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Form Alternation of the Gonopod and Chela from Breeding to Non-breeding Season in Males of the Crayfish *Cambaroides dauricus* (Decapoda: Cambaroididae)

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The occurrence of cyclic morphological alternation in male crayfish of the family Cambaridae following molting is widely acknowledged. However, there remains a contentious issue within the genus *Cambaroides*: Some previous studies have proposed that male *Cambaroides* exhibit a pair of non-corneous Form II gonopods during the non-breeding season, while others argue that these species lack Form II in adult males. This study examined the color and shape of the corneous tips on the gonopod to determine its form. Additionally, morphometric methods were utilized to distinguish between Form II adults and juveniles. The results of the study confirm the presence of Form II adults in *Cambaroides dauricus* and *Cambaroides similis*. The Form I gonopod is characterized by four golden-colored corneous terminal elements, whereas the Form II gonopod features blunt, non-corneous terminal elements, on which the color is the same as that of the surrounding surface. Furthermore, cyclic dimorphism on the gonopod of *C. dauricus* was observed seasonally, and distinct morphological differences in the chela were noted between Form I and Form II adults.

Key words: Cyclic dimorphism, Cornification, Life history, East Asian freshwater crayfish, Juvenile, Adult

BACKGROUND

The presence of cyclic dimorphism in male crayfish of the family Cambaridae and the genus *Cambaroides* has been a subject of prolonged debate (Fitzpatrick 1995). Male cambarid crayfish are categorized as Form I adults, Form II adults, and Juveniles based on the morphology and functionality of the gonopod, chela and other characters (Faxon 1884; Hobbs 1945; Scudamore 1948). Form I adult male crayfish molt into Form II during the non-breeding season and revert to Form I during the subsequent molt into the breeding season (Scudamore 1948). The Form I gonopod exhibits corneous terminal elements, while the Form II gonopod features blunt and non-corneous terminal elements. The juvenile gonopod is morphologically similar to that of Form II (Hart 1953). Scudamore (1948) substantiated the idea that sex hormones may regulate the cycle of changes in male sexual form during molting. Within the family Cambaridae, the Form I gonopod was believed to signify sexual maturity or seasonal reproductive activity of males (Larson and Magoulick 2011). The concept of dimorphism of the gonopod was first introduced by Hagen (1870). Faxon (1885) hypothesized the existence of a cycle: Form I in the breeding season would molt into Form II in the non-breeding season. To date, cyclic dimorphism has only been proven in members of the family Cambaridae (Faxon 1885; Hart 1953; Shen et al. 2000; Kawai et al. 2013). Additionally, dimorphism in the chela of male crayfish has been recorded in some American cambarids (Faxon 1884). It was observed that the Form I male of the family Cambaridae

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possesses larger hooks on ischia compared to the Form II male (Faxon 1884; Hobbs 1945). The hook on ischia also exhibits cyclic dimorphism, considered a common characteristic of *Cambaroides* and the family Cambaridae (Hobbs 1974). Nevertheless, several studies suggested that the conversion from Form I to Form II does not occur universally (Taylor 1985). In recent years, the cyclic dimorphism of female Cambaridae has been discovered and studied (Wetzel 2002; Hamasaki et al. 2020; Schuster et al. 2022; Kawai and Mclay 2023), whereas it has not been observed in female *Cambaroides*. This distinction arises from the fact that, based on Kawai et al. (1994) and my own unpublished data, adult female *Cambaroides* molt only once a year in their natural habitat.

Genus *Cambaroides* has six known species. Among them, *Cambaroides dauricus* is one of the most widespread East Asian crayfish (Kawai et al. 2015). It is found across northeast China, Mongolia, the far east of Russia, and North Korea, extending from the Yalu River basin in the south to the Heilongjiang River basin in the north (Kawai et al. 2015). Localities of *C. similis*, situated farther south, encompass the Liaodong Peninsula and the Korean Peninsula (Kawai and Min 2005). *Cambaroides japonicus* is restricted to Hokkaido and the northeast of Honshu (Kawai et al. 2015). *Cambaroides schrenckii* is distributed in both China and Russia, primarily in the Ussuri River basin, the Lower Songgari River, the Lower Heilongjiang River, and Sakhalin Island (Kawai and Tudge 2008; Kawai et al. 2013 2015; Alda and Kawai 2022). While the life histories of *C. japonicus* and *C. similis* have been studied over the last three decades (Kawai et al. 1994 1995; Kawai and Saito 1999 2001; Kawai and Scholtz 2002; Jung et al. 2009), the absence of Form II in adult males of *Cambaroides* has been a subject of scrutiny. Kawai et al. (2015) proposed the lack of cyclic dimorphism in adult males within the genus *Cambaroides*, establishing a characteristic that supports the group's classification as an independent monophyletic family Cambaroididae, beneath the superfamily Astacoidea. The study also revealed that there is no difference in the hooks on ischia of *C. japonicus* between the breeding and non-breeding seasons (Kawai and Saito 1999). However, other researchers had reported that the adult male of *Cambaroides* exhibited an alternate form of the gonopod (Faxon 1884; Hobbs 1974). Faxon (1884) also provided a description based on just four specimens. He described Form I *C. dauricus* with strongly developed hooks on ischia, while Form II *C. japonicus* exhibited weaker ones. My earlier collection of specimens (unpublished) also provides evidence supporting the presence of Form II in adult males of *C. dauricus* and *C. similis*. To validate the occurrence of form alternation in these *Cambaroides* species, a comprehensive morphological study was conducted on collected samples. This investigation unequivocally confirmed the presence of such dimorphism in adult males of *C. dauricus*. The principal emphasis of this study lay in the meticulous examination of *C. dauricus* specimens, supplemented by a detailed examination of a limited number of *C. similis* specimens and an exceedingly sparse sampling of *C. schrenckii* specimens.

MATERIALS AND METHODS

Study area

The study was conducted in the Hunehe River basin in Fushun City, Liaoning Province, China. Considering the low population density of every species of the genus *Cambaroides* in China, I collected samples from different sampling sites along branches of the river basin of the Hunehe River to avoid catching excessive samples at one site (Fig. 1). The total length of the Hunehe River is 415 km; the major tributaries in the Fushun City area are the Yingge River, Zhangdang River, Suksuhu River, She River, Dongzhou River, and Sarhv River. The sources of these rivers are all mountain streams; the width of the streams ranges from 0.5–10 m. The altitude of the sites ranges from 498–562 m (Jiubing Town), 355–410 m (Tangtu Town), 219–265 m (Zhangdang Town), 289–316 m (Shangjiahe Town), 544–582 m (Wandianzi Town), and 318–354 m (Qingyuan Town). Given the limited variations in altitude, latitude, longitude, and habitat across these sites (Fig. 1), it is reasonable to assume the absence of significant environmental or climatological distinctions.

