

## The morphological distinction of *Ralfsia expansa* and *R. hancockii* (Ralfsiaceae, Phaeophyta) from Mexico

DANIEL LEÓN-ÁLVAREZ<sup>1\*</sup> AND JORGE GONZÁLEZ-GONZÁLEZ<sup>2</sup>

<sup>1</sup>Sección de Algas, Herbario, Facultad de Ciencias, Universidad Nacional Autónoma de México, 04510 DF México, México

<sup>2</sup>Laboratorio de Ficología, Facultad de Ciencias, Universidad Nacional Autónoma de México, 04510 DF México, México

D. LEÓN-ÁLVAREZ AND J. GONZÁLEZ-GONZÁLEZ. 2003. The morphological distinction of *Ralfsia expansa* and *R. hancockii* (Ralfsiaceae, Phaeophyta) from Mexico. *Phycologia* 42: 613–621.

The brown alga *Ralfsia expansa* described by Børgesen (1912) and Tanaka & Chihara (1980a) may actually be two different species. To evaluate this possibility, a comparison of *R. expansa* with the related species *R. hancockii* was made. The following specimens were examined: the holotype of *R. hancockii* and several other specimens of this species from 11 localities along the Mexican tropical Pacific coast, and three specimens of *R. expansa* from the type locality (Veracruz, Mexico). The observations confirm the diagnostic value of: (1) the number of cells per stalk of the unilocular reproductive structures; and (2) the presence of a cortical layer of several cells differentiated from the medulla; they also confirm the lack of diagnostic value of certain other features such as the symmetry of the thallus in longitudinal radial section. We consider *R. expansa sensu* Børgesen to be a distinct species, typically found on the tropical Atlantic coast. Specimens of *R. expansa sensu* Tanaka & Chihara, described in previous reports from the Pacific coast of Mexico, are actually *R. hancockii*, which appears to be restricted to the Gulf of California and the Pacific coast of Mexico.

### INTRODUCTION

The holotype of the marine brown crustose alga *Ralfsia expansa* (J. Agardh) J. Agardh [Agardh 1847, as *Myrionema* (?) *expansum*] was first collected by Liebman from an unspecified area on the east coast of Mexico in Veracruz. The type specimen was sterile, and an incomplete description of the taxon was published by Agardh in 1848. Two subsequent studies described the species and included the reproductive structures: Børgesen (1912), using material collected from the Danish West Indies, and Tanaka & Chihara (1980a), with material from Tahiti, several Japanese islands, and Florida. However, these descriptions differ from one another and may indicate different species.

On the Mexican tropical Pacific (MTP) coast, we frequently see *R. hancockii* Dawson (León-Álvarez & González-González 1993, 1995), originally described by Dawson (1944, p. 223, pl. 31, figs 6, 7, pl. 54, fig. 2) from the Gulf of California. The descriptions of this species and *R. expansa sensu* Tanaka & Chihara (1980a, pp. 231–233, figs 1B, 2C, D) suggest that these two species may be identical. On the other hand, the description of *R. expansa sensu* Børgesen (1912, pp. 1–4, figs 1–2) differs from those of *R. hancockii* and *R. expansa sensu* Tanaka & Chihara, suggesting that *R. expansa* may actually be two separate species.

This article discusses the diagnostic value of characteristics that have been used to identify these taxa (Børgesen 1912; Weber van Bosse 1913; Dawson 1944; Tanaka & Chihara 1980a). These characteristics are re-evaluated on the basis of a morphological analysis of specimens from the MTP.

### MATERIAL AND METHODS

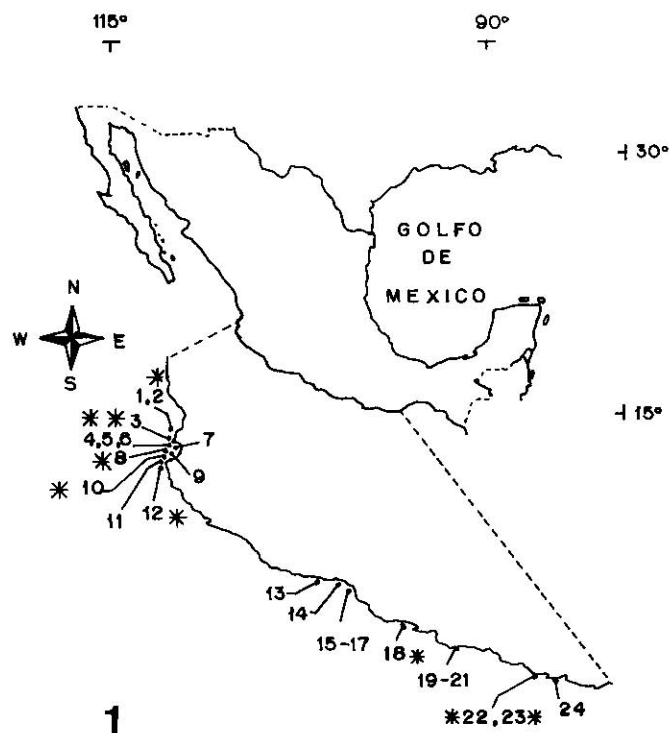
A total of 25 samples of *Ralfsia* from 11 sites throughout the MTP were examined in detail. We also looked at previously collected samples (León-Álvarez & González-González 1995) from 24 sites in the region (Fig. 1) that had been catalogued for the continuity of surface coverage and shape of the crusts, and for environmental features. For this study, samples were collected by breaking off pieces of rock to which the specimens adhered. The thalli were detached while under a stereo microscope, with care being taken so as not to destroy the basal parts of the crusts. Radial longitudinal sections were prepared by hand, stained with malachite green, and mounted with glycerinated gelatine (González-González & Novelo-Maldonado 1986). One to three slides were prepared for each sample.

In order to compare features uniformly, specimens were described in accordance with criteria used by the authors Areschoug (1843), Agardh (1848), Børgesen (1912), Setchell & Gardner (1924, 1925), Hollenberg (1969), and Tanaka & Chihara (1980a, b, c, 1981a, b, c). Each characteristic was measured in 10 microscope fields, and the arithmetic mean and the standard deviation (SD) were calculated. Because the exact nature of the reproductive cells (i.e. gametes or spores) produced by unilocular and plurilocular structures in *R. expansa* and *R. hancockii* could not be established with certainty, we use the morphological terms unangium and plurangium instead of unilocular or plurilocular sporangia.

