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MOLECULAR GENETIC AND DEVELOPMENTAL STUDIES ON MALACOSTRACAN CRUSTACEA

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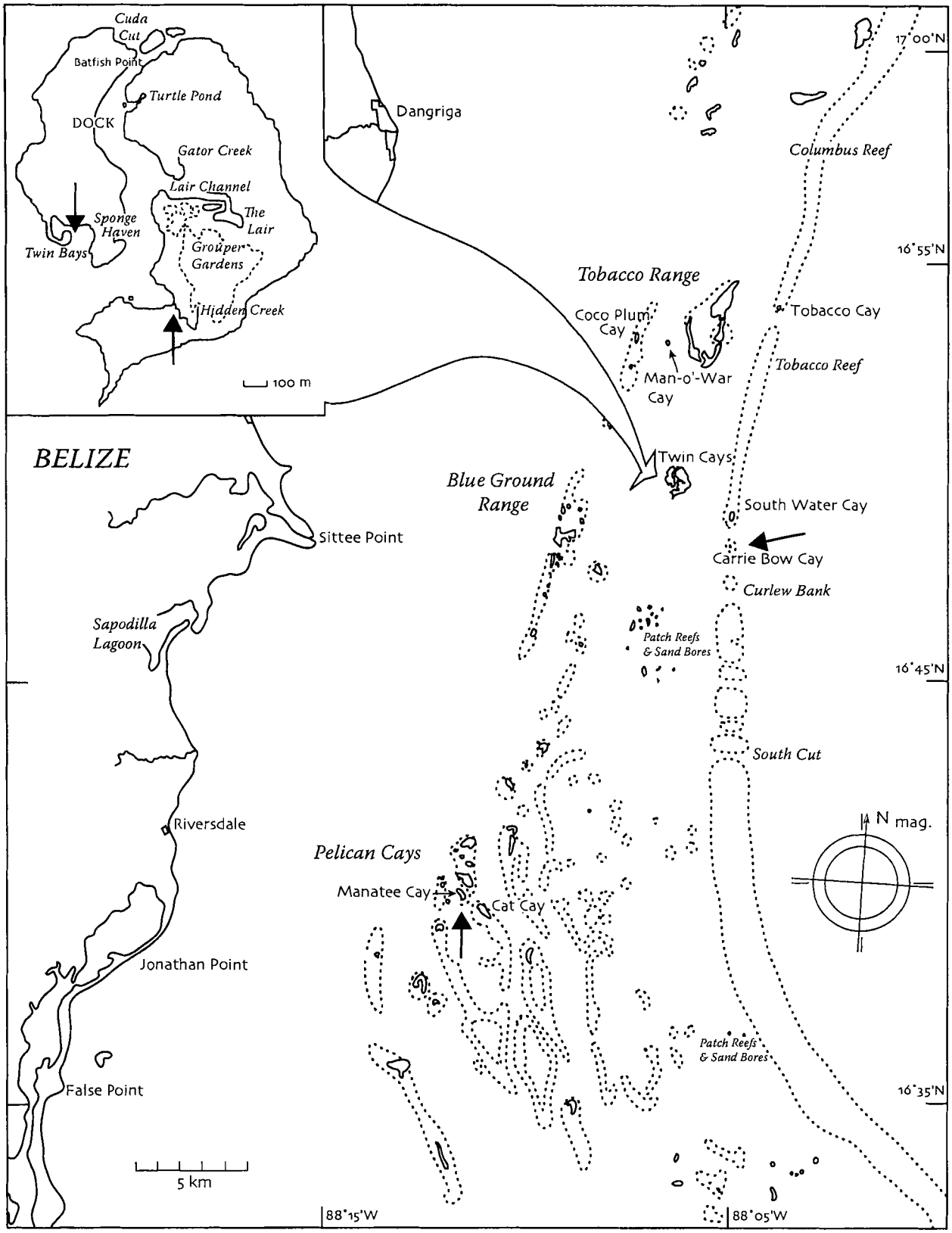

Figure 1. Collecting localities (black arrows) at Twin Cays, off Carrie Bow Cay, and in the Pelican Cays

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ABSTRACT

Arthropods dominate our seas, land, and air and have done so for hundreds of millions of years. Among the arthropods the crustaceans present us with an extremely rich history of morphological change, much of which is still represented among extant forms (morphological disparity among the crustaceans is much higher than in any other group of arthropods). With regard to the Crustacea, several characteristics of the amphipod crustacean embryo make it particularly well suited to embryological manipulations. These include early holoblastic (complete) cleavage coupled with early cell division asymmetries that facilitate microinjection. The high diversity of crustacean taxa near Carrie Bow Cay presents a unique opportunity to extend previous findings in laboratory strains of the amphipod *Parhyale hawaiensis*. In addition, the exploration of standing genetic variation in natural populations may yield important clues in the search for mechanisms by which genes influence organismal development and sculpt morphology through time. The principal collection sites are at south Twin Cays (Twin Bays, Hidden Creek), Manatee Cay (Pelican Cays), and outside the barrier reef near Carrie Bow Cay.

INTRODUCTION

Our ongoing studies of crustacean development and molecular genetics depend greatly on field observations and new samples for laboratory analysis. The Carrie Bow Cay surroundings offer a multitude of habitats from bluewater to coral reefs and mangrove islands within a radius of a few kilometers. The collection sites that we visit regularly include Twin Bays located on the south-west tip of Twin Cays, Hidden Creek on the south end of East Twin Cays, Ctenophore Ridge located off the south tip of Manatee Cay (Pelican Cays), and the water column outside the barrier reef, one-half mile east of Carrie Bow Cay where we make plankton tows at night (Fig. 1).

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Crustacean Diversity

A conservative estimate of the number of extant arthropod species is 1,097,289 (85% of described extant invertebrates). Crustaceans, which (again conservatively) currently number 68,171 extant species, are second only to hexapods in metazoan species diversity. However, the total number of crustaceans, both described and undescribed, is estimated to be 5-10x higher than the current species count (Brusca and Brusca, 2003). For example, peracaridan crustaceans occupying coral reefs alone are thought to number ~54,500 species (Kensley, 1998). Clearly identification of extant crustaceans is far from saturation, and thus the observed diversity of morphological form among crustaceans can only continue to expand.

