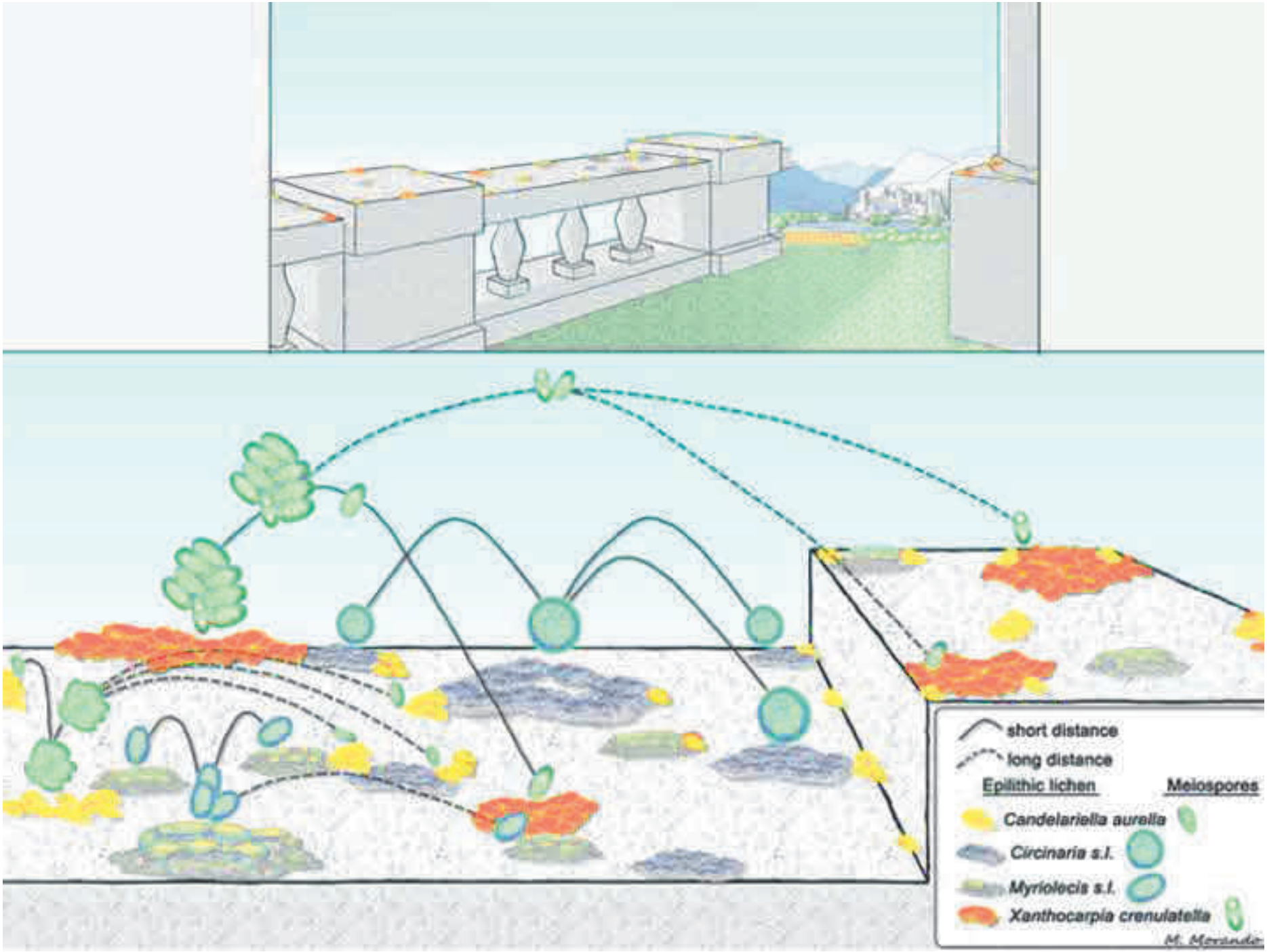
Effectiveness of aerobiological dispersal and microenvironmental requirements together influence spatial colonization patterns of lichen species on the stone cultural heritage

*Graphical Abstract



Highlights

- Relationship of lichen patterns on heritage stones and propagule dispersal is tested
- We combined analyses of the spatial population structure with aerobiological analyses
- Dispersal patterns and microenvironment co-drive specific abundance and distribution
- Knowledge on lichen reproductive patterns is crucial for stone heritage conservation

Abstract

Dispersal patterns of lichen species in monumental and archaeological sites and their relationships with spatial population structure are almost unknown, hampering predictions on colonization dynamics that are fundamental for planning conservation strategies.

In this work, we tested if the local abundance and distribution pattern of some common lichen species on carbonate stones of heritage sites may be related to their patterns of propagule dispersal. We combined analyses of the spatial population structure of eight species on the calcareous balustrade of a heritage site in Torino (NW Italy) with aerobiological analyses. In situ and laboratory analyses were mainly focused on the ejection of ascospores and their air take-off and potential dispersal at short and long distance. Results indicate that the spatial distribution of lichens on the stone surfaces is influenced by both species-specific patterns of propagule dispersal and microenvironmental requirements. In particular, apotheciate species that have a higher ejection of ascospores with higher potential for long range dispersal are candidate for a much aggressive spreading on the monumental surfaces. Moreover, their occurrence on natural or artificial stone surfaces in the surroundings of the stone monumental surface may easily support recolonization dynamics after cleaning interventions, as an effective supply of propagules is expected. On the other hand, species with a lower dispersal rate have a more clustered distribution and are less effective in rapid recolonization, thus representing a minor threat for cultural heritage conservation. These results support the idea that information on the reproductive strategy and dispersal patterns of lichens should be coupled with traditional analyses on stone bioreceptivity and microclimatic conditions to plan effective restoration interventions of stone surfaces.

Keywords

calcareous stone; cultural heritage conservation; lichen colonization dynamics; ascospore ejection; reproductive strategy; spatial population structure

1. Introduction

Since the 1990s, scientists dealing with the conservation of stone cultural heritage focused their attention on bioreceptivity, i.e. the ability of a material to be colonized by living organisms (Guillitte, 1995; Miller et al., 2012). Stone bioreceptivity depends on intrinsic properties of the lithology, including physical and chemical features (primary receptivity), and may also be affected by the influence of early colonizers, starting colonization successions (secondary receptivity), or by human interferences, as the application of restoration products (tertiary receptivity) (Guillitte, 1995; Guillitte and Dreesen, 1995). However, lithobiontic colonization also depends on extrinsic factors, as microclimate conditions and the supply of reproductive structures, for which atmospheric aerosol is generally considered the main vector (Caneva et al., 2003a; De Nuntiis and Palla, 2017).

