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Macro- and microscopic brain anatomy of the amazon lava lizard (*Tropidurus torquatus*) (WIED, 1820)

Anatomia macro- e microscópica do encéfalo do calango (***Tropidurus torquatus***) (WIED, 1820)

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Abstract
Reptiles have a key role in understanding amniotes' reproductive independence of water. Many adaptations arose, including in locomotor patterns and behaviours, and the nervous system adapted to those new habits. We have described the macroscopic anatomy and cytoarchitecture of the Amazon Lava Lizard brain (*Tropidurus torquatus*), an abundant lizard in South America. Fifteen specimens were captured, euthanized and their brains were dissected, eight of these were processed and stained in haematoxylin-eosin. Their main areas of the brain are the telencephalon and diencephalon, in the forebrain, tectum and tegmentum, in the midbrain and bulbota and cerebellum, in the hindbrain. The main and accessory olfactory bulbs are the most rostral structure of the brain and are composed of six layers. Brain hemispheres compose the telencephalon and are divided in pallium and subpallium. Medial, dorsomedial, lateral and dorsal cortices are part of the pallium. Striatum, pallidum and septum compose the subpallium. The diencephalon is composed of thalamus, epithalamus and hypothalamus. The midbrain has a ventral tegmentum, composed of torus semicircularis and a dorsal 14 layered optic tectum. Most part of the hindbrain is composed of the bulbo, and the cerebellum arises from it, forming a three-layered plate like structure. In general, the brain of *Tropidurus torquatus* resembles those of other lizards, with its own adaptations.
Keywords: histology; lizard; morphology; reptile; nervous system.

Resumo
Os répteis têm um papel fundamental para a compreensão da independência reprodutiva da água que surgiu nos amniotas. Várias adaptações ocorreram, inclusive em padrões e comportamentos locomotores, e o sistema nervoso se adaptou a esses novos hábitos. Descrevemos a anatomia macroscópica e a citoarquitetura do encéfalo do calango (*Tropidurus torquatus*), um lagarto abundante na América do Sul. Quinze espécimes foram capturados, eutanasiados e seus encéfalos dissecados, oito destes foram processados e corados em hematoxilina-eosina. As principais áreas do cérebro são o telencéfalo e o diencéfalo, na parte anterior do encéfalo, teto e tegmento, no mesencéfalo e bulbo e cerebelo, na parte posterior do encéfalo. Os bulbos olfatórios principais e acessórios são as estruturas mais rostrais do cérebro e são compostos por seis camadas. Os hemisférios cerebrais compõem o telencéfalo e são divididos em pálio e subpálio. Os córtices medial, dorsomedial, lateral e dorsal fazem parte do pálio. Estriado, pálido e septo compõem o subpálio. O diencéfalo é composto pelo tálamo, epitálamo e hipotálamo. O mesencéfalo possui um tegmento ventral, composto de torus semicircularis e um tecto óptico dorsal com 14 camadas. A maior parte da parte posterior do encéfalo é composta pelo bulbo, e o cerebelo surge como uma projeção dessa estrutura, em formato plano, com três camadas. Em geral, o encéfalo de *Tropidurus torquatus* se assemelha ao de outros lagartos, com suas próprias adaptações.
Palavras-chave: histologia; lagarto; morfologia; réptil; sistema nervoso.

1. Introduction

Reptiles' embryos present the amniotic membrane, an adaptation that arose with water independence in reproduction. But this feature is not the only one associated with the transition from water to land that happened in tetrapods. Adaptations to the head to accommodate the differences in feeding, hearing and other behaviours and specialized limbs to support the body off the ground were some of the changes that happened, with equivalent changes in the nervous system(1).

Survival and reproduction of organisms in the environment in which they live is important to a species' success and the nervous system coordinate activities towards these goals. Mammalian and reptile brain share ancestry and a number of functional attributes and since the reptile brain is simpler, it may provide invaluable help in deciphering modern neuroscience questions(2).

Lizards have been identified as model organisms for various types of studies due to their easy observation, capture and handling. One of these species, the amazon lava lizard (*Tropidurus torquatus*) (Wied, 1820) has been explored in several studies, including temperature(3), diet(4), reproduction(5) and embryonic development studies(6). Its specimens are extremely abundant, being distributed from Brazil to Argentina,

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with a seasonal reproductive cycle in the rainy season. The species is diurnal and preferentially inhabits open environments, feeding on invertebrates, flowers and fruits(3, 5).

We aimed to describe the macroscopic anatomic and cytoarchitecture of the brain of *T. torquatus*, highlighting its main regions and structures.

2. Materials and methods

2.1 .Macroscopic analysis

This research is part of supported by the Biodiversity Authorization and Information System of Brazil (Sistema de Autorização e Informação em Biodiversidade - SISBIO), protocol number - SISBIO 61909-1, and by ethics committee of the Federal University of Goiás (Universidade Federal de Goiás - UFG / Regional Jataí), protocol number - CEUA 013/18, both of which permitted the collection, transportation and care of the animals.

Fifteen juvenile and adult specimens of *T. torquatus* were used. Animals of both sexes were collected with a noose at the Universidade Federal de Goiás - Regional Jataí. They were euthanized with an intraperitoneal lethal dose of bupivacaine hydrochloride (100 mg/kg) and dissected with the help of tweezers, scissors and a dissecting microscope(7). The skin of the head was removed, followed by removal of eyes and muscles around the brain. Next the bones protecting the brain and the dura mater were extracted, exposing the structure. Finally, the brain was carefully removed from the remaining brain case and severed at the spinal cord and fixed in 10% formalin for 2 weeks. The terminology for the areas and structures was used according with Wright et al.(8) and Naumann et al.(2) for the reptile brain, and the Nomina Anatomica Veterinarian(9).

2.2. Histologic analysis

Eight brains were stained with haematoxylin-eosin (HE). The material was dehydrated in a series of alcohol 100% (5 baths, 50 min each), followed by submersion in xylol (2 baths / 50 min each) and paraffin inclusion (3 baths / 50 min each). The brains were included in three sectioning planes (sagittal, frontal and transversal) and then sectioned with microtome at 5 µm. For the staining protocol the paraffin was melted in an incubator (1 hour) and the remaining paraffin was removed with xylene (2 baths, 20 min each). Then it was passed through a series of alcohol solutions (100, 90, 70 and 50%, 5 min each) and bathed in distilled water (10 min) before the haematoxylin staining (5 min). Next it was submitted to running tap water (10 min) and counter-stained with eosin (4 min). It was dehydrated through alcohol 70% (5 quick immersions), alcohol 80% (1 min), alcohol 90% (2 min), alcohol 100% (5 min) and finally submersed in xylene (2 baths, 5 min each) and mounted with entellan.

