IJIE Int. J. Indust. Entomol. 45(2) 115-125 (2022)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2022.45.2.115

ISSN 1598-3579 (Print)
ISSN 2586-4785 (Online)

Developmental characteristics and genetic diversity of the two-spotted cricket *Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae) in South Korea

Gyu-Dong Chang, Su Hyun Yum, and Jeong-Hun Song*

Industrial Insect and Sericulture Division, Department of Agricultural Biology, National Institute of Agricultural Sciences, Wanju 55365, South Korea

Abstract

In this study, we investigated the developmental characteristics and genetic diversity of seven populations of two-spotted crickets (*Gryllus bimaculatus* De Geer, 1773 (Orthoptera: Gryllidae)) raised in South Korea. Regarding the developmental characteristics of the species, we observed no statistically significant difference in the weight of the nymphs in the six populations we tested. After molting, although weight differences were observed between the populations in each stage of the developmental period, the average weight for each developmental stage was constant. We also analyzed mitochondrial *COI* gene sequences (DNA barcoding region) of the reared crickets collected from five insect farms and two national insect rearing facilities and the resultant sequences were analyzed together with the 12 sequences from foreign countries specimens obtained from public data. We detected six haplotypes from 111 specimens, indicating a low intraspecific genetic distance (~1.8%). The most dominant haplotype was overwhelmingly haplotype 1, which was found in all South Korean specimens and four specimens from China, Indonesia, and Germany. These findings indicate that the low genetic diversity of South Korean specimens can be explained by the fact that the *G. bimaculatus* population imported for feed from Japan in the early 2000s became a maternal group that spread throughout cricket farms in South Korea. In order to breed healthy cricket strains, it is necessary to increase genetic diversity by importing them from other countries through appropriate quarantine procedures.

Int. J. Indust. Entomol. 45(2), 115-125 (2022)

Received : 28 Nov 2022
Revised : 08 Dec 2022
Accepted : 15 Dec 2022

Keywords:
Gryllus bimaculatus,
genetic diversity,
COI,
haplotype,
developmental
characteristics,
Korea

Introduction

Gryllus (*Gryllus*) *bimaculatus* De Geer (GB, English name: two-spotted cricket, Korean name: Ssang-byeol-gwi-ttu-ra-mi) belongs to the family Gryllidae (Orthoptera), and the subfamily Gyllinae and is the only species in South Korea belonging to the genus *Gryllus* Linnaeus (Korean Society of Applied Entomology, 2022). GB is easily distinguished from other cricket species by its morphological feature of a yellow dot at the base of the forewing (Song *et al*., 2016). The size of this pattern and the color of their body, however, vary according to the environment (Iba *et al*., 1995).

***Corresponding author.**
Jeong-Hun Song, Ph.D.
Industrial Insect and Sericulture Division, Department of Agricultural Biology, National Institute of Agricultural Sciences, Wanju 55365, South Korea
Tel: +82-63-238-2956 / FAX: +82-63-238-3833
E-mail: jeonghuns@korea.kr

GB is not native to South Korea but has been developed through indoor breeding (Jung and Bae, 2007). Predominantly distributed in Laos, Indonesia, Malaysia, and Africa (Jung and Bae, 2007; Keum *et al*., 2012), GB was introduced to South Korea from Japan for pet food in the early 2000s (Jung and Bae, 2007; Park *et al*., 2013; Rural Development Administration, 2014; 2017).

Unlike the domestic species *Teleogryllus* (*Brachyteleogryllus*) *emma* Ohmachi & Matsuura (English name: emma field cricket, Korean name: Wang-gwi-ttu-ra-mi), GB can reproduce in all seasons, as long as the temperature is suitable, without experiencing a wintering period, and can also be bred in groups. In addition, GB has a shorter breeding period than other edible insects such as *Tenebrio molitor* (Linnaeus) and *Protaetia brevitarsis seulensis* (Kolbe) (Rural Development Administration, 2017) and has the advantage of a high protein content (Ghosh *et al*., 2017; Phesatcha *et al*., 2022). To date, approximately 2,000 types of insects have been identified as edible insects worldwide (Ghosh *et al*., 2017; Wilkinson *et al*., 2018); however, GB is commonly used in the global food industry because of its physiological advantages described above (van Huis, 2020). On account of its commercial advantages, GB was the first cricket species to be registered as a raw food ingredient by the Ministry of Food and Drug Safety in South Korea in 2016, and since then, GB has become the most widespread edible insect in South Korea and is bred at farms in various regions for commercial use and supply (Kim *et al*., 2019; Kim *et al*., 2022; Song *et al*., 2016).

Research on GB has been mainly conducted for commercial purposes to investigate the differences in feeding ability according to cricket weight, size, fertility, and status under various breeding conditions. Iba *et al*. (1995) reported that the growth, behavior, and levels of biogenic amines in GB differ according to the density of individuals during breeding, whereas Song *et al*. (2016) reported a change in the survival rate and body weight of GB depending on the type of food. Tsukamoto *et al*. (2014) revealed that female GBs tended to ingest a larger amount of food, particularly protein, after mating. In addition, several publications have reported beneficial effects of GB consumption on human physiological activity, mainly through protective effects against alcoholic and non-alcoholic liver disease, anti-obesity effects, anti-aging effects, and immunomodulatory effects (Ahn *et al*., 2015; 2020; Hwang *et al*., 2019; Im *et al*., 2018; Seo *et al*., 2004). However, no previous studies have attempted to select superior lines of GB or analyzed the genetic diversity of GB reared in cricket farms.

