Environmental Research 160 (2018) 347–352

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Molecular characterization of *Cryptococcus neoformans* and *Cryptococcus gattii* from environmental sources and genetic comparison with clinical isolates in Apulia, Italy

Maria Teresa Montagna[a], Antonella De Donno[b], Giuseppina Caggiano[a,*], Francesca Serio[b], Osvalda De Giglio[a], Francesco Bagordo[b], R. D'Amicis[c], Shawn R. Lockhart[d], Massimo Cogliati[c]

[a] Department of Biomedical Science and Human Oncology, University of Bari 'Aldo Moro', Bari, Italy
[b] Department of Biological and Environmental Science and Technology, University of Salento, Lecce, Italy
[c] Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milano, Italy
[d] Fungal Reference Laboratory, Centers for Disease Control and Prevention, Atlanta, GA, USA

ABSTRACT

The present study investigated the environmental distribution of *Cryptococcus neoformans* and *C. gattii* species complex molecular types, mating types and sequence types in Apulia, a region of Southern Italy. A total of 2078 specimens from arboreal and animal sources were analyzed. The percentage of positive samples was similar among both arboreal and animal specimens: 4.2% vs. 5.1% for *C. neoformans* species complex and 0.6% vs. 1.4% for *C. gattii* species complex. Molecular typing identified 78 isolates as VNI (76 αA and two aA), one as AD-hybrid αADa, and 16 as VGI aB. VNI isolates presented 10 different sequence types (STs) and VGI isolates two. The most frequent STs among *C. neoformans* and *C. gattii* species complex isolates were ST23 (51%) and ST156 (90%), respectively. Comparison with molecular types and STs results obtained from 21 clinical isolates collected in Apulia showed that one *C. neoformans* VNI clinical isolate shared an identical sequence type of one arboreal isolate (ST61) and that one *C. gattii* VGI clinical isolate matched with the main ST (ST156) present in the environment. In addition, molecular type VNIV was found only among clinical isolates and was absent in the investigated environmental area. In conclusion, the present study identified which *C. neoformans* and *C. gattii* species complex genotypes are circulating in Apulia, defined their ecological niches and revealed the relationship with clinical cases. It represents a basal study for addressing future investigations and public health interventions in the region.

1. Introduction

Cryptococcus neoformans and *C. gattii* are two basidiomycetes species complexes associated with life-threatening infections in humans and other animals (Heitman et al., 2011). *C. neoformans* species complex represents the major opportunistic pathogen among an increasing number of immune-compromised individuals, whereas *C. gattii* species complex is gaining prominence as a primary cause of diseases (Rajasingham et al., 2017; Chen et al., 2014).

Recently, taxonomy of *C. neoformans* and *C. gattii* has undergone important changes due to the rapid development of molecular biology techniques, which have shown that these species are actually two "species complexes" (Kwon-Chung et al., 2017) including several cryptic species according to a new proposed taxonomy (Hagen et al., 2015). *C. neoformans* species complex includes two varieties or two species according to the new proposed taxonomy: *C. neoformans* var. *grubii* (*C. neoformans*) identified by molecular types VNI, VNII and VNB, *C. neoformans* var. *neoformans* (*C. deneoformans*) identified by molecular type VNIV, and inter-varietal hybrids (diploids or aneuploids) identified by molecular type VNIII. *C. gattii* species complex includes four molecular types VGI, VGII, VGIII, and VGIV (Meyer et al., 2003, 2009). The two species complexes differs in ecological, biochemical and genetic characteristics as well as in host preference and manifestations of disease. *C. neoformans* var. *grubii* (*C. neoformans*) has a worldwide distribution, occurring naturally in avian excreta and trees, and C. *neoformans* var. *neoformans* (*C. deneoformans*) occurs mainly in Europe (Cogliati, 2013a) and, with a lower frequency, in other continents. *C. gattii* species complex is mainly associated with trees and presents an endemic distribution in tropical and subtropical areas (Ellis and Pfeiffer, 1990; Chen et al., 2014), but in last decade its area of

* Corresponding author.
E-mail address: giuseppina.caggiano@uniba.it (G. Caggiano).

http://dx.doi.org/10.1016/j.envres.2017.09.032
Received 16 June 2017; Received in revised form 28 September 2017; Accepted 30 September 2017

distribution has been shown to include temperate areas across the world as well, including Europe (Cogliati et al., 2016a, 2016b; Hagen et al., 2012; Bartlett et al., 2012; Springer and Chaturvedi, 2010; Kidd et al., 2004).

Apulia represents the Eastern most region of Southern Italy with a surface of 19,358 km^2 and four million inhabitants, mostly dedicated to tourism, agriculture and livestock rearing. It is characterized by wide coastal development (approximately 860 km coastline) and by the Mediterranean macroclimate (mild winters, and hot and dry summers), more or less significantly modified by the influence of several geographic factors and the complex surface morphology.

