3D visualisation and volumetric analysis of the ascending olfactory pathway in two groups of fishes using diceCT imaging

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The size (volume or mass) of the olfactory bulbs in relation to the whole brain is currently used as a neuroanatomical proxy for olfactory ability in a range of vertebrates, including fishes. While iodine stains are commonly used to enhance contrast in a range of biological tissues for X-ray imaging, diffusible iodine-based contrast-enhanced computed tomography (diceCT) provides a more rigorous approach to optimizing contrast in soft tissues using Lugol's solution (I_2KI) (Gignac *et al*. 2016). This stain exhibits high rates of penetration and can often provide good contrast between and within soft tissues. Combining diceCT with non-destructive X-ray imaging provides a powerful tool for undertaking *in situ* anatomical investigations at high resolution, particularly in the case of fragile tissues such as the nervous system. Here, we present a two-step study (stain optimisation and volumetric analysis) of the olfactory system of the brownbanded bamboo shark, *Chiloscyllium punctatum*, and the common goldfish, *Carassius auratus*, using diceCT. In total, six fish specimens were used in this study; three goldfish, 9-9.5cm in total length (TL) and 8.5-12g in body weight (BW), and three brownbanded bamboo sharks, 31-36.5cm in TL and 89-114g in BW. Adapting the method outlined by Gignac *et al.* (2016), Lugol's iodine solution - 1% I_2, 2% KI (Culling 1963) - was used for all samples and stain times were optimised for each species with the aim of providing suitable contrast for the entire brain. Both species were scanned prior to staining (T=0h) and then scanned every 24h or 48h for the goldfish and sharks, respectively. To compare stain intensities, raw X-ray CT data were reconstructed using air and water calibration phantoms scanned under identical conditions to the samples. Optimal contrast across the olfactory bulbs was achieved at T=96h for the goldfish and T=240h for the shark. Higher resolution scans of the whole brain were obtained at the two optimised staining times for all specimens. The volume of the olfactory cavities, olfactory bulbs, the telencephalon and the whole brain, as well as the length and diameter of the olfactory nerves and tracts were measured after segmentation of the brain data using Avizo (Standard 8.1.1). We found that the bamboo shark has a relatively larger olfactory bulb and a very different organisation in the olfactory pathway, both in the nerves and tracts, compared to the goldfish. Additionally, the hydrodynamics within the olfactory cavities were simulated into STAR-CCM+ using segmentations of the snout, nares and olfactory cavities prepared as 3D mesh models with Avizo, MIMICS (19.0 Research) and Materialise 3-matic (12.0), to assess the functional morphology of the olfactory organs. The use of this developing technique in our comparative study has been crucial to visualise differences in the morphological organisation of the olfactory system of both model species and infer novel hypotheses to test in the future. Ultimately, such neuroanatomical comparisons are fundamental approaches to improving our understanding of the evolution of the olfactory system in early vertebrates and the neural basis of olfactory abilities in cartilaginous and bony fishes.

