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The brain of *Brycon orbignyanus* (Valenciennes, 1850) (Teleostei: Characiformes: Bryconidae): gross morphology and phylogenetic considerations

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The brain of *Brycon orbignyanus* is described as a model for future studies of the gross morphology of the central nervous system in Characiformes. The study of brain gross morphology of 48 distinct taxa of Characiformes, one of Cypriniformes, two of Siluriformes and two of Gymnotiformes, allowed us to propose, for the first time, six putative brain synapomorphies for the Characiformes and also two possibly unique gross brain morphology characters for the Siluriformes. A detailed protocol for the extraction of the brain in Characiformes is also provided.

O encéfalo de *Brycon orbignyanus* é descrito como um modelo para futuros estudos da anatomia externa do Sistema Nervoso Central de Characiformes. O estudo da morfologia externa de 48 táxons distintos de Characiformes, um de Cypriniformes, dois de Siluriformes e dois de Gymnotiformes, permitiu-nos propor, pela primeira vez, seis prováveis sinapomorfias encefálicas e também duas possíveis características encefálicas para Siluriformes. Um protocolo detalhado para a dissecção e extração do encéfalo de Characiformes é também apresentado.

Keywords: Comparative morphology, Encephalon, Ostariophysi, Otophysi, Putative synapomorphies.

Introduction

During the last two centuries, diagnoses of fish taxa and hypotheses of their evolutionary relationships were almost exclusively based on osteological attributes (see Wiley & Johnson, 2010; Datovo & Vari, 2014). This extensive exploration of osteological features in bony fishes was really efficient in the delimitation of major Teleostei clades; notwithstanding, this almost exclusive focus on osteological features resulted in the "relatively minor attention" to other anatomical systems (Datovo & Vari, 2014), and very few studies have even tried to describe and analyze other major anatomical systems in fishes, such as the neuroanatomy, which according to Datovo & Vari (2014), based mostly on Wiley & Johnson (2010), represents approximately 1% of the synapomorphies currently recognized for teleosteans fishes.

Studies of comparative brain anatomy of teleosts focusing on phylogenetic relationships are scarce, and the first to combine brain features and cladistic methods was Northcutt (1984, 1985), who has shown that cladistics analytical tools could be used to find out the patterns resulting from the evolution of vertebrate brains (Abrahão & Pupo, 2014; Striedter, 2005). Previous to Northcutt's (1984, 1985) publications, studies of fish brain anatomy were focused on the relationship between ecological attributes and brain gross morphology, or simple descriptions – either total or partial - of chondrichthyan and teleostean brains (Ewart, 1888; Herrick, 1899, 1901; Evans, 1931, 1940; Miller & Evans, 1965; Nieuwenhuys, 1967).

Despite the scarcity of neuroanatomical features in phylogenetic analyses, a few neuroanatomical characters were found to be synapomorphic for some lineages of Teleostei. Some examples include the anterior brain position in relation to the cranial cavity in Gadiformes: Melanonoidei (Howes, 1993); the olfactory sensory epithelium arranged in sensory islets and absence of the *saccus vasculosus* in the Cyprinodontiformes and Atheriniformes (Yamamoto, 1982; Parenti, 1993, 2005); and the large dorsal *telencephalon* of the anterior portion of central nucleus and small medial portion, together with the absence of accessory optic tract and nucleus (except for the Sternopygidae), in addition to ampullary organs organized in rosettes in Gymnotiformes (Albert *et al*., 1998; Wiley & Johnson, 2010).

Characiformes contains approximately 2,100 valid living species, occurring mostly in the freshwaters of the Neotropical region (19 families) and less so in the African Sub-Saharan region of Africa (four families) (Reis *et al*., 2003; Oliveira *et al*., 2011). As with other teleostean groups, the clades within

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the Characiformes are diagnosed and have their evolutionary interrelationships hypothesized almost exclusively with osteological characters (Fink & Fink, 1981, 1996). The great diversity of lifestyles found in Characiformes is most probably reflected in the organization of their central nervous system (see Nieuwenhuys *et al*., 1998), thus, we hypothesize that the study of the brain gross morphology of characiforms can provide important insights into their biology, ecology, behavior, evolution and phylogenetic relationships, as pointed by Lisney & Collin (2006) for the Animal Kingdom, and their diversity of brain morphologies as a whole.

Among the Otophysi (composed by the Orders Cypriniformes, Characiformes, Siluriformes and Gymnotiformes), the study of the brain gross morphology has recently been addressed for gymnotiforms (Albert *et al*., 1998; Albert, 2001), in addition to the siluriforms Callichthyidae and Pseudopimelodidae (Abrahão & Shibatta, 2015), the Callichthyidae which was the subject of a Master's dissertation (Pupo, 2011) and the Characiformes studied in a Doctoral thesis (Pereira, 2014), both unpublished at this moment. These Otophysi brain studies have unequivocally shown that the central nervous system can be an important source of phylogenetically informative morphological characters, although relatively unexplored. Bearing that in mind, we have chosen *Brycon orbignyanus*, a member of the putative generalized and phylogenetically basal family Bryconidae (Roberts, 1969; Mirande, 2009, 2010; Oliveira *et al*., 2011), to be described as an example of a generalized Characiformes brain.

Material and Methods

Specimen preparation and brain dissection. The examined specimens belong to the following institution fish collections: LBP (Laboratório de Biologia e Genética de Peixes, Universidade Estadual Paulista "Júlio de Mesquita Filho"); LIRP (Laboratório de Ictiologia de Ribeirão Preto, Universidade de São Paulo); MZUSP (Museu de Zoologia da Universidade de São Paulo); and UNT (Laboratório de Sistemática Ictiológica da Universidade Federal do Tocantins). The complete list of examined specimens is summarized in Table 1.

All specimens examined in the present study were adults to avoid the potentially confusing effects of developmental changes (Huber & Rylander, 1992). Standard length (SL) and head length (HL) were taken point to point with digital calipers on the left side of the specimens. Specimens were stained following the musculature dissection technique proposed by Datovo & Bockmann (2010), which allows a better visualization of cranial bones and its sutures in prepared specimens without any undesirable changes caused to their brains. The brains were then removed from the braincase using a protocol specifically developed and described below for bony fishes with laterally compressed skulls, like the characiforms.

Brain dissection protocol. To extract the brain of characiform fishes, the following dissection procedure was applied on both sides of the specimens heads:

To remove the lateral bones, branchial-basket and eyes, first scrape the epidermal layer (skin) on the opercle, orbital, facial, maxilla, premaxilla and dentary bones - scraping the epidermal layer (skin) and fat (*e.g.* Anostomoidea) of the neurocranium roof allows a better visualization of the cranial sutures. Remove the eyeball and associated musculature (*Musculus rectus superior*, *Musculus rectus externus*, *Musculus rectus inferior*, *Musculus rectus internus*, *Musculus obliquus superior* and *Musculus obliquus inferior*), making a severing incision in the proximal region of eyeball muscles, as well as in the *pedunculi bulbi* (anterior portion of *nervus opticus*). Cut free the maxillary, premaxillary and dentary bones. During the process, remove the anterior portion of the olfactory epithelium, located nearby the maxillary and premaxillary bones.

Remove the epaxial musculature located near the Weberian Apparatus and supraneural bones. Make an incision on the mid-posterior surface portion of the supraoccipital bone, and proceed to the first dorsal-fin ray removing the epaxial musculature until reaching the neural tube.

