignis)*, grey potoo *(Nyctibius griseus)*, tawny frogmouth *(Podargus strigoides)* and oilbird *(Steatornis caripensis)* and the linear discriminants plotted against one another.

We also performed a cluster analysis, which enables the examination of groups of species (i.e., clusters) that are not widely separated. The cluster analysis provides a representation of the similarity and dissimilarity among species in multivariate space that is easier to interpret than other multivariate methods, such as principal component analysis, and includes all of the inherent variation. Cluster analyses were performed using the hierarchical cluster function (hclust) algorithm [Murtagh, 1985] in R [R Development Core Team, 2004] on both the midsagittal and the volumetric measures. We used the Ward's linkage method because it optimizes the minimum variance within clusters [Ward, 1963] and has been previously used in comparisons of brain composition in birds [Rehkämper et al., 2003].

Results

Macromorphology

Figure 2 shows photographs of the lateral aspect of the cerebellum of: pigeon *(Columba livia*, A); saw-whet owl *(Aegolius acadicus*, B); oilbird (C); tawny frogmouth (D); spotted nightjar *(Eurostopodus argus*, E); feline owlet-nightjar (F); glossy swiftlet *(Collocalia esculenta*, G) and rufous-breasted hermit *(Glaucis hirsuta*, H). The pigeon cerebellum can be described as 'generic' or representative of most birds: ten primary folia and their associated fissures are clearly present. Note that the external appearance of the cerebellum of the frogmouth, oilbird and owls is not that dissimilar from that of the pigeon. The profile of the pigeon is round, whereas both the frogmouth and the saw-whet have a 'spade-shaped' profile, which is also present in all other species of owl [also see Larsell, 1967]. In contrast, the cerebellum of the apodiforms is quite unique: folia I–III are very much reduced such that the overall profile is shaped like a kidney bean. The nightjar and the owlet-nightjar are similar to the apodiforms with folia I–III reduced but visible. Overall, it appears that the anterior lobe is scoop-shaped, with a large, rounded indentation that forms a 'pocket' for the optic lobe. Although not shown, this unique anterior lobe morphology is also present in the grey potoo and pauraque *(Nyctidromis albicollis)*.
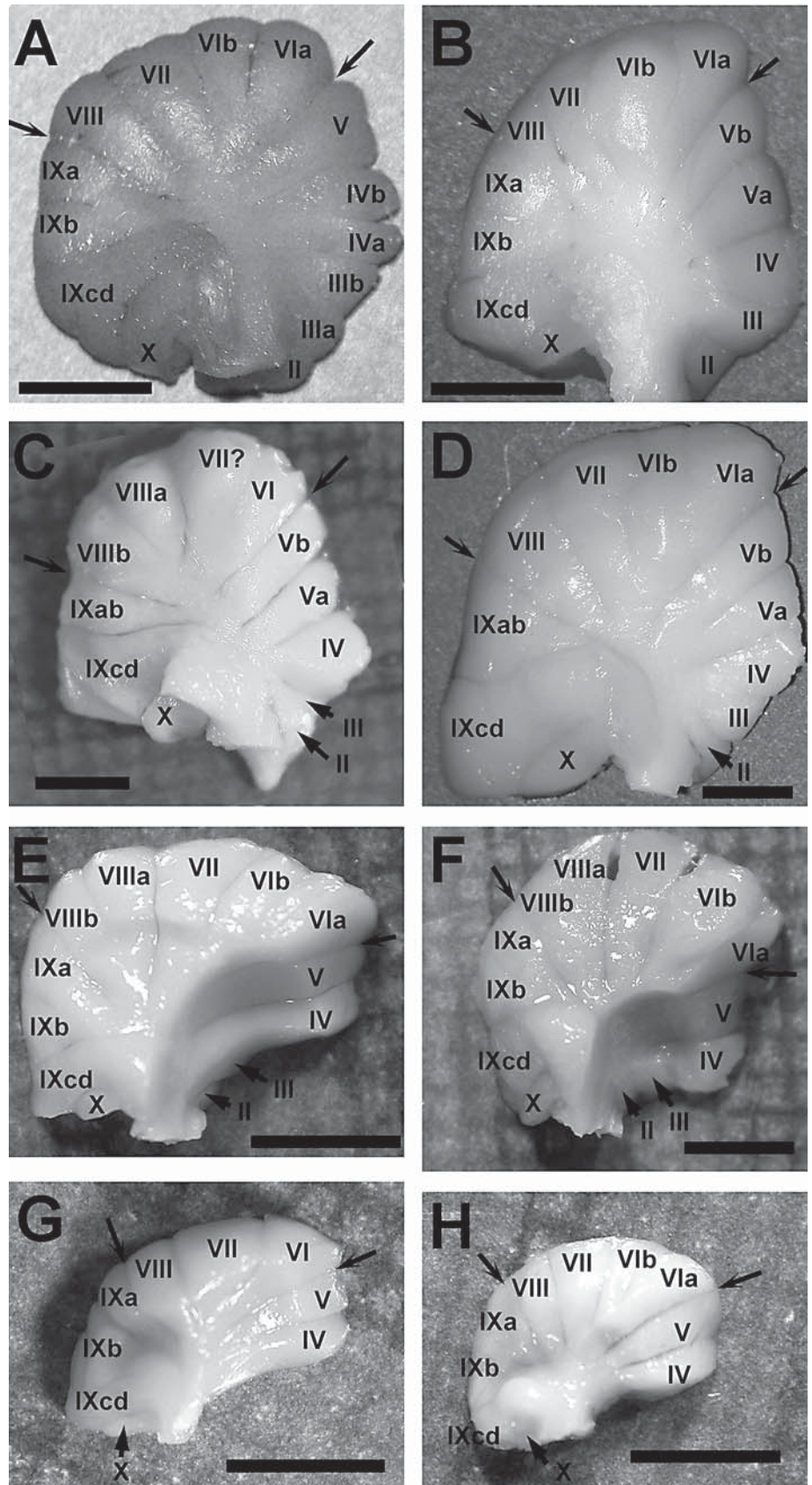
Fig. 2. Photos of the lateral aspect of cerebella removed from the rest of the brain are shown for: **A** Pigeon *(Columba livia)*; **B** Saw-whet Owl *(Aegolius acadicus)*; **C** Oilbird (USNM 431365, *Steatornis caripensis)*; **D** Tawny Frogmouth *(Podargus strigoides)*; **E** Spotted Nightjar *(Eurostopodus argus)*; **F** Feline Owlet-nightjar (BBM-NG 101365, *Aegotheles insignis)*; **G** Glossy Swiftlet (USNM 20281, *Collocalia esculenta*); and **H** Rufous-breasted Hermit (USNM 616825, *Glaucis hirsuta)*. The folia are numbered from rostral (I) to caudal (X) using Larsell's [1967] taxonomy. The arrows indicate the primary and secondary fissures. Scale bars = 3 mm.
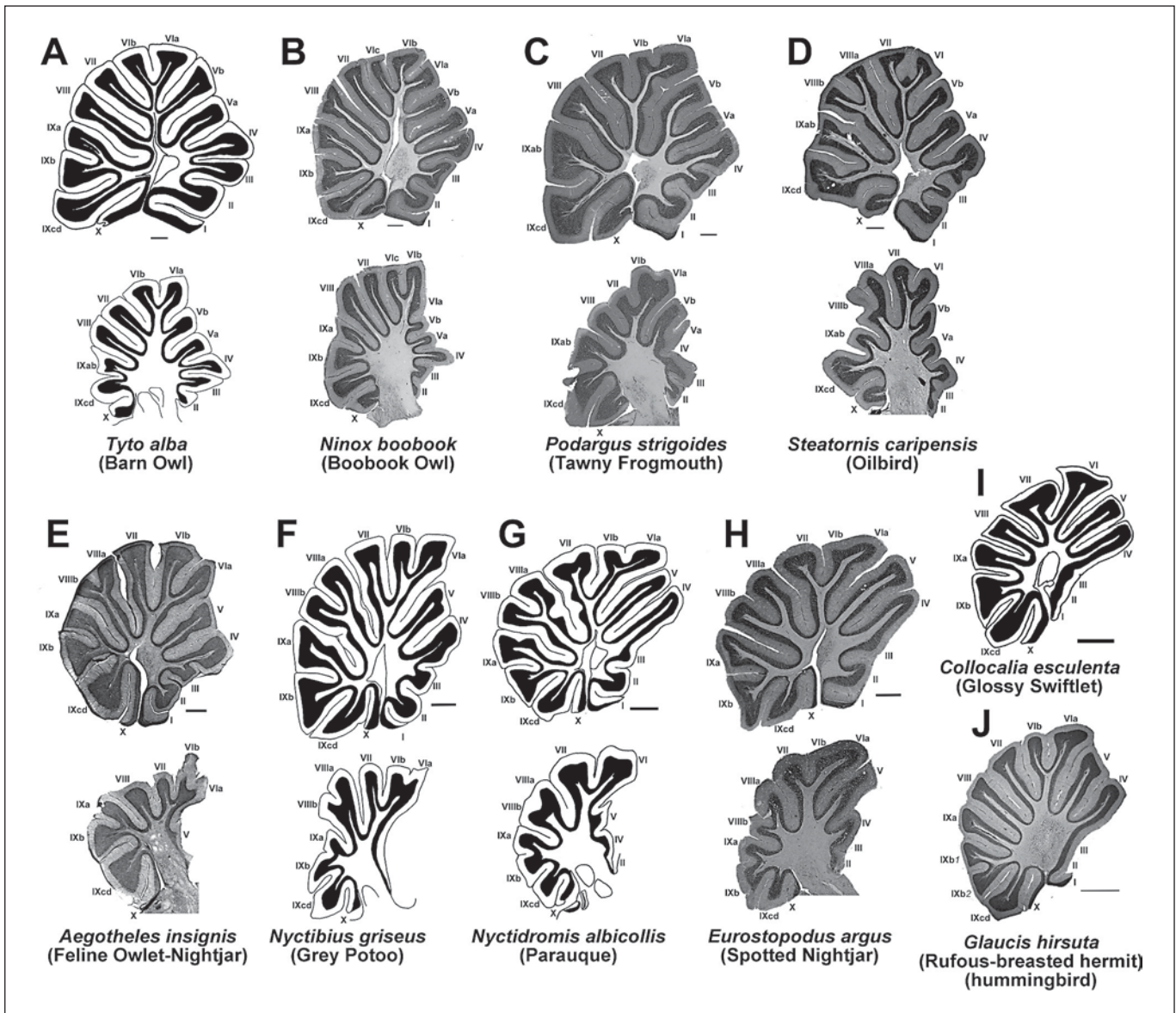
Fig. 3. Nissl-stained sagittal cerebellum sections are shown for: **A** Barn owl *(Tyto alba)*; **B** Boobook Owl *(Ninox boobook)*; **C** Tawny Frogmouth *(Podargus strigoides)*; **D** Oilbird (USNM 431365, *Steatornis caripensis)*; **E** Feline Owlet-nightjar (BBM-NG 101365, *Aegotheles insignis)*; **F** Grey Potoo (USNM 504185, *Nyctibius griseus)*; **G** Pauraque (USNM 504211, *Nyctidromus albicollis)*; **H** Spotted Nightjar *(Eurostopodus argus)*; **I** Glossy Swiftlet (USNM 20281, *Collocalia esculenta)*; and **J** Rufous-breasted Hermit (USNM 616825, *Glaucis hirsuta)*. For all caprimulgiforms and owls, two sections are shown for each species. The top section is a mid-sagittal section through the ventricle. The bottom section is a sagittal section taken half way between the mid-sagittal section (top section) and the lateral-most extent of the cerebellum. For each species, the folia are numbered from rostral (I) to caudal (X) using Larsell's [1967] taxonomy. Scale bar = 1 mm.

