

Detection and identification of Ephedra Herba seed pests based on X-rays and DNA barcodes

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Abstract

As an important natural medicinal resource for humans, *Ephedra sinica* Stapf often suffers from various biological stresses during its growth process. One of the pests that pose a threat to Ephedra is the Ephedra seed pest. Its primary mode of damage is laying eggs inside the Ephedra seeds, where they develop and eventually emerge as adult wasps, causing significant damage to the seeds. This study aimed to investigate the effectiveness of X-ray for Ephedra seed pest detection, the impact on the use value of Ephedra seeds infested by pests, and the further confirmation of pest species information. The mature Ephedra seeds from the Inner Mongolia Autonomous Region were taken as the teste object. The results showed that the germination rates of three different batches of seeds were 46%, 40%, and 38%, while the seedling emergence rates were only 36%, 30%, and 32%, respectively, significantly lower than that of control healthy seeds 66% and 52%. The phylogenetic tree Neighbor-Joining (NJ) was constructed by extracting the COI sequences of the pest samples, and was identified as a new species of Eurytoma genus based on morphological characteristics. X-ray detection is a commonly used, non-invasive method. Based on non-destructive X-ray testing, the quality of Ephedra seeds could be classified into three types: healthy plump seeds, seeds infested by pests, and underdeveloped hollow seeds. In conclusion, the combined use of X-ray and DNA barcoding methods can achieve rapid and accurate detection and identification of *E. sinica* seed pests, which is of great significance for the management and control of Ephedra seed production.

Introduction

Ephedra sinica Stapf is a perennial herbaceous shrub belonging to the Ephedraceae family in the gymnosperm division. It is one of the sources of the traditional Chinese medicine Ephedra herba listed in the Chinese Pharmacopoeia (2020 edition) (Zheng et al., 2023). Ephedra has well-developed roots that allow it to adapt to harsh environments such as extreme cold, drought, and poor soil conditions. Its above-ground parts contain abundant energy and fiber, making it a valuable forage grass (Xiao et al., 2022). Ephedra has been recorded as a medicinal herb in the "Shennong's Herbal Classic" and has a history of over four thousand years in China (Tang et al., 2020). China is a major producer of Ephedra herba raw materials. However, the discovery of the bioactive component ephedrine in recent times has led to a continuous increase in market prices, resulting in extensive exploitation of natural Ephedra herba resources and a drastic decline in reserves, causing significant environmental damage (Hong et al., 2011). To ensure the sustainable use of Ephedra resources and conserve the ecological environment, artificial cultivation of Ephedra has been carried out in an extensive area covers Ningxia, Gansu, and Inner Mongolia with the support and assistance of relevant departments, achieving preliminary success (Li et al., 2000). Among the three medicinal *Ephedra* species (*E. equisetina*, *E. intermedia*, and *E. sinica*), *E. sinica* exhibits certain advantages in terms of alkaloid content and harvesting and processing methods, making it the main species for Ephedra herba commercialization and artificial cultivation (Cha et al., 2002). With the ongoing expansion of Ephedra herba cultivation areas, the Ephedra seed production has been growing, accompanied by an increasing problem of seed pests. Surveys have found that seeds from

various Ephedra-producing areas in Inner Mongolia suffered from infestation by seed pests to varying degrees. The peak period of pest occurrence was from June to August, and the occurrence of Ephedra seed pests was highly consistent with its seed growth period, with severe infestation rates exceeding 50% (An et al., 2000). The eggs can hatch at the time of seed development of the nutrients of the seeds to complete their own growth and development. It has a serious impact on the quality of seeds, resulting in a decrease in the germination rate of seeds, which not only hinders the expansion of Ephedra cultivation, but also impeding the industrial production of Ephedra seeds. Currently, the severity of Ephedra seed pest infestations is generally assessed through random sampling and seed quality inspections.

However, Ephedra seeds are small volume and large in quantity, requiring a significant amount of time and labor for sampling and quality inspection. The commonly used dissecting method for quality inspection is destructive and adds additional costs. Ephedra seeds, being gymnosperms, which not only forms a natural protective barrier for the propagation and growth of the parasitic pest, effectively deterring predation and competitors, but also makes it difficult to distinguish and screen from appearance during general inspection in the production and processing process. As a result, parasitic eggs and larvae can be spread through the circulation of seeds, posing a risk of the spread of harmful organisms. Therefore, it is crucial to develop effective methods for detecting infested seeds and further determining the species information of the pests.

X-rays are high-energy electromagnetic radiation with extremely high frequencies (30 PHz to 30 EHz), short wavelengths (0.01 nm to 10 nm), and significant energy (100 eV to 100 keV). X-rays undergo attenuation when passing through materials, and the total attenuation coefficient depends on the material density and thickness. The grayscale value of an X-ray image is inversely proportional to the degree of attenuation of X-rays passing through the material. Different substances will have different grayscale values, allowing for differentiation. Based on this characteristic, X-rays have played a significant role in medical diagnosis, airport security screening, non-destructive testing, industrial inspection, and other fields. X-rays can be classified into two categories: hard X-rays and soft X-rays, based on their wavelength penetration capabilities (SIMAK et al., 1953). In the mid-20th century, soft X-ray radiography has been widely used as a fundamental inspection technique in foreign countries for assessing the quality of tree seeds (Chen et al., 1979). It can detect mechanical damage, empty seeds, and pest infestations, as well as determine germination capacity (Wang et al., 1988). However, the effectiveness of soft X-ray radiography is influenced by various factors, with exposure parameters and sample properties being the main factors. Only when these two factors are coordinated can clear images be obtained. With the advancement of X-ray technology, the next-generation X-ray radiography system (Faxitron MX-20) has effectively addressed these limitations. The exposure parameters can be automatically adjusted based on the sample's properties to select the appropriate voltage and current. Additionally, the exposure time has been greatly reduced, with higher resolution and images can be obtained immediately after capturing, equipped with image processing tools, significantly improving inspection efficiency and avoiding labor-intensive destructive testing. X-ray non-destructive testing can detect pests hidden inside seeds, but it cannot further provide rapid and accurate identification of the species information of the pests.

