Anatomical Studied on the Cranial Nerves of *Liza Ramada* (Family: Mugilidae) Nervus Glossopharyngeus

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Abstract: This study deals with the nervus glossopharyngeus of *Liza ramada.* The microscopic observations showed that, the nervus glosspharyngeus arises by one root and leaves the cranial cavity through its own foramen. It gives visceromotor fibres for the first levator arcualis branchialis muscles. It has single extracranially located epibranchial (petrosal) ganglion. The ramus pretrematicus carries general viscerosensory fibres for the epithelial lining of the pharynx and special ones for the taste buds.The ramus posttrematicus carries both general viscerosensory fibres for the epithelial lining of the pharynx and special ones for the gill filaments, as well as visceromotor fibres for the first adductor arcualis branchialis and the first obliquus ventralis muscle.

[Dakrory, A.I.; Ali, R.S. and Issa, A.Z. **Anatomical Studied on the Cranial Nerves of *Liza Ramada* (Family: Mugilidae) Nervus Glossopharyngeus**] Life Science Journal 2012;9(2):86-93]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 15

Key Words: Nervus glossopharyngeus- *Liza ramada*.

1. Introduction:

The study of the cranial nerves is important because their distribution is correlated with the habits and habitats of animals and also because they show an evolutionary trend among animals of the same group. The cranial nerves connect the brain with all the important centers of perception of the outer surface of the head, as well as the inner surface of the buccopharyngeal and other visceral regions, so that they seem to be important in determining the animal's behavior (**Shaheen, 1987**).

The sensory systems (receptors, their nerves as well as their canters) play a major and sometimes a decisive role in many fish behavioral patterns (feeding, defense, spawning, schooling orientation, migration, etc..).

Although there are several classical studies on the cranial nerves of fishes, yet they are still useful to the investigators. The most valuable works from these early ones were those carried out by **Allis (1897, 1903, 1909 & 1922)** and **Herrick (1899 & 1901).**

Several authors published their works on the cranial nerves of bony fishes. The most recent of them are the studies of **Northcutt and Bemis (1993)** and **Piotrowski and Northcutt (1996)** on *Latimeria chalumnae* and *Polypterus senegalus*, respectively and **Dakrory (2000)** on *Ctenopharyngodon idellus*. Other works on the nerve or group of nerves were performed. **De Graaf (1990)** studied the innervation of the gills in *Cyprinus carpio* by dissection. Also, **Song and Northcutt (1991)** gave a detailed description on the morphology, distribution and innervation of the lateral line receptors in the holostean, *Lepisosteus platyrhincus*. **Dakrory (2003)** studied the ciliary ganglion and its anatomical relations in some bony fishes.

It is quite evident from the above historical review that there are numerous works on the cranial nerves of fishes, but a few studies have been made concerning the cranial nerves of some species belonging to Mugilidae. Although the previously mentioned studies of different authors may threw light on the subject of the cranial nerves of fishes, yet it cannot be stated that the cranial nerves of a Mugilidae is similar to other fishes; and what are the differences if present? Thus it was suggested that a detailed microscopic study on the glossopharyngeal nerve in *Liza ramada* will be very useful.

The main and fine branches of this cranial nerve, its distribution, its relation with other nerves and with the other structures of the head, their analysis and the organs they innervate are studied thoroughly, hoping that it may add some knowledge on this important subject and also to the behaviour and phylogeny of this group of fishes.

2.Material and Methods

The species under investigation of the marine water bony fish *Liza ramada* belongs to family Mugilidae.

Liza ramada is a diurnal fish inhabiting shallow areas. It feeds on epiphytic algae, detritus and small benthic or planktonic organisms, pelagic eggs and larvae. It is an economically important fish as a source of proteins.