Key Words: comparative neuroanatomy, fish, olfaction, hydrodynamics, diceCT

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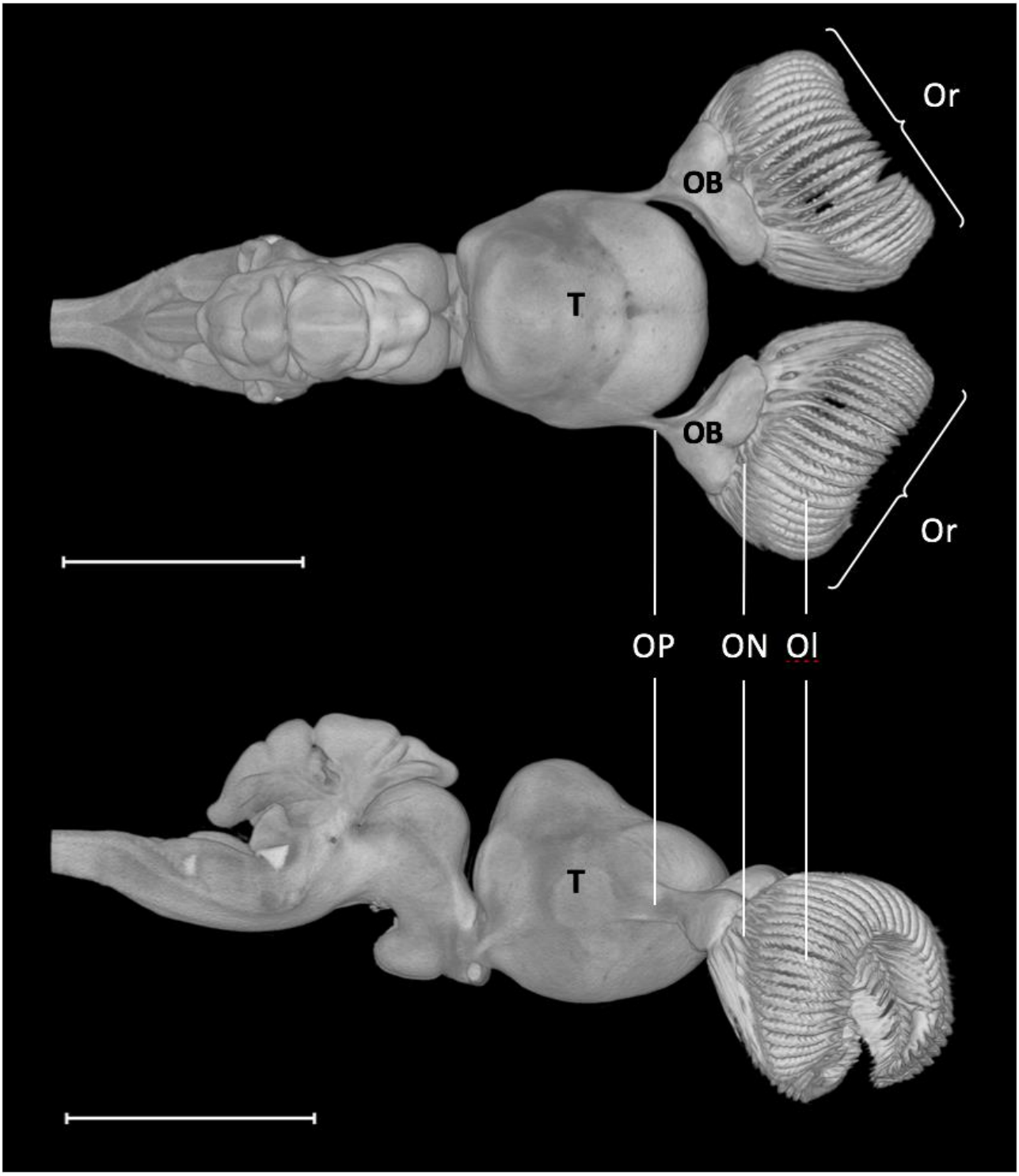

Figure. The dorsal view (top) and lateral view (bottom) of the whole brain and peripheral olfactory system of a brownbanded bamboo shark, *Chiloscyllium punctatum*, segmented using Avizo 8.1.1. From the frontal side on the right to the caudal side on the left, both images show the ascending olfactory pathway in three dimensions. The paired olfactory organs (rosettes, OR) are made up of primary folds (olfactory lamellae, Ol), which bear secondary folds in the shark. Olfactory lamellae give rise to the olfactory nerves (ONs) that terminate within the olfactory bulbs (OBs). And, the olfactory peduncles (OPs), comprised of two internal olfactory tracts, project from the olfactory bulbs to the telencephalon (T). Or; olfactory rosette, Ol; olfactory lamella, ON; olfactory nerve, OB; olfactory bulb, OP; olfactory peduncle, T; telencephalon. Scale bar: 1cm.