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RESEARCH ARTICLE

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Micro-morphological identification study on *Cordyceps sinensis* (Berk.) Sacc. and its adulterants based on stereo microscope and desktop scanning electron microscope

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Abstract

The Chinese Materia Medica, *Cordyceps sinensis* (called "Dongchongxiacao" in Chinese), used as a tonic for nearly 600 years by Traditional Chinese Medicine, which has been recorded by Chinese Pharmacopoeia. This drug is rare and precious, which in turn lead to the emergence of adulterants derived from the same genus of *Cordyceps*. The adulterants which can be commonly found in the market are *Cordyceps gunnii* (called "Gunichongcao" in Chinese), *Cordyceps liangshanensis* (called "Liangshanchongcao" in Chinese), and *Cordyceps gracilis* (called "Xinjiangchongcao" in Chinese). This study combined a desktop scanning electron microscope and stereo microscope to distinguish *C. sinensis* from the above three adulterants especially on their different characters of caterpillar parts. Referring to the professional entomological literature, the micro-morphological features including the cuticle of the abdomen and the planta of abdomen prolegs were observed, photographed, and expressed based on the description of macroscopic characters. The identification method studied in this article is more convenient, quick, and environmental friendly.

KEYWORDS
adulterant, *Cordyceps sinensis*, desktop scanning electron microscope, micro-morphological identification, stereo microscope

1 | INTRODUCTION

Cordyceps sinensis (Berk.) Sacc. (Family Hypocreaceae), called "Dongchongxiacao" in Chinese, is a composite consisting of the stroma of fungus, parasitized on the larva of some species of insects (Family Hepialidae), and the dead caterpillar. It is one of the famous and valuable Chinese Materia Medica. This herb is distributed in the Qinghai-Tibet plateau and its surrounding high-altitude areas (Zhang, Liu, & Huang, 2008). According to Traditional Chinese Medicine, it has the action of tonifying kidney, replenishing lung, stanching bleeding, and resolve phlegm (Chinese Pharmacopoeia Commission, 2015). Modern pharmacological studies showed that *C. sinensis* mainly contains the components of polysaccharides, sugar alcohols, amino acids and nucleosides, sterols, fatty acids, and so forth, and has the immunomodulatory, antioxidant, and antitumor activities, and so forth (Chen, Wang, Nie, & Marcone, 2013; Olatunji et al., 2018; Qiu, Cao, & Han, 2016; Wang et al., 2017; Yue, Ye, Zhou, Sun, & Lin, 2013). "Dongchongxiacao" is mainly collected from the wild, and is the only accepted species in the genus of *Cordyceps* that has been recorded officially as a herbal drug in Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015). With the overharvesting of this species and dramatic rise in price, the resource of *C. sinensis* is decreasing year by year, which in turn has led to the emergence of adulterants derived from the same genus of *Cordyceps* (Zhuo & Da, 2014).

Cordyceps gunnii (Berk.) Berk. is one of the most commonly found adulterant of "Dongchongxiacao" in the market (Liang, 1983; Liang, 2007),

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called Gunichongcao in Chinese and distributed in low altitude areas (200-800 m) of some southern provinces in China (Wu et al., 1997; Zhu, Wang, & Han, 2004). *Cordyceps liangshanensis* Zang, Liu and Hu and *Cordyceps gracilis* (Grev.) Dur. et Mont. are the other two adulterants which were commonly used as "Dongchongxiacao" in Sichuan and Xinjiang provinces respectively (Hu, 1983; Pu, 1983).

Although these adulterants have some similar chemical components with *C. sinensis* (Chen, Tian, Meng, & Fu, 2003; Wu & Tang, 2011), but they are different in the source of fungi, the host larva, producing areas, and growing environment. On the other hand, some wholesalers sometimes used the dyed and processed adulterants to sold as "Dongchongxiacao"(Zeng, 2009). In particular, *C. gunnii* has toxicity and adverse reactions reports (Xu, 2009). These adulterants have important implications for safety and efficacy in Morden clinical practice. Therefore, it is necessary to carry out research on identification methods in order to prevent confusion.

Microscopic identification by a light microscope is the most practical method to authenticate *C. sinensis* from its adulterants or counterfeits (Au et al., 2012; Chan et al., 2011; Gao, Wang, Zeng, Mai, & Ma, 2011; Hu, Kang, & Zhao, 2003; Kang, Luo, Zheng, & Lin, 2011; Liu, Hua, Chu, Li, & Li, 2011), which is considered as a more convenient, low-cost and environment-friendly method when compared with chemical components analysis (Deng, Cheong, Wang, Zhao, & Li, 2018; Guo, Li, Huang, Liang, & Chen, 2006; Wang et al., 2013; Zuo et al., 2013) and DNA sequencing analysis (Duan, Shang, Zhang, & Zheng, 2017; Wong, Wong, & Shawa, 2015; Zhang, Kang, Wei, & Ma, 2015).

Microscopic identification study of *C. sinensis* mainly focuses on fungal characters such as the embedded type of perithecia and the shape of the ascus, which is found only in the mature individuals (He & Zhang, 2000; Liang, Liu, & Liu, 1995). However, *C. sinensis* is mostly harvested with a short stroma, and mature individuals are difficult to find in the commodity. Therefore, the studies on microscopic identification methods have gradually focused on the characters of caterpillar parts (Chan et al., 2011; Kang, Zhang, & Lin, 2013; Ye et al., 2016). But most of the features reported in this literature could not be clearly characterized under the light microscope and correctly expressed using professional terms.

