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Corresponding author:






Nuno Simões;

Email: ns@ciencias.unam.mx

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Sea slugs (Gastropoda: Heterobranchia) associated with Autonomous Reef Monitoring Structures (ARMS) in southern Gulf of Mexico and Mexican Caribbean Sea

Xochitl G. Vital^{1,2} , Lilian A. Palomino-Alvarez^{2,3} , Deneb Ortigosa^{4,5} ,
Edlin J. Guerra-Castro^{5,6}  and Nuno Simões^{2,5,7} 

¹Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México, Unidad de Posgrado, Edificio D, 1^o Piso, Circuito de Posgrados, Ciudad Universitaria, Alcaldía Coyoacán, C.P. 04510, Ciudad de México, Mexico;

²Unidad Multidisciplinaria de Docencia e Investigación Sisal (UMDI-SISAL), Facultad de Ciencias, Universidad Nacional Autónoma de México, Sisal, Yucatán, Mexico; ³Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Av. Ciudad Universitaria 3000, C.P. 04510, Coyoacán, Ciudad de México, Mexico;

⁴Colección Nacional de Moluscos, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, Mexico; ⁵Laboratorio Nacional de Resiliencia Costera, CONACYT, Ciudad de México, Mexico; ⁶Departamento de Sistemas y Procesos Naturales, Escuela Nacional de Estudios Superiores Unidad Mérida, Universidad Nacional Autónoma de México, Mérida, Yucatán, Mexico and ⁷Harte Research Institute for Gulf of Mexico Studies, Texas A&M University–Corpus Christi, Corpus Christi, TX, USA

Abstract

Cryptic species in coral reefs, such as sea slugs, represent an important portion of their biodiversity, which is usually underestimated. Autonomous Reef Monitoring Structures (ARMS) have been implemented to estimate cryptic diversity in coral reefs. Therefore, this research aimed to contribute to the southern Gulf of Mexico (GM) and the Caribbean Sea (CAR) coral reefs' sea slugs' diversity and distribution using ARMS as a collection method. Fifty-eight ARMS were placed at three coral reefs in the GM and CAR, recovered after 1–2 years and then, disassembled at the laboratory. Plates were individually placed in trays with seawater, where we searched for sea slugs. A total of 242 organisms were found belonging to 31 species; 20 of them were identified to the species level, while 11 were determined up to genus or family. More than half of the species (19) were found in Bajo de 10 (GM), while 15 species were found in the CAR localities. Unlike previous studies, we assessed sea slugs' diversity exclusively by an indirect sampling method. In this work, we found 9.4% of the sea slug diversity recorded in the Caribbean, and we report four determined species for the first time in the country. New records of species, and even one family for the GM stress the gap of information that we still need to fulfil in the area. We recognize ARMS as a useful tool to find juvenile, cryptic and rare species of sea slugs, as well as to standardize their quantification and record their diversity.

Introduction

Invertebrates in coral reefs represent a critical ecological role in biodiversity because they can be engaged in intricate relationships, impacting the reef integrity and structure (Glynn and Enochs, 2011). To know these complex interactions and how they can affect the coral reefs, we need more information regarding invertebrates, including describing their diversity. Diversity in these ecosystems has been measured mostly with well-studied (e.g. corals) or visible macrofauna, but few studies address small invertebrates, which are harder to find, and whose taxonomy is less known (Plaisance *et al.*, 2011). The biodiversity associated with coral reefs is usually miscalculated because numerous areas are poorly studied or sampled, and most of the reef species are cryptic, small-sized organisms living in holes or cracks, sometimes being nocturnal and/or well camouflaged in their habitat, then unnoticed (Glynn and Enochs, 2011).

Cryptic species in coral reefs, such as sea slugs, represent an important portion of reef biodiversity (Plaisance *et al.*, 2011; Jensen, 2013). Describing the diversity of sea slugs is difficult since their recording and identification require experience in collecting techniques, and a trained eye to locate them, as they are very small and well-camouflaged (Jensen, 2013; Goodheart *et al.*, 2016). Worldwide, most of the recorded species of sea slugs are located in coral reefs of the Indo-Pacific Ocean, where it is estimated that 15–40% of the species are still undescribed (Gosliner *et al.*, 2018). In the Gulf of Mexico (GM) and the Caribbean Sea (CAR), *circa* 350 species are present (Valdés *et al.*, 2006; García and Bertsch, 2009; Rosenberg *et al.*, 2009; Redfern, 2013); the Caribbean is considered one of the richest regions regarding sea slugs (García and Bertsch, 2009). Many species complex have been described recently in the region and divided into new species for different groups of sea slugs (e.g. Ornelas-Gatdula *et al.*, 2012; Krug *et al.*, 2016; Ghanimi *et al.*, 2020; García-Méndez *et al.*, 2022). Records in the Mexican Atlantic coast are mostly from the southern GM (Campeche

Bank) on the reefs: Cayo Arcas and Cayo Arenas (Ortigosa and Simões, 2019), Sisal, Madagascar and Serpiente (Ortigosa *et al.*, 2013) and Alacranes (Sanvicente-Añorve *et al.*, 2012b; Ortigosa *et al.*, 2015); while in the Mexican CAR coast records are scattered in a few localities from new species publications, sea slug guides or books from the tropical northwestern Atlantic (Ortea and Espinosa, 1996; Valdés *et al.*, 2006; Ortea and Bacallado, 2007; Ortea *et al.*, 2014) and molluscan inventories for the Yucatan Peninsula (Vokes and Vokes, 1983).

Two common collecting methods for this group of gastropods have been used: (1) a direct method, where a visual and careful search for sea slugs is made on suitable substrates preferred by these animals. This method is dependent on observation time and expertise. Also, (2) an indirect method that involves collecting all potential substrates where the slugs could be found and left in white trays until the level of oxygen decreases, and then, they are reviewed thoroughly to find cryptic as well as non-cryptic species (Goodheart *et al.*, 2016). However, the diversity found with the latter method is strongly dependent on the type of substrate, and it implies potential damage to the ecosystem. An indirect method that could be an alternative for the study of the diversity of sea slugs is the Autonomous Reef Monitoring Structures (ARMS). These structures mimic reef complexity and are easy to manipulate and collect (Ransome *et al.*, 2017); also, they are considered a non-invasive standardized sampling method enabling the comparison of results between different regions (Pearman *et al.*, 2020).

ARMS have been implemented to estimate cryptic diversity in coral reefs (Zimmerman and Martin, 2004; Ip *et al.*, 2022). They provide an ideal artificial substrate for the settlement of benthic groups, such as sponges, tunicates, bryozoans and algae (known substrates for sea slugs), and consequently, could increase the incidence of these gastropods (Palomino-Alvarez *et al.*, 2021a, 2021b). Nevertheless, this methodology has never been considered as an indirect approach to assess the diversity of this group of molluscs. Therefore, this research aimed to contribute to the southern GM and the Mexican CAR coral reefs' sea slugs' diversity and distribution records using ARMS as an indirect, standardized and comparable collection method.

Materials and methods

A total of 58 ARMS were placed at a depth of 3–7 m for 1–2 years between October 2018 and July 2021 at Bajo de 10 reef in the southern GM (21°20'53.82" N, 90°8'45.48" W), La Bonanza reef in Puerto Morelos (20°57'53.54" N, 86°48'52.194" W) and Mahahual reef in the Mexican CAR (18°37'24" N, –87°43'32" W) (Figure 1). All sampling units were retrieved covering them with a 500 µm mesh underwater, bringing them to the surface and transported to the laboratory in individual boxes with aerated seawater for processing. Each ARMS was disassembled, and each plate was placed in a tray with seawater, where we searched exhaustively for sea slugs (Palomino-Alvarez *et al.*, 2021a).