Aerobiology applied to cultural heritage examines airborne biological particulates which may be a potential cause of biodeterioration, when the characteristics of the substrate and the surrounding (micro-)environmental conditions are compatible with the ecological and nutritional needs of the lithobiontic microorganisms (Caneva et al., 2003a). In this context, most of investigations dealt with indoor environments, where the characterization of bioaerosols was combined with the control of microclimatic conditions (Skóra et al., 2015; De Nuntiis and Palla, 2017). A minor attention has still been devoted to outdoor environments, where knowledge on the load of biodeteriogens in the bioaerosol may possibly support a better understanding of population dynamics and consequent threats to heritage conservation (Caneva et al., 2003b; Piervittori et al., 2007). In particular, lichens, which are among the most aggressive biodeteriogens of the outdoor stone cultural heritage (Seaward, 2015; Salvadori and Casanova-Municchia, 2016), have been rarely considered in aerobiological investigations (Favero-Longo et al., 2014; Banchi et al., 2018). In this perspective, the possibility to characterize lichen ascospores in the air spora and to explore relationships

between specific dispersal ability and local distribution patterns on heritage surfaces has been recently demonstrated (Morando et al., 2017). However, dispersal patterns of lichen species most frequently found in monumental and archaeological sites, and their relationships with spatial population structure, are almost unknown, hampering predictions on colonization dynamics that are fundamental for planning conservation strategies.

In this work, we tested the null hypothesis that the local abundance and distribution pattern of common lichen species on carbonate stones of heritage sites are independent from their microclimate requirements and/or their patterns of propagule dispersal. We combined analyses of the spatial population pattern of eight species on the calcareous balustrades of a heritage site in Torino (NW-Italy) with aerobiological analyses. Establishment and growth of lichens imply the success of the whole aerobiology pathway (Scheidegger and Werth, 2009), including propagule discharge, take-off, transport (dispersal *s.s.*), deposition and impact (Lacey, 1996; Magyar et al., 2016), and, in the case of asymbiotic propagation, the acquisition of a compatible photobiont (Honegger, 2012). In situ and laboratory analyses were mainly focused on the ejection of ascospores (and the detachment of vegetative propagules) and their air take-off and potential dispersal at short and long distance.

2. Material and Methods

2.1 Study sites and lichen survey

Investigations were carried out on the balustrade of the podium of the Basilica of Superga (WGS 84: N 45°4.826' - E 7°46.047'), symbol of Baroque architecture in NW Italy (Fig. 1a; Corino et al. 1990), located on a top of the hill (672 m a.s.l.) on the east side of the Torino metropolitan area. In this area, mean annual temperature is 12.5 °C and mean annual rainfall is 900 mm. The balustrade (Fig. 1b), approx. 100 m long and 30 cm wide, borders the podium of the Basilica and is made of a local biocalcirudite known as Gassino stone, the carbonate

sedimentary rock most widely used in Torino architecture (Borghi et al., 2014). Last cleaning intervention on the balustrade dates back to 1990 (Corino et al., 1990).

Lichen colonization on the top horizontal surfaces (capstones) of the balustrade was recorded in March 2016 by surveying rectangular plots (30 × 150 cm) distributed in the South (n=5), South-West (n=4), North-West (n=4) and North sides (n=5; Fig. 1c-d).

All the plots shared lithology, horizontal orientation, surface micromorphology and conservation history. The solar exposure of each plot, related to different levels of shading by the Basilica, was estimated from hemispheric photographs, taken with a LG 360 CAM (R-105; LG Electronics inc., South Korea), processed with CAN-EYE software (v. 6.495; Weiss and Baret, 2017) and evaluated with regard to the portion (%) of the sky non-covered by the building (Supplementary note S1). Distance from the nearest trees (meters) was also calculated, as increasing proximity could be related to increasing eutrophication of the balustrade surface (Nascimbene et al. 2009a).

Each plot was surveyed using a grid divided into three lines of 15 quadrats (10 × 10 cm). Small samples of lichen thalli were collected by non-destructive techniques to check in the laboratory provisional identifications assigned during fieldwork. Lichen species were identified using Clauzade and Roux (1985), Smith et al. (2009) and monographic descriptions. Nomenclature follows Nimis (2016). Sample vouchers were deposited at the Cryptogamic Herbarium of the University of Torino (HB-TO Cryptogamia).

2.2 Population pattern analyses

Population pattern analyses were carried out for the eight more abundant species: *Candelariella aurella* (Hoffm.) Zahlbr., *Circinaria calcarea* (L.) A. Nordin, Savić & Tibell, *Circinaria contorta* (Hoffm.) A. Nordin, Savić & Tibell, *Myriolecis albescens* (Hoffm.) Śliwa, Zhao Xin & Lumbsch, *Myriolecis* gr. *dispersa* (Pers.) Śliwa, Zhao Xin & Lumbsch,

101 *Pyrenodesmia erodens* (Tretiach, Pinna & Grube) Søchting, Arup & Frödén, *Verrucaria*
102 *macrostoma* DC., and *Xanthocarpia crenulatella* (Nyl.) Frödén, Arup & Søchting.

103 For each species, the following abundance (i-ii) and distribution (iii-iv) parameters were
104 considered:
105 (i) total frequency, as % occurrence in the 18 rectangular plots throughout the balustrade;
106 (ii) average frequency, as mean % occurrence in the 10 × 10 cm quadrats of each plot;
107 (iii) Moran's I coefficient (Moran, 1950), to evaluate the species distribution pattern along the
108 balustrade (distance matrix in Table S1) according to a clustered or diffuse/random spatial
109 structure, and computed using the R-software Version 3.5.1 (The R Foundation for Statistical
110 Computing, 2018), ape package (Paradis et al., 2004);
111 (iv) an average Spread Index (SpIn), here proposed to evaluate whether the species have a
112 clustered or diffuse distribution at the scale of each plot, and computed for each plot as
113 follows:

114 $SpIn = Av_{full}/Av_{empty}$

115 where Av_{full} is the average number of colonized quadrats surrounding each colonized quadrat,
116 and Av_{empty} is the average number of colonized quadrats surrounding each uncolonized
117 quadrat (details on the SpIn calculation in Supplementary note S2).

118 Differences among species in terms of average frequency per plot and Spread Index were
119 tested by ANOVA with post-hoc Tukey's test using SYSTAT 10 ($P<0.05$ as significant).
120 Potential relationships of population patterns with the microenvironmental parameters varying
121 along the balustrade were also considered. In particular, Pearson correlation of species
122 frequency with solar exposure (CorrSol) and distance from nearest trees (CorrTree) were
123 calculated, on the basis of data quantified for each plot, using SYSTAT 10 (post-hoc
124 Bonferroni's test; $P<0.05$ as significant). Ecological indicator values proposed by Nimis

125 (2016) on the tolerance range of each species with regard to light and eutrophication were
126 considered for comparison.
127
128 *2.3 Dispersal analysis*
129 The aerobiology pathways of the species were examined in situ with regard to the release of
130 reproductive structures, and in the laboratory considering their air take-off and potential
131 dispersal at short and long distance.
132 The ejection of ascospores and, in the case of *P. erodens*, the release of soredia (i.e. vegetative
133 propagules) were evaluated directly on the balustrade by placing overturned Petri dishes
134 containing agar medium (15 g l^{-1} Agar A1296, Sigma Aldrich Chemie, Steinheim, Germany,
135 in deionized water; Petri diameter = 5.5 cm; agar layer 6 mm high) on both areas colonized by
136 the different species and areas lacking colonization (control). Petri dishes were positioned
137 after that thalli had been hydrated for one hour with patches of sterile paper soaked with
138 deionized water. Agar surfaces were exposed on the balustrade surface for three hours, during
139 which air flow allowed the progressive desiccation of thalli. For each species, replicates
140 (n=15) were performed in autumn-winter 2016 and in summer 2018, placing the Petri dishes
141 in different parts of the balustrade. The number of apothecia covered by each Petri dish was
142 counted by image analysis of high quality photographs taken with a Nikon Coolpix AW100,
143 using ImageJ (1.51g; US National Institutes of Health, Bethesda, USA). The ascospores were
144 counted under a Nikon Eclipse 50i microscope (400× magnification).
145 Air take-off and the consequent short and long distance potential dispersal of reproductive
146 structures were evaluated following the protocol described by Favero-Longo et al. (2014) and
147 Morando et al. (2017). Apothecia of the epilithic species *C. aurella, C. calcarea, C. contorta,*
148 *M. albescens,* and *X. crenulatella*, and of the endolithic *M. dispersa,* were collected from the
149 balustrade and pasted on an adhesive tape at the bottom of Petri plates in groups of 100-400

