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Determination of geographic origin of Chinese mitten crab (*Eriocheir sinensis*) using integrated stable isotope and multi-element analyses

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ABSTRACT

Geographical traceability is critical to the commercial viability of the highly valued Chinese mitten crab (*Eriocheir sinensis*). This study examined the possibility of utilizing a combination of multi-element and stable isotope analysis, together with multivariate statistical authenticity analysis, to identify the origins of 164 commodity crabs (using the third pereiopod as the sample material) from eight sites around China. The $\delta^{13}C$, $\delta^{15}N$ values and the Na, Mg, Al, K, Ca, Mn, Cu, Zn, Sr, Ba contents differed significantly depending on the crabs' origin. The linear discriminant analysis showed that 99.4% of the samples were correctly classified and the cross-validated accuracy rate was 98.2%. Meanwhile, the support vector machine exhibited high discrimination ability with 99.4% accuracy. This study provides a non-conventional, integrated approach for determining the traceability of *E. sinensis.* This method offers the potential of being able to identify an even more diverse geographical range of *E. sinensis* origins.

1. Introduction

The Chinese mitten crab (*Eriocheir sinensis* Milne-Edwards, 1853) is native to northeast and southeast Asia, and has also spread to Europe, the Middle East, and North America (Fialho, Banha, & Anastácio, 2016). In China, the major natural habitats are located in the river basins of the Yangtze, Huanghe, and Liaohe rivers, while the dominant culture areas are located within the Yangtze delta (Zhang, Li, Wu, Liu, & Cheng, 2017). Given its unique flavor and high nutritional content, with a good balance of essential amino acids, fatty acids, and minerals (Chen, Zhang, & Shrestha, 2007), *E. sinensis* is one of the most important aquaculture species to the Chinese aquatic food industry, with annual production in 2016 reaching 812,103 tons (MOAC, 2017). To enhance the social and economic benefits of agricultural products, the Chinese government is promoting the "Protection of Geographical Indication (PGI) Products". To date, *E. sinensis* from 24 well-known origins have been registered by the General Administration of Quality Supervision, Inspection, and Quarantine of China (AQSIQ). Due to its high commercial value (especially PGI products), the rapidly developing *E. sinensis* industry continues to be plagued with mislabeled, fake, and counterfeit crabs, which can seriously undermine the rights and interests of consumers while adversely affecting the market. For example, a famous PGI example of *E. sinensis,* originating from Yangcheng Lake, has long been the target of crab adulteration (such as the so-called "bathed crabs"). Attempts have been made, to differentiate between populations of *E. sinensis* originating from the same or different river systems, based on the morphological features of the crabs (Wang, Xu, Zhang, & Ma, 2007), microsatellite markers (Herborg, Weetman, Van, & Hänfling, 2007; Wu, et al., 2007), fatty acid content, (Zhu & Bai, 2007) and flavor profiles (Gu, Wang, Zhang, & Mao, 2014; Kong et al., 2012; Wang et al., 2016). Our previous pilot studies involved taking morphological truss network measurements, and/or multi-elemental contents, in the third pereiopod of crabs from three or four different locations (Yang, Su, Liu, & Yang, 2012; Zhao, Su, Liu, Chen, & Yang, 2014). The discrimination rates of these previous attempts varied between 70 and 100%, pointing to the possibility of being able to pinpoint the origin of a crab, although the accuracy was higher for crabs from different river systems (macro-scale geographical regions) but lower for those from the same river (micro-scale geographical regions). Therefore, it is worth exploring more effective ways of overcoming this challenge of increasing the discernibility rate of the geographic origins for *E. sinensis* to be close to 100%, especially for a given river system, through the combined use of the aforementioned approaches with other existing technologies.