All sea slugs were photographed, when possible, and then anaesthetized in seawater with magnesium chloride (5–7%) for at least 1 h; later, they were preserved in glass containers with 70 or 96% ethanol. Specimens were deposited at Colección Regional de Moluscos de la Península de Yucatán (SEMARNAT: YUC-INV-240-01-11) in Facultad de Ciencias, UNAM, Sisal, Yucatan, Mexico. Species determination of live organisms was made with identification guides or recent literature (Valdés *et al.*, 2006; Redfern, 2013; Krug *et al.*, 2016; Ghanimi *et al.*, 2020); nomenclature follows MolluscaBase eds. (2023), except for Fionidae (Cella *et al.*, 2016). Results include the number of organisms found, approximate maximum body length,

localities where they were recorded, collection numbers, a brief description of the external morphology and remarks for all the undetermined species, and which need further confirmation. The distribution of all recorded species is shown in Table 1.

Results

A total of 242 organisms were found in 58 ARMS, belonging to 31 species; 20 were identified to the species level, while 11 were adult specimens determined up to genus or a higher taxonomic level. Juvenile organisms were found and were identified to a possible genus, representing seven potential additional species (Table 1). Nudibranchia was the group with the largest number of species recorded (58%), followed by Sacoglossa (22.6%), Cephalaspidea and Anaspidea (6.4%), and Pleurobranchida and Runcinida (3.2%); the juveniles were not considered in these percentages to reduce bias. More than half of the species (19) were found in Bajo de 10 (GM), while 15 were found in the Caribbean: 10 in Puerto Morelos and 5 in Mahahual. The only species simultaneously recorded in the three reefs was *Elysia velutinus* Pruvot-Fol, 1947. Even though Puerto Morelos and Mahahual belong to the same region, they only shared *Elysia flava* Verrill, 1901 and possibly *Jorunna* cf. *spazzola* (Er. Marcus, 1955), as potential juveniles of the species were found in both localities. The remaining species were recorded exclusively in one locality (Table 1).

Phylum MOLLUSCA Linnaeus, 1758
 Class GASTROPODA Cuvier, 1795
 Subclass HETEROBRANCHIA Burmeister, 1837
 Order CEPHALASPIDEA P. Fischer, 1863
 Family RETUSIDAE Thiele, 1925
 Genus *Retusa* T. Brown, 1827
Retusa sp.
 (Figure 2A, B)

Material Examined

Ten organisms (3 mm), Bajo de 10 (CMPY-005728, CMPY-005740-41, CMPY-005755).

Description

Body colour translucent white with a dark area showing through the shell. Head with a cephalic shield, two lateral lobes and a frontal notch. Shell oval, wider in the centre with convex sides, of white colour; apex with a narrow umbilicus; aperture lip with a wing connected to the columellar margin; aperture has the length of the shell; sculpture reticulated, with spiral lines crossed by growth lines.

Remarks

Specimens resemble *Retusa* sp. 1 in Valdés *et al.* (2006) and *Retusa* sp. in Redfern (2013).

Family HAMINOEIDAE Pilsbry, 1895
 Genus *Haminoea* Turton & Kingston (in Carrington), 1830
Haminoea sp.
 (photo not available)

Material Examined

One organism (3 mm), Bajo de 10 (CMPY-004455).

Description

Body colour opaque white. Shell translucent white, globose and fragile, with light spiral grooves crossed by fine axial growth lines; apex with a depression; wide aperture.

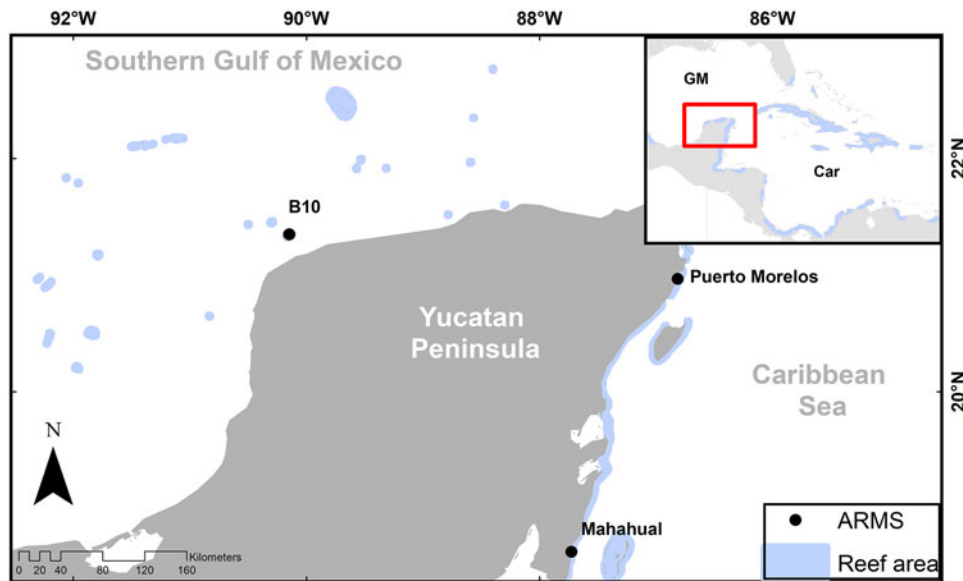


Figure 1. Localities where ARMS were implemented in southern GM (Bajo de 10 = B10) and Mexican Caribbean (Puerto Morelos and Mahahual). Reef area data are from Burke and Maidens (2004).

Remarks

Specimen similar to *Haminoea* sp. D in Redfern (2013). Could be a juvenile specimen.

Order RUNCINIDA Burn, 1963
 Family RUNCINIDAE H. Adams & A. Adams, 1854
 Genus *Lapinura* Er. Marus & Ev. Marcus, 1970
Lapinura sp.
 (Figure 2D)

Material Examined

Five organisms (2 mm), Puerto Morelos (CMPY-005770, CMPY-005838).

Description

Body elongated and narrow except in the centre which is slightly wider; background colour dark brown, the largest specimen (2 mm) had white iridescent blotches in the middle and margin of the notum; a green specimen was found with an orange tint in the posterior area of the notum (Figure 2D). Dorsum smooth. Posterior end of the body with a notch, and a small external translucent shell. In the green specimen, gill was visible in the notch and had at least four leaves, same coloration as the body. Eyespots visible.

Remarks

A *Lapinura* undescribed species of black colour had been previously recorded in Cozumel, Mexico by Valdés *et al.* (2006) as *Runcina*; no detailed description was provided, and it does not coincide with the specimens found in this study. Description and photographs of *Lapinura divae* in Redfern (2013) resemble our specimens; however, the original description of this species does not mention any blotches or spots over the dorsum or in any part of the body (Ev. Marcus and Marcus, 1963).

Order PLEUROBRANCHIDA
 Family PLEUROBRANCHIDAE Gray, 1827
 Genus *Berthella* Blainville, 1824
Berthella sp.
 (Figure 2H)

Material Examined

One organism (3 mm), Mahahual (CMPY-004515).

Description

Body oval with an internal plate-like shell translucent white, very fragile; body colour translucent white. Rhinophores rolled, V-shaped. Gill on the right side of the body.

Remarks

This was a juvenile organism. Recently, Ghanimi *et al.* (2020) described two *Berthella*'s species distributed in the Caribbean: *Berthella vialactea* and *Berthella nebula*, and mentioned some morphological differences that can help discern between them. However, the juvenile characteristics and the partial damage of this organism when it was found on the tray did not allow the identification to the species level.

Order NUDIBRANCHIA Cuvier, 1817
 Family AEGIRIDAE P. Fischer, 1883
 Genus *Aegires* Lovén, 1844
Aegires cf. *ortizi* Templado, Luque & Ortea, 1987
 (Figure 2I)

Material Examined

One organism (6 mm), Puerto Morelos (CMPY-005771).

Description

Body elongated, broader at the centre and with a head relatively rounded; background colour greyish brown, covered with white dots and brown patches. Dorsum with many spiculate tubercles almost arranged in lines, and spicules visible throughout the body. Rhinophores smooth, of the same colour of the body, with a brown ring located $\frac{1}{4}$ before the distal end; each protected by three anterior tubercles. Gills covered by processes, with white tips. Eyespots visible in front of rhinophores at the base of tubercles.

