

Comparative brain morphology of Neotropical parrots (Aves, Psittaciformes) inferred from virtual 3D endocasts

Julieta Carril,^{1,2} Claudia Patricia Tambussi,^{2,3} Federico Javier Degrange,^{2,3,4} María Juliana Benitez Saldívar^{2,5} and Mariana Beatriz Julieta Picasso^{2,6}

¹*Cátedra de Histología y Embriología Animal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina*

²*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*

³*Centro de Investigaciones en Ciencias de la Tierra (CICTERRA), CONICET-UNC, Córdoba, Argentina*

⁴*Centro de Investigaciones Paleobiológicas (CIPAL), Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina*

⁵*Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina*

⁶*División Paleontología Vertebrados, Museo de La Plata, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Buenos Aires, Argentina*

Abstract

Psittaciformes are a very diverse group of non-passerine birds, with advanced cognitive abilities and highly developed locomotor and feeding behaviours. Using computed tomography and three-dimensional (3D) visualization software, the endocasts of 14 extant Neotropical parrots were reconstructed, with the aim of analysing, comparing and exploring the morphology of the brain within the clade. A 3D geomorphometric analysis was performed, and the encephalization quotient (EQ) was calculated. Brain morphology character states were traced onto a Psittaciformes tree in order to facilitate interpretation of morphological traits in a phylogenetic context. Our results indicate that: (i) there are two conspicuously distinct brain morphologies, one considered walnut type (quadrangular and wider than long) and the other rounded (narrower and rostrally tapered); (ii) Psittaciformes possess a noticeable notch between hemisphaeria that divides the bulbus olfactorius; (iii) the plesiomorphic and most frequently observed characteristics of Neotropical parrots are a rostrally tapered telencephalon in dorsal view, distinctly enlarged dorsal expansion of the eminentia sagittalis and conspicuous fissura mediana; (iv) there is a positive correlation between body mass and brain volume; (v) psittacids are characterized by high EQ values that suggest high brain volumes in relation to their body masses; and (vi) the endocranial morphology of the Psittaciformes as a whole is distinctive relative to other birds. This new knowledge of brain morphology offers much potential for further insight in paleoneurological, phylogenetic and evolutionary studies.

Key words: 3D brain reconstructions; Aves; character mapping; endocranial morphology; geometric morphometrics.

Introduction

Among non-passeriform birds, Psittaciformes are one of the most species-diverse clades (Mayr, 2010). They are characterized by their advanced cognitive abilities, and highly developed locomotor and feeding behaviours (Iwaniuk et al.

2005). Psittaciformes differ morphologically in many respects from other birds. The orbital rings, peculiarities in the shape of the beak and mandible, and the shape of the quadrate are some of the exceptional characters of the skull. Moreover, the few anatomical studies on the brain of Psittaciformes have identified a number of features that provide a general characterization: relatively larger brains and telencephala than other non-passerines birds (Iwaniuk et al. 2005), which is possibly related to their advanced cognitive abilities, including vocal learning and communication (Iwaniuk & Hurd, 2005; Iwaniuk et al. 2005). In comparison with other birds, Psittaciformes show a high degree of

Correspondence

Julieta Carril, Calle 64 N°3, B1900BVA La Plata, Buenos Aires, Argentina. T: +54-011-1559520903; E: julyetacarril@gmail.com

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cerebellum foliation (Iwaniuk et al. 2006, 2007), and smaller diencephala, myelencephala, mesencephala, optic tecta and cerebellum (Mlíkovský, 1989; Iwaniuk et al. 2004, 2005). In only one species, the Kakapoo (*Strigops habroptilus*; Gray, 1845), the olfactory bulbs are large (Corfield et al. 2011).

Using computed tomography (CT) and three-dimensional (3D) visualization software, we studied the endocranial anatomy of representatives of Neotropical parrots whose monophyly has been supported by morphological and molecular studies (Smith, 1975; Sibley & Alquist, 1990; de Kloet & de Kloet, 2005; Tavares et al. 2006). Neotropical parrots comprise a total of 167 species (Forshaw, 2010), which vary greatly in body mass, but have homogeneous morphological characters and behavioural habits (Collar, 1997).

The use of CT scans has gained in importance as an exploratory tool in the field of biology. The study of 3D models generated from CT scans opens a range of possibilities never before investigated in the field. This methodology presents obvious advantages. Materials usually do not need treatment before entering the scanner, no deterioration occurs during the scan, and the results allow the reconstruction of high-quality 3D models. This includes modelling of both hard and soft tissues that can be used for different purposes, such as anatomical and biomechanical studies. The use of this technology has been a breakthrough in studies of various types, both in fossil (Cunningham et al. 2014; Sutton et al. 2014) and extant organisms, resulting in both descriptive (Lautenschlager et al. 2013; Cox & Faulkes, 2014; Quayle et al. 2014; Racicot & Rowe, 2014) and/or ontogenetic studies (Picasso et al. 2010; Fisher et al. 2014; Rücklin et al. 2014). The datasets obtained by the scans are long-lasting and easily portable, facilitating the comparison and study of the 3D models obtained, which are easily manipulated, sectioned and redescribed. More importantly, this methodology provides data on extant taxa that are characterized as 'very rare', endangered or rare in the fossil record.

Although it has been stated that endocranial morphology varies across Aves, variation among closely related species remains largely unknown (Smith & Clarke, 2012). The aim of this work is to explore the brain morphology of Neotropical parrots and to identify potential differences and similarities with other Psittaciformes. Moreover, we intended to provide a database of brain anatomy of living Neotropical Psittaciformes that will contribute to functional, behavioural, evolutionary and/or paleoneurological studies. For these purposes, we employed 3D digital endocast reconstructions, and we explored endocast shape variation using 3D geometric morphometric methods. The latter approach has recently been used by other researchers (Kawabe et al. 2014), but this is the first time it has been applied to Psittaciformes. We also trace the evolution of morphological traits over a molecular phylogeny to infer the characteristics at ancestral nodes. The strength of this analysis lies in the tree, the taxa included and the evolutionary liability of the

characters analysed. This study represents an excellent opportunity to examine in detail the history of certain important neurological characteristics. Previous hypotheses about the evolution of brain morphology are scarce, and primarily focused on the initial transformations at the origin of birds and not within a particular clade of Neornithes. We expected that the morphology of Neotropical parrots (according to their ecological and behavioural similarities) would be more conservative in comparison with non-Neotropical Psittaciformes. We also predicted that, as noted for other birds (Jerison, 1973; Iwaniuk et al. 2005; Balanoff et al. 2013), there would be a positive correlation between body mass and brain volume and, given their complex cognitive capacities (Iwaniuk et al. 2005), the Neotropical Psittaciformes would have high encephalization quotient (EQ) values.