Samples from the MTP were fixed in a 4% formalin solution and were kept together with slides and descriptions in the Herbarium of the Facultad de Ciencias, Universidad Nacional Autónoma de México (FCME) (Table 1). Samples from Veracruz were dried and incorporated without registry numbers into the FCME collection: GM-5/Oct/2001, GM-14/Feb/2002.

The herbaria of the Natural History Museum in Los An-

\* Corresponding author (dla@hp.ciencias.unam.mx).



**Fig. 1.** Localities studied. \* = Sites intensively examined. **Nayarit State:** 1. Sayulita; 2. Los Muertos; 3. Careyerros; 4. Las Cuevas; 5. Las Manzanillas; 6. Embarcadero, Punta Mita; 7. El Tizate; 8. Isla Larga. **Jalisco State:** 9. Boca Tomatlán; 10. Colemilla; 11. Corrales; 12. Playitas, Cabo Corrientes. **Michoacán State:** 13. Caleta de Campos; 14. Lázaro Cárdenas. **Guerrero State:** 15. Las Cuatas, Zihuatanejo; 16. Las Gatas, Zihuatanejo; 17. La Ropa, Zihuatanejo; 18. Puerto Escondido; 19. Muelle del puerto, Acapulco; 20. El Corsario, Acapulco; 21. El Yunque, Acapulco. **Oaxaca State:** 22. La Cruz, Barra Santa Elena; 23. Zona de plataformas, Barra Santa Elena. 24. Barra Copalita, Huatulco.

geles (LAM), the University of California at Berkeley (UC), the Escuela Nacional de Ciencias Biológicas of the Instituto Politécnico Nacional in Mexico (ENCB), and the National Museum of Natural History of the Smithsonian Institute, Washington, DC (US) were visited, and the following specimens were reviewed: the holotype and isotype of *R. hancockii* Dawson (D640, HAHF9 in LAM500460 and UC700549, respectively), the holotype of *R. integra* Hollenberg (US61155), the lectotype of *R. pacifica* Hollenberg (US61158), two samples of *R. expansa*, one from Veracruz, Mexico (ENCB4257) and the other from Tahiti (collected by W.A. Setchell, UC261260), a sample designated by Dawson as *R. occidentalis* Hollenberg (UC925766) from Acapulco, Guerrero State, and a sample determined by Hollenberg as *R. pacifica* (collected by Dawson: HAHF48148 in LAM599699).

## OBSERVATIONS

### A specimen of *R. expansa* from the type locality

**SPECIMEN DETAILS:** Punta Limón, Veracruz. Rocky littoral. Collected by Ernesto Chávez, 9 November 1976. Catalogue number 4257 (ENCB). Determined by Araceli Ramírez.

*Ralfsia expansa* from Veracruz is a dark brown, rugose, coriaceous crust when dry, without growth lines but with a conspicuous margin without lobulations. The thickness of the vegetative thallus is 180–252  $\mu\text{m}$  (without rhizoids) and 315–450  $\mu\text{m}$  in the reproductive parts. Single hyaline hairs are found in pits or clefts, originating on the thallus surface from the medullar filaments. Rhizoids are distributed irregularly in isolated areas. In a longitudinal radial view, the filaments are bilaterally symmetrical mainly in areas that coincide with irregularities of the substrate, although in other regions, the filaments grow unilaterally. Medullar filaments arise at acute angles to the basal prostrate filaments that are not evidently in layers, and curve and thin towards the apex as a result of repeated branching. These are tightly united throughout their length and are not surrounded by a gelatinous matrix. Basal cells are polyhedral to irregular in shape, 24.1–52.5  $\mu\text{m}$  long  $\times$  10.5–17.8  $\mu\text{m}$  in diameter, with a length–diameter ratio (l:d) of 1.6–4.2; subcortical botuliform cells are 17.8–42  $\mu\text{m}$   $\times$  12.6–18.9  $\mu\text{m}$ , with an l:d of 1.4–3.3, with thin walls (less than a third of the cell diameter) and abundant physodes like hyaline discoid granules. The cortical layer is three–five cells thick, morphologically differentiated from the medulla, with cubic to cylindrical cells, 3.1–5.6  $\mu\text{m}$   $\times$  4.2–5.7  $\mu\text{m}$ , with an l:d of 0.7–1.2, and a parietal laminar chloroplast. The apical cells are dome-shaped. Pyrenoids are absent.

Plurangia are found in sori distributed irregularly throughout the thallus and covered by a gelatinous layer. They are mature, originate terminally from reproductive erect medullar filaments, and are not lateral to paraphyses. Generally, one–two plurangia form a filament. The plurangia are cylindrical, 25.2–87.5  $\mu\text{m}$   $\times$  4.2–7.5  $\mu\text{m}$ , and consist of a series of cylindrical cells and one terminal sterile dome-shaped cell with dimensions 5.7–8.4  $\mu\text{m}$   $\times$  4.2–6.3  $\mu\text{m}$  and an l:d of 1.2–1.7. Unangia are absent.

### The type specimen of *R. hancockii*

**TYPE LOCALITY:** San Jose del Cabo, Baja California, Mexico, 16 February 1940. 'growing over bare rock surfaces in middle littoral'. (sic Dawson 1944, p. 223).

The holotype of *R. hancockii* Dawson [as *R. hancockii*, Dawson (1944, p. 223, pl. 31, figs 6, 7, pl. 54, fig. 2); D640 in the Natural History Museum, Los Angeles (HAHF9 in LAM500460)] is associated with crustose corallines. It is light reddish brown with several darker zones (corresponding to the

