

Molecular variations in *Lenzites* species collected from Nigeria and other parts of the world using Internal Transcribed Spacers (ITS) regions of Ribosomal RNA

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Abstract

The phylogeny of six specimens of *Lenzites* species collected from Akure Nigeria was studied using molecular data obtained by sequencing the internal transcribed spacers (ITS) regions of ribosomal RNA using universal primers (ITS4 and ITS5). Preliminary basic local alignment search tool (BLAST) revealed the identity of the specimens from Akure as *Lenzites* species. The percentage relationship between rRNA gene sequences of *Lenzites* species from Akure and *Lenzites* species sequences in NCBI GenBank database showed 99 to 100 similarity. Phylogenetic tree generated using MEGA 4 software strongly supported *Lenzites* clade that includes most *Lenzites* species together with species of *Trametes* and *Daedalea* which are considered as synonyms of *Lenzites* species. The rRNA gene sequences of three of the *Lenzites* species designated specimens 1, 3 and 6 were in the same clade with most of the *Lenzites* species collected from NCBI GenBank. Generally, specimens 1 to 6 form a monophyletic group with *Lenzites* species collected from other parts of the world. The results revealed the relationship of *Lenzites* species collected from Nigeria with *Lenzites* species with other parts of the world.

Key words – Polyporoid, *Lenzites* species, Phylogeny, rDNA, ITS.

Introduction

Macrofungi are known to be rich sources of bioactive compounds with great therapeutic properties (Liu 2007). Several bioactive compounds with positive physiological properties had been found in macrofungi. Some of these bioactive compounds are glycolipids, compounds derived from shikimic acid, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides, nucleosides, sesterterpenes, polysaccharides and many other substances of different origins (Lorenzen and Anke 1998; Wasser and Weis 1999; Mizuno 1999 and Liu 2007). They are known to be medically active in several therapies, such as antioxidant, antitumor, antibacterial, antiviral, hematological and immunomodulation (Wasser and Weis 1999; Lindequist *et al.* 2005).

It has been reported that 140,000 macrofungi are on earth and out of these only 14,000 (10%) are known (Lindequist *et al.* 2005). Presently, most macrofungi in Africa are yet to be correctly identified. Specifically, the pore fungi of tropical areas remain under studied (Douanla-Meli *et al.* 2007). This is as a result of lack of well trained manpower and state of the earth

equipment. Previous identification is based on morphological description of the fruiting bodies, host specificity, and geographical distribution (Seo and Kirk 2000). Morphologically, mushrooms belonging to the same and even different genera may look similar. Hence, morphological characteristics have their limitations in allowing a reliable distinction of intra-specific characteristics.

Lenzites spp are characterized by the combination of pileate basidiocarps, poroid hymenophore, trimitic hyphal system, thin-walled smooth basidiospores not reacting in presence of Melzer's reagent and production of a white-rot type of wood decay (Gilbertson and Ryvarden 1987). Two common synonyms of the genera *Lenzites* are *Daedalea* and *Trametes*. Members of the polyporoids are similar morphologically. However, recent molecular studies have helped to clarify the higher level of and generic relationship of polyporoid (Justo and Hibbett 2011). Ribosomal RNA (rRNA) gene sequences have provided a wealth of information concerning phylogenetic relationships (Hillis and Dixon 1991), and studies of rRNA gene sequences have been used to infer phylogenetic history across a very broad spectrum, from studies among the basal lineages of life to relationships among closely related species and populations (Yang 2011). The objectives of the present study are to ascertain the phylogenetic relationship between *Lenzites* species collected in Akure Nigeria by sequencing of the ITS region of the rRNA gene and comparing the gene sequences of these *Lenzites* species with sequences obtained from the NCBI Genbank.

Materials and Methods

Fungal material

Fresh fruiting bodies of *Lenzites* species were collected from Akure, Nigeria. The fruitbodies were kept dry in tissue papers that were placed in a polythene paper containing silica gel. The polythene bags containing the samples were well labeled for easy identification and taken to the laboratory for further examination. Herbarium samples of *Lenzites* fruitbodies were kept at the herbarium of Institute of Microbiology, Chinese Academy of Sciences, Beijing.

