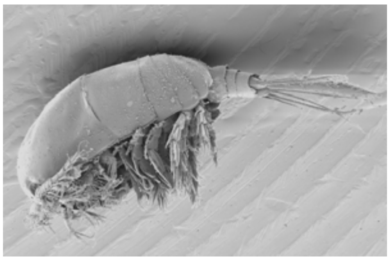
SHORT MOBILITY REPORT



OCCURRENCE OF *PSEUDOCYCLOPS XIPHOPHORUS* DIFFERENT MORPHOTYPES IN LAKE FARO (NORTH-EASTERN SICILY, MEDITERRANEAN SEA): PHYLOGENETIC, TAXONOMIC AND BIOLOGICAL IMPLICATIONS.

Dr. Cinzia Brugnano

Tutor:
Prof. Geoff Boxshall

Il proponente
Dr. Pasquale Trotta

Il fruitore
Dr. Cinzia Brugnano

INTRODUCTION

Hyperbenthic calanoid copepods represent a particular ecological group of Copepoda Calanoida that are of interest for a variety of ecological, biogeographic and taxonomic reasons. Despite the small number of species and the low abundance, this ecological group plays an important role since it increases the functional diversity of marine and brackish coastal ecosystems.

From a biogeographic point of view, the hyperbenthic Copepoda Calanoida recorded in the Mediterranean provide an important contribution to knowledge of the origin of the Mediterranean fauna and of the relationships of the Mediterranean basin with the Atlantic and Indo-Pacific Oceans over geological time frames. From the taxonomic point of view, the hyperbenthic Copepoda include the most ancestral families of the order Calanoida, such as Pseudocyclopidae and Ridgewayiidae, or little known ones like Stephidae and Arietellidae. The observation of new hyperbenthic species and their developmental stages (obtained in the laboratory) provides an important contribution to knowledge of the evolution of copepods.

Pseudocyclops xiphophorus, originally found along the coast of Inhaca Island (Monzambique, Indian Ocean) by Wells, (1967), was reported in Lake Faro for the first time in Mediterranean Sea and redescribed by Zagami *et al.* (2005). In Lake Faro, this species was found in fouling attached to submerged ropes and mooring posts. *P. xiphophorus* was included by Ohtsuka *et al.* (1999) in a species group, referred to as the *magnus*-group. The *magnus*-group shares an important synapomorphy: seta V on the caudal ramus is spatulate in the female (only in *P. magnus*, *P. xiphophorus* and *P. bilobatus*). Within the *P. xiphophorus* population in Lake Faro, there are females and males in which caudal seta V is spatulate, but in other specimens this seta is thin. Occasionally a specimen may be asymmetrical, with a spatulate seta on one side and an unmodified seta on the other. In addition to this variation in caudal seta form, the Lake Faro population displays variation in other characters:

- male body total length greater than female;
- larger males show sexual dimorphism on the right leg 2:
    1. the outer spine of the second exopodal segment and first spine of the third exopodal segment form a pincer shape.
    2. the spine of the second exopodal segment carries a row of denticles on the inferior margin, and first spine of the third segment upwards.

Adopting a traditional taxonomic point of view, the scale of these differences between morphotypes might lead to their treatment as different species, but interbreeding experiments were performed in the laboratory between females and males showing different combination of these characters.

AIMS

Morphological observations alone cannot always be sufficient to characterise very similar species belonging to the same genus. Therefore, morphological methods would have to be supplemented by fecundity experiments in the laboratory, and by the adoption of bio-molecular techniques. A more comprehensive species diagnosis could obtained from comparison between these methods.

The aim of this project is to confirm morphological observations on the morphotypes found in Lake Faro, using differential interference contrast light microscopy (DIC) and scanning electron microscopy (SEM), and to improve techniques of preservation and inclusion techniques, dissection methods and, if possible new drawing instruments and software will also be explored.

MATERIAL AND METHODS

Fouling samples were collected by hand. In order to limit escape of organisms, submerged ropes to which fouling was attached, were placed directly in a plastic container with environmental water and whole ropes were subsequently cut.

