

***Padina ditristromatica* and *Padina pavonicoides*
(Dictyotales, Phaeophyceae): two new records
for the marine benthic flora
of the Mediterranean Spanish coasts**

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Abstract – *Padina ditristromatica* Ni-Ni-Win & H. Kawai and *Padina pavonicoides* Ni-Ni-Win & H. Kawai, two species recently described from the Mediterranean Sea, are reported for the first time from the coasts of the Iberian Peninsula, and the former also from the Balearic Islands. In this paper new data are provided for both species, especially cell dimensions. The indusium features, which are proposed as new taxonomical character for *P. ditristromatica*, as well as the lack of stolon-like structures in this species, are considered useful features for distinguishing *P. ditristromatica* from *P. pavonica*. A distribution map of both species in the Spanish coasts is also provided.

***Padina* / Dictyotales / Phaeophyceae / Iberian Peninsula / Balearic Islands / Mediterranean Sea**

INTRODUCTION

Padina pavonica (Linnaeus) Thivy is the type species of the genus *Padina* Adanson (Dictyotales, Phaeophyceae). *Padina boergesenii* Allender & Kraft, *Padina boryana* Thivy, *Padina gymnospora* (Kützing) Sonder, *Padina tenuis* Bory de Saint-Vincent and *P. pavonica* have been reported from the Mediterranean Sea (Cormaci *et al.*, 2012). According to South & Skelton (2003) and Abbott & Huisman (2004) *P. tenuis*, a species widely cited on the Mediterranean (Nizamuddin, 1981; Ribera *et al.*, 1992), is a synonym of *P. boryana*. On the other hand, Ni-Ni-Win *et al.* (2011) described two new species, *Padina ditristromatica* Ni-Ni-Win & H. Kawai and *Padina pavonicoides* Ni-Ni-Win & H. Kawai, from the Mediterranean Sea. These authors point out that the references of *P. boryana* and *P. gymnospora* from the Mediterranean Sea must correspond to misidentifications with *P. boergesenii*. Recently, Cormaci *et al.* (2012), according to Ni-Ni-Win *et al.* (2011), considered that the citations of *P. boergesenii* from the Mediterranean Sea must be misidentifications. Consequently, Cormaci *et al.* (2012) propose that

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nowadays the genus *Padina* is represented in this geographical area by only three species: *P. pavonica*, *P. pavonicoides* and *P. ditristromatica*. *P. pavonica* is considered a circumtropical species (Furnari *et al.*, 2010) which is widely distributed in the Mediterranean (Ribera *et al.*, 1992), and very frequent along the Spanish coasts (Barceló *et al.*, 1998). *Padina ditristromatica* is cited from Italy, Greece and Turkey (Ni-Ni-Win *et al.*, 2011; Tsiamis *et al.*, 2013). *Padina pavonicoides* is cited from the Balearic Islands, France and Turkey (Ni-Ni-Win *et al.*, 2011; Taskin, 2013). *Padina ditristromatica* presents a flabelliform-lobulated frond fixed by a stupose rhizoidal disc, a mixture of two and three cell layers in the middle and basal zones of the frond, a heavy calcification on both faces of the plant, conspicuous broad-depressed hair lines that alternate between both surfaces and are spaced unequally, and sporangial sori arranged in patches, or sometimes in broken lines (Ni-Ni-Win *et al.*, 2011). *Padina pavonicoides* is more similar to *P. pavonica* concerning the habit and the degree of calcification, however it differs from the latter in its anatomy of three cell layers along the entire frond, as well as in other aspects (the structure of hair lines, the structure and arrangement of the sori and the reproductive system). Like *P. ditristromatica*, it presents half-immersed conspicuous broad-depressed hair lines that alternate between both surfaces and are spaced at equal distances, and the sporangial sori are arranged in patches (Ni-Ni-Win *et al.*, 2011). As both species have been recently described, there are few publications concerning them (Cormaci *et al.*, 2012; Taskin, 2013), as well as some unpublished research papers (Gómez Gómez, 2012; Peralta Poch, 2013; Riera, 2014).