Materials and methods

Dry skulls of 14 specimens of extant Neotropical Psittaciformes at the osteological collections of the Museo de La Plata (MLP) and of the Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN; Argentina) were scanned. The species were: *Amazona aestiva*, *Amazona vinacea*, *Anodorhynchus hyacinthinus*, *Ara chloropterus*, *Ara* sp., *Aratinga leucophthalma*, *Cyanoliseus patagonus*, *Enicognathus ferrugineus*, *Myiopsitta monachus*, *Nandayus nenday*, *Pionopsitta pileata*, *Pionus maximiliani*, *Primolius auricollis* and *Pyrrhura frontalis* (Table 1). Also, in our analysis, a *Cacatua galerita*

Table 1 Endocranial volume, body mass and EQ of the brains of Neotropical parrots.

Species	Specimen No.	ECV (cm ³)	BM (g)	EQ
<i>Amazona aestiva</i>	MLP 621	7.237	451	1.642
<i>Amazona vinacea</i>	MACN 1032	8.399	254	2.640
<i>Anodorhynchus hyacinthinus</i>	MACN 2355a	23.715	1331	2.909
<i>Ara chloropterus</i>	MACN 1479a	23.418	1214	3.027
<i>Ara</i> sp.*	MLP 943	15.906	923	2.402
<i>Aratinga leucophthalma</i>	MLP 407	2.932	158	1.207
<i>Cyanoliseus patagonus**</i>	MLP 65	6.383	278	1.906
<i>Enicognathus ferrugineus</i>	MACN 1426a	4.911	160	2.007
<i>Myiopsitta monachus</i>	MLP 79	3.258	120	1.568
<i>Nandayus nenday</i>	MLP 405	4.008	128	1.859
<i>Pionopsitta pileata</i>	MLP 67	2.769	119	1.339
<i>Pionus maximiliani</i>	MLP 343	5.257	293	1.523
<i>Primolius auricollis</i>	MACN 2518	7.780	245	2.496
<i>Pyrrhura frontalis**</i>	MLP 351	2.394	80	1.450
<i>Cacatua galerita</i>	MLP 755	11.604	723.5	2.012

BM, body mass; ECV, endocranial volume; EQ, encephalization quotient.

*Average between body mass of all species of *Ara*.

**Average between female and male body mass.

endocast reconstruction was included in order to compare the shape of Neotropical parrots with a representative of a non-Neotropical Psittaciformes.

Scans were taken in the transverse plane using a General Electric Bright Speed HiSpeed CT scanner at 140 kV and 300 mA with 0.62 mm slice thickness at the Hospital San Juan de Dios, La Plata, Argentina. The DICOM images were processed and reconstructions of the 3D models of the endocast were made with the open source software 3D-SLICER (Fedorov et al. 2012; Fig. 1).

Additionally, in order to corroborate the relationship of endocast features to brain structures, we dissected out the brain of a monk parakeet *Myiopsitta monachus* (Fig. 2). Specifically, we evaluated the foliation of the surface of the brain and the cerebellum. In extant birds, the cranial cavity is almost completely filled by the brain, so the internal surface of the cranial bones closely reflects brain anatomy (Edinger, 1951; Elzanowski & Galton, 1991; Iwaniuk & Nelson, 2002; Osmólska, 2004; Evans, 2005; Picasso et al. 2010). This makes it possible to build casts of the endocranial volume (i.e. endocasts), and study the development of different traits and regions of this 'brain'.

For descriptions of the endocasts, the osteological nomenclature proposed by Baumel & Witmer (1993) is used, and brain anatomical terminology for the central nervous system follows Breazile & Kuenzel (1993).

The EQ (Jerison, 1973) for each specimen was calculated according to the following equation: $EQ = ECV/0.137 W^{0.568}$ (Smith & Clarke, 2012), where ECV is the endocranial volume (cm^3) calculated using the 3D-SLICER software and W is the body mass (g) obtained from Dunning (2008). Species with endocranial volumes larger than expected for their body mass have $EQ > 1$, whereas species with endocranial volumes smaller than expected have EQ values < 1 .

We used PAST 3.02a software (Hammer et al. 2001) to test a linear regression between \log_{10} -transformed body mass and brain volume.

For the 3D geomorphometric analysis, 25 homologous landmarks were digitized from the endocast (Fig. 3; Table 2) using AMIRA (v.5.1 trial version; Mercury Computer Systems, San Diego, CA, USA). The

dataset was analysed by applying generalized least-squares Procrustes analysis (Rohlf & Slice, 1990a,b) using the MORPHOJ software package (Klingenberg, 2011). This process works by translating, rotating and scaling all specimens to a common reference, minimizing the distance between homologous landmarks. As a consequence, the effects of size, position and orientation are eliminated, and the remaining information reflects shape disparity alone (Procrustes shape coordinates). The absolute size of the specimen is preserved as centroid size, which is calculated as the square root of the sum of squared distances of landmarks from their centroids (Bookstein, 1991). To explore the patterns of major morphological variation of the brains, we applied a principal component (PC) analysis to the Procrustes shape coordinates using MORPHOJ. MORPHOLOGIKA software was used to illustrate 3D shape change.

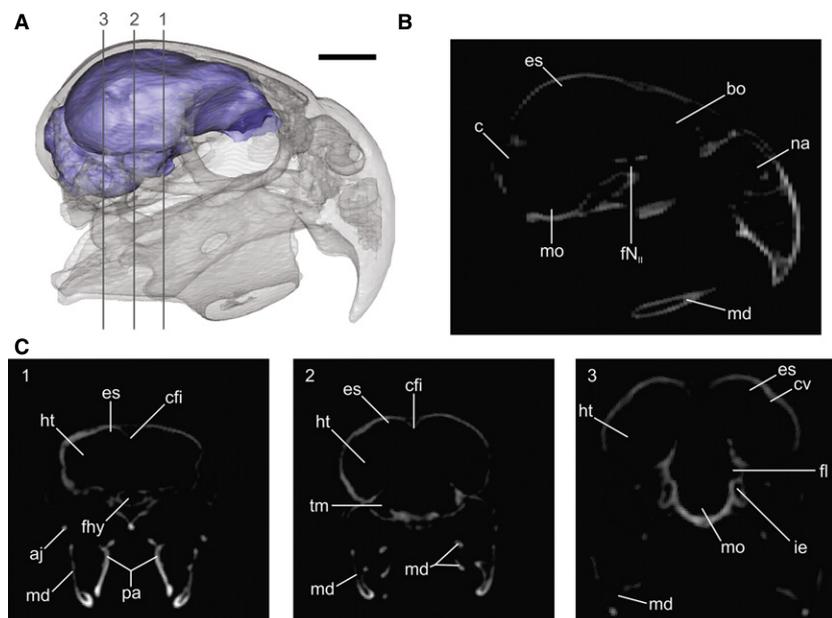
To evaluate the evolutionary history of morphological characters that have been proposed as diagnostic (the dorsal shape of the telencephalon, the dorsal expansion of the eminentia sagittalis, the relative size of the optic lobes and the conspicuity of the fissura mediana; characters 4, 5, 11 and 21 of Smith & Clarke, 2012 respectively), we reconstructed ancestral states using the MESQUITE package Version 3.01 (Maddison & Maddison, 2014) and a molecular phylogeny (Tavares et al. 2006). Characters were considered as unordered. Parsimony and Maximum Likelihood (Markov-K-state1, with equal probability for any particular character change) approaches were applied.

