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**INDUSTRY STANDARD OF THE
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DNA barcoding method - Part 8: Cuscuta

DNA条形码方法 第8部分：菟丝子属

(English Translation)

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Table of Contents

Foreword	
1 Scope	
2 Normative references	
3 Terms and definitions	
4 Basic information of Cuscuta	
5 General principles	
6 Principles of the method	
7 Instruments and reagents	
7.1 Apparatus and utensils	
7.2 Main reagents	
8 Screening and identification methods	
8.1 Sample processing	
8.2 Preparation of nucleic acid	
8.3 Amplification of DNA barcode fragments	
8.4 Detection of PCR products	
8.5 Sequencing and sequence processing	
9 Results judgment	
9.1 Judgment of China's quarantine pest DNA barcode identification system	
9.2 Judgment of plant pest quarantine and identification system	
9.3 Judgment of international general database	
10 Storage of voucher specimens and original data	
10.1 Storage of voucher specimens	
10.2 Save the original data	

Foreword

This document is drafted in accordance with GB/T 1.1-2020.

SN/T 4876 "DNA Barcoding Method" is a series of standards and has been published the following parts.

--Part 1: Quarantine Cactitermites

--Part 2: Quarantine beetle

--Part 3: Quarantine Moth

--Part 4: Quarantine Sorghum

--Part 5: Datura

--Part 6: The genus Tubula

--Part 7: Poinsettia subgenus

--Part 8: Cuscuta

This document is part 8 of SN/T 4876 this document was proposed and managed by the General Administration of Customs of the People's Republic of China.

1 Scope

This document specifies the DNA barcode detection and identification method of Cuscuta.

This document is applicable to phytosanitary laboratories conducting molecular identification of Cuscuta at border ports.

2 Normative references

The contents of the following documents constitute the indispensable clauses of this document through normative references in the text. Among them, dated quotations Only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable Used in this document.

SN/T 1385 Quarantine and identification method of Cuscuta

3 Terms and definitions

The following terms and definitions apply to this document.

DNA barcode

A short, standardized gene segment used to distinguish different species. This fragment has obvious genetic variation and differentiation among species it is easy to carry out PCR amplification. DNA barcode technology is mainly used for species identification and new species discovery.

Voucher specimen

A specimen that provides physical evidence for identification or research and has sufficient archived information (collection and identification information, etc.) for long-term preservation, it can be a complete organism or a part of it, or it can be the genetic material of an organism. The archived information of the voucher specimen record should have it is retrospective. The preservation of voucher specimens is very important. It is a physical

reference voucher for DNA barcodes. The voucher specimen information includes collection information, Collect information and appraisal information for review.

Nuclear ribosome DNA nuclear ribosome DNA; nrDNA all the genetic information contained in the nuclear chromosomes of eukaryotes, namely base pairs, is different from plastids such as chloroplasts and mitochondria DNA, as well as other forms of DNA such as cosmids and plasmids.

Chloroplast DNA chloroplast DNA; cpDNA DNA present in the chloroplast. The DNA of higher plant chloroplasts is a double-stranded covalently closed circular molecule, and its length depends on the biological species.

However, the size is between 120 kb and 217 kb, which is equivalent to the size of the phage genome.

Internal transcribed spacer; ITS in eukaryotic ribosomal DNA, 18S rRNA and 28S rRNA gene spacer sequences are called internal transcribed spacers. Inward turn the ribosomal genome sequence where the recording spacer is located from 5'to 3'are. external transcribed spacer (external transcribed spacer, ETS), 18S gene, internal transcribed spacer 1 (ITS1), 5.8S gene, internal transcribed spacer 2 (ITS2), 28S gene and genes Intergenic spacer (IGS). That is, the internal transcribed spacer contains ITS1, 5.8S gene and ITS2. Ribosomal RNA the 18S, 5.8S and 28S genome sequences tend to be conserved in most organisms, with little variation among organisms, while the internal transcribed spacer ITS1 and ITS2, as non-coding regions, bear less selective pressure, relatively change greatly, and can provide detailed systematic analysis the required heritable traits.

1.5-Ribulose biphosphate carboxylase large subunit gene RubisCo_large; rbcL

1.5-Ribulose diphosphate carboxylase (RubisCo) is an enzyme with the dual functions of carboxylation and oxygenation. It plays an important role in photosynthesis and photorespiration.

Sucking plays an important role. RubisCo enzyme is composed of 16 subunits, including 8 large subunits and 8 small subunits. Of which large subunit the gene (rbcL) is encoded by the chloroplast genome.

4 Basic information of Cuscuta

Chinese name. Cuscuta Scientific name. Cuscuta spp.

Taxonomic status. Angiosperm (Magnoliophyta), Magnolia (Magnoliatae), Solanaceae (Solanales), Convolvulaceae (Convolvulaceae) Cuscuta is a parasitic herb, has no roots, and has no hair on the whole. The stem is entwined, slender, linear, yellow or red, not green, Fix the host with the aid of a suction device. No leaves, or degenerated into small scales. The flowers are small, white or light red, sessile or short-stemmed, spike-like, total Shaped or clustered into head-like inflorescence; bracts are small or absent; flowers are 5

to 4 out of number; sepals are nearly equal in size, more or less connected at the base; corolla tubular, Pot-shaped, globose, or bell-shaped, with marginally split or fringe-shaped scales under the stamens of the inner surface of the corolla tube; the stamens are born in the throat of the corolla or adjacent corolla lobes, usually slightly protruding, with short filaments and inward anthers; pollen grains are oval, without spines; ovary 2 compartments, each Chamber 2 Ovule, style 2, completely separated or somewhat commissural, stigma globose or elongated. Capsule spherical or ovoid, sometimes slightly fleshy, perilobate or Irregular rupture. Seeds 1 to 4, glabrous; embryo in the fleshy endosperm, linear, disc-shaped curved or spiral, without cotyledons or thin with tiny scaly scars. There are about 170 species of plants in this genus, which are widely distributed in the warm temperate zone of the world, mainly producing America.

Cuscuta plants are easy to spread with the host and its products. They are malignant weeds that can cause serious economic losses and are difficult to control. Therefore, Many countries in the world classify it as a prohibited or restricted pest.

5 General principles

The method established in this standard should not completely replace the morphological identification method, but should complement each other with the morphological identification method SN/T 1385.

Mutual corroboration.

6 Principles of the method

According to the ITS sequence of the genus Cuscuta and the characteristics of the rbcL gene, the DNA barcoding technology was used to screen and identify the genus Cuscuta.

7.1 Apparatus and utensils

Conventional PCR instrument, micro spectrophotometer, electrophoresis instrument, gel imaging system, sequencer, high-speed refrigerated centrifuge, biological safety Cabinet, oven, autoclave, microscope, dissecting mirror, balance (1/10 000 g), mortar, shaker, water bath, ice maker, pure Water meter, vortex shaker, refrigerator, refrigerated mixing mill, micropipette (2 µL, 10 µL, 20 µL, 100 µL, 200 µL, 1 000 µL), centrifuge tube (2 mL), PCR reaction tube (0.2 mL, 0.5 mL).

7.2 Main reagents

Unless otherwise specified, all reagents are analytical reagents or biochemical reagents.

Plant genome extraction kit, ultrapure water, DNA Marker, agarose, nucleic acid gel dye, liquid nitrogen, deoxyribonucleoside Triphosphates (dNTPs), Taq polymerase, 10xPCR buffer. CTAB extraction reagent (see Appendix A in A.1).