**Figs 2–6.** *Ralfsia hancockii*, type specimen. Light microscope (LM).

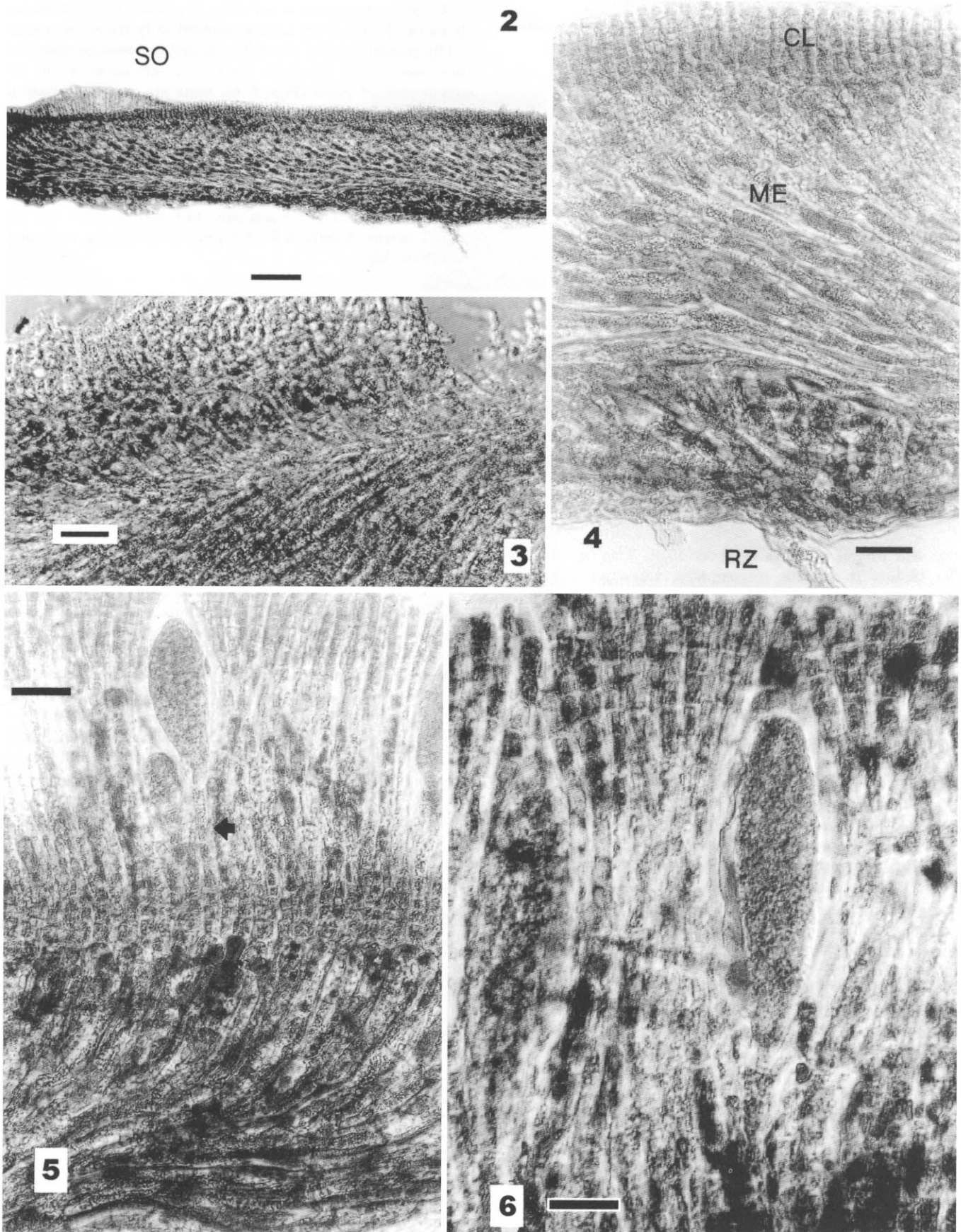
**Fig. 2.** Unilateral growth. Thallus with young sorus (SO). Scale bar = 92  $\mu\text{m}$ .

**Fig. 3.** Filaments diverging upwards and downwards. Scale bar = 23  $\mu\text{m}$ .

**Fig. 4.** Cortical layer (CL) differentiated from the medulla (ME). Part of rhizoid (RZ). Scale bar = 23  $\mu\text{m}$ .

**Fig. 5.** Unangia with more than one stalk cell (arrow). Scale bar = 23  $\mu\text{m}$ .

**Fig. 6.** Stalked unangia, terminally inserted. Scale bar = 15  $\mu\text{m}$ .



**Table 1.** Specimens examined from FCME.

Catalogue number	Slide number	Description number
PTM3326	53–54	10
PTM3541	121	11
PTM2852	25	59
PTM2213	248	131
PTM3005	255	132
PTM3381	241	124
PTM3588	135	28
PTM3330	56	80
PTM2814	6	37
PTM3336	57	8
PTM2215	83	9
PTM3773	140	29
PTM3571	132	22
PTM3544	123	16
PTM2817	9	61
PTM3547	105	13
PTM3540	120	7
PTM3621	266	125
PTM3622	243	127
PTM3539	114	5
PTM3538	113	4
PTM3376	239	123
PTM3562	104	20
PTM3614	242	126
PTM3641	119	6

sori) and irregular specks or spots up to 7 cm in diameter. The thallus is smooth (rugose and verrucose in isotype UC700549), with a skin-like aspect, radial and concentric growth lines, a surface with many regularly distributed pores, and an unlobed conspicuous margin.

The thickness of the vegetative thallus is 108–306  $\mu\text{m}$ , and 198–360  $\mu\text{m}$  in the reproductive parts. Growth in radial longitudinal sections is mainly unilateral (Fig. 2), although in some thick parts of the thallus the filaments also diverge downwards resulting in bilateral symmetry (Fig. 3). Tightly united, thick-walled medullar filaments arise from several basal layers. They curve upwards as a result of repeated ramification, becoming erect and thinner towards the apices. These filaments have irregular basal cells, 25.2–42.0  $\mu\text{m}$  in length  $\times$  10.5–14.7  $\mu\text{m}$  in diameter, with an l:d of 2.0–2.8, and cylindrical to subspherical subcortical cells, 22–31.5  $\mu\text{m}$   $\times$  10.5–17.8  $\mu\text{m}$ , with an l:d of 1.6–2.6. The cortical layer is four–seven cells thick and is morphologically differentiated from the medulla (Fig. 4); the cortical layer is composed of simple anticlinal filaments with regular cuboidal cells and dome-shaped superficial cells, 6.3–9.4  $\mu\text{m}$   $\times$  5.7–8.4  $\mu\text{m}$ , with an l:d of 1.1–1.6, each with a parietal chloroplast. Hyaline hairs are grouped in pits or cryptostomata. Rhizoids are distributed throughout the thallus but in certain parts they are grouped and are longer (the isotype lacks rhizoids). Pyrenoids are absent.

