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First record of *Perinereis macropus* and *Perinereis cultrifera* (Annelida, Polychaeta) from rocky shores east Algeria, (SW Mediterranean sea)

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Abstract

Polychaetous from Algeria are little studied, despite their importance, they comprise diverse, and ecologically significant species, in the present study individuals of two Nereididae species, *Perinereis macropus* and *Perinereis cultrifera* were sampled on the rocky shores of Collo and Skikda Coasts, and were described for the first time with biometric measurements, morphological variations and reproduction aspects, in attempt to provide general information about this group from Algeria, necessary for other future works, especially those on marine biodiversity; we marked the absence of *P. macropus* at the second site, affected by pollution, as well as the difference between algae associated with each species; weight and size variables show differences intersites and among species; oogenesis is asynochronous, mature oocytes occur during July and May for *P. macropus* and *P. cultrifera* respectively, and only adults of *P. macropus* show epitokal modifications, the semelparity character is observed in both sexes of the two species.

Keywords: Nereididae, Ecology, Biodiversity, Anatomy, Reproduction, Algeria

1. Introduction

At present, marine biodiversity studies are a matter of high interest to scientists and decision makers focusing on the current impacts generated by human activities on ecosystem processes and climate change ^[1], unlimited number of recent ones confirms that data of the actual situation of marine biodiversity must have a high priority, as a baseline for the indication of actual ecosystem's health, and the evaluation of future changes ^[2], in order to protect, and valorize high quality ecosystems, and if possible rehabilitate and restore degraded ones ^[3].

In this context, the marine benthic macrofauna is particularly suitable for monitoring environmental long term changes at the ecosystem level ^[4]; among this benthic groups, ^[5] confirm that any long term changes assessment of the benthos should be reflected in the polychaete community; as they play a key role in ecosystems they colonize, they are widely used in the environmental quality assessments, using indicators that vary from biomarkers measured at different levels of biological organization or at the whole organism ^[6-8], to benthic indices used and developed to evaluate the ecological quality into different degrees of disturbance, based on the community diversity, and the classification of taxa to ecological, or trophic groups ^[9, 10].

In Algeria, compared with other countries of the Western Mediterranean sea, such growing interest to environmental quality assessment, especially coastal zones confront scarce information on marine biodiversity, which contributes with 21% of the Mediterranean Sea global biodiversity, and does not reflect the real state ^[11].

Among the 85 families of polychaeta known to occur worldwide ^[12], Nereididae ^[13] is a significant component of communities occupying diverse habitats, from the intertidal to the deep sea, and represents a large family of approximately 44 genus and 677 species ^[14]; one of these diverse genera is *Perinereis* ^[15] represented by around 74 species worldwide ^[14], but only 04 species *Perinereis cultrifera*, *Perinereis macropus*, *Perinereis marionii*, and *Perinereis oliveirae* have been recorded from Algerian waters ^[16, 17], view of recent literature from Algerian coasts conducted especially on this genus, shows an interest about the value of a single species *Perinereis cultrifera* as bioindicator of anthropogenic impact, and a few studies focusing on its biology, but highlights like said above, the lack of documented information in terms of inventory, ecology, description and illustration of this group from Algeria, necessary

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to provide a valuable interpretation of numerous study fields that can be conducted in the future on this genus.

Perinereis cultrifera [18] is known to have a wide geographical distribution, and has a promoting role in economic sector as fishing baits [19], as well as feed in aquaculture [20], and belong to a species complex [21]. While for *Perinereis macropus* [22] information about its geographic distribution, biology and ecology still poorly documented, in the Mediterranean Sea, this species was also described by [23] (Naples, Monaco).

The current study presents a description in terms of biometric measurements and illustration of the two polychaetes species *P. macropus* and *P. cultrifera* from Algeria, and attempts to increase our current knowledge about their distribution,

habitat, identification and biology in general.

2. Materials and Methods

2.1 Sampling

Examined individuals were sampled at three occasions July 2013, May and June 2014, at low tide in the intertidal zone of a rocky shore from two stations north east Algeria, the first is located on the eastern part of Collo coast, whereas the second on the western part of Skikda coast (Figure 1). Worms were forced out from hard substrates by using bleaching liquid (10% in sea water) and collected gently by a pincer to avoid damaging them.



Fig 1: Location of the sampling sites.

