



# 数字化DNA技术在食品真实性中的应用

## Application of Digital DNA Technology in Food Authenticity

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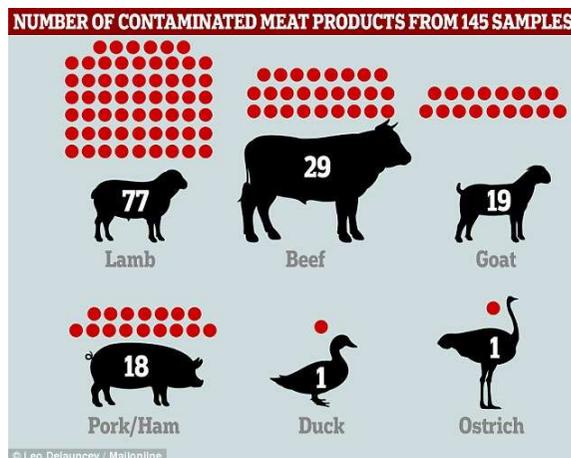
# 国内外食品掺假现状 Current Authenticity Issues

食品掺假和欺诈是个全球性问题 Food fraud has been a global issue



# 国内外食品掺假现状 Current Authenticity Issues

## UK Food Standards Agency Says 1/5 of Meat It Tested Contained Mystery DNA



- 2018年9月，英国食品标准局（FSA）披露的一份调查报告显示，抽检的665份含肉食品五分之一掺假；
- One in five of 665 tested samples “were partly or wholly made up of unspecified meat.”

<https://www.bbc.co.uk/news/uk-45371852>

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Analytical Methods

DNA barcoding and mini-barcoding in authenticating processed animal-derived food: A case study involving the Chinese market

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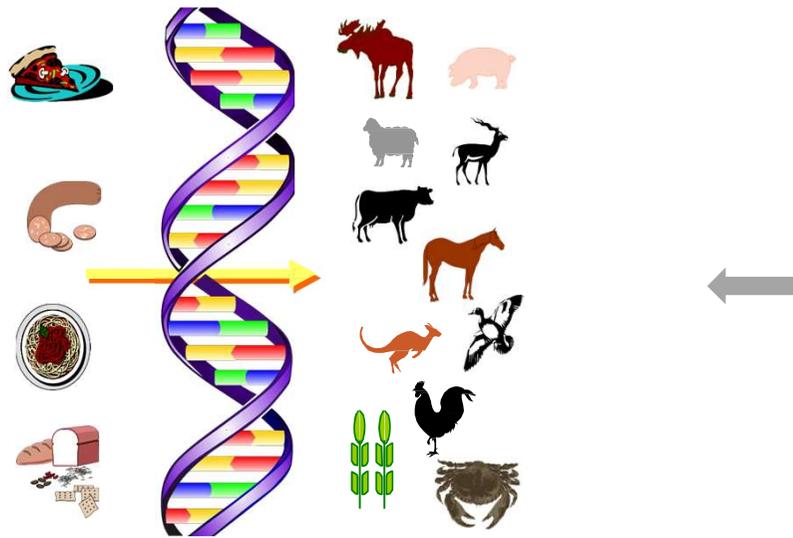


- 本研究团队利用DNA条形码和微条形码技术对所选市售肉制品进行检测，发现接近23%的肉制品出现掺假或标签不符现象。
- Approximately 23% of selected commercial samples were determined to be mislabeled.

Xing, Ran-Ran, et al. Food Chemistry (2019): 125653.

# 食品真实性鉴别新技术

## Advanced technologies for food authenticity identification



### 基于DNA的检测技术 DNA-based methods

即使经过加工，形态发生变化，DNA序列信息也不会改变。

同时样本部分受损也不会影响识别结果。

- DNA is more stable, and is not affected by external factors.

### 食品真实性鉴别新技术

#### Advanced technologies for food authenticity identification

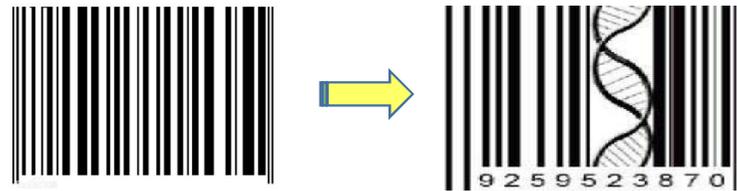




# DNA条形码技术 DNA barcoding

2003年，加拿大动物学家Paul Hebert在DNA分类学的基础上，将超市用以区分成千上万种不同商品的条形码概念引入，首次明确提出了DNA条形码的概念。

In 2003, the Canadian zoologist Paul Hebert assumed DNA barcode.



## DNA条形码DNA barcoding

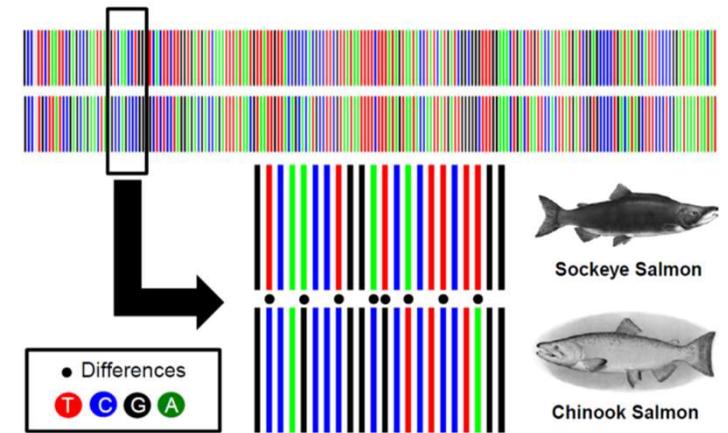
■ DNA条形码是指生物体内能够代表该物种的、标准的、有足够变异的、易扩增且相对较短的DNA片段。

Paul D. N. Hebert, et al., Proc. R. Soc. Lond. B , 2003, 270, 313–321.

# DNA条形码技术 DNA barcoding

## 原理

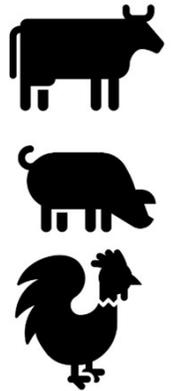
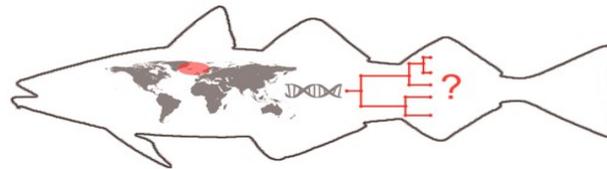
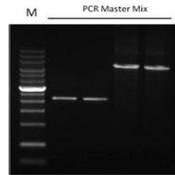
- 每种生物物种的DNA序列都是**唯一**的，给DNA条形码提供了物质基础。
- The DNA sequence of each biological species is unique, giving the DNA barcode its material basis.
- 对于食品的真伪鉴别，从本质上可追溯为鉴定其生物成分。
- The food authentication can be traced essentially to the identification of its biological composition.



通过序列比对进行物种鉴定

# 为什么选择DNA条形码? Why DNA barcode ?

- **准确性高。** 特定的物种具有特定的DNA序列信息，避免了形态学鉴别特征会因趋同和变异导致物种的鉴定误差。
- DNA is more stable, avoiding morphological identification that can lead to errors in species identification due to external factors.
- **可以建立数据库，成为永久性资料。**
- Databases can be created and become permanent information.





# DNA条形码数据库 DNA barcode databases

数据库	网址
GenBank	<a href="http://www.ncbi.nlm.nih.gov/genbank/">www.ncbi.nlm.nih.gov/genbank/</a>
BOLD	<a href="http://www.boldsystems.org/index.php/IDS_OpenIdEngine">http://www.boldsystems.org/index.php/IDS_OpenIdEngine</a>
BOMMD	<a href="http://www.dnabarcode.net/bomm">http://www.dnabarcode.net/bomm</a>
UNITE	<a href="http://unite.ut.ee/">http://unite.ut.ee/</a>
RSSL (FDA)	<a href="http://www.accessdata.fda.gov/scripts/fdcc/?set=seafood_barcode_data">http://www.accessdata.fda.gov/scripts/fdcc/?set=seafood_barcode_data</a>
FISH-BOL	<a href="http://www.fishbol.org">www.fishbol.org</a>
MarBOL	<a href="http://www.marinebarcoding.org/">www.marinebarcoding.org/</a>

international  
BARCODE  
OF LIFE



国际生命条形码计划



生命条形码联盟



GenBank

EMBL

DDBJ

BOLD SYSTEMS

BOLD

# 测序技术 Sequencing technologies

## •1<sup>st</sup> Generation = Sanger Sequencing

- 700 bps – 1000 bps **Golden standard**
- Low throughput
- High sequencing quality

