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A short note on the terminal nerve in *Callorhinchus milii* (Callorhinchidae, Holocephali, Chondrichthyes)

by

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Résumé. – À propos du nerf terminal de *Callorhinchus milii* (Callorhinchidae, Holocephali, Chondrichthyes)

Le nerf terminal a été décrit récemment en détail chez les holocéphales. Cependant, cette étude portait uniquement sur deux espèces (*Hydrolagus colliei* et *Chimaera monstrosa*) de Chimeridae, la famille la plus dérivée parmi les holocéphales actuels. Nous complétons ici le précédent travail en décrivant, pour la première fois, le nerf terminal chez la chimère éléphant (*Callorhinchus milii*) dans son dernier stade ontogénétique, à partir de coupes sériées d'un spécimen. Le trajet du nerf terminal de *C. milii* ressemble à celui des autres holocéphales. Nous proposons que la position plutôt ventrale et médiane soit la condition de base pour les holocéphales actuels et peut-être pour les gnathostomes.

Keywords. – Chimaeriformes – Callorhinchidae - *Callorhinchus milii* - Neuroanatomy - Olfactory epithelium - *Nervus terminalis*.

Extant holocephalan fish are an ancient group among recent gnathostomes and are considered sister-group to Elasmobranchii (sharks, batoids, and rays) (e.g. Zangerl, 1981; Maisey, 1986). Thanks to their exceptional morphology, a unique combination of primitive gnathostome and spectacular derived characters, they have been studied for a long time and much work has concentrated on their cranium (e.g. Cole, 1896; Dean, 1906; Allis, 1917; Kesteven, 1933; De Beer and Moy-Thomas, 1935; Stahl, 1967; Didier, 1995). Information of morphology and biology comes mainly from two species, *Hydrolagus colliei* (Lay & Bennett, 1839) and *Callorhinchus milii* Bory de St. Vincent, 1823. Particular interest on the morphology and genetics of *C. milii* arose during the last years, which is not only because this species is more easily accessible but also because *C. milii* belongs to Callorhinchidae, the basal sister family of the other two extant families Chimaeridae and Rhinochimaeridae (e.g. Didier, 1995; Licht *et al*., 2012; Pradel *et al*. 2013; Jerve *et al*., 2014; Ryll *et al*., 2014; Venkatesh *et al*., 2014).

Some investigations have been performed on the cranial nerves of holocephalans (e.g. Cole, 1896; Liu, 2001) and the cranial nerves of holocephalan fish still conceal unknown information about gnathostome evolution (Pradel *et al*. 2013). Though the cranial nerves have been often examined under different aspects, the *nervus terminalis* was described for holocephalans only recently, in 2012 by Licht and Bartsch, thereby correcting a doubtful statement that this nerve is not present in Holocephali (Liu, 2001). But the terminal nerve has never been described for *Callorhinchus* and even Schauinsland (1903) missed it in his otherwise very informative 3-D reconstruction of the head and brain of *C. milii*.

That this nerve should not be present in all holocephalan families was particularly surprising, considering that it is present and to some extent prominent in the Elasmobranchii (e.g. Locy, 1899, 1905; Norris and Hughes, 1920; Demski *et al*., 1987). Nevertheless, Licht and Bartsch (2012) had only access to two species (*Hydrolagus colliei* and *Chimaera monstrosa* Linnaeus, 1758), which just represent the most derived family (Chimaeridae) of extant holocephalans and were not able to demonstrate the presence of the terminal nerve in Callorhinchidae. However, it would be particularly interesting if the pattern of the *nervus terminalis* in Callorhinchidae, was similar to that in Chimaeridae (i.e. an entirely ventro-mesial position relative to the *bulbus* and *tractus olfactorius* and elongate forebrain hemispheres), or showed a more dorsal course as in elasmobranchs (e.g. Locy, 1899, 1905). Here, we now supplement our study and demonstrate a ganglionated terminal nerve, well-separated from the fibre bundles of the olfactory nerve and tract in the holocephalan *Callorhinchus milii*.

MATERIAL AND METHODS

This study is based on a series of histological transverse sections of a pre-hatchling of *Callorhinchus milii* (NMNZ No. 1670), stored at the Museum für Naturkunde (Berlin). The specimen was caught in April 1955 in Day's Bay (Wellington, New Zealand): total length = 72 mm; head length = 19 mm. The head was embedded in Paraffin, and serially cut into 10 μm-thick sections, stained in Heidenhain's AZAN, examined in a Leica DME microscope, and photographed with a Zeiss AxioCam MRc 5.

RESULTS

Though histological preservation of the specimen is far from ideal, it was easy to follow the course of the terminal nerve along the telencephalic hemispheres. The *nervus terminalis* has its anterior origin at the olfactory epithelium and nasal capsule in the region of the anteriormost fibres of the *nervus olfactorius*. The following

† Note: Martin Licht, the first author of this study, friend, colleague and a gifted and enthusiastic younger researcher in ichthyology and paleontology, passed away on Friday, September 11, 2015 all of a sudden. Sadly, he did neither see this study published, nor the prints of his recent work on *Tetragonurus*. Our thoughts are with him, and his family he leaves behind.