2.3. Image capturing and processing

The macroscopic external morphology and topography of the brain were described and documented with a dissecting microscope (Leica ICC50 HD®). Histological images were analysed and photographed using an optic microscope (LEICA DM750®) with an embedded camera (LEICA ICC50 HD®), with objective lens 4x (0.10), 10x (0.22), 20x (0.40) and 40x (0.65). After capture, macro- and microscopic images were processed using the software Affinity Photo® v1.5.2.69 to merge continuous pictures of the same region, Adobe Photoshop CS6® v13.0 for background processing and adjusting tone and light and CorelDRAW X7® v17.1.0.572 to assemble images and point structures.

3.Results and discussion

The brain of *T. torquatus* was smooth and it extended from the medulla oblongata to the olfactory bulbs. It was limited caudally from the spinal cord by the foramen magnum and rostrally by olfactory capsules (Fig. 1A). The olfactory bulbs were located rostrally to the eyes, being connected to the brain by the olfactory peduncles. It was composed of forebrain (telencephalon and diencephalon), midbrain (tectum and tegmentum) and hindbrain (medulla oblongata and cerebellum) (Fig. 1B, 2, 5-7). These divisions are found in reptiles in general, though each species presents their own adaptations, as will be presented below(2, 10, 11).

A system of ventricles was associated with every region of the brain, and its ependyma formed an inner-most layer in all regions containing ventricles (Fig. 3, 4). The choroid plexus was located inside the ventricles (Fig. 4D). Only two meninges were present covering the brain, pia and dura mater, and they were closely associated with the ventricular system.

3.1. Olfactory bulbs

Olfactory nerves entered the ventromedial surface of the main and accessory olfactory bulbs of *T. torquatus*, coming from the nasal capsule and vomeronasal organ, respectively, which is a feature present in lizards(8, 12-14). In general, the olfactory bulbs are described as small and oval or pear-shaped, as was observed in *T. torquatus* and also described previously in *Anolis garmani, Anolis grahami, Anolis lineatopus. Chameleon Vulgaris, Tupinambis teguixin* (= *Tupinambis nigropunctatus*) and *Salvator merianae* (= *Tupinambis teguixin*)(11, 12, 15-17). They were more triangular-shaped in *Iguana iguana iguana*(13).

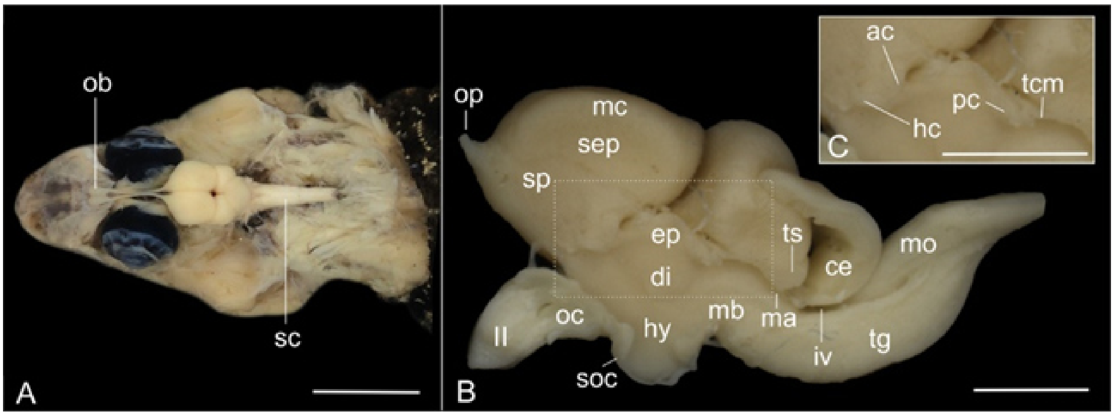

Figure 1. A – Topography of the brain of *Tropidurus torquatus*. B – Macroscopic anatomy of the brain of *Tropidurus torquatus*. C – detail of picture B. A – dorsal view; B, C - medial view. INDEX of structures: ac – anterior commissure; ce – cerebellum; di – diencephalon; ep – epithalamus; hc – hippocampal commissure; hy – hypothalamus; II – optic nerve; iv – fourth ventricle; ma – mesencephalic aqueduct; mb – midbrain; mc – medial cortex; mo – medulla oblongata; ob – olfactory bulb; oc – optic chiasm; op – olfactory penducle; pc – posterior commissure; sc – spinal cord; sep – septum; soc – supraoptical commissure; sp – subpallium; tcm – tectal commissure; tg – tegmentum; ts – torus semicircularis. Scale Bar (A): 2 cm; Scale Bar (B, C): 5 mm.

Main (rostral) and accessory (caudomedial) olfactory bulbs comprised the olfactory bulbs in *T. torquatus*, as was seen in *A. garmani, A. grahami, A. lineatopus, Gekko gecko, I. iguana iguana* and *Podarcis hispanica*(13-14, 18). The accessory olfactory bulbs in *G. gecko* were thinner and more distinct from the main bulbs than in *T. torquatus*, as well in *A. garmani, A. grahami, A. lineatopus, Gekko gecko, I. iguana iguana* and *Podarcis hispanica*(18).

In *T. torquatus*, the olfactory fibers were directed to the brain hemispheres through thin, long and cylindrical olfactory peduncles, which entered the brain hemispheres near their rostral ends, with some of its fibers being directed to the olfactory tubercles, as described in other lizards (*A. garmani, A. grahami, A. lineatopus, C. Vulgaris, G. gecko, I. iguana iguana,* P. hispanica and *T. teguixin*)(12-18).

In *T. torquatus,* the olfactory peduncles became thicker as they reached the brain hemispheres (Fig. 2), which was also observed in *G. gecko, I. iguana iguana* and *T. teguixin*(13, 15, 18) and this structure appeared to be significantly thinner in *A. garmani*(12). Olfactory ventricles were present and connected to lateral ventricles in *T. torquatus*, as reported in *G gecko* and *I. iguana iguana*(13, 18). While there is no information for most species, Shanklin(17) noted the lack of an olfactory ventricle in *C. Vulgaris*.