To completely disjoint and remove the cranial roof, first cut open the mesethmoid sutures with adjacent bones and remove it; then cut lengthwise the soft tissue of the frontal fontanel (the Erythrinidae and Lebiasinidae both have a completely ossified fontanel and thus bone will be cut instead of soft connective tissue); proceed backwards separating the paired parietal and supraoccipital bones, and then remove them totally. At this point the posterior portion of the brain *corpus cerebelii* and *rhombencephalon* should be visible. Cut free the pterotic and sphenotic bones, taking into account that both bones encase the lateral sides of the brain, making their inadequate removal prone to damage the brain. At the end of the aforementioned dissecting steps the brain should be almost completely exposed laterally and dorsally, and intact.

To completely extract the brain from the neurocranium, begin by making a severing incision on the posterior portion of *medulla spinalis*, posterior to the root the *nervus vagus* near and anterior to the vertical passing through the middle of the Weberian Apparatus and posterior to the imaginary line on the ventral surface of the insertion of the complex of the spino-occipitales nerves; the subsequent cuts of the cranial nerves must proceed in the following postero-anteriorly sequence, to avoid breaking inadvertently the anterior cranial nerves: sever the efferent (n. X) of *lobus vagi*; the slim *nervus abducens* (n. VI) on the floor of the neurocranium; nerves from *octavolateralis* area (*nervus trigeminus* – n. V, *nervus facialis* – n. VII, *nervus octavus* – n. VIII, *nervus linea lateralis anterior* – nlla and *nervus linea lateralis posterior* – nllp); the *nervus opticus* (n. II) at the middle portion of the *nervus* passing through the floor of neurocranium where the *chiasma opticum* is located, and finally the *nervus olfactorius* (n. I). When the described procedure is complete, the brain should be completely free from the neurocranium.

Table 1. Material examined in the present study. Asterisk represents type species of genus; SL = Standard length; HL = Head length.

Taxa	Catalog #	Morphometric data				
		Total	Brain	Range (SL)	SL	HL
Acestrorhynchidae						
Acestrorhynchinae						
*Acestrorhynchus falcatus**	LIRP 7639	12	2	(130.1-152.4)	130.24	39.86
Heterocharacinae						
Heterocharax leptogrammus	MZUSP 55725	92	2	(22.7-32.1)	24.80	6.9
Anostomidae						
Schizodon nasutus	LIRP 7151	2	1	(211.8-251.0)	211.80	48.17
Bryconidae						
Bryconinae						
Brycon orbignyanus	LIRP 6309	4	2	(170.8-183.4)	175.25	41.90
Salmininae						
Salminus hilarii	LIRP 7084	2	1	(215.6-237.1)	237.10	65.07
Chalceidae						
Chalceus erythrurus	LIRP 5955	2	1	(175-192)	192.00	50.40
Chalceus guaporensis	LIRP 8625	15	2	(140.1-152.7)	146.37	37.53
Characidae						
Acestrorhamphinae						
Oligosarcus pintoi	LIRP 7615	13	2	(75.6-83.3)	76.4	22.1
Aphyocharacinae						
Aphyocharax dentatus	LIRP 2018	16	2	(27.8-57.0)	45.88	11.68
Aphyoditeinae						
Microschemobrycon callops	LIRP 7544	200	5	(24.4-27.0)	25.86	6.82
***Astyanax* clade**						
Astyanax lacustris	LIRP 3243	7	2	(71.8-96.0)	85.4	20.9
Astyanax jordani	LBP 4586	5	1	(56.5-68.7)	61.0	15.9
Characinae						
Charax leticiae	MZUSP 89106	119	2	(30.8-88.8)	88.11	27.63
*Roeboexodon guyanensis**	MZUSP 94250	-	1	(57.2-51.8)	53.2	15.1
*Exodon paradoxus**	LIRP 7535	174	2	(44.0-52.1)	51.30	14.90
Roeboides descalvadensis	LIRP 7624	11	2	(66.4-73.1)	68.54	17.89
Roeboides myersi	MZUSP 85208	96	1	(66.13-110.29)	76.00	23.10
Roeboides prognathus	MZUSP 6536	10	1	(72.35-89.09)	76.50	19.6
Cheirodontinae						
Serrapinnus notomelas	LIRP 1819	64	2	(20.9-27.1)	26.53	6.37
Pristelinae						
Hemigrammus marginatus	LIRP 4272	56	2	(19.6-27.9)	27.7	6.9
Moenkhausia sanctafilomenae	LIRP 2385	9	2	(37.2-57.3)	37.9	10.0
Rhoadsiinae						
*Rhoadsia altipinna**	LIRP 8157	16	1	(25.0-79.8)	72.83	21.79
Stervadiinae						
Mimagoniates rheocharis	LIRP 6127	12	2	(29.6-51.1)	51.11	11.39
Stethaprioninae						
Gymnocorymbus ternetzi	LIRP 6018	23	2	(27.1-41.7)	40.97	11.03
Tetragonopterinae						
*Tetragonopterus argenteus**	LIRP 5779	10	2	(48.9-57.0)	57.12	17.21
*Probolodus heterostomus**	MZUSP 7904	30	1	(40.2-58.8)	46.6	12.2
Incertae sedis						
*Stygichthys typhlops**	LBP 8107	4	1	-	33.9	11.0
Chilodontidae						
*Caenotropus labyrinthicus**	LIRP 7537	76	2	(54.7-78.3)	70.36	18.71
Crenuchidae						
*Characidium fasciatum**	LIRP 10	24	2	(30.3-54.8)	39.68	9.27

Table 1. (conclusion).

Taxa	Catalog #	Morphometric data				
		Total	Brain	Range (SL)	SL	HL
Ctenoluciidae						
Boulengerella cuvieri	LIRP 7536	5	1	(150.1-266.7)	156.86	50.36
Curimatidae						
Steindachnerina brevipinna	LIRP 7505	56	2	(47.5-92.1)	78.22	19.92
Cynodontidae						
*Cynodon gibbus**	UNT 11753	04	1	(176.2-189.3)	188.10	41.55
Erythrinidae						
*Hoplerythrinus unitaeniatus**	LIRP 750	8	1	(97.1-134.5)	117.33	35.32
Gasteropelecidae						
Carnegiella marthae	LBP 4199	211	2	(20.9-29.7)	28.62	7.61
Hemiodontidae						
Hemiodus sterni	LIRP 7636	9	1	(96.2-135.0)	112.56	25.18
Iguanodectidae						
Iguanodectinae						
Iguanodectes spilurus	MZUSP 109455	100	2	(47.4-53.3)	51.30	10.00
Lebiasinidae						
Pyrrhulininae						
Pyrrhulina australis	LIRP 6049	10	2	(30.9-34.3)	32.91	7.99
Parodontidae						
Apareiodon affinis	LIRP 7613	8	2	(98.8-101.1)	100.03	23.51
Prochilodontidae						
Prochilodus lineatus	LIRP 7321	5	2	(135.6-185.6)	139.27	36.93
Serrasalmidae						
Acnodon normani	UNT 2022	1	1	-	90.00	27.23
*Catoprion mento**	MZUSP 8451	76	1	(29.74-107.45)	66.4	21.1
Serrasalmus maculatus	LIRP 8013	8	1	(80.7-84.2)	82.72	28.29
*Utiaritichthys sennaebragai**	LIRP 8158	10	2	(30.4-114.5)	53.73	14.08
Triportheidae						
Agoniatinae						
*Agoniates halecinus**	UNT 8759	2	1	(193.9-233.4)	193.90	39.62
Triportheinae						
Triportheus nematurus	LIRP 7800	2	1	(79.8-106.8)	79.87	20.65
Citharinidae						
*Citharinus latus**	MZUSP 84480	17	1	(88.2-114.9)	104.70	33.45
Distichodontidae						
*Neolebias unifasciatus**	MZUSP 84476	223	2	(18.6-29.7)	26.6	7.5
Hepsetidae						
*Hepsetus odoe**	MZUSP 84469	6	1	(111.7-144.2)	112.4	38.2
Outgroups						
Cypriniformes						
Cyprinidae						
Cyprininae						
*Cyprinus carpio**	LIRP 8923	2	2	(72.3-99.4)	72.3	23.8
Gymnotiformes						
Gymnotidae						
Gymnotus carapo	LIRP 7767	6	1	(129.0-135.8)	129.0	16.1
Sternopygidae						
*Sternopygus macrurus**	LIRP 4918	8	1	(127.3-115.3)	115.3	13.1
Siluriformes						
Diplomystidae						
Diplomystes mesembrinus	LBP 449	21	1	(71.9-126.8)	72.62	18.4
Pimelodidae						
*Pimelodus maculatus**	LIRP 6012	3	1	(67.4-86.2)	67.4	19.3

