



## Ultraviolet sunscreen compounds in epiphytic red algae from mangroves

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### Abstract

Epiphytic red algae of the order Ceramiales from mangroves and salt marshes (nine species from *Bostrychia*, three from *Stictosiphonia* and four from *Caloglossa*) produce varying levels of the UV-absorbing compounds mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330 and palythinol, a suite of substances chemically assigned as mycosporine-like amino acids (MAAs). Mean MAA levels varied from 0.02 to 12.8 mg g<sup>-1</sup> DW in field-collected and laboratory cultured specimens. While in field samples of *Bostrychia montagnei* Harvey, *Bostrychia radicans* (Montagne) Montagne and *Caloglossa apomeiotica* J. West et G. Zuccarello MAA concentrations were generally higher compared to cultured plants of the same taxa, *Bostrychia tenella* (Lamouroux) J. Agardh did not show such a difference. *Catenella caespitosa* (Withering) L. Irvine, *Catenella impudica* (Montagne) J. Agardh and *Catenella nipae* Zanardini (Gigartinales, Caulacanthaceae) produce two novel UV-absorbing compounds: MAA-1 (1.4–4.3 mg g<sup>-1</sup> DW) and MAA-2 (0.1–1.0 mg g<sup>-1</sup> DW), which absorb at 334 nm and 320 nm, respectively. In laboratory culture of *Bostrychia moritziana* when photosynthetically active radiation (PAR) was increased from 20 to 40 μmol photons m<sup>-2</sup> s<sup>-1</sup>, the total level of palythinol increased by 85% (from 2.0 to 3.7 mg g<sup>-1</sup> DW). In a culture of *Caloglossa leprieurii* when PAR was increased from 40 to 80 μmol m<sup>-2</sup> s<sup>-1</sup> the porphyra-334 content increased by 77% (from 3.1 to 5.5 mg g<sup>-1</sup> DW). Extremely high MAA contents of >30 mg g<sup>-1</sup> DW were detected in mature tetrasporangial sori prepared from two isolates of laboratory-cultured reproductive *Caloglossa apomeiotica* compared to vegetative plants (about 10 mg MAAs g<sup>-1</sup> DW) indicating tetraspores loaded up with UV-sunscreens. All data demonstrate that mangrove red algae contain high MAA concentrations, particularly the reproductive structures, and hence these compounds may act as biochemical photoprotectants against exposure to UV-radiation.

### Introduction

The tropical mangrove ecosystem provides habitat, shelter and food for many marine organisms such as crabs, molluscs and fish. The pneumatophores, prop roots and basal trunks of the mangrove trees are very often densely covered with epiphytic red algae of the so-called *Bostrychia-Caloglossa* association (Post, 1968), a group of species representing an important source of primary production for this system. The algal taxa primarily include *Bostrychia*, *Stictosiphonia*, *Caloglossa* (Order Ceramiales) and *Catenella* (Order Gigartinales) (King & Puttock, 1989, 1994;

Dawes, 1996). Some representatives of these genera also occur as epiphytes in temperate salt marshes or epilithically growing on exposed rocky shores. However, most of these marine plants exist in almost 'terrestrial' conditions in the upper littoral zone of the mangroves (Post, 1968), and due to tidal immersion-emersion cycles, inhabit a highly variable environment (Karsten et al., 1992). Diurnal and seasonal changes of abiotic parameters include strong fluctuations in salinity, temperature, nutrient concentrations and the light regime.

Enhanced solar ultraviolet (UV) radiation due to the depletion of the stratospheric ozone layer has been proposed as a major stress factor for many photo-

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trophic organisms in aquatic ecosystems (Franklin & Forster, 1997). Even under normal stratospheric ozone concentrations in warm-temperate to tropical regions, UV-radiation is strong enough to affect marine organisms in the intertidal zone (Fleischmann, 1989; Madronich, 1993).

Multiple harmful effects of UV-radiation on marine primary producers have been reported, and include the direct influences of UV-B (280–320 nm) on molecular targets such as DNA, RNA and proteins, on physiological processes such as photosynthesis, growth and on community structures (Smith et al., 1992; Buma et al., 1995; Davidson et al., 1996; Franklin & Forster, 1997; Häder & Figueroa, 1997; Yakovleva et al., 1998; Aguilera et al., 1999). Although UV-A (320–400 nm) may also have a net damaging influence on photosynthesis (Cullen et al., 1992), this waveband in general exerts a more positive effect on physiology by inducing photobiological events such as photolyase activity to repair DNA damage (Buma et al., 1997).

Of major interest is the identification of repair and/or protective mechanisms that allow phototrophic organisms living in high-light habitats to survive and reproduce. Photoreactivation and excision repair of DNA, accumulation of water- and lipid-soluble antioxidants and the activation of antioxidant enzymes represent typical biochemical defenses against UV-induced photochemical damage (Britt, 1995; Dunlap & Shick, 1998).

Another important physiochemical mechanism against biologically harmful UV-radiation involves the biosynthesis and accumulation of UV sunscreens. Typically absorbing in the UV-A and UV-B wavebands, these compounds were invoked to function as passive shielding substances by dissipating the absorbed radiation energy in form of harmless heat without generating photochemical reactions (Bandaranayake, 1998) or photosensitising the formation of reactive oxygen species (Dunlap & Yamamoto, 1995).

The most common substances with a potential role as UV-sunscreens in marine organisms are the mycosporine-like amino acids (MAAs), a suite of chemically closely related, water-soluble compounds. MAAs have been identified in taxonomically diverse marine organisms including bacteria, cyanobacteria, micro- and macroalgae, invertebrates and fish (for review see: Dunlap & Shick, 1998; Sinha et al., 1998). Typically absorbing between 310 and 360 nm (Karentz et al., 1991), their function as intracellular screening agents has been inferred from a decrease in concentra-

tion with increasing depth as observed in corals (Dunlap et al., 1986) and macroalgae (Karsten et al., 1999). In addition, macroalgae from South Europe contained up to 2-fold higher MAA contents compared to similar species from higher latitudes indicating a positive relationship with the natural solar UV-radiation of the respective biogeographic region, i.e. the higher the UV-dose the more MAAs are formed and accumulated (Karsten et al., 1998a). MAA-formation and accumulation can be species-specifically induced by PAR and/or UV-A/B (Helbing et al., 1996; Hannach & Sigleo, 1998; Neale et al., 1998; Karsten et al., 1998b, 1999; Karsten & Wiencke, 1999; Franklin et al., 1999). The sunscreen function of MAAs has mainly been demonstrated for cyanobacteria. Using growth as an indicator for physiological fitness under UV-stress, Garcia-Pichel et al. (1993) reported that MAA-loaded cells showed much less UV-induced inhibition compared with unloaded cells. In more recent studies on eukaryotic algae, Riegger & Robinson (1997) calculated sunscreen factors for Antarctic phytoplankton due to the presence of MAAs of up to 0.72, i.e. 72% of harmful UV quanta were absorbed before hitting intracellular molecular targets. In the red-tide dinoflagellate *Gymnodinium sanguineum* Hirasaka, MAAs prevent, at least partially, UV-induced inhibition of photosynthesis (Neale et al., 1998).

Although MAAs are widely present in various types of marine organisms, few data exist of their type and quantity in macroalgae (Tsujino et al., 1978, 1980; Nakamura et al., 1982; Karentz et al., 1991; Karsten et al., 1998a,b; Yakovleva, 1999), in particular from tropical high-radiation ecosystems such as mangroves. In a preliminary study, we measured for the first time in 14 macroalgal samples from mangroves the MAA composition and found generally high concentrations compared to temperate species (Karsten et al., 1998c). In the present investigation, a qualitative and quantitative inventory was made of MAAs in mangrove and salt marsh macroalgae collected from around the world. For some species, reproductive and vegetative tissues were compared. In addition, the effect of different intensities of photosynthetic active radiation (PAR, 400–700 nm) on the MAAs in selected species was studied.