On the Spanish coasts, the genus *Padina* was represented solely by *P. pavonica* (Guiry & Guiry, 2014; Gallardo *et al.*, 1985) until the publication of the study by Ni-Ni-Win *et al.* (2011). In the framework of the *Flora phycologica iberica* project, we revised several herbarium specimens of *P. pavonica* from the Spanish Mediterranean coasts in order to know if any of these samples corresponded to either of the two species recently described by Ni-Ni-Win & Kawai. The revision of these herbarium samples, as well as the study of new *Padina* material, resulted in the detection of the presence of some specimens of *P. ditristromatica* and *P. pavonicoides* on these coasts.

MATERIAL AND METHODS

The study was carried out from 142 herbarium specimens (sheets and formalin preserved material) from the Mediterranean coast of the Iberian Peninsula (Girona to Cádiz) and the Balearic Islands stored at the BCN-Phyc herbarium (Centre de Documentació de Biodiversitat Vegetal de la Universitat de Barcelona). In addition, new specimens of *Padina* were collected at several locations in Girona and Tarragona in 2012 and 2013. For the anatomical study, frond sections of the margin, middle and basal parts of all studied specimens were made by hand with a razor blade. Photographs were taken with a Carl Zeiss camera (AxioCam ERc 5s) coupled with either a Nikon Eclipse 50i microscope or a Zeiss Stemi 2000-C stereomicroscope. All new studied specimens were stored at the BCN-Phyc Herbarium.

RESULTS

Of the 142 herbarium samples studied, 111 corresponded to *Padina pavonica*, 28 to *P. ditristromatica*, and 3 to *P. pavonicoides*. In the same way, among the *Padina* material collected in 2012 and 2013, some specimens of *P. ditristromatica* were also detected. From all of these specimens, morpho-anatomic studies of vegetative and reproductive structures were carried out.

