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Details on the morphological characteristics of marine copepods, *Copilia mirabilis, Sapphirina stellata* and *Macrosetella gracilis*, in Iligan and Sarangani bays, Mindanao, southern Philippines

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## Abstract

*Copilia mirabilis, Sapphirina stellata* and *Macrosetella gracilis* belonging to Families Sapphirinidae and Miraciidae, respectively, were described in detail. Specific morphological characters such as body segmentation, number of segments, setae and spines on the antennule, antenna, maxilla, maxillipeds, paired legs, numbers of spines on the caudal ramus, color and body shape of the organism were used to identify and illustrate these three copepod species. Outlines of male and female body forms and its measurements were included.

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#### Introduction

Copepods group of minute aquatic are crustaceans that closely resembles the shrimps. The body is divided into a wider prosome, located anteriorly and comprised of the head and the segmented metsome or thorax, and a narrower urosome located posteriorly (Mauchline, 1998). They are often semi-transparent, although some species may vary from reddish to green or iridescent depending on the pigments that they accumulate from their food. Their size ranges from 0.2 to 10mm in length, but adults are typically between 1-2mm in length.

They colonized all types of bodies of water including freshwater environments, the surface layers of the sea down to the abyss, and even in leaves of bromeliads (Huys and Boxshall, 1991). Copepods are categorized as one of the important constituents of the zooplankton community and form 75% of the major bulk in the zooplankton biomass (Lacuna *et al.*, 2014; Angara *et al.*, 2013) such that they are considered to be the most abundant planktonic metazoans in the sea and, maybe, on the whole globe (Humes, 1994).

Ecologically, copepods play a crucial role in the marine food webs since they function as link between microscopic unicellular algae to higher trophic level consumers such as fish and are also direct food source of many larvae of fish and other marine animals (Möllmann et al., 2004). In temperate and even some Asian countries (i.e., Japan, Taiwan, Australia), copepods are now identified and used as potential aquaculture live feeds for marine fish because they possess high concentrations of essential nutrients like DHA (Dososahexaenoic acid), EPA (Eicosapentaenoic acid), ARA (Arachidonic acid) that are optimal for the normal development, growth and survival of various fish larvae (Shields et al., 1999; McKinnon et al., 2003; Rajkumar and Vasagam, 2006; Liu and Xu, 2009). On the contrary, the application of copepods as live feeds in Philippine aquaculture industry remains elusive, mainly because taxonomic works are usually incomplete and sometimes unreliable.

This is partly because taxonomy involves tedious work so that this particular field in science is unpopular in the country.

Although copepod taxonomy has been well documented in temperate countries and even in some Asian countries, efforts on marine pelagic copepod taxonomy in the Philippine Archipelago remain ignored by local scientists.

The existing accounts on Philippine marine pelagic copepods were limited to the reports of Lacuna *et al.* (2013a, b, and c), Walter *et al.* (2006), Suarez-Morales (2000), Schizas and Shirley (1994), Walter (1984) and Wilson (1950). To address this gap, this study was carried out in order to increase the information on marine planktonic copepods with the aim in providing accurate description of the morphology of these organisms including drawings and measurements.

Hence, this study was envisioned in order to increase the information on marine planktonic copepods with the aim in providing accurate description of the morphology of these organisms including drawings and measurements.

#### Materials and methods

#### Study Areas

Collection of plankton samples were obtained offshore from several sampling stations established within Iligan and Sarangani Bays (Fig. 1). Iligan bay, which is known for its "U" formation, lies 8°30'31" North Latitude and 123°43'15" East Longitude. Gingoog and Macajalar bays separate Iligan bay from its southern portion, while on the north it is separated by Bohol Sea.

The bays' mouth extends 560 kilometers and has an area of 2,000 square kilometers. Iligan bay holds important marine resources, such as fish, shellfish and algae that are used as food sources to nearby residents occupying the surrounding area of the bay. Sarangani bay, recognized as the richest tuna fishing ground in the country, lies 6°07' North Latitude and 125°06' East Longitude.

