Evaluating Invasive Marbled Crayfish as a Potential Livestock for Sustainable Aquaculture

Sina Tönges¹, Karthik Masagounder², Frank Lenich³, Julian Gutekunst¹, Marvin Tönges⁴, Jasmin Lohbeck⁵, Aubry K. Miller⁵, Florian Böhl² and Frank Lyko¹*

¹ Division of Epigenetics, DKFZ-ZMBH Alliance, German Cancer Research Center (DKFZ), Heidelberg, Germany, ² Evonik Operations GmbH, Hanau, Germany, ³ Independent Researcher, Weidenberg, Germany, ⁴ Independent Researcher, Kassel, Germany, ⁵ Cancer Drug Development, German Cancer Research Center (DKFZ), German Cancer Consortium (DKTK), Heidelberg, Germany

The marbled crayfish (Procambarus virginalis) is a recently discovered freshwater crayfish species, which reproduces by apomictic parthenogenesis, resulting in a monoclonal, and all-female population. The animals were widely distributed through the aquarium trade and have established numerous stable wild populations through anthropogenic releases. They are highly prevalent in Madagascar, where they have become a popular source of nutritional protein. As freshwater crayfish aquaculture in open systems is a thriving, but ecologically damaging global industry, alternatives are urgently needed. Although marbled crayfish are often branded by their invasive mode of reproduction, their overall invasiveness is not higher than for other cultured crayfish species. Furthermore, their resiliency and high adaptability provide a strong rationale for evaluating them for closed, and environmentally safe aquaculture approaches. Here we describe a novel population of marbled crayfish in a former German coal mining area that is characterized by acid and polluted water. Even under these adverse conditions, animals grew to sizes, and weights that are comparable to commercially farmed freshwater crayfish. Tailored feed development and laboratory testing demonstrated highly efficient feed conversion, suggesting a considerable capacity for sustainable production in closed systems. We further show that marbled crayfish meat can be readily introduced into European meals. Finally, chemical analysis of marbled crayfish exoskeletons revealed comparably high amounts of chitin, which is a valuable source for the synthesis of chitosan and bioplastics. Our results thus suggest that production of marbled crayfish in closed systems may represent a sustainable alternative for crayfish aquaculture.

Keywords: livestock, invasive species, feed conversion, chitin, sustainability, aquaculture, marbled crayfish, tailored feeds

INTRODUCTION

The marbled crayfish (Procambarus virginalis) is a recently discovered freshwater crayfish species that emerged in the aquarium trade about 25 years ago (Scholtz et al., 2003; Lyko, 2017). The animals quickly established themselves as popular ornamental pets, which promoted their wide distribution (Patoka et al., 2014; Faulkes, 2015). Notably, the marbled crayfish is the only known...
crayfish species that reproduces by obligate apomictic parthenogenesis, a mechanism that results in the formation of an all-female, and globally monoclonal population (Martin et al., 2007; Vogt et al., 2008; Gutekunst et al., 2018; Hossain et al., 2018). Through anthropogenic releases, marbled crayfish have been introduced to various freshwater systems, where they have formed numerous stable populations (Chucholl et al., 2012). Stable wild populations of marbled crayfish have been described in a diverse range of habitats (Andriantsoa et al., 2019; Maiakovska et al., 2021). The animals have also shown the capacity to rapidly form large colonies consisting of 100 of 1,000 of animals (Andriantsoa et al., 2019; Maiakovska et al., 2021). High tolerance to various habitat parameters and high population densities are important features of invasive species.

While the introduction of marbled crayfish raises considerable ecological concerns, it also creates opportunities for human exploitation. This is exemplified by the spread of the animals on Madagascar, where their distribution area has increased 100-fold over 10 years (Jones et al., 2009; Kawai et al., 2009; Gutekunst et al., 2018). This dramatic increase was largely fueled by anthropogenic distribution, as marbled crayfish have developed into a valuable source of dietary protein (Andriantsoa et al., 2019, 2020). However, their potential benefits need to be carefully balanced against their potential negative ecological impacts (Vogt, 2021). And while marbled crayfish are subject to regulation in the European Union (Regulation No. 1143/2014 on the prevention and management of the introduction, and spread of invasive alien species), the corresponding measures have been found to be ineffective (Patoka et al., 2018). As such, it is important to develop ecologically safe frameworks for the culture of marbled crayfish.

