



# The cephalic lateral line system and its innervation in *Pardachirus pavoninus* (Soleidae: Pleuronectiformes): comparisons between the ocular and blind sides

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## Abstract

The cephalic lateral line system and its innervation were examined and compared between the ocular and blind sides in *Pardachirus pavoninus* (Soleidae). On the ocular side, the otic and preopercular canals were partly (posteriorly and dorsally, respectively) formed by canalized scales (one and five, respectively), each containing a canal neuromast (i.e., “lateral line scales”) and innervated by the anterior lateral line nerve (otic and mandibular rami, respectively). The canal neuromasts of the five scales were recognized as homologous with superficial neuromasts in other taxa based on innervation. The scales, each with a canal perpendicular to the long axis of the scale, bridged the wide gap between the otic region of the cranium and preopercle. The superficial ophthalmic ramus was bifurcated on both sides, the dorsal ramule emerging from the cranium via a frontal foramen. The buccal ramus on the blind side was intensively ramified in the area made available by migration of the eye to the ocular side. The numbers of canal and superficial neuromasts differed greatly between the sides, being 19 and 173 on the ocular side, and 1 and 465 on the blind side, respectively. Sensory strips of superficial neuromasts on the blind side had clear long and short axes. Numerous dermal papillae occurred on the blind side, forming complex channels, according to directions of the long axes.

**Keywords** Flatfishes · Scales · Nerves · Neuromasts · Asymmetry

## Introduction

The lateral line system, a receptor system for sensing water motion, comprises neuromasts as functional units, located either in canals (canal neuromasts: CNs) or on the body surface (superficial neuromasts: SNs). Reflecting the evolutionary history and ecological characteristics of a specific taxon, the arrays of CNs and SNs are diverse (for review, see Coombs et al. 1988), their study being critical for an understanding of phylogeny and adaptations to water environments. Flatfishes (Pleuronectiformes) provide interesting questions for laterality of the system, with an asymmetrical

body in which the ocular side faces the water column and the blind side faces the bottom sediments after metamorphosis and subsequent settlement. Accordingly, the system is expected to show different properties in morphology and functions between the sides. The homology of lateral line components should be questioned, recognizing that even homologous components may function differently.

In pleuronectiforms, the lateral line canals (generally reduced on the blind side compared with those on the ocular side), including supporting bony elements, have been relatively well studied, with summary works available for some families (i.e., Sakamoto 1984; Voronina 2002, 2003, 2007, 2009; Voronina and Dias de Astarloa 2007). In Soleidae, however, the canals have been rarely studied, although reduced infraorbital and preopercular canals on the blind side have been considered as a characteristic of the family (Chapleau 1993).

Overall, descriptions of CN and SN patterning are generally unavailable in pleuronectiforms. With the exception of Pleuronectidae (Cole and Johnston 1901), Rhombosoleidae (Roper 1981) and Soleidae (see below), SNs have

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been examined mostly by developmental biologists focusing on larval and juvenile stages (Kawamura and Ishida 1985; Neave 1986; Pankhurst and Butler 1996; Otsuka 2003; Ma et al. 2016). In Soleidae, Cunningham (1890) noted the abundance of SNs (as superficial epidermal sense organs of unknown function) on the skin of the (blind side) head in *Solea vulgaris*. Appelbaum and Schemmel (1983) estimated such SNs to number between 400 and 500 in that species, whilst arguing that the functions of numerous associated dermal papillae were to protect the SNs and regulate flow. Ochiai (1966) reviewed the cephalic lateral line system of Japanese soleids and showed that the lateral lines (roughly approximating rows of SNs in this study) were better developed on the blind side than on the ocular side. Regarding larval and juvenile stages, Harvey et al. (1992) traced SN development on the head of *Solea solea*, demonstrating higher numbers on the future blind side than on the future ocular side.

In pleuronectiforms, innervation of the lateral line system has also been rarely studied, comprehensive descriptions being limited to three species, one each in Pleuronectidae (Cole and Johnston 1901), Paralichthyidae and Bothidae (Sasaki et al. 2007). In Soleidae, Cunningham (1890) briefly commented on the subject, and Fukuda et al. (2010) described and illustrated the hypertrophy of the supratemporal ramus of the posterior lateral line nerve. Because the asymmetric lateral line system poses problems in determining the homology of the SN rows between the sides, innervation of the system is critical for such an evaluation.

Soleids are non-visual, nocturnal feeders, relying almost exclusively on a diet of small benthic invertebrates (Ochiai 1966). Of these, *Pardachirus pavoninus* (Lacepède, 1802), the Peacock sole (an Indo-Pacific species), is characterized by poison glands arranged along the bases of the dorsal, anal and pelvic fins on both the ocular and blind sides (Ochiai 1957). Clark and George (1979) suggested that toxin from the blind side may be used to paralyze or kill prey, fish having to detect the presence of prey by utilizing undermined sensors, prior to discharging the toxin. The cephalic lateral line system and its innervation are examined in this species, with an emphasis on comparisons between the ocular and blind sides, the latter having numerous papillae on the surface of the skin (see above). Lateral line scales incorporated into the cephalic lateral line system are also reported for the first time in bony fishes and are described in detail.

## Materials and methods

The cephalic lateral line system is here defined as including canals (either bony or cutaneous), canalized scales, channels on the blind side (see below), and canal (CNs) and superficial (SNs) neuromasts, primarily innervated by