Several recent studies examining the evolutionary relationships among the major groups of arthropods suggest two possible relationships between the Hexapoda (including insects) and the Crustacea (Fig. 2). One possibility is that the two groups are sister taxa (Boore et al., 1995; Friedrich et al., 1995; Eernisse, 1997; Boore et al., 1998; Giribet et al., 2001) (Fig. 2B). The other possibility is a 'Pancrustacea' clade in which the insects branch from within a paraphyletic Crustacea (Reiger and Shultz, 1997; Hwang et al., 2001) (Fig. 2A). In this scenario, insects would represent a terrestrialized branch of crustaceans.

Under either of these two hypotheses of insect-crustacean relationships, the Crustacea bear the closest affinity to insects among the arthropods. Thus exploration of evolutionary transformations within the crustaceans, and between the crustaceans and insects, should be a high priority for biologists interested in understanding the connections between development and evolution within and between these two clades.

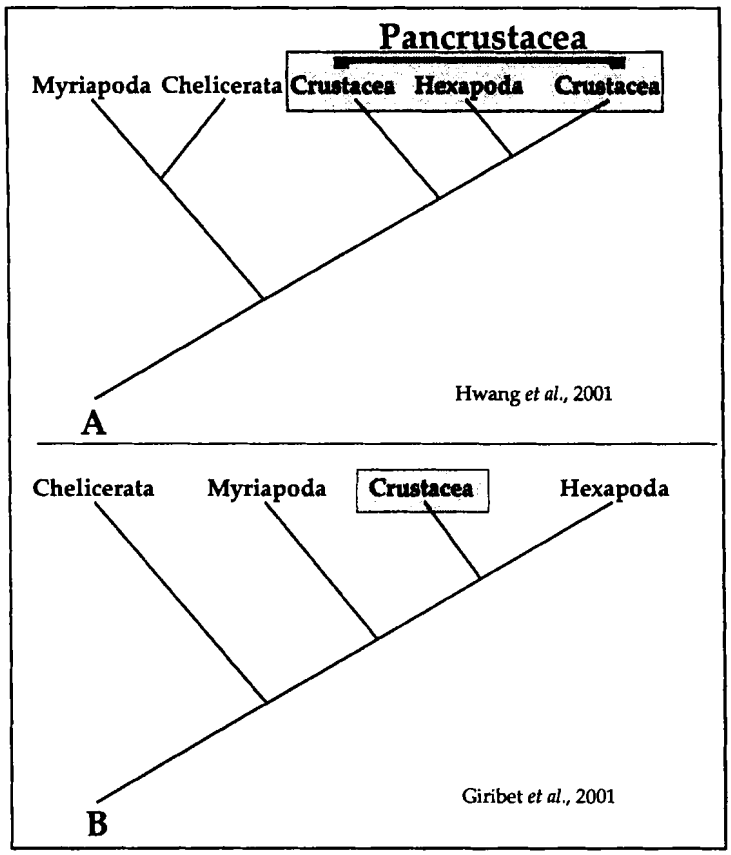

Figure 2. Current hypotheses regarding the Crustacean-Insect relationship.
(A) The 'Pancrustacea' hypothesis places the hexapod lineage within the Crustacea. Under this scenario current morphologic classification schemes of the Crustacea are paraphyletic and are grades. The monophyletic Pancrustacea presents the hexapods as a terrestrialized branch of crustaceans. Data from Hwang et al., 2001 suggests that the more basal Myriapoda and Chelicerata are sister taxa. (B) The competing hypothesis suggests current classification schemes correctly identify a monophyletic Crustacea. Data from Giribet et al., 2001 suggests that the Crustacea and Hexapoda are sister taxa. Their work places the Myriapoda + Crustacea + Hexapoda in a monophyletic clade with chelicerates as the basal outgroup.

Crustacean Appendages: comparative morphology meets comparative gene expression

The Crustacea largely interact with their environment via their appendages; thus vast amounts of variation exist between the different appendages of a single individual as well as between appendages from different species. Comparative studies of crustacean appendage development present an important story regarding the evolution of morphology over both relatively short (a few million years) and relatively long (a few hundred million years) evolutionary time scales. Comparisons of appendage development utilizing molecular and genetic data garnered from *Drosophila* appendage development have been a recurrent theme in recent comparative work in an attempt to understand the molecular basis for some of the variation seen in crustacean limbs (e.g. Williams, 1998; Nulsen and Nagy, 1999; Abzhanov and Kaufman, 2000; Browne and Patel, 2000; Williams et al., 2002).

Generally crustacean limbs fall between two morphological extremes (Fig. 3). At one extreme is the lobed phyllopodous appendage composed of limb branches that are broad and laterally compressed (e.g. *Artemia*, *Eubranchipus*, *Triops*) (Fig. 3A). At the other extreme is the seemingly uniramous appendage which appears to be one multiarticulated rod (all other limb branches have been eliminated or greatly reduced) (e.g. *Stenorhynchus*) (Fig. 3C). The ancestral state of the crustacean limb most likely was neither a strictly phyllopodous limb nor a strictly uniramous limb but a biramous limb composed of two primary branches (Fig. 3B) (Schram, 1986).

Despite the variation seen in crustacean limbs, a consistent nomenclature allows us to compare the different limb morphologies (Fig. 3). The region of the limb most proximal to the body wall is termed the coxopodite (historically termed the 'protopod') (blue shading in Fig. 3). The coxopodite may consist of up to three articulating elements (Fig. 3B, 3C) or be a simple fused structure (Fig. 3A). Distal to the coxopodite is the telopodite (lighter shading in Fig. 3). The telopodite includes the main limb branches termed the endopod and exopod (Fig. 3B). The principal ventral branch is the endopod. The principal dorsal branch is the exopod. Additional cuticular structures may be present on the coxopodite; however, they are not multi-jointed structures. Cuticular structures arising ventral and medial to the endopod are termed endites (Fig. 3A); for example, the crustacean gnathobase is often thought to be an elaborated endite. Cuticular structures arising dorsal and lateral to the exopod are termed exites (Fig 3A and 3B). A common exite structure is the epipod that usually serves a respiratory function (Schram, 1986; Manton, 1977; McLaughlin, 1982; Williams and Nagy, 1996).

