Pakistan J. Zool., vol. 49(4), pp 1223-1230, 2017. DOI: http://dx.doi.org/10.17582/journal.pjz/2017.49.4.1223.1230

A Study on Structural Characteristics of Intestinal Tract of the Air-Breathing Loach, *Paramisgurnus dabryanus* (Sauvage, 1878)

YaQiu Liu and ZhiJian Wang*

Key Laboratory of Freshwater Fish Reproduction and Development, Ministry of Education, The Key Laboratory of Aquatic Science of Chongqing, School of Life Science, Southwest University, Chongqing 400715, China

Article Information
Received 04 August 2016
Revised 20 December 2016
Accepted 16 January 2017
Available online 14 June 2017

Authors' Contribution
YL and ZW designed the study. YL performed the experiments and analyzed the data. ZW helped in experimental work and data analysis. YL and ZW wrote the article.

Key words
Intestinal tract, *Paramisgurnus dabryanus*, Microstructure, Ultrastructure, Mucous epithelial cells, Air-breathing loach.

ABSTRACT

This study aimed to observe morphology and ultrastructure of intestinal tract of *Paramisgurnus dabryanus* with light and electron microscopies. Intestinal tract was divided into three parts. Morphologically complex folds were formed on surfaces of anterior and middle intestines where many secretory cells were present. Highly developed junction complex were seen in anterior intestine. Cytoplasm contained abundant mitochondria and pinocytotic vesicles. Epithelium of posterior intestine was thin, translucent and squamous with dense blood capillary network underground, while there were no folds on mucous membrane surface. Distance between blood capillary network and intestinal cavity was 1.95±0.34 *μm*. Respiration epithelial cells of posterior intestine are a kind of modified squamous epithelial cells which show the ultrastructural characteristics of both types I and II pneumocyte of lung alveoli in mammals. The blood is composed of three layers: the thin layer of the extended cytoplasm of respiratory epithelial cells (1.47±0.23 *μ*m), the thin sheet of cytoplasm of endothelial cells (0.21±0.03 *μ*m) and the basement membrance (0.27±0.05 *μ*m) between them is similar to that of the respiratory organs in other vertebrates. Intestinal tract has digestion and respiration functions. Anterior intestine performs digestion/absorption. Posterior intestine performs gas exchange/accessory respiration. In middle intestine, digestion and respiration overlap.

INTRODUCTION

A large majority of teleostean fish generally rely on their gill to carry out gas exchange in water. However, some types of fish live in static water where they frequently face periodical drought. Their living environment is seriously hypoxia. Under such hypoxia environment, some types of fish can start using their accessory organs to carry out respiration for meeting their physiological oxygen requirement. To date, it has been reported that approximately 400 species of fish among 50 families can carry out respiration via their accessory organs (Graham, 2011). These types of fish can carry out lung respiration (Moraes *et al*., 2005), skin respiration (Mittal and Munshi, 1971; Park *et al*., 2006), swim bladder respiration (Graham, 2011), air bladder respiration (Hughes *et al*., 1974); supra-branchial organ respiration (Hakim *et al*., 1978; Baloch and Jafri, 2004) or digestive track respiration (Park and Kim, 2001; Podkowa and Goniakowska-Witalińska, 2002; Cruz *et al*., 2009). Among the types of fish that rely on digestive track as the accessory organ to carry out respiration, the fish species in the families of *Cobitidae* and *Callichthyidae* etc. are the major members. Under hypoxia condition, they continuously swallow air through mouth and then expel the air via cloaca. Currently, the studies on the digestive track respiration of fish have been mainly focused on a few types of fish such as *Lepidocephalichthys guntea*, *Misgurnus anguillicaudatus*, *Misgurnus mizolepis* (Moitra *et al*., 1989; Park and Kim, 2001; Park *et al*., 2003; Ghosh *et al*., 2011; Zhang *et al*., 2014).

Paramisgurnus dabryanus, a small-sized fish belonging to the family of *Cobitidae* of the order Cypriniformes, is which is unique in China. In China, it is mainly distributed in the middle and lower streams of Yangzi River and the valley of Pearl River. Its meat is delicious and rich in nutrition. *P. dabryanus* is a polyphagia and economic fish living in benthos (You *et al*., 2009). *P. dabryanus* frequently occurs in the hypoxia environment such as swamp, paddy field, and ponds. It prefers to swallow air bubble and relies on intestinal tract as the accessory organ to carry out respiration. However, the structures of intestinal tissues that possess the dual functions of digestion and respiration in *P. dabryanus* within the genus of *Paramisgurnus* have not been reported yet.

Therefore, in this study, we conducted a investigation

* Corresponding author: wangzj1969@126.com
0030-9923/2017/0004-1223 $ 9.00/0

on the structural characteristics and the formation/development of the respiratory function of the intestinal tract of *P. dabryanus* through observing the microstructure and ultrastructure of its intestinal tissues in relation with the characteristics of its survival environment, aiming to provide the basic knowledge about the structure-function relationships of three sections of its intestinal tract.