Hence, this study aimed to research the differences of *C. sinensis* and its adulterants on their micro-morphological characteristics of the caterpillar parts using stereo microscope (SM) and desktop scanning electron microscope (SEM), and describe the characteristics based on professional entomological literatures (Gullan & Cranston, 2014; Lu, Guan, & Wu, 1951; Zhu, 1965; Zhu et al., 2004), then establish a quick, simple, and environmental friendly method to distinguish *C. sinensis* from its three adulterants.

2 | MATERIALS AND METHODS

2.1 | Materials

Different batches of samples from *C. sinensis* and its adulterants-*C. gunnii*, *C. liangshanensis*, and *C. gracilis* were investigated (Table 1). The voucher specimens were identified by the authors and preserved in the National Institutes for Food and Drug Control (China).

TABLE 1 List of the tested samples

No.	Latin name	Chinese phonetic name	Source	Collection time
A1	*C. sinensis*	Dongchongxiacao	Changdu, Xizang	2015.6
A2	*C. sinensis*	Dongchongxiacao	Guoluo, Qinghai	2015.6
A3	*C. sinensis*	Dongchongxiacao	Yushu, Qinghai	2015.6
A4	*C. sinensis*	Dongchongxiacao	Biru, Xizang	2015.5
A5	*C. sinensis*	Dongchongxiacao	Deqin, Yunnan	2015.6
B1	*C. gunnii*	Gunichongcao	Huaihua, Hunan	2010.6
B2	*C. gunnii*	Gunichongcao	Hanshou, Hunan	2010.7
B3	*C. gunnii*	Gunichongcao	Xinyang, Henan	2015.5
B4	*C. gunnii*	Gunichongcao	Qiandongnan, Guizhou	2015.5
B5	*C. gunnii*	Gunichongcao	Lushan, Jiangxi	2019.6
C1	*C. liangshanensis*	Liangshanchongcao	Luzhou, Sichuan	2017.10
C2	*C. liangshanensis*	Liangshanchongcao	Luzhou, Sichuan	2017.10
C3	*C. liangshanensis*	Liangshanchongcao	Liangshan, Sichuan	2017.9
C4	*C. liangshanensis*	Liangshanchongcao	Liangshan, Sichuan	2017.9
C5	*C. liangshanensis*	Liangshanchongcao	Liangshan, Sichuan	2017.9
D1	*C. gracilis*	Xinjinagchongcao	Aletai, Xinjiang	2010.7
D2	*C. gracilis*	Xinjinagchongcao	Altai, Xinjiang	2015,6
D3	*C. gracilis*	Xinjinagchongcao	Aletai, Xinjiang	2015,7
D4	*C. gracilis*	Xinjinagchongcao	Aletai, Xinjiang	2019,6
D5	*C. gracilis*	Xinjinagchongcao	Urumqi, Xinjiang	2019.6

2.2 | Reagents and apparatus

A digital single-lens reflex (DSLR) camera (Canon 5D4, Japan) was used for acquiring photographs of the samples. A stereo microscope (SM) (Zeiss SteREO Discovery V12, Germany) equipped with a digital camera (Zeiss Axiocam 506, Germany) and a desktop scanning electron microscope (SEM) (Phenom Pure desktop SEM, Netherlands) were used for acquiring photographs of the micro-morphological characters.

3 | METHODS

A soft bristle brush was dipped in little water and 95% alcohol separately to remove the dust and residual hyphae membrane present on the surface of each batch of the sample. The samples were photographed using the DSLR camera. In addition, the characters of *C. sinensis* from its three adulterants, including shape, color, size, and so forth, were described and compared.

Then the samples were placed on the microscope stage to observe the characteristics under visible light, which included the cuticle of the abdomen segments and the planta of the abdomen proleg. Then, the function of extended depth of focus was used to capture and merged the images. The blade was held to scrape the body wall and the proleg on the abdomen of the caterpillar part. Then the material was adhered to the carbon conductive tape (25 mm W x 5 ml, Tedpella) and treated them by spray-gold. This was followed by observation under the desktop SEM with 10 kV, and 25% spot size was selected. A temperature-controlled SEM stage was used to freeze all samples below 20°C in each case to maintain the shape of the samples in a high vacuum SEM environment.

4 | RESULTS

4.1 | Description

4.1.1 | *C. sinensis*

A caterpillar sclerotium is joined with a stroma growing out from the vertex of the head. The stroma is mostly single, slenderly cylindrical, slightly twisted, unbranched, 4–7 cm long, and 2–3 mm in diameter; externally dark brown to brown in color, with fine longitudinal wrinkles; and the texture is a flexible and whitish fracture (Figure 1a). The caterpillar part is slightly cylindrical, slightly curved, 2.0–4.8 cm long, and 3–6 mm in diameter, including the head, thorax, and abdomen (front to back); the head is small, slightly shrunken, yellowish-brown to reddish-brown; the thorax is 0.6–0.7 cm long, pale yellow to yellow, with relatively fine annulations at the dorsal side and three pairs of jointed legs at the ventral side; the abdomen is 2.0–4.0 cm long, dark yellow to yellowish-brown, including 10 segments, with significant annulations at the dorsal side and five pairs of significant protuberant prolegs at the ventral side; the texture is fragile, easily broken, slightly even fracture, yellowish-white, and with dark brown stripes (residual internal organs) in the center (Figure 2a).