Microscopically in *T. torquatus*, both olfactory bulbs presented six layers: olfactory nerve fibers, glomerular, external plexiform, mitral, internal plexiform and granular layers. In the main bulb the layers were located concentrically around the ventricle, while in the accessory one it was mainly on the medial wall because the ventricle was located laterally (Fig. 3). This is similar to *A. garmani, A. grahami, A. lineatopus* and *P. hispanica*(12, 14) and distinct from the findings in *I. iguana iguana*, for which only three cell layers were described: external granular, mitral and internal granular layers(13) and in *C. Vulgaris*, where the cells are very little differentiated and appear to be more like granular cells, without distinct layers(17). This divergence could be due to different staining methods, which may not have detailed the cytoarchitecture of the olfactory bulb in these species.

3.2. Telencephalon

Macroscopically, the brain hemispheres of *T. torquatus* presented cordiform shape (as in *A. garmani, Ctenophorus decresii, G. gecko, I. iguana iguana, P. hispanica, T. teguixin* and *S. merianae*) and were visually larger than the oval shaped optic tectum. Brain hemispheres in reptiles are composed of a superficial pallium and a subpallium(11-14, 16, 18-20). The pallium component of *T. torquatus* was comprised of medial, dorsomedial, lateral and dorsal cortex, and the ventrolateral dorsal ventricular ridge. The dorsomedial cortex was continuous with the medial cortex, but its cells were larger and less densely packed.

Three layers are presented in the cortices of *T. torquatus*, external and internal plexiform layers with scarce cells and an organized cell layer in between. This disposition was also reported in *A. garmani, A. grahami, A. lineatopus, C. Vulgaris, C. decresii, G. gecko, I. iguana iguana, T. teguixin, S. merianae*(8, 12, 13, 16-23). The dorsal cortex of *T. torquatus* presented a less organized cell layer and it was partially overlapped by the dorsomedial and lateral cortices (Fig. 4) whereas in *C. decresii,* the lateral cortex presented the most indistinct plexiform layer (19). In *G. gecko* and *T. teguixin,* the internal plexiform layer was described as a subcortical layer of scattered cells and a fiber layer(18, 23), we did not notice any distinct fiber layer in *T. torquatus.*

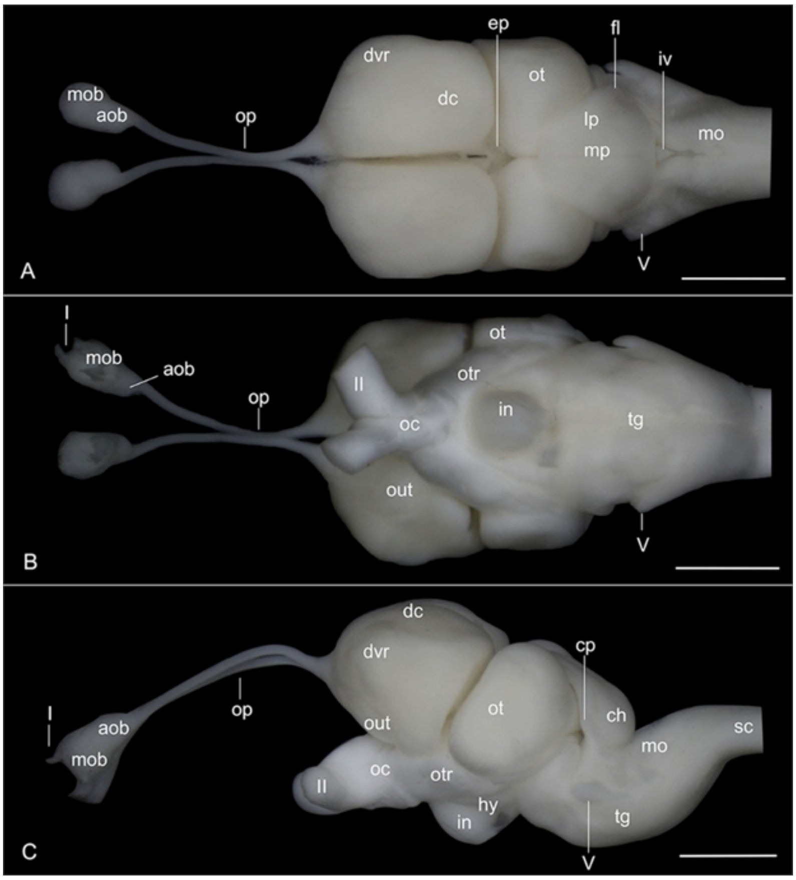

Figure 2. Macroscopic anatomy of the brain of *Tropidurus torquatus*. A – dorsal view; B – ventral view; C – left lateral view. INDEX of structures: aob – acessory olfactory bulb; ch – cerebellar hemisphere; cp – cerebellar penducle; dc – dorsal cortex; dvr – dorsal ventricular ridge; ep – epithalamus; fl – flocculus; hy – hypothalamus; I – olfactory nerve; II – optic nerve; in – infundibulum; iv – fourth ventricle; lp – lateral part of cerebellar hemisphere; mo – medulla oblongata; mob – main olfactory bulb; mp – median part of cerebelar hemisphere; oc – optic chiasm; op – olfactory penducle; ot – optic tectum; otr – optic tractum; out – olfactory tubercle; sc – spinal cord; tg – tegmentum; V – trigeminal nerve. Scale Bar: 5 mm.

