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First record of sex reverses in two Indo-Pacific mytildes (*Brachidontes variabilis* and *Modiolus arcutulus*) in the Great Bitter Lake, Egypt

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Abstract
Two Indo-Pacific mytilides; *Brachidontes variabilis* and *Modiolus arcuatulus,* were collected monthly from the Great Bitter Lake during the period from December 2015 to March 2016. The two species are dioecious. Females significantly outnumbered males. X^2 test = 8.042 ($P < 0.005$) and 7.100 ($P < 0.01$) for *B. variabilis* and *M. arcuatulus*, respectively. Hermaphroditic individuals were found in each species. The present study suggests, perhaps surprisingly, the presence of sex reverse for the first time in the two mytilides in the Great Bitter Lake. This characteristic is not progressive but limited to a very few individuals. The rapid transition of the gonads from one cycle to the next in spent stage has been suggested as the reason for the presence of low frequencies of hermaphroditism.

Keywords: Sex ratio, Sex reverse, *Brachidontes variabilis, Modiolus arcutulus,* Great Bitter Lake, Egypt

1. Introduction

The Mytilidae is a family of considerable antiquity dating back to the Devonian era and includes many important genera such as *Mytilus*, *Modiolus,* and *Brachidontes* [1]. Mussels belonging to this family dominate in much of the low and mid intertidal regions in temperate seas of the northern and southern hemispheres [2]. They are suspension feeders and are held in place by strong byssus threads.

Mussel larvae settlement and firm attachment of young mussels are facilitated by rough textured rock surfaces which reduce the effective current velocity. Many man-made structures, the hulls of ships, cooling systems of power plants, surfaces of docks, piers, buoys, mariculture cages and offshore platforms all attract diverse communities of sessile fauna and flora to settle on them. These communities are known in general as "fouling communities".

Fouling communities consist of a mixture of algae and suspension-feeding invertebrates including mussels. Fouling on ships increases drag, thereby reducing speed, and growth of barnacles may increase the possibility of corrosion. Growth of fouling organisms on offshore buoys also increases drag and may eventually result in the buoy dipping below the water surface in strong currents. Military vessels, water treatment plants, water intake pipes and cooling water systems of industrial plants and power stations often experience fouling problems caused by mussels in Suez Canal water system (pers. observ.) and many parts of the world [3-5]. Settlement and growth of the mussels on these utilities result in blockage of free flow of water in the conduits and clogging of condenser tubes.

With the opening of the Suez Canal in 1869, the waters of the subtropical Mediterranean and the tropical Red Sea came into direct contact. A considerable number of bivalve species of the Red Sea and Mediterranean migrated to the Suez Canal lakes. This migration resulted in a mixed population of bivalves from two different marine environments. Por [6] studied the theory of species migration through the Suez Canal and suggested the term "Lessepsian migration" for the movement of species from the Red Sea to the Mediterranean. The number of bivalve species migrated from the Red Sea to the Suez Canal lakes (Indo-Pacific species) is higher than those moved from the Mediterranean in the opposite direction [7-8]. Fouda and Abou-Zied [7] recorded 35 species of Indo-Pacific bivalves including 4 species of mytilids; *Brachidontes variabilis* (Krauss, 1848), *Arcuatulus arcuatulus* (=*Modiolus arcuatulus,* Barash and Danin, 1972), *Septifer bilocularis* Linnaeus 1758 and *Modiolus auriculatus* (Krauss, 1848).

The available information about the ecology and biology of Indo-Pacific bivalves in the Suez Canal lakes are very limited [9]. Thus, the main aim of this work is to investigate sex ratio and its variation among different size groups of two Indo-Pacific mytilids; *Brachidontes variabilis* (Krauss, 1848) and *Modiolus arcutulus*, Barash and Danin 1972, in the Great Bitter Lake. The possibility of the presence of sex reverses in the two mussels.

2. Materials and methods

Brachidontes variabilis (Krauss, 1848) sampling was carried out by quadrates; each measuring 10 x 10 cm. Mussels inhabiting the area of the quadrates were scraped from the rock surface using a sharp knife. For *Modiolus arcutulus;* Barash and Danin, 1972, core samples of sediment were taken from the surface of the mussel bed using 14 cm diameter circular plastic pipe. Samples were washed out carefully in situ through one mm size sieve. Collection of samples was carried out from the Great Bitter Lake during the period from December 2015 to March 2016. Collected samples were kept in labeled containers filled with 6% formaldehyde-seawater solution and then transported to the laboratory. Some specimens were fixed in Bouin's solution for histological preparation.

