Phylogenetic analysis and genetic diversity of the xylariaceous ascomycete Biscogniauxia mediterranea from cork oak forests in different bioclimates

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Cork oak is a tree species with ecological importance that contributes to economic and social development in the Mediterranean region. Cork oak decline is a major concern for forest sustainability and has negative impacts on cork oak growth and production. This event has been increasingly reported in the last decades and seems to be related with climate changes. Biscogniauxia mediterranea is an endophytic fungus of healthy cork oak trees that turns into a pathogen in trees weaken by environmental stress. Understanding the drivers of B. mediterranea populations diversity and differentiation is expected to allow a better control of cork oak decline and preserve forest sustainability. Endophyte isolates from different cork oak forests were identified as B. mediterranea and their genetic diversity was evaluated using phylogenetic and microsatellite-primed PCR analyses. Genetic diversity and variability of this fungus was correlated with environmental/phytosanitary conditions present in forests/trees from which isolates were collected. High genetic diversity and variability was found in B. mediterranea populations obtained from different forests, suggesting some degree of isolation by distance. Bioclimate was the most significant effect that explained the genetic variability of B. mediterranea, rather than precipitation or temperature intensities alone or disease symptoms. These findings bring new implications for the changing climate to cork oak forests sustainability, cork production and quality.

Cork oak (Quercus suber L.) is an evergreen oak species with high economic, ecological, and social importance in the Mediterranean region¹². This species is mainly distributed throughout the Mediterranean Basin, where is well adapted to the climate. Despite that, global climate changes can induce abiotic stress on trees, impacting negatively cork oak growth and productivity¹³. Indeed, cork oak decline has been increasingly reported in the last decades and seems to be linked to climate changes, which can increase susceptibility to pathogen attack and facilitate infection by opportunistic pathogens⁵. Biscogniauxia mediterranea is an opportunistic pathogen with an endophytic lifestyle that has been associated with cork oak decline in the Mediterranean region⁷–⁹. This fungus causes charcoal disease that leads to an extensive inner bark and xylem necrosis, often associated with a blackish exudation on the outer bark¹⁰,¹¹. As the appearance of B. mediterranea-related symptoms are mainly associated with weakened trees (e.g., by drought), the incidence of this disease increased over the last years and was recently reported on young trees¹²,¹³.

Molecular markers are widely used to study population genetics of phytopathogenic fungi¹⁴. Early studies on B. mediterranea populations used random amplified polymorphic DNA (RAPD), being able to detect a high genetic variability for geographically close populations⁸,¹⁵. These results indicated a high rate of sexual reproduction and heterothallic mating system displayed by this species. More recently, high genetic variability was reported within a single stromata using microsatellite-primed PCR primers¹⁶. Also, B. mediterranea populations

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from different Mediterranean countries displayed high genetic diversity (by using sequence analysis of Internal Transcribed Spacer of ribosomal RNA gene, translation elongation factor 1-α and β-tubulin, as well as microsatellite-primed PCR primers)\textsuperscript{25}, but studied isolates were not phylogenetic associated with host species or age, geographic position, or charcoal canker expression. However, the analysis of Tunisian populations of \textit{B. mediterranea} by using microsatellite-primed PCR primers suggested a positive association of their morphological variability and ecological factors, such as altitude, rainfall, and temperature\textsuperscript{19}. Altogether, these findings pointed to the high plasticity and adaptability of this species to the environment\textsuperscript{15,17}. In this work, we aimed to understand the correlation of \textit{B. mediterranea} genetic populations with environmental factors and disease symptoms found on cork oak trees from different bioclimates. For increasing the significance of molecular characterization of \textit{B. mediterranea} populations we used two different typing analyses, a multilocus (6 loci) and a microsatellite (4 primers) approach. This work will increase the current knowledge about drivers of \textit{B. mediterranea} genetic variability and contribute for the formulation of appropriate disease management.

Results

The molecular characterization of \textit{B. mediterranea} endophytic isolates from cork oak trees was performed using a multilocus sequence analysis and microsatellite-primed PCR fingerprinting. From all analyzed loci, partial glutamine synthetase (GS) revealed the highest variability with 19.2% of identical sites (including the outgroup sequences, \textit{B. atropunctata}, \textit{B. nummularia} and \textit{Xylaria hypoxylon}), followed by actin (ACT; 27.6%), translation elongation factor 1-α (TEF; 53.2%), β-tubulin (TUB2; 57.7%), chitin synthase 1 (CHS; 61.4%) and internal transcribed spacer (ITS; 66%). As expected, the nucleotide datasets displayed higher percentage of identical sites without outgroups. The corresponding values ranged from 36.9% (GS) to 94.7% (TUB2). The multilocus alignment was in line with this trend (23.4% of identical sites with outgroups; 66.4% without outgroups).

Phylogenetic trees of each single locus were not able to individually resolve \textit{B. mediterranea} isolates for any factors of interest (forest, bioclimate, disease severity index or disease symptoms; results not shown). The concatenated dataset comprised sequences from 52 \textit{B. mediterranea} isolates and from the three outgroup species (\textit{B. atropunctata}, \textit{B. nummularia} and \textit{Xylaria hypoxylon}). From a total of 2874 characters, 1775 were constant, 815 parsimony-uninformative and 284 parsimony-informative. Maximum Likelihood (ML) tree produced clades of \textit{B. mediterranea} isolates with moderate [ML bootstrap (BS) 70–80; BI posterior probability (PP) 0.8–0.9], high (BS 81–95; PP 0.91–0.95) or very high (BS > 95; PP > 0.95) support values\textsuperscript{19,20}, but no evident patterns regarding factors of interest were exposed using this approach (Fig. 1). Isolates obtained from cork oaks growing in different locations and distinct bioclimates did not clustered together; and visible tree disease symptoms did not contribute for the distribution of isolates. For example, Clade A comprised isolates (\textit{e.g.} Bm25, Bm57, Bm37) from different forests and bioclimates (from the most humid to the driest), also presenting isolates obtained from trees with different disease severity levels. The same was observed for Clade C (with 6 isolates; \textit{e.g.} Bm36, Bm66, Bm08), which even included an isolate (Bm79) obtained from an olive tree. Moreover, some information can be inferred from the tree. Group III (with moderate ML support) displayed a higher number of isolates from trees with mild symptoms (21 out of the 31 found in Group III, contrasting with 7 isolates in the remaining 19). Clade B (in Group I) only included isolates (Bm30, Bm54 and Bm69) from declining trees, while Clade D (Group III) only included isolates (Bm55, Bm11 and Bm03) collected from mild diseased trees, though both clades presented isolates from different forests and bioclimates. Overall, isolates from different locations were distributed along the phylogenetic tree, with few of them being placed together. The same distribution pattern was observed regarding isolates from different bioclimates, even though the sequences in Group VII refer to isolates (Bm49, Bm47, Bm46 and Bm64) obtained from two forests (GV and GR) with the same bioclimate (sub-humid). Also, a clear distribution pattern was not observed concerning the presence of exudates on the \textit{B. mediterranea} host trees. However, subclade A1 only encompassed isolates (Bm25, Bm23, Bm57 and Bm37) obtained from cork oak trees not producing trunk exudates. The opposite was observed for Clade B (Bm50, Bm54 and Bm69) and Group VI (Bm55, Bm11, Bm03 and Bm15), where all isolates were recovered from trees producing exudates, even if from dissimilar bioclimatic locations (sub-humid and humid). In line with the low resolution of \textit{B. mediterranea} isolates by any of the studied factors (forest, bioclimate, disease severity index or disease symptoms), the majority of \textit{B. mediterranea} isolates collected from the same tree were not clustered together. For instance, although pairwise identity within those isolates was high, isolates from a single tree, such as Bm60, Bm61, Bm63 and Bm67 (pairwise identity of 95.4%) and Bm46, Bm56 and Bm58 (99.6%), were found to cluster better with isolates obtained from other forests and/or were placed apart in the phylogenetic tree. Similarly, isolates collected on olive trees (Bm79 and Bm80) were distantly placed from each other, in separate lineages.

