COMMENTS ON GNATHIID ISOPODS

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A favorite Gary Larsen cartoon shows an undersea view of a number of jellyfish floating over two outhouses, one labeled "Boys", one "Girls". The caption reads "Only they know for sure". Sex discrimination in many dioecious species is difficult or impossible without microscopic examination of gametes. Wouldn't it be nice if males and females clearly differed externally and macroscopically? That is the case in most crustaceans, and the old adage "Be careful what you ask for, you just might get it" applies. The result is yet another kind of problem; connecting males and females when they don't look alike. Among the clearest examples of this are the gnathiid isopods. Connecting the two sexes of a species of gnathiid is a problem often considered daunting by even the most observant taxonomists.

Many authors admit their inability to distinguish any specimens but adult males. Thus Müller 1988 states "No notes on females and praniza-larvae are included in this paper because no reliable characters could be found for their identification to species level." Similarly Cohen and Poore (1994) in their family level review state "Only males were described because of the difficulty of identifying praniza stages and females. In the species-rich environment of south-eastern Australia, dredge samples with more than one species of gnathiid were common, therefore association of females and pranizas with males was not considered a sufficient criterion for identification. No obvious characters were found that enabled females or pranizas to be accurately identified to species and this problem was not explored. Identification of females and pranizas to the species level would be a major project and was not attempted here." The authors explicitly acknowledge that co-occurrence is the main tool which has been used to associate male and other forms at the species level. In Monod (1926), the initial broad-scale monograph on the family, many species have descriptions of both males and females, and some also pranizas. Monod does not state how between-sex connections were established, and we are left to conclude that they are based on sample co-occurence.

The Immediate Problem

Identification of gnathiid isopods in Southern California Bight monitoring efforts has presented problems in data analysis. Different agencies pursue different reporting strategies with their data. In most cases only adult male gnathiids are identified to species, while females and juveniles are left at genus or family level. In other cases, females and juveniles are identified to species at least some of the time. Consequently data gathered by different groups is not fully comparable in a united analysis. This situation has been exacerbated by presence of two gnathiid genera in our waters. The generic key currently available applies only to adult males. Thus most individuals are not separable to genus unless their specific identity is known. Identifications of females and larvae must either be dropped, or placed on a firmer footing before this situation can be improved.

Co-occurence has been used by some agencies to associate males and females. In CSDLAC data, where 30 years of monitoring at the same sites yielded males of only *Caecognathia crenulatifrons*, females and pranizas have also been identified as this species. Other agencies report a broader spectrum of males, and are faced with the uncertainty described by Cohen & Poore. There are eight described species of gnathiids in the Southern California Bight: *Caecognathia crenulatifrons*, *C. sanctaecrucis*, *Gnathia clementensis*, *G. coronadoensis*, *G. productitridens*, *G. steveni*, *G. tridens*, and *G. trilobata* (Wetzer & Brusca 1997). We will demonstrate that characters allowing connection of the various forms of a species on bases other than co-occurrence can be found. This is of interest as there are three distinct morphologies for each gnathiid species; larval [both zuphea and praniza], adult female, and adult male. (Figure 1).

