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Hermaphroditism in two populations of *Chiton articulatus* (Mollusca: Polyplacophora) from the eastern tropical coast of México

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Through histological analyses, this study reveals an unusually high incidence of hermaphroditism in *Chiton articulatus*. Specimens were sampled every 30 days between September 2010 and September 2011 at two locations (Las Brisas and Jaramillo beaches, Acapulco) on the tropical eastern Pacific coast of Mexico. At both sites, hermaphroditism was found throughout the year, although in varying proportions. Higher percentages of hermaphroditism were found during the pre-spawning summer months (Las Brisas Beach 63%, Jaramillo Beach 68%). Two different kinds of hermaphroditic gonads were found, showing a preponderance of either male or female tissues but, commonly, female tissues occupied the greatest part of the gonad cross sections. Similar to other species of polyplacophorans, there was a predominance of males, although M:F sex ratios ranged from 0.7 to 4.5: 1 at Las Brisas Beach and 0.3–8: 1 at Jaramillo Beach.

Keywords: chiton; sexuality; eastern Pacific

Introduction

Most chitons (Mollusca: Polyplacophora) are gonochoric (Eernisse and Reynolds 1994; Ruppert and Barnes 1996) and only two hermaphrodite species are known. Heath (1907) was the first to report hermaphroditism in polyplacophorans, observing that small *Cyanoplax dentines* (Gould 1846) (= *Cyanoplax caverna* according to Eernisse (1986)) from the north Pacific coast of the USA were females, while larger specimens were hermaphrodites. Eernisse (1988), Buckland-Nicks and Eernisse (1993), and Eernisse and Reynolds (1994) reported *Cyanoplax fernaldi* (Eernisse 1986) (= *Lepidochitona fernaldi*) as a simultaneous hermaphroditic species. Eernisse (1988) confirmed that regular self-fertilization occurs in both *C. caverna* and *C. fernaldi*. Ishiyama et al. (1994), while studying the reproductive biology of *Enoplochiton niger* (Barnes, 1824) from Peru, found occasional hermaphrodites (two specimens). Scarano and Ituarte (2009) also reported occasional hermaphroditism in *Plaxiphora aurata* Spalowsky 1795, from the southwestern Atlantic coast at Puerto Madryn, Chubut, Argentina.

Chiton articulatus Sowerby in Broderip & Sowerby, 1832 is a medium sized species (maximum length reported, 86.8 mm), common along the northeastern Pacific coast. In Mexico, this species occurs from north of Mazatlán, Sinaloa to Puerto Angel and Salina Cruz, Oaxaca (Reyes-Gómez 2004). This species is consumed as food along the Mexican tropical Pacific. The current status of its fisheries and distribution from Acapulco, Guerrero, and Revillagigedo Islands were reviewed by Rojas-Herrera (1988) and Holguin-Quiñones and Michel-Morfín (2002). According to the latter authors, *C. articulatus* has been overexploited by subsistence fisheries leading to a decline in its populations.

In this paper, we report, for the first time, the occurrence of hermaphroditism in *C. articulatus* found during a study of its reproductive biology. For the populations studied, patterns of temporal variation in the percentage of hermaphrodites are also described.

Materials and methods

Specimens were collected monthly from September 2010 to September 2011 from rocky shores at two localities on the tropical Eastern Pacific coastline of Mexico: at Las Brisas Beach (16°49′43.91″ N, 99°52′02.25″ W) and Jaramillo Beach (16°52′23.95″ N, 99°56′24.94″ W), Acapulco. Gonads were obtained by dissection, fixed in 10% formalin in sea water, dehydrated in an ethanol series, and embedded in paraffin wax. Sections (6 μm thick) were obtained with a rotary microtome and, after paraffin

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wax removal, were stained with hematoxylin and eosin (Humason 1979). Photographs were taken with a Leica DFC290 camera. Sex identification was based on histological observation; the sex ratio was determined by dividing the number of males and of females without considering hermaphrodites. To evaluate the proportion of male and female tissues in the gonads of the hermaphrodites, the software Sigma Scan Pro 5.0 was used to estimate the area of each sex in 25 slides from each sampling site.

