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Organization of the cephalic lateral-line canals in *Electrophorus varii* de Santana, Wosiacki, Crampton, Sabaj, Dillman, Mendes-Júnior & Castro e Castro, 2019 (Gymnotiformes: Gymnotidae)

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Electrophorus spp. generate high-voltage electric discharges for defense and hunting, and low-voltage electric discharges (as other Gymnotiformes) for electrolocation and communication. Despite intense interest in the unusual electrogenic and electroreceptive capacities of electric eels, the other sensory systems of *Electrophorus* spp. are relatively poorly known. Here we describe the ontogenetic development and organization of the cephalic lateral-line canals in the lowland electric eel, *Electrophorus varii*. Preserved specimens of larvae, juveniles, and adults were examined to describe the spatial distribution of the canals and pores. Ontogenetic shifts of the cephalic lateral line formation were observed for each canal and support a hypothesis of non-synchronized development. The morphogenesis of cephalic canals in larvae and juveniles begins just before the onset of exogenous feeding. In adults, the cephalic sensory canals are formed separately from the skull and overlay cranial and mandibular bones and muscles. This study provides the first detailed description of the development and organization of the cephalic lateral-line system in *Electrophorus varii.*

Keyword: Electric eel, Life history, Mechanoreception, Ontogenetic shifts, Sensory system.

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Electrophorus spp. geram descargas elétricas de alta voltagem, usadas para defesa e caça, e (como os demais Gymnotiformes) descargas elétricas de baixa voltagem para eletrolocalização e comunicação. Apesar do grande interesse nas capacidades eletrogênicas e eletrorreceptivas incomuns dos poraquês, os outros sistemas sensoriais de *Electrophorus* spp. são relativamente pouco conhecidos. Aqui nós descrevemos o desenvolvimento ontogenético e a organização dos canais da linha lateral cefálica no poraquê da planície *Electrophorus varii*. Exemplares preservados de larvas, juvenis e adultos foram analisados para descrever a distribuição espacial dos canais e poros. Variações ontogenéticas na formação da linha lateral cefálica foram observadas para cada canal, apresentando um desenvolvimento não-sincronizado. Variações ontogenéticas da formação da linha lateral cefálica foram observadas para cada canal e suportam uma hipótese de desenvolvimento assincrônico. A morfogênese dos canais cefálicos em larvas e juvenis precede o início da alimentação exógena. Nos adultos, os canais sensoriais cefálicos se formam separadamente do crânio e sobrepõem os ossos e músculos cranianos e mandibulares. Este estudo fornece a primeira descrição detalhada do desenvolvimento e organização do sistema da linha lateral cefálica de *Electrophorus varii*.

Palavras-chave: História de vida, Mecanorrecepção, Mudanças ontogenéticas, Poraquê, Sistema sensorial.

INTRODUCTION

The genus *Electrophorus* comprises freshwater electric eels (known as poraquês in the Brazilian Amazon) that are able to generate high-voltage discharges of up to 860V (Crampton, 2019; de Santana *et al*., 2019) for defense and hunting (Catania, 2019). Like other Gymnotiformes, they also generate low-voltage electric discharges for electrolocation, prey detection, and communication with conspecifics (Moller, 1995; Crampton, Albert, 2006). During early life stages, however, electric eels are unable to generate high discharges to stun prey. This, in part, may drive ontogenetic changes in diet (Assunção, Schwassmann, 1995; Schwassmann *et al*., 2014). *Electrophorus* larvae are known to feed on eggs provided by an adult while still in the nest, and, as they grow up, the small eels start to eat small aquatic invertebrates (Assunção, Schwassmann, 1995).

Recently, de Santana *et al.* (2019) described two new species of *Electrophorus*, *E. varii* de Santana, Wosiacki, Crampton, Sabaj, Dillman, Mendes-Júnior & Castro e Castro, 2019, and *E. voltai* de Santana, Wosiacki, Crampton, Sabaj, Dillman, Castro e Castro, Bastos & Vari, 2019, based on molecular, anatomical and ecological evidence. This unexpected diversity suggests that, although electric eels have been known for almost 250 years, their basic biology may exhibit interspecific variation that is still poorly comprehended. Also, while many studies have focused on the electrogenic-electrosensory attributes of electric eels, the anatomy, physiology, and ecological relevance of their non-electrical sensory systems and their functional relevance have been overlooked (*e.g*., Hagiwara *et al*., 1965; Szabo, 1974; Gotter *et al*., 1998).

