First Natural Endocranial Cast of a Fossil Snake (Cretaceous of Patagonia, Argentina)

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ABSTRACT

In this study, we describe a natural endocranial cast included in a partially preserved medium-sized skull of the Upper Cretaceous South American snake *Dinilysia patagonica*. The endocast is composed of sedimentary filling of the cranial cavity in which the posterior brain, the vessels, the cranial nerves, and the inner ear surrounded by delicate semicircular canals, are represented. It is simple in form, with little differentiation between the three main areas (Forebrain, Midbrain, and Hindbrain), and without flexures. The nervous system is well preserved. The posterior brain surface is smooth, except for two small prominences that make up the cerebellum. A large inner ear is preserved on the right side; it consists of a voluminous central mass, the vestibule, which occupies most of the space defined by the three semicircular canals. In particular, the lateral semicircular canal is very close to the vestibule. This characteristic, in combination with the medium to large body size of *Dinilysia*, its large skull and dorsally exposed orbits, and vertebrae bearing a rather high neural spine on a depressed neural arch, suggests that this snake would have had a semifossorial lifestyle. Anat Rec, 301:9–20, 2018. © 2017 Wiley Periodicals, Inc.

Key words: snakes; Cretaceous; *Dinilysia patagonica*; palaeoneurology

INTRODUCTION

Although for a long time Palaeoneurology has been based exclusively on natural or artificial endocasts (Jerison, 1973; Edinger, 1975; Hopson, 1979), the use of high resolution X-ray computed tomography has increased the number of artificial endocranial casts of the nervous system in a wide range of extinct vertebrates, especially mammals and archosaurs (Witmer et al., 2008 and references cited there). Studies on Palaeoneurology in snakes were nonexistent (Hopson, 1979), but Yi (2013, 2015) and Yi and Norell (2015) recently analyzed some aspects of the inner ear anatomy in a variety of snakes through X-ray computed tomography which builds three-dimensional models that are virtual endocasts of the bony inner ear labyrinth. The materials analyzed by these authors included a skull of the extinct snake *Dinilysia patagonica* (MACN-RN 1014).
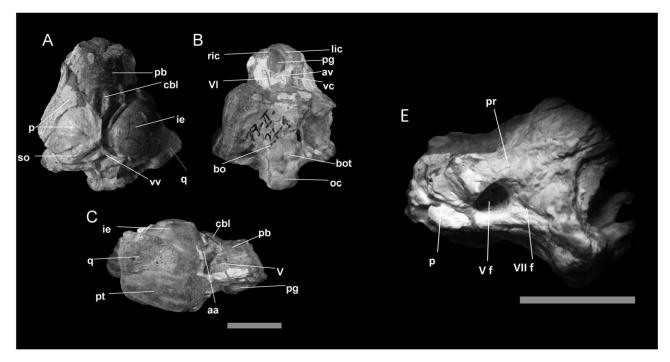
Fig. 1. Skull of *Dinilysia patagonica*, MLP 79-II-27-1. Scale bar equals 10 mm. (**A**) Dorsal view, (**B**) ventral view, (**C**) right lateral view and (D) left lateral view. Abbreviations: aa, anterior ampulla; av, aortic vessels; bo, basioccipital; bot, basioccipital tubers; cbl, cerebellum; ie, inner ear; lic, left inner carotid; oc, occipital condyle; p, parietal; pb, posterior brain; pg, pituitary gland; pr, prootic; pt, pterigoid; q, quadrate; ric, right inner carotid; so, supraoccipital; V, trigeminal nerves; VI, abducens nerves; vc, V f, foramen of the trigeminal nerves; VII f, foramen of the facial nerves, vidian canal; vv, vascular vessels.

Dinilysia patagonica is an Upper Cretaceous snake from Argentina, known for exceptionally well preserved cranial and postcranial remains recovered in northwest Patagonia (Smith-Woodward, 1901; Estes et al., 1970; Hecht, 1982; Rage and Albino, 1989; Albino and Caldwell, 2003; Albino, 2007; Scanferla and Canale, 2007; Caldwell and Calvo, 2008; Filippi and Garrido, 2012; Zaher and Scanferla, 2012; Triviño and Albino, 2015). Taking into account the osteological anatomy, *Dinilysia patagonica* is considered basal in most ophidian cladograms (Caldwell, 1999; Rieppel and Zaher, 2000; Scanlon and Lee, 2000; Tchernov et al., 2000; Lee and Scanlon; 2002; Zaher and Scanferla, 2012; Hsiang et al., 2015); thus, relevant neuroanatomical data of this species could add substantial information about the early evolution of snakes.

In this paper, a natural cranial endocast of an extinct snake is described for first time. The material corresponds to a fragmentary skull of *Dinilysia patagonica* (MLP 79-II-27-1) previously reported by Caldwell and Albino (2002) and Albino (2007). This skull includes an endocast preserved by sedimentary filling of the cranial cavity in which the posterior brain, the vessels, the cranial nerves, the inner ear and the semicircular canals are preserved (Figs. 1 and 2). Both the osteology and the soft tissue cast of this unpublished skull are also described herein. The results of this study are compared with the conclusions published by Yi (2013, 2015) and Yi and Norell (2015) in order to test the hypothesis about the burrowing origin of modern snakes.

MATERIALS AND METHODS

What exactly does a natural endocranial cast represent? The nervous system of reptiles is tubular, linear in organization, and has some degree of dorsoventral flexure along its length (Wyneken, 2007). The brain cavity is limited by a tubular cranium composed rostrally by the cartilaginous ethmoids, laterally by the bony otic series, ventrally by the basisphenoid and laterosphenoids, and caudally by the occipital series (Wyneken, 2007). The tubular cranium is covered by the supraoccipital, parietal, and frontal bones, and there is a subdural space (below the dura mater) and an epidural space (above the dura mater). An endocast is the sedimentary infill of a cavity that forms a three-dimensional structure. Therefore, what it is known as a natural cranial endocast is the filling of the intracranial cavity that contains the brain with its cranial nerves, the meninges that cover and protect them, and the blood vessels. These casts may provide approximations of the brain morphology, with the possibility to see details of some superficial structures (Macrini et al., 2006).