Midsagittal Measures – Qualitative Observations

We examined the midsagittal sections of the cerebellum from 20 species (see table 1). Figure 3 shows midsagittal sections through the cerebellum from owls (A, B), caprimulgiforms (C–H) and apodiforms (I, J). In figure 3A–H, a second sagittal section is shown: a lateral section that was located halfway between the midline and the lateral edge of the cerebellum. There are several

noticeable differences among the caprimulgiforms, apodiforms and owls. Overall, the owls resemble a 'typical' bird (fig. 3A, B). There are ten clearly defined primary folia. The apodiforms are clearly different (fig. 3I, J). Most obvious is the reduction of folia I–III, but there are also other notable differences. Folium V is sub-divided into Va and Vb in all six owls, whereas the apodiforms have a single folium V. Furthermore, the owls' folium X is obliquely oriented and contains a ventral lamella, but in the apodiforms folium X contains only a dorsal lamella which is vertically oriented. The ventral lamella is small in the barn owl *(Tyto alba)*; (fig. 3A), but in all other species it resembled that of the boobook owl (fig. 3B).

Figures 3E–H shows that the cerebellum of the nightjars, the owlet-nightjar and the potoo resembles that of the apodiforms more so than the owls. Most noticeably, the anterior lobe is reduced in size. In the two nightjars, folium III is quite small, appearing as a minute swelling at the base of IV. Folium III in the owlet-nightjar and potoo is also small, but not as small as the nightjars. Folium II in the midsagittal sections of these four species is not as reduced as in the apodiforms. The lateral sections emphasize that the folia of the anterior lobe do not extend as far laterally as in the owls. Folium V is not bifurcated as in the owls; only a single folium is present, as in the apodiforms. Folium VIII, on the other hand, is bifurcated such that two distinct subfolia can be differentiated. Finally, X does not have a ventral lamella and it is vertically oriented as in the apodiforms.

