© 2021. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2021) 224, jeb242490. doi:10.1242/jeb.242490

RESEARCH ARTICLE

Diversity of lateral line patterns and neuromast numbers in the genus *Oryzias*

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ABSTRACT

A remarkable diversity of lateral line patterns exists in adult teleost fishes, the basis of which is largely unknown. By analysing the lateral line patterns and organ numbers in 29 *Oryzias* species and strains we report a rapid diversification of the lateral line system within this genus. We show a strong dependence of lateral line elaboration (number of neuromasts per cluster, number of parallel lateral lines) on adult species body size irrespective of phylogenetic relationships. In addition, we report that the degree of elaboration of the anterior lateral line, posterior lateral line and caudal neuromast clusters is tightly linked within species, arguing for a globally coordinated mechanism controlling lateral line organ numbers and patterns. We provide evidence for a polygenic control over neuromast numbers and positioning in the genus *Oryzias*. Our data also indicate that the diversity in lateral lines can arise as a result of differences in patterning both during embryonic development and post-embryonically, where simpler embryonic patterns generate less complex adult patterns and organ numbers, arguing for a linkage between the two processes.

KEY WORDS: Medaka, Body size, Patterning, Phenotypic diversity

INTRODUCTION

The lateral line system is a sensory system specific to fishes and amphibians (Pichon and Ghysen, 2004; Coombs et al., 2014). Its main function is to sense water flow changes and relay the information back to the brain. It does so by relying on specialized organs (neuromasts) located either superficially or within canals over the body surface in species-specific patterns (Ledent, 2002; Sapede et al., 2002; Ghysen and Dambly-Chaudière, 2007). This sensory system is important for fish shoaling, predator avoidance and rheotaxis (Ghysen and Dambly-Chaudière, 2007; Montgomery et al., 1997; Suli et al., 2012; Coombs et al., 2014). It is composed of two major parts that have different developmental origins (Nikaido et al., 2017; Piotrowski and Baker, 2014): the anterior lateral line (aLL) system and the posterior lateral line (pLL) system. The aLL consists of neuromasts around the head, jaw and opercle of fish (Piowtroski and Baker, 2014; Ghysen and Dambly-Chaudière, 2007); developmentally, these arise from ectodermal placodes (Piowtroski and Baker, 2014). The pLL consists of neuromasts positioned along the trunk and tail (Kimmel et al., 1995; Ghysen and Dambly-Chaudière, 2007); these arise from migrating primordia along the anterior–posterior axis during development (Ghysen and Dambly-Chaudière, 2007; Lecaudey et al., 2008; Nechiporuk and Raible, 2008). The aLL has received scant attention from the scientific community and very little is known about it (Ghysen and Dambly-Chaudière, 2007), whereas the pLL has been systematically studied in a number of teleost species, primarily so in the zebrafish. There, a wide literature covers embryonic development of the pLL in molecular detail (Aman and Piotrowski, 2008; David et al., 2002; Grant et al., 2005; Haas and Gilmour, 2006; Hernández et al., 2006; Lecaudey et al., 2008; López-Schier and Hudspeth, 2005, 2006; Lush and Piotrowski, 2014; Ma and Raible, 2009; Nechiporuk and Raible, 2008; Sánchez et al., 2016) and post-embryonic growth mechanisms of neuromasts have also been analysed (Nuñez et al., 2009; Ledent, 2002; Sapede et al., 2002).

Teleost fishes grow throughout life (Karkach, 2006; Johns and Easter, 1977) and more neuromasts are added to cope with a constantly growing body (Ghysen and Dambly-Chaudière, 2007; Sapede et al., 2002; Nuñez et al., 2009; Wada et al., 2008; Coombs et al., 2014; Becker et al., 2016). The basis of the reported diversity of lateral line patterns in adult teleost fishes (Webb, 1989; Coombs et al., 2014; Coombs et al., 1988) remains poorly understood. Because of the exposed location and accessibility of superficial neuromasts (SNs), the system has been recognized as an especially attractive model to address phenotypic diversity in vertebrate peripheral nervous systems (Dijkgraaf, 1963; Coombs et al., 1988; Wada et al., 2008; Becker et al., 2016; Nakae and Sasaki, 2010; Sato et al., 2019). Indeed, important comparative work on the pLL of distantly related teleosts argues that differences in patterns arise post-embryonically (Ghysen et al., 2012, 2010; Pichon and Ghysen, 2004; Sapede et al., 2002; Becker et al., 2016). In addition, the formation of the caudal lateral line (cLL), also known as the caudal neuromast cluster (CNC), on the caudal fin of teleosts has been shown to harbour species-specific patterns and neuromast numbers (Wada et al., 2008), and it has been used as a model to study pattern evolution and diversification (Wada et al., 2008). More recently, work on threespined sticklebacks has argued for a genetic basis for differences in neuromast numbers between different populations

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Received 25 February 2021; Accepted 19 November 2021

(Wark and Peichel, 2010; Wark et al., 2012). So far no study has attempted to address lateral line system pattern and neuromast number diversity as a combined system (aLL, pLL and CNC), or to analyse the degree of evolutionary diversification of lateral line systems within one genus and to explain its possible origins.

Here, we analysed the complete adult lateral line system (aLL, pLL and CNC) of 29 species and strains within the genus *Oryzias* and specifically focused on the most divergent among them. We included species from the Sulawesi lakes, which represents a hotspot of morphological and genetic diversity for *Oryzias* (Hilgers, and Schwarzer, 2019; Ansai et al., 2021). Our results show that a diversity of lateral line patterns and organ numbers exists within this genus. In addition, our data indicate that despite differences in developmental origin, the number of organs and the organization of patterns in the aLL, on the one hand, and the pLL and CNC on the other, are tightly linked in adult *Oryzias* fish. We quantified the number of neuromasts, number of neuromasts per cluster and number of lateral lines as a proxy for lateral line system elaboration. Species with elaborated aLL patterns consistently also displayed elaborated pLL and CNC patterns and vice versa. These results argue that a common logic (and possibly mechanism) governs post-embryonic organ numbers and pattern distribution in the entire lateral line system, despite their diverse embryonic trajectories from different sensory placodes. Interestingly, we show that the degree of elaboration of the lateral line system is linked to adult species body size and not phylogenetic relationships. Larger species consistently had more elaborated lateral line systems while smaller species had the least elaborated systems. In addition, we report evidence for an expansion of the lateral line system in *Oryzias* species endemic to the Sulawesi islands. We show that most of the diversity of lateral line systems we report in the *Oryzias* genus arises as a result of post-embryonic differences in patterning and organ addition but that in certain cases differences can be traced back to changes in developmental processes during embryogenesis.

MATERIALS AND METHODS

Animal ethics and species used

Oryzias latipes (medaka) and *Oryzias nigrimas* were maintained as closed stocks at the Centre of Organismal Studies of the University of Heidelberg (Tierschutzgesetz §11, Abs. 1, No. 1). The different strains used for *O. latipes* were all inbred for more than 9 generations. All other *Oryzias* species were maintained as closed stocks at National BioResources Project (NBRP) Medaka at the National Institute for Basic Biology, Okazaki, Japan. Medaka and *O. nigrimas* husbandry and experiments were performed according to local animal welfare standards (Tierschutzgesetz 111, Abs. 1, No. 1, Haltungserlaubnis) and in accordance with European Union animal welfare guidelines. The fish colony was maintained under standard recirculating aquaculture conditions, 14 h of light and 10 h of darkness. The strains and species used in this study were: *Oryzias latipes* Cab strain, *Oryzias mekongensis* (Kalasin), *Oryzias mekongensis* (Udon Thani), *Oryzias mekongensis* (Nakhon Phanom), *Oryzias minutillus*, *Oryzias curvinotus* (Hanoi), *Oryzias curvinotus* (Hong Kong), *Oryzias profundicuola*, *Oryzias marmoratus*, *Oryzias sarasinorum*, *Oryzias javanicus*, *Oryzias matanensis*, *Oryzias dancena* (RS832), *Oryzias dancena* (marine), *Oryzias dancena* (freshwater CB), *Oryzias dancena* (Phuket), *Oryzias woworae* (Indonesian), *Oryzias celebensis* (Malino), Hybrid *Oryzias woworae*×*celebensis* (Ujung Pandang) F1, *Oryzias hubbsi*, *Oryzias nigrimas*, *Oryzias luzonensis* and *Oryzias celebensis* (Ujung Pandang). Information in parentheses after the species name above refers to the locale where these fish occur. *Oryzias latipes* strains were as follows: *Oryzias latipes* (HB32D, inbred strain), *Oryzias latipes* (HB11A, inbred strain), *Oryzias latipes* (HSOK), *Oryzias latipes* (Hi3E, inbred strain), *Oryzias latipes* (HNCMH2, inbred strain), *Oryzias latipes* (HO4C, inbred strain). Species and strains are summarized in Tables S1 and S2. Because of limited availability of females in the fish facility, we preferentially used adult males for our study on the aLL, pLL and CNC. We used larvae of both sexes for our analysis of the larval pLL.

