

Article

DNA Barcoding Data Reveal Important Overlooked Diversity of *Cortinarius sensu lato* (*Agaricales*, *Basidiomycota*) in the Romanian Carpathians

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Abstract: *Cortinarius* s.l. is a globally distributed agaricoid genus that has been well studied in Europe with over 1000 described species. However, the information about their taxonomy and diversity in eastern Central Europe is still limited. Only 124 species have been reported so far from Romania, based solely on morphological observations. The aim of this study was to re-examine the diversity of the genus *Cortinarius* s.l. in the Romanian Carpathian area, employing molecular phylogenetic and morphological methods. During intensive field work in the period 2017–2020, a total of 234 *Cortinarius* s.l. specimens were collected and studied with integrative taxonomic methods. For all the samples, we amplified and sequenced the nrDNA ITS region, which is the widely used official barcode marker of fungi. These sequences were compared to the data found in public databases (GenBank, UNITE, BOLD). Based on phylogenetic analyses, we identified 109 *Cortinarius* s.l. species, which represent 40 sections and 3 clades. Out of these species, 43 have previously been documented from Romania based on morphological identification methods, while 66 species are reported as new to the country.

Keywords: Eastern Europe; MrBayes; nrDNA ITS; RAxML; phylogeny; revision; taxonomy



Citation: Szabó, E.; Dima, B.; Dénes, A.L.; Papp, V.; Keresztes, L. DNA Barcoding Data Reveal Important Overlooked Diversity of *Cortinarius sensu lato* (*Agaricales*, *Basidiomycota*) in the Romanian Carpathians. *Diversity* **2023**, *15*, 553. <https://doi.org/10.3390/d15040553>

Academic Editor:
Stephan Koblmüller

Received: 14 March 2023
Revised: 3 April 2023
Accepted: 9 April 2023
Published: 13 April 2023



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1. Introduction

Cortinarius (Pers.) Gray s.l. (*sensu lato*) has been considered to be the biggest genus of the order *Agaricales* [1], with a cosmopolitan distribution of over 3000 described species [2]. *Cortinarius* species are important ectomycorrhizal fungi (EcM) associated with different trees and shrubs, belonging to the order *Fagales*, families *Caesalpiniaceae*, *Cistaceae*, *Dipterocarpaceae*, *Myrtaceae*, *Pinaceae*, *Polygonaceae*, *Rhamnaceae*, *Rosaceae* and *Salicaceae* as well as a few herbaceous plants in the *Cyperaceae*. Because of their EcM nutritional mode, they also play a key role in carbon cycling, especially in boreal forests. Several groups have narrow ecological preferences, and they are sensitive to environmental changes; therefore, some species have been used as indicators for valuable natural environments [3]. Species belonging to *Cortinarius* have a highly variable appearance, from mycenoid to tricholomatoid basidiomata. Their color can be uniformly brown or colorful, and the

surface of the pilei is dry, fibrillose, silky, squamose or viscid [4]. Another common feature of *Cortinarius* is a cobweb-like partial veil, the usually rusty-brown spore print and the lack of a germ pore and perisporium [5]. In the beginning of the 21st century, molecular phylogenetic studies showed that the genus also contains several species (mainly extra-European) with sequestrate fruiting body forms [6]. According to IndexFungorum (<http://www.indexfungorum.org>, accessed on 7 March 2023), 5819 *Cortinarius* names have been published worldwide (accessed 2 March 2023). However, this number includes all infrageneric taxa, and still the number of existing species names are estimated to be over 5000. Many of these names were inconsistently used in the literature, and there is also a high number of synonyms. One of the most challenging parts of *Cortinarius* taxonomy is to examine which species have already been described [3].

Since the early 19th century, many researchers have focused on the genus *Cortinarius* in Europe, e.g., [7–9]. From the 20th century onwards, several fundamental morphological works were published from Europe, e.g., [10–18]. From the late 1980s, two taxonomic schools have spread in Europe, representing the South European (especially French, Italian and Spanish) and the Scandinavian directions. During this period, the Atlas des Cortinaires [19–45] described approximately 1500 new species, while the more conservative species concept of the Scandinavian school, the *Cortinarius*, Flora Photographica [46–49], dealt with only 300 species. The introduction of molecular tools in addition to the macro- and micromorphological character-based identification led to a more precise and reliable classification and helped avoid misunderstandings in the taxonomy and nomenclature of the genus. Northern Europe became one of the most investigated and well-studied regions [4,50–63], but in the last few decades, several other studies focused also on the Central European region [64–70]; however, the information from this region is still limited. Similarly, there is a lack of information regarding the southern parts of Europe [3], with only a few papers [71–73] published from the Mediterranean area so far. Depending on the identification and classification concepts, the genus *Cortinarius* s.l. was separated into several subgenera by different authors. Moser [15] named seven subgenera, while Moëgne-Loccoz et al. [19,20] and Bidaud et al. [25,26] mentioned six subgenera in their works. Scandinavian researchers [46–49] separated the genus into five subgenera. In the molecular era, phylogenetic analyses showed that these subgenera are mostly artificial, e.g., [3,70]. New infrageneric classifications of *Cortinarius* based on multigene phylogenetic analyses were proposed by Garnica et al. [74] and Soop et al. [75]. Recently, Liimatainen et al. [76] has split the genus *Cortinarius* into 10 genera based on genomic and multi-gene sequence data. This new classification, however, is not adopted in the current study, due to practical reasons.

Fungal research in Romania has received increasing interest since 1950, leading to 8727 reported fungal species [77,78], including both macro- and microfungi. Although there are a number of publications that mention the genus *Cortinarius* and provide scarce species lists from few areas in the Carpathians [79–89], systematic study that only focuses on this genus is still lacking. Based on an extensive literature search, we know about 124 reported *Cortinarius* species from Romania, identified based only on their morphological characteristics. This is a relatively low number when compared to the more than 1000 known European *Cortinarius* species [90].

Using solely morphological characteristics to identify *Cortinarius* species is very challenging and needs a lot of experience and solid knowledge. During the development of the basidiocarps, several characteristics may change significantly and overlap with other species, i.e., their intraspecific variability is high. Micromorphological characteristics, such as the size, shape or ornamentation of the basidiospores, also play an important role in the identification process, e.g., [4]. Nonetheless, DNA-sequence-based analyses (e.g., DNA barcoding) are the most reliable identification methods to date, allowing the investigation of the differences between species and varieties too. The nrDNA ITS (Internal Transcribed Spacer) region has been proposed and started to be widely used in molecular taxonomy as the universal and official barcoding region for fungi [91]. ITS is composed of three

subregions: ITS1, 5.8S and ITS2, of which the ITS1 and ITS2 spacers show higher evolutionary rates than the 5.8S; therefore, these are the best suited for studying interspecific level differences [92]. This region evolved relatively rapidly compared to the mitochondrial genes; therefore, in the case of *Cortinarius* too, it is useful and widely used for species identification purposes [3,56,74,93,94].

To study fresh material, we based our study mainly on sampling in the Apuseni Mts, which is a prominent forested area of Romania [95] and located in northern part of the Western Romanian Carpathians. The mountain range is regarded as an important biogeographical area between the Pannonian Plain and the Transylvanian Plateau as well as in the Southern and Eastern Carpathians. Due to its location, several biogeographical regions are present in the area (e.g., alpine, arctic, Mediterranean, etc.), which has resulted in it being one of the regions in Europe with high biodiversity [96]. Several suitable forest types and hot spots for *Cortinarius* are represented in the area, both on calcareous and siliceous bedrocks. The forest vegetation is dominated by oak (*Quercus petraea* agg.), mixed hornbeam–beech (*Carpinus betulus*, *Fagus sylvatica*), beech (*F. sylvatica*), mixed beech and spruce (*F. sylvatica*, *Picea abies*) and spruce (*P. abies*) forests [97]. Additionally, we also extended our sampling to some localities in the Eastern Carpathians (Ținutul Sării, Baraolt and Liban).

Due to the limited knowledge and the lack of molecular genetic data of the genus *Cortinarius* in Romania, the aims of this study were to make a pilot revision of the diversity, taxonomy and distribution of *Cortinarius* s.l. species, using DNA barcoding combined with morphological methods, and update the list of species of this important ectomycorrhizal genus.

2. Materials and Methods

2.1. Taxon Sampling

For our pilot survey, samples were collected from sites including the main habitat types, characteristic for the Transylvanian part of Romania, such as broad-leaved forests (40%), coniferous forests (35%), mixed forests (11%) and other habitat types (14%; e.g., transitional woodland–shrub, pastures, natural grasslands). The collections were made from 2017 to the autumn of 2020, with the professional collaboration of K. Babos, L. Bartha, G. Bélfenyéri, A. Dénes, R. Erös, K. Fehér, L. Gál, B. Jancsó, K. Macalik and Cs. Szabó.

A total of 234 *Cortinarius* samples were collected (Table 1). Every sample has a unique code (e.g., C001, where the C means the first letter of the genus name), and in every case the collection coordinates were recorded. The coordinates were registered in the decimal degrees (DD) format, with six decimals (Table 1). The majority of the samples were photographed in the field (Figure 1), and gross morphology was noted. All samples were dried with a dehydrator at 45 °C. Samples were deposited at the Faculty of Biology and Geology, Babeş-Bolyai University, Cluj-Napoca, Romania.