In extant reptiles, the volume of the brain does not determine the topographic relationships between brain and skull (Starck, 1979). The size of the brain is determined by the body size whereas the volumes of individual cerebral segments are dependent on the development of sense organs (Starck, 1979). In most reptiles, the brain is smaller than the intracranial cavity. Starck (1979) reminds us that these relationships not only show group specific differences, but also ontogenetic and possibly
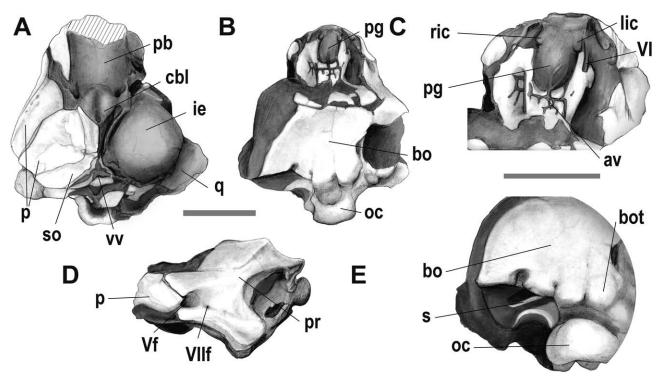
Fig. 2. Skull of *Dinilysia patagonica*, MLP 79-II-27-1. Scale bar equals 10 mm. (**A**) Dorsal view, (**B**) ventral view, (**C**) ventroanterior view zoom, (**D**) lateral view, (**E**) ventroposterior view zoom. Abbreviations: av, aortic vessels; bo, basioccipital; bot, basioccipital tubers; cbl, cerebellum; ie, inner ear; lic, left inner carotid; oc, occipital condyle; p, parietal; pb, posterior brain; pg, pituitary gland;; pr, prootic; q, quadrate; ric, right inner carotid; s, stapes; so, supraoccipital; V f, foramen of the trigeminal nerve; VII f, foramen of the facial nerve; VI, abducens nerves; vv, vascular vessels.

sexual ones. Wyneken (2007) suggests that the nervous system of reptiles is relatively simple in anatomical structure yet allows greater functional diversity in species-specific behaviors and adaptation to diverse niches.

According to Hopson (1979), the reptile brain does not fill the brain cavity and the extra neural elements occupy part of the intracranial space. This would means that the endocast shows the place previously occupied by the brain and intracranial space, providing only a superficial overview of the topography of the brain (Jerison, 1969; Hopson, 1979; Norman and Faiers, 1996; Larsson et al, 2000; Wyneken, 2007). Although several groups of reptiles present mostly ossified braincases, the brain still does not completely occupy the cranial cavity; thus endocasts would neither be representative of the brain topography in these cases. In this sense, a well-developed subdural space is observed in marine turtles, *Sphenodon* and many lizards. Most Testudines and Crocodylia present a moderate subdural space. However, this space is very narrow in snakes and amphisbaenians (Starck, 1979). Because of this, Wyneken (2007) considers that endocasts of snakes would allow us to hope a good morphological copy of their brains. Recently, Olori (2010) studied a digitized endocast of an uropeltid and enunciated the hypothesis that the relationship between the cranium and the endocraneal cavity is similar to that of mammals, involving the possibility that endocraneal space is completely occupied by the brain. This condition could be a consequence of the high degree of fusion in the bones of the uropelid skull, thus determining a completely closed cranial cavity (Olori, 2010).

For this paper, several skulls of Dipsadidae (Colubroidea) were dissected to test the hypothesis of Olori (2010) in other extant snakes, focusing on the disposition of the brain and subdural space in the cranial cavity. These snakes were collected after they died flattened by cars on the road. The heads of some of these snakes were sectioned in sagital plane and others in longitudinal plane (Table 1), to observe the disposition of brain regions, the cranial space occupying these regions, and the arrangement of the veins and cranial nerves. As Figures 3 and 4 shows, the cranial bones surround the tissue of the nervous system very closely, leaving a very small space between the bone and the nervous tissue for the blood vessels to pass through. Thus, the brain fills around 90% of the endocranial cavity in snakes, leaving a very narrow intracavitary space.

Fossil specimens examined. An exceptionally well-preserved specimen of *Dinilysia patagonica* (MLP 79-II-27-1) was used for the present study. The material was collected by Santiago Roth at the end of XIX century in Boca del Sapo, Neuquén city, at the confluence of the Neuquén and Limay Rivers, Neuquén province, Argentina. The specimen was recovered from rocks belonging to the Bajo de La Carpa Formation (Santonian), Río Colorado Subgroup, Neuquén Group. It is represented by the posterior portion of a well preserved skull including the corresponding fragment of a natural endocast

TABLE 1. Extant dissected individuals for comparative purpose

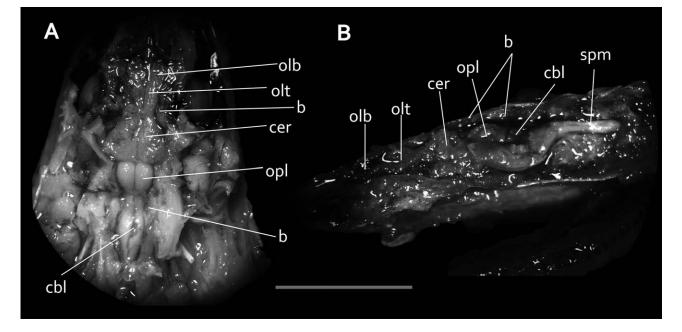| Specie	Collection number	Provenance	Date of collection	Colector	Dissection mode
Erythrolamprus poecilogyrus	MLP –R 6462	Ruta provincial 11, La Plata- Magdalena	11/14/2015	Jorge Williams	Section longitudinal to the main axis of the body
Erythrolamprus poecilogyrus	MLP –R 6463	Ruta provincial 11, La Plata- Magdalena	11/14/2015	Jorge Williams	Section parallel to the main axis of the body
Thamnodynastes strigatus	MLP –R 6464	Ruta provincial 11, La Plata- Magdalena	11/14/2015	Jorge Williams	Dorsal dissection and left lateral
Lygophis anomalus	MLP –R 6465	Ruta provincial 11, La Plata- Magdalena	11/20/2016	Laura Triviño	Bone conservation, observation of cranial nerves
Lygophis anomalus	MLP –R 6466	Ruta provincial 11, La Plata- Magdalena	12/09/2016	Laura Triviño	Bone conservation, observation of cranial nerves
Philodryas patagoniensis	MLP –R 6467	Ruta provincial 11, La Plata- Magdalena	11/20/2016	Laura Triviño	Section longitudinal to the main axis of the body
Philodryas patagoniensis	MLP –R 6468	Ruta provincial 11, La Plata- Magdalena	11/20/2016	Laura Triviño	Dorsal dissection and right lateral
Xenodon dorbignyi	MLP –R 6469	Ruta provincial 11, La Plata- Magdalena	12/09/2016	Laura Triviño	Dorsal dissection and right lateral

Fig. 3. Dissection of skull of *Erythrolamprus poecilogyrus*. Scale bar equals 10 mm. (**A**) Dorsal view, (**B**) left lateral view. Abbreviations: cbl, cerebellum; cer, cerebrum; spm, spinal medulla; olb, olfactory bulb; olt, olfactory tract; opl, optic lobe.

exposed on the dorsal right side. The specimen was mechanically prepared through the extraction of the following bones to have better endocast exposure: parietal, prootic, a supraoccipital segment, and a portion of the right otooccipital. The extraction of these bones exposed the sedimentary fillings that occupied the place of the soft tissues of the brain, nerves, blood vessels, and inner ear, forming the first natural endocranial cast of a snake.