Results

Brain–body relationship

As predicted, we found a strong positive correlation between body mass and brain volume ($r^2 = 0.92$, $P < 0.05$; Fig. 4). All the Neotropical Psittaciformes studied here had an EQ higher than 1 (Table 1). *A. leucophthalma* showed the smallest value of EQ (1.207), and *A. chloropterus* reached the highest EQ values (3.027). Five species,

Fig. 1 Three-dimensional reconstruction of *Cyanoliseus patagonus* endocast, generated from CT scans. (A) Lateral view of the skull in transparency showing the endocast; (B, C) tomographs of the skull in (B) sagittal and (C) coronal planes (1–3) showing the spaces occupied by the brain and its different regions/structures. aj, arcus jugalis; bo, bulbus olfactorius; c, cerebellum; cfi, crista frontalis interna; cv, crista vallearis; es, eminentia sagittalis; fhy, fossa hypophysialis; fl, floculus; fNII, foramen nervus opticum; ht, hemisphaeria telencephali; ie, inner ear; md, mandible; mo, medulla oblongata; na, apertura nasi ossea; pa, ossa palatina; tm, tectum mesencephali. Scale bar: 1 cm.



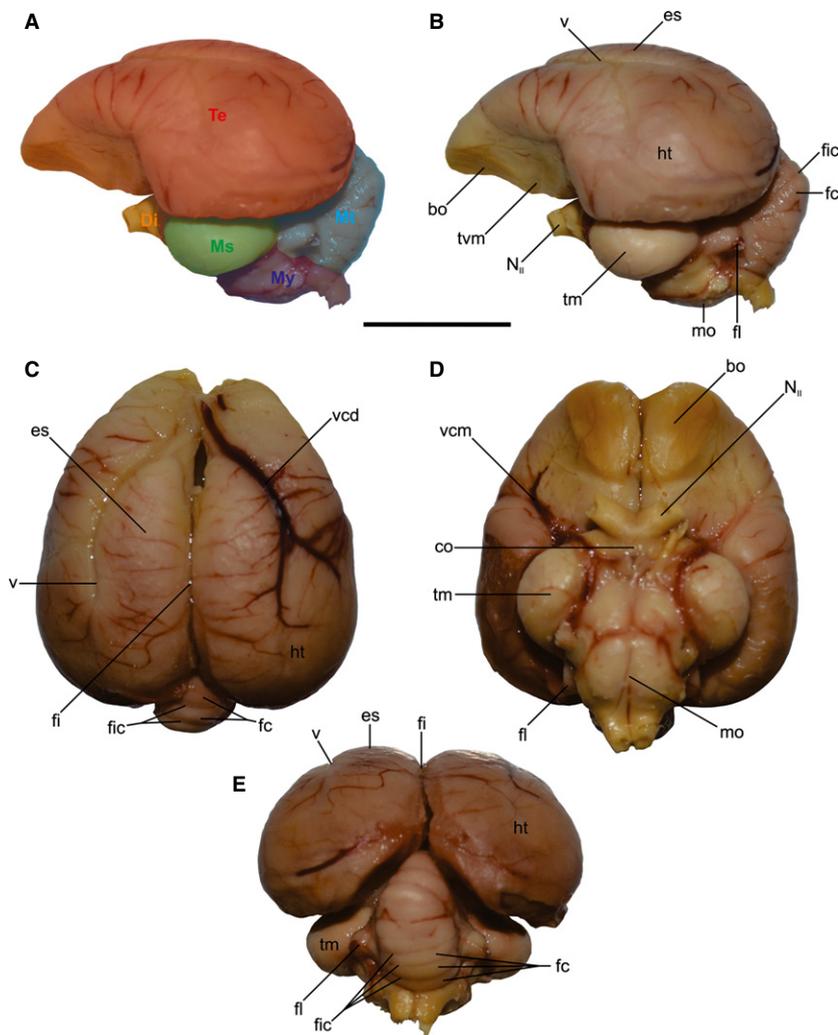


Fig. 2 Dissection of *Myiopsitta monachus* brain. (A) Regions of the brain in lateral view, (B) left lateral, (C) dorsal, (D) ventral and (E) caudal views showing the main structures of the brain. bo, bulbus olfactorius; co, chiasma opticum; Di, diencephalon; es, eminentia sagittalis; fc, folia cerebeli; fi, fissura interhemispherica; fic, fissura cerebeli; fl, flocculus; ht, hemisphaeria telencephalica; mo, medulla oblongata; Ms, mesencephalon; Mt, metencephalon; My, myelencephalon; NII, nervus opticus; Te, telencephalon; tm, tectum mesencephali; tv, tuber ventromediale; v, vallecula; vcd, vena cerebri dorsorostralis; vcm, vena cerebri dorsorostralis media. Scale bar: 1 cm.

A. vinacea, *A. hyacinthinus*, *Ara* sp., *E. ferrugineus* and *P. auricollis* had EQs higher than 2.

Description of the endocasts

General morphology

Fresh material studies confirm the similarity between the actual brain and the virtual endocast (Fig. 2). Virtual cranial endocasts are hereafter referred to as brain models or simply brains. In the brain of Psittaciformes, the telencephalon conspicuously overlaps the cerebellum, and therefore the brain is high and short in general appearance (Figs 2B,C and 5A,C,F,H). All brains showed a noticeable notch located rostrally between hemisphaeria, originating two expansions of the most rostral portions of the telencephalon and dividing the bulbus olfactorius (Fig. 5). The overall shape of the brain is either quadrangular (*A. hyacinthinus* and *Ara*), or more rounded and rostrally tapered (*C. patagonus*, *P. auricollis*, *N. nenday*, *P. maximiliani*, *Amazona*, *E. ferrugineus*, *P. pileata*, *P. frontalis*, *A. leucophthalma* and *M. monachus*). In the former, the brain is similar in shape to a walnut

when viewed dorsally (Fig. 5A), the notch aforementioned wider and more pronounced, the fissura interhemispherica broader, and the telencephalon more rostrally and caudally expanded (Fig. 5F). In the rounded morphotype (Fig. 5F), the notch is less excavated, the fissura interhemispherica narrower, and the telencephalon rostrally and caudally less expanded. Another difference between the brain morphotypes is the lateral extension of the tectum mesencephali: rounder brains present more laterally expanded tecta. A significant difference between both morphotypes is that walnut brains present a more longitudinally linear arrangement, while rounder brains are folded more cranio-caudally, giving them an S-shape in which the optic lobes are more rostrally positioned than in the walnut brain. The cerebellum is more caudally positioned in the rounded brain.

