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Abstract

On the stone cultural heritage, the influence of architecture-related microenvironmental features on lichen diversity, abundance and consequent threats for conservation has been still poorly characterized to support management plans. Such relationships were here investigated on the vertical surfaces of the House of the Ancient Hunt in Pompeii, archaeological site in S-Italy where the variability of lichen saxicolous communities has been still completely neglected despite their widespread occurrence. Lichen colonization in semiconfined rooms was sporadic and limited to *Dirina massiliensis*, while a remarkable turnover of six communities, encompassing 22 species, characterized mortar, painted and plastered surfaces in outdoor environments, with local covers up to 80%. Microscopic and spectroscopic analyses displayed the deteriogenic potential of three dominant species, due to hyphal penetration within paint and plaster layers (*Verrucaria macrostoma*) and the release of oxalic acid and/or secondary metabolites with acidic and chelating functions (*D. massiliensis*, *Lepraria lobificans*). A higher vertical distance of surfaces from the ground and a larger room dimension were the main conditional factors related to a higher lichen abundance and the distribution of the different communities. Such knowledge on architecture-related microenvironmental features driving lichen distribution and biodeterioration threats may contribute to address restoration priorities and conservation strategies.

Keywords: archaeological areas; biodeterioration; community variability; environmental factors; lichens; stone cultural heritage

1. Introduction

The growth of lithobiontic communities on the stone cultural heritage depends on complex relationships among (micro-)organisms, materials and the environment (Pinna 2017). Physical and chemical properties of a stone substrate determine its bioreceptivity, i.e. its attitude to be colonized by one or several groups of living organisms (Guillitte 1995; Miller et al. 2012). However, patterns of biological diversity and abundance on a certain material vary with its geographic location, related to, e.g., macroclimate and pollution, and may further depend on local factors determining distinct microniches (Caneva and Ceschin 2008; Pinna 2017). Parameters controlling microbial growths, as light intensity, water availability, and temperature, relate to regional climate conditions, but also to extrinsic and intrinsic features of each stone surface, as aspect, shading rates, ventilation, vertical distance from the ground and other properties related to architectural geometries (Cutler et al. 2013; Caneva et al. 2015; Ahmad 2015). Such local factors affect the suitability of each surface to be colonized by a certain lithobiont species -depending on its autoecological requirements-, but they may also influence the external propagule supply which triggers colonization (De Nuntiis et al. 2003).

In the case of lichens, biodeteriogens on a wide spectrum of stone surfaces, the presence of different species was related to biogeophysical and biogeochemical processes with diverse deterioration impact (Gazzano et al. 2009; Salvadori and Casanova-Municchia 2015; Seaward 2015). With this regard, for different materials and climatic areas, the understanding of factors controlling colonization patterns of lichen species may guide the identification of dangerous microclimatic conditions for stone conservation and the definition of restoration priorities. In the case of stone monuments in tropical area, the forest canopy gradient was related to different lichen-dominated communities, with different degrees of aggressiveness (Caneva et al. 2015). In the Mediterranean region, the variability of epilithic communities with respect to gradients of environmental variables was examined on natural rock outcrops, highlighting the importance of solar radiation and water availability at the micro-scale (Giordani et al. 2014). The rich literature on biodeterioration in cultural heritage sites along the Mediterranean basin highlights a high level of lichen diversity and a wide range of lichen-related biodeterioration issues (Nimis et al. 1987; Piervittori 2004; Seaward 2015 with refs. therein). The influence of environmental parameters on lichen diversity in monumental areas was also remarked (e.g. Ariño et al. 1995; Nimis et al. 1998; Nascimbene and Salvadori 2008; McIlroy de la Rosa et al. 2013). However, relationships between architecture-related microenvironmental factors, lichen diversity and abundance, and consequent deterioration threats have been still poorly supported with numerical analyses.

Surprisingly, at the best of our knowledge, lichens have been quite completely neglected in studies on ancient Pompeii, one of the most important archaeological sites in the world, although lichen occurrence is evident on many natural and artificial stone materials (*sensu* Caneva et al. 2008), affecting their aesthetic value and potentially threatening their conservation (Fig. 1A-D). Information has been recently provided on the influence of regional-scale climatic factors on the presence/distribution of biodeterioration phenomena in the archaeological area (Traversetti et al. 2018). However, knowledge on lichen diversity, species distribution, and related deterioration issues is still lacking.

In this paper, we characterized lichen diversity and distribution on masonries and wall paintings of the Pompeian House of the Ancient Hunt (*Casa della caccia antica*). The study aims (a) to verify if the lichen presence is homogeneously distributed through the different rooms of the House, and (b) to test the hypothesis that the distribution patterns of lichen species are related to different materials, aspect and other architecture-related features of stone surfaces, considered as proxies of different microclimatic conditions. Deterioration patterns related to dominant lichen species are also preliminary assayed and discussed with regard to their significance for conservation.

2. Materials and methods

2.1. *Investigation site*

Since its first excavations, the ancient city of Pompeii has begun to display a wide variety of conservation problems, which are particularly difficult to control also due to the extension of the archaeological area (more than 66 hectares), the massive tourist presence, management difficulties, and uncautious or outmoded restoration operations (Wollner 2013). Since 2012, the *Grande Progetto Pompei* aims to enhance the effectiveness of the activities for protecting the archaeological area and to address a transition from extraordinary interventions to a continuous and planned conservation maintenance (Osanna and Rinaldi 2018). In the framework of this and related side projects, several Pompeian monuments were and still are object of restoration and supportive diagnostic investigations.
The House of the Ancient Hunt is now the focus of a two year project (*Da Pompei a Venaria. Per un progetto di conoscenza, valorizzazione, divulgazione: la Casa della Caccia antica*, directed by D. Elia and supported by *Fondazione CRT,* Italy) which aims at a systematic reappraisal of the *domus* in a multisciplinary perspective (Elia and Meirano in press). Stratigraphical verifications, archaeological investigations and studies on building techniques have been made in order to achieve a better knowledge and to allow for revisited interpretations of the phases which characterized the long life of the *domus*. An intensive diagnostic program involving chemists, physicists, geologists, botanists, etc. has been launched aiming at the recognition of building and decoration materials and at supporting conservation operations. Meanwhile, a selection of the wall paintings and a mosaic are the object of practical activities of the Master Degree in Conservation and Restoration of Cultural Heritage of the University of Torino, in agreement with the Foundation Centre for Conservation and Restoration of Cultural Heritage “La Venaria Reale”.

The House (VII.4.48) is a *domus* of approximately 600 m^2 (Allison and Sear 2002) at less than 200 m from the Pompeii *forum*. It was built around the middle of the II century BC and its internal organization was modified in the various phases of use. The House displays an *atrium* and a *peristylium* surrounded by several rooms, distinguishable as outdoor or semi-confined environments for the presence/absence of modern protective roofs, for a total of 24 rooms (Fig. 1E).