Unangia are present in sori (Fig. 2) and are scattered sparsely on the thallus. They arise subsuperficially and are surrounded by paraphyses that are differentiated morphologically from the reproductive filaments. Single unangia occur at the terminal ends of stalks (Figs 5, 6); these are ovoid, ellipsoid to claviform, 62.5–92.5  $\mu\text{m}$   $\times$  22.5–42.5  $\mu\text{m}$ , with an l:d of 1.8–3.1. Stalks consist of four–five cells that are 7.5–12.5  $\mu\text{m}$  in diameter and morphologically differentiated from the reproductive filament. Slender claviform paraphyses, 100–150  $\mu\text{m}$  in length, contain 10–13 (18) cells: basal cylindrical cells, 9.4–17.8  $\mu\text{m}$   $\times$  3.1–7.3  $\mu\text{m}$  with an l:d of 2.2–3.7; and ob-ovoid subapical cells, 6.3–11.5  $\mu\text{m}$   $\times$  6.3–8.4  $\mu\text{m}$  with an l:d of 0.9–1.8.

### Specimens from the MTP

The following is a compilation of the descriptions of the specimens collected from the MTP. The alga is crustose and forms orbicular or irregular spots 0.5–15 cm in diameter, dark brown, yellowish brown or greenish to cherry-coloured when moist, dark brown to reddish brown when dry, and yellowish brown after being fixed in formalin, with or without radial growth lines. The surface of the thallus is smooth, rugose or verrucose. Adherence to the substratum is total or partial (in the centre or margin); thalli do not disintegrate when detached from the substratum or when rubbed between the fingers. The margin is prominent and smooth or lobed, the lobes overlapping the thallus or not.

The vegetative part of the thallus is 100–810  $\mu\text{m}$  thick, the reproductive parts 279–810  $\mu\text{m}$  thick. Hyaline hairs are grouped on the thallus in spheroidal cryptostomata or in pits or clefts, arising at half thickness from the thallus in the medullar filaments, or hyaline hairs are absent. Rhizoids are distributed over the entire lower surface of the thallus, or irregularly distributed or absent. Ascocysts are absent.

In radial longitudinal sections, filaments grow unilaterally throughout the crust (Figs 7, 8), bilaterally with little ventral development (Figs 9, 10), bilaterally with an evident central axis in the whole crust, or unilaterally and bilaterally in different parts of the same thallus, and with different degrees of bilateral development. Medullar filaments, tightly united throughout their entire length, arise from prostrate filaments that are arranged irregularly or in some cases regularly in one–five layers; prostrate filaments consist of irregularly shaped cells. Medullar filaments curve as a result of repeated ramification, sometimes tortuously, and usually become thinner towards the apex, although in some cases their diameter increases, branching three or more times; they are not surrounded by a gelatinous matrix. Erect filaments consist of irregular cubic, cylindrical, or polyhedral cells in the basal part, 11.0–87.5  $\mu\text{m}$  long  $\times$  5.2–27.5  $\mu\text{m}$  in diameter, with an l:d of 0.5–5.2; their subcortical cells are botuliform to cylindrical, obovoid

**Figs 7–12.** Specimens from the MTP, now identified as *Ralfsia hancockii*. LM.

**Fig. 7.** Thallus with unilateral growth and sorus (SO). Scale bar = 92  $\mu\text{m}$ .

**Fig. 8.** Thallus with unilateral growth. Scale bar = 92  $\mu\text{m}$ .

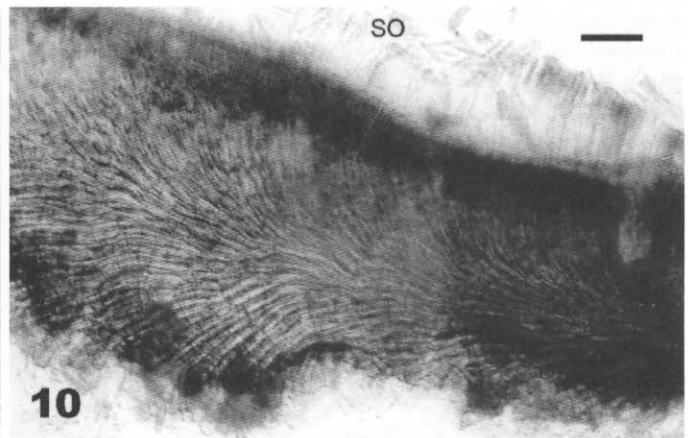
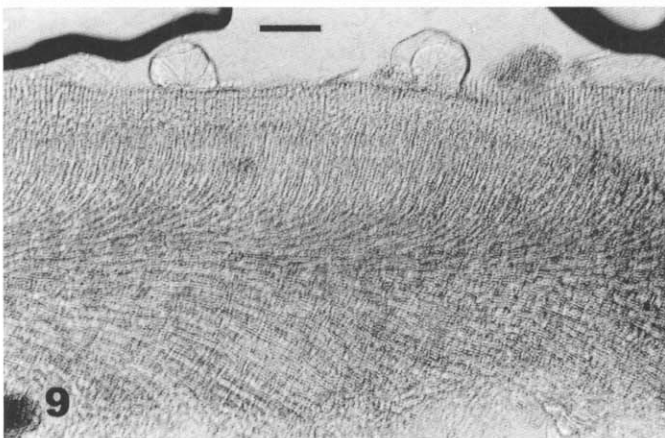
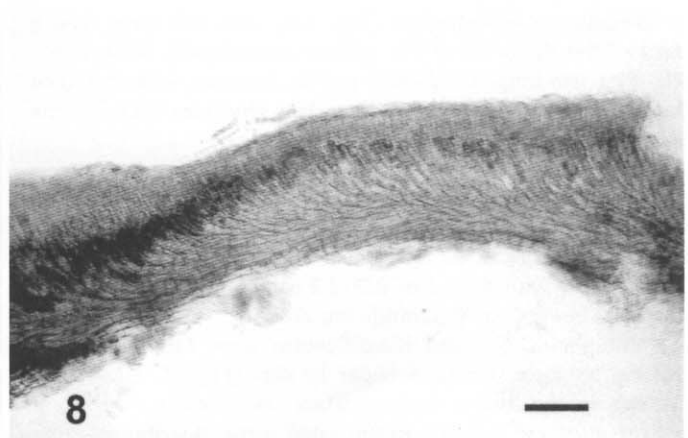
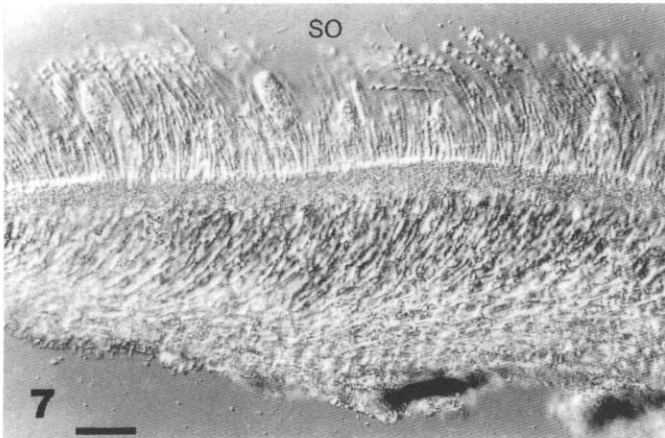
**Fig. 9.** Bilateral thallus. Scale bar = 92  $\mu\text{m}$ .