150 units, to be used for the experiments. Fertile thalli of the epilithic peritheciate *V. macrostoma*
151 and the endolithic *P. erodens* were instead collected from natural outcrops due to difficulties
152 in sampling intact reproductive structures from the balustrade without affecting the stone
153 substrate. In the laboratory, for each species, apothecia (or whole thalli of *V. macrostoma* and
154 *P. erodens*) were immersed for 15 min in deionized water and incubated for three days within
155 a perspex box (120 x 70 x 60 cm) including a fan (FT30-G1, Viglietta SPA, Italy) moving the
156 air at about 4 m sec^{-1}, 10 Petri dishes (diameter = 5.5 cm) containing agar medium and a
157 Hirst-type volumetric sampler VPPS 2010 (Lanzoni, Italy), typically used for aerobiological
158 monitoring. The Petri dishes, encircling the rocks at a distance of 5-15 cm from the exposed
159 reproductive structures, were used as passive traps to capture short-range dispersed
160 propagules. With the VPPS, located with the suction nozzle at 40 cm from the ground layer
161 and at 50-70 cm from the apothecia, the propagule dispersion in the air volume was examined,
162 the spore take-off and entering the turbulent air being considered a requisite for long-range
163 dispersal. For each species, the experiment was replicated two times, due to limitations in the
164 sampling of intact apothecia from the balustrade. The ascospores that impacted on the VPPS
165 adhesive tape and on the agar surfaces were quantified at 400x magnification according to
166 Favero-Longo et al. (2014).

167 Population structure parameters, dispersal parameters (maxima values of ejected spores, and
168 spores involved in short- and long distance dispersal) and microenvironment-related
169 parameters (CorrSol, CorrTree) calculated for each species were indexed and processed
170 through a Redundancy Analysis (RDA) to summarize the joint variations, choosing forward
171 selection of variables option (Legendre et al., 2011). Each parameter was indexed with
172 reference to an ordinal scale (from 1 to 10), with ranges defined dividing in ten parts the
173 interval between the maximum and minimum observed values. In the case of micro-
174 environmental parameters, the absolute values were used for the indexing procedure, as both a

positive or a negative correlation indicated the influence of the variable conditions on the population patterns. RDA was performed using CANOCO 4.5 (Ter Braak & Šmilauer, 2002).

3. Results

3.1 Population patterns

The balustrade of the podium of the Basilica was abundantly colonized by lichens (11 species in total), with the 8 selected species occurring in at least 50% of the plots (Fig. 2a). With reference to the ecological indicator values (Table 1), the requirement of all these species with respect to the light factor ranges from plenty of diffuse light (light index=3) to a very high direct solar radiation (5). With respect to their tolerance to eutrophication, *Circinaria calcarea* and *Pyrenodesmia erodens* are only resistant to a weak eutrophication (max eutrophication index=3), while other species occur in rather to highly eutrophicated conditions (max eutrophication index=4-5). In substantial agreement, from a syntaxonomic point of view all the species are diagnostic of the class Verrucarietea nigrescentis, including calcicolous (hemi-)nitrophilous lichen associations, with the exception of *P. erodens*, which is diagnostic species of Clauzadeetea immersae, including non or slightly nitrophilous associations (Roux et al. 2009; syntaxonomic scheme in Table S2).

The population structure reflects approx. 25 years of recolonization dynamics following the last cleaning intervention. *Candelariella aurella* and *Myriolecis dispersa* were the most abundant in terms of both total frequency and average frequency per plot (100% of the plots and >60% of quadrats per plot, respectively; Fig. 2b-c). With regard to this latter parameter, the other species were in a range between 7% (*Verrucaria macrostoma*) and 31% (*Xanthocarpia crenulatella*) and most of the slight differences were not statistically

198 significant. All the thalli exhibited a healthy appearance, with the exception of those of *P.*
199 *erodens*, most of which were ailing and partially covered by cyanobacteria.
200 All species occurred in at least one plot of each plot group, at the differently exposed sides of
201 the balustrade, with the exception of *C. calcarea* which was absent in the S-exposed corner.
202 Moran I' coefficients for *C. calcarea*, *X. crenulatella*, *M. albescens*, *M. dispersa*, and *P.*
203 *erodens* indicated a clustered distribution pattern even if the values were low (<0.3),
204 suggesting that the spatial autocorrelation was not strong (Fig. 2d). At the plot level, values of
205 the Spread Index were significantly higher for *C. aurella* than for *C. calcarea* (0.8) and *P.*
206 *erodens* (0.5), indicating diffuse and clustered distribution respectively (Fig. 2e). The other
207 species had intermediate SpIn values in the range 0.9-1.1.
208 Correlation coefficients higher than |0.5| were calculated for frequencies per plot and solar
209 exposure (CorrSol) with regard to *C. calcarea* (-0.765), *M. dispersa* (-0.637) and *M.*
210 *albescens* (-0.560), and for distance of nearest trees (CorrTree) in the case of *X. crenulatella*
211 (-0.524; Table 1). However, only the negative CorrSol of *C. calcarea*, indicating its higher
212 frequency in more shaded plots, resulted significant (P<0.05).

213 *3.2 Specific reproductive potential*

214 Significant differences in the quantity of reproductive structures dispersed by the eight species
215 were found, with remarkable variability for both the ejection and the short- and long-distance
216 dispersal steps (Fig. 3).
217 In situ tests showed that all the six apotheciate species and the peritheciate *V. macrostoma*
218 ejected ascospores (Fig. 3a). The strong variability between different replicates seems to be a
219 prominent trait of some species. Most of the ascospores were still arranged in packages (in
220 most cases still including eight ascospores), while the ejection of single spores was a less
221 frequent phenomenon (Fig. 4a-c). *C. aurella* displayed the highest values of maximum, 75th
222 percentile and median ejection, accounting for an ejection capability significantly higher than
223 that of the other species. The 75th percentiles of ejection values recorded for *X. crenulatella*,