The fully formed larvae of this species were collected from the coast of the Mediterranian Sea at Kafer El-Sheykh Governorate, during August 2006. The heads of the fully formed larvae were fixed in aqueous Bouin for 24 hours. After that the heads were washed several days with 70% alcohol. Decalcification was necessary before cutting and staining *in toto* for this bony species. This was carried out by placing the heads in EDTA solution for about 40 days, changing the solution every 3 days.

The heads were sectioned transversely (10 microns in thickness), after embedding in paraffin. The serial sections were stained with Mallory's triple stain (**Pantin, 1946**). The serial sections were drawn with the help of the projector. From these sections, an accurate graphic reconstruction for the glossopharyngeal nerve was made in a lateral view. In order to show the position of the nerve, and its relations to the other different structures of the head, several serial sections were photomicrographed.

3. Results

In *Liza ramada* studied the nervus glossopharyngeus arises from the ventrolateral side of the medulla oblongata by a single root (Figs. 1 & 2, RO.IX). Directly after its origin, this nerve runs posteriorly and ventrolaterally passing dorsal and then dorsolateral to both th saccular ramus of the nervus octavus and the sacculus and ventromedial and ventral to the utricle. At the end of this course, this nerve passes outside the cranial cavity through its own foramen (Fig. 3, F.GPH) which is located on the ventrolateral side of the auditory capsule penetrating the exoccipital bone (Fig. 3, EXO).

Extracranially, the nervus glossopharyngeus joins directly the head of sympathetic trunk forming a common nerve (Fig. 1, N.COM). This nerve extends forwards lateral to the auditory capsule and dorsomedial and then medial to the internal jugular vein. After a considerable course in this position, it becomes ventromedial to the latter vein. Here, the common neve separates into the head sympathetic trunk dorsomedially and the nervus glossopharyngeus ventrolaterally (Figs. 1 & 4, N.IX). Shortly forwards the nervus glossopharyngeus carries the petrosal "Epibranchial"ganglion (Figs. 1 & 5, G.EB.IX). From the anterior end of the ganglion the nervus glossopharyngeus (Fig. 1) originates and continues forwards passing dorsomedial and then medial to the first levator arcus branchialis muscle. It gives off a lateral branch for the latter muscle (Fig. 1, N.LB.I). More forwards, the nervus glossopharyngeus turns its course ventrally and posteriorly to enter the first hollobranch. Here, it gives off a lateral motor branch for the first levator arcum branchialis muscle (Fig. 1, N.LB.I). At its enterance the first hollobranch, it runs ventral to the second levator arcus branchialis muscle dorsolateral and dorsal to the first efferent branchial vessel and medial to the first levator arcus branchialis muscle. Here it gives off a motor branch for the latter muscle (Fig. 1, N.LB.I). The main nerve runs dorsolateral, lateral and then ventral to the first efferent branchial vessel and lateral to the epibranchial bone. Here, the nervus glossopharyngeus (Fig. 1, N.IX) divides into a medial pretrematic and lateral posttrematic rami.

Pretrematic ramus

Directly after its separation from the posttrematic ramus, the pretrematic one (Fig. 1, R.PR.IX) extends anteroventrally passing lateral and then ventral to the epibranchial bone and ventromedial to the ramus posttrematic. The ramus pretrematic continues anteroventrally passing ventral and ventromedial to the epibranchial bone and dorsomedial to the ceratobranchial bone (Fig. 6, R.PR.IX). After a short forward course, it gives off a ventral branch for the gill rackers (Fig. 1, N.GR) and their covering epithelium. This ramus continues forwards dorsal and medial to the ceratobranchial for a long course giving off numerous twigs for the gill rackers and epithelium covering these rackers (Fig. 1, N.GR).

