Sex with the lights off: Can a morphological structure reveal the sex and functional sexual maturity in the genus *Plesionika*?

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Abstract

The present work describes a taxonomic trait able to determine sex in pandalid shrimps of the genus *Plesionika* and an indicator of functional maturity in females. This structure is described as formed by two teeth with abundant setae present in all males of the nine *Plesionika* species examined. This morphological trait has been ignored across the taxonomy in pandalids. Thoracic teeth were located on the 7th thoracic sternite. Each species presents a characteristic trait of this structure, although *P. williamsi* has a particular pair of thoracic teeth compared with their congeners. This structure could be used as a tool in the systematics of this genus. Sex determination through thoracic teeth shall represent a faster alternative to the classical appendix masculina sexing method. Functional maturity of females is linked to the fading of immature thoracic teeth. The observation of living specimens in tanks has allowed for the finding that males of *P. narval* and *P. edwardsii* actively seek (search for) females and use their third pair of multi-articulate legs to locate the teeth of the thoracic region, which implicates these structures in the activity of "pure searchers" to locate mature females. Thoracic teeth open a new window to different fields of research, such as taxonomy, systematics, and sexual behaviour, and are a tool to determine the first maturity of the *Plesionika* species.

Key words: sexual dimorphism, *Plesionika*, deep-sea pandalids, thoracic teeth, sexual maturity, pure searchers, secondary sexual characters.

1. Introduction

Since the pioneering research of King and Moffit (1984), sex determination in deep-sea pandalids has been based on two types of taxonomic evidence: i) the morphology of the first and second pleopods (with endopods modified according to sex), and ii) the presence of male gonopores located on the coxa of the 5th pereiopod. The transition of individuals from juveniles to adults is a crucial phase of the reproductive process in decapod species, and many of them show morphological changes when reaching sexual maturity (Petriella and Boschi, 1997). Changes in the relative growth of some morphological structures with body growth when the animal reaches the pubertal moult are often used as a tool for estimating sexual maturity in decapods (Hartnoll, 1974). The growth of a morphological structure, such as the appendix masculina, in relation to the appendix interna located on the 2nd pair of pleopods has often been used as a proxy for first maturity in pandalid males (King and Moffit, 1984; Thessalou-Legaki, 1989). On the other hand, the change in relative growth between carapace length and the width of the abdomen is used as a proxy for morphological maturity in *Plesionika* females (Ahamed and Ohtomi, 2014).

Sexual interactions among shrimps may be mediated by combinations of several signals or a single signal (Hughes, 1996). Among caridean shrimps, at least 4 of 11 known species showing a pure-searching mating pattern belong to the family Pandalidae (Correa and Thiel, 2003). In these species, the use of contact pheromones may play a crucial role in mate recognition (Bauer, 2010. The individuals of *Plesionika* species generally live aggregated in groups formed by small males and large females, with a high frequency of contact among them (King and Moffitt, 1984). Females of pure-searching mating patterns “hide” their reproductive condition as much as possible until the moult, perhaps to prevent male harassment before they are receptive (Bauer and Abdalla, 2001; Díaz and Thiel, 2004). In pure-searching mating species, there are no complex behaviours, such as the courtship of receptive females or the presence of aggressive encounters between males (see Correa and Thiel, 2003 and papers cited therein). Therefore, in this context of an aggregated species with a pure-searching male mating strategy, the receptive sexual condition of the female is perceived by males by sex pheromones (Caskey and Bauer, 2005). The real mechanism of reproductive appendages is still poorly known in crustaceans and remains a fertile topic for study

(Bauer, 2013). The exact receptor involved and contact pheromone associated with the social recognition still remains unidentified in caridean shrimps (Chak *et al.*, 2015).

Plesionika Spence Bate, 1888 is the most taxonomic speciose genus within the family Pandalidae, containing 93 species (Cardoso, 2011; De Grave and Fransen, 2011; Li and Chan, 2013; Ahamed *et al.*, 2017). This genus has a widespread distribution around the world in subtropical, tropical and in some temperate waters (Holthuis, 1980; Chace, 1985). The misidentification of *Plesionika* species may occur sometimes (Shanis *et al.*, 2014) due to scarce differences in the current diagnostic morphological characteristics among similar species (Crosnier and Forest, 1973; Chan and Yu, 1990, 2000; Chan and Crosnier, 1997; Li and Chan, 2013). Moreover, molecular systematics of the genus *Plesionika* indicate that although it is genetically distinct, this genus is not monophyletic as it currently is defined, highlighting some discrepancies between their current taxonomy and systematics (da Silva *et al.*, 2013; Chakraborty *et al.*, 2015). In addition, from the point of view of systematic use, the morphology of the pleopods and pereiopods in *Plesionika* species is a useful tool to determine sex and maturity (King and Moffit, 1984; Thessalou-Legaki, 1989; Ahamed and Ohtomi, 2014).

The aim of this study is to describe a morphological structure present in *Plesionika* species that can be used in several ways: i) for sexual assignment in both juveniles and adults, ii) to estimate sexual maturity conditions of females at any time of the year, iii) to estimate the mean size at sexual maturity for females, according to the receptive sexual condition and developmental stage of the ovaries, iv) to contribute in new taxonomic tools to clarify possible future discrepancies between taxonomy and molecular systematics, and v) to study mating interactions and reproductive strategies in aquarium. These goals are relevant because this structure can contribute to improve the biological knowledge of these species, helping to obtain better biological parameters for *Plesionika* shrimps, which constitute a valuable fishing resource on continental/insular shelves and slopes of tropical and subtropical areas (Cartes *et al.*, 1994; Vafidis *et al.*, 2005; Pajuelo *et al.*, 2015; González *et al.*, 2016).