In the laboratory, shell lengths (SL); (maximum distance on the anterior-posterior axis) of the entire specimens were measured to the nearest 0.1 mm by using a Vernier caliper. Smears of the gonadal tissue from each animal collected was examined microscopically to confirm its sex. Sex ratio expressed as the number of females per males (M: F) was determined. Statistically significant deviations from the expected sex ratio of 1:1 were assessed by a Chi-square (X^2) analysis with one degree of freedom [10]. Gonads of mature individuals fixed in Bouin's solution were dissected, dehydrated in alcohol, cleared in xylene, embedded in paraffin wax and sectioned (6 – 8 µm) at a standard point (Fig 2A & 3A). Tissue sections were stained in Ehrlich's hematoxylin and Eosin Y following Howard and Smith [11].

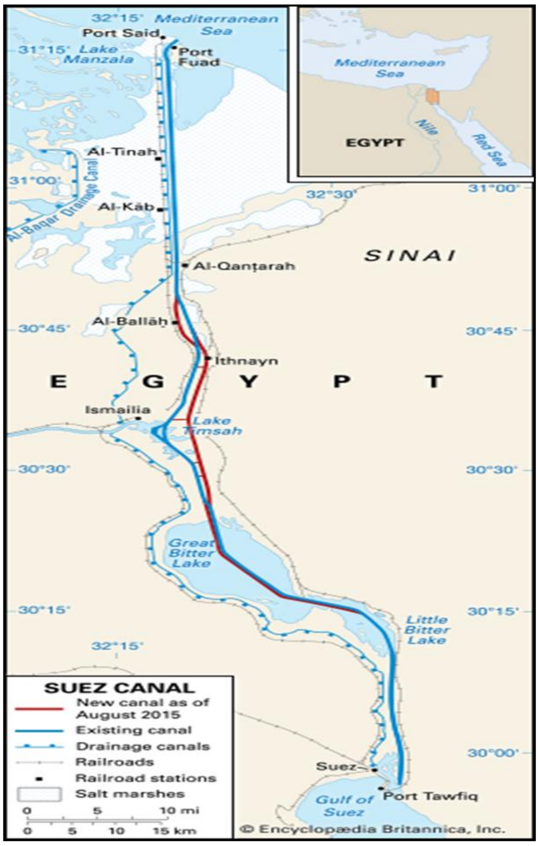
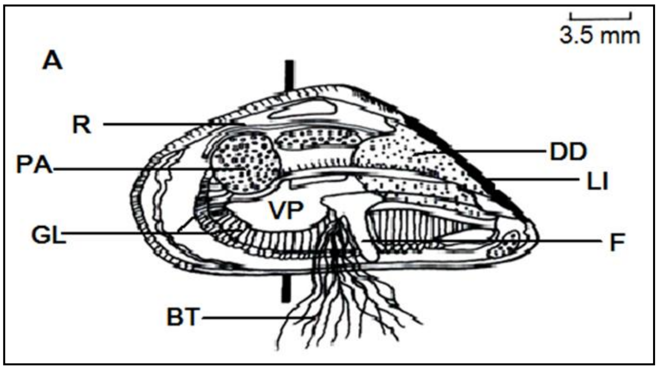


Fig 1: Map of the Suez Canal showing mussel collection area; Great Bitter Lake.



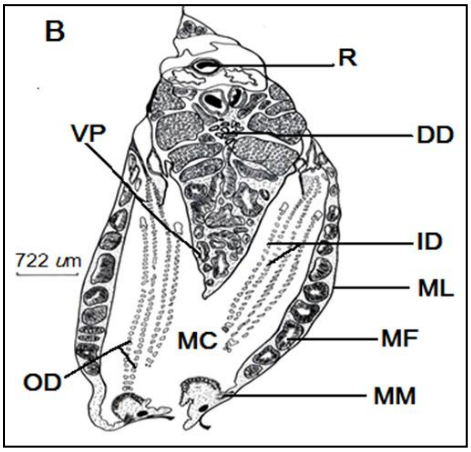


Fig 2: *B. variabilis*, (A) Gross anatomy after the removal of the right mantle lobe, the right ctenidium and the right labial palps. The line shows the location where histological cross-sections were taken place. (B) Slide microprojector drawing of a transverse section showing the position of the gonad. Abyssal threads (AT), Digestive diverticula (DD), Female follicles (FF), Foot (F), Gill lamella (GL), Inner demibranch (ID), Ligament (L), Male follicles.(MF), Mantle cavity (MC), Mantle lope (ML), Mantle margin (MM), Outer demibranch (OD), Posterior adductor mussel (PA), Rectum (R), Right mantle (RM),Ventral part of visceral mass (VP) and Visceral mantle (VM).

