IMMUNE SYSTEMS

The immunogenetics of sexual parasitism

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Sexual parasitism has evolved as a distinctive mode of reproduction among deep-sea anglerfishes. The permanent attachment of males to host females observed in these species represents a form of anatomical joining, which is otherwise unknown in nature. Pronounced modifications to immune facilities are associated with this reproductive trait. The genomes of species with temporarily attaching males lack functional *aicda* genes that underpin affinity maturation of antibodies. Permanent attachment is associated with additional alterations, culminating in the loss of functional *rag* genes in some species, abolishing somatic diversification of antigen receptor genes, the hallmark of canonical adaptive immunity. In anglerfishes, coevolution of innate and adaptive immunity has been disentangled, implying that an alternative form of immunity supported the emergence of this evolutionarily successful group of vertebrates.

Lophiiform fishes constitute a morphologically diverse assemblage of taxa that share a peculiar and distinctive mode of feeding; it is characterized most notably by a luring apparatus (illicium) derived from a modified first dorsal-fin spine, located on the tip of the snout (Fig. 1A) (*1*, *2*). Of the five suborders found within the lophiiform fishes, the Ceratioidei, or deep-sea anglerfishes, is the most diverse, with 168 known species divided among 11 families and 35 genera (*2*). Deep-sea anglerfish are found distributed throughout the world's oceans at depths below 300 m and have evolved several adaptations that facilitate their deep-sea lifestyle, chief among which are their distinctive reproductive habits, which involve extreme sexual dimorphism and physical pair formation. Male deep-sea anglerfish are dwarfed compared with their mates—those of some species grow to only 6 to 10 mm standard length (*2*–*4*)—and attach themselves (either temporarily or permanently) to the bodies of relatively gigantic females (Fig. 1A). In some taxa, attachment is followed by fusion of epidermal and dermal tissues and, eventually, by connection of the circulatory systems so that the male becomes permanently dependent on the female for nutrients, with the pair becoming a kind of self-fertilizing chimera (*2*, *5*–*8*).

Sexual parasitism represents a form of naturally occurring anatomical joining (parabiosis), which is otherwise known in nature only from the rare instances of genetically identical conjoined twins (*9*). From an immunological perspective, the formation of permanent physical attachments between anglerfish pairs is notable: In all other vertebrate species, such a tissue fusion event would be expected to provoke a potent immune response directed against the "nonself" major histocompatibility complex (MHC) antigens. Typically, successful transplantation of tissues requires the careful cross-matching of donor and recipient MHC haplotypes, together with immunosuppressive drugs to ensure long-term survival of the allogeneic graft (*10*, *11*). Given that some anglerfish species can undergo tissue fusion without eliciting a destructive immune response, we set out to explore immunological adaptations that are associated with sexual parasitism within this suborder of lophiiform fishes. To this end, we considered several possibilities. First, that ceratioid species may consist of genetically identical or closely related clones, in which case the barrier of allorecognition would be removed, allowing successful tissue fusion between individuals of the same species. Second, we hypothesized that ceratioids may be able to identify suitable mates through olfactory assessment of MHC genotype, which we previously found to contribute to mate choice in aquatic and terrestrial species (*12*, *13*). Selecting matching MHC haplotypes before parasitization would be a mechanism to circumvent allogeneic rejection and facilitate long-term attachment and/or tissue fusion. To examine these first two hypotheses, our interest initially focused on the extent of MHC diversity, both among individuals of the same species and between corresponding female and male pairs. We also considered a third hypothesis, that ceratioids might enable sexual parasitism by specific adaptations of the genes underpinning adaptive immune responses, perhaps functionally similar in outcome to the immunological interactions that occur at the feto-maternal interface of the mammalian placenta (*14*). To examine this latter possibility, we focused our attention on genes with unequivocal orthologies and specific roles in the vertebrate immune system, such as those encoding the CD4 and CD8 coreceptor molecules and the components associated with the antigen receptors of B cells and T cells, as well as genes required for the generation of their somatically diversified receptor repertoires.

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Reproductive modes in ceratioids

The reproductive modes in ceratioids fall into two broad categories. In the first group of species, males attach temporarily to females but never become parasitic; in the second group, males attach permanently to females (*2*, *4*, *8*). An important distinguishing feature among sexually parasitizing species is the number of males simultaneously attached to females (*2*) (Fig. 1B). Females of the three *Ceratias* species studied here (*Ceratias holboelli*, *C. tentaculatus*, and *C. uranoscopus*) almost always have only one attached male; only 2 out of 32 parasitized females have two males attached (1.06 ± 0.06 males per female, mean ± SEM). By contrast, females of *Cryptopsaras couesii*, a species closely related to *Ceratias*, often ($P = 0.038$; two-tailed t test, when compared to *Ceratias*) carry more than one male (1.60 ± 0.20 males per female; $P = 0.014$; two-tailed t test, when compared with *Ceratias*), with 12 out of 47 parasitized females having two or more, and up to eight, males attached. Multiple attachments are also frequently observed in the two species of the family Linophrynidae studied here, *Photocorynus spiniceps* [1.76 ± 0.22 males per female ($n = 25$ females)] and *Haplophryne mollis* (1.76 ± 0.22 males per female; $n = 5$ females).

Study population and design

We generated whole-genome shotgun sequences of 31 specimens representing 10 species of Ceratioidei, covering a broad phylogenetic spectrum of this suborder (*15*) (tables S1 and S2). Among the species known to exhibit temporary attachment, we studied one species each of Himantolophidae (*Himantolophus appelii*), Melanocetidae (*Melanocetus johnsonii*), Diceratiidae (*Diceratias pileatus*), and Gigantactinidae (*Gigantactis vanhoeffeni*); for reference, we use the subscript [TA] as an identifier when discussing the immune genotype of these species. *C. holboelli*, *C. tentaculatus*, and *C. uranoscopus* are identified by [PA1], indicating that males permanently attach to females in a 1:1 mode; *C. couesii*, *P. spiniceps*, and *H. mollis* are identified by [PAn] to indicate that often multiple males permanently attach to a single female. As controls for the 10 species of the suborder Ceratoidei described above, we examined the genomes of specimens from two further suborders of lophiiform fishes (tables S1 and S2). The suborder Antennarioidei is represented by specimens from two families (Tetrabrachiidae, *Tetrabrachium ocellatum*; and Antennariidae, *Antennarius commerson*), and the suborder of Chaunacoidei is represented by *Chaunax abei*. Because males never attach to females in these species, they