The House was excavated between 1833 and 1835. Around the middle of the XIX century, some rooms were covered with sloping roofs in tiles and wood. In the 40s-50s of the XX century, rooms 4 and 15 were covered with roofs in concrete. Interventions of wall integration were led in 1978. The last consolidations were performed between 2009 and 2010. Nowadays, the House displays a wide range of materials, environmental and conservation conditions. In semi-confined environments, wall paintings, plaster of the preparation layer (*arriccio*) and mortar between stone blocks are often well conserved, while in outdoor environments they are generally more deteriorated, with detachments, swelling, discoloration, lichens and biofilms containing cyanobacteria and, subordinately, microcolonial fungi and green algae. Stone blocks of Sarno limestone, volcanic and pyroclastic rocks, composing the structure of ancient walls and modern integrations, are well preserved, with their surface poorly characterized by lithobiontic communities. In most of the rooms, the floor is covered with soil for protective purposes, leading the presence of spontaneous vascular plants.

2.2. Sampling design and environmental parameters

Lichen presence/absence was surveyed on the vertical stone surfaces of the 24 rooms of the House, including: (a) ancient Roman and (b) modern mortars binding the tuff and limestone blocks, (c) plastered and (d) painted surfaces. Observations were run with the aid of a hand-lens in June 2017 from the ground level up to 3.5 m in height, while higher levels (where present) were only visually observed from the distance. The maximum lichen cover (%) within each room was evaluated by visually surveying a single 50 × 50 cm plot, selectively placed on the most colonized surface.

Diversity relevés were performed through the rooms where lichens occurred with a maximum cover higher than 1% (Fig. 1E-H), with the exception of rooms 16 and 17, where most of lichen colonization was located at a height higher than 3.5 m and was not accessible for detailed observations and sampling. In these rooms (n=10), independent 50 × 50 cm plots were preferentially distributed to represent the maximum colonization per material (a-d) per aspect. Each plot was surveyed using a square grid divided into 25 quadrats (10 × 10 cm). The cover of lichen species within each plot and their presence within each quadrat were estimated visually. The frequency of each species within each plot was calculated as the sum of their occurrences within the grid quadrats. Lichens were identified using Clauzade and Roux (1985), Smith et al. (2009), McCune (2016) and monographic descriptions. Nomenclature follows Nimis (2016). Sample vouchers were deposited at the Cryptogamic Herbarium of the University of Torino (HB-TO Cryptogamia).

For each quadrat, beside the material (MAT) and the aspect (ASP), the following features were evaluated, categorized and indexed: vertical distance from the ground (HEI), horizontal distance from the nearest wall corner (DNC), room dimension (ROD). Materials were categorized on the basis of different grain size (see Piovesan et al. 2009) -related to porosity and water retention-, as follows: ancient Roman mortars (4), modern mortars (3), plastered surfaces (2), painted surfaces (1). Rock blocks of ancient and modern walls, on which the colonization was subordinated to and driven by that of the ancient and modern binding mortars, respectively, were not separately categorized, but considered with these latter. Aspect, related to aridity, was categorized as follows (modified from Pharo et al. 1999): SSE (4), WSW (3), ENE (2), NNW (1). The vertical distance from the ground -related to capillary water rise (Hall and Hoff 2007)- was categorized as follows: ≤50 cm (1), 51-150 cm (2), 151-250 cm (3), >250 cm (4). The horizontal distance from the nearest corner -related to humidity stagnation (Abuku et al. 2009)- was categorized as follows: ≤50 cm (1), 51-100 cm (2), ≥101 cm (3). The room dimension -related to ventilation (Zhang and Chen 2006)- was categorized on the basis of the floor area as follows: <5 m^2 (1; rooms 5a, 5c), 5-10 m^2 (2; rooms 1, 9, 21); 11-35 m^2 (3; rooms 7, 11, 14), >35 m^2 (4; room 12).

2.3. Statistics

The relative importance of components of γ-diversity [i.e. similarity (S), relativized richness difference (D), and relativized species replacement (R)] was evaluated for all combinations of plots through the overall rooms and for each separate room by analysing the matrix of species presence/absence with SDR Simplex software (2001) using the Simplex method (SDR Simplex; Podani and Schmera 2011). Similarity (S) was calculated following the Jaccard coefficient of similarity:

$S_{Jac} = a/n$

where *a* is the number of species shared by the two plots, and *n* is the total number of species.
The relativised richness difference (D) was calculated as the ratio of the absolute difference between the species numbers of each site (*b*, *c*) and the total number of species, *n*:

$D = |b-c|/n$

Relativised species replacement (R) was calculated as:

$R = 2 * \min \{b, c\}/n$

A relativised β-diversity as the sum of R+D, a relativised richness agreement as the sum of R+S, and a relativised nestedness as the sum of S+D were also calculated for each pair of areas following Podani and Schmera (2011).

A first Principal Coordinate Analysis (PCoA-I; symmetric scaling with species score divided by standard deviation, centring samples by samples, centring species by species; Ter Braak and Šmilauer 2002) was performed on the matrix of species frequencies at the plot level to visualize the relatedeness of communities through the House. A second ordination of plots by Principal Coordinate Analysis (PCoA-II) was based on a matrix including microenvironmental features (HEI and DNC of the central quadrat of each plot, prevalent MAT for each plot, ROD, ASP) and overall lichen abundance (LICH, as total of specific lichen frequecies per plot) to visualize their correlation.

Quadrats 10 × 10 cm were classified (UPGMA, Sokal-Sneath as dissimilarity coefficient, arbitrary resolution of ties; Podani 2001) on the basis of species presence/absence. The matrices of species presence/absence and microenvironmental features at the quadrat level were processed through a canonical correspondence analysis (CCA), which partitions variation explained by each variable and constructs a model of significant variables (CCA using biplot scaling for interspecies distances, Hill's scaling for inter-sample distances; choosing forward selection of variables option; performing Monte Carlo permutation test on the first and all ordination axes) (Ter Braak and Verdonschot 1995).
Classification analyses were performed using SYN-TAX 2000 - Hierarchical Classification (Podani 2001), while ordinations were performed using CANOCO 4.5 (Ter Braak and Šmilauer 2002).

2.4. Spectroscopic, chromatographic and microscopic analyses

Millimetric fragments of thalli of dominant species in the House (*Verrucaria macrostoma*, *Dirina massiliensis*, *Lepraria lobificans*) were collected using lancets and inoculation needles, without affecting the colonized substrate, for performing analyses on the lichen potential biodeteriogenic activity (Gazzano et al. 2009). In particular, (i) the production of metabolites chemically affecting mineral stability through acidolytic or chelating actions, as oxalic acid and secondary metabolites, and (ii) the hyphal penetration through the substrate, acting physical disaggregation, were evaluated.

Oxalic acid production was assessed with reference to the occurrence of oxalate deposits in the thalli, evaluated by µ-Raman spectroscopy. Raman spectra were collected with a micro-spectrometer Horiba Jobin Yvon HR800 equipped with an HeNe laser at an excitation wavelength of 632.8 nm, a CCD air-cooled detector operating at -70°C, and an Olympus BX41 light microscope. The spectra were compared with oxalate spectra reported by Edwards et al. (2003).