Live imaging and sample preparation

Larvae (2 days post-hatching) and 1 year old adults of each species and strain were anaesthetized in 1× ERM (embryo rearing medium) supplemented with Tricaine (0.03%) (Sigma-Aldrich, A5040). The 100× ERM stock is composed of 100 g NaCl, 3 g KCl, 4 g $CaCl_2{\cdot}2H_2O$, 16 g $MgSO_4{\cdot}7H_2O$ and was made up to 1 litre with dH_2O). Fish were mounted laterally on standard Petri dishes. Larvae screening and image acquisition were done on a fluorescence stereomicroscope (Olympus SZX16) with a digital CCD camera (Olympus DP71). Hair cells in neuromasts were visualized using the vital dye 4-Di-2-ASP (Sigma-Aldrich) as previously described (Sapede et al., 2002; Seleit et al., 2017a). Live samples were incubated for 5–10 min in a 5 mmol l^{-1} 4-Di-2-ASP solution, washed in ERM and analysed using a fluorescence stereomicroscope (Olympus SZX16, equipped with 0.5× and 1× objectives). We used 2–8 adult males per species for our analysis (Table S2).

Image and data analysis

All image analysis was done using FIJI and ImageJ (Schindelin et al., 2012). Image stitching utilized 2D and 3D stitching plug-ins on ImageJ. All neuromast counting was done manually. All boxplots were generated using PlotsOfData (Postma and Goedhart, 2019). For adult size measurements, data were analysed from publicly available information on the NBRP medaka website (https://shigen.nig.ac.jp/medaka/strainListAction.do;jsessionid=3BFC5AAA304AF190AC9D0BD2D78536AA?renderDownloadButton=false&strainTypeId=2&listTitle=strain.type.related) and from previous studies (Parenti, 2008; Parenti et al., 2013). Measurements of size were done from the tip of the snout to the tip of the tail, i.e. total fish length (TL). For correlation analysis, a Pearson's product moment correlation test was used, and the data were analysed and plotted using R software.

Phylogenetic analysis

The mitochondrial DNA (mtDNA) phylogeny of the 15 adrianichthyids used in this study was estimated using sequences of the *NADH dehydrogenase subunit 2* (*ND2*) and *cytochrome b* (*cyt b*) genes, with two species of Beloniformes (*Hyporhamphus sajori* and *Cololabis saira*) as outgroups. All sequences were downloaded from the DNA Data Bank of Japan (DDBJ) (Table S1) and aligned separately for *ND2* and *cyt b*, using the ClustalW option in MEGA7 version 7.0.26 (Kumar et al., 2016). The aligned sequences of *ND2* (1046 bp) and *cyt b* (1141 bp) were concatenated into a single sequence. A maximum-likelihood (ML) phylogeny among these species was estimated using raxmlGUI version 1.31 (Silvestro and Michalak, 2012), where the codon-specific GTR+I+G model was assigned to each gene. A rapid bootstrap analysis of 10,000 replicates was conducted to calculate branch support, and the bootstrap values were mapped onto the best-scoring ML tree.

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RESULTS

The present study involved 15 species of the genus *Oryzias* (Table S1). All species and strains analysed were maintained as closed stock under the same laboratory conditions. These species occupy a broad geographical zone that covers a large swathe of Southeast Asia (Fig. 1A). They are distributed in the Indochina Peninsula and East Asia, throughout Southeast and South Asia, as well as Sulawesi and its satellite islands. Members of the genus *Oryzias* therefore live in diverse ecological habitats (Fig. S1). The phylogenetic tree that we used for the genus *Oryzias* (Fig. 1B) is based on mtDNA analysis (see Materials and Methods for details) and largely agrees with previously reported analysis (Takehana et al., 2005; Sumarto et al., 2020). Species are grouped in three main clades, which correspond to the '*latipes* species group', the '*javanicus* species group' and the '*celebensis* species group' (Takehana et al., 2005). The monophyly of each species group was supported by ML bootstrap values of 78–100%. For the morphological analysis, we report data obtained for the lateral line system on lateral views of adult males (Table S2) and male and female larvae. Our analysis, therefore, does not include SNs located in the dorsal part of the head and around the mandible. The data for adults are grouped in three main sections (Table S3), corresponding to the different LL systems: the aLL (Fig. 2), the pLL (Fig. 3) and the CNC (Fig. 4; Fig. S3). For each, we present a scheme corresponding to the wild-type pattern observed in the transgenic *Oryzias latipes* line Tg(*K15*:H2B-EGFP) (Seleit et al., 2017b), which we use as a reference species.

Number and organization of neuromasts in the aLL

The aLL comprises neuromasts around the head, jaw and operculum of fish and amphibia (Piotrowski and Baker, 2014; Ghysen and Dambly-Chaudière, 2007) (Fig. 2Ai,ii). Little is known, however, about the diversity of aLL patterns of SNs between different teleost fishes. A classification exists for the different lines in *O. latipes*, which we have used as a reference (Fig. 2Aii) (Ishikawa, 1994). As not every species shows the exact same distribution of clusters, we have focused on those located posterior to the retina, corresponding to the dorsal group (DG), horizontal line (hl) and lower opercular line (lol) in *O. latipes*. Our results show that both neuromast cluster distribution and organ numbers within clusters, vary markedly between the different *Oryzias* species and strains analysed (Fig. 2B–E). Organ numbers in clusters located around the head range between 1 and 16 (Fig. 2Bi; Table S3). The miniature species *O. mekongensis* (Nakohn Phanom) has the least elaborated aLL, with a reduced number of clusters and a reduced number of SNs within each cluster (Fig. 2Bi,D). The medium-sized *O. latipes* and *O. hubbsi* have intermediate aLL cluster numbers and SNs within each cluster (Fig. 2Bi). Larger species such as *O. sarasinorum* have the most elaborated aLL clusters in terms of both SN numbers and number and distribution of clusters (Fig. 2B). Interestingly, we observed that species with similar body sizes (Fig. 1C) tended to have a similar aLL organization (Fig. 2Bii). The trend that emerges from our data is that larger species consistently display more elaborated aLLs while the smallest species have some of the least elaborated aLLs. All in all, aLL complexity is more strongly correlated to body size than to phylogenetic relationships between the different *Oryzias* species.

Number of lines in the pLL

It has previously been shown that adult pLL patterns (distribution of SNs and number of lines on the trunk) vary in different teleosts and it has been hypothesized that this is a result of post-embryonic differences in patterning strategies (Sapede et al., 2002; Pichon and Ghysen, 2004; Ghysen et al., 2010, 2012). However, it is still unclear whether such a high diversity of the pLL exists within one genus. By looking at the pLL (Fig. 3Ai,ii) of the different adult *Oryzias* species and strains, we discovered a substantial diversity of pLL patterns, organ numbers and distributions (Fig. 3B–G). The middle-sized *O. latipes* (Cab) displays a pLL system with four lines of SNs running along the anterior–posterior axis of the tail (Fig. 3Aii). The dorsal pLL extends up to the dorsal fin, the midline pLL runs close to the horizontal myoseptum, and two ventral pLLs complete the pattern (Fig. 3Aii). Neuromasts that belong to each of these lines are organized in clusters (Fig. 3Aii, enlarged region), each containing 2–7 SNs. The consistent number of pLLs and the range of SNs per cluster that we found in *O. latipes* varied among the different species and strains analysed (Fig. 3B; Table S3). On the lower end of the spectrum, we found the small-sized *O. mekongensis* (Udon Thani), which has a rudimentary pLL system with only one complete ventral pLL and clusters that contained only 2–3 SNs (Fig. 3B,C). On the upper end of the spectrum, *O. sarasinorum* displays a pLL system with 5 lines of SNs running along the anterior–posterior axis of the tail. As we report for the aLL, body size and not phylogenetic relationships is a better predictor of the elaboration of the pLL system, with smaller species having fewer pLLs and fewer SNs per cluster than larger species (Fig. 3C). For example, the pLL systems of the similar-sized *O. minutillus* and *O. mekongensis* (Udon thani) (Fig. 3C,D) are nearly identical despite their distant phylogenetic relationship. Likewise, the similarly sized *O. latipes* and *O. dancena* (Fig. 3B) have very close pLL organization despite a distant phylogenetic relationship.

Organization of neuromast clusters in the pLL

The organization of adult pLL neuromasts in clusters (2–20 neuromasts with the same innervation) has been reported in other species of teleosts such as *Danio rerio* and *Astyanax mexicanus* (Ledent, 2002; Sapede et al., 2002; Ghysen and Dambly-Chaudière, 2007; Nuñez et al., 2009; Pichon and Ghysen, 2004). Neuromasts in the clusters are arranged vertically in both species, and clusters that are arranged this way are referred to as stitches. We noticed that among the genus *Oryzias*, neuromasts within a cluster are arranged in diverse patterns. In *O. latipes*, the clusters are arranged in a dorso-ventral, vertical pattern along 4 pLL lines (Fig. 3Ai,ii); however, species such as *O. celebensis* (Pandang) and *O. woworae* display a different arrangement of neuromasts within a cluster and, in addition, harbour a different number of pLL lines. Five pLLs run along the tail in *O. woworae* and the neuromasts at the midline and ventral pLLs are arranged vertically along the scales (Fig. 3E), resembling stitches. *Oryzias celebensis* has only 3 pLLs running along the tail, and neuromasts are arranged in circular clusters (Fig. 3G). We decided to exploit the ability of these species to form viable hybrids (Myosho et al., 2018) to address a possible genetic contribution to pLL numbers and the distribution of neuromasts within clusters. The F1 hybrid progeny (*O. celebensis* female×*O. woworae* male) showed an intermediate phenotype, with 4 pLLs running along the tail, while neuromast arrangement within the cluster was neither purely *celebensis* nor purely *woworae* (Fig. 3F).

