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SPECIES RECOGNITION AMONG THE PEDUNCULATE BARNACLES (CIRRIPEDIA: THORACICA) ON THE MANGROVE CRAB, *SCYLLA SERRATA*

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ABSTRACT. - Barnacles of the genus *Octolasmis* commonly found in the branchial chambers of the mangrove crab, *Scylla serrata,* were examined using traditional diagnostic characters as well as new features and measurements never before applied to the group. We found two distinguishable clusters within the complex on the basis of labrum tooth counts, capitular variations (curvature along the occludent margin, ratio of anterior posterior measurements to capitulum length, area of the scutum, and the angle between the arms of the scutum), and fecundity. Experiments which dealt with nutrition and ontogeny further characterized the two groups. Four hypotheses put forth to explain observed differences in capitular plates among the barnacles can be found in the literature: 1. ontogeny, 2. nutrition, 3. gas exchange, and 4. separate taxa. These hypotheses were examined in the light of the new findings and it was concluded that the data fit the fourth hypothesis best, and that two barnacle species, *O. cor* and *O. angulata*, are the primary inhabitants of the branchial chambers of mangrove crabs.

INTRODUCTION

Pedunculate barnacles of the genus *Octolasmis* are frequently found in the branchial chambers of decapod Crustacea. *Octolasmis cor* is a common inhabitant (Aurivillius, 1892, 1894) of the wide- ranging mangrove crab, *Scylla serrata* (Forskål, 1755), gill chambers. However, there are in the same gill chambers octolasmids which on the basis of external anatomy are distinct from *O. cor* as described by Aurivillius (1892).

Over the past 90 years several authors have inquired into the *O. cor* variants. Gruvel (1902), Annandale (1909), Venkateswaran and Fernando (1982) recognized three varieties of *O. cor* whereas Bullock (1964) and Newman (1960) noted four variants or stages respectively. Barnard (1924) and Arudpragasam (1967) noted variations in form without describing specific variants. Four explanations for these variations have been proposed: 1. ontogenetic stages (Gruvel,1902; Newman, 1960): 2. unequal nutrition (Gruvel, 1902): 3. unequal gas exchange (Bullock, 1964; Arudpragasam, 1967): 4. two separate taxa (Jeffries *et. al.* 1982).

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The research reported in this paper was conducted over a period of several years and is based on data from observations on morphology, reproduction, and ecology of octolasmids obtained from the branchial chambers of the mangrove crab, *S. serrata*. It argues that three of the published hypotheses are wrong in interpreting all observed variants as *O. cor* and asserts that two species, *O. cor* and *O. angulata* (Aurivillius, 1892), inhabit the gill chambers of *S. serrata.*

MATERIALS AND METHODS

Mangrove crabs, *S. serrata* (Forskål, 1755), air-freighted from southeast Asia, from Indonesia and Sri Lanka, and received dry were examined live and preserved for barnacle symbionts at the National University of Singapore and at Dickinson College. The methods employed were mostly those described earlier (Jeffries *et al.*, 1982, 1985; Jeffries & Voris, 1983).

In addition, camera lucida drawings were made of 278 preserved octolasmids (85.3% gravid) from one female pre-molt crab, crab No. 8, (96.5 mm carapace width). For each specimen drawings were made of left and right side views of the capitulum. Planimeter measurements of the scutal plates (right and left sides were averaged) provided the basis for the area comparisons. A subset of 83 drawings was used to obtain protractor measurements of the angle between the arms of the scutum, a measure of compactness (a ratio of capitular depth along the anterior posterior axis to its length), and the curvature of the occludent margin of the capitulum (fig. 1c).

Weights of *O. cor* and *O. angulata* with and without ovigerous lamellae, used in the fecundity comparisons were obtained using a precision torque balance after drying the specimens for at least 20 hours.