## Materials and methods

The locations and dates of collection for the macroalgal species studied are listed in Table 1. Plants

Table 1. Location and date of red macroalgae investigated growing as epiphytes in the intertidal zone of mangroves. Some species were collected from salt marshes and anthropogenic hard substrata

<i>Bostrychia calliptera</i> (Montagne) Montagne I	Field	Guatemala, Likin, 22 Mar. 1993
<i>Bostrychia calliptera</i> II	Field	Brasil, Ilha do Cardoso, 4 Apr. 1990
<i>Bostrychia harveyi</i> Montagne I	Field	Australia, Tasmania, Huonville, 2 Mar. 1993
<i>Bostrychia harveyi</i> II	Field	Australia, Tasmania, Port Arthur, 27 Feb. 1993
<i>Bostrychia harveyi</i> III	Field	New Zealand, Wellington, 17 Oct. 1964
<i>Bostrychia montagnei</i> Harvey I	Field	Brasil, Ilha do Cardoso, 5 Apr. 1990
<i>Bostrychia montagnei</i> II	2438	Bermuda, 16 Sep. 1980
<i>Bostrychia moritziana</i> (Sonder ex Kützinger) J. Agardh I	Field	Brasil, Ilha do Cardoso, 5 Apr. 1990
<i>Bostrychia moritziana</i> II	Field	Venezuela, Rio El Pilar, 23 Apr. 1992
<i>Bostrychia pinnata</i> J. Tanaka et Chihara	Field	Australia, Darwin, 18 Jul. 1987
<i>Bostrychia radicans</i> (Montagne) Montagne I	Field	Mexico, La Paz, 18 May 1992
<i>Bostrychia radicans</i> II	Field	Mexico, Loreto, 23 May 1992
<i>Bostrychia radicans</i> III	Field	Mexico, Mulege, 24 May 1992
<i>Bostrychia radicans</i> IV	Field	Mexico, Teacapan, 20 Mar. 1992
<i>Bostrychia radicans</i> V	Field	Mexico, San Blas, 22 Mar. 1992
<i>Bostrychia radicans</i> VI	Field	Mexico, Jalisco, 19 Mar. 1992
<i>Bostrychia radicans</i> VII	Field	Mexico, Bahia Magdalena, 24 Mar. 1991
<i>Bostrychia radicans</i> VIII	Field	Guatemala, Likin, 22 Mar. 1993
<i>Bostrychia radicans</i> IX	Field	South Africa, Durban, 4 Oct. 1991
<i>Bostrychia radicans</i> X	3144	Venezuela, Edo Sucre, 11 Apr. 1991
<i>Bostrychia radicans</i> XI	3228	USA, York River, Virginia, 6 Nov. 1991
<i>Bostrychia radicans</i> XII	2879	USA, Mullica River, New Jersey, 17 Jul. 1973
<i>Bostrychia radicans</i> XIII	2928	USA, Tampa, Florida, 16 Oct. 1988
<i>Bostrychia radicans</i> XIV	2973	Australia, Darwin, 4 Jun. 1989
<i>Bostrychia radicans</i> XV	3003	Micronesia, Kosrae, 2 Sep. 1989
<i>Bostrychia radicans</i> XVI	3017	Brasil, Ilha do Cardoso, 24 Nov. 1989
<i>Bostrychia radicans</i> XVII	2832	Australia, Magnetic Island, 4 Jun. 1987
<i>Bostrychia radicans</i> XVIII	3043	Peru, Puerto Pizarro, 10 Feb. 1990
<i>Bostrychia scorpioides</i> (Hudson) Montagne. I	Field	France, Brittany, 13 Jul. 1990
<i>Bostrychia scorpioides</i> II	Field	The Netherlands, Osterschelde, 8 Sep. 1983
<i>Bostrychia simpliciuscula</i> Harvey ex J. Agardh.	Field	Japan, Okinawa, 7 Sep. 1993
<i>Bostrychia tenella</i> (Lamouroux) J. Agardh. I	Field	Australia, Broome, 8 Dec. 1987
<i>Bostrychia tenella</i> II	Field	Indonesia, Batam Island, 16 Jun. 1989
<i>Bostrychia tenella</i> III	Field	Japan, Okinawa, 7 Sep. 1993
<i>Bostrychia tenella</i> IV	2756	Puerto Rico, La Parguera, 2 Nov. 1986
<i>Bostrychia tenella</i> V	2978	Belize, Twin Cays, 10 Jul. 1989
<i>Bostrychia tenella</i> VI	2764	Philippines, Initao, 13 May 1987
<i>Caloglossa apomeiotica</i> J. West et G. Zuccarello I	Field	Mexico, La Paz, 18 May 1992
<i>Caloglossa apomeiotica</i> II	3025	Mexico, Bahia Magdalena, 22 May 1992
<i>Caloglossa apomeiotica</i> III	Field	Mexico, Jalisco, 19 Mar. 1992
<i>Caloglossa leprieurii</i> (Montagne) J. Agardh. I	Field	Guatemala, El Manchon, 23 Mar. 1993
<i>Caloglossa leprieurii</i> II	Field	Mexico, Esteros las Garzas, 25 Mar. 1993
<i>Caloglossa leprieurii</i> III	Field	Australia, Botany Bay, 7 Sep. 1991
<i>Caloglossa leprieurii</i> IV	Field	Australia, Georges River, 7 Sep. 1991
<i>Caloglossa leprieurii</i> V	Field	Australia, Port Jackson, 8 Sep. 1991
<i>Caloglossa leprieurii</i> VI	Field	Australia, Wilsons Promontory, 2 Oct. 1991
<i>Caloglossa leprieurii</i> VII	Field	Australia, Port Arthur, 27 Feb. 1993
<i>Caloglossa ogasawaraensis</i> Okamura	Field	Japan, Eno Island, 31 Aug. 1993
<i>Caloglossa stipitata</i> Post	3375	Guatemala, Likin, 22 Mar. 1993
<i>Catenella caespitosa</i> (Withering) L. Irvine I	Field	Japan, Fukidou River, 11 Sep. 1993

Continued on p. 162

Table 1. contd.

<i>Catenella caespitosa</i> II	Field	Japan, Miyara River, 11 Sep. 1993
<i>Catenella impudica</i> (Montagne) J. Agardh.	Field	Japan, Okinawa, 7 Sep. 1993
<i>Catenella nipae</i> Zanardini I	Field	Australia, Georges River, 21 Jan. 1993
<i>Catenella nipae</i> II	Field	Australia, Harrington, 13 Mar. 1993
<i>Catenella nipae</i> III	Field	Australia, Wapengo Creek, 13 Jan. 1993
<i>Catenella nipae</i> IV	Field	Australia, Salamander Bay, 13 Mar. 1993
<i>Catenella nipae</i> V	Field	Australia, Moruya River, 12 Jan. 1993
<i>Catenella nipae</i> VI	Field	Australia, Nelson Lagoon, 13 Jan. 1993
<i>Catenella nipae</i> VII	Field	Australia, Hawkesbury Bridge, 14 Mar. 1993
<i>Catenella nipae</i> VIII	Field	Australia, Newcastle, 13 Mar. 1993
<i>Catenella nipae</i> IX	Field	Australia, Port Macquarie, 13 Mar. 1993
<i>Stictosiphonia arbuscula</i> J.D. Hooker et Harvey	Field	New Zealand, Dunedin, 13 Mar. 1988
<i>Stictosiphonia intricata</i> (Bory de Saint Vincent) P.Silva I	Field	Australia, Crystal Head, 11 Jul. 1987
<i>Stictosiphonia intricata</i> II	Field	Australia, Sydney, 24 Oct. 1992
<i>Stictosiphonia intricata</i> III	Field	Australia, Whiskey Bay, 26 Nov. 1994
<i>Stictosiphonia intricata</i> IV	Field	South Africa, Durban, 10 Aug. 1989
<i>Stictosiphonia tangatensis</i> Post	3242	South Africa, St. Lucia, 22 Dec. 1991

were air-dried in the sun within 30–45 min, a time period too short to induce any changes in intracellular MAA concentration. Samples were stored in sealed plastic bags under cool, dry and dark conditions until analysis. Some samples were kept under these conditions for up to several years without any detectable loss of MAAs as indicated by repeated quantitative determination of extracts made from the same algal material over a period of 18 months ( $p < 0.01$ , 1-way ANOVA). In addition, some samples originated from unialgal culture material. These were isolated from field collections and established in laboratory culture under the following conditions: 30–32 ppt PES/2 culture medium (Starr & Zeikus, 1993), 22–25 °C, 10–40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR, cool-white fluorescent lighting, 12 h light:12 h dark photoperiod. The algae were maintained in continuous immersion culture with changes of medium at intervals of 2–10 weeks.