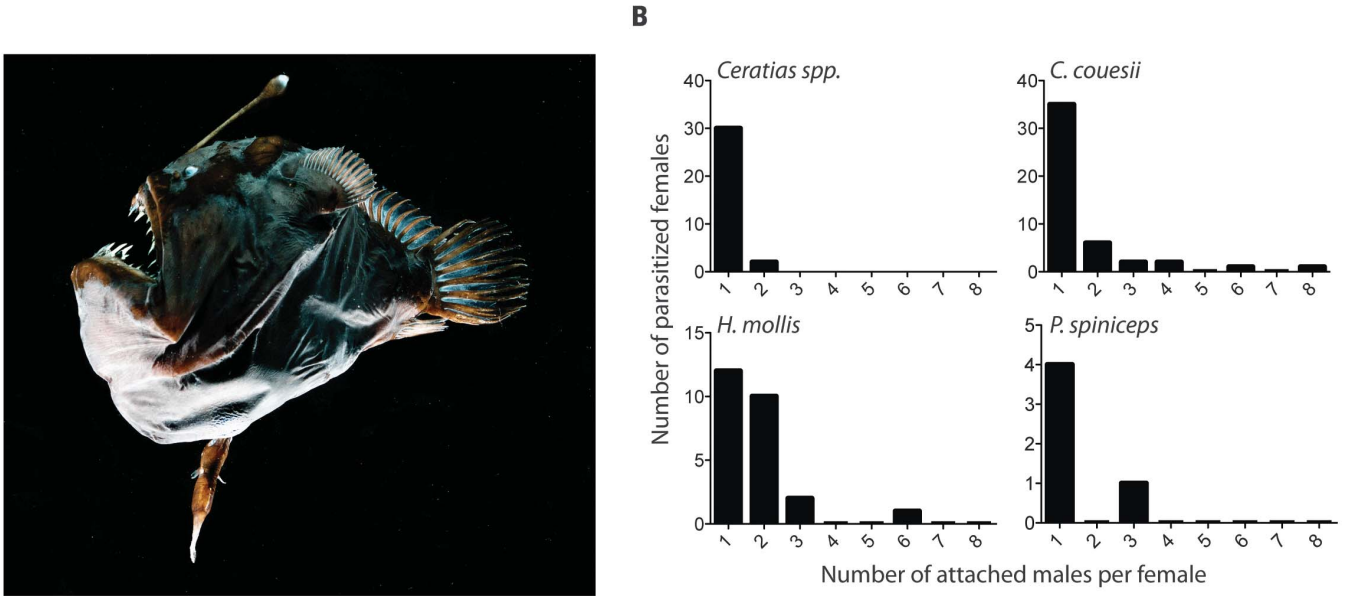

Fig. 1. Sexual parasitism in ceratioid anglerfishes. (**A**) Parasitized female of *M. johnsonii*. Note the illicium at the forehead and the attached male at the belly. [Photo credit: Edie Widder] (**B**) Exclusive ([PA1]) and consortial ([PAn]) modes of male parasitism. Data are compiled from (*2*); for *P. spiniceps*, the parasitized female described here is included.

are given the [NA] identifier. The scarcity of parasitized females, lack of fresh material, and poor quality of archival ceratioid specimens poses a considerable challenge for molecular analysis. We were unable to obtain intact RNA from the specimens available to us and therefore focused our analysis on genomic DNA (tables S1 and S2). Using gene-centered local assemblies (in selected cases confirmed by Sanger sequencing of amplified genomic fragments), we examined the structures and syntenic relationships of a collection of genes with unequivocal orthologies and well-established roles in the vertebrate immune system (table S3).

Diversity of MHC genes in lophiiform fishes

Histocompatibility in jawed vertebrates is governed by haplotype variability in MHC genes, and we therefore began our examination of anglerfish immune systems by exploring the MHC gene content of the various species in our collection, with particular emphasis on male-female pairs where possible. We found that the three nonparasitizing species (*T. ocellatum*$_{[NA]}$, *A. commerson*$_{[NA]}$, and *C. abei*$_{[NA]}$) and three of four temporarily attaching species (*D. pileatus*$_{[TA]}$, *H. appelii*$_{[TA]}$, and *M. johnsonii*$_{[TA]}$) each possess large numbers of different MHC class I genes of the U type (*mhc1u*) but only few genes of the Z type (*mhc1z*) (table S4), which is typical of the situation in Teleostei (*16*). This diverse *mhc1* repertoire observed in nonattaching and temporarily attaching species was complemented by multiple *mhc2a* alleles (table S4), indicating that a diverse *mhc* gene content does not represent an immunological barrier to the formation of temporary attachments in most instances.

By contrast, we observed profound changes in the MHC gene status of the six species exhibiting permanent attachment of males to females. Among the three species (*C. holboelli*$_{[PA1]}$, *C. tentaculatus*$_{[PA1]}$, and *C. uranoscopus*$_{[PA1]}$) that practice permanent exclusive attachment, we found that whereas *mhc2* diversity was maintained (table S4), the *mhc1* gene complement was substantially altered. We detected at most two *mhc1u* genes per *Ceratias* species, whereas an unusually large number of distinct *mhc1z* gene sequences were recovered (table S4). The importance of the apparent contraction of *mhc1u* and expansion of *mhc1z* is, as of now, unknown; however, the substantial shift in *mhc1* gene diversity in anglerfish species that form permanent, exclusive attachments suggests that alteration of antigen-presentation pathways may be required to facilitate tissue fusion.

Loss of MHC diversity was much more substantial among anglerfish species capable of forming permanent, consortial matings. We found no functional versions of *mhc1u* and *mhc1z* genes in the four *C. couesii*$_{[PAn]}$ specimens sequenced, and although we could detect one or two *mhc2aa* sequences per individual, no *mhc2ab* genes were found in in these individuals. In *H. mollis*$_{[PAn]}$, no functional *mhc1u* and *mhc2a* sequences were found, and a maximum of two alleles each for exons 2 and 3 of *mhc1z* genes were recorded. Of the four specimens of *P. spiniceps*$_{[PAn]}$ (one female, three attached males) examined, we noted a lack of detectable *mhc1u* and *mhc2a* sequences; however, each individual possessed one or two alleles of *mhc1z*. Collectively, these results indicate that anglerfish that use permanent consortial pairings are largely depauperate in MHC genes.