Number and arrangement of neuromasts in the CNC

It has been reported that neuromasts located at the caudal fin have defined species-specific numbers and distributions in different teleosts (Wada et al., 2008). The medaka CNC (Fig. 4Ai,ii) has been

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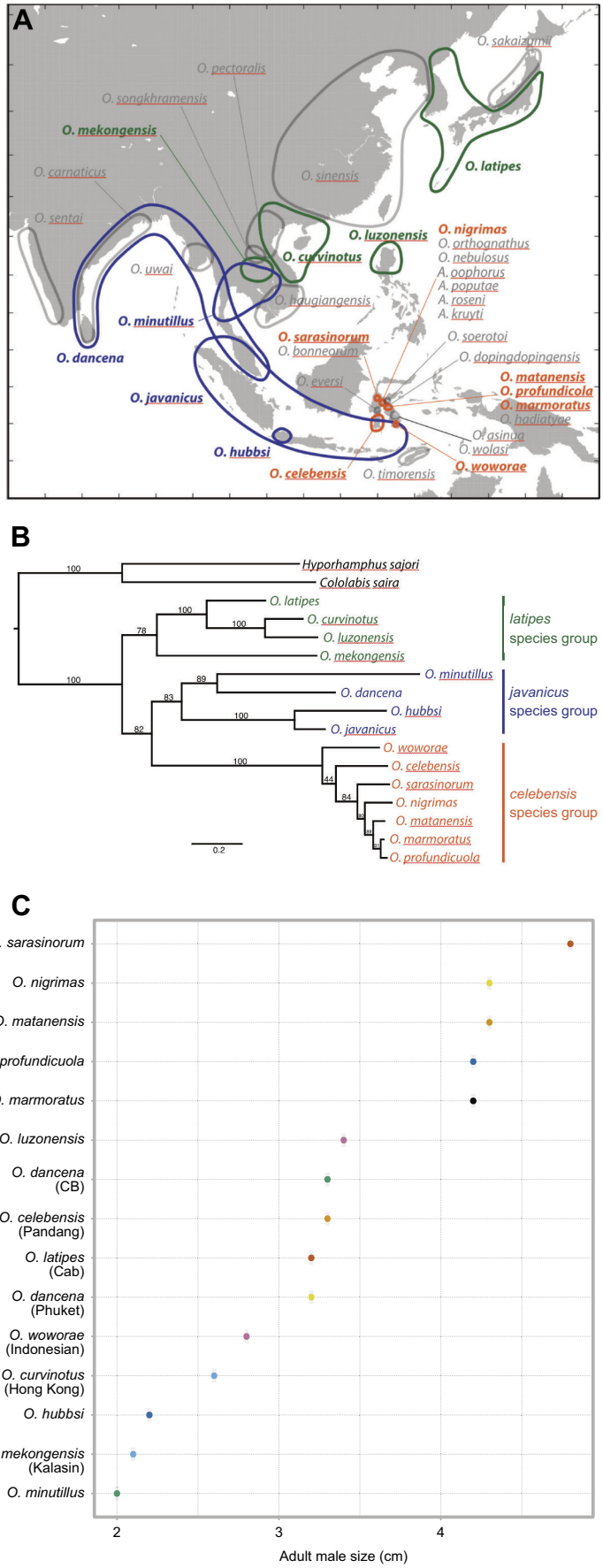

Fig. 1. Geographical location, phylogenetic relationships and adult body size in the genus *Oryzias*. (A) Geographical range of members of the *latipes*, *celebensis* and *javanicus* groups in South East Asia. Notice that members of the *celebensis* group (orange) are based only in Sulawesi, Indonesia, while the *latipes* and *javanicus* groups (green and blue, respectively) cover a broader geographical area. (B) Phylogenetic relationship between members of the genus *Oryzias*. The three main groups are *latipes* (green), *celebensis* (orange) and *javanicus* (blue). (C) Size of adult males for a number of selected *Oryzias* species arranged from smallest to largest. Data are based on publicly available adult measurements of size on the NBRP medaka website (see Materials and methods). The size of adult species ranges from the miniature *O. mekongensis* and *O. minutillus* to the large *O. sarasinorum*.

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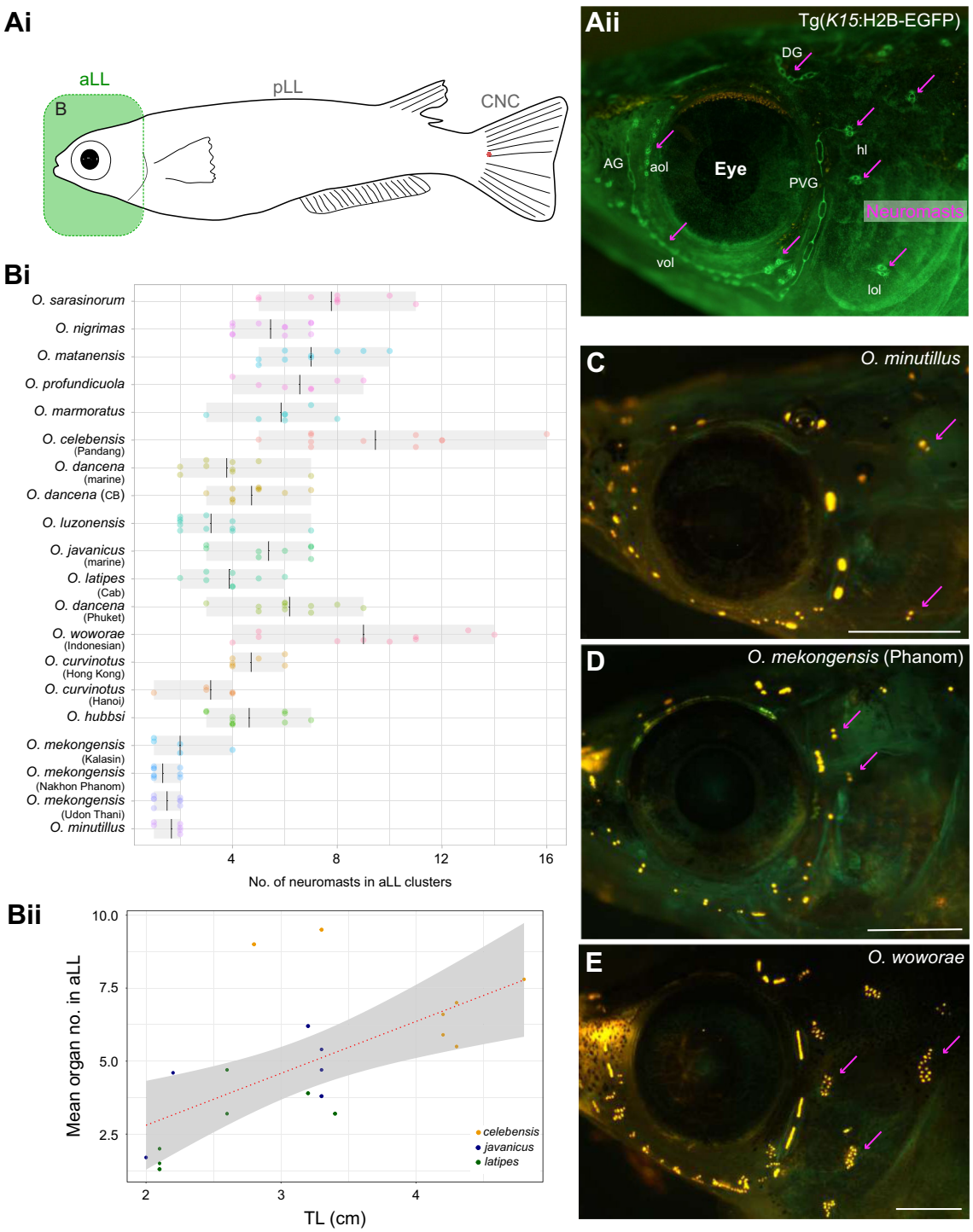

Fig. 2. Anterior lateral line (aLL) neuromast numbers and distribution in selected *Oryzias* species. (Ai) Schematic diagram of an adult *Oryzias latipes* fish highlighting the position of the aLL (green). pLL, posterior lateral line; CNC, caudal neuromast cluster. (Aii) Fluorescence image of the lateral view of aLL neuromasts in a transgenic *Oryzias latipes* line [Tg(*K15*:H2B-EGFP)] adult with neuromast positions highlighted by magenta arrows. AG, anterior group; vol, ventral orbital line; aol, anterior orbital line; lol, lower opercular line; PVG, posteroventral group; hl, horizontal line; DG, dorsal group. (Bi) Graph of the number of neuromasts in aLL clusters of different *Oryzias* species. Data are arranged by adult species size, from the smallest to the largest. Notice that the range is quite wide (1–16 SNs per cluster). Species such as *O. mekongensis* (Udon Thani) and *O. minutillus* have a very limited number of superficial neuromasts (SNs) in their aLL clusters, whereas *O. celebensis*, *O. nigrimas* and *O. sarasinorum* have expanded aLLs. The vertical black line indicates the mean, the shaded grey area is the range. (Bii) There is a strong positive correlation between fish total length (TL) and mean organ number in the aLL of the different *Oryzias* groups (Pearson correlation coefficient R=0.64, P=0.0024). Members of the *latipes*, *javanicus* and *celebensis* sub-groups are indicated by different colours. (C–E) Fluorescence images of 4-Di-2-ASP-stained adult (1 year) aLL patterns and organ numbers in (C) *O. minutillus*, (D) *O. mekongensis* (Nakhon Phanom) and (E) *O. woworae*, arranged from the least elaborated aLL to the most elaborated. Magenta arrows indicate the location of aLL clusters. Notice the wide differences in organ number and cluster distribution between *O. minutillus* and *O. mekongensis* (Nakhon Phanom) on the one hand and *O. woworae* on the other. Note, for *O. woworae*, the infraorbital canal neuromasts are quite large and the aLL clusters are larger than those of similarly sized species. Anterior is to the left and dorsal is up in all panels. Scale bars: 1 mm.