We removed *O. cor* and *O. angulata* live from the gills of several *S. serrata* in Singapore and cultured them in artificial sea water and fed them on brine shrimp for nearly eight months in 1981. Exuviae of 25 *O. cor* and 34 *O. angulata* thus obtained were used in the study of taxonomic characters associated with the mouthparts, penis and cirri. At the same time growth and copulatory behavior were studied in the two species.

In Phuket, Thailand in 1990 gills were removed from several recently sacrificed mature mangrove crabs (from natural populations) to dishes containing aerated sea water. Barnacles to be used in experiments on ontogeny and nutrition effects were carefully removed from those gills with a jeweler's forceps.

The youngest available live *O. cor* (24; initial capitular lengths 1.430-2.145 mm) and *O. angulata* (31; initial capitular lengths 1.430-2.145 mm) were handled according to size in groups of 4-10 in glass tumblers (inside diameter 40 mm and height 55 mm). The tumblers were covered with Nitex mesh and immersed in running sea water at 29^{o}C, with plankton as food for the barnacles to be used in the ontogeny experiment.

Only the smallest live barnacles, of uncertain species and with undeveloped captiular plates, were selected from the same source for use in the experiment on nutrition effects. The barnacles were segregated according to their original locations on the outside (49; initial capitular lengths 0.858-1.573 mm) or inside (43; initial capitular lengths 0.715-1.144 mm) gill surfaces. They were handled according to size in groups of 4 - 15 and otherwise treated the same as the barnacles in the ontogeny experiment.

RESULTS

Distribution. - *O. cor* has been reported on two decapod families, five genera, and seven species, whereas *O. angulata* has been reported on four decapod families, 13 genera, and 17 species (see Appendix A).

Within hosts, *O. cor* and *O. angulata* are located primarily on the gills, except in cases of overwhelming infestations (e.g. more than 300 per chamber), in which they may inhabit the roof and floor of the branchial chamber as well. The two species are distributed over the gills differently. Of 278 Octolasmids in the two gill chambers of crab No. 8, two *O. cor* and 104 *O. angulata* were on the outer surfaces of the gills whereas 172 *O. cor* and no *O. angulata* were on the inner surfaces of the gills.

Morphology. - Careful examination of 26 *O. cor* and 34 *O. angulata* exuviae with light microscopy allowed qualitative and quantitative comparisons of characters. Among several traditional features examined the two species were indistinguishable. The palpus inner margin bears setae and there are scales on the outer margin. The mandibles bear five teeth and denticles are associated with teeth three, four, and five; the inner and outer surfaces are covered with comb-like clusters of bristles; and the convex and concave margins bear setae in tufts. Maxilla I bears 10 to 12 stout spines and lateral and medial bristles. Maxilla II bears setae on the margins. The caudal appendages are single-jointed and bear setae on the distal and posterior margins. The cirri bear inner and outer arms of nearly equal length, pair I consisting of five segments each, the others of 10 to 12 each. The proximal two-thirds of the penis is covered with scales directed basally, the distal one-third is ringed with scattered three-pronged spines, and overall there are scattered setae.

The average number of teeth on the labrum is different between the two species (fig. 2), on *O. cor* it is 19.1 ($n = 16$, range 17 to 20, $s^2 = 1.476$), and on *O. angulata* it is 14.4 ($n = 7$, range 11 to 16, $s^2 = 2.529$). These means are statistically different ($p<.001$, $t = 6.96$, d.f. = 21).

Scutal area measurements for the left and right sides of each of the 278 individuals were averaged. The relationship between overall size (as measured by the length of the capitulum) and the area that the scutal plates cover differs between the two species (fig. 3). The regression of scutal plate area on capitular length for *O. cor* is $Y = 105.08X - 125.31$ and $Y = 28.02X - 18.52$ for *O angulata*. These two slopes are significantly different ($p<.001$, $t = -7.47$, d.f.= 274).

