REVIEW

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The paradise fish, an advanced animal model for behavioral genetics and evolutionary developmental biology

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Abstract

Paradise fish (*Macropodus opercularis*) is an air-breathing freshwater fish species with a signature labyrinth organ capable of extracting oxygen from the air that helps these fish to survive in hypoxic environments. The appearance of this evolutionary innovation in anabantoids resulted in a rewired circulatory system, but also in the emergence of species-specific behaviors, such as territorial display, courtship and parental care in the case of the paradise fish. Early zoologists were intrigued by the structure and function of the labyrinth apparatus and a series of detailed descriptive histological studies at the beginning of the 20th century revealed the ontogenesis and function of this specialized system. A few decades later, these fish became the subject of numerous ethological studies, and detailed ethograms of their behavior were constructed. These latter studies also demonstrated a strong genetic component underlying their behavior, but due to lack of adequate molecular tools, the fine genetic dissection of the behavior was not possible at the time. The technological breakthroughs that transformed developmental biology and behavioral genetics in the past decades, however, give us now a unique opportunity to revisit these old questions. Building on the classic descriptive studies, the new methodologies will allow us to follow the development of the labyrinth apparatus at a cellular resolution, reveal the genes involved in this process and also the genetic architecture behind the complex behaviors that we can observe in this species.

KEYWORDS
behavioral genetics, evo-devo, paradise fish

1 | INTRODUCTION

"The golden-green only reaches small dimensions: it is usually only one or two decimeters long: but it is very pleasant to see; its colors are magnificent, its movements light, its evolutions varied; it animates and decorates in a charming way the limpid water of the lakes," so begins one of the first scientific description of the paradise fish (*Macropodus opercularis*) by the French naturalist Bernard Germain de Lacépède in his landmark study "Histoire Naturelle Des Poissons" (Lacépède, 1802). The scientific name of this tropical freshwater fish has changed over time (from *Labrus opercularis* [Linné, 1758] and *Macropodus viridauratus* [Cuvier, 1831; Lacépède, 1802]) but its popularity as an ornamental species has been almost constant since before the 18th century (Figure 1a–c).
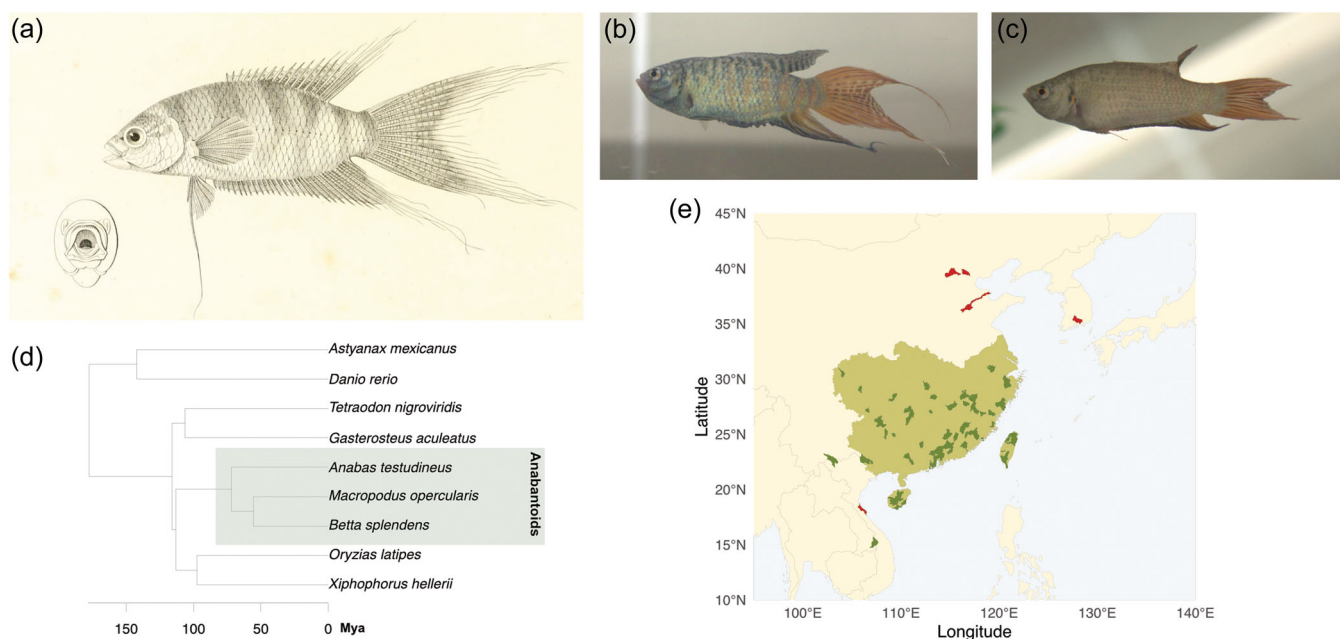
FIGURE 1 Phylogeny and distribution of the paradise fish (*Macropodus opercularis*). (a) One of the first scientific depictions of the paradise fish by Cuvier (Cuvier, 1831). (b, c) Sexually dimorphism in adult paradise fish: a typical male (b) and female (c) fish are shown. (d) The phylogeny of paradise fish relative to other popular model species and other anabantoids (extracted from Fishtree [Chang et al., 2019]). (e) The geographic distribution of paradise fish (dark green—resident populations, olive green—probably extant resident populations, red—introduced populations) (source of data: [IUCN, 2022]).