Crustacean limbs also can be grouped according to their organization along the A-P axis of the body. Different regions (tagmata) of the body possess characteristic types of limbs with characteristic functions. Cephalic appendages typically include two pairs of antennae (an1 and an2) involved in sensory, and often motor, functions. The gnathal region contains the mandibles (mn) and two pairs of maxillary appendages (mx1 and mx2) that are primarily associated with feeding functions. Thoracic and abdominal appendages are of variable numbers and morphologies and are variably involved in feeding, respiration, and locomotion.

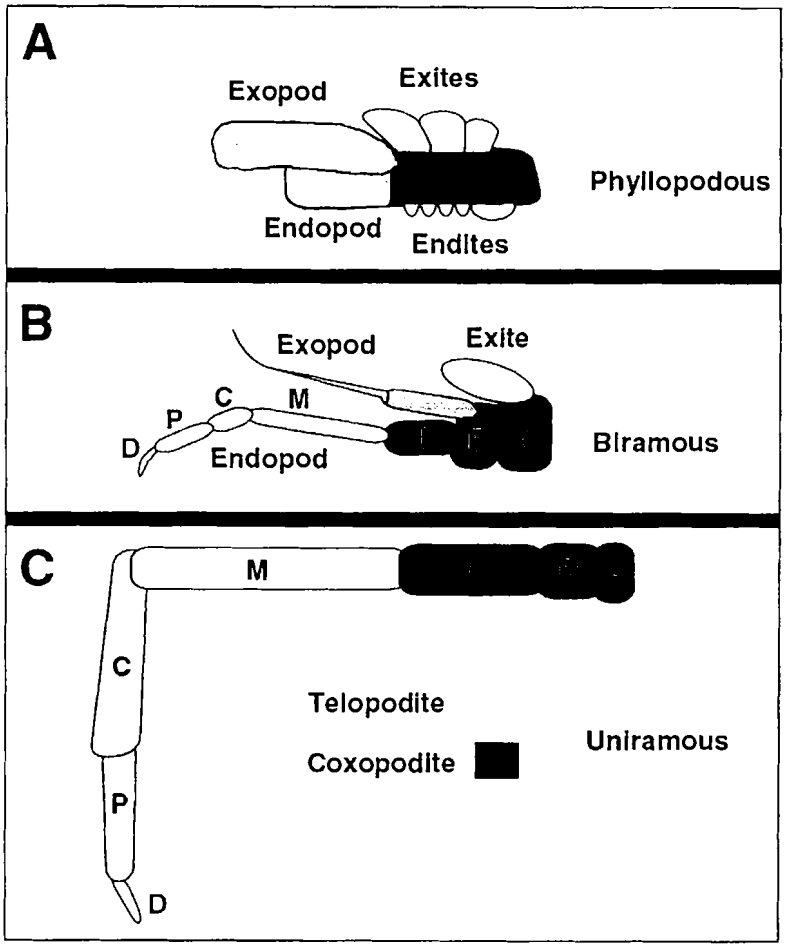

Figure 3. Crustacean appendage morphology.
Ventral is down, dorsal is up. Dark shading indicates the coxopodite, which can be a fused structure (A) or composed of up to three jointed, articulating elements (B and C). Proximal to distal, the three elements are: C-coxa, B-basis, and I-ischuim. Light shading indicates the telopodite, which can be unbranched (C) or include two major distal branches emanating from the coxopodite (A and B). The ventral-most branch is the endopod; the dorsal-most branch is the exopod. While these branches can exhibit considerable variation, the endopod typically constists of four jointed, articulating elements. Proximal to distal, these four elements are: M-merus, C-carpus, P-propodus, and D-dactyl. The coxopodite may also possess a number of cuticular projections that may articulate at the junctions with the coxopodite but are non-jointed. Projections arising ventral and medial to the endopod are endites (A). Projections arising dorsal and lateral to the exopod are exites (A and B).

A crustacean 'model' system for the study of embryonic development and evolution

The use of model systems in developmental biology has played a crucial role in advancing our understanding of biological phenomena in complex multi-cellular organisms such as the metazoans. The six major metazoan model systems in use (the fly *Drosophila melanogaster*, the nematode *Caenorhabditis elegans*, the frog *Xenopus laevis*, the chicken *Gallus domestica*, the zebrafish *Danio rerio* and the mouse *Mus musculus*) share several experimental characteristics that have made them workhorses for developmental and genetic investigation. In all six, the use of forward and reverse

genetic techniques can be employed to alter normal gene expression both temporally and spatially. In addition, techniques for cell lineage analysis and methods of micromanipulation have been developed including microinjection, transplantation, cell explantation, and cell ablation. The wide breadth of experimental techniques available in these systems allows for complex developmental questions regarding gene function, cell fate, and pattern formation to be explored. The results from these studies can be used as starting points for broader investigation of metazoan pattern formation and changes in both morphology and gene function through evolutionary time.

There are currently ~1.33 million described species of metazoans. Each, of course, bears a unique genome shaped by a unique evolutionary history. Of this number, the invertebrate grade represents 96% of metazoan species. Vertebrates represent the remaining 4% of metazoans (Brusca and Brusca, 2003). Of the six major model systems, four are vertebrates. A realistic understanding of biological diversity is further hindered by the fact that identification of extant invertebrates is far from saturation, whereas identification of new vertebrate species has slowed and is likely close to complete. While significant data has been obtained from each of the major model systems in use, comparisons to other non-model taxa are necessarily constrained by the current limited, and skewed, sample size. In particular, comparative data to date has largely been informative strictly with regard to issues of conservation of gene expression and/or gene function. This is due to the vast evolutionary distances that exist between the current model systems. Extrapolations from model system data sets can be problematic. For example the two invertebrate model systems, *C. elegans* and *Drosophila*, share a common ancestor well over 550 million years ago, time enough to mask the evolutionary transitions that have crafted nematodes in one case and flies in the other case.