The angle subtended by lines originating at the narrowest point where the occludent and basal arms of the scutum connect and tangential to the inner margins of the arms (see fig. 1c) is different for the two species (fig. 4). The angle averages 38.3 degrees for 40 *O. cor* (fig. 1b) and 62.7 degrees for 43 *O. angulata* (fig. 1a). These means are significantly different ($p<.001$, $t = 13.1$, d.f. = 70).

The shape of the occludent margin of the capitulum as seen in side view varies between the two species and was scored on a scale 1 to 5, concave to convex (fig. 1c). *O. cor* ($n = 40$) is more convex than *O. angulata* ($n = 43$) as indicated by a highly significant Chi-square ($p<.01$, $X^2 = 54.4$, d.f.= 8).

The capitulum is less compact in *O. cor* than in *O. angulata* as demonstrated by comparing ratios (capitular depth, A-P, divided by capitular length). For 40 *O. cor* and 43 *O. angulata* the average ratios were 0.851 and 0.950 respectively. These means are significantly different ($p<.001$, $t = 9.2$, d.f. = 81).

Fecundity. - A subset of 19 *O. cor* and 9 *O. angulata* of 2.28 mm capitular length were compared. Average body weights were different, 0.45 mg for *O. cor* and 0.24 mg for *O. angulata*. These means are significantly different ($p<.001$, $t = 7.0$, d.f.= 23).

Average weights of ovigerous lamellae as a percent of body weight were 9.23 and 14.66 respectively for *O. cor* and *O. angulata*. These means are significantly different ($p<.001$, $t=2.3$, d.f.= 26).

Ontogeny. - Among the *O. cor* and *O. angulata* from the gills of *S. serrata* cultured in artificial sea water on brine shrimp larvae from 30 January 1981 until 3 September 1981, barnacle vigor was indicated by periodic molting. For example, nine *O. cor* were observed to molt six to twelve times and twelve *O. angulata* molted seven to twelve times. Growth was evident although no measurements were recorded. In another experiment eight *O. cor* were paired in close confinement, from 4 April 1981 until 16 July 1981, and capitular growth increments ranged from 0.143 to 0.572mm, copulatory behavior was common, and one became gravid. Similarly, ten *O. angulata* were paired, growth increments ranged from 0.143 to 1.144mm, copulatory behavior was common, three became gravid, and two became gravid a second time. Live nauplius I were released from both species and comparative studies of the nauplius stages are underway.

Barnacles from natural *S. serrata* populations in Phuket, Thailand in 1990 were cultured in tumblers in the laboratory for sixteen to seventeen days. 24 *O. cor* with initial capitular lengths 1.430-2.145 mm reached final sizes of 2.145-3.575 mm. 31 *O. angulata* with initial capitular lengths of 1.430-2.145 mm reached final sizes of 2.145-4.004 mm. Newman (1960, 1967) suggested that the variation in plate morphology observed among octolasmids on *S. serrata* was a result of ontogenetic differences. If this were the case, one would expect that the increases in plate area and the changes in plate proportions associated with the variants could be observed in animals grown in the laboratory. Of 39 *O. angulata* placed in tumblers 31 survived 16 to 17 days (Table 1). Although these barnacles grew an average of 1.5mm in capitular length during this period, none showed any noticeable changes in capitular plate morphology. Nor were any differences observed among 24 *O. cor* that were maintained in dishes for 17 days and grew an average of 1.1mm (Table 1). During these extended periods of almost daily observations, there were no perceptible changes in the capitular plates of either species.

Nutrition and Gas Exchange. - Bullock (1964) and Arudpragasam (1967) suggested that the variation in plate morphology observed among octolasmids on *S. serrata* was ecophenotypic. Specifically, the variants with heavier capitular valves were said to be correlated with maximum respiratory currents on the inside gill surfaces whereas the forms with less conspicuous valves correlated with reduced current conditions on the outside gill surfaces. Differences in flow rates clearly affect both food availability and gas exchage. One would expect that if small barnacles of uncertain identity were removed from the two regions and grown under constant conditions the resulting phenotypes of the barnacles would be similar regardless of origin. Of 43 small barnacles of uncertain identity removed from the inside gill surfaces and placed in tumblers in the laboratory 29 survived 17 days (Table 2). At the end of 17 days four were still of uncertain identity, 24 were clearly *O. cor* and 1 was identified as an *O. angulata*. Of 49 small barnacles