Telencephalon

The most noticeable feature of the telencephalon was the pronounced fissura interhemispherica (Figs 2C,E and 5A,D, F,I). In all brains compared, the eminentia sagittalis

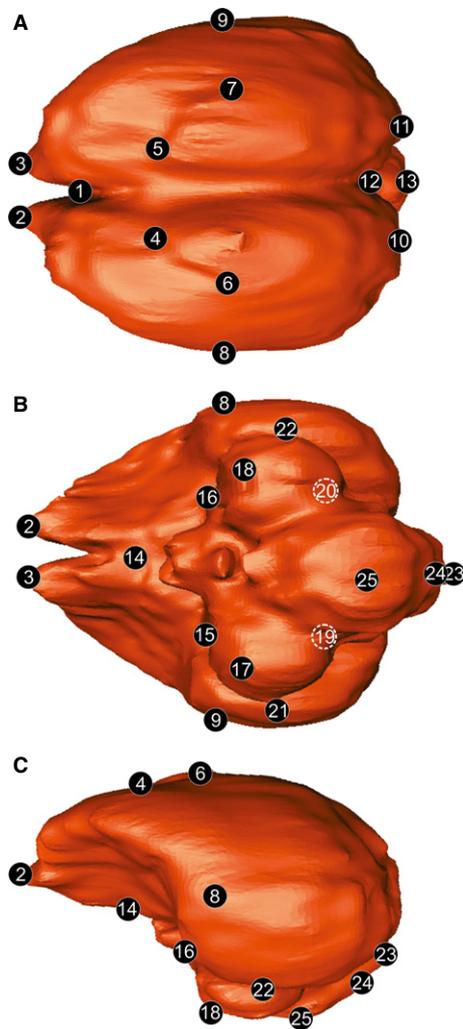


Fig. 3 The 3D brain landmarks used for shape analysis shown in *Enicognathus ferrugineus* brain. (A) Dorsal, (B) ventral, (C) lateral view. See Table 2 for landmark description.

originates behind the notch, extending to the cerebellum and occupying more than half of the total length of the brain (Fig. 5A,F). The caudally positioned eminentia sagittalis corresponds to type B of Stingelin (1957; in type A, the eminentia sagittalis is rostrally positioned; Milner & Walsh, 2009). The eminentia sagittalis is prominent except in *P. maximiliani* and *Amazona* (Fig. 5F,H). In all specimens, the bulbus olfactorius was simple, corresponding to the one-lobe type of Cobb (1959), being small and ventrally oriented (Fig. 5C,D,H,I).

Diencephalon

In just a few cases, CT data allowed us to reconstruct the exit of the optic nerve (Fig. 5B,G). The nerves are transversely thick and diverge widely in a rostral direction. The chiasma opticum, where the optic nerves partially cross, is not clearly delimited in the CT images but is easily observed in the fresh material (Fig. 2D).

Table 2 Landmarks used and anatomical description (Fig. 3).

Landmark	Anatomical description
1	Most rostral point of the fissura interhemispherica
2–3	Most rostral point of the hemisphaeria telencephalica
4–5	Most rostral point of the eminentia sagittalis
6–7	Most lateral point of the eminentia sagittalis
8–9	Most lateral point of the widest part of the telencephalon*
10–11	Most caudal point of the hemisphaeria telencephalica
12	Median junction between telencephalon and cerebellum*
13	Most caudal point of the cerebellum
14	Most caudal point of the tuber ventromediale
15–16	Intersection of telencephalon, tectum mesencephali and diencephalon*
17–18	Most rostral point of the optic lobes (tectum mesencephali)
19–20	Intersection of telencephalon, tectum mesencephali and cerebellum*
21–22	Most lateral point of the optic lobes (tectum mesencephali)
23	Median dorsal point of foramen magnum*
24	Median ventral point of foramen magnum*
25	Most ventral point of the medulla oblongata

*Taken from Kawabe et al. (2013, 2014).

Mesencephalon

The tecta mesencephali (optic lobes) are prominent, but small relative to the telencephalon. They are proportionally larger in rounded brains. They have a crescent shape, and they are noticeable in lateral and caudal views (Fig. 5C,E,H, J). They are covered by the telencephalon in dorsal view (Fig. 5A,F).

Metencephalon

The boundary between the cerebellum and telencephalon is easily distinguishable on the dorsal surface, although in *P. frontalis* and *A. leucophthalma* the cerebellum is partially covered by the telencephalon. The cerebellum shows a cranio-caudal elongation, and no folia cerebelli can be distinguished in the endocast (Fig. 5A,E,F,J). This is because the sinus/meningial tissue surrounding the cerebellum is sufficiently thick to prevent the cerebellum from contacting the endocranium. Like other living birds, parrots have folia cerebelli (Fig. 2) and, in fact, they show a high degree of cerebellar foliation (Iwaniuk et al. 2006). The sinus occipitalis could not be differentiated in the endocast. Regrettably, the extension and development of the flocculi could not be established in most of the 3D reconstructions. However, the

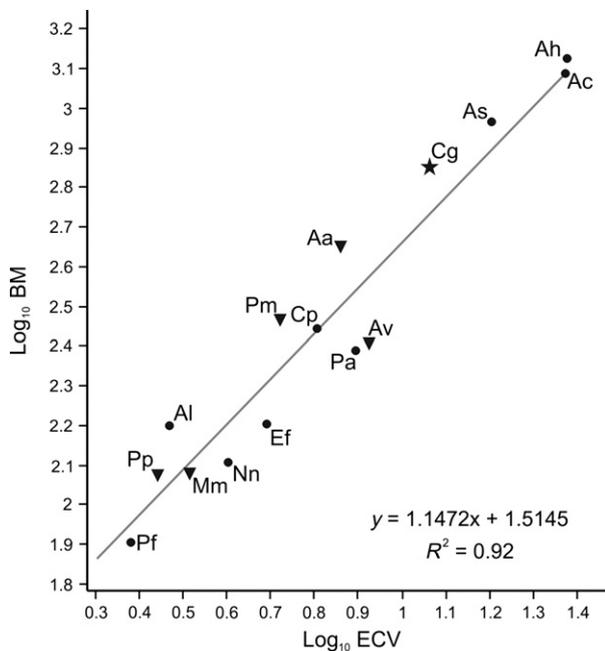


Fig. 4 Scatterplot of log-transformed brain volume vs. body mass in parrots. Solid star indicates cockatoos; solid dots indicate Macaws and allies; and inverted solid triangles indicate Amazons and allies, according to Tavares et al. (2006). Aa, *Amazona aestiva*; Av, *Amazona vinacea*; Ah, *Anodorhynchus hyacinthinus*; Ac, *Ara chloropterus*; As, *Ara* sp.; Al, *Aratinga leucophthalma*; Cg, *Cacatua galerita*; Cp, *Cyanoliseus patagonus*; Ef, *Enicognathus ferrugineus*; Mm, *Myiopsitta monachus*; Nn, *Nandayus nenday*; Pp, *Pionopsitta pileata*; Pm, *Pionus maximiliani*; Pa, *Primolius auricollis*; Pf, *Pyrrhura frontalis*.

dissection of *Myiopsitta* (Fig. 2) shows that the flocculi are well developed, with a wide base, directed and tapering caudally.

Myelencephalon

The medulla oblongata is elongated (Fig. 5B,G) and wider in the rounded brains. It shows a poorly marked fissura mediana.

