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"Comunità microbiche e fungine associate a due pesci mesopelagici bioluminescenti (*Argyropelecus hemigymnus* e *Maurolicus muelleri)*"

"Microbial and mycobial assemblages associated with two bioluminescent mesopelagic fishes (*Argyropelecus hemigymnus* and *Maurolicus muelleri*)"

Tesi di Laurea Magistrale
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Riassunto

I microorganismi trovati in associazione a pesci di ambienti profondi, sono essenziali per fornire nutrimento, crescita, sviluppo e protezione all'ospite. La composizione del microbioma di questi pesci è probabilmente unica, date le sfide ambientali affrontate sia dall'ospite che dai microbi, in particolare in termini di temperatura, pressione e disponibilità di nutrienti. Inoltre, l'intestino dei pesci va a costituire una delle fonti di nutrienti più dense e abbondanti in ambiente profondo, rappresentato da comunità batteriche più concentrate rispetto all'ambiente circostante, vasto e altamente diluito. Diversi studi hanno indagato la composizione del microbioma intestinale nei pesci marini, ma questi si sono concentrati su pesci di interesse commerciale e a profondità relativamente basse. Infine in ambiente profondo (e non solo), molte associazioni simbiotiche svolgono determinate funzioni caratteristiche, come la bioluminescenza. Questo studio fornisce nuovi spunti alla conoscenza del microbioma e del mycobioma mondiale, indagando per la prima volta la biodiversità di batteri e funghi associati a due pesci mesopelagici bioluminescenti (*Maurolicus muelleri* e *Argyropelecus hemigymnus*), campionati nello Stretto di Messina. Inoltre, il progetto ha come scopo quello di valutare le potenziali funzioni dei microorganismi associati ed evidenziare possibili connessioni tra microbioma e mycobioma associati e la capacità bioluminescente delle due specie di pesci. L'integrazione dell'analisi colturale

con l'analisi molecolare è stata eseguita per ottenere un'ampia panoramica della diversità associata, in particolare di quelle specie batteriche potenzialmente bioluminescenti. Differenze e somiglianze dei microbiomi e mycobiomi sono state valutate dal livello interspecifico (tra le due diverse specie) al livello intraspecifico (tra i diversi individui della stessa specie) al livello intra-individuale (dato dal confronto dei microbiomi nelle tre parti del corpo di ogni esemplare). I risultati sulla diversità hanno rivelato che la composizione tassonomica dei microbiomi è simile a livello interspecifico, spiegato dalla presenza di taxa batterici condivisi. La difformità tra le due specie di pesci è principalmente guidata dalla presenza di alcuni generi batterici trovati in alcuni individui, indicando anche un'alta variabilità intraspecifica. A livello intra-individuale, i microbiomi non sono stati porzionati tra le diverse parti anatomiche del corpo: non è presente un microbioma specializzato che descriva ogni diversa parte del corpo. Le principali funzioni potenziali batteriche del microbioma erano simili nelle due specie di pesci, forse a causa dello stesso habitat specifico in cui vivono. L'indagine sulla biodiversità dei mycobiomi ha rivelato che la composizione tassonomica è dominata da funghi non identificati in entrambe le specie, indicando che i pesci potrebbero ospitare un'alta ricchezza di nuove linee genetiche fungine. Considerando i funghi identificati, sono state trovate differenze significative tra le due specie di pesci, con una maggiore diversità nel mycobioma di *A. hemigymnus* rispetto a *M. muelleri*. A livello intra-individuale, i mycobiomi non sono stati porzionati tra le diverse

parti anatomiche del corpo, non mostrando alcuna specializzazione, e le alte percentuali di funghi non identificati ci impediscono di approfondire la nostra comprensione. Infine, la diversità e le funzioni putative dei microbiomi non hanno rivelato una connessione con il fenomeno della bioluminescenza dei pesci, ma le analisi al SEM hanno sicuramente mostrato un'abbondanza di batteri specificamente localizzati all'interno del fotoforo di entrambe le specie bioluminescenti. Inoltre, le informazioni ancora limitate disponibili sui funghi bioluminescenti marini lasciano la questione aperta.

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1. INTRODUCTION

1.1 The important role of bacteria and fungi associated with marine organisms

Microbial communities are defined as multi-species assemblages, in which microorganisms interact with each other in a sharing environment (Konopka 2009). The term microbiome was first defined in 1988, by Whipps and colleagues, in a study carried out on the microorganisms of the rhizosphere (soil area surrounding plant roots). They described the 'microbiome' as a combination of the words *'micro'* and *'biome'*, defining it as a 'characteristic microbial community' in a 'reasonably well-defined habitat with specific chemical and physical properties' (Whipps et al. 1988). This definition represents a substantial advancement in the concept of microbial community, as it considers the also distinct properties and functions, that derived from the interactions with the environment, resulting in the formation of specific ecological niches. However, many other definitions of the microbiome have been published in recent decades. The currently most cited describes the microbiome in an ecological context, as a community of commensal, symbiotic and pathogenic microorganisms within a body space or different environment (Lederberg and Mccray, 2001). Other studies consider 'microbiome' the combination of set of genes and genomes (i.e., 'metagenome') with the environment; the analysis of microbiome is thus characterised by the use of

metagenomic, metabolomic, metatranscriptomic, metaproteomic approaches combined with environmental metadata (Marchesi and Ravel, 2015).

In the marine environment, every millilitre of the 1.3 billion km^3 of water contains millions of cells of microorganisms such as protists, bacteria, archaea, fungi and viruses (Eakins and Sharman, 2010). These are few micrometres or smaller in size, but collectively their roles in oxygen production, nutrient cycling and degradation of organic matter, provide critical functions for the oceans and Earth (Arrigo 2005; Falkowski et al. 2008). The microorganisms that reside on or within animals make up the animal's microbiome, and they may be acquired from the surrounding environment, horizontally transmitted; or they may have strict inheritance patterns, passed down through generations of the host, often transferred through the female germ line (Nussbaumer, et al. 2006; Sharp et al. 2007; Vijayan., et al. 2019). Host-microbiome dynamics are generally described as falling into two main categories: symbiosis, where the organisms are involved in normal metabolic and immune signalling interaction; and secondly dysbiosis, where the relationships or interactions are severely altered, possibly linked to a major stress or infection event (Apprill 2017). Symbiotic relationships are those that occur between two or more organisms that live in close physical association over time, as described by Anton de Bary, 1979 and reported in Brinkmann et al. 2017. These relationships are vast and diverse in the marine environment and can provide support and protection both to the symbiotic microbes and the host organism. In fact, symbiosis can also

contribute to host defence mechanisms (Lindquist et al. 2005; Paul et al. 2007; Thomas et al. 2010, Webster and Taylor 2012; Ansorge, et al. 2019; Hudspith, et al. 2021): symbiotic microorganisms can produce specific compounds to protect themselves and the host from pathogens and predators (Haygood et al. 1999; Lopanik 2014; Brinkmann, et al. 2017; Kusmita, et al. 2021). Although host-microbiome symbiosis and dysbiosis have been extensively studied and considered for human beings, many of the same concepts are applicable to organisms in the sea. The exact factors and mechanisms that may affect symbiosis are likely to depend on the complexity of host anatomy and physiology (e.g., simplistic sponges and corals versus more complex fish and sharks; Apprill 2017); in addition, the 'symbiotic state' is subject to a variety of environmental fluctuations, which are generally defined by habitat characteristics and could lead to altered host-microbiome relationships. For example, in Sullam et al. (2012), it is shown how the trophic level and phylogeny of the host, the habitat is a determining factor for the diversity and structure of gut microbiome in fish. Stress is another factor that more complex animals encounter on a daily basis (e.g., squid, crabs, fish); it could be related to social/territorial encounters, chasing or fleeing, and the short-term production of stress hormones such as cortisol can influence host-microbiome relationships (Moloney, et al. 2014). There are also events in animal life, such as the development, the ageing and the reproduction, that lead to drastic changes; Causing a state of 'altered symbiosis' (Bakke, et al. 2015; Egerton, et

al. 2018; Butt and Volkoff 2019). Fluctuations in the microbial community and associated genes, as well as normal animal life events, can be considered 'healthy' fluctuations (Nicholson et al. 2012). While symbiosis and altered symbiosis are considered normal host-microbiome variations over the course of an organism's life, dysbiosis is the breakdown of the relationship, usually linked to one or more major stressors, and can significantly alter host health and lead to a disease state (Holmes, et al. 2011). The stressor may come from an external source, such as a pollutant, an infectious agent, or a long-term natural environmental change - and there are probably countless other factors that could fall into this category (Apprill 2017). Symbiotic microbial communities of marine microorganisms can confer different metabolic traits to their hosts, increasing the attention of scientists towards the potential production of bioactive compounds of biotechnological importance (Brinkmann et al. 2017).

Currently, science is focused on identifying the core members of the microbiome (Shade and Handelsman 2012). After gaining an understanding of 'who's there', typically using diversity-based investigations targeting ribosomal RNA (rRNA) genes, these microbiomes are often examined as a whole or in smaller units to know cell functions, nature of the associations and ultimately find out the role of the microbiome in animal health, physiology, ecology, and behaviour (Ezenwa et al. 2012; Mcfall-Ngai et al. 2013). In addition, the oceans are changing at unprecedented rates due to climate change and human-induced

impacts, and the microbiome is being studied for its possible role as a sentinel of a changing host (Ainsworth and Gates 2016; Corinaldesi et al. 2022).

Among marine animals, fish, which comprise almost half of the total vertebrate diversity with an origin dating back 600 million years and about 34,600 species described to date (Nelson 2006; FishBase 2021), are associated with complex assemblages of microorganisms, which reside in almost all fish organs such as the skin, gills, digestive tract, internal organs such as the liver, kidneys, spleen and light-emitting organs (Austin 2002). In fish, the microbial assemblage is mainly studied at the gut level because of its significant contribution to important physiological functions of the host such as digestion, metabolism, reproduction, development and immune response (Butt & Volkoff 2019). Furthermore, the composition of the gut microbiome is influenced by a plethora of factors such as habitat salinity, temperature, trophic level, taxonomy, feeding habits and selective host pressures, leading to the development of a unique and diverse microbial community (Bevins and Salzman 2011; Sullam et al. 2012).

The fungal kingdom exhibits an immense diversity of life forms and, nutritional strategies, profoundly influencing and shaping ecosystems, by producing and channelling nutrients through different trophic levels and the entire food web (Peay, et al., 2016). Although fungal diversity on the planet is estimated at up to 3.8 million species, probably less than 10% of fungi have been identified (Hawksworth and Lücking, 2017). Until today, a relatively small proportion of the described species are associated with marine environments, with

around~1,100 exclusive marine species (Amend, et al., 2019). It's estimated that there are more than 10,000 species of marine fungi (Jones, 2011), with new and undescribed species richness at relatively high taxonomic levels (Picard, 2017). Several fungi found in the sea are also found in terrestrial environments, indicating remarkably effective adaptive capabilities within the fungal kingdom (Comeau, et al. 2016). Fungi have been found in almost all marine habitats examined from the deep sea to surface waters, including sediment (Orsi, et al. 2013), water column (Taylor and Cunliffe 2016), driftwood (Rämä, et al. 2014), sessile and mobile invertebrates (Yarden 2014), algae (Wainwright et al. 2017), and marine mammals (Pollock, et al. 2000). Marine fungi live associated with a wide range of organisms in the oceans, such as sponges, corals, invertebrates, macroalgae and seagrasses (Jones, 2011). DNA-based environmental surveys, and in particular the sequencing of genetic markers such as the internal transcribed spacer (ITS) region, small subunit (SSU) and large subunit (LSU) (Nilsson, et al. 2019), have made it possible to investigate marine fungal diversity in a wider range of habitats, indicating a broad and phylogenetically distinct mycobiomes, probably differentiated by geography, substrate and environmental conditions (Amend, et al., 2019). The investigation of the mycobiome, or fungal microbiota within a host, is an important but understudied component of the marine microbial ecosystem. Recent studies focused the attention on the fungal diversity of Antarctic marine environments, where at least one representative of all known fungal phyla has been reported

in different substrates or hosts (Ruisi et al., 2007, Bridge and Spooner 2012; Kochkina et al., 2012). Recently in Godinho et al. (2019), a special focus has been devoted to marine fungal diversity associated with marine animals especially sponges. The results indicate endemic, psychrophilic and cosmopolitan mesophilic fungi, which may present an interesting ecological symbiosis with their hosts (Godinho, et al. 2019). Sponges and particularly the fungi associated with them represent one of the most appropriate sources of defence molecules and could represent a promising biomass for the delivery of new antifouling compounds (Bovio, et al. 2019).

1.2 Bioluminescence in ocean

Bioluminescence refers to the phenomenon whereby certain living organisms emit light by converting the chemical energy of certain cells into light energy (Hastings 2003; Shimomura 2012; Carrasco-Lopez, et al. 2021). Eighty % of bioluminescent creatures live in the oceans and most of them are abyssal animals, living below 1000 metres; they hide in the depths during the day and come to the surface at night for feeding (Davis, et al. 2016; Shimomura 2016). Therefore, they spend most of their lives in the dark and bioluminescence is essential (Warrant and Locket 2004). The chemical principle behind bioluminescence involves molecules that are in an excited electronic state, emit energy in the form of photons, and then return to their fundamental state.

Bioluminescent reactions involve an oxygen environment and the interaction of an organic substrate that emits light (luciferin) and an enzyme that catalyses the reaction (luciferase). The terms luciferin and luciferase are used loosely, without specific chemical identity, because they change in each different light-emitting system (Shimomura 2012). The colour of bioluminescence is often determined by the structure of the luciferase involved in the process: small structural variations in these proteins can result in significant differences in emission wavelength (Nakatsu, et al. 2006). Sometimes luciferin, luciferase and oxygen are bound together to form a single element called a 'photoprotein', in which case light is produced when calcium ions join the system (Widder 2010). In general, luminescence in the marine environment occurs in three ways: extracellular secretion, intracellular processes and bacterial symbiosis. Extracellular secretion involves the release of luminescent material into the external environment; the secretion becomes luminous on contact with seawater. This type of bioluminescence is controlled by the concentration of the luminescent substrate (e.g., Osborn, et al. 2009). Intracellular bioluminescence is typical of those species that have luminous organs, glandular in nature, located on the whole surface of the body, made up of cells called photocytes that are responsible for the secretion of the chemiluminescent substrate. In this case, organs are called photophores (Mensinger and Case 1990; Herring 1978). In the bacteria luminescence, bacterial form symbiotic associations with animals such as teleosts and cephalopods by providing them

the light in exchange for a substrate to grow (Osman and Weinnig, 2021). The most studied case of bioluminescent marine symbiosis is that between the squid *E. scolopes* and the bioluminescent bacterium *Vibrio fischeri*, which inhabits a special light organ in the mantle of the squid (Tischler, et al. 2021). The bacteria are fed by the squid with a solution of sugars and amino acids and in return help to conceal its silhouette through the phenomenon of counter-illumination. In addition, the presence of the bacteria induces the morphological development of the squid light organ (McFall-Ngai and Ruby 1998; Visick, et al. 2021). The host squid also controls the luminescent performance of the symbiont and bacteria that fail to maintain adequate light production are expelled out of the luminous organ (Nyholm et al. 2004). The functions of bioluminescence for marine organisms are manifold. First, as intraspecific communication (a): the light signal is used to attract a mate by means of species-specific signals. For this reason, the structures associated with bioluminescence show a marked sexual dimorphism (Herring 2007). In fact, the light-emitting organs may be in different positions number or may have been lost in one of the sexes (usually in males). Second, as interspecific communication (b): the light can be used as a method of defence. In fact, many animals such as annelids, squid, crustaceans, jellyfish and fish release bioluminescent chemicals that serve to distract or blind the predator (Herring 2007).

Other animals mark the predator with a kind of bioluminescent slime, making them easy targets for secondary predators (Mesinger and Case 1992; Fleisher and Case 1995). Still others use it as a warning that their prey is unassailable, and thus with an aposemantic function (Herring and Widder 2004). Another important function is the ability to camouflage themselves through bioluminescence. In this process, called counter-illumination, many deep-sea species, particularly crustaceans, cephalopods and fish, have bioluminescent organs on their bellies that exactly match the colour and intensity of sunlight so that they disappear to predators watching them from below (Johnsen, et al. 2004; Jones and Nishiguchi 2004). Bioluminescence is not used only as defence; in fact, many animals use it during predation to localize or attract their prey (**Fig. 1**).

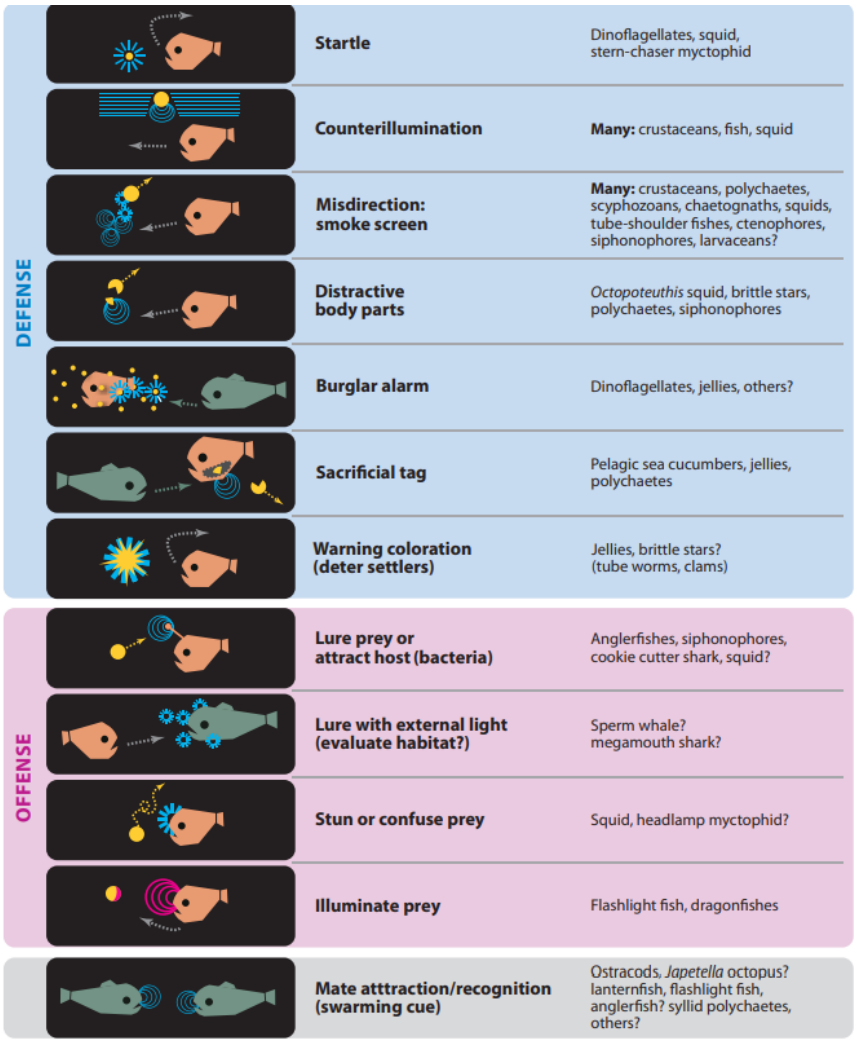


Fig. 1: Schematic diagram showing the functions of bioluminescence (Haddok et al. 2010)

Bacterial bioluminescence

Bacterial luminescence involves the oxidation of $FMNH_2$ together with a long-chain aldehyde and a two-subunit luciferase. The *lux* gene is responsible for the production of light (Meighen, 1991). Bioluminescent bacteria are common in the ocean, especially in temperate and warmer waters. Mutualistic associations

are known mainly in a variety of marine species of fish and squid (Haddock, et al. 2010). Bacteria are not luminous until they have reached high enough abundance to start the quorum sensing (Nealson and Hastings, 2006; Waters and Bassler, 2005), and once induced, they glow continuously in the presence of oxygen rather than producing discrete flashes. These properties are specific to bacteria, which makes them particularly suitable as photogenic symbionts and can lead to spectacular marine phenomena such as 'milky seas' (Miller et al., 2005). Among prokaryotes, light production is only known from so-called bacteria, gram-negative γ-proteobacteria, and not from Archaea (Haddock, et al. 2010). The most studied symbiotic bacteria are in the genus Vibrio, including the predominantly free-living species *V. harveyi*, although the genus *Shewanella* also includes a bioluminescent species (Makemson et al., 1997). Recently, it has been shown that many new strains of luminous bacteria are present in the deep sea, including some bacteria related to them, which have traditionally been affiliated to *Photobacterium phosphoreum* (Gentile et al. 2009). Although there are many exceptions, *Vibrio fischeri* (*Aliivibrio fischeri*) is considered part of the species typically involved in symbiosis with sepiolid and loliginid squid and monocentric fishes, while *Photobacterium leiognathi* and its relatives are mainly symbionts of Leiognathous, Apogonid and Morid fishes (Kaeding et al., 2007).

Bioluminescence in fish

Bioluminescence is found in at least 42 families in 11 orders of bony fishes, plus one family of sharks (Suntsov and Brodeur, 2008). In contrast to invertebrate taxa, many of these groups use bacterial symbionts for light production, as studied in Pietsch (2009) for Anglerfish, in Haygood & Distel (1993) for torch fishes such as *Photoblepharon spp*. and in Ikejima et al., (2008) for shallow pony fishes such as *Leiognathus spp*. Other luminous fishes have intrinsic luminescence using coelenterazine or some other uncharacterised light emitters (Mallefet and Shimomura 1995). Fish photophores are often highly modified and adapted to control not only the intensity of light but also its angular distribution, according to their specific function (Cavallaro et al. 2004). Myctophids, or lantern fish, are extremely abundant in midwater and migrate close to the surface at night. They have small photophores facing downwards and sideways, as well as large photophores on the tail, which can produce bright and fast flashes (Mensinger & Case 1990). The order Stomiiformes includes some of the most elaborate arrangements of photophores, including barbels, ventral arrays and red and blue suborbital photophores (Herring and Cope, 2005). Within the family Stomiidae, there are several new species, which are distinguished partly on the basis of their barbels and light organs (Kenaley, 2009, 2008). Their diversification may be related to feeding ecology, as Stomiid species have a fairly high degree of prey specificity, and the preferred

prey of various species includes copepods, euphausiids, decapod shrimps, fish and squid (Sutton 2005). In the order Chondrichthyes, the Squalidae is a family of luminous lantern sharks that use ventral countershading for both defensive and offensive purposes (Mizuno et al., 2021).

Argyropelecus hemigymnus and *Maurolicus muelleri* are two species of mesopelagic fishes, belonging to the family *Sternoptychidae*, order Stomiiformes, subclass Teleostei. The family *Sternoptychidae*, according to the phylogenetic analysis of Weitzman, 1974, comprehends 10 genera, including *Maurolicus* and *Argyropelecus*. Among the many synapomorphisms that these taxa share, the presence of Alpha-type photophores and their location in glandular groups are the most important ones.

Maurolicus muelleri (Gmelin, 1789; **Fig. 2**) is a small, short-lived, fast-growing fish. Its maximum size is about 7 cm, but fish longer than 5 cm are rare (Gjøsaeter, 1981). Among mesopelagic species, *Maurolicus* is one of the most abundant and potentially accessible to commercial fishing, as it often resides near the sea surface (Godø et al., 2009) The genus *Maurolicus* has a circum-global distribution, being found in all non-polar oceans, where they maintain enormous population sizes (Norris 2000). *Maurolicus* species are distributed throughout the open oceans, along continental shelves and escarpments, around isolated seamounts and in some cases in fjord systems and inland seas (Landaeta et al., 2015). This group has a complex taxonomic history, due in part to the lack of variation in photophores, used as dichotomous key in other

mesopelagic fishes (Rees et al., 2020). It migrates in the water column at depths of 150-250 m during the day and about 50 m at night (Okiyama 1971; Banon, et al. 2016). It feeds mainly on copepods and euphausiids (Weitzman 1974). They become sexually mature at the age of 1 year and spawn in March - September, producing 200-500 eggs that float to the surface in the Mediterranean where salinity is higher (Daan, et al. 1990).

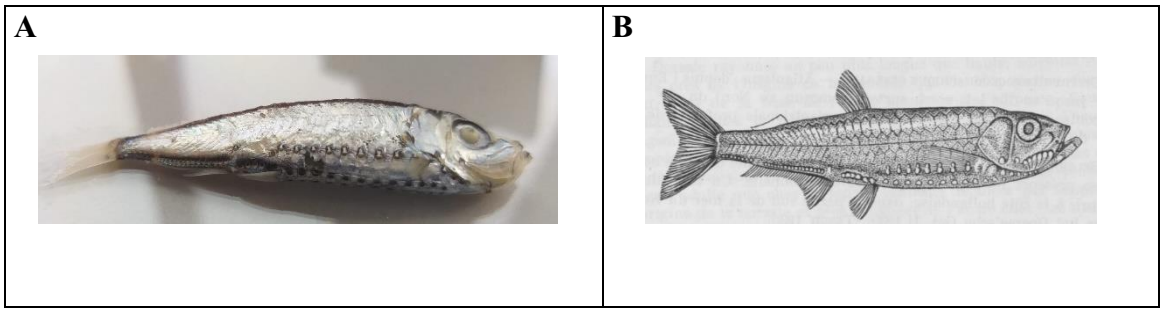


Fig. 2: a) Image of *Maurolicus muelleri*; b) Illustration of *Maurolicus muelleri* (Poll, 1947)

Argyropelecus hemigymnus (Cocco, 1929; **Fig. 3**) has an unmistakable morphology, typical of abyssal fish. It has a highly laterally compressed body, with upturned tubular eyes, measuring up to 6 cm. It is found between 200 and 700 m deep during the day, concentrated between 350 and 550 m, and between 100 and 650 m at night, preferring a depth between 150 and 380 m (Mytilineou, et al. 2005). Adults make marked vertical migrations, may be solitary or in small groups, and feed opportunistically at dusk on calanoid copepods or small fish (Miller, et al. 1986). In Eduardo et al., (2020) they investigated the

distribution and trophic ecology of the hatchet fish in the western tropical Atlantic, defining five functional groups with different food preferences, isotopic composition, and peak abundance, revealing a possible high distribution of resources. There is a slight sexual dimorphism in body size, with males being slightly smaller than females. Reproduction is oviparous, with planktonic eggs and larvae (Eduardo, et al. 2020).

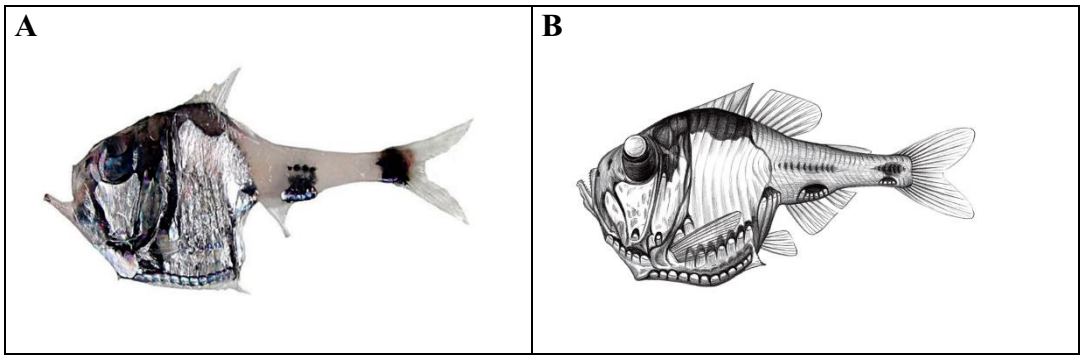


Fig. 3: a) Image of *Argyropelecus hemigymnus* (Luciano Gomes Fischer. fishbase.org); b) Illustration of a *Argyropelecus hemigymnus*.

As first described in Bassot, 1960 (a,b), there are three types of photophores: alpha, beta and gamma types. Both *A. hemigymnus* and *M. muelleri* possess alpha-type photophores. The structural and ultrastructural study of the photophores of *A. hemigymnus* and *M. muelleri*, in Cavallaro et al. 2004 allowed to understand the articulated organisation of these alpha-type organs, supporting the hypothesis that the emitted luminescence is intracellular and glandular type and is produced by the continuous and intense activity of photocytes. In *A. hemigymnus*, the photocytes are grouped inside a chamber,

known as the "photogenic chamber", at the mouth of which is a lens or "filter" (**Fig. 4**). The function of the lens is to concentrate the light emitted by the organ and to guide the light towards the mouth of the photophore (Denton et al., 1970). The entire photogenic chamber is surrounded by a layer of cells, rich in guanine crystals, immersed in an amorphous matrix (Denton et al. 1969), known as the "reflector". The function of the reflector is to reflect the light emitted and direct it towards the lens. The wall of the reflector is covered by a layer of iridocytes containing melanin granules known as the "pigmented layer" (Bassot, 1959). Ventrally, each photophore is bounded by a thick layer of cells, known as the "gelatinous layer", which has dioptric properties. In *M. muelleri* the photocytes in the single photophores are arranged in two well separated chambers called " tank" and "projector". The photophores are located in two depressions running longitudinally on the ventral surface of the fish. The part supplied by the dioptric structures runs towards the outer surface. Structural differences have also been identified in the lens-filter: in *M. muelleri* it consists of one or two cell layers, whereas in *A. hemigymnus* it is thicker (Denton et al, 1970; Denton & Herring, 1978). It should be noted that in *M. muelleri* the reflector is completely absent in the photophores of the preocular series in which the light cells are directly enclosed in the pigmented layer. This does not occur in Sternoptychidae to which *A. hemigymnus* belongs.

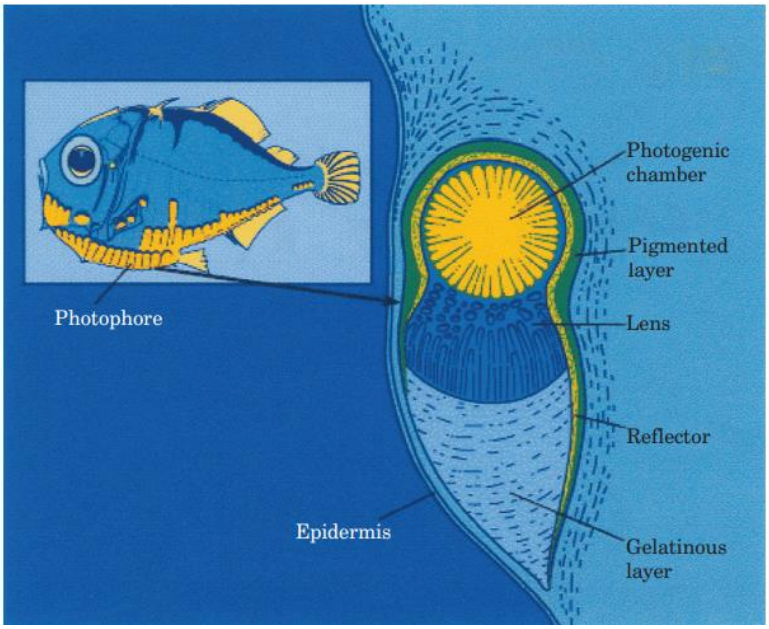


Fig. 4: General distribution and structure of photophores in fishes (Cavallaro, et al. 2004).

Furthermore, the bioluminescent substrate used by *A. hemigymnus* is known to be coelenterazine (Baguet & Marechal, 1974), while the mechanism in *M. muelleri* is unknown. Studies on the nervous control of photophores of deep-sea luminescent fish are limited to a pharmacological approach: light organs isolated from *A. hemygimnus* and *M. muelleri* show a much higher sensitivity to adrenaline than to noradrenaline (Baguet & Marechal, 1978; Baguet & Christophe, 1983). More recently in Krönström et al., 2005, immunohistochemical studies revealed the presence of neuromodulatory action of NO (Nitric Oxide) in *A. hemigymnus*, a mechanism that seems more common, since it has been detected in other luminal deep-sea fish (Krönström

and Mallefet, 2010). Furthermore, in Zaccone et al., (2011a, b) adrenaline was localised in the photophores of *A. hemigymnus*. Nevertheless, in a study carried out by Foran, (1991), on Myctophids and Stomiiphormes, it was shown that, probing for bacterial luciferase genes, a strong signal was obtained directly on specific regions of the fish light organs, while no signal was found on other internal or epidermal tissues of the fish. These data provide the first indication that bacterial luminescent symbionts exist in Myctophids and Stomiiformes and that they are responsible for luminescence. This observation is consistent with luminous bacteria acting as a light source in Myctophids and Stomata and argues that the genes required for luminescence do not reside in fish chromosomes (Foran, 1991). In contrast, in Haygood et al., (1994), the presence of bioluminescent symbionts was disproved through bacterial luciferase assays and *lux* DNA hybridisation of photophores. Photophore assays from six species of Myctophids and two species of Stomiiphormes showed that the highest levels detected of bacterial luciferase activity were 3-4 orders of magnitude lower than the activity of control luminal bacteria, and they were less than or equal to the activity of intestinal homogenates, which were up to 10 times higher than the highest photophore activities. In addition, hybridisation with *lux* probes did not detect *lux* sequences in the photophore DNA of species of myctophids and stomata (species not specified).