Freshwater crayfish are increasingly popular livestock for aquaculture with a global value that now exceeds 10 billion US dollars (FAO, 2016). They are a rich source of nutritional protein, which contributes to their high global demand. Although there are more than 670 known crayfish species (Crandall et al., 2015), only a small number of them, including the noble crayfish (Astacus astacus), the narrow-clawed crayfish (Pontastacus leptodactylus), the red swamp crayfish (Procambarus clarkii), the signal crayfish, (Pacificastacus leniusculus), the yabby (Cherax destructor), the red claw (Cherax quadricarinatus), and the marron (Cherax tenuimanus). P. clarkii, which is native to Mexico and United States (Loureiro et al., 2015), now accounts for more than 95% of the global crayfish aquaculture production and its production volume has rapidly increased over the past decade (FAO, 2016). P. clarkii aquaculture is usually done in rice fields, with a target size of 20–25 grams and is based on the omnivorous feeding patterns of the animals (Wang et al., 2018). China currently produces and consumes most of the farmed P. clarkii, and with a production of more than 720,000 tons in 2015 (Wang et al., 2018).

However, P. clarkii is considered highly invasive and the farming of the animals in open culture has created significant ecological problems, including competition with other species, and the potential transmission of pathogens (Gherardi, 2006; Putra et al., 2018; Haubrock et al., 2021). Furthermore, animals that are farmed in open culture can be dispersed by birds, which further increases their invasive spread (Anastácio et al., 2014). Alternative species and approaches are therefore urgently needed. A transition to closed-system aquaculture, which is ecologically safe, would require the development of a corresponding technical solution. This includes tailored feeds that meet specific needs of the cultured animals, while targeting specific production parameters (e.g., growth, feed efficiency, and economics). Fish meal is often used as a preferred source of dietary protein in aquaculture, but its use is ecologically unfavorable. Plant-based ingredients, such as soybean and rapeseed meal, are common alternatives, but they lack methionine and lysine. Methionine is a limiting amino acid for many animals and its deficiency can directly affect animal growth (Bulbul et al., 2015). Supplementation with crystalline amino acids is therefore required to meet the amino acid demands of aquaculture livestock, including crustaceans (Nunes et al., 2014; Tan et al., 2018). As inadequate protein and feed utilization causes high ammonia production, tailored feeds also contribute to maintaining high water quality (Cowey and Walton, 1989; Council, 2011). However, marbled crayfish are currently fed with various empirically determined diets and tailored feeds are not available.

The marbled crayfish is very closely related to P. clarkii (Owen et al., 2015; Lyko, 2017), indicating that it may represent a viable alternative as an aquaculture livestock. While it has been argued that poor growth and conservation concerns should preclude any further consideration (Vogt, 2021), marbled crayfish culture conditions have not been optimized yet, and their potential for closed-system aquaculture has not been developed. Here we present data showing that the animals can grow to sizes that match and exceed the commercial target weight of P. clarkii. We also developed tailored feeds for closed-system aquaculture and found that feed conversion was highly efficient. Furthermore, we show that marbled crayfish exoskeletons are rich in chitin, an important raw material for the production of biodegradable plastics. Our study thus defines an integrated approach toward the sustainable utilization of marbled crayfish.

**MATERIALS AND METHODS**

**Animal Collection and Morphometric Analysis**

Marbled crayfish (N = 768) were collected between October 2019 and September 2020 by hand catching at Murner See, Germany (49.348875N and 12.206505E). The crayfish plague infection status of 20 animals from the population was molecularly tested by local authorities and found to be negative. Visual inspection showed that animals were generally in good condition, without any detectable damage. Total length (rostrum to uropods) was measured using a 15 cm caliper. After standard excess water removal, animal wet weight was determined (in grams) using an SJS 60007 scale (BASETech). Data were grouped and plotted in histograms. Chemical water analysis was provided by Raiffeisen-Laborservice (Ormont, Germany).