Geomorphometric analysis

More than half of the variation is contained in the first three components (PC1, 35.68%; PC2, 21.09%; PC3, 8.4%).

The plot of PC1 and PC2 (Fig. 6A) shows that most taxa are grouped together with negative values for PC1. Separate from the majority are *A. hyacinthinus* and *C. galerita*, with positive values for PC1, and *E. ferrugineus*, with positive values for PC2, although the latter shows lower values for PC1 and higher values for PC2 than the first two taxa. Also, *A. aestiva* and *A. chloroptera* are separated by positive values for PC1 and negative values for PC2.

The PC2 vs. PC3 plot does not allow groups to be discriminated, while the PC1 vs. PC3 plot separates two groups: most of the taxa are located in the positive space for PC1,

whereas species with negative values of PC1 include *E. ferrugineus*, *A. aestiva*, *A. chloroptera*, *A. hyacinthinus* and *C. galerita*.

Principal component 1 is strongly influenced by brain width, the length and width of the fissura interhemispherica, the rostral arrangement of the tectum mesencephali and the caudal extension of the cerebellum (Fig. 6B). Positive values correspond to rostrally wide brains, with deep and wide fissura interhemispherica, rostrally directed tectum mesencephali and caudally extended cerebellum. Also, both hemispheres are rostrally directed. Linear regression between PC1 and the body mass logarithm ($r=0.68$; $r^2=0.46$; $P=0.005$) shows that PC1 is poorly correlated with size. PC2 is influenced by the height and width of eminentia sagittalis, the width of fissura interhemispherica, the dorso-ventral development of the cerebellum and the location where maximum brain width occurs (Fig. 6B). Positive values correspond to brains with narrower and higher eminentia sagittalis, narrower fissura interhemispherica and higher cerebellum. Maximum brain width is more rostrally located. Lastly, PC3 is influenced by the width and length of the fissura interhemispherica, the dorso-ventral development of the hemispheres in the lateral view, the width of the cerebellum, the caudal extension of the medulla oblongata, and the orientations of the foramen magnum and the tectum mesencephali. Positive values indicate narrow fissura interhemispherica, greater ventral extension of the hemispheres, narrower cerebellum, shorter medulla oblongata, ventro-caudally directed foramen magnum and more caudo-ventrally directed tectum mesencephali.

Character plotting and ancestral state reconstruction

Brain morphological character states are traced onto the Psittaciformes phylogenetic hypothesis of Tavares et al. (2006) in Fig. 7. From the 28 characters of Smith & Clarke (2012), features 18–20 (flocculi), 22 (hypophysis), 23 (carotid artery) and 24–28 (inner ear) could not be encoded here and are therefore not plotted into a cladogram. *Strigops* was excluded from this analysis because we lacked an endocast for this species. All Neotropical parrot endocasts showed: (i) reduced, simple and ventrally positioned bulbus olfactorius; (ii) eminentia sagittalis caudally positioned, not laterally extended; (iii) large tectum mesencephali (but small relative to the telencephalon), not visible in dorsal view, with a curved contact with the telencephalon; (iv) expanded telencephalon; (v) short and narrow cerebellum, with a v-shaped contact with the telencephalon and indistinct fissura cerebelli; and (vi) not distinguishable sinus occipitalis. However, some variations among Neotropical parrots can be diagnosed in the eminentia sagittalis (characters 4 and 5 of Smith & Clarke, 2012), the size of the optic lobes relative to the rhombencephalon (character 11) and recognition of the fissura mediana (character 21).

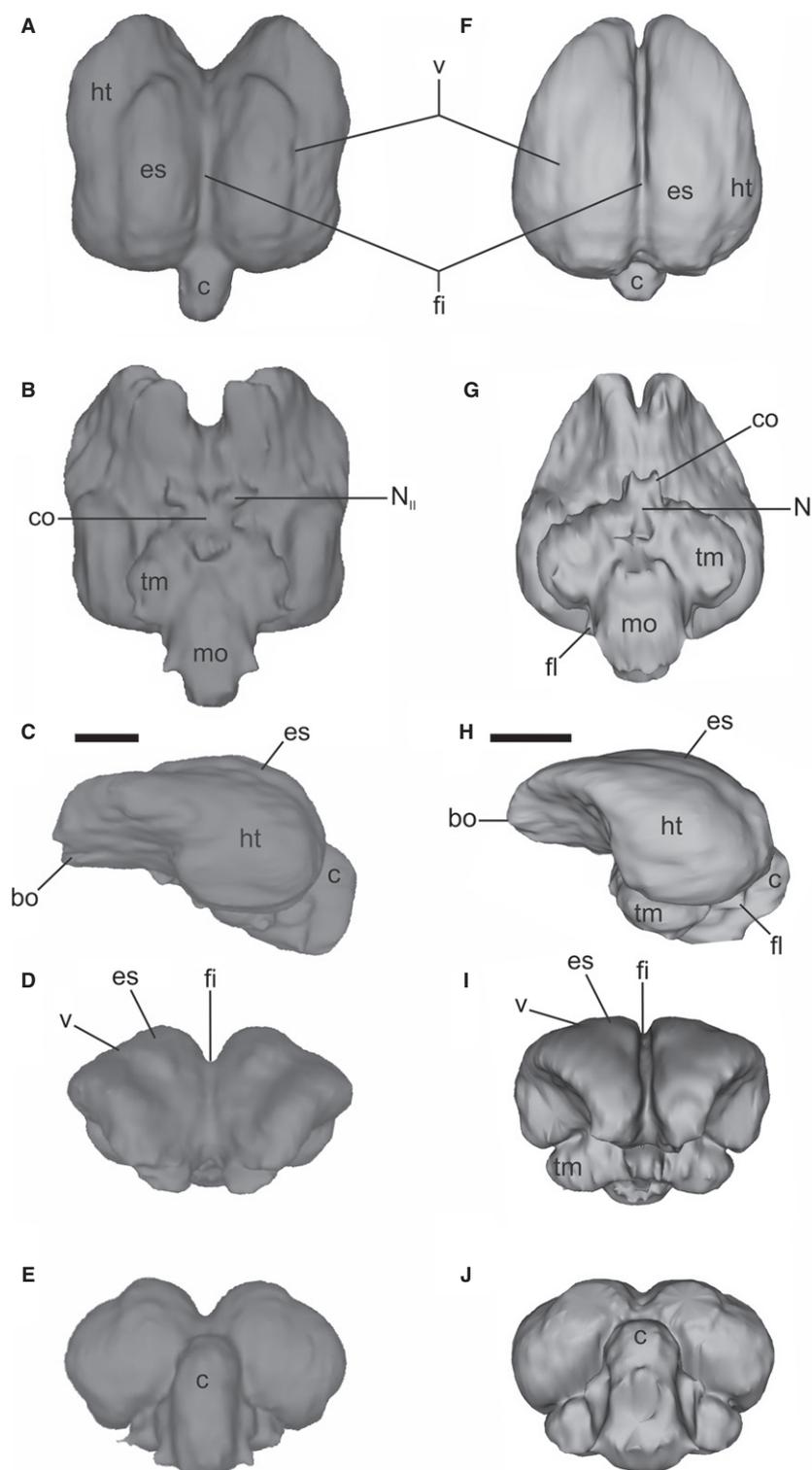


Fig. 5 Endocast of adult *Anodorhynchus hyacinthinus* (A–E) and *Pionus maximiliani* (F–J) in dorsal, ventral, left lateral, cranial and caudal views. bo, bulbus olfactorius; c, cerebellum; co, chiasma opticum; es, eminentia sagittalis; fi, fissura interhemispherica; fl, flocculus; ht, hemispherium telencephali; mo, medulla oblongata; NII, nervus opticus; tm, tectum mesencephali; v, vallicula. Scale bar: 1 cm.