**Fig. 10.** Bilateral growth with central symmetric axis and sorus (SO). Same specimen as in Fig. 8. Scale bar = 92  $\mu\text{m}$ .

**Fig. 11.** Subcortical cylindrical cells and cortical layer (CL). Scale bar = 23  $\mu\text{m}$ .

**Fig. 12.** Unangium terminally inserted on stalk (arrow). Scale bar = 23  $\mu\text{m}$ .





or irregular to subspherical (Fig. 11), with thin (less than a third of the diameter of the cell) or occasionally thick walls, 9.0–67.5  $\mu\text{m}$  long  $\times$  7.5–32.5  $\mu\text{m}$  in diameter, with an l:d of 0.8–4.5, and frequently with abundant physodes (like hyaline discoid granules).

The cortical layer (Fig. 11) is two–nine cells thick and is clearly morphologically differentiated from the medullar cells. Cortical cells are mainly irregular in form, or commonly doliform to cylindrical, or obovoid to dome shaped, 3.17  $\mu\text{m}$   $\times$  2.5–9.4  $\mu\text{m}$ , with an l:d of 0.7–2.2 (up to 3.4), and a parietal laminar chloroplast. Pyrenoids are absent.

Plurangia of doubtful identification were found on thalli lacking unangia. Unangia occur in sori (Figs 7, 10) spread throughout the thallus surface. They are juvenile, mature, or both mature and juvenile in the same sorus, distributed irregularly, covered by a mucilaginous layer or not, and arising subsuperficially in the thallus, lateral to filaments that are morphologically differentiated from the vegetative ones (paraphyses). The unangia are inserted at the ends of stalks (Fig. 12) that are mainly, but not always, differentiated morphologically from the filament from which they originated. One unangium is present per stalk or reproductive filament. Unangia are ellipsoidal to ovoid, subspherical, or claviform to subclaviform, 37.5–112  $\mu\text{m}$   $\times$  12.5–40.0  $\mu\text{m}$ , with an l:d of 1.2–5.6. Stalks commonly contain three–seven cells (sometimes 2 or 10) that are 5–15  $\mu\text{m}$  in diameter. Slender claviform paraphyses are 55–225  $\mu\text{m}$  in length, with 7–19 basal cylindrical to cubic cells (7.3–20.0  $\mu\text{m}$   $\times$  2–9  $\mu\text{m}$ , with an l:d of 1–7); apical doliform cells are cubic, obovoid, ovoid, or rectangular (dorsoventrally flattened), 4.5–10  $\mu\text{m}$   $\times$  0.5–2.8  $\mu\text{m}$ .

## DISCUSSION

Børgeesen (1912) found that brown algal specimens from the West Indies were quite similar to the sterile type specimen of *R. expansa* [*Myrionema* (?) *expansum* J. Agardh], then in the Botanical Museum in Copenhagen. Although we have not seen the type specimen our observations of specimens from the type locality (Veracruz) wholly agree with the vegetative characteristics of *R. expansa* described by Børgeesen. Several diagnostic vegetative features are consistent for all specimens of this species from Veracruz seen by us or reported by others. These characters include the presence of medullar filaments arising at acute angles relative to the basal prostrate filaments that are not in evident layers, curving and thinning towards the apex as a result of repeated branching, and the presence of a cortical layer of small cells clearly differentiated from the medullary cells (see figures in Weber van Bosse 1913; Schnetter 1976; Tanaka & Chihara 1980a; Lawson & John 1982).

However, some diagnostic characters are known to vary. In Børgeesen's (1912) study of *R. expansa*, it was observed that 'often the leaf is more or less bilateral', similar to the form of *R. verrucosa* (Areschoug) Areschoug illustrated by Le Jolis (1864, t10, no. 37) and Reinke (1889a). Børgeesen even suggested the possibility that *R. expansa* could be a form of *R. verrucosa* worthy of further study. Despite Børgeesen's comments about the growth in *R. expansa*, his drawings of a thallus section with bilateral symmetry were, directly or indirectly, the starting point for many reports in which the species

was identified by this character [compare Børgeesen (1912) with Weber van Bosse (1913), Børgeesen (1914), Taylor (1960), Joly (1965), Earle (1969), Schnetter (1976), Tanaka & Chihara (1980a), and Lawson & John (1982)]. Tanaka & Chihara (1980a) valued symmetry for delimiting *R. expansa* and *R. verrucosa*; nevertheless, they recognized that in the latter species, 'the symmetrically bilateral structure is rarely found' (*ibid.* p. 233), implying that it can also be present. Børgeesen's (1912) comments regarding the variation in growth symmetry of *R. expansa* from the Danish West Indies, and our observations of symmetry in *R. expansa* from the type locality Veracruz (specimen ENCB4257), are sufficient to undermine the diagnostic value of this character in the species.

