

A citizen science contribution to the knowledge of the fungal diversity in Italy

Arnold Knijn¹, Amalia Ferretti¹, Irja Saar²

¹Via Arno 36, 00198 Roma, Italy

E-mail: knijn_roma@hotmail.com

²Institute of Ecology and Earth Sciences, University of Tartu, J. Liivi 2, 50409 Tartu, Estonia

Abstract: In May 2017, UNITE invited citizen scientists to send fungal collections for DNA sequencing. We describe the results of one response to this call: the findings of eight years of collecting fungi in Italy, mainly from urban parks in the Metropolitan city of Rome and forests and urban parks north of Rome in the Latium region. Between 2018 and 2025, authors AK and AF collected 486 fungal specimens, which they sent to the University of Tartu in Estonia. Overall, of the 233 distinct species identified by molecular analysis, 34 were rare, seven a first vouchered and phylogenetically confirmed record for Italy, and one a new species to science (*Agaricus hortusdamarum*). Urban habitats yielded 218 specimens, representing 165 species, advancing the knowledge of fungal diversity in these understudied environments.

Keywords: citizen science, biodiversity, barcoding; urban parks, *Tremella laurisolvae*, *Cyanoboletus mediterraneensis*, *Rhodotus asperior*

INTRODUCTION

Knowledge of biodiversity status and evolution is recognised as crucial information for the planning and evaluation of habitat conservation and regeneration projects (Luiselli, 2009; Banks-Leite et al., 2020; Brancalion et al., 2025). Monitoring biodiversity gives insight into the effects of human intervention, climate change and other variables on a given ecosystem. Although the importance of information on fungal diversity and occurrence has been well documented (Rathnayaka et al., 2025; Han et al., 2025; Verma, 2025), fungi are still frequently omitted from biodiversity studies, especially in urban settings. Regional census is the foundation of fungal diversity research. Successive investigators have reported that “citizen scientists” can amass large fruitbody collections with minimal or no time expenditure from mycologists, permitting the latter to devote their expertise to specialised work (Heilmann-Clausen et al., 2019; Crous et al., 2021; Shumskaya et al., 2023). Phylogenetic analysis reinforces contributions by citizen scientists, because it defines evolutionary pathways beyond that possible using morphological characters alone. Hence, citizen science projects have proliferated around the globe, producing descriptions of new species as well as precious information on fungal biodiversity. For example, almost half (47%) of the fungal

species occurrence data available through the Global Biodiversity Information Facility (GBIF) platform comes from citizen science contributions (Chandler et al., 2017).

The term “citizen science” was coined by the British sociologist Alan Irwin (1995) to describe the role of science in a society where scientific enquiry is guided by the public to respond to societal needs, often with public participation. “Citizen science” was also used by the American ornithologist Rick Bonney (1996), apparently unaware of its 1995 appearance, to describe a traditional practice in ornithology, the use of data generated by volunteer non-ornithologists (birdwatchers) for scientific purposes. Some form of Bonney’s concept has become the current sense of the term. “Citizen science” was added to the Oxford English Dictionary in 2014, with the definition, “scientific work undertaken by members of the general public, often in collaboration with or under the direction of professional scientists and scientific institutions”. Unfortunately, the exact interpretation of both “citizen” and “science” vary with the situation, making scientists in one setting citizens in another, and vice versa. A single all-encompassing definition must be circumscribed extremely widely, e.g., “... an umbrella term for many very different practices which only have in common that they involve people in research who come from different professions or disciplines than

[those the] projects deal with” (Strähle & Urban 2021). It seems that in mycological practice a somewhat narrower definition may suffice, as most reports involving citizen science projects describe a collaborative relationship between relatively few mycologists and more numerous members of (mostly) amateur mushroom clubs (Heilmann-Clausen et al., 2019; Shumskaya et al., 2023; ISPRA 2025), or even schoolchildren (Crous et al., 2021).