Table 1. Metadata of *Cortinarius* samples studied and identified during this work. All sequences are newly generated.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. alboviolaceus</i>	C213	04.10.2020	Sovata	46.662003	25.215961	OP099671
<i>C. anfractoides</i>	C361	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099771
<i>C. anfractoides</i>	C363	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099773
<i>C. anomalus</i>	C145	30.08.2020	Liban	46.552611	25.525509	OP099625
<i>C. anomalus</i>	C160	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP099635
<i>C. anomalus</i>	C324	07.11.2020	Cheile Someşului Cald	46.67071058	22.81810123	OP099745

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. anomalus</i>	C159 *	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP132853
<i>C. aureopulverulentus</i>	C338	07.11.2020	Cheile Someșului Cald	46.62928894	22.7806518	OP099756
<i>C. balteatocumatilis</i>	C290	01.11.2020	Chinteni	46.895154	23.520302	OP099723
<i>C. balteatocumatilis</i>	C291	01.11.2020	Chinteni	46.895154	23.520302	OP099724
<i>C. balteatus</i>	C103	10.07.2020	Zetea	46.4733373	25.3454161	OP099607
<i>C. bergeronii</i>	C223	24.10.2020	Cheile Vârghișului	46.183191	25.590322	OP099680
<i>C. brunneus</i>	C246A	24.10.2020	Statiunea Muntele Baisoara	46.50627098	23.26640838	OP099695
<i>C. bulliardii</i>	C359	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099769
<i>C. caesiophylloides</i>	C009	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099571
<i>C. calochrous</i>	C221	24.10.2020	Cheile Vârghișului	46.205054	25.558222	OP099678
<i>C. calochrous</i>	C233	24.10.2020	Cheile Vârghișului	46.203207	25.559007	OP099685
<i>C. camphoratus</i>	C097	19.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099603
<i>C. camphoratus</i>	C153	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP099630
<i>C. camphoratus</i>	C202	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099661
<i>C. caninus</i>	C040	22.09.2018	Valea Ierii	46.524239	23.275145	OP099579
<i>C. caninus</i>	C146	30.08.2020	Liban	46.552103	25.524967	OP099626
<i>C. caninus</i>	C149	03.09.2020	Mărtiniș	46.2647	25.355	OP099627
<i>C. caninus</i>	C189	04.10.2020	Chiheru de Jos	46.686903	25.040178	OP099648
<i>C. caninus</i>	C192	04.10.2020	Pădurea Buneților	46.081617	25.034936	OP099651
<i>C. caninus</i>	C193	04.10.2020	Pădurea Buneților	46.081617	25.034936	OP099652
<i>C. caninus</i>	C194	04.10.2020	Pădurea Buneților	46.081617	25.034936	OP099653
<i>C. caninus</i>	C199	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099658
<i>C. caninus</i>	C200	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099659
<i>C. caninus</i>	C206	04.10.2020	Chiheru de Jos	46.681697	25.035186	OP099665
<i>C. caninus</i>	C207	04.10.2020	Chiheru de Jos	46.681697	25.035186	OP099666
<i>C. caninus</i>	C255	01.11.2020	Harghita	46.538506	25.612982	OP099701
<i>C. caperatus</i>	C074	13.10.2019	Statiunea Muntele Baisoara	46.522375	23.273463	OP099594
<i>C. caperatus</i>	C092	19.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099598
<i>C. caperatus</i>	C093	26.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099599
<i>C. caperatus</i>	C120	28.07.2020	Cheile Someșului Cald	46.626862	22.788439	OP099617
<i>C. caperatus</i>	C143 *	08.08.2020	Statiunea Muntele Baisoara	46.53262527	23.28001233	OP132852
<i>C. catharinae</i>	C089	02.12.2019	Făgetul Clujului	46.735133	23.539162	OP099596
<i>C. cinereobrunneolus</i>	C343	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099759
<i>C. cinnamomeus</i>	C105	10.07.2020	Zetea	46.4733373	25.34546	OP099608

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. claricolor</i>	C094	26.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099600
<i>C. collinitus</i>	C003	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099565
<i>C. collinitus</i>	C011	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099573
<i>C. collinitus</i>	C045	22.09.2018	Valea Ierii	46.525571	23.274678	OP099580
<i>C. collinitus</i>	C048	22.09.2018	Valea Ierii	46.524456	23.274586	OP099583
<i>C. collocandoides</i>	C349	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099760
<i>C. colymbadinus</i>	C114	23.06.2020	Liban	46.553983	25.520218	OP099613
<i>C. corrosus</i>	C263	01.11.2020	Ghimes	46.528277	26.033167	OP099704
<i>C. corrosus</i>	C273	01.11.2020	Ghimes	46.528277	26.033167	OP099710
<i>C. croceus</i>	C108 *	23.06.2020	Liban	46.553983	25.520218	OP132850
<i>C. croceus</i>	C115	23.06.2020	Liban	46.553983	25.520218	OP099614
<i>C. croceus</i>	C116	23.06.2020	Liban	46.553983	25.520218	OP099615
<i>C. croceus</i>	C124	28.07.2020	Cheile Someșului Cald	46.6342004	22.7476044	OP099618
<i>C. daulnoyae</i>	C175	18.10.2020	Făgetul Clujului	46.738306	23.539194	OP099639
<i>C. daulnoyae</i>	C176	18.10.2020	Făgetul Clujului	46.738306	23.539194	OP099640
<i>C. daulnoyae</i>	C286	01.11.2020	Chinteni	46.895154	23.520302	OP099720
<i>C. daulnoyae</i>	C298 *	01.11.2020	Făgetul Clujului	46.732552	23.543583	OP142445
<i>C. daulnoyae</i>	C307	01.11.2020	Făgetul Clujului	46.729857	23.254839	OP099735
<i>C. daulnoyae</i>	C310	01.11.2020	Făgetul Clujului	46.734696	23.540594	OP099737
<i>C. daulnoyae</i>	C360	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099770
<i>C. delibutus</i>	C253	01.11.2020	Harghita	46.538506	25.612982	OP099699
<i>C. delibutus</i>	C254	01.11.2020	Harghita	46.538506	25.612982	OP099700
<i>C. aff. delibutus</i>	C110	23.06.2020	Liban	46.553983	25.520218	OP099612
<i>C. elatior</i>	C352	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099762
<i>C. elegantior</i>	C322	07.11.2020	Cheile Someșului Cald	46.67071058	22.81810123	OP099743
<i>C. elegantissimus</i>	C241	24.10.2020	Cheile Vârghișului	46.201743	25.560039	OP099690
<i>C. eliae</i>	C293	01.11.2020	Chinteni	46.895154	23.520302	OP099725
<i>C. eliae</i>	C294	01.11.2020	Chinteni	46.895154	23.520302	OP099726
<i>C. eliae</i>	C295	01.11.2020	Chinteni	46.895154	23.520302	OP099727
<i>C. fraudulosus</i>	C257	01.11.2020	Harghita	46.538506	25.612982	OP099702
<i>C. fulminoides</i>	C334	07.11.2020	Cheile Someșului Cald	46.64369044	22.7320642	OP099753
<i>C. gallurae</i>	C379	21.10.2020	Bătarci	48.014053	23.146755	OP099780
<i>C. geniculatus</i>	C357	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099767
<i>C. gentilis</i>	C051	22.09.2018	Valea Ierii	46.524888	23.274323	OP099585
<i>C. glaucopus</i>	C012	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099574
<i>C. glaucopus</i>	C248	02.11.2020	Mănăstireni	46.792118	23.153491	OP099697
<i>C. glaucopus</i>	C328	07.11.2020	Cheile Someșului Cald	46.64377853	22.73244046	OP099748

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. glaucopus</i>	C329	07.11.2020	Cheile Someșului Cald	46.64377853	22.73244046	OP099749
<i>C. glaucopus</i>	C332	07.11.2020	Cheile Someșului Cald	46.64369044	22.7320642	OP099751
<i>C. glaucopus</i>	C335	07.11.2020	Cheile Someșului Cald	46.64420997	22.72738871	OP099754
<i>C. aff. glaucopus</i>	C326	07.11.2020	Cheile Someșului Cald	46.6310984	22.76807258	OP099746
<i>C. aff. glaucopus</i>	C331	07.11.2020	Cheile Someșului Cald	46.64369044	22.7320642	OP099750
<i>C. aff. glaucopus</i>	C341 *	07.11.2020	Cheile Someșului Cald	46.64060986	22.81455206	OP132857
<i>C. hadrocroceus</i>	C106	10.07.2020	Zetea	46.4733373	25.34546	OP099609
<i>C. hadrocroceus</i>	C140	08.08.2020	Statiunea Muntele Baisoara	46.51092527	23.27111959	OP099621
<i>C. hillieri</i>	C171	18.10.2020	Făgetul Clujului	46.738306	23.539194	OP099637
<i>C. hinnuleus</i>	C377	21.10.2020	Bătarci	48.014053	23.146755	OP099779
<i>C. holoxanthus</i>	C046	22.09.2018	Valea Ierii	46.524456	23.274586	OP099581
<i>C. holoxanthus</i>	C052	22.09.2018	Valea Ierii	46.524888	23.274323	OP099586
<i>C. holoxanthus</i>	C095	19.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099601
<i>C. huronensis</i>	C016	15.08.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099575
<i>C. huronensis</i>	C021	15.08.2018	Statiunea Muntele Baisoara	46.537253	23.305079	OP099578
<i>C. huronensis</i>	C151	03.09.2020	Mărtiniș	46.2647	25.355	OP099629
<i>C. hydrotelamonioides</i>	C205	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099664
<i>C. incognitus</i>	C004	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099566
<i>C. lacustris</i>	C387	21.10.2020	Bătarci	48.014053	23.146755	OP099783
<i>C. largus</i>	C232	24.10.2020	Cheile Vârghișului	46.199804	25.583554	OP099684
<i>C. largus</i>	C242	24.10.2020	Cheile Vârghișului	46.630342	25.835442	OP099691
<i>C. largus</i>	C385 *	21.10.2020	Bătarci	48.014053	23.146755	OP132858
<i>C. leproleptopus</i>	C356	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099766
<i>C. lilacinovelatus</i>	C305	01.11.2020	Făgetul Clujului	46.729734	23.548487	OP099734
<i>C. luridus</i>	C390	21.10.2020	Bătarci	48.014053	23.146755	OP099785
<i>C. luridus</i>	C123 *	28.07.2020	Cheile Somesului Cald	46.643648	22.7281299	OP132851
<i>C. luridus</i>	C289 *	01.11.2020	Chinteni	46.895154	23.520302	OP132856
<i>C. masseei</i>	C388	21.10.2020	Bătarci	48.014053	23.146755	OP099784
<i>C. aff. magicus</i>	C285	01.11.2020	Chinteni	46.895154	23.520302	OP099719
<i>C. aff. magicus</i>	C303	01.11.2020	Făgetul Clujului	46.729599	23.548782	OP099732
<i>C. multiformis</i>	C001	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099564
<i>C. multiformis</i>	C008	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099570
<i>C. multiformis</i>	C138	08.08.2020	Statiunea Muntele Baisoara	46.50627098	23.26640838	OP099619