MATERIALS AND METHODS

Materials

The adult individuals of *P. dabryanus* were obtained from Dianjiang County Fishery Station of Chongqing City (32°20′N; 107°21′E) in April, 2014 and were temporally reared in the circulating water vat in the laboratory and provided with natural light and continuing aeration. The water quality indexes were as follows: water temperature, 21-23^{o}C; dissolved oxygen, 7.21±0.63 (mg L^{-1}); and pH, 7.5-8.2. A total of 30 mature individuals of *P. dabryanus* (half males and half females) were selected. Their body lengths and body weights were 105.45-113.73 mm and 8.4-11.3g, respectively.

Methods

Direct anatomy was conducted on the fresh and live fish body and the morphological features of its intestinal tract were observed under the Nikon SMZ1000 anatomical lens. The length and weight of intestinal tract were measured and the ratio of intestinal length/body length and the ratio of intestinal weight/body weight were calculated.

The intestinal tract of adult fish was fixed with Bouin's fixing solution and preserved in 70% ethanol. The intestinal

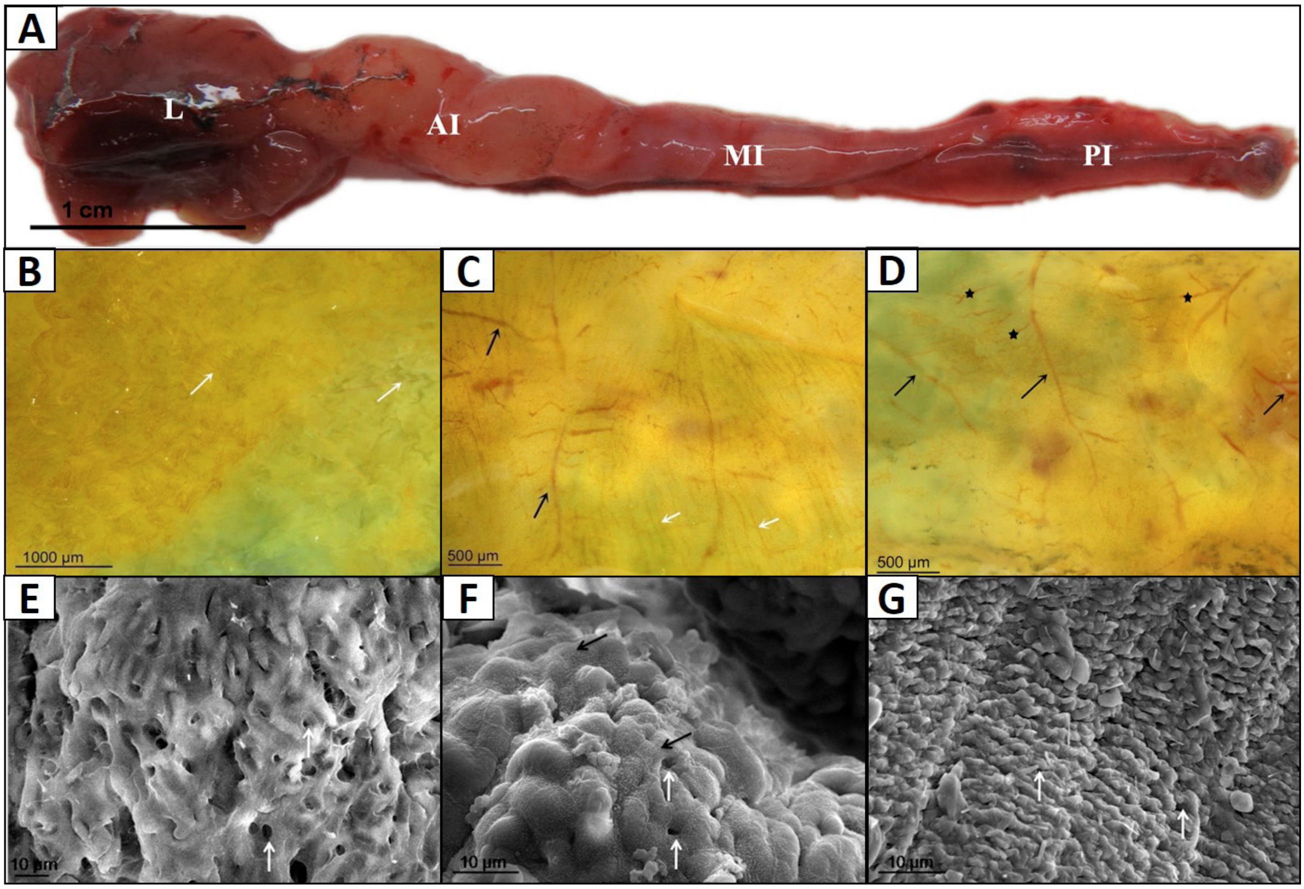

Fig. 1. Morphology observation on the surface of the intestinal tract of *P. dabryanus*. **A,** intestinal tract of *P. dabryanus*; **B,** the anatomy of anterior intestine, the white arrow notes the intestinal folds; **C,** the anatomy of middle intestine, the white arrow notes the intestinal folds; the black arrow notes the blood vessels underneath the intestinal epithelial tissue; **D,** the anatomy of posterior intestine, black arrow indicate the main blood vessels underneath the intestine epithelial tissue, * notes the branching blood vessel network; **E,** the white arrow notes the secretory hole of the secretory cells; the black arrow indicate the microvillus; **F,** the white arrow notes the secretory hole of the secretory cells; the black arrow indicate the microvillus; **G,** the white arrow notes the secretory hole of the secretory cells; AI, anterior intestine; L, liver; MI, middle intestine; PI, posterior intestine

samples were gradually dehydrated with ethanol gradient, embedded with routine paraffin, sectioned into 5-7 μm pieces, and stained with haematoxylin-eosin (H&E). The slide sections were visualized with micro-imaging system Eclipse 80i-Nikon Instruments and photographed. Three slides were prepared for each intestinal section of *P. dabryanus* and 10 fields were randomly selected to observe. The related data were measured with IPP imaging analysis software.

The intestinal tissue samples were pre-fixed with 2.5% glutaraldehyde, washed with phosphate buffered saline (PBS) three times, fixed with 1% osmic acid, dehydrated with ethanol gradient, displaced with acetone, embedded with epoxy agent 650 polymer and sectioned with LKB-5 ultramicrotome. The ultra-thin sections were double stained with uranyl acetate and lead citrate and visualized under H-7500 TEM and photographed.

