The morphologies of mudskipper pelvic fins in relation to terrestrial and climbing behaviour

Citation for published version:
Hidayat, S, Wicaksono, A, Raharjeng, A, Jin, DSM, Alam, P & Retnoaji, B 2022, 'The morphologies of mudskipper pelvic fins in relation to terrestrial and climbing behaviour', *Proceedings of the Zoological Society*, vol. 75, no. 1, pp. 83-93. https://doi.org/10.1007/s12595-021-00422-1

Digital Object Identifier (DOI):
10.1007/s12595-021-00422-1

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Proceedings of the Zoological Society

The Morphologies of Mudskipper Pelvic Fins in Relation to Terrestrial and Climbing Behaviour

Saifullah Hidayat[1,2] • Adhityo Wicaksono[1,3,4] • Anita Raharjeng[1] • Desmond Soo Mun Jin[5] • Parvez Alam[6] • Bambang Retnoaji[1]

Abstract Two species of mudskipper are identified with different behaviours, which are related to their pelvic fin morphologies. *Periophthalmus variabilis* has unfused pelvic fins and is capable to climb on the vertical substrate. Another species, *Boleophthalmus boddarti* has fused pelvic fins which supports the fish mobility across the muddy substrate. In context of anatomy, both pelvic fins are composed of a *frenum* which covers the pelvic girdle and pelvic fin rays (*lepidotrichia*). The unfused pelvic fin of *P. variabilis* has split rays that are not interconnected, whereas the pelvic fins of *B. boddarti* are fused completely and the fin rays are merged to the skin. The pelvic fin ray bones of *B. boddarti* are composed of large bone structure, allowing it to function as a strong sitting pad on a semi-terrestrial substrate. Comparatively, in *P. variabilis*, the ray bones are shorter, hence provides flexibility to grip more surface area for the fish to stick during vertical locomotion. Histologically, the epidermal layer of *B. boddarti* pelvic fins has lower quantities of mucous cell than in *P. variabilis*, by 14.33 ± 1.53 and 33.33 ± 1.53 mucous cells per 100 epithelial cells, respectively. This difference reveals that more mucus is produced in *P. variabilis*, hence possibly giving this fish an enhanced capacity for Stefan adhesion on a terrestrial substrate than in *B. boddarti*. From this, the terrestrial locomotion and climbing ability are more supported in *P. variabilis*, compared to the more aquatic *B. boddarti*.

Keywords *Boleophthalmus* · *Periophthalmus* · Biomechanics · Histology · Anatomy

Saifullah Hidayat and Adhityo Wicaksono: shared first authorship.

Bambang Retnoaji
bambang.retnoaji@ugm.ac.id

1 Laboratory of Animal Structure and Development, Faculty of Biology, Gadjah Mada University, Yogyakarta 40135, Indonesia

2 Department of Biology Education, Faculty of Science and Technology, Universitas Islam Negeri Walisongo, Semarang 50185, Indonesia

3 Laboratory of Natural Material Technology, Department of Chemical Engineering, Åbo Akademi University, Porthaninkatu 3, 20500 Turku, Finland

4 Division of Biotechnology, Generasi Biologi Indonesia (Genbinesia) Foundation, Jl. Swadaya Barat no. 4, Gresik Regency 61171, Indonesia

5 Rapid Gain Global, Singapore, Singapore

6 Institute of Material and Processes, School of Engineering, University of Edinburgh, Edinburgh EH9 3FB, UK

Introduction

The blue-spotted mudskipper (*Boleophthalmus boddarti* (Pallas 1770)) and the slender mudskipper (*Periophthalmus variabilis* (Eggert 1935)) are both amphibious fishes adapted for terrestrial environment (Gordon et al. 1969). *B. boddarti* has a larger and heavy body with a fused pelvic fin, relatively compared to *P. variabilis*, which has a smaller and lighter body weight with an unfused pelvic fin. The carnivorous mudskippers, *Periophthalmus* prefers the higher grounds to improve their way to hunt crabs and gastropods while in contrast, the herbivorous *Boleophthalmus* spends two-thirds of its active time grazes upon surface sediments with help of its caudal fin to scrapes diatoms or algae (Patzner et al. 2011). Due to the diet preference of *Periophthalmus*, specific biomechanical adaptations are required to improve its locomotion system.

The mudskipper adaptations on the pelvic fin structures are influenced by their habitats and locomotion behaviours. The hydrodynamic forces are dominating in the aquatic environment, compared to the gravity in the dry terrestrial environment, and these differences require specific biomechanical adaptations for loads in muscle, bones, and joints for each environment (Pace and Gibb 2009). Two mudskipper genera, *Periophthalmus* and *Periophthalmodon*, are known for their highly terrestrial kinematic behaviour compared to other mudskipper genera (Zhang et al. 2003). *P. variabilis* is able to climb, attaches itself to the vertical substrate like mangrove roots or rocks, and perches using both pectoral and pelvic fins (Polgar and Crosa 2009), while on horizontal substrate, it ''crutches'' using its pectoral fins to move parallel to the substrate (Harris 1960). The pectoral fins of *P. variabilis* assist the mudskipper to move forward like a pair of front limbs, acting like a pair of crutches, while the unfused pelvic fins stabilise their movement and act like hind limbs (Gibson 1986; Sayer 2005; Kutschera and Elliott 2013). The pectoral fins of *P. variabilis*, also deployed mid-air during the unique locomotion of water-hopping to soften the landing (Wicaksono et al. 2020). Unlike the amphibians and higher vertebrates, the *P. variabilis* unfused pelvic fins deployed instantly in a piston-like movement as the result of pressure from the pectoral musculature into the lateral muscle which presses the pelvic fins downward (Wicaksono et al. 2017). On vertical substrate, the unfused pelvic fins of *Periophthalmus* provide better attachment due to greater Stefan adhesion in comparison to the *Boleophthalmus* fused pelvic fins (Wicaksono et al. 2016).

Structurally, the pelvic fins are composed by the pelvic disc or plate and the rays (Standen, 2010). The pelvic disc is composed of the bone frame: the symphysis process (*processus symphysicus*) and the basipterygia bones (*os basipterygium*) (Lundberg and Dahdul 2008). The fin rays of teleostei reveals that each of the ray bones or *lepidotrichia* consists of cylindrical tubes in a form of a pair of crescent-shaped bones facing each other; a series of parallel crescent-shaped bones called *hemitrichia*, which are in turn conjoined by dense fibrous connective tissues (collagen fibres) in the whole length of *lepidotrichium* (single *lepidotrichia*) and filled with loose connective tissue, nerve bundles and blood vessels (Genten et al. 2009; Pfefferli and Jaźwińska 2015). There are two *hemitrichia* extended from the pelvic disc in parallel: the dorsal and the ventral *hemitrichia*, and these *hemitrichia* are connected to six muscles in teleostei (Farell 2011). These muscles are both abductor and adductor muscles attached in the frame of *os basipterygium* that controls the fin movements (Standen 2010).

