

RESEARCH NOTE

The mytilid plicate organ: revisiting a neglected organ

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Mytilid bivalves are among the most widespread of marine organisms. They range from the deep sea to the intertidal, and the poles to the tropics. They live in or on both hard and soft substrates, as well as epibiotically on host organisms (Bhaduri *et al.*, 2017). A few species have even entered brackish-water estuaries (Morton, 2015 and references therein) and two have invaded freshwater (Morton & Dinesen, 2010). These mussels thrive under diverse abiotic conditions due to various adaptations of the mytilid body plan and evolution of distinct physiological features. Members of the Mytiloidea most likely descend from a common modioline ancestor and are morphologically differentiated by their shell shape and musculature complex (Morton, 2015; Oliver, 2015). Numerous, detailed studies have described mytilid-specific anatomical characteristics of general lamellibranch organs such as the foot, mantle, labial palps, gill, kidney and alimentary channel (e.g. Pieri & George, 1979; Dinesen & Morton, 2014; Morton, 2015; Oliver, 2015; Morton & Puljas, 2018). Mytilids have, however, developed a unique organ which is not known in other lamellibranches—the plicate organ (PO). Its morphology and localization have been described in detail in classical anatomical studies on *Mytilus edulis* by Sabatier (1875, 1877) and Purdie (1887). Based on its morphology, the PO also has been called the frilled organ, plaited organ, plicate channels or in French organes godronnés (Sabatier, 1875, 1877; Field, 1922; Wilson, 1979). The organ is composed of foliated unicellular membranous structures that surround a haemolymph space (Sabatier, 1875; Fig. 1). Its lamellae receive seawater that circulates through mantle cavity, in the current that is generated for filter feeding. The PO is located on the inner and outer sides of the ctenidial base; however, authors disagree on whether these morphologically comparable structures represent two parts of the same organ or need to be considered as independent of each other. In contrast to Sabatier (1875), Purdie (1887) considered both parts to constitute a single organ, a conclusion he based on the overall similar morphology in *M. edulis*. In this work, we will differentiate between the inner (IPO) and outer (OPO) plicate organs (Figs 1, 2).

Based on the organ's shape, enlarged surface and location, Sabatier (1875) suggested a role in respiration. This is of particular importance as the mantle tissues typically contain the gonads and

thus require efficient ventilation prior to spawning (Sabatier, 1875; Purdie, 1887). Gas exchange is most likely enhanced by motile cilia on the organ's surface, which facilitate water flow and increase turbulence in the boundary layer (Sabatier, 1875). Commonly, the ctenidia have been assumed to be the main site of respiratory gas exchange in bivalves. This, however, has not been supported by any data and is most likely based on the assumption that ctenidia have a function similar to that of gills in fish and crustaceans. Nevertheless, the primary role of the ctenidia is food acquisition (Field, 1922). Furthermore, haemolymph circulation in the ctenidia is slow and probably discontinuous, which would limit oxygen transport (Sabatier, 1875; Booth & Mangum, 1978). In fact, integumentary gas exchange seems to be of high importance for respiration in all molluscs, bivalves in particular (Graham, 1988). Thus, enlargement of the body surface by the PO may support O₂ uptake and CO₂ release.

Recent findings also suggest a central role for the PO in ammonia excretion, as immunohistochemistry has revealed pronounced Rh-like protein abundance in the apical membrane (Thomsen *et al.*, 2016). This protein is known as a common NH₃ gas channel in aquatic animals and molluscs (Wehrauch, Joseph & Allen, 2018; Hu *et al.*, 2017). High abundance of the Rh-like protein was, however, detected in the OPO but not in the IPO (Thomsen *et al.*, 2016), indicating that the physiological function of these organs may differ despite their similar morphology (Figs 1, 2B). This needs to be examined further by the sequencing of tissue-specific gene-expression patterns. The Rh-like protein was also present in the ctenidia, although the protein concentration was much lower compared with the OPO (Thomsen *et al.*, 2016). Thus, the large surface area of the ctenidia may still contribute to the excretion of ammonia and exchange of O₂ and CO₂.

