# Diatoms (Bacillariophyta) associated with lichens from Ulu Peninsula (James Ross Island, NE Antarctic Peninsula)

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## Abstract
Since 2000, the entire Antarctic diatom flora is being revised using a more fine-grained taxonomy based on a better analysis and interpretation of the morphological and molecular observations. Despite the increased diatom research and efforts, the diversity and ecology of diatoms of lichen inhabiting flora of James Ross Island weren't studied yet. To reveal the actual diatom diversity, samples were collected during February and March 2018 from lichens on the Ulu Peninsula, James Ross Island, a 2,450 km$^2$ large island, situated in the north-western part of the Weddell Sea, close to the northern tip of the Antarctic Peninsula. The analysis of 29 lichen samples revealed the presence of 56 diatom taxa belonging to 17 genera. The most abundant species were *Luticola muticopsis*, *Hantzschia amphioxys* f. *muelleri*, *Pinnularia borealis* var. *scalaris*, *Luticola* aff. *pusilla* and *Achnanthes muelleri*. Biogeographically, the lichen-inhabiting diatom flora of the Ulu Peninsula is composed of cosmopolitan, Antarctic and endemic elements. The present study is the first focusing on the diversity of lichen-inhabiting diatom communities on James Ross Island, revealing the presence of a rather species rich diatom flora.

***Key words:*** Antarctica, diatoms, diversity, James Ross Island, lichens, Ulu Peninsula

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## Introduction

Diatoms are eukaryotic unicellular microorganisms, characterised by its unique, silica-impregnated cell wall known as a frustule and are one of the most abundant groups of micro-algae present in the Antarctic Region (Jones 1996, Van de Vijver et Beyens 1999a). Although the majority of diatom species are bound to aquatic habitats, a large number of diatom taxa are able to survive in non-submerged or even dry habitats such as dry mosses, humid rocks, soils and lichens (Van de Vijver et Beyens 1999b, Zidarova et al. 2010). Lichen communities represent an important part of the Antarctic vegetation and belong to principal components of the terrestrial ecosystem

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of James Ross Island (Bohuslavová et al. 2012). Diatoms have shown the ability to colonize lichens via aerial dispersal (Bertrand et al. 2016), but the diatom diversity has been rarely studied and papers focusing on lichen inhabiting diatom flora are extremely scarce. Dodd et Stoermer (1962) were the first to investigate diatom flora associated to a lichen *Collema* sp. in Boone Country, Iowa. In 2004 Lakatosh et al. conducted a research on diatoms living inside the thallus of adnate corticolous lichens in neotropical lowland rain forests and finally in 2016 Bertrand et al. published a preliminary study on diatoms associated with five lichen genera. The most recent ecological surveys on James Ross Island were dealing with the freshwater, semi-aquatic, moss-inhabiting and soil diatom communities of the Ulu Peninsula (Kopalová et al. 2012, Kopalová et al. 2013, Kopalová et al. 2014, Chattová et al. 2016). Despite the increased diatom research and efforts, the diversity and ecology of lichen inhabiting flora of James Ross Island weren't studied yet. One of the main objectives of this study is to bring new information about diatoms associated with lichens from Ulu Peninsula.

## Material and Methods

*Study site*

James Ross Island (64°10'S, 57°45'W), a large island with a total surface area of 2450 km², is located in the northwestern part of the Weddell Sea, close to the northern tip of the Antarctic Peninsula (Fig. 1). The mean annual temperature is around -7.0°C (Ambrožová et Láska 2016). More than 75% of its area is covered with a glacier (Rabassa et al. 1982). The largest ice-free area, Ulu Peninsula (310 km$^2$) is located in the northern part of the island (Kavan et al. 2017) and forms the largest ice-free area in the Antarctic Peninsula region (Hrbáček et al. 2017). Vascular plants are absent, the vegetation is limited to a bryophyte and lichen tundra (Barták et al. 2016). The human presence on the island is limited to its northern part, where the Johann Gregor Mendel Czech Antarctic Station is located since 2006.