In May 2017, recognising the large potential of citizen science to help fungal diversity research, UNITE (<https://unite.ut.ee/>), made an appeal to citizen scientists: “Call for Citizen Scientists: Submit specimens for sequencing! UNITE is opening a free service for the sequencing of fungal specimens and/or living cultures. We target mostly Citizen Scientists who often collect important and rare species, but which are rarely sequenced.”

The present study describes the results of one response to this citizen science invitation.

MATERIAL AND METHODS

This study was carried out by one mycologist in Estonia (IS), and two citizen scientists in Italy, experts in other life science fields (AK & AF). Both citizen scientists had taken an especial interest in fungi for several years, collecting and studying them in the field, thereby acquiring some mycological knowledge. Between 2018 and 2025, AK and AF sent 486 specimens to the University of Tartu for sequencing, collected primarily from urban parks in the Metropolitan city of Rome and forests and urban parks north of Rome in the Latium region. Standard field collecting techniques were used, and specimens preserved by air drying at low heat. AK and AF performed a preliminary identification of the specimens on-site and microscopy on fresh samples. IS performed the deposit of specimens in the TUF fungarium and database entry in the PlutoF database (Abarenkov et al., 2010). IS also performed microscopy on dry samples, editing the raw sequences and molecular identifications. In parallel, AK performed molecular phylogenetic analyses.

Ribosomal DNA-based analysis was performed on the specimens in the frame of the UNITE project (Köljalg et al., 2013, Abarenkov et al.,

2024). DNA extraction, PCR amplification of SSU partial, ITS1, 5.8S, ITS2, LSU partial regions and sequencing were performed as in Voitek et al. (2020). Initial molecular analyses consisted in alignment of the obtained sequences against the Full UNITE+INSD v9.0 dataset for Fungi (Abarenkov et al., 2022). Further analyses were performed engaging Maximum Likelihood and Bayesian inference with opportunistic sets of sequences. After identification of the species, a search was conducted on the UNITE web page (<https://unite.ut.ee/search.php#findtn-panel2>) and the number of sequences of the same species was recorded for both the UNITE (Abarenkov et al., 2024) and INSD (International Nucleotide Sequence Database, Karsch-Mizrachi et al., 2018) datasets. The indicative prevalence of each species in Italy was determined by a combination of searches on the internet, the GBIF database (<https://www.gbif.org/>), the UNITE/INSD sequence databases and the checklist of Italian fungi by Onofri et al. (2005). Afterwards, in Italy, only checklists on specific species and/or local regions have been published.

RESULTS

Of 486 submitted collections (Figure 1), 458 arrived in satisfactory condition, and were deposited in the TUF fungarium (mycological collections of the University of Tartu, <https://app.plutof.ut.ee/study/view/81151>). Of these, 406 specimens yielded DNA with high-quality ITS sequences, which were added to the UNITE database. Alignment and phylogenetic analyses using sequences from the UNITE and INSD databases were performed for taxonomic classification. Together with macroscopic and microscopic observations, these analyses assigned 355 collections to 233 distinct species, including one new species, *Agaricus hortusdamarum* A. Ferretti, I. Saar & A. Knijn. Also, one undetermined *Entoloma* subgenus *Cyanula* specimen (Knijn et al., 2021) turned out to be a species new to Italy: *Entoloma dislocatum* thereafter described by Noordeloos et al. (2022). Fifty-one fungi could be identified to genus only, due to current lack of correspondence with sequences of classified specimens. The association evolved into a collegial relationship with the depositors (AK & AF) and the sequencer (IS), which led to brief collaborative reports of