Having characterized the *mhc1* and *mhc2* content of the various species in our collection, we turned our attention to the permanently attached pairs from which we were able to obtain DNA from the fusion partners. Examination of the *mhc* haplotypes of exclusive pairs of *C. holboelli*$_{[PA1]}$ and *C. uranoscopus*$_{[PA1]}$ revealed that although some overlap in *mhc1* and *mhc2* alleles was detected between the fusion partners, each individual had several specific *mhc1* and *mhc2* alleles not found in their attached mate (Fig. 2A). These results clearly demonstrate that MHC haplotype matching is not essential for tissue fusion between anglerfish. The lack of MHC diversity detected among the anglerfish species that form permanent consortial pairs renders the assessment of MHC haplotype matching between fusion partners largely redundant; however, we note that for the *P. spiniceps*$_{[PAn]}$ female with three attached males, all members of the consort had the same *mhc1z* allele, with the female having one additional allele. For the *H. mollis*$_{[PAn]}$ pair, the *mhc1z* alleles differed between the partners.

Overall, our results suggest that anglerfish species that use mating strategies involving permanent attachment have undergone substantial changes to their MHC gene content:

Permanent exclusive pairings are associated with restructuring of the *mhc1* system, whereas the use of a consortial mating strategy is associated with an almost complete loss of both *mhc1* and *mhc2* haplotype diversity. One outlier in our analysis was *G. vanhoeffeni*$_{[TA]}$; this species exhibits a general reduction in both *mhc1z* and *mhc2a* diversity relative to other temporarily attaching or nonattaching species (table S4). The reason for this reduction in MHC diversity in a temporarily attaching species is unknown; however, *G. vanhoeffeni*$_{[TA]}$ exhibits a number of additional immunological abnormalities, setting it apart from other temporarily attaching species (see below).

Pseudogenization of CD8 co-receptor genes

MHC class I–restricted CD8$^+$ cytotoxic T cells are key mediators of allograft rejection (*17*). Given that permanent attachment is associated with loss of *mhc1u* diversity (table S4), we next examined the *cd8a* and *cd8b* genes, which encode the heterodimeric CD8αβ co-receptor that interacts with MHC class I molecules (*18*). Notably, functional *cd8a* and *cd8b* genes were absent in all six permanently attaching species; in two cases, remnants of the *cd8a* gene could be found in the interval between the genes flanking the tandemly arranged *cd8a* and *cd8b* genes (Fig. 2B and figs. S1 to S3). Moreover, both *cd8* genes were also missing in the genome of *G. vanhoeffeni*$_{[TA]}$, in line with the lack of functional *mhc1u* genes in this species (fig. S1 and table S4), further strengthening its distinctive immunogenetic constellation among the species with a temporary attachment mode. Collectively, these findings suggest that the canonical antigen-specific cytotoxic pathway is impaired in all species distinguished by permanent male attachment. However, the *perforin* gene (table S3), which encodes a key element of the cytotoxic pathway, is present in all genomes analyzed here, suggesting the presence of cytotoxic innate lymphoid cells in these species.

Loss of the CD4 pathway

MHC class II–restricted CD4$^+$ helper T cells are also known to play an important role in

