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Genome rearrangements, male pregnancy and immunological tolerance – the curious case of the syngnathid immune system

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The syngnathid fish group (seahorses, pipefishes and seadragons) is a fascinating lineage associated with an array of evolutionary peculiarities that include diverse morphologies and their unique male pregnancy. These oddities also extend to their immune systems, with a growing body of research highlighting a range of intriguing immunological characteristics and genomic rearrangements, which pose questions regarding their evolutionary history and immune strategies. The functional loss of the major histocompatibility complex class II pathway (MHC II) in the *Syngnathus* genus and related pathway components in the seahorse (*Hippocampus*) were two discoveries that initially piqued interest. This sparked discussions concerning immune capabilities, possible facilitative roles in advanced male pregnancy evolution through means of evoking immunological tolerance, as well as a general re-evaluation of how we interpret vertebrate immunological plasticity. Experimental approaches have attempted to clarify further the impact of immune repertoire loss on the efficacy of the syngnathid immune response, specificities regarding the pathways in play during pregnancy as well as the concept of immunological inheritance. The first characterization of the immune cell repertoire of *Syngnathus typhle* using scRNA-seq represents the latest step to understanding the immune dynamics of these enigmatic fish. This report serves as a review for the immunological insights into the fascinating syngnathid fish group; encompassing their evolutionary history, immune cell populations, links to male pregnancy, and sex specificity, in addition to highlighting future research opportunities in need of investigation.

KEYWORDS

syngnathidae, immunity, male pregnancy, review, seahorse, pipefish, genome, evolution

1 Introduction

Facilitated by the evolution of self-non-self recognition mechanisms, the immune system provides crucial protection against harmful pathogens as well as maintaining immune homeostasis. Over time, the immune system has evolved into a diverse set of specific, rapid and modulatory pathways facilitated by multifunctional cell types and chemical signals. Innate immunity represents the first line of defence, a rapid, generally non-specific response

that initiates antigen-presentation, inflammation and activation of the complement and adaptive immune systems (Medzhitov and Janeway, 2000). The adaptive immune system provides highly specific responses upon pathogen re-exposure and is chief orchestrator of immune memory (Bonilla and Oettgen, 2010). Assisted by the major histocompatibility complexes (MHC I and II), antigen presentation processes must equally be able to determine self from non-self in order to avoid autoimmune related responses (Ljunggren and Kärre, 1990; Edwards and Hedrick, 1998). Forms of the evolutionary conserved innate immunity are found in all vertebrates and most invertebrates, evolving prior to the adaptive branch, which first emanated in primordial jawed vertebrates and has since become a hallmark of vertebrate evolution (Flajnik and Kasahara, 2010). Adaptive immune components are well conserved among gnathostomes from sharks to mammals, and cases of genomic immune system plasticity were deemed rare among this lineage. This has since been refuted with marine species such as the elephant shark (Venkatesh et al., 2014) and coelacanth (Amemiya et al., 2013), as well as teleost fishes including anglerfish (Dubin et al., 2019; Swann et al., 2020), Gadiformes (Star et al., 2011; Malmström et al., 2016) and several representatives of syngnathids (Haase et al., 2013; Roth et al., 2020) exhibiting remarkable cases of adaptive immune system remodelling. These cases raise questions concerning the conventions of vertebrate immunity and the underlying requirements for a functional immune system.

One group in particular that has attracted significant interest is the syngnathid fish group comprising seahorses, pipefishes, pipehorses and seadragons (Herald, 1959; Dawson, 1986). The bizarre and diverse morphologies held among syngnathid teleosts are emblematic of their peculiar evolutionary path, having also evolved the sole instance of male pregnancy in the animal kingdom (Stöltzing and Wilson, 2007). Recent discoveries have highlighted the occurrence of adaptive immune system remodelling in some pipefish and seahorse species, giving rise to a convoluted and drastically alternative set of immune defences (Bahr and Wilson, 2011; Haase et al., 2013; Roth et al., 2020). Alongside and succeeding these studies, research has delved further into the molecular underpinnings that shaped these enigmatic fish defences; exploring the links with male pregnancy evolution, immunological tolerance, transgenerational immune priming and alternative immune strategies (Figure 1) (Roth et al., 2012; Whittington et al., 2015; Beemelmans and Roth, 2016a; Keller and Roth, 2020; Whittington and Friesen, 2020; Parker et al., 2022). This review attempts to summarise the growing body of research concerning the syngnathid immune system, its evolution, and associations with other facets of their physiology and reproduction.

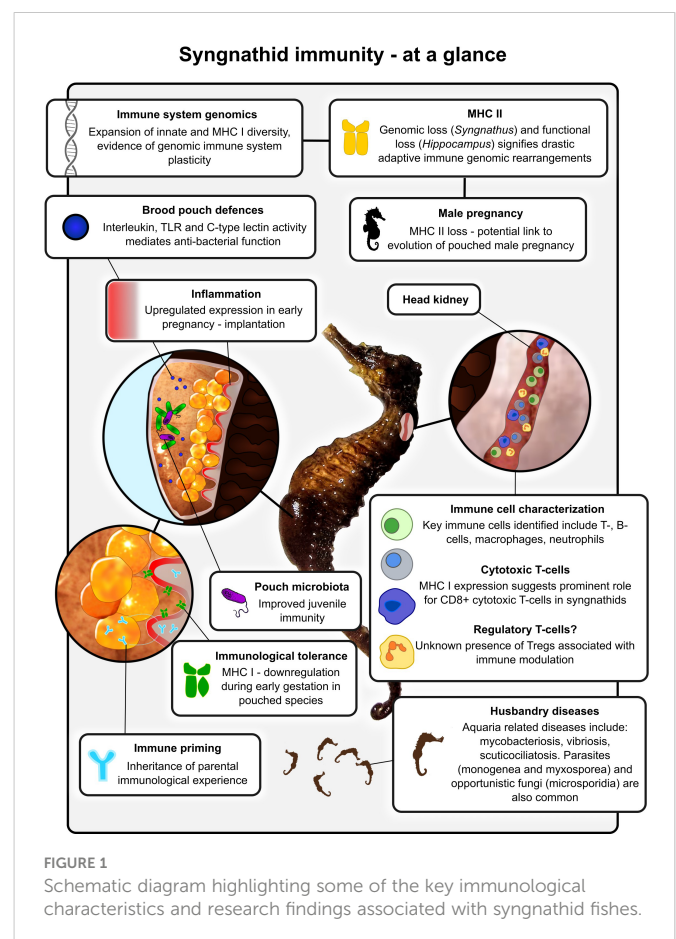
2 Genomics of the immune system

The last decade has seen the release of several high quality syngnathid genomes. Most of the studies focused around the genetics underlying syngnathid unique morphology and male pregnancy evolution (Small et al., 2016; Roth et al., 2020; Zhang et al., 2020; He et al., 2021; Li et al., 2021; Qu et al., 2021; Small et al., 2022). The genomes were fundamental to linking the loss of particular genes to a number of morphological traits such as the loss of

mineralized teeth (loss of P/Q-rich *scpp* genes), pelvic fins (loss of *tbx4*) and ribs (HOX gene losses) (Lin et al., 2016; Small et al., 2016; Zhang et al., 2020; Qu et al., 2021). This research also paved the way towards the realization that the genetics of male pregnancy are highly complex. The diversity of intricate brooding structures, such as the *Hippocampus* pouch and *Syngnathus* skin folds, as well as behaviour and immune system adaptations make it difficult to disentangle the evolutionary path and genetic foundations of male pregnancy. Nonetheless, some advancements in the understanding of male pregnancy evolution have been made. For example, syngnathid genome studies reported expansions of patristacin (*pastn*) genes, metalloproteases that are involved in egg hatching (Small et al., 2016; Lin et al., 2016). This, together with pregnancy-related expression patterns suggests a role of *pastn* genes in male pregnancy evolution. The immune gene repertoire of syngnathids also appears to be modified when compared to other teleost fish, showing expansions and contractions of certain crucial gene families and pathways, as well as the loss of some key adaptive immune system components (Roth et al., 2020).