*Fieldwork and sample preparation*

29 lichen samples were collected during a field campaign in February and March 2018. The samples were collected in PVC bottles. Each sample was geographically localized using GPS and was accompanied by a detailed site description. Table 1 lists all samples together with their geographic co-ordinates. Photographs of selected sampling sites with the lichens are presented at Fig. 2. Light microscope observations directly at the Mendel station were conducted using an Olympus CX31 microscope. All samples were scanned under a microscope before fixing with 3% formaldehyde and the dominating cyanobacterial and algal flora was listed. The fixed material was taken into Czech Republic, where the permanent diatom slides were prepared for further analysis following the method described in Van der Werff (1955): small parts of the samples were cleaned by 37% $H_2O_2$ and heated to 80°C for about 1 h.

The reaction was completed by addition of $KMnO_4$. Following digestion and centrifugation (five times 10 min. at 3500 × g), cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom valves were mounted in Naphrax®.

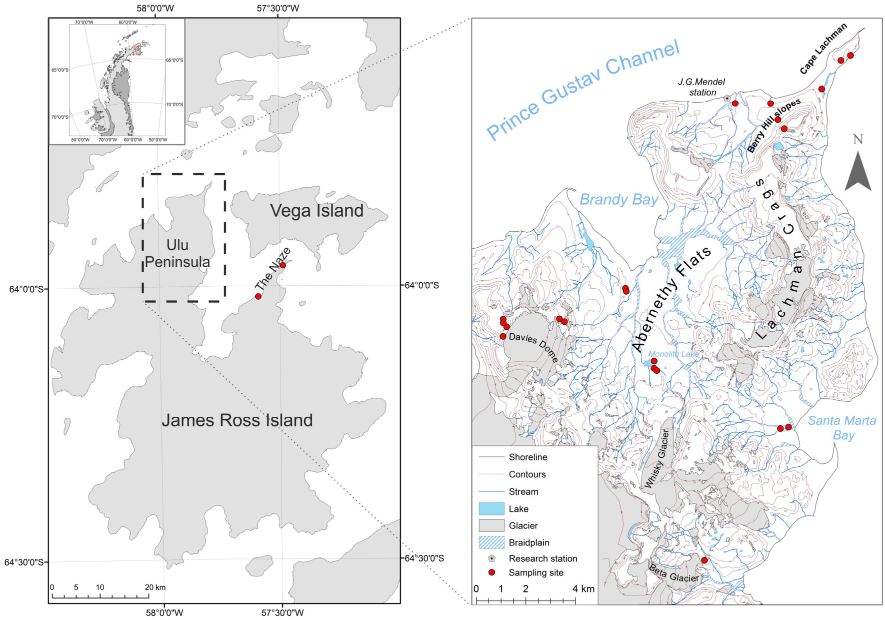


**Fig. 1.** Map of James Ross Island ([1] - Antarctic Digital Database 2018) and a modified map of James Ross Island-Northern part ([2] - Czech Geological Survey 2009) with indication of sampling sites. Credits: Filip Hrbáček.


**Fig. 2.** General view on sampling site No. 26 *Xanthoria* sp. (A), sampling site No. 9 with *Usnea antarctica* (B), sampling site No. 6 with *Candelariella* aff. *flava* (C), sampling site No. 13 with *Xanthoria* sp. (D).