Sampling

A total of 177 male *Cambaroides dauricus* specimens were collected during the study, with 172 samples preserved in 95% ethanol. The remaining 5 individuals were not subjected to ethanol fixation. Additionally, 15 male *Cambaroides similis* were collected in Liaoning Province, China (Table 1), and 2 male *Cambaroides schrenckii* were acquired from the Wutong River, Hebei Town, Hegang City, Heilongjiang Province, China. All samples were obtained between April and October, spanning the years 2017 to 2022 (Table 1). Each 1–2 km line transect involved nocturnal manual collection of visible crayfish during stream tracing. Simultaneously, a rudimentary recording of the spermatophores and spawns of females was conducted. Crayfish collection was suspended from November to March due to frozen streams and extremely low

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temperatures (*e.g.*, averaging -10.7°C during the 2020 winter to 2021 spring) in the Fushun City area. Additionally, molting does not occur in winter months (Mitchell and Smock 1991; Kawai et al. 1994).

In October 2021, 17 Form I adult male *C. dauricus* specimens were collected from Fushun County, Fushun City, Liaoning Province, China. These specimens were kept in aquariums (1200 mm * 400 mm * 250 mm, L * W * D), with a controlled water depth of 100 mm. Plastic nets were employed to segregate every crayfish, although they shared the same water supply. The diet comprised fish, shrimp, and *Elodea* aquatic plants. Water temperature remained uncontrolled, varying from 0 to 25.9°C with seasonal changes, and natural light

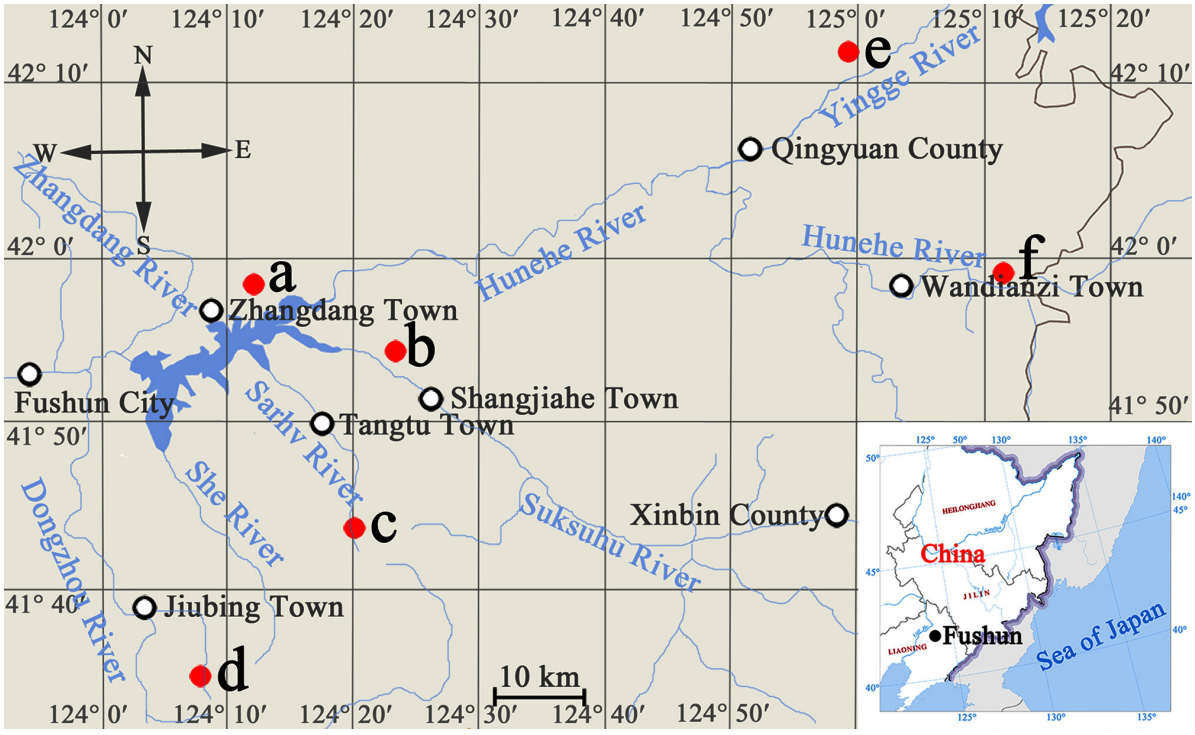

Fig. 1. Sample Site Localities: Zhangdang Town Site (a), Shangjiahe Town Site (b), Tangtu Town Site (c), Jiubing Town Site (d), Qingyuan Town Site (e), Wandianzi Town Site (f).

Table 1. Details of sample collection

Species	Month	Number	Site
Cambaroides dauricus	Apr.	15	Zhangdang Town, Fushun County
	May.	18	Zhangdang Town, Fushun County
	Jun.	27	Wandianzi Town, Qingyuan County; Qingyuan Town, Qingyuan County; Tangtu Town, Fushun County
	Jul.	28	Wandianzi Town, Qingyuan County; Tangtu Town, Fushun County
	Aug.	26	Shangjiahe Town, Xinbin County
	Sept.	12	Jiubing Town, Fushun County
	Oct.	51	Zhangdang Town, Fushun County; Shangjiahe Town, Xinbin County
	Total	177	106 corneous, 71 non-corneous
Cambaroides similis	Jul.	7	Gushanzi Town, Haicheng City
	Sept.	6	Anbo Town, Pulandian District, Dalian City
	Oct.	2	Gushanzi Town, Haicheng City
	Total	15	5 corneous, 10 non-corneous

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illuminated the laboratory setting.

Morphometric measurements and statistical analyses

One hundred and seventy two *C. dauricus* (101 corneous and 71 non-corneous) and fifteen *C. similis* (5 corneous and 10 non-corneous) samples were measured. Morphometric measurements were conducted using a vernier caliper on the following characters: POCL (postorbital carapace length), GL (gonopod length), PL (propodus length of chela), PaL (palm length of chela), PaW (palm width of chela), PaD (palm depth of chela), and DL (dactyl length of chela).

Analysis focused on 172 *C. dauricus* samples, considering ratios such as DL/PL, PaL/PL, PaW/PL, PaD/PL, PaD/PaL, PaW/PaL, PaD/PaW, PL/POCL, DL/POCL, PaL/POCL, PaW/POCL, and PaD/POCL for chela morphometrics, and the ratio GL/POCL for gonopod morphometrics. A one-way ANOVA was conducted on these morphometric ratios, with "Form of male crayfish" as the factor, and Tukey tests for pairwise differences, to discern variations in chela and gonopod dimensions among the forms. The allometric growth equation (y = a*x^b) was applied to model the relative dimensions of these chela morphometric characters.

Method of form confirmation

Male crayfish were classified into three distinct forms based on the size of the chela, gonopod, carapace, and the cornification of the gonopod in the present study. These forms are delineated as Form I adults, Form II adults, and Juveniles. The determination of form is facilitated by the examination of the shape and color of cornification on the surface of gonopod terminal elements, which encompass the mesial process, centrocaudal process, centrocephalic process, and caudal process (Fig. 2). Crayfish exhibiting more sharp terminal elements with a distinctive golden color, differing from the surrounding surface of the gonopod's tip, are classified as corneous Form I adults. Conversely, crayfish lacking sharp terminal elements and displaying a color congruent with the surrounding surface of the gonopod's tip are identified as non-corneous Form II adults or Juveniles (Fig. 2).

