

# High levels of endemism among Galapagos basidiolichens

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**Abstract** This study is a re-assessment of basidiolichen diversity in the Galapagos Islands. We present a molecular phylogenetic analysis, based on 92 specimens from Galapagos, using two nuclear ribosomal DNA markers (ITS and nuLSU). We also re-examined the morphology and anatomy of all sequenced material. The molecular results confirm our previous assessment that all Galapagos basidiolichens belong to the *Dictyonema* clade, which in Galapagos is represented by four genera: *Acantholichen*, *Cora*, *Cyphellostereum*, and *Dictyonema*. Most species previously reported from Galapagos in these genera were at the time believed to represent widely distributed taxa. This conclusion, however, has changed with the inclusion of molecular data. Although almost the same number of species is distinguished, the phylogenetic data now suggest that all are restricted to the Galapagos Islands. Among them, six species are proposed here as new to science,

namely *Cora galapagoensis*, *Cyphellostereum unoquinoum*, *Dictyonema barbatum*, *D. darwinianum*, *D. ramificans*, and *D. subobscuratum*; and four species have already been described previously, namely *Acantholichen galapagoensis*, *Cora santacruzensis*, *Dictyonema pectinatum*, and *D. galapagoense*, here recombined as *Cyphellostereum galapagoense*. Our analysis is set on a very broad phylogenetic framework, which includes a large number of specimens (N = 826) mainly from Central and South America, and therefore strongly suggests an unusually high level of endemism previously not recognized. This analysis also shows that the closest relatives of half of the basidiolichens now found in Galapagos are from mainland Ecuador, implying that they reached the islands through the shortest route, with all species arriving on the islands through independent colonization events.

**Keywords** Lichens · Systematics · Biodiversity · Evolution · Lichenized basidiomycetes · Galapagos

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## Introduction

The Galapagos Islands represent an isolated ecosystem long considered a living laboratory of evolution (Darwin 1859; Carlquist 1974; Williamson 1981; Bensted-Smith 2002). Assuming that species only rarely managed to colonize the islands, presumably from South and Central America, when they did arrive they filled unoccupied ecological niches and evolved independently from their mainland relatives. Many groups of organisms therefore exhibit high levels of endemism in the archipelago: 32–43% for vascular plants, ~50% for invertebrates (excluding insects), 47% for insects, and 59% for land vertebrates (Tye et al. 2002). However, endemism in lichens has typically been considered to be much lower, around 3–10% (Weber 1986; Yáñez et al. 2013).

Until 2005, when the Charles Darwin Foundation (CDF) initiated a new, comprehensive species inventory, William Weber was responsible for gathering most of the modern lichen collections from the Galapagos, resulting in a checklist including 196 species (Weber 1986). Since then, there have been three updated checklists: two by Elix and McCarthy (1998, 2008), who included 229 and 253 species, respectively, and the third by Bungartz et al. (2013c), which included 579 accepted species, along with several reports of names still under revision.

As part of the general species inventory of lichens in the Galapagos, several revisionary treatments dedicated to particular groups have been published recently (Aptroot and Bungartz 2007; Aptroot and Sparrius 2008; Aptroot et al. 2008; Bungartz 2008; Bungartz et al. 2010, 2013a, b, 2015, 2016a, b; Tehler et al. 2009; Yáñez et al. 2013). These also include a first treatment of Galapagos basidiolichens (Yáñez et al. 2012), which was based only on morphological and anatomical studies.

Prior to the treatment of Yáñez et al. (2012), four basidiolichen taxa had been reported from the Galapagos Islands, partially under different names, all members of the *Dictyonema* sensu lato clade:

1. *Cora pavonia* (Sw.) Fr. in Dodge (1935), also cited in Weber (1966), later moved to *Dictyonema montanum* (Sw.) Parm. by Weber (1986), which was followed by Elix and McCarthy (1998), although the correct name at that time was either *D. pavonium* (Sw.) Parm. (Parmasto 1978) or subsequently *D. glabratum* (Spreng.) D. Hawksw. (Hawksworth 1988).
2. *Dictyonema sericeum* (Fr.) Mont. by Linder (1934), changed to *Dictyonema guadalupense* (Rabenh.) Zahlbr. by Weber (1986), which was followed by Elix and McCarthy (1998), although the accepted name at the time was *D. sericeum* (Parmasto 1978).

3. *Dictyonema* sp. (Weber 1993) (an unidentified, appressed filamentous, crustose species).
4. *Acantholichen pannarioides* P. M. Jørg. (Jørgensen 1998; Lawrey et al. 2009; Lücking et al. 2009).

These four species include one foliose growth form (*Dictyonema glabratum*), one filamentous, shelf-forming morphotype (*Dictyonema sericeum*), an appressed-filamentous, crustose form (*Dictyonema* sp.), and a microsquamulose taxon (*Acantholichen pannarioides*). All specimens subsequently collected were generally assigned to one of these taxa based on their morphology. In addition, *Dictyonema moorei* (Nyl.) A. Henssen and *D. membranaceum* C. Agardh. were used as working names for specimens collected in the islands, but in the first revision of the group published by Yáñez et al. (2012), these names were added to the list of rejected taxa for the archipelago (Bungartz et al. 2013c).

Yáñez et al. (2012) were the first to document the diversity of basidiolichens in the Galapagos using a revised genus concept based on Lawrey et al. (2009), who recognized four genera within the *Dictyonema* clade: *Acantholichen* P.M. Jørg., *Cora* Fr., *Cyphellostereum* D.A. Reid, and *Dictyonema* C. Agardh ex Kunth sensu stricto (s.str.). Yáñez et al. (2012) used a conservative approach when identifying Galapagos material based on this revised concept, recognizing new species only where no available name could be applied. This led to the distinction of eight taxa in the *Dictyonema* clade for the islands: *Acantholichen pannarioides*, *Cora glabrata* (Spreng.) Fr., *Cyphellostereum imperfectum* Lücking, Barillas & Dal-Forno, *Cyphellostereum* sp., *Dictyonema galapagoense* Yáñez, Dal Forno & Bungartz, *Dictyonema pectinatum* Dal Forno, Yáñez-Ayabaca & Lücking, *Dictyonema sericeum*, and *Dictyonema schenkianum* (Müll. Arg.) Zahlbr. Of these, *D. galapagoense* and *D. pectinatum* were proposed as new to science based on Galapagos specimens and *C. imperfectum* was described as new, based on material from Guatemala, citing also material from the Galapagos. As a result, of the seven species formally recognized (excluding *Cyphellostereum* sp.), two (29%) were considered endemic, whereas prior to that treatment, none of the four recognized taxa had been considered endemic.

Based on molecular, anatomical and morphological evidence, Dal-Forno et al. (2016a) recently discovered that specimens traditionally identified as the widespread neotropical *Acantholichen pannarioides* include at least six distinct species. Specimens collected in the Galapagos Islands are all genetically and morphologically distinct from *A. pannarioides*, and are now considered to belong to a species considered endemic to the archipelago, accordingly named *A. galapagoensis* Dal-Forno, Bungartz & Lücking.

Given the extensive collections representing the *Dictyonema* clade gathered by us in the Galapagos Islands and throughout the Neotropics and other regions of the world, and reports of high levels of regional and local endemism in this clade from other Neotropical areas (Dal-Forno et al. 2013, 2016a; Lücking et al. 2013a, b, 2014a, 2016; Vargas et al. 2014), we set out to test the following hypotheses:

- (1) Are all species of the *Dictyonema* clade now reported from the Galapagos endemic to the archipelago?
- (2) If these species are endemic, have they evolved as a result of separate colonization events or as a result of a single colonization followed by adaptive radiation (a scenario often considered typical for island evolution)?
- (3) Have present-day species in the Galapagos derived from continental lineages that originated in the north, i.e., Central America, or have they reached the archipelago following the shortest distance, i.e., directly from continental Ecuador, or else from southern South America?

To accomplish these objectives, we included in our analysis sampling from the archipelago, mainland South and Central America, and additional localities outside the Neotropics. Morphological and anatomical characters were examined in combination with our extensive phylogenetic datasets, using two nuclear markers of the rDNA cistron.

## Materials and methods

### Taxon sampling

Our present study focused on re-examining material collected as part of the Galapagos Lichen Inventory by Yáñez et al. (2012). Our specimens from the sampled islands are distributed as follows: Floreana (11), Isabela (20), Pinta (1), San Cristóbal (4), Santa Cruz (53), and Santiago (3), for a total of 92 specimens (Table 1). No specimens belonging to the *Dictyonema* clade were found on Española. We restricted our research to specimens for which we had molecular data available, and here include only abbreviated collection information. More detailed collection data can be found at the Charles Darwin Foundation Collections Database, available online at <http://www.darwinfoundation.org/datazone/collections/>.

To test our hypotheses, we augmented the Galapagos dataset with an additional 826 specimens belonging to the *Dictyonema* clade from 19 countries, mainly from the Neotropics (Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guatemala, Mexico, Peru, Puerto Rico, Venezuela), but also outside of Latin America (e.g., Fiji, Philippines, United States, among several others).

Information for all taxa not from the Galapagos is provided in Suppl. Tables 1 (concatenated dataset), 2 (*Cora*), 3 (*Cyphellostereum*), and 4 (*Dictyonema*). For *Acantholichen* this information has already been published by Dal-Forno et al. (2016a) and therefore it is not repeated here.

### Morphological and anatomical studies

Re-examination of the Galapagos specimens was performed using standardized protocols previously published by Lücking et al. (2013a). For the descriptions we also adopted a standardized format, which recognizes a distinct number of characters for species in each genus. Morphological measurements and observation are taken from dry, rehydrated and/or fresh specimens. Selected specimens were documented by photographs including a measuring scale. Regarding lobe dimensions, no differences were found in dry versus wet state. However, differences in color were notable and whenever possible documented in both states. Whenever applicable, differences in texture were recorded, as these can be substantially distinctive in the dry versus wet state. Finally, the marginal structure of the hymenophore often differs in both states: in some species the hymenophore patches become involute when dry.

Specimens were examined with a LEICA MS5, an OLYMPUS SZX12 and a ZEISS Stemi DV4 dissecting microscope, and a ZEISS Axioskop 2, an OLYMPUS BH-2 and ZEISS Axio Imager A1 compound microscope. Macrophotos were taken either in the laboratory or in the field with a SONY Alfa 33 DSLR, a CANON Powershot SX20IS, a NIKON F301, a NIKON D300, a Nikon D800E and a NIKON D7000, the latter three with 62 mm Nikkor Micro Lens and R1C1 macro flash; for images of herbarium specimens we used a Novoflex macro-table and for photographic magnifications higher than 1:1 an extension tube or Novoflex bellows, controlling the camera via ControlMyNikon v. 5.2 (<http://www.controlmynikon.com/>). For microphotos, the aforementioned microscopes were equipped with a DAGE MTI DC-330 3CCD, JENOPTIK ProgRes C3 and C5, a 1401KEM 10x Eyepiece 2.07 Megapixel PupilCam, or a NIKON DSLR phototube. Photos were processed with ADOBE Photoshop CS2 or CS5.1 or ADOBE Photoshop Elements 10.

Secondary metabolites were examined only for a few selected Galapagos specimens, and no substances could be detected. Dal-Forno et al. (2013) previously reported a complete absence of any acetone-soluble compounds.

### Molecular data, sequence alignment and phylogenetic analysis

DNA was extracted, amplified and sequenced according to Dal-Forno et al. (2013). When high quality sequences from

**Table 1** Specimens from Galapagos included in this study, including the island they came from

| Species                             | Collector                      | Island                   | Control#       | ITS GB#            | LSU GB#         |
|-------------------------------------|--------------------------------|--------------------------|----------------|--------------------|-----------------|
| <i>Acantholichen galapagoensis</i>  | F. Bungartz 5593               | Galapagos, Santa Cruz    | DIC064         | EU825953           | EU825953        |
| <i>Acantholichen galapagoensis</i>  | M. Dal-Forno 1204              | Galapagos, Santa Cruz    | MDF057         | KT429784           | KT429799        |
| <i>Acantholichen galapagoensis</i>  | M. Dal-Forno 1205 <sup>a</sup> | Galapagos, Santa Cruz    | MDF058         | KT429785           | KT429800        |
| <i>Acantholichen galapagoensis</i>  | A. Aptroot 64679               | Galapagos, Santa Cruz    | MDF089         | KT429786           | KT429801        |
| <i>Acantholichen galapagoensis</i>  | F. Bungartz 4125               | Galapagos, Isabela       | MDF090         | KT429787           | KT429802        |
| <i>Acantholichen galapagoensis</i>  | A. Aptroot 65187               | Galapagos, Isabela       | MDF091         | KT429788           | –               |
| <i>Acantholichen galapagoensis</i>  | A. Aptroot 65554               | Galapagos, Santiago      | MDF092         | KT429789           | –               |
| <i>Acantholichen galapagoensis</i>  | F. Nugra 400                   | Galapagos, Santa Cruz    | MDF093         | KT429790           | KT429803        |
| <i>Acantholichen galapagoensis</i>  | F. Nugra 379                   | Galapagos, Santa Cruz    | MDF094         | KT429791           | KT429804        |
| <i>Acantholichen galapagoensis</i>  | F. Bungartz 8152               | Galapagos, Santa Cruz    | MDF100         | KT429792           | KT429805        |
| <i>Acantholichen galapagoensis</i>  | F. Bungartz 8577               | Galapagos, San Cristóbal | MDF101         | KT429793           | –               |
| <i>Cora galapagoensis</i>           | A. Aptroot 65557               | Galapagos, Santiago      | DIC343         | KX772453           | KY861707        |
| <i>Cora galapagoensis</i>           | F. Bungartz 4831               | Galapagos, Santiago      | DIC345         | KX772454           | KY861709        |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1180 A            | Galapagos, Santa Cruz    | MDF033a        | KJ780545           | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1187 A            | Galapagos, Santa Cruz    | MDF040a        | KJ780546           | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1192              | Galapagos, Santa Cruz    | MDF045         | KJ780547           | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1196              | Galapagos, Santa Cruz    | MDF049         | KJ780548           | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1199 A            | Galapagos, Santa Cruz    | MDF052, MDF123 | KJ780549, KJ780559 | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1206              | Galapagos, Santa Cruz    | MDF059         | KJ780550           | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1218              | Galapagos, Santa Cruz    | MDF068         | KJ780551           | –               |
| <i>Cora galapagoensis</i>           | M. Dal-Forno 1223 <sup>a</sup> | Galapagos, Santa Cruz    | MDF073         | KJ780552           | –               |
| <i>Cora galapagoensis</i>           | A. Yáñez 1509                  | Galapagos, Santa Cruz    | MDF124         | KJ780560           | –               |
| <i>Cora galapagoensis</i>           | A. Yáñez 1508                  | Galapagos, Santa Cruz    | MDF139         | KJ780561           | –               |
| <i>Cora galapagoensis</i>           | A. Yáñez 1513                  | Galapagos, Santa Cruz    | MDF140         | KJ780562           | –               |
| <i>Cora galapagoensis</i>           | A. Yáñez 1538                  | Galapagos, Santa Cruz    | MDF142         | KJ780563           | –               |
| <i>Cora galapagoensis</i>           | A. Yáñez 1540                  | Galapagos, Santa Cruz    | MDF143         | KJ780564           | –               |
| <i>Cora galapagoensis</i>           | F. Nugra 437                   | Galapagos, Santa Cruz    | MDF147         | KJ780566           | –               |
| <i>Cora galapagoensis</i>           | F. Bungartz 3322               | Galapagos, Santa Cruz    | MDF148, MDF418 | KX772617, KJ780567 | –               |
| <i>Cora galapagoensis</i>           | M. Herrera-Campos 10546        | Galapagos, Isabela       | MDF149         | KJ780568           | –               |
| <i>Cora galapagoensis</i>           | F. Nugra 1034                  | Galapagos, Isabela       | MDF405         | KJ780623           | –               |
| <i>Cora galapagoensis</i>           | F. Nugra 1098                  | Galapagos, Isabela       | MDF406         | KJ780624           | –               |
| <i>Cora galapagoensis</i>           | F. Bungartz 10325              | Galapagos, Isabela       | MDF407         | KJ780625           | –               |
| <i>Cora santacruzensis</i>          | F. Bungartz 5594               | Galapagos, Santa Cruz    | DIC348         | KX772455           | <b>KY861711</b> |
| <i>Cora santacruzensis</i>          | A. Yáñez 1547 <sup>a</sup>     | Galapagos, Santa Cruz    | MDF144         | KJ780565           | –               |
| <i>Cyphellostereum galapagoense</i> | F. Bungartz 8517 <sup>a</sup>  | Galapagos, San Cristóbal | MDF120         | <b>KY861477</b>    | <b>KY861720</b> |
| <i>Cyphellostereum galapagoense</i> | A. Yáñez 1545                  | Galapagos, Santa Cruz    | MDF126         | <b>KY861478</b>    | –               |
| <i>Cyphellostereum unoquinoum</i>   | F. Bungartz 9475 <sup>a</sup>  | Galapagos, Floreana      | MDF176         | <b>KY861495</b>    | <b>KY861721</b> |
| <i>Dictyonema barbatum</i>          | F. Bungartz 8363 <sup>a</sup>  | Galapagos, Isabela       | DIC341         | <b>KY861430</b>    | <b>KY861705</b> |
| <i>Dictyonema barbatum</i>          | F. Bungartz 6852               | Galapagos, Isabela       | DIC342         | <b>KY861431</b>    | <b>KY861706</b> |
| <i>Dictyonema barbatum</i>          | F. Bungartz 8576               | Galapagos, San Cristóbal | DIC344         | <b>KY861432</b>    | <b>KY861708</b> |
| <i>Dictyonema barbatum</i>          | A. Aptroot 65186               | Galapagos, Isabela       | DIC346         | <b>KY861433</b>    | <b>KY861710</b> |
| <i>Dictyonema barbatum</i>          | C. Truong 1275                 | Galapagos, Isabela       | DIC349         | <b>KY861434</b>    | <b>KY861712</b> |

