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Comparative studies on the life history characteristics of two *Brachionus calyciflorus* strains belonging to the same cryptic species

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ABSTRACT

The life history characteristics of rotifers usually vary at different temperatures, depending on their species or strains. However, differences in the adaptability of rotifers sampled from two climatic zones and belonging to the same cryptic species in response to temperature changes remain undescribed. In this study, the phylogenetic relationships of *Brachionus calyciflorus* collecting from two climatic zones were reconstructed, and two clones of the same potential cryptic species were selected as representatives of two populations. The life history characteristics of the two *B. calyciflorus* strains were comparatively studied at four different temperatures (20 °C, 24 °C, 28 °C and 32 °C). The results showed that the responses of the life history traits of *B. calyciflorus* to temperature were strain-dependent. The Xishuangbanna strain is more suitable for growth at the higher temperature and that the Kunming strain grows preferentially at the lower temperature. Temperature significantly affected the durations of all developmental stages, offspring production, life expectancy at hatching, age-specific fecundity and survivorship of the two strains, which adopted the identical life history strategy (high population growth and low survivorship with increasing temperature). The discrimination of cryptic species is necessary to test the ecological or evolutionary characteristics of species.

1. Introduction

Environmental variation across both space and time is universal in nature, with various climatic regimes and habitat features that are transforming the fitness of millions of species at a low rate. The evolutionary responses of species to their environment are important because the elicited adaptive strategies, such as shifts in ecological niches and adjustments of life history traits, could contribute to the population dynamics (Halsband-Lenk et al., 2004), which could increase the extinction risk of endemic populations due to the strong selection imposed by local environmental factors. Vertebrates have responded to environmental stress via migration and adaptation; however, habitat fragmentation and priority effects of the founder proposed by "Monopolization Hypothesis" (De Meester et al., 2002) are likely to impede migration and colonization in

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zooplanktons. Thus, temperature has been ascribed a major role in restricting the occurrence and activities of zooplankton (Halbach, 1973). The thermal history to which an organism has been exposed influences survival, fecundity and the timing of the development and reproduction of most, if not all, zooplankton species (Halbach, 1973; Xiang et al., 2016), from the level of the individual to population, including rotifers, cladocerans and copepods (Gama-Flores et al., 2014). Thus, the persistence of populations or strains in different temperature zones may depend more heavily on adaptive evolution to environmental fluctuation with different life history strategies, and as a result life history patterns are finely tuned to local environmental conditions.

Rotifers are minute aquatic invertebrates that occur in almost all types of bodies of water worldwide and play a key role between phytoplankton and planktivorous fish in freshwater ecosystems (Wallace et al., 2006). The presence of potential cryptic species has been shown to be widespread in zooplankton, which has been inferred for monogont rotifers based on the application of molecular phylogenetics, coalescent theory and mating tests (Gómez et al., 2002; Gilbert and Walsh, 2005; Xiang et al., 2011). Different cryptic species tend to be characterized by different ecological features, allowing their adaptation to disparate ecological niches. However, the effects of such hidden diversity are usually overlooked in ecological studies. To date, several empirical studies are available regarding the responses of the life history characteristics among *Brachionus calyciflorus* populations or strains to changing temperature (Halbach, 1973; Awaïss and Kestemont, 1992; Xi et al., 2005; Gama-Flores et al., 2014). However, most of these cases, if not all, represent populations that were sampled either from the same locations or from near sites belonging to the same climatic zone. Consequently, the contrasting population ecological traits could not reflect the differences in adaptive evolutionary abilities in response to the temperature change. In addition, pre-investigation and the definition of cryptic species were not determined before the ecological experiment. Accordingly, further studies should be conducted to explore the underlying adaptation and response mechanisms of rotifer dwelling in different climatic zones in response to fluctuations in temperature and even to global warming.

Life table demography is an important approach to study the life history strategy and population dynamics of zooplankton under continuously changing environmental conditions. It presents birth rates and death rates in an age-specific way, and life history variables such as average lifespan, generation time and population growth rate related to survivorship and reproduction, can be quantified (Wallace et al., 2006). Thus, the results of life tables provide valuable insights into the suitability of the ambient conditions for the zooplankton.

On the southwest border of China, two cities, Kunming and Xishuangbanna, lie in close proximity with a distance of 400 km, but they have significant differences in climate characteristics. Kunming is located in the subtropical zone, with an annual temperature of 15 °C and moderate seasonality. In comparison, Xishuangbanna belongs to the typically tropical monsoon zone. The weather is rather changeable, with more sunshine and rainfall, and the annual average temperature is 21 °C. Differences in adaptability of rotifers from these two distinct climatic zones would provide interesting information. In the present study, we collected *B. calyciflorus* from a subtropical (Kunming) and a tropical (Xishuangbanna) environment, and we compared the life history characteristics of these two rotifer strains in response to changing temperature using a life table demographic approach. This study will elucidate the adaption of these rotifers to water environment habitats as well as the response strategies over the life history of two rotifer strains in response to temperature changes.

2. Materials and methods

2.1. Sample collection and clonal culture

B. calyciflorus individuals were collected from Lake Dianchi in Kunming (N24°95′, E102°65′) and waters in the Xishuangbanna Tropical Botanical Garden (N21°55′, E101°15′). The local water temperature was 19 °C and 25 °C, respectively. After transferring them back to the laboratory, the rotifers were clonally cultured under natural light in a homothermal incubator with EPA medium (pH 7.4–7.8) (Peltier and Weber, 1985). The culture temperatures were set at 20 ± 1 °C and 24 ± 1 °C, which are close to the water temperatures during sampling. The rotifers were fed daily with the algae *Scenedesmus obliquus* (2.0×10^6 cells·ml^{-1}), which was semi-continuously cultured in HB-4 medium with a 16:8 light: dark photoperiod of 3000 lx fluorescent light (Li et al., 1959). Before feeding the rotifers, the algae were precipitated by centrifugation and resuspended in EPA medium and then stored at 4 °C. The density of the stock algae was estimated using a hemocytometer (Tiefe, 0, 100 mm, 1/400 qmm, Germany). Finally, 60 clones of *B. calyciflorus* were successfully established, including 31 Kunming (KM) clones and 29 Xishaungbanna (BN) clones.

2.2. Phylogenetic analyses and discrimination of cryptic species

According to the procedures detailed by Xiang et al. (2011), DNA was extracted from the 60 clones of *B. calyciflorus*, and the partial mitochondrial COI gene fragment was amplified and sequenced (GenBank No. KT161884, KT161885, KT161886, KT161887, KT161888, KT161889, KT161890, KT161891, KT161892, KT161893, KT161894, KT161895, KT161896, KT161897, KT161898, KT161899, KT161900, KT161901, KT161902, KT161903, KT161904, KT161905, KT161906, KT161907, KT161908, KT161909, KT161910, KT161911, KT161912, KT161913, KT161914 for KM clones and KJ862219–KJ862247 for BN clones). A phylogenetic tree was reconstructed with 12 haplotypes, defined by 60 clones, using the maximum likelihood method in PAUP* 4.0b10 (Swofford, 2002). The most likely model, GTR + I, was indicated by the Akaike Information Criterion using Modeltest 3.7 (Posada and Buckley, 2004). Four clades were confirmed, and the sequence divergences of the percent were all