As paradise fish and its close relatives in the Anabantiformes order (Figure 1d) typically live in brackish lagoons, ditches, ponds with dense vegetation and other hypoxic environments of their natural range in Southeast Asia (Figure 1e) (Graham, 1997; Henninger, 1907), one might think that this species became a popular model of laboratory research due to its peculiar physiological adaptations, like obligate air breathing. Yet this is only part of the truth: while in the early years of the 20th century some scientists were indeed fascinated by these adaptations, by the mid-century the interest in paradise fish has faded and the renaissance of paradise fish research after the 1950s was due to this species' undemanding nature and complex social behavior which made it a good and hardy laboratory model for behavioral research (Bush & Wilson, 1956).

While hardiness is hardly an irrelevant trait in a model species, we will argue in this review that the choice of paradise fish for ethological research was an inspired one, as this species has a complex behavioral repertoire that makes it a prime subject for behavioral genetic research (Csányi, 1993). Furthermore, the multiple adaptations derived from its special natural history make it an exciting model for evo-devo and eco-evo-devo studies, which is also ideally suited to answer some questions with biomedical relevance.

1.1 | Anatomical adaptations to air breathing

Just as life in general, the first vertebrates evolved in water. In this medium gills were generally evolutionary adaptive as they facilitated gas exchange. This organ alone, however, is only sufficient for gas exchange if the water has an adequate oxygen concentration. Aquatic habitats, on the other hand, also include waters with low dissolved oxygen concentrations, and as soon as the landmasses (and later the air) started to teem with life, they too became frontiers to be conquered by vertebrates. Adapting to these novel environments, where oxygen uptake from the water was insufficient or outright impossible, necessitated the evolution of specialized organs that can help gas exchange from the air as well. Accordingly, we can document numerous evolutionary events that led to the origin of air breathing: a recent analysis of extant vertebrates suggests that convergent evolution of air breathing species from water breathing ancestors happened more than 80 times (Damsgaard et al., 2020). Numerous anatomical structures have evolved to be used for aerial gas exchange during this process, including the swim bladder, the skin, the gastrointestinal tract and the mouth (Graham, 1997).