In the past 15 years the number of non-model taxa in which descriptive analyses of gene expression have been made has steadily increased. Thus far, the interpretable data has largely served to reinforce concepts related to conservation of expression (e.g. Patel et al., 1989). Again this is due, in large part, to the paucity of data reported in non-model organisms. Among these non-model taxa currently being utilized, a small number can now be considered as 'minor' model systems in which techniques for reverse genetics are beginning to be successfully applied and some micromanipulations have proven to be feasible (for example, the long history of the sea urchin, *Strongylocentrotus purpuratus*, as a developmental system and the more recent history of the flour beetle, *Tribolium castaneum*, as a developmental and potential genetic system).

Evolution acts on species at the level of the population and, as barriers to gene exchange arise, independent lineages are generated. Each isolated lineage, or species, can then be described by virtue of unique characters not shared with other lineages or species (Harrison, 1998; de Queiroz, 1998; Shaw, 1998). Evolution by the process of lineage splitting generates differences between extant species and the relationships between groups of extant species are largely assessed via extrapolation from comparative observations between living representatives of a given lineage. This is particularly the case with regard to recent examinations of embryological phenomena. While this type of observation, in particular of gene expression patterns, in non-model systems provides suggestive data for conservation and, more recently, the divergence or convergence of specific characters, processes, and/or mechanisms (e.g. Patel et al., 1989; Davis et al.,

2001; Abouheif and Wray, 2002), it is only functional data generated in the small number of major and minor model system taxa that allow for secure and robust interpretations regarding any observed changes in gene expression.

Thus, we are currently presented with many critical unanswered questions regarding the tempo (rate or pace) and mode of evolutionary change over time, as well as how evolution has generated the full range of extant biological diversity, that cannot be addressed with the current complement of model systems for embryonic development. This problem is being addressed by 'gap-filling' with new systems more closely related to model systems currently in use by researchers. Optimally these new systems are taxa in which functional studies can be feasibly designed and implemented. In this way we are beginning to identify important differences between species that can be shown, by functional experimentation, to have evolutionary significance.

The Amphipoda

The amphipods [Peracarida; Malacostraca; Crustacea] are commonly referred to as beachhoppers or scuds. Within the Crustacea, amphipods rank as one of the most ecologically successful and speciose extant orders and occur in nearly all known marine, fresh, and brackish water environments as well as in high-humidity terrestrial ecosystems (such as tidal zones, coastal flood plains, and forest leaf litter) (e.g. Barnard and Karaman, 1991; Vinogradov et al., 1996; Lindeman, 1991; Sherbakov et al., 1999; Kamaltynov, 1999; Vainola and Kamaltynov, 1999; Sheader et al., 2000; Poltermann et al., 2000). They have predominately exploited scavenging niches and thus an apt description for the group would be 'the flies of the sea'. The ecological diversity represented in the group is reflected in similarly high levels of morphological disparity. Several thousand amphipod species have been described (>7000), and the current rate of several new species descriptions per year suggests that the upper limit of extant amphipod species is far higher than the current species count. Phylogenetic relationships among amphipods remain poorly resolved with the current suites of morphological characters in use by systematists (Fig. 4) (Martin and Davis, 2001; Kim and Kim, 1993). However there are distinct characters that unite amphipods as a natural, monophyletic group. Most recognizable among these characters are lateral compression of the body, sessile compound eyes, and the orientation of the thoracomere appendages (periopods) to the body axis (periopods 1-2 orient anteriorly, periopods 3-5 orient posteriorly, thus the name for the group, amphipod) (Plate 1, A-B). Additionally amphipod thoracic appendages bear two dorsal branches of interest, large coxal plates that have become flattened, heavily cuticularized, protective sheets attached dorsally to the base of thoracic appendages (Plate 1, A-B) and the gills that are also laterally compressed but have a highly complex internal network of branching tubes used for gas exchange. The cephalon has a unique organization in which thoracomere 1 (t1), bearing the maxillipeds, is fused to the head. This fusion is accompanied by a close arrangement of the gnathal appendages, including the maxillipeds, in a basket shape around the mouth to form a highly compact buccal mass (Plate 1, B). The Amphipoda are a monophyletic, species-rich, assemblage and amphipod groups contain a web of complex relationships between members. These are the hallmarks of a highly successful evolutionary lineage.

The marine amphipod, *Parhyale hawaiensis* (Dana, 1853), is well suited for both mechanistic and functional genetic studies within crustaceans (Browne, 2003; Browne and Patel, 2000; Gerberding et al., 2002). Several aspects of *Parhyale* embryological development are derived when compared to other peracaridian species such as isopods and mysids (cell lineage and early cell-cleavage patterns, as well as later gene expression correlating with the development of specific morphological structures). Examples of the types of characters we are actively exploring are variations in early embryonic cleavage patterns and associated cell lineages (Gerberding et al., 2002) and changes in the expression patterns of genes involved in embryonic segmentation, limb patterning, and nervous system patterning (Browne, 2003; Browne and Patel, 2000; Duman-Scheel and Patel, 1999; Averof and Patel, 1997).