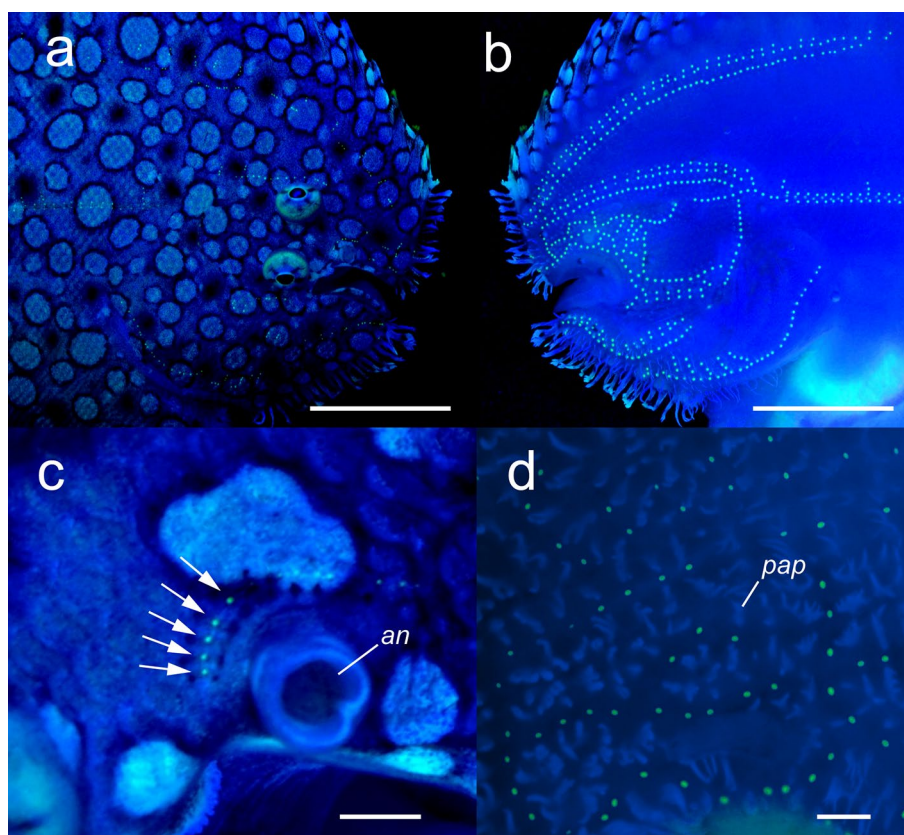
the superficial ophthalmic, buccal, otic and mandibular rami of the anterior lateral line nerve, and supratemporal ramus of the posterior lateral line nerve, which also extends posteriorly over the anterior part of the trunk. The SNs innervated by dorsal ramules derived from the lateral ramus of the posterior lateral line nerve are also included in the system, as they occur continuously along the anterodorsal margin of the body (i.e., head and anterior trunk; see Fig. 1b).

In *Pardachirus pavoninus*, dermal papillae are numerous and quite dense on the blind side of the head, with fringing narrow strips of the skin of variable lengths and directions (see Fig. 7). These strips, each associated with a row of SNs or a single SN, are referred to here as “channels”. The specimens examined in this study are listed below. Measurements (in mm) were of standard length (SL). Abbreviations in parentheses in “*Specimens examined*” refer to the method of examination: DA, 4-Di-2-ASP treated (neuromasts, but note that this imaging reveals only the location of hair cells; Fig. 1; Nakae et al. 2012); SB, cleared and stained by Sudan Black B protocol (nerves; Filipinski and Wilson 1984); AL, stained by Alizarin Red S (scales and cranial skeleton, including canal structures); CY, stained by Cyanine Blue (SNs and dermal papillae). The illustrations and descriptions of CNs, SNs, canals, scales and dermal papillae were primarily based on a single 46.1 mm SL specimen (see below), although additional specimens were used to check for intraspecific variation. Nerves were observed in six specimens because only some of the nerves could be traced in each specimen. The terminology for cephalic canals, nerves and bones generally follows Webb (1989a), Northcutt et al. (2000) and Chappleau (1993), respectively. Nerves are generally described according to the following descending hierarchy: ramus, ramule and element; the term “branch” is used in all hierarchical levels for convenience. Superficial neuromasts (SNs) were categorized into groups (based on topology) for the purpose of descriptions.

The sensory strip is the population of hair cells, occurring centrally in the neuromast (see Coombs et al. 1988). Measurements of sensory strips were taken from photographs (Fig. 1; bright dots) of the 46.1 mm specimen treated with 4-Di-2-ASP.

*Specimens examined.* All specimens were collected from the coast of Okinawa Island, southern Japan. BSKU (Laboratory of Marine Biology, Faculty of Science, Kochi University) 123065 (1 individual; 46.1 mm SL; DA), BSKU 123105 (1; 133; DA; SNs measured), BSKU 123066 (1; 121; AL), BSKU123067 (1; 160; AL), BSKU 123068 (1; 215; CY), BSKU 119433 (1; 131; SB), BSKU 119434 (1; 121; SB), BSKU 119435 (1; 165; SB), BSKU 119436 (1; 141; SB), BSKU 123063 (1; 141; SB), BSKU 123064 (1; 133; SB).

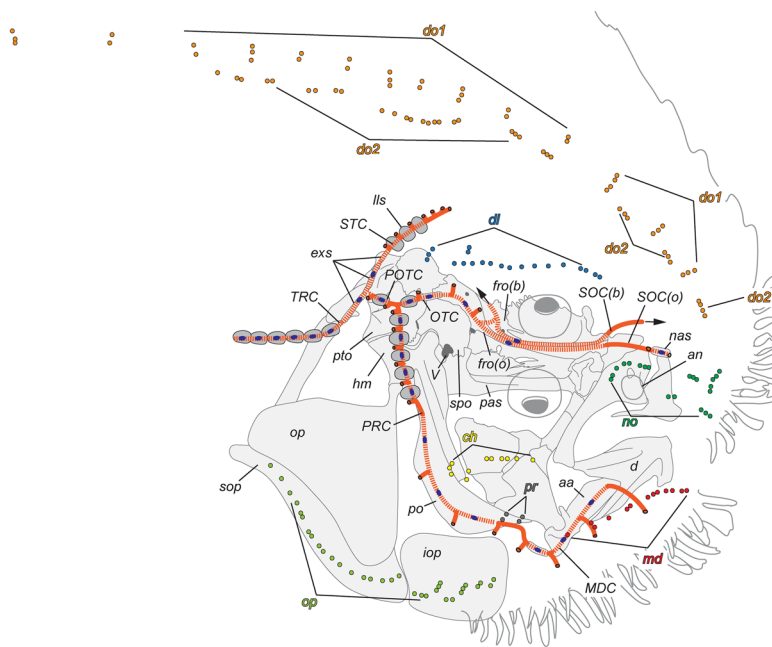
**Fig. 1** Superficial neuromasts (small dots) stained with 4-Di-2-ASP in *Pardachirus pavoninus*. **a** Ocular and **b** blind sides (BSKU 123065; 46.1 mm SL). **c, d** Area around the anterior nostril on the ocular and blind sides, respectively (BSKU 123105; 133 mm SL). *an* anterior nostril, *pap* papillae. Arrows indicate superficial neuromast. Bars indicate 5 mm (**a, b**) and 1 mm (**c, d**)