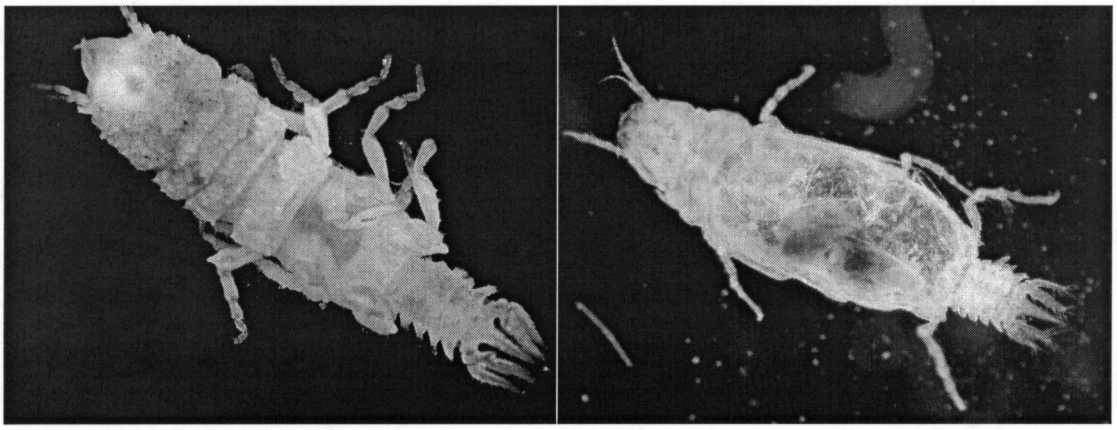

Figure 1 (a) Adult male and (b) adult female morph of *Gnathia* sp CS1

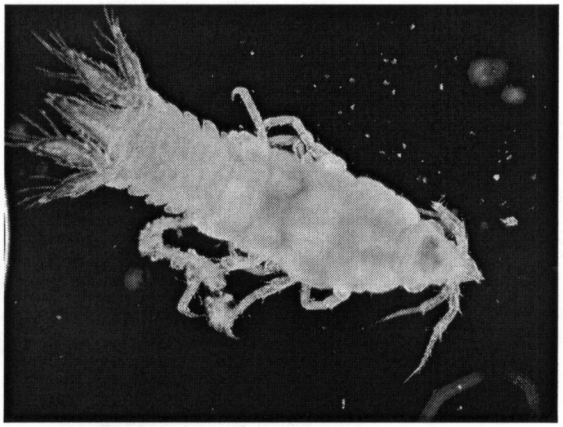

Figure 1(c) Zuphea of *Gnathia* sp CS1

Three Morphs = One Species

The three forms in each gnathiid species are directly related to parasitism in the larvae, and sexual dimorphism in the non-feeding adults. Gnathiid larvae parasitize fish both externally and within the oral and opercular cavities (Monod 1926). Distinctions between

zuphea [pre-feeding] and praniza [post-feeding] larvae are functional rather than morphological. There are apparently three moult cycles for the larvae, each consisting of a zuphea and a praniza (Tanaka & Aoki 1998, Upton 1987). As praniza finish feeding on the host fish they drop off and moult into a larger zuphea, beginning the next larval cycle. Morphology remains the same, with moult cycle membership indicated solely by size (Figure 2). After the third cycle is complete the praniza moult into one of the adult forms. Upton (1987) indicates an additional moult between 3rd cycle praniza and adult male in *Paragnathia formica*. This free living transitional moult may also occur in other gnathiid genera. Sex is evident by the last larval moult cycle, so both juvenile male and female third cycle praniza can be distinguished based on gonadal development.

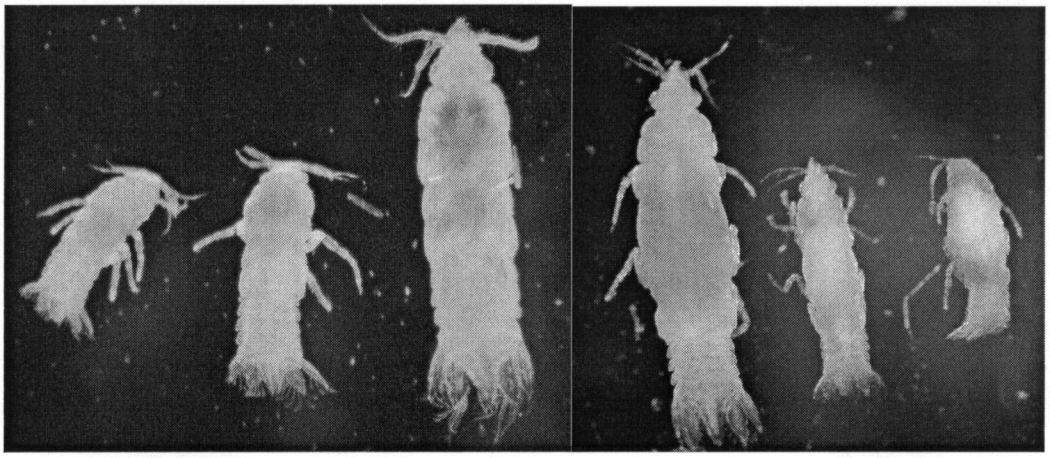

Figure 2 Zupheas cycles 1, 2, 3, and pranizas cycles 3, 2, 1 of *Gnathia* sp CS1

Larvae (both zupheas and pranizas) resemble the adult female in having small heads. The relatively compact pereonites of the larvae also become large membranous coverings for the brood in the adult female. Female adults are hardly more than a brood sack. Once the brood is released, females survive for just a day or so (pers. comm. R. Brusca to L. Haney), with the musculature of the abdomen the only evidence they are not shed moults. As the larvae reach their largest, the resemblance to adult females becomes closer. They can still be easily separated by the presence of styliform mouthparts, which are lacking in both adult females and adult males.