At present, insect species identification strategies mainly include traditional identification and molecular identification. Traditional identification classifies species step by step according to their external and subtle morphological characteristics through anatomical and microscopic observation, which is applicable to known insect species, while other methods are required for unknown species. With the continuous development of DNA barcoding technology in recent years, specific DNA sequences representing specific biological information can be used to differentiate and identify species (Hu et al., 2019; Liao et al., 2022). This method has advantages such as simplicity, accuracy, rapidity, and independence of the developmental stage of the species, individual morphology, and the expertise of the researcher. It fills the gaps in traditional identification methods. Modern research has shown that the COI (cytochrome oxidase subunit I) gene sequence has low intraspecific variation but high interspecific variation (Jing et al., 2018). It also rarely experiences insertions and deletions. As a result, it has been widely accepted as a universal barcode for animal identification by biologists. In this study, a combination of traditional morphological identification and DNA barcoding is used for pest identification (Zhou et al., 2023). These two approaches complement and validate each other, further ensuring the accuracy of species identification.

In this study, we focused on mature Ephedra seeds from three different batches sourced from the Inner Mongolia Autonomous Region. Due to the habits of certain pests, the Ephedra seeds were found to be infested with harmful insects, making it difficult to differentiate through visual inspection alone. Therefore, we propose a rapid identification and detection method for hidden pests in traditional Chinese medicinal materials. We utilize the next-generation X-ray radiography system (Faxitron MX-20) to detect insect-infested Ephedra seeds and further employ DNA barcoding to rapidly identify the species information of the pests. This method plays a practical role in rapid detect the quality of Ephedra seeds, reducing cultivation costs, and increasing economic benefits for businesses. Additionally, it provides valuable insights for understanding and addressing the issue of pest species that pose a threat to traditional Chinese medicinal materials.

Materials and Methods

Sample Collection

The maturation period of Ephedra seeds is typically around mid-July each year (Si et al., 2009). In this study, from July 14th to 16th, 2022, our research team collected mature Ephedra cones in three different areas in Etuoke Front Banner, Ordos City, Inner Mongolia Autonomous Region (107°48'E, 38°18'N; 107°32'E, 38°63'N; 107°84'E, 37°83'N) at an altitude of 1296 m in a sandy dune area (Fig. 1A). The mature Ephedra cones are oval-shaped and have red fleshy bracts (Fig. 1B). As confirmed by Dr. Hu Haoyu from the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, the plant was identified as *E. sinica* Stapf, a species of the Ephedra genus. The red fleshy bracts were removed, and the plant material was cleaned and air-dried at room temperature. Seeds of *E. sinica* weighing over 50 g per batch were obtained and kept for further use. Larval samples of the parasitic

insects on Ephedra seeds were collected through inspection, and the larvae were allowed to hatch to obtain adult specimens.

Seed Inspection

Seed Characteristics 50 seeds were randomly selected from each of the three batches of *E.sinica* seeds. A caliper was used to measure the length, width, and thickness of the seeds, repeated three times, and the average values were recorded. Additionally, a random selection of 1000 seeds was weighed using a QL-E210A analytical balance with a precision of one thousandth, repeated three times, and the average values were obtained to calculate the thousand-grain weight of *E.sinica* seeds from different batches. The seeds were dissected and observed under a dissecting microscope to describe their characteristics.

Seed Germination

By examining randomly selected seeds with intact seed coats from each batch, 50 seeds were chosen for each group. The seeds in all three groups were washed and disinfected with 75% ethanol for 30 seconds, followed by three rinses with distilled water. They were then disinfected with 1% NaClO for 5 min, rinsed three times with distilled water, and soaked in distilled water for 24 h to fully absorb water and expand. Finally, the seeds were evenly distributed in sterilized Petri dishes with a diameter of 90 mm, which were lined with 2 layers of filter paper. Each Petri dish was filled with 5 mL of distilled water. The Petri dishes were placed in a climate-controlled incubator for germination, with the following parameters set: humidity at 25%, temperature at $25 \pm 1^\circ\text{C}$, light intensity at level 5, and a light/dark cycle of 16 h/8 h. During the germination process, water was added as needed to maintain the moisture of the germination bed. Germination was defined as the emergence of a root approximately 1 cm long from the seed, and seedling emergence was defined as the unfolding of green cotyledons (Liu et al., 1997). The germination progress of the Ephedra seeds was recorded and monitored every 2 d, with the final record taken on the 21th d.