Illustration and description. Brain illustrations were made using a pen tablet digital interface and image editing softwares applied to digital photographs made with a stereomicroscope and an attached digital camera. Colors in all illustrations are entirely arbitrary, not corresponding to the real colors of the anatomical structures illustrated. Brain descriptions were based on Meek & Nieuwenhuys (1998) and Striedter (2005), with a single modification: fish brains descriptions usually follow the posteroanterior direction (*e.g.*, Meek & Nieuwenhuys, 1998), but herein we chose to follow Striedter (2005) in adopting the anteroposterior direction, the most commonly used for vertebrates as a whole (see Bauchot *et al.*, 1989; Striedter, 2005; Eastman & Lannoo, 2007, 2008 for fishes; ten Donkelaar, 1998a for amphibians; ten Donkelaar, 1998b for reptiles and Walsh & Milner, 2011 for avians). For illustration and description purposes, the brains were divided into *telencephalon*, *mesencephalon*, *diencephalon*, *rhombencephalon* and *medulla spinalis* (Fig. 1).

The anatomical brain nomenclature follows Meek & Nieuwenhuys (1998) and Striedter (2005); bone nomenclature follows Weitzman (1962), with the modifications proposed by Castro & Vari (2004) and musculature terminology follows Datovo & Vari (2013; 2014).

Results

Brain gross morphology of *Brycon orbignyanus* (Figs. 1 and 2). The brain limits established for *Brycon orbignyanus* and also applied to other Characiformes examined in the present study are: the anterior portions of the *bulbus olfactorius* anteriorly, usually ending at the oval olfactory epithelium and the insertion of the complex of the *spino-occipitales* nerves on the ventral surface of the *medulla spinalis*, posteriorly. The encephalon is slightly elongate and narrow; slightly wider in its middle portion near the *mesencephalon* (*tectum opticus*) and *diencephalon*. The brain occupies the cranial cavity almost entirely, from the region near the mesethmoid and lateral ethmoid to the region anterior to the third neural arch, not contacting the Weberian apparatus.

The *olfactory epithelium* is not considered as part of the *telencephalon* properly. In *Brycon orbignyanus* it is oval with a narrow support rod surrounded by 25 to 30 lamellae similar in size. The most rostral component of the *telencephalon* is the *bulbus olfactorius*. In *B. orbignyanus*, each *nervus tractus olfactorius* is composed of a slender and relatively elongated olfactory peduncle with a terminal expansion. The olfactory bulb is oval and elongate, narrower proximally and enlarged distally. The *nervus tractus olfactorius* is inserted directly on the ventral surface of the *telencephalon*.

The *telencephalon* is divided in two distinct parts: a conspicuous and well-developed *area dorsale* on its dorsal surface, and a small narrow *area ventrale* on its ventral surface, both parts widely and closely interconnected.

The *diencephalon* is well developed, located between the *telencephalon* and *rhombencephalon*, and composed of the following parts: the *epithalamus* and a pineal gland, both of which are inconspicuous and easily lost during dissection and located on the dorsal surface, but not in contact with the *telencephalon* and *corpus cerebelli*; an oval and extremely reduced *saccus vasculosus*; a vertically developed *hypothalamus* that is seen as a slight prominence on the ventral surface of the *diencephalon*, in ventral and lateral views; an oval *lobus inferior hypothalami*, kidney-shaped in ventral view and smaller than the *hypothalamus*; and an oval small *hypophysis* stalked on the *hypothalamus*.

The *thalamus dorsalis* arises from grooves between the *tecta optici* and the *telencephalon*, being moderately developed along their extension and thicker than the olfactory tract. The *chiasma opticum* is located anteriorly to the posterior *telencephalon* margin.

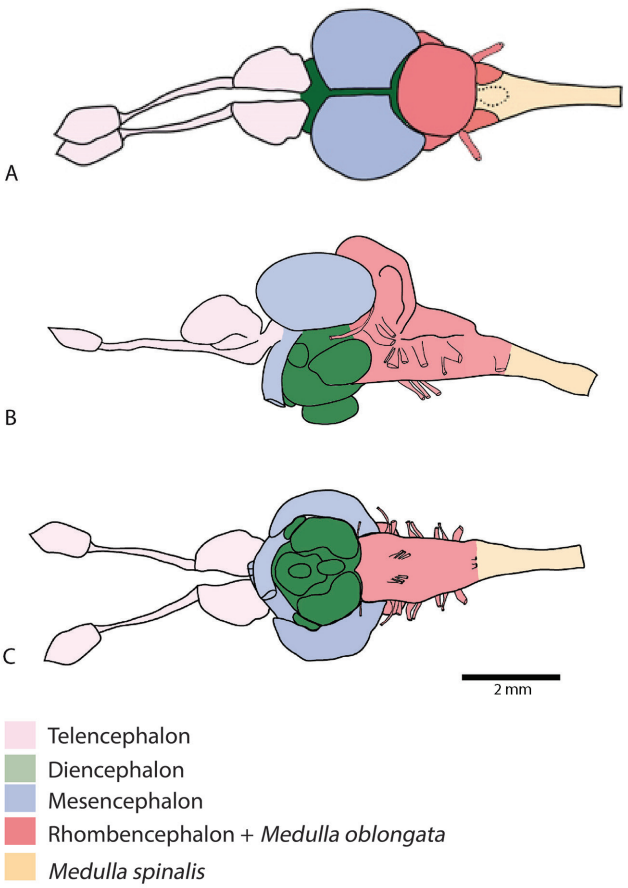

Fig. 1. Brain of *Brycon orbignyanus* (Characiformes: Bryconidae), LIRP 6309, 175.5 mm SL. Main encephalic divisons (*Telencephalon*, *Diencephalon*, *Mesencephalon*, *Rhombencephalon + Medulla oblongata* and *Medulla spinalis*) in different colors. a. dorsal; b. lateral and c. ventral views.