## •2<sup>nd</sup> Generation = Next Generation Sequencing

- ~ 600 bps **Start from 2005**
- High throughput
- High sequencing quality

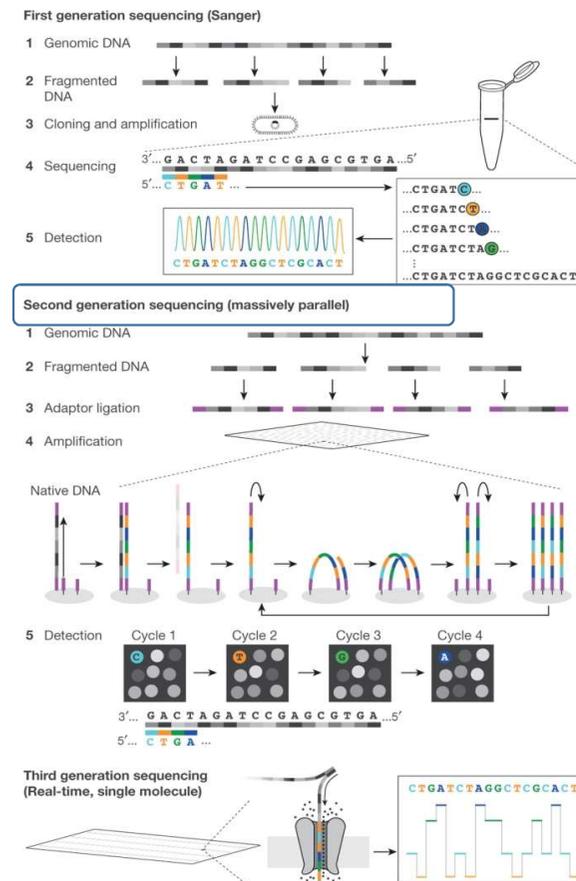
## •3<sup>rd</sup> Generation = Single Molecule Sequencing

- ~ 10K – 1 M bps **No PCR**
- Medium throughput
- Acceptable sequencing quality

### NGS also known as

高通量测序 High throughput sequencing

超深度测序 Ultra-deep sequencing



J Shendure *et al.* *Nature* 1–9 (2017)  
doi:10.1038/nature24286

**nature**



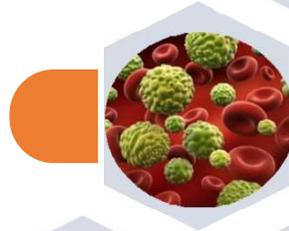
# 测序技术应用领域 Sequencing technologies application

**人类遗传学研究**  
**Human Genetics Research**  
法医基因组学  
Forensic Genomics



**宏基因组学研究**  
**Metagenomics Research**  
微生物基因组  
Microbial Genomics  
微生物群  
Microbiota

**复杂疾病基因组学**  
**Complex Disease Genomics**  
肿瘤研究  
Oncology



**农业基因组学研究**  
**Agri-genomics Research**  
农业育种  
Applying agricultural genomics  
to improve the food supply

**生殖健康**  
**Reproductive Health**  
无创产前检测(NIPT)



**药物开发**  
**Drug Development**



**食品真伪鉴别**  
**Food Authenticity**



# DNA条形码在食品真实性鉴别中的应用

## Application of DNA barcoding in food authenticity identification

本研究团队目前开展的基于DNA条形码技术的研究



肉类食品物种鉴别  
Species  
identification in  
meat products



海珍品真伪鉴别  
Species  
identification in  
seafood



食用香辛料物种鉴别  
Species identification  
in  
spices



小浆果及果汁真伪鉴别  
Authentication of  
small berries and  
juices



有毒蘑菇鉴别  
Species identification  
in  
poisonous mushrooms



## 应用案例 Applications



### 食品中动物源性物种成分鉴别

Animal Species Identification in Food Products

# 各类动物产品的基因条码检测

## DNA barcoding in authenticating animal-derived food



标准动物样品基因条码鉴定

样品	鉴定结果	名称	覆盖度	匹配率
绵羊	<i>Ovis aries</i>	绵羊	100%	99%
山羊	<i>Capra hircus</i>	山羊	99%	97%
狗	<i>Canis lupus</i>	狗	100%	98%
貉	<i>Nyctereutes procyonoides</i>	貉	99%	99%
鹿	<i>Cervus elaphus</i>	马鹿	100%	99%
骆驼	<i>Camelus bactrianus</i>	双峰驼	99%	99%
猫	<i>Felis catus</i>	猫	99%	92%
大鼠	<i>Rattus norvegicus</i>	大鼠	100%	99%
小鼠	<i>Mus musculus</i>	小鼠	99%	99%
兔	<i>Oryctolagus cuniculus</i>	兔	100%	99%
马	<i>Equus caballus</i>	马	98%	99%
驴	<i>Equus asinus</i>	驴	98%	98%
猪	<i>Sus scrofa</i>	猪	100%	99%
黄牛	<i>Bos taurus</i>	黄牛	99%	99%
牦牛	<i>Bos grunniens</i>	牦牛	98%	98%

### 《动物制品中动物源性成分基因条码检测方法 Sanger测序法》

样品名称	鉴定结果	拉丁名	匹配率
鮫鰵鱼	黄鮫鰵	<i>Lophius litulon</i>	99%
白鲟鱼	石首鱼	<i>Cynoscion jamaicensis</i>	93%
鳊鱼	武昌鱼 (又称鳊鱼)	<i>Megalobrama amblycephala</i>	99%
草鱼	草鱼	<i>Ctenopharyngodon idella</i>	98%
长江回鱼	钳鱼	<i>Ictalurus punctatus</i>	98%
达尖犁头鳊	鳊	<i>Rhynchobatus cf</i>	97%
大马哈鱼俄罗斯	大马哈鱼	<i>Oncorhynchus keta</i>	99%
大马哈鱼黑龙江	大马哈鱼	<i>Oncorhynchus keta</i>	99%
大西洋鲑鱼群岛	绵鲑	<i>Zoarces gillii</i>	90%
东星斑	鳧棘鲈 (又称东星斑)	<i>Plectropomus leopardus</i>	99%
鳊鱼	大眼鳊	<i>Siniperca kneri</i>	100%
黑鱼	乌鳢 (又称黑鱼)	<i>Channa argus</i>	96%
鲫鱼	鲫鱼	<i>Carassius gibelio</i>	99%
剑鱼	剑鱼	<i>Xiphias gladius</i>	97%
接吻鱼	白沙鲈	<i>Sillago sihama</i>	95%
金线鱼	金线鱼	<i>Nemipterus japonicus</i>	99%
孔鳊	缸属	<i>Dasyatis bennetti</i>	99%
鲤鱼	鲤鱼	<i>Cyprinus carpio</i>	99%
罗非鱼	罗非鱼	<i>Oreochromis</i>	99%
南非无须鳕鱼	南非无须鳕鱼	<i>Merluccius capensis</i>	97%
青斑鱼	青石斑鱼	<i>Epinephelus awoara</i>	98%
青鳉	绿青鳉	<i>Pollachius virens</i>	99%
秋刀鱼	乔氏吻鳐	<i>Rhynchorhamphus georgii</i>	98%
沙鱼	低眼无齿	<i>Pangasianodon hypophthalmus</i>	99%
鲟鱼	云鲟鱼	<i>Tenualosa ilisha</i>	95%
笋壳鱼	斑驳尖塘鳢 (又称笋壳鱼)	<i>Oxyeleotris marmorata</i>	99%
太平洋鳕鱼	黄线狭鳕	<i>Gadus chalcogrammus</i>	99%
台鱼	白腹鲭	<i>Scomber japonicus</i>	98%
竹荚鱼	月银汉鱼	<i>Menidia menidia</i>	100%
鳟鱼智利	虹鳟	<i>Oncorhynchus mykiss</i>	99%
黄鳝	黄鳝	<i>Monopterus albus</i>	98%

样品	鉴定结果	名称	覆盖度	匹配率
鹌鹑	<i>Coturnix</i>	鹌鹑	98%	98%
大雁	<i>Anser</i>	大雁	98%	96%
鸽子	<i>Columba livia</i>	鸽子	98%	97%
火鸡	<i>Meleagris gallopavo</i>	火鸡	98%	98%
原鸡	<i>Gallus gallus</i>	原鸡	98%	99%
沙鸡	<i>Syrhaptus paradoxus voucher</i>	沙鸡	100%	98%
山鸡	<i>Phasianus colchicus</i>	雉鸡	100%	96%
乌鸡	<i>Gallus gallus domesticus</i>	乌骨鸡	98%	98%
鸭	<i>Anas platyrhynchos breed Shaoxing</i>	绿头鸭	98%	98%

ICS 07.080  
A 40



中华人民共和国国家标准

GB/T 35918—2018

### 动物制品中动物源性检测基因条码技术 Sanger 测序法

Identification of animal origin in animal products by DNA barcoding—  
Sanger sequencing