*Ralfsia hancockii* was not mentioned by Tanaka & Chihara in their review (Tanaka & Chihara 1980a, b, c, 1981a, b, c). In the MTP, this species develops two morphologies (León-Alvarez & González-González 1995), one with variable symmetry, tending to be bilateral, and the other one tending to grow unilaterally. Symmetrical variation also occurs in the type specimen (Figs 2, 3). Our observations indicate that symmetry varies not only between specimens but also within the same MTP specimen (Figs 8, 10). Variation in symmetry has also been reported by Hollenberg (1969) for other *Ralfsia* species such as *R. hesperia* Setchell & Gardner and *R. occidentalis* Hollenberg in Taylor (1945) [included in Hollenberg (1969) in the synonymy of *R. pacifica* Hollenberg], further decreasing the diagnostic value of symmetry in these *Ralfsia* species.

The diagnostic value of the reproductive characters in *R. expansa* is also controversial because of the sterility of Agardh's type specimen. Børgeesen (1912, p. 4) considered the presence of unicellular stalks as an essential difference between *R. expansa* and *R. verrucosa*. By contrast, Tanaka & Chihara (1980a) observed in *R. expansa* unangial stalks or 'pedicells' of three–six cells, but suggested that the difference was 'a result of differences in strains' (Tanaka & Chihara 1980a, p. 233). Over the years, we have not observed unangia in specimens of *R. expansa* collected in Veracruz; unicellular stalks have been also reported for this species by Schnetter (1976) from the Atlantic coast of Colombia.

*Ralfsia hancockii* was originally described as having unangial stalks formed by several cells and 'unknown' plurangia (Dawson 1944). The descriptions of *R. hancockii* and *R. expansa sensu* Tanaka & Chihara with unangia are therefore practically identical. If the cell number of the stalk is a diagnostic feature, then *R. expansa sensu* Tanaka & Chihara could be assigned to *R. hancockii*; both are described as having at least three cells per stalk (Table 2). Otherwise, *R. hancockii* could be a synonym of *R. expansa*.

Although there is variation in the morphology of the specimens of *R. hancockii* from the MTP (León-Alvarez & González-González 1995), they all have unangia with three–seven stalk cells (in rare cases when two stalk cells are present, the same sorus has up to six) with little variation (SD 1.3; mean 4.7), and never zero–one cells (as for *R. expansa sensu* Børgeesen).

The presence of two or more stalk cells (an attribute confirmed in the type material with four cells) (Fig. 5) appears to be unique to *R. hancockii*, whereas the presence of just one stalk cell characterizes *R. expansa sensu* Børgeesen (*non* Tanaka & Chihara), whose description is most frequently fol-

**Table 2.** Diagnostic characteristics of *Ralfsia expansa* and *R. hancockii*.

	<i>R. expansa sensu</i> Børgesen <sup>1</sup>	<i>R. expansa sensu</i> Tanaka & Chihara <sup>2</sup> = <i>R. hancockii</i>	<i>R. hancockii</i> Dawson <sup>3</sup>
Thickness (µm)	ni <sup>4</sup>	200–800 (–1100)	175–200–306 <sup>5</sup>
Symmetry	frequently bilateral	bilateral	mainly unilateral <sup>5</sup>
Epithallous cells			
Diameter (µm)	ni	15–25	10–15 <sup>5</sup>
Length–diameter ratio	ni	2.5–4	2–2.8
Cortical layer	present	present	present <sup>5</sup>
Cell diameter (µm)	—	4–6	5.7–8.4 <sup>5</sup>
Cell length–diameter ratio	—	1–1.2	1.1–1.6 <sup>5</sup>
Plurangia	present	present	not in the type specimen <sup>5</sup>
Insertion	terminal	intercalary	—
Unangia			
Length (µm)	75–120	75–95 (110)	62.5 <sup>5</sup> –90–110
Diameter (µm)	30	23–35	20–42.5 <sup>5</sup>
Cells (no. per stalk)	1 (0, very seldom)	3–6	4–5 <sup>5</sup>
Paraphyses			
Length (µm)	100–170	100–190 (220)	100 <sup>5</sup> –150–175
Basal cells			
Diameter (µm)	ca. 3	2.5–3	3.1–7.3
Length–diameter ratio	ca. 4 (Fig. 2)	4–7	2.2–3.7
Unangia and plurangia	on different thalli	sometimes on the same thallus	only unangia in the type specimen <sup>5</sup>

<sup>1</sup> Børgesen (1912, pp. 1–4, figs 1, 2).

<sup>2</sup> Tanaka & Chihara (1980b, pp. 231–233, figs 1B, 2C, D).

<sup>3</sup> Dawson (1944, p. 223, pl. 31, figs 6, 7, pl. 54, fig. 2).

<sup>4</sup> ni, not indicated; —, not applicable.

<sup>5</sup> Holotype D640, LAM500460.

lowed. With our present knowledge of the species, the diagnostic significance of the absence of plurangia in *R. hancockii* from the MTP is uncertain.

The high similarity between *R. hancockii* and *R. expansa* suggests that both species could be considered subspecies under *R. expansa*. However, because our knowledge of *R. expansa* and its reproductive structures is incomplete, we believe it is better to consider it as distinct from *R. hancockii*.

*Ralfsia hancockii* and *R. expansa* can be distinguished from

other species of the genus [those included in subgenus *Eu-ralfsia* Batters (Batters 1890)] by the combination of characters shown in Table 3.

### Biogeographic considerations

*Ralfsia expansa* has frequently been reported on the Atlantic coast of Mexico and in many sites outside Mexico (Agardh 1847, 1848; Kim 1964; Garza-Barrientos 1976; Huerta-Múz-

**Table 3.** Characteristics distinguishing *Ralfsia expansa* and *R. hancockii* from other species of the genus (subgenus *Eu-ralfsia*), including the type species *R. fungiformis*.

	<i>R. hancockii</i>	<i>R. expansa sensu</i> Børgesen	<i>R. verrucosa</i>	<i>R. integra</i>	<i>R. pacifica</i>	<i>R. hesperia</i>	<i>R. fungiformis</i> (Gunnerus) Setchell & Gardner
Cortical layer	present	present <sup>1</sup>	absent <sup>2</sup>	absent <sup>3</sup>	absent <sup>4</sup>	ni <sup>5,6</sup>	present <sup>7,8</sup>
Unangial stalk	present	present or absent (very seldom)	absent <sup>2,9</sup>	present or absent <sup>3,8</sup>	absent <sup>4</sup> (usually) <sup>8</sup>	present or absent <sup>8</sup>	absent <sup>10</sup>
Cell number	> 1 (usually 4)	1 (0)	0	0–?	0	0–16	0

<sup>1</sup> Børgesen (1912, fig. 1b).