2. Materials and methods

Nine of the thirteen *Plesionika* species occurring in the north-eastern Atlantic waters, i.e., *Plesionika antigai* (Zariquiey Álvarez, 1955), *Plesionika edwardsii* (Brandt, 1851), *Plesionika ensis* (A. Milne-Edwards, 1881), *Plesionika gigliolii* (Senna, 1902), *Plesionika heterocarpus* (A. Costa, 1871), *Plesionika longicauda* (Rathbun, 1901), *Plesionika martia* (A. Milne-Edwards, 1883), *Plesionika narval* (Fabricius, 1787), and *Plesionika williamsi* (Forest, 1964), were examined. *Plesionika* species occurring in the North Atlantic but not available in the present study are *P. acanthonotus* (Smith, 1882), *P. geniculatus* (Milne-Edwards, 1883), *P. holthuisi* Crosnier & Forest, 1968, and *P. rossignoli* Crosnier & Forest, 1968. The individuals studied came from decapod collections of the University of Las Palmas de Gran Canaria (namely, the ICCM study collection) and of the Funchal Natural History Museum (MMF). The number and geographical sampling location of individuals examined were as follows: *P. antigai* (n=46; Madeira), *P. edwardsii* (n=129; Cape Verde and Canary Islands, Mediterranean Sea, Madeira, Azores, Agadir), *P. ensis* (n=53; Agadir, Cape Verde and Canary Islands, Madeira), *P. gigliolii* (n=64; Mediterranean Sea), *P. heterocarpus* (n=45; Agadir, Mediterranean Sea), *P. longicauda* (n=20; Cape Verde Islands), *P. martia* (n=23; Canary Islands, Madeira, Mediterranean Sea), *P. narval* (n=60; Cape Verde and Canary Islands, Madeira) and *P. williamsi* (n=83; Cape Verde and Canary Islands, Madeira). The carapace length (CL) was measured with digital callipers (0.01 mm). Individuals were sexed under a binocular microscope based on the presence or absence of the appendix masculina on the endopod of the 2nd pair of pleopods and on the examination of the endopod shape of the 1st pair of pleopods (King and Moffitt, 1984).

Shape and position of thoracic teeth for each *Plesionika* species have been described for the first time based on a combination of features, which include the form of the outer and inner sides of teeth, the shape of the tip, the separation between both teeth and its height referred to the male gonopore.

The time consumed by using the 2nd pair of pleopods sexing technique was measured in a sample of 50 non-ovigerous specimens of *P. edwardsii*. Individuals of *P. edwardsii* were randomly selected in 10 successive samplings by two trained samplers. Then, following the same procedure, specimens were sexed based on the presence or absence of the teeth structure on the 7th thoracic sternite, between the 5th pair of pereiopods. Pearson's correlation was used to establish the degree of success between techniques.

An analysis of variance (ANOVA) was also applied to test differences in the samplers and sex determination methods (Underwood, 1997).

Females of *P. edwardsii* from the Canary Islands (n=150, 10 for each 1-mm CL) between 10 and 25 mm CL were dissected, and the maturity stage was histologically assessed following Triay-Portella *et al*. (2014, 2017). The histological morphology of the ovary stages and the oocytes were described following the scale proposed by ICES (2010) and adapted for *P. edwardsii* in Triay-Portella *et al.* (2017).

The teeth structures of the 7th thoracic sternite of 10 males and females for each 1-mm CL between 10 and 25 mm in CL were recorded and photographed. The percentage of females without thoracic teeth for each 1-mm CL class was calculated for *P. edwardsii, P. narval, P. gigliolii* and *P. williamsi*. In these species, all sizes between the transitional point were represented, particularly in sizes in which the degeneration of the thoracic teeth occurs in the females. Size at sexual maturity ($CL_{m50\%}$; the carapace length at which 50% of females have no thoracic teeth) was estimated by fitting data to the logistic equation $P=100/1+\exp(r(CL-CL_{m50\%})$ by a non-linear regression, where $CL_{m50\%}$ is the size at 50% maturity, P the proportion of individuals without thoracic teeth in each size class, and r is the parameter of the model (González *et al*., 2016).

Other samples of 45 individuals of *P. edwardsii* and 45 individuals of *P. narval* were kept alive for one month in two tanks of 1000 l and two observation tanks of 500 l in dark conditions with a water temperature of 14°C and salinity of 35.8 ppt, following the recommendations of Landeira *et al.* (2009). Males and females (with and without the teeth structures of the 7th thoracic sternite) were marked with fluorescent paint to be identified. These conditions were the same as those recorded with a XR-420-CTD sensor at the capture area at 300 m in depth. Living individuals (n=15) were filmed using an HD camera with red light during the night when the shrimps showed increased activity. A total of 381 interactions were recorded (n=129, male-male; n=135 male-female; n=117, male-immature females). An analysis of variance (ANOVA) was also applied to test differences in the contact time and interaction groups (Underwood, 1997).

3. Results

3.1. New secondary sexual characters

A new distinguishing feature located on the 7th thoracic sternite between the 5th pair of pereiopods was observed (Fig. 1a, 1b). This structure showed a clear morphological difference between males and females from all *Plesionika* species examined. In males and immature females, this morphological structure consists of two teeth located on the 7th thoracic sternite, specifically placed between the 5th pereiopods (Fig. 1b, 1c). Thoracic teeth from immature females (incisor shape) can be differentiated from thoracic teeth from males by a more pointed shape and the absence of male gonopores in immature females (Fig. 2).

However, this structure was absent in mature females in all *Plesionika* species studied. Indeed, females presented short denticles on the coxa of 5th pereiopods (Fig. 1d). In addition, the gonopores of both sexes were photographed; female gonopores were on the 3rd pair of pereiopods (Fig. 1e), and male gonopores were on the coxa of 5th pereiopod and devoid of denticles on coxa (Fig. 1f). In males, teeth on the 7th thoracic sternite were always present in the entire size range in all species of *Plesionika* examined in the present study.

Differences between the thoracic teeth for each species can be observed (Fig. 3a-c). Teeth descriptions are as follows: *P. antigai*: Both sides of teeth symmetrical, with a slight inclination to the inside. Teeth obovate with a constriction in the middle, well separated, its height clearly exceeding the male gonopores (constriction coincides with the top of the gonopore) (Fig. 3a3). *P. edwardsii*: Both sides of teeth symmetrical, appearing as a triangle with a slight inclination to the inside. Tip pointed (the most pointed within the studied *Plesionika* species). Inner side slightly concaved near top. Teeth clear and progressively more separated from the base to the top, its height slightly exceeding the male gonopores (Fig. 3b3). *P. ensis*: Outer side of teeth draws an irregular and pronounced slope. Tip small and rounded. Inner side of teeth with overhanging slope. Both teeth joined at the top and appearing progressively separated to the base, its height barely reaching the male gonopores (Fig. 3c3). *P. gigliolii*: Both sides of teeth symmetrical. Tip squared-off in the outer side. Inner side of teeth with a less pronounced tip and ending in flat back. Teeth clearly separated, its height barely exceeding the male gonopores (Fig. 3d3). *P. heterocarpus*: Outer side of teeth draws a gentle slope to its end, where the slope is more pronounced. Tip rounded. Inner side of teeth flat back. Teeth clearly separated, its height not reaching half of the male gonopores (Fig. 3e3). *P. longicauda*: Outer side of teeth has a pronounced concave slope ending in a rounded tip. Inner side draws a complex slope: a rounded convex slope from the top to the middle followed by an overhanging slope to the base. Teeth separated by a narrow space, its height clearly exceeding the male gonopores (Fig. 3f3). *P. martia*: Outer side of teeth draws a curving slope, with rounded tip. Inner side has a flat back or a slightly overhanging slope. Teeth clearly separated, its height nearly reaching the top of male gonopores (Fig. 3g3). *P. narval*: Outer side of teeth with steep slope. Tip squared-off in the outer side. Inner side of teeth with a less pronounced tip and ending in flat back. Teeth separated by a narrow space, its height clearly reaching male gonopores (Fig. 3h3). *P. williamsi*: Outer side of teeth with smooth slope until the tip. Inner side with rounded tip and pronounced slope. Teeth joined at level of the base, its height not reaching half of the male gonopores (Fig. 3i3).