Posttrematic ramus

The posttrematic ramus of the nervus glossopharyngeus of *Liza ramada* (Figs. 1 & 7, R.PT.IX) extends lateroventrally passing ventrolateral to the first efferent branchial vessel and lateral to the epibranchial bone. Here, it gives off a fine nerve for the epithelium of the upper gill filaments (Fig. 1, N.GF). At the point of the articulation of the epi- and ceratobranchial bones of the gill arch, the posttrematic ramus turns anteriorly to run ventrolateral to the latter bone and dorsal to the efferent branchial vessel. Thereafter, it continues forwards extending ventrolateral to the ceratobranchial, lateral to the first efferent branchial vessel and dorsal to the gill filament (Fig. 1, R.PT.IX). During this course it gives off numerous branches for the muscles and epithelium of the filament. More forwards, the posttrematic ramus extends ventral and ventromedial to the ceratobranchial bone and dorsal and then dorsomedial to the first efferent branchial vessels. Reaching the point of the attachment of the first holobranch with the isthmus, this ramus continues anteromedially passing ventromedial to the first oblique ventral muscle and medial to the first afferent branchial vessel.Here, it gives off a branch for the latter muscle (Fig.1, N.OV.I). Anterior to the articulation between the hypobranchial and ceratobranchial, the ramus posttrematic continues anterodorsally passing lateral to the hypobranchial to ramify and end in the epithelium and the pharyngeal taste buds of the roof of the isthmus.

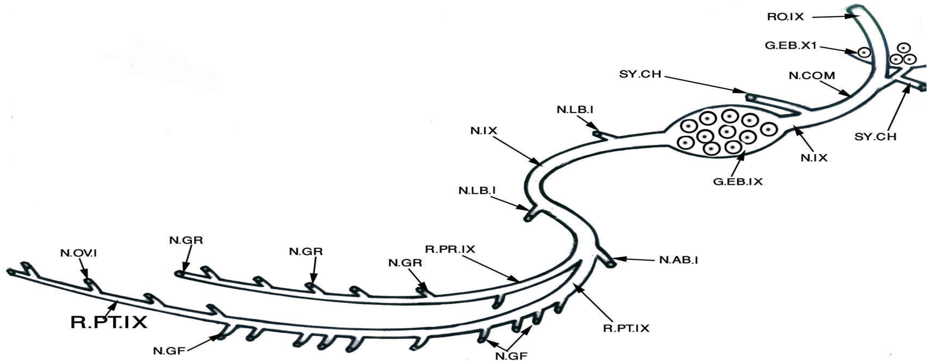

Fig. 1: Graphic reconstruction of the glossopharyngeal nerve in a lateral view. G.EB.IX: Epibranchial ganglion of the nervus glossopharyngeus; G.EB.X_1: Epibranchial ganglion of the first vagal branchial trunk; N.AB.I: Nerve to the first adductor arcus branchialis muscle; N.COM: Common nerve; N.GF: Nerve to the gill filament; N.GR: : Nerve to the gill rackers; N.LB.I: Nerve to to the first levator arcum branchialis muscle; N.OV.I: nerve for first obliquus ventralis muscle; N.IX: nervus glossopharyngeus; R.PH.X_1: Ramus pharyngeus of the first branchial vagal trunk; R.PR.IX: Pretrematic ramus of the glossopharyngeal nerve; R.PT.IX: Posttrematic ramus of the glossopharyngeal nerve; RO.IX: Root of nervus glossopharyngeus; SY.CH: Sympathetic chain.

Fig. 2: Photomicrograph of a part of transverse section passing through the otic region showing the root of both the nervus glossopharyngeus. AC**:** Auditory capsule; ALLN: Anterior lateral line nerve; B: Brain; G.EPB.X_1: Epibranchial ganglion of the first vagal branchial trunk; R.LG: Lagenar ramus; R.PH.X_1: Ramus pharyngeus of the first branchial vagal trunk; R.PT.X_1: Posttrematic ramus of the fisrt vagal branchial trunk.

