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REVIEW

A review of research on Chinese *Tuber* species

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Abstract Truffles are abundant in some regions of China. Nevertheless, it was not until the 1980s that *Tuber* species were discovered by Chinese mycologists. In recent years, international truffle markets have shown an increasing interest in the import of Chinese *Tuber*. These truffles serve as a complement to European truffles due to their lower prices and their greater availability in a deficit market. However, Chinese truffles have been the subject of fraudulent commercial practices, and these could have a negative effect on truffle culture. These concerns have been behind numerous recent studies designed to obtain detailed information about Chinese *Tuber* species. Unfortunately, many of these studies are not published in English, and are dispersed throughout the specific local or national bibliography and proceedings of specialized truffle conferences. In view of the need to expand current knowledge of Chinese *Tuber* species, we present a comprehensive summary of the taxonomy, ecology, mycorrhizae, genetics, biochemistry, and cultivation of Chinese *Tuber* species. We also provide a synthetic taxonomy and morphological characterization of 16 Chinese *Tuber* species in order to assist in their verification and monitoring.

Keywords Chinese *Tuber* · Ectomycorrhizae · Mushroom yield · Truffle culture · Truffle ecology

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Introduction

Truffles (*Tuber* Micheli ex Wiggers) are an important resource in some regions of China (Wang et al. 1998; Wang 2008). In the last 14 years, there have been regular shipments of Chinese truffles from China to Europe, Japan, United States, and Australia (Rey 2001; Wang and Hall 2001; Yamanaka et al. 2001; Yang 2001). Rey (2001) states that Chinese truffle exports are increasing in popularity, as is the consumption of these truffles worldwide, due in part to the fact that they are three to five times cheaper than European truffles. He further explains that Chinese truffles act as a complement to the market for *T. melanosporum* Vittad. (Périgord black truffle) and *T. aestivum* Vittad. because they are cheaper, have a milder aroma, and the carpophores are of good quality for culinary purposes.

Chinese truffles have been the subject of fraudulent commercial practices in European and Japanese markets, and have been incorrectly marketed as *Tuber melanosporum* and other truffle species. Moreover, the importation of large quantities of Chinese truffles could have a negative effect on truffle cultivation in these countries (García-Montero et al. 1997a, 2008; Di Massimo et al. 1998; Yamanaka et al. 2000, 2001; Ferrara and Palenzona 2001). This scenario has stimulated numerous recent studies designed to obtain detailed information on Chinese truffles versus European truffles. Unfortunately, many of the studies on Chinese truffle that might be useful to international truffle culture are not published in English and are dispersed throughout the specific local or national bibliography and proceedings of specialized truffle conferences.

Chen and Gong (2000) stated that 26 *Tuber* species have been recorded in China since 1985. Wang (2008) indicated that 24 species of *Tuber* have been found in China in the last 20 years, half of which are new species. Ren et al. (2005) have recently proposed the first literature reviews of *Tuber* species located in China, and they present 17 Chinese *Tuber* species. Although truffles have been found in many provinces and regions (Yunnan, Sichuang, Xizang, Xinjiang, Shanxi, Hebei, Beijing, Liaoning, Jilin, Inner Mongolia, Hubei, Hunan, Gansu, and Taiwan) throughout the country, their distribution is mostly limited to the regions which have been searched. Wang et al. (2007) subsequently reported that at least 31 *Tuber* species have been described from China in recent years; however, he has focused his work on only 17 Chinese *Tuber* species and debates the validity of these species. Ren et al. (2005) and Wang et al. (2007, 2008a, b) also list 18 European and American *Tuber* species that have been cited in China (in alphabetical order): *T. aestivum* Vittad., *T. asa* (Lespiault) Tul. and C. Tul., *T. borchii* Vittad., *T. brumale* Vittad., *T. californicum* Harkn., *T. dryophilum* Tul. and C. Tul., *T. excavatum* Vittad., *T. foetidum* Vittad., *T. lyonii* Butters, *T. maculatum* Vittad., *T. melanosporum*, *T. nitidum* Vittad., *T. oligospermum* (Tul. and C. Tul.) Trappe, *T. puberulum* Berk. and Broome, *T. rapaeodorum* Tul., *T. rufum* Pico, *T. shearii* Harkn. and *T. texense* Heimsch. However, they indicate that the validity of several taxa is questionable (*T. borchii*, *T. brumale*, *T. californicum*, *T. dryophilum*, *T. excavatum, T. nitidum*, *T. oligospermum*, *T. rapaeodorum*, *T. rufum, T. shearii* and *T. texense*). Moreover, Chen et al. (2008) have confirmed the presence of *T. excavatum* and *T. borchii* var. *sphaerospermum* Malençon in China.

In view of the need to expand current knowledge of Chinese *Tuber* species, we present a discussion of the taxonomy, ecology, mycorrhizae, genetics, biochemistry, and cultivation of these species, and we analyze their impact on international truffle culture. We also provide a synthetic taxonomy and morphological characterization of Chinese *Tuber* species in order to assist in the verification and monitoring of Chinese *Tuber* species. We located and acquired several original articles written in Chinese through the Technical University of Madrid's (UPM) inter-library lending service. We used the taxonomic citations, the species distribution maps, their English abstracts, the descriptions in Latin of the new species, and the bibliographic references (written in Latin) from these original manuscripts written in Chinese. We have only located and analyzed the available scientific information on 24 Chinese *Tuber* taxa (in alphabetical order): *T. formosanum* Hu, *T. furfuraceum* Hu and Y. Wang, *T. gigantosporum* Y. Wang and Z. P. Li, *T. himalayense* B. C. Zhang and Minter, *T. huangshawanense* Wang, *T. huidongense* Y. Wang, *T. indicum* Cooke and Massee, *T. indicum* var. *yunnanense* Yamanaka, *T. latisporum* Juan Chen and P. G. Liu, *T. leptoperidium* Ren, *T. liaotongense* Wang, *T. liui* A. S. Xu, *T. mindense* Wang, *T. pseudoexcavatum* Wang, G. Moreno, L. G. Riousset, J. L. Manjón and G. Riousset, *T. pseudohimalayense* G. Moreno, Manjón, Díez and García-Montero, *T. sinense* Tao and Liu, *T. taiyuanense* Liu, *T. tianshanense* Tao, *T. turbinatosporum* Zhang, *T. umbilicatum* Juan Chen and P. G. Liu, *T. verrucosum* Ren, *T. xizangense* A. S. Xu, *T. zhongdianense* He, Li and Wang, and a new Chinese *Tuber* species located in Italy being sold designated with the code C-11.

Taxonomy

The first Asian truffle, *Tuber indicum*, was discovered near Mussoorie in the northwest Himalayas in India in the nineteenth century (Cooke and Massee 1892). Ninety-three years later, in the 1980s, five new *Tuber* species were discovered in Asia: a species from India, *T. himalayense* cited in China and four new Chinese species, *T. taiyuanense*, *T. tianshanense*, *T. liaotongense*, and *T. sinense*. Wang et al. (2007) indicated that *T. tianshanense* was described by Tao (1988) in his MSc thesis dissertation. This species was mentioned later by Liu and Liu (1994) and Chen and Gong (2000) but was never formally published, as pointed out by Ren et al. (2005).

In the 1990s, 10 Chinese *Tuber* species were proposed: *T. huangshawanense*, *T. mindense*, *T. turbinatosporum*, *T. gigantosporum*, *T. formosanum*, *T. pseudoexcavatum*, *T. pseudohimalayense*, a new Chinese *Tuber* in Italy (C-11 code), *T. liui*, and *T. xizangense*.

Tuber turbinatosporum was described by Zhang (1990) in his MSc thesis dissertation. However, Wang et al. (2007) explained that the holotype appears to have been lost, and this species was never formally published. Hu (1992) proposed *T. formosanum* from Dung-Pu Mountain in central Taiwan and Hu et al. (2005) report that this truffle is endemic to Taiwan. However, Ren et al. (2005) and

Wang et al. (2007) stated that no holotype was deposited in a public herbarium, and they indicate that *T. formosanum* is invalid. Wang et al. (2008a, b) remark that *T. formosanum* is an invalid species closely related to *T. indicum*, found only in Taiwan. Ren et al. (2005) and Wang et al. (2007) also reported that there are no valid descriptions for *T. huangshawanense* and *T. mindense*, and that holotypes were not deposited in public herbaria. Therefore, these species are invalid.

Wang et al. (1998) reported that, in 1995, J.L. and G. Riousset located an unknown Chinese *Tuber* species in a French market. This unknown species appeared to be new and conspecific with a new species that Y. Wang had found in Huidong, Sichuan, China, provisionally named *T. pseudoexcavatum* in his unpublished paper. This species was also found in the Spanish market by researchers at the University of Alcalá. These discoveries led the authors to agree to jointly publish the results (Wang et al. 1998). In Spain, Moreno et al. (1997) proposed *T. pseudohimalayense* sp. nov. Wang, and Hall (2001) indicated that *T. pseudohimalayense* was very similar to *T. sinense*. Zhang et al. (2005) and Wang et al. (2006b) proposed that *T. pseudohimalayense* should be regarded as a synonym of *T. indicum* based on morphological studies and molecular analyses. Manjón et al. (2009) have evaluated the taxonomic validity of *T. pseudohimalayense* species on the basis of genetic studies and the morphological comparison of ectomycorrhizae. They show *T. pseudoexcavatum* and *T. pseudohimalayense* belong to a single taxon, unrelated to *T. indicum*. In this regard, Wang et al. (2008a, b) indicated that *T. pseudohimalayense* and *T. pseudoexcavatum* have been proposed as co-species in the PhD thesis of Chen (2007).

From 2000 to the present, at least eight additional Chinese *Tuber* species have been proposed: *Tuber indicum* var. *yunnanense*, *T. huidongense*, *T. leptoperidium*, *T. verrucosum*, *T. zhongdianense*, *T. furfuraceum*, *T. umbilicatum*, and *T. latisporum*.

Tuber leptoperidium was described by Ren (2003), but never formally published. *Tuber verrucosum* was proposed by Ren (2003) as sp. nov, but without a valid description (*T. verrucosum* Pers. is not the species of *T. verrucosum* proposed by Ren; the latter name is invalid and a synonym of *T. pseudoexcavatum*).

Some of these authors report several phylogenetic relationships among Chinese truffles. Wang and He (2002) indicated that almost all *Tuber* species collected and described from Huidong County, such as *T. sinense*, *T. pseudoexcavatum*, *T. gigantosporum*, and *T. huidongense* have spiny-reticulate ascospores, which imply that they may be closely related phylogenetically.

He et al. (2004) indicated that *T. zhongdianense* ascospores closely resemble those of *T. gigantosporum*, also characterized by having very low ornamentation, which suggests that they may be closely related phylogenetically.