| | Locality | Date | GPS | Eleva-tion | Type of lichen |
|---|---|---|---|---|---|
| Lich1* | Abernethy Flats | 3.2.2018 | 63°52'15" S 57°58'26" W | 69 | *Usnea antarctica* |
| Lich2* | Abernethy Flats | 3.2.2018 | 63°52'16" S 57°58'27" W | 69 | *Usnea antarctica* |
| Lich3 | Abernethy Flats | 3.2.2018 | 63°53'59" S 57°57'16" W | 74 | *Xanthoria* sp. |
| Lich4 | Monolith Lake | 3.2.2018 | 63°53'58" S 57°57'16" W | 71 | *Xanthoria* sp. |
| Lich5* | Under Davies Dome Glacier | 5.2.2018 | 63°52'52" S 58°01'54" W | 397 | *Usnea antarctica* |
| Lich6* | Under Davies Dome Glacier | 5.2.2018 | 63°52'52" S 58°01'44" W | 397 | *Candelariella* aff. *flava* |
| Lich7 | Davies Dome Mesa | 5.2.2018 | 63°53'20" S 58°04'33" W | 426 | *Usnea antarctica* |
| Lich8* | Near OTC Davies Dome Mesa | 5.2.2018 | 63°53'02" S 58°04'17" W | 512 | *Candelariella* aff. *flava* |
| Lich9* | Near Meteo station Davies Dome | 5.2.2018 | 63°52'49" S 58°04'37" W | 496 | *Usnea antarctica* |
| Lich10* | Davies Dome Glacier | 5.2.2018 | 63°52'48" S 58°04'40" W | 534 | *Candelariella* aff. *flava* |
| Lich11* | Monolith Lake, near dead seal T3 | 9.2.2018 | 63°53'49" S 57°56'58" W | 86 | *Candelariella* aff. *flava* |
| Lich12 | Monolith Lake, near dead seal T3 | 9.2.2018 | 63°53'49" S 57°56'58" W | 86 | *Xanthoria* sp. |
| Lich13 | Monolith Lake, next to dead seal T3 | 9.2.2018 | 63°53'53" S 57°56'58" W | 81 | *Xanthoria* sp. |
| Lich14 | St. Martha Cove | 10.2.2018 | 63°55'28" S 57°50'19" W | 73 | *Xanthoria* sp. |
| Lich15* | St. Martha Cove | 10.2.2018 | 63°55'28" S 57°50'53" W | 62 | *Usnea antarctica* |
| Lich16 | Cape Lachman | 15.2.2018 | 63°47'02" S 57°47'02" W | 85 | *Xanthoria* sp. |
| Lich17* | Top of Berry Hill | 16.2.2018 | 63°48'48" S 57°50'43" W | 364 | *Usnea antarctica* |
| Lich18* | Cape Lachman | 15.2.2018 | 63°47'02" S 57°47'02" W | 85 | *Xanthoria* sp. |
| Lich20* | Cape Lachman | 15.2.2018 | 63°47'12" S 57°47'32" W | 122 | *Usnea antarctica* |
| Lich21* | Cape Lachman | 15.2.2018 | 63°47'35" S 57°47'02" W | 105 | *Usnea antarctica* |
| Lich22* | Cape Lachman | 15.2.2018 | 63°47'22" S 57°47'72" W | 53 | *Xanthoria* sp. |
| Lich23 | The Naze | 27.2.2018 | 63°55'50" S 57°30'55" W | 65 | *Xanthoria* sp. |
| Lich24 | Interlagos Lachman | 11.3.2018 | 63°47'56" S 57°48'39" W | 14 | *Xanthoria* sp. |
| Lich25 | Berry Hill Slopes | 11.3.2018 | 63°48'17" S 57°50'97" W | 46 | *Xanthoria* sp. |
| Lich26 | Under Terrapin Hill | 27.2.2018 | 63°58'41" S 57°35'32" W | 4 | *Xanthoria* sp. |
| Lich27* | Near Beta Glacier | 26.2.2018 | 63°57'58" S 57°54'58" W | 199 | *Usnea antarctica* |

| | | | | | |
|---|---|---|---|---|---|
| Lich28 | Berry Hill Slopes | 7.3.2018 | 63°48'25" S 57°50'33" W | 146 | *Xanthoria* sp. |
| Lich29 | Berry Hill Slopes | 7.3.2018 | 63°48'24" S 57°50'32" W | 143 | *Xanthoria* sp. |
| Lich30 | Near JGM station and Bohemian stream | 9.3.2018 | 63°48'13" S 57°52'57" W | 31 | *Xanthoria* sp. |

**Table 1.** List of samples collected on JRI, samples without diatoms are marked with an asterisk*.

In each sample, 200 diatom valves were identified, if possible and enumerated on random transects at 1000× magnification using an Olympus BX50 microscope (Japan), equipped with Differential Interference Contrast (Nomarski) optics. The diatom taxa were identified as much as possible up to species level or variety. When the taxonomic status of a taxon was uncertain, abbreviations 'cf.' (confer: probably belongs to the species identified), 'aff.' (affinis: it bears some similarity to this taxon, but it is not conspecific), or 'sp.' (species of genera given) were used. For identification of Antarctic species mainly the following publications were consulted: Zidarova et al. 2010, 2016, Van de Vijver et al. 2010, 2011, 2014).