The intestinal tissue samples were pre-fixed with 2.5% glutaraldehyde, washed with PBS three times, dehydrated with ethanol gradient, displaced with acetone, frozen at -80^{o}C and freezing dried with Freeze Dryer FDU-2200, spayed with Jeoljec-3000FC and visualized with scanning electron microscope (SEM) JSM-6510LV.

Data analysis

The recorded data were statistically analyzed with MS Excel (2010) and spss19.0 software. All the experimental data were expressed as mean value ± standard deviation (SD). The significant analyses for the difference in the experimentally recorded data were conducted with one-way ANOVA. If the difference was significant, the differences in the mean values were compared with multiple comparison methods. The difference with $P<0.05$ was regarded as statistically significant.

RESULTS

Anatomy of intestinal tract

P. dabryanus does not have stomach and its esophagus is relatively shorter. The initial terminal of its intestinal tract is swallowing and in a long-tubulose shape. The intestinal tract can be divided into anterior, middle and posterior intestines. The helix and cured anterior section of intestine is known as anterior intestine. The section from anterior intestine to the second slightly cured part is known as middle intestine. The section from the cured part to cloaca is known as posterior intestine (Fig. 1A). The anterior intestine was highly folded (Fig. 1B) and the middle intestine was moderately folded and a few blood vessels appeared in it (Fig. 1C). The lumen of posterior intestine was narrow and the intestinal wall was thin and transparent with fully distributed blood vessels (Fig. 1D).

Table I.- Comparison of histological structure of intestine tract of *P. dabryanus*.

Intestine regions	n	Height of mucous folding (μm)		Thickness of mucous layer (μm)		Thickness of submucosa (μm)		Thickness of muscular layer (μm)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Anterior intestine	30	$435.32±65.21^{c}$	280.53-635.65	$51.23±6.23^{c}$	29.32-92.12	$31.25±2.31^{a}$	12.31-55.13	$89.23±8.59^{c}$	59.53-120.23
Middle intestine	30	$272.25±50.45^{b}$	159.31-367.42	$30.56±4.21^{b}$	15.31-50.63	$45.31±3.98^{b}$	21.31-65.19	$42.98±5.46^{b}$	26.31-75.42
Posterior intestine	30	$110.37±32.24^{a}$	80.21-130.35	$21.35±3.15^{a}$	9.81-30.53	$60.78±5.21^{c}$	26.37-95.03	$24.56±2.92^{a}$	16.25-57.11

Note: Different superscripts (letters) in the same row indicate the significant differences ($P<0.05$).

SEM of intestinal mucosal layer

Visualization of mucosal layer of intestinal tract with SEM under higher magnification revealed that the microvilli were densely arranged on the surface of mucosal membrane of anterior intestine and abundant secretory gland cells were be mingled with columnar cells. Semi-globularly-shaped projections appeared on the surface of middle intestine where microvilli were densely gathered (Fig. 1E, F). The columnar cells on the surface of mucosal membrane of posterior membrane exhibited irregular shapes and their outlines were not obvious, mostly were epithelial pavement cells. Fewer secretory cells were present there and the secretory holes were smaller. The microvilli were shorter and sparse (Fig. 1G).