The production of secondary metabolites was qualitatively evaluated by Thin Layer Chromatography (TLC). At least three specimens per species were extracted with acetone. Silicagel SIL G-25 UV254 (Macherey-Nagel; Düren, Germany) was used as the support on glass plates and a solution of toluene and acetic acid (170:30) was used as the solvent ('Solvent C' sensu Orange et al. 2010) for compound separation. The developed chromatograms were examined using a Spectroline Longlife UV lamp (254 and 365 nm wavelengths; Spectronics Corporation, Westbury, NY, USA) with fluorescent analysis cabinet (without spray reagents). The Retention factor (Rf) of the observed spots were defined in relation to reference compounds [i.e. norstictic acid extracted from *Pleurosticta acetabulum* (Neck.) Elix and Lumbsch and usnic acid produced by Sigma-Aldrich (St Louis, MO, USA)].

Finally, one fragment of painted surface (20 × 10 × 5 mm) colonized by *V. macrostoma*, already detached and lying out of context on the ground of room 12, was used to examine the lichen-substrate interface under reflected light (RLM) and scanning electron (SEM) microscopy. RLM observations were carried out using an Olympus SZH10 on a cross section stained using the periodic acid - Schiff (PAS) to visualize the biological component within the lithic substrate (Favero-Longo et al. 2005). The hyphal penetration component (*sensu* Favero-Longo et al., 2005) was characterized in terms of structural organization and depth of penetration through the paint and plaster layers. SEM observations were carried out with a scanning electron microscope JEOL JSM IT300LV in the secondary electon mode (High Vacuum - Low Vacuum 10/650 Pa - 0.3–30 kV) on a second carbon-coated cross section. The cross-sections are conserved in the Lichen-Petrographic Collection of the Herbarium of the University of Torino (Gazzano et al. 2007).

3. Results

3.1. Lichen diversity and distribution

Lichen colonization characterized 12 out of the 24 rooms of the House (Fig. 1E). Eleven out of seventeen outdoor environments (65%) displayed maximum lichen cover higher than 1%, with values ranging from 2 to 80% (Figs. 1F-H and 2A). Few thalli sparcely occurred in the remnant outdoor environments (maximum cover <1%), appearing negligible for conservation issues. Only one of the seven semi-confined environments displayed colonized surfaces (12% max. cover in room 6; Fig. 1I).

Twenty-two lichen species were found through 52 out of the 75 plots in the 10 rooms surveyed in detail (Table 1), where 31% of plots was instead uncolonized. Diversity in outdoor rooms ranged from 11 to 3 species, with maximum species diversity per plot varying from 5 to 1, while only *Dirina massiliensis* colonized the back wall of the semi-confined room 6 (Fig. 1I). This latter species was the most widespread through the House, occurring in 80% of rooms, followed by *Verrucaria macrostoma* (70%) and *Lepraria lobificans* (70%). These three species also displayed both the highest frequencies per plot (>20%) and per quadrat (>8%), and their cover values were higher than 1% in at least 10% of plots (Fig. 2B, left side). *V. macrostoma* and *D. massiliensis*, in particular, displayed maximum cover values of 80% and 55%, respectively. Other seven species only locally (<3% of plots) displayed remarkable covers up to 10% (Fig. 2B, centre), while remnant species only punctually occurred (Fig. 2B, right side).

The evaluation of the relative importance of components of γ-diversity, i.e., S, D and R, for all pairs of plots showed a low species similarity through the House (S=15.8%), whereas the species replacement was the major component (R=52.8%). Relativized β-diversity (R + D) was 84.2% (Table 2). The SDR analyses performed separately for each room also showed relatively low similarity (av. S= 21.5%) and high species replacement (av. R= 48.7%), with the exception of room 5a and the semi-confined room 6, where two and one species only occurred, respectively. Accordingly, in Principal Coordinate Analysis-I (PCoA-I), which explained 84.9% of total variance of species frequency values, plots of each room were sparcely distributed through the diagram (Fig. S1 and Table S1a).

PCoA-II (Fig. 3A), which ordinated plots on the basis of microenvironmental features and the overall lichen abundance (LICH, as total of specific lichen frequencies) explained 89.1% of total variance (details in Table S1b). LICH was positively correlated, along the first axis (35.8%), with ROD and, subordinately, HEI. Plots with no lichens (n=23) clustered in the left of the diagram, characterized by higher values of MAT and ASP.

The classification of quadrats on the basis of the species presence resulted in the separation of six main groups (*i-vi*), characterized by the combination of the three dominant species (*V. macrostoma, L. lobificans, D. massiliensis*) with one or more subordinate species (Table S2; Fig. S2). The Canonical Correspondence Analysis (CCA; Fig. 3B and C) displays the ordination of clusters *i-vi* with respect to species and microenvironmental features at the quadrat scale. The analysis extracted four main axes which explained 93.0% of species-environmental relationships. The first and all canonical axes were significant (Monte Carlo test, P-value=0.002). All microenvironmental features exhibited significant conditional effect according to forward selection ($P<0.002$; details in Table S3). The first (41.3% of species-environmental correlation) and second (24.2%) axes were characterized by HEI (weighted correlation, w.c., -0.85 with axis 1) and ROD, (w.c. -0.72 with axis 2), respectively, which displayed the higher conditional effects (F-value: 21.2 and 14.2, respectively). MAT, mainly correlated with axis 3 (w.c. 0.75), and the other microenvironmental factors exhibited lower conditional effects (F-values: MAT 9.18, ASP 7.94, DNC 7.30). The three dominant species separately scattered in the diagram, with *V. macrostoma* and *D. massiliensis* positively and negatively correlated with HEI, respectively, and *L. lobificans* negatively correlated with ROD and positively with MAT. In quadrats of cluster *i* (n= 242), characterizing the left side of the diagram (higher height from the ground, dry exposition, far from the humid corners, larger room dimension, fine-grained materials: painted and plastered walls), *V. macrostoma* occurred alone and associated with *F. citrina* or *C. aurella*, both sharing its photophytism, xerophytism and tolerance of high eutrophication (Nimis 2016), and/or *D. massiliensis*. The presence of this latter species alone or associated with species less tolerant of eutrophication (*L. lobificans*, *V. muralis*, *X. lactea*, *P. chalybeia*, *A. calcarea*, *P. incrustans*) characterized cluster *ii* (n= 127), positively correlated with axis 1. Clusters *iii* (n= 69) and *iv* (n= 24), characterized by *F. coronata* and *V. muralis*, respectively, alone or in association with the three dominant and other species, scattered in the center of the diagram. Clusters *v* (n= 146) and *vi* (n= 16) were characterized by *L. lobificans* alone and associated with other species sharing mesophytism and poor tolerance of eutrophication (*v*: *T. incavatum*, *Scytinium* sp., *X. lactea*; *vi*: *S. calcarea*; quadrats scattered in the right side) or with *V. macrostoma* (quadrats scattered in the left side). Basal branches of the classification represented scattered occurrence of rare species, here considered as cluster *vii* (n= 22).

