Clinical Toxicology (2010) **48**, 545–549

ISSN: 1556-3650 print / 1556-9519 online
DOI: 10.3109/15563650.2010.495346

informa
healthcare

ARTICLE

Ingestion of a newly described North American mushroom species from Michigan resulting in chronic renal failure: *Cortinarius orellanosus*

BRYAN S. JUDGE[1], JOSEPH F. AMMIRATI[2], GARY H. LINCOFF[3], JOHN H. TRESTRAIL III[4], and P. BRANDON MATHENY[5]

[1]*Department of Emergency Medicine, Michigan State University College of Human Medicine, Spectrum Health-Toxicology Services, Grand Rapids, MI, USA*
[2]*Department of Biology, University of Washington, Seattle, WA, USA*
[3]*The New York Botanical Garden, Bronx, NY, USA*
[4]*Center for the Study of Criminal Poisoning, Grand Rapids, MI, USA*
[5]*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA*

Background. Some mushrooms in the genus *Cortinarius* are well known to cause acute and chronic renal failure. Until now, there have been no confirmed cases of renal failure due to the ingestion of a *Cortinarius* mushroom in North America. We describe a case of a woman who ingested mushrooms found under an oak tree in western Michigan and developed chronic renal failure. *Methods.* Phylogenetic analysis of the internal transcribed spacer (ITS) regions of nuclear-encoded ribosomal RNA was performed between an unconsumed sample of the Michigan specimens, a control sample of *Cortinarius orellanus* (JFA9859) from Europe, and other closely related ITS sequences of *Cortinarius* retrieved from GenBank. An additional gene region, *rpb*2, was also sequenced for comparison. *Results.* Phylogenetic analysis revealed the Michigan material to be closely related to, but distinct from, other ITS sequences of the Orellani clade in *Cortinarius*. Divergence is less at the *rpb*2 locus. No historical taxa from North America are known to match the identification of the Michigan material. *Conclusion.* The mushrooms ingested by the patient were confirmed to be a new species of *Cortinarius* closely related to *C. orellanus*. We introduce a newly described North American species, *Cortinarius orellanosus*, capable of causing renal failure after ingestion.

Keywords Cortinarius; Mushroom; Renal failure; Poisoning; Orellanine

Introduction

Orellanine-containing mushrooms in the genus *Cortinarius*, including *Cortinarius orellanus* Fries and *Cortinarius rubellus* Cooke, can result in acute and chronic renal failure after ingestion. Numerous human poisonings from *Cortinarius* have been documented in Europe.[1,2] However, confirmed cases of renal failure in North America attributable to the ingestion of *Cortinarius* mushrooms have not been well documented. A case of mushroom ingestion in the Pacific Northwest causing renal failure has been previously ascribed to *C. orellanus*,[3] since *C. orellanus* and *C. rubellus* were the only-known nephrotoxic mushrooms. However, one of the authors of that report felt that the ascription was erroneous.[4] It is likely that the mushroom responsible was *Amanita smithiana*, which is endemic to western North America, and has been implicated in several cases of delayed-onset renal failure.[5] Another report from Canada describes a patient who developed renal failure after eating "magic" mushrooms. Although the patient's renal failure was attributed to the ingestion of *Cortinarius* mushrooms, no mycological evidence was provided to confirm this association.[6] Finally, several cases have been mentioned in the National Poison Data System about exposures to mushrooms containing orellanine; however, detailed information regarding the exposure and mushroom(s) involved is not readily available from these reports.[7,8]

Case report

A 53-year-old female presented to the emergency department (ED) with an inability to urinate. Nine days before presentation, she had ingested the caps and stems of six mushrooms that she had picked in the summertime under an oak tree in her backyard in western Michigan. The mushrooms were cooked in vegetable oil with some onions and shared with a male companion, who did not consume as many of the mushrooms

Received 26 February 2010; accepted 18 May 2010.
Address correspondence to Bryan S. Judge, Toxicology Services, Grand Rapids Medical Education Partners, Michigan State University Program in Emergency Medicine, 1900 Wealthy Street SE, Grand Rapids, MI 49506, USA. E-mail: bryan.judge@spectrum-health.org

as the patient did. Three days after consumption she developed vomiting and diarrhea and was treated with promethazine suppositories that were prescribed by her physician via telephone. Her friend had diarrhea that resolved, but no other symptoms. The patient denied having abdominal pain, back pain, or myalgias. She experienced diminishing urine output over a period of 4 days before coming to the ED for evaluation.