Finally, Hu and Wang (2005) supposed that *Tuber furfuraceum* resembles *T. huidongense* but differs in its yellowish, scurfy peridial surface and larger but narrower ascospores. Its ascospore length—width ratio of (1.3) 1.7 (2.3) µm—contrasts with that of *T. huidongense* of 1.1–1.4 µm. It is interesting that, although the Taiwanese black truffle, *T. formosanum*, resembles *T. indicum* and other black truffles from southwest China (Yamanaka et al. 2001), *T. furfuraceum* (from Taiwan) and *T. huidongense* (from China) fruit in the same habitats as their Taiwanese and Chinese black truffle associates. This supports the morphological evidence that *T. furfuraceum* and *T. huidongense* are closely related. Other work with Chinese *Tuber* species include the discovery of truffles in the Yunnan province by Pu (1989), and truffle harvesting in Sichuan by Wang (1995).

In this section, we present, in alphabetical order, taxonomic descriptions of each valid Chinese *Tuber* species, i.e., 16 species and varieties (Tables 1 and 2). All information is taken from published descriptions. Below, we provide a key for Chinese *Tuber* determination based on the features we have compiled for these species, after consideration of the *Tuber* key published by Pegler and Spooner (1993) (Fig. 1), and a synthetic ecology and mycorrhizae description of the Chinese *Tuber* taxa (Table 3).

Tuber furfuraceum Hu and Y. Wang

Hu and Wang (2005) proposed *Tuber furfuraceum* from Nan-Tou, Taiwan. This species has small brown ascomata (0.5–2.5 cm), rough with patches or stripes covered with a sort of yellowish scurfy substance, no hairs; faint odor, not distinctive; 340–480 µm thick peridium, two layers; the outer layer complex, 170–270 µm thick, with a crust of subglobose, brown cells, up to 22 µm, 2 µm thick-walled cells, below the outermost layer, with more or less globose cells, of 10–20 µm diam.; 170–210 µm thick inner layer, composed of colorless, interwoven hyphae of 3–4 µm in diam. with swollen cells, up to 10 µm, which gradually merge into the gleba tissue. Gleba brown; veins distinct, whitish, branched; asci (1) 2–4 (5) spored, stalked, up to 45 µm long; ascospores dark brown, ellipsoid, of (36) 43–46×23–27 µm (excluding ornamentation); spiny-reticulate with bent tips, irregular in mesh shape and size, meshes numbering (3) 4–5 (7) down and 3–5 across the spore with ornamentation up to 5 µm in height.

Table 1 Macroscopic characters of Chinese *Tuber* species (in alphabetical order)

Tuber species	Carpophore color	Carpophore size (cm)	Peridium	Peridium thickness (µm)	Gleba
T. furfuraceum	Brown	0.5–2.5	Rough with patches covered with a yellowish scurfy substance, no hairs	340–480	Brown, whitish veins
Paradoxa gigantospora (= *T. gigantosporum*)	Yellowish brown	1.5	Densely tomentose	190–230	Dark brown, white veins
T. himalayense	Black	2–2.5	Flattened warts	700–800	Graayish gleba, very thin white veins
T. huidongense	Yellowish brown with a red tint	0.5–2.5	Rough with scattered hairs	430–500	Purplish brown, veins white, branched
T. indicum	Black	1–7	Warts irregularly polygonal	550–700	Purplish black gleba, very thin whitish veins
T. indicum var. yunnanense	Black	2–8	Flattened warts	–	–
T. latisporum	Whitish	2–2.5	Conspicuously pubescent	200–500	Black, with a few whitish or creamy veins
T. liaotongense	Brownish yellow	0.6–2	Slightly warted	–	Gray lilac, with white veins
T. liui	Reddish brown	1.7–3.5	Pubescent	300–700	Brownish, white veins
T. pseudohimalayense (= *T. pseudoexcavatum*)	Brown to brownish orange	3–10	Wide and flat warts	290–500	Gray brown, white veins
T. sinense	Brown	Can be >10	Verrucose to pyramidal warts	550–1500	Pale grayish brown, cream to grayish white veins
T. taiyuanense	Brown	0.7–1.5	Glabrous	–	White external veins and brown internal veins
T. umbilicatum	Ochre	1.2–1.9	Smooth or minute papillae, with umbilicate depression	320–500	Purple brown or gray-brown, with pink tint at maturity, narrow branching white veins
T. xizangense	Reddish brown	1.7–1.2	Pubescent	250–330	Brownish, white veins
T. zhongdianense	Pale brown	Up to 1.5	Puberulent	220	Brown, white, branching veins

Tuber furfuraceum resembles *T. huidongense* but differs in its yellowish, scurfy peridial surface and larger but narrower ascospores.

Tuber gigantosporum Y. Wang and Z. P. Li

Wang and Li (1991) proposed *Tuber gigantosporum* from Huidong County, Sichuan province. This species has small yellowish brown ascomata (1.5 cm); densely tomentose; peridium of 190–230 µm of interwoven, colorless, hyphae of 3 µm in diam., with brownish pigment in the outer hyphae, surface cells developing into septate hairs up to 5 µm in diam., forming a crowded turf on the peridium surface; gleba solid, dark brown; marbled with white veins; 1 (2)-spored asci; ascospores ellipsoid (occasionally globose), brown to almost black, of 105–115 (120)×70–75 (80) µm (excluding ornamentation); ornamentation reticulate; with 7–8 meshes along their length, spines up to 4 (5) µm tall; 14 µm thick-walled.

He et al. (2004) confirmed *Tuber gigantosporum* from China. Wang et al. (2007) indicated that this species was probably invalidly published, and Wang and Hu (2008) have transferred this species from *Tuber* to *Paradoxa*. These authors indicated that *P. gigantospora* (Y. Wang and Z. P. Li) Y. Wang comb. nov. is distinct in that it invariably has only one spore per ascus, a defining character for the genus *Paradoxa*.

Tuber himalayense B. C. Zhang and Minter

Tuber himalayense is a species that was discovered by Zhang and Minter (1988) while studying the type collection of *T. indicum* in the herbarium of the Royal Botanical Garden at Kew. *Tuber himalayense* was also located by J. F. Duthie in Mussoorie, India. Zhang and Minter (1988) indicated that *T. himalayense* is very similar in external appearance to *T. indicum*. *Tuber himalayense* has small black ascomata (2–2.5 cm); however, it has less regularly flattened warts; 700–800 µm thick peridium, composed of

Table 2 Microscopic characters of Chinese *Tuber* species

Tuber species	Spore number in ascus	Ascospore color	Ascospore shape	Ascospore size[a] (μm)	Ornamentation height (μm)	Ornamentation
T. furfuraceum	2–4	Dark brown	Ellipsoid	43–46×23–27 e.o	Up to 5	Spiny-reticulate with bent tips, irregular meshes
Paradoxa gigantospora (= *T. gigantosporum*)	1	Brown to black	Ellipsoid	105–115×70–75	Up to 4	Reticulate
T. himalayense	2–4	Brown to dark reddish brown	Ellipsoid to globose	28–45×23–40 i.o	–	Variable, reticulate to spiny (tending to reticulate)
T. huidongense	2–4	Brown	Ellipsoid	37–40×28 e.o	Up to 6	Spiny-reticulate, with bent tips, irregular meshes
T. indicum	3–5	Darker	Ellipsoid	22–32×15–22 e.o	3–5	Spiny, slightly hooked, free, sparse
T. indicum var. yunnanense	2–5	–	–	28–35×19–25 e.o	4–6	Spiny, partial and irregular reticulation
T. latisporum	1–4	Yellowish brown to reddish brown	Ellipsoid	40–49×31–40 e.o	3–5	Alveolate reticulum
T. liaotongense	2–4	Brown	Ellipsoid to subglobose	29–40×26–35 i.o	–	Reticulate or alveolate-reticulate
T. liui	1–4	Brownish	Ellipsoid	60–78×40–55 i.o	3–8	Spiny-reticulate
T. pseudohimalayense (= *T. pseudoexcavatum*)	1–8	Brown to dark brown	Ellipsoid	24–28×16–19 e.o	Up to 5	Spiny-reticulate
T. sinense	1–4	Yellowish brown to brown	Ellipsoid	32–36×43–49 i.o	3–6	Spiny-reticulate
T. taiyuanense	2–4	Brown	Ellipsoid to globose	28–37×18–26	3.8–4	Spiny-reticulate, spines hooked
T. umbilicatum	1–4	Yellow brown	Ellipsoid	33–40×20–32 e.o	3–5	Spiny-reticulate
T. xizangense	1–3	Brownish	Ellipsoid	35–50×46–55 i.o	2–7	Spiny-reticulate
T. zhongdianense	1–2	Brown	Ellipsoid	50–63×38–44 e.o	2	Reticulate

[a] *io* Including ornamentation, *eo* excluding ornamentation

two layers; outer layer complex (200–500 μm including warts which are 200–400 μm high) with a crust of nearly globose, reddish black outermost cells, 1–3 μm thick-walled; immediately below, cells are paler and more elongated (20×10 μm) with thinner walls; below these are other thin-walled cells which are paler brown, less elongated, of 15–25 (30) μm, forming a textura angularis; inner layer (300–500 μm thick) with faintly pigmented, thin-walled, 3–5 μm cells, forming a textura intricata; gleba grayish, marbled with numerous, very thin, white veins; asci 2–4 (5)-spored, apparently lacking a stalk; ascospores ellipsoid to globose, brown to dark reddish brown, of 28–45 (50)×23–40 (45) μm (including ornamentation); with highly variable ornamentation, mostly reticulate, very irregular mesh shape and size, often with free spines mixed with the mesh; some ascospores predominantly spiny, but tending to form a reticulation.

Tuber indicum by comparison has spiny ascospores, a purplish gleba and polygonal peridial warts (Manjón et al. 1995). The peridium of *T. himalayense* is similar in appearance to *T. indicum*, but without the innermost layer of thick-walled, dark brown cells (Di Massimo et al. 1998). We have studied the type collections of both species and agree with their descriptions. Ren et al. (2005) indicated that the presence of *T. himalayense* in China requires further confirmation owing to the lack of specimen citation and locality. Roux et al. (1999), He et al. (2004), Zhang et al. (2005) and Wang et al. (2006a, b) have cited *T. himalayense* from China. However, Wang et al. (2008a, b) indicated that *T. himalayense* has not been found in China.