To maximize the benefits of the edible insect industry, it is necessary to establish mass production technology, which in turn requires developing and distributing insect lines with excellent quality and developing a model for each development stage that can be installed in an automated breeding device. To this end, several populations of the species must be collected to understand their basic genetic characteristics and select superior lines according to their growth characteristics. In this study, we collected multiple populations of GB specimens raised in public institutions specializing in breeding and insect farms, then compared the developmental characteristics between populations and evaluated the genetic diversity of GB raised in South Korea.

Materials and Methods

Collection methods and breeding conditions

Eight GB populations (B101–B106, B111–B112) were obtained: the National Institute of Agricultural Sciences (B101); the Silkworm and Insect Management Center, Gyeongbuk Provincial Government (B111); and six insect farms in South Korea such as Thegonchung (Gyeonggi, B102), 153Farm (Gyeonggi, B103), Jipyeongseon (Jeonbuk, B104), Saramgwagonchung (Gangwon, B105), Ramiaenbyeori (Gangwon, B106), and Hanbangmeogingonchung (Chungbuk, B112). In the population label, the first digit 'B' denotes *Gryllus bimaculata* and the second digit '1' indicates the population with traits related to developmental characteristics and fertility. The third and fourth digits together represent the number assigned by the National Institute of Agricultural Sciences (Wanju, South Korea). Six populations (B101–B106) were selected to compare differences in the developmental characteristics between populations of GB (developmental period and weight of the nymph stage, weight of adults, body length and width, survival rate between populations, and period from hatching to adults). After hatching, we compared the developmental characteristics from the first-instar nymph stage to the adult stage through two successive experiments. In the first experiment, we investigated the characteristics of each developmental stage. In the second experiment, group B105 was excluded because of death. To ensure equal growth conditions for all collected populations, we maintained the collected populations under specific breeding

conditions (i.e., 26 ± 1.0 °C, 60 ± 5% relative humidity, 9:15 h light:dark photoperiod) and compared the developmental characteristics of the second generation of offspring. Eggs were collected from 15 pairs of males and females per group three times per day. Eggs from the second and third sampling times were selected for the experiments. Forty individuals from each group were individually bred in insect-breeding jars (cat. No. 310122, 120 × 80 (mm); ventilation hole size, 40 mm; mesh hole size, 0.053 mm; SPL Life Science Co., Pocheon, South Korea) and predominantly fed a mixture of wheat bran and fishmeal (1:1) and bran (100%) alternately every three days (0.1 g for 14 days after hatching, 0.2 g from day 15 to day 35, and 0.7 g after day 36). For 35 days after hatching, hydration was provided by moistening the oasis with water to prevent drowning, and after 35 days, by moistening cotton with water. Radishes were provided as a supplementary food source.

Growth trial

The developmental period and weight of each stage were investigated in each group by checking the specimens daily for molting and then weighing the nymphs of each instar one day after molting to prevent mortality. The weight, body width, and length of the nymphs and adults were measured weekly.

Statistical analysis

Statistical analyses were performed using SPSS 27.0 statistical software (SPSS Inc., Chicago, IL, USA). Significant differences among the six populations (B101–B106) were analyzed by one-way analysis of variance (ANOVA). If any significant differences were found, the means were then compared using a post-hoc Scheffe or Games-Howell test (depending on the homogeneity of variance).

Molecular analysis

A total of 99 individuals, with 10 to 18 per group, were selected from seven populations (B101–B104, B106, B111–B112, excluding B105) to analyze the genetic diversity; one field cricket (*Teleogryllus emma*) was included as the external group. For molecular analysis, total genomic DNA was extracted from the tarsi of the GBs, which were previously stored at −18 °C in a freezer in 20-ml tubes, using QuickExtract DNA Extraction Solution (QE09050, Lucigen, WI, USA).

For amplification of the mitochondrial cytochrome c oxidase subunit I (*COI*) gene, 1 µl of forward primer, 1 µl of reverse primer, 16–17 µl of distilled water, and 1–2 µl of DNA template were added to the analysis site with 5X PCR Premix (TP1230, GenomicsONE, Seoul, South Korea), which contains a tracking dye (bromophenol blue), recombinant Taq DNA polymerase, reaction buffer, $MgCl_2$, dNTPs mixture, and enzyme stabilizer. We used the universal primer set LCO1490 / HCO2198 (Folmer *et al*., 1994) and set the PCR cycle as follows: 95 °C for 5 min, (95 °C for 30 s, 48 °C for 25 s, 72 °C for 45 s) × 35 cycles, and 72 °C for 5 min. The PCR products were sent to Macrogen (Daejeon, South Korea) for purification and sequence analysis. All sequences obtained were aligned to approximately 658 bp by removing the primer binding site after aligning the forward and reverse sequences using Geneious Prime software (Geneious, CA, USA). Twelve foreign GB specimens available from GenBank and the Barcode of Life Data Systems (BOLD) were also analyzed. Pairwise genetic distance and neighbor-joining trees were calculated using the Kimura 2-parameter model (Kimura, 1980) in MEGA X (Kumar *et al*., 2018). Bootstrap support values for each node were evaluated using MEGA X based on 1,000 replicates. Genetic distances were calculated using an uncorrected pairwise distance method (Srivathsan and Meier, 2012). Further, we constructed a median-joining haplotype network based on the COI gene sequences (COI barcoding region) using PopART (Leigh and Bryant, 2015). The output was processed in Adobe Illustrator.