Adult males are, like adult females, non-feeding. They bear large mandibles, ostensibly for use in competition with other males for territory or mates, and resemble soldier ants. Harem formation and territory maintenance has been demonstrated for some gnathiids (e.g. *Paragnathia formica*; Upton 1987), and is suspected for most. The large mandibles and robust heads of males clearly separate them from females and immature forms.

Larvae and Females in Previous Literature

Praniza and zuphea larvae are not illustrated for any of the California species. Females have been partially illustrated for *C. crenulatifrons* (Monod, 1926 – head and urosome; Menzies and Barnard, 1959 – head only) and *G. tridens* (Menzies and Barnard, 1959 head only; Wetzer and Brusca 1997 whole body). The question of how the males and females were connected is left unanswered by these authors. Published illustrations of *Gnathia tridens* females differ in the character of the frons, which may indicate that some specimen attributions are incorrect.

Menzies and Barnard (1959) illustrated a female with a centrally concave frons in the original description. They did not indicate if the illustrated specimen was part of the type lot, or even from the same station. Wetzer and Brusca (1997) illustrate a female *G. tridens* paratype with a rounded frons which differs little from that figured both by Monod (1926) and by Menzies and Barnard (1959) for female *C. crenulatifrons*. This specimen may have been inappropriately assigned to *G. tridens* by Menzies and Barnard. Further study is needed to clarify the correct frons structure for female *G. tridens*. Connections between male and female in this species must be placed on a firmer basis than co-occurrence.

Potentially Useful Characters

Neutral characters, unrelated to secondary sexually differences, are needed to connect specimens of the two sexes and juveniles as members of the same species. In *Gnathia tessieri* (Cals 1972) males and females have similar setal types on their pereopods. Such setal characters may prove of value in other gnathiid species as well. While characteristics of the frons will differ in males and females of a given species, they should be useful in separating females of several species within a sample. Pigmentation, abdominal epimeres, telson shape, uropod setation and tuberculation, relative intensity of body setation, and general body proportions are also of potential use, both for separating females and recognizing common morphology between morphs within a species. Some of these proved of value in separating the specimens from our multiple species sample from the San Pedro Sea-shelf. Unlike Cohen & Poore (1994) we view samples with multiple species as an opportunity rather than as a problem. If both sexes of multiple species are present, a sample can serve as a "Rosetta Stone" assisting recognition of subtle morphological characters.

Our Rosetta Stone Sample

We recently recovered 16 gnathiid specimens representing two species, *Caecognathia crenulatifrons* and *C. sanctaecrucis* from 87m depth on the San Pedro Seashelf. Note *C. sanctaecrucis* replaces the preoccupied name *Gnathia hirsuta* Schultz, 1966. This collection contained adults of both sexes, as well as four praniza.

Four females were collected with six male *C. crenulatifrons* and two male *C. sanctaecrucis* (Figure 3). All the females were spent, having recently released their

