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An Investigation and Invasiveness Analysis of Two Species of Giant African Snail in a Coastal City of Southern China

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Abstract: Investigations and research on the giant African snail (GAS) mainly focus on *Achatina fulica*. However, in recent years, a more harmful GAS, *Achatina immaculata*, has been reported. In order to understand the invasive status of *A. immaculata* in China, we take Shenzhen, a coastal city in Southern China, as an example to carry out an ecological survey on the field populations of the two species of GAS in various districts. We explore the differences in the invasive characteristics of the two species of snails in terms of their dietary intake, cold adaptation and drought resistance. The results indicate that, based on the phylogenetic tree analysis, more than half of the sampled snails exhibit higher similarity to *A. immaculata*. The number of wild *A. immaculata* is significantly greater than that of *A. fulica*, and 70.64% of the 746 GAS are *A. immaculata*. At the same time, it is also found that the maximum shell length of *A. immaculata* is 135.83 mm, with an average shell length of 76.00 mm, which is significantly different from the average shell length of *A. fulica* (56.57 mm, $p < 0.01$). The food intake assay shows that there is no difference in the food preferences of the two species, but the food demand of *A. immaculata* is significantly greater than that of *A. fulica* (2.32 fold, $p < 0.01$). In the cold adaptation assay, *A. immaculata* recovers from the cold dormancy state significantly faster than *A. fulica* (1.92 fold, $p < 0.05$), and the speed with which *A. immaculata* enters the dormancy state in the drought environment is significantly slower than that of *A. fulica* (0.706 fold, $p < 0.05$). With the characteristics of a large body size, large food intake and strong resistance to cold and drought resistance, *A. immaculata* has the potential to be dominant in competition with *A. fulica* in the same ecological niche, and it has become the main invasive species of GAS in Shenzhen.

Keywords: *Achatina immaculata*; *Achatina fulica*; giant African snail; invasiveness; East Asia

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Citation: Zhang, Y.; Wang, X.; Tang, Y.; Wang, L.; Han, R.; Qiao, X.; Wan, F.; Qian, W.; Liu, C. An Investigation and Invasiveness Analysis of Two Species of Giant African Snail in a Coastal City of Southern China. *Agriculture* **2024**, *14*, 1217. https://doi.org/10.3390/agriculture14081217

Academic Editor: Mahyar Mirmajlessi

Received: 25 June 2024
Revised: 19 July 2024
Accepted: 23 July 2024
Published: 24 July 2024

1. Introduction

The International Union for the Conservation of Nature (IUCN) has listed *Achatina fulica*, often referred to as the giant African snail (GAS), as one of the world's top 100 most invasive species of shellfish organisms. *A. fulica* originates from East Africa and has a history of over 90 years of invasion in China [1,2]. In 1931, the GAS was first reported in Xiamen, Fujian Province, China. Subsequently, it gradually spread throughout Southern Fujian [3,4]. Currently, it has expanded to the Pearl River Delta region of Guangdong Province, the Leizhou Peninsula, Hainan Island, and the southern part of Guangxi Province [5,6]. Once the large-scale invasion of the GAS is achieved, by virtue of its large body size, large feeding capacity, fast growth rate and strong reproduction, it can harm more than 500 types of plants, which seriously affects the development of agriculture and forestry [7,8]. Furthermore, as an exotic invasive species, the GAS engages in competitive or predatory interactions with native species. This poses a significant threat to indigenous snails and plants in the invaded ecosystem, potentially leading to the extinction of local species [9]. More importantly, the GAS serves as an intermediate host for *Angiostrongylus* (*Angiostrongylus cantonensis*), contributing to the transmission of eosinophilic meningitis in humans. This has the potential to adversely impact public health [10–12]. In view of its harmful effects and the huge agricultural losses that it causes, some countries have spent huge sums of money on the fight against the GAS. For example, in the United States of America, the state government of Florida spent USD 23 million on GAS control when the snail was rediscovered in 2011, after having been declared eradicated in 1975 [13]. Therefore, once the GAS establishes a significant invasion, it becomes challenging to control, and its eradication becomes nearly impossible [14].