Microsatellite-primed PCR fingerprinting of 68 \textit{B. mediterranea} isolates generated different banding patterns. Primer (GTG\textsubscript{3}) generated 15 bands (from 0.25 to 1.5 kb), (CAG\textsubscript{5}) generated 17 bands (ranging from 0.3 to 2 kb, in which one was monomorphic), (ACAC\textsubscript{5}) generated 17 bands (from 0.25 to 1 kb) and M13 generated 26 bands (from 0.15 to 1.5 kb). The monomorphic band was removed and a binary dataset with the remaining (74) band positions was used for molecular analysis. Using this approach, the genetic diversity of \textit{B. mediterranea} varied within different factors of interest/populations (Table 1). Considering total populations, the number of alleles (Na) varied from 1.40 to 1.97 and the number of effective alleles (Ne) from 1.45 to 1.57, which were found considering ‘forest’ and ‘exudates’ factors, respectively. These results were corroborated by the genetic diversity found within populations (Hs; Table 2). ‘Exudates’ factor (total population) also displayed the highest Shannon’s information index (I = 0.50), Nei’s gene diversity (h = 0.33) and percentage of polymorphic loci (PPL = 98.7), while ‘forest’ factor showed the lowest genetic diversity (I = 0.38; h = 0.26; PPL = 67.9). In addition to ‘exudates’, other disease-related factors also revealed high genetic diversity levels for total populations, in particular when considering ‘cankers’ and ‘disease severity levels’ factors (Tables 1 and 2). The genetic diversity of \textit{B. mediterranea} also differed among populations when considering individually each environmental/disease-related factor.
Considering the 'bioclimate' factor, isolates obtained from sub-humid forests revealed the highest genetic diversity and those from hyper-humid the lowest. While Grândola (GR, a sub-humid forest) was the forest with the highest *B. mediterranea* genetic diversity, PG-ER (hyper-humid) and AL (humid) were the forests with the lowest diversity. Isolates collected from healthy trees (low disease severity level) revealed the lowest genetic diversity and declining trees were associated with more diverse *B. mediterranea* isolates. This result is in line with the highest genetic diversity found among *B. mediterranea* isolates collected from trees producing trunk exudates. However, the opposite was found for trees with trunk cankers, in which higher genetic diversity...
Table 1. Genetic diversity of *B. mediterranea* populations. *Na* represents the number of alleles, *Ne* the effective number of alleles, *I* the Shannon’s information index, *h* the Nei’s gene diversity and PPL the percentage polymorphism loci.

| Population       | Na      | Ne      | I       | h       | PPL  |
|------------------|---------|---------|---------|---------|------|
| Bioclimate       | 1.64 ± 0.09 | 1.51 ± 0.04 | 0.44 ± 0.03 | 0.30 ± 0.02 | 81.1 |
| Hyper-humid      | 1.76 ± 0.07 | 1.47 ± 0.04 | 0.43 ± 0.03 | 0.28 ± 0.02 | 86.5 |
| Humid            | 1.97 ± 0.03 | 1.59 ± 0.04 | 0.51 ± 0.02 | 0.34 ± 0.02 | 98.7 |
| Sub-humid        | 1.73 ± 0.08 | 1.52 ± 0.04 | 0.47 ± 0.03 | 0.31 ± 0.02 | 86.5 |
| Semi-arid        | 1.77 ± 0.04 | 1.52 ± 0.02 | 0.46 ± 0.01 | 0.31 ± 0.01 | 88.2 |
| Total            | 1.77 ± 0.04 | 1.52 ± 0.02 | 0.46 ± 0.01 | 0.31 ± 0.01 | 88.2 |
| Forest           | 0.91 ± 0.11 | 1.32 ± 0.05 | 0.26 ± 0.04 | 0.18 ± 0.03 | 40.5 |
| PG-ER            | 1.47 ± 0.10 | 1.50 ± 0.04 | 0.42 ± 0.03 | 0.28 ± 0.02 | 73.0 |
| PG-RC            | 1.62 ± 0.09 | 1.46 ± 0.04 | 0.41 ± 0.03 | 0.27 ± 0.02 | 79.7 |
| LI               | 0.99 ± 0.11 | 1.33 ± 0.05 | 0.27 ± 0.04 | 0.18 ± 0.03 | 43.2 |
| AL               | 1.51 ± 0.09 | 1.48 ± 0.04 | 0.41 ± 0.03 | 0.28 ± 0.02 | 74.3 |
| GV               | 1.92 ± 0.05 | 1.56 ± 0.03 | 0.50 ± 0.02 | 0.33 ± 0.02 | 96.0 |
| GR               | 1.42 ± 0.10 | 1.47 ± 0.04 | 0.40 ± 0.03 | 0.28 ± 0.02 | 68.9 |
| HC-CT            | 1.39 ± 0.10 | 1.45 ± 0.04 | 0.38 ± 0.03 | 0.26 ± 0.02 | 67.6 |
| HC-MA            | 1.40 ± 0.04 | 1.45 ± 0.02 | 0.38 ± 0.01 | 0.26 ± 0.01 | 67.9 |
| Total            | 1.70 ± 0.08 | 1.54 ± 0.04 | 0.46 ± 0.03 | 0.31 ± 0.02 | 85.1 |
| Disease severity level | 2.00 ± 0.00 | 1.55 ± 0.04 | 0.50 ± 0.02 | 0.33 ± 0.02 | 100 |
| Healthy          | 1.97 ± 0.03 | 1.59 ± 0.04 | 0.52 ± 0.02 | 0.34 ± 0.02 | 98.7 |
| Mild             | 1.89 ± 0.03 | 1.56 ± 0.02 | 0.49 ± 0.01 | 0.33 ± 0.01 | 94.6 |
| Declining        | 0.97 ± 0.11 | 1.41 ± 0.06 | 0.28 ± 0.04 | 0.20 ± 0.03 | 40.5 |
| Moderate damage  | 1.60 ± 0.09 | 1.53 ± 0.04 | 0.45 ± 0.03 | 0.30 ± 0.02 | 77.0 |
| Light damage     | 1.92 ± 0.05 | 1.56 ± 0.04 | 0.50 ± 0.02 | 0.33 ± 0.02 | 96.0 |
| No damage        | 1.57 ± 0.10 | 1.53 ± 0.04 | 0.45 ± 0.03 | 0.30 ± 0.02 | 78.4 |
| Total            | 1.61 ± 0.04 | 1.52 ± 0.02 | 0.43 ± 0.01 | 0.29 ± 0.01 | 78.4 |
| Defoliation      | 2.00 ± 0.00 | 1.58 ± 0.04 | 0.51 ± 0.02 | 0.34 ± 0.02 | 100 |
| Yes              | 1.95 ± 0.04 | 1.55 ± 0.04 | 0.49 ± 0.02 | 0.33 ± 0.02 | 97.3 |
| No               | 1.97 ± 0.02 | 1.57 ± 0.03 | 0.50 ± 0.02 | 0.33 ± 0.01 | 98.7 |
| Cankers          | 1.76 ± 0.08 | 1.55 ± 0.04 | 0.47 ± 0.03 | 0.32 ± 0.02 | 87.8 |
| Yes              | 2.00 ± 0.00 | 1.57 ± 0.04 | 0.51 ± 0.02 | 0.33 ± 0.02 | 100 |
| No               | 1.88 ± 0.04 | 1.56 ± 0.03 | 0.49 ± 0.02 | 0.33 ± 0.01 | 93.9 |