**Table 1** continued

| Species                         | Collector                      | Island                   | Control#       | ITS GB#                   | LSU GB#         |
|---------------------------------|--------------------------------|--------------------------|----------------|---------------------------|-----------------|
| <i>Dictyonema barbatum</i>      | F. Bungartz 6906               | Galapagos, Isabela       | DIC350         | <b>KY861435</b>           | <b>KY861713</b> |
| <i>Dictyonema barbatum</i>      | C. Truong 1259                 | Galapagos, Isabela       | MDF131         | <b>KY861479</b>           | –               |
| <i>Dictyonema barbatum</i>      | C. Truong 1533                 | Galapagos, San Cristóbal | MDF132         | <b>KY861480</b>           | –               |
| <i>Dictyonema barbatum</i>      | A. Aptroot 63148               | Galapagos, Santa Cruz    | MDF133         | <b>KY861481</b>           | –               |
| <i>Dictyonema barbatum</i>      | F. Bungartz 6849               | Galapagos, Isabela       | MDF136         | <b>KY861482</b>           | –               |
| <i>Dictyonema barbatum</i>      | A. Yáñez 1550                  | Galapagos, Santa Cruz    | MDF138         | <b>KY861483</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Herrera-Campos 10560        | Galapagos, Isabela       | DIC347         | <b>KY861401</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1171              | Galapagos, Santa Cruz    | MDF026         | <b>KY861459</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1174              | Galapagos, Santa Cruz    | MDF028         | <b>KY861460</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1177              | Galapagos, Santa Cruz    | MDF030         | <b>KY861461</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1178              | Galapagos, Santa Cruz    | MDF031         | <b>KY861462</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1179              | Galapagos, Santa Cruz    | MDF032         | <b>KY861463</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1182 A            | Galapagos, Santa Cruz    | MDF035         | <b>KY861465</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1191              | Galapagos, Santa Cruz    | MDF044         | <b>KY861466</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1208              | Galapagos, Santa Cruz    | MDF061         | <b>KY861467</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1209 <sup>a</sup> | Galapagos, Santa Cruz    | MDF062         | <b>KY861468</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1211              | Galapagos, Santa Cruz    | MDF064         | <b>KY861469</b>           | 1               |
| <i>Dictyonema darwinianum</i>   | A. A. Spielmann 8249           | Galapagos, Santa Cruz    | MDF076         | <b>KY861472</b>           | –               |
| <i>Dictyonema darwinianum</i>   | M. Dal-Forno 1183              | Galapagos, Santa Cruz    | MDF086         | <b>KY861475</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Yáñez 1828                  | Galapagos, Floreana      | MDF156         | <b>KY861484</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Yáñez 1842                  | Galapagos, Floreana      | MDF157         | <b>KY861485</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Yáñez 1541                  | Galapagos, Santa Cruz    | MDF159, MDF195 | <b>KY861486, KY861504</b> | –               |
| <i>Dictyonema darwinianum</i>   | A. Yáñez 1956                  | Galapagos, Floreana      | MDF160         | <b>KY861487</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Aptroot 64519               | Galapagos, Santa Cruz    | MDF168         | <b>KY861488</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Aptroot 65037 A             | Galapagos, Isabela       | MDF169         | <b>KY861489</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Bungartz 3276               | Galapagos, Santa Cruz    | MDF171         | <b>KY861490</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Bungartz 3956               | Galapagos, Santa Cruz    | MDF172         | <b>KY861491</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Bungartz 5746               | Galapagos, Pinta         | MDF173         | <b>KY861492</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Bungartz 6883               | Galapagos, Isabela       | MDF174         | <b>KY861493</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Bungartz 8350               | Galapagos, Isabela       | MDF175         | <b>KY861494</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Bungartz 9476               | Galapagos, Floreana      | MDF177         | <b>KY861496</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Yáñez 1507                  | Galapagos, Santa Cruz    | MDF184         | <b>KY861498</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. Yáñez 2062                  | Galapagos, Floreana      | MDF197         | <b>KY861506</b>           | –               |
| <i>Dictyonema darwinianum</i>   | F. Nugra 1051                  | Galapagos, Isabela       | MDF409         | <b>KY861556</b>           | –               |
| <i>Dictyonema darwinianum</i>   | A. A. Spielmann 10621          | Galapagos, Isabela       | MDF410         | <b>KY861557</b>           | <b>KY861726</b> |
| <i>Dictyonema darwinianum</i>   | F. Nugra 1096                  | Galapagos, Isabela       | MDF411         | <b>KY861558</b>           | –               |
| <i>Dictyonema pectinatum</i>    | M. Dal-Forno 1170 <sup>a</sup> | Galapagos, Santa Cruz    | MDF025         | <b>KY861458</b>           | <b>KY861716</b> |
| <i>Dictyonema pectinatum</i>    | M. Dal-Forno 1221              | Galapagos, Santa Cruz    | MDF071         | <b>KY861471</b>           | <b>KY861719</b> |
| <i>Dictyonema ramificans</i>    | M. Dal-Forno 1214 <sup>a</sup> | Galapagos, Santa Cruz    | MDF066         | <b>KY861470</b>           | <b>KY861718</b> |
| <i>Dictyonema ramificans</i>    | A. Yáñez 1517                  | Galapagos, Santa Cruz    | MDF187         | <b>KY861499</b>           | –               |
| <i>Dictyonema ramificans</i>    | A. Yáñez 1518                  | Galapagos, Santa Cruz    | MDF188         | <b>KY861500</b>           | –               |
| <i>Dictyonema ramificans</i>    | A. Yáñez 1521                  | Galapagos, Santa Cruz    | MDF190         | <b>KY861501</b>           | –               |
| <i>Dictyonema ramificans</i>    | A. Yáñez 1534                  | Galapagos, Santa Cruz    | MDF193         | <b>KY861502</b>           | <b>KY861723</b> |
| <i>Dictyonema ramificans</i>    | A. Yáñez 1539                  | Galapagos, Santa Cruz    | MDF194         | <b>KY861503</b>           | –               |
| <i>Dictyonema subobscuratum</i> | M. Dal-Forno 1181              | Galapagos, Santa Cruz    | MDF034         | <b>KY861464</b>           | –               |
| <i>Dictyonema subobscuratum</i> | F. Bungartz 9549 <sup>a</sup>  | Galapagos, Floreana      | MDF179         | <b>KY861497</b>           | <b>KY861722</b> |



**Table 1** continued

| Species                         | Collector                     | Island              | Control# | ITS GB#         | LSU GB#         |
|---------------------------------|-------------------------------|---------------------|----------|-----------------|-----------------|
| <i>Dictyonema subobscuratum</i> | F. Bungartz 9550 <sup>b</sup> | Galapagos, Floreana | MDF180   | –               | –               |
| <i>Dictyonema subobscuratum</i> | F. Bungartz 9551 <sup>b</sup> | Galapagos, Floreana | MDF181   | –               | –               |
| <i>Dictyonema subobscuratum</i> | F. Bungartz 9552 <sup>b</sup> | Galapagos, Floreana | MDF182   | –               | –               |
| <i>Dictyonema subobscuratum</i> | A. Yáñez 2058 A               | Galapagos, Floreana | MDF196   | <b>KY861505</b> | <b>KY861724</b> |

A few specimens were sequenced twice, and therefore more than one ITS was included. New sequences are in bold. Previously published sequences are from Lawrey et al. (2009) for GenBank numbers starting with EU, Dal-Forno et al. (2016a) for GenBank numbers starting with KT, Lücking et al. (2016) for GenBank numbers starting with KX, and Lücking et al. (2014a) for GenBank numbers starting with KJ

<sup>a</sup> Type specimens

<sup>b</sup> Specimens with only partial ITS, not included in the phylogenetic trees, but identified with molecular data

the internal transcribed spacer ribosomal RNA (ITS) could not be generated through Sanger sequencing, barcoded amplicon sequencing using the 454 platform was adopted (Lücking et al. 2014b).

Multiple subsets of data were generated for the phylogenetic analyses presented here: Subset 1 shows the general placement of the Galapagos species using two markers (ITS and nuclear large ribosomal subunit, nuLSU,  $N = 131$ ) while Subsets 2, 3 and 4 show the delimitation of several hundred species using ITS sequences only, the barcoding locus for Fungi (Schoch et al. 2012). Subset 2 includes 651 sequences from *Cora*, the largest clade in *Dictyonema* s.l. (plus two *Corella* outgroups); subset 3 depicts a phylogeny for which 57 sequences of *Cyphellostereum* were analyzed, and 222 sequences of *Dictyonema* s.str. were used to generate subset 4.

For the *Cora* dataset (subset 2), we used the tree file from Lücking et al. (2016), which followed the same specifications below. All sequences were processed either in Geneious 7.1.7 (<http://www.geneious.com>) or in BioEdit 7.09 (Hall 1999). Individual ITS and nuLSU fasta files, as well as the concatenated dataset, were subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al. 2010a, b), using MAFFT (Katoh and Toh 2010; Katoh et al. 2005) as the alignment option, with 100 bootstrap iterations to calculate the Guidance scores.

Alignments were subjected to ML search using RAxML 7.2.6 or 8.2.0 (Stamatakis 2006; Stamatakis et al. 2005), with nonparametric bootstrapping using 500 replicates under the GTRGAMMA model in the CIPRES Science Gateway V. 3.3 (Miller et al. 2010).

For the concatenated dataset, each gene region was first analyzed separately, and then combined after analysis for potential conflict in the individual gene trees (Mason-Gamer and Kellogg 1996). Since no conflict was detected, we combined the two loci and subjected the combined

dataset to maximum likelihood analysis (ML) as described above. The datasets were not partitioned for the individual or concatenated analyses.

All trees were visualized in FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>) and edited in ADOBE Illustrator CS5.1.

## Results

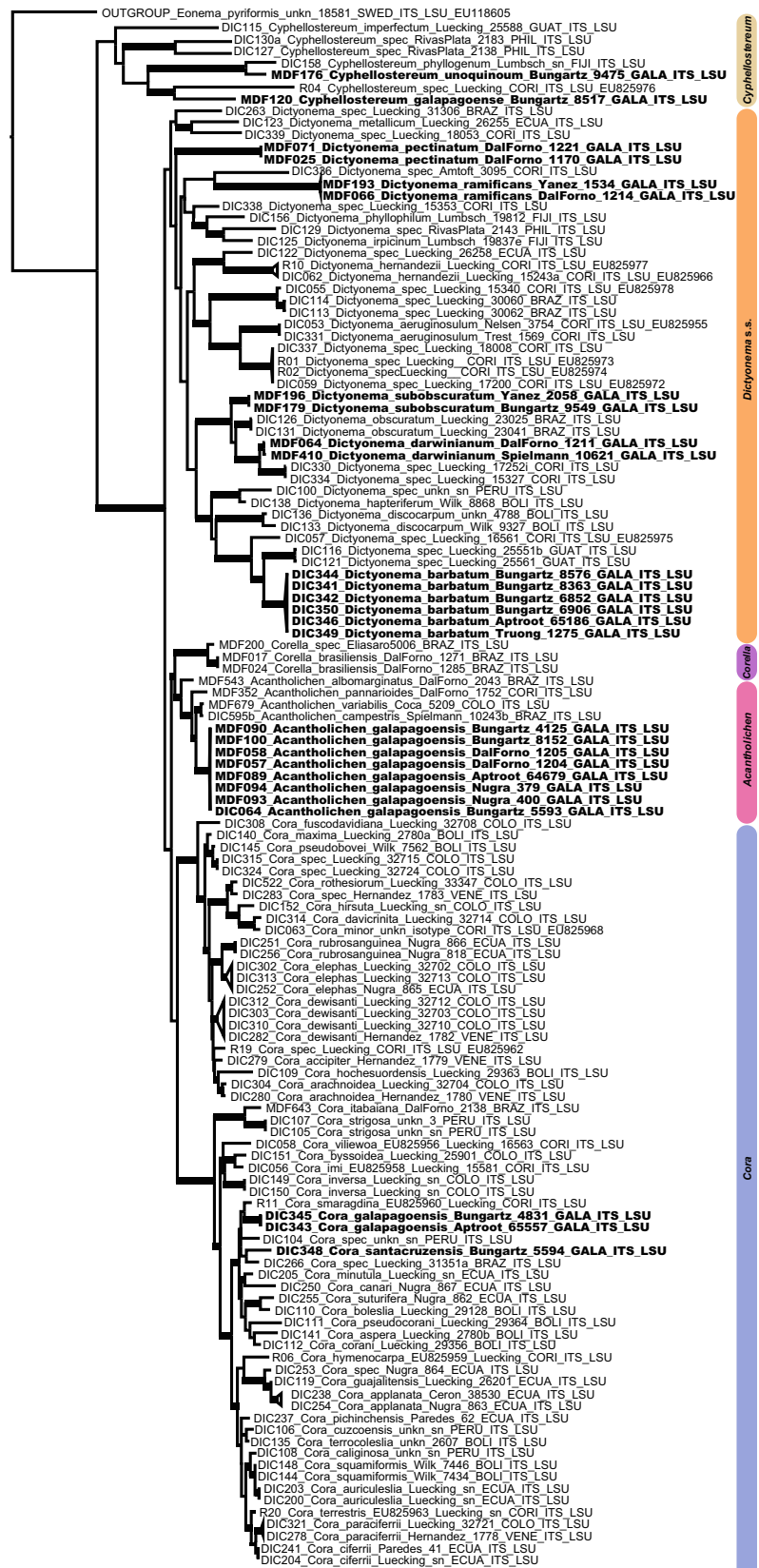
### Phylogenetic studies

The aligned concatenated dataset included 2368 base pairs, 934 of which correspond to the ITS and 1434 to the nuLSU. For the ITS trees, the alignment length was 852 bp for *Cora*, 911 bp for *Cyphellostereum*, and 1011 bp for *Dictyonema*. All alignments are available at TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S20812>).

For all four phylogenetic trees (combined all genera: Fig. 1; individual genus ITS: *Cora* Fig. 2 and Suppl. Fig. 1, *Cyphellostereum* Fig. 3, and *Dictyonema* Fig. 4 and Suppl. Fig. 2), well-supported clades indicate the presence of ten species of *Dictyonema* s.l. in Galapagos. Given these results, we conclude that all species of the target group in Galapagos are endemic.

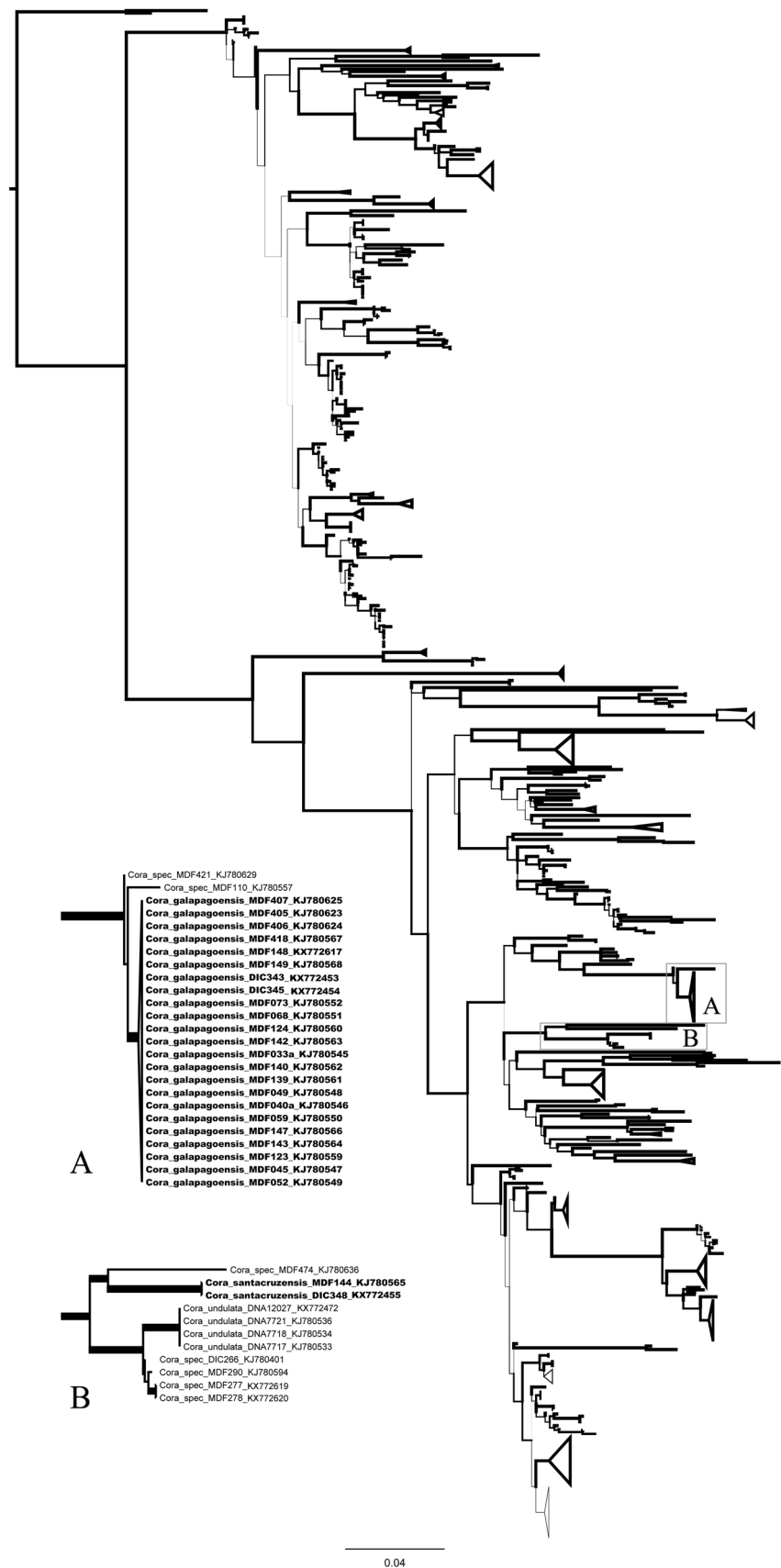
Out of the ten species, five (*Cora santacruzensis*, *Cyphellostereum galapagoense*, *Dictyonema ramificans*, *D. pectinatum*, and *D. subobscuratum*) have an Ecuadorian sister species and four (*Acantholichen galapagoensis*, *Cora galapagoensis*, *D. barbatum*, and *D. darwinianum*) have a broader continental context, which is strong evidence for colonization from mainland Ecuador in most cases. *Cyphellostereum unoquinoum* appears in all phylogenetic trees to be most closely related to material labeled *C. phyllogenum* from Fiji. In all cases, the