3.2. Time consumption in sex determination related to sampling strategies

The percentage of males and females correctly identified using the teeth located on the 7th thoracic sternite was 100% correlated with the sex assigned by using traditional secondary sexual characteristics, such as female gonopores on the 3rd pair of pereiopods, differences in the morphology of endopods on the 1st pair of pleopods, and the presence of appendix masculina on the endopod of the 2nd pleopod in males but the absence on the endopod in females.

The mean time consumed in the sexing technique based on 2nd pair of pleopods was 6.28±0.76 min/50 shrimps. On the other hand, the mean time consumed in sex determination based on thoracic teeth was 2.21±0.08 min/50 shrimps. The sexing technique based on teeth was faster than the 2nd pleopod technique (Table A.1; F=674.305, $p<0.001$); in fact, there was a reduction in time consumed of more than 60% (t=25.12, $p<0.001$, Fig. A.1).

3.3. Changes in the thoracic teeth and functional maturity in females

The “disappearance” (degeneration) of thoracic teeth in females of all *Plesionika* species examined does not occur abruptly. Some morphological changes occur in the thoracic teeth prior to pubertal moult, in which they degenerate progressively until their disappearance (Fig. 2). However, males always present thoracic teeth without the changes associated with moult. The fading in the teeth prior to pubertal moult is linked to the functional ovarian maturation of females. Prior to pubertal moult, during the period in which teeth are degenerating progressively until their disappearance, *P. edwardsii*, *P. narval* and *P. williamsi* showed histologically in all cases that females with thoracic teeth always had immature ovaries in which the germinal zone was not totally apparent (Stage I). Macroscopically, at this stage, gonads were characterized for their thin and translucent aspect (Fig. 4). However, females without thoracic teeth always had mature ovaries. Although, macroscopically, the resting phase (Stage II) could be confused with immature ovaries, there are some characteristics that can differentiate them. At Stage II, gonads were reddish-yellow and filled less than half of the cephalothoracic cavity. Histologically, ovaries were disorganized, with abundant connective tissue and a well-defined germinal zone (Fig. 4). The length at sexual maturity, which was based on the fading of thoracic teeth in females, was estimated to be 18.11 mm CL in *P. edwardsii* (Canary Islands population) (18.56), 12.01 mm CL in *P. gigliolii* (Mediterranean population), 13.85 mm CL in *P. narval* (Canary population), and 18.70 mm CL in *P. williamsi* (Canary population) (Fig. 5). The similar sizes at maturity were obtained when estimated by using the histological stage of ovaries.

3.4. Role of thoracic teeth in the pure-searcher reproductive strategy

It was not possible to make quantitative observations regarding the reproductive behaviour between specimens of *P. edwardsii* and *P. narval* because all females died during the ecdysis process, as they were unable to harden the exoskeleton. However, observations of contacts between males and females were recorded. The contacts were made at the thoracic region where thoracic teeth are located. Contact between males (2.63±0.57 seconds) or between males and immature females was abruptly interrupted (2.59± 0.46 seconds). Contact between males and mature females (170.18±0.93 seconds) was more prolonged (ANOVA test F=6.04, p=0.0026), since males were more active and conducted several contacts, always at the thoracic zone of females, where the 3rd pair of pereiopods are located (Fig. 6).

4. Discussion

The correct identification of some species of the genus *Plesionika* remains difficult using the current taxonomic traits due to their similarity (Holtuis, 1951; Chace, 1985; Chan and Crosnier, 1991, 1997; Chan and Yu, 2000; Shanis *et al.*, 2014; Ahamed *et al.*, 2017). Current works on genetics indicate that the genus *Plesionika* is not monophyletic as currently defined (da Silva *et al.*, 2013, Chakraborty *et al.*, 2015), highlighting some discrepancies between the current taxonomy and molecular systematics. In females of *Plesionika*, thoracic teeth constitute a taxonomic trait that could be used to separate groups according to the morphology or shape of teeth. Systematics in *Plesionika* reveals the existence of morphological variations among specimens from different localities (Shanis *et al.*, 2014; Centelles *et al.*, 2016). A first preliminary morphological analysis showed clear differences in the shape of thoracic teeth in *P. williamsi* in comparison with the other species examined. Both taxonomy and molecular systematics stress the importance of the accumulation of new data on morphology and colouration of *Plesionika* shrimps to clarify the taxonomy of some species of this genus (da Silva *et al.*, 2013; Centelles *et al.*, 2016; Ahamed *et al.*, 2017).

Since the publication by King and Moffitt (1984), the direct sex determination of deep-sea pandalids has consisted of three types of taxonomic evidence: i) the shape of the endopod of the first pair of pleopods, ii) the coxa of the fifth pair of pereiopods (male gonopores located on the coxal segments of the 5th pair of pereiopods), and iii) the appendix masculina on the endopod of the second pair of pleopods in males. The present results showed that the examination of thoracic teeth can advantageously replace two methods regularly used in the field of reproductive biology in *Plesionika* species, such as the examination of traditional secondary sexual characteristics (female gonopore, morphology of endopods or presence/absence of appendix masculina) and the histological examination of ovaries (Fanelli and Bellucio, 2003; Consoli *et al.*, 2014; Triay-Portella *et al.*, 2017). The gradual disappearance of thoracic teeth is linked to the functional maturity and development of the female ovaries and the time of reaching maturity, indicating their complete disappearance. Additionally, sex assignment by the presence/absence of thoracic teeth is an enhanced method because its application is faster than the aforementioned techniques (saving more than 60% of the time). Indeed, sex assignment by the thoracic teeth method does not require the use of a magnifying glass for sex classification of individuals with sizes above average at sexual maturity, which is indispensable when sex determination is conducted by the presence/absence of appendix masculina on a second pair of pleopods, as it is usually done. The absence of male gonopores in the 5th pereiopods, together with the presence of teeth is an unequivocal combination for the female's immature determination. The combination of both methods, i.e. the presence of immature teeth and the absence of male gonopores on the 5th pair of pereiopods, is a robust technique and it does not lead to error. Time consumption in case of mature size is more valuable than sex determination, based on the fact that gonadal histology techniques are not necessary to estimate the size of first functional maturity in these species.