Table 2. Genetic differentiation coefficients of *B. mediterranea* populations. *Ht* represents total genetic diversity, *Hs* the mean within-population genetic diversity, *Gst* the genetic differentiation coefficients among different populations and *Nm* the gene flow number.

| Population             | *Ht*         | *Hs*         | *Gst*     | *Nm*     |
|------------------------|--------------|--------------|-----------|----------|
| Bioclimate             | 0.34 ± 0.018 | 0.308 ± 0.015 | 0.097    | 4.670    |
| Forest                 | 0.339 ± 0.019 | 0.258 ± 0.011 | 0.239    | 1.593    |
| Disease severity level | 0.344 ± 0.018 | 0.328 ± 0.017 | 0.045    | 10.526   |
| Defoliation            | 0.345 ± 0.018 | 0.293 ± 0.014 | 0.151    | 2.819    |
| Exudates               | 0.343 ± 0.018 | 0.332 ± 0.017 | 0.032    | 15.248   |
| Cankers                | 0.342 ± 0.020 | 0.327 ± 0.018 | 0.046    | 10.305   |
was associated with isolates from trees without this symptom. Regarding defoliation, the isolates obtained from trees with very accentuated damage were also less genetically diverse, while those from trees with moderate and light damages presented higher genetic diversity.

In agreement with phylogenetic tree analysis from multilocus sequence analysis, principal coordinates analysis (PCoA) did not revealed clusters of cork oak isolates, according to the factors of interest (Fig. S1, both axes only capturing around 15% of data variation). Also, isolates from olive tree did not cluster all together and were closer to isolates from healthy and mild diseased trees. However, certain populations revealed a higher differentiation in allele frequencies than others, as evaluated by $G_{st}$ coefficient (Table 2). There was a higher genetic differentiation among ‘forest’ ($G_{st} = 0.239$), followed by ‘defoliation’ ($G_{st} = 0.151$), and ‘bioclimate’ ($G_{st} = 0.097$) populations. The lowest genetic differentiation was found in ‘cankers’ ($G_{st} = 0.046$), ‘disease severity levels’ ($G_{st} = 0.045$) and ‘exudates’ ($G_{st} = 0.032$) populations. As expected, gene flow ($N_{m}$) values were opposite to $G_{st}$, being higher among populations with lower genetic differentiation and vice-versa (Table 2). These results agree with the genetic pairwise distances found in such populations (Table S1). Pairwise genetic distances in ‘forest’ achieved higher values (ranged from 0.244 to 0.067), than ‘defoliation’ (0.200 to 0.019), or bioclimate (0.085 to 0.053). Other disease-related variables (‘cankers’, ‘disease severity levels’ and ‘exudates’) never achieved more than 0.048 genetic distances. Among populations, isolates obtained from sub-humid bioclimate were the most genetically close from the ones obtained in the other forests (Table S1). Indeed, GR (a sub-humid forest) displayed the least genetic distance from all other forests. In contrast, isolates from humid bioclimate (in particular from AL forest) displayed the highest genetic distance from all other forests. Interestingly, isolates obtained from very accentuated defoliated trees revealed a high genetic distance from other isolates. AMOVA results revealed that variation within populations was always higher than among populations, which reinforces the high variability of $B. mediterranea$ composition. In any case, variation among populations was higher for ‘forest location’ (10%), followed by ‘bioclimate’ (6%), ‘cankers’ (4%) and ‘exudates’ (3%), all at $p < 0.001$ (Table 3), suggesting higher genetic differentiation between regions and bioclimates. Accordingly, Mantel test shown significant correlation between $B. mediterranea$ genetic diversity and geographic distance of cork oak forests ($R = 0.105, p = 0.001$). For further understanding the relative contribution of forest location, environmental, and disease-related factors in driving the genetic diversity of $B. mediterranea$ populations, a redundancy analysis was performed (Fig. 2). The variables that explained the variation of $B. mediterranea$ genetic diversity were ‘forest location’ and ‘bioclimate’ factors, followed by ‘exudates’ and ‘cankers’ (all at $p < 0.001$), being all the others (temperature, precipitation, disease severity levels, and defoliation) not significant. The combination of all significant variables explained 9.602% ($p = 0.001$) of $B. mediterranea$ genetic variance. Most of this variation is due to the ‘bioclimate’ and ‘forest

