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„Get small if it gets uncomfortable. Character shift and phenotypic plasticity of *Ninnigobius canestrinii* (Ninni, 1883) introduced in a shallow freshwater lake"

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TABLE OF CONTENTS

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ABSTRACT

Ninnigobius canestrinii (formerly *Pomatoschistus canestrinii*) is an epibenthic, euryhaline sand goby inhabiting brackish and freshwater habitats along the Adriatic coast. Past morphological studies revealed that sand gobies which face the same environmental conditions like a similar habitat and life history combined with a low dispersal rate tend to exhibit the same morphological features: a reduction of head canals, squamation and body size. In this study, a natural population from river Jadro (Croatia) was compared to a recently introduced population of lake Trasimeno (Italy) regarding these three morphological traits. This offered the opportunity to assess (1) the phenotypic diversity between natural and introduced populations and (2) how the squamation and the head lateral line canal system of this benthic species responds to a shift along a hydrodynamic gradient (lake vs. stream). Because both traits are key taxonomic tools, the introduction offered also the opportunity to assess (3) the strength of these two morphological traits as taxonomic tools. Specimens from the introduced population showed a smaller body size, a higher variability and more reductions in head canal and squamation types than the natural population of river Jadro. The reason seems to be adaptation to a new habitat and consequently to different environmental and hydrodynamic conditions. Since recent phylogenetic studies revealed cryptic diversity in Adriatic sand gobies, these morphological results could perhaps be used for future species identification.

Zusammenfassung

Ninnigobius canestrinii (vormals *Pomatoschistus canestrinii*) ist eine epibenthische, euryhaline Sandgrundel, die sowohl brackische, als auch Süßwasserhabitate entlang der Adriatischen Küste bewohnt. Morphologische Studien an Sandgrundeln haben gezeigt, dass Sandgrundel, die denselben Umweltbedingungen ausgesetzt sind, wie etwa einem gleichen Habitat, einem gleichen Lebenszyklus sowie einer geringen Verbreitung, dieselben morphologischen Merkmale aufweisen: eine Reduktion der Kopfkanäle, der Beschuppung und der Körpergröße. In dieser Masterarbeit wird eine natürliche Fischpopulation des Flusses Jadro (Kroatien) mit einer eingesetzten Fischpopulation im Trasimenischen See (Italien) hinsichtlich dieser drei Merkmale verglichen. Dadurch kann (1) die phänotypische Diversität zwischen einer natürlichen und einer eingesetzten Population verglichen und untersucht werden, (2) inwiefern sich die Beschuppung und die Kopfkanäle entlang eines unterschiedlichen hydrodynamischen Gradienten (See vs. Fluss) verändern. Da beide Merkmale eine wichtige Rolle bei der Artbestimmung spielen, wurde auch untersucht (3) inwiefern sich diese morphologischen Merkmale zur Artenidentifikation eignen. Die Fische der eingesetzten Population wiesen eine geringere Körpergröße sowie eine höhere Variabilität und mehr Reduktionen im Kopfkanalsystem und der Beschuppung auf als die natürliche Population. Gründe dafür könnten die Anpassung an ein neues Habitat und an die damit einhergehenden neuen Umwelt- sowie hydrodynamischen Bedingungen sein. Neueste Studien zeigten erst kürzlich eine kryptische Diversität bei den Adriatischen Sandgrundeln auf. Die morphologischen Ergebnisse könnten somit potenziell für spätere Artidentifikation genutzt werden.

INTRODUCTION

Gobiidae

Gobiiformes, formerly assigned to the suborder Gobioidei, are an order of teleost fishes comprising of eight families: Rhyacichthyidae, Myleringidae, Odontobutidae, Eleotrididae, Butidae, Thalasseleotrididae, Gobionellidae and Gobiidae. All in all, they include about 321 genera and about 2167 species worldwide. The knowledge of the systematics of Gobiiformes and its diversity is recently expanding. But still, at present, there is no clear consensus on the familial classification of gobioid fishes (Nelson et al. 2016).

One of these families, Gobiidae, are a family of generally benthic and small fishes with a worldwide distribution. They include more than 1120 species with 170 genera and are known to inhabit marine, freshwater or brackish waters in temperate as well as in tropic regions (Thacker et al. 2011). Some authors consider Gobiidae as a subfamily within a larger Gobiidae (Nelson et al. 2016) and according to Thacker (2009) the family Gobiidae includes the subfamily Gobiidae and Gobionellidae.

Most of the species belonging to the family Gobiidae are of small body size and bear pelvic fins which are either wholly or partially joined to a ventral disc(Thacker et al 2011). Scales (ctenoid as well as plasmoid scales) are rarely absent. Some species have prominent barbels.

Gobiidae are one of the most species-rich marine fish families and often are considered the most abundant fishes in freshwaters on oceanic islands (Nelson et al. 2016). In tropical coral reefs, Gobiidae make 35% of the total fish fauna and 20% of the local biodiversity there (Thacker et al. 2011). Some species are catadromous and very often the dominant element of the benthic fish fauna in coral reefs (Nelson et al. 2016).

Sand gobies

A very complex goby fauna can be found in European freshwaters and seas. This includes marine, euryhaline, as well as geographically restricted endemic freshwater species. Among all European goby species, there are five genera which are collectively known as sand gobies. These five genera are: *Knipowitschia*, *Pomatoschistus*, *Economidichthys*, *Ninnigobius* and *Orsinigobius*. Their distribution reaches from the coastal Atlantic of western Europe, through the Mediterranean, including many of its associated freshwaters, till drainages of the Black Aral and the Caspian Sea (Thacker et al. 2019).

Phylogenetic studies by Agorreta et al. (2013) and Thacker et al. (2019) revealed that sand gobies are part of the family Gobionellidae. This confirmed the assumption of McKay and Miller (1997) that sand gobies also show affinity to Gobionellidae based on their morphology and on isozymes. Sensu (Thacker 2009) Gobionellidae are a subfamily of Gobiidae.

Sand gobies are seen as a well-supported monophyletic group, based on morphological traits and also on phylogenetic studies (*mitochondrial 12S/16S* and *rhodopsin*) (Thacker et al. 2019).

The Adriatic Sea as well as the Adriatic Sea drainage system is known to maintain a high biodiversity in fishes. Above all occurring fishes in the Adriatic region, the family Gobiidae (Gobiidae + Gobionellidae sensu Thacker (2009)) are the most diversified group.

There are a few reasons to explain this high biodiversity: past geological events such as the closure of the Tethys, the Messinian salinity crisis and glaciation processes during the Pleistocene divided the Mediterranean Sea into different bioregions including the Adriatic Sea and the Adriatic Sea drainage system. As a result of these events the Adriatic Sea became a semi-enclosed shallow sea, connected with the Ionian Sea which allowed isolation (Tougard et al. 2021).

Molecular data revealed that diversification most probably occurred during climatic cycles of the Pleistocene and intraspecific diversification of the Adriatic sand gobies occurred during different periods of glaciation. The sea level drop might have forced some isolated populations to adapt to fluvio-lacustrine environments, rather than tracking marine habitats. Studies suggested that the adaption to freshwater habitats evolved several times and this would explain the fact that most of the Adriatic sand gobies are freshwater species (Tougard et al. 2021).

Starting off with the description of four gobiid species in the middle of the 19th century, until now, 52 species inhabiting either marine, brackish or freshwater habitats in the Adriatic region have been described (Tougard et al. 2021). The two sand goby genera occurring in the Adriatic region are *Pomatoschistus* and *Knipowitschia* (Tougard et al. 2021). Both genera are ecologically distinct from each other. While *Pomatoschistus* species are very often found in inshore mud, sand or rubble habitats occupying marine waters and occasionally estuarine waters, *Knipowitschia* species prefer estuarine waters and freshwaters (Thacker et al. 2019).

Ahnelt et al. (1995) discovered that different populations of the primary freshwater fish *Knipowitschia caucasica*, a sand goby occurring in freshwater lakes, rivers as well as lagoons in the region of the Aegean Sea, show intraspecific variability between populations concerning the head lateral line system, meristic features and colour patterns. They discovered a

relationship between the biogeographic occurrence and the reduction or completeness of head canals. While western Aegean populations of *Knipowitschia caucasica* showed a complete head canal type, only a few lacking the posterior oculoscapular canal, the eastern populations showed more reductions in the development of head lateral line canals, including the reduction of the frontal parts of the anterior oculoscapular canal.

This morphological variation can be explained by geographic isolation due to past glacial and interglacial periods which let the sea level rise and drop repeatedly (Ahnelt et al. 1995). Further morphological studies by Ahnelt (2011) on other Aegean freshwater sand gobies (*Knipowitschia byblisia* and *Knipowitschia caunosi*) revealed that there seems to be a tendency towards reducing squamation and head canals as well as body size when gobiid fishes are facing the same environmental conditions: a similar habitat and life history, combined with a low dispersal (Ahnelt 2011).

Besides Aegean *Knipowitschia* species, this scenario can also be found in Adriatic and even in Pacific gobies (Ahnelt et al. 2004, Kovačić 2005). Because a small body size is very often associated with short generation time, a tendency to reduce squamation and the lateral line head canal in gobiid fishes could be explained by adapting to a shorter life cycle (Ahnelt 2011).

Small sized fish species, like gobies, often correlated with a high abundance, are known to play an important role in ecological food webs (Pockberger et al. 2014). Gobiid fishes like *Pomatoschistus microps*, which occur in intertidal near shore areas, estuaries, lagoons and brackish waters along the Atlantic coast, the North and the Baltic Sea as well as the western part of the Mediterranean Sea even fulfill the role of an important keystone species. They are an important prey for larger predators and thus play an important role on the size of species in lower and higher trophic levels such as macrozoobenthos, birds, mammals and bigger fishes. Furthermore, a high abundance of small sized fishes also triggers the competition for other species which feed on the same resource (Pockberger et al. 2014).

Study object: *Ninnigobius canestrinii* (Ninni 1883)
Ninnigobius canestrinii (formerly *Pomatoschistus canestrinii*) is an epibenthic, euryhaline sand goby which inhabits brackish and freshwater habitats along the Adriatic coast. It preferably inhabits estuaries and lagoons, but can also be found in rivers or marine areas adjoining brackish habitats (fig. 1) (Miller et al. 2004; Kovačić 2005).

Figure 1: *Ninnigobius canestrinii* (fishbase.se; accessed 13th of October, 2021).

The Canestrini´s goby occurs in the northern Adriatic from Monfalcone to the Po Delta (Italy) and also in Dalmatian localities like the River Jadro, Zymanja and Split. Recently, it was introduced into freshwater as a result from transfer via ballast water and therefore it can also be found in the Italian Lake Trasimeno in central Italy (Ahnelt et al. 2018). *Ninnigobius canestrinii* feeds on copepods, polychaete worms, amphipods, chironomid larvae, ostracods, barnacle larvae, isopods, molluscs and fish eggs and its main predators are young eels and other piscivorous vertebrates. The life-span of this species is one year: juveniles reach maturity usually by nine to 12 months. The spawning season begins at the end of March and ends in June, sometimes in July. Eggs are attached on suitable stones, submerged wood, branches or any other suitable objects in shallow waters. The egg patch is then protected and fanned by the male until they hatch (Miller et al. 2004).

Morphology

There are three morphological traits which are commonly used to distinguish between sand gobies: (1) the presence and location of scales on the body surface, (2) the occurrence of dark spots among the body and (3) reductions of the head canal system (Kovačić 2005, Tougard et al. 2021).

Studies on the morphology of sand gobies by Ahnelt (2011), Ahnelt et al. (1995) and Ahnelt and Göschl (2003) revealed that gobiid fishes, facing the same environmental conditions like a similar habitat and life history, combined with a low dispersal, tend to reduce squamation and the lateral line head canals.

The same scenario has also been found in various populations of *Ninnigobius canestrinii* Kovačić (2005). This author compared morphological features like the pattern of squamation, the reduction of head canals as well as sensory papillae (neuromasts) of different populations of *Ninnigobius canestrinii* in different localities of Croatia and Montenegro. It turned out that *Ninnigobius canestrinii* as well seems to be highly morphologically variable concerning squamation and the reduction of head canals (Kovačić 2005).

Although there are several keys to identify sand gobies from the Adriatic Sea based on morphological characters, at present a lot of difficulties in species identification exist (Tougard et al. 2021).

Recent molecular studies in order to investigate the phylogenetic relationships between Adriatic sand gobies revealed a highly resolved phylogeny on the interspecific level. Phylogenetical results highlight cryptic diversity within the Adriatic sand goby group which means that new species might be described within the next years (Tougard et al. 2021). At present, it is even not clear if *Ninnigobius canestrinii* and *Pomatoschistus montenegrensis* belong to the genus *Ninnigobius* or if these species even belong to a single genus (Tougard et al. 2021). Additionally, the results of these authors split the Adriatic populations of *Ninnigobius canestrinii* into three different lineages belonging to three different geographic areas: Raša (Croatia), Baćinska lakes (Croatia) and Venice (Italy) (Tougard et al. 2021).

Lateral line system and head canals

The lateral line is a highly specialised mechanosensory system in all classes of fishes and is crucial for a number of behaviours like prey detection, schooling, holding position in water currents and predator avoidance (Kasumyan 2003, Fuiman et al. 2004, Ahnelt et al. 2021). In bony fishes the lateral line is composed of neuromast receptors which are either located freely on the skin or in a pored lateral line canal on the head and the trunk. Typically, in canals one neuromast is located between two canal pores (Bird and Webb 2014).

Neuromasts

Neuromasts serve as the functional units of the lateral line system. These epidermal structures detect mechanical stimuli by detecting shift waves of water oscillations (Engelmann et al. 2002, Kasumyan 2003, Fuiman et al. 2004).

Neuromasts consist of two basic cell types: (1) hair cells and (2) supporting cells. Each hair cell (fig. 2) is composed of a high kinocilium and shorter stereocilia. In the basal part are afferent and efferent nerve fibres. Kinocilia and stereocilia of hair cells are embedded in a cupula filled with gel like media. Hair cells are surrounded by supporting cells which separate the sensory field of the neuromast from the epithelium. They are always oriented in two opposite directions in free as well as in canal neuromasts (Kasumyan 2003).
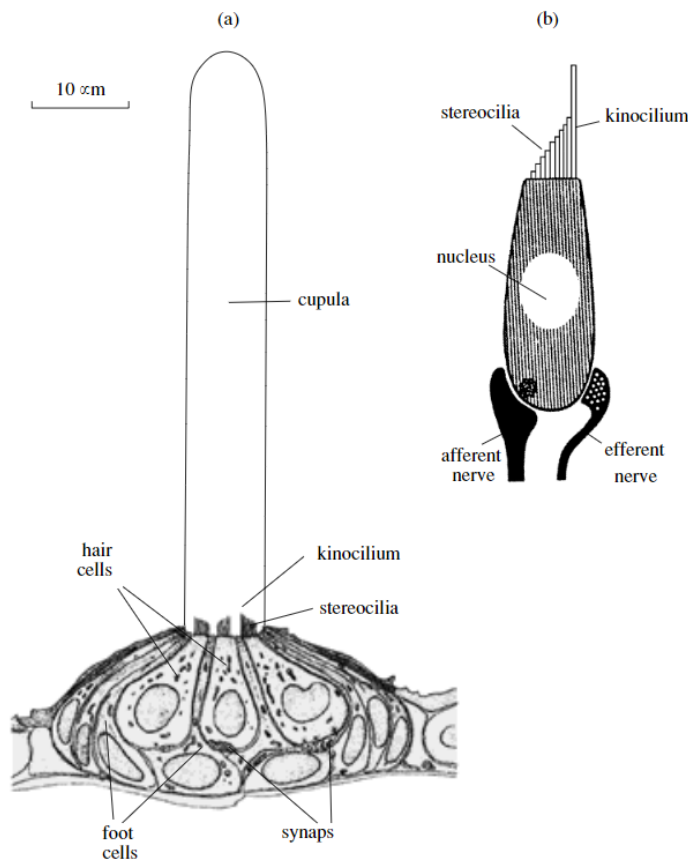
Figure 2: Structural components of canal neuromasts (a) and the scheme of hair cells (b) (Kasumyan 2003).

The mechanoreceptors detect shift waves of underwater oscillations. An incoming shift wave deflects the cupula: if stereocilia are detected towards the kinocilium, the nerve cell gets excited. If stereocilia are detected into the other direction, the nerve cell gets depolarized and the receptors are suppressed (Kasumyan 2003, Fuiman et al. 2004).

Normally, external water and canal fluid motions displace the cupula of the lateral line and cause a deflection of the ciliary couples. The flow in the canals depends on pressure differences between neighbouring canals (Engelmann et al. 2002, Bleckmann and Zelick 2009).

Superficial neuromasts and canal neuromasts

There are two types of neuromasts: (1) Free or superficial neuromasts which are located freely on the body surface and (2) canal neuromasts which are enclosed in fluid-filled canals (Ahnelt et al. 2021). These two types of neuromasts are adapted to different hydrodynamic stimuli and complement each other in the detectable frequency band: while superficial neuromasts are sensitive to velocity, engage in velocity-sensitive responses and are insensitive to oscillatory water motions, canal neuromasts respond to flow acceleration and motions under a unidirectional flow and are involved in determining alterations in the hydrodynamic situation, also in a constant current (Engelmann et al. 2002, Kasumyan 2003; Sato 2022). The canals of canal neuromasts are components of the dermatocranium and are known to demonstrate phenotypic variation within bony fishes (Bird and Webb 2014).

Variations in the structure of the lateral line have been documented recently. This includes variation in the number and spatial distribution of superficial neuromasts or variation in structure and size of a lateral line canal, their orientation and shape of both (Engelmann et al. 2002). There also seems to be a strong relationship between the morphology and the mechanical filter properties of the lateral line system (Bleckmann and Zelick 2009). The placement and the extent of canals among the body as well as the number of superficial neuromasts are convergent across taxa and have been linked to specific habitat-related life styles of the fish (Engelmann et al. 2002). Also, the pattern of the lateral line system is known to be linked to the demands of the environment and the hydrodynamic conditions as well as a specific type of locomotion (Ahnelt et al. 2021).

Each component of the lateral line, is it for example canals or neuromasts, is known to vary in its morphology among fishes, as it is seen as an adaption to their respective habitats, habits and also phylogenetic backgrounds (Sato 2022). Fishes living in habitats with divergent hydrodynamic conditions face different demands of the mechanosensory lateral line. Species living in still, slow moving water often have a high number of superficial neuromasts and a reduced lateral line system, while fishes living in running waters generally have a well-developed lateral line canal system and very few superficial neuromasts (Ahnelt and Bohacek 2004, Ahnelt et al. 2021). This even goes for closely related fish species (Engelmann et al. 2002).

An increase of superficial neuromasts in teleost fishes has evolved many times independently, but there seems to be a tendency towards increasing the number of superficial neuromasts in fishes which inhabit environments without turbulent flows (Sato 2022).

Superficial neuromasts are usually involved in obtaining information about the presence and the direction of water currents and in obtaining information about oscillation which are caused by slow water movements, while canal neuromasts are adapted to detect high frequency stimuli in turbulent waters (Kasumyan 2003, Ahnelt et al. 2021).

By moving through turbulent waters, a rheophilic fish`s lateral line is automatically exposed to self-generated noise. Generally, these fish are constantly exposed to large background water motions and pressure fluctuations. If and how these hydrodynamic noises interfere with the own lateral line perception is still not known (Bleckmann and Zelick 2009). For example, midwater fishes are known to use their lateral line to detect moving objects. By doing so, they do not only discriminate the direction of the moving object, but they are also able to detect their speed, their size and even the shape (Bleckmann and Zelick 2009).

