Data Article

Phylogenetic and distributional data on boletoid fungi (Boletaceae) in Cyprus and description of a new sampling methodology

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1. Data

The high-profile family of Boletaceae accommodates rare as well as economically important terrestrial fungi with tubular hymenophores and dark, usually fusiform or subfusiform spores [2–4]. Although the family has been intensively studied in recent years and extensive systematic re-
arrangements have been proposed, boletoid fungi in Mediterranean and insular ecosystems remain poorly documented. The data presented here is supplementary to the research paper “Present status and future of boletoid fungi (Boletaceae) on the island of Cyprus: cryptic and threatened diversity unravelled by ten-year study” [1], and was obtained during a 10-year macromycete inventory on the Mediterranean island of Cyprus. A new, rainfall-based sampling methodology is introduced and described in detail, designed to produce maximum yields of fungal diversity in Mediterranean ecosystems, where rainfall is unpredictable and uneven in distribution, and fungal fruitings are consequently prolific but localized and brief (Table 3). Thirty representative sites dominated by ectomycorrhizal trees and shrubs, were preselected and systematically surveyed, following rainfall episodes (Tables 1 and 2, Fig. 1). In addition, the known distribution of boletoid species documented on the island is depicted (Fig. 2), and the ITS polymorphism within the /Butyriboletus fechtneri sensu lato clade is demonstrated (Table 4).

2. Experimental design, materials and methods

2.1. Data collection and sampling methodology

Data on fungi belonging to the Boletaceae family was gathered during a decade-long macromycete inventory on the island of Cyprus, between 2006 and 2017, following a modified protocol based on
Table 1
Permanent sites: Thirty sites pre-selected and regularly surveyed between seasons 2007/08 and 2016/17, including approximate area (m²) for each site, elevation, ectomycorrhizal tree composition (habitat), elevation and number of visits per season between 2007/08 and 2016/17.

| Locality                          | District                  | Area m² | Elevation (m) | Habitat (EcM)                                                                 | 07/08 | 08/09 | 09/10 | 10/11 | 11/12 | 12/13 | 13/14 | 14/15 | 15/16 | 16/17 | Av. |
|-----------------------------------|---------------------------|---------|---------------|------------------------------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| Troodos East (Makria Kontaria)    | Nicosia                   | ~1km²   | 1600–1750     | Pinus nigra ssp. pallasiana, Quercus alnifolia, Arbutus andrachne, Cistus creticus | 4     | 5     | 2     | 1     | 2     | 1     | 1     | 6     | 1     | 1     | 2.4 |