224 *M. albescens*, *M. dispersa*, and *V. macrostoma* (in the range 0.13-0.16 ejected spores per
225 apothecium, sp·ap^{-1}) were 2.6 to 5.3 times higher than those of *C. contorta* (0.05 sp·ap^{-1}) and
226 *C. calcarea* (0.03 sp·ap^{-1}). High values of ascospore ejection, however, were also observed
227 for two replicates of this latter species (up to 0.7 sp·ap^{-1}). No soredia, characterizing the
228 asexual reproduction of *P. erodens*, were captured or observed.
229 In laboratory assays, all the apotheciate species displayed potential for both short- and long-
230 distance dispersal, while dispersed reproductive structures were not collected for the
231 peritheciate *V. macrostoma* and the sorediate *P. erodens* (Fig. 3b). For all the species, single
232 ascospores rather than ascospore packages, were collected by both the passive traps and the
233 volumetric sampler (Fig. 4d-g). Short distance dispersal was mostly recorded on Petri dishes
234 in lee position with respect to the exposed apothecia, indicating that ascospore split and
235 deposition immediately followed the package discharge (data not shown). Notwithstanding
236 that only two replicates for each species were performed, some differences among the
237 apotheciate species were worth noting. *M. albescens* showed the highest value of spores
238 involved in short-range dispersal (1.2 sp·ap^{-1}), approx. the double of the maxima observed for
239 *M. dispersa* and *X. crenulatella*, and three and four times higher than those of *C. aurella* and
240 *Circinaria* sp. pl. respectively. With regard to spores available for long-range dispersal, *M.*
241 *dispersa* (1.3 sp·ap^{-1}) and *C. aurella* (1.2 sp·ap^{-1}) had the highest values, followed by *X.*
242 *crenulatella* (0.7 sp·ap^{-1}) and, with approx. three times lower values, *M. albescens* and
243 *Circinaria* sp. pl. (0.4 sp·ap^{-1}).
244 In the RDA (Fig. 5a-b), the first three axes explained 97.1% of the variance of relationship
245 between population structure, dispersal and microenvironment-related parameters. Total
246 frequency through the plots, average frequency per plot, Spread Index (for which higher
247 values indicate a higher species diffusion) were positively related along the first axis (66.3%
248 of total variance) with ejected spores, spores available for long-distance dispersal and
249 CorrTree. The Moran I's coefficient was positively related with CorrSol and the spores

involved in short-distance dispersal along the second (25.7%) and third (5.1%) axes, respectively. Spores available for long-distance dispersal and CorrSol displayed the highest conditional factor (F=5.30 and F=4.33, respectively; P<0.05) according to forward selection, followed by CorrTree (F=2.67), spores involved in short-distance dispersal (F=1.50) and ejected spores (F=1.13), which were not significant (P>0.05). *C. aurella* and *M. dispersa*, scattered in the right side of the diagrams (Fig. 5a-b), were positively associated with spores available for long-distance dispersal. In the left upper side, characterized by the Moran I's coefficient, *C. calcarea* and *M. albescens* related with CorrSol and spores involved in short-distance dispersal, respectively. *P. erodens*, *V. macrostoma*, and *C. contorta* scattered in the left lower side of the diagrams, oppositely to spores available for long-distance dispersal and CorrSol. Similar relationships were observed in two other RDAs considering the median and 75th percentile values of spore ejection rather than the maximum (data not shown).

4. Discussion

Our findings reject the tested null hypothesis and indicate that both microclimate requirements and specific patterns of propagule dispersal contribute to determine the local abundance and spatial patterns of epilithic and endolithic lichens encrusting calcareous heritage surfaces. Since in our study site the last cleaning intervention dates back to 1990 (Corino et al., 1990), these results may provide a keystone reference for elucidating recolonization dynamics following restoration activities.

In previous literature (Ariño et al., 1995; De los Ríos et al., 2009; McIlroy de la Rosa et al., 2013), spatial distribution of lichens on heritage structures was related to microenvironmental conditions. This could apply also to our balustrade where species frequencies per plot were partially related to different levels of solar exposure and distance from nearest trees, responsible for eutrophication (Nascimbene et al. 2009a). The significant negative CorrSol of

C. calcarea, and the highest value of CorrTree of *X. crenulatella*, likely reflect different microenvironmental requirements which may account for the spatial autocorrelation recorded for these species. Higher frequency of *X. crenulatella* with increasing proximity to trees fits the high nutrient requirement of this nitrophilous species (Nimis 2016). Instead, despite the significant negative value of CorrSol for *C. calcarea*, ecological indicator values suggest for this species a higher tolerance of direct light radiation than that of *V. macrostoma* and *X. crenulatella*, which show positive CorrSol values. Both these incongruencies -for which it should be taken into account that ecological indicator values are expert assessments (Nimis 2016)- and the weak statistical support to the calculated CorrSol and CorrTree (mostly with $P>0.05$) reflect the fact that the eight species have comparable microenvironmental requirements. Moreover, these evidences suggest that species spatial patterns may be also related to other factors as in the case of the quantified differences in the reproductive potential (Ellis, 2019). Actually, RDA results showed that both spores available for long-distance dispersal, i.e. a reproduction-related factor, and CorrSol, i.e. a microenvironment-related factor, were significant determinants of the population structure. In particular, the positive correlation of species abundance with spores available for long-distance dispersal and ejected spores underlines the need of a high release of reproductive structures to successfully establish and spread colonization (Scheidegger and Werth, 2009). Species ejecting a higher number of ascospores, which are also more easily taken off and dispersed, as *C. aurella* and *M. dispersa*, are the most abundant on the balustrade and are therefore the main candidate as deterioration agents. They have great potential for effective and rapid recolonization after cleaning interventions if fertile thalli occur in the surroundings of the heritage site, even at long distance. For other species, as *M. albescens*, the patchy and clustered distribution, is related to a high number of spores involved in short-distance dispersal, but with a low potential for long-distance dispersal. Such species, even if present in the nearby of restored

heritage surfaces, are expected to be less aggressive in terms of recolonization potential and more easily controlled.

As ascospore packages were mostly detected in ejection tests, while single spores were collected in dispersal assays, the air movement was likely responsible for a drying effect determining the package disaggregation. It is worth noting that experiments without air movement generally yielded the discharge at a short distance of spores still organized in packages (Garrett, 1971; Ostrofsky and Denison, 1980). Maxima values of spores available for long-distance dispersal were observed for the small spores of *C. aurella* (major axis 10-12 µm) and *M. dispersa* (major axis 8-14 µm), according to the fact that particles with lower diameters always show lower deposition rate and higher life times in the air than larger ones (De Nuntiis et al., 2003). Similarly, higher colonization rates and effective dispersal were related to a lower propagule size for epiphytic lichens (Hilmo et al., 2012; Johansson et al., 2012) as well as for crustose aquatic lichens (Nascimbene et al., 2009b). However, ascospore size may be not the only factor determining the amount of spores available for long-distance dispersal, as values measured for the small spores of *M. albescens* (major axis 8-14 µm) are lower than those for the large spores of *Circinaria* sp. pl. (major axis 20-30 µm). With this regard, ascospores of similar size were shown to be dispersed at different distances in laboratory experiments in absence of air movement (Bailey and Garrett, 1968). Nevertheless, this phenomenon would require further clarification.

In the case of *P. erodens*, the release of soredia was related to its active dissolution of the substratum and the consequent surface exposure to dispersal vectors (Tretiach et al., 2003). However, dispersal of soredia is usually associated to dry surface conditions or to the impact of rain drops (Armstrong, 1991), while the washing strategies we adopted *in situ* and in the lab, stimulating ascospore discharge, may have instead prevented the discharge of propagules of *P. erodens*. Because of their larger size (av. diameter approx. 50 µm) than ascospore (max lentgh: 30 µm in *C. calcarea*) the soredia should be suitable for short distance dispersal, as

generally reported for asexual propagules (Scheidegger and Werth, 2009). This would account for the clustered distribution of this species at both the whole balustrade and plot scale. In this perspective, this species could be critical for heritage conservation due to its endolithic growth (Pinna and Salvadori, 2000) and resiliance to restoration interventions (Sohrabi et al., 2017), rather than for its dispersal and recolonization potential. In the case of the Basilica of Superga, in particular, the unhealthy condition of *P. erodens* suggests that current environmental conditions are less favourable for the species than in the past and that its thalli may be remnants dating back the last cleaning intervention, and not removed because of their endolithic growth.