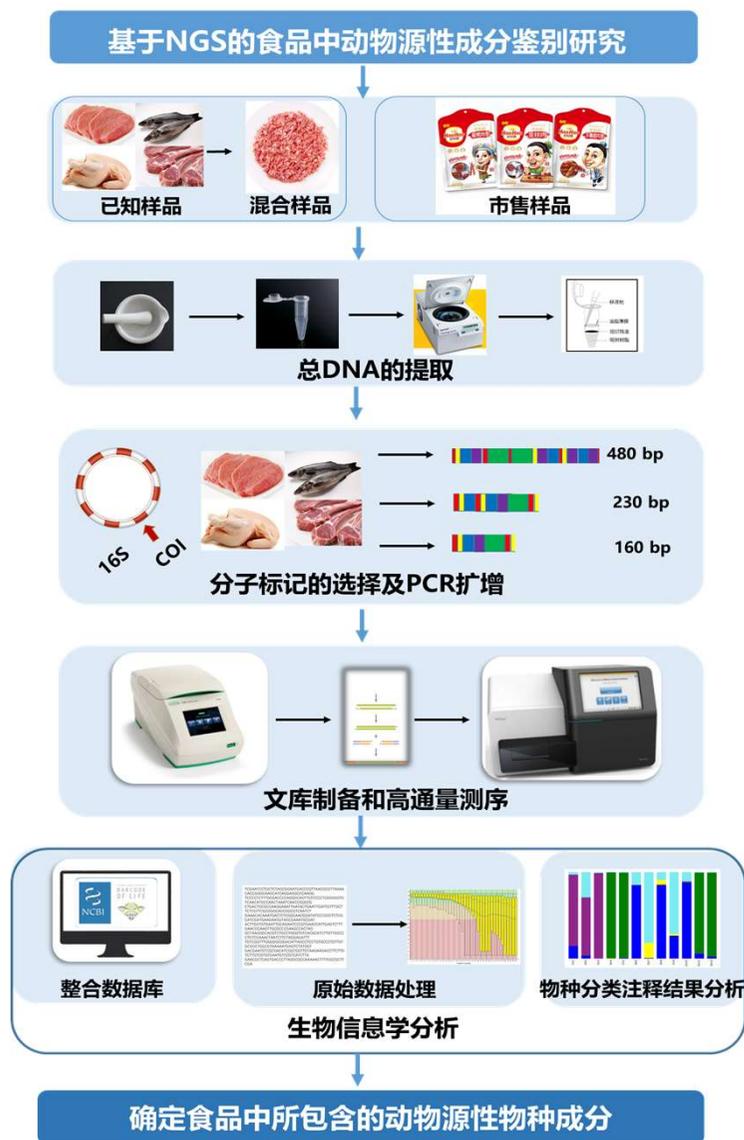
2018-02-06 发布

2018-09-01 实施

中华人民共和国国家质量监督检验检疫总局  
中国国家标准化管理委员会 发布

# 技术流程 Workflow

Application of NGS for species identification in meat and poultry products: A DNA metabarcoding approach



Sample Preparation

DNA Extraction

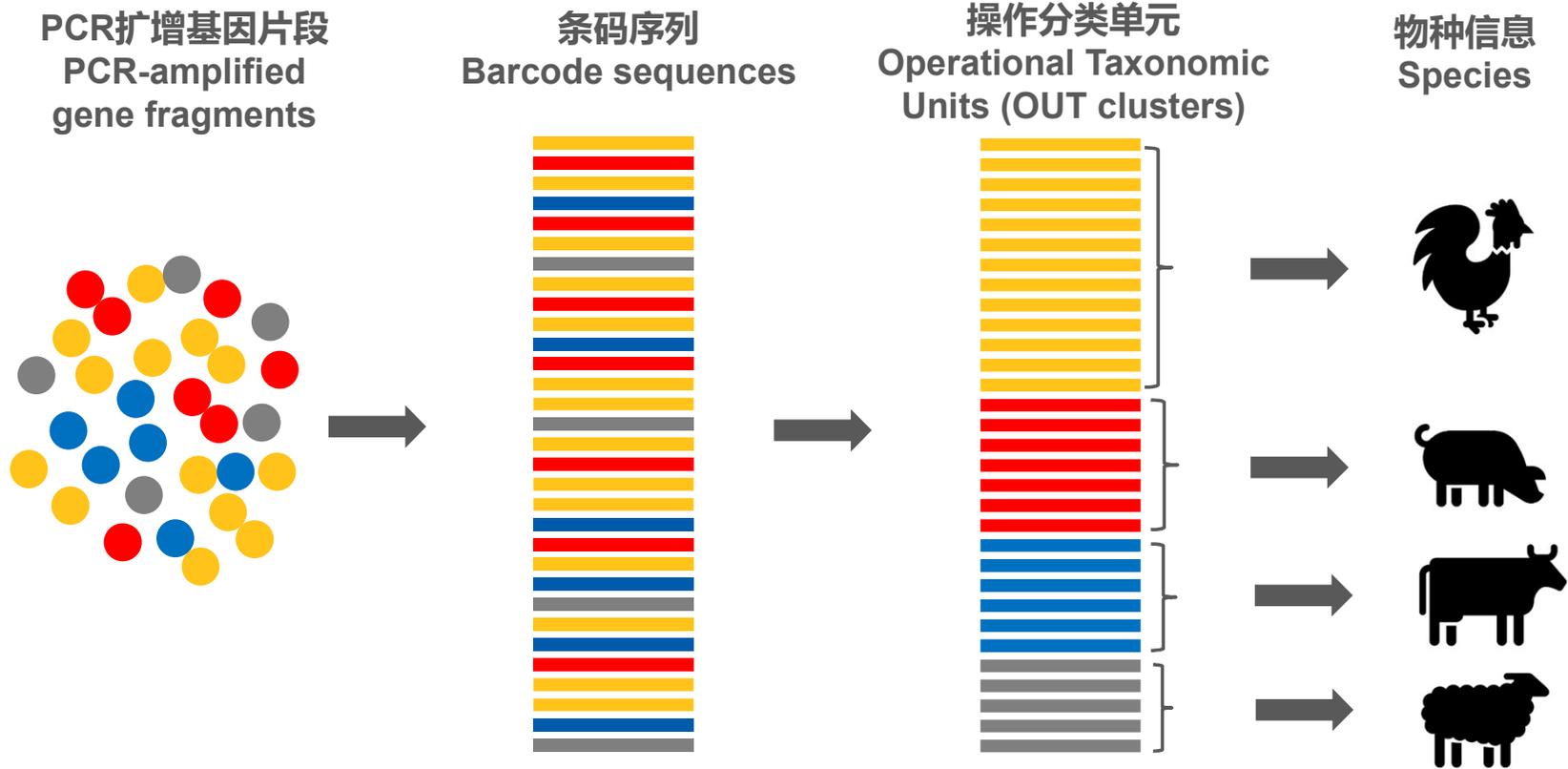
Markers & Primers  
PCR

High-throughput Sequencing

Data Analysis

Accredited Results

# 物种鉴定 Species identification



# 市售食品中动物源性物种成分鉴别

## Species Identification of Commercial Food Products



从各大电子商务平台、连锁超市、农贸市场、餐馆购买不同类型的动物源性食品，包括：鲜肉、肉片、肉干、肉酱、卤肉、肉松、肉串、肉馅、肉罐头、肉丸、宠物食品等 Different commercial animal origin food representing a variety of product types and species were obtained from supermarket, local market, restaurants and three online retail sources in China.



本研究选取的所有样品标注产品中包含一种或多种动物物种。Listed one or several animal species



共收集27份市售畜禽类样品，11份市售鱼肉制品  
Twenty-seven commercial meat and poultry products, 11 fish products