In the case of labyrinth fishes (or anabantoids) a specific modification of the first gill epibranchial results in a characteristic structure, the labyrinth organ (LO) (Graham, 1997; Tate et al., 2017). The LO fills an enlarged suprabranchial cavity (SBC), a hallmark structure of the group (Figure 2a,b). This cavity can be sealed off from the mouth cavity by interlocking outgrowths (clefts) of the roof and the first gill (Henninger, 1907; Peters, 1978). Both the LO and the SBC are covered by a fine epithelial membrane, which has essential roles in gas exchange (see below) and which is sometimes referred to as the "labyrinth pocket" (Zograff, 1886).
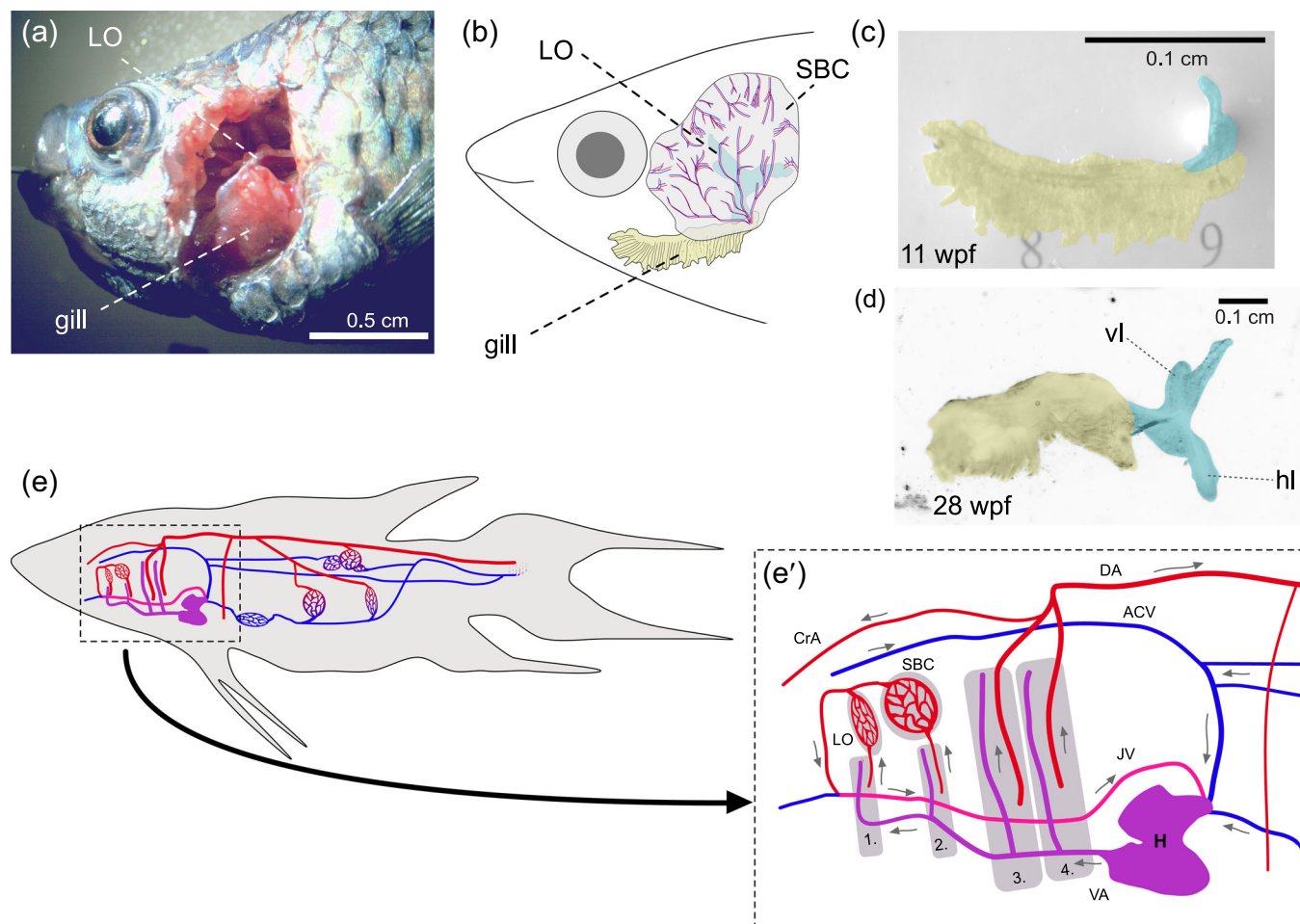
FIGURE 2 Development of the LO and mixed circulation in post-metamorphic paradise fish. (a) Well developed LO and gills are shown in situ in the labyrinth pocket. (b) Schematic view of the paradise fish head region: the walls of the SBC (light gray) are highly capillarized, and the LO (cyan) extends into the SBC. (c) Gill (yellow) and LO (cyan) dissected from an 11 weeks post fertilization (wpf) fish shows only a rudimentary trunk being present at this stage. (d) At 28 wpf, the trilamellar organization of the paradise fish LO can be observed. (e, e') In postmetamorphic paradise fish the circulation through the first and second gill arches is rewired towards the labyrinth apparatus. This way, after gas exchange occurs within the 1st and 2nd gill, oxygenated blood (red) enters the capillary system of the LO and SBC, respectively. Reoxygenated blood is then collected into vessels that joins the jugular vein (JV). The jugular, therefore, carries a mixture of oxygenated and unoxygenated blood (pink) back to the heart, where it is mixed with unoxygenated blood (blue) coming from other veins (e.g. anterior cranial vein—ACV). The mixed blood (purple) enters the ventral aorta (VA) from where it flows into the afferent arteries of the gills. The efferents exiting the 3rd and the 4th gill arch fuse to form the dorsal aorta (DA). A detour from the efferent of the 3rd arch forms the carotid artery (CrA). (Based on [Bader, 1937]). Gray arrows denote the direction of the blood flow within the blood vessels.

Even the earliest descriptions of anabantoids have made a link between the presence of the LO and the extreme adaptations to land-dwelling of some anabantoid species (e.g. the climbing perch [*Anabas testudineus*]). The early zoologists, however, thought that the LO functions as a sponge that retains water to aid gas exchange over the gills and keep them moist during the spells on the land (Cuvier, 1831). It was only during the late 19th and early 20th century when more detailed observations of living specimens (Das, 1928; Zograff, 1888, 1886) lead to the recognition that the LO's principle role in anabantoids is of "a supplementary respiratory apparatus, helping the fish to breathe while in open air" (Zograff, 1888), and the relative simplicity of the paradise fish LO (especially compared with those of climbing perches), was an important consideration that helped to refute the "sponge-theory" of LO

function (Zograff, 1886). When anabantoids exhale, water enters the operculum and expels the air from the SBC through the buccal cavity into the atmosphere (sometimes air might be seen escaping through the operculum in the form of bubbles). Later, during inhalation, the gulped air flows through the buccal cavity into the SBC, displacing the water in the chamber, which then flows out under the operculum (Peters, 1978).

1.1.1 | Development of the labyrinth apparatus

The development of the LO starts only in the post-metamorphic stage and it reaches its final, tri-lamellar structure gradually (Figure 2a) (Bader, 1937; Das, 1928; Henninger, 1907).

One of the earliest hallmarks of labyrinth development in juvenile fish is an epithelial proliferation in the dorsal gill cavity from the pseudobranch, to the point of entry of the first gill arch in the oral cavity roof. This stage is followed by the proliferation of the underlying mesenchyme and a concomitant loss of the basal membrane that previously separated the epithelium and the mesenchyme. At the end of this proliferative burst, numerous blood lacunae appear in the mesenchymal tissue that will become capillaries, and the basal membrane between the epithelium and the underlying mesenchyme is reformed. Differentiation results in the transformation of cells between individual capillaries into pillar cells and the flattening of overlying epithelial cells into a single layer of stretched, very thin epithelium. By the end of the differentiation process the SBC forms and becomes separated from the pseudo-branch (Bader, 1937).