Determination of Seed Germination Indices

The germination rate and seedling emergence rate of the seeds were determined using the paper plate method. The data were analyzed and processed using Microsoft Excel and IBM SPSS Statistics software (26 versions), following the formulas below:

Germination Rate (%) = (Number of seeds germinated / Total number of seeds tested) $\times$ 100%

Seedling Emergence Rate (%) = (Number of seedlings emerged / Total number of seeds tested) $\times$ 100%

X-ray Detection

X-ray Image Acquisition

X-ray images were acquired using the next-generation X-ray radiation system (Faxitron MX-20). The system consisted of a detection device (sample inspection chamber, X-ray source, X-ray detector, and shielding device), a power supply, and a computer operating system (used to control and manage the X-

ray detection system, as well as process and analyze the collected image data) (Fig. 2). Place the Ephedra seeds with the abdomen facing down in the scanning area of the sample testing room, and perform X-ray scanning in the scanning area. The X-ray source emitted the rays, and the X-ray detector captured the signals. The voltage and current settings of the X-ray source were set to 20 kV and 21 mA, respectively. The detector was based on a linear CCD (charge-coupled device) imaging system, which captured grayscale lines generated when X-rays passed through the objects. The image processing system stitched these lines together at high speed on the computer to form the image. In this study, the following parameters were used:

X-ray Source: Faxitron MX-20 X-ray System (Output kV: 20–80 kV, Output mA: 0.2–0.7 mA, Continuous Maximum Output Power: 50 W). Linear Array Detector: XNDT Technology XNDT-04 Data Acquisition System (Pixel Size: 50 μ m – 0.8 mm, Scanning Range: 400 mm-600 mm, Electronic Signal-to-Noise Ratio: 50,000:1, System Signal-to-Noise Ratio: 25,000:1).

To this end, using X-ray non-destructive testing, we selected 50 healthy and plump seeds for germination experiments as controls, following the procedure outlined in 2.2.2, to investigate the impact of pests on seed germination rate. Furthermore, we randomly selected 50 seeds from each of the three different batches of samples for testing and calculated the parasitic rate of pests using the following formula:

Parasitic Rate of Small Wasps (%) = (Number of seeds infested by pests / Total number of tested seeds) $\times$ 100%.

X-ray Image Processing

After X-ray examination, the X-ray image files obtained were processed and analyzed using image processing techniques on the computer operating system. The original X-ray images were enhanced in terms of brightness and contrast to facilitate identification. The X-ray images of Ephedra seeds were manually annotated and differentiated into three groups: "healthy and plump seeds," "underdeveloped and shriveled seeds," and "seeds infested by pests." The internal X-ray images of the seeds appeared uniformly grayish-white, indicating they were classified as "healthy and plump seeds." Additionally, if the image shows signs of pests (eggs, larvae, pupae, or adult insects), it is classified as "seed infested by pests."

Morphological Identification

The seeds were dissected using double-sided razor blades and insect pins to collect samples of pest larvae and adult specimens after cultivation and hatching. Photographs were taken under a Leica stereomicroscope (LED5000 SLI), and the main external morphological characteristics of the larvae and adults were described.

DNA Molecular Identification

Adult specimens and larvae were separately taken for total DNA extraction using an Animal Genomic DNA Kit (Tiangen Biotech Co., Ltd., Beijing, China), and the DNA concentration was measured using a Qubit 3. PCR amplification was performed in a 25 μ L reaction system, including 12.5 μ L PCR MasterMix (Aidlab Biotech Co., Ltd., Beijing, China), 8.5 μ L ddH₂O, 1 μ L each of forward and reverse primers (LCOI1490: 5'-GGTCAACAAATCATAAAGATATTGG-3'; HCO2198: 5'-TAAACTTTCAGGGTGACCAAAAAATCA-3') (2.5 μ M, synthesized by China Shenggong Company), and 2 μ L DNA template. The PCR reaction conditions were as follows: initial denaturation at 94°C for 1 min; denaturation at 94°C for 1 min, annealing at 45°C for 1.5 min, extension at 72°C for 1.5 min, for 5 cycles; denaturation at 94°C for 1 min, annealing at 50°C for 1.5 min, extension at 72°C for 1 min, for 35 cycles; final extension at 72°C for 5 min, and then storage at 4°C. The PCR products were analyzed by agarose gel electrophoresis, and the qualified PCR products were sent to Beijing Ruibo Biotech Co., Ltd. for Sanger sequencing. The obtained sequences were compared with sequences in the NCBI (National Center for Biotechnology Information) database using BLAST to determine homology with known species sequences. Clustal W alignment and construction of a NJ phylogenetic tree were performed using MEGA 6.0 software to determine the species information.

Results

The damage of seed pests

Mature *E. sinica* seeds are covered by a hard, brown-black, or black-brown semi-leathery testa. The seeds are approximately 4 mm long, 2.5 mm wide, and 1.5 mm thick, with a triangular oval shape. The ventral side is flat or concave, while the dorsal side is convex. There are ridges in the middle of the convex surface, and the hilum is prominent (Fig. 3A, B). The length, width, thickness, and thousand-grain weight of the tested Ephedra seeds from different batches are shown in Table 1. The results indicate no significant differences among the different batches ($P > 0.05$).

Table 1 Basic Characteristics of Different Batches of *E. sinica* Seeds.

No.	Length (mm)	Width (mm)	Thickness (mm)	Thousand-grain weight (g)	Purity (%)
1	4.05±0.45	2.48±0.31	1.43±0.15	7.78±0.10	95.3
2	4.14±0.35	2.54±0.33	1.49±0.22	8.09±0.08	96.1
3	4.23±0.38	2.65±0.42	1.54±0.18	8.25±0.15	97.2

A total of 33 seeds with a diameter of approximately 0.5 mm were examined from the three batches, and more than 90% of the insect holes were found on the dorsal median ridge (DR) of the seeds (Yong, 2011). Very few insect holes were observed on the ventral side (Fig. 3C).