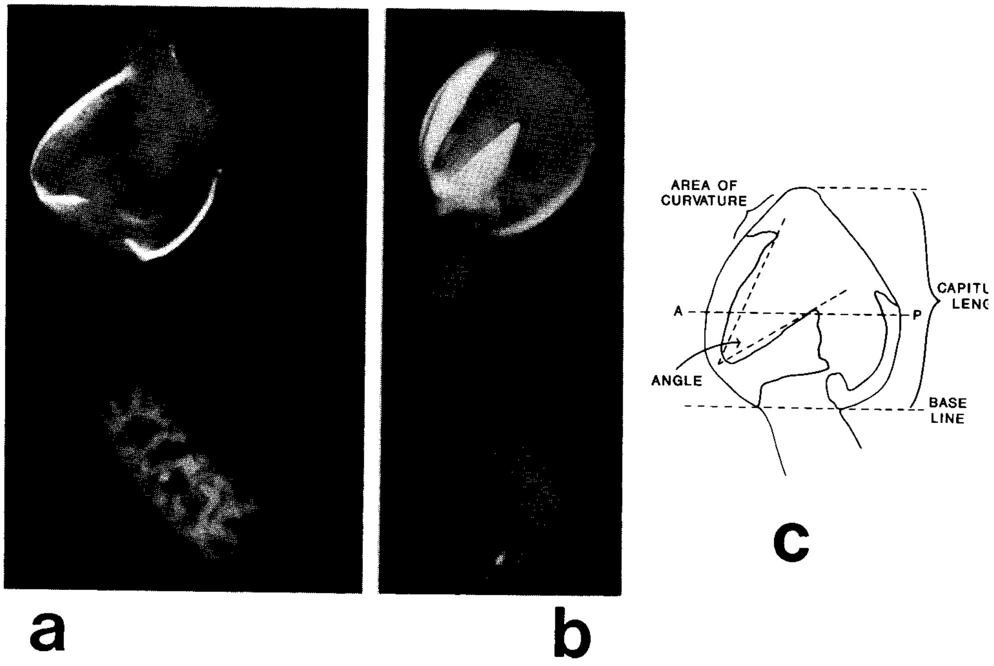


Fig. 1. a, b, photographs of the left sides of *O. angulata* and *O. cor* respectively, showing the scutum an carina; c, a drawing of the left side of a typical *O. cor* showing the angle, length, and anterior-posterio measurements, as well as the area of curvature.

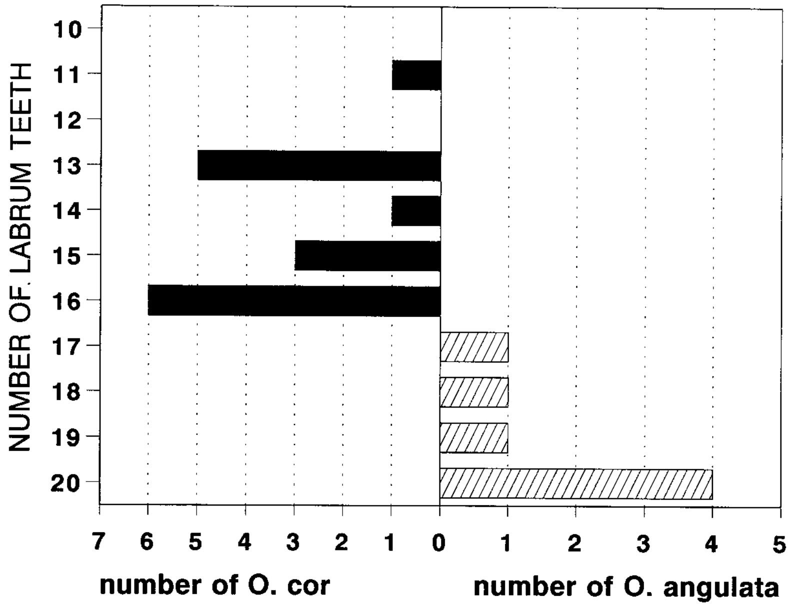


Fig. 2. Labrum teeth counts obtained from exuviae of 16 *O. cor* and seven *O. angulata*.