The Form II adults and Juveniles were determined through the following morphological analysis and statistical methods: Crayfish smaller than the smallest Form I adult were identified as juveniles (Kawai and Saito 1999; Jones and Eversole 2011). However, it's imperative to note that a Form II crayfish, surpassing the size of the smallest Form I, doesn't automatically qualify as an adult. In this study, for non-corneous samples, crayfish with < 16.22 mm POCL (the POCL of the smallest Form I male *C. dauricus* in this study, refer to results, Fig. 5) were categorized as Juveniles, while those with > 16.22 mm POCL encompassed oversized Juveniles and Form II adults. Principal Component Analysis (PCA) was initially conducted on POCL, PL, PaL, PaW, PaD, DL, and GL (Fig. 3). Subsequently, utilizing the score of PC1 (Eigenvalue = 6.8738, Variance = 98.20%, refer to results, Fig. 3) and GL/POCL, Hierarchical Cluster Analysis (HCA) was executed (Mean, Euclidean distance). The samples that clustered together on one branch, which contained those smaller than 16.22 mm POCL, were designated as Juveniles, while the remaining samples were classified as Form II adults.

RESULTS

The Juveniles and Form II adults

In 71 non-corneous samples of *C. dauricus*, with a minimum size of 16.22 mm observed in the smallest Form I POCL, it was challenging to completely distinguish between Juveniles and Form II adults (Fig. 3). Several sampling points exhibited overlap within the 95% confidence ellipse. Hierarchical cluster analysis (HCA) revealed the convergence of these non-corneous *C. dauricus* samples, encompassing both Form II adults and Juveniles, into three distinct branches (Fig. 4). Samples situated within the branch corresponding to POCL measurements < 16.22 mm were identified as Juveniles (n = 28). The remaining two branches represented Form II adults (n = 43). Notably, five oversized samples (POCL: 17.02, 17.18, 17.28, 17.30, and 17.68 mm) were still classified as Juveniles. Within the Form II adult category, a distinction was made between branches indicative of "moderate size" and "large size" individuals (Fig. 4).

Gonopod form alternation adjusted for POCL

In this study, *C. dauricus* (n = 172) measured 10.24–37.92 mm POCL (mean = 22.39 mm, SD = 5.52) and 2.30–14.74 mm GL (mean = 9.12 mm, SD = 2.23). *C. similis* (n = 15) measured 12.84–38.16 mm POCL (mean = 20.95, SD = 6.49) and 8.82–15.78 mm GL (mean = 8.38, SD = 3.07). The smallest corneous gonopods are presented at 16.22 mm POCL for *C. dauricus* and 20.64 mm POCL for *C. similis* (Fig. 5). Samples with a POCL larger than 16.22 mm (*C. dauricus*) and 20.64 mm (*C. similis*) in corneous specimens suggest Form I adults. This POCL (16.22 mm) could also be regarded as the size of the

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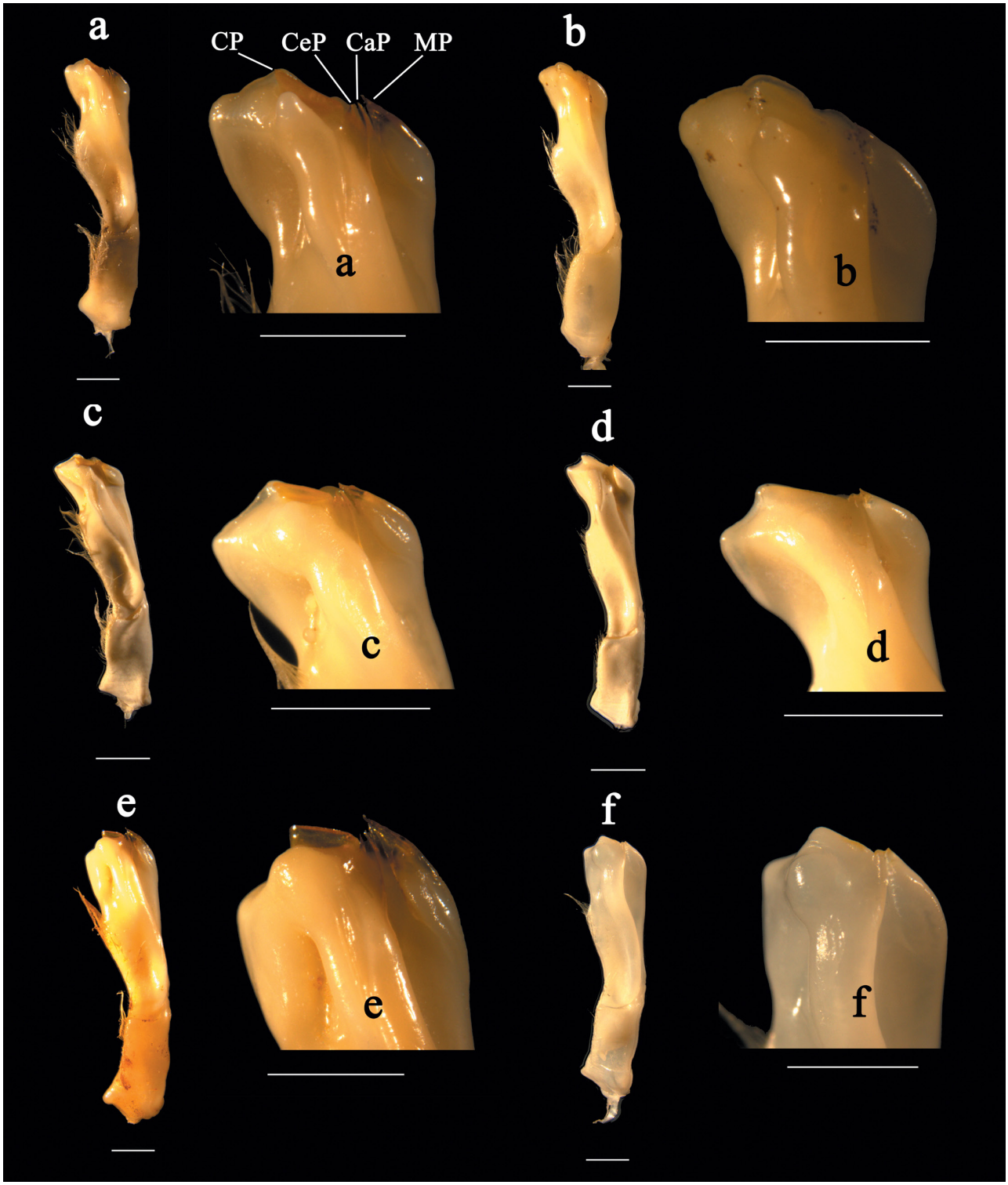

Fig. 2. Gonopod of *Cambaroides dauricus*: Form I, 28.38 mm POCL, 10.60 mm GL (a); Form II, 30.18 mm POCL, 11.92 mm GL (b). Gonopod of *Cambaroides similis*: Form I, 20.64 mm POCL, 8.76 mm GL (c); Form II, 22.18 mm POCL, 8.98 mm GL (d). Gonopod of *Cambaroides schrenckii*: Form I, 27.14 mm POCL, 11.64 mm GL (e); Form II, 27.08 mm POCL, 11.48 mm GL (f). The terminal elements: mesial process (MP); central projection: centrocaudal process (CaP), centrocephalic process (CeP); caudal process (CP). Scale bars = 1 mm.