Further investigations have to be carried out to better comprehend the functions of bacterial symbionts associated with the abyssal fishes and their potential role in the light emission.

1.3 Upwelling in the Strait of Messina

The strong tidal currents in the Strait of Messina (**Fig. 5**) are well known since ancient times, as reported by the Homeric poem 'Odyssey' in Greek mythology, which represented the local currents as the monsters 'Scylla' and 'Charybdis'.

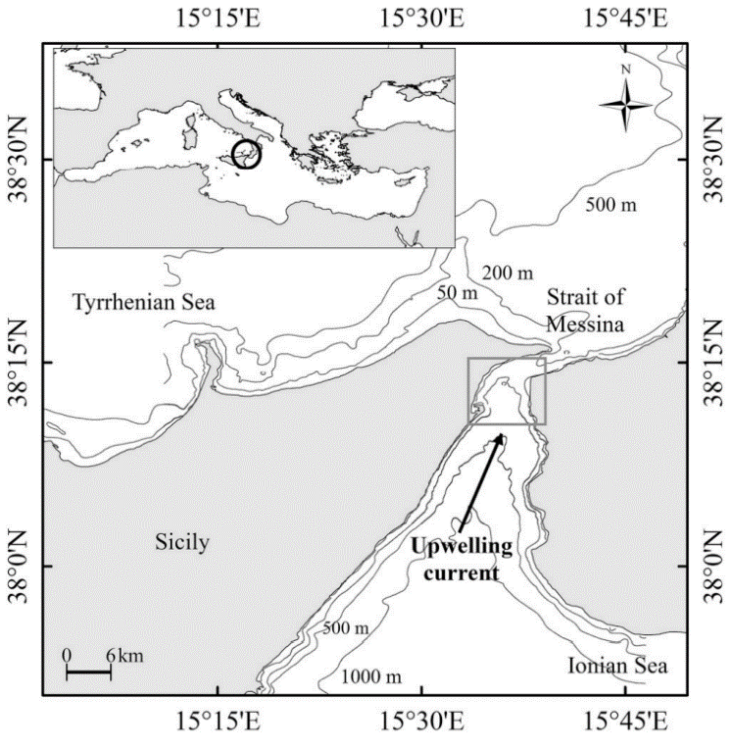


Fig. 5: Strait of Messina (black row: direction of upwelling current), (Battaglia et al. 2020).

The Strait of Messina morphologically has the shape of an inverted funnel, with a width of 3.3 km in the north (from the Sicilian coast, Capo Peloro, to the Calabrian coast, Punta Pezzo) and about 17 km in the south (from Capo Alì in Sicily to Capo Armi in Calabria). In addition, geographic conformation of the coast, the profile of the seabed (which is about 1500 m and rises very rapidly northward to 80 m), and the different density of water between adjacent seas play an essential role in the formation of unique hydrodynamic phenomena (Battaglia et al., 2020). One of the effects of these events is the upwelling of deep water from the Ionian basin to surface waters (i.e., upwelling is the rising of deeper, colder, salty and nutrient rich waters, coming from Levantine Intermediate Water, creating turbulent phenomena with horizontal and vertical movements of the water masses (Vercelli, 1925; Vercelli and Picotti, 1926). The primary cause of this phenomenon is the very intense hydrodynamic regime that develops in this area, determined by tidal currents, which about every six hours, causes the occurrence of a reversal of the current flow between the basins of the Ionian Sea and the Tyrrhenian Sea (Vercelli, 1925; Vercelli & Picotti, 1926). Indeed, the tidal cycles of the Tyrrhenian and Ionian seas have opposite phases, and currents flooding alternately through the sill reach velocities higher than 6 knots (=300 cm/s) (Cescon et al., 1997). There are several factors, in addition to the hydrodynamic regime and tidal currents, which can promote or influence the upwelling phenomenon: wind direction, lunar cycle, atmospheric pressure, seasonality, ecological and biological

parameters. In fact, the intensity of these dynamics depends on the phases of the moon which influence the intensity of the tidal currents. These, can reach speeds of 300 cm/s during new and full moons, also reach the enormous values of 500 cm/s in special cases, i.e., in combination with other factors such as wind drift, meteorological peaks, atmospheric pressure and turbulence (Vercelli 1925, Mosetti 1995).

One of the most interesting aspects related to hydrodynamic phenomena in the Strait of Messina is the stranding on the shoreline of numerous species of marine organisms, belonging to different animal and plant phyla (Battaglia et al., 2017). Since the 19th century, scientific interest in deep-sea fishes of the Mediterranean Sea has been stimulated by tales of fishermen and sailors describing monstrous and luminescent fishes, often referred to as "devil fish" in slang. Thanks to these strandings, Anastasio Cocco (1799-1854) published the first contribution to the knowledge and taxonomy of the Mediterranean deep-sea fauna (Ammendolia, Cavallaro, & Rao, 2014). In particular, with regard to mesopelagic organisms, the habit of performing nictimeric migrations can amplify the beaching phenomenon. In fact, the daily vertical migration of zooplankton in the night time hours towards the surface triggers the displacement of many mesopelagic species that for trophic reasons follow their prey in the surface water layers. The ascent to shallower bathymetric layers exposes these species (very often of small size, i.e., juveniles) to the risk of being intercepted by the upwelling current (Battaglia et al., 2017). When strong

upwelling currents occur, the rapid rising of deep water causes the malfunction of swim bladder of these fish, that thus are unable to cope with the sudden change in pressure. These individuals are then transported towards the shore, where they strand (Battaglia et al., 2010; Battaglia et al., 2015). Mesopelagic fishes are the most important fraction of the pelagic nekton in oceanic waters that, despite their small size, compose high biomasses and represent a significant source of energy for a large number of predators (Mann, 1984). In particular, they join the first levels of the marine food web to the upper levels and contribute significantly to the transfer of energy from epipelagic layers to deep waters, due to their behaviour of performing vertical diel migrations (Gjøsaeter and Kawaguchi, 1980). The concentration of mesopelagic fauna in the Strait of Messina due to updrafts has significant ecological implications in the trophic network as an important food source for several predators including the opportunists such as Atlantic bluefin tuna (Battaglia et al., 2013). In recent years, interest in Mediterranean mesopelagic fishes has been reawakened, thanks to the contribution of some authors who investigated their species composition (Olivar et al., 2012), genetic aspects (Bernal et al., 2014), luminescence (Cavallaro et al., 2015) and diet (Bernal et al., 2013; Battaglia et al., 2014, Battaglia et al., 2016)

2. **OBJECTIVES**

The main objectives of this project are:

1. Investigating the microbial and fungal assemblages associated with two bioluminescent mesopelagic fish species and in their different body parts (photophores, gut, epithelial tissue);

2. Evaluating the putative functions of microbiomes associated with two bioluminescent mesopelagic fish species and in their different body parts (photophores, gut, epithelial tissue);

3. Investigating the role of the microbiome and mycobiome in the bioluminescent of mesopelagic fish species.

3. MATERIALS AND METHODS

3.1 Study area and samples collection

Samples of *Argyropelecus hemigymnus* and *Maurolicus muelleri* were collected along the coasts of the Strait of Messina, during two different sampling campaigns: 1) in March 2017, on the Calabrian coast side, at Scilla (RC) and 2) in April 2021, on the Sicilian coast side, at Torre faro (ME; **Fig. 6**).

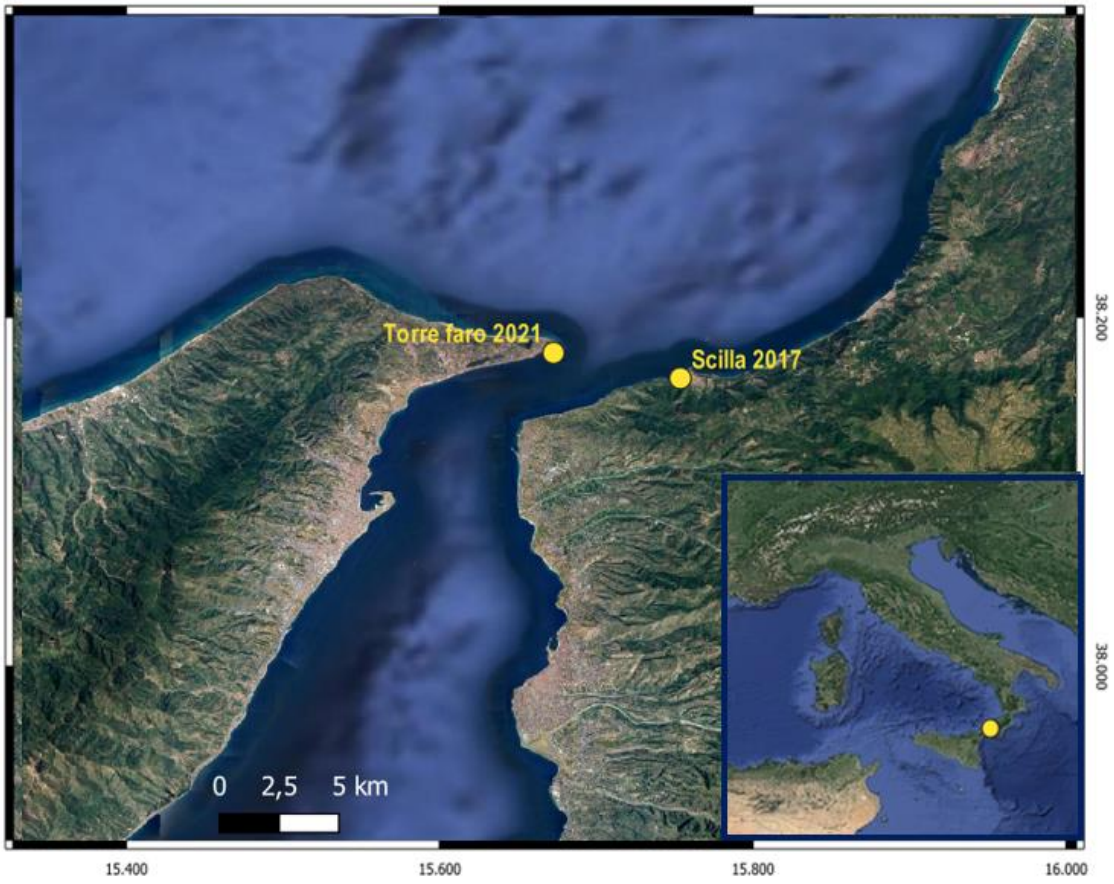


Fig. 6: Map showing the sampling areas where fishes were collected.

The specimens found during the two campaigns were used for different types of analyses, as shown in **Tab. 1**. Samples collected in March 2017 were immediately submerged in sterile falcon tubes with RNAlater for storage without jeopardizing the quality or quantity of RNA and DNA and were used for molecular analyses. RNAlater is an aqueous, non-toxic tissue storage reagent that rapidly permeates tissue to stabilize and protect cellular RNA in situ in unfrozen specimens (Larsen, et al. 2015; Jensen, et al. 2021). Samples collected in April 2021 were used fresh for microbiological analyses and then preserved in RNAlater. All samples were stored at -20°.

Table 1. Report on fish specimens collected during two sampling campaigns and the different types of analyses carried out.

Samples	**Data**	**Location**	**Longitude**	**Latitude**	**Growth Stage**	**Typology of analysis**
1 *Argyropelecus hemigymnus* (1AH)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
2 *Argyropelecus hemigymnus* (2AH)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
3 *Argyropelecus hemigymnus* (3AH)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis

4 *Argyropelecus hemigymnus* (4AH)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
5 *Argyropelecus hemigymnus* (5AH)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
6 *Argyropelecus hemigymnus* (6AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation
7 *Argyropelecus hemigymnus* (7AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation
8 *Argyropelecus hemigymnus* (8AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation
9 *Argyropelecus hemigymnus* (9AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation
10 *Argyropelecus hemigymnus* (10AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation, Scanning electron microscope
11 *Argyropelecus hemigymnus* (11AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation, Scanning electron microscope
12 *Argyropelecus hemigymnus* (12AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation, Scanning electron microscope

13 *Argyropelecus hemigymnus* (13AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Microbial and Mycobial assembly cultivation, Scanning electron microscope
14 *Argyropelecus hemigymnus* (14AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Adult	Microbial and Mycobial assembly cultivation, Scanning electron microscope
15 *Argyropelecus hemigymnus* (15AH)	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Adult	Microbial and Mycobial assembly cultivation
1 *Maurolicus muelleri* (1MM)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
2 *Maurolicus muelleri* (2MM)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
3 *Maurolicus muelleri* (3MM)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
4 *Maurolicus muelleri* (4MM)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
5 *Maurolicus muelleri* (5MM)	March 2017	Scilla (RC)	15°42'3.7993"	38°15'9.496"	Adult	Phylogenetic host analysis, Microbiome and Mycobiome analysis
6 *Maurolicus muelleri*	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Adult	Scanning electron microscope

7 *Maurolicus muelleri*	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Adult	Scanning electron microscope
8 *Maurolicus muelleri*	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Adult	Scanning electron microscope
9 *Maurolicus muelleri*	Aprile 2021	Torre Faro (ME)	15°39'0.527"	38°15'53.554"	Juvenile	Scanning electron microscope

*the abbreviations indicated in the brackets identify each specimen of fish and will be used it indicate samples in the graphs and results.

3.2 Molecular identification of fish species

Specimens were identified through the sequencing of Cytochrome Oxidase I (COI) and 12S mitochondrial genes.

Total genomic DNA was extracted from a small portion of body tissue (ca. 0,5 mg) from all fishes, using the Qiagen DNeasy Blood and Tissue Kit (Gallet, et al. 2019). Manufacturer's instructions were followed, extending the incubation time with proteinase K at 56°C overnight. Genomic DNA quantification and quality control were performed with a Thermo Fisher Scientific Nanodrop TM 1000 spectrophotometer by using 260/280 and 260/230 ratios.

The identification and the phylogenetic relationship of fish species was demonstrated with two different mitochondrial markers: the coding mitochondrial 12S rDNA genes (Dudu, et al. 2015), chosen for species discrimination, and the COI gene, chosen as it is a suitable universal barcoding gene, rapidly evolving, and able to detect an intraspecific variability. COI is

widely used by the Barcode of Life Data System (BOLD), an informatics programme aiding the acquisition, storage, analysis, and publication of DNA barcode records.

The amplification of the mtDNA COI fragments was performed using Fish F1 (5'TCAACCAACCACAAAGACATTGGCAC3') and Fish R1 (5'TAGACTTCTGGTGGCCAAAGAATCA3'), a primers combination described by Ward et al (2005). The Fish F1/R1 pair was selected because of producing the longest and clearest amplicons for most species (Pegg, et al. 2006). The amplification of mtDNA 12S was performed using the primer combination L1085/L1478 (5'TAAACCAGGATTAGATACCC3'; 5'GAGAGTGACGGGCGATGTGT3'), previously described by Bernal et al. (2014).

The reaction mixtures consisted of 5 µl of 5x My Taq Reaction Buffer (Meridian Bioscience), 0,4 µl of each primer (0,4 µM), 0,5 µl of My Taq DNA polymerases (5 U/µl concentration), 1µl of DNA template and a quantity of filtered and autoclaved Milli-Q water to reach a final volume of 25µl.

The thermal cycling profiles consisted in an initial denaturation of 5 min at 95°, followed by 30 cycles of 30 s at 95°, 45 s at 50° (annealing step, both for COI and 12S), 1 min at 72°, and a final extension of 10 min at 72°. Successful DNA amplification was verified by 1,5% agarose gel electrophoresis using 10.000x GelRed Nucleic Acid Stain (Biotium), 1,2 gr agarose, 80 ml of TE Buffer for

the gel preparation and 1 µl of 5x GelPilot DNA Loading Dye (Quiagen), 1,5 µl of Gene Ruler 100bp DNA Ladder (Bioline) for the electrophoresis.

Successful PCR products were purified using Qiagen PCR Purification Kit and sequenced using the Sanger method (Sanger et al. 1977) with the same primers as in the PCR protocols, through an Applied Biosystems 3730 DNA Analyzer 48 capillaries (Life Technologies) at Molecular Facility of Stazione Zoologica Anton Dohrn.

Sequences obtained were analyzed using the software Geneious 7.0.6 (Kearse et al. 2012). Before to proceed with the assembling of the two strands into consensus sequences, each sequence was cleaned, removing the terminal low quality parts and primers sections. Multiple alignments were performed using MUSCLE algorithm (Edgar, 2004). Searches were performed using BLAST, checking for significant similarity with other known sequences (https://www.ncbi.nlm.nih.gov/), and results with a low percentage coverage (<90%) and low percentage identity (<95%) were not considered. Homologous sequences were downloaded and added to the alignment to test the consistency of the groups obtained at species level. Evolutionary analyses of the COI haplotypes were performed using the neighbor-joining, procedure run on Geneious 7.0.6. Phylogenetic analyses were conducted in IQtree (Nguyen, et al., 2015), a fast and effective stochastic algorithm based on the Maximum Likelihood method, applying the best-fit nucleotide substitution model. The most appropriate model suggested for the COI dataset was the Hasegawa-

Kishino-Yano model (Hasegawa, et al. 1985), with equal substitution rates and equal base frequencies. Evaluation of the statistical confidence in the tree nodes was based on 1000 nonparametric bootstrap replicates. The species *Electrona risso* (Cocco, 1929) was chosen as the outgroup taxon of early teleost to root evolutionary tree.

Due to the low quality of the obtained 12S obtained and due to the absent of known 12S *A. hemigymnus* and *M.muelleri* sequences on the GeneBank, the 12S mithocondrial gene was excluded from the study.

3.3 Scanning electron microscopy (SEM) analyses

For SEM analyses specimens collected in April 2021 fixed in RNA later were used. The photophores were still brightening at the time of collection. Samples were prepared for the analysis with an initial immersion in a glutaraldehyde 2,5% solution for 48h. Then, they were subsequently dehydrated in ascending grades of ethanol (20% x 3h; 50% x 3h; 70% x 3h, 80% x 3h 90% x 3h; 95% x 3h; 99% over night) followed by critical point drying in carbon dioxide (Cavallaro, et al. 2019; Cavallaro, et al. 2021). Then, the samples were coated with gold and examined with Scanning Electron Microscope TESCAN and FESEM ZEISS SUPRA 40 (CISMIR- Centre for Nanostructure Microscopy Research and Service). To better investigate the internal structure of

photophores, specimens were gently cut in the surface with sterile scalpel in the area of photophores before the procedure.

3.4 Microbiomes and mycobiomes associated with mesopelagic fishes

The investigation of the microbial and fungal assemblages was carried out using two different approaches: molecular and microbiological analyses.

3.4.1 Molecular analysis

Specimens selected for this investigation were sampled in March 2017. Total genomic DNA was extracted from three different body sections in both species: Photophores (P), Gut (G), Tissue (T). The Qiagen DNeasy Blood and Tissue kit (Gallet et al. 2019) was used according to the manufacturer's instructions, extending the incubation time with proteinase K at 56°C overnight. Genomic DNA quantification and extraction quality control, using 260/280 and 260/230 ratios, were performed with a Thermo Fisher Scientific Nanodrop TM 1000 spectrophotometer.

Amplification was performed on an approximately 550bp fragment of the 16S rDNA genes, using the primer set Bakt_805R (5'-GACTACHVGGGTATCTAATCC-3') and Bakt_341F (5'-CCTACGGGNGGCWGCAG-3') specific for the bacteria (Herlemann et al. 2011). The reaction mixtures consisted of 5 µl of 5x My Taq Reaction Buffer (Meridian Bioscience), 0,125 µl of each primer (100 µM), 0,5 µl of My Taq

DNA polymerases (5 U/µl concentration), 1ul of DNA template and a quantity of filtered and autoclaved Milli-Q water to reach a final volume of 25µl. The thermal cycling consisted in 2 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 53°C, 45 s at 72°C, with a final extension of 5 min at 72°C. DNA amplification products were checked with 1,5% agarose gel electrophoresis using 10.000x GelRed Nucleic Acid Stain (Biotium), 1,2 gr agarose, 80 ml of TE Buffer for the gel preparation and 1 µl of 5x GelPilot DNA Loading Dye (Quiagen), 1,5 µl of Gene Ruler 100bp DNA Ladder (Bioline) for the electrophoresis.

Amplification was performed on the region 2 of internal transcribed spacer (ITS2) of ribosomal DNA. The Internal Transcribed Spacer region has become the most sequenced region used as DNA barcoding marker to identify fungal taxonomy at species level, and even within species (Blaalid, et al. 2013; Fajarningsih 2016). The primers used were ITS 3F (5'-GCATCGATGAAGAACGCAGC-3') and ITS 4R (5'-TCCTCCGCTTATTGATATGC-3') (Gao, et al., 2008). The reaction mixtures consisted of 5 µl of 5x My Taq Reaction Buffer (Meridian Bioscience), 0,4 µl of each primer (25 µM), 0,5 µl of My Taq DNA polymerases (5 U/µl concentration), 1µl of DNA template and a quantity of filtered and autoclaved Milli-Q water to reach a final volume of 25µl. The thermal cycling consisted in 5 min at 95°C, followed by 32 cycles of 40 s at 94°C, 40 s at 55°C, 90s at 72°C,

with a final extension of 7 min at 72°C. The DNA amplification products was checked in the same way described above.

Amplified microbial and fungal DNA was sequenced on an Illumina MiSeq sequencer using V3 technology (2 x 300 paired-end), with primers targeting the Bacterial V4 region and Fungal ITS2 region at Genomix4Life.

Raw sequences were analysed through the QIIME2 pipeline (version 2021.11; https://qiime2.org/). Forward and reverse sequence files were loaded, and paired-end sequences were formed by Casava 1.8 paired-end demultiplexed fastq. Sequences pairs were analysed by means of DADA2 plugin (Callahan et al. 2016), which infers community composition in each sample by partitioning sequences according to the respective error models, thus filtering for erroneous reads and chimeras and resolving minimal variation between prokaryotic and fungal taxa. Paired sequences were merged by the pipeline before producing an Amplicon Sequence Variant (ASV) table. From the resulting ASV table, each sample was subsampled to 22750 sequences for the bacterial dataset and to 220 sequences for the fungal dataset, resulting in a normalised ASV table. The depth of subsampling was chosen as a compromise between the highest number of sequences fully describing the biodiversity of the samples and the lowest loss of samples. No samples were discarded after normalisation for bacteria. 4MMP-1AHT-2AHG-4AHT samples for fungi were discarded because they had <220 sequences. The normalized ASV table was used for calculation of rarefaction curves and as input for the subsequent analyses, such as the

determination of alpha and beta diversity indices (Shannon and Evennes indices, Bray Curtis dissimilarity and Unweighted UniFrac distance). To infer the bacterial taxonomy affiliation of ASVs, a taxonomic classifier was first trained on the SSU bacterial region amplified by the primers on the Silva reference database 138 (Dueholm, et al. 2020); the classifier was then used on the ASVs identified. Amplicon Sequence Variants (ASVs) of fungal dataset were compared against the UNITE database (Version: 8.3; Last updated: 2020-12-11; Nilsson et al. 2018) for the taxonomic affiliation. Taxonomic affiliation was performed through the USEARCH SINTAX procedure (Edgar et al., 2018) using default parameters.

To further predict the relevant potential function of microbiomes a functional annotation was carried out on identified bacterial taxa using FAPROTAX dataset (Louca et al., 2016). Significant differences (p-value <0,05) in the richness and in the taxonomic composition of microbiomes and mycobiome were highlighted through a permutational analysis of variance (PERMANOVA) and Multi-Dimensional Scale (MDS) representations; similarities among the different group were evaluated by classification-clustering based on the Bray Curtis similarity of transformed quantity data with and identification of the main responsible taxa describing the differences was done with SIMPER analysis, both included in the PRIMER-E-6 software (Anderson et al., 2008).

3.4.2 Microbiological analysis in mesopelagic fish

Argyropelecus specimens sampled in April 2021 were used to investigate microbial and mycobial assemblages through the cultivation of microorganisms on culture media. The sampled specimens were transported as fresh to the laboratory in a sterile environment. Specimens were washed for 2 min in sterile seawater. Both tissue and photophores sections were plated on specific culture media to support the growth of bacteria and fungi associated with them. The culture media used were three, one for bioluminescent bacteria and two for generic marine bacteria and fungi. The specific medium for bioluminescent bacteria used is BOSS, consisting of 20 g NaCl; 2,5 g glycerol; 1,0 g peptone (Bacto-Peptone); 1 g beef extract; 15 g Bacto-Agar; MilliQ up to 1000 ml (Hassan, et al., 2010). This is the special microbiological medium used for the cultivation of bioluminescent bacteria and their growth rate on this medium is fast (Ansari 2012). The generic medium for bacteria used is Zobell Marine Agar 2216 (Cho, et a., 2004; Gentilhomme, 2020). The generic medium for fungi used is Marine Agar (MA, Difco) containing 10g peptone, 20g d-glucose, 20g yeast, 10g agar. The plates were incubated for 20 days at 15°C. The grown colonies were isolated in new Petri dishes with the same medium. The contaminated plates were re-isolated and incubated for further 20 days.

The genomic DNA of isolated colonies was extracted by the Qiagen DNeasy Blood and Tissue Kit (Gallet et al., 2019), following the manufacturer

instructions with an extended incubation with proteinase K at 56°C overnight. Genomic DNA quantification and quality control, using 260/280 and 260/230 ratios, were performed with a Nanodrop TM 1000 Spectrophotometer from Thermo Fisher Scientific.

The bacterial 16S rDNA gene and the fungal internal transcribed spacer region 2 (ITS2) were amplified, using the primers Bakt_805R/341F and ITS_4R/3F, respectively. PCR amplification protocols are the same described above (3.4.1 section).

DNA amplification products were checked with 1,5% agarose gel electrophoresis using 10.000x GelRed Nucleic Acid Stain (Biotium), 1,2 gr agarose, 80 ml of TE Buffer for the gel preparation and 1 µl of 5x GelPilot DNA Loading Dye (Quiagen), 1,5 µl of Gene Ruler 100bp DNA Ladder (Bioline) for the electrophoresis. Successful PCR products were purified using Qiagen PCR Purification Kit and sequenced using the Sanger method (Sanger et al. 1977) with the same primers as in the PCR protocols, through an Applied Biosystems 3730 DNA Analyzer 48 capillaries (Life Technologies) at Molecular Facility of Stazione Zoologica Anton Dohrn.

The resulting sequences were analysed using Geneious 7.0.6 software (Kearse et al. 2012) Before to proceed with the assembling of the two strands into consensus sequences, each sequence was cleaned, removing the terminal low quality parts and primers sections. Some sequences were discarded due to the low quality. To identify the isolated bacteria and fungi associated with the

mesopelagic fishes, the sequences were put on the BLAST platform, checking for significant similarity to known sequences deposited in GenBank (https://www.ncbi.nlm.nih.gov/) with a high percentage of coverage (>99%) and a hight percent identity (>98%).

4. RESULTS

4.1 Molecular identification of fish species

Molecular and phylogenetic analyses

The primer set used to amplify the mitochondrial gene 12S has generated low quality DNA sequences. In addition, the known 12S sequences of previous identified individuals as *A. hemigymnus* and *M. muelleri* were not present in the NCBI database, and no search could conduct good results. For these reasons, the 12S gene sequences have been excluded from the study.

The primer set used to amplify the fragment of mitochondrial COI gene has successfully generated good quality DNA sequences. Low quality was obtained only for the reverse of sample *A. hemigymnus 4*, therefore the consensus sequence was built on the high-quality forward sequence.

Sequences with percentage coverage <90% and percentage identity >95%, resulting from the searches on BLAST platform, and additional known sequences downloaded from GenBank and used for the construction of the phylogenetic tree are listed in **Tab. 1 SM (Supplementary material)** and **Tab. 2 SM.** The alignment of COI sequences and the subsequent phylogenetic tree revealed that all the 5 specimens of *Maurolicus muelleri* were identical to *Maurolicus muelleri_ Atlantic_Ocean* (from GenBank), confirming the identity to the samples (**Tab. 1 SM**). The 5 COI specimens of *A. hemigymnus* revealed among them from 1 to 6 nucleotide mutations, but all of them clustered

together with the sequence of *Argyropelecus_Atlantic_Ocean* from GenBank (**Tab. 1 SM**), revealing a possible intra-specific variability in the COI gene of this specie but confirming the identity. The addiction of known sequences of other species within the genera *Maurolicus* and *Argyropelecus* have helped to create a more complete phylogenetic tree with high percentages of bootstrap as support (**Fig. 7**: blue and yellow boxes). The two large groups represent a genus level division.

The minimum distance between the *M. muelleri* group and the *A. hemigymnus* group is 101 nucleotide mutations. The distance matrix used to build the phylogenetic tree is shown on **Tab. 3 SM**.

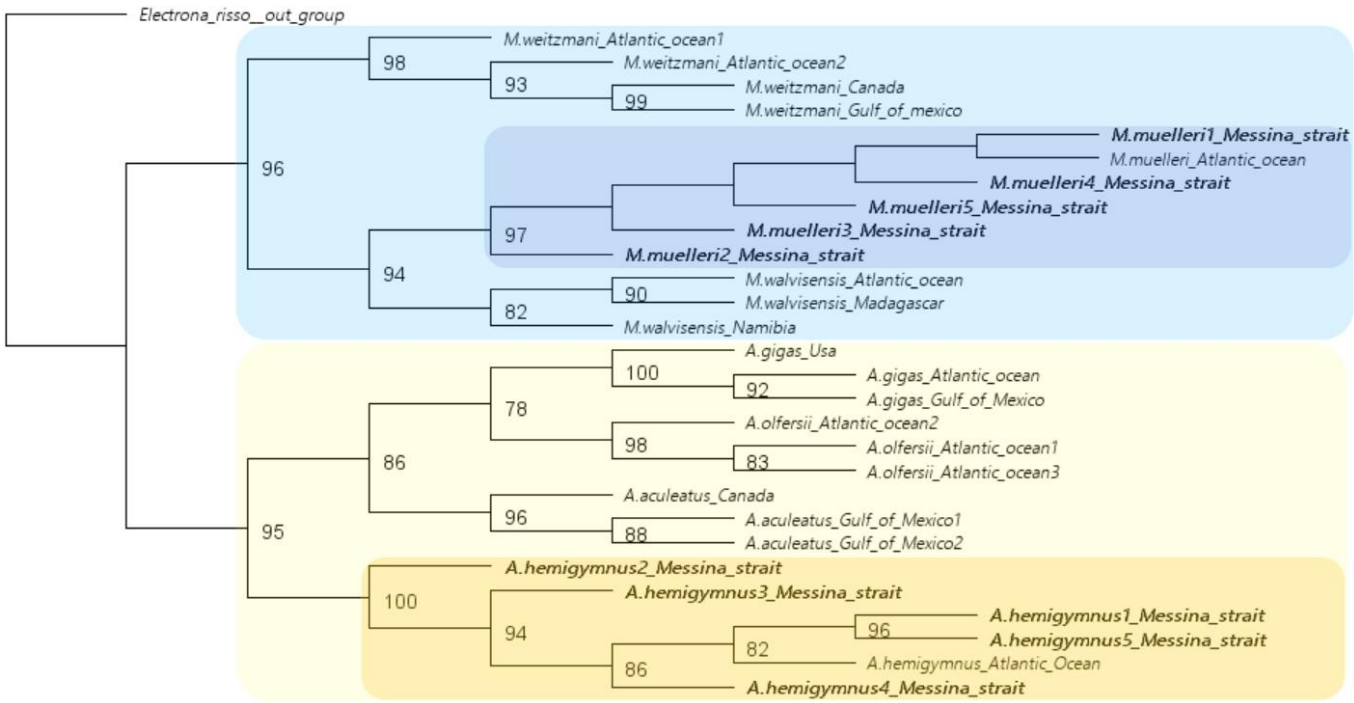
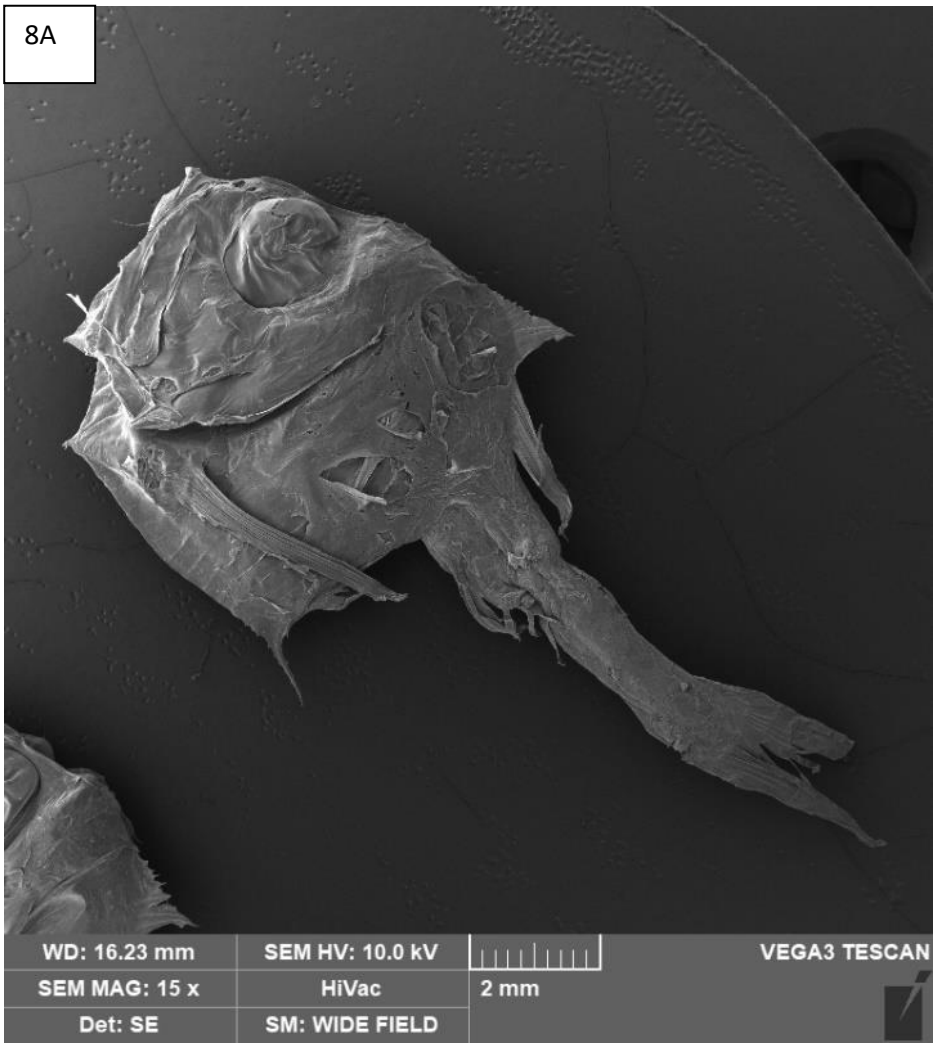


Fig. 7: Phylogenetic tree of COI mitochondrial gene carried out on sequences of *M.muelleri* and *A.hemigymnus* obtained in this study and downloaded from GenBank.

