# Postembryonic Development of the Korean Crayfish, Cambaroides similis (Decapoda, Cambaridae) Reared in the Laboratory

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### **ABSTRACT**

The postembryonic development of the Korean crayfish, *Cambaroides similis* (Koelbel, 1892), was described in detail and compared with those of other known species of Astacidae and American Cambaridae, and phylogenetic relationship of the genus *Cambaroides* was discussed. Ovigerous females of the Korean crayfishes appear in May. Durations of juvenile stages 1 and 2 are two and four weeks at  $15.0 \pm 0.5^{\circ}$ C, respectively. From juvenile stage 3, it leaves from its mother. It is considered that *C. similis* is very closely related more to the Astacidae than to the Cambaridae in the characteristics of the maxillule of juvenile stage 1 and the antenna, maxilla, pleopod, and telson of juvenile stage 2.

Key words: Korean crayfish, Cambaridae, Cambaroides similis, Juvenile stage

#### INTRODUCTION

The crayfish in the northern hemisphere (superfamily Astacoidea) includes two families, the Astacidae and the Cambaridae, and both families were distinguished by external features, gill number, and ecological features (Hobbs, 1974). The Far Eastern Asian crayfish of the genus Cambaroides belongs to the family Cambaridae and includes four species: C. dauricus (Pallas,

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1772), C. schrenkii (Kessler, 1874), C. japonicus (De Haan, 1841), and C. similis (Koelbel, 1892) (see Fitzpatrick 1985). Although, the genus Cambaroides belonged to the family Cambaridae, on the basis of the gill number and the ecological features, it could be placed into the Astacidae by Hobbs (1974)'s suggestion. Up to now, the phylogenetic relationship of it is not settled and it has been placed either in Astacidae or Cambaridae. Therefore, some information about the postembryonic development of the Korean crayfish, C. similis, may be important for understanding a taxonomic position of the Cambaroides.

Postembryonic development of the *Cambaroides* has been studied only in Japanese crayfish, *C. japonicus* (see Kurata, 1962; Yamanaka *et al.*, 1997). Little is known about the Korean crayfish, *C. similis*. The only report is Kim (1977) and he reported morphological features, distribution, habitats, and breeding season. But its postembryonic development is not known yet. Recently, abundance of Korean crayfish has been declined, probably as a result of environmental changes and water pollution. At present, the population protection of this species is needed. The aim of this paper is (1) to describe and illustrate the postembryonic development of the *C. similis*, (2) to compare it with those from other known species of Astacidae and Japanese crayfish, *C. japonicus*, and to discuss the phylogenetic relationship of the genus *Cambaroides*.

#### **MATERIALS AND METHODS**

In May 1999, ovigerous crayfishes of *Cambaroides similis* (Koelbel, 1892) were collected in Milyang of the southern part of Korea. A number of ovigerous females (mean post orbital carapace length,  $20.2\pm1.8$  mm) were brought to the laboratory, where egg developmet, juvenile growth, and duration of each stage were monitored. The females were held in environmental chambers in which the photoperiod (12L:12D), air, and water temperatures were controlled. Water temperature during postembyonic development was  $15.0\pm0.5^{\circ}C$ . The development of embryo and juveniles was checked weekly. Some of it were removed for detailed description and preserved in 10% neutral formalin. At least five juveniles in each stage were observed. Its morphology was described and illustrated by using Leitz Laborlux s microscope with a *camera lucida*. The setal terminology used throughout this paper closely follows that of Thomas (1970). Specimens of juveniles and ovigerous females were deposited in Silla University, Republic of Korea.

### **RESULTS**

When the ovigerous crayfishes were collected in May, the size of eggs was  $2.8\pm0.01$  mm. The number of eggs was 48-62 per female. The eggs were very dark brown in colour and attached to the pleopods. The developmental stage was corresponded to the embryonized mysis (zoea) stage, and the heartbeat could be discerned. As the embryo grew, the carapace, antenna, antennule, abdomen, and walking legs were fully developed just before hatching. After hatching, the eyes were prominent and pigmented. The embryo occupied about half of the total egg volume.