Results and discussion

Gonads from hermaphrodites had either a preponderance of male or female tissues but, commonly, female tissue occupied the greatest part of the gonad's volume (Figure 1). In gonads with a preponderance of female tissue, the percentage of male tissue was 0.024–8.44% (mean = 1.95%) in animals from Jaramillo Beach, and 0.059–15.88% (mean = 3.58%) from Las Brisas Beach. In gonads with a preponderance of male tissue, the percentage of female tissue was 0.24–2.07% (mean = 1.26%) in animals from Jaramillo Beach, and 0.12–2.4% (mean = 1.39%) from Las Brisas Beach.The percentage of hermaphrodites at each sampling site varied during the study period (Tables 1 and 2, Figure 2). At Las Brisas Beach, 362 specimens ranging in length from 30 to 78 mm were studied. The lowest percentages of hermaphrodites were found between December 2010 and April 2011 (except for September 2010 when no hermaphrodites were found). At Jaramillo Beach, 369 specimens ranging in length from 34 to 69 mm were studied. The lowest percentages of hermaphroditism were found between December 2010 and April 2011 (except for September 2011 when no hermaphrodites were found). Overall, temporal patterns of hermaphroditism were similar at both study sites. There was a tendency for the percentage of hermaphrodites to increase from December 2010 to July 2011; with values varying from 20 to 40%.The percentage of hermaphrodites peaked in October at Las Brisas Beach and in August at Jaramillo Beach.

The expression of hermaphroditism in the *C. articulatus* populations studied here seems to differ from other hermaphroditic species from the eastern Pacific coast. In the case of *C. caverna*, Eernisse (1988) described a spatial segregation of male and female tissues in the gonad, with the male portion being much reduced in size and

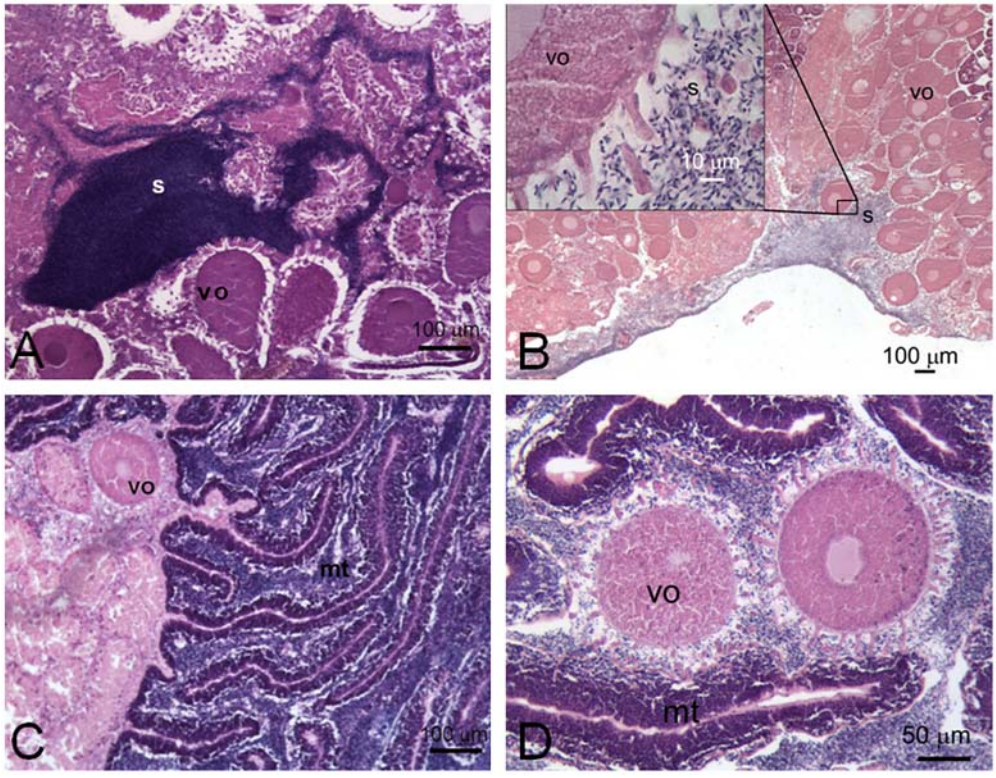

Figure 1. Thin sections of the gonad of *C. articulatus*; (A) Gonad with predominance of female tissues. Female fraction with vitellogenic oocytes (vo). The small male fraction shows an advanced stage of development, with a mass of sperm (s) in the center of the acini; (B) Female tissue in an advanced stage of development with vitellogenic oocytes (vo) surrounded by a mass of sperm (s); (C) Gonad with predominance of male tissue. Male tissues (mt) shows an advanced stage of spermatogenesis and spermiogenesis adjacent to a small female fraction with vitellogenic oocytes (vo); and (D) Gonad with predominance of male tissues. Two vitellogenic oocytes (vo) are surrounded by male tissues (mt). Stain: Hematoxylin and eosin.

Table 1. *C. articulatus* at Las Brisas.