The ratio between appendix masculina and appendix interna is used as a proxy of morphological maturity in mid- and deepwater pandalids. Similarly, changes in the relative growth of the abdomen with body size in *Plesionika* species were used as a proxy of size morphological maturity in females (Ahamed and Ohtomi, 2014). However, this relative growth has been rarely verified with ovarian development (Triay-Portella *et al*., 2017). Reviewing the literature, the most extended method to obtain size at physiological maturity in females of *Plesionika* is based on the ovigerous condition of females (Table A.2). This method, which is based on the percentage of females carrying eggs by size during the breading season, is strongly biased because it is not able to differentiate mature females without eggs from immature females, yielding an overestimated size at maturity. The use of thoracic teeth gives a new way to estimate maturity in females based on percentages of individuals with or without thoracic teeth by size. The advantages of this method seem to be considerable, since the identification of the maturity condition is not linked to the ovigerous condition or breeding season, and it is correlated with the ovarian maturation and development, which does not need the use of histological techniques to verify the physiological maturity condition in species of *Plesionika*.

Recent research reveals that some of the pereiopodal setae in pandalids present differences between sexes, while some specific setae only present in male pereiopods

(Wortham *et al*., 2014). In this sense, female cuticle setae on the coxa of the 5th pair of pereiopods could be related to the spermatophore fixation on the ventral thoracic area (Bauer, 1976). Setae structure attached to pereiopods and antenna in decapods are vital in overall mechanical, sensory reception, feeding and morphological functions, including sensory mating and grooming activities (Bauer, 2013). In terrestrial arthropods such as Trichoptera, the 5th sternite is related to the occurrence of a female sex and aggregation pheromones (Löfstedt *et al*., 1994). Observations done in tanks with living individuals under dark conditions have allowed for the finding that males of *P. narval* and *P. edwardsii* actively seek females and use the third pair of their multi-articulate legs for it, making contact mainly at the thoracic region where teeth are located. When a male detects the presence of thoracic teeth during interactions between male and male or between male and immature female, the contact was abruptly interrupted. However, when thoracic teeth were not detected during male-female interaction, the interaction was more prolonged. This suggests that these thoracic teeth play a role in the recognition of sex and the sexual condition of *Plesionika* in deep dark environments. The role of thoracic teeth and their importance in the reproductive biology of these species promise to be an interesting field of study. Additionally, the presence of thoracic teeth in close relatives and closely related taxa remains unknown.

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Figure captions

Figure 1. Location of secondary sexual characters in *Plesionika edwadsii* as a model of genus *Plesionika* (a). Thoracic teeth in males (b) and immature females (c), short denticles on coxa of mature females (d), female gonopore (e), male gonopore (f) and devoid coxa denticles (f). Black arrow indicates the presence of the character; white arrow indicates the absence of the character.

Figure 2. Progressive degeneration of immature thoracic teeth in *Plesionika edwardsii* females until pubertal moult (right), and the prevalence of thoracic teeth in males (left). From top to bottom, there is an increase in carapace size.

Figure 3. Thoracic teeth present in males (1,3), absent in females (2) for *Plesionika antigai* (a), *Plesionika edwardsii* (b), *Plesionika ensis* (c), *Plesionika gigliolii* (d), *Plesionika heterocarpus* (e), *Plesionika longicauda* (f), *Plesionika martia* (g), *Plesionika narval* (h) and *Plesionika williamsi* (i).

Figure 4. Histological sections of immature *Plesionika edwadsii* with thoracic immature teeth present, while immature ovaries (a) and mature *P. edwardsii* without teeth present while resting ovaries (b).

Figure 5. Maturity ogive estimated by $\mathrm{CL}_{\mathrm{m}50\%}$ (the CL at which 50% of females without thoracic teeth). Fitted to a logistic equation of *Plesionika edwardsii*, *Plesionika gigliolii*, *Plesionik anarval* and *Plesionika williamsi*.

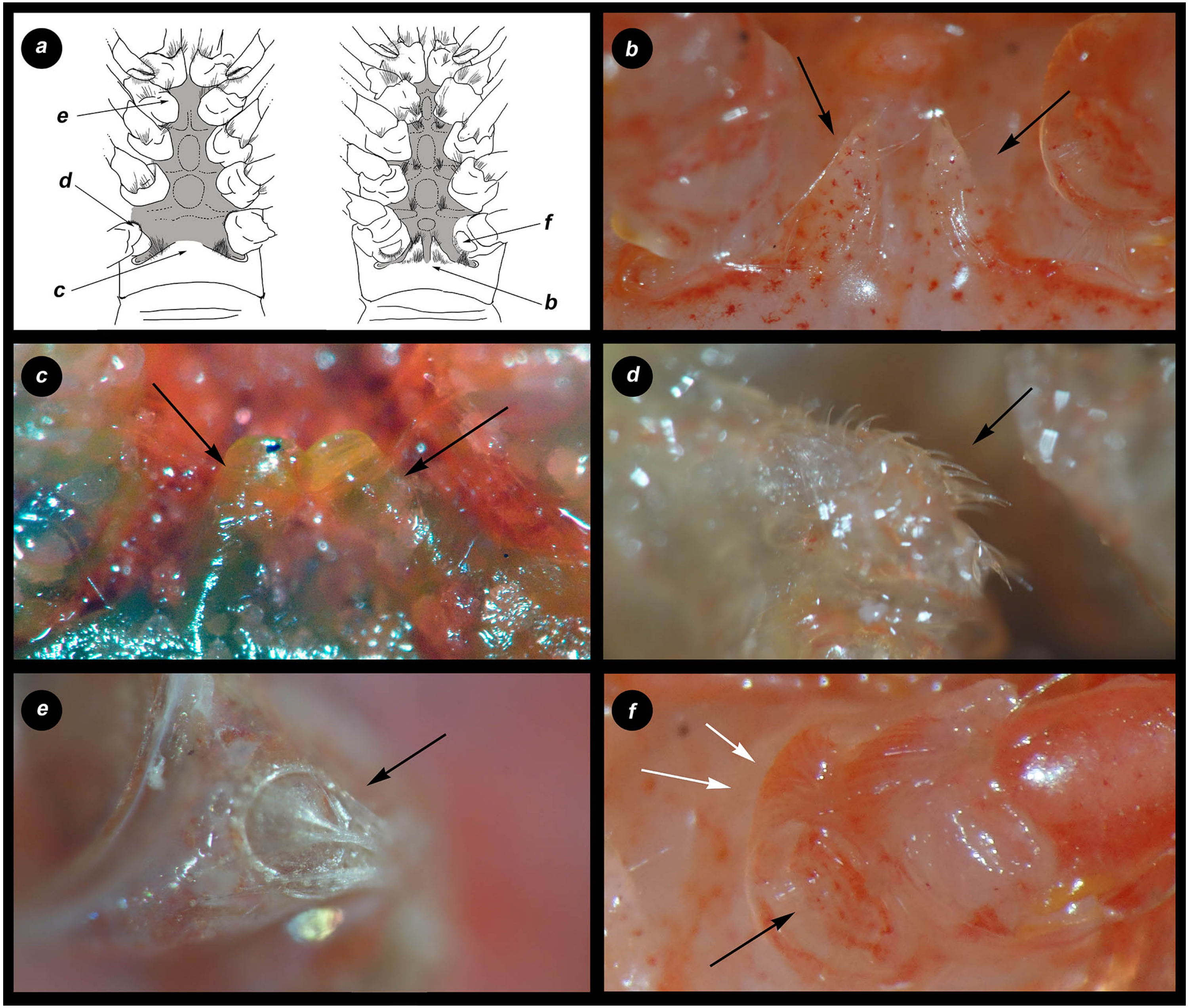
Figure 6. Social interactions related to thoracic teeth in observation tanks between male-female *Plesionika edwardsii* (a) and *Plesionika narval* (b).

