Acta Pharmaceutica Sinica B 2015;5(4):358–366

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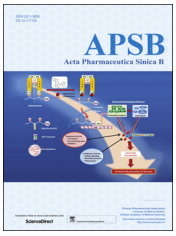

ORIGINAL ARTICLE

Morphological and microscopic characterization of five commonly-used testacean traditional Chinese medicines

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Received 21 September 2014; received in revised form 4 December 2014; accepted 14 March 2015

KEY WORDS

Testacean traditional Chinese medicine;
Morphological characteristics;
Microscopic characteristics;
Normal light microscopy;
Polarized light microscopy

Abstract Testacean traditional Chinese medicine (TTCM), derived from the outer shell of sea or freshwater mollusks, is a special and important category of Chinese medicinal materials. To ensure the effective use of TTCM, a comparative identification study was performed on five commonly-used testacean drugs, including Haliotidis Concha, Arcae Concha, Meretricis Concha, Ostreae Concha and Margaritifera Concha (Shijueming, Walengzi, Geqiao, Muli and Zhenzhumu in Chinese, respectively). Typical morphological photographs of the crude drugs were acquired, and the key microscopic characteristics of the derived powders under normal light microscope and polarized light microscope were summarized. The major results can be concluded as follows: (1) the original species involved in the five TTCMs could be distinguished by their respective interspecies morphological characteristics; (2) the key identification characteristics of the five powdered crude drugs were mainly crystal fragments, with the fragment features under both normal light and polarized light microscope providing powerful points for differentiating the five commonly-used testacean drugs. This study demonstrated that it is feasible to provide authentication for these five kinds of TTCMs by the combination of morphology with microscopy.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2015.03.014

1. Introduction

Testacean traditional Chinese medicine (TTCM) is mainly from the outer shell of sea or freshwater mollusks, with properties of both mineral and animal drugs. In China, use of this kind of medicine can be traced back to 200 BC in Shen Nong Ben Cao Jing, the first traditional Chinese medicine book. It is estimated that about 100 kinds of testacean drugs are documented in Chinese Marine Materia Medica, a contemporary monograph descriptive of marine drugs[1]. Recently, abundant shell resources are attracting more and more scientists to investigate their medicinal value.

Haliotidis Concha, Arcae Concha, Meretricis Concha, Ostreae Concha and Margaritifera Concha are recorded in the Chinese Pharmacopoeia, with Chinese names of Shijueming, Walengzi, Geqiao, Muli and Zhenzhumu, respectively. The five kinds of TTCMs are used extensively as effective medicines to induce sedation, calm the nerves, improve eyesight or to soften hard masses and eliminate nodulations[2–6]. However, commercial shell drugs are mostly applied in coarse powder, which is prone to give rise to species confusion due to their similarity in appearance and color. What is worth noting is that authentication work for the five kinds of TTCMs has not been established.

These testacean medicinal materials are composed mainly of calcium carbonate or calcium sulfate, in hard texture. For mollusk shells, previous research was mainly on their formation, structure and organic matrix[7–9]. Although some methods including X-ray diffraction, infrared and atomic absorption spectrometry have been applied to identification, expensive instruments are needed[10–12]. It is well known that microscopic techniques are fast, accurate, economical as well as simple, and have been used widely for the authentication of herbal, animal and even mineral medicines[13–15]. A few microscopic studies[1,16] on TTCMs have been reported, however, only cursory hand-drawing pictures on cross section were provided and none of them showed detailed color illustrations of the characteristics of powder. Herein we perform the identification of Haliotidis Concha, Arcae Concha, Meretricis Concha, Ostreae Concha and Margaritifera Concha by morphological and microscopic study. Morphological characteristics are depicted with color photographs and powder microscopic characteristics using normal light microscopy combined with polarized light microscopy.

2. Materials and methods

2.1. Materials

Twenty-five samples were collected from different main production areas. Among these samples, there were 7 batches of Haliotidis Concha, 5 batches of Arcae Concha, 4 batches of Meretricis Concha, 4 batches of Ostreae Concha and 5 batches of Margaritifera Concha. All these shells were obtained from coastal areas and used as crude experimental materials without calcination after rinsing, cracking and powdering. The details of each sample are presented in Table 1. These samples were authenticated by Prof. Huijun Li (China Pharmaceutical University, Nanjing, China) and deposited at the State Key Laboratory of Natural Medicines of China Pharmaceutical University.

2.2. Apparatus and software

A digital camera (Cannon EOS 500D, Japan) was used for taking morphological photographs of the typical shell samples. A TCM grinder (TAISITE FW135, China) was used to prepare the powdered crude drugs. An optical microscope (Nikon E200, Japan) equipped with a digital camera (Nikon Smart V550D, Japan) was used to observe the powder features and Smart V550D Capture software to record the photographs.

2.3. Reagents

Dilute glycerin was prepared according to procedures described in Appendix XV B, Pharmacopoeia of the People's Republic of China, 2010[17].