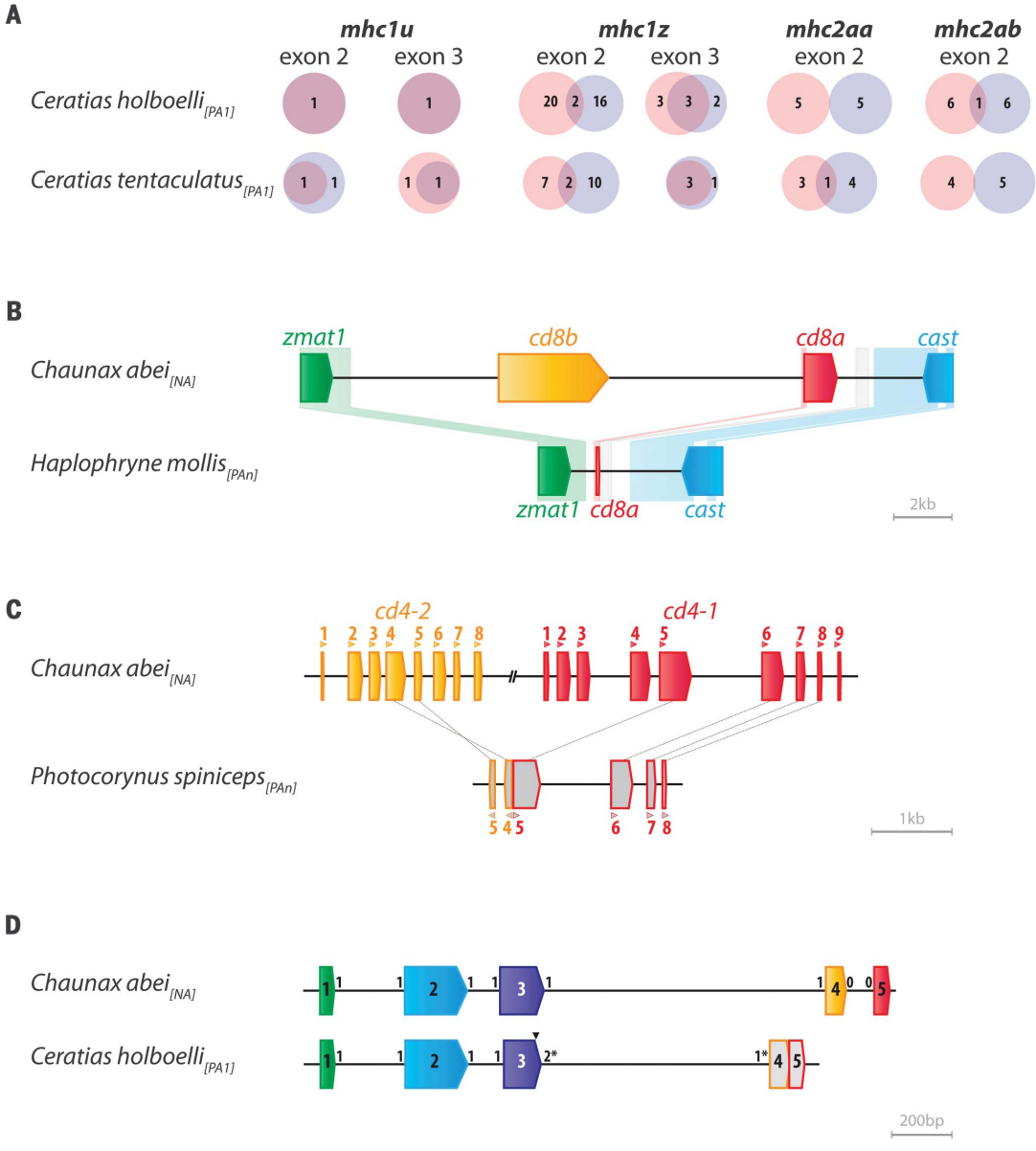

Fig. 2. Immunogenomic features of lophiiform fishes. (**A**) Number of shared and distinct *mhc* gene alleles in female-male pairs of the two indicated species of *Ceratias*. Blue indicates the male genotype, and pink indicates the female genotype. (**B**) Tandem arrangement of *cd8b* and *cd8a* genes and their flanking genes *zmat1* and *cast*. *H. mollis*$_{[PAn]}$ retains a short sequence of the 5′ end of *cd8a*. The structure of the locus of *C. abei*$_{[NA]}$ serves as a reference; sequence homologies are indicated as shaded blocks. (**C**) Gene models of *cd4-2* and *cd4-1*. The relative orientation of exons is indicated by arrowheads. Gray lines connect equivalent exons between species. In *P. spiniceps*$_{[PAn]}$, the *cd4-2* gene has lost all exons except exons 4 and 5, which are found in an inverse orientation; the first four exons of *cd4-1* are deleted, and remnants of others exhibit deleterious sequence alterations. Oblique parallel black lines indicate the omission of intergenic sequence to highlight coding features. (**D**) Gene model of the *cd3gd* gene, which exhibits deleterious changes in *Ceratias* species. Exons are depicted as shaded blocks; internal numbers indicate exon number, whereas numbers flanking the exons indicate the splice phase. A 23–base pair deletion (indicated by a black arrowhead) in exon 3 of the *Ceratias* gene causes a splicing defect, and mismatched splice sites are indicated with an asterisk. In addition, the *Ceratias cd3gd* gene exhibits the loss of the intron between exons 4 and 5.

allograft rejection (*17*), so we next turned to the *cd4-1* and *cd4-2* genes (*19*) that encode the characteristic co-receptors of the helper T cell lineage (*18*). We also examined the status of the *cd74* gene, which encodes a specialized chaperone molecule (also known as invariant chain) that facilitates the transport of MHC class II molecules to endosomes, which is an important prerequisite for antigen presentation (*20*). In all species with a diverse set of *mhc2a* genes (table S4), functional *cd4-1*, *cd4-2*, and *cd74* genes are readily identifiable (figs. S4 to S11). By contrast, *G. vanhoeffeni*$_{[TA]}$, *C. couesii*$_{[PAn]}$, *P. spiniceps*$_{[PAn]}$, and *H. mollis*$_{[PAn]}$ lack functional versions of the *cd4-1* and *cd4-2* genes (Fig. 2C and figs. S4 to S12); with the exception of *G. vanhoeffeni*$_{[TA]}$, which possesses a single *mhc2a* gene, the other three species lack functional *mhc2a* genes (table S4). We failed to detect *cd74*-related genes in *P. spiniceps*$_{[PAn]}$ and *H. mollis*$_{[PAn]}$; the *cd74* gene structures in *G. vanhoeffeni*$_{[TA]}$ and *C. couesii*$_{[PAn]}$ predict an unusual form of the protein, whose functional properties are unclear (fig. S12). Collectively, it appears that the canonical MHC class II pathway is unlikely to play an important role in immune defense in these four species.

Alterations of the T cell receptor signaling complex

The substantial changes in MHC gene content and the frequent loss of MHC co-receptors among anglerfish that practice permanent attachment suggest that the T cell compartment in these animals is likely to have also undergone considerable changes. In mammals, the heterodimeric T cell receptor, which recognizes peptides presented by MHC molecules with the aid of CD4 or CD8 co-receptors, consists of α and β chains encoded by the *Tcra* and *Tcrb* genes. This antigen-recognition module then associates with a hexameric signaling complex consisting of polypetides encoded by the *Cd3g*, *Cd3d*, *Cd3e*, and *Cd3z* genes (*21*); in fishes, the mammalian *Cd3g* and *Cd3d* genes are represented by a single precursor gene, *cd3gd* (*22*). On binding of the T cell receptor to MHC-peptide complexes, the CD3 complex initiates a signaling cascade that encompasses protein tyrosine phosphorylation of its immunoreceptor tyrosine-based activation motifs (ITAMs) (*23*). Intact *cd3gd* genes were found in *T. ocellatum*$_{[NA]}$, *A. commerson*$_{[NA]}$, *C. abei*$_{[NA]}$, *H. appelii*$_{[TA]}$, *M. johnsonii*$_{[TA]}$, *D. pileatus*$_{[TA]}$, and *G. vanhoeffeni*$_{[TA]}$; however, they could not be detected in *C. couesii*$_{[PAn]}$, *P. spiniceps*$_{[PAn]}$, and *H. mollis*$_{[PAn]}$ (figs. S13 to S15). The three *Ceratias*$_{[PA1]}$ species share a variant of the *cd3gd* gene that is predicted to encode a protein lacking the characteristic cytoplasmic ITAM (Fig. 2D and figs. S13 to S15). Although it is unknown whether this truncated protein is produced (the internal deletion in the *cd3gd* gene may lead to nonsense-mediated degradation of the transcript), the variant CD3 signaling complexes of *Ceratias*$_{[PA1]}$ is predicted to reduce the T cell receptor's overall signaling capacity (*24*). Because essential components of the cytotoxic pathway (*mhc1u*, *cd8a*, *cd8b*) appear to be missing in *Ceratias*$_{[PA1]}$, the altered T cell receptor signaling most likely affects the function of the T helper lineage by reducing the magnitude of the antigen-specific antibody responses in these species. We were unable to substantiate the presence of *cd3e* genes in *C. couesii*$_{[PAn]}$, *P. spiniceps*$_{[PAn]}$, and *H. mollis*$_{[PAn]}$; however, intact genes were identified in all other species, with the exception of *G. vanhoeffeni*$_{[TA]}$, in which a truncated cd3e protein is predicted to lack the ITAM (fig. S16). All species were found to possess intact *cd3z* genes, with the exception of *P. spiniceps*$_{[PAn]}$, in which an internal deletion leads to the absence of one of the three ITAMs (fig. S17). We presume that retention of the *cd3z* gene in *H. mollis*$_{[PAn]}$ and *P. spiniceps*$_{[PAn]}$ is due to a requirement in other signaling complexes, such as those used by innate immune cells (*25*).