In many fishes, the lateral line canals are located on the body and on the head (Bird and Webb 2014). Gobiidae are known to have a high amount of free neuromasts, which are not enclosed in sensory canals on their head and on their body. The whole taxon lacks a lateral line canal on the trunk and displays a highly modified cephalic canal (Scatollin et al. 2007).

Head canals in teleost fishes usually are either on the skin or embedded within dermatocranial bones (Bird and Webb 2014). These highly conservative canals can be divided into following sections: the supraorbital canal (SO), the infraorbital canal (IO), the otic canal, the preopercular canal (PO), the postotic canal, the supratemporal canal (ST) and the mandibular canal (MD). (Bird and Webb 2014; Sato 2022). Parts of the supraorbital canal (SO) are contained in the nasal bone and the frontal bone, which form parts of the neurocranium and the dorsal edge of the orbit. The infraorbital canal (IO) lies within the infraorbital bone series, which borders the ventral half of the orbit (Bird and Webb 2014). The otic canal extends from the interorbital junction to the pteroic (Sato 2022). The preopercular canal (PO) lies in the preopercular bone which is part of the opercular apparatus which is critical for gill ventilation and feeding (Bird and Webb 2014). It passes through the dentary and the anguloarticular bone. The postotic canal lies between the otic-preopercular and the postotic-supratemporal junction and passes through the pteroic and the extrascapular bones. The supratemporal canal (ST) reaches dorsally from the postotic-supratemporal junction through the extrascapular bones and terminates at the parietal region (Sato 2022). The mandibular canal (MD) contains the two parts of the lower jaw

which is the dentary bone and the anguloarticular bone. The SO, IO and the PO all meet caudally to the eye and continue as the otic and post-otic canals in the pterotic and extrascapular bones (Bird and Webb 2014).

Formation of the lateral line and canal morphogenesis

The formation of the lateral line system is induced by lateral line nerves, which derive from respective placode. Free neuromasts appear first. Size and number of superficial neuromasts increases rapidly during ontogeny: after several days or even a few hours after their emergence they can be found on the whole body of a fish. First, they usually develop at the anterior part of the body and then reach back to the caudal fin until the whole body is covered. Free neuromasts have a proliferation ability, which means that they are able to develop new free neuromasts during their whole life or rather during a certain period in life than in only one ontogenetic period (Kasumyan 2003).

Parts of interspecific variation in the lateral line system are produced by heterochronic differences in the development of canals and neuromasts. Since the number of neuromasts increases with growth of the fish, interspecific differences in canal complexity thus can be, among other causes, led back to differences in the timing of the serial canal formation (Sato 2022).

The rate of superficial neuromast formation also depends on the hydrodynamic conditions (Kasumyan 2003). The morphology of the lateral line is known to vary interspecifically under different environmental conditions. Fish species which live in turbulent conditions usually exhibit more pores and canal neuromasts than superficial neuromasts. While superficial neuromasts get overstimulated in turbulent waters, canal neuromasts remain their function as they are sheltered by the canal (Vanderpham et al. 2013). The lateral line canals develop later, but have the same vector: head canals appear first and then the formation of the lateral line system continues to the posterior body part (Kasumyan 2003).

The canal development is induced by neuromasts: first, free neuromasts appear in the location of the later canal formation, their number increases and they form rows which go down into the epidermal groove. The roof of the canal develops later: first the roofs appear in the anterior part of the body and then reach back to the posterior part. Canal neuromasts become larger with the growth of the fish (Kasumyan 2003).

The morphogenesis of the individual canal segment generally occurs in four stages (fig. 3). Stage one: cells differentiate within the epithelium. Stage two: a presumptive canal sinks in and

canal walls ossify within the dermis on either side. Stage three: soft tissue fuses over the neuromast and form a canal segment. Stage four: The ossified canals extend over the neuromasts and fuse to form a roof of the canal segment. Canal segments grow towards another, fuse and leave a pore between them. Canal segments then become incorporated into the dermal bone underneath (Bird and Webb 2014).
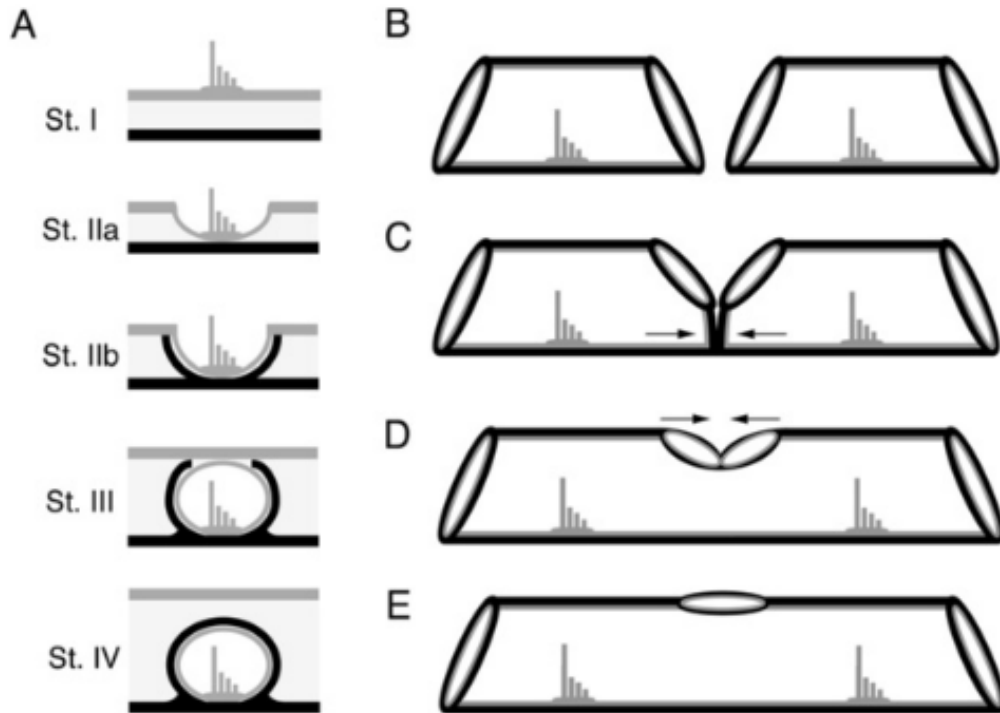

Figure 3: The neuromast-centred canal morphogenesis occurs in four stages (St. I.- St. IV). Stage I: Cells differentiate within the epithelium. Stage II: The presumptive canal sinks in, canal walls ossify within the dermis. Stage III: Soft tissue fuses over the neuromast and forms a canal segment. Stage IV: The ossified canals extend over the neuromasts and fuse to form a roof of the canal segment (Bird and Webb 2014).

The reduction of head canals starts first with shortening of the canal, then the adjacent canal is displaced and new pores are developed. The reduction finishes with the loss of the last part of canals and the pores themselves (Ahnelt et al. 2004, Stelbrink and Freyhof 2006).

Head canal system in Gobiidae and *Ninnigobius canestrinii*
The degree of head canal reductions as well as the arrangements of superficial neuromasts are a commonly used diagnostic feature for species of Gobioidei: almost all species own either a completely developed or partially reduced head canal system combined with many superficial neuromasts (Sato 2022).

In many gobiid taxa these canals are completely reduced, just developed as short canal remnants, or, at the best, be developed as three canals, the anterior oculoscapular canal (AOS), the posterior oculoscapular canal (POS) and the preopercular canal (POC) (Miller 2004, Scattolin et al. 2007). Superficial neuromasts on the head are considered to be homologous to canal neuromasts based on their innervation. They derive from original canal neuromasts. An increase of superficial neuromasts is achieved by dividing each canal homologue into two or more canals (Sato 2022).

Figures 4 and 5 show a general head canal system of Gobiidae. Gobiidae usually have three developed canals, which are more or less developed or reduced: the anterior oculoscapular canal (AOS) with pores B (paired), C (unpaired), D (unpaired), E (paired), F (paired), G (paired) and H (paired), the posterior oculoscapular canal (POS) with pores K and L (both paired) and the preopercular canal (POC) with pores M, N and O (all three paired). Infraorbital, postotic, supratemporal and mandibular canal are absent. The horizontal part of the POS is missing, only the vertical part is present. The AOS in Gobiidae comprises of the supraorbital and the otic canal, the POC of Gobiidae is equal to the posterior temporal canal (Ahnelt 2001).
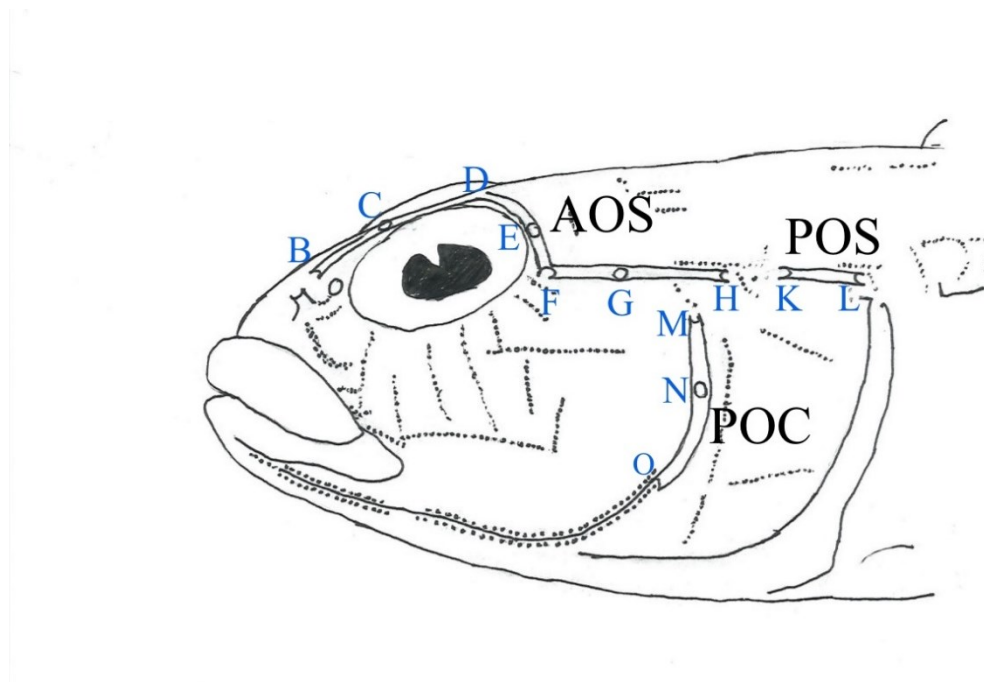

Figure 4: Location of the head canals on *Gobius sp.* AOS: Anterior oculoscapular canal with pores B (paired), C (unpaired), D (unpaired), F (paired) and H (paired). POS: posterior oculoscapular canal with pores K and L (both paired). POC: preopercular canal with pores M, N and O (both paired) (Miller 1986).
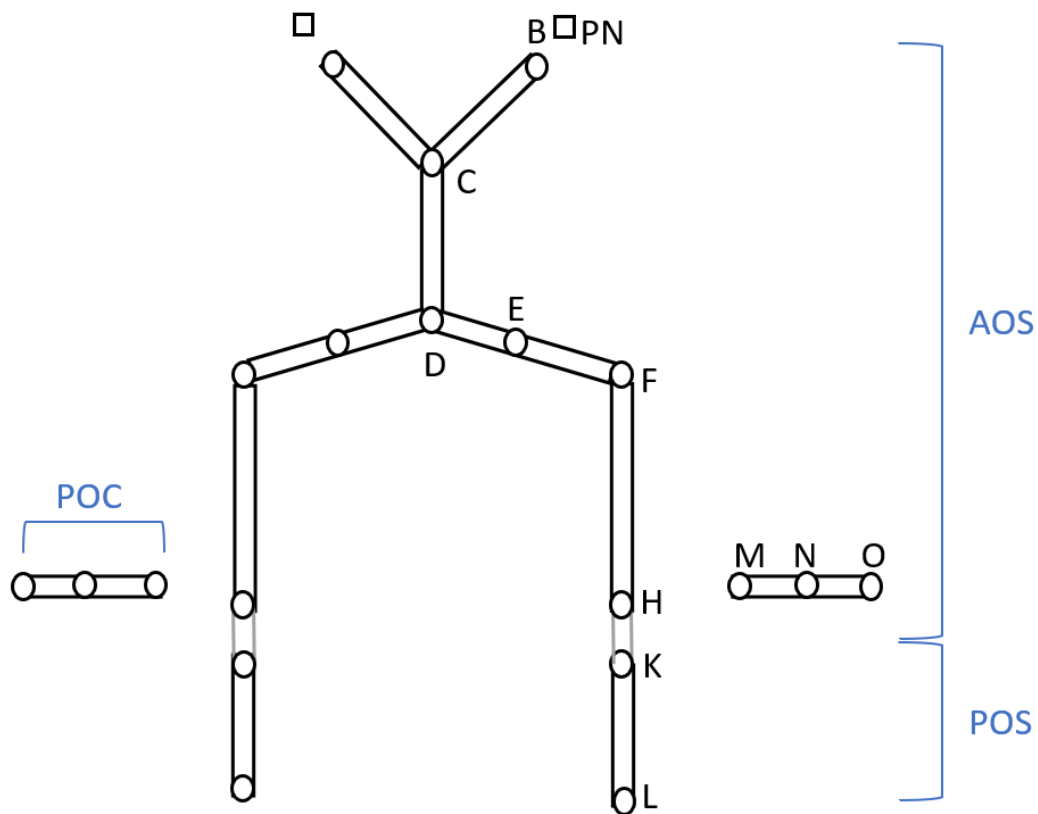
Fig. 5: Head canal system of *Gobius sp.* AOS: Anterior oculoscapular canal with pores B (paired), C (unpaired), D (unpaired), F (paired) and H (paired). POS: posterior oculoscapular canal with pores K and L (both paired). POC: preopercular canal with pores M, N and O (both paired).

Kovačić (2005) and Stelbrink and Freyhof (2006) independently investigated the head canals of different populations of *Ninnigobius canestrinii* in different localities of Croatia and Montenegro. It turned out that *Ninnigobius canestrinii* seems to be highly morphologically variable concerning the reduction of head canals with the canals partly or completely reduced.

Nevertheless, it has to be mentioned that recent phylogenetic studies on the phylogeny of Adriatic sand gobies split up the populations of *Ninnigobius canestrinii* into three lineages and it is difficult to assign these lineages to the species *Ninnigobius canestrinii* (Tougard et al. 2021).

The reduction of head canals and squamation in sand gobies in general could be an adaption to the invasion of freshwater (Miller 2004, Ahnelt 2011).

Squamation

Scales are dermis-derived structures within the fish skin and have several functions: physical defence, serving as a calcium reservoir to prevent possible folding of the skin and the alternation of water flow (Sire et al. 2004; Fletcher et al. 2014).

Development

The development of the squamation pattern has been described in several teleost fishes: in all fishes studied so far scales appear right after metamorphosis. In Osteichthyans, one single scale appears first and acts as an "initiator" to constitute the pattern. Scales are then added successively in regular rows from posterior to anterior (fig.6) until the squamation pattern is completely developed (Sire et al. 2004).
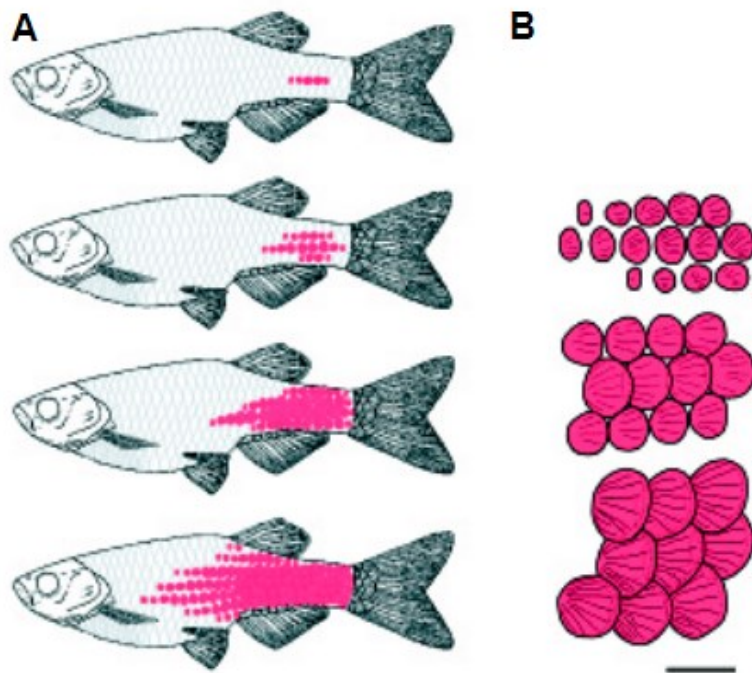

Figure 6: Development of the squamation pattern in the zebra fish (*Danio rerio*). The development starts with one single scale acting as "initiator" and then successively regular rows of scales are developed from posterior to anterior (Sire et al. 2004).

Squamation in Gobiidae and *Ninnigobius canestrinii*

The presence or absence of scales are a morphological trait used for species identification in gobies (Ahnelt and Kovačić 1997, Ahnelt 2011). Morphological studies on sand gobies revealed that gobies tend to reduce their squamation in order to adapt to a freshwater environment, where

gobiid fishes face the same environmental conditions like a similar habitat and life history, combined with a low dispersal (Miller 2004). A similar scenario has also been found in various populations of *Ninnigobius canestrinii* (Kovačić 2005, Stelbrink and Freyhof 2006). These authors found the squamation variously developed from a complete state reduced to patches.

In this study a natural, riverine population of *Ninnigobius canestrinii* from the Croatian river Jadro was compared to a recently introduced population of *Ninnigobius canestrinii* from the Italian lake Trasimeno concerning morphological traits as size, pattern of squamation and pattern of lateral line head canals.

The introduction of Canestrini's goby into a new and for the riverine species not natural environment, a lake, offered the opportunity to assess (1) the phenotypic diversity between natural and introduced populations of this gobiid species in a lake and (2) how the squamation and the head lateral line canal system of this benthic species responds to a shift along a hydrodynamic gradient. Because both traits are key taxonomic tools for gobiid fishes (Miller, 2004) the introduction into a new environment offers also the opportunity to assess (3) the strength of these two morphological traits as taxonomic tools.

MATERIAL AND METHODS

188 fishes from both, natural and introduced populations, were examined and compared with each other concerning morphological traits such as the pattern of squamation, the pattern of head canals, size and standard length.

Sampling sites

Lago Trasimeno

Located in Central Italy (Fig. 7), Lake Trasimeno is one of the largest basins in western Europe and the fourth largest lake in Italy (Ahnelt et al. 2018, Frondini et al. 2019). It is characterized by a shallow depth, relatively high salinity and no natural outflows. The lake has a surface area of about 120.5 km² and is with an average depth of 4.7m relatively shallow (Frondini et al. 2019). Due to its shallow depth thermal stratification is usually absent and therefore water temperature is about the same as air temperature (Ahnelt et al. 2018). The catchment area of Lake Trasimeno has been artificially enlarged between 1957-1962, to overcome remaining periods of high and low water level and droughts (Frondini et al. 2019) (Fig. 8).