Morphological observations

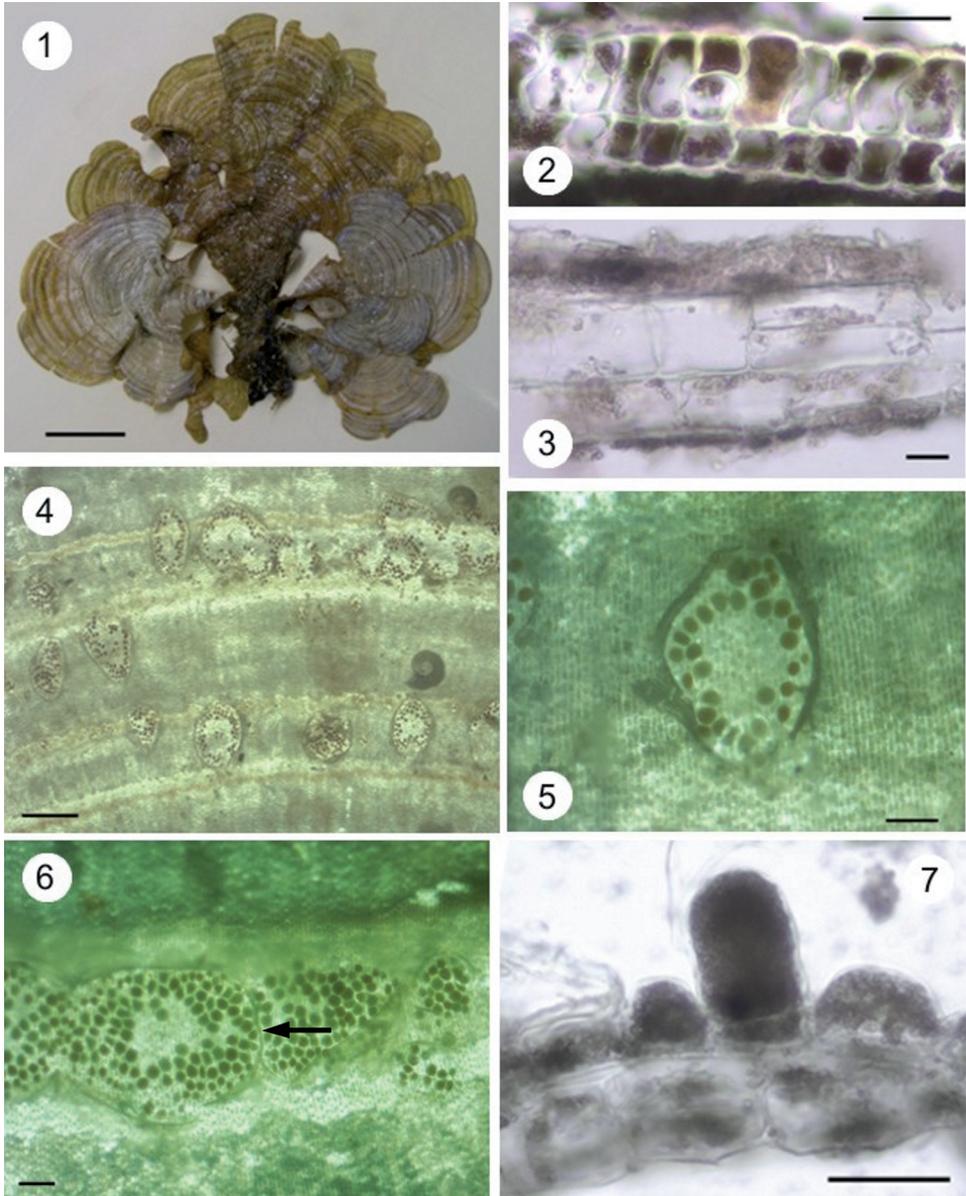
Padina ditristromatica

Our specimens of *Padina ditristromatica* consists of erect, flabelliform to semicircular, often segmented blades up to 11.5 cm high, usually attached to the substratum by a stupose rhizoidal basal disc of 0.5–2 cm in diameter (Fig. 1); the rhizoids are uniseriate and non-ramificated. No stolon-like structures (prostrate rhizomes) were observed. The specimens are moderate to heavily calcified on both surfaces except in the concentric hair lines (occasionally completely calcified on the upper surface). Concentric hair lines are common on the lower surface, and less frequent on the upper one; they alternate on both surfaces and are spaced unequally. Blades are 92.9–143.9 μm thick and consist of 2 to 3 cell layers in the middle and basal parts (2 cell layers at the margins) (Figs 2, 3). In cross section, cells are square, 15–27(48) μm wide by 14–29(38) μm high, and in areas with 2 cell layers, one of the two cells is higher than the other, 19–44(53) μm high. In longitudinal section, cells are rectangular, 56–89(102) μm long by 15–30 μm high (the higher cell is 27–52 μm high in areas of 2 cell layers).

Only fertile sporophytes and sterile specimens were found. Sporangia mainly occur in rounded or anticlinally-elongated sori that are 200–500 (1000) μm in length, although some of them can be transversely-elongated; sori are between the hair lines, isolated (forming patches) or sometimes grouped in discontinuous bands (broken lines) parallel to the hair lines (Fig. 4). Indusium is present and persistent after aperture, with a clear longitudinal opening in anticlinally-elongated sori (Figs 4, 5). When sori fuse, longitudinal remnants of indusium may disappear, forming an irregular sorus (Fig. 4). Sometimes, indusium limits between joined sori can be observed (Fig. 6). In any case, the indusium of the sori is independent of the hair lines. Sporangia are rounded on surface view, 72–96 μm in diameter, usually undivided. In cross section, the sporangia are obovate, (72)108–144 \times (57)72–92 μm , supported by a pedicel consisting of a single rectangular cell, 32–56 \times 14–28 μm (Fig. 7).

Our specimens of *Padina ditristromatica* occurred in the upper infralittoral zone, from the surface to a depth of 7(15) m, mainly in well-illuminated and sheltered places. They occur on substrate rich in sediment, or on dead *Posidonia oceanica* (Linnaeus) Delile matte. On Spanish Mediterranean coasts, *P. ditristromatica* has been found in Catalonia, the Valencian Country and in the Balearic Islands (Fig. 8). According to our present observations, both sterile specimens and fertile sporophytes occur from May to November; however, some individuals (sterile) of this species were also recorded in January.