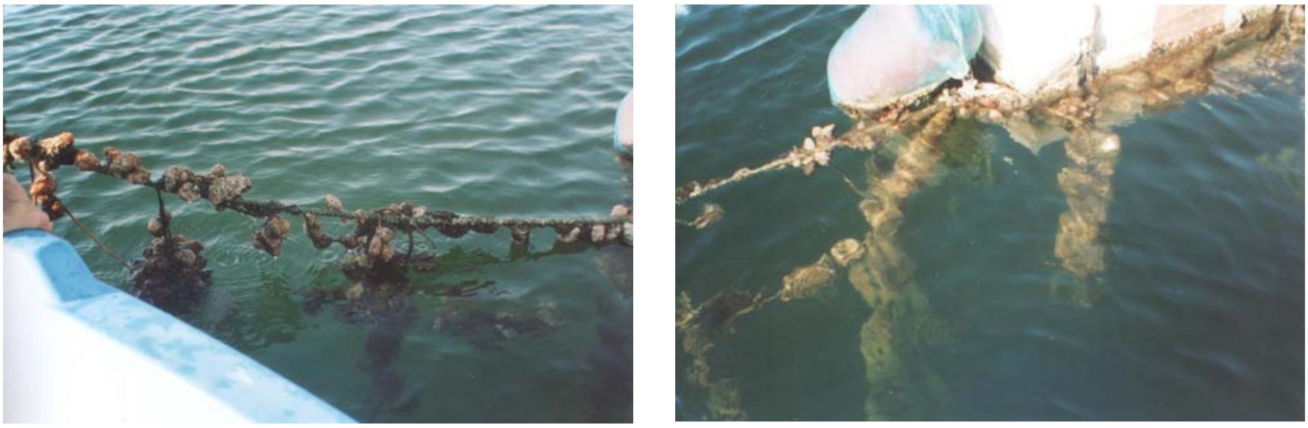


Fig. 1. Fouling samples.

Fouling samples (fig. 1) were immediately carried to the laboratory. Live *P. xiphophorus* specimens were counted and sorted from fouling washing-water under a stereo-microscope and transferred to an aquarium containing 100 ml of filtered sea water. In the aquarium, specimens of *P. xiphophorus* were reared on a mixed diet of three phytoplankton species: *Tetraselmis suecica*, *Pavlova lutheri* and *Isochrysis galbana*. Egg production was monitored daily for each pair of males and females placed individually into 50 ml crystallizing dishes containing filtered sea water enriched with the same diet given in excess concentrations. After death of the female, each couple was preserved in 4% formaldehyde. Egg production and the life cycle of *P. xiphophorus* were studied under different temperature conditions by Brugnano *et al*. (2006).

Experimental crosses were carried out between:

♀ (with seta V spatulate) and ♂ (with P2 not modified, and seta V thin);

♀ (with seta V thin) and greater ♂ (with P2 modified, and seta V spatulate);

♀ (with seta V thin) and ♂ (with P2 not modified, and seta V spatulate);

♀ (with seta V spatulate) and greater ♂ (with P2 modified, and seta V thin);

About twenty males of the pairs breeding in the laboratory and preserved in a 4% buffered formaldehyde and sea water solution, were measured under light microscopy. Some of them were dissected to observe legs 2 and 5. Five modified and four unmodified males were processed for SEM.

STUDY AREA

Fig. 2. Study Area

Lake Faro (surface area 263,600 m^2, max depth 28 m) is a coastal lagoon located on the north-eastern tip of Sicily (fig. 2). It has typical features of a meromictic basin, i.e. an oxic epilimnion and an anoxic hypolimnion, characterized by large fluctuation in physico-chemical parameters, especially temperature (10-28 °C), salinity (34-37 PSU) and dissolved oxygen (ranging from absent, near the bottom in the central area, to 8.3 mg/l in surface along the shore). These layers are separated by a metalimnion, within which strong blooms of anoxygenic phototrophic bacteria cause the periodic development of a red water layer (Genovese, 1963; Truper & Genovese, 1968).

MORPHOLOGICAL RESULTS

Body length of male *P. xiphophorus* with modified leg 2 ranged from 0.634 to 0.652mm, with a mean of 0.639mm, compared with the morphotype with an unmodified leg 2 with a range from 0.580 to 0.616 mm and a mean of 0.598mm. The abdomen, antennules and mouthparts are as in Zagami et al. (2005). In both morphotypes, the caudal ramus bears six setae, with seta II spiniform and flattened with a terminal flagellum; seta V is the longest and typically has a sword-shaped structure. Caudal seta V showed variations in shape both in females and males: It was spatulate (fig. 3) in the type material (Wells, 1967) and in Zagami's redescription. However, here we report the presence of both males and females which possess thin, unmodified caudal seta V (fig. 4a;b).

Fig. 3. *P. xiphophorus* female with spatulate caudal setae V.