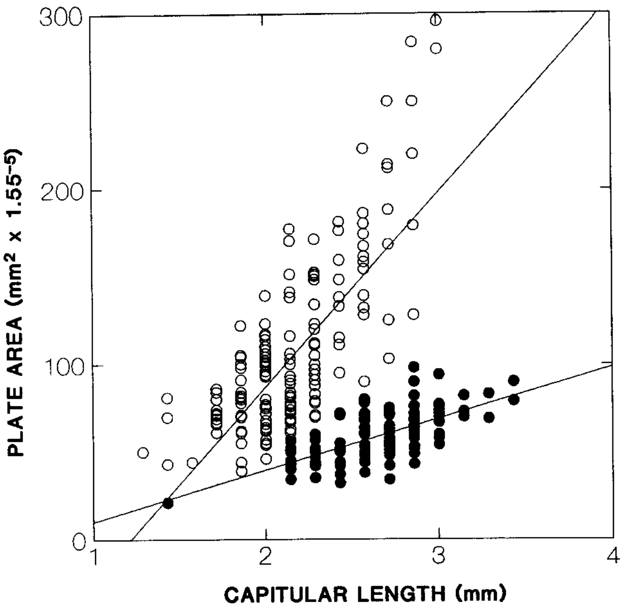


Fig. 3. Scutal areas in mm² of 174 *O. cor* (open circles) and 104 *O. angulata* (closed circles) plotted against capitular length in mm. The regression lines of scutal plate area on capitular length for *O. cor* is Y = 105.08X -125.31 and Y = 28.02X -18.52 for *O angulata*. The two slopes are significantly different (p<.001, t = -7.47, d.f.= 274).