The described specimen was directly compared with the following specimens of *Dinilysia patagonica*: MLP 26–410 (holotype), MACN-PV RN 1013, and MACN-PV RN 1014.

Living specimens examined. The fossil specimen was also compared with skulls of the extant *Boa constrictor occidentalis* (UNMdP-O 44), *Salvator merianae* (MLP-R 5969, MLP-R 6029), *Broghammerus reticulatus* (MLP-R 6030), and *Bothrops alternatus* (MLP-R 6031).

Moreover, for comparative studies, latex endocasts (CNP-ME 146, CNP-ME 147, CNP-ME 148) were obtained from skulls of the extant snake species *Boa constrictor occidentalis* (UNMdP-O 50, UNMdP-O 47),
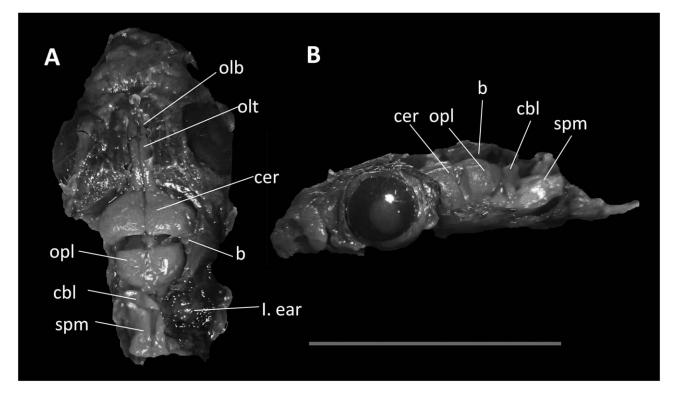
Fig. 4. Dissection of skull of *Thamnodynastes strigatus*. Scale bar equals 10 mm. (**A**) Dorsal view, (**B**) left lateral view. Abbreviations: b: bones; cbl, cerebellum; cer, cerebrum; i ear, inner ear; spm, spinal medulla; olb, olfactory bulb; olt, olfactory tract; opl, optic lobe.

and *Hydrodynastes gigas* (UNMdP-O 54). Table 1 details the dissected skulls of Dipsadidae used in this study to see the relationships among the brain and the braincase.

The material was studied using a stereoscopic microscope and was photographed with digital cameras.

Abbreviations. CNP-ME: Colección de moldes endocraneanos del Centro Nacional Patagónico (CENPAT), Puerto Madryn, Argentina; MACN-PV RN: Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Sección Paleontología de Vertebrados, Colección Río Negro, CABA, Argentina; MLP: Museo de La Plata, División Paleontología de Vertebrados, La Plata, Argentina; MLP-R: Museo de La Plata, sección Zoología de Vertebrados, División Herpetología, Reptiles, La Plata, Argentina; UNMdP-O: Universidad Nacional de Mar del Plata, Colección Herpetológica, Sección Osteología, Mar del Plata, Argentina.

Systematic Paleontology
REPTILIA Linnaeus, 1758
SQUAMATA Oppel, 1811
SERPENTES Linnaeus, 1758
DINILYSIA Smith-Woodward, 1901
DINILYSIA PATAGONICA Smith-Woodward, 1901
Figures (1 and 2), 5–9, and Figure 12

Referred specimen. MLP 79-II-27-1 posterior portion of an articulated skull including the endocast.

Provenance. Boca del Sapo, Neuquén city Neuquén province, Argentina.

Horizon. Neuquén group, Río Colorado Subgroup, Bajo de la Carpa Formation (Santonian, upper Cretaceous).

Description
Braincase and Basicranium

Among elements of the braincase, the parietal, prootic, supraoccipital and otooccipital bones are well preserved on the left side (Figs. 1 and 2). The parietal bone forms the roof as well as the lateral walls of the braincase, and makes up the anterior limit of the trigeminal foramen. Furthermore, the parietal surrounds the dorsal part of the inner ear and posterior brain. On the left wall of the braincase, behind the parietal bone, the prootic forms the posterior margin of the trigeminal foramen. Posterior to the trigeminal foramen there is a small foramen for the facial nerve. Both foramina are separately found, each one having a sole opening where nerves pass through.

In the occipital region, the supraoccipital, otooccipital, and basioccipital bones form the dorsal and posterior portion of the braincase and basicranium. The supraoccipital is located dorsally and posteriorly to the parietal, united by a zig-zag suture. This bone covers the posterior and dorsal extreme of the myelencephalon. The otooccipitals and the basioccipital constitute the ellipsoidal occipital condyle. A small part of the supraoccipital and otooccipitals forms the foramen magnum. In the otooccipital portion of the spinal canal, near the foramen magnum, there is a pair of foramina; the anterior is the exit of the vagus nerve and the posterior foramen is for the hypoglossal nerve. The metotic foramen opens externally, near the neck of the occipital condyle and over the otooccipitals. The glossopharyngeal, vagus and hyploglossal nerves, as well as the jugular vein, exit the cranial cavity through this foramen.

The basicranium is formed by the basioccipital and basiparasphenoid (Figs. 1 and 2). Ventrally, the
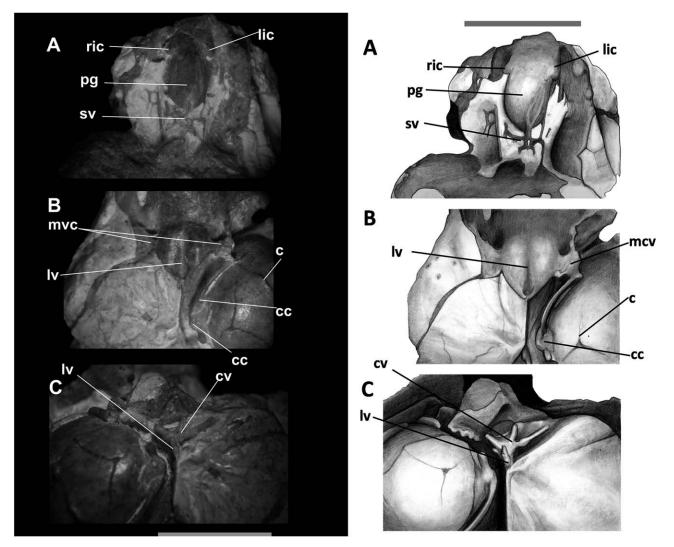
Fig. 5. Blood vessels. Scale bar equals 10 mm. (**A**) posterior view of the venous vessels, (**B**) medial view of the venous vessels, (**C**) arterial vessels, (**D**) arterial vessels, (**E**) medial view of the venous vessels, (F) posterior view of the venous vessels. Abbreviations: c, capillaries; cc, common crus; cv, cerebral vein; lic, left inner carotid; lv, longitudinal vein; mcv, median cerebral vein; pg, pituitary gland; sv, venous sinuses; ric, right inner carotid.