The low abundance of the peritheciate *V. macrostoma* on the balustrade is consistent with poor successes in the ejection tests and the absence of spores in laboratory dispersal assays. The absence of ascospores of *V. macrostoma* even in the passive traps may indicate a limitation of the distance of discharge within the 5-15 cm from the nearest source, which is compatible with the weak discharge capability quantified for some species (Bailey and Garrett 1968). Nevertheless, we cannot rule out that dispersal by *V. macrostoma* may depend on vectors other than air, as animals, feeding of its thalli (which do not contain deterrent secondary metabolites) and thus becoming responsible of zoochory (Baur et al. 1995; Asplund 2010). The low abundance of *V. macrostoma* on the balustrade may also depend on a low availability of compatible photobionts (Spitale and Nascimbene 2012): *V. macrostoma* has photobiont partners (e.g. *Coccobotrys*, *Protococcus*; Stocker-Wörgötter and Turk 1989) different from those of the genus *Trebouxia* that are shared by the other species (Smith et al. 2009). However, the fact that *V. macrostoma* is more abundant on other calcareous balustrades of cultural heritage sites in Torino (Supplementary note S3), suggests that local conditions may affect fertility and reproductive performance of the species (Monte, 1993).

5. Conclusive remarks

Results of our quantitative analyses indicate that the spatial distribution of lichens on the balustrade of the Basilica of Superga -representative of calcareous heritage surfaces in the dry submediterranean area of Italy (sensu Nimis 2016)- is correlated with species-specific patterns of propagule dispersal, as revealed by aerobiological experiments, and microenvironmental requirements. In particular, apotheciate species that have a higher ejection of ascospores with higher potential for long range dispersal are candidate for a much aggressive spreading on the monumental surfaces. Moreover, their occurrence on natural or artificial stone surfaces in the surroundings of the stone monumental surface may easily support recolonization dynamics after cleaning interventions, as an effective supply of propagules is expected (Favero-Longo et al., 2014). When these species occur in the surroundings, a restoration plan more effective in the long term should thus consider the adoption of protocols to decrease the bioreceptivity of the monumental surface (Pinna, 2017; Fidanza and Caneva, 2019) and/or, if practicable (e.g. in the case of a localized occurrence), their removal. On the other hand, species with a lower dispersal rate have a more clustered distribution and are less effective in rapid recolonization, thus representing a minor threat for cultural heritage conservation. When they occur on surfaces adjacent to or in the close proximity of the monument (at a distance in the order of few meters or less), their removal appears similarly recommendable to drastically reduce the risk of recolonization. When they occur at greater distances, their presence may be instead safely considered as a biodiversity value for the often invoked joined valorization of natural and cultural heritage (Nimis et al., 1992).

All these results strongly support the idea that information on the reproductive strategy of lichens, and, in particular, the evaluation of their local dispersal patterns should be coupled with traditional analyses on stone bioreceptivity and microclimatic conditions to plan effective restoration interventions of stone surfaces.

Acknowledgements

Field and laboratory activities were carried out thanks to funds from the 'Università di Torino' and 'Compagnia di San Paolo' (Project CSTO169034/2016: 'From rocks to stones, from landforms to landscapes – geoDIVE' - PROactive management of GEOlogical heritage in the PIEMONTE Region). MM was a recipient of a PhD fellowship funded by INPS (Istituto Nazionale di Previdenza Sociale). The authors are grateful to Comune di Torino, Soprintendenza Archeologia Belle Arti e Paesaggio per la Città Metropolitana di Torino and Direzione del Polo Museale del Piemonte for permission to access the monumental sites. The authors are also thankful to Irene Franciosa, Chiara Michelis and Giulio Borghi (Università di Torino) for assistance during fieldwork. The authors are grateful to the Editor E. Paoletti and anonymous reviewers for the valuable suggestions which improved the effectiveness of the study.

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549

550 **Figure captions**

551 **Fig. 1.** Study site: **a** the Basilica of Superga (Torino, NW Italy) viewed from SW; **b** the
552 balustrade of the podium of the Basilica; **c** distribution of the relevés (n=18, dots) along the
553 balustrade (scale bar = 5 m); **d** lichens on the top horizontal surface of the balustrade, covered
554 with the 10 × 10 cm square grid.

555 **Fig. 2.** Lichen abundance and distribution on the balustrade of the Basilica of Superga. **a**
556 Presence/absence of the surveyed species (*Candelariella aurella*, Can.aur; *Myriolecis* gr.
557 *dispersa*, Myr.dis; *Myriolecis albescens*, Myr.alb; *Verrucaria macrostoma*, Ver.mac;
558 *Circinaria calcarea*, Cir.cal; *Circinaria contorta*, Cir.con; *Xanthocarpia crenulatella*,
559 Xan.cre; *Pyrenodesmia erodens*, Pyr.ero;) through the quadrats of the 30×150 cm plots (1-18;
560 black squares indicate the species occurrence). **b** Total frequency of the species through the
561 balustrade (% of plots which host the species; contribution to the species abundance from
562 plots in the S, dark grey, SW, grey, NW, light grey, N, white, parts of the balustrade). **c**
563 Average frequency per plot of each species (av. % of quadrats which host the species;
564 according to Tukey's test, columns which do not share at least one letter are statistically
565 different, $P<0.05$). **d** Moran I's coefficient (*, $P<0.05$). **e** Spread Index (according to Tukey's
566 test, columns which do not share at least one letter are statistically different, $P<0.05$).

567 **Fig. 3.** Dispersion of reproductive structures by the surveyed species (abbreviations as in Fig.
568 2). **a** Ejected ascospores (per apothecium, as evaluated directly on the balustrade of the
569 Basilica of Superga): boxplots showing maxima values (upper whisker), 75th percentile (top
570 box), median (transversal line), 25th percentile (bottom box), minimum (lower whisker),
571 outliers (circles). **b** Ascospores dispersed at short distance and long distance (per apothecium,
572 as evaluated in the laboratory microcosm): maximum (black symbols) and minimum (white
573 symbols) values of ascospores collected within the passive traps at short distance from the

incubated ascocarps (squares) and ascospores taken off and collected by the Hirst-type volumetric sampler, available for long distance dispersal (circles). Reproductive structures of *Pyrenodesmia erodens*, lacking ascocarps, but showing soredia, were never collected, neither in ejection nor in dispersal assays.

Fig. 4. Ascospores collected in ejection and dispersal assays. **a-c** Packages of spores collected in ejection tests with *Candelariella aurella* (**a**), *Verrucaria macrostoma* (**b**) and *Xanthocarpia crenulatella* (**c**). Bars = 20 μm. **d-e** Ascospores of *Circinaria contorta* within an ascocarp (*, **d**) and collected with the passive traps at a short distance from the reproductive structures incubated in the laboratory microcosm (**e**). **f-g** Ascospores of *Xanthocarpia crenulatella* collected by the Hirst volumetric sampler in the laboratory microcosm (**f**) and within an ascocarp (*, **g**). Scale bars: 20 μm (a-e, g), 10 μm (f).

