

# 动物药残留毛的显微鉴定研究——蝉蜕及其混淆品的鉴别

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**摘要:**目的 以动物药残留毛的显微特征为依据, 对中药蝉蜕及其易混淆品进行鉴别。方法 对黑蚱、螳螂、焰螳螂的干燥蜕壳粉末进行显微制片观察, 结合图像测量软件提取刚毛的直径、长度、毛窝直径等显微特征参数, 进行比较分析。结果 黑蚱、螳螂、焰螳螂的虫体体表刚毛在形态、颜色、直径、长短等方面以及毛窝的形态、颜色、大小等方面均具鉴别特征, 并将之汇集成蝉蜕及混淆品的刚毛鉴定检索表。结论 利用动物药残留毛的显微特征差异, 可以达到蝉蜕真伪鉴别的目的。

**关键词:**蝉蜕; 黑蚱; 螳螂; 焰螳螂; 金蝉衣; 刚毛; 显微鉴定

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## 1 Introduction

Periostracum Cicadae is the larva slough of *Cryptotympana pustulata* (Fabricius). It was first reported in the book of "Separate Collection of Famous Doctors"<sup>[1]</sup>. Periostracum Cicadae is one of the commonly used Chinese medicines in China with the effect of dispersing wind-heat, promoting eruption, improving eyesight, dispelling wind and relieving spasm<sup>[2]</sup>. In modern pharmacological studies, it has been proved that Periostracum Cicadae possesses anti-convulsions, tranquil, anti-allergic and anticancer functions<sup>[1]</sup>. For the various species of cicada and the collectors' lack of experience in identification, there are many other species were reported being confused with Periostracum Cicadae in the Chinese medicinal materials market, such as the larva slough of *Platypleum kaempferi* (Fabricius). Moreover, Periostracum Tibicen, the larva slough of *Tibicen flammat*

(Dist.), has been regarded as a high-quality species of Periostracum Cicadae, which is usually exported to Southeast Asia<sup>[1-2]</sup>. Those species were often used in powder form or put into capsules and unable to be identified by naked eyes. Thus a research on identification of Periostracum Cicadae and its two counterfeits were conducted based on the characteristics of different polypide setae contained by the three kinds of insects.

## 2 Materials and Methods

**Materials:** Five passels of *Cryptotympana pustulata* (Fabricius) (A) as reference materials, and four passels of two other kinds of counterfeits, *Platypleum kaempferi* (Fabricius) (B) and *Tibicen flammat* (Dist.) (C), were purchased from different markets in China respectively. All the samples were authenticated by Prof. KANG Ting-guo and deposited in college of Chinese Materia Medica of Liaoning university of T. C. M. in China. The details are shown as Table 1.

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Tab 1 Origins of commercial crude drugs periostracum cicadae ( A ) and its two counterfeit s ( B and C )

表 1 蝉蛻 ( A )及其混淆品 ( B、C )的商品来源

Sample	Sample Number	Date Collected	Collecting areas
<i>Cryptotympana pustulata</i> ( Fabricius ) ( A ) ( Periostracum Cicadae )	P. C. I	Feb, 2003	Hospital Affiliated to Henan College of T. C. M. , Henan, China
	P. C. II	Mar, 2003	Chinese Medicinal Materials Market of Xi' an, Shanxi, China
	P. C. III	Mar, 2003	Chinese Medicinal Materials Market of Jinan, Shandong, China
	P. C. IV	May, 2003	Chinese Medicinal Materials Market of Anguo, Hebei, China
	P. C. V	May, 2003	Chengdafangyuan Pharmacy of Shenyang, Liaoning, China
<i>Platypleum kaempferi</i> ( Fabricius ) ( B ) ( counterfeit of Periostracum Cicadae )	P. K. I	Mar, 2003	Chinese Medicinal Materials Market of Jinan, Shandong, China
	P. K. II	May, 2003	Chinese Medicinal Materials Market of Chengdu, Sichuan, China
<i>Tibicen flammatus</i> ( Dist ) ( C ) ( Periostracum Tibicen )	P. T. I	May, 2003	Chinese Medicinal Materials Market of Chengdu, Sichuan, China
	P. T. II	May, 2003	Chinese Medicinal Materials Market of Chengdu, Sichuan, China

Apparatus: Olympus BX50 System Biologic Microscope;  
Olympus DP10 Camera.