Tuber huidongense Y. Wang

Wang and He (2002) described *Tuber huidongense* from Huidong County, Sichuan. This species has small, yellowish brown ascomata with a red tint (0.5–2.5 cm); surface is rough with scattered hairs; 430–500 μm thick peridium, composed of two layers; the outer layer complex,

Fig. 1 Key for Chinese *Tuber* determination based on the features we have compiled for these species and on the *Tuber* key of Pegler and Spooner (1993)

Key to the species of Chinese *Tuber*

(Ascospore size = including ornamentation)

1.a	Peridium with flat to pyramidal warts	2
1.b	Peridium slightly warted to minutely papillate or smooth; glabrous or pubescent	5
2.a	Peridium black	3
2.b	Peridium from brown to brown orange but never blackish when fresh	4
3.a	Spores with variable ornament: reticulate to spiny; asci 2-4-spored; spores 28-45 x 23-40 µm; ascomata up to 2.5 cm; flattened warts	*T. himalayense*
3.b	Spores with spiny ornament; asci 3-5-spored; spores 30-42 x 23-32 µm; ascomata up to 7 cm; warts irregularly polygonal	*T. indicum*
4.a	Asci 1-8-spored; ascomata deeply excavated; gleba grey brown, marbled only with white veins	*T. pseudohimalayense* (= *T. pseudoexcavatum*)
4.b	Asci 1-4-spored; ascomata not deeply excavated; gleba pale greyish brown, marbled with two types of veins: colourless and brown veins	*T. sinense*
5.a	Spores with a reticulate ornament	6
5.b	Spores with a spiny-reticulate ornament	8
6.a	Asci 1-2-spored; spores 52-65 x 40-46 µm; ornamentation reticulate with 7-11 meshes along their length	*T. zhongdianense*
6.b	Asci 1-4 or 2-4-spored; spores shorter than 55 µm; ornamentation not reticulate or meshes in different number or arrangement	7
7.a	Asci 1-4-spored; spores 43-54 x 34-45 µm; ornamentation with 3-5 meshes along their length; peridium whitish; gleba black with whitish or cream veins	*T. latisporum*
7.b	Asci 2-4-spored; spores 29-40 x 26-35 µm; ornamentation with 5-6 meshes along their length; peridium brownish yellow; gleba grey lilac with white veins	*T. liaotongense*
8.a	Asci (at least some) with more than 2 spores; spores shorter than 100 µm	9
8.b	Asci 1-spored; spores 105-115 x 70-75 µm	*Paradoxa gigantospora* (= *T. gigantosporum*)
9.a	Asci 2-4-spored; spores from brown to dark brown	10
9.b	Asci 1-3 or 1-4-spored; spores brownish	12
10.a	Peridium glabrous; ascomata brown	11
10.b	Peridium rough with scattered hairs; ascomata yellowish brown	*T. huidongense*
11.a	Spores 45-51 x 25-32 µm; ornamentation up to 5 µm high; gleba with whitish veins only; peridium rough with patches covered with a sort of yellowish scurfy substance	*T. furfuraceum*
11.b	Spores 28-37 x 18-26 µm; ornamentation up to 4 µm high; gleba with white veins of outer portions of the ascoma and brown veins of inner portions of the ascoma; peridium glabrous	*T. taiyuanense*
12.a	Ascomata without umbilicate depression; peridium pubescent; peridium peeling hardly from the gleba	13
12.b	Ascomata with umbilicate depression; peridium smooth or minute papillae; peridium peeling easily from the gleba	*T. umbilicatum*
13.a	Spores 35-50 x 46-55 µm; ornamentation up to 7 µm high; asci 1-3-spored	*T. xizangense*
13.b	Spores 60-78 x 40-55 µm; ornamentation up to 8 µm high; asci 1-4-spored	*T. liui*

190–200 µm thick, with a crust of globose, brown, thick-walled cells, below the outermost cells is a pseudoparenchymatous layer, the cells more or less globose, of 10–15 µm in diam., brown, 1–2 µm thick-walled; 250–300 µm thick inner layer, composed of colorless, interwoven hyphae, of 2–3 µm in diam., gradually merging into the glebal tissue. Gleba purplish brown; veins distinct, white, branched; inter-ascal hyphae colorless, interwoven and thin-walled; asci 2–4-spored with an apparent stalk; ascospores brown, ellipsoid, of 37–40×28 µm (excluding ornamentation); spiny-reticulate, with bent tips, irregular in mesh shape and size, (3) 4–5 (7) meshes in length, 3–5 meshes in width, the ornamentation up to 6 µm tall.

Tuber huidongense is rare compared to *T. sinense* and *T. pseudoexcavatum*; only two collections have been found. *Tuber huidongense* belongs to the *T. rufum* group (Wang et al. 2007) and is closely related to *T. borchii* and *T. maculatum*, but differs in that it has smaller ascospores with bent tips and much smaller asci with apparent stalks. This species is also noted for its two layered peridium and the ornamentation spiny-reticulate of the ascospore (Wang and He 2002). He et al. (2004) cited *T. huidongense* from China.

Tuber indicum Cooke and Massee

Tuber indicum has medium-sized, black ascomata (1–7 cm); warts irregularly polygonal; peridium of 550–700 µm composed of two layers; outer layer (500–600 µm including warts) with a crust of nearly globular, reddish black, outermost cells, 1–3 µm thick-walled; immediately below, cells are paler and more

Table 3 Some ecological parameters of Chinese *Tuber* species

Tuber species	Potential mycorrhizal partner	Soil type	Altitude (m a.s.l.)	Fruiting season
T. furfuraceum	*Cyclobalanopsis glauca*	Calcareous	1,200–1,300	–
T. formosanum	*Cyclobalanopsis glauca*	Calcareous	1,200–1,300	–
Paradoxa gigantospora (= *T. gigantosporum*)	*Pinus yunnanensis*	–	2,000–2,300	August to November
T. himalayense	*Quercus incana*, *Q. pubescens*	–	–	–
T. huidongense	*Pinus yunnanensis*, *P. armandii*	–	2,070	–
T. indicum	*Pinus armandii*, *P. yunnanensis*, *P. pinea*, *Quercus incana*, *Q. cerris*, *Q. ilex*, *Q. ilex* subsp. *ballota*, *Q. pubescens*	Calcareous to no calcareous	2,000–3,000	–
T. indicum var. yunnanense	*Pinus yunnanensis*, *P. armandii*	Calcareous	1,600–3,000	–
T. latisporum	*Pinus armandii*, *Pinus* sp.	–	2,000	Octuber to December
T. liaotongense	*Pinus koraiensis*, *Larix olgensis*, *Quercus mongolica*	–	–	August to September
T. liui	*Quercus aquifolioides*	–	3,000	–
T. pseudohimalayense (= *T. pseudoexcavatum*)	*Pinus yunnanensis*, *Quercus ballota*	Calcareous	2,000–2,300	August to november
T. sinense	*Pinus yunnanensis*, *P. armandii*, *Quercus* sp., *Alnus cremastogyne*	Calcareous and clay soils, high pH	1,600–3,000	–
T. taiyuanense	*Pinus* sp.	–	–	–
T. umbilicatum	*Pinus yunnanensis*	–	1,900–2,000	–
T. xizangense	*Rhododendron* sp.	–	3,200	–
T. zhongdianense	*Quercus pannosa*	–	3,100	–

elongated (20×10 μm) with thinner walls, in places somewhat resembling a palisade; below these are other thin-walled cells which are paler brown, less elongated (10 μm) forming a textura angularis; inner layer (50–200 μm) with faintly pigmented, thin-walled 3–5 μm cells, forming a textura intricata; gleba purplish black, marbled with numerous, very thin, whitish veins; the paraphyses bordering the aeripheral veins are persistent; asci 3–5 (6)-spored, stalks rarely observed; ascospores ellipsoid, dark, of (15) 22–32 (45)×(12) 15–22 (30) μm (excluding ornamentation), or 30–42 (55)×23–32 (40) μm (including ornamentation); Ceruti's (1960) measurements were 24–46×16–24 μm; ornamentation of spines 3–5 μm tall, 1–3 μm wide at the base, slightly hooked, free, sparse (Bardet et al. 1995; Manjón et al. 1995; Fourré et al. 1996; Janex-Favre et al. 1996).

Zhang and Minter (1988) indicated that *T. indicum* appears to have a wider distribution than that described by Cooke and Massee (1892). Roux et al. (1999), Riousset et al. (2001), He et al. (2004), Ren et al. (2005), Zhang et al. (2005) and Wang et al. (2006a, b) cited *T. indicum* from China.

Tuber indicum var. *yunnanense* Yamanaka

Yamanaka et al. (2000, 2001) described *Tuber indicum* var. *yunnanense* from Yunnan. This species has medium-sized, black ascomata, of 2–8 (10) cm in diam.; with irregular, polygonal, flattened warts with poorly-defined radial splits. Asci (1) 2–5 (6)-spored. Ascospores of (22) 28–35 (40)× (16) 19–25 (30) μm (excluding spines) in size. However, the spore size is extremely variable in this species. Spines are 4–6 μm tall, broad at the base and often slightly hooked at the apex. The ascospores are partially and irregularly reticulate (pseudo-reticulum) by anastomosis of broad bases of the few spines.

Tao et al. (1989) described a species harvested in southwest China as *T. sinense*, but this species shows the spore with typical reticulation on the surface. *Tuber indicum* var. *yunnanense* differs from *T. himalayense*, *T. pseudohimalayense* and the Japanese *T. indicum* (Imazeki et al. 1988) in the morphology of the peridium and ascospore. *Tuber indicum* var. *yunnanense* differs from the Indian *T. indicum* described by Zhang and Minter (1988) in the irregular polygonal, flattened warts with poorly defined radial splits.

Tuber latisporum Juan Chen and P. G. Liu

Chen and Liu (2007) cited *Tuber latisporum* from Yunnan province, southwest China. This species has whitish, subglobose or irregularly lobed ascomata (2–2.5 cm), surface conspicuously pubescent; peridium of 200–500 µm, with two layers: 150–320 µm thick outer layer, pseudoparenchymatous with subglobose to subangular, pale yellow or colorless cells of 6–27×5–22 µm, 1–3 µm thick-walled, hairs abundant, of 60–127 µm long, and 4–6 µm diam. at base; inner layer of 150–250 µm, of intricately interwoven, colorless hyphae of 2–5 µm diam., the walls thin to somewhat thickened; gleba black at maturity, marbled with a few whitish or cream veins; asci sessile, 1–4 spored; ascospores broadly ellipsoid, yellowish brown to reddish brown, walls up to 2 µm, of (37) 40–49 (51)×(28) 31–40 (44) µm (excluding ornamentation); ornamentation an alveolate reticulum, the mesh of 7–15 (16)×(6) 7–12 µm, 3–5 µm deep, 3–5 meshes across the spore width.

Tuber latisporum is closely related to *T. liui*, *T. zhongdianense*, *T. borchii*, *T. puberulum*, *T. rapaeodorum*, *T. maculatum*, *T. dryophilum* and *T. magnatum* Pico. However, *T. latisporum* differs from these especies in the combined characters of structure of peridium, the shape and reticulum of the spores (Chen and Liu 2007).

Tuber liaotongense Wang

Wang (1990) proposed *Tuber liaotongense* from Fushun, east Liaoning province in northeast China. This species has small brownish yellow ascomata (0.6–2 cm); slightly warted; peridium pseudoparenchymatous in outer layer and prosenchymatous in inner layer, the 2–3 outer cell layers brown; gleba gray lilac, marbled with white veins; asci 2–4-spored, with conspicuous remains of crosiers, then shortly stalked; ascospores ellipsoid to subglobose, brown, of 29–40×26–35 µm (including ornamentation); reticulate or alveolo-reticulate with 5–6 meshes along length.

Tuber liaotongense belongs to the *T. rufum* group (Ren 2003; Wang et al. 2007).