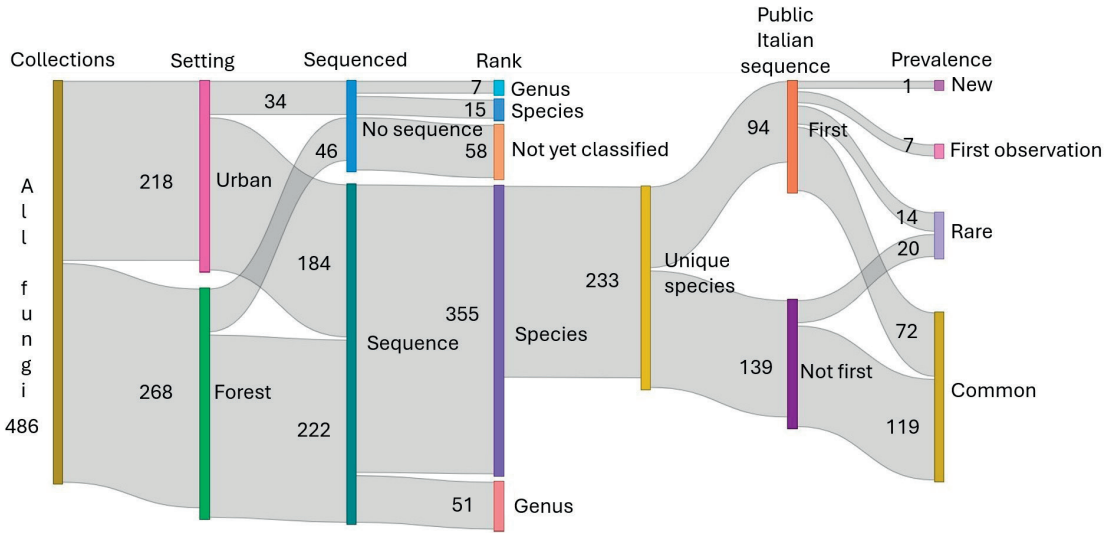


Fig. 1. Sankey diagram illustrating the breakdown of the fungal collections under review. The levels indicate respectively: Collections = Number of specimens collected; Setting = Surroundings of collection; Sequenced = has the specimen been sequenced; Rank = level of classification reached; Public Italian sequence = is it the first time a sequence of this species has been published for Italy; Prevalence = regional prevalence of the species as estimated from a search in UNITE, INSD, GBIF

noteworthy finds, summarised here (Knijn & Ferretti, 2018; Knijn et al., 2019, 2021, 2025; Ferretti et al., 2020, 2022, 2024, 2026).

Of the 233 distinct species for which barcode ITS sequences were obtained, 187 were common, 34 rare, seven a first vouchered observation for Italy, and one new; 218 specimens were collected in urban settings, and 268 in forests (Figure 2). Furthermore, 14 species were at some stage of assessment in the International Union for Conservation of Nature (IUCN) Global Fungal Red List Initiative (Table 1), of which three were assessed as Vulnerable and one as Near Threatened.

Twenty species collected from urban settings are reputed to be rare and/or threatened. Among these, the subtropical to tropical *Rhodotus asperior* Li Ping Tang, Zhu Liang Yang & Bau Tolgor (Figure 3a) was found in two sites within the urban park of Villa Ada in Rome. This apricot pink saprotroph is macroscopically identical to *Rhodotus palmatus* (Bull.: Fr.) Maire which is included as Vulnerable in the IUCN Red List and protected by law in several European countries (Iršénaitė et al., 2019). In

the same urban park, we found *Cyanoboletus mediterraneensis* Biketova, A. Rinaldi & Simoni (Figure 3b) under *Quercus ilex* L. and *Laurus nobilis* L., previously reported only from Israel and the Italian isles of Sardinia by molecular analysis, and Sicily based on morphology (Biketova et al., 2022).