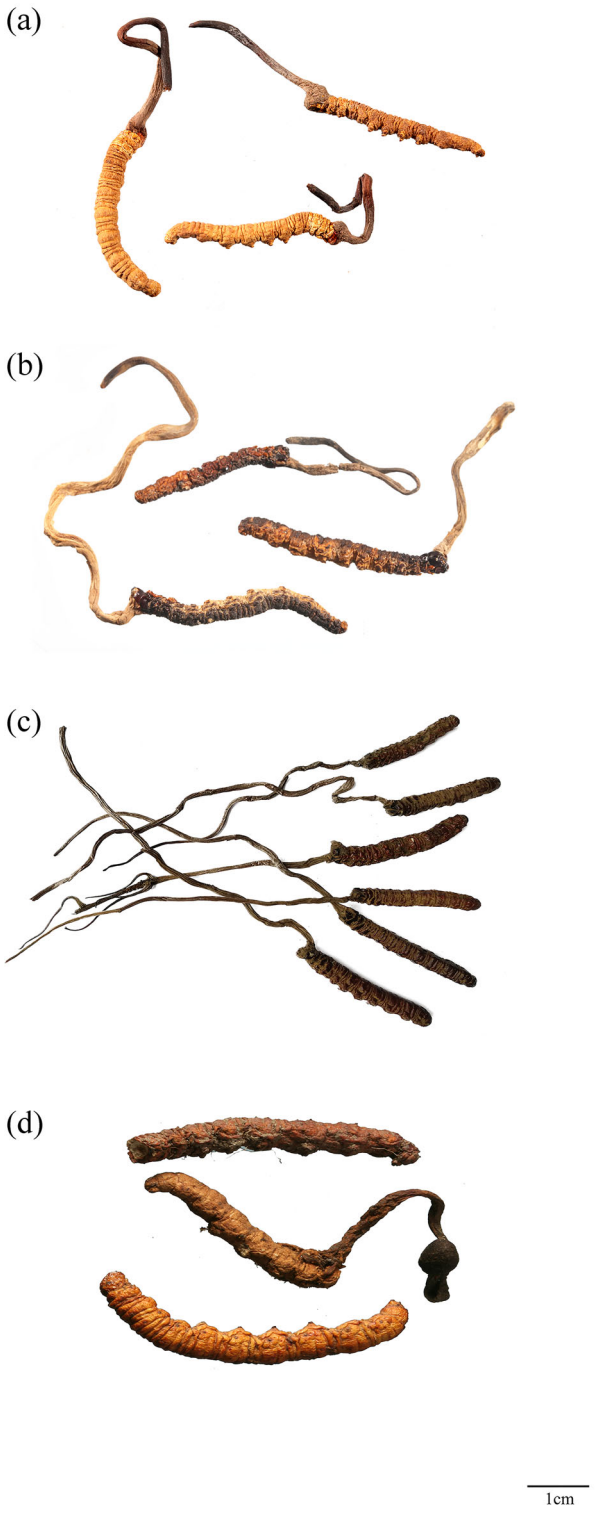

FIGURE 1 Crude drugs of *Cordyceps sinensis* (a), *Cordyceps gunnii* (b), *Cordyceps liangshanensis* (c), and *Cordyceps gracilis* (d)

4.1.2 | *C. gunnii*

A caterpillar sclerotium is joined with a stroma which is grown out from the vertex of the head. The single or multiple stroma is slightly