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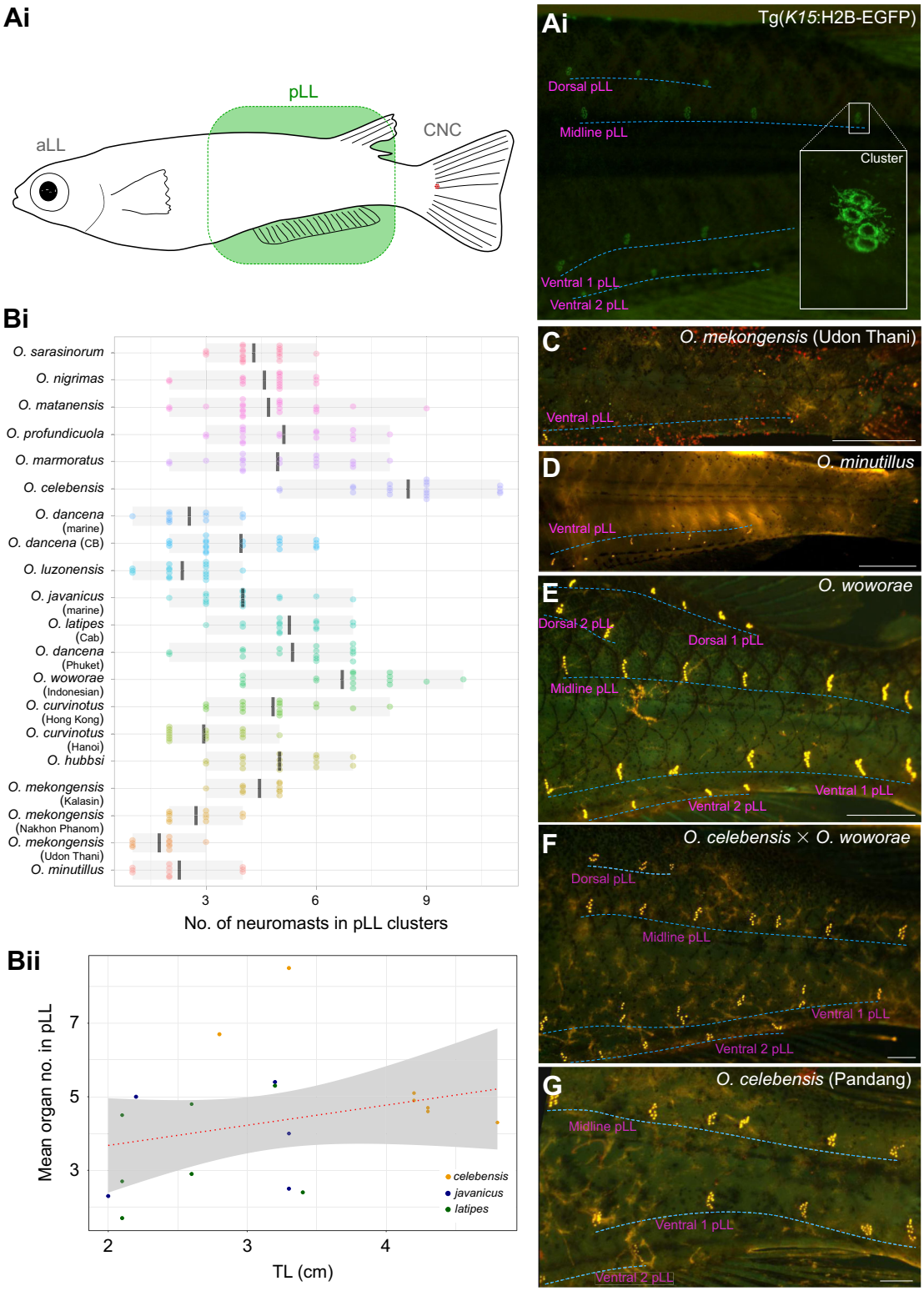

Fig. 3. See next page for legend.

shown to originate post-embryonically from a single founder neuromast (Wada et al., 2008; Seleit et al., 2017b), offering us a quantitative trait of post-embryonic organogenesis. We decided to analyse the number of SNs in, and the morphology of, the CNC in 1 year old adults of the different *Oryzias* species and strains available. Our results show a wide range of phenotypes in the CNCs of the different fishes analysed (Fig. 4B–F; Fig. S3). The number of neuromasts per CNC ranges from as few as 2 in miniature species such as *O. mekongensis* (Fig. S3) to 17 neuromasts in larger species such as *O. sarasinorum* (Fig. 4F; Fig. S3). The distribution of neuromasts in the cluster also varies markedly between different *Oryzias* species and can broadly be divided into three categories; the

Fig. 3. Posterior lateral line (pLL) organ numbers and distribution in selected *Oryzias* species. (Ai) Schematic diagram of an adult *Oryzias latipes* fish highlighting the position of the pLL (green). (Aii) Fluorescence image of the lateral view of pLL neuromasts in a Tg(*K15*:H2B-EGFP) adult with each lateral line indicated with a dashed blue line. The enlarged boxed region shows a cluster of SNs in one of the neuromast positions highlighted by magenta arrows. (Bi) Graph of the number of SNs in pLL clusters of different *Oryzias* species. Data are arranged by adult species size, from the smallest to the largest. Species such as *O. mekongensis* (Udon Thani) and *O. minutillus* have a very limited number of SNs in their pLL clusters, whereas *O. celebensis and O. sarasinorum* have much larger pLL clusters. (Bii) There is a weak positive correlation between fish TL and mean organ number in the pLL of the different *Oryzias* groups (Pearson correlation coefficient R=0.289, P=0.2151). Members of the *latipes*, *javanicus* and *celebensis* sub-groups are indicated by different colours. (C–G) Fluorescence images of 4-Di-2-ASP-stained adult (1 year) pLL patterns and organ numbers in (C) *O. mekongensis* (Udon Thani), (D) *O. minutillus*, (E) *O. woworae*, (F) hybrid F1 adult progeny of a cross of *O. celebensis* (Ujung Pandang) to *O. woworae* and (G) *O. celebensis* (Pandang). *Oryzias mekongensis* (Udon Thani) and *O. minutillus* have only 1 pLL line, *O. woworae* has 5 pLL lines along the trunk and *O. celebensis* (Pandang) has only 3, whereas the hybrid F1 fish has 4 pLL lines. Clusters along the midline pLL are arranged in a dorsal–ventral manner (known as 'stitches') while *O. celebensis* (Ujung Pandang) SNs are clustered together in a circular distribution and the F1 hybrid shows an intermediate organization and number of pLL lines between those of the two parental species. Anterior is to the left and dorsal is up in all panels. Scale bars: 1 mm.

first and most abundant group comprises tight circular clusters of SNs [e.g. *O. latipes* (Cab), *O. hubbsi*, *O. woworae*], the second group contains CNCs that are arranged along the anterior–posterior axis [e.g. *O. dancena* (marine, HK-1RS832) and *O. javanicus* (marine)] and the third group has CNCs arranged dorso-ventrally [*O. mekongensis* (Nakohn Phanom)] (Fig. S3).

As above, we observed that species with similar body sizes tend to have a similar number of neuromasts in their CNC (Fig. 4Bi,ii; Table S3). The largest species by body size in our dataset is *O. sarasinorum* and it also has the most elaborated CNC structure (with up to 17 SNs) (Fig. 4F). *Oryzias latipes* (Cab) has an intermediate CNC that falls between those of *O. mekongensis* and the elaborated *O. sarasinorum* (Fig. 4Bi), and an intermediate body size as well (Fig. 1C). *Oryzias dancena* (Chidambaram) and *O. celebensis* (Ujung Pandang) are among the species with large CNCs (Fig. 4C; Fig. S3); they have a similar body size (3.3 cm) and their CNC is composed of many neuromasts (mean±s.d 10.5±1 and 13.5±1.5, respectively). Phylogenetically, however, *O. dancena* (Chidambaram) belongs to the *javanicus* group while *O. celebensis* (Pandang) belongs to the *celebensis* sub-group (Fig. 1B). *Oryzias mekongensis* (Udon Thani) and *O. minutillus* are among the smallest species we analysed; they have a similar body size (2.1 and 2.0 cm, respectively) and their CNCs are among the least elaborated (mean±s.d 1.75±1.1 and 2.5±0.7, respectively) (Fig. S3). Phylogenetically those two species are among the most distantly related in our dataset (Fig. 1B), yet they occur in close geographical proximity (Fig. 1A). These results mirror those we reported above for the aLL and the pLL, and suggest that a similar logic might govern the post-embryonic expansion of all lateral lines in the different *Oryzias* species. Our data argue that body size is a better predictor of the degree of elaboration of the adult pLL system.