Remarks

This specimen matched most of *Aegires ortizi*'s description, including the structures protecting the rhinophores and gills, except by the distribution of tubercles and shape, as well as the

Table 1. Sea slug fauna found in ARMS in coral reefs from the GM (Bajo de 10) and the Caribbean (Puerto Morelos and Mahahual)

| Taxon | Number of organisms | | | Recorded distribution | References and notes |
|--|---------------------|----|-----|---|--|
| | B10 | PM | MAH | | |
| Cephalaspidea | | | | | |
| <i>Retusa</i> sp. | 10 | | | Bahamas, Mexico (GM*), Venezuela | Valdés <i>et al.</i> (2006) as <i>Retusa</i> sp. 1, Redfern (2013) as <i>Retusa</i> sp. Appears to be an undescribed species |
| <i>Haminoea elegans</i> (Gray, 1825) | | 3 | | Bahamas, Belize, Bermuda, Bonaire, Brazil, Colombia, Costa Rica, Cuba, Curaçao, Grenada, Guadeloupe, Honduras, Jamaica, Martinique, Mexico (GM, CAR*), Puerto Rico, St. Lucia, St. Vincent and the Grenadines, Tobago, Trinidad, United States, Venezuela, Virgin Islands | Chávez <i>et al.</i> (1970); Ekdale (1974); Hicks <i>et al.</i> (2001); Valdés <i>et al.</i> (2006); Ortea <i>et al.</i> (2012); Sanvicente-Añorve <i>et al.</i> (2012b); Zamora-Silva and Ortigosa (2012); Redfern (2013); Lamy and Pointier (2017); Ortea and Buske (2018) |
| <i>Haminoea</i> sp. | 1 | | | Not included because it is an undetermined species | Resembles <i>Haminoea</i> sp. D in Redfern (2013). Could represent a juvenile |
| Runcinida | | | | | |
| <i>Lapinura</i> sp. | | 5 | | Mexico (CAR*) | Appears to be an undescribed species, no previous records found |
| Anaspidea | | | | | |
| <i>Stylocheilus polyomma</i> (Mörch, 1863) | | | 1 | Aruba, Bahamas, Barbados, Belize, Bermuda, Bonaire, Brazil, Cayman Islands, Colombia, Costa Rica, Curaçao, Guadeloupe, Grenada, Jamaica, Martinique, Mexico (GM, CAR*), Panama, Puerto Rico, St. Vincent and the Grenadines, United States, Venezuela, Virgin Islands | Valdés <i>et al.</i> (2006); Ortea <i>et al.</i> (2012); Sanvicente-Añorve <i>et al.</i> (2012b); Ortigosa <i>et al.</i> (2013); Ortea and Buske (2018) as <i>Stylocheilus striatus</i> ; Bazzicalupo <i>et al.</i> (2020) |
| <i>Notarchus punctatus</i> Philippi, 1836 | 1 | | | Bahamas, Belize, Costa Rica, Guadeloupe, Jamaica, Martinique, Mexico (GM*), St. Vincent and the Grenadines | Valdés <i>et al.</i> (2006); Rosenberg <i>et al.</i> (2009); Ortea <i>et al.</i> (2012); Redfern (2013); Camacho-García <i>et al.</i> (2014); Lamy and Pointier (2017); Ortea and Buske (2018) |
| Pleurobranchida | | | | | |
| <i>Berthella vialactea</i> Ghanimi, Schrödl, Goddard, Ballesteros, Gosliner & Valdés, 2020 | | 1 | | Jamaica, Martinique, Mexico (CAR*), Panama, Puerto Rico | Ghanimi <i>et al.</i> (2020) |
| <i>Berthella</i> sp. | | | 1 | Not included because it is an undetermined species | Juvenile (found in February) |
| Nudibranchia | | | | | |
| <i>Aegires cf. ortizi</i> Templado, Luque & Ortea, 1987 | | 1 | | Bahamas, Cayman Islands, Cuba, Guadeloupe, Mexico (CAR*), Panama, Venezuela | Ortea <i>et al.</i> (1990, 2012); Valdés <i>et al.</i> (2006); Goodheart <i>et al.</i> (2016) |
| <i>Felimida clenchi</i> (H. D. Russell, 1935) | 4 | | | Bermuda, Cayman Islands, Colombia, Costa Rica, Curaçao, Jamaica, Mexico (GM), Panama, St. Lucia, St. Vincent and the Grenadines, United States, Venezuela | Valdés <i>et al.</i> (2006); Ortigosa <i>et al.</i> (2013); Caballer-Gutiérrez <i>et al.</i> (2015); Goodheart <i>et al.</i> (2016); Padula <i>et al.</i> (2016) |
| <i>Felimida</i> sp. 1 | 1 | | | Mexico (GM) | Ortigosa <i>et al.</i> (2013) as <i>Chromodoris regalis</i> . Appears to be an undescribed species |
| <i>Felimida</i> sp. 2 | 2 | | | Not included because it is an undetermined species | Juveniles (found in October) |
| <i>Felimare bayeri</i> Ev. Marcus & Er. Marcus, 1967 | 1 | | | Belize, Cuba, Guadeloupe, Mexico (GM, CAR), Panama, United States | Valdés <i>et al.</i> (2006); Ortea <i>et al.</i> (2012); Ortigosa <i>et al.</i> (2015) |
| <i>Felimare ruthae</i> (Ev. Marcus & Hughes, 1974) | | 3 | | Antigua, Aruba, Bahamas, Barbados, Costa Rica, Cuba, Curaçao, Grenada, Guadeloupe, Jamaica, Martinique, Mexico (GM, CAR*), Puerto Rico, St. Lucia, St. Martin, Venezuela, Virgin Islands | Ortea <i>et al.</i> (1996); Valdés <i>et al.</i> (2006); Rosenberg <i>et al.</i> (2009); Ortigosa <i>et al.</i> (2013); Redfern (2013); Ortigosa <i>et al.</i> (2015); Ortea and Buske (2018) |
| <i>Felimare</i> sp. 1 | 2 | | | Not included because it is an undetermined species | Juveniles (found in August) |