In two light experiments with *Bostrychia radicans* (culture no. 3144, Venezuela) and *Caloglossa leprieurii* (culture no. 2937, Victoria, Australia), the algae were exposed for 4 weeks to 2-fold higher irradiances (40 and 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Afterwards, the algal samples were oven-dried at 70 °C overnight.

Vegetative and reproductive plants of two isolates of *Caloglossa apomeiotica* (culture no. 3244, Mexico and culture no. 3421, Florida, U.S.A.) were compared for their qualitative and quantitative MAA concentrations. Tetrasporangial sori were excised from mature plants under a stereomicroscope using razor-blades.

Whole plants or a set of reproductive tissue of about 10–20 mg dry weight (DW) were extracted for 1.5–2 h in screw-capped centrifuge vials filled with 1 mL 25% aqueous methanol (v/v) and incubated in a waterbath at 45 °C. After centrifugation at 5000  $g$  for 5 min, 800  $\mu\text{L}$  of the supernatants were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 800  $\mu\text{L}$  100% methanol and vortexed for 30 s. After passing through a 0.2  $\mu\text{m}$  membrane filter, samples were analysed with a Waters HPLC system according to the method of Karsten & Garcia-Pichel (1996), modified as follows. MAAs were separated on a stainless-steel Knauer Spherisorb RP-8 column (5  $\mu\text{m}$ , 250  $\times$  4 mm I.D.) protected with a RP-8 guard cartridge (20  $\times$  4 mm I.D.). The mobile phase was 5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow rate of 0.7 ml min<sup>-1</sup>. MAAs were detected at 330 nm and absorption spectra (290–400 nm) were recorded each second directly on the HPLC-separated peaks. Identification was done by spectra, retention time and by co-chromatography with standards extracted from the marine red macroalgae *Chondrus crispus* Stackhouse (Karsten et al., 1998b) and *Porphyra umbilicalis* (Linnaeus) Kützting, which were kindly provided by Dr L. A. Franklin, Biologische Anstalt Helgoland, Germany, as well as from ocular lenses of the coral trout *Plectropomus leopardus* (Lacepède, 1802), kindly sent by Dr David Bellwood, James Cook University, Townsville, Australia. Quan-

tification was made using the following molar extinction coefficients: shinorine:  $\epsilon_{334}=44\,700$  (Tsuji et al., 1980), palythine:  $\epsilon_{320}=36\,200$  (Takano et al., 1978a), palythinol:  $\epsilon_{332}=43\,500$  (Dunlap et al., 1986), porphyra-334:  $\epsilon_{334}=43\,300$  (Takano et al., 1978a), mycosporine-glycine:  $\epsilon_{310}=28\,100$  (Gleason, 1993), asterina:  $\epsilon_{330}=43\,500$  (Gleason, 1993), palythene:  $\epsilon_{360}=50\,000$  (Takano et al., 1978b). Except two MAAs (in the text referred as MAA-1 and MAA-2), all other compounds could be easily identified. For quantification of both unknown MAAs, an average molar extinction coefficient of all published values ( $=41\,300$ ) was used. All amounts are given as mean of four replicates ( $\pm$ SD) based on separate extracts from separate algae, randomly collected from the respective habitat or from cultures and expressed as concentration on a dry weight basis.

## Results

Ultraviolet absorbing MAAs extracted from air- and oven-dried mangrove macroalgae were characterised by HPLC (Table 2), and identified and quantified according to their retention times, absorption spectra, co-chromatography with standards and molar extinction coefficients. Eight different MAAs could be detected within all samples investigated, of which six were identified as mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330 and palythinol. Two unknown UV-absorbing compounds were found in all species and isolates of the genus *Catenella* (Table 2) exhibiting absorption spectra with a maximum at 334 nm and 320 nm, respectively, the former designated as MAA-1 at 2.5 min and the latter as MAA-2 at 3.0 min in the present study (Figure 1b). A typical HPLC chromatogram of a *Caloglossa* extract is shown in Figure 1a indicating two major peaks of shinorine at 3.2 min and of porphyra-334 at 3.5 min. Co-chromatography of a *Catenella* and a *Caloglossa* extract resulted in four distinctly separated peaks (Figure 1c) indicating different compounds.

Within the red algae investigated, all samples contained MAAs, with many species accumulating high concentrations of up to  $12.77\text{ mg g}^{-1}$  DW (Table 2). In terms of quantity, the sum of all MAAs in the various samples ranged from 0.02 to  $12.77\text{ mg g}^{-1}$  DW, with most plants containing between 1 and  $3\text{ mg g}^{-1}$  DW. Most species showed a high degree of MAA diversity. However, there was some variability in occurrence of specific MAAs among the isolates of each species.

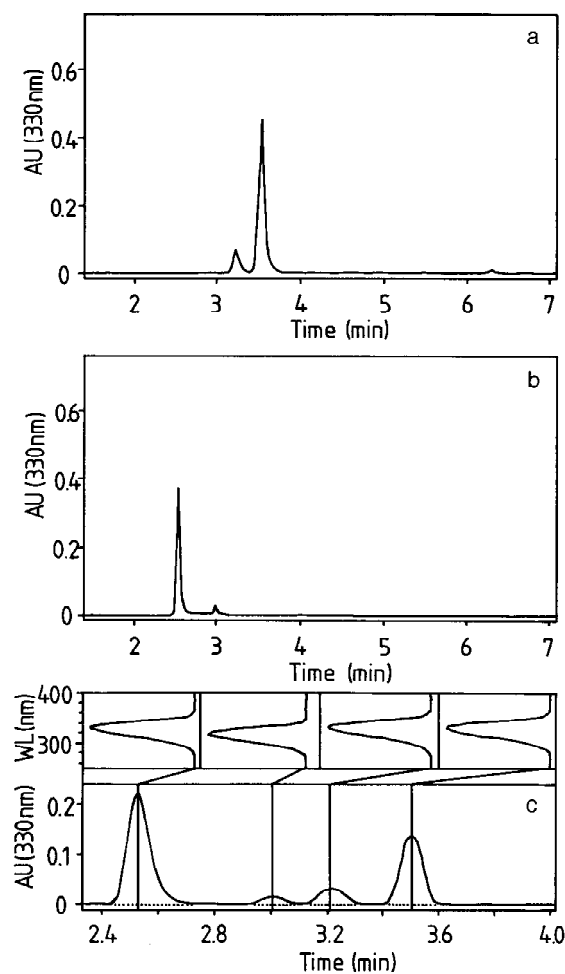


Figure 1. Typical high-performance liquid chromatography chromatograms of *Caloglossa leprieurii* (a) *Catenella nipae* (b) and a mixture of both species (c). In *C. leprieurii*, two major peaks at 3.2 min and 3.5 min representing shinorine and porphyra-334, respectively, could be identified. In *C. nipae* two unknown MAAs designated as MAA-1 at 2.5 min and as MAA-2 at 3.0 min were determined. Co-chromatography of a *Caloglossa* and a *Catenella* extract resulted in four distinguished and separated peaks indicating different compounds. AU: absorbance units; WL: wavelength.