### **Postembryonic Development**

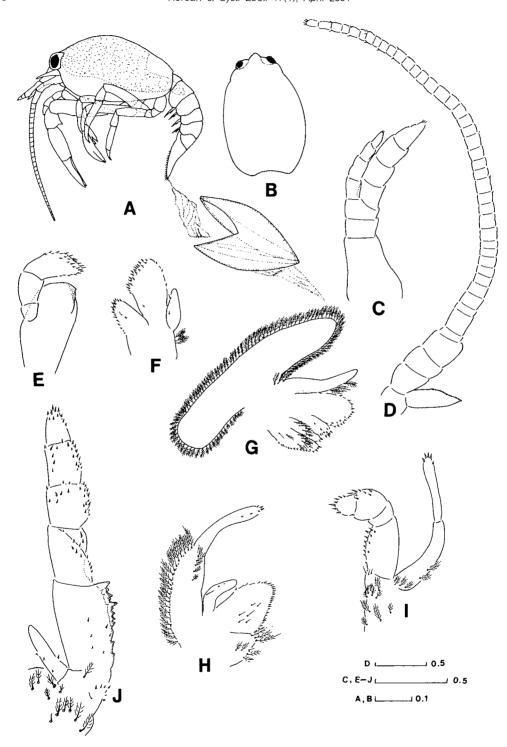
Juvenile stage 1 (Figs. 1, 2)

Hatching involves splitting of egg capsule on dorsal side of embryo, and juvenile emerges with appendages and abdomen. Outer egg capsule splitted usually remains attached to egg stalk. Freshly emerged juvenile remains attached to inner lining of egg capsule by thin thread connected to tip of its telson. Telson thread formed by twisting of inner egg capsule lining, acts as safety line during hatching, thus preventing juvenile from detached away from pleopod (Fig. 1A). Telson thread is usually broken within a few days after hatching. Post-orbital carapace length is  $3.63\pm0.07$  mm. It is not active, has rounded carapace filled with yolk, short down-curved rostrum, and sessile eyes (Fig. 1B). Red pigments are present on lateral and posterior parts of carapace. Antennule (Fig. 1C) has 4 segments in exopodite and endopodite. It has no visible setae except on distal segments, which bear 2 or 3 minute setae. Antenna (Fig. 1D) is bent downward, basal segment has scale-like exopodite which outer margin is slightly serrated, flagellum consists of 39 segments, and its distal segment bears 4 minute setae. Mandible (Fig. 1E) has 3-segmented palp. Its distal segment bears more than 24 setal buds. Maxillule (Fig. 1F) has numerous setal buds on coxal and basal endites. Coxa has 3 plumose setae. Endopod bears minute setae on medial surface and on distal outer margin. Maxilla (Fig. 1G) has bilobed coxal and basal endites. Coxal endite bears numerous setal buds and some plumose setae, and basal endite has numerous setal buds. Endopod has 3 plumose setae on outer margin and on proximal outer margin. Scaphognathite bears more than 100 plumose setae. First maxilliped (Fig. 1H) has coxal endite with 7 plumose setae and simple seta. Basal endite is with numerous setal buds on outer margin and 6 simple setae. Endopod bears simple seta and 2 setal buds. Basal portion of exopod has 22 plumose setae on outer margin and 6 plumose setae on medial surface. Distal portion of exopod is with 6 setal buds. Second maxilliped (Fig. 11) has 4-segmented endopod. Several setal buds are present on segments 1, 3, and 4. Segment 2 is naked. Exopod is 2-segmented. Basal segment has 5 plumose setae proximally. Distal segment has 5 setal buds terminally. Third maxilliped (Fig. 1J) has 5-segmented endopod. Segment 1 bears cutting edge. Segments 1-5 are with several setal buds. Exopod has 4 setal buds.