Multi-elemental analysis has been used to trace the origins of clams, shrimp, and crabs (Iguchi, Isshiki, Takashima, Yamashita, & Yamashita,

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2014; Li, Boyd, & Odom, 2014; Yang et al., 2012). Stable isotope analysis is also used widely for determining the authenticity of the geographical origins of aquatic and animal food products (e.g., fish, shellfish, sea cucumber, pork, beef, poultry, milk, butter, cheese) (Camin, Bontempo, Perini, & Piasentier, 2016; Sant'Ana, Ducatti, & Ramires, 2010; Turchini, Quinn, Jones, Palmeri, & Gooley, 2009; Zhang, Liu, Li, & Zhao, 2017). Nevertheless, to the best of the authors' knowledge, no studies investigating the traceability of the origins of the Chinese mitten crab based on stable isotope ratio analysis have been published. Given the results reported for the multi-elemental and stable isotope ratio analyses, it is possible that their combination could enable the accurate determination of the geographical origins of fish, shrimp, lamb, beef, poultry, cheese, olive oil, and wine (Curtis, Stunz, Overath, & Vega, 2014; Franke, Gremaud, Hadorn, & Kreuzer, 2005; Kelly, Heaton, & Hoogewerff, 2005; Ortea & Gallardo, 2015).

Accordingly, in this study, we hypothesized that the combination of multi-elemental analysis and stable isotope analysis, using the third pereiopods as simple, non-lethal sample material, would provide an effective means of identifying the geographical origin of *E. sinensis*. To this end, we used elemental analysis and stable isotope data in combination with multivariate statistical analysis methods (Stanimirova et al., 2010) based on linear discriminant analysis (LDA) and the support vector machine (SVM), to assess the feasibility of authenticating and tracing the PGI and non-PGI origin of *E. sinensis* crabs obtained from different areas within the Yangtze River system. That is, the present study set out to establish a non-conventional, integrated approach and provide a theoretical basis for tracing the origins of *E. sinensis*.

2. Materials and methods

2.1. Sample collection

E. sinensis crabs ($n = 164$) were collected from October 2015 to January 2016 from eight different sites located across the lower reaches of the Yangtze River, China (Fig. 1). The geographical locations included five lake habitats, namely, Changdang Lake (CD), Gaoyou Lake (GY), Hongzhe Lake (HZ), Taihu Lake (TH), Yangcheng Lake (YC); one Yangtze estuary natural habitat (Dongtan, DT); and two aquaculture pond habitats (Bacheng, BC; Honggao, HG). Among these, GY, HZ, TH, YC, DT, and BC are PGI locations while CD and HG are non-PGI locations. For each sample site, 20 (CD, TH, YC, DT, BC, HG) or 22 (GY, HZ) commercially sized crabs were selected, with the specimens all being of a similar size, with an equal number of male and female specimens so as to reduce the effects of intrinsic factors (e.g., (species, size, age, sexual maturity) (Siano et al., 2016). The wet weight of the collected crabs was in a range of 54.9 ± 15.4 g to 161.4 ± 25.8 g. All the samples were sealed in individual plastic bags and stored at −20 °C in a laboratory freezer.

2.2. Sample pretreatment

Employing the methodology described in one of our Chinese Patents (Code: ZL 201010220154.3), the entire left third pereiopod of each *E. sinensis* specimen, with weights ranging from 2.1 ± 0.5 g to 5.3 ± 0.7 g (wet weight basis), was used as the source material for the chemical analyses. The samples were defrosted, washed with ultrapure water, and then baked at 80 °C for 24 h in an oven until a constant weight was attained. The water contents of the samples after the baking step were 55 ± 1.6%. The dried samples were pulverized using a carnelian mortar and pestle, and then stored under dry conditions prior to analysis.