In relation to the climate change aspect and presence of tropical-subtropical mycological species in the Mediterranean environment, we discovered *Tremella laurisilvae* J. Kout (Figure 3c), on a rotten trunk in a *L. nobilis* wood in the Villa Ada urban Park (November 2024). *Tremella laurisilvae* is a species relatively new to science, described from the evergreen laurel forests in the archipelagos of Macaronesia (Kout et al., 2015), and ours is the first observation of the species in Europe. It uses a parasitic strategy in hyperparasitism, developing on other parasitic *Biscogniauxia* fungi and on infected dead wood (Vujanovic et al., 2020; Patejuk et al., 2022). We have previously published reports of *Punctularia atropurpurascens* (Berk. & Broome) Petch from the same urban park (Knijn & Ferretti, 2018; Knijn et al., 2019). In

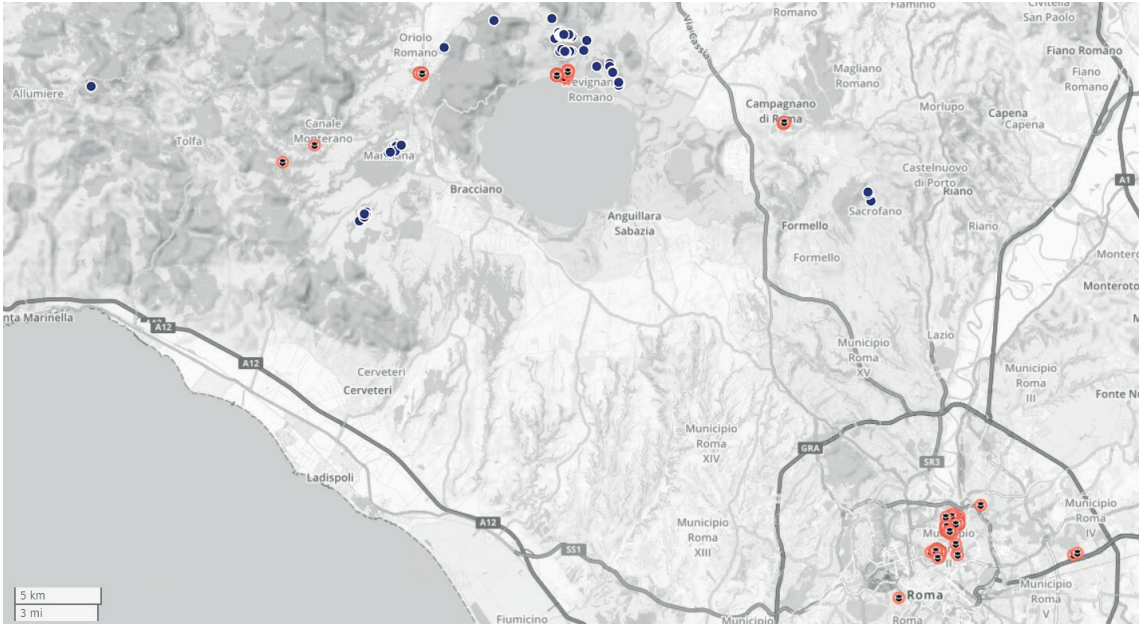


Fig. 2. OpenstreetMaps map created with umap indicating the locations in the central Latium region of the specimens collected in the period 2018-2025. The blue circles with a white ring indicate specimens collected from forests, circles with a red ring and a plant icon indicate specimens collected from urban settings. Swiss style monochrome Data © OpenStreetMap (<https://openstreet-map.org/copyright>) contributors ODbL (<https://opendatacommons.org/licenses/odbl/>) | Map by SOSM (<https://sosm.ch/projects/tile-service/>) Elevation: ASTER GDEM, EarthEnv-DEM90, CDEM contains information under OGL Canada. Powered by Leaflet (<http://leafletjs.com/>) and Django (<https://www.djangoproject.com/>), glued by uMap project (<https://umap-project.org/>) (version 3.7.3).

this case, molecular analyses proved essential to distinguish the specimens from *Hypochnella violacea* Auersw. ex J. Schröt and confirm the identification of this subtropical rare species, previously documented only on the basis of morphology in three collections from Sicily, Italy. These findings are significant because the Mediterranean regions are hotspots for climate change, for which fungi serve as indicator species.