Software: Win-measure

Reagent: Chloral hydrate<sup>[4]</sup>

Dilute glycerin ( prepared according to procedures described in Appendix 102, Pharmacopoeia of the People's Republic of China, part I , 2005 )

Methods: Powder of every sample, was washed and flushed 3 times by chloral hydrate, and then sealed with dilute glycerin. Each sample was observed under the optical and the system biologic microscope. Chose representative dimensions with respect to the middle diameter of seta shaft, the middle diameter of medullar and/or the length of 10 ~ 20 setae measured by win-measure, and the medullar index ( = diameter of medulla ÷ diameter of seta shaft × 100 ) for differentiation.

3 Observation and Results

3.1 Exterior Morphology

A) *Cryptotympana pustulata* ( Fabricius ):

The larva slough of *Cryptotympana pustulata* ( Fabricius ) is slightly elliptic and curving, about 3.5 cm long, and 2 cm in diameter. The external surface is yellowish-brown, translucent, and shiny. The head is of a pair of filiform antennae and the protruded compound eyes. The front jaw is protruded. The mouth is full-grown. The upper labrum is short. The lower labrum is like long tube-shaped. The dorsal surface is split like crisscross, and curled inwards. The thorax is left with scars of two pairs of wings on both sides, attached with 3 pairs of legs. The abdomen is blunt round. With a little soil, it is light, hollow and crispy. It is odourless and tasteless. ( Fig 1. A )

B) *Platypleum kaempferi* ( Fabricius ):

The larva slough of *Platypleum kaempferi* ( Fabricius ), one of the counterfeit s of Periostracum Cicadae, is smaller than that of *Cryptotympana pustulata* ( Fabricius ). Wrapped up with lots of soil, the external surface is translucent, grayish-yellow, 1.5 ~ 2 cm long and 0.8 ~ 1 cm in diameter. ( Fig 1. B )

C) *Tibicen flammatus* ( Dist ):

The larva slough of *Tibicen flammatus* ( Dist ) is longer, thinner and flatter than that of *Cryptotympana pustulata* ( Fabricius ). With little soil, the external surface is translucent, brown or reddish-brown, 3.5 ~ 3.8 cm long and 1.2 ~ 1.5 cm in diameter. ( Fig 1. C )

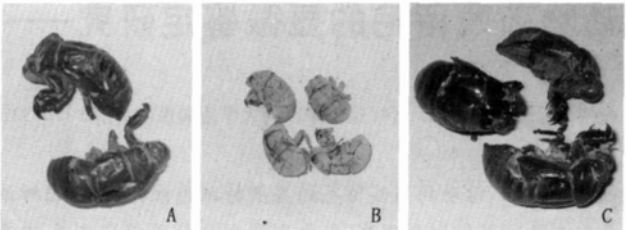


Fig 1 Raw Medicinal Materials of Periostracum Cicadae and that of its two Counterfeits

A - *Cryptotympana pustulata* ( Fabricius ); B - *Platypleum kaempferi* ( Fabricius ); C - *Tibicen flammatus* ( Dist )

图 1 蝉蛻及其混淆品外形图

A - 黑蛻; B - 蟋蛻; C - 焰蛻蝉

3.2 Microscopic Characteristics

A) *Cryptotympana pustulata* ( Fabricius ):

The long yellowish-brown or reddish-brown setae are 171 ~ 596 μm long, 5 ~ 95 μm in diameter, 2 ~ 15 μm in medullar diameter and 37.13 ± 8.24 in medullar index. The seta tip is blunt. The medullar cavity is rough on inner fringe, almost filling the whole seta shaft. The medullar is particulate on surface, reddish-brown, bended in wave-like line in the cavity frequently. The portion between the root and the basal part shrink unsymmetrically, 9 ~ 23 μm in diameter and 11 ~ 33 μm in basal portion diameter. The complete root is helmet-shaped, 15 ~ 29 μm in diameter and 6 ~ 13 μm in height. ( Fig 2. A<sub>1</sub> )