The bay is located at the southernmost tip of Mindanao and opens up to the Celebes Sea in the Pacific Ocean. The length of its coastline measures 79 kilometers (from Glan to Maasim) with a depth averaging to about 350 meters and an area of 449.22 square kilometers.

The bay is bordered by lush stands of mangroves and coral reefs which provide covers and protection for invertebrates, permanent and temporary juvenile and adult fish of marketable and recreational value.





Collection, Processing, and Identification of Samples Plankton samples were gathered from the established stations in Iligan and Sarangani bays employing conical plankton net (length: 1.8 m; mouth diameter: 0.45 m; mesh size opening: 300 µm). Several horizontal tows (5 minutes towing) and vertical hauls (from 50m depth) of the net were made and the collected plankton samples were immediately and carefully transferred to polyethylene bottles which were then preserved with 5% buffered formalin/seawater solution. Copepods were taxonomically identified using the following morphological attributes: body segmentation, number of segments, setae and spines on the antennules, antenna, maxillipeds and paired legs.

Copepod specimens were classified down to species level through dissection following the methods of Huys and Boxshall (1991). Separate mounts on the dorsal body and the dissected parts such as the urosome, antennules, antennae, maxillipeds and legs 1-5 were prepared for male and female species utilizing Hoyer's medium as mountant. Measurement of each prepared mounts and counts on the number of segments of the body, urosome, antennules, antennae as well as the number of setae and spines were done by means of a dissecting microscope. Afterwards, drawings were made for each mounted parts that were projected from the micro projector.

Celebes Sea

C

For the measurement, prosome length was evaluated dorsally beginning from the anterior tip of the head to the posterior margin of the last metasomal somite, whereas those of the urosome was quantify commencing from the anterior margin of the genital somite down to the posterior tip of the caudal rami excluding the setae. Each copepod species was classified following the accounts and taxonomic keys of Dakin and Colefax (1940), Kasturirangan (1963), Owre and Foyo (1967), Boxshall and Halsey (2004) and Al-Yamani *et al.* (2011).

### **Results and discussion**

Two pelagic copepods (*Copilia mirabilis* and *Sapphirina stellata*) under Order Cyclopoida of Family Sapphirinidae and one copepod (*Macrosetella gracilis*) under Order Harpacticoida from Family Miraciidae were documented. Comprehensive account on the female and male *Copilia mirabilis*, while female only of the species *Sapphirina stellata* and *Macrosetella gracilis* are presented together with the illustrations and measurements.

Morphological Characteristics Family Sapphirinidae Copilia mirabilis Dana, 1849



**Fig. 3.** (A) Ventral view of female urosome and (B) dorsal view of male urosome of *C. mirabilis* Dana. AS: Abdominal segments, CR: Caudal rami.



**Fig. 4.** (A) female antennule, (B) right and (C) left antennules of male *C. mirabilis* Dana.



**Fig. 2.** Dorsal view of (A) female and (B) male *C*. *mirabilis* Dana. Red arrow points to the median dorsal spine on the 4<sup>th</sup> metasomal segment.

**Fig. 5.** (A) Female and (B) male antenna of *C. mirabilis* Dana. Red arrow points to the claw-like appearance of the  $4^{\text{th}}$  segment.



**Fig. 6.** Male maxilliped of *C. mirabilis* Dana. B: Basis. Red arrow points to the swelling on the inner margin of the basis.



**Fig. 7.** Female swimming (A) leg 1, (B) leg 2, (C) leg 3 and (D) leg 4 of *C. mirabilis* Dana. Red arrow points to the 1-segmented endopod.



**Fig. 8.** Male swimming (A) leg 1, (B) leg 2, (C) leg 3 and (D) leg 4 of *C. mirabilis* Dana. Red arrow points to the 1-segmented endopod.

## 94 | G. Lacuna et al.

### Synonym

Copelia mirabilis, Saraswathy 1966.