| | | | | | |
|--|-----|---|--|---|---|
| <i>Felimare</i> sp. 2 | 1 | | Not included because it is an undetermined species | Juvenile (found in April) | |
| Hallaxa sp. | 1 | | Mexico (GM ¹) | The genus <i>Hallaxa</i> and the family it belongs to (Actinocyclusidae) had not been recorded previously in the GM | |
| Discodorididae sp. | | 1 | Bahamas, Mexico (CAR*) | Redfern (2013) as Discodoridid sp. A | |
| Jorunna cf. spazzola (Er. Marcus, 1955) | 6 | 1 | 1 | Barbados, Brazil, Cuba, Curaçao, Honduras, Mexico (GM, CAR*), Turks and Caicos, United States, Virgin Islands | Valdés <i>et al.</i> (2006); Ortigosa <i>et al.</i> (2013); Ortea and Buske (2018). Specimens from the CAR appear to be juveniles (found in February and April) |
| Taringa telopia Er. Marcus, 1955 | 1 | | | Aruba, Bahamas, Barbados, Brazil, Cuba, Dominican Republic, Martinique, Mexico (GM ¹), Panama, St. Lucia | Valdés <i>et al.</i> (2006); Redfern (2013); Ortea <i>et al.</i> (2017); Ortea and Buske (2018); Donohoo and Gosliner (2020). The genus <i>Taringa</i> had not been recorded previously in the GM |
| Doto sp. | 112 | | | Bahamas, Mexico (GM*) | Redfern (2013) as <i>Doto</i> sp. D. Appears to be an undescribed species |
| <i>Flabellina</i> dushia (Ev. Marcus & Er. Marcus, 1963) | 2 | | | Bahamas, Curaçao, Jamaica, Martinique, Mexico (GM), United States | Valdés <i>et al.</i> (2006); Ortigosa <i>et al.</i> (2013); Redfern (2013) |
| <i>Flabellina</i> engeli Ev. Marcus & Er. Marcus, 1968 | 1 | | | Barbados, Colombia, Costa Rica, Cuba, Curaçao, Grenada, Martinique, Mexico (GM), Puerto Rico, St. Lucia, United States | Valdés <i>et al.</i> (2006); Ortigosa <i>et al.</i> (2013); de la Cruz-Francisco <i>et al.</i> (2017) |
| Learchis sp. 1 | 8 | | | Mexico (GM*) | Appears to be an undescribed species, no previous records found |
| <i>Learchis</i> sp. 2 | 5 | | | Mexico (GM) | Ortigosa <i>et al.</i> (2013) as <i>Aeolidiella</i> sp. 1. Appears to be an undescribed species or juveniles (found in April and November) |
| <i>Phidiana</i> lynceus Bergh, 1867 | 43 | | | Aruba, Bahamas, Barbados, Bonaire, Brazil, Colombia, Costa Rica, Curaçao, Guadeloupe, Jamaica, Martinique, Mexico (GM, CAR), Panama, St. Lucia, St. Martin, St. Vincent and the Grenadines, United States, Venezuela, Virgin Islands | Valdés <i>et al.</i> (2006); Ortea <i>et al.</i> (2012); Sanvicente-Añorve <i>et al.</i> (2012b); Redfern (2013); Ortigosa <i>et al.</i> (2015); Vital <i>et al.</i> (2015); Ortea and Buske (2018) |
| Tenellia cf. tina (Er. Marcus, 1957) | 1 | | | Barbados, Bonaire, Brazil, Costa Rica, Curaçao, Jamaica, Mexico (GM ¹), Panama, St. Kitts, United States | Edmunds and Just (1983); Valdés <i>et al.</i> (2006) as <i>Cuthona tina</i> |
| Tenellia sp. 1 | 1 | | | Barbados, Mexico (GM*) | Edmunds and Just (1983) as <i>Cuthona</i> . Appears to be an undescribed species |
| Tenellia sp. 2 | 1 | | | Mexico (GM*) | Appears to be an undescribed species, no previous records found |
| Berghia cf. creutzbergi Er. Marcus & Ev. Marcus, 1970 | 1 | | | Bahamas, Barbados, Brazil, Cayman Islands, Costa Rica, Cuba, Curaçao, Martinique, Mexico (GM ¹), United States, Venezuela | Valdés <i>et al.</i> (2006); Carmona <i>et al.</i> (2014); Grune Löffler <i>et al.</i> (2014); Ortea and Buske (2018) |
| Sacoglossa | | | | | |
| Hermaea sp. | 1 | | | Mexico (GM*) | Appears to be an undescribed species, no previous records found |
| Polybranchia schmekelae Medrano, Krug, Gosliner, Biju Kumar & Valdés, 2018 | | 1 | | Barbados, Bonaire, Canary Islands, Costa Rica, Curaçao, Jamaica, Florida, Mexico (CAR*), Panama, Virgin Islands | Rosenberg <i>et al.</i> (2009) as <i>Polybranchia viridis</i> ; Medrano <i>et al.</i> (2018) |
| <i>Elysia</i> crispata Mörch, 1863 | | 1 | | Antigua, Barbuda, Bahamas, Barbados, Belize, Bonaire, Cayman Islands, Colombia, Costa Rica, Cuba, Curaçao, Dominica, Guadeloupe, Haiti, Honduras, Jamaica, Mexico (GM, CAR), Panama, St. Lucia, St. Martin, Trinidad and Tobago, United States, Venezuela, Virgin Islands | Valdés <i>et al.</i> (2006); Krug <i>et al.</i> (2016); Ortea and Buske (2018) |
| Elysia flava Verrill, 1901 | 7 | | 2 | Western Atlantic: Bahamas, Belize, Bermuda, Brazil, Costa Rica, Cuba, Curaçao, Grenada, Guadeloupe, Honduras, Jamaica, Mexico (GM, CAR*), Panama, Puerto Rico, St. Lucia, St. Vincent and the Grenadines, Venezuela | Valdés <i>et al.</i> (2006); Ortigosa <i>et al.</i> (2015); Caballer-Gutiérrez <i>et al.</i> (2015); Krug <i>et al.</i> (2016); Ortea and Buske (2018); Ortigosa and Simões (2019) |
| Elysia patina Ev. Marcus, 1980 | | 1 | | Bahamas, Mexico (GM, CAR*), United States | Ortigosa <i>et al.</i> (2013); Ortigosa <i>et al.</i> (2015); Krug <i>et al.</i> (2016) |

(Continued)

Table 1. (Continued.)

| Taxon | Number of organisms | | | Recorded distribution | References and notes |
|--|---------------------|----|-----|--|--|
| | B10 | PM | MAH | | |
| <i>Elysia velutinus</i> Pruvot-Fol, 1947 | 2 | 1 | 1 | Bahamas, Barbados, Belize, Bermuda, Brazil, Cayman Islands, Costa Rica, Cuba, Curaçao, Dominica, Jamaica, Mexico (GM, CAR), Panama, St. Thomas, United States, Union Islands, Virgin Islands, St. Vincent and the Grenadines | Valdés et al. (2006); Ortigosa et al. (2013, 2015); Krug et al. (2016) |
| <i>Elysia</i> sp. 1 | | 1 | | Not included because it is an undetermined species | Juvenile (found in April) |
| <i>Elysia</i> sp. 2 | | 1 | | Mexico (CAR*) | Appears to be an undescribed species; no previous records found |

B10, Bajo de 10; PM, Puerto Morelos; MAH, Mahahual; CAR, Caribbean; GM, Gulf of Mexico. The number of organisms found and locality is given. Month of collection for juvenile specimens is provided. Recorded distribution is given, except for undetermined species. New records of species are in bold, an asterisk (*) indicates if the new record is for the Mexican coast or a dagger (†) refers if the new record is for the GM, in the recorded distribution.

shining white spots, which were both similar to those of *Aegires gomezi* (Ortea et al., 1990).

Family CHROMODORIDIDAE Bergh, 1981

Genus *Felimida* Ev. Marcus, 1971

Felimida sp. 1

(Figure 2K)

Material Examined

One organism (30 mm), Bajo de 10 (CMPY-005747).

Description

Body oval elongated; background colour orange with white spots, it has a white margin with purple dots and a yellow border. Dorsum soft with several irregular papillae, the largest located at each side of the body. Rhinophores orange with purple tips, and around 16 lamellae. Branchial sheath with 13 leaves, translucent orange and purple lines in the tip. Foot and ventral area white. Tail with a V shape, as wide as the body, of the same colour as the foot but with orange and purple tints.

Remarks

This species is similar to *Felimida regalis*; however, it presents a purple pattern in the gills and surrounding the mantle and has a different number of branchial leaves (Padula et al., 2016).

Felimida sp. 2

(Figure 2L)

Material Examined

Two organisms (4 mm), Bajo de 10 (CMPY-005742).

Description

Body oval elongated; background colour white with light purple, it has a white margin bordered by a thin orange line that broadens in front of the head; a white band extends from in between the rhinophores to the base of the branchial sheath; right in the centre of the body, there is a blue patch and orange dots. Rhinophores with five visible lamellae, whitish at the base and with purple tips. At least three branchial leaves of the same colour of the body and purple lines. Eyespots visible behind rhinophores. Tail V-shaped, narrow, with the same translucent white colour of the body.

Remarks

This specimen was a juvenile of the *Felimida clenchi-binza* chromatic group. Colour pattern is not a reliable element to identify juvenile members of this group to the species level (Padula et al., 2016). The smallest organism (<2 mm) did not have the gills developed yet.

Genus *Felimare* Ev. Marcus & Er. Marcus, 1967

Felimare sp. 1

(Figure 3B)

Material Examined

Two organisms (2.5 mm), Bajo de 10 (CMPY-005745).