| Troodos Central (Kaledonia trail) | Nicosia                   | ~2km²   | 1600–1700     | P. nigra ssp. pallasiana, Q. alnifolia, Alnus orientalis, A. andrachne, C. creticus | 7     | 8     | 8     | 1     | 4     | 5     | 3     | 6     | 3     | 1     | 4.6 |
| Troodos Central (Kyvernitikes katoikies 1) | Nicosia                   | ~1km²   | 1700–1750     | P. nigra ssp. pallasiana | 8     | 16    | 7     | 1     | 8     | 5     | 7     | 9     | 6     | 3     | 7   |
| Troodos Central (Kyvernitikes katoikies 2) | Nicosia                   | ~1km²   | 1600–1700     | P. nigra ssp. pallasiana, Q. alnifolia, A. andrachne, C. creticus | 7     | 6     | 3     | 1     | 4     | 3     | 2     | 5     | 1     | 2     | 3.4 |
| Troodos South (Kataskoinoseis)    | Nicosia                   | ~2km²   | 1400–1600     | P. nigra ssp. pallasiana, Q. alnifolia, A. andrachne, C. creticus | 3     | 4     | 3     | 1     | 3     | 4     | 4     | 3     | 1     | 1     | 2.7 |
| Troodos North (Almyroloivado/Livadi Pasia) | Nicosia                   | ~2km²   | 1600–1700     | P. nigra ssp. pallasiana, Cedrus brevifolia, Q. alnifolia | 1     | 1     | 5     | 1     | 2     | 2     | 3     | 1     | 1     | 3     | 2   |
| Prodromos South (Kampou Kalogiro) | Nicosia                   | ~700m²  | 1300–1400     | P. nigra ssp. pallasiana, Q. alnifolia, A. andrachne, C. creticus | 1     | 1     | 3     | 1     | 4     | 2     | 2     | 1     | 1     | 2     | 1.8 |
| Prodromos North (Prodromos dam)   | Nicosia                   | ~1km²   | 1400–1500     | P. nigra ssp. pallasiana, Q. alnifolia, A. andrachne, C. creticus | 2     | 5     | 10    | 1     | 12    | 9     | 4     | 8     | 5     | 1     | 5.7 |
| Platania/Karvounas                | Nicosia                   | ~2 km   | 1000–1100     | P. brutia, Q. alnifolia, A. andrachne, C. creticus | 1     | 3     | 3     | 1     | 3     | 3     | 1     | 2     | 2     | 1     | 2     | 1.9 |
| Amiantos                          | Limassol                  | ~500m²  | 1200–1400     | P. brutia, Q. alnifolia, A. andrachne, Quercus infectoria ssp. veneris, Cistus spp. | 2     | 1     | 2     | 2     | 6     | 4     | 7     | 1     | 4     | 6     | 3.5 |
| Trooditissa                       | Limassol                  | ~2km²   | 1300–1400     | P. nigra ssp. pallasiana, Q. alnifolia, A. andrachne, C. creticus | 9     | 4     | 5     | 4     | 7     | 6     | 2     | 5     | 5     | 7     | 5.4 |
| Platres/Caledonian Falls          | Limassol                  | ~2km²   | 1200–1300     | P. brutia, Q. alnifolia, A. andrachne, C. creticus | 7     | 11    | 3     | 5     | 3     | 7     | 4     | 1     | 1     | 1     | 4.3 |