#### Occurrence

All stations in Iligan bay contain the male and female species, whereas only the females where observed in Sarangani bay.

#### Description of adult female and male

Body length of female 2.8926 mm, while the male is 3.8 mm. Female specimens (Fig. 2A) slightly depressed and less cylindrical in shape with a quadrangular head outline. The prosome consist of a cephalosome and 5 metasomal segments. The cephalosome is fuse with the 1<sup>st</sup> metasomal segment so that it is broader when compared to the successive metasomal segment where the width of the  $2^{nd}-4^{th}$ metasomal segments decreases progressively.

The 4<sup>th</sup> metasomal segment bears a median dorsal spine pointing downwards (Fig. 2A as indicated by the red arrow). The head or cephalosome bears a pair of frontal lenses located near its corners (Kasturirangan, 1963) and separated by at least twice as the diameter of one lens (Owre and Foyo, 1967). Male samples (Fig. 2B) have flattened deeply depressed, broad body and phylloid or leaf-like shape (Kasturirangan, 1963). The prosome include the cephalosome and 4 metasomal segments with no frontal lenses present on the head. Live specimens were transparent in both sexes at the time of collection. The urosome in female is 5-segmented (Fig. 3A) with abdominal/urosomal segments 1-3 forming the genital somites. In male, the urosome has 5 abdominal segments where the widths gradually decrease. Caudal rami are elongated to a great extent and styliform for both sexes (Fig. 3A,B).

Antennule is uniramous and is less than 10 segments in both sexes. In female, both antennules are 7segmented (Fig. 4A) with the 1<sup>st</sup> segment bears 1 outer seta, the 2<sup>nd</sup> segment has 2 outer setae, the 3<sup>rd</sup> segment bears 3 outer setae, the 4<sup>th</sup> segment bears 2 setae, the 5<sup>th</sup> segment has 3 setae, the 6<sup>th</sup> segment has 2 setae while the last segment has 4 setae. The left and right antennules measures 0.3999 mm and 0.4132 mm, respectively. In male, both antennules are 5-segmented (Fig. 4B, C). On the right antennule (Fig. 4B), 1<sup>st</sup>-5<sup>th</sup> segments include 3, 3, 2, 2, and 4 setae, respectively. For the left antennule (Fig. 4C), 1<sup>st</sup>-5<sup>th</sup> segments have 2, 11, 2, 1 and 3 setae, respectively. Both antennules measure 0.33 mm.

The female antenna is uniramous, slender, prehensile and 4-segmented (Kasturirangan 1963) where the 4<sup>th</sup> segment has a claw-like appearance (Fig. 5A). One large spine and ornamentation of spinules (22) are visible along the inner margin of the 1st segment (Boxshall and Halsey, 2004). A spinulate spine (about 3 spines) is prominent at the base of the 2<sup>nd</sup> segment. Three smaller spines are also noticeable on the 3rd segment. The male antenna is uniramous, 4segmented and thinner than the female (Fig. 5B). All segments are devoid of seta and spine except for the 2<sup>nd</sup> segment where a single spine is present. Male antenna is longer than the female and measures 1.3 mm and 0.9998 mm, respectively. Maxilliped is only present in male (Fig. 6). It is 2-segmented with robust protuberance distally on the inner margin of the basis (Dakin and Colefax, 1940; Owre and Foyo, 1967; Boxshall and Halsey, 2004).

Swimming legs 1-4 of both sexes are biramous (Fig. 7 A-D and 8 A-D) with legs 1-3 having 3-segmented exopodites and endopodites, whereas leg 4 comprise of 3-segmented exopod and 1-segmented endopod (Boxshall and Halsey, 2004). The endopod in leg 4 (Fig. 7D and 8D) of both sexes has the same length with the 1<sup>st</sup> segment of the exopod (Kasturirangan, 1963). The morphological characters illustrated and described for *C. mirabilis* are comparable with the works of Lehnhofer (1926), Dakin and Colefax (1940), Kasturirangan (1963), Owre and Foyo (1967), Ramirez (1971), Chen and Zhang (1974), Boxshall and Halsey (2004) and Al-Yamani *et al.* (2011).