2.4. Methods

The exterior features of each authenticated sample were examined by observing, measuring and touching. The color digital photographs of typical samples were taken with a digital camera. Each sample of these crude drugs was powdered using a grinder and passed through a 300 μm sieve, sealed with dilute glycerin when observed under the microscope. The powder of each sample was observed for at least 10 slides. The distinctive representative characteristics under normal light and polarized light microscope were chosen and imaged.

3. Results

3.1. External morphology

The typical color photos and external morphological characteristics of the 5 kinds of TTCMs (15 species in total) are given in Fig. 1 and Table 2. *Haliotis diversicolor* Reeve, *Haliotis discus hannai* Ino, *Haliotis ruber* (Leach), *Haliotis asinina* Linnaeus and *Haliotis laevigata* (Donovan), as the original species of Haliotidis Concha; *Arca subcrenata* Lischke, *Arca inflata* Reeve, *Arca granosa* Linnaeus as the species of Arcae Concha; *Meretrix meretrix* Linnaeus, *Cyclina sinensis* Gmelin as the species of Meretricis Concha; *Ostrea talienwhanensis* Crosse, *Ostrea rivularis* Gould, as the species of Ostreae Concha; *Hyriopsis cumingii* (Lea), *Cristaria plicata* (Leach), *Pteria martensii* (Dunker), as the species of Margaritifera Concha, were studied in this paper.

3.2. Powder microscopy

The key microscopic characteristics of the five powdered crude drugs under normal and polarized light microscopy are given in Fig. 2 and Fig. 3. The detailed descriptions of each drug powder composed of their respective original species are described below. The transverse section view of prismatic layer fragments can be observed in the powder of Haliotidis Concha, Ostreae Concha and Margaritifera Concha, of which the comparison data are displayed in Table 3.

3.2.1. Microscopic characteristics of Haliotidis Concha

Nacreous layer fragments are visible everywhere, differing in size, mostly in polygonal or irregular shapes; surfaces are with slightly granular nature. These fragments are usually imbricate or in dune shape, some with a distinct wavy margin; under the polarized microscope, they show dark blue centrally and yellow at the margin. Polygonal dark brown or tan fragments can be observed, opaque under the polarized microscope. Prismatic layer fragments

Table 1 Collection data of five kinds of testacean drugs.

Testacean drug	Source	Sample number	Species	Collection area
Haliotidis Concha	Coastal area	SJM-1	*H. diversicolor*	Yulin, Guangxi
		SJM-2	*H. discus*	Yulin, Guangxi
		SJM-3	*H. discus*	Anguo, Hebei
		SJM-4	*H. ruber*	Yulin, Guangxi
		SJM-5	*H. ruber*	Anguo, Hebei
		SJM-6	*H. asinina*	Guangzhou, Guangdong
		SJM-7	*H. laevigata*	Guangzhou, Guangdong
Arcae Concha	Coastal area	WLZ-1	*A. subcrenata*	Zhuhai, Guangdong
		WLZ-2	*A. subcrenata*	Qingdao, Shandong
		WLZ-3	*A. subcrenata*	Nanning, Guangxi
		WLZ-4	*A. inflata*	Putian, Fujian
		WLZ-5	*A. granosa*	Ningbo, Zhejiang
Meretricis Concha	Coastal area	GQ-1	*M. meretrix*	Qingdao, Shandong
		GQ-2	*M. meretrix*	Wenchang, Hainan
		GQ-3	*M. meretrix*	Xiamen, Fujian
		GQ-4	*C. sinensis*	Tianjin
Ostreae Concha	Coastal area	ML-1	*O. talienwhanensis*	Qingdao, Shandong
		ML-2	*O. talienwhanensis*	Lianyungang, Jiangsu
		ML-3	*O. rivularis*	Zhanjiang, Guangdong
		ML-4	*O. rivularis*	Wenchang, Hainan
Margaritifera Concha	Coastal area	ZZM-1	*H. cumingii*	Nanjing, Jiangsu
		ZZM-2	*H. cumingii*	Anqing, Anhui
		ZZM-3	*C. plicata*	Zhuji, Zhejiang
		ZZM-4	*C. plicata*	Nanjing, Jiangsu
		ZZM-5	*P. martensii*	Beihai, Guangxi