Results

Developmental characteristics

We investigated the developmental period and weight of each nymphal instar stage and the weight, body length, and width of the adult stage for 145 individuals in six populations (Table 1). The average period for first-instar nymphs to reach the adult stage was 63.3 ± 4.9 days, or approximately two months. The developmental characteristics of the nymphs were also compared for each instar stage between the populations. All developmental stages showed statistically significant differences in the length of the developmental period between populations, except for the third and fourth nymphal instars (Fig. 1). However, we observed no statistically significant difference in the weight of each nymphal instar stage between populations (Fig. 2).

We observed a statistically significant difference in the survival rate between populations in the first experiment but not in the

Table 1. Developmental characteristics of *Gryllus bimaculatus* in each developmental stage

Developmental stage		Developmental period (days)	Weight (mg)	Body length (mm)	Body width (mm)
Nymphal instar	1	6.8±0.9	-	-	-
	2	5.9±0.8	2.6±0.6	-	-
	3	5.9±0.8	7.1±1.3	-	-
	4	6.4±0.7	14.6±3.3	-	-
	5	7.0±1.0	33.5±8.1	-	-
	6	7.9±1.2	77.9±18.1	-	-
	7	9.9±1.9	168.8±38.1	-	-
	8	12.4±2.3	361.2±81.9	-	-
	9	14.6±1.0	429.6±75.6	-	-
Adult (from 1st instar to the day of adult emergence		63.3±4.9	670.9±119.0	24.3±1.2	6.7±0.5

Developmental period, weight, and adult body length and width are based on measurements of total 145 individuals from six populations (B101–B106). Values are the mean ± SD. Dash indicates not measured.

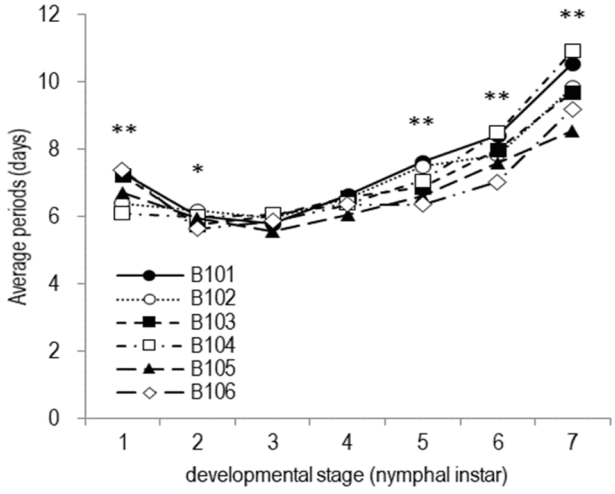

Fig. 1. Comparison of average nymphal periods among B101–B106 populations of *Gryllus bimaculatus*. Values are the means; ** $p < 0.01$; * $p < 0.05$ (determined by one-way analysis of variance).

second. The survival rate was highest for populations B103 and B104 in the first experiment and dropped sharply in group B105 from the third week (Fig. 3). The period from hatching to adulthood differed significantly between the populations in the first experiment, with populations B105 and B106 reaching the adult stage in the shortest time (Table 2). However, in the second

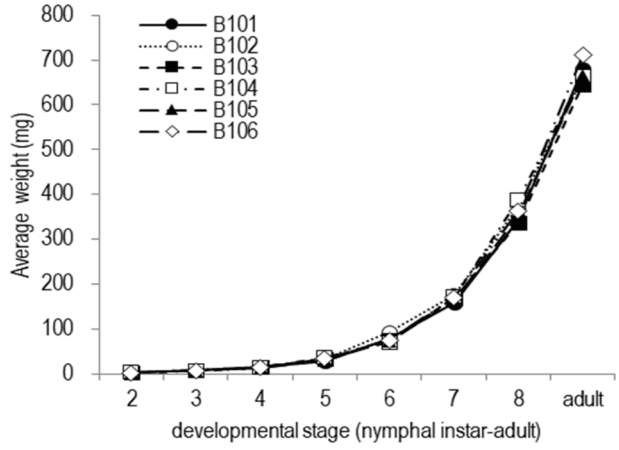

Fig. 2. Comparison of average nymph and adult weights by developmental stage for B101–B106 populations of *Gryllus bimaculatus* (Values are the means).

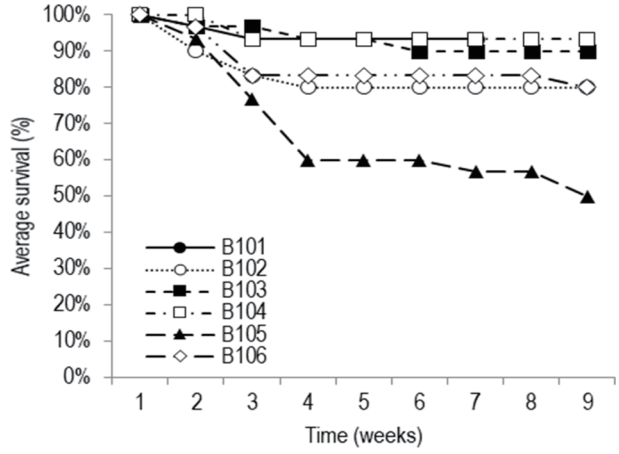

Fig. 3. Comparison of average nymphal survival rate among B101–B106 populations of *Gryllus bimaculatus* as a percentage of the total number of nymphs in that week (n = 30).

experiment, no statistically significant differences were observed between populations (Table 2). In the first experiment, no significant difference was found between populations regarding the weight of adults, whereas in the second experiment, B103 had the highest weight, with a statistically significant difference between populations (Table 2). The weight of nymphs by week showed a significant difference in almost all weeks, except for three weeks during the first experiment and two weeks during the second experiment (Figures 4 and 5). Around the eighth week, when the first adult was observed, B105 and B106 exhibited the highest weights in the first experiment (Fig. 4), whereas B103 and B104 exhibited the highest weights in the second experiment (Fig. 5).