### Remarks

*C. mirabilis* was also recorded in the following Philippine Islands: Off Southern Luzon, off Santa Cruz, West coast Luzon, off Corregidor, East of Mindoro, South of Romblon, Marinduque to Luzon, between Panay and Negros, between Panay and Guimaras, between Cebu and Leyte, between Bohol and Leyte, East Coast of Negros, Pujada Bay Mindanao, Tawi-Tawi Group in Sulu Archipelago, off Jolo Sea in Mindanao (Wilson, 1950).

Sapphirina stellata Giesbrecht, 1891.



**Fig. 9.** (A) Dorsal view and (B) urosome of female *S. stellata* Giesbrecht. Red arrow points to the tooth-like projection of the caudal ramus.



**Fig. 12.** Female swimming (A) leg 1, (B) leg 2, (C) leg 3, (D) leg 4 of *S. stellata* Giesbrecht.



**Fig. 10.** (A) Left and (B) right antennule of female *S*. *stellata* Giesbrecht. Red arrow points to the strong, curved spine on segments 3 and 4.



**Fig. 11.** (A) Left and (B) right antenna of female *S*. *stellata* Giesbrecht.

## Synonym

Saphirina ovalis Brady, 1883; Sapphirina setellata Lan et al., 2004.

#### Occurrence

Female species were commonly encountered in the vertical waters in all stations in Sarangani bay. Males were not observed in the samples.

## Description of adult female

Body length of female is 1.6663 mm. The body is obovate and flat. Live samples during collections are semi-transparent. The prosome is made up of cephalosome and 5-segmented metasomal segments (Fig. 9A). The cephalosome or head contain a pair of small lenses and with a width that is almost the same with the 1<sup>st</sup> metasomal segment that gradually decreases as it reach the 4<sup>th</sup> segment. The urosome is 5-segmented (Fig. 9B). The 1<sup>st</sup> segment have no curved or pointed posterior margin unlike the rest of the urosomal segments. The width of the 2<sup>nd</sup> segment down to the anal segment decreases gradually. The caudal ramus is relatively broad but is not truncate distally and bears a minute semi-sharp marginal point or tooth-like projection on the inner portion (indicated by a red arrow) situated below the apex and not reaching the terminal setae (Boxshall and Halsey, 2004). Each caudal ramus bears 2 outer and 2 terminal setae that are slender. The urosome measures 0.2666 mm.

The antennules are uniramous and lack any geniculation. It is 5-segmented on both sides (Fig. 10 A,B ). For the left antennule, the 1<sup>st</sup> segment have no seta or spine, the 2<sup>nd</sup> segment have 10 setae, both the 3<sup>rd</sup> and 4<sup>th</sup> segments bears 1 seta and 1 strong, curved spine (indicated by a red arrow, Owre and Foyo, 1967; Boxshall and Halsey, 2004) while the 5<sup>th</sup> segment bears 2 terminal setae. On the right antennule, the 1<sup>st</sup> segment have no seta or spine, the 2<sup>nd</sup> segment has 10 setae, both the 3<sup>rd</sup> and 4<sup>th</sup> segments bear 1 seta and 1 strong, curved (indicated by a red arrow, Owre and Foyo, 1967; Boxshall and Halsey, 2004) while the 5<sup>th</sup> segment has 10 setae, both the 3<sup>rd</sup> and 4<sup>th</sup> segments bear 1 seta and 1 strong, curved (indicated by a red arrow, Owre and Foyo, 1967; Boxshall and Halsey, 2004) while the 5<sup>th</sup> segment bears 4 terminal setae. The left and right antennules measure 0.2533 mm and 0.2666 mm, respectively.

The antenna is uniramous and looks like a maxilliped. Both sides are 4-segmented (Fig. 11 A,B) with the 1<sup>st</sup> and 3<sup>rd</sup> segments without a seta, the 2<sup>nd</sup> segment bears 2 inner spines while at the terminal end of the 4<sup>th</sup> segment is a claw. The 2<sup>nd</sup> segment is notably larger than the rest of the segments.