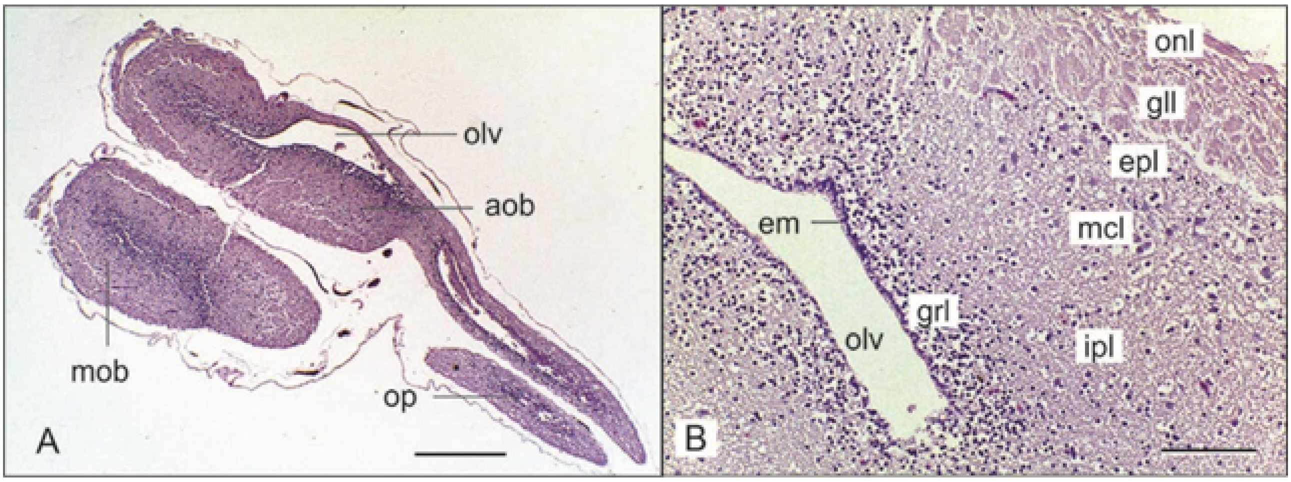

Figure 3. Olfactory bulb of *Tropidurus torquatus.* Frontal sections, HE staining. INDEX of structures: aob – acessory olfactory bulb; em – ependyma; epl – external plexiform layer; gll – glomerular layer; grl – glanular layer; ipl – internal plexiform layer; mcl – mitral cell layer; mob – main olfactory bulb; olv – olfactory ventricle; onl – olfactory nerve layer; op – olfactory penducle. Scale Bar (A): 500 µm; Scale Bar (B): 100 µm.

A clear distinction between rostral and caudal parts of the dorsal ventricular ridge were not visible with H.E. staining, and it presented as uniform distributed cells in *T. torquatus* (Fig. 5-7). Some parts of the ventricular ridge were covered by the cortices but lateral parts of the ventricular ridge were covered by a layer of pia mater and ependyma called pallial membrane (Fig. 5A), which was also found in *A. garmani*, *A. grahami* and *A. lineatopus*[(12)].

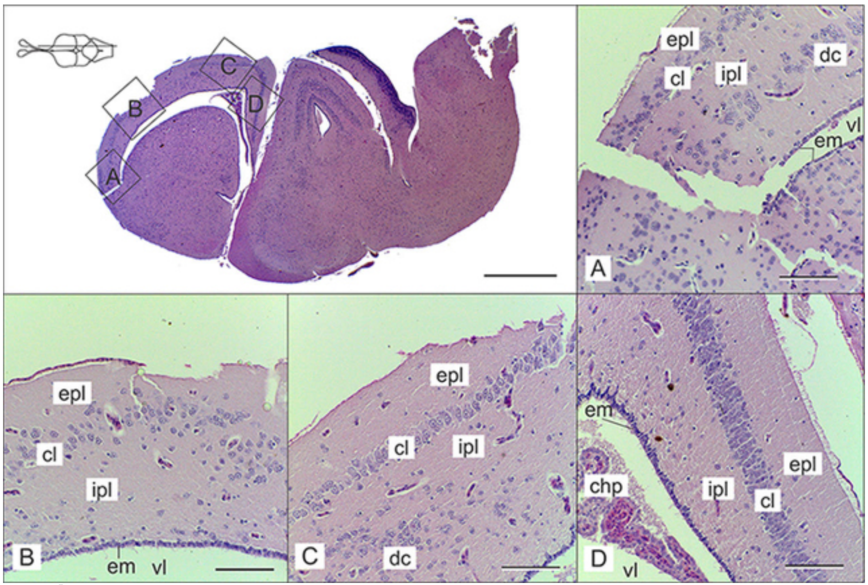

Figure 4. Cortices of *Tropidurus torquatus*. Sagittal sections. A – lateral cortex; B – dorsal cortex; C – dorsomedial cortex; D – medial cortex. INDEX of structures: chp – choroid plexus; cl – cell layer; dc – dorsal cortex; em – ependyma; epl – external plexiform layer; ipl – internal plexiform layer; lv – lateral ventricle. Scale Bar: 100 µm.

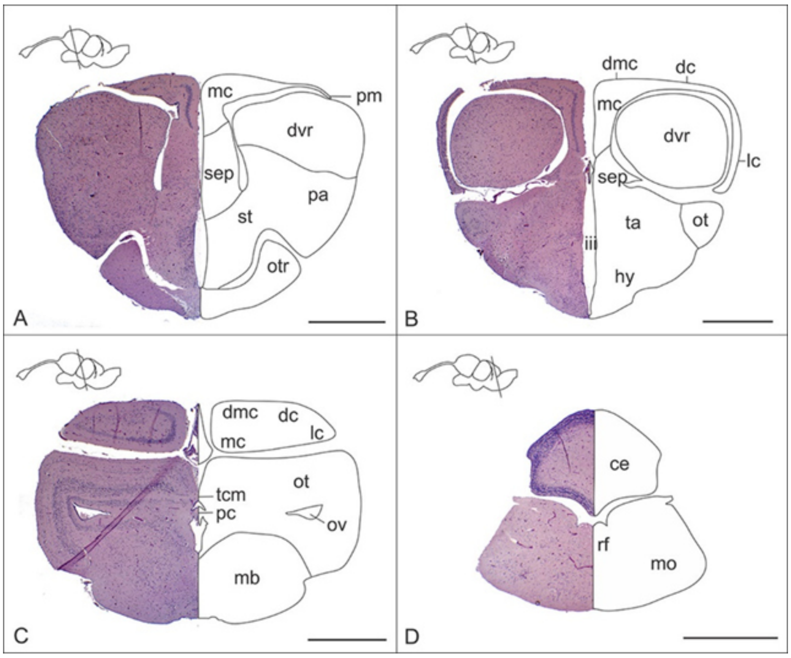

Figure 5. Transversal sections and scheme of the brain of *Tropidurus torquatus.* HE staining. INDEX of structures: ce – cerebellum; dc – dorsal cortex; di – diencephalon; dmc – dorsal medial cortex; dvr – dorsal ventricular ridge; ep – epithalamus; hy – hypothalamus; lc – lateral cortex; lv – lateral ventricle; mb – midbrain; mc – medial cortex; mo – medulla oblongata; oc – optic chiasm; ot – optic tectum; pa – pallidum; pm – pallial membrane; rf – reticular formation; sep – septum; sp – subpallium; st – striatum; ta – thalamus; tg – tegmentum; ts – torus semicircularis;. Scale Bar: 1 mm.