Table 2. Comparison of survival rate, developmental period, and adult weight among six GB populations (B101–B106) in two experiments

Population	Survival rate (%)		Developmental period (days)		Adult weight (mg)	
	1st/	2nd	1st	2nd	1st	2nd
B101	87^{ab}	83	67.6±5.9^{c}	60.6±3.3	680.2±128.7	679.0±148.8^{ab}
B102	80^{ab}	83	63.5±2.4^{b}	61.4±5.3	657.7±100.5	663.9±139.9^{ab}
B103	90^{a}	90	64.3±3.9^{bc}	58.4±4.0	642.6±125.3	769.2±166.1^{a}
B104	93^{a}	90	64.1±3.5^{b}	60.7±3.6	664.9±118.7	616.2±121.1^{b}
B105	50^{b}	-	58.9±4.1^{a}	-	673.2±115.8	-
B106	80^{ab}	83	59.9±2.7^{a}	61.0±2.7	711.1±120.6	623.5±124.8^{b}
p-value	**	0.816	**	0.097	0.467	**

Values are the mean ± SD. Dash indicates not applicable.** $p < 0.01$, * $p < 0.05$ (obtained by one-way analysis of variance); a–c superscript letters indicate significant differences according to Scheffe's multiple range comparison test at $\alpha = 0.05$

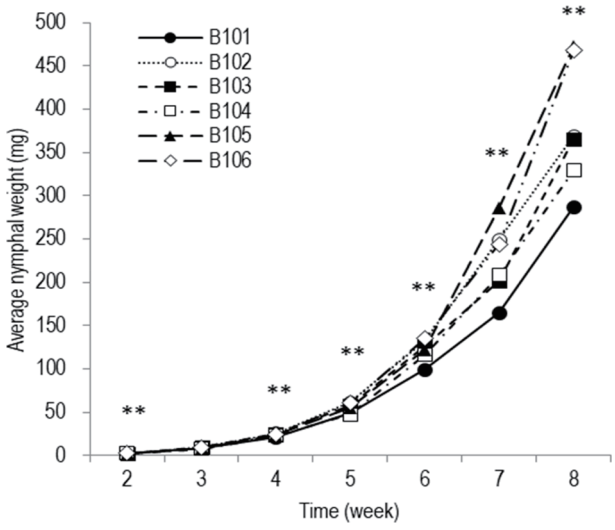

Fig. 4. Average nymphal weight among B101–B106 populations of *Gryllus bimaculatus*, determined weekly until eight weeks (when the first emergence of adults was observed). Values are the means; ** $p < 0.01$ (obtained by one-way analysis of variance).

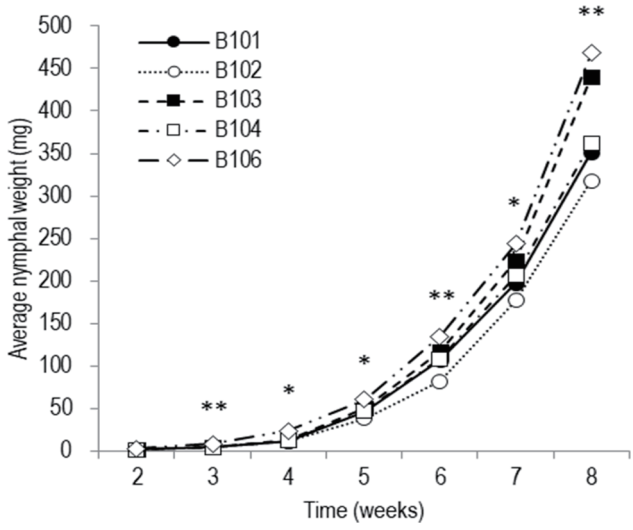

Fig. 5. Average nymphal weight among B101–B104 and B106 populations of *Gryllus bimaculatus*, determined weekly until eight weeks (when the first emergence of adults was observed). Values are the means; ** $p < 0.01$; * $p < 0.05$ (obtained by one-way analysis of variance).

Molecular characteristics

In total, we obtained 100 new sequences from seven populations (B101–B104, B106, B111–B112) of GB and one new sequence from *Teleogryllus emma* as an outgroup (653 bp of the partial COI gene region). All new sequences were deposited in GenBank (accession numbers: OP142775–OP142874, Table 3).

Within the 112 GB individuals, intraspecific genetic distances were low, ranging from 0.0% to 1.3%, as shown by the neighbor-joining tree (Fig. 6A, Table 4). In particular, GBs of the seven populations from South Korea had no genetic differences, with an intraspecific genetic distance of 0.0% (Table 4), and were genetically consistent with those collected from Germany, China, and Indonesia, forming a lineage in the neighbor-joining tree (Fig. 6A). A genetic difference of 0.0–1.8% was observed between the GB populations from South Korea and those from other countries, with the largest between those collected from Pakistan and India (2.3 %, Table 4).