**Fig. 1** Phylogeny (ITS + nuLSU) of *Dictyonema* s.l. obtained under ML. Branches are thickened for all bootstrap (BS) values  $\geq 70$

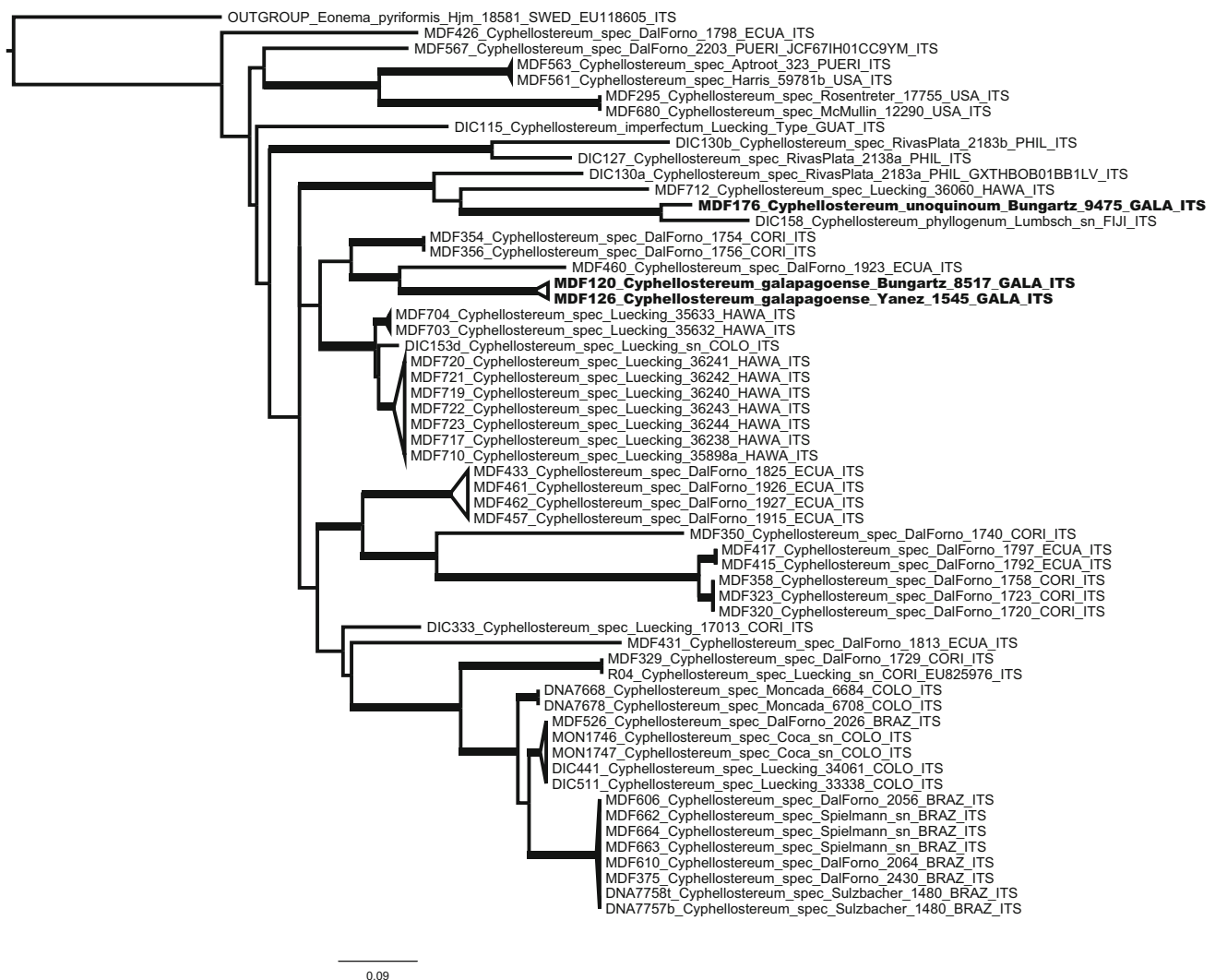


0.04

**Fig. 2** Phylogeny (ITS) of *Cora* obtained under ML. Modified from Lücking et al. (2016). Branches are widened according to bootstrap value in FigTree v1.4.2. Placement of Galapagos species are outlined. For full tree consult Suppl. Fig. 1. a, b—*Inlets* showing *Cora galapagoensis* and *C. santacruzensis* positions in the *Cora* tree, with branches thickened for all bootstrap (BS) values  $\geq 70$



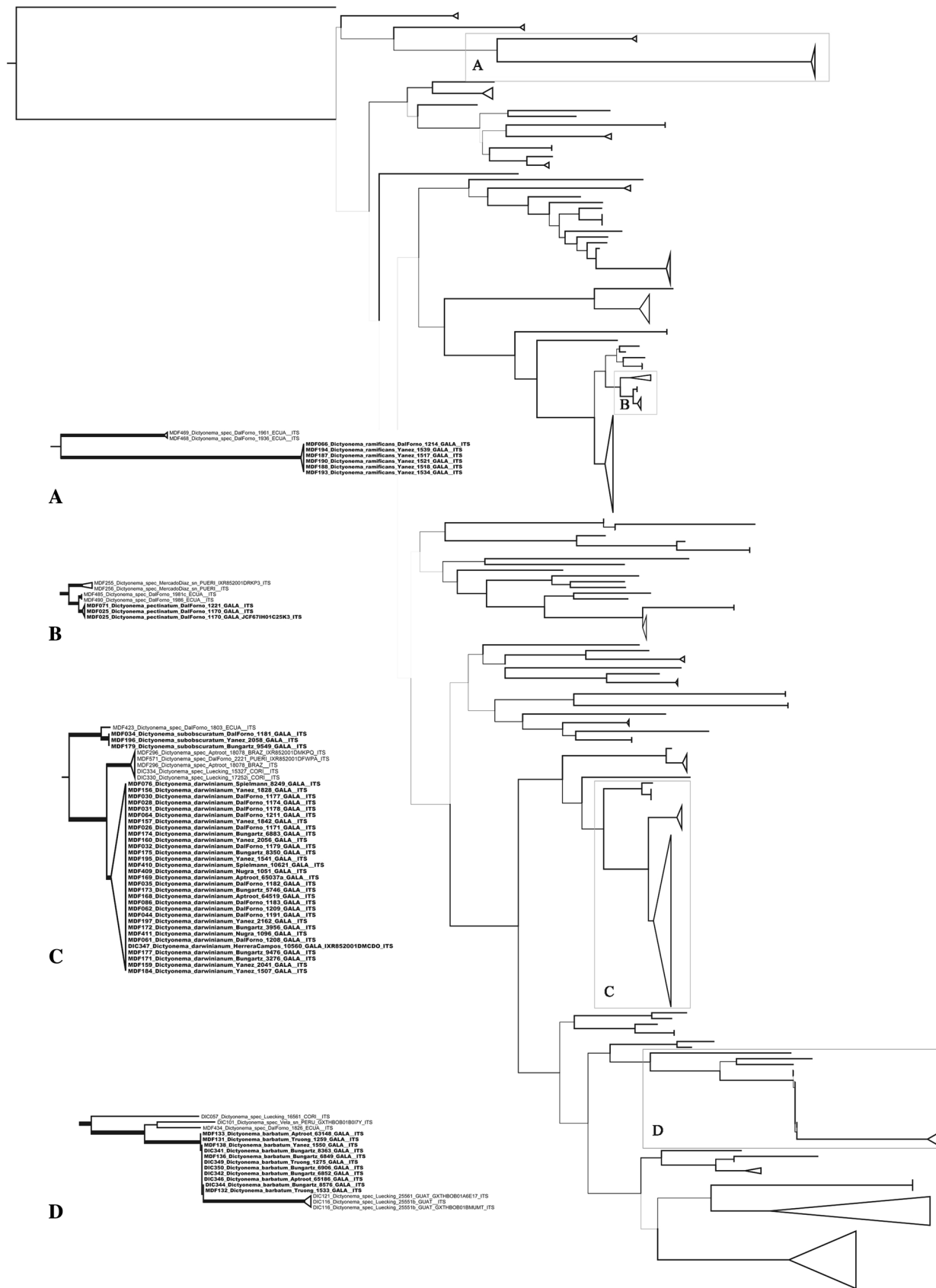


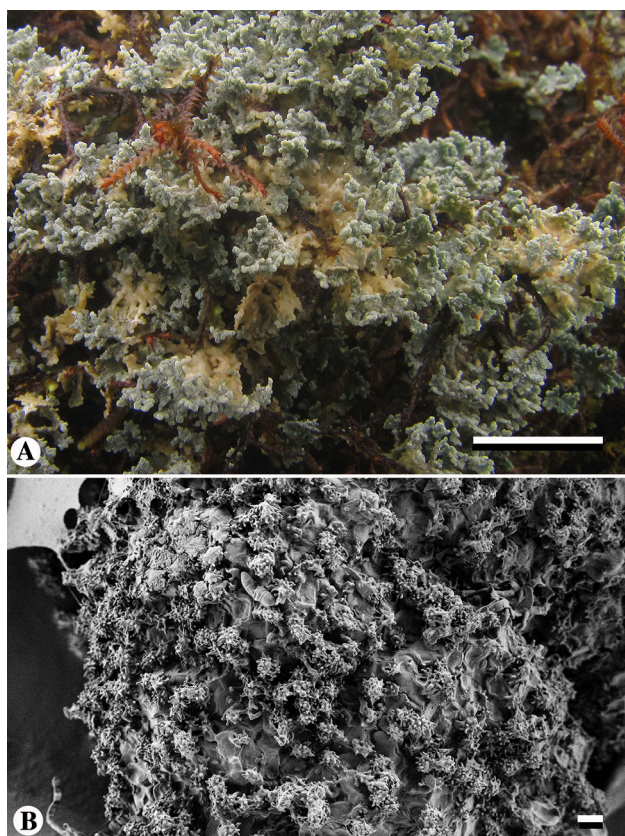


**Fig. 3** Phylogeny (ITS) of *Cyphellostereum* obtained under ML. Branches are thickened for all bootstrap (BS) values  $\geq 70$

Galapagos lineages are monophyletic and supported, even if the continental lineages appear close in the phylogeny. Since in the large *Cora* (Suppl. Fig. 1) and *Dictyonema* (Suppl. Fig. 2) ITS trees there are similar cases of allopatric or parapatric cryptic species, i.e., close sister species but each supported and from different geographic areas (Lücking et al. 2016), we consider all Galapagos lineages to be endemic.

**Fig. 4** Phylogeny (ITS) of *Dictyonema* obtained under ML. Branches are widened according to bootstrap value in FigTree v1.4.2. Placement of Galapagos species are outlined. For full tree consult Suppl. Fig. 2. **a–d**—Inlets showing *D. ramificans*, *D. pectinatum*, *D. subobscuratum*, *D. darwinianum*, and *D. barbatum* in the *Dictyonema* tree, with branches thickened for all bootstrap (BS) values  $\geq 70$





**Fig. 5** *Acantholichen galapagoensis* (holotype, M. Dal-Forno 1205). **a** Habit of the species (scale bar 1 cm); **b** SEM image showing thallus surface covered with acanthohyphidia (scale bar 10 μm). Photo **b** by Morgan Gostel

## Taxonomic studies

The taxonomic data are highly congruent with the molecular data, with all species having distinctive morphological and/or anatomical characters that are also monophyletic in phylogenetic reconstructions. These differences are presented in the resulting key below and in the accompanying species descriptions and notes.

## Key to genera and species of the *Dictyonema* clade in the Galapagos Islands

1. Thallus filamentous.....2  
Thallus squamulose or foliose.....8
2. Fibrils thin, cyanobacterial cells up to 11(–12) μm broad; hyphal sheath variable, with thin and long cells (*Cyphellostereum*)\*.....3  
Fibrils thicker, cyanobacterial cells broader than 11 μm, appearing vertically compressed; hyphal sheath made of jigsaw puzzle-shaped cells (*Dictyonema*).....4

\*Note: If the material has thin fibrils, resembling a *Cyphellostereum*, but it lacks a discernible fungal sheath, even though it may contain loosely associated hyphae, this possibly might be a free-living cyanobacterium or green algae. In Yáñez et al. (2012), we were considering this form to be a possible undescribed species of *Cyphellostereum*, but at moment we are rejecting the possibility of belonging to that genus.

3. Fibrils mostly erect, hyphal sheath composed of tightly packed cells with elongated jigsaw pattern, sheath completely covering the cyanobacterial filaments inside (similar to that of *Dictyonema*, but narrower overall) ..... *Cyphellostereum galapagoense*  
Fibrils not distinctly erect (thallus mostly horizontally orientated), hyphal sheath composed of sinuous cells, typically leaving at least a few spaces, thus not completely covering the fibrils inside .....  
.....*Cyphellostereum unoquinum*
4. Thallus forming semi-circular shelves.....  
..... *Dictyonema barbatum*  
Thallus not forming shelves but a more or less continuous mat growing closely attached to the substrate .....5
5. Thallus fibrils mostly horizontally arranged, appearing as if combed or in delicate appressed intricate-arachnoid pattern, with distinctive lighter hues towards the tips, microscopic papillae present.....  
..... *Dictyonema pectinatum*  
Thallus fibrils arranged irregularly, lighter tips absent or not easily detectable, microscopic papillae absent...  
.....6
6. Fibrils interconnected, especially towards their tips, forming vertical net-like structures, fibrils anatomically abundantly branched.....  
.....*Dictyonema ramificans*  
Fibrils not interconnected, independent, fibrils anatomically not or very rarely branched.....7
7. Cyanobacterial cells often longitudinally divided, hyphae from fungal sheath angular, not wavy.....  
.....*Dictyonema subobscuratum*  
Cyanobacterial cells rarely longitudinally divided, hyphae from fungal sheath wavy.....  
.....*Dictyonema darwinianum*
8. Thallus microsquamulose, with acanthohyphidia (spiny apical cells on both thallus surfaces, giving it a white-pruinose appearance).....  
.....*Acantholichen galapagoensis*\*\*  
Thallus foliose, without acanthohyphidia .....9
9. Lobes imbricate, with long conspicuous branching sutures, light to olive green when wet, forming up to 1 m large thalli, texture of the upper cortex roughened, resembling an elephant skin, very common throughout

the islands.....*Cora galapagoensis*  
 Lobes not imbricate, with short inconspicuous branching sutures, emerald-green when wet, forming small thalli, texture of the upper cortex more or less smooth, not resembling an elephant skin, uncommon.....*Cora santacruzensis*\*\*

\*\*For detailed descriptions of *Acantholichen galapagoensis* and of *Cora santacruzensis*, see Dal-Forno et al. (2016a) and Lücking et al. (2016), respectively.

## The species

*Acantholichen galapagoensis* Dal-Forno, Bungartz & Lücking, Mycologia 108 (1): 44 (2016) Fig. 5

**Distribution and ecology:** We found this species on several islands of the Galapagos, namely Isabela, San Cristóbal, Santa Cruz and Santiago. In general, specimens do not grow directly on trees or shrubs, but typically on epiphytic liverworts and mosses, on introduced (*Cinchona pubescens*, *Psidium guava*), native (*Zanthoxylon fagara*) and endemic trees (*Scalesia pedunculata*, *Psychotria* spp.).

**Remarks:** All *Acantholichen* specimens collected in Galapagos belong to a single, endemic species. In Galapagos this taxon is easy to recognize, because no other species observed there has the rather typical minute, microsquamulose and light blue-gray to olivaceous thallus.

No genetic differences were observed from material originated from different islands.

*Cora galapagoensis* Dal-Forno, Bungartz & Lücking sp. nov. Fig. 6

**Mycobank:** MB 820713 **GenBank:** KJ780552 (ITS)

**Diagnosis:** A small to mid-sized, epiphytic *Cora* with light green-grey to olive sutured lobes with undulate surface and more or less concentrically arranged hymenophore patches. Endemic to Galapagos.

**Type:** Ecuador: Galapagos: Santa Cruz Island, along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park, 0°39'56"S, 98°19'31"W, alt. 502 m, *Miconia robinsoniana* shrubland, on bryophytes, growing on *Frullania* sp., 23-Jun-2010, M. Dal-Forno 1223 (CDS 44748, holotype; GMUF, isotype).