Histology of intestinal tract

The lumen of anterior intestine was relatively wide and the lumen wall was relatively thick (Fig. 2A). Simple columnar epithelium cells were arranged tightly. On the surface of the relatively well developed intestinal villi, they were clustered. But on the surface of intestinal villi, they were lobulated (Fig. 2A). The lumen wall of the middle intestine was thinner than that of the anterior intestine. The intestinal cavity became narrow and the villus height was reduced (Fig. 2B). Erythrocytes began to appear in epithelium mucosae (Fig. 2B). Compared with those of anterior and middle intestines, the mucous layer, muscular layer and mucous folds of posterior intestine were all significantly reduced ($P<0.05$) (Table I) and the mucous cells were also significantly reduced as well ($P<0.05$) (Table II). The submucosa was consisted of a large number of connective tissue networks and was highly vascularized (Fig. 2C). In the mucous membrane, a large number of erythrocytes were seen to extend to the intestinal cavity (Fig. 2C).

Ultrastructure of intestinal epithelium

The mucosa epithelium of anterior intestine was mainly consisted of columnar absorptive cells and goblet cells. The microvilli of mucous epithelium cells were orderly and densely arranged on the juxtaluminal surface. The cytoplasmic membrane of the absorptive cells was notched toward cytoplasm where a large number of multi-vesicular bodies were distributed and the nucleus was located on the proximal base. The neighboring absorptive cells were connected through junction complexes (Fig. 2D). The cytoplasm of mucous epithelium cells of the intestinal track was full of a large number of highly electron-dense and obvious mitochondria as well as abundant pinocytotic vesicle bodies (Fig. 2E). The respiratory epithelium cells of the posterior intestine were of capillarization. The nucleus-containing section of the flat epithelium pavement cells were inlaid into the interspace of the neighboring capillaries and their nucleus shapes were irregular. The edges were stained more deeply and rich in heterochromatin. The microvilli were short and irregular. The cytoplasmic membrane covered the external wall of capillaries (Fig. 2F). The cytoplasm of the pavement epithelium cells contained a large number of multi-lamellar bodies, rough endoplasmic reticulum, and mitochondria. Highly electron-dense vesicles were distributed in the cytoplasmic section nearby the external edge (Fig. 2G, H). The size of the lamellar bodies was about 0.52-1.18 μm. There was obvious boundary between the concentrically circular lamellar bodies and the interspace section became liquidized (Fig. 2I) Underneath the flat epithelium layer were blood capillaries whose cross-sections were irregular and contained many erythrocytes (Fig. 2F). The capillary wall was consisted of a monolayer of endothelium cells, whose cytoplasm was extremely extended to become flat cytoplasmic lamellar. The nuclei of the endothelium cells displayed irregular shapes and were located inside the blood capillaries (Fig. 2H). Air-blood barriers were present in epithelium cells (1.47±0.23 μm) and cytoplasmic lamella of endothelium cells (0.21±0.03 μm) and thin glomerular basement membrane (0.27±0.05 μm) between them. The gas-blood diffusion distance between blood capillary and intestinal cavity of *P. dabryanus* was 1.95±0.34 μm.

Table II.- The densities of mucous cells and blood capillaries in intestinal tract of *P. dabryanus*.

Intestine regions	n	No. of mucous cells per 100 µm		No. of blood capillaries per 100 µm	
		Mean ± SD	Range	Mean ± SD	Range
Anterior intestine	30	10.32 ± 3.98^{a}	7-14	-	-
Middle intestine	30	7.18 ± 2.5^{9a}b	4-10	4.16 ± 1.21^{b}	3-6
Posterior intestine	30	5.15 ± 1.45^{b}	3-7	7.21 ± 2.15^{a}	5-10

Different superscripts (letters) in the same row indicate the significant differences ($P<0.05$); "-" notes blood capillaries are not found.

DISCUSSION

P. dabryanus mainly uses its posterior intestine to carry out air-breathing and surface of posterior intestine was covered by a layer of highly capillarilalized respiratory epithelium cells. These structural features are similar to those reported by the studies on some types of teleostean that rely on digestive tract to carry out accessory respiration (Reifel and Travill, 1979; McLeese and Moon, 1989; Podkowa and Goniakowska-Witalińska, 2002; Cruz *et al*., 2009). These structural characteristics are similar to those of intestinal tissues of *M. mizolepis* and *M.anguillicaudatus* air-breathing organs (McMahon and Burggren, 1987; Park and Kim, 2001; Park *et al*., 2003; Goncalves *et al*., 2007). It has been suggested by previous studies that the functions of microvilli in the respiratory epithelium of the air-breathing organs of fish were to increase the surface area of its gas exchange (Huges and Munshi, 1973; Maina and Maloiy, 1986). However, we believed that posterior intestine was the main section for gas exchange, *i.e.,* the gas exchange occurred in a few microvilli in the extended ultrathin cytoplasmic lamella, indicating that microvilli are not directly related to the increased respiratory surface area but mainly plays a role in accessory digestion.