basioccipital has a pair of basal tubercles anterior to the occipital condyle, which also appears in other specimens of *Dinilysia*. The floor of the basiparasphenoid is lost, thus, it is possible to see the elements that run within this bone. The cast shows the pituitary gland surrounded by a groove that corresponds to the Vidian Canal. The internal carotid and the dorsal branch of the facial nerve (palatine ramus or Vidian nerve) pass through the Vidian Canal. A portion of nerve VI and several blood vessels, which would have irrigated the base of the brain, are also recognized.

Natural Endocranial Cast

The natural endocast preserved in the specimen MLP 79-II-27-1 exhibits the posterior brain, that is, the hindbrain (cerebellum and medulla oblongata). The forebrain is exposed in ventral view; it is represented by the diencephalon with the ventrally extended pituitary gland.

The brain is horizontal, without flexures between regions. The natural endocast also includes sedimentary fillings corresponding to some cranial nerves, the right ear (middle and inner), and the impressions related to venous and arterial craniocerebral circulatory elements (Figs. 1 and 2).

Brain

In ventral view, the pituitary gland cast is observed as an expansion of the diencephalon. The position of this gland is posterior, next to the output of the trigeminal nerve (Figs. 1 and 2). Vessels related to the ventral circulatory system of the skull are immediately behind the gland. The Abducens nerve (VI) surrounds the vessels, and external to this nerve are the natural casts of the Vidian Canal system. The internal carotids, emerging from their respective Vidian Canals, enter at the most anterior part of the pituitary gland.
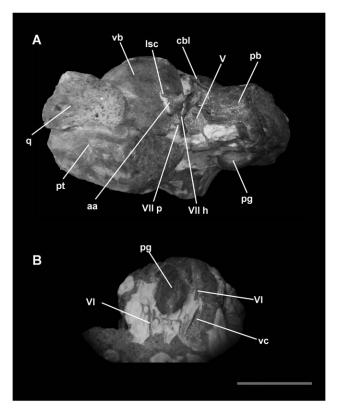
Fig. 6. Natural endocranial cast. Scale bar equals 10 mm. (**A**) right lateral view, (**B**) ventral view. Abbreviations: aa, anterior ampulla; cbl, cerebellum; lsc, lateral semicircular canal; pb, posterior brain; pg, pituitary gland; pt, pterigoid; q, quadrate; V, trigeminal nerves; VI, abducens nerves; VII h, hyomandibular facial nerves; VII p, palatine facial nerves; vb, vestibule; vc, vidian canal.
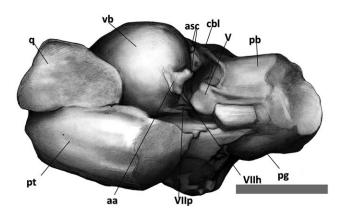

Fig. 7. Diagram lateral view of natural endocranial cast. Scale bar equals 10 mm. Abbreviations: aa, anterior ampulla; asc, anterior semicircular canal; cbl, cerebellum; pb, posterior brain; pg, pituitary gland; pt, pterigoid; q, quadrate; V, trigeminal nerves; VII h, hyomandibular facial nerves; VII p, palatine facial nerves; vb, vestibule.

The hindbrain is observed in dorsal view; it is formed by two smaller regions, the metencephalon and the myelencephalon. The structures observed in the hindbrain of the endocast are the following: cerebellum, cranial nerves V, VI and VII, and medulla oblongata. The cerebellum and cranial nerves V, VI, and VII are observed forming part of the metencephalon. Next to the inner ear and branching outwards from the lateral wall of this region are the trigeminal nerve (V) and the facial nerve (VII). The root of the trigeminal nerve is bigger than the facial nerve and is located in front of it. The abducens nerve (VI) is noted in the floor of the metencephalon, posterior to the exit of the trigeminal nerve and it runs near the pituitary gland, internal to the Vidian canal. The medulla oblongata is observed in the ventral face of the myelencephalon, where nerves IX, X and XII exit.

The cast of the cerebellum is located in the medial region of the parietal, in the area where this bone begins to taper laterally. The cerebellum consists of a simple structure (corpus cerebelli) separated into two hemispheres by an anteroposterior groove where the dorsal longitudinal venous sinus that drains the brain would have run (Figs. 1, 2, and 5).

The natural endocast only preserves the corresponding filling of the trigeminal (V), abducens (VI) and facial (VII) nerves. The trigeminal nerve (V) originates in the metencephalon and exits the braincase through a large and single foramen anterior to the inner ear (displayed on the left side) (Figs. 1D and 2). This nerve innervates the muscles of the jaw and the eye regions. The trigeminal ganglion corresponding to this nerve [=gasser (gasserian) or lunate] is located outside the braincase; it is a motor-sensitive element which is divided into four branches (Figs. 6A and 7). The abducens nerves (VI) exit from the floor of the metencephalon, posterior to the trigeminal nerve (Figs. 2C and 6B) and runs parallel to the pituitary gland in the ventral face of the basicranium. The facial nerves (VII) originate on the lateral wall of the metencephalon, posterior to the root of the trigeminal nerve (Figs. 6A and 7). They have a dorsal branch (hyomandibular branch) that goes lateroventrally to the external edge of the endocast immediately before the inner ear and the ventral branch (palatine branch), which is directed towards the posterior foramen of the Vidian canal and enters the channel. Externally, in the prootic bone, it is possible to see a single foramen that is independent from the trigeminal recess.

Ear

An area crossed by the massive columella (stapes) is located ventrolaterally, viewed from the occipital plane (Figs. 2E and 8A). The distal end of this element contacts the quadrate. Its major axis is directed towards the ventral part of the inner ear. The stapes is in contact with the inner ear through the footplate that rests in the oval window.