Figure A.1. Comparative time consuming (min50 shrimps) using thoracic teeth and appendix masculina sexing technique for 50 non-ovigerous specimens of *Plesionika edwardsii*.

Table captions

Table A.1. Results of the two-way ANOVA testing differences between samplers (sampler 1 and 2), sexing methods (thoracic teeth and appendix masculina) and interaction between factors (*, $p < 0.05$; **, $p < 0.001$).

Table A.2. Synopsis of the main reproductive ecology studies on the genus *Plesionika*. Minimum carapace length (CL min), maximum carapace length (CL max), minimum length of the smallest female carrying eggs (mm CL) and with mature ovaries (mm CL), size at first functional maturity based on mature ovaries (CL m 50%), size at first maturity based on % of females carrying eggs (CL50% ovigerous), first size of morphological maturity in males based on the ratio of appendix masculina and appendix interna (CL appendix masculina), and first size of morphological maturity in females based on the relationship between CL and abdomen width (CL abdomen width).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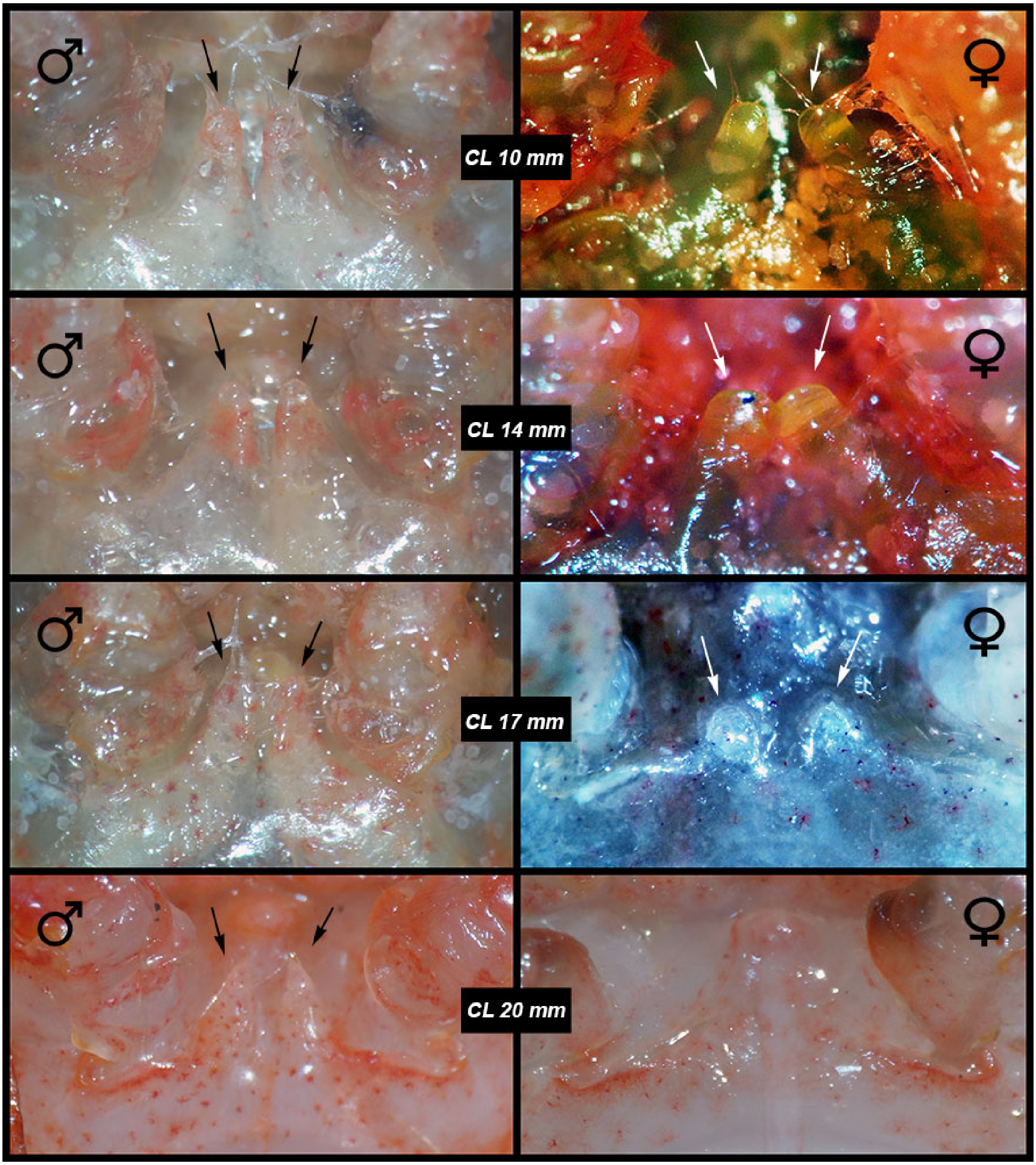
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CL 10 mm
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CL 14 mm
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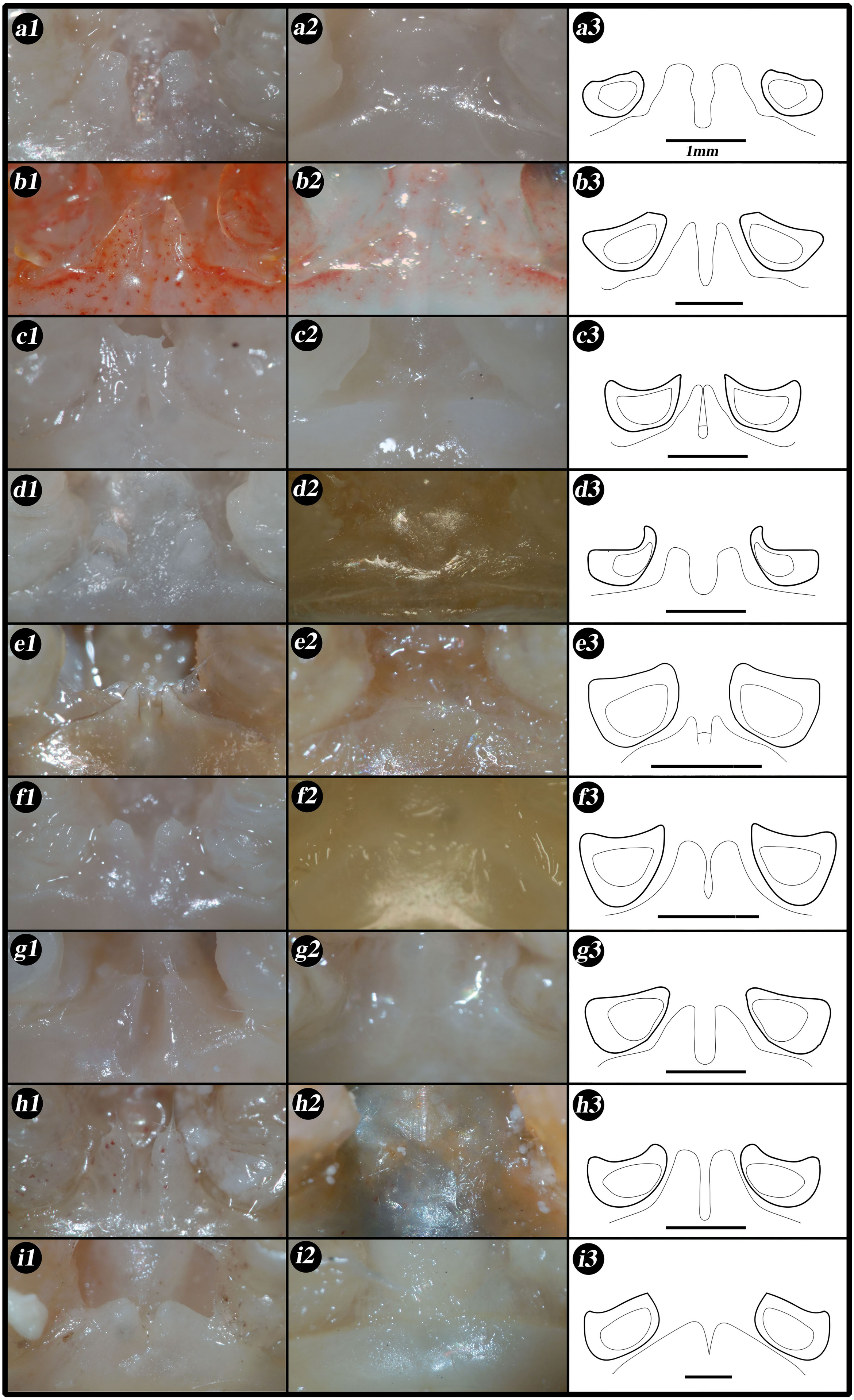
CL 17 mm
♂
♀
CL 20 mm