The lateral line system is responsible for detecting water movements generated by potential predators, prey and conspecifics (*e.g*., Coombs, Braun, 2003). In electric eels, it is presumed to play an important role in early life stages, before the electrogenic system is fully functional (Schwassmann *et al*., 2014). The distribution of the lateral line canals is also important as a morphological character used for taxonomic studies and phylogenetic relationships inferences (*e.g*., Arratia, Huaquín, 1995; Di Dario, de Pinna, 2006; de Santana, Crampton, 2010; Stephens, 2010; Crampton *et al*., 2016).

Despite its evolutionary and adaptive importance, little is known about the lateral line structure in *Electrophorus* species, with the main studies on the topic dating back to the 1970s and focusing on the electroreceptive organs associated with the mechanosensory system (*e.g*., Szabo, 1965, 1974). The only detailed anatomical description of the lateral-line canals of *Electrophorus* is provided by Pastana (2014) who included cephalic lateral-line features as characters in a study of characiform phylogeny (the study included *Electrophorus electricus* and some other gymnotiform species as outgroups).

The spatial distribution of lateral-line canals and pores on the cephalic surface has been broadly related to the habitats use and feeding habits in fishes (*e.g*., Arratia, Huaquín, 1995). Nonetheless, despite numerous investigations of the importance and function of their electric organs (Szabo, 1966; Schwassmann *et al*., 2014) the mechanoreceptive and other non-electrical sensory systems of *Electrophorus* species are poorly known. Likewise, most investigations of predatory behavior in *Electrophorus* species have focused on the strength of the high-voltage electric discharges for striking prey, mostly fishes and invertebrates (Catania, 2014; Oliveira *et al*., 2020; Bastos *et al*., 2021). However, the feeding behavior of early ontogenetic phases have been neglected and there is a lack of information on how the electric eels search and locate their prey. Due to the highly reduced visual systems of most gymnotiforms (Albert, Crampton, 2006), the mechanosensory lateral line system may supplement the electrosensory system in facilitating prey localization (Westby, 1988). Here we describe the early development and organization of cephalic lateral-line canals in *Electrophorus varii* and explore correlations between lateral line ontogeny and larval and juvenile feeding behavior.

MATERIAL AND METHODS

Sampling sites. Specimens of *E. varii* were collected in two different sites of the Brazilian Amazon Basin: a *terra-firme* stream at Adolpho Ducke Forest Reserve (Rio Negro drainage, 2°59'02.4"S 59°55'55.2"W), located at the northeastern outskirts of the city of Manaus, and a whitewater lake, Purupuru Lake (Rio Solimões drainage, 3°24'03.6"S 59°51'03.6"W), in the Careiro da Várzea municipality (Fig. 1). Procedures for sampling followed INPA's ethics committee rules (protocol number 044/2016).

Adolpho Ducke Forest Reserve. Nursery sites were monthly monitored from July 2017 to September 2018 at Igarapé Bolivia, a *terra-firme* stream in the reserve, using electric fish finders (Crampton *et al*., 2007). Five nests were located under stream banks, where the development of the nestlings was monitored. The nests were visited every two-three days for behavioral record-taking and to sample up to five specimens per nest and event; these were used to build an ontogenetic series. To collect specimens in the

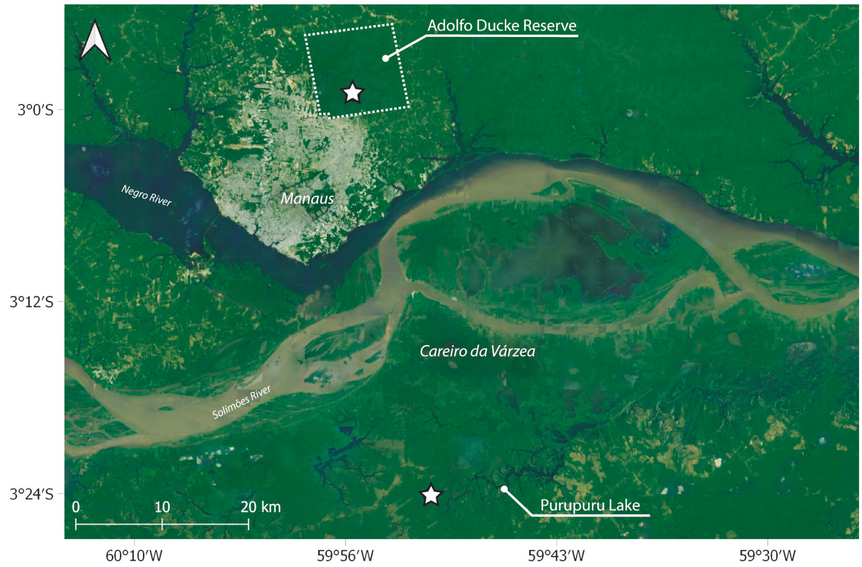

FIGURE 1 | Sampling areas (Adolpho Ducke Reserve and Purupuru Lake, Amazonas, Brazil) where individuals of *Electrophorus varii* were captured. The white stars represent the exact sampling spots.