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. multiformis</i>	C144	08.08.2020	Statiunea Muntele Baisoara	46.50358372	23.264886235	OP099624
<i>C. multiformis</i>	C181	04.10.2020	Sovata	46.699756	25.173806	OP099642
<i>C. multiformis</i>	C184	04.10.2020	Sovata	46.699756	25.173806	OP099645
<i>C. multiformis</i>	C096	19.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099602
<i>C. multiformis</i>	C006	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099568
<i>C. napus</i>	C214	04.10.2020	Sovata	46.662003	25.215961	OP099672
<i>C. neofurvolaeus</i>	C047	22.09.2018	Valea Ierii	46.524456	23.274586	OP099582
<i>C. neofurvolaeus</i>	C049	22.09.2018	Valea Ierii	46.524456	23.274586	OP099584
<i>C. ochraceopallescens</i>	C055	22.09.2018	Valea Ierii	46.524076	23.274521	OP099587
<i>C. ochraceopallescens</i>	C056	18.11.2018	Făgetul Clujului	46.720612	23.5606	OP099588
<i>C. ochraceopallescens</i>	C283	01.11.2020	Făgetul Clujului	46.717317	23.536817	OP099717
<i>C. odoratus</i>	C284	01.11.2020	Borsa	46.951797	23.60519	OP099718
<i>C. odoratus</i>	C299	01.11.2020	Făgetul Clujului	46.734583	23.543577	OP099730
<i>C. odoratus</i>	C304	01.11.2020	Făgetul Clujului	46.729743	23.54856	OP099733
<i>C. olearioides</i>	C354	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099764
<i>C. olearioides</i>	C355	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099765
<i>C. olidoamarus</i>	C288	01.11.2020	Chinteni	46.895154	23.520302	OP099722
<i>C. olidoamarus</i>	C296	01.11.2020	Chinteni	46.895154	23.520302	OP099728
<i>C. olidoamarus</i>	C381	21.10.2020	Bătarci	48.014053	23.146755	OP099781
<i>C. ominosus</i>	C182	04.10.2020	Sovata	46.699756	25.173806	OP099643
<i>C. pallidostriatus</i>	C315	06.11.2020	Cheile Someșului Cald	46.67023386	22.81820682	OP099741
<i>C. pelerinii</i>	C219 *	24.10.2020	Brăduț	46.201631	25.598088	OP132854
<i>C. persoonianus</i>	C174	18.10.2020	Făgetul Clujului	46.738306	23.539194	OP099638
<i>C. pilatii</i>	C070	13.10.2019	Statiunea Muntele Baisoara	46.6776035	23.4564326	OP099591
<i>C. pruinatus</i>	C391	21.10.2020	Bătarci	48.014053	23.146755	OP099786
<i>C. pseudodaulnoyae</i>	C311	01.11.2020	Almașu	46.869822	23.146055	OP099738
<i>C. pseudodaulnoyae</i>	C386	21.10.2020	Bătarci	48.014053	23.146755	OP099782
<i>C. pseudoferoidus</i>	C142	08.08.2020	Statiunea Muntele Baisoara	46.5113753	23.27106945	OP099623
<i>C. pseudoferoidus</i>	C141	08.08.2020	Statiunea Muntele Baisoara	46.51101007	23.277106504	OP099622
<i>C. pseudonaevosus</i>	C007	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099569
<i>C. purpurascens</i>	C208	04.10.2020	Sovata	46.662003	25.215961	OP099667
<i>C. purpurascens</i>	C327	07.11.2020	Cheile Someșului Cald	46.63110059	22.76854548	OP099747
<i>C. purpurascens</i>	C367	18.11.2020	Romuli	47.560767	24.530413	OP099775
<i>C. purpurascens</i>	C370	19.11.2020	Parva	47.44473	24.64913	OP099777
<i>C. purpurascens</i>	C371	20.11.2020	Rebrișoara	47.44884	24.58896	OP099778

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. radicosissimus</i>	C313A	06.11.2020	Cheile Someșului Cald	46.67023386	22.81820682	OP099740
<i>C. renidens</i>	C019	15.08.2018	Statiunea Muntele Baisoara	46.538338	23.305955	OP099577
<i>C. renidens</i>	C109	23.06.2020	Liban	46.553983	25.520218	OP099611
<i>C. renidens</i>	C119	28.07.2020	Cheile Someșului Cald	46.626862	22.788439	OP099616
<i>C. rubellus</i>	C098	05.08.2017	Ponok	46.638228	22.815111	OP099604
<i>C. rubricosus</i>	C281	01.11.2020	Făgetul Clujului	46.717317	23.536817	OP099715
<i>C. rubrophyllus</i>	C102	10.07.2020	Zetea	46.56182	25.3733821	OP099606
<i>C. rufoallutus</i>	C005	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099567
<i>C. saginus</i>	C150	03.09.2020	Mărtiniș	46.2647	25.355	OP099628
<i>C. salor</i>	C297	01.11.2020	Rediu	46.733722	26.537746	OP099729
<i>C. saporatus</i>	C369	19.11.2020	Parva	47.440112	24.650527	OP099776
<i>C. scaurocaninus</i>	C222	24.10.2020	Cheile Vârghișului	46.203991	25.558544	OP099679
<i>C. scaurocaninus</i>	C230	24.10.2020	Cheile Vârghișului	46.20322	25.559007	OP099683
<i>C. scaurocaninus</i>	C239	24.10.2020	Cheile Vârghișului	46.205054	25.558222	OP099688
<i>C. scaurocaninus</i>	C240	24.10.2020	Cheile Vârghișului	46.215552	25.5473	OP099689
<i>C. semisanguineus</i>	C010	27.07.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099572
<i>C. semivelatus</i>	C350	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099761
<i>C. sodagnitus</i>	C280	01.11.2020	Făgetul Clujului	46.717317	23.536817	OP099714
<i>C. sodagnitus</i>	C282	01.11.2020	Făgetul Clujului	46.717317	23.536817	OP099716
<i>C. spadicellus</i>	C154	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP099631
<i>C. spadicellus</i>	C155	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP099632
<i>C. spadicellus</i>	C157	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP099633
<i>C. spadicellus</i>	C158	10.10.2020	Statiunea Stana de Vale	46.697647	22.626014	OP099634
<i>C. spadicellus</i>	C211	04.10.2020	Sovata	46.662003	25.215961	OP099670
<i>C. spilomeus</i>	C196	04.10.2020	Chiheru de Jos	46.672231	25.051561	OP099655
<i>C. subargyronotus</i>	C358	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099768
<i>C. subdecolorans</i>	C229	24.10.2020	Cheile Vârghișului	46.205947	25.557701	OP099682
<i>C. subfoetens</i>	C333	07.11.2020	Cheile Someșului Cald	46.64369044	22.7320642	OP099752
<i>C. aff. sublilacinopes</i>	C309	01.11.2020	Făgetul Clujului	46.738275	23.537126	OP099736
<i>C. subparvannulatus</i>	C071	13.10.2019	Statiunea Muntele Baisoara	46.6776035	23.4564326	OP099592
<i>C. subporphyropus</i>	C178	10.10.2020	Groșii Țibleşului	47.52174	24.15647	OP099641
<i>C. subpurpurascens</i>	C091	30.11.2019	Făgetul Clujului	46.698086	23.587791	OP099597
<i>C. subpurpurascens</i>	C195	04.10.2020	Chiheru de Jos	46.672231	25.051561	OP099654
<i>C. subpurpurascens</i>	C216	19.10.2020	Feleacu	46.695459	23.58806	OP099674
<i>C. subpurpurascens</i>	C217	19.10.2020	Feleacu	46.695459	23.58806	OP099675
<i>C. subpurpurascens</i>	C237	24.10.2020	Cheile Vârghișului	46.199842	25.583542	OP099686

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. subtortus</i>	C017	15.08.2018	Statiunea Muntele Baisoara	46.5382	23.3071099	OP099610
<i>C. subtortus</i>	C075	13.10.2019	Statiunea Muntele Baisoara	46.522375	23.273463	OP099595
<i>C. subtortus</i>	C099	19.08.2017	Statiunea Muntele Baisoara	46.520139	23.271811	OP099605
<i>C. subtortus</i>	C139	08.08.2020	Statiunea Muntele Baisoara	46.50358372	23.262648623	OP099620
<i>C. subtortus</i>	C243	24.10.2020	Statiunea Muntele Baisoara	46.50627098	23.26640838	OP099692
<i>C. subtortus</i>	C245	24.10.2020	Statiunea Muntele Baisoara	46.50627098	23.26640838	OP099694
<i>C. subtortus</i>	C247	24.10.2020	Statiunea Muntele Baisoara	46.50627098	23.26640838	OP099696
<i>C. subtortus</i>	C073	13.10.2019	Statiunea Muntele Baisoara	46.522375	23.273463	OP099593
<i>C. sulphurinus</i>	C318	06.11.2020	Cheile Someșului Cald	46.67071058	22.81810123	OP099742
<i>C. talimultiformis</i>	C185	04.10.2020	Sovata	46.699756	25.173806	OP099646
<i>C. talimultiformis</i>	C201	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099660
<i>C. talimultiformis</i>	C312	06.11.2020	Cheile Someșului Cald	46.67023386	22.81820682	OP099739
<i>C. talimultiformis</i>	C323	07.11.2020	Cheile Someșului Cald	46.67071058	22.81810123	OP099744
<i>C. testaceomicaceus</i>	C342	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099758
<i>C. tirolianus</i>	C218	24.10.2020	Cheile Vârghișului	46.204486	25.558374	OP099676
<i>C. tirolianus</i>	C259 *	01.11.2020	Harghita	46.538506	25.612982	OP132855
<i>C. tirolianus</i>	C268	01.11.2020	Ghimes	46.528277	26.033167	OP099707
<i>C. tirolianus</i>	C269	01.11.2020	Ghimes	46.528277	26.033167	OP099708
<i>C. tirolianus</i>	C276	01.11.2020	Ghimes	46.528277	26.033167	OP099712
<i>C. tirolianus</i>	C277	01.11.2020	Ghimes	46.528277	26.033167	OP099713
<i>C. torvus</i>	C353	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099763
<i>C. traganus</i>	C183	04.10.2020	Sovata	46.699756	25.173806	OP099644
<i>C. traganus</i>	C210A	04.10.2020	Sovata	46.662003	25.215961	OP099669
<i>C. traganus</i>	C244	24.10.2020	Statiunea Muntele Baisoara	46.50627098	23.26640838	OP099693
<i>C. trivialis</i>	C220	24.10.2020	Brăduț	46.201631	25.598088	OP099677
<i>C. trivialis</i>	C366	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099774
<i>C. aff. trivialis</i>	C225	24.10.2020	Cheile Vârghișului	46.203991	25.5585445	OP099681
<i>C. aff. trivialis</i>	C362	07.11.2020	Făgetul Clujului	46.6993848	23.5488839	OP099772
<i>C. turgidus</i>	C064	04.10.2019	Valea Bratcutei	46.886256	22.58647	OP099590
<i>C. turgidus</i>	C163	19.10.2020	Făgetul Clujului	46.738306	23.539194	OP099636
<i>C. turmalis</i>	C203	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099662
<i>C. turmalis</i>	C209	04.10.2020	Sovata	46.662003	25.215961	OP099668
<i>C. turmalis</i>	C215	19.10.2020	Feleacu	46.695459	23.58806	OP099673
<i>C. ultrodistorus</i>	C107	10.07.2020	Zetea	46.4733373	25.34546	OP099610

Table 1. Cont.