Description

Body slender; background colour blue with a whitish-transparent margin and an inner yellow margin, surrounding the blue area; a yellow line crosses the notum from head to tail in the centre. Ventral part of the body translucent white. Rhinophores with five lamellae of the same colour of the body, with an anterior yellow line and a posterior white line measuring half the length of the rhinophore. A yellow dot is almost located between the bases of rhinophores. With at least four branchial leaves of the same blue colour

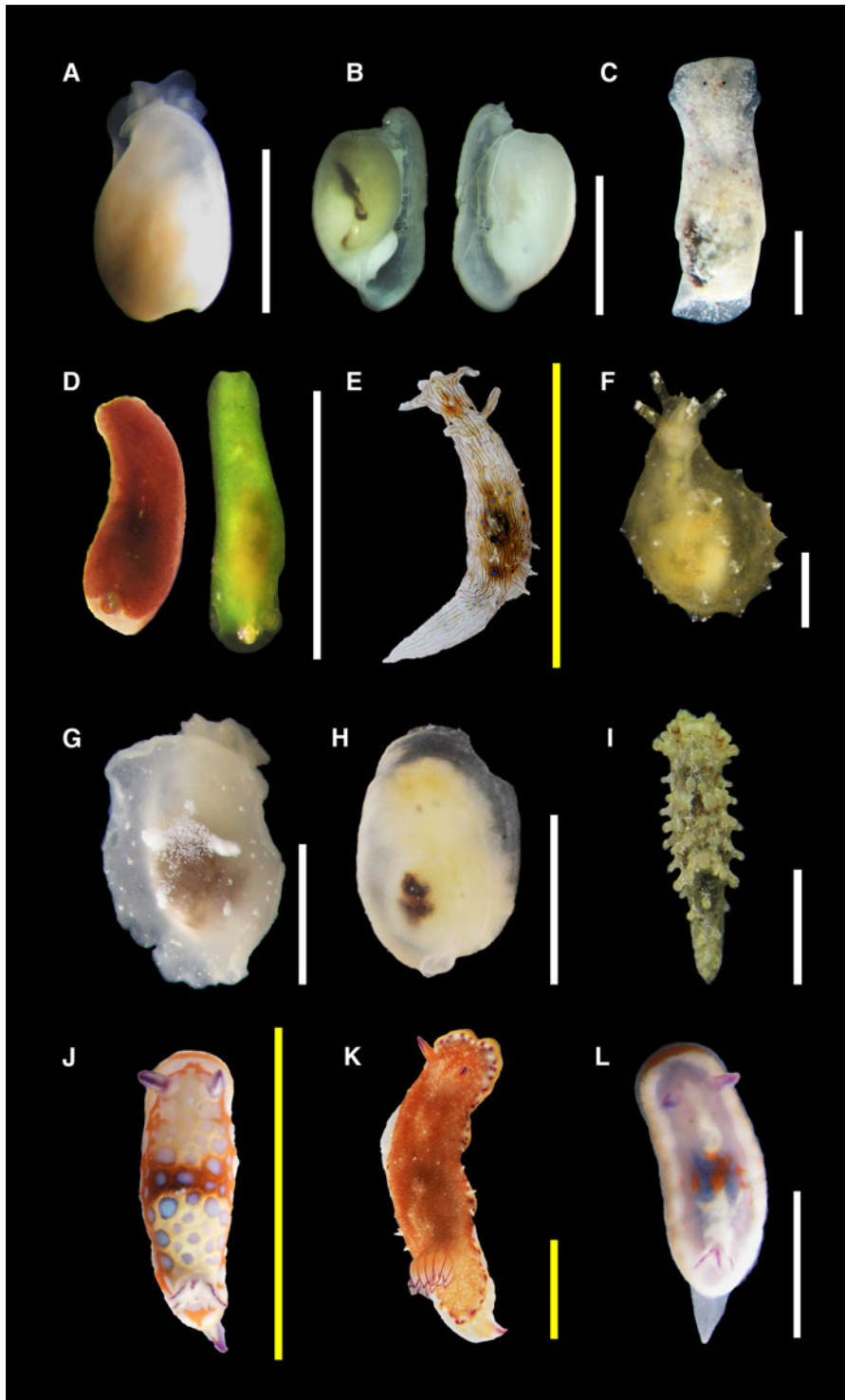


Figure 2. Species found in this study (authorities are given in Table 1): (A, B) *Retusa* sp.; (C) *Haminoea elegans*; (D) *Lapinura* sp.; (E) *Stylocheilus polyomma*; (F) *Notarchus punctatus*; (G) *Berthella vialactea*; (H) *Berthella* sp.; (I) *Aegires* cf. *ortizi*; (J) *Felimida clenchi*; (K) *Felimida* sp. 1; (L) *Felimida* sp. 2. Scale bars: A–D, F–I, L (white), 2 mm; E, J, K (yellow), 10 mm. H and L are considered juvenile specimens.

with yellow dots in the exterior area, forming a line. Four mantle dermal formations were visible in the ventral area of the mantle located at each side of the head and four in the posterior end.

Remarks

Organisms were juveniles from the blue chromatic group defined by Ortea *et al.* (1996).

Felimare sp. 2
(Figure 3C)

Material Examined

One organism (1.3 mm), Puerto Morelos (CMPY-005773).

Description

Body slender; background colour blue with a broad white margin. A thin yellow line crosses most of the dorsum from head to tail in the centre and some yellow patches are present in the white margin. Ventral part of the body translucent white. Rhinophores smooth, of the same colour as the body. With branchial leaves of the same blue colour. We were not able to see mantle dermal formations.

Remarks

This was a juvenile from the blue chromatic group defined by Ortea *et al.* (1996). *Felimare* sp. 2 is different from *Felimare* sp. 1 in the colour pattern of the body and the shape of rhinophores.