a1
a2
a3
1mm
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d1
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f1
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f3
g1
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i1
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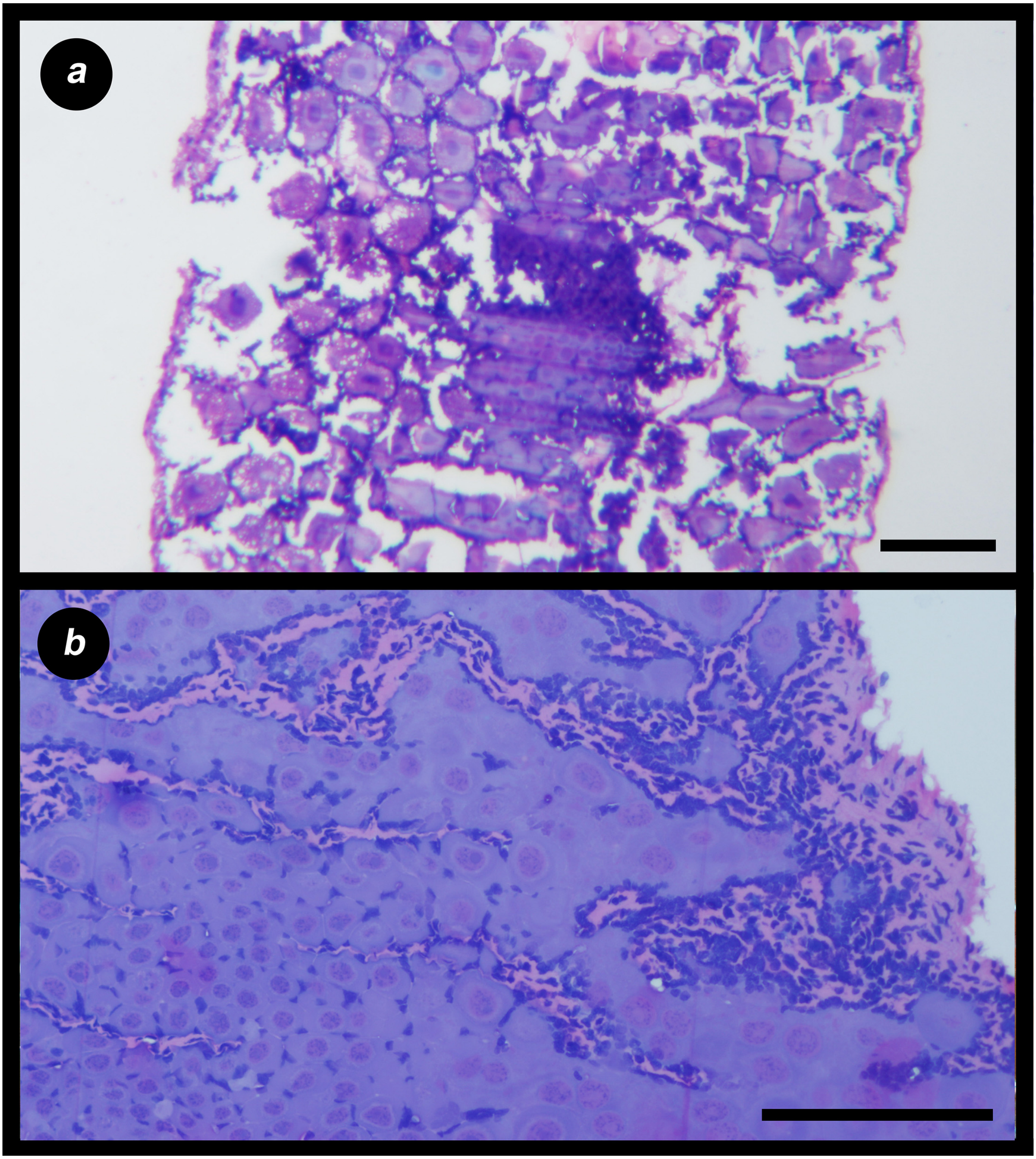
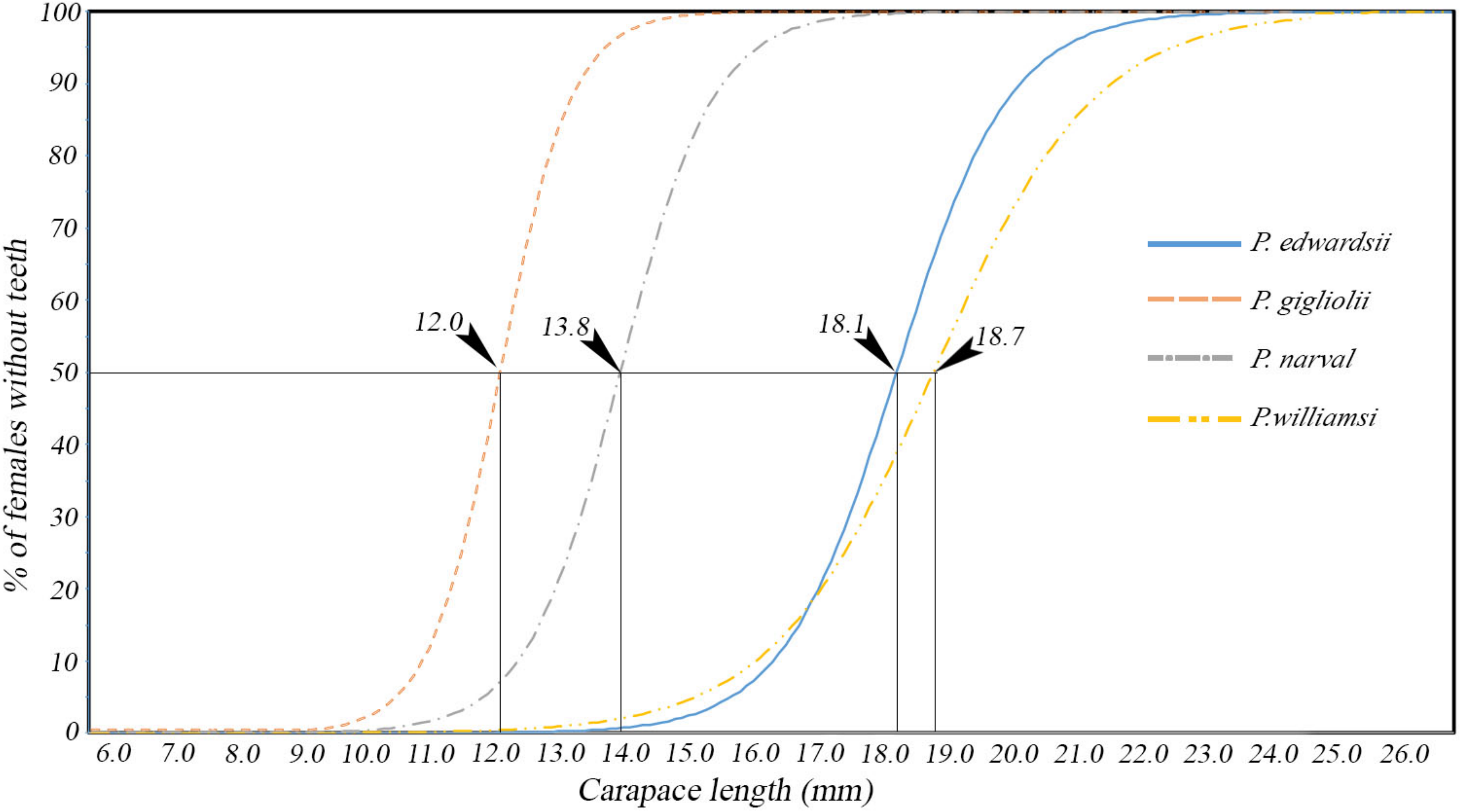
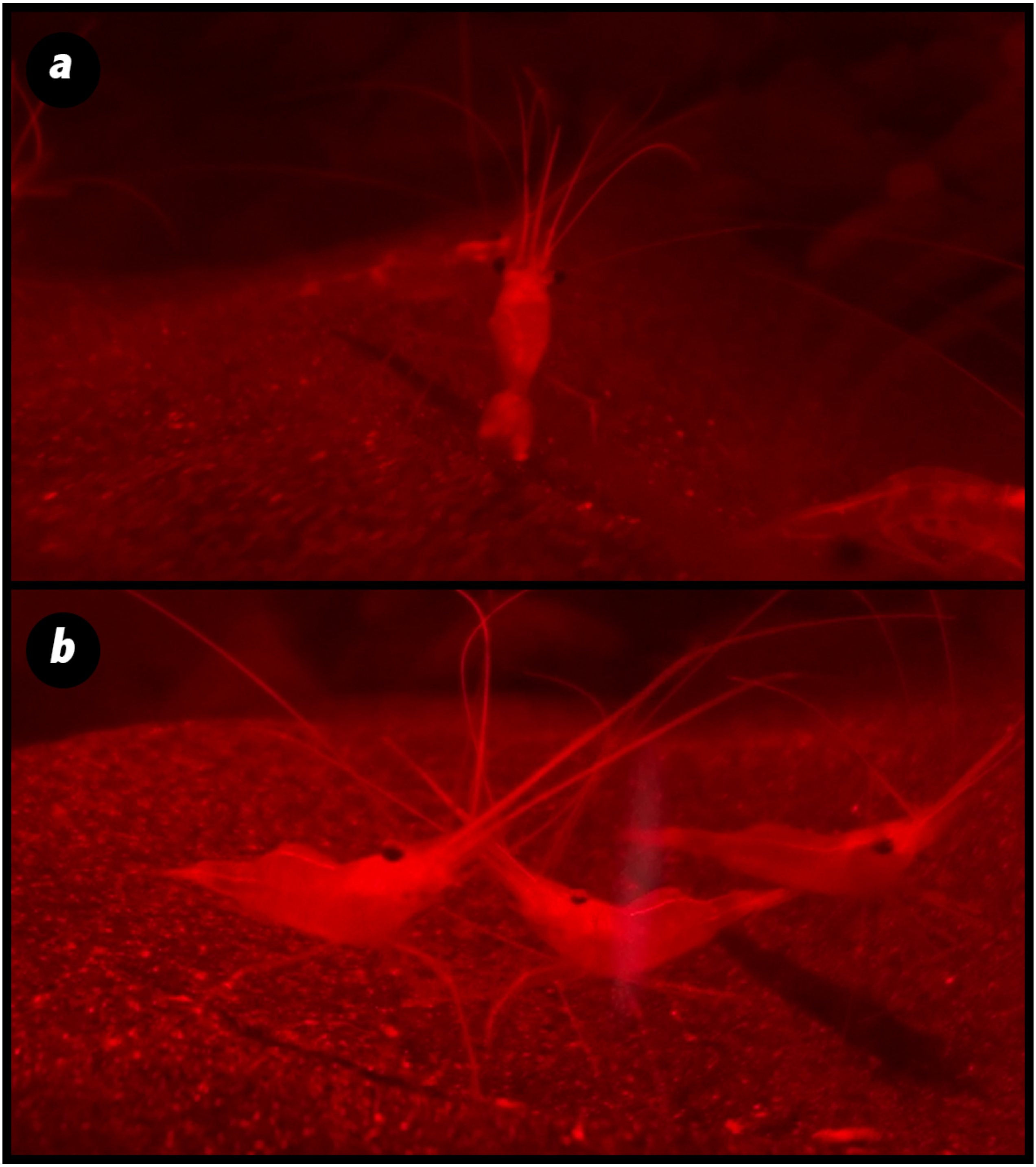