Tuber liui A. S. Xu

Xu (1999) proposed *Tuber liui* from Miling, Xizang (Tibet), China. This species has small reddish brown ascomata (1.7–3.5 cm), pubescent; 300–700 µm thick peridium, comprising a distinct outer and inner layer, the outer layer of 200–250 µm, pseudoparenchymatous; the inner layer composed of interwoven, colorless hyphae of 5–14 µm in diam.; gleba brownish with white veins; asci 1–4-spored; ascospores ellipsoid, brownish, of 60–78 (94)×40–55 µm (including ornamentation); ornamentation spiny-reticulate, with spines located at the nodes of the reticulum; spines 3–8 µm tall; with 6–11 meshes along their length; meshes of 5–10×10–14 µm.

Ascospores are similar in size to *T. macrosporum* Vittad. *Tuber liui* is also close to *T. puberulum*, *T. rapaeodorum* and *T. borchii*. However, *T. liui* differs from *T. macrosporum* in having no polygonal verrucae on the surface of the ascoma; *T. rapaeodorum* has a gleba with distinct veins, and ellipsoid to subglobose ascospores, and a fairly strong smell; *T. borchii* has a peridium up to 500 µm thick and hyphae of the inner peridium are up to 9 µm in diam. He et al. (2004) and Chen and Liu (2007) confirmed *T. liui* from China.

Tuber pseudoexcavatum Wang, G. Moreno, L. G. Riousset, J. L. Manjón and G. Riousset

Wang et al. (1998) and Wang and Hall (2001) described *Tuber pseudoexcavatum* with medium-size ascomata (3–10 cm), subglobose and deeply excavated; with more or less pyramidal, wide, brown to brown orange, flat warts; 290–500 µm thick peridium, comprising distinct outer and inner layers. The outer layer is 190–350 µm thick including warts, formed by subglobose cells, of 10–20 µm in diam. (textura angularis), brown reddish, outermost cells mainly 1–3 µm thick-walled; 100–140 µm thick inner layer (texture intricata), composed of colorless, interwoven hyphae, of 3–9 µm in diam.; gleba gray brown, marbled with white veins of interwoven, colorless hyphae; mild odor; asci 1–8-spored, with short remains of crosiers; ascospores ellipsoid, brown to dark brown of (23) 24–28 (35)×16–19 (−22) µm (excluding ornamentation); spines up to 5 (8) µm tall; ornamentation spiny-reticulate, formed by spines with broad basal connections to form a reticulum composed of variable meshes.

Tuber pseudoexcavatum ascomata are macroscopically similar to *T. excavatum* but with a brownish red peridium and spiny-reticulate ascospores. *Tuber pseudoexcavatum* has been found in Sichuan and Yunnan province, southwest China. He et al. (2004), Zhang et al. (2005) and Wang et al. (2006b) also cited *T. pseudoexcavatum* from China. Manjón et al. (2009) have proposed that *T. pseudoexcavatum* is a synonym of *T. pseudohimalayense* according to the law of priority, sharing rather similar taxonomic and molecular characters.

Tuber sinense Tao and Liu

Tao et al. (1989) described *Tuber sinense* with brown ascomata which are highly variable; the surface ranges from verrucose to pyramidal warts; peridium with two layers; 350–800 (1,000) µm thick outer layer with yellow brown to yellow, irregular cells of 5–15 µm in diam., 1–2 µm thick-walled; 200–500 µm thick inner layer, of pale yellow to colorless hyphae of 3–5 µm in diam.; gleba pale grayish brown, marbled with cream to grayish white veins; asci 1–4-spored; ascospores ellipsoid, yellowish brown to brown, of 32–36.5×43–49.5 µm (including ornamentation); ornamentation spiny-reticulate, which also varies from spiny to almost reticulate, 3–6 (7) µm tall; 3–4.5 µm thick-walled (Tao et al. 1989).

Wang and Hall (2001) indicated that *T. gigantosporum* and an undescribed *Tuber* sp. are closely associated with *T. sinense* in southwest China. These authors explained that *T. sinense* can be distinguished from other species because it has two types of veins: colorless veins composed of interwoven hyphae mixed together with inflated cells, and brown veins composed only of thick-walled, inflated cells up to 15–20 µm in diam., similar to those in the peridium which can open into furrows on the surface. These characters can also be used to separate *T. sinense* from *T. indicum* which has veins composed only of colorless, thin-walled cells of 5–10 µm. Tao et al. (1989) proposed *T. sinense* from Sichuan province, China. Wang and Hall (2001) stated that *T. sinense* is the most important of the Chinese truffles, as its ascomata can be more than 10 cm in diam. He et al. (2004) and Wang et al. (2006a, b) also cited *T. sinense* from China.

Tuber taiyuanense Liu

Liu (1985) proposed *Tuber taiyuanense* from Taiyuan City in Shanxi province. This species has small brown ascomata (0.7–1.5 cm); glabrous; white external veins and brown internal veins; asci 2–4-spored, with stalk; ascospores brown, ellipsoid to globose, of 28.4–37.8×18.9–26.5 µm; ornamentation spiny-reticulate with 3.8–4 µm tall spines, hooked; reticulum of 2.8–4.7 µm in diam.; 2–2.2 µm thick-walled.

Wang (1990) and Wang et al. (2007) considered *T. taiyuanense* closely related to the *T. rufum* group. These authors reported that the holotype was deposited in the herbarium of the Department of Biology, Taiyuan, Shanxi, China. Unfortunately it has since been destroyed. A neotype was selected by Wang and Pei (2001) and deposited in the herbarium of HMAS, Chinese Academy of Sciences (HMAS 75888).

Tuber umbilicatum Juan Chen and P. G. Liu

Chen et al. (2005a, b) proposed *Tuber umbilicatum* from Yunnan province, southwest China. This species has small ochre ascomata 1.2–1.9 cm broad, with an umbilicate depression at the base, surface smooth or with minute papillae up to 50 µm tall; peridium peeling easily from the gleba, 320–500 µm thick with two layers: outer layer of 90–250 µm, pseudoparenchymatous, with subglobose to subangular, yellow brown cells of 7–16×5–11 µm, 1–3 µm thick-walled; inner layer 150–400 µm, of intricately interwoven, colorless hyphae of 2–5 µm diam., the walls thin to somewhat thickened; gleba purple brown or gray brown with pink tint at maturity, marbled with numerous, narrow branching white veins radiating from the basal cavity; asci 1–4 (6) spored sessile or sometimes with a short stalk; ascospores ellipsoid, yellow brown, of (28) 33–40×20–32 µm (excluding ornamentation), walls up to 2 µm wide; ornamentation of spines 3–5 (6) µm tall connected by an alveolate reticulum < 1 µm tall, alveoli of 3–6 (7)×2.5–5 µm, 6–8 across the spore width and (6) 7–10 along the spore length.

Chen et al. (2005a, b) proposed a key to seven *Tuber* species bearing spiny-reticulate ascospores.

Tuber xizangense A. S. Xu

Xu (1999) proposed *Tuber xizangense* from Miling, Xizang (Tibet), China. This species has small reddish brown ascomata (1.7–1.2 cm), pubescent; 250–330 µm thick peridium comprising a distinct outer and inner layer; 75–120 µm thick outer layer, pseudoparenchymatous; the inner layer composed of interwoven, colorless hyphae of 2–6 µm in diam.; gleba brownish with white veins; asci 1–3-spored; ascospores ellipsoid, brownish of 35–50 (29)×46–55 µm (including ornamentation); ornamentation spiny-reticulate, with spines located on the nodes of the reticulum; spines 2–7 µm tall; with 4–12 meshes along their length; meshes of 2–12.5×10–14 µm.

Tuber xizangense also differs from *T. rapaeodorum* and *T. borchii* in the color of the peridium, unpitted surface of ascomata, and obtuse apex of hairs. He et al. (2004) confirmed this species from China. Wang et al. (2007) indicated that the validity of *T. xizangense* is questionable and stated that holotypes were subsequently studied by Ren (2003), who did not recognize it as a new species.

Tuber zhongdianense He, Li and Wang

He et al. (2004) described *Tuber zhongdianense* from Zhongdian (Shangri-La), Yunnan, southwest China. This species has puberulent, pale brown ascomata (up to 1.5 cm); faint odor; 220 µm thick peridium, composed of two layers; outer layer complex, 60–70 µm thick, pseudoparenchymatous, with vertically-arranged, globose to rectangular, thin-walled cells of 19–32×13–15 µm; scattered, large, inflated, colouless cells of 50×34 µm; 2-µm thick walls giving rise at the surface to hairs in two layers (two kinds of hairs: tapered and obtuse); 150–160 µm thick inner layer, with interwoven, colorless hyphae of 2–4 µm in diam.; gleba brown; veins distinct, white, branching; asci

1–2 (3–4)-spored; ascospores brown broadly ellipsoid, of 50–63×38–44 µm (excluding ornamentation); reticulate with a regular mesh, normally 7–11 meshes long, 6–9 meshes wide, ornamentation of 2 µm (6–7 µm) tall.

This species is closely related to *T.borchii*, *T. rapaeodorum* and *T. puberulum*. *Tuber zhongdianense* differs in having two kinds of hairs and larger ascospores. This species differs from *T. liui* in that it has smaller ascospores with shorter ornamentation. *Tuber zhongdianense* is also notable for having a pseudoparenchymatous epicutis composed of vertically-arranged, globose to rectangular cells. Chen and Liu (2007) confirmed *T. zhongdianense* from China.

Tuber C-11 code

In Italy, Di Massimo et al. (1998) described a Chinese truffle found in the truffle market (C-11 code), which may be a new species. This species presents ascomata which ranges from light to dark brown with a reddish tint, 30–50 g in weight; verrucose; peridium with two layers; the outer layer formed by globose, brown cells, underlain by a layer of globular and elongated thin-walled cells, which in some places have a palisade arrangement; the inner layer is composed of colorless hyphae which are intertwined and give rise to an intricate structure; asci 1–4 (5)-spored, non-pedunculated; ascospores ellipsoid, reddish brown of 48 (56)×35 (42.5) µm (mean value, including ornamentation); ornamentation formed by spines 4 (2–6) µm tall, with connections to form an irregular reticulum with incomplete alveolus (5–10 µm). At the two poles of the spore, there is a complete alveolus which is larger that the rest of the alveoli, ending in 6–7 crests.

This species has great variability, above all at the spore level; this has led us to err on the side of caution and to await new studies before proposing it as a new species.

Other important contributions to Chinese *Tuber* taxonomy are the PhD theses of Song (2005) and Chen (2007). Wang et al. (2008a, b) highlighted some of the contributions of these theses, such as the proposal of new species of Chinese *Tuber* that have not been described in the literature (*T. chenggonense and T. pseudorufum*) (Song 2005), the inclusion of *T. sinense* in the *T. indicum* complex (Chen 2007), and the cited relationship between *T. pseudohimalayense* and *T. pseudoexcavatum* (Chen 2007).