The presence of *C. neoformans* species complex in the environment of Apulia was previously reported (Cogliati et al., 2016a, 2016b; Cafarchia et al., 2006; Montagna et al., 2003, 1997b, 1996) and several cryptococcosis cases have been recorded in the main hospitals of the region (FIMUA Cryptococcosis Network, 2002). The discovery of *C. gattii* species complex in Apulia and its first environmental isolation dates back to 1997 and was concurrently reported with an autochthonous human case of meningitis (Montagna et al., 1997a, 1997b). Recent studies (Romeo et al., 2012, 2011; Iatta et al., 2012) confirmed the presence of *C. gattii* species complex in Apulia and other areas of Southern Italy indicating that it has adapted to the environmental conditions existing in the Mediterranean area (Cogliati et al., 2016a, 2016b).

In the present study, we performed a meta-analysis using all data recorded during several environmental surveys carried out in Apulia. Molecular typing and multi-locus sequence typing (MLST) analysis were also applied in order to identify the different genotypes and their distribution in the investigated area. Finally, we compared the MLST profiles of clinical and environmental isolates to determine genetic relatedness.

2. Materials and methods

2.1. Environmental surveys in Apulia

C. neoformans and *C. gattii* species complex isolates and data were collected from previously reported environmental surveys carried out in Apulia (Cogliati et al., 2016a, 2016b; Cafarchia et al., 2006; Montagna et al., 2003), as well as from other surveys performed in this area but not yet reported. Sampling covered a large portion of the territory and took place in Northern, Central and Southern Apulia, and sampled both animals and trees (Supplementary data, Table 1). Samples were processed and cultured on Niger seed agar as previously reported (Cogliati et al., 2016a, 2016b; Cafarchia et al., 2006; Montagna et al., 2003). One or more brown colonies from each positive sample were collected and processed for molecular typing.

2.2. Cryptococcosis cases in Apulia

In the present study isolates from 21 cases of cryptococcosis recorded in Apulia since 1991 were identified by molecular typing and then compared with the environmental isolates (Supplementary data, Table 2). All patients but one were HIV positive and all cases were diagnosed at hospital “Azienda Ospedaliero Universitaria Policlinico”of Bari, the main town of the region. Molecular type and mating type of all isolates was available but MLST profile was determined in only six isolates (three VNI, one VNIV and two VGI).

2.3. Molecular analysis

Mating type and molecular type of *C. neoformans* and *C. gattii* species complex isolates was determined by multiplex PCR as previously described (Cogliati et al., 2000, 2015; Esposto et al., 2004; Feng et al., 2013).

Multi-locus sequence typing was performed using the consensus ISHAM protocol (Meyer et al., 2009) by sequencing seven loci: *CAP59,* IGS1*, GPD1, LAC1, PLB1, SOD1,* and *URA5*. All sequences are available at the *Cryptococcus* MLST database (ww.mycologylab.net).

Concatenate sequences were aligned by Clustal Omega software (http://www.ebi.ac.uk/tools/msa/clustalo). A minimun spanning tree was constructed using Phyloviz software (www.phyloviz.net) and a maximum likelihood tree with Mega software (www.megasoftware.net). Strains IUM 01–4726 (VNII), IUM 97–4515 (VNB), and IUM 98–2742 (VNIV), previously isolated from Italian patients (Cogliati et al., 2016a, 2016b, 2013), were used as outgroups.

2.4. Statistic analysis

Percentage of positive samples from Northern, Central and Southern Apulia as well as from animal and arboreal origin were compared using chi square statistics.

3. Results

3.1. Distribution of the environmental isolates

In Apulia, from 1994 to present, 2078 samples from both animal and arboreal sources were collected. The arboreal samples consisted of 1320 specimens from 799 trees, and were represented by swabs of trunk hollows (45%), soil near the tree (19%), leaves (17%), bark (16%) and flowers (3%). Forty-five percent of sampled trees were *Eucalyptus* trees followed by pine trees (18%), olive trees (12%), *Prunus*, *Quercus,* and *Cupressus* (4% each), and another 30 tree genera with less than 2% each. The percentage of trees colonized by *C. neoformans* species complex was 4.2% (34/799) and by *C. gattii* species complex 0.6% (5/799). *C. neoformans* species complex was recovered from 18 olive trees, five *Eucalyptus* trees, five pine trees, three almond trees, one carob trees, one pear tree, and one apricot tree, whereas *C. gattii* species complex was isolated from one *Eucalyptus* and one olive tree. This latter olive tree harbored both *C. gattii* and *C. neoformans* species complex strains. The animal samples (n = 758) were mainly correlated to birds (bird excreta, cages, water near birds) but there were also some samples from mammals (hair, feces, water). The percentage of positive samples was 5.1% (39/758) for *C. neoformans* species complex and 1.4% (11/758) for *C. gattii* species complex. Comparison of the percentage of positive samples in animal and arboreal samples did not show any statistically significant difference.