2.1 The major histocompatibility complex

The major histocompatibility complex (MHC) represents a group of adaptive immune system genes. In tetrapods and chondrichthyes, the MHC genes are closely linked on the same chromosomal region. However, in teleosts this region is split between different chromosomes



and lacks defined synteny. The MHC is usually divided into two major classes that represent two arms of the adaptive immune system - I and II. The MHC class I pathway is responsible for protection against viruses and malfunctioning cells, while the MHC class II pathway defends against extracellular threats, like bacteria and parasites. Both are also crucial mediators of self-non-self recognition and maintenance of self-tolerance (Cooper and Alder, 2006; Neeffes et al., 2011; Grimholt, 2016; Roth et al., 2020). The genes that encode MHC class I and II molecules are present in multiple copies within a genome and often are highly polymorphic. These genes are further grouped into classical and non-classical subsets, depending on the structure and binding capacities of the encoded proteins. The latter is often deduced based on homology. Classical molecules are highly expressed and polymorphic; they bind peptide antigens and present them to immune cells. Non-classical molecules vary in their roles, from binding of non-peptide antigens to accessory molecules (Dijkstra et al., 2013; Grimholt et al., 2015; Grimholt, 2016). Five MHC class I lineages exist in teleosts - U, Z, S, L, and P, with all classical MHC I molecules in teleosts belonging to the U lineage (Grimholt et al., 2015; Grimholt, 2016). Teleost MHC class II molecules are divided into three lineages - A, B and E, with classical teleost MHC II molecules belonging to the A lineage, which seem to be present in all species (with a few exceptions) (Dijkstra et al., 2013; Grimholt, 2016).

Following the report in cod (Star et al., 2011) it was discovered that the pipefish *Syngnathus typhle* lacks expression of the *mhc II α/β* , *cd4* and the MHC class II transactivator (*ciita*) genes, and express a truncated version of MHC II invariant chain gene (*cd74*) (Haase et al., 2013). Consequently, it was hypothesized that like cod, *S. typhle* had lost the MHC II adaptive immune system component. The loss of these genes along with the activation induced cytidine deaminase (*aicda*) gene was then confirmed with genome studies, first in *Syngnathus scovelli* (Small et al., 2016) and then in other *Syngnathus* species (Roth et al., 2020). To date, there are only four vertebrate clades that have lost the conventional MHC class II pathway. The *Callorhynchus milii* (elephant shark) genome lacks *cd4* and related transcription factors, but contains polymorphic MHC II genes (Venkatesh et al., 2014). The *Syngnathus* genus, the entire Gadiformes order, *Lophius piscatorius* and some ceratioid species, all appear to have lost key MHC II pathway genes rendering it non-functional (Star et al., 2011; Haase et al., 2013; Malmstrøm et al., 2016; Small et al., 2016; Dubin et al., 2019; Swann et al., 2020; Roth et al., 2020).

The *Hippocampus* species have modifications in the autoimmune regulator (*aire*), *cd74*, and *ciita* genes, which also suggests an altered adaptive immune system. CIITA regulates the expression of MHC I and MHC II genes in antigen-presenting cells, and AIRE is responsible for negative selection on self-recognizing T cells. Genes encoding for AIRE and CIITA in *Hippocampus* have highly divergent exons compared to other teleost species, while CD74 has modifications in the CLIP (class II-associated invariant chain peptide) region (Roth et al., 2020). In the mammalian adaptive immune system, the CLIP region blocks the peptide-binding groove of a classical MHC class II molecule until it reaches the MHC class II compartment (MIIC) of the late endosome. The CLIP then is removed from the peptide-binding groove through the interaction with the non-classical MHC II DM molecule, allowing other peptides (e.g. antigens) to bind to it. In mammals, both the CD74 with intact CLIP and the MHC II DM are crucial for normal MHC II pathway

function. Curiously, all teleosts appear to lack the aforementioned MHC II DM molecule (Neeffes et al., 2011; Dijkstra et al., 2013). To date the mechanism with which CLIP dissociates from the MHC II peptide-binding groove in teleosts is unknown. Dijkstra and colleagues suggested that the accessory molecule might not be needed at all, since some MHC II molecules bind CLIP with low affinity and that CLIP was observed to disassociate rapidly at low pH, or other non-classical MHC II molecules could functionally replace the DM (Dijkstra et al., 2013). However, the fact that CD74 with intact CLIP is preserved in most sequenced teleost species suggests that either it is homologous in function to mammalian CD74, and thus species that lost it have a modified MHC II pathway, or it has functions outside of the pathway or even the immune system.

In syngnathids, the MHC I pathway also appears to be modified. When compared to Syngnathiformes without male pregnancy, species with pregnancy (Nerophinae, *Hippocampus*, and *Syngnathus*) were estimated to have an expansion of genes encoding the MHC I U lineage molecules (Roth et al., 2020), though another study reports smaller numbers in some *Syngnathus* and *Hippocampus* species (11/30 vs 7/11) (Qu et al., 2021). Surprisingly, these expanded genes form distinct clusters on a gene tree. Such clustering might represent sub- or neofunctionalization, serving as an adaptation to male pregnancy and a compensatory mechanism for the MHC II loss, where certain MHC I lineages specialise on the cross-presentation pathway or perform an entirely different immune function. Similar patterns of MHC I expansions and clustering can be observed within the Gadiformes order (Star et al., 2011; Malmstrøm et al., 2016).

2.2 Alternative immune pathways

Despite high metabolic costs, the gnathostome adaptive immune system offers a tremendous advantage in a form of highly specific immune responses and immune memory. The key components of the system are highly conserved throughout the gnathostome lineage and thus it seems highly unusual for a taxon to lose a core part of its functionality (Flajnik, 2018).

Since the first report in cod there was a debate concerning the reasons and mechanisms of the MHC II loss in certain teleost clades. Two broad scenarios have been proposed so far. The first scenario simply proposes that the MHC II pathway is dispensable in teleosts and was lost through genetic drift. The second suggests that the loss was mediated by directional selection. The authors then proposed two hypotheses for the second scenario: the metabolic shift hypothesis and the functional shift hypothesis (Star and Jentoft, 2012). Though originally discussed for cod, these hypotheses can be applied to all clades that have lost the MHC II pathway.

The metabolic shift hypothesis describes a situation where under particular environmental conditions the metabolic gains of losing the MHC II pathway would outweigh the protection it provides. The mutations that hamper expression would then be favoured, resulting in the gradual loss of the pathway and its core genes. Here the loss of MHC II pathway is independent of any other potential modifications to the immune system (e.g. expansion of innate immune receptors). On the contrary, the functional shift hypothesis suggests that certain environments could favour the development of alternative adaptive or innate immune pathways that make the MHC II pathway redundant.

Selection on the pathway would then be relaxed and through genetic drift, it would slowly degenerate. In a functional shift hypothesis, the emergence of these alternative immune pathways would predate or occur concurrently with the loss of MHC II. Thus, if the hypothesis is supported, in the species that lost MHC II pathway we can expect to observe an alternative immune gene profile (e.g. *via* MHC I cross-presentation). It is important to note that these hypotheses are not mutually exclusive and could act on the immune system simultaneously (Star and Jentoft, 2012).