Figure 8 shows ancestral morphological states inferred from reconstructions of character history. In all but one of the characters (optic lobes, Fig. 8C), Parsimony and Maximum Likelihood models have no conflicts in the consequent interpretations. Mapping of endocranial character changes on a phylogeny of Psittaciformes allowed for inferences

regarding morphological changes of the endocranium in the Neotropical parrots (Arini) relative to the remainder Psittaciformes.

The reconstruction of character evolution revealed that the rounded telencephalon in dorsal view is the ancestral condition for Neotropical psittaciforms (Fig. 8A), and it is

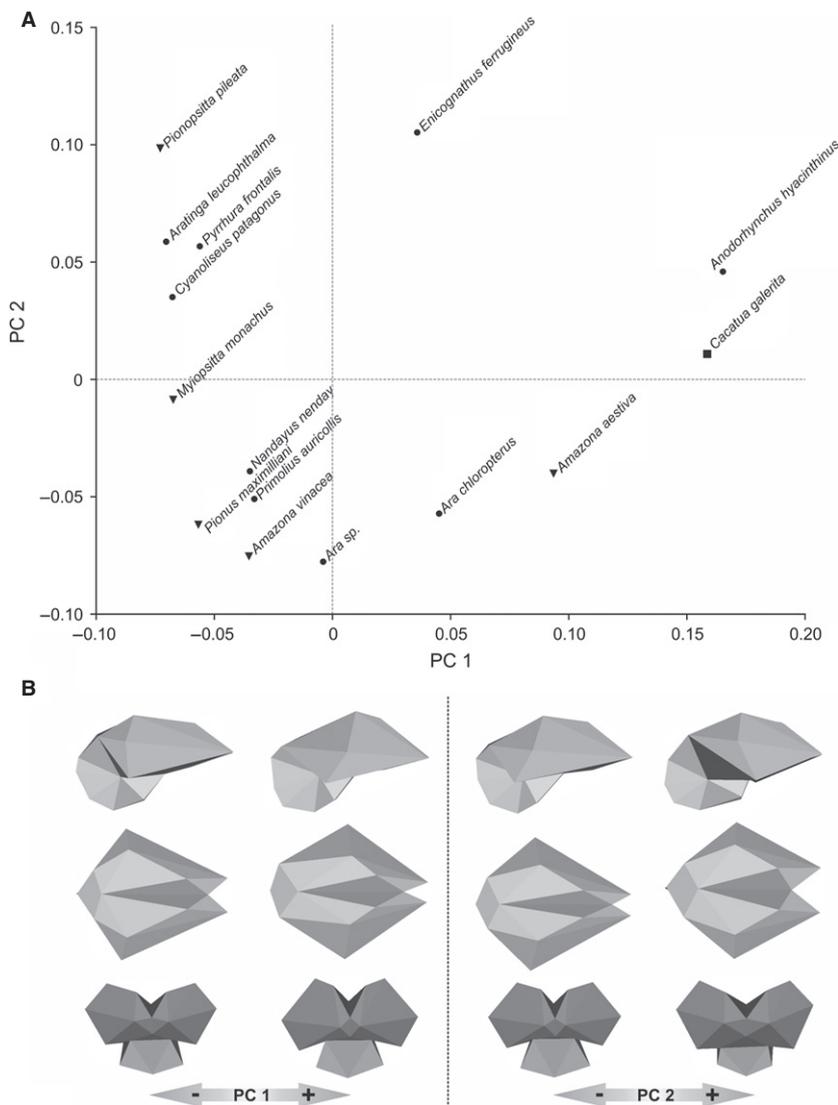


Fig. 6 Principle component (PC) analysis of the brain shape of parrots. (A) Plot of PC1 against PC2. (B) Brain shape variation along PC1 and PC2 axis. Solid dots indicate Macaws and allies; inverted solid triangles indicate Amazons and allies; and solid squares indicate cockatoos, according to Tavares et al. (2006).

maintained in most Arini studied here. Several previous studies have shown that the rounded condition is likely plesiomorphic for Aves (Corfield et al. 2008; Kawabe et al. 2009; Smith & Clarke, 2012). Both *Ara* and *Anodorhynchus* are the only Arini that change to the quadrangular condition, and the same state is present in *Cacatua*. It appears that a potential parallelism or convergent evolution happened at least once.

According to the ancestral reconstruction, the distinctly enlarged dorsal expansion of the eminentia sagittalis is the plesiomorphic condition (Fig. 8B). This trait is maintained in all the Arini, except in the cluster formed by *Amazona* and *Pionus* that have an unexpanded condition. There is considerable variation in the dorsal expansion of the eminentia sagittalis within birds. Birds with different ecological or behavioural attributes show similar expansion of the eminentia sagittalis (Walsh & Milner, 2011), whereby the relationship between both is not clear.

Concerning the question about the evolution of the size of the optic lobes, the situation is not resolved. A parsimony model (Fig. 8C) proposes that small optic lobes is the ancestral state that evolved to the large condition in *Primolius*, *Nandayus*, *Aratinga*, *Enicognathus* and the clade of all Amazon parrots. Reversals happened in *Ara*, and remain in the ancestral state in *Anodorhynchus*, *Cyanoliseus*, *Pyrrhura* and *Cacatua*. The likelihood approach reconstructed the evolutionary history of this trait with greater vagueness. Small or large optic lobes have about the same chance (0.52 and 0.48, respectively) of being the ancestral condition of Neotropical parrots, pointing to the need of further detailed investigations on this structure. Optic lobes are relatively small for example in the kiwi, owls, some charadriiforms and several songbirds (Corfield et al. 2012; Smith & Clarke, 2012) and, for the moment, there is no biological or ecological correlation that would help to understand the evolutionary history shown in our models.

