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HIMANTORMIA LUGUBRIS, AN ANTARCTIC ENDEMIC ON THE EDGE OF THE
LICHEN SYMBIOSIS

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20

21 Abstract:

22 *Himantormia lugubris* is an Antarctic endemic with a distribution restricted to the
23 northwest tip of Antarctic Peninsula, adjacent islands and South Georgia Island. In this
24 region *H. lugubris* is an important component of the epilithic lichen community. The
25 species has a fruticose thallus with usually simple and flattened branches whose grey
26 surface is often disrupted exposing the black and dominant chondroid axis. Because the
27 photobiont cells are mainly restricted to the patchy grey areas, positive carbon balance
28 seems to be rather difficult for this species. Therefore, the aim of this paper is to
29 elucidate which functional strategy, possibly linked with thallus anatomy, is used by *H.*
30 *lugubris* that enables it to be a successful species in the maritime Antarctic. To achieve
31 this goal, we constructed a picture of the lichen's physiological, anatomical and
32 morphological characteristics by using a broad range of modern technologies, such as
33 chlorophyll fluorescence, CO₂ exchange and electron microscopy. We found that *H.*
34 *lugubris* has a very low net photosynthesis, apparently restricted to the grey areas, but
35 high respiratory rates. Therefore, positive net photosynthesis is only possible at low
36 temperatures. Chlorophyll content is also low but is present in both gray and black areas
37 although it seems not to be functional for photosynthesis in the latter. Our conclusion is
38 that the only possibility for this species to achieve a positive carbon balance is to be
39 active for long periods under optimal conditions, that means, wet, cold and with enough
40 light, a common combination in this region of Antarctica. Given these constraints, we
41 suggest that *H. lugubris* is likely to be especially sensitive species to predicted climate
42 warming in the maritime Antarctic.

43

Introduction:

Antarctic climate is usually described as extremely cold and dry, which is true for most of the Antarctic continent but not for the north-west tip of the Antarctic Peninsula and adjacent islands, an area known as maritime Antarctic (Convey 2013). Here, strong and humid westerly winds from the Pacific Ocean are the main feature of the atmospheric circulation. Despite the climate being much more habitable than in continental Antarctica, the prevailing cold temperatures in summer, typically below 5°C, prevent the growth of the vast majority of vascular plants. As a result, a rich and abundant cryptogamic tundra flourishes (Green et al. 2007) with around 400 lichen species and 100 bryophytes having been recorded in this relatively small biogeographic region (Olech 2004; Peat et al. 2007; Ochyra et al. 2008).

Wet and cloudy weather, accompanied by moderately cold temperatures, a favourable combination for poikilohydric organisms that greatly benefit from long periods of activity, especially during the growing season, coinciding with the austral summer (Green et al. 2007). Lichen species in this region are growing at the highest rate in the whole of Antarctica (Sancho et al. 2007; 2017) reflecting the calculated high annual net photosynthetic productivity (NPP) (Schroeter et al. 2017).

Fruticose macrolichens are dominant in many epilithic communities, giving the landscape a distinctive appearance, especially on the South Shetland Islands (Rodriguez et al. 2018). In this extensive tundra community, the blackish-brown endemic species *Himantormia lugubris* (Hue) I.M. Lamb often plays a co-dominant role together with different *Usnea* spp (Kappen and Redon 1987). In comparison to several, spatially close, co-occurring *Usnea* species, the species is exceptional because of its extremely low photosynthetic rates (A_{max}) which appear to hardly allow for any net carbon uptake even under optimal conditions (Kappen et al. 1991) as well as having very low photobiont populations (Sojo et al. 2003) and thallus chlorophyll contents (Kappen et al. 1991). As a result, *H. lugubris* could be considered a highly vulnerable species, being at risk of a negative carbon budget with only minor changes in environmental conditions.