More than 19 fish species occur in the lake. Most of them got introduced and are alien species: e.g., carp (*Cyprinus carpio* Linneaus, 1758), goldfish (*Carassius sp.)*, rudd (*Scardinius hesperidicus* Bonaparte, 1845), southern pike (*Esox cisalpinus* Bianco and Delmastro, 2011), perch (*Perca fluviatilis* Linneaus, 1758), largemouth bass (*Micropterus salmoides* (Lacepéde1802)) or sandsmelt (*Atherina boyeri* Risso, 1810) and also two gobiid species: *Knipowitschia panizzae* (Verga, 1841) and *Ninnigobius canestrinii* (Ninni, 1883) (Ahnelt et al. 2018).

Figure 7: Sampling sites in Italy and Croatia. Red square: Lake Trasimeno (IT) represents the introduced population. Blue spot: Jadro River (CR) represents the natural population. (Vidas 2009).

Samples were taken from the south shore (43°8′N, 12°6′E) by Dr. Laura Pompei and Prof. Dr. Massimo Lorenzoni (University of Perugia, Italy) (Fig. 8). Fishes were sampled during breeding season (May 2015) by landing nets with a mesh width of approximately 5mm. In the laboratory they were anesthetized and euthanized by an overdose of MS-222 (tricaine methanesulfonate) and stored in 6% formalin for several weeks. Subsequently the specimens were transferred into 70% ethanol after passing through an ascending alcohol series.

Figure 8: Lago Trasimeno and its catchment area. Light grey: natural catchment of the lake. Dark grey: river basins which were artificially connected to the lake. Red spot: sampling site (Frondini et al. 2019).

River Jadro

River Jadro (43°30′N, 16°26′E) is a short river on the east coast of the Adriatic Sea, close to Split (Croatia) (Fig. 9). It´s total length is 4.3 km. The river originates in the foothills Mosor, 34.2 m above mean sea level and discharges in the Adriatic Sea near Split (Figs. 7 and 9) (Ljubenkov 2015).

The Jadro River is a karst river and has supplied the city of Split with drinking water from Roman times to the present day. The upper parts of the river preserve the river's natural characteristics. The lower parts of the river are regulated and polluted by anthropogenic impact (Rađa and Rađa 2008).

The estuary of the river has a stratified character: it is separated into two layers: the upper layer is characterized by low salinity and the lower layer is characterized by an almost constant sea water salinity (Ljubenkov 2015).
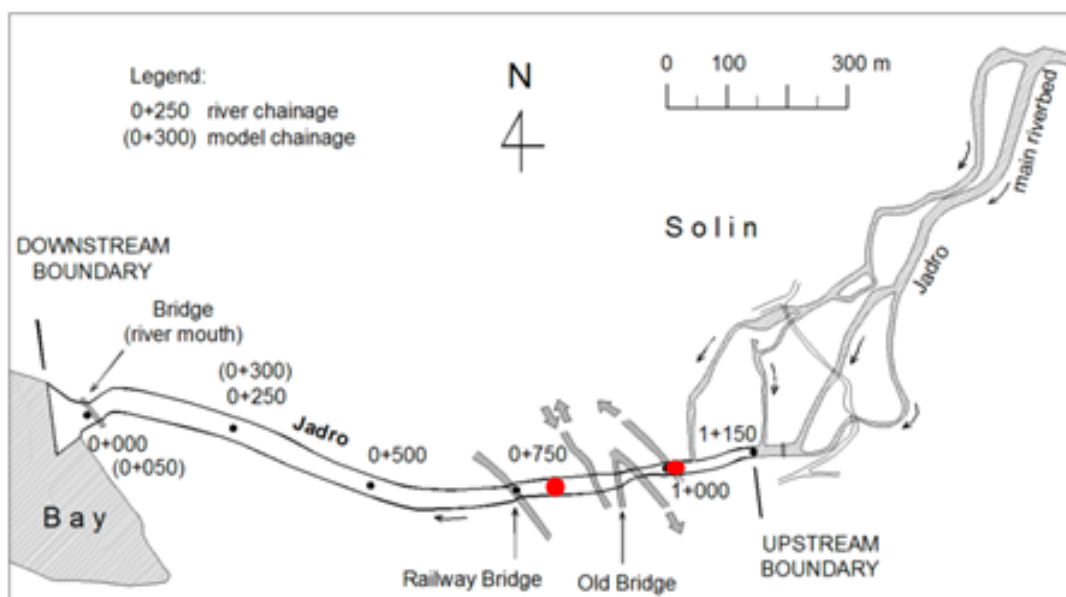
Figure 9: Lower course of the Jadro River (0 km – 1,150 km), the habitat of *Ninnigobius canestrinii*. Between 750 km and 1000 km: sampling sites (red spots) (Ljubenkov 2015).

Material examined

All in all, 188 specimens of *Ninnigobius canestrinii* (Teleostei, *Gobiidae*) from 5 populations have been examined (Table 1). Only specimens of an adequate body size were used for the examination of the head lateral line canals and the squamation. At a minimum standard length of 22.6 mm, we considered the developmental processes for developing squamation and head canals to be completed.

Lake Trasimeno (Italy, collection H. Ahnelt, 2015) total 140 specimens (46 females, 94 males): 140 spec.; mean standard length females (SL): 26.80mm; mean standard length males (SL): 27.95 mm (Table 1).

Jadro River (Croatia), total 48 specimens (8 females, 34 males, 6 unidentified sex): 4 spec. NMW 30632 (1881); 15 spec. NMW 30635 (1882); 14 spec. NMW 30612 (1883); 9 spec. NMW 78700 (1886); 6 spec. NMW 79816 (1892); mean standard length females (SL): 37.98mm; mean standard length males (SL): 42.10 mm (Table 1).

Samples from Lake Trasimeno (Italy) represent the introduced population. According to Miller (2004), *Ninnigobius canestrinii* was introduced to the lake recently. Nevertheless, it is not clear how often this species got introduced.

The historic samples from River Jadro (Croatia) represent the natural population. It is a native stream population in a lotic habitat and deposited at the Ichthyological collection of the Museum

of Natural History in Vienna (NMW). The specimens were already preserved and stored in 70% ethanol. This historical sample was selected to avoid possible bias by anthropogenic activities because *Ninnigobius canestrinii* was introduced in several water bodies in Croatia during the second half of the 20th century (Miller 2004, Jelić et al. 2016).

Table 1: Material examined. 1 Population from Lake Trasimeno (IT). 5 Populations from River Jadro (CR).

Locality	Coordinates	Number	Habitat	Year	Individuals (n)	Sex	Size (mm)
Lago Trasimeno	43°8′N, 12°6′E	Coll. H. Ahnelt	lake	2015	140	46 females 94 males	22.64-38.28 mm
River Jadro	43°30′N, 16°26′E	NMW 30632	stream	1881	4	4 males	34.72-44.08 mm
River Jadro	43°30′N, 16°26′E	NMW 30635	stream	1882	15	1 female 11 males 3 unknown	38.16-46.78 mm
River Jadro	43°30′N, 16°26′E	NMW 30612	stream	1883	14	11 males 3 unknown	35.48-51.78 mm
River Jadro	43°30′N, 16°26′E	NMW 78700	stream	1886	9	4 females 5 males	30.16-44.98 mm
River Jadro	43°30′N, 16°26′E	NMW 79816	stream	1892	6	3 females 3 males	30.52-46.16 mm

Methods

All fishes were examined with the aid of a binocular microscope. For each specimen the following morphological traits were examined: sex, standard length (SL) in mm, pattern of head canals and pattern of squamation (Figs. 10 and 11).

The patterns of head canals and squamation were assorted to different types of reduction: head canal types (type A-J) and squamation types (type A-F), starting with type A as the most complete pattern and ending with type J and F as the most reduced patterns that were found. Sex was determined by the shape of the urogenital papilla. The urogenital papilla of the males is longer than wide, those of the females is about as long as wide.

Specimens with a standard length smaller than 22.60 mm were not included into the data as the development of squamation and head canals was considered to be complete only at a body size over 22.60 mm. Calculations and the evaluation of the data were done via Microsoft Excel.

Locality ....TRASIMENO SEE.......... Date ....

Number .....TRA-P2015-05:27............

| SL	24,15 mm	Notes
Sex	♀	
Squamation	continuous ✓ in patches	
Scales reach dorsal midline	before origin of D1 at origin of D1 before middle of D1 before end of D1 before D2 (interdorsal) ✓ at origin of D2	
Scales reach ventral midline	Before anus at origin of anal fin before middle of anal fin before end of anal fin posterior to anal fin ✓	continuous
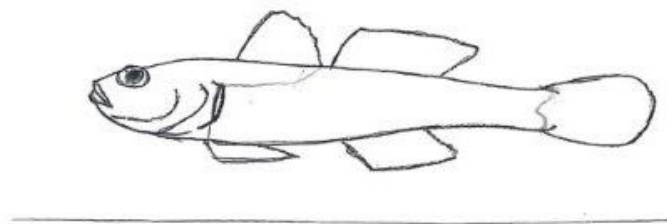
Figure 10: Datasheet which was used to collect data on the squamation pattern. This datasheet was used for each specimen examined.

GOBIUS QUAGGA / N. canestrinii

Project Knipowitschia/Pomatoschistus

L. Koch, H. Ahnelt

Locality SPALATO	Date 17.4.19
Collection NMW 30612	Number NMW 30612; 04
SL (mm) 44,90 mm	Sex ♂

AOS left	Pores C̶ D̶ / F̶ / H̶ / present	B ✓
AOS right	Pores C̶ / D̶ / F̶ / H̶ / present	B ✓
AOS	Additional pores B(r.+l.) Missing pores	
POS	Present Y/N Pores Furrow left right	
POC	Complete with pores M̶ O̶ Furrow left ✓ right	
POC	Incomplete/ furrow	
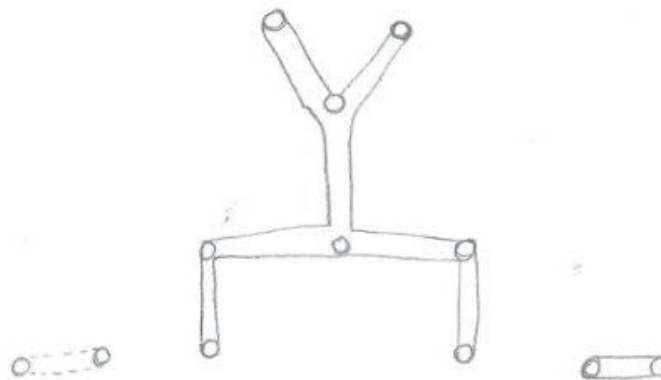
Figure 11: Datasheet which was used to collect data on the pattern of head canals. This datasheet was used for each specimen examined.

RESULTS

Fishes from Lake Trasimeno

The introduced population of Lake Trasimeno showed a very high variation of head canal patterns. All in all, 12 different types of head canal patterns have been found. The majority showed a reduction of the anterior part of the anterior oculoscapular canal (AOS).

The pattern of squamation was very homogenous within the lake population. The majority of all types have been assorted to two types (type B and C), while only a few specimens were assorted to another type.

Size

Altogether 140 specimens from Lake Trasimeno were examined. Males tend to be bigger than females (table 2). The range of standard length is about the same in both sexes, 22.64 mm and 38.28 mm in females and a range between 22.60 mm and 37.11 mm in males. The mean standard length of females is 26.80 mm, which is 5.86% smaller than in males (table 2). The standard deviation for males (3.11) is higher than for females (3.06).

Table 2: Standard length and number of examined males and females of the population from Lake Trasimeno.

Sex	females	males
Total number (n)	46	94
Standard length (range) in mm	22.64 - 38.28	22.60 - 37.11
Mean Standard length in mm	26.80	28.47
Standard Deviation	3.06	3.11

Head Canals

Twelve different types of head canals were found in the lake population (fig. 12, table 3). The most frequent types were type H, which 28.99 % of all specimens had, followed by type F with a share of 18.84 % and type G with a share of 15.22 %. The vast majority of all head canal types showed reductions. Only 5.07% had a complete or almost complete head canal system. Also, the majority lacks the anterior part of the AOS (between pores B and C), from type F on, all types show this reduction (fig. 12).

Table 3: Number (n = 138) and percentage (%) of the 12 head canal types in the population of Lake Trasimeno. Type A: most complete type. Type L: most reduced type.

Canal types	number (n)	percentage (%)
A	5	3.60
B	2	1.44
C	2	1.44
D	1	0.72
E	1	0.72
F	26	18.71
G	21	15.11
H	40	28.78
I	8	6.47
J	11	7.91
K	17	12.23
L	4	2.88
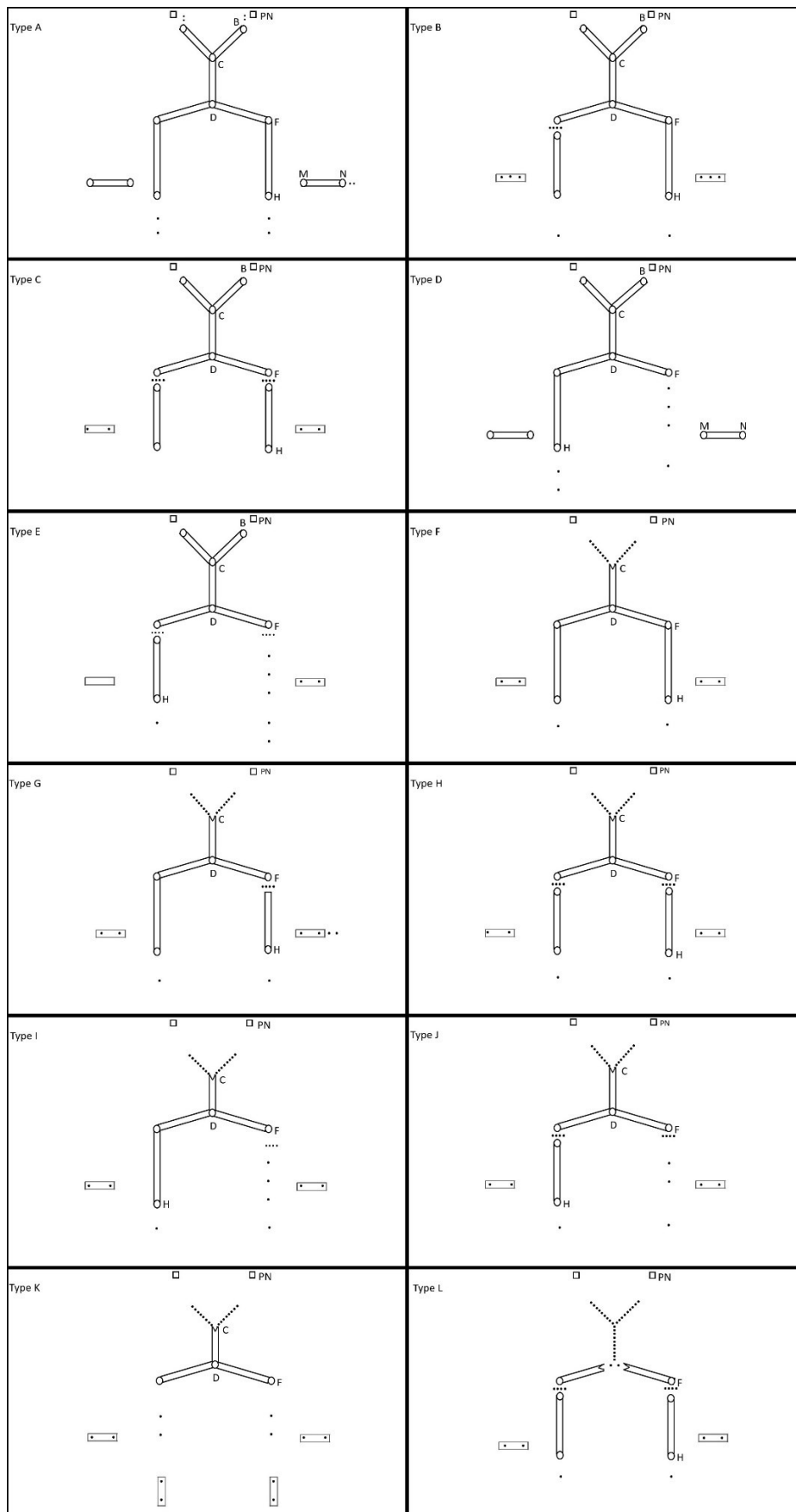
Figure 12: The patterns of head canals found in the introduced population of Lake Trasimeno. Type A: most complete, type L: most reduced type.
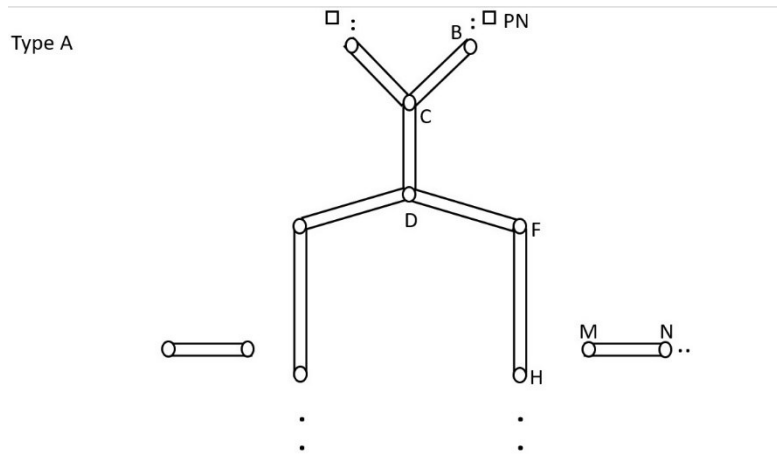
Figure 13: Head canal type A found in the population of lake Trasimeno. (PN: posterior nasal pore; B-N: pores).

Type A: Five specimens showed a complete head canal system (fig. 13). The anterior oculoscapular canal (AOS) is completely developed, carrying pores B (paired), C, D (unpaired) and F and H (both paired). The posterior oculoscapular canal (POS) is absent, just neuromasts are present: either one or two neuromasts. The preopercular canal (POC) is either developed on both sides with pores M and N respectively, or just on one side (with pores M and N) or reduced to a furrow (in three specimens) and replaced by superficial neuromasts: one specimen has three neuromasts in a furrow, one specimen has four neuromasts besides a completely developed preopercular canal (POC) and two specimens have two neuromasts within a furrow.
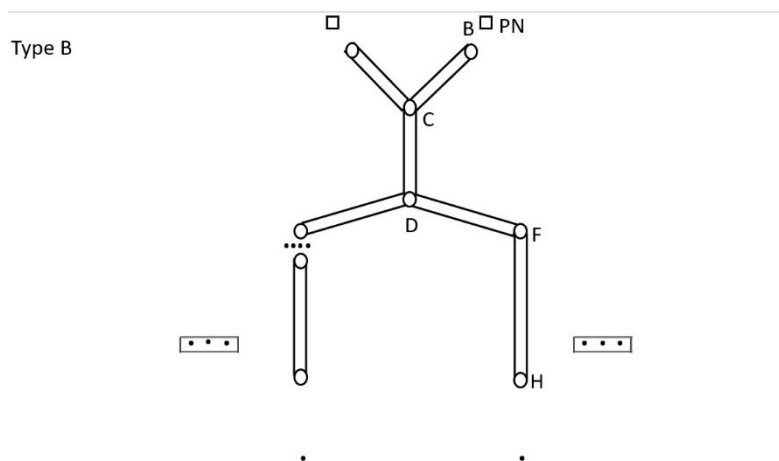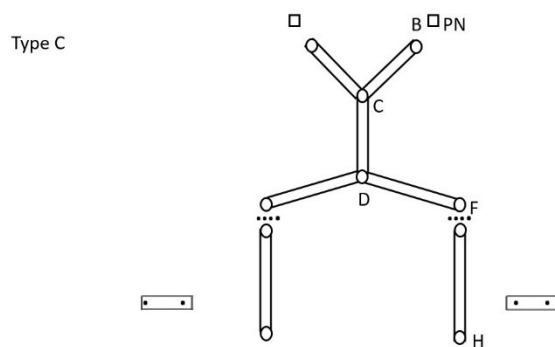

Figure 14: Head canal type B found in the population of lake Trasimeno. (PN: posterior nasal pore; B-H: pores).