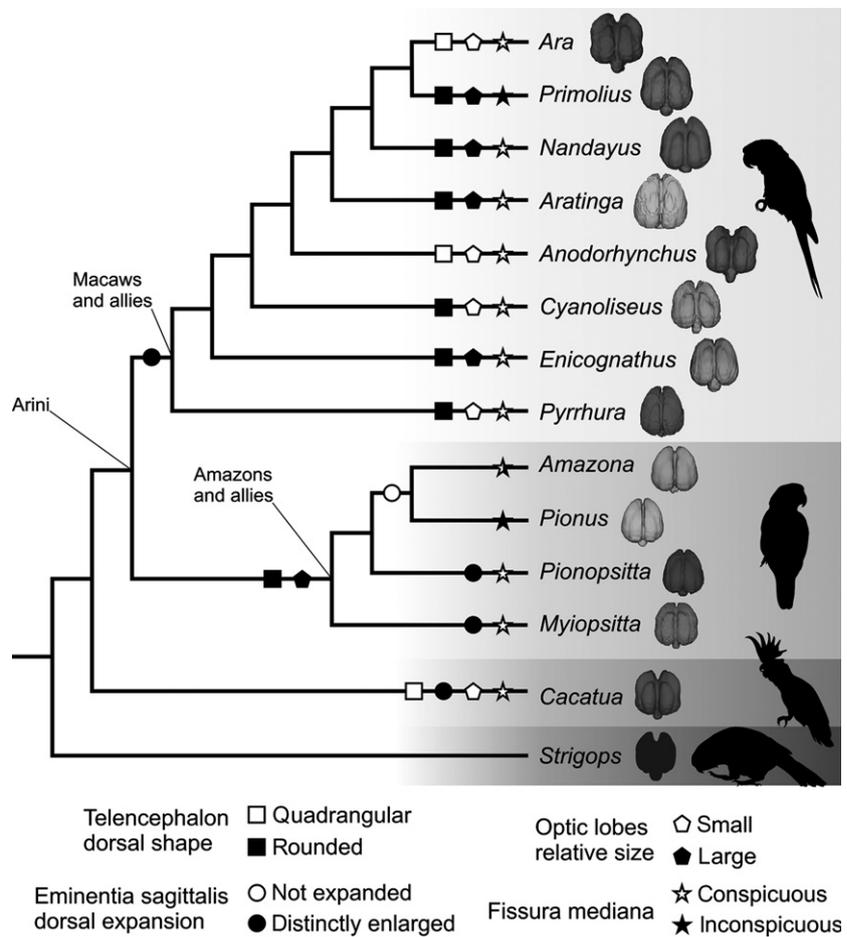


Fig. 7 Phylogenetic diagram plotting the occurrence of some brain morphological traits in Neotropical parrots. Phylogenetic hypothesis modified from Tavares et al. (2006). Characters from Smith & Clarke (2012).

Conspicuous fissura mediana is the ancestral state (Fig. 8D). Most taxa conserved this trait that evolves into inconspicuous fissura only in *Primolius* and *Pionus*. Potential advantages of one or another state remain unclear at this time.

Discussion

Brain morphology often reflects the influence of ecological, behavioural or phylogenetic factors (Lefebvre et al. 2002, 2004). Because, in extant birds, the cranial cavity is almost completely filled by the brain, studying brain endocasts allows us to study brain morphology, and to test correlations between sensorial or cognitive capabilities and the development of some brain regions (Edinger, 1951; Elzanski & Galton, 1991; Iwaniuk & Nelson, 2002; Osmólska, 2004; Evans, 2005; Picasso et al. 2010).

Both qualitative (descriptions) and quantitative (3D geomorphometrics analysis; Fig. 6) methods differentiate two conspicuously distinct brain morphologies, mainly based on the maximum width of the hemisphaeria. Walnut brains are associated with a higher and/or narrower eminentia sagittalis, a more extended cerebellum and wider fissura interhemispherica than rounded brains. For

example, *A. hyacinthinus* is characterized by a walnut brain shape with a high and narrow eminentia sagittalis, a high and caudally extended cerebellum, and a wide and deep fissura interhemispherica (Fig. 5A–E). In contrast, *P. maximiliani* has a more rounded brain, with a low and wide eminentia sagittalis, a low cerebellum, and a narrow and shallow fissura interhemispherica (Fig. 5F–J). Moreover, brain characters traced onto a cladogram show that brain morphologies in the Neotropical parrots are heterogeneously distributed in the clade (Fig. 7). We find no functional or phylogenetic explanation for this heterogeneity. However, it seems plausible that the primitive condition for brain morphology is the walnut type, as more basal Psittaciformes such as *Nestor notabilis* and *Cacatua galerita* exhibit this morphology (Corfield et al. 2011; Gsell, 2012). The walnut type could be then synapomorphic for the clade Psittaciformes as it is distinctive relative to other birds. According to this hypothesis, the rounded type seems to be the primitive condition of the Neotropical Arini, and the walnut brain type has appeared more than once in the Neotropical parrots (Figs 7 and 8).

Except for *P. maximiliani* and *Amazona*, the eminentia sagittalis occupies a caudal position of the telencephalon in Psittaciformes. This feature, formed by the hyperpallium, is