Given this counterintuitive background, the aim of this paper is to elucidate which functional strategy *H. lugubris* utilises to be a successful species in the maritime Antarctic and, also, if this lifestyle is associated with increased risk for survival in future climate change scenarios. Therefore, we constructed a picture of the lichen's physiological, anatomical and morphological characteristics by using a range of technologies, such as chlorophyll fluorescence, CO₂ exchange and electron microscopy.

Material and methods

Species description

Himantormia lugubris (Hue) I.M. Lamb is one of the most typical Antarctic endemic lichens (Lamb 1964). Its distribution ranges from 65°S in the western side of Antarctic Peninsula to the South Shetland, South Orkney and South Georgia Islands, 54°S (Figure 1). *H. lugubris* has a fruticose thallus with usually simple and flattened branches, forming cushions strongly attached on acidic rocks (Figure 2). Although, in young specimens, the surface is regularly smooth and grey-cream coloured, in older specimens the surface is often disrupted exposing the black and dominant chondroid axis. Flat, brown apothecia are common on the top of the branches (Figure 2).

H. lugubris is usually associated with the fruticose lichens *Usnea aurantiaco-atra* and *U. antarctica* and the mosses *Andreaea gainiii* and *A. regularis*, forming one of the most typical and abundant epilithic community of the maritime Antarctic (Lindsay 1971).

Study site

The research was carried out during February and March 2018 in the vicinity of the Spanish Juan Carlos I Station at South Bay, Livingston Island, South Shetland Islands (62°39'46"S, 60°23'20"W; see map in Laguna-Defior et al. (2016). South Bay is known to be a rich area for vegetation and 187 lichen and 59 bryophyte species have been reported from the area (Sancho et al. 1999; Søchting et al. 2004). On the raised terraces over the beach, regular hydration allows the development of a lush cryptogamic

tundra with a broad variety of mosses and mainly fruticose lichens, many of them being cosmopolitan (e.g, *Sphaerophorus globosus*, *Cladonia* spp.) or bipolar (e.g. *Stereocaulon alpinum*; *S. glabrum*) species. Drier and rockier sites are dominated by fruticose lichens and dark brown moss cushions from the genus *Andreaea*. *H. lugubris* occurs within this community and becomes more frequent with altitude along the altitudinal gradient from the coast to the summit of Reina Sofia Mountain (275m a.s.l.).

Climate

This corner on the northwest side of South Bay has a comparatively mild weather within the South Shetland Islands. The annual average temperature recorded here, -0.9 °C (Bañón and Vasallo 2016) is above those recorded in other South Shetland stations (Olech 2004). The climate is strongly affected by the maritime conditions and the area is not particularly cold with the coldest month, July, averaging -6.1 °C and the warmest, February, 2.2 °C. Absolute minimum temperatures rarely fall below -15 °C and are often close to the freezing point even in winter. Temperature range is also very low, e.g. mean temperature for January 2001 was $2.56 \pm 0.03^{\circ}\text{C}$ and this is a consequence of the very high cloud cover which is a characteristic feature of this region (Bargagli 2005).

For comparison, information is available from a nearby station, Arctowski, (King George Island, 110 km distant) which has a very similar climate. Mean annual temperature is -1.6°C. The sky is totally overcast on 213 days of the year and sunny periods occur for only 0.4% days per month. In the summer months, November to February, inclusive, sunny periods are even rarer with a monthly average is 0.125 days, or 9 hours per month. Annual precipitation, mostly as rain over the austral summer, is around 800 mm per year and is very evenly spread across the year with a mean value of 69.3 ± 2.1 mm and 19.7 ± 0.3 days per month (all data are from Meteoblue, www.meteoblue.com). The climate can be summarised as consistently cool, moist and dull.