Externally, the mudskipper possess specific adaptation to breathe by using moist, highly vascularised skin with capillaries near its superficial epidermal layer and its outermost layer (Park 2002). Typical characteristics of the mudskipper skin are the dermal bulges, thick connective tissue containing blood vessels, and the mucous cells, despite the dermal bulges only exist in *Boleophthalmus* and *Scartelaos* genera of the mudskipper (Zhang et al. 2000). Comparatively, the skin layer located in the fin of *B. pectinirostris* (Linnaeus, 1758) consists of a thin epithelial layer without dermal bulges, undifferentiated blood vessels on the dermis, and no mucous secreting cells (Park et al. 2003). The mucous producing cells are important component to produce hyaluronic acid mucous which assists the fish to adhere onto the vertical substrate (Wicaksono et al. 2016).

In this paper, the main highlight are the anatomical, morphological and histological analyses for the pelvic fins of two mudskipper species, *B. boddarti* and *P. variabilis*. The general anatomical study of both species has been conducted in our study, documented in a thesis (Hidayat 2015), while we want to highlight our analysis regarding the pelvic fins in this paper. The *B. boddarti* is more aquatically adapted despite its ability of terrestrial locomotion. In comparison, *P. variabilis* is more terrestrially adapted, and able to climb onto vertical substrates. Morphologically, *B. boddarti* has fused pelvic fins, while the pelvic fins of *P. variabilis* is unfused. We hypothesise that the physical adaptations of the pelvic fin correlate to their behaviour and our objective is to connect any anatomical, morphological and histological differences found in our observation into the specific function related to the behaviours of each mudskipper.

Materials and Methods

Fish Sampling and Observations

All fishes used in this study were shared as in Wicaksono et al (2016). Fishes for each of two species of mudskippers were used for this research, *B. boddarti* (mean body length 13.1 cm, height 1.7 cm, weight 18.5 g) and *P. variabilis* (mean body length 7.4 cm, height 0.8 cm, and weight 3.7 g) were collected in the coastal regions of Kaliwungu, Kendal Regency, Central Java, Indonesia between October and December 2014. Each specimens were caught using a fishnet and handled with care, and then kept into separated, aerated aquaria for each species. The fish behaviours were observed on five fishes per species (in total observation) in both their natural habitats and in glass aquaria, using a Samsung HMX-F90, HD camera to video and photograph the fishes. The rest of the fishes were taken for later analysis. All glass aquaria were kept in Laboratory of Animal Structure and Development, Universitas Gadjah Mada, Yogyakarta, Indonesia. Three fishes per species were used for histological analysis and five fishes per species for bone structure analysis. Two of the entire fishes of each species were modelled for the study in Wicaksono et al (2016).

Staining Preparation and Procedures for Histological Analysis

The histological preparation of pelvic follows the adapted standard paraffin methods from Gray in 1954 (Gray 1954). Three fishes per species were euthanized with 70% ethanol. Both of the pelvic fin types (fused and unfused, from *B. boddarti* and *P. variabilis*, respectively) were cut in the same anatomical positions and rinsed using phosphate buffer solution (PBS) after which they were fixed using Bouin solution (combination of picric acid, acetic acid, and formal-dehyde in aqueous solution) for 48 h. Samples were then stained using hematoxylin and eosin (HE) for nucleus shape identification process. The modified acid-fast staining (MAF) was used to identify connective tissue, and periodic acid-Schiff staining (PAS) was also used to identify the mucous secretion. These staining methods are the modified version of the Bancroft and Cook protocol (Bancroft and Cook 1984).

Bone Structure Analysis

The observation of the pelvic fin bone-cartilage structure was performed after staining using the modified Alizarin Red/Alcian Blue staining protocol from Inouye (Inouye 1976). Five fishes per species euthanized with 70% ethanol for bone staining. The samples were fixed in 99% ethanol for 72 h and were then treated with acetone for 1 week. Following the treatment, the samples were incubated in the staining solution (0.015% Alcian Blue, 0.015% Alizarin Red in 70% ethanol) for 3 days. After washing with water, the fish were rinsed using 1% KOH for 48–72 h, and followed by incubation in the solution of 1% KOH in 20% glycerol and 0.01% KOH in 20% glycerol. The stained fishes were stored in 50% glycerol, and the sections of samples were then analysed from photographic images of the staining result.

Results and Discussions

The Behaviour of the Mudskippers and the Movements of Pelvic Fins

Both species are seemingly active in the mangrove area. However, *P. variabilis* spent more time in terrestrial and drier regions, while *B. boddarti* spent more time in the aquatic and muddy region. This is consistent to the information provided by Patzner et al. (2011). The *P. variabilis* waits for its food near mangrove trees and uses its pectoral fins for locomotion (propulsive and recovery motions) while its unfused pelvic fin to support its body, consistent to information by Pace and Gibb (2009). Climbing behaviour was also observed on *P. variabilis*. During climbing, this mudskipper moves on a surface of highly-inclined or as in this study, on a vertical substrate using its pectoral fins to move towards the cranial direction (forward) and the unfused pelvic fins keep the mudskipper body on the substrate as adhesive pad, and locking the body to the surface. Upon vertical locomotion or climbing, the pelvic fins are similarly expanded and retracted in cyclic manner as in the horizontal locomotion. This role of the extended unfused pelvic fins is significant, as *P. variabilis* can stick to the aquarium glass by completely relying the extended pelvic fins without the pectoral fins being extended (Fig. 1).

Comparatively, *B. boddarti* was unable to climb the vertical substrate of the glass aquarium and it could only walk in slightly inclined and horizontal substrate only. For locomotion, *B. boddarti* used its pectoral fins which acted like the forelimbs in tetrapods to walk on the substrate and its fused pelvic fins underneath its body served as cushion pad when the fish landed after hopping (Fig. 2).

Fig. 1 *P. variabilis* climbs on the aquarium glass (vertical substrate) using both pelvic fins and pectoral fins extended a, and only pelvic fins are extended, while the pectoral fins are retracted b

Fig. 2 Sequential pictures of *Boleopthalmus boddarti* hopping from the muddy substrate (ex situ observation) and followed by landing (from 00.00 to 00.14 ms). Time frame showed in milliseconds

Both fish species used their pelvic fins in the same way for swimming, for stability during terrestrial locomotion, and as landing cushion after hopping. However, some difference of fin activities can be observed in correspondence to the different behaviours, such as serves a stabilising pad during terrestrial locomotion and as an adhesive pad while climbing on inclined or vertical substrates (Table 1). This function of pelvic fins as a cushion of point where the body weight is taken upon locomotion is described by Sayer (2005), hence it can be referred that the pelvic fins could work as the body stabilizer.