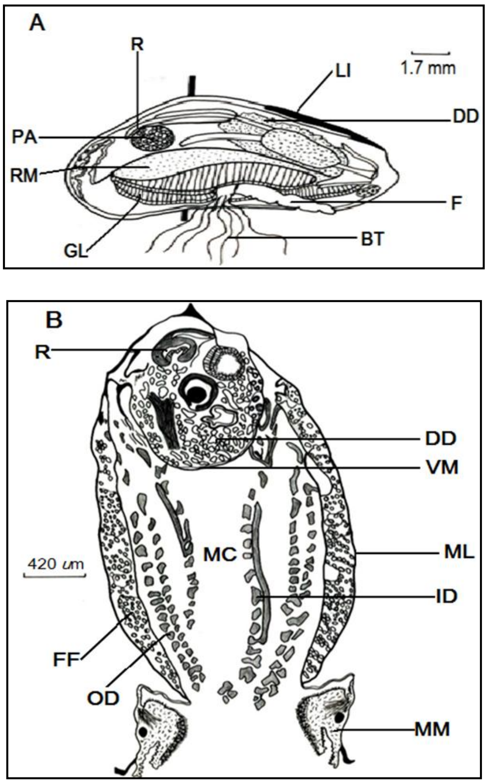


Fig 3: *M. arcuatulus*, (A) Gross anatomy as seen from the right side. The line shows the location where histological cross-sections were taken place. (B) Slide microprojector drawing of a transverse section showing the position of the gonad. Abyssal threads (AT), Digestive diverticula (DD) Female follicles (FF), Foot (F), Gill lamella (GL), Inner demibranch (ID), Ligament (L), Male follicles (MF), Mantle cavity (MC), Mantle lope (ML), Mantle margin (MM), Outer demibranch (OD), Posterior adductor mussel (PA), Rectum (R), Right mantle (RM), Ventral part of visceral mass (VP) and Visceral mantle (VM).

3. Results

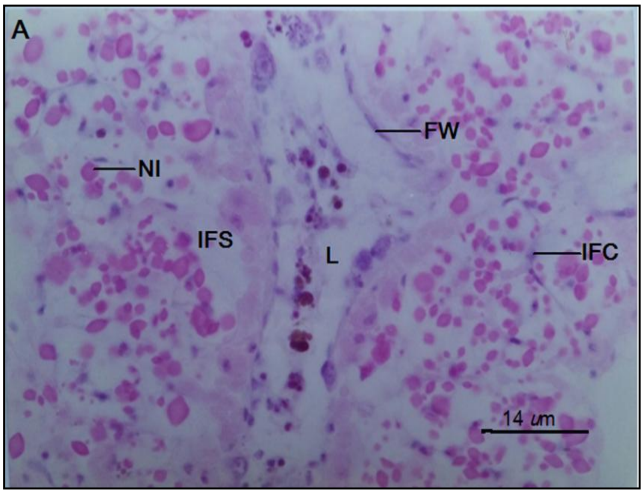
3.1 Gonad position

The gonads of *Brachidontes variablis* and *Modiolus arcuatulus* enclose the digestive diverticula and may intersperse among other regions of the visceral mass depending on the maturity state of the species. In *B. variabilis*, the ventral part of the visceral mass posterior to the foot was entirely filled with gonadal materials forming a triangular lobe in the midline between the gills (Fig 2 A & B). This lobe was not found in *M. arcuatulus* (Fig 3 A & B). Gonads also extend into both left and right mantle lobes. The number of follicles present in transverse sections of the mantle varies considerably according to the state of gonad development.

