Deinagkistrodon (Agkistrodon)

Figure 1 A photograph of Deinagkistrodon (Agkistrodon)

A. External surface of Deinagkistrodon (Agkistrodon)
B. Internal surface of Deinagkistrodon (Agkistrodon)
C. Magnified image of head with upturned snout
D. Magnified image of fang
E. Magnified image of dorsal scale
F. Magnified image of tail with horny scale

1. NAMES

Official name: Deinagkistrodon (Agkistrodon)

Chinese name: 蕲蛇

Chinese phonetic name: Qishe

2. SOURCE

Deinagkistrodon (Agkistrodon) is the dried body of *Deinagkistrodon acutus* (Güenther) [*Agkistrodon acutus* (Güenther)] (Viperidae). It is collected in summer or autumn. Cut open the abdomen, the viscera removed, washed clean, held open with bamboo pieces, coiled up in disc-shape with head at the centre, dried over a stove or under the sun, then removed the bamboo pieces to obtain Deinagkistrodon (Agkistrodon).

3. DESCRIPTION

It coiled up in a disc shape, 200-420 mm in diameter; body 5-14 cm wide, 0.7-1.3 m long, up to 2 m long. Head locates at the centre of the disc, slightly raised upward, triangular and flattened, with a prominent upturned snout, commonly known as “Qiaobitou (upward nose)”. Each side of the upper jaw possesses 1 long tubular fang, sharp and hollow, often with 1-3 spare fangs behind. Dorsal scales possess strong keels and small tubercular warts. Back greyish-brown to dark brown, or brownish-black, back of head darker. Each side of the dorsum bears 16-22 V-shaped stripe patterns alternated blackish-brown with greyish-brown, both upper ends of the “V” stripe patterns usually unite at the mid-vertebral line, forming large squarish blotches, commonly known as “Fangshengwen”, some of the “V” stripe patterns do not exactly contact with those on the other side but arranged alternatively. Abdomen opened, greyish-white or yellowish-white, scales relatively large, a row of large black subrounded spots extends along the latero-ventral junction at each side, commonly known as “Lianzhuban (a patch like a string of beads)”. Inner wall of abdomen yellowish-white or greyish-yellow; spinous process of vertebrae relatively high, distinct; numerous ribs on both sides, mostly falcate, incline posteriorly. Tail tapers gradually, with a dark grey triangular horny scale at the end, commonly known as “Fuzhijia (Buddha’s nail)”. Odour stinky; taste slightly salty (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Powder

Colour pale yellow to yellowish-white. Keratinized scales nearly colourless or pale yellow, showing semi-circular or papillary protrusions laterally, subrounded, oval or polygonal in surface view, imbricate, 9-53 μm in diameter, distributed with pale grey or pale brown fine granular matters. Epidermal cells nearly colourless or pale yellow, cell boundaries indistinct, dark brown pigment granules densely distributed, mostly aggregated irregularly, reticulated or branched. Striated muscle fibres relatively abundant, colourless, often broken, mostly flaky, edges relatively straight, intact fibres 15-289 μm in diameter; with fine and dense stripes, straight or slightly wavy, some not clear. Bone fragments relatively abundant, nearly colourless or pale grey, in irregular pieces; bone lacunae subrounded or fusiform, mostly arranged in same direction, few irregularly arranged; bone canaliculi relatively small (Fig. 2).