4.2 Scanning electron microscopy

Considering their large size (around 5 cm), the *Maurolicus muelleri* specimens were difficult to observe through the TESCAN VEGA3 scanning microscope. A general panoramic photo was obtained only for *Argyropelecus hemigymnus* (Fig. 8A). SEM observation of specimens revealed the presence of bacteria within the ventral photophores and on the surface skin, in both species of fishes. In particular, bacteria of various shapes and sizes were found on the external body surface of both species, sometimes covered by a biofilm matrix (**Fig.: 8A; 9A; 10A; 8M; 9M; 10M**). An assemblage of eukaryotes belonging to the fungal kingdom was found on a sample of *Argyropelecus hemigymnus* (**Fig. 11A**). In the skin surface of a sample of *Maurolicus muelleri* a group of 10 µm long cells was found, but with no clear identification (**Fig. 11M**). Conspicuous bacterial associations were found within the photophores of both species (**Fig.: 12A; 12M**). These are smaller in size than bacteria located at the skin surface. In fact, the size of the bacteria observed inside the luminal organ never exceeds 1µm in both species (**Fig: 13A; 14A; 15A; 16A; 13M; 14M; 15M; 16M**).



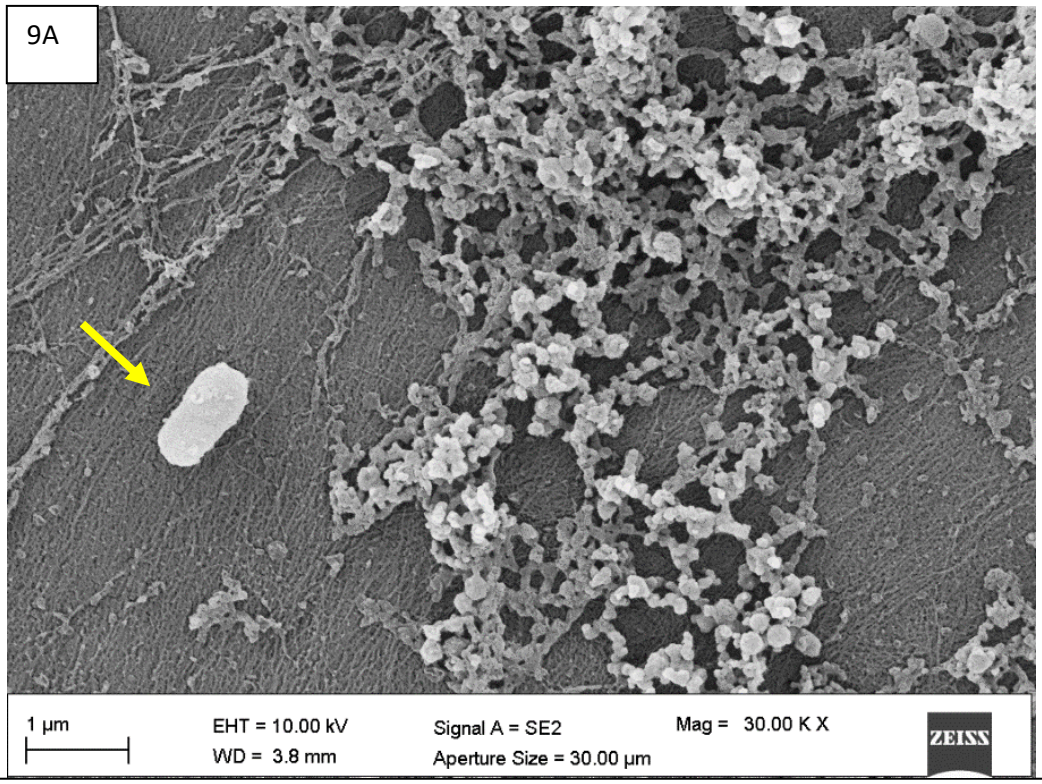
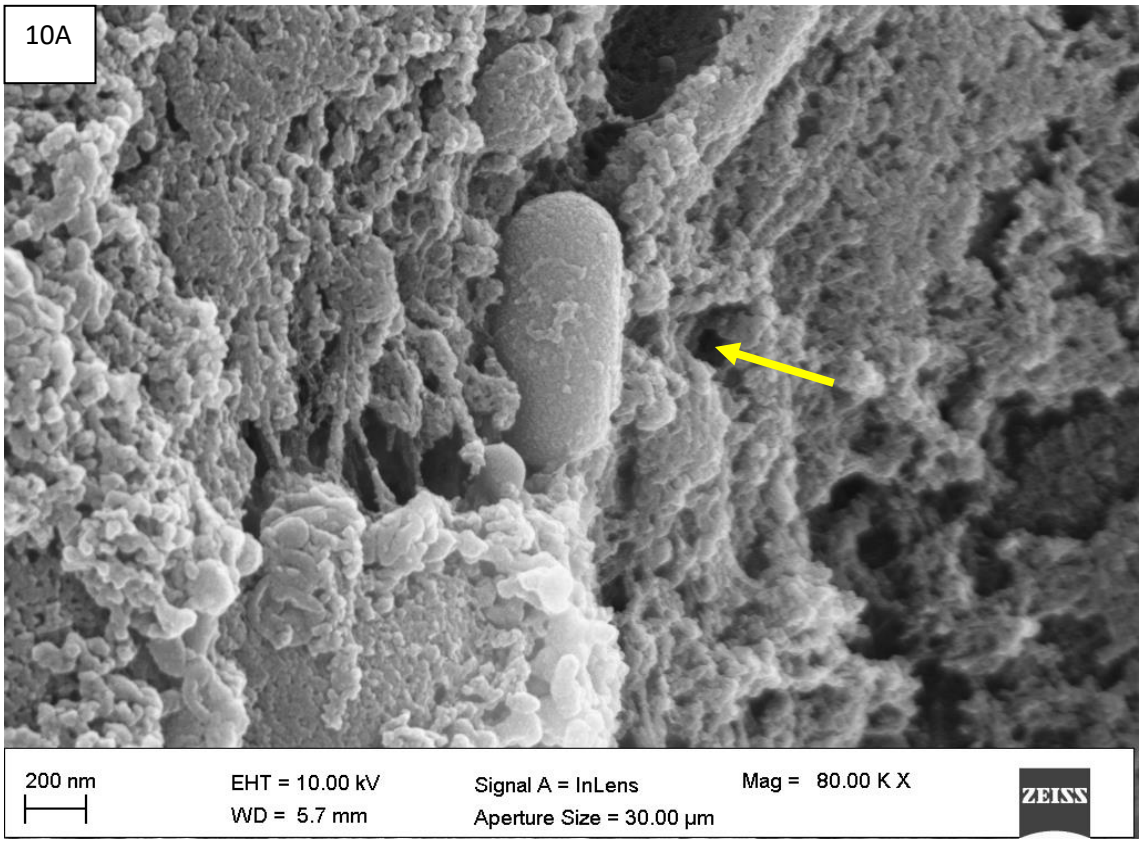


Fig. 8A: Entire view of *Argyropelecus hemigymnus* through TESCAN VEGA3.
Fig. 9A: Bacteria associated with the skin surface of *Argyropelecus hemigymnus*.



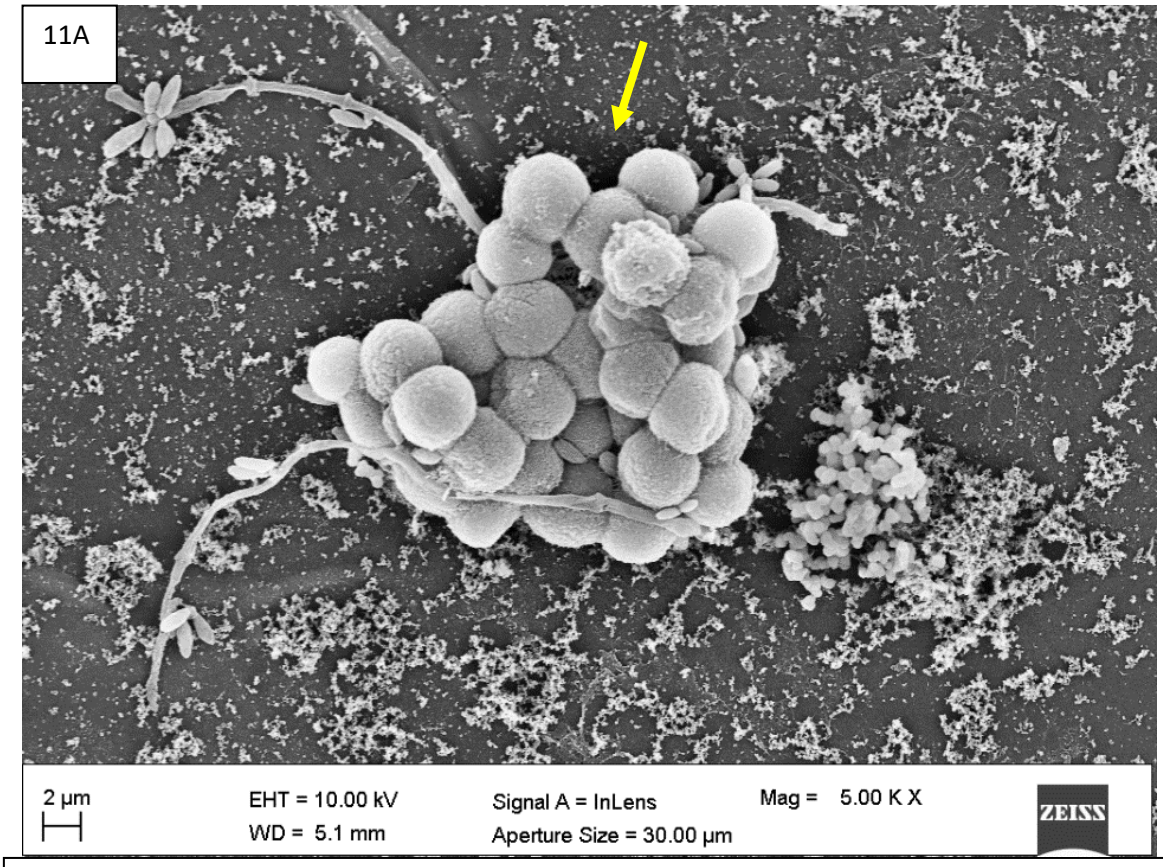
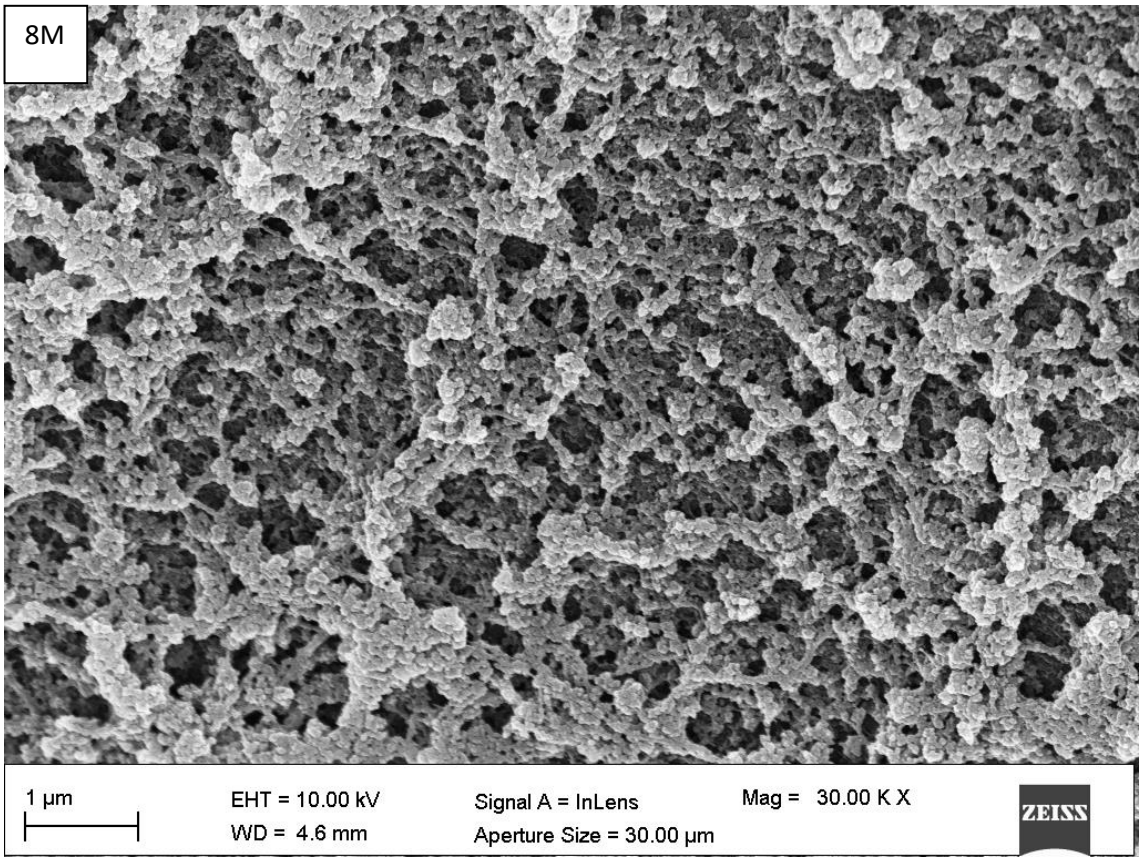


Fig. 10A: Bacteria and biofilms associated with the skin surface of *Argyropelecus hemigymnus*

Fig. 11A: Fungal association found on the skin surface of *Argyropelecus hemigymnus*



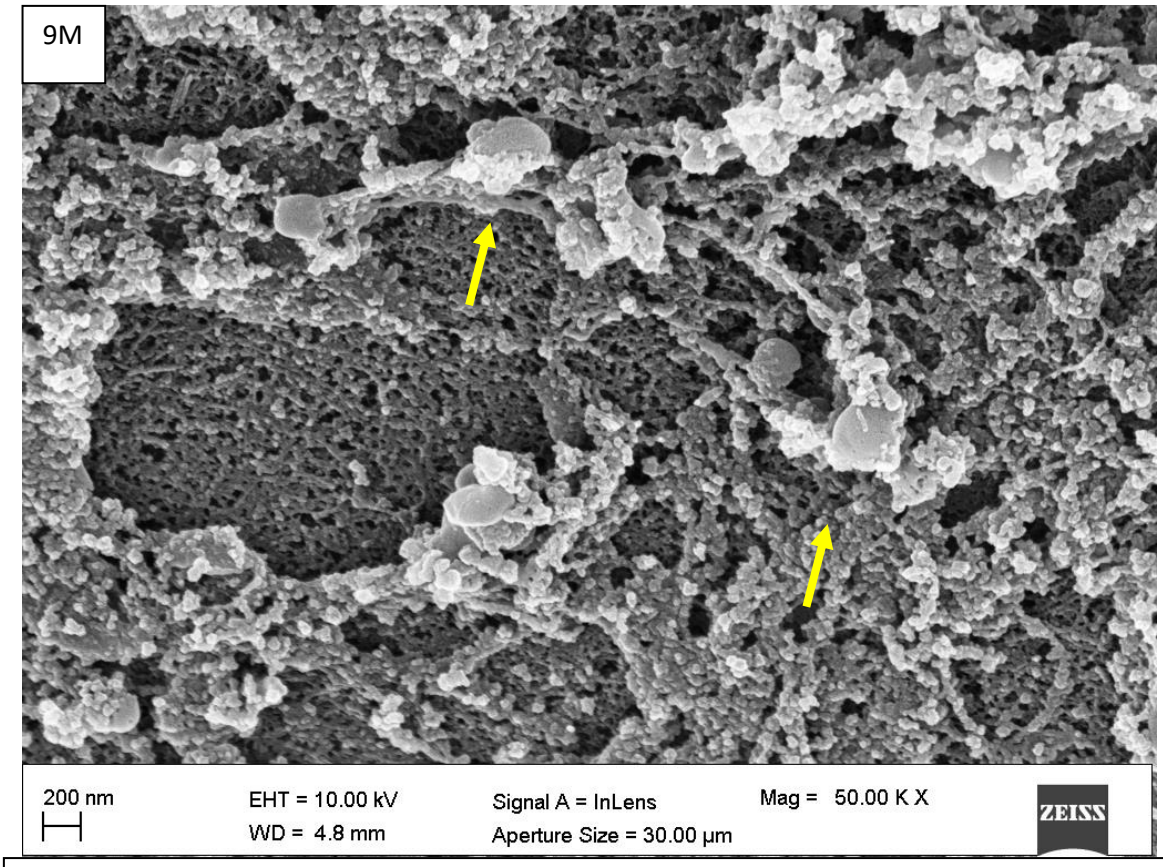
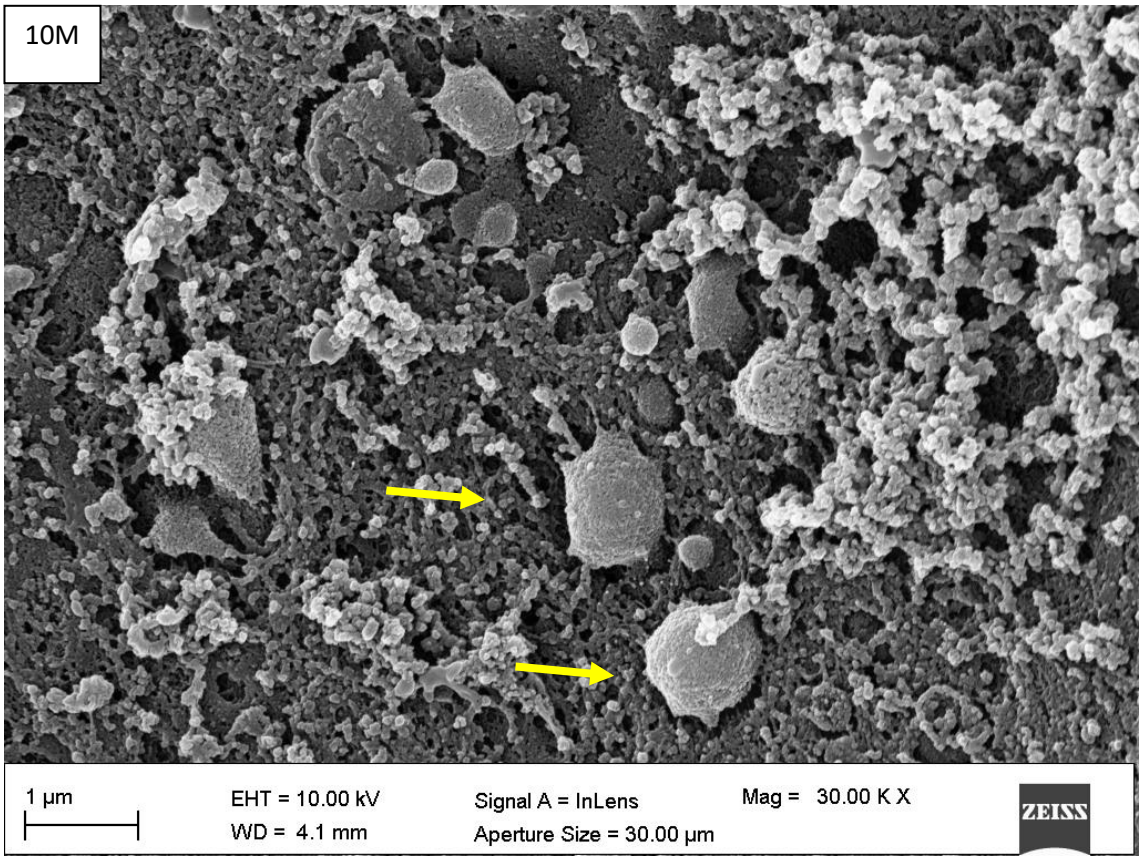


Fig. 8M: Biofilms associated with the skin surface of *Maurolicus muelleri*
Fig. 9M: Zoom of the external part of biofilm of *Maurolicus muelleri*, where bacteria are present.



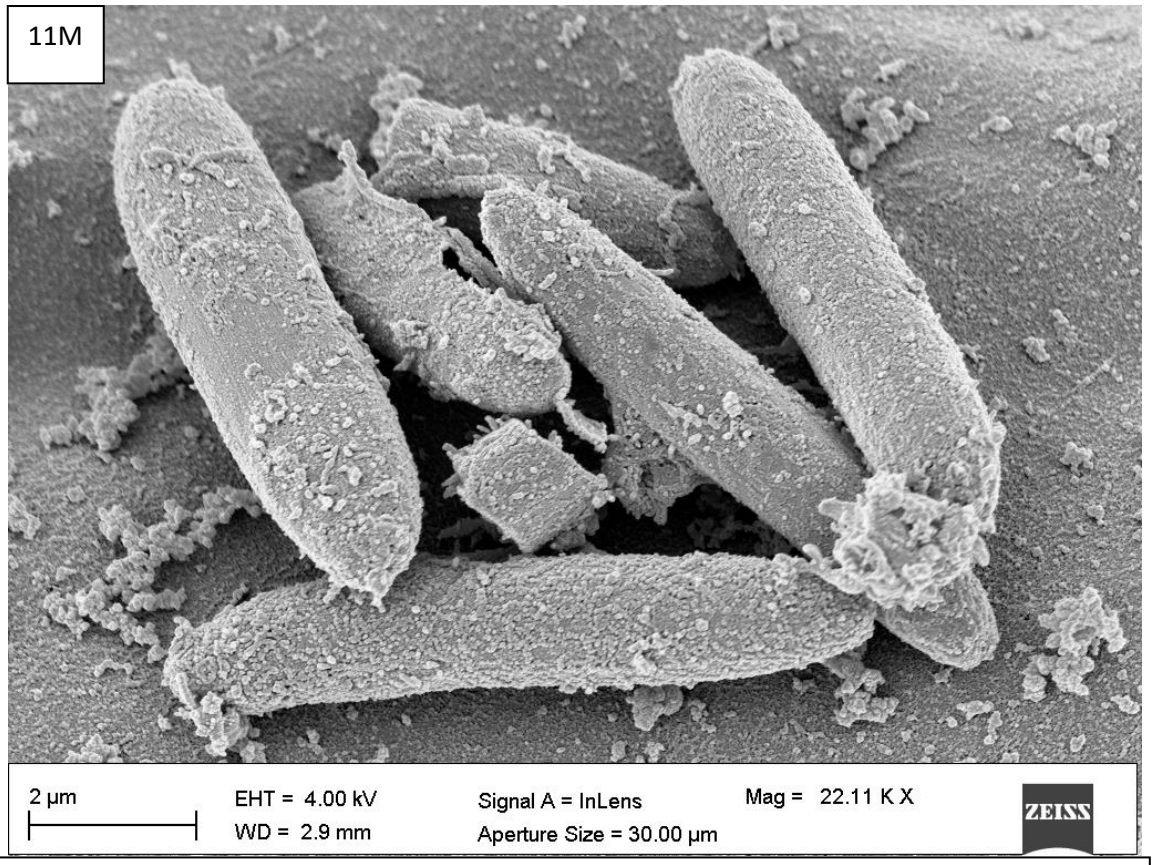
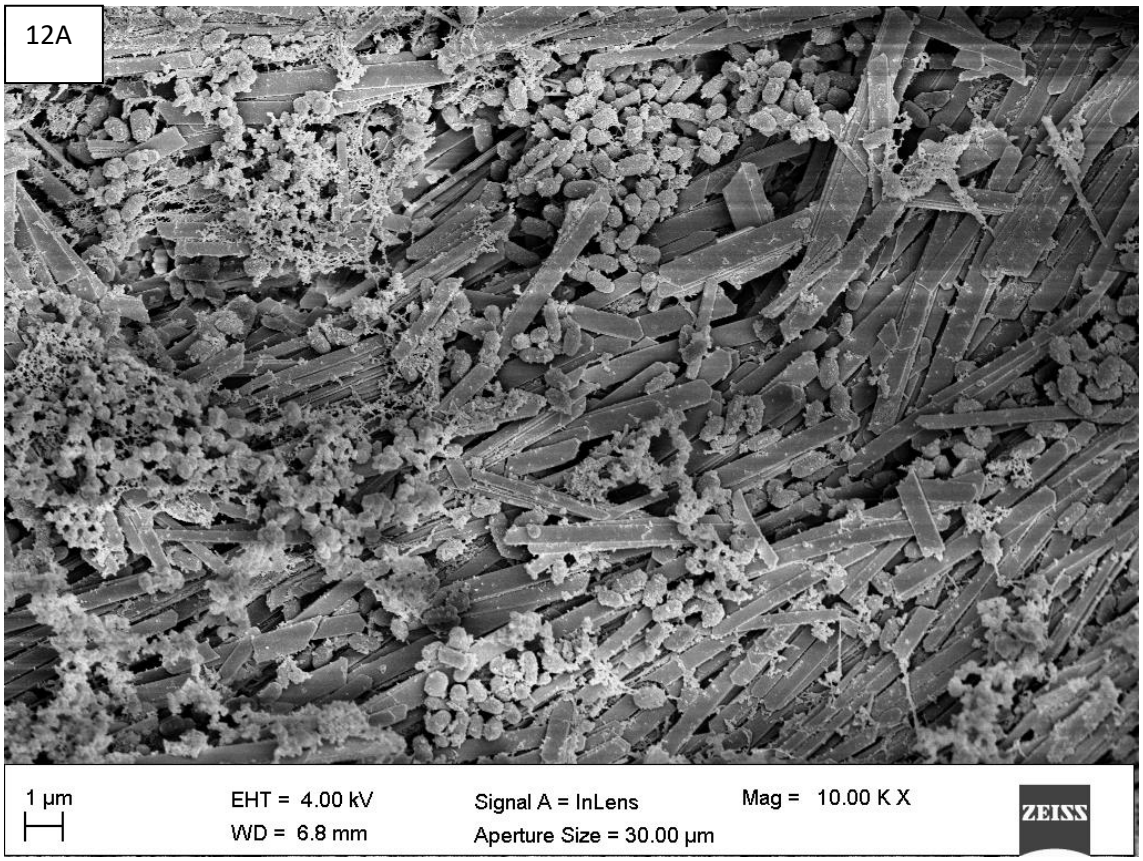


Fig. 10M: Bacteria associated with the skin surface of *Maurolicus muelleri*
Fig. 11M: Assembly of cells (size 10 µm) in the external surface of *Maurolicus muelleri*



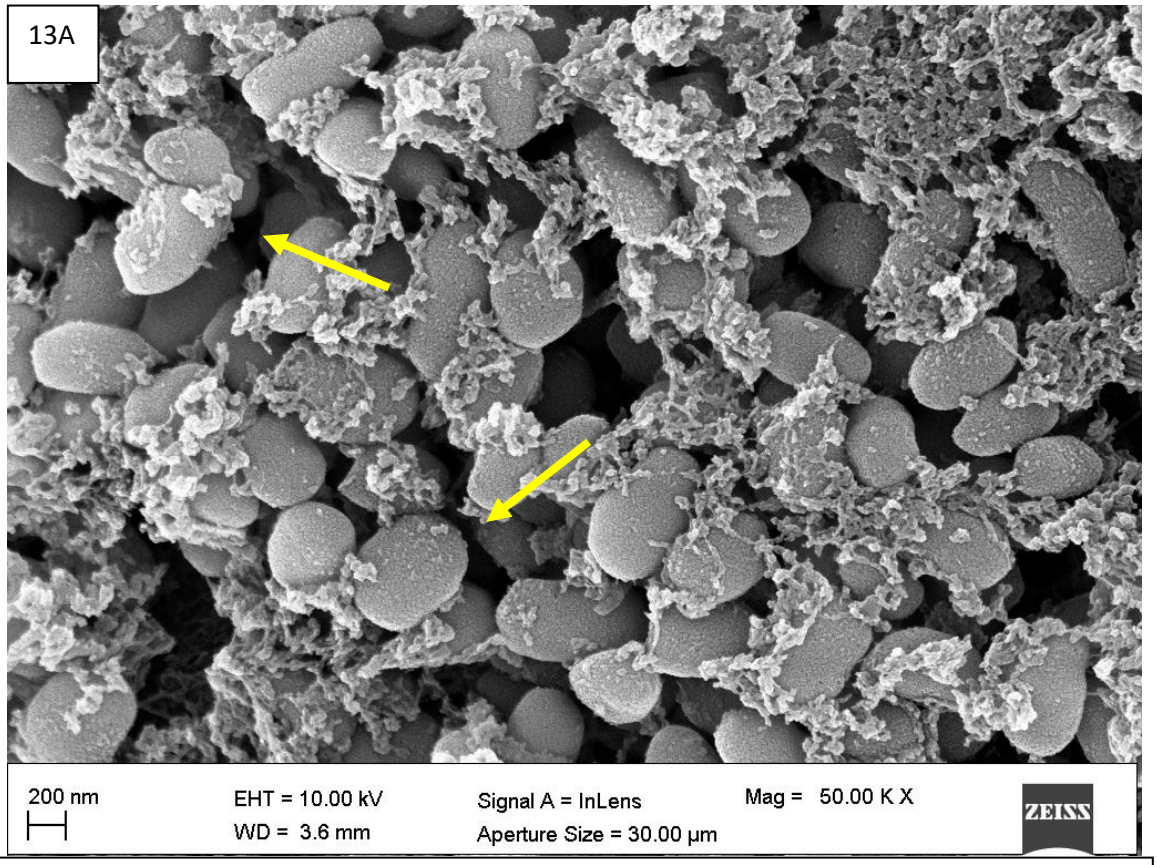
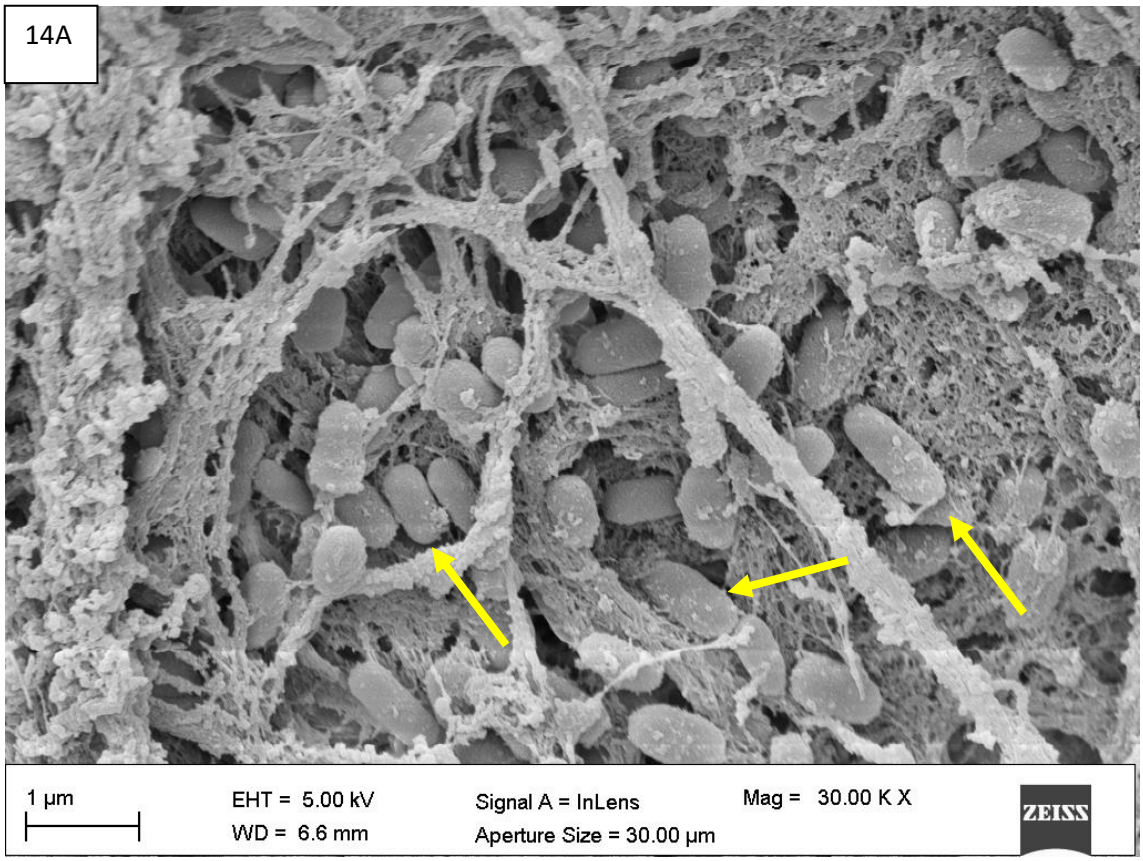