The geographical distribution of environmental sampling is shown in Fig. 1. Thirty-six trees and animal specimens were sampled in Northern, 1093 in Central, and 428 in Southern Apulia, and the positive rate was 17.6%, 5.8%, and 6.1%, respectively. Statistical analysis showed no significant difference between the Central and Southern areas. By contrast, the percentage of positive samples was significantly higher in the North although the number of specimens sampled was considerably lower.

3.2. Molecular types and mating types of the environmental and clinical isolates

A total of 45 arboreal isolates from 39 trees were collected during the environmental surveys carried out in Apulia. Fourty isolates (88%) were *C. neoformans* var. *grubii* belonging to molecular type VNI, 39 of which were mating type αA and one mating type aA, the remaining five isolates (12%) were *C. gattii*, VGI, mating type aB. Among animal origin isolates (n = 50), 37 (74%) were VNI, mating type αA, one (2%) VNI mating type aA, one (2%) was a VNIII AD-hybrid, mating type αADa, and 11 (22%) were *C. gattii*, VGI, mating type aB (Supplementary data, Table 1).

Eleven out of the 21 clinical isolates (52%) were *C. neoformans* var. *grubii*, VNI, αA, five (24%) were *C. neoformans* var. *neoformans*, VNIV, αD, three (14%) were VNIII AD hybrids (2 aADα and 1 αDDα), and the

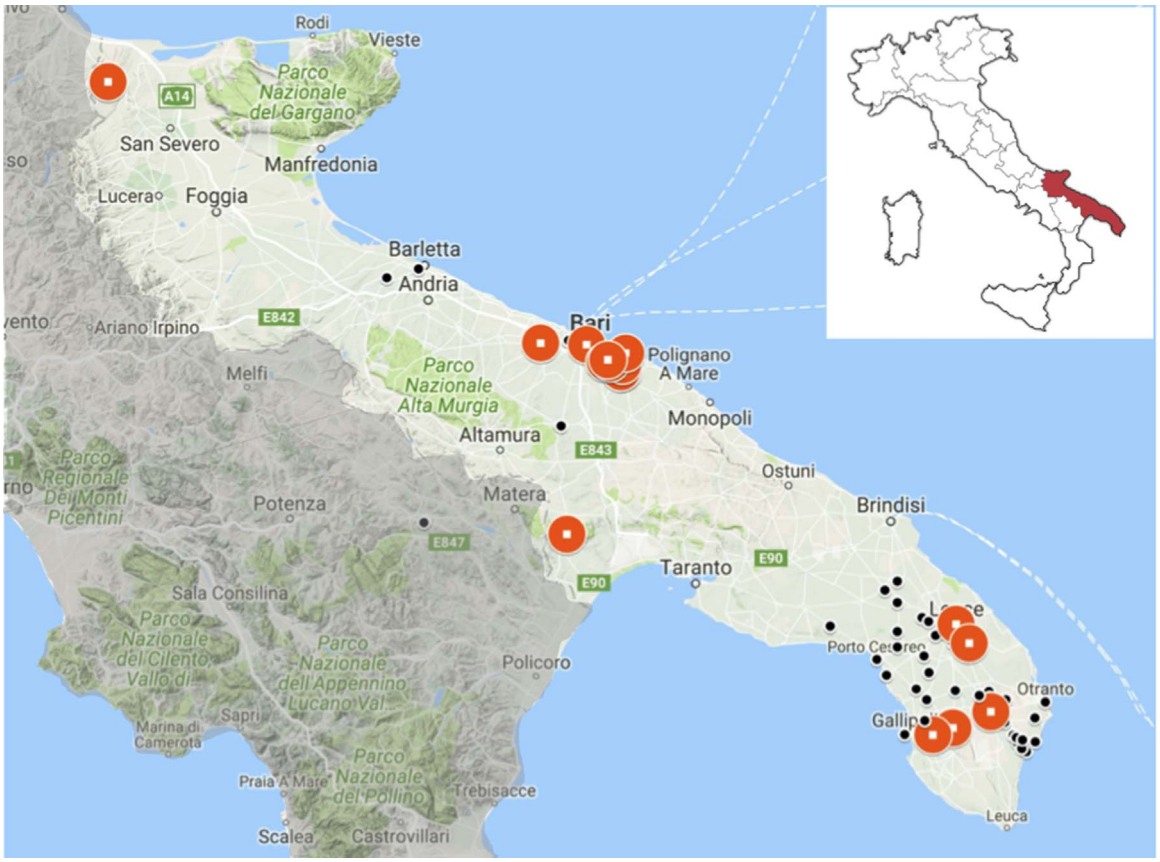

Fig. 1. Geographical distribution of samplings (black dots) and positive samples (red circles) in Apulia region. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

remaining two (10%) were *C. gattii* VGI, one mating type aB and the other mating type αB (Supplemetary data, Table 2).