Extraction of DNA

Standard DNA isolation methods employing cetyltrimethylammonium bromide (CTAB) lysis buffer (Zolan and Pukkila 1986) was used. Briefly, dried portions of *Lenzites* fruitbodies (2g) were ground with a mortar and pestle. The grounded materials were transferred into well labeled microtubes. Prewarmed (60°C) extraction buffer (CTAB) was added and the tubes were incubated at 65°C for 30 to 60 minutes. Equal volume of chloroform and alcohol (24:1) was added and mixed by inverting tubes for 15 minutes. The tubes were centrifuge for 10 minutes at 10,000g (13000rpm). The process was repeated but the time of mixing was 3 minutes and time of centrifugation was 5 minutes at the same speed as above. Upper aqueous layers were removed into clean tubes and 40µl NaAc was added followed by 260µl of cold isopropanol. This was gently mixed by inverting tubes. The tubes were incubated at -20°C overnight. On the second day, the mixture was centrifuged at 10,000g (13000rpm) for 10 minutes. The supernatant was discarded and pellets rinsed with 70% alcohol and mixed for sometimes. This procedure was repeated three times. After discarding the supernatant, the sample was dried in a dryer for 20 minutes at room temperature. Pellets were resuspended in 30µl TE. DNA concentration and quality was checked by observing the band on an ethidium-stained agarose gel (0.7%) using 0.2µl of each sample.

PCR amplification of the ITS region

The entire region of ITS4 and ITS5 were amplified by PCR using the universal primers (Gardes and Bruns 1993). The reaction mix was made up to a total volume of 25 µl, composed of 23 µl of *Taq* polymerase "Ready to Go" mixture (Pharmacia, mention place first time) with 0.2 µl of each primer (100 pM) and 2 µl of DNA solution (here you need to give the concentraion,

even if only roughly). The tubes were placed in a thermal cycler (GenAmp PCR System 2400, Perkin–Elmer, place) for amplification under the following conditions: 30 cycles of (1) denaturation at 95°C for 30 s, (2) annealing at 50°C for 1 min, (3) extension at 72°C for 1 min were these steps repeated?. The amplification products were purified using a PCR Purification Kit (company?) and electrophoresed on ethidium-stained agarose gel (0.7%) to check the purity. DNA sequencing was performed using the primers the same primer pair used in the PCR reactions (ITS 4 and ITS 5) in an Applied Biosystem DNA Analyser.

Alignment of sequence

Alignments (pairwise and multiple alignment) were performed with the Clustal W package (Thompson *et al.* 1997). The aligned sequences were corrected manually, focusing on gap positions. DNA sequence data were analyzed to provide pairwise percentage sequence divergence. The data obtained from the sequence alignment were used to plot a tree diagram (MEGA 4 Software).

Results

The ITS primer produced single PCR product for the *Lenzites* species designated specimens 1 to 6 with sequence length that ranged from 600 to 620bp (Table 1). The percentage level of relationship of the six *Lenzites* species collected from Nigeria to existing *Lenzites* species ranged from 99 to 100% (Table 2). The results of the ITS gene sequence obtained from NCBI GenBank BLAST discriminated between all the *Lenzites* species. *Lenzites* species designated specimen 1 collected from Nigeria was 100% to homologous *Lenzites* species with ascension number JX082361.1 collected from French Guiana (Table 2). The phylogenetic tree generated placed specimens 1, 3 and 6 in the same clade with *Lenzites* species and *Daedaleopsis flavida* (Fig. 1).

Table 1 Length of ITS region of *Lenzites* species collected in Akure, Nigeria.

Code	Tentative Identity	Length of ITS Region (bp)
Specimen1	<i>Lenzites</i> species	610
Specimen2	<i>Lenzites</i> species	620
Specimen3	<i>Lenzites</i> species	600
Specimen4	<i>Lenzites</i> species	600
Specimen5	<i>Lenzites</i> species	600
Specimen6	<i>Lenzites</i> species	610

Bp: Base pair

Table 2 Genomic identification obtained from NCBI BLAST search based on the ITS rDNA sequences of *Lenzites* species collected in Akure, Nigeria.