Haplotype network analysis detected a total of six haplotypes, and all populations collected in South Korea were identified as

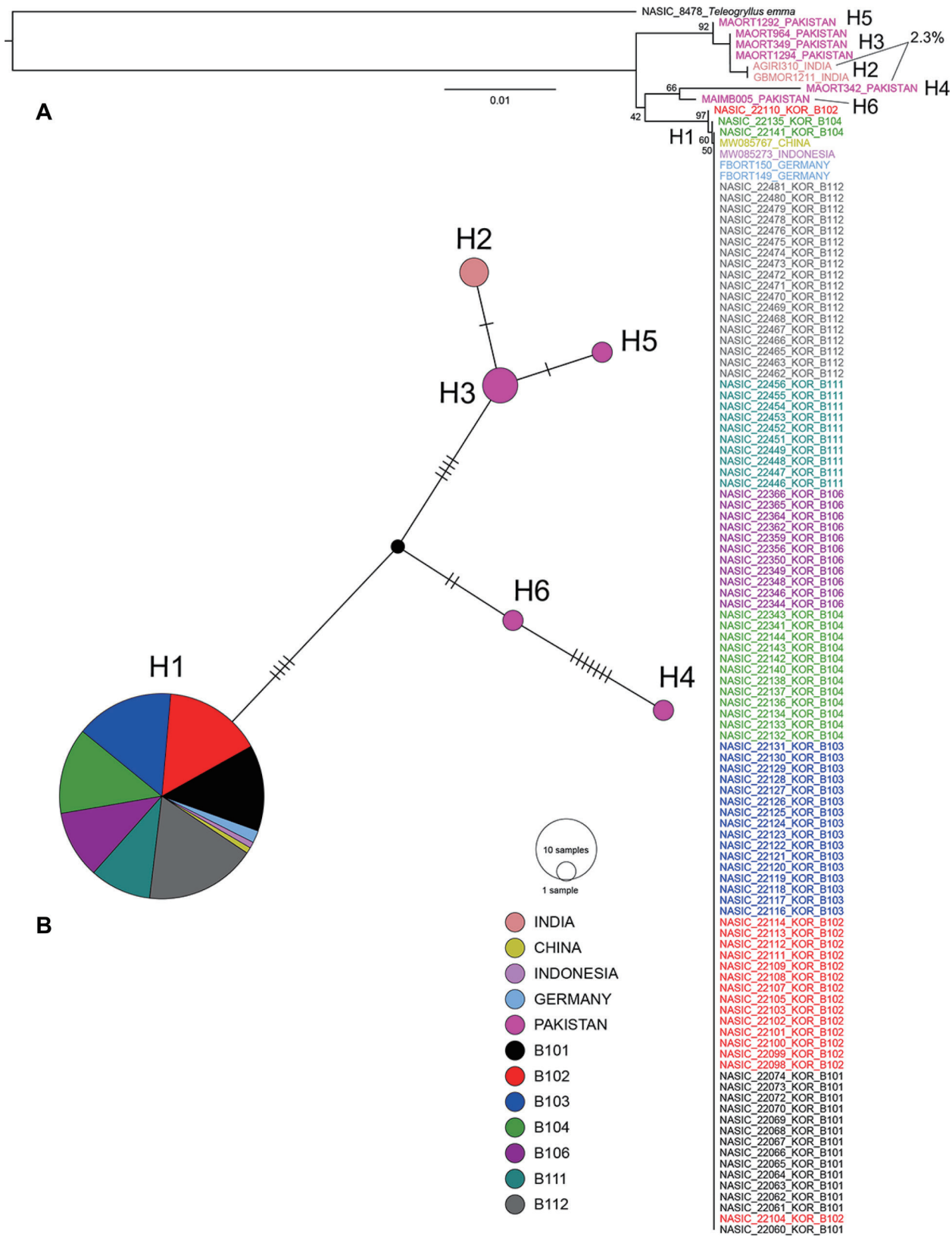

Fig. 6. (A) Neighbor-joining tree of 111 samples of *Gryllus bimaculatus* collected from five insect farms and two national insect rearing facilities, including 12 foreign samples. The tree shows six haplotypes distributed within the maximum intraspecific genetic distance of 2.3%. (B) Median-joining haplotype network for the *COI* gene. Populations from South Korea (B101–B104, B106, B111–B112) are differentiated by color, whereas other populations are separated by country.

Table 3. List of specimens investigated in this study, showing their locality data, population code, haplotype, voucher code, and GenBank accession number for COI.

Species	Population	Haplotype	NASIC voucher code	Collection locality	*COI*
Gryllus bimaculatus	B101	H1	22060–22074	Wanju, Jeonbuk, Korea	OP142775– OP142788
G. bimaculatus	B102	H1	22098–22114	Yangju, Gyeonggi, Korea	OP142789– OP142804
G. bimaculatus	B103	H1	22116–22131	Goyang, Gyeonggi, Korea	OP142805– OP142820
G. bimaculatus	B104	H1	22132–22343	Gimje, Jeonbuk, Korea	OP142821– OP142834
G. bimaculatus	B106	H1	22344–22366	Goseong, Gangwon, Korea	OP142835– OP142845
G. bimaculatus	B111	H1	22446–22456	Sangju, Gyeongbuk, Korea	OP142846– OP142855
G. bimaculatus	B112	H1	22462–22481	Jecheon, Chungbuk, Korea	OP142856– OP142873
G. bimaculatus	-	H1	-	Germany	FBORT149*
G. bimaculatus	-	H1	-	Germany	FBORT150*
G. bimaculatus	-	H6	-	Pakistan	MAIMB005*
G. bimaculatus	-	H5	-	Pakistan	MAORT1292*
G. bimaculatus	-	H3	-	Pakistan	MAORT1294*
G. bimaculatus	-	H4	-	Pakistan	MAORT342*
G. bimaculatus	-	H3	-	Pakistan	MAORT349*
G. bimaculatus	-	H3	-	Pakistan	MAORT964*
G. bimaculatus	-	H1	-	Indonesia	MW085273**
G. bimaculatus	-	H1	-	China	MW085767**
G. bimaculatus	-	H2	-	India	GBMOR1211*
G. bimaculatus	-	H2	-	India	AGIRI310*
Teleogryllus emma	-	-	8478	Wanju, Jeonbuk, Korea	OP142874

All sequences of *G. bimaculatus* and *Teleogryllus emma* in South Korea were newly generated for this study. *Sequences were downloaded from BOLD (Barcode of Life Data Systems). **Sequences were downloaded from GenBank.