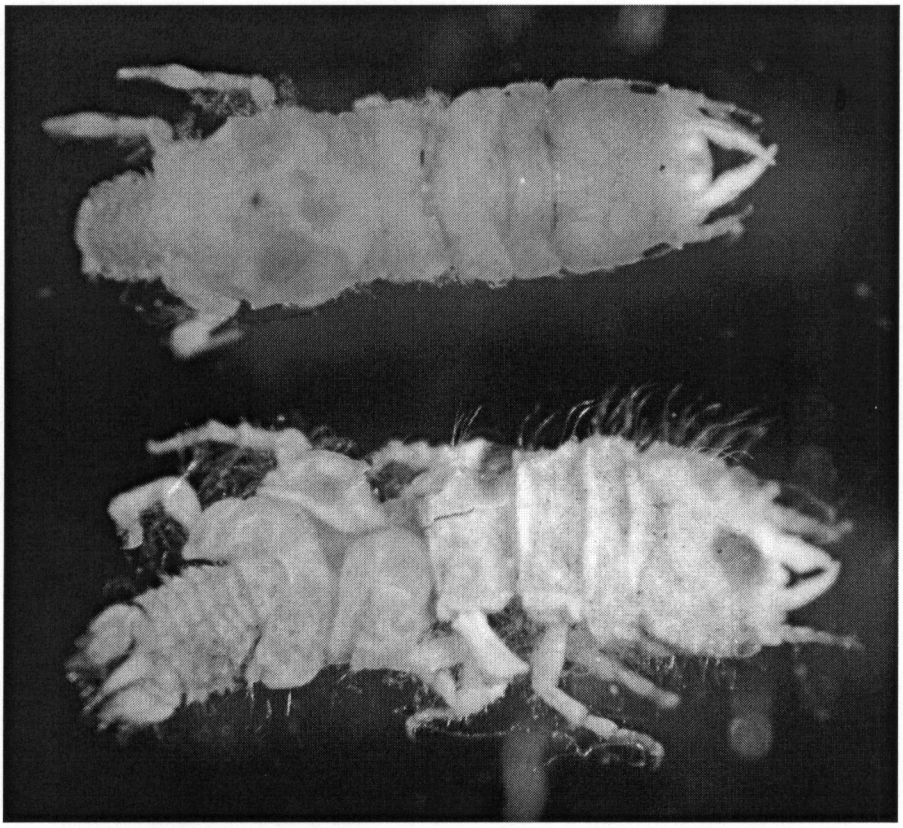

Figure 3 *Caecognathia* males: *crenulatifrons* (above) and *sanctaecrucis*

brood. Superficially all four females appeared similar. Closer scrutiny allowed us to distinguish them based on color pattern of the posterior part of the body, and one as a bit stockier than the other three. The cephalon of this female had a bisinuate rather than a bluntly rounded anterior margin. A small granulose tubercle was also observed between her eye and the base of her antennae. She had long scattered setae on her cephalon and along her abdomen margins. Lastly her abdominal epimera were truncate and not posterio-dorsally subacute (Figure 4).

Great!!! Two different females...so who goes with who? This question proved easier to solve than we had anticipated. Body form was the initial clue; *C. crenulatifrons* is not as broad as *C. sanctaecrucis*. We hypothesized that the broader, stockier males were associated with the stocky female, then looked for other characters to support this association. Male *C. sanctaecrucis* are very hirsute, with many long simple setae all over the body. While the setose female had far fewer setae, their presence supported relationship with our hirsute males (*C. crenulatifrons* has almost no setae in either sex).

The *C. sanctaecrucis* males and female also had laterally truncate rather than backswept abdominal epimera (as in *C. crenulatifrons*), although those of the male were in double pairs on each segment and the female's were single.