Fig. 3: Photomicrograph of a part of transverse section passing through the otic region showing the glossopharyngeal foramen in the exoccipital bone, the rami posttrematic, pharyngeus as well as the epibranchial ganglion of the first vagal branchial trunk; AJV: Anterior jugular vein ; ALLN: Anterior lateral line nerve; B: Brain; EB.II: Epibranchial of second holobranch; EXO: Exoccipital bone; F.GPH: Glossopharyngeal foramen; G.EB.X_1: Epibranchial ganglion of the first vagal branchial trunk; N.IX: Nervus glossopharyngeus; R.AM.PO: : Ramus ampullaris posterior; R.LG: Lagenar ramus; R.PH.X_1: Ramus pharyngeus of the first branchial vagal trunk; R.PT.X_1: Posttrematic ramus of the fisrt vagal branchial trunk.

Fig. 4: Photomicrograph of a part of transverse section passing through the otic region showing the position of the glossopharyngeal nerve. AC**:** Auditory capsule; AJV: Anterior jugular vein ; B: Brain; ; N.IX: Nervus glossopharyngeus; M.LB.I: First levator arcus banchialis muscle; PSC: Posterior semicircular canal ; R.AM.PO: : Ramus ampullaris posterior; R.LG: Lagenar ramus

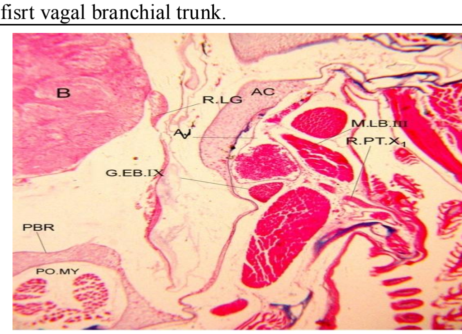

Fig. 5: Photomicrograph of a part of transverse section passing through the otic region showing epibranchial ganglion of the glossopharyngeal nerve and the posttrematic ramus of the first branchial vagal trunk. AC**:** Auditory capsule; B: Brain; G.EB.IX: Epibranchial ganglion of the nervus glossopharyngeus; M.LB.III: Third levator arcus branchialis muscle; PBR: Prootic bridge; PO.MY: Posterior myodome; R.LG: Lagenar ramus.

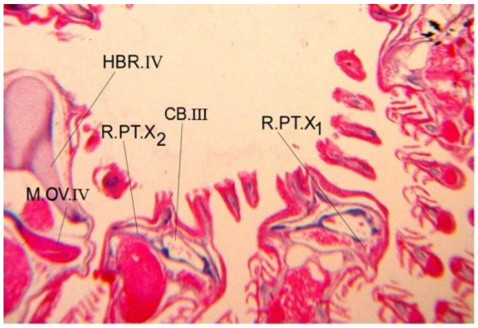

Fig. 6: Photomicrograph of a part of transverse section passing through the otic region showing the pretrematic ramus of the glossopharyngeal nerve and the posttrematic rami of the first and second branchial vagal trunks. CB.III: Third ceratobranchial; HBR.IV: Fourth hypobranchial cartilage; M.OV.IV: Fourth obliquus ventralis muscle; R.PT.X1: Posttrematic ramus of the fisrt vagal branchial trunk; R.PT.X2: Posttrematic ramus of the second vagal branchial trunk.

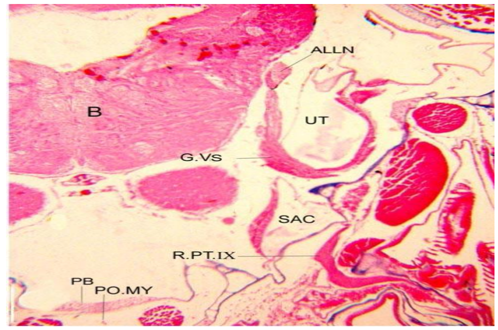

Fig. 7: Photomicrograph of a part of transverse section passing through the otic region showing the posttrematic ramus of the nervus glossopharyngeus. ALLN: Anterior lateral line nerve; B: Brain; G.VS: Ganglion visceralis; PB: Prootic bone; PO.MY: Posterior myodome; SAC: Sacculus; R.PT.IX: Posttrematic ramus of the glossopharyngeal nerve.

