

***TOMOPHAGUS CATTIENENSIS* SP. NOV., A NEW GANODERMATACEAE
SPECIES FROM VIETNAM: EVIDENCE FROM MORPHOLOGY
AND ITS DNA BARCODES**

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Ganoderma Karsten and allies (Ganodermataceae Donk, Polyporales, Agaricomycetidae) are a group of polypore fungi of significant economic importance. Several species cause severe losses to perennial crops in many tropical countries (Flood *et al.* 2000) and some are of great interest for medicinal and pharmaceutical purposes, particularly in Eastern Asia (Jong & Birmingham 1992, Lin & Zang 2004). Members of the Ganodermataceae are characterized and easily recognized by the unique presence of double-walled basidiospores. The monophyly of the group was confirmed in molecular phylogenetic analyses (Hibbett *et al.* 1997, Moncalvo *et al.* 2002). However, both generic and specific circumscription within the family have been difficult and controversial (Furtado 1965, Steyaert 1972, 1980, Corner 1983, Zhao 1989, Moncalvo & Ryvardeen 1997, Moncalvo 2000). Murrill (1905a,b) created the genus *Tomophagus* to segregate *G. colossus* (Fr.) C.F. Baker from *Ganoderma* based on its unusually thick and pale context that becomes soft and light when dry. *Tomophagus* was not accepted by Furtado (1965), Steyaert (1972, 1980), Corner (1983), Ryvardeen (1991), and many other authors. Steyaert (1980) even suggested that *G. colossus* could be a tropical variant of the temperate western North American species *G. oregonense* Murrill that also has a soft and pale context. However, recent molecular phylogenetic studies that used sequence data from the internal transcribed spacers of the nuclear ribosomal DNA repeats (ITS rDNA; Moncalvo *et al.* 1995, Moncalvo 2000) and nearly complete mitochondrial small-subunit ribosomal DNA sequences (Hong & Jung 2004) indicate that *G. colossus* and *G. oregonense* are distantly related, supporting the recognition of *Tomophagus* as a distinct genus.

Tomophagus colossus was described by Fries (1851) from Costa Rica as *Polyporus colossus* and later transferred to *Ganoderma* by Baker (1918). It is a rare species but it has been reported throughout the tropics, except from East Africa (Ryvardeen & Johansen 1980, Ofodile *et al.* 2005). In Vietnam, *T. colossus* was first recorded by Patouillard (1897) as *G. obokense* Pat., a species described from Somalia and later synonymized with *G. colossus* (Furtado 1965, Steyaert 1972, Ryvardeen & Johansen 1980, Ryvardeen 2004). This rare species was only recently rediscovered in Vietnam (Ngo Anh *et al.* 2001). In this paper we report a second species of *Tomophagus* from Cat Tien National Park in southern Vietnam (Cat Tien is a designated UNESCO Biosphere Reserve; The MAB Programme 2007). The new species reported here is distinguished from *T. colossus* on the basis of combined evidence from morphology, cultural characteristics, and ITS rDNA barcodes.

I. METHODS

Organisms studies and morphological examinations: Four *Tomophagus* collections were obtained from Vietnam between 2000 and 2008. Macroscopic and anatomical examinations of basidiomata were made as described in Le Xuan Tham (1998). Basidiospore structure was described according to Pegler & Young (1973) and Perreau (1973). Data were compared with the detailed description of the type specimen of *T. colossus* in Steyaert (1972), Ryvar den & Johansen (1980), and Ryvar den (2004). Descriptions of this species in Corner (1983), Gilbertson & Ryvar den (1986), and Wu & Zhang (2003) were also consulted.

Cultivation: Context tissues from fresh collection of strains CT99, CT119, HCMC10 and ANH s.n. were used for isolation in pure culture in a homemade broth of Potato-Glucose-Agar (PGA: Le Xuan Tham *et al.* 1999). Mycelial characteristics were observed on that medium, particularly for the presence/absence of chlamydospores as reported in Peng (1990). *In vitro* basidioma induction was conducted at room temperature on rubber tree sawdust supplemented with 5% rice bran in 2.5 L plastic bags (Le Xuan Tham *et al.* 1999).