Many reports equate the GAS with *A. fulica*, but, strictly speaking, GAS is a generic term for several species of agate snails, including *A. fulica* and the more recently discovered and more damaging *Achatina immaculata* (Figure 1b). *A. immaculata* is native to Southeastern Africa and was first discovered in Taiwan in 2011 [15]. In 2014, *A. immaculata* was detected in logs imported from Mozambique at the port of Dongguan in Guangdong, China. This was the first recorded interception of this species in China [16]. Greater reproductive potential and greater environmental tolerance have been reported for *A. immaculata*, and these traits may make *A. immaculata* more invasive than *A. fulica* [17]. For example, in Mauritius, *A. immaculata* was found to have driven *A. fulica* into unsuitable areas at altitudes of 1200 to 2000 ft in order to occupy suitable environments [18]. Therefore, *A. immaculata* may gradually replace *A. fulica* in the same ecological niche as it is a more damaging GAS due to its greater adaptability. However, less research has been conducted on *A. immaculata* in China, and, given its very similar appearance to *A. fulica*, confusion between the two may affect the assessment of trends in the spread of GAS and the occurrence of hazards, interfering with the development of "one policy for one species" prevention and control strategies.

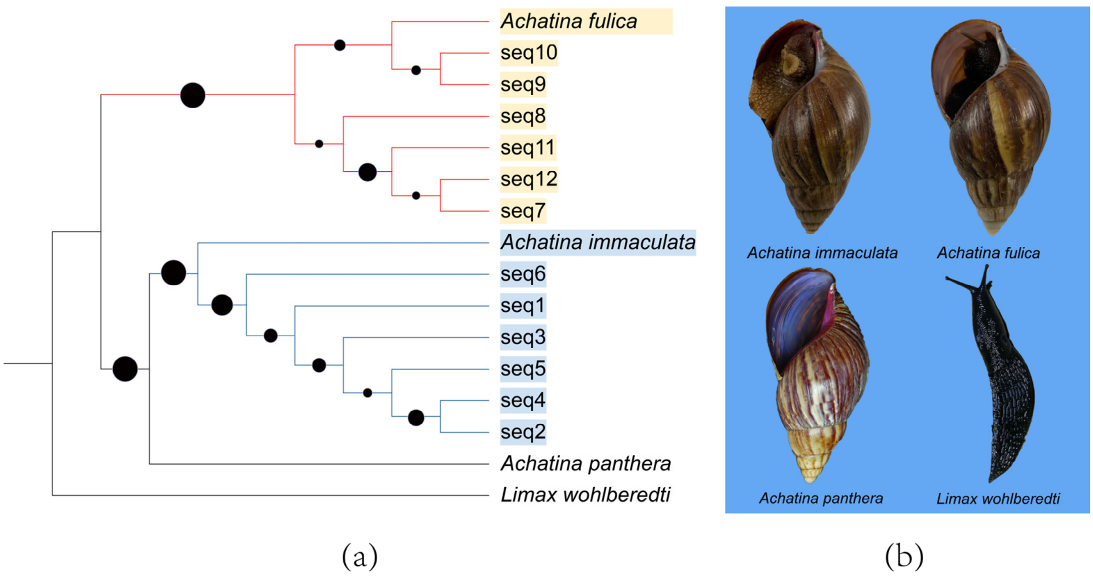

Figure 1. (**a**) Phylogenetic tree construction using MEGA software (the size of the black circles indicates the support values). (**b**) Comparison of the morphologies of the four species.

Shenzhen, an East Asian city adjacent to the Pacific Ocean, provides suitable environmental conditions for the colonization, spread and outbreak of invasive species due to its suitable natural environment and climatic factors. The Shenzhen area has a subtropical maritime climate with mild seasons and abundant rainfall, which are suitable for the growth and reproduction of invasive organisms [19]. In Shenzhen, we have observed the substantial presence of *A. immaculata*, and the population continues to gradually increase. Currently, research on the invasive GAS in our country primarily focuses on *A. fulica*, with only limited reports on the more destructive *A. immaculata*. There is a significant gap in the study of its population ecology and biological characteristics. The suspected presence of *A. immaculata* has been observed in 79 locations [20]. This study is intended to examine the gene sequences, population size, individual differences and environmental tolerance of these two species of GAS, in order to investigate the population and individual characteristics of *A. immaculata*, gain a better understanding of the invasive characteristics of *A. immaculata* and provide a reference for the prediction of possible trends in *A. immaculata* populations.