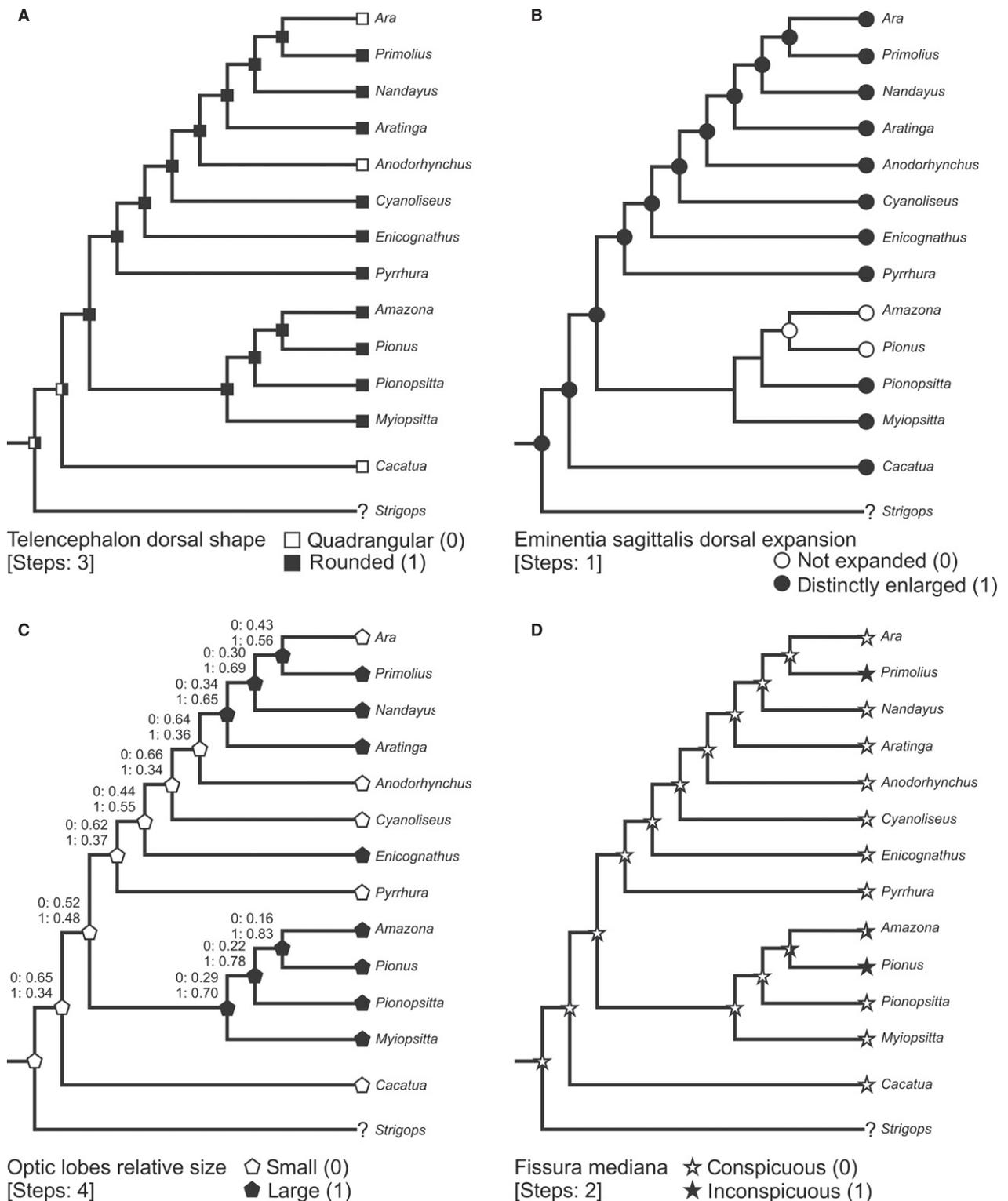


Fig. 8 Ancestral-state reconstruction of endocast in Arini (Psittaciformes) based on Parsimony analysis (steps are indicated between brackets). Additionally in (C), Maximum Likelihood state inferences are indicated by numbers. Characters were taken from Smith & Clarke (2012) and considered as unordered. Phylogenetic proposal modified from Tavares et al. (2006).

mostly involved in integrating and processing the visual signals (Reiner et al. 2004; Reiner, 2005; Walsh & Milner, 2011) and somatosensory inputs (Medina & Reiner, 2004; Mouritsen et al. 2005). The caudal position of this structure (also seen in Anseriformes and Apterygiformes) could be related to the increase in the rostral-most portions of the telencephalon (Walsh & Milner, 2011).

The noticeable notch between hemisphaeria that separates the two lobes of the bulbi olfactorius is related to the presence of a septum osseum fossae bulbi, a bony septum present only in parrots, albatrosses and kiwis (Baumel & Witmer, 1993). This notch, termed the 'inter-hemispheric septum' *sensu* Iwaniuk et al. (2001), varies in size and shape within psittaciforms, depending on body size and the size of the novel ethmomandibularis muscle (which attaches to the septum), and it could play a role in protecting the brain during feeding and locomotion, which often involve strong bite forces (Iwaniuk et al. 2001). Interestingly, according to our observations, the walnut type only occurs in large parrots, such as *Strigops*, *Cacatua*, *Anodorhynchus* and *Ara* (i.e. taxa with body mass ranging 800–2000 g). The walnut-shaped brains of these taxa reflect the presence of a very pronounced interhemispheric sulcus coinciding with an interhemispheric bony septum. To our knowledge, the ethmomandibularis muscle (that elevates the lower jaw) is particularly noticeable in two of these larger taxa, *Ara* and *Anodorhynchus* (Porto, 2004). It could be assumed that the size of the muscle associated with the shape of the septum would sculpture the brain. Unfortunately, we have no information on how this muscle is in the other two taxa. For now, this may not be more than an assumption. On the contrary, the m. ethmomandibularis is also big in smaller parrots such as *Myiopsitta* (Carril, Degrange & Tambussi, in press), whose brain is rounded. The potential relationship between brain types with behavioural variables is a question that remains to be explored.

Except for the kiwis, vultures and seabirds, the bulbus olfactorius of most living birds (including parrots) are small (Walsh & Milner, 2011), mainly because their herbivorous diet allows them to locate food by sight (Fernandez et al. 1997). Bulbus with a relatively small size had been previously observed for parrots and passerines in the classical work of Cobb (1959).

The tecta mesencephali are prominent in Neotropical parrots, as has been described for other diurnal Psittaciformes (eg. kea and cockatoo; Corfield et al. 2011; Gsell, 2012). In the only parrot with nocturnal habits, the kakapoo *Strigops habroptilus*, they are extremely small and partially obscured by the lateral aspect of the cerebral hemisphaeria (Corfield et al. 2011; Gsell, 2012). Strikingly, the kakapoo shows a relatively large bulbi olfactorius. Only few Neotropical parrots (such as *A. hyacinthinus*) show relatively large bulbi compared with the rest of the Psittaciformes, but they are as large of those of *C. galerita* (or smaller).

Parrots have a relatively small cerebellum but significantly more folia than other groups of birds (Iwaniuk et al. 2005, 2006). Unfortunately, the development of the folia cerebelli could not be distinguished in the 3D reconstructions, but they can be seen in the fresh brain (Fig. 2E). It has been suggested that the morphology of the endocranial cavity containing the cerebellum could be constrained in size and shape in Psittaciformes by the large jaw adductor muscles attached to the braincase surrounding this cavity (Iwaniuk et al. 2006). Studies on the mandibular muscles in Neotropical parrots show a well-developed adductor mandibulae complex, including the m. adductor mandibulae externus profundus, origin of which is located on a deep fossa temporalis (Carril, Degrange & Tambussi, in press).

The EQ values of Neotropical parrots (Table 1) suggest they possess brain volumes higher than expected for their body masses, reaffirming that Psittaciformes has significantly larger brains than other birds (Iwaniuk et al. 2005). Large brain size could play an important role in the evolution of advanced cognitive abilities (Iwaniuk et al. 2005), and it has been demonstrated that species with larger brains have greater behavioural flexibility (Gsell, 2012), resulting in more successfully adapting to changing environments. However, it is important to consider brain organization as well as brain size in assessing the effect of brain morphology on cognitive abilities (Iwaniuk et al. 2005). Despite this caveat, it can be assumed that larger regions of the brain are associated with more complexity in the functions controlled by or related to that region (Jerison, 1973; Healy & Rowe, 2007). Parrots have a large tecta mesencephali and small bulbi olfactorii, which could be related to their highly versatile behaviour during arboreal locomotion. Arboreal locomotion involves perching, climbing, hanging and moving easily among trees using both the head (jaws, tongue and neck) and hindlimbs (Carril et al. 2014). All these activities demand a big compromise between visual acuity and gaze stabilization.

Conclusions

Based on our CT scan data, Neotropical parrots show two conspicuously distinct brain morphologies (walnut type and rounded), mainly based on the shape and maximum width of the hemisphaeria, with a striking rostral notch. The functional interpretation of this distinction is pending.

Given a phylogeny and an evolutionary hypothesis, it is possible to reconstruct histories of character change and to infer models of character evolution. Rounded telencephalon in dorsal shape, distinctly enlarged dorsal expansion of the eminentia sagittalis and conspicuous fissura mediana are the ancestral conditions and retained throughout most Neotropical parrots.

This new knowledge of brain morphology provides a basis for further insights regarding paleoneurological, phylogenetic and evolutionary studies.

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