In the absence of complementary transcriptomes, the complexity of the T cell receptor gene loci confounded a definitive inventory of *tcr* genes in anglerfishes by analysis of genomic DNA sequences alone. In particular, unambiguous identification of *trdc* and *trgc* constant-region genes was not possible because of their similarities with other immunoglobulin domain–encoding genes. However, we were able to definitively identify *tcra* and *tcrb* constant-region genes (designated *trac* and *trbc*, respectively) in all species with non-attaching males ([NA]) and temporarily attaching males ([TA]), as well as in species exhibiting permanent exclusive attachments ([PA1]) (figs. S18 and S19). By contrast, we were unable to confidently identify *trac* and *trbc* genes in any of the species of anglerfishes that exhibit permanent consortial ([PAn]) pairings. (A poorly supported candidate *trac* gene was detected in two of four *C. couesii*$_{[PAn]}$ specimens, a *trbc* fragment was detected in *H. mollis*$_{[PAn]}$, and no evidence of either the *trac* or *trbc* gene was found in *P. spiniceps*$_{[PAn]}$ genome sequences.) We therefore provisionally conclude that the assembly of a functional αβ T cell receptor heterodimer is unlikely in anglerfish species that form permanent consortial ([PAn]) pairings.

Impaired antibody-mediated immunity

Antibody-mediated allograft rejection is a known complication in transplant settings (*17*, *26*) and suggests that in addition to the defects in cellular immunity described above, anglerfish species that use temporary or permanent attachment modes must have evolved mechanisms to avoid humoral rejection of mates. We therefore examined key genetic elements of the humoral arm of adaptive immunity and began by searching for genes encoding three essential components of the B cell receptor signaling complex, namely, *ighm*, *cd79a*, and *cd79b* (*27*). We found that with the exception of *H. mollis*$_{[PAn]}$ and *P. spiniceps*$_{[PAn]}$, all species possess functional *ighm* (fig. S20), *cd79a*, and *cd79b* genes (Fig. 3, A and B, and figs. S21 to S30). These results indicate that most anglerfish species are expected to be capable of mounting antibody-mediated immune responses; however, at least some species that practice consortial pairings lack an adaptive humoral defense system.

Class switching and germinal center reactions are thought to be critical features of antibody-mediated allograft rejection (*28*). We therefore investigated the *aicda* gene, which encodes a cytidine deaminase required for immunoglobulin gene hypermutation and class switch recombination (*29*). Notably, although we found intact *aicda* genes in all three anglerfish species that never form attachments, all 10 ceratioid species ([TA], [PA1], and [PAn]) were found to be *aicda*-deficient (Fig. 3C and figs. S31 and S32). However, remnants of the *aicda* gene could be identified situated between *nat14* and *necap1* flanking genes in several instances, providing evidence that this gene was pseudogenized by deletional mechanisms (Fig. 3C and figs. S31 and S32). By contrast, all species retain two paralogs of *apobec2* (table S3), the members of the cytidine deaminase family most closely related to *aicda* (*30*), supporting the notion that the loss of *aicda* in ceratioids is a selective event. The loss of *aicda* may represent an immunogenetic adaptation to reduce the risk of allo-immunization reactions (*31*), thereby facilitating successful repeated temporary attachments by the same or different males; moreover, this modification of the immunogenome may also be beneficial in the situation of permanent attachment.

Collectively, our findings suggest that attenuation of antibody responses may be required to facilitate temporary attachments; however, complete abrogation of antibody-mediated immunity is not required for permanent exclusive ([PA1]) and consortial ([PAn]) attachments to occur. In the two linophrynid species, *H. mollis*$_{[PAn]}$ and *P. spiniceps*$_{[PAn]}$, the facility of antibody-mediated immunity appears to have been lost, as is evident from the presence of pseudogenized forms of the *cd79a* (Fig. 3A) and *cd79b* (Fig. 3B) co-receptor genes and by our inability to identify sequences related to the constant region of *igm* (fig. S20 and table S3).

Loss of RAG-based somatic diversification of antigen receptors

The findings described above indicate that the immunogenomes of *H. mollis*$_{[PAn]}$ and

P. spiniceps[PAn] have undergone substantial changes, leading to the absence of canonical antigen-specific T cell receptor and B cell receptor signaling pathways, the cornerstones of the prototypical adaptive immune systems of jawed vertebrates for the past 500 million years (*32*–*35*). In the context of the mammalian immune system, the immunogenetic constellation in *H. mollis*[PAn] and *P. spiniceps*[PAn] would be consistent with the lethal condition of severe combined immunodeficiency (*36*). Because this is evidently not the case, we entertained the possibility that the alternative γδ T cell lineage might play a dominant role in the immune defense system of the two linophrynid species and, by inference, perhaps also for *C. couesii*[PAn]. However, the lack of crucial elements of the cd3 complex would argue against the presence of a canonical γδ T cell receptor–CD3 signaling complex in these species.

Whereas compensatory activity of the γδ T cell lineage cannot be excluded for *C. couesii*[PAn], it can be confidently ruled out for both *H. mollis*[PAn] and *P. spiniceps*[PAn]. This conclusion is based on the identification of pseudogenized versions of both *rag1* and *rag2* genes (*37*) in the latter two species (Fig. 4 and figs. S33 to S42). The *rag1* gene, which is encoded in three exons, is disrupted by the invasion of transposable element–related and other types of repetitive sequences in both *H. mollis*[PAn] and *P. spiniceps*[PAn] (Fig. 4 and figs. S33 to S41). The *rag2* gene, which consists of a single coding exon and is located immediately adjacent to the *rag1* gene in tail-to-tail configuration in all known jawed vertebrates, has lost most of its coding capacity in *P. spiniceps*[PAn] because of an interstitial deletion; this leaves behind only short segments of an upstream noncoding segment and a pseudogenized stretch of downstream coding sequences (Fig. 4B). No remnants of *rag2* coding sequences could be found in *H. mollis*[PAn] (figs. S33 and S42). Collectively, these findings demonstrate that *P. spiniceps*[PAn] and *H. mollis*[PAn] have lost the key facilities that characterize classical adaptive immunity of jawed vertebrates (*32*–*35*). They lack genes underlying MHC-based antigen-presentation pathways, genes encoding antigen receptors, and the key elements of the machinery required for somatic assembly of antigen receptor genes. Because these species lack functional *aicda* genes, they cannot use an alternative activation-induced cytidine deaminase–mediated diversification mechanism, a process important for antibody repertoire formation in birds and ruminants (*38*) and assembly of *VLR* antigen receptor genes in lampreys (*39*, *40*). Moreover, we have been unable to find sequences related to distant members of the *Rag*-like gene family, such as the transib transposase from the insect *Helicoverpa zea* (*41*), which might be able to cooperate with an endogenous Rag2-like activity to achieve V(D)J recombination (*42*).