DNA analyses: ITS sequences from collecting ANH and HCMC10 were produced at the Royal Ontario Museum. Sequences from CT99 and CT119 were produced at the DNA sequencing facility of the Institute for Microorganism and Biotechnology, National Hanoi University. A sequence of *T. colossus* CBS 216.36 (Philippines) was retrieved from Moncalvo *et al.* (1995). A BLAST search in the NCBI database using TRTC157076 as the query sequence was used to identify additional sequences from closely related taxa to include in our dataset. Taxa used for DNA analyses are listed in Table 1, along with their source and GenBank accession numbers. Sequences were aligned manually and analyzed in PAUP* (Swofford 2003) using maximum parsimony and 1,000 nonparametric bootstrap replicates, with 100 heuristic searches of random addition sequence and TBR branch-swapping (MP). All gaps in the alignment consisted of single deletion/insertion and were treated as a "fifth base" in our analyses. Trees were midpointrooted.

II. RESULTS

DNA analyses: BLAST searches returned 99% similarity to *Ganoderma* sp. kk-02 (AJ749970) from India (Sharma *et al.* 2005), and < 92% similarity to other *Ganoderma* sequences in the database. Therefore, we only included AJ749970 in our molecular analyses. After removing residual SSU and LSU sequences at the 3'- and 5'- end, respectively, ITS sequences were aligned in 558 positions. Thirty-five characters were variable, of which 27 were parsimony-informative. Maximum-parsimony analysis returned a single tree of length 35 (CI=1.000, RI=1.000). Nonparametric bootstrapping strongly supported (100%) the reciprocal monophyly of *T. cattienensis* sp. nov. and *T. colossus* (Fig. 1).

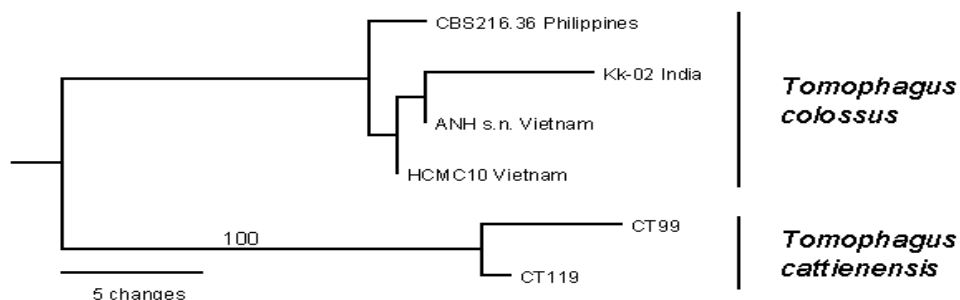


Figure 1: Phylogenetic relationship between *T. cattienensis* and *T. colossus* races, based on rDNA

Taxonomy

***Tomophagus cattienensis* Le Xuan Tham & J.M. Moncalvo, spec. nov. (Fig. 2)**

MycoBank no. MB 561806

Etymology: Named after the locality of the original collections: Cat Tien National Park, Vietnam.

Holotype: Pham Ngoc Duong & Le Xuan Tham, collection CT99; 6 June 2007; on the trunk of a broad leaf tree, Cat Tien National Park, Dong Nai Province, Vietnam; voucher deposited in the mycology herbarium of the Cat Tien National Park; fragments (*isotypes*) in the Royal Ontario Museum, Toronto, Canada (TRTC 161191), and in the National Museum of Natural Science, Taichung, Taiwan.