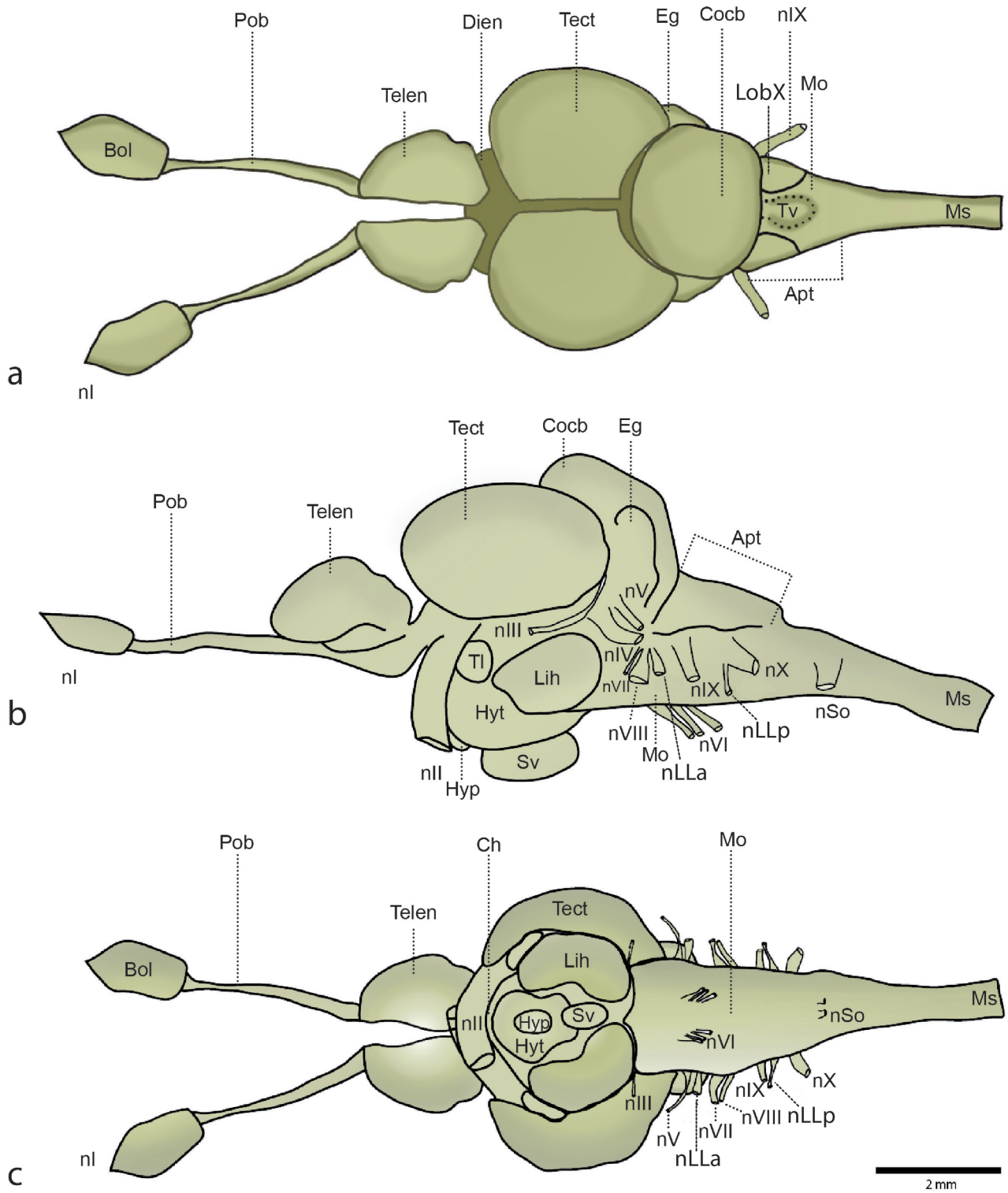

Fig. 2. Brain of *Brycon orbignyanus* (Characiformes: Bryconidae), LIRP 6309, 175.5 mm SL. a. dorsal; b. lateral and c. ventral views. **Apt** = *Area postrema*; **Bol** = *bulbus olfactorius*; **Ch** = *chiasma opticum*; **Cocb** = *corpus cerebelli*; **Dien** = *diencephalon*; **Eg** = *eminentia granularis*; **Hyp** = *hypophysis*; **Hyt** = *hypothalamus*; **Lih** = *lobus inferior hypothalami*; **LobX** = *lobus vagi*; **Mo** = *medulla oblongata*; **Ms** = *medulla spinalis*; **Pob** = *nervus tractus olfactorius*; **Sv** = *saccus vasculosus*; **Tect** = *tectum opticum*;; **Telen** = *Telencephalon* **Tl** = *Torus lateralis*; **Tv** = *tela ventriculi*; **Cranial Nerves**: **nI** = *nervus olfactorius*; **nII** = *nervus opticus*; **nIII** = *nervus oculomotorius*; **nIV** = *nervus trochlearis*; **nV** = *nervus trigeminus*; **nVI** = *nervus abducens*; **nVII** = *nervus fascialis*; **nVIII** = *nervus octavus*; **nLLa** = *nervus lineae lateralis anterior*; **nLLp** = *nervus lineae lateralis posterior*; **nIX** = *nervus glossopharyngeus*; **nX** = *nervus vagus* and; **nSo** = *nervus spino-occipitales*.

The *mesencephalon* is composed of the *tecta optici*, *tegmentum*, *torus longitudinalis*, and the *torus lateralis*. The *tecta optici* are well developed, divided in two symmetrical rounded halves that equal to approximately one-third of the brain length; the *tegmentum* is small and totally covered by the *tectum opticus*, both in dorsal and lateral views and inconspicuous; the *torus longitudinalis* is totally inconspicuous (visible only in stained histological sections) located between the *tecta optici*, near the *telencephalon* complex; the *torus lateralis* is reduced and located in front of the *lobus inferior hypothalami* and the anterior portion of *tectum opticum*, with undefined limits, both in lateral and ventral views.

The *rhombencephalon* comprises the associated cerebellar complex and medullary areas; the *crista cerebellaris* region is almost inconspicuous, being just a slight prominence, when visible; the *eminentia granularis* is connected exclusively to the medial region of the *corpus cerebelli*; the *corpus cerebelli* is spherical and smaller than the *tectum opticum*, being the dorsalmost structure of the brain; the *lobus vagi* is moderately developed and shaped like lateral wings attached to the basis of the *corpus cerebelli*; the *lobus facialis* is inconspicuous and of very difficult visualization.

The *medulla spinalis* is cylindrical throughout its length, except for its anterior portion, near the *corpus cerebelli*, where it is flattened and cone-shaped, in dorsal view; the *area postrema,* usually is approximately trapeze-shaped in dorsal view, but its precise delimitation is possible only in stained histological preparations; the *ventriculi quarti* region is a semicircular area in dorsal view, with a slight concavity that abuts the *corpus cerebelli* posterior margin.

The cranial nerves are: rostrally, the *nervus olfactorius* (nI), ending in the *bulbus olfactorius*; the moderately thick *nervus opticus* (nII), ending in the eyeball; the *nervus oculomotorius* (nIII), arising from the base of the midbrain *crista cerebellaris* region; followed by the moderately thick *nervus trochlearis* (nIV) innervating the eye extrinsic muscle (Meek & Nieuwenhuys, 1998); and the posteriormost *nervus trigeminus* (nV), extremely slender, with ramifications not amenable to observation by us. Also in the midbrain section, arising laterally from the base of the *rhombencephalon*, are the anteriormost *nervus fascialis* (nVII), followed by the *nervus octavus* (nVIII), *nervus linea lateralis anterior* (nlla), *nervus glossopharyngeus* (nIX), *nervus linea lateralis posterior* (nllp), and the posteriormost *nervus vagus* (nX). Also arising from the *rhombencephalon*, although from its ventral surface, is the *nervus abducens* (nVI) (Meek & Nieuwenhuys, 1998). The posteriormost nerve is the *nervus spino-occipitalis*, arising from the medullary base, wide at its origin and progressively narrow distally. It is probably not a real cranial nerve, but a ganglion located between the ear and the eye, being the anteriormost part of a prominent line of neuromasts that extends from head to tail (Ghysen & Dambly-Chaudière, 2004).