Nidulariopsis iowensis (L.B. Walker) Zeller (Figure 3d) belongs to the family *Geastraceae* close to *Sphaerobolus stellatus*, first collected on conifer trunks in Iowa, USA and subsequently in Europe (Germany). Although little studied, species of *Nidulariopsis* are rare with a limited distribution, and characterised by significant saprophytic activity. In the Villa Ada urban park, some specimens grew on a fallen

trunk in a forest of *Pinus pinea* L., *Robinia pseudoacacia* L., and *Q. ilex*.

The historical Villa Borghese in the centre of Rome proved to be another interesting urban garden for fungal diversity. In fact, in the Villa gardens called “Parco dei Daini”, created in the seventeenth century, the new species *A. hortusdamarum* (Ferretti et al., 2022) was found under *Cupressus sempervirens* L. and *P. pinea* close to *Cedrus libani* A. Rich. In the same gardens, but in *Q. ilex* woods, specimens of *Lactarius mediterraneensis* Listosella & Bellù were found, a rare ectomycorrhizal fungus growing in evergreen oak woods with a Mediterranean distribution, assessed as Near Threatened in the IUCN Red List (Table 1). Within Boletales we identified *Alessioporus ichnusanus* (Alessio, Galli & Littini) Gelardi, Vizzini & Simonini assessed as Vulnerable in the IUCN Red List

Table 1. Species collected in this study, included in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species with their current status (<https://www.iucnredlist.org/> [Accessed 24 May 2026]).

Species	Red List Category	Setting
<i>Alessioporus ichnusanus</i> (Alessio, Galli & Littini) Gelardi, Vizzini & Simonini	Vulnerable	Urban park Villa Borghese
<i>Podoscypha multizonata</i> (Berk. & Broome) Pat.	Vulnerable	Urban park Villa Ada
<i>Tricholoma acerbum</i> (Bull.) Vent.	Vulnerable	Forest
<i>Lactarius mediterraneensis</i> Llistos. & Bellù	Near Threatened	Urban park Villa Borghese
<i>Agaricus campestris</i> L.	Least Concern	Urban park Villa Ada
<i>Amanita caesarea</i> (Scop.) Pers.	Least Concern	Forest
<i>Cantharellus alborufescens</i> (Malençon) Papetti & S. Alberti	Least Concern	Urban park Villa Ada, Villa Borghese
<i>Cantharellus cinereus</i> Pers.	Least Concern	Forest
<i>Cantharellus pallens</i> Pilát	Least Concern	Forest
<i>Craterellus calicornucopioides</i> D. Arora & J.L. Frank	Least Concern	Forest
<i>Russula vesca</i> Fr.	Least Concern	Forest
<i>Suillus granulatus</i> (L.) Roussel	Least Concern	Forest
<i>Volvariella bombycina</i> (Schaeff.) Singer	Least Concern	Urban park Villa Ada, Villa Borghese; Forest
<i>Leucocoprinus ariminensis</i> (Dovana, Angeli, Contu & Brandi) M. Asif, Saba & Vellinga, listed as <i>Leucoagaricus ariminensis</i> Dovana, Angeli, Contu & Brandi	Data Deficient	Urban alley

(Table 1) and *Rheubarbariboletus persicolor* (H. Engel, Klofac, H. Grünert & R. Grünert) Vizzini, Simonini & Gelardi. This latter species is considered rare in Mediterranean habitats and particularly present in protected reserves, ensuring a certain level of protection.