The proliferative burst in the gill cavity also results in the elongation of the articular process of the epibranchial segment of the first gill arch. For a while it remains in contact with the respective pharyngobranchial, therefore, it will span the forming SBC. As the SBC grows and develops, the articular process also becomes progressively longer. After the SBC reaches a certain size, however, the connection with the pharyngobranchial is severed and the elongated process continues to grow within the SBC and will ultimately become the labyrinth trunk (Figure 2b,c) (Bader, 1937).

During normal development, after the animal reaches a certain size (~15−20 mm) two easily distinguishable lamellae will start to grow on the trunk: a caudally directed one that grows in the horizontal plane, and an anterior, ventricular lamella (Figure 2d). The trunk and the lamellae together will form the LO and their surface (similarly to the wall of the SBC) will be covered by capillaries lying under a thin epithelial layer (Bader, 1937).

A peculiar feature of LO development is its plasticity. When blocked from accessing the water surface, adult paradise fish can only survive in well oxygenated water, otherwise they die in a matter of hours (Bader, 1937; Henninger, 1907). However, juvenile paradise fish without well-developed LOs can be raised without surface access for months. A close observation suggests, however, that in such fish the capillarization of the SBC and LO is severely reduced, villous elevations can be observed in the walls of the labyrinth cavity and the development of the lamellae is also affected. Interestingly, when such fish are let to surface, the developmental anomalies of the labyrinth system will disappear, and in a matter of weeks a normal LO develops (Bader [1937] and our unpublished observations). These observations suggest that the latter aspects of LO development are under environmental control and air-breathing itself exerts a regulatory influence on the development of the air-breathing apparatus.

1.1.2 | Vasculature of the LO

As expected in the case of a breathing organ, the surface of the LO serves as a conduit for gas exchange. The specialized blood supply of the LO emanates from the efferent branchial arteries of the first gill

arches. The labyrinthine artery (*arteria labyrinthica*) sequentially branches first into the individual lamellae then within the lamellae themselves, finally forming a dense network of capillaries. The walls of the SBC are also enmeshed by capillaries that originate from the efferent branchial arteries of the first and second gill arches (*arteria sacculi externa* and *arteria sacculi interna*, respectively) (Bader, 1937; Henninger, 1907). These capillaries are covered by a thin epithelium and individual capillary territories ("fields," according to Henninger) are separated by capillary-free epithelial strips ("streets") made up of multilayered epithelium, rich in mucous-secreting cells (Bader, 1937; Grigorian, 1900; Henninger, 1907; Zograff, 1888, 1886). After gas exchange occurs, oxygenated blood is collected into ducts that converge into the major veins of the LO and SBC which merge prior entering the jugular vein (Bader, 1937; Henninger, 1907). It is worth highlighting that in this specialized circulatory system the descending aorta only receives oxygenated blood from the efferent arteries of gill arches 3 and 4, whereas oxygenated blood that passes through gill arches 1 and 2 and then the labyrinth system is diverted to the jugular vein where it mixes with the venous blood of the anterior cardinal veins before it re-enters the heart (Figure 2e,e') (Bader, 1937; Henninger, 1907).

Interestingly, during the early stages of development the circulatory system of the larval and juvenile paradise fish follows an archetypical teleost ground plan, with the efferents of gill arches 1 and 2 also contributing to the descending aorta. The development of the LO and SBC, with their specialized, capillary networks will result in the significant remodeling of this pre-existing circulatory framework, and will lead to the development of the anabantoid-specific circulation outlined above (Bader, 1937).

1.1.3 | Evolution of the LO

The gill-like structure of the lining covering the LO and the walls of the SBC has attracted considerable speculation. While some early descriptions called this the "labyrinth gill," there is no direct evidence to support the homology of gills and LO lining (Bader, 1937). Yet, it is still noteworthy that the proliferative origins of the labyrinth apparatus lie in the proximity of the pseudobranch and the epibranchial of the first gill arch. Further studies will be needed to show whether this is just pure coincidence, or indeed, these structures have some inductive effects in the process.

To clarify the true evolutionary origins of the labyrinth apparatus, it will be also important to perform fate-mapping experiments and compare the gene expression programs that are activated during the formation of the LO and SBC with those observed during standard gill development. It is noteworthy that the modular activation of the gill developmental program can be also observed during the development of the pseudobranch itself (Thiruppathy et al., 2022). As demonstrated recently, this structure, which is derived from the mesenchyme of the mandibular arch, does not only show anatomical resemblance to gill filaments, but also shares a gene expression

program with those (Hirschberger & Gillis, 2022; Thiruppathy et al., 2022).

The appearance of evolutionary novelties often involve repurposing of existing genes, signaling pathways or complete signaling modules by the evolution of novel enhancer sequences in their proximity (Rebeiz & Tsiantis, 2017; True & Carroll, 2002). The LO and the complete labyrinth apparatus are excellent examples for a true evolutionary novelty, therefore, it will be very revealing to uncover which developmental modules are being reutilized during their ontogeny.