The long brownish-yellow setae are 104 ~ 408 μm long, 6 ~ 11 μm in diameter, 1 ~ 4 μm in medullar diameter and 28.08 ± 6.63 in medullar index. The seta tip is blunt. The medullar cavity is rough on inner fringe, almost filling the whole seta shaft. The medullar is yellowish-brown, filling up the cavity

cotton-likely frequently, bended in wave-like line occasionally. The portion between the root and the basal part shrink unsymmetrically,  $7 \sim 10 \mu\text{m}$  in diameter and  $9 \sim 15 \mu\text{m}$  in basal portion diameter. (Fig 2.  $A_2$ )

The light yellow slender setae are broken and bended frequently,  $4 \sim 12 \mu\text{m}$  in diameter,  $1 \sim 3 \mu\text{m}$  in medullar diameter and  $28.33 \pm 9.31$  in medullar index. The medullar is near colorless. The very narrow cavity is rough on inner fringe usually. (Fig 2.  $A_3$ )

Occasionally the setae are almost colorless, slender, broken and bended,  $4 \sim 6 \mu\text{m}$  in diameter, about  $1 \mu\text{m}$  in medullar diameter and  $15.9 \pm 4.41$  in medullar index. The medullar appears light brown. The medullar cavity is narrow. (Fig 2.  $A_4$ )

The stubby setae are brownish-yellow or reddish-brown,  $57 \sim 167 \mu\text{m}$  long,  $11 \sim 21 \mu\text{m}$  in diameter,  $3 \sim 5 \mu\text{m}$  in medullar diameter and  $21.90 \pm 3.50$  in medullar index. The seta tip is blunt. The medullar is reddish-brown usually, near black occasionally, and particulate on surface. The medullar cavity is smooth on inner fringe. The portion between the root and the basal part shrink unsymmetrically,  $16 \sim 24 \mu\text{m}$  in diameter and  $13 \sim 33 \mu\text{m}$  in basal portion diameter. (Fig 2.  $A_5$ )

There are three kinds of setae sacs and all appear circular-shaped. The smaller are yellow on outer fringe,  $6 \sim 10 \mu\text{m}$  in diameter. The black marks, from which the setae fall off, are visible. The greater are brownish-yellow ~ yellowish-brown on outer fringe,  $12 \sim 29 \mu\text{m}$  in diameter. The yellowish-brown marks, from which the setae fall off, are visible. Another kind of setae sacs is yellow, with yellow and globularity-shaped newly bom setae in the center. (Fig 2.  $A_6 \sim 8$ )

#### B) *Platypleum kaempferi* (Fabricius):

The long yellow setae are broken usually. The setae without medullar are  $6 \sim 7 \mu\text{m}$  in diameter. The setae with medullar are  $5 \sim 19 \mu\text{m}$  in diameter,  $1 \sim 5 \mu\text{m}$  in medullar diameter and  $28.43 \pm 5.53$  in medullar index. The medullar is slightly dark, and distributed with black shot-like particles. (Fig 2.  $B_1 \sim 2$ )

The long yellowish-brown setae all contain medullar, which are  $7 \sim 23 \mu\text{m}$  in diameter,  $2 \sim 9 \mu\text{m}$  in medullar diameter and  $34.19 \pm 5.68$  in medullar index. The medullar is slightly dark, distributed with black substance or shot-like particles. (Fig 2.  $B_3$ )

The yellow stubby setae usually have a complete root. The seta tip is sharp comparatively, and the broadish basal portion shrink symmetrically to a bottleneck-like shape. The setae without medullar are  $52 \sim 122 \mu\text{m}$  long,  $6 \sim 19 \mu\text{m}$  in diameter, and  $8 \sim 19 \mu\text{m}$  in basal portion diameter. The setae with medullar are  $68 \sim 133 \mu\text{m}$  long,  $9 \sim 28 \mu\text{m}$  in basal portion diameter,  $2 \sim 6 \mu\text{m}$  in medullar diameter, and  $25.7 \pm 7.89$  in medullar index. The yellowish-brown medullar usually appears in line, and dis-

tributed with black shot-like particles. (Fig 2.  $B_4 \sim 5$ )

