CONSTRUCTION OF A DATASET FOR THE GENE *nrLSU* TO SUPPORT THE IDENTIFICATION OF *CANTHARELLUS* AT LANGBIAN MOUNTAIN, LAM DONG

TRINH VAN HANH, VU TIEN LUYEN
University of Science, VNU-HCM, Viet Nam

PHAN HUU HUNG
Tay Nguyen Institute of Scientific Research, Vietnam Academy of Science and Technology

TRUONG BINH NGUYEN
Da Lat University, Viet Nam

TRAN DUC VINH, LAO DUC THUAN, LE HUYEN AI THUY*
Ho Chi Minh City Open University, Viet Nam - *thuy.lha@ou.edu.vn

(Received: February 06, 2018; Revised: May 07, 2018; Accepted: May 14, 2018)

ABSTRACT

Fungal species of *Cantharellales* order are found worldwide including Europe, Africa, Asia, and North America. This clade includes two smaller, separate, but closely related genera *Cantharellus* and *Craterellus*. *Cantharellus* was firstly identified in the middle of the 17th century. Besides traditional classification based on morphology, molecular phylogeny of the nuclear ribosomal large subunit gene (*nrLSU*) can be an effective tool to provide more information during species identification for this group. In this current report, a dataset of the *nrLSU* gene including 60 sequences of *Cantharellus* species and 1 sequence belonging to *Craterellus* (outgroup), was obtained from Genbank. Phylogenetic analyses by MEGA 6.0 showed a monophyletic relationship between the six well-established clades, namely *Cantharellus* (Clade 1), *Rubrinus* (Clade 2), *Cinnabarinus* (Clade 3), *Parvocantharellus* (Clade 4), *Pseudocantharellus* (Clade 5) and subgenus *Afrocantharellus* (Clade 6) similar to that of morphological classification. Therefore, this dataset is efficient to support the identification of the fungal samples, collected at the Langbian Mountain, Lam Dong.

Keywords: *Cantharellus*; *Large subunit (LSU) of rDNA*; *The phylogenetic tree.*

1. Introduction

Cantharellus Adans.: Fr (Family Cantharellaceae J. Schröt.). as circumscribe in the middle of the 17th century, belong to *Cantharellales* order (*Cantharallaceae*, Cantharelloid clade), collected from Europe, Africa, Asia and North America, in detail, 23 species in North America, 7 species in South America, 7 species in Australia, 9 species in Europe, 3 species in New Zealand, 46 species in Africa, and 19 species in Asia (Tibuhwa et al., 2012; Eyssartier et al., 2009; Buyck et al., 2011; Shao et al., 2014). Morphology of *Cantharellus* was characterized as the overall vase-like or infundibuliform shaped, the superficially resemble agarics (gilled mushrooms), but the surface is smooth, wrinkled, or follicle-shaped (Kumari et al., 2011). These species are mostly ectomycorrhizal fungi, forming a mutually beneficial relationship with the roots and other trees, primarily within the beech family and pine family. *Cantharellus* has been considered having high economic value, such as *Craterellus cornucopioides*, *Cantharellus* *formosus*, *Craterellus tubaeformis*, *Cantharellus minor* (Redhead et al., 1997; Arora et al., 2008), with significant levels of proteins, lipids, minerals, vitamins and some neutraceutical compounds. (Pilz et al., 2003; et al., 2011).

The *Cantharellus* and *Craterellus* have been recognized as two closely related genus (Persoon, 1979; Fries, 1838), which may be recognized by their lack of division into cap and stipe, and their rudimentary. Recent phylogenetic studies from sequence analyzes suggested to be useful to study fungal taxonomy and diversity. These include the small subunit (SSU), large subunit (LSU) of rRNA, the internal transcribed spacer region (ITS), etc (Kumari et al., 2011; Feibelman et al., 1997; Buyck et al., 2011) Among that, phylogenetic analysis of the nuclear large subunit rRNA (LSU) in *Cantharellus* has supported to clarify the identity of different species (Redhead et al., 1997; Dunham et al., 2003a; Arora and Dunham, 2008). In order to resolve the internal or external evolutionary relationship of Cantharellaceae, Feibelman et al (1997) studied the phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of nuclear large subunit rDNA. Sequence analyses demonstrated that *Cantharellus* and *Craterellus* should be treated as distinct genera on the classification of *Cantharellus* clade (Buyck et al., 2011).