Distinctus ex Polyporus colossus Fr. *quo lux lucis rutilus frons superficies, leviter congelata, contextu frons si quando siccus, quod amplius basidiosporae. Ordines genorum ITS competentes ordinum depositorum NCBI (GenBank) JN184397.*

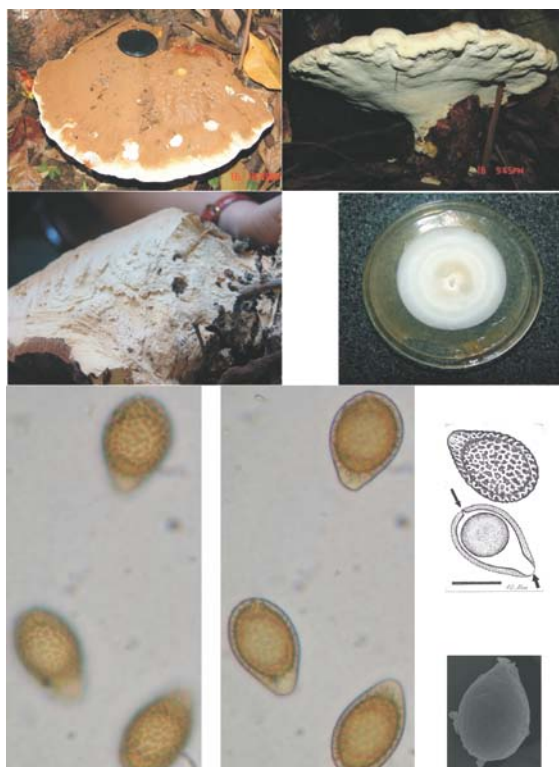


Figure 2: Holotypus of *T. cattienensis* (fruitbody, basidiospores and cultured mycelia)

Pileus bulky, thick, flabelliform, dimidiate (~29 cm in diameter and ~16 cm long) with a very short cylindrical stipe, perennial, occurring singly on rotting trunks of hardwood trees. Crust red-brown or red-coffeaate, laccate at first, but later becoming dull brown by a dusty covering of basidiospores, thin, easily broken when cracking or pressing with fingernails. Margin obtuse, rugose, very thick (~3-4 cm), white creamy or pale yellow brown. The crust of the stipe is thicker, more laccate, glossy red-brown. *Context* thick ~3.7-7.7 cm, up to 11 cm at the base, homogeneous (without stratifications), soft, light, creamy white becoming pale brown upon drying, somewhat cheesy and powdery, slightly striate above the tubes, composed of dimitic hyphae. *Tube layer* thin (0.8-1.4 cm), grey brown to cinnamon brown, unstratified. *Pores* round or angular, small (2-3/mm) with the surface creamy turning pale grey or dark grey when touched. *Basidiospores* typically ganodermoid, honey yellow, 17.5-21.5 x 11.5-14.5 µm, truncate-ovate with a yellow round

guttule in the center. Basidiospore surface coarse verrucose, pits connected with each other or not completely reticulate (labyrinthine) with thin pillar layer from inner wall. Apex (= germ pore aperture) hyaline, very thick, concave (truncate) or convex, opposite to the hilum (= the attachment of spore to sterigma). ITS sequence accession number of the type specimen in the NCBI (GenBank) database: JN184397.

Additional specimens examined: Vietnam: Dong Nai Province, Cat Tien National Park, on the trunk of broad leaf tree. 2 August 2008, Pham Ngoc Duong & Le Xuan Tham, coll. CT119; deposited in the mycology herbarium of the Cat Tien National Park. ITS sequence accession number in the NCBI (GenBank) database: JN184398.

***Tomophagus colossus* (Fr.) Murrill**

The two collections of *T. colossus* in Vietnam examined in this study (Table 1) fully correspond to the morphological descriptions of the type specimen in Steyaert (1972), Ryvardeen & Johansen (1980), and Ryvardeen (2004). In addition, the collection of ANH s.n. was described in detail in Ngo Anh *et al.* (2001). This species is easily recognized from its thin yellow crust and a pale and chalky context. It is rarely found but widespread in the tropics. It was reported in the Neotropics including Mexico, Florida, and Brazil (Steyaert 1972, Gilbertson & Ryvardeen 1986, Gomes-Silva *et al.* 2011), tropical Africa (Steyaert 1972, Ryvardeen & Johansen 1980), the Arabian Peninsula (Al Bahry *et al.* 2005), India, Pakistan and Sri Lanka (Steyaert 1972), Malaysia and Indonesia (Corner 1983), and South China and Taiwan (Wu & Zhang 2003). Our ITS analyses also indicate that the strain *Ganoderma* sp. kk-02 reported from India by Sharma *et al.* (2005) corresponds to *T. colossus*. Based on the literature cited above, *T. colossus* has a wide host range on woody angiosperms and monocotyledons. Wide host range and pantropical distribution of a fungal morphotype is uncommon. Future comprehensive studies of this species using mating and molecular data from a broad geographic sampling may reveal the existence of cryptic species within this unique pantropical morphotype.