Development of the Chinese export market

Fourré et al. (1996) stated that exports of Chinese truffles to Europe began in 1990 through German sales representatives. Wang and Hall (2001) described a trial shipment of Chinese truffle species closely resembling *Tuber melanosporum* that was sent to Germany for appraisal in the late 1980s, although it was not until about 1993 that regular shipments began between China and the European markets. In 1994, substantial amounts of imported Chinese truffles began to arrive, and since then Chinese truffle exports have increased considerably. However, there is little information available as to the exact quantity of Chinese truffles marketed in international truffle markets.

Bardet et al. (1995) and Fourré et al. (1996) confirmed that, in 1994, *Tuber indicum* was collected in the provinces of Yunnan and Sichouan. These truffles were first sent to Hong Kong and from there they were exported to France at the rate of 600 kg a week during winter. They indicate that, between September 1994 and April 1995, 23,800 kg of Chinese truffles were imported into France and were sold at a price of US\$67–92 kg^{-1}, whereas the price of *T. melanosporum* was US\$714–816 kg^{-1} during the same period.

In Spain, García-Montero et al. (1997b, 2005) reported that 1995 was the first year in which a major quantity (5,000 kg) of Chinese truffles was imported into the region. Most of these truffles were imported in January and February. At first, market prices were between US\$100 and 120 kg^{-1} but they subsequently fell until they stabilized at around US\$50 kg^{-1}. Ninety percent of the volume of these Chinese imports to Spain was probably from French markets, while the remaining 10% appear to be imported from Italian and Chinese markets.

In Italy, Di Massimo et al. (1998) indicated that the importation of Chinese truffles began in 1994, with a total import of 3,500 kg (fresh only). These authors reported that these imports increased to 6,519 kg in 1995 and then decreased in 1996 to 500 kg, although they suspect that Chinese truffle imports in Italy were in fact higher than these official figures. During this time, prices reached US \$100 kg^{-1}, but they subsequently fell until stabilizing at around US\$50 kg^{-1}.

Yamanaka et al. (2001) indicate that the importation of Chinese truffles to Japan began in 1994, and that between 1994 and 1997 the import of Chinese truffles to Japan increased every year, reaching 4.76 tons in 1997 compared to 4.25 tons from France (fresh weight). Recently Asian truffles have been imported into Japan from Thailand and Bhutan (Yamanaka et al. 2001). There is therefore a strong probability that black truffles are also found in countries in eastern and western Asia and may be exported to Japan and to Europe and America in the near future.

Chinese truffles have been the subject of fraudulent commercial practices in European and Japanese markets where they may have been sold as *Tuber melanosporum* and other truffle species. García-Montero et al. (2005) recently reported that Chinese truffle species were found in canned truffle products purporting to be *T. aestivum* as well as in products labeled *T. melanosporum* which were sold at high prices in the delicatessen section of a well-known department store in Madrid, Spain. These facts highlight the necessity for European mycologists to investigate Chinese *Tuber* spp. to obtain detailed information on their taxonomy in order to distinguish them from European black truffles.

Bardet et al. (1995), Manjón et al. (1995), Fourré et al. (1996), Di Massimo et al. (1996), and Montecchi (1996) have identified *Tuber indicum* as the main species present in the first commercial batches of Chinese truffles which arrived in Europe. They indicate that the identification of *T. indicum* was done based on the hypothesis that this species presents a wide variability in both its micro- and macroscopic morphological characteristics. The same authors, as well as Janex-Favre et al. (1996) and Riousset et al. (2001), have compared in detail the taxonomy of *T. indicum* and *T. melanosporum* and indicated that both species are very similar although the aroma and taste of *T. indicum* is milder and sweeter. The peridium of *T. indicum* is smoother and its warts are more flattened and smaller. The asci of *T. indicum* may contain up to 6 spores whereas this is rare in *T. melanosporum*. The spines on the spores of *T. indicum* are longer (3–5 µm) than those of *T. melanosporum* (2.5–3 µm) and wider at the base, and are also less numerous. Spores of *T. indicum* are more often short, ellipsoid and more rounded than those of *T. melanosporum*. Di Massimo et al. (1998) and García-Montero (2000) proposed the peridium structure and the distribution of the percentage of asci with a different number of spores (number of asci with *n* spores / total number of asci × 100) as taxonomic characteristics of significant interest for distinguishing Chinese truffles from European species. The ascomata of *T. indicum* and *T. melanosporum* can only be clearly distinguished at the microscopic level, primarily by observation of the glebal structure, number of ascospores per asci, constancy in the size of the spores of *T. indicum*, and spore wall ornamentation.

Some authors and sales representatives have reported that *Tuber himalayense* may also be present in batches of Chinese truffles imported into Europe. Fourré et al. (1996) describe a possible example of *T. himalayense* from a Chinese salesman resident in Bordeaux in 1993. Comandini and Pacioni (1997) have synthesized and described the mycorrhizae of *T. himalayense* in Italy, together with the mycorrhizae of *T. indicum*. These authors indicated that the ascomata of *T. himalayense* and *T. indicum* are very difficult to separate morphologically. Only the spore ornamentation, ascospores with free spines (*T. indicum*) or spines connected by a basal reticulum (*T. himalayense*), are considered to be clearly diagnostic. However, these authors indicated that both kinds of spores can sometimes be observed within a single ascus. Zhang and Minter (1988) have also observed both kinds of spore in a single ascus in the case of *T. himalayense*. Comandini and Pacioni (1997) have compared the mycorrhizae of both species, and they indicated that these species are difficult to separate on the basis of morphological differences between their mycorrhizae (see below).

Montecchi (1996) has observed collections of Chinese truffles in Italy which present characteristics corresponding to the diagnosis of both *Tuber indicum* and *T. himalayense*. He considered that Cooke and Massee may have defined the species *T. indicum* based on a type collection formed by ascomata which were not fully mature (spores of 15–18 × 10–12 µm). Montecchi argued that Ceruti (1960) studied other collections of Cooke and Massee with much larger ascospores (24–46 × 16–24 µm). He also referred to the work of Zhang and Minter (1988). However, Montecchi (1996) does not indicate whether he has studied the type of *T. himalayense* or *T. indicum*. He stated that all the Chinese truffles he studied correspond to *T. indicum* and that the variability he observed in *T. indicum* can be explained by the intraspecific variability inherent in the genus *Tuber* due to the maturity of the ascomata. Riousset et al. (2001) reported that certain *T. himalayense* spores are similar to immature spores of *T. indicum*. Montecchi (1996) indicated that in order to recognize a species of *Tuber* it is also necessary to have detailed knowledge of the variations in its taxonomic characteristics throughout the maturation of its ascomata.

Chevalier et al. (1995), Fourré et al. (1996), Moreno et al. (1997), Di Massimo et al. (1998), García-Montero (2000), and Zhang et al. (2005) have studied the type collections of *Tuber indicum* and *T. himalayense*. These authors agree with the proposal of Zhang and Minter (1988), which clearly distinguishes both species based on their spore ornamentation, glebal color, peridium, and wart morphology. These authors have not found examples of *T. himalayense* in the truffles imported from China and therefore consider that *T. himalayense* is not present in the European markets or is very uncommon. They consider that it is not enough merely to use the commercial term “Himalayan truffles” or *T. himalayense* to designate the Chinese truffles which reach the European markets.

Other Chinese truffles have also been detected in European truffle markets (Moreno et al. 1997; Di Massimo et al. 1998). In Spain, García-Montero et al. (1997b) found *Tuber aestivum* and *T. melanosporum* ascomata in shipments imported from China. Wang et al. (2007) stated that *T. aestivum* was described by Mao (1998) but without any indication of location. Moreover, there are no collections deposited in any Chinese herbarium and this taxon was not mentioned in a more recent publication by the same author (Mao 2000). Ren et al. (2005) also cited *T. aestivum* in

China, and Wang et al. (2008a, b) reported that some Chinese authors have found small amounts of *T. aestivum* in the Chinese black truffle market (Chen et al. 2005a, b). The presence of *T. aestivum* in China was confirmed by Song et al. (2005) based on collections from under *Quercus* sp. in Sichuan province. These authors provided a full description of this species based on these Chinese collections and compared them with European collections of *T. aestivum* and related species (*T. mesentericum*, *T. melanosporum*, and *T. brumale*). They indicated thatd although there are a few differences between the Chinese and European collections (peridial warts are blunt and also lower than those of European collections) they can be considered to be conspecific.

Fourré et al. (1996) indicated that *Tuber sinense* had not yet arrived in French markets. In 1995, however, J. L. Riousset acknowledged that *T. sinense* had been marketed in France (Wang et al. 1998). Wang and Hall (2001) confirmed that in Europe some of the Chinese truffles that were similar to *T. melanosporum* were originally identified as *T. indicum* or *T. himalayense*, while others similar to *T. excavatum* were considered to be a new species, *T. pseudoexcavatum* (Wang et al. 1998). However, the same authors, together with Wang and He (2002), believed that most of the truffles similar to *T. melanosporum* and *T. excavatum* currently being exported in hundreds of tons a year from southwest China to Europe are *T. sinense* and *T. pseudoexcavatum*. *Tuber pseudoexcavatum* has also been detected in Italian (Di Massimo et al. 1998) and Japanese (Yamanaka et al. 2001) truffle markets.

Genetic studies

Ceruti et al. (2003) and Wang et al. (2007) indicated that the morphological classification within *Tuber* using ascoma and ascospore characters has led to controversy. Bardet et al. (1995), Janex-Favre et al. (1996), Di Massimo et al. (1996, 1998), and other authors have indicated the need to develop cytological, biochemical and molecular genetic studies to apply to the study of the carpophores and mycorrhizae of Chinese *Tuber*.

Wang (2008) indicated that China might be one of centers of *Tuber* phylogeny and distribution in the world. Roux et al. (1999) studied the phylogenetic relationships among *Tuber* species from Europe and China through parsimony analysis of ITS (Internal Transcribed Spacer) sequences. These authors obtained three major clades among the species analyzed. The black truffles, *T. brumale*, *T. melanosporum*, *T. indicum*, and *T. himalayense*, were grouped in an independent clade. *Tuber melanosporum*, *T. indicum*, and *T. himalayense* clade, were very closely related, but considered as distinct species.

Zhang et al. (2005) stated that *Tuber pseudohimalayense* and *T. sinense* should be regarded as synonyms of *T. indicum* based on ITS analysis. Wang et al. (2006a) considered *Tuber indicum*, *T. himalayense*, *T. sinense*, and *T. pseudohimalayense* to be one species, *T. indicum* complex, according to ITS and beta-tubulin sequences. The work of Wang et al. (2006b) with ITS sequences did not permit the separation of *Tuber indicum* from *T. sinense* and *T. himalayense*, suggesting that they belong to the same species.