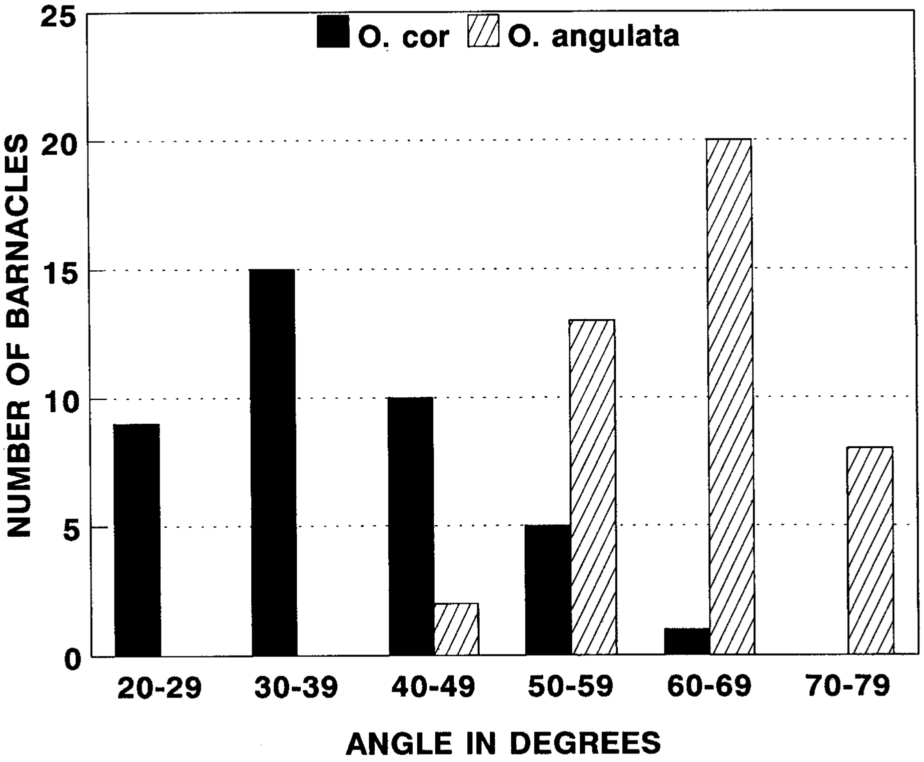
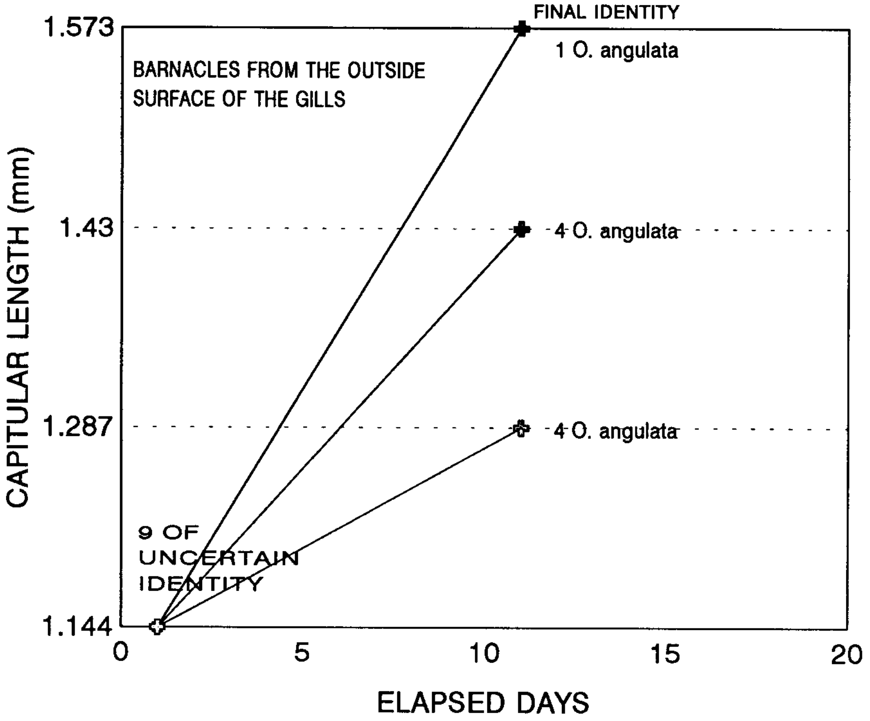


Fig. 4. Comparison of angle measurements between the arms of the scutum for 40 *O. cor* (solid bars) and 43 *O. angulata* (hatched bars).