## Results

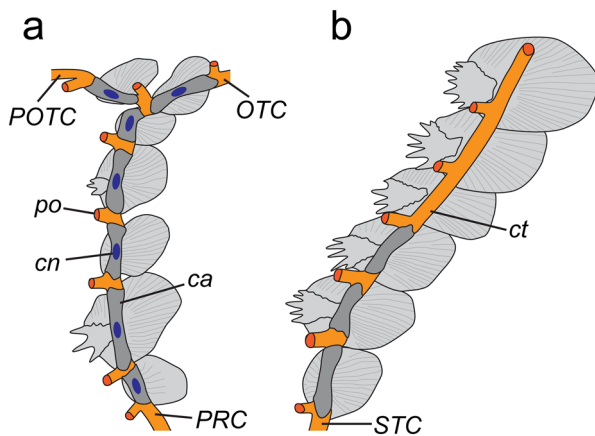
**Ocular side. Canals and canal neuromasts** (Figs. 2, 3; Table 1). Canals were formed by bones (including tubular ossicles), scales and cutaneous (soft tissue) tubules (including outer extensions, each with a terminal pore). The bones and scales housed canal neuromasts (CNs). The cephalic lateral line system comprised 6 canals: supraorbital (SOC; 3 CNs, 4 pores), otic (OTC; 2, 2), preopercular (PRC; 9, 11), mandibular (MDC; 2, 2), postotic (POTC; 1, 2) and supratemporal (STC; 1, 7) canals; an infraorbital canal was absent, owing to the lack of the infraorbitals. The SOC extended from the posterior part of the ocular side frontal (2 CNs) to the anterior opening of the nasal (1 CN), with the frontal and nasal being connected by a cutaneous tubule, with a pore immediately behind the nasal; SOCs of the ocular and blind sides were closely parallel between the eyes and interconnected transversely through a short canal posterior to the upper eye; posterior to the transverse canal, 2 short extensions were given off. The OTC occurred between the posterior end of the SOC (determined by the position of the 2 CNs innervated by the otic ramus) and the PRC–POTC commissure, comprising a canalized scale (1 CN) posteriorly and tubular ossicle (1 CN) anteriorly (both in the dermis covering muscle tissue), with 2 extensions. The PRC extended downward, subsequently curving anteriorly

to connect with the MDC; the upper part of the PRC comprised 5 canalized scales (Figs. 2, 3a) in the dermis covering the muscle tissue lateral to the hyomandibular (note the wide separation of the otic region of the cranium from the preopercle), each with a canal (1 CN) perpendicular to its long axis and ctenii along the posterior margin (presence or absence of ctenii variable intraspecifically; Figs. 2, 3a); the canals of neighboring scales were linked to one another by a cutaneous tubule with a posterior extension (total of 6 extensions along scales; number variable, see Figs. 2, 3a); below the scales, the PRC passed through the preopercle, with 3 cutaneous extensions each protruding from a pore in the bone, and subsequently, extended as a cutaneous tubule which terminated at a tubular ossicle (1 CN) with 2 cutaneous extensions. The MDC coursed through the lower jaw (2 CN), projecting 2 cutaneous extensions ventrally (each extending from a pore in the anguloarticular). The POTC occurred between the OTC–PRC and STC–TRC commissures, comprising 1 scale (1 CN), with a canal parallel to the long axis of the scale and 2 associated extensions. The STC coursed anterodorsally from the POTC–TRC commissure, comprising 2 dorsal extrascapulae (1 CN in the second), 3 scales (each with a canal perpendicular to the long axis; CN absent) and (dorsally) a cutaneous tubule, with a total of 6 extensions (subject to intraspecific variation, scales numbering up to 6 with 3 sharing a common cutaneous tubule;



**Fig. 2** Lateral line canals (orange), neuromasts (dots; illustrated as roughly approximating sensory strip shape) and cranial skeleton (gray) on the ocular side of *Pardachirus pavoninus* (BSKU 123065; 46.1 mm SL; bones from BSKU 123066; 121 mm SL). *aa* Anguloarticular, *an* anterior nostril, *d* dentary, *exs* extrascapula, *fro* (*o*, *b*) frontals of ocular (*o*) and blind (*b*) sides, *hm* hyomandibular, *iop* interopercle, *lls* lateral line scale, *LR* lateral ramus, *MDC* mandibular canal, *nas* nasal, *op* opercle, *OTC* otic canal, *pas* parasphenoid, *po* preopercle, *POTC* postotic canal, *PRC* preopercular canal, *pto* pter-

otic, *SOC(o, b)* supraorbital canals of ocular (*o*) and blind (*b*) sides, *sop* subopercle, *spo* sphenotic, *STC* supratemporal canal, *TRC* trunk lateral line canal, *V* trigeminal foramen. Large navy and small dots indicate canal and superficial neuromasts, respectively. Blue, dark green, green, gray, orange, red and yellow dots indicate *dl*, *no*, *op*, *pr*, *do*, *md* and *ch* groups, respectively. See text for abbreviations of neuromast groups. Broken lines indicate paths through bony canal structures (including scale canals). Arrows indicate extension to blind side



**Fig. 3** Dorsal parts of preopercular (a) and supratemporal (b) canals on the ocular side of *Pardachirus pavoninus* (BSKU 123067; 160 mm SL). *ca* Canal, *cn* canal neuromast, *ct* cutaneous tubule, *po* pore. See Fig. 2 for other abbreviations

Fig. 3b). The anteriormost part of the TRC was formed by the ventralmost (third) extrascapula (1 CN).