Pulsating refers to fin expansion and followed by retraction in cyclic manner underwater, normally observed during swimming. Curving refers to the pelvic fins form a perfect curvature while fully extended in both fused and unfused to aid as the mudskipper stood still on the substrate. Flattening as a pad, the pelvic fins went visually from a cusp into a perfect flat upon fish landing from terrestrial hopping possibly to reduce the impact force and this was observed in both *B. boddarti* and *P. variabilis*. Specifically in *B. boddarti*, the fused pelvic fins were also flattened, fully in contact to the substrate of its ventral when the mudskipper performed terrestrial locomotion (crutching). The other flattening action, which works as a stabilizer for terrestrial locomotion of the pelvic fins was not observed in *P. variabilis*, which instead overtaken by pelvic fins retraction-extension in cyclic manner when it moved forward in terrestrial locomotion. A study by Harris (1960) shows that the pelvic fins work in coordination with the pectoral fins (in a study with *P. koelreuteri* (Pallas, 1770)), in these following cycle: (1) The pectoral fins work as crutches that propel the body forward in a single stroke, and during this progress, the pelvic fins are folded or retracted, and (2) upon the pectoral fins recovery stroke, the mudskipper body weight is rested and supported by the now retracted pelvic fins as the pectoral fins are not in contact to the substrate until they return to the initial state and the cycle returns. Compared to this study result, the *P. variabilis* pectoral and pelvic fins are working similarly with the cycle of extension and retraction during the locomotion over the substrate. Specifically in *P. variabilis*, the unfused pelvic fins was also fully extended in contact to the substrate during climbing. The special morphology of the pelvic fins in *P. variabilis*, which are flat and flexible, compared to *B. boddarti* cup-like but more rigid fused pelvic fins (Wicaksono et al. 2016) are greatly contributed gripping the substrate and providing Stefan adhesion, a form of wet-assisted adhesion between two parallel plates that in this case allows the *P. variabilis* to climb through the vertical substrate that is not occurring in *B. boddarti*. The cup-like pelvic fins of *B. boddarti*, on the other hand, due to its curvature of shape, works as either suction pad or

Table 1 Pelvic fin activities in both species with respect to specific behaviour

No	Fin activities	*P. variabilis*	*B. boddarti*
1	Pulsating (during swimming)	*	**
2	Curving (as sitting pad)	**	*
3	Flattening (as cushion pad)	*	*
4	Flattening (during terrestrial locomotion)	−	*
5	Retracting (during terrestrial locomotion)	*	−
6	Extending (as adhesive pad)	*	−

No activity (−), less activity (*), more activity (**)

just a sitting pad to the substrate instead of providing Stefan adhesion.

From these observations we assumed that the pelvic fin of *B. boddarti* is more useful for underwater locomotion, and adapted to movements like swimming rather than standing still on the substrate. In contrast, the pelvic fins of *P. variabilis* hardly contributes to underwater locomotion, and are more often used as stabilising pad when the fish stops on the horizontal substrate, as well as in climbing on inclined or vertical substrates. During aquatic locomotion (e.g. swimming or diving), *P. variabilis* relies completely on its caudal fins and sometimes pectoral fins (Wicaksono et al. 2020). Therefore, in regards to pelvic fin actions during terrestrial locomotion, the pelvic fin of *B. boddarti* appears to work by curving (retraction during swimming), flattening (upon extension), and drags along the ventral face of the fish in cycle, which is possibly to hold on a watery/muddy surface to allow the *B. boddarti* to stand still in the substrate as a sitting pad. Comparatively, *P. variabilis* unfused pelvic fins undergo the cycle of curving as the initial form, followed by retraction, which suggested to decreases drag between the skin and the substrate during the movements on drier terrestrial locomotion (this effective retraction is supported by the *P. variabilis* pelvic fins flexibility, described in Wicaksono et al. (2016)) that might damages the fish skin, and later back to the extension of the pelvic fins.

Morphological Structures of the Mudskipper Pelvic Fins

Morphologically in general, there are in general two types of mudskipper pelvic fin: fused and unfused. The levels of fins fusion may varied within species. *P. variabilis* has an unfused pelvic fin (split rays which are not interconnected) (Fig. 3b) while *B. boddarti* has a fused pelvic fin (the fin rays are connected to the skin) (Fig. 3a). Species from the genus *Periophthalmus* exhibit several types of pelvic fins including; fully fused (*Periophthalmus chrysospilos* (Bleeker, 1853)), partially fused (*Periophthalmus variabilis*, fish used in this study) and fully unfused (*Periophthalmus gracilis* (Eggert, 1935)) (Polgar and Crosa 2009).

The individual pelvic fins of a mudskipper is composed the membrane of skin connected to the pelvic rays for supporting the movement of fin rays (*frenum*) and fin rays (*lepidotrichia*), with the majority of the *frenum* of the pelvic fin is located anterior to the pectoral fin (*cranial*), similarly to cod (Gadidae) (Lagler et al. 1977). The *frenum* of the pelvic fin in *P. variabilis* is located closer to the cranium, but not parallel to the pectoral fin, while the pelvic fin *frenum* in *B. boddarti* is located farther to the cranium and parallel to the pectoral fin (Fig. 3). This positional arrangement provides for greater kinematic flexibility in *P. variabilis* whilst using its pelvic fins, and concurrently giving improved stabilisation and support to its body in comparison with the pelvic fin of *B. boddarti* since the unfused fin is more stretchable to a relatively greater width.

Pelvic Fin Bone Structure

The pelvic fin of the mudskipper consisted of bones (Susanto 2012), which can be seen more clearly in Fig. 4. In a pelvic muscular-bone system, the anterior symphysis process (*processus symphysicus anterior*) connects the