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Determination of Amino Acid Requirements Using Factorial Modeling

The amino acid profile of marbled crayfish (laboratory stock, DKFZ Heidelberg) was determined using ion-exchange chromatography (AMINOLab®, Evonik Nutrition and Care, Germany), except for tryptophan, and which was estimated using high-performance liquid chromatography (HPLC). In addition, the amino acid content of a commonly used marbled crayfish feed (NovoPleco, JBL, Neuhofen, Germany), which was used as a control feed (D1), was also analyzed. Amino acid requirements were calculated with a factorial modeling approach (Council, 2011) and are presented in Supplementary Table 1 on digestible basis and total basis, and assuming 85% digestibility for amino acids (Nunes et al., 2014). The utilization of different amino acids absorbed across the gut was assumed to be 50–60% and the maintenance requirements for different amino acids were considered to be 15–25% of the amino acids absorbed (i.e., on digestible basis).

Experimental Diets

The detailed ingredient and nutrient composition of the tailored feeds in this study are provided in Supplementary Table 1. A basal feed was formulated using soybean meal, soy protein concentrate, fish meal and krill meal as the main protein sources, and contained 29% crude protein, and 18.31 MJ/kg gross energy. The amino acid profile of the diet (Supplementary Table 1) was balanced (except for methionine) considering the requirements predicted by the factorial modeling approach. The basal tailored feed (D2) was formulated with low levels of methionine (0.45%) and methionine + cysteine (0.86%) and then supplemented with increasing levels of methionine dipeptide (AQUAVI® Met-Met): 0.07% (D3), 0.15% (D4), and 0.25% (D5). As such, D2 represents a methionine-deficient tailored feed, while D3 is a tailored feed with a methionine level that was matched to the (non-tailored) control feed. D4 was matched to the amino acid profile of the crayfish, while D5 was designed to have a methionine surplus. Feed contents were validated by amino acid analysis (Supplementary Table 1). The D2–D5 feeds were produced in pellets of 2 mm size.

Laboratory Testing of Tailored Feeds

In total, 100 adolescent animals (mean total length: 1.75 cm, SD: 0.25 cm; mean weight: 0.11 g, and SD: 0.05 g) were fed for 3 months. Per feed, 20 animals were kept in 4 tanks (25.6 × 18.1 × 13.6 cm), with five animals per tank, at 20°C under natural daylight. The animals were fed daily at 17:00 with 0.08 g × 13.6 cm), with five animals per tank, at 20°C under natural daylight. The animals were fed daily at 17:00 with 0.08 g of feed, as higher amounts of feed were refused by the animals. Daily mean feed intake per tank was calculated by dividing the amount of feed placed in a tank on a given day by the number of animals surviving on that day. The calculated daily mean feed intake value was totaled over the entire experimental period to calculate the total mean feed intake in each tank. Severely injured (e.g., loss of both chelae) or dead animals were removed from the trial immediately to prevent confounding effects related to cannibalistic feeding. All animals were weighed once per week (independently of their molt frequencies) to determine weight gains over time. Water parameters (Supplementary Table 2) were monitored daily for temperature and weekly for NH₄, NO₃, NO₂, and O₂ (JBL Test sets, Neuhofen, Germany). After the weekly measurement of chemical water parameters, water was exchanged with tap water of the same temperature.

Data Analysis

Survival probabilities for all animals and the groups D1–D5 were calculated in R using the CRAN packages survival (version 2.44-1.1) and survminer (version 0.4.6). Kaplan–Meier plots were generated, and survival probabilities were calculated for each feed group and for all animals (Supplementary Figure 1). To investigate differences in survival between feeds pairwise p-values were calculated using a log-rank test. Growth curves were plotted for the five different feeds (D1–D5) by the average weight of animals distributed over 14 weeks. To statistically evaluate the different feeds, we built a generalized estimating equation model (GEE) as implemented in the geeglm function of the R package geepack. The model considers weight gain per feed over the time of 14 weeks and accounts for an autoregressive first-order correlation structure with a Gaussian error distribution, and link function. The Wald statistic was used to estimate levels of significance.