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smallest adult male of *C. dauricus*. Non-corneous samples beyond 16.22 mm POCL (*C. dauricus*) and 20.64 mm POCL (*C. similis*), without an absence in large size (Fig. 5), indicate the presence of Form II adults. Simultaneously, a Form II adult of *C. schrenckii*, with a POCL of 27.08 mm, is depicted in the photograph (see Fig. 2). Form I adult *C. dauricus* measured 16.22–37.92 mm POCL (mean = 23.85 mm, SD = 4.73, n = 101). Form II adult *C. dauricus* measured 17.76–35.02 mm POCL (mean = 24.08 mm, SD = 4.38, n = 43). Juvenile *C. dauricus* measured 10.24–17.68 mm POCL (mean = 14.54 mm, SD = 1.96, n = 28).

The annual cyclic life history of adult males and females of *Cambaroides dauricus* in the Hunehe River basin

The percentage of Form I in total adult males remained consistently high in April, May, September, and October (Fig. 6). April (water temperature: 4.9°C)

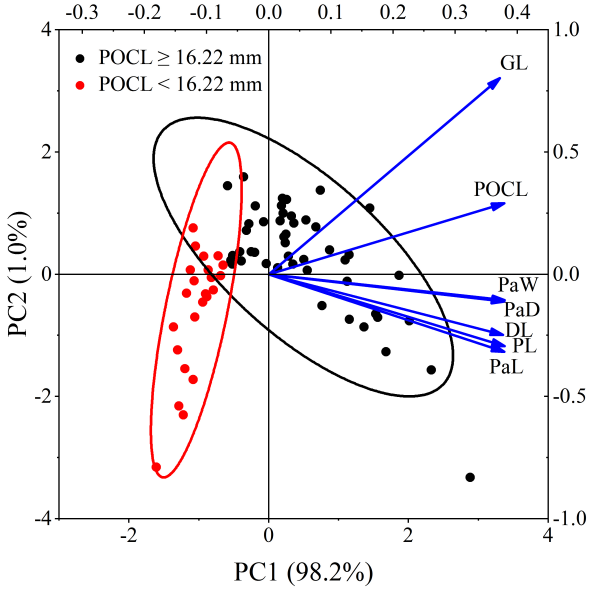

Fig. 3. The Principal Component Analysis (PCA) Biplots of non-corneous *C. dauricus* samples (n = 71). Employing a threshold of POCL exceeding 16.22 mm as a basis for grouping, the analysis reveals a discernible clustering pattern, where data points are predominantly segregated into two distinct clusters. Notably, a small number of points lie within the overlapping region of the 95% confidence ellipses delineating these clusters. This observation indicates that solely relying on the POCL criterion above 16.22 mm is insufficient for categorical differentiation. Specifically, these points may pertain to either the Juveniles category, characterized by POCL values below 16.22 mm, or the Form II adults group. The axes correspond to the principal components, where PC1 elucidates 98.20% of the total variance (Eigenvalue: 6.8738), while PC2 accounts for 1.05% of the variance (Eigenvalue: 0.0732).

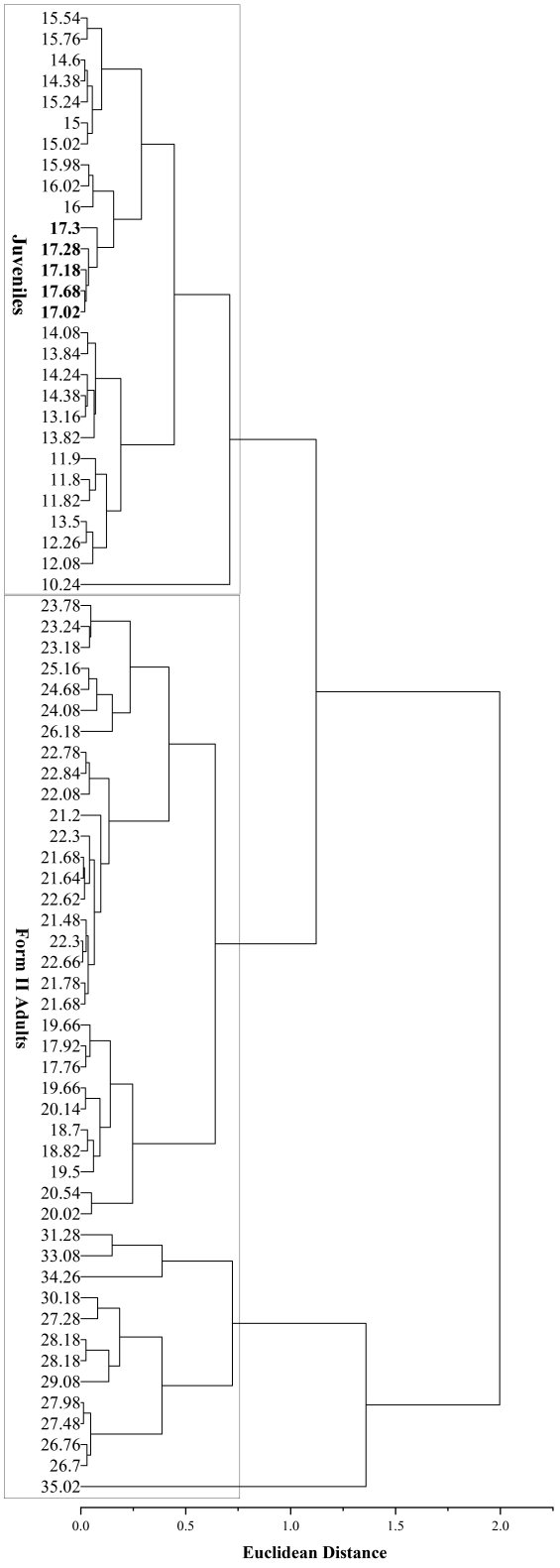

Fig. 4. Hierarchical Cluster Analysis Tree depicting 71 non-corneous samples, encompassing 43 Form II adults and 28 Juveniles. The left side of the tree displays the POCL of the Form II adults and Juveniles. Notably, Juvenile samples with a POCL larger than 16.22 mm are highlighted with bold typeface.

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and May are early spring in the northeast of China, and Form I adults reached 100%. In late May, the water temperature was still very cold (7.7°C), Form I adult males had kept corneous throughout winter. Some females had spawned, while others still carried spermatophores. June was the transition period from late spring to summer (water temperature: 13.7°C), and the proportion of Form I began to decline. During this period, a substantial number of adult males molted, and most adult females brought spawns on their swimming legs. July marked the entrance into summer (water temperature: 16.6°C), and the proportion of Form I was the lowest (Fig. 6). The proportion of Form I significantly increased as the season progressed from late summer to early autumn in August. During this period, males and females prepared for mating; Form II adult males molted back to Form I, and adult females also molted. On August 23rd, 2022 (temperature: 19.2°C), I observed five females that had completed mating, carrying spermatophores on their annulus ventralis and the base between the fourth and fifth pereopods. September (temperature: 10.2°C) and October (temperatures: 9.5°C and 3.4°C) corresponded to mid-autumn to late autumn, with the proportion of Form I nearly reaching 100%. Autumn contained numerous matings as the water temperature dropped below 3.4°C. Winter, lasting from November to March of the following year, saw no molting (Mitchell and Smock 1991; Kawai et al. 1994), and the proportion of Form I remained constant. The form alternation of adult male *C. dauricus* aligned with the seasonal shifts between breeding and non-breeding periods.