In order to quantify the absolute and relative contribution of each potential respiratory organ, we measured the surface areas of the ctenidia, as well as the IPO and OPO. Freshly collected *M. edulis* (5–50 mm shell length, SL) were opened by cutting the posterior adductor muscle and inspected under a stereomicroscope (Leica M165 FC). Pictures were taken using a Leica DFC 310 FX camera and analysed with ImageJ v. 1.50i. The surface area of the ctenidia was calculated from the total measured area of one

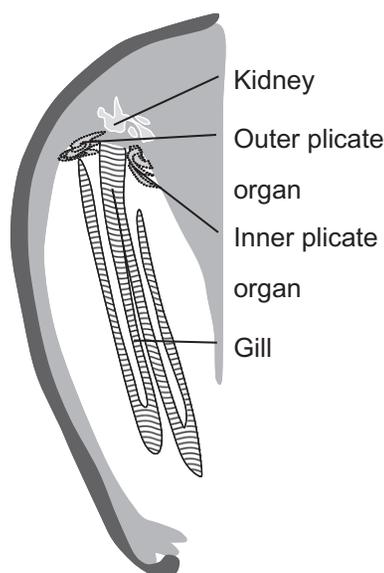


Figure 1. Schematic transverse section of a representative member of Mytilinae, depicting the position of the outer and inner plicate organs, kidney and ctenidia (modified after Thomsen *et al.*, 2016).

demibranch and extrapolated to account for all eight lamellae. Areas of IPO and OPO were assessed by measuring mean height and diameter of at least 15 lamellae and counting their total number. Total surface area was calculated based on approximation by a cylindrical shape and multiplication by the number of lamellae. The surface area of all three organs increased with SL of the inspected animals, and the correlation between SL and ctenidial area corresponded to measurements published for *M. edulis* (Jones, Richards & Southern, 1992). For example, a *M. edulis* individual

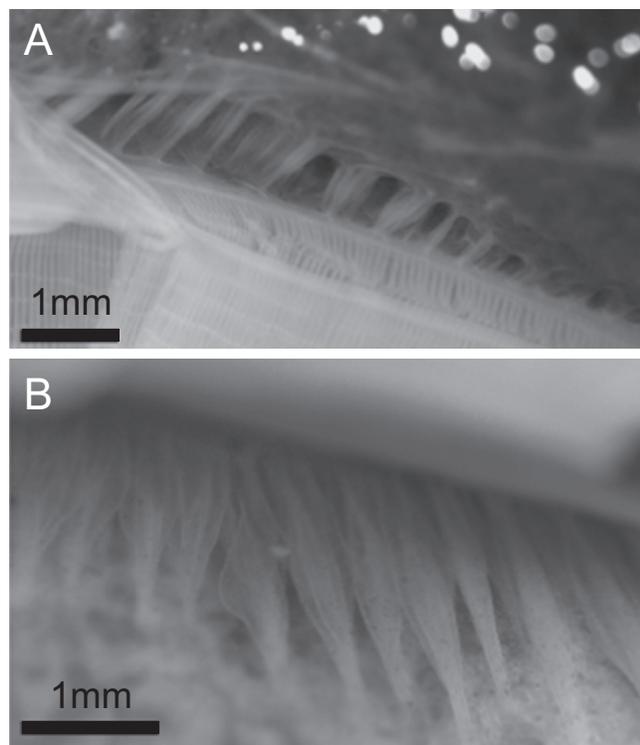


Figure 2. Photographs of the plicate organs of *Mytilus edulis*. **A.** Inner plicate organ. **B.** Outer plicate organ.

of 30-mm SL had a ctenidia surface area of about 1,000 mm² (Fig. 3A). In comparison, the surfaces of the IPO and OPO were much smaller, 20 and 40 mm², respectively (Fig. 3B–D). Importantly, the relative contribution of each organ to total respiratory surface (IPO + OPO + gill area) was almost constant within the inspected size spectrum, and the PO contributed $4.6 \pm 0.65\%$ (mean \pm SE, $n = 7$) to this. The area of the plicate organ thus appears to be quite small compared with the overall surface available for gas exchange in *M. edulis*. The actual contribution to respiration and nitrogenous waste excretion could, however, be much higher, given the previously mentioned physiological features.