| Source          | df  | SS      | MS      | Est. var | %    | PhiPT     |
|-----------------|-----|---------|---------|----------|------|-----------|
| Bioclimate      |     |         |         |          |      | 0.062***  |
| Among pops      | 3   | 75.087  | 25.029  | 0.815    | 6    |           |
| Within pops     | 61  | 748.328 | 12.268  | 12.268   | 94   |           |
| Total           | 64  | 823.415 | 13.083  | 100      |      |           |
| Forest          |     |         |         |          |      | 0.103***  |
| Among pops      | 7   | 155.291 | 22.184  | 1.347    | 10   |           |
| Within pops     | 57  | 668.124 | 11.721  | 11.721   | 90   |           |
| Total           | 64  | 823.415 | 1.069   | 100      |      |           |
| Disease severity level |   |         |         |          |      | 0.008     |
| Among pops      | 2   | 29.700  | 14.850  | 0.105    | 1    |           |
| Within pops     | 62  | 793.715 | 12.802  | 12.802   | 99   |           |
| Total           | 64  | 823.415 | 12.907  | 100      |      |           |
| Defoliation     |     |         |         |          |      | 0.006     |
| Among pops      | 4   | 54.603  | 13.651  | 0.076    | 1    |           |
| Within pops     | 60  | 768.813 | 12.814  | 12.814   | 99   |           |
| Total           | 64  | 823.415 | 12.889  | 100      |      |           |
| Exudates        |     |         |         |          |      | 0.032***  |
| Among pops      | 1   | 26.030  | 26.030  | 0.414    | 3    |           |
| Within pops     | 63  | 797.386 | 12.657  | 12.657   | 97   |           |
| Total           | 64  | 823.415 | 13.071  | 100      |      |           |
| Cankers         |     |         |         |          |      | 0.040***  |
| Among pops      | 1   | 22.945  | 22.945  | 0.523    | 4    |           |
| Within pops     | 63  | 800.470 | 12.706  | 12.706   | 96   |           |
| Total           | 64  | 823.415 | 13.229  | 100      |      |           |

Table 3. Analysis of molecular variance (AMOVA) among and within $B. mediterranea$ populations (Pops). Asterisks on population differentiation (PhiPT) values represent significance of PhiPT values as ***$p < 0.001$. df, degree of freedom; SS: sum of squares; MS: mean squares; Est. Var: estimated variance component; %: percentage of genetic variation.
which is concordant with other reports\(^9,15,17,18\), even when isolates come from the same stroma\(^16\). Furthermore, the B. \(B.\) location of cork oak forests from which they were obtained. This contrasts with previous studies, where trees\(^17\). However, the contribution of geographic isolation in terranea and semi-arid bioclimates was significantly different from 0, which indicates linkage disequilibrium (Table S2).

The genetic variability of \(B.\) \(B.\) mediterranea \(B.\) populations, indicating short-distance dispersal. The clonal structure of the populations from the majority of bioclimates and the finding that PG-ER gest low migration rates between geographically distant populations. The unique and shared contribution of variables is detailed. Percentage of variation was calculated by adjusted \(R^2\). ***represent \(p < 0.001\) and **\(p < 0.01\) for statistical significance of model.

**Figure 2.** Contribution of the most significant variables for explaining genetic variability in \(B.\) \(B.\) mediterranea \(B.\) populations. The unique and shared contribution of variables is detailed. Percentage of variation was calculated by adjusted \(R^2\). ***represent \(p < 0.001\) and **\(p < 0.01\) for statistical significance of model.

Discussion

In this study, the genetic diversity of endophytic \(B.\) \(B.\) mediterranea \(B.\) isolates was evaluated by multilocus phylogeny and microsatellite fingerprinting (MSP-PCR). The evaluated isolates comprised those obtained from cork oak trees, thriving in different forests, bioclimates, and displaying distinct charcoal disease severity levels and symptoms. This experimental approach is expected to provide new information about which factors/variables contribute the most to this species diversity and variability. Other isolates obtained from olive trees were also analyzed to evaluate host-specificity. As expected, all studied \(B.\) \(B.\) mediterranea \(B.\) isolates were phylogenetically apart from other Biscogniauxia species included in this study as outgroups, revealing the genetic divergence of \(B.\) \(B.\) mediterranea \(B.\) species. In general, individual (i.e., single-locus) and concatenated (i.e., multi-locus) phylogenetic analyses did not correlate \(B.\) \(B.\) mediterranea \(B.\) isolates with any of the studied environmental/disease variables. Also, after determining the microsatellite polymorphic patterns, \(B.\) \(B.\) mediterranea \(B.\) isolates did not cluster according to the factors of interest. These results suggest a high genetic diversity and variability of \(B.\) \(B.\) mediterranea \(B.\) populations, which is concordant with other reports\(^13,16\), even when isolates come from the same stroma\(^16\). Furthermore, the inability to resolve \(B.\) \(B.\) mediterranea \(B.\) isolates obtained from cork oak and those obtained from olive tree reinforces current knowledge about the high adaptability of this fungus to different hosts, as suggested by\(^17\).

The genetic variability of \(B.\) \(B.\) mediterranea \(B.\) isolates was mostly explained by 'forest location' (6.9%) and 'bioclimates' (4.6%). Indeed, when these factors were combined, they explained 8.8% of \(B.\) \(B.\) mediterranea \(B.\) genetic variation. Considering 'forest location', \(B.\) \(B.\) mediterranea \(B.\) populations demonstrated the lowest genetic diversity within population, while also revealing the highest genetic differentiation and lowest gene flow among populations. These results suggest some isolation by distance of \(B.\) \(B.\) mediterranea \(B.\) communities, which agrees with the significant correlation (Mantel test) found between the \(B.\) \(B.\) mediterranea \(B.\) genetic diversity and forest geographic location of cork oak forests from which they were obtained. This contrasts with previous studies, where \(B.\) \(B.\) mediterranea \(B.\) intraspecific polymorphism and genetic diversity were not associated with geographic position of host trees\(^17\). However, the contribution of geographic isolation in \(B.\) \(B.\) mediterranea \(B.\) genetic differentiation was suggested for cork oak populations in Tunisia\(^16\). The high genetic variability of \(B.\) \(B.\) mediterranea \(B.\) has been related with the heterothallic mating system of this species\(^16\) and sexual reproduction with the production of a high number of variable ascospores\(^15\). Our results suggest that global \(B.\) \(B.\) mediterranea \(B.\) population displays linkage disequilibrium and a clonal genetic structure. Despite that, populations from the majority of forests (PG-ER, PG-RC, AL, GR, HC-CT and HC-MA) demonstrated to have random mating with frequent sexual reproduction. \(B.\) \(B.\) mediterranea \(B.\) ascospores are primarily dispersed by wind after the occurrence of precipitation\(^11,12\), although insects could also play a role for their spreading in short- and long- distances, depending on their bioecology\(^11,12\). Our results suggest low migration rates between geographically distant \(B.\) \(B.\) mediterranea \(B.\) populations, indicating short-distance dispersal. The clonal structure of the populations from the majority of bioenvironments and the finding that PG-ER
and AL forests presented the lowest genetic diversity among all other sampled forests reinforces this suggestion. In contrast with other sampled forests, both are characterized by a high density of mixed forest trees and low anthropogenic interference, which may restrain spore dispersal to and from distant locations. Indeed, canopy architecture and the use of mixed tree species have been reported as a management strategy to reduce spore dispersal of pathogenic fungi\textsuperscript{23-25}. In addition, high genetic differentiation among forest populations can be a result of random events but there is enough gene flow to refute the effects of genetic drift\textsuperscript{26}. While a significant variation was found among forest populations, high variability of \textit{B. mediterranea} was also described within populations. This result concurs with the high intraspecific genetic variability found within populations described by Henriques et al.\textsuperscript{27}, indicating an adaptation of \textit{B. mediterranea} to the environment and ensuring species long-term survival.