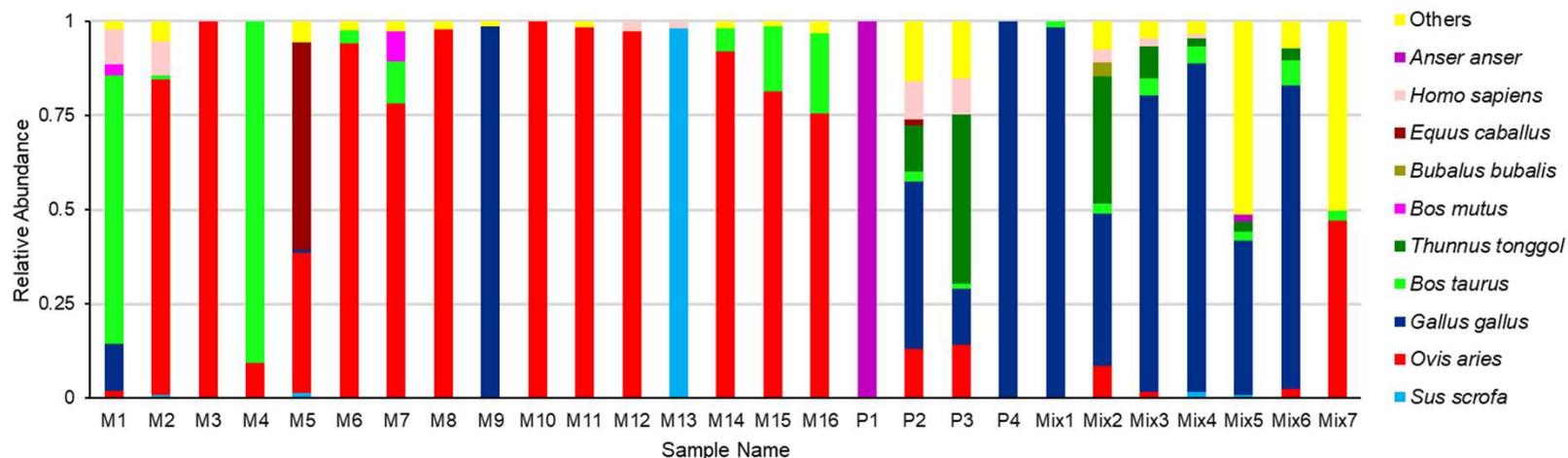
### 市售样品收集

### Commercial Food Products





# 市售样品检测结果 Results of Commercial Products



畜禽类样品在种水平上的物种相对丰度柱形图

Relative species composition in meat samples based on sequence read counts

分类 classification	举例 Sample	产品描述 Product	标签列出的动物物种 Species listed	检出动物物种 Species identification	是否相符 Yes/No?
物种信息与标签相符 Correct labeled	M3	羊肉 mutton	羊 sheep	羊 sheep	√
标签1种物种, 测序结果大于1种 Mislabeled	M4	牛肉馅	牛 cattle	牛、鸡 Cattle, chicken	×
标签中包含多个物种, 检测结果与标签不一致 Mislabeled	Mix1	狗指挥棒 Dog food	鸭、鸡、牛、羊、鹿 Dark, chicken, cattle, sheep, deer	鸡、牛 Chicken, cattle	×
检测到的物种信息与标签标识完全不一致 Mislabeled	M4	烤骆驼肉 roasted camel meat	骆驼 camel	牛、羊 Cattle, chicken	×

Xing, R.-R., et al. (2019). Application of next generation sequencing for species identification in meat and poultry products: A DNA metabarcoding approach. Food Control, 101, 173-179.



## 应用案例 Applications



### 海参制品物种鉴别

Species Identification in Sea cucumber Products

# 世界主要商业海参分布

## Distribution of major commercial sea cucumbers



# 海参概况

## Overview of sea cucumber

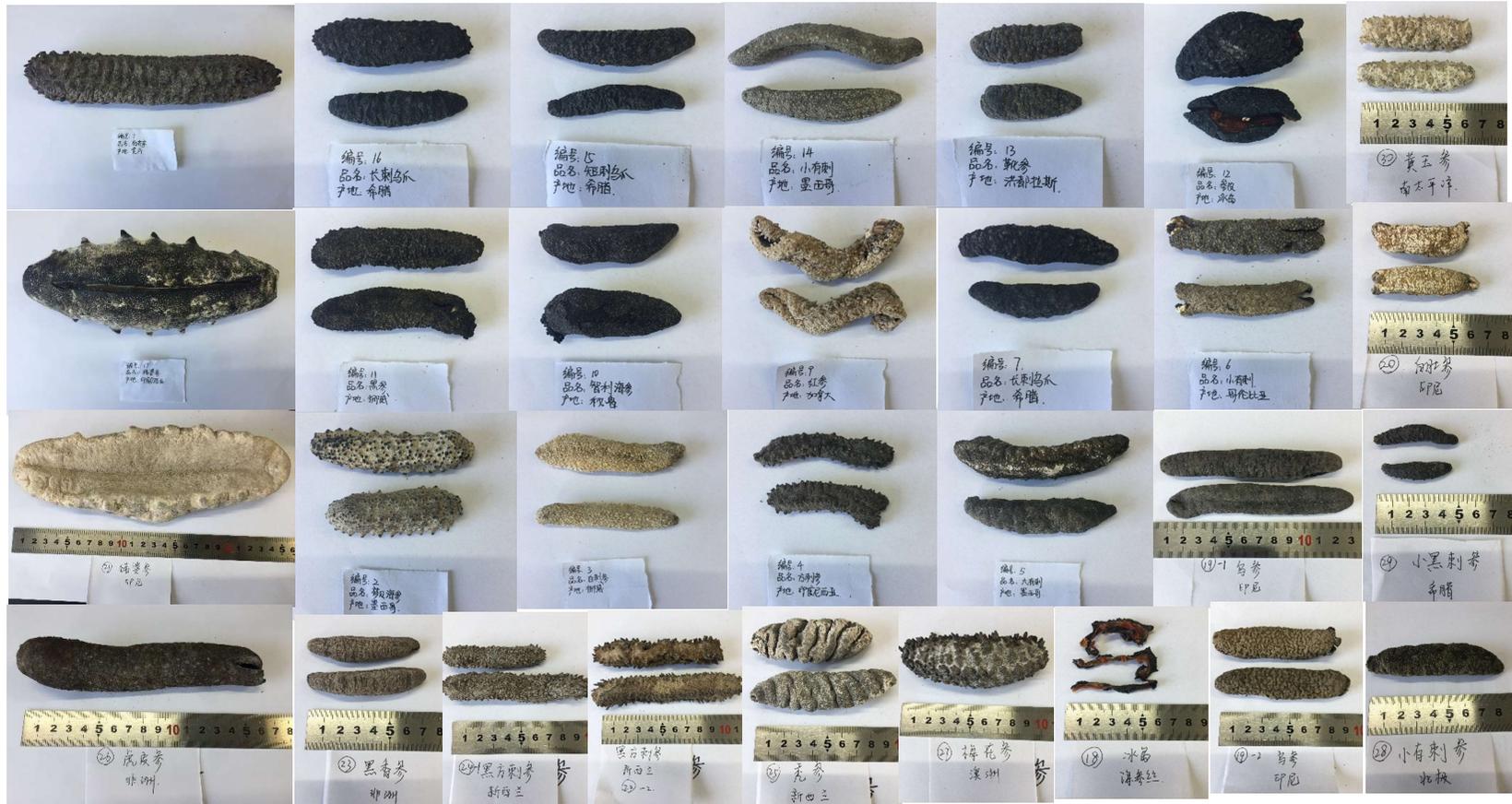
- 全世界约有1700多种海参，绝大多数海参不能食用。
- There are more than 1,700 species of sea cucumbers around the world
- 据统计，全世界仅有60多种可食用海参。分属于海参纲的3目，4科，14属。Among these, about 60 species of edible sea cucumbers are edible.
- 全球捕捞的海参几乎90%供给中国、韩国、日本、东南亚等亚洲地区。Almost 90% of the sea cucumbers harvested worldwide are supplied to China, Korea, Japan, Southeast Asia and other Asian regions.
- 中国是最大的海参消费市场。China has been the largest market for sea cucumbers.



目	科	属	主要的种			
指手目 Aspidochirotida	海参科 Holothuridae	辐肛参属 <i>Actinopyga</i>	白底辐肛参 <i>Actinopyga mauritiana</i> (Quoy and Gaimard, 1833) 乌皱辐肛参 <i>Actinopyga miliaris</i> (Quoy and Gaimard, 1833) 等 白尼参属 <i>Bohadschia</i> 蛇目白尼参 <i>Bohadschia argus</i> (Jaeger, 1833) 图纹白尼参 <i>Bohadschia marmorata</i> (Jaeger, 1833) 等			
		海参属 <i>Holothuria</i>	黑海参 <i>Holothuria atra</i> (Jaeger, 1833) 黄乳海参 <i>Holothuria fuscogлива</i> (Cherbonnier, 1980) 玉足海参 <i>Holothuria leucospilota</i> (Brandt, 1835) 墨西哥海参 <i>Holothuria mexicana</i> (Ludwig, 1875) 糙海参 <i>Holothuria scabra</i> (Jaeger, 1833) 等 格皮氏海参 <i>Pearsonothuria gareffei</i> (Semper, 1868)			
		皮氏海参属 <i>Pearsonothuria</i>				
		刺参科 Stichopodidae	刺参属 <i>Stichopus</i>	绿刺参 <i>Stichopus chloronotus</i> (Brandt, 1835) 花刺参 <i>Stichopus variegatus</i> (Semper, 1868) 糙刺参 <i>Stichopus horrens</i> (Selenka, 1868) 等 仿刺参 <i>Apostichopus japonicus</i> (Selenka, 1867)		
			仿刺参属 <i>Apostichopus</i>			
			梅花参属 <i>Thelenata</i>	梅花参 <i>Thelenata ananas</i> (Jaeger, 1833) 等		
			暗刺参属 <i>Isostichopus</i>	慢步等刺参 <i>Isostichopus badionotus</i> (Selenka, 1867) 暗色等刺参 <i>Isostichopus fuscus</i> (Ludwig, 1875)		
			拟刺参属 <i>Parastichopus</i>	加州拟刺参 <i>parastichopus californicus</i> (Stimpson, 1857)		
			类刺参属 <i>Astichopus</i>	毛刺参 <i>Astichopus multifidus</i> (Sluiter, 1910)		
			澳洲刺参属 <i>Australostichopus</i>	软刺参 <i>Australostichopus mollis</i> (Hutton, 1872)		
			指手目 Dactylochirotida	瓜参科 Cucumariidae	瓜参属 <i>Cucumaria</i>	大西洋瓜参 <i>Cucumaria frondosa</i> (Gunnerus, 1767)
					圆瓜参属 <i>Athyonidium</i>	智利瓜参 <i>Athyonidium chilensis</i> (Semper, 1868)
					海地瓜属 <i>Acaudina</i>	海地瓜 <i>Acaudina molpadoides</i> (Semper, 1868)
		芋参目 Molpadida	尻参科 Caudinidae			