The two field samples of *Bostrychia calliptera* were somewhat different in MAA composition, one contained MAA-1, shinorine, porphyra-334 and asterina-330, whereas the second contained shinorine, porphyra-334, palythine and asterina-330. *Bostrychia harveyi* field samples analysed here are from cool temperate areas of Tasmania and New Zealand and contain only porphyra-334 and palythine at the lowest MAA levels ( $0.02\text{--}0.06\text{ mg g}^{-1}$  DW) seen in our results (Table 2). The field sample of *Bostrychia montagnei* contained shinorine, porphyra-334, palythine,

Table 2. Mycosporine-like amino acid (MAA) concentrations in red macroalgae growing as epiphytes in the intertidal zone of mangroves from all over the world. Most samples were collected in the field, others, as indicated by the culture number, were taken from continuous growth cultures, which had been previously isolated from mangroves. The three samples of *Bostrychia harveyi* were epilithically growing on anthropogenic substrata such as piles underneath a bridge or brick walls of a channel. Both samples of *Bostrychia scorpioides* originated from European salt marshes. Values are given as mean  $\pm$  standard deviation ( $n=4$ ) and expressed as mg per g dry weight; all MAAs are listed in terms of retention time; Myco-Gly: Mycosporine-Glycine; n.t.: no trace