first weeks after hatching, we dug small openings on the stream bank above the nest cavities. After the second month of life, the eels were collected at night as they foraged outside the nest. All specimens were preserved in 4% buffered formalin and conserved in 75% ethanol solution.

Purupuru Lake. Sampling in Purupuru Lake focused on adult individuals and was conducted in November 2018, using hook-and-line. The specimens were preserved in 10% formalin solution and conserved in 75% ethanol solution.

Ontogenetic series and staging. The number and distribution of lateral-line pores and canals were examined in 22 larvae and 10 juvenile specimens, ranging from 1.5 to 38.0 cm TL, and two adults of 148.0 and 111.5 cm TL. The distinction between larvae and juveniles was made following a staging protocol based on anatomic and behavioral characteristics (Verçoza, 2020), where larvae are recognized as the individuals measuring between 1.0 to 15.0 cm TL under parental care, juveniles as the specimens without parental care ranging from 15.0 to 85.0 cm, and adults as the mature specimens above 85.0 cm TL (Mendes-Júnior *et al*., 2016) (shown in the Fig. **S1**).

Larval and juvenile feeding behavior. The larval and juvenile observations were carried out only at the Ducke Reserve due to environmental conditions such as transparent water and nest location. Field observations were made from both outside water (from the banks) and underwater (using a GoPro Hero 3+ and a Panasonic HX-A1 camera, as well as a portable borescope) at the same days of specimens collection for anatomical analyses. To obtain images from the interior of the nest cavity, one of the image-capturing devices was inserted next to the foam structure that composes the upper layer of the nest. The feeding behavior was classified in two phases following

Bastos (2020): I – larval feeding inside the nest by eggs provided by the adult eel(s), and II – juvenile foraging outside the nest, consuming small invertebrates.

Anatomical analysis. Larvae and juvenile specimens were examined under a stereomicroscope (16x). Adult specimens were examined both by naked eye and using a stereomicroscope, after the removal of the epidermis of the head and the anterior surface of the trunk to expose the cephalic (or cranial) lateral-line canal's structure (**S2**). Three-dimensional visualization of the cranium as well as the analysis of the reconstructed data was performed using VGStudio MAX 2.2.3 64 bit (Volume Graphics GmbH, Heidelberg, Germany).

Pores were quantified by counting on both left and right sides of the head and for each canal. Organization and connections of the canals were observed by injecting a dye (0.5% toluidine blue solution) into the pores. The terminology of cephalic lateral line canals followed Northcutt (1989): infraorbital (IO), mandibular (MD), otic (O), pre-opercular (PO), supraorbital (SO) and supratemporal (ST). Some other terms used in the current study followed Webb (2013). "Groove" refers to the canal structure before its enclosure, when the canal neuromasts are totally exposed. "Segment of canals" refers to a short section of canals that after the enclosure process becomes fused to the adjacent segment, resulting in a lateral-line pore. "Pore" refers to the canal opening on the skin surface that serves to maintain canal neuromasts in contact with the external environment.

Voucher specimens of *E. varii* were catalogued at Fish Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil (larvae and juveniles: INPA-ICT 059502 – 059510; adults: INPA-ICT 059511 – 059513).

RESULTS

Lateral-line canals in larval and juvenile specimens. Although the hatchlings range from 0.8 to 1.1 cm TL, the occurrence of externally visible lateral-line canals was firstly detected in individuals of 1.9 cm TL. At this size, short grooves start to appear on the head surface, marking the beginning of canal morphogenesis and originating the presumptive infraorbital and mandibular canals (Fig. 2A). This event occurs after consumption of the yolk and just before the individuals initiate the exogenous feeding on provided eggs.

At 2.5 cm TL, the grooves become deeper into the skin, clearly marking the supraorbital, infraorbital, mandibular, otic and pre-opercular canals (Fig. 2B). At this size, the individuals start exhibiting more directional, oriented swimming and are able to actively avoid being caught by a hand net.