**Superficial neuromasts** (Figs. 1, 2; Table 2). Sensory strips of superficial neuromasts (SNs) were rounded and

0.06–0.10 mm in diameter ( $n = 20$ ;  $\bar{x} = 0.08$ ;  $SD \pm 0.01$ ). Seven groups of SNs were present on the epidermis (total 173 SNs), including the dorsal (*do*), dorsolateral (*dl*), nostril (*no*), cheek (*ch*), preopercular (*pr*), mandibular (*md*) and opercular (*op*) groups. The *do* group (71 SNs) comprised 2 subgroups (*do1*, 2); the *do1* subgroup (36 SNs) included 14 transverse rows (2–4 SNs per row) occurring separately from one another along the dorsal margin of the head and anterior trunk, with SNs being absent from the margin posterior to the subgroup; the *do2* subgroup (35 SNs), comprising ca. 9 separated longitudinal rows (number highly subjective), extended ventrally to the *do1* subgroup. The *dl* group (20 SNs) included 1 longitudinal row (above the upper eye) and 2 short transverse rows (posterodorsal to the former). The *no* group (21 SNs) occupied the space dorsal to the upper jaw, comprising 1 transverse, 3 longitudinal and 1 curved rows. The *ch* group (11 SNs) occurred as 1 horizontal row (below the lower eye) and 2 short transverse rows (more posteriorly). The *pr* group (4 SNs) included 2 short transverse rows on the ventrolateral surface of the preopercle. The *md* group (13 SNs) formed a longitudinal row along the lateral surface of the lower jaw. The *op* group (33 SNs) extended along



**Table 1** Number of canal (CN) and superficial (SN) neuromasts innervated by each ramus in *Pardachirus pavoninus*

Ramus <sup>a</sup>	Ocular side				Ramus	Blind side			
	Branch <sup>b</sup>	CN (canal <sup>c</sup> )	SN	SN <sup>d</sup>		Branch <sup>b</sup>	CN (canal <sup>c</sup> )	SN	SN <sup>d</sup>
SOR	SORd	0	31	(47)	SOR	SORd	0	55	(61)
	SORv	3 (SOC)	16			SORv	1 (SOC <sup>e</sup> )	6	
BR		0		11	BR	0		100	
MDR	MDRp	6 (PRC)	33	(55)	MDR	MDRp	0	63	(133)
	MDRd	2 (MDC)	22			MDRd	0	57	
	MDRv	3 (PRC)	0			MDRv	1 (PRC)	13	
STR		3 (POTC, STC, TRC)		24	STR	0		78	
OR		2 (OTC)		0	OR	0		13	
LR	DR1–3	0		36	LR	DR1	0	80	
Total		19		173	Total	2		465	

CN and SN numbers from BSKU 123065, 46.1 mm SL; nerves from six specimens (see “Materials and methods”)

<sup>a, b</sup>BR buccal ramus, DR dorsal ramule of lateral ramus, MDR mandibular ramus; MDRd dorsal ramule of mandibular ramus; MDRp posterior ramule of mandibular ramus; MDRv ventral ramule of mandibular ramus, LR lateral ramus, OR otic ramus, SOR superficial ophthalmic ramus; SORd dorsal ramule of superficial ophthalmic ramus; SORv ventral ramule of superficial ophthalmic ramus, STR supratemporal ramus

<sup>c</sup>See text for abbreviations

<sup>d</sup>Total number in parentheses if two or more rami (or ramules) are involved

<sup>e</sup>Twisted to ocular side

**Table 2** Number and innervation of superficial neuromasts (SNs) in each group of *Pardachirus pavoninus*

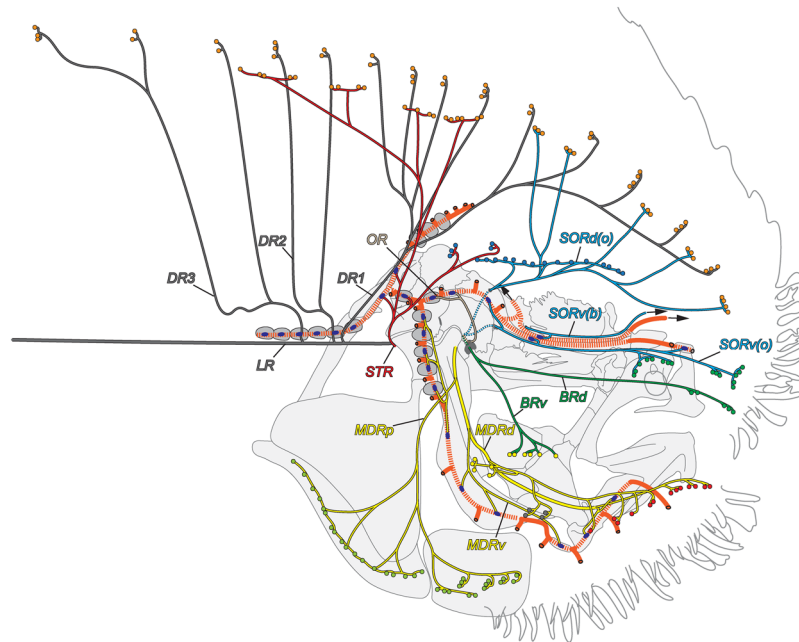
Group <sup>a</sup>	Ocular side				Blind side			
	Subgroup	Branch <sup>b</sup>	n	n <sup>c</sup>	Subgroup	Branch <sup>b</sup>	n	n <sup>c</sup>
do	do1	DR1–3	36	(71)	do1	DR1	80	(136)
	do2	SORd	16		do2	SORd	23	
		STR	19		STR	33		
dl		SORd	15	(20)	dl1	STR	45	(90)
		STR	5		dl2	SORd	32	
no					dl3	OR	13	
		SORv	16	(21)	no	SORv	6	(95)
		BR	5		BR	89		
ch		BR	6	(11)	ch1	BR	11	(29)
		MDRd	5		ch2	MDRd	18	
pr		MDRd		4	pr1	MDRp	14	(41)
					pr2	MDRd	14	
					pr3	MDRv	13	
md		MDRd		13	md1	MDRd	12	(25)
					md2	MDRd	13	
op		MDRp		33		MDRp		49
Total				173	Total			465

SN number from BSKU 123065, 46.1 mm SL; nerves from six specimens (see “Materials and methods”)

<sup>a</sup>See text for abbreviations

<sup>b</sup>Abbreviations as in Table 1

<sup>c</sup>Total number in parentheses if two or more rami (or ramules) are involved



**Fig. 4** Innervation of lateral line system on the ocular side of *Parachirus pavoninus* (nerves from six specimens; see “Materials and methods”). BR buccal ramus (green), DR dorsal ramule of lateral ramus (gray), MDR mandibular ramus (yellow); MDRd dorsal ramule of mandibular ramus (yellow); MDRp posterior ramule of mandibular ramus (yellow); MDRv ventral ramule of mandibular ramus (yellow), OR otic ramus (beige), SOR superficial ophthalmic ramus (blue);

SORd(o) dorsal ramule of superficial ophthalmic ramus on ocular side (blue); SORv(o, b) ventral ramule of superficial ophthalmic ramus on ocular (o) and blind (b) sides (blue), STR supratemporal ramus (red). Large navy and small dots indicate canal and superficial neuromasts, respectively. See Fig. 2 for groups and colors of superficial neuromasts

the interopercle and subopercle, with 4 short oblique rows anteriorly.