Fig.4. *P. xiphophorus* female (a) and male (b) with thin caudal seta V.

Thin or spatulate caudal seta V is thus, a variable character in Lake Faro population. Rarely, both setal forms co-occurred in the same specimen (fig. 5): with one caudal ramus carrying a spatulate seta V and the other one carrying a thin seta V.

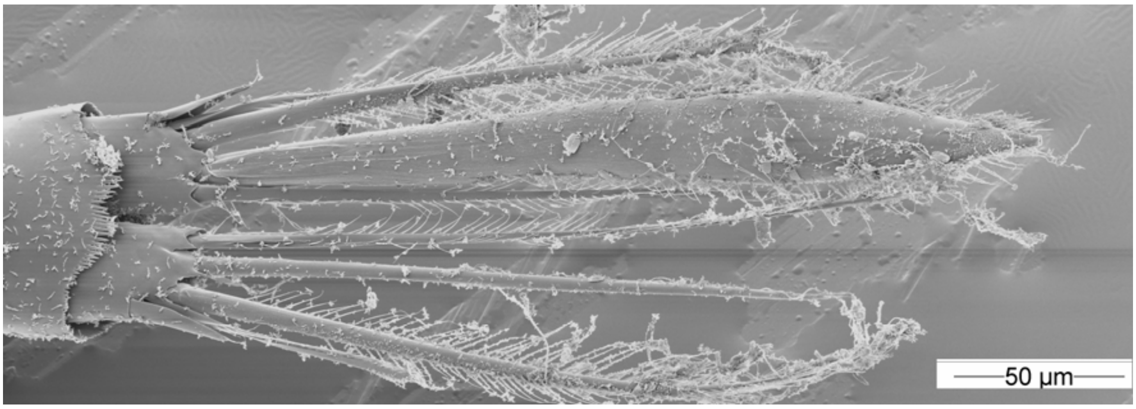


Fig. 5. Thin and spatulate setae on each caudal ramus in the same female.

The swimming leg formula is identical in the two morphotypes and as follows :

	Coxa	Basis	Exopod segment	Endopod segment
Leg 1	0-1	0-0	I-1; I-1; II, I, 4	0-1; 0-2; 1, 2, 3
Leg 2	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 4
Leg 3	0-1	I-0	I-1; I-1; III, I, 5	0-1; 0-2; 2, 2, 4
Leg 4	0-1	1-0	I-1; I-1; III, I, 5	0-1; 0-2; 2, 2, 3

However, leg 2 showed great variability in the extent of the modifications on the second and third exopodal segments and their outer spines. We compared male right leg 2 of *P. xiphophorus* male paratype (fig 6A), of the two morphotypes from Lake Faro (fig 6B), (BMNH Reg. N° 284), and of a *P. xiphophorus* specimen collected from Hong Kong (fig 6C),(BMNH Reg. N° 1989.136).