Fig. 12A: Panoramic view of the bacterial association present inside the photophores in *Argyropelecus hemigymnus*.
Fig. 13A: Zoom of bacterial cells found inside the photophore of *Argyropelecus hemigymnus*



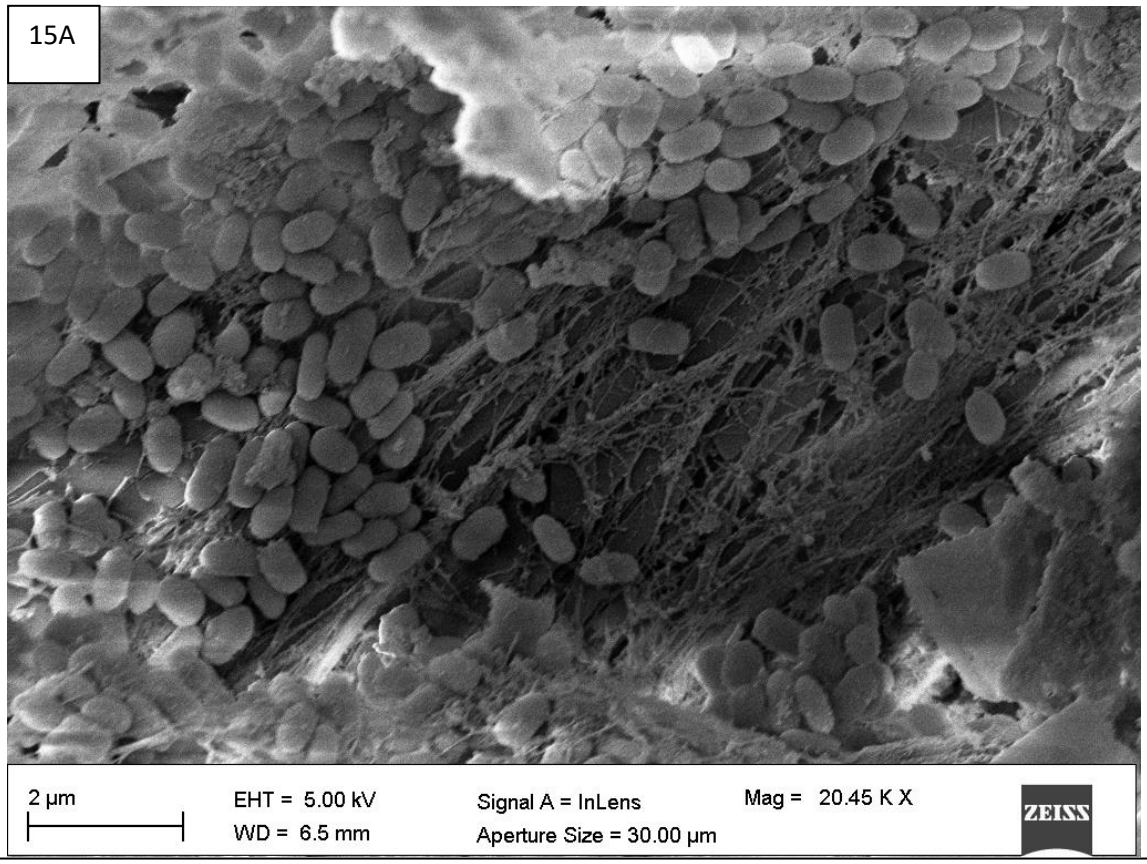
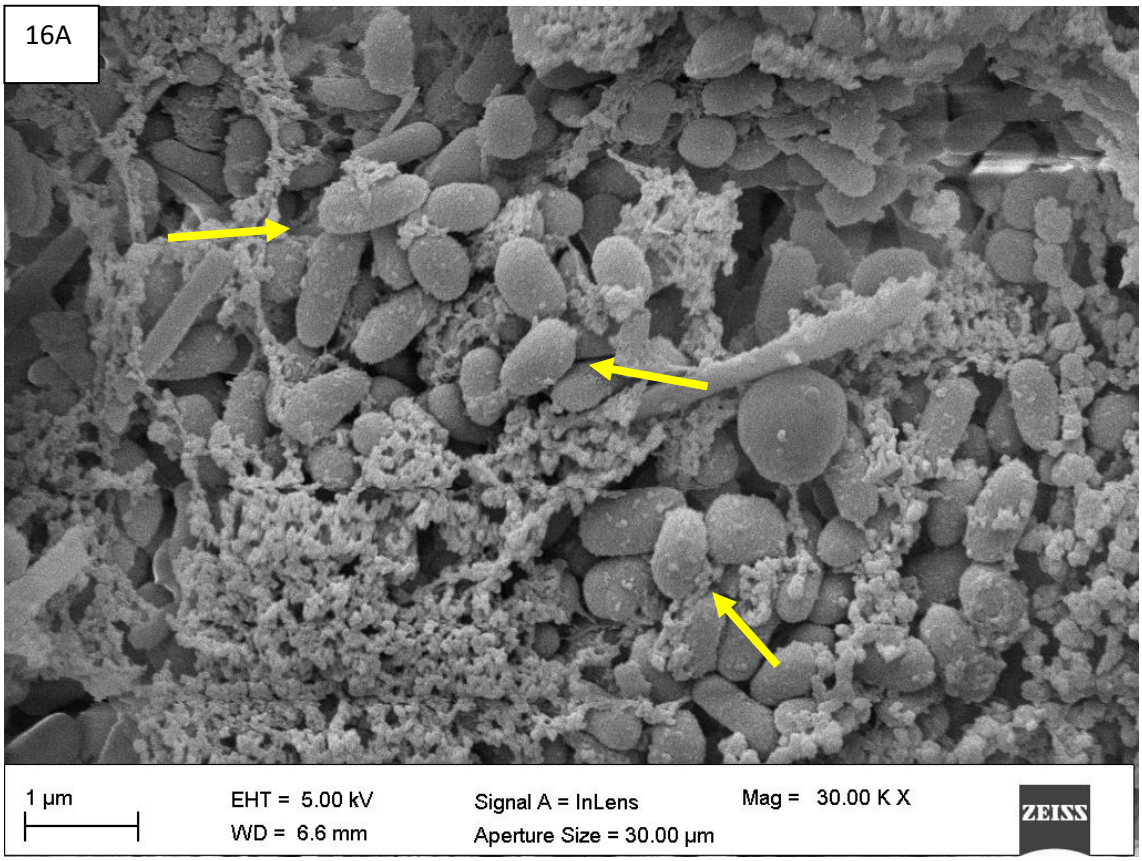


Fig. 14A and **Fig. 15A**: Imagine of bacterial cells found inside the photophore of *Argyropelecus hemigymnus*



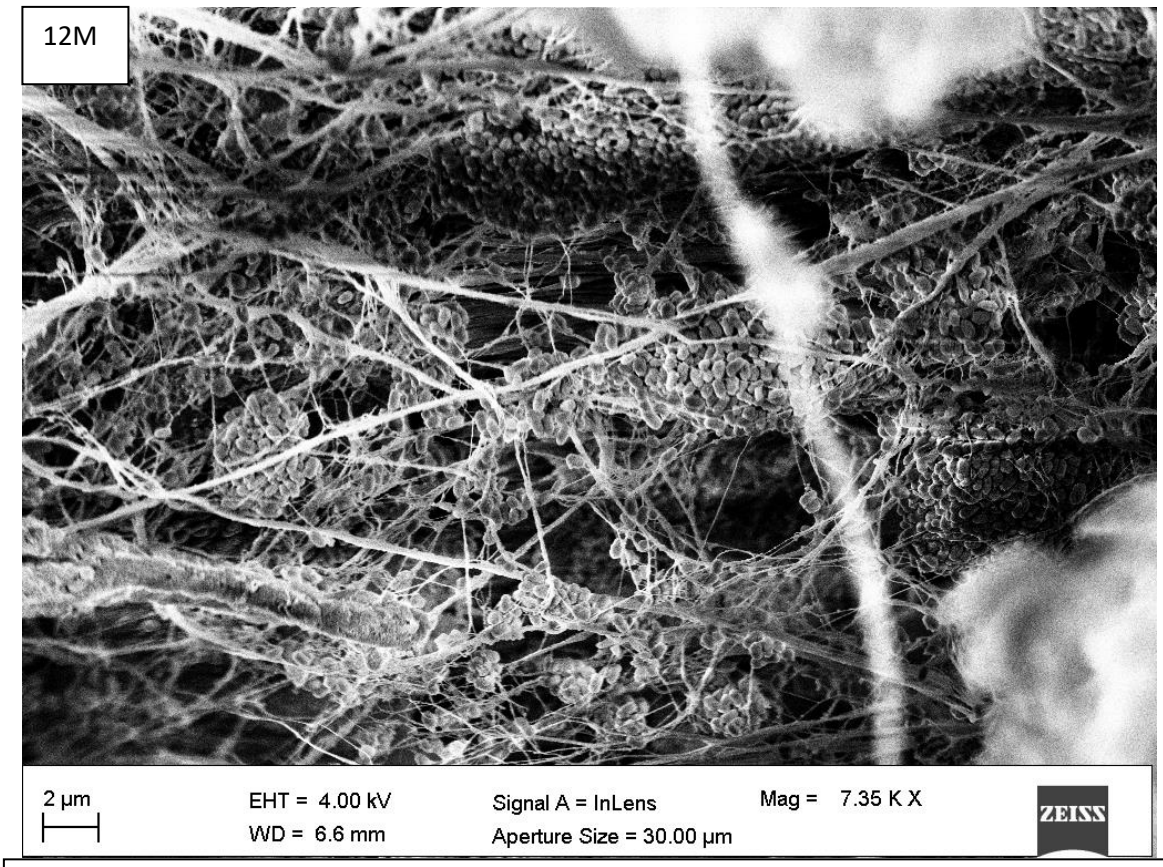
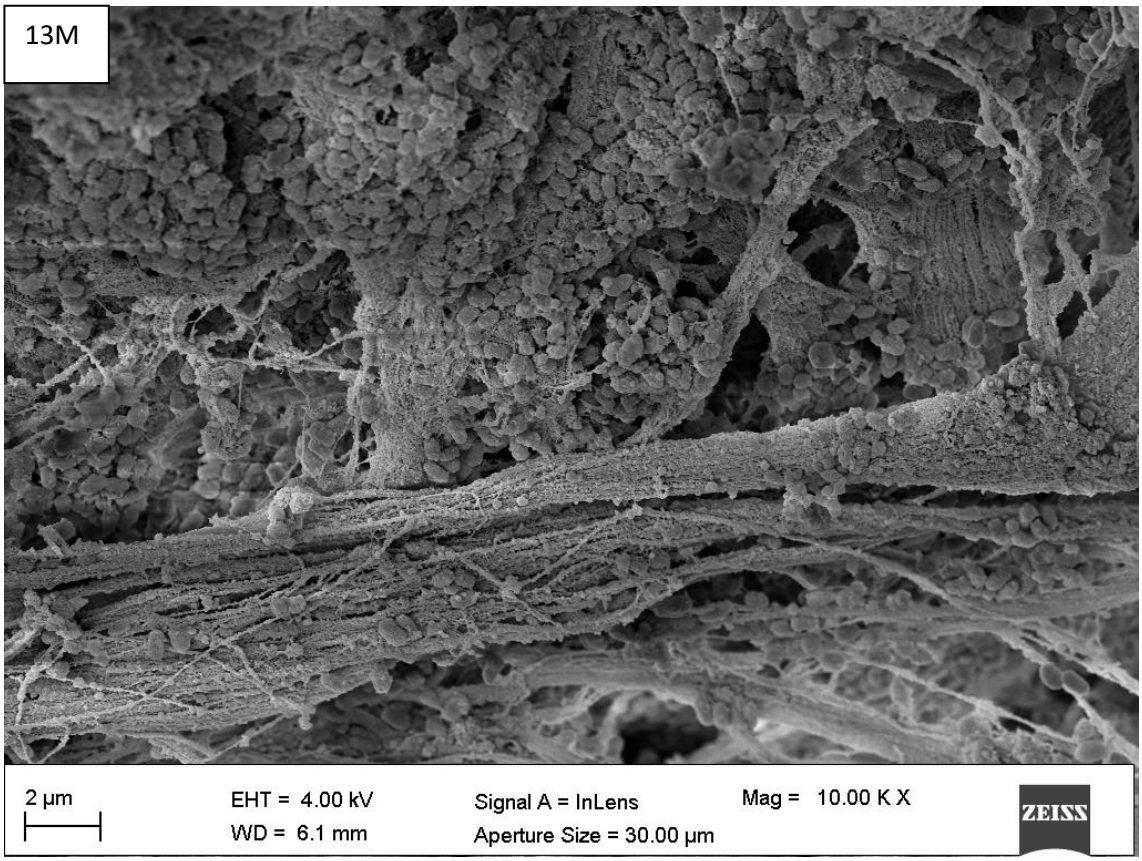


Fig. 16A: Zoom of bacterial cells found inside the photophore of *Maurolicus muelleri*
Fig. 12M: Panoramic view of the bacterial association present within the body portion of photophores in *Maurolicus muelleri*



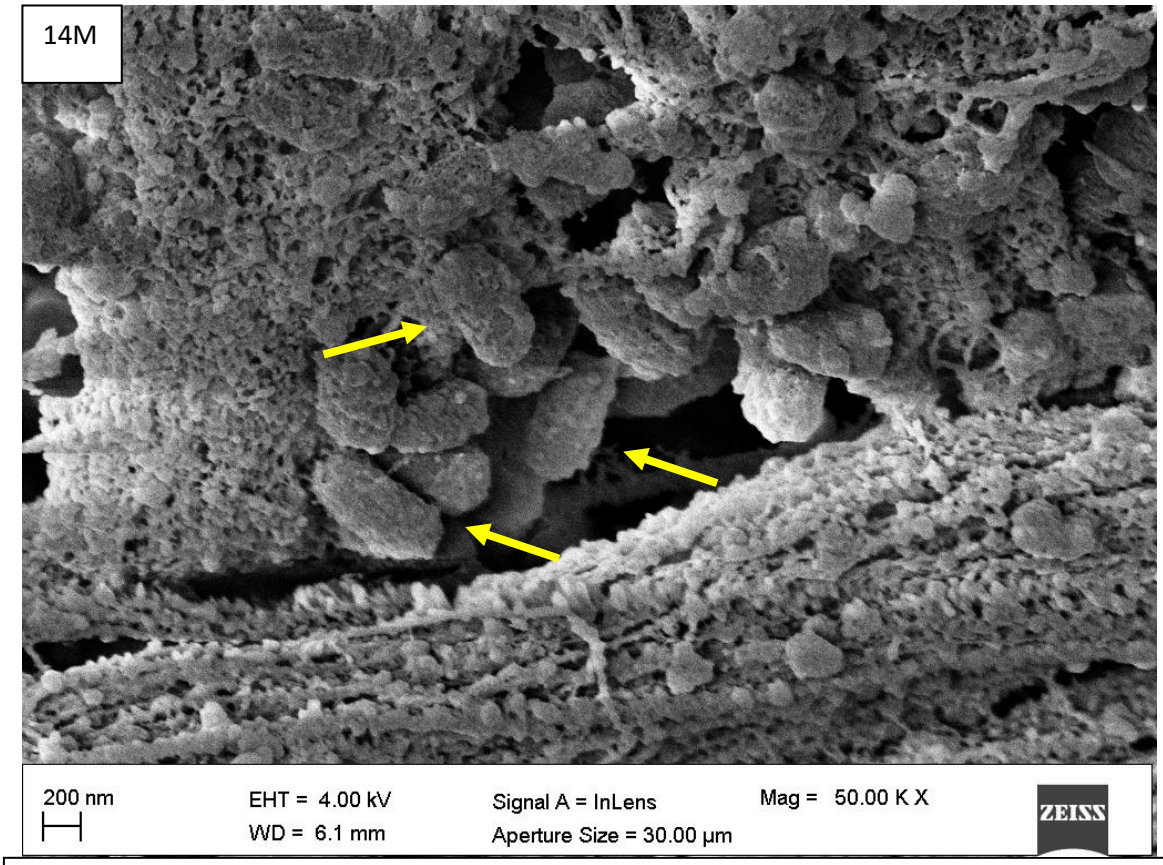
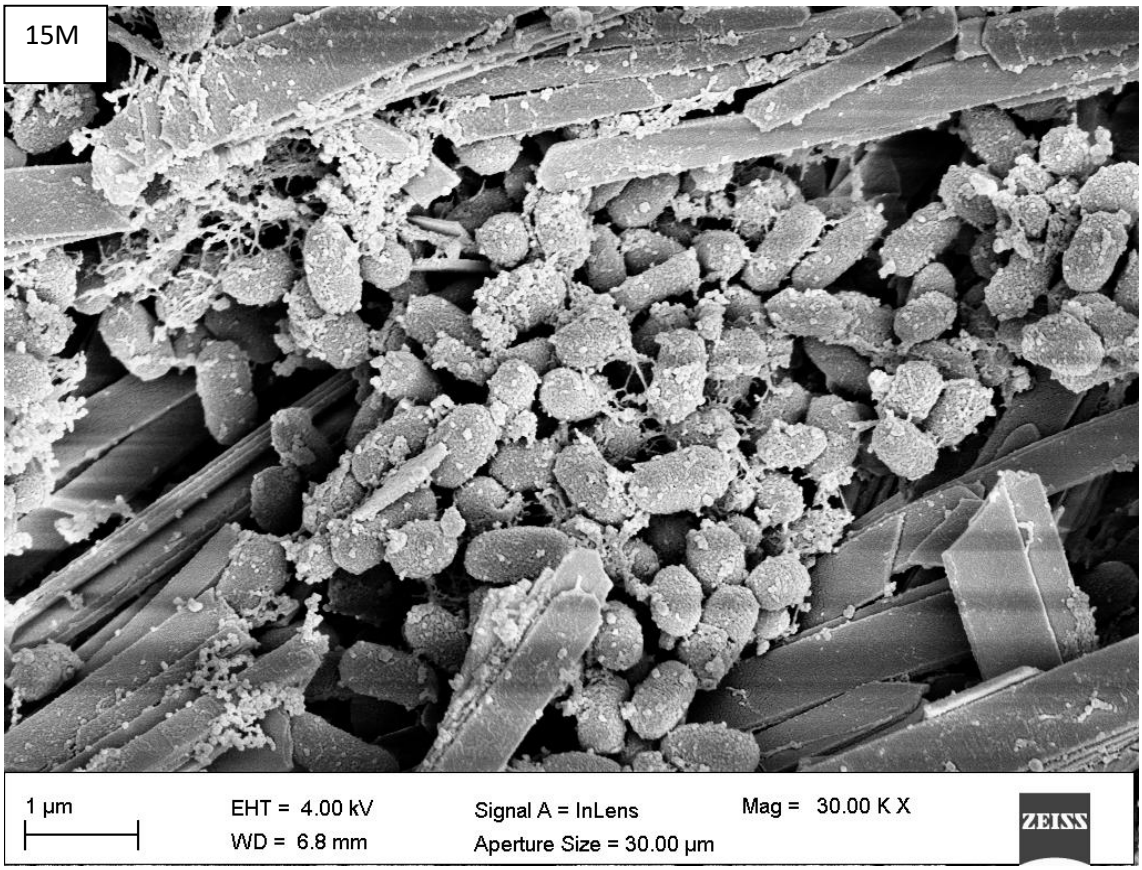


Fig. 13M and **Fig. 14M**: Imagine of bacterial cells found inside the photophore of *Maurolicus muelleri*



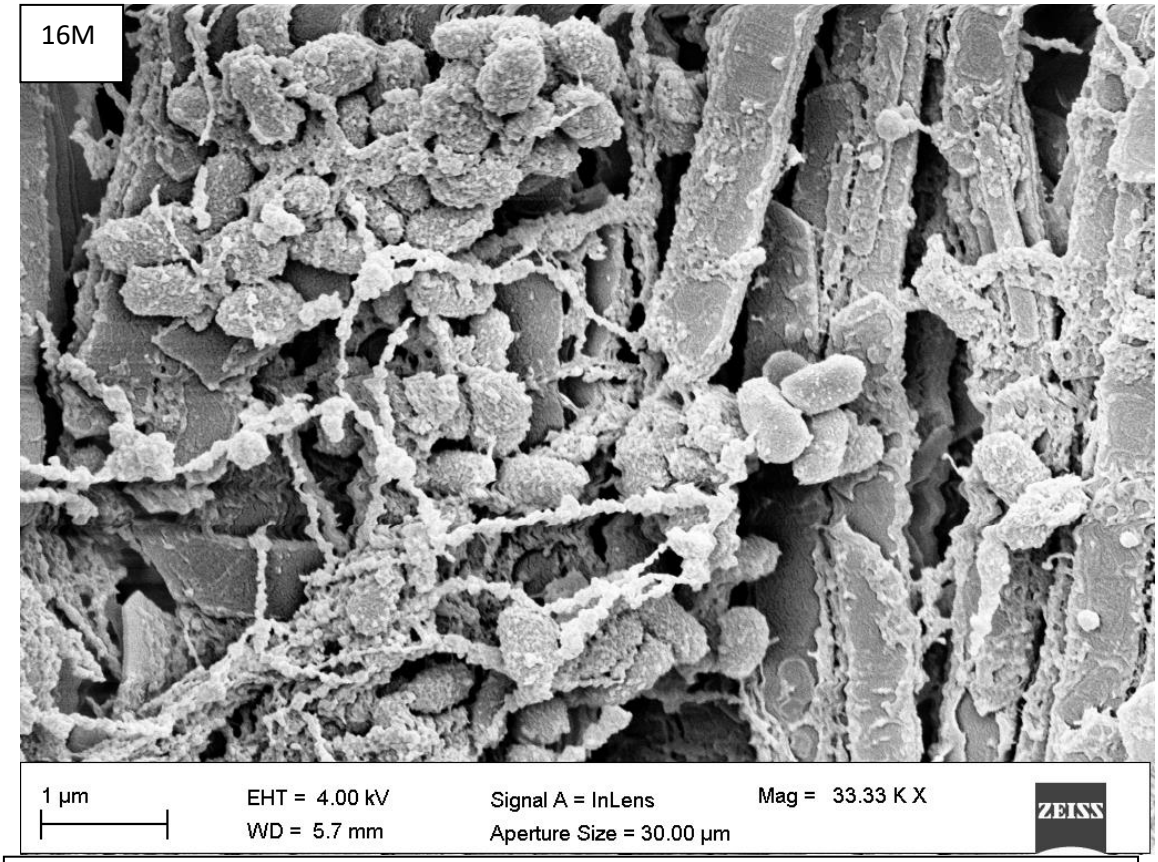


Fig. 15M and **Fig. 16M**: Images of bacterial cells found inside the photophore of *Maurolicus muelleri*

4.3 Microbiome and mycobiome associated with mesopelagic fishes

4.3.1 Molecular analysis

α and β diversity of microbial assemblages associated with different body parts of both fishes.

The rarefaction curve, considering the same number of sequences for all the samples, reached a plateau in the microbiomes of each body part of the fishes, demonstrating that the bacterial diversity of samples was completely covered (**Fig. 17**). Total ASV richness was calculated of each sample and validated with statistical analysis.

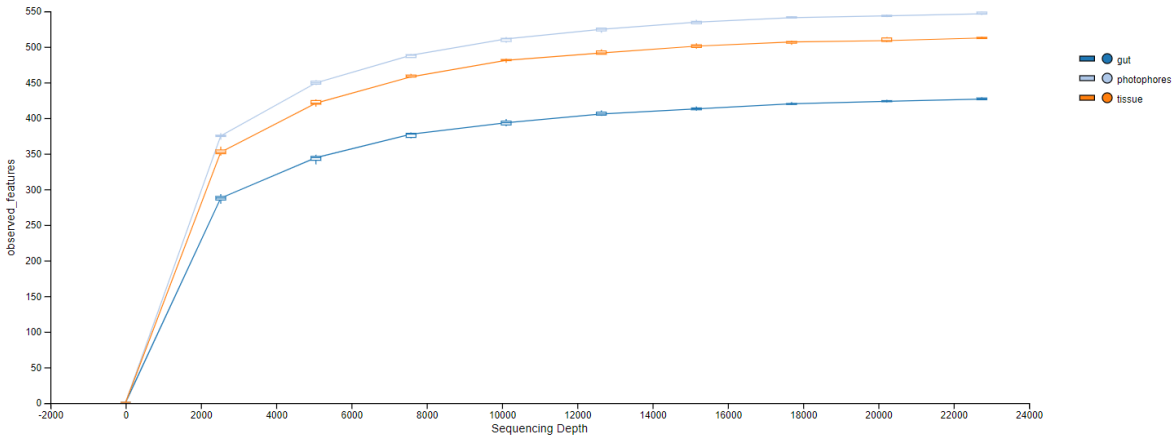


Fig.17: Rarefaction curve of samples after a normalization of 22750 sequences for the different body parts of *M. muelleri* and *A. hemigymnus*

The number of ASVs in *M. muelleri* ranged from 148 to 182 in the photophore, from 147 to 158 in the gut and from 141 to 179 in the tissue samples. The ASVs richness in *A. hemigymnus* varied from 126 to 167 in the photophore, from 57 to 143 in the gut and from 97 to 149 in the tissue samples (**Fig. 18**). The lowest

value of ASV richness in *M. muelleri* was found in the gut (3MM) and the highest value was found in the photophores (5MM) while in *A. hemigymnus* the lowest value was discovered in the gut (1AH) and the highest value in the photophores (4AH). Significant differences were found in the number of ASVs between the two different species of fishes, nevertheless no significant differences were found the different body parts of the specimens (**Tab. 4 SM**).

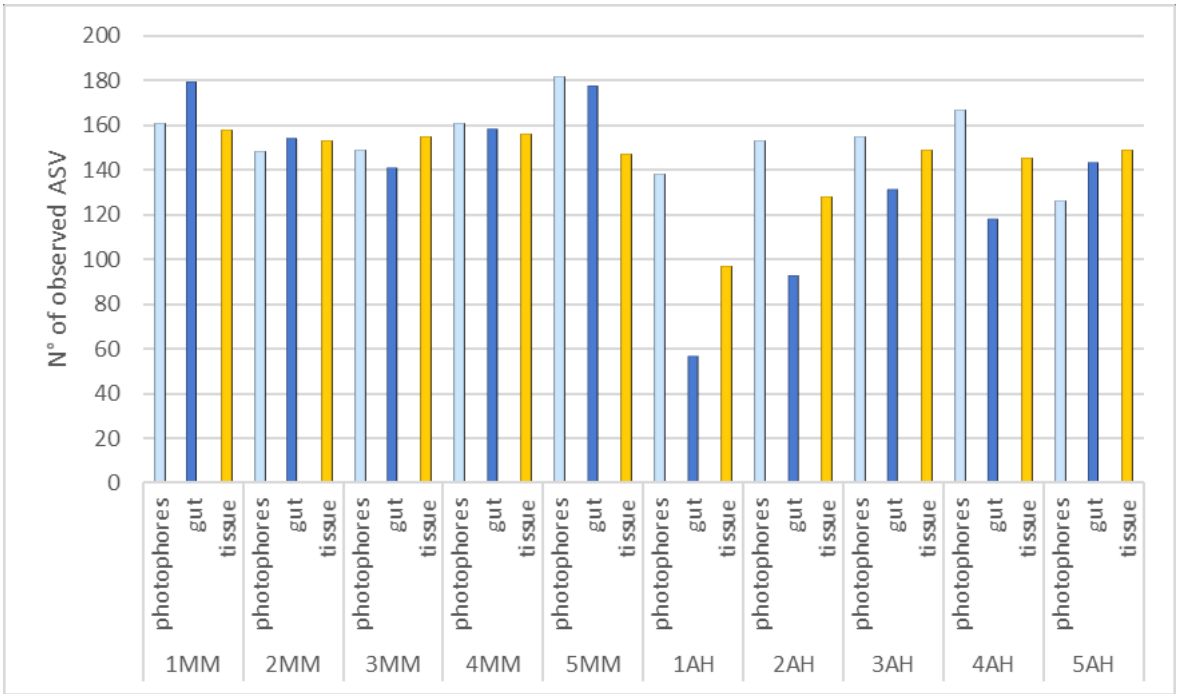


Fig. 18: Numbers of ASVs observed in the different parts of the body of *M. muelleri* and *A. hemigymnus* specimens

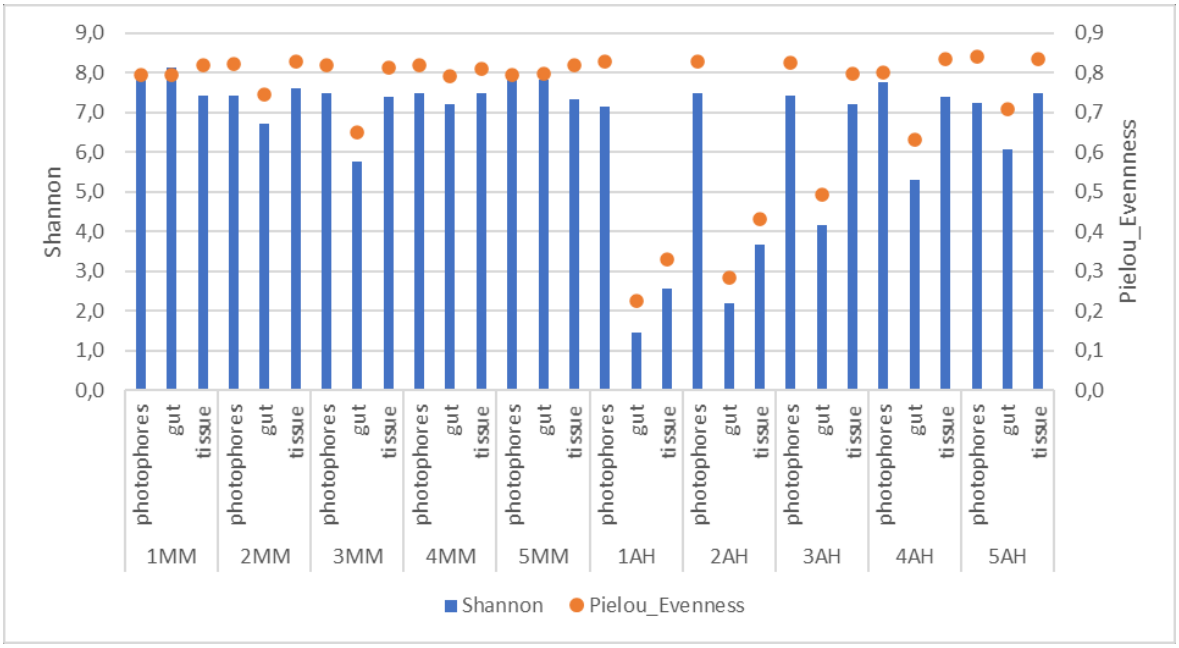


Fig. 19: Shannon and Evenness indices of microbial community associated with the different parts of *M. muelleri* and *A. hemigymnus* individuals.

High variability was found in Shannon and Evenness indices among all samples, with significant differences considering the microbiomes associated with both the two different species and the different body parts of fishes (**Fig. 19**; **Fig. 4 SM**).