After reaching nearly 3.0 cm TL, the anteriormost grooves of the infraorbital, mandibular and supraorbital canals start their closure, forming a series of short canal segments. Lastly, the fusion between two canal segments is accompanied by the appearance of spots that represent where the lateral-line pores will be located (Fig. 2C). At this stage the otic and pre-opercular canals are still open and the supratemporal canal becomes visible. The otic canal is located posterior to the confluence of the infraorbital and supraorbital canals on the cephalic surface. A small pit is formed at the end of the

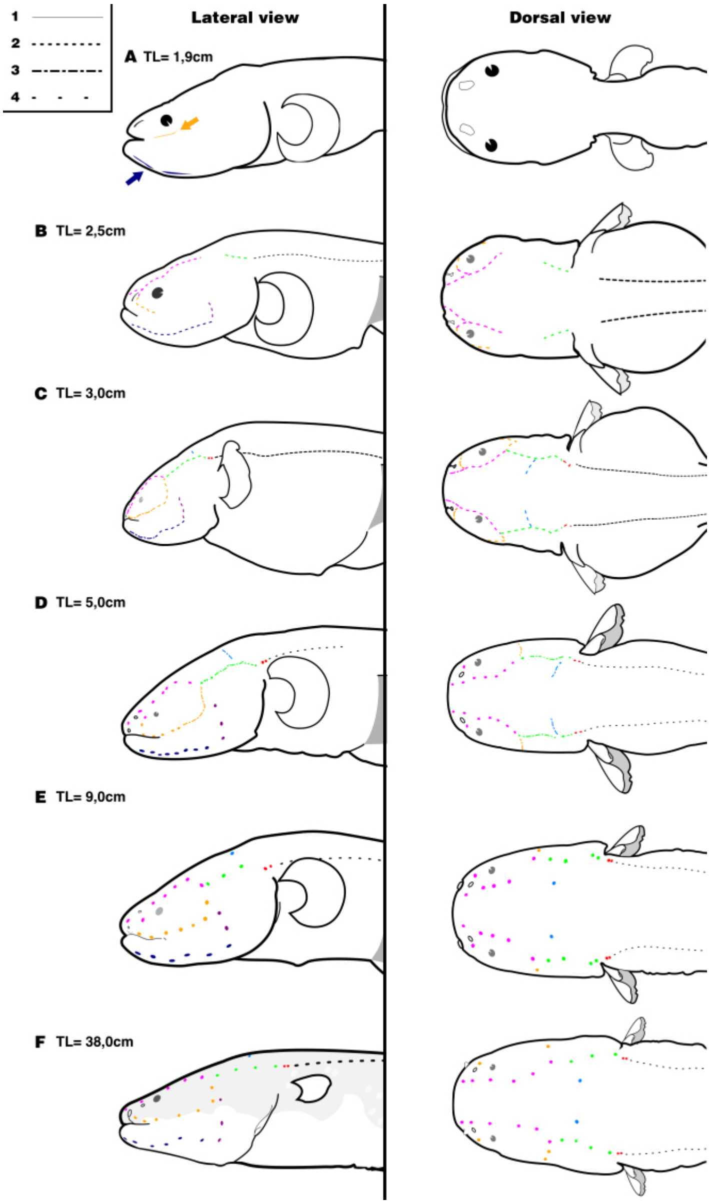

FIGURE 2 | Ontogenetic development of the lateral-line canals in larvae and juveniles of *Electrophorus varii*, in lateral and dorsal views. **A.** 1.9 cm TL; **B.** 2.5 cm TL; **C.** 3.0 cm TL; **D.** 5.0 cm TL; **E.** 9.0 cm TL; **F.** 38 cm TL. Types of dashes indicate the (1) shallow and short grooves on the skin; (2) deep grooves; (3) segments of grooves start enclosure; (4) pores of canals on the skin. Yellow: infraorbital canal; navy blue: mandibular canal; green: otic canal; purple: pre-opercular canal; pink: supraorbital canal; light blue: supratemporal canal; grey: posterior lateral-line canal of the trunk and; red: otic pit between cephalic and trunk canals. Arrows indicate short grooves of the presumptive infraorbital and mandibular canals.

otic canal, posteriorly in the skull, marking the limit to the trunk lateral-line canal. Laterally positioned on the head, the pre-opercular canal forms a continuum connected to the posterior section of the mandibular canal. Ventrally, the anteriormost pores of the mandibular canal are located at the tip of the jaws, just below the dentaries symphysis.