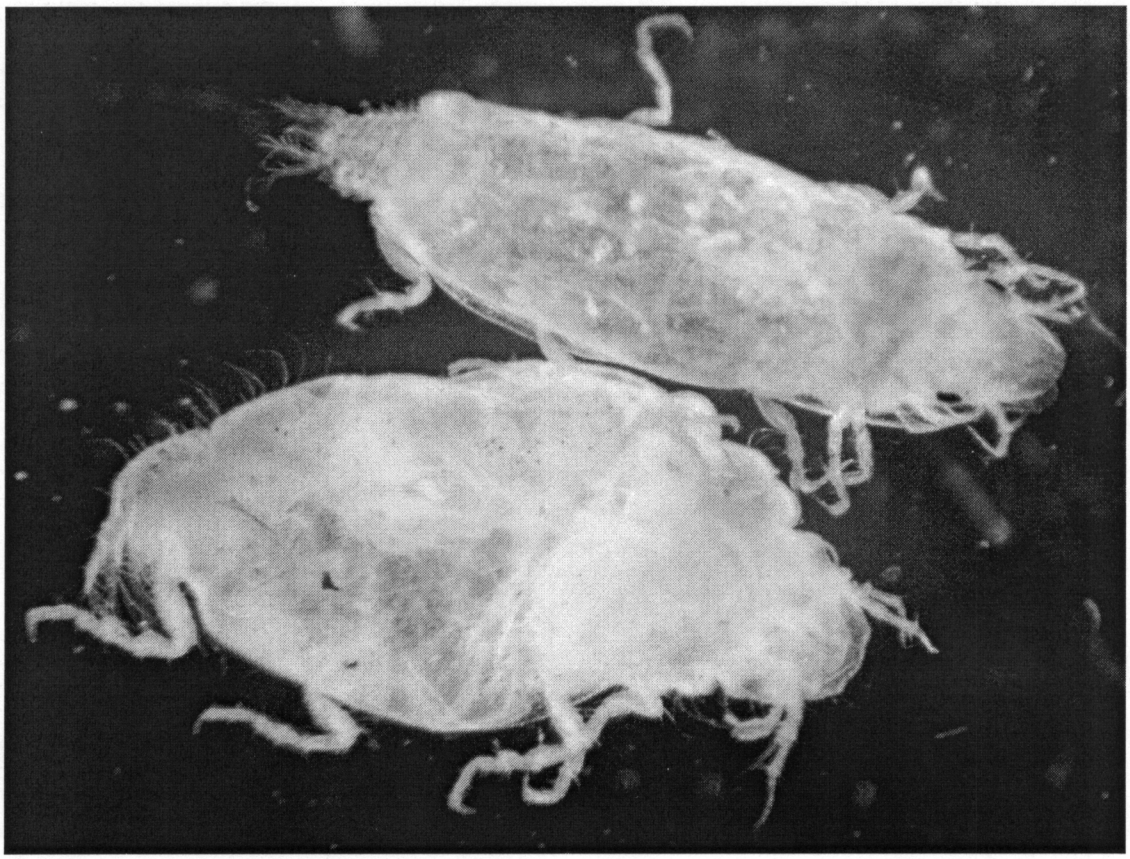

Figure 4 *Caecognathia* females: *crenulatifrons* (above) and *sanctaecrucis*

Presence of a granulose knob in front of the eye was the final connection. This was smaller in our stocky female than in either of the two *C. sanctaecrucis* males, but was lacking in both male *C. crenulatifrons*, and in the second female form (Figure 5).

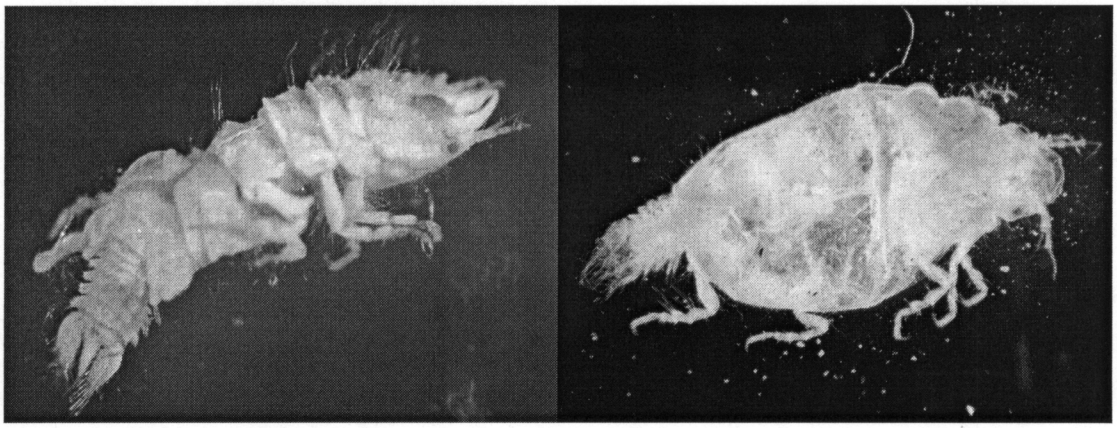

Figure 5 *Caecognathia sanctaecrucis* male and female

OK, so we seem to have been able to match one female with two males of *C. sanctaecrucis*. That left four pranizas, three females and 6 male *C. crenulatifrons*. Were they all *C. crenulatifrons*? We concluded that they were based on a common

pigmentation pattern of the abdomen. Males of *C. crenulatifrons* have small speckles of reddish brown pigment expressed as two broad bands. These bands extend along the long axis from the thorax to the posterior portion of the abdomen. A similar pattern was seen in the three females and four pranizas. Additionally, in these females and pranizas abdominal epimera are backswept posterio-distally into blunt points, a pattern like that of *C. crenulatifrons* males (Figure 6).