Species	DNA-Codes	Collection Date	Location	Latitude	Longitude	GenBank acc. no.
<i>C. uraceonemoralis</i>	C238	24.10.2020	Cheile Vârghişului	46.199056	25.573537	OP099687
<i>C. variicolor</i>	C057	06.09.2019	Demsus	45.5623456	22.700035	OP099589
<i>C. variicolor</i>	C186	04.10.2020	Chiheru de Jos	46.686903	25.040178	OP099647
<i>C. variicolor</i>	C197	04.10.2020	Chiheru de Jos	46.672231	25.051561	OP099656
<i>C. variicolor</i>	C198	04.10.2020	Chiheru de Jos	46.672231	25.051561	OP099657
<i>C. variicolor</i>	C204	04.10.2020	Chiheru de Jos	46.679428	25.035528	OP099663
<i>C. varius</i>	C275	01.11.2020	Ghimes	46.528277	26.033167	OP099711
<i>C. varius</i>	C337	07.11.2020	Cheile Someşului Cald	46.62928894	22.7806518	OP099755
<i>C. varius</i>	C340	07.11.2020	Cheile Someşului Cald	46.62928894	22.7806518	OP099757
<i>C. venetus</i>	C251	01.11.2020	Harghita	46.538506	25.612982	OP099698
<i>C. venetus</i>	C260	01.11.2020	Harghita	46.538506	25.612982	OP099703
<i>C. venetus</i>	C265	01.11.2020	Ghimes	46.528277	26.033167	OP099705
<i>C. venetus</i>	C266	01.11.2020	Ghimes	46.528277	26.033167	OP099706
<i>C. venetus</i>	C271	01.11.2020	Ghimes	46.528277	26.033167	OP099709
<i>C. aff. vibratilis</i>	C188	04.10.2020	Chiheru de Jos	46.686903	25.040178	ON832643
<i>C. violaceus</i>	C190	04.10.2020	Chiheru de Jos	46.686903	25.040178	OP099649
<i>C. violaceus</i>	C191A	04.10.2020	Pădurea Buneţilor	46.081617	25.034936	OP099650
<i>C. xanthochlorus</i>	C287	01.11.2020	Chinteni	46.895154	23.520302	OP099721
<i>C. xanthochlorus</i>	C301	01.11.2020	Făgetul Clujului	46.729576	23.548556	OP099731

* Short sequences excluded from the phylogenetic analyses.



Figure 1. Basidiomata of some *Cortinarius* s.l. species new to Romania: (A,B) *C. catharinae* C089 (sect. *Calochroi*), (C) *C. daulnoyae* C298 (sect. *Phlegmacioides*), (D) *C. fulminoides* C334 (sect. *Aureocistophili*), (E) *C. hadrocroceus* C140 (sect. *Dermocybe*), (F) *C. lacustris* C387 (sect. *Hinnulei*), (G,H) *C. masseei* C388 (sect. *Obtusi*), (I) *C. subdecolorans* C229 (singleton), (J) *C. subfoetens* C333 (sect. *Glaucopodes*), Photos. (A,C,E,F,I,J) E. Szabó; (B,D) A. Dénes, (G,H) K. Babos and K. Fehér.

2.2. Micromorphological Study

We studied the basidiospores of our collections (Figure 2). In general, the size of the spores are $5\text{--}15 \times 3\text{--}8 \mu\text{m}$. Basidiospores were studied in 3% KOH or Melzer's reagent, the latter to observe the dextrinoid reaction of the spore wall in some groups [98]. The analyses were performed with an Olympus CX23 microscope with $1000\times$ magnification using immersion oil and an oil immersion lens. The photographs were taken with a Canon 700D camera attached to the microscope. Photos were stacked with Zerene Stacker (zerenesystems.com/cms/home), and the size of the spores was measured with Piximètre (<http://ach.log.free.fr/Piximetre/>) software. In addition to the basidiospore sizes, coloration and the ornamentation were also noted. From the measured parameters, the Q value (length/width ratio) was calculated, which indicates the shape of the spores (Q = 1.01–1.05: globose, Q = 1.05–1.15: subglobose, Q = 1.15–1.30: broadly ellipsoid, Q = 1.30–1.60: ellipsoid, Q = 1.60–2.0: oblong, Q = 2.0–3.0: cylindrical). Basidiospore ranges for the species new to Romania are given in Table 2. To exclude aberrant spores, the values are based on spores within the 0.75 confidence interval.

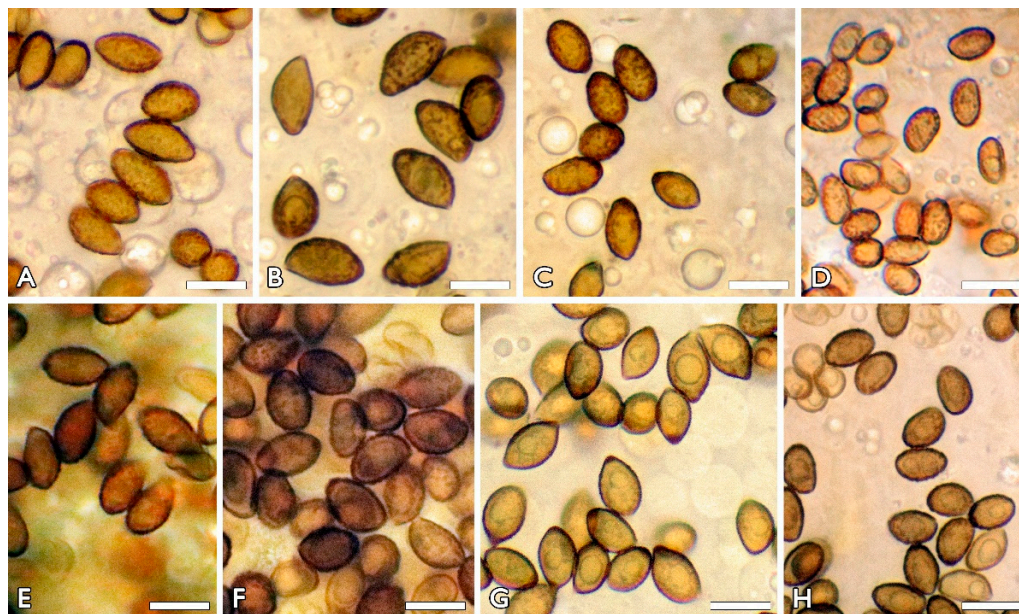


Figure 2. Basidiospores of some *Cortinarius* s.l. species new to Romania: (A) *C. catharinae* C089 (sect. *Calochroi*), (B) *C. daulnoyae* C298 (sect. *Phlegmacioides*), (C) *C. fulminoides* C334 (sect. *Aureocistophili*), (D) *C. subdecolorans* C229 (singleton), (E) *C. subfoetens* C333 (sect. *Glaucopodes*), (F) *C. hadrocroceus* C140 (sect. *Dermocybe*), (G) *C. masseei* C388 (sect. *Obtusi*), (H) *C. lacustris* C387 (sect. *Hinnulei*). Scale bar: 10 μm . Photos: E. Szabó.

Table 2. *Cortinarius* species discovered new to Romania. Infrageneric classification, habitat types as well as basidiospore measurements and Q values are given for each species. BLf—broad-leaved forest; Cf—coniferous forest; Mf—mixed forest; P—pastures; TWS—transitional woodland-shrub; NG—natural grasslands.

Species	Section/Clade	Habitat	No. of Collected Samples	Basidiospores
<i>Cortinarius anfractoides</i> Rob. Henry and Trescol 1987	<i>Infracti</i>	BLf	2	$8.8\text{--}9.5 \times 7.2\text{--}7.4 \mu\text{m}$ Q = 1.2–1.3
<i>Cortinarius aureopulverulentus</i> M.M. Moser 1952	<i>Calochroi</i>	Cf	1	$12.4\text{--}13.1 \times 8.0\text{--}8.7 \mu\text{m}$ Q = 1.5–1.6

Table 2. Cont.