Upon presentation, the patient had normal vital signs and physical examination findings. She denied any prior medical problems, had no known drug allergies, did not smoke, rarely drank alcohol, took a multivitamin on a daily basis, and was a strict vegetarian. Four years before the ingestion, the patient's creatinine and blood urea nitrogen were 1.0 and 20 mg/dL, respectively. Pertinent initial serum chemistries included sodium 126 mEq/L, potassium 6.4 mEq/L, bicarbonate 18 mEq/L, chloride 90 mEq/L, anion gap 18 mEq/L, glucose 109 mg/dL, creatinine 13.8 mg/dL, blood urea nitrogen 88 mg/dL, AST 15 IU/L, and ALT 8 IU/L. Urinalysis revealed specific gravity 1.010, pH 8.0, glucose 70 mg/dL, sodium 113 mEq/L, creatinine 18.3 mg/dL, protein 1,088.0 mg/dL, 38 white blood cells/HPF, and 18 red blood cells/HPF.

An electrocardiogram demonstrated no findings consistent with hyperkalemia. The patient was treated with 50 mEq of sodium bicarbonate intravenously and 15 g of sodium polystyrene sulfonate orally. She was admitted in the hospital for urgent hemodialysis, further evaluation, and treatment. Intact samples of the mushrooms ingested were brought to the hospital, photographed, and dried (Fig. 1). Based on the photograph, the mushrooms were preliminarily identified as a *Cortinarius* mushroom species by a mycologist. Dried specimens were sent to the University of Washington and the University of Tennessee for further analysis.

On the fifth day of hospitalization (14 days post-ingestion), a renal biopsy was performed and demonstrated severe interstitial edema, moderate interstitial nephritis, and acute tubular necrosis (Fig. 2). Nine days after presentation, the patient was discharged from the hospital but remained anuric, and on hemodialysis. One year after ingestion her creatinine was 6.9 mg/dL, and she reported that she was suffering from depression and requiring peritoneal dialysis 5 times a week.

Fig. 1. *Cortinarius orellanosus* – aspect and coloration of mature specimens.

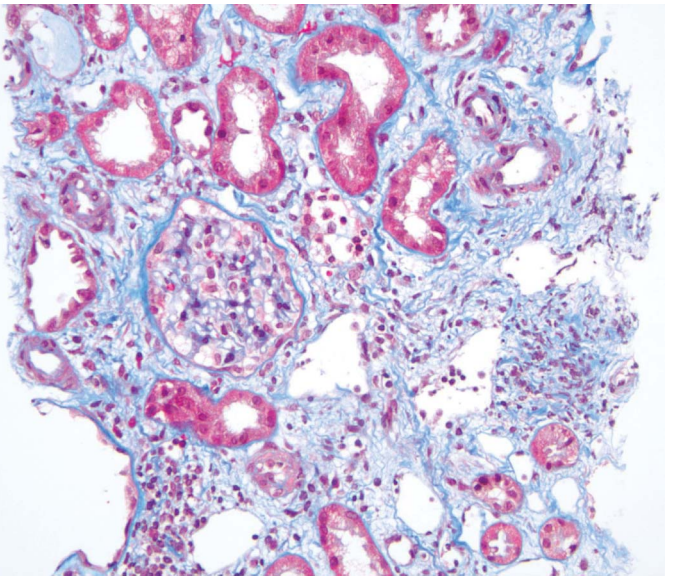

Fig. 2. Renal biopsy 14 days post-ingestion: Tubulointerstitial lesions with tubular degeneration, interstitial edema, and inflammatory infiltrates (Masson's trichrome × 200 magnification).

Materials and methods

An unconsumed sample of the Michigan specimens and the control sample of *C. orellanus* (JFA9859) collected in Italy were sent to the University of Tennessee for comparative DNA sequence analysis. The fungal collections reside at the University of Washington Herbarium.

DNA extraction was performed by removing 10–20 mg of dried material (a pileus wedge) for grinding in a 1.5-mL microtube with 80–100 mg of sterilized sand, a micropestle, and liquid nitrogen. DNA was extracted using a fungal DNA extraction kit manufactured by Omega Bio-Tek Inc. (Norcross, Georgia). Genomic DNA was serially diluted in two successive 1 : 10 dilutions with sterile water. PCR amplifications of the internal transcribed spacer (ITS) region, functionally the bar code locus of choice in fungal molecular systematics, were performed on a C1000 thermocycler manufactured by Bio-Rad (Hercules, CA, USA). A mixture of sterile water and 5X buffer, GoTaq, and dNTPs supplied by Invitrogen Corp. (Carlsbad, CA, USA) was prepared for each dilution of DNA and controls following manufacturer protocols. ITS1F and ITS4 primers were used for PCR amplification.[9,10] Products of a single-copy nuclear protein-coding gene, *rpb2*, were also produced using primers b6F and b7.1R.[11] Amplicons were screened in 1% agarose gels with ethidium bromide and viewed on a UV transilluminator.