Distinction between *T. cattienensis* and *T. colossus*

Wild collections of *T. cattienensis* clearly differ morphologically from *T. colossus* by having glossy light red-brown basidiomata (rather than yellow), a slightly harder crust, a context that turns pale brown upon drying (instead of remaining creamy white), and slightly larger basidiospores (17.5-21.5 x 11.5-14.5 μm versus 14-20 x 9-14 μm). These characteristics are retained in basidiomata produced in artificial cultivation (Fig. 3). In artificial culture, basidiomata of *T. cattienensis* isolates develop and mature in 60-75 days, which is 20-25 days longer than for our *T. colossus* strains. We also observed the difference between the two species in the shape of chlamydospores produced in cultures grown on PGA medium (Fig. 3). *Tomophagus cattienensis* also grows more slowly than *T. colossus* on PGA medium.

Phylogenetic analysis of ITS rDNA barcodes clearly distinguishes between the two species (Fig.1). MP analyses yielded a single tree and indicated 100% bootstrap support for the distinction between *T. cattienensis* and *T. colossus*. Pairwise inter-specific difference was observed in 27- 33 nucleotide positions (4.83 - 5.91%), whereas intra-specific divergence was much lower: the two *T. cattienensis* collections from Vietnam differ in only 6 nucleotide positions (1.08%), and our four Asian samples of *T. colossus* differ in 1-10 positions (0.18-1.8%). These values for intra- and inter-specific variation in fungal ITS sequences are in agreement with the study by Nilsson *et al.* (2008). The present work therefore confirms the view that ITS can be a good molecular marker (DNA barcode) for species identification in fungi (Seifert 2009), although it may not work in all taxa (Seifert *et al.* 2007, Nilsson *et al.* 2008).