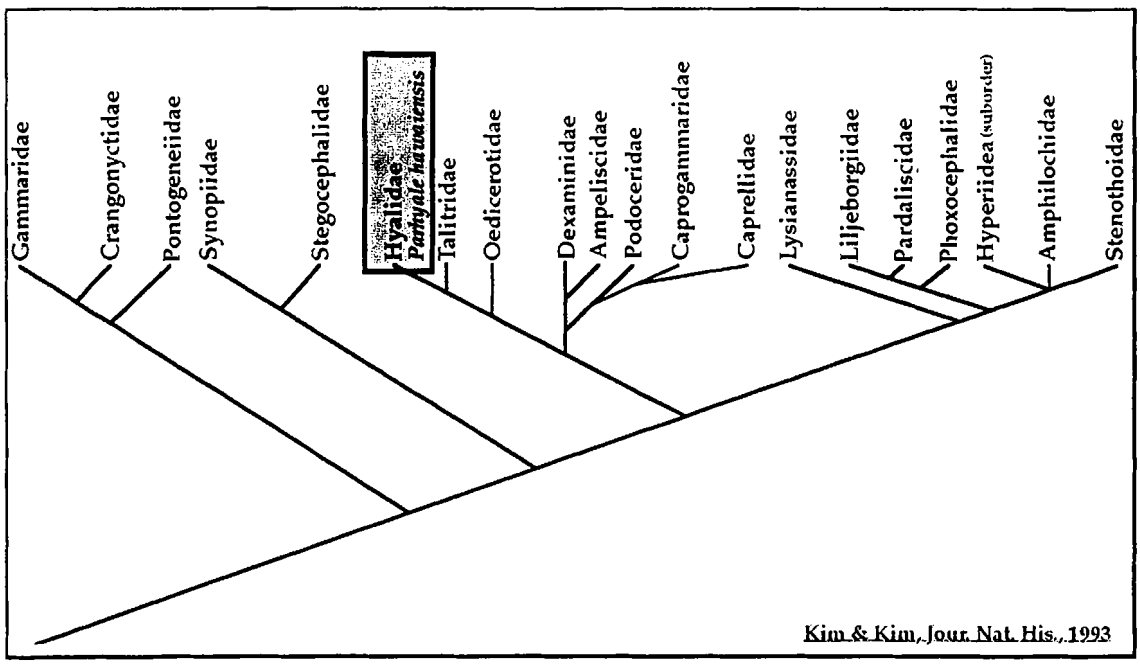

Figure 4. Proposed relationships among some of the major groups of amphipods.
The Kim and Kim, 1993 phylogenetic analysis of several morphological characters propose a monophyletic Hyalidae (indicated by red box). *Parhyale hawaiensis* is a member of this large family of amphipods. The Talitridae are considered sister taxa to the hyalids. The talitrids include *Orchestia cavimana*, an amphipod in which cell lineage and gene expression analyses have been reported (e.g. Wolff et al., 2002; Scholtz et al., 1994). It is important to note that the affinities among most amphipod groups are far from resolved and this represents only a first approximation of relationships within the Amphipoda.

FIELD WORK AND LABORATORY STUDIES

How to explain the circumtropical distributions of *Parhyale hawaiensis* and *Stenopus hispidus*: Ecotypes or Species Complexes?

Both *Parhyale hawaiensis* and the coral-banded shrimp, *Stenopus hispidus,* are present in both Atlantic and Pacific Oceans. Both have largely overlapping circumtropical distributions based on their respective morphological descriptions. Their

life histories, however, are vastly different from one another. Ecologically, *Parhyale hawaiensis* is a detritovour that has a circumtropical, worldwide, intertidal, and shallow-water marine distribution (Shoemaker, 1956; Barnard, 1965) (Fig. 5), possibly existing as a species complex (Myers, 1985).

Figure 5. Geographical distribution of *Parhyale hawaiensis*.
Light-shaded region indicates the approximate north-south boundaries capable of supporting *Parhyale hawaiensis*. The range of *P. hawaiensis* is extensive; they inhabit shallow water environments and are found associated with continental coastlines (including bays and estuaries), mangrove forests, shallow reefs, marine atolls, seamounts, etc. Black markings indicate known ranges [Atlantic: Texas, Florida, North Carolina, Bermuda, Haiti, Dominican Republic, Puerto Rico, St. Croix, Curacao, Bonaire, Panama, Colombia, Venezuela, Brazil, Democratic Republic of the Congo] [Pacific: Lower California, Costa Rica, Panama, Ecuador, Galapagos Islands, Hawaii, Johnston Island, throughout Oceanica, Polynesia, Micronesia, Bay of Bengal, India, Arabian Sea, Red Sea, East Africa]. Type locality, Maui, is indicated with a black/white bull's-eye.

In the case of *Parhyale hawaiensis*, though widely distributed, they inhabit shallow waters and are a benthic species. In addition, embryonic development occurs in a protected environment, is direct, and the juveniles are benthic. Thus, dispersal across large bodies of water for this species is presumably a significant problem. The broad distribution of *Parhyale hawaiensis* in the face of this dispersal problem suggests that many populations of *Parhyale hawaiensis* may be relatively isolated from one another. Gene-flow analysis is a useful tool to employ to attempt to determine the degree of genetic exchange between population 'islands'. A possible scenario would be that *Parhyale hawaiensis* exists as groups of loosely connected ecotypes and/or as a species complex. Importantly, if morphological variation were found to correlate with observed population structure, the connection between genetic change and morphological change within a single species could be addressed (or very closely related species, if a species flock exists). Work is in progress regarding population genetics in *Parhyale hawaiensis*.

In contrast to *Parhyale*, *Stenopus hispidus* (Plate 1, C) possesses a benthic adult reproductive phase coupled with a pelagic larval phase. In this case a potential mechanism for wide dispersal of *Stenopus hispidus* would appear to exist. Interestingly observations of adult populations at locations in both the Atlantic and Pacific suggest that juveniles are often settling at depths in excess of 35-40 ft along less protected outer-reef walls, while larger reproductive adults in pairs predominate in less exposed, shallow, inner reefs. In habitats that are exposed to significant disturbance for extended periods no stratification of *Stenopus hispidus* by age is observed. This is suggestive of a recruitment regime that could be in part deciphered with robust gene-flow information regarding the connections between populations. Currently several *Stenopus hispidus* genetic loci are being assayed to address these questions including the CO1, ITS-1, 12S, and cytb genes.

Parhyale hawaiensis: Early Development and Lineage Analysis

Observations of cell lineages resulting from holoblastic cell cleavage have been made in just a few animals. These observations of the differential movement of cell populations relative to one another have been made utilizing simple visual discrimination techniques as well as by injection of tracers designed to label a specific cell and its resulting progeny. Invariant cell-lineage patterns have been described in the nematode *C. elegans* (Sulston et al., 1981), the ascidian *H. roretzi* (Nishida, 1987), and the annelid *H. triserialis* (Weisblat et al., 1984).

Among the arthropods, most insects examined, such as *Drosophila*, have superficial cleavage early in development and thus appear to lack invariant cell lineages during early development. Early cell fates in *Drosophila* embryogenesis appear to be governed in large part by the regulation of positional information cascades arrayed along the embryonic anterior-posterior and dorsal-ventral axes (summarized in Fig. 6). The presence of identifiable, invariant, cell lineages in insects, such as *Drosophila*, are restricted to specific tissues such as the nervous system later in development.