Dimorphism of the Chela

A total of 172 *C. dauricus* samples, including 144 adult specimens, underwent thorough examination through one-way ANOVA. The results indicate that, relative to the overall body size, Form I chela exhibited greater length, width, and depth compared to Form II (refer to Table 2 and Fig. 7). However, minimal alterations were observed in the ratios between the various morphological characteristics within the chela. This suggests that while the chela vary in size between Form I and Form II, the fundamental shape of the chela remains unchanged. Further elaboration on the distinct

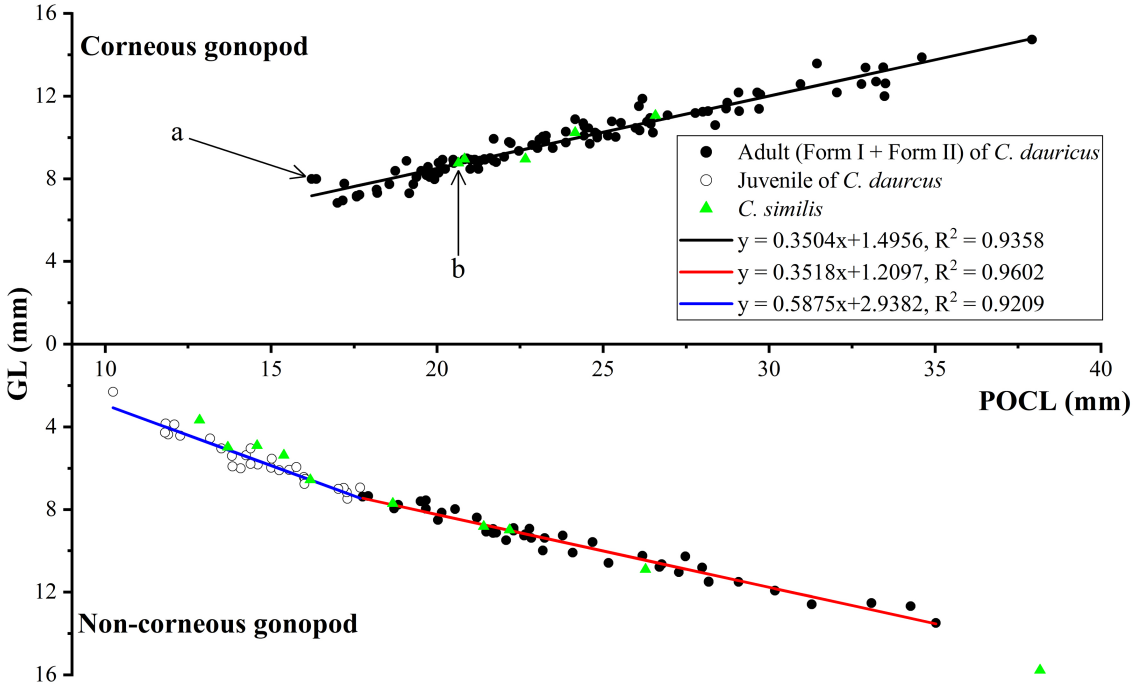

Fig. 5. GL fitted for POCL. The upper section of the scatter plot features corneous gonopods, indicative of the Form I adults, while the lower section includes non-corneous gonopods, encompassing the Form II adults and Juveniles. The corneous gonopod's minimum POCL is 16.22 mm for *C. dauricus* (a) and 20.64 mm for *C. similis* (b). In *C. dauricus*, the black line illustrates the curve of the Form I adult GL fitted for POCL (n = 101), with GL/POCL ranging from 0.3584 to 0.4932 (mean = 0.4153, SD = 0.0219). The red line represents the curve of the Form II adult GL fitted for POCL (n = 43), with GL/POCL ranging from 0.3701 to 0.4305 (mean = 0.4034, SD = 0.0150). Meanwhile, the blue line signifies the curve of the juvenile GL fitted for POCL (n = 28), featuring GL/POCL ranging from 0.2246 to 0.4324 (mean = 0.3813, SD = 0.0424). Significantly, differences in GL/POCL among the Form I, Form II, and Juvenile were observed in *C. dauricus* ($F_{2, 169}$ = 20.653, P = 0.000), with the Form I adult displaying a longer GL than the Form II adult when adjusted for POCL ($F_{1, 142}$ = 10.568, P = 0.001).

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relationships is provided in the subsequent section (Table 2). After adjusting for POCL, significant differences were observed in PL among Form I, Form II, and Juvenile ($F_{2, 169}$ = 50.673, P = 0.000). Specifically, the PL of Form I exceeded that of Form II ($F_{1, 142}$ = 12.987, P = 0.000). Similarly, significant differences were found in PaL ($F_{2, 169}$ = 50.764, P = 0.000), with Form I surpassing that of Form II ($F_{1, 142}$ = 9.711, P = 0.002). The trend continued with PaW ($F_{2, 169}$ = 81.610, P = 0.000), where Form I exhibited greater width than Form II ($F_{1, 142}$ = 10.980, P = 0.001), and PaD ($F_{2, 169}$ = 71.714, P = 0.000), where Form I had a deeper dimension compared to Form II ($F_{1, 142}$ = 12.717, P = 0.000). Additionally, DL displayed significant differences ($F_{2, 169}$ = 28.885, P = 0.000), with Form I surpassing Form II ($F_{1, 142}$ = 14.577, P = 0.000). Upon adjusting for PL, significant differences persisted among Form I, Form II, and Juvenile for PaL ($F_{2, 169}$ = 10.976, P = 0.000), PaW ($F_{2, 169}$ = 16.234, P = 0.000), PaD ($F_{2, 169}$ = 2.845, P = 0.000), and DL ($F_{2, 169}$ = 29.576, P = 0.000). However, no significant distinctions emerged between Form I and Form II for PaL ($F_{1, 142}$ = 0.143, P = 0.706), PaW ($F_{1, 142}$ = 0.140, P = 0.709), PaD ($F_{1, 142}$ = 1.051, P = 0.307), and DL ($F_{1, 142}$ = 0.021, P = 0.886). Upon adjusting for PaL, no significant difference was observed in PaW among Form I, Form II, and Juvenile ($F_{2, 169}$ = 2.759, P = 0.066). However, significant differences were noted in PaD ($F_{2, 169}$ = 9.082, P = 0.000). PaW ($F_{1, 142}$ = 0.448, P = 0.504) and PaD ($F_{1, 142}$ = 0.828, P = 0.364) of Form I were not larger than for Form II. Upon adjusting for PaW, significant differences were detected among Form I, Form II, and Juvenile for PaD ($F_{2, 169}$ = 14.369, P = 0.000), with Form I displaying greater depth than Form II ($F_{1, 142}$ = 9.361, P = 0.003).