The yellowish-brown stubby setae usually have a complete root. The seta tip is sharp comparatively, and the broadish basal portion shrink symmetrically to a bottleneck-like shape. The setae without medullar are  $50 \sim 124 \mu\text{m}$  long,  $8 \sim 16 \mu\text{m}$  in diameter, and  $11 \sim 22 \mu\text{m}$  in basal portion diameter. The setae with medullar are  $60 \sim 181 \mu\text{m}$  long,  $8 \sim 24 \mu\text{m}$  in diameter,  $1 \sim 6 \mu\text{m}$  in medullar diameter, and  $22.73 \pm 5.86$  in medullar index, the basal portion of which are  $13 \sim 89 \mu\text{m}$  in diameter,  $2 \sim 12 \mu\text{m}$  in medullar diameter and  $26.09 \pm 7.35$  in medullar index. (Fig 2.  $B_6 \sim 7$ )

The reddish-brown or grayish-yellow setae sacs appear circular or oval in shape and are  $14 \sim 29 \mu\text{m}$  in diameter. The remnant setae roots are visible in center of sacs. The outer surface around sacs is concave sometimes, and  $44 \sim 79 \mu\text{m}$  in diameter. (Fig 2.  $B_8$ )

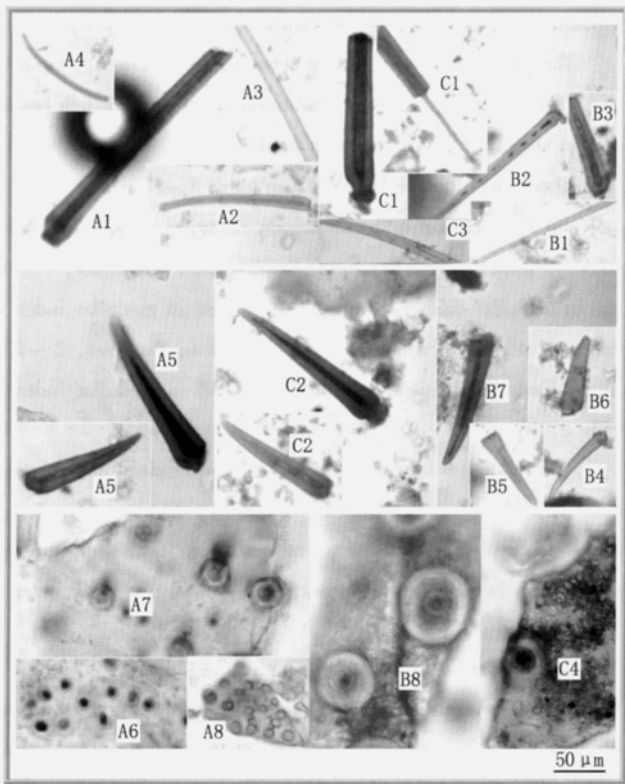
#### C) *Tibicen flammatus* (Dist.):

The long yellowish-brown or reddish-brown setae are broken usually,  $179 \sim 387 \mu\text{m}$  long,  $4 \sim 24 \mu\text{m}$  in diameter,  $4 \sim 11 \mu\text{m}$  in medullar diameter, and  $40.54 \pm 7.71$  in medullar index. The seta tip is blunt. The medullar cavity is rough on inner fringe, almost filling the whole seta shaft. The medullar is dark, particulate on surface, bended in wave-like line in the cavity frequently and stretched out of the cavity occasionally, the portion between the root and the basal part shrink unsymmetrically,  $9 \sim 31 \mu\text{m}$  in diameter and  $11 \sim 41 \mu\text{m}$  in basal portion diameter. The flank of setae roots are oval in shape and  $11 \sim 34 \mu\text{m}$  in longitude diameter. (Fig 2.  $C_1$ )