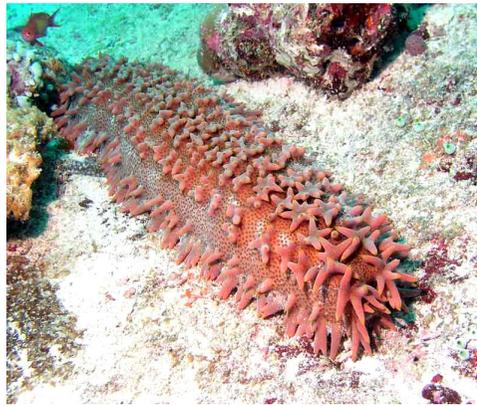
# 海参概况 Overview of sea cucumber



# 海参概况 Overview of sea cucumber



**California fish**



**Prickly redfish**



**Floridana**



**Cucumeria**



**Sand fish**



**Foscali**



# 海参市场现状 Sea Cucumber Market Status

- 目前海参在贸易中多以干海参、水发海参、精深加工的海参制品等形式流通，鲜活和冻品仅占一小部分
- 全球有90%的鲜海参被加工成各种干制品，包括盐干海参、淡干海参、真空冷冻干燥海参
- 海参胶囊、海参口服液、海参罐头、海参饮料、海参丸等深加工制品
- Most commercially available sea cucumbers are dry-processed, or highly-processed, such as powdered, solution-processed



# 海参市场现状 Sea Cucumber Market Status

海参种类繁多，不同品种的海参经济价值不同，价格可相差数十倍  
The price of edible sea cucumbers varies largely based on species

## 标签混乱、贴错标签 mislabeling

- 同一物种标注名称不同、不同物种同一标注名称、根据颜色或产地给物种标名称等



## 以次充好 substitution

- 低值海参假冒高值海参



## 食品欺诈 food fraud

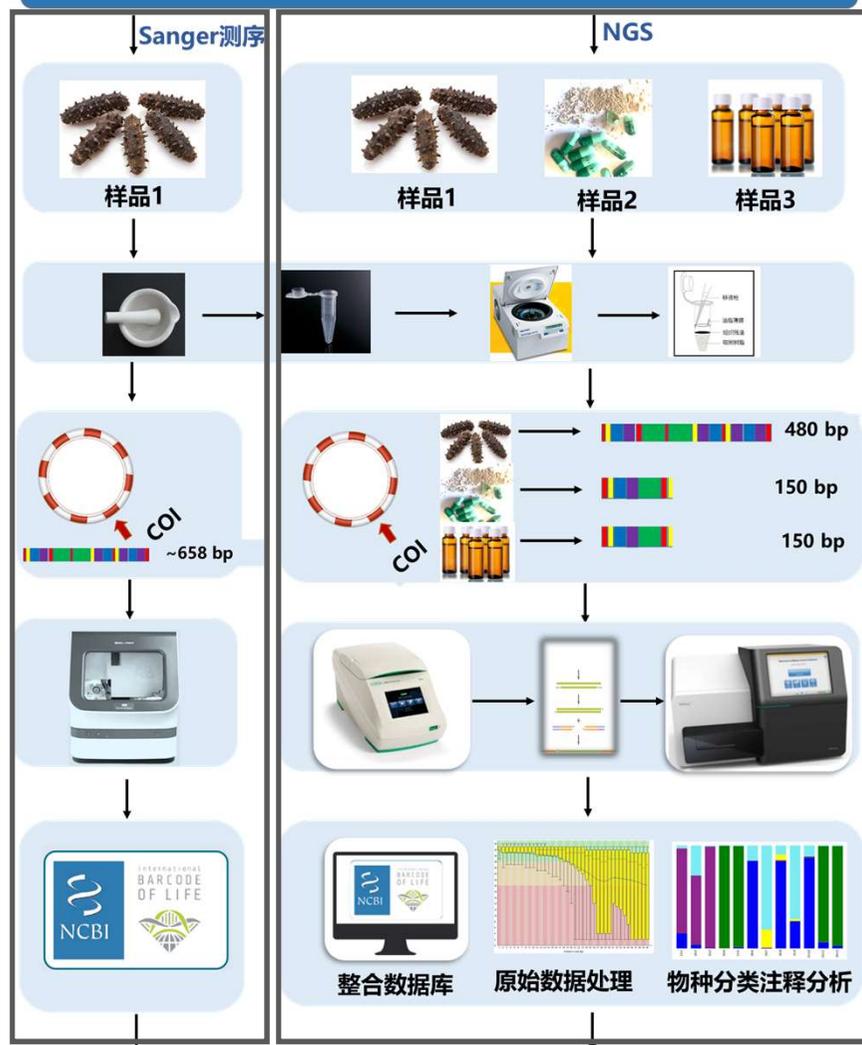
- 损害消费者利益，冲击海参市场，对保护濒危物种带来危害等



亟需建立准确、有效的海参真实属性鉴别方法，为企业、监管部门提供技术支持

# 技术流程 Workflow

## 基于DNA条形码技术的食用海参物种鉴定研究



确定海参产品中的物种成分

Sample Preparation

DNA Extraction

Markers & Primers  
PCR

High-throughput Sequencing

Data Analysis

Accredited Results





# 基于Sanger测序的海参物种鉴定Species identification of sea cucumbers based on Sanger sequencing

市售海参样品标签符合性Identification results of commercial sea cucumber samples

编号	标签名称 (对应的中文名称)	DNA长条形码鉴定结果	标签 符合性	编号	标签名称 (对应的中文名称)	DNA长条形码鉴定结果	标签 符合性
1	秃参	<i>Holothuria mexicana</i> (墨西哥海参)	/	17	俄罗斯参 (仿刺参)	<i>Apostichopus japonicas</i> (仿刺参)	+
2	小有刺参	<i>Holothuria mexicana</i> (墨西哥海参)	/	18	山东刺参 (仿刺参)	<i>Stichopus horrens</i> (糙刺参)	-
3	小有刺参	<i>Holothuria grisea</i> (I)	/	19	黑方刺参 (绿刺参)	<i>Stichopus chloronotus</i> (绿刺参)	+
4	小黑刺参	<i>Holothuria tubulosa</i> (长刺乌爪)	/	20	花刺参 (花刺参)	<i>Apostichopus japonicas</i> (仿刺参)	-
5	短刺乌爪	<i>Holothuria poli</i> (短刺乌爪)	+	21	白刺参 (花刺参)	<i>Parastichopus tremulus</i> (挪威拟刺参)	-
6	长刺乌爪	<i>Holothuria tubulosa</i> (长刺乌爪)	+	22	福建刺参 (仿刺参)	<i>Apostichopus japonicas</i> (仿刺参)	+
7	米刺参	<i>Holothuria tubulosa</i> (长刺乌爪)	/	23	梦贝海参	<i>Isostichopus badionotus</i> (慢步等刺参)	/
8	黑参 (黑海参)	<i>Holothuria forskali</i> (I)	-	24	梅花参 (梅花参)	<i>Thelenota ananas</i> (梅花参)	+
9	猪婆参 (黄乳海参)	/	-				
10	黑香参	<i>Holothuria isuga</i> (I)	/				
11	靴参 (白底辐肛参)	<i>Actinopyga agassizi</i> (阿氏辐肛参)	-				
12	海参丝	<i>Cucumaria frondosa</i> (大西洋瓜参)	/				
13	獐子岛刺参 (仿刺参)	<i>Apostichopus japonicas</i> (仿刺参)	+				
14	山东刺参 (仿刺参)	<i>Apostichopus japonicas</i> (仿刺参)	+				
15	同仁堂刺参 (仿刺参)	<i>Apostichopus japonicas</i> (仿刺参)	+				
16	辽宁刺参 (仿刺参)	<i>Apostichopus japonicas</i> (仿刺参)	+				