The main objective of the current study is to establish a dataset to support the identification of *Cantharellus*. In further study, it will be applied in the identification of the *Cantharellus* clade at Langbian mountain, Lam Dong province in following publications.

2. Materials and Methods

2.1. Taxa and sequence collection

Sequence of *nrLSU* were collected from Genbank (NCBI) and previous published data. The data set was constructed according to Fig. 1. The *nrLSU* is noted with strain, accession number, name of taxon and locality.

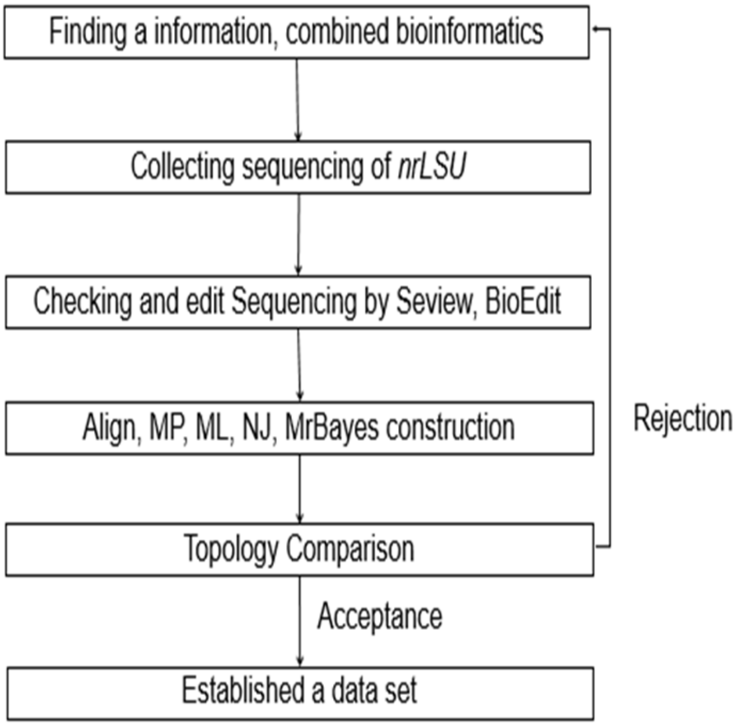

Figure 1. The protocol for construction of *nrLSU* data set

2.2. Phylogenetic analysis

The phylogenetic tree was constructed, based on the neighbor-joining (NJ) and maximum parsimony (MP) maximum likelihood (ML) methods, by using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0. Additionally, the best evolution model was predicted by using jModelTest.

3. Results and Discussion

3.1. The systematic dataset

The final *nrLSU* rDNA sequence data set, including 60 sequences of *Cantharellus* and 1 sequence belonged to *Craterellus* (served as outgroup), were collected from Genbank (NCBI) (listed in Table 1). In detail, the *nrLSU* rDNA was collected from different countries; included 7 sequences in Tanzania, 21 sequences in Madagascar, 2 sequences in Zambia, 17 sequences in USA, 6 sequences in Slovakia, 3 sequencing in France, notably, only 4 sequences in Malaysia; within average length of 1424 nucleotides. The best substitution for phylogenetic analysis was predicted by using j Model Test.

Table 1

The data of *nrLSU* gene was used for construction of phylogenetics trees

No.	Accession	Taxon	Strain	Locality
1	KF294621	*Cantharellus addaiensis* Henn. 1898	BB 98.057	Tanzania
2	KF294667	*Cantharellus addaiensis*	BB 98.033	Tanzania
3	KF294606	*Cantharellus aff. congolensis*	BB 06.176	Madagascar
4	KF294608	*Cantharellus aff. congolensis*	BB 06.197	Madagascar
5	KF294603	*Cantharellus aff. decolorans*	BB 06.146	Madagascar
6	KF294653	*Cantharellus aff. decolorans*	BB 08.234	Madagascar
7	KF294654	*Cantharellus aff. decolorans*	BB 08.278	Madagascar
8	KF294604	*Cantharellus aff. tanzanicus*	BB 06.148	Madagascar
9	KF294605	*Cantharellus aff. tanzanicus*	BB 06.149	Madagascar
10	KF294668	*Cantharellus afrocibarius*	BB 96.235	Zambia
11	KF294669	*Cantharellus afrocibarius*	BB 96.236	Zambia
12	KF294645	*Cantharellus albidolutescens*	BB 08.057	Madagascar
13	KF294646	*Cantharellus albidolutescens*	BB 08.070	Madagascar
14	KF294627	*Cantharellus altipes*	BB 07.019	USA
15	KF294636	*Cantharellus altipes*	BB 07.162	USA
16	KF294656	*Cantharellus ambohitantelyi*	BB 08.336	Madagascar
17	KF294639	*Cantharellus amethysteus*	BB 07.284	Slovakia
18	KF294642	*Cantharellus amethysteus*	BB 07.309	Slovakia
19	KF294635	*Cantharellus appalachiensis*	BB 07.123	USA
20	KF294663	*Cantharellus cerinoalbus*	AV 06.501	Malaysia
21	KF294641	*Cantharellus cibarius*	BB 07.300	Slovakia
22	KF294658	*Cantharellus cibarius*	GE 07.025	France
23	JN940601	*Cantharellus cinnabarinus*	BB 07.120	USA
24	KF294624	*Cantharellus cinnabarinus*	BB 07.001	USA