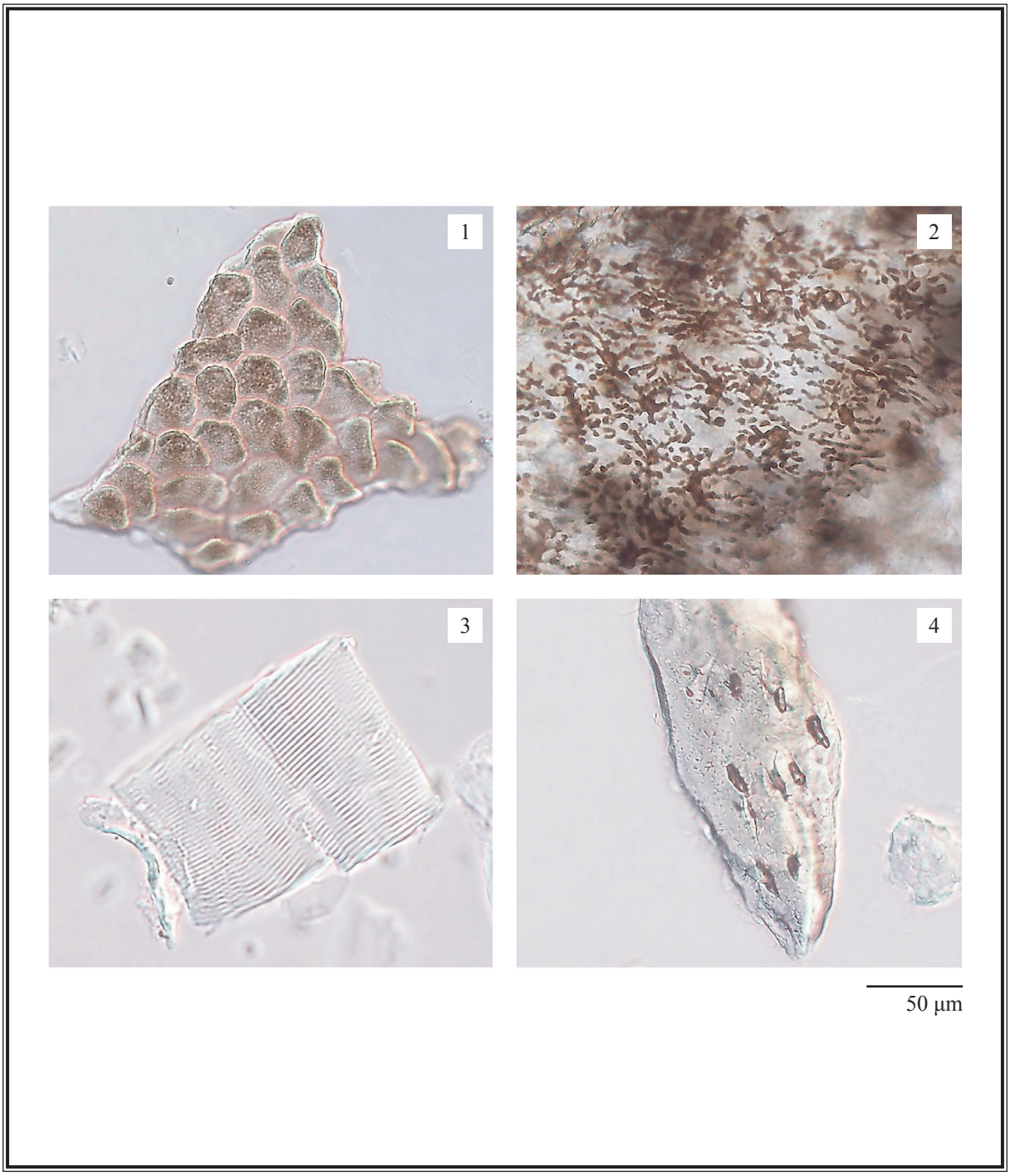

Figure 2 Microscopic features of powder of Deinagkistrodon (Agkistrodon) (under the light microscope)

1. Keratinized scale 2. Epidermal cells 3. Striated muscle fibres 4. Bone fragment

4.2 Deoxyribonucleic acid (DNA) Identification by Specific Polymerase Chain Reaction (PCR)

Carry out the method as directed in Appendix XIV.

Procedure

Weigh 10.0 mg of the powdered sample, sample duplicate and extraction positive control(s) into separate microtubes. Prepare blank tube(s) as extraction negative control(s). Perform DNA extraction using animal genomic DNA purification kit according to the manufacturer's manual.

Prepare a polymerase chain reaction (PCR) reagent mixture including reaction buffer containing 1.5 mM $MgCl_2$ (Taq buffer), 0.5 µM of primer Deinagkistrodon-S (5'-GGC AAT TCA CTA CAC AGC CAA CAT CAA CT-3') and Deinagkistrodon-AS (5'-CCA TAG TCA GGT GGT TAG TGA TAC-3'), 0.2 mM deoxyribonucleotides mix (dNTP), and 0.5 U DNA polymerase. For sample, sample duplicate and extraction positive control(s), add 200 ng of template DNA into the PCR reagent. For extraction negative control(s) and PCR negative control(s), add the eluted solution from DNA extraction process and sterile water, respectively, to the PCR reagent. The PCR condition is shown in Table 1. Identify the PCR products by electrophoresis analysis shown in Table 2. Measure the size of DNA fragments by referring to the DNA size marker.