<i>Bostrychia calliptera</i> I	1.06 $\pm$ 0.12	n.t.	n.t.	0.03 $\pm$ 0.01	0.09 $\pm$ 0.02	n.t.	0.02	n.t.	1.21 $\pm$ 0.15	Field
<i>Bostrychia calliptera</i> II	n.t.	n.t.	n.t.	0.05 $\pm$ 0.02	0.46 $\pm$ 0.09	1.65 $\pm$ 0.33	0.07 $\pm$ 0.02	n.t.	2.24 $\pm$ 0.38	Field
<i>Bostrychia harveyi</i> I	n.t.	n.t.	n.t.	n.t.	0.03	0.03 $\pm$ 0.01	n.t.	n.t.	0.06 $\pm$ 0.01	Field
<i>Bostrychia harveyi</i> II	n.t.	n.t.	n.t.	n.t.	0.02	0.01	n.t.	n.t.	0.03	Field
<i>Bostrychia harveyi</i> III	n.t.	n.t.	n.t.	n.t.	0.01	0.01	n.t.	n.t.	0.02	Field
<i>Bostrychia montagnei</i> I	n.t.	n.t.	n.t.	0.10 $\pm$ 0.02	0.17 $\pm$ 0.04	0.25 $\pm$ 0.04	0.19 $\pm$ 0.03	0.59 $\pm$ 0.05	1.30 $\pm$ 0.12	Field
<i>Bostrychia montagnei</i> II	n.t.	n.t.	n.t.	0.06 $\pm$ 0.01	0.32 $\pm$ 0.13	0.04 $\pm$ 0.01	0.12 $\pm$ 0.03	n.t.	0.54 $\pm$ 0.13	Culture
<i>Bostrychia moritziana</i> I	n.t.	n.t.	n.t.	0.13 $\pm$ 0.03	0.16 $\pm$ 0.03	n.t.	2.85 $\pm$ 0.29	0.71 $\pm$ 0.11	3.85 $\pm$ 0.38	Field
<i>Bostrychia moritziana</i> II	n.t.	n.t.	n.t.	0.17 $\pm$ 0.05	0.39 $\pm$ 0.16	0.04 $\pm$ 0.02	n.t.	2.06 $\pm$ 0.61	2.66 $\pm$ 0.77	Field
<i>Bostrychia pinnata</i>	n.t.	n.t.	n.t.	n.t.	n.t.	0.77 $\pm$ 0.09	n.t.	n.t.	0.77 $\pm$ 0.09	Field
<i>Bostrychia radicans</i> I	n.t.	0.55 $\pm$ 0.08	n.t.	n.t.	0.85 $\pm$ 0.10	0.08 $\pm$ 0.02	0.08 $\pm$ 0.03	4.16 $\pm$ 0.66	5.72 $\pm$ 0.8	Field
<i>Bostrychia radicans</i> II	n.t.	0.21 $\pm$ 0.03	n.t.	n.t.	0.36 $\pm$ 0.07	0.02	0.04 $\pm$ 0.02	2.29 $\pm$ 0.45	2.92 $\pm$ 0.55	Field
<i>Bostrychia radicans</i> III	n.t.	0.19 $\pm$ 0.04	n.t.	n.t.	0.70 $\pm$ 0.01	0.03 $\pm$ 0.01	0.07 $\pm$ 0.01	2.66 $\pm$ 0.51	3.65 $\pm$ 0.49	Field
<i>Bostrychia radicans</i> IV	n.t.	0.08 $\pm$ 0.02	n.t.	n.t.	0.10 $\pm$ 0.03	0.05 $\pm$ 0.01	0.08 $\pm$ 0.02	3.27 $\pm$ 0.75	3.58 $\pm$ 0.88	Field
<i>Bostrychia radicans</i> V	n.t.	0.07 $\pm$ 0.02	n.t.	n.t.	0.15 $\pm$ 0.03	0.02	0.07 $\pm$ 0.02	2.65 $\pm$ 0.68	2.96 $\pm$ 0.70	Field
<i>Bostrychia radicans</i> VI	n.t.	0.07 $\pm$ 0.03	n.t.	n.t.	0.59 $\pm$ 0.12	0.04 $\pm$ 0.01	0.11 $\pm$ 0.03	4.11 $\pm$ 0.66	4.92 $\pm$ 0.81	Field
<i>Bostrychia radicans</i> VII	n.t.	0.09 $\pm$ 0.02	n.t.	n.t.	0.66 $\pm$ 0.09	n.t.	0.12 $\pm$ 0.04	3.23 $\pm$ 0.83	4.10 $\pm$ 0.95	Field
<i>Bostrychia radicans</i> VIII	n.t.	0.28 $\pm$ 0.11	n.t.	n.t.	4.35 $\pm$ 1.03	n.t.	0.19 $\pm$ 0.03	7.95 $\pm$ 1.69	12.77 $\pm$ 2.01	Field
<i>Bostrychia radicans</i> IX	n.t.	n.t.	n.t.	n.t.	0.19 $\pm$ 0.02	n.t.	0.24 $\pm$ 0.06	2.63 $\pm$ 0.54	3.06 $\pm$ 0.60	Field
<i>Bostrychia radicans</i> X	n.t.	n.t.	n.t.	n.t.	0.32 $\pm$ 0.05	n.t.	0.26 $\pm$ 0.04	3.48 $\pm$ 0.41	4.06 $\pm$ 0.43	Culture
<i>Bostrychia radicans</i> XI	n.t.	n.t.	n.t.	n.t.	1.36 $\pm$ 0.47	0.09 $\pm$ 0.02	0.03 $\pm$ 0.01	2.66 $\pm$ 0.72	4.14 $\pm$ 0.59	Culture
<i>Bostrychia radicans</i> XII	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	1.57 $\pm$ 0.37	1.57 $\pm$ 0.37	Culture
<i>Bostrychia radicans</i> XIII	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.10 $\pm$ 0.02	0.88 $\pm$ 0.13	0.98 $\pm$ 0.18	Culture
<i>Bostrychia radicans</i> XIV	n.t.	n.t.	n.t.	n.t.	0.13 $\pm$ 0.04	n.t.	0.11 $\pm$ 0.03	1.60 $\pm$ 0.41	1.84 $\pm$ 0.49	Culture
<i>Bostrychia radicans</i> XV	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.04 $\pm$ 0.01	0.84 $\pm$ 0.09	0.88 $\pm$ 0.13	Culture
<i>Bostrychia radicans</i> XVI	n.t.	n.t.	n.t.	n.t.	0.34 $\pm$ 0.15	n.t.	0.10 $\pm$ 0.03	1.16 $\pm$ 0.46	1.60 $\pm$ 0.56	Culture
<i>Bostrychia radicans</i> XVII	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	0.04 $\pm$ 0.01	1.14 $\pm$ 0.27	1.18 $\pm$ 0.31	Culture
<i>Bostrychia radicans</i> XVIII	n.t.	n.t.	n.t.	n.t.	0.10 $\pm$ 0.03	n.t.	0.06 $\pm$ 0.01	0.90 $\pm$ 0.12	1.06 $\pm$ 0.17	Culture
<i>Bostrychia scorpioides</i> I	n.t.	0.20 $\pm$ 0.07	n.t.	1.03 $\pm$ 0.28	n.t.	0.24 $\pm$ 0.05	0.08(0.02)	n.t.	1.55 $\pm$ 0.41	Field
<i>Bostrychia scorpioides</i> II	0.23 $\pm$ 0.06	n.t.	n.t.	1.19 $\pm$ 0.34	n.t.	0.29 $\pm$ 0.06	0.21 $\pm$ 0.03	n.t.	1.92 $\pm$ 0.46	Field
<i>Bostrychia simpliciuscula</i>	n.t.	n.t.	n.t.	0.09 $\pm$ 0.02	0.14 $\pm$ 0.04	n.t.	2.12 $\pm$ 0.38	0.64 $\pm$ 0.17	2.99 $\pm$ 0.53	Field
<i>Bostrychia tenella</i> I	n.t.	n.t.	n.t.	n.t.	0.02	n.t.	n.t.	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	Field
<i>Bostrychia tenella</i> II	n.t.	n.t.	n.t.	0.03 $\pm$ 0.01	0.39 $\pm$ 0.08	0.03 $\pm$ 0.01	0.02	0.14 $\pm$ 0.04	0.61 $\pm$ 0.11	Field
<i>Bostrychia tenella</i> III	n.t.	n.t.	n.t.	n.t.	0.35 $\pm$ 0.10	1.18 $\pm$ 0.45	n.t.	0.09 $\pm$ 0.02	1.62 $\pm$ 0.58	Field
<i>Bostrychia tenella</i> IV	n.t.	n.t.	n.t.	0.06 $\pm$ 0.02	0.95 $\pm$ 0.27	0.27 $\pm$ 0.04	n.t.	0.18 $\pm$ 0.03	1.46 $\pm$ 0.39	Culture
<i>Bostrychia tenella</i> V	n.t.	n.t.	n.t.	0.15 $\pm$ 0.04	1.54 $\pm$ 0.43	0.11 $\pm$ 0.02	n.t.	0.55 $\pm$ 0.09	2.35 $\pm$ 0.62	Culture
<i>Bostrychia tenella</i> VI	n.t.	n.t.	n.t.	0.03 $\pm$ 0.01	1.23 $\pm$ 0.40	n.t.	0.10 $\pm$ 0.02	0.65 $\pm$ 0.10	2.01 $\pm$ 0.47	Culture
<i>Caloglossa apomeiotica</i> I	n.t.	n.t.	n.t.	0.50 $\pm$ 0.11	3.06 $\pm$ 0.66	n.t.	0.06 $\pm$ 0.01	n.t.	3.62 $\pm$ 0.80	Field
<i>Caloglossa apomeiotica</i> II	n.t.	n.t.	n.t.	0.28 $\pm$ 0.09	2.05 $\pm$ 0.49	n.t.	0.08 $\pm$ 0.02	n.t.	2.41 $\pm$ 0.53	Culture
<i>Caloglossa apomeiotica</i> III	n.t.	n.t.	n.t.	0.51 $\pm$ 0.13	3.29 $\pm$ 0.75	n.t.	0.28 $\pm$ 0.06	n.t.	4.08 $\pm$ 0.84	Field
<i>Caloglossa leprieurii</i> I	n.t.	n.t.	n.t.	0.36 $\pm$ 0.07	4.62 $\pm$ 0.81	n.t.	0.16 $\pm$ 0.03	n.t.	5.14 $\pm$ 0.94	Field
<i>Caloglossa leprieurii</i> II	n.t.	n.t.	n.t.	0.69 $\pm$ 0.06	5.45 $\pm$ 0.62	n.t.	0.39 $\pm$ 0.09	n.t.	6.53 $\pm$ 0.77	Field
<i>Caloglossa leprieurii</i> III	n.t.	n.t.	n.t.	0.06 $\pm$ 0.02	3.24 $\pm$ 0.38	n.t.	0.10 $\pm$ 0.02	n.t.	3.40 $\pm$ 0.48	Field
<i>Caloglossa leprieurii</i> IV	n.t.	n.t.	n.t.	0.09 $\pm$ 0.02	3.85 $\pm$ 0.43	n.t.	0.09 $\pm$ 0.02	n.t.	4.03 $\pm$ 0.61	Field
<i>Caloglossa leprieurii</i> V	n.t.	n.t.	n.t.	0.13 $\pm$ 0.03	4.11 $\pm$ 0.67	n.t.	0.07 $\pm$ 0.01	n.t.	4.31 $\pm$ 0.75	Field
<i>Caloglossa leprieurii</i> VI	n.t.	n.t.	n.t.	0.28 $\pm$ 0.07	4.92 $\pm$ 0.79	n.t.	0.04 $\pm$ 0.01	n.t.	5.26 $\pm$ 0.83	Field
<i>Caloglossa leprieurii</i> VII	n.t.	n.t.	n.t.	0.03 $\pm$ 0.01	2.03 $\pm$ 0.46	n.t.	0.05 $\pm$ 0.02	n.t.	2.11 $\pm$ 0.52	Field
<i>Caloglossa ogasawaraensis</i>	n.t.	n.t.	n.t.	0.17 $\pm$ 0.04	2.56 $\pm$ 0.40	n.t.	0.06 $\pm$ 0.02	n.t.	2.78 $\pm$ 0.44	Field
<i>Caloglossa stipitata</i>	n.t.	n.t.	n.t.	0.86 $\pm$ 0.16	2.51 $\pm$ 0.28	n.t.	0.06 $\pm$ 0.02	n.t.	3.43 $\pm$ 0.39	Culture
<i>Catenella caespitosa</i> I	1.60 $\pm$ 0.49	n.t.	0.11 $\pm$ 0.01	n.t.	n.t.	n.t.	n.t.	n.t.	1.71 $\pm$ 0.52	Field
<i>Catenella caespitosa</i> II	2.41 $\pm$ 0.33	n.t.	0.16 $\pm$ 0.03	n.t.	n.t.	n.t.	n.t.	n.t.	2.57 $\pm$ 0.64	Field
<i>Catenella impudica</i>	4.21 $\pm$ 0.64	n.t.	0.96 $\pm$ 0.22	n.t.	n.t.	n.t.	n.t.	n.t.	5.17 $\pm$ 0.77	Field
<i>Catenella nipae</i> I	4.27 $\pm$ 0.76	n.t.	0.36 $\pm$ 0.12	n.t.	0.04 $\pm$ 0.01	n.t.	n.t.	n.t.	4.67 $\pm$ 0.80	Field
<i>Catenella nipae</i> II	1.80 $\pm$ 0.37	n.t.	0.10 $\pm$ 0.03	n.t.	n.t.	n.t.	n.t.	n.t.	1.90 $\pm$ 0.46	Field

Continued on p. 165

Table 2. contd.