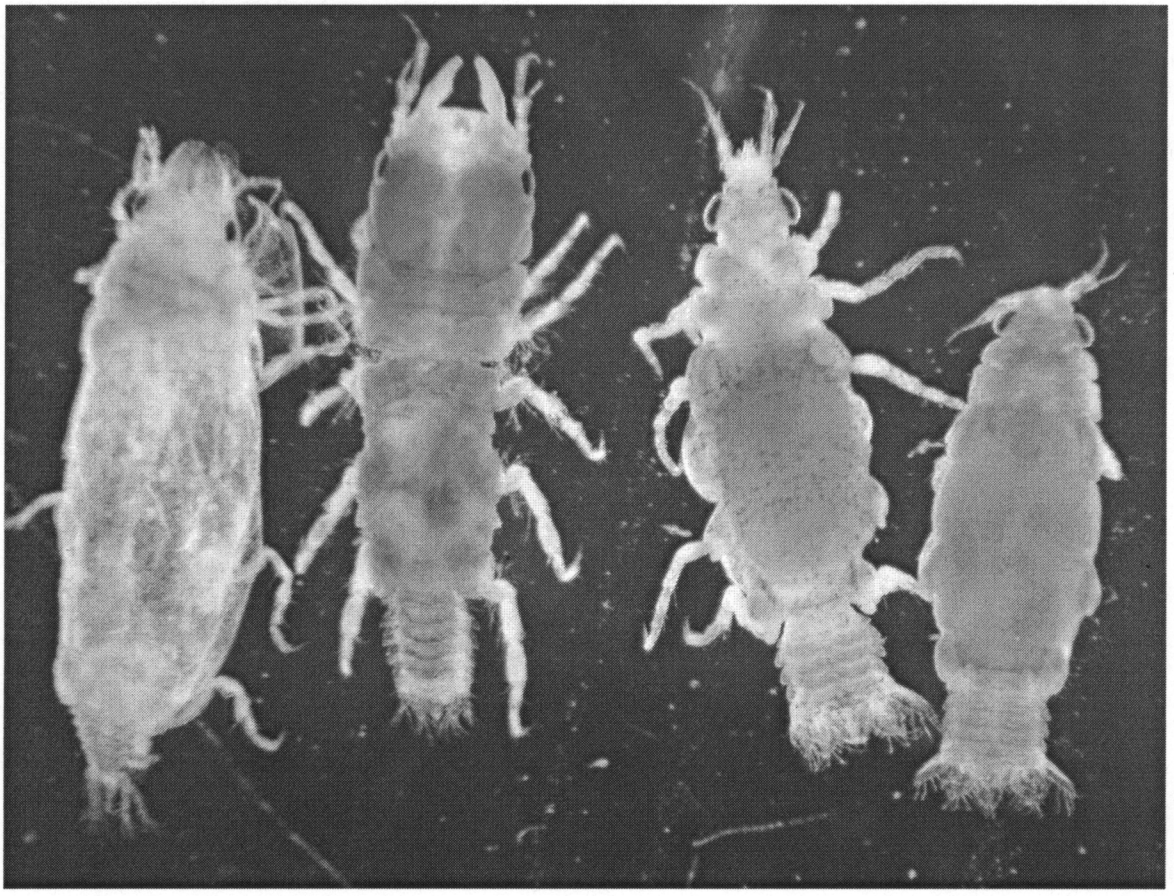

Figure 6 *Caecognathia crenulatifrons* female, male, and two cycle 3 praniza larvae

Other C. sanctaecrucis Material

While our material matched the description of male *C. sanctaecrusis*, we thought it wise to compare with other specimens. Lisa Haney examined the *C. sanctaecrucis* type specimen at the Natural History Museum of Los Angeles County (the *C. crenulatifrons* type material is located elsewhere). The cephalon and telson of the male holotype were no longer associated with the specimen, are missing, and could not be reevaluated. She also examined non-type *C. sanctaecrucis* specimens collected during the MMS Santa Marina Basin study. Both mature males and females, collected in samples of rock retrieved during submersible dives, were consistent with the males and females from our San Pedro Sea-shelf sample.

Non-morphological Methods

By far the most convincing method of relating the adult males with females of a species is by rearing and observing metamorphosis. Smit *et al.* (2002) reared pranizas and observed developing adult males and female morphologies. They also verified conspecificity by breeding the resultant adults to produce viable offspring. Grutter *et al.* (2000) demonstrated relatedness of juveniles and adults by rearing and subsequent DNA analysis. They are secondarily determining morphological characters which should allow identification of all life stages to species. To date this work remains in progress and unpublished. Unfortunately, these techniques have not been applied to species in Southern California. In cases where gnathiids occur within direct view (intertidal or in diving depths), females can be behaviorally connected with the male who is guarding them if the harem is intact and not disturbed prior to examination.

Hope for California Gnathia?

Despite the absence of definitive rearing or DNA based information, our observations demonstrate that differentiation of females and juveniles based on morphology is possible. More careful examination may allow future development of keys and other aids to identification of all life stages in the local species of *Gnathia*. We think our two *Caecognathia* species can be reliably identified using the characters we discuss, but the praniza of *C. sanctaecrucis* remains unknown. It is likely that mixed species catches will allow identification of character states connecting males, females and juveniles of other southern California gnathiid species.

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