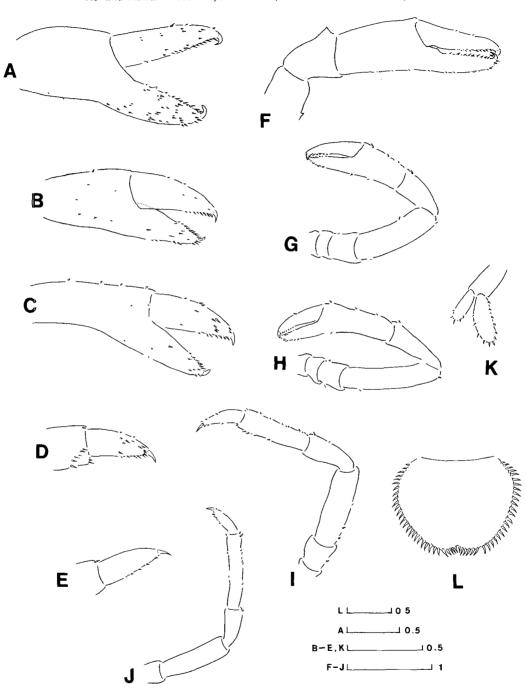
Soon after hatching, juveniles attach to mother's long pleopodal setae (oosetae) by specialized recurved spines on tips of its cheliped (dactylopodite and propodite) (Fig. 2A, F). Second (Fig. 2B, G) and third (Fig. 2C, H) pereopods possess curved spines on tips of dactylopodite and propodite. Biting edges of first three pairs of pereopods bear several setal buds. Fourth (Fig. 2D, I) and fifth (Fig. 2E, J) pereopods have slightly curved spines at apex of dactylopodite. Third pleopod (Fig. 2K) has 5 setal buds on endopod and 12 setal buds on exopod. Tail fan (Fig. 2L) is undifferentiated, with 58 conate setae on its outer margin.

# Juvenile stage 2 (Figs. 3, 4)

After 2 weeks, juvenile molts into juvenile stage 2. Mean post-orbital carapace length was  $4.32 \pm 0.17$  mm. Carapace is more elongated and contains less yolk. Eyes are stalked (Fig. 3A, B). Antennule (Fig. 3C) has 6-segmented endopodite and 4-segmented exopodite. Exopodite is with 2 and 4 aesthetascs on segments 3 and 4, respectively. Antenna (Fig. 3D) has increased in length. Scale-like exopodite is with 17 long plumose setae on outer margin. Flagellum consists of 50 segments, and each segment usually has 2-7 minute setae. Mandible (Fig. 3E) has 23 simple setae

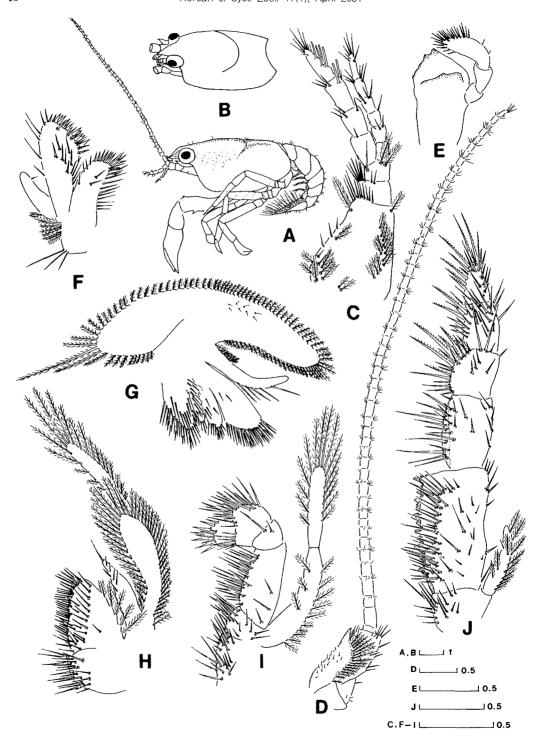


**Fig. 1.** Juvenile stage 1 of *Cambaroides similis*. A, lateral view, egg capsule and telson thread; B, dorsal view of carapace; C, antennule; D, antenna; E, mandible; F, maxillule; G; maxilla; H, first maxilliped; I, second maxilliped; J, third maxilliped. Unit of scale bar is in mm.