2. Materials and Methods

2.1. Survey Sample Area

The survey sites covered all districts in Shenzhen City, Guangdong Province, China (22°38′17.54″ N, 114°05′52.35″ E). Due to the nocturnal and moisture-loving nature of GAS, the survey was conducted between 20:00 and 22:00, when the temperature was 25 °C to 30 °C and the humidity was 60% to 85%, and the days before and after sunny weather were avoided as much as possible. We selected Dapeng New District (Centipede Ridge, 22°35′26.80″ N, 114°29′32.61″ E), Bao'an District (Tiegang Reservoir, 22°36′19.39″ N, 113°53′24.08″ E), Nanshan District (Talent Park, 22°30′39.56″ N, 113°56′54.74″ E), Guangming District (New City Park, 22°44′51.03″ N, 113°56′36.97″ E), Yantian District (Meisha Bay Park, 22°35′112.73″ N, 113°56′36.97″ E), Futian District (Xiangmihu Park, 22°32′47.90″ N, 114°01′19.48″ E) and Longhua District (Guanlan River Wetland Park, 22°41′1.73″ N, 114°02′39.88″ E) as the seven survey sites, counting the number of snails of both species within 500 meters of each survey site. Using the quarantine identification method for *Achatina immaculata* (DB35/T 1883—019) and the phylogenetic tree of cytochrome c oxidase subunit I(COX1) as the standard, we measured the shell length and compared the morphological characteristics and sequencing results with the sequence from GenBank (SUB14156589) to confirm the species.

2.2. Test Snail

The experimental site was located in the negative-pressure breeding room at the Agricultural Genomics Institute at Shenzhen Chinese Academy of Agricultural Sciences (AGIS-CAAS), without direct sunlight. The test specimens of *A. fulica* and *A. immaculata* were collected from the farm of AGIS-CAAS (22°35′50.01″ N, 114°30′13.38″ E). After collecting the two types of GAS, they were kept in plastic pots covered with coconut soil at a temperature of 25 °C to 30 °C and indoor humidity of about 65% to 75% and fed regularly with fresh vegetable leaves for one week to allow them to adapt to the environment for the experiment.

2.3. Phylogenetic Analysis

The GAS was first screened initially on the basis of standardized samples (Achatina panthera: ZGSDBWG0374; *A. fulica*: TCFCDO150; *A. immaculata*: TCFCDO005) in terms of its appearance characteristics [15], and further identification was carried out using mitochondrial DNA [21]. Based on the instructions of the DNeasy Blood & Tissue Kit, some experimental conditions were slightly adjusted to extract the genomic DNA of the GAS. Universal primers LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (TAA ACT TCA GGG TGA CAA AAA AAT CA) [22] were used to amplify the COX1 gene of the GAS. The extracted products were sequenced, and the sequencing results were subjected to sequence alignment with data from the NCBI database, including MH898869.1 (*Achatina panthera*), OR234677.1 (*A. fulica*), FJ606481.1 (*Limax wohlberedti*) and data from GeneBank (SUB14156589). Using the MEGA11 [23] software and the maximum likelihood method, we assessed the robustness of the tree through 1000 bootstrap analyses.

The resulting tree was visualized and annotated using MEGA11. The final phylogenetic tree provided a clear representation of the evolutionary relationships among the species.

2.4. Food Intake and Preference

According to a summary of the main host plants of GAS from the CABI website (https://www.cabidigitallibrary.org/ (accessed on 25 August 2022)) [24], we selected seven of the most commonly reported plants from the list for feeding [25–28], including cabbage (*Brassica oleracea* var. *Capitata*), cauliflower (*Brassica oleracea* var. *Botrytis*), cucumber (*Cucumis sativus*), marrow (*Cucurbita pepo*), banana (*Musa nana*), pawpaw (*Carica papaya*) and French marigold (*Tagetes patula*) (Table 1). We selected fifteen *A. fulica* and fifteen *A. immaculata* snails with shell lengths of 4–5 cm. Each species was divided into three groups, with five snails in one group. Each group of snails was placed in an experimental container and fed with the seven different types of food, with feedings conducted twice daily at 8 AM and 8 PM. This setup allowed for the comparison of the food preferences between the two snail species. After identifying their favourite foods, we grouped the two GAS as described above. The snails were fed with the food preferred by both species to measure their food intake. Feeding was conducted twice daily at 8 AM and 8 PM. Daily intake was analyzed in the food preference test and food intake was analyzed in the food intake comparison test for three consecutive days, with the intake in each group calculated by subtracting the amount of bait left over from the amount fed. Statistical analyses of the data were performed by one-way ANOVA using SPSS to determine the significance of differences.

Table 1. Selected food for determination of food preference.