Besides ‘forest location’ factor, a significant variation on \textit{B. mediterranea} populations occurred in response to ‘bioclimate’, although precipitation and temperature alone were not significantly correlated with \textit{B. mediterranea} variability, as revealed by variation partitioning redundancy analysis. In contrast, other studies indicated a positive correlation between \textit{B. mediterranea} genetic diversity with temperature and rainfall\textsuperscript{30}. The ability of \textit{B. mediterranea} to develop in a wide range of temperatures\textsuperscript{27}, associated with the significant variability of this fungus in different bioclimates may represent a problem for charcoal cork oak disease management. This will be further challenged by the effect of combined bioclimate and charcoal symptoms (exudates in cork oak trunk) in increasing \textit{B. mediterranea} variability. Indeed, in Tunisia, the correlation between bioclimate and charcoal disease development has been suggested\textsuperscript{7}. In any case, the variability promoted by disease symptoms (exudates and cankers presence) was better explained when taking into consideration the forest location, suggesting that other factors characteristic of each forest (i.e., silvicultural practices not included in this study) are contributing to \textit{B. mediterranea} variability. Moreover, \textit{B. mediterranea} isolates collected from declining trees or trees with charcoal symptoms, like trunk exudates, presented a higher genetic diversity than those collected from healthier trees.

Conclusions

Several reports suggested that \textit{Quercus suber} populations and their associated microbiota are vulnerable to different bioclimates\textsuperscript{28-30} and will be affected by the predicted climate changes\textsuperscript{31}. Therefore, as cork oak forests currently displaying a moist bioclimate become more arid, they will be increasingly affected by environmental stressors. Taking all together, the results obtained in this work support the previous suggestions that \textit{B. mediterranea} isolates have facilitated adaptation. Our findings reinforce the previous knowledge of \textit{B. mediterranea} opportunistic behavior and reveal the importance of bioclimate as a source of \textit{B. mediterranea} variability, exacerbating the implications of a changing climate on cork oak forests sustainability which will affect cork production and quality.

Methods

\textit{Biscogniauxia mediterranea} isolates. \textit{Biscogniauxia mediterranea} isolates were obtained from cork oak twigs as endophytes\textsuperscript{32}. Twigs were sampled, during 2017, from eight Portuguese cork oak forests with different bioclimates and bioclimatic classifications (Fig. S2; Table S3). Bioclimate for each forest was determined using Emberger index and Emberger climatogram\textsuperscript{33,34} and ranged from hyper-humid to semi-arid bioclimates. If existent, trees with different disease severity levels were collected from each location. Disease symptoms included defoliation (5 levels: 0–10%—no damage; 11–25%—light damage; 26–50%—moderate damage; 51–90%—severe damage; > 90%—extreme damage), cankers, and trunk symptoms (3 levels: 0—no damage; 1—moderate damage; 2—severe damage) (Table S4). Disease damages included dry, wilting and decolorated leaves, presence of cankers, decolorated trunk and presence of exudates (Fig. 3). Disease severity levels were grouped into three categories (healthy, mild, and declining), considering the combination of different symptoms and corresponding levels, as described elsewhere\textsuperscript{2}. Endophytic isolates were obtained by sterilizing the surface of cork oak twigs, placing twigs onto Potato Dextrose Agar (PDA) medium and obtaining pure cultures through re-plating outgrowing fungi into fresh PDA medium\textsuperscript{2}. DNA of pure cultures was extracted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA) and \textit{B. mediterranea} isolates were identified using universal primer pairs ITS1-F (5’-CTTGGTGCATTAGGAGGATTA-3’) and ITS4 (5’-TCCTCCTGATTGATATGC-3’). A total of 74 \textit{B. mediterranea} isolates obtained from cork oak were distinguished and identified by sequencing (Table 4). Three other isolates were obtained from \textit{Olea europaea} twigs (Bm78\textsuperscript{96} and olives (Bm79 and Bm80\textsuperscript{97}), collected from cvs. \textit{Cobrançosa} (Bm78 and Bm80) and \textit{Madural} (Bm79). These \textit{O. europaea} derived isolates were included in this study to evaluate host-speciality in \textit{B. mediterranea}. All methods complied with relevant institutional, national, and international guidelines and legislation.

Multilocus sequence analysis. Multilocus phylogenetic analysis was performed by targeting several loci: internal transcribed spacer (ITS), translation elongation factor 1-α (TEF), partial glutamine synthetase (GS), actin (ACT), chitin synthase 1 (CHS) and β-tubulin 2 (TUB2). PCR amplifications were performed according to each pair of primers and target region (Table 5). PCR reactions were prepared for all loci in 10 µl volume using 0.2 U/µl of NZYTag II 2× Green Master Mix (NZYTech, Portugal), 0.5 µM of each primer and 1 µl of DNA. PCR products were run on a 1% (w/v) agarose gel, stained with Green Safe Premium (NZYTech, Portugal). Isopropanol 75% (v/v) was used to purify PCR products and sequencing was performed by Macrogen, Inc services (Madrid, Spain).

DNA sequences were processed using AB1 trace files in Geneious version 2010.4.8.5 (Biomatters, New Zealand), unless stated otherwise. For each isolate and molecular marker, forward and reverse sequences were trimmed (0.05 error probability limit), assembled and consensus sequences were created. Consensus sequences obtained in this study were deposited in GenBank (for accession numbers, see Table 4). Multiple sequence
alignments of each region were performed by using MUSCLE version 3.5 algorithm with a maximum of 10 iterations. Distance measure used for 1st iteration was kmer4_6 and subsequent iterations were run with pctid_kimura. All iterations were performed using UPGMB clustering method and CLUSTALW sequence weighting scheme. If needed, alignments were manually edited and Gblocks (web-based, version 0.91b, January 2002, http://molevol.cmima.csic.es/castresana/Gblocks_server.html, last accessed date: 08/02/2021) was used to eliminate poorly aligned positions and divergent regions, allowing smaller final blocks. Geneious version 2010.4.8.5 was then used to concatenate alignments. Some isolates were not sequenced with enough quality in some targeted loci and were excluded from individual alignments before concatenation. DNA sequences from closely related taxa—Biscogniauxia atropunctata, B. nummularia and Xylaria hypoxylon—were used as outgroups and were retrieved from GenBank (Table 4).

