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Use of mandibular tusks as weapons in the aggressive behavior of the burrowing mayfly *Rhoenanthus coreanus* (Yoon and Bae, 1985) (Ephemeroptera: Potamanthidae)

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**ABSTRACT**

In larvae of burrowing mayflies (Ephemeroidea), mandibular tusks are generally associated with burrowing and/or feeding. This study reports, for the first time, the use of mandibular tusks as weapons in the aggressive behavior of the burrowing mayfly *Rhoenanthus coreanus* (Yoon and Bae, 1985) (Potamanthidae). The larvae used their tusks as weapons in combat behaviors such as tusking, which was observed in 60.81% and 62.98% of the studied males and females, respectively. Most cases involved combat by tusking between females (70.45%), followed by combat between males (21.60%), and combat between male and female individuals (3.97%). Aggressive behavior was more common in female larvae with longer mandibular tusks. Positive relationships were observed between the number of cases of aggressive behavior (i.e., tusking) and the size of mandibular tusks in both male and female larvae \((p < 0.001)\). Female larvae with large mandibular tusks \((5.00 \pm 0.50 \text{ mm})\) could use their tusks to lift their opponents in a manner similar to horned beetles. Contests typically occurred as a result of territorial conflict and served as a means to find or retain occupied shelters for feeding and hiding.

**Introduction**

Aggressive behavior is often critical for animal success and survival. By exhibiting aggressive behavior, animals can access resources, compete for mates, defend, or expand territories, and defend both themselves and their offspring (Hardy and Briffa 2013; Rillich and Stevenson 2019; Sherer and Certel 2019). However, aggressive behavior can also be expensive with regard to energy and metabolism, especially in animals that develop exaggerated structures (e.g., horns, fangs and tusks that can
be used as weapons) (Palestrini, Rolando, and Laiolo 2000; Altuzar-Molina and Aluja 2016; Rillich and Stevenson 2019). In general, larger and more aggressive individuals are considered more successful than smaller and less aggressive ones with regard to both territory acquisition and fitness (Brown, Smith, Moskalik, and Gabriel 2006; Whitman 2008). However, a large body size can also incur costs, such as increased rates of energy use, predation, injury, and even mortality (Blanckenhorn 2000; Whitman and Vincent 2008; Rillich and Stevenson 2019).

In mayflies, the occurrence of aggressive behavior has rarely been reported and has not been thoroughly studied or documented. Additionally, most reports of aggressive behavior in mayflies have been associated with reproductive competition in adults or larvae under high-density conditions (Corkum 1978; Harker 1992; Peckarsky, McIntosh, Caudill, and Dahl 2002; Grether 2019). Interestingly, larvae of burrowing mayflies (Ephemeroptera: Ephemeroidea) possess mandibular tusks, which are outgrowths of the mandibles (Bae and McCafferty 1993). In 1993, Bae and McCafferty published a comprehensive study of the functions and evolutionary trends of mandibular tusks in the superfamily Ephemeroidea, stating that mandibular tusks are primarily used for burrowing and feeding, as reported in previous studies (Keltner and McCafferty 1986; Bae and McCafferty 1991; McCafferty and Bae 1992).

Among burrowing mayflies, the family Potamanthidae, a basal lineage of Ephemeroidea, represents a functional group of interstitial burrowers in terms of the burrowing behavior of the larvae (McCafferty 1991; Bae and McCafferty 1993). The potamanthid mayfly *Rhoenanthus coreanus* (Yoon and Bae, 1985) is distributed across Northeast Asia, including the Korean Peninsula, northeastern China, and the Russian Far East. The larvae of *R. coreanus* are commonly found in lowland streams and rivers and prefer mixed substrates of sand and silt embedded with large stones (Bae and McCafferty 1991; Lee, Bae, and Yoon 1999; Figure 1). As no additional studies have comprehensively evaluated the functions of mandibular tusks in burrowing mayflies, the present study aimed to observe and describe the use of exaggerated mandibular tusks in *R. coreanus*, which are associated with aggressive behavior in this species.

**Material and methods**

**Larval sampling and rearing**

Larvae of *R. coreanus* were collected from three different sites in South Korea (Hongcheon River: 37°45'37.6"N, 127°57'23.7"E, 14.VI.2021; Jojong Stream: 37°44'03.1"N, 127°24'51.3"E, 08.IV.2022 and 12.V.2022; Seomjin River: 35°11'35"N, 127°22'37"E, 3.V.2021) from late spring to early summer in 2021 and 2022. The sediment and water from the collection sites were enriched with organic matter (Figure 1). Water, sediment, and stones from the collection sites were collected together with the larvae and transported to the laboratory in an effort to maintain natural conditions and food sources for the live larvae (Calvin 1967; McCafferty and Bae 1992).