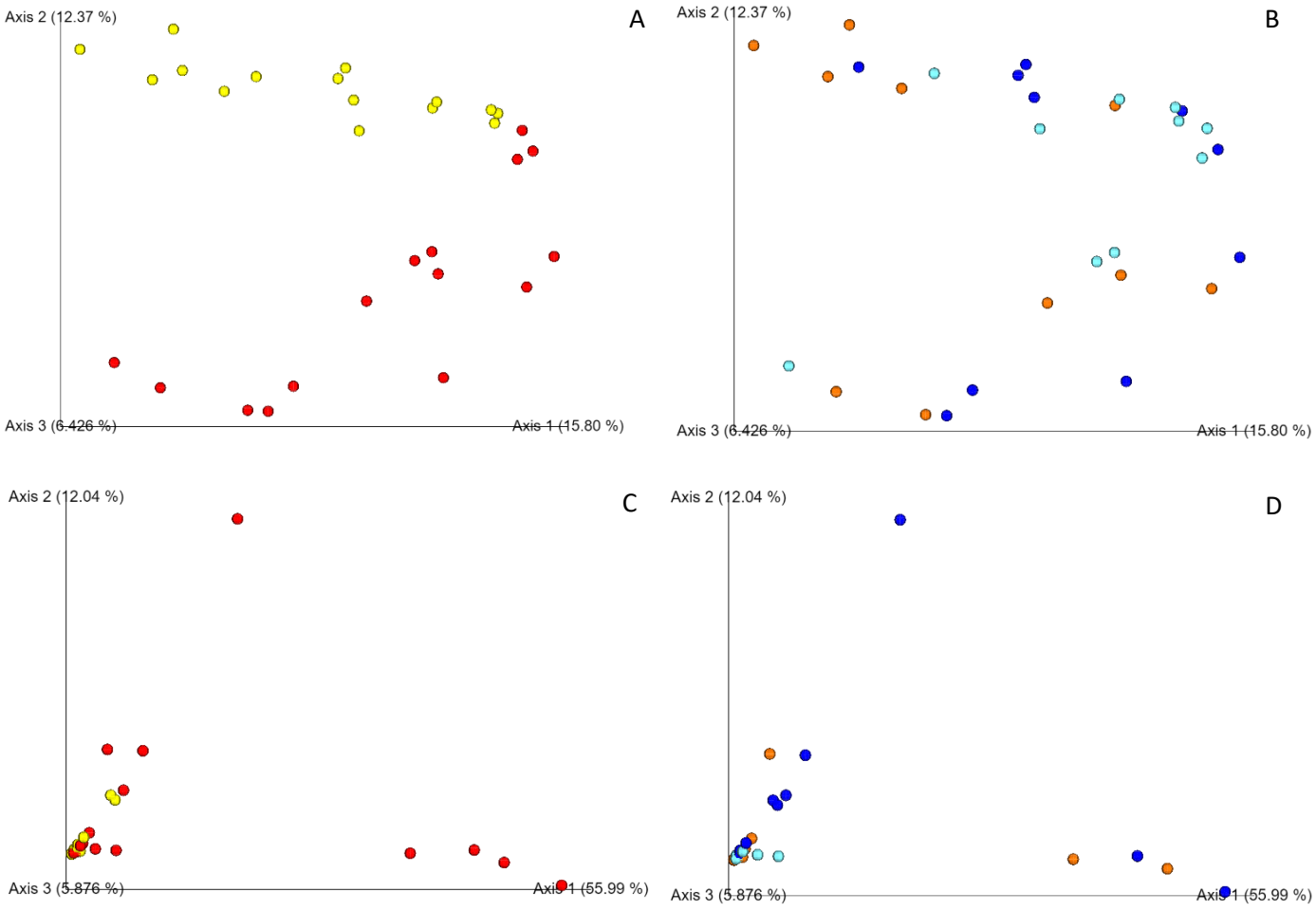


Fig. 20: PCoA plot of Beta-diversity of microbiomes carried out on unweighted UniFrac distance (**A-B**) and Bray Curtis dissimilarity (**C-D**) among different fish species (**A-C**) and among different body parts (**B-D**), respectively. (Red: *M. muelleri*; Yellow: *A. hemigymnus*; Blue: Gut; Light blue: Photophores; Orange: Tissue)

The Beta diversity analysis revealed significant differences in terms of both composition and phylogenetic diversity of the microbial communities between the two fish species (**Fig. 20**, (**A-C**)). No significant differences were found among microbiomes associated with the different body parts (**Fig. 20**, (**B-D**).

Microbiome diversity associated with different species and different body-parts of fishes

The results of the analysis on the microbiomes' taxonomic composition revealed a total of 230 genera, 143 families, 87 orders, 31 classes, 18 phyla. Significant taxonomic differences were found between samples belonging to different species, showing a dissimilarity of 29,5 % (**Tab. 4SM**; **Tab. 5SM**). Significant taxonomic differences were found also among all samples belong to the same body parts, showing a dissimilarity of 30,3% among photophores vs gut, of 20,3% among photophores vs tissue and of 32,4% among gut vs tissue (**Tab. 4SM**; **Tab. 5SM**). Taxonomic analyses show that a core of bacterial members (at genus level) were found shared among all the specimens of M.Muelleri **(Fig. 21A)** and A.hemingus **(Fig. 21B)** and similar bacterial genera were revealed between two cores. *Bacteroides* (*Bacteroidaceae*), *Faecalibacterium* and *Subdoligranulum* (*Ruminococcaceae*), *Alistipes* (*Rikinella*), *Clostridia UCG-014*, *Roseburia* and *Lachnospira* (*Lachnospiraceae*) genera were found in all samples. The core differed only in the presence of *SUP05_cluster* (*Thioglobaceae)*, *Agathobacter* (*Lachnospiraceae*) and the family *Enterobacteriaceae* (unidentified genus) (**Fig.21**). In particular, the genus *SUP05_cluster* was predominantly found in the gut and tissues of 1AH (95,8% and 84,5%, respectively) and 2AH samples (80% and 64%, respectively) and with lower percentages also in the tissues of 3AH, 4AH, 5AH (1,6%, 1,6% 1,1%, respectively; **Fig. 22**).

Entherobacteriaceae family was present in all samples of *A. hemigymnus* with low percentages (from 0,01% to 2,4%), showing an increase in 3AHG-T and 5AH G samples (67%, 15% and 36,8%, respectively). The genus Vibrio was found with the highest percentage in 3MMG sample, with lower percentages in the *Argyropelecus* samples (1AHP-T, 2AHP-T, 3AHT, 4AHP-G-T and 5AHT with 3,5%-1,1%, 2,9%-1,7%, 4,7% and 1,2%-3,6%-4,8% and 2,5% respectively) and with percentage below the 1% in the others *Maurolicus* specimens (**Fig. 22**). *Mycoplasma* genus was found with the highest percentage in 3MM G and 4AH G (35% and 41%, respectively).

Simper analysis revealed that among specimens of *M. muelleri*, samples belonging to the same body part showed a high similarity (89,7%, 80,7%, 88,8% of photophores, gut and tissue samples, respectively), mainly driven by the presence of the dominant genera. On the other hand, simper analysis revealed that among specimens of *A. hemigymnus*, gut is those that showed the higher diversity (only 55,3%, of similarity), followed by tissue (66,5% of similarity) and then photophores samples (87,9% of similarity; **Fig.22**; **Tab. 5SM**).

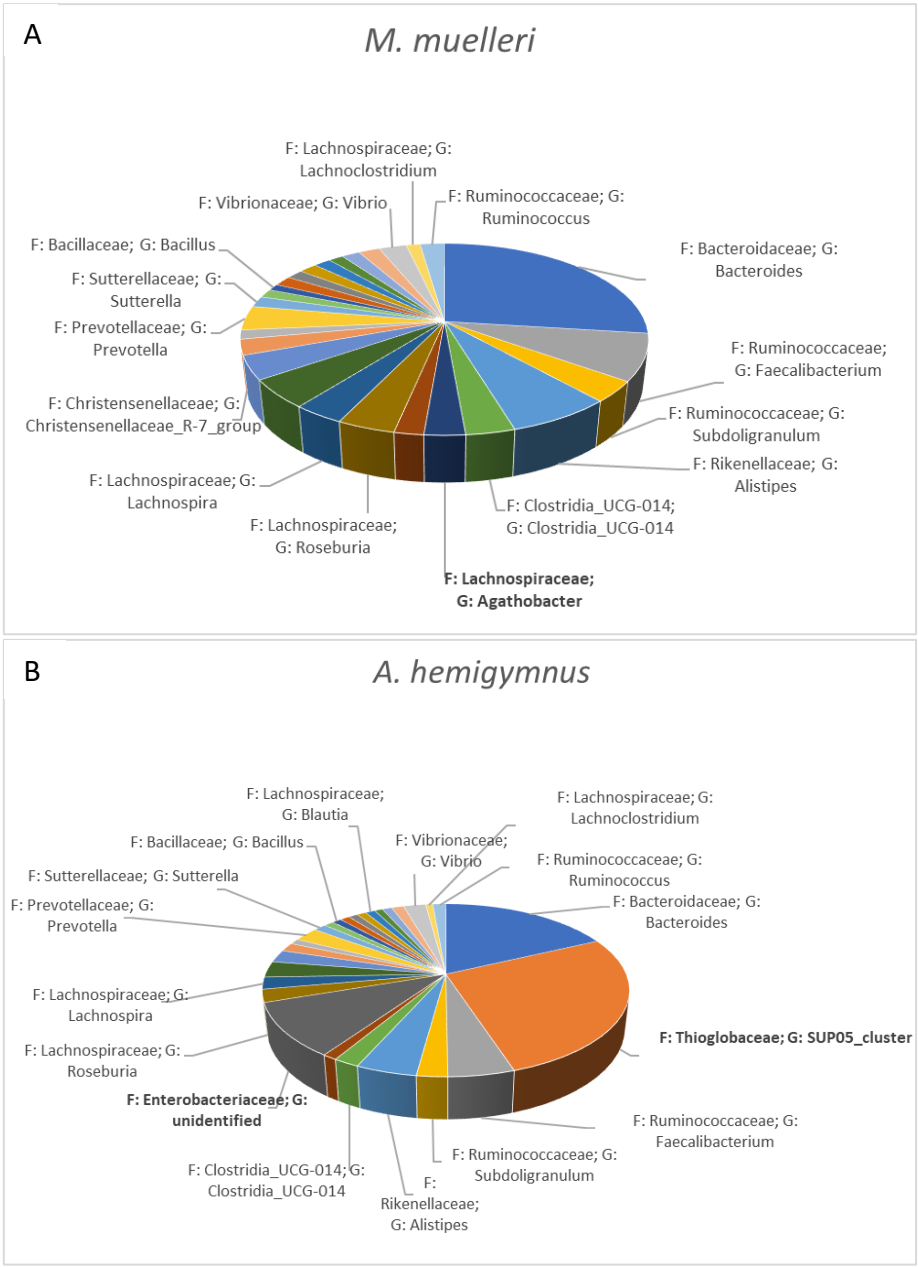


Fig. 21: Core microbiomes formed by all the bacterial genera (0,8%) shared among all the specimens of *M.Muelleri* (A) and *A.hemingus* (B).

Fig. 22: Taxonomy composition of microbial associated with different body parts of *Maurolicus muelleri* and *Argyropelecus hemigymnus*

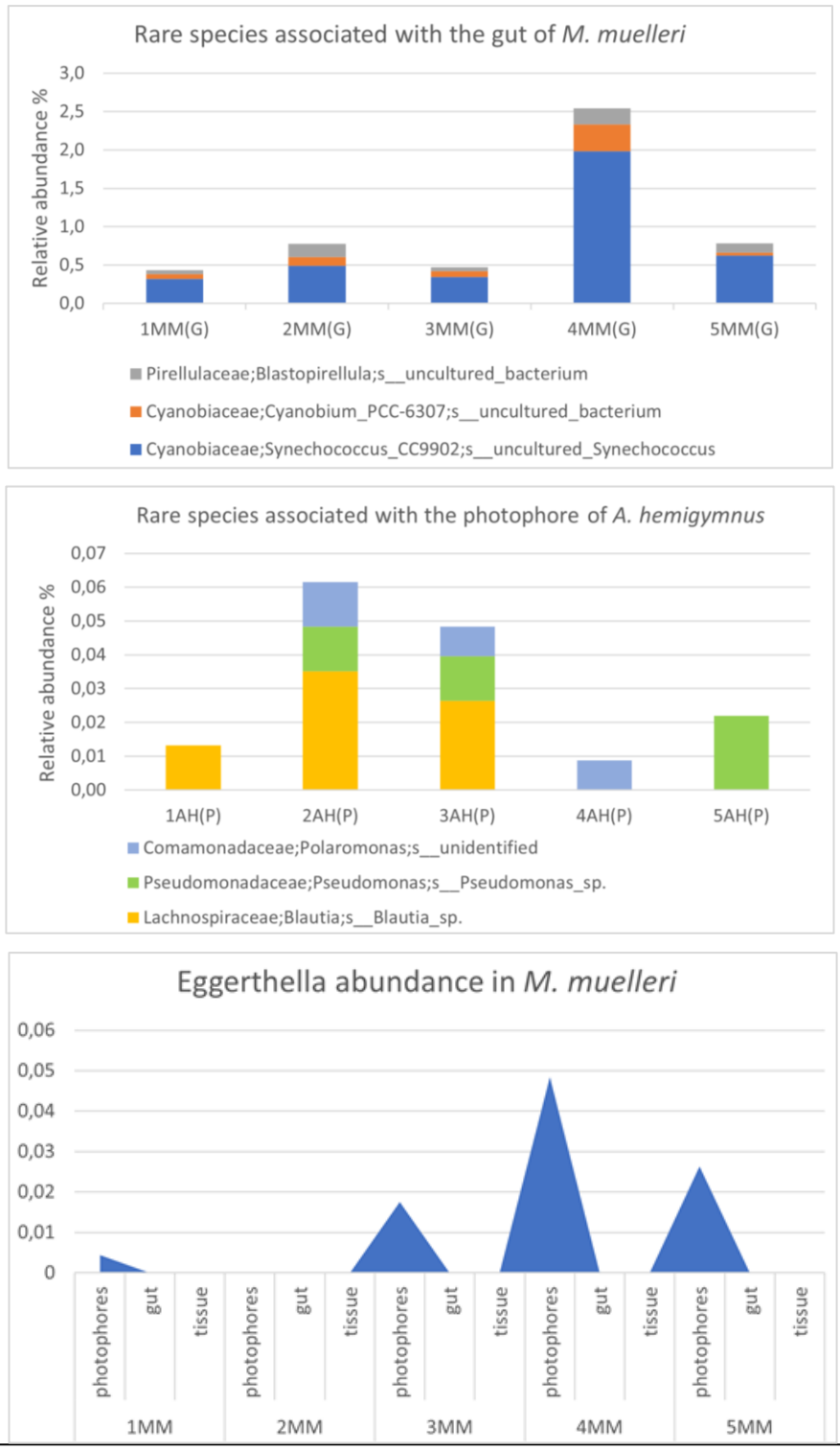


Fig. 23: Bacterial rare species exclusively associated with specific parts of the body of the two fishes.

Among the rare bacterial taxa (with relative abundance less than 1% in all samples *Synechoccu CC9902*, *Cyanobium PCC-6307* and *Blastopirellula* were

found exclusively in all the gut sample of *M. muelleri; Polaromonas, Psudomonas sp* and *Blautia sp* was exclusively found in some of the photophores sample of *A. hemigymnus*; and finally, the *Eggerthella* genus was found exclusively in different parts of the body of *M. muelleri* with the higher percentages in the photophores (**Fig. 23**).

The cluster analysis carried out on the taxonomic composition of microbiomes associated with all specimens do not reveal a clear separation among the two species and the three different body parts (**Fig. 24**). Nevertheless, the result highlights a low similarity among the sample 1AHG and the group of 2AHG-T, 3AHF, 1AHT and the others, due to high percentages of Enterobacteriaceae and Thioglobaceae (*SUP05*) bacterial families (**Fig. 21**; **Fig. 24**).

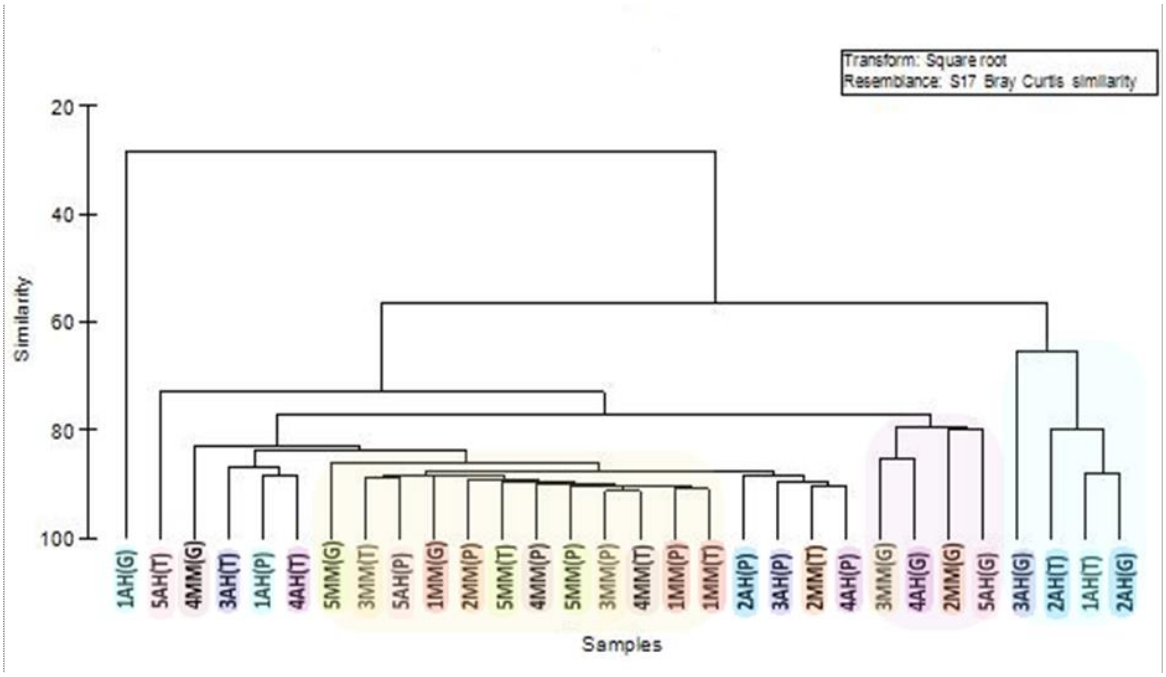


Fig. 24: Cluster analysis carried out on the taxonomic composition of microbiomes associated with specimens of *A. hemigymnus* and *M. muelleri*. Samples representing microbiome collected in different parts of the same individual were highlighted with the same colour.

Putative functions of microbiomes associated with the different body parts of *A. hemigymnus* and *M. muelleri*

Significant differences were found in the putative functions between microbiomes associated with individuals of *A. hemigymnus* and *M. muelleri* (**Fig. 25**; **Fig. 26**; **Tab. 4 SM**). The most important bacterial functions in all samples were fermentation and chemoheterotrophy, with contributions from 0.5%(1AHG) to 44% (3MMP) and 2% (1AHG) to 44% (3MMG), respectively. Nitrate-reducing bacteria were found in almost all samples, with high percentage values in 1AH (19,8% in gut and 19,2% in tissue) and 2AH (18,8% in gut and 17,7% in tissue).

Dissimilarities between the two species were mainly determined by the bacteria responsible of the dark oxidation of the sulfide/sulfur compounds and the nitrate/nitrogen respiration, present in *M. muelleri* with an average abundance of 0,2% and 0,4% in *A. hemigymnus* with 11,5% and 19,9%, respectively (**Fig. 25**; **Fig. 26**).

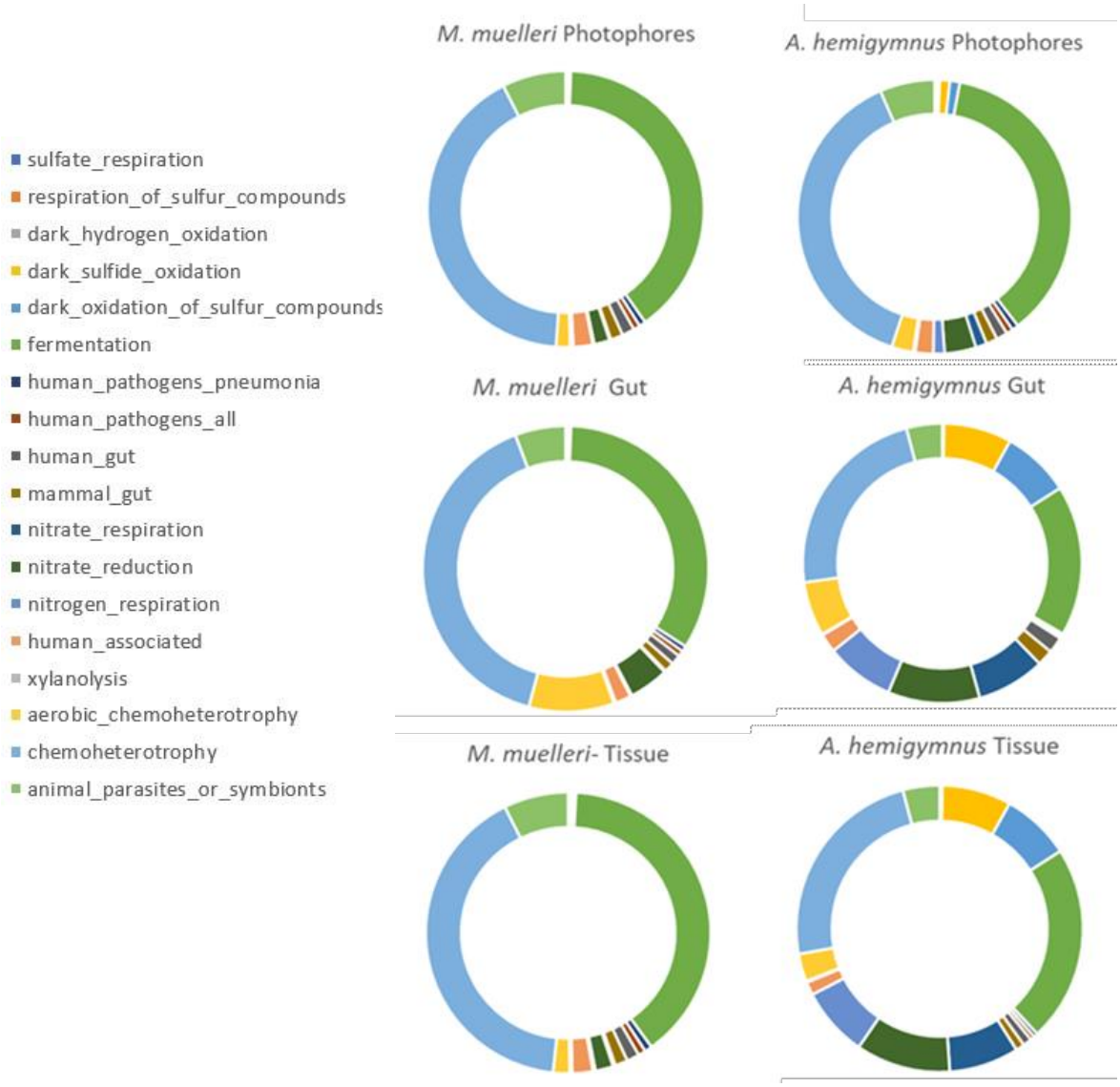


Fig. 25: Putative functions developed by microbiomes associated with different body parts of *M. muelleri* and *A. hemigymnus*

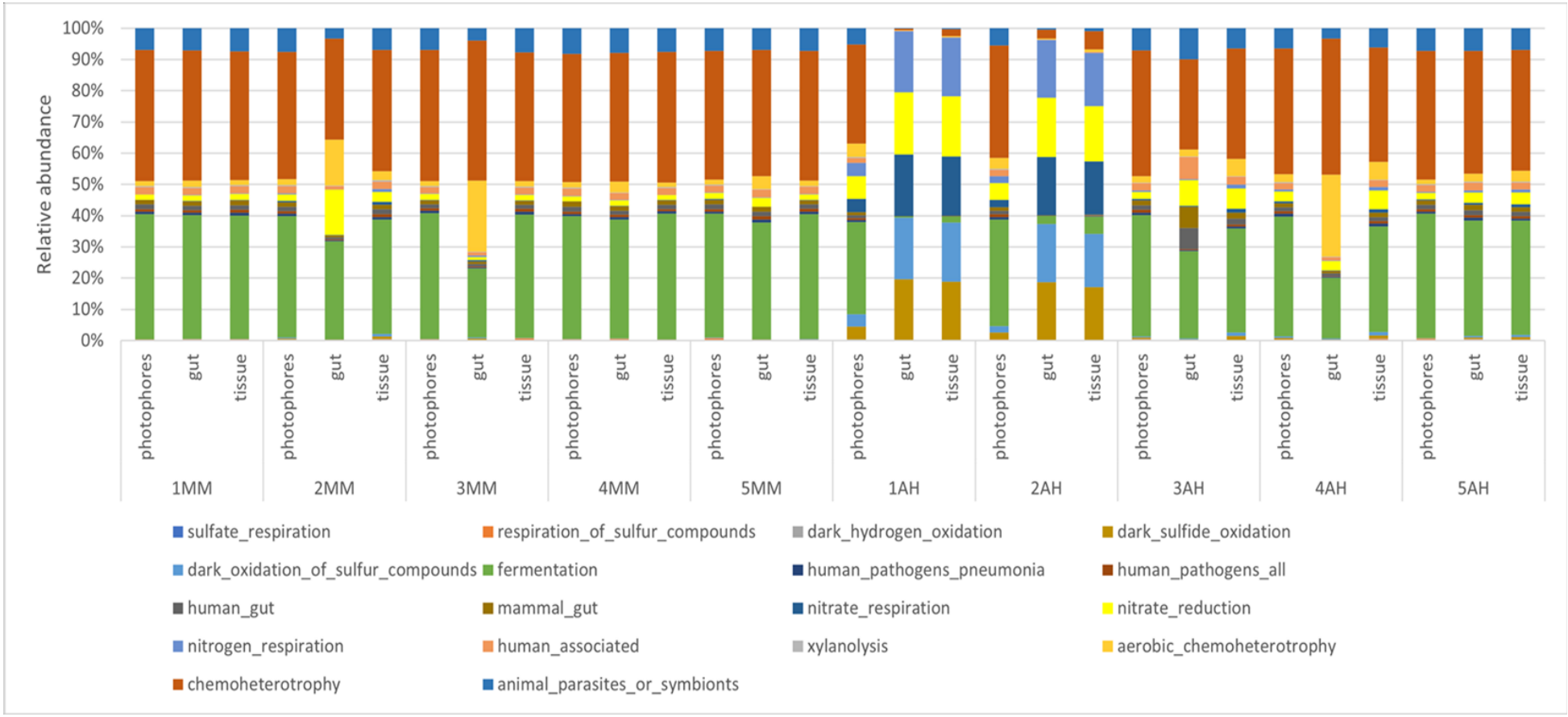


Fig. 26: Putative functions developed by microbiomes associated with the different body parts of *A. hemigymnus* and *M. muelleri*

Bacteria responsible for parasitism or symbiosis in animals were found especially in photophores with percentage from 6,9% (3MMP) to 8,2% (4MMP) in *M. muelleri* and from 5,2 (1AHP) to 7,2% (5AHP) in *A. hemigymnus* but no significant differences were found in the putative functions of the microbiomes associated with the different body parts.

The cluster analysis carried out on putative functions developed by microbiomes associated with all the specimens do not reveal clear separation among the two species and the 3 different group of body parts, but between samples 1AHG-T, 2AHG-T and the others due to high percentages of bacteria involved in the dark sulfide/sulfur compound oxidation and nitrate/nitrogen respiration (**Fig. 27**).

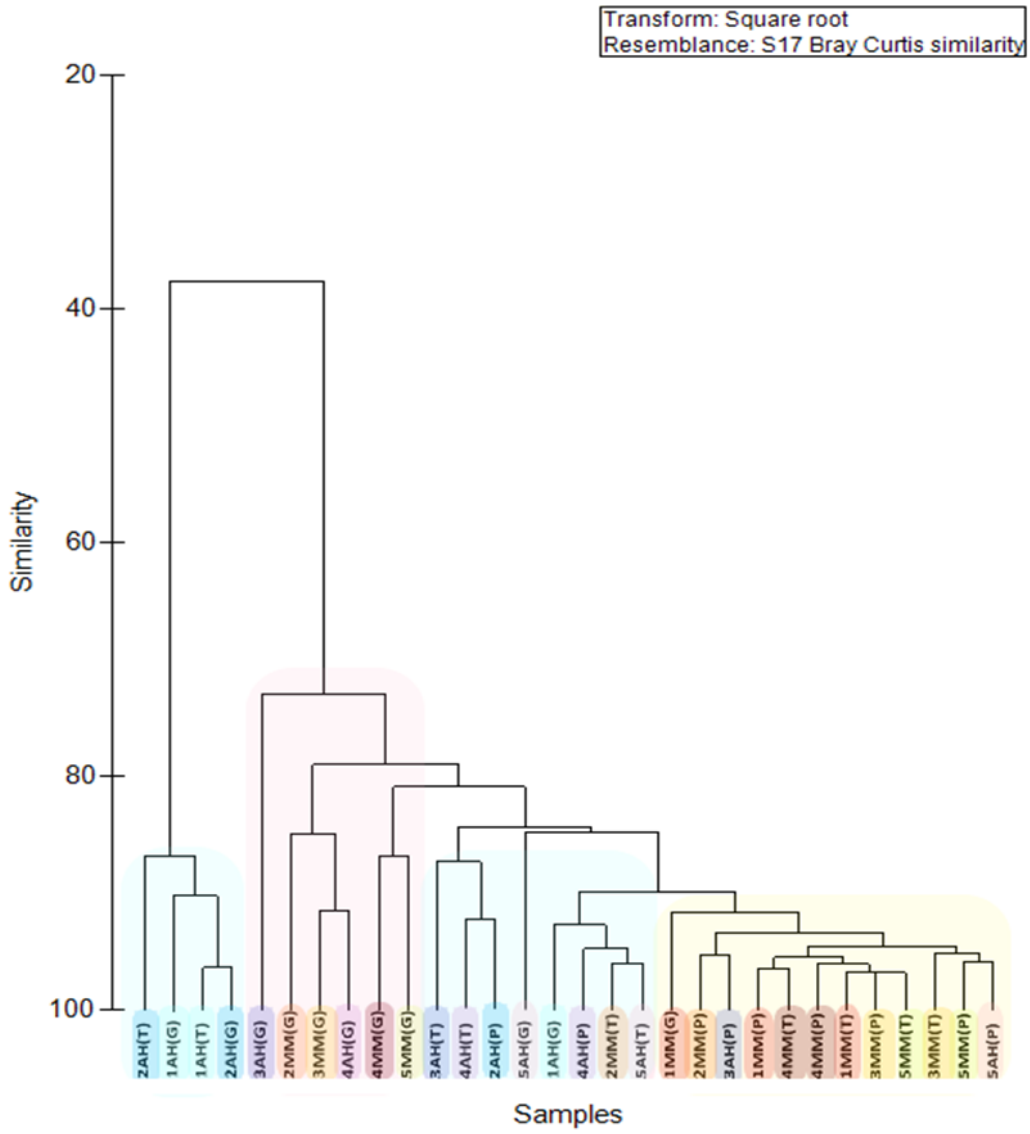


Fig. 27: Cluster analysis performed on the putative functions of microbiomes associated with *A. hemigymnus* and *M. muelleri* specimens and different body parts.

α and β diversity of mycobial assemblages associated with different body parts of both fishes

The rarefaction curve, normalizing all samples to the same number of sequences, reached a plateau in all mycobiomes associated with body parts of both fishes, demonstrating that the fungal diversity of samples was completely covered (**Fig. 28**). Total ASV richness was calculated for each sample and validated with statistical analysis.

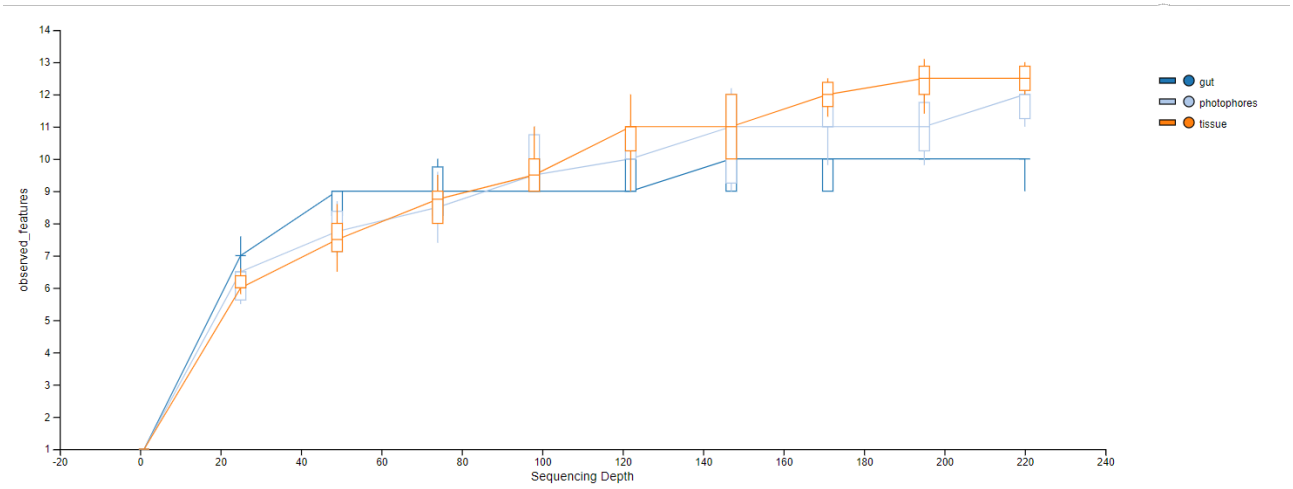


Fig. 28: Rarefaction curve of samples after a normalization of 220 sequences for the different body parts of *M. muelleri* and *A. hemigymnus*

ASVs richness in *M. muelleri* varied from 1 to 4 in the photophores and gut; and from 2 to 5 in the tissue samples. The ASVs richness in *A. hemigymnus* varied from 4 to 11 in the photophores, from 2 to 7 in the gut and from 1 to 6 in the tissue samples (**Fig. 29**). The lowest values of ASV richness were found in *M. muelleri* (1MM P-G, 4MMG, 5MMT), while the highest value was found

in photophore sample of *A. hemigymnus* (2AH). Significant differences were found in the number of ASVs between the two different species of fishes, nevertheless no significant differences were found considering the different body parts of the specimens (**Tab. 4SM**).