Chlorophyll fluorescence

The activity status of the photobionts within a complete thallus was monitored by Chl_a fluorescence using images obtained with a pulse amplitude modulated fluorometer (IMAGING-PAM; H. Walz, Effeltrich, Germany). The images provide F_o (basal

fluorescence), F_m' maximal fluorescence measured during a saturation light flash, and photosynthetic yield of PSII, $\phi PSII = (F_m' - F')/F_m'$. Measurements were made at an actinic light level that replicated natural light levels in the area (Schroeter et al. 2017). A total of three independent, fully hydrated thalli were screened, immediately after their collection. In order to take into consideration thallus internal variability, five homogeneous areas of interest were selected on the regularly smooth and grey-cream coloured parts of the lichen, and five on the black and dominant chondroid axis of each thallus.

In order to check for differences between the individuals a one-factor ANOVA test was conducted, and residuals tested for normal distribution with a Q/Q plot. To test for differences between the grey and black thallus areas a student t-test was conducted, following a Shapiro Wilk test for normal data distribution. Both tests were conducted using R studio (Version 1.1.463 – © 2009-2018 RStudio, Inc.).

Gas-exchange:

CO₂ gas exchange measurements were conducted under controlled light and temperature conditions using a minicuvette system (CMS400, Walz, Germany) installed in the lab facilities of the Juan Carlos I Station. CO₂ exchange was measured as the difference between the air passed through the cuvette with the sample and the ambient air (Schroeter et al. 1994; Pannowitz et al. 2006). Complete thalli were used for experiments, including holdfast. Experiments were designed to mimic natural conditions as much as possible in order to prevent any artificial treatment effects. Therefore, the samples were stored outdoors, in a position similar to their natural arrangement and only natural hydration was used for reactivation (hydrating). This was achieved by coupling the experiments with periods of rain, strong enough to soak the thalli. Before each experiment, samples were gently shaken to remove part of the adherent water and weighed to determine maximum thallus water content. Thallus water content as well as chlorophyll content and CO₂ exchange were calculated on a dry weight basis (DW). DW was determined after all measurements for a particular experiment had been completed by placing a known proportion of the samples in an oven at 65 °C for 24h. The other part of the samples were reserved for anatomical and

ultrastructural survey and were preserved at -20 °C until processing in Museo Nacional de Ciencias Naturales in Madrid.

The response of CO₂ exchange to photosynthetic photon flux density (PPFD) was determined from measurements made at 0, 50, 100, 200, 400, 800 and 1200 μmol photon m⁻² s⁻¹ and repeated at 10, 5 and 0 °C and at 10, 15, and 20 °C. All measurements were made at the optimal water content which had been determined previously by monitoring the CO₂ exchange as the sample dried from maximum water content to a water content below the maximal NP at 10 °C and 400 μmol photon m⁻² s⁻¹. The light source was a KL 2500 LCD (Schott) cold light which avoided heating the samples inside the cuvette (Schroeter et al. 2020, submitted). The PPFD response curves were obtained by statistical fitting of a Smith function, as detailed in Green et al. (1997), which provided the light compensation point (LCP; the minimal light intensity at which positive net photosynthesis is reached) as the point where intersection between the calculates curve and the X axis occurs. A_{max} (the maximum net photosynthesis rate) as the average of the maximum net photosynthesis rate obtained for each of the studied samples. PPFD_{sat}, the light intensity at which 90 % of maximal net photosynthesis (NP) is reached was then calculated from the response curve. Dark respiration (DR) was determined for each temperature level 15 minutes after sample was enclosed in the darkened cuvette. Four replicates of similar size and weight were used in the gas exchange experiments.

Q₁₀ at a given temperature was calculated as:

$$Q_{10} = \left(\frac{R_2}{R_1} \right)^{\left(\frac{10}{T_2 - T_1} \right)}$$

- *Q₁₀* is the *factor* by which the reaction rate increases when the temperature is raised by ten degrees. *Q₁₀* is a unitless quantity.
- *R₁* is the measured reaction rate at temperature *T₁* (where *T₁* < *T₂*).
- *R₂* is the measured reaction rate at temperature *T₂* (where *T₂* > *T₁*).
- *T₁* is the temperature at which the reaction rate *R₁* is measured (where *T₁* < *T₂*).
- *T₂* is the temperature at which the reaction rate *R₂* is measured (where *T₂* > *T₁*).