Since then evidence supporting the metabolic cost hypothesis was obtained in cod-like fishes demonstrating that the loss of MHC II predated expansions of MHC I genes, hinting that the latter might be a compensatory response rather than a competing alternative immune strategy (Malmström et al., 2016). The Atlantic cod also shows a distinct Toll-like receptor (TLR) profile. TLRs represent a large family of Pattern Recognition Receptors (PRRs) and are one of the key components of the innate system (Brubaker et al., 2015). The expansion of *tlr7*, 8, 9, 22 and 25, and contractions of *tlr2* and 5 in cod is hypothesized to be a consequence of the MHC II loss and greater reliance on the innate immune system (Solbakken et al., 2016).

In syngnathids, only one genome study has mentioned the TLR repertoire and related pathways (He et al., 2021). The researchers reported species-specific contractions of TLR 2, 4 and 5 cascades in *Hippocampus abdominalis* using Gene ontology (GO) analysis approach. The expanded genes that belong to the aforementioned GO terms are *mapk14a*, *mapk3*, *s100b*, *tab2*, *ikbkq*, *peli1b*, *irak1* and *dusp3a* (He et al., 2021). While these genes do indeed belong to the TLR signalling pathways, the results have to be treated with caution as they represent only a small fraction of the GO term size (~50 genes) and at least some of these genes also belong to other pathways that are unrelated to the immune system. Strangely enough, the TLR10 cascade term is not mentioned in the manuscript, but it also appears to be enriched because of the same eight genes. However, the TLR1, TLR2, and TLR9 related genes that appear to be contracted within the whole Syngnathidae lineage were only referred to in the supplementary materials. *Tlr18* is reported to be expanded in the ancestor of Syngnathidae, yet no additional information is provided (i.e. copy number information) (He et al., 2021). In turn, investigating the diversity of TLRs in syngnathids is crucial as sub/neofunctionalization of the expanded TLR lineages could serve as an alternative to the lost MHC II pathway.

A number of genome studies have used similar approaches, casting a wide net and looking at enriched GO terms or expanded protein families, rather than at selected individual genes or groups of genes (Small et al., 2016; Zhang et al., 2020; Qu et al., 2021; Small et al., 2022). Such an approach helps to look at a broader picture of syngnathid genome evolution, but cannot substitute a more detailed investigation of immune system components. In order to trace the immune system evolution alongside the male pregnancy gradient a thorough genome scan of all sequenced syngnathids is needed. The genes encoding innate and adaptive immune system receptors, co-receptors, accessory, regulatory and signalling molecules should be evaluated for presence-absence and copy number variations. So far, only a fraction of immune pathways have been evaluated.

In the genomes of the leafy seadragon (*Phycodurus eques*) and “weedy” seadragon (*Phyllopteryx taeniolatus*) seven gene families related to innate immunity experienced contractions, among them contractions in tripartite motif-containing (TRIM), immune-associated nucleotide-binding (IAN), and mannose receptor (MRC) gene families were identified, confirming previous reports in syngnathids (Small et al., 2022). The TRIM family of proteins are involved in many cellular processes, including within the immune system. Expansions of certain genes encoding for TRIM sub-families have been described in teleosts and shown to have strong antiviral activities (Ozato et al., 2008; Van Der Aa et al., 2009; Langevin et al., 2017). Mannose receptor family (C-type lectin superfamily) is a multifunctional protein family with roles within adaptive and innate immune systems (Vasta et al., 2011). Interestingly, a Manado pipefish (*Microphis manadensis*) genome study also reports contractions within nine C-type lectin-domain containing families (Zhang et al., 2020). The IAN/GTPase of the immunity-associated protein (IAN/GIMAP) family genes were shown to be upregulated in zebrafish during viral infections (Balla et al., 2020). GIMAP and GIMAP-like genes have also been identified in invertebrates and hypothesized to have immune functions (Weiss et al., 2013; Milan et al., 2018; Lu et al., 2020; Limoges et al., 2021). The *gimap4* gene was shown to be upregulated during pouch development and late pregnancy in *S. typhle*, which is suggested to suppress lymphocyte maturation and proliferation protecting the eggs (Roth et al., 2020). Despite the overall IAN/GIMAP family contraction, *gimap4* seems to remain intact throughout the Syngnathidae lineage (Small et al., 2022). In addition to the aforementioned C-type lectin family contraction, contractions of NACHT-domain and immunoglobulin-domain containing families in *M. manadensis* have been reported (Zhang et al., 2020).

Alternative immune strategies could develop not only *via* expansions/contractions of certain receptor molecules, but also *via* modifications of regulatory pathways. Interleukins are a group of short protein cytokines that represent promising candidates for such investigation, owing to their important involvement in innate and adaptive immune system regulation (Secombes et al., 2011).

3 Male pregnancy

3.1 Immunological tolerance

The evolution of the unique male pregnancy can only be attributed to the syngnathid fish group, of which there are a number of varying brooding strategies and physiological adaptations, some of which are similar to female amniotes (Stölting and Wilson, 2007; Whittington and Friesen, 2020). Syngnathid brooding forms range from simplified external egg-attaching integument tissue (Nerophinae), egg-retaining inverted skin flap extensions, to the advanced fully enclosed marsupium-like brood pouches with placenta-like structures (Wilson et al., 2001; Carcupino et al., 2002; Ripley et al., 2010). These brooding differences between closely related species allow for the examination of evolutionary change and the potential drivers or crucial adaptations that culminate in advanced forms of pregnancy. In turn, pregnancy

evolution is heavily linked with the evolution of the adaptive immune system, and therefore syngnathids provide scope to understanding immune system evolution and its relevance within the realm of pregnancy. Currently, it is generally accepted that the expansion of a number of vertebrate systems accommodating organism physical growth, likely coincided with adaptive immune system evolution (Kasahara, 2000).

The evolution of gestation requires specialized morphological and immune gene expression changes (Moffett-King, 2002; Zenclussen et al., 2006; Hedlund et al., 2009). However, the co-evolution of gestation and the immune system creates a dilemma, regarding the avoidance of embryonic rejection *via* immune modulation and still maintaining maternal immune vigilance (La Rocca et al., 2014). In mammals, these problems have been solved through gene expression changes during pregnancy and at its onset, specific immune cell activities and specialized uterine/placental tissues (Moffett and Loke, 2006; Hedlund et al., 2009). In a general sense, immunological function in syngnathids is found to be disparate between pregnant and non-gravid individuals (Small et al., 2016; Roth et al., 2020; Parker et al., 2022). Similar suppression of the adaptive immune system has been noted in syngnathid pregnancy. This is through the diversity downregulation of MHC I genes and the functional (*Hippocampus* spp.) and complete genomic loss of MHC II (*Syngnathus* spp.), which appears a striking potential solution to immune regulation in pouched syngnathids when compared with the less drastic gene downregulation (Roth et al., 2020; Parker et al., 2022). MHC I pathway related downregulation was found to occur specifically during early gestation in syngnathids with a defined brood pouch, contrary to pouchless species (Parker et al., 2022). Immunological activity differences between brooding strategies have also been observed in *S. typhle* (inverted brood pouch), which exhibited a greater immune investment during pregnancy compared with *Nerophis ophidion* (pouchless) (Keller and Roth, 2020). These reports suggest that the evolution of the more 'intimate' brooding strategies required the coevolution of immune suppressive measures to accommodate the progeny.

Following mammalian coitus, sperm containing seminal fluid enters the female reproductive tract (Poiani, 2006). Seminal plasma is enriched with signalling molecules that have been shown to influence successful pregnancy establishment and implantation (Robertson et al., 2011; Robertson et al., 2013). It is also posited to act as an immunological tolerance primer for the receiving female to avoid embryo rejection (Tremellen and Robertson, 1999; Robertson et al., 2018). Mucus-like fluid has been reported to surround deposited syngnathid eggs (Carcupino et al., 1997; Watanabe, 1999), however, its significance or function is unknown. Whittington and Friesen (2020) have posited that the fluid could be a female equivalent to seminal fluid that potentially influences the onset and immunological homeostasis of male pregnancy. This is a deeply interesting concept that should be explored in more depth in the future.