Species	Family	Reference
Cabbage (*Brassica oleracea* var. *Capitata*)	Brassicaceae	Maheshini et al. [25]
Cauliflower (*Brassica oleracea* var. *Botrytis*)	Brassicaceae	Reddy et al. [26]
Cucumber (*Cucumis sativus*)	Cucurbitaceae	Maheshini et al. [25]
Marrow (*Cucurbita pepo*)	Cucurbitaceae	Thakur et al. [27]
Banana (*Musa nana*)	Musaceae	Aregowda et al. [28]
Pawpaw (*Carica papaya*)	Caricaceae	Thakur et al. [27]
French marigold (*Tagetes patula*)	Asteraceae	Thakur et al. [27]

2.5. Cold Adaptation Treatment

According to reports, the minimum adaptive temperature of the GAS is 15 °C, below which it enters a dormant state [7]. Therefore, we selected *A. immaculata* and *A. fulica* with shell lengths of 4–5 cm. Ninety *A. immaculata* were divided into three groups, with each group consisting of thirty snails, and three replicate experiments were conducted in total. The selection of *A. fulica* was performed as above. They were placed in a low-temperature incubator and kept at a stable temperature of 10 °C. After 24 h of low-temperature treatment, all snails became dormant and they were transferred to room temperature, and the recovery rate of the two species of snails from dormancy was calculated at 25°C for 24 h. The recovery rate of the two species of snails from dormancy was determined by their recovery from dormancy. Recovery from dormancy was judged by the snails' gastropods protruding from the shell and whether they were feeding normally. Statistical analyses of the data were performed by one-way ANOVA using SPSS to determine the significance of differences.

2.6. Drought Resistance Treatment

In the literature, the minimum adapted humidity for GAS is reported as 45% [7], below which they enter dormancy. Therefore, *A. fulica* and *A. immacula* with a shell length of 4–5 cm were selected. Ninety *A. immaculata* were divided into three groups, with each group consisting of thirty snails, and three replicate experiments were conducted in total. The selection of *A. fulica* was performed as above. They were placed in an incubator and kept at room temperature with humidity below 20%. The experiment lasted for 7 d and the dormancy rate of the two snails was calculated on the 1st, 3rd, 5th and 7th days. The dormancy of the snails was judged by the fact that all snails withdrew into the shell and secreted mucus to close the shell mouth and isolate it from the outside world. The statistical analysis of the data was carried out using one-way ANOVA with SPSS to determine the significance of differences.

3. Results

3.1. Phylogenetic Analyses

In one report, the axial lip and inner lip of *A. panthera* and *A. immaculata* are pink, while those of *A. fulica* are pale white or bluish white. As for the shell surface, *A. panthera* has reddish-brown longitudinal stripes or flame-like markings, *A. fulica* has brown longitudinal stripes or scorched brown misty markings, and *A. immaculata* does not have any longitudinal stripes or markings. Therefore, based on the morphological characteristics, samples 1 to 6 were *A. immaculata* and the rest were *A. fulica* [15]. By amplifying and sequencing the COX1 gene of the GAS, we successfully obtained a series of gene sequences of GAS. Using the MEGA phylogenetic analysis software, we constructed an evolutionary tree of the snail. In the comparison results (Figure 1a), samples 7 to 12 showed evolutionary similarity to *A. fulica*, with the sequence similarity ranging from 93% to 95% (Table 2) and with sample 9 having the highest similarity to *A. fulica* at 93.59%. However, the similarity to *A. immaculata* was less than 90%. Samples 1 to 6 showed similarity to *A. immaculata*, with the sequence similarity ranging from 95% to 98% and with sample 6 having similarity of 97.63%. However, the similarity to *A. fulica* was less than 85%. All samples showed less than 80% similarity to *Achatina panthera* and *Limax wohlberedti.*

Table 2. Sequence comparison.

	Similarity to *Achatina fulica*	Similarity to *Achatina immaculata*	Similarity to *Achatina panthera*
seq1	74.42%	97.33%	83.89%
seq2	73.33%	96.90%	83.38%
seq3	74.49%	97.05%	83.94%
seq4	73.33%	96.47%	83.55%
seq5	74.76%	97.05%	84.06%
seq6	76.41%	97.63%	84.58%
seq7	93.18%	75.60%	72.34%
seq8	93.17%	75.32%	72.06%
seq9	93.59%	75.46%	72.20%
seq10	93.87%	75.95%	72.59%
seq11	93.18%	76.10%	72.83%
seq12	93.48%	76.45%	72.18%

3.2. Distribution and Population Survey

In the seven districts of Shenzhen, a total of 746 GAS were collected, of which 527 were *A. immaculata*, accounting for 70.64% (Figure 2a), significantly outnumbering *A. fulica* ($p < 0.001$). In each district, *A. immaculata* accounted for more than half of the collected specimens, with the highest prevalence in Guangming District, where *A. immaculata* accounted for 84.62% (Figure 2b). Measuring the shell lengths of these snails (Figure 2c), it was found that the maximum shell length of *A. immaculata* was 135.83 mm, while *A. fulica* had a maximum shell length of only 80.18 mm. Overall, the mean shell length of *A. immaculata* (76.00 mm) compared to *A. fulica* (56.57 mm) showed a highly significant difference ($p < 0.001$). Specifically, *A. immaculata*'s shell lengths were predominantly distributed in the 60–90 mm range, whereas *A. fulica*'s shell lengths were more concentrated in the 50–70 mm range (Figure 2d).