**Description:** Thallus epiphytic, growing on bryophytes over branches and trunks, with other lichens and bryophytes, foliose, parallel to the substrate when on branches to completely perpendicular to the substrate when on trunks; lobes 0.5–1.5 (–2) cm wide (delimited by sutures) and 1–3 cm long, densely branched, mostly white to light grey when found dry in nature, grey, light green or olive when wet, to bluish green to grey when rehydrated (4.5 years after

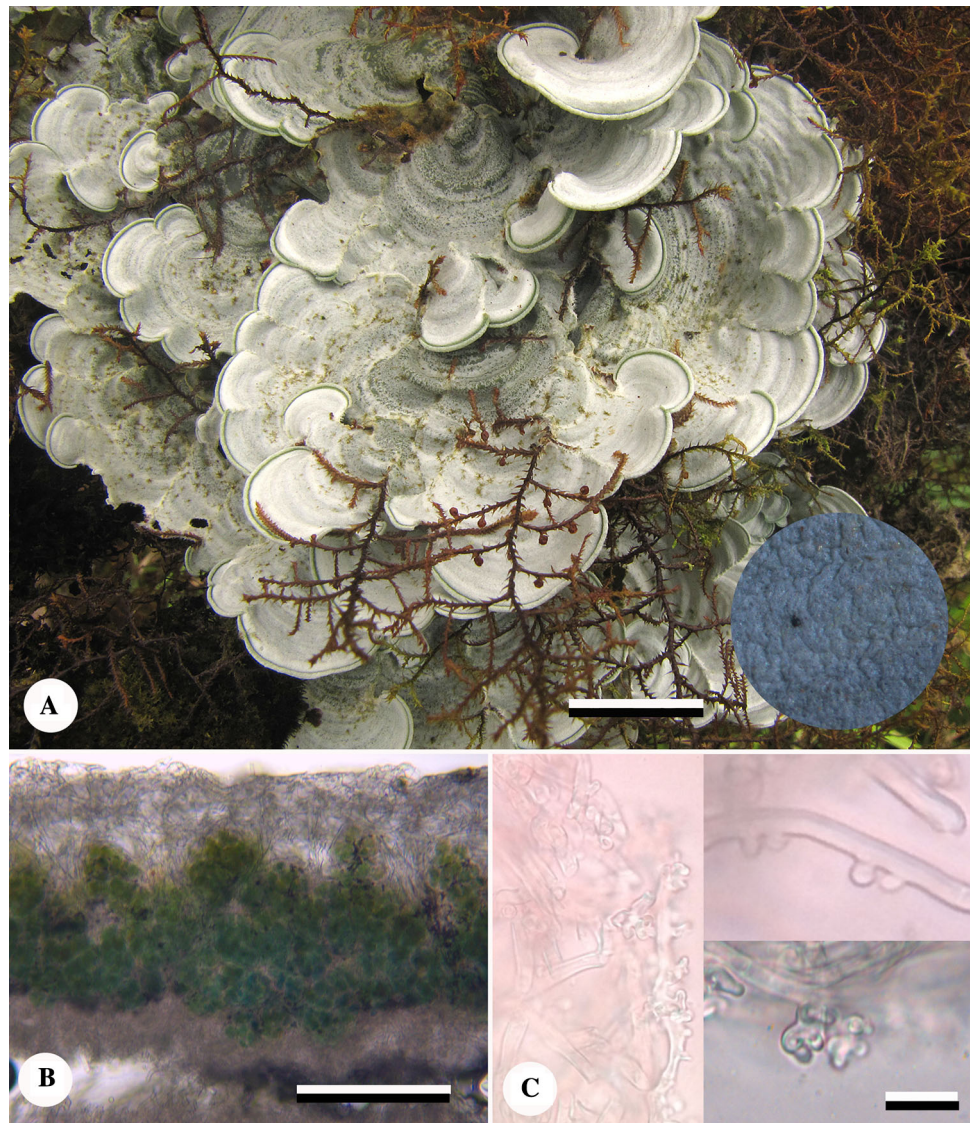
collecting), becoming white to light grey in the herbarium (similar to when found dry in nature), with concolorous margins, thin with a papery texture. Upper surface glabrous, roughened (like “elephant skin”), with pronounced and shallow ridges, 3–5 per cm<sup>2</sup> and 10–12 per cm<sup>2</sup>, respectively; lower surface ecorticate, mostly glabrous, rarely with few hairs where the thallus attaches to its substrate or along sutures, hairs white when fresh, not usually darkening with storage in the herbarium. Margins involute, indistinct, thin. Thallus in section 220–320 (–360) µm thick, with distinct upper cortex, photobiont layer, and medulla; upper cortex viaduct-shaped formed by a 10–25 µm thick layer of densely packed, periclinial, 4–6 µm thick hyphae supported by a 50–105 µm high ‘medullary’ layer of spaced groups of irregularly arranged to anticlinal, 4–6 µm thick hyphae; photobiont layer 50–160 (–200) µm thick, irregular, composed of clusters of short, coiled cyanobacterial filaments wrapped in a dense hyphal sheath formed by jigsaw puzzle-shaped cells, clusters 15–35 µm diam., individual photobiont cells 5–8 µm broad and 7–12 µm long, blue-green, penetrated by tubular fungal hyphae; heterocytes sparse, pale yellow, 5–7 µm diam; cells of hyphal sheath wavy in lateral outline, 5–6 µm thick; medulla 25–50 (–70) µm thick, composed of loosely woven, irregularly arranged to more or less periclinial hyphae 3–5 µm thick; clamp connections absent but lower medullary hyphae with numerous small papilliform, unbranched to branched hyphae, 3–5 µm thick.

Hymenophore developed as linear to reticulate, large, steroid patches dispersed on the underside, patches 0.5–2 mm long and 0.5–3 mm broad, pale yellow, smooth surface and strongly involute, smooth margins; hymenophore in section 80–100 µm thick, composed of a paraplectenchymatous layer resting on loose, 4–6 µm thick, generative medullary hyphae and supporting the hymenium; hymenium composed of numerous, palisade-like basidioles and scattered basidia; basidioles 10–15 × 5–6 µm; basidia 20–30 × 6–8 µm, basidiospores ellipsoid to fusiform, non-septate, hyaline, 5–7 × 4–7 µm.

**Distribution and ecology:** This species is known from multiple collections in Galapagos, from three islands lichenologists have visited: Isabela, Santa Cruz and Santiago. It is a very common basidiolichen in the archipelago. There are several habitats where this species grows, such as in endemic *Miconia* shrublands, secondary forest of introduced *Cinchona pubescens* and *Psidium guajava*, tall forest of *Persea americana*, and forests of the endemic *Scalesia pedunculata*, among others. Most specimens, unless stated otherwise below, are growing on bryophytes over branches and tree trunks.



**Fig. 6** *Cora galapagoensis* (multiple specimens). **a** Habit of the species (M. Dal-Forno 1180a, scale bar 2 cm), inset showing the texture of the upper surface (elephant skin). **b** Cross section showing the thallus layers, note the loose hyphae from the cortex (F. Nugra 1034, scale bar 100  $\mu$ m). **c** Montage showing the different hyphae “sprouting”, multiple specimens (scale 6  $\mu$ m)



**Etymology:** The epithet refers to the whole archipelago, since this is the most common *Cora* found across the islands.

**Remarks:** This is a new, yet well-known species found across the Galapagos Islands in the genus *Cora*. It is not the only *Cora* species on the islands, but the one most common and widespread. It is very easily recognized by its light grey to almost white color when dry, growing mostly on bryophytes over trees. This species can form up to 1 m broad colonies, and its most recognizable characteristic are the imbricate lobes (closely adjoining, fused lobes), which appear as if “sewn together” by long sutures. Among the many neotropical species sampled by us, *C. galapagoensis* is most closely related to two, yet undescribed species from Brazil (MDF110, M. Dal-Forno 1267) and Ecuador (MDF421, M. Dal-Forno 1800), which differ morphologically by not having the characteristic imbricate lobes. The

species was previously identified as *Cora glabrata* (Yáñez et al. 2012; Bungartz et al. 2013c), *Cora pavonia* (Dodge 1935), and *Dictyonema montanum* (Weber 1986), the latter being a homotypic synonym of *Cora pavonia*. However, these names (*C. glabrata* and *C. pavonia*) apply to species probably endemic to the Caribbean Islands (Lücking et al. 2014a).

According to Lücking et al. (2016), the following key characters are important and may be included in the table presented there: substrate: epiphytic; lobe width: small-medium (to 2 cm); soredia: absent; surface trichomes: absent; sutures: long; hymenophore: concentric; color: olive; papillae: present; cortex: viaduct; surface: undulated; margin trichomes: absent; distribution: Galapagos Islands; habitat: montane forest; species: *C. galapagoensis*. *Cora santacruzensis* agrees with *C. galapagoensis* in most of these characters, but forms small

thalli with only short to indistinct branching sutures, has an emerald-green color when wet and produces a diffusely viaduct-shaped upper cortex, giving it a more smooth surface (Lücking et al. 2016). *Cora galapagoensis* by comparison has a viaduct-shaped upper cortex, which resembles an “elephant skin” under a stereoscope.

**Additional material examined** (20): Ecuador: Galapagos: Santiago Island (2), along the trail from Cerro Gavilán to La Central, alt. 890 m, on soil, 24-Mar-2006, *F. Bungartz* 4831 (CDS 29005, GMUF); near permanent plot # 11 Pampa, alt. 870 m, on soil, 24-Mar-2006, *A. Aptroot* 65557 (CDS 32145, GMUF). -Isabela Island (4), Volcán Sierra Negra, alt. 580 m, on bark, 14-Aug-2008, Herrera-Campos 10546 (CDS 40282, GMUF); Volcán Cerro Azul, alt. 456 m, 3-May-2012, *F. Nugra* 1034 (CDS 52198, GMUF), alt. 655 m, on rock, 7-May-2012, *F. Nugra* 1098 (CDS 52261, GMUF), alt. 767 m., 3-May-2012, *F. Bungartz* 10325 (CDS 52298, GMUF). —Santa Cruz Island (14), along trail from Bellavista to El Puntudo, alt. 469 m, 23-June-2010, *M. Dal-Forno* 1180a (CDS 44714, GMUF), alt. 502 m, 23-June-2010, *M. Dal-Forno* 1187A (CDS 47764, GMUF), *M. Dal-Forno* 1192 (GMUF), *M. Dal-Forno* 1196 (CDS 44728, GMUF), *M. Dal-Forno* 1206 (GMUF), *M. Dal-Forno* 1218 (CDS 44741), alt. 684 m, *M. Dal-Forno* 1199a (CDS 44752, GMUF); N of Bellavista, alt. 555 m, 28-Oct-2010, *A. Yáñez-Ayabaca* 1508 (CDS 44999), *A. Yáñez-Ayabaca* 1509 (CDS 45000), *A. Yáñez-Ayabaca* 1513 (CDS 45004); below El Puntudo, alt. 762 m, 28-Oct-2010, growing over *Cladonia confusa* on the ground, *A. Yáñez-Ayabaca* 1538 (CDS 45031), growing over *Campylopus anderssonii* on front of boulder, *A. Yáñez-Ayabaca* 1540 (CDS 45033); vía Media Luna, lindero del del Parque Nacional Galapagos, alt. 500 m, 23-Aug-2007, *F. Nugra* 437 (CDS 36189); on the north-western fork of the way from the parking lot to Caseta, near Media Luna, alt. 600 m, on rock, 28-Dec-2005, *F. Bungartz* 3322 (CDS 26988).

***Cora santacruzensis*** Dal Forno, Bungartz & Yáñez-Ayabaca, Fungal Diversity Online First: 56 (2016) Fig. 7

**Distribution and ecology:** This species is known from a couple of specimens in what seems to be a single population in Santa Cruz. The unusual habitat around an old farm with a plantation of many invasive species (for example: avocado trees, yucca, pineapple), and its restricted distribution makes us wonder if this species is truly endemic or if it might be a species introduced along with the introduced plants. Nonetheless, the site is rather unique in Galapagos. The area is characterized by some of the largest, oldest *Scalesia* trees in the archipelago, today only a vegetation fragment of an ancient forest, characterized by an unusually high diversity of lichens, including many rare species. Therefore it seems equally possible that *C.*

*santacruzensis* is an endemic relict of these *Scalesia* forest relicts. We cannot reject either hypothesis since the type specimen was growing on an avocado tree, but the other specimen grows on the endemic guava (*Psidium galapageium*) in a forest of *Scalesia pedunculata*, another plant endemic to the Galapagos.

**Remarks:** This is another, much rarer species of *Cora* that can be found in the Galapagos. It has no unique characteristic when compared to other species of *Cora*; however, it can be differentiated from *C. galapagoensis* by its darker thallus in the herbarium and the absence of imbricate lobes with long branching sutures, among other characters (see above). In addition, *C. santacruzensis* is so far known from a single population in Santa Cruz, located north of the collection sites of *Cora galapagoensis* in this area.

In addition to the two species of *Cora* for which we have morphological, anatomical, ecological and molecular data, another specimen, quite different in appearance, has been collected, so far only once. This specimen grows directly on rock (*F. Bungartz* 3983, CDS 27913). Though it had been filed at the herbarium as *Cora glabrata* (Yáñez et al. 2012), the sample has unfortunately not been included in our molecular analysis. However, now with a better understanding of the ecological characteristics of individual species in this genus, we believe the specimen might belong to a third, so far undescribed species occurring in the islands. The lichen grows on exposed basaltic rocks, at a geological distinct site, where giant lava bubbles, some collapsed, form a thin rim of basaltic rock upon which thalli of this *Cora* grow abundantly. Using molecular markers, we look forward to testing our hypothesis if the specimen indeed belongs to a third new species.

***Cyphellostereum galapagoense*** (Yáñez, Dal Forno & Bungartz) Dal-Forno, Bungartz & Lücking, comb. nov. Fig. 8  
= *Dictyonema galapagoense* Yáñez, Dal Forno & Bungartz, Fungal Diversity 52: 234 (2012)

**Mycobank:** MB 820714 **GenBank:** KY861477 (ITS)

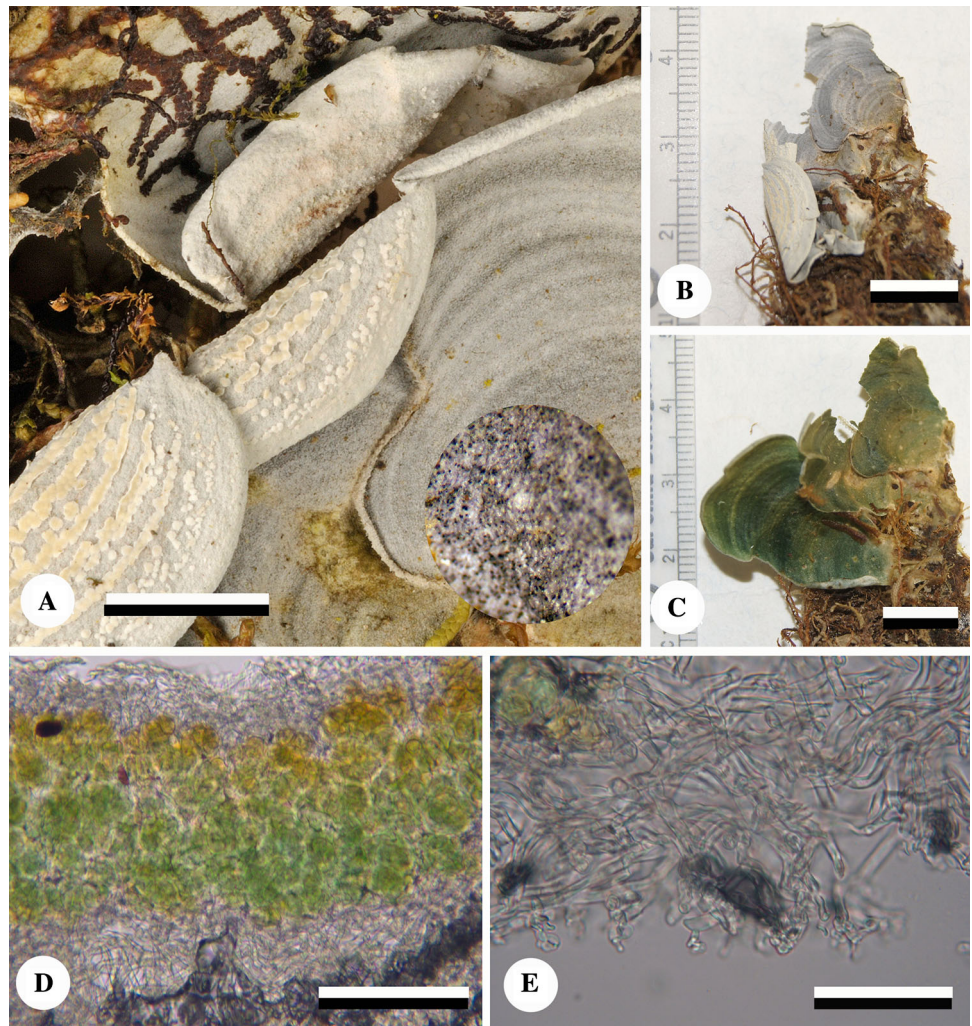
**Type:** Ecuador: Galapagos: San Crisóbal Island, trail from Cerro Pelado to El Ripioso, 0°51'41"S, 89°27'39"W, alt. 392 m, *Psidium guajava* forest with some old *Hippomane mancinella* trees and dense understory of *Rubus niveus*, *Tournefortia rufo-sericea* and *Zanthoxylum fagara*, on bryophytes, growing over mosses on bark of *Hippomane mancinella*, upper side of inclined branch (ca. 20 cm in diam.), SW-exposed; semi-shaded, wind- and rain-sheltered, 23-Aug-2008, *F. Bungartz* 8517 (CDS 41163 holotype, GMUF isotype)

**Description:** Thallus growing on and among bryophytes, epiphytic on tree branches, filamentous, in a more or less confluent patch of irregularly erect, blue green fibrils closely interwoven, forming a dense mat. Prothallus absent; hypothallus rarely present, formed by a thin layer of white



**Fig. 7** *Cora santacruzensis* (all A. Yáñez-Ayabaca 1547).

**a** Close up showing upper and lower sides, the later with hymenophores, *inlet* showing the texture of the smooth upper surface (scale bar 1 cm). **b** Isotype dry (scale bar 1 cm). **c** Isotype wetted in the laboratory after 4 years of collecting (scale bar 1 cm). **d** Cross section showing the layers, note the thinner cortex (scale bar 100  $\mu$ m). **e** Medullar hyphae “sprouting” (scale bar = 25  $\mu$ m)



hyphae, not easily visible. Thallus lacking discernible layers; photobiont composed of numerous *Rhizonema* cyanobacterial filaments wrapped in an closed hyphal sheath; cyanobacterial filaments composed of 7–10 (–12)  $\mu$ m wide and 5–10  $\mu$ m high, uniseriate, green cells, mostly square to elongate-cylindrical, in chains, penetrated by tubular fungal hyphae; heterocytes frequent, pale yellow, 7–10  $\mu$ m wide and 5–10  $\mu$ m high; cells of hyphal sheath with jigsaw pattern, somehow resembling those of *Dictyonema*, but thinner and less sinuous on the margins, 2.5–3.5  $\mu$ m thick; with some occasional 3  $\mu$ m thick hyaline additional hyphae associated, lacking clamp connections. Hymenophores not observed.