## 检测结果:

- 24份样品中, 10份样品标签名称相符; 6份标签名称不符; 其余8份海参通过标签无法得到海参明确物种信息, 利用DNA条形码对其鉴定可得到明确的物种信息。

注: “+”表示物种鉴定结果与标签名称相符, “-”表示物种鉴定结果与标签名称不符。 “/”表示标签不明确无法判断标签符合性



# 基于NGS的海参物种鉴定

## Species identification of sea cucumbers based on NGS

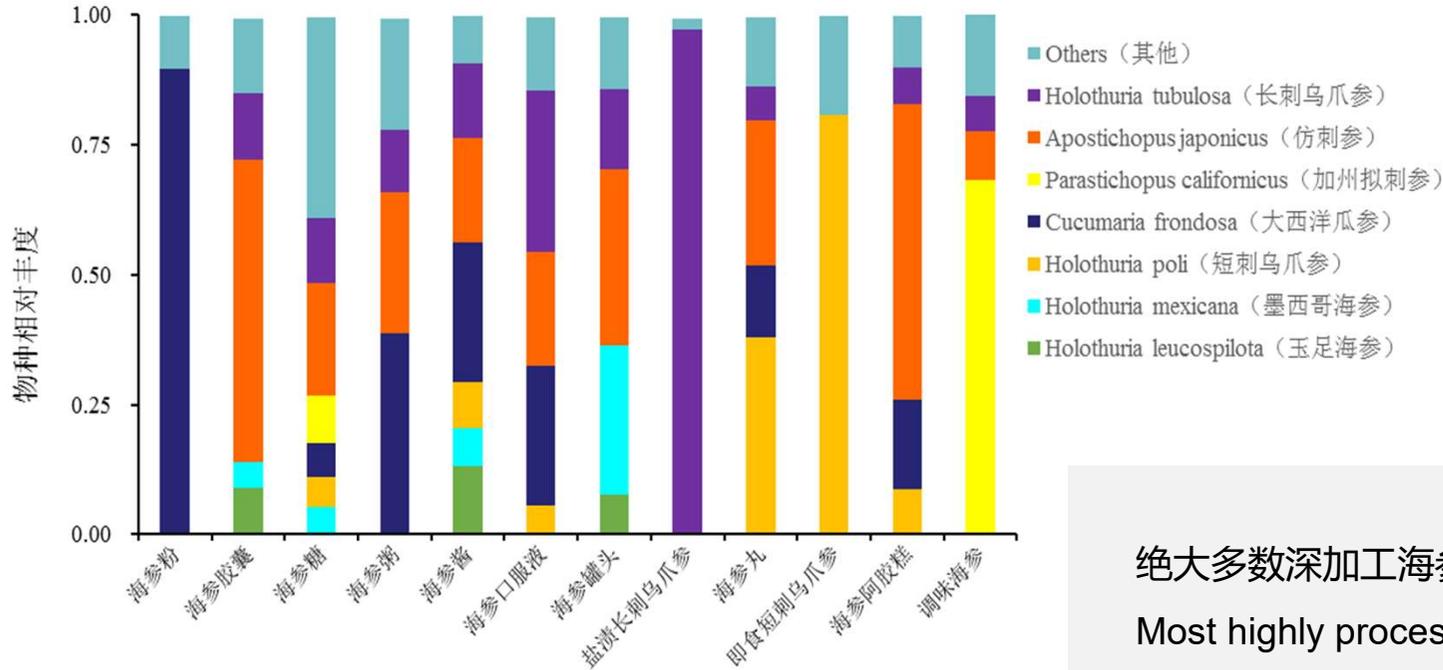
### 海参制品的信息

Sample information of 12 commercially processed sea cucumber products

Sample ID	产品描述 Product	Product description	标签列出的物种 Species listed
H1	辽参海参粉	Sea cucumber powder	辽刺参 ( <i>Apostichopus japonicus</i> )
H2	海参胶囊	Sea cucumber capsule	海参Sea cucumber
H3	海参糖	Sea cucumber sugar	海参Sea cucumber
H4	海参粥	Sea cucumber porridge	海参Sea cucumber
H5	海参酱	Sea cucumber sauce	刺参 ( <i>Apostichopus japonicus</i> )
H6	海参口服液	Sea cucumber oral solution	威海刺参 ( <i>Apostichopus japonicus</i> )
H7	海参罐头	Canned sea cucumber	俄罗斯刺参 ( <i>Apostichopus japonicus</i> )
H8	盐渍长刺乌爪参	Salted sea cucumber	长刺乌爪参 ( <i>Holothuria tubulosa</i> )
H9	海参丸	Sea cucumber ball	海参Sea cucumber
H10	即食短刺乌爪参丝	Frozen ready-to-eat sea cucumber	短刺乌爪参 ( <i>Holothuria poli</i> )
H11	海参阿胶糕	Sea cucumber cake	海参Sea cucumber
H12	阿拉斯加调味海参	Flavored sea cucumber	阿拉斯加红参 ( <i>Parastichopus californicus</i> )

# 基于NGS的海参物种鉴定

## Species identification of sea cucumbers based on NGS



市售海参制品的物种相对丰度柱形图

Relative species abundance of commercial sea cucumber products

绝大多数深加工海参制品检出不止一种海参成分  
 Most highly processed sea cucumber products were identified to contain more than one sea cucumber species.



# 基于NGS的海参物种鉴定

## Species identification of sea cucumbers based on NGS

举例 Sample	产品描述 Product	标签列出的动物物种 Species listed	检出动物物种 Species identification	是否相符 Yes/No?
H6	海参口服液 Sea cucumber oral solution	刺参 <i>A. japonicas</i>	长刺乌爪、大西洋瓜参、仿刺参、短刺乌爪 <i>H. tubulosa</i> , <i>C. frondosa</i> , <i>A. japonicus</i> , <i>H. poli</i>	×
H7	海参罐头 Canned sea cucumber	刺参 <i>A. japonicas</i>	墨西哥海参、长刺乌爪、仿刺参、玉足海参 <i>H. mexicana</i> , <i>H. tubulosa</i> , <i>A. Japonicus</i> , <i>H. leucospilota</i>	×
H2	海参胶囊 Sea cucumber capsule	海参Sea cucumber	仿刺参、长刺乌爪、玉足海参、墨西哥海参 <i>A. Japonicus</i> , <i>H. tubulosa</i> , <i>H. Leucospilota</i> , <i>H. mexicana</i>	√
H9	海参丸 Sea cucumber ball	海参Sea cucumber	短刺乌爪、大西洋瓜参、仿刺参、长刺乌爪 <i>H. poli</i> , <i>H. tubulosa</i> , <i>A. Japonicus</i> , <i>H. tubulosa</i>	√
H8	盐渍长刺乌爪参 Salted sea cucumber	长刺乌爪参 <i>H. tubulosa</i>	长刺乌爪参 <i>H. tubulosa</i>	√
H10	即食短刺乌爪参丝 Frozen ready-to-eat sea cucumber	短刺乌爪参 <i>H. poli</i>	短刺乌爪参 <i>H. poli</i>	√

# 应用案例 Applications



## 食用香辛料物种鉴别

## Species Identification in Dietary Spices



# Overview of Dietary Spices

## Single Spices

## Compound Spices



### Example: Chinese five spice powder

- Chinese five spice is a traditional blend of ingredients balancing five basic tastes; sweet, sour, salty, bitter and umami.
- The ingredients are complex and the formulation of commercially available five spice powder varies among manufacturers.
- According to the survey, there are about 19 raw materials (star anise, cinnamon, cumin).