Table 4. Inter- and intraspecific genetic differences in the two species *Gryllus bimaculatus* and Teleogryllus emma for COI (658 bp) calculated using p-distance.

	Individual	Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	AGIRI310_INDIA	H2														
2	FBORT149_GERMANY	H1	0.015													
3	FBORT150_GERMANY	H1	0.015	0.000												
4	GBMOR1211_INDIA	H2	0.000	0.015	0.015											
5	MAIMB005_PAKISTAN	H6	0.011	0.010	0.010	0.011										
6	MAORT1292_PAKISTAN	H5	0.003	0.011	0.011	0.003	0.011									
7	MAORT1294_PAKISTAN	H3	0.002	0.013	0.013	0.002	0.010	0.002								
8	MAORT342_PAKISTAN	H4	0.023	0.018	0.018	0.023	0.011	0.023	0.022							
9	MAORT349_PAKISTAN	H3	0.002	0.013	0.013	0.002	0.010	0.002	0.000	0.022						
10	MAORT964_PAKISTAN	H3	0.002	0.013	0.013	0.002	0.010	0.002	0.000	0.022	0.000					
11	MW085273_INDONESIA	H1	0.015	0.000	0.000	0.015	0.010	0.011	0.013	0.018	0.013	0.013				
12	MW085767_CHINA	H1	0.015	0.000	0.000	0.015	0.010	0.011	0.013	0.018	0.013	0.013	0.000			
13	B101–112_KOREA	H1	0.015	0.000	0.000	0.015	0.010	0.011	0.013	0.018	0.013	0.013	0.000	0.000		
14	NASIC8478_*Teleogryllus emma*	-	0.120	0.120	0.120	0.120	0.122	0.120	0.122	0.128	0.122	0.122	0.120	0.120	0.120	

haplotype H1 (Fig. 6B), along with the German, Chinese, and Indonesian specimens (Fig. 6B). Specimens collected from Pakistan were identified as four different haplotypes (H3 to H6), and H3 was close to H2, which was the haplotype of specimens collected in India (Fig. 6, Table 3–4).

Discussion

To understand the developmental characteristics of GB, we measured the breeding performance of six populations grown in South Korea, compared the developmental characteristics among breeding populations, and performed preliminary experiments to select superior lines. Performance was compared by using two experiments for each developmental characteristic. Characteristics that differed between the populations included weekly nymph weight in the first and second experiments, developmental period and survival rate in the first experiment, and adult weight in the second experiment. Characteristics that did not differ between populations included the developmental period and survival rate in the second experiment and adult weight in the first experiment. As such, some characteristics exhibited statistically significant differences only in the first experiment or only in the second experiment. The only characteristic that showed a statistically significant difference in both experiments was the weight of the nymphs in each week, with group B106 exhibiting the highest nymph weight in week 8 (Figs. 4 and 5). Furthermore, regarding the developmental characteristics of nymphs by instar stage, which were investigated based on the molting date, we observed that the developmental period of each instar varied between populations, except for instars 3 and 4 (Fig. 1); however, no statistically significant difference in instar weight was found in any of the six populations (Fig. 2). Therefore, we believe that the difference in nymph weight between the populations results from the different developmental period for each nymphal instar. That is, populations B105 and B106 had a shorter development period for instar stages 5 to 7 than the other populations (Fig. 1), as well as higher growth rates at week 8 (Fig. 4). However, the results were not consistent, as differences in adult weight between populations were only observed in the second experiment (Table 2).

In recent years, as insects have emerged as a solution to the global shortage of protein sources, edible insects have begun to attract the attention of many countries (van Huis *et al*., 2013; Liu *et al*., 2020; Moruzzo *et al*., 2021; Riekkinen *et al*., 2022; Rodríguez-Rodríguez *et al*., 2022; Veldkamp *et al*., 2022; Verneau *et al*., 2021; Żuk-Gołaszewska *et al*., 2022). As such, there are growing demands for high-performance lines that exhibit disease resistance (Grau *et al*., 2017) and excellent growth to maximize the output (van Broekhoven *et al*., 2015; Lee and Roh, 2010; Song *et al*., 2018) of the edible food industry. In addition, the distribution of such lines to farms is very important in improving quality and increasing uniformity. In this respect, a superior line of GB requires the following characteristics: adult weight must be high at the time of shipment, the developmental period must be short to accelerate harvesting time, and the survival rate must be high to increase the production rate (Table 2). However, it was difficult to determine a superior group in this experiment. To solve this problem, more populations should be collected to verify the developmental characteristics of each generation through passage breeding. Despite differences between populations regarding the developmental period of each GB instar raised in South Korea, the weight was constant for each developmental stage. Therefore, we suggest measuring nymph weight as a method of identifying the corresponding instar stage and using the data to develop a GB growth model that can be installed in an automated rearing system.