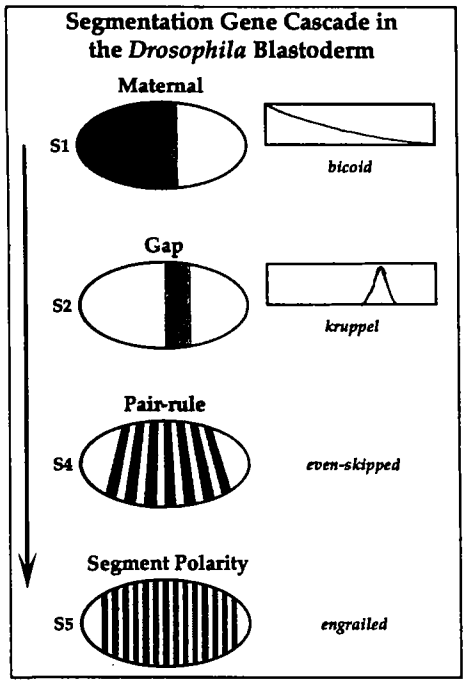

Figure 6. Schematic Representation of the Segmentation Gene Cascade in *Drosophila*. The arrow indicates the time axis. Embryos are oriented anterior to the left and dorsal up. The metameric organization of *Drosophila* is generated via deployment of a hierarchical gene cascade. First the anterior-posterior axis is established by opposing maternal gradients (for example the *bicoid* gene) in stage 1 embryos (S1). The relative concentrations of these maternal gradients (schematized in block form to the right of the embryo) regulate zygotic expression of the next group of genes in the cascade, gap genes (for example *hunchback, kruppel, giant, knirps*, etc.). This group of genes is deployed in stage 2 (S2) embryos. The wide gap gene expression domains are used as cues for the next group of genes to be expressed in the cascade, pair-rule genes (for example *hairy, fushi tarazu, even-skipped, paired, runt*, etc.). This group of genes is deployed in the syncytial blastoderm of stage 4 (S4) embryos. The pair-rule genes pattern the A-P axis with a two-segment periodicity. This two-segment periodicity of gene expression is followed by expression of segment polarity genes (for example *engrailed, gooseberry, patched, wingless*, etc.). This group of genes is deployed in the cellular blastoderm of stage 5 (S5) embryos. Segment polarity genes define compartment boundaries within each developing parasegment. The parasegment unit serves as the reiterated metamer upon which morphological segments are patterned.

A number of crustaceans have total cleavage in early embryogenesis; however, only a small number of studies have attempted to determine whether invariant cell lineages occur (Bigelow, 1902; Hertzler et al., 1992; Hertzler et al., 1994; Gerberding et al., 2002, Wolff et al., 2002). The unique early blastomere arrangement in amphipods has been well described (Langenbeck, 1898; Weygoldt, 1958; Scholtz, 1990) but only two lineage studies have been completed (Gerberding et al., 2002; Wolff et al, 2002). Historically, the literature has suggested that crustaceans exhibit spiral cleavage (e.g. Shiino, 1957; Anderson, 1969; Anderson, 1973; Nielsen, 2001).

To the contrary, in *Parhyale* we find a very clear radially based early cleavage program. At the eight-cell stage, *Parhyale* embryos have four macromeres and four micromeres and each blastomere lineage is restricted to a single germ layer. There is no obvious resemblance between lineage patterns observed in *Parhyale* and those described

among spiralians, nematodes, and deuterostomes. In addition, the *Parhyale* lineage maps unexpectedly differ substantially from the few partial lineage maps described for most other crustaceans (Gerberding et al., 2002).

Head Gap Gene Ortholog Expression and Function in *Parhyale hawaiensis* Neurogenesis

Disparity of form within the crustacean is intimately associated with the ability of the crustacean nervous system to interface with the local environment, integrate information, and respond to changing conditions. A large body of work exists regarding the neuroanatomy of the Crustacea (e.g. Sandeman et al., 1992; Harzsch et al., 1999; Harzsch, 2001; Harzsch and Glotzner, 2002). In addition, recent comparative studies between crustaceans and insects have suggested both strong similarities and notable differences in neuronal morphology (Whitington et al., 1993; Whitington, 1996). These initial studies have been extended to suggest the homology between a small number of specific neuronal identities by correlating similarities in neuronal morphology with the expression of molecular markers (Duman-Scheel and Patel, 1999).

The crustacean brain possesses a great deal of variation that would seem to correlate with changes in the degree of terrestrialization, dependence on visual stimuli, and feeding habits (Schmitz, 1992; Thompson et al., 1994). Clearly anterior head development is quite different between *Parhyale* and the fly *Drosophila*. Current work exploring the dynamics of gene expression in the head and brain of *Parhyale* (Plate 1, D) seeks to explore the role of these regulatory genes in crustacean brain and nervous system development. The natural outgrowth of this data, in an evolutionary and developmental context, is to look at these patterning mechanisms in different, but related, head/brain and nervous systems. Within the Amphipoda the hyperiids demonstrate dramatic changes in head morphologies (Vinogradov et al., 1996).

Relationships Among and Within the Amphipoda, Pelagic Hyperiids: 'Cracking' the Amiphipod Code

As things stand now the Amphipoda are generally organized into two groups, the largely benthic gammarids (to which *Parhyale hawaiensis* belongs) and the exclusively pelagic hyperiids (it is highly likely that the hyperiids, as currently recognized, are a polyphyletic assemblage). Phylogenetic resolution among the Amphipoda is currently poor. Notably, gammarid and hyperiid amphipods have very sharp differences in the organization of their heads and anterior nervous systems, which are most likely due to constraints imposed by their very different respective life histories. Detailed studies of hyperiids are very few due to their exclusively pelagic life history.