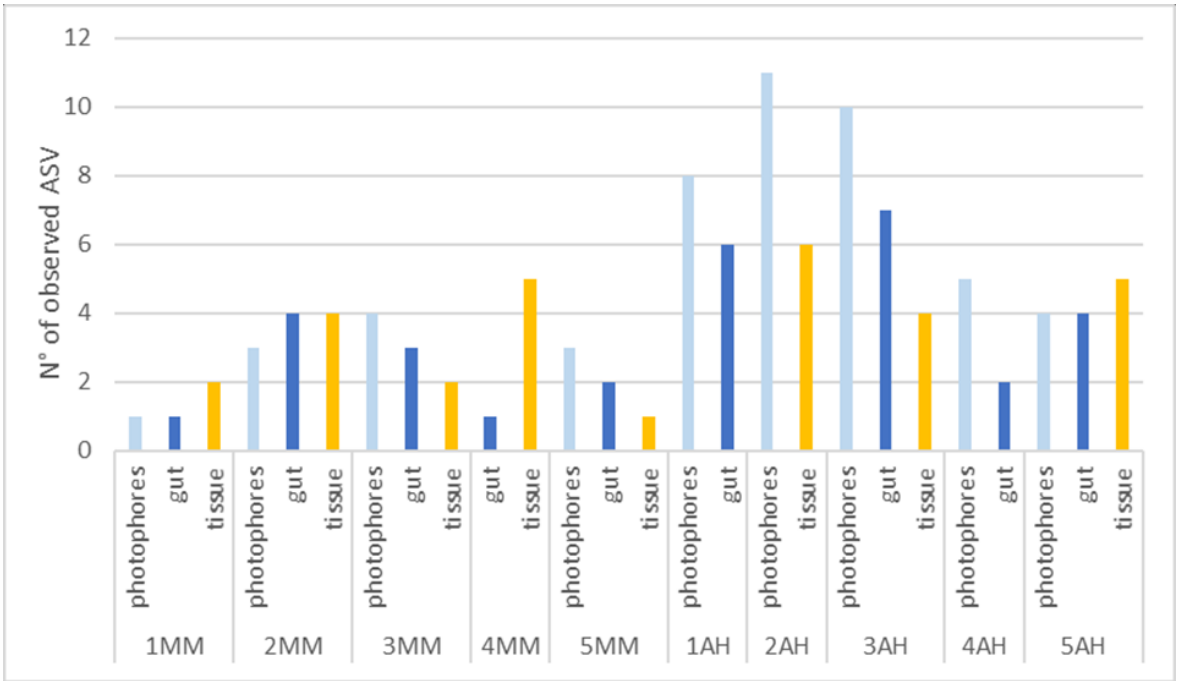


Fig. 29: Number of ASV observed in different part of *M. muelleri* and *A. hemigymnus* specimen

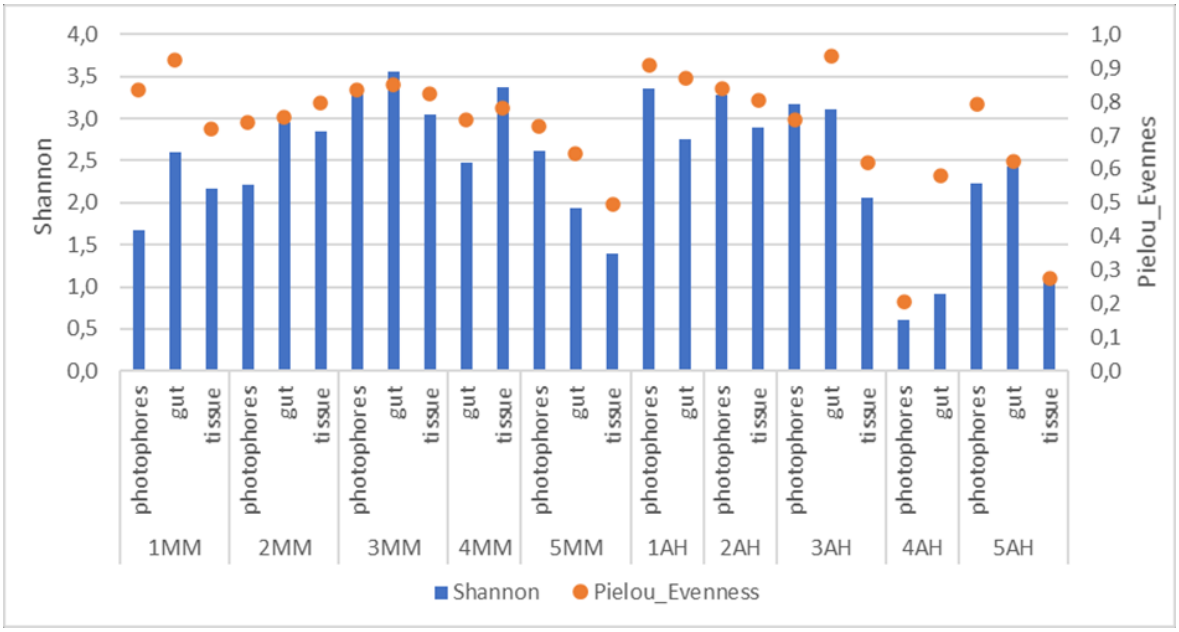


Fig. 30: Shannon and Evenness indices of mycobial community associated with the different body parts of *M. muelleri* and *A. hemigymnus* specimens.

High variability was found in the Shannon index among all samples, with significant differences considering the two different species (**Fig. 30**; **Fig. 4 SM**). Nevertheless, no significant differences were found in samples considering the Evenness index values.

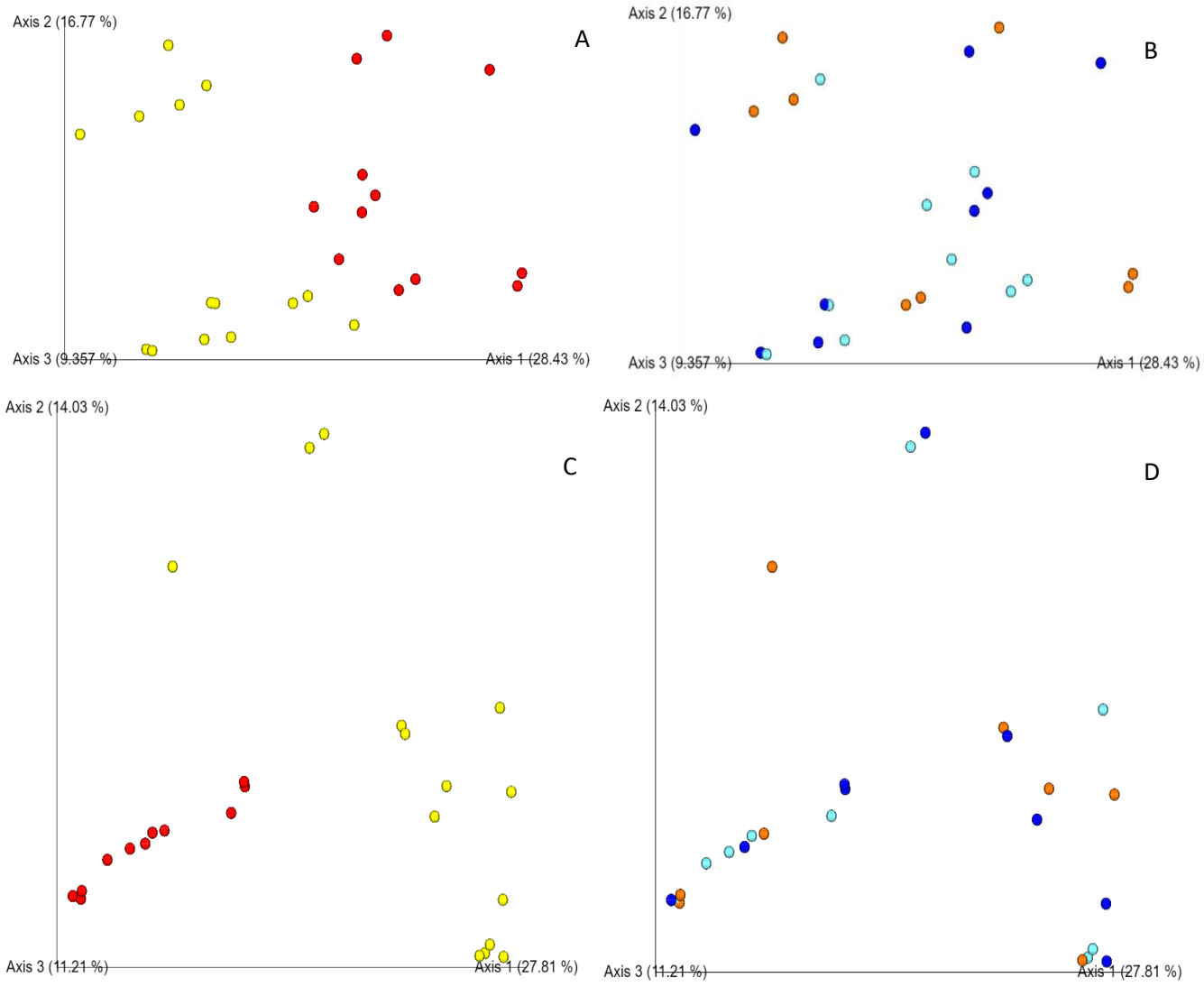


Fig. 31: PCoA plot of Beta-diversity of mycobiome carried out on unweighted UniFrac distance (A-B) and Bray-curtis dissimilarity (C-D) between the two fish species (A-C) and among the different body part (B-D). (Red: *M. muelleri*; Yellow: *A. hemigymnus*; Blue: Gut; Light blue: Photophores; Orange: Tissue).

The Beta diversity analysis revealed significant differences in terms of both composition and phylogenetic diversity of the mycobial communities associated with the two different fish species (**Fig.31**, (**A-C**)). No significant differences were found among mycobiomes of the different body parts (**Fig. 31**, (**B-D**)).

Mycobiome diversity associated with body parts of fish species

The results of the analysis of the taxonomic composition of the microbiome revealed a total of 26 genera, 28 families, 23 orders, 13 classes grouped in a total of 4 phyla (*Ascomycota*, *Basidiomycota*, *Rozellomycota* and *Chrytridiomycota*; **Fig. 32**). Significant taxonomic differences were found among samples belonging to different species, highlighting a dissimilarity of 43% (**Tab. 4SM; Tab. 5SM**). In fact, the higher diversity of fungal phyla was found in different body parts of three specimens of *A. hemigymnus* (1AH P, G; 2AH P, T; 3AH P, G; **Fig. 32**). Nevertheless, in the other samples a great percentage of sequences (from 79,5% in 2MMG to 100% in 1MMP, G; 4MMG; 5MMT) was attributed to fungi that haven't yet been described and/or identified (**Fig. 32**; **Fig 33A**; **Fig. 34A**). To better investigate the fungal diversity and its distribution in the samples, the unidentified fungi were excluded, uncovering the identified fungal taxa (**Fig. 33B**; **Fig. 34B**).

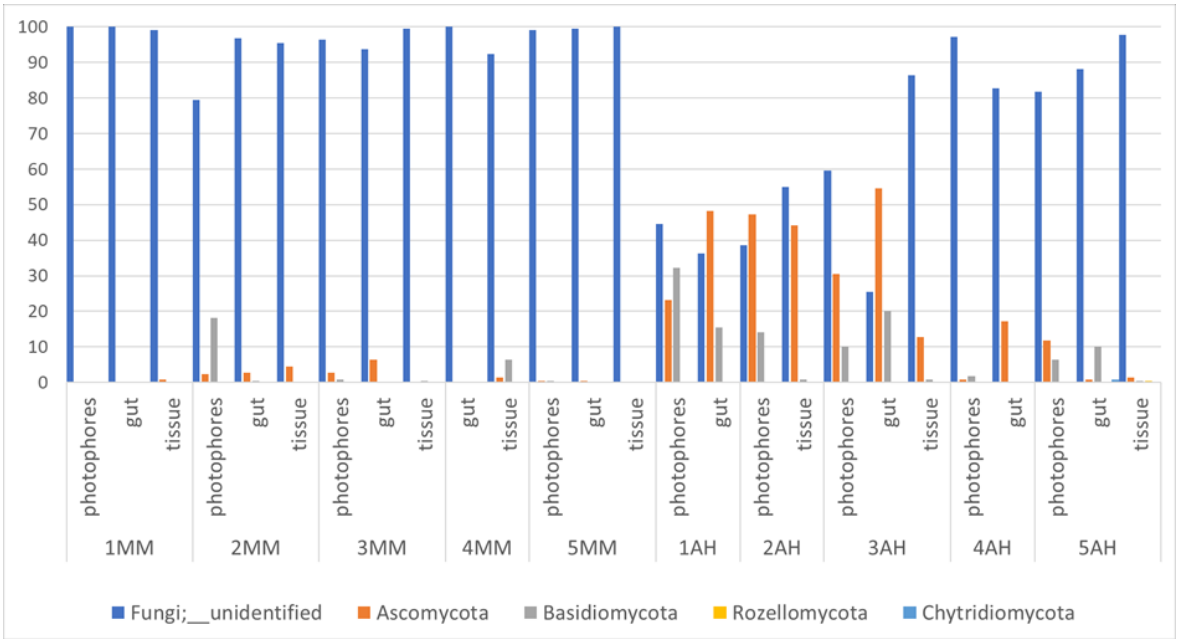
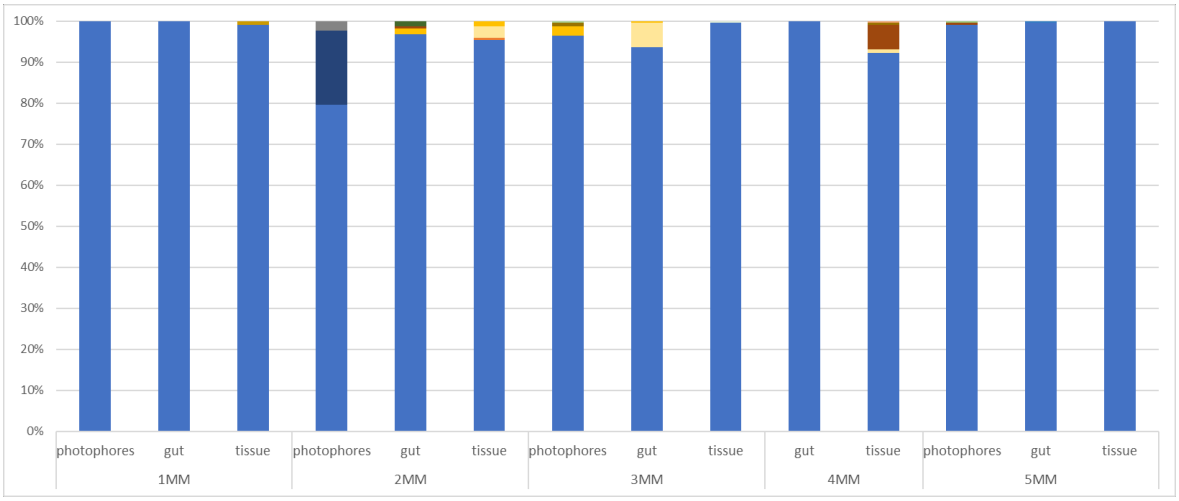


Fig. 32: Abundance of the different phyla of fungi found in association with specimens of *M. muelleri* and *A. hemigymnus.*

Unidentified fungal sequences described the *M. muelleri* diversity exclusively in some samples (1MMP, 1MMG, 4MMG, 5MMT) and covering more than 80% in the others (**Fig. 33A**).

A



B

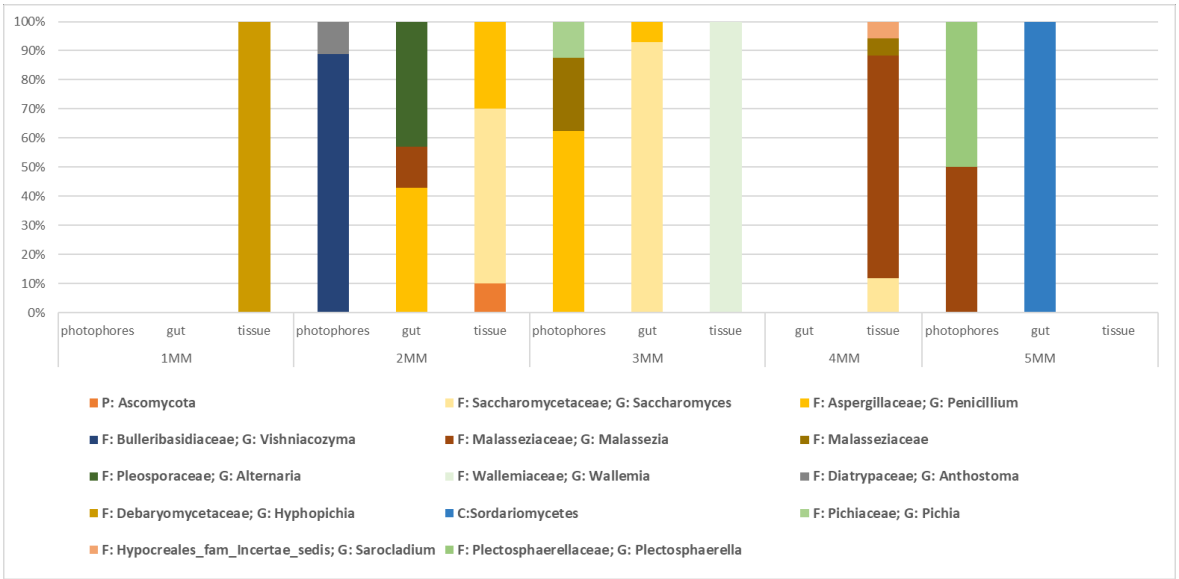
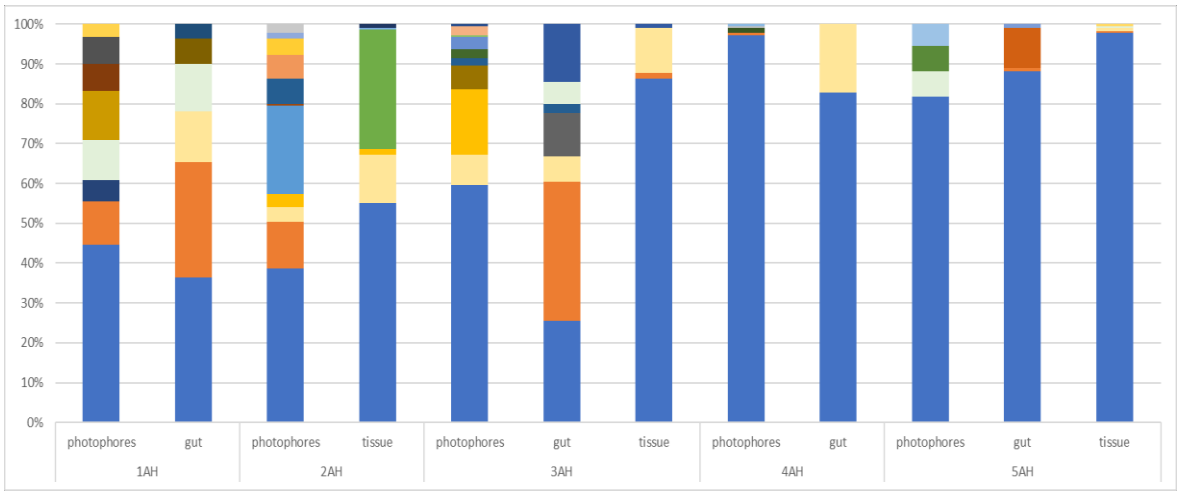


Fig. 33: Taxonomic composition of mycobial families found in *Maurolicus muelleri* specimens, including (**A**) and excluding (**B**) the percentages of unidentified fungal taxa.

Excluding the unidentified fungi, some samples showed the exclusive presence of *Debaryomycetaceae* (*Hyphopichia* genus*)* (1MMT) and the *Wallamiaceae (Wallemia* genus*)* (3MMT) families and the *Sordariomycetes* order (5MMG). All the remaining samples highlighted associations with 2 or 3 different fungal families as the *Aspergillaceae* (*Penicillum*), the *Saccharomycetaceae* (*Saccharomyces*) and the *Malasseziaceae* (*Malassezia*) (**Fig. 33B**). Simper analysis revealed that among specimens of *M. muelleri*, samples belong to the same body part showed a high similarity (77,5%, 88,3%, 83,9% of photophores, gut and tissue samples, respectively), mainly driven by the great presence of unidentified fungi (**Fig.34; Tab. 5SM).**

A



B

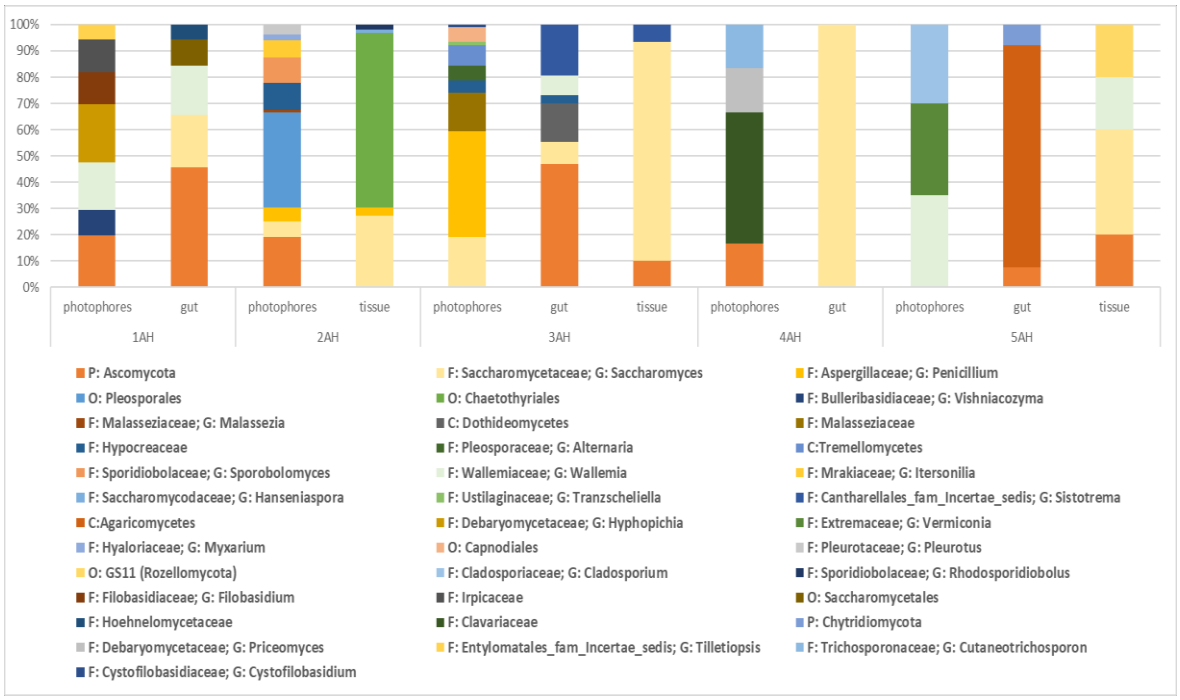


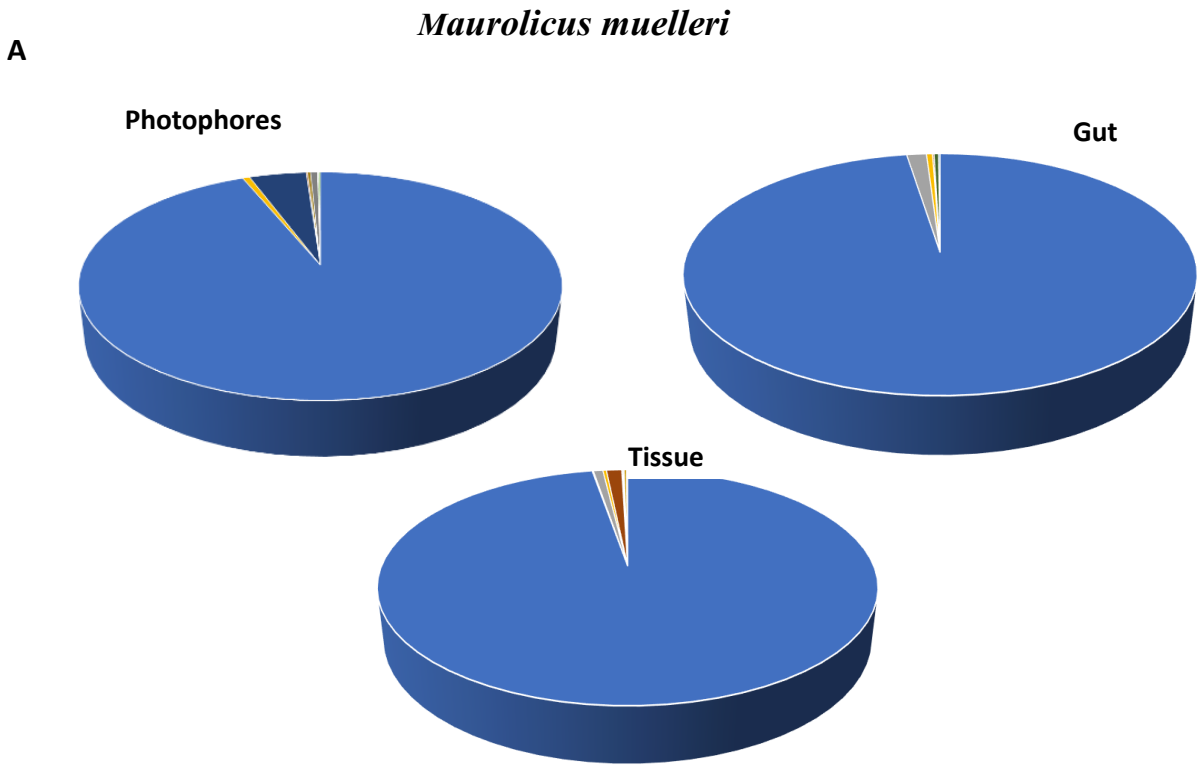
Fig. 34: Taxonomic composition of mycobial families found in *Argyropelecus hemigymnus* specimens, including (**A**) and excluding (**B**) the percentages of unidentified fungal taxa.

A. hemigymnos samples showed a higher diversity than *M. muelleri* samples. Sequences of unidentified fungi covered from the 25% to the 97% of the total taxonomic composition (**Fig. 34A**). Excluding the unidentified fungi, diversified fungal associations were uncovered in the samples. In fact, except

for the sample 4AHG, exclusively represented by the *Saccharomycetaceae* family (*Saccharomyces* genus), from 3 to 10 fungal families were present in each sample. The highest diversities of taxonomic composition were found in the specimens 1AH, 2AH, 3AH, where the most abundant fungal families were the *Saccharomycetaceae (Saccharomyces)*, the *Wallemiaceae (Wallemia)*, the *Aspergillaceae* (*Penicillum*) and other fungal taxa of the phylum *Ascomycota*.

Simper analysis revealed that among specimens of *A. hemigymnus*, photophores are those that showed the higher diversity (only 38,6%of similarity), followed by gut (48,7% of similarity) and then tissues samples (64,9% of similarity; **Fig.35**, **Table 5SM**).

No significant differences were found among all samples belong to the same body parts (**Tab. 4SM**).



B *Argyropelecus hemigymnus*

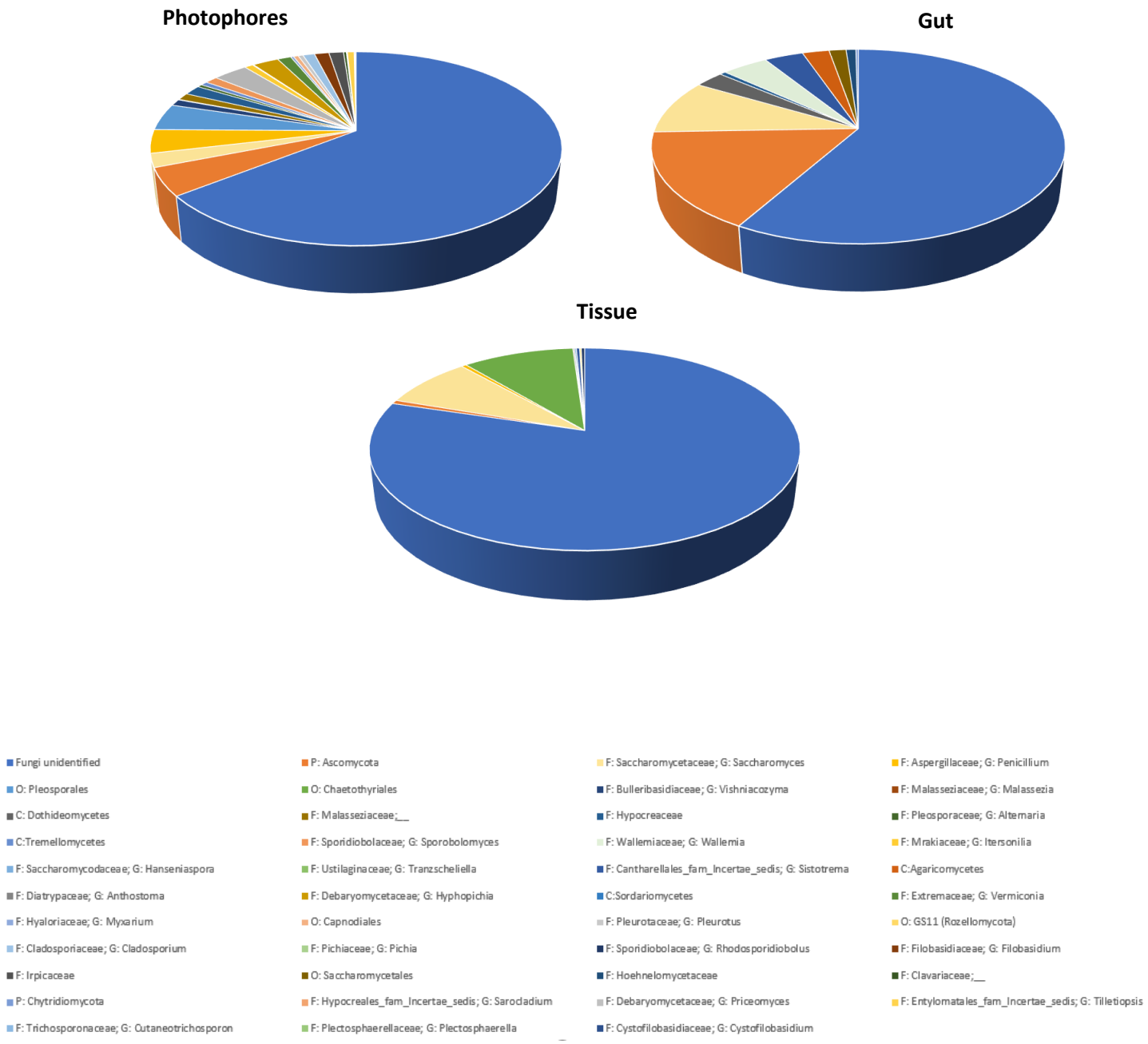


Fig. 35: Abundance of the different phyla of fungi found in association with specimens of *M. muelleri* (**A**) and *A. hemigymnus* (**B**).

The cluster analysis showed that there is not a clear division between samples belong to the same species o samples belong to the same body parts. In fact, the analysis divided samples in two main groups represented by the 4 highest taxonomic diversified samples (2AHP, 1AHG, 1AHP, 3AHG) and all the others (**Fig. 36**).