**Distribution and ecology:** This species is known now from an additional specimen from Santa Cruz; the type is from San Cristóbal. Both grow in humid environments, as do all basidiolichens in the archipelago.

**Remarks:** Aside from sharing characteristics with the genus *Dictyonema*, such as the similar pattern of hyphae composing the fungal sheath around the cyanobacteria and haustoria, the reason that led us to originally describe it in

that genus, molecular data indicates that this species belongs to *Cyphellostereum*. As a typical *Cyphellostereum*, the cyanobacterial cells are mostly square in shape, and much narrower, usually 10  $\mu$ m broad or less, rarely up to 12  $\mu$ m broad. Both specimens of this species show a rather unique growth habit with fibrils forming a filamentous mat that also proliferates vertically, giving an erect-suberect aspect to the thallus. Anatomically, these erected compacted fibrils resemble hairs of *Cora* species.

**Additional material examined** (1): Ecuador: Galapagos: Santa Cruz Island, abandoned farm behind El Puntudo, alt. 729 m, on bryophyte, 28-Oct-2010, A. Yáñez-Ayabaca 1545 (CDS 45039, GMUF).

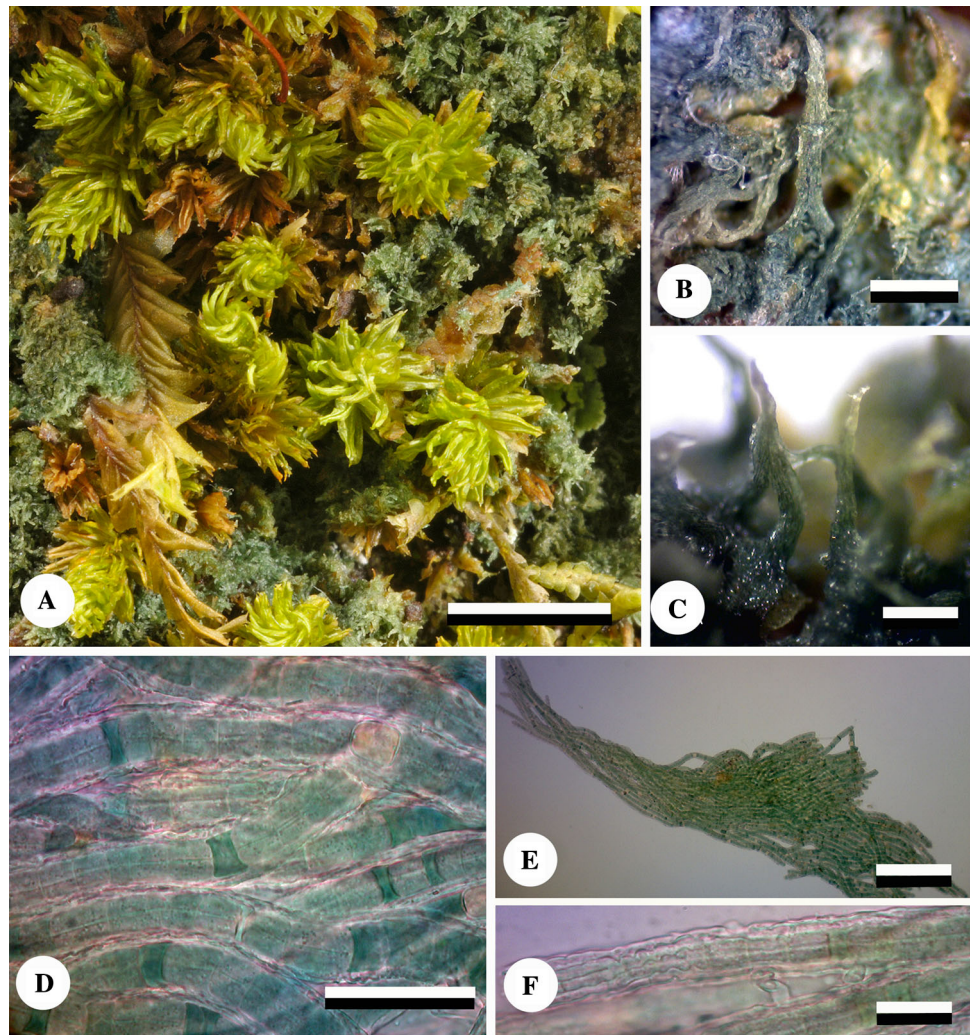
***Cyphellostereum unoquinoum*** Dal-Forno, Bungartz & Lücking sp. nov. Fig. 9

*Mycobank*: MB 820715 *GenBank*: KY861495 (ITS)

**Diagnosis:** Differing from *Cyphellostereum galapagoense* in the lower density of fibrils and by the green hue. The fungal sheath in this species is distinctive in that it does not completely surround the cyanobacteria.



**Fig. 8** *Cyphellostereum galapagoense* (all F. Bungartz 8517). **a** Habit of the species (scale bar 5 mm). **b**, **c** Dry and wetted specimen, respectively, showing fibrils condensed (scale bar 0.5 mm). **d** Arrangement of the fibrils 40 × (scale bar 60 μm). **e** Arrangement of the fibrils 10 × (scale bar 200 μm). **f** Close up showing fungal sheath (scale bar 20 μm)



**Type:** Ecuador: Galapagos: Floreana Island, on top of Cerro Asilo de la Paz, permanent plot 25, 1°19'1.7"S, 90°27'7.5"W, 531 m, humid zone, mixed dense *Scalesia pedunculata* forest with trees of *Zanthoxylum fagara* and *Psidium guajava*, *Tournefortia rufo-sericea* and *Lantana camara* in shrub layer; trees and shrubs covered with large curtains of bryophytes, growing on bryophytes over bark of branches of *Psidium guajava*; shaded, wind- and rain-sheltered, 13-Jan-2011, F. Bungartz 9475 (CDS 46556, isotype GMUF).

**Description:** Thallus growing on and among bryophytes, epiphytic on tree branches, loose filamentous, in a confluent patch, forming a loose mat of irregular and more or less individual to slightly interwoven, green fibrils. Prothallus and hypothallus absent. Thallus lacking discernible layers; photobiont composed of numerous *Rhizonema* cyanobacterial filaments wrapped in an almost entirely closed hyphal sheath formed by sinuous cells; cyanobacterial filaments composed of 7.5–11 μm wide and 5–7 μm high, green cells; heterocytes frequent, pale to bright yellow, 7–10 μm wide and 5–7.5 μm

high; cells of hyphal sheath sinuous and irregular, 2–3 (–4) μm thick; no additional hyphae associated, lacking clamp connections. Hymenophores not observed.

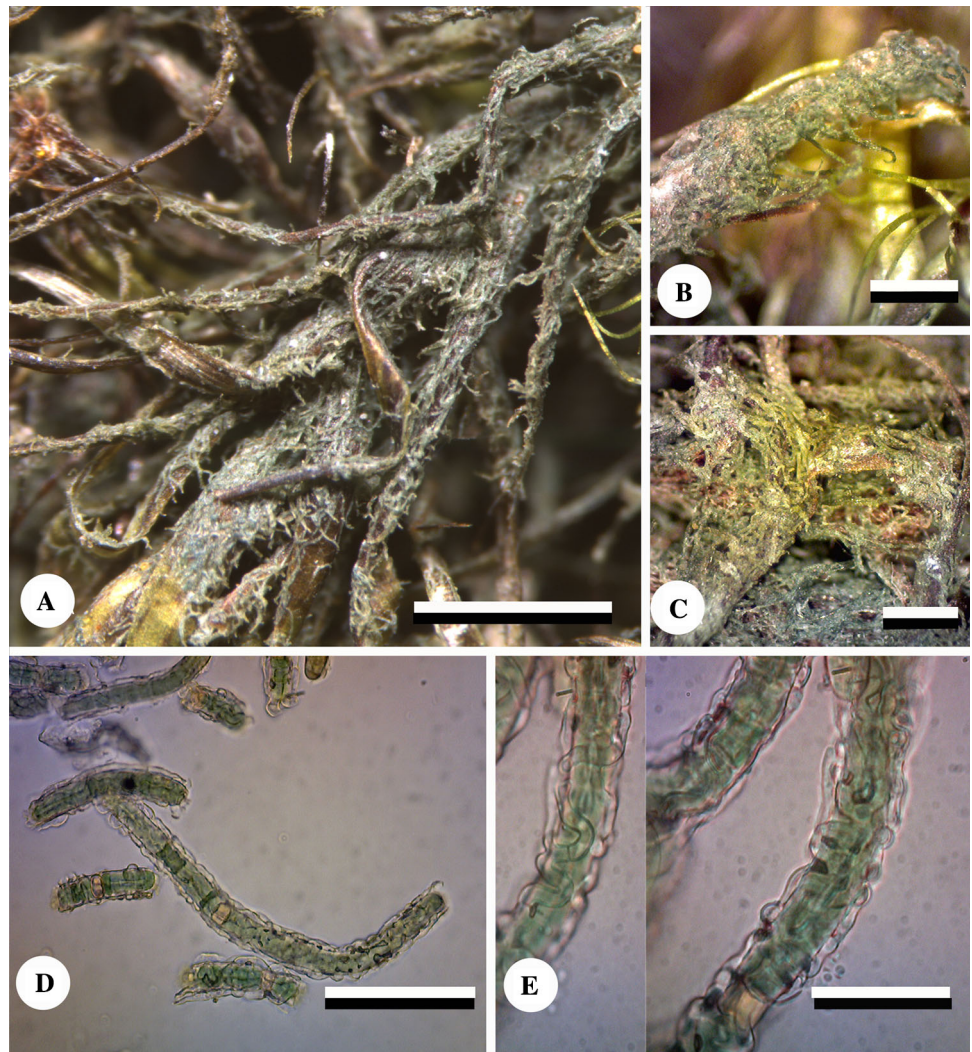
**Distribution and ecology:** This species is known from a single collection from the humid zone in Floreana Island.

**Etymology:** Named in honor of the Galapagos National Park, commemorating the year 1959 when the National Park was founded (*u-num* = 1, *no-vem* = 9, *quinque* = 5, *no-vem* = 9). Without their continued support, the discovery of an enormous lichen biodiversity in this unique archipelago would not be possible.

**Remarks:** The material had been previously identified by us with the name *D. galapagoense* (= *Cyphellostereum galapagoense*) in Bungartz et al. (2013c), but it actually represents a different taxon, not previously recognized. Apart from its phylogeny, the differences lay mainly in the much denser fibrils and a more bluish hue of *C. galapagoense* in comparison to this new species. In addition, *C. unoquinoum* does not present the somewhat erect arrangement of the bundles of fibrils found in *C.*



**Fig. 9** *Cyphellostereum unoquinoum* (holotype, F. Bungartz 9475). **a** Habit of the species, growing on bryophytes (scale bar 1 mm). **b, c** Close up of the thallus, showing fibrils loosely attached and not very dense (scale bar 1 mm). **d** Fibrils general appearance  $40\times$  (scale bar  $60\ \mu\text{m}$ ). **e** Fibrils showing the fungal sheath around the cyanobacteria, note that despite dense, fungal sheath do not completely cover the cyanobacterial cells  $100\times$  (scale bar  $40\ \mu\text{m}$ )



*galapagoense*. *Cyphellostereum unoquinoum* is more closely related to, and morphologically similar to, *C. phyllogenum* (based on material from Fiji).

In our previous paper (Yáñez et al. 2012), we included a possible additional species of *Cyphellostereum*, referred as *Cyphellostereum* sp. A couple of specimens under this identification were revised and one (A. Yáñez-Ayabaca 1545) is now included in *Cyphellostereum galapagoense*, while others (M. Dal-Forno 1180b and 1190) were not successfully amplified with regular PCR with the ITS or nuLSU primers commonly used. We also performed PCR with multitag pyrosequencing (MTPS) primers for ITS1F and ITS2 and despite effective amplification, the fungal reads came up as uncultured fungi in the Helotiales (Ascomycota) in BLAST (Altschul et al. 1990). We also amplified 16S, and the bacteria we amplified did not belong to *Rhizonema* or any other group of cyanobacteria based on searches in the RDP10 database (Cole et al. 2014). We therefore

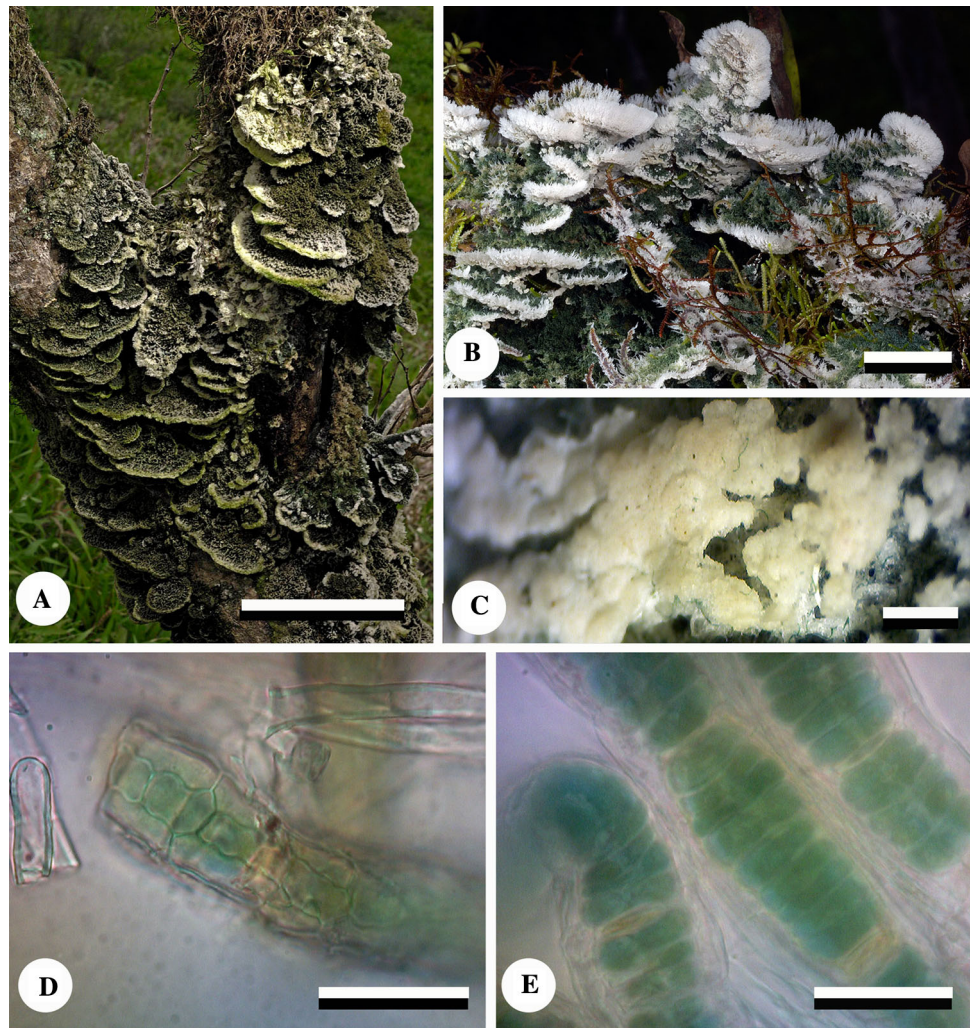
believe that this taxon needs to be further sampled and analyzed to establish its identity. Possible reasons for our identification failure are due to the size of these samples, which are less than 5 mm, and the fact that they are indiscernible in the field, usually only found in the laboratory under a stereoscope (for figures, see Yáñez et al. 2012, Fig. 1g–h). Nonetheless, we are rejecting the idea of these specimens belong to *Cyphellostereum* or any genera in the *Dictyonema* clade.

We are also now rejecting the presence of *Cyphellostereum imperfectum*, a species from Guatemala included in our previous assessment of the island basidiolichens (Yáñez et al. 2012). We believe that the intermixed filaments of *Cyphellostereum* found with *Dictyonema pectinatum*, originally identified as *C. imperfectum*, may potentially be one of the two species now confirmed with molecular data to be endemic of the archipelago. However, they have not been observed again and therefore cannot be further identified.