1.2 | Specialized behavior of paradise fish

Writing about labyrinth fishes in 1888 Nicholas Zograff noted: "we learn from the accounts of travelers that these fishes develop more mental activity than any other species of the same class; such instances we see, for example, in their mode of nest building (Macropodus, Trichogaster), their changes of basins (Anabas, Ophiocephalus), and, lastly, in their way of getting food (Toxotes)" (Zograff, 1888). And indeed, further rigorous experiments supported this observation, demonstrating that the paradise fish is an inherently curious species, with a complex behavioral repertoire. Some elements of this repertoire are directly related to its special physiology and adaptations to its environment, while others derive from the complex social networks these fish can develop in their natural habitats (Csányi, 1993; Rácz et al., 2021; Tate et al., 2017).

1.2.1 | Behavioral adaptation to air breathing

The evolution of the LO and SBC were necessary preconditions for air breathing, but in the absence of appropriate physiological and behavioral adaptations would have been hardly relevant (Damsgaard et al., 2020).

Breathing activities in all fishes are driven by their oxygen demands, and as the oxygen content of the air is higher than that of the water, aquatic surface respiration can be observed during aquatic hypoxia even in those species that have no adaptations and normally only rely on unimodal water breathing (Florindo et al., 2018; Milsom et al., 2022). The control of these breathing activities in typical teleosts is debated: recent evidence has highlighted the importance of neuroepithelial cells, located in the gills, as peripheral chemoreceptors driving breathing activities. Yet, overall the regulation of ventilation in fish is still poorly understood and in some species emerging evidence also suggests the presence of central chemoreceptors and/or central circuits controlling breathing activities (Damsgaard et al., 2020; Florindo et al., 2018; Milsom et al., 2022; Pan & Perry, 2023; Zaccone et al., 2018).

Fish with bimodal (air and water) respiration, such as the paradise fish are especially interesting as their study could reveal details about how the different modalities of breathing can be coordinated and how peripheral and central chemoreceptors can cooperate to achieve this.

In adult paradise fish kept under standard conditions, a regular air breathing activity of ~1 per minute can be observed. Early observations of labyrinth fish already provided evidence about the importance of surface breathing: adult fish being completely blocked from accessing the surface will struggle and die in a matter of hours unless their water is well oxygenated. Increased activity results in higher oxygen demand, while elevated water temperatures lower oxygen solubility, consequently both will increase air-breathing frequency in labyrinth fish (Bader, 1937; Henninger, 1907). When blocked from accessing the surface, fish will compensate with more intense and faster water breathing. The frequency of these ventilatory movements can increase up to one order of magnitude (Bader, 1937).

The onset of air breathing activity starts around 1 month of age, thus somewhat concomitantly with the development of the LO and the rewiring of the circulation in the 1st and 2nd gill arches (Bader, 1937). We do not have any direct evidence about peripheral or central chemoreceptors having a role in the regulation of surface breathing, but the fact that males can suppress air breathing for a while during dominance fights suggests a central regulation of this behavior (Damsgaard et al., 2020). It is also worth emphasizing that air-breathing is an evolutionarily risky behavior, as during surface access fish are more exposed to their predators.

1.3 | Sexual behavior

Paradise fish are territorial species, with complex courting and brood care behaviors. Adult fish are sexually dimorphic both in their appearance and behavior. Males sport long dorsal, caudal and anal fins, and a bright coloration with vibrant blue and red stripes, while females have rounder bellies and are significantly paler (Rácz et al., 2021). Territorial males also show a higher level of aggression towards conspecifics than females, especially towards other males (Davis et al., 1974).