## Results

During this study, 29 samples from three different lichen species - *Usnea antarctica* Du Rietz, *Xanthoria* sp. and *Candelariella* aff. *flava* (C.W. Dodge & Baker) Castello & Nimis have been analysed, resulting in the observation of a total number of 56 diatom taxa, belonging to 17 genera. Fifteen samples contained (almost) no diatoms, even after counting an entire slide. Subsequently, these samples have been removed from further analysis. Species richness per sample ranged from 7 to 23 with an average number of taxa per sample of 15. The highest species richness was recorded in sample Lich 25 (23 taxa), a wet *Xanthoria* sample collected on the north-facing slopes of Berry Hill. This area is supplied by melt water from annual snow deposition and frozen ground. The dominant species were *Luticola muticopsis* (Van Heurck) D. G. Mann with more than 13% of all counted valves, followed by *Hantzschia amphioxys* (Ehrenberg) Grunow f. *muelleri* Ts. Kobay. (12%), *Pinnularia borealis* var. *scalaris* (Ehrenberg) Rabenhorst (10%), *Luticola* aff. *pusilla* Van de Vijver, Kopalová, Zidarova & Levkov (9%) and *Achnanthes muelleri* Carlson (8%). Table 2 provides an alphabetical list of all observed species together with their biogeographical distribution. For selected species see Fig. 3. The genera *Luticola* (fourteen taxa), *Humidophila* (seven taxa), *Nitzschia* (six taxa) *Hantzschia* (five taxa) and *Pinnularia* (four taxa) were the most species rich genera. Other important genera include *Achnanthes*, *Muelleria* and *Stauroneis* (three taxa). Table 3 lists all genera arranged according to their species number. The majority of diatom taxa were identified from lichens of the genus *Xanthoria*. Only two single valves of *Hanzschia amhioxys* and *Pinnularia borealis* var. *scalaris* were observed on the thallus of *Usnea antarctica.* No diatoms were found on *Candelariella* aff. *flava,* most likely because the lichen was growing on a dry rock in the most recently deglaciated area of Davies Dome Mesa. Diatoms in the samples were frequently accompanied by various species of genera *Leptolyngbya*, *Oscillaltoria*, *Cyanothece*, *Klebsormidium* and *Nostoc*. From a biogeographical point of view, 26% of the taxa have a typical cosmopolitan distribution, with another 5% species having a restricted distribution to Southern Hemisphere. More than a half of the species (55%) are confined to the Maritime Antarctic Region, with additional 14% showing a restricted distribution to the Antarctic Continent.

| **Species** | ***Xanthoria* sp.** | ***Usnea antarctica*** | **Distribution** |
|---|---|---|---|
| *Achnanthes coarctata* (Brébisson ex W.Smith) Grunow | x | | C |
| *Achnanthes muelleri* Carlson | x | | SH |
| *Achnanthes taylorensis* D.E.Kellogg, Stuiver, T.B.Kellog & G.H.D.Denton | x | | MA/CA |
| *Brachysira minor* (Krasske) Lange-Bertalot | x | | C |
| *Caloneis australis* Zidarova, Kopalová & Van de Vijver | x | | MA |
| *Denticula jamesrossensis* Van de Vijver, Kopalová, Ector & Kociolek | x | | MA |
| *Fragilaria* cf. *parva* Tuji & Williams | x | | C |
| *Halamphora oligotraphenta* Lange-Bertalot & Levkov | x | | C |
| *Halamphora* sp. (Cleve) Levkov | x | | U |
| *Hantzschia abundans* Lange-Bertalot | x | | C |
| *Hantzschia amphioxys* (Ehrenberg) Grunow *f. muelleri* Ts. Kobay. | x | x | MA/CA |
| *Hantzschia* cf. *acuticapitata* Zidarova & Van de Vijver | x | | MA |
| *Hantzschia hyperaustralis* Van de Vijver & Zidarova | x | | MA/CA |
| *Hantzschia incognita* Zidarova & Van de Vijver | x | | MA |
| *Humidophila australis* (Van de Vijver & Sabbe) R.L. Lowe et al. | x | | MA/CA |
| *Humidophila contenta* group (Grunow) Lowe et al. | x | | C |
| *Humidophila inconspicua* (Kopalová & Van de Vijver) Lowe et al. | x | | MA |
| *Humidophila* aff. *ingeae* (Van de Vijver ) Lowe et al. | x | | SH |
| *Humidophila keiliorum* Kopalová | x | | MA |
| *Humidophila sceppacuersiae* Kopalová | x | | MA |
| *Humidophila vojtajarosikii* Kopalová, Zidarova & Van de Vijver | x | | MA |
| *Chamaepinnularia australomediocris* (Lange-Bertalot &Smidt) Van de Vijver | x | | MA/SA |
| *Luticola amoena* Van de Vijver, Kopalová, Zidarova & Levkov | x | | MA |
| *Luticola australomutica* Van de Vijver | x | | MA |
| *Luticola austroatlantica* Van de Vijver, Kopalova Spaulding et Esposito | x | | MA/CA |
| *Luticola doliiformis* Kopalová & Van de Vijver | x | | MA |
| *Luticola evkae* Kopalová | x | | MA |
| *Luticola gigamuticopsis* Van de Vijver | x | | MA |