Type B: Two specimens were assorted to type B (fig. 14). The anterior oculoscapular canal (AOS) carries pores B (paired), C, D (unpaired) and F and H (both paired). Porus C is either

reduced (one specimen) or not present (one specimen). The left canal section between porus F and H is separated from the anterior oculoscapular canal (AOS). The posterior oculoscapular canal (POS) is absent, just neuromasts are present: one neuromast left and right. The preopercular canal (POC) is reduced to a furrow with neuromasts (in both specimens) on both sides: one specimen has three neuromasts (left) and six neuromasts (right) within a furrow and the other specimen has three neuromasts on each side.

Figure 15: Head canal type C found in the population of lake Trasimeno. (PN: posterior nasal pore; B-H: pores).

Type C: Two specimens were assorted to type C (fig. 15). The AOS carries pores B (paired), C, D (unpaired) and F and H (both paired). Porus C is either reduced (one specimen) or not present (one specimen). Porus E was present on one side in one specimen. The left and the right canal section between porus F and H are separated from the anterior oculoscapular canal (AOS). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast left and right. The preopercular canal (POC) is reduced to a furrow (in both specimens) with two neuromasts on both sides.
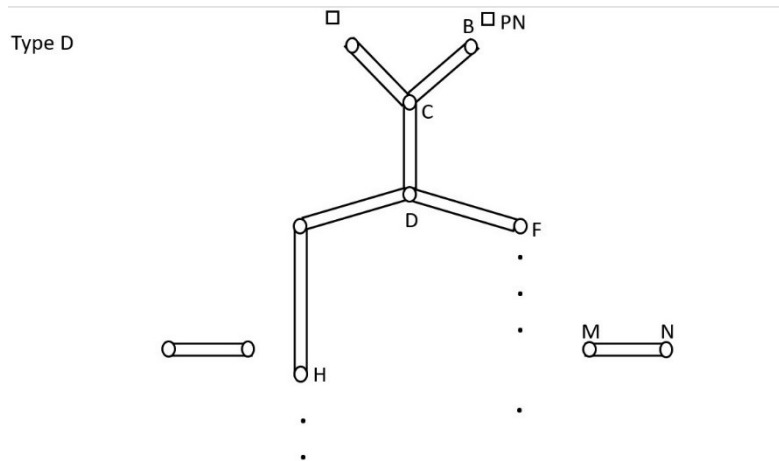
Figure 16: Head canal type D found in the population of lake Trasimeno. (PN: posterior nasal pore; B-N: pores).

Type D: One specimen was assorted to type D (fig. 16). The AOS carries pores B (paired), C, D (unpaired) and F (paired) and H (left side only). The right canal section between porus F and H is absent and reduced to three neuromasts on the right side. The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and two neuromasts left. The preopercular canal (POC) is developed on both sides with pores M and N.
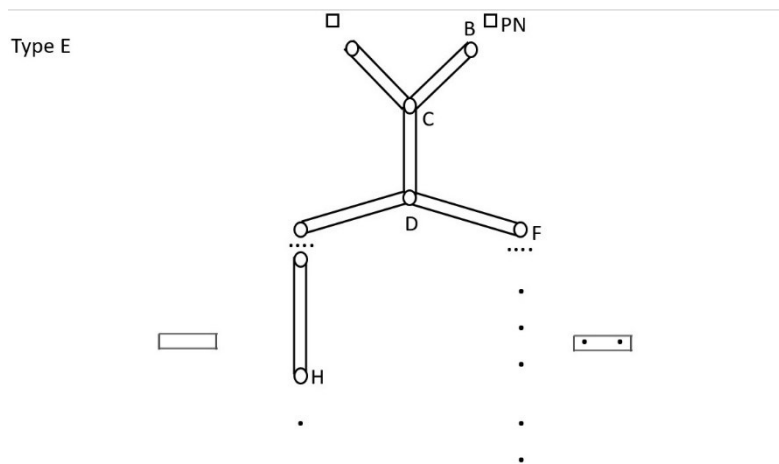

Figure 17: Head canal type E found in the population of lake Trasimeno. (PN: posterior nasal pore; B-N: pores).

Type E: One specimen was assorted to type E (fig. 17). The AOS carries pores B (paired), C, D (unpaired) and F (paired) and H (left side only). The left canal section between porus F and H is separated from the anterior oculoscapular canal (AOS). The right canal section between porus F and H is absent and reduced to three neuromasts on the right side. The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast left and two

neuromasts right. The preopercular canal (POC) is developed on both sides with pores M and N.
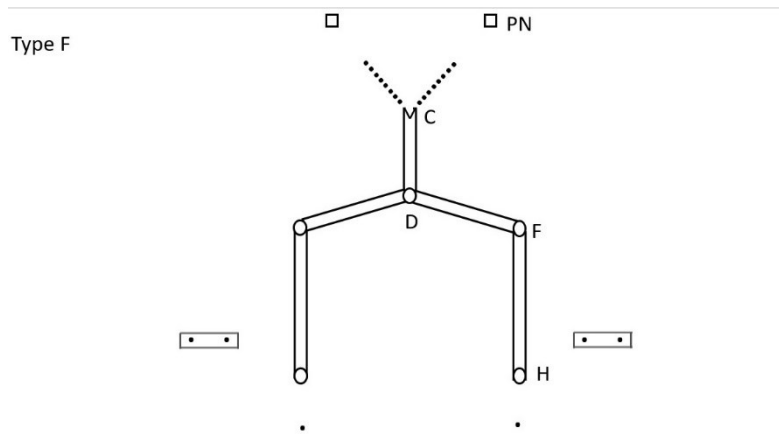

Figure 18: Head canal type F found in the population of lake Trasimeno. (PN: posterior nasal pore; C-H: pores).

Type F: 26 specimens were assorted to type F (fig. 18). The anterior oculoscapular canal (AOS) carries pores B (paired, in one specimen) C, D (unpaired) and F and H (both paired). Pores B (paired) are absent (in 23 specimens). three specimens have developed porus B either on the left side (one specimen) with one neuromast on the right side or on the right side (two specimens) with either one neuromast (one specimen) or a furrow (one specimen) on the left side. Porus C is absent in one specimen and reduced to a furrow with one neuromast right and left. The anterior canal section between pores B and C of the AOS is absent, only neuromasts are present: either one neuromast right and left (16 specimens), two neuromasts right and one neuromast left (three specimens) or two neuromasts left and one neuromast right (two specimens) or two neuromasts right and one neuromast left (three specimens). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (17 specimens), two neuromasts right and left (four specimens), one neuromast right and two neuromasts left (one specimen), one neuromast right and three neuromasts left (one specimen), one neuromast only right (one specimen), one neuromast only left (one specimen) or no neuromasts at all (one specimen).

The preopercular canal (POC) is either developed on both sides with pores M and N respectively (one specimen), developed on one side only (in four specimens) or reduced to a furrow (in 19 specimens) and replaced by superficial neuromasts: two neuromasts right and left

(13 specimens), three neuromasts right and left (one specimen), two neuromasts right and three left (one specimen), two neuromasts left and three right (two specimens), three neuromasts left and five right (one specimen) or three neuromasts left and one right (one specimen). Additional neuromasts to a completely developed POC were found (in two specimens): either with one additional neuromast right (one specimen) or one additional neuromast left (one specimen). four specimens have a completely developed POC on one side only: three specimens on the right and one specimen on the left side. The other side, either left or right, is reduced to a furrow and replaced by superficial neuromasts: developed POC right with an additional neuromast right and two neuromasts left (one specimen), developed POC right and two neuromasts left (two specimens) or developed POC left and two neuromasts right (one specimen).
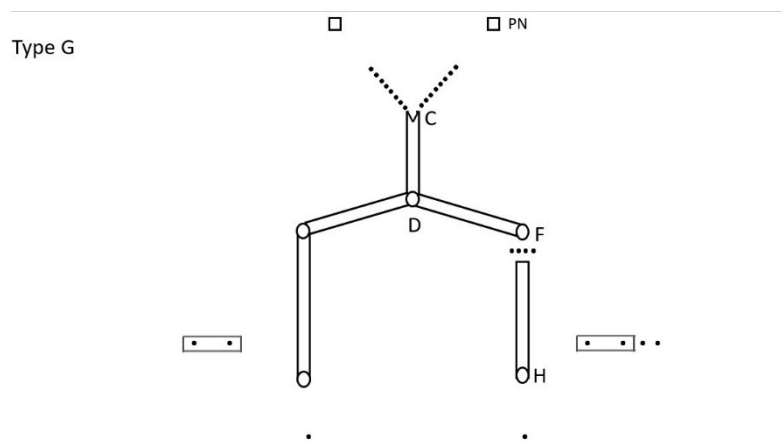

Figure 19: Head canal type G found in the population of lake Trasimeno. (PN: posterior nasal pore; C-H: pores).

Type G: 21 specimens were assorted to type G (fig. 19). The anterior oculoscapular canal (AOS) carries pores C, D (unpaired) and F and H (both paired). Pores B (paired) are absent (in 16 specimens). five specimens have developed pores B either on the left side (one specimen) with one neuromast right or on the right side (four specimens) with either one neuromast (three specimens) or a furrow (one specimen) on the left. The anterior canal section between pores B and C of the AOS is absent, only neuromasts are present: either two neuromasts right and left (17 specimens), two neuromasts right and one neuromast left (one specimen) or two neuromasts left and one neuromast right (one specimen). The left (in 11 specimens) or the right canal section (in 10 specimens) between porus F and H is separated from the anterior oculoscapular canal (AOS). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one

neuromast right and left (13 specimens), two neuromasts right and left (one specimen), one neuromast right and two neuromasts left (two specimens), one neuromast left and two neuromasts right (three specimens), two neuromasts left and three neuromasts right (one specimen) or no neuromasts at all (one specimen). The preopercular canal (POC) is developed on one side only (in one specimen) or reduced to a furrow (in 20 specimens) and replaced by superficial neuromasts: two neuromasts right and left (15 specimens), two neuromasts right and three left (three specimen), two neuromasts left and four right (one specimen) or four neuromasts left and two right (one specimen).one specimen has a completely developed POC on the right side only. The other side is reduced to a furrow and replaced by two superficial neuromasts.
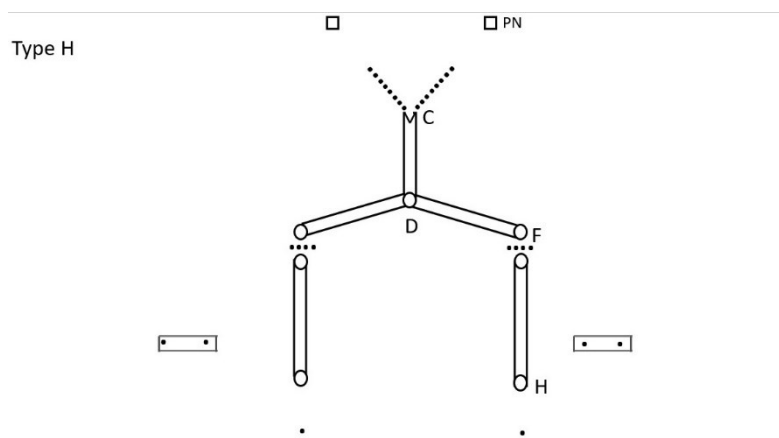

Figure 20: Head canal type H found in the population of lake Trasimeno. (PN: posterior nasal pore; C-H: pores).

Type H: The majority of all specimens (40) were assorted to type H (fig. 20). The anterior oculoscapular canal (AOS) carries pores C, D (unpaired) and F and H (both paired). Pores B (paired) are absent (in 37 specimens). three specimens have developed pores B either on the left side (two specimens) with one neuromast right or on the right side (one specimen) with either one neuromast on the left. The anterior canal section between pores B and C of the AOS is absent, only neuromasts are present: either one neuromast right and left (25 specimens), two neuromasts right and left (three specimens), two neuromasts right and one neuromast left (five specimens), two neuromasts left and one neuromast right (two specimens), two neuromasts left and three neuromasts right (one specimen) or no neuromasts at all (one specimen). The right and left canal section between porus F and H is separated from the anterior oculoscapular canal (AOS). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (18 specimens), two neuromasts right and left (four specimens), one

neuromast right and two neuromasts left (four specimens), one neuromast left and two neuromasts right (four specimens), three neuromasts left and one neuromasts right (one specimen), one neuromast only left (four specimens) or no neuromasts at all (four specimens). The preopercular canal (POC) is either developed on both sides with pores M and N respectively (two specimens) or reduced to a furrow (in 38 specimens) and replaced by superficial neuromasts: one neuromast right and left (one specimen), two neuromasts right and left (28 specimens), five neuromasts right and left (one specimen) or three neuromasts left and one right (three specimens).
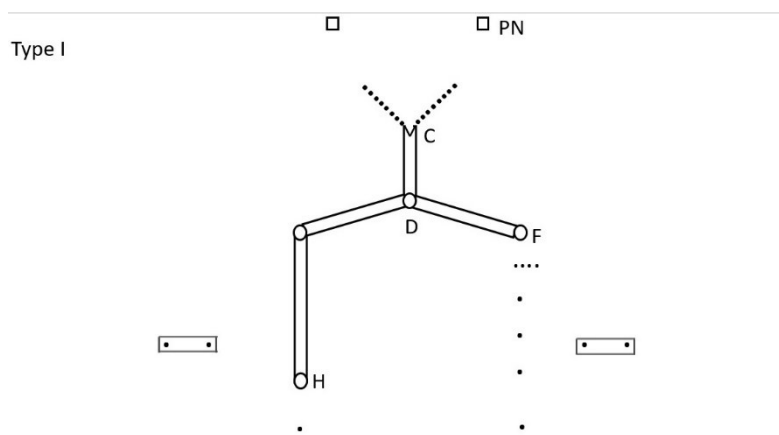

Figure 21: Head canal type I found in the population of lake Trasimeno. (PN: posterior nasal pore; C-H: pores).

Type I: Nine specimens were assorted to type I (fig. 21). The anterior oculoscapular canal (AOS) carries pores C (unpaired, but paired in one specimen), D (unpaired) and F (paired) and H (only right). Pores B (paired) are absent (in six specimens). three specimens have developed pores B either on the left side (one specimen) with one neuromast right or on the right side (two specimens) with either one or two neuromasts on the left. The anterior canal section between pores B and C of the AOS is absent, only neuromasts are present: either one neuromast right and left (four specimens) or two neuromasts right and one neuromast left (two specimens). The left (in five specimens) or right (in four specimens) canal section between pores F and H of the AOS is absent, only neuromasts are present: either one (two specimens) or two (three specimens) neuromasts left, two neuromasts right (two specimens), three neuromasts right (one specimen) or no neuromasts at all (one specimen). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (one specimen), two neuromasts

right and left (one specimen), one neuromast right and two neuromasts left (three specimens), one neuromast left and two neuromasts right (two specimens) or no neuromasts at all (two specimens). The preopercular canal (POC) is reduced to a furrow (in nine specimens) and replaced by superficial neuromasts: two neuromasts right and left (five specimens), three neuromasts right and left (one specimen), eight neuromasts right and left (one specimen), two neuromasts right and three left (one specimen) or six neuromasts right and left (one specimen).
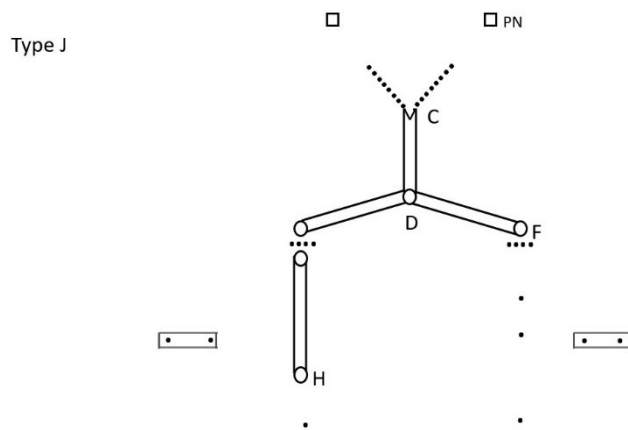

Figure 22: Head canal type J found in the population of lake Trasimeno. (PN: posterior nasal pore; C-H: pores).

Type J: 11 specimens were assorted to type J (fig. 22). The anterior oculoscapular canal (AOS) carries pores C, D (unpaired) and F and H (both paired). Pores B (paired) are absent. The anterior canal section between pores B and C of the AOS is absent, only neuromasts are present: either one neuromast right and left (seven specimens), two neuromasts right and left (one specimen), two neuromasts right and one neuromast right (one specimen) or two neuromasts left and one neuromast left (one specimen) or with one neuromast only left (one specimen). The left (in six specimens) or the right (in five specimens) canal section between porus F and H is separated from the anterior oculoscapular canal (AOS). The right canal section (six specimens) or the left canal section (five specimens) between porus F and H is absent, just neuromasts are present: one neuromast left (one specimen), two neuromasts left (two specimens), three neuromasts left (two specimens), one neuromasts right (one specimen), two neuromasts right (three specimens) or three neuromasts right (two specimens). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (four specimens), one neuromast right and two neuromasts left (two specimens), two neuromasts right and one neuromast left (two specimens), two neuromasts right and three neuromasts left (one specimen), two neuromasts left and three neuromasts right (one specimen) or no neuromasts at all (one

specimen). The preopercular canal (POC) is reduced to a furrow (in 11 specimens) and replaced by superficial neuromasts: two neuromasts right and left (seven specimens), two neuromasts right and three left (one specimen), two neuromasts left and three right (one specimen) or two neuromasts left and seven right (two specimens).
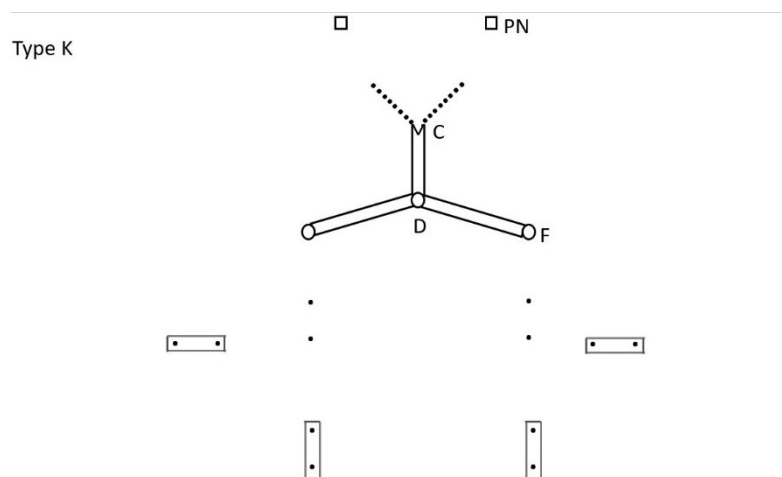

Figure 23: Head canal type K found in the population of lake Trasimeno. (PN: posterior nasal pore; C-F: pores).