Previous work has already described the ethogram of the species, defining several characteristic elements of its complex behavioral repertoir regarding either social or non-social contexts (Csányi, 1993; Csányi et al., 1985a, 1985b; Rácz et al., 2021). For example, during aggressive male-male encounters usually a combination of the following behavioral elements can be observed: head-tail display (Figure 3a), head-head display (Figure 3b), parallel swimming, flaring of the gill covers (opercula). If territorial disputes cannot be solved with these ritualized display behaviors, physical aggression follows. This could involve shaking, biting and mouth locking (Figure 3c). Opercular flares and the subsequent mouth locks are highly costly behaviors for the involved males, as they impede ventilation and lead to hypoxia when, in natural habitats, the water is already low in dissolved oxygen. For this reason, these elements are only used briefly and rarely (Tate et al., 2017).
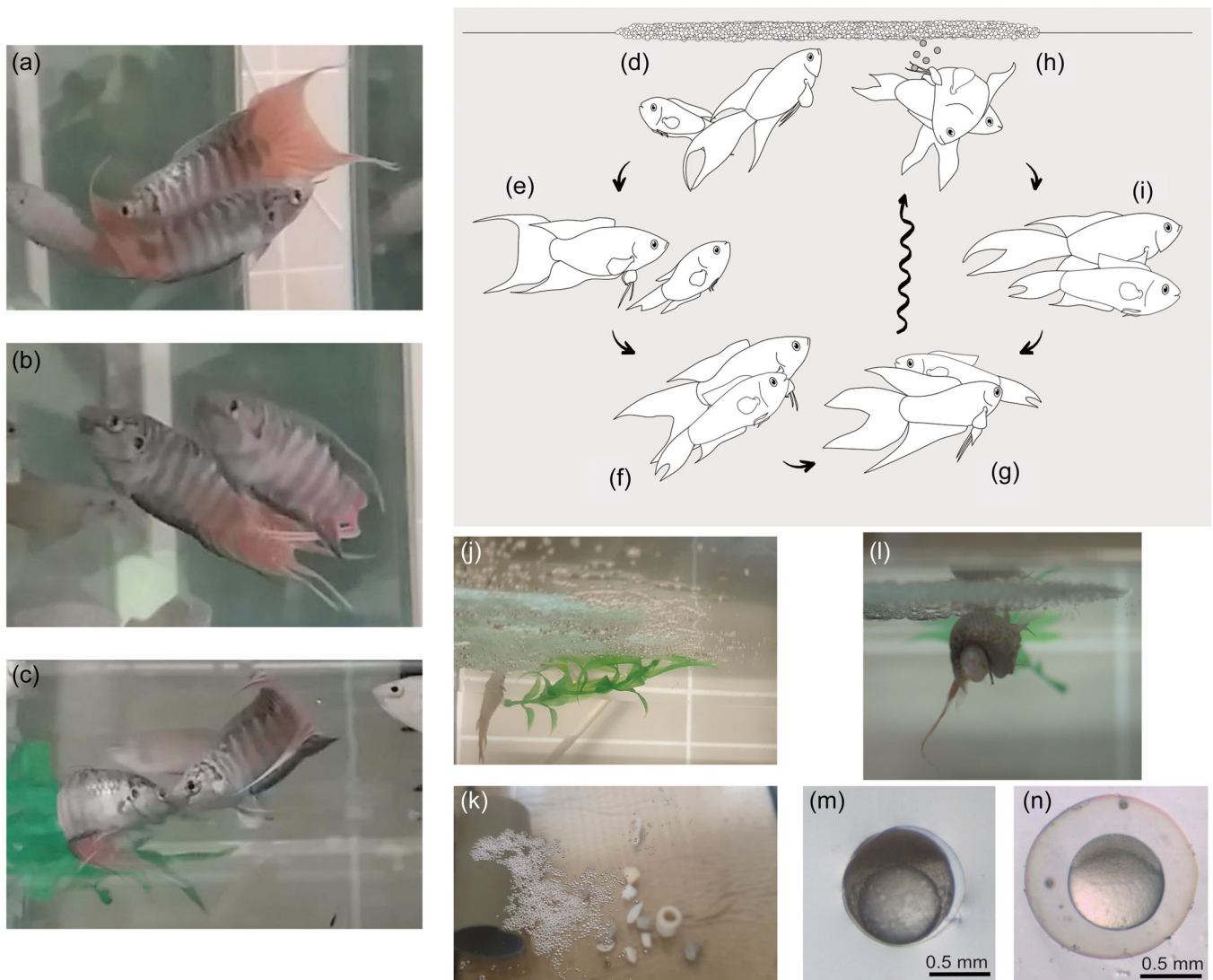
FIGURE 3 Paradise fish behavior. (a) Male paradise fish performing a head-tail display. (b) Males in head-head display position. (c) Paradise fish males in mouthlock. (d−i) Steps of paradise fish courtship: (d) nest building, (e) male fish performs lateral spread display, (f) male presents the bubble nest to the female, (g) female and male swim in circles under the nest, (h) spawning with anabantoid, (i) swimming inhibition and sinking. (Figure from: [Rácz et al., 2021]). (j, k) Close-up views of a typical bubble nest from the bottom (j) and the top (k). (l) Breeding paradise fish are shown during a typical "anabantoid embrace". (m, n) Comparison of paradise fish (m) and zebrafish (*Danio rerio*) embryos (n) at the beginning of gastrulation. Note the large oil droplet within the yolk of the paradise fish embryo and also the significantly larger perivitellinar space surrounding the zebrafish embryo.

Courtship (Figure 3d–i) and parental care in paradise fish are even more remarkable (and interlinked) behaviors, which made this species a popular target for ethological observations (Hall, 1968). Breeding behavior is initiated with nest building by males. This peculiar behavioral feature, observed in some other anabaontoids as well, is made possible by the special morphological adaptations of these fish, namely air-breathing using its LO, filled with mucous-producing cells.

Bubble nests are built using mucous-covered air bubbles that are positioned under floating plant leaves near the air-water surface. Once a nest is built, the male presents it to the female (Figure 3e,f), which will either accept it or reject it (rarely, the female can also join the nest building effort). If the female approves the nest, they will

swim under it (Figure 3g). The male extends its caudal fin and then approaches the female, pointing sideways and vibrating his whole body. The female then moves closer to the male, also pointing to the side, and they both "cuddle" in a C shape. The male turns the female around (this is the stereotypical "anabantoid embrace" [Figure 3h,j]), at which point egg-release and external fertilization occurs. Lastly, the pair sinks together without moving (Figure 3i). The procedure can be repeated several times—on average 15–16 bouts per spawning sequence occur in paradise fish which last for several hours and will result in ~900 eggs (Hall, 1968; Rácz et al., 2021; Vierke, 1975).