Wang et al. (2007) described the taxonomic position of several Chinese *Tuber* species belonging to the *T. rufum* and *T. puberulum* groups by analyzing ITS and mitochondrial large ribosomal RNA sequences of several dried ascomata harvested all over China in the last 20 years, and then comparing them with American and European samples. In China, three species belonging to the *T. rufum* group could be validly recognized: *T. huidongense*, *T. liaotongense*, and *T. taiyuanense*. However, these authors indicated that one question remained: are *T. huidongense*, *T. liaotongense*, and *T. taiyuanense* different species or are they three subspecies? They considered that only a study at the population level can answer this question. Within the *T. puberulum* group, the Chinese specimens could not be separated clearly from the American and European ones. They concluded that the existence of the European species, *T. borchii*, *T. dryophilum*, *T. oligospermum*, and *T. rapaeodorum*, or the American species *T. californicum* and *T. shearii*, is doubtful in China. Nevertheless, at least three new species or subspecies belonging to the *T. puberulum* group are present in China. The *T. puberulum* group would have four subgroups: a subgroup with four European species and at least one American species, two subgroups comprising only Chinese samples which probably have to be reclassified as two new Chinese species or subspecies, and a fourth subgroup comprising two European species and two Chinese samples described as *T. asa*. The European species *T. asa* is probably absent from China, although it was recorded by Wang (1990). *Tuber liui*, which belongs to this fourth subgroup, appears to be closely related to the European species *T. puberulum*.

Chen and Liu (2007) reported that phylogenetic analysis of ITS sequences revealed three major clades: *Tuber latisporum, T. borchii*, *T. puberulum*, *T. zhongdianense*, and *T. liui* in clade I; clade II comprises *T. rapaeodorum*, *T. foetidum*, and *T. maculatum*; clade III was basal to clades I and II, and comprises *T. magnatum* and *T. aestivum*. These results show that *T. magnatum* did not cluster with the other white truffles but instead formed an independent phylogenetic lineage with the black truffle, *T. aestivum*. They concluded that white and black truffles are not natural groups, nor do they form a monophyletic lineage, and that combined molecular phylogenetic, morphological, and anatomical observations clearly separate *T. latisporum* from other white truffles (*T. borchii*, *T. puberulum*, *T. zhongdianense*, *T. liui*, *T. dryophilum*, *T. magnatum*, *T. rapaeodorum*, *T. foetidum*, and *T. maculatum*).

Jeandroz et al. (2008) have combined various data sets and methods of analysis to produce the first comprehensive molecular phylogeny of the *Tuber* genus and to analyze its biogeography. Phylogenetic relationships among *Tuber* species from Europe, North Africa, China, Asia, and North America were reconstructed based on a data set of ITS sequences. The resulting molecular phylogeny divided the *Tuber* genus into five distinct clades, in agreement with previous published studies (*Puberulum*, *Melanosporum*, *Rufum*, *Aestivum*, and *Excavatum* groups). However, these authors showed several discrepancies with the classical taxonomy of the genus, and proposed a new phylogenetic classification. The radiation of the *Tuber* genus could have started between 271 and 140 million years ago, with two equally probable scenarios of intra- and inter-continental diversification of the genus according to the geographic distribution of the most recent common ancestor in Europe or Eurasia. The biogeographical patterns imply intra-continental dispersal events between Europe and Asia and inter-continental dispersal events between North America and Europe or Asia, which are compatible with land connections during the Tertiary period.

Recently, Huang et al. (2009) used the internal transcribed spacer (ITS) and b-tubulin gene sequences to generate the phylogenetic relationship of *Tuber formosanum* and *T. furfuraceum* with other Chinese and European taxonomic relatives. They report that phylogenetic analysis of ITS sequences revealed five major clades: *T. excavatum* in clade I. Clade II comprises *T. aestivum*, *T. uncinatum*, *T. mesentericum*, *T. panniferum*, *T. magnatum*, and *T. macrosporum*. Clade III comprises *T. furfuraceum*, *T. rufum*, *T. huidongense*, *T. ferrugineum*, and *T. candidum*. Clade IV was divided into two subclades: *T. maculatum*, *T. rapaeodorum*, *T. foetidum*, *T. scruposum*, and *T. whetstonensis* formed subclade 1; while *T. oligospermum*, *T. borchii*, *T. puberulum*, *T. zhongdianense*, *T. liui*, and *T. latisporum* were grouped into subclade 2. The truffles in this clade have been known as the '*puberulum*' group. Clade IV was divided into two subclades: *T pseudoexcavatum* and *T. brumale* formed subclade 1; while *T. formosanum* and black truffles such as *T. indicum*, *T. himalayense*, *T. sinense*, and *T. melanosporum* were grouped into subclade 2. Huang et al. (2009) concluded that *T. formosanum* highly resembles the *T. indicum* complex, while *T. furfuraceum* is most similar to *T. huidongense*. They explained that *T. furfuraceum* and *T. formosanum* would have diverged from their close relatives in mainland China between 10.2 and 4.1 million years ago, respectively, and proposed that these two *Tuber* species found in Taiwan might originate from common ancestors with some truffle species in China. However, due to the long period of divergence and geographical separation, they have evolved into indigenous species of Taiwan.

Aroma and biochemical studies

Bardet et al. (1995), Fourré et al. (1996), and Di Massimo et al. (1998) reported that *Tuber indicum* has a less pronounced, sweeter and less pleasant flavor than *T. melanosporum*. Chevalier et al. (1995) indicated that *T. indicum* has an aroma reminiscent of "mushroom, and may bring to mind that of a scleroderma". They also indicated that, at the end of the harvesting period (February, March), extreme maturity or the delays caused by transport often cause a rapid degeneration in their organoleptic characteristics and that either fresh or cooked, *T. indicum* has a more bitter, less pleasant taste than *T. melanosporum*. The main volatile compound of the *T. indicum* odor was determined by Bellesia et al. (2002) as 3-methylbutanol. Unlike other *Tuber* species, no sulfur compound was found, and the composition of the volatile compound changed for the worse during storage. Yang (2001) indicated that *T. sinense* has a more delicate flavor that *T. melanosporum*. Saltron et al. (1997) analyzed the volatile compounds of the truffle juices of *T. melanosporum*, *T. brumale*, *T. aestivum*, and *T. indicum* by dynamic headspace and gas chromatography-mass spectrometry (GC-MS). Splivallo et al. (2007) applied stir bar sorptive extraction in headspace mode (HS-SBSE), coupled with GC-MS, to compare the aroma profile of *T. indicum* and other truffles. A clear distinction was obtained between *T. indicum* and *T. melanosporum* based on the VOCs (volatile organic compounds) pattern.

Molecular species of ceramides, sphingolipids, and a novel sterol have been isolated from the fruiting bodies of *Tuber indicum*. This is the first example of the isolation of a polyhydroxylated ergosterol glucoside from higher fungi in nature, and the first time sphingolipids have been reported from truffles (Gao et al. 2001a, b, 2004a, b). Yang (2001) tested the vitamin, mineral, and amino acid content of *T. sinense* compared with *T. melanosporum*, and reported that both truffles have about the same nutritional value. Moreover, studies have also been carried out on the effects of polysaccharide from *T. sinense* on the tumor and immune system of mice (Hu et al. 1994).

Recently, some Chinese authors have developed studies on the underground cultivation processes, fermentation conditions, and production of biochemical compounds of Chinese *Tuber* (Tang et al. 2007, 2008a, b, 2009; Liu et al. 2008; Wang et al. 2008a, b).

Biogeography and ecology

Roux et al. (1999) and Zhang et al. (2005) studied the phylogenetic relationships among *Tuber* species from Europe and China. The Périgord black truffle *T. melanosporum* and the Chinese black truffles *T. indicum* and *T. himalayense* were very closely related. Wang et al. (2006a) also studied the phylogenetic relationships between Chinese and European truffles. These authors obtained four phylogenetic clades, which made it possible to differentiate a black truffle clade, composed of two subclades; one comprising the Asian black truffles (*T. indicum*, *T. sinense*, *T. himalayense*) and *T. melanosporum*, the second comprising *T. pseudoexcavatum* and *T. brumale*. In terms of evolutionary history, these authors were able to propose a common ancestor located somewhere between Europe and China, probably in India, for all the species belonging to the *T. melanosporum* group. A first speciation may have led to the differentiation of two subclades. The *T. brumale/pseudoexcavatum* subclade would have started to migrate first, *T. brumale* towards Europe and *T. pseudoexcavatum* towards China. Later, the *T. melanosporum* subclade would have started to migrate along the same route, *T. melanosporum* towards Europe and *T. indicum* towards China. These two successive migrations towards Europe and China possibly took place before the climatic oscillations of the Pleistocene era.

Wang et al. (2006b) indicated that the *Tuber indicum* complex displayed a higher level of genetic differentiation among regional populations, compared with the European *T. melanosporum* (Murat et al. 2004), and the phylogenetic distances between the Chinese populations of *T. indicum* complex confirm the existence of a phylogeographical structure in China with two groups. They proposed tracing an imaginary line running west to east between the two groups, with the first located in the south and the second in the north. The existence of a sinuous limit between the two groups suggests that at least two factors could be involved in their differentiation: a northwards migration and a possible recolonization from the bottom of the valleys. During the last glaciation, *T. indicum* possibly survived in southern China or at lower altitudes. Paleobotanical reconstructions obtained from fossil pollen samples show that post-glaciation colonization took place from the south to the north, and 6,000 years ago forest vegetation extended to higher elevations than today. Successive decreases and increases in temperature since the last glacial maximum to the present day may have led to population fragmentation which could have resulted in a high level of genetic differentiation in populations within the *T. indicum* complex. Murat et al. (2004) indicated that glaciations and post-glacial migrations are major factors responsible for the present patterns of genetic variation seen in natural populations in Europe. For ectomycorrhizal fungi, escape from refuge can only follow range expansion by their specific hosts.

At present, Wang et al. (2008a, b) explained that Chinese black truffles grow predominantly in southwest and south China, and the “light-colored truffles” (such as *Tuber maculatum* and *T. huidongense*) are found both in south China and in north China. In southwest China, truffle-producing forests are subtropical with calcareous soils. These forests are being displaced by coniferous forests or pine plantations. In northeast China, truffles grow under temperate forests with acidic or calcareous soils (mixed forests of pines and deciduous broad-leaved trees or their secondary-growth forests).

From the vegetation aspect, Yang (2001) indicated that Chinese truffles species are mainly associated to forest woods with the following plants: *Pinus tabulaeformis* Carr. var. *yunnanensis* (Franch.) Shaw, *P. armandii* Franch., *Quercus acutissima* Carr., *Q. pannosa* Hand-Mazz., *Viburnum cylindricum* Buch., *Alnus cremastogyme* Burk., *Coriaria nepalensis* Wall., *Camptotheca acuminata* Decne., *Prunus mume* Siebt., *Pyrus ashia* Buch.-Ham. ex. D. Don., and *Berberis poiretii* Schneid. In these woods, most truffle species are harvested from November to the following March.