The endocast of the right inner ear is preserved. It is a compact and massive structure located behind the base of the trigeminal nerve. The inner ear is predominantly represented by a large element in the center, the vestibule, which would have contained a large central mass, the statolith, when the animal was alive (Figs. 3, 4, 7, 8B, and 9). This structure is ellipsoid shaped (although in lateral view it is spherical) and is compact in appearance. It is directed towards the midplane of the skull, passing over the medulla oblongata and behind the small lobes of the cerebellum. The vestibule is surrounded by very thin and close semicircular canals:
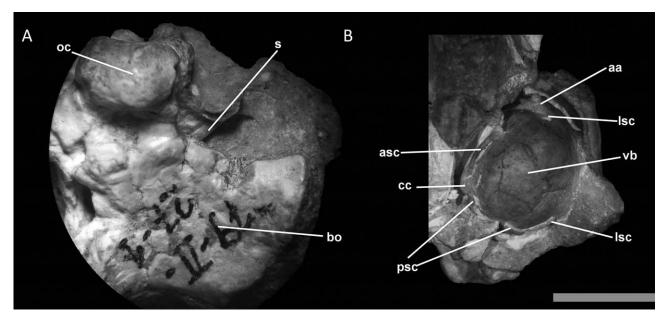
Fig. 8. (**A**) Ventral view of the middle ear. (**B**) Inner ear. Scale bar equals 10 mm. Abbreviations: aa, anterior ampulla; asc, anterior semicircular canal; bo, basioccipital; cc, common crus; lsc, lateral semicircular canal; oc, occipital condole; psc, posterior semicircular canal; s, stapes; vb, vestibule.

anterior, posterior and lateral. These delicate canals maintain their continuous diameter and are smoothly curved around the vestibule. The lateral canal is long and positioned in a horizontal plane close to the vestibule and the quadrate. Between the lateral semicircular canal and the vestibule there is no bone separates them and the two structures are very close to each other. Also, the lateral canal follows the contour of the vestibule. The anterior and posterior semicircular canals originate from the foremost and hindmost ends of the vestibule, respectively. They are directed vertically and laterally to the median plane of the skull. In dorsal view, these canals form a flattened cone with a hemispherical end at the base, around the vestibule. The anterior and posterior canals form the sides of the cone, whereas the lateral canal produces the contour of the hemisphere. The anterior ampulla is also preserved, connecting the anterior canal and the small fragment of the lateral canal. The ampulla is a compact structure with an anterior prominence; it is preserved at the front end of the vestibule, very close to the exit of the trigeminal nerve. At the junction between the anterior and posterior semicircular canals, a very small common crus is observed. The semicircular canals are connected together forming different angles: the angle is greater than 90° between the anterior and posterior canals, whereas it is close to 90° between the posterior and lateral canals, as well as between the anterior and lateral canals.

Venous and Arterial Craniocerebral Circulation

In the endocast of *Dinilysia*, the casts of the venous vessels run along the dorsal surface of the brain (Fig. 5). These vessels runs along the midline of the skull, and the branches in the posterior region are transformed into the posterior cerebral veins that exit the braincase through the jugular foramen. From the inner ear exit vessels that drain the vestibule (these vessels are thin and branched, and appear on the surface of the vestibule), and in the rostral end of the cerebellum they join the dorsal longitudinal venous sinus.

On the ventral part of the cast, the mark left by the Vidian canal is observed with the corresponding refill of the internal carotid artery (which forms the cerebral carotid artery when it enters the brain) (Fig. 5). This artery reaches the front end of the pituitary gland, entering and irrigating the brain from the ventral region. The left carotid artery has a slightly larger diameter than the right one.

Three thin unidentified vessels are observed behind the pituitary gland, the middle one enters the gland whereas the other two surround it laterally.

DISCUSSION

The material studied here is the first record of a natural endocranial cast of an extinct snake. Studies on the central nervous system of fossil snakes are nonexistent whereas literature on this issue in recent snakes is scarce. The earliest work on the nervous system of recent snakes dates from the late nineteenth century by Edinger (1896), who made descriptions of fibers of the telencephalon of reptiles and included images of fiber tracts and cell masses of the forebrain of pythons. Auen and Langebartel (1977) described for the first time all cranial nerves in two species of colubrids (*Elaphe obsolete quadrivittata* and *Thamnophis ordinoides*), identifying eleven cranial nerves. The spinal accessory nerve (XI) is absent in snakes. The trigeminal nerve contains four-branches. Branch V_1 is a sensory nerve and reaches the jaw's anterior end. Branch V_4, like branches V_2 and V_3, has a motor component. This pterygoid branch (V_4) is small to branched and innervates five muscles of the upper jaw. The output of the cranial nerves is evident in
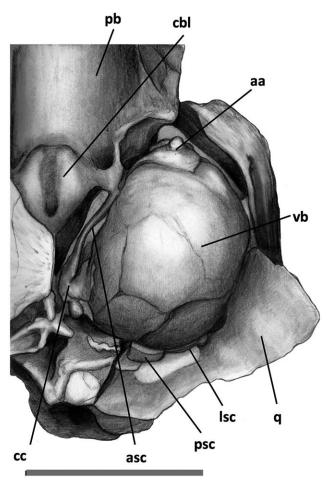
Fig. 9. Diagram Inner ear. Scale bar equals 10 mm. Abbreviations: aa, anterior ampulla; asc, anterior semicircular canal; cbl, cerebellum; cc, common crus; lsc, lateral semicircular canal; pb, posterior brain; psc, posterior semicircular canal; q, quadrate; vb, vestibule.
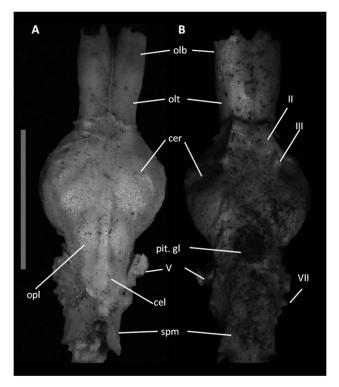

Fig. 10. Latex endocast, *Hydrodynastes gigas*. Scale bar equals 10 mm. (**A**) Dorsal view, (**B**) ventral view. Abbreviations: cbl, cerebellum; cer, cerebrum; i ear, inner ear; spm, spinal medulla; olb, olfactory bulb; olt, olfactory tract; opl, optic lobe; opt ch, optic chiasma; pit gl, pituitary gland; II, optic nerves; V, trigeminal nerves; X, vagus nerves.

the arrangement of the skull bones of all reptiles, allowing identification both in existing and extinct taxa (Romer, 1956; Breazile, 1979; Saveliev, 2008; Carabajal, 2009). Rieppel and Zaher (2000) have noted in recent snakes that the two branches of the facial nerve usually exit the braincase through two separate foramina, except in those cases where the palatine branch pursues an intracranial course into the Vidian canal. In the studied specimen of *Dinilysia patagonica*, one foramen is observed for the facial nerve, which is consistent with the condition observed in other skulls of this snake (Estes et al, 1970; Zaher and Scanferla, 2012).