**Innervation** (Fig. 4; Tables 1, 2). The anterior lateral line nerve comprised the superficial ophthalmic (SOR), buccal (BR), otic (OR) and mandibular (MDR) rami, and the posterior lateral line nerve comprised the supratemporal (STR) and lateral (LR) rami. These nerves were clearly distinguishable from other cranial nerves, because of the terminal innervation of the neuromasts. The SOR (innervating 3 CNs, 47 SNs) was divided into single dorsal (SORd) and ventral (SORv) ramules with a common root located intracranially; the SORd (31 SNs) coursed dorsally within the cranium, emerging from a foramen in the ocular side frontal and radiating in a fan-like manner to innervate the longitudinal row of the *dl* group (15 SNs) and anterior half (excepting the anteriormost row) of the *do2* subgroup (16 SNs); the SORv (3CNs, 16 SNs) passed anterodorsally within the cranium, exiting externally through a foramen between the sphenotic and frontal, subsequently innervating 1 CN of the SOC posterodorsally, and 2 CNs of the SOC and the dorsal part of the *no* group (16 SNs) anteriorly. The BR (11 SNs) emerged from the cranium through the trigeminal (V) foramen between the sphenotic and parasphenoid, immediately dividing into dorsal (BRd) and ventral (BRv) ramules; the BRd (5 SNs) coursed anteriorly to innervate

the ventral part of the *no* group; and BRv (6 SNs) coursed obliquely downward to innervate the *ch* group ventral to the lower eye. The OR, sharing the trigeminal (V) foramen with the BR for its exit, innervated 2 CNs of the OTC. The MDR (11 CNs, 55 SNs) emerged from the cranium through the facial (VII) foramen in the prootic, coursing anteroventrally to reach the tip of the lower jaw; it gave off a ramule posterolaterally to the hyomandibular (MDRp; opercular ramule of the MDR sensu Freihof 1978) to innervate 6 CNs (5 on 5 scales dorsally; 1 on preopercle ventrally) of the dorsal part of the PRC and all SNs (33) of the *op* group. More ventrally, the MDR split into the dorsal (MDRd) and ventral (MDRv) ramules; the MDRd (2 CNs, 22 SNs) further split into 2 elements, the dorsal element (15 SNs) innervating 2 transverse rows (5 SNs) of the posterior *ch* group, the *pr* group (4 SNs) and the posterior 6 SNs of the *md* group, and the ventral element innervating 2 CNs of the MDC and the anteriormost 7 SNs of the *md* group; the MDRv innervated 3 CNs of the PRC. The STR (3 CNs, 24 SNs) emerged from the cranium through the vagal (X) foramen in the exoccipital and divided into 2 ramules. The anterior ramule was short, innervating 1 CN of the POTC (on the scale) and 2 transverse rows (5 SNs) of the posterior part of the *dl* group. The posterior ramule was long and serially ramified, basally innervating the anteriormost CN of the TRC and 1 CN of the STC, and

**Fig. 5** Lateral line canals, neuromasts (illustrated as roughly approximating sensory strip shape) and cranial skeleton on blind side of *Pardachirus pavoninus* (BSKU 123065; 46.1 mm SL; bones from BSKU 123066; 121 mm SL). See text for abbreviations of groups. Abbreviations and colors as in Fig. 2



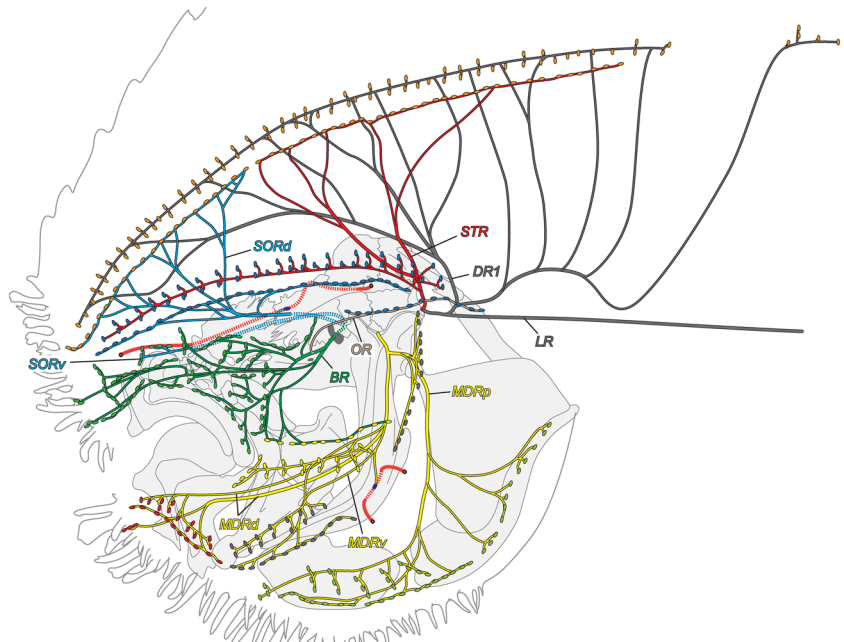
dorsally the posterior half of the *do2* subgroup (19 SNs). The LR gave off 3 DRs (36 SNs) dorsally, DR1–3 innervating 24, 5 and 7 SNs of the *do1* subgroup, respectively.

**Blind side.** Canals and canal neuromasts (Fig. 5; Table 1). Only 2 canals (SOC and PRC) occurred on the blind side. The SOC (1 CN) extended along the entire length of the blind side frontal, running closely parallel to the ocular side SOC between the eyes (twisted to the ocular side owing to the eye migration), terminating anteriorly as a long cutaneous extension (to the surface of the blind side) and posteriorly as a short extension (also to the surface of that side). The PRC (1 CN) was short, being restricted to the middle part of the preopercle with a pair of cutaneous extensions.