3.2 Brood pouch defences

Unlike mammals, pouched syngnathids have had to overcome another immunological quandary when it comes to internal gestation

relating to the inner pouches and progeny exposure to environmental water and pathogens (Fiedler, 1954; Whittington and Friesen, 2020; Parker et al., 2022). Balancing the activity of immune system defences and concurrent immunological tolerance measures is a challenging proposition and is yet to be fully understood. Immunological function during male pregnancy is found to be disparate to non-gravid individuals (Small et al., 2016; Roth et al., 2020; Parker et al., 2022), with immunological factor concentrations at their greatest during pregnancy in seahorses (Lin et al., 2017). Bacterial activity and growth is thought to be facilitated in the sealed brood pouch particularly during the later gestation stages (Whittington and Friesen, 2020) and are likely a key driver of these immune disparities between pregnancy statuses. Uterine flushing in some gestating shark species is believed to assist with gas exchange and waste disposal around the time of parturition (Burger, 1967; Evans et al., 1982; Tomita et al., 2016; Tomita et al., 2017). A consequential upregulation of immune processes is also suggested to counter the influx of harmful pathogens (Sunyem and Vooren, 1997; Ellis and Otway, 2011; Buddle et al., 2020). Related upregulated expression of immune genes during parturition have also been described in seahorses with studies advocating the occurrence of similar brood pouch flushing (Whittington et al., 2015; Parker et al., 2022). Brood pouch flushing and its role in immune defence is an intriguing concept that will require further experimental studies.

A number of specific immunological defence components within syngnathid brooding structures have been proposed. Transcriptomic evidence of interleukin release (Whittington et al., 2015; Jiang et al., 2022), TLR gene expression (Whittington et al., 2015; Zhang et al., 2019; Wu et al., 2021) and C-type lectin activity (Melamed et al., 2005; Small et al., 2013; Whittington et al., 2015) in syngnathid brood pouches pertain to anti-bacterial function. The co-option of the antifungal *hepcidin* genes in seahorses is believed to have dampened their antimicrobial potential in the brood pouch to assist with immune homeostasis, but could still play a minor defence role against pouch dwelling pathogens (Whittington et al., 2015; Xiao et al., 2022). Bulk RNA-seq studies have reported the upregulated expression of genes coding for phospholipase sPLA2-IB and the macroglobulin A2M in the brood pouch during pregnancy in *Hippocampus* species (Wu et al., 2021; Parker et al., 2022; Xiao et al., 2022). Both are suggested to provide antimicrobial assistance, however, their presence and specific function within the brood pouch would benefit from further functional experimentation. Retinoic acid has a number of physiological functions, many of which revolve around immune system regulation (Larange and Cheroutre, 2016). In turn, retinoic acid concentration stability was suggested to be important for avoiding oxidative stress during male pregnancy (Li et al., 2020). Prolactin has been shown to influence growth, skin secretion regulation and immunological function in teleost fishes (Páll et al., 2004; Richards et al., 2009). It has also been isolated in the seahorse brood pouch during pregnancy and is thought to contribute to pouch osmoregulation; however, its potential immunological role is yet to be properly defined (Boisseau, 1967; Patron et al., 2008; Scobell and MacKenzie, 2011; Whittington and Wilson, 2013; Clarke and Bern, 2012). Upregulated seahorse pouch-derived genes with implicated immune roles are regularly identified, however, a clear understanding of the functional relationships connecting most of the aforementioned components is still lacking. In time, condensing

molecular and gene expression findings into a comprehensible network should help discern pregnancy immune modulation processes from pathogen protective measures.

3.3 Inflammation

The inflammatory function of the uterine tissues during early mammalian pregnancy is associated with tissue swelling which supports egg implantation (Mor and Abrahams, 2002; Dekel et al., 2010; Chavan et al., 2017). The fleshiness of mature male syngnathid brood pouches has been observed, in preparation for the deposition of eggs (Harlin-Cognato et al., 2006; Whittington and Friesen, 2020; Parker et al., 2022). These visual observations were recently corroborated by the upregulation of inflammation-related genes during the early stages of gestation in syngnathids of external, inverted brooding and advanced brooding forms (Parker et al., 2022), and during pouch development (Roth et al., 2020). Inflammatory pathway induction stimulates immune cell recruitment, which consequently causes cytokine release leading to tissue reshaping/remodelling (Granot et al., 2012). Therefore, it is conceivable that the extension of tissue folds in the syngnathid pouch is influenced by inflammatory pathways, and in turn aids egg immersion in pouched syngnathids. The molecular triggers for this inflammation are unknown; however, it could in part be induced by a seminal-like substance coating the deposited eggs. Seminal fluid is known to trigger inflammation upon entering the female reproductive tract in mammals (Robertson, 2005). A similar function may be found in syngnathids with female egg-coating fluid serving as the stimulant, however, this would require extensive work to clarify. Inflammation exhibited in the integument tissue of pouchless syngnathid species such as *N. ophidion* is suggested to be representative of the evolutionary root of this form of egg retention assistance (Parker et al., 2022). Similarities drawn with reproductive strategies in ricefishes (Hilgers et al., 2022), support the idea of inflammation assisting with the instigation of evolutionary modification and tissue specialisation. A number of brooding strategies have evolved within the lineage that occupy morphological gaps between the inverted dual skin-flapped pouch of *S. typhle* and the pouchless *N. ophidion*. For example, *Stigmatopora* pipefish species have pouch extensions akin to *S. typhle*, but without complete egg envelopment, while *Doryrhamphus dactyliophorus* have evolved thinner membranous egg capsules to retain the growing embryos (Wilson et al., 2001). Exploring the expression profiles of such phylogenetic representatives could provide a clearer understanding of the influence of inflammatory processes on the evolution of brood retention.

4 Sex specificity

Across a number of species, distinct differences have been documented concerning the immune capabilities of the respective sexes, with males generally possessing a less efficient immune system compared with females (Hamilton, 1948; Møller et al., 1998; Kurtz et al., 2000; Falagas et al., 2007; Roth et al., 2008; Abdullah et al., 2012). In humans, this evolutionary disparity is in part associated with female pregnancy, with an increase in immune potential linked

to the parent providing the highest degree of investment (Rolf, 2002). Therefore, the strength of parental immunity appears to depend on the life-history strategy; however, within the syngnathid lineage changes in sex roles and the degrees of parental investment vary depending on the species, rendering the immunological activity and concept of sex-role reversal difficult to disentangle. Despite this, in some cases of induced parental care, sex role reversal in syngnathids appears to have potentially led to distinct sexual immune dimorphism, with males adopting the role with greatest immunological and parental responsibility, while females are tasked with attracting mates. Studies on *Hippocampus comes* and *S. typhle* support this difference, with paternal immune response efficiencies appearing greater than in females (Roth et al., 2011; Lin et al., 2016a). These reports also suggest that competition for mates reduced immunity and that the adoption of parental care during pregnancy likely has a positive effect on the parent's immunity. Experimental exposure to water contaminants further support this sex distinction with immunocompetence in males greatly exceeding that of females (Jiang et al., 2019).