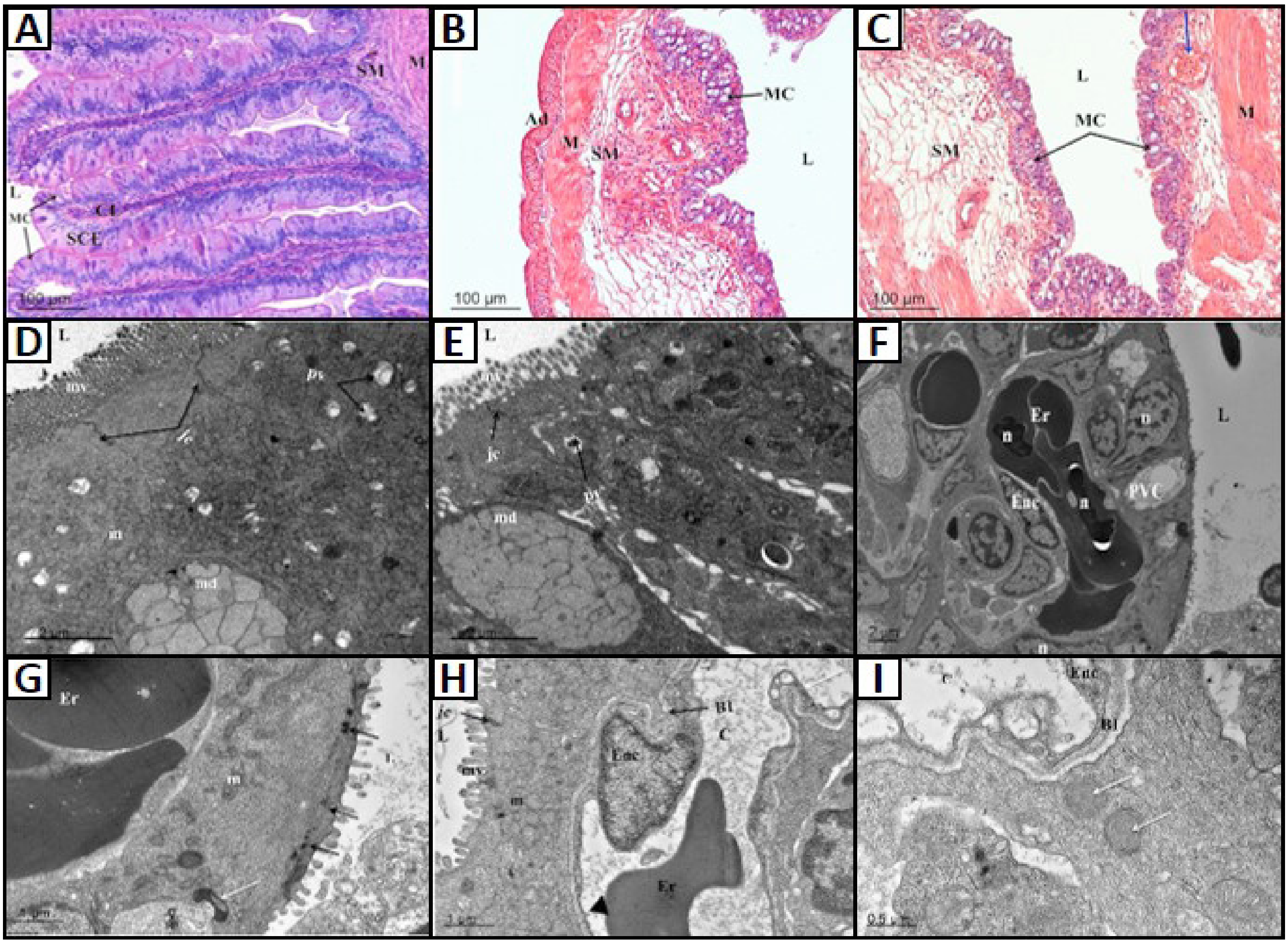

Fig. 2. Histological structure and ultrastructure of the intestinal tract of *P. dabryanus.* **A,** transverse-section of anterior intestine: simple columnar epithelium, goblet cells in epithelial mucous membrane of anterior intestine, submucosa and muscular layer were shown; **B,** transverse-section of middle intestine: columnar epithelium and goblet cells in the epithelial mucous membrane of middle intestine, submucosa and muscular layer were shown; **C,** transverse-section of posterior intestine: columnar epithelium, goblet cells in epithelial mucous membrane of posterior intestine, submucosa and muscular layer were shown. The blue arrow notes the blood vessels; **D,** showing the mucous epithelial cell of anterior intestine; **E,** showing the mucous epithelium cell of middle intestine; **F,** showing the mucous epithelium cell of posterior intestine and blood capillary; **G,** showing epithelial cell in mucous membrane of posterior intestine adjacent to a capillary. Black arrow notes the electron-dense vesicles; White arrow notes the lamella bodies; **H,** showing the epithelium in mucous membrane of posterior intestine and endothelial cell of capillary. Black arrowhead notes pore; and White arrow notes pyramidal projection of endothelial cell protruding into the capillary lumen; **I,** showing thin layers of endothelial cell. White arrows indicate the lamella bodies. Ad, adventitia; BI, basal lamina; C, capillary; CL, central lacteal; Er, erythrocyte; EnC, endothelial cell; jc, junctional complex; L, lumen; M, mucous layer; m, mitochondria; MC, mucous cell; md, mucus droplet; mv , microvillus; n , nucleus; pv, pinocytotic vesicle; PVC, pavement epithelial cell; SCE, simple columnar epithelium; SM, submucosa.

Observation on the ultrastructure of mucous epithelium cells present in various sections of intestinal tract with TEM revealed that the epithelium cells of posterior intestine were extremely flat. Cytoplasmic thin covered the outer of blood capillary. The nuclei of epithelium cells displaced oval shape or irregular shapes and contained obvious heterochromatin. Cytoplasm contained osmiophilic lamellar bodies, mitochondria and Golgi body. Most of the epithelial cells of *P. dabryanus* posterior intestine resemble both type I and II pneumocyte in mammals alveolar epithelium (Satora, 1998). These structural characteristics are also similar to those of the air-breathing teleostean (Podkowa and Goniakowska-Witalińska, 2002, 2003; Cruz *et al*., 2009). Lamellar bodies are a type of surfactants which are usually present in respiratory organs such as animal lungs and air-sac and have the functions of carrying out gas exchange and antibacterial role (Daniels *et al*., 1995; Rubio *et al*., 1996; Daniels and Brauner, 2004). Fish live in water and the surface of their respiratory epithelium is easily polluted by water and other substances. The surfactants secreted by lamellar bodies are beneficial for maintaining the stability of the entire respiratory surface and for performing their normal functions.