Date	Females	Males	Indet.	Herm.	*N*	% Herm.
Sept-10	14	10	0	0	24	0
Oct-10	0	11	0	19	30	63.3
Nov-10	0	18	0	12	30	40
Dec-10	12	12	2	3	27	11.1
Jan-11	6	12	0	6	26	23.1
Feb-11	12	14	0	4	30	13.3
Mar-11	8	15	0	7	30	23.3
Apr-11	12	14	0	4	30	13.3
May-11	4	18	0	8	30	26.7
Jun-11	6	12	0	12	30	40
Jul-11	10	6	0	9	25	36
Aug-11	7	9	0	9	25	36
Sept-11	8	14	0	3	25	12

Note: *N* = number of specimens; Indet.= sex indeterminate; Herm. = number of hermaphrodites;% Herm. = percentage of hermaphrodites.

Table 2. *C. articulatus* at Jaramillo.

Date	Females	Males	Indet.	Herm.	*N*	% Herm.
Sept-10	6	10	0	6	22	27.3
Oct-10	2	16	1	11	30	36.7
Nov-10	4	13	0	13	30	43.3
Dec-10	9	12	5	3	29	10.3
Jan-11	4	6	14	4	28	14.3
Feb-11	13	16	0	1	30	3.3
Mar-11	7	16	0	7	30	23.3
Apr-11	9	15	0	6	30	20
May-11	6	12	0	12	30	40
Jun-11	9	12	0	9	30	30
Jul-11	9	13	0	8	30	26.7
Aug-11	6	2	0	17	25	68
Sept-11	7	18	0	0	25	0

Note: *N* = number of specimens; Indet. = sex indeterminate; Herm. = number of hermaphrodites; % Herm. = percentage of hermaphrodites.

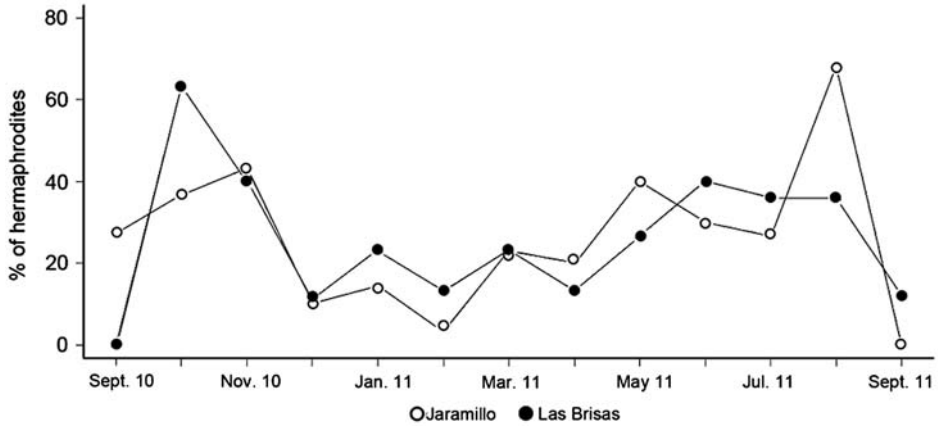

Figure 2. Temporal variations in the percentage of hermaphrodite individuals at Jaramillo and Las Brisas beaches.

peripheral to the female tissues, which occupied the greatest part of the gonad. This spatial pattern was not observed in *C. articulatus*. Eernisse (1988) also reported that only one out of 10 individuals of *C. fernaldi* produced a large testis, without evidence of female tissues, while most individuals developed ovaries with very small amounts of male tissue. In this regard, our findings in *C. articulatus* are similar, with female tissues being commonly

preponderant in hermaphrodite gonads. On the other hand, Eernisse (1988) reported, based on histological observations on *C. caverna*, five "males" (i.e. gonads lacking eggs) out of 94 animals with detectable gonads.

As ripe oocytes and sperm were found together in gonads of adult hermaphrodite *C. articulatus*, self-fertilization may be possible. To date we have not found evidence for this.

Overall, sex ratios showed a predominance of males (Tables 1 and 2). This is a common characteristic in many gonochoric chiton species (Pearse 1979). Values of the male/female ratio varied greatly throughout the study period ranging from 0.7 to 4.5: 1 at Las Brisas Beach and 0.3–8:1 at Jaramillo Beach, with peaks in October–November.

Unlike other polyplacophorans, annual variability in the sexuality of *C. articulatus* seems to be a characteristic of this species, as the proportion of hermaphrodites varied with the time of the year, being higher in the summer months (June–September) before spawning. During the rest of the year, there were varying percentages of males, females, and hermaphrodites.

In many invertebrates, chitons included, brooding has also been related to hermaphroditism (Strathmann and Strathmann 1982; Strathmann et al. 1984; Eernisse 1988), however this not seems to be the case of *C. articulatus*. During the study period, brooding was never found; therefore, we consider *C. articulatus* a non-brooding species. Whilst the few hermaphroditic chiton species are brooders, most brooding species are gonochoric (Eernisse 1988).

Little is known about the reproductive biology of *C. articulatus*, and studies on the type of hermaphroditism (sequential or simultaneous) and relationship between size of specimens and hermaphroditism are now required.

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