Products were screened and purified using QIAGEN PCR purification columns (Valencia, CA, USA). A BigDye Terminator 3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) was used to produce the sequence reactions that were subsequently purified in 96-well Sephadex G-50 columns (General Electric Healthcare, Piscataway, NJ, USA) using separator strips manufactured by Princeton Separations (Freehold, NJ, USA). Sequencing was performed on an ABI 3730 48-capillary electrophoresis genetic analyzer at the Molecular Biology Resource Facility at the University of Tennessee. Sequence chromatograms were inspected and edited using Sequencher 4.8 software (Gene Codes Corp, Ann Arbor, MI, USA). GenBank (NCBI) accession numbers for ITS sequences of both the sample involved in the poisoning case (FJ263280) and the *C. orellanus* control (FJ263279) were released immediately to the public.

BLASTn comparisons of query sequences were performed with sequences available at GenBank, a database of publicly available DNA sequences. Seven taxonomic and environmental ITS sequences were downloaded that were found to correspond to members of the Orellani clade[12] or were highest matches to our query sequences: these included accessions-labeled *C. rubellus*, *Cortinarius orellanoides* Rob. Henry, and *C. orellanus*, species involved in delayed-onset renal failure.[13] Two sequences of *C. rubellus* from Scotland were also downloaded from the UNITE database.[14] Sequences of *Cortinarius bolaris* (Pers.: Fr.) Fries were used as outgroups for rooting purposes in phylogenetic analyses of the ITS regions, which were performed using Bayesian Inference in MrBayes 3.1.2, maximum parsimony (MP) in PAUP* [Phylogenetic Analysis using Parsimony (*and Other Methods)] portable version 4.0b10 for Unix, and maximum likelihood (ML) using GARLI (Genetic Algorithm for Rapid Likelihood Inference) v0.951.[15–17] Model test 3.7 was used to determine a best-fit model of nucleotide evolution under the Akaike information criterion for Bayesian and ML analyses.[18]

Thirteen ITS sequences of the Orellani clade, *Cortinarius canarius* (E. Horak) G. Garnier and two accessions of *C. bolaris* were aligned using ClustalX 2.0.9 and default alignment settings.[19] Output was saved as a nexus file and minor manual alignment adjustments made in MacClade 4.08.[20] The final version of the nexus file included 728 nucleotide sites and is available upon request. After excluding the first 52 sites due to unevenness of character sampling, the model best-fit to the alignment was a HKY+I model (Hasegawa–Kishino–Yano model with a proportion of invariable sites), a nucleotide transformation matrix that allows a different substitution rate for transitions relative to transversions (Ti : tv ratio = 1.5469), a proportion of invariable sites (I) = 0.6241, an equal rates parameter for all sites (gamma), and estimated base frequencies. In the Bayesian analysis, tree sampling occurred for one million generations logging trees every 100 generations from two independent runs. The average SD of split frequencies between the two runs reached less than 0.01 after 90,000 generations, an indicator used to determine similarity of tree sampling from different runs. The first 20% of trees sampled (2,000) were burned, which left 8,001 trees from each run. A total of 16,002 trees was combined and used to reconstruct a summary tree and calculate posterior probabilities for each internode. An internode recovered more than 95% of the time has a posterior probability >0.95, an indicator of statistical significance.

Statistical strength for internodes was also assessed using MP and ML bootstrapping. In the former, 1,000 bootstrapped pseudoreplicate datasets were created. A heuristic algorithm under the parsimony criterion was used to search for the optimal tree for each replicate with 10 random-addition-sequence replicates using stepwise addition to obtain starting trees for branch swapping. The tree bisection–reconnection algorithm was used for branch swapping with MulTrees set to no, which saves only one of the best trees found during branch swapping. One hundred ML bootstraps were also performed using Genetic Algorithm for Rapid Likelihood Inference following a previously outlined procedure.[21] Consensus trees were then created from bootstrapped trees, and 70% recovery for a given internode was considered significant.