Gas-blood barrier is the only way for gas exchange. The structures of gas-blood barrier of *P. dabryanus* are similar to those of a majority of air-breathing fish and consisted of the extended ultrathin cytoplasmic layer of the epithelium cells, the similar cytoplasmic layer of endothelium cells and the underneath layer between them. The frequency of gas exchange is closely related to the thickness of gas-blood barrier (Maina and Maloiy, 1985). The gas-blood diffusion distance of intestinal tract of *P. dabryanus* was 1.95 μm, which was longer than that of air-sac *of Heteropneustes fossilis* (1.60 μm) (Hughes *et al*., 1974) but far shorter than that of skin respiration *of Mastacembelus pancalus* (34.0 μm) (Mittal and Munshi, 1971) and close to that of supra-branchial chamber of *Channa punctata* (0.78 μm) (Hakim *et al*., 1978). It has been indicated that in order for benefitting gas exchange, the gas-blood diffusion distances in the respiratory organs such as lungs of vertebrate animals, are similar and they are all shorter than 3.0 μm (Klika and Lelek, 1967; Weibel, 1973; Goniakowska-Witalinska, 1995).

Many air-breathing fish are distributed in various parts in the world. For example, lungfish is distributed in South America, Africa and Australia. The respiratory organs of air-breathing fish are the accessory organs of gill respiration. The gills of *P. dabryanus* are located in the back-end of pharyngeal portion, which are similar to the intestinal epithelium of the air-breathing fish in that they both originate from endoblast endoderm during the embryo stage. In term of its ultrastructure, its organelles such as air-breathing epithelium cells, gas-blood barrier, and microvilli, and lamella bodies are all similar to those of pulmonary alveoli of higher mammals (Weibel, 1973). In term of individual development, the air-breathing of *P. dabryanus* appears earlier and usually appears in period of metamorphosis when its gills start falling off (Liang *et al*., 1988). Similar to a majority of air-breathing teleostean, *P. dabryanus* has gills with complete structural and functions. Its two respiratory ways are necessary and can adjust the proportions of these two respiratory ways according to the changes in oxygen contents in the environment. During evolution, in order to land, certain parts of the ancient fish in water gradually evolved into respiratory organs that can adapt to the terrestrial environment.