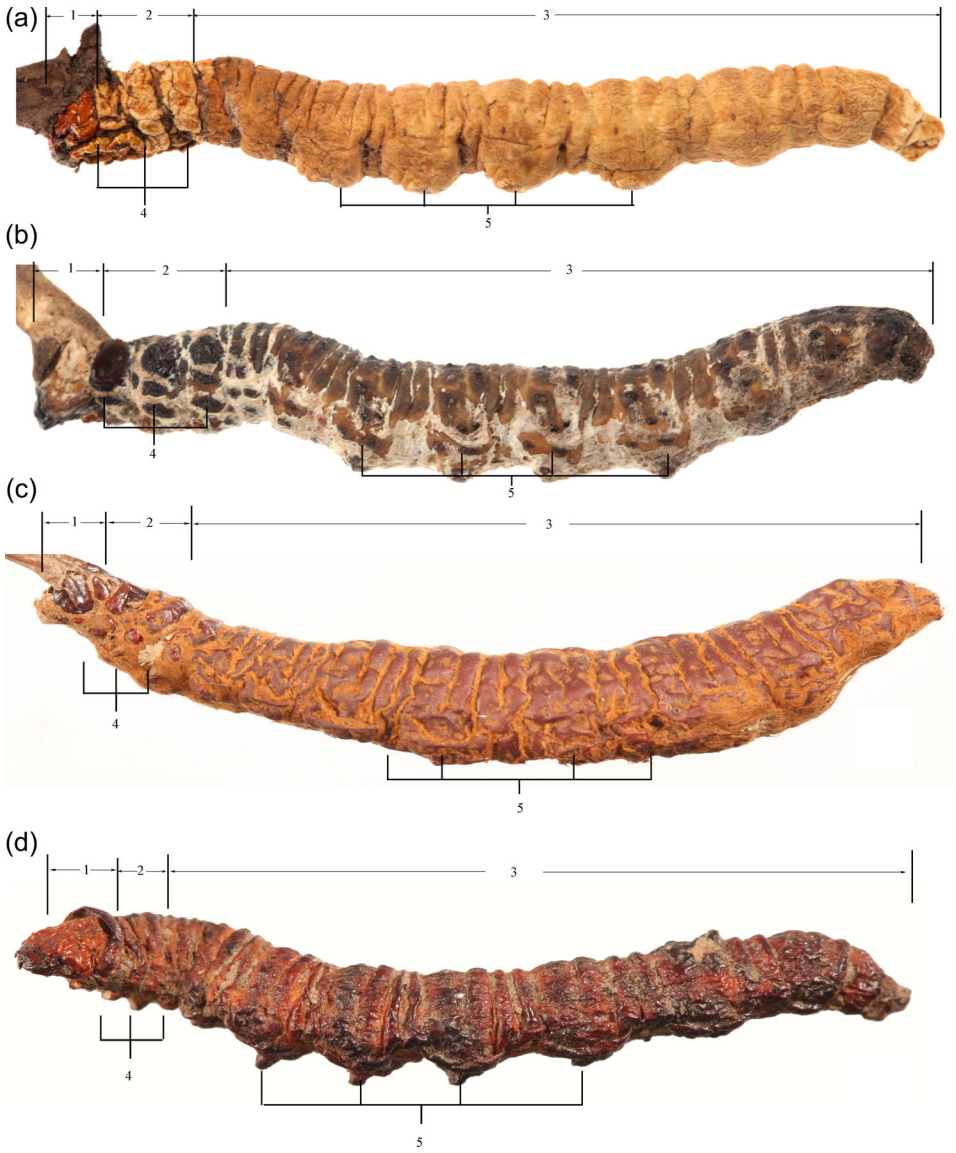

FIGURE 2 Caterpillar part of *Cordyceps sinensis* (a), *Cordyceps gunnii* (b), *Cordyceps liangshanensis* (c), and *Cordyceps gracilis* (d). 1. Head; 2. Thorax; 3. Abdomen; 4. Thorax leg; 5. Abdomen prolegs

cylindrical, slightly twisted, sometimes branched, 2–8 cm long, and 2–4 mm in diameter, externally gray to grayish-brown (Figure 1b). The caterpillar part is slightly cylindrical, slightly curved, 2.5–5.2 cm long, and 4–7 mm in diameter; most include a whitish hyphae membrane on the surface; the head is slightly enlarged, smooth and lustrous, dark brown; the thorax is 0.4–0.8 cm long, dark brown, with relatively sparse annulations at the dorsal side and three pairs of jointed legs at the ventral side; the abdomen is 1.8–4.0 cm long, brown to dark brown, with shallow annulations at the dorsal side and five pairs of slightly protuberant prolegs at the ventral side (Figure 2b).

4.1.3 | *C. liangshanensis*

A caterpillar sclerotium is joined with a stroma which is grown out from one side of the head. Stroma is almost single, slender cylindrical, unbranched or upper branched, irregularly curved or twisted, 9–26 cm long, 1.5–2.8 mm in diameter, externally yellowish-brown to dark brown (Figure 1c). The caterpillar sclerotium is slightly spindle-shaped and twisted, 2.5–4.3 cm long and 5.3–8.3 mm in diameter, densely covered with yellowish-brown to brownish hyphae membrane; the head is smaller, 2.4–3.3 mm wide, smooth and lustrous, dark brown; the thorax is 0.3–0.5 cm long, reddish-brown, with relatively sparse annulations at the dorsal side; the abdomen is 1.5–3.8 cm long, reddish-yellow, with irregular annulations at the dorsal side and 5 pairs of flat prolegs at the ventral side (Figure 2c).

4.1.4 | *C. gracilis*

Stroma is rarely found, almost single, slender cylindrical, almost unbranched, 1.8–2.6 cm long, 1.0–1.5 mm in diameter; externally brown to yellowish-black (Figure 1d). The caterpillar sclerotium is sub-cylindrical, slightly curved, 2.3–3.8 cm long and 3.2–5.2 mm in diameter; the head is small, 2.8–3.3 mm wide, slightly shrunken, yellowish-brown to dark brown; the thorax was 0.3–0.6 cm long, yellowish to reddish-brown, with relatively fine annulations at the dorsal side; the abdomen is 1.9–3.2 cm long, yellowish to reddish-brown,

TABLE 2 Comparison of the descriptive characteristics of *Cordyceps sinensis* and its adulterants

			C. sinensis	*C. gunnii*	*C. liangshanensis*	*C. gracilis*
Stroma	Number		Mostly single	Single or multiple	Mostly single	Rare
	Branched		Unbranched	Sometimes branched	Unbranched or upper branched	Unbranched
	Color		Dark brown to brown	Gray to grayish-brown	Yellowish-brown to dark brown	Brown to yellowish-black
Caterpillar	Surface		Without hyphae membrane	With whitish hyphae membrane	With yellowish-brown to brownish hyphae membrane	Without hyphae membrane
	Head	Shape	Small, slightly shrunk,	Enlarged, smooth and lustrous	Small, smooth and lustrous	Small, slightly shrunken
		Color	Yellowish brown to reddish brown	Dark brown	Dark brown	Yellowish brown to dark brown
	Thorax	Color	Pale yellow to yellow	Dark brown	Reddish-brown	Yellowish to reddish-brown
		Annulations	Fine	Sparse	Sparse	Fine
	Abdomen	Color	Dark yellow to yellowish brown	Brown to dark brown	Reddish-yellow	Reddish-brown
		Annulations	Significant	Shallow	Irregular	Significant
		Prolegs	Significant protuberant	Slightly protuberant	Flat	Significant protuberant

with significant annulations at the dorsal side and five pairs of significant protuberant prolegs at the ventral side (Figure 2d; Table 2).

4.2 | Micro-morphological characteristics

4.2.1 | Cuticle of the abdomen segments

C. sinensis

Under stereo microscope: The surface is rough, dark yellow to yellowish-brown (Figure 3a1). Multiple pinaculums are arranged regularly in each abdominal segment. The pinaculum is subrounded, 300–500 μm in diameter, yellow, with a smooth or slightly wrinkled surface, slightly lustrous, with one or two rounded nests in the center (Figure 4a1).