Embryonic pLL

Intrigued by the differences observed in the adult pLL patterns among *Oryzias* species and strains, we decided to look at pLL patterns at the end of embryogenesis, using a number of representative species to gain a better understanding of the origin of those differences. We had previously reported that different embryonic pLL patterns exist in different teleost species (Seleit et al., 2017a, 2022), but whether they also differ within the *Oryzias* clade had not been addressed before. We analysed the final embryonic pLL pattern by looking at recently hatched larvae (N=10 for each species), within 24 h of exiting the chorion, and we noticed two different scenarios. On the one hand, we observed a common embryonic pLL pattern in species that show different complexities as adults. These species include *O. latipes*, *O. nigrimas*, *O. hubbsi* and *O. celebensis* (Fig. 5), whose larvae display two parallel pLL – the previously reported ventral pLL (vpLL) and myoseptum pLL (mpLL) (Fig. 5A,B) (Seleit et al., 2017a). On the other hand, we noticed cases in which there were clear differences in patterning at the larval stage. Among these, *O. latipes* (HB32D) larvae showed just one line of SNs at the midline at the end of embryonic development (mpLL) (Fig. 5G), suggesting a reduced output of the embryonic pLL system in this strain. Another interesting case is *O. mekongensis*, a species in which adults had a missing midline pLL (Fig. 3C). The analysis of *O. mekongensis* at larval stages suggests that the mpLL absence is developmentally encoded, as they showed only one ventral pLL at the end of development as opposed to two for most other species analysed (Fig. 5H). Taken together, these data suggest that there are mechanisms that act specifically post-embryonically to shape pLL patterns and organ numbers, as has been previously suggested for other species (Nuñez et al., 2009; Pichon and Ghysen, 2004). But they also reveal that the origin of differences in patterning in certain cases can be traced back to differences during embryonic development (Seleit et al., 2017a, 2022). The genetic basis of those differences remains to be elucidated, but our results uncover a diversity of pLL patterns that is encoded at the level of developmental processes in *Oryzias* species and strains.

DISCUSSION

Teleost fishes are the most diverse clade of vertebrates in the animal kingdom (Nelson, 2006; Moriyama et al., 2012). Different species come in a wide array of shapes and sizes (Nelson, 2006), which continue to grow over their lifetime (Karkach, 2006; Johns and Easter, 1977). This demands a constant scaling of sensory systems. The wide diversity of lateral line patterns existing among teleost fishes (Webb, 1989; Coombs et al., 1988; Coombs et al., 2014) encouraged us to study variations in the aLL, pLL and CNC among species within one genus. We report that overall body size is a good predictor of neuromast numbers in the aLL, pLL and CNC of the different *Oryzias* species. Our results reveal a hitherto unrecognized link between species body size and not only organ number but also the degree of elaboration of the lateral line system. Larger species consistently had more elaborated lateral line systems and smaller species had less elaborated systems regardless of phylogenetic relatedness. Variations in organ number are best seen when comparing the CNC, as this cluster occurs and can be recognized unambiguously in all *Oryzias* species. We have previously reported that in *O. latipes*, all neuromasts in the CNC originate from a common embryonic neuromast (Seleit et al., 2017b), therefore providing an accurate value of post-embryonic growth. The case of the pLL, in contrast, is a better example for an increase in complexity. Indeed, our results show that the linear correlation between body size and the number of SN per cluster is weaker in the pLL than in the aLL or CNC. This is related to the fact that the pLL displays a different number of lines running along the trunk in the different species. In this way, even if the number of neuromasts per cluster does not differ greatly, the total number of neuromasts in the pLL does vary because of the additional lines, i.e. complexity. It is

Journal of Experimental Biology (2021) 224, jeb242490. doi:10.1242/jeb.242490

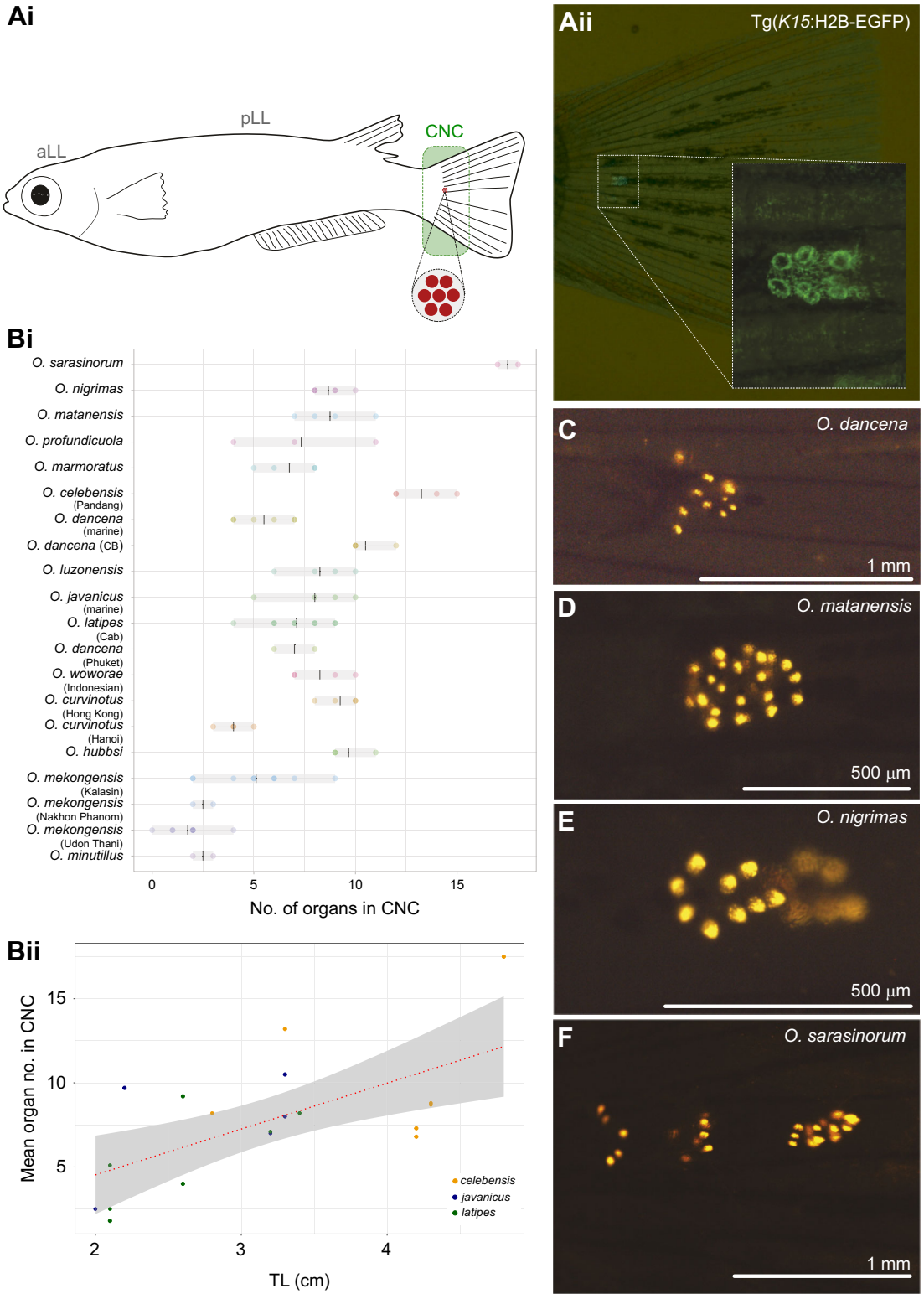

Fig. 4. Caudal neuromast cluster (CNC) neuromast numbers and distribution in the *Oryzias* group. (Ai) Schematic diagram of an adult *Oryzias latipes* fish highlighting the position of the CNC (green). (Aii) Fluorescence image of the lateral view of the CNC in a Tg(*K15*:H2B-EGFP) adult. The enlarged boxed region shows the CNC neuromasts. (Bi) Graph of the number of SNs in CNCs of different *Oryzias* species. Data are arranged by adult species size, from the smallest aLL to the largest. Notice that the range is quite wide (2–17 SNs). Small species such as *O. mekongensis* (Udon Thani) and *O. minutillus* have a very limited number of SNs in their CNCs, whereas the large *O. celebensis* and *O. sarasinorum* have much bigger CNCs. (Bii) There is a strong positive correlation of TL and mean organ number in the CNC of the different *Oryzias* groups (Pearson correlation coefficient R=0.6388, P=0.0024). Members of the *latipes*, *javanicus* and *celebensis* subgroups are indicated by different colours. (C–F) Fluorescence images of 4-Di-2-ASP-stained adults showing a close-up of CNC organization in (C) *O. dancena* (Phuket), (D) *O. matanensis*, (E) *O. nigrimas* and (F) *O. sarasinorum*. Notice the diversity of SN numbers and pattern of CNC organization in the different *Oryzias* species and strains; extended examples are given in Fig. S3.