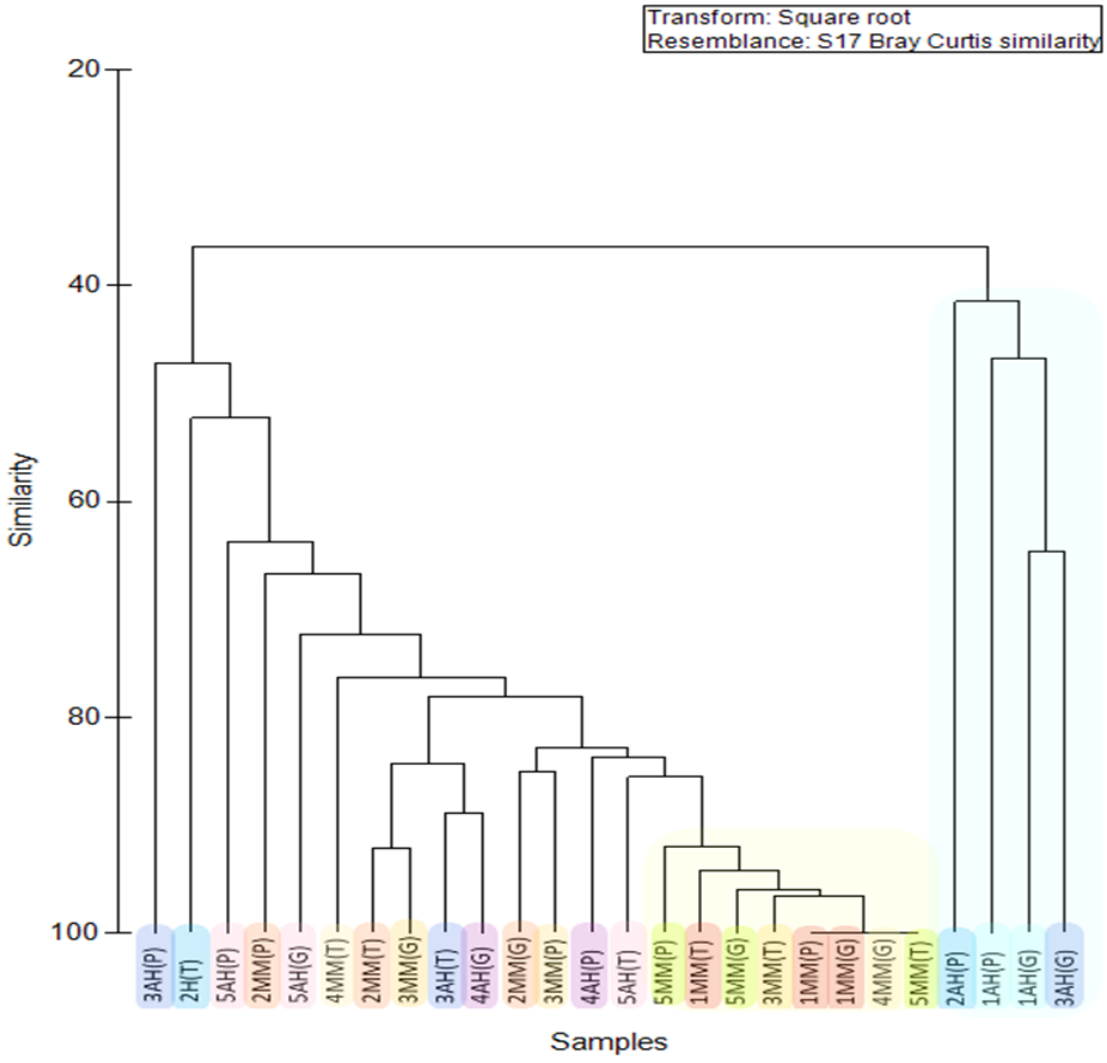


Fig. 36: Cluster analysis carried out on the taxonomic composition of mycobiomes associated with specimens of *A. hemigymnus* and *M. muelleri.* Samples representing mycobiomes collected in the different body parts of the same individual were highlighted with the same colour.

4.3.2 Microbiological analysis

Cultivation of bacterial species from photophores and tissue of A. hemigymnus

Among plates with the specific media for bioluminescent bacteria in which the photophores were plated, only 6 plates showed bacterial colonies, and among these only 12 bacterial species were isolated and sequenced (**Fig. 37 (A-B-C-D)**; **Tab. 2**). Among plates with the generic media for bacteria in which tissues were plated, only 3 plates showed bacterial colonies, and among these only 1 bacterial species was isolated and sequenced (**Fig. 37 (E-F)**; **Tab. 3**). Among all the DNA sequences obtained, samples 10AH(P)2 and 6AH(T)2 showed a low quality also in the second essay of isolation, therefore were excluded (**Tab. 2**; **Tab.3**).

The identity of the good quality sequences obtained was investigated using BLAST on NCBI database (**Tab. 4**). The bacterial species obtained from the tissue was *Nocardioides salarius*, while those obtained from photophores were: *Oceanicaulis alexandrii, Alcanivorax sp, Alteromonas australica, Dokdonia donghaensis, Paracoccus homiensis, Sulfitobacter pontiacus, Cellulophaga sp, Alteromonas sp. Sulfidobacter pontiacus* and *Oceanicaulis alexandrii* were found in three and two samples, respectively.

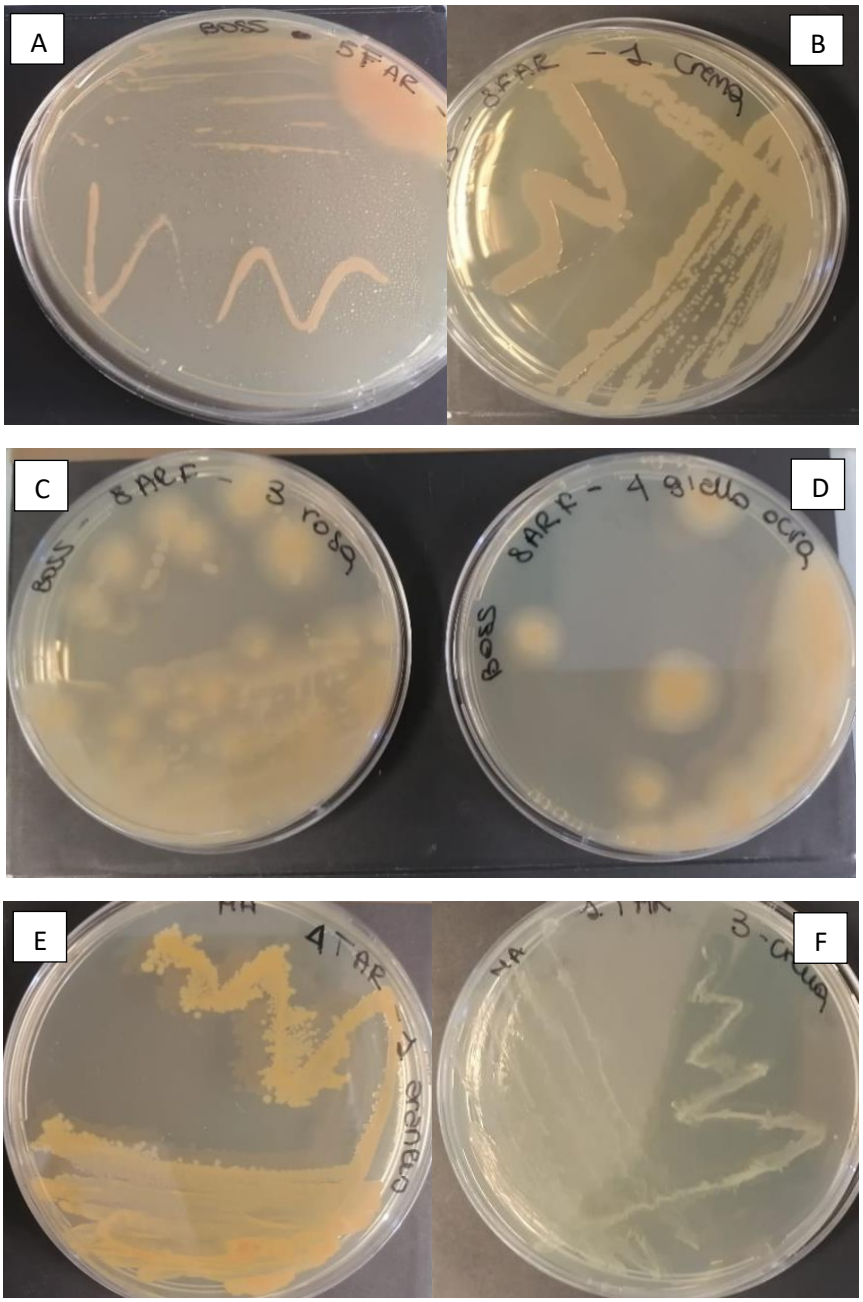


Fig. 37: Images of bacterial isolates from photophores (**A-B-C-D**) and tissue (**E-F**) of *A. hemigymnus*

Tab. 2: List of *A. hemigymnus* photophore samples plated on specific media for bioluminescent bacteria, isolated and sequenced.

Body part body part plated	Growth on culture medium (Y/N)	Number of colonies grown	Sample ID	Sequencing success (Y/N)
6AH(Photophores)	Y	2	6AH(P)1	Y
			6AH(P)2	N
7AH(Photophores)	Y	2	7AH(P)1	N
			7AH(P)2	Y
8AH(Photophores)	N			
9AH(Photophores)	Y	1	9AH(P)1	Y
10AH(Photophores)	Y	2	10AH(P)1	Y
			10AH(P)2	N
11AH(Photophores)	Y	1	11AH(P)1	Y
12AH(Photophores)	N			
13AH(Photophores)	Y	7	13AH(P)1	Y
			13AH(P)2	Y
			13AH(P)3	Y
			13AH(P)4	Y
			13AH(P)5	Y
			13AH(P)6	Y
			13AH(P)7	Y
14AH(Photophores)	N			
15AH(Photophores)	N			

Tab. 3: List of *A. hemigymnus* tissue samples plated on fungal media, isolated and sequenced

Body part plated	Growth on culture medium (Y/N)	Number of colonies grown	Sample ID	Sequencing success (Y/N)
6AH(Tissue)	Y	2	6AH(T)1	Y
			6AH(T)2	N
7AH(Tissue)	Y	1	7AH(T)1	N
8AH(Tissue)	N			
9AH(Tissue)	Y	1	9AH(T)	N
10AH(Tissue)	N			
11AH(Tissue)	N			
12AH(Tissue)	N			
13AH(Tissue)	N			
14AH(Tissue)	N			
15AH(Tissue)	N			

Tab.4: Identification of bacterial strains found from GenBank (BLAST-NCBI)

Sample ID	HQ%	Consensus sequence length	Taxonomy NCBI	Query cover (%)	Percent identity (%)
6AH(T)1	90,60%	417	*Nocardioides salarius*	100	100
6AH(P)1	92,20	397	*Oceanicaulis alexandrii*	100	99,75
7AH(P)2	86,80	174	*Alcanivorax sp*	100	98,85
9AH(P)1	94,40	432	*Alteromonas australica*	100	100
10AH(P)1	93,80	417	*Oceanicaulis alexandrii*	100	99,76
11AH(P)1	78,50	433	*Dokdonia donghaensis*	99	100
13AH(P)1	91,40	417	*Paracoccus homiensis*	100	99,76
13AH(P)2	96,30	408	*Sulfitobacter pontiacus*	100	100
13AH(P)3	93,10	423	*Cellulophaga sp*	100	100
13AH(P)4	95,10	365	*Sulfitobacter pontiacus*	100	100
13AH(P)5	72,60	321	*Sulfitobacter sp*	100	99,96
13AH(P)6	82,10	273	*Alteromonas sp*	100	100
13AH(P)7	97,10	411	*Sulfitobacter pontiacus*	100	100

Cultivation of fungal species from photophores and tissue of *A. hemigymnus*

Among all plates with the fungal medium where photophores and tissues were plated, only 1 and 3 showed fungal growths, respectively; 4 mycobial species from these were isolated and sequenced (**Fig. 38 (A-B-C-D)**; **Tab. 5**; **Tab. 6**). The identity of the good quality sequences obtained was investigated using BLAST on NCBI database (**Tab.7**).

The fungal species obtained from the tissue of fish were: *Cladosporium sp, Cryptococcus sp, Microstroma sp;* the unique species obtained from photophores was *Arthinium sp.*

Sample 13AH(P)7 showed the presence of two different colonies on the plate: the two colonies were isolated, sequenced and identified as *Emericellopsis maritima* together with *Sulfidobacter*.

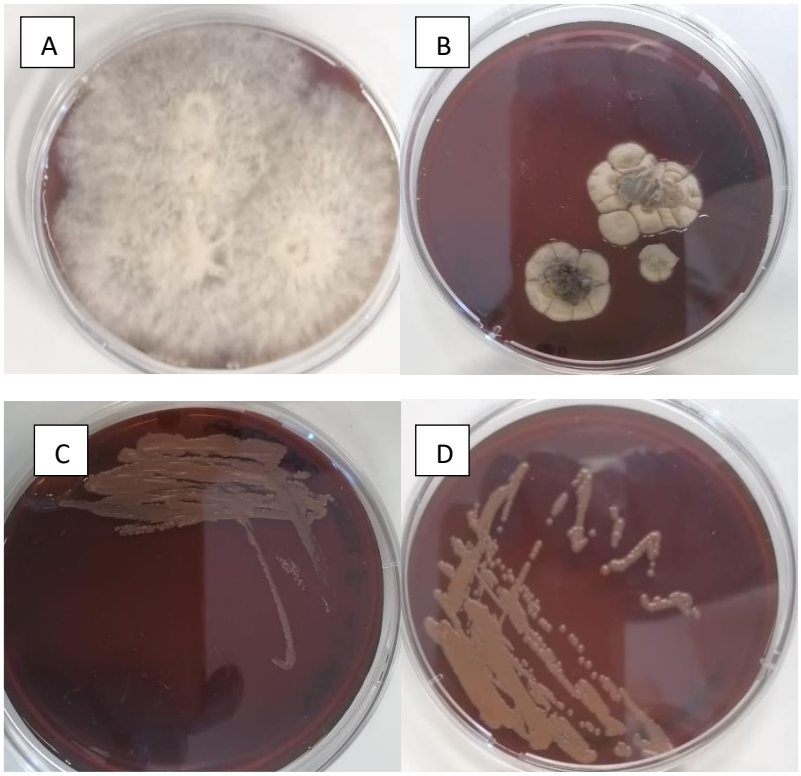


Fig. 38: Images of fungal isolation from photophores (A) and tissue (B-C-D) of *A. hemigymnus*

Tab. 5: List of *A. hemigymnus* photophore samples plated on fungal media, isolated and sequenced.

Mycobial cultivation from Photophores	Growth on culture medium (Y/N)	Number of colonies grown	Sample ID	Sequencing success (Y/N)
6AH(Photophores)F	N			
7AH(Photophores)F	N			
8AH(Photophores)F	N			
9AH(Photophores)F	N			
10AH(Photophores)F	N			
11AH(Photophores)F	N			
12AH(Photophores)F	N			
13AH(Photophores)F	Y	2	13AH(P)F1	N
			13AH(P)F2	Y
14AH(Photophores)F	N			
15AH(Photophores)F	N			

Tab.6: List of *A. hemigymnus* tissue samples plated on fungal media, isolated and sequenced.

Mycobial cultivation from Tissue	Growth on culture medium (Y/N)	Number of colonies grown	Sample ID	Sequencing success (Y/N)
6AH(Tissue)F	Y	1	6AH(T)F	Y
7AH(Tissue)F	Y	1	7AH(T)F	Y
8AH(Tissue)F	N			
9AH(Tissue)F	N			
10AH(Tissue)F	N			
11AH(Tissue)F	N			
12AH(Tissue)F	N			
13AH(Tissue)F	N			
14AH(Tissue)F	Y	1	14AH(T)F	Y
15AH(Tissue)F	N			

Tab. 7: Identification of fungi found from GenBank using BLAST tools

Sample ID	HQ%	Consensus sequence length	Taxonomy NCBI	Query cover	% Ident
6AH(T)F	94,40%	533	*Cladosporium sp*	100%	99,81%
7AH(T)F	87%	491	*Cryptococcus sp*	100%	100%
14AH(T)F	81,90%	238	*Microstroma sp*	100%	100%
13AH(P)F2	91%	645	*Arthinium sp*	100%	91,32%
13AH(P)7	93%	544	*Emericellopsis maritima*	100%	100%

5. DISCUSSION

Inter-specific, intra-specific and intra-individual biodiversity of microbiomes associated with mesopelagic fish *A. hemigymnus* and *M. muelleri*.

The deep sea, defined as all the environments beyond the continental shelf depth (> 200 m), represents the largest biome in the world, reaching more than 65% of the Earth's surface and including more than 95% of the global biosphere (Herring, 2001). Despite this, it remains still out of reach for many investigations (Rex, et al. 2010; Danovaro, et al. 2014; Corinaldesi, et al. 2015; Danovaro, et al. 2020). Deep-sea habitats are characterized by the lack of light, low temperatures, high pressures, low nutrient levels; and it has concealed for long time novel life forms both at macroscopic and microscopic scale. It has been estimated that the deep sea harbours one of the largest pools of microbes in aquatic systems, accounting for almost 75% of the oceanic prokaryotic biomass (Jorgensen and Boetius, 2007; Aristegui, et al. 2009; Collins, et al. 2021) and its diversity and potential are vastly underexplored compared to surface water and terrestrial environments (DeLong et al. 2006; Vitorino, et al. 2018). Several studies have investigated the composition of the intestinal microbiome in marine fish, but these have primarily focused on commercial interest fish at relatively shallow depths (Star, et al. 2013; Dehler et al. 2017). The microbiome of fish undoubtedly plays a vital role in its health and

development, symbiotically contributing through microbial digestion releasing metabolites that can be utilized by the host (Egerton, et al. 2018). Nevertheless, the complexity and spatial organization of the deep-sea microbiomes, especially those living associated with mesopelagic fish, is little investigation for now, and many questions remain still opened.

This study represents the first investigation on the microbial assemblages associated with *M. muelleri* and *A. hemigymnus* (mesopelagic fish), even comparing specific microbiomes of their different body parts (photophores, gut, tissue). Differences and similarities of microbiomes were assessed from the interspecific (between the two different species) to the intraspecific (among the different individuals of the same species) to the intraindividual levels (given by the comparison of microbiomes in the three body parts of every specimen). Results of our investigation highlight significant differences in terms of ASV richness between the microbiomes of the two species with the highest value found in *M. muelleri* and the lowest in *A. hemigymnus*. The investigation on the taxonomy composition of microbiomes revealed a 70% of similarity between the two fish species. This resulted by the presence of bacterial genera shared among all samples with similar abundances in both species as *Bacteroides* (*Bacteroidaceae*), *Faecalibacterium* and *Subdoligranulum* (*Ruminococcaceae*), *Alistipes* (*Rikinella*), *Clostridia UCG-014, Roseburia* and *Lachnospira* (*Lachnospiraceae*), *Parabacteroides* (*Tannerellaceae*), *Prevotella* (*Prevotellaceae)*, *Sutterella* (*Sutterellaceae*) and others.

Bacteroides genus and *Lachnospiraceae* family genomes contain many genes belonging to CAZyme families, which can break down a wide variety of indigestible (for the host) polysaccharides and play important roles in the fermentation of dietary fibers (El Kaoutari et al. 2013; Schwalm and Groisman, 2017). Besides, *Lachnospiraceae* and *Ruminococcaceae* families are obligate anaerobes well known for their associations with several herbivorous hosts (Llewellyn et al., 2014; Moran et al.,2019) including fishes (Sullam et al. 2012; Jones et al. 2018).

The only bacterial taxa that diversified the two core microbiomes are the genus SUP05 (Thioglobaceae) and the family *Enterobacteriaceae* (genus unidentified) found in *A. hemigymnus* and the *Agathobacter* genus (Lachnospiraceae) found in *M. muelleri*.

The *SUP05* clade comprises both primary producers and primary consumers of organic carbon; they are characterized by chemosynthetic and sulphur-oxidizing bacteria that dominate the deep-sea hydrothermal vents microbial communities around the world, and commonly form symbioses with invertebrate organisms (Zhou et al. 2019; Morris, 2021). Instead, members of Enterobacteriaceae family are pathogens and can be responsible for a wide spectrum of diseases (Mitchell et al. 2014).

All the individuals of *A. hemigymnus* showed a 65,7% similarity of their microbiomes, with some bacterial genera present only in some individuals. In particular, the gut and tissue samples of *A. hemigymnus* showed 55,3% and

66,5% of similarity, respectively, in contrast with the 87,9% of similarity found in the photophores samples. This is due to the presence of *SUP05* genus (*Thioglobaceae* family), which had a higher contribution in both gut and tissue of only two specimens and high abundances of the *Enterobacteriaceae* family in the gut of other two individuals of *A. hemigymnus*.

The genus *Mycoplasma* was found with high abundances only in the gut of an individual in both species. Studies on microbiomes associated with salmons show that *Mycoplasma* genus characterize gut of healthy individuals and that there is a positive correlation between its relative abundance and the fish weight, potentially indicating a beneficial effect for its host (Bozzi et al. 2021). All the individuals of *M. muelleri* showed an 85% similarity in their microbiomes, explained by the high similarity among samples of each body part (photophores: 86,7%; gut: 80,7%; tissue: 88,8%) and low dissimilarity between the photophores vs gut (17%), photophores vs tissue (10%) and gut vs tissue (17%). Comparing the taxonomic composition of microbiomes of the different anatomic parts of individuals of a same species, significant differences were found between photophores vs gut and gut vs tissue in *M. muelleri*, due to the presence of *Vibrio*, *Bacillus* and *Mycoplasma* genera only in the gut of two specimens. Some species of *Bacillus* control the growth of opportunistic pathogens and produce anti-viral compounds (Arena et al. 2006; Xu, H. et al. 2014), for this reason they are used like probiotics in aquaculture (Tarnecki et al. 2019). The microbiomes of the body parts showed a higher value of

dissimilarity between photophore vs gut and photophore vs tissue in *A. hemigymnus*. This is undoubtedly due to the presence of bacterial genera such as *SUP05* (*Thioglobaceae*) and *Enterobacteriaceae* family in the gut and tissue of *A. hemigymnus* specimens, described above. Within the individual, the similarity across microbiomes of photophores, gut and tissue changed depending on everyone (from 80% to 89% for *M. muelleri* and from 55% to 88% for *A. hemigymnus*).

Therefore, the results indicate a microbial assemblage structure that does not describe each anatomical part specifically nor each species of fish. Differences observed among microbiomes of individuals of *A. hemigymnus* could be also due to the different stability or temporal variability of bacterial assemblage inhabiting organisms. In fact, pathogens, environmental stressors and immune dysfunctions can lead to increased stochasticity in the microbiome (Star et al. 2013, Hong, et al. 2015, Flórez et al. 2015). Presence of *Streptooccaceae* and *Pasteurellaceae* families (even if under the 1%) in some fish specimens, increasing the intraspecific variability, could indicate altered conditions in the healthy status of species, as bacteria belong to these families are usually considered potential pathogens of human and animals (Rosemberg, et al. 2014). In addition, multiple evidence has reported the importance of rare or transient bacterial taxa, that could have an influence disproportionate to their abundance in the microbiome (Shade, et al. 2014: Shi et al. 2016). This could be the case of all the bacterial species found with an abundance less than 1% and

representing the 88% of the total number of genera found, both in *M. muelleri* and *A. hemigymnus*. In particular, the rare bacterial species exclusively found in specific body parts, such as *Synechococcus sp, Cyanobium sp* and *Blastopirellula sp* in gut of *M. muelleri* and *Blautia sp, Pseudomonas sp* and *Polaromonas sp* in photophore of *A. hemigymnus*.

Synechococcus is an important member of the photosynthetic picoplankton and has an efficient mechanism to adapt to salinity and light intensity variations (Kim, et al. 2018). Together with Cyanobium, it is widely distributed in ocean ecosystems as one of the most common picoplanktonic marine Cyanobacteria genera (Palenik, et al. 2003; Callieri 2017). Le and Wang (2021) suggest that the presence of these bacteria in the gut microbiome can be due to the ingestion by the fish during feeding (Le and Wang 2021). Other studies highlight, instead, that cyanobacteria are the most commonly observed symbionts of marine sponges, besides heterotrophic bacteria (Regueiras, et al. 2017.; Konstantinou, et al. 2018), and in some cases, they are the most conspicuous component (Pfannkuchen, et a. 2010). Their role in association with sponges is wide: they can provide up to 80% of the host's energy requirements through photosynthesis, they also assist in the removal of nitrogenous wastes (Pagliara, et al. 2020) and they provide biochemical defense throughout the production of bioactive metabolites (Thacker and Freeman 2012). The ability of cyanobacteria to produce molecules with antibacterial, antiviral and antifungal properties, as well as to exert anti-proliferative or toxic activities on cancer cells

results of particular interest to date (Regueiras, et al. 2018; Demay, et al. 2019; Huang and Zimba 2019). Over 400 biomolecules produced by marine cyanobacterial strains have recently been reported (Mi, et al. 2017).

Members of *Polaromonas* genus (*Comamonadaceae*) are Gram-reaction-negative, aerobic and non-motile, and have been isolated from various environmental sources, such as marine water (Irgens, et al.1996), tap water (Kämpfer, et al. 2006), sediment (Jeon,et al. 2004; Choi, et al. 2018) and soil (Weon, et al. 2008). *Polaromonas* species have been found in contaminated environments, where they may take part in degradation of hydrocarbons and xenobiotics and in pristine environments (Jeon,et al. 2004; Yagi, et al. 2009). They are among the dominant bacteria of glacial ice and glacial sediment worldwide, including polar and high-elevation environments (Darcy, et al. 2011, Gawor, et al. 2016).

Finally, the results of microbiological cultivation of bacteria associated with different parts of the body of *A. hemigymnus* showed bacterial genera that were not detected by molecular analysis on taxonomic composition, as *Sulfitobacter sp.* (in particular, *Sulfitobacter pontiacus*), *Alteromonas* sp. (in particular, *Alteromonas australica*), *Oceanicaulis alexandrii*, *Alcanivorax sp*, *Dokdonia donghaensis*, *Paracoccus homiensis* and *Cellulophaga sp* associated with photophores and *Nocardioides* salaries associated with tissues, through BOSS (medium for bioluminescent bacteria) and MA (generic marine agar medium) media, respectively. All these bacteria were found associated with different

marine organisms and are involved in important metabolic functions, useful in a potential host-association. In particular, *Sulfitobacter* is obligately chemoorganotrophic but can use energy from the oxidation of thiosulfate and sulfite (Sorokin, et al. 2015). *Sulfitobacter pontiacus* is well known to produce a soluble AMP-independent sulphite oxidase of high activity. This enzyme can be of great help in creating biosensor systems for detecting sulphite in food and beverages (Muffler, et al. 2008); together with *Alteromonas* genera, it has described as an important component of the microbiome of the defence tissue (*Acontia*: threadlike tissues composed of nematocyst-containing cnidocytes) in the anemone *Exaiptasia diaphana* and other marine invertebrates (Maire et al. 2021). *Oceanicaulis alexandrii* and *Cellulophaga* have been isolated from associations with the marine dinoflagellate *Alexandrium tamarense* and the Antarctic sponge *Iophon* sp. (class *Demospongiae*), respectively (Strompl et al. 2003; Moreno, et al. 2021). Indeed, *Alcanivorax sp* is considered alkane-degrading bacteria, showing a role in the first steps of crude oil biodegradation in marine environments (Golvshin et al. 2003; Cappello et al. 2007); *Paracoccus homiensis*, instead, has shown an algicidal activity against harmful algal blooms (Ding et al. 2021); *Dokdonia donghaensis* is a strictly aerobic, gram-negative, phototrophic bacterium that thrives in marine environments, able to form biofilms, as a survival strategy that allows the organism to grow while being protected from environmental stresses and increasing their

resistance to antimicrobial agents, desiccation, and grazing (Jefferson, 2004; DeLong et al. 2010).

Summarizing, microbiomes associated with two mesopelagic fish investigated in this study show a high diversity of bacterial taxa, most of which potentially involved in important functions also for the host metabolism. No specific microbiomes were found in the different part of the body or describing specifically each fish species, but a high individual variability was detected. Despite this, no evidence in literature were found on a potential role of bacterial species found in fish photophores in the bioluminescence phenomenon.

Inter-specific, intra-specific and intra-individual biodiversity of mycobiomes associated with mesopelagic fish *A. hemigymnus* and *M. muelleri*.

Marine fungi form morphologically, phylogenetically, and ecologically diverse groups (Shearer, et al. 2007). Several studies highlighted that fungi are present from coastal waters to the deep-sea environments across a variety of ecosystem types spanning from hypersaline anoxic basins (Edgcomb et al., 2016; Raghukumar 2017; Barone, et al. 2019) to hydrothermal vents (Xu et al., 2017; Xu et al., 2018), from cold seeps (Thaler et al., 2012; Wang, et al. 2014; Nagano, et al. 2017) to surface and subsurface sediments (Barone, et al. 2018; Vargas-Gastélum, et al 2020; Yang, et al. 2020). Polar environments are also

characterized by a considerably high number of fungal taxa (Zhang, et al. 2015; Ogaki, et al. 2020), which can be involved in organic matter degradation and nutrient cycling (Amend, et al. 2019; Gladfelter, et al. 2019). Fungi have been isolated from marine animals, with which they can interact as pathogens, parasites, or symbionts (Alker, et al. 2001; Raghukumar et al. 2008; Nguyen, et al. 2018). Although culture-based studies allowed isolating and investigating a variety of fungal taxa (in sponges: Bovio, et al. 2018, crustaceans: Cui, et al. 2016, molluscs and echinoderms: Godinho et al. 2019), molecular tools have allowed the identification of a larger fraction of the fungal diversity provided (Nilsson, et al. 2019; Vargas-Gastélum, et al. 2020; Varrella, et al. 2021). Nevertheless, the nature and the strength of the relationships between the host and the associated fungi have yet to be fully understood. Moreover, previous investigations have revealed an important role of the gut mycobiome as a source of novel yeasts with potential industrial applications, as probiotics (Gatesoupe, 2007). Furthermore, recent studies indicate that fungi are effective in metabolising hydrocarbons and can be used in the bioremediation of contaminated sites following oil or petroleum spills, polycyclic aromatic hydrocarbons and metals (Bovio et al. 2017; Dell'Anno, et al. 2020). Even without active management, fungi rapidly dominated sediments in the Gulf of Mexico after the Deepwater Horizon oil spill (Bik, et al. 2012; Mahmoudi, et al. 2013). The greatest effort in applied marine fungal research relates to the discovery and isolation of novel natural products, including secondary

metabolites with antibacterial, cosmeceutical and cosmetic properties (Corinaldesi, et al. 2017; Agrawal, et al. 2018).

This study represents the first investigation of mycobiomes associated with mesopelagic fish *M. muelleri* and *A. hemigymnus*. Results of our investigations showed higher diversity of mycobiomes, in terms of ASV richness, among fish individuals of *A. hemigymnus* than in *M. muelleri*. Four fungal phyla (*Ascomycota, Basidiomycota, Rozellomycota, Chytridiomycota*) were found, although most of the ASVs were classified only to the fungal kingdom (Fungi), indicating that fish mycobiomes might harbour a higher richness of novel fungal lineage. The percentage of unidentified Fungi sequences cover the total abundance of diversity in some samples. Taxonomic composition of mycobiomes revealed a 58% similarity between the two fish species, but it could be different, considering the unidentified species. Ascomycota and Basidiomycota are the most common phyla found in the marine environment (Amend, 2019), while *Chytridiomycota* and *Rozellomycota* were found in mycoplankton and associated in deep-sea hadal sediment (Grossart, et al. 2019; Yang, et al. 2021; Xu, et al. 2019). *Saccharomyceae* and *Aspergillaceae* families, particularly *Saccharomyces* and *Penicillum* genera, constitute the highest abundance of the sequences found in two individuals of *M. muelleri* and 4 individuals of *A. hemigymnus*. Several studies on human gut mycobiome highlights that *Saccharomyces* and *Malassezia* genera dominate mycobial diversity (Nash, et al. 2017). Previous studies have observed high levels of

Malassezia at different body sites, describing it as a prominent commensal of the skin and oral mycobiomes (Theelen, et al. 2018; Park, et al. 2021). Our results showed Malassezia genus only in the tissue of one specimen of *M. muelleri.* Indeed, *Saccharomyces* produces some energy substrates for intestinal cells, which contribute to gut health, for this it's the most common yeast incorporated into aquatic feeds (probiotic) to effectively reduce the need for animal protein (Jones, et al. 2020), where an improvement in growth performance and feed efficiency rate has been demonstrated in many farmed fish (carp: Abdulrahman 2016; Dawood, et al. 2016; sea bass: Dawood, et al. 2021; tilapia: Abu-Elala; 2013; sea bream: Zaineldin, et al. 2021, rainbow trout fry: Gonçalves, et al. 2017). *Penicillium* genus include several ubiquitous species with worldwide distribution, which have already been detected in many Antarctic substrates (macroalgae: Godinho et al., 2013; invertebrates: Godinho et al., 2019, sediments: Gonçalves et al., 2013, and seawater: Gonçalves, V et al., 2017), indicating its high capability to survive in extreme conditions of marine environment.

Among all the individuals of each fish species, the diversity of the mycobial assemblages of photophores, gut and tissue (body parts) show an overall similarity of 85% in *M. muelleri* individuals and 55% in *A. hemigymnus*. In the *M. muelleri* specimens the mycobiomes of the body parts exhibited a higher similarity to each other (photophores: 77%, gut: 88%, tissue: 84%), than in the *A. hemigymnus* specimens (photophores: 38%, gut: 48%, tissue: 65%). The

photophores of the *A. hemigymnus* species revealed a greater diversity of fungal genera, including the *Cladosporium*, that has been reported to have antibiotic and antifungal properties (Xiong, et al. 2009; Mohamed, et al. 2021). Moreover, a high variability at the intra specific level was found among fungal genera, with high abundances only in some individuals as *Vermiconia* (*Extremaceae*), *Agaricomycetes* class (genus unidentified), *Chaetothyriales* order (genus unidentified), *Pleosporales* order (genus unidentified) in *A. hemigymnus*. *Agaricomycetes is a* class previously detected in deep-sea environments (Singh, et al. 2012; Xu, et al. 2014) and reported as a dominant fungal class in mangrove habitats (Arfi, et al. 2012).