3.2 Gonad organization

Males and females are similar in gross anatomy of their reproductive system. Gonads are ramified organs composed of numerous follicles, which are of various shapes. Most of the spaces between the follicles (interfollicular spaces) are filled with connective tissue which is well developed in *B. variabilis* and scanty in *M. arcuatulus* (Fig 4).

The follicles contain germ cells at different stages of development depending upon the reproductive condition of the species (Fig 5 & 6). Oocytes in either the early or late developmental stage arise along the walls of the ovarian follicles Testicular follicles showed spermatogenic cells; spermatogonia, spermatocytes, spermatids and sperms in the center of the follicles. In *B. variabilis* and *M. arcuatulus* the formation of germ cells follows the same basic pattern of other pelecypods [12-15]. The gametes arise by proliferation from the wall of the follicles and when they become mature, they were pushed towards the center of the follicles.



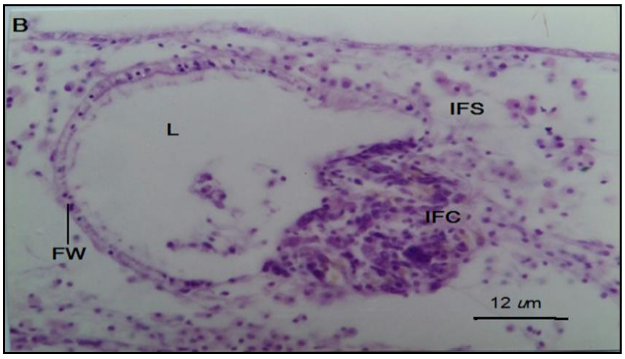


Fig 4: Histological photomicrographs of gonad in spent stage for *B. variabilis* (A) and *M. arcutulus* (B). Follicle wall (FW), Interfollicular connective tissue (IFC), Interfollicular space (IFS), Lumen (L) and Nutritive inclusion (NI).

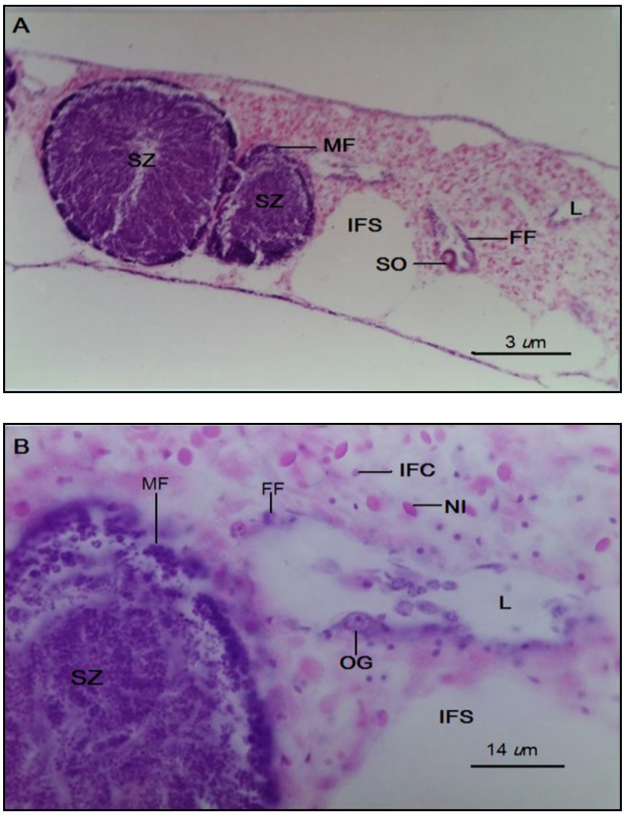


Fig 5: Histological photomicrographs of hermaphrodite gonad of *B. variabilis* (A), (B) is a magnified portion of (A). Feale follicle (FF), Interfollicular connective tissue (IFC), Interfollicular space (IFS), Lumen (L), Male follicle (MF), Nutritive inclusion (NI). Oogonia (OG), Small oocytes (SO) and Spermatozoa (SZ),