Although the PO was first described in detail almost 150 years ago, its distribution within the family is unclear. The first studies focused on *M. edulis* only (Sabatier, 1875, 1877). Purdie (1887) investigated two additional species in his New Zealand study area. These were originally considered to be *M. magellanicus* and *M. latus* which, however, are now regarded as synonyms of *Perna perna* and *P. canaliculus*, respectively. Although the PO was present in all three species, its dimension differed among them, being much smaller in *P. canaliculus* (Purdie, 1887). This revealed that the dimensions of the PO are not necessarily uniform within the family. The same might be true for the presence or absence of the PO within the mytilids. Our study provides a first, short overview of the presence or absence of the PO from diverse phylogenetic branches of the Mytilidae. For this purpose, we performed a literature review and investigated ethanol-preserved specimens, aside from *M. edulis*. Importantly, although anatomical descriptions are available for numerous mytilid species, the PO has rarely been mentioned. This could mean that the PO is either absent, or simply overlooked due to its delicate structure—it is only clearly distinguishable in water when its lamellae are not collapsed. The phylogeny of mytilids changes as a result of progress in sequencing efforts and molecular data do not always agree with findings based on morphological work. For this comparison, the investigated species were assigned to one of the seven recognized mytilid subfamilies (World Register of Marine Species, <http://www.marinespecies.org/>; Table 1).

Both IPO and OPO are present in *Modiolus modiolus* the only investigated representative of the Modiolinae. Similarly, both organs are present in the Mytilinae, including *M. edulis*, *M. trossulus*, *M. galloprovincialis* and *M. californianus* (Sabatier, 1875, 1877; Thomsen *et al.*, 2016) and *P. perna* and *P. canaliculus* (Purdie, 1887). A study of *Aulacomya atra* showed only an OPO (Zaixso, 2003). However, as the study was not focused on the PO, the fact that IPO was not mentioned does not prove its absence in this species. Only the OPO is visible in *Limnoperna fortunei* and the IPO is not present (Table 1). This is the only freshwater species of the Mytilinae, which has recently been supported by molecular data (Leoni *et al.*, 2017). The same situation, the absence of the IPO and presence of the OPO, was observed in *Xenostrobus securis*. These observations reveal a uniform presence of at least the OPO in this subfamily.

The situation in the Brachidontinae is far less clear. In *Brachidontes pharaonis*, the IPO and OPO are present, whereas in *B. striatulus* and *B. puniceus* only the IPO could be detected (Table 1). In *Mytilaster minimus*, no PO was present. These observations indicate that even closely related species (Terranova *et al.*, 2007; Trovant *et al.*, 2016) can differ substantially in specific anatomical characteristic such as presence of the PO.

The anatomy of the Lithophaginae, including that of the PO, has been studied in detail by Wilson (1979), who at the time considered them all as *Lithophaga* species. Subsequently, a group was differentiated as the genus *Leiosolenus* (supported by molecular data; Owada, 2007; Owada & Hoeksema, 2011; Liu, Liu & Zhang, 2018). *Lithophaga teres* possessed the IPO and *L. antillarum* the OPO (Wilson, 1979). In addition, low ridges were present in *L. antillarum* at the location of the IPO, which could represent a

reduced and less distinct IPO in this species (Wilson, 1979). Similarly, although the PO was missing in all investigated *Leiosolenus* species, they contain a series of pockets at the position of the IPO. These potentially enlarge the respiratory surface in a similar to the PO and may thus represent a reduced form of the organ.

In the Septiferinae two species, *Mytilisepta virgata* and *Septifer bilocularis*, have been investigated, but no PO was present. In the

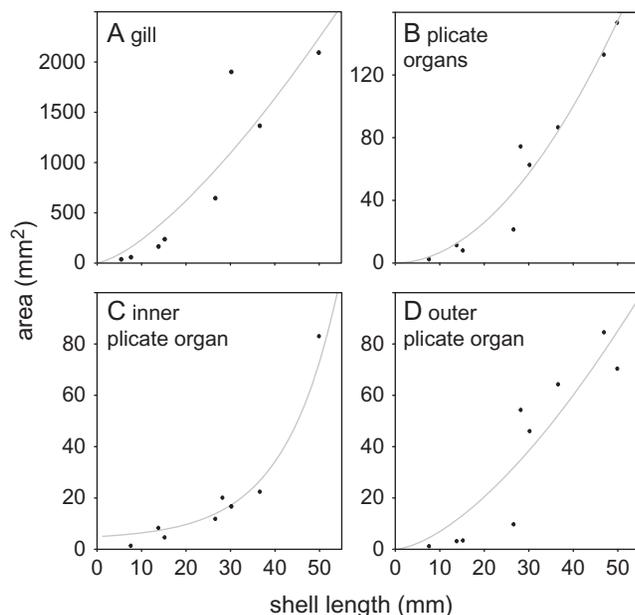


Figure 3. Areas of potential respiratory surfaces of *Mytilus edulis* in relation to shell length. **A.** Ctenidia. **B.** Plicate organs. **C.** Inner plicate organ. **D.** Outer plicate organ.