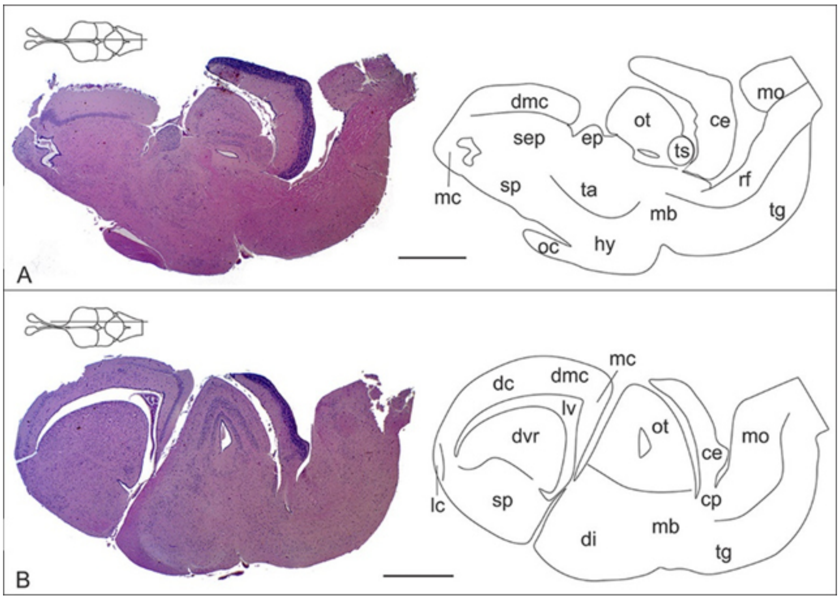

Figure 6. Sagittal sections and scheme of the brain of *Tropidurus torquatus.* HE staining. INDEX of structures: ce – cerebellum; dc – dorsal cortex; dmc – dorsal medial cortex; dvr – dorsal ventricular ridge; hy – hypothalamus; lc – lateral cortex; mb – midbrain; mc – medial cortex; mo – medulla oblongata; ot – optic tectum; rf – reticular formation; sep – septum; sp – subpallium; ta – thalamus; tcm – tectal commissure. Scale Bar: 1 mm.

Subpallium was composed of the septum, striatum, and pallidum, from which the first was located medially between medial cortex and striatum. The amygdaloid complex was identified in the subpallium of *G. Gecko*(18), but it was not possible to distinguish it in *T. torquatus.* The lateral ventricle is associated with the telencephalon and was located between the cortices and dorsal ventricular ridge in *T. torquatus* (Fig. 5). The hippocampal and anterior commissures cross the hemispheres and were identified in *T. torquatus* (Fig. 1C).

3.3. Diencephalon

In *T. torquatus*, four regions composed the diencephalon: epithalamus, thalamus (dorsal and ventral), and hypothalamus, as in *C. Vulgaris, G. gecko* and *T. teguixin*(15, 17, 21). The diencephalon is associated with the third ventricle (Fig. 5-7), a median ventricle located between both sides of the diencephalon, in *T. torquatus*. The diencephalon was almost completely covered by the hemispheres on the dorsal surface, with only part of the epithalamus being exposed (Fig. 2A).

Most of the epithalamus was composed of the habenula in *T. torquatus*, from which some fibers crossed over at the habenula commissure. Structures of the pineal complex projected from the epithalamus: the pineal organ, dorsal sac, paraphysis and parietal eye (Fig. 8). The pineal organ was oval shaped in *T. torquatus* and triangular shaped in *S. merianae*(11). The pretectum lies at the transition of the diencephalon and the mesencephalon(15, 17, 21) and could not be distinct in *T. torquatus.*

The thalamus was only visible in the sagittal section. In its dorsal part the posterior commissure was identified in *T. torquatus* (Fig. 1C, 5C). The largest part of the diencephalon was composed by the hypothalamus, part of which was visible on the ventral surface, the protruding infundibulum was medially located in this region. Also, ventrally, thick optic nerves intersected at the optic chiasm and entered the brain through optic tracts, surrounding the infundibulum, where the hypophysis was connected to the brain (Fig. 2). A supraoptical commissure was present ventrally in the hypothalamus, caudal to the optic chiasm (Fig. 1B), that is in agreement with literature data(15, 17, 21).

3.4. Mesencephalon

The mesencephalic tectum and tegmentum comprised the mesencephalon. The tegmentum was located ventrally, continuous with the hindbrain. Its tectum had an optic tectum and torus semicircularis (Fig. 5-7). Macroscopically, the optic tectum was oval shaped and noticeably smaller than the brain hemispheres and partially covered by the cerebellum (also described in *S. merianae*(11)), while the torus semicircularis was a small median structure located caudoventrally to the optic tectum, completely covered by the cerebellum (Fig. 1B, 2)

The torus semicircularis was funnel shaped and it was larger in its medial part, which in both antimeres are partly fused at the midline. It thins out gradually as it extends laterally in *T. torquatus,* as also seen in *Gallotia galloti* and *T. teguixin*(24, 25). A band of fibers crossed both parts of the tectum forming the tectal commissure (Fig. 1C, 5C). The cerebral aqueduct passed through the midbrain toward the fourth ventricle (Fig. 1B).