Code	Tentative Identity	Culture of Closest relative	Ascension number of relative	Origin	% Level of relationship
Specimen1	<i>Lenzites</i> species	BRFM 1079	JX082361.1	French Guiana	100
Specimen 2	<i>Lenzites</i> species	BRFM 1079	JX082361.1	French Guiana	99
Specimen3	<i>Lenzites</i> species	BRFM 1079	GU731566.1	Marseille	99
Specimen4	<i>Lenzites</i> species	BRFM 1079	JX082361.1	French Guiana	99
Specimen5	<i>Lenzites</i> species	BRFM 1079	JX082361.1	French Guiana	99
Specimen6	<i>Lenzites</i> species	BRFM 1079	JX082361.1	French Guiana	99

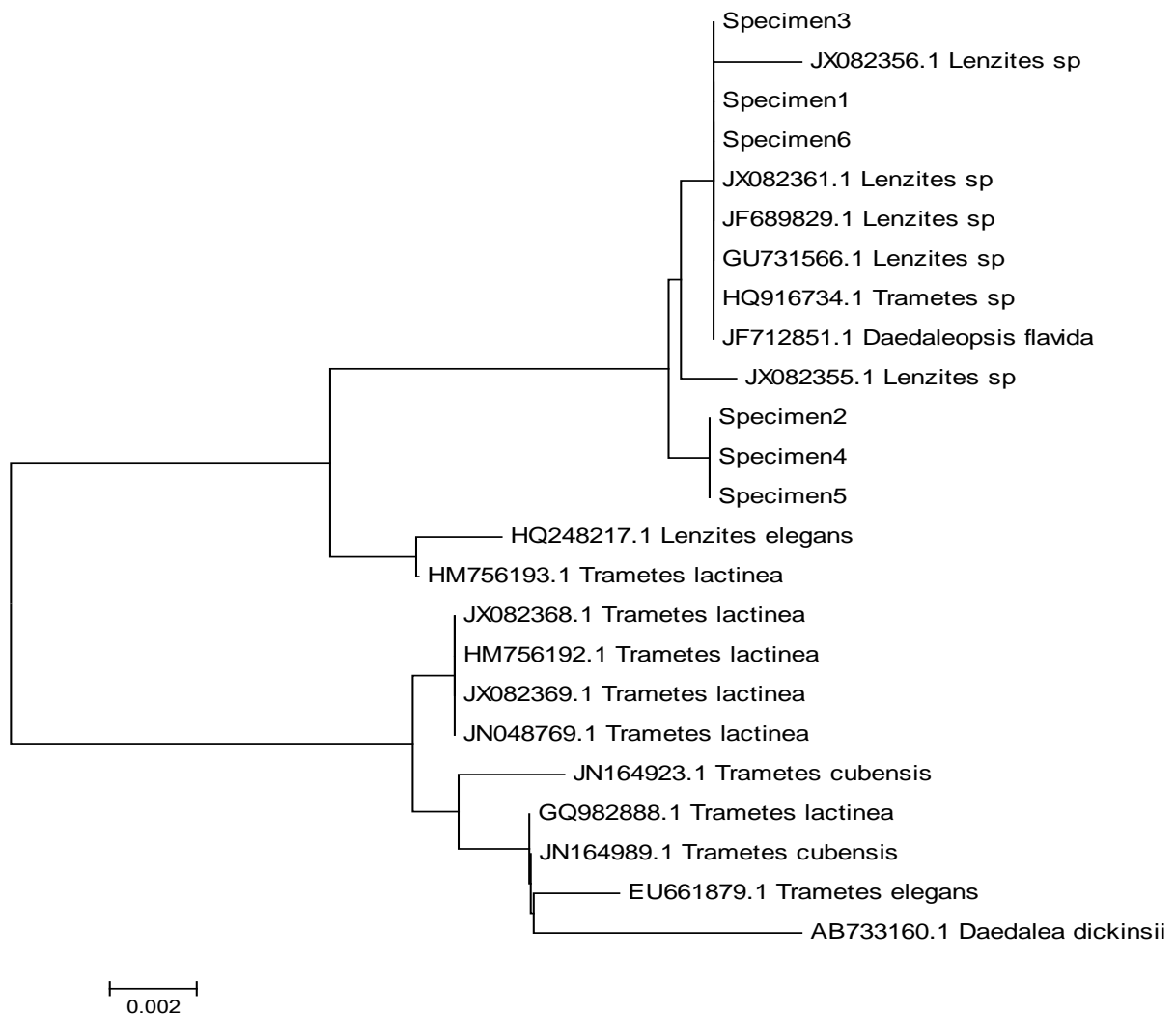


Fig. 1 - Phylogenetic tree of the Genus *Lenzites* collected from Nigeria based on nucleotide sequences of the ITS region.

Discussion

Correct identification of macrofungi had been a challenge in most developing Countries. This is as a result of the use of morphological description mostly employed by mycologist in this part of the world. It has been observed that morphological description have limitation in not allowing a reliable distinction of intraspecific characteristics (Seo and Kirk 2000). Molecular techniques could be used to adequately characterize and identify intra and inter specific characteristics (Zakaria *et al.* 2009). Hence, a molecular technique was employed in this study to resolve conflicting data from morphological characteristics in the identification of *Lenzites* species collected from Nigeria.

The sequence length obtained ranged from 600 to 620bp (Table 1). Min and Hickey (2007) had earlier reported that the standard short barcode sequences (~600 bp) are sufficient for species identification among the fungi. It has also been reported that Ribosomal DNA (rDNA) sequences provides a wealth of information concerning phylogenetic relationships (Hillis and Dixon 1991), and studies of rDNA sequences have been used to infer phylogenetic history across