Wang (1990) has gathered more than 50 collections of *Tuber* species in northeast China in plantations (20–30 years old) of *Pinus koraiensis* Sieb. and Zucc., *P. densiflora* Sieb. and Zucc., *P. tabulaeformis*, *Larix olgensis* A. Henry, and *L. kaempferi* (Lamb.) Carr. *Tuber taiyuanense* grows at a depth of up to 10 cm in soil under pines (Liu, 1985). *Tuber gigantosporum* and *T. pseudoexcavatum* occur under *Pinus yunnanensis* at elevations of 2,000–2,300 m a.s.l., fruiting from August to November (Wang and Li 1991; Riousset et al. 2001; Wang and Hall 2001). *Tuber indicum* occurs under *Pinus armandii* and *P. yunnanensis* at 2,000–2,500 m a.s.l. (Riousset et al. 2001; Granetti et al. 2005). *Tuber liaotongense* has been associated with 25- to 30-year-old plantations of *Pinus koraiensis* and *Larix olgensis* (Wang 1990). *Tuber sinense* and *T. indicum* var. *yunnanense* have been collected buried deep under *Pinus yunnanensis* and *P. armandii*, at an altitude of 1,600–3,000 m a.s.l. (Tao et al. 1989; Zhang and Wang 1990; Yamanaka et al. 2000, 2001; Wang and Hall 2001; Yang 2001; Wang et al. 2006b). *Tuber huidongense* has also been found associated with pines at an altitude of 2,070 m a.s.l. (Wang et al. 2007). *Tuber umbilicatum* occurs with *Pinus yunnanensis* at 1,900–2,000 m a.s.l. (Chen et al. 2005a, b) and *Tuber latisporum* occurs under *Pinus armandii* at 2,000 m a.s.l., and fruits from October to December (Chen and Liu 2007).

Other Chinese *Tuber* species have been collected under broad-leaved trees and mixed forests of pines and broad-leaved trees. *Tuber indicum* and *T. himalayense* occur under

Quercus incana Bartr. (Cooke and Massee 1892; Zhang and Minter 1988). *Tuber liui* occurs under *Quercus aquifolioides* Rehder and Wilson at 3,000 m a.s.l., and *Tuber xizangense* under *Rhododendron* sp. at 3,200 m a.s.l. in Tibet (Xu 1999). *Tuber zhongdianense* is associated with *Quercus pannosa* at 3,100 m a.s.l. (He et al. 2004). *Tuber sinense* is found in mixed forests of pines and broad-leaved trees (*Quercus* sp., *Alnus cremastogyne*, etc.). *Tuber formosanum* and *T. furfuraceum* are associated with *Quercus* subgenus *Cyclobalanopsis glauca* (Thunb.) Oerst. at 1,200–1,300 m a.s.l. in Taiwan (Hu and Wang 2005). *Tuber liaotongense* is associated with *Quercus mongolica* Fisch. (Wang 1990).

Wang et al. (2008a, b) summarized the mean truffle-producing forest vegetation. Most Chinese black truffles (*Tuber indicum* complex, *T. aestivum*, and *T. pseudoexcavatum*) are produced from coniferous forests resulting from secondary growth that forms 10- to 40year-old *Pinus yunnanensis* forest (1,000–2,500 m a.s.l.), *P. armandii* forest (1,500–2,600 m a.s.l.), and *Keteleeria evelyniana* Mast. forest (below 1,500 m a.s.l.). The secondary-growth coniferous forests developed following the destruction of tropical evergreen broad-leaved forests of *Castanopsis*, *Lithocarpus*, and *Cyclobalanopsis* (1,400–2,500 m a.s.l.).

Soil requirements

Some Chinese *Tuber* species, as the *T. indicum complex* species, have been found in calcareous soils. Some fruit on the surface of the forest floor while others grow underground at depths of 5–15 cm or more. *Tuber furfuraceum* and *T. pseudoexcavatum* live in calcareous soils (Wang and Li 1991; Riousset et al. 2001; Wang and Hall 2001; Hu and Wang 2005). *Tuber sinense* has been found in the upper 30 cm of calcareous and clay soils (Zhang and Wang 1990; Wang and Hall 2001). *Tuber indicum* var. *yunnanense* has been found at a depth of 2–12 cm in very poor, calcareous soils (Yamanaka et al. 2000, 2001). *Tuber formosanum* is found only in calcareous soils with a wide range of soil parameters (pH, total nitrogen, carbon, sulfur, and available nutrients), suggesting that *T. formosanum* can adapt to a wide range of soil conditions (Hu et al. 2005).

Riousset et al. (2001) reported that *T. indicum* fruits at a depth of 30–40 cm. under a mulch of pine needles in pine mountain woods, in soils free from calcium carbonate and with a moderate pH, rich in organic matter and with a high C/N ratio; however, other authors have indicated that *T. indicum* inhabits calcareous soils. Fourré et al. (1996) report that *T. indicum* occurs on calcareous soils at 2,000–3,000 m a.s.l. in Yunnan. Granetti et al. (2005) indicate that *T. indicum* inhabits calcareous soil with a pH between 5.5 (due to organic matter) and 8.5. García-Montero et al. (2008) confirm that *T. indicum* and *T. pseudoexcavatum* mycorrhizae grow well in calcareous substrates rich in active carbonate, which is similar to the requirements for the development of *T. melanosporum*.

Mycorrhiza formation

In recent years, some authors have cited the presence of various naturally-occurring Chinese *Tuber* mycorrhizae in different forest types: *T. liaotongense* forms mycorrhizae with *Pinus koraiensis*, *Larix olgensis*, and *Quercus mongolica* (Wang 1990); *T. zhongdianense* with *Quercus pannosa* (He et al. 2004); *Tuber furufuraceum* with *Cyclobalanopsis glauca* (Hu and Wang 2005); *Tuber sinense* and *T. huidongense* with *Pinus yunnanensis* and *P. armandii*; and *T. latisporum* possibly with *Pinus* (Chen and Liu 2007). Wang and Hall (2001) reported that mycorrhizae of *T. sinensis* are whitish when young have short projections and became pale brown to dark brown with age. They are monopodial or dichotomous, finally becoming tuberculate with age.

Zambonelli et al. (1996, 1997) and Di Massimo et al. (1998) synthesized *Tuber indicum* mycorrhizae with *Pinus pinea* L., *Quercus cerris* L., and *Q. ilex* L. Comandini and Pacioni (1997) synthesized and compared *Tuber indicum* and *T. himalayense* mycorrhizae with *Quercus pubescens* Willd. These authors also reported that these mycorrhizae were very similar to *Tuber melanosporum* mycorrhizae, as both species have a puzzle-like hyphal pattern and cystidia occasionally with a single orthogonal branch. Zambonelli et al. (1997) state that the only difference between *T. indicum* and *T. melanosporum* mycorrhizae is that the pseudocells of the mantle of *T. indicum* have a more regular and polygonal shape and are slightly smaller than the pseudocells of the mantle of *T. melanosporum*.

Comandini and Pacioni (1997) reported that from a macroscopic point of view there are no apparent differences between *T. himalayense* and *T. indicum* mycorrhizae on *Quercus pubescens*, but explained that macroscopic features can be inconsistent and influenced by aging and soil conditions. These authors also reported that, when the microscopic characters are considered, only the outermost part of the mantle is likely to show slight differences between *T. himalayense* and *T. indicum* mycorrhizae. Comandini and Pacioni indicated that the surface view of *T. himalayense* mycorrhizae is quite well-defined (the hyphal pattern always has the same features despite the large number of specimens that they observed; the forms and dimensions of hyphal cells are constant). The case of *T. indicum* is rather different, as it has a very heterogeneous mantle surface structure. Even in the same sample, it is not uncommon to find parts in which the hyphal pattern is quite similar to that of *T. himalayense*. Several of the specimens observed show forms with intermediate features in between the two types.

Manjón et al. (1998) and García-Montero et al. (2008) synthesized *T. pseudoexcavatum* and *T. indicum* mycorrhizae with *Quercus ilex* L. subsp. *ballota* (Desf.) Samp. They also compared the morphological and anatomical characteristics of *T. pseudoexcavatum*, *T. indicum*, and *T. himalayense* versus *T. melanosporum* mycorrhizae on *Quercus ilex* subsp. *ballota*. Chinese *Tuber* ectomycorrhizae are anatomically very similar to the *T. melanosporum* mycorrhizae due to the presence of cystidia with right-angle-like ramifications and cells on the outer mantle with a sinuous puzzle-like form on both *Tuber* species.

Yamanaka et al. (2000) showed that *Tuber indicum* var. *yunnanense* formed dichotomous ectomycorrhizae with *Pinus armandii*, and the mycelial colony of this species did not develop in soil under carpophores or in the surrounding pine roots. Yamanaka et al. (2001) gave a general description and provided photographs of these mycorrhizae: emanating hyphae with septa are seen on the mantle surface, albeit infrequently; mantle layer is differentiated into three layers; tannin cells and a well-developed Hartig-net in inter-cortical cellular spaces can be observed in the longitudinal section; there is a pseudoparenchymatous outermost mantle layer with septate hyphae, and a plectenchymatous innermost layer.

Chen et al. (2001) provide the first report on the mycorrhizal synthesis of *T. melanosporum* in native Chinese tree species through inoculation techniques.

Hu and Wang (2005) demonstrated that *T. formosanum* forms ectomycorrhizae with *Cyclobalanopsis glauca*. Hu (1987) used a spore suspension to successfully colonize *C. glauca* seedlings with *T. formosanum*. Hu et al. (2005) indicate that these mycorrhizae were present on 45% of the root tips of 4-year-old *Cyclobalanopsis glauca* seedlings, 2 years after planting.

Wang et al. (2008a, b) indicate that *Pinus yunnanensis*, *P. armandii*, *Castanea mollisima* BL.. and *Quercus franchetii* Skan in F. B. Forbes & Hemsley have been successfully mycorrhized by *T. indicum* under greenhouse conditions. However, the production of truffle seedlings has not been commercialized.

Geng et al. (2009) reviewed the synthesis of Chinese *Tuber* mycorrhizae with different host plants, and remark that many of these mycorrhizal investigations were carried out with exotic trees. They report that Asian black truffles were found in association with a variety of indigenous species, including *Alnus nepalensis* D. Don., *Cyclobalanopsis glauca*, *Pinus armandii*, *P. yunnanensis*, *P. taiwanensis* Hayata, *Quercus* spp., *Keteleeria evelyniana* (Zhang and Minter 1988; Zhang and Wang 1990; Hu 1992; Zang et al. 1992; Chen et al. 1998; Chen 2007; Wang et al. 2007). Moreover, Geng et al. (2009) cite the synthesis of *T. indicum* with its possible indigenous hosts in southwestern China, *Castanea mollissima*, *Pinus massonia* Lamb., *P. armandii*, and *P. yunnanensis* by Chen (2003) and Hu et al. (2004, 2006). Geng et al. (2009) also synthesized *Tuber indicum* mycorrhizae with *Pinus armandii* and *Castanea mollissima* and confirmed the *T. indicum* mycorrhizae by ITS-rDNA sequence analyses, as well as providing a detailed description of the mycorrhizae morphology. They concluded that the morphology of emanating hyphae and epidermoid-like mantle appearance is similar to the *T. indicum* mycorrhizae obtained with some European trees.