| | | | |
|---|---|---|---|
| *Luticola higlerii* Van de Vijver, Van Dam & Beyens | x | | MA |
| *Luticola katkae* Van de Vijver & Zidarova | x | | MA |
| *Luticola muticopsis* (Van Heurck) D.G.Mann | x | | SH |
| *Luticola permuticopsis* Kopalová & Van de Vijver | x | | MA |
| *Luticola pusilla* Van de Vijver, Kopalová, Zidarova & Levkov | x | | MA |
| *Luticola tomsui* Kopalová | x | | MA |
| *Luticola truncata* Kopalová & Van de Vijver | x | | MA |
| *Luticola vermuelenii* Van de Vijver | x | | MA |
| *Muelleria austroaltlantica* Van de Vijver & S.A. Spaulding | x | | MA |
| *Muelleria luculenta* S.A.Spaulding & J.P.Kociolek | x | | MA |
| *Muelleria regigeorgiensis* Van de Vijver & Spaulding | x | | MA |
| *Navicula gregaria* Donkin | x | | C |
| *Navicula romanedwardii* Zidarova, Kopalová & Van de Vijver | x | | MA |
| *Nitzschia commutata* Grunow | x | | C |
| *Nitzschia homburgiensis* Lange-Bertalot | x | | C |
| *Nitzschia paleacea* Grunow | x | | C |
| *Nitzschia perminuta* (Grunow) M. Peragallo | x | | C |
| *Nitzschia soratensis* E. Morales & Vis | x | | C |
| *Nitzschia vancauwenberghiana* Hamsher et al. | x | | MA |
| *Orthoseira roeseana* (Rabenhorst) O'Meara | x | | C |
| *Pinnularia australoschoenfelderi* Zidarova, Kopalová & Van de Vijver | x | | MA |
| *Pinnularia borealis* var. *pseudolanceolata* B. Van de Vijver & R.Zidarova | x | | MA |
| *Pinnularia borealis* var. *scalaris* (Ehrenberg) Rabenhorst | x | x | C |
| *Pinnularia subaltiplanensis* Zidarova, Kopalová & Van de Vijver | x | | MA |
| *Psammothidium rostrogermainii* Van de Vijver, Kopalová & Zidarova | x | | MA/CA |
| *Stauroneis latistauros* Van de Vijver & Lange-Bertalot | x | | MA/CA |
| *Stauroneis pseudoschimanskii* Van de Vijver & Lange-Bertalot | x | | MA |
| *Staurosirella antarctica* Van de Vijver & E. Morales | x | | MA |

**Table 2.** List of all species observed in this study together with their biogeographical distribution. *Distribution*: CA - Antarctic Continent, MA - Maritime Antarctic Region, SH - Southern Hemisphere, C - Cosmopolitan, U - Unknown.