No.	Accession	Taxon	Strain	Locality
25	KF294630	*Cantharellus cinnabarinus*	BB 07.053	USA
26	KF294609	*Cantharellus congolensis*	BB 98.039	Tanzania
27	KF294661	*Cantharellus diminutivus*	DS 06.033	Malaysia
28	KF294638	*Cantharellus ferruginascens*	BB 07.283	Slovakia
29	KF294674	*Cantharellus fistulosus*	DT 43	Tanzania
30	KF294650	*Cantharellus ibityi*	BB 08.196	Madagascar
31	KF294651	*Cantharellus ibityi*	BB 08.203	Madagascar
32	KF294628	*Cantharellus lateritius*	BB 07.025	USA
33	KF294633	*Cantharellus lateritius*	BB 07.058	USA
34	JN940597	*Cantharellus lewisii*	BB 07.003	USA
35	KF294623	*Cantharellus lewisii*	BB 02.197	USA
36	KF294637	*Cantharellus lilacinopruinatus*	BB 07.221	Slovakia
37	KF294625	*Cantharellus minor* Peck 1872	BB 07.002	USA
38	KF294632	*Cantharellus minor*	BB 07.057	USA
39	KF294655	*Cantharellus paucifurcatus*	BB 08.320	Madagascar
40	KF294647	*Cantharellus platyphyllus subsp. Bojeriensis*	BB 08.158	Madagascar
41	KF294648	*Cantharellus platyphyllus subsp. Bojeriensis*	BB 08.160	Madagascar
42	KF294617	*Cantharellus platyphyllus*	BB 98.012	Tanzania
43	KF294620	*Cantharellus platyphyllus*	BB 98.126	Tanzania
44	KF294644	*Cantharellus quercophilus*	BB 07.097	USA
45	KF294649	*Cantharellus sebosus*	BB 08.162	Madagascar
46	KF294652	*Cantharellus sebosus*	BB 08.234	Madagascar
47	JN940604	*Cantharellus tricolor*	BB 06.180	Madagascar
48	KF294607	*Cantharellus* sp	BB 06.179	Madagascar
49	KF294664	*Cantharellus subamethysteus*	DS 06.218	Malaysia
50	KF294601	*Cantharellus subincarnatus* subsp	BB 06.080	Madagascar
51	KF294602	*Cantharellus subincarnatus* subsp	BB 06.096	Madagascar
52	KF294660	*Cantharellus subpruinosus*	GE 07.080	France
53	KF294631	*Cantharellus tabernensis*	BB 07.056	USA
54	KF294634	*Cantharellus tabernensis*	BB 07.119	USA
55	KF294622	*Cantharellus tanzanicus*	BB 98.040	Tanzania
56	KF294629	*Cantharellus tenuithrix*	BB 07.035	USA
57	KF294626	*Cantharellus texensis*	BB 07.018	USA
58	KF294659	*Cantharellus friesii*	GE 07.077	France
59	KF294662	*Cantharellus cuticulatus*	DS 06.283	Malaysia
60	KF294640	*Craterellus tubaeformis*	BB 07.293	Slovakia