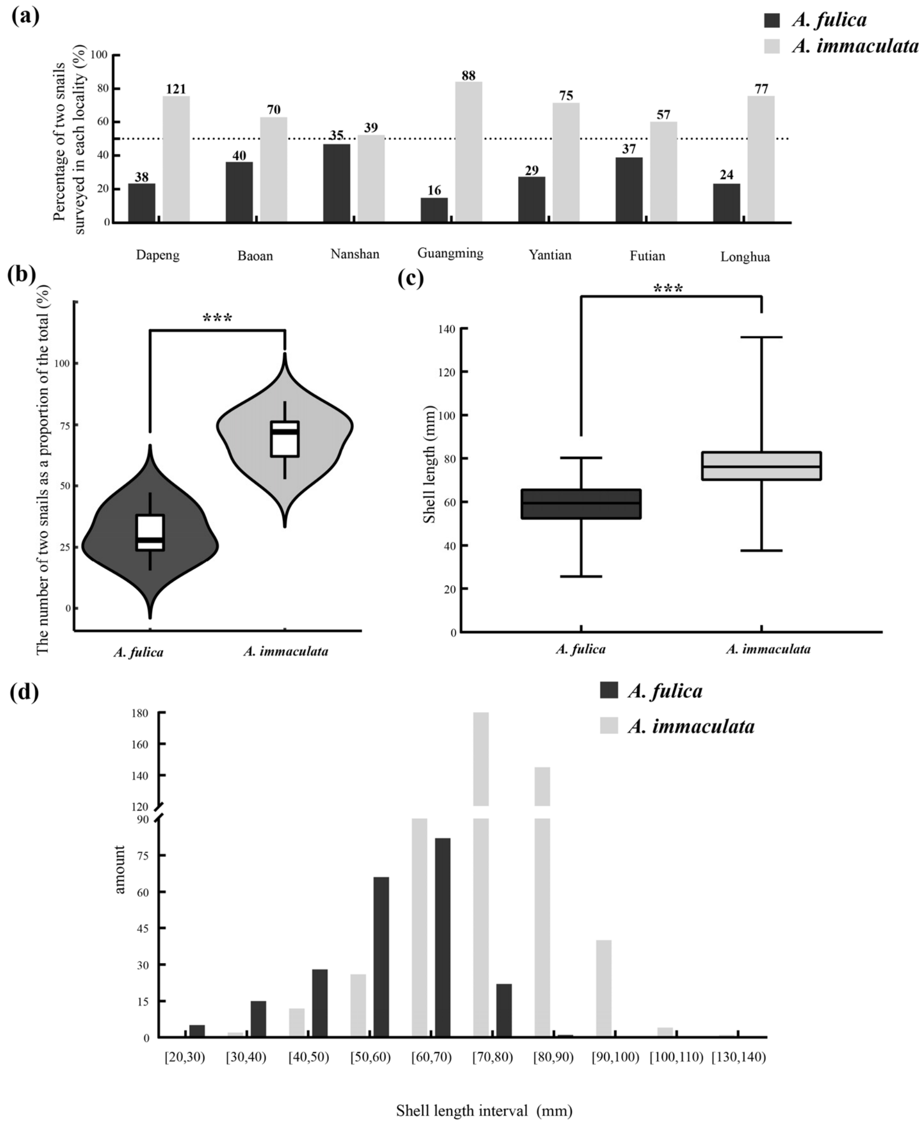

Figure 2. Population survey results of *A. immaculata* and *A. fulica* in 7 districts of Shenzhen city. (**a**) Proportions of *A. immaculata* and *A. fulica* in each district; (**b**) proportions of the two snails among the total number; (**c**) shell lengths of *A. immaculata* and *A. fulica*; (**d**) shell length distribution for *A. immaculata* and *A. fulica* (*** represents $p < 0.01$; the numbers on the bars represent the number of snails).

3.3. Food Intake and Dietary Preferences

In terms of food preferences, the two snail species showed no significant difference in their preferences, with the top three preferred foods being cabbage, pawpaw and cucumber, while showing a relatively lower affinity for marrow, cauliflower, banana and French marigold (Figure 3a). However, in the comparative food consumption experiment, *A. immaculata* showed a higher food demand than *A. fulica*. On the first day, the average daily food intake per group of *A. immaculata* was 26.625 g, significantly higher than the daily food intake of *A. fulica* (11.072 g). Over the course of three days, the total food consumption of *A. immaculata* was 2.32 times that of *A. fulica*, a highly significant difference ($p < 0.001$) (Figure 3b).