4. Discussion

The nervus glossopharyngeus of *Liza ramada* arises from the medulla oblongata by a single root; a case which is found in several bony fishes. The nervus glossopharyngeus, however, arises by two roots in *Ameiurus* (Herrick, 1901), *Polyoden* (Norris, 1925), *Mastacembelus armatus* (Maheswari, 1965) and *Amphipnous cuchia* (Saxena, 1967). On the other hand, Norris (1925) recorded four or five groups of rootlets for the nervus glossopharyngeus in *Scaphirynchus*.

Among cartilaginous fishes, a single root for the nervus glossopharyngeus was recorded in *Dasyatis rafinesque* (Chandy, 1955) and *Pteroplatea altavela* (Mazhar, 1979). However, in *Rhinobatus halavi*, the nervus glossopharyngeus arises by means of three rootlets, which soon unite into one root (Dakrory, 2000).

In Agnatha, there is a single postotic nerve (glossopharyngeal-vagal nerve) which issues from the vagal lobe and passes to the pharynx and gills (Matsuda *et al*., 1991). This is the case found in the lamprey *Ichthyomyzon unicuspis* (Wicht, 1996) and the hagfishes *Eptatretus stoutii* and *Myxine glutinosa* (Braun, 1998). On the other hand, separate glossopharyngeal and vagal nerves were mentioned by Jollie (1968) and Fritzsch and Northcutt (1993) in lampreys and by Kuratani *et al*. (1997) in embryos of *Lampetra japonica*.

Among Amphibia, the nervus glossopharyngeus arises separately from the medulla oblongata as a single trunk and enters the anterior part of a large ganglion shared by the nervi glossopharyngeus and vagus in *Salamander salamandra* and *Plethedon cinereus* (Wake *et al*., 1983) and *Bufo regularis* (Shaheen, 1987). In *Bufo viridis* (Soliman and Mostafa, 1984), however, the nervi glossopharyngeus and vagus arise together by three roots, which enter a common ganglion.

In *Liza ramada*, there is a separate foramen in the exoccipital bone, for the glossopharyngeal nerve. This is also the case found in *Lampanyctus leucopsarus* (Ray, 1950), *Cyprinus carpio* (de Graaf, 1990), *Gnathonemus petersii* (Lazar *et al*., 1992), *Polypterus senegalus* (Piotrowski and Northcutt, 1996), *Clarias gariepinus* (Adriaens and Verraes, 1998), *Ctenopharyngodon idellus* (Dakrory, 2000) , *Tilapia zillii* (Ali, 2005; Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010).

Among cartilaginous fishes, the nervus glossopharyngeus passes through a glossopharyngeal canal, which is communicated with the cavity of the auditory capsule. This was confirmed by Allis (1923) in *Chlamydoselachus anguineus*, de Beer (1931) in *Scyllium canicula* and El-Toubi (1949) in *Acanthias vulgaris*. This canal was also found by Hamdy (1960), El-Toubi and Hamdy (1959 & 1968), Hamdy and Hassan (1973), El-Satti (1982) and by Dakrory (2000). Hence, this canal appears to be a common feature for the Chondrichthyes while it is absence in bony fishes.

In the amphibians so far described, the nervi glossopharyngeus and vagus pass outside the cranial cavity through the jugular foramen (Sokol, 1981; Soliman and Mostafa, 1984; Shaheen, 1987; Haas, 1995; Reiss, 1997; Hall and Larson, 1998). Thus, the presence of a single foramen for the exit of the nervi glossopharyngeus and vagus appears to be a general rule in amphibians.