Chlorophyll extraction and quantification

Determination of the chlorophyll content of the lichen material followed Barnes et al. (1992). Six replicates of 20 mg for each sample were repeatedly washed with CaCO₃ saturated acetone to remove lichen substances, and pigments extracted in CaCO₃ saturated DMSO. Absorbance of the extracts was measured using a UV-visible spectrophotometer (Uvikon XL, NorthStar Scientific, UK). Turbidity was checked at 750 nm and only samples with turbidity lower than 0.010 absorbance units were used. Absorbance was measured at 664.9, 648.2, 480, 435 and 415 nm, and chlorophyll concentrations calculated (expressed in mg g⁻¹ dry weight) of Chl_a and Chl_b using the formulas provided in Barnes et al. (1992).

Anatomy and photobiont ultrastructure

Samples for ultrastructural and anatomical study were prepared following de los Ríos and Ascaso (2002). In brief, small moist young lacinia fragments were initially fixed in glutaraldehyde (2.5% v/v in phosphate buffer), then postfixed in osmium tetroxide (1% w/v in phosphate buffer) and finally dehydrated in a graded ethanol series before embedding in Spurr's resin. Ultrathin sections (70 nm) were cut on a Reichert Ultracut-E ultramicrotome (using a diamond knife). Ultrathin sections were post-stained with lead citrate (Reynolds 1963) and observed in JEOL JEM-2100 and JEM-1011 transmission electron microscopes. For anatomical studies, once the ultrathin sections were taken, the surface of the resin block with the sample remnant was carbon coated and studied by scanning electron microscopy in back-scattering electron mode with a FEI Inspect scanning electron microscope.

Results

Chlorophyll fluorescence

Photosynthetic activity, detectable by chlorophyll fluorescence, was restricted to the grey areas of the lichen branches (Figure 3). In these areas, the yield (quantum efficiency of photosystem II, ϕ PSII) was almost constant at 0.556 ± 0.05 (Fig. 3). Individual algal clusters could be identified in the chlorophyll fluorescence images (Fig. 3) and their locations coincided with those identified via electron-microscopy (Fig. 6).

Both steady-state terminal fluorescence (F') as well as the maximal fluorescence of the light-adapted sample (F_m') were significantly higher in the grey part of the lichen thallus in comparison to the black chondroid axis (Figure 4).

Gas exchange

Net photosynthetic rates were very low even at the optimal combination of light and temperature (Table 1). $A_{\max}$ ranged between 0.28 to 0.33 $\mu\text{mol CO}_2/\text{Kg s}^{-1}$ at 5 and 10 °C respectively and 1200 PPFD. Light compensation points were also high and increasing with rising temperature till 20 °C, where no compensation could be achieved even at the highest light intensity tested (Table 1; Fig 5). This strong dependence on temperature is also clear when Q_{10} (5-15°C) of $A_{\max}$ and Q_{10} (5-15 °C) of dark respiration are compared: 0.86 and 3.53, respectively. NP tends to saturation with PPFD between 400 and 800 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at lower temperatures, and over 800 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ at 15 and 20 °C (Fig. 5).

Table 1. Gas exchange and light compensation points of *Himantormia lugubris* at different temperatures. In all cases $n = 4$. Standard deviation is shown in brackets.