Under desktop scanning electron microscope: The surface is densely covered with microthichiae, and scattered or aggregated in granular-like protuberance; conical microthichiae, irregularly arranged, 2–35 μm long, 1–2 μm wide at the base, with a sharp terminal (Figure 3a2); the protuberance agglomerate is 25–60 μm in diameter, with a rough surface and small microthichiae (Figure 3a3). The pinaculum has many significant tiny holes on the surface and a ring-shaped trichopore near the center; the trichopore is 75–105 μm in diameter, with a protuberant edge; the visible seta residue is in the sunken center (Figure 4a2,a3).

C. gunnii

Under stereo microscope: The surface is smooth, lustrous, dark brown (Figure 3b1). The pinaculum is subrounded, 770–950 μm in diameter, with a smooth or slightly wrinkled surface, lustrous, sometimes damaged, brownish-black, with one or two rounded nests in the center (Figure 4b1).

Under desktop scanning electron microscope: Most of the cuticle surface is uneven, with glyph-like striation and many fine tiny holes, scattered with subrounded warty protuberance (Figure 3b2,b3); The surface of the pinaculum is smooth, with many insignificant tiny holes and a ring-shaped trichopore near the center; the trichopore was 100–120 μm in diameter, had a slightly protuberant edge (Figure 4b2,b3).

C. liangshanensis

Under stereo microscope: The surface is rough, lustrous, dark brown (Figure 3c1). The pinaculum was subrounded, 500–600 μm in diameter, yellowish-brown, with the slightly wrinkled surface, slightly lustrous, with one or two rounded nests in the center (Figure 4c1).

Under desktop scanning electron microscope: The surface is uneven, with the hyphae spread under the cuticle; some hyphae protrude from the surface, long tubular, hollow (Figure 3c2,c3). The pinaculum had many insignificant tiny holes on the surface and a ring-shaped trichopore near the center; the trichopore was 50–75 μm in diameter, with a protuberant edge (Figure 4c2,c3).

C. gracilis

Under stereo microscope: The surface is smooth, slightly lustrous, yellowish to reddish-brown (Figure 3d1). The pinaculum is reddish-yellow, with an unclear edge and one or two rounded nests in the center (Figure 4d1).

Under desktop scanning electron microscope: The surface is smooth or uneven, with many significant tiny holes (Figure 3d2,d3); The surface of the pinaculum is wrinkled, with many significant tiny holes and a ring-shaped trichopore near the center; the trichopore is 80–120 μm in diameter, with a slightly protuberant edge (Figure 4b2, b3; Table 3).