The brain surface of reptiles is typically smooth in dorsal view, presenting different structures that are placed consecutively one after the other. They are: the Forebrain, associated with the sensory organs developing in the nasal capsule, and related to smelling and sensory-motor integration; the Midbrain, associated with the optical capsule, which carries out visual processing as well as neuroendocrine roles; and the Hindbrain, associated with the otic capsule, which carries out the role of hearing and balance, and also makes role of homeostasis (Wyneken, 2007). The dorsal view of the

specimen MLP 79-II-27-1 studied in this paper presents the Hindbrain, which consists of two smaller regions: the metencephalon and the myelencephalon. The metencephalon contains the cerebellum dorsally; this has a very simple structure formed by two longitudinal lobes. Ventral to the cerebellum are the pons, the medulla oblongata, and the fourth ventricle. The trigeminal (V), abducens (VI), and facial (VII) nerves exit from the medulla. The vestibulocochlear nerve (VIII) is not observed in this material. The myelencephalon is formed by the medulla oblongata, and is the source of the remaining posterior cranial nerves, i.e., the glossopharyngeal (IX), vagus (X), spinal accessory (XI) (nerve not present in snakes), and the hypoglossal (XII). In the endocast of *Dinilysia patagonica* the pituitary gland, which is a ventral projection of the diencephalon (posterior region of the forebrain) (Butler and Hodos, 2005; Wyneken, 2007), is located near the exit of the trigeminal nerve. It is interpreted as the ventral projection of the gland that is in contact with the base of the hindbrain.

According to Wyneken (2007) the shape and size of both the brain and the sense organs are possibly related to the lifestyle of the snakes. Olori (2010) reconstructed the endocast of a recent fossorial snake (*Uropeltis woodmasoni*), which is simple in form, with little differentiation between the three main areas (Forebrain, Midbrain and Hindbrain). During the observation of latex endocasts and the sections of skulls of recent semiaquatic and terrestrial colubrids and boids (Figs. 3, 4, 11, and
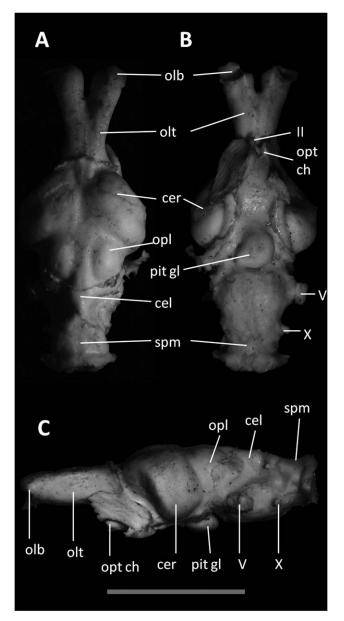
Fig. 11. Latex endocast, *Boa constrictor occidentalis*. Scale bar equals 10 mm. (**A**) Dorsal view, (**B**) ventral view. Abbreviations: cbl, cerebellum; cer, cerebrum; i ear, inner ear; spm, spinal medulla; olb, olfactory bulb; olt, olfactory tract; opl, optic lobe; pit gl, pituitary gland; II, optic nerves; III, oculomotor nerves; V, trigeminal nerves; VII, facial nerves.

Fig. 12. Reconstruction of *Dinilysia patagonica*.

12), it was possible to recognize marked flexures between the metencephalon and myelencephalon (pontine flexure, where the myelencephalon is exposed slightly towards a more dorsal position), as well as between the midbrain and the forebrain (cephalic flexure, where the forebrain is exposed towards a dorsal position). Also, in dorsal view, there is a marked differentiation between areas, where the lobes of the cerebrum (telencephalon) are larger in comparison with the optic lobes (Figs. 3, 4, 11, and 12). In the natural endocast of *Dinilysia* the morphology is very similar to what Olori (2010) observed in her studies on *Uropeltis*. That
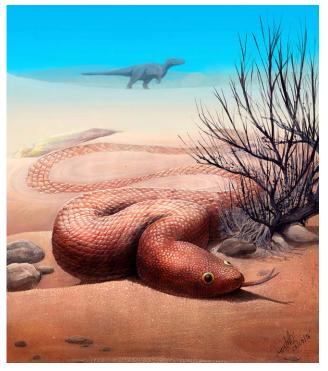
is, the brain does not present strong differentiation between the size of the lobes, and in lateral view, it is developed in horizontal plane, without flexures. Taking into account that these two snakes are placed in a basal position in recent phylogenetic analyses (Hsiang et al., 2015), this similarity could be related to the primitive condition of the nervous system in both

Uropeltis and Dinilysia

According to Yi (2013, 2015) and Yi and Norell (2015), the degree of vestibular expansion in the inner ear is related to the lifestyle of snakes. These authors analyzed the inner ear of 34 species of modern and fossil snakes, and 10 species of lizards and amphisbaenians. The sample included modern snakes in three habit groups: aquatic, terrestrial generalists, and burrowing. According to Yi and Norell (2015), the burrowing species show a lateral semicircular canal partly fused with the vestibule; in contrast, aquatic species show an expanded distance between the lateral semicircular canal and the vestibule. Nonburrowing terrestrial snakes display an intermediate state between these two types. Analyzing the virtual endocast of the extinct snake *Dinilysia patagonica*, Yi and Norell (2015) concluded that this snake shares with modern burrowing squamates a large spherical vestibule, a large foramen ovale, and slender semicircular canals in the inner ear. The vestibule occupies most of the space defined by the three semicircular canals and contacts the lateral canal (Yi and Norell, 2015). Because this morphology only appears in squamates that actively burrow underground, Yi and Norell (2015) conclude that *Dinilysia* has a burrowing lifestyle.

The present study of the natural cranial endocast of *Dinilysia patagonica* and the inner ear allow us to

confirm that this snake has an expanded and ellipsoid vestibule almost entirely occupying the space enclosed by the semicircular canals. The lateral canal is very close to the vestibule, there is not bone between them, and these elements are not fused together. This morphology coincides with that described by Yi and Norell (2015) for burrowing snakes on the basis of the 3-D reconstruction of the specimen MACN-RN 1014.