The formation of canal segments continues until fish reach approximately 5.0 cm TL. At this length, the posterior portion of the supraorbital and mandibular canals, as well the dorsal portion of the pre-opercular, are closed. The infraorbital, otic and supratemporal canals already have the grooves edges fused, but most parts of those canals remain open. The surrounding epithelium covers the otic pit, forming a double-pored structure dorsally to the base of the pectoral fins (Fig. 2D).

The complete set of pores and fused canal segments is present at nearly 9.0 cm TL, when individuals show a cephalic lateral-line morphology similar to that of the adults, with large, well-separated pores over the skin surface (Figs. 2E–F). Nevertheless, pores may be commonly found doubled in all canal series. Individuals of this size start to forage outside the nest, feeding on small invertebrates associated with the substrate.

The number of pores also varies on the mandibular canal, specifically for the symphyseal pores (located on the symphysis of the mandible) that connect both left and right branches of the canal. In small specimens, there are two pores on this region that seem to fuse, forming an arch-like pattern (Fig. 3C). The number of pores or short segments since the first appearance to the end of morphogenesis in each canal is shown in Tab. 1.

Cephalic lateral-line canals in adults. The cephalic lateral-line canals in adults of *Electrophorus varii* are positioned beneath the thick epidermis and pores are visible on the head surface. All canals present tubules terminating in large pores with stiffened edges (Fig. 3). The canal walls are relatively rigid and become partially ossified in adult stages (Fig. 4).

The supraorbital canal stretches along the frontals and extends towards the tip of the snout. At midway between the posterior nare and the orbit, the supraorbital receives a branch of the infraorbital canal. The infraorbital is a long canal, running along a large stretch from the back of the skull, surrounding the eye, until deviating dorsally towards the supraorbital canal. Thus, infraorbital and supraorbital canals are connected anteriorly and posteriorly, making a closed circuit. Both canals branch off small tubules that open in the skin pores. The posterior confluence of the supraorbital and infraorbital is connected to the otic canal, forming a triple joint.

TABLE 1 | Number of pores of cephalic lateral-line canals during the ontogeny of *Electrophorus varii*. Mean number of segments or pores are presented for each canal in larval/juvenile and adult specimens.

Canals	First appearance (TL, cm)	End of morphogenesis (TL, cm)	N° of pores or segments	N° of pores (adults)
Infraorbital (IO)	1.9	8.4	6 – 8	8
Mandibular (MD)	1.9	5.1	6 – 7	6
Otic (O)	2.5	8.7	4 – 5	3 – 4
Preopercular (PO)	2.5	5.0	3 – 4	3
Supraorbital (SO)	2.5	5.1	7 – 10	7 – 8
Supratemporal (ST)	2.8	7.0	1 – 3	1

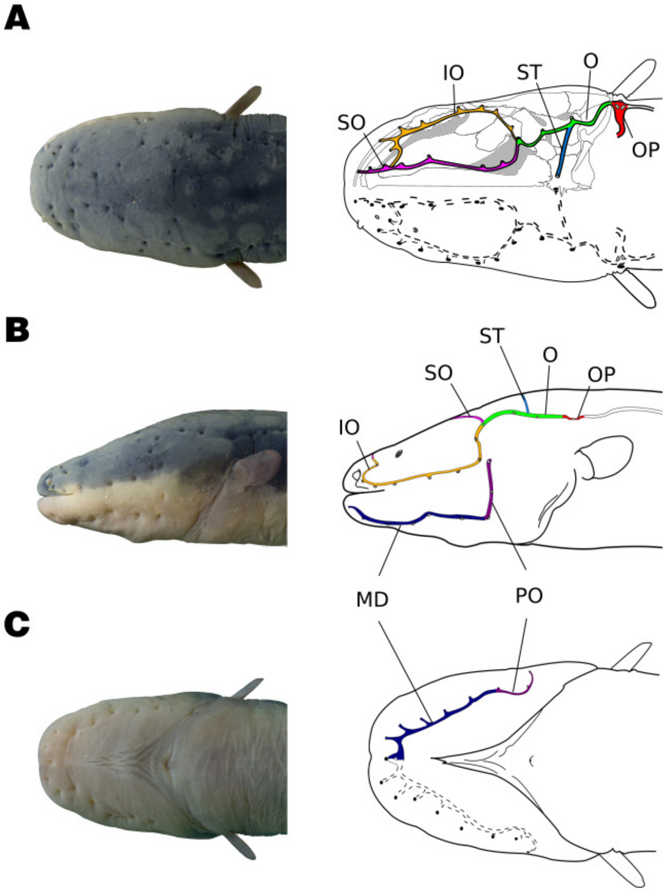

FIGURE 3 | Organization of the cephalic lateral-line canals in adult *Electrophorus varii*. Arrangement of all canals in **A.** dorsal, **B.** lateral and **C.** ventral views. Yellow: infraorbital canal; navy blue: mandibular canal; green: otic canal; purple: pre-opercular canal; pink: supraorbital canal; light blue: supratemporal canal; grey: posterior lateral-line canal of the trunk; red: otic pit (OP) between cephalic and trunk canals. The photographs are from a juvenile that completed the development and is morphologically similar to an adult. The drawings are from an individual of 148 cm TL.