Molecular taxonomical and phylogenetical results

The ITS sequence of FJ263279 *C. orellanus* JFA9858 from Italy matched GenBank accession AF389164 *C. orellanus* from Austria at all nucleotide sites except for one gapped position (99% similarity). The two-spacer regions of the Michigan material implicated in the poisoning case differed at 16 sites (97% similarity) with respect to Austrian *C. orellanus*. Eight of the site differences represent nucleotide substitutions and eight represent insertion–deletion (indels) events. No closely related *rpb2* sequences were detected after BLASTn searches. A pairwise comparison of *rpb2* sequences of the Michigan material [GU462004] and *C. orellanus* from Italy [GU462003] reveals four nucleotide differences between them at three coding sites (all synonymous) and one intron site (in intron4). The Bayesian summary tree of ITS data (Fig. 3) illustrates that the Michigan *Cortinarius* sample clusters with other species of the Orellani clade and occupies a branch sister to European species of *C. orellanus*, from which it is genetically distinct.

Taxonomy

As per the International Code of Botanical Nomenclature, for the name of a new species to be validly published, it must be printed in hard copy and described in Latin. A holotype must be designated and its place of deposit given. Please see the text available at http://informahealthcare.com/doi/suppl/10.3109/15563650.2010.495346 for English version.

Cortinarius orellanosus Ammirati and Matheny, sp. nov.
Pileus 3–5 cm, umboconvexus, disco latescente et scutescente, superficie sicca, fibrillosus vel squamaceofibrillosus,

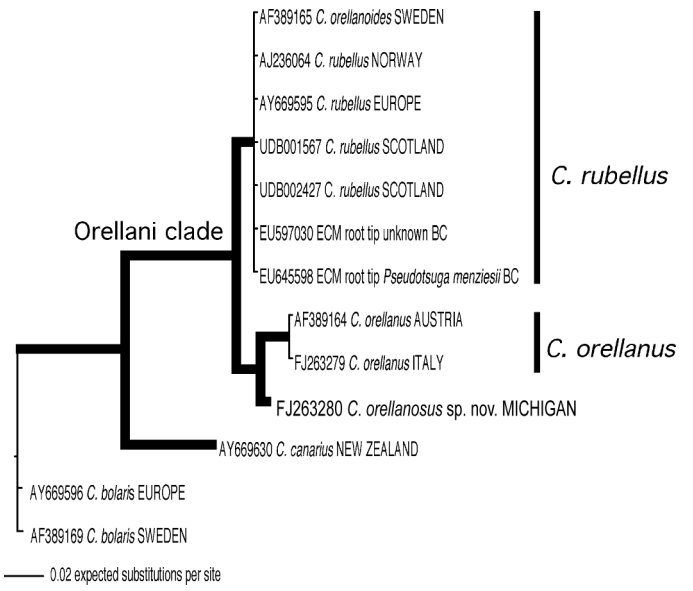

Fig. 3. Phylogenetic tree estimate of evolutionary relationships of isolates in the Orellani clade of *Cortinarius* inferred by Bayesian analysis. Thick branches indicate significant measures of branch support inferred by Bayesian posterior probabilities and MP and ML bootstrapping.

aurantiacobrunneus vel rubrobrunneus vel flavobrunneus, atrescens cum aetate. Lamellae subdistantes vel distantes, sane crassae, subauranticobrunneae vel brunneae, atrescentes cum aetate. Stipes 6.5–7.5 × 0.8–1.3 cm, basi aucta, superficie sicca, quibusdam fibrilibus superficialibus ad vela, pallidoflavus vel pallido-ochraceus, brunneus vel rubrobrunneus cum aetate. Contextus albidoflavus vel languidochraceus. Sporae (9) 9.5–10 (10.5) × (5.5) 6.5–7 µm, ellipsoideae vel late ellipsoideae, manifeste verrucosae. Terrestris sub quercu.
Typus: USA. Michigan. Kent County. Ada, under oak, July 11, 2008, John H. Trestrail III, 07112008 (Holotypus, WTU).

Discussion

Cortinarius orellanosus is a medium size gill mushroom that is characterized by a dome-shaped, dry, orange red brown to yellow brown cap; thick, well-spaced, orange brown gills; and a dry, yellowish to reddish brown stalk that is tapered below with some veil fibrils on the surface. The flesh of the mushroom is whitish to yellowish. Species in this group have rust brown spore prints and the spores are ellipsoid and distinctly ornamented.