Phylogenetic trees for each individual locus were generated using sequences from 74 B. mediterranea isolates for ITS, 70 for TEF, 68 for GS, 66 for ACT, 69 for CHS and 71 for TUB2 (Table 4). The final concatenated alignment used to build the multilocus phylogenetic tree included sequences from 52 B. mediterranea isolates and from the three outgroup species (isolates marked with * in Table 4). PartitionFinder2 version 2.1.1 was run on CIPRES Science Gateway (web-based, version 3.3, https://www.phylo.org/portal2/, last accessed date: 17/02/2021) to find best-fit partition schemes of each loci. Bayesian inference (BI) trees were computed using MrBayes version 3.2.7. Models (lset) and prior probability distributions (prset) were set according to PartitionFinder2 results. Two independent runs were performed with one million generations and four chains in each run. The two runs were converged with a burnin of 25% and tree with posterior probabilities was generated. Maximum Likelihood (ML) trees were generated using W-IQ-TREE (http://iqtree.cibiv.univie.ac.at/, last accessed date: 17/02/2021), a web server for IQ-TREE. Best-fit model was computed using ModelFinder version 1.4.2, with an edge-linked partition model. Branch support analysis was performed using 1000 ultrafast bootstrap replicates and minimum correlation coefficient of 0.99. Phylogenetics trees were visualized in FigTree version 1.4.4. ML bootstrap (BS) and BI posterior probability (PP) values and topologies obtained by both phylogenetic inference methods were compared using TreeGraph 2 version 2.0.40. This allowed the distinction of clades with reliable support values or groups of sequences showing limited support. Additional data from environmental/disease factors of interest were added using the vector image editor Inkscape version 0.92.

Microsatellite-primed PCR fingerprinting. The molecular diversity of 68 B. mediterranea isolates was also evaluated by using microsatellite-primed PCR (MSP-PCR) with four sets of primers, (GTG)₃, (CAG)₃, (ACAC)₃ and M13 (phage M13 core sequence; 5'-GAGGTTGNNGGNTCT)¹⁷,¹⁸. PCR reactions were prepared...
| Isolate | Location  | Forest | Tree number | Bioclimate | Disease severity level | GenBank accession numbers |
|---------|-----------|--------|-------------|------------|------------------------|--------------------------|
| Bm02    | Alcobaça  | AL     | 4           | Humid      | Mild                   | MZ502502 MZ713533         |
| Bm03*   | Alcobaça  | AL     | 4           | Humid      | Mild                   | MZ502503 MZ713531 MZ713581 |
| Bm04    | Alcobaça  | AL     | 2           | Humid      | Healthy                | MZ502504 MZ713498 MZ713565 |
| Bm05*   | Alcobaça  | AL     | 2           | Humid      | Healthy                | MZ502505 MZ713499         |
| Bm06    | Alcobaça  | AL     | 2           | Humid      | Healthy                | MZ502506 MZ713490 MZ713608 |
| Bm07*   | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502509 MZ713500 MZ713579 |
| Bm08*   | Limãos    | LI     | 2           | Humid      | Declining              | MZ502510 MZ713521 MZ713572 |
| Bm09*   | Limãos    | LI     | 3           | Humid      | Mild                   | MZ502511 MZ713520 MZ713569 |
| Bm10*   | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502512 MZ713491 MZ713574 |
| Bm11*   | Limãos    | LI     | 3           | Humid      | Healthy                | MZ502513 MZ713493 MZ713609 |
| Bm12*   | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502514 MZ713528 MZ713568 |
| Bm13    | Limãos    | LI     | 2           | Humid      | Declining              | MZ502515 MZ713501 MZ713607 |
| Bm14    | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502516 MZ713545 MZ713567 |
| Bm15*   | Limãos    | LI     | 3           | Humid      | Mild                   | MZ502517 MZ713502 MZ713614 |
| Bm16*   | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502518 MZ713503 MZ713606 |
| Bm17*   | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502519 MZ713542 MZ713581 |
| Bm18*   | Limãos    | LI     | 5           | Humid      | Mild                   | MZ502520 MZ713548 MZ713588 |
| Bm19*   | Limãos    | LI     | 3           | Humid      | Mild                   | MZ502521 MZ713503 MZ713607 |
| Bm20*   | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502522 MZ713490 MZ713580 |
| Bm21    | Limãos    | LI     | 1           | Humid      | Mild                   | MZ502523 MZ713504 MZ713621 |
| Bm22*   | Limãos    | LI     | 6           | Humid      | Mild                   | MZ502524 MZ713544 MZ713619 |
| Bm23*   | Herdade   | HC-MA  | 4           | Semi-arid  | Declining              | MZ502525 MZ713550 MZ713622 |
| Bm24*   | Herdade   | HC-MA  | 6           | Semi-arid  | Healthy                | MZ502526 MZ713551 MZ713585 |
| Bm25*   | Herdade   | HC-MA  | 6           | Semi-arid  | Healthy                | MZ502527 MZ713505 MZ713600 |
| Bm26    | Herdade   | HC-MA  | 5           | Semi-arid  | Mild                   | MZ502528 MZ713548 MZ713605 |
| Bm27*   | Herdade   | HC-MA  | 5           | Semi-arid  | Mild                   | MZ502529 MZ713534 MZ713570 |
| Bm28    | Herdade   | HC-MA  | 6           | Semi-arid  | Healthy                | MZ502530 MZ713506 MZ713595 |
| Bm29*   | Herdade   | HC-MA  | 2           | Semi-arid  | Mild                   | MZ502531 MZ713489 MZ713612 |
| Bm30*   | Herdade   | HC-MA  | 2           | Semi-arid  | Mild                   | MZ502532 MZ713524 MZ713616 |
| Bm31    | Peneda Gerês | PG-RC | 4           | Hyper-humid | Healthy                | MZ502533 MZ713507 MZ713586 |
| Bm32    | Peneda Gerês | PG-RC | 3           | Hyper-humid | Healthy                | MZ502534 MZ713508 MZ713587 |
| Bm33    | Peneda Gerês | PG-RC | 4           | Hyper-humid | Healthy                | MZ502535 MZ713509 MZ713588 |
| Bm34    | Peneda Gerês | PG-RC | 2           | Hyper-humid | Healthy                | MZ502536 MZ713510 MZ713589 |
| Bm35    | Peneda Gerês | PG-RC | 1           | Hyper-humid | Mild                   | MZ502537 MZ713511 MZ713590 |
| Bm36*   | Peneda Gerês | PG-RC | 5           | Hyper-humid | Healthy                | MZ502538 MZ713512 MZ713591 |
| Bm37*   | Peneda Gerês | PG-RC | 2           | Hyper-humid | Healthy                | MZ502539 MZ713513 MZ713592 |
| Bm38    | Peneda Gerês | PG-RC | 2           | Hyper-humid | Healthy                | MZ502540 MZ713514 MZ713593 |
| Bm39*   | Peneda Gerês | PG-ER | 4           | Hyper-humid | Healthy                | MZ502541 MZ713515 MZ713594 |
| Bm41*   | Peneda Gerês | PG-ER | 4           | Hyper-humid | Healthy                | MZ502542 MZ713516 MZ713595 |
| Bm42*   | Peneda Gerês | PG-ER | 3           | Hyper-humid | Healthy                | MZ502543 MZ713517 MZ713596 |
| Bm43*   | Grândola   | GR     | 1           | Sub-humid  | Declining              | MZ502544 MZ713518 MZ713597 |
| Bm44*   | Grândola   | GR     | 2           | Sub-humid  | Declining              | MZ502545 MZ713520 MZ713598 |
| Bm45    | Grândola   | GR     | 4           | Sub-humid  | Mild                   | MZ502546 MZ713521 MZ713599 |
| Bm46*   | Grândola   | GR     | 3           | Sub-humid  | Mild                   | MZ502547 MZ713522 MZ713600 |
| Bm47*   | Grândola   | GR     | 4           | Sub-humid  | Mild                   | MZ502548 MZ713523 MZ713601 |
| Bm48*   | Grândola   | GR     | 4           | Sub-humid  | Mild                   | MZ502549 MZ713524 MZ713602 |
| Bm49*   | Grândola   | GR     | 2           | Sub-humid  | Declining              | MZ502550 MZ713525 MZ713603 |
| Bm50*   | Grândola   | GR     | 1           | Sub-humid  | Declining              | MZ502551 MZ713526 MZ713604 |
| Bm51    | Grândola   | GR     | 3           | Sub-humid  | Mild                   | MZ502552 MZ713527 MZ713605 |
| Bm52*   | Grândola   | GR     | 1           | Sub-humid  | Declining              | MZ502553 MZ713528 MZ713606 |