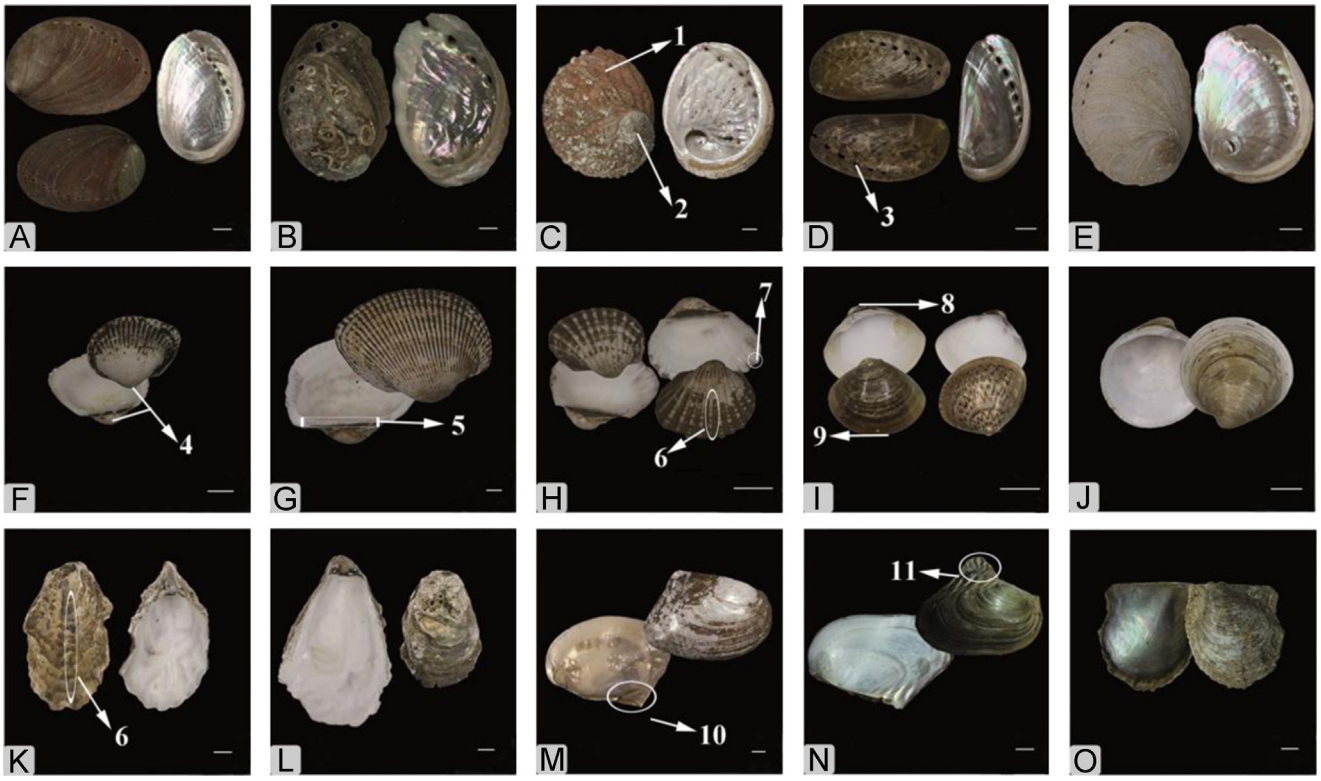

Figure 1 Typical color photographs of intact shells. Shells of (A) *H. diversicolor*; (B) *H. discus*; (C) *H. ruber*; (D) *H. asinina*; (E) *H. laevigata*; (F) *A. subcrenata*; (G) *A. inflata*; (H) *A. granosa*; (I) *M. meretrix*; (J) *C. sinensis*; (K) *O. talienwhanensis*; (L) *O. rivularis*; (M) *H. cumingii*; (N) *C. plicata*; (O) *P. martensii*.1, Body whorl part; 2, spiral part; 3, protuberance with an open; 4, umbo; 5, hinge and a row of denticles; 6, radiated rib; 7, protruding long tooth; 8, dorsal margin; 9, ventral margin; 10, triangular sail-like hind wing; 11, large crest.

Table 2 Morphological characteristics of the 5 TTCMs (15 species in total).