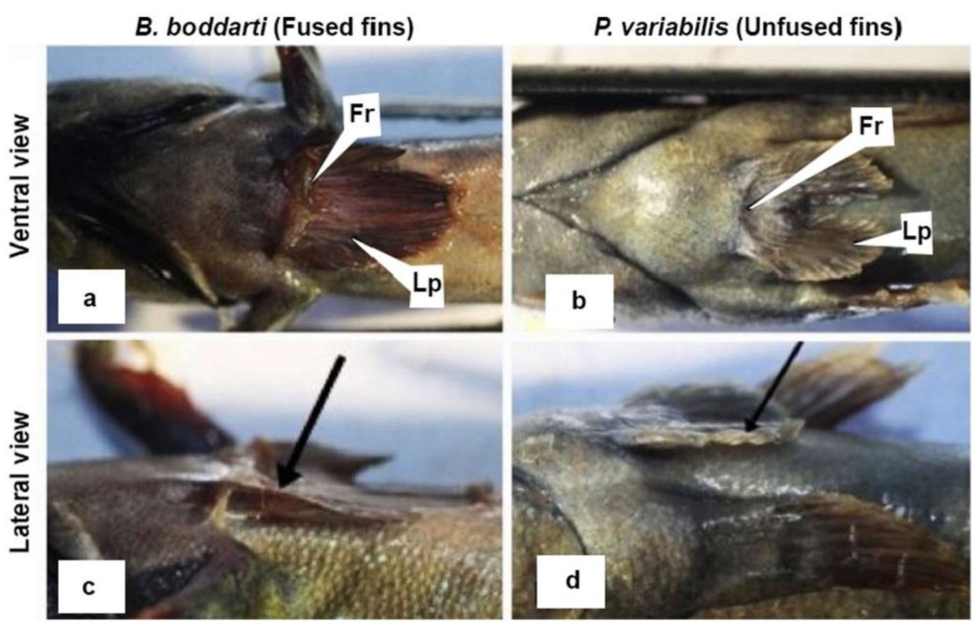

Fig. 3 Morphological structure of pelvic fins from ventral (bottom) views a, b and lateral (side) views c, d of *B. boddarti* and *P. variabilis*, respectively. In these plates, the *frenum* is shown in (Fr) and the *lepidotrichia* in (Lp). The pelvic fin ray outer linings from the lateral view are marked with the black arrows. Note the position differences of the pelvic fins relative to the pectoral fins radii and the crania: *B. boddarti* pelvic fins are farther from the cranium but parallel to the pectoral fins radii, while *P. variabilis* pelvic fins are closer to the cranium but unparallel to the pectoral fins radii

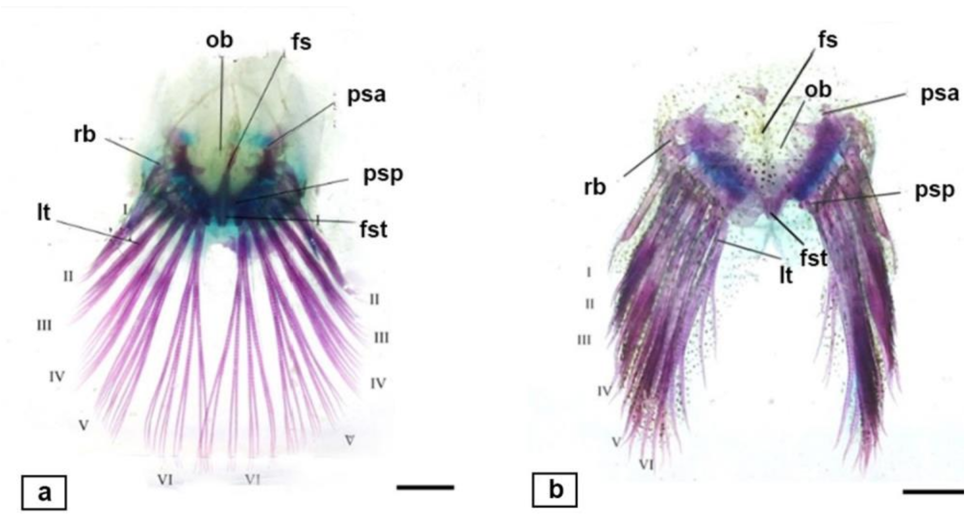

Fig. 4 Pelvic fin skeletal system of *B. boddarti* fused pelvic fins, a, and *P. variabilis* unfused pelvic fins, b (viewed under direct imaging by camera with Alizzarin Red-Alcian Blue (ARAB) staining). *Notes*: Radial bone (rb), ray bone (*lepidotrichia*) (lt, I through VI), basipterygia bone (*os basipterygium*) (ob), anterior symphysis process (*processus symphysicus anterior*) (psa), posterior symphysis process (*processus symphysicus posterior*) (psp), facial symphysis (*facies symphysica*) (fs), and tubercle of the facial symphysis (*facies symphysica tuberculum*) (fst). Scale bars = 5 mm

pelvic bones to the body lateral muscles while the posterior symphysis process (*processus symphysicus posterior*) connects the two laminae/plates of the basipterygia bones (*os basipterygium*), and these laminae play an important role in structural support of the pelvic fin where material strength is mainly required to assist the terrestrial locomotion. The dual basipterygia plate bones are held together by a cartilage coupling that shapes pelvic girdle (Lagler et al. 1977).

The fin ray bones (*lepidotrichia*) are the primary components that shapes the fin rays. Here in mudskipper there are 6 pairs of rays with cartilage bone on each tip. The radial region of ray bones sticks to the posterior of basipterygia bone at its base. Transversely, the fin ray bones retain a ring-like structure that allows the pelvic ray to adhere rigidly onto the basipterygia bone.

The pelvic fins for both *P. variabilis* and *B. boddarti* have the same structures. The primary difference is the pelvic fins of *P. variabilis* are unfused with not-interconnected, full-split rays, whereas the pelvic fin of *B. boddarti* is fully fused and the fin rays are interconnected. In qualitative observation, angle that interconnects the posterior symphysis process and the pelvic ray bones on *P. variabilis* is appeared to be larger than *B. boddarti*. The larger the angle, the pelvic fins are becoming unfused and spread freely to both lateral orientations (left and right). This angle allows the mobility in the pelvic fin of *P. variablis* in the front and back (mediocranial and mediocaudal) sections to improve. A potential consequence of this enhanced mobility is that the pelvic fin of *P. variabilis* has a widened area that improves its ability to adhere to substrates by enlarging the contact surface which facilitates the Stefan adhesion (Wicaksono et al. 2016).

The structures and sizes of the ray fin bones on *B. boddarti* are different to those of *P. variabilis* (Fig. 5). The size of longest pelvic fin rays bone of *B. boddarti* is 7.8 mm, while on *P. variabilis* is 4.2 mm. The average lower surface area of the pelvic fin bone on *B. boddarti* is 0.026 mm^2 while average lower surface area of the pelvic fin bone on *P. variabilis* is 0.009 mm^2. Closely viewed, the thickness of the individual segment bone of the ray is narrow and rigged on *P. variabilis*, while broader, smoother, and each interconnected directly to one another on *B. boddarti* (see Fig. 5a, b; compare individual segments, ''is'').