The swimming legs 1-4 are biramous and consist of a coxa, a basis, a 3-segmented exopod and endopod (Fig. 12 A-D). The 1<sup>st</sup> leg (Fig. 12A) include a coxa without any spine or seta and a basis with only 1 outer seta.

The 1<sup>st</sup> exopodal segment comprise of 1 outer spine, the 2nd segment bears 1 outer spine and 1 inner seta while the 3rd segment have 2 outer spines, 1 terminal spine and 5 inner setae. For the endopod, the 1st and 2<sup>nd</sup> segments contain 1 inner seta while the 3<sup>rd</sup> segment bears 5 inner setae and 1 terminal spine. For the 2<sup>nd</sup> leg, the coxa and the basis are devoid of seta or spine (Fig. 12B). The 1st and 2nd exopodal segments are the same with that of the 1st leg but the 3rd exopodal segment carry 2 outer spines, 1 terminal spine and 6 inner setae. For the endopod, the 1st segment is the same with the 1st leg but the 2nd segment have 2 inner setae while the 3rd segment include 4 inner setae, 1 terminal seta and 1 outer seta. For the 3<sup>rd</sup> leg, the coxa consists of 1 inner seta but the basis doesn't bear any seta or spine (Fig. 12C). The 1st and 2<sup>nd</sup> exopodal segments are the same with that of the 1st leg but the 3rd exopodal segment have 2 outer spines, 1 terminal spine and 6 inner setae. For the endopod, the 1st segment is the same with the 1st leg but the 2nd segment comprise of 2 inner setae. The 3rd segment includes 2 inner setae and 1 terminal spine. The 4<sup>th</sup> leg bears a coxa and a basis without any projection (Fig. 12D). The endopod is shorter than the exopod. The exopod measures 0.2000 mm while the endopod is only 0.1733 mm.

The exopodal  $1^{st}$  and  $2^{nd}$  segments and the  $1^{st}$  endopodal segment are the same with  $3^{rd}$  leg. The  $3^{rd}$  exopodal segment consists of 1 outer spine, 1 terminal spine and 6 inner setae. The  $1^{st}$  - $3^{rd}$  endopodal segments include only 1 inner seta.

The taxonomic characteristics presented for *S. stellata* are comparable with the descriptions of Lehnhofer (1929), Dakin and Colefax (1940), Owre and Foyo (1967) and Chen and Zhang (1974).

#### Remarks

*S. stella* was also recorded in the following Philippine Islands: Off Santa Cruz, Pujada Bay in Mindanao (Wilson, 1950).

Family Miraciidae Macrosetella gracilis Dana, 1848.



**Fig. 13.** (A) Dorsal view and (B) urosome of female *M. gracilis* Dana.



**Fig. 14.** (A) Left and (B) right antennule of female *M*. *gracillis* Dana.



**Fig. 15.** (A) Left and (B) right antenna of female *M*. *gracilis* Dana.



**Fig. 16.** (A) Left and (B) right maxilliped of female *M. gracilis* Dana.



Fig. 17. Swimming (A) leg 1, (B) leg 2, (C) leg 3, (D) leg 4 and (e) leg 5 of female M. gracilis Dana.

## 98 | G. Lacuna et al.

The urosome is 4-segmented (Fig. 13B) where caudal rami much longer than the last 2 abdominal/ urosomal somites combined (Al-Yamani *et al.*, 2011) and apical setae as long as the whole body (kasturirangan, 1963, Al-Yamani *et al.*, 2011).

The antennules are short and at most 9-segmented (Fig. 14 A,B), a characteristic distinguishable among harpacticioids. On the left antennule (Fig. 14A), the 2<sup>nd</sup> segment bears 2 setae, the 3<sup>rd</sup> segment have 1 seta, the 4<sup>th</sup> segment includes 2 setae while the 5<sup>th</sup> segment contain 1 seta. The rest of the segments lack any seta. For the right antennule (Fig. 14B), the 4<sup>th</sup> and last segments bear 1 seta, the 8<sup>th</sup> segment is made up of 2 setae while the rest of the segments are devoid of setae.