Gross brain morphology in other characiforms. Some features related to the forebrain (*telencephalon* and *diencephalon*) *rhombencephalon* (*corpus cerebelli*) and the *tecta optici* are conserved in the examined taxa, thus allowing us to compare and perceive their variation within 48 taxa of Characiformes and five other non-characiform otophysan taxa herein studied (Table 1). The following similar areas of the gross brain morphologies across all the examined taxa have been considered as clearly perceived and homologous areas: 1) presence or absence of the *lobus facialis*; 2) degree of development of the *lobus vagi*, *corpus cerebelli* and *tecta optici*; 3) width of *rhombencephalon*; and 4) external morphology of *area postrema* (see Discussion).

The *telencephalon* (*pallium*) is the most variable brain area in terms of size and shape, as observed in several other actinopterygian taxa (see Northcutt, 1981), due to the formation of a highly differentiated superficial layer of gray matter after the eversion of *pallium* (Northcutt, 1981), as we have also observed both in Characiformes and outgroup taxa, making almost impossible to identify a generalized morphological pattern for the area. The *corpus cerebelli* together with the *telencephalon* are the most variable parts of the actinopterygian brains regarding their size and shape (Nieuwenhuys, 1982) and present a unique additional structure known as *eminentia granularis* in teleosteans (Nieuwenhuys, 1967), as observed in characiforms.

On the other hand, in Characiformes the *rhombencephalon* is moderately developed, (*i.e.* length and height) with a modest *corpus cerebelli*, different from the *rhombencephalon* observed by us in the Cypriniformes, Siluriformes and Gymnotiformes taxa, which present comparatively larger *corpus cerebelli*.

The *area postrema* region on the dorsal surface of *rhombencephalon* presents a unique shape in all the characiform taxa examined, usually with a slight depression, quite different from its shape in the comparative taxa of Cypriniformes, Siluriformes and Gymnotiformes examined (Figs. 2-5).

In the present study, we have observed a considerable diversity in the *corpus cerebelli* of the Characiformes, always much reduced when compared to the *corpus cerebelli* of the examined Gymnotiformes and Siluriformes.

The *tecta optici* form the roof of the midbrain in teleostean fishes and are considered the main visual center in fishes (Northmore, 2011). In the examined taxa, it showed an enormous variation of its relative size, probably related to the relative importance of vision in the various taxa. Nevertheless, several authors affirmed that, in vertebrates, the relative size of parts of vision apparatus is totally correlated to size of the image on retina and visual information reaching the brain (Garamszegi *et al*., 2002; Howland *et al*., 2004). According to our results, Characiformes and Cypriniformes usually present normally developed and similar optic structures, differing mostly in size, while the examined Siluriformes and Gymnotiformes taxa possess much reduced optic structures.

The *lobus vagi* is inconspicuous or almost absent in almost all Characiformes examined. When visible, the *lobus vagi* is shaped as small lateral wings emerging from the *rhombencephalon* base, as in *Brycon orbygnianus* (Figs. 1 and 2), except in the Chilodontidae which have a large *lobus vagi*, very similar in shape and size to the one found in our representative taxon of the Cypriniformes.

Remarkably the *lobus facialis* in the Characiformes is barely observable, clearly different from the conspicuous structure found in all the Cypriniformes, Siluriformes and Gymnotiformes examined.

In addition, we also propose herein two apparently exclusive brain features of the Siluriformes among all the examined taxa, but we consider that more studies must be done to establish their real phylogenetic signals, increasing the number of taxa representing this order (Figs. 1-3 and 5): (1) olfactory rosette elongate and well developed (Fig. 6c) *vs*. the olfactory rosette approximately circular and moderately developed in Characiformes, Cypriniformes and Gymnotiformes (Fig. 6a, b and d); and (2) 60 olfactory lamellae present *vs*. a comparatively reduced number of lamellae, not surpassing 30, in the representatives of the Cypriniformes (16 lamellae), Characiformes (25-30 lamellae), and Gymnotiformes (15 lamellae) (Fig. 6a-d). Taking into account the well-known fact that the Cypriniformes and Characiformes are usually primarily diurnal and visually oriented, whereas the Siluriformes and Gymnotiformes are primarily nocturnal and/or inhabitants of very turbid waters, being oriented mainly by the senses of smell and tact in the case of siluriforms (see Caprio, 1978), and almost exclusively by electroreception, in the case of gymnotiforms (Albert *et al*., 1998; Albert, 2001), it is to be expected the presence of more complex and developed olfactory rosettes in the Siluriformes.

Discussion

As pointed by Striedter (2005), even homologous brain regions differ in size, shape, position, cytoarchitecture, histochemistry, connections, and/or function across the major vertebrates groups. Striedter (2005), nevertheless, also pointed that the forebrain, *corpus cerebelli* and *tectum opticum* are conserved homologous regions of vertebrate brains. Thus, it is not unexpected that we have found the same unequivocally identifiable and conserved homologous regions in the brains of representatives of the 22 families of Characiformes analyzed in this study.

Due to the taxonomic diversity and ecological plasticity of characiform fishes, combined with our aim to make available a first description of the external brain morphology of a characiform, we chose a representative of this order possessing putatively generalized and ancestral-like external brain morphology, since the external brain form of *Brycon* probably resembles that of the ancestral of all characiforms. Taking these considerations together with the availability of specimens for the inevitable and destructive brain removals, we have opted to use specimens of *Brycon*. Among the 48 taxa of Characiformes examined by us (Table 1), *B. orbignyanus* possess the external brain morphology more similar to the representative of the Cypriniformes, the sister order of all remaining Otophysi, especially regarding their respective olfactory bulbs, *telencephalon* and *corpus cerebelli* (Figs. 1-3). Also, in many significant past papers the genus *Brycon* was considered "primitive", "generalized" or phylogenetically basal in the Neotropical Characiformes, or at least in relation to the Characidae, sharing several osteological features with basal African groups (see Weitzman, 1962; Buckup, 1998; Mirande, 2009, 2010; Malabarba & Weitzman, 2003 and Oliveira *et al*., 2011).

The use of *B. orbignyanus* external brain morphology as a surrogate of the hypothetical ancestral external brain morphology of the Characiformes should be done warily, since ours is a preliminary analysis encompassing just part of the many known taxa of Characiformes. Notwithstanding the presence of conserved homologous regions in the brains of all taxa of Characiformes, variation found in the characiform brain gross morphology allows us to consider their brain as a rich source of phylogenetically useful characters.