**Fig. 10** *Dictyonema barbatum* (multiple specimens).

**a** Specimen growing conspicuously in Isabela (F. Bungartz 4127, scale bar = 15 cm). **b** General aspect of shelves of the species (F. Bungartz 6906, scale bar 3 cm). **c** Close up showing the white to beige continuous hymenophore (C. Truong 1259, scale bar 1 mm). **d** Loose hyphae and fungal sheath hyphae, note the different shapes 100 × (F. Bungartz 6849, scale bar = 30 μm). **e** Cyanobacterial cells involved in fungal sheath 100 × (F. Bungartz 6849, scale bar 25 μm)



***Dictyonema barbatum*** Dal-Forno, Bungartz & Lücking sp. nov. Fig. 10

*Mycobank*: MB 820716 *GenBank*: KY861430 (ITS)

**Diagnosis:** Characterized by semicircular, shelf-like, filamentous lobes similar to *Dictyonema sericeum*, but forming mostly continuous hymenophores.

**Type:** Ecuador, Galapagos, Isabela, Volcán Sierra Negra, close to the southern crater rim, along the trail to Alemania, 0°51'12"S, 91°8'40.5"W, alt. 1055 m, pampa of *Pteridium arachnoideum*, *Pernettya howellii*, *Lycopodium* sp., and with occasional tree ferns (*Cyathaea weatherbyana*) and *Psidium guajava* shrubs, on bark, branch of *Psidium guajava*; semi-shaded, wind- and rain-sheltered, 16-Aug-2008, F. Bungartz 8363 (CDS 41009, holotype; GMUF, isotype).

**Description:** Thallus epiphytic on bark of branches and trunks, shelf-like filamentous, up to 60 cm across, with single lobes up to 8 cm wide, composed of loosely interwoven, dark green to bluish green fibrils leaving interspaces and bordered by a broad, irregularly interwoven, white to pale beige margin (prothallus). Fibrils arranged

more or less horizontally, but also vertically. Thallus in section from 0.8 up to 5 mm thick, composed of a thick photobiont layer and a thick medulla forming a white hypothallus, this latter structure also with some green fibrils (with photobiont) on the underside; photobiont layer composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed by jigsaw puzzle-shaped cells; cyanobacterial filaments composed of 15–20 (–22) μm wide and 3–4 (–8) μm high, bluish green cells penetrated by tubular fungal hyphae; heterocytes frequent, pale to slightly bright yellow, 9–15 μm wide and 2–5 (–7) μm high; cells of hyphal sheath wavy in lateral outline, 3–4 μm thick, center hyphae reaching 9 μm thick; hyphae associated with hyphal sheath straight, hyaline, 4–7 μm thick, lacking clamp connections; hypothallus and prothallus formed by interwoven, strongly agglutinate, generative hyphae.

Hymenophores developed and frequently present, corticioid-steroid, forming irregular, reticulated, resupinate patches dispersed on the underside, patches up to 1.5 (–2) mm broad and 6 mm long, with white (when fresh) to pale



yellow (in herbarium), smooth surface and sometimes minutely tomentose-fuzzy involute margins; hymenophore in section 80–130  $\mu\text{m}$  thick, composed of a paraplectenchymatous layer resting on strongly agglutinated, 4–6  $\mu\text{m}$  thick, generative hyphae emerging from the supporting thallus; hymenium composed of numerous, palisade-like basidioles and scattered basidia; basidioles  $15\text{--}21 \times 5\text{--}6 \mu\text{m}$ ; basidia  $20\text{--}30\text{--}(35) \times 5\text{--}6 \mu\text{m}$ , 4-sterigmate; basidiospores ellipsoid to fusiform, non-septate, hyaline,  $7\text{--}8 \times 3\text{--}4 \mu\text{m}$ .

**Distribution and ecology:** This species is rather conspicuous and common, which has frequently been collected on introduced substrates such as guava and avocado trees. It is usually present in open areas receiving both high amounts of rainfall and being exposed to the light; natural habitats of this species are probably forest fragments of once widely abundant *Frullania*-covered *Zanthoxylum* forests (the so-called “brown-zone”).

**Etymology:** The epithet meaning “bearded” or “having a beard” is a reference to the fuzzy white to beige appearance of the shelf-like morphology of this lichen, each shelf resembling a bearded chin. The characteristic white hairs causing this unique appearance are a result of the fungal sheath lacking the photobiont filaments inside, a character already recognized previously and illustrated in detail by Yáñez et al. (2012).

**Remarks:** This new species is represented by numerous specimens, uniform overall, but with minor variation in the thickness of the thallus and the color of the prothallus (old specimens with a darkened hue, yellow to light brown). All specimens form filamentous shelves, a morphotype unique among *Dictyonema* s.str. in the Galapagos. Aside from horizontal fibrils, there are also vertical fibrils compacted becoming erect structures; these can, but not necessarily always, form a prothallus also. The new species belongs to the *Dictyonema sericeum* clade, which includes multiple, distinct species, all forming shelf-like thalli (Dal-Forno et al. 2013; Lücking et al. 2013b). Among these, *D. barbatum* presents a mostly continuous hymenophore, different from *D. giganteum* L. Vargas, Moncada & Lücking, *D. discocarpum* Lücking, Dal-Forno & Wilk, and *D. hapteriferum* Lücking, Dal-Forno & Wilk, all new species recently segregated from *D. sericeum*. *Dictyonema giganteum* (Vargas et al. 2014) presents very small individual hymenophores, *D. discocarpum* has more or less disc-shaped hymenophores, and *D. hapteriferum* has hymenophores that resemble hapteres found in some lichens (Lücking et al. 2013b). The importance of the hymenophore shape as diagnostic character has also been pointed out in the genus *Cora* (Lücking et al. 2016). *Dictyonema barbatum* has close, yet undescribed relatives from mainland Ecuador, Peru and Guatemala.

**Additional material examined** (10): Ecuador, Galapagos. —San Cristóbal Island (2), Cerro San Joaquín, alt. 681 m, on bark, among mosses on branches of *Psidium guajava* shrubs, 24-Aug-2008, C. Truong 1533 (CDS 39844, GMUF), alt. 771 m, on bark of branches and twigs of *Miconia robinsoniana*, 24-Aug-2008, F. Bungartz 8576 (CDS 41222, F); —Isabela Island (6), Volcán Sierra Negra, alt. 924 m, on *Polypodium* stems among mosses on the ground, 16-Aug-2008, C. Truong 1259 (CDS 39570, GMUF), alt. 939 m, on *Frullania* sp. on *Psidium guajava* branch; 08-Sep-2007, F. Bungartz 6849 (CDS 36297, GMUF), alt. 550 m, on branches of *Inga* sp., 09-Sep-2007, F. Bungartz 6852 (CDS 36301, F), alt. 579 m, on bark of *Psidium guajava* branches, 09-Sep-2007, F. Bungartz 6906 (CDS 36398, F), alt. 580 m, on bark of branches of *Psidium guajava* with mosses (together with *Cora galapagoensis*), 14-Aug-2008, C. Truong 1275 (CDS 39586, F); Volcán Alcedo, alt. 1100 m, on bark of *Zanthoxylum*, 07-Mar-2006, A. Aptroot 65186 (CDS 31770, F); —Santa Cruz Island (2), Bellavista, alt. 400 m, on bark of *Miconia*, 27-May-2005, A. Aptroot 63148 (CDS 29878, GMUF); abandoned farm behind El Puntudo, alt. 729 m, on bryophyte, growing over hepatics on branch of *Persea americana*; 28-Oct-2010, A. Yáñez-Ayabaca 1550 (CDS 45044, GMUF).

***Dictyonema darwinianum*** Dal-Forno, Bungartz & Lücking sp. nov. Fig. 11

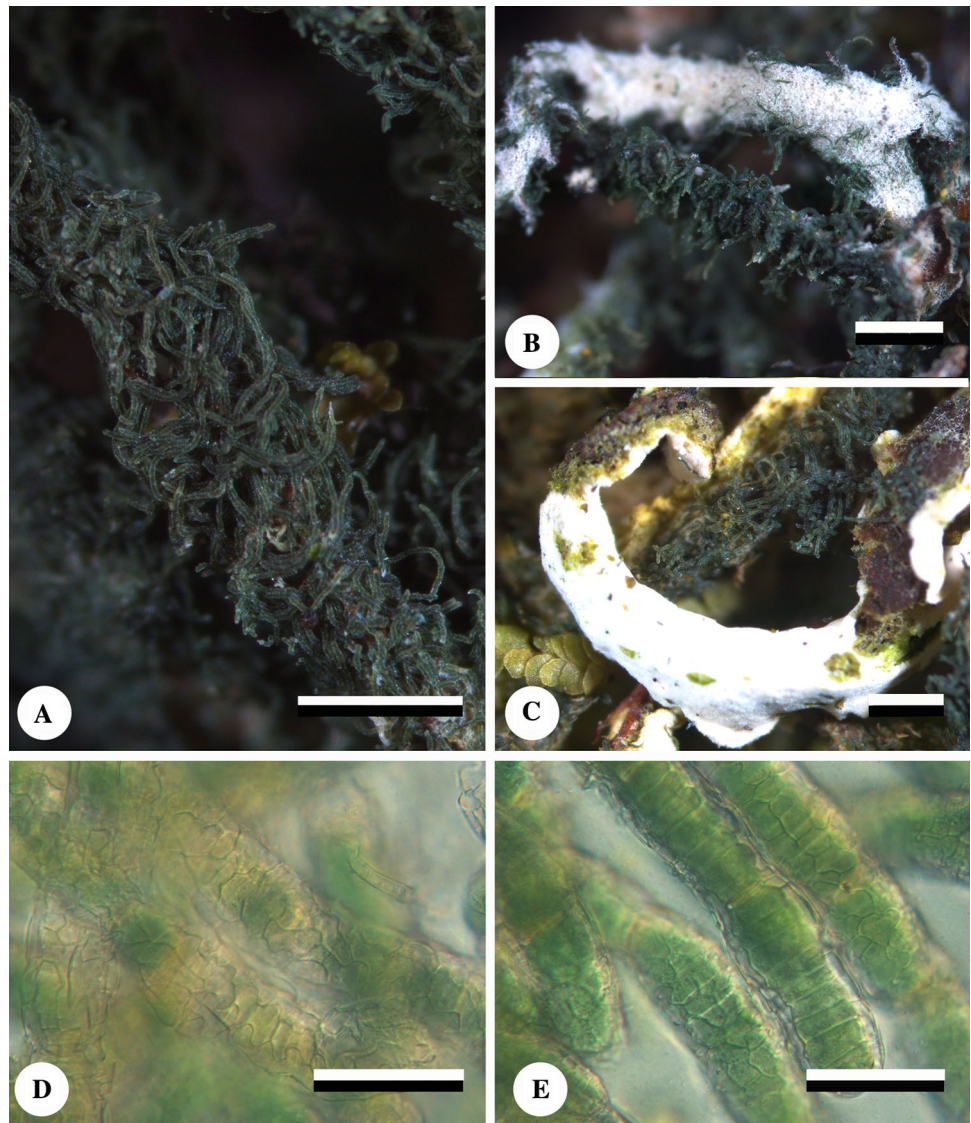
**Mycobank:** MB 820717 **GenBank:** KY861468 (ITS)

**Diagnosis:** An epiphytic, common species of *Dictyonema* forming filamentous-crustose mats on *Frullania* and overall detritus; characterized by mainly horizontal, loosely interwoven, mostly dark blue green fibrils sitting on top of a white hypothallus and a discrete but discernible prothallus.

**Type:** Ecuador, Galapagos, Santa Cruz Island, along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park,  $0^{\circ}39'56.8''\text{S}$   $98^{\circ}19'31.4''\text{W}$ , alt. 502 m, *Miconia robinsoniana* shrubland, growing over *Frullania* sp. and fern fronds, 23-Jun-2010, M. Dal-Forno 1209 (CDS 44733, holotype; GMUF, isotype).

**Description:** Thallus epiphytic on tree trunks and branches, mostly overgrowing bryophytes, but also on detritus among bryophytes, slightly appressed filamentous, in irregular, confluent patches, up to several centimeters across and entire thallus eventually covering larger areas of the substrate (largest specimen so far observed: 10 cm in diam.), forming a strongly compressed mat of mainly horizontal, loosely interwoven, mostly dark blue-green fibrils developing on top of a white hypothallus, along the margin forming a discrete, but discernible prothallus. Thallus with a photobiont layer and a “medulla” (hypothallus); photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed

**Fig. 11** *Dictyonema darwinianum* (multiple specimens). **a, b** General aspect of the erect fibrils of the species (A—M. Dal-Forno 1182, scale bar 1 mm, B—M. Dal-Forno 1183, scale bar 2 mm). **c** Stereoid hymenophore (M. Dal-Forno 1209, scale bar 2 mm). **d, e** Cyanobacterial cells involved in fungal sheath  $40\times$  (M. Dal-Forno 1174, scale bar  $50\text{ }\mu\text{m}$ )



by jigsaw puzzle-shaped cells, sometimes thinner towards their tips; cyanobacterial filaments composed of (12–)15–20  $\mu\text{m}$  wide and 4–8 (–9)  $\mu\text{m}$  high, bluish green cells penetrated by tubular fungal hyphae (haustoria), uniseriate (rarely biseriate); heterocytes sparse, pale yellow, 8–16  $\mu\text{m}$  wide and 4–8  $\mu\text{m}$  high; cells of hyphal sheath variably wavy in lateral outline, but showing the very typical jigsaw puzzle-shaped pattern characteristic of *Dictyonema*, 5–8  $\mu\text{m}$  thick, very rarely branching; hyphae associated with hyphal sheath straight, 5–7  $\mu\text{m}$  thick, lacking clamp connections.

Hymenophores developed as bulging, stereoid patches from the underside of the thallus margins, white patches up to 1.2 mm broad and 1 cm long, with white (when fresh) to pale yellow (in herbarium), smooth surface; hymenophore in section 50–85  $\mu\text{m}$  thick, composed of a paraplectenchymatous layer connected to loose medullary

hyphae; hymenium composed of numerous, palisade-like basidioles and basidia; basidioles 20–30  $\times$  5–7  $\mu\text{m}$ ; basidia 25–35  $\times$  5–8  $\mu\text{m}$ ; basidiospores ellipsoid to narrowly drop-shaped, non-septate, hyaline, 5–7  $\times$  2–3  $\mu\text{m}$ .

**Distribution and ecology:** This represents the most common *Dictyonema* species in the Islands, being represented by many collections from Santa Cruz and Isabela, as well as one from Pinta.

**Etymology:** Named in honor of Charles Darwin and the Charles Darwin Foundation in the Galapagos.

**Remarks:** This common and widespread species is a rather typical *Dictyonema*, with a wavy fungal sheath, mainly uniseriate cyanobacterial cells, dense fibrils forming a crustose mat, otherwise without particularly distinctive features. *Dictyonema darwinianum* does not form net-like or combed fibrils, such as *D. ramificans* or *D. pectinatum*, respectively. It also does not form bracket-like



thalli, as does *D. barbatum*. It is, however, very similar to *D. subobscuratum*, another species found in the Galapagos. The easiest way to distinguish both of these species is by comparing their fibril anatomy: *D. subobscuratum* differs by the usually muriform aspect of its photobiont cells, while *D. darwinianum* has mainly uniseriate photobiont cells. This character makes the fibrils of *D. subobscuratum* slightly ticker than those of *D. darwinianum*. On the other hand, fibrils of *D. darwinianum* are mostly erect and much denser than *D. subobscuratum*. Nonetheless, only the very trained taxonomist can reach these conclusions after comparing sufficient material of both species under the stereoscope. Therefore, microscopic examination of photobiont cells remains the only distinctive character to separate these two species reliably without molecular data. The closest relative of *D. darwinianum* is an undescribed taxon thus far known from Costa Rica, Puerto Rico and Brazil.

**Additional material examined** (30): Ecuador: Galapagos: —Pinta Island (1), on top of the highest point of the island, alt. 625 m, on plant debris and bryophytes, 26-Feb-2007, *F. Bungartz* 5746 (CDS 33400, GMUF). —Santa Cruz Island (17), along the trail to El Puntudo, alt. 698 m, on bryophytes (*Frullania aculeate*) growing epiphytically on *Cinchona pubescens* branches, 28-Dec-2005, *F. Bungartz* 3276 (CDS 26918, GMUF); alt. 469 m, growing on *Frullania* sp., 23-Jun-2010, *M. Dal-Forno* 1177 (CDS 44711, GMUF); alt. 502 m, on bryophytes, 23-Jun-2010, *M. Dal-Forno* 1182 (CDS 44717, GMUF), *M. Dal-Forno* 1183 (CDS 44718, GMUF), *M. Dal-Forno* 1191 (CDS 44724, GMUF), *M. Dal-Forno* 1208 (CDS 44732, GMUF), *M. Dal-Forno* 1211 (CDS 44735, GMUF); alt. 684 m, A.A. *Spielmann* 8249 (CDS 44757, GMUF); along the road from Bellavista to Los Gemelos, alt. 574 m, on bryophytes over bark, 23-Jun-2010, *M. Dal-Forno* 1171 (CDS 44706, GMUF), *M. Dal-Forno* 1174 (CDS 44709, GMUF), *M. Dal-Forno* 1178 (CDS 44712, GMUF), *M. Dal-Forno* 1179 (CDS 44713, GMUF); between El Puntudo and Cerro Crocker, alt. 760 m, growing on *Frullania* sp. on *Cinchona* trunk, 28-Oct-2010, A. Yáñez-Ayabaca 1541 (CDS 45035, GMUF); N of Bellavista, alt. 555 m, *Miconia* shrubland, growing on *Frullania* sp. on branches of *Miconia robinsoniana*, 28-Oct-2010, A. Yáñez-Ayabaca 1507 (CDS no. 44998), Steve Divine's Farm at the end of Tortoise Road, alt. 364 m, Feb. 23, 2006, A. *Aptroot* 64519 (CDS 31091, GMUF), *F. Bungartz* 3956 (CDS 27838, GMUF). —Isabela Island (7), Volcán Alcedo, alt. 1089 m, on bark of *Tournefortia*, 05-Mar-2006, A. *Aptroot* 65037a (CDS 31619, GMUF); Volcán Sierra Negra, alt. 550 m, growing over hepatics on top of *Psidium guajava* branches, 09-Sep-2007, *F. Bungartz* 6883 (CDS 36362, GMUF); alt. 980 m, on bryophytes on branches of *Psidium guajava*, 14-Aug-2008, M.A. *Herrera-Campos* 10560 (CDS 40297, GMUF); alt. 1055 m, growing over dead plant material and

*Lycopodium* and fern stems near the ground; 16-Aug-2008, *F. Bungartz* 8350 (CDS 40996, GMUF); Volcán Cerro Azul, 917 m, on basaltic rock, 7-May-2012, *F. Nugra* 1096 (CDS 52259, GMUF), alt. 767 m, on soil, May 3, 2012, *F. Nugra* 1051 (CDS 52215, GMUF), alt. 902 m, on bryophytes on fern stems, 7-May-2012, A.A. *Spielmann* 10621 (CDS 51988, GMUF). —Florea Island (5), on top of Cerro Asilo de la Paz, permanent plot 25, alt. 531 m, on bryophytes, Jan. 13, 2011, A. Yáñez-Ayabaca 1828 (CDS 46566, GMUF), A. Yáñez-Ayabaca 1842 (CDS 46565, GMUF), *F. Bungartz* 9476 (CDS 46557, GMUF); Cerro Verde, alt. 348 m, on bryophytes, Jan. 15 2011, A. Yáñez-Ayabaca 1956, along trail from Crucecita Farm going East, at the base of Cerro de los Suspiros. alt. 414 m, Jan. 22, 2011, on roots, A. Yáñez-Ayabaca 2062 (CDS 46571, GMUF).