We also analyzed the genetic diversity in seven GB populations raised in South Korea. All South Korean GB specimens belonged to a single haplotype, and no genetic differences was found (Fig. 6, Table 4). Compared to individuals from other countries, only a small difference of approximately 2% was observed, which can be attributed to genetic variations between individuals within the order Orthoptera (Kim *et al*., 2022; Tantrawatpan *et al*., 2011). We suggest that the low genetic diversity of Korean GB specimens is because the group imported from Japan for animal breeding in the early 2000s served as a maternal group for the current GB bred in South Korean farms and institutions (Park *et al*., 2013; Rural Development Administration, 2014; 2017). As previously mentioned, this low genetic diversity may explain the lack of distinct developmental characteristics between populations. Even in the case of *Protaetia brevitarsis seulensis*, the most cultivated domestic edible insect, the genetic diversity of the farm breeding group is very low. Han *et al*. (2022) suggested that this phenomenon occurred because most breeding insects in farm households are obtained through exchange and

distribution between farms rather than collection, even for native domestic species. As the GB population size was small at the time of its introduction to South Korea, and its introduction period was relatively short, we believe that the low genetic diversity can be attributed to the breeding of a small number of individuals and their distribution between farms.

This low genetic diversity may be one of the factors that make domestic GB susceptible to disease. Indeed, the lower the genetic diversity within a species, the higher the probability of disease (Hughes and Boomsma, 2004; King and Lively, 2012; Spielman *et al*., 2004). Therefore, it is necessary to increase the genetic diversity of GB, which is currently a promising insect resource in the domestic insect industry (van Huis, 2020; Kim *et al*., 2019; Kim *et al*., 2022; Song *et al*., 2016), to maintain a healthy population. To this end, resources native to foreign countries should be secured and introduced into the country according to relevant quarantine procedures to ensure diversity of the group. Based on the result of this study, we first suggest Pakistan and India as candidates form GB-importing countries because these regions have haplotypes that have not been found in South Korea and have various haplotypes (Fig. 6, Table 3). Interestingly, Panagiotopoulou *et al*. (2016) previously reported the genetic hybridization of GB and *G. campestris* Linnaeus. Thus, crossbreeding of related species should be considered to increase the genetic diversity of domestic GB, which is estimated to be a single maternal line, as long as it poses no risks to the environment, economy, or public health.

In conclusion, Korean GB do not consistently differ in developmental characteristics between populations, which is thought to be related to their low genetic diversity. This study is the first attempt to compare developmental characteristics between populations of GB and understand their genetic diversity together and provides the basis for further research on selecting industrially useful, superior strains of GB.

Acknowledgments

We are grateful to H.G. Kim (Department of Agricultural Biology, National Institute of Agricultural Sciences, Korea) for their valuable assistance in maintaining colonies of two-spotted crickets. This work was carried out with the support of "The Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01355902)" Rural Development Administration, Republic of Korea.

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

References

Ahn MY, Hwang JS, Yun EY, Kim MJ, Park KK (2015) Anti-aging effect and gene expression profiling of aged rats treated with *G. bimaculatus* extract. Toxicol Res 31, 173–180.

Ahn MY, Kim BJ, Kim HJ, Jin JM, Yoon HJ, Hwang JS, *et al*. (2020) Anti-diabetic activity of field cricket glycosaminoglycan by ameliorating oxidative stress. BMC Complement Med Ther 20, 232.

van Broekhoven S, Oonincx DGAB, van Huis A, van Loon JJA (2015) Growth performance and feed conversion efficiency of three edible mealworm species (Coleoptera: Tenebrionidae) on diets composed of organic by-products. J Insect Physiol 73, 1–10.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Marine Biol Biotechnol 3, 294–299.

Ghosh S, Lee SM, Jung C, Meyer-Rochow VB (2017) Nutritional composition of five commercial edible insects in South Korea. J Asia Pac Entomol 20, 686–694.

Grau T, Vilcinskas A, Joop G (2017) Probiotic *Enterococcus mundtii* isolate protects the model insect, *Tribolium castaneum*, against *Bacillus thuringiensis*. Front Microbiol 8, 1261.

Han T, Park SC, Park H (2022) Genetic diversity of insect farm populations of *Protaetia brevitarsis seulensis* (Kolbe, 1886) (Coleoptera, Scarabaeidae) in South Korea inferred from *COI gene sequences.* J Natl Park Res 13, 83–103.

Hughes WHO, Boomsma JJ (2004) Genetic diversity and disease resistance in leaf-cutting ant societies. Evolution 58, 1251–1260.

van Huis A (2020) Edible crickets, but which species? J Insects Food Feed 6, 91–94.

van Huis A, Itterbeeck J van, Klunder H, Mertens E, Halloran A, Muir G, *et al*. (2013) Edible insects: Future prospects for food and feed security. FAO Forestry Paper 171. FAO, Italy.

Hwang BB, Chang MH, Lee JH, Heo W, Kim JK, Pan JH, *et al*. (2019) The edible insect, *Gryllus bimaculatus*, protects against gut derived inflammatory responses and liver damage in mice after acute alcohol exposure. Nutrients 11, 857.

Iba M, Nagao T, Urano A (1995) Effects of population density on growth, behavior, and levels of biogenic amines in the cricket,

Gryllus bimaculatus. Zool Sci 12, 695–702.

Im AR, Yang WK, Park YC, Kim SH, Chae S (2018) Hepatoprotective effects of insect extracts in an animal model of non-alcoholic fatty liver disease. Nutrients 10, 735.

Jung CE, Bae YH (2007) Oviposition and temperature-dependent egg hatching by the field cricket, *Gryllus bimaculatus* De Geer (Orthoptera: Gryllidae). Korean J Soil Zool 12 (1–2), 28–32.

Keum ES, Kim JW, Park DS, Jung CE (2012) Morphological and genetic comparison of *Telegryllus emma* and *Gryllus bimaculatus* (Orthoptera: Gryllidae) used for feed insect industry. Korean J Soil Zool 16 (1–2), 42–46.