Fairly stable populations of the hyperiid *Glossocephalus milneedwardsi* and its host ctenophore, *Mnemiopsis sp* (Plate I, E) can be found at shallow depths along the submerged ridge (Ctenophore ridge) extending from the southern tip of Manatee Cay (Pelican Cays). This area is somewhat sheltered from open water by Cat Cay to the east; however, a strong upwelling current here brings large numbers of ctenophores to the surface. The vast majority of the ctenophore swarms are composed of *Mnemiopsis*

with some *Beroe*. Approximately 10-15% of the *Mnemiopsis* individuals carry the associated *Glossocephalus*. The availability of large numbers of *Glossocephalus* adults, juveniles, and embryos, with their host *Mnemiopsis* along the shallow ridges in the Pelican Cays represents a unique and rare opportunity to observe hyperiid behavior *in situ*, and to obtain high quality embryonic material for molecular work. The Pelican Cay population of *Glossocephalus* is extremely compelling in this regard and continued work on this species can fill a void in current knowledge regarding hyperiid amphipods.

Glossocephalus appears to have a non-parasitic relationship with *Mnemiopsis*, involved perhaps with cleaning the host surface at regular intervals (observations from both the field and animals observed in holding tanks at Carrie Bow Cay). The adult, juvenile, and embryonic stages of *Glossocephalus* have the same optically transparent properties of the *Mnemiopsis* host. Interestingly, newly fertilized single-celled *Glossocephalus* embryos appear to have a large lipid droplet sequestered within the yolk. Light microscopy examination of the first few cell cleavage events in live embryos allows tracking of the lipid droplet as it is progressively compartmentalized to one side of the embryo (the transparent quality of the embryo precludes direct observation of cell cleavage planes). Later in development the droplet is sequestered in the developing midgut. As the midgut begins digesting remaining yolk reserves, the droplet is observed breaking down in the digestive ceacum and anterior region of the maturing midgut.

Three lines of investigation are currently being undertaken in *Glossocephalus*. As their head morphology is radically different from that of *Parhyale*, I am interested in embryonic patterning events during early head ectoderm development and brain development that differ between the two species. Formal lineage analysis in *Glossocephalus* will provide an important contrast with that of *Parhyale* (Gerberding et al., 2002) and *Orchestia* (Wolff et al, 2002) regarding the evolution of the invariant cell lineage observed in these two species of gammarid amphipods. Finally the hyperiid amphipod life history and behavioral aspects of host interaction make the population of *Glossocephalus* found near Carrie Bow Cay an important study group, particularly since there is a marked paucity of data in the literature on this group. A number of modifications to various limb appendages appear to directly support their interactions with host ctenophores. Additional behavioral documentation along with morphological analysis of appendage morphology should shed light on the host/symbiont relationship.

CONCLUDING REMARKS

The intense examination of laboratory strains of *Parhyale hawaiensis* in combination with comparative studies will yield important clues in the search for mechanisms by which genes influence organismal development and sculpt morphology. Ongoing comparative investigations of crustacean brain/nervous system development, appendage development, cell lineage analysis, and population structure in related taxa will provide invaluable information regarding how these patterning mechanisms change through time. The unique mangrove Cay/barrier reef environment near Carrie Bow Cay, in combination with the field station facilities, provides easy access to a number of crustacean species important to these comparative investigations.

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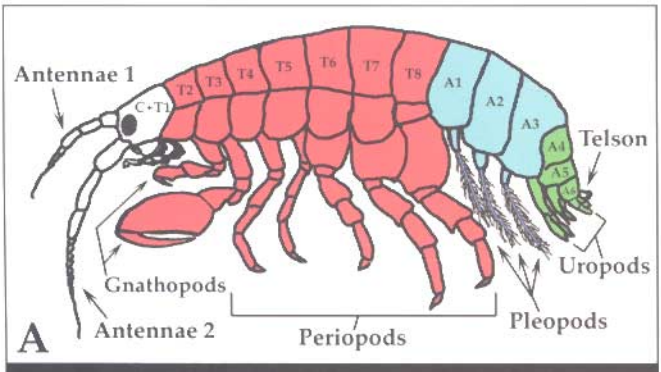
PLATE I

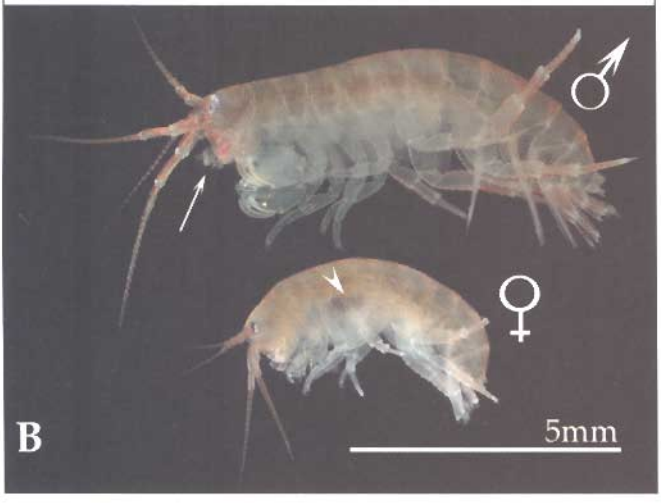
A-B. The *Parhyale hawaiensis* body plan.
(A) Schematic of adult body plan. The cephalon (head) is in white and consists of the first six segments plus the first segment of the pleon (thoracomere 1). All segments from the second cephalic segment posterior bear a pair of appendages. For the cephalon these appendages from anterior to posterior are; antennae 1 (an1), antennae 2 (an2), mandibles (mn), first maxillaries (mx1), second maxillaries (mx2), and the maxillipeds of thoracomere 1 (t1). The pereon, composed of thoracomeres 2-8 (t2-t8), is coded red. Each thoracomere of the pereon possesses paired appendages. The proximal most element of each appendage, the coxa, has a dorsal branch which is compressed and expanded into a structure called the coxal plate which closely follows the margin of its associated thoracomere body wall. The appendages of thoracomeres 2 and 3 are distinctly subchelate in form and termed gnathopods. Thoracomeres 4-8 possess appendages termed periopods. The first two pairs of periopods are oriented anteriorly whereas periopods on thoracomeres 5-8 are oriented posteriorly. The first three segments of the abdomen (a1-a3) are grouped into the pleon. Each bears a pair of appendages termed pleopods. The final three segments of the abdomen (a4-a6) are grouped into the urosome. Each urosome segment bears a pair of uropods. The animal terminates along its anterior-posterior axis with a telson, which is a cleft flap of cuticle posterior and dorsal of the anus. (B) Sexually mature animals possess a number of dimorphic characters. Males are larger than females. The second pair of gnathopods (t3) is enlarged in males. Females possess a ventral brood pouch in which they incubate eggs until hatching (arrowhead). All amphipods retain a highly compressed arrangement of mouthparts into a compact basket termed the buccal mass (arrow).