Concerning the case in Reptilia, the nervus glossopharyngeus passes outside the cranial cavity through an apertura medialis recessus scalae tympani, and enters the latter recessus which represents the anterior part of the fissura metotica. Then, it leaves the recessus through the apertura lateralis recessus scalae tympani (de Beer, 1937; Dakrory, 1994). In such case, this nerve passes outside the cavity of the auditory capsule.

In the present study, there is only one glossopharyngeal ganglion, the petrosal (epibranchial) ganglion, which is located extracranially. Among bony fishes, the nervus glossopharyngeus has a single extracranial petrosal ganglion in *Polypterus senegalus* (Lehn, 1918), *Lampanyctus leucopsarus* (Ray, 1950), *Trichiurus lepturus* (Harrison, 1981), *Cyprinus carpio* (de Graaf, 1990), *Ctenopharyngodon idellus* (Dakrory, 2000) , *Tilapia zillii* (Ali, 2005; Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010).

On the other hand, a medial sensory intracranial ganglion, in addition to the lateral extracranial petrosal (epibranchial) one, were found for the nervus glossopharyngeus in *Ameiurus* (Herrick, 1901), *Scorpaena scrofa* and *Polypterus* (Allis, 1909, 1922, respectively), *Parasilurus asotus* (Atoda, 1936), *Latimeria chalumnae* (Northcutt and Bemis, 1993) and *Polypterus senegalus* (Piotrowski and Northcutt, 1996).

Among cartilaginous fishes, a single, extracranially located petrosal ganglion was found in *Squalus acanthias* (Norris and Hughes, 1920) and *Dasyatis rafinesque* (Chandy, 1955).

Among Amphibia, Wake *et al*. (1983) mentioned that only one ganglion is present for the nervi glossopharyngeus and vagus in *Salamandra salamandra* and *Plethodon cinereus*. This was also the case described by Norris (1908) in *Amphiuma means*, Paterson (1939) in *Xeonpus laevis*, Soliman and Mostafa (1984) in *Bufo viridis*. In this respect, Northcutt (1992) stated that, in *Ambystoma trigrinum* and other salamanders, the glossopharyngeal ganglion fuses with all the other sensory ganglia of the postotic cranial nerves, forming postotic ganglionic complex. On the other hand, Shaheen (1987) stated that each of the glossopharyngeal and vagal nerves has its own separate ganglion.

Dealing with reptiles, the nervus glossopharyngeus has a well distinct petrosal ganglion, as described by many authors. In addition to this ganglion, a root ganglion (ganglion superius) may be present in some forms. Such ganglion superius was observed in *Cerastes vipera* (Hegazy, 1976), *Agama pallida* (Soliman *et al*., 1990).

In the present study, the nervus glossopharyngeus, proximal to the petrosal ganglion is connected with the cranial sympathetic nerve. This is the case found in *Tilapia zillii* (Ali, 2005; Dakrory andAli, 2006) and in *Mugil cephalus* (Hussein, 2010). On the other hand, in *Cyclothone acclinidens*, Gierse (1904) stated that the nervus glossopharyngeus fuses intracranially with the root of the posterior lateral line nerve and, probably, anastomoses with the ramulus ampularis posterior. Similarly, Allis (1909, 1922) found a communicating branch arising from the intracranial ganglion cells of the nervus glossopharyngeus to the root of the nervus vagus in *Scorpaena scrofa*, and from the root of the former nerve to that of the latter one in *Polypterus*, respectively. This was confirmed by Lehn (1918) and Saxena (1967) in *Polypterus senegalus* and *Amphipnous cuchia*, respectively. On the other hand, Handrick (1901) stated that, the roots of the nervi glossopharyngeus and vagus join and emerge together through the same foramen, but without apparent interchange of fibres in *Argyropelecus hemigymnus*. Dakrory (2000) also found a connection between the nervus glossopharyngeus and the middle lateral line nerve in *Ctenopharyngodon idellus*.