a
b


100
90
80
70
60
50
40
30
20
10
0
% of females without teeth
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
20.0
21.0
22.0
23.0
24.0
25.0
26.0
Carapace length (mm)
12.0
13.8
18.1
18.7
P. edwardsii
P. gigliolii
P. narval
P.williamsi


a
b

two-way ANOVA	SS	df	SM	F	p-value
Sampler	0.59	1	0.59	2.18	0.15
Sexing method	182.59	1	182.59	674.31	0.000**
Sampler * sexing mehod	0.66	1	0.66	2.44	0.13
Error	9.75	36	0.27		
Total	948.92	40			

Species	Region	sex	LC min (mm)	LC max (mm)	Smallest Ovigerous (mm)	Smallest mature (mm)	CL50 mature ovaries (mm)	CL50 %ovigerous (mm)	Morphometric (CL-apendix masculina)	Morphometric (CL-abdomen Width)	From:
***Plesionika acanthonotus* (S.I. Smith, 1882)**	Mediterranean				9.6						Company and Sardà, 1997
								9.9			Abelló *et al*., 2002
***Plesionika antigai* (Zariquiey Alvarez, 1995)**	Mediterranean	females						9.52			Abelló *et al*., 2002
	Sardinian Sea	females	2.5	17.5	7.8			9.7			Campisi *et al*., 1998
		males	2.4	16.9				10			Campisi *et al*., 1998
***Plesionika edwardsii* (Brand, 1851)**	Canary Islands	females	8	40	13						Santana *et al*., 1997
		males	8	34							Santana *et al*., 1997
		females	7.06	30.45			18.56				Triay-Portella *et al*., 2017
		males	7.94	28.29					17.4		González *et al*., 2016
	Cape Verde Islands	females	10.6	29.1			16.39				Triay-Portella *et al*., 2017
		males	10.95	26.86					16.4		González *et al*., 2016
	Madeira	females	12.06	33.28			19.73				Triay-Portella *et al*., 2017
		males	12.5	30.85					19.3		González *et al*., 2016
	Mediterranean	females			18.3						Company and Sardà, 1997
		females			10			16.34			Abelló *et al*., 2002
		females	7	30	15.5			18			Colloca, 2002
		males	7	28							Colloca, 2002
		females	9.9	29.1	13.6						García-Rodriguez *et al*., 2000
		males	10.1	28.8							García-Rodriguez *et al*., 2000
	Tyrrhenian Sea	females	10	29	15	13	18.5	20.2			Possenti *et al*., 2007
		males	11	26							Possenti *et al*., 2007
***Plesionika gigliolii* (Senna, 1903)**	Mediterranean	females			9.3						Compañ and Sardà, 1997
		females			7.8			8.33			Abelló *et al*., 2002
***Plesionika heterocarpus* (A. Costa, 1871)**	Mediterranean	females			11.16						Company and Sardà, 1997
		females			8.5			10.16			Abelló *et al*., 2002
***Plesionika izumiae* Omori 1971**	Japan	female			6.0-14.7			8.5			Ahamed and Ohtomi, 2011
	Japan	female								8.6	Ahamed and Ohtomi, 2014
		male							7.3		Ahamed and Ohtomi, 2014
***Plesionika martia* (A. Milne-Edwards, 1883)**	Adriatic	females						17.1			Pipitione and Tumbiolo, 1993
		females						13.7			Marsan *et al*., 2000
	Aegean Sea	females	11.5	24.9	<13						Koçac *et al*., 2012
		males	12.4	21.1							Koçac *et al.,* 2012
	Canary Islands	females						17.9			González *et al.,* 2001
	Ionian sea	females	9.3	29.1	11.8			16.16			Chilari *et al*., 2005
		males	8	23.5							Chilari *et al*., 2005
		females						12			Maiorano *et al.,* 2002
		females						11.8			Chiliari *et al*., 2005
	Mediterranean	females			14.2						Company and Sardà, 1997
		females			10			15.58			Abelló *et al*., 2002
								16.9			Maiorano *et al*., 2002
		females			12		12	15.5			Maiorano *et al*., 2002
		males									Maiorano *et al*., 2002
***Plesionika narval* (J.C. Fabricius, 1787)**	Canary Islands	females	7	30	8			11.96			González *et al*., 1996
		males	6.3	28							González *et al*., 1996
		Indeterminates	2								González *et al*., 1996
	Madeira	females	6.72	28.61	6.72			14.61			Sousa *et al*., 2014
		males	2.45	27.72							Sousa *et al*., 2014
Plesionika quasigrandis Chace, 1985	South-west cost of India	females	7.8	11.8							Chakraborty *et al*., 2014
		males	7.3	11.3							Chakraborty *et al*., 2014
Plesionika semilaevis Spence bate, 1881	East China	females	6	19.2	10.4						Ohtomi, 1997
		males	6.35	17							Ohtomi, 1997
			females>males			**Sexual maturity**	**3**	**20**	**2**	**1**	**Total=26**

			total studies per technique

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