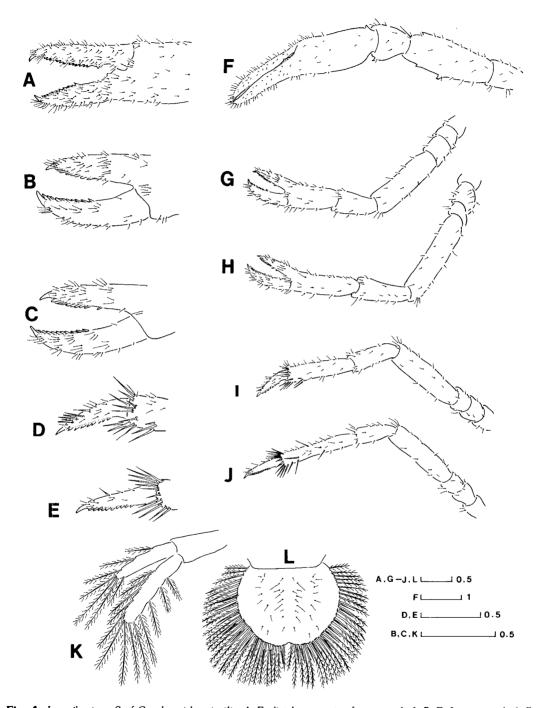


**Fig. 2.** Juvenile stage 1 of Cambaroides similis. A-E, distal segments of pereopods 1-5; F-J, pereopods 1-5; K, third pleopod; L, telson. Unit of scale bar is in mm.

on distal segment of palp. Maxillule (Fig. 3F) has numerous simple and denticulate cuspidate setae on coxal and basal endites. Coxal epipod has 7 plumose and 3 simple setae. Endopod is

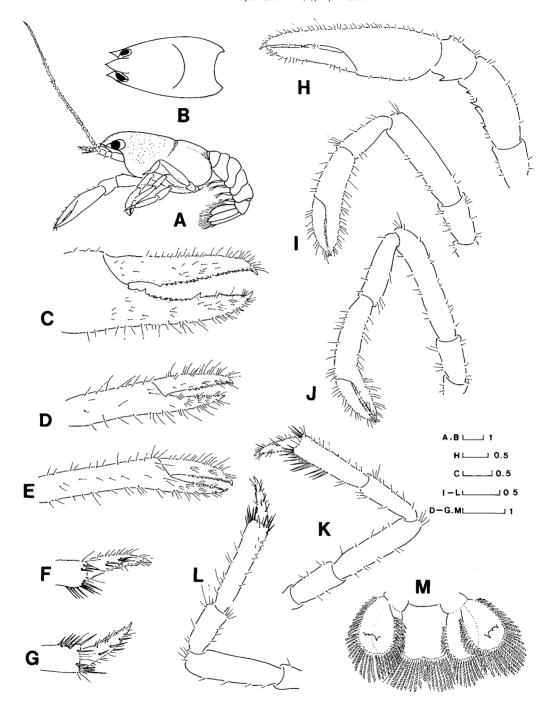


**Fig. 3.** Juvenile stage 2 of *Cambaroides similis*. A, lateral view; B, dorsal view of carapace; C, antennule; D, antenna; E, mandible; F, maxillule; G; maxilla; H, first maxilliped; I, second maxilliped; J, third maxilliped. Unit of scale bar is in mm.