Both *C. orellanus* and *C. orellanosus* occur on the ground in association with oak trees, but *C. orellanus* also occurs with beech and hazel. Additional study is needed to determine the distribution of *C. orellanosus* in North America. It has likely been previously collected by other workers but not published to date. There are no collections of North American material in the University of Michigan herbarium under the name *C. orellanus*, and it has not been reported from North America. An excellent description and photograph of European *C. orellanus* can be found in *Cortinarius, Flora Photographica*.[22] *Cortinarius rubellus* Cooke has been reported from North America[23] and is also featured in *Cortinarius, Flora Photographica*.[22]

This report details a confirmed case in North America of the ingestion of a *Cortinarius* mushroom species resulting in renal failure. Phylogenetic analysis revealed that the mushroom ingested is genetically similar to *C. orellanus* but distinct at two loci. Thus, the Orellani clade comprises at least three species involved in delayed-onset renal failure: a widespread species in Europe and western North America, *C. rubellus*; a European species, *C. orellanus*; and a unique North American species, *C. orellanosus*, implicated in the mushroom poison case reported here.

The clinical features described in this case are similar to the main characteristics of *Cortinarius* species poisoning summarized by Danel et al.[2] Our patient experienced vomiting and diarrhea 3 days after ingestion and undoubtedly developed nephrotoxicity in the preceding days before coming to ED. Typically gastrointestinal symptoms develop a few days (median 3 days) after ingestion, followed by delayed acute renal failure that occurs 4–15 days (median 8.5 days) following consumption. It is unclear what role, if any, the patient's usage of a daily multivitamin had on the development of her renal failure in conjunction with the consumption of the mushrooms.

Glucosuria, hematuria, leukocyturia, proteinuria, increased serum creatinine and serum potassium, and metabolic acidosis were present upon admission. A renal biopsy performed 14 days after ingestion showed interstitial edema, interstitial nephritis, and tubular necrosis. These laboratory and histopathological abnormalities are consistent with the primary biological features of the renal phase of *Cortinarius* species poisoning.[2] Additionally, the patient had renal failure 1 year after ingestion that necessitated peritoneal dialysis. Although complete recovery can occur, many patients who have been poisoned by a *Cortinarius* mushroom species have developed chronic renal failure requiring intermittent renal replacement therapy or a renal transplant.[1]

It is unknown whether the patient's companion developed nephrotoxicity. We assume that he did not since he consumed fewer mushrooms than the patient presented here and to our knowledge never sought medical care. The toxic effects of *Cortinarius* mushroom species seem to be dose-dependent;[2] however, there is considerable inter-individual variability in the susceptibility to the toxic effects of these mushrooms.[24] Interestingly, an animal study demonstrated that females appear to be more resistant than males to the toxic effects of *Cortinarius speciosissimus*.[25]

Our report is limited by the fact that we did not attempt to isolate orellanine from the sample of mushrooms. We felt that this was unnecessary for several reasons. First, most of the available analytical methods to detect this toxin are difficult to perform. Second, these methods are subject to false positives and lack sensitivity.[2] Third, we had a limited

amount of material and felt that it was necessary to preserve the remaining specimens; additional *C. orellanosus* mushrooms need to be collected before analysis of orellanine can be performed. Finally, the genetic similarity of *C. orellanosus* to other *Cortinarius* mushrooms within the Orellani clade implicates orellanine as the putative toxin in this case.

Description of *C. orellanosus* as a new species is supported by the degree of dissimilarity (3%) at the ITS locus. A 3% threshold is regarded as an approximate minimum indicator for species recognition.[26] Several but fewer nucleotide differences exist at the *rpb*2 locus as well. Although it would be desirable to assess the degree of interspecific variation in *C. orellanosus*, we were not permitted to collect additional specimens from the patient's backyard.

Conclusions

The mushrooms ingested by the patient were confirmed to be a new species of *Cortinarius* closely related to *C. orellanus*. We introduce a newly described North American species, *C. orellanosus*, capable of causing renal failure after ingestion. The suspected toxin responsible for the patient's renal failure is orellanine because of the genetic similarity of *C. orellanosus* and other mushrooms within the Orellani clade. Further study is necessary to determine the distribution of *C. orellanosus* in North America.