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| Localities/Monoiatis | District | Area m² | Elevation | Habitat (EcM) | 07/08 | 08/09 | 09/10 | 10/11 | 11/12 | 12/13 | 13/14 | 14/15 | 15/16 | 16/17 | Av. |
|----------------------|----------|---------|-----------|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|------|
| Saittas/Moniatis     | Limassol | ~2km²   | 600–750 m | *P. brutia*, Quercus coccifera ssp. calliprinos, C. salviifolius, C. creticus | 11    | 5     | 5     | 4     | 5     | 3     | 5     | 2     | 2     | 4    | 4.6  |
| Pera Pedi/Mandria    | Limassol | ~2km²   | 750–850 m | *P. brutia*, Quercus infectoria ssp. veneris, Q. coccifera ssp. calliprinos, Cistus spp. | 8     | 15    | 10    | 8     | 5     | 4     | 3     | 3     | 2     | 2    | 6.0  |
| Trimiklini           | Limassol | ~500m²  | 600–700 m | *P. brutia*, Q. coccifera ssp. calliprinos, C. salviifolius, C. creticus | 7     | 8     | 4     | 4     | 5     | 4     | 5     | 2     | 2     | 2    | 4.3  |
| Mesa Potamos         | Limassol | ~2km²   | 750–1000 m| *P. brutia*, Q. amnifolia, Q. infectoria ssp. veneris, A. andrachne, Cistus spp. | 4     | 1     | 1     | 2     | 1     | 1     | 4     | 1     | 2     | 2    | 1.8  |
| Ayia Paraskevi       | Limassol | ~2km²   | 550–700 m | *P. brutia*, Q. infectoria ssp. veneris, Q. coccifera ssp. calliprinos, A. andrachne, Cistus spp. | 1     | 2     | 5     | 7     | 3     | 2     | 2     | 2     | 2     | 3    | 2.9  |
| Germasogeia          | Limassol | ~500m²  | 120–150 m | *Q. coccifera* ssp. *calliprinos, C. creticus, Q. salviifolius, C. parviflorus* | 2     | 2     | 1     | 2     | 2     | 2     | 1     | 1     | 1     | 1    | 1.6  |
| Asgata               | Limassol | ~500m²  | 150–200 m | *C. salviifolius, C. creticus* | 2     | 3     | 2     | 7     | 4     | 1     | 2     | 1     | 1     | 1    | 2.4  |
| Kalavasos            | Limassol | ~1km²   | 150–200 m | *C. salviifolius, C. creticus* | 1     | 1     | 1     | 3     | 2     | 1     | 2     | 1     | 2     | 1    | 1.5  |
| Pissouri             | Limassol | ~800m²  | 200–250 m | *P. brutia* | 3     | 3     | 2     | 4     | 1     | 2     | 2     | 3     | 1     | 1    | 2.2  |
| Alassa               | Limassol | ~500m²  | 400–450 m | *Salix alba* | 1     | 4     | 1     | 2     | 2     | 2     | 4     | 1     | 1     | 1    | 1.9  |
| Akrotiri             | Limassol | ~2km²   | 0–10 m    | *Pinus halepensis*, *P. brutia*, Cistus parviflorus, C. salviifolius | 1     | 5     | 1     | 2     | 13    | 4     | 5     | 8     | 3     | 2    | 4.4  |
| Fassouri             | Limassol | ~500m²  | 0–5 m     | *Eucalyptus gomphocephala, E. camaldulensis* | 2     | 6     | 3     | 3     | 3     | 3     | 4     | 1     | 1     | 1    | 2.7  |
| Agios Nikolaos South (Arminou dam) | Paphos | ~1km² | 450–600 m | *P. brutia*, Q. coccifera ssp. calliprinos, C. creticus, C. salviifolius | 3     | 4     | 6     | 6     | 2     | 8     | 3     | 4     | 1     | 2    | 3.9  |
| Agios Nikolaos North (Kelefos bridge) | Paphos | ~1km² | 450–500 m | *P. brutia*, Q. coccifera ssp. calliprinos, A. orientalis, C. creticus, C. salviifolius | 3     | 4     | 6     | 6     | 1     | 3     | 3     | 4     | 1     | 1    | 3.2  |
| Akamas               | Paphos   | ~2km²   | 100–250 m | *P. brutia*, *Monspeliensis*, C. salviifolius | –     | 1     | 1     | 1     | 1     | –     | –     | 3     | 1     | 2    | 1.0  |
| Cedar Valley         | Paphos   | ~1km²   | 1000–1200 m| *Cedrus brevifolia*, *P. brutia*, A. andrachne, Q. amnifolia | 1     | 1     | 1     | 3     | 2     | –     | –     | 1     | 1     | –    | 1.0  |
| Stavros tis Psokas/Kanaviou | Paphos | ~2km² | 1000–1200 m | *P. brutia*, A. andrachne, Q. amnifolia, Q. infectoria ssp. veneris, Cistus spp. | 1     | 1     | 3     | 2     | 2     | –     | –     | –     | 1     | 1    | 1.1  |
Table 3
Climatological data: Precipitation records per month between 2007 and 2016, based on official data retrieved from the Cyprus Department of Meteorology. Column A: indicates the month for the studied period; Column B: indicates the rainfall average during the 30-years long period 1961–1990, considered as ‘normal’ in the current meteorological database; Column C: indicates the measured rainfall for the considered month of the survey; Column D: indicates the ratio between the two previous columns, demonstrating deficit/excess of rainfall between the observed data and the expected (averages).