**Fig. 4.** Juvenile stage 2 of *Cambaroides similis*. A-E, distal segments of pereopods 1-5; F-J, pereopods 1-5; K, third pleopod; L, telson. Unit of scale bar is in mm.

unchanged. Maxilla (Fig. 3G) has numerous simple setae on bilobed coxal and basal endites. Endopod has 5 setae on outer margin and 6 plumose setae on proximal outer portion. Four pairs



**Fig. 5.** Juvenile stage 3 of *Cambaroides similis*. A, lateral view; B, dorsal view of carapace; C-G, distal segments of pereopods 1-5; H-L, pereopods 1-5; M, telson and uropod. Unit of scale bar is in mm.

of setae now present on surface of scaphognatite. Its distal part has 2 longer plumose setae. First maxilliped (Fig. 3H) has numerous simple and plumose setae on coxal and basal endites, endopod,

and exopod. Second maxilliped (Fig. 3I) has numerous simple, cuspidate, and serrate setae on endopod. Its segment 2 is now with 1 cuspidate and 1 simple setae. Basal and distal segments of exopod are with 13 and 15 plumose setae, respectively. Third maxilliped (Fig. 3J) has numerous simple, cuspidate, and serrate setae on 5-segmented endopod. Serrate setae are prominent on segments 3-5. Exopod has 12 plumose and 1 simple setae.

Juvenile stage 2 attaches to mother's pleopodal setae by curved spine at terminal of dactylopodite and propodite of cheliped (Fig. 4A, F). Tips of dactylopodite and propodite at second (Fig. 4B, G) and third pereopods (Fig. 4C, H) are equipped with curved spines. Biting edges of first three pairs of pereopods bear several stout toothed setae with 3-5 denticulations. Fourth (Fig. 4D, I) and fifth (Fig. 4E, J) pereopods have slightly curved spines at apex of dactylopodite, which bears several stout toothed setae on ventral margin. Third pleopod (Fig. 4K) has 7 long plumose setae on endopod and 13 long plumose setae on exopod. First pleopod in male is absent. Tail fan (Fig. 4L) shows round shape of large telson with two kinds of setae, about 60 long plumose and 20 acuminate setae, on outer margin and simple shorter setae on surface. Anal thread is not observed.

## Juvenile stage 3 (Fig. 5)

After another 4 weeks, the juvenile molts into stage 3. It is similar to adult (Fig. 5A, B). Cheliped (Fig. 5C, H) has hooklike (curved) spine at end of dactylopodite and propodite. Hooks of all pereopods are less curved or slightly curved than previous stage (Fig. 5C-G, 5H-L). Mean post-orbital carapace length was  $4.77\pm0.23\,\mathrm{mm}$ . Body and all appendages are covered with numerous small setae. Yolk is still present, but its quantity is small. First pair of pleopod in male is still lacking. Uropod is completely developed, same length as telson and having heavily setose edges, exopod of uropod has three spines on ventral side (Fig. 5M). Juvenile is active and leaves permanently from its mother.

### **DISCUSSION**

In Cambaroides similis (Koelbel, 1892) and C. japonicus (De Haan, 1841), the number of egg is within 100, which is much fewer than that of *Procambarus clarkii* (Girard, 1852), an introduced species. Egg size and fecundity of the three species were compared in Table 1.

Embryonic development of *C. similis* is similar to those described for Astacidae, American Cambaridae, and Parastacidae (Wood-Mason, 1876; Rudolph and Rios 1987; see Celada *et al.*, 1991). The early stage of the larva was entirely embryonized mysis (zoea) stage and hatched as juvenile stage 1. Three different types in the characteristics of the telson thread, anal thread, and spines on pereopods have been reported for the attchments of the juveniles to their mothers (Scholtz, 1995). The Cambaridae and Astacidae juveniles of the northern hemisphere use a recurved spine at the tip of chela and curved pereopods to attach to the mother, whereas the Parastacidae juvenile of the southern hemisphere is equipped with the recurved spine on the tips of pereopods 4 and 5 for attachment. It is revealed that the juvenile of *C. similis* has a telson thread and attaches to the mother by using a recurved spine at the tip of chela and curved spines of the pereopods 2 and 3.