Under appropriate air, moisture, and temperature conditions, the front end of the *E. sinica* seeds gradually cracks after 2 d, exposing the embryo; after 3 d, the radicle begins to grow, reaching a length of up to 4

cm; after 6 d, cotyledons start to grow, with two green needle-shaped leaflets; germination is completed after 20 d. The seed germination rates of the three batches of experimental groups were 46%, 40%, and 38%, respectively, and the control group was 66%; the seedling emergence rates were 36%, 30%, and 32%, respectively, and the control group was 52% (Fig. 5A, B). The seedling emergence rate and germination rate of the experimental group were significantly lower than those of the control group.

X-ray detection

As described in Section 2.3.2, by X-ray image analysis, *E. sinica* seeds can be divided into three categories: full seeds, empty seeds, and insect-eaten seeds, with three manifestations. We randomly inspected and selected a certain number of seeds with complete shapes, took pictures and records under the type microscope, and then conducted X-ray detection. X-ray images could clearly identify whether there were pests in the seeds, and further anatomical examination verified the accuracy of X-ray detection.

The pest parasitism rate was 14%, 8%, and 20%, and the proportion of underdeveloped empty seeds was 20%, 28%, and 18%, as shown in Figure 6. The seeds under conventional inspection can only observe whether the appearance of the arils is intact and complete and cannot judge whether the interior of the seeds is healthy. Through X-ray detection, we can see that the gray value of the inner seed kernel of a healthy Ephedra seed (representing number) in the sample is uniform. The insect-eaten seeds (representing number) appear clearly hollow in the middle of the X-ray image, which is very obvious in contrast to the healthy seeds. The naked eye can see the larval form of an invasive pest, and the seeds have been completely eaten. The gray value of the X-ray image of the empty seed kernel (representing the number) is not uniform, and there are obvious differences. The difference in gray value can be explained by the X-ray imaging principle, under the same X-ray irradiation conditions, the gray value of the X-ray image can be compared with the value of the material density and thickness. The light gray area in the X-ray image is caused by the lower than normal tissue density of the seed kernel.

Thus, healthy seeds, empty seeds, and insect-eaten seeds can be identified by X-ray images.

Identification

Species identification and morphological characteristics

The adult pest was observed as being small, with transparent membranous wings, which is characteristic of Hymenoptera, Eurytomidae (Zang et al., 2008). China wide shoulder Eurytomidae: 12 genus and 61 species were recorded, China's Inner Mongolia region distribution of nine, the world record of 1267, less than 5% of the world's record. Eurytomidae; can be divided into three species: phytophagous, parasitic, and predatory. The phytophagous species are represented by *Bruchophagus* and *Eurytoma*, and their larvae feed on plant seeds, such as *Bruchophagus gibbus* and *Eurytoma larici* et al. (Zhang et al., 2019). There are many species of Eurytomidae, their classification is complex, and identification is difficult. In

this paper, by checking relevant literature (Liu et al., 2013), the morphological characteristics of its adults and larvae (Fig. 6), are described as follows:

The adult body length is about 3 mm, the head is black, the back view is wider than the chest, and the front view is oval, slightly forward. Compound eyes are red; Antennae linear black, 7 segments with brown villous, located in the middle of the face, antennae deeply depressed, marginal ridge on both sides; The chest is black, and the knees, tibial ends, and tarsus of each foot are light yellow. Short ventral stalk; The wings are transparent, and the veins are yellowish. The abdomen is dark brown, slightly dark green, and oval, with white bristles at the end, and the ovipositor is exposed.

The larva is oval, 2-3 mm long, 1 mm wide, milky white, translucent skin, no feet, a total of 13 sections of the body, divided into three parts: head, chest, and abdomen. The head is oblate, the mouthparts are pale brown and spiny, and the body has a black blind sac. The pupa is a spindle-shaped orange-yellow, about 3 mm in length, with a raised back and black back before emergence. The egg is a long, tadpole-shaped, pale yellow egg composed of an egg stalk, egg filament, and egg body.

COI sequence analysis

In Hymenoptera, Eurytomidae accounts for only 1%, with 88 genera, among which 628 species of Eurytomidae are the most documented genus (data images from <http://www.sp2000.org.cn/browse/>) (Fig. 7A, B). In this paper, the COI gene sequence was obtained by sequencing, the length of which was 691 bp. The NCBI database was used to analyze and compare the results of BLASTN analysis, and no similar sequence was found in the Eurytomidae. Combined with the published species data from NCBI, a phylogenetic tree was constructed using the adjacency method. The numbers on the branches represented the bootstrap value based on 1000 repetitions, and the values on the branches only showed the self-developing support rate of $\geq 50\%$. Intraspecific Kimura-2-parameter genetic distance analysis showed that the intraspecific variation of the species was small, the K2P genetic distance of the species ranged from 0 to 0.005, and the K2P genetic distance between the species and other closely related species ranged from 0.02 to 0.045. The referenced sequences are from the GenBank database; the relevant entry numbers are indicated after the species name; and the species sequences obtained in this study are marked with black dots. NJ phylogenetic tree results indicate (Fig. 7C) that the species clustered together with *Eurytoma rosae* Nees and separated into a clade. DNA barcoding combined with morphological identification supported this species as a newly differentiated *Eurytoma* species.

