

# A phylogenetic study of commercial Chinese truffles and their allies: Taxonomic implications

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## Abstract

Phylogenetic relationships of commercial Chinese truffles and their allies were investigated mainly by morphological studies and analyses of the sequences of ITS regions of nuclear ribosomal DNA. Two species, *Tuber indicum* and *T. himalayense*, closely related to the European *T. melanosporum* (the Perigord Truffle), are recognized among commercial Chinese black truffles. Both *T. pseudohimalayense* and *T. sinense* should be regarded as synonyms of *T. indicum*. *Tuber* species producing excavated ascomata are not monophyletic, suggesting that excavation of ascomata may have evolved more than once, or evolved once during the evolution of truffle species and then was lost once during the evolution of *Tuber* species.

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## 1. Introduction

Truffles are very renowned and are of high economic importance because of their culinary value. Although the Chinese truffles, resembling *Tuber melanosporum* Vitt. (the European prized Perigord Truffle), are inferior in taste and odor, truffles exported from China to Europe have increased dramatically since about 1993 [1]. For a long time, commercial Chinese truffles were lumped as *T. indicum* Cooke and Masee [2], but in recent years, several new species have been recognized: *T. himalayense* Zhang and Minter [3], *T. pseudohimalayense* Moreno et al. [4], *T. sinense* Tao and Liu [5]. The taxa mentioned above are similar to each other and it seems to be challeng-

ing to identify them based on subtle morphological characters alone. Previous molecular studies [6–10] have repeatedly found two main monophyletic groups or haplotypes (A and B) among the Chinese truffles identified as *T. indicum*. It was unclear whether such a simple, two-part partitioning was related to differences in populations of diverse collecting sites as proposed by Paolucci et al. [7] or the partitioning reflected repeated misidentifications of several taxa as *T. indicum* [8]. Another opinion is that most of the truffles exported from southwestern China to Europe similar to *T. melanosporum* and regarded as *T. indicum* or *T. himalayense* were *T. sinense*, while *T. pseudohimalayense* was considered to be very similar to *T. sinense* [1]. These controversial views made it worthwhile to clarify the taxonomy of commercial Chinese truffles similar to *T. melanosporum*.

*T. pseudoexcavatum* Wang et al., another newly published commercial species from China, is macroscopically

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similar to *T. excavatum* Vitt. and *T. mesentericum* Vitt. from Europe [1,11]. These three species are characterized by their deeply excavate ascomata (with an evident basal cavity), but their phylogenetic relationships have never been considered.

In this study a phylogenetic investigation of commercial Chinese truffles (including *T. indicum*, *T. himalayense*, *T. pseudohimalayense*, *T. sinense* and *T. pseudoexcavatum*) and their relative taxa was conducted based both on morphological and molecular analyses. Parsimony, likelihood and distance inferences were applied on a large number of internal transcribed spacer (ITS) sequences of Chinese truffles with different geo-

graphical origins in order to evaluate their relationships, the interspecific and/or intraspecific divergence in the *T. indicum* “complex,” and to propose phylogenetic relationships between *T. pseudoexcavatum* and phenetically similar species.

## 2. Materials and methods

### 2.1. Morphological studies and sample source

Standard techniques were employed [11,12] for macro-morphological and anatomical studies. Types of

Table 1  
Taxa included in DNA analysis

Taxon	Voucher	Geographic origin	GenBank	
<i>Tuber indicum</i> in Group A	HKAS 39501	Kunming, Yunnan, China under <i>Pinus armandii</i>	<sup>b</sup> AY514305	
	HKAS 39506	Chuxiong, Yunnan, China under <i>Pinus yunnanensis</i>	<sup>b</sup> AY514306	
	HKAS 39515	Kunming, Yunnan, China	<sup>b</sup> AY514307	
	HKAS 39516	Kunming, Yunnan, China	<sup>b</sup> AY514308	
	HKAS 39507	Gongshan, Yunnan, China	<sup>b</sup> AY514309	
	HKAS 38933	Kunming, Yunnan, China under <i>Pinus yunnanensis</i>	<sup>b</sup> AY773357	
	–	Imported from China	Y09791	
	–	Imported from China	Y09792	
	–	Imported from China	AF106881	
	–	Imported from China	AF300822	
	–	Imported from China	AF300824	
	–	Imported from China	U89362	
	<i>T. indicum</i> in Group B [ <i>T. himalayense</i> ] <sup>a</sup>	–	Huili, Sichuan, China	AF132502
		–	Imported from China	U89360
–		Imported from China	AF106882	
–		Imported from China	AF106883	
–		Imported from China	AF300823	
–		Imported from China	AF106884	
–		Imported from China	AF132503	
<i>T. himalayense</i> [ <i>T. indicum</i> ] <sup>a</sup>	–	Huidong, Sichuan, China	AF132504	
<i>T. himalayense</i>	HKAS 25689	Huize, Yunnan, China under <i>Pinus yunnanensis</i>	<sup>b</sup> AY773356	
<i>T. pseudohimalayense</i> ? [ <i>T. indicum</i> ] <sup>a</sup>	–	Imported from China	U89361	
<i>T. melanosporum</i>	–	Italy	AF106873	
	–	Italy	AF106874	
	–	Italy	AF106875	
	–	Italy	AF106876	
	–	Spain	AF106877	
	–	France	AF106878	
	–	France	AF106879	
	–	France	AF132501	
	–	Unknown	AF167096	
	–	Unknown	AF167097	
	–	France or Italy	AF300825	
	–	France or Italy	AF300826	
	–	France or Italy	AF300827	
–	Unknown	U89359		
<i>T. pseudoexcavatum</i>	HKAS 39504	Chuxiong, Yunnan, China	<sup>b</sup> AY514310	
<i>T. excavatum</i>	–	Hungary	AJ557545	
<i>T. mesentericum</i>	–	Italy	AF106887	
<i>T. brumale</i>	–	Unknown	AF106880	
	–	Unknown	AF132504	
<i>T. brumale</i> f. <i>moschatum</i>	–	Unknown	AF001010	
<i>T. borchii</i>	–	Unknown	AF132505	
	–	Unknown	AJ557538	
–	Unknown	AJ557536		

<sup>a</sup> Recent epithets denoted within brackets are according to our conclusions.

<sup>b</sup> Sequences obtained in this study. The others were from GenBank.

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