The juvenile of *C. similis* has three stages and from the third stage it leaves its mother. According to Table 2, the morphological characteristics of juvenile stage 1 of *C. similis* coincide well with those of the first and second larval stages of *C. japonicus* described by Yamanaka *et al.* 

**Table 1.** Comparisons diameter and number of eggs between *Cambaroides similis*, *Procambarus clarkii*, and *Cambaroides japonicus*.

| Species               | Diameter of egg (mm) | Number of eggs | Authors              |
|-----------------------|----------------------|----------------|----------------------|
| Procambarus clarkii   | 2.0                  | 200-1,000      | Miyake 1973          |
| Cambaroides japonicus | 2.5                  | 30-40          | Yamanaka et al. 1997 |
| Cambaroides japonicus | 2.5                  | 30-100         | Miyake 1973          |
| Cambaroides similis   | 2.8                  | 48-62          | Present study        |

**Table 2.** Morphological characteristics of each postembryonic stages of *Cambaroides japonicus* and *Cambaroides similis*.

|                   | C. japonicus<br>(Yamanaka et al., 1997)                       | C. similis<br>(The present study)   |  |  |
|-------------------|---|---|--|--|
| Stage 1           | as Ist larval stage   | as juvenile stage 1   |  |  |
| Rostrum           | small, rounded at tip, bent downward, without setae           | small, down-curved, without setae   |  |  |
| Pleopod           | biramous with setal buds                                      | biramous with setal buds  |  |  |
| Telson            | a round shape, with conate setae on its outer margin          | a round shape, with conate setae on its outer margin  |  |  |
| Duration          | 2-3 days  | 2 weeks   |  |  |
| Stage 2           | as 2nd larval stage   | as juvenile stage 2   |  |  |
| Rostrum           | slightly developed, without setae                             | with a triangular tip with setae  |  |  |
| Pleopod           | biramous with setal buds                                      | with long plumose setae   |  |  |
| Telson            | a round shape, with conate setae on its outer margin          | a round shape, with marginal plumose and acuminate setae                                    |  |  |
| Duration          | 2-3 days  | 4 weeks   |  |  |
| Stage 3           | as 3rd larval stage   | as juvenile stage 3   |  |  |
| Rostrum           | with a triangular tip with setae                              | developed with more extent, broad   |  |  |
| Pleopod           | with long plumose setae                                       | with long plumose setae   |  |  |
| Telson and uropod | a round shape, with marginal plumose and acuminate setae      | uropod is completely developed, same<br>length as telson and having heavily<br>setose edges |  |  |
| Duration          | 10-14 days  |   |  |  |
| Stage 4           | as juvenile stage 1   |   |  |  |
| Rostrum           | developed with more extent, broad                             |   |  |  |
| Pleopod           | with long plumose setae                                       |   |  |  |
| Telson and uropod | five palms by telson and uropods, with marginal plumose setae |   |  |  |

(1997). Also, its juvenile stages 2 and 3 corresponds to the third larval stage and juvenile stage 1 of *C. japonicus*, respectively. In Kurata (1962)'s brief description on *C. japonicus*, juvenile stage 3 was essentially adult in the external morphology. Considering that crayfishes of Cambaridae, Astacidae, and Parastacidae, all have three stages before they leave their mother (Andrew, 1907; Hopkins, 1967; Suter, 1977; Price and Payne, 1984; Hamr, 1992; Rudolph and Iracabal, 1994), the four stages (three larvae and one juvenile) of *C. japonicus* are questionable. Except in number of postembryonic stage, it is recognized that the characteristic of juveniles *C. similis* almost resembles to those of *C. japonicus*. They show small rostrum, short antenna, specialized recurved spine on tip of the cheliped, the telson with conate setae on outer margin, and no anal thread (stage 1).