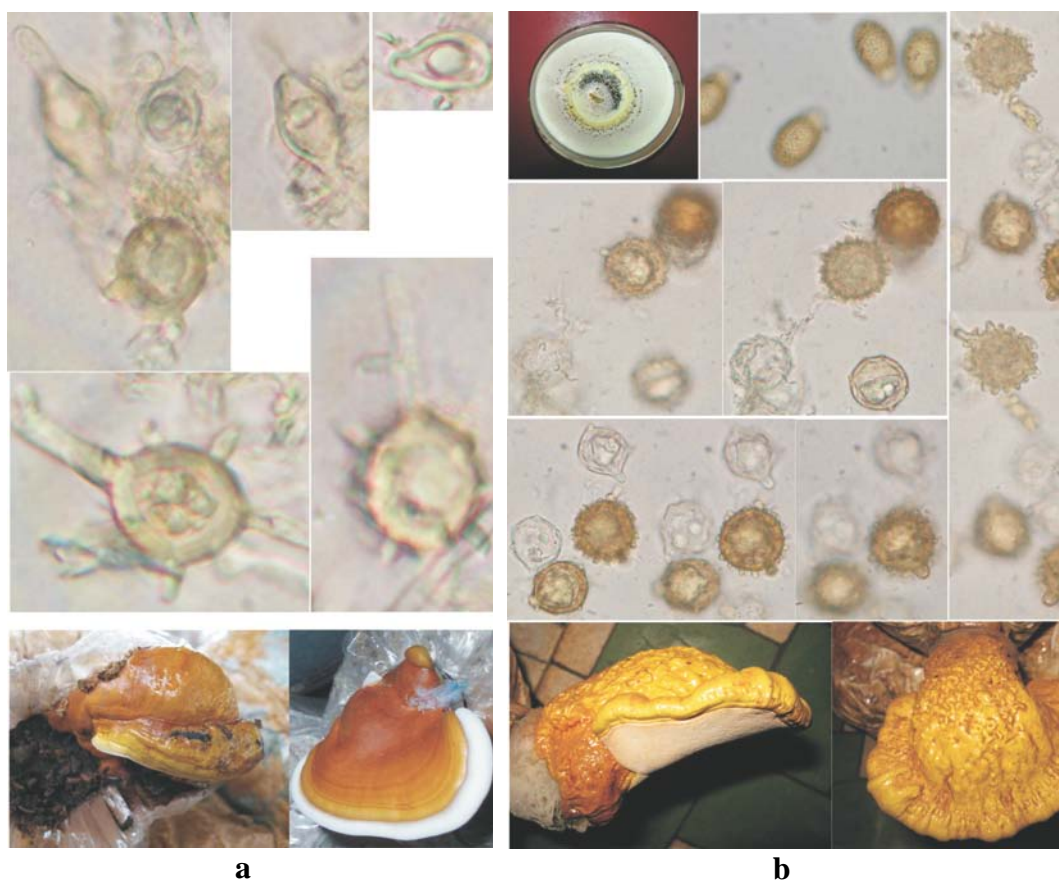


Figure 3: *Chlamydospores* and cultured fruitbodies of *T. cattienensis* (a) and *T. colossus* (b)

Practical implications of the discovery of *T. cattienensis*

Members of the Ganodermataceae have been shown to be pharmacologically active and their therapeutic use is being investigated (Dzubak *et al.* 2006, Liby *et al.* 2007). Recent studies have reported several new lanostane triterpene lactones (“colossolactones”) from the cultivated *T. colossus* strain ANH s.n. from Vietnam (Kleinwachter *et al.* 2001, El Dine *et al.* 2008). Colossactones were also present in a Nigerian strain of that species (Ofodile *et al.* 2005). *Tomophagus colossus* is also known for its excellent delignification activity (Adaskaveg *et al.* 1990, 1995). Therefore, the discovery of a novel species of *Tomophagus*, *T. cattienensis*, may have implications for the discovery of novel bioactive compounds for pharmaceutical use and/or the pulp and biofuel industries.

Acknowledgments: We thank Dr. Ngo Anh (University of Hue, Vietnam) for providing us with a specimen of *T. colossus* from Thua Thien Hue. Dr. Dao Thi Luong (National University of Hanoi) and Ms. Leena Rivzi (Royal Ontario Museum and University of Toronto) produced DNA sequence data. Mycological research at Cat Tien National Park was supported by a grant from the Dong Nai Province Department of Science and Technology to LXT. This work was also supported from the Natural Science and Engineering Research Council and Genome Canada for funding the Canadian Barcode of Life Network, and a ROM Fieldwork grant, to JMM.

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**TOMOPHAGUS CATTIENENSIS SP. NOV., MỘT LOÀI LINH CHI
GANODERMATACEAE MỚI Ở VIỆT NAM: BẰNG CHỨNG HÌNH THÁI HỌC
VÀ TRÌNH TỰ ITS DNA**

**LÊ XUÂN THÁM, NGUYỄN LÊ QUỐC HÙNG, BÙI HOÀNG THIÊM, PHẠM NGỌC DƯƠNG,
DƯƠNG VĂN HỢP, BRYN T. M. DENTINGER, JEAN-MARC MONCALVO**

TÓM TẮT

Chi *Tomophagus* được xác lập tách biệt khỏi chi *Ganoderma* với một loài đặc sắc, *Ganoderma colossus*. Các dẫn liệu sinh học phân tử gần đây xác nhận tính hữu hiệu của chi đơn loài này. Chúng tôi báo cáo phát hiện một loài thứ hai của *Tomophagus*, *T. cattienensis* sp.nov., ở Vườn Quốc gia Cát Tiên, Nam Việt Nam, một vùng rừng thấp - Khu Dự trữ sinh quyển của UNESCO. Việc công nhận loài mới này dựa trên bằng chứng hình thái học, phân tử, các đặc trưng nuôi cấy và trình tự ITS rDNA. Việc phát hiện loài mới này có triển vọng tìm được các hoạt chất mới cho ứng dụng dược học và cho công nghiệp giấy.