Type K: 17 specimens were assorted to type K (fig. 23). The anterior oculoscapular canal (AOS) carries pores C, D (unpaired) and F (paired). Pores B and H (paired) are absent (in 17 specimens). two specimens carry pores E (paired). One specimen has developed pore B either on the right side with one neuromast left. The anterior canal section between pores B and C of the AOS is absent (in 16 specimens), only neuromasts are present: either one neuromast right and left (12 specimens), three neuromasts right and left (one specimen), two neuromasts right and one neuromast left (two specimens) or two neuromasts left and one neuromast left (one specimen). The canal section between pores F and H of the AOS is absent, only neuromasts are present: either one neuromast right and left (one specimen), two neuromasts right and left (five specimens), three neuromasts right and left (one specimen), two neuromasts right and one neuromast right (one specimen), two neuromasts right and three neuromasts left (four specimens), one neuromast left and three neuromasts right (two specimens) or two neuromasts left and three neuromast right (three specimens). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (five specimens), two neuromasts right and left (four specimens), one neuromast right and two neuromasts left (one specimen), one neuromast right and three neuromasts left (one specimen), two neuromasts right and three neuromasts left (two specimens), one neuromast left and two neuromasts right (three specimens), or two neuromasts left and three neuromasts right (one specimen). The

preopercular canal (POC) is reduced to a furrow (in 11 specimens) and replaced by superficial neuromasts: two neuromasts right and left (six specimens), three neuromasts right and left (three specimens), five neuromasts right and left (one specimen), two neuromasts right and three left (three specimens), one neuromast left and two neuromasts right (one specimen) or two neuromasts left and three right (two specimens).
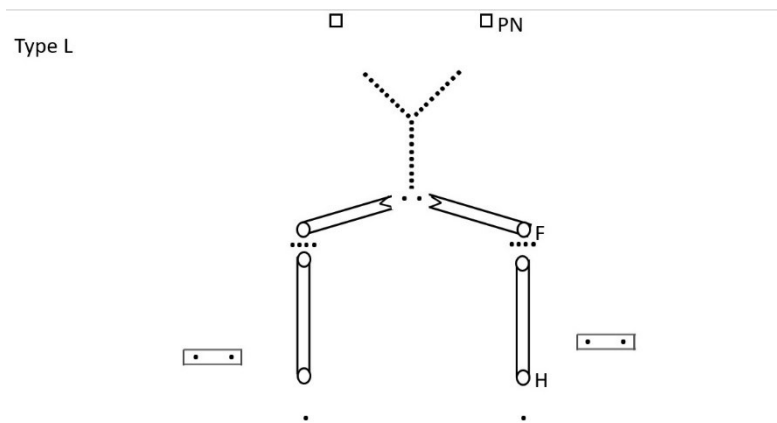

Figure 24: Head canal type L found in the population of lake Trasimeno. (PN: posterior nasal pore; C-F: pores).

Type L: Four specimens were assorted to type L (fig. 24). The anterior oculoscapular canal (AOS) carries pores F and H (both paired). Pores B (paired) (four specimens), porus C (paired) (four specimens) and porus D (one specimen) are absent. The anterior canal section between pores B and D of the AOS is absent, only neuromasts are present: at the region of pores B, either one neuromast right and left (three specimens) or no neuromasts at all (one specimen). At the region of porus C, either one neuromast right and left (two specimens) or no neuromasts at all (two specimens). The canal section between porus F and H is separated from the anterior oculoscapular canal (AOS) (one specimen), connected to the AOS (one specimen) or absent, just neuromasts are present: either four neuromasts right and left (one specimen) or no neuromasts at all (one specimen). The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast left and right (two specimens) or no neuromasts at all (two specimens). The preopercular canal (POC) is either developed on both sides with pores M and N respectively (one specimen) or reduced to a furrow (in three specimens) and replaced by superficial neuromasts: two neuromasts right and left (one specimen) or three neuromasts right and two neuromasts left (two specimens).

Squamation types

Six different types of squamation were found in the lake population (table 4). The most frequent types were type C, which more than half (58.16 %) of all specimens had, followed by type B with 37.59 %. The majority showed a patchy squamation (type C, fig. 25), followed by a continuous squamation with naked areas along the ventral midline (type B). The squamation types in the lake population are quite homogenous, as only a few (either one or three specimens) were assorted to a different squamation type than type B and C.

Table 4: Number (n = 141) and percentage (%) of the six squamation types found in the population of lake Trasimeno. Type A: most complete type. Type F: most reduced type.

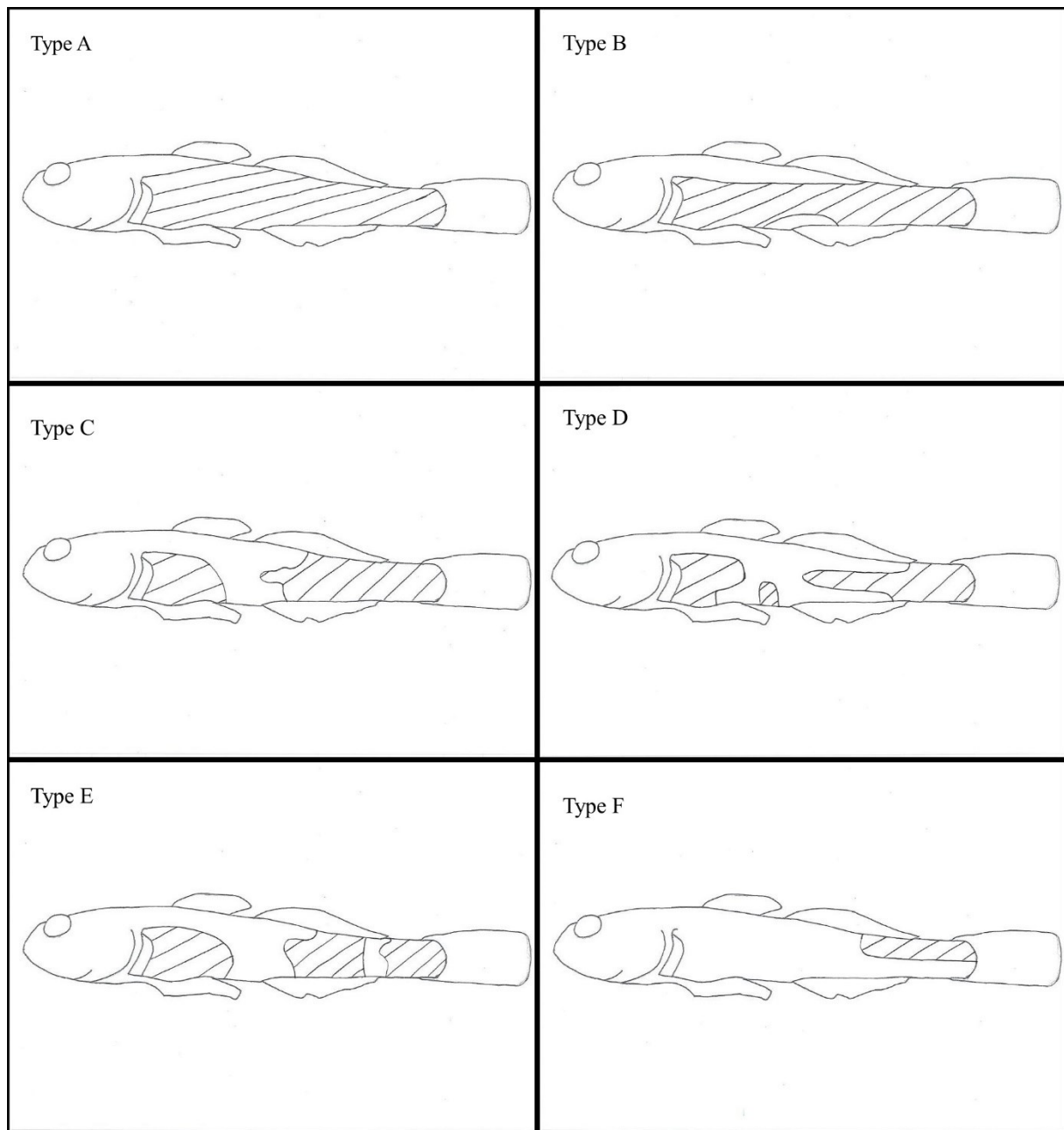| Squamation types	number (n)	Percentage (%)
A	1	0.71
B	53	37.59
C	82	58.16
D	1	0.71
E	3	2.13
F	1	0.71

Type A

Type B

Type C

Type D

Type E

Type F

Figure 25: All patterns of squamation found in lake Trasimeno. Type A: most complete, type F: most reduced type.
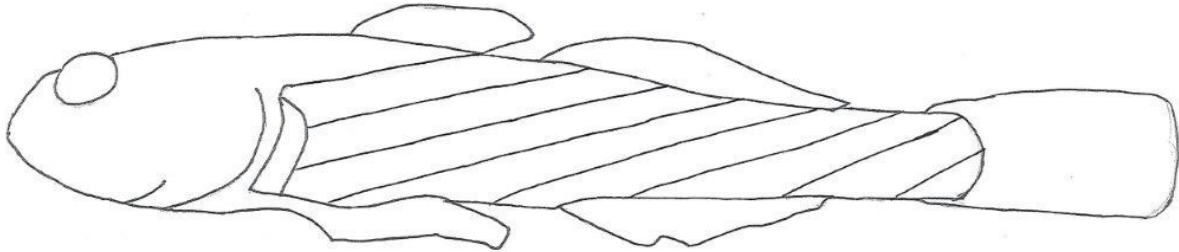
Type A

Figure 26: Squamation type A found in the population of lake Trasimeno.

Type A: One specimen was assorted to squamation type A. The squamation is continuous, reaching from the origin of the pectoral fin to the origin of the caudal fin (fig. 26). The dorsal part of the body (predorsal area) before the end of D1 is scale-free. Scales reach the dorsal midline at the end of D1 and the ventral midline underneath the pectoral fin.
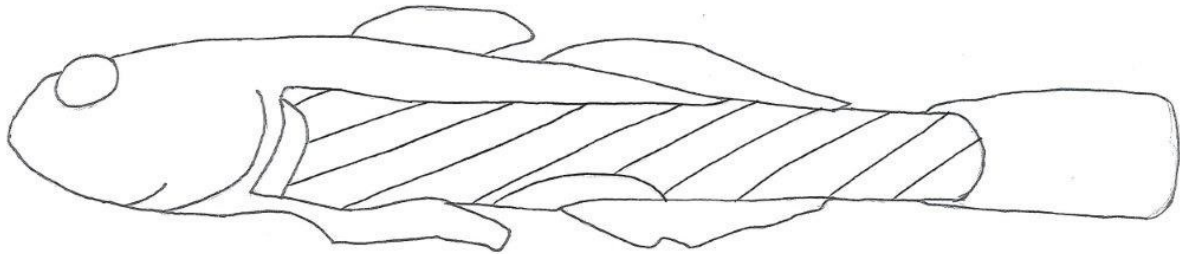
Type B

Figure 27: Squamation type B found in the population of lake Trasimeno.

Type B: 53 specimens were assorted to squamation type B. The squamation is continuous, reaching from the origin of the pectoral fin to the origin of the caudal fin or shortly before the origin of the caudal fin. The dorsal part of the body is scale-free. Size and length of the dorsal scale-free area can vary, as the region, where scales reach the dorsal midline varies (fig. 27). Scales reach the dorsal midline either before the middle of dorsal fin 2 (D2), before or at the end of D2. The ventral midline shows one scale-free area around the origin of the anal fin. One specimen had even three scale-free areas around the ventral midline of the body: one near the origin of the pelvic fin, the second around the anal fin and the third one near the caudal fin. Scales reach the ventral midline either before the anus, at the origin of the anal fin, before the middle of the anal fin, before the end of the anal fin or posterior to the anal fin.
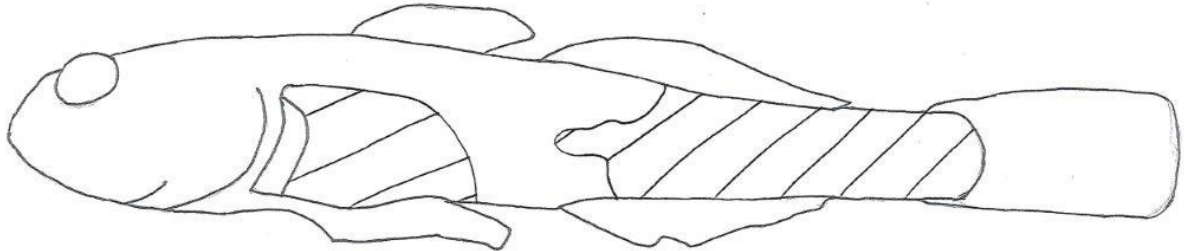
Type C

Figure 28: Squamation type C found in the population of lake Trasimeno.

Type C: The most common squamation type of the lake population was type C. The squamation is patchy and divided into two patches: one is located around the pectoral fin and the other at the posterior part of the body, reaching either from the middle or the posterior third of the body back to the origin of the caudal fin (fig. 28). Scales reach the dorsal midline either before the middle of dorsal fin 2 (D2), at the middle of D2, before the end of D2, at the end of D2 or posterior to D2. The ventral region around the anus is always scale-free. Scales reach the ventral midline either before the anus, at the origin of the anal fin, before the end of the anal fin or before the middle of the anal fin.
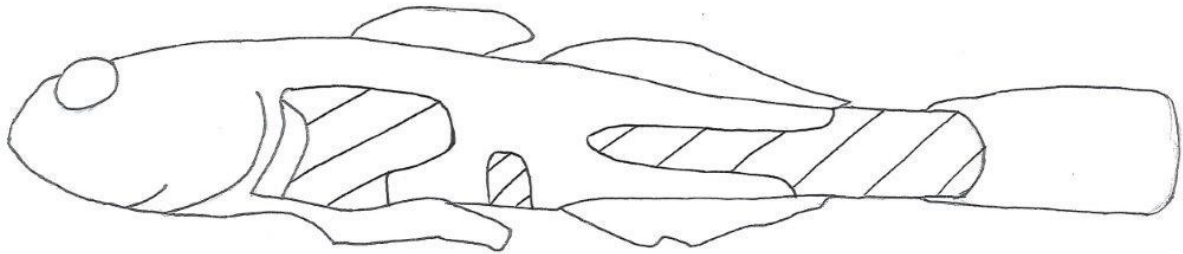
Type D

Figure 29: Squamation type D found in the population of lake Trasimeno.

Type D: One specimen was assorted to squamation type D. The squamation is patchy and divided into three patches: one is located around the pectoral fin and the second between D2 and the anal fin and the third at the posterior part of the body, reaching from posteriorly to the anal fin and D2 back to the origin of the caudal fin (fig. 29). Scales reach the dorsal midline at the middle of dorsal fin 2 (D2), before the end of D2 and posterior to D2. Scales reach the ventral midline before the anus, before the middle of the anal fin, before the end of the anal fin or posterior the anal fin.
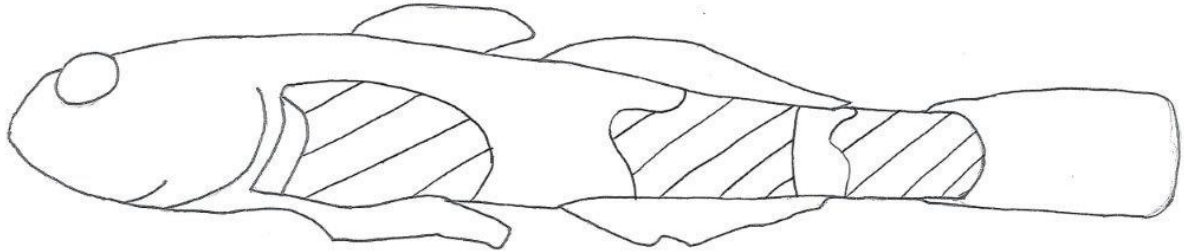
Type E

Figure 30: Squamation type E found in the population of lake Trasimeno.

Type E: Three specimens were assorted to squamation type E. The squamation is patchy and divided into two or three patches: one patch is located around the pectoral fin and the second patch is located between the pelvic and the anal fin and the third patch is located at the posterior part of the body, reaching either from the middle or from the posterior third of the body back to the origin of the caudal fin (fig. 30). Scales reach the dorsal midline either before the middle of dorsal fin 2 (D2), at the end of D2 or posterior to D2. Scales reach the ventral midline before the end of the pelvic fin, before the anus or before the end of the anal fin.
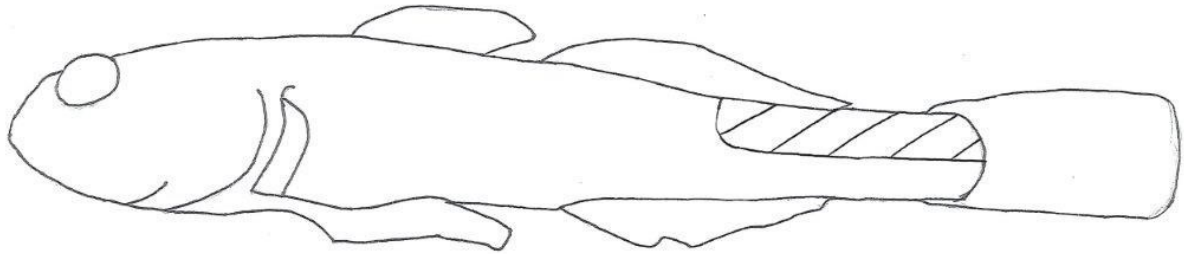
Type F

Figure 31: Squamation type F found in the population of lake Trasimeno.

Type F: One specimen was assorted to squamation type F. The squamation is patchy, consisting of one patch only which is located dorsally at the posterior part of the body (fig 31). Scales reach the dorsal midline before the end of dorsal fin 2 (D2). Scales do not reach the ventral midline at all.

Jadro River

The natural population of river Jadro showed a high variation of head canal patterns. All in all, nine different types of head canal patterns have been found, where exact 50% of all specimens have been assorted to only one type (type C).

The pattern of squamation was also quite homogenous within the natural population. The majority of all types could have been assorted to one type (type C).

Size

Altogether 48 specimens from river Jadro were examined. In six specimens the sex determination was not possible. According to table 5, we found that males tend to be bigger than females and they also show a bigger range of standard length than female specimens. The mean standard length of females is 37.98 mm, which is 4. 31 mm smaller than in males (table 5). Also, the standard deviation for males (5.55) is higher than for females (5.14).

Table 5: Standard length and number of examined males and females of the population from River Jadro.

Sex	females	males
Total number (n)	8	34
Standard length (range)	45.28 mm- 32.09 mm	51.70 mm- 30.16 mm
Mean Standard length	37.98 mm	42.10 mm
Standard Deviation	5.14	5.55

Head Canal Types

Nine different types of head canals in the Jadro River population were found (table 6). Only one specimen showed a complete head canal system (type A). The most frequent types were type C, which was found in 50 % of all specimens, followed by type G with 14.58 % and type D with 10.42 %. All other types were equally distributed among the examined specimens with two or three individuals per type.

Table 6: Number (n) and percentage (%) of head canal types found in the natural population of river Jadro. Type A: most complete type. Type I: most reduced type (n= 48).