Hormones represent a complicated but compelling set of factors charged with mediating many important steps in mammalian pregnancy. Hormonal dynamics are intrinsically different between males and females in humans, and endocrine processes dictate immune shifts in females during pregnancy (Robinson and Klein, 2012). A number of endocrine-related studies in syngnathids have been conducted to date, highlighting their importance in syngnathid pregnancy, parturition and pouch development (Boisseau, 1967; Mayer et al., 1993; Ripley and Foran, 2010; Scobell and MacKenzie, 2011; Whittington and Wilson, 2013; Paul et al., 2020; Dudley et al., 2021). Others have identified sexual dimorphism in the pipefish liver, suggesting that estrogen in pipefish regulates reproductive physiology similarly to fish without reversed sex roles (Rose et al., 2015). However, the significance of sex specific hormonal activity in the realm of syngnathid male pregnancy, sex role reversal and specifically immune function still remains relatively unknown, but is an interesting proposition for future investigation.

5 Immune priming

5.1 Transgenerational immune priming

Transgenerational immune priming (TGIP) describes the transfer of parentally derived immunological experience to the progeny (Grindstaff et al., 2003). TGIP has a crucial influence on offspring survival and *via* the maternal line is a phenomenon well reported across the animal kingdom (Roth et al., 2018). The unique male pregnancy provided the mechanistic opportunity for a transfer of maternal experience *via* the egg in combination with a transfer of paternal experiences provided *via* the paternal brood pouch during male pregnancy (Roth et al., 2012; Beemelmans and Roth, 2016a). The parental investment dynamics are found to be asymmetric with maternal immune benefits only persisting during the early life stages, compared with the paternal immune influence which was suggested to be long-lasting (Roth et al., 2012; Beemelmans and Roth, 2016a). In addition, maternal priming is suggested to benefit the offspring's adaptive immune system, while the paternal influence rather

influences the innate branch (Roth et al., 2012; Beemelmans and Roth, 2016a). This is in contrast to the investment dynamics of conventional mammalian sex roles and is one of the very few instances of paternally derived TGIP in the animal kingdom. Sex-specific grandparental immune priming has also been determined in concert with the male pregnancy system, with F2 offspring benefitting from the immunological experiences of the grandparents (Beemelmans and Roth, 2017). Both these reports further support the influence of TGIP on the co-evolutionary arms race between pathogens and their hosts and that sex-role reversal still maintains the typical immune priming customary to mammals where the female primarily supports the offspring. Under changing environmental conditions, when parents are exposed to an additional environmental stressor (i.e., a temperature shift), the transfer of immunity from parents to offspring is hampered, implying that trans-generational plasticity reaches its limits when multiple stressors occur during the parental generation and offspring environments become unpredictable (Roth and Landis, 2017). TGIP might be influenced or partly maintained by a specific community of maternal and paternal microbes (Beemelmans et al., 2019).

5.2 Brood pouch microbiota

Host-associated microbiota are integral for a number of physiological processes, including nutritional uptake, development and immunity; colonizing vulnerable regions such as the skin and gut in many vertebrates (Robinson et al., 2010; Hooper et al., 2012; Hacquard et al., 2015). Recently there has been a surge in research relating to the composition, evolutionary characteristics and function of the syngnathid brood pouch microbiota (Beemelmans and Roth, 2016b; Beemelmans et al., 2019; Wagner, 2019). It has been observed that upon immune system activation in pregnant males, there is an upward turn in microbial community richness (Beemelmans et al., 2019). This is proposed to coincide with larval mouth opening and consequent microbial colonisation of the progeny. The establishment of a cohesive, functional microbiome is widely recognized as a crucial player in immune system development and efficiency (Gómez and Balcázar, 2008; Belkaid and Hand, 2014). Pouch microbial community changes during pregnancy, environmental influences, as well as diversity differences between pouch types and species are all topics that would benefit from further investigation. These along with future functional experimentation should improve the understanding of the functional relationships that exist between microbes, male pregnancy and immunity.

6 Immune cells

6.1 Syngnathid immune organs

The major immune organ and the first to develop in syngnathids is the head kidney (Tort et al., 2003; Falk-Petersen, 2005). Splenic presence and functionality among syngnathids is largely unsubstantiated with no spleen identifications in pipefish, while in seahorses its presence is contentious, with a number of contrasting reports (Matsunaga and Rahman, 1998; Novelli et al., 2015; Luo et al.,

2016; Ofelio et al., 2018; Wijerathna et al., 2022). In seahorses, developmental studies report the presence of a spleen during early juvenile development (Novelli et al., 2015; Ofelio et al., 2018), but adult spleens are seldom reported. This could be an indication of splenic shrinkage during adulthood, which in turn could render them functional redundant, however, this speculation would require further experimental clarification. The gut-associated lymphoid tissue (GALT) is a mucosal region located in the intestines, commonly found in humans and other animals, performing an important immunological role in the gut maintaining and developing immune cells in preparation for a response (Haley, 2017). In seahorses and pipefish, the GALT has been deduced missing or at the very least reduced to a vestigial level, with immune cells primarily stemming from the main head kidney (Matsunaga and Rahman, 1998; Roth et al., 2020). This loss was proposed to be an ancestral change in predatory activity, reducing the need for gut related immunological reserves (Matsunaga and Rahman, 1998). As with other teleost species, syngnathids likely possess gill-associated lymphoid tissue (GIALT) that offers mucus derived immunological protection from external pathogens encountered during oxygen uptake (Salinas, 2015). This is a clear sign of immunological activity in the tissue (Roth et al., 2012; Birrer et al., 2012; Luo et al., 2016). Nasopharynx-associated lymphoid tissue (NALT) and skin-associated lymphoid tissue (SALT) are equally important mucosal immune hubs, serving to protect the skin and olfactory organs, respectively (Salinas, 2015). Interestingly, research pertaining to the presence of SALT and NALT in syngnathids is yet to materialise but should be encouraged as it could hold the answers for many immune related knowledge gaps across the lineage.

6.2 Immune cell populations

The identification of immune cell types in teleost fishes in recent years has been assisted by the development of efficient cell sorting machinery and the advent of single-cell RNA sequencing methodologies (Islam et al., 2014; Chen et al., 2019). These methods are at the forefront of transcriptome research, providing a high-resolution investigative assessment of cell types that transcends traditional bulk-RNA sequencing techniques. Among the fish species that have so far undergone immune cell characterizations or specific cell isolations are zebrafish (*Danio rerio*) (Dee et al., 2016; Athanasiadis et al., 2017; Carmona et al., 2017; Tang et al., 2017; Hernández et al., 2018; Ferrero et al., 2020; Loes et al., 2021), Atlantic cod (*Gadus morhua*) (Guslund et al., 2020; Guslund et al., 2022), Atlantic salmon (*Salmo salar*) (Smith et al., 2021), Nile tilapia (*Oreochromis niloticus*) (Niu et al., 2020; Wu et al., 2021), Mexican tetra (*Astyanax mexicanus*) (Peuß et al., 2020) and rainbow trout (*Oncorhynchus mykiss*) (Perdiguerro et al., 2021). Recently, the first syngnathid immune cell repertoire was characterized in *S. typhle* providing a crucial baseline for future immune cell studies within the lineage (Parker et al., 2022). This report described the presence of a number of key immune cell types and their associated gene identifiers including macrophages (*mrc1*, *mpeg1*), neutrophils (*cebpe*, *lce*, *ncf4*), B- (*iglc1*, *cd53*, *cd79b*) and T-cell lymphocytes (*cd2*, *cd3e*, *v-tcr*). Interestingly, no signs of CD4⁺ T-cell types were observed, which is in line with the loss of MHC II in the species (Haase et al., 2013; Roth et al., 2020). However, perhaps the most interesting discoveries

concerned two genes within the T-cell cluster, *ilr2rb* and *gzma*, suggesting the potential presence of regulatory T-cells (Tregs) and cytotoxic T-lymphocytes (CTLs), respectively. Immunosuppressive Treg populations and their function remain elusive in syngnathids but if their identification can be confirmed, it would be a fascinating addition to the immunological tolerance discussion surrounding the lineage. The identification of CTLs in this study, along with their prominence in another recent study in the same species, suggest that they may be at the forefront of the syngnathid immune response (Parker and Roth, 2022). Elevated CTL activity in the MHC II/CD4⁺ cell devoid *S. typhle* could also hint at a potential compensatory measure that has evolved, however, these deductions likely require further substantiation.