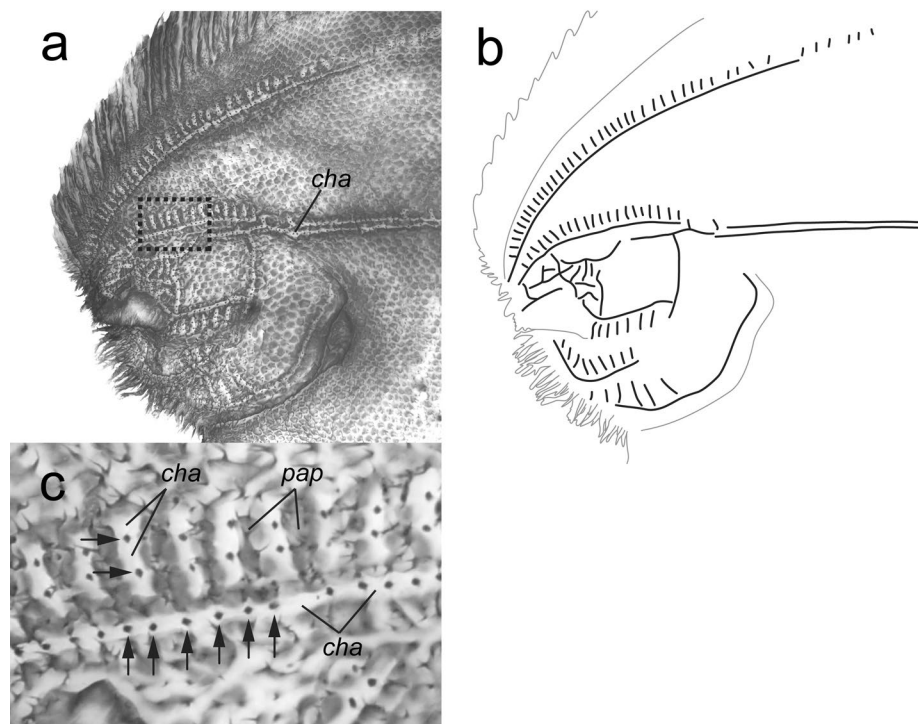
**Superficial neuromasts** (Figs. 1, 5, 7; Table 2). Sensory strips of superficial neuromasts (SNs) were elliptical and their longer axes were 0.11–0.15 mm long ( $n = 20$ ;  $\bar{x} = 0.12$ ;  $SD \pm 0.01$ ), 1.18–1.59 times ( $\bar{x} = 1.41$ ;  $SD \pm 0.11$ ) the length of the shorter axes. Numerous dermal papillae occurred on the epidermis, with fringing narrow strips of the skin. These strips were each associated with a row of SNs or single SN (channels; see “Materials and methods”). Six groups of SNs were located on the blind side (total 465 SNs; the number was highly stable among individuals, 468 in 215 mm specimen): the dorsal (*do*), dorsolateral (*dl*), nostril (*no*), cheek (*ch*), preopercular (*pr*), mandibular (*md*) and opercular (*op*) groups. The *do* group (136 SNs) comprised 2 subgroups (*do1*, 2): the *do1* subgroup (80 SNs), a narrow band (long axis direction of sensory strips—transverse: Tv) mostly comprising 2 rows (49 channels), extending from the snout to the anterior trunk along the dorsal margin of the body (SNs absent from the margin posterior

to the subgroup), and the *do 2* subgroup (56 SNs), a long row (long axis direction of sensory strips—longitudinal: Lt; 1 channel) extending below the *do1* subgroup. The *dl* group (90 SNs) comprised 3 subgroups (*dl1*–3): the *dl1* subgroup (45 SNs; Tv), a narrow band, mostly comprising 2 rows (25 channels), extending from the snout to the otic region, the *dl2* subgroup (32 SNs; Lt), a long longitudinal row below the *dl1* subgroup (1 channel), and the *dl3* subgroup (13 SNs; Lt), a longitudinal row (1 channel) posteroventral to the *dl2* subgroup. The *no* group (95 SNs), forming a large patch occupying the snout and anterior cheek, comprised ca. 13 rows (see Figs. 5, 7 for rows, and directions of sensory strips and channels) in a complex reticulated pattern. The *ch* group (29 SNs) comprised 2 subgroups (*ch1*, 2): the *ch1* subgroup (11 SNs; Lt), a longitudinal row (1 channel) on the lower cheek, and the *ch2* subgroup (18 SNs; Tv), a narrow band below the *ch1* subgroup, comprising 8 short transverse rows (2–4 SNs each; 8 channels). The *pr* group (41 SNs) included 3 subgroups (*pr1*–3): the *pr1* subgroup (14 SNs; Tv) occurring transversely as a row (1 channel) posterior to the cheek, the *pr2* subgroup (14 SNs; Tv), a narrow band on the ventral part of the preopercle, comprising 6 transverse rows (2 SNs per row, each associating a channel) and 2 posterior single SNs (Tv; 2 channels), and the *pr3* subgroup (13 SNs; Lt) extending obliquely as a row (1 channel) below the *ch1* subgroup. The *md* group (25 SNs) included 2 subgroups (*md1*, 2): the *md1* subgroup (12 SNs) forming a narrow band along the middle of the lower jaw, including 4 single SNs (Tv; 4 channels) and 4 transverse rows (2 SNs [Tv] per row, each associating a channel), and the *md2* subgroup (13 SNs; Lt) including single long (10 SNs; 1 channel) and short (3 SNs; 1 channel) rows below the *md1* subgroup. The *op* group (49

**Fig. 6** Innervation of lateral line system on blind side of *Pardachirus pavoninus* (nerves from six specimens; see “Materials and methods”). Abbreviations and colors as in Fig. 4



**Fig. 7** Channels (a) and their line illustration (b) on blind side of *Pardachirus pavoninus* (BSKU 123068; 215 mm SL). c Close up of dotted box in a. cha channel, pap papillae. Arrows indicate superficial neuromasts



SNs) formed a row (30 SNs; Lt; 1 channel) along the subopercle and interopercle, with an SN and 6 short transverse rows (19 SNs; Tv; 1 channel per SN and row) anteriorly.