Testacean drug	Original species	Morphological characteristics	
		Shape and size	Shell surface
Haliotidis Concha	*H. diversicolor*	Elongated ovoid, inner sideear-like	The outer dark red or brown, with yellowish-brown cloudiness, spiral ribs and fine growth lines. The spiral part small, the body whorl large; dozens of protuberances arranged towards right from the second spiral whorl to the edge of the body whorl part, usually the last 6–8 open. The inner smooth, with pearly luster.
	H. discus	Long oval	The outer brown or turquoise, with many coarse and irregular wrinkles, usually with attachments such as bryozoans; the last 3–5 protuberances open. The inner showing turquoise pearly luster.
	H. ruber	Flat ovoid, large	The outer usually brick-red, with attachments similar to white lime; the spiral ribs and growth lines ridged in undulation. The last 7–9 protuberances open. The inner with nacreous luster.
	H. asinina	Long and narrow, ear-like, slightly small and curved	The outer smooth, greyish- or yellowish-green, with triangular or irregular jade green, brown or apricot speckles. The last 5–7 protuberances, mostly ellipsoidal, open on the same level with the shell surface.
	H. laevigata	Oval, somewhat large and flat	The outer ecru, growth lines relatively conspicuous.
Arcae Concha	*A. subcrenata*	Long oval and oblique fan-shaped	The shell surface bulgy, the umbo curling inwards to the ligament. The outer white, with brownish coat and minute hairs; radiated ribs distinct. The inner white, with depressions at the margin corresponding to the ribs on the outer surface, forming protruding long teeth. The hinge straight, bearing a row of denticles.
	A. inflata	Large	Similar to the shell of *A. subcrenata*.
	A. granosa	Somewhat small, long oval	The outer surface with brownish coat but no minute hairs; radiated ribs distinct, with extremely obvious granular protuberances.
Meretricis Concha	*M. meretrix*	Somewhat triangular, the ventral side arch-like	The outer smooth, with a coat like oil paint, mostly in yellowish-brown to dark brown; serrated or wavy brown stripes forming circular figured-belt, varying with individual differences. The inner white. The hinge wide, the right shell with three cardinal teeth and two front lateral teeth; the left shell with three cardinal teeth and one front lateral teeth.
	C. sinensis	Subrounded	The outer usually in pale yellow; concentric growth ring pulses above the shell surface, forming ring fine ribs. The inner white, the edge occasionally in purple, with a row of denticles. Both the right and left shells with three cardinal teeth, without lateral teeth.
Ostreae Concha	*O. talienwhanensis*	Subtriangular, the right shells somewhat flat, while the left concaved in box-shape	The outer in pale yellow or purple-brown; concentric scales arranged loosely and undulated up and down; the left shell with thick radiated ribs. The inner white, trace of adductor unobvious.
	O. rivularis	Oval or subtriangular	The outer pale yellow, brown or greyish-green; concentric growth scales overlapped one another. The inner white; trace of adductor mostly in half-moon-shape, in light yellow or purple.
Margaritifera Concha	*H. cumingii*	Large, somewhat flat, in scalene triangular. Dorsal posterior margin projected upward, forming a large triangular sail-like wing; ventral margin slightly arch-like	The outer with a layer of brownish-yellow to pitchy horn, concentric growth lines distinct. The inner opalescent, with pearly luster, trace of mantle visible; both the right and left shells with two pseudo-cardinal teeth, the left with two slat lateral teeth, the right with one slat lateral tooth.
	C. plicata	Large, somewhat in scalene triangular. Dorsal posterior margin expanded upward, forming a large crest; ventral margin straight	The outer in tan or pitchy, margin mostly in brownish-yellow; several brownish-yellow or greenish-yellow stripes radiated from the umbo to the ventral margin visible; growth lines distinct; longitudinal ribs gradually thick arranged from the

Table 2 (*continued*)

Testacean drug	Original species	Morphological characteristics	
		Shape and size	Shell surface
			umbo to the back of the shell. The inner lustrous, trace of mantle visible; both the right and left shells with two lateral teeth.
	P. martensii	Small, somewhat flat, in oblique square. The posterior ear large, while the anterior ear small. Dorsal margin straight, ventral margin rounded	The outer with fine concentric growth lines, forming slices. The inner with strong luster.