Specimens examined: **Girona**: Cala Gras (Llançà), 10-09-2012, BCN-Phyc 6235; *ibid.*, 26-10-2013, BCN-Phyc 6236; Cala Canyelles (Llançà), 12-10-2004, BCN-Phyc 1027; *ibid.*, BCN-Phyc 1028; *ibid.*, BCN-Phyc 1029; *ibid.*, 21-11-2004, BCN-Phyc 1609; *ibid.*, BCN-Phyc 1610; *ibid.*, BCN-Phyc 1611; *ibid.*, 26-06-2005, BCN-Phyc 3022; *ibid.*, 26-06-2008, BCN-Phyc 5644;



Figs 1-7. *Padina ditristromatica*. **1.** Habit; scale bar = 2 cm. **2.** Cross section of the blade at the middle zone; scale bar = 50 μ m. **3.** Longitudinal section of the blade at the middle zone; scale bar = 20 μ m. **4.** Sporangial sori with longitudinal opening of the indusium; scale bar = 1 mm. **5.** Detail of a sorus; scale bar = 200 μ m. **6.** Detail of a broken irregular line of sori showing the indusium remnant perpendicular to hair lines (arrow); scale bar = 200 μ m. **7.** Detail of a sporangium with a single cell pedicel; scale bar = 50 μ m.

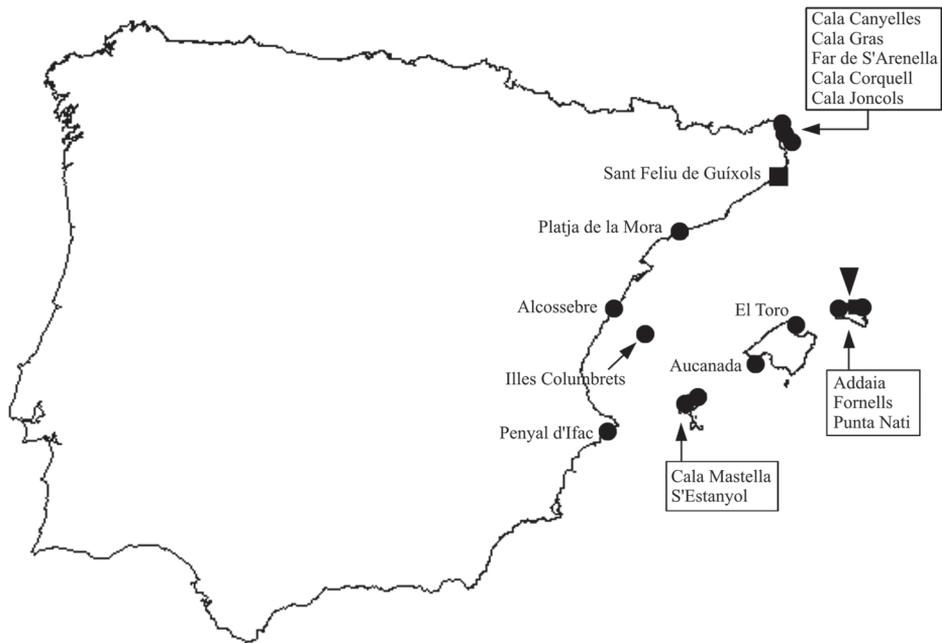
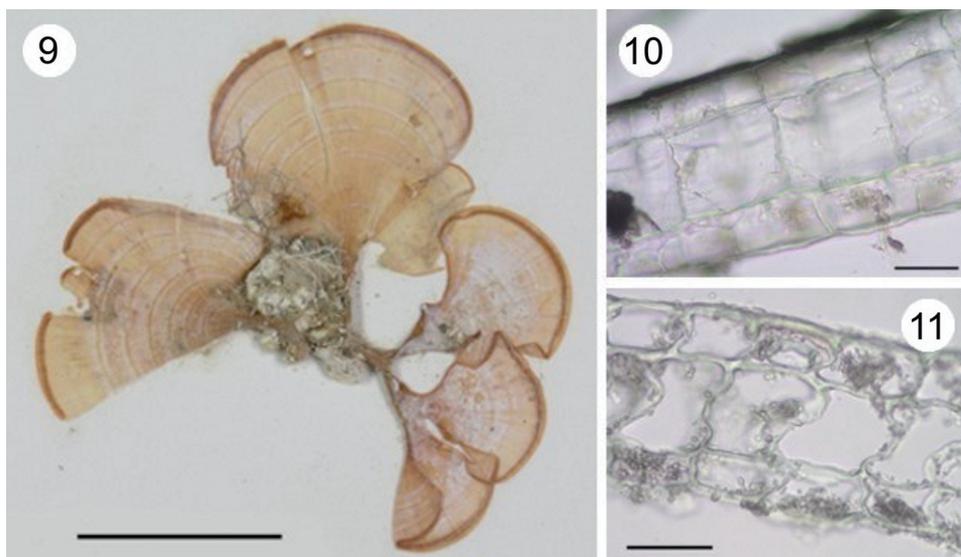