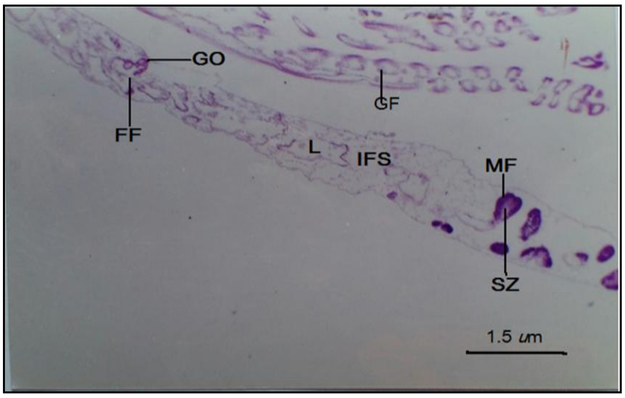


Fig 6: Histological photomicrographs of hermaphrodite gonad of *M. arcuatulus*. Feale follicle (FF), Gill-fillaments (GF), Interfollicular space (IFS), Lumen (L), Male follicle (MF), Growing oocytes (GO) and Spermatozoa (SZ).

3.3 Hermaphroditism

Microscopic examination of *B. variabilis* and *M. arcuatulus* revealed the presence of hermaphrodite gonads. Male and female gonadal follicles were found in different regions of the same gonad (Fig 5 & 6).There is no boundary between the two follicles. Each follicle is either all male or all female; the absence of ambisexal follicles. Both male and female follicles are not equally developed. Lumina of the testicular follicles are filled with a dense mass of morphologically ripe spermatozoa. On the other hand, ovarian follicles were in early active stage. Oogonia, small oocytes and growing oocytes were attached with the follicle wall.

3.4 Sex ratio

The sexes in *B. variablis* and *M. arcuatulus* are separate and no external signs of sexual dimorphism are apparent. Out of 652 individuals examined microscopically for *B. variablis*, 232 (35.6%) were sexed as males, 286 (43.9%) as females and 134 (20.5%) as unsexable. The overall sex ratio (1.0 M: 1.2 F) differed significantly (X^2 = 32.0, P<0.001) from the expected 1:1 ratio indicating that females outnumbered males. Of the 253 animals collected for *M. arcuatulus*, 35.2% were males, 46.6% were females and 18.2% were sexually undefined. The departure from equal proportions of males and females was significant (X^2 = 11.7, P < 0.001) indicating a greater number of females than males.

The changes of the proportion of males, females and sexually undefined individuals at different shell lengths (2 mm size groups) are represented in Table 1 for *B. variablis* and in Table 2 for *M. arcuatulus*. Sexually undefined were recorded in the majority of size groups; 8 out of 10 and 6 out of 7 for the two mussels, respectively. The deviation at 95% level of significance from the expected 1: 1 ratio occurred in 16.0 – 25.9 and in 10.0 – 11.9 shell length size groups for *B. variablis* and *M. arcuatulus*, respectively. In these size groups, females outnumbered males.

Table 1: *B. variabilis*. Percentage and ratio of male, female and sexually undefined individuals at different shell lengths (2 mm size groups) in the Great Bitter Lake.

Shell length	Sample size (N)				X^2 value	Percentage			Ratio		
	Males	Females	Undf.	Total number		Males	Females	Undf.	Males	Females	Undf.
8.0-9.9	12	8	-	20	0.8	60.0	40.0	-	1	0.7	-
10.0-11.9	26	24	-	50	0.8	52.0	48.0	-	1	0.9	-
12.0-13.9	22	34	4	60	2.7	36.7	56.7	6.6	1	1.5	0.2
14.0-15.9	28	34	12	74	0.7	37.8	45.9	16.3	1	1.2	0.4
16.0-17.9	32	48	16	96	5.3*	33.3	50.0	16.7	1	1.5	0.5
18.0-19.9	28	38	26	92	8.4*	30.4	41.3	28.3	1	1.4	0.9

20.0-21.9	34	40	32	106	10.0*	32.1	37.7	30.2	1	1.2	0.9
22.0-23.9	26	26	28	80	9.8*	32.5	32.5	35.0	1	1	1.1
24.0-25.9	18	26	14	58	4.5*	31.1	44.8	24.1	1	1.4	0.8
26.0-27.9	6	8	2	16	0.5	37.5	50.0	12.5	1	1.3	0.3
Total	232	286	134	652	32.0*	35.6	43.9	20.5	1	1.2	0.6

*The critical value for X^2 goodness of fit test of equal numbers of males and females (1 df) at 95% significance is 3.841

Table 2: *M. arcuatulus*. Percentage and ratio of male, female and sexually undefined individuals at different shell lengths (2 mm size groups) in the Great Bitter Lake.