Discussion

After long-term natural selection and interaction between parasitic insects and host plants in nature, mutual adaptability and synchronicity have emerged in growth and development (Hu et al., 2022). For example, in *Bruchophagus glycyrrhizae* (Qian et al., 2008) and *Bruchophagus huonchei* (Fan et al., 1991), the larval emergence period coincides with the flowering period of plants, and the larval growth period coincides with seed development and maturity. The same is true for Ephedra seed pests. Seed quality determines the reproduction and survival of plant populations, as well as the time when plants enter the

natural ecosystem, and has a direct impact on yield (Xu et al., 2014). It was found that the germination rate and emergence rate of Ephedra seeds were decreased by 24.7% and 19.4%, respectively, which seriously affected the germination ability and the speed and uniformity of seed germination. The germination rate of the experimental group was low. The author believed that on the one hand, it was caused by the destruction of seeds by pests, insect-eaten seeds were detected in 14% of the ungerminated seeds; on the other hand, there were underdeveloped empty seeds, proportion 20%, which have lost the ability to germinate, possibly because Ephedra herba, through selective abortion, eliminated bad genotypes to improve the individual fitness of offspring, forming a reproductive protection mechanism to cope with a resource-poor and arid environment. Subsequently, ephedra genome information can be studied and mined at present. Genome research is the most effective way to analyze all the genetic resources of a species, and it is also an important strategic resource for the Chinese medicine industry (Liao et al., 2022; Xu et al., 2022; Chen et al., 2022; Sun et al., 2023; Xu et al., 2023). Through X-ray detection and seed quality inspection, the pest rate of Ephedra seed in the samples of this study reached more than 20%, indicating that the harm of Ephedra seed pests in this area is still very serious.

Pests hide in the seeds of plants and develop with the development of seeds, and it is difficult to detect from the appearance of seeds in the early stage of shell emergence. The traditional control methods are difficult to deal with, and the effect is not good. For example, the early seed quality inspection was mainly conducted by the anatomical method, which has been used ever since, which is characterized by high workload, low efficiency, and damage to seeds, and the dissected seeds can no longer be used for actual production. In addition, both the rolling method and the water separation method adopted by experience have certain limitations. In this regard, in the 1950s of the last century, soft X-ray imaging technology was applied in forest seed inspection abroad, which could distinguish empty, deformed, and full seeds, achieving non-destructive testing with high accuracy. In 1973, China successfully developed the first DGX-4 soft X-ray machine and used it for the detection of forest seeds, which can accurately see whether there are pests in the seeds and the development status of the seeds. However, the shooting effect is affected by many factors, and different materials require different exposure conditions. The X-ray radiation system used in this study has the advantages of a simpler operation process, shorter time consumption, and no pollution compared with a soft X-ray machine. With the rapid development and iteration of artificial intelligence, automatic sorting systems, and deep learning-based inspection models, this intelligent technology has been widely used to improve productivity and product quality. In view of the problem of detecting internal defects, X-ray based non-destructive testing technology has been combined with deep learning. In this regard, this paper provides a strong basis for the subsequent development of classification models for Ephedra seed quality detection (Xue et al., 2023).

For the identification of unknown species, we use traditional morphological identification combined with DNA barcoding to check the literature reference information to ensure the accuracy of the identification of species. Based on these results, we speculate that this pest belongs to a new group of Eurytoma genus, which can further determine its taxonomic status and enrich the research on *Eurytoma* species in China.

Conclusion

In this study, it was found that after Ephedra seeds were subjected to pest infestation, the seed germination rate was only 41.3%, which was 24.7% lower than that of healthy seeds, and the seed pest infestation rate was more than 20%. In addition, empty seeds were also one of the reasons for the low germination rate. We confirmed that X-ray technology can be used to detect Ephedra seeds with internal pests and can also distinguish between empty and dented seeds, which provides the possibility for the development of an intelligent sorting system for Ephedra seed quality. By morphological identification and DNA barcoding, it was confirmed that Ephedra seed pests belong to the Eurytoma genus and may be a new species.

Declarations

Author Contribution Statement

HHX drafted, revised the manuscript. YRG analyzed the data, revised the manuscript. LYZ performed the experiments. LL and WGW correct grammar. JHG provided figures of the paper. WS, FX and GC provide experimental materials. HYH experiments design for the research. JX critical revision of the manuscript. SLC supervised the research. All authors read and approves the final manuscript.

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Conflict of interest The authors declare that they have no conflict of interest.

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Figures

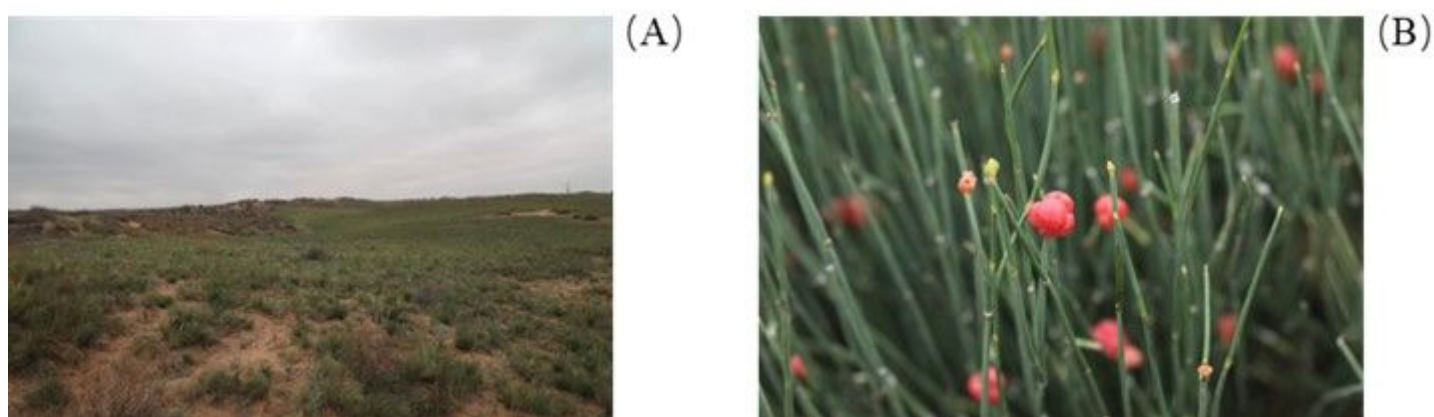


Figure 1

Ephedra herb growing environment. **A** sandy dune area; **B** Mature grass *E. sinica* cones.