The mean weight gain per tank was determined by calculating the mean final body weight per tank minus the mean initial body weight per tank. Feed conversion ratios (FCR) were calculated as:

\[
FCR = \frac{\text{total feed intake per animal [g]}}{\text{mean weight gain [g]}}
\]

Mean daily feed intakes (FI) were calculated as:

\[
FI = \frac{\text{amount of feed placed in a tank per day [g]}}{\text{number of surviving animals on that day [n]}}
\]

To account for different feeding behaviors within a group of animals, we calculated mean values for daily feed intake. These values were summed for all days to calculate the total mean feed intake per animal. Subsequently, mean FCRs were calculated for each trial group. Data was checked for normality by using the Shapiro–Wilk test (W = 0.93947, p = 0.2579). Homogeneity of variances was checked by Bartlett’s test (Bartlett’s K-squared = 1.5781, p = 0.8127) before applying parametric tests. The R internal function aov was used with the model of feed conversion ratio by feed (RES = 0.731). As the total variances between groups were statistically significant (p-value = 0.00239) a more detailed pairwise comparison between all groups was performed using the Tukey HSD test in R.

Isolation of Chitin

The following procedure is based on slightly modified literature protocols (Percot et al., 2003a,b) to remove CaCO₃ and protein from shellfish shells. Peeled exoskeletons from pre-cooked marbled crayfish (wild catch from Murner See, Germany, TL 4–14 cm) and the current industry standard, the whiteleg shrimp (Litopenaeus vannamei, commercially obtained from Scheck-in Center, Heidelberg, Germany, TL 8–15 cm) were collected, and air dried. Exoskeletons were subsequently ground to powder with
RESULTS

Sizes and Weights of Marbled Crayfish From an Oligotrophic Lake

Murner See in Germany is a former brown coal mining site that was flooded in 1982 to create a recreational lake with a water surface of 94 ha (Figure 1A). Water quality is characterized by substantial acidity and pollution with Aluminum and Manganese (Figure 1B). As a consequence, the lake is very oligotrophic, with limited amounts of plant detritus, mosses, leaves, and insect larvae available for the support of aquatic life. Nevertheless, the lake supports a large marbled crayfish colony (Figure 1C). To characterize this population, 768 marbled crayfish were collected by hand catching and their total lengths, and weights were determined. This revealed that the majority of animals had sizes between 4 and 12 cm (Figure 2A). Weights usually ranged from 5 to 30 g, with several animals weighing > 35 g (Figure 2B). Furthermore, wild-caught animals that were transferred to a laboratory culture system continued to grow noticeably (Figure 2C), suggesting considerable potential for commercial aquaculture. Systematic variation of key parameters, such as water temperature, stocking density, and feeding will be required to fully evaluate the production potential of marbled crayfish.

Formulation and Testing of Tailored Feeds

To support the development of a closed marbled crayfish culture system, we used a factorial modeling approach to formulate tailored feeds (see section “Materials and Methods” for details). These feeds were then used to determine the optimum level of methionine supplementation for marbled crayfish growth (Supplementary Table 1). We subsequently tested the tailored feeds in multiple independent groups of adolescent marbled crayfish (N = 100) over 3 months (see section “Materials and Methods” for details). Survival analysis showed no significant differences between the groups (Supplementary Figure 1), thus allowing direct comparisons. Analysis of size and weight data revealed a noticeable effect of the tailored feeds on the growth of the animals: when compared to the non-tailored control feed (D1), feed D3 (tailored, matched methionine) resulted in a significantly higher growth compared to D2 (methionine deficient), and D4 (methionine matched to control) showed a faster growth gain compared to D2 (methionine deficient), and D4 (methionine matched to requirement). During the early stages of the trial (until week 8), D5 showed the best performance, and while D3 showed the best performance during the late stages of the trial (Figure 3). These findings demonstrate the importance of tailored feeds and proper methionine supplementation for the optimum growth of marbled crayfish.

Determination of Feed Conversion Ratios

At the end of the trial, a mean weight gain of 2.81 g was observed for the tailored diet D3, while a mean gain of 1.43 g was observed for the control diet D1 (Table 1). Subsequent determination of FCR revealed that the FCR for D3 was significantly lower (p < 0.05, ANOVA, and RES = 0.731) better than for D1 (1.38–2.41, Table 1). These results again illustrate the superior performance of tailored feeds. Also, the methionine-deficient diet D2 showed the poorest FCR among all feeds (Table 1), which illustrates the importance of methionine supplementation for marbled crayfish feeds. The best FCR was observed for D3 (1.38, Table 1), and suggests that marbled crayfish are highly efficient feed converters.