a very broad spectrum, from studies among the basal lineages of life to relationships among closely related species and populations (Yang 2011). In the present study, sequences obtained from rDNA of the six macrofungi specimens collected in Nigeria tentatively confirm the identity as *Lenzites* species collected from Nigeria and the relationship with existing *Lenzites* species from other parts of the world (Table 2). It is a well known fact that members of the polyporoids are similar morphologically. However, molecular studies can help to clarify the higher level of and generic relationship of polyporoid (Justo and Hibbett 2011).

Daedaleopsis has been described as synonym of *Lenzites* (Kirk *et al.* 2008). In a study, Justo and Hibbett (2011) proposed that the genera *Artolenzites*, *Corioloopsis*, *Coriolus*, *Cubamyces*, *Cyclomycetella*, *Lenzites*, *Poronidulus*, *Pseudotrametes* and *Pycnoporus* are considered synonyms of *Trametes*. The phylogenetic tree (Fig. 1) generated also revealed that *Lenzites* specimens 1 to 6 form a monophyletic group with existing *Lenzites* species obtained from NCBI GenBank. The monophyletic nature of specimens 1 to 6 and *Lenzites* species from the GenBank indicates that they are from the same ancestral stock. *Lenzites* species designated specimens 2,4 and 5 form a separate clade within the monophyletic tree housing the *Lenzites* species. These three *Lenzites* species (specimens 2,4 and 5) may be new species of *Lenzites*. Earlier information on *Lenzites* species shows that it is a genus of fungi in the family Polyporaceae. It was circumscribed by Elias Magnus Fries in 1835. *Lenzites* species is widespread and it is made up of six species (Kirk *et al.*, 2008).

Conclusively, phylogenetic analysis of the ITS region of the rDNA of specimens 1 to 6 clearly resolved the identity of macrofungi designated specimens 1 to 6 collected from Nigeria as *Lenzites* species. Moreover, the percentage relationship of these *Lenzites* species (specimens 1 to 6) and their relationship with *Lenzites* species from other parts of the world was also revealed.

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