Journal of Experimental Biology (2021) 224, jeb242490. doi:10.1242/jeb.242490

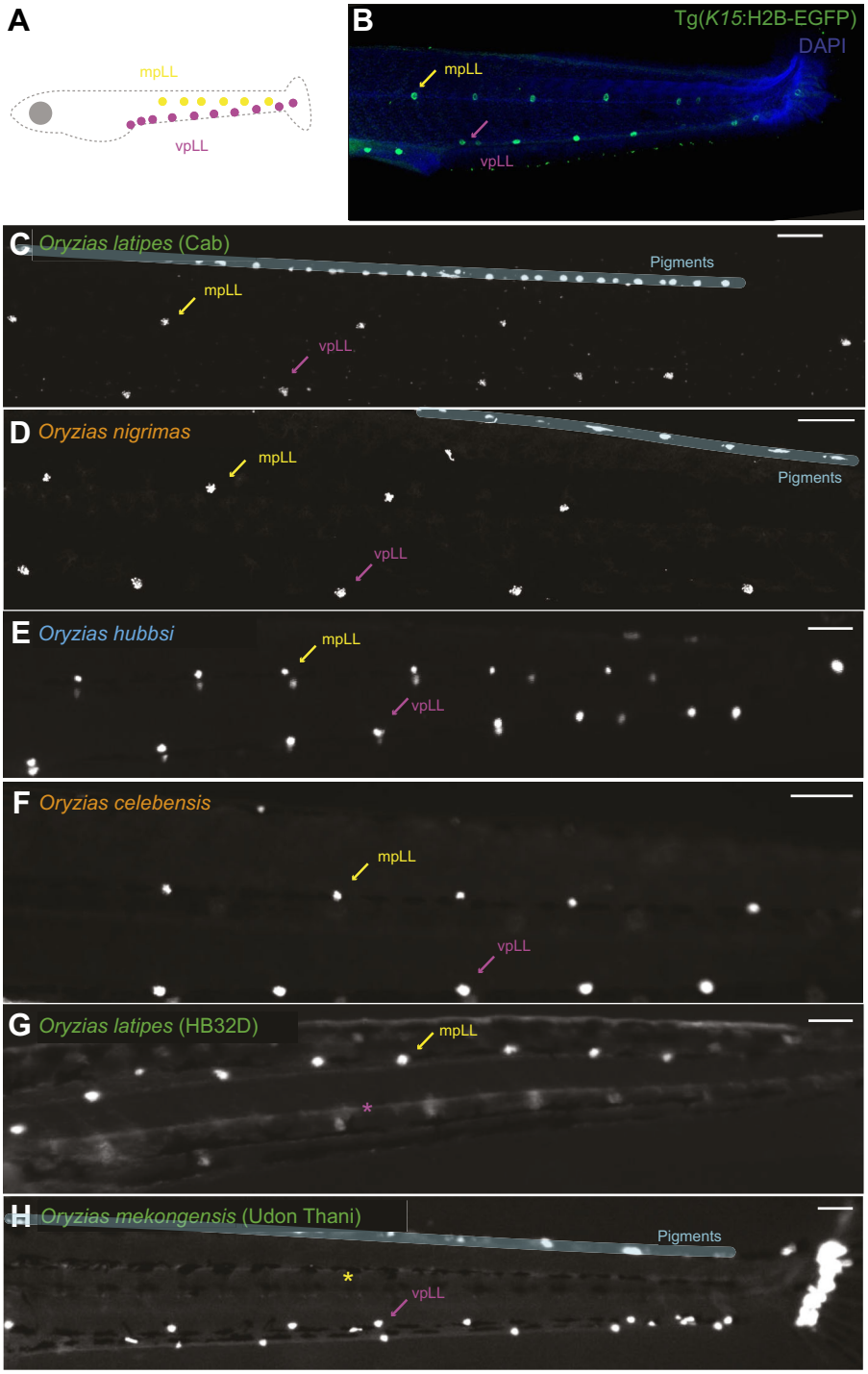

Fig. 5. Larval pLL patterns in a selected number of *Oryzias* species and strains. (A) Schematic diagram of an *Oryzias latipes* larvae highlighting the position of the two pLLs at the end of embryogenesis, the myoseptum pLL (mpLL, yellow) and the ventral pLL (vpLL, magenta). (B) Fluorescence image of the lateral view of the pLL in Tg(*K15*:H2B-eGFP) larvae stained with DAPI (blue). (C–H) Fluorescence images of 4-Di-2-ASP-stained larvae showing pLL patterns and organ numbers at the end of embryogenesis (hatching) in (C) *O. latipes* (Cab), (D) *O. nigrimas*, (E) *O. hubbsi*, (F) *O. celebensis*, (G) *O. latipes* (HB32D) and (H) *O. mekongensis* (Udon Thani). Magenta arrows in all panels indicate the pLL lines, yellow arrows indicate the myoseptum pLL lines; the magenta asterisk indicates the absence of a vpLL and the yellow asterisk indicates the absence of the mpLL. *N*=10 for all fish larvae. Two parallel neuromast lines (one at the midline and one at the ventral line) are conserved in *O. latipes* (Cab), *O. nigrimas*, *O. hubbsi* and *O. celebensis*, whereas *O. latipes* (HB32D) has one line of SNs at the midline and *O. mekongensis* (Udon Thani) shows only one ventral line and no midline neuromasts. Anterior is to the left and dorsal is up in all panels. Scale bars: 100 µm. Species names are colour coded according to sub-group (as in Fig. 1): *latipes* complex in green, *celebensis* complex in orange and *javanicus* complex in blue. Auto-fluorescent pigments located at the dorsal side of the larvae were covered by a partially transparent grey line, to avoid mixing them up with neuromasts in C, D and H.

important to mention, however, that size alone cannot be the sole determinant of lateral line (aLL, pLL and CNC) morphology. It is instead likely that a combination of habitat, geography, hydrodynamic stimuli and phylogeny contributes to the final lateral line morphology of each species. In the absence of accurate data to assess the contribution of each parameter, we have focused here on the relationship between neuromast number/LL complexity and body size.

Even though the aLL and pLL are derived from different embryonic placodes (Nikaido et al., 2017), post-embryonic neuromast number and distribution control seem to share a conserved logic across the aLL and pLL system of *Oryzias* species. Recent data from QTL mapping in sticklebacks have shown that a genetic basis does exist for differences in neuromast numbers (not patterns) in the lateral line (Wark et al., 2012). The generation of *O. celebensis*×*O. woworae* hybrids indicates a polygenic control for neuromast number and organization in the pLL; our data argue for multiple QTLs controlling patterning and neuromast numbers in the *Oryzias* LL system. As size seems to be a good predictor of pLL elaboration (Fig. 3Bii) it would be interesting to try hybrids from species of markedly different sizes, such as *O. sarasinorum* (largest by overall body size) and *O. mekongensis* (miniature by overall body size) (Fig. 1C; Fig. S2). Unfortunately, previous attempts at generating such hybrids by natural mating or by *in vitro*

fertilization approaches proved unsuccessful. We believe that the use of medaka in-bred strains could be a particularly powerful tool in the future for understanding the underlying genetics behind differences in lateral line patterning (Hyodo-Taguchi, 1996; Kinoshita et al., 2009; Spivakov et al., 2014). Crosses among these strains were shown to be viable, and with the continued decrease in whole genome sequencing costs, genome-wide QTL linkage analysis can be performed to uncover the genetic basis of the pattern and neuromast differences in the lateral line of *Oryzias* species, possibly with implications across all teleosts.

The wide range of CNC organ number and distribution among members of the genus *Oryzias* suggests a rapid evolutionary diversification of this system. We consistently observed that larger *Oryzias* species (*O. nigrimas*, *O. sarasinorum*) had more elaborated (in terms of both number and organization) CNC patterns as compared with smaller species (*O. mekongensis*, *O. minutillus*). On the one hand this could reflect genetic differences in 'budding speed' (accessory organ addition rate) (Wada et al., 2008; Ghysen and Dambly-Chaudière, 2007); on the other hand these differences could reflect different ecological constraints. The latter view is supported by our data, where phylogenetically divergent species (*O. mekongensis and O. minutillus*) occurring in a similar geographical location develop very similar CNCs. In addition, data from other teleosts suggest that CNC distribution reflects ecology rather than phylogeny (Wada et al., 2008). Previous work in *Astyanax mexicanus* has shown that neuromast numbers differ between cave and surface populations (Sumi et al., 2015). It has also been shown that in threespine stickleback, the number of neuromasts differs in adults living in different habitats (Wark and Peichel, 2010; Wark et al., 2012), but two independent populations in the same lake show similar neuromast numbers, arguing for parallel evolution under a similar ecological selective pressure (Wark and Peichel, 2010; Wark et al., 2012). Our work on aLL and pLL patterns in a number of species, but particularly on the miniature *O. mekongensis* and *O. minutillus*, argues that a similar explanation could account for the concurrence of lateral lines in these species. It is certainly possible that similar selective pressures could exploit standing genetic variation similarly, leading to the same phenotypic outcomes (Rohner et al., 2013; Chan et al., 2010). However, data acquired on superficial neuromast distribution in 12 common European Cypriniform species show no relationship between total number of superficial neuromasts and habitat (Beckmann et al., 2010), highlighting the complexity and case-specific nature of the link between habitat and lateral line morphology. It is clear, however, that more ecological data are needed in the case of the *Oryzias* genus to further our understanding of that link. We noticed that species endemic to the Sulawesi lakes tended to have an expanded lateral line system as compared with other members of the genus *Oryzias*. While this could reflect the fact that many species endemic to the Sulawesi region are larger in size than other *Oryzias* genus members, our data on *O. woworae* and *O. celebensis* show that they have highly elaborated aLL and pLL systems despite being intermediately sized. The ecology of the Sulawesi lakes differs markedly from the natural habitats of other members of the genus *Oryzias*. Indeed, it has been reported recently that the Sulawesi region represents a hotspot of genetic and morphological diversity of ricefish (Hilgers, and Schwarzer, 2019; Ansai et al., 2021). This region and its diversity could therefore be an interesting avenue to explore the ecological basis of lateral line diversification in teleosts. That said, it is clear that neither size nor ecology alone can explain the diversification of lateral line morphology, and special attention must also be paid to the hydrodynamic stimuli/noise that fish are exposed to in their natural habitats (Herzog et al., 2017; Brown et al., 2013). It is formally possible that the remarkable diversity of lateral line patterns in teleosts is not under any selective pressure and is simply a by-product of variation in developmental strategies between species, with no explicit adaptive value (Ghysen and Dambly-Chaudière, 2016). However, we speculate that a functional and adaptive value reflecting particular morphologies, life histories and ecological niches of teleost fishes is instead likely (Wark and Peichel, 2010; Wark et al., 2012; Wada et al., 2008; Braun and Grande, 2008; Sumi et al., 2015).