Natural yield, truffle culture and markets

Wang (1990) and Wang et al. (2008a, b) reported that the Chinese historical record mentions only one entry for truffles, by Chen (1245). This truffle species was called “Mai Xun” which is possibly one of species of *Rhizopogon* (*R. rubescens*), often fruiting in the loose sandy soil near the river and had a unique flavor. Chamberlain (1996), in her ethnomycological experiences in southwest China, recounts that she found, dried or tinned, wild *Tuber* sp. cooked in a Zhongdian restaurant in northwest Yunnan. Wang et al. (1998), Riousset et al. (2001), and Wang and Hall (2001) indicated that many truffles species, in particular *T. indicum* and the large carpophores of *T. sinense*, are abundant and have been commercially collected as food products for a long time in southwest China. Wang and Hall (2001) and Wang et al. (2008a, b) also indicated that several species of *Tuber* are used as tonics by the Yi and Han people in China, and that the local names are “Wuniang Teng” (fruit without mother, Sichuan), “Song-Mao Fuling” (fungal fruting body underneath pine needles) and “Zhugong-Jun” (pig-digging fungus, Yunnan). Riousset et al. (2001) stated that *T. indicum* can either be used fresh or preserved dried in slices or in brine, and is eaten cooked with meat and vegetables. In years of drought when *T. indicum* is scarce, the carpophores are grated and used as a condiment or as a medicinal product.

Fourré et al. (1996) stated that *Tuber indicum* is harvested in Yunnan using pigs. However, Yamanaka et al. (2000, 2001), Riousset et al. (2001), Wang and Hall (2001), Granetti et al. (2005), and Wang et al. (2008a, b) reported that truffles are not found using trained truffle dogs or pigs, but by digging up the soil with small adzes or pickaxes or ploughs which causes severe damage to the habitat, and often results in the harvest of many immature truffles. The truffle forest floor is entirely ploughed to a depth of 30 cm or more and tree roots are exposed to the air. Damaged truffle forests take years to recover and may never produce any truffles again. Moreover, the Chinese harvest immature carpophores out of season–for instance in August–although the season to collect mature fruit bodies is November to March. These authors indicated that random digging and out-of-season harvesting are the reasons why Chinese truffles are of inferior quality and have a faint aroma.

Therefore, in China, many truffle woods are environmentally disturbed year after year by random digging and harvesting of immature carpophores. In the last four years, black truffle harvesting has been moving to forests in the interior of the district (Yamanaka et al. 2000, 2001). Wang (2008) and Wang et al. (2008a, b) explained that until now the total production of Chinese black truffles is still increasing because new regions of production are continually being found and exploited. However, local production is declining sharply due to destructive harvesting methods. Wang et al. (2008a, b) showed as an example that more than 20 tons of Chinese black truffles were produced annually from Huidong County, Sichuan, before 1993, but only 4–5 tons in 2003. They explained that it is crucial that Chinese farmers should be educated to take care of the truffle habitats, and also indicated that management and legislation regulating the commercial harvesting and conservation of truffles in China is urgently needed.

Liu et al. (2003) have classified *Tuber sinense*, *T. himalayense*, and *T. pseudoexcavatum* as "endangered fungi taxa", as their populations have sharply decreased due to excessive collection by humans. These authors have also classified *T. sinense* as "fungi taxa with economic importance", and stated that the key fungal taxa proposed, including these Chinese truffles, constitute a basic foundation for the research, application, and conservation of fungi from China.

Yang (2001) stated that local consumption of truffles in southwest China is negligible, and that most truffles are exported. Of these, the majority are fresh, while smaller amounts are frozen, sun-dried, or freeze-dried. This author explained that, as truffles have only recently been exported for a very short time in China, many aspects of the business still require special consideration: (1) the method of collecting truffles is primitive and damages truffle production; (2) there is insufficient research into certain varieties of truffle; (3) there are insufficient export markets for processed truffles; that is, the supply of high-quality processed truffles far exceeds demand (although freeze-dried truffle retains the full truffle flavor and fragrance, it is presently exported in small quantities); (4) there is a lack of comprehensive truffle research within China; (5) producers have little access to information regarding quality, standards, and market requirements; there is no research or development of truffle farming in southwest China; (6) natural resources are underused; (7) due to insufficient export markets, there is a truffle surplus in China and presently no use for this surplus; and (8) as truffles do not constitute a large source of revenue in the Chinese economy, truffle producers received little consideration from local governments and related departments.

Yao et al. (2004) described the recent progress in research into cultivation of edible truffles in terms of species, seedling cultivation and orchard management. These authors reported on the existing problems in truffle cultivation in China and proposed some suggestions for the protection and natural and artificial cultivation of truffles in China.

Recently, Wang (2008) and Wang et al. (2008a, b) stated that harvesting and trading in Chinese black truffles are becoming a multi-million dollar industry that has brought good income to the local economy and farmers. The total export of Chinese black truffles have been estimated at between 80 tons (Wang et al. 2008a, b) and 800 tons worth over US$20 million in 2006 (Wang 2008).

Hu et al. (2005) and Wang (2008) indicated that the first truffle plantation was established in Taiwan in 1989, and in 1996 produced *Tuber formosanum* carpophores associated to *Cyclobalanopsis glauca*. Hu et al. (2005) described the ecological conditions and cultivation procedures of this truffle plantation. Wang (2008) indicated that two other truffle plantations were established in Hunan and Guizhou Province recently but have not yet produced. Wang et al. (2008a, b) described the truffle plantation located in Guizhou, established with *Quercus aliena* BL. mycorrhized by *Tuber indicum*, *T. melanosporum*, and *T. magnatum* in calcareous soils. Moreover, they indicated that research on field inoculation of existing pine trees using spores to increase the yield of Chinese black truffles is underway. In 2007, a trial in which *T. indicum* spores were applied to an existing *Pinus yunnanensis* plantation was established by the Kumming Institute of Botany (Chinese Academy of Sciences in Yongshen, Yunnan). Wang (2008) concluded that the production of truffle-colonized trees and establishment of truffle plantations are underway and have considerable potential in China.

Conclusions

Chinese truffles are a very important complement to traditional truffle culture due to their lower prices, their good quality aroma, and their greater availability in a deficit market. However, the Chinese truffle market poses risks to truffle culture and ecosystems due to the possible introduction of Chinese *Tuber* into other countries, either accidentally or intentionally (García-Montero et al. 1997a; Di Massimo et al. 1998; Ferrara and Palenzona 2001). Murat et al. (2008) have confirmed the appearance of *T. indicum* DNA in root tips and soil samples from a *T. melanosporum* plantation in Piedmont (Italy). It is therefore important to develop protocols of quality control for plants inoculated with truffles or other edible fungi, in order to ensure that plants do not become contaminated during production. The potential use of bar coding as a means of identifying the truffle species that are showing up in the international markets could add significant security on these topics. These concerns have been behind numerous recent studies on Chinese *Tuber* species.

Regarding *Tuber* taxonomy studies, Janex-Favre et al. (1996) indicated that the classic analysis of the morphology of the carpophores and the ornamentation of the ascospores of *Tuber* constitute reliable criteria for identifying truffle species, but with certain limitations. The use of these morphological characteristics requires an extensive study of the inter- and intraspecies variability within each region. Montecchi (1996) indicated that detailed knowledge is also required of the variations in morphological characteristics of each species of *Tuber* throughout its maturing period. All *Tuber* species require 2–3 months to complete maturity of their carpophores. During this long maturing period, the microscopic characters vary widely. For these reasons, molecular genetics have played an increasing role in the study of the taxonomy of *Tuber*. However, the morphological and genetic studies must be complemented with research on *Tuber* ecology, mycorrhizae, cytology, and biochemistry (Calonge 1990).

Wang et al. (2007) reported that about 140 species and 65 subspecies and varieties of *Tuber* have been reported worldwide. These authors stated that currently there are only 70–75 validly described *Tuber* species. Chen and Liu (2007) put forward a similar analysis. The development of numerous studies on the taxonomy, ecology, pedology, cytology, and mycorrhizae in many populations from different geographic areas, linked to recent studies on biochemistry and molecular genetics, has led to greater knowledge of this complex genus. Riousset et al. (2001) provided an example of the integration of different disciplines applied to the study of *Tuber* to analyze European *Tuber* populations based on morphological and ecological studies, polymorphism of proteins, isoenzymes and nucleic acids, and others.

The present review of research on Chinese *Tuber* highlights the imbalance between the number of morphological and molecular taxonomic studies, which have been relatively intense, as compared to the paucity of studies on ecology, soils, mycorrhizae, cytology, biochemistry, and other aspects of Chinese *Tuber*. This imbalance limits the scope of the preliminary conclusions provided by the modern lines of Chinese truffle research. Further research is required into ecological patterns and processes and the biological variability of the Chinese populations of *Tuber*, by conducting long-term studies covering large territorial units in a country such as China, which has one of the highest levels of biodiversity in the world. In this regard, new populations of *Tuber* may well be found in China and other Asian countries which may provide new taxonomic, ecological, and commercial discoveries on this valuable product.

Until 2000, Chinese research on *Tuber* species was very limited (19 references). However, in the last 10 years, Chinese research has produced at least 33 references on *Tuber* taxonomy, ecology, phylogeny, and truffle culture: 3 doctoral theses, 14 scientific papers and 16 articles in international journals listed in the Journal Citation Report (JCR). A comparative analysis was done of Chinese *Tuber* research by Chinese authors versus authors from other countries. An analysis of the trend in Chinese *Tuber* research is shown in Fig. 2. In the first period, the number of Chinese references is clearly lower than published references from other countries (Fig. 2). However, the last 10 years have seen an increase in Chinese publications, and these results indicate a higher scientific production in comparison with other countries. Nevertheless, recently, Wang (2008) explained that China is still well behind the Western world in terms of both research on and cultivation of truffles, and also indicated that careful management, regulation, and conservation of truffles in China are urgently needed.

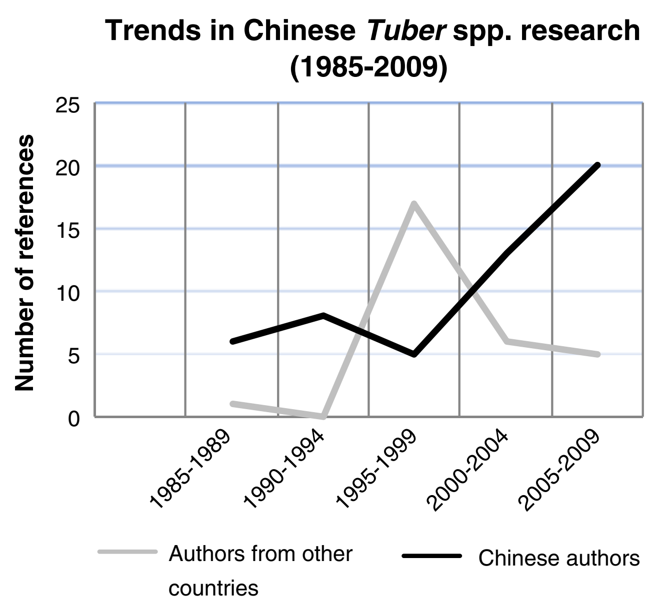

Fig. 2 Comparative analysis between Chinese *Tuber* research (on taxonomy, ecology, phylogeny, and truffle culture) developed by Chinese authors versus authors from other countries

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