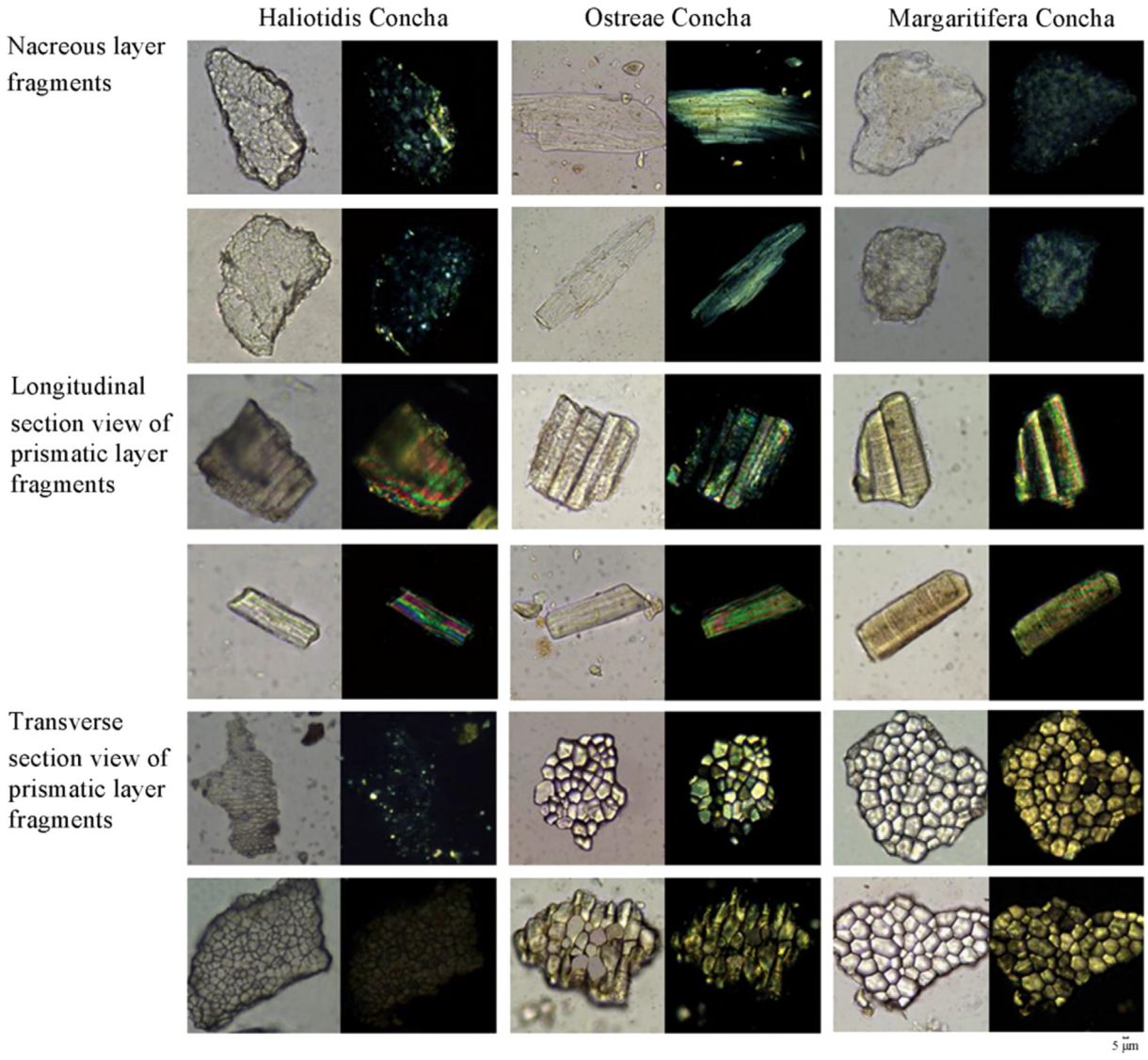

Figure 2 The key microscopic characteristics of Haliotidis Concha, Ostreae Concha and Margaritifera Concha.

are rare, and single ones are usually prismatic in longitudinal section view; sometimes several arranged regularly, polygonal in the transverse section view, polychromic and dark yellow when observed under the polarized microscope.

3.2.2. Microscopic characteristics of Arcae Concha

Fragments differ in size, in strips and irregular polygons; larger ones are mostly yellowish-brown, smaller ones pale yellow. Layering of crystal fragment margins is not obvious. Smaller fragments in light yellow usually show peacock-feather-like polychromicity under the polarized microscope. Some brown fragments with bulgy radiated ribs can be seen. Fragments with minute hair fractures are often visible. Occasionally, stripes of some brown fragments can be observed and quadrangular yellowish-brown fragments of the shell coat can be viewed.