Comparison of prevalence of the different molecular types between clinical and environmental isolates showed that the percentage of isolates of *C. gattii* species complex did not differ significantly in the two groups. By contrast, the percentage of isolates of *C. neoformans* var. *grubii,* and *C. neoformans* var. *neoformans* and AD hybrids, among clinical isolates were lower and higher, respectively, than among environmental isolates ($p > 0.05$) (Fig. 2).

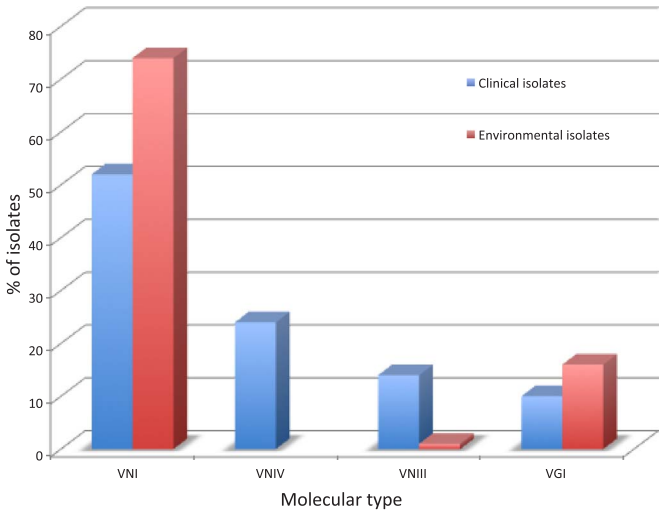

Fig. 2. Percentage of isolates belonging to the different molecular types in clinical and environmental isolates.

3.3. MLST analysis

MLST analysis revealed that VNI isolates presented 10 different sequence type (ST) profiles, and that ST23 and ST63 were the most representative STs including 51% and 13% of VNI isolates, respectively. Four STs (ST493, ST495, ST544, ST545) were not present in the global MLST database and were classified as new STs. All *C. gattii* VGI environmental isolates belonged to ST156, except one which was ST199, a genotype very similar to the former. The three clinical VNI isolates belonged to ST61 (one isolate) and ST69 (two isolates), whereas the two clinical VGI isolates belonged to ST156 and ST232. The unique VNIV isolate with known ST was IUM 98–2742 and belonged to ST112 (Supplementary data, Table 3).

3.4. Phylogenetic analysis

Maximum likelihood phylogenetic reconstruction identified one main cluster among the investigated VNI isolates, which grouped ST23, ST56, and ST61 including a total of 26 isolates, whereas the remaining seven STs (ST63, ST69, ST264, ST493, ST495, ST544 and ST545) were weakly correlated with one another and with the main cluster (Fig. 3). The clinical isolate IUM 99–2359 shared the same ST (ST61) with an arboreal isolate recovered from an olive tree in Southern Apulia. By contrast, no environmental isolate was found that belonged to the same ST (ST69) as the other two clinical isolates. The minimum spanning tree showed that ST61 could represent the ancestral genotype, sharing identical loci with all the other STs present in Apulia (Fig. 4). Isolates from Central Apulia had higher genotypic diversity represented by seven different STs compared to Northern and Southern isolates represented by four STs (Fig. 4). Both ST63 and ST23 included isolates from Northern, Central and Southern Apulia. *C. gattii* VGI clinical