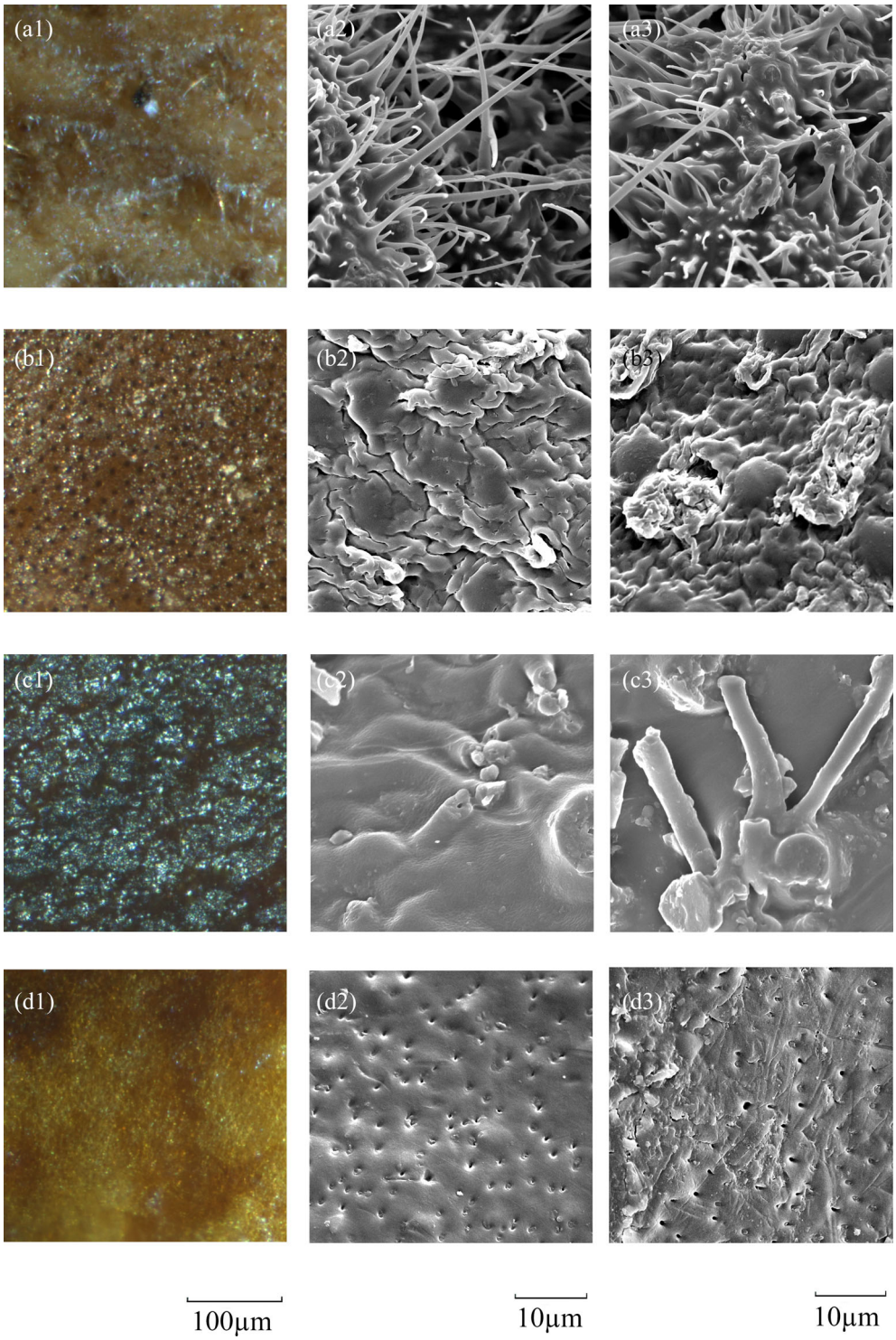

FIGURE 3 Micro-morphological characteristics of the cuticle surface (a) *Cordyceps sinensis*, (b) *Cordyceps gunnii*, (c) *Cordyceps liangshanensis*, (d) *Cordyceps gracilis*. 1. Observed under stereo microscope (×150); 2 and 3. Observed under desktop scanning electron microscope (×6500)

4.2.2 | Planta of the abdomen proleg

C. sinensis

Under stereo microscope: The planta is located on the top of the abdomen proleg, subrounded, with many brownish-yellow crochets on the surface, arranged in 5–6 circular rings, gradually shorter from inside to outside (Figure 5a1).

Under desktop scanning electron microscope: The crochet is conical, with smooth surface and an obtuse terminal; the inner ones is 35–65 μm long, and is 10–15 μm wide at the base, and curved outwards (Figure 5a2); the outer ones is 20–25 μm long, and 8–10 μm wide at the base, and upstanding or curved outwards (Figure 5a3).

C. gunnii

Under stereo microscope: The planta is oblate, with many brownish-yellow to brown crochets on the surface, arranged in 4–7 oblate rings; and the innermost ones are more than twice as long as the outer ones (Figure 5b1).

Under desktop scanning electron microscope: The crochet is conical, with longitudinal striation on the surface and an shape

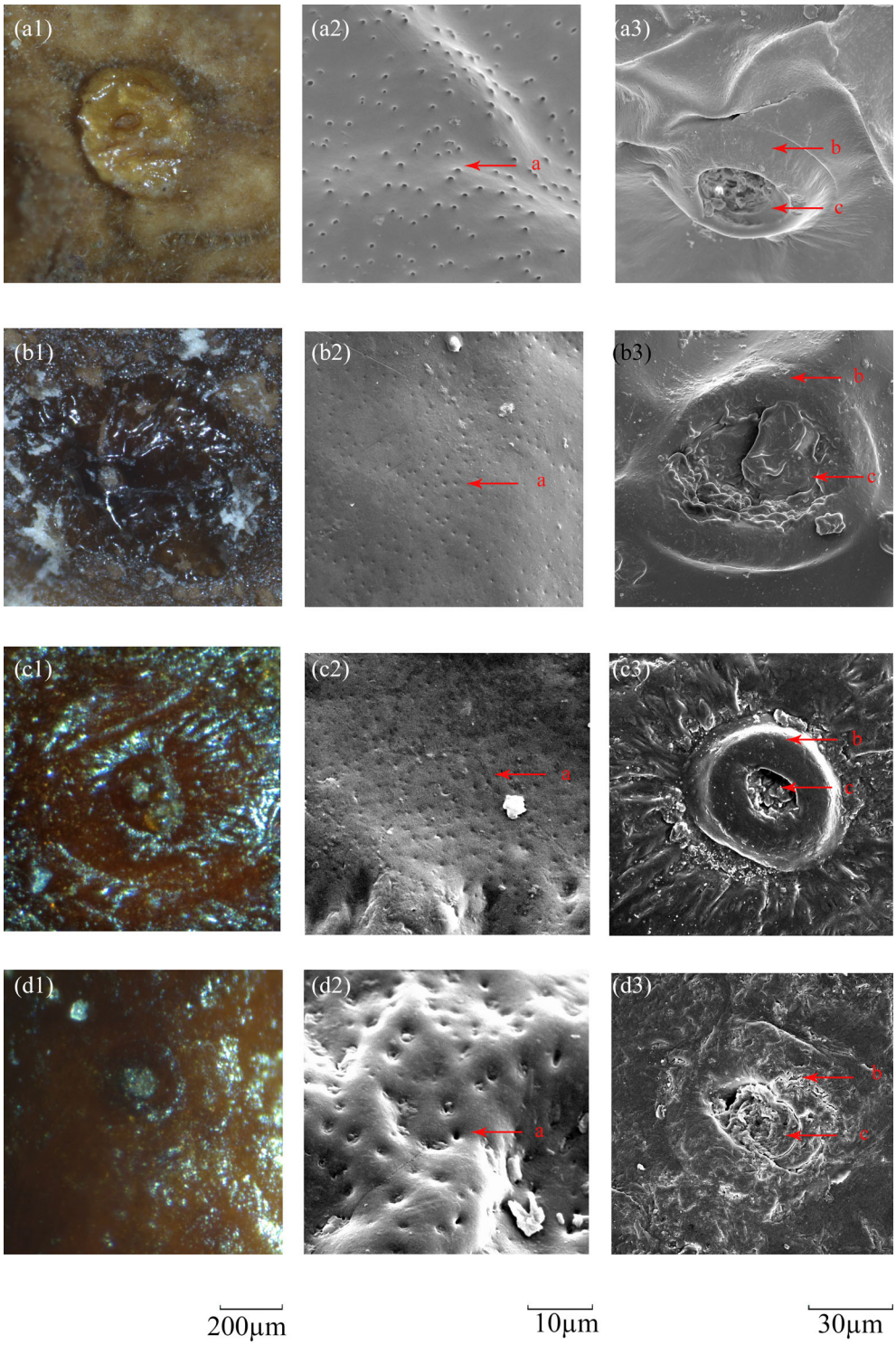

FIGURE 4 Micro-morphological characteristics of the pinaculum (a) *Cordyceps sinensis*, (b) *Cordyceps gunnii*, (c) *Cordyceps liangshanensis*, (d) *Cordyceps gracilis*. 1. Observed under stereo microscope (×100); 2 and 3. Observed under desktop scanning electron microscope (2—×2000; 3—×6500). a. Fine and tiny holes; b, The edge of trichopore; c, The center of trichopore