3.2. Phylogenetic analysis

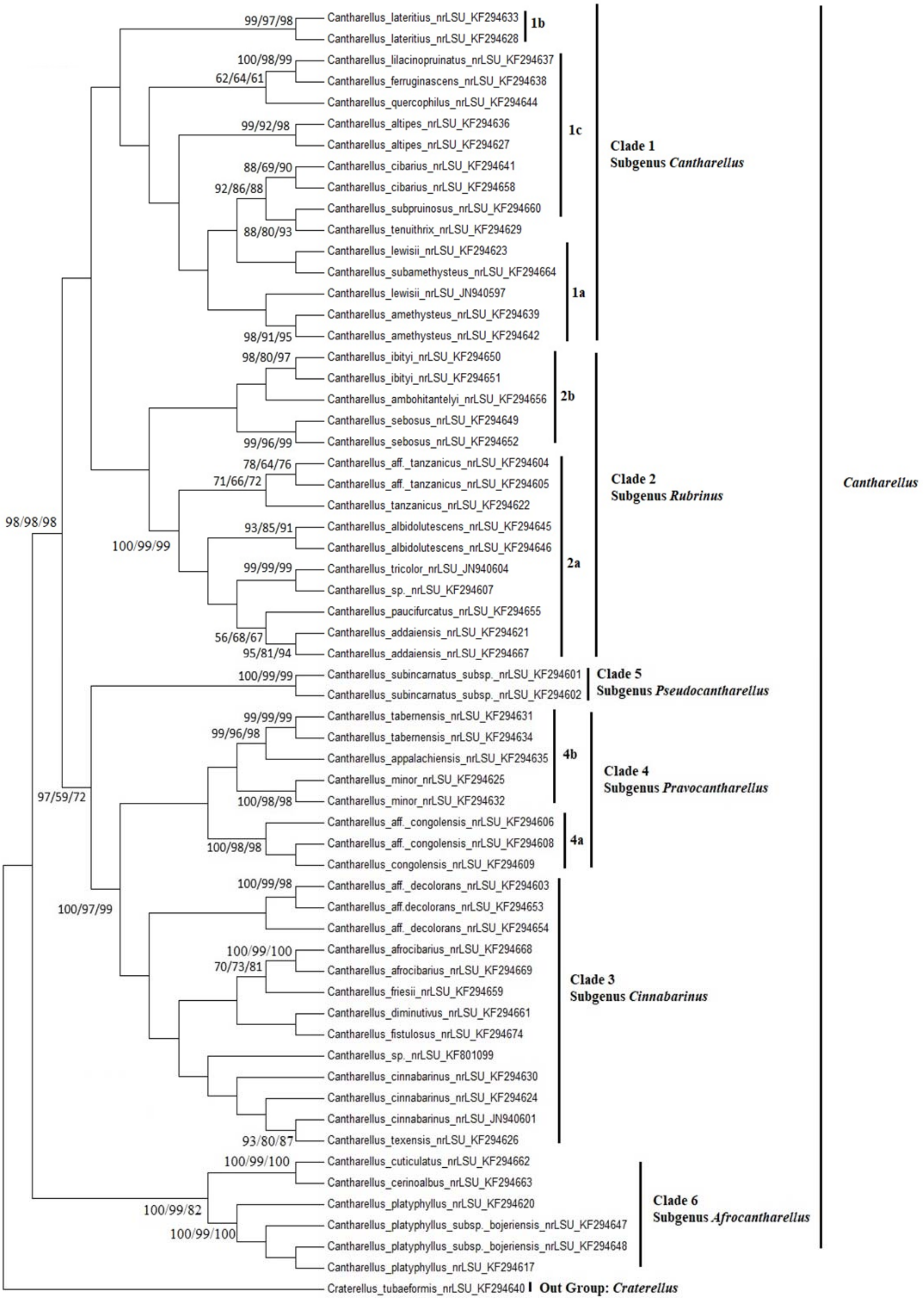

Figure 2. A phylogenetic tree was constructed via maximum parsimony method. The Maximum Parsimony tree is shown with bootstrapping value of NJ/MP/ML.

The evolution model that was most fit with the observed data set was Kimura 2-parameter, with following parameters: parameters = 121, BIC = 7891.413, lnL = -3301.880, (+I) = n/a, (+G) = 0,24, R = 2.10, f(A) = 0,250, f(T) = 0,250, f(G) = 0,250, f(C) = 0,250, r(AT) = 0,040, r(AC) = 0,040, r(AG) = 0,169, r(TA) = 0,040, r(TC) = 0,169, r(TG) = 0,040, r(CA) = 0,040, r(CT) = 0,169, r(CG) = 0,040, r(GA) = 0,169, r(GT) = 0,040, r(GC) =0,040.