Temperature °C	Light compensation point* ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	$A_{\max}$ $\mu\text{mol CO}_2/\text{kg s}^{-1}$	$A_{\max}$ $\text{mg CO}_2/\text{mg Chlh}^{-1}$	Respiration $\mu\text{mol CO}_2/\text{kg s}^{-1}$
0	52	0.2340 (0.04)	0.1489 (0.02)	-0.12 (0.03)
5	85	0.2800 (0.04)	0.1748 (0.05)	-0.19 (0.02)
10	173	0.3330 (0.07)	0.2089 (0.06)	-0.39 (0.04)
15	330	0.2400 (0.06)	0.1570 (0.09)	-0.67 (0.08)
20	No compensation	-0.0660 (0.01)	-0.0547(0.11)	-1.15 (0.33)

* These values have not been directly measured but calculated from the curves (see Material and Methods).

Chlorophyll content

The only statistically significant ($P < 0.001$) difference in chlorophyll content between grey and black areas of the thallus was for chlorophyll a, which was twice as high in the grey areas (Table 2). Chlorophyll content (Chl a+b) for the whole thallus was 0.31 mg g^{-1} dry weight and the Chla/Chlb ratio was 1.31 (Table 2). Chlorophyll a + b in grey and black areas was 0.35 and 0.26 mg g^{-1} dry weight, respectively ($P = 0.105$), and the Chla/Chlb ratios were 1.62 and 0.63. Turbidity values (below 0.010) were low and allowed accurate chlorophyll a and chlorophyll b measurements.

Table 2: Chlorophyll content and ratio Chla/Chlb. Asterisk indicates that differences between grey and black parts of the thallus are statistically significant ($P < 0.001$). In all cases $n = 6$. Standard deviation is shown in brackets.

Part of the thallus	Chl. a mg g^{-1} dry weight	Chl. b mg g^{-1} dry weight	Chl. a+b mg g^{-1} dry weight	Ratio a/b
Grey areas	0.21* (0.01)	0.14 (0.03)	0,35 (0.04)	1,62
Black areas	0,10* (0.05)	0.16 (0.05)	0,26 (0.11)	0,63
Whole thallus	0,16 (0.03)	0,15 (0.04)	0,31 (0.09)	1,31

Thallus anatomy and photobiont ultrastructure

Scanning electron microscopy study revealed that internally the young branches (laciniae) are composed of a massive central chondroid axis consisting of longitudinally-running conglutinated hyphae and thin algal-medullary and cortex layers (Fig. 6A). Cortex was always observed in these young branches but their density and thickness were variable (Fig. 6B). *Trebouxia* cells showing a massive chloroplast with a single pyrenoid and numerous lipid globules in the cytoplasm were observed by transmission electron microscopy in the algal-medullary layer along the branches (Fig. 6C-D). However, different ratio of mycobiont to photobiont between grey and black areas of the branch was found (Fig. 6A-6B). While grey areas had a thin upper cortex, an algal layer with a more or less continuous presence of *Trebouxia* algal cells (Fig. 6C-D), only scattered groups of small *Trebouxia* algal cells were detected at black areas (Fig. 6B). Ultrastructural study revealed that the majority of algal cells from the black

areas corresponded to aplanospores (Fig. 6E-F). Differences are also detected with respect to the physical relationships established between symbionts. While clear interactions were established between symbiont cells in grey areas with algal cells surrounded by mycobiont hyphae (Fig. 6A, 6C), these were scarce in the black areas because the aplanospores remain mostly in the mother cell (Fig. 6F).