| Month       | Normal (mm) | Actual (mm) | Actual/normal |
|-------------|-------------|-------------|---------------|
| October 2007| 32.70       | 10.20       | 0.31          |
| November 2007| 53.30     | 39.90       | 0.75          |
| December 2007| 105.60    | 90.00       | 0.85          |
| January 2008| 102.40      | 38.30       | 0.37          |
| February 2008| 81.60      | 35.60       | 0.44          |
| March 2008| 61.90       | 21.50       | 0.35          |
| April 2008| 29.90       | 2.30        | 0.08          |
| May 2008| 19.60       | 9.50        | 0.48          |
| June 2008| 6.00        | 0.20        | 0.03          |
| July 2008| 2.60        | 0.20        | 0.08          |
| August 2008| 2.90        | 2.20        | 0.76          |
| September 2008| 4.50    | 22.40       | 4.98          |
| October 2008| 32.70      | 22.70       | 0.69          |
| November 2008| 53.30     | 21.50       | 0.40          |
| December 2008| 105.60    | 95.40       | 0.90          |
| January 2009| 102.40      | 108.80      | 1.06          |
| February 2009| 81.60      | 106.30      | 1.30          |
| March 2009| 61.90       | 75.00       | 1.21          |
| April 2009| 29.90       | 22.60       | 0.76          |
| May 2009| 19.60       | 29.20       | 1.49          |
| June 2009| 6.00        | 0.50        | 0.08          |
| July 2009| 2.60        | 1.60        | 0.62          |
| August 2009| 2.90        | 6.10        | 2.10          |
| September 2009| 4.50    | 37.80       | 8.40          |
| October 2009| 32.70      | 40.30       | 1.23          |
| November 2009| 53.30     | 44.50       | 0.83          |
| December 2009| 105.60    | 152.10      | 1.44          |
| January 2010| 102.40      | 149.50      | 1.46          |
| February 2010| 81.60      | 107.50      | 1.32          |
| March 2010| 61.90       | 2.60        | 0.04          |
| April 2010| 29.90       | 20.20       | 0.68          |
| May 2010| 19.60       | 14.10       | 0.72          |
| June 2010| 6.00        | 9.20        | 1.53          |
| July 2010| 2.60        | 5.40        | 2.08          |
| August 2010| 2.90        | 0.00        | 0.00          |
| September 2010| 4.50    | 1.00        | 0.22          |
| October 2010| 32.70      | 9.20        | 0.28          |