**Innervation** (Fig. 6; Tables 1, 2). As on the ocular side, the anterior and posterior lateral line nerves on the blind side comprised 4 and 2 rami, respectively. The SORd (innervating 55 SNs) extended anteriorly within the cranium and emerged from a foramen in the blind side frontal, thereafter

radiating extensively (but becoming merged into a common longitudinal element distally) to innervate the *dl2* subgroup (32 SNs) and anterior part of the *do2* subgroup (23 SNs). The SORv (1 CN, 6 SNs) passed anteriorly within the cranium and exited from a foramen between the sphenotic and frontal. It extended anteriorly along the SOC of the blind side frontal (twisted to the ocular side) to innervate 1 CN, thereafter coursing to the blind side to innervate



the anterodorsal part of the *no* group. The BR (100 SNs) emerged from the cranium through the trigeminal (V) foramen between the sphenotic and parasphenoid, thereafter ramifying extensively to innervate most SNs of the *no* group (89 SNs) and all SNs of the *ch1* subgroup (11 SNs). The OR (13 SNs), merged with the BR proximally and turned posteriorly to innervate the *dl3* group. The MDR (1 CN, 133 SNs) gave off a ramule posteriorly at the dorsal part (MDRp; 63 SNs), which supplied the *pr1* subgroup (14 SNs) and the *op* group (49 SNs). Further ventrally, the MDR ramified into 3 ramules, the dorsal ramule (MDRd; 44 SNs) innervating the *ch2*, *pr2* and *md1* subgroups (18, 14, 12 SNs, respectively), the middle ramule (also treated as MDRd based on bifurcation of ocular side MDRd; 13 SNs) innervating the *md2* subgroup and the ventral ramule (MDRv; 1 CN, 13 SNs) innervating 1 CN of the PRC and the *pr3* subgroup. The STR (78 SNs) innervated the *dl1* subgroup (45 SNs) proximally and the posterior part of the *do2* group (33 SNs) distally, the latter sharing a common longitudinal element for its innervation. The LR gave off the DR1 (80 SNs) to innervate the *do1* subgroup by extensive proximal ramification and distal unification into a common longitudinal element (excepting the posteriormost 5 SNs).

## Discussion

To date, lateral line scales have been linked conceptually only with the trunk canal (TRC) in bony fishes, essentially being one and the same (see Webb 1989b; Voronina and Hughes 2018). In the cephalic lateral line system of *Pardachirus pavoninus*, however, additional “lateral line scales” were recognized as lateral line components on the ocular side (Figs. 2, 3). Such scales constituted the posterior part of the otic canal (OTC) and dorsal parts of the preopercular (PRC) and supratemporal (STC) canals; their canal neuromasts (CNs; absent from STC) were supplied by the otic and mandibular rami of the anterior lateral line nerve, respectively (Fig. 4). This is the first documentation of neuromasts in “lateral line scales” on the head, and innervated by a nerve other than the posterior lateral line nerve. Although serial homology of the scales with those of the TRC may be questioned if based solely on innervation, they are similar in size and shape, both have ctenii along the posterior margin (albeit not on all scales; Fig. 3) and tubed canals centrally (albeit cutaneous in the dorsal part of the STC; Fig. 3), suggesting that the tubular head scales are formed like the trunk lateral line scales (see Webb 1989a, c). Therefore, the term “lateral line scales” is applied here based on their putative homology, although convergence (and necessity of a new descriptive term) cannot be ruled out entirely. The homology of the scales with the two tubular ossicles on the anterior parts (one each) of the OTC and PRC (Fig. 2) is unknown.

In Pleuronectiformes, lateral line scale formation begins on the caudal peduncle and progresses anteriorly to the postotic region (Fukuhara 1986, 1988; Able and Lamonaca 2006), as is common in bony fishes (Sire and Akimenko 2004; for exceptions, see Webb 1990). The general absence of scales in association with the cephalic lateral line system indicates that such progression terminates in the postotic region. If so, in *P. pavoninus*, formation of the “lateral line scales” may extend beyond the postotic region, reaching as far as the temporal region (STC) dorsally, the otic region (OTC) anteriorly and the dorsalmost part of the preopercle (PRC) ventrally. If this extension of the formation to the head is demonstrated, the serial homology of the lateral line scales between the head and trunk will be further supported.

In *P. pavoninus*, three “lateral line scales” of the STC lacked CNs (Figs. 2, 3), despite their close structural similarities to those of the POC. Wonsettlar and Webb (1997) found in two species of *Hexagrammos* (Hexagrammidae) that four of the five trunk lateral lines were devoid of CNs, being inconsistent with the traditional (and most recent; see Wada et al. 2014) view that a presumptive CN induces canal morphogenesis. The absence of CNs from lateral line scales in a taxon with a single trunk lateral line (one CN per two or more scales) seems to be relatively common in percormorphs (i.e., carangid *Trachurus japonicus* and pomacentrid *Amphiprion ocellaris*; personal observations). Therefore, the mechanism of canal morphogenesis may not be so simple as presently understood (see discussion in Wonsettlar and Webb 1997).

On the ocular side of *P. pavoninus*, the preopercle was widely separated from the otic region of the cranium (Fig. 2), the dermis occupying the gap between the two. It differs significantly from the typical teleostean condition in which the two are closely contiguous, providing no space of the dermis between the two. In *P. pavoninus*, five scales bridged this gap, each with a canal perpendicular to the long axis of the scale, to form a continuous canal connection (i.e., dorsal part of PRC) between the upper part of the preopercle and the otic region (Figs. 2, 3). Five CNs of these scales and the dorsalmost CN of the preopercle were innervated by the posterior ramule of the mandibular ramus (MDRp; Fig. 4). In a typical teleost, however, the MDRp innervates a vertical SN row on the anterior margin of the opercle and the dorsalmost CN of the preopercle (see, for example, Sato et al. 2017). Therefore, the dorsalmost CN of the bone in the respective taxa is homologous, and the five CNs of the scales represent the transformation from SNs.

In *P. pavoninus*, the dermis covered the space dorsal to the preopercle (see above). This condition most likely allows development of the scales of the PRC and OTC, leading to the transformation of SNs on the opercle into CNs, with shift of the SNs from a location posterior to the preopercle (see *pr1* in Figs. 5, 6 for this condition) to mostly dorsal

to the bone (PRC; Figs. 2, 4), and the transformation of an SN, otherwise destined to be a CN in the bony canal of the pterotic, to the CN on the scale (OTC; Figs. 2, 4).