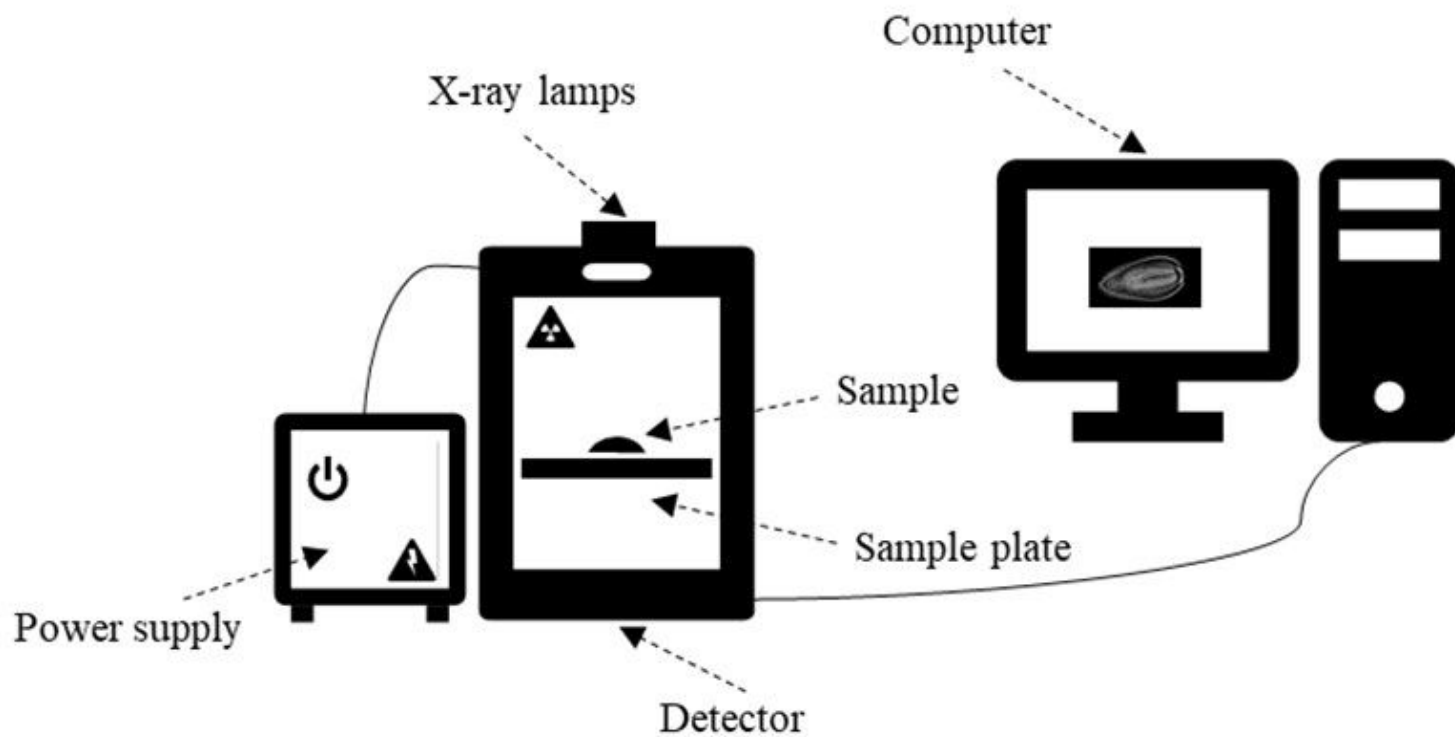


Figure 2

Schematic diagram of the X-ray system image acquisition process

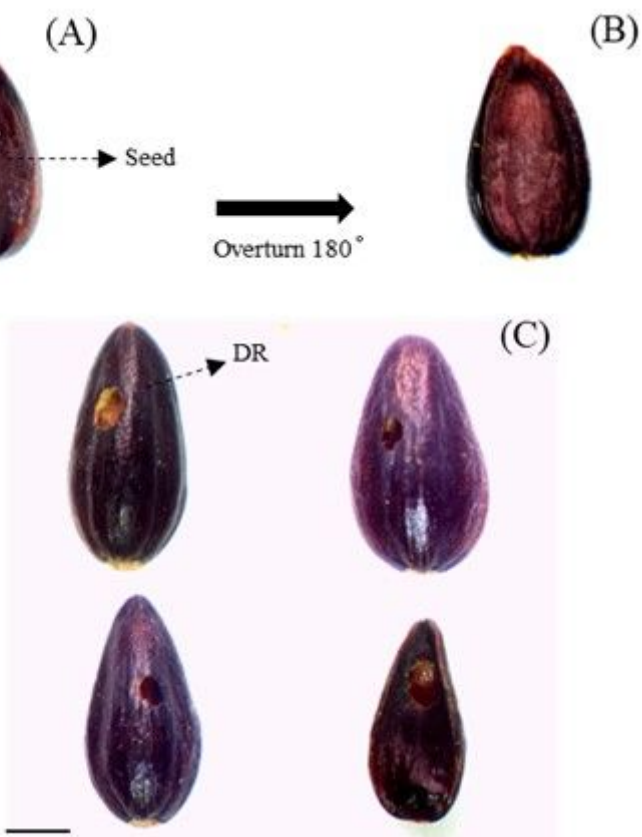


Figure 3

Mature seed state. **A** Back view of mature *E. sinica* seed; **B** Abdomen view of mature *E. sinica* seed; **C** The location where the pests bite through the *E. sinica* seeds after their metamorphosis into adults. Scale bar: 1 mm.