After the end of the spawning sequence the pair will retrieve and relocate the eggs that missed the nest. Soon, however, the males will start guarding the nest and do not allow the females near their nests

any longer. Paternal care will last for several days, until the hatched larvae are old enough to leave the nest (Hall, 1968; Rácz et al., 2021).

While our understanding about the neural networks involved in these behaviors is scant at the moment, it is noteworthy that early ablation experiments support the central role of the telencephalon in courtship and nest building (Davis et al., 1976; Kassel & Davis, 1977; Schwagmeyer et al., 1977).

To our knowledge, the adaptive value of building bubble nests was not addressed directly, but it is likely that the proximity of air will help with the gas exchange of developing embryos in the natural hypoxic waters. Gas exchange is also aided by the increased buoyancy of the eggs achieved by the enlarged oil droplets found in them (Figure 3k,l) (Yu & Guo, 2018). These droplets are not universal within anabantoids (e.g., they are not present in the eggs of Bettas) and will keep the embryos and young larvae floating in the proximity of the surface (Vierke, 1975, 1991). Of note, this enlarged early buoyancy also results in a peculiar upside-down, "hanging-larval" position of the developing embryos and young larvae (Vierke, 1975), and most likely requires the remodeling of neural networks involved in posture stabilization as the oil is being slowly absorbed and the swim bladder develops at later stages of larval development.

The scientific relevance of such a complex behavioral repertoire becomes more apparent when we compare it with that of zebrafish (Danio rerio). Zebrafish is the most widely used fish model in biomedical research and despite the undisputable advantages of observing this species at larval stages, the behavior of these larvae is limited to basic survival responses that are often assessed in ethologically irrelevant contexts, taking the individuals out of their social environment. In contrast, paradise fish follow a specialized and different path during the development of their behaviors (our unpublished results). Also, due to the solitary nature of paradise fish the experimentally favorable individual testing would have a lesser effect on their behavioral responses compared with zebrafish. Over the past years highly specific and sensitive manipulating and monitoring strategies have been developed for observing larval zebrafish (Portugues et al., 2013). Now we have the opportunity to apply these methodologies to understand the background of various internal states and behaviors of other species, such as paradise fish. The list of possibilities is long, but it is important to highlight the potential of in vivo imaging of the whole brain to assess the neurological background of complex contextual responses, such as an aggressive encounter or a cognitive task. Longer behavioral sequences also give us the opportunity to better understand the neural underpinnings of more behavioral elements, their transitions and their specific roles.

1.4 | Genetics

The paradise fish genome is relatively compact, it is ~485 Mb long and spreads over 23 chromosomes (unpublished observation of our group and [Abe, 1975]). Unsurprisingly, given their phylogenetic proximity, the paradise fish genome also shows high synteny to the recently published Betta splendens genome (~450 Mb on 21 chromosomes) (Fan et al., 2018; Kwon et al., 2022; Wang et al., 2021; Zhang et al., 2022). Most extant paradise fish populations (both resident and recently introduced) studied so far show very low levels of genetic diversity, which probably indicates habitat fragmentation coupled with high levels of inbreeding (Kano et al., 2018; Tzeng et al., 2006; Wang et al., 1999). (This finding also coincides with our unpublished observations of domesticated and wild-type strains).

The combination of a compact genome with well documented, complex social behaviors provides an ideal base to develop a model for behavioral genetics. Indeed, existing evidence supports a strong genetic component for some of the signature behaviors paradise fish display. For example, an early comparison of domesticated and wild-type populations showed that key components of their sexual behavior differ significantly: wild-type males are more aggressive and breed faster, whereas animals observed in captivity after many generations build larger nests and have a larger brood (Ward, 1967). It has been also noted that different inbred paradise fish strains created through gynogenesis, and the recombinant strains derived from them can show quite differing behaviors under identical conditions (Csányi, 1993; Csányi & Gervai, 1986; Gervai & Csányi, 1985).

2 | DISCUSSION

A combination of anatomical and behavioral peculiarities and a small genome make anabantoids an especially attractive complementary model both for evo-devo research and behavioral genetics.

For example, uncovering the evolutionary and developmental origins of the labyrinth apparatus will reveal the genetic changes behind the emergence of an evolutionary novelty. While recent evo-devo studies have uncovered numerous examples for the co-option of genes and gene-networks during the evolution of new innovations in invertebrates (e.g. the evolution of the treehopper helmet, the beetle gin-trap, or the mollusc radula [Fisher et al., 2020; Hilgers et al., 2018; Hu et al., 2018]), our understanding about similar processes in vertebrates is significantly scanter. The developmental remodeling of the circulation in parallel with the emergence of the labyrinth apparatus during metamorphosis could also offer a new paradigm for understanding dynamic changes within the context of functional circulatory system.

Given the large number of interesting questions to be tackled, well-established breeding protocols (Rácz et al., 2021) and a high-quality reference genome (Fodor et al., 2023), the paradise fish is well on track to become a new, complementary genetic model organism (Matthews & Vosshall, 2020).