Bearing that in mind, we herein propose six putative synapomorphic brain features for the Characiformes, not found in the examined Cypriniformes, Siluriformes and Gymnotiformes (Figs. 3-5, respectively): (1) *area postrema* shaped as an inverted triangle, wider anteriorly and narrowing posteriorly (Figs. 1-2) *vs*. *area postrema* equally narrow throughout its full length, with inconspicuous limits in the Cypriniformes, Siluriformes and Gymnotiformes (Figs. 3-5, respectively); (2) width of the *rhombencephalon* not exceeding the width of the midbrain, both in dorsal and ventral views (Figs. 1-2) *vs*. *rhombencephalon* wider than midbrain due to the larger size of the *corpus cerebelli* in the Cypriniformes, Siluriformes and Gymnotiformes (Figs. 3-5, respectively); (3) *lobus vagi* less developed (except in the Chilodontidae) (Figs. 1-2) *vs*. *lobus vagi* well-developed in the Cypriniformes, Siluriformes and Gymnotiformes (Figs. 3-5, respectively); (4) *lobus facialis* inconspicuous; when visible, a small oval structure attached to the basis of the *corpus cerebellaris* (Figs. 1-2) *vs*. *lobus facialis* well-developed and visible in Cypriniformes and Siluriformes (Figs. 3-4, respectively), and developed but completely hidden by the uniquely large *corpus cerebellaris* in the Gymnotiformes (Fig. 5); (5) *corpus cerebelli* rounded, and elongate vertically (Figs. 1-2) *vs*. *corpus cerebelli* horizontally elongate in the Siluriformes and Gymnotiformes (Figs. 4 and 5, respectively) and moderately developed and dorsally pointed in the Cypriniformes (Fig. 3); (6) *tectum opticum* horizontally elongate, in contact with the anterior margin of the *corpus cerebelli* except in *Stygichthys typhlops* (a blind troglobitic species) (Figs. 1-2) *vs*. *tectum opticum* in the Cypriniformes vertically elongate and not in contact with the anterior margin of the *corpus cerebelli*, and relatively reduced in Siluriformes and Gymnotiformes (Figs. 3-5).

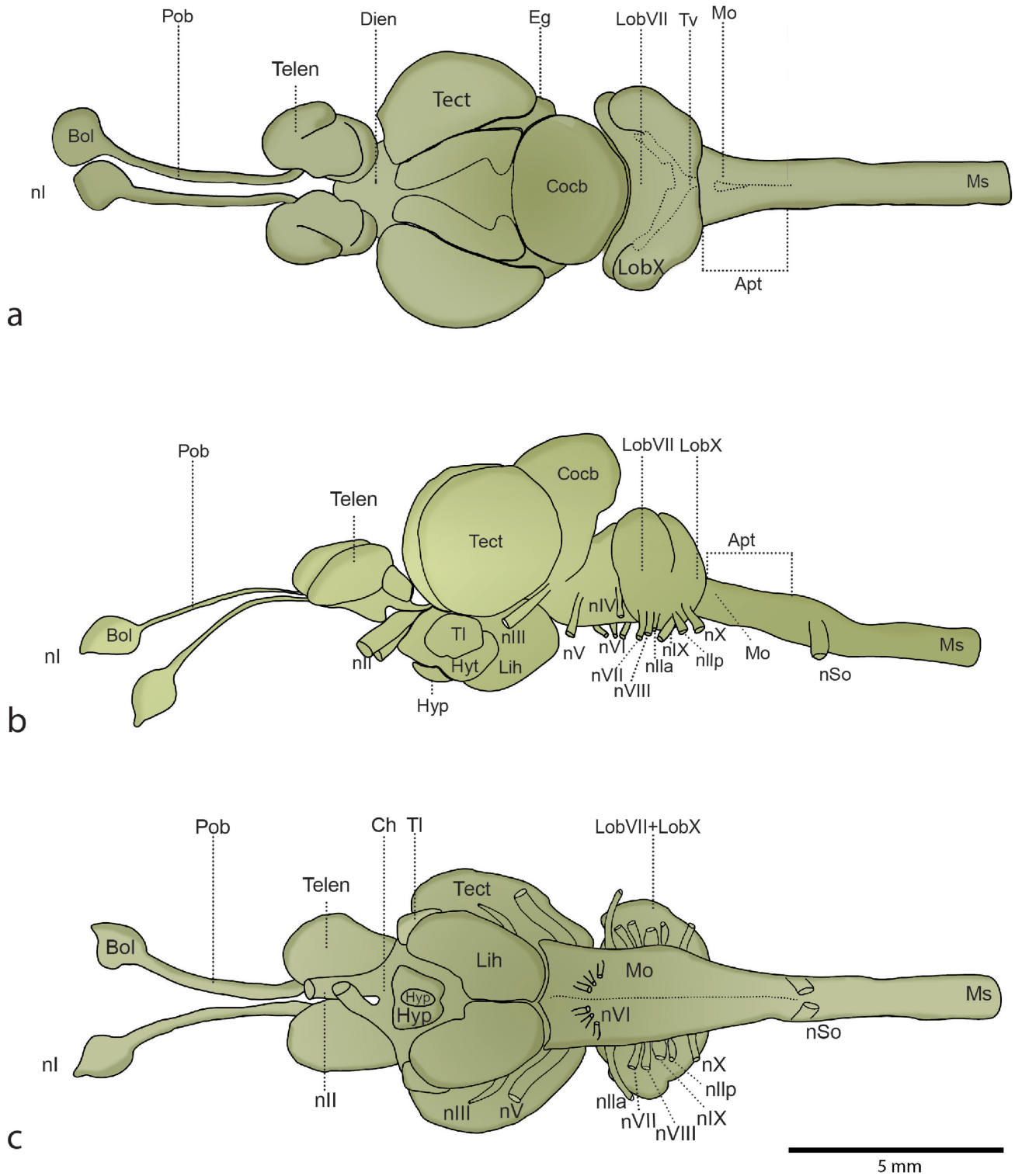

Fig. 3. Brain of *Cyprinus carpio* (Cypriniformes: Cyprinidae), LIRP 8923, 72.3 mm SL. a. dorsal; b. lateral and c. ventral views. **Apt** = *Area postrema*; **Bol** = *bulbus olfactorius*; **Ch** = *chiasma opticum*; **Cocb** = *corpus cerebelli*; **Dien** = *diencephalon*; **Eg** = *eminentia granularis*; **Hyp** = *hypophysis*; **Hyt** = *hypothalamus*; **Lih** = *lobus inferior hypothalami*; **LobVII** = *lobus facialis*; **LobX** = *lobus vagi*; **Mo** = *medulla oblongata*; **Ms** = *medulla spinalis*; **Pob** = *nervus tractus olfactorius*; **Tect** = *tectum opticum*; **Telen** = *Telencephalon*; **Tl** = *Torus lateralis*; **Tv** = *tela ventriculi*. **Cranial Nerves**: **nI** = *nervus olfactorius*; **nII** = *nervus opticus*; **nIII** = *nervus oculomotorius*; **nIV** = *nervus trochlearis*; **nV** = *nervus trigeminus*; **nVI** = *nervus abducens*; **nVII** = *nervus fascialis*; **nVIII** = *nervus octavus*; **nIIa** = *nervus lineae lateralis anterior*; **nIIp** = *nervus lineae lateralis posterior*; **nIX** = *nervus glossopharyngeus*; **nX** = *nervus vagus* and; **nSo** = *nervus spino-occipitales*.