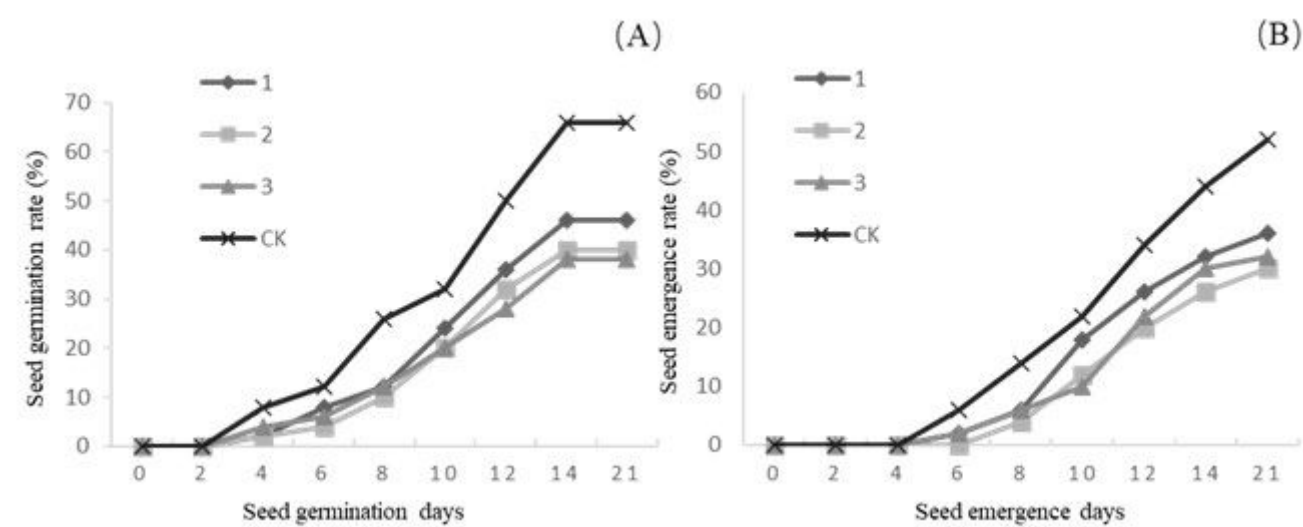


Figure 4

A Germination rate of different batches; **B** Different batch emergence rate

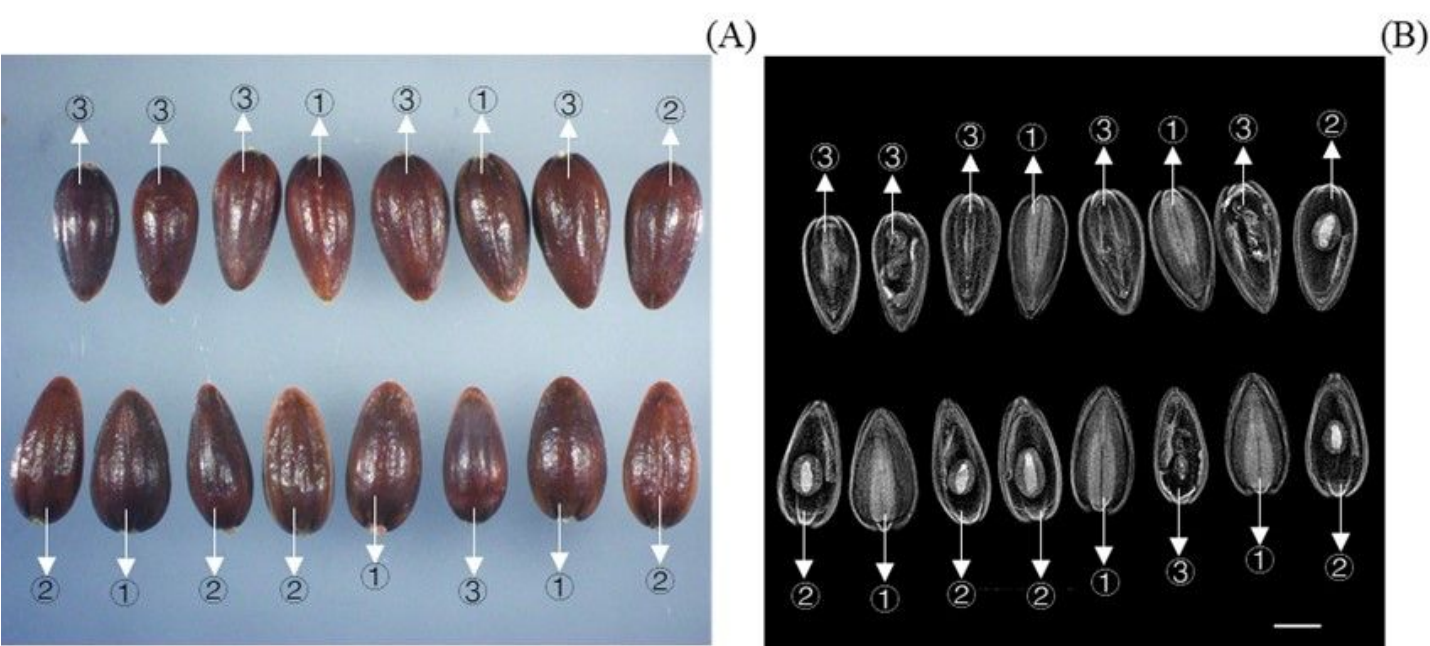


Figure 5

Appearance and X-ray images of *E. sinica* seeds. **A** Anatomical mirror perspective; **B** X-ray images. representing healthy Ephedra seed , representing insect-eaten seeds, representing empty seed kernel. Scale bar: 2 mm.