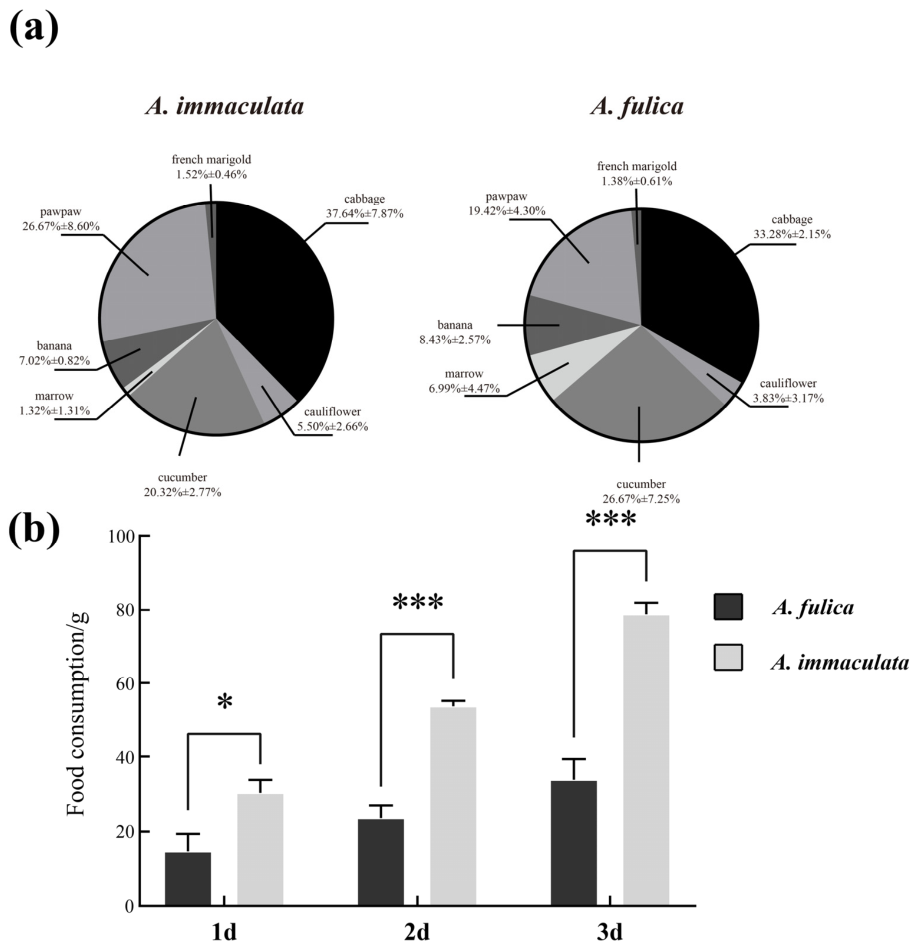

Figure 3. Comparison of feeding differences between *A. immaculata* and *A. fulica*. (**a**) Preferences of *A. immaculata* and *A. fulica* for food types; (**b**) food intake of *A. immaculata* and *A. fulica* (* represents $p < 0.05$; *** represents $p < 0.01$).

3.4. Cold Adaptation

In the context of low-temperature stress, there are significant differences in the low-temperature tolerance between *A. immaculata* and *A. fulica*. When exposed to a low temperature of 10 °C, both snail species entered dormancy at the same rate, with all snails entering dormancy 24 h later. However, after transferring the dormant snails to room-temperature conditions for 24 h, 55% of the *A. immaculata* had recovered from dormancy and showed normal mobility and feeding. In contrast, only 28.9% of the *A. fulica* had recovered, indicating a significantly slower rate of recovery from low-temperature dormancy compared to *A. immaculata* ($p < 0.1$) (Figure 4).

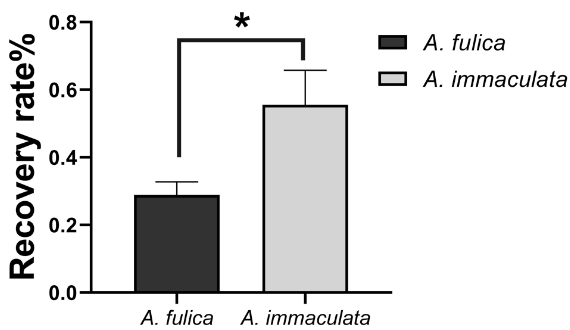

Figure 4. Recovery rates of two snail species from cold dormancy within 24 h after cold adaptation treatment. (* represents $p < 0.05$).