2.2 Lab procedures

Worms were fixed in 4% formaldehyde, sorted under a stereomicroscope then preserved in 70% ethanol; all lab procedures were performed on collected individuals of the two species *P. macropus* and *P. cultrifera*.

Identification and description were basically focused on paragnaths' count and shape from all areas of everted pharynx, so worms were forced to evert their pharynx by generating a little pressure at the third chaetigerous toward the head; in addition of microscopical observations of jaws, parapodia and chaeta after performing sections.

Wet weight (g), and four parts of the animal's body were measured (mm), namely total length of the worm, length of the three first segments prostomium, peristomium and first chaetigerous (L_3), length of the head and ten first chaetigerous ($H+10$), and width at chaetigerous 10 (excluding parapodia), by using an ocular micrometer mounted on a stereo microscope; beside total length, the number of chaetigerous was also counted only in complete worms.

In order to determine the sex and describe the reproductive products of collected worms, each individual was opened approximately at the 10th–20th segment, the coelomic fluid contents were released and deposited on a glass slide, then examined under an optic microscope, for females the mean oocytes size was estimated using the average value of the diameter from thirty oocytes, using an eyepiece graticule.

3. Results and Discussion

3.1 Material examined

Perinereis macropus (Claparède, 1870) Fig. 2 (A-E)

Nereis (*Lipephile*) *macropus* Claparède 1870, p 80, Pl VIII, Fig. 1 (A-F).

Specimens of *P. macropus* were sampled among Rhodophyta corallinaceae *Lithophyllum byssoides* and vermetid reef formed by an association between Rhodophyta corallinaceae and Mollusca vermetidae *Dendropoma petraeum*; station 01: In July, 2013 individuals occur with posteriorly epitokal modification, (modification starts at 16-18 chaetigerous), two *Heteronereis* males largest complete 0.0872 g, 25 mm long, L_3 3.4 mm, $H+10$ 7 mm long and 2 mm wide, with 90 chaetigerous; seven heteronereis females largest complete 0.0941 g, 28 mm long, L_3 3 mm, $H+10$ 7 mm long and 2 mm wide, with 96 chaetigerous. In May 2014, three males, largest complete 0.2688 g, 58 mm long, L_3 4 mm, $H+10$ 9 mm long and 2.4 mm wide, with 100 chaetigerous; three females largest incomplete 0.2423 g, L_3 3.6 mm, $H+10$ 9 mm long and 3.4 mm wide. In June 2014, eleven males, largest complete 0.4240 g, 72 mm long, L_3 5 mm, $H+10$ 10 mm long and 2.2 mm wide, with 97 chaetigerous; one specimen regenerated posteriorly 0.1556 g, 49 mm long, L_3 3.4 mm, $H+10$ 8 mm long and 2 mm wide, with 70 chaetigerous (last 16 chaetigerous regenerated); four females, largest complete 0.4380 g, 90 mm long, L_3 4.2 mm, $H+10$ 10 mm long and 3 mm wide, with 95 chaetigerous. Station 02: we have noticed the absence of *P. macropus* during the period of sampling that might be a consequence of the very restricted area of vermetid reef, and the total absence of *Lithophyllum byssoides*, in addition of pollution known to affect this site, that may have a consequence on habitat and related species biodiversity.

Perinereis cultrifera (Grube, 1840) Fig. 2 (F-I)

Nereis cultrifera Grube 1840, p 74, Fig. 6

Specimens of *P. cultrifera* were collected among rhodophyta corallinaceae *Jania rubens* and different species of the genus *Coralina* present at the site sampling; station 1: In May 2014,

three males, largest complete 0.4171 g, 52 mm long, L₃ 5.2 mm, H+10 10 mm long and 3.2 mm wide, with 55 chaetigerous; one female largest incomplete 0.4929 g, L₃ 6 mm, H+10 14 mm long and 3 mm wide. *In June 2014*, four males largest complete, regenerated posteriorly 0.5152 g, 70 mm long, L₃ 6.8 mm, H+10 12 mm long and 4 mm wide, with 56 chaetigerous (last 10 chaetigerous regenerated); another specimen regenerated posteriorly 0.2123 g, 44 mm long, L₃ 4.6 mm, H+10 12 mm long and 3 mm wide, with 47 chaetigerous (last 4 chaetigerous regenerated); four females, largest incomplete 0.4151 g, L₃ 7.1 mm, H+10 12 mm long and 4 mm wide.