The antennae are uniramous and 2-segmented (Fig. 15 A,B) each of which has 4 apical setae with the 2 being much shorter. Both antennae do not have exopod. The maxillipeds are 3-segmented with the last segment forming a claw. The 2<sup>nd</sup> segment is setiferous (Fig. 16).

The swimming legs 1-5 are biramous. The first leg (Fig. 17A) include a 3-segmented exopod and a 2segmented endopod. The 1st and 2nd exopodal segments bear 1 outer seta while the 3rd segment has 1 small outer seta and 2 terminal setae. For the endopod, the 1st segment is slender and longer, as long as 2 exopodal segments combined (indicated by red arrows). The 2<sup>nd</sup> segment bears 1 inner seta and 2 apical setae of unequal length. For the 2<sup>nd</sup> leg (Fig. 17B), it is comprised of a 3-segmented exopod and endopod. The 1st exopodal segment is devoid of any seta, the 2<sup>nd</sup> segment bears 2 outer setae while the 3<sup>rd</sup> has 2 outer setae and 1 inner seta. For the endopod, the 1st segment contain 1 inner seta, the 2nd segment possess 1 outer seta and 1 inner setae while the 3rd segment bears 3 outer setae, 1 inner seta and 2 apical setae. For the 3<sup>rd</sup> leg (Fig. 17C), it is composed of a 3-segmented exopod and endopod. The 1st exopodal segment bears 1 outer seta, the 2<sup>nd</sup> segment have an outer and inner seta, while the 3rd segment include 2 small outer setae, 4 inner setae and 1 apical seta.

The 1st and 2nd endopodal segments bear 1 inner seta while the 3rd segment contains 1 small outer seta, 3inner setae and 1 apical seta. For the 4th leg (Fig. 17D), it has a 3-segmented exopod and endopod. The 1<sup>st</sup> exopodal segment 1 possess 1 outer seta, the 2<sup>nd</sup> segment comprise of an outer and inner seta, while the 3rd segment bears 2 outer and 5 inner setae. The 1st and 2nd endopodal segments bear 1 inner seta, while the 3rd segment includes 4 inner setae, an outer and apical seta. For the 5th leg (Fig. 17E), the basis and endopod are fused as baseoendopod (indicated by a red arrow, Al-Yamani et al., 2011). The basal segment, which display an inner growth or expansion (Kasturirangan, 1963, Al-Yamani et al., 2011), possess 4 apical setae of different length with the second inner seta being the longest. The exopod segment is slender and elongated and possesses 3 outer edge setae and 3 apical setae with the 2 inner setae of the same length. The taxonomic characteristics presented for *M. gracilis* are comparable with the descriptions of Kasturirangan (1963), Owre and Foyo (1967), Chen et al. (1974) and Al-Yamani et al. (2011),

## Remarks

*M. gracilis* was also recorded in the following Philippine Islands: Off Romblon, Mindoro Strait, Malampaya Sound in Palawan, Eastern Palawan, off Western Samar, North of Cebu, between Leyte and Mindanao, between Siquijor and Bohol, West Mindanao, Jolo Sea in Mindanao (Wilson, 1950).

## Conclusion

*Copilia mirabilis* of both sexes were one of the frequently seen copepod species in the waters of Iligan and Sarangani Bays. However, only the female species of *Macrosetella gracilis and Sapphirina stellata* were encountered in the vertical waters of Sarangani bay and even rarely sampled in Iligan Bay. The copepod species we identified were similar morphologically with those reported in other bodies of water such as China Sea, Japan Sea, Australia, Indian Coast, the Caribbean and Northwestern Arabian Gulf indicating exchange and mixing of large masses of water occurring in these bodies of water brought about by major ocean currents.

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