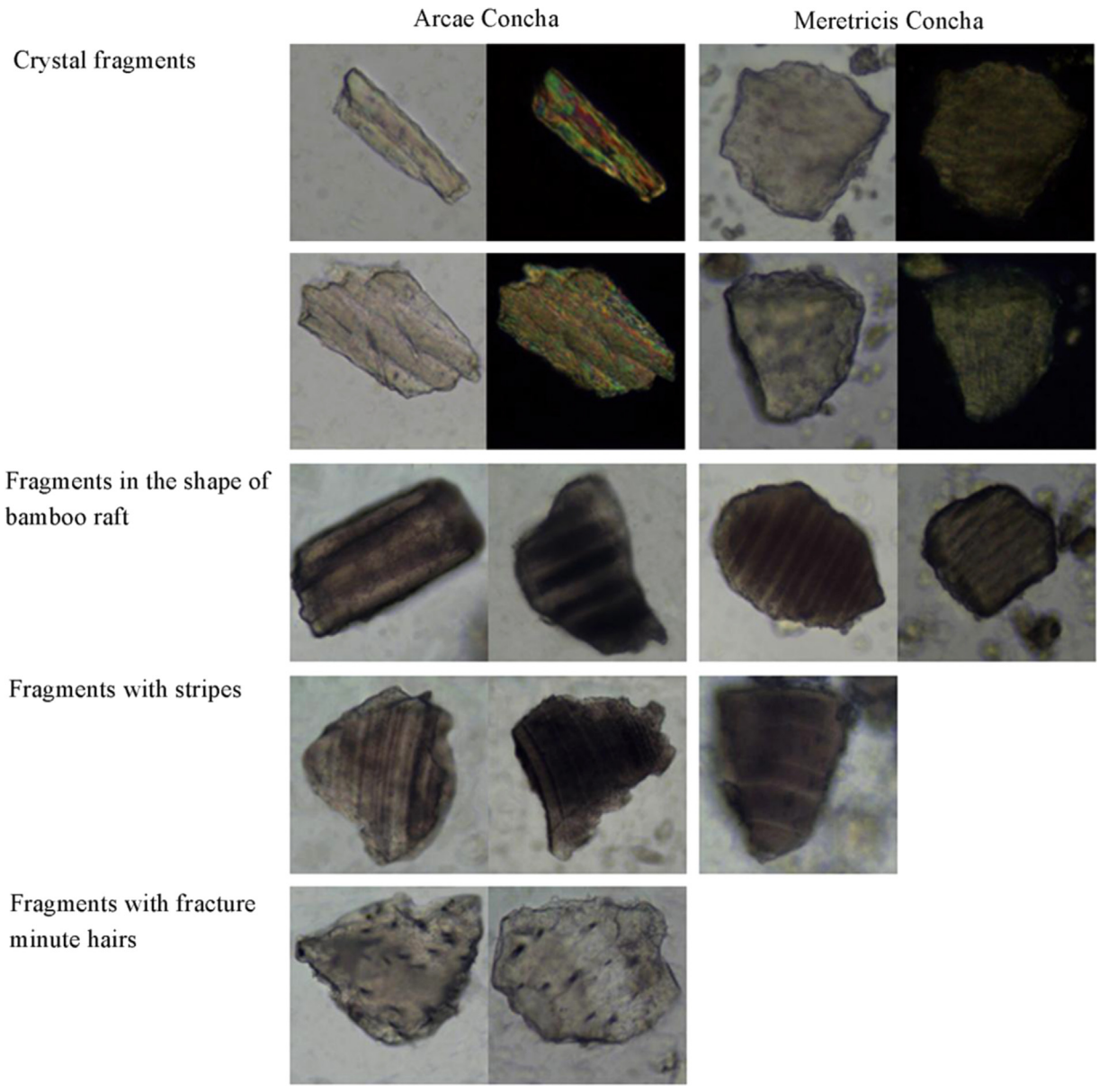

Figure 3 The key microscopic characteristics of Arcae Concha and Meretricis Concha.

Table 3 Comparison data of transverse section view of prismatic layer fragments.

Testacean drug	Species	Transverse section view of prismatic layer fragments	
		Length (μm)	Width (μm)
Haliotidis Concha	*H. diversicolor*	18–52	6–20
	H. discus	15–50	4–12
	H. ruber	17–40	7–20
	H. asinina	25–85	5–17
	H. laevigata	20–70	6–12
Margaritifera Concha	*H. cumingii*	40–112	8–23
	C. plicata	45–200	17–50
	P. martensii	24–78	6–30
Ostreae Concha	*O. talienwhanensis*	33–72	6–22
	O. rivularis	15–100	7–20

3.2.3. Microscopic characteristics of Meretricis Concha
Fragments differ in size, mostly in sub-squares; surface color is dark, and usually brown, opaque, or dark yellow when observed under the polarized microscope. Crystal fragments are with unobvious layer structure, some fragments in the shape of a bamboo raft. Some fragments are brown with light and dark stripes occasionally seen.

3.2.4. Microscopic characteristics of Ostreae Concha
Nacreous layer fragments are mostly irregular, larger in long strip or plate-like structures; surface is uneven and fine striations are nearly visible, pale yellow or translucent, showing yellow and blue color when observed under the polarized microscope. Prismatic layer fragments are occasionally noticeable, some appearing in rows. Longitudinal section view of the prismatic layer fragments shows prisms, while the transverse section view is polygonal under the polarized microscope, showing polychromicity or dark yellow and yellow.

3.2.5. Microscopic characteristics of Margaritifera Concha
Nacreous layer fragments are visible everywhere, dissimilar in size, mostly in polygonal or irregular shapes, with obvious granularity on the surface. These fragments are usually imbricate or in dune shape, some with serrated margins; under the polarized microscope, they show dark blue centrally and yellow at the margin. Prismatic layer fragments are rare. Longitudinal section views show obvious transverse striations, with prismatic shapes, mostly truncated at the fracture-end, polygonal in transverse section view. These fragments show polychrome and dark yellow when observed under the polarized microscope.

4. Discussion and conclusions

In this paper 25 authenticated samples gathered from different areas were studied, including 7 species of Haliotidis Concha, 5 of Arcae Concha, 4 of Meretricis Concha, 4 of Ostreae Concha, and 5 of Margaritifera Concha (Table 1).

In the morphological studies, different species of the 5 TTCMs can be distinguished from each other by their respective interspecies morphological features (Fig. 1 and Table 2). Different species of Haliotidis Concha show different shell surface colors. The outer surface of the shell of *H. diversicolor* is dark red or brown, with yellowish-brown or green cloudiness; *H. discus* has many coarse and irregular wrinkles on the shell surface, in brown or turquoise; the outer shell surface of *H. ruber* is brick-red, bearing attachments similar to white lime. Scattered triangular or irregular apricot or dark green cloudiness can be found on outer surface of shell of *H. asinina*, which is greyish-green or yellowish-green; green stripes are usually visible on pale yellow and white shell surface of *H. laevigata*. Three original species of Arcae Concha share similar external features. Compared to the shells of *A. subcrenata* and *A. inflata*, *A. granosa* is small, without minute hairs, and radiated ribs have distinct granular protuberances; *A. inflata* is larger than *A. subcrenata*. The shells of *M. meretrix* and *C. sinensis* are easily distinguished by their external morphology, for example, the shell outer surface of *M. meretrix is* often yellowish-brown, with serrated stripes, while *C. sinensis* usually pale yellow, with fine ribs. When it comes to Ostreae Concha, several distinct ribs radiated from the umbo of the left shell of *O. talienwhanensis,* and can be used to differentiate from *O. rivularis*. The key macroscopic feature of Margaritifera Concha is a hinge tooth.