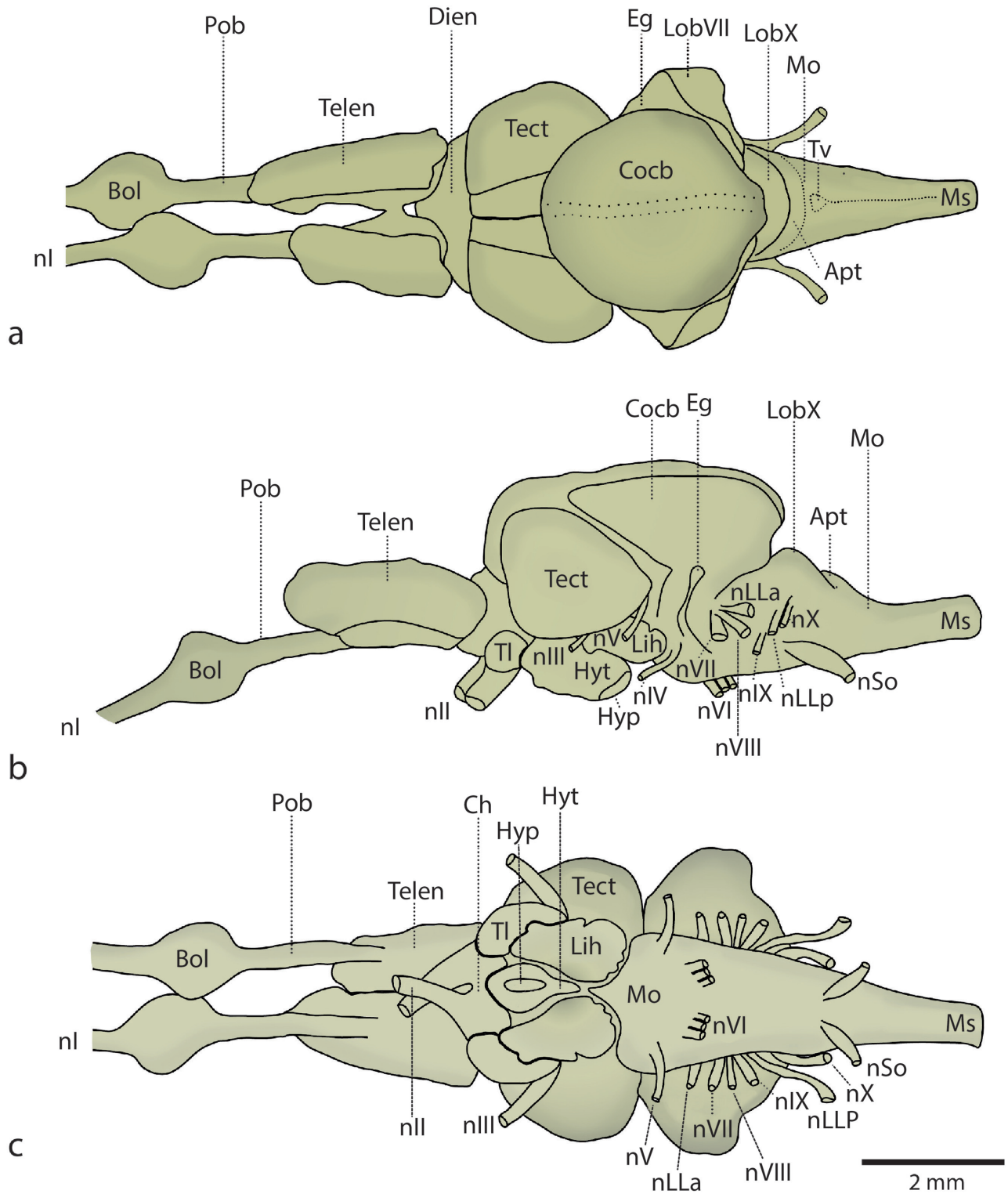

Fig. 4. Brain of *Diplomystes mesembrinus* (Siluriformes: Diplomystidae), LBP 449, 72.62 mm SL. a. dorsal; b. lateral and c. ventral views. **Apt** = *Area postrema*; **Bol** = *bulbus olfactorius*; **Ch** = *chiasma opticum*; **Cocb** = *corpus cerebelli*; **Dien** = *diencephalon*; **Eg** = *eminentia granularis*; **Hyp** = *hypophysis*; **Hyt** = *hypothalamus*; **Lih** = *lobus inferior hypothalami*; **LobVII** = *lobus facialis*; **LobX** = *lobus vagi*; **Mo** = *medulla oblongata*; **Ms** = *medulla spinalis*; **Pob** = *nervus tractus olfactorius*; **Tect** = *tectum opticum*; **Telen** = *Telencephalon*; **Tl** = *Torus lateralis*; **Tv** = *tela ventriculi*. **Cranial Nerves**: **nI** = *nervus olfactorius*; **nII** = *nervus opticus*; **nIII** = *nervus oculomotorius*; **nIV** = *nervus trochlearis*; **nV** = *nervus trigeminus*; **nVI** = *nervus abducens*; **nVII** = *nervus fascialis*; **nVIII** = *nervus octavus*; **nlla** = *nervus lineae lateralis anterior*; **nllp** = *nervus lineae lateralis posterior*; **nIX** = *nervus glossopharyngeus*; **nX** = *nervus vagus* and; **nSo** = *nervus spino-occipitales*.