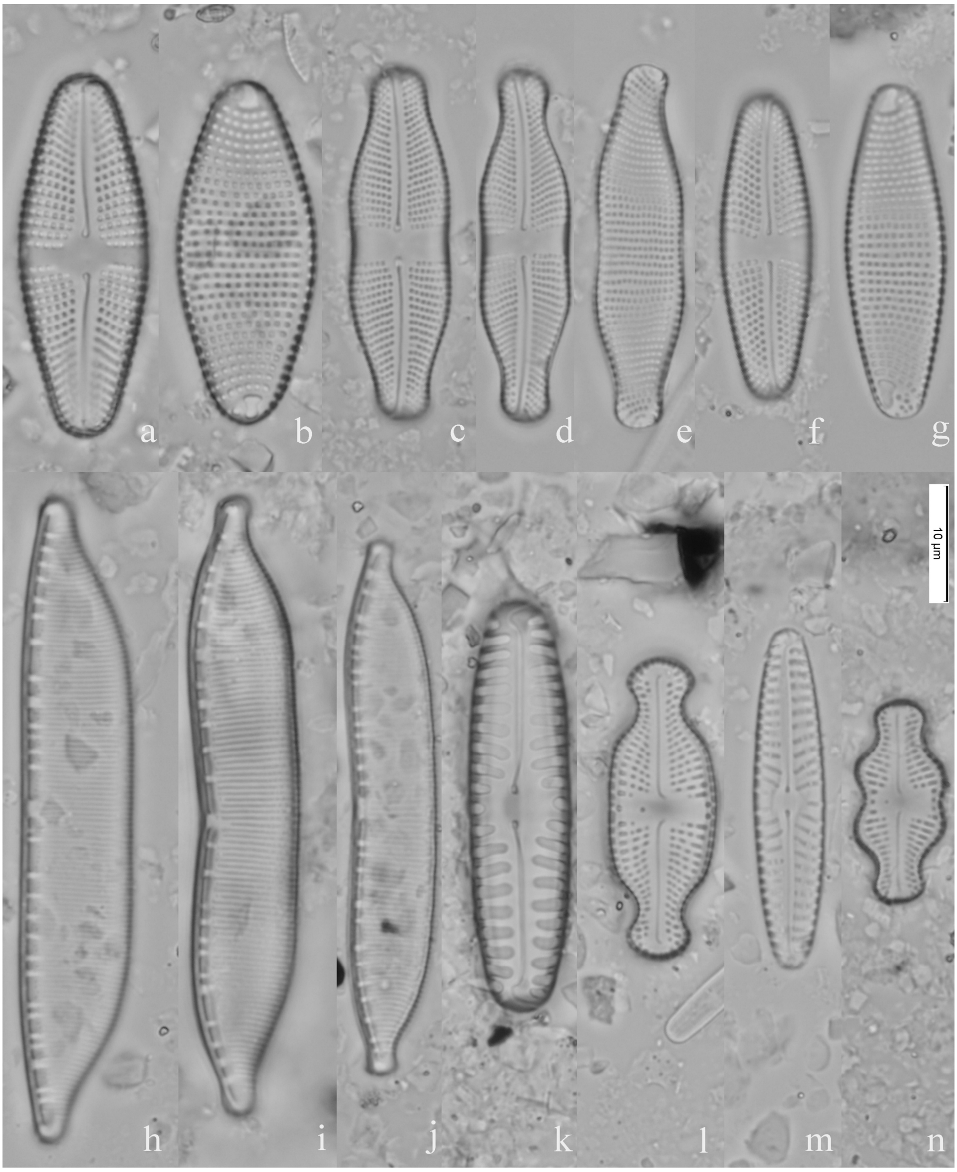


**Fig. 3.** a - *Achnanthes muelleri* raphe valve, b - *Achnanthes muelleri* rapheless valve, c, d - *Achnanthes coarctata* raphe valve, e - *Achnanthes coarctata* rapheless valve, f - *Achnanthes taylorensis* raphe valve, g - *Achnanthes taylorensis* rapheless valve, h - *Hantzschia hyperaustralis*, i - *Hantzschia abundans*, j - *Hantzschia amphioxys* f. *muelleri*, k - *Pinnularia borealis*, l - *Luticola muticopsis*, m -*Navicula romanedwardii*, n - *Luticola tomsui*.