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Table 3 (continued)

| Month          | Normal (mm) | Actual (mm) | Actual/normal |
|----------------|-------------|-------------|---------------|
| November 2010  | 53.30       | 0.10        | 0.00          |
| December 2010  | 105.60      | 109.90      | 1.04          |
| January 2011   | 102.40      | 105.90      | 1.03          |
| February 2011  | 81.60       | 73.60       | 0.90          |
| March 2011     | 61.90       | 68.80       | 1.11          |
| April 2011     | 29.90       | 42.80       | 1.43          |
| May 2011       | 19.60       | 24.30       | 1.24          |
| June 2011      | 6.00        | 9.50        | 1.58          |
| July 2011      | 2.60        | 0.00        | 0.00          |
| August 2011    | 2.90        | 0.60        | 0.21          |
| September 2011 | 4.50        | 20.20       | 4.49          |
| October 2011   | 32.70       | 14.50       | 0.44          |
| November 2011  | 53.30       | 80.30       | 1.51          |
| December 2011  | 105.60      | 117.20      | 1.11          |
| January 2012   | 102.40      | 238.40      | 2.33          |
| February 2012  | 81.60       | 99.40       | 1.22          |
| March 2012     | 61.90       | 39.20       | 0.63          |
| April 2012     | 29.90       | 18.60       | 0.62          |
| May 2012       | 19.60       | 30.60       | 1.56          |
| June 2012      | 6.00        | 9.00        | 1.50          |
| July 2012      | 2.60        | 4.00        | 1.54          |
| August 2012    | 2.90        | 2.90        | 1.00          |
| September 2012 | 4.50        | 0.20        | 0.04          |
| October 2012   | 32.70       | 53.40       | 1.63          |
| November 2012  | 53.30       | 84.40       | 1.58          |
| December 2012  | 105.60      | 209.40      | 1.98          |
| January 2013   | 102.40      | 59.20       | 0.58          |
| February 2013  | 81.60       | 41.70       | 0.51          |
| March 2013     | 61.90       | 11.80       | 0.19          |
| April 2013     | 29.90       | 48.50       | 1.62          |
| May 2013       | 19.60       | 27.10       | 1.38          |
| June 2013      | 6.00        | 0.00        | 0.00          |
| July 2013      | 2.60        | 0.40        | 0.15          |
| August 2013    | 2.90        | 0.00        | 0.00          |
| September 2013 | 4.50        | 7.00        | 1.56          |
| October 2013   | 32.70       | 16.10       | 0.49          |
| November 2013  | 53.30       | 25.10       | 0.47          |
| December 2013  | 105.60      | 58.00       | 0.55          |
| January 2014   | 102.40      | 36.90       | 0.36          |
| February 2014  | 81.60       | 41.50       | 0.51          |
| March 2014     | 61.90       | 27.20       | 0.44          |
| April 2014     | 29.90       | 13.10       | 0.44          |
| May 2014       | 19.60       | 62.90       | 3.21          |
| June 2014      | 6.00        | 15.50       | 2.58          |
| July 2014      | 2.60        | 3.70        | 1.42          |
| August 2014    | 2.90        | 4.50        | 1.55          |
| September 2014 | 4.50        | 10.80       | 2.40          |
| October 2014   | 32.70       | 45.00       | 1.38          |
| November 2014  | 53.30       | 48.30       | 0.91          |
| December 2014  | 105.60      | 84.20       | 0.80          |
| January 2015   | 102.40      | 168.10      | 1.64          |
| February 2015  | 81.60       | 104.60      | 1.28          |
| March 2015     | 61.90       | 62.30       | 1.01          |
| April 2015     | 29.90       | 16.10       | 0.54          |
| May 2015       | 19.60       | 20.10       | 1.03          |
| June 2015      | 6.00        | 5.80        | 0.97          |
| July 2015      | 2.60        | 1.80        | 0.69          |
| August 2015    | 2.90        | 0.90        | 0.31          |
| September 2015 | 4.50        | 4.80        | 1.07          |
| October 2015   | 32.70       | 54.70       | 1.67          |
| November 2015  | 53.30       | 10.20       | 0.19          |
| December 2015  | 105.60      | 34.30       | 0.32          |