Acknowledgements
We would like to thank The Company of Biologists for a travel grant that made this work possible. We would also like to thank Jochen Wittbrodt for generous support and access to equipment; Karen Gross for the adult fish drawing; NBRP medaka for providing fishes; and the entire staff at the Naruse Laboratory for their hospitality and help. We are grateful to Koji Inoue (University of Tokyo), Masato Kinoshita (Kyoto University) and Yusuka Takehana (Nagahama Institute of Bio-Science and Technology) for sharing pictures of *Oryzias* natural habitats.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: A.A.S., L.C.; Validation: A.A.S.; Formal analysis: A.A.S., K.Y., K.N., L.C.; Investigation: A.A.S.; Resources: S.A., K.Y., K.W.M., K.N., L.C.; Data curation: A.A.S.; Writing - original draft: A.A.S., L.C.; Writing - review & editing: A.A.S., S.A., K.Y., K.W.M., K.N., L.C.; Visualization: A.A.S., S.A., K.Y., K.N., L.C.; Supervision: K.N., L.C.; Project administration: K.N., L.C.; Funding acquisition: K.N., L.C.

Funding
This work was funded by a Company of Biologists travel grant to A.S., National Institute for Basic Biology Collaborative Research Program (18-342 to L.C.) and the Deutsche Forschungsgemeinschaft via the Collaborative Research Centre SFB873 (subproject A11 to L.C.). Deposited in PMC for immediate release.

References
Aman, A. and Piotrowski, T. (2008). Wnt/β-catenin and Fgf signaling control collective cell migration by restricting chemokine receptor expression. *Dev. Cell* **15**, 749-761. doi:10.1016/j.devcel.2008.10.002

Ansai, S., Mochida, K., Fujimoto, S., Mokodongan, D. F., Sumarto, B. K. A., Masengi, K. W. A., Hadiaty, R. K., Nagano, A. J., Toyoda, A., Naruse, K. et al. (2021). Genome editing reveals fitness effects of a gene for sexual dichromatism in Sulawesian fishes. *Nat. Commun.* **12**, 1350. doi:10.1038/s41467-021-21697-0

Becker, E. A., Bird, N. C. Webb, J. F. (2016). Post-embryonic development of Canal and Superficial Neuromasts and the generation of two CRANIAL lateral line phenotypes. *J. Morphol.* **277**, 1273-1291. doi:10.1002/jmor.20574

Beckmann, M., Eros, T., Schmitz, A. and Bleckmann, H. (2010). Number and distribution of superficial neuromasts in twelve common european cypriniform fishes and their relationship to habitat occurrence. *Int. Rev. Hydrobiol.* **95**, 273-284. doi:10.1002/iroh.200911185

Braun, C. B. and Grande, T. (2008). Evolution of peripheral mechanics for the enhancement of sound reception. In *Fish Bioacoustics* (ed. J. F. Webb, R. R. Fay and A. N. Popper), pp. 99-144. New York: Springer.

Brown, A. D., Sisneros, J. A., Jurasin, T., Nguyen, C. and Coffin, A. B. (2013). Differences in lateral line morphology between hatchery- and wild-origin steelhead. *PLoS ONE* **8**, e59162. doi:10.1371/journal.pone.0059162

Chan, Y. F., Marks, M. E., Jones, F. C., Villarreal, G., , Jr, Shapiro, M. D., Brady, S. D., Southwick, A. M., Absher, D. M., Grimwood, J., Schmutz, J. et al. (2010). Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. *Science* **327**, 302-305. doi:10.1126/science.1182213

Coombs, S., Janssen, J. and Webb, J. F. (1988). Diversity of lateral line systems: Phylogenetic and functional considerations. In *Sensory Biology of Aquatic Animals* (ed. J. Atema, R. R. Fay, A. N. Popper and W. N. Tavolga), pp. 553-593. New York: Springer.

Coombs, S., Bleckmann, H., Fay, R. R. and Popper, A. N. (2014). *The Lateral Line System*. New York: Springer.

David, N. B., Sapede, D., Saint-Etienne, L., Thisse, C., Thisse, B., Dambly-Chaudiere, C., Rosa, F. M. and Ghysen, A. (2002). Molecular basis of cell

migration in the fish lateral line: role of the chemokine receptor CXCR4 and of its ligand, SDF1. *Proc. Natl. Acad. Sci. USA* **99**, 16297-16302. doi:10.1073/pnas.252339399

Dijkgraaf, S. (1963). The functioning and significance of the lateral-line organs. *Biol. Rev.* **38**, 51-105. doi:10.1111/j.1469-185X.1963.tb00654.x

Ghysen, A. and Dambly-Chaudière, C. (2007). The lateral line microcosmos. *Gene Dev* **21**, 2118-2130. doi:10.1101/gad.1568407

Ghysen, A. and Dambly-Chaudière, C. (2016). Development vs. behavior: a role for neural adaptation in evolution? *Int. J. Dev. Biol* **60**, 77-84. doi:10.1387/ijdb.160097ag

Ghysen, A., Schuster, K., Coves, D., de la Gandara, F., Papandroulakis, N. and Ortega, A. (2010). Development of the posterior lateral line system in Thunnus thynnus, the Atlantic blue-fin tuna, and in its close relative Sarda sarda. *Int. J. Dev. Biol.* **54**, 1317-1322. doi:10.1387/ijdb.103102ag

Ghysen, A., Dambly-Chaudière, C., Coves, D., de la Gandara, F. and Ortega, A. (2012). Developmental origin of a major difference in sensory patterning between zebrafish and bluefin tuna. *Evol. Dev.* **14**, 204-211. doi:10.1111/j.1525-142X.2012.00529.x

Grant, K. A., Raible, D. W. and Piotrowski, T. (2005). Regulation of latent sensory hair cell precursors by glia in the zebrafish lateral line. *Neuron* **45**, 69-80. doi:10.1016/j.neuron.2004.12.020

Haas, P. and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev. Cell* **10**, 673-680. doi:10.1016/j.devcel.2006.02.019

Hernández, P. P., Moreno, V., Olivari, F. A. and Allende, M. L. (2006). Sub-lethal concentrations of waterborne copper are toxic to lateral line neuromasts in zebrafish (*Danio rerio*). *Hear. Res.* **213**, 1-10. doi:10.1016/j.heares.2005.10.015

Herzog, H., Klein, B. and Ziegler, A. (2017). Form and function of the teleost lateral line revealed using three-dimensional imaging and computational fluid dynamics. *J. R. Soc. Interface* **14**, 20160898. doi:10.1098/rsif.2016.0898

Hilgers, L. and Schwarzer, J. (2019). The untapped potential of medaka and its wild relatives. *eLife* **8**, e46994. doi:10.7554/elife.46994

Hyodo-Taguchi, Y. (1996). Inbred strains of the medaka, *Oryzias latipes*. *Fish Biol. J. Medaka* **8**, 11-14.

Ishikawa, Y. (1994). Innervation of lateral line system in the medaka, *Oryzias latipes*. *Fish Biol. J. Medaka* **6**, 17-24.