In *Liza ramada*, two rami arise from the petrosal ganglion; the rami pretrematic and posttrematic.The same was recorded in *Mugil cephalus* (Hussein, 2010). Herrick (1899) stated that in *Menidia* the nervus glossopharyngeus shows a considerable reduction in its peripheral branches; there is no ramus pharyngeus and the pretrematic ramus is reduced to a tiny remnant. Still further redution for the ramus pretrematicus was found by Gierse (1904) in *Cyclothone acclinidens*. Moreover, this nerve, in *Gnathonemus petersii*, is small both peripherally and centrally (Lazar *et al*., 1992). On the other hand, the pretrematic ramus is completely lacking in *Esox* and *Silurus* according to Stannius (1849). Moreover, *Ctenopharyngodon idellus* has two rami only, the rami pharyngeus and posttrematicus emerge from the nervus glossopharyngeus (Dakrory, 2000). The nervus glossopharyngeus has all the rami of a typical branchial nerve in *Amia calva, Scomber scomber* and *Scorpaena scrofa* (Allis, 1897, 1903, 1909, respectively), *Polycentrus schomburgkii* (Freihofer, 1978), *Trichiurus lepturus* (Harrison, 1981), *Cyprinus carpio* (de Graaf, 1990) and *Polypterus senegalus* (Piotrowski and Northcutt, 1996).

Among cartilagenous fishes, three rami arise from the petrosal ganglion; the rami pharyngeus, pretrematic and posttrematic. This is the typical condition found in elasmobranches as reported by Norris and Hughes (1920) in *Squalus acanthias*, Chandy (1955) in *Dasyatis rafinesque* and Dakrory (2000) in *Rhinobatus halavi*.

In *Liza ramada,* there is no connection between the nervus glossopharyngeus and the nervus facialis; i.e. there is no Jacobson's anastomosis. The same was recorded in *Tilapia zillii* (Ali, 2005, Dakrory and Ali, 2006) and in *Mugil cephalus* (Hussein, 2010). On the other hand, a Jacobson's anastomosis was found in *Amphipnous cuchia* (Saxena, 1967) and *Trichiurus lepturus* (Harrison, 1981). However, another Jacobson's anastomosis was found between the ramus pharyngeus of the nervus glossopharyngeus and the posterior palatine ramus of the nervus facialis in *Menidia* and *Ameiurus* (Herrick, 1899, 1901), *Polycentrus schomburgkii* (Freihofer, 1978) and

Ctenopharyngodon idellus (Dakrory, 2000). In this respect, Piotrowski and Northcutt (1996) described a connection between the ramus pharyngeus of the nervus glossopharyngeus and the rostral pole of the facial ganglion in *Polypterus senegalus*.

Among Amphibia, the ramus hyomandibularis facialis is communicated with the nervus glossopharyngeus in *Xenopus laevis* (Paterson, 1939), *Bufo viridis* (Soliman and Mostafa, 1984) and *Bufo regularis* (Shaheen, 1987). In *Amphiuma means* (Norris, 1908), however, the nervus glossopharyngeus anastomoses with the ramus alveolaris (chorda tympani).

In Reptilia, Jacobson's anastomosis is carried either through the medial cranial sympathetic ramus, which connects the nervi glossopharyngeus and vagus (Willard, 1915; Soliman *et al*., 1974; Dakrory, 1994), or through the lateral cranial sympathetic ramus (Soliman, 1969).

In the present study, the ramus posttrematicus of the nervus glossopharyngeus is not divided into two parts anterior and posterior. It is also found in both *Tilapia zillii* (Ali, 2005; Dakrory & Ali, 2006a) and *Mugil cephalus* (Hussein, 2010). On the other hand, this ramus is divided into pars anterior and pars posterior in all the ray-finned fishes (Norris, 1925), *Latimeria chalumnae* (Northcutt and Bemis, 1993) and *Polypterus senegalus* (Piotrowski and Northcutt, 1996) and the shark, *Squalus acanthias* (Norris and Hughes, 1920).

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