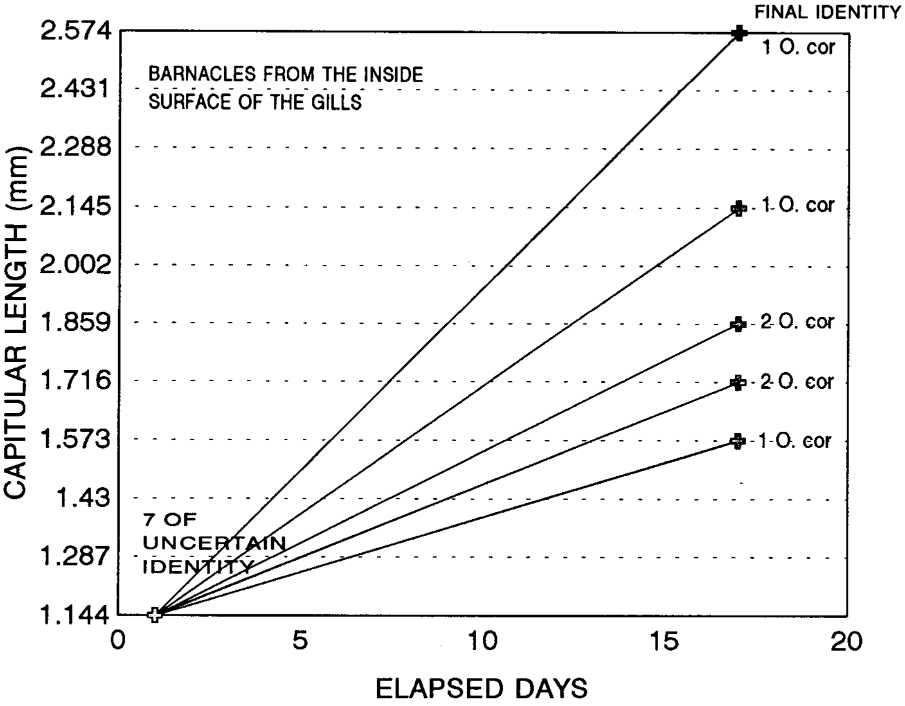


Fig. 5. Graphs showing the final sizes and identities of those barnacles with initial capitular lengths of 1.144 taken from outside gill surfaces (top graph) and inside gill surfaces (bottom graph).

of uncertain identity removed from the outside gill surfaces and placed in tumblers in the laboratory 26 survived 11 days. At the end of 11 days 7 were still of uncertain identity, none were *O. cor* and 19 were identified as *O. angulata* (Table 2). Thus under constant laboratory conditions barnacles from the two gill regions did not grow up to have similar morphology but rather each exhibited the morphology usually associated with that gill region (Fig. 5).

DISCUSSION

Distribution. - *Octolasmis cor* shares seven of the 18 hosts occupied by *O. angulata* (see Appendix A). These data confirm widely overlapping distributions over many host species.

Since 1964 *O. cor* variants have been identified with subregions of the crab gill chamber. Bullock (1964) reported on four variants of which A (equivalent to our *O. angulata*), comprised 99 per cent of the population in the epibranchial chamber, whereas no C and D and less than one per cent of the population of variant B (B, C, and D = our *O. cor*) were found there. Arudpragasam (1967: 106-107) reported, "The upper surfaces of the gills carried cirripedes only vary rarely. Not more than one in a hundred individuals was found in these regions. The least development of the plates was seen on cirripedes attached to the upper quarters [of the gills]." Venkateswaran & Fernando (1982: 244-246) reported, "Varieties of a and b of *O. cor* were abundant on the ventral faces of the gills whereas var. c dominated the dorsal face." "However, there were specimens of *O. cor* var. c (least developed scuta) that occur on ventral side where vars. a and b predominate and specimens of vars. a and b (well developed scuta) on the dorsal side." Their var. c is the same as our *O. angulata.* In addition, we found that on ten *S. serrata*: seven *O. cor* were on the outer surface of the gills whereas 1016 were on the inside; 727 *O. angulata* (*O.* sp. c) were on the outside surface of the gills and 450 were on the inside (Jeffries *et al*, 1982).

Thus agreement may be recorded on these facts: many more barnacles are found on the ventral surfaces of the gills; *O. angulata* greatly outnumbers *O. cor* on the dorsal surfaces of the gills; and *O. cor* greatly outnumbers *O. angulata* on the ventral surfaces of the gills.

Morphology. - Among the conventional species characters of the mouthparts and appendages we found *O. cor* and *O. angulata* to differ only in the number of teeth on the labrum (fig. 2). Our average of 14.4 for *O. angulata* agrees with the 10 to 15 strong teeth reported by Hiro (1937) for *O. angulata.* Unfortunately, we have been unable to find labrum tooth counts for *O. cor* in the literature.