Morphology of juvenile crayfish has been described in some species of the families Astacidae (see Andrews, 1907), Cambaridae (see Price and Payne, 1984) and Parastacidae (see Suter, 1977). An elongated telson with small number of plumose setae in juvenile stage 2, an anal thread in juvenile stage 2, and erected the first pleopod of male in juvenile stage 3 are apomorphy among the

**Table 3.** Comparisons of the juvenile stages of Astacus leniusculus, Cambarus affinis, and Cambaroides similis.

|                | Family Astacidae  | Family Cambaridae  |   |
|----------------|---|--|---|
|                | Astacus leniusculus<br>(Andrew, 1907)                               | Cambarus affinis<br>(Andrew, 1907)                                   | Cambaroides similis<br>(The present study)                          |
| Juvenile stage | 1   |  |   |
| Antennule      | endopodite and exopodite both with 5 segments                       | endopodite and exopodite both with 4 segments                        | endopodite and exopodite both with 4 segments                       |
| Antenna        | flagellum with 50 segments  | flagellum with 25 segments   | flagellum with 39 segments  |
| Maxillule      | coxal epipod with 3 plumose setae                                   | coxal epipod without setae   | coxal epipod with 3 plumose setae                                   |
| Telson         | 66 marginal conate setae  | 26 marginal conate setae   | 58 marginal conate setae  |
| Juvenile stage | 2   |  |   |
| Antennule      | endopodite and exopodite<br>both with 5 segments                    | endopodite and exopodite<br>each with 4, 5 segments,<br>respectively | endopodite and exopodite each with 6, 4 segments, respectively      |
| Antenna        | flagellum with 54 segments,<br>scale with marginal<br>plumose setae | flagellum with 39 segments, scale serrated                           | flagellum with 50 segments,<br>scale with marginal<br>plumose setae |
| Maxilla        | scaphognathite with 1 longer plumose seta on distal part            | scaphognathite without<br>longer setae on distal part                | scaphognathite with 2 longer plumose setae on distal part           |
| Pleopod        | with well-developed plumose setae                                   | with setal buds  | with well-developed plumose setae                                   |
| Telson         | about 46 marginal plumose and 20 acuminate setae                    | about 30 marginal conate setae                                       | about 60 marginal plumose and 20 acuminate setae                    |

American Cambaridae. Whereas, a round telson with numerous plumose setae marginally in juvenile stage 2, no anal thread in juvenile stage 2, and less erected first pleopod of male in juvenile stage 3 are commonly observed among the Astacidae members. Several postembryonic characteristic of *C. similis* more resembles that described for representative of the Astacidae and all these differ from the corresponding characteristic of American Cambaridae. Moreover, in the characteristic of the maxillule of juvenile stage 1 and the antenna, maxilla, and pleopod of juvenile stage 2, it is considered that *C. similis* is very closely related to the Astacidae than to the Cambaridae (Table 3). Consequently, the morphological characteristic of juveniles of *C. similis* does not support the recent phylogenetic relationship of the genus *Cambaroides* with the Cambaridae. In order to make a better understanding of phylogenetic relationship of *Cambaroides*, various taxonomic studies should be necessary.

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### 한국산 가재 Cambaroides similis (십각목, 가재과)의 후기배발생

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#### 요 약

한국산 가재(Cambaroides similis)의 후기배발생과정을 상세히 기재하고, Astacidae와 미국산 가재과(Cambaridae)에서 후기배발생과정이 알려진 종들과 아시아산 가재속(Cambaroides)의 종들에서, 그 계통적 유연관계를 비교 조사하였다. 한국산 가재의 포란한 암컷은 5월에 출현하였다. 제1,2 어린시기는 15.0±0.5°C에서 각기 2주일과 4주일이 소요되었다. 제3 어린시기에는 모체로 부터 이탈하였다. 제1어린시기의 제1소악과 제2어린시기의 제2촉각, 제2소악, 복지, 그리고 미지에서 그 형태적 특징들이 가재과(Cambaridae)보다 Astacidae와 매우 유사함을 알 수 있었다.