The ray bone segments in *P. variabilis* appeared to be more condensed and packed, with jagged structures. These features probably useful to assist its terrestrial locomotion as it provides a grip the substrate as it stabilises the body during the movements of the pectoral fins during the crutching process as referred by Harris (1960), although to note. In comparison, the smooth ray bone segments in *B. boddarti* as it used more for swimming, the segment smoothness probably contributes on lower hydrodynamic drag. The drag is an important element during a contact between the mudskipper fish body to the substrate as it allows the fish to get a control during terrestrial or semi-terrestrial locomotion. In a terrestrial locomotion, a perfect amount of drag allows the fish to grip the substrate, but if too much, it may damage the skin (hence the reason why, in this case the fins of the *P. variabilis* have to be retracted as well). In a semi-terrestrial (i.e. locomotion on a muddy

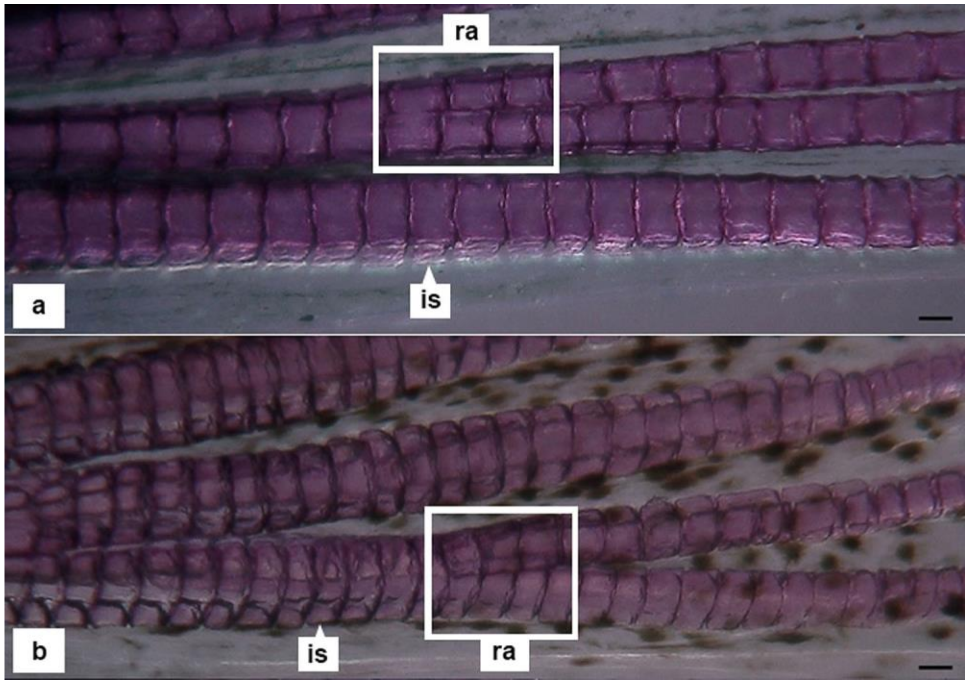

Fig. 5 Structures of the ray bones (*lepidotrichia*) of *B. boddarti* fused pelvic fins, a, and *P. variabilis* unfused pelvic fins, b. *Notes*: The ramified segments (ra), a group of segments which split the ray bones into two rays. Note that the individual ray bones segments (is) in *B. boddarti* are broader than in *P. variabilis*. Magnification: 10 9 10. Scale bars = 100 um

substrate or on a pond benthic substrate), a perfect amount of drag on the body surface holds the fish on the substrate against the water flow. *B. boddarti,* as its pelvic fins are fused and are unable to retract, by altering its shape might be the only way to make it work as a suction pad or sitting pad in the muddy or watery substrate, while its smooth surface topography is useful upon swimming (aquatic) locomotion to increase hydrodynamics.

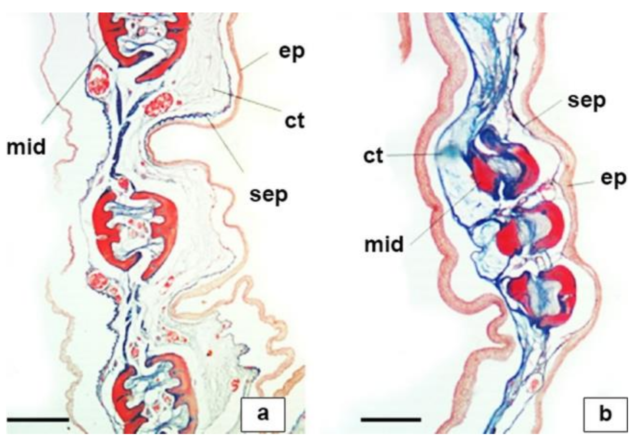

Fig. 6 Transverse cross-sectional perspective of pelvic fin histology (under MAF staining) of *B. boddarti* fused pelvic fins, a, and *P. variabilis* unfused pelvic fins, b, reveals the thickness difference between two mudskipper on each histological layers. *Notes*: epithelial (ep), subepithelial (sep), connective tissue (ct), and middle layer (mid). Magnification: 2 9 10. Scale bars = 50 um

Histological Structure of the Pelvic Fin

By observing the histological structures from a cross sectional perspective (on a transverse sectioning), it is noted that the fins of both *B. boddarti* and *P. variabilis* are composed by an epithelial layer, a sub-epithelial layer, connective tissue, and a middle layer (Fig. 6).

The surface of the pelvic fin outer epithelial layer consists of the layered, flat-shaped cells. In *B. boddarti* the flat-shaped epithelium is made up of 4 layers, while in *P. variabilis* there are only 3 layers, which is similar to observations by Park et al. (2003). The thinner layer epithelial layer in *P. variabilis* might corresponds to the terrestrial nature of *P. variabilis* and with thinner layer, it would be easier to oxygen to diffuse through the skin into the bloodstream (especially when the blood vessels are found in the outer epithelial layer with more mucous cell, further discussed later). Under HE staining, the nuclei of epithelial cells appear dark. Additionally, in the epithelial layer, there are mucous cells which appear due to the MAF staining as round and ovoid in shape, in adjacent amongst the flat epithelial cells. The secreted mucous is visible in magenta colouration under PAS staining (Fig. 7). The mudskippers use a mucous layer that serves as an anti-friction protectant for the surface epithelial layer (Zhang et al. 2000; Kardong 2009) and to assist attachment to the substrate by mediating Stefan adhesion, which is greatly increased in higher viscosity by the presence of mucus (Wicaksono et al. 2016). Mucous in the skin epidermal layer is an aid to respiration, in maintaining an ionic and