Richard et al. (2004) [5]. Thirty loosely delimited sites were pre-selected and regularly surveyed (see Table 1), in addition to other less frequently visited localities. Fungal diversity was for the most part undocumented on the island prior to this inventory, therefore the sampling strategy was designed to cover as large an area as possible and yield the maximum possible number of species. As a result, pre-selected sites were consequently large, ranging in size from ~500m² to ~2km². Selection of the permanent sites included all major habitat types formed by ectomycorrhizal (EcM) trees and shrubs on the island, and preliminary observations, anecdotal reports, altitudinal range, accessibility and mean annual precipitation were also taken into consideration. Single-tree communities are rare on Cyprus, therefore the majority of sites were comprised of mixed-tree communities. Of these, mixed *Pinus brutia*/*Quercus alnifolia* habitats are the most widely distributed woodland habitats on the island and as such were better represented among the permanent sites, but mixed *P. brutia/Q. coccifera* subsp. *calliprinos*, and *P. nigra* subsp. *pallasiana/Q. alnifolia* habitats were also well-represented (see Table 1). Because fungal fruiting episodes in the Mediterranean region are typically prolific but brief, and seasonal rainfall in Cyprus is uneven in distribution and highly unpredictable, surveys within permanent sites systematically followed rainfall episodes. Precipitation data for each locality was retrieved at least three times a week from the Cyprus Department of Meteorology official website http://www.moa.gov.cy/moa/ms/ms.nsf/DMIndex_en/DMIndex_en?OpenDocument, and forays were planned accordingly. Surveying usually spanned between September and April, 18–20 days following the first substantial rainfall of the season (>20 mm) and regularly thereafter, usually 1–2 days following subsequent rainfall episodes, or 2–4 times a week. In a typical season, surveying begun from the higher elevations of the Troodos massif (1,200–1,950 m above sea level) and, as temperatures dropped and precipitation increased, surveys gradually shifted to the lower elevations, where most of the fruiting occurs during the colder winter months. Surveying for spring species followed the opposite pattern, beginning from the lowlands in late winter and gradually extending to the higher elevations, until mid-to late spring. Exceptionally, surveys were also carried out in the summer months, following substantial precipitation (>30 mm) at the higher elevations of the Troodos mountains (>1,400 m a.s.l.), where brief localized fruitings sometimes occurred. Collection of specimens within the permanent sites was mostly opportunistic and followed fructification patterns, though identified hotspots within each site and certain tree-hosts of interest were regularly checked. Surveys usually lasted 2–4 hours on each site, with 1–4 sites visited in each foray. Highly productive seasons with abundant precipitation and prolific fructifications were more intensively surveyed than seasons with low precipitation and poor fructifications. Overall, a total of 767 forays were carried out during the
Fig. 1. Selection of representative habitats dominated by ectomycorrhizal (EcM) trees and shrubs: (A) *Pinus nigra* supsp. *pallasiana* forest in the oromediterranean belt (1850 m a.s.l.) at Chionistra; (B) *Pinus nigra* supsp. *pallasiana* forest in the supramediterranean belt (1600 m a.s.l.) at Troodos; (C) *Pinus nigra* supsp. *pallasiana* and *Quercus alnifolia* forest in the oromediterranean belt (1750 m a.s.l.), at Troodos; (D) *Pinus brutia* forest in the mesomediterranean belt (1000 m a.s.l.), at Stavros tis Psokas; (E) *Pinus brutia* forest in the thermomediterranean belt (200 m a.s.l.) at Akamas; (F) *Pinus brutia* and *Quercus coccifera* subsp. *calliprinos* forest in the thermomediterranean belt (450 m a.s.l.) at Kelefos; (G) *Quercus infectoria* subsp. *veneris* stand in the thermomediterranean belt (500 m a.s.l.), at Aya Paraskevi; (H) *Quercus coccifera* subsp. *calliprinos* stand in the thermomediterranean belt (400 m a.s.l.), at Arminou; (I) *Quercus alnifolia* matorral in the mesomediterranean belt (800 m a.s.l.), at Palaichori; (J) *Cistus salvifolius* and *C. cretecus* matorral in the thermomediterranean belt (200 m a.s.l.), at Kalavasos; (K) *Cistus salvifolius* matorral in the thermomediterranean belt (200 m a.s.l.), at Asgata; (L) *Cistus monspelliensis* matorral in the thermomediterranean belt (400 m a.s.l.), at Akamas; (M) *Cedrus brevifolia* forest in the mesomediterranean belt (1200 m a.s.l.), at Tripilos; (N) Riparian *Alnus orientalis* forest in the thermomediterranean belt (400 m a.s.l.), at Kelefos; (O) *Eucalyptus camaldulensis* and *E. gomphocephala* plantation in the dunal belt (5 m a.s.l.), at Fassouri.
decade, with a minimum of 42 and a maximum of 129 forays taking place annually, averaging 76.7 forays per season (see Table 2). Over this period, more than 3,500 vouchered collections belonging to over 1,200 species were gathered and archived, from which all relevant data to Boletaceae fungi was extracted and analyzed separately.