Evolution of sexual parasitism

Of note, no sex-related differences in immune gene content were detected between male and female fish of a given species, indicating that

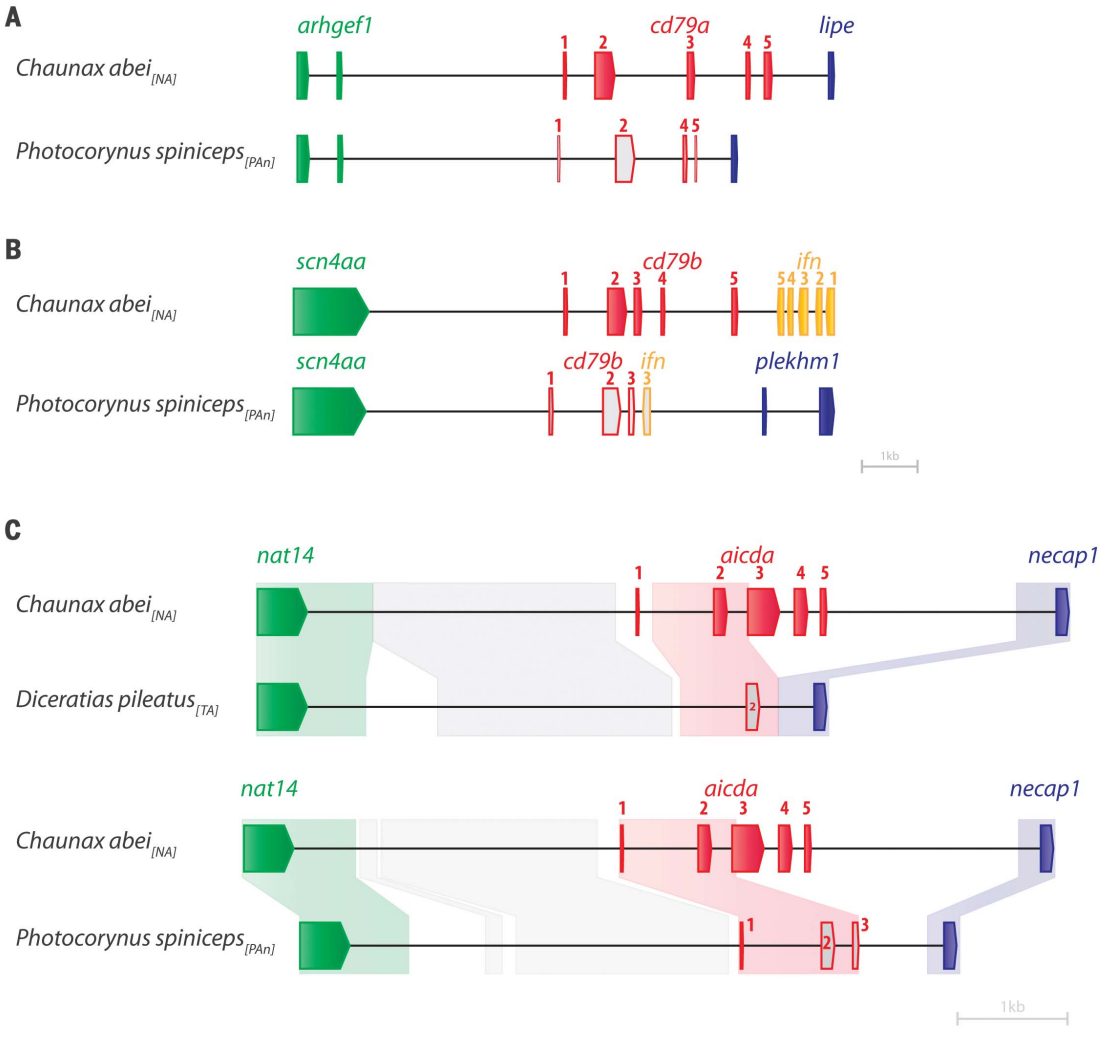

Fig. 3. Structure of genes in the humoral arm. (**A**) Pseudogenization of the *cd79a* gene in *P. spiniceps*[PAn] through deleterious mutations in exon 2, loss of exon 3, and truncation of exon 5. (**B**) Pseudogenization of the *cd79b* gene in *P. spiniceps*[PAn] through deleterious mutations in exons 2 and 3 and an internal deletion affecting a neighboring type I *ifn* gene. (**C**) Pseudogenization of the *aicda* gene in *D. pileatus*[TA] and *P. spiniceps*[PAn] through multiple deletions across the gene and deleterious mutations in several exons; sequence homologies are indicated as shaded blocks.

these changes are species-specific and that the evolution of sexual parasitism does not involve the asymmetrical loss of immune genes in different sexes of the same species. Collectively, our work identifies four types of immunogenetic adaptations associated with sexual parasitism in anglerfishes (Fig. 5A). (i) Temporary attachment ([TA]) appears to minimally require reduced antibody responses in the form of absent affinity maturation (*H. appelii*$_{[TA]}$, *M. johnsonii*$_{[TA]}$, and *D. pileatus*$_{[TA]}$). (ii) Permanent exclusive attachment ([PA1]) is additionally associated with impaired T cell–mediated immune facilities, possibly abolishing cytotoxic immune responses through $CD8^+$ αβ T cells and reducing the magnitude of a helper T cell function through alterations of T cell receptor signaling. (iii) In the situation of permanent consortial ([PAn]) attachments, it appears that canonical cytotoxicity must be completely eliminated and antibody responses be severely blunted through loss of both aicda-mediated affinity maturation and helper T cell function (*C. couesii*$_{[PAn]}$). (iv) In an extreme variation on this theme, the immunogenomes of *P. spiniceps*$_{[PAn]}$ and *H. mollis*$_{[PAn]}$ lack all defining features of RAG-mediated adaptive immunity of jawed vertebrates. *G. vanhoeffeni*$_{[TA]}$ defies simple classification in this system. The immunogenome of this species appears more aligned with that of permanently attaching species than with that of the other temporary attachers examined here; however, to date, no instances of permanently attached males have been documented for this species, despite relatively extensive sampling (*2*). The distinctive immunological changes observed in *G. vanhoeffeni*$_{[TA]}$ could conceivably support a more prolonged period of temporary attachment in this species, or may even be indicative of an intermediate stage on an evolutionary trajectory toward permanent attachment (either [PA1] or [PAn]). At present, it is not possible to resolve the question of whether the observed pattern of gene loss drives attachment mode or vice versa, because a generalized linear model analysis (taking species phylogeny into account) did not reach statistical significance (supplementary methods).