The optic tectum had 14 layers starting from the ventricle and can be organized into six strata in *T. torquatus*

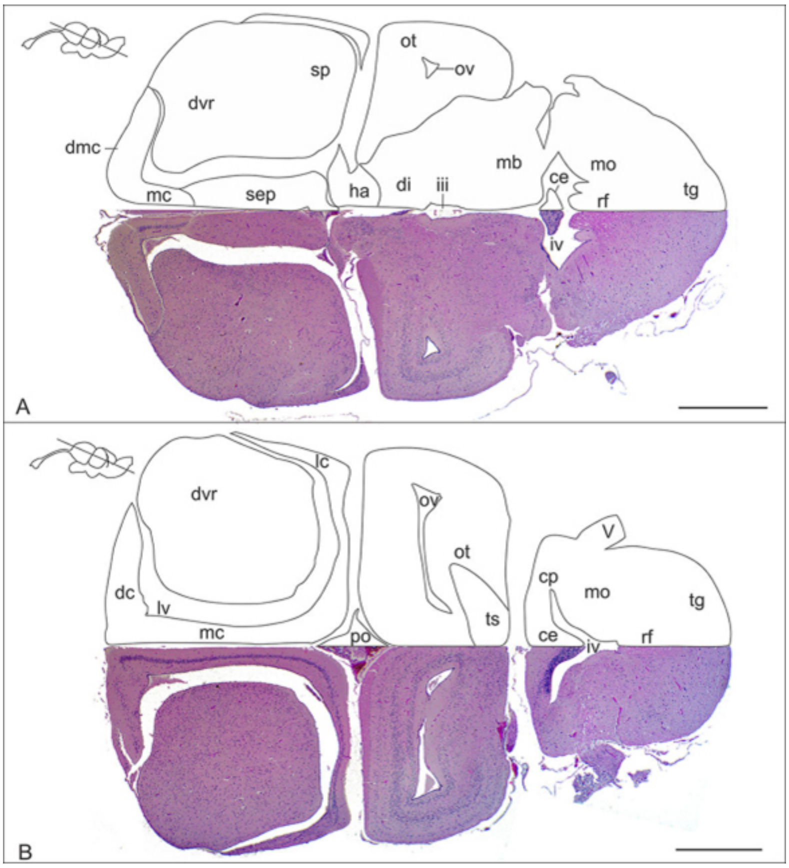

Figure 7. Frontal sections and scheme of the brain of *Tropidurus torquatus.* HE staining. INDEX of structures: ce – cerebellum; cp – cerebellar penducle; dc – dorsal cortex; di – diencephalon; dmc – dorsal medial cortex; dvr – dorsal ventricular ridge; ha – habenula; iii – third ventricle; iv – fourth ventricle; lc – lateral cortex; lv – lateral ventricle; mb – midbrain; mc – medial cortex; mo – medulla oblongata; ot – optic tectum; ov – optic ventricle; po – pineal organ; rf – reticular formation; sep – septum; sp – subpallium; tg – tegmentum; ts – torus semicircularis. Scale Bar: 1 mm.

(Fig. 9), as also seen in *C. decresii*, *I. iguana iguana* and *T. teguixin*(19, 26, 27). This organization was first described by Ramón, as shown by Huber & Crosby(28): stratum fibrosum periventriculare [ependyma / epithelial zone (1); molecular zone (2)], stratum griseum periventriculare [cellular zone (3); molecular zone (4); cellular zone (5)], stratum album centrale [central fiber zone (6)], statum griseum centrale [central cellular zone (7)], stratum fibrosum and griseum superficiale [cellular zone (8); molecular zone (9); cellular zone (10); molecular zone (11); cellular and optic fiber zone (12); molecular zone (13)] and stratum opticum [optic fiber zone (14)]. Layer 7 was thicker than the other cell layers and layer 6 was the largest fiber layer in *T. torquatus*, and also layer 14 was thicker rostrally and layers 7-11 were sparser, with a less distinct organization than in *I. iguana iguana*(27).

3.5. Hindbrain

The medulla oblongata and cerebellum composed the hindbrain. The ventral part of the medulla oblongata was composed of the tegmentum, which was continuous with the tegmentum of the midbrain. One structure of the tegmentum was the reticular formation, distinct for its appearance of loose fibers (Fig. 6). The medulla oblongata was large lateral-laterally and it caudally tapered toward its division with the spinal cord, which also appeared larger rostrally. Laterally the medulla oblongata had a curved appearance and several roots of nerves protruded from its surface, both laterally and ventrally.

Dorsally in the medulla oblongata, part of the rhomboid fossa was visible, being covered rostrally by the cerebellum, it was formed by the fourth ventricle, with the choroid plexus laying over it (Fig. 2). The rhomboid fossa in *A. garmani*, *G. gecko* and, *T. teguixin*(12, 16, 18) is longer (caudo-caudally) than in *T. torquatus*, and present a more cylindrical shape, while in the latter it is triangular shaped. It appeared to be more similar between *I. iguana iguana*, *S. merianae*(11, 13) and *T. torquatus*. It was oblique in *Anniella nigra*, mainly covered by nerve roots, with only a vertical slit being visible in dorsal view(29).

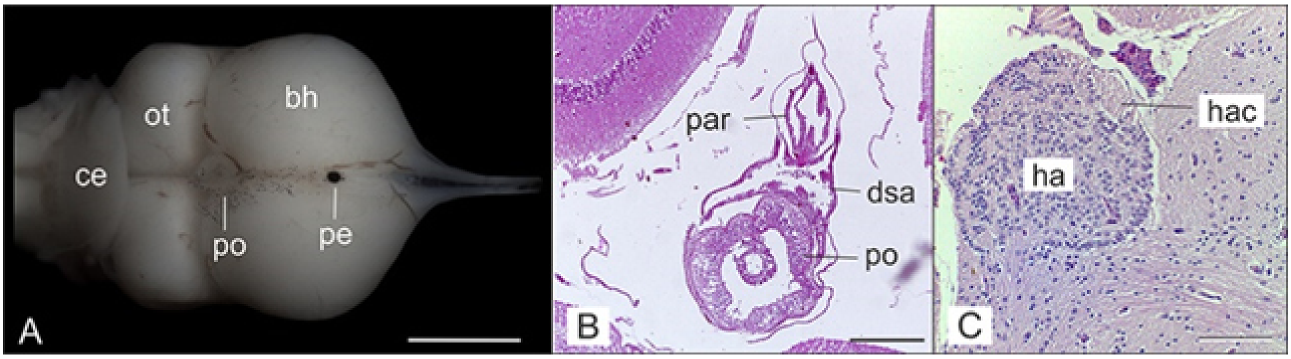

Figure 8. Epithalamus of *Tropidurus torquatus* and associated structures. A – dorsal view of the brain; B – frontal section of pineal complex -; C – sagittal section of habenula. INDEX of structures: bh – brain hemisphere; ce – cerebellum; dsa – dorsal sac; ha – habenula; hac – habebular commissure; ot – optic tectum; par – paraphysis; pe – parietal eye; po – pineal organ. Scale Bar (A): 5 mm; Scale Bar (B): 200 µm; Scale Bar (C): 100 µm.