Station 2: *In May 2014*, three males, largest complete 0.3258 g, 60 mm long, L₃ 5 mm, H+10 11 mm long and 2.4 mm wide, with 68 chaetigerous; four females largest complete 0.2192 g, 46 mm long, L₃ 5.4 mm, H+10 8 mm long and 3.2 mm wide, with 56 chaetigerous.

In June 2014, two males, largest complete 0.5190g, 60 mm long, L₃ 5.8 mm, H+10 11.2mm long and 3mm wide, with 64

chaetigerous; four females, largest incomplete L₃ 6 mm, H+10 12 mm long and 3 mm wide. *In July 2013*, individuals were absent at the two stations both, as a result of the semelparous characters of these species, adults died in days after reproduction.

3.2 Description and identification traits

The two species are recognized by their elongated and multi segmented form, and by the presence simultaneously of two antennae, two biarticulated palps, four pairs of tentacular cirri, four eyes, and a pair of dark or light brown of serrated jaws, with 4-6 teeth on an eversible pharynx, these characters agree with the description of Nereididae by [24], thus the general form is the same with segments along the body all alike, except at the anterior end where there is the head formed by prostomium and peristomium, and at the posterior extremity the pygidium, with two anal cirri that vary in length (Figure 2).



Fig 2: *P. macropus* (A) Dorsal view of whole animal; (B) Dorsal view of anterior region; (C) Ventral view of anterior region; (D) Dorsal view of details of the head; (E) Ventral view of pygidium; *P. cultrifera* (F) Dorsal view of anterior and middle region; (G) Dorsal view of anterior region; (H) Ventral view of anterior region; (I) Dorsal view of details of the head; (J) Ventral view of pygidium, (TC) Tentacular cirri, (P) Biarticulated palp, (Ey) Four eyes, (bar scale: 1mm).

Thus all nereids were easy to separate from other Polychaetes, but the difficulty was that *P. macropus* and *P. cultrifera* coexist with two other closer nereids *Nereis falsa* and *Platynereis dumerilii*, so we had followed the usual method for description, based on the number and shape of paragnaths on maxillary and oral ring of pharynx, divided into areas from

I to VIII [25], so first, we had been interested in area VI key element for description of the genus *Perinereis*, represented by a large transverse smooth bar, then identification to species level was conducted according to [24, 26] as summarized in Table 01.

Table 1: Number and shape of paragnaths on maxillary and oral rings on everted pharynx according to [24, 26].

	Areas	<i>P. macropus</i>	<i>P. cultrifera</i>
Maxillary ring	I	3-7 cones rarely 2	1-3 cones in longitudinal line
	II	each side triangular clusters of cones	each side cluster of cones in oblique rows
	III	rectangular cluster of cones flanked with 3- 5 ones	rectangular cluster of cones in 2 rows
	IV	each side triangular clusters of cones	each side triangular clusters of cones
oral ring	V	1 large and 5-12 (usually 10) sub equal in irregular line, or in a group	3 cones in a triangle or 1 single (floridana)
	VI	each side 1 large transverse bar-shaped	each side 1 large transverse bar-shaped
	VII-VIII	4-5 transverse rows of sub equal cones	2 transverse rows of equal cones

Morphological characters of parapodia, and chaetae structure are also important, and were examined for both species after performing cross sections at different region of the body; parapodia of the two first chaetegerous are uniramous with absence of notochaeta, whereas the rest are biramous, having two parts, the first is the notopodium at the dorsal side with a dorsal cirrus, and two conical ligules, between which we found notochaetae and a notoaciculum, the second is the neuropodium at the ventral one, with a ventral cirrus, one conical ligule, well developed tuft of neurochaetae and a neuroaciculum; chaetae are compounds homogomph spinigers, heterogomph spinigers and heterogomph falcigers, also in heteronereis species we noticed the natatory chaetae (Figure 5: J, K, L), (Figure 6: G).

3.3 Morphological variations

With more practice, the two species were first separated on the basis of color which is bright green and green bronze for *P. macropus*, and *P. cultrifera* respectively; also in preserved worms, we can notice a pigmentation pattern present dorsally in *P. macropus* (Figure 2: A, B), that varies from light to dark green bands, while for *P. cultrifera* it can be noticed on the prostomium and palps, and it appears to be easy to fade very quickly after preservation (Figure 4: D, E), this character is

useful for identifying criptic nereidid species [27].