Table 4 Morphological diagnostic keys to each species of the five TTCMs.

1. The original species with one shell, the outer surface of shell with three spiral whorls.
  2. The outer surface usually with attachments.
    3. The outer brown or turquoise, usually with attachments such as bryozoans……..….*H. discus.*
    3. The outer usually brick-red, with attachments similar to white lime……..……..…*H. ruber.*
  2. The outer surface without attachments.
    4. The outer not smooth.
      5. The outer dark red or brown, with yellowish-brown cloudiness……*H. diversicolor.*
      5. The outer ecru……...*H. laevigata.*
    4. The outer smooth……..*H. asinina.*
1. The original species with one right and one left shells, without spiral whorls.
  6. The outer surface with distinct radiated ribs.
    7. The inner surface of shell with a row of hinge denticles.
      8. The outer with brownish coat and minute hairs
        9. The shell small……*A. subcrenata.*
        9. The shell large……...*A. inflata.*
      8. The outer without minute hairs……*A. granosa.*
    7. The inner surface of shell without hinge teeth
      10. Trace of adductor unobvious……..…*O. talienwhanensis.*
      10. Trace of adductor mostly in half-moon-shape……..…….*O. rivularis.*
  6. The outer surface without distinct radiated ribs.
    11. The right shell with one or two lateral teeth.
      12. The right shell with one lateral tooth……..….. *H. cumingii.*
      12. The right shell with two lateral teeth.
        13. The left shell with one lateral tooth……..…… *M. meretrix.*
        13. The left shell with two lateral teeth…….…… *C. plicata.*
    11. The right shell without lateral teeth.
      14. The outer with concentric growth ring pulses…… *C. sinensis.*
      14. The outer with fine concentric growth lines, forming slices…… *P. martensii.*

Table 5 Microscopic diagnostic keys to the five TTCMs.

1. The drug powder with prismatic layer fragments and nacreous layer fragments.
   2. Nacreous layer fragments imbricate or in dune shape.
      3. Transverse section view of prismatic layer fragments 15–85 μm in length, 4–20 μm in width…….….. Haliotidis Concha.
      3. Transverse section view of prismatic layer fragments 24–200 μm in length, 6–50 μm in width……..…….. Margaritifera Concha.
   2. Nacreous layer fragments in long strip or plate-like.
      4. Transverse section view of prismatic layer fragments 15–100 μm in length, 6–22 μm in width……..…… Ostreae Concha.
1. The drug powder without distinct prismatic layer fragments and nacreous layer fragments.
   5. Some fragments with bulgy radiated ribs and fracture minute hairs……..…… Arcae Concha.
   5. Some fragments in shape of bamboo raft……..... Meretricis Concha.

The left shell of *H. cumingii* has two pseudo-cardinal teeth and one slat lateral tooth; the left shell of *C. plicata* is the same as right shell, has one anterior lateral tooth and one posterior lateral tooth, and no pseudo-cardinal teeth; *P. martensii* has no teeth.

The morphological diagnostic keys to each species of the five TTCMs are established in Table 4. As regarding powder microscopic characteristics of the five TTCMs, the key identification points are summarized in Table 5. Due to the powder of Arcae Concha and Meretricis Concha lacking obvious structure in the nacreous and prismatic layers, Haliotidis Concha, Ostreae Concha and Margaritifera Concha will be mainly discussed in powder microscopic identification. In our normal light microscopic observation, fragments of nacreous layer showed laminated structure, while the prismatic layer showed prismatic structure, and transverse section views revealed polygonal structures. Nacreous layer fragments of Haliotidis Concha had closely arranged lamellae, somewhat thin, with the fracture margin undulating up and down, while some of *H. discus*, *H. asinina* margins were slight serrated; Ostreae Concha fragments appeared in long strips or were plate-like, often broom-like at one end, with a straight fracture margin, showing fine stripes; Margaritifera Concha with fine little sawteeth and lamellae arrange closely. Prismatic layer fragments of Haliotidis Concha are rare and smaller, several often in rows, and transverse section views are difficult to find, 15–85 μm in length and 4–20 μm in width; prismatic fragments of Ostreae Concha often truncate in fracture-end, some taper at the other end, with transverse section views 15–100 μm in length and 6–22 μm in width. Obvious growth lines can be observed on prismatic fragments of Margaritifera Concha, with surface views generally 24–200 μm in length and 6–50 μm in width. Colors changes due to polarization state variation after polarized light passes through mineral crystals were studied in this paper[18]. Under the polarized light microscope, nacreous layer fragments are usually in blue at the central part, while prismatic layer fragments are often polychromatic. Nacreous layer fragments of Haliotidis Concha and Margaritifera Concha show dark blue at the center and yellow at the margin, while Ostreae Concha shows yellow at the center and blue at the margin. Longitudinal section views of prismatic layer fragments of these crude drug powders usually show bright polychromicity, sometimes in dark yellow.