<sup>2</sup> Reinke's (1889b) and Fletcher's (1987) detailed illustrations.

<sup>3</sup> US61155 holotype.

<sup>4</sup> US61158 lectotype.

<sup>5</sup> Setchell & Gardner (1924, 1925).

<sup>6</sup> ni, not indicated.

<sup>7</sup> Tanaka & Chihara (1980a) and Fletcher (1987).

<sup>8</sup> Hollenberg (1969).

<sup>9</sup> Womersley (1987).

<sup>10</sup> Tokida (1954), Edelstein *et al.* (1968), and Tanaka & Chihara (1980a).

quiz & Garza-Barrientos 1980; Sánchez-Rodríguez 1980; Huerta-Múzquiz *et al.* 1987), including the tropical and subtropical Atlantic coast (Taylor 1960; Joly 1965; Schnetter 1976) and Africa (Lawson & John 1982). *Ralfsia expansa* was reported on the Pacific coast of México by Chávez (1972); however, the specimens were not documented, and therefore the identification could not be substantiated.

*Ralfsia hancockii* Dawson has been reported only in the Gulf of California (Dawson 1944) and in the Pacific coast of Mexico (Dawson 1954, 1961; León-Alvarez & González-González 1993, 1995), from Bahía de Banderas (Nayarit and Jalisco States) to Santa Elena (Oaxaca State) (Fig. 1). We have also observed in some samples from Pacific Mexico, specimens of *R. hancockii* that had been mistaken for another species. One specimen from Acapulco, Guerrero State (February 1947; UC925766), was determined by Dawson to be *R. occidentalis* (= *R. pacifica* Hollenberg 1969), and another sample was specified as *R. pacifica* by Hollenberg (HAHF48148 in LAM599699) with the following note: '*R. hancockii* except for color. . . Un. sporangia on (2)–4–6 celled pedicels'. A report of *R. pacifica* in Michoacan was based on sterile specimens that were drawn with a cortex (Dreckman-Estay 1987; Dreckman *et al.* 1990). These observations suggest that *R. hancockii* has also been confused with *R. pacifica* (*R. verrucosa* *sensu* Womersley 1987). *Ralfsia hancockii* is a typical algal species from the Mexican Pacific that may have been derived (and changed little since) from the closely related *R. expansa* from the Gulf of Mexico, when the Tehuantepec isthmus was closed during the Tertiary period 3.5–4 million years ago (Tamayo 1949).

## CONCLUSIONS

From our evaluation of the diagnostic characteristics and the biogeographic affinity of our *Ralfsia* specimens, we conclude that *R. expansa sensu* Tanaka & Chihara (1980a) previously reported from the MTP is actually *R. hancockii*. We consider *R. expansa sensu* Børgesen *non* Tanaka & Chihara to be restricted to the Atlantic, from where it has frequently been reported.

## ACKNOWLEDGEMENTS

We are indebted to Drs Michael Wynne, Paul Silva and Marilza Cordeiro† for their valuable comments; to Drs James Norris, Robert Sims, Valery Anderson and Dick Moe for their hospitality during our visits to consult collections; to Cheryl Patten for checking our English; to Alejandro Martínez Mena and José Antonio Hernández for taking photographs; and to Gayle I. Hansen and Robert Waaland for reviewing the manuscript.

## REFERENCES

AGARDH J.G. 1847. Nya alger från Mexico. *Öfversigt af Kongliga Vetenskaps-Akademiens Förhandlingar* 4: 5–17.

- AGARDH J.G. 1848. *Species, genera et ordines algarum. Algas fucoideas complectens*, vol. 1. Gleerup, Lund, Sweden. 363 pp.
- ARESCHOUG J.E. 1843. Algarum (Phycearum) minus rite cognitarum pugillus secundus. *Linnaea* 17: 257–269.
- BATTERS E.A.L. 1890. A list of the marine algae of Berwick-on-Tweed. *History of the Berwickshire Naturalist's Club* 12: 221–392.
- BØRGESSEN F. 1912. Two crustaceous brown algae from the Danish West Indies. *Nuova Notarisa* 23: 123–129.
- BØRGESSEN F. 1914. The marine algae of the Danish West Indies, vol. 1, part. 2. Phaeophyceae. *Dansk botanisk Arkiv* 2: 157–226.
- CHÁVEZ M.I. 1972. Estudio de la flora marina de la Bahía de Zihuatanejo y lugares adyacentes. In: *Memorias IV Congreso Nacional de Oceanografía*, México (Ed. by J. Carranza), pp. 265–271. Sociedad Mexicana de Oceanografía, México.
- DAWSON E.Y. 1944. The marine algae of Gulf of California. *Allan Hancock Pacific Expeditions* 3(10): 189–454.
- DAWSON E.Y. 1954. The marine flora of Isla San Benedicto following the volcanic eruption of 1952–1953. *Allan Hancock Foundation Publications Occasional Papers* 16: 1–25.
- DAWSON E.Y. 1961. A guide to the literature and distributions of Pacific benthic algae from Alaska to the Galapagos Islands. *Pacific Science* 15: 370–461.
- DRECKMAN K.M., PEDROCHE E.F. & SENTÍES A. 1990. Lista florística de las algas marinas bentónicas de la costa norte de Michoacán México. *Boletín de la Sociedad Botánica de México* 50: 19–42.
- DRECKMAN-ESTAY K.M. 1987. *Algas marinas bentónicas de Playa San Telmo, Michoacán, México*. Tesis Profesional. Facultad de Ciencias, Universidad Nacional Autónoma de México, México D.F. 170 pp.
- EARLE S.A. 1969. Phaeophyta of the Eastern Gulf of Mexico. *Phycologia* 7: 71–254.
- EDELSTEIN T., CHEN L. & MCLACHLAN J. 1968. Sporangia of *Ralfsia fungiformis* (Gunn.) Setchell et Gardner. *Journal of Phycology* 4: 157–160.
- FLETCHER L.R. 1987. *Seaweeds of the British Isles*, vol. 3. British Museum, London. 330 pp.
- GARZA-BARRIENTOS M.A. 1976. Primeras consideraciones referentes a la flora marina del sureste de la República Mexicana. In: *Memorias de la Iª Reunión Latinoamericana sobre Ciencia y Tecnología de los Océanos* (Ed. by R. Perez Rodríguez & M.R. Suárez Zozaya), pp. 210–239. Secretaría de Marina, Veracruz, México.
- GONZÁLEZ-GONZÁLEZ J. & NOVELO-MALDONADO E. 1986. Algas. In: *Manual de herbario, administración y manejo de colecciones, técnicas de recolección y preparación de ejemplares botánicos* (Ed. by A. Lot & F. Chiang), pp. 47–54. Consejo Nacional de la Flora de México, México D.F. 142 pp.
- HOLLENBERG G.J. 1969. An account of the Ralfsiaceae (Phaeophyta) of California. *Journal of Phycology* 5: 290–301.
- HUERTA-MÚZQUIZ L. & GARZA-BARRIENTOS A. 1980. Contribución al conocimiento de la flora marina de la zona sur del litoral de Quintana Roo, México. *Anales de la Escuela Nacional de Ciencias Biológicas (México)* 23: 25–44.
- HUERTA-MÚZQUIZ L., MENDOZA-GONZÁLEZ C. & MATEO-CID L.E. 1987. Avance sobre un estudio de las algas marinas de la Península de Yucatán. *Phytologia* 62: 23–53.
- JOLY A.B. 1965. Flora marinha do litoral norte do Estado de São Paulo e regiões circunvizinhas. *Boletim da Faculdade de Filosofia, Ciências e Letras da Universidade de São Paulo (Serie Botânica)* 294(21): 5–393.
- KIM C.S. 1964. *Marine algae of Alacran Reef, Southern Gulf of Mexico*. Unpublished PhD thesis. Department of Botany, Graduate School of Arts and Sciences, Duke University, Durham, North Carolina. 213 pp.
- LAWSON G.W. & JOHN D.M. 1982. The marine algae and coastal environment of Tropical West Africa. *Nova Hedwigia* 70: 1–455.
- LE JOLIS A. 1864. Liste des algues marines de Cherbourg. *Mémoires de la Société Impériale des sciences naturelles de Cherbourg* 10: 1–168.
- LEÓN-ALVAREZ D. & GONZÁLEZ-GONZÁLEZ J. 1993. Algas costrosas del Pacífico tropical mexicano. In: *Biodiversidad marina y costera de México* (Ed. by S.I. Salazar-Vallejo & N.E. González), pp. 456–