The otic canal is relatively long, extending from its joint to the supraorbital and infraorbital confluence until the otic pit, bearing three to four pores. The supratemporal canal is represented by a single long tubule, derived from the otic canal mid area, oriented mesially on the parietal portion, and terminating in a single pore. The posterior edge of the otic canal ends on the otic pit, which is connected to the lateral-line canal on the trunk and extends towards the end of the body. The otic pit is a depression on the skin that is also observed in the larval phase (Fig. 2C, the early stage of its appearance is represented by two dots in red), but much more conspicuous in adults. The otic pit, underneath the skin, shows three exits: more anteriorly, it is the exit of the end of the otic canal, more posteriorly, it is the surfacing of the body lateral line, and another in the middle. In fact, the otic pit represents a deep penetration of the otic canal into the body before surfacing as the body lateral line: opposite to the deep external pore, the thin and flexible canal wall is connected to an internal tubule that penetrates behind the head between the epaxial and hypaxial (*obliquus superioris*) muscles and expands as

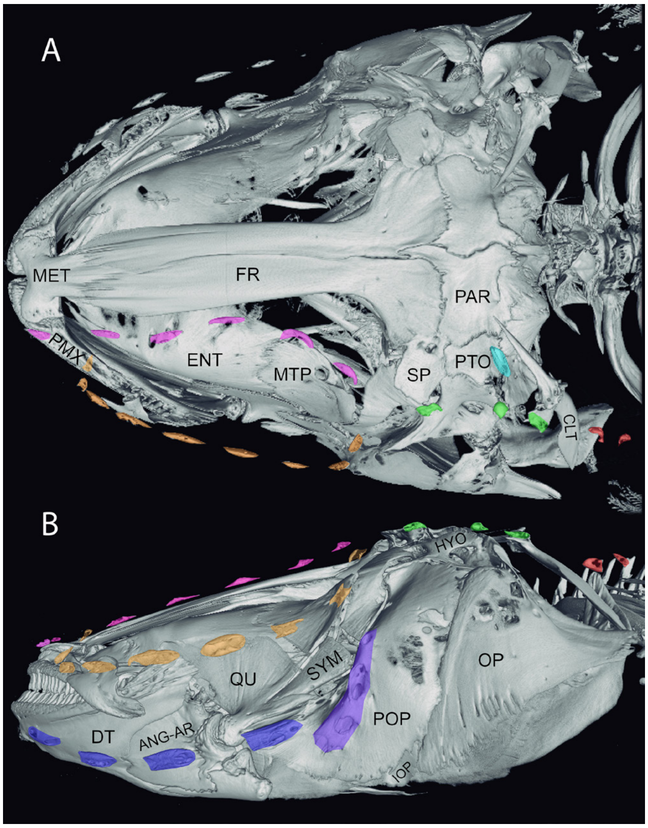

FIGURE 4 | Images of CT scan of the head of *Electrophorus varii.* **A.** dorsal and **B.** left lateral views of the cranium show the ossification of the lateral-line canals. The ossified segments of canals are marked by colors. Yellow: infraorbital canal; navy blue: mandibular canal; green: otic canal; purple: pre-opercular canal; pink: supraorbital canal; light blue: supratemporal canal; red: otic pit ossicles. ANG-AR: Angulo-articular; CLT: cleithrum; DT: dentary; ENT: entopterygoid; FR: frontal bone; HYO: Hyomandibular; IOP: interopercle; MET: mesethmoid; MTP: metapterygoid; OP: opercle; PAR: parietal; PMX: premaxila; POP: preopercle; PTO: pterotic; QU: quadrate; SP: sphenotic; SYM: sympletic. Specimen size = 82 cm TL.

a chamber. This expanded chamber-like tubule presents a conspicuous sinuosity and is apparently connected to the anterior portion of the swim bladder by a short chamber with a flexural wall (Fig. 3A).