Unfortunately, Yi and Norell (2015) overlooked the previous suggestion of a possible semifossorial mode of life for this snake given by Albino and Caldwell (2003). That is, the possibility that *Dinilysia* was a partly surface-active snake, which spent a part of its time below a nonconsolidate ground excavated by itself. Extreme fossorial habits (active burrowers that live underground most of their time, as scolecophidians, uropeltids, anilioids, and amphisbaenians) are accompanied by relevant body features completely absent in *Dinilysia patagonica*, such as small size, reduction and lateralization of the eyes, and vertebrae with reduced or absent neural spines on depressed neural arches (Albino and Caldwell, 2003). In contrast, *Dinilysia* is characterized by a medium to large body size (more than 1.50 m according to Albino and Caldwell, 2003; exceeding 1.80 m according to Yi and Norell, 2015), large and dorsally exposed orbits, and neural spines of vertebrae relatively high in depressed neural arches; all characteristics compatible with both semifossorial or semiaquatic lifestyles (Albino and Caldwell, 2003). Besides, the skull of *Dinilysia* is large (around 10 cm long), with an expanded posterior region, whereas the anterior region seems to be more delicate due to a probable loosely connected joint between the premaxilla and maxilla. This condition would not be compatible with an active excavation of nearly compact ground with the skull, as is the case of true fossorial snakes that have small skulls with a consolidate and rigid snout (Cundall and Rossman, 1993). Nevertheless, the widened posterior region of the skull of *Dinilysia* would have been probably associated with a little neck delineation, providing a good adaptation to move unconsolidated ground easily. Thus, the very large inner ear, with the lateral semicircular canal close to the vestibule as in burrowing snakes could be explained considering a semifossorial lifestyle for this snake.

The stapes of *Dinilysia patagonica* is large and massive, and connects the quadrate with the oval window, making possible transmission of environment vibration to the inner ear through it. This characteristic was also observed by Frazzetta (1999) in *Xenopeltis unicolor*, where the stapes has a foot plate of large diameter, which is greater than the total length of the bone. In addition, *X. unicolor* is a medium-sized snake that grows up to 1 m (Greene, 2000), and has a relatively robust body, a posteriorly wide braincase, with short and triangular quadrates, and relatively large orbits in dorsal position (Frazzetta, 1999), all characteristics also found in *D. patagonica*. Taking into account that *X. unicolor* is one of the largest semifossorial extant snake; thus, *D. patagonica* could have developed similar habits with a partly fossorial lifestyle.

In conclusion, the characteristics found in the skull, vertebrae and natural endocast of *Dinilysia patagonica* as a whole support that this snake had a semifossorial mode of life. It is possible that these snakes maintained

their voluminous bodies resting under unconsolidated sediment, leaving their large dorsal eyes exposed on the outside and feeling the ground vibrations through their enormous inner ear waiting to hunt any possible prey (Fig. 10).

The basal position of *Dinilysia patagonica* in most phylogenetic analyses contradicts with the widespread view that all primitive snakes were small, burrowing forms that are gape-limited and eat small invertebrate prey (Gauthier et al., 2012; Hsiang et al., 2015; Yi and Norell, 2015). The alternative phylogenetic positions of *Dinilysia* (Zaher, 1998; Longrich et al., 2012; Reeder et al., 2015) demonstrate that large semifossorial snakes, which likely consumed prey of diverse shapes and sizes and spent part of its time below a nonconsolidate ground excavated by itself, would have appeared early in snake phylogeny (premacrostomatan) (Albino and Caldwell, 2003; Albino, 2007, 2011; Albino and Brizuela, 2014). This does not provide specific support to the presumption of a subterranean origin of snakes as opposed to the hypothesis of an aquatic origin for this group, but, together with the record of madtsoiids, indicate that the earliest diversification of terrestrial snakes (i.e., not strictly aquatic nor fully subterranean) probably occurred in Gondwana (Albino, 2011; Albino and Brizuela, 2014).

ACKNOWLEDGMENTS

LITERATURE CITED

Albino AM. 2007. Lepidosauromorpha. In: Gasparini Z, Salgado L, Coria RA, editors. Patagonian mesozoic reptiles. Indiana: Indiana University Press. p 87–115.

Albino AM. 2011. Evolution of Squamata Reptiles in Patagonia based on the fossil record. Biol J Linn Soc 103:441–457.

Albino AM, Brizuela S. 2014. An overview of the South American fossil squamates. The Anat Rec p 349–368.

Albino AM, Caldwell MW. 2003. Hábitos de vida de la serpiente Cretácica *Dinilysia patagonica* Woodward. Ameghiniana 40:407–414.

Auen EL, Langebartel DA. 1977. The cranial nerves of the colubrid snakes *Elaphe* and *Thamnophis*. J Morphol 154:205–222.

Breazile J. 1979. Systema nervosum centrale. In: Baumel J, King A, Lucas A, Breazile J, Evans H, editors. Nomina anatomica avium, an annotated anatomical dictionary of birds. London: Academic Press. p 417–472.

Butler AB, Hodos W. 2005. Comparative vertebrate neuroanatomy: evolution and adaptation. New York: John Wiley & Sons.

Caldwell MW. 1999. Squamate phylogeny and the relationships of snakes and mosasauroids. Zool J Linn Soc 125:115–147.

Caldwell MW, Albino AM. 2002. Exceptionally preserved skeletons of the Cretaceous snake *Dinilysia patagonica* Smith Woodward, 1901. J Vert Paleontol 22:861–866.

Caldwell MW, Calvo J. 2008. Details of a new skull and articulated cervical column of *Dinilysia patagonica* Woodward, 1901. J Vert Paleontol 28:349–362.

Cundall D, Rossman DA. 1993. Cephalic anatomy of the rare Indonesian snake Anomochilus weberi. Zool J Linn Soc 109:235–273.

Edinger L. 1896. Untersuchungen über die vergleichende Anatomie des Gehirns. Neue Studien Über das Vorderhirn der Reptilien. Abh Sneckenb naturf Ges 19:313–386.

Edinger T. 1975. Paleoneurology 1804–1966. An annotated bibliography. Adv Anat Embryol Cell Biol 49:1–258.

Estes R, Frazzetta TH, Williams EE. 1970. Studies on the fossil snake *Dinilysia patagonica* Smith Woodward: Part I. Cranial morphology. Bull Mus Comp Zool Harv 140:25–74.

Filippi LS, Garrido AC. 2012. Nuevo registro del género *Dinilysia* (Squamata, Serpentes) para la formación Anacleto (Campaniano inferior-medio), Rincón de Los Sauces, Neuquén, Argentina. Ameghiniana 49:132–136.