Sixteen commercial crude samples of the five TTCMs bought from drug stores were examined using powder microscopy. The results indicated different TTCMs could be easily differentiated from each other under the guidance of Table 5, although the original species of each TTCM was not accurately authenticated.

In conclusion, despite the fact that the 5 commonly-used and easily confused TTCMs have similar chemical constituents, differences in powder fragment features under normal and polarized light microscope still exist. Based on our experimental results, the combination of morphological and microscopic characteristics of these drugs can be used as a simple and especially economic method to distinguish one from another. Further research on other TTCMs is needed.

Acknowledgments

Great appreciation is expressed for Hainan University's and Peking University's provision of samples of Margaritifera Concha and Haliotidis Concha. Financial support for this study was provided by the 863 Program of Ministry of Scientific and Technology of China (No. SS2013AA093002).

References

1. Guan HS, Wang SG. *Chinese marine materia medica*, vol. 3. Shanghai: Scientific and Technical Press; 80–427.
2. Ma AC, Yang X, Jiang Y, Li HH, Guan HS, Liu HB. Studies on the angiotensin-converting enzyme inhibitory activity of marine TCM Shi-Jue-Ming. *Period Ocean Univ China* 2012;**42**:135–7.
3. Tie BR. Comparison of trace elements in muli before and after processing. *China J Chin Mater Med* 1993;**18**:342–3, 381.
4. Xu GX, Lin Y, Wang TT, Wu YB. Pharmacological study of shijueming and its application progress in ophthalmology. *Int J Ophthalmol* 2009;**9**:2389–90.
5. Yang CL, Chen JT, Jin DD, Wang JJ, Zhao Y. The bone induction effect of water soluble matrix from nacre powder on human marrow stroma cells. *Chin J Clin Anat* 2007;**25**:190–3.
6. Zhang H, Zhang L, Liu Y. Studies on chemical components and pharmacological activities of Os Draconis (Longgu) and Ostreae Concha. *China J Chin Mater Med* 2011;**36**:1839–40.
7. Kamat S, Su X, Ballarini R, Heuer AH. Structural basis for the fracture toughness of the shell of the conch *Strombus gigas*. *Nature* 2000 1036–40.
8. Nudelman F, Gotliv BA, Addadi L, Weiner S. Mollusk shell formation: mapping the distribution of organic matrix components underlying a single aragonitic tablet in nacre. *J Struct Biol* 2006;**153**: 176–87.
9. Osuna-Mascaró A, Cruz-Bustos T, Benhamada S, Guichard N, Marie B, Plasseraud L, et al. The shell organic matrix of the crossed lamellar queen conch shell (*Strombus gigas*). *Comp Biochem Physiol B Biochem Mol Biol* 2014;**168**:76–85.
10. Shao JJ, Zhong JW, Chen JW, Li X, Cai BC. X-ray diffraction analysis and establishment of fingerprints of Ostreae Concha. *China Pharm* 2011;**22**:3697–9.
11. Li XY, Wang HZ, Sun SL, Zhang JB. Analysis on Fourier transform infrared and graphite furnace atomic absorption spectrometry of pearls with different colors. *J Gems Gemmol* 2007;**9**:15–8.
12. Bourrat X, Qiao L, Feng QL, Angellier M, Dissaux A, Beny JM, et al. Origin of growth defects in pearl. *Mater Charact* 2012;**72**:94–103.
13. Li Q, Chu C, Wang YQ, Chen HB, Li P, Zhao ZZ. Authentication of the 31 species of toxic and potent Chinese Materia Medica by microscopic technique assisted by ICP-MS analysis, Part 4: four kinds

of toxic and potent mineral arsenical CMMs. *Microsc Res Tech* 2011;**74**:1–8.
14. Yu KZ, Liu J, Guo BL, Zhao ZZ, Hong H, Chen HB, et al. Microscopic research on a multi-source traditional Chinese medicine, Astragali Radix. *J Nat Med* 2014;**68**:340–50.
15. Yan J, Fang B, Zhang SS, Hu XC, Zhang GS. Study on three-dimensional microstructure and chemical composition of bivalve shells of *Hyriopsis Cumingii* (Lea). *Mater Rev* 2013;**27**:108–12.
16. Lu HQ. Identification study of the medicinal shells. *Acta Pharma Sinica* 1980;**15**:495–501.
17. The State Pharmacopoeia Committee of China. *Pharmacopoeia of the People's Republic of China*. vol. 1. Beijing: China Medical Science Press; 2010; p. 33–6.
18. Kong FM, Li GH, Peng HD. Theoretical analysis of polarization state for polarizing microscope. *J Appl Opt* 2008;**29**:821–4.