The cerebella of the oilbird and frogmouth (fig. 3C, D) are more similar to the owls than to the apodiforms. They do, however, also have some characteristics that are found in the nightjars and other characters that are unique. In both the oilbird and the frogmouth, the anterior lobe is not reduced, and is clearly visible in the midsagittal and lateral sections. Folium III does, however, appear smaller than in the owls, and in the frogmouth III branches from the base of IV as in the other caprimuligiforms (fig. 3E–H). Like the owls, V in both the oilbird and the frogmouth has two subfolia and X is obliquely oriented and has a ventral lamella. The branching pattern of V is, however, slightly different between the frogmouth and the oilbird. In the oilbird (fig. 3D), Vb branches from the white matter giving rise to VI and VII as in the owls (fig. 3A, B), whereas Vb branches off of Va in the frogmouth (fig. 3C). In fact, the profile of VI and VII of the frogmouth resembles that of the nightjars more so than the owls. Unlike all of the other species examined, the oilbird has a dramatically reduced VI/VII. The fissure between folia VI and VII does not extend as far laterally as it does in other species. For this reason, VI and VII might actually be folia VIa and VIb respectively. Folium VIII also differs between the oilbird and the frogmouth. The oilbird shares with nightjars a bifurcated VIII, but the frogmouth has a single VIII as in the owls. Finally, unlike the owls, nightjars and apodiforms, folium IXa and IXb were not separated in either the oilbird or the frogmouth.

In summary, the qualitative observations of sagittal sections indicate that the cerebellum of the nightjars, the owlet-nightjar and the potoo are similar to one another and share many characteristics with apodiforms. In contrast, the cerebellum of the oilbird and frogmouths are quite different from other caprimulgiforms and share many characteristics with owls as well as some unique features (i.e., VI and VII in the oilbird).

Midsagittal Measures – Quantitative Analysis

Figure 4A is a scatter plot of the proportional size of the posterior lobe (i.e., length of folia VI–IXcd) against the anterior lobe (folia I–V). Corroborating our qualitative observations, the apodiforms, nightjars, potoo and owlet-nightjar all have relatively small anterior lobes and relatively large posterior lobes, whereas the opposite is true of the frogmouth, oilbird and owls (fig. 4A). When broken down into individual folia, shown in the histograms in figure 4B–D, considerable variation in relative size is present both within and across all folia. For example, within the anterior lobe folium I is fairly constant in relative size across all species (fig. 4B). Marked differences among taxa are, however, present within folia II, III and V. Folium II is much smaller in apodiforms; III is smallest in the apodiforms and more than three times larger in the owls and the frogmouth. The nightjars, potoo and owlet-nightjar and oilbird all have an intermediately-sized III. Folium V is clearly larger in all those species in which V was divided into Va and Vb: frogmouth, owls and oilbird. Within the posterior lobe, the small folium VI observed in the oilbird midsagittal section (fig. 3D) is much smaller than that of all the other species sampled (fig. 4C) whereas VII retains a similar size across all species. However, it is unclear whether VII is absent in the oilbird and should be included as part of VI (see above), in which case VI would be of average size. Folium VIII is larger in all those species in which it was bifurcated: nightjars, potoo and oilbird (fig. 4D). With the exception of the large IXcd in the oilbird, both IXcd and X did not vary much among the species sampled.
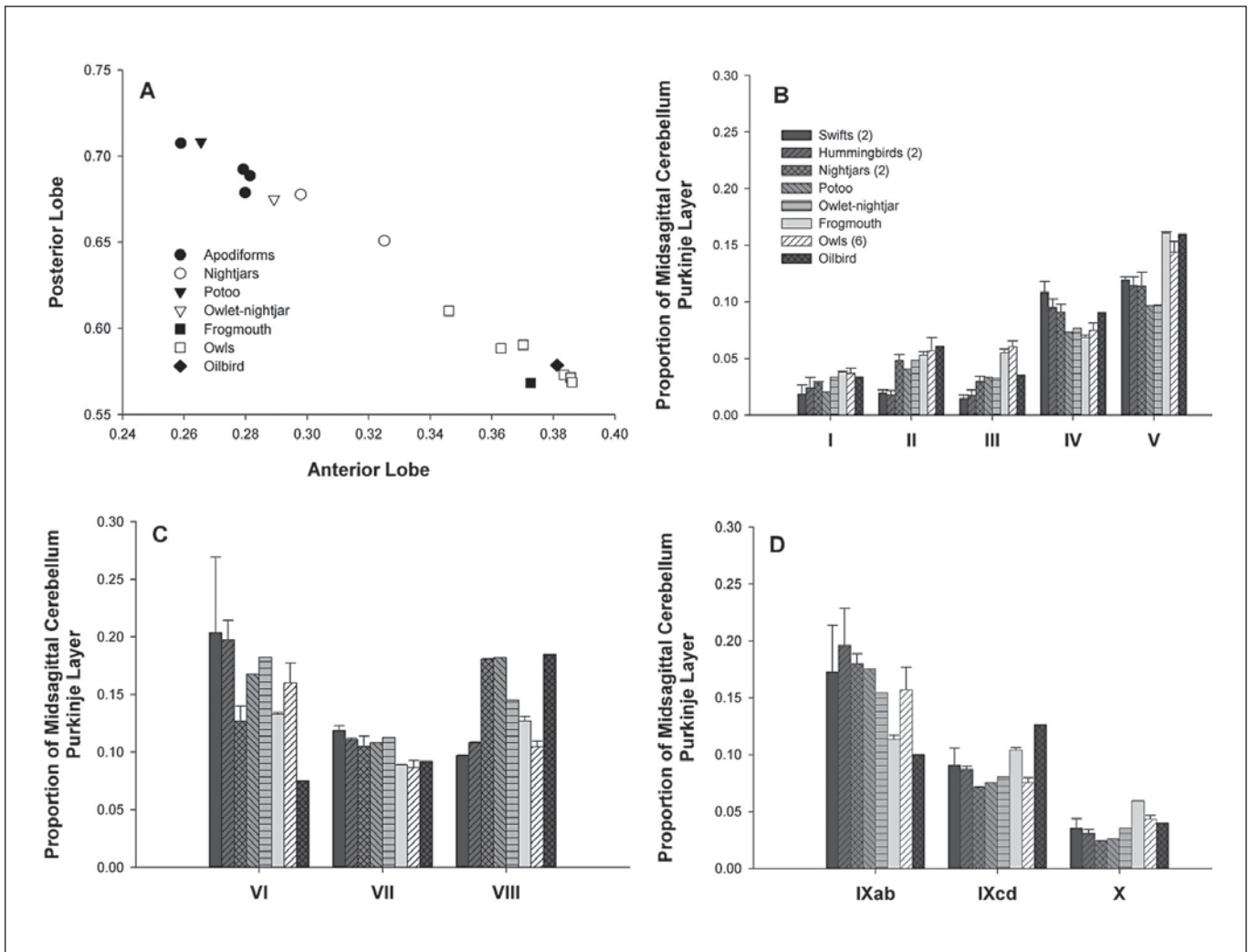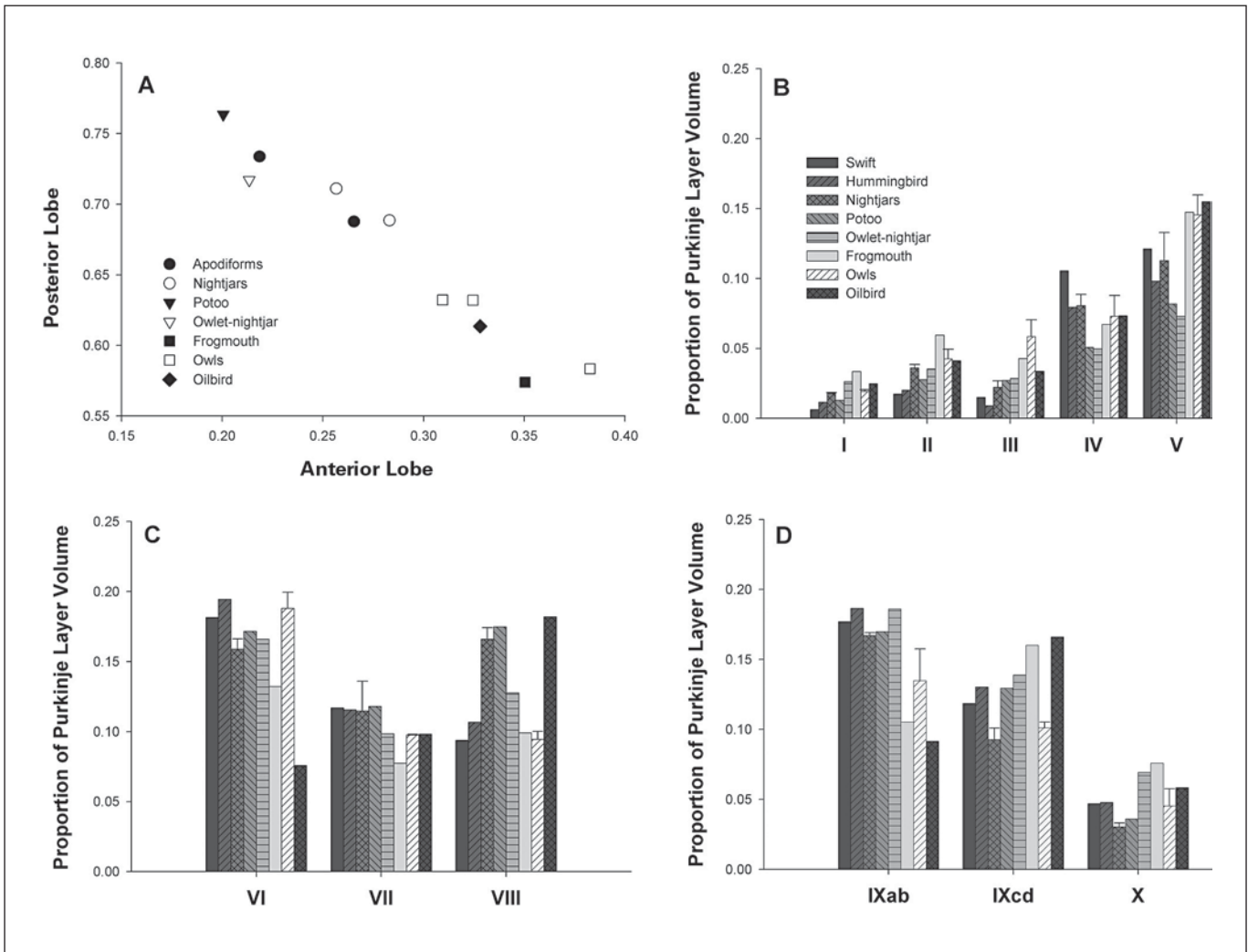
Fig. 4. The graphs shown here indicate the relative size of the Purkinje cell layer of each of the cerebellar folia as expressed as a proportion of total Purkinje cell layer length of midsagittal sections. The first plot (**A**) is a plot of the proportion of anterior (I–V) versus posterior lobes (VI–IXcd). The next three histograms show the relative size of each folium expressed as a proportion of the total Purkinje cell length: **B** anterior lobe (folia I–V); **C** folia VI–VIII; and **D** folia IXab, IXcd and X. Where more than one species or specimen was examined, a standard error bar is provided. Numbers in parentheses refer to the number of species sampled for those groups with error bars.