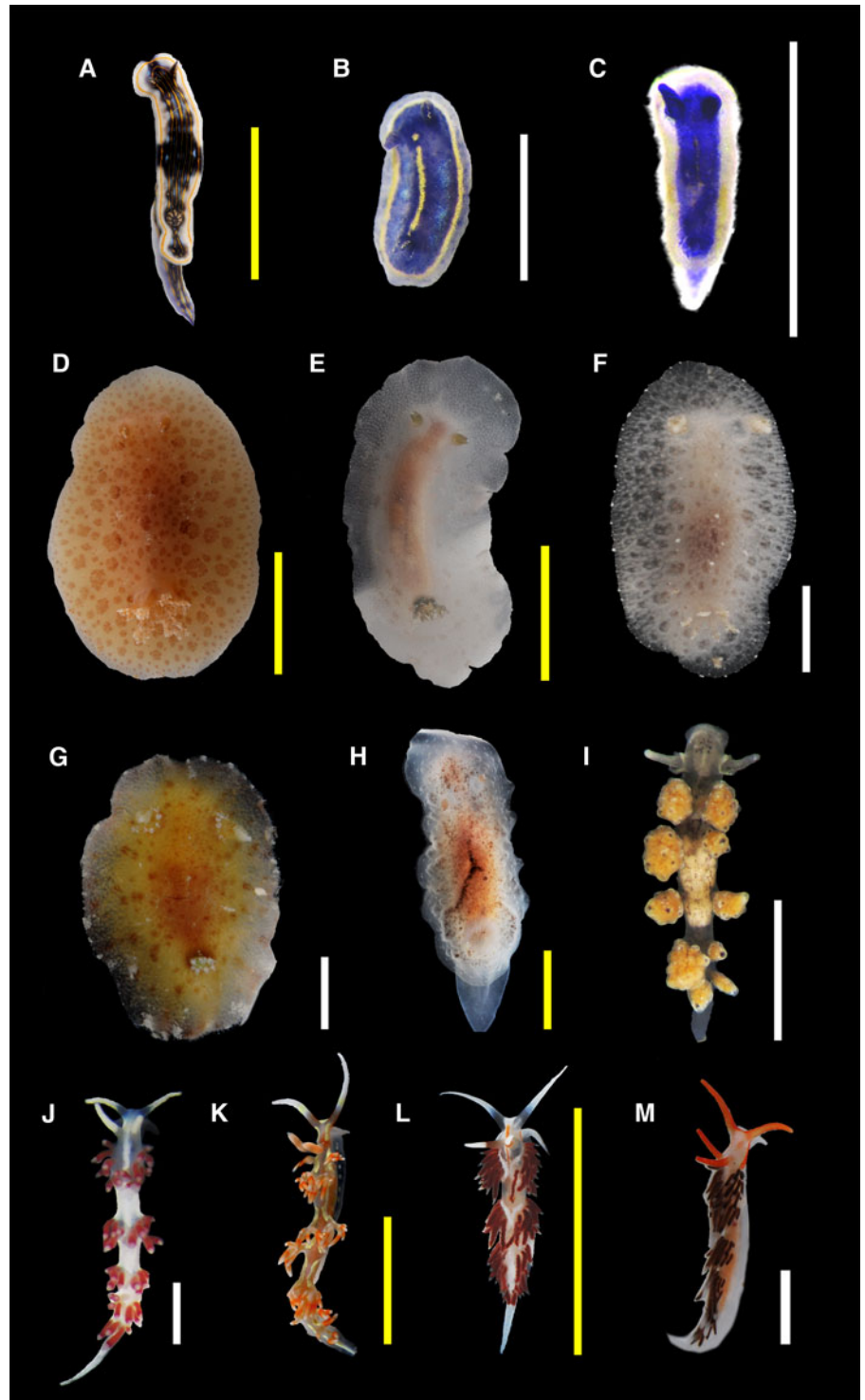


Figure 3. Species found in this study (authorities are given in Table 1): (A) *Felimare ruthae*; (B) *Felimare* sp. 1; (C) *Felimare* sp. 2; (D) *Discodorididae* sp.; (E) *Jorunna* cf. *spazzola*; (F) *Jorunna* cf. *spazzola* (juvenile); (G) *Taringa telopia*; (H) *Hallaxa* sp.; (I) *Doto* sp.; (J) *Flabellina dushia*; (K) *Flabellina engeli*; (L) *Learchis* sp. 1; (M) *Learchis* sp. 2. Scale bars: B, C, F, G, I, J, M (white), 2 mm; A, D, E, H, K, L (yellow), 10 mm. B, C, F and M are considered juvenile specimens.

Family DISCODORIDIDAE Bergh, 1981
Discodorididae sp.
 (Figure 3D)

Material Examined

One organism (27 mm), Mahahual (CMPY-005833).

Description

Body oval; background colour light brown, many darker brown dots on dorsum, most of them forming circles of different sizes, with the smallest in the centre and the border. Small caryophyllidia present, spicules visible throughout the body. Rhinophores of

the same colour of the body, with a white tip and white dots making an anterior line, and around 20 lamellae. Six three-pinnate branchial leaves of the same colour of body, but with brown dots randomly distributed and white tips.

Remarks

This organism resembles *Discodoridid* sp. A from Redfern (2013), but with a yellowish-brown colour.

Genus *Jorunna* Bergh, 1876
Jorunna cf. *spazzola*
 (Figure 3E, F)

Material Examined

Five organisms (26 mm), Bajo de 10 (CMPY-005835), one organism (26 mm), Bajo de 10 (CMPY-005834); one organism (7.5 mm), Mahahual (CMPY-005842); one organism (7 mm), Puerto Morelos (CMPY-005777).

Description

Body oval; background colour white with grey circles and a light brownish area in the centre; margin with white widely spaced dots. Ventral area white with brown dots (except in juveniles) and a brown patch in the middle of the foot. Spicules visible throughout the body. Rhinophores light brown at the base and white tips, with at least five lamellae in juveniles (Mahahual and Puerto Morelos specimens) and at least 14 lamellae in adults (most of Bajo de 10 specimens). Gill with six retractile, bi-tripinnate branchial leaves of the same colour as mantle.

Remarks

These organisms resemble the description of *J. cf. spazzola*, which has been recorded in the Caribbean (Valdés *et al.*, 2006), and in the southern GM (Ortigosa *et al.*, 2013). However, adults do not correspond with the maximum recorded size (9 mm) and have more lamellae in rhinophores (14) (Alvim and Pimenta, 2013). Also, juveniles of the Caribbean specimens from this work had a marked white-beige tip in rhinophores and branchial leaves that do not have the juveniles shown in Valdés *et al.* (2006). The mitochondrial cytochrome c oxidase I (COI) gene sequences were obtained from two specimens (CMPY-005834-35), but they did not match >90% any data in GenBank (National Center for Biotechnology Information, 2023).

Family ACTINOCYCLIDAE O'Donoghue, 1929
Genus *Hallaxa* Eliot, 1909
Hallaxa sp.
(Figure 3H)

Material Examined

One organism (35 mm), Bajo de 10 (CMPY-005739).

Description

Body oval elongated; background colour translucent white, with an orange patch in the centre of dorsum and brown dots scattered randomly; mantle with a brown-yellowish margin. Anterior part of the mantle is slightly wider. Dorsum soft with many papillae of different sizes, distributed throughout the notum. Ventral area and foot white with very small brownish dots. Rhinophores with 11–12 lamellae of the same colour as the body. Eight branchial bipinnate leaves. Tail wide and tongue-shaped.

Remarks

Most of *Hallaxa* spp. have been described for the Indo-Pacific Ocean; however, only one species is present in the Atlantic, *Hallaxa apefae*, described by Er. Marcus (1957) and cited by Ortea and Buske (2018). Descriptions from those publications did not match the organism found in this work, suggesting an undescribed species.

Family DOTIDAE Gray, 1853
Genus *Doto* Oken, 1815
Doto sp.
(Figure 3I)

Material Examined

One hundred and twelve organisms (4.5 mm), Bajo de 10 (CMPY-005730, 5733-35).

Description

Body slender with a narrow tail; background colour translucent white with black patches scattered in the entire body. Rhinophores smooth in a cup-like base, with black dots and some white patches, especially in the tips. Cerata grape-shaped with white dots and a characteristic black dot at the tip of most of the tubercles they had; digestive glands visible through the cerata and have different colours (orange, yellowish, white, grey). Most specimens had five pairs of cerata. Eyespots between rhinophores or in front of them. Foot of the same colour as the body.

Remarks

Organisms resemble *Doto* sp. D in Redfern (2013).

Facelinidae Bergh, 1889
Genus *Learchis* Bergh, 1896
Learchis sp. 1
(Figure 3L)

Material Examined

Eight organisms (11 mm), Bajo de 10 (CMPY-005756).

Description

Body elongated with long and curved foot corners; background colour translucent white, white lines from the base of cerata draw an 'x' curved pattern in the dorsum; a white band with an orange line crosses the head and back, in between rhinophores and reaches the middle of oral tentacles, which are long and white, except in the base; an orange line is also present at each side of the head, between the base of tentacles and the first group of cerata. Rhinophores smooth, reddish-orange at the base and with a white distal half. Red cerata with white tips, long, slender and pointed; at least three groups of cerata are present. Long and thin tail of the same white colour of the foot.

Remarks

Specimens very similar to *Learchis evelinae*. However, the original description of this species does not mention the orange line between rhinophores (Edmunds and Just, 1983), and the tail of our specimens is longer and thinner (Redfern, 2013).

Learchis sp. 2
(Figure 3M)

Material Examined

Five organisms (8 mm), Bajo de 10 (CMPY-005724, 5726, 5753).

Description

Body elongated with short, curved foot corners, background colour translucent white; the head is white with orange oral tentacles, a line of the same orange colour is formed between oral tentacles and the base of tentacles and the first group of cerata; two blurred white lines are below the base of cerata. Rhinophores are smooth, of the same orange colour as oral tentacles. Cerata are slender and relatively short, brown with white tips; cerata are aligned and hard to count, two spaces between groups of them are notorious. Short and thin tail of white colour, the same as the foot.

Remarks

These organisms could be juveniles. As they had shorter oral tentacles, cerata, tail and distinct colour patterns from the smallest specimens of *Learchis* sp. 1, we recognize them as a different species.