# DNA barcodes selection and validation

## Identification of Botanicals in Dietary based on 4 candidate DNA barcodes

No.	Species Name	Latin name	ITS2 gene		rbcL gene	trnL (UAA) gene	psbA-trnH gene	
			GeneBank database (similarity)	Chinese herbal medicine DNA barcoding system (similarity)	GeneBank database (similarity)	GeneBank database (similarity)	GeneBank database (similarity)	Chinese herbal medicine DNA barcoding system (similarity)
1	granule	Amomum villosum	A. villosum (100.0%)	A. villosum (100.0%)	M	M (Amomum sp.)	N	N
2	strawberry	Amomum tsao-ko	A. tsao-ko (100.0 per cent)	A. tsao-ko (100.0 per cent)	--	--	--	--
3	cardamom (Elettaria cardamomum)	Amomum krervanh	A. compactum (100.0 per cent) A. krervanh (99.2 per cent)	A. compactum (100.0 per cent) A. krervanh (99.0 per cent)	M	M	A. compactum (99.9%) A. krervanh (99.5 per cent)	A. compactum (99.9%)
4	dried ginger	Zingiber officinale	Z. officinale (99.2%)	Z. officinale (98.5 per cent)	M	Z. officinale (100.0 per cent) Roscoea bhutanica(99.2%) Roscoea tumjensis (99.1%)	Z. officinale (100.0 per cent)	Z. officinale (100.0 per cent)
5	lesser galangale (Kaempferia galanga)	Alpinia galanga	--	--	M	M	A. galanga (99.8 per cent)	A. galanga (99.7 per cent)
6	cyanide (loanword)	Kaempferia galanga	K. galanga (100.0%)	K. galanga (100.0%)	M	M	K. galanga. (99.3%)	K. galanga. (99.3%)
7	cumin (Cuminum cyminum)	Cuminum cyminum	C. cyminum (100.0%)	C. cyminum (99.6%)	M	M	C. cyminum (100.0%)	N
8	fennel	Foeniculum vulgare	F. vulgare (100.0%)	F. vulgare (100.0%)	M	M	F. vulgare (99.6%)	F. vulgare (100.0%)
9	coriander seeds	Coriandrum sativum	C. sativum (100.0%)	C. sativum (100.0%)	M	C. sativum (98.6%)	C. sativum (99.4%)	N
10	Angelica dahurica (botany)	Angelica dahurica	A. dahurica (100.0 per cent)	A. dahurica (100.0 per cent)	M	M	A. anomala (100.0 per cent) A. dahurica (98.8 per cent)	A. dahurica (100.0 per cent)
11	cinnamon	Cinnamomum cassia	--	--	M (Cinnamomum sp.)	--	C. Cassia (100.0 per cent)	C. Cassia (100.0 per cent)
12	laurel leaf	Laurus nobilis	N	N	M	M	L. nobilis (98.9%)	L. nobilis (99.2%)
13	nutmeg (Myristicaceae)	Myristica fragrans	--	--	M	M	M. fragrans (99.1%)	M. fragrans (100.0%)
14	green pepper	Zanthoxylum schinifolium	Z. schinifolium (100.0%)	Z. schinifolium (100.0%)	M	M (Zanthoxylum sp.)	Z. schinifolium (100.0%)	N
15	dried orange peel	Citrus reticulata	C. Reticulata (100.0 per cent)	C. Reticulata (100.0 per cent)	M	M (Citrus sp.)	C. Reticulata (99.4 per cent) C. aurantium (99.6%)	C. Reticulata (99.8 per cent) C. aurantium (99.3%)
16	clove (Eugenia aromatica)	Syzygium aromaticum	S. aromaticum (100.0%)	S. aromaticum (100.0%)	--	/	--	--
17	anise	Illicium verum	--	--	M (Illicium sp.)	M	I. verum (99.5 per cent)	I. verum (99.7 per cent)
18	licorice	Glycyrrhiza uralensis	G. uralensis (100.0%)	G. uralensis (100.0%)	M (Glycyrrhiza sp.)	M	G. glabra (100.0 per cent) MG. inflata (99.8%) G. uralensis (99.8%)	G. pallidiflora (100.0%) G. uralensis (100.0%)
19	white pepper	Piper nigrum	P. nigrum (99.2%)	P. nigrum (99.3%)	M (Piper sp.)	M (Piper sp.)	P. nigrum (100.0%)	P. nigrum (100.0%)

"-" indicates no target band; "N" indicates that there is no sequence match in the database; "/" indicates that sequencing has failed; "M" indicates that five or more species (similarity ≥ 98%, coverage ≥ 98%) have been matched.

# Identification of Botanicals in Dietary Spices

- 12 **common raw materials** of Chinese five spice powder + 3 **common adulterants** (in powder form) + commercial Chinese five spice powder
- Preparation of **known mixtures** and DNA extraction
- Library preparation and high- throughput sequencing

Preparation protocol for mixed samples for next-generation sequencing.

Sample name	Sample composition and proportion
Authentic five spice powder	RF1 60 g groundnuts, 7 g cardamom, 12 g shanai, 12 g cloves, 7 g cinnamon
	RF2 20 g star anise, 8 g cumin, 6 g tangerine peel, 5 g dried ginger, 43 g cinnamon, 18 g peppercorns.
	RF3 52 g star anise, 10 g shanai, 4 g grits, 7 g licorice, 7 g cinnamon, 3 g white pepper, 17 g dried ginger.
Adulterated five spice powder	R1 RF1 / wheat (99:1)
	R2 RF1 / wheat (90:10)
	R3 RF1 / wheat (80:20)
	R4 RF2 / rice (99:1)
	R5 RF2 / rice (90:10)
	R6 RF2 / rice (80:20)
	R7 RF3 / maize (99:1)
	R8 RF3 / maize (90:10)
	R9 RF3 / maize (80:20)







## 应用案例Applications



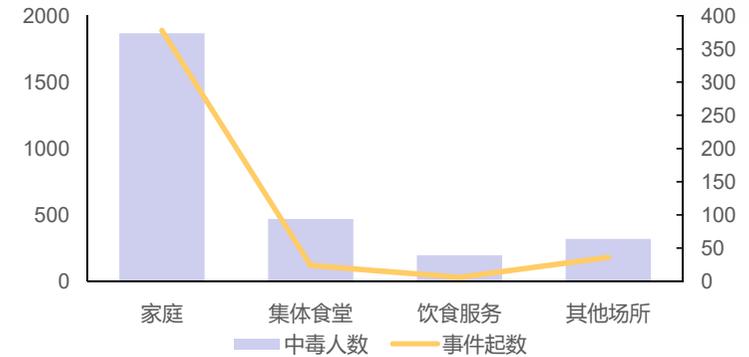
### 基于DNA条形码技术的有毒蘑菇鉴别

Species identification in poisonous mushrooms



# 有毒蘑菇概况 Poisonous mushrooms

- 毒蘑菇是指含有毒素的个体肉质的一类**大型真菌**，多处于野生状态；
- 我国有毒蘑菇约**480种**，主要隶属于39科、112属；
- 是造成**食物中毒及死亡**的主要原因之一。



蘑菇中毒事件发生场所

2019年中国蘑菇中毒事件中蘑菇种类分布

Mushroom species	Number of incidents	Number of patients	Deaths	Deaths Mortality
<i>Russula</i>	65	195	1	4.55%
<i>Amanita</i>	60	147	20	90.91%
<i>Chlorophyllum</i>	58	142	0	0.00%
Other mushrooms	22	115	0	0.00%
<i>Entoloma</i>	12	38	0	0.00%
<i>Galerina</i>	6	14	1	4.55%
<i>Agaricus</i>	6	15	0	0.00%
<i>Scleroderma</i>	6	10	0	0.00%
<i>Lepiota</i>	5	11	0	0.00%
<i>Gymnopilus</i>	5	6	0	0.00%
<i>Inocybe</i>	4	7	0	0.00%
<i>Psilocybe</i>	4	17	0	0.00%
<i>Lactarius</i>	3	12	0	0.00%
<i>Omphalotus</i>	3	19	0	0.00%
Others	11	19	0	0.00%
Total	270	767	22	100.00%