<i>Catenella nipae</i> III	1.43±0.35	n.t.	0.12±0.02	n.t.	n.t.	n.t.	n.t.	n.t.	1.55±0.43	Field
<i>Catenella nipae</i> IV	1.44±0.28	n.t.	0.12±0.03	n.t.	n.t.	n.t.	n.t.	n.t.	1.56±0.25	Field
<i>Catenella nipae</i> V	1.97±0.29	n.t.	0.17±0.03	n.t.	n.t.	n.t.	n.t.	n.t.	2.14±0.38	Field
<i>Catenella nipae</i> VI	1.35±0.47	n.t.	0.11±0.02	n.t.	n.t.	n.t.	n.t.	n.t.	1.46±0.61	Field
<i>Catenella nipae</i> VII	2.82±0.56	n.t.	0.14±0.03	n.t.	n.t.	n.t.	n.t.	n.t.	2.96±0.57	Field
<i>Catenella nipae</i> VIII	3.18±0.59	n.t.	0.18±0.04	n.t.	n.t.	n.t.	n.t.	n.t.	3.36±0.88	Field
<i>Catenella nipae</i> IX	3.22±0.41	n.t.	0.24±0.04	n.t.	n.t.	n.t.	n.t.	n.t.	3.46±0.65	Field
<i>Stictosiphonia arbuscula</i>	n.t.	n.t.	n.t.	0.10±0.02	3.26±1.04	1.31±0.58	1.46±0.44	n.t.	6.13±1.51	Field
<i>Stictosiphonia intricata</i> I	n.t.	n.t.	n.t.	0.17±0.03	2.46±0.44	0.15±0.04	0.10±0.02	0.34±0.10	3.22±0.60	Field
<i>Stictosiphonia intricata</i> II	n.t.	n.t.	n.t.	0.14±0.03	1.53±0.56	0.27±0.06	0.13±0.03	n.t.	2.07±0.68	Field
<i>Stictosiphonia intricata</i> III	n.t.	n.t.	n.t.	0.02	0.90±0.19	n.t.	0.12±0.02	0.24±0.07	1.28±0.27	Field
<i>Stictosiphonia tangatensis</i>	n.t.	n.t.	n.t.	0.09±0.02	1.05±0.25	0.27±0.04	0.12±0.02	0.05±0.02	1.58±0.34	Culture

asterina-330 and palythanol totaling 1.30 mg g<sup>-1</sup> DW, whereas the culture specimen contained all MAAs except palythanol and totaled only 0.54 mg g<sup>-1</sup> DW.

One field specimen of *Bostrychia moritziana* is from a freshwater river in Venezuela and contained shinorine, porphyra-334, palythine and palythanol. The other field specimen from an estuarine mangrove in Brazil contained shinorine, porphyra-334, asterina-330 and palythanol.

*Bostrychia radicans* field samples (I–IX) generally contained significantly higher levels of all MAAs than laboratory cultured samples (X–XVIII) (mean value: 4.85 mg g<sup>-1</sup> DW vs. 1.92 mg g<sup>-1</sup> DW; *t*-test, *P*<0.02). The highest recorded content of a specific MAA was 7.95 mg g<sup>-1</sup> DW palythanol in *B. radicans* field specimens from Guatemala. Shinorine was absent in all *B. radicans* (Table 2). The eight field specimens contained mycosporine-glycine, whereas the 10 culture specimens did not contain any trace of this MAA. In addition, most isolates of *B. radicans* usually had porphyra-334, palythine, asterina-330 and palythanol, but in the sample from New Jersey, U.S.A., only palythanol could be detected (Table 2).

The two field samples of *Bostrychia scorpioides* from temperate salt marshes of Europe were quite different in composition. The Netherlands sample contained MAA-1 (the *Catenella* compound), shinorine, palythine, and asterina-330 whereas the French sample lacked MAA-1 but contained mycosporine-glycine, shinorine, palythine and asterina-330.

*Bostrychia tenella* is widespread in tropical coral reef and mangrove habitats with minimal shade. These specimens are often yellow in colour because of higher irradiance. Using a *t*-test (*p*>0.24) indicated that the total MAA levels in field specimens (0.76 mg g<sup>-1</sup> DW) were statistically not significant differ-

ent from cultured specimens (1.94 mg g<sup>-1</sup> DW). In *B. tenella*, mycosporine-glycine was not evident although porphyra-334, palythine and palythanol were common to all.

The one field sample of *Stictosiphonia arbuscula* from the cool temperate rocky coast of New Zealand contained shinorine, porphyra-334, asterina-330 and palythanol. Porphyra-334 comprised 53% of the total 6.13 mg g<sup>-1</sup> DW which seems quite high for these latitudes (46° S).

*Stictosiphonia intricata* is also present in open rocky habitats of cool temperate coastlines of the southern hemisphere (30–54° S) where it is likely to receive high UV exposure and most samples contained shinorine, porphyra-334, palythine, asterina-330 and palythanol. The cultured specimens of *Stictosiphonia tangatensis* (from tropical coastline in Natal, South Africa) exhibited the same five UV-absorbing compounds as *S. intricata* although total MAAs were slightly lower in *S. tangatensis* than in *S. intricata* (1.58 mg g<sup>-1</sup> DW vs 2.19 mg g<sup>-1</sup> DW).

The one cultured specimen and two field samples of *Caloglossa apomeiotica* contained shinorine, porphyra-334 and asterina-330. Porphyra-334 constituted 60–85% of the total (2.41–4.08 mg g<sup>-1</sup> DW). A similar composition was evident in 6 field samples of *Caloglossa leprieurii* with porphyra-334 comprising 83–96% of the total MAAs (2.11–6.53 mg g<sup>-1</sup> DW).

One field sample of *Caloglossa ogasawaraensis* and one culture specimen of *Caloglossa stipitata* have an overall composition similar to that seen in the other *Caloglossa* species.

The distribution patterns of the various MAAs within the species reported here may indicate some chemotaxonomic relationships (Table 3). *Caloglossa* species always contain high concentrations

Table 3. Relative distribution of mycosporine-like amino acids (MAAs) in red macroalgae growing as epiphytes in the intertidal zone of mangroves from all over the world. *Bostrychia harveyi* was collected from anthropogenic substrata such as piles underneath a bridge or brick walls of a channel. *Bostrychia scorpioides* originated from salt marshes.

Species	MAA-1	Myco-Gly	MAA-2	Shinorine	Porphyra-334	Palythine	Asterina-330	Palythiol
<i>Bostrychia calliptera</i>	***	n.t.	n.t.	*	*	***	*	*
<i>Bostrychia harveyi</i>	n.t.	n.t.	n.t.	n.t.	**	**	n.t.	n.t.
<i>Bostrychia montagnei</i>	n.t.	n.t.	n.t.	*	*	*	*	**
<i>Bostrychia moritziana</i>	n.t.	n.t.	n.t.	*	*	*	**	***
<i>Bostrychia pinnata</i>	n.t.	n.t.	n.t.	n.t.	n.t.	***	n.t.	n.t.
<i>Bostrychia radicans</i>	n.t.	*	n.t.	n.t.	**	*	*	***
<i>Bostrychia scorpioides</i>	*	*	n.t.	***	n.t.	*	*	n.t.
<i>Bostrychia simpliciuscula</i>	n.t.	n.t.	n.t.	*	*	n.t.	***	**
<i>Bostrychia tenella</i>	n.t.	n.t.	n.t.	*	**	**	*	**
<i>Caloglossa apomeiotica</i>	n.t.	n.t.	n.t.	*	***	n.t.	*	n.t.
<i>Caloglossa leprieurii</i>	n.t.	n.t.	n.t.	*	***	n.t.	*	n.t.
<i>Caloglossa ogasawaraensis</i>	n.t.	n.t.	n.t.	*	***	n.t.	*	n.t.
<i>Caloglossa stipitata</i>	n.t.	n.t.	n.t.	**	***	n.t.	*	n.t.
<i>Catenella caespitosa</i>	***	n.t.	*	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Catenella impudica</i>	***	n.t.	*	n.t.	n.t.	n.t.	n.t.	n.t.
<i>Catenella nipae</i>	***	n.t.	*	n.t.	*	n.t.	n.t.	n.t.
<i>Stictosiphonia arbuscula</i>	n.t.	n.t.	n.t.	*	***	**	**	n.t.
<i>Stictosiphonia intricata</i>	n.t.	n.t.	n.t.	*	***	*	*	*

\*\*\*: high concentration; \*\*: medium concentration; \*: low concentration; n.t.: no trace

of porphyra-334 together with some minor levels of shinorine and asterina-330. These MAAs are also present in similar amounts in *Stictosiphonia* species, but palythine and, in most cases, palythiol occur as well. *Catenella* can be characterized by the presence of both unknown MAA-1 and MAA-2 (Table 3). In contrast to these genera, the MAA distribution patterns within *Bostrychia* are not homogeneous. Shinorine, palythine, asterina-330 or palythiol can all be the quantitatively dominant MAAs in different *Bostrychia* species.