3.2. Lichen potential deterioration activity

Raman spectroscopy displayed spectra attributable to calcium oxalate dihydrate ($CaC_2O_4{\cdot}2H_2O$) within the thalli of *D. massiliensis* (Fig. S3): bands with wavenumber at 1476, 910 and 504 cm^{-1} were assignable to $\nu(CO_2)$ sym, $\nu(CC)$ and $\delta(CO_2)$ sym vibrational modes, respectively (Edwards et al. 2003). Similar spectra were not detected in correspondence of *L. lobificans* and *V. macrostoma*.

TLC on *D. massiliensis* displayed the occurrence of erythrin ($C_{20}H_{22}O_{11}$), also preliminary detected with spot tests (C+ red; *sensu* Orange et al. 2010), and other unidentified susbtances (see Smith etal. 2009). In *L. lobificans*, the occurrence of atranorin ($C_{19}H_{18}O_8$), stictic ($C_{19}H_{14}O_9$), constictic ($C_{19}H_{14}O_{10}$) and (±) roccellic ($C_{17}H_{32}O_4$) acids was detected. No secondary metabolites were found in *V. macrostoma* (data not shown).

On the other hand, *V. macrostoma* displayed a remarkable hyphal penetration within the substrate. RLM (Fig. 4A-D) displayed the continuous presence of a network of hyphae and hyphal bundles (diameter up to 40 µm) through the paint layer and the upper, fine part of plaster, down to 1.0 mm. Hyphal penetration also sparcely affected the deeper part of plaster, with maximum penetration down to 2.0-2.5 mm. SEM observations indicated both the micron-scale porosity of mortar matrix (Fig. 4E and F) and the boundaries of sub-millimetric clasts (Fig. S4) as passageways for the hyphal growth.

4. Discussion

Although lithobiontic communities are remarkable threats for the conservation of cultural heritage, scientific knowledge on biodeteriogens in the archeological area of Pompeii is limited to reports on vascular plants (Ciarallo and D'Amora 1990) and few investigations on microbial patinas of fungi and bacteria, responsible for discoloration and deterioration of mural paintings, respectively (Veneranda et al. 2017; Tescari et al. 2018). Residual occurrence of biogenic pigments causing aesthetic decay was also characterized (Maguregui et al. 2012). A role of wind direction at the regional scale has recently been reported to affect the distribution of biological patinas on differently exposed vertical surfaces of architectural elements (Traversetti et al. 2018).

This study first informs about and quantifies lichen diversity and abundance in a Pompeian House, addressing relationships between microenvironmental features and dominant species and giving an insight into lichen-driven deterioration issues.

4.1. Lichen diversity and deteriogenic potential

Archaeological sites in the Mediterranean region were recognized as hotspots of saxicolous lichen diversity: they are often characterized by a higher co-occurrence of different stone materials and heterogeneous microenvironmental conditions than the surrounding areas, thus favouring the co-occurrence of different communities and, definitely, more species (Nimis et al. 1987). The number of 22 species on the vertical surfaces of artificial stone materials in the 600 m^2 House of the Ancient Hunt (Table 1) is analogous to that reported for the horizontal sandstone flagstones of the approx. 1000 m^2 *forum* of Baelo Claudia (Spain), while 77 species were found on siliceous and calcareous pebbles cemented with mortar in the approx. ten times wider area of the Roman Amphitheatre of Italica (Nimis et al. 1998). The fact that different substrates were considered in the House (mortars, painted and plastered surfaces), but sharing the same carbonate chemistry, likely explains the relatively low number of species. Moreover, it is worth noting that the blocks of both Sarno limestone and volcanic and pyroclastic rocks in the ancient walls and modern integrations were quite uncolonized, revealing a lower bioreceptivity with respect to the surface of artificial stone materials. Accordingly, mortars and building materials were already reported as highly bioreceptive for calcicolous and rather nitrophilous species (Ariño et al. 1995). These latter also characterize the investigated House, with the dominant *V. macrostoma* and, subordinately, *F. citrina*, *F. coronata* and other species which typically occur in rather to highly eutrophicated situations (Table 1; ecological indicator values by Nimis 2016, in S2). Within the other dominant species, for which a lower nitrophytism is reported, *D. massiliensis* often characterizes artificial stone materials, including frescoes, in both outdoor and semi-confined environments (Edwards et al. 1991, 1997; Seaward 2004; Nugari et al. 2009). Accordingly, only this species was found on painted surfaces of the semi-confined room 6, confirming the poor bioreceptivity of semi-confined environments for most lichen species (Roccardi et al. 2008). *L. lobificans* gathers to the *L. nivalis* group, which was already reported on mortars in archaeological sites of Southern Spain (Ariño and Saiz-Jimenez 2004). The dominance of few species, in terms of cover and frequency, and the local or rare occurrence of others (Fig. 2B), generally characterize lichen communities in anthropic habitats, especially at early successional stages (Nascimbene and Salvadori 2008). In terms of maximum abundance per room, values above 80% quantified in two rooms (Fig. 2A; Table 1), on painted surfaces in particular, are similar to cover values reported for calcicolous lichen communities in archaeological sites of Central Italy (Nimis et al. 1987).

Lichen communities in the Pompeian House are thus generally congruent, in terms of diversity and abundance, with those reported in other archaeological areas of the Mediterranean basin. In particular, their abundance, at least at a local scale, accounts for a remarkable potential threat for conservation. With this regard, our findings show for all the three dominant specie in the House, *V. macrostoma*, *D. massiliensis* and *L. lobificans*, patterns of physical or chemical interaction with the substrate which account for potential deteriogenic effects. The hyphal penetration component of *V. macrostoma*, which thoroughly affects the upper layers of the painted surfaces, including the paint layer and the upper fine part of plaster (Fig. 4A-D), promote their physical disaggregation down to 1 mm in depth. Although early stages of hyphal penetration within the rock substrates is related to the intrinsic availability of discontinuities, as pores and fractures (Favero-Longo et al. 2009), pressures subsequently exerted by hyphal structures, during their development and because of expansion and contraction of thalli according to water availability, increase discontinuities between clasts and thus favor their detachment (Ascaso and Wierzchos 1995; Salvadori and Casanova-Municchia 2015). The hyphal growth around matrix fragments and along the boundaries of sub-millimetric clasts (SEM images in Fig. 4E and F, and Fig. S4), may temporarily contribute to their coherence, by adhering to and keeping them together, but is likely to have very negative consequences after the natural decay of thalli or even before, if cleaning interventions are planned without care for biodeterioration patterns (Pinna 2017; Casanova-Municchia et al. 2018). Moreover, hyphal penetration may contribute to the formation of microhabitats and chemical microenvironments within the substrate and thus support the endolithic growth of other lithobiontic microorganisms having biodeteriorative effects (de los Ríos et al. 2002; Sohrabi et al. 2017).