† Deceased.



474. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad y Centro de Investigaciones de Quintana Roo, México D.F.
- LEÓN-ALVAREZ D. & GONZÁLEZ-GONZÁLEZ J. 1995. Characterization of the environmental distribution and morphs of *Ralfsia hancockii* Dawson (Phaeophyta) in the Mexican Tropical Pacific. *Botanica Marina* 38: 359–367.
- REINKE J. 1889a. Algenflora der Westlichen Ostsee Deutschen Antheils. Eine systematisch-pflanzengeographische Studie. *Bericht der Commission für wissenschaftlichen Untersuchung der deutschen Meere in Kiel für die Jahre 1887 bis 1889* 6: 1–101.
- REINKE J. 1889b. *Atlas deutscher Meeresalgen*, part 1, pls 1–25. Paul Parey, Berlin. 34 pp.
- SÁNCHEZ-RODRÍGUEZ M.E. 1980. Ficoflora del sustrato rocoso dentro de las Costas del Golfo de México. *Boletim Instituto Oceanográfico de São Paulo* 29: 347–350.
- SCHNETTER R. 1976. *Marine Algen der karibischen Küsten von Kolumbien*, vol. 1. Phaeophyceae, Band 24. J. Cramer, Germany. 125 pp.
- SETCHELL W.A. & GARDNER N.L. 1924. Phycological contributions VII. *University of California Publications in Botany* 13: 1–13.
- SETCHELL W.A. & GARDNER N.L. 1925. The marine algae of the Pacific coast of North America. Part III. Melanophyceae. *University of California Publications in Botany* 8: 383–898.
- TAMAYO J.L. 1949. *Geografía general de México*, vol. 1. Geografía física. Cooperativa de los Talleres Gráficos de la Nación, México, D.F. 628 pp.
- TANAKA J. & CHIHARA M. 1980a. Taxonomic study of the Japanese crustose brown algae (2) *Ralfsia* (Ralfsiaceae, Ralfsiales), part 1. *Journal of Japanese Botany* 55: 225–236.
- TANAKA J. & CHIHARA M. 1980b. Taxonomic study of the Japanese crustose brown algae (1). General account and the order Ralfsiales. *Journal of Japanese Botany* 55: 193–201.
- TANAKA J. & CHIHARA M. 1980c. Taxonomic study of the Japanese crustose brown algae (3). *Ralfsia* (Ralfsiaceae, Ralfsiales), part 2. *Journal of Japanese Botany* 55: 337–342.
- TANAKA J. & CHIHARA M. 1981a. Taxonomic study of the Japanese crustose brown algae (4). *Ralfsia* (Ralfsiaceae, Ralfsiales), part 3. *Journal of Japanese Botany* 56: 97–104.
- TANAKA J. & CHIHARA M. 1981b. Taxonomic study of the Japanese crustose brown algae (5). *Endoplura* and *Diplura* (Ralfsiaceae, Ralfsiales). *Journal of Japanese Botany* 56: 153–160.
- TANAKA J. & CHIHARA M. 1981c. Taxonomic study of the Japanese crustose brown algae (6) *Pseudolithoderma* (Lithodermataceae, Ralfsiales). *Journal of Japanese Botany* 56: 77–381.
- TAYLOR W.R. 1945. Pacific marine algae of the Allan Hancock Expeditions to the Galapagos Islands. *Allan Hancock Pacific Expeditions* 12: 1–528.
- TAYLOR W.R. 1960. *Marine algae of the eastern tropical and sub-tropical coasts of the Americas*. The University of Michigan Press, Ann Arbor. 870 pp.
- TOKIDA J. 1954. The marine algae of Southern Saghalien. *Memoirs of the Faculty of Fisheries Hokkaido University* 2: 1–264.
- WEBER VAN BOSSE A. 1913. Liste des algues du Siboga. *Siboga Expedition Monographie* 59a, pp. 1–186.
- WOMERSLEY H.B.S. 1987. *The marine benthic flora of Southern Australia. Part II*. South Australian Government Printing Division, Adelaide. 484 pp.

Accepted 2 April 2003