Fig. 8. Distribution of *Padina ditristromatica* (dots) and *P. pavonicoides* (squares) in the Iberian Peninsula and Balearic islands; arrowhead shows the previous record of *P. pavonicoides* in the Spanish coasts (Ni-Ni-Win et al., 2011).

Far de S'Arenella (Port de la Selva), 11-09-2012, BCN-Phyc 6237; *ibid.*, 14-10-2012, BCN-Phyc 6238; Cala Corquell (Port de la Selva), 07-09-2012, BCN-Phyc 6239; Cala Joncols (Roses), 17-05-1994, BCN-Phyc 3396. **Tarragona:** Platja de la Mora (Tamarit), 22-09-2013, BCN-Phyc 6240. **Castelló de la Plana:** El Navarrete, Illes Columbrets, 31-07-2014, BCN-Phyc 1612; Els Bruts, Alcossebre, 08-07-2004, BCN-Phyc 1613. **Alicante:** Penyal d'Ifac, 01-08-1993, BCF-Phyc 3410. **Balearic Islands:** Addaia (Menorca), 30-01-1979, BCN-Phyc 3405; Fornells (Menorca), 30-09-1979, BCN-Phyc 2263; Punta Nati (Menorca), 11-09-1995, BCN-Phyc 3422; Aucanada (Mallorca), 1-08-1978, BCN-Phyc 3420; *ibid.*, 15-09-1978, BCN-Phyc 3391; *ibid.*, 9-10-1979, BCN-Phyc 3390; El Toro (Mallorca), 17-07-1994, BCN-Phyc 3388; *ibid.*, 3-06-1997, BCN-Phyc 3399; Cala Mastella (Ibiza), 15-09-1998, BCN-Phyc 3424; S'Estanyol (Ibiza), 15-09-1998, BCN-Phyc 3423.

Padina pavonicoides

Our specimens of *Padina pavonicoides* consist of erect, semicircular, rarely segmented blades up to 3 cm high, usually attached to the substratum by a stupose rhizoidal base without forming a clear basal disc (Fig. 9); no stolon-like structures were observed. Blades are slightly calcified on the upper surface with no or little calcification on the lower surface. Concentric hair lines are common on the lower surface, and less frequent on the upper one; they alternate on both blade sides. In surface view, the cells are rectangular, 28-37 × 60-75 μm. In cross and longitudinal sections, blades consist of 3 cell layers in the middle and basal parts (2 cell layers at margins), the central cell being double the height of the exterior cells (Figs 10, 11). In cross section, exterior cells are square, 21-27 μm wide by 23-36 μm high, and central cells are rectangular, 19-23 μm wide by



Figs 9-11. *Padina pavonicoides*. **9.** Habit; scale bar = 2 cm. **10.** Longitudinal section of the blade at the middle zone; scale bar = 50 μm . **11.** Cross section of the blade at the middle zone; scale bar = 50 μm .

48-62 μm high. In longitudinal section, external cells are rectangular, 60-112 μm long by 24-31 μm high.