Transcriptome assessments of the leafy appendages possessed by the seadragon, *P. taeniolatus*, uncovered inflammation-related gene expression, suggesting a potential immunological role (Qu et al., 2021). The defensive addition of highly upregulated MHC I gene expression in the leafy extensions supports the theory that due to the appendages being crucial to seadragon camouflage and therefore survival, there is added importance in its protection and regeneration.

7 Husbandry pathology

The demand for syngnathid species, in particular seahorses, has increased drastically over the last decade as they have become a prominent feature in traditional Chinese medicine and ornamental fish collections (Vincent, 1996). These practices rely on prolific husbandry set-ups, which in turn increase the demand for information on efficient aquaculture rearing methods (Koldewey and Martin-Smith, 2010). Syngnathid fish husbandry is associated with elevated infection risks due to excessive fish handling, lower water quality and higher stock densities than those found in the natural environment (Prosser et al., 2011). Even factors such as ambient aquaria noise are believed to induce stress and impact immunological efficiency in syngnathids (Anderson et al., 2011). Syngnathid fishes raised in aquaria systems are subjected to numerous pathogenic challenges which are often exacerbated due to imperfections in husbandry rearing conditions (Sanaye et al., 2013). Here is a brief overview of some of the more common pathogens encountered by syngnathids within the aquaria trade, and their related diseases. A more comprehensive list of harmful pathogens and health issues is also included (Table 1).

Mycobacteria related infections can lead to mycobacteriosis, a disease that reared seahorses are particularly susceptible to (Koldewey, 2005). Infection has been shown to stimulate atypical lesions on a number of organs and body parts such as the tail, spleen, liver and kidney in seahorse cultures (Bombardini et al., 2006; Balcázar et al., 2011; Fogelson et al., 2017; Fogelson et al., 2018), while infections in seadragon and pipefish species have also been cited (Bombardini et al., 2006; Bonar et al., 2013).

A number of flavobacteria strains such as *Tenacibaculum* spp., *Cellulophaga fucicola* and *Flavobacterium columnare* have all been isolated previously from pipefish, seahorse and seadragon aquaria stocks (Bombardini et al., 2006; LePage, 2012; Declercq et al., 2014; LePage et al., 2015). Targeted tissues can vary greatly among fish species,

with these syngnathid studies highlighting signs of necrosis in the gills, tail, skin and muscle.

Vibrio strains are the most abundant, diverse opportunistic marine pathogens and regularly used experimentally to assess syngnathid fish immunity (Thompson et al., 2004; Birrer et al., 2012; Landis et al., 2012; Roth et al., 2012; Goehlich et al., 2021). When conditions suit, they are a common cause of disease in aquaria raised syngnathids (Alcaide et al., 2001; Balcázar et al., 2010; Balcázar et al., 2010; Wang et al., 2016). Symptoms can include lethargy, skin spots, loss of appetite and tail necrosis (Balcázar et al., 2010). *Vibrio* are also implicated in opportunistic secondary infections, with recent findings suggesting that gas bubble disease (GBD) associated with syngnathid husbandry (Lin et al., 2010; LePage et al., 2015; Zhang et al., 2015), renders fish susceptible to vibronic invasion (Kang et al., 2022). Moreover, juvenile seahorses are particularly susceptible to *Vibrio* strains, which can lead to a number of physiological and developmental issues (Lin et al., 2016b; Shao et al., 2019).

Scuticociliates are a group of parasitic unicellular marine organisms and renowned causative agents of scuticociliatosis, another common disease among aquaria fish species such as seadragons, seahorses and pipefishes (Marcer et al., 2005; Garner et al., 2008; Rossteuscher et al., 2008; Sang et al., 2011; Bonar et al., 2013; Di Cicco et al., 2013; Ofelio et al., 2014; Armwood et al., 2021). Often scuticociliatosis leads to severe skin lesions and necrosis, while internal organs, blood vessels and gills are also regularly affected (Cheung et al., 1980; Woo and Buchmann, 2012; Bonar et al., 2013; Ofelio et al., 2014).

Monogenean flatworms are another common parasite often reported among syngnathids including many from the *Syngnathus* pipefish group (Bombardini et al., 2006; Williams et al., 2008; Vaughan et al., 2010; Paladini et al., 2010). They have been shown to parasitize a number of anatomical regions including the brood pouch, skin and gills (Williams et al., 2008; Paladini et al., 2010; Cone et al., 2013). Myxospora are microscopic parasites from the cnidaria phylum that commonly infiltrate seahorses (Vincent and Clifton-Hadley, 1989; Sears et al., 2011), seadragons (Garner et al., 2008; Bonar et al., 2013) and pipefish (Longshaw et al., 2004). The parasites often reside in the gall bladder of the infected individuals (Vincent and Clifton-Hadley, 1989; Longshaw et al., 2004; Bombardini et al., 2006).

A number of fungal pathogens including microsporidia, primarily of the genus *Glugea*, and melanized fungi, have been isolated from syngnathid fishes (Bombardini et al., 2006; Nyaoke et al., 2009). *Glugea* strains are spore-forming organisms capable of transforming infected fish cells into proliferating masses known as xenomas a key symptom of microsporidiosis (Lom and Dyková, 2005; Dyková and Lom, 2007). In *Hippocampus erectus*, *Glugea* had a particular tendency to corrupt skin and connective tissues (Blasiola, 1979; Vincent and Clifton-Hadley, 1989; Bombardini et al., 2006). Phaeohyphomycosis stems from the infection of opportunistic melanized fungi such as those from the *Exophiala* genus, and has been documented a number of times, particularly in weedy and leafy seadragons where they had a tendency to infect vascular tissues (Nyaoke et al., 2009; Bonar et al., 2013).

Viral pathogens and their aquacultural impacts are under-researched in syngnathids, with very little information documented on the topic. Suspected viral induced lesions have been identified in *H. abdominalis* previously without conclusively identifying the specific culprit (LePage et al., 2015), while a more recent paper has isolated and characterized a

TABLE 1 Harmful pathogens identified in aquaria bred or raised syngnathid fishes.