Utilization of Marbled Crayfish for Protein and Chitin Production

We also explored the suitability of marbled crayfish as a source of nutritional protein in European meals. Indeed, we found fried marbled crayfish tails (Figure 4A) to be suitable for crayfish risotto, and for appetizers (Figure 4B). Furthermore, the shell waste that was generated in this process, could be easily collected, and preserved by air-drying (Figure 4C).

To determine the chitin content of marbled crayfish shell waste, we used a chemical extraction protocol (see section “Materials and Methods” for details). Because they represent the major source of commercially extracted chitin and because data or material from P. clarkii were not available, we used similarly processed whiteleg shrimp (L. vannamei) shells for comparisons. This revealed a significantly higher chitin content for marbled crayfish than for L. vannamei (2.60% vs. 0.85%, p < 0.05,
and Figure 4D). These findings suggest that marbled crayfish represent a valuable source of chitin.

**DISCUSSION**

The increasing demand for nutritional protein and bioplastics requires the development of sustainable, and ecologically friendly production strategies. Our size and weight data of marbled crayfish revealed comparable features to commercially harvested *P. clarkii*, which is the dominant stock in crayfish aquaculture (Wang et al., 2018). Our findings thus contradict conclusions based on morphometric data from wild populations and laboratory colonies (Vogt, 2021). Differences in morphometric data analysis can be explained by the fact that previously reported populations were usually analyzed without any size selection and included the full spectrum of developmental stages, while our dataset is based on hand-catching, which selects bigger animals. As aquaculture production would also selectively harvest bigger animals, we consider our data more informative, and relevant. Furthermore, many of the populations that were analyzed by Vogt (2021) were located in challenging environments (cold climate, presence of predators, and lack of nutrients), while aquaculture is designed to improve and accelerate growth. Further work will be required to define optimized conditions and environments, and to determine the time that is needed to reach marketable sizes under optimized conditions.

Methionine is often the limiting amino acid to promote growth in aquatic animals, if feeds contain mainly plant proteins (Bulbul et al., 2015). Indeed, we observed the lowest growth rate for the feed with the lowest methionine level. This is consistent with the notion that methionine is a growth-limiting nutrient for marbled crayfish, similar to other crayfish, and shrimps and fish (Yan et al., 2007; Gu et al., 2013; Tan et al., 2018). Interestingly, the feed with the highest methionine level mainly promoted growth at earlier developmental stages, while later stages performed best with a feed that had an intermediate methionine level. These observations suggest different needs of methionine in different stages of crayfish development. Studies in other aquatic animals show that growth rates reach a plateau (Harding et al., 1977; Alam et al., 2001), or even decrease when the methionine requirements of the animals are overfed (Millamena et al., 1996; Sveier et al., 2001). Our results further suggest that methionine levels of 0.7% optimally support the growth of juvenile and early adolescent
FIGURE 3 | Growth of laboratory-cultured, adolescent marbled crayfish fed with tailored feeds. Growth curves are shown for diets D1–D5, with different methionine concentrations, as indicated. A generalized estimating equations model revealed a statistically significant weight gain for diet D3 ($p = 0.02$) and D5 ($p = 0.03$) when compared to diet D1. Error bars indicate the standard error.

TABLE 1 | Weight gains and feed conversion ratios (FCR) of crayfish fed with different diets.

| Feed   | Description (methionine content in brackets) | Mean weight gain (per animal) | Mean feed intake (per animal) | FCR  |
|--------|-----------------------------------------------|------------------------------|-------------------------------|------|
| D1     | Control (0.52%)                               | 1.43 g                       | 3.62 g                        | 2.41 |
| D2     | Tailored, met-deficient (0.45%)               | 0.97 g                       | 3.29 g                        | 3.35 |
| D3     | Tailored, met like control (0.52%)            | 2.81 g                       | 3.81 g                        | 1.38 |
| D4     | Tailored, met-matched (0.60%)                 | 1.56 g                       | 3.11 g                        | 2.22 |
| D5     | Tailored, met surplus (0.70%)                | 2.63 g                       | 4.29 g                        | 1.72 |

FCR comparisons: D1:D3 $p = 0.041$, D2:D3 $p = 0.003$, and D2:D5 $p = 0.012$.

marbled crayfish. A balanced methionine supplementation is particularly relevant in closed aquaculture systems, where it supports rapid growth while maintaining high water quality.