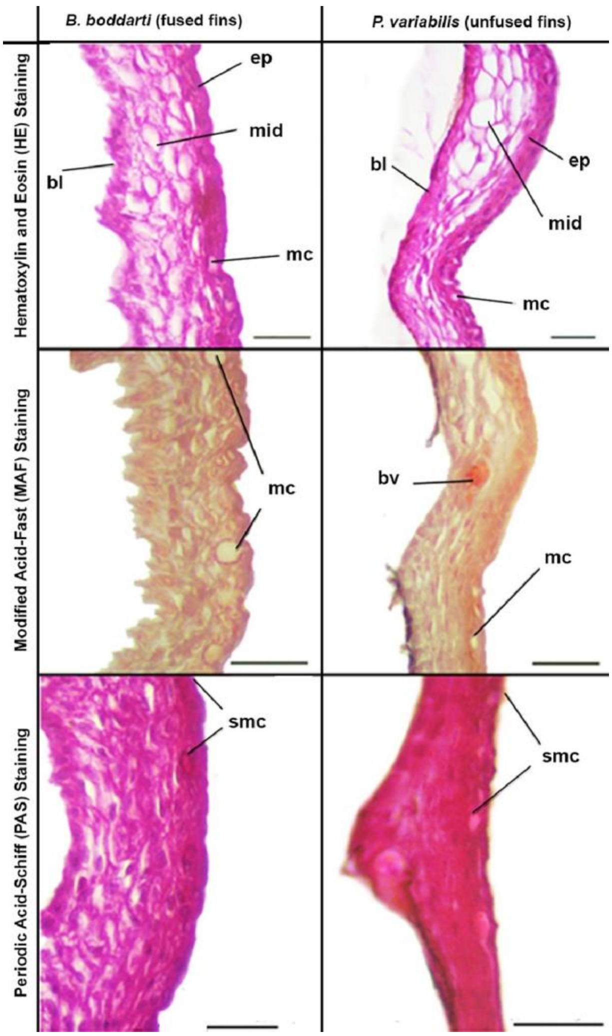

Fig. 7 The epithelial layer of the *B. boddarti* fused pelvic fins and *P. variabilis* unfused pelvic fins, stained using hematoxylin and eosin (HE), modified acid-fast (MAF), and periodic acid-Schiff (PAS) shows the size differences in two mudskipper species and histological cell components. *Notes*: surface of epithelia consisted of flat-shaped cells (ep), middle layer consists of swollen cell with big vacuoles (mid), basal layer consists of cylindrically-shaped cells (bl), mucous cell (mc), secretory mucous cells (smc), and blood vessel, f. Magnification: 20 9 10. Scale bar = 20 um

osmotic balance, for protection, and used as attachment (Shepard 1994; Wicaksono et al. 2016).

The middle section of epithelial layer consists of cells with large-sized vacuoles (under HE staining) known as the swollen cells that are reported to occupy 4 layers of the whole epithelial layer of *B. pectinirostris* (Park et al. 2003). These swollen cells prevent dehydration of the mudskipper skin while it walks on land (Zhang et al. 2000). Blood vessels exist between the middle parts of the epithelial cells of *P. variabilis*. Similarly located, the blood vessels have been classified as intra epithelial blood capillaries in *P. modestus* (Suzuki 1992). Using MAF staining, the blood vessels are shown with orange coloration, which implies the existence of erythrocytes. The lower or, basal section of epithelial layer consists of elongated or cylindrical cells and cuboidal cells in densely packed arrangements. Under HE staining, this region appears dark with nuclei arranged in the centre of each cell. The epithelial layer of the pelvic fin is slightly thicker in *P. variabilis* (43.6 um) than in *B. boddarti* (33.1 um). The difference of thickness between the two species occurs most prominently in the middle section of the epidermal layer, which is abundant with swollen cells (Fig. 7). This thicker epithelial layer in the process of *P. variabilis* is assumed to provide greater protection from friction while walking on land and when climbing trees, and is thus effectively works as a friction barrier than in aquatic *B. boddarti*.

The mucous secreting cells are found most abundantly in the pelvic fins of *P. variabilis* within the epidermal layers (Fig. 7; Table 2). The extraneous mucous cells could imply to more secretion of mucous in *P. variabilis*, to reduce the surface damages during terrestrial locomotion in both crutching and climbing in drier terrestrial area. Contrast to *B. boddarti*, which has fewer mucous cells but retains epidermal hydration by remaining in muddy and aquatic areas for the majority of its time. In most fishes, the mucous contains glycoproteins or mucins which have adhesive properties (Asakawa 1970), and it is shown in previous study that *P. variabilis* produces hyaluronic acid (a polymer of D-glucuronic acid and N-acetyl-D-glucosamine) mucous to assist itself adhering to the substrates when climbing on inclined or vertical substrates (Wicaksono et al. 2016).

In *P. variabilis*, blood vessels are found in the mid-section of the pelvic fin epithelial layer, whereas in *B. boddarti*, no blood vessels are found in this section but rather in the sub-epithelial section. Intra-epithelial blood capillaries located under the skin epidermal layer in *P. modestus* (Cantor, 1842) are reported to be vital for subcutaneous respiration support by diffusion (Suzuki 1992). The location of the blood vessel which is located in epithelial layer in *P. variabilis* is possibly to enhance the chance to obtain oxygen in terrestrial condition (in the open air instead of water), compared to the *B. boddarti* which can rely mostly on its gills and water-bound oxygen.

Table 2 The quantity of the mucous cells per 100 cells on the epithelial layer of *B. boddarti* versus *P. variabilis*

Species	Cell quantity per 100 epithelial cells
Periophthalmus variabilis	33.33 ± 1.53
Boleophthalmus boddarti	14.33 ± 1.53

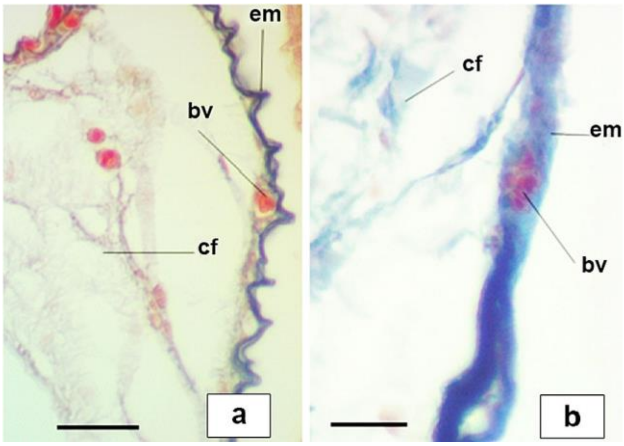

Fig. 8 Cross-sectional view of sub-epithelial layer (under MAF staining) in the fused pelvic fin of *B. boddarti*, a, and the unfused fin of *P. variabilis*, b, shows the thickness difference in the membrane between two mudskipper species. *Notes*: Elastic membrane (em), blood vessel with erythrocytes apparent inside (bv), and collagen fibre (cf). Magnification: 20 9 10. Scale bar = 20 um

Below the epithelium is the sub-epithelial layer made up of elastic membranes (*membrana elastica*), Fig. 8, that stretches and folds over connective tissue. The membrane can be observed as a dark blue band under MAF staining (Fig. 8, showed in a). When this membrane forms through fibroblastic secretions, it is defined as the granular layer (Suzuki 1992). Between these elastic membranes there are blood vessels with small lumens, with erythrocytes inside. The elastic membrane in *P. variabilis* is seemingly thicker than in *B. boddarti* which probably provides more barrier in terrestrial environment as in the epithelial layer.

Below the sub-epithelial layer is the dermal layer, which is comprised of connective tissue, Fig. 9. This layer consists of densely packed collagen fibres that appears grey under MAF staining. In this layer several fibroblasts can also be seen. In a previous study on *P. modestus*, it was reported that fibroblasts within the dermal layer are responsible for secreting granules that form collagen (Suzuki 1992). Blood vessels with erythrocytes can be seen (red colour) between the collagen fibres. These blood vessel act as nutritional and oxygen support to the tissue, in addition to the facilitation of subcutaneous respiration.