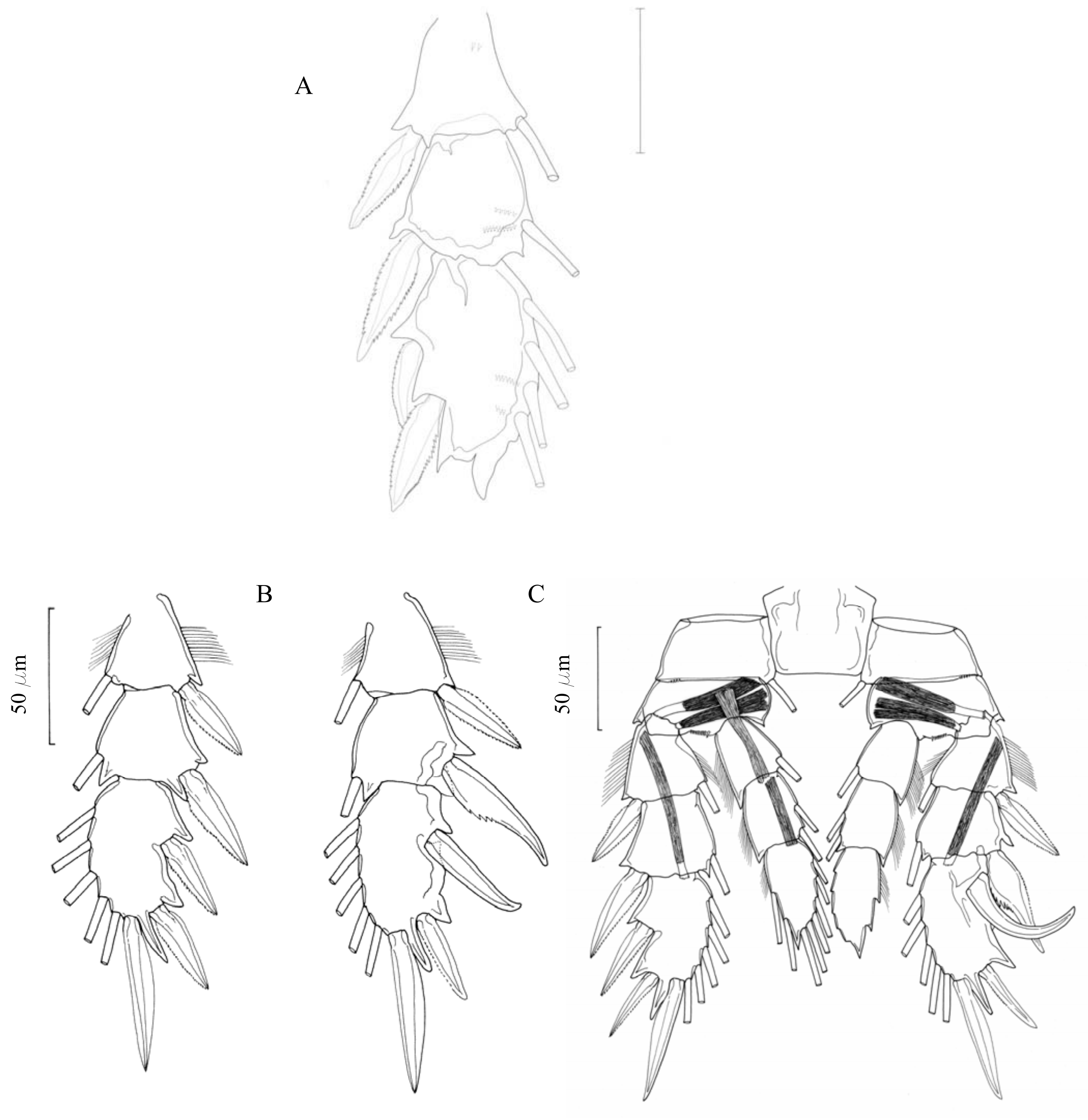


Fig. 6. *P. xiphophorus* male leg 2: A) Wells' paratype: anterior view; B) unmodified type and slightly modified: posterior views; C) highly modified: posterior view.

As revealed by SEM, the outer spine on the second exopodal right leg 2 differed in shape from the corresponding spine on the left leg. It is a normal bipinnate spine on the left leg (fig. 7A), whereas the spine on the right leg is larger and has a well developed row of denticles on the inferior margin. The proximal spine on the third exopodal segment of the right leg 2 was also variable, and it was typically larger and curved upward, in modified males (fig. 7B). The outer spine was also positioned more proximally on the segment than the corresponding spine on left side. This strong asymmetry in modified males is unique in the Calanoida.