2.3. Stable isotope ratio analysis

A small quantity (0.2 mg) of the dry sample was placed in a tin cup and the $\delta^{13}C$ and $\delta^{15}N$ values were measured using a stable isotope ratio mass spectrometer (Delta V Advantage; Thermo Fisher Scientific Inc., Waltham Inc., USA). According to the IUPAC guidelines (Brand, Coplen, Vogl, Rosner, & Prohaska, 2014; Murray et al., 2013), the carbon/nitrogen stable isotope natural abundance is expressed, as:

$$\delta E = [(R_{sample}/R_{standard}) - 1]$$

where E represents ^{13}C or ^{15}N, and R represents the $^{13}C/^{12}C$ ratio for C or the $^{15}N/^{14}N$ ratio for N. The accuracies of the $\delta^{13}C$ and $\delta^{15}N$ isotopic analyses were monitored against a commercially available isotope standard (IAEA-600 Caffeine) with certified $\delta^{13}C$ values and informative $\delta^{15}N$ values of −27.771 ± 0.043‰ Vienna Pee Dee Belemnite standard (VPDB) and 1.0 ± 0.2‰ atmospheric N_2 (air N_2), respectively. The standard deviations (precision) for the analysis of IAEA-600 (caffeine) were 0.06‰ for $\delta^{13}C$ and 0.06‰ for $\delta^{15}N$ between 10 replicates. During the analysis, the standard material was inserted into 20 samples for quality control. To correct the data, the reference

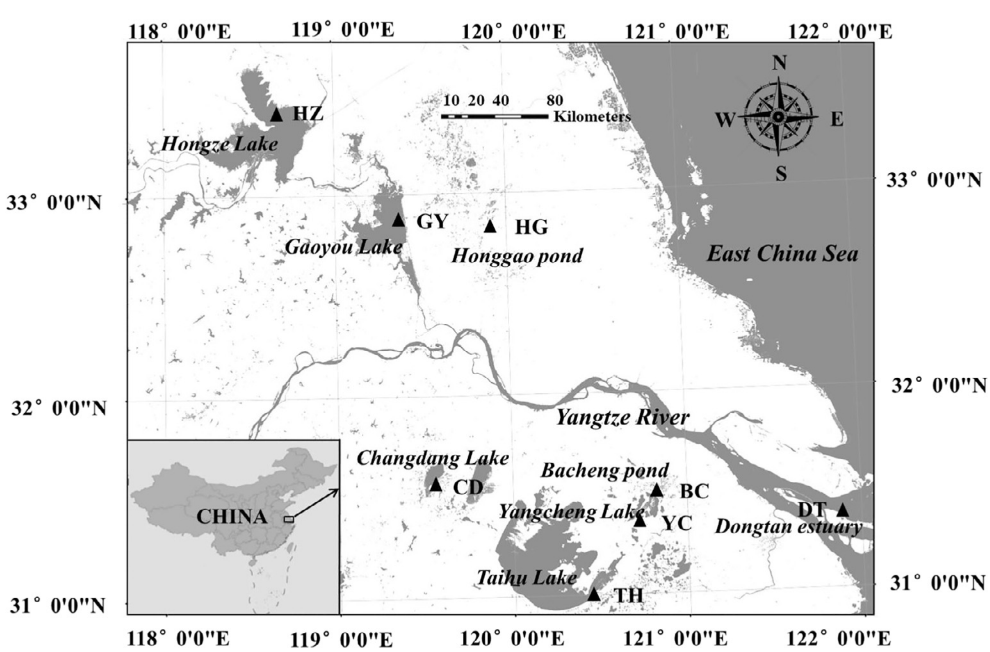

Fig. 1. Sampling locations of *Eriocheir sinensis* in lower reaches of the Yangtze River, China. Bacheng aquaculture pond (BC; N31°28.689′, E120°55.157′); Changdang Lake (CD; N31°36.514′, E119°35.551′); Dongtan area of Yangtze estuary (DS; N31°04.235′, E120°28.699′); Gaoyou Lake (GY; N32°56.074′, E119°24.253′); Honggao aquaculture pond (HG; N32°50.367′, E119°52.481′); Hongzhe Lake (HZ; N33°26.781′, E118°40.187′); Taihu Lake (TH; N31°27.140′, E121°56.036′); and Yangcheng Lake (YC; N31°26.278′, E120°44.917′).