How did sexual parasitism evolve? Are temporary attachment and facultative parasitism precursors to obligate parasitism, or are the former more derived states of the latter? Did obligate parasitism arise more than once? Previous attempts to address these issues have generated phylogenies based on either morphological characters (*43*) or analysis of mitochondrial genomes (*15*). Although these distinct approaches yielded phylogenetic trees with differing topologies, both studies surprisingly concluded that permanent sexual parasitism has evolved in multiple instances, with the temporary attachment mode serving as the ancestral state to obligate parasitism. Our new genetic resources provided the opportunity to readdress these findings, this time from the perspective of nuclear-encoded genes. To this end, we turned to the dataset of orthologous paralogy-free exon markers established by Hughes *et al*. in their reconstruction of the phylogeny of ray-finned fishes (*44*). A total of 946 exons, corresponding to about 86% of the Hughes dataset, were successfully identified in all species included in our study. Phylogenetic reconstruction by Bayesian inference (Fig. 5B) supports the notion that temporal attachment is the ancestral state in Ceratioidei (*15*, *43*). Moreover, grouping the consortial permanent attaching Linophrynidae species, *P. spiniceps*$_{[PAn]}$ and *H. mollis*$_{[PAn]}$, with the temporarily attaching *G. vanhoeffeni*$_{[TA]}$ is in line with the phylogenetic reconstruction of Pietsch and Orr that was based on morphological characters (*43*); this association is also supported by their unusual immune gene constellations. By contrast, the three other temporarily attaching species—*D. pileatus*$_{[TA]}$, *M. johnsonii*$_{[TA]}$, and *H. appelii*$_{[TA]}$—form a separate monophyletic group, in accordance with their distinct immune genotype, characterized by the lack of antibody affinity maturation.

The paraphyletic constellation of *G. vanhoeffeni*, *P. spiniceps*, and *H. mollis* and the four Ceratiidae species supports the conclusions by Pietsch and Orr (*43*) and Miya *et al.* (*15*) of the independent acquisition of permanent attachment of males to females; however, the analysis of additional genomes of other species of this family will be required to strengthen this conclusion.

The sequence of events leading to sexual parasitism and the driving forces behind them remain speculative; however, we view modification of the immune system to curtail allogeneic reactions to be an essential component of the process. Whether the evolutionary advantage of sexual parasitism is itself sufficient to drive changes in the immune system or whether other evolutionary forces first lead to changes in the immune system, which are then exploited for the evolution of sexual parasitism, is an important question to resolve in future studies. The results presented here would suggest that the latter scenario is more likely. For example, *G. vanhoeffeni*$_{[TA]}$ forms only temporary attachments but exhibits losses of a number of genes (e.g., *cd4*, *cd8*) that could potentially allow the formation of more permanent pairings. The lack of the MHC class II pathway per se is not associated with sexual parasitism, because it has also been described in other species, such as cod (*45*), the nonparasitizing anglerfish *Lophius piscatorius* (*46*), and seahorses and pipefishes, which practice male pregnancy (*47*, *48*). We therefore conclude that it is the unprecedented loss of other components of adaptive immunity, as described here, that is associated with the emergence of obligate sexual parasitism in ceratioids.

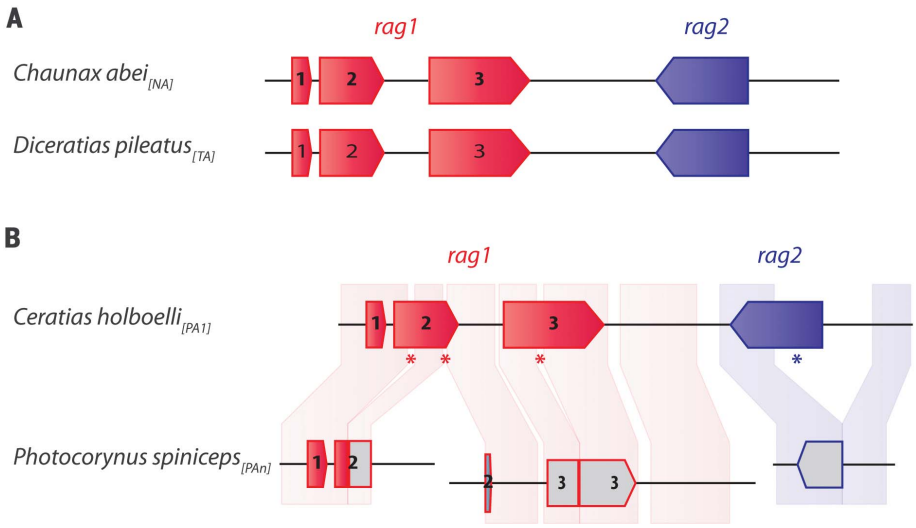

Fig. 4. Deleterious mutations in *rag* genes in species with consortial attachment. (**A**) Configurations of the intact *rag* loci in *C. abei*$_{[NA]}$ and *D. pileatus*$_{[TA]}$, demonstrating the conserved tandem arrangement of *rag1* (red) and *rag2* (blue) genes that is observed in the genomes of all jawed vertebrates reported to date. (**B**) Compared with the intact *rag1* gene in exclusively permanently attaching *C. holboelli*$_{[PA1]}$, the *rag1* gene of *P. spiniceps*$_{[PAn]}$ exhibits a number of deleterious insertions and deletions, resulting in defective coding sequences (indicated by gray shading of exons); the same is true for the pseudogenized *rag2* gene. Deletions resulting in the loss of >100 base pairs of coding sequence (relative to *C. holboelli*$_{[PA1]}$) are highlighted by an asterisk underneath the reference gene; sequence homologies are indicated as shaded blocks.

New types of vertebrate immune systems

The deep sea is known to harbor rich microbial communities (*49*) and is also home to unicellular parasites capable of infecting at least some anglerfish species (*50*). How can fish species subscribing to permanent male parasitism survive despite the loss of antigen-specific cytotoxic activity and antibody affinity maturation on one end of the spectrum and complete loss of RAG-dependent adaptive immunity on the other end? Although we consider it unlikely, we cannot exclude the possibility that anglerfishes have evolved an entirely new type of adaptive immune system. Rather, we propose that the evolutionary emergence of exclusive and consortial parasitization, and the associated loss of several aspects of classical adaptive immunity, was accompanied by reorganization of innate immune defenses to provide efficient protection against pathogens. All ceratioid species possess at least one gene encoding type I interferon (fig. S43), as well as at least one gene encoding interferon γ (fig. S44); likewise, *tnfa* genes were readily identifiable (fig. S45). These findings suggest that key elements of innate immunity are intact. The identification of functional *prf1* (fig. S46) genes suggests that innate cytotoxic functions are present, perhaps mediated by natural killer–like or innate lymphoid cell–like cells (*51*). In addition, the presence of an ortholog of the *il10* gene (fig. S47), as well as of the gene encoding the common γ chain of the IL2 receptor (*il2rg*) (fig. S48), suggests that important regulatory and effector cytokine–mediated regulatory networks may still be in place. Moreover, the identification of genes encoding effector molecules of the Toll signaling pathway [*myd88* (fig. S49) and *ticam1* (fig. S50)] support the notion of intact innate defenses in ceratioids. It is possible that the immunogenetic adaptations in these fishes represent hard-wired versions of the altered immune response profiles seen in *rag1*-mutant zebrafish (*52*).