The stubby setae are yellowish-brown or reddish-brown,  $88 \sim 102 \mu\text{m}$  long,  $15 \sim 31 \mu\text{m}$  in diameter,  $3 \sim 10 \mu\text{m}$  in medullar diameter and  $31.87 \pm 8.96$  in medullar index. The seta tip is blunt. The medullar is dark, almost black occasionally, and particulate on surface. The medullar cavity is smooth on inner fringe. The portion between the root and the basal part shrink unsymmetrically,  $16 \sim 31 \mu\text{m}$  in diameter and  $21 \sim 43 \mu\text{m}$  in basal portion diameter. The flank of setae roots are oval in shape and  $19 \sim 38 \mu\text{m}$  in longitude diameter. (Fig 2.  $C_2$ )

The long light yellow slender setae are broken and bended frequently,  $7 \sim 11 \mu\text{m}$  in diameter,  $2 \sim 4 \mu\text{m}$  in medullar diameter and  $34.43 \pm 5.28$  in medullar index. The medullar is dark, particulate on surface, bended in wave-like line in the cavity sometimes, almost filling the whole seta shaft. The medullar cavity is rough on inner fringe. (Fig 2.  $C_3$ )

The yellow setae sacs appear circular,  $19 \sim 26 \mu\text{m}$  in diameter. The reddish-brown marks, from which the setae fall off, are visible. (Fig 2.  $C_4$ )



**Fig 2** Microscopic identification on setae of periostracum cicadae and that of its two counterfeit

A - *Cryptotympana pustulata* (Fabricius): 1 ~ 4 - long seta, 5 - stubby seta, 6 ~ 8 - setal sac; B - *Platypleum kaempferi* (Fabricius): 1 ~ 3 - long seta, 4 ~ 7 - stubby seta, 8 - setal sac; C - *Tibicen flammatus* (Dist): 1, 3 - long seta, 2 - stubby seta, 4 - setal sac

**图 2** 蝉蛻及其混淆品粉末中刚毛显微特征图

A - 黑蚱: 1 ~ 4 - 长刚毛, 5 - 短刚毛, 6 ~ 8 - 毛窝; B - 螳螂: 1 ~ 3 - 长刚毛, 4 ~ 7 - 短刚毛, 8 - 毛窝; C - 焰螳螂: 1, 3 - 长刚毛, 2 - 短刚毛, 4 - 毛窝

#### 4 Discussion

In exterior morphology, *Platypleum kaempferi* (Fabricius) can be easily identified from *Cryptotympana pustulata* (Fabricius) and *Tibicen flammatus* (Dist), since it is only 1.5 ~ 2 cm long, while the others are beyond 3 cm long.

Though they are similar in size and shape, *Cryptotympana pustulata* (Fabricius) and *Tibicen flammatus* (Dist) can be

identified from the color. The former often appears yellowish-brown, while the latter is reddish-brown usually.

In microscopic observation, Periostracum Cicadae can be differentiated from the two of counterfeits by the different characteristics of the setae. The microscopic identification key for their setae were shown in Table 2.

**Tab 2** Microscopic Identification Key for Setae of Periostracum Cicadae (A) and that of its two Counterfeits (B and C)

**表 2** 蝉蛻 (A) 及其两种混淆品 (B、C) 的刚毛显微鉴定检索表

1. the tip of the stubby seta is sharp comparatively and the medullar is unclear ..... B) *Platypleum kaempferi* (Fabricius)
1. the tip of the stubby seta is blunt and the medullar is obvious
2. the bended light yellow or colorless slender setae are visible frequently ..... A) *Cryptotympana pustulata* (Fabricius)
2. the bended light yellow or colorless slender setae are invisible ..... C) *Tibicen flammatus* (Dist)

Following the previous studies on *Cordyceps Sinensis* and so on<sup>[3]</sup>, the method of identifying animal drugs from their counterfeits by the remnant hair is further confirmed.

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