Acknowledgments

This work was supported partly by funding from Daniel E. Stuntz Memorial Foundation. The Latin description was prepared by Owen M. Ewald, Assistant Professor, Foreign Languages and Literature, Seattle Pacific University. We thank Aaron Wolfenbarger for producing the *rpb*2 sequences. Molecular laboratory work was funded by the University of Tennessee.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Karlson-Stiber C, Person H. Cytotoxic fungi–an overview. Toxicon 2003; 42:339–349.
2. Danel VC, Saviuc PF, Garon D. Main features of *Cortinarius* spp. poisoning: a literature review. Toxicon 2001; 39:1053–1060.
3. Moore B, Burton BT, Lindgren J, Rieders F, Kuehnel E, Fisher P. *Cortinarius* mushroom poisoning resulting in anuric renal failure. Vet Hum Toxicol 1991; 33:369.
4. West PL, Lindgren J, Horowitz BZ. *Amanita smithiana* mushroom ingestion: a case of delayed renal failure and literature review. J Med Toxicol 2009; 5:32–38.
5. Warden C, Benjamin D. Acute renal failure associated with suspected *Amanita smithiana* ingestions: a case series. Acad Emerg Med 1998; 5:808–812.
6. Raff E, Halloran PF, Kjellstrand CM. Renal failure after eating "magic" mushrooms. Can Med Assoc J 1992; 147:1339–1341.
7. Litovitz TL, Smilkstein M, Felberg L, Klein-Schwartz W, Berlin R, Morgan JL. 1996 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am J Emerg Med 1997; 15(5):447–500.
8. Bronstein AC, Spyker DA, Cantilena LR, Green JL, Rumack BH, Giffin SL. 2008 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 26th annual report. Clin Toxicol 2009; 47(10):911–1084.
9. White TJ, Bruns TD, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: A Guide to Methods and Applications. New York: Academic Press; 1990:315–322.
10. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes–application to the identification of mycorrhizae and rusts. Mol Ecol 1993; 2:113–118.
11. Matheny PB. Improving phylogenetic inference of mushrooms using RPB1 and RPB2 sequences (Inocybe, Agaricales). Mol Phylogenet Evol 2005; 35:1–20.
12. Peintner U, Moncalvo J-M, Vilgalys R. Toward a better understanding of the infrageneric relationships in *Cortinarius* (Agaricales, Basidiomycota). Mycologia 2004; 95:1042–1058.
13. Benjamin DR. Mushrooms Poisons and Panaceas: A Handbook for Naturalists, Mycologists, and Physicians. New York: W.H. Freeman & Company; 1995.
14. Kõljalg U, Larsson K-H, Abarenkov K, Nilsson RH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Vrålstad T, Ursing B. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol 2005; 166:1063–1068.
15. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 2003; Aug 12:1572–1574.
16. Swofford DL. PAUP*. Phylogenetic Analysis Using Parsimony (* and other Methods). Version 4. 4th ed. Sunderland, MA: Sinauer Associates; 2003.
17. Zwickl DJ. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion [Ph.D. dissertation]. Austin, TX: The University of Texas at Austin; 2006.
18. Posada D, Crandall KA. Modeltest: Testing the model of DNA substitution. Bioinformatics 1998; 14(9):817–818.
19. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 1997; 25:4876–4882.
20. Maddison DR, Maddison WP. MacClade 4.08: Analysis of Phylogeny and Character Evolution. Sunderland, MA: Sinauer Associates; 2005.
21. Matheny PB, Moreau P-A. A rare and unusual lignicolous species of Inocybe (Agaricales) from eastern North America. Brittonia 2009; 61:163–171.
22. Brandrud TE, Lindström H, Marklund H, Melot J, Muskos S. Cortinarius, Flora Photographica. Matfors, Sweden: Cortinarius HB; 1998.
23. Robertson CP, Wright L, Gamiet S, Machnicki N, Ammirati J, Birkebak J, Meyer C, Allen A. *Cortinarius rubellus* Cooke from British Columbia, Canada and Western Washington, USA. Pac Northwest Fungi 2006; 1:1–7.
24. Bouget J, Bousser J, Pats B, Ramee MP, Chevet D, Rifle G, Giudicelli CP, Thomas R. Acute renal failure following collective intoxication by *Cortinarius orellanus*. Intensive Care Med 1990; 16:506–510.
25. Nieminen L, Pyy K. Sex differences in renal damage induced in the rat by the Finnish mushroom, *Cortinarius speciosissimus*. Acta Pathol Microbiol Scand 1976; [A] 84:222–224.
26. Ryberg M, Nilsson RH, Kristiansson E, Töpel M, Jacobsson S, Larsson E. Mining metadata from unidentified ITS sequences in GenBank: a case study in Inocybe (Basidiomycota). BMC Evol Biol 2008; 8:50.