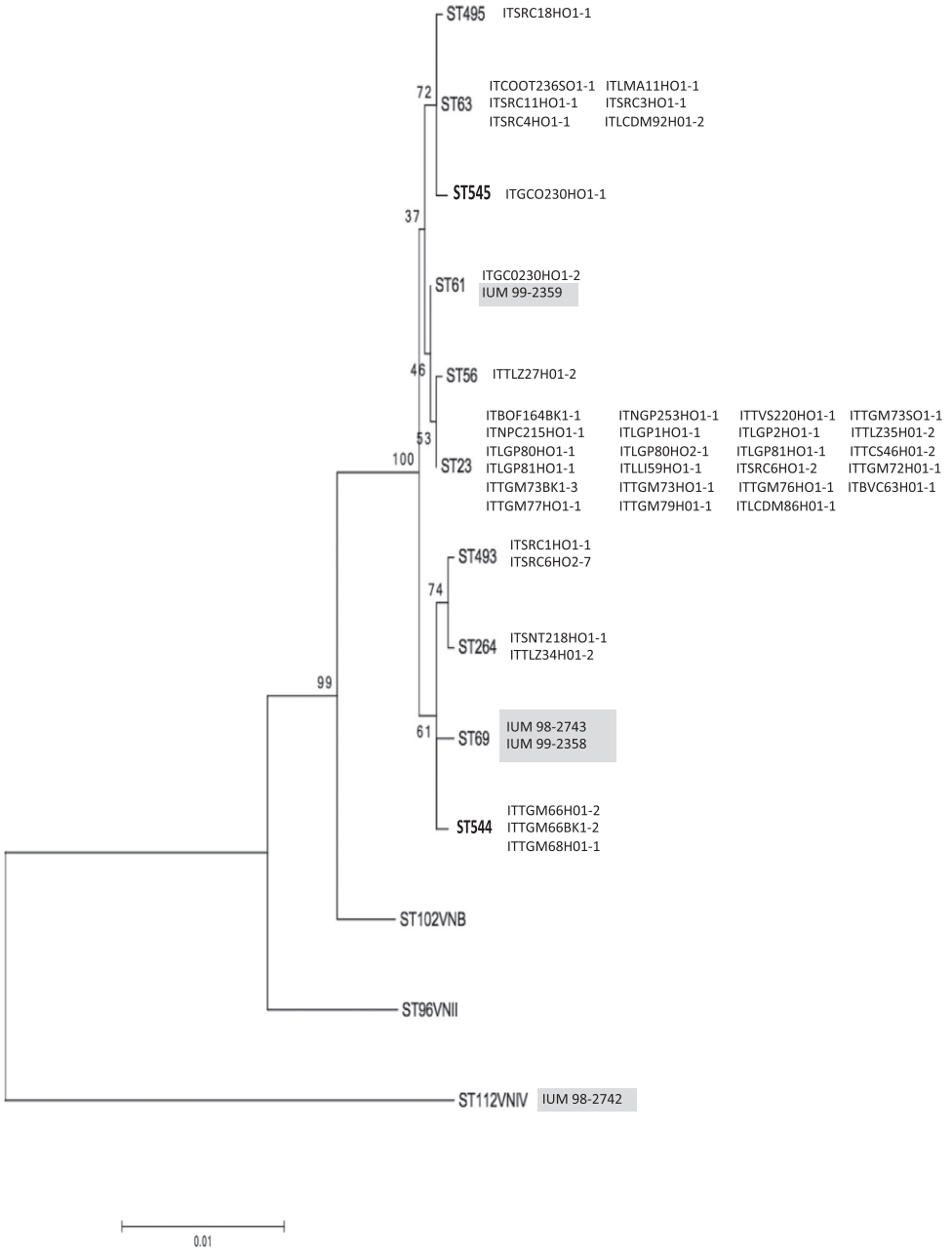

Fig. 3. Maximum likelihood phylogenetic reconstruction based on concatenated sequences of seven loci: *CAP59, GPD1,* IGS1, *LAC1, PLB1, SOD1,* and *URA5*. Numbers near the nodes represent the bootstrap values obtained by 1000 repetitions. Clinical isolates are highlighted in grey.

isolate IUM 95–4427 presented the same ST (ST156) of most of the environmental isolates, whereas the ST (ST232) of other clinical isolates was not found.

4. Discussion

The present study confirms the presence of *C. neoformans* and *C. gattii* species complexes from both animal and arboreal sources throughout Apulia with a high prevalence in Northern Apulia, although this last result is biased by the low number of sites and samples investigated.

A recent European environmental survey reported the percentage of positive trees to be 4.6% and 0.4% for *C. neoformans* and *C. gattii* species complexes, respectively, with a high prevalence of colonized olive trees (Cogliati et al., 2016a, 2016b). In Apulia we found a similar percentage of positive trees (4.2% and 0.6%) among which 43% were olive trees, confirming the important role of this tree as an ecological niche for both *Cryptococcus* species complexes. This represents a major risk factor for cryptococcal disease in Apulia, which is the Italian region with the highest percentage of territory covered by olive cultivation (32%). This study also reports the results of a high number of samples from animal sources allowing an estimate, for the first time in Europe, of the percentage of positivity in these samples. In addition, a comparison of the animal results with those obtained from arboreal samples revealed that the two *Cryptococcus* species complexes are equally distributed in plants and animals, the formers likely acting as reservoirs and the latter as vectors for environmental spread.