| Genus | n | % |
|---|---|---|
| *Luticola* | 14 | 25 |
| *Humidophila* | 7 | 13 |
| *Nitzschia* | 6 | 11 |
| *Hantzschia* | 5 | 9 |
| *Pinnularia* | 4 | 7 |
| *Achnanthes* | 3 | 5 |
| *Muelleria* | 3 | 5 |
| *Stauroneis* | 3 | 5 |
| *Halamphora* | 2 | 3 |
| *Navicula* | 2 | 3 |
| *Brachysira* | 1 | 2 |
| *Caloneis* | 1 | 2 |
| *Denticula* | 1 | 2 |
| *Fragilaria* | 1 | 2 |
| *Chamaepinnularia* | 1 | 2 |
| *Orthoseira* | 1 | 2 |
| *Psammothidium* | 1 | 2 |
| **Total** | **56** | **100** |

**Table 3.** Genera ordered by decreasing percental portion (%) calculated on the number of the taxa (n).

## Discussion

Based on the relative abundance data, the principal taxon was *Luticola muticopsis,* a cosmopolitan species, frequently reported from Antarctica and typically found in terrestrial habitats influenced by sea birds and sea sprays (Zidarova et al. 2016). The dominant taxa are typical terrestrial species reported to be frequent also in microbial mat samples on JRI (Skácelová et al. 2015) and soils, where Chattová et al. (2016) found 86 diatom taxa. Such relatively high number of typical terrestrial species of diatoms found in the lichen samples is not surprising, considering the fact that the lichen microhabitat can be characterized by extreme environmental conditions, mainly by unstable moisture regime. The diatom diversity of the James Ross Islands lichen inhabiting diatom flora is comparable with streams and seepages of James Ross Island where Kopalová et al. (2012) found 69 taxa. When compared with diatom communities reported from lake ecosystems by Kopalová et al. (2013), samples in this study show somewhat lower species richness.

Dodd et Stoermer (1962) list in the first study focused on lichen-inhabiting diatom flora thirteen diatom species inhabiting the surface of a lichen identified as *Collema* sp. The samples were dominated by typical terrestrial species *Achnanthes coarctata* (Brébisson ex W. Smith) Grunow and *Hantzschia amphioxys*, similar communities characterized by the dominance of the species of genera *Achnanthes* and *Hantzschia* were reported also from James Ross Island. Lakatosh et al. (2004) identified eighteen diatom species belonging to nine genera inside the thallus of the three crustose lichens *Thelotrema alboolivaceum* Vain., *Cryptothecia rubrocincta* (Ehrenb.) Thor and *Phylopsora corallina* (Eschw.) Müll. Arg in neotropical lowland rain forests and discuss the potential benefits both diatoms and lichens could derive from symbiosis. They report typical cosmopolitan terrestrial taxa and six unidentified species, which can be probably new to science. Four of the typical cosmopolitan taxa are shared with the James Ross Islands lichen diatom flora- *Humidophila contenta* (Grunow) Lowe et al. (former *Diadesmis contenta*), *Orthoseira roeseana* (Rabenhorst) O'Meara, *Pinnularia borealis* and *Hantzschia amphioxys*. The most species rich lichen-inhabiting diatom communities (313 species) report Bertrand et al. (2016) from five lichen genera- *Evernia*, *Usnea*, *Ramalina*, *Cladonia* and *Pseudevernia*, collected in France. The communities were generally dominated by *Pinnularia borealis* and *Hantzschia amphioxys*, species playing an important role also on James Ross Island. However, differences can be found in the associated diatom flora of the dominant species, Bertrand et al. report *Achnanthidium minutissimum* (Kützing) Czarnecki, *Humidophila gallica* (W. Smith) Lowe et al. and *Luticola goeppertiana* (Bleisch) D. G. Mann ex J. Rarick et al., species absent in the lichen inhabiting flora of JRI.

The obtained results confirm the presence of a typical and highly specific non-marine diatom flora in the Antarctic Region. For future research, the author suggests a more extensive sampling campaign, including a low temperature SEM observation and detection of diatoms within the thallus, between lichen filaments.

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