terminal; and the inner ones is 55–100 μm long, and 15–20 μm wide at the base, curved outwards, (Figure 5b2); the outer ones is 20–25 μm long, and 7–10 μm wide at the base, and also curved outwards (Figure 5b3).

C. liangshanensis

Under stereo microscope: The planta is oblate, with many yellow to reddish-yellow crochets on the surface, arranged in a single ring or 2–3 oblate rings; and the inner crochets are nearly as long as the outer ones (Figure 5c1).

Under desktop scanning electron microscope: The crochet is conical, with a smooth surface and an acute terminal; and the inner crochets are similar sharp with the outer ones, 20–25 μm long, and 7–10 μm wide at the base, and also curved outwards (Figure 5c2,c3).

C. gracilis

Under stereo microscope: The planta is subrounded, with many brownish-yellow crochets on the surface, arranged in 3–5 circular rings, and is gradually shorter from inside to outside (Figure 5d1).

TABLE 3 Comparison of micro-morphological characteristics of *Cordyceps sinensis* and its adulterants

			C. sinensis	*C. gunnii*	*C. liangshanensis*	*C. gracilis*
Cuticle	Under SM	Surface	Rough, lustrousless	Smooth, lustrous	Rough, lustrous	Smooth, slightly lustrous
		Pinaculum size	300–500 μm in diameter	770–950 μm in diameter	500–600 μm in diameter	500–600 μm in diameter
		Pinaculum color	Yellow	Brownish-black	Yellowish-brown	Yellowish to reddish-brown
	Under desktop SEM	Surface	Densely covered with microthichiae	Covered with glyph-like striation	With the hyphae spread under the cuticle	Smooth or uneven
		Microthichiae	Conical, 2–35 μm long	–	–	–
		Tiny holes on the surface	Insignificant	Insignificant	Insignificant	Significant
		Trichopore of the Pinaculum	75–105 μm in diameter	100-120 μm in diameter	50–75 μm in diameter	80–120 μm in diameter
Planta	Under SM	Shape	Subrounded	Oblate	Oblate	Subrounded
		Crochets	5–6 circular rings	4–7 oblate rings	Single ring or 2–3 oblate rings	3–5 circular rings
		Changes of crochets	Gradually shorter from the inside to the outside	The innermost ones more than twice as long as the secondary outer ring	The inner crochets are nearly as long as the outer ones	Gradually shorter from the inside to the outside
	Under desktop SEM	Surface of crochet	Smooth	With longitudinal striation	Smooth	Smooth
		Size of the inner crochets	35–65 μm long	55–100 μm long	20–25 μm long	25–45 μm long
		Shape of the outer crochets	Upstanding or curved outwards	Curved outwards	Curved outwards	Upstanding or curved outwards

Under desktop scanning electron microscope: The crochet is conical, with smooth surface and an obtuse terminal; the inner ones is 25–45 μm long, and is 8–15 μm wide at the base, and curved outwards (Figure 5d2); the outer ones is 18–25 μm long, and 8–12 μm wide at the base, and upstanding or curved outwards (Figure 5d3).

5 | DISCUSSION

Traditional macroscopic identification is one of the important methods for the authentication of Chinese Materia Medica (Zhao, 2011). However, it is very difficult to distinguish the fine morphological differences between Chinese Materia Medica and its adulterants. With the help of stereo microscope and scanning electron microscope, the micro-morphological characteristics of Chinese Materia Medica can be observed. At the same time, clear images can be obtained by the digital camera system. Through the comparison of the digital micro-morphological characteristics, the origin of Chinese Materia Medica can be identified more accurately.

In this article, we studied some important identification characteristics of *C. sinensis* emphasizing on its caterpillar sclerotium parts, such as the cuticle of the abdomen and the planta on the abdomen prolegs, based on the reference of professional entomological literature using desktop scanning electron microscope and stereo microscope. The comparison study between *C. sinensis* and its adulterants indicated that they are different from each other. The differences are mainly associated with the different host insects. The host of *C. sinensis* and *C. gracilis* are different species from the genus of Hepialus (Family Hepialidae). Nevertheless, the host of *C. gunnii* and *C. liangshanensis* are separately derived from two different genus of Hepialidae family (Liang, 1983; Liang, 2007; Zhang et al., 2008).

In this study，the micro-morphological characteristics of the caterpillar parts have been studied to identify *C. sinensis* and its adulterants

The cuticle of the larvae acts as a barrier between the living tissues and the environment. It is thin and flexible in many larvae, but its structure remains complex and varies among different taxonomic groups (Gullan & Cranston, 2014). The microtrichiae on the cuticle of the abdomen is considered as a very important identification character to distinguish *C. sinensis* from its adulterants.