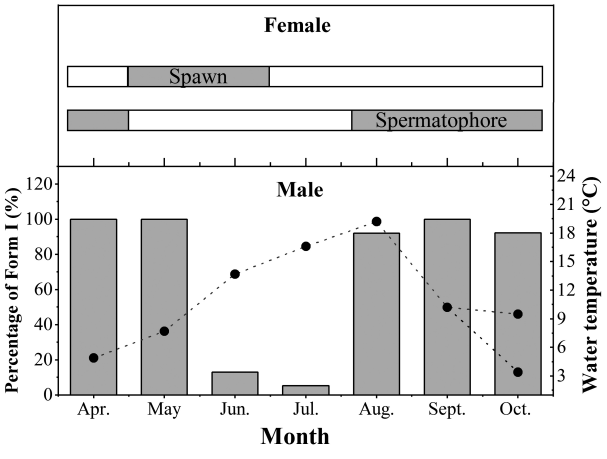

Fig. 6. The annual cyclic life history of adult male and female *C. dauricus* in the Upper Hunehe River basin. The top half illustrates spermatophores and spawns on the female in various months. The bottom half features a histogram displaying the proportion of Form I males (n = 106) among all adult male *C. dauricus* (n = 149) across different months. The accompanying dot plots provide information on water temperature.

Molting, form alternation, and death of adult male *Cambaroides dauricus* during feeding in the aquarium

From October 2021 to October 2022, 17 adult male *C. dauricus* samples were kept in aquariums with water temperatures ranging from 0 to 25.9°C. Spring molting occurred between 18.5 and 23.0°C during the late spring and early summer, with 10 individuals successfully molting in June and July (58.82% of total samples). Mortality peaked at temperatures from 22.2 to 25.9°C, with 11 individuals (64.71% of total samples) succumbing between July 25th and August 12th. Notably, 4 individuals (23.53% of total samples and 36.36% of dead samples) died post-molting, and 7 (41.18% of total samples and 63.64% of dead samples) died without molting. Among the survivors, one individual died on August 28th, foregoing autumn molting, while 5 re-molted in September (29.41% of total samples, 83.33% of survivors). All Form I crayfish, which successfully molted in June and July, altered into Form II after spring molting. Meanwhile, Form II crayfish that successfully molted and survived in September all reverted to Form I after autumn molting. 12 individuals (70.59% of total samples) died during feeding (Table 3). No instances were observed of adult forms changing from Form II to Form II simultaneously.

DISCUSSION

Kawai and Saito (1999) reported the absence of the Form II gonopod in adult male *C. japonicus* and detected no difference in chela length between breeding and non-breeding seasons (Kawai and Saito 2001). As a result, they suggested the lack of cyclic dimorphism in *C. japonicus*. Relying on specimens of *C. japonicus*, *C. similis*, and *C. schrenckii*, previous studies have consistently shown that the gonopod of the genus *Cambaroides* maintains Form I consistently throughout both breeding and non-breeding seasons (Kawai and Min 2005; Kawai and Tudge 2008; Kawai et al. 2013). Nevertheless, reports of Form II adult males in the genus *Cambaroides* also exist. Faxon (1884) described Form II *C. japonicus* with smaller and non-corneous terminal elements of the gonopod, suspecting a dual form in males of *Cambaroides*, similar to *Cambarus*. Kawai (unpublished), upon inspecting Faxon's specimens, noted their dry or ethanolic preservation for hundreds of years, causing the color of the terminal elements to

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become indistinguishable. Given the presence of sharp apexes on the gonopod in all specimens, he dismissed their classification as Form II. Hart (1953) mentioned Form II *C. similis* and *C. schrenckii* and Form I *C. japonicus* in his examined specimens. The current study supports the occurrence of the Form II gonopod in adult male *C. dauricus*, *C. similis*, and *C. schrenckii*. Building on prior studies and additional evidence presented in the current study, the prevailing opinion is that cyclic dimorphism occurs in adult male *C. dauricus*, *C. similis*, and *C. schrenckii*, while adult male *C. japonicus* only exhibits Form I.

Form alternation absence

The alternation from Form I to Form II in males of the family Cambaridae has been generally accepted and discussed, while some investigations have shown that a complete alternation between Form I and Form II did not necessarily occur (Taylor 1985). Another pattern of dimorphism absence is observed in the family Astacidae, where the gonopod of the adult male consistently remains non-corneous with a membranaceous tip (Hagen 1870; Faxon 1885). Incidentally, Schuster et al. (2022) mentioned that many crayfishes in the southern USA do not strictly obey the rules of form alternation. Their observations in Alabama revealed instances where many crayfishes remained in Form I throughout the year.

The absence of Form II in adult *C. japonicus* may be attributed to its annual molting cycle. Kawai et al. (1994) substantiated that the molting season of *C. japonicus* occurs from June to October, exhibiting a singular peak. In laboratory rearing, Kawai et al. (1995) observed that both male and female adult *Cambaroides* molt only once a year, suggesting an annual molting cycle for *C. japonicus*. *C. japonicus* is primarily restricted to islands, and predominantly found in Hokkaido. The limited and northern distribution range of *C. japonicus* localities may further inhibit the occurrence of a second molting event in