Fig. 5. Factorial map of RDA carried out on population structure parameters (total frequency through the plots, av. frequency per plot; Moran I's coefficient; Spread Index), dispersal (maxima values of ejected spores, and spores involved in short- and long distance dispersal) and microenvironment-related (CorrSol, CorrTree) parameters. **a** Axes 1 and 2. **b** Axes 1 and 3. Species abbreviations as in Fig. 2.

Table 1. Ecological indicator values (Nimis 2016) of lichen species recorded on the balustrade of the Basilica of Superga, and Pearson correlation coefficients (ρ) of species frequency per plot with solar exposure (CorrSol) and distance from nearest trees (CorrTree). Indicator scale for Light (Nimis 2016): 1 - in very shaded situations, 2 - in shaded situations, 3 - in sites with plenty of diffuse light but scarce direct solar irradiation, 4 - in sun-exposed sites, but avoiding extreme solar irradiation, 5 - in sites with very high direct solar irradiation; indicator scale for Eutrophication (Nimis 2016): 1 - not resistent to eutrophication; 2 - resistent to a very weak eutrophication; 3 - resistent to a weak eutrophication; 4 - occurring in rather eutrophicated situations; 5 - occurring in highly eutrophicated situations. Significant correlations according to Bonferroni's test ($P<0.05$) are reported in bold.

	Ecol. indicator values		CorrSol		CorrTree	
Species	Light	Eutrophication	ρ	P	ρ	P
Candelariella aurella	3-5	2-4	-0.273	1.000	-0.463	0.422
Myriolecis gr. *dispersa*	3-5	2-4	-0.637	0.160	-0.405	0.766
Myriolecis albescens	3-5	3-4	-0.560	0.566	-0.226	1.000
Verrucaria macrostoma	3-4	3-5	0.333	1.000	-0.208	1.000
Circinaria calcarea	4-5	2-3	**-0.765**	**0.008**	0.060	1.000
Circinaria contorta	3-4	3-5	-0.437	1.000	-0.223	1.000
Xanthocarpia crenulatella	4	4	0.440	1.000	-0.524	0.205
Pyrenodesmia erodens	4-5	2-3	0.493	1.000	0.111	1.000

Figure1
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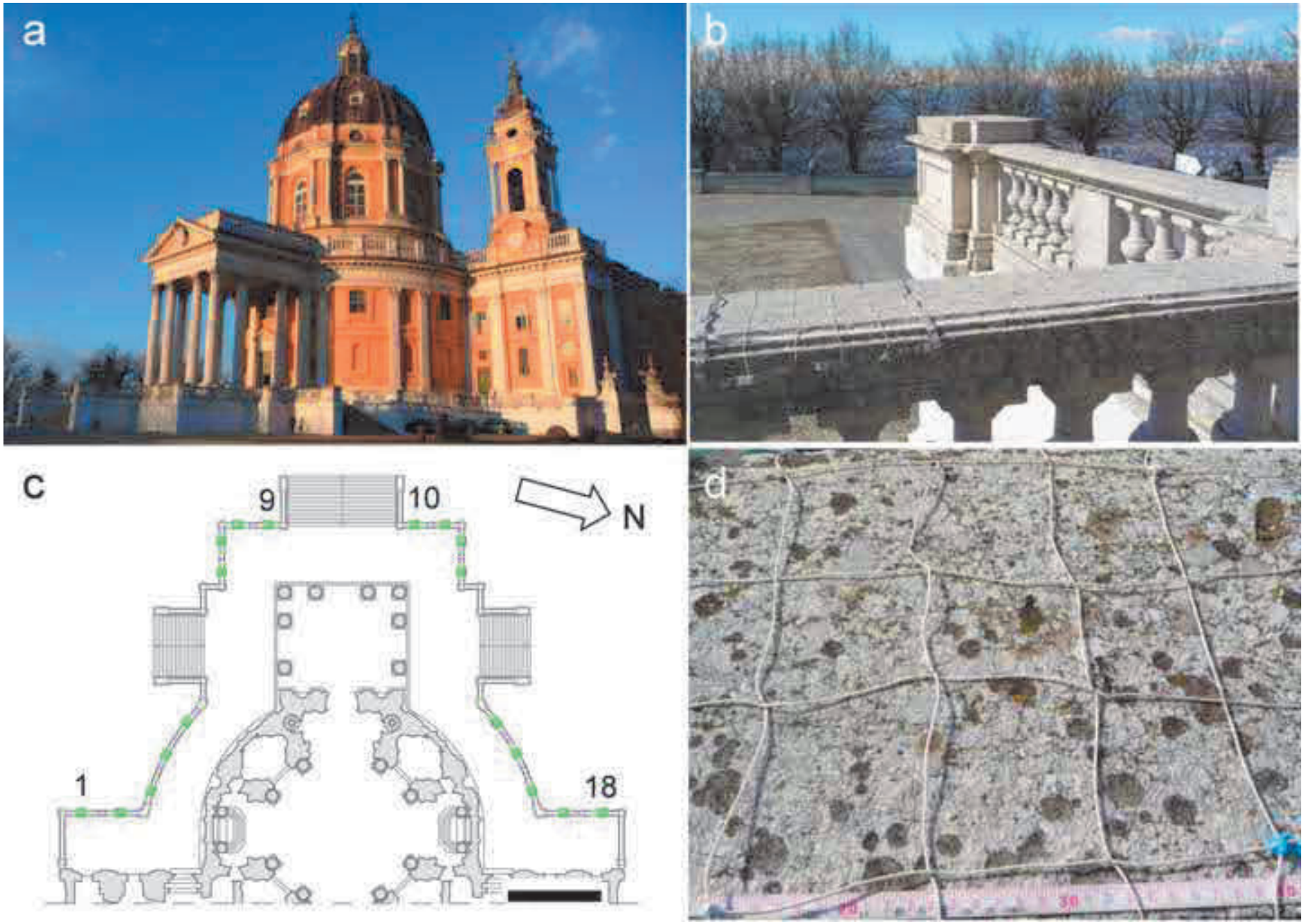