Species	Section/Clade	Habitat	No. of Collected Samples	Basidiospores
<i>Cortinarius balteatocumatilis</i> Rob. Henry 1939	<i>Phlegmacioides</i>	P	2	8.4–9.2 × 5.2–5.9 μm Q = 1.5–1.7
<i>Cortinarius brunneus</i> (Pers.) Fr. 1838	<i>Brunnei</i>	Cf	1	8.2–9.5 × 6.1–6.8 μm Q = 1.2–1.5
<i>Cortinarius caesiophylloides</i> Kytöv., Liimat., Niskanen, Brandrud and Frøslev 2014	<i>Multiformes</i>	Cf	1	10.4–11.0 × 6.2–6.6 μm Q = 1.57–1.73
<i>Cortinarius catharinae</i> Consiglio 1997	<i>Calochroi</i>	BLf	1	9.7–10.5 × 5.8–6.3 μm Q = 1.6–1.8
<i>Cortinarius cinereobrunneolus</i> Chevassut and Rob. Henry 1982	<i>Urbici</i>	BLf	1	7.7–8.4 × 5.0–5.2 μm Q = 1.5–1.7
<i>Cortinarius claricolor</i> (Fr.) Fr. 1838	<i>Claricolores</i>	Cf	1	6.4–7.2 × 3.5–3.7 μm Q = 1.8–1.9
<i>Cortinarius colymbadinus</i> Fr. 1838	<i>Uracei</i>	Mf	1	8.5–9.4 × 5.4–5.8 μm Q = 1.5–1.7
<i>Cortinarius corrosus</i> Fr. 1838	<i>Calochroi</i>	Cf	1	12.5–14.1 × 7.5–7.9 μm Q = 1.6–1.9
<i>Cortinarius daulnoyae</i> (Quél.) Sacc. 1910	<i>Phlegmacioides</i>	Cf	7	12.4–13.2 × 7.3–7.7 μm Q = 1.7–1.8
<i>Cortinarius</i> aff. <i>delibutus</i>	<i>Delibuti</i>	Mf	1	8.5–8.9 × 7.0–7.4 μm Q = 1.2–1.3
<i>Cortinarius eliae</i> Bidaud, Moënné-Locc. and Reumaux 1996	<i>Phlegmacioides</i>	P	3	11.5–12.5 × 6.8–7.1 μm Q = 1.7–1.8
<i>Cortinarius fraudulosus</i> Britzelm. 1885	<i>Arguti</i>	Cf	1	15.9–16.6 × 8.6–9.0 μm Q = 1.8–1.9
<i>Cortinarius fulminoides</i> (M.M. Moser) M.M. Moser 1967	<i>Aureocistophili</i>	Mf	1	9.4–10.2 × 5.9–6.5 μm Q = 1.5–1.6
<i>Cortinarius gallurae</i> D. Antonini, M. Antonini and Consiglio 2005	/Gallurae	BLf	1	8.6–9.4 × 5.7–6.5 μm Q = 1.4–1.6
<i>Cortinarius geniculatus</i> Bidaud 2014	<i>Bovini</i>	BLf	1	10.7–12.0 × 6.4–6.7 μm Q = 1.6–1.8
<i>Cortinarius glaucopus</i> aff.	<i>Glaucopodes</i>	Cf	2	8.5–9.3 × 5.4–5.7 μm Q = 1.6–1.7
<i>Cortinarius hadrocroceus</i> Ammirati, Niskanen, Liimat. and Bojantchev 2014	<i>Dermocybe</i>	Cf	2	7.3–7.8 × 4.3–4.7 μm Q = 1.6–1.8
<i>Cortinarius hillieri</i> Rob. Henry 1938	<i>Bovini</i>	BLf	1	10.7–11.8 × 6.1–6.7 μm Q = 1.7–1.8
<i>Cortinarius holoxanthus</i> (M.M. Moser and I. Gruber) Nezdjdm. 1980	<i>Dermocybe</i>	NG	3	9.3–9.9 × 4.8–5.2 μm Q = 1.8–2.1
<i>Cortinarius huronensis</i> Ammirati and A.H. Sm. 1972	<i>Dermocybe</i>	Cf	3	8.0–8.5 × 4.9–5.4 μm Q = 1.47–1.65
<i>Cortinarius hydrotelamonioides</i> Rob. Henry 1970	<i>Firmiores</i>	BLf	1	9.3–10.3 × 5.1–5.8 μm Q = 1.7–1.9
<i>Cortinarius incognitus</i> Ammirati and A.H. Sm. 1972	<i>Dermocybe</i>	Cf	1	7.4–8.0 × 5.2–5.4 μm Q = 1.3–1.6
<i>Cortinarius lacustris</i> Moënné-Locc. and Reumaux 1997	<i>Hinnulei</i>	BLf	1	9.6–11.1 × 5.9–6.6 μm Q = 1.5–1.8

Table 2. Cont.

Species	Section/Clade	Habitat	No. of Collected Samples	Basidiospores
<i>Cortinarius leproleptopus</i> Chevassut and Rob. Henry 1988	<i>Leprocye</i>	BLf	1	7.9–8.5 × 6.8–7.3 μm Q = 1.1–1.2
<i>Cortinarius lilacinovelatus</i> Reumaux and Ramm 2001	<i>Calochroi</i>	BLf	1	11.1–11.7 × 6.1–6.5 μm Q = 1.7–1.8
<i>Cortinarius luridus</i> Rob. Henry 1969	<i>Hinnulei</i>	BLf	3	8.9–9.7 × 6.3–6.7 μm Q = 1.4–1.5
<i>Cortinarius masseei</i> Bidaud, Moëgne-Locc. and Reumaux 1993	<i>Obtusi</i>	BLf	1	7.0–7.8 × 4.9–5.3 μm Q = 1.4–1.6
<i>Cortinarius</i> aff. <i>magicus</i>	<i>Glauropodes</i>	Cf	2	7.6–8.0 × 5.0–5.3 μm Q = 1.5–1.6
<i>Cortinarius neofurvolesus</i> Kytöv., Niskanen, Liimat. and H. Lindstr. 2005	<i>Bovini</i>	NG	2	8.5–9.5 × 5.1–5.9 μm Q = 1.5–1.8
<i>Cortinarius ochraceopallenscens</i> Moëgne-Locc. and Reumaux 2001	<i>Calochroi</i>	BLf	3	12.4–13.2 × 6.4–6.9 μm Q = 1.8–2.0
<i>Cortinarius odoratus</i> (Joguet ex M.M. Moser) M.M. Moser 1967	<i>Calochroi</i>	BLf	3	11.6–12.5 × 6.8–7.2 μm Q = 1.7–1.8
<i>Cortinarius olidoamarus</i> A. Favre 1986	<i>Glauropodes</i>	P	3	8.4–9.2 × 5.2–5.9 μm Q = 1.5–1.7
<i>Cortinarius ominosus</i> Bidaud 1994	<i>Dermocybe</i>	TWS	1	6.8–7.7 × 4.4–4.7 μm Q = 1.5–1.6
<i>Cortinarius pallidostriatus</i> Rob. Henry 1968	<i>Hydrocybe</i>	Cf	1	8.6–9.6 × 5.5–6.0 μm Q = 1.5–1.7
<i>Cortinarius pelerinii</i> Bellanger, Carteret and Reumaux 2013	<i>Anomali</i>	BLf	1	8.8–9.4 × 6.3–7.0 μm Q = 1.3–1.4
<i>Cortinarius persoonianus</i> Bidaud 2009	<i>Infracti</i>	BLf	1	8.7–9.5 × 7.2–7.6 μm Q = 1.2–1.3
<i>Cortinarius pilatii</i> Svrček 1968	<i>Flexipedes</i>	Cf	1	9.0–9.7 × 6.0–6.5 μm Q = 1.4–1.6
<i>Cortinarius pruvinatus</i> Bidaud, Moëgne-Locc. and Reumaux 1993	<i>Obtusi</i>	BLf	1	10.5–11.7 × 6.2–7.3 μm Q = 1.5–1.7
<i>Cortinarius pseudodaulnoyae</i> Rob. Henry and Ramm 1991	<i>Phlegmacioides</i>	BLf	2	12.7–13.2 × 7.2–7.7 μm Q = 1.7–1.8
<i>Cortinarius pseudoferoidus</i> Niskanen, Liimat., Ammirati and Kytöv. 2014	<i>Dermocybe</i>	NG	2	6.9–7.6 × 4.3–4.9 μm Q = 1.4–1.7
<i>Cortinarius pseudonaevosus</i> Rob. Henry 1957	<i>Phlegmacioides</i>	Cf	1	12.9–14.4 × 7.5–8.3 μm Q = 1.7–1.8
<i>Cortinarius radicosissimus</i> Moëgne-Locc. 1997	<i>Hinnulei</i>	Cf	1	8.6–9.4 × 6.8–8.0 μm Q = 1.2–1.3
<i>Cortinarius renidens</i> Fr. 1838	<i>Renidentes</i>	Cf	3	7.8–8.1 × 5.6–6.0 μm Q = 1.3–1.4
<i>Cortinarius rubricosus</i> (Fr.) Fr. 1838	<i>Rubricosi</i>	BLf	1	10.0–10.9 × 7.0–7.5 μm Q = 1.4–1.6
<i>Cortinarius rubrophyllus</i> (Moëgne-Locc.) Liimat., Niskanen, Ammirati and Dima 2014	<i>Dermocybe</i>	Cf	1	6.0–6.3 × 3.8–4.3 μm Q = 1.5–1.6
<i>Cortinarius rufoallutus</i> Rob. Henry ex Bidaud and Reumaux 2006	<i>Multiformes</i>	Cf	1	9.7–10.3 × 5.7–5.93 μm Q = 1.7–1.8

Table 2. Cont.