Type	Class	Strains	Tissue	Species	Reference
Bacterial	Actinomycetia	<i>Mycobacterium poriferae</i>	K, SB	<i>Hippocampus erectus</i>	(Anderson and Petty, 2013)
		<i>Mycobacterium syngnathidarum</i>	O	<i>Syngnathoides biaculeatus</i>	(Fogelson et al., 2018)
			M	<i>Hippocampus erectus</i>	(Fogelson et al., 2018)
		<i>Mycobacterium chelonae</i>	S	Unspecified	(Koldewey, 2005)
		<i>Mycobacterium marinum</i>	S	Unspecified	
		<i>Mycobacterium fortuitum</i>	S	Unspecified	
		<i>Mycobacterium</i> spp.	S	<i>Hippocampus erectus</i>	(Bombardini et al., 2006)
			S	<i>Syngnathus typhle</i>	
			S	<i>Syngnathus scovelli</i>	
			S	<i>Syngnathoides biaculeatus</i>	
			SB, G	<i>Phycodurus eques</i>	
			SB, G	<i>Phyllopteryx taeniolatus</i>	
		<i>Nocardia nova</i>	S	<i>Hippocampus reidi</i>	(Dill et al., 2017)
	<i>Gordonia</i> sp.	K, SB	<i>Hippocampus erectus</i>	(Anderson and Petty, 2013)	
	<i>Tsakamurella paurometabola</i>	K, M, S	<i>Hippocampus barbouri</i>	(Florio et al., 2004; Bombardini et al., 2006)	
	Bacilli	<i>Bacillus subtilis</i>	T	<i>Hippocampus kuda</i>	(Sanaye et al., 2013)
	Chlamydiia	Chlamydiales spp.	S, G	<i>Phycodurus eques</i>	(Langdon et al., 1991; Meijer et al., 2006)
			S, G	<i>Syngnathus acus</i>	(Longshaw et al., 2004; LePage et al., 2015)
	Flavobacteriia	<i>Cellulophaga fucicola</i>	S	<i>Hippocampus kuda</i>	(LePage, 2012)
		<i>Flavobacterium columnare</i>	G	<i>Hippocampus abdominalis</i>	(LePage et al., 2015)
<i>Hippocampus kuda</i>					
<i>Phyllopteryx taeniolatus</i>					
<i>Tenacibaculum maritimum</i>		S, G	<i>Syngnathus typhle</i>	(Bombardini et al., 2006)	
<i>Tenacibaculum mesophilum</i>		S	<i>Hippocampus kuda</i>	(LePage et al., 2012)	
<i>Tenacibaculum aestuarii</i>	T	<i>Hippocampus kuda</i>	(Declercq et al., 2014)		
Gammaproteobacteria	<i>Aeromonas</i> spp.	S	<i>Hippocampus erectus</i>	(Bombardini et al., 2006)	
		S	<i>Syngnathus scovelli</i>		
	<i>Pseudoalteromonas spongiae</i>	T	<i>Hippocampus kuda</i>	(Sanaye et al., 2013)	
	<i>Vibrio harveyi</i>	S	<i>Hippocampus kuda</i>	(Alcaide et al., 2001; Tendencia, 2004; Raj et al., 2010; Binh et al., 2016; Xie et al., 2020)	
		S	<i>Hippocampus spinosissimus</i>	(Binh et al., 2016)	
		S	<i>Hippocampus comes</i>		
		S, L, Mo	<i>Hippocampus</i> sp.	(Alcaide et al., 2001)	

(Continued)

TABLE 1 Continued

Type	Class	Strains	Tissue	Species	Reference		
			S, K	<i>Hippocampus erectus</i>	(Qin et al., 2017; Li et al., 2020)		
			S	<i>Hippocampus kuda</i>	(Binh et al., 2016; Xie et al., 2020)		
			S	<i>Hippocampus spinosissimus</i>	(Binh et al., 2016)		
			S	<i>Hippocampus comes</i>			
			S, T	<i>Hippocampus gutturalis</i>	(Balcázar et al., 2010)		
			S, T	<i>Hippocampus hippocampus</i>			
			G, K, L	<i>Hippocampus reidi</i>	(Martins et al., 2010)		
			S, G	<i>Hippocampus erectus</i>	(Bombardini et al., 2006)		
			S, G, K, SP, B	<i>Hippocampus barbouri</i>	(Florio et al., 2004; Bombardini et al., 2006)		
			S, G	<i>Hippocampus abdominalis</i>	(Bombardini et al., 2006)		
			S, G	<i>Hippocampus zosterae</i>			
			S, G	<i>Syngnathus scovelli</i>			
			<i>Vibrio vulnificus</i>		S, H	<i>Hippocampus kuda</i>	(Binh et al., 2016; Jiang et al., 2020)
					S	<i>Hippocampus spinosissimus</i>	(Binh et al., 2016)
					S	<i>Hippocampus comes</i>	
			<i>Vibrio splendidus</i>		S, T	<i>Hippocampus gutturalis</i>	(Balcázar et al., 2010)
					S, T	<i>Hippocampus hippocampus</i>	
			<i>Vibrio fortis</i>		I	<i>Hippocampus erectus</i>	(Wang et al., 2016)
			<i>Vibrio tubiashii</i>		L, K	<i>Hippocampus erectus</i>	(Shao et al., 2019)
			<i>Vibrio rotiferianus</i>		T	<i>Hippocampus erectus</i>	(Yang et al., 2017)
			<i>Vibrio</i> sp.		S	<i>Hippocampus kuda</i>	(Sanaye et al., 2013)
			<i>Photobacterium ganghwense</i>		T	<i>Hippocampus kuda</i>	
			<i>Photobacteria</i> sp.		I, L, K	<i>Hippocampus erectus</i>	(Zhang et al., 2022)
S	<i>Hippocampus kuda</i>	(Sanaye et al., 2013)					
Fungal	Chaetothyriomycetes	<i>Exophiala angulospora</i>	D, BV	<i>Phyllopteryx taeniolatus</i>	(Nyaoke et al., 2009)		
			D, BV	<i>Phycodurus eques</i>			
		<i>Exophiala aquamarina</i>	D, BV	<i>Phycodurus eques</i>			
		<i>Exophiala lecanii-corni</i>	F, M, K, S, SB	<i>Hippocampus erectus</i>	(Armwood et al., 2021)		
		<i>Exophiala</i> sp.	S, T	<i>Hippocampus erectus</i>	(Blazer and Wolke, 1979; Armwood et al., 2021)		
			L	<i>Hippocampus</i> sp.	(Stoskopf, 1993)		
		Melanized fungi (Unspecified)		BV	<i>Phycodurus eques</i>	(Bonar et al., 2013)	
BV	<i>Phyllopteryx taeniolatus</i>						
Dothideomycetes		<i>Cladosporium</i> spp.	U	<i>Hippocampus erectus</i>	(Bombardini et al., 2006)		

(Continued)