Discussion

In the environmental conditions of the maritime Antarctic, it appears that, in order to be successful, fast growth (high photosynthetic rates and chlorophyll contents) are less important than structural stability and hardness. Being at the far end of the stress-gradient for many other autotrophic organisms we would expect little competition with faster growing plants so that the optimal resource partitioning within a single thallus could come into play. In this case, the important drivers are not biotic but abiotic factors such as temperature and mainly wind. Survival is targeted rather than rapid growth and *Himantormia lugubris* has achieved this with a remarkable morphology. Adult thallus of *H. lugubris* has reduced drastically the areas with well-developed algal layer (the grey areas) in favour of a strong, black, fungal axis. Accordingly, when photosynthetic rates are presented on a whole thallus basis, it shows one of the lowest $A_{\max}$ ever measured for a macrolichen, including comparison with foliose and fruticose species from all over the world (Matthes and Feige 1983; Schroeter et al 1995; Palmqvist et al. 2002) and even with those particularly unproductive species from continental Antarctica (Kappen et al. 1991). The very high light compensation points, especially at temperatures above 10 °C, and the exceptional low Q_{10} (5-15 °C), well below 1 both mean that the highest productivity will occur at low temperatures when the photobiont can compensate respiration (Friedman and Sun 2005). Respiratory losses strongly increase with temperature exhibiting an extremely high value for Q_{10} of 3.53, comparable to those obtained from some plants living in very high elevations (Larigauderie and Körner 1995). In plants a Q_{10} of 2 (i.e. a doubling the metabolic rate with temperature increase of 10 °C) is usually considered as normal (Ryan 1991; Rasmusson et al 2019).

This extreme physiological behaviour is in part due to the patchy distribution of the photobiont cells within the thallus with chlorophyll content in the grey areas around 50% higher than in the black areas, coinciding with a higher abundance of healthy and mature algal cells. When the entire thallus is considered, the chlorophyll content is at the bottom the range measured in other fruticose and foliose lichens from Antarctica, such as *Usnea aurantiaco-atra* (Laguna-Defior et al. 2016), *Usnea antarctica* (Schroeter et al. 1995), *Cetraria aculeata* (Domaschke et al. 2013), *Umbilicaria aprina* (Sancho et al. 2003), *U. decussata* or *Parmelia saxatilis* (Sancho et al. 1997). Similar type of lichenization in patches or rings along the thallus branches can be also observed

in Antarctic *Usnea* species (Walker 1985; Laguna-Defior et al. 2016) yet the chlorophyll content as well as the net photosynthesis are considerably higher than in *H. lugubris*. The connection between this morphology and the Antarctic environmental conditions is still an open question but could be related with the building of a stronger structure to cope with high wind speeds and their abrasive effect on fruticose thalli. In *Himantormia deusta*, a sister species from the Tierra del Fuego mountains (Thell et al. 2007), the axis is not as strong and dominant in the thallus morphology as in *H. lugubris* (Elvebakk et al. 2014). It has been reported that the fungal cell wall rigidity is due to a molecular complex formed by chitin and α -1,3-glucan (Kang et al. 2018). Given the low nitrogen availability in this area (Benabent et al. 2018) it seems that the strategy of *H. lugubris* would be to allocate this nutrient preferably in chitin, increasing thallus strength, than in algal cells (chlorophyll and rubisco, mainly), reducing productivity.

Previous studies reported a lack of algal cells in black areas of *H. lugubris* thallus (Lamb 1964; Sojo et al. 2003). However, our electron microscope observations show algal cells in both the grey and the black areas of analysed young branches, albeit in black areas they were scarce often present as aplanospores. This is in good agreement with the lower fluorescence signal of these black areas and the Fv/Fm close to 0 as measured with the Imaging-PAM system, suggesting that the chlorophyll contained in these cells is not functionally active for photosynthetic activity, at least on the detection level used in this work. Nevertheless, the lack of algal cells in older branches where chondroid axis is directly exposed (Lamb 1964) cannot be discarded.