Table 1 Recommended PCR cycling conditions

PCR system	PCR machine		
Temperature ramp rate	2.2°C/second		
Initial denaturation	95°C, 5 min		
Number of cycles	30		
PCR profile of each cycle	**Temperature (°C)**	**Time (min)**	**Remarks**
	95	0.5	Denaturation
	63	0.5	Annealing
	72	0.5	Extension
Final extension	72°C, 5 min		

Table 2 Recommended electrophoresis and detection conditions

Separation unit	1.5% agarose gel with 0.001% SYBR dyes DNA gel stain
Loading volume	5 μL
DNA size marker	100 bp DNA ladder
Detector	UV transilluminator
Running voltage	80 V
Running time	30 min

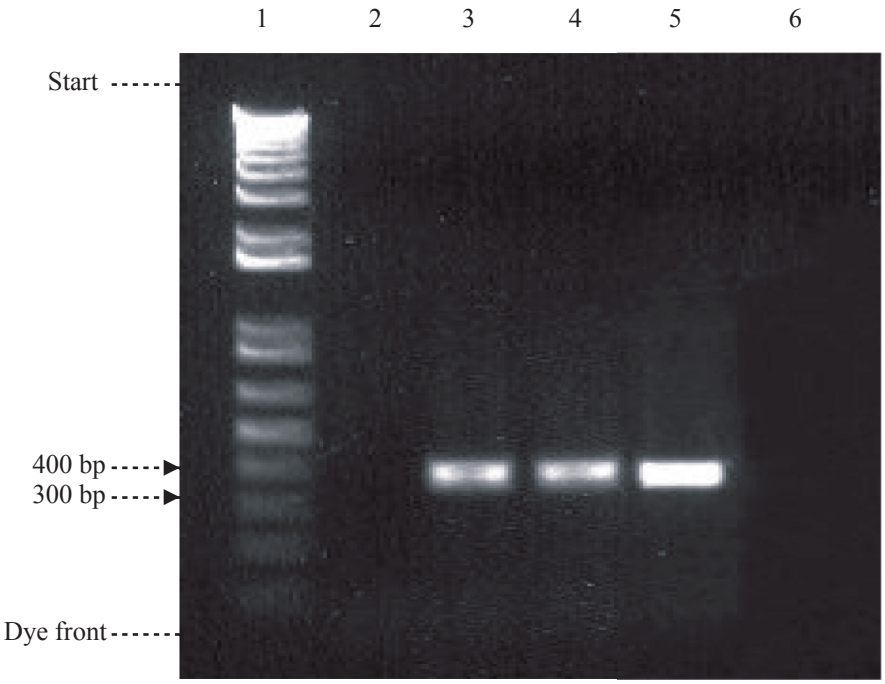

Figure 3 Reference gel electropherogram of Deinagkistrodon (Agkistrodon) under the recommended PCR conditions and electrophoresis analysis conditions

1. 100 bp DNA ladder
2. Extraction negative control
3. Sample
4. Sample duplicate
5. Extraction positive control
6. PCR negative control

For positive identification, the PCR products of sample, sample duplicate and extraction positive control(s) shall give a DNA band in between 300 and 400 bp under the recommended electrophoresis detection (Fig. 3).

5. TESTS

5.1 Heavy Metals *(Appendix V)*: The CMM shall meet the requirements for arsenic, cadmium and lead as specified in Appendix V. For mercury, Deinagkistrodon (Agkistrodon) should meet the specified limit of not more than 1.0 mg/kg, when the CMM will be processed as a decoction in the final consumption form; otherwise, the limit for mercury specified in Appendix V shall be applied.

5.2 Pesticide Residues *(Appendix VI)*: meet the requirements.

5.3 Mycotoxins *(Appendix VII)*: meet the requirements.

5.4 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

5.5 Foreign Matter *(Appendix VIII)*: not more than 1.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 32.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 8.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. CAUTION

This CMM should be used after proper processing (such as decoction).