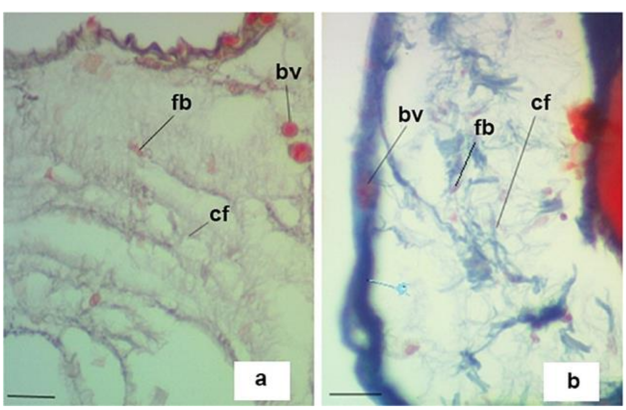

Fig. 9 Cross-sectional view of dermal or connective tissue layer (under MAF staining) in fused pelvic fin of *B. boddarti*, a, and unfused pelvic fin of *P. variabilis*, b. *Notes*: Collagen fibres (cf), blood vessel with an erythrocyte inside (bv), and fibroblast (fb). The images on this figure are the extension, and located in the same specimen as in Fig. 8. Magnification: 20 9 10. Scale bars = 20 um

The sub-epithelial layer or elastic membrane (*membrana elastica*) and dermal layer of connective tissue (collagen fibres and blood vessels) in *B. boddarti* and *P. variabilis* have supportive properties on the fin flexibility which allow them to curve. This curvature adjustment in the pelvic fins shape might assist *B. boddarti* to sit onto the muddy substrate as the fused pelvic fins has a slightly concave morphology to function as cushion pad underneath the body, while in *P. variabilis* the flexibility helps the mudskipper to moves in solid terrestrial substrate. Pelvic fin flexion is important in view of improving Stefan

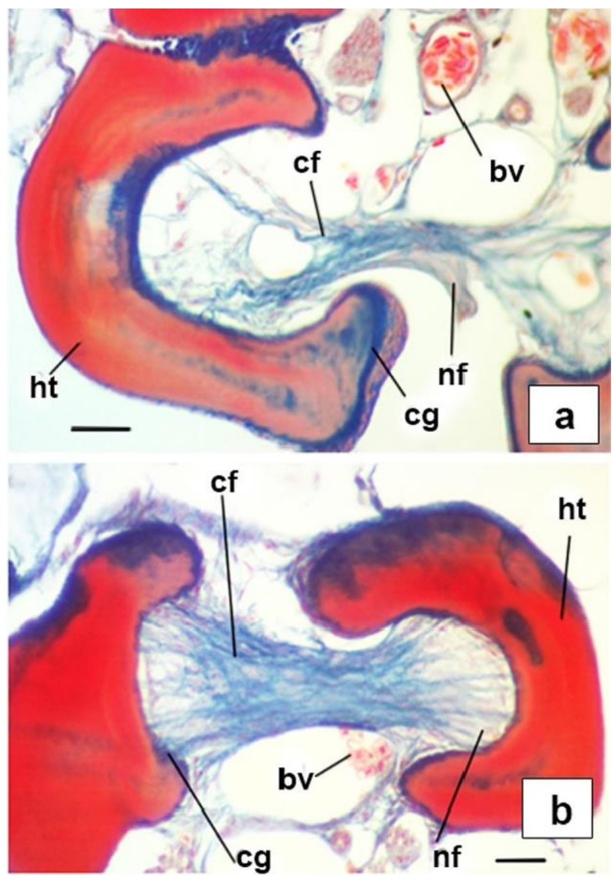

Fig. 10 The *hemitrichia* cross-sections (under MAF staining) of *B. boddarti* fused pelvic fins, a, and *P. variabilis* unfused pelvic fins, b. *Notes*: *Hemitrichia* in bright red (ht), collagen fibres in bluish streaks (cf), nerve fibres shown in faint red (nf), blood vessels with red erythrocytes inside (bv), and (e) cartilage in dark blue, outlining the *hemitrichia*. Magnification: 20 9 10. Scale bars = 20 um

adhesion while the fin makes contact to the substrate surface during terrestrial locomotion (Wicaksono et al. 2016).

The ray bones (*lepidotrichia*) of pelvic fin rays consists of two crescent-shaped structures called *hemitrichia* and the *hemitrichia* possesses a hard bony structure (red colour in MAF staining) and cartilage (blue colour in MAF staining) as shown in Fig. 10. This outer-lining of the *hemitrichia* with cartilage provides soft body structure to prevent the paired *hemitrichia* to crash upon contact, especially more cartilaginous layer is seen in the proximal part of the *hemitrichia* rather than in the distal part. The cross sectional area of *hemitrichia* in *B. boddarti* is 8456 um^2 and is larger than in *P. variabilis* (7922 um^2). If compared against body size, the larger *hemitrichia* of *B. boddarti* may be more effective in supporting the body weight of the mudskipper, which is substantially heavier than *P. variabilis*.

Conclusion

In summary, *P. variabilis* and *B. boddarti* have identifiably different pelvic fin structures which appear to be optimised somewhat to their individual environmentally driven behaviour between terrestrial and aquatic environments. *P. variabilis* has a flexible unfused fin, a small body size, a high ratio of pelvic fin surface area to body size and an abundance of mucus secreting mucous cells. These factors collectively aid the climbing behaviour in the inclined or vertical substrates observed for this species of mudskipper in terrestrial environments. Contrarily, *B. boddarti* is unable to climb steeply inclined or even vertical substrates, and correspondingly, this species of mudskipper lacks the high-mobility unfused pelvic fin, has large body size, has a lower ratio of pelvic fin surface area to body size than *P. variabilis*, and has less mucous secreting mucous cells. We conclude that these two species might have adapted and evolved differently, in greater accordance to their preferred environments and subsequent kinematic behaviours.

Acknowledgements The author wishes to extend profound and sincere appreciation to Luke Daniel the college of Idaho for assistance during the writing of the manuscript. We also thank CIMO, Finland to support this project.

Author Contributions AW and SH performed experiment under supervision of PA and BR. All authors contributed equally in the writing of the article manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Asakawa, M. 1970. Histochemical Studies of the Mucus on the Epidermis of Eel, *Anguilla japonica*. *Bulletin of the Japanese Society of Scientific Fisheries* 36: 83–87.

Bancroft, J.D., and H.C. Cook. 1984. *In Manual of Histological Technique*. Edinburgh: Churchill Livingstone.

Farrell, A.P. 2011. *Encyclopedia of Fish Physiology: From Genome to Environment*. Elsevier: Academic Press.

Genten, F., E. Terwinghe, and A. Danguy. 2009. *Atlas of Fish Histology*. Enfield: Science Publisher.