Not surprisingly, the prevalent molecular type among clinical and environment isolates was VNI, and it represented high genotypic variability especially in Central Apulia, the area with the highest population density. Molecular type VNIV was identified among clinical isolates but was not recovered from the environment. This finding could reflect its presence in areas or niches that were not sampled. Previous studies showed that *C. neoformans* var. *neoformans* (VNIV) could be able to survive in European areas with a sub-continental climate (Cogliati et al., 2016a, 2016b), therefore it is likely that this yeast has a higher

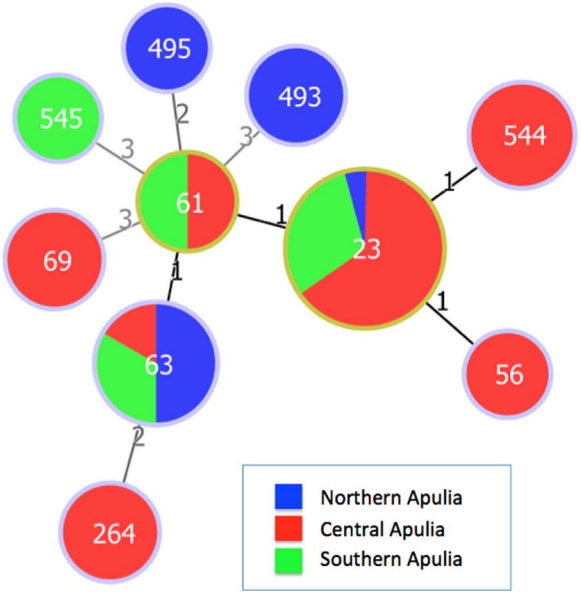

Fig. 4. Minimum spanning tree of VNI isolates. Numbers inside the circles represent the sequence types code, whereas those on the branches the number of different loci. The circle size is proportional to the number of isolates.

probability to be recovered in inlands of Apulia near Appenines mountains such as the Murgie highlands (Fig. 1) that are located not far from Bari, the main town of the region. The presence of VNIV in the environment of Southern Italy was shown by other authors who reported the isolation of this yeast in the nostrils of squirrels living in the Appenine area of Calabria (Iatta et al., 2015), and in an olive tree on the Eastern face of mount Etna in Sicily (Cogliati et al., 2016a, 2016b).

MLST analysis shows that some *C. neoformans* STs are present in both environmental and clinical isolates suggesting that the infections were likely acquired in Apulia. However, in this study we identified only one ST61 environmental isolate and a previous study reported that ST61, ST69 are the prevalent molecular types in Italy (Cogliati et al., 2013), therefore infection outside the region could not be excluded. In addition, our results showed that ST61 has likely been present in Apulia for a long time, and may be the progenitor of the new genotypes found in this study. The most prevalent ST among the isolates investigated in the present study was ST23 which was also identified in seven cases of cryptococcosis recorded in other regions, as well as ST56 was identified in two patients in Milano (Cogliati et al., 2013). However, the low number of environmental samplings performed in the other Italian regions does not allow to determine if these genotypes are present only in the environment of Apulia or elsewhere in the country.

The clinical *C. gattii* VGI isolates belonging to ST156 was clearly correlated to the environmental *C. gattii* VGI isolates present in Apulia, sharing the same rare mating type aB, since only strains belonging to *C. gattii* VGI mating type αB with a different ST (ST197) were isolated in previous studies carried out in Italy (Romeo et al., 2011; Maestrale et al., 2015; Cogliati et al., 2016a, 2016b).

In conclusion, the present study identified which *C. neoformans* and *C. gattii* species complex genotypes are circulating in Apulia, defined their ecological niches and revealed the relationship with clinical cases. Furthermore, it represents a basal study for addressing future investigations and public health interventions in the region.

Acknowledgements

We thank Dr. K.J. Kwon Chung and Dr. A. Varma (NIH, Bethesda, USA), and Dr. V. Rickerts (RKI, Berlin, Germany) for providing technical support on sequencing.

This study was partially supported by the Gilead Fellowship Program 2015 (# 19400).

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2017.09.032.

References

Bartlett, K.H., Cheng, P.Y., Duncan, C., Galanis, E., Hoang, L., Kidd, S., Lee, M.K., Lester, S., MacDougall, L., Mak, S., Morshed, M., Taylor, M., Kronstad, J., 2012. A decade of experience: cryptococcus gattii in British Columbia. Mycopathologia 173 (5–6), 311–319.

Cafarchia, C., Romito, D., Iatta, R., Camarda, A., Montagna, M.T., Otranto, D., 2006. Role of birds of prey as carriers and spreaders of *Cryptococcus neoformans* and other zoonotic yeasts. Med. Mycol. 44 (6), 485–492.

Chen, S.C., Meyer, W., Sorrell, T.C., 2014. Cryptococcus gattii infections. Clin. Microbiol. Rev. 27, 980–1024.

Cogliati, M., 2013a. Global molecular epidemiology of Cryptococcus neoformans and Cryptococcus gattii: an atlas of the molecular types. Scientifica 2013, 675213.