D. massiliensis may similarly or even more deeply penetrate the substrate, as maximum penetration depths of 20 mm were recorded within carbonatic substrates (Seaward and Edwards 1995). In comparison to *V. macrostoma*, which is not known to secrete metabolites with acidic and chelating functions, *D. massiliensis* is recognized as a remarkable agent of biogeochemical processes at the thallus-substrate interface because of its secretion of oxalic acid (Edwards et al. 1997; Salvadori and Casanova-Municchia 2015). Accordingly, *D. massiliensis* thalli contained deposits of calcium dehydrate oxalates ($CaC_2O_4{\cdot}2H_2O$), for which a physiological role in storing and releasing water to counter arid conditions was suggested (Edwards et al. 1997; Adamo and Violante 2000). In terms of biodeterioration of mural paintings, the lichen released oxalic acid, responsible for acidolysis and complexolysis, dissolves calcite and metal-containing pigments, and reacts with free Ca^{2+} forming the oxalate deposits (Unković et al. 2017). Such process leads to pigment discoloration and long-term aesthetic disturbance because of the calcium oxalate insolubility (Adamo and Violante 2000; Rosado et al. 2013), and may account for a lichen origin of oxalate deposits previously reported on Pompeii ruins (Maguregui et al. 2012). Beside oxalic acid, *D. massiliensis* also produces erythrin, which is sufficiently soluble in water (57 mg l^{-1}) to function as metal-chelating agent and further promote chemical deterioration (Iskandar and Syers 1971).

Similarly, *L. lobificans* release atranorin and stictic acid, potentially exerting a deteriogenic role (Ascaso and Galvan 1976). The occurrence in ortho (adjacent) positions of these polyphenolic compounds of certain electron donors polar groups, such as -OH, -COOH and -CHO, largely determines their water solubility and metal complexing capacity (Adamo and Violante 2000). However, *Lepraria* thalli rarely grow directly on the lithic surface, but on soil deposits or mosses, reasonably filtering their interaction with the substrate.

Different levels of potential deteriogenic effect may be thus recognized for the three dominant species, with the threats by *D. massiliensis* > *V. macrostoma* > *L. lobificans* [quantitative estimations using the Index of Lichen Potential Biodeteriogenic Action (Gazzano et al. 2009) in Table S4]. Knowledge on their distribution and the understanding of conditional factors may be thus crucial to face biodeterioration hotspots, establish restoration priorities and plan preventive strategies.

4.2. Community variability and microenvironmental factors

The regional climate primarily influences the environmental conditions of open-air archaeological sites (Caneva and Pacini 2008). In this context, remarkable weather fluctuations characterizing the climate of Pompeii are generally detrimental to conservation (Pérez et al. 2013). In parallel, investigations on other Pompeian Houses showed that different microclimate conditions can be detected between and within the rooms of a single House (Merello et al. 2014), which may be crucial to drive the distribution of different lichen communities.

A microclimate sensor-based monitoring is not available for the House of the Ancient Hunt. Nevertheless, our findings show that architecture-related environmental features as material (MAT), aspect (ASP), vertical distance from the ground (HEI), horizontal distance from the nearest wall corner (DNC) and room dimension (ROD), easily evaluable and related (as proxies) to microclimatic features, are significant conditional factors to drive the distribution of different lichen communities in the House.

The high values of species turnover displayed by SDR analysis both between and within rooms (high R and R+D values in Table 2) and the plot variability within each single room (PCoA-I in Fig. S1) confirm archaeological sites as hotspots of biodiversity because of the occurrence of different microniches (Nimis et al. 1987). According to the multivariate analyses, HEI and ROD are main conditional factors driving lichen distribution (CCA in Fig. 4B and C) and are positively related to lichen abundance (LICH in PCoA-II in Fig. 4A). The higher the surface and the larger the room, the more the lichens: as vertical distance from the ground and room dimension are related to wind velocity and ventilation patterns (Britter and Hanna 2003; Zhang and Chen 2006), influencing particle life times in the air and deposition rates (De Nuntiis et al. 2003), parameters HEI and ROD likely influence the propagule supply necessary to start colonization. Similarly, they influence the deposition of nutrients (Britter and Hanna 2003) which support the occurrence of species rather to highly tolerant of eutrophication (including *V. macrostoma*) at higher distance from the ground and in larger rooms (clusters *i* and *ii*, positively related with HEI and ROD). The same factors may also influence the impact of wind-driven rain as relevant bioclimatic factor driving biological covers in Pompeii (Traversetti et al. 2018). With this regard, high colonization on surfaces with northern and western aspects, favored by West winds influencing wind-driven rain, is here confirmed: LICH opposed to ASP in PCoA-II (Fig. 4A) indicate a positive correlation between high lichen frequencies and NNW exposition. However, maximum cover values were observed on surfaces with SSE aspect at high distance from the ground. Increasing HEI also implies lower capillary water rise, and thus lower water availability (Hall and Hoff 2007), which agrees with the xerophytic trait of species of clusters *i* and *ii*, and it is also congruent with their drier southern exposition (higher ASP values) and their higher distance from wall corners, i.e. from humidity stagnation (Abuku et al. 2009). By contrast, species of clusters *v* and *vi*, including *L. lobificans*, grow at lower HEI, ROD, ASP and DNC values, according to their mesophyly and poor tolerance of eutrophication. Accordingly, factors regulating humidity, solar radiation and temperature were already shown to drive the distribution of lichen communities on the stone cultural heritage in the tropical area (Caneva et al. 2015), but also lichen distribution at the micro-scale on natural outcrops in the Mediterranean region (Giordani et al. 2014). Water availability, in particular, has recently been confirmed as critical factor to promote microbial colonization and improve biodeteriorative effects on the stone cultural heritage (Caneva et al. 2016; Liu et al. 2018).

The substrate material (MAT) also significantly affects the distribution of lichen communities, with the less porous, fine-grained painted and plastered surfaces revealing even higher receptivity to lichen colonization than both ancient and modern mortars (PCoA-II in Fig. 4A). The exposure of raw walls and related mortars generally characterize areas where paint and plaster layers were not recovered or conserved, implying general surface instability and conservation difficulties and thus also justifying lower lichen occurrence (Favero-Longo et al. 2015). In this sense, surfaces close to the ground, where *L. lobificans* and related species prevail (see Smith et al. 2009), seem more threatened by physical factors potentially determining instability (as capillary water rise) than by biodeterioration. By contrast, a priority focus should be rather posed on the still conserved paint and plaster layers, having their value threatened by lichen communities dominated by the highly deteriogenic *V. macrostoma* and *D. massiliensis*. A significant reduction in precipitation is expected in southern areas of Europe, associated with a lower biomass accumulation on the stone cultural heritage (Gómez-Bolea et al. 2012). Nevertheless, such a new climate scenario may even imply a higher success of the lichen communities already adapted to xeric and eutrophicated conditions of Pompeian surfaces.