These parameters were applied to MEGA 6.0 to construct phylogenetic trees. As the results, the topology of neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) trees were similar to each other and to the trees from Buyck et al. (2014). 58 referent sequences from *Cantharellus* clade with 16 sequences for Subgenus *Cantharellus* (Clade 1), 15 for Subgenus *Rubrinus* (Clade 2), 12 for Subgenus Cinnabarinus (Clade 3), 8 for Subgenus *Parvocantharellus* (Clade 4), 2 for Subgenus *Pseudocantharellu*s (Clade 5), 5 for Subgenus *Afrocantharellus* (Clade 6).

4. Conclusion

In general, we have successfully applied the bioinformatics for construct a dataset basing on *nrLSU* gene to support the identification of *Cantharellus*. This dataset is efficient to support the identification of the fungal samples, collected at the Langbian Mountain, Lam Dong in the future■

Acknowledgments

We wish to express our thanks to the research project sponsored by Tay Nguyen Institute of Scientific Research.

References

Arora, D., Dunham, S. M. (2008). A new, commercially valuable chanterelle species, Cantharellus californicus sp. nov., associated with live Oak in California, USA. *Econ Bot, 62*, 376-391.

Buyck, B., Kauff, F., Eyssartier, G., Couloux, A., Hofstetter, V. (2013). Molecular evidence for novel *Cantharellus* (*Cantharellales*, Basidiomycota) from tropical African miombo woodland and a key to all tropical African chanterelles. *Fungal Diversity, 58,* 281-298.

Buyck, B., Hofstetter, V. (2011). The contribution of tef-1 sequences to species delimitation in the Cantharellus cibarius complex in the southeastern USA. *Fungal Divers, 49,* 35-46.

Buyck, B., Kauff, F., Eyssartier, G., Couloux, A., Hofstetter, V. (2014). A multilocus phylogeny for worldwide *Cantharellus* (*Cantharellales*, Agaricomycetidae). *Fungal Diversity, 64,* 101.

Dahlman, Mattias; Danell, Eric; Spatafora, Joseph W. (2000). Molecular systematics of *Craterellus*: cladistic analysis of nuclear LSU rDNA sequence data. *Mycological Research, 104*(4), 388-394.

Dunham, O'Dell T. E., Molina R. (2003). Analysis of nrDNA sequences and microsatellite allele frequencies reveals a cryptic chanterelle species *Cantharellus cascadensis* sp. nov. from the American Pacific Northwest. *Mycological Research, 107*(107), 1163-1177.

Eyssartier, G., Stubbe, D., Walleyn, R., Verbeken, A. (2009). New records of Cantharellus speceis (Basidiomycota, Cantharellaceae) from Malaysian depterocarp rainforest. *Fungal Divers, 36,* 57-67.

Feibelman, T. P., Doudrick, R. L., Cibula, W. G. And Bennett, J. W. (1997). Phylogenetic relationships within the Cantharellaceae inferred from sequence analysis of the nuclear large subunit rDNA. *Mycological Research, 101*(12), 1423-1430.

Fries, E. M. (1838). Epicrisis systematis mycologici, seu synopsis Hymenomycetum", Annals of natural history, 1838.

Kumari, D., Upadhyay, R.C., Reddy, M.S. (2011). *Cantharellus pseudoformosus,* a new species associated with Cedrus deodara from India. *Mycoscience, 52,* 147-151.

Kumari, D. (2011). Studies on the diversity and nutritional value of Cantharellaceae of Western Himalayas, India.

Persoon, C.H. (1797). Commentatio de fungis clavaeformibus sistens specierum hus usque notarum descriptiones cum differentiis specificis, nec non auctorum synonymis, Apud Petr. Philipp. Wolf.

Pilz, D., Norvell L., Danell E., Molina R. (2003). Ecology and management of commercially harvested chanterelle mushrooms, Gen. *Tech. Rep, 83*.

Redhead, S.A., Norvell, L., Danell, E. (1997). Cantharellus formosus and the Pacific golden chanterelle harvest in western North America, *Mycotaxon, 65,* 285-322.

Shao, C. S., Buyck, B., Hofstetter, V., Tian, X. F., Geng, Y. H., Yu F. Q & Liu, G. P. (2014). Source Cantharellus hygrophorus, a New Species in Subgenus Afrocantharellus from Tropical Southwestern China. *Cryptogamie, Mycologie, 35*(3), 283-291.

Tibuhwa, D. D., Saviæ S., Tibell, L., Kivaisi, A. K. (2012). *Afrocantharellus* gen. stat. nov. is part of a rich diversity of African Cantharellaceae. *IMA Fungus, 3*(1), 25-38.