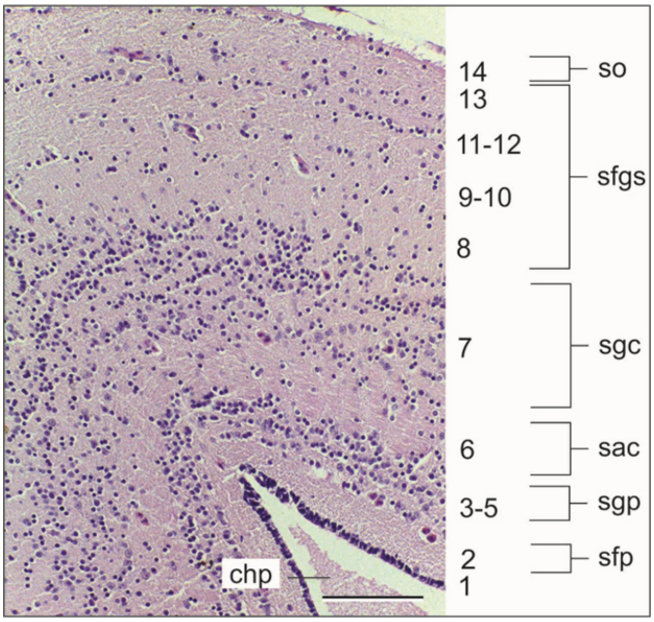

Figure 9. Optic tectum of *Tropidurus torquatus.* Sagittal section. Numbers represents layers. INDEX of structures: 1-14 – layers; sac – stratum album centrale; sfgs – stratum fibrosum and griseum superficiale; sfp – stratum fibrosum periventriculare; sgc – statum griseum centrale; sgp – stratum griseum periventriculare; so – stratum opticum; Scale Bar: 100 µm.

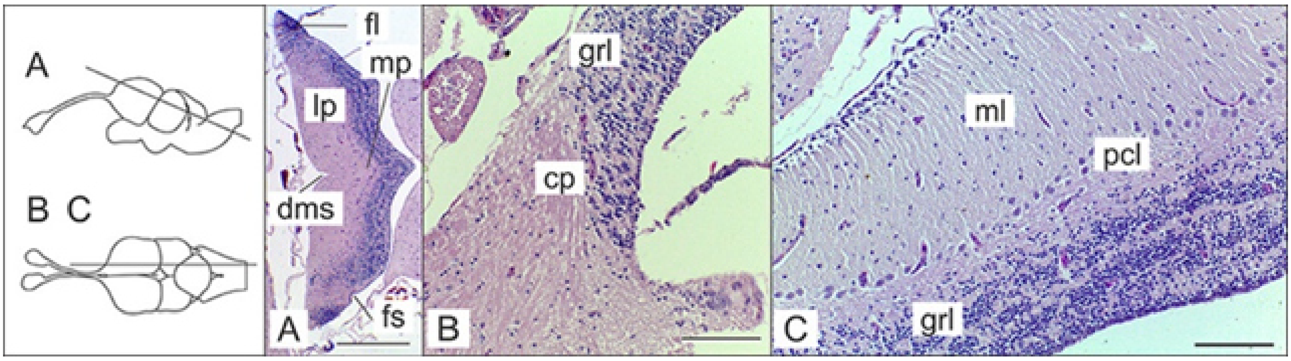

Figure 10. Cerebellum of *Tropidurus torquatus.* A – frontal section; B, C – sagittal sections. INDEX of structures: cp – cerebellar penducle; dms – dorsal median sulcus; fl – flocculus; fs – floccular sulcus; grl – glanular layer; lp – lateral part of cerebellar hemisphere; ml – molecular layer; mp – median part of cerebelar hemisphere; pcl – purkinje cell layer. Scale Bar (A): 500 µm; Scale Bar (B, C): 100 µm.

In *T. torquatus*, the cerebellum protruded from the dorsorostral part of the medulla oblongata, connected to it through the cerebellar peduncle (Fig. 10B). It had a plate shape and it curved rostrally (Fig. 2), divided into two lateral flocculi (also observed in *S. merianae*(11)) and hemispheres with a median part and two lateral parts (Fig. 10A).

The curved plate shape of the cerebellum, which covered the optic tectum, is shared among other lizards, including *A. garmani, C. decresii, I. iguana iguana, Phrynosoma douglasii, T. teguixin, S. merianae, Sceloporus biseriatus* and *Sceloporus graciosus*(11-13, 15, 16, 19, 27, 29). However, in the lizards *G. gecko* and *P. hispanica*, it didn't cover the optic tectum and it appeared to be slightly curved backwards in the former and smaller in the latter(17, 18).

In the lizard *A. nigra*, a legless lizard, the cerebellum was small in size, compared to the rest of the brain. It was almost hidden from view, lying in a depression formed by the midbrain, medulla oblongata and nerve roots. The cerebellum of *Gerrhonotus principis* presented interesting features. In dorsal view it presented a triangular shape, its median part had a tongue-like structure projecting backwards following the contour of the rhomboid fossa. The lateral parts were curved forward, similar to the cerebellum of other lizards mention above(29).

The cerebellar cortex in *T. torquatus* was formed by three layers: granular layer, Purkinje cell layer and molecular layer. The most external and dorsocaudal layer was the granular layer, composed of small and densely packed granule cells and larger Golgi cells. The molecular layer was the most ventrorostral and inner layer, with the presence of many dendrites from the adjacent Purkinje cells and axons from granule cells, and few basket and stellate cells. The Purkinje cell layer was located between the other two layers, formed by a single line of cells (Fig. 10C). These layers and cells were very similar to the ones found in *C. decresii, C. vulgaris, P. douglasii,* S. *biseriatus* and *S. graciosus*(17, 19, 29).