Species	Section/Clade	Habitat	No. of Collected Samples	Basidiospores
<i>Cortinarius saginus</i> (Fr.) Fr. 1838	<i>Phlegmacium</i>	TWS	1	10.5–10.8 × 6.0–6.5 μm Q = 1.7–1.8
<i>Cortinarius saporatus</i> Britzelm. 1897	<i>Calochroi</i>	Mf	1	10.5–11.7 × 6.4–7.6 μm Q = 1.5–1.8
<i>Cortinarius scaurocaninus</i> Chevassut and Rob. Henry 1982	<i>Glauropodes</i>	Cf	4	8.7–9.3 × 5.2–5.4 μm Q = 1.6–1.8
<i>Cortinarius semivelatus</i> Rob. Henry 1970	<i>Squalidi</i>	BLf	1	8.0–8.7 × 4.9–5.7 μm Q = 1.4–1.7
<i>Cortinarius sodagnitus</i> Rob. Henry 1935	<i>Calochroi</i>	BLf	2	11.5–12.5 × 6.3–7.1 μm Q = 1.6–1.9
<i>Cortinarius spadacellus</i> Brandrud 1997	<i>Phlegmacioides</i>	Mf	5	10.9–11.5 × 6.9–7.1 μm Q = 1.5–1.7
<i>Cortinarius subargyrotus</i> Niskanen, Liimat. and Kytöv. 2014	<i>Uracei</i>	BLf	1	9.5–10.3 × 5.9–6.4 μm Q = 1.5–1.8
<i>Cortinarius subdecolorans</i> M. Langl. and Reumaux 2000	/Dionysae	BLf	1	9.8–10.6 × 6.2–6.6 μm Q = 1.5–1.6
<i>Cortinarius subfoetens</i> M.M. Moser and McKnight 1995	<i>Glauropodes</i>	Mf	1	8.3–8.8 × 5.4–5.7 μm Q = 1.5–1.6
<i>Cortinarius</i> aff. <i>sublilacinopes</i>	<i>Calochroi</i>	BLf	1	11.7–12.2 × 7.0–7.4 μm Q = 1.6–1.7
<i>Cortinarius subporphyropus</i> Pilát 1954	<i>Purpurascetes</i>	Mf	1	11.1–12.1 × 6.6–7.3 μm Q = 1.6–1.7
<i>Cortinarius subpurpurascens</i> (Batsch) J. Kickx f. 1867	<i>Purpurascetes</i>	BLf	5	10.6–11.2 × 6.0–6.5 μm Q = 1.7–1.8
<i>Cortinarius testaceomicaceus</i> Bidaud 2014	<i>Exsulares</i>	BLf	1	9.5–11.2 × 6.8–7.5 μm Q = 1.3–1.6
<i>Cortinarius tirolianus</i> Bidaud, Moëne-Locc. and Reumaux 2005	<i>Glauropodes</i>	Cf	6	7.5–7.9 × 5.0–5.2 μm Q = 1.5–1.6
<i>Cortinarius</i> aff. <i>trivialis</i>	<i>Myxacium</i>	BLf	2	13.4–14.7 × 8.0–8.8 μm Q = 1.6–1.7
<i>Cortinarius ultrodistorus</i> Rob. Henry and Vagnet 1992	/Ultrordistorus	Cf	1	7.4–8.8 × 4.5–5.0 μm Q = 1.5–1.9
<i>Cortinarius uraceonemoralis</i> Niskanen, Liimat., Dima, Kytöv., Bojantchev and H. Lindstr. 2014	<i>Uracei</i>	BLf	1	9.1–10.3 × 5.5–6.0 μm Q = 1.58–1.87
<i>Cortinarius</i> aff. <i>vibratilis</i>	<i>Vibratiles</i>	BLf	1	8.4–8.9 × 5.2–5.6 μm Q = 1.6–1.7

2.3. Molecular Genetic Analysis

DNA extractions, PCR amplifications, gel electrophoreses, and the purification of the PCR products were performed in the molecular laboratory at the Interdisciplinary Research Institute on Bio-Nano-Sciences of Babeş-Bolyai University, Cluj-Napoca, Romania.

Genomic DNA was extracted from a small piece of the lamella (10–15 mg) under sterile conditions using the ISOLATE II Genomic DNA Kit (Bioline Meridian Bioscience, Inc. Cincinnati, OH, USA) following a modified protocol: (1) After the crushing the dried samples, 180 μL of Lysis Buffer GL and 25 μL of Proteinase K solution were added to the sample and were mixed using Thermoblock for 3 h at 56 °C for better amalgamation; (2) the elution of the DNA was performed in two steps with 30–30 μL of Elution Buffer G solution

(before spinning, the elution buffer stayed in the column for 3 min). The concentration of the DNA solutions was measured with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). All DNA concentrations fell between 30 and 100 ng/ μ L.

The target region was amplified using the following primers: ITS1F (5'-CTTGGTCATT-TAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') [94]. The PCR was performed in a volume of 50 μ L of the reaction mixture, which contained 0.5 μ L of MyTaq™ DNA Polymerase (Bioline Reagents Ltd., London, UK), 10 μ L of 5 \times MyTaq™ Reaction Buffer, 1 μ L of the primer pairs (20 μ M each, from the following primer mix: 10 μ L ITS1F + 10 μ L ITS4 + 80 μ L PCR-grade water), 1 μ L of DNA solution and 37.5 μ L of PCR-grade water. The PCR conditions included an initial 5 min denaturation step at 95 °C, followed by 35 cycles of denaturation of 1 min at 95 °C, 1 min of annealing at 52 °C and 1 min of elongation at 72 °C. Finally, there was a 10 min final extension at 72 °C.

The success of the PCR was confirmed with a 1% agarose gel electrophoresis of 2 μ L from each sample, performed at 120 V for 20 min. The gel was dyed with ethidium-bromide and was examined under UV light. After the run, the positive samples were purified using Wizard SV Gel and a PCR Clean-Up System (Promega, Madison, Wisconsin, USA USA) following the provided protocol, with one modification: for a more concentrated solution of the PCR product, the elution of the PCR product was performed in two steps with 20–20 μ L of nuclease free water (before spinning, the buffer stayed in the column for 3 min). The purity of the PCR product and the concentration of the solution was measured with a spectrophotometer.

The PCR products were sent to MacroGen Europe (Amsterdam, The Netherlands) for Sanger sequencing with ITS1F and ITS4 primers.

2.4. Phylogenetic Analysis

The results of the sequencing were downloaded from the MacroGen Europe website. The quality of the sequences was analyzed using Trev from the Staden Program Package [99], and the primers' connection region was cut manually. Homologous sequences were searched by using the BLASTn algorithm [100] both in public, non-curated database (GenBank; <http://www.ncbi.nlm.nih.gov/>, accessed on 12 November 2021), and in curated public databases (UNITE; <http://unite.ut.ee/>, BOLD; <http://www.boldsystems.org/>). Reference and type sequences (Table S1) were downloaded and added to our own dataset.

The sequences were aligned in BioEdit [101] using MAFFT with the E-INS-I strategy [102]. To refine the nucleotide alignment, the phylogenetically informative indel positions were coded in FastGap 1.2 [103], and the binary matrix was added to the nucleotide alignment (ITS1, 5.8S, ITS2) in SeaView 4 [104]. Our dataset was analyzed based on Maximum Likelihood (ML) and Bayesian Inference (BI) methods. The ML phylogenetic reconstruction was performed in raxmlGUI [105] using rapid bootstrap analysis with 1000 replicates. Three nucleotide partitions (ITS1, 5.8S, ITS2) were set to the GTRGAMMA substitution model in addition to one binary partition (indel characters) that was set to default. The BI analysis was inferred in MrBayes 3.2.6 [106], with a MCMC (Markov chain Monte Carlo) algorithm, and the GTR + Γ substitution model with gamma distribution. The nucleotide partitions and the binary matrix from gap coding were treated as a mixed data type. The analysis ran for 10,000,000 generations with 25% burn-in. The resulting phylogenetic tree was visualized in FigTree v1.4.2 [107], MEGA 7 [108] and Adobe Illustrator CS4.

3. Results

In total, 234 sequences were analyzed (Table 1). BI and ML analyses of the nrDNA ITS region revealed topologically similar phylograms. A preliminary survey was conducted to see whether the 10 different genera of *Cortinariaceae* established by Liimatainen et al. [76] could be reconstructed based only on single gene analysis, but ITS was not found to be suitable for recognizing these genera. Therefore, we decided to treat *Cortinarius* in a classical sense. In order to achieve a better overview of the morphological similarities of the studied species, the dataset was divided into two parts. The first alignment was composed of 760 characters. After gap coding, a binary set of 267 characters was added to the nucleotide alignment, leading to a mixed data matrix containing 1027 characters. The alignment contained ITS sequences of leprocyboid, dermocyboid, rozitoid, telamonioid, and myxacioid species, i.e., from the classical morphological groups such as subgen. *Cortinarius*, subgen. *Telamonia*, and subgen. *Myxacium*. Our results showed (Figure 3) that the studied species belonged to sections *Anomali*, *Bovini*, *Brunnei*, *Camphorati*, *Cortinarius*, *Defibulati*, *Delibuti*, *Dermocybe*, *Exulares*, *Firmiores*, *Flexipedes*, *Hinnulei*, *Hydrocybe*, *Leprocycybe*, *Myxacium*, *Obtusi*, *Orellani*, *Renidentes*, *Rozites*, *Rubricosi*, *Spilomei*, *Squalidi*, *Telamonia*, *Tragani*, *Uracei*, *Urbici* and *Vibratiles*, as well as to the clades /Gallurae and /Ultrodistortus. The second alignment included ITS sequences of species from the morphological subgen. *Phlegmacium* and was composed of 712 characters. In this case, 185 binary characters from the gap coding were added to the nucleotide characters, resulting in a final alignment of 897 characters. Our results showed (Figure 4) that the studied species belonged to sections *Arguti*, *Aureocistophili*, *Calochroi*, *Claricolores*, *Glaucopodes*, *Infracti*, *Multiformes*, *Phlegmacioides*, *Phlegmacium*, *Purpurascetes*, *Subtorti*, *Turmales* and *Varii*, and the clade /Dionysae (= *Phlegmacium* sect. *Dionysae*). Most sections and clades are well-supported with ML bootstrap support values between 80–100% and BI posterior probabilities between 0.92–1. For the sectional names, the taxonomic work of Liimatainen et al. [3,56,76] were followed.

Based on our study, we were able to recognize 109 *Cortinarius* s.l. species from Romania, belonging to 40 sections and 3 clades. Out of these, 43 species have been previously documented, but their identifications were based only on morphology (Table S2). The remaining 66 species are reported here as new to Romania (Table 2). Among these, we were not able to link any of the described *Cortinarius* names to six phylogenetically well-separated species; therefore, we used the 'aff.' prefix before the epithets which link them to their closest phylogenetic or morphological species (Figures 3 and 4). These are *C. aff. delibutus* in sect. *Delibuti*, *C. aff. glaucopus* and *C. aff. magicus* in sect. *Glaucopodes*, *C. aff. sublilacinopes* in sect. *Calochroi*, *C. aff. trivialis* in sect. *Myxacium* and *C. aff. vibratilis* in sect. *Vibratiles*. Concerning the ongoing *Cortinarius* studies in Europe, our unpublished results (data not shown) indicate that these species are likely taxonomic novelties, but unveiling their taxonomy and nomenclature needs further analyses and the results will be included in different publications.