Canal types	number (n)	percentage (%)
A	1	2.08
B	2	4.17
C	24	50.00
D	5	10.42
E	3	6.25
F	2	4.17
G	7	14.58
H	2	4.17
I	2	4.17

Figure 32 shows all patterns of head canals which were found in the Jadro river population (fig.32). beginning with type A as the most complete and ending with type I as the most reduced head canal system.
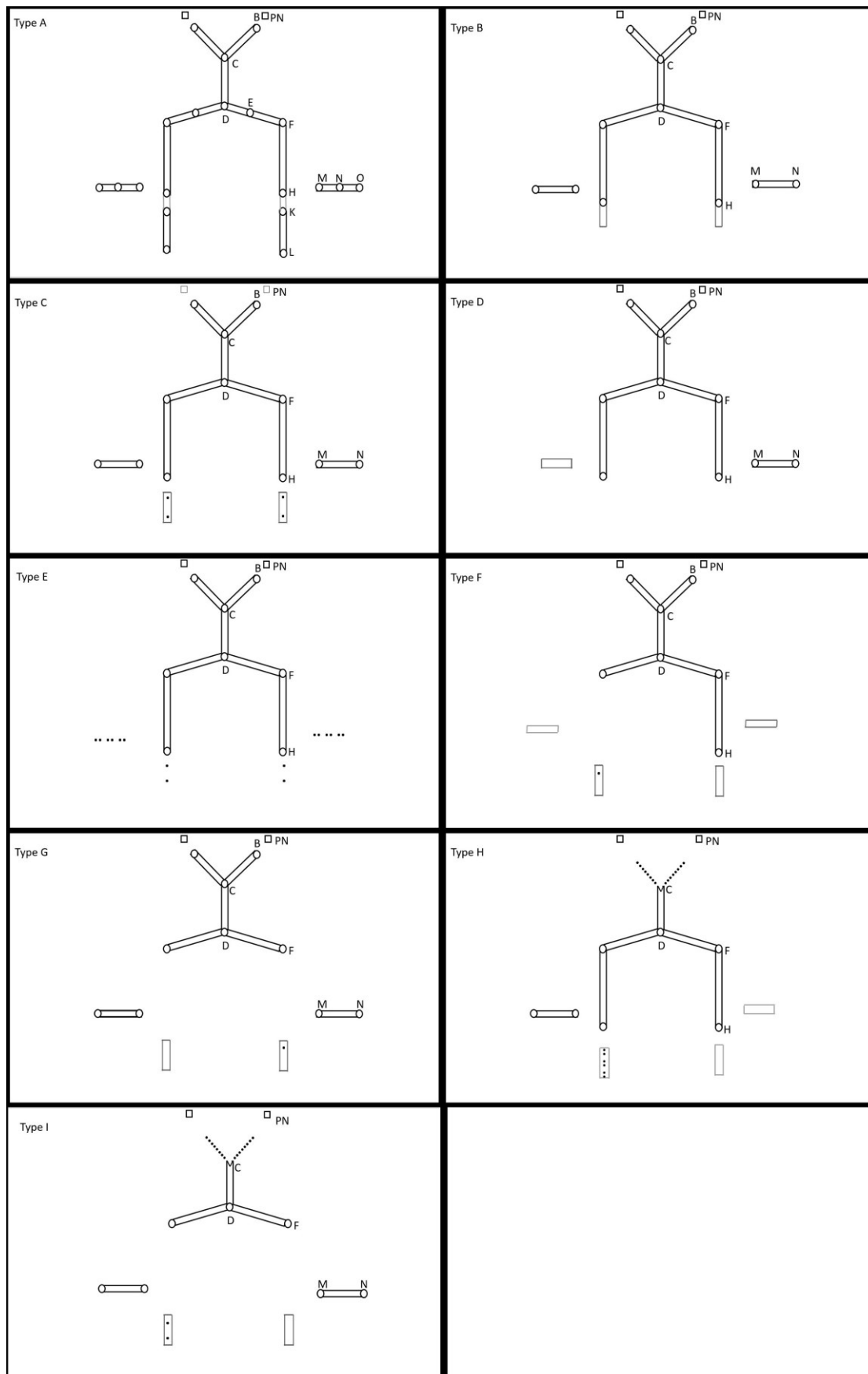
Figure 32: Patterns of head canals found in the natural population of river Jadro. Type A: most complete. type I: most reduced type.
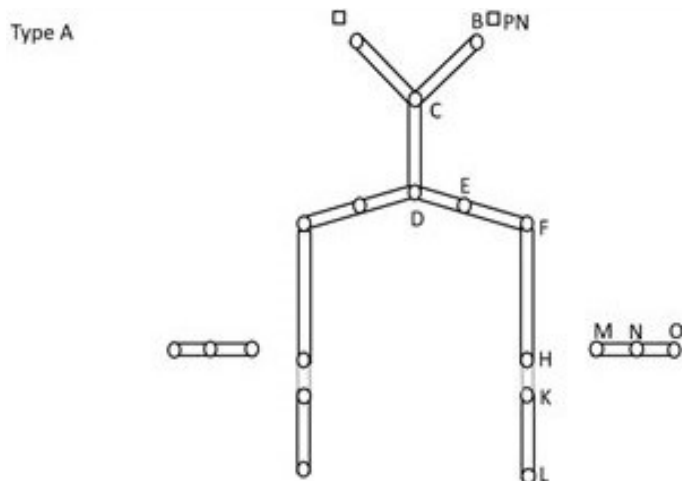
Figure 33: Head canal type A found in the Jadro river population. (PN: posterior nasal pore; B-O: pores).

Type A: One specimen showed a complete head canal system (fig. 33). The anterior oculoscapular canal (AOS) is completely developed, carrying pores B (paired), C, D (unpaired) and F, E and H (both paired). The anterior oculoscapular canal (AOS) is connected to the posterior oculoscapular canal (POS) with a furrow. The posterior oculoscapular canal (POS) is completely developed, carrying pores K and L (both paired). The preopercular canal (POC) is developed on both sides with pores M, N and O (all paired).
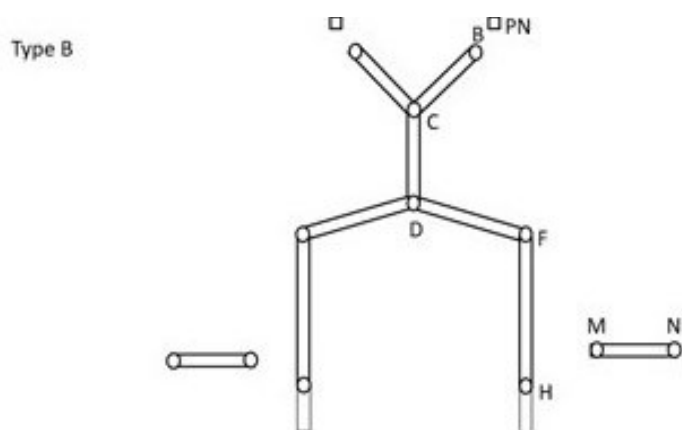

Figure 34: Head canal type B found in the Jadro river population (PN: posterior nasal pore; B-N: pores).

Type B: Type B was found in two specimens (fig. 34). The anterior oculoscapular canal (AOS) is completely developed, carrying pores B (paired), C, D (unpaired) and F and H (both paired).

The posterior oculoscapular canal (POS) is absent, just neuromast are present: two neuromasts left (one specimen) or no neuromasts at all (one specimen). The preopercular canal (POC) is either developed on both sides with pores M and N (both paired).
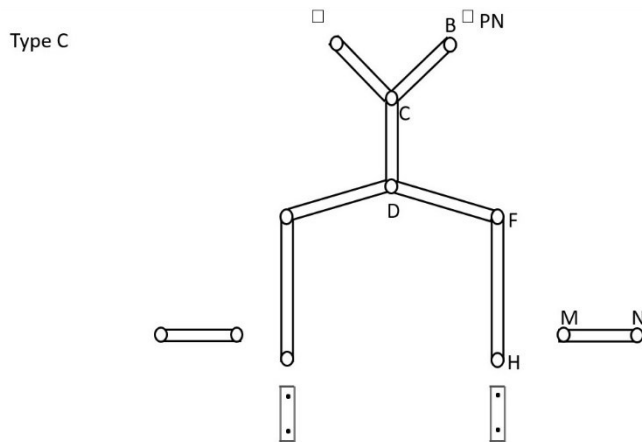

Figure 35: Head canal type C found in the Jadro river population (PN: posterior nasal pore; B-N: pores).

Type C: Type C was the most common type (24 specimens) (fig. 35). The anterior oculoscapular canal (AOS) is completely developed, carrying pores B (paired), C, D (unpaired) and F and H (both paired). Porus B is absent in one specimen on the right and reduced to a furrow with no neuromasts at all. one specimen has an open interorbital section between porus C and D. The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (one specimen), two neuromasts right and left (three specimens), four neuromasts right and left (one specimen), one neuromast right and three neuromasts left (one specimen), two neuromasts right and four neuromasts left (one specimen), two neuromasts right and five neuromasts left (one specimen), four neuromasts right and five neuromasts left (one specimen), two neuromasts left and four neuromasts right (one specimen), five neuromasts left and seven neuromasts right (one specimen), two neuromasts only right (two specimens), two neuromasts only left (one specimen), or no neuromasts at all (10 specimens). The preopercular canal (POC) is developed on both sides with pores M and N (both paired).
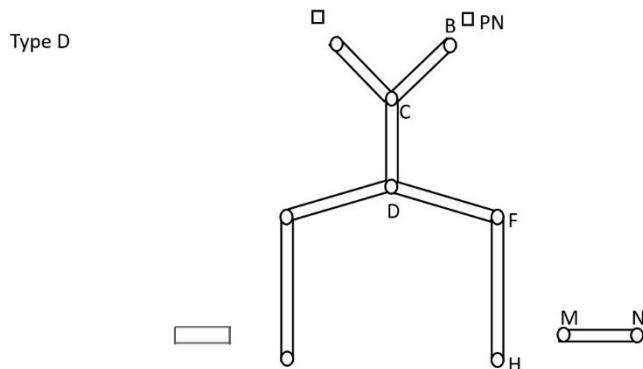
Figure 36: Head canal type D found in the Jadro river population (PN: posterior nasal pore; B-N: pores).

Type D: Type D was found in five specimens (fig. 36). The anterior oculoscapular canal (AOS) is completely developed, carrying pores B (paired), C, D (unpaired) and F and H (both paired). The posterior oculoscapular canal (POS) is absent, just neuromast are present: two neuromasts right and left (one specimen), three neuromasts right and four neuromasts left (one specimen), four neuromasts left and six neuromasts right (one specimen) or no neuromasts at all (two specimens). The preopercular canal (POC) is either developed on both sides with pores M and N respectively (one specimen), or just on the right side (with pores M and N) (four specimens) with a furrow on the left, replaced by superficial neuromasts: four neuromasts left (one specimen), nine neuromasts left (one specimen) or no neuromasts at all (two specimens).
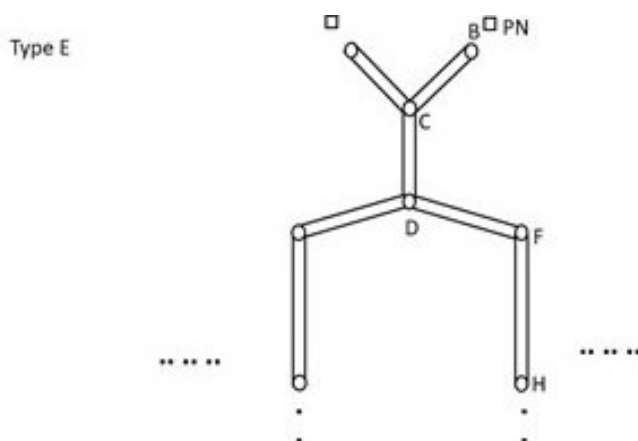

Figure 37: Head canal type E found in the Jadro river population (PN: posterior nasal pore; B-H: pores).

Type E: Type E was found in three specimens (fig. 37). The anterior oculoscapular canal (AOS) is completely developed, carrying pores B (paired), C, D (unpaired) and F and H (both paired). The posterior oculoscapular canal (POS) is either developed with pores K and L (both paired) (in one specimen) or absent, just neuromast are present: four neuromasts right and left (one specimens) or four neuromasts left and two neuromasts right (one specimen). The preopercular canal (POC) is reduced to a furrow and replaced by superficial neuromasts: two neuromasts right and left (one specimen), six neuromasts right and left (one specimen) or two neuromasts left and seven right (one specimen).
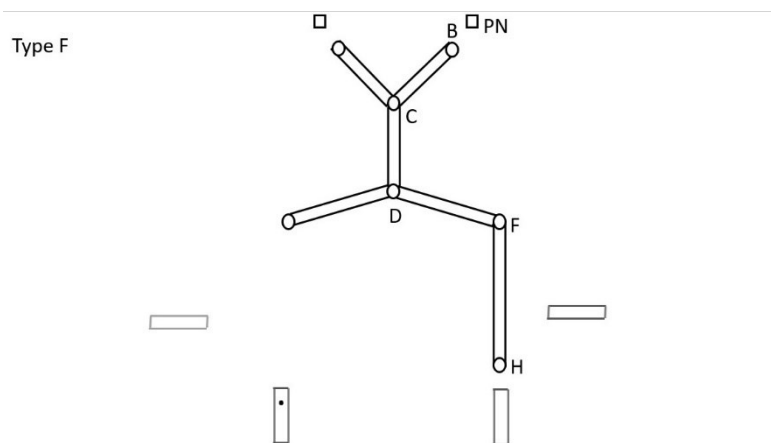

Figure 38: Head canal type F found in the Jadro river population (PN: posterior nasal pore; B-H: pores).

Type F: Type F was found in two specimens (fig. 38). The anterior oculoscapular canal (AOS) carries pores B (paired), C, D (unpaired) and F and H (only left). The right canal section between porus F and H is absent with no neuromasts. The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right (one specimen) or no neuromasts at all (one specimen). The preopercular canal (POC) is either developed on both sides with pores M and N respectively (one specimen) or reduced to a furrow (in one specimen) and replaced by superficial neuromasts: four neuromasts only right (one specimen).
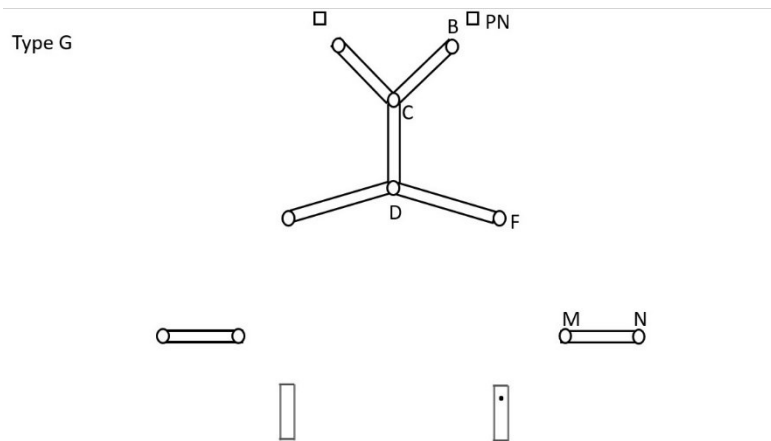
Figure 39: Head canal type G found in the Jadro river population (PN: posterior nasal pore; B-N: pores).

Type G: Type G was found in seven specimens (fig. 39). The anterior oculoscapular canal (AOS) carries pores B (paired), C, D (unpaired) and F (paired). Pores H (paired) are absent (in all specimens). The anterior canal section between pores F and H of the AOS is reduced to a furrow in one specimen. The posterior oculoscapular canal (POS) is absent, just neuromast are present: one neuromast right and left (one specimen), one neuromast right and two neuromasts left (one specimen), one neuromast only right (one specimen), five neuromasts only left (one specimen) or no neuromasts at all (three specimens). The preopercular canal (POC) is developed on both sides with pores M and N.
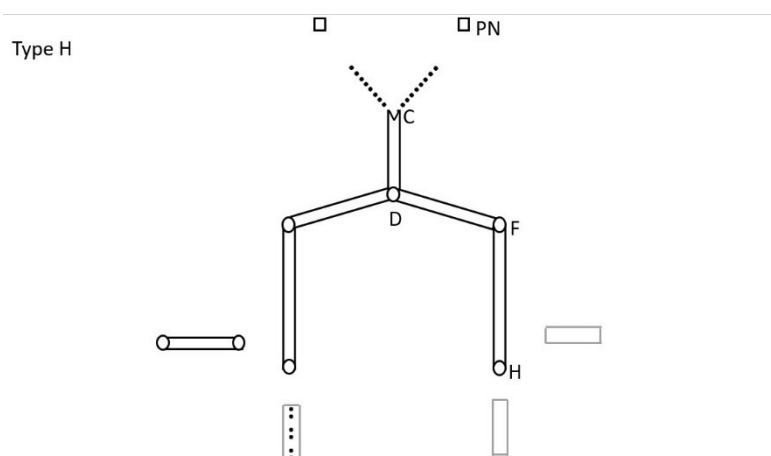

Figure 40: Head canal type H found in the Jadro river population (PN: posterior nasal pore; C-H: pores).

Type H: Type H was found in two specimens (fig. 40). AOS carries pores C, D, F (right and left) and H (right and left). The anterior part of the AOS (canal from porus B to porus C) is reduced to neuromasts. The POS is reduced to furrows on both sides with neuromasts on one side. The POC is either complete on both sides. carrying pores M (right and left) and N (right and left) or reduced to a furrow on one side (fig.27).
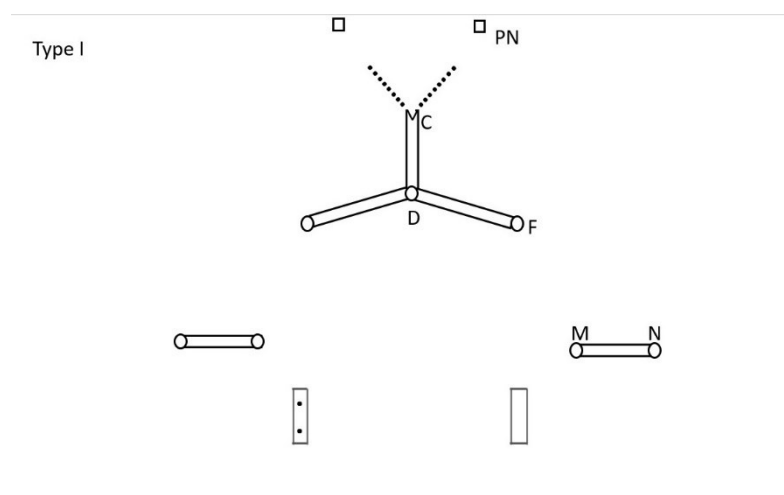

Figure 41: Head canal type I found in the Jadro river population (PN: posterior nasal pore; C-N: pores).

Type I: Type I was found in two specimens (fig. 41). The anterior oculoscapular canal (AOS) carries pores C, D (unpaired) and F and H (both paired). Pores B (paired) and porus C (in one specimen) are absent. The anterior canal section between pores B and C of the AOS is absent, only neuromasts are present: either three neuromast right and left (one specimen) or six neuromasts right and left (one specimen). The posterior oculoscapular canal (POS) is absent, just neuromast are present: four neuromasts right and six neuromasts left (one specimen) or two neuromasts only right (one specimen). The preopercular canal (POC) is either developed on both sides with pores M and N respectively (one specimen) or developed on one the right side only (in one specimen) with four superficial neuromasts.

Squamation Types

We found six different types of squamation in the natural riverine population (table 7, fig. 42). The most frequent type was type C, which 69.44 % of all specimens from river Jadro had. The results of the squamation types in the riverine population are quite homogenous, as only a few (either one or 4 specimens) were assorted to a different squamation type than type C.

Table 7: Number (n) and percentage (%) of squamation types found in the population of river Jadro. Type A: most complete type. Type F: most reduced type (n= 36).