TABLE 1 Continued

Type	Class	Strains	Tissue	Species	Reference	
			U	<i>Hippocampus zosterae</i>		
			U	<i>Hippocampus barbouri</i>		
	Microsporea		<i>Glugea heraldii</i>	C	<i>Hippocampus erectus</i>	(Blasiola, 1979; Vincent and Clifton-Hadley, 1989)
			<i>Glugea</i> sp.	S	<i>Hippocampus erectus</i>	(Bombardini et al., 2006)
	Sordariomycetes		<i>Fusarium solani</i>	S	<i>Hippocampus erectus</i>	(Salter et al., 2012; Brown et al., 2020)
	Terrasporidia		<i>Nucleospora hippocampi</i>	I	<i>Hippocampus erectus</i>	(Wang et al., 2022)
Parasitic	Cestoda		<i>Proteocephalidae</i> sp.	I	Unspecified	(Bombardini et al., 2006)
	Chromadorea		<i>Anisakis simplex</i>	V	<i>Syngnathus acus</i>	(Longshaw et al., 2004)
	Conoidasida		<i>Cryptosporidium</i> sp.	I	<i>Hippocampus kuda</i>	(LePage et al., 2015)
			<i>Eimeria phyllopterycis</i>	I	<i>Phyllopteryx taeniolatus</i>	(Osborn et al., 1999; Upton et al., 2000)
			<i>Eimeria syngnathi</i>	I	<i>Syngnathus abaster</i>	(Yakimoff and Gousseff, 1936)
			Coccidian protozoa (undefined)	I	<i>Phyllopteryx taeniolatus</i>	(Bonar et al., 2013)
	Kinetoplastida		<i>Ichthyobodosp.</i>	G	Unspecified	(Koldewey, 2005)
	Monogenea		<i>Gyrodactylus corleonis</i>	S, F, G	<i>Syngnathus typhle</i>	(Paladini et al., 2010)
			<i>Gyrodactylus eyipayipi</i>	U	<i>Syngnathus acus</i>	(Vaughan et al., 2010)
			<i>Gyrodactylus neretum</i>	U	<i>Syngnathus typhle</i>	(Paladini et al., 2010)
			<i>Gyrodactylus pisculentus</i>	S	<i>Syngnathus fuscus</i>	(Williams et al., 2008)
			<i>Gyrodactylus shorti</i>	U	<i>Syngnathus scovelli</i>	(Holliman, 1963)
			<i>Gyrodactylus leptorhynchi</i>	BP, S	<i>Syngnathus leptorhynchus</i>	(Cone et al., 2013)
			<i>Gyrodactylus syngnathi</i>	U	<i>Syngnathus rostellatus</i>	(Appleby, 1996)
			<i>Gyrodactylus</i> sp.	S	<i>Syngnathus typhle</i>	(Bombardini et al., 2006)
		S	<i>Syngnathus scovelli</i>			
	Myxozoa		<i>Sinuolinea phyllopteryxa</i>	U	<i>Phyllopteryx taeniolatus</i>	(Garner et al., 2008)
			<i>Sphaeromyxidae</i>	GB	<i>Hippocampus erectus</i>	(Vincent and Clifton-Hadley, 1989; Sears et al., 2011)
			<i>Sphaeromyxa sabrezesi</i>	U	<i>Hippocampus brevirostris</i>	(Bellomy, 1969)
				U	<i>Hippocampus guttulatus</i>	
			<i>Ceratomyxa</i> sp.	GB	Unspecified	(Bombardini et al., 2006)
			<i>Myxidium incurvatum</i>	GB	<i>Syngnathus acus</i>	(Longshaw et al., 2004)
			<i>Kudoa quadratum</i>	M	<i>Syngnathus acus</i>	
	Myxozoa sp.			K, BV	<i>Phyllopteryx taeniolatus</i>	(Bonar et al., 2013)
				K, BV	<i>Phycodurus eques</i>	
	Oligohymenophorea		<i>Philasterides dicentrarchi</i>	S	<i>Phycodurus eques</i>	(Rossteuscher et al., 2008)
				S	<i>Phyllopteryx taeniolatus</i>	
				V	<i>Hippocampus abdominalis</i>	(Marcer et al., 2005; Di Cicco et al., 2013)
				I	<i>Hippocampus kuda</i>	(Sang et al., 2011)

(Continued)

TABLE 1 Continued

Type	Class	Strains	Tissue	Species	Reference
		<i>Porpostoma notatum</i>	S	<i>Hippocampus hippocampus</i>	(Ofelio et al., 2014)
		<i>Uronema marinum</i>	G, M	<i>Hippocampus erectus</i>	(Cheung et al., 1980)
			BV, N, Bl, K	<i>Hippocampus kuda</i>	
		<i>Uronema</i> sp.	T	<i>Hippocampus kuda</i>	(Declercq et al., 2014)
		<i>Uronema</i> -like	E, S, L	<i>Phycodurus eques</i>	(Bombardini et al., 2006)
			E, S, L	<i>Phyllopteryx taeniolatus</i>	
			S, L	<i>Syngnathoides biaculeatus</i>	
		Scuticociliatida (no species)	D	<i>Phyllopteryx taeniolatus</i>	(Umehara et al., 2003)
			S, M	<i>Doryrhamphus dactyliophorus</i>	(Armwood et al., 2021)
			S, BV, G, M	<i>Phyllopteryx taeniolatus</i>	(Bonar et al., 2013)
			S, BV, G, M	<i>Phycodurus eques</i>	
		<i>Miamiensis avidus</i>	U	<i>Hippocampus</i> sp.	(Thompson and Moewus, 1964)
		<i>Trichodina</i> sp.	G	<i>Syngnathus acus</i>	(Longshaw et al., 2004)
	Palaeacanthocephala	<i>Corynosoma australe</i>	U	<i>Hippocampus</i> sp.	(Braicovich et al., 2005)
	Prostomatea	<i>Cryptocaryon irritans</i>	S	<i>Hippocampus kuda</i>	(Sanaye et al., 2013)
	Phyllopharyngea	<i>Brooklynella hostilis</i>	S	<i>Hippocampus kuda</i>	(Blasiola, 1983)
	Spirotrichea	<i>Licnophora hippocampi</i>	G, S	<i>Hippocampus trimaculatus</i>	(Meng and Yu, 1985)
	Trematoda	<i>Cryptocotyle lingua</i>	C	<i>Syngnathus acus</i>	(Longshaw et al., 2004)
		<i>Dictysarca virens</i>	K, SB	<i>Hippocampus erectus</i>	(Anderson and Petty, 2013)
		<i>Digenea</i> sp.	I	Unspecified	(Bombardini et al., 2006)
Viral	Magsaviricetes	<i>Betanodavirus</i>	N	<i>Hippocampus abdominalis</i>	(Chen et al., 2022)
Other	External gas-bubble disease		S	<i>Syngnathus schlegeli</i>	(Sanaye et al., 2013; Kang et al., 2022)
			S	<i>Hippocampus haema</i>	
	Gas-bubble disease		S, BP, H	<i>Hippocampus erectus</i>	(Bombardini et al., 2006; Lin et al., 2010; Sanaye et al., 2013)
			SB, I	<i>Hippocampus abdominalis</i>	(Woods, 2000; Sanaye et al., 2013)
	Fibrosarcoma		BP	<i>Hippocampus erectus</i>	(Willens et al., 2004)
	Angioma/lymphangioma		S	<i>Hippocampus erectus</i>	(Boylan et al., 2015)
	Cardiac rhabdomyosarcoma		He	<i>Phyllopteryx taeniolatus</i>	(LePage et al., 2012)
Pancreatic islet cell neoplasia		P	<i>Phyllopteryx taeniolatus</i>	(Bonar et al., 2013)	

Infected tissues include: B (brain), Bl (bladder), BP (brood pouch), BV (blood vessel), C (connective tissue), D (disseminated), De (dermis), E (eye), F (fin), G (gills), H (head), He (heart), I (intestine), K (kidney), L (liver), M (muscle), Mo (mouth), N (neuronal), O (ovary), S (skin), SB (swim bladder), SP (spleen), T (tail), U (unspecified) and V (viscera).

new strain of virus called the seahorse nervous necrosis virus (SHNNV) (Chen et al., 2022). Extracted from the brain and eye, SHNNV is deduced to cause harmful vacuolations in the organs and based on infection experiments is more virulent among juveniles. In *H. abdominalis*, *viperin*, an antiviral related gene was identified and upregulated in intestinal and kidney tissues when exposed to infection, suggesting it could have a role in modulating syngnathid antiviral responses (Tharuka et al., 2019). These limited findings strengthen the need for further research focused on elucidating the pathology, diversity and general relevance of viruses in the syngnathid aquaculture trade.

8 Conclusion

Syngnathids are some of the most fascinating subjects for evolutionary and immunological research due to their unique male pregnancy and intriguing immunological rearrangements. Molecular based studies highlighted here provide an ideal platform for future experimental work, which should focus on understanding the functional properties and mechanisms at play and how they relate to syngnathid evolution and physiology. By sharing a recent common ancestor and exhibiting diverse brooding strategies, syngnathids are useful candidates for comparative work and interpreting the nuances of evolutionary adaptation. Understanding the intricate inner workings of syngnathid immune function, immunological tolerance and pregnancy, should provide a useful alternative perspective to model species research and could prove vital for the development of applied autoimmune and other medical related practices.

Author contributions

JP, AD and OR contributed to the conception of the manuscript. JP wrote the first draft of the manuscript, JP, AD and OR wrote

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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