*Bostrychia radicans* 3144 cultured for several months at  $20 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  contained high concentrations of palythiol together with some asterina-330 (Figure 2). Doubling this irradiance to  $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  resulted in a 85% increase ( $2.0\text{--}3.7 \text{ mg g}^{-1} \text{ DW}$ ) in palythiol, the quantitatively dominant MAA (Figure 2). In addition, a 73% increase ( $0.15\text{--}0.26 \text{ mg g}^{-1} \text{ DW}$ ) occurred in asterina-330 together with the appearance of mycosporine-glycine, shinorine and porphyra-334 in significant amounts ( $0.11\text{--}0.30 \text{ mg g}^{-1} \text{ DW}$ ).

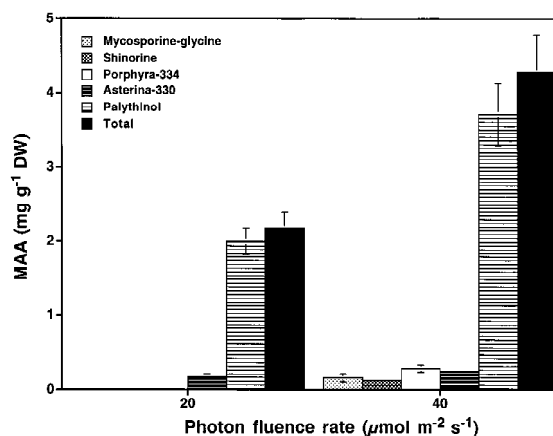


Figure 2. MAA biosynthesis in *Bostrychia radicans* 3144 after 4 weeks in 20 and 40  $\text{mmol m}^{-2} \text{s}^{-1}$  PAR. Mean values  $\pm$  standard deviation ( $n=4$ ) are given.

In a similar experiment *Caloglossa leprieurii* 2937 normally cultured at  $40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  was exposed to  $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  for 4 weeks, resulting in a 77% increase in porphyra-334 ( $3.09\text{--}5.48 \text{ mg g}^{-1} \text{ DW}$ ), while total MAA concentration in-

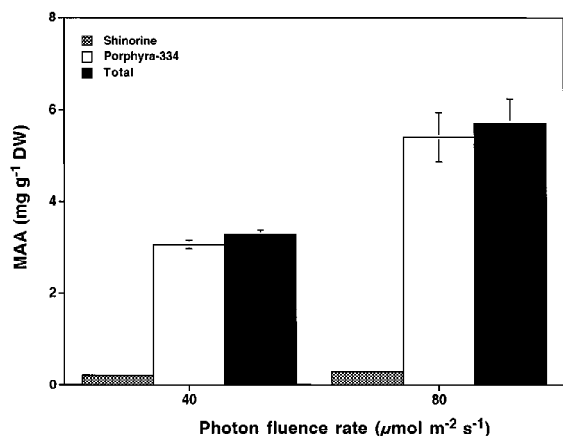


Figure 3. MAA biosynthesis in *Caloglossa leprieurii* 2937 after 4 weeks in 40 and 80  $\text{mmol m}^{-2} \text{s}^{-1}$  PAR. Mean values  $\pm$  standard deviation ( $n=4$ ) are given.

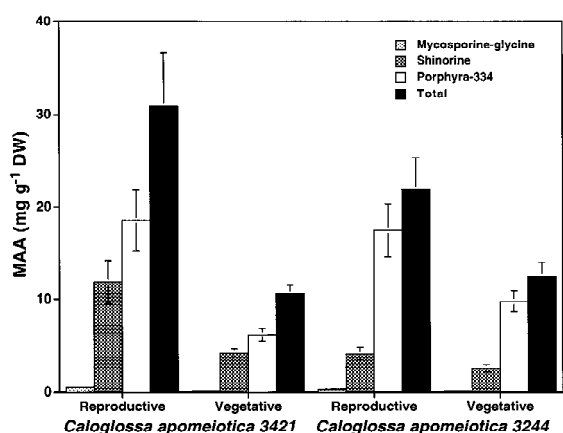


Figure 4. Qualitative and quantitative MAA distribution in reproductive and vegetative plants of culture-grown *Caloglossa apomeiatica* 3244 and *Caloglossa apomeiatica* 3421. Tetrasporangial sori were excised from mature plants. Mean values  $\pm$  standard deviation ( $n=4$ ) are given.

creased by 75% (Figure 3). In this species, only shinorine and porphyra-334 occurred.

Reproductive tissue of *Caloglossa apomeiatica* 3421 exhibited much higher MAA contents compared to vegetative tissue (Figure 4). In tetrasporangial stichidia, up to  $30.9 \text{ mg g}^{-1} \text{ DW}$  total MAAs could be measured which were quantitatively dominated by shinorine and porphyra-334. Vegetative samples of this isolate contained only  $10.6 \text{ mg g}^{-1} \text{ DW}$  total MAAs (Figure 4). The same difference in MAA content between reproductive and vegetative tissues could be seen in *C. apomeiatica* 3244. While tetrasporangial tissue showed  $21.9 \text{ mg g}^{-1} \text{ DW}$  total MAAs, veget-

ative plants exhibited  $9.8 \text{ mg g}^{-1} \text{ DW}$  total MAAs (Figure 4).

## Discussion

The present investigation provides a comprehensive inventory of the qualitative and quantitative occurrence of UV-absorbing MAAs in tropical/subtropical red macroalgae of the so-called *Bostrychia-Caloglossa* association, a group of unique taxa typically growing as epiphytes on mangroves (Post, 1968). Due to the tidal flows, the algae are regularly exposed to various abiotic factors in the mangrove habitat, in many cases even for long periods up to several weeks and hence they are often living under 'atmophytic' or nearly 'terrestrial' conditions (Post, 1963, 1968). As a consequence, the mangrove algae experience strong amplitudes of all prevailing environmental parameters including solar radiation. Although many species of the genera *Bostrychia* and *Caloglossa* have been reported to be shade-adapted in laboratory studies (Karsten & West, 1993; Karsten et al., 1993), they do not always grow in the field under a protective canopy. Extensive floristic studies in Pacific Mexico and Guatemala clearly proved the occurrence of many mangrove red algae under full solar radiation (West et al., 1992; Pedroche et al., 1995). Thus far, any protective mechanism against the harmful effects of naturally high doses of UV-radiation in members of this unique flora have been unexplored.

The results here clearly demonstrate high MAA concentrations for most mangrove algal species, which is in good agreement with earlier reports on the occurrence of UV-absorbing compounds particularly in members of the Rhodophyceae (Sivalingam et al., 1974; Sivalingam & Nisizawa, 1990; Karentz et al., 1991; Maegawa et al., 1993; Molina & Montecino, 1996; Karsten et al., 1998a,c). Karsten et al. (1998a) reported that MAA contents in Rhodophyceae from Arctic to cold-temperate localities were normally only half (ranging from 0 to  $3.5 \text{ mg total MAAs g}^{-1} \text{ DW}$ ) of those in species from warm-temperate regions (ranging from 0.2 to  $7.8 \text{ mg total MAAs g}^{-1} \text{ DW}$ ). In the present study, the MAA concentrations measured in the tropical mangrove algae are approximately 30–50% higher (ranging from 0.1 to  $12.8 \text{ mg total MAAs g}^{-1} \text{ DW}$ , in reproductive tissue up to  $30 \text{ mg g}^{-1} \text{ DW}$ ) compared to warm-temperate species and this is in good agreement with our preliminary investigation on 14 macroalgal samples from mangroves and

47 species collected on the tropical island Hainan, People's Republic of China (Karsten et al., 1998c). Taking all these data together, it seems that the MAA concentration is an important factor controlling the biogeographic distribution of these algae, since species from lower, high-solar latitudes always exhibit more MAAs than species from higher, low-solar latitudes. These observations indicate that the higher the natural solar UV-radiation of the respective habitat the more MAAs are formed and accumulated in these algae. In addition, most investigated field samples generally contained higher levels of all MAAs than the respective laboratory cultured samples, and this may be attributed to the lower PAR (20–40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and/or absence of UV in which the cultures were usually grown compared with the high natural solar radiation (PAR <2000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) including UV measured in many field conditions (Pedroche et al., 1995).