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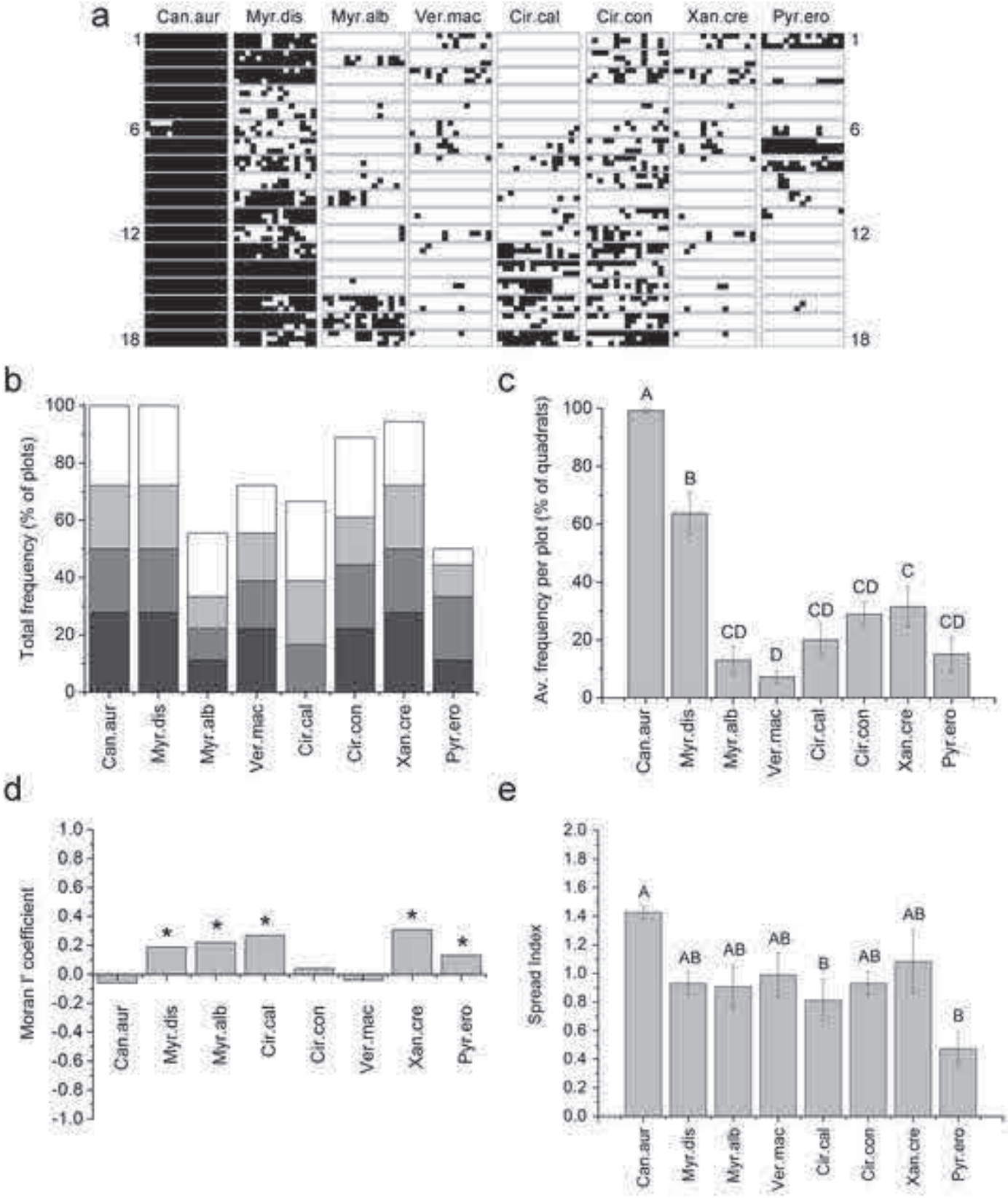

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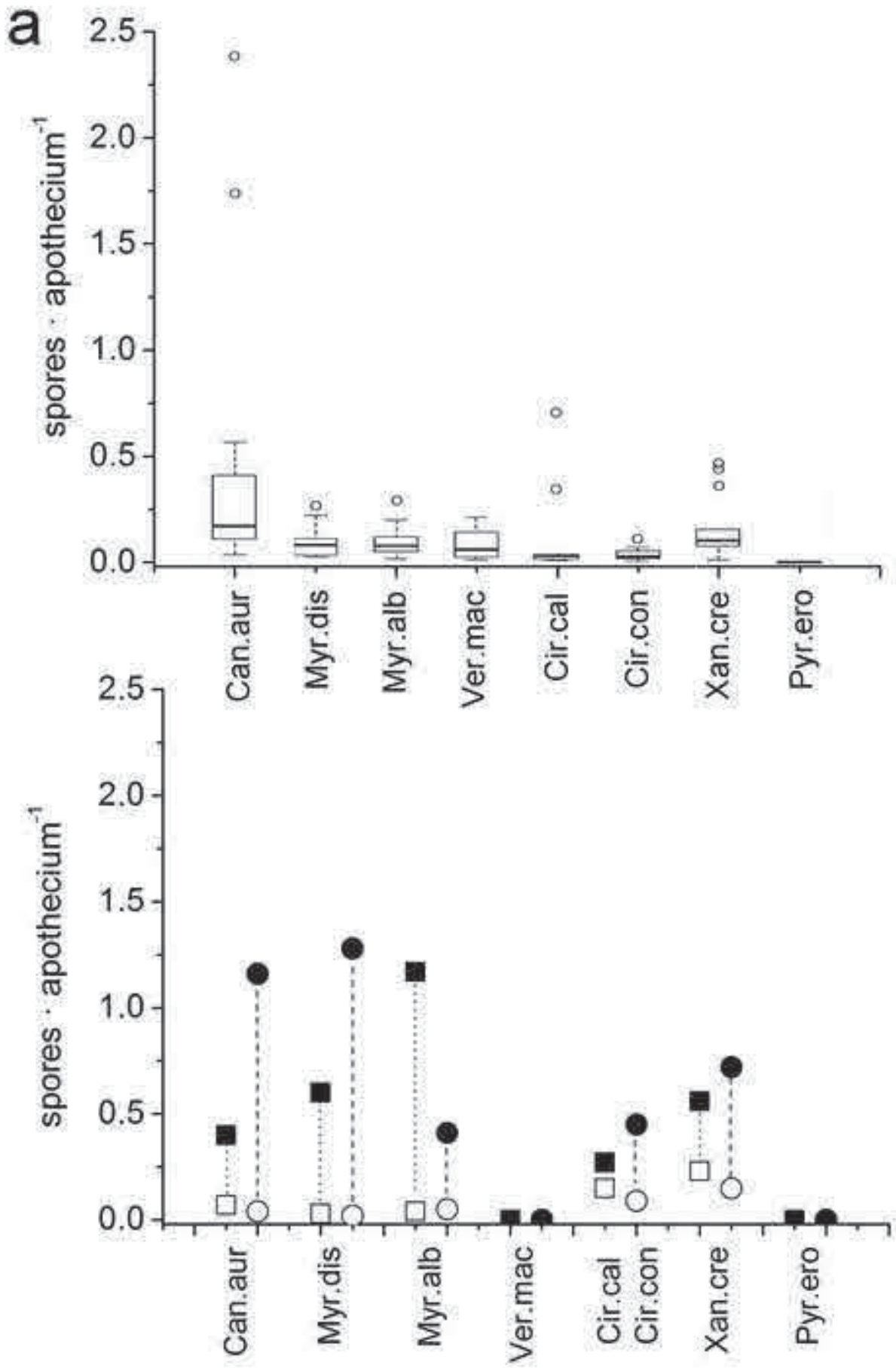