Several features suggest that *H. lugubris* can be best understood as being extremely shade-adapted despite growing in the fully exposed lithic habitat. Features typical of such an adaptation are the low $A_{\max}$ for photosynthesis and the very low Chla/Chlb ratios. The latter was 1.31 for the whole thallus. These values are very much at the low end for plants, for example, 3.9 and 3.0 for sun and shade leaves of *Fagus sylvatica* and a mean value of around 3.8 for lichens (Matsubara et al. 2005). *H. lugubris* also shows large shifts in quantity of the carotenoid lutein epoxide (Czeczuga et al. 1986). This carotenoid is involved in a cycle that protects against high light during photosynthesis (Ladygin 2008). It is known sporadically throughout plants and seems to provide rapid protection and is found predominantly in shade-adapted tree leaves in deep shade environments. It appears that *H. lugubris* is utilising this cycle as a rapid response to changes in light intensity; similar to sunflecks in a forest is the sudden sunshine in

habitats in which clouds predominate. The chlorophyll content of *H. lugubris* is low whereas high contents are normal for shade plants, however, when related to the very low A_{max} the content can be seen as high (Table 1).

In spite of its physiological limitations, *H. lugubris* can grow well in the maritime Antarctic because of the unusual climate of the area, temperatures are low and very constant, drying pressure will be low because of the cloudiness and hydration will occur often due to the regular precipitation. Its natural distribution is strongly tied to these climatic conditions (Fig. 1). The species will benefit from long periods of activity during the growth season (Schroeter et al. 2010; Schlensog et al. 2013; Pintado et al. forthcoming paper) and from the steady low temperatures that reduces respiration rates. It has been shown that a small warming has enhanced the net primary production (NPP) of several lichen species on Livingston Island (Sancho et al. 2017, 2019), including *Usnea aurantiaco-atra* that very often shares the habitat with *H. lugubris*. Short-term acclimatisation is suggested to be difficult in Antarctic lichens (Colesie et al. 2018). It is, however, arguable whether this species would benefit from any increase in temperature in the maritime Antarctic. Its extreme dependence on low temperatures and high precipitation rates makes it very fragile in a climate warming scenario, especially considering the possible competition with other fruticose lichens. We hypothesize the migration of this species to higher altitude and eventually its disappearance of many of its present localities if climate significantly warms in the next decades. Even worse would be a combination of warming with a shift in the location of the cloud belt that dominates the climate in this region.

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378

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Figure 1. Distribution map of *Himantormia lugubris* (UK Polar Data Centre, Antarctic Plant Database:
<https://data.bas.ac.uk/metadata.php?id=GB/NERC/BAS/AEDC/>)

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388 Figure 2. Natural habitat of *Himantormia lugubris*. Several brown apothecia are
389 visible at the top of many branches. It is growing together with *Usnea*
390 *aurantiaco-atra* (on the right).

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393 Figure 3. Image composition of *H. lugubris* including: thallus with normal light
394 (upper left); quantum efficiency of photosystem II (Yield II); maximal
395 fluorescence (Fm') and steady-state terminal fluorescence (F).

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Figure 4 Fluorescence signal obtained from the grey and black areas of the *Himantormia lugubris* branches : F, steady-state terminal fluorescence; Fm maximal fluorescence; Y (II), quantum efficiency of photosystem II (Yield) (n=15, error is given as standard deviation).

Figure 5. Net photosynthesis versus light intensity of *Himantormia lugubris* for different temperatures. Each point represents the measurement of a single thallus.

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415 Fig. 6: Anatomy and photobiont ultrastructure of *Himantormia lugubris*. (A-B)
416 Scanning electron image in back scattered mode (SEM-BSE) of transversal
417 section of grey (A) and black areas (B). CL: cortical layer; AL: algal layer; Ax:
418 central axis. (C) Transmission electron image (TEM) of the alga layer from grey
419 area. F: fungal cell; P: pyrenoid; L: lipid globules (D) TEM image of mycobiont-
420 photobiont contacts in the algal layer of grey area showing mycobiont hyphae
421 penetration of the mother cell wall (arrows). (E) TEM image of algal cells
422 forming part of aplanospores from a black area. A: algal cell; F: fungal cell; CL:
423 cortical layer. (F) TEM image of an algal cell from a black area noted by a white
424 arrow in (E). A: algal cell; F: fungal cell; cw: cell wall; mcw: mother cell wall.

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