As a somewhat parallel surge of interest in the biology and genomics of the Siamese fighting fish has produced several high-quality genomic datasets in the past few years (Fan et al., 2018; Kwon et al., 2022; Wang et al., 2021; Zhang et al., 2022), the comparative genomic analysis of M. opercularis and B. splendens can

also add interesting insights into genome evolution in general and the potential genetic underpinnings of divergent features in these two species (e.g., oil metabolism necessary for egg development).

These studies can be extended to interspecific comparisons of transcriptome changes during specific behaviors. Transcriptomic studies have been already performed to uncover neurogenomic states in *B. splendens* during aggressive encounters (Vu et al., 2020, 2021). Comparing the transcriptome changes in two closely related species under similar circumstances will help us understand whether the regulation of these behaviors is robust (i.e., the same genes are involved) or plastic (expression of somewhat different gene sets leads to convergent outcomes). These comparative studies might also help us understand why the domestication of Bettas led to the emergence of hyperaggressive subtypes, whereas the same process in the case of the paradise fish did not produce similar exaggerated territorial behaviors (i.e., they are significantly more tolerant to their tankmates). Interspecies comparison might also reveal the molecular origins of divergent parental behaviors: why do Siamese fighting-fish males care for the eggs and fry alone, whereas in case of the paradise fish both parents might be involved in guarding their offspring.

Decades of ethological observations have already created a vast literature about paradise fish behavior, defining the paradise fish ethogram under diverse conditions (Csányi, 1993; Miklósi et al., 1995, 1997; Rácz et al., 2021). Recent methodological advances, such as the adoption of novel, open-source methods for automated video analysis and pose estimation, such as DeepLabCut, DeepPoseKit or PoseR, will enable high-throughput data analysis, introducing new quantification standards into ethology (Graving et al., 2019; Luxem et al., 2023; Mullen et al., 2023; Nath et al., 2019). We posit that adopting these tools to paradise fish will reignite the interest in the complex behaviors this species manifests. Ethograms for different wild-type populations and later their genomic comparisons could also help identifying genetic changes relevant for differing behaviors. For such studies it would be especially helpful to use isogenic strains, so it is especially noteworthy that the low genetic diversity in natural paradise fish populations suggests a tolerance for inbreeding. If multiple isogenic strains could be established in paradise fish, similarly to medaka (*Oryzias latipes*) (Fitzgerald et al., 2022), the untangling of more complicated gene-environment interactions (GxE) would also become a lot easier.

The expansion of the molecular toolkit will be also crucial. While no successful manipulation of the paradise fish genome has been hitherto published, the recently reported successful genetic manipulation in the closely related *B. splendens* (Palmiotti et al., 2023) suggests that the manipulation of anabantoid genomes is feasible. The creation and ethological analysis of genome-engineered lines that either carry mutations in relevant neural genes or reporters for neural function, such as GCaMP, widely adopted in zebrafish (Portugues et al., 2013), will make further progress possible and could extend behavioral research in paradise fish to larval stages as well.

It is worthwhile to emphasize that the aforementioned anatomical, developmental and behavioral peculiarities will also make the paradise fish an excellent model for a number of research programs with biomedical interest. For example, understanding the dynamics and molecular prerequisites of vasculature reorganization in vivo could help to deepen our understanding of vascular remodeling in cancer, which is important both for the diagnosis and therapy of malignant tumors (Farnsworth et al., 2014). Further studies on paradise fish could also provide clues for successful tissue engineering treatments in regenerative medicine, where the development of functional microvascular networks is of utmost importance (Meng et al., 2021).

Oocyte lipidation observed in paradise fish eggs could provide a new model for the study of lipid transport through the circulation, as lipoproteins deposited in the oocytes are produced in other tissues of the female fish and transported through the maternal circulation (Hiramatsu et al., 2015). The transport of lipids and lipoproteins is an important factor in cardiovascular disease (Bhargava et al., 2022), therefore, a better understanding of the physiology of lipid transport regulation in female paradise fish could reveal novel therapeutical targets.

Paradise fish, similarly to many other air-breathing fish, evolved to cope with hypoxic environments (Graham, 1997). Thus, a detailed understanding of the physiological and molecular changes that occur in these fish when they encounter environments with low oxygen concentration will help us devise better strategies for humans who need to cope with similar conditions (e.g., patients with anemia, heart failure or pulmonary diseases).

Finally, our preliminary observations suggest that the behavioral responses of paradise fish larvae kept in isolation are much more robust than those of their zebrafish peers. This finding might be unsurprising given the substantial differences between the social structure of the natural populations of the two species, yet it also suggests that paradise fish larvae are much better suited to be used in the testing of drugs that affect behavior.

In summary, we believe that the time is ripe for "rediscovering" paradise fish. Many of the biological questions that excited zoologists a century ago or the ethologists working with this species in the late 20th century are still relevant. But now we also have the molecular methods at our hands to answer these questions and expand the use of this model into biomedical research as well.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

No new data were created or analyzed in this study, therefore, data sharing is not applicable to this article.

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