Cogliati, M., Allaria, M., Tortorano, A.M., Viviani, M.A., 2000. Genotyping Cryptococcus neoformans var. neoformans with specific primers designed from PCR-fingerprinting bands sequenced using a modified PCR-based strategy. Med. Mycol. 38 (2), 97–103.

Cogliati, M., Zamfirova, R.R., Tortorano, A.M., Viviani, M.A., FIMUA Cryptococcosis Network, 2013b. Molecular epidemiology of Italian clinical Cryptococcus neoformans var. grubii isolates. Med. Mycol. 51 (5), 499–506.

Cogliati, M., D'Amicis, R., Tortorano, A.M., 2015. Cryptococcus gattii sero-mating type allelic pattern determined by multiplex PCR. Clin. Microbiol. Infect. 21 (190), e1–e4.

Cogliati, M., Zani, A., Rickerts, V., McCormick, I., Desnos-Ollivier, M., Velegraki, A., Escandon, P., Ichikawa, T., Ikeda, R., Bienvenu, A.L., Tintelnot, K., Tore, O., Akcaglar, S., Lockhart, S., Tortorano, A.M., Varma, A., 2016a. Multilocus sequence typing analysis reveals that Cryptococcus neoformans var. neoformans is a recombinant population. Fungal Genet. Biol. 87, 22–29.

Cogliati, M., D'Amicis, R., Zani, A., Montagna, M.T., Caggiano, G., De Giglio, O., Balbino, S., De Donno, A., Serio, F., Susever, S., Ergin, C., Velegraki, A., Ellabib, M.S., Nardoni, S., Macci, C., Oliveri, S., Trovato, L., Dipineto, L., Rickerts, V., McCormick-Smith, I., Akcaglar, S., Tore, O., Mlinaric-Missoni, E., Bertout, S., Mallié, M., Martins, M.D., Vencà, A.C., Vieira, M.L., Sampaio, A.C., Pereira, C., Criseo, G., Romeo, O., Ranque, S., Al-Yasiri, M.H., Kaya, M., Cerikcioglu, N., Marchese, A., Vezzulli, L., Ilkit, M., Desnos-Ollivier, M., Pasquale, V., Korem, M., Polacheck, I., Scopa, A., Meyer, W., Ferreira-Paim, K., Hagen, F., Theelen, B., Boekhout, T., Lockhart, S.R., Tintelnot, K., Tortorano, A.M., Dromer, F., Varma, A., Kwon-Chung, K.J., Inácio, J., Alonso, B., Colom, M.F., 2016b. Environmental distribution of Cryptococcus neoformans and C. gattii around the Mediterranean basin. FEMS Yeast Res. 16 (7), fow086.

Ellis, D.H., Pfeiffer, T.J., 1990. Natural habitat of Cryptococcus neoformans var. gattii. J. Clin. Microbiol. 28, 1642–1644.

Esposto, M.C., Cogliati, M., Tortorano, A.M., Viviani, M.A., 2004. Determination of Cryptococcus neoformans var. neoformans mating type by multiplex PCR. Clin. Microbiol. Infect. 10 (12), 1092–1094.

Feng, X., Fu, X., Ling, B., Wang, L., Liao, W., Pan, W., Yao, Z., 2013. Rapid differentiation of cryptic species within Cryptococcus gattii by a duplex PCR assay. J. Clin. Microbiol. 51, 3110–3112.

FIMUA Cryptococcosis Network, 2002. European Confederation of Medical Mycology (ECMM) prospective survey of cryptococcosis: report from Italy. Med. Mycol. 40 (5), 507–517.

Hagen, F., Colom, M.F., Swinne, D., Tintelnot, K., Iatta, R., Montagna, M.T., Torres-Rodriguez, J.M., Cogliati, M., Velegraki, A., Burggraaf, A., Kamermans, A., Sweere, J.M., Meis, J.F., Klaassen, C.H., Boekhout, T., 2012. Autochthonous and dormant Cryptococcus gattii infections in Europe. Emerg. Infect. Dis. 18, 1618–1624.

Hagen, F., Khayhan, K., Theelen, B., Kolecka, A., Polacheck, I., Sionov, E., Falk, R., Parnmen, S., Lumbsch, H.T., Boekhout, T., 2015. Recognition of seven species in the *Cryptococcus neoformans/C. gattii* species complex. Fungal Genet. Biol. 78, 16–48.

Heitman, J., Kozel, T.R., Kwon-Chung, K.J., Perfect, J.R., Casadevall, A., 2011. Cryptococcus: From Human Pathogen to Model Yeast. ASM Press, Washington, DC, USA.

Iatta, R., Hagen, F., Fico, C., Lopatriello, N., Boekhout, T., Montagna, M.T., 2012. Cryptococcus gattii infection in an immunocompetent patient from Southern Italy. Mycopathologia 174 (1), 87–92.