In typical teleosts, the superficial ophthalmic ramus (SOR) is a single branch, running closely parallel to the SOC. In *P. pavoninus*, however, the SOR was bifurcated (i.e., SORd and SORv) on both the ocular and blind sides (Figs. 4, 6, respectively), an apparently common condition in Pleuronectiformes, although available information, in particular for the blind side, is limited (see citation below). The SORd was correctly termed as a branch of the anterior lateral line nerve for the first time in this study. In all pleuronectiform species reported thus far, the SORd closely resembles the ramus lateralis accessorius (RLA; also termed “ramus recurrence facialis”: Freihofner 1963; Zottoli and Van Horne 1983) in its intracranial dorsal path and its exit from the dorsal surface of the cranium (parietal for RLA, see below). It should be noted that application of the term RLA to a lateral line nerve element is not presently acceptable, because the former innervates taste buds (Freihofner 1963, 1970). In *Pleuronectes platessa* (Pleuronectidae), Cole and Johnston (1901) showed that the “RLA” on the ocular side innervated “pit organs” posterior to the upper eye, after emergence from the cranium via a foramen in the frontal. This “RLA” is identical to the SORd in *Pardachirus pavoninus*, because both share a root with the SORv (see Cole and Johnston 1901). Cole (1898) identified “the right dorsal branch of the fifth” described by Cunningham (1890) on the ocular side of *Solea vulgaris*, as the “RLA”. In that species, the branch emerged via a foramen in the frontal to supply the skin of the extreme anterior end of the dorsal fin (Cunningham 1890). We consider “the skin” to actually be SNs, since the latter occur on the skin in that region in *Solea solea* (see Harvey et al. 1992); hence, our recognition of “the right dorsal branch” as the SORd. Sasaki et al. (2007) wrongly applied the term “parietodorsal branch of the RLA” (following Freihofner 1963) to the SORd, the nerve occurring on each side in two pleuronectiforms (*Pseudorhombus pentophthalmus* [Paralichthyidae] and *Engyprosopon grandisquama* [Bothidae]), although it exits differently from the cranium (via parietal vs. frontal in the two species). Therefore, it appears that the RLA is absent in pleuronectiforms (known thus far; see also Freihofner 1963). The path of the SORv along the SOC, typical in bony fishes, indicates that the SORv is plesiomorphic and the SORd apomorphic, the latter being a putative synapomorphy of the order.

In *P. pavoninus*, rami on the blind side (Fig. 6) were generally better developed than those on the ocular side (Fig. 4), innervating a greater number of SNs (Table 2). Most notable was the BR, ramifying intensively over the snout and most of the anterior cheek dorsal to the upper jaw to innervate the *no* and *chl* groups. This condition contrasted sharply with the two simple branches on the ocular side, with the migration

of the incipient upper eye apparently providing the surface area necessary for such ramification. Considering the position of the eye in the initial (symmetrical) phase, the ventral branches are expected to have developed initially below the eye, followed by subsequent ramification of the dorsal branches in accordance with migration to the ocular side. In fact, an SN row (apparently innervated by BR) occurs along the ventral margin of the future upper eye in the symmetrical phase in *Solea solea* (see Harvey et al. 1992).

In *P. pavoninus*, the SORd and STRs on both sides had a radiating pattern of ramification. However, the branches on the blind side were distally interconnected, unifying into a common longitudinal element, to innervate a linear SN row by each element (two rows forming the *do2* group; Fig. 6), whereas those on the ocular side were distally separated, innervating many short SN rows (also as in the *do2*; Fig. 4). Such distal interconnection on the blind side also characterizes the DR1 on that side in its innervation of the *do1* group (Fig. 6), reflecting functional differentiation between the sides as discussed below.

In *P. pavoninus*, the number of SNs differed greatly between the sides, being 173 on the ocular side and 465 on the blind side (Table 2). Because only one CN of the PRC occurs on the blind side (Table 1; note 1 CN of the blind side SOC twisted to the ocular side), the greater number has been partly the result of replacement of CNs by SNs, involving a predicted budding process of founder neuromasts (Wada et al. 2010). However, because CNs totaled only 19 (plus 1; see above) on the ocular side, the significant difference in number is largely due to the overall hypertrophy of the blind side lateral line system associated with the novel structures (channels; see below). Asymmetry of the SN numbers has also been reported in some soleid species (Appelbaum and Schemmel 1983; Harvey et al. 1992), because the higher number is functionally important in the search for benthic prey (Harvey et al. 1992). In Soleidae, numerous dermal papillae also occur densely on the blind side head (Ochiai 1966) (Figs. 1d, 7), functioning partly to protect SNs from damage (Appelbaum and Schemmel 1983) and also in the formation of channels as discussed below.

In *P. pavoninus*, sensory strips of SNs on the ocular side (without papillae) were small and rounded (Figs. 1, 2). However, those on the blind side (with papillae) were large and elliptical, each with clear long and short axes (Figs. 1, 5). In Soleidae, elliptical sensory strips on the blind side have also been reported in *Solea vulgaris* (see Appelbaum and Schemmel 1983) and *S. solea* (see Harvey et al. 1992). In both species, the axis of best sensitivity of each hair cell is parallel to the long axis of the strip, as seen in other pleuronectiforms (Rhombosoleidae; Roper 1981). This correspondence also occurs in non-pleuronectiforms (see Becker et al. 2016). Because the long axis is always parallel to the direction of the channel in *P. pavoninus*, the hair cell orientation is most

likely parallel to the long axis. This is in accordance with Appelbaum and Schemmel's (1983) suggestion that, in *S. vulgaris*, papillae enhance the directional sensitivity of the SNs (i.e., sensory strips) by forming an arch on each side of the SNs.

In *P. pavoninus*, channels were longitudinal, transverse and oblique to the body axis with very variable lengths (Fig. 7). On the dorsal surface of the head, the *do1* and *dl1* groups were associated with numerous short transverse channels and the *do2*, *dl2* and *dl3* groups were each arranged along long longitudinal channels (Fig. 7). Accordingly, channels were generally aligned in two directions in that region of the head. On the snout and upper cheek, however, directionality of the channels was more complex, forming a reticulated network. On the ventral surface of the head, channel orientation was similar to that on the dorsal surface, with long longitudinal (*ch1*, *pr3*, *md2* and *op* groups) and short transverse (*ch2*, *pr2* and *md1* groups) channels (Figs. 5, 7). The presence of long longitudinal channels suggests that the neuromasts function as accelerometer as flows increase proportionally with swimming speed. We also suggest that the reticulated channels on the snout and cheek (*no* group) comprise an additional sensor for the location of prey, since water motion generated by prey are likely to be multidirectional. However, the foraging behavior of *P. pavoninus* is unknown, so these suggestions should be tested by underwater observations and experimentation.

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