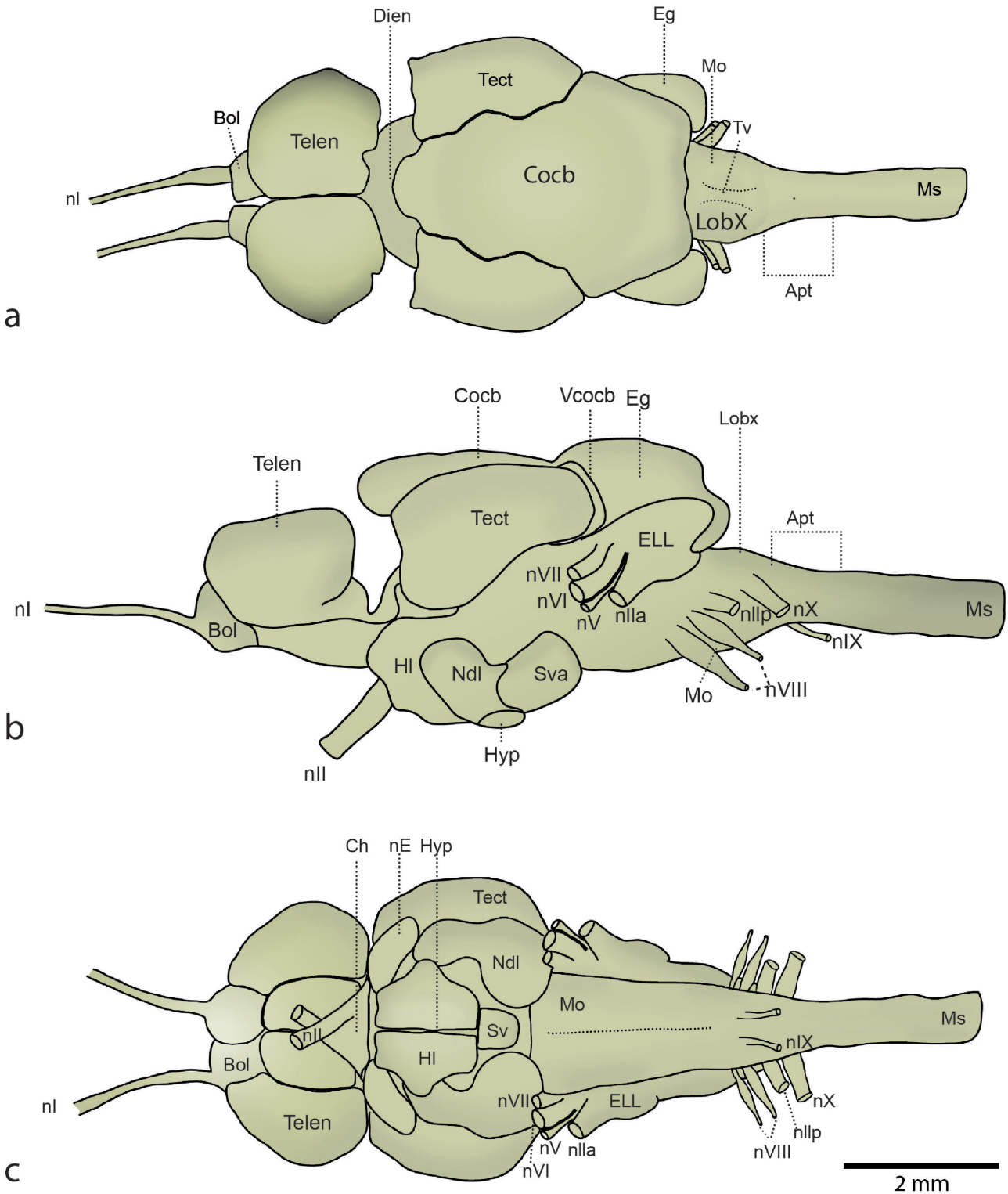

Fig. 5. Brain of *Gymnotus carapo* (Gymnotiformes: Gymnotidae), LIRP 7767, 129.0 mm SL. a. dorsal; b. lateral and c. ventral views. **Apt** = *Area postrema*; **Bol** = *bulbus olfactorius*; **Ch** = *chiasma opticum*; **Cocb** = *corpus cerebelli*; **Dien** = *diencephalon*; **Eg** = *eminentia granularis*; **ELL** = electrosensory lateral line lobus; **Hl** = *lateral nucleus* of *hypotalhamus*; **Hyp** = *hypophysis*; **LobX** = *lobus vagi*; **Mo** = *medulla oblongata*; **Ms** = *medulla spinalis*; **ndl** = lateral portion of *nucleus diffusus*; **nE** = *nucleus electrosensorius*; **Sv** = *saccus vasculosus*; **Tect** = *tectum opticum*; **Tv** = *tela ventriculi*; **Vcocb** = valvula cerebellum. **Cranial Nerves**: **nI** = *nervus olfactorius*; **nII** = *nervus opticus*; **nV** = *nervus trigeminus*; **nVI** = *nervus abducens*; **nVII** = *nervus fascialis*; **nVIII** = *nervus octavus*; **nIIa** = *nervus lineae lateralis anterior*; **nIIp** = *nervus lineae lateralis posterior*; **nIX** = *nervus glossopharyngeus*; **nX** = *nervus vagus*.

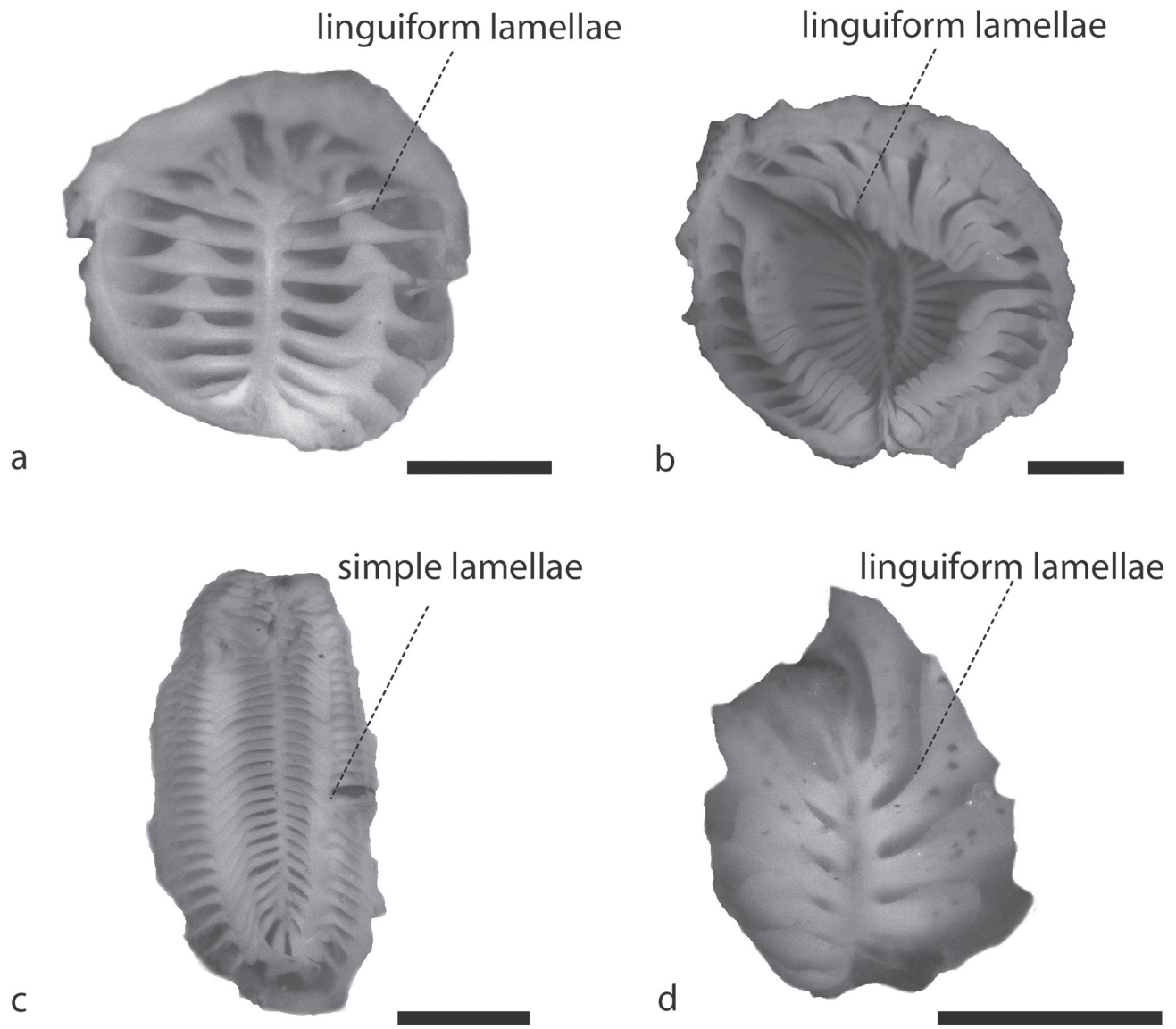

Fig. 6. Olfactory epithelium of representatives of Otophysi. a. *Cyprinus carpio* (Cypriniformes); b. *Brycon orbignyanus* (Characiformes); c. *Pimelodus maculatus* (Siluriformes) and; *Sternopygus macrurus* (Gymnotiformes). Scale bars = 1 mm.

Conclusions

The initial premise in our comparative study of the brain gross morphology of the Characiformes reflected the commonly held belief that the Central Nervous System in fishes was highly conserved throughout their evolution (*e.g.*, Northcutt, 1984, 2002; Butler & Hodos, 2005). That was not what we found in our study, where the form of the brains has shown surprisingly variation among the examined taxa of Characiformes, having provided six phylogenetically useful characters. Thus, the CNS morphology in the Characiformes, as most probably also in other otophysan orders, is unquestionably worth exploring, as was the case for other non-osteological sources for morphological information (*e.g.*, Datovo & Castro, 2012; Datovo & Vari, 2014).

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