Family FIONIDAE Gray, 1857
Genus *Tenellia* A. Costa, 1866
Tenellia cf. *tina* (Er. Marcus, 1957)
(Figure 4B)

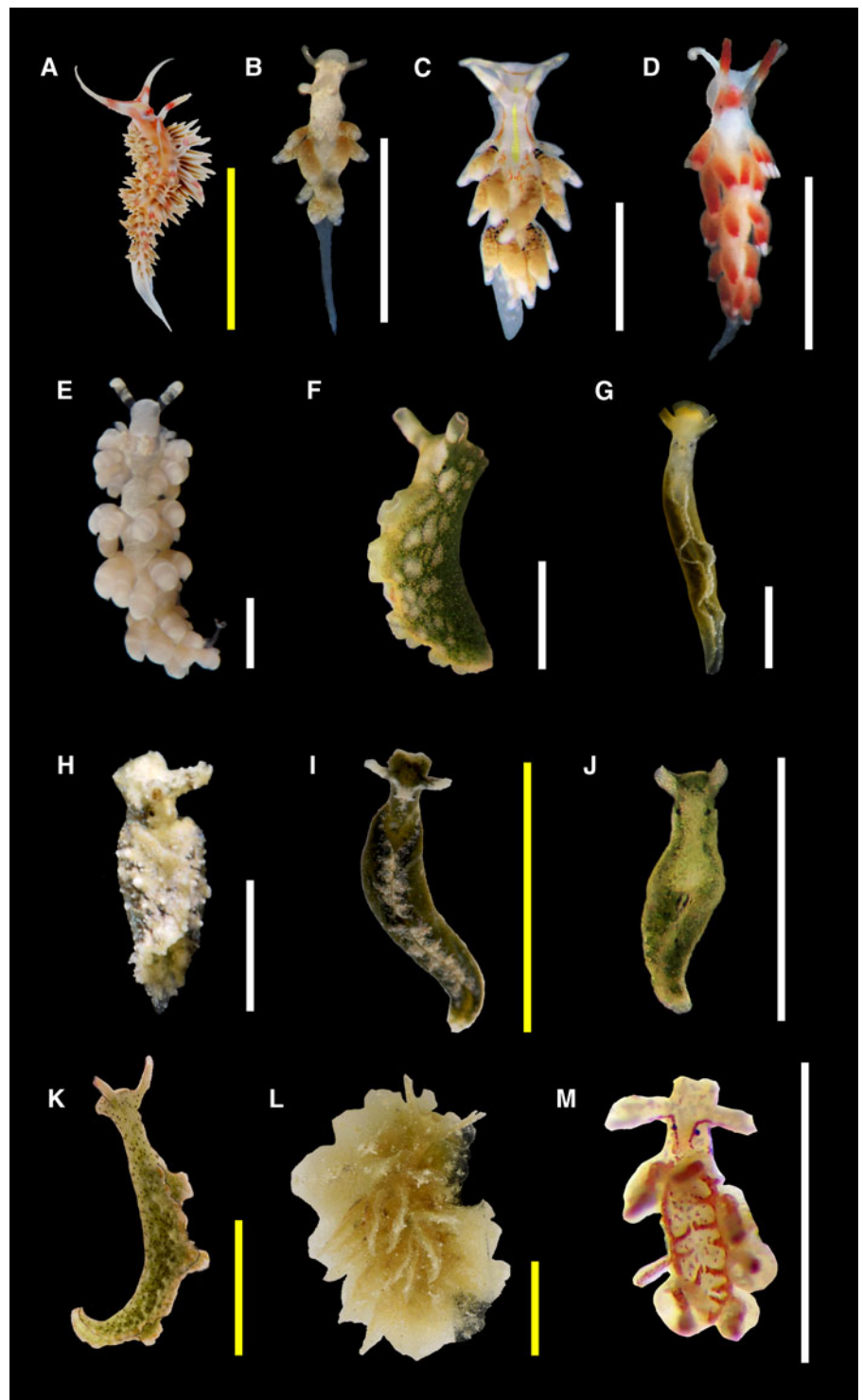


Figure 4. Species found in this study (authorities are given in Table 1): (A) *Phidiana lynceus*; (B) *Tenellia* cf. *tina*; (C) *Tenellia* sp. 1; (D) *Tenellia* sp. 2; (E) *Berghia* cf. *creutzbergi*; (F) *Elysia crispata*; (G) *Elysia flava*; (H) *Elysia patina*; (I) *Elysia velutinus*; (J) *Elysia* sp. 1; (K) *Elysia* sp. 2; (L) *Polybranchia schmekelae*; (M) *Hermaea* sp. Scale bars: B–H, J, M (white), 2 mm; A, I, K, L (yellow), 10 mm. J is considered a juvenile specimen.

Material Examined

One organism (2.5 mm), Bajo de 10 (CMPY-005718).

Description

Body slender with a long tail, almost half of the size of the body; background colour opaque white, with some shiny dots in the entire body; each oral tentacle has a white dot near the tip. A white glistening patch in the shape of W is in the dorsum, between the first group of cerata. Rhinophores smooth, with two white bands: the distal band is longer than the proximal band. Cerata elongated but relatively globose and pointed, light brown with white tips; there are two groups of cerata

along the body. Eyespots in the base of rhinophores. Foot white transparent.

Remarks

Individual very similar to *Tenellia tina*. However, the coloration pattern of rhinophores, dorsum, cerata and oral tentacles do not fully correspond (Er. Marcus, 1957). Recently, Cella *et al.* (2016) studied the systematics of the family Tergipedidae, where *Cuthona/Catriona tina* was previously assigned. They found that this family is not monophyletic, and as it is part of a larger clade, they reassigned most of the members to Fionidae because it is the oldest name of the taxon. Also, the same authors

considered that species previously recognized as *Cuthona*, should be changed to *Tenellia* (Cella *et al.*, 2016).

Tenellia sp. 1
(Figure 4C)

Material Examined

One organism (4.5 mm), Bajo de 10 (CMPY-005737).

Description

Body slender with a wide and short tail that has white dots; background colour opaque white with orange spots scattered in the dorsum; white patches in oral tentacles, and at each side of the body; a dashed white line at each side of the foot. A yellow line crosses the head in the centre and reaches the first group of cerata, as well as two parallel orange lines that start at the base of oral tentacles and continue at each side of the head; an orange line of the same colour is found dorsally between oral tentacles. Rhinophores smooth and white, with a brownish ring in the second proximal quarter. Cerata are relatively globose and pointed, light brown with white tips and a black base; presents at least four groups of cerata. Eyespots in the base of rhinophores. Foot of the same colour as the body.

Remarks

External morphology almost identical to *Cuthona* sp. recorded in Barbados by Edmunds and Just (1983). See remarks of *T. cf. tina* for details about family and genus.

Tenellia sp. 2
(Figure 4D)

Material Examined

One organism (4.5 mm), Bajo de 10 (CMPY-005723).

Description

Body slender with a narrow tail; background colour opaque white; head with an orange area near the base of rhinophores. Rhinophores smooth, half white at the base and half red at the top. Cerata elongated and pointed, orange at the base, reddish at the top and with white tips; four groups of cerata are present. Eyespots at the base of rhinophores. Oral tentacles and foot of the same colour as the body.

Remarks

See remarks of *T. cf. tina* for details about family and genus.

Family AEOLIDIIDAE Gray, 1827

Genus *Berghia* Trinchese, 1877

Berghia cf. creutzbergi Er. Marcus & Ev. Marcus, 1970
(Figure 4E)

Material Examined

One organism (8 mm), Bajo de 10 (CMPY-005722).

Description

Body slender, long; background colour beige-whitish, with dense white pigmentation on the notum and head. Oral tentacles with a white dashed coloration, and a transparent base; presents foot corners almost triangular-shaped, with a rounded tip. Rhinophores covered by tubercles with slight brownish pigmentation, a white tip and a transparent base. Cerata globose, translucent white with notorious white rings in the middle of the cerata and a vanilla coloration at the tips and the base. Foot translucent white. Tail narrow, same colour as the foot.

Remarks

Specimen very similar to *Berghia creutzbergi*, but with a whiter coloration and more globose cerata; the white ring in the middle of cerata has not been mentioned in this species (Carmona *et al.*, 2014). Cerata move from side to side when crawling (Carmona *et al.*, 2014; Goodheart *et al.*, 2016).