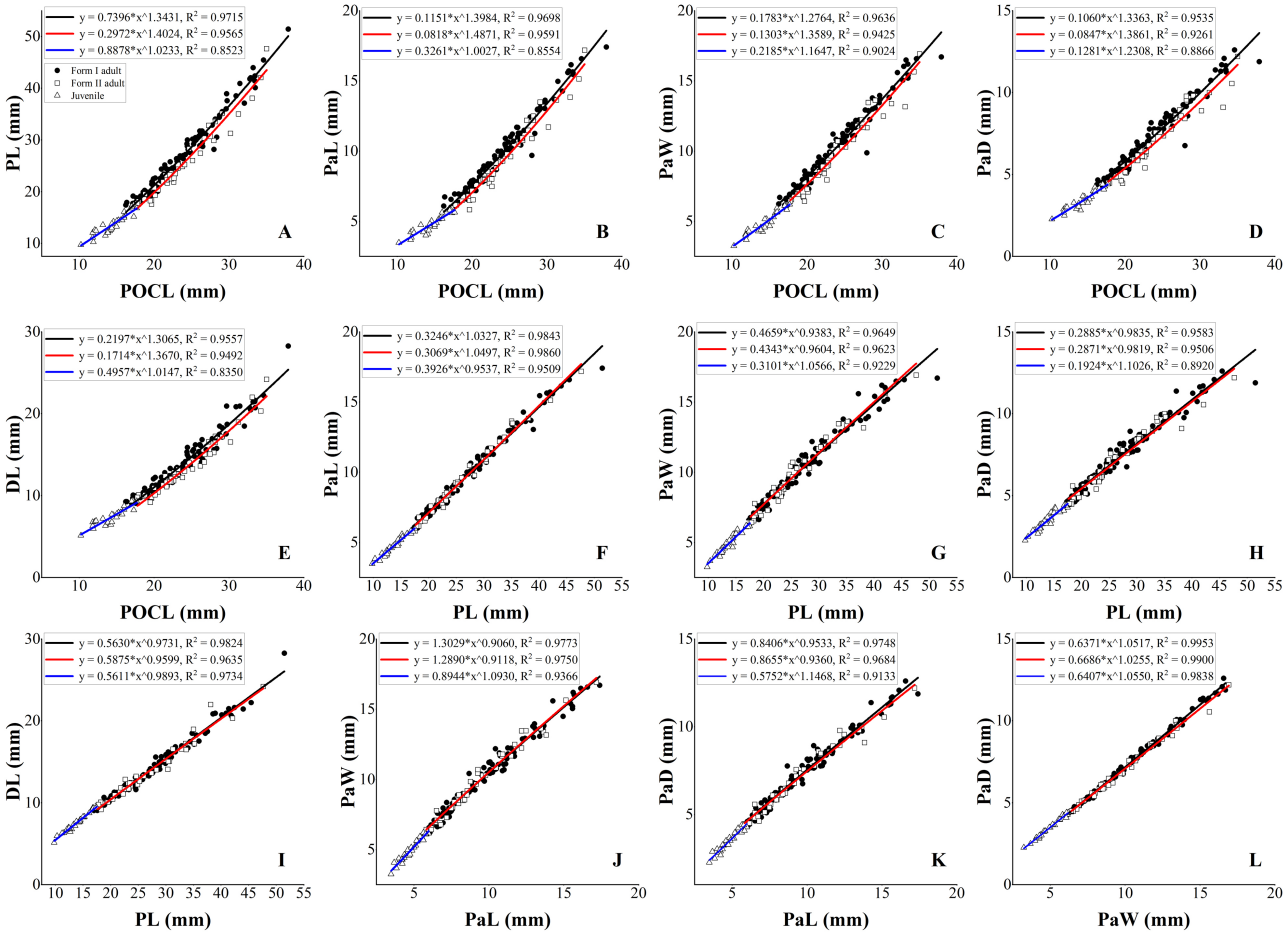

Fig. 7. The association among morphometric characteristics of the chela in *C. dauricus*. The legend conventions remain consistent across all figures, with the solid circles representing the Form I adults, the hollow boxes denoting the Form II adults, and the hollow triangles indicating the Juveniles. Additionally, the black curves correspond to the Form I adults, the red curves to the Form II adults, and the blue curves to the Juveniles.

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Table 2. Pairwise comparison (Tukey test). In the present study, PL/POCL, employed to represent the relative chela length adjusted to the carapace length; PaL/POCL, employed to represent the relative palm length adjusted to the carapace length; PaW/POCL, employed to represent the relative palm width adjusted to the carapace length; PaD/POCL, employed to represent the relative palm depth adjusted to the carapace length; and DL/POCL, employed to represent the relative finger length adjusted to the carapace length, were utilized to demonstrate changes in chela size relative to the entire body. Additionally, PaL/PL, employed to represent the relative palm length adjusted to the chela length; PaW/PL, employed to represent the relative horizontal sectional shape adjusted to the chela length; PaD/PL, employed to represent the relative longitudinal sectional shape adjusted to the chela length; DL/PL, employed to represent the relative finger length adjusted to the chela length; PaW/PaL, employed to represent the relative horizontal sectional shape of the palm; PaD/PaL, employed to represent the relative longitudinal sectional shape of the palm; and PaD/PaW, employed to represent the relative cross-sectional shape of the palm, were utilized to indicate the relative alterations among the various morphological characteristics of the chela

Ratio of the chela's morphometric measurements	Mean		
	Form I	Form II	Juvenile
PL/POCL	1.1238[a]	1.0660[b]	0.9454[c]
PaL/POCL	0.4055[a]	0.3838[b]	0.3287[c]
PaW/POCL	0.4263[a]	0.4059[b]	0.3393[c]
PaD/POCL	0.3061[a]	0.2875[b]	0.2373[c]
DL/POCL	0.5798[a]	0.5502[b]	0.5159[c]
PaL/PL	0.3606[a]	0.3597[a]	0.3479[b]
PaW/PL	0.3810[a]	0.3797[a]	0.3593[b]
PaD/PL	0.2724[a]	0.2696[a]	0.2513[b]
DL/PL	0.5164[a]	0.5169[a]	0.5458[b]
PaW/PaL	1.0596[aa]	1.0538[ab]	1.00334[bc]
PaD/PaL	0.7556[a]	0.7498[a]	0.7226[b]
PaD/PaW	0.7172[a]	0.7077[b]	0.6990[c]

Table 3. Form alternation in male *Cambaroides dauricus* fed in aquariums

ID	POCL when caught (mm)	Form when caught	Date of spring molting	Temperature at spring molting (°C)	Form after spring molting	Date of autumn molting	Form after autumn molting	Temperature at autumn molting (°C)
LF01	24.16	I	2022.06.06	18.5	II	died on 2022.08.06; temperature 24.9		
LF02	19.16	I	2022.06.06	18.5	II	died on 2022.08.05; temperature 24.5		
LF03	19.52	I	2022.06.10	18.5	II	died on 2022.08.05; temperature 25.6		
LF04	19.72	I	2022.06.10	18.7	II	2022.09.11	I	20.4
LF05	26.18	I	2022.06.11	19.0	II	2022.09.11	I	20.4
LF06	20.52	I	2022.06.12	19.3	II	2022.09.13	I	20.5
LF07	19.08	I	2022.06.14	19.2	II	died on 2022.08.28; temperature 17.7		
LF08	34.60	I	2022.06.26	20.8	II	died after spring molting soon		
LF09	16.36	I	2022.07.02	22.6	II	2022.09.14	I	20.7
LF10	21.24	I	2022.07.07	23.0	II	2022.09.14	I	20.7
LF11	23.88	I	died without molting on 2022.07.08; temperature 22.2					
LF12	22.02	I	died without molting on 2022.07.16; temperature 22.6					
LF13	19.92	I	died without molting on 2022.08.03; temperature 25.7					
LF14	28.74	I	died without molting on 2022.08.04; temperature 25.9					
LF15	27.78	I	died without molting on 2022.08.05; temperature 24.5					
LF16	33.48	I	died without molting on 2022.08.08; temperature 24.3					
LF17	20.48	I	died without molting on 2022.08.12; temperature 23.0					

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a year. Conversely, other species of *Cambaroides* predominantly inhabit mainland areas. This broader range might facilitate the possibility for adult males of these species to undergo two moltings per year. The geographic disparities influencing the number of molts are likely associated with climatic factors, particularly the annual frequency of rainy seasons in each respective area. In northeast China, the periods of concentrated precipitation in late spring and late summer nearly coincide with the molting periods of crayfish. Further studies are needed to substantiate the correlation between these factors.

A significant number of male Cambaridae experience mortality after mating (Andrews 1904; Taylor 1985). A similar case of mortality was observed in the present study. During the aquarium feeding period, 70.59% of adult *C. dauricus* individuals either died in Form I or after molting in the summer. Possible reasons for these deaths include senescence, malnutrition, or water overheating. Kawai et al. (1994) reported a water temperature range of 0.8 to 21.4°C at the locality of *C. japonicus*. In this study, field records indicated a water temperature range of 3.4 to 19.2°C. Due to unusually high temperatures in the summer of 2022, the aquarium's highest water temperature reached 25.9°C, significantly surpassing the stream temperature. Some of the deceased crayfish were of a large size. While laboratory feeding may not fully represent crayfish life history stages in natural water, similar occurrences have been noted in other studies. Deventer (1937) described adult *Faxonius propinquus* (= *Cambarus propinquus* Deventer, 1937) that, having finished reproducing, died following molting when attaining maximum size. Tack (1941) reported in *Faxonius immunis* (= *Cambarus immunis* Tack, 1941) that a substantial number of larger Form I males died during the breeding season in August and September. Smith (1953) documented some Form I individuals of *Faxonella clypeata* (= *Orconectes clypeatus* Smith, 1953) that died after the reproductive season without molting. These instances highlight that Form I male crayfish, upon reaching maximum size, may die before or following molting. However, these reports do not negate the existence of Form I to Form II alternation in these species, as all adult males alter from Form I to Form II when normally molting into the non-breeding season. Therefore, these deaths are attributed to crayfish senescence rather than the absence of dimorphism.