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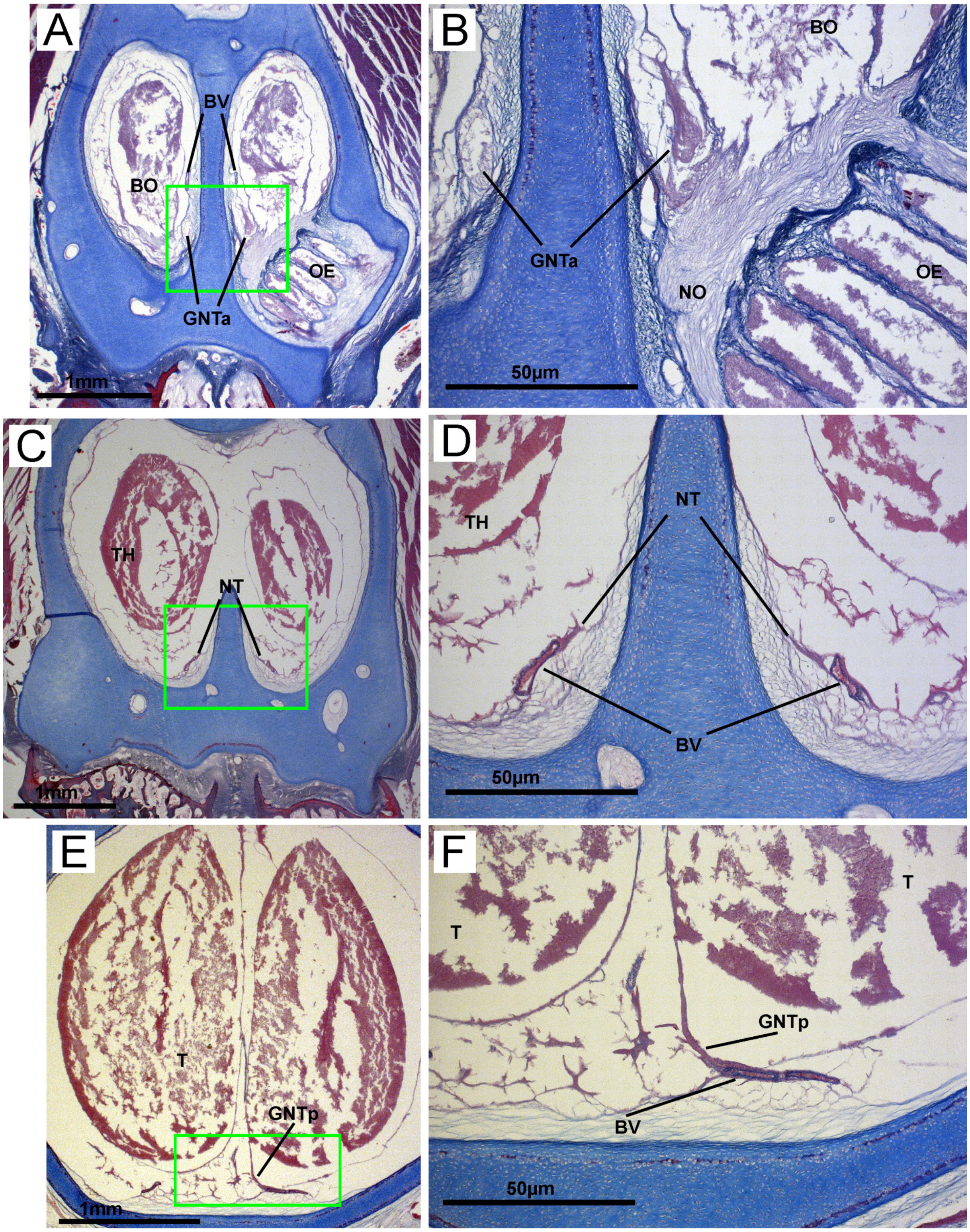

Figure 1. - Histological transverse sections through nasal sac and forebrain of a pre-hatchling of *Callorhinchus milii* (NMNZ No 1670), respectively. **A**: Anterior ganglion of the *nervus terminalis*, close to the olfactory epithelium and nasal capsule in the region of the fibres of the *nervus olfactorius*. **B**: Larger magnification of squared region in figure 1A. **C**: Relative position of the terminal nerve close to the nasal septum on its course posterior along the *bulbus* and *tractus olfactorius*. **D**: Larger magnification of squared region in figure 1C. **E**: Posterior ganglion of the *nervus terminalis* at the medioventral part of the telencephalon in *C. milii*. **F**: Larger magnification of squared region in figure 1E. Abbreviations: (BO) *bulbus olfactorius*, (BV) blood vessel, (GNTa) anterior ganglion of *n. terminalis*, (GNTp) posterior ganglion of *n. terminalis*, (NO) *n. olfactorius*, (NT) *n. terminalis*, (OE) olfactory epithelium, (T) telencephalon, (TH) telencephalic hemisphere.