Squamation types	number (n)	percentage (%)
A	2	5.56
B	4	11.11
C	25	69.44
D	3	8.33
E	1	2.78
F	1	2.78
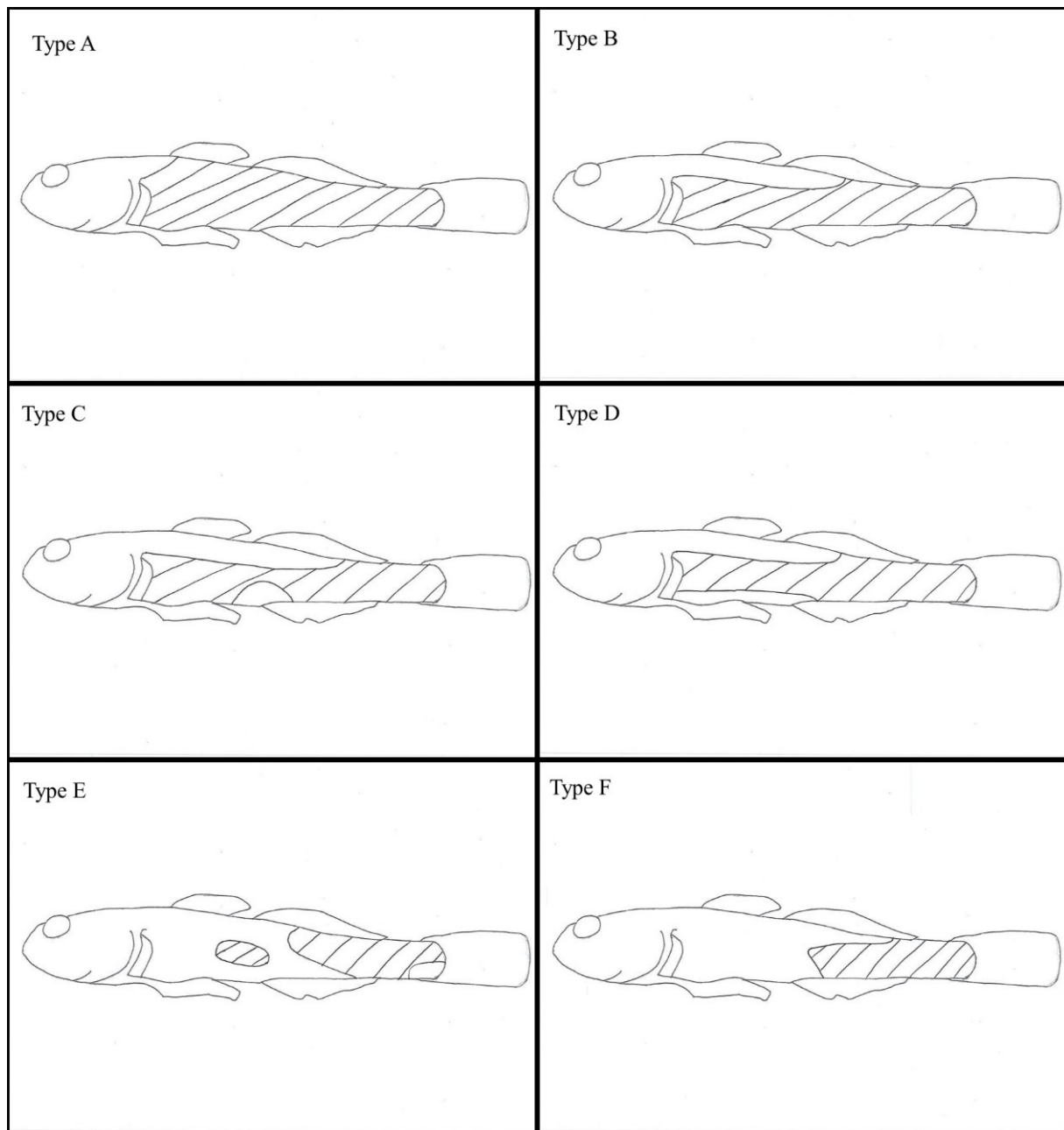
Figure 42: All patterns of squamation found in river Jadro. Type A: most complete, type F: most reduced type.

Type A

Figure 43: Squamation type A found in the population of river Jadro.

Type A: Two specimens were assorted to squamation type A. The squamation is continuous, reaching from the origin of the pectoral fin to the origin of the caudal fin (fig. 43). Scales reach the dorsal midline at the beginning of dorsal fin 1 (D1) and the ventral midline underneath the pectoral fin.

Type B

Figure 44: Squamation type B found in the population of river Jadro.

Type B: Four specimens were assorted to squamation type B. The squamation is continuous, reaching from the origin of the pectoral fin to the origin of the caudal fin (fig. 44). The upper dorsal part of the body before the end of D1 is scale-free. Scales reach the dorsal midline either at the beginning or in the middle of dorsal fin 1 (D1) and the ventral midline underneath the pectoral fin.
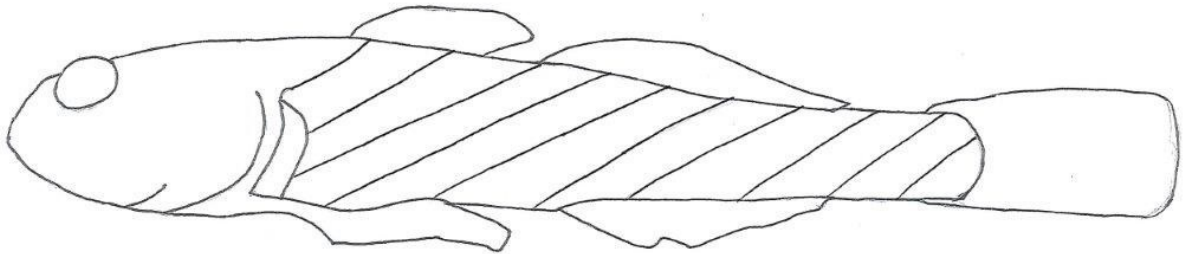
Type C

Figure 45: Squamation type C found in the population of river Jadro.

Type C: 25 specimens were assorted to squamation type C. The squamation is continuous, reaching from the origin of the pectoral fin to the origin of the caudal fin. The upper dorsal part of the body is scale-free. Size and length of the dorsal scale-free area can vary, as the region, where scales reach the dorsal midline varies (fig. 45). Scales reach the dorsal midline either between dorsal fin 1 (D1) and dorsal fin 2 (D2), at the origin of D2, before the middle of D2, at the middle of D2 or before the end of D2. The ventral midline shows one scale-free area around the origin of the anal fin. In a few specimens, the ventral scale free area can extend to the origin of the pectoral fin. Scales reach the ventral midline either before the origin of the anal fin, at the origin of the anal fin, before the middle of the anal fin or at the middle the anal fin.
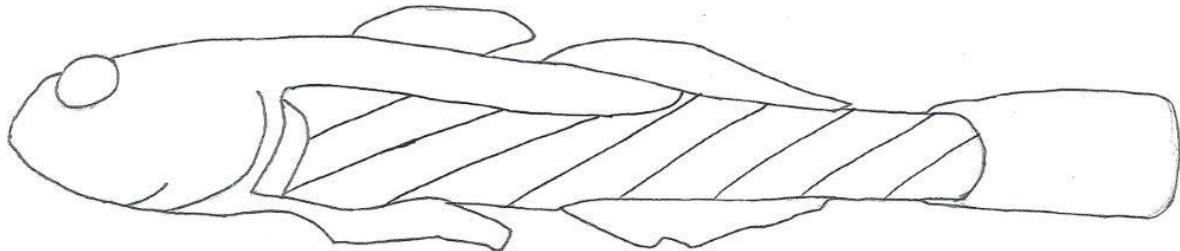
Type D

Figure 46: Squamation type D found in the population of river Jadro.

Type D: Three specimens were assorted to squamation type D. The squamation is continuous, reaching from the origin of the pectoral fin to the origin of the caudal fin. The upper dorsal part of the body is scale-free (fig. 46). Scales reach the dorsal midline either at the middle of D2 or before the end of D2. The lower ventral part shows a scale-free area until scales reach the ventral midline before the middle of the anal fin.

Type E
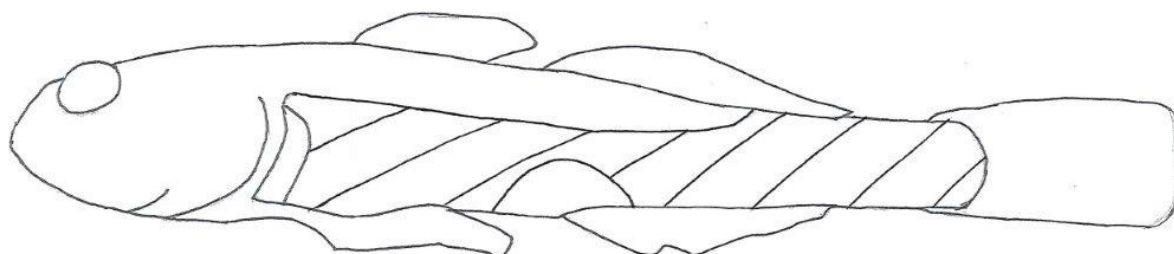

Figure 47: Squamation type E found in the population of river Jadro.

Type E: One specimen was assorted to squamation type E. The squamation is patchy and divided into two patches: one patch is located in the middle of the body and the other patch is located at the posterior part of the body, reaching from the posterior third of the body to the origin of the caudal fin (fig. 47). Scales reach the dorsal midline either before the middle of dorsal fin 2 (D2) and the ventral midline before the end of the anal fin.

Type F
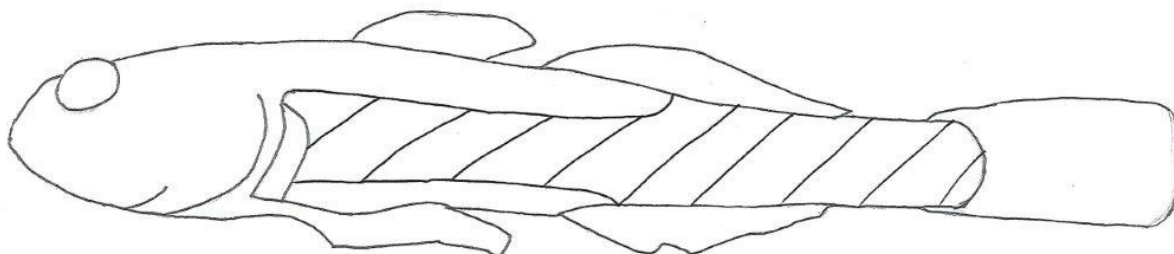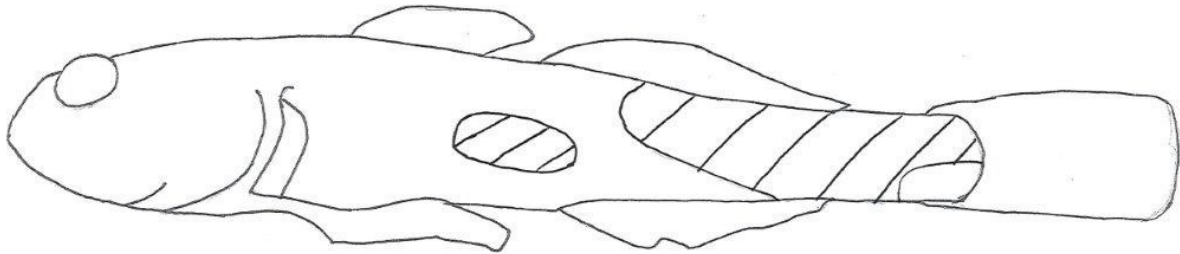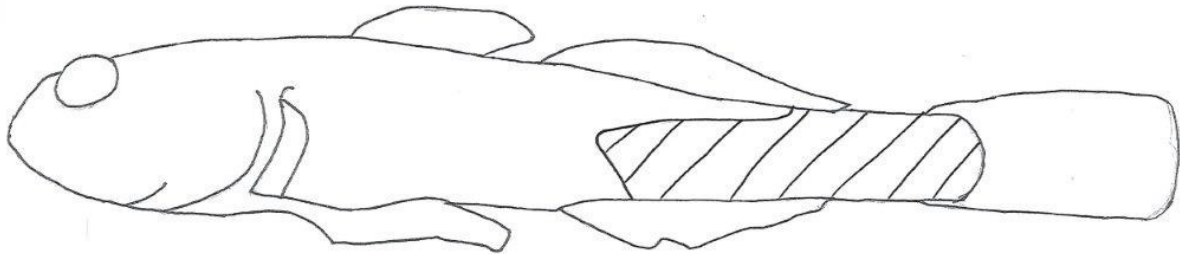

Figure 48: Squamation type F found in the population of river Jadro.

Type F: One specimen was assorted to squamation type F. The squamation is patchy consisting of one patch only, which is located at the posterior part of the body (fig. 48). Scales reach the dorsal midline before the end of dorsal fin 2 (D2) and reach the ventral midline before the middle of the anal fin.

Comparison of both populations

Size

Altogether 188 specimens from lake Trasimeno and river Jadro were examined. In six specimens the sex determination was not possible. In both populations males are bigger than females. In general, specimens from lake Trasimeno are smaller than specimens from river Jadro (table 8). Females from lake Trasimeno are 29.44% smaller than females from river Jadro. Males from lake Trasimeno are 32.38% smaller than males from river Jadro. The range of standard length is about the same in both sexes in both populations.

Table 8: Comparison of standard length and number of both populations.

	Lake Trasimeno		River Jadro	
sex	females	males	females	males
Total number (n)	46	94	8	34
Standard length (range)	26.80 mm	28.47 mm	37.98 mm	42.10 mm
Mean Standard length	22.64 mm- 38.28 mm	22.60 mm- 37.11 mm	45.28 mm- 32.09 mm	51.70 mm- 30.16 mm
Standard Deviation	3.06	3.11	5.14	5.55

Head Canal Types

In the riverine, natural population from Jadro River, nine different types of head canals were found (types A-I). Type A with a complete head canal system and type I showing the most reductions. The introduced population from Lake Trasimeno showed 12 different types of head canals (types A-J).

Comparing the head canal patterns of both populations, altogether 20 types of head canals were found. There is only one type which was found in both populations (table 9). Type A of the lake population is equal to type C of the riverine population. All the other types can either be found within the riverine population or within the lake population.

Both populations seem to differ a distinctly concerning this morphological trait. While the lake population shows a high variability and more than half of the specimens show reductions, especially in the anterior part of the anterior oculoscapular canal (AOS), fishes from the natural, riverine population tend to have less reductions and do not vary so much. The head canal types of lake Trasimeno specimens showed more neuromasts than those of the river Jadro (fig. 49 and fig. 50).

Nevertheless, more specimens (n= 144) from the lake population than from the riverine population (n= 44) have been examined.

Table 9: Number (n = 168) and percentage (%) of head canal types found in the population of lake Trasimeno and in the population from river Jadro. Type A: most complete type. Type T: most reduced type.

Head Canal Types	n (Trasimeno Lake)	n (Jadro River)	Total	Percentage (%)
A		1	1	0.54
B		2	2	1.08
C	5	24	29	15.59
D		5	5	2.69
E		3	3	1.61
F	2		2	1.08
G	2		2	1.08
H	1		1	0.54
I		2	2	1.08
J	1		1	0.54
K		7	7	3.76
L		2	2	1.08
M	26		26	13.98
N	21		21	11.29
O	40		40	21.51
P	8		8	4.30
Q	11		11	5.91
R		2	2	1.08
S	17		17	9.14
T	4		4	2.15
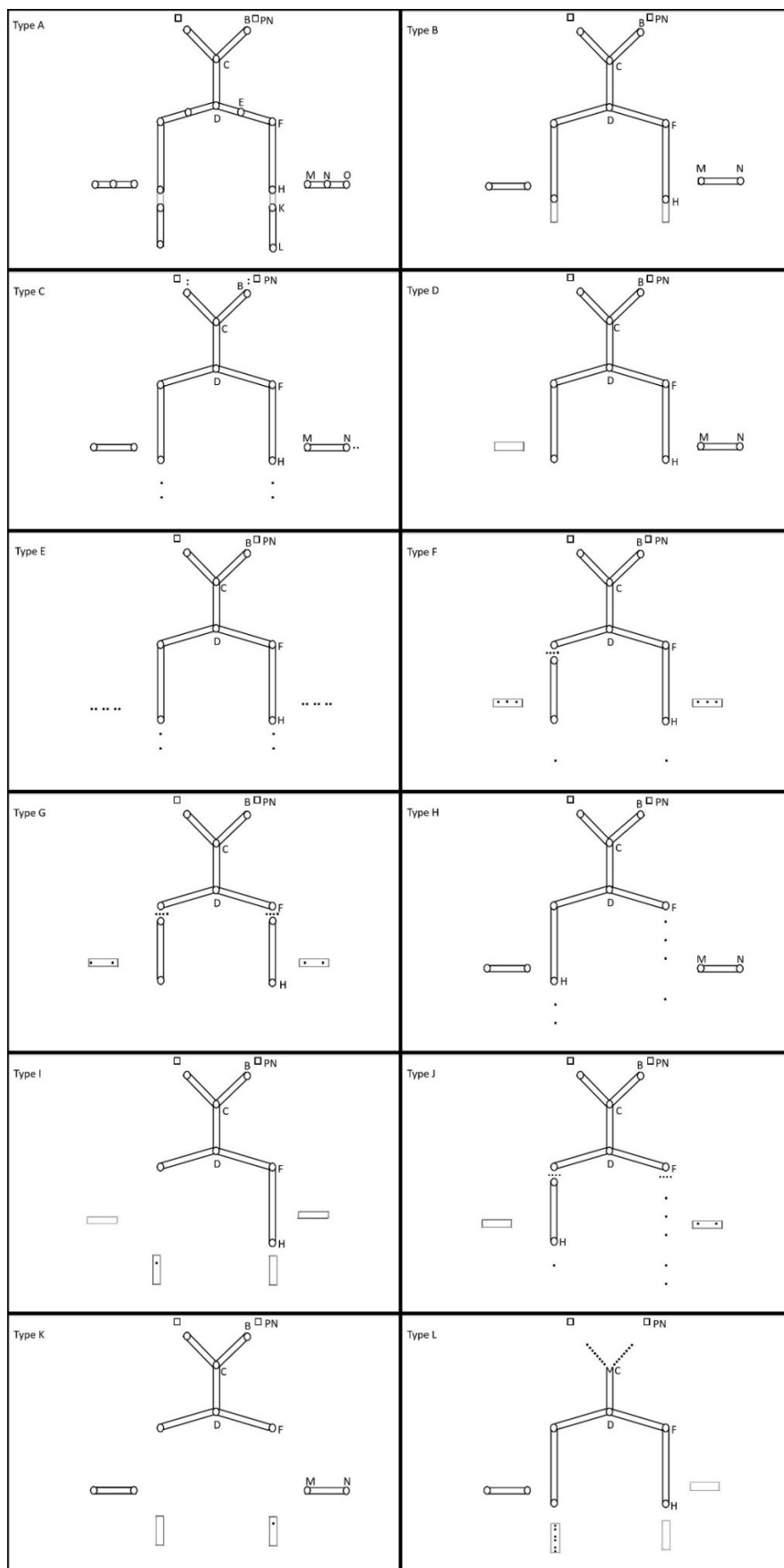
Fig. 49: Comparison of ally head canal types (types A- L). A: most complete type, type T: most reduced type found in river Jadro and Lake Trasimeno.
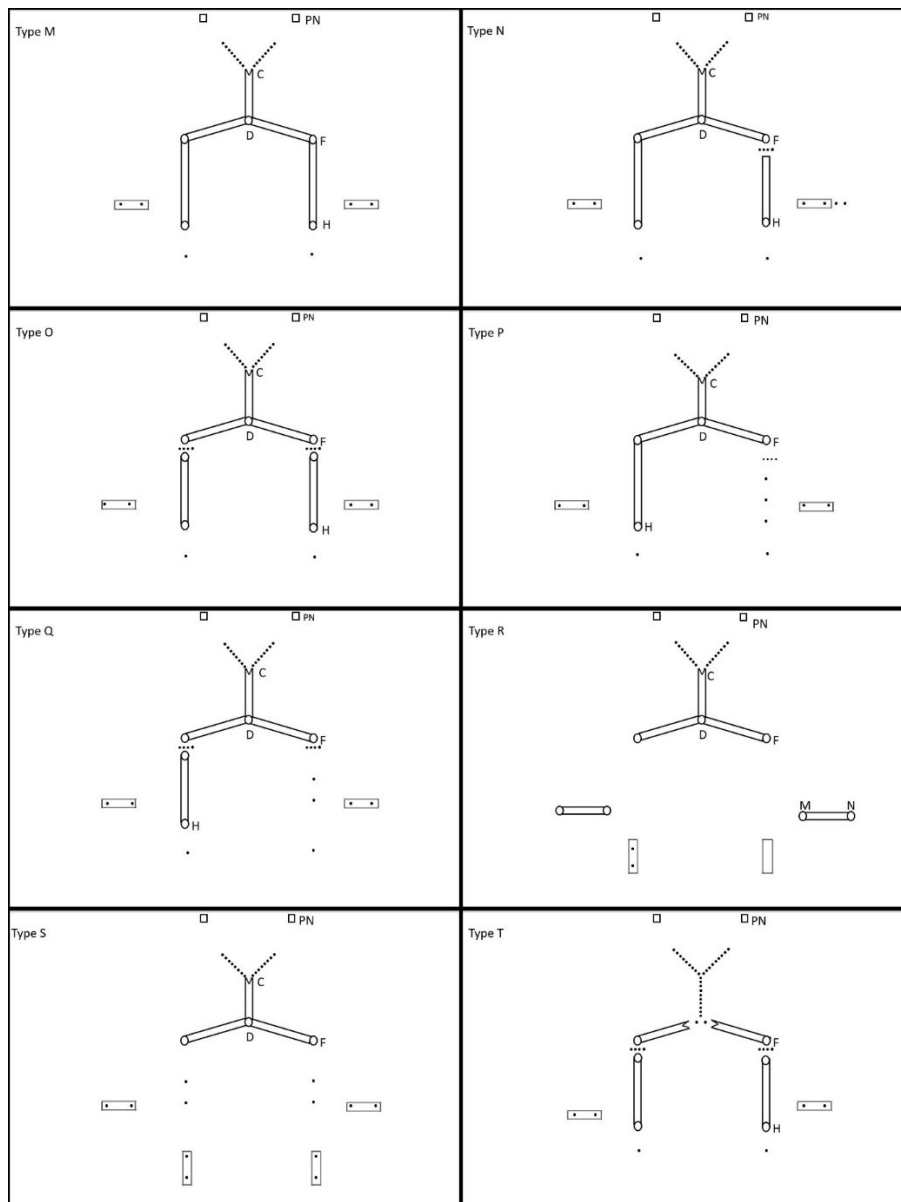
Fig. 50: Comparison of all head canal types (types M-T). A: most complete type, type T: most reduced type found in river Jadro and Lake Trasimeno.