Method of confirming the form of the gonopod

Hagen (1870) characterized the Form I gonopod with more acute, long, and separated terminal elements, or profuse setae, while the Form II gonopod had slightly broader and obtuse terminal elements. Faxon (1884) described that in the first form gonopod of *C. dauricus*, the teeth at the tip apex were brown and corneous, and in the second form gonopod of *C. japonicus*, the terminal teeth were smaller and not corneous. Deventer (1937) thought the Form II gonopod "lost the hard texture, sharp points, and peculiar sculpturing." Hobbs (1940) mentioned that identifying the processes in the second form was sometimes difficult. Hart (1953) distinguished Form I and Form II by "the presence of a corneous condition of the terminal elements of the first pleopod" and "the terminal processes of the first pleopod are blunt and never corneous." Jones and Eversole (2011) determined the form based on the shape and color of the cornification of the gonopod: "in Form I males, the tips of the gonopods were corneous, yellow or golden-colored, and blade-like; in Form II males, the tips of the gonopods were bulbous, not cornified, and usually white or cream-colored." From these studies, we can infer that gonopods in Form I and Form II can be distinguished by the shape and color of the terminal elements. Characters with sharp, acute, blade-like, and more colorful terminal elements can be judged to be in the corneous Form I, while those with no cornification, blunt, obtuse, and single-color terminal elements can be judged to be in the non-corneous Form II.

The present investigation identified the Form I adults based on their cornification, which exhibited a golden or brown color, contrasting with the surrounding surface. The cornification covered the tips of the sharp mesial process, centrocaudal process, centrocephalic process, and the blade-like caudal process. Conversely, the Form II adults and the Juveniles were distinguished by the non-sharp mesial process, central projection, and the blunt caudal process, exhibiting the same color as the surrounding surface. However, Kawai and Saito (1999) determined the gonopod form solely by observing the morphology of the terminal elements (three tiny spines), whether sharply acute (corneous) or blunt (non-corneous), without considering the caudal process. In Cambaridae, researchers typically overlook the caudal process when confirming the gonopod form, possibly because some cambarid crayfish always have a specialized caudal process (or caudal knob), or it is simply non-corneous in Form I or absent (Table 4). However, in East Asian crayfishes, the caudal process occupies a substantial portion of the gonopod's tip (Fig. 2), making it conspicuous. While the shape of the three tip elements (the mesial process and central projection) may sometimes be difficult to distinguish between different forms, the color of the cornification and the shape of the caudal process are evidently distinct between the Form I adult and both the Form II adult and the Juvenile.

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Corneous and non-corneous terminal elements of the Form I gonopod in different groups

The gonopod of *Cambaroides* is morphologically generalized, and the gonopod of the family Cambaridae is more differentiated (Hobbs 1942; Hart 1953). This renders the gonopod of *Cambaroides* more original. Within the family Cambaridae, at least six types exist in the gonopods, which are generalized or lacking terminal elements (Hobbs 1940). The cornification covering the terminal elements of the Form I gonopod vary between different groups. With reference to Hobbs (1940 1974), the gonopods of Cambaridae and *Cambaroides* were categorized into five patterns based on whether the Form I terminal element is corneous or non-corneous and present or absent. As listed in table 4, in the subfamily Cambarellinae, exemplified by the genus *Cambarellus*, the cephalic process is absent, the mesial process is non-corneous, the caudal process is either corneous or non-corneous, the central projection is corneous in Form I, and all three terminal elements are non-corneous in Form II (Albaugh and Black 1973). In some groups of the subfamily Cambarinae, exemplified by the genus *Cambarus*, *Lacunicambarus*, and *Creaserinus*, the cephalic process is absent, the central projection and caudal process or caudal knob are corneous, and the mesial process is consistently non-corneous in the Form I gonopod. In their Form II gonopod, the central projection and caudal process are non-corneous, or the caudal knob is not visible (Jones and Eversole 2015; Glon et al. 2020; Johnson et al. 2021). Another group in the subfamily Cambarinae, represented by the genus *Faxonius* and *Orconectes*, features the absence of the cephalic process and caudal process, a corneous central projection, and a non-corneous mesial process in Form I. In Form II, the terminal elements are all non-corneous (Taylor et al. 2016; Fetzner and Taylor 2018). The genus *Procambarus*, the other group of the subfamily Cambarinae, exhibits the most variable terminal elements of the gonopod in the family Cambaridae (Hobbs 1974). In some groups of this genus, such as the subgenus *Scapulicambarus*, *Leconticambarus*, *Austrocambarus*, *Girardiella*, and *Hagenides*, the cephalic process may be present or weak, while in some species like *P. spiculifer*, the cephalic process is absent (Hobbs 1940 1972; Schuster et al. 2015). In *Procambarus*, the central projection and caudal process (if present) are corneous, the mesial process and cephalic process (if present) are non-corneous in Form I, and in Form II, all elements of the gonopod are non-corneous, significantly reduced in size, and exhibit a distinct structure (Reimer 1975; Pedraza-Lara et al. 2021). Contrastingly, in *C. dauricus* and *C. similis*, the cephalic process is absent, and the mesial process, central projection (centrocaudal process, centrocephalic process), and caudal process are all corneous in Form I (Table 4, Fig. 2). In Form II, they are both non-corneous. Consequently, the present study contends that the characteristic "all the terminal elements are corneous in Form I" is an original feature of the gonopod in the genus *Cambaroides*, and the "corneous mesial process in the Form I gonopod" serves as a characteristic for distinguishing the family Cambaroididae from the family Cambaridae.

CONCLUSIONS

In the results, the persistence of Form II gonopods was observed even beyond the smallest adult POCL for both *C. dauricus* and *C. similis*. Additionally, photographs displaying Form I and Form II gonopods of *C. schrenckii* were presented (see Fig. 2, e, f). Furthermore, distinct morphological differences in the chela of *C. dauricus* were identified between Form I and Form II adults. These meticulous observations provide compelling evidence supporting the hypothesis of cyclic dimorphism within these three crayfish species, while it

Table 4. Cornification of terminal elements on the Form I gonopod across various crayfish groups. Use common genus in each family or subfamily as examples

Family (Subfamily)	Genus *e.g.*	Terminal elements of gonopod			
		Mesial process	Cephalic process	Central projection	Caudal process or caudal knob
Cambaroididae	*Cambaroides*	corneous	absent	corneous	corneous
Cambaridae (Cambarellinae)	*Cambarellus*	non-corneous	absent	corneous	non-corneous or corneous
Cambaridae (Cambarinae)	*Cambarus*	non-corneous	absent	corneous	corneous
	Faxonius	non-corneous	absent	corneous	absent
	Procambarus	non-corneous	non-corneous or absent	corneous	corneous or absent

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is noteworthy that adult male *C. japonicus* exclusively manifests Form I.

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