CONCLUSION

The intestinal tract of *P. dabryanus* has dual functions, digestion and respiration. Anterior intestine is the region performing the functions of digestion and absorption. Posterior intestine is the region for gas exchange and accessory respiration. The structure of middle intestine is between anterior intestine and posterior intestine Middle intestine is a region where digestion and respiration overlap. The formation of intestinal respiratory functions may be related to its survival in the hypoxia environment in static water.

ACKNOWLEDGEMENTS

Authors would like to thank two anonymous reviewers for their constructive and helpful advice to the manuscript. This work was financially supported by the Special Fund for Agro-scientific Research in the Public Interest under Grant number 201203086.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Cruz, A.L.D., Pedretti, A.C.E. and Fernandes, M.N., 2009. Stereological estimation of the surface area and oxygen diffusing capacity of the respiratory stomach of the air-breathing armored catfish *Pterygoplichthys anisitsi* (Teleostei: Loricariidae). *J. Morphol*., **270**: 601–614. https://doi.org/10.1002/jmor.10708

Daniels, C.B., Orgeig, S. and Smits, A.W., 1995. The evolution of the vertebrate pulmonary surfactant. *Physiol. Zool*., **68**: 539–566. https://doi.

org/10.1086/physzool.68.4.30166344

Daniels, C.B. and Brauner, C.J., 2004. The origin and evolution of the surfactant system in fish: insights into the evolution of lungs and swim bladders. *Physiol. Biochem. Zool.*, **77**: 732–749. https://doi.org/10.1086/422058

Ghosh, S.K., Ghosh, B. and Chakrabarti, P., 2011. Fine anatomical structures of the intestine in relation to respiratory function of an air-breathing loach, *Lepidocephalichthys guntea* (actinopterygii: cypriniformes: cobitidae). *Acta Ichthyol. Piscat.*, **41**: 1–5. https://doi.org/10.3750/AIP2011.41.1.01

Goncalves, A., Castro, L., Pereira-Wilson, C., Coimbra, J. and Wilson, J., 2007. Is there a compromise between nutrient uptake and gas exchange in the gut of *Misgurnus anguillicaudatus*, an intestinal air-breathing fish? *Comp. Biochem. Physiol. D.*, **2**: 345–355. https://doi.org/10.1016/j.cbd.2007.08.002

Goniakowska-Witalinska, L., 1995. The histology and ultrastructure of the amphibian lung. In: *Histology, ultrastructure and immunohistochemistry of the respiratory organs in non-mammalian vertebrates* (ed. L. M. Pastor), University of Murcia in Spain. pp. 75–112.

Graham, J.B., 2011. The biology, diversity, and natural history of air-breathing fishes: an introduction. In: *Encyclopedia of fish physiology* (ed. A.P. Farrell), Academic Press, New York, pp. 1850–1860. https://doi.org/10.1016/B978-0-12-374553-8.00044-7

Hakim, A., Munshi, J.S.D. and Hughes, G.M., 1978. Morphometries of the respiratory organs of the Indian green snake-headed fish, *Channa punctata*. *J. Zool.*, **184**: 519–543. https://doi.org/10.1111/j.1469-7998.1978.tb03305.x

Hughes, G.M., Singh, B.R., Guha, G., Dube, S.C. and Munshi, J.S.D., 1974. Respiratory surface areas of an air-breathing siluroid fish *Saccobranchus* (*Heteropneustes*) *fossilis* in relation to body size. *J. Zool.*, **172**: 215–232. https://doi.org/10.1111/j.1469-7998.1974.tb04103.x

Hughes, G.M. and Munshi, J.S.D., 1973. Nature of the air-breathing organs of the Indian fishes *Channa*, *Amphipnous*, *Clarias* and *Saccobranchus* as shown by electron microscopy. *J. Zool.*, **170**: 245–270. https://doi.org/10.1111/j.1469-7998.1973.tb01377.x

Liang, Z.X., Liang, J.Y., Chen, C., Li, Z.J., Lin, J.H. and Zhang, J.J., 1988. The embryonic development and fingerling culture of loach, *Paramisgurnus dabryanus* Sauvage. *Acta Hydrobiol. Sin.,* **12**: 27-42 (In Chinese).

Klika, E. and Lelek, A., 1967. A contribution to the study of the lungs of the *Protopterus annectens* and *Polypterus senegalensis*. *Folia Morphol.*, **15**: 168–175.

Maina, J.N. and Maloiy, G.M.O., 1985. The morphometry of the lung of the African lungfish (*Protopterus aethiopicus*): Its structural-functional correlations. *Proc. R. Soc. Lond. B. Biol.*, **224**: 399–420.

Maina, J.N. and Maloiy, G.M.O., 1986. The morphology of the respiratory organs of the african air-breathing catfish (*Clarias mossambicus*): a light, electron and scanning microscopic study, with morphometric observations. *J. Zool.*, **209**: 421–445. https://doi.org/10.1111/j.1469-7998.1986.tb03602.x

Baloch W.A. and Jafri S.I.H., 2004. New record of a catfish, *Clarias batrachus* (Linn. 1758) from Pakistan. *Pakistan J. Zool.*, **36**:167-169.