Kim CH, Park SY, Lee YC, Kim JH, Byun BK (2019) Mass rearing conditions for the production of *Gryllus bimaculatus* De Geer (Orthoptera: Gryllidae). Korean J Appl Entomol 58(1), 69–76.

Kim WT, Kim SY, Ji SM, Chang GD, Song JH (2022) Current status and future perspective of industrial insect use in South Korea. Korean J Appl Entomol 61, 221–227.

Kim DY, Kim BM, Park TYS, Cho GH, Kim TW (2022) First record of *Teleogryllus* (Brachyteleogryllus) *marini* Otte & Alexander, 1983 (Orthoptera: Gryllidae) in Korea and discussion of its continued misidentification using DNA barcoding. J Asia Pac Entomol 25, 1–8.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16, 111–120.

King KC, Lively CM (2012) Does genetic diversity limit disease spread in natural host populations? Heredity 109, 199–203.

Korean Society of Applied Entomology, Entomological Society of Korea (2022) Check List of Insects from Korea. The 60th anniversary of Korean Society of Applied Entomology, The 50th anniversary of The Entomological Society of Korea.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35, 1547–1549.

Lee KP, Roh C (2010) Temperature-by-nutrient interactions affecting growth rate in an insect ectotherm. Entomol Exp Appl 136, 151–163.

Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. Methods Ecol Evol 6, 1110–1116.

Liu AJ, Li J, Gómez MI (2020) Factors influencing consumption of edible insects for Chinese consumers. Insects 11, 10.

Moruzzo R, Mancini S, Guidi A (2021) Edible insects and sustainable development goals. Insects 12, 557.

Panagiotopoulou H, Baca M, Baca K, Sienkiewicz P, Ślipiński P, Żmihorski M (2016) Genetic identification of a non-native species introgression into wild population of the field cricket, *Gryllus campestris* (Orthoptera: Gryllidae) in Central Europe. Eur J Entomol 113, 446–455.

Park YK, Lee HG, Choi YC (2013) Effects of rearing density on food consumption, adult mortality, and mean number of hatchlings of *Gryllus bimaculatus* (Orthoptera: Gryllidae). J Sericul Entomol Sci 51, 89–94.

Phesatcha B, Phesatcha K, Viennaxay B, Matra M, Totakul P, Wanapat M (2022) Cricket meal (*Gryllus bimaculatus*) as a protein supplement on *in vitro* fermentation characteristics and methane mitigation. Insects 13, 129.

Riekkinen K, Väkeväinen K, Korhonen J (2022) The effect of substrate on the nutrient content and fatty acid composition of edible insects. Insects 13, 590.

Rodríguez-Rodríguez M, G. Barroso F, Fabrikov D, Sánchez-Muros MJ (2022) *In vitro* crude protein digestibility of insects: A review. Insects 13, 682.

Rural Development Administration (2014) Guidebook of Industrial Insects. Rural Development Administration, Wanju, Korea. (In Korean)

Rural Development Administration (2017) Standards and Specifications for Breeding of Industrial Insects. Rural Development Administration, Wanju, Korea. (In Korean)

Seo DH, Hwang SY, Han J, Koh SK, Kim I, Ryu KS, *et al*. (2004) Immune-enhancing activity screening on extracts from two crickets, *Gryllus bimaculatus* and *Teleogryllus emma*. Entomol Res 34, 207–211.

Song MH, Han MH, Kwak KW, Lee S, Kim ES, Park KH, *et al*. (2016) Effect of different diets on growth and development of the two-spotted cricket, *Gryllus bimaculatus* (Orthoptera: Gryllidae). Int J Indust Entomol 33, 59–62.

Song MH, Lee HS, Park K (2018) Effects of dietary animal feed on the growth performance of edible insects. J Life Sci 28(5), 563–568.

Spielman D, Brook BW, Briscoe DA, Frankham R (2004) Does inbreeding and loss of genetic diversity decrease disease resistance? Conserv Genet 5, 439–448.

Srivathsan A, Meier R (2012) On the inappropriate use of Kimura-2-parameter (K2P) divergences in the DNA-barcoding literature. Cladistics 28, 190–194.

Tantrawatpan C, Saijuntha W, Pilab W, Sakdakham K, Pasorn P, Thanonkeo S, *et al*. (2011) Genetic differentiation among populations of *Brachytrupes portentosus* (Lichtenstein 1796) (Orthoptera: Gryllidae) in Thailand and the Lao PDR: the Mekong River as a biogeographic barrier. Bull Entomol Res 101, 687–696.

Tsukamoto Y, Kataoka H, Nagasawa H, Nagata S (2014) Mating

changes the female dietary preference in the two-spotted cricket, *Gryllus bimaculatus*. Front Physiol 95, 1–6.

Veldkamp T, Meijer N, Alleweldt F, Deruytter D, Van Campenhout L, Gasco L, *et al*. (2022) Overcoming technical and market barriers to enable sustainable large-scale production and consumption of insect proteins in Europe: A SUSINCHAIN perspective. Insects 13, 281.

Verneau F, Amato M, La Barbera F (2021) Edible insects and global food security. Insects 12, 472.

Wilkinson K, Muhlhausler B, Motley C, Crump A, Bray H, Ankeny R (2018) Australian consumers' awareness and acceptance of insects as food. Insects 9, 44.

Żuk-Gołaszewska K, Gałecki R, Obremski K, Smetana S, Figiel S, Gołaszewski J (2022) Edible insect farming in the context of the EU regulations and marketing—An overview. Insects 13, 446.