Volumes Measures – Quantitative Analysis

Volume measures were obtained from 15 species (see table 1). Variation in the volume measures largely supports our observations based upon midsagittal sections only (see above). Figure 5A is a scatter plot of the proportional sizes of the posterior lobe volumes against the anterior lobe volumes. Corroborating our observations of the midsagittal measures, the apodiforms, nightjars, potoo and owlet-nightjar all have relatively small anterior lobes and relatively large posterior lobes, whereas the op-

posite is true of the oilbird, owls and frogmouth. The histograms of the proportions of the individual folia also corroborate the midsagittal measures. Folium II, and to a lesser degree I, is markedly smaller in the swift and hummingbird than in the other species (fig. 5B). With respect to III, it is smallest in the apodiforms and largest in the frogmouth and owls. There is extensive variation in the size of folium V ranging from a small folium in the owlet-nightjar and potoo to a large folium in the frogmouth, owls and oilbird. Within the posterior lobe, VI is excep-

Fig. 5. The graphs shown here indicate the relative size of the Purkinje cell layer of each of the cerebellar folia expressed as a proportion of total Purkinje cell layer volume measured by the length of the Purkinje cell layer throughout the medio-lateral extent of the cerebellum. The first plot (**A**) are the mean proportions of the total volume of the Purkinje cell layer of the anterior (I–V) and posterior lobes (VI–IXcd). The next three histograms show the mean proportions of the total volume of the Purkinje cell layer that each folia comprises within the following: **B** anterior lobe (folia I–V); **C** folia VI–VIII; and **D** folia IXab, IXcd and X. Where more than one species or specimen was examined, a standard error bar is provided. Numbers in parentheses refer to the number of species sampled for those groups with error bars.

tionally small in the oilbird (fig. 5C, but see above). The nightjars, potoo and oilbird all have a large VIII, whereas the owls and the swift and hummingbird have a small VIII. In contrast to the midsagittal measures, where IXab was smallest in apodiforms (fig. 5D), the volume measures of IXab demonstrated that it is slightly larger in the apodiforms, nightjars, owlet-nightjar and potoo, relative to the frogmouth, oilbird and owls (fig. 5D). Within the VbC, the oilbird has a large IXcd and X is relatively large

in the owlet-nightjar and frogmouth and relatively small in the nightjars.