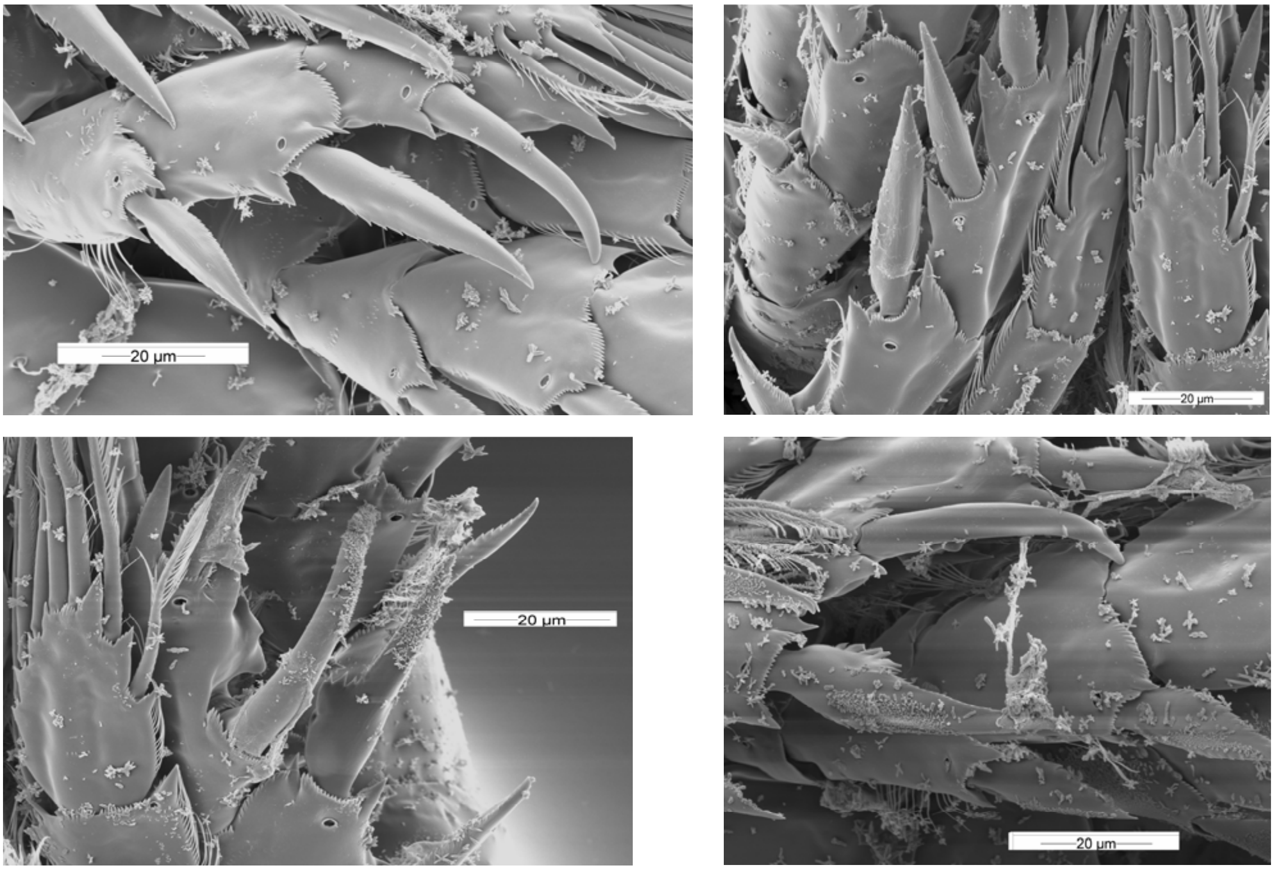


Fig. 7. SEM images. *P. xiphophorus* male leg 2: A) unmodified types; B) modified: different views.

Dissection and electron microscopy revealed no substantial differences on leg 5 between two male morphotypes. Leg 5 (fig. 8) is complex, with the coxa and basis completely fused on the left leg and separated on the right. On the right leg, the basis bears a minute seta laterally on the posterior surface. The right exopod comprises a proximal segment bearing an outer bipinnate spine, and two distal spinous processes which represent the distal exopodal segments. The endopod is elongate naked and has two small lobes. It is ornamented with a small pore. The left leg with a protopod bears an elongate, tapering process on its anterior surface, and this is ornamented with fine setules on its anterior surface (fig. 8). The exopod is indistinctly segmented. The first segment bears one bipinnate spine between two inner and one outer processes. The distal part of the exopod is complex, composed of two laminar hyaline plates that probably represent the second and third exopodal segments. The endopod is one-segmented and naked, but is ornamented with one poredistally.

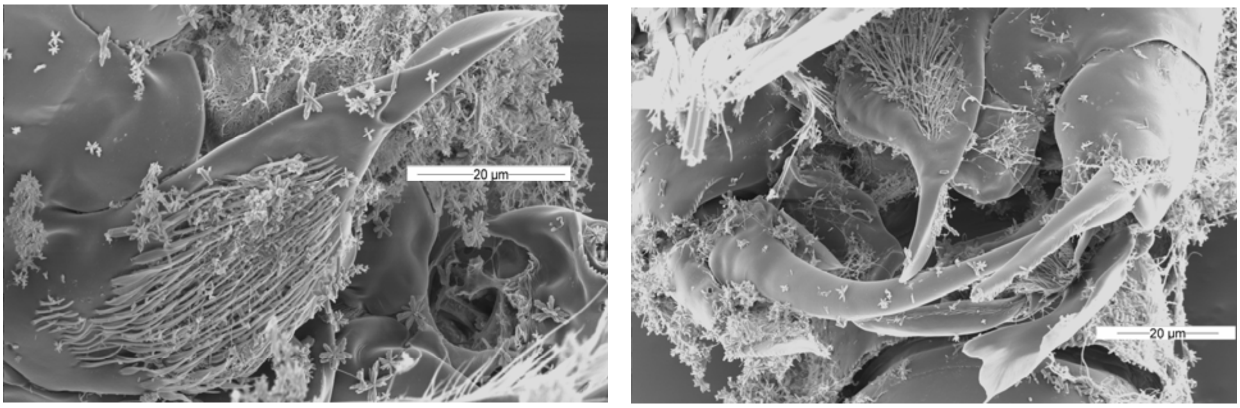


Fig. 8. SEM images of *P. xiphophorus* male leg 5: process on protopod; Leg 5.