C. The coral banded shrimp, *Stenopus hispidus*.
Stenopus hispidus typically occupies obstructed overhang habitats such as mangrove prop root junctions and spaces between and under plate corals. The figure shows the typical upside down posture. This individual is a mature female. The yellow arrow indicates developing embryos held ventrally by the swimmerets. The turquoise arrowhead indicates the dorsal position of the ovaries, in this case full of developing oocytes.

D. Expression of *Ph otd1* in *Parhyale hawaiensis*.
Anterior is up, blue staining is the fluorescent marker DAPI and indicates the position of each cell nucleus, red staining is digoxogenin labeled probe to *Ph otd1* mRNA and indicates cells expressing the *Ph otd1* gene. This particular embryo is in the germband stage of development. During this stage of embryonic development in *Parhyale* anterior *Ph otd1* expression has resolved into two ectodermal bilateral clusters that will become the future anteriormost brain neuromere, the protocerebrum. The single, more posterior and medial, column of *Ph otd1* expressing cells mark cells fated to become the ventral midline.

E. The hyperiid amphipod, *Glossocephalus milneedwardsi* and host ctenophore, *Mnemiopsis*.
Animals in this photo are in holding tanks at Carrie Bow Cay. *Glossocephalus milneedwardsi* is an exclusively pelagic amphipod that is known to associate with the ctenophore *Mnemiopsis*. The photo shows the typical types of positions *Glossocephalus* occupies on the outer surface of the ctenophore host. Red arrows indicate male *Glossocephalus*. The lower male is in a 'cleaning' position with the ventral aspect of the head in close proximity to the host. The white arrows indicate female *Glossocephalus*. Embryos in the ventral brood pouch are visible as opaque, white light scatter in this photo.


Antennae 1
C+T1
T2
T3
T4
T5
T6
T7
T8
A1
A2
A3
A4
A5
A6
Telson
Uropods
Pleopods
Gnathopods
Antennae 2
Periopods
A


♂
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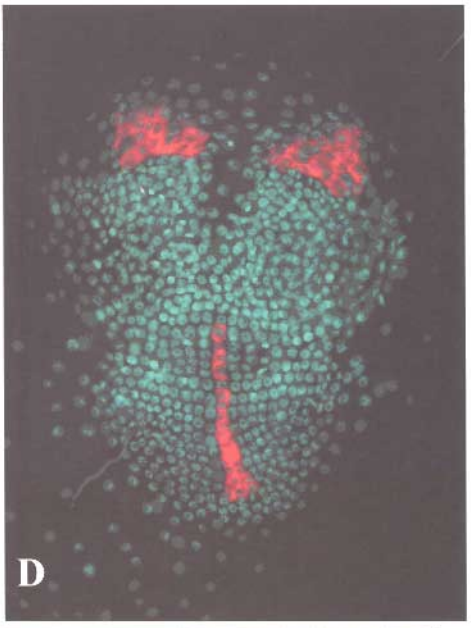
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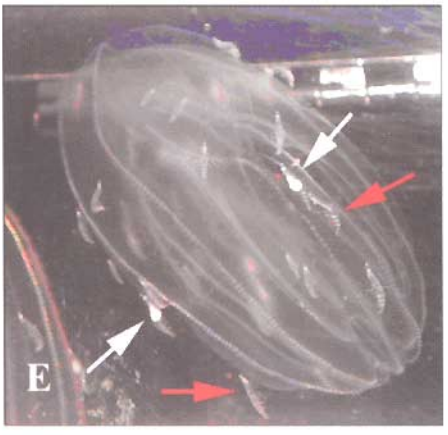
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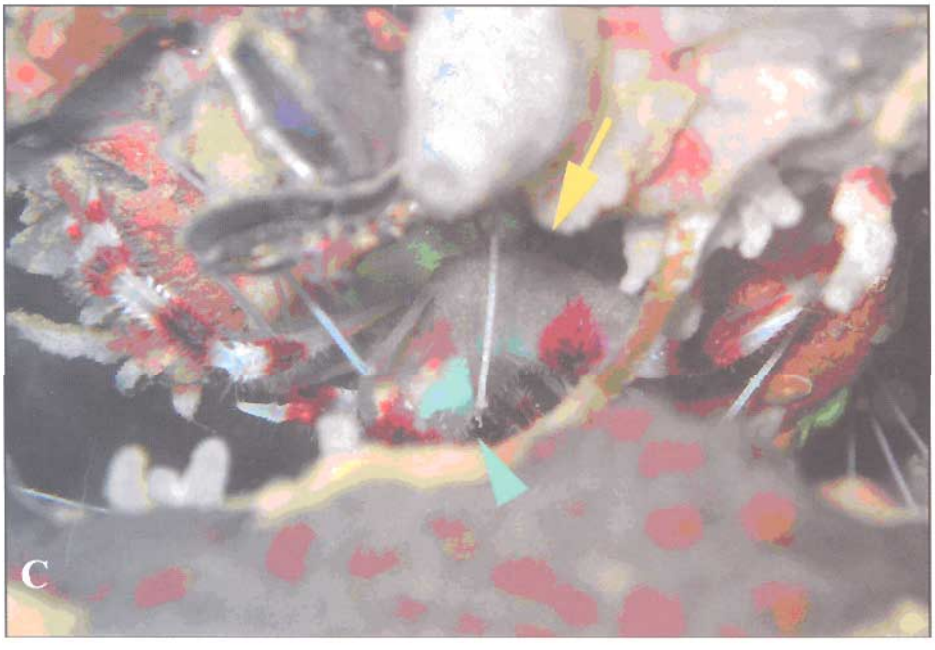
C

PLATE 1