Multiple Linear Discriminant Analysis (MDA)

As discussed above, a MDA was applied to the midsagittal measures using apodiforms, nightjars and owls as training groups. The MDA distinguished among the apodiforms, nightjars and owls based upon two linear discriminants (table 2). The first linear discriminant (LD1)
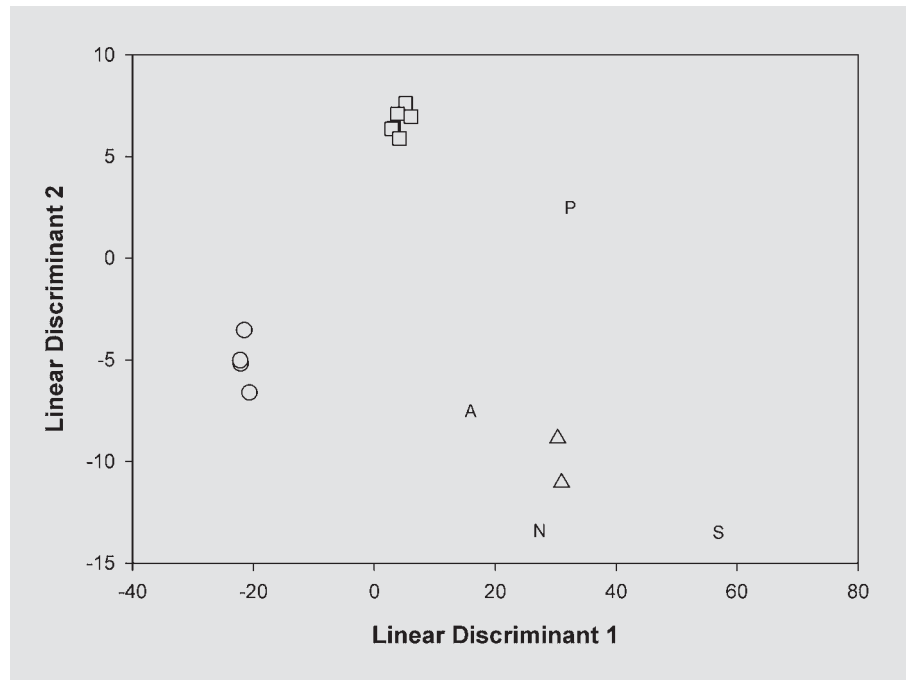
Fig. 6. A scatterplot of the linear discriminant 1 versus linear discriminant 2 resulting from a discriminant function analysis of folia proportions of mid-sagittal cerebellum sections. The symbols refer to the following groups: circles = apodiforms; triangles = nightjars and potoo; and squares = owls. The letters indicate the following caprimulgiforms: 'N' – grey potoo *(Nyctibius griseus)*; 'S' – oilbird *(Steatornis caripensis)*; 'P' – tawny frogmouth *(Podargus strigoides)*, and 'A' – feline owlet-nightjar *(Aegotheles insignis)*.

is most strongly correlated with the relative size of I, VIII and X and accounts for 87.1% of the variation. LD2 is most strongly correlated with VIII, IXcd and X and accounts for 12.9% of the variation. Figure 6 is a plot of LD2 against LD1, including the locations of the frogmouth, owlet-nightjar, potoo and oilbird as calculated with the MDA. Clearly, the owlet-nightjar and potoo are closest to the nightjars and the frogmouth is closest to the owls. The oilbird, however, is quite distant from all three training groups.
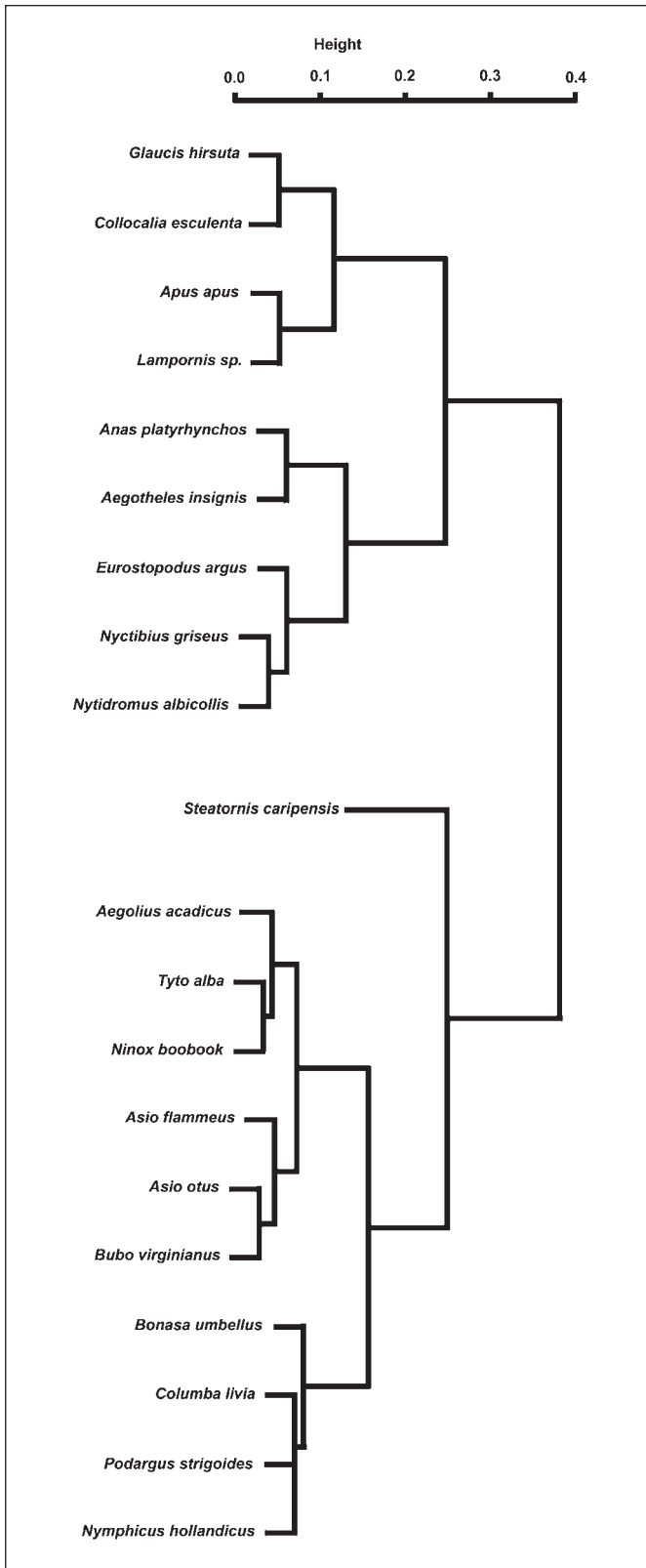
Cluster Analyses

Cluster analysis of the midsagittal measures yielded a dendrogram with two main clusters (fig. 7). The top cluster contains all of the apodiforms, nightjars, the potoo, owlet-nightjar and the mallard *(Anas platyrhynchos)*. In contrast, the bottom cluster comprises all of the owls, frogmouth, oilbird, cockatiel *(Nymphicus hollandicus)*, ruffed grouse *(Bonasa umbellus)* and pigeon. The oilbird is in a basal or out-group position within this bottom cluster, supporting our observations of its similarity with owls and unique VI/VII morphology. Although not shown, the position of the oilbird remained unchanged regardless of whether we considered folium VII absent or not.