Gibson, R.N. 1986. Intertidal Teleosts: Life in a Fluctuating Environment. In *The Behaviour of Teleost Fishes*, ed. T.J. Pitcher. Berlin: Springer. https://doi.org/10.1007/978-1-4684-8261-4_15.

Gordon, M.S., I. Boetius, D.H. Evans, RMc. Carthy, and L.C. Oglesby. 1969. Aspects of The Physiology of Terestrial Life in Amphibious Fishes. I. The Mudskipper, *Periophthalmus sobrinus*. *The Journal of Experimental Biology* 50: 141–149.

Gray, P. 1954. *The Microtomist's Formulary and Guide*. New York: The Blakiston Co.

Harris, V.A. 1960. On the locomotion of the mud-skipper *Periophthalmus koelreuteri* (Pallas) (Gobiidae). In *Proceedings of the Zoological Society of London*, vol. 134(1), 107–135. Oxford: Blackwell Publishing Ltd.

Hidayat, S. 2015. Struktur histologi dan morfokinetik sirip perut ikan gelodok *Periophthalmus variabilis* (Eggert, 1935) dan *Boleopthalmus boddarti* (Pallas, 1770). Master Thesis, Universitas Gadjah Mada, Indonesia

Inouye, M. 1976. Differential Staining of Cartilage and Bone in Mouse Skeleton by Alcian Blue and Alizarin Red. *S. Congenital Anomalies* 16: 171–173. https://doi.org/10.24540/cgafa.16.3_171.

Pfefferli, C., and A. Jaźwińska. 2015. The Art of Fin Regeneration in Zebrafish. *Regeneration* 2: 72–83. https://doi.org/10.1002/reg2.33.

Kutschera, U., and J.M. Elliott. 2013. Do Mudskippers and Lungfishes Elucidate the Early Evolution of Four-Limbed Vertebrates? *Evolution: Education and Outreach* 6: 1–8. https://doi.org/10.1186/1936-6434-6-8.

Kardong, K.V. 2009. *Vertebrae Comparative Anatomy Function Evolution*, 5th ed. Singapore: McGraw-Hill Companies, Inc.

Lagler, K.F., J.E. Bardach, R.R. Miller, and D.R.M. Passino. 1977. *Ichtyology*, 2nd ed. New York: Wiley.

Lundberg, J.G., and W.M. Dahdul. 2008. Two new Cis-Andean Species of the South American Catfish Genus Megalonema Allied to Trans-Andean *Megalonema xanthum*, with Description of a New Subgenus (Siluriformes: Pimelodidae). *Neotropical Ichthyology* 6: 439–454. https://doi.org/10.1590/S1679-62252008000300018.

Pace, C.M., and A.C. Gibb. 2009. Mudskipper Pectoral Fin Kinematic in Aquatic and Terestrial Enviroments. *Journal of Experimental Biology* 212: 2279–2286. https://doi.org/10.1242/jeb.029041.

Park, J.Y. 2002. Structure of the Skin of an Air-Breathing Mudskipper, *Periophthalmus magnuspinnatus*. *Journal of Fish Biology* 60: 1543–1550. https://doi.org/10.1111/j.1095-8649.2002.tb02446.x.

Park, J.Y., J.Y. Lee, S.I. Kim, and Y.S. Kim. 2003. A Comparative Study of the Regional Epidermis of an Amphibious Mudskipper Fish, *Boleophthalmus pectinirostris* (Gobidae, Pisces). *Foloi Zool* 52: 431–440.

Patzner, P., J.L. van Tassel, M. Kovacic, and B.G. Kapoor. 2011. *The Biology of Gobies*. New Hampshire: CRC Press.

Piper, R. 2007. *Extraordinary Animals: An Encyclopedia of Curious and Unusual Animals*. Connecticut: Greenwood Publication.

Polgar, G., and G. Crosa. 2009. Multivariate Characterization of The Habitat of Seven Species of Malayan Mudskippers (Gobiidae: Oxudercinae). *Marine Biology* 156: 1475–1486. https://doi.org/10.1007/s00227-009-1187-0.

Sayer, M.D.J. 2005. Adaptation of Amphibious Fish for Surviving Life Out of Water. *Fish* 6: 186–211. https://doi.org/10.1111/j.1467-2979.2005.00193.x.

Shepard, K.L. 1994. Function for Fish Mucus. *Reviews in Fish Biology and Fisheries* 4: 401–429. https://doi.org/10.1007/BF00042888.

Standen, E.M. 2010. Muscle Activity and Hydrodynamic Function of Pelvic Fins in Trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology* 213: 831–841. https://doi.org/10.1242/jeb.033084.

Susanto, G. N. 2012. Struktur Skeleton dan Otot Alat Gerak Serta Mekanisme Gerak Ikan Amfibi Periophthalmus gracilis Eggert (Mudskipper) dan Andamia heteroptera Bleeker (Rockskipper). Thesis. Faculty of Biolgy, Gadjah Mada University. Yogyakarta (in Indonesian).

Suzuki, N. 1992. Fine Structure of the Epidermis of the Mudskipper, *Periophthalmus modestus* (Gobiidae). *The Japanese Journal of Ichthyology* 38: 379–386. https://doi.org/10.11369/jji1950.38.379.

Zhang, J., T. Taniguchi, T. Takita, and B.A. Ahyaudin. 2000. On The Epidermal Structure of *Boleophthalmus and Scartelaos* Mudskippers with Reference to Their Adaptation to Terrestrial Life. *Ichthyological Research* 47: 359–366. https://doi.org/10.1007/BF02674263.

Zhang, J., T. Taniguchi, T. Takita, and A.B. Ali. 2003. A Study on the Epidermal Structure of *Periophthalmodon* and *Periophthalmus* Mudskippers with Reference to Their Terrestrial Adaptation. *Ichthyological Research* 50: 310–317. https://doi.org/10.1007/s10228-003-0173-7.

Wicaksono, A., S. Hidayat, Y. Damayanti, D.S.M. Jin, E. Sintya, B. Retnoaji, and P. Alam. 2016. The Significance of Pelvic Fin Flexibility for Tree Climbing Fish. *Zoology* 119: 511–517. https://doi.org/10.1016/j.zool.2016.06.007.

Wicaksono, A., S. Hidayat, B. Retnoaji, A. Rivero-Müller, and P. Alam. 2017. A Mechanical Piston Action May Assist Pelvic–Pectoral Fin Antagonism in Tree-Climbing Fish. *Journal of the Marine Biological Association of the United Kingdom* 98: 2121–2131. https://doi.org/10.1017/S0025315417001722.

Wicaksono, A., S. Hidayat, B. Retnoaji, and P. Alam. 2020. The Water-Hopping Kinematics of the Tree-Climbing Fish, *Periophthalmus variabilis*. *Zoology* 139: 125750. https://doi.org/10.1016/j.zool.2020.125750.