The MAA content in Rhodophyceae is also influenced by vertical distribution, since species from deeper waters lack these compounds (Maegawa et al., 1993; Karsten et al., 1998a). In the endemic Arctic red alga *Devaleraea ramentacea* (L.) Guiry a strong linear decrease in MAA concentration occurs with increasing depth, at least in shallow waters (2–5 m) (Karsten et al., 1999). The authors concluded that the lower MAA contents in isolates from deeper waters correlate with lower natural solar UV-irradiance in the respective subtidal growth position. These results confirm that increasing UV-radiation typically stimulates MAA concentrations, and indicate that both geographical and vertical distribution of macroalgal species influence the UV-radiation levels that determine the MAA content (Helbing et al., 1996; Hannach & Sigleo, 1998; Karsten et al., 1999; Karsten & Wiencke, 1999).

With the exception of *Catenella*, all other mangrove algal samples investigated here contained MAAs which typically occur in many other Rhodophyceae (Karentz et al., 1991; Karsten et al., 1998a,c; Sinha et al., 1998). However, the UV-absorbing compounds designated as MAA-1 and MAA-2 in our present study have never been reported before. The absorption characteristics of MAA-1 and MAA-2 are similar to shinorine (maximum at 332 nm) and palythine (maximum at 320 nm), respectively, but the retention times are completely different (Figure 1). Therefore, we speculate that both substances represent either chemical isomers of known MAAs such as shinorine and palythine or undescribed compounds. It

is interesting to note that only the *Catenella* samples contain both MAA-1 and MAA-2 with no other detectable MAA, except a trace of porphyra-334 in one specimen of *C. nipae*. The combination of these unknown compounds may be a chemotaxonomical marker for *Catenella* species. In the cyanobacterial genus, *Microcoleus* patterns of MAAs and carotenoids were used to resolve low-rank taxonomy (Karsten & Garcia-Pichel, 1996).

Exposing *Bostrychia radicans* for 4 weeks under twice the photon fluence rate of artificial radiation in the PAR range compared with the control led to approximately 2-fold accumulation in total MAA concentration (Figure 2). Although MAAs in many marine organisms are generally positively correlated with natural doses of UV-radiation (Shick et al., 1995), naturally enhanced doses of PAR can also induce the formation and accumulation of MAAs in the red alga *Chondrus crispus* from Helgoland after transplantation from subtidal (4–6 m) to shallow waters (Karsten et al., 1998b). While in this species the quantitatively most important MAA, shinorine, was much more stimulated by UV-radiation, the amounts of palythine, palythanol and palythene were more affected by high PAR. In a transplantation experiment with *Devaleraea ramentacea*, only the UV-B-waveband was effective in stimulating biosynthesis and accumulation of various MAAs (Karsten et al., 1999). In contrast, in similar experiments on *Palmaria palmata* (Linnaeus) O. Kuntze, treatment with PAR, PAR plus UV-A and total solar spectrum led under all conditions to a waveband-specific stimulation in the formation of different MAAs (Karsten & Wiencke, 1999). While PAR mainly stimulated the synthesis of porphyra-334, UV-A and UV-B predominantly led to the accumulation of shinorine and palythine, respectively. In *Caloglossa leprieurii* at 40  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , only two main MAAs, namely shinorine and porphyra-334, could be detected. Doubling the PAR intensity to 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was accompanied with a stimulation of the porphyra-334 biosynthesis only (Figure 3). All data available on the photophysiology of MAAs in Rhodophyceae are contradictory in terms of effectiveness of various spectral wavebands and in terms of individual MAA-responses. However, these discrepancies can be explained by species-specific responses in MAA-induction and accumulation under different spectral wavebands.

Comparison between reproductive and vegetative tissues of two *Caloglossa apomeiotica* isolates clearly demonstrate total MAA concentrations of up

to 30 mg g<sup>-1</sup> DW for tetrasporangial tissue (Figure 4). To our knowledge, such a high MAA value has never been reported before in the literature. However, since the reproductive tissue prepared contained some vegetative cells as well, the specific accumulation of MAAs in the sori must be even higher. The sum of all MAAs represents at least 3% of the dry biomass, quite a serious metabolic investment, which speaks for the ecological importance of UV-radiation and the use of sunscreens in the ecophysiology of these red algae, particularly to protect reproduction. In a recent study on the impact of UV-radiation on brown algal zoospores, a drastic loss of viability due to DNA- and photo-damage of the photosynthetic apparatus could be demonstrated (Wiencke et al., 2000). Consequently, the upper depth distribution of these plants seems to be mainly determined by the UV tolerance of the zoospores which, therefore, were regarded as critical life stage in terms of dispersal and survival capacity. On the other hand, loading-up of red algal tetraspores with MAAs can be an effective photoprotective strategy to counteract harmful effects of UV-radiation and hence guarantees reproduction and long-term survival of the population.

Thus far, relevant action spectra for the induction and biosynthesis of MAAs to assess physiological radiation effects in any organism are missing. However, preliminary experiments with red algae indicate the effectiveness of the short UV-A waveband (Kräbs et al., unpublished results). Consequently, it is reasonable to assume the presence of cryptochrome and/or a UV-photoreceptor in MAA-producing macroalgae to explain these photophysiological effects. Many cellular processes such as photosynthesis are stimulated by blue-light (e.g. Forster & Dring, 1993), and hence solar radiation not only provides energy for photosynthesis, but simultaneously controls metabolism, for example via light/dark modulation of enzymes, and development of plants (Scheibe, 1990).

MAAs are one of nature's sunscreens, with 19 structurally distinct compounds so far identified in marine organisms (Dunlap & Shick, 1998). Although MAA levels in macroalgae show a decrease in concentration with increasing growth depth and are in general positively correlated with natural doses of UV-radiation (Karsten et al., 1998b), experimental evidence for the role of MAAs as UV-protectants in these plants is still circumstantial. Nevertheless, the presence of increasing MAA contents in *D. ramentacea* with decreasing depth strongly correlated with a more insensitive photosynthetic capacity under UV expos-

ure (Karsten et al. 1999). In *C. crispus*, photoinhibition of photosynthesis under UV-B treatment became less pronounced with increasing MAA concentrations (Van de Poll et al., University of Groningen, unpublished results). Photosynthetic experiments on the unicellular microalga *Gymnodinium sanguineum* proved that MAAs act as spectrally specific UV-sunscreens (Neale et al., 1998). In another study, Riegger & Robinson (1997) reported that for various Antarctic phytoplankton species high MAA concentrations correlated with an increased, albeit not complete, resistance to UV photodamage. These authors estimated that due to the presence of MAAs up to 72% of harmful UV quanta were absorbed before hitting cytoplasmic molecular targets in *Phaeocystis* sp. colonies. Moreover, Dionisio-Sese et al. (1997) showed that the presence of MAAs in the surface tunic of the colonial ascidian *Lissoclinum patella* Gottschaldt protect its photosynthetic symbiont, *Prochloron* sp., from UV-induced photodamage. Recent publications on marine microalgae and invertebrates strongly support the photobiological function of MAAs as a cellular defense system against the harmful effects of UV-radiation (Dunlap & Shick, 1998). Therefore, we conclude that the physiological capability of mangrove red algae to synthesise and accumulate high MAA concentrations, particularly in the reproductive structures under elevated solar radiation plays a vital role as biochemical adaptation ensuring survival under the environmental extremes in the tropical habitat.

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