The cluster analysis of the volume measures yielded similar results (fig. 8). Only two main clusters are appar-

Table 2. The coefficients of the two linear discriminants resulting from our multiple linear discriminant analysis of midsagittal cerebellum measures

Folium	Linear discriminant 1	Linear discriminant 2
I	190.01	85.19
II	122.72	−8.66
III	39.37	98.38
IV	149.66	33.34
V	50.24	94.40
VI	0.04	−12.58
VII	−127.64	19.63
VIII	674.27	−109.57
IXab	−114.62	0.96
IXcd	−120.56	−219.14
X	882.32	209.64

ent, both of which support the differences between the oilbird and frogmouth and the other caprimulgiforms described previously. As in the cluster analysis of the midsagittal measures, the top cluster is composed of the apodiforms, nightjars, owlet-nightjar, potoo and mallard. The bottom cluster contains the owls, frogmouth, oilbird, pigeon and grouse. The only noticeable differences between this clustering pattern and that of the previous

Fig. 7. A dendrogram resulting from a Ward's cluster analysis of the proportions of each folia (I–X) of midsagittal cerebellum sections.
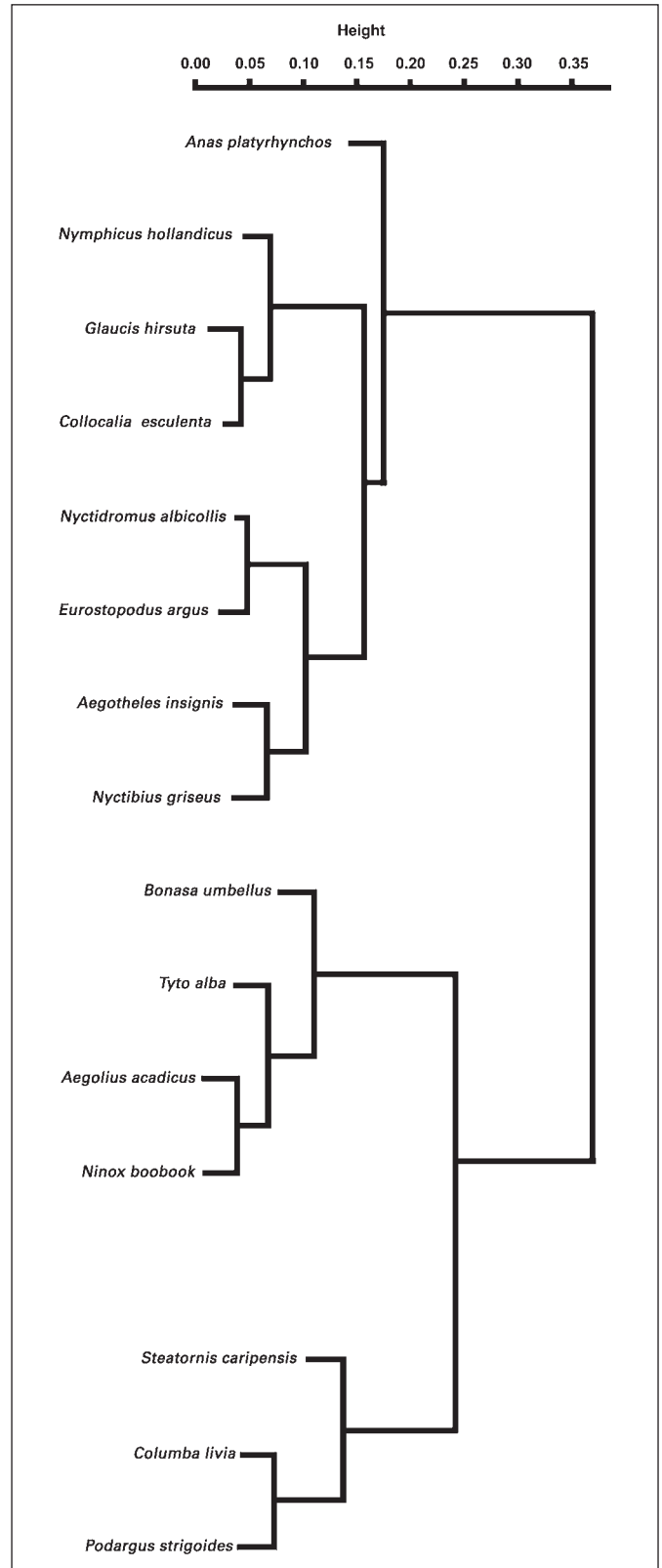
Fig. 8. A dendrogram resulting from a Ward's cluster analysis of the proportions of the volumes of each folia (I–X).

analysis are: the oilbird is no longer an 'out-group'; the grouse is clustered with the owls; and the cockatiel is within the top cluster rather than the bottom cluster. Again, the position of the oilbird remained unchanged regardless of whether we considered folium VII as absent or not. Overall, the cluster analyses corroborate all of our other observations and analyses: the owlet-nightjar, potoo and nightjars are more similar to the apodiforms than any other group and the oilbird and frogmouth are more like the owls than they are the apodiforms or other caprimulgiforms.

Discussion

The cerebellar morphology of caprimulgiform birds varies among the five families. The nightjars, owlet-nightjar and potoo all share similar cerebellar morphology. The frogmouth, however, resembles an owl far more than any of the other caprimulgiform families. Lastly, the oilbird shares some traits with the nightjars, some with owls and at least one, the apparent reduction of folia VI or VII, which is not shared with any other bird. This variation in cerebellar morphology within a putatively monophyletic group has important implications for understanding the evolution of caprimulgiform birds and the functional organization of the avian cerebellum.

Although it could be suggested that much of this variation in cerebellar foliation reflects body, brain and/or cerebellum size [Senglaub, 1963; Pearson and Pearson, 1976], this is unlikely to be the case for apodiform and caprimulgiform birds. Both apodiforms and caprimulgiforms have relatively small brains, but so do galliforms, and pigeons [Iwaniuk, 2003] that have 'typical' cerebella [fig.1; Senglaub, 1963; Larsell, 1967]. Species that are larger or similar in body size to both apodiforms and caprimulgiforms, such as songbirds, shorebirds and smaller parrots, also have ten discrete folia and lack a reduced anterior lobe [Senglaub, 1963; Larsell, 1967]. Apodiforms and caprimulgiforms tend to have relatively large cerebella [Boire and Baron, 1994; Iwaniuk and Hurd, 2005] despite having a relatively small anterior lobe. We therefore suggest that the cerebellar diversity in caprimulgiform birds reflects, to some degree, behavioral and/or ecological variation rather than some scaling relationship with body, brain or cerebellum size.