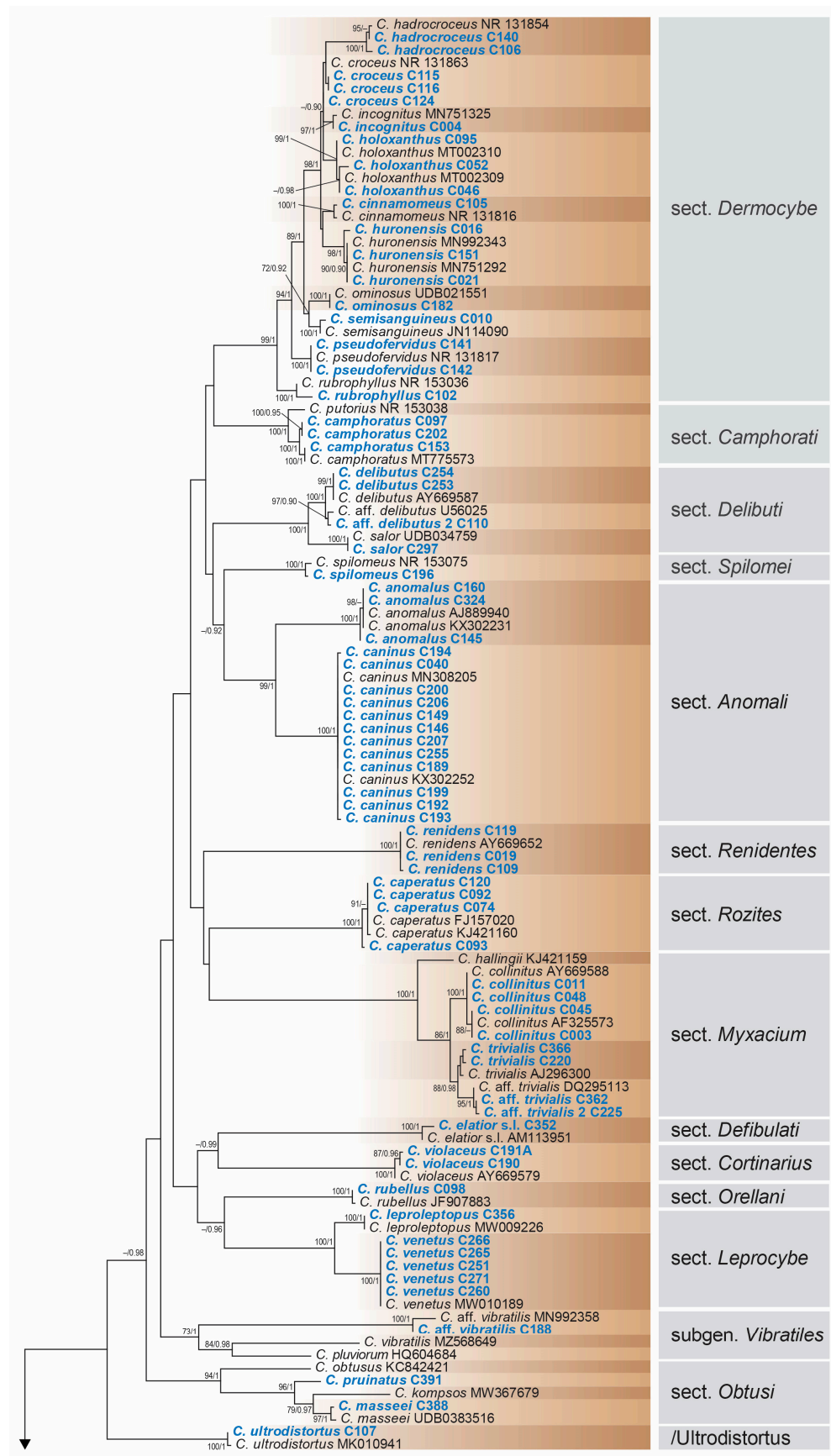


Figure 3. Cont.

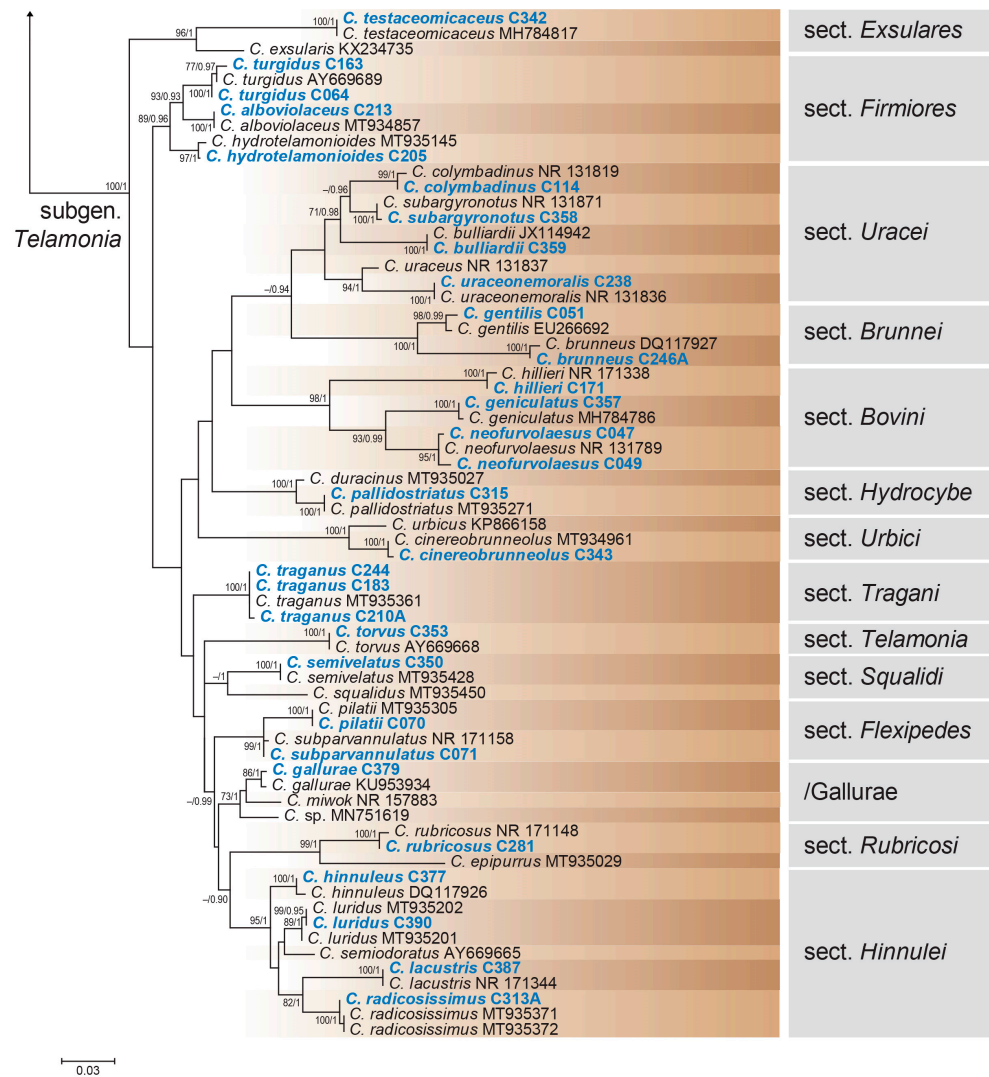


Figure 3. Maximum likelihood phylogenetic tree of the dermocyboid, leprocyboid, rozitoid, telamonioid, and myxacioid sections/clades of the genus *Cortinarius* s.l. based on nrDNA ITS sequence analyses with gap coding. Sequences produced in this study are in blue and boldface and labelled with their voucher numbers. Sequences from public repositories are marked with their GenBank/UNITE accession numbers. ML bootstrap values of >70% as well as Bayesian posterior probabilities of >0.9 are placed above or below branches. The scale bar indicates 0.03 expected change per site per branch.