5. Conclusions

In the House of the Ancient Hunt in Pompeii, lichens display remarkable cover values and a high deteriogenic potential, due to hyphal penetration within the painted and plastered surfaces and/or the release of metabolites with acidic and chelating functions. Architecture-related microenvironmental features drive the species distribution. A higher vertical distance from the ground (HEI) and a larger room dimension (ROD) are the main conditional factors related to a higher lichen abundance and the occurrence of the potentially more deteriogenic species. A focus on microenvironmental parameters may thus support the management of biodeterioration issues, addressing restoration priorities and the definition of preventive conservation strategies.

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Tables

Table 1. Lichen diversity and abundance in the House of the Ancient Hunt.

							Rooms									
							12	**14**	**21**	**7**	**1**	**11**	**5a**	**9**	**5c**	**6**
Plots per room (n)							12	5	5	6	9	14	12	5	4	3
Max. total cover per plot (%)							80.2	80.0	30.0	12.4	12.2	8.0	8.0	5.1	2.3	2.0
Species per room (n)							11	4	3	11	5	10	3	7	4	1
Max. species per plot (n)							4	2	2	5	3	5	1	4	4	1
Av. species per plot (n)							2.3	1.6	0.8	2.5	1.1	1.7	0.4	1.4	1.3	1.0
Species		Colonized rooms (n out of 10)	Max. species cover (%)	Cover ≥1% (% of plots)	Frequency per plot (%)	Frequency per quadrat (%)	Max. species cover per room (%)									
Verrucaria macrostoma DC.	Vm	7	80.0	18.7	28.00	14.13	70.0	80.0	0.1	12.0	12.0	6.0	-	0.1	-	-
Dirina massiliensis Durieu & Mont.	Dm	8	55.0	12.0	28.00	12.05	55.0	0.1	30.0	-	0.1	0.1	1.0	0.1	-	2.0
Lepraria lobificans Nyl.	Lm	7	8.0	13.3	20.00	8.21	1.0	-	2.0	6.0	-	2.0	8.0	0.1	2.0	-
Flavoplaca coronata (Körb.) Arup, Frödén & Søchting	Fo	5	5.0	1.3	12.00	4.05	0.1	0.1	-	0.1	-	0.1	-	5.0	-	-
Flavoplaca citrina (Hoffm.) Arup, Frödén & Søchting	Fc	3	1.0	1.3	6.67	2.19	1.0	-	-	-	-	0.1	-	0.1	-	-
Arthonia calcarea (Sm.) Ertz & Diederich	Ac	3	10.0	2.7	4.00	1.71	4.0	-	-	10.0	-	-	-	-	0.1	-
Verrucaria muralis Ach.	Vu	5	8.0	2.7	10.67	1.49	0.1	-	-	8.0	-	2.0	-	0.1	0.1	-
Thelidium incavatum Mudd	Ti	2	6.0	1.3	4.00	1.17	-	-	-	0.1	-	-	6.0	-	-	-
Lecidella cfr. *asema* (Nyl.) Knoph & Hertel var. *asema*	La	2	5.0	1.3	4.00	0.85	5.0	-	-	-	-	0.1	-	-	-	-
Strigula calcarea Bricaud & Cl. Roux	Sc	2	10.0	2.7	4.00	1.01	-	10.0	-	4.0	-	-	-	-	-	-
Candelariella aurella (Hoffm.) Zahlbr.	Ca	2	0.1	-	4.00	0.37	-	-	-	-	0.1	0.1	-	-	-	-
Toninia aromatica (Sm.) A. Massal.	Ta	1	0.1	-	1.33	0.37	-	-	-	0.1	-	-	-	-	-	-
Myriolecis albescens (Hoffm.) Sliwa, Zhao Xin & Lumbsch	My	2	0.1	-	4.00	0.27	-	-	-	0.1	0.1	-	-	-	-	-
Xanthocarpia lactea (A. Massal.) A. Massal.	Xl	2	0.1	-	2.67	0.21	-	-	-	-	-	0.1	-	0.1	-	-
Pyrenodesmia chalybaea (Fr.) A. Massal.	Pc	1	0.1	-	1.33	0.16	-	-	-	-	0.1	-	-	-	-	-
White squamulose R-	Ws	1	0.1	-	1.33	0.11	-	-	-	-	-	-	-	-	0.1	-
Calogaya pusilla (A. Massal.) Arup, Frödén & Søchting	Cp	1	0.1	-	1.33	0.05	0.1	-	-	-	-	-	-	-	-	-
Scytinium sp.	Sy	1	0.1	-	1.33	0.05	-	-	-	0.1	-	-	-	-	-	-
Acarospora fuscata (Schrad.) Arnold	Af	1	0.1	-	1.33	0.05	-	-	-	0.1	-	-	-	-	-	-
Catapyrenium cfr. *daedaleum* (Kremp.) Stein	Cd	1	0.1	-	1.33	0.05	0.1	-	-	-	-	-	-	-	-	-
Protoblastenia cfr. *incrustans* (DC.) J. Steiner	Pi	1	0.1	-	1.33	0.05	-	-	-	-	-	0.1	-	-	-	-
Lecania sylvestris(Arnold) Arnold var. sylvestris	Ls	1	0.1	-	1.33	0.05	0.1	-	-	- -	-	-	-	-	-	-

Table 2. Percentage contribution from the SDR Simplex analyses of lichen communities through the overall rooms and in each room. S (relative similarity), R (relative replacement), D (relative richness difference), R+D (relative β-diversity), S+R (relative richness agreement), S+D (relative nestedness).

Rooms	S	D	R	R+D	S+R	S+D (-anti nestedness)	Matrix fill
All	15.8	31.5	52.8	84.2	68.5	31.6	9.4
12	18.5	31.0	50.5	81.5	69.0	37.1	20.5
14	20.0	23.3	56.7	80.0	76.7	30.0	40.0
21	33.3	22.2	44.4	66.7	77.8	33.3	44.4
7	10.3	38.8	50.8	89.7	61.2	22.7	27.3
1	30.8	27.5	41.7	69.2	72.5	55.0	40.0
11	15.7	36.4	47.8	84.3	63.6	39.3	26.7
5a	20.0	0.0	80.0	80.0	100.0	20.0	25.0
9	0.0	34.4	65.6	100.0	65.6	0.0	33.3
5c	25.0	75.0	0.0	75.0	25.0	100.0	62.5
6	100.0	0.0	0.0	0.0	100.0	100.0	100.0
Av. all rooms	21.5	29.9	48.7	78.5	70.1	36.2	33.1
Av. outdoor rooms	19.3	32.1	48.6	80.7	67.9	37.5	35.5