Cerebellar Diversity in Caprimulgiformes

As mentioned previously, there is considerable debate regarding the inter-familial relationships within the order Caprimulgiformes [see reviews in Sibley and Ahlquist, 1990; Cleere, 1998; Mayr, 2002; Cracraft et al., 2004]. Some authors have even questioned whether this order is indeed monophyletic [Mayr, 2002; Cracraft et al., 2004]. Our analysis of caprimulgiform cerebellar morphology does not resolve this debate, but it does indicate that the cerebellar structure varies considerably among caprimulgiform families. If we were to argue paraphyly based upon the cerebellum, we would group the oilbird and frogmouth outside of the other caprimulgiforms and apodiforms [sensu Mayr, 2002].

In accordance with the observations of Portmann and Stingelin [1961], we found that nightjars possess a markedly reduced anterior lobe. Specifically, folium III is particularly small relative to the rest of the cerebellum. This was also true of the owlet-nightjars and potoos. In fact, the cerebella of all three families are remarkably similar to one another both qualitatively and quantitatively. It is therefore not surprising that they also clustered together in both the discriminant function analysis and cluster analyses. Behaviorally, these three families also share more with one another than they do with the other families within the order: frogmouths and the oilbird. Potoos and nightjars feed primarily upon small invertebrates that are captured in the air [Cleere, 1998; del Hoyo et al., 1999]. Owlet-nightjars also feed on small invertebrates, but they capture them by swooping onto the ground as well as by aerial hawking [Higgins, 1999; del Hoyo et al., 1999]. Phylogenetically, however, there is some debate as to whether these three families are closely related or not. Potoos and nightjars are generally regarded as close relatives of one another [Mariaux and Braun, 1996; Brumfield et al., 1997; Mayr, 2002; Mayr et al., 2003; Cracraft et al., 2004; Fidler et al., 2004], but a number of recent studies indicate that owlet-nightjars are more closely related to hummingbirds and swifts than they are to nightjars and potoos [Mayr, 2002; Mayr et al., 2003; Cracraft et al., 2004; Fain and Houde, 2004]. Despite this proposed paraphyly within the Caprimulgiformes, the clade composed of the apodiforms and owlet-nightjars is still considered to be closely related to the nightjars and potoos [Mayr, 2002; Mayr et al., 2003; Cracraft et al., 2004], which suggests that a massive reduction of folia I–III of the anterior lobe has evolved only once.

Despite being a caprimulgiform, there were marked differences between the frogmouth cerebellum and that of the nightjars, owlet-nightjar and potoo. Specifically, in frogmouths, folia V was a doublet and X was obliquely oriented and had a ventral lamella as in the owls. There are few phylogenetic analyses that have not placed the

frogmouths as a close relative of other caprimulgiform birds. Generally, frogmouths are considered a basal clade within the Caprimulgiformes [Mariaux and Braun, 1996; Livezey and Zusi, 2001; Mayr, 2002; Mayr et al., 2003; Fain and Houde, 2004; Cracraft et al., 2004]. In addition, frogmouths have a number of behavioral differences that set them apart from other caprimulgiform birds. For example, in addition to invertebrates, they eat small vertebrates that are captured by swooping down onto the ground from a perch [Higgins, 1999]. Related to this owl-like form of prey capture, frogmouths also possess stereoscopic vision [Pettigrew, 1986], a large area of binocular overlap in the visual field [Wallman and Pettigrew, 1985] and an enlarged Wulst [Iwaniuk and Hurd, 2005]. Although these features also appear to be true of the owlet-nightjars [Pettigrew, 1986; Iwaniuk, Pettigrew and Wylie, unpubl. data], they differ from frogmouths in both their prey and hunting behavior (see above). Thus, behaviorally and neuroanatomically, the frogmouth is more owl-like than it is nightjar-like [Iwaniuk and Hurd, 2005]. It is therefore not surprising that most of our analyses demonstrated a close similarity between owls and frogmouths. Although it could be suggested that this similarity is indicative of a close phylogenetic relationship between owls and frogmouths, it is more likely that this is a case of convergent evolution. Most phylogenetic analyses have demonstrated that caprimulgiform birds are not closely related to owls [Cracraft, 1988; McKitrick, 1991; Mindell et al., 1997; van Tuinen et al., 2000; Johansson et al., 2001; Mayr and Clarke, 2003; Mayr et al., 2003; Prychitko and Moore, 2003; Poe and Chubb, 2004; Fain and Houde, 2004; Cracraft et al., 2004; but see Sibley and Ahlquist, 1990; Bleiweiss et al., 1994]. In fact, Fain and Houde [2004] and Fidler et al. [2004] both suggested that frogmouths and owls represent an example of convergent evolution into a nocturnal, predatory niche. Given the breadth of morphological and behavioral similarities between owls and frogmouths, convergent evolution of overall brain composition [Iwaniuk and Hurd, 2005] and cerebellar morphology is not unexpected.

The oilbird cerebellum was unique among the species examined. Although some cerebellar folia are nightjar-like (e.g., folium VIII), other parts are owl-like (e.g., folia V and X) and the unique morphology of VI and VII was not observed in any other species. Most of the qualitative features align the oilbird with the owls (fig. 2, 3), but quantitative measurements of the folia (fig. 4–8) indicate that there are just as many differences as there are similarities. A likely explanation for the apparently aberrant cerebellum of the oilbird is its unique life history. Unlike all

other caprimulgiforms, the oilbird is the only species that nests in dimly lit caves, echolocates and feeds exclusively on fruit [del Hoyo et al., 1999]. In addition to a unique life history, the oilbird possesses a combination of morphological features unlike any other family of birds, which has made its phylogenetic position difficult to resolve [Sibley and Ahlquist, 1990; Livezey and Zusi, 2001]. Whatever the actual phylogenetic relationships of the oilbird to other birds, it is clear that its' cerebellar morphology is as unique as its life history.