肉褐鳞环柄菇



铅绿褶菇



亚稀褶红菇



灰花纹鹅膏菌

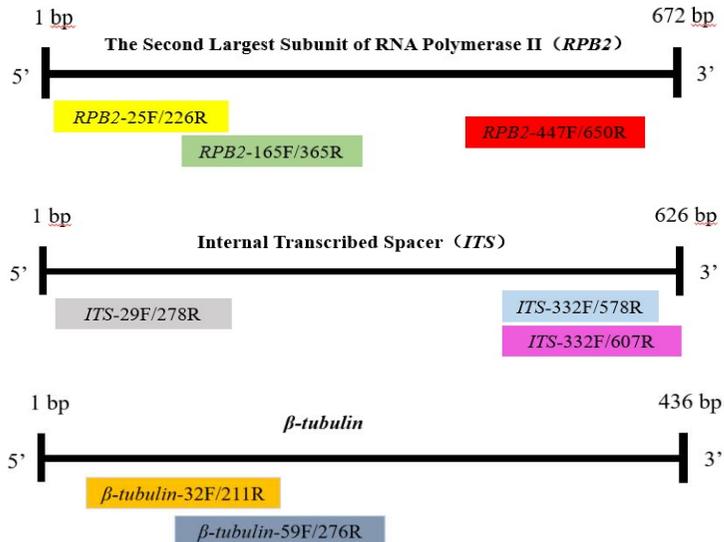
我国常见的四种毒蘑菇



# 基于DNA条形码技术的有毒鹅膏菌属物种鉴别 Species identification of *Amanita* by DNA barcoding

## DNA条形码引物设计与筛选

DNA barcode primer design and screening



## 实验样品信息

序号	分类地位	样品编号	物种名称	拉丁名	是否有毒
1	伞菌目鹅膏菌科鹅膏菌属	CAIQ12001	朽柄鹅膏菌	<i>Amanita sinocitrina</i>	是
2	伞菌目鹅膏菌科鹅膏菌属	CAIQ12002	小毒蝇鹅膏菌	<i>Amanita melleiceps</i>	是
3	伞菌目鹅膏菌科鹅膏菌属	CAIQ12003	红托鹅膏菌	<i>Amanita rubrovolvata</i>	是
4	伞菌目鹅膏菌科鹅膏菌属	CAIQ12004	草鸡枞鹅膏菌	<i>Amanita caojizong</i>	否
5	伞菌目鹅膏菌科鹅膏菌属	CAIQ12005	草鸡枞鹅膏菌	<i>Amanita caojizong</i>	否
6	伞菌目鹅膏菌科鹅膏菌属	CAIQ12006	草鸡枞鹅膏菌	<i>Amanita caojizong</i>	否
7	伞菌目鹅膏菌科鹅膏菌属	CAIQ12007	异味鹅膏菌	<i>Amanita kotohiraensis</i>	是
8	伞菌目鹅膏菌科鹅膏菌属	CAIQ12008	欧氏鹅膏菌	<i>Amanita oberwinklerana</i>	是
9	伞菌目鹅膏菌科鹅膏菌属	CAIQ12009	欧氏鹅膏菌	<i>Amanita oberwinklerana</i>	是
10	伞菌目鹅膏菌科鹅膏菌属	CAIQ12010	灰花纹鹅膏菌	<i>Amanita fuliginea</i>	是
11	伞菌目鹅膏菌科鹅膏菌属	CAIQ12011	灰花纹鹅膏菌	<i>Amanita fuliginea</i>	是
12	伞菌目鹅膏菌科鹅膏菌属	CAIQ12012	灰花纹鹅膏菌	<i>Amanita fuliginea</i>	是
13	伞菌目鹅膏菌科鹅膏菌属	CAIQ12013	球基鹅膏菌	<i>Amanita subglobosa</i>	是
14	伞菌目鹅膏菌科鹅膏菌属	CAIQ12014	假黄盖鹅膏菌	<i>Amanita pseudogenmata</i>	是
15	伞菌目鹅膏菌科鹅膏菌属	CAIQ12015	锥鳞白鹅膏菌	<i>Amanita virgineoides</i>	是
16	伞菌目鹅膏菌科鹅膏菌属	CAIQ12016	锥鳞白鹅膏菌	<i>Amanita virgineoides</i>	是
17	伞菌目鹅膏菌科鹅膏菌属	CAIQ12017	拟卵盖鹅膏菌	<i>Amanita neoovoidea</i>	是
18	伞菌目鹅膏菌科鹅膏菌属	CAIQ12018	鹅膏一种菌	<i>Amanita sp.</i>	是
19	伞菌目鹅膏菌科鹅膏菌属	CAIQ12019	致命鹅膏菌	<i>Amanita exitialis</i>	是
20	伞菌目鹅膏菌科鹅膏菌属	CAIQ12020	土红鹅膏菌	<i>Amanita rufoferruginea</i>	是
21	伞菌目鹅膏菌科鹅膏菌属	CAIQ12021	刻鳞鹅膏菌	<i>Amanita sculpta</i>	是
22	伞菌目鹅膏菌科鹅膏菌属	CAIQ12022	裂皮鹅膏菌	<i>Amanita rimosa</i>	是
23	伞菌目鹅膏菌科鹅膏菌属	CAIQ12023	裂皮鹅膏菌	<i>Amanita rimosa</i>	是
24	伞菌目鹅膏菌科鹅膏菌属	CAIQ12024	东方褐盖鹅膏菌	<i>Amanita orientifulva</i>	是
25	伞菌目鹅膏菌科鹅膏菌属	CAIQ12025	黄柄鹅膏菌	<i>Amanita flavipes</i>	是



# 基于DNA条形码技术的有毒鹅膏菌属物种鉴别

## Species identification of *Amanita* by DNA barcoding

DNA条形码扩增序列比对结果

序号	样品编号	物种名称	拉丁名	ITS序列		
				NCBI鉴定结果序列相似度	收录号	长度bp
1	CAIQ12001	杵柄鹅膏菌	<i>Amanita sinocitrina</i>	+	MN622709.1	290
2	CAIQ12002	小毒蝇鹅膏菌	<i>Amanita melleiceps</i>	+	KF017939.1	290
3	CAIQ12003	红托鹅膏菌	<i>Amanita rubrovolvata</i>	+	JN943178.1	290
4	CAIQ12004	假褐云斑鹅膏菌	<i>Amanita pseudoprinceps</i>	+	NR_159588.1	290
5	CAIQ12005	草鸡枞鹅膏菌	<i>Amanita caojizong</i>	+	NR_159571.1	290
6	CAIQ12006	袁氏鹅膏菌	<i>Amanita yuaniana</i>	+	MH508654.1	290
7	CAIQ12007	异味鹅膏菌	<i>Amanita kotohiraensis</i>	+	MH508414.1	290
8	CAIQ12008	裂皮鹅膏菌	<i>Amanita rimosa</i>	+	MN061275.1	290
9	CAIQ12009	欧氏鹅膏菌	<i>Amanita oberwinklerana</i>	+	MH508446.1	290
10	CAIQ12010	灰花纹鹅膏菌	<i>Amanita fuliginea</i>	+	MN061271.1	290
11	CAIQ12011	灰花纹鹅膏菌	<i>Amanita fuliginea</i>	+	FJ176717.1	290
12	CAIQ12012	灰花纹鹅膏菌	<i>Amanita fuliginea</i>	+	KU356799.1	290
13	CAIQ12013	球基鹅膏菌	<i>Amanita subglobosa</i>	+	KF017947.1	290
14	CAIQ12014	假黄盖鹅膏菌	<i>Amanita pseudogemmata</i>	+	MK239258.1	290
15	CAIQ12015	锥鳞白鹅膏菌	<i>Amanita virgineoides</i>	+	KP004958.1	290
16	CAIQ12016	锥鳞白鹅膏菌	<i>Amanita virgineoides</i>	+	MG383668.1	290
17	CAIQ12017	拟卵盖鹅膏菌	<i>Amanita neoovoidea</i>	+	MK279377.1	290
18	CAIQ12018	鹅膏一种	<i>Amanita minutisquama</i>	+	NR_159582.1	290
19	CAIQ12019	致死鹅膏菌	<i>Amanita exitialis</i>	+	KC755037.1	290
20	CAIQ12020	土红鹅膏菌	<i>Amanita rufoferruginea</i>	+	KU497532.2	290
21	CAIQ12021	刻鳞鹅膏菌	<i>Amanita sculpta</i>	N		290
22	CAIQ12022	裂皮鹅膏菌	<i>Amanita rimosa</i>	+	MN061275.1	290
23	CAIQ12023	裂皮鹅膏菌	<i>Amanita rimosa</i>	+	N061275.1	290
24	CAIQ12024	东方褐盖鹅膏菌	<i>Amanita orientifulva</i>	+	FJ441035.1	290
25	CAIQ12025	黄柄鹅膏菌	<i>Amanita flavipes</i>	+	MH508346.1	290
26	CAIQ12026	假淡红鹅膏菌	<i>Amanita subpallidrosea</i>	+	KP691679.1	290
27	CAIQ12027	假淡红鹅膏菌	<i>Amanita subnallidrosea</i>	+	KP691681.1	290

# 基于DNA条形码技术的鹅膏菌属物种鉴别

## Species identification of *Amanita* by DNA barcoding

[www.crazyblack.cn](http://www.crazyblack.cn)



鹅膏菌属物种信息网页效果图



# 食品追溯 Food products traceability

## “法国原产牛肉” “法国原产猪肉” 认证溯源体系 “French-origin beef” “French-origin pork” Certified Traceability System

2019年第二届中国国际进口博览会启动，实现了法国原产猪肉全链条追溯，保障了从进口到消费者餐桌的质量安全。

The 2nd China International Import Expo (CIIE) 2019 has enabled the full chain traceability of French-origin pork and beef.



## 燕窝追溯体系 Bird Nest Traceability System



### 二维码

使用微信，支付宝扫一扫，获取全程追溯信息。

### 燕窝码

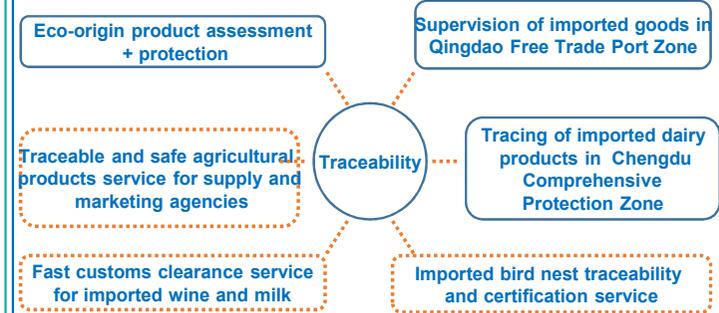
由16位数字组成，每枚溯源标签拥有唯一身份编码。

### 防伪码

刮开溯源标签的下部涂层，获取防伪码激活产品，验证产品真伪。



## 大数据中心 Big Data Center





# Thanks for your attention

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