Bathymodiolinae, neither *Bathymodiolus childressi* nor *Idas washingtonia* had a PO. The same was observed for the Crenellinae (see Samadi *et al.*, 2007; Liu *et al.*, 2018, for phylogeny) in which three species, *Musculus discors*, *M. subpictus* and *Musculista senhousia*, were examined, and did not possess either a PO or a comparable structure.

Our comparative study of the distribution of the PO has revealed a diverse picture of the absence or presence of the organ within the Mytiloidea. The IPO and OPO are present in the Modiolinae, which have been considered as the basal group as it has the longest fossil record (Soot-Ryen, 1969). However, this position is questioned by recent molecular data, which subdivide the subfamily and allocate them separately to the two major mytilid lineages (Liu *et al.*, 2018). Nevertheless, the presence of either an IPO or OPO is verified for at least four more subfamilies. This suggests that the organ represents an ancestral character of mytilid anatomy, which is possibly secondarily reduced in some branches of the family. Furthermore, the comparison clearly demonstrates the need to differentiate between the IPO and OPO, as both only occur in some species, whereas one of the organs is reduced in others. The classification of species into subfamilies will potentially change in the future as a result of new genetic information. Inconsistencies with regard to the organs' presence or absence in certain groups could potentially vanish and may thus modify these preliminary conclusions.

Regardless, although the PO is present in some species and therefore fulfills physiological functions such as gas exchange, it is absent in others. This suggests that these latter species have developed alternative mechanisms or sites for efficient gas exchange, or that the ctenidia have assumed potentially larger roles. Although there is no clear pattern, the IPO and/or OPO seem to be commonly present in larger species (adult maximum SL >6 cm), but are more often absent in smaller species (SL <1 cm), which have a more advantageous surface-to-body-mass relationship, allowing for more efficient gas exchange. For example, in the larger *Brachidontes pharaonis*, both organs are present, whereas the smaller *B. puniceus* and *B. striatulus* possess the IPO only. Furthermore, no

Table 1. List of mytilid species with status of the plicate organ and reference of the first description.

Subfamily	Species	Inner plicate organ	Outer plicate organ	Reference
Modiolinae	<i>Modiolus modiolus</i>	X	X	This study
Mytilinae	<i>Mytilus edulis</i>	X	X	Sabatier (1877, 1880)
	<i>Mytilus galloprovincialis</i>	X	X	This study
	<i>Mytilus californianus</i>	X	X	Thomsen <i>et al.</i> (2016)
	<i>Perna perna</i>	X	X	Purdie (1887)
	<i>Perna carnalliculus</i>	X	X	Purdie (1887)
	<i>Aulacomya atra</i>	?	X	Zaixso (2003)
	<i>Limnoperna fortunei</i>	–	X	This study
Brachidontinae	<i>Xenostrobus securis</i>	–	X	This study
	<i>Brachidontes pharaonis</i>	X	X	This study
	<i>Brachidontes striatulus</i>	X	–	This study
	<i>Brachidontes puniceus</i>	X	–	This study
Lithophaginae	<i>Mytilaster minimus</i>	–	–	This study
	<i>Lithophaga teres</i>	X	–	Wilson (1979)
Septiferinae	<i>Leiosolenus</i> sp.	–	–	Wilson (1979)
	<i>Septifer bilocularis</i>	–	–	This study
Bathymodiolinae	<i>Mytilisepta virgata</i>	–	–	This study
	<i>Bathymodiolus childressi</i>	–	–	This study
Crenellinae	<i>Idas washingtonia</i>	–	–	This study
	<i>Musculus discors</i>	–	–	This study
	<i>Musculus subpictus</i>	–	–	This study
	<i>Musculista senhousia</i>	–	–	This study

Abbreviations: X, present; –, absent; ?, not stated.

PO has been detected in the large deep-sea *Bathymodiolus childressi*. As this species is adapted to low temperatures, however, metabolism and consequently respiratory rates will be lower in comparison with that of its shallow-water relatives (Khripunoff *et al.*, 2017). In order to evaluate the detailed function of the PO within the context of the whole animal's performance, comparative physiological studies need to evaluate whether metabolic rates differ between similar-sized species with or without the PO, in comparable habitats. Furthermore, in species without the organ, alternative sites for respiration and ammonia excretion need to be located.

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