2.2. Ecological, morphological, phenological and chorological analyses

Over 200 Boletaceae collections were gathered and analyzed during this ten-year-inventory. All specimens were photographed in situ, the altitude and soil characteristics were annotated, and the host plant was assigned based on analysis of plant community composition. For collections found in mixed stands, the putative host-plant was assigned based on analysis of the fruiting pattern, spatial distribution of ECM plants, and known host preferences for each species following original descriptions and monographic works [3,6–11]. When the precise ectomycorrhizal symbiont was uncertain, no host-plant was assigned. For the purpose of evaluating estimated abundance, collections found >25 m apart from one-another were considered as fruiting from different mycelia following Dahlberg & Stenlid 1994 [12], and Hirose et al. 2004 [13]. Detailed macromorphological observations were made on fresh fruit bodies, when possible from various developmental stages. Oxidation of the context was observed after bruising the hymenium and longitudinally slicing one or more fresh fruit bodies from each collection. Microscopic studies were performed on both fresh and dried material under a Leica BME binocular, an AmScope T360B trinocular plan achromatic, and a Zeiss axioskop microscopes at ×100, ×400 and ×1000 magnifications. For spore study, normal tap water was used as a mounting medium. A
minimum of 30 naturally discharged, normally developed spores were measured from each basidio-
carp, after placing fragments of the pileus on a glass slide overnight. When fresh material was not
available, naturally discharged spores deposited on the stipe apex were measured. The Me (average
length and width), Q (minimum and maximum length/width ratio) and Qm (average length/width
ratio) were calculated for each collection, based on methods described by Peintner et al. (2003)
[14], and Assyov (2012) [15]. Melzer’s solution was used to observe possible amyloidity of the hyphae at the
stipe base, following Singer (1965) [6], and Ladurner & Simonini (2003) [10]. Congo red in 10%
ammonia (NH4OH), lactophenol cotton blue (LPCB), and 5% potassium hydroxide (KOH) were used to
highlight the basidia, cystidia and pileipellis. All climatological data cited in this study (including
normal, actual and cumulative actual/normal monthly precipitation), was retrieved from Cyprus
Department of Meteorology. Correlation between climatological variables (monthly, seasonal and
annual precipitation levels), and fruiting abundance of boletoid fungi, was performed using Pearson’s
product-moment tests in R 3.2.4 (R Core Team 2016). Distribution maps were compiled in QuarkXPress
14.2.1, based on records reported in Loizides et al. (2019) [1].

2.3. DNA extraction, amplification and sequencing

Following morphological studies, representative specimens identified to belong to distinct species
were selected for molecular analysis. A number of collections from atypical habitats or displaying
unusual features were also molecularly analyzed, along with comparative collections from Bulgaria,
Croatia, France, Greece and Switzerland. DNA extraction and PCR amplification were conducted with the
REDExtract-N-Amp™ Plant PCR Kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufac-
turer’s instructions. The internal transcribed spacers and 5.8S rDNA (ITS) were amplified from each
collection, with the ITS-1F/ITS-4b primer pair, as described in Richard et al. (2015) [16]. When no band
was detected by agarose-gel electrophoresis analysis, 1 μL of the PCR product was used as template in a
second PCR using the ITS1F/ITS4 primer pair [17]. Amplicons were purified and sequenced by Eurofins
Genomics, Ebersberg, Germany. Raw sequence data were edited and assembled with Codon Code
Aligner 4.1.1 (CodonCode Corp., Centerville, MA, USA), and deposited in Genbank under the accession
numbers indicated in Table 1 [1].

2.4. Phylogenetic analyses

Phylogenetic analyses were performed online at www.phylogeny.lirmm.fr. Multiple sequence
alignment was carried out with MUSCLE 3.7 [18], using full processing mode and 16 iterations.
When required, alignments were edited manually or with Gblocks 0.91b, set to lowest stringency in the selection of conserved blocks [19,20]. Maximum likelihood (ML) phylogenetic analyses were performed with PhyML 3.0 [21], using the GTR + I + Γ model of evolution. Branch support was assessed using the non-parametric, Shimodaira–Hasegawa, version of the approximate likelihood-ratio test (SH-aLRT), implemented in the latest release of PhyML and which ensures high accuracy when SH-aLRT > 0.8 [22,23]. Phylogenies were built using FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and edited with Inkscape 0.91 (https://inkscape.org/fr/).

**Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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