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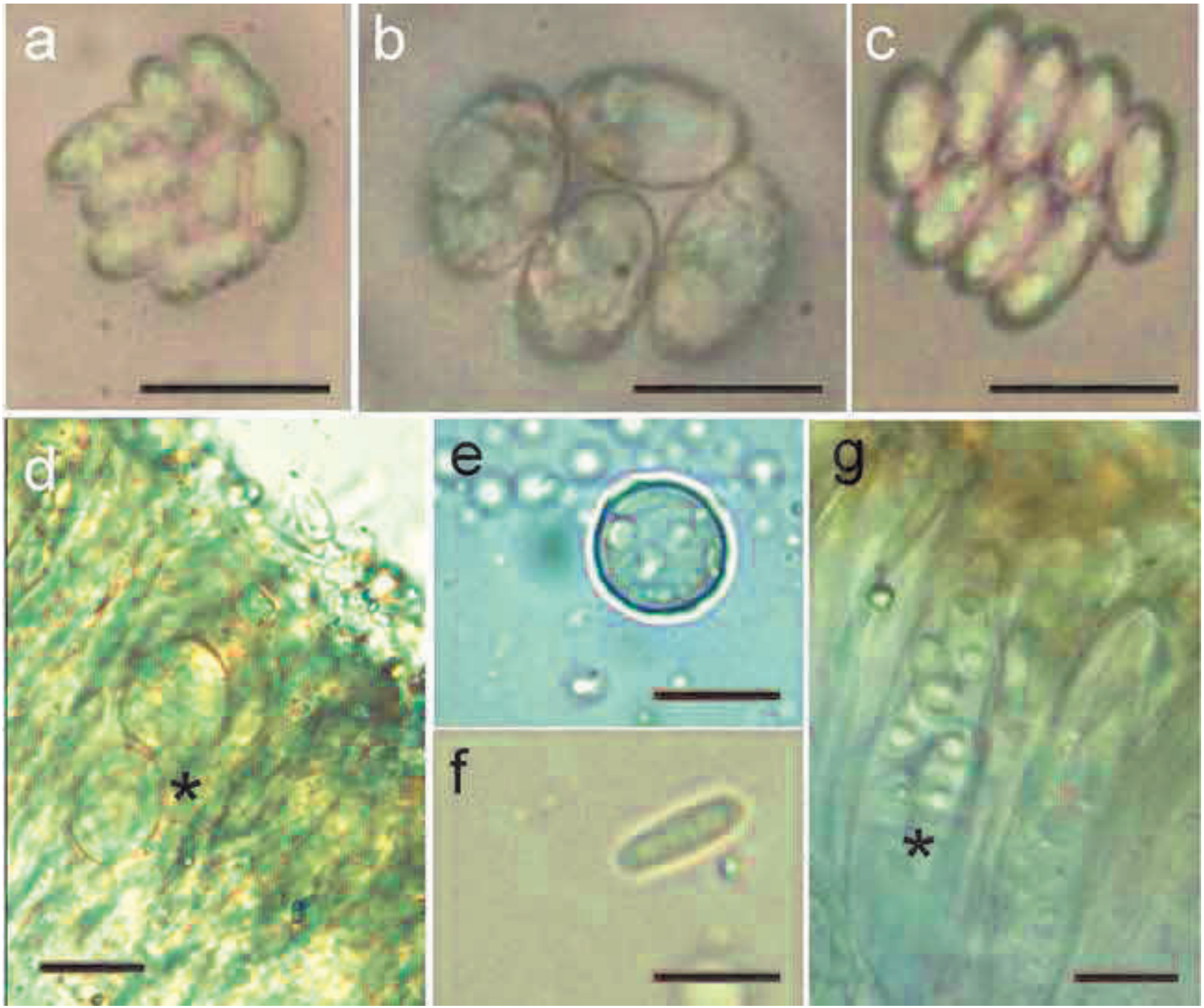

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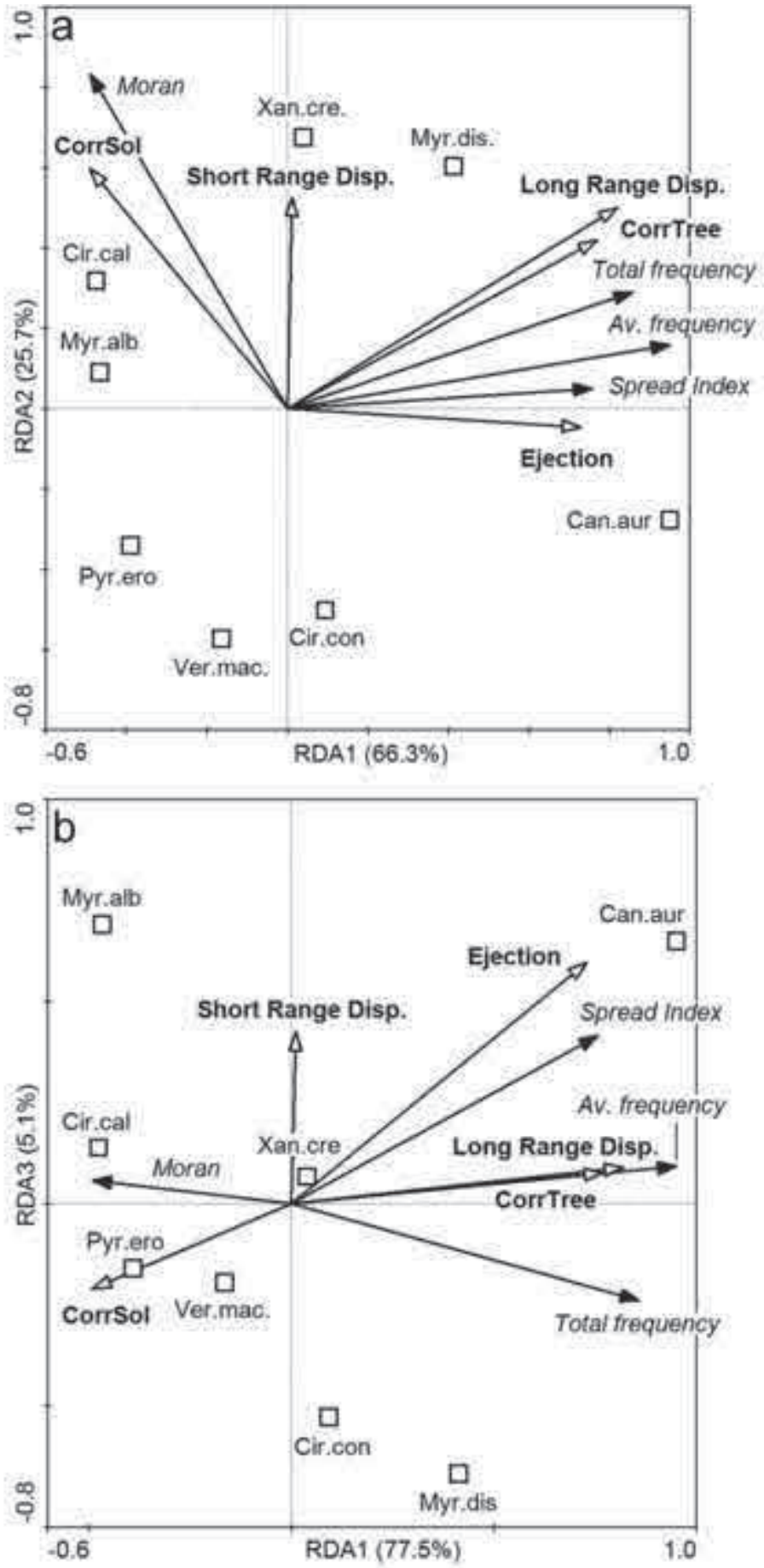

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