Shell length	Sample size (N)				X^2 value	Percentage			Ratio		
	Males	Females	Undf.	Total number		Males	Females	Undf.	Males	Females	Undf.
6.0-7.9	7	14	5	26	3.0	26.9	53.9	19.2	1	2.0	0.7
8.0-9.9	19	26	12	57	3.4	33.3	45.6	21.1	1	1.4	0.6
10.0-11.9	20	25	15	60	4.1*	33.3	41.7	25.0	1	1.3	0.8
12.0-13.9	18	19	8	45	1.4	40.0	42.2	17.8	1	1.1	0.4
14.0-15.9	12	12	5	29	0.8	41.4	41.4	17.2	1	1.0	0.4
16.0-17.9	9	12	1	22	0.5	40.9	54.5	4.6	1	1.3	0.1
18.0-19.9	4	10	-	14	2.6	28.6	71.4	-	1	2.5	0.0
Total	89	118	46	253	11.7*	35.2	46.6	18.2	1	1.3	0.5

*The critical value for X^2 goodness of fit test of equal numbers of males and females (1 df) at 95% significance is 3.841

4. Discussion

4.1 Sex ratio

Bivalves exhibit wide variation in the expression of sexuality, ranging from strictly gonochoristic species to those that are invariably functional hermaphrodites [16]. Female biased sex ratio was recorded for *B. variabilis* and *M. arcuatulus* as stated by Kandeel [9], Ghobashy, *et al.*, [13] and Morton [17]. However, the sex ratio of the mussels *Mytilus galloprovincialis*, *M. edulis*, *Mytella guyanensis* and *Margaritifera margaritifera* does not significantly deviate from 1:1 [18-21].

The gonad cycle of *B. variabilis* and *M. arcuatulus* in Lake Timsah and the Bitter Lakes was described by Kandeel [9]. There was no well-defined resting period in the transition between the successive gonad cycles as described for the cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia, N. W. Spain [19]. In this study, samples were collected during the period from December 2015 to March 2016. This period was characterized by the occurrence of completely spawning and spent stages. The spent gonad contained very few follicles which were sparse and very few in diameter. Early stages of gametogenesis (oogonia and spermatogonia) were sometimes observed around the periphery of gonadal follicles (Fig 4). The majority of spent mussels were sexually undefined because it was impossible to distinguish between the two early stages of gametogenesis

Spent gonad of *B. variabilis* contained a large abundance of interfollicular connective tissue (Fig 4). This tissue serves as a storage tissue utilized during gametogenesis. The association between gonad and storage tissue cycles in mussels is well known [19, 22-24]. However, an obvious storage tissue was not observed in *M. arcuatulus* (Fig 4).

4.2 Sex reverse

The occasional found of gametocytes or follicles of the opposite sex in the gonads of *B. variabilis* and *M. arcuatulus* (Fig 5 & 6) may be signs of the capacity of the two mussels for hermaphroditism or sex reversal. This characteristic is not progressive but limited to a very few individuals. True sex reversal was found in *Mytilus viridis* [25].

Males changed their sex between two successive gametogenic cycles. Kandeel [9] reported that the population of the two mussels had 4 and 3 gametogenic cycles per year, respectively. Each population passed rapidly from one cycle to the next without resting stage (i.e. continuous reproduction). This may explain the presence of low frequencies of cases of hermaphroditism (two individuals from each species).The low frequencies of hermaphroditism detected in this study agree with those found by Lubet [18] for both *M. edulis* and *M. galloprovincialis,* Villalba [19] for *M. galloprovincialis,* Sunila [26] for *Mytilus edulis*, and Bauer [27] for *Margaritifera margaritifera.* According to Bauer [27], females of *M. margaritifera* in some rivers in Germany became hermaphrodites and on the other hand some hermaphrodites became females. Occasional functional hermaphroditism among mytilids is fairly common [19, 26, 28-32]. In contrast, *Margaritifera falcate* (Gould) is normally monoecious according Heard [33]. Although the cause of hermaphroditism is unclear, an unbalanced sex ratio has been suggested as a possible cause [27].

5. Conclusion

It can be concluded that female biased sex ratio was recorded for *B. variablis* and *M. arcuatulus.* The presence of hermaphroditic individuals suggested the presence of sex reverse. This data may provide valuable information upon which further researches can be carried out.

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