3.5. Drought Resistance

There are also significant differences in drought resistance between the two GAS species. At 20% humidity, *A. immaculata* remained dormant for the first three days, whereas *A. fulica* exhibited a dormancy rate of 4.44% on the first day, increasing to 24.44% on the third day. On the fifth day of drought resistance, the dormancy rate of *A. fulica* was significantly higher (68.89%) compared to *A. immaculata* (46.67%) ($p < 0.1$). By the end of the seventh day, all *A. fulica* had entered dormancy, while 11.11% of the *A. immaculata* remained non-dormant (Figure 5).

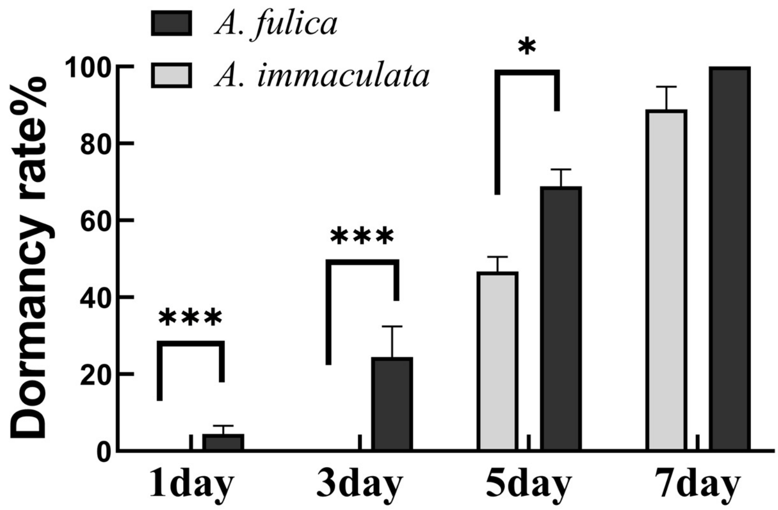

Figure 5. Dormancy rates of *A. immaculata* and *A. fulica* under drought resistance. (* represents $p < 0.05$, *** represents $p < 0.01$).

4. Discussion

The members of Achatina are a large group of land snails with more than 100 species, including *A. fulica* [29]. According to reports, it has been discovered that, in addition to *A. fulica*, the invasive GAS also include *A. immaculata* [2]. Currently, research on the invasive GAS in China is mainly focused on *A. fulica*, with very few reports addressing the more harmful *A. immaculata* [17]. Therefore, compared to other invasive mollusks, research on *A. immaculata* in China is still in its infancy. Its invasion trends, population numbers and living habits remain unknown, posing challenges for the control of GAS. This study investigates the population numbers and individual trait differences of two species of GAS, *A. immaculata* and *A. fulica*, in the wild in Shenzhen. The results indicate that *A. immaculata* has established a stable invasive population in Shenzhen, with its population size already surpassing that of *A. fulica*. Additionally, due to its larger size, stronger environmental adaptability and greater food consumption, *A. immaculata* has supplanted *A. fulica* as the predominant and more harmful invasive GAS species in Shenzhen. These results lay the foundation for the exploration of the competition between the two GAS during the invasion process and provide a theoretical basis for the development of specific biological control strategies.

The three species of GAS are morphologically extremely similar, while, in size, *A. immaculata* is larger than *A. fulica* [12]. The major difference between the three different large snails is that *A. fulica* has a pale white or bluish-white axial lip and inner lip, whereas *A. panthera* and *A. immaculata* have a pink axial lip and inner lip. In terms of the shell surface, the three species of large snails are quite different, e.g., *A. panthera* has a reddish-brown longitudinal or flame-like mottled shell surface, *A. fulica* has brown longitudinal or burnt-brown misty mottling, and *A. immaculata* does not have any longitudinal or mottling patterns [15]. This study also found significant differences in body size between the two species, with the mean shell length of *A. immaculata* being significantly greater than that of *A. fulica*, with shell lengths ranging from 5 to 8 cm and with the largest shells reaching over 13 cm in length. This study found that the body size is related to the ecological population and environmental adaptation of the species, such as resource acquisition, environmental adaptation and interspecific fighting [30]. Individuals with larger body sizes can gain advantages in interspecific competition for the same resources, thereby enhancing their resource acquisition efficiency [31]. For instance, larger-sized linyphiid spiders such as *Bathyphantes pallida* can outcompete ecologically similar competitors like *Meioneta unimaculata*, thereby occupying the optimal predation territories [32]. Therefore, we hypothesize that the larger body size of *A. immaculata* confers a competitive advantage, gradually displacing *A. fulica* and acquiring more survival resources. Because of the similarity in appearance, it is very easy to experience confusion in re-testing and they can be identified more accurately by molecular identification. Many studies have shown that mitochondrial DNA is a suitable genetic molecular marker for intraspecific and interspecific variation, as well as for species identification [21]. Therefore, the comparison of COX1 allows the more precise identification and estimation of the form of invasion as well as the degree of harm [33].