In general *P. macropus* worms were clearly more elongated, they were longer with a higher chaetegerous number, compared with *P. cultrifera* worms, characterized by a longer L_3 and larger width size, beside longer tentacular cirri extending to the 5th-6th chaetegerous, while for *P. macropus* the longest ones can reach the 3rd-4th chaetegerous; the maximum number of chaetegerous of the two species is lower than that cited in [24], in Polychaetes, chaetegerous' number can vary considerably, in fact they can easily lose the posterior part of the body by autotomy, and regenerate it by addition of new segments from the posterior end of the body, called the posterior growth zone [28], In the present study worms with regenerating few or many chaetegerous are observed, from either the middle or posterior part of the body; thus the number of lost and replaced chaetegerous, affect even the total length, that sometimes does not correlate with other biometric measurements; also biometry of individuals *P. cultrifera* vary according to geographic location [29].

Parapodia from *P. cultrifera* shows no differences along the body, while for *P. macropus* we clearly notice a modified form of parapodia, that began from the posterior region and extend to the pygidium, with elongated and enlarged notopodial ligule and short dorsal cirrus (Figure 5: A-E, H, I).

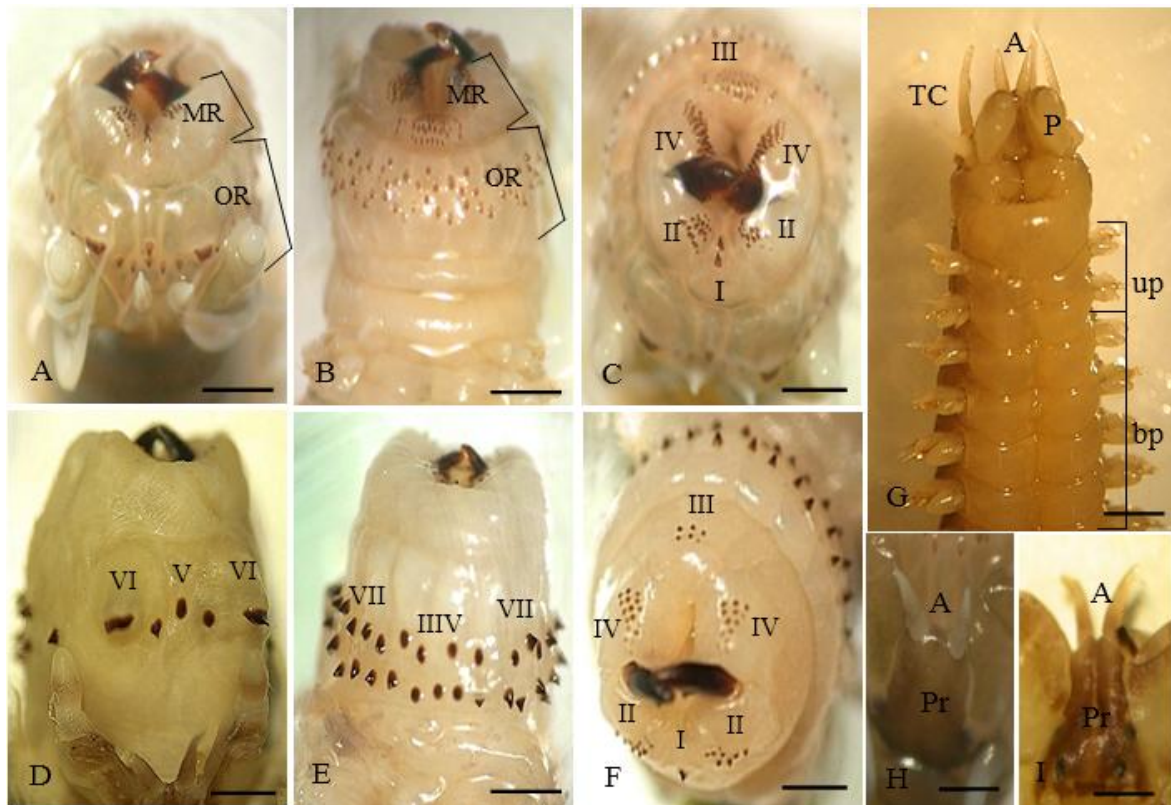


Fig 3: Details of paragnaths' shape and arrangement on everted pharynx; *P. macropus* (A-C) Dorsal, ventral, and top view; *P. cultrifera* (D-F)

Dorsal, ventral, and top view; (G) General aspect of the anterior ventral part of a worm showing devagenated pharynx and parapodia;

Prostomium and antenna for (H) *P. macropus*, and (I) *P. cultrifera*; (MR) Maxillary ring, (OR) Oral ring, (A) Antenna, (P) Biarticulated palp, (TC) Tentacular cirri, (up) Uniramous parapodia, (bp) Biramous parapodia, (Pr) Prostomium, (bar scale: 1mm).