Iatta, R., Immediato, D., Puttilli, M.R., Danesi, P., Passantino, G., Parisi, A., Mallia, E., Otranto, D., Cafarchia, C., 2015. *Cryptococcus neoformans* in the respiratory tract of squirrels, *Callosciurus finlaysonii* (Rodentia, Sciuridae). Med. Mycol. 53, 666–673.

Kidd, S.E., Hagen, F., Tscharke, R.L., Huynh, M., Bartlett, K.H., Fyfe, M., 2004. A rare genotype of Cryptococcus gattii caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc. Natl. Acad. Sci. USA 101, 17258–17263.

Kwon-Chung, K.J., Bennett, J.E., Wickes, B.L., Meyer, W., Cuomo, C.A., Wollenburg, K.R., Bicanic, T.A., Castañeda, E., Chang, Y.C., Chen, J., Cogliati, M., Dromer, F., Ellis, D., Filler, S.G., Fisher, M.C., Harrison, T.S., Holland, S.M., Kohno, S., Kronstad, J.W., Lazera, M., Levitz, S.M., Lionakis, M.S., May, R.C., Ngamskulrongroj, P., Pappas, P.G., Perfect, J.R., Rickerts, V., Sorrell, T.C., Walsh, T.J., Williamson, P.R., Xu, J., Zelazny,

A.M., Casadevall, A., 2017. The case for adopting the “species complex” nomenclature for the etiologic agents of cryptococcosis. mSphere 2 (1) (e00357-1).
Maestrale, C., Masia, M., Pintus, D., Lollai, S., Kozel, T.R., Gates-Hollingsworth, M.A., Cancedda, M.G., Cabras, P., Pirino, S., D'Ascenzo, V., Ligios, C., 2015. Genetic and pathological characteristics of *Cryptococcus gattii* and *Cryptococcus neoformans* var. *neoformans* from meningoencephalitis in autochthonous goats and mouflons, Sardinia, Italy. Vet. Microbiol 177, 409–413.
Meyer, W., Castañeda, A., Jackson, S., Huynh, M., Castañeda, E., IberoAmerican Cryptococcal Study Group, 2003. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg. Infect. Dis. 9 (2), 189–195.
Meyer, W., Aanensen, D.M., Boekhout, T., Cogliati, M., Diaz, M.R., Esposto, M.C., Fisher, M., Gilgado, F., Hagen, F., Kaocharoen, S., Litvintseva, A.P., Mitchell, T.G., Simwami, S.P., Trilles, L., Viviani, M.A., Kwon-Chung, J., 2009. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. Med. Mycol. 47, 561–570.
Montagna, M.T., Mele, M.S., De Donno, A., Marcuccio, C., Pulito, A., 1996. Criptococcosi e AIDS. Nota I: Indagini sulla diffusione di Cryptococcus neoformans nelle città di Bari e Lecce. Riv. Ital. Ig. 56, 69–77.
Montagna, M.T., Tortorano, A.M., Fiore, L., Ingletti, A.M., Barbuti, S., 1997a. Cryptococcus neoformans var. gattii en Italie. Note I. Premier cas autochtone de me´ningite de se´rotype B chez un sujet VIH positif. J. Mycol. Med. 7, 90–92.
Montagna, M.T., Viviani, M.A., Pulito, A., Aralla, C., Tortorano, A.M., Fiore, L., Barbuti, S., 1997b. Cryptococcus neoformans var. gattii in Italy. Note II. environmental investigation related to an autochtonous clinical case in Apulia. J. Mycol. Med 7, 93–96.
Montagna, M.T., Santacroce, M.P., Caggiano, G., Tatò, D., Ajello, L., 2003. Cavernicolous habitats harbouring Cryptococcus neoformans: results of a speleological survey in Apulia, Italy, 1999–2000. Med. Mycol. 41 (5), 451–455.
Rajasingham, R., Smith, R.M., Park, B.J., Jarvis, J.N., Govender, N.P., Chiller, T.M., Denning, D.W., Loyse, A., Boulware, D.R., 2017. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect. Dis. 17 (8), 873–881.
Romeo, O., Scordino, F., Criseo, G., 2011. Environmental isolation of Cryptococcus gattii serotype B, VGI/MATα strains in southern Italy. Mycopathologia 171 (6), 423–430.
Romeo, O., Scordino, F., Chillemi, V., Criseo, G., 2012. Cryptococcus neoformans/Cryptococcus gattii species complex in southern Italy: an overview on the environmental diffusion of serotypes, genotypes and mating-types. Mycopathologia 174 (4), 283–291.
Springer, D.J., Chaturvedi, V., 2010. Projecting global occurrence of Cryptococcus gattii. Emerg. Infect. Dis. 16, 14–20.