Continued
in 10 µl volume using 0.2 U/µl of NZYTaq II 2× Green Master Mix (NZYTech, Portugal), 1 µM of each primer and 1 µl of DNA. The amplifications with (GTG)5 were performed using the following PCR program: initial denaturation at 94 °C, for 4 min; 35 cycles of 45 s at 94 °C, 45 s at 56 °C and 30 s at 72 °C; and final elongation at 72 °C for 10 min. Amplifications with (CAG)5, (ACAC)5 and M13 primers comprised the following PCR program: initial denaturation at 94 °C, for 2 min; 40 cycles of 30 s at 93 °C, 60 s at 53 °C and 30 s at 72 °C; and final elongation at 72 °C for 10 min. Amplifications with each primer were performed in duplicate for reproducibility. PCR products were run on a 1.5% (w/v) agarose gel, stained with Green Safe Premium (NZYTech, Portugal). The visualization and image acquisition were performed using an UV transilluminator (VWR Genosmart, United Kingdom). DNA fingerprinting gel images were analyzed using GelAnalyzer version 19.1 (http://www.gelalyzer.com) and each band was scored as 0 (absence) or 1 (presence).

Molecular diversity of B. mediterranea isolates was evaluated by grouping samples into populations, based on their host origin, such as climate parameters (including bioclimate), forest provenience, or disease-related

| Isolate | Location | Forest | Tree number | Bioclimate | Disease severity level | GenBank accession numbers |
|---------|----------|--------|-------------|------------|------------------------|--------------------------|
| Bm53    | Grândola | GR     | 5           | Sub-humid  | Declining              | MZ502551 MZ713536 MZ713559 – MZ700470 MZ713423 |
| Bm54*   | Grândola | GR     | 1           | Sub-humid  | Declining              | MZ502552 MZ713510 MZ713578 MZ700366 MZ700450 MZ713480 |
| Bm55*   | Grândola | GR     | 4           | Sub-humid  | Mild                   | MZ502553 MZ713541 MZ713575 MZ700386 MZ700456 MZ713485 |
| Bm56*   | Grândola | GR     | 3           | Sub-humid  | Mild                   | MZ502554 MZ713511 MZ713596 MZ700387 MZ700439 MZ713479 |
| Bm57*   | Grândola | GR     | 2           | Sub-humid  | Declining              | MZ502555 MZ713523 MZ713611 MZ700377 MZ700436 MZ713418 |
| Bm58*   | Grândola | GR     | 3           | Sub-humid  | Mild                   | MZ502556 MZ713495 MZ713593 MZ700416 MZ700487 MZ713444 |
| Bm59    | Grândola | GR     | 1           | Sub-humid  | Declining              | MZ502557 – – – – – – |
| Bm60*   | Gavião   | GV     | 1           | Sub-humid  | Declining              | MZ502558 MZ713530 MZ713576 MZ700360 MZ700438 MZ713433 |
| Bm61*   | Gavião   | GV     | 1           | Sub-humid  | Declining              | MZ502559 MZ713537 MZ713573 MZ700379 MZ700469 MZ713441 |
| Bm62    | Gavião   | GV     | 3           | Sub-humid  | Mild                   | – – MZ713610 MZ700421 – – MZ713443 |
| Bm63*   | Gavião   | GV     | 1           | Sub-humid  | Declining              | MZ502560 MZ713519 MZ713601 MZ700398 MZ700451 MZ713458 |
| Bm64*   | Gavião   | GV     | 2           | Sub-humid  | Declining              | MZ502561 MZ713554 MZ713589 MZ700390 MZ700455 MZ713452 |
| Bm65    | Gavião   | GV     | 4           | Sub-humid  | Mild                   | MZ502562 – – MZ713563 MZ700358 MZ700425 – – |
| Bm66*   | Gavião   | GV     | 4           | Sub-humid  | Mild                   | MZ502563 MZ713513 MZ713615 MZ700368 MZ700465 MZ713434 |
| Bm67*   | Gavião   | GV     | 1           | Sub-humid  | Declining              | MZ502564 MZ713547 MZ713562 MZ700385 MZ700424 MZ713417 |
| Bm68*   | Herdade Contenda | HC-CT | 5 | Semi-arid | Mild | MZ502565 MZ713538 MZ713566 MZ700396 MZ700435 MZ713470 |
| Bm69*   | Herdade Contenda | HC-CT | 6 | Semi-arid | Declining | MZ502566 MZ713525 MZ713580 MZ700367 MZ700428 MZ713429 |
| Bm70    | Herdade Contenda | HC-CT | 2 | Semi-arid | Declining | MZ502567 – – MZ713564 – – – – |
| Bm71    | Herdade Contenda | HC-CT | 5 | Semi-arid | Mild | MZ502568 MZ713540 – – – – – – |
| Bm72*   | Herdade Contenda | HC-CT | 5 | Semi-arid | Mild | MZ502569 MZ713512 MZ713625 MZ700422 MZ700452 MZ713428 |
| Bm73    | Herdade Contenda | HC-CT | 5 | Semi-arid | Mild | MZ502570 – – MZ713624 MZ700384 MZ700434 MZ713424 |
| Bm74    | Herdade Contenda | HC-CT | 5 | Semi-arid | Mild | MZ502571 – – MZ713592 MZ700388 – – MZ713478 |
| Bm75*   | Herdade Contenda | HC-CT | 2 | Semi-arid | Declining | MZ502572 MZ713488 MZ713598 MZ700391 MZ700454 MZ713472 |
| Bm76    | Herdade Contenda | HC-CT | 3 | Semi-arid | Mild | MZ502573 MZ713546 MZ713582 – – MZ700478 MZ713471 |
| Bm78a   | Mirandela | – | – | – | – | MZ502574 MZ713539 MZ713594 MZ700380 MZ700433 – – |
| Bm79a   | Mirandela | – | – | – | – | MZ502575 MZ713494 MZ713618 MZ700373 MZ700453 MZ713442 |
| Bm80a   | Mirandela | – | – | – | – | MZ502576 MZ713532 MZ713597 MZ700397 MZ700486 MZ713435 |