Finally, the results of the microbiological cultures, through generic medium for fungi (MA, Difco), show the presence of *Cladosporium*, *Cryptococcus*, *Microstoma* in the tissues and *Arthrinium* in the photophores of the *Argyropelecus hemigymnus* specimens. The most reported *Basidiomycota* pathogen of marine animals is *Cryptococcus* which contains three species that parasitize Carnivora and Cetacea (Huckabone et al., 2015). This basidiomycetous genus causes cryptococcosis, a disease infecting mainly mammalian lungs. *Cryptococcus* species have a dimorphic lifecycle that reproduces as a yeast asexually and as a hyphal form when meiotic basidia and basidiospores are produced (Zhao et al., 2019). Many *Arthrinium* species has gained a wide popularity due to their ability to produce bioactive compounds with important pharmacological and medicinal, and industrial applications

(Tsukada, et al 2011; Crous et al. 2013). More recently, isolation from various marine environments, including seawater, seaweed, and the inner tissues of marine sponges, were reported (Miao et al. 2006; Tsukamoto et al. 2006; Suryanarayanan 2012; Flewelling et al. 2015; Hong et al. 2015; Wei et al. 2016; Elissawy et al. 2017; Li et al. 2017). *Arthrinium* species isolated from sponges, egg masses of sailfin sandfish, and seaweeds showed promising bioactive properties, including high enzymatic activity, antifungal activity, and antioxidant capacity (Elissawy et al. 2017; Li et al. 2017; Park et al. 2018; Heo et al. 2018). Moreover, a new pyridone alkaloid from a deep sea *Arthrinium sp*, inhibits proliferation of Osteosarcoma cells by inducing cell cycles arrest and apoptosis (Zhang, et al. 2021).

Overall results of the present study indicate that the mycobiomes of mesopelagic fish show abundant and diversified fungal assemblages, although most of them were still unidentified in marine environments and their diversity and functions remain unknown for now.

Assessing the diversity and putative functions of microbiomes associated with different parts of a host's body (photophores, gut, epithelial tissue).

The functions and role of the microbiome community are linked to metabolic activities and to the final products that result from their activity (Nicholson et al. 2012). The fish gut microbiome contributes to digestion and can affect the

nutrition, growth, reproduction, overall population dynamics and vulnerability of the host fish to disease (Ghanbari, et al. 2015). Gut microbial diversity is species-specific and can vary to changing of physiological factors such as temperature, pH, nutrients availability and environmental conditions (Sehnal, et al. 2021). Many studies have shown that a high microbial diversity increases the resistance and resilience to stressors and promotes high functionality for the host, too (Wittebolle et al. 2009; Mendes et al. 2015). In fact, because microbiome can react more rapidly to environmental changes, it has been suggested that it may facilitate host adaptation (Song et al. 2016; Moeller, et al. 2020) Our results showed that the microbial assemblages that live associated with fish covered similar functional patterns, not diversified in the different anatomical parts: the highest percentages of bacterial ASVs found in all fish samples are potentially involved in fermentation and chemoheterotrophy suggesting an important fraction of commensalist and symbiotic bacteria. Similar functional classes were found in farmed fish, where *Gammaproteobacteria, Alphaproteobacteria, Firmicutes* and *Bacteroidetes* were the main executors of chemohetherotrophy and aerobic chemoheterotrophy (Sun, et al. 2021). In a healthy animal state, bacteria provide nutrients and energy to hosts via fermentation of indigestible food, thus maintaining a balanced metabolism and immune system (Flint, et al. 2012).

A high contribution of aerobic chemoheterotrophic functions was found in the gut of 3 specimens of fishes investigated, but it can be developed by different

bacteria group. Indeed, numerous studies showed that microbes living in the gut of marine organisms exert similar ecological functions although their assemblage is highly diversified (Gibbons, 2017).

Significant differences in the putative functions of the microbial assemblages associated with the two fish species were observed. Presence of bacteria involved in the nitrate/nitrogen respiration, in the nitrogen compounds degradation and in the dark sulfide/sulfur compounds oxidation were found mainly in *A. hemigymnus* samples (1AH, 2AH). Similar functional classes have been found in highly productive environments where bacterial assemblages control organic carbon turnover and nitrogen and sulphur cycling, such as estuaries and chemosynthetic ecosystems (Baker et al. 2015; Ding, et al. 2017). Indeed, studies have shown that the *Thioglobaceae* found within sponges (*Cladorhiza E2* specimens and *Endeavour Spinularia sp*.) are likely capable of sulfide oxidation and may be responsible for the observed depleted carbon values (Georgieva, et al. 2020). Furthermore, similar results were found by Peng and colleagues (2021), where dominant functional groups in fish included chemoheterotrophy (26.01%), fermentation (25.26%), aerobic chemoheterotrophy (20.77%), nitrate reduction (13.19%), animal parasites or symbionts (4.71%) and human pathogens (4.45%) (Peng, et al. 2021). Our results showed a lower abundance of human pathogens (0.6%). Several members of the families *Vibrionaceae*, *Enterobacteriacea*, *Mycoplasmataceae*

are considered human and animal pathogens (Hurst, et al. 2018; Diwan, et al. 2021).

Information on microbiomes that drive functional processes in mesopelagic fish is really limited; further investigation is needed to deepen our understanding of these important associations.

Unravelling possible connections between microbiome, mycobiome and bioluminescence.

Bioluminescent bacteria with a variety of bioluminescence emission have been identified in *Vibrionaceae, Shewanellaceae* and *Enterobacteriaceae* families, specifically in five main genera: *Vibrio, Photobacterium, Aliivibrio, Photorhabdus* and *Shewanella* (Urbanczyk, et al. 2008; Dunlap, et al. 2014) and other species, where DNA and protein sequences of most genes of the *lux* operon are available (**Tab 6SM**, Brodl, et al. 2018). It has now been demonstrated repeatedly that many micro and macro-organisms can be transformed into a luminous state through genetic engineering by the use of genes responsible for light emission. For instance, many non-luminous bacterial and fungal species are known to produce luminescence upon the cloning of *lux* and *luc* genes (Ramesh 2021). But there are, however, exceptional cases in the natural environment where, as an example, a freshwater

bacterium known as *Vibrio cholerae biovar albensis* produces luminescence due to two nucleotide deletions in the *luxO* gene (Kasai 2007). Conversely, many non-luminous bacterial members of the genus *Vibrio* and *Photobacterium* are known to become luminous by acquiring *lux* genes through the phenomenon of horizontal gene transfer (HGT) phenomenon (Dunlap 2014) or by losing or modifying certain specific genes (Ramesh and Mohanraju 2017). To date, these processes have only been identified in *Vibrio* and *Photobacterium* species; thus, it remains an open question as to whether other groups of bacteria could turn into emitting species by *lux* gene acquisition.

Our results show the presence of the genus *Vibrio* in most samples, but with high abundance only in the gut of an individual of *M. mulleri*. Recently, Brodl et al. (2018) reported the formation of a biofilm *in vitro* by the strains of bioluminescent bacteria isolated from the intestines of marine fish. These bacteria exhibit co-aggregation and tend to attach to solid surfaces. Moreover, Burtseva et al. (2020) attributed bioluminescence to the *Enterobacteriaceae member, Kosakonia cowanii* in finfishes from White Sea, although it was not been previously described as a luminescent bacterium.

In addition, terrestrial bioluminescent fungi are widely known. All bioluminescent fungi described to date, except for some phytopathogens (e.g., *Armillaria mellea* and *Mycena citricolor*) are saprotrophic (Desjardin, et al. 2008; Mahish, et al. 2021). They can be found in tropical and temperate areas,

where the high humidity and warm climate favors their reproduction, growth, and survival (Oliveira, et al. 2013). The distribution of luminous tissues among fungal species is not uniform, in some species both mycelium and the whole basidiome are luminescent, while in other species only certain parts emit light (Waldenmaier, et al. 2012). The structure of fungal luciferin and its precursor were identified in 2015 (Purtov 2015) as 3-hydroxyhispidin and hispidin, respectively, in four bioluminescent fungi: *Neonothopanus nambi*, *Mycena citricolor*, *Panellus stipticus*, and *Armillaria borealis*. In 2017, the structure of the emitter oxyluciferin was demonstrated from the finding that the compound hydrolyzes enzymatically into caffeic acid (Kaskova et al. 2017). This diversity and evolution cover diverse fungi that exhibit different emission patterns, including emitted tissues. The first comprehensive review in 2008 covered 64 luminescent species (Desjardin, 2008); today, scientists have recognized a total of 109 luminescence fungi, which can be classified into four molecular lineages (Chew, et al. 2015; Cortés-Pérez, et al. 2008; Chang, et al. 2020; Dutta, et al. 2020): 12 in the *Omphalotus* lineage, 10 in the *Armillaria* lineage, 85 in the *Mycenoid* lineage (mostly *Mycenaceae*) and two in the *Lucentipes* lineage. The last lineage represents an unnamed family (Desjardin, et al. 2010; Oliveira, et al. 2012) with an insufficient molecular sequence (Ke, et al. 2022).

No bioluminescent marine fungi are reported in the scientific literature, although in Mashukova, et al. (2016), they studied for the first-time

luminescence characteristics of the some micromycetes, isolated from the bottom sediments of the Black Sea. Luminescence parameters were registered in laboratory using mechanical and chemical stimulations. Culture of *Penicillium commune* gave no light emission with any kind of stimulation. Culture of *Acremonium sp.* has shown luminescence in the blue - green field of spectrum. Culture of *Aspergillus fumigatus* owned the most expressed properties of luminescence. Moreover, action of ethyl alcohol (chemical stimulation) to culture also stimulated signals, but intensity of light emission was 3-4 times lower than under mechanical stimulation. For sure the given studies will permit to evaluate contribution of marine fungi into general bioluminescence of the sea (Mashukova, et al. 2016). Also for this reason, no correspondence has been found, for now, between the fungal taxa identified in association with the mesopelagic fishes investigated in this study and potential bioluminescence phenomena.

Despite our investigations on the 16S rDNA and on putative functions did not revealed presence of bioluminescent bacteria, SEM investigations has clearly showed significant abundances of prokaryotic cells grouped exclusively in the photophore sections. Further investigation is needed, particularly through metagenomic or metatrascriptomic analysis, to answer more thoroughly the questions about who these bacteria are and what they do.

There are thousands of unexplored animals whose luminescence is yet to be determined naturally or using stimulation techniques (Chatragadda, 2020). In

the current scenario of global climate change, horizontal gene transfer (HGT) of *lux* genes from luminous bacteria to non-luminous bacterial members is remaining a question mark (Urbanczyk et al. 2008). Therefore, understanding these concepts is important for analyzing ecological and biological influences on luminescence. Research on luminous organisms would certainly improve our understanding of known bioluminescence systems, as well as to identify and characterize novel bioluminescent systems for developing new bioluminescence-based monitoring tools (Yeh, et al.2019).

6. **CONCLUSIONS**

This study provides the first insights on the diversity of bacteria and fungi associated with the mesopelagic fish *Maurolicus muelleri* and *Argyropelecus hemigymnus*, expanding our knowledge on their microbiomes and mycobiomes. Considering the microbiome, each species of fish revealed a few specific dominant genera shared among all members of the species and some rare exclusive genera. Despite this, results revealed that the taxonomy composition of microbiomes is similar at the interspecific level, and possibly explained by the presence of shared bacterial taxa as *Bacteroides*; *Lachnospiraceae* family, *Roseburia* and *Lachnospira* genera; *Alistipes*; *Clostridia* and others. Dissimilarity between the two fish species is mainly driven by the presence of some bacterial genera found in some individuals (i.e., *SUP05* genus, *Thioglobaceae* family), indicating a high intraspecific variability, which can be driven by biological factors as the healthy status. At the intra-individual level, microbiomes were not portioned among the different anatomic parts of the body: a specialized microbiome describing each different body part is not present. The integration of culture-bases analyses to the molecular analyses was performed to obtain a broader overview of the diversity associated with the fishes, in particular of those potentially bioluminescent; the cultivable bacterial species found were not detected by the molecular analysis.

The main putative bacterial functions (chemoheterotrophy, fermentation and presence of symbionts or parasites) obtained by the analysis of the microbiomes revealed that these were similar in the two fish species, probably because the two species live in a similar environment (mesopelagic waters) and have similar trophic strategies. An exception was found in 2 individuals of *A. hemigymnus*, which showed a high contribution of bacteria involved in the dark sulfide/sulfur compounds oxidation and the nitrate/nitrogen respiration, highlighting again a high intraspecific variability.

Mycobiome diversity in different body parts are well studied in humans, but little is known in marine organisms. Our results reveal that the taxonomy composition of mycobiomes is dominated by unidentified fungi in both species, indicating that these fishes might harbour a high richness of novel fungal lineages. Considering the identified fungi, significant differences were found between the two fish species, with a higher diversity in *A. hemigymnus* mycobiome than in *M. muelleri,* but in both fishes the photophore is the body part with the highest fungal variability (at the genus level). At the intra-individual level, the mycobiomes were not portioned among the different anatomical parts of the body, showing no specialization, and with a high percentage of unidentified fungi.

7. **Supplementary material**

Table 1 SM: List of additional sequences retrieved from GenBank using BLAST tools

Marker	Haplotype	Description	Samples site	Query Cover (%)	Percent identity (%)	NCBI CODE
COI	*M.muelleri 1; M.mulleri 2; M.mulleri 3; M.muelleri 4; M.muelleri 5*	*Maurolicus muelleri*	Atlantic ocean	100	100	MT132239.1
	A.hemigymnus 1	*Argyropelecus hemigymnus*	Atlantic ocean	100	99,26	EU148086.1
	A.hemigymnus 2			100	99,26	
	A.hemigymnus 3			100	99,26	
	A.hemigymnus 4			100	99,82	
	A.hemigymnus 5			100	99,63	

Table 2 SM: List of additional sequences of genera (*Maurolicus* and *Argyropelecus*) and out-group sequences extracted from GenBank.

Order	Family	SubFamily	Genera	Description	Sampling site	Accession number
Stomiiformes	*Sternoptychidae*	*Maurolicinea*	*Maurolicus*	*Maurolicus weitzmani*	Atlantic ocean 1	KU958023.1
				Maurolicus weitzmani	Atlantic ocean 2	KU958021.1
				Maurolicus weitzmani	Canada	KY033669.1
				Maurolicus weitzmani	Gulf of Mexico	MT132201.1
				Maurolicus walvisensis	Atlantic ocean	KU958029.1
				Maurolicus walvisensis	Madagascar	MT132321.1
				Maurolicus walvisensis	Namibia	MT132287.1
		ternoptychina	*Argyropelecus*	*Argyropelecus gigas*	Usa	MG856721.1
				Argyropelecus gigas	Atlantic ocean	EU148084.1
				Argyropelecus gigas	Gulf of Mexico	MT323748.1
				Argyropelecus aculeatus	Canada	KY033552.1
				Argyropelecus aculeatus	Gulf of Mexico	MF041221.1
				Argyropelecus aculeatus	Gulf of Mexico	MT323550.1
Myctophiformes	*Myctophidae*	-	*Electrona*	*Electrona risso*	Tasman sea	HQ564142.1

Table 3 SM: Estimates of evolutionary divergence between COI sequences.

Samples: 1*: Electrona_risso__out_group*, 2: *M.weitzmani_Atlantic_ocean1*, 3: *A.hemigymnus2_Messina_strait*, 4: *M.weitzmani_Atlantic_ocean2*, 5: *M.muelleri2_Messina_strait,* 6: *M.walvisensis_Namibia*, 7: *A.aculeatus_Canada,* 8: *A.hemigymnus3_Messina_strait*, 9: *M.weitzmani_Canada*, 10: *M.weitzmani_Gulf_of_mexico*, 11: *M.muelleri3_Messina_strait*, 12: *M.walvisensis_Atlantic_ocean*, 13: *M.walvisensis_Madagascar*, 14: *A.gigas_Usa*, 15: *A.olfersii_Atlantic_ocean2*, 16: *A.aculeatus_Gulf_of_Mexico1*, 17: *A.aculeatus_Gulf_of_Mexico2*, 18: *A.hemigymnus4_Messina_strait*, 19: *M.muelleri5_Messina_strait*, 20: *A.gigas_Atlantic_ocean*, 21*: A.gigas_Gulf_of_Mexico*, 22: *A.olfersii_Atlantic_ocean1*, 23: *A.olfersii_Atlantic_ocean3*, 24: *A.hemigymnus_Atlantic_Ocean*, 25: *M.muelleri4_Messina_strait*, 26*: A.hemigymnus1_Messina_strait*, 27: *A.hemigymnus5_Messina_strait*, 28: *M.muelleri1_Messina_strait*, 29: *M.muelleri_Atlantic_ocean*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1																													
2	4.0																												
3	4.0																												
4	5.0	3.0	7.0																										
5	5.0	5.0	7.0	6.0																									
6	5.0	5.0	7.0	6.0	4.0																								
7	5.0	7.0	5.0	8.0	8.0	8.0																							
8	5.0	7.0	3.0	8.0	8.0	8.0	6.0																						
9	6.0	4.0	8.0	3.0	7.0	7.0	9.0	9.0																					
10	6.0	4.0	8.0	3.0	7.0	7.0	9.0	9.0	2.0																				
11	6.0	6.0	8.0	7.0	3.0	5.0	9.0	9.0	8.0	8.0																			
12	6.0	6.0	8.0	7.0	5.0	3.0	9.0	9.0	8.0	8.0	6.0																		
13	6.0	6.0	8.0	7.0	5.0	3.0	9.0	9.0	8.0	8.0	6.0	2.0																	
14	6.0	8.0	6.0	9.0	9.0	9.0	5.0	7.0	10.0	10.0	10.0	10.0	10.0																
15	6.0	8.0	6.0	9.0	9.0	9.0	5.0	7.0	10.0	10.0	10.0	10.0	10.0	4.0															
16	6.0	8.0	6.0	9.0	9.0	9.0	3.0	7.0	10.0	10.0	10.0	10.0	10.0	6.0	6.0														
17	6.0	8.0	6.0	9.0	9.0	9.0	3.0	7.0	10.0	10.0	10.0	10.0	10.0	6.0	6.0	2.0													
18	6.0	8.0	4.0	9.0	9.0	9.0	7.0	3.0	10.0	10.0	10.0	10.0	10.0	8.0	8.0	8.0	8.0												
19	7.0	7.0	9.0	8.0	4.0	6.0	10.0	10.0	9.0	9.0	3.0	7.0	7.0	11.0	11.0	11.0	11.0	11.0											
20	7.0	9.0	7.0	10.0	10.0	10.0	6.0	8.0	11.0	11.0	11.0	11.0	11.0	3.0	5.0	7.0	7.0	9.0	12.0										
21	7.0	9.0	7.0	10.0	10.0	10.0	6.0	8.0	11.0	11.0	11.0	11.0	11.0	3.0	5.0	7.0	7.0	9.0	12.0	2.0									
22	7.0	9.0	7.0	10.0	10.0	10.0	6.0	8.0	11.0	11.0	11.0	11.0	11.0	5.0	3.0	7.0	7.0	9.0	12.0	6.0	6.0								
23	7.0	9.0	7.0	10.0	10.0	10.0	6.0	8.0	11.0	11.0	11.0	11.0	11.0	5.0	3.0	7.0	7.0	9.0	12.0	6.0	6.0	2.0							
24	7.0	9.0	5.0	10.0	10.0	10.0	8.0	4.0	11.0	11.0	11.0	11.0	11.0	9.0	9.0	9.0	9.0	3.0	12.0	10.0	10.0	10.0	10.0						
25	8.0	8.0	10.0	9.0	5.0	7.0	11.0	11.0	10.0	10.0	4.0	8.0	8.0	12.0	12.0	12.0	12.0	12.0	3.0	13.0	13.0	13.0	13.0	13.0					
26	8.0	10.0	6.0	11.0	11.0	11.0	9.0	5.0	12.0	12.0	12.0	12.0	12.0	10.0	10.0	10.0	10.0	4.0	13.0	11.0	11.0	11.0	11.0	3.0	14.0				
27	8.0	10.0	6.0	11.0	11.0	11.0	9.0	5.0	12.0	12.0	12.0	12.0	12.0	10.0	10.0	10.0	10.0	4.0	13.0	11.0	11.0	11.0	11.0	3.0	14.0	2.0			
28	9.0	9.0	11.0	10.0	6.0	8.0	12.0	12.0	11.0	11.0	5.0	9.0	9.0	13.0	13.0	13.0	13.0	13.0	4.0	14.0	14.0	14.0	14.0	14.0	3.0	15.0	15.0		
29	9.0	9.0	11.0	10.0	6.0	8.0	12.0	12.0	11.0	11.0	5.0	9.0	9.0	13.0	13.0	13.0	13.0	13.0	4.0	14.0	14.0	14.0	14.0	14.0	3.0	15.0	15.0	2.0	

Table 4 SM: Result of PERMANOVA main test carried out on:

ASV richness of microbiomes of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	233,47	233,47	11,435	0,001	998
bo	2	91,301	45,65	2,2359	0,137	999
spxbo	2	110,68	55,342	2,7106	0,055	999
Res	24	490,01	20,417			
Total	29	925,46				

Taxonomy composition of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	2950,7	2950,7	8,0782	0,001	998
bo	2	2002,3	1001,1	2,7408	0,026	998
spxbo	2	1311,8	655,88	1,7956	0,097	999
Res	24	8766,4	365,27			
Total	29	15031				

Evenness index of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	364,93	364,93	8,0707	0,01	998
bo	2	394,76	197,38	4,3652	0,03	999
spxbo	2	255,64	127,82	2,8269	0,079	999
Res	24	1085,2	45,216			
Total	29	2100,5				

Shannon index of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	621,21	621,21	9,7935	0,005	998
bo	2	536,66	268,33	4,2303	0,027	998
spxbo	2	363,81	181,9	2,8677	0,074	998
Res	24	1522,3	63,431			
Total	29	3044				

Putative function of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	3903,1	3903,1	9,0391	0,002	999
bo	2	1442,6	721,29	1,6704	0,182	999
spxbo	2	1200,7	600,36	1,3904	0,256	999
Res	24	10363	431,8			
Total	29	16910				

ASV richness of mycobiomes of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	2531,9	2531,9	13,9	0,001	998
bo	2	347,86	173,93	0,95487	0,386	998
spxbo	2	97,309	48,655	0,26711	0,839	998
Res	20	3643	182,15			
Total	25	6918,7				

Taxonomy composition of mycobiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	4503,9	4503,9	5,9334	0,002	999
bo	2	1586,2	793,1	1,0448	0,386	996
spxbo	2	1184,5	592,27	0,78025	0,688	998
Res	20	15181	759,07			
Total	25	22580				

Evenness index of mycobiomes among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	153,9	153,9	1,976	0,207	999
bo	2	124,54	62,268	0,79948	0,51	999
spxbo	2	46,781	23,391	0,30032	0,749	998
Res	20	1557,7	77,885			
Total	25	1840,3				

Shannon index of microbiome among different body parts and species (sp= Species; bo= Body parts)

Source	df	SS	MS	Pseudo-F	P(perm)	Unique perms
sp	1	3903,1	3903,1	9,0391	0,002	999
bo	2	1442,6	721,29	1,6704	0,182	999
spxbo	2	1200,7	600,36	1,3904	0,256	999
Res	24	10363	431,8			
Total	29	16910				

Table 5SM: Result of SIMPER analysis carried out on:

Taxonomy composition of microbiome among the species

Group	Avarege similarity	Avarege dissimilarity
M. muelleri *A. hemigymnus*	85,46% 65,77%	
M. muelleri vs A. hemigymnus		29,49%

Taxonomy composition of microbiome among different body parts

Group	Avarege similarity	Avarege dissimilarity
Photophores Gut Tissue	87,52 62,14 73,50%	
Photophores vs Gut Photophores vs Tissue Gut vs Tissue		30,23% 20,32% 32,44%

Taxonomy composition of microbiomes among different body parts within fish species

Species	Body parts	Avarege similarity (%)	Avarege dissimilarity (%)
M. muelleri *M. muelleri* *M. muelleri*	Photophores Gut Tissue	89,69% 80,70% 88,79%	
M. muelleri *M. muelleri* *M. muelleri*	Photophores vs Gut Photophores vs Tissue Gut vs Tissue		16,98% 10,72% 17,03%
A. hemigymnus *A. hemigymnus* *A. hemigymnus*	Photophores Gut Tissue	87,91% 55,28% 66,51%	
A. hemigymnus *A. hemigymnus* *A. hemigymnus*	Photophores vs Gut Photophores vs Tissue Gut vs Tissue		42,14% 27,98% 37,96%

Putative function of microbiome among different species

Group	Avarege similarity	Avarege dissimilarity
M. muelleri	87,39%	
A. hemigymnus	66,48%	
M. muelleri vs A. hemigymnus		29,31%

Putative function of microbiome among different body parts

Group	Avarege similarity	Avarege dissimilarity
Photophores	90,15%	
Gut	61,13%	
Tissue	73,75%	
Photophores vs Gut		29,16%
Photophores vs Tissue		18,78%
Gut vs Tissue		32,76%

Putative function of microbiome among different body parts within fish species

Species	Body parts	Avarege similarity	Avarege dissimilarity
M. muelleri	Photophores	94,17%	
M. muelleri	Gut	81,86%	
M. muelleri	Tissue	93,42%	
M. muelleri	Photophores vs Gut		17,51%
M. muelleri	Photophores vs Tissue		5,90%
M. muelleri	Gut vs Tissue		17,34%
A. hemigymnus	Photophores	88,74%	
A. hemigymnus	Gut	51,98%	
A. hemigymnus	Tissue	65,12%	
A. hemigymnus	Photophores vs Gut		38,37%
A. hemigymnus	Photophores vs Tissue		26,99%
A. hemigymnus	Gut vs Tissue		37,74%

Taxonomy composition of mycobiome among the species

Group	Avarege similarity	Avarege dissimilarity
M. muelleri	84,42%	
A. hemigymnus	48,75%	
M. muelleri vs A. hemigymnus		42,77%

Taxonomy composition of mycobiome among different body parts

Group	Avarege similarity	Avarege dissimilarity
Photophores	52,14%	
Gut	63,24%	
Tissue	78,81%	
Photophores vs Gut		41,58%
Photophores vs Tissue		37,60%
Gut vs Tissue		29,99%

Taxonomy composition of mycobiomes among different body parts within fish species

Species	Body parts	Avarege similarity (%)	Avarege dissimilarity (%)
M. muelleri	Photophores	77,56%	
M. muelleri	Gut	88,25%	
M. muelleri	Tissue	83,92%	
M. muelleri	Photophores vs Gut		16,14%
M. muelleri	Photophores vs Tissue		18,22%
M. muelleri	Gut vs Tissue		12,71%
A. hemigymnus	Photophores	38,63%	
A. hemigymnus	Gut	48,65%	
A. hemigymnus	Tissue	64,91%	
A. hemigymnus	Photophores vs Gut		54,72%
A. hemigymnus	Photophores vs Tissue		50,24%
A. hemigymnus	Gut vs Tissue		42,28%

Table 6SM: List of bioluminescent bacterial strains with available *lux* operon sequence (Brodl, et al. 2018).

No	strain	GenBank accession no	available sequence information
1	*Photobacterium leiognathi subsp. mandapamensis* ATCC 27561	DQ988878	*luxC*DABFEG, ribEBHA**
2a	*Photobacterium mandapamensis* ajapo.4.20_copy1	EU122285	*luxCDABFEG, ribEBHA*
2b	*Photobacterium mandapamensis* ajapo.4.20_copy2	EU122286	*luxCDABEG, ribEBHA*
3	*Photobacterium leiognathi subsp. mandapamensis* PL-721	DQ988877.2	*luxC*DABFEG, ribEBHA**
4	*Photobacterium phosphoreum* ATCC 11040	DQ988873	*luxCDABFEG, ribBHA**
5	*Photobacterium phosphoreum* (NBRC 104104)	AB367391.1	*luxCDABFEG*
6	*Photobacterium phosphoreum* FS-1.1	AY849486.2	*luxC*DABFEG, ribB*
7	*Photobacterium phosphoreum* (NBRC 13896)	AB104437.1	*luxCDABFEG, lumP*
8	*Photobacterium phosphoreum*	LC144829.1	*luxCDABFEG*
9	*Photobacterium leiognathi* ATCC 25521	M63594	*luxCDABEG*
10a	*Photobacterium leiognathi* lelon.2.1_copy1	EF536333.1	*luxCDABEG, ribEBHA, lumQ*
10b	*Photobacterium leiognathi* lelon.2.1_copy2	EF536334.1	*luxC*DABEG, ribEBHA*
11a	*Photobacterium leiognathi* lnuch.21.1	EF536335.1	*luxCDABEG, ribEBHA, lumQ*
11b	*Photobacterium leiognathi* lnuch.21.1	EF536336.1	*luxCDABEG, ribEBHA*
12	*Photobacterium aquimaris* (NBRC 104633)	JQ229765.1	*luxCDABFEG, ribEBHA**
13	*Photobacterium aquimaris* BS-1	JQ229766.1	*luxCDABFEG, ribEBHA, lumP*
14	*Photobacterium kishitanii* (pjapo.1.1)	DQ988874	*luxCDABFEG, ribEBHA**
15	*Photobacterium kishitanii* (NCIMB 844)	AY341064.2	*luxCDABFEG, ribEB*
16	*Photobacterium damselae* BT-6	EU122290.1	*luxD*ABE*
17a	*Photobacterium* lnuch.13.1_copy1	EF536338	*luxCDABEG, ribEBHA, lumQ*
17b	*Photobacterium* lnuch.13.1_copy2	EF536332	*luxCDABEG, ribEBHA, lumQ*
18	*Aliivibrio salmonicida*	AF452135	*luxCDABE, luxR, luxI, luxR2, ribG*
19	*Aliivibrio salmonicida* LFI1238	FM178380.1	*luxCDABEG, luxR, luxI*
20	*Aliivribrio fischeri* ATCC 7744	AY341062.2	*luxC*DABE**
21	*Aliivibrio fischeri* MJ-1	AF170104.1	*luxCDABEG, luxR, luxI*
22	*Aliivibrio fischeri* MJ11	CP001133.1	*luxCDABEG, luxR, lux, ribG*
23	*Aliivibrio logei* KCh1	HQ450520.1	*luxCDABE, luxR, luxI, luxR2*
24	*Vibrio vulnificus* VVL1 ATCC 43382	EU122289	*luxC*DABEG*
25	*Vibrio orientalis* ATCC 33934	EU556495.1	*luxCDABEG, luxH*
26	*Vibrio albensis* (NCIMB 41)	AB115761	*luxCDABEG*
27	*Vibrio harveyi* ATCC 14126	EU122288	*luxCDABEG, ribB**
28	*Vibrio harveyi* BCB440	EU192082.1	*luxCDABEG, luxH*
29	*Vibrio harveyi* ATCC 33843	CP009468.1	*luxCDABEG*
30	*Vibrio chagasii* 21N-12	EU122293.1	*luxD*ABE**
31	*Vibrio chagasii* SB-52	EU122294.1	*luxD*ABE**
32	*Vibrio campbellii* ATCC BAA-1116_1	CP006606.1	*luxCDABEG, luxH*
33	*Vibrio campbellii* ATCC BAA-1116_2	CP000790.1	*luxCDABEG, ribB*
34	*Vibrio campbellii* LA16-V1	CP021146.1	*luxCDABEG*
35	*Vibrio azureus* LC2-005 (NBRC 104587)	CP018617.1	*luxCDABEG*
36	*Vibrio* sp. BCB494	EU192084.1	*luxC*DABEG, luxH*
37	*Photorhabdus luminescens subsp. luminescens* ATCC 29999	M90093.1	*luxCDABE*
38	*Photorhabdus luminescens* Hw	M90092.1	*luxCDABE*
39	*Photorhabdus luminescens* ZM1	AF403784.1	*luxCDABE*
40	*Photorhabdus luminescens* HW	M62917.1	*luxCDABE**
41	*Photorhabdus luminescens subsp. laumondii* TTO1	BX571866.1	*luxCDABE*
42	*Photorhabdus asymbiotica* ATCC 43949	FM162591.1	*luxCDABE*
43	*Photorhabdus temperata subsp. thracensis* (DSM 15199)	CP011104.1	*luxCDABE*
44	*Shewanella hanedai* ATCC 33224	AB058949	*luxC*DABEG*
45	*Shewanella hanedai* (NCIMB 2157)	AB261992.1	*luxCDABEG, luxR, luxI*
46	*Shewanella woodyi*	AB368544.1	*luxCDABE*
47	*Shewanella woodyi* ATCC 51908	CP000961.1	*luxCDABEG*
48	*Candidatus* Photodesmus katoptron (Akat2007.1.1)	HQ333499.1	*luxCDABEG*
49	Bacterium symbiont of Cryptopsaras couesii	CP020663.1	*luxCDABEG*

*Indicates partial sequences; a, b (in numbering) Indicates two copies of the *lux* operon in the same bacterial strain.

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