McLeese, J.M. and Moon, T.W., 1989. Seasonal changes in the intestinal mucosa of winter flounder, *Pseudopleuronectes americanus* (Walbaum), from Passamaquoddy Nay, New Brunswick. *J. Fish Biol.*, **35**: 381–393. https://doi.org/10.1111/j.1095-8649.1989.tb02990.x

McMahon, B.R. and Burggren, W.W., 1987. Respiratory physiology of intestinal air breathing in the teleost fish *Misgurnus anguillicaudatus*. *J. exp. Biol.*, **133**: 371–393.

Mittal, A.K. and Munshi, J.S.D., 1971. A comparative study of the structure of the skin of certain air-breathing fresh-water teleosts. *J. Zool.*, **163**: 515–532. https://doi.org/10.1111/j.1469-7998.1971.tb04547.x

Moitra, M., Singh, O.K. and Munshi, J.S.D., 1989: Microanatomy and cytochemistry of the gastro-respiratory tract of an air-breathing cobitid fish, *Lepidocephalichthys guntea*. *Jpn. J. Ichthyol.*, **36**: 227–231. https://doi.org/10.1007/BF02914326

Moraes, M.F., Holler, S., Da, C.O., Glass, M.L., Fernandes, M.N. and Perry, S.F., 2005. Morphometric comparison of the respiratory organs in the south american lungfish *Lepidosiren paradoxa* (Dipnoi). *Physiol. Biochem. Zool.*, **78**: 546–559. https://doi.org/10.1086/430686

Park, J.Y. and Kim, I.S., 2001. Histology and mucin histochemistry of the gastrointestinal tract of the mud loach, in relation to respiration. *J. Fish Biol.*, **58**: 861–872. https://doi.org/10.1111/j.1095-8649.2001.tb00536.x

Park, J.Y., Kim, I.S. and Kim, S.Y., 2003. Structure and mucous histochemistry of the intestinal respiratory tract of the mud loach, *Misgurnus anguillicaudatus* (Cantor). *J. appl. Ichthyol.*, **19**: 215-219. https://

doi.org/10.1046/j.1439-0426.2003.00452.x

Park, J.Y., Kim, I.S. and Lee, Y.J., 2006. A Study on the vascularization and structure of the epidermis of the air-breathing mud-skipper, *Periophthalmus magnuspinnatus* (Gobiidae, Teleostei), along different parts of the body. *J. appl. Ichthyol*., **22**: 62–67. https://doi.org/10.1111/j.1439-0426.2006.00696.x

Podkowa, D. and Goniakowska-Witalińska, L., 2002: Adaptations to the air breathing in the posterior intestine of the catfish (*Corydoras aeneus*, callichthyidae). a histological and ultrastructural study. *Folia Biol*., **50**: 69–82.

Podkowa, D. and Goniakowska-Witalińska L., 2003: Morphology of the air-breathing stomach of the catfish *Hypostomus plecostomus*. *J. Morphol*., **257**: 147–163. https://doi.org/10.1002/jmor.10102

Reifel, C.W. and Travill, A.A., 1979. Structure and carbohydrate histochemistry of the intestine in ten teleostean species. *J. Morphol*., **162**: 343–360. https://doi.org/10.1002/jmor.1051620305

Rubio, S., Chailley-Heu, B., Ducroc, R. and Bourbon, J.R., 1996. Antibody against pulmonary surfactant protein a recognize proteins in intestine and swim bladder of the freshwater fish, carp. *Biochem. biophys. Res. Commun*., **225**: 901–906. https://doi.org/10.1006/bbrc.1996.1270

Satora, L., 1998. Histological and ultrastructural study of the stomach of the air-breathing *Ancistrus multispinnis* (Siluriformes, Teleostei). *Canadian J. Zool*., **76**: 83–86. https://doi.org/10.1139/cjz-76-1-83

Weibel, E.R., 1973. Morphological basis of alveolar-capillary gas exchange. *Physiol. Rev.*, **53**: 419–495.

You, C., Yu, X. and Tong, J., 2009. Detection of hybridization between two loach species (*Paramisgurnus dabryanus* and *Misgurnus anguillicaudatus*) in wild populations. *Environ. Biol. Fish*., **86**: 65–71. https://doi.org/10.1007/s10641-007-9282-x

Zhang, J., Yang, R., Yang, X., Fan, Q., Wei, K. and Wang, W., 2014. Ontogeny of the digestive tract in mud loach *Misgurnus anguillicaudatus* larvae. *Aquacul. Res*., **1**: 1-11. https://doi.org/10.1016/j.aquaculture.2014.04.017