The mandibular canal is positioned latero-ventrally to the mouth and follows underneath and along the jaw and branches off in six to seven tubules that terminate in pores. Both sides of the mandibular canal are connected by a junction of two tubules that end in a pore in the symphysis of the jaw. Posteriorly, the pre-opercular canal forms a continuum with the mandibular canal, makes a turn on the opercular elements and turns dorsally, towards the cranium. In contrast to all other canals, the pre-opercular canal is partially embedded in the underlying dermal bone, with pores located at the point of junction between two segments of canals. Pores are present in all canals and their number may vary between individuals due to cases of 'pore doubling', *i.e.*, where one tubule opens in two pores (Tab. 1).

DISCUSSION

The morphogenesis of cephalic sensory canals in larvae and juveniles of *E. varii* begins just before the onset of the exogenous feeding. This timing is also observed in other sensory structures, such as the electric organ (Schwassmann *et al*., 2014), which in some fishes occurs synchronically to the yolk depletion and a rapid skull development. These synchronous changes are commonly associated with the start of exogenous food sources consumption (*e.g*., Mathias, Li, 1982).

Unlike most fishes, where the morphogenesis of the cephalic lateral-line canals is closely related to dermatocranial bones (Webb, Shirey, 2003), *E. varii* presents ossified lateral-line canals separately formed from the skull and overlying cranial and mandibular bones and muscles. The pre-opercular canal, however, is an exception, as it is embedded in the underlying bone. Similar morphology of cephalic lateral-line canals has also been observed in some apteronotid species (Albert, 2001) and Ophidiiformes (Pastana *et al*., 2020). The tubules of *E. varii* are arranged in the sub-epidermal layer and terminated in pores on the skin surface. Nevertheless, ontogenetic shifts of the formation of lateral-line canals are similar to those observed for other teleost fish (Webb, Shirey, 2003; Mukai *et al*., 2008; Pastana *et al*., 2020). Early larvae of *E. varii* present canal segments turning into deeper depressions and arranged mostly as conspicuous longitudinal grooves. As already described in some teleosts (*e.g*., Tarby, Webb, 2003), asynchronous development of the cephalic lateral line canals is also observed in *E. varii*. While infraorbital, mandibular and supraorbital canals are completed from rostral to caudal direction, the opposite orientation (caudal to rostral) was observed for the otic canal. No evidence of development orientation could be detected for the pre-opercular and supratemporal canals. However, this should be verified with histological analysis.

The overall development and ossification patterns of the cephalic lateral-line canals of larval phase of *E. varii* are similar to those reported in the literature for most bony fishes (Tarby, Webb, 2003; Webb, Shirey, 2003; Pastana *et al*., 2020; Rizzato *et al*., 2020). However, in *E. varii* all canals lie in the skin, completely independent of, and located over cranial and mandibular bones and muscles. This total lack of bony connection of the canals with the cranium is an unusual feature among teleosts (Webb, 2013). The integration of cranial lateral-line canals into the underlying bones has been thoroughly examined in fishes and classified as 'two-component' (when there is a fusion of two minor ossifications) or 'one-component' (where the underlying bones remain independent throughout the fish's life) (Lekander, 1949). However, a distinct and unusual pattern was observed in *E. varii*. The cephalic lateral-line canals are immersed into skin, without a direct association with the cranial bony elements during the canals formation. This situation precludes classifying *E. varii* as possessing either a two-component or one-component process of lateral line bones development (Lekander, 1949; Tarby, Webb, 2003; Webb, Shirey, 2003). Instead, canal formation in *E. varii* occurs independently of the underlying bones. As mentioned above, an exception was observed in the pre-opercular canal, which has the lateral line dependent on the underlying bone; this being a more typical case of developmental integration between lateral line canals and dermatocranial bones.

Electrophorus varii begins voracious predatory feeding in areas close to the nest at around 60 days after hatching. The larvae were seen foraging close to the substrate searching for prey (Bastos, pers. obs.), such as invertebrates and fishes (Assunção, Schwasmann, 1995). The robust and thick skin of the head of electric eels possibly help support injuries caused by objects in the water such as those caused by pieces of palm spines that were commonly found inserted in their epidermis. Along the ontogenetic development of *E. varii*, the lateral-line canals become progressively ossified in segments; this segmented ossification is maintained throughout the adults. Tomographic images show a restricted laminar dermal ossification on the infraorbital and mandibular canals, a condition similar to some Apteronotidae (Bernt *et al*., 2018). The pores present stiffened edges on the skin surface but structural composition analysis is required to explore whether this stiffening is the result of keratinization or mineralization.