Squamation types

In the riverine, natural population from river Jadro, as well as in the lake population six different types of squamation were found. Comparing these results with the results of the head canal types, the squamation seems to be more homogenous in both populations. In both populations more than the half of all squamation types found have been assorted to either one or two types (table 9).

Comparing the squamation patterns of both populations, altogether 11 squamation types were found. There is only one type which occurred in both populations (table 10). Type B of the lake

population is equal to type C of the riverine population. All the other types can either be found within the riverine population or within the lake population.

Comparing the both populations, the squamation of the lake population seems to be more reduced than the natural population. Only two specimens of the riverine population showed a patchy squamation. In the majority of specimens, the squamation was continuous from the base of the pectoral fin to the origin of the caudal fin. In the lake population the majority had a patchy squamation (fig. 51).

Nevertheless, more specimens (n= 141) from the lake population than from the riverine population (n= 36) have been examined. Thus, the percentage of the 11 different squamation type results can have a bias.

Table 10: Number (n) and percentage (%) of squamation types found in the population of lake Trasimeno and in the population from river Jadro. Type A: most complete type. Type K: most reduced type (n= 177).

| Squamation types	n (Lake Trasimeno)	n (Jadro)	Total	percentage (%)
A		2	2	1.13
B	1		1	0.56
C		4	4	2.26
D	53	25	78	44.07
E		3	3	1.69
F	82		82	46.33
G	1		1	0.56
H	3		3	1.69
I		1	1	0.56
J		1	1	0.56
K	1		1	0.56

Fig. 51: Comparison of all squamation types (types A- K). A: most complete type; type L: most reduced type found in river Jadro and Lake Trasimeno.
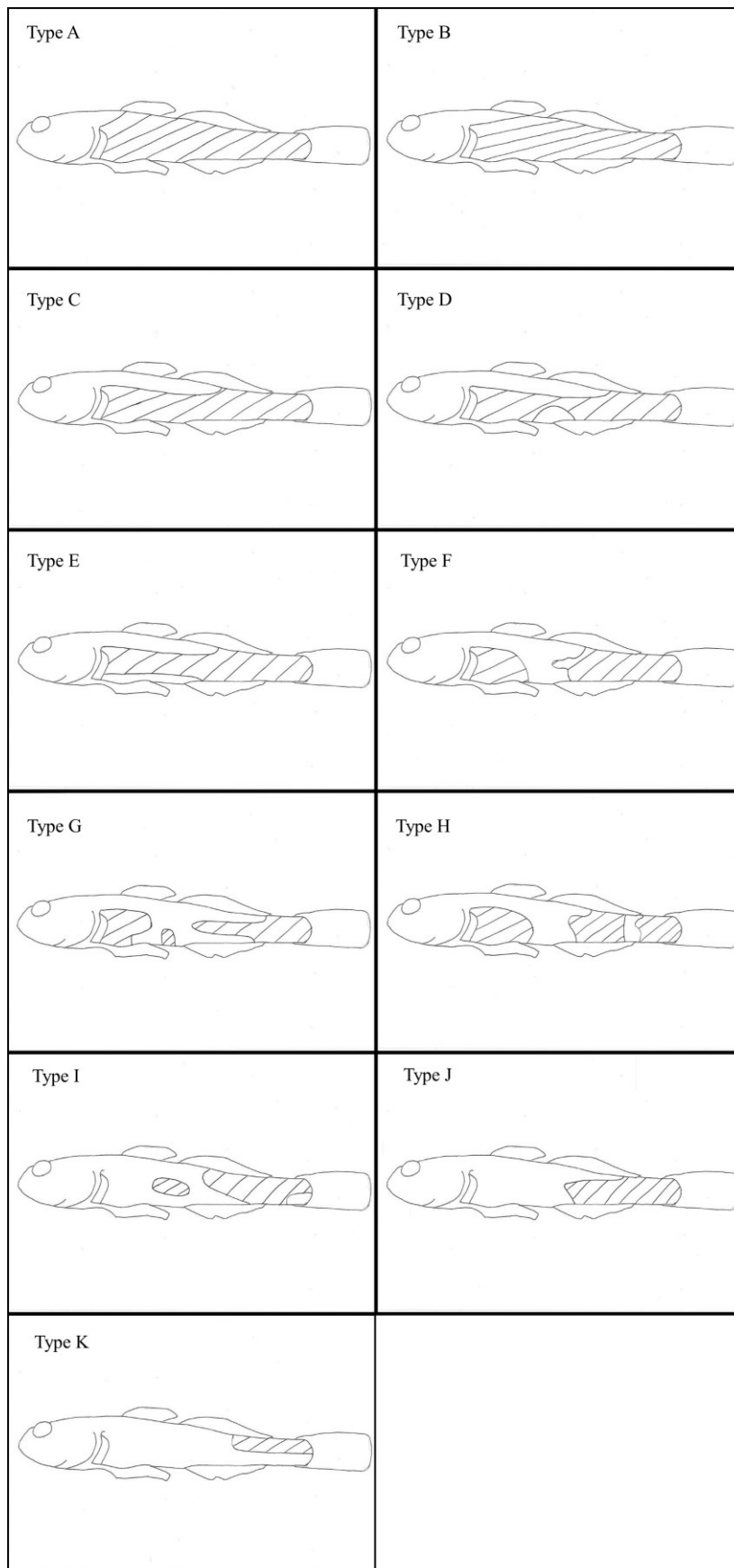
DISCUSSION

An introduced population of *Ninnigobius canestrinii* from Lake Trasimeno (Italy) was compared to a natural population of River Jadro concerning size, the pattern of the head lateral line canals and the pattern of the squamation. The two populations differ in all these three characters with the lake population showing distinct reductions compared to the river population.

The introduction of Canestrini's goby into a new and for this riverine species (Miller 2004) not natural environment, stagnant water, offered the opportunity to assess (1) the phenotypic diversity between natural and introduced populations in a lake and (2) how the squamation and the head lateral line canal system of this benthic species responds to a shift along a hydrodynamic gradient. Because both traits are key taxonomic tools for gobiid fishes (Miller 2004) the introduction into a new environment offered also the opportunity to assess (3) the strength of these two morphological traits as taxonomic tools.

Specimens from the introduced population of Lake Trasimeno showed a smaller body size, a higher variability and more reductions in head canal and squamation types than the natural population of river Jadro. Generally, the introduced population showed more reductions in all three examined morphological traits. Females from lake Trasimeno were 29.44% smaller than females from river Jadro and males were 32.38% smaller than males from river Jadro. While in the riverine population the majority of individuals had a complete head canal system excluding the preopercular canal section, this was the case only in a minority of specimens in the lake population. The same goes for the pattern of squamation: it seemed to be more homogenous, since in both populations the same number of squamation types (six) was found. Still, these types varied enormously between the two populations: while in the riverine population the majority had a continuous squamation, in the lake this is the case for a minority.

Sand gobies sharing the same environmental conditions such as a similar habitat and life history combined with a low dispersal rate are already known to exhibit the same morphological features as found in this study: a reduction of head canals, squamation and body size (Ahnelt 2011). These paedomorphic features have inter alia already been found in various gobiid fishes: *Knipowitschia byblisia*, *Knipowitschia caunosi* and *Knipowitschia caucasica* from the Aegean region, *Knipowitschia ephesi*, *Knipowitschia mermere* from Anatolia or even *Eucyclogobius newberryi*, which inhabits lagoons and estuaries in California (Ahnelt 1995, 2011, Ahnelt et al. 1995, 2004). These species all inhabit a similar habitat: either a shallow freshwater lake or estuaries and lagoons (Ahnelt 1995, 2011, Ahnelt et al. 1995).

The same goes for the fishes which have been examined in this study. Comparing both sampling sites, the lake is a standing water characterized by no natural outflows and the river is a running water characterized by brackish conditions and different salinity gradients resulting in higher salinity on the ground. Both habitats are more or less isolated habitats: the freshwater lake has no natural outflows and the riverine fishes are more or less isolated within the estuary of the river due to different salinity gradients (Ljubenkov 2015, Frondini et al. 2019).

Geographic isolation subsequently leads to reproductive isolation and speciation (Rocha and Bowen 2008, Matute 2013). By colonizing new habitats, such as the lake, species are subject to new environmental conditions and consequently to new selective pressures which lead to adaptions to the new environment. The loss of genetic variation caused by reproductive isolation is known as the founder effect. A small body size in the founding population can be a sign of suffering from the effects of inbreeding (Matute 2013). Gobiid fishes adapting to habitats characterized by stagnant or very slow flowing waterbodies are generally characterized by reduction in size, reduction or loss of squamation and a reduced head lateral line canal system (Ahnelt 1995, 2011, Ahnelt et al. 1995, 2004, Kovačić 2005, Stelbrink and Freyhof, 2006).

Body size

Fishes from river Jadro were larger than fishes from Lake Trasimeno. Females from lake Trasimeno were 29.44% smaller than females from river Jadro and males from lake Trasimeno were 32.38% smaller than males from river Jadro.

Ninnigobius canestrinii is a fish species known to prefer salinities of 5-20‰, typically inhabiting estuaries or lagoons. Although it is known to occur also in freshwaters, longevity is reduced in such habitats. Lake Trasimeno is a freshwater lake although with a low salinity of 1‰ and fishes got introduced into this habitat. The brackish water of river Jadro in which the gobies occur naturally therefore suits the preferences of this species better (Miller 2004, Ljubenkov 2015, Frondini et al. 2019).

There are various factors known to influence body size in fishes: predatory pressure, fishing, warming temperatures and the adaption to a small habitat (Ahnelt et al. 1995, 2011, Baudron et al. 2014, Forestier et al. 2020). A small body size can also be a sign of suffering from the effects of inbreeding (Matute 2013). Fishing and predatory pressure can influence body size in that way that fishes select for earlier maturation at a smaller body size (Forestier et al. 2020). Lake Trasimeno is not only known to harbour piscivorous fishes like the pike (*Esox lucius* Linnaeus, 1758) or the southern pike (*Esox cisalpinus* Bianco and Delmastro, 2011), but is also known to

be an important site for commercial fishing in Italy (Lorenzoni et al. 2002, 2010, Ahnelt et al. 2018). Since *Ninnigobius canestrinii* is an epibenthic, small fish, it is of no commercial interest, but due to its size and if it occurs in large numbers, it possibly plays an important role in ecological food webs as has been shown for other sand goby species (Miller 2004, Pockberger et al. 2014).

Warming temperatures, caused by climate change, make respiring underwater more challenging for fishes. Since the anabolic oxygen demand is increasing, the oxygen solubility is at the same time decreasing. A smaller body size improves the ability to balance the demand for oxygen and the uptake of oxygen as the surface area to volume ratio is increased (Baudron et al. 2014). The riverine gobies were caught downstream at the rivermouth of river Jadro where the population of this benthic species is under a constant marine influence. Since the Mediterranean Sea is usually characterized by relatively small tides, estuaries of rivers which debouch into the Adriatic Sea, like river Jadro, have a stratified character: a thin layer of freshwater lies above a bigger and denser layer of salt water (Ljubenkov 2015). Nevertheless, with each tide well oxygenated marine water floods the estuary and the lower reaches of the river Jadro. Additionally, the mean annual water temperature of this river in its lower course does not exceed 22° C. Due to the dynamics of estuaries, temperature fluctuates. During increasing sea water influence (high tide), the temperature of the river rises in the estuary (Divić et al. 2020).

Contrary, Lake Trasimeno is with an average depth of 4.7m relatively shallow (Frondini et al. 2019). Due to this shallow depth, thermal stratification is usually absent and therefore water temperature is about the same as air temperature (Ahnelt et al. 2018) and can reach 30° C during the summer months. From 1988 until 2006 the average water temperate increased by 1.3°C during spring and about 1°C in summer (Ludovisi et al. 2010).

Additionally, a smaller body size, as found in the lake population, gives not only the opportunity to subdivide in the environment, it also enables the survival in habitats where resources are limited and is often linked with a short generation time which however favours speciation and diversity (Ahnelt et al. 1995, Ahnelt 2011).

Head canals and squamation
Specimens from the introduced population of Lake Trasimeno showed a higher variability and more reductions in head canal types, squamation types and body size than the natural population of river Jadro. Generally, the introduced population showed more reductions in all three examined morphological traits. The pattern of squamation seemed to be more homogenous,

since in both populations the same number of squamation types (six) was found. Still, these types vary enormously between the two populations: while in the riverine population the majority (94%) had a continuous squamation, in the lake the minority (38%) had a continuous squamation. The same goes for the head canal types: while in the riverine population 80% (individuals from type A-E) had a complete head canal system excluding the preopercular canal section, in the lake population only 3% (only individuals from type A) had a complete head canal system excluding the preopercular system.

More reductions and therefore a higher variability of head canals in the lake population can be led back to different hydrodynamic conditions in both habitats: While Lake Trasimeno is a freshwater lake, the population of river Jadro inhabits an area of the river influenced by marine conditions. Therefore, latter population faces more denser salt water and is exposed to, although generally low, currents (Ljubenkov 2015, Ahnelt et al. 2018, Frondini et al. 2019).

Intraspecific phenotypic variation in the structure of the mechanosensory lateral line system is related to environmental conditions and more specifically to habitat: fishes living in habitats with high turbulences like a coastal river tend to have a higher number of canal neuromasts and generally a more well-developed lateral line canal system than fishes living in still, slow moving water like a lake (Vanderpham et al. 2013, Ahnelt et al. 2021). Since superficial neuromasts are sensitive to velocity and insensitive to oscillatory water motions, fishes living in standing waters tend to have numerous superficial neuromasts and fishes inhabiting turbulent waters tend to have only a low number of neuromasts (Engelmann et al. 2002, Kasumyan 2003). Consequently, by comparing the results of the lake population to the results of the riverine population, the lake population seems to face less turbulent waters than the riverine population which is clearly the case in the shallow lake.

Generally, the pattern of squamation of the lake population showed more reductions than the natural riverine population. The majority of fishes from Lake Trasimeno had a discontinuous squamation. The reduction of squamation might be a result of the adaption to new, small freshwater habitats. Gobies tend to reduce their squamation in order to adapt to a freshwater environment, where gobiid fishes face the same environmental conditions like a similar habitat and life history, combined with a low dispersal (Miller 2004). Furthermore, fishes from the lake also had a smaller body size than the riverine population. Since during the development of the squamation, scales are added successively in regular rows from posterior to anterior, the squamation of fishes from the lake population might not be developed completely (Sire et al. 2004).

Phylogeny

Recent phylogenetical studies on Adriatic sand gobies revealed cryptic diversity of *Ninnigobius canestrinii* and other sand gobies (Thacker et al. 2019, Tougard et al. 2021). The populations of *Ninnigobius canestrinii* are split into three different lineages belonging to three different geographic areas: Raša (Croatia), Baćinska lakes (Croatia) and Venice (Italy) (Tougard et al. 2021) but the populations of Lake Trasimeno and the Jadro river were not included in this study and especially latter remains undescribed yet (Ahnelt et al. 2021).

To identify the examined population correctly, morphological traits such as the degree of head canal reductions as well as the arrangements of superficial neuromasts can be used as diagnostic feature for species of Gobioidei (Sato 2022). Because both traits, the head canal system as well as the squamation pattern are key taxonomic tools for gobiid fishes (Miller 2004), the introduction into a new environment offered also the opportunity to assess the strength of these two morphological traits as taxonomic tools. According to the results of this study and comparing a natural population to an introduced population, the pattern of the squamation as well as the pattern of head canals showed a high degree of reductions in the introduced population. Both traits are very variable and rather linked to environmental conditions than to serve as a strong taxonomic tool (Ahnelt 1995, 2011, Ahnelt et al. 2004, Kovačić 2005).

All in all, the results also correspond to those of Kovačić (2005) and Stelbrink et al. (2006), who also found high morphological variability concerning the reduction of head canals and squamation in *Ninnigobius canestrinii* of the Adriatic region (Kovačić 2005, Stelbrink et al. 2006). Kovačić (2005) already demanded further studies on the phylogeny on the genus *Pomatoschistus*, a demand which regarding to the new phylogenetic studies, seems to be even more necessary to understand the phylogenetic relationships among Adriatic sand gobies better. The found morphological results within this study could perhaps be used as taxonomic tool in future studies for species identification within the Adriatic sand gobies.

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