Captions

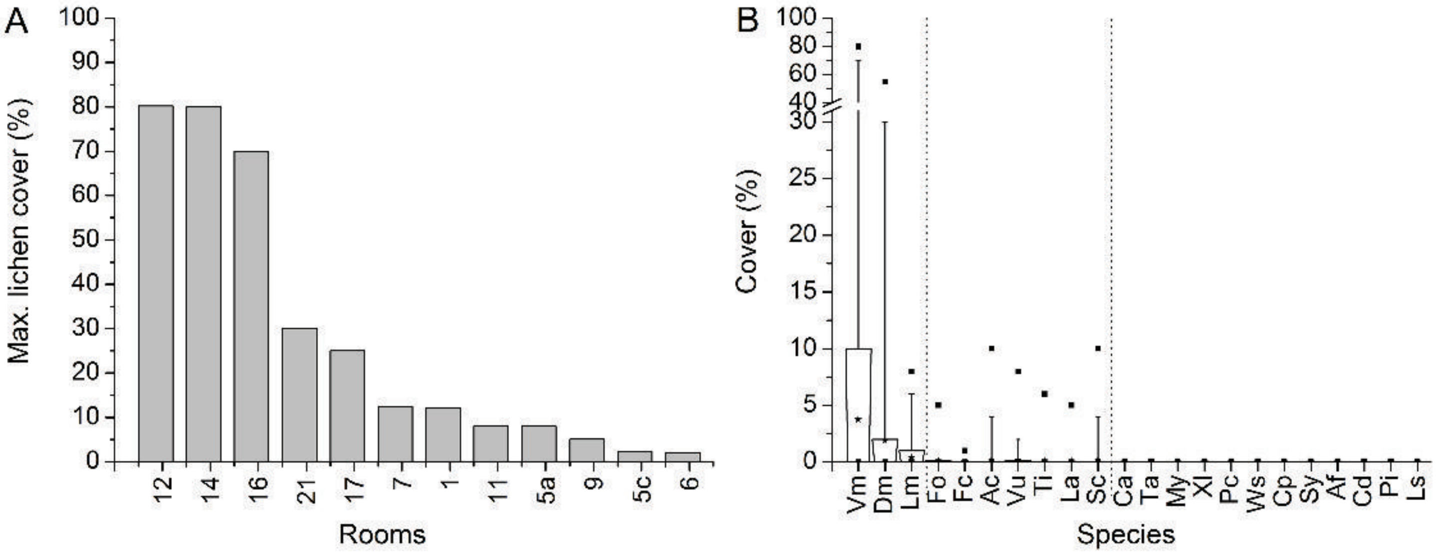
Fig. 1. Lichen colonization on different stone substrates of the ruins of Pompeii (Italy). (A) Tuff blocks of an *opus reticulatum* near the *Antiquarium*. (B) Carbonatic rock slates and terracotta jars in a *thermopolium*. (C) Millstones in the *pistrinum* (bakery) of Popidius Priscus. (D) Corner between Via della Fortuna and Vicolo Storto. (E-I) House of the Ancient Hunt: map (E; semi-confined rooms are crossed; numbers of rooms with maximum lichen cover higher than 1% are circled; scale bar: 5 m) and wall paintings in the outdoor environment of room 12 (F; relevés on the SSE and ENE-facing walls in G and H, respectively) and in the semi-confined room 6 (I). Asterisks highlight the localization of lichen communities.

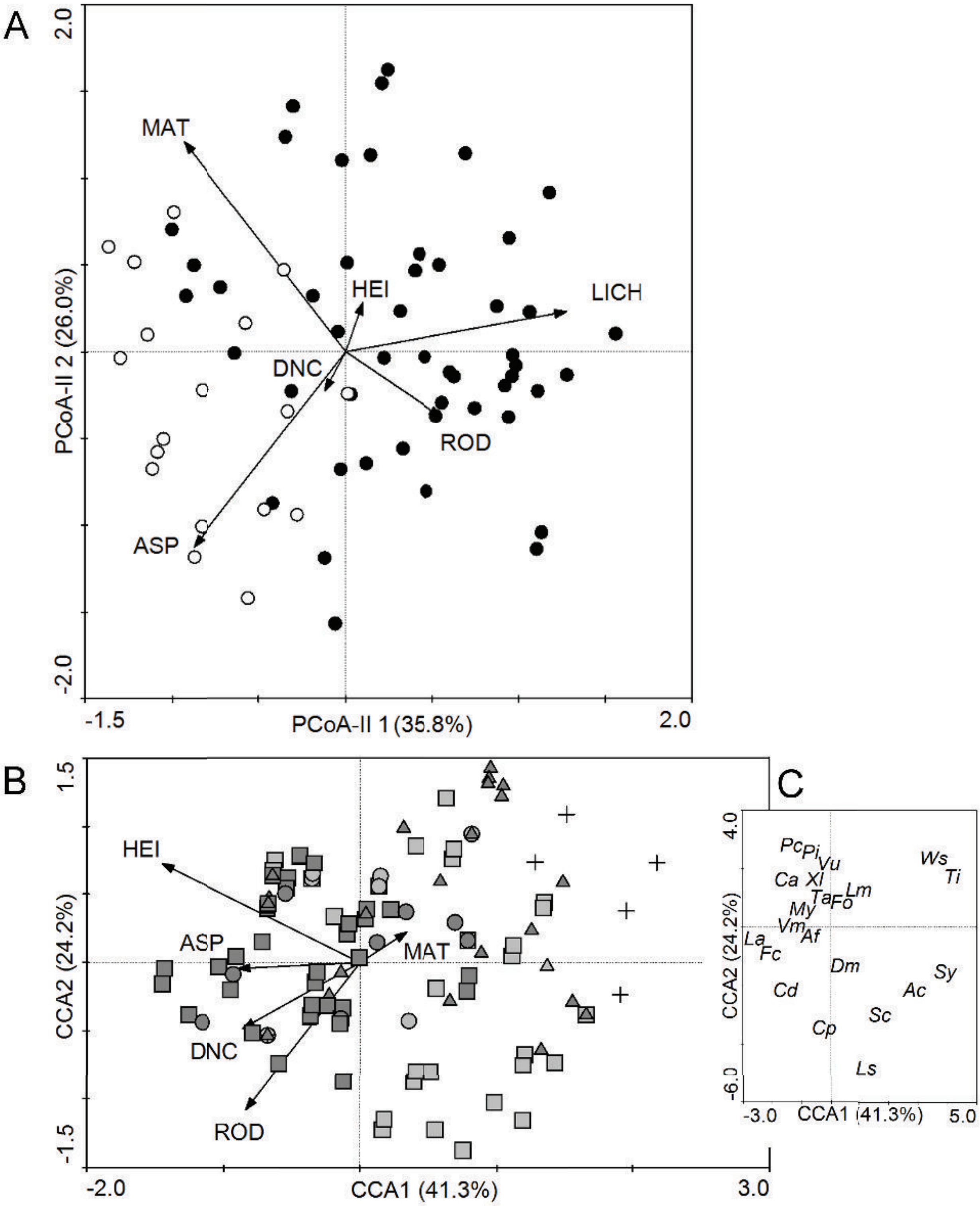
Fig. 2. Lichen colonization through the House. (A) Maximum lichen cover in the rooms with maximum cover value higher than 1%. (B) Ranges of species cover (%): dominant species (left), locally abundant species (centre), punctually occurring species (right). Maximum (▪), 99th percentile (upper whisker), 75th percentile (top box), average (star) cover. Abbreviations of species names in Table 1.