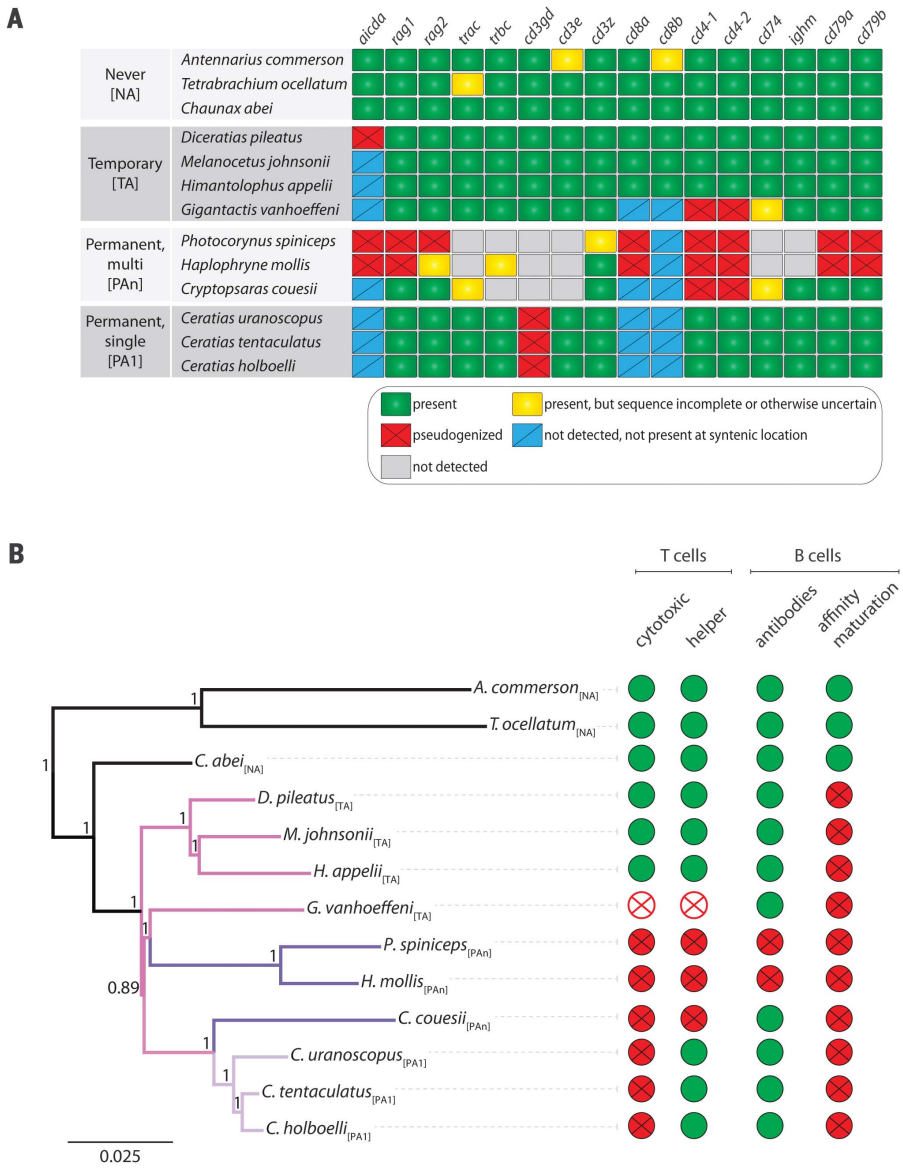

Fig. 5. Immune gene content and phylogenetic relationship of the various anglerfish species examined. (**A**) Tabular summary of gene content. Green shading indicates the presence of a functional gene, yellow indicates gene models that are incomplete or otherwise uncertain, red indicates the presence of a defective gene, blue indicates genes that were not detected and were additionally demonstrated to be absent from their expected syntenic location, and gray indicates genes for which no evidence was detected. An extended version of the table with notes can be found in the supplementary materials. (**B**) Bayesian reconstruction of ceratioid phylogeny based on the analysis of 946 orthologous exons (*44*). The attachment modes are noted next to the species names and are color coded in the phylogenetic tree. Also indicated are the presumed immunological consequences of genetic aberrations. Solid green circles indicate the presence of immune facility, and solid red circles with X's indicate the absence; open red circles with X's indicate that T cell receptors are present but lymphocyte lineage-specific co-receptors are missing.

Conclusions

Our study uncovers a surprising degree of evolvability of immune defenses in vertebrates. Our findings challenge the view that once innate and adaptive immune systems have engaged in coevolution, neither arm can be eliminated without detrimental consequences. The nature of immunogenetic adaptations that are associated with such substantial impairments of adaptive immunity are of particular interest from an evolutionary point of view. Key nodes of evolvability of innate immune gene networks may be revealed by a comparison of the components of innate immune systems before (as present in invertebrates), during (as present in the majority of vertebrates), and after (as present in anglerfishes) coevolution with adaptive immunity. Moreover, such studies also hold promise to learn about potential strategies to enhance innate immune defenses in immune-deficient patients. On a more general level, our study highlights the advances that can be made by exploring the vast phenotypic space of vertebrates more fully. We predict that additional variations on the general design of vertebrate immunity will be discovered among the animals occupying the extraordinarily diverse ecological niches and habitats of this planet.

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SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/369/6511/1608/suppl/DC1
Materials and Methods
Figs. S1 to S53
Tables S1 to S8
References (*54–80*)
MDAR Reproducibility Checklist
Data S1

View/request a protocol for this paper from *Bio-protocol*.

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The immunogenetics of sexual parasitism

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Reconfiguring an immune response

The deep sea is a vast and generally empty environment. Finding a mate can thus be difficult. In response to this situation, one group of deep-sea denizens, the anglerfishes, have evolved a system in which males attach to females, in some cases permanently, through fusion of tissues and connection of circulatory systems. Such attachment greatly challenges the immune systems of the fish. Swann *et al.* found that these challenges have been met by the evolution of increasingly reduced immune responses among anglerfish species, including the loss of what have been considered essential vertebrate responses. These shifts suggest that vertebrate immune systems may be more flexible over evolutionary time than was previously thought.

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