The primary agricultural threat posed by GAS stems from their broad diet [34], substantial food consumption and the greater food requirements of larger individuals such as *A. immaculata*. In food intake, it was observed that both species of *Achatina* preferred cabbage, pawpaw and cucumber. However, regarding snails of a similar body size, *A. immaculata* exhibited significantly greater food intake than *A. fulica*. This research indicates that their food intake is positively correlated with their growth rate, with individuals that exhibit greater food intake experiencing faster growth [35]. Therefore, we speculate that the larger food intake of *A. immaculata* may contribute to its faster growth and provide it with a competitive advantage over *A. fulica* in resource competition with similar life histories. Furthermore, research conducted in Wenshan Prefecture, Yunnan Province, China found that the density of *A. fulica* ranged from 10 to 30 individuals per cubic meter, with crop damage reaching up to 80% [7]. Therefore, compared to *A. fulica*, the larger food intake of *A. immaculata* may lead to the formation of larger population densities in a shorter time frame, resulting in a greater area of plant damage and causing greater economic losses to the agriculture and forestry sectors [36].

Broad ecological tolerance to environmental factors is a significant factor contributing to the successful invasion and establishment of populations [37]. In this study, it was found that following cold adaptation treatment, the recovery rate from cold dormancy in *A. immaculata* was significantly faster than that in *A. fulica*. Under drought conditions, *A. immaculata* entered dormancy significantly sooner than *A. fulica*. These results indicate that, compared to *A. fulica*, *A. immaculata* exhibits greater tolerance to low temperatures and drought. Studies have reported that *A. fulica* prefers temperatures ranging from 15 to 38 °C and soil humidity between 45% and 85% [7]. When the environmental conditions exceed this range, GAS enter a wax-sealed dormancy state. Therefore, it is speculated that *A. immaculata* possesses stronger environmental adaptability. When the environmental suitability is low, *A. immaculata* is more likely to survive compared to *A. fulica*. Research has found that when two species in competition simultaneously coexist, if one species has stricter requirements for a certain environmental condition, it will be at a disadvantage in the competition. For example, in invasive mosquitoes, compared to *Anopheles stephensi*, the higher temperature tolerance and faster growth rate of *Aedes aegypti* under the same temperature conditions make it the competitively dominant species [38]. Therefore, the higher environmental tolerance of *A. immaculata* poses a threat to the survival of *A. fulica*, giving it a greater advantage in population competition. Moreover, the results also indicate stronger stress resistance, granting *A. immaculata* greater capabilities to withstand low temperatures and expand its invasive range.

In summary, the large body size, substantial food intake and strong stress resistance of *A. immaculata* confer advantages in environmental adaptation, resource acquisition and population expansion. Leveraging this competitive advantage, *A. immaculata* has displaced *A. fulica* from its ecological niche and has emerged as the most harmful invasive species of

GAS in Shenzhen. There are large areas in China with climates similar to that of Shenzhen. However, data on the invasion numbers, distribution areas and severity of damage caused by *A. immaculata* are relatively scarce. This study, using Shenzhen as a case study, provides a reference for other regions with similar climates. Additionally, it lays the foundation for further improvements in the risk monitoring and early warning system for GAS and for the development of tailored control strategies based on the principle of "one species, one strategy".

Author Contributions: C.L. and W.Q. designed the research; Y.Z., X.W. and Y.T. performed the research; Y.Z. and X.W. analyzed the data; Y.Z. wrote the article with input from the other authors; Y.Z., L.W. and R.H. reviewed and edited the article; supervision, F.W., C.L., X.Q. and W.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by grants from the National Key R&D Program of China (2021YFC2600100 and 2021YFC2600101), the National Natural Science Foundation for Young Scientists of China (31901950) and the Key Program in AGIS under Grant No. AGIS-ZDXM-202304.

Institutional Review Board Statement: The animal protocols used in this work were evaluated and approved by Animal Use and Ethic Committee (CEUA) of the Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultur, permission code is 4-5-2022.

Data Availability Statement: In this study, the four sequences for sequence comparison were obtained from the NCBI database (MH898869.1 (*Achatina panthera*), OR234677.1 (*A. fulica*), FJ606481.1 (*Limax wohlberedti*)) and GenBank (SUB14156589 (*A. immaculata*)), and the rest were extracted by the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

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