Even though the lateral-line canal of the trunk has not been studied in detail in this paper, we noted that its wall is more flexible than in the cephalic branches (although this remains to be confirmed with histological analysis). In general, the evolutionary significance of the partial ossification of cephalic lateral-line canal' walls and its disconnection of the cranial bones in *E. varii* is not clear among Gymnotiformes or other Ostariophysi, and deserves further study (see *e.g*., Arratia, Huaquín, 1995; Britz, Moritz, 2007).

Complete formation of the canal pores was detected only in late larvae (~9.0 cm TL) when foraging behavior had already begun. Foraging behavior in (adult) Gymnotiformes is usually associated only (or mostly) to the electrosensory system. However, considering that feeding is a multisensory task (Liang *et al*., 1998) and given the known role of the lateral-line canals in both prey detection (Coombs *et al*., 2001) and schooling (Blaxter, Fuimann, 1989), the ontogenetic timing of the finalization of the canal pores highlights the likelihood of a strong role for mechanoreception in the foraging behavior of *E. varii* in individuals of *ca*. 9+ cm size range. Because parental guarding still occurs at this size, we conjecture that the lateral-line system may also act as an additional source of parent-offspring communication through intraspecific mechanical signals, which occurs during mating and spawning behaviors in some other fish groups (*e.g*., Satou *et al*., 1994; Coombs, Braun, 2003).

Pores of the lateral-line canals system open to the skin surface by short tubules that extend from the main canal through the thick epidermis. All pores in *E. varii* adults are large and clearly visible on the skin surface, as is common in other fishes occupying habitats with limited visibility and low levels of acoustic noise (Branson, Moore, 1962; McAllister, 1968; Hassan, 1989).

Despite being well developed, the cephalic lateral-line canals of *E. varii* are relatively simple in comparison to those of other Otophysi (*e.g*., Blaxter, 1987). All the dorsal cephalic canals (SO, IO, ST, and O) are connected to each other and are linked to the ventral canals (MD and PO) by a connection between PO and IO. Connection of canals of both left and right sides of the head was observed only for the mandibular canal. Although the supratemporal commissure is present in other fishes (Arratia, Huaquín, 1995; Webb, 2013), the lack of connection between the left and right sides of the head probably provides more accuracy for the localization of mechanical disturbances in the water. The anterior and posterior connections between supra- and infraorbital have been also observed in some species of Characiformes (*e.g*., Pastana, 2020), and Siluriformes (*i.e*., *Rhamdella* Eigenmann & Eigenmann, 1888, in Bockmann, Miquelarena, 2008 and *Pimelodella* Eigenmann & Eigenmann, 1888 by Slobodian, Pastana, 2018). For Gymnotiformes, anterior and/or posterior connections were described for *Orthosternarchus* species (Albert, 2001) and for some other apteronotids (Bernt *et al*., 2018) and appear to be an uncommon but phylogenetically widely-distributed feature in Otophysi.

A single pore dorsally located on the basis of the pectoral fin, the otic pit, sunk in the surrounded skin is of particular interest since it delimits an internal cavity that comprises the interface of the cephalic lateral-line canals and the trunk (posterior lateral-line) canal. In a deeper dissection, we noted that the internal wall, just opposite the pore of the cavity, has a thin membrane that covers a large lateral chamber filled with a lymph-like fluid, similar to those observed in many other fishes, such as the catfish *Ancistrus* sp. and cobitids (Bleckmann *et al*., 1991; Kratochvil, Ladich, 2000).

The otic pit cavity is located in the same position of the structure described as a pseudotympanum by Dutra *et al.* (2015) for a 28.2 cm TL specimen of *E. varii* (listed therein as *Electrophorus electricus* (Linnaeus, 1766)). A pseudotympanum has been described as a hiatus devoid of musculature that is thought to enhance the reception of external body vibration (Birindelli, Shibatta, 2011; Dutra *et al*., 2015). In our analyses we have not found any evidence of an empty area in adult specimens, but instead observed the expansion of the sinking canal of the otic pit in a walled chamber at this location (**S2C–D**).

Moreover, the connection of cranial canals (SO and IO via O, and OP) to the trunk lateral-line canal and to a lateral chamber, supposedly related to the auditory region, resembles the *recessus lateralis* of the lateral-line system described for clupeomorph fishes (Blaxter, 1987; Di Dario, 2004). By unraveling novelties about cephalic lateral line structure and organization on *E. varii*, this work marks the starting point of a detailed investigation to understand the functional relevance of the cephalic lateral line system in those organisms and its connections with the other sensorial systems in *Electrophorus varii*.

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COMPETING INTERESTS

The authors declare no competing interests.

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