Frazzetta TH. 1999. Adaptations and significance of the cranial feeding apparatus of the sunbeam snake (*Xenopeltis unicolor*): Part I. Anatomy of the skull. J Morphol 239:27–43.

Gauthier JA, Kearney M, Maisano JA, Rieppel O, Behlke AD. 2012. Assembling the squamate tree of life: perspectives from the phenotype and the fossil record. Bull Peabody Mus Natl Hist 53:3–308.

Hecht MK. 1982. The vertebral morphology of the cretaceous snake *Dinilysia patagonica* Woodward. N Jb Geol Paläont Mlt 1982:523–532.

Hopson JA. 1979. Paleoneurology. In: Gans, C, Northcutt RG, Ulinski P, editors, Biology of the Reptilia, v. 9, Neurology A. London: Academic Press.

Hsiang AY, Field DJ, Webster TH, Behlke ADB, Davis MB, Racicot RA, Gauthier JA. 2015. The origin of snakes: revealing the ecology, behavior, and evolutionary history of early snakes using genomics, phenomics, and the fossil record. BMC Evol Biol 15:87.

Jerison HJ. 1969. Brain evolution and dinosaur brains. Am Nat 103:575–588.

Jerison HJ. 1973. Evolution of the brain and intelligence. New York: Academic Press.

Larsson HCE, Sereno PC, Wilson JA. 2000. Forebrain enlargement among non avian theropod dinosaurs. J Vert Paleontol 20:615–618.

Lee MSY, Scanlon JD. 2002. Snake phylogeny based on osteology, soft anatomy and ecology. Biol Rev 77:333–401.

Linnaeus C. 1758. Systema Naturae per Regna tria Naturae secundum Classes, Ordines, Genera, Species, cum Characteribus, Differentiis, *Synonyms*, *Locus*, volume 1. Laurenti Salvii, Stockholm, 824 pp.

Longrich NR, Bhullar BAS, Gauthier JA. 2012. A transitional snake from the Late Cretaceous period of North America. Nature 488:205–208.

Macrini TE, Rowe T, Archer M. 2006. Description of a cranial endocast from a fossil platypus, *Obdurodon dicksoni* (Monotremata, Ornithorhynchidae), and the relevance of endocranial characters to monotreme monophyly. J Morphol 267:1000–1015.

Norman DB, Faiers T. 1996. On the first partial skull of an ankylosaurian dinosaur from the Lower Cretaceous of the Isle of Wight, southern England. Geol Mag 133:299–310.

Olori JC. 2010. Digital endocasts of the cranial cavity and osseous labyrinth of the burrowing snake *Uropeltis woodmasoni* (Alethinophidia: Uropeltidae). Copeia 1:14–26.

Oppel M. 1811. Die Ordnungen, Familien, und Gattungen der Reptilien als Prodrom einer Naturgeschichte derselben. München, Joseph Lindauer. p 87.

Paulina Carabajal A. 2009. El neurocráneo de los dinosaurios Theropoda de la Argentina. Osteología y sus implicancias filogenéticas. Tesis Doctoral, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata. p 540.

Rage JC, Albino AM. 1989. *Dinilysia patagonica* (Reptilia, serpents): matériel vertébral additionnel du Crétacé supérieur d'Argentine. Etude complémentaire des vertèbres, variations intraspécifiques et intracolumnaires. Neues Jahrb Geol Paläontol Monatsh 1989:433–447.

Reeder TW, Townsend TM, Mulcahy DG, Noonan BP, Wood PL Jr., Sites JW Jr., Wiens JJ. 2015. Integrated analyses resolve conflicts over squamate reptile phylogeny and reveal unexpected placements for fossil taxa. PLOS One 10:e0118199.

Rieppel O, Zaher H. 2000. The braincases of mosasaurs and Varanus, and the relationships of snakes. Zool J Linn Soc 129:489–514.

Romer AS. 1956. Osteology of the Reptiles. Chicago, Univ. Chicago Press. p 772.

Saveliev SV. 2008. Neurobiological approaches in vertebrate paleontology. Paleontol J 42:573–580.

Scanferla CA, Canale JI. 2007. The youngest record of the Cretaceous snake genus *Dinilysia* (Squamata, Serpentes). S AM J Herpetol 2:76–81.

Scanlon JD, Lee MSY. 2000. The Pleistocene serpent *Wonambi* and the early evolution of snakes. Nature 403:416–420.

Smith-Woodward A. 1901. On some extinct reptiles from Patagonia, of the genera *Miolania*, *Dinilysia* and *Genyodectes*. Proc Zool Soc Lond 70(2):169–184.

Starck D. 1979, Cranio-cerebral Relations in recent reptiles. In: Gans C, Northcutt RG, Ulinski P, editors. Biology of the Reptilia, v. 9. Neurology A. London: Academic Press, p. 1–38.

Tchernov E, Rieppel O, Zaher H, Polcyn MJ, Jacobs IJ. 2000. A new fossil snake with limbs. Science 287:2010–2012.

Triviño LN, Albino AM. 2015. Hallazgo de restos de la serpiente *Dinilysia patagonica* Smith-Woodward 1901 en una nueva localidad del Santoniano de Patagonia, Argentina. Est Geol 71:e033. https://doi.org/10.3989/egeol.41858.347.

Witmer LM, Ridgely RC, Dufeau DL, Semones MC. 2008. Using CT to peer into the past: 3D visualization of the brain and ear regions of birds, crocodiles, and non avian dinosaurs. In: Endo H, Frey R, editors. Anatomical imaging: towards a new morphology. Tokyo, Japan: Springer. p. 67–88.

Wyneken J. 2007. Reptilian neurology: anatomy and function. Vet Clin N Am Exot Anim Pract 10:837–853.

Yi H. 2013. Inner-ear morphology suggests burrowing behaviour in early snakes. 73rd Society of Vertebrate Paleontology Meeting. Los Ángeles, EE.UU. October, 242.

Yi H. 2015. Habitat specialization of early snakes: insights from the Late Cretaceous Gondwanan species. Libro de resúmenes, V Congreso Latinoamericano de Paleontología de Vertebrados (Colonia de Sacramento), 3.

Yi H, Norell MA. 2015. The burrowing origin of modern snakes. Sci Adv 1:e1500743.

Zaher H. 1998. The phylogenetic position of Pachyrhachis within snakes (Squamata, Lepidosauria). J Vert Paleontol 18:1–3.

Zaher H, Scanferla CA. 2012. The skull of the Upper Cretaceous snake *Dinilysia patagonica* Smith-Woodward, 1901, and its phylogenetic position revisited. Zool J Linn Soc 164:194–238.