Behavioral Correlates of Cerebellar Morphology

Larsell [1967] suggested that the significant reduction of the anterior lobe in hummingbirds was due to their 'poor' hindlimb musculature. Our findings agree with his original interpretation. Both swifts and hummingbirds possess relatively small hindlimb muscles [Zusi and Bentz, 1984] and a markedly reduced anterior lobe. Nightjars also have relatively small hindlimb muscles [Cleere, 1998] and a small anterior lobe. Although potoos and owlet-nightjars both have relatively larger hindlimb muscles than apodiforms and nightjars, the hindlimb muscles are still weak compared to other taxa [Cleere, 1998; del Hoyo et al., 1999; Iwaniuk, pers. obs]. Lastly, the oilbird and frogmouth have slightly larger hindlimb muscles than the other caprimulgiforms, but not as large as the raptorial hindlimbs of owls. Taken as a whole, there is a correlation in the size of folia I–III and the apparent size of the hindlimb musculature in these species that strongly suggests that this part of the anterior lobe is involved in the coordination and innervation of the hindlimbs. In owls, tactile stimulation of the tail and leg resulted in electrophysiological responses in folium III [Whitlock, 1952], but in pigeons, folium III responded primarily to deep somatosensory stimulation of the wings [Schulte and Necker, 1998]. Both of these studies, however, recorded from relatively few leg-responsive cells compared to the total sampled (29% and 23.9%, respectively). Because of the contradictory data provided by these two studies, it is uncertain what role folium III might play in hindlimb function, but based upon our data it is likely that the size of folium III reflects the relative musculature of the hindlimbs.

The functional implications of a relatively small folium II in apodiforms are uncertain. Previous electrophysiological studies have recorded from very few sites within folium II [Whitlock, 1952; Gross, 1970; Clarke, 1974; Schulte and Necker, 1998]; however, it does receive input from the spino-cerebellar tracts [Whitlock, 1952]. Intracranial lipomas in crested ducks (*Anas platyrhynchos* f.

dom.) that drastically reduced the sizes of folia I and II result in head and neck ataxia and torticollis [Bartels et al., 2002] which suggests that folium II is related to some aspect of postural maintenance. The small II in apodiforms might therefore reflect some aspect of postural behavior that is not present in the other species sampled. Alternatively, this may be an example of correlated evolution between folia II and III. That is, selection for a smaller folium III might have also resulted in a small folium II due to connectivity, similarity in function or constraints [anatomical, physiological and/or developmental; Striedter, 2004].

Larsell [1967] also suggested that folia IV–VI were particularly large in hummingbirds because of their flying abilities. Folia IV and V receive input from the wings [Whitlock, 1952; Gross, 1970; Schulte and Necker, 1998], but we did not find that they are particularly large in hummingbirds. Folium VI receives input from the legs in pigeons [Schulte and Necker, 1998] and the face and wings in owls [Whitlock, 1952]. Folia IV–VI tended to be largest in owls and the frogmouth, neither of which are maneuverable or agile fliers. Rather, both the frogmouth and owls have broad, rounded wings that provide slow, expensive flight, but with a lot of lift [Norberg, 1990]. Galliforms and swifts also have relatively large folia IV–VI, but the former have broad, rounded wings that enable fast flight and vertical take-offs, whereas the latter have long, narrow wings and are highly maneuverable [Norberg, 1990]. So, it would appear that the expansion of folia IV–VI is not reliably correlated to any obvious feature of wing morphology or flight behavior.

Both folia VII and VIII respond strongly to visual and auditory stimuli of tectal origin [Whitlock, 1952; Gross, 1970; Clarke, 1974]. Folium VII is primarily visual [Whitlock, 1952; Gross, 1970; Clarke, 1974] and appears to be quite large in species with good visual acuity, such as raptors [Larsell, 1967]. Although VIII appears to be primarily auditory [Whitlock, 1952; Gross, 1970], it is relatively large in the nightjars, oilbird and potoo (fig. 3, 4). A relatively large VIII in the oilbird is not unexpected as it might reflect the presence of echolocation in this species, but why it is also enlarged in non-echolocating caprimulgiforms is unclear. The apparent absence of VII in the oilbird is also interesting because they have a highly rod-dominated retina [Martin et al., 2004; Rojas et al., 2004] and lack stereoscopic vision despite a large binocular visual field [Pettigrew and Konishi, 1984; Martin et al., 2004]. Surprisingly, neither folium VII nor VIII were especially large in the owls, which possess both global stereopsis [van der Willigen et al., 1998] and numerous au-

ditory specializations [Konishi, 2003]. Given the inconsistent relationships between visual and hearing abilities and the relative size of folia VII and VIII, we cannot determine how these folia might be involved in visual and/or auditory processing.

Whether similar correlations between folia size and behavior can be made across a wider range of birds is uncertain because it is unclear whether foliar divisions are actually representative of functional divisions within the cerebellum. Whitlock [1952] and Gross [1970] both report extensive overlap among tactile, auditory and visual stimuli within folia IV–IX. This is especially true for folia VI, VII and VIII within the posterior lobe. Functional divisions among folia are found in mammals. For example, subtle alterations in the fissuration pattern of the cerebellum in inbred mouse *(Mus musculus)* strains are correlated with significant differences in behavior [Cooper et al., 1991; Le Roy-Duflos, 2001]. If subtle differences can have significant behavioral effects, then larger differences in foliation pattern, as we observed among caprimulgiforms, should reflect correspondingly larger behavioral effects.

Nonetheless, the functional implications of foliar divisions are not well understood. Nishiyama and Linden [2004] have shown that there are marked differences in the innervation and electrophysiological properties of Purkinje cells between sulcal and bank Purkinje cells. Over the past 20 years, the dominant discourse with respect to cerebellar organization has emphasized the importance of parasagittal zones that cut across the lobules [e.g., Voogd and Bigare, 1980; Voogd and Glickstein, 1998; Apps and Garwicz, 2000; Ruigrok, 2003; Sillitoe et al., 2003; Sugihara and Shinoda, 2004]. It has even been suggested that the development of zonal boundaries occurs independently of cerebellar lobules and fissures [Sillitoe et al., 2003]. Zones are also present within the avian cerebellum [Wold, 1981; Arends and Zeigler, 1991; Feirabend et al., 1996; Winship and Wylie, 2003; Wylie et al., 2003a, b], so it is equally likely that functional differences in cerebellar anatomy among birds occurs at the level of zones (i.e., number and/or size of zones) rather than at foliar divisions.

Two questions then arise: why does foliar structure and folium size vary among species; and why does at least some of it (e.g., folium III), appear to be correlated with specific behavioral and/or other morphological differences? It is possible that changes in the size and number of folia in the avian cerebellum reflects changes in the size and/or number of zones as described above. Foliar structure might also vary according to the architecture of the

brain case and/or overall brain morphology that affects the way the cerebellum can develop. Although the optic tectum is not exceptionally large in apodiforms [Boire and Baron, 1994; Iwaniuk and Hurd, 2005], the orientation of the optic lobes could certainly affect how the anterior lobe of the cerebellum develops. Differences in the number and morphology of the folia could result from allometric scaling as well. Lastly, foliar variation could result from a combination of some or all of these factors. Determining which of these factors affect the number of structure of folia and their relative strength will have significant implications for understanding the organization and evolution of the avian cerebellum.

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