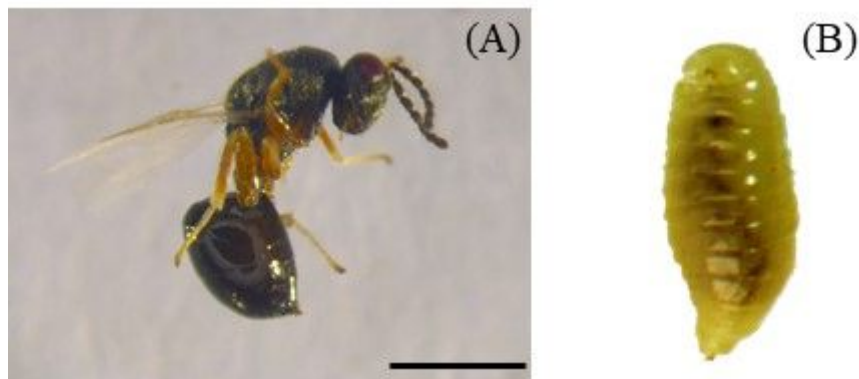


Figure 6

A Adult of *Eurytoma* sp.; **B** larva of *Eurytoma* sp. Scale bar: 1 mm.

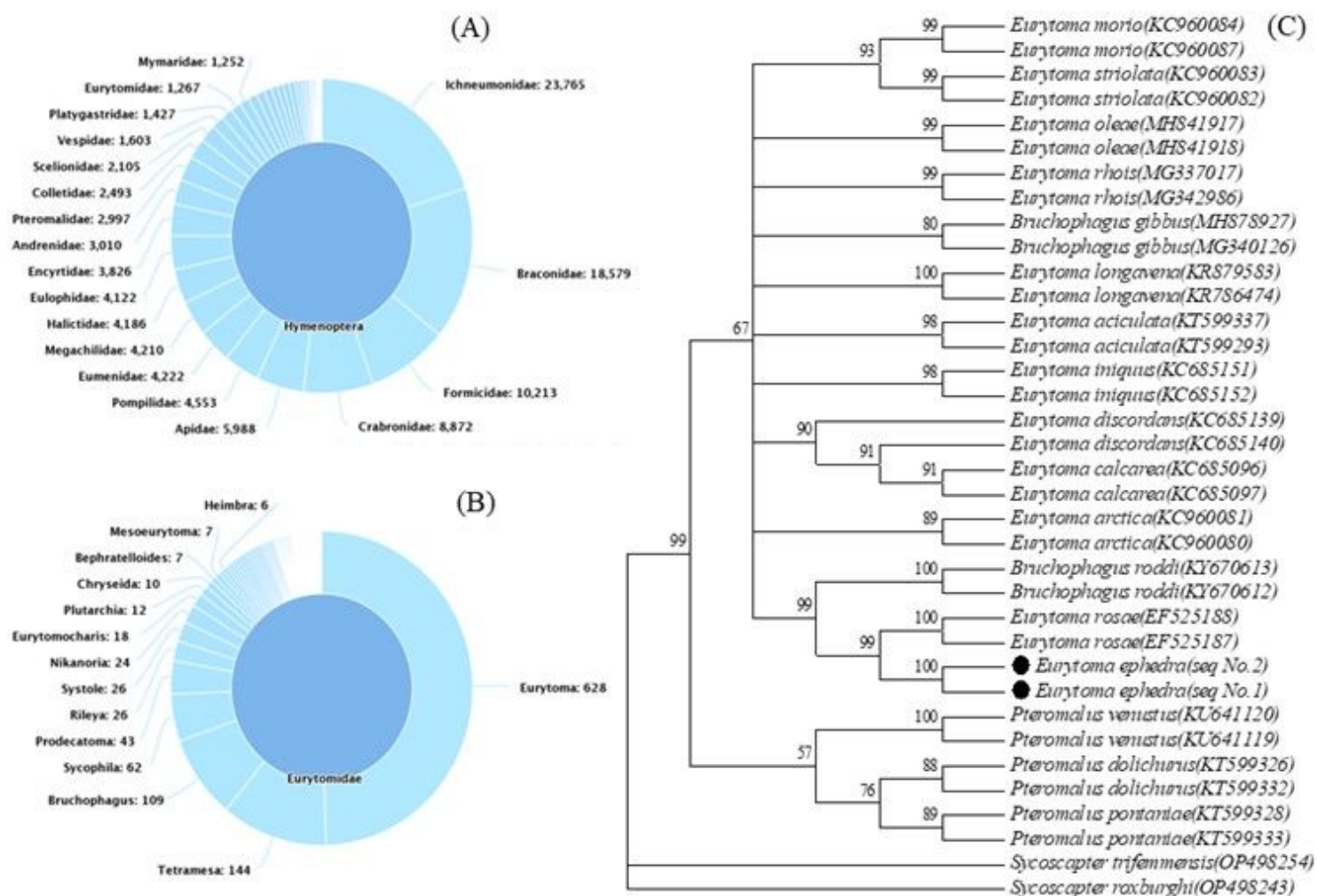


Figure 7

A Circular statistical scale diagram of *Eurytoma* species; **B** Circular statistical scale diagram of Hymenoptera species **C** Phylogenetic tree of the species NJ of *Eurytoma* species.