INTERBREEDING EXPERIMENTS

The results of these experiments are given in Brugnano et. al., (2005) and demonstrated that these different morphotypes reproduced successfully and that their offspring were able to produce fertile eggs. There are no substantial differences in egg production between two male morphotypes.

In according to the definition of a biological species, these morphotypes should be regarded as the same species.

DISCUSSIONS

The *P. xiphophorus* population in Lake Faro exhibits a great variability in the form of male leg 2 and in the form of the caudal setae in both sexes, but gene flow is not interrupted and reproductive segregation doesn't occur. Many studied reported intraspecific morphological variations on copepods in relation to geographic distribution and environmental plasticity of the species (Elgmork and Halvorsen, 1997) or problems related to morphological stasis due to maintenance of a standard morphology over vast periods of time in populations that are reproductively isolated (Lee and Frost, 2002). But few previous studies have referred to this kind of intraspecific variability. Modifications of caudal setae in the same genus were reported by Sewell (1932), who described a form bearing a modified caudal seta V as *Pseudocyclops obtusatus* var. *latisetosus*. Sewell distinguished it from the type form which had normal, slender caudal setae, in which the 2nd and 3rd setae were longer than the others but not appreciably stouter. In his new variety these two setae are very considerably thickened and flattened; but about half way along their length, the width suddenly decreases. The lateral margins of the proximal portions of the setae are ornamented with numerous small pinnules, while the distal part is plumose. In other planktonic copepod genera, the presence of modified oar-shaped or club-shaped setae on the appendages and caudal rami of *Oncaea* has been reported by Boxshall and Böttger, (1987). They were not only found on *O. atlantica* and *O. platysetosa*, as described by these authors but also on female *O. vodjianitski* that Shmeleva and Dedalo (1965) caught in the same series of samples from Red Sea. The outer seta on the basis of both the first and the second swimming legs was oar-shaped in *O. vodjianitski*. These modified setae were not illustrated by Shmeleva and Delalo (1965) in their original description of this species nor were they found by Krišnić and Malt (1985) in their redescription of Mediterranean material. It is possible that there is intra-specific variation in setal morphology as Nishida (1985) found a modified club-shaped seta on the swimming legs of some specimens of the planktonic cyclopoid *Oithona setigera* (Dana) but a typical tapered seta on others. Modified and transformed into a thickish is also seta V on the caudal ramus of the genus *Paracartia* constituted by the two species *P. grani* (Sars, 1904; Labbé, 1929) and *P. latisetosa* (Giesbrecht, 1892).

Modifications on right leg 2 of males probably represent sexual dimorphism between male and female, but which is its real function is unknown. This kind of modified leg 2 could be implicated in copulation processes, maybe, in cutting and transfer of the spermatophores.

The same variation was found in a *P. xiphophorus* specimen from Hong Kong, but it didn't occur in Wells' paratypes and Zagami et al. (2005) didn't refer to it in their redescription. A similar modification was described by Vervoort, (1964) in the *Pseudocyclops pacificus* male. In this species, it consisted of an external marginal spine greatly developed, curved backward on the third segment of right leg 2. This strong asymmetry in modified males is unique in the Calanoida and it has never considered as sexual dimorphism before (Ohtsuka and Huys, 2001), even if male fifth leg had been the only part implicated in mating and copulation.

No significant differences were found on leg 5 between two *P. xiphophorus* male morphotypes. But a more careful study on this structure allowed us to understand the complex leg 5 of this species and provide a more complete description.

Species criteria are not necessarily mutually exclusive, and alternate criteria typically capture different aspects of the biological information present in a particular data set. We suggest that rather than use a single criterion specified a priori for diagnosing species boundaries, using multiple criteria simultaneously can produce species identifications that are more robust to the assumptions employed by different investigators. Therefore, the next step will be to perform a molecular analysis in order to estimate genetic differences between *P. xiphophorus* morphotypes.

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