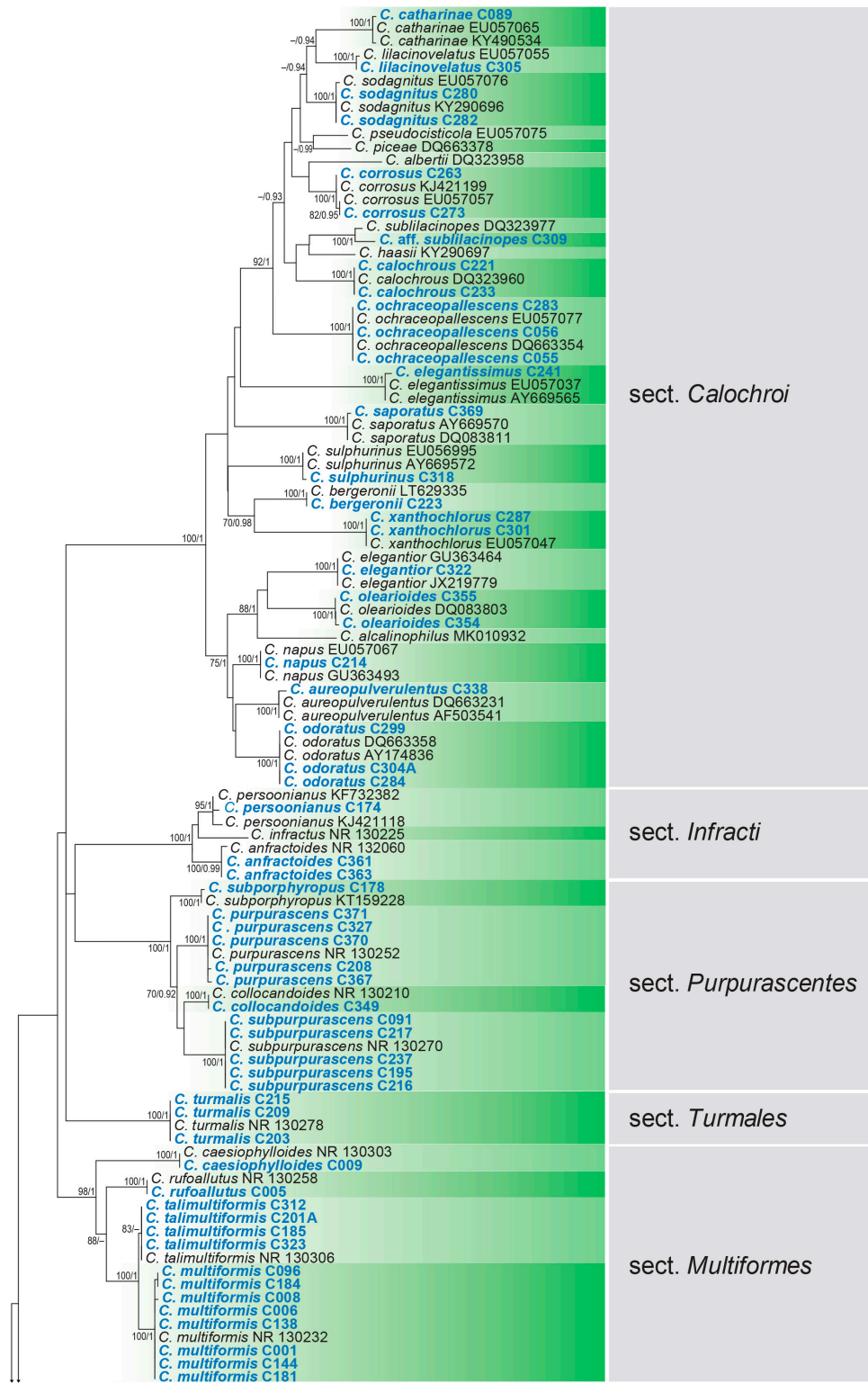


Figure 4. Cont.

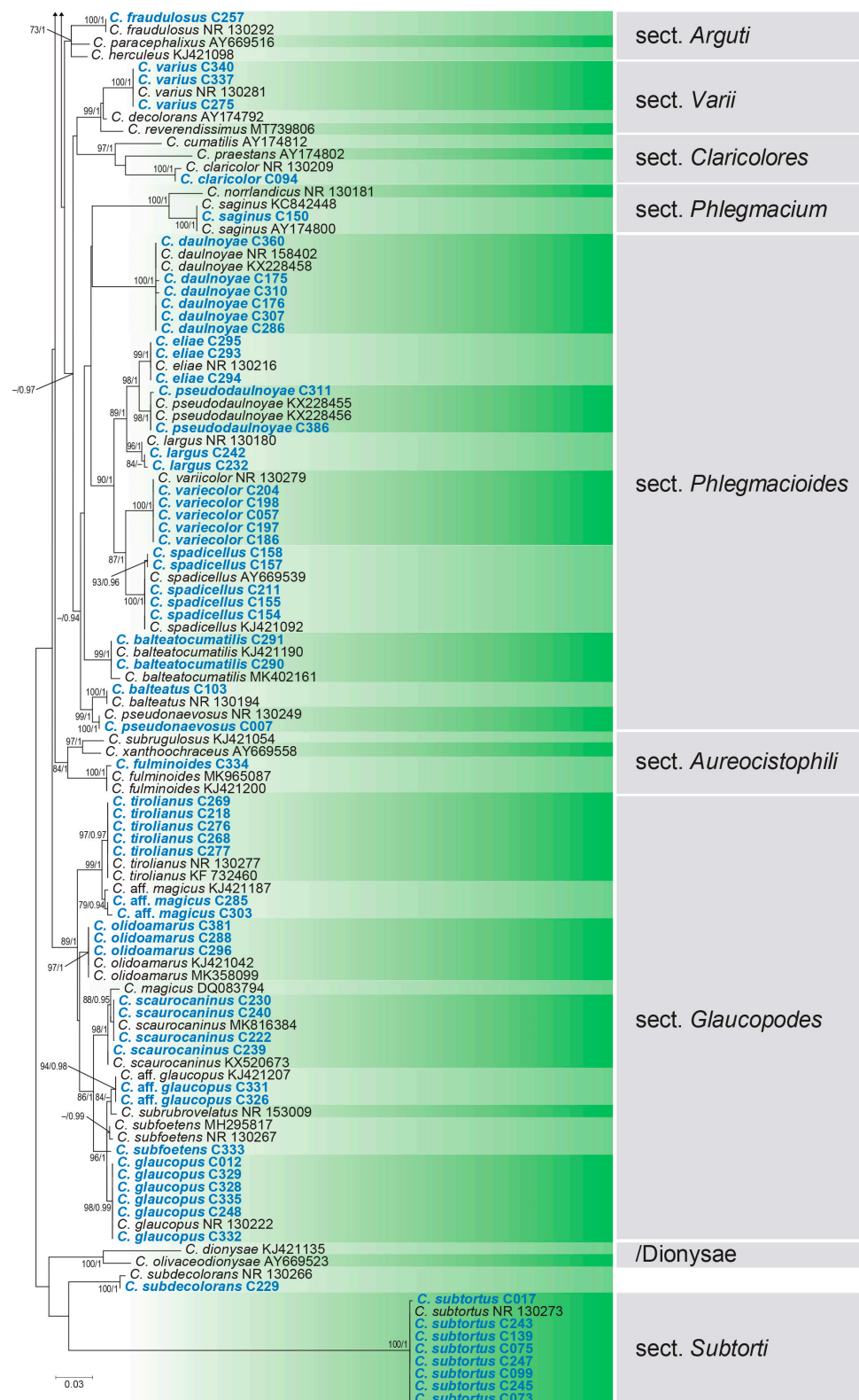


Figure 4. Maximum likelihood phylogenetic tree of the phlegmacioid sections/clades of the genus *Cortinarius* s.l. based on nrDNA ITS sequence analyses with gap coding. Sequences produced in this study are in blue and boldface and labelled with their voucher numbers. Sequences from public repositories are marked with their GenBank/UNITE accession numbers. ML bootstrap values of >70% as well as Bayesian posterior probabilities of >0.9 are placed above or below branches. The scale bar indicates 0.03 expected change per site per branch.

4. Discussion

In this study, we updated the checklist of the largest agaric genus, *Cortinarius* s.l., in Romania, employing nrDNA ITS barcoding. Macrofungi, including *Cortinarius*, were only studied using classical morphological methods in Romania to date; thus, our work presents the first biodiversity study using the combination of molecular phylogenetic and morphological methods in the country. Similar to the results of other national barcoding projects in Europe, e.g., in Norway [109–111], Finland [112], the Netherlands [113] or in Austria (https://www.abol.ac.at/en/project/higher_fungi/, accessed on 1 July 2022), our results showed a high number of previously not reported taxa at the country level. From the 109 *Cortinarius* s.l. species identified in the course of this study, 66 species are reported here as new to Romania, raising the known number of species up to ca. 190. This number is, however, lagging far behind that from Western and Northern European countries, where species observation activities and DNA barcoding campaigns and databases are in a more advanced stage compared to Romania or its neighboring countries, e.g., in Bulgaria or Hungary. For example, in Norway, the listed number of *Cortinarius* species is over 550 [114], while based on data from the older literature, the number of species in Bulgaria is 105 [115], and in Hungary, there are 169 species [116]; however, these numbers are hardly comparable with each other due to the different methods used in species identification (i.e., integrative taxonomy vs. morphological species recognition).

The majority of the species discovered as new to Romania belongs to phlegmacioid lineages/sections, especially to sect. *Calochroi* (nine species) as well as sect. *Glaucopodes* and sect. *Phlegmacioides* (six species in either section). Sect. *Dermocybe* is represented with 7 new species to the country, whereas 29 other sections/clades are represented by only 1–3 species in our dataset. Altogether, the 109 identified species in this study belong to 43 sections/clades. After actualizing the species list of genus *Cortinarius* s.l. with our new data, we assessed ca. 190 species now known in Romania. However, we are aware of the fact that among the previously published data, there could be wrongly identified and named collections; thus, without taxonomic revision of these materials, the correct number of *Cortinarius* species cannot be accurately established. Based on the various valuable habitats and nature types in the Carpathian Mountains and adjacent regions in Romania (out of which we only conducted samplings from the Apuseni Mts, and few sites in the Eastern Carpathians), we anticipate discovering an even larger diversity of *Cortinarius* s.l. species than that established in this work, when the sampling is extended to all areas with suitable habitats for *Cortinarius* in Romania. We also believe that other groups of macrofungi will be shown to be more species-rich when accurate investigations combining molecular techniques (e.g., DNA barcoding) together with morphological identification methods will be applied in the future.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15040553/s1>, Table S1: *Cortinarius* sequences from public databases used in this study, Table S2: *Cortinarius* taxa published to date from Romania and identified with morphological methods. References [53,79,81,85,87,117–129] are cited in Supplementary Materials.

Author Contributions: Conceptualization, E.S., B.D. and L.K.; methodology, E.S. and B.D.; molecular analysis, E.S., B.D. and A.L.D., resources, L.K. and V.P., data curation, E.S. and B.D., writing—original draft preparation, E.S. and B.D., writing—review and editing, E.S., B.D., A.L.D., L.K. and V.P., supervision B.D. and L.K., funding acquisition L.K. and V.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partially funded by the Collegium Talentum Program of Hungary. The work of B.D. was funded by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences; the National Research, Development and Innovation Office of Hungary (OTKA FK-143061); and the ELTE Thematic Excellence Programme 2020 (TKP2020-IKA-05), financed by the National Research, Development and Innovation Office of Hungary. The support of the János Bolyai Research Scholarship of the Hungarian Academy of Sciences to V.P. is highly appreciated.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within the article and Supplementary Materials. Some data can also be found in publicly available datasets: <https://www.ncbi.nlm.nih.gov/>; <http://www.mycobank.org/>; <http://www.indexfungorum.org/>, accessed on 2 March 2023.

Acknowledgments: We would like to thank Krisztina Babos, László Bartha, Gábor Bélfenyéri, Anna Dénes, Réka Erős, Kinga Fehér, László Gál, Boróka Jancsó, Kunigunda Macalik and Csilla Szabó for their help with the sample collection, Boróka Jancsó for her help with the microscopical spore photos, and Ágota Szabó for her help in recording habitat composition. We are grateful to the three anonymous reviewers (especially to one of them) for their valuable comments which greatly improved our manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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