On the other hand, the Lepidopteran caterpillars are characterized with polypod larvae, cylindrical bodies, short thoracic legs, and abdominal prolegs (pseudopods). The prolegs on the abdomen are usually lobe-like and have a planta each on the top. The planta bears

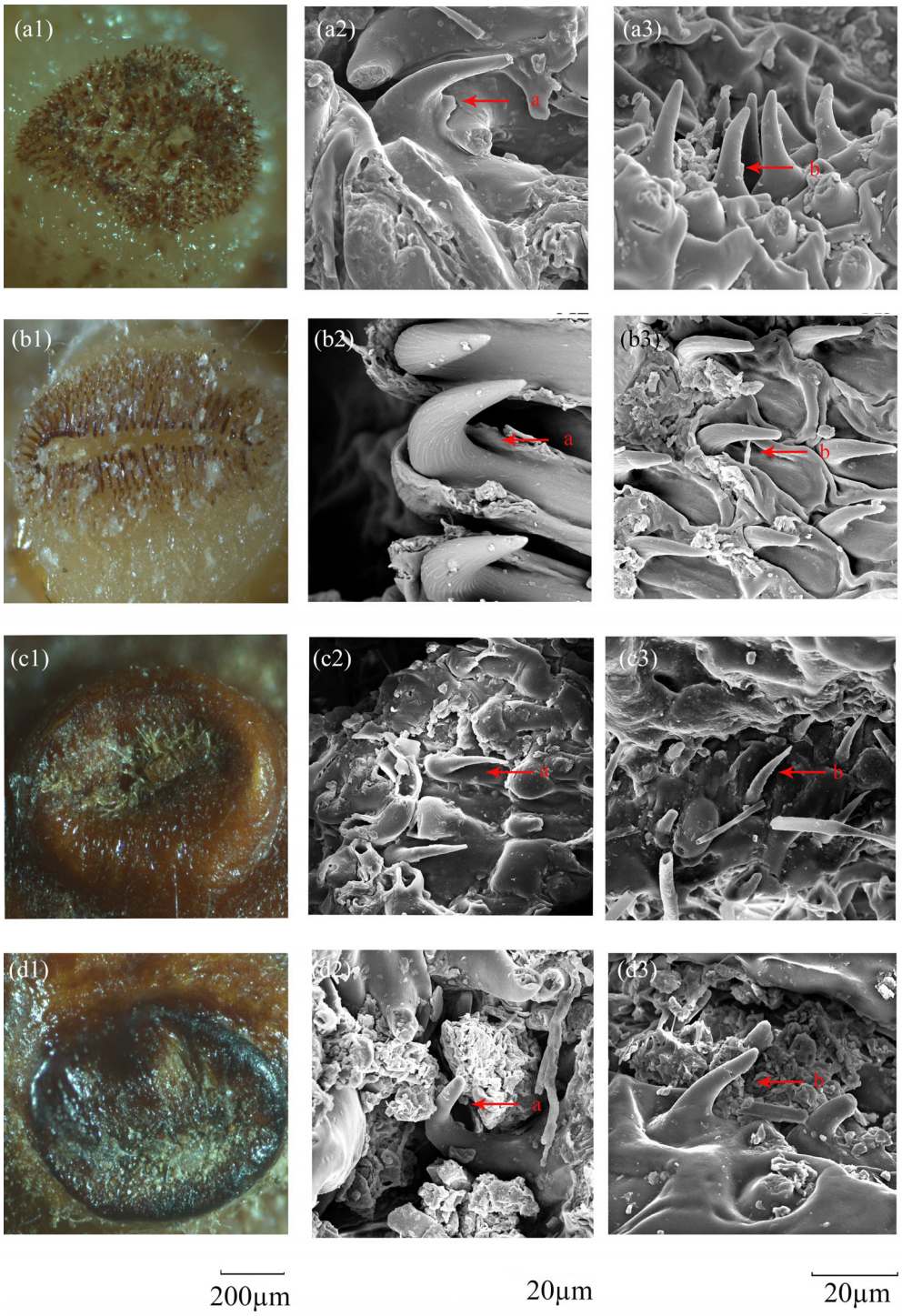

FIGURE 5 Micro-morphological characteristics of the planta of the abdomen proleg (a) *Cordyceps sinensis*, (b) *Cordyceps gunnii*, (c) *Cordyceps liangshanensis*, (d) *Cordyceps gracilis*. 1. Under stereo microscope (×150); 2 and 3. Under desktop scanning electron microscope (×4000). a, The inner crochets; b, The outer crochets

crochets, which is an important feature that distinguishes the larvae of lepidoptera from other insect larvae. The arrangement of crochets is also often used as a basis for the classification of lepidoptera (Lu et al., 1951; Zhu, 1965; Zhu, Wang & Ha, 2004). As our study report, the surface of crochets on the planta and their changes from inside to outside is different among *C. sinensis* and its adulterants.

For the first time, the use of desktop SEM for identification of "Dongchongxiacao" was reported in this article. The micro-morphological characteristics could be observed clearly under desktop SEM, which has advantage in particularly observing the surface characteristics of biological samples

Compared with the floor SEM modules, the Phenom desktop SEM can be used easily. Meanwhile, it has very good signal performance owing to the adoption of CeB6 electron ejection source. Taking the advantage of a set of dedicated designed vacuum differential system inside the column, desktop SEM has shortened the outgassing time significantly to 15 s, which normally need 5–10 min in traditional floor SEM modules. More importantly, this vacuum differential system

assists in observing the fragile samples a lot. When traditional floor SEM were used, the sample must be well prepared by following a series of complicated sample preparation procedures like chemical fixation and gradient dehydration to maintain the plumpness in ultra-high vacuum level (normally 10−5 Pa). However, in desktop SEM, the sample chamber was designed to work at 0.1 Pa, and this prevents the samples from narrowing even without the involvement of those pre-preparation procedures.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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