Fig. 3. Relationships between lichen colonization and microenvironmental features. (A) PCoA-II: ordination of plots on the basis of their microenvironmental features (dominant material, MAT; aspect, ASP; vertical distance from the ground, HEI; horizontal distance from the nearest wall corner, DNC; room dimension, ROD) and the overall lichen abundance (LICH). Black and white dots indicate plot with and without lichens, respectively (PCoA-II scores in Table S1B). (B-C) Factorial maps in the canonical correspondence analysis (CCA) showing (B) the position of quadrats (symbols according to UPGMA classification in Fig. S1: *i*, dark grey square; *ii*, light grey square; *iii*, dark grey circle; *iv*, light grey circle; *v*, dark grey triangle; *vi*, light grey triangle; *vii*, cross) together with the contributions of microenvironmental features and (C) of different species (abbr. in Table 1) All the extracted axes displayed in the figure were significant according to Monte Carlo test (CCA scores in Table S3).

Fig. 4. Hyphal penetration component of *Verrucaria macrostoma* within the paint and plaster layers (A-D: cross section stained by PAS and observed by RLM; E and F: cross section observed by SEM). (A) Overview of hyphal penetration through the different layers (pa, paint layer; pl, plaster layer). (B) Magnification of the network of hyphal bundles (arrow) and hyphae (asterisks) at the paint layer. (C) Magnification of hyphal bundles (arrows) in the upper part of the plaster layer. (D) Network of hyphae (asterisks) growing through the mortar matrix. (E, and magnification in F) Hyphal growth through the porosity of the mortar matrix. Scale bars: 1 mm (A), 500 μm (B, E), 250 μm (C, D), 100 μm (F).


A
B
C
D
E
F
G
H
I
N
1
2
3
4
5a
5b
5c
6
7
7a
8
9
10
11
12
12a
13
14
15
16
17
18
20
21


A
100
90
80
70
60
50
40
30
20
10
0
Max. lichen cover (%)
12
14
16
21
17
7
1
11
5a
9
5c
6
Rooms
B
100
80
60
40
30
25
20
15
10
5
0
Cover (%)
Vm
Dm
Lm
Fo
Fc
Ac
Vu
Ti
La
Sc
Ca
Ta
My
Xl
Pc
Ws
Cp
Sy
Af
Cd
Pi
Ls
Species


A
2.0
-2.0
-1.5
2.0
PCoA-II 2 (26.0%)
PCoA-II 1 (35.8%)
MAT
HEI
LICH
DNC
ROD
ASP
B
1.5
-1.5
-2.0
3.0
CCA2 (24.2%)
CCA1 (41.3%)
HEI
ASP
MAT
DNC
ROD
C
4.0
-6.0
-3.0
5.0
CCA2 (24.2%)
CCA1 (41.3%)
Pc
Pi
Vu
Ws
Ti
Ca
Xl
Ta
Fo
Lm
My
Vm
Af
La
Fc
Dm
Sy
Ac
Cd
Sc
Cp
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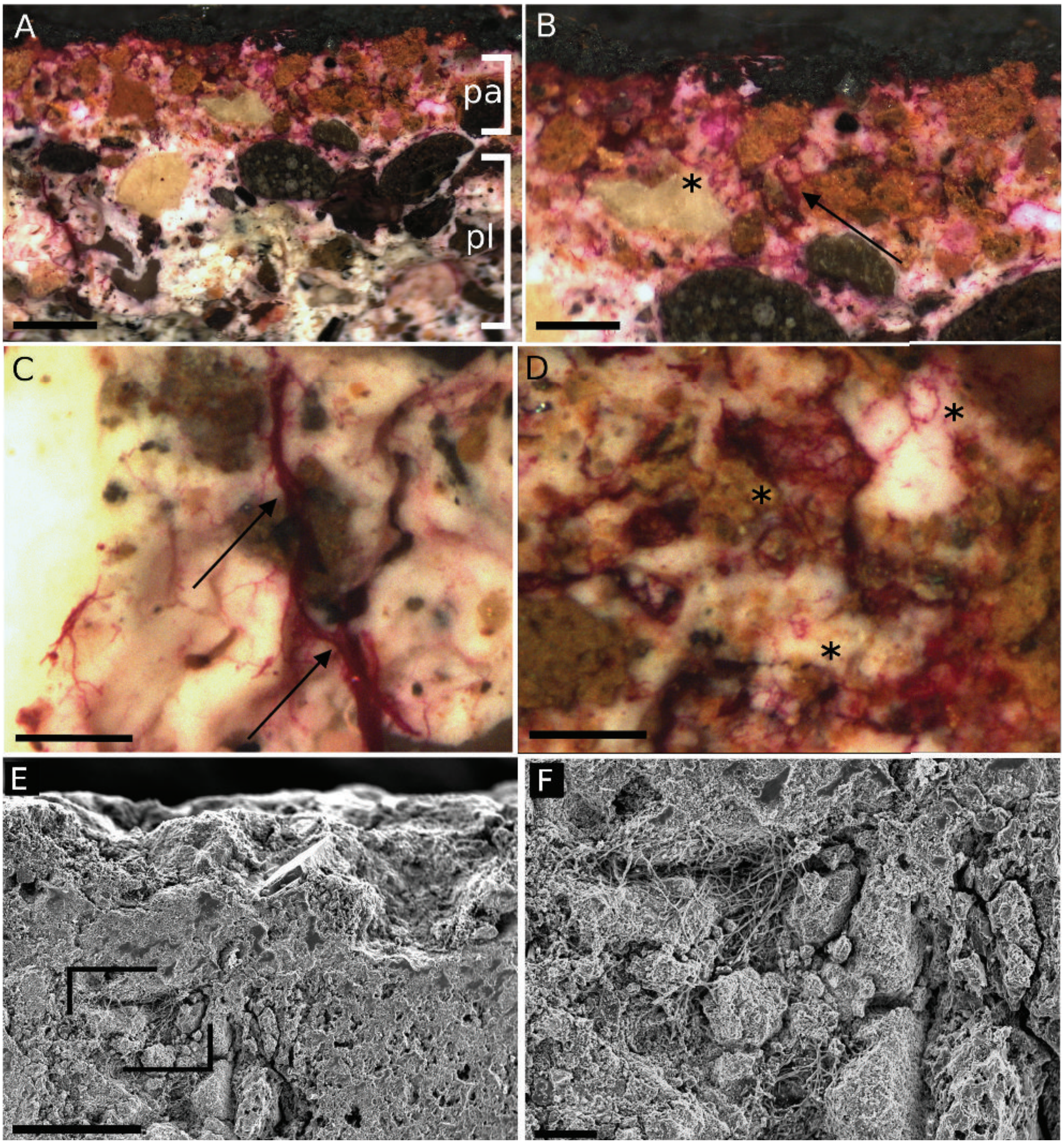
A
pa
pl
B
*
C
D
*
*
*
E
F