Our specimens of *P. pavonicoides* occurred in the infralittoral zone, between 10 and 25 m depth. On Spanish Mediterranean coasts, *P. pavonicoides* was known from the Balearic Islands (Ni-Ni-Win *et al.*, 2011) and has been found in Girona (Catalonia) (Fig. 8). The three specimens found corresponded to May-June and they are non-fertile.

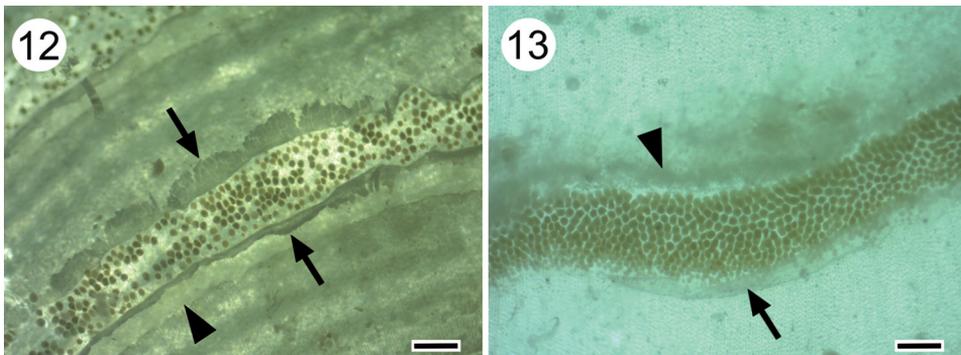
Specimens examined: **Girona:** Sant Feliu de Guixols, 04-07-1993, BCN-Phyc 6241; *ibid.*, 07-05-1994, BCN-Phyc 3398; *ibid.*, BCN-Phyc 6241.

DISCUSSION

In this paper, the first citation of *Padina ditristromatica* for the Spanish coasts is reported. Presently, all Spanish citations of this species correspond to the Mediterranean coasts (Catalonia, the Valencian Country and the Balearic Islands), however, the future revision of *Padina* samples from other regions of Spain will probably contribute new records of the species on these coasts. Similarly, the large number of locations where this species is present could indicate that *P. ditristromatica* is a frequent species in the Mediterranean Sea. On the other hand, *P. pavonicoides* was previously reported in Spain from the Balearic Islands (Ni-Ni-Win *et al.*, 2011), and therefore our citations of Sant Feliu de Guixols (Girona) expand its distribution to the peninsular Spanish coasts. Like in *P. ditristromatica*, the distribution of *P. pavonicoides* in our coasts could expand in subsequent revisions, although it seems to be a less frequent species.

Our specimens of *P. ditristromatica* are consistent with the description of this species provided by Ni-Ni-Win *et al.* (2011), confirming most of the characters proposed by these authors, such as: the habit, the attachment type, the calcification degree on both blade surfaces, the mixture of 2 to 3 cell layers in the middle area of the blades (although not always easy to observe), the presence of hair lines on both surfaces in alternate disposition and spaced unequally, as well as the arrangement in patches of the sporangial sori. However, in the present paper, we provide new morpho-anatomic details of both the vegetative and reproductive structures, and some of these are proposed as new taxonomical characters. The cell measurements, in surface view, as well as in transversal and longitudinal sections, are indicated here for the first time. Likewise, the description of the sporangia including their shape and size are also reported for the first time. Ni-Ni-Win *et al.* (2011) described the species on the basis of female gametophytes and tetrasporophytes, nevertheless they did not provide any information about the sporangia in the text. The authors only indicated in a comparative table that the sporangial sori are indusiate, occur on the lower surface of the blade, are arranged in broken lines or patches and are located half-immersed in the cuticle layer. According to our observations, the sporangia show a single cell pedicel which lies at the same level as the external vegetative cells of the lower surface of the blade.