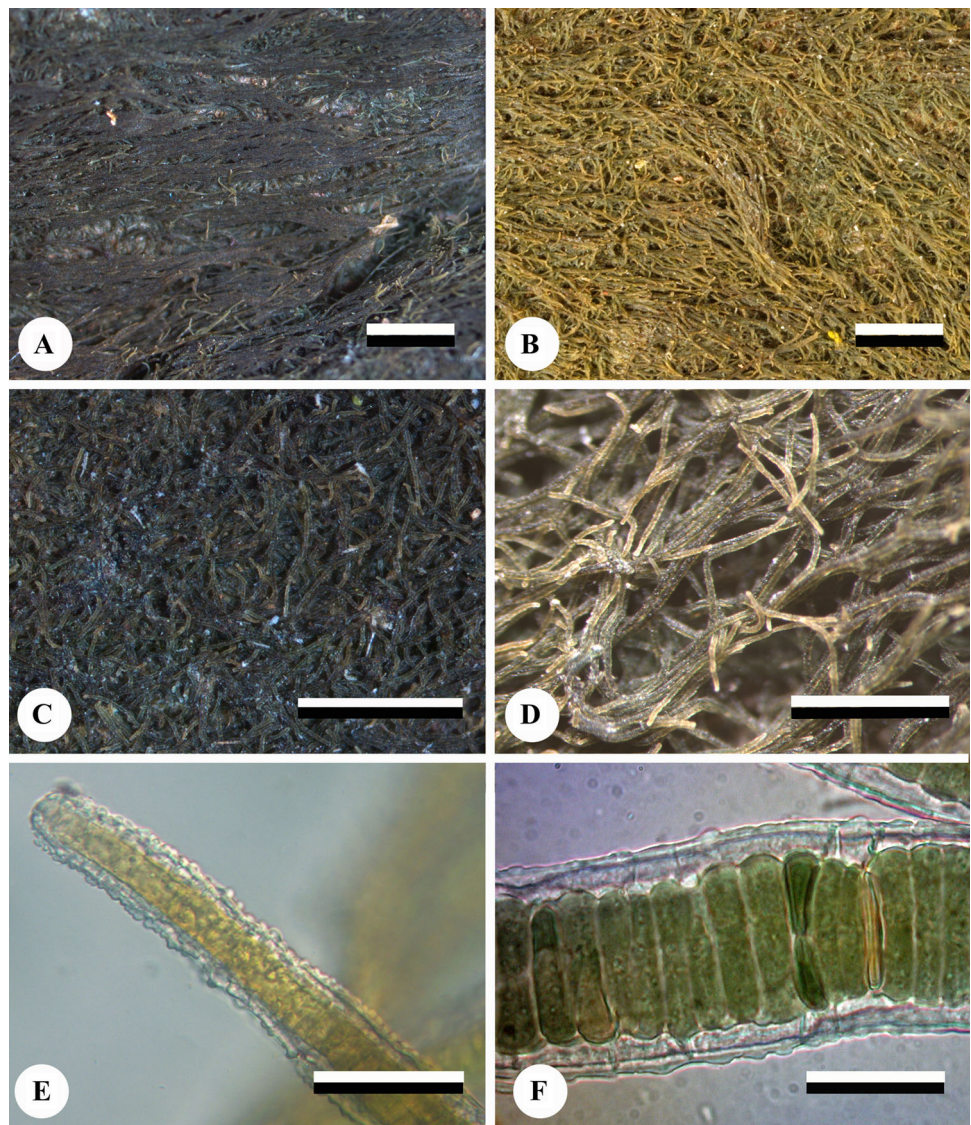
***Dictyonema pectinatum*** Dal Forno, Yáñez-Ayabaca & Lücking, Fungal Diversity 52: 234 (2012) Fig. 12

**Description:** Thallus epiphytic on tree trunks and branches, appressed filamentous, in irregular, confluent patches, up to several cm across and covering larger areas of the substrate, forming a mat of horizontally arranged, as if “combed,” loosely interwoven, deep bluish green to somewhat olive fibrils sitting on top of a white indistinct hypothallus, sometimes extending to form a discrete prothallus. Fibrils that do not get overly long to form the characteristic “combed” aspect of the thallus, are of delicate intricate-arachnoid appearance, but still show a tendency to become distinctly elongated and lighter towards the tips. Thallus with a thick photobiont layer and a thin medulla; photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath formed by jigsaw puzzle-shaped cells; cyanobacterial filaments composed of 16–21  $\mu\text{m}$  wide and 5–9  $\mu\text{m}$  high, blue green cells penetrated by tubular fungal hyphae; heterocytes sparse to frequent, yellow, 12–13  $\mu\text{m}$  wide and 2–3  $\mu\text{m}$  high; cells of hyphal sheath wavy in lateral outline, 5–9  $\mu\text{m}$  thick; hyphae associated with hyphal sheath straight, 4–6  $\mu\text{m}$  thick, lacking clamp connections. Hymenophore not observed.

**Distribution and ecology:** This is the only *Dictyonema* in Galapagos with all specimens growing directly on bark, as previously mentioned by Yáñez et al. (2012). All specimens have been collected on *Psidium guajava*, the common guava, a tree introduced to the archipelago for its fruits. Considering that *D. pectinatum* is so far known only from the bark of a tree not native to the archipelago, we initially believed the species would probably occur on the continent as well (see also the comments below). However, until now the species appears to be restricted to the Galapagos Islands, where the species is known from one small population in Santa Cruz only. As far as we know, structural bark characteristics of *Psidium guajava*, the introduced tree, are no



**Fig. 12** *Dictyonema pectinatum* (multiple specimens). **a, b** General aspect of combed fibrils of the species in two different lighting conditions (M. Dal-Forno 1170, scale bar 4 mm). **c** General aspect of the fibrils in parts of the thallus where the fibrils do not have the combed appearance, note the lighter tips (M. Dal-Forno 1221, scale bar 2 mm). **d** Close up showing the lighter tips of the fibrils in stereoscope (M. Dal-Forno 1170, scale bar 500  $\mu$ m). **e** Close up showing the lighter tips of the fibrils in light microscope (M. Dal-Forno 1170, scale bar 25  $\mu$ m). **f** Cyanobacterial cells involved in fungal sheath 100  $\times$  (M. Dal-Forno 1221, scale bar 30  $\mu$ m)



different from those of *Psidium galapageium*, the Galapagos Guava, a very close endemic relative. This might help to explain, why this corticolous, endemic basidiolichen has been found on the bark of an introduced tree.

**Remarks:** *Dictyonema pectinatum* is another species fairly easy to identify, even in the field, due to the “combed” appearance of its fibrils. The appearance of this species, with the parallel-horizontal long arrangement of the fibrils, is quite unique within the genus. However, not all thalli present this distinctive character. In that instance, fibrils grow in an arachnoid and intricate pattern and primarily horizontally, tightly appressed to the substrate. Beyond that, most of the fibrils also have lighter tips and the fungal sheath has “papillae,” a very conspicuous feature, observed mostly under a light microscope (although the lighter tips may also be observed under a stereoscope).

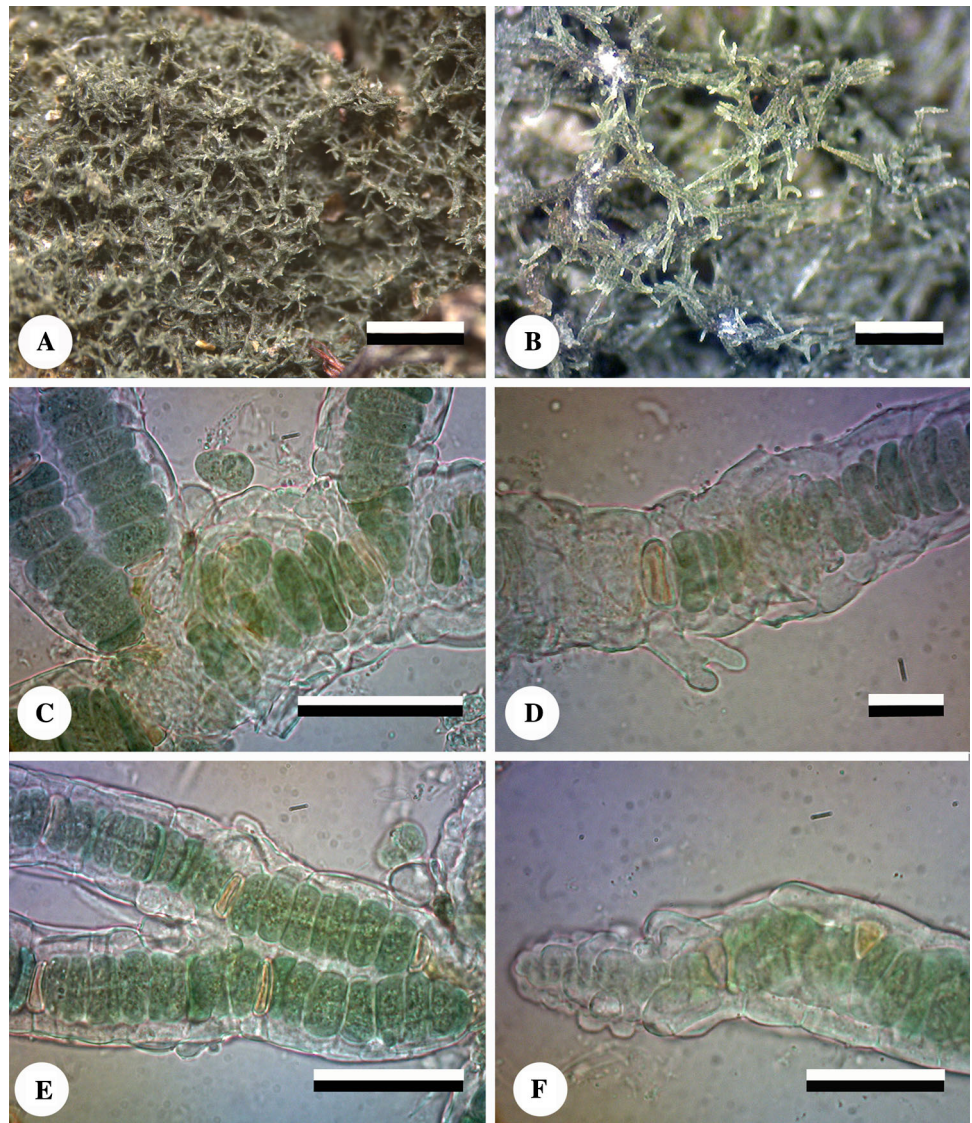
The papillae follow the natural wavy outline of the fungal sheath and become denser towards the tips.

Hymenophore-like structures have been observed in one of the specimens (M. Dal-Forno 1221, CDS 44744); however, these structures resembling resupinate patches of hymenophores turned out to be an old decomposing specimen of a chlorolichen, evidenced by the many green algae surrounding its hyphae in cross section.

Originally we thought the species also occurs in mainland Ecuador, with two specimens collected in Mindo, a high altitude humid zone in Pinchincha Province. However, the Ecuadorian and Galapagos material form two supported sister clades, with the ITS sequences from Galapagos differing in seven nucleotides. We therefore consider these two lineages an example of cryptic speciation with allopatric distribution.



**Fig. 13** *Dictyonema ramificans* (M. Dal-Forno 1214). **a** General aspect of fibrils (scale bar 1 mm). **b** Arachnoid arrangement of the fibrils (scale bar 0.5 mm). **c** Many fibrils growing together 100 × (scale bar 50 μm). **d** The beginning of a lateral ramification 100 × (scale bar 15 μm). **e** Two fibrils connected by fungal sheath, also note the haustoria 100 × (scale bar 50 μm). **f** Ornamented hyphae from fungal sheath towards the tip of a fibril 100 × (scale bar 25 μm)



**Material examined** (2): Ecuador—Galapagos, Santa Cruz Island, along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park, 0°39'56.8"S, 98°19'31.4"W, alt. 502 m, *Miconia robinsoniana* shrubland, on bark of *Psidium guajava*, 23-Jun-2010, *M. Dal-Forno* 1221 (CDS 44744, GMUF); 0°40'48"S, 90°19'26"W, alt. 469 m, on bark of *Psidium guajava*, 23-Jun-2010, *M. Dal-Forno* 1170 (CDS 44705, holotype; GMUF, isotype).

***Dictyonema ramificans*** Dal-Forno, Yáñez-Ayabaca & Lücking sp. nov. Fig. 13

**Mycobank:** MB 820718 **GenBank:** KY861470 (ITS)

**Diagnosis:** A *Dictyonema* species with a unique net-like pattern formed by branching fibrils.

**Type:** Ecuador, Galapagos, Santa Cruz Island; along trail from Bellavista to El Puntudo, behind the park fence, close to the border of the National Park 0°39'56"S,

98°19'31"W, alt. 502 m, *Miconia robinsoniana* shrubland, on bryophytes, growing over *Frullania* sp., 23-Jun-2010, *M. Dal-Forno* 1214 (CDS 44738, holotype; GMUF, isotype).

**Description:** Thallus epiphytic on bryophytes, but also on leaves, filamentous, in irregular, confluent patches, each up to 10 cm across, forming a dense mat of more or less horizontal but also vertical, loosely interwoven, bluish green to brownish olive, developing on top of a white hypothallus extending along the margin to form a prothallus. Fibrils forming an arachnoid pattern, formed by interconnected fibrils in a more or less erect structure. Thallus organized in photobiont layer and “medulla” (hypothallus), photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath; cyanobacterial filaments uni- or biseriate, composed of up to 15 μm wide and up to 10 μm high, thinner towards their tips, bluish green cells often longitudinally



divided, penetrated by tubular fungal hyphae; heterocytes sparse to frequent, mostly central, rarely lateral, pale yellow, 10–15  $\mu\text{m}$  wide and 5–10  $\mu\text{m}$  high; cells of hyphal sheath highly variable in shape and size, angular to wavy in lateral outline, highly ornamented and sinuous towards the tips of the fibrils, up to 15  $\mu\text{m}$  thick, ramification present and frequent, connecting different fibrils; hyphae associated with hyphal sheath straight, 5–7  $\mu\text{m}$  thick, lacking clamp connections.