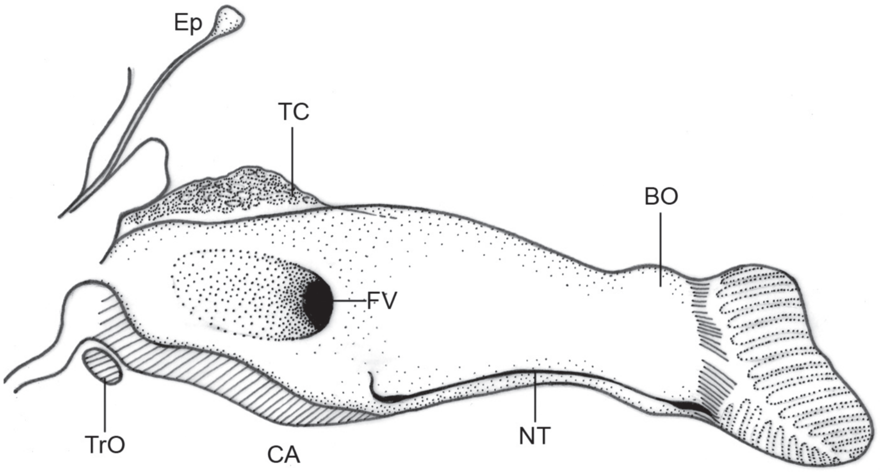

Figure 2. - Sketch of the forebrain and course of the terminal nerve of the pre-hatchling of *Callorhinchus milii* in sagittal section showing the left hemisphere. Abbreviations: (BO) *bulbus olfactorius*, (CA) anterior commissure, (Ep) epiphysis, (FV) ventricular foramen, (NT) *nervus terminalis*, (TC) tela choroidea (of the median forebrain ventricle), (TrO) *tractus opticus*.

course of the terminal nerve in *C. milii* is also consistent with the course in *Chimaera* and *Hydrolagus*. Licht and Bartsch (2012) described the course as follows: its anterior ganglion lies on the inner ventral side of the first cranial nerve close to the nasal septum and sends afferent branches to the *nervus olfactorius* and/or close to the olfactory epithelium (Fig. 1A, B). The relative position of the nerve does not change, following its course along the olfactory nerve posteriorly towards the *bulbus* and *tractus olfactorius* (Fig. 1C, D). A second ganglion is situated at the medioventral part of the telencephalic hemisphere (Fig. 1E, F). Finally, the *nervus terminalis* enters the telencephalon mediodorsally (Fig. 1E). During its course, the terminal nerve is closely associated with blood vessels, in particular a distinct anterior branch of the inner carotid artery (Fig. 1D, F).

DISCUSSION

It is generally known that the terminal nerve is present in most, if not all, vertebrates (e.g. Demski and Schwanzel-Fukuda, 1987) and also in holocephalan fish (Licht and Bartsch, 2012). But it has not been detected in the most basal extant family Callorhinchidae. Licht and Bartsch (2012) stated the question that it would be interesting whether the course of the terminal nerve in the Callorhinchidae resembles more those of the related elasmobranchs, or if it is like in the more derived extant holocephalans *H. colliei* and *C. monstrosa*. In elasmobranch fish, the terminal nerve rests on the rostrodorsal surface of the olfactory bulb and runs dorsal or medial to the *bulbus olfactorius* and *tractus olfactorius* (e.g. Locy, 1905; Demski, 1987), while in *C. milii* the terminal nerve has its entire course ventral and medial to the *bulbus* and *tractus olfactorius* as in other holocephalans (e.g. Licht and Bartsch, 2012) (Fig. 2). Because of the same pattern of the *nervus terminalis* in both extant holocephalan families, we conclude that this more ventral and median course is basic for extant Holocephali. However, the terminal nerve is still undescribed for Rhinochimaeridae, and also for fossil holocephalans with a different cranial anatomy. Possibly, the course of the terminal nerve coincides with the special cranial morphology and far ventral position of nasal sac and capsule, and the elasmobranch condition is the more basal one also for the holocephalan clade. In Dipnoi adults and larvae, as shown in Pinkus (1895) and Bartsch (1993), the course is more similar to that in elasmobranch fish. But we assume that the ventral and median position, as well as the course to the olfactory tract and bulb, is a plesiomorphic character for gnathostomes, as also shown in Actinopterygians (Allis, 1897: plate XXXVIII, fig. 64; Brookover, 1910) or in extinct placoderms (e.g. Long, 1988: fig. 12; Dupret *et al.*, 2014: Extended data figs 3e, f). Not surprisingly, this delicate nerve is only known in a few of those fossil taxa in which an ossified and well-preserved endoskeleton of the neurocranium exactly moulds nerve branches, vessels and nasal capsules. Nevertheless, the potential phylogenetic and developmental implications of its presence and course in holocephalans are yet to be determined. But, our findings further confirm its general presence in Holocephali and that the pattern of the *nervus terminalis* in extant Holocephali differs from that of their next extant relatives, the Elasmobranchii. Certainly, there is some correlation with the type of development and invagination of the telencephalic hemispheres in these groups. The results for holocephalan fish may thus trigger new questions and interest in the comparative anatomical study of this structure, its development and significance in vertebrate evolution.

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