Our experiments also provided first estimates for the FCR of marbled crayfish. The FCR is an important parameter for evaluating the potential of an organism for the sustainable production of biomass. In this context, it is notable that we observed FCRs as low as 1.4, which compares favorably to other species that are known as efficient feed converters, such as *L. vannamei*, and some insects (Hung and Quy, 2013; Ooninxcx et al., 2015). In comparison, the FCR of *P. clarkii* with similar sizes to the animals used in our study was found to be 2.8 with standard feed and could be improved to 1.9 by the addition of flavonoid feed supplements (Liu et al., 2020). Additional research is required to further optimize marbled crayfish feed conversion with less refined feeds and different culture conditions to fully realize their potential for the sustainable production of nutritional protein.

The sustainability of marbled crayfish aquaculture could be further improved by a zero-waste processing approach, which makes use of the considerable chitin content of their exoskeletons. Chitin and its derivatives, such as chitosan, are important raw materials for many industries and have found frequent use in wound bandages, filter materials, and for the production of biodegradable plastics (Park and Kim, 2010). Shrimp and crab shell waste currently provide most of the raw material for chitin extraction (Younes and Rinaudo, 2015), while the chitin content of *P. clarkii* shells remains unknown. Our results show that marbled crayfish exoskeletons contain 3 times more chitin per animal compared to whiteleg shrimp.
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Marbled Crayfish Utilization

FIGURE 4 | Utilization of marbled crayfish as a source for dietary protein and chitin. (A) Marbled crayfish tails with herbs, fried in butter. (B) Marbled crayfish tail meat on Avocado mousse. (C) Dried exoskeletons (shell waste) from marbled crayfish. (D) Comparison of chitin content per animal in marbled crayfish (P. vir) exoskeletons and whiteleg shrimp (L. van) shell waste. An unpaired two-tailed t-test showed that the difference between the two groups is highly significant (p = 0.0016).

While RAS-based production of marbled crayfish has already been described as ecologically safe (Vogt, 2021), it has also been argued that the approach might be unprofitable. However, profitable processes for RAS-based mass production of shrimps have been established and are being further optimized (Shinji et al., 2019). As chitin extraction from shell waste could provide a significant increase in revenue, we suggest that RAS-based production of marbled crayfish might be a profitable and environmentally safe alternative to the current practices of P. clarkii farming in rice paddies. RAS-based aquaculture also allows for stringent pathogen monitoring (Ahmed and Turchini, 2021) and would thus prevent the transmission of infectious agents that threaten native crayfish populations (Keller et al., 2014; Mrugała et al., 2015). Similarly, stocking from laboratory populations, which are known to be free of crayfish plague (Vogt, 2021), would exclude a key pathogen associated with crayfish aquaculture.

Finally, it is important to notice that the role of invasive species in human livelihoods is dynamic in space and time (Shackleton et al., 2019). This is exemplified by the introduction and anthropogenic distribution of marbled crayfish in Madagascar, where the animals have had a demonstrable positive impact on household economy, and food security (Andrianjato et al., 2020). Robustness, high adaptability and efficient feed conversion are important traits that can be found both in invasive species and in successful livestock. Given the possibilities to minimize...
environmental risks through the use of closed systems, it will be important to fully evaluate the potential of marbled crayfish as a livestock for sustainable aquaculture.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

**ETHICS STATEMENT**

The experiments described in this study are not subject to ethics committee approval and consent procedures. Laboratory stocks were described previously (Vogt et al., 2015) and handled according to institutional guidelines for animal care. Wild animals were collected in compliance with local fishery regulations.

**AUTHOR CONTRIBUTIONS**

ST performed the experiments and analyzed the data. KM designed the feeds and the feed trial, and analyzed the data. JG performed statistical analyses. FLe collected animals and prepared morphometric analyses. MT prepared marbled crayfish meals. JL and AM performed the chitin extraction. FB and FLY conceived the study. ST and FLY wrote the manuscript with input from the other authors. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.651981/full#supplementary-material

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