In our specimens, the sori are indusiate and usually rounded or slightly elongated longitudinally, although in some specimens transversely elongated sori were observed. They often form irregular broken bands, usually above the hair lines, nevertheless sometimes they can cover the entire surface comprised between two hair lines, especially in the old areas of the frond. The indusium is independent of the hair lines and persists as marginal remnants in the mature sori; in rounded or anticlinally-elongated sori, it tears longitudinally, their remnants remaining perpendicular to the hair lines. In transversely-elongated sori, most indusium remnants appear parallel to the hair lines, nonetheless they are clearly visible and perpendicular to the hair lines at their ends, even when the sori merge in broken lines (Fig. 6). Moreover, the indusium remnants occur in both sides of the sorus, so they are visible between the sporangia and the hair lines (Fig. 12). No information about the indusium aperture in other *Padina* species is found. However, our observations in *P. pavonica* have shown that in this species the



Figs 12-13. Indusium of the sporangial sori. **12.** *Padina ditristromatica*; scale bar = 400 μ m. **13.** *Padina pavonica*; scale bar = 400 μ m. The arrows indicate the indusium remnants and the arrowheads show the hair lines.

indusium is related to the hair lines, since it covers not only the sporangia but also the hairs before its aperture. When the indusium splits, it does it transversally and its remnants stay parallel to the hair lines; unlike in *P. ditristromatica*, no indusium remnants occur between the hair lines and the sporangia (Fig. 13). According to these observations, we propose the indusium independence from the hair lines, as well as its longitudinal aperture in both rounded and anticlinally-elongated sori, as new taxonomical characters for *P. ditristromatica* and useful features to distinguish this species from *P. pavonica*.

On the other hand, Ni-Ni-Win *et al.* (2011) comment that *P. ditristromatica* attaches to the substrate by means of a stupose base lacking a “*Vaughaniella*” stage. According to several authors (Cribb, 1951; Boergesen, 1951; Allender & Kraft, 1983; Abbot & Huisman, 2004; Ni-Ni-Win *et al.*, 2010), the “*Vaughaniella*” stage corresponds to the creeping proliferations occurring at the base of some *Padina* species. Although the presence of stolon-like structures is not always indicated in the descriptions of *P. pavonica*, Peralta (2013), on the basis of a phenological study of this species, corroborates that these structures effectively occur at the base of this alga, though in some months they were very short. However, they were lacking in young specimens of *P. pavonica*, in which only rhizoids occur. Therefore, according to these results, we propose the base without stolon-like proliferations (“*Vaughaniella*” stage) as another useful character to distinguish adult specimens of *P. ditristromatica* from those of *P. pavonica*.

Until now, *Padina ditristromatica* had been cited from the intertidal to subtidal up to 3 m deep. Our results show that this species can reach a depth of 7 m, and according to Peralta (2013) it coexists temporally with *P. pavonica* in the same habitat.

Our specimens of *Padina pavonicoides* agree with the description of this species provided by Ni-Ni-Win *et al.* (2011). As is typical of this species, they show a scarce calcification and a blade with three cell layers, the central one being double the height of the two external layers. Concerning this fact, some authors (Ni-Ni-Win *et al.*, 2011; Cormaci *et al.*, 2012) point out that *P. pavonica* can also present one taller central cell (sometimes up to double height) in the part of the frond with three cell layers. In our observations of *P. pavonica*, we also have found specimens showing this character, but not constant along the cross section, since in some places the central cell divides to form four cell layers. Otherwise, we have observed some specimens of *Padina* showing the typical cross section of *P. pavonicoides*, although they presented several cell layers at the base, unlike this species. Therefore, in our opinion this is a feature that should be reinvestigated.

On the other hand, as the three specimens found in this study were sterile, we cannot provide data on the reproductive structures. Knowledge of these structures needs to be increased, because the data included in the original description of this species are not very exhaustive and they are similar to those reported for *Padina ditristromatica*.

Concerning the habitat, *Padina pavonicoides* seems to live at a greater depth than *P. ditristromatica*, because all specimens found until now live at between 10 and 25 meters depth. Our *Padina* specimens collected in Menorca, at the same location and depth that Ni-Ni-Win *et al.* (2011) found *P. pavonicoides*, corresponded with *P. pavonica*, showing that both species can coexist.

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