Hymenophores developed as bulging, stereoid, resupinate patches, 0.1–1 (–2) mm broad and up to 6 mm long, with white (when fresh) to slightly yellowish white (in herbarium), smooth surface; hymenophore in section 62–75  $\mu\text{m}$  thick, composed of a paraplectenchymatous layer connected to loose medullary hyphae; hymenium composed of scattered, palisade-like basidioles and basidia; basidioles 15–20  $\times$  4–6  $\mu\text{m}$ ; basidia 20–25  $\times$  5–7  $\mu\text{m}$ ,

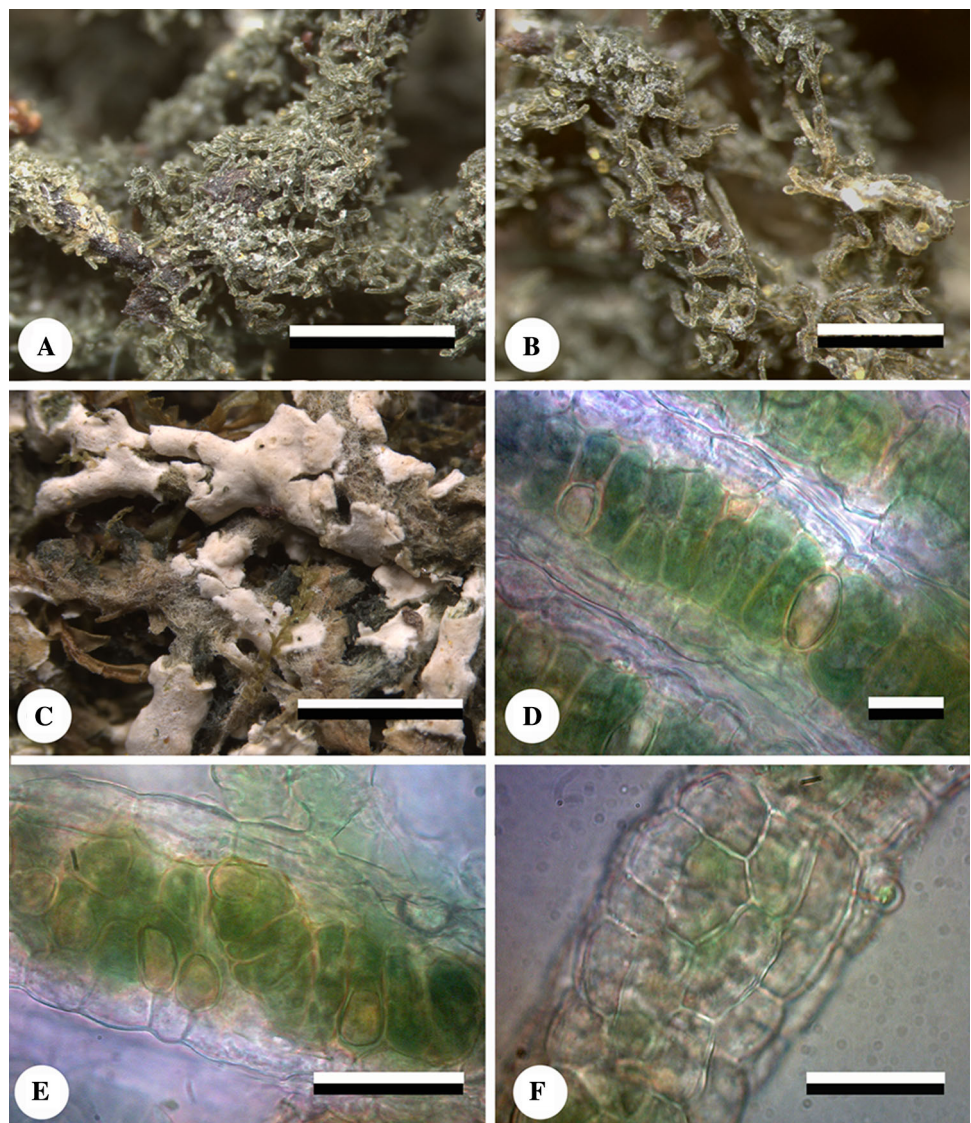
basidiospores ellipsoid to narrowly drop-shaped, non-septate, hyaline, 7–8  $\times$  2–4  $\mu\text{m}$ .

**Distribution and ecology:** All specimens of this species grow on bryophytes, so far only found in the humid zone of Santa Cruz.

**Etymology:** This epithet refers to the branching pattern of the fibrils.

**Remarks:** This species is characterized by the formation of an erect arachnoid pattern on top of a white hypothallus, due to the branching pattern of its fibrils. The cyanobacterial filaments are organized inside their fungal sheath as a single chain; however, the fungal sheaths branch and eventually form secondary chains. Although *D. pectinatum* may also form an arachnoid pattern, it differs in this species by fibrils being appressed on the substrate and intricate overall, not forming a net-like erect structure. In *D. pectinatum*, the arachnoid pattern is formed by irregular

**Fig. 14** *Dictyonema subobscuratum* (multiple specimens). **a, b** General aspect of fibrils (F. Bungartz 9549, scale bar 1 mm and 0.5 mm, respectively). **c** Thallus growing on bryophytes showing abundant white hymenophore (F. Bungartz 9550, scale bar 2 mm). **d, e** Close up showing the longitudinally divided cells 100  $\times$  (F. Bungartz 9551, scale bar 15 and 25  $\mu\text{m}$ , respectively). **f** Angular variable hyphae from fungal sheath 100  $\times$  (F. Bungartz 9551, scale bar 25  $\mu\text{m}$ )





tangling fibrils, while in *D. ramificans* by the branching fibrils. Additionally, in *D. pectinatum* the hyphae of the fungal sheath are ornamented with papillae, especially towards their tips. The closest relative of *D. ramificans* included in our studies is from mainland Ecuador, a lineage still formally undescribed.

**Additional material examined** (5): Ecuador, Galapagos, Santa Cruz Island, path from Media Luna to El Puntudo, alt. 684 m, on *Frullania* sp. on branch of *Cinchona pubescens*, 28-Oct-2010, A. Yáñez-Ayabaca 1517 (CDS 45008, GMUF), A. Yáñez-Ayabaca 1518 (CDS 45009, GMUF), A. Yáñez-Ayabaca 1521 (CDS 45012, GMUF), alt. 684 m, on bryophyte and on trunk of *Cinchona pubescens*, 28-Oct-2010, A. Yáñez-Ayabaca 1534 (CDS 45027, GMUF), alt. 762 m, on *Frullania* sp. on the ground, 28-Oct-2010, A. Yáñez-Ayabaca 1539 (CDS 45032, GMUF).

***Dictyonema subobscuratum*** Dal-Forno, Bungartz & Lücking sp. nov. Fig. 14

**Mycobank:** MB 820719 **GenBank:** KY861497 (ITS)

**Diagnosis:** Morphologically similar to *Dictyonema obscuratum*, but genetically different and geographically restricted to the Galapagos Islands.

**Type:** Galapagos, Floreana Island; along rim trail of Cerro Pajas, eastern part of rim, 1°17'44.8"S 90°27'15.6"W, alt. 442 m, humid zone, low dense vegetation of *Zanthoxylum fagara*, *Scalesia pedunculata*, *Macraea laricifolia*, *Croton scouleri* and ferns (*Polypodium tridens*) over lava boulders; bryophytes hanging in curtains from trees, growing over hepatics (*Frullania* sp.) hanging from *Zanthoxylum* twigs; semi-shaded, wind- and rain-exposed, on bryophytes, 26-Jan-2011, F. Bungartz 9549 (CDS 46559, holotype; GMUF, isotype).

**Description:** Thallus epiphytic on bryophytes, filamentous, in irregular, confluent patches, each up to 5 cm across, forming a mat of more or less irregular, loosely interwoven, mostly individual, not dense, dark blue-green to dark green fibrils, developing on top of a white hypothallus sometimes forming a discrete prothallus. Thallus organized in photobiont layer and medulla (hypothallus), photobiont composed of numerous cyanobacterial filaments wrapped in a closed hyphal sheath; cyanobacterial filaments composed of up to 24 µm wide and up to 10 µm high, bluish green cells, often longitudinally divided, penetrated by tubular fungal hyphae; heterocytes frequent, central or lateral, pale yellow, 6–17 µm wide and 5–10 µm high; cells of hyphal sheath highly variable in shape and size, mostly angular and straight in lateral outline, not wavy, without the typical jigsaw-puzzle pattern that is otherwise characteristic of *Dictyonema*, 5–20 µm thick; hyphae associated with hyphal sheath straight, 5–7 µm thick but also angular, similar to the

hyphal sheath, forming almost a connecting tissue between fibrils, lacking clamp connections.

Hymenophores developed as bulging, stereoid patches, patches up to 1 mm broad and 3.5 mm long, with white (when fresh) to yellowish white (in herbarium), smooth surface; hymenophore in section 75–100 µm thick, composed of a paraplectenchymatous layer connected to loose medullary hyphae; hymenium composed of scattered, palisade-like basidioles; basidioles 20–30 × 5–7 µm; basidia and basidiospores not observed.

**Distribution and ecology:** This species grows mostly on bryophytes over branches and trunks in humid zones of Santa Cruz and Floreana in the Galapagos Islands.

**Etymology:** The epithet refers to the similarity to another species described recently as *Dictyonema obscuratum*.

**Remarks:** Widened tips or other areas of the fibril with a muriform aspect are common in this species, which is caused by the often longitudinally divided cyanobacterial cells, especially towards the tips. This character is similar to *Dictyonema obscuratum*, a species from Brazil, which in addition to this feature, also forms sheaths composed of angular cells as *D. subobscuratum*, both lacking the typical jigsaw puzzle aspect generally characteristic of the genus *Dictyonema*. Compared to *D. subobscuratum*, however, hyphal sheath cells of *D. obscuratum* are quite wide. In Galapagos *D. subobscuratum* is the species observed to have the highest frequency of heterocytes along the filaments (one for every 3–7 cyanobacterial cells). The two taxa (*D. obscuratum* and *D. subobscuratum*) are in sister clades in the trees based on our concatenated dataset (Fig. 1) and on ITS (Fig. 4, Suppl. Fig. 4).

One Galapagos specimen appears to be infected by a parasite, with many cyanobacterial cells completely brown and dead (A. Yáñez-Ayabaca 2058 A).

**Additional material examined** (5): Ecuador, Galapagos, —Floreana Island (4), along rim trail of Cerro Pajas, alt. 504 m, on bryophytes, 26-Jan-2011, F. Bungartz 9550 (CDS 46560, GMUF), F. Bungartz 9551 (CDS 46561, GMUF), F. Bungartz 9552 (CDS 46560, GMUF); at SE-base of Cerro de los Suspiros, permanent plot 22, alt. 342 m, on tree trunk, 22-Jan-2011, A. Yáñez-Ayabaca 2058 A (CDS 48407, GMUF). —Santa Cruz Island (1), along trail from Bellavista to El Puntudo, growing over *Frullania* sp., 23-Jun-2010, M. Dal-Forno 1181 (CDS 44716, GMUF).

## Discussion

Prior to our study, there had been no attempt to infer the evolutionary origins of basidiolichens on the Galapagos. The ten species now recognized for the Galapagos did not

evolve from a single colonization event but originated independently from multiple colonizations. As a consequence, basidiolichen diversity in the islands is not the result of adaptive radiation, as found in many plant lineages such as the genus *Scalesia* (Asteraceae), and animals such as the Darwin's finches, the giant tortoises and snails of the family Bulimulidae (Darwin 1859; Losos and Ricklefs 2009; Tye et al. 2002).

A previous study of diversification in the lichen fungal genus *Roccella* in the Galapagos (Tehler et al. 2009) found that of the five species in the *Roccella galapagoensis* aggregate, four are endemic to Galapagos, while the other one is endemic to Peru. The Galapagean species likely have a single common ancestor, suggesting a single colonization with subsequent radiation, which contrasts with the absence of radiation events in the evolution of Galapagos basidiolichens. This difference is perhaps explained by different ecological requirements of the two groups: *Roccella* is a genus characteristic of dry, coastal habitats, whereas lichenized Hygrophoraceae are generally confined to the humid shrubland and cloud forests of the Galapagos highlands. Humid vegetation in the archipelago is restricted to the southern, rain-exposed slopes of the higher volcanoes and therefore isolated by large areas of dry lowland. Ali and Aitchison (2014) developed a model of a dynamically changing Galapagos map according to the variation in sea level. They described a scenario affecting most strongly the highlands where, with sea level rise, the humid vegetation would shrink and only expand again when sea level lowers. These oscillations presumably resulted in high evolutionary pressure for the species restricted to the humid highlands, reducing their potential for local adaptive radiation. Populations that managed to survive when the humid zone was “constricted” would be much less exposed to habitat differentiation, and their opportunity for adaptive radiation during those periods would be severely limited. Notably, the situation is very similar in Lobariaceae, members of which in Galapagos also prefer humid habitats and exhibit a high level of endemism with no evidence for radiation (Moncada et al. 2016).

Gradstein and Weber (1982) speculated that lichens adapted to dry habitats in the Galapagos lowlands originated in similar habitats along the coast of South America, particularly Peru and Chile, brought to the islands by the trade winds from the south that follow the Antarctic Humboldt current along Chile and Peru, then below the equator veering off towards the Galapagos. This hypothesis seems to be supported for the genera *Roccella* (Tehler et al. 2009) and *Redonographa* (Lücking et al. 2013b). In contrast, the same authors (Gradstein and Weber 1982) predicted that lichens characteristic of the Galapagos humid highlands, such as Lobariaceae and the basidiolichens studied here, would have reached the archipelago from

Central America in the north, following the Panama current. This hypothesis is not supported by our data; on the contrary, in most cases where a closely related sister taxon was sampled, this taxon was from Ecuador, suggesting that dispersal of these lichens is limited by distance. Long-distance dispersal to the Galapagos would involve some form of transport from the mainland, including wind and bird vectors, or rafting on vegetation islands, which can carry along vegetative diaspores (soredia, isidia and thallus fragments that contain both lichen partners) or fungal spores. In the case of dispersal by spores, the mycobiont would require a suitable photosynthetic partner to successfully colonize, a requirement that Weber (1966) suggested would limit lichens dependent on this mode of dispersal to arrive and survive in the islands. The basidiolichens studied here may associate with a few photobiont species in the cyanobacterial genus *Rhizonema* (Dal-Forno et al. 2016b) and can share or even steal photobionts from other basidiolichens, or even unrelated ascolichens, such as the genus *Coccocarpia*, as shown for other lichenized fungi (Goward 1994; Piercey-Normore and DePriest 2001; Lücking et al. 2009; Cornejo and Scheidegger 2016; Cornejo et al. 2016). Therefore, dispersal by fungal spores alone is not necessarily an ecological constraint to colonization for these lichens. The fact that the ten species of basidiolichens now recognized in the Galapagos Islands can be traced back to ten separate colonization events actually suggests that successful colonization via fungal spores could have happened quite frequently in this clade. On the other hand, such dispersal appears to be sufficiently rare to prevent continuous gene exchange with mainland lineages, leading to reproductive isolation and divergence in the Galapagos populations.

We previously did not anticipate the high level of endemism now documented in Galapagos basidiolichens. What was known as *Dictyonema glabratum* or more recently as *Cora glabrata*, assumed to be an almost subcosmopolitan taxon (Lücking et al. 2014a), corresponds to two species endemic to the Galapagos: *C. galapagoensis* and *C. santacruzensis*. Similarly, lichens previously identified as the presumably widespread *Dictyonema sericeum* and *D. schenkianum* are now distinguished as *D. barbatum* and *D. darwinianum*, *D. subobscuratum*, and *D. ramificans*, respectively. All Galapagos material previously identified as *Acantholichen pannarioides* represents the endemic *A. galapagoensis* (Dal-Forno et al. 2016a). Only two of the species recognized by Yáñez et al. (2012) were at the time considered to be endemic: *Dictyonema galapagoense*, now placed in *Cyphellostereum*, and *D. pectinatum*.

In comparison to our previous assessment (Yáñez et al. 2012), only a small increase in the overall number of taxa is documented here for the *Dictyonema* clade, which encompasses all known basidiolichens in the archipelago:

from eight (formally seven) to ten species now being reported from the islands. Prior to 2012, only four species had been recognized, and our first assessment had doubled this number (Yáñez et al. 2012). The addition of molecular data increased this number by a further 25%, and we expect that more sampling may lead to further discoveries. More than an accurate assessment of species richness, the molecular data revealed evolutionary relationships and a striking level of endemism, initially considered to be zero, then in our 2012 treatment estimated at 29%, and finally with molecular evidence now suggested to be as high as 100%. Although species may eventually be discovered that are not endemic to the archipelago or some of the taxa currently known from the Galapagos may turn up elsewhere, we believe that our extensive data set available from across the Neotropics, including material from outside the Americas, indicates that this is relatively unlikely.

As the studies on *Rocella* (Tehler et al. 2009), Lobariaceae (Moncada et al. 2016), and basidiolichens have shown, estimates of endemism in other lichen groups in the Galapagos will likely change once molecular studies are more widely employed. However, even without the use of genetic data, several recent studies include a high proportion of species from Galapagos described as new to science, indicating potentially endemic taxa, such as in the genus *Ramalina* (Aptroot & Bungartz 2007). Bungartz et al. (2015) suggest that endemism in the genus *Pertusaria* could be as high as 30–37%, or even 46% if all taxa newly described are considered endemic. In *Rinodina* the degree of endemism could be as high as 37% (Bungartz et al. 2016a) and in *Diploicia* all species now reported from the Galapagos are known only from this archipelago (Bungartz et al. 2016b).

Compared to other oceanic volcanic islands, such as Hawaii and St. Helena, high endemism in Galapagos lichens is not necessarily unexpected. For example, Aptroot (2008) reported that out of the ten *Ramalina* species found in St. Helena, four may be endemic (40%), while in the Galapagos (Aptroot & Bungartz 2007) four out of the 15 may be endemic (27%). The basidiolichens of St. Helena are not thoroughly known, with a single species reported from this island, *Cora sanctae-helenae* Lücking, based on historical material, thought to be endemic and possibly extinct (Aptroot 2008; Lücking et al. 2015). Other archipelagos and oceanic island groups have not been as well studied for basidiolichens as the Galapagos, but unpublished data (Dal Forno et al., in prep.) available from Hawaii suggest that basidiolichen diversity is comparatively low, although with a potentially high level of endemism. Recently collected specimens representing the *Dictyonema* clade in Hawaii, in combination with

phylogenetic data, indicate that there is only one species of *Dictyonema*, *D. moorei* (Nyl.) Henssen, and a few undescribed *Cyphellostereum* species. *Dictyonema moorei* has been cited for Hawaii by Elix and McCarthy (1998), but based on our preliminary molecular data is also found in Korea and Brazil, indicating that this species is one of the few exceptions in the *Dictyonema* clade in possibly being pantropical, having arrived in Hawaii relatively recently. A similar exception is known from Lobariaceae with the pantropical species *Crocodia aurata* (Moncada et al. 2014): populations from Hawaii are somewhat derived phylogenetically but still fit within the species; however, the material from Galapagos resembling that species is a separate taxon (Moncada et al. 2016).

## Conclusions

Overall there is strong evidence that basidiolichen species arrived on the islands during independent colonization events and that all species are endemic. For this group of species, and possibly other groups adapted to the humid highland habitat, endemism appears not to be the result of adaptive radiation. For these species, not only the sea, but also the dry Galapagos lowlands present a colonization barrier. Half of the species apparently reached the archipelago via the shortest route from mainland Ecuador and not as previously suggested from Central America. Colonization success appears to have been limited especially by their capacity to reach suitable habitat and perhaps also by finding an appropriate photobiont.

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