The shape and distribution of paragnaths in the two species show rectangular-base paragnaths (bar-shaped), with pointed or rounded apex named Shield-shaped bars, (Figure 3: A, D) for area VI; this type is present in *Perinereis* species having a single area VI paragnaths, and *Pseudonereis* species [25]; the remaining areas are characterized by uniform-base paragnaths, with circular base, and are tapered towards an apex named conical paragnaths (Figure 3: B, C, E, F); size height and color of paragnaths in *P. cultrifera*, present

differences, first on the oral ring, are particularly larger, higher and brown darker, while those on the maxillary one are comparatively small and faint, second in contrast with *P. macropus* where paragnaths are almost similar in size, smaller and less darker, except those on the maxillary ring that are more slender and acutely pointed; unlike these types we found another type of the uniform-base paragnaths in *P. dumerelii*, with a circular base, which are long and slender, called Rod-like paragnaths.

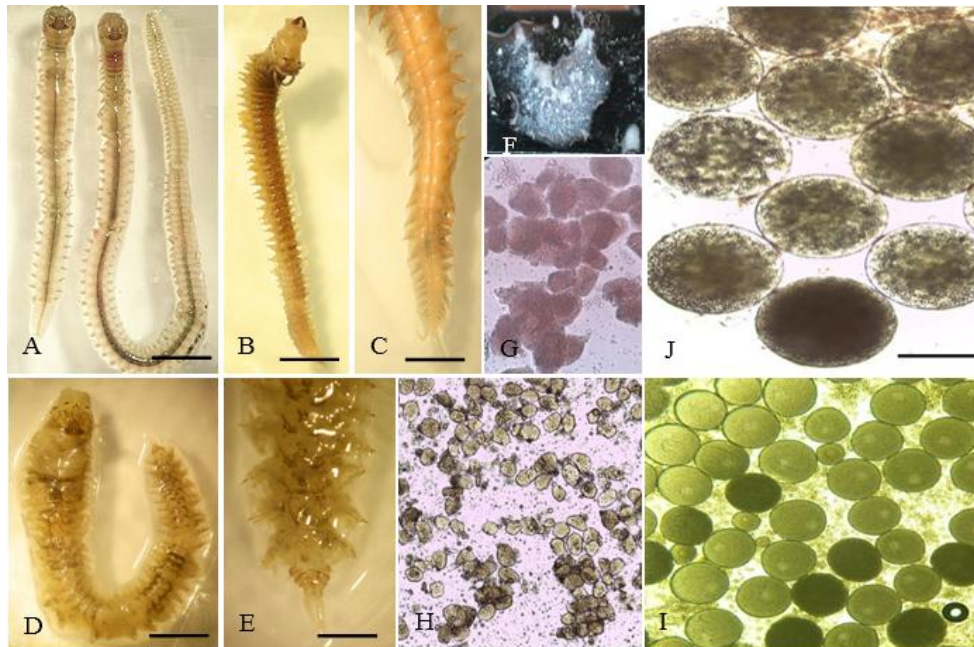


Fig 4: (A) Two individuals *P. macropus* showing differences in total length caused by autotomy, despite regenerating lost chaetigerous; (B) Regenerated chaetigerous beginning from the middle region of the body; (C) Close up of many regenerating chaetigerous ; (D) Individual *P. cultrifera* showing a regenerating posterior region and a pigmentation pattern; (E) Close up of few regenerating chaetigerous; (F) Normal aspect of coelomic fluid containing sperm aggregates; (G-H) Sperm aggregates (I) Asynchronous oocytes; (J) Mature oocytes, (bar scale: 1mm, J: 200 μ m), (G: x 100), (H, I, J: x 40).