The sixteenth-century Palazzo Altieri, with its park, resides in the centre of Oriolo Romano, in the province of Viterbo in Latium. The eight-hectare park is a mixed Italian and English garden with a main avenue, intersected by avenues with *Prunus laurocerasus* L., that borders the meadows, flanked by *Q. ilex* and *Abies alba* Mill., and surrounded by *L. nobilis*. Specimens of the rare species *Russula stenocystidiata* (Sarnari) Donelli were found in these meadows of the Villa. These findings represent an example of the importance of obtaining DNA sequences of all specimens because *R. stenocystidiata* shows, like other *Russula* in subsection *Amoeninae*, a large variability in morphology and colouration (Figure 4), and could be misidentified.

Other interesting species collected in the same meadows, are *Amanita griseocaerulea* Migl., & Donato, a species recently described by

Migliozzi and Donato (2022) from the Riserva Naturale della Sughereta of Pomezia in Latium and *Leucocoprinus crystallifer* (Vellinga) Migl., & Donato, described as rare in Italy (Della Maggiora, 2023).

DISCUSSION

Our review confirms that bulk DNA sequencing of local fungi provides significant knowledge about regional fungal diversity, especially in understudied specialised habitats. Our review also reaffirms that bulk sequencing can be done in much greater numbers and lesser time with citizen science collaboration. Because at least over 80–90% of the world’s estimated several million fungal taxa are unknown (Rathnayaka et al., 2025 and references within), and the number of trained mycologists is in the low thousands, a large pool of citizen scientists is a major resource for mycological diversity and distribution work. In return for this collaboration, mycologists provide teaching and expert identification help to citizen scientists, creating a mutualistic relationship not unlike that of many of the organisms that they study.



Fig. 3. Photographs of selected interesting species reported from urban settings: a) *Rhodotus asperior*; b) *Cyanoboletus mediterraneensis*; c) *Tremella laurisilvae*; d) *Nidulariopsis iowensis*.

UNITE's "Call for Citizen Scientists", one such global project, provided the spark that resulted in this work, and supported the cost of sequencing, without which the findings would lack scientific significance. The direct contribution of citizen scientists to UNITE's goals was summarised in the November 2023 update of the project (<https://unite.ut.ee/posts.php?id=2>): 37 citizen scientists submitted 1–1315 specimens for sequencing. Of the total 2099 specimens submitted, 1815 were successfully sequenced for rDNA ITS. These contributions have introduced over 800 distinct taxa to the UNITE database, many new to science. In addition, the submitted material remains available for all investigators for future studies. All submitted collections, including over ten type specimens, are accessible to workers from the mycological collections of the University of Tartu.

Collections not identified to species level can become useful in future investigations, when more sequences will be available. Photographs and metadata are available in the PlutoF biodiversity platform (<https://plutof.ut.ee/>; Abarenkov et al., 2010) and are also published to the GBIF network (<https://www.gbif.org/>). ITS sequences are uploaded to the UNITE public database, to serve as important references for the BLAST and phylogenetic analyses of future research.

Even this small study has made some notable contributions. The highlights (a new species to science, first reports of species in Italy, and the finding of rare species) have been published as they were discovered, and have been reviewed here, but the overview revealed worthwhile additions to the knowledge of fungal diversity



Fig. 4. Photographs of *Russula stenocystidiata* specimens from Villa Altieri, illustrating pronounced variation in fruitbody morphology, particularly in the colour of the stipe, gills and pileus cuticle.

in the generally understudied urban parkland habitat (Newbound et al., 2010; Zhang et al., 2019). Although the important role of fungi in environment health has been well demonstrated, generally, fungi have not been considered in decisions of park maintenance. In this study, a relatively high number of fungal taxa from urban parks proved to be rare. Many of these parks began as centenary Roman villas, with some tracts transformed into traditional public parks by landscape design, and others allowed to develop into veritable urban forests. That these special enclaves harbour uncommon species should be very helpful knowledge to persons and organizations charged with maintaining these lands. Thus, citizen science efforts not only benefit amateurs and professional investigators, but can also, albeit in a small

way, make a direct and palpable contribution to society in general.

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