Superorder SACOGLOSSA
Family PLAKOBRANCHIDAE Gray, 1840
Genus *Elysia* Risso, 1818
Elysia sp. 1
(Figure 4J)

Material Examined

One organism (2 mm), Puerto Morelos (CMPY-005775).

Description

Body slender; background colour beige-white with green areas, especially around the head and in parapodia; two black-blue marks are visible on notum, below the white pericardium. Moustache in upper lip is present. Rhinophores rolled, of the same colour of body and dark-green in their anterior base. Parapodia smooth, without papillae, greenish with a beige glistening border. Eyespots behind the base of rhinophores. Foot translucent, with green digestive diverticula showing through.

Remarks

The single organism found was a juvenile and does not resemble any of the *Elysia* species from the Caribbean (Ortea *et al.*, 2014; Krug *et al.*, 2016; Ortea, 2018).

Elysia sp. 2
(Figure 4K)

Material Examined

One organism (19 mm), Puerto Morelos (CMPY-005766).

Description

Body slender with tiny white papillae scattered in most of the body; background colour beige with olive-green and white-cream patches, as well as black and white spots. Rhinophores rolled, with white patches, an orange coloration and a black edge. Parapodia forming three siphonal openings when closed, they have the same colour of the body, a black margin and a submarginal orange band. Eyespots behind the base of rhinophores are hard to distinguish from black spots. Foot same colour as the body, with a green net of digestive diverticula showing through.

Remarks

This organism had some resemblance to *Elysia ornata* (Krug *et al.*, 2016) but had tiny papillae and white-cream patches all over the body, among other differences mentioned in the description.

Family HERMAEIDAE H. Adams & A. Adams, 1854
Genus *Hermaea* Lóven, 1844
Hermaea sp.
(Figure 4M)

Material Examined

One organism (1.5 mm), Bajo de 10 (CMPY-005736).

Description

Body slender; background colour translucent white with red dots in the whole body and the red digestive system showing through; a regular pattern is observed in the dorsum. Rhinophores rolled, of

the same body colour with the exception of two red lines going parallel from the proximal half of rhinophores, to each side of the head. Cerata elongated, transparent, with the vertical and red digestive glands visible, some of them were no longer embedded in the body. Eyespots behind the base of rhinophores. Foot of the same colour as the body.

Remarks

None of the described *Hermaea* species from the Caribbean resembles this organism regarding its characteristics or coloration pattern (Valdés et al., 2006; Caballer-Gutiérrez and Ortea, 2013).

Discussion

In this study, we found 31 species of heterobranch sea slugs using ARMS as an indirect collection method to study one locality from the southern GM and two from the Mexican CAR. This diversity represents 9.4% of the sea slug diversity recorded in the CAR (329 spp.) by García and Bertsch (2009). Even though some studies have addressed part of the study area (Valdés et al., 2006; Ortigosa et al., 2013), here we report four determined species for the first time in the country. Further, *B. cf. creutzbergi*, *Taringa telopia* and *T. cf. tina* represent their first record in the GM. Also, *Taringa* and the family Actinocyclusidae, represented in our study by *Hallaxa* sp., had not been previously recorded in the GM.

Most species were represented by only one specimen, limiting the identification to the species level. These numbers are frequently recorded for the group, as low abundances are found per species in the inventories in the CAR region (Valdés et al., 2006; Ortigosa et al., 2013; Camacho-García et al., 2014; Goodheart et al., 2016) and the Campeche and Yucatan Bank (Sanvicente-Añorve et al., 2012b; Ortigosa et al., 2013, 2015; Ortigosa and Simões, 2019). Nevertheless, some species had high abundances in our samplings, such as *Doto* sp. (91 in one recovery event) or *Phidiana lynceus* (43 in eight recovery events), clearly demonstrating the potential of this collection method to study recruitment patterns, temporal population dynamics and substrate preferences of common and abundant sea slug species. Unlike the studies mentioned above, which used direct and indirect methods, here we assessed sea slugs' diversity exclusively by an indirect method and found similar results.

As with other indirect methods, where substrates are collected, we found juvenile specimens of small sizes, such as *Felimida* sp. and *Felimare* sp. However, we did not observe their adult state, which might have been searching for other suitable habitats. Very small individuals (<10 mm in total length as adults) and remarkably cryptic are usually found by indirect methods (Goodheart et al., 2016); ARMS proved their utility to find tiny organisms and could represent a methodological option for studying juvenile stages or life cycles of this group of molluscs.

Macroalgae is the most common substrate collected when studying sea slugs indirectly (Sanvicente-Añorve et al., 2012a). Then, herbivore groups are usually more abundant when using this method; species feeding on substrates attached to algae (e.g. hydroids and bryozoans) can also be present. In ARMS, several food sources for heterobranch sea slugs are found, such as sponges, ascidians, bryozoans, hydroids and algae, because the structures are used as a hard substrate base (Palomino-Alvarez et al., 2021a, 2021b), thus increasing the possibility of finding specimens of different feeding habits.

Other studies in the area have documented 13–16% of undetermined species (Sanvicente-Añorve et al., 2012a, 2012b). In contrast, in our study 47% were not identified at the species level. This result is related to the (1) juvenile condition, (2) lack of description of species previously recorded elsewhere and (3)

potentially undescribed new species. The diversity of colour patterns at the juvenile stage in *Felimida* sp. 2, *Felimare* sp. 1, *Felimare* sp. 2 and *Elysia* sp. 1 is not reliable to provide a confirmation (Krug et al., 2016; Padula et al., 2016). Also, five undetermined species from our research, including *Retusa* sp. and *Doto* sp., seem to have been formerly recorded in other localities but not described (Valdés et al., 2006; Redfern, 2013). Having genetic data of the mentioned species could have helped in the identification, as molecular techniques are currently used to confirm species; however, the worldwide health situation due to COVID-19, along with our budget constraints, did not allow the sequencing of specimens. The only exceptions were two *J. cf. spazzola* specimens and one organism identified as *T. telopia*; their mitochondrial COI sequence was obtained before the mobility restrictions arrived at our country in early 2020. While *J. cf. spazzola* sequences did not match >90% any data in GenBank (National Center for Biotechnology Information, 2023), *T. telopia*'s sequence coincided with the specimen recorded (accession no. MN720291) by Donohoo and Gosliner (2020).

Typically, the specific amounts of substrates collected in sea slugs' inventories using indirect methods are not mentioned. Most studies using the direct method report a great variation in the species richness and abundance (Thompson, 1976; Nybakken, 1978; Sanvicente-Añorve et al., 2012a, 2012b; Goodheart et al., 2016). For example, collecting effort (total searching time through direct observations for all observers) in the cited works consisted of 0.2–5.3 h invested for recording a species per locality (Goodheart et al., 2016). Even though collecting methods and their efforts are not comparable, it should be considered that a large underestimation in the species richness of the group remains a constant issue due to the strong dependence on sampling method and expertise (Nichols et al., 1998; Jensen, 2013). The new records on both coasts (southern GM and Mexican CAR), especially of genera and even family, stress the gap of information that we still need to fulfil in the area. Therefore, we recognize ARMS as a non-invasive useful sampling method to find juvenile, cryptic and rare species of sea slugs and other small or cryptic invertebrates, as well as to standardize their quantification and record their diversity.

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Author's contribution. L. A. P.-A., E. J. G.-C. and N. S. designed research. X. G. V. and L. A. P.-A. involved in material preparation and data collection. E. J. G.-C. and N. S. obtained funding for field trips and laboratory requirements. X. G. V. and D. O. analysed data. X. G. V., L. A. P.-A. and

D. O. wrote the first draft of the manuscript and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest. The authors declare none.

Ethical standards. Collection and preservation of organisms were conducted according to all applicable institutional, local and international regulations.

Data. All data generated or analysed during this study are included in this published article. A dataset is available at Ocean Biodiversity Information Systems (<https://doi.org/10.15468/kfzrq4>). GenBank (<https://www.ncbi.nlm.nih.gov/>) accession number for *Taringa telopia* (CMPY-005840) is OQ606964; sequences from two organisms of *Jorunna* cf. *spazzola* (CMPY-005834-35) are available upon request.

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