In *A. nigra*, the layers were more similar to that of anurans, with the granular layer located ventrocaudally and the molecular layer dorsorostrally. The Purkinje cell layer was also formed by a single line of cells. The median part of the cerebellum of *G. principis* presented the granular layer ventrocaudally, similar to *A. nigra*, while it bended rostrally as it extended laterally, like that of most lizards (e.g. *T. torquatus*). The author proposed that this lateral part is what predominated in other lizards with the common conformation to the cerebellum(29).

4. Conclusions

Generally speaking, the brain of *Tropidurus torquatus* was similar to that of other lizard species, with their adaptations to live in the environment they are inserted. Their brain possessed a large area dedicated to processing olfactory and visual stimuli, which are necessary to survive, either foraging and hunting for prey or hiding from predators.

Conflict of interest

Authors declare no Conflict of Interests for this article.

Author contributions

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References

1. Shedlock AM, Edwards SV. Amniotes (amniota). In: Hedges SB, Kumar S (eds.). The timetree of life. Oxford: Oxford University Press; 2009. p. 375-379.

2. Naumann RK, Ondracek JM, Reiter S, Shein-Idelson M, Tosches MA, Yamawaki TM, Laurent G. The reptilian brain. Current Biology. 2015;25(8):R317-R321.

3. Kiefer MC, Van Sluys M, Rocha CF. Body temperatures of *Tropidurus torquatus* (Squamata, Tropiduridae) from coastal populations: Do body temperatures vary along their geographic range? Journal of Thermal Biology. 2005;30:449-456.

4. Siqueira CDC, Kiefer MC, Sluys MV, Rocha CFD. Plant consumption in coastal populations of the lizard *Tropidurus torquatus* (Reptilia: Squamata: Tropiduridae): how do herbivory rates vary along their geographic range? Journal of Natural History. 2010;45:171-182.

5. Ortiz MA, Boretto JM, Piantoni C, Álvarez BB, Ibargüengoytía NR. Reproductive biology of the Amazon Lava Lizard (*Tropidurus torquatus*) from the Wet Chaco of Corrientes (Argentina): congeneric comparisons of ecotypic and interspecific variations. Canadian Journal of Zoology. 2014;92:643-655.

6. Py-Daniel TR, De-Lima AKS, Lima FC, Pic-Taylor A, Rodrigues RPJ, Sebben A. A Staging Table of Post-Ovipositional Development for the South American Collared Lizard *Tropidurus torquatus* (Squamata: Tropiduridae). The Anatomical Record. 2017;300:277-290.

7. Hoops D. A perfusion protocol for lizards, including a method for brain removal. MethodsX. 2015;2:165-173.

8. Wright KP, et al. E. In: Binder MD, Hirokawa N, Windhorst

U. (eds.) Encyclopedia of Neuroscience. Berlin: Springer; 2008. p. 1029-1548.

9. World Association of Veterinary Anatomists. Nomina anatomica veterinaria. 6th ed. Columbia: International Committee on Veterinary Gross Anatomical Nomenclature; 2017. 160 p.

10. Senn DG. Embryonic development of the central nervous system. In: Gans C (ed.). Biology of the Reptilia, Volume 9. Neurology A. London: Academic Press; 1979. p. 173-244.

11. Reis LTMD. Estudo morfológico do encéfalo de répteis (Chordata: Reptilia) (Doctoral dissertation). Uberlândia: Universidade Federal de Uberlândia. 2017.

12. Armstrong JA, Gamble HJ, Goldby F. Observations on the olfactory apparatus and the telencephalon of Anolis, a microsmatic lizard. Journal of anatomy. 1953;87:288-307.

13. Northcutt RG. Architectonic studies of the telencephalon of *Iguana iguana*. Journal of Comparative Neurology. 1967;130:109-147.

14. Llahi S, García-Verdugo JM. Neuronal organization of the accessory olfactory bulb of the lizard *Podarcis hispanica*: Golgi study. Journal of Morphology. 1989;202:13-28.

15. Cruce JAF. A cytoarchitectonic study of the diencephalon of the tegu lizard, *Tupinambis nigropunctatus*. Journal of Comparative Neurology. 1974;153:215-238.

16. Curwen AO. The telencephalon of *Tupinambis nigropunctatus*. I. Medial and cortical areas. Journal of Comparative Neurology. 1937;66:375-404.

17. Shanklin WM. The central nervous system of *Chameleon vulgaris*. Acta zoologica. 1930;11:425-490.

18. Smeets WJ, Hoogland PV, Lohman AH. A forebrain atlas of the lizard *Gekko gecko*. Journal of Comparative Neurology. 1986;254:1-19.

19. Hoops D, et al. A 3D MRI-based atlas of a lizard brain. Journal of Comparative Neurology. 2018;526(16):2511-2547.

20. Lohman AHM, Van Woerden-Verkley I. Further studies on the cortical connections of the Tegu lizard. Brain research. 1976;103:9-28.

21. Bruce LL, Butler AB. Telencephalic connections in lizards. I. Projections to cortex. Journal of Comparative Neurology. 1984;229:585-601.

22. Butler AB. Telencephalon of the lizard *Gekko gecko* (Linnaeus): some connections of the cortex and dorsal ventricular ridge. Brain, Behavior and Evolution. 1976;13:396-417.

23. Lohman AHM, Mentink GM. Some cortical connections of the tegu lizard (*Tupinambis teguixin*). Brain research. 1972;45:325-344.

24. Browner RH, Rubinson K. The cytoarchitecture of the torus semicircularis in the Tegu lizard, *Tupinambis nigropunctatus*. Journal of Comparative Neurology. 1977;176:539-557.

25. Díaz C, Yanes C, Trujillo CM, Puelles L. Cytoarchitectonic subdivisions in the subtectal midbrain of the lizard *Gallotia galloti*. Journal of Neurocytology. 2000;29:569-593.

26. Butler AB, Ebbesson SO. A Golgi study of the optic tectum of the Tegu lizard, *Tupinambis nigropunctatus*. Journal of Morphology. 1975;146:215-227.

27. Foster RE, Hall WC. The connections and laminar organization of the optic tectum in a reptile (*Iguana iguana*). Journal of Comparative Neurology. 1975;163:397-425.

28. Huber G, Crosby EC. The reptilian optic tectum. Journal of Comparative Neurology. 1933;57:57-163.

29. Larsell O. The cerebellum of reptiles: lizards and snake. Journal of Comparative Neurology. 1926;41:59-94.