For nearly 90 years the form of the capitular plates has been used to classify the variants of *O. cor*. Gruvel (1902) described three varieties of *O. cor*, sometimes found on the same crab, using as discriminating characters the nature and disposition of the scuta and the carina. He proposed two hypotheses to explain what he observed, i.e., that they represent ontogenetic stages, or that they are morphologic variations based on unequal nutrition. Annandale (1909: 104) did agree and wrote, "Too much stress must not be laid on the differences between them, for many intermediate forms occur." Monod (1922) pointed out the similarities between variety A (*sensu* Gruvel) and *O. angulata* (Aurivillius, 1892). He concluded that *O. cor* and *O. angulata* are the same species. Barnard (1924: 58) wrote of *O. cor*, "The specimens vary greatly in the shape of the basal portion of the scutum..." Newman (1960: 104) illustrated four examples of *O. cor* from a single crab and wrote, "Form of valves variable, for the most part dependent upon age." Other than the drawings, he did not present any data to support this assertion. In 1967 (p.18) he again asserted "...these were actually ontogenetic stages rather than phenotypic variants...", citing his 1960 paper. Bullock (1964) illustrated four variants of *O. cor* which he suggested are related to their positions in the branchial chamber of *S. serrata*; he concluded that they are not separate species. Arudpragasam (1967: 115) examined variation in form of *O. cor* on *S. serrata* and concluded that it was "ecophenotypic." The results of Venkateswaran & Fernando (1982) agreed in part with those of Bullock and Arudpragasam about the sites on the host gills preferred by the variants, but contradicted any further interpretation about the ecophenotypic nature of the variations in plate morphology.

Consideration of Hypotheses.- Four hypotheses have been put forth to explain the variants observed: 1. they represent ontogenetic stages of the same species; 2. they represent nutritional variants of the same species; 3. they represent variants of the same species resulting from different gas exchange conditions; 4. they represent different species.

The data that we have presented here do not support the first three hypotheses. Specifically, it is highly improbable that the number of teeth on the labrum (fig. 2) would vary in response to growth, nutrition or current flow patterns. It is also unlikely that aspects of fecundity and capitular features including scutal area (fig.3), scutal angle (fig. 1 a,b), capitular curvature and compactness would all vary simultaneously in response to growth, nutrition or gas exchange conditions. In addition, our closely monitored studies of animals in culture, in which no perceptible changes from one body morph to another were detected fail to corroborate an ontogenetic, nutritional or gas exchange hypothesis. It should be noted that Bullock (1964) also rejected the growth hypothesis on the basis of comparisons of valve shape and disposition among small sized adults. We are also in strong agreement with the observation of Venkateswaran & Fernando (1982), that discounted the current flow explaination, since both species occasionally occur side by side in the epibranchial (outside gill surface) as well as the hypobranchial (inside gill surface) chambers.

It is our view that the hypothesis of separate taxa is most consistent with the available data.

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APPENDIX A

Octolasmis cor has been reported on Decapod Crustacea: *Scylla serrata* and *Panulirus* sp. (fide Annandale, 1909); from the Portunidae: *S. serrata, Charybdis cruciata, Podophthalmus vigil, Thalamita crenata, Thalamita spinimana,* and *Thalamita sima* (fide Jeffries *et al.*, 1982).

Octolasmis angulata has been reported on four families, 13 genera, and 17 species, including: the Palinuridae, *Panulirus* spp.; Portunidae, *Portunus (Neptunus) pelagicus, P. (Neptunus)* *sanguinolentus, Goniosoma crucifer* (fide Annandale, 1909), *Charybdis cruciata, C. natator, Podophthalmus vigil, Thalamita crenata, T. spinimana,* and *T. danae*; the Majidae, *Camposcia retusa*; the Xanthidae, *Atergatis integerrimus, Etisus utilis, Leptodius exaratus, Lophozozymus pictor, Menippe rumphii,* and *Myomenippe hardwicki* (fide Jeffries *et al.*, 1982). To these we would add a host, *Thalamita sima,* previously designated for *O.* sp. c (fide Jeffries *et al.*, 1982).

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