Johns, P. R. and Easter, S. S., Jr (1977). Growth of the adult goldfish eye. II. increase in retinal cell number. *J. Comp. Neurol.* **176**, 331-341. doi:10.1002/cne.901760303

Karkach, A. (2006). Trajectories and models of individual growth. *Demogr. Res.* **15**, 347-400. doi:10.4054/DemRes.2006.15.12

Kimmel, C. B., Ballard, W. W., Kimmel, S. R., Ullmann, B. and Schilling, T. F. (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* **203**, 253-310. doi:10.1002/aja.1002030302

Kinoshita, M., Murata, K., Naruse, K. and Tanaka, M. (2009). *A Laboratory Manual for Medaka Biology*. Iowa: Wiley-Blackwell.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**, 1870-1874. doi:10.1093/molbev/msw054

Ledent, V. (2002). Postembryonic development of the posterior lateral line in zebrafish. *Development* **129**, 597-604. doi:10.1242/dev.129.3.597

Lecaudey, V., Cakan-Akdogan, G., Norton, W. H. J. and Gilmour, D. (2008). Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135**, 2695-2705. doi:10.1242/dev.025981

López-Schier, H. and Hudspeth, A. J. (2005). Supernumerary neuromasts in the posterior lateral line of zebrafish lacking peripheral glia. *Proc. Natl. Acad. Sci. USA* **102**, 1496-1501. doi:10.1073/pnas.0409361102

López-Schier, H. and Hudspeth, A. J. (2006). A two-step mechanism underlies the planar polarization of regenerating sensory hair cells. *Proc. Natl. Acad. Sci. USA* **103**, 18615-18620. doi:10.1073/pnas.0608536103

Lush, M. E. and Piotrowski, T. (2014). ErbB expressing Schwann cells control lateral line progenitor cells via non-cell-autonomous regulation of Wnt/β-catenin. *eLife* **3**, e01832. doi:10.7554/eLife.01832

Ma, E. Y. and Raible, D. W. (2009). Signaling pathways regulating zebrafish lateral line development. *Curr. Biol.* **19**, R381-R386. doi:10.1016/j.cub.2009.03.057

Montgomery, J. C., Baker, C. F. and Carton, A. G. (1997). The lateral line can mediate rheotaxis in fish. *Nature* **389**, 960-963. doi:10.1038/40135

Moriyama, Y., Kawanishi, T., Nakamura, R., Tsukahara, T., Sumiyama, K., Suster, M. L., Kawakami, K., Toyoda, A., Fujiyama, A., Yasuoka, Y. et al. (2012). The medaka zic1/zic4 mutant provides molecular insights into teleost caudal fin evolution. *Curr. Biol.* **22**, 601-607. doi:10.1016/j.cub.2012.01.063

Myosho, T., Takahashi, H., Yoshida, K., Sato, T., Hamaguchi, S., Sakamoto, T. and Sakaizumi, M. (2018). Hyperosmotic tolerance of adult fish and early embryos are determined by discrete, single loci in the genus oryzias. *Sci. Rep.* **8**, 1-8. doi:10.1038/s41598-018-24621-7

Nakae, M. and Sasaki, K. (2010). Lateral line system and its innervation in Tetraodontiformes with outgroup comparisons: descriptions and phylogenetic implications. *J. Morphol.* **271**, 559-579. doi:10.1002/jmor.10817

Nechiporuk, A. and Raible, D. W. (2008). FGF-dependent mechanosensory organ patterning in zebrafish. *Science* **320**, 1774-1777. doi:10.1126/science.1156547

Nelson, J. (2006). *Fishes of the World*, 4th edn. New York: Wiley.

Nikaido, M., Navajas Acedo, J., Hatta, K. and Piotrowski, T. (2017). Retinoic acid is required and Fgf, Wnt, and Bmp signaling inhibit posterior lateral line placode induction in zebrafish. *Dev. Biol.* **431**, 215-225. doi:10.1016/j.ydbio.2017.09.017

Nuñez, V. A., Sarrazin, A. F., Cubedo, N., Allende, M. L., Dambly-Chaudière, C. and Ghysen, A. (2009). Postembryonic development of the posterior lateral line in the zebrafish. *Evol. Dev.* **11**, 391-404. doi:10.1111/j.1525-142X.2009.00346.x

Parenti, L. R. (2008). A phylogenetic analysis and taxonomic revision of ricefishes, Oryzias and relatives (Beloniformes, Adrianichthyidae). *Zool. J. Linn. Soc.* **154**, 494-610. doi:10.1111/j.1096-3642.2008.00417.x

Parenti, L. R., Hadiaty, R. K., Lumbantobing, D. and Herder, F. (2013). Two new ricefishes of The Genus oryzias (atherinomorpha: BELONIFORMES: ADRIANICHTHYIDAE) augment the ENDEMIC freshwater fish fauna of Southeastern Sulawesi, Indonesia. *Copeia* **2013**, 403-414. doi:10.1643/CI-12-114

Pichon, F. and Ghysen, A. (2004). Evolution of posterior lateral line development in fish and amphibians. *Evol. Dev.* **6**, 187-193. doi:10.1111/j.1525-142X.2004.04024.x

Piotrowski, T. and Baker, C. V. H. (2014). The development of lateral line placodes_ Taking a broader view. *Dev. Biol.* **389**, 68-81. doi:10.1016/j.ydbio.2014.02.016

Postma, M. and Goedhart, J. (2019). PlotsOfData—a web app for visualizing data together with their summaries. *PLoS Biol.* **17**, e3000202. doi:10.1371/journal.pbio.3000202

Rohner, N., Jarosz, D. F., Kowalko, J. E., Yoshizawa, M., Jeffery, W. R., Borowsky, R. L., Lindquist, S. and Tabin, C. J. (2013). Cryptic variation in morphological evolution: HSP90 as a capacitor for loss of eyes in cavefish. *Science* **342**, 1372-1375. doi:10.1126/science.1240276

Sánchez, M., Ceci, M. L., Gutiérrez, D., Anguita-Salinas, C. and Allende, M. L. (2016). Mechanosensory organ regeneration in zebrafish depends on a population of multipotent progenitor cells kept latent by Schwann cells. *BMC Biol.* **14**, 27. doi:10.1186/s12915-016-0249-2

Sapede, D., Gompel, N., Dambly-Chaudiere, C. and Ghysen, A. (2002). Cell migration in the postembryonic development of the fish lateral line. *Development* **129**, 605-615. doi:10.1242/dev.129.3.605

Sato, M., Nakae, M. and Sasaki, K. (2019). Convergent evolution of the lateral line system in Apogonidae (Teleostei: Percomorpha) determined from innervation. *J. Morphol.* **280**, 1026-1045. doi:10.1002/jmor.20998

Schindelin, L., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B. et al. (2012). Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9**, 676-682. doi:10.1038/nmeth.2019

Seleit, A., Krämer, I., Ambrosio, E., Dross, N., Engel, U. and Centanin, L. (2017a). Sequential organogenesis sets two parallel sensory lines in medaka. *Development* **144**, 687-697. doi:10.1242/dev.142752

Seleit, A., Krämer, I., Riebesehl, B., Ambrosio, E., Stolper, J., Lischik, C. Q., Dross, N. and Centanin, L. (2017b). Neural stem cells induce the formation of their physical niche during organogenesis. *eLife* **6**, e29173. doi:10.7554/eLife.29173

Seleit, A., Gross, K., Onistschenko, J., Hoang, O. P., Theelke, J. and Centanin, L. (2022). Tissue interactions govern pattern formation in the posterior lateral line of medaka. *Dev. Biol.* **481**, 1-13. doi:10.1016/j.ydbio.2021.09.002

Silvestro, D. and Michalak, I. (2012). RaxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* **12**, 335-337. doi:10.1007/s13127-011-0056-0

Spivakov, M., Auer, T. O., Peravali, R., Dunham, I., Dolle, D., Fujiyama, A., Toyoda, A., Aizu, T., Minakuchi, Y., Loosli, F. et al. (2014). Genomic and phenotypic characterization of a wild medaka population: towards the establishment of an isogenic population genetic resource in fish. *G3 (Bethesda)* **4**, 433-445. doi:10.1534/g3.113.008722

Suli, A., Watson, G. M., Rubel, W. and Raible, D. W. (2012). Rheotaxis in larval zebrafish is mediated by lateral line mechanosensory hair cells. *PLoS ONE* **7**, e29727. doi:10.1371/journal.pone.0029727

Sumarto, B. K. A., Nofrianto, A. B., Mokodongan, D. F., Lawelle, S. A., Masengi, K. W. A., Fujimoto, S. and Yamahira, K. (2020). Variation in mating behaviors between a tropical and a temperate species of medaka fishes. *Zool Sci.* **38**, 45-50. doi:10.2108/zs200114

Sumi, K., Asaoka, R., Nakae, M. and Sasaki, K. (2015). Innervation of the lateral line system in the blind cavefish *Astyanax mexicanus* (Characidae) and comparisons with the eyed surface-dwelling form. *Ichthyol. Res.* **62**, 420-430. doi:10.1007/s10228-015-0458-7

Takehana, Y., Naruse, K. and Sakaizumi, M. (2005). Molecular phylogeny of the medaka fishes genus *Oryzias* (Beloniformes: Adrianichthyidae) based on nuclear and mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **36**, 417-428. doi:10.1016/j.ympev.2005.01.016

Wada, H., Hamaguchi, S. and Sakaizumi, M. (2008). Development of diverse lateral line patterns on the teleost caudal fin. *Dev. Dyn.* **237**, 2889-2902. doi:10.1002/dvdy.21710

Wark, A. R. and Peichel, C. L. (2010). Lateral line diversity among ecologically divergent threespine stickleback populations. *J. Exp. Biol.* **213**, 108-117. doi:10.1242/jeb.031625

Wark, A. R., Mills, M. G., Dang, L.-H., Chan, Y. F., Jones, F. C., Brady, S. D., Absher, D. M., Grimwood, J., Schmutz, J., Myers, R. M. et al. (2012). Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. *G3* **2**, 1047-1056. doi:10.1534/g3.112.003079

Webb, J. F. (1989). Developmental constraints and evolution of the lateral line system in teleost fishes. In *The Mechanosensory Lateral Line* (ed. S. Coombs, P. Gorner and H. Munz), pp. 79-97. New York: Springer-Verlag.