3.4 Reproduction

The two species are gonochoric, males present sperm aggregates within coelomic cavity, whereas females present spherical or oval free oocytes within parapodia also, when females were completely packed with mature ones, this finding is similar to literature on oogenesis in nereids, gonads had never been localized, and oocytes grow free in the coelomic cavity [30], observations show that oocytes maturation is asynchronous in the two species (Figure 4: I), this agree with earlier studies where a clear heterogeneous size of oocytes occurs [31, 32], unlike in *Platynereis dumerelii* [33] and *Nereis falsa* [34]. where oocytes maturation is

synchronous; for *P. macropus* mature oocytes can reach a maximum diameter of $268.76 \pm 11.68 \mu\text{m}$ in July; for *P. cultrifera* maximum oocyte diameter showed a similar value $334.69 \pm 19.55 \mu\text{m}$ and $345.33 \pm 13.82 \mu\text{m}$ in May at both stations Collo and Skikda respectively, these values are similar of those mentioned in [29, 31, 32-35] suggested that oocyte growth follows an initial phase of very slow growth, a phase of rapid growth, and a final phase of egg differentiation with little growth, likewise [30]. Reported that oocytes proliferation and growth occur over an extended period of time, where smaller oocytes catch up the larger ones, to reach a uniform size, when spawning is imminent [36]. (Figure 4: J).



Fig 5: (A, B) *P. macropus* showing a modified form of parapodia of the posterior part; *P. cultrifera* with unmodified form along the whole body (C); Close up of parapodia from posterior region (D) *P. macropus*, (E) *P. cultrifera*; (F) Jaw; (G) Cross section of an anterior segment; (H) Uniramous parapodium; (I) Biramous parapodium from anterior region; (J) Biramous parapodium from posterior region; (K) Homogomph spiniger chaeta; (L) Heterogomph falciger chaeta; (M) Heterogomph spiniger chaeta; (DC) Dorsal cirrus, (NtDL) Notopodial dorsal ligulle, (NtC) Notochaeta, (NtVL) Notopodial ventral ligulle, (NrC) Neurochaeta, (NrVL) Neropodial ventral ligulle, (VC), (ventral cirrus), (bar scale: 3 mm, bar scale F: 0.5mm), (G, H, I: x 10), (J, K, L: x 40).

In Nereididea, the sexual reproduction is accompanied or not by morphological changes, called epitoky or atoky respectively, in the present study these changes were recorded only for *P. macropus*, the heteronereis stage is fully developed with strong epitokous modifications, greatly enlarged black eyes, flattened posterior parapodia as a result of changes in the parapodial lobes' morphology, with natatory chaetae and paddle for swimming, a change of the body coloration, and a reduction in worms' length and width, resulting from histolysis of correlated segments' muscles (Figure 6), this metamorphosis is a special reproductive form in Nereididae, during which in sexually maturing worms, new tissues differentiate, while old ones degenerate or

transdifferentiate [37], These observations agree with literature on other closer nereids species, where morphological, behavioral and physiological changes correlate with metabolic and biochemical ones [38], epitokous worms are prepared for a short pelagic existence during swarming, where they swim and release gametes on the water surface, days after spawning individual of the two sexes die, this agrees with our observations, the two species are known to be strictly semelparous, they breed once in their life cycle; literature on *P. cultrifera* along the Algerian coast, reported two reproductive patterns, with atokous or epitokous type, according to geographic location [39].



Fig 6: (A) Heteronereis specimen showing modification of the body color; (B) Another one showing morphological modifications of epitoky; Enlarged eyes, and the beginning of a modified region; (D) Close up of flattened parapodia, arrows indicate start of the first modified parapodium; (E) Parapodium from anterior region; (F) Parapodium from posterior region; (G) Natatory chaeta, (DCLL) Dorsal cirrus lamella, (PNOL) Post-chaetal neuropodial lamella, (NtAc) Notoaciculum, (PNLL) Post-chaetal neuropodial lamella, (NrAc) Neuroaciculum, (VCLL) Ventral cirrus lamella, (bar scale: 5 mm); (E, F: x 10), (G: x 40).

4. Conclusion

Individuals of *P. macropus* and *P. cultrifera* were sampled at two stations Collo and Skikda, from hard substrates, to investigate their ecology, biology and reproduction in general, and also to give a description based on these aspects; our observations had clarified a difference between the associated algae species that might explain the absence of *P. macropus*, in Skikda, results of biometric measurements vary between the two species and among sites, some clear morphological variations can be detected such as color, paragnathes' shape and arrangement, and parapodia forms along the body, others require several macro and microscopic observations; oocytes' diameter values and their aspects indicate the maturation's degree of females, where we had easily noticed a heterogamous diameter in maturing females, indicating that oogenesis is asynchronous; concerning epitokal modifications adults of *P. cultrifera* show an atokous character of reproduction, and the two species are semelparous.

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