Table 4. GenBank accession numbers for each sequenced locus [internal transcribed spacer (ITS), translation elongation factor 1-α (TEF), partial glutamine synthetase (GS), actin (ACT), chitin synthase 1 (CHS) and β-tubulin 2 (TUB2)] of endophytic B. mediterranea isolates obtained from cork oak twigs and other fungi used in this study. Information is given concerning the sampled forests and disease severity level of sampled host trees. Isolates without GenBank accession number were only used for microsatellite-primed PCR fingerprinting. *Refers to sequences used for multilocus analysis. **Refers to B. mediterranea specimens obtained from olive trees.
parameters (including severity level, defoliation, and presence of exudates). Frequency- and distance-based genetic diversity of each population was evaluated using GenALEX version 6.51b2. Frequency-based genetic diversity was evaluated considering the number of different alleles (Na), number of effective alleles (Ne), Shannon’s Information index (I), diversity \([h = 1 − (q^2 + q)]\) and percentage of polymorphic loci (PPL). Distance-based genetic diversity was assessed using different analyses: Principal Coordinates Analysis (PCoA) to find patterns within dataset; Nei’s pairwise genetic distance to calculate genetic distance between populations; and Mantel test to calculate statistical correlation between genetic diversity and geographic distance (999 permutations). F-statistics to analyze diversity and genetic differentiation among populations were calculated in POPGENE version 1.32. Given the reduced sample size, \(B. \text{mediterranea}\) iso-lates collected from olive tree (Bm78, Bm79 and Bm80) were included only in phylogenetic and PCoA analyses.

Redundancy analysis (RDA) was used to explore responses of \(B. \text{mediterranea}\) composition to environmental (bioclimate, mean maximum and minimum temperatures and mean total precipitation for the 10 years previous to sampling collection) and disease variables (disease severity level, exudates, cankers, and defoliation), by making use of the R version 4.0.2. Analyses were performed using the package vegan version 2.5–75, except when stated otherwise. Spatial trend was included in RDA using a trend surface analysis. Latitude–longitude data was stated otherwise. Spatial trend was included in RDA using a trend surface analysis. Latitude–longitude data was referred as ‘forest location’ variable in sections related with spatial variables.

**Table 5.** Locus regions amplified for \(B. \text{mediterranea}\) phylogenetic analyses and correspondent PCR conditions. The corresponding amplicons size are shown in brackets.

| Locus | Primers | PCR program | Refs. |
|-------|---------|-------------|-------|
| ITS (600 bp) | ITS1-F \(5'\)-CTTGGTCATTAGGAGGAAGTAA \(3'\) | Initial denaturation: 94 °C for 5 min 35 cycles: 40 s at 94 °C; 30 s at 54 °C; 60 s at 72 °C Final elongation: 72 °C for 10 min | 35 |
| | ITS4 \(5'\)-TCCGCCCTTAAGGATATGC | | |
| TEF (350 bp) | EF 1-728 F \(5'\)-CATCGGAAAGGTCCAGAAGG \(3'\) | Initial denaturation: 96 °C for 3 min 40 cycles: 30 s at 95 °C; 45 s at 54 °C; 45 s at 72 °C Final elongation: 72 °C for 7 min | 57 |
| | EF 1-986 R \(5'\)-TACCTGGAAGAACCTTTACC | | |
| GS (700 bp) | GSFI \(5'\)-ATGGCCGAGTACATCCTGG \(3'\) | Initial denaturation: 95 °C for 4 min 35 cycles: 30 s at 95 °C; 30 s at 54 °C; 45 s at 72 °C Final elongation: 72 °C for 7 min | 58 |
| | GSRJ \(5'\)-GAACCGTGAAAGTTCCAG | | |
| ACT (900 bp) | ACT-1 \(5'\)-TGGGGAGCTAGTGAIAATCTGG \(3'\) | Initial denaturation: 94 °C for 5 min 35 cycles: 30 s at 95 °C; 30 s at 57 °C; 60 s at 72 °C Final elongation: 72 °C for 7 min | 58 |
| | ACT-4R \(5'\)-TCTTCGATTTCTCTGGAIAATCCACAT | | |
| CHS (300 bp) | CHS-79 F \(5'\)-TGGGGCAAGAGTGCTTGGAAAGG \(3'\) | Initial denaturation: 95 °C for 2 min 40 cycles: 60 s at 95 °C; 30 s at 62 °C; 20 s at 72 °C Final elongation: 72 °C for 5 min | 57 |
| | CHS-354 R \(5'\)-TGGAAAGACCATCTTGAGGATTG | | |
| TUB2 (500 bp) | Bt2a \(5'\)-GATACCAAAATCGGTGCTCCTTC \(3'\) | Initial denaturation: 95 °C for 5 min 35 cycles: 30 s at 95 °C; 30 s at 58 °C; 60 s at 72 °C Final elongation: 72 °C for 7 min | 60 |
| | Bt2b \(5'\)-AACCATTAGTCTGACCCCTGGC | | |

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**Author contributions**

D.C. contributed to study design, performed the experiments, analyzed data, and contributed to manuscript writing. V.R. contributed to study design, analyzed data, and contributed to manuscript writing. R.M.T. contributed to study design and manuscript editing. P.B. contributed to study design and manuscript editing. T.L.N. contributed to study design and manuscript writing.

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**Competing interests**

The authors declare no competing interests.

**Additional information**

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