In the laboratory, approximately 50 larvae from each experimental site were transferred to transparent aquariums (length × width × height: 25 × 15 × 15 cm) and
acclimated to laboratory conditions for 48 h with water (20°C), substrate (mixed sand and silt), and stones from the collection sites (Figure 2). The aquariums were co-located inside a larger transparent glass aquarium (60 × 30 × 30 cm; Figure 2) that contained fresh, clean water at a temperature of 18–21°C, which is recommended for the rearing of this species (Lee, Bae, and Yoon 1999). Each aquarium contained ∼50 larvae and eight stones (three large and five medium/small) on a substrate of mixed sand and silt to provide microhabitats similar to those found in the natural habitat of the larvae.

**Behavioral observation**

Behavioral observations were performed in the laboratory within a period of 2–4 wk for the larvae collected from each of the three sampling sites, and only fully
matured active larvae including last-instar larvae were used for observation. No larvae were used repeatedly for observation. The larvae were sexed on the basis of external morphology (e.g., eye size and genitalia), and pronotum width (PW) was used as a reference for total body size to the nearest 0.01 mm (Mayorga and Bae 2021; Figure 3).

In the laboratory, behavioral interactions between the same or different sexes were observed using a cellphone camera with a macro lens (iPhone X Camera with 0.45× Super Wide-Angle Lens, 12.5× Macro Lens, and 20× Macro Lens; Criacr, Shenzhen Amier Technology Co., Ltd., USA) (Figure 2). More detailed observations of larval morphology and behavior were conducted using a stereomicroscope (SZ51; Olympus, Tokyo, Japan). For single larval behavioral observations, one or two larvae were placed in a small circular transparent container (10 × 5 cm) that held sediment (2.5 cm depth) and one small round stone (4.7 × 2.8 cm) from one of the collection sites. Individual larvae were observed for approximately 30 min. All observations were recorded on video using the cellphone.

Figure 2. The scheme of aquarium setup used to rear and observe *Rhoenanthus coreanus* (Yoon and Bae, 1985) larvae: (A) main aquarium containing clean, fresh water; (B, C) small aquariums larvae.

Figure 3. Average-sized *Rhoenanthus coreanus* (Yoon and Bae, 1985) specimens: (A) male; (B) female. Scale bar = 1 mm. PW: Pronotum width.
camera, as indicated above, for detailed analysis under slow-motion playback (0.25, 0.50 and 0.75× speeds).

Statistical analyses were performed to compare significant differences in body size, length of the mandibular tusks, and aggressive behaviors between males and females using R Studio (R Development Core Team 2017), as described by Mayorga and Bae (2021).

**Results**

**Larval observation**

The 282 larvae collected in the present study included 74 males and 208 females, and with females being larger than males (a mean body size (PL) for males: 2.48 ± 0.42 mm; females: 3.58 ± 0.74 mm; \( t = p < 0.001 \); Table 1).

We observed that the larvae that occupied their shelters always clung to the stones, with their dorsal surfaces facing downward, used their middle and hind legs to cling to the stones, and usually only allowed their head and forelegs to remain visible outside the shelters (Figure 4). In the present study, ‘shelter’ is used to refer to the area created by the larvae by excavating and sweeping sediment in an interstitial space under the stones (Figure 4).

<table>
<thead>
<tr>
<th></th>
<th>Mean PW</th>
<th>CV%</th>
<th>SD</th>
<th>n</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2.48</td>
<td>25.32</td>
<td>0.42</td>
<td>74</td>
<td>11.64*</td>
</tr>
<tr>
<td>Female</td>
<td>3.58</td>
<td>21.14</td>
<td>0.74</td>
<td>208</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CV%, coefficient of variation; SD, standard deviation; n, number of individuals; \( t \), t-test; *= \( p < 0.001 \).
Aggressive behavior

Two main tusk-using behaviors (tusking and lifting) have been recognized as aggressive behaviors in combat (Figure 5), and a no-tusk-using behavior (tailing) has also been observed in larvae with aggressive behavior.

Tusking

Tusking behavior was observed in 60.81% of the collected male individuals ($n = 74$) and 62.98% of female individuals ($n = 208$), with 21.60%, 70.45% and 3.97% of all tusking instances being observed in male vs. male, female vs. female, and male vs. female interactions, respectively. During tusking, mandibular tusks were used directly as weapons in combat between larvae, especially during apparent territorial conflict when outsider larvae attempted to enter the shelter of other larvae (Figure 4, Supplementary video 1).

The observed instances of combat varied in both duration (several to 90 s) and sequence of motions (Figure 5). The larvae initially confronted each other using their forelegs to slap, embrace, and push each other (Figure 5), and if the confrontation persisted, they would often tusk each other (Figure 5). Dominant larvae, which exhibited more aggressive behavior and were mostly victorious in combat, held their rivals by the exterior-lateral and basal parts of the rival’s tusks. Thus, the dominant larvae controlled their rivals by using their own tusks to press their opponents’ tusks. Because mandibular tusks are moveable, they can be used as forceps while the larvae move forward and backward using their forelegs (Figure 5). The matches concluded when a less-dominant larva left, when an opponent larva was cornered, or when an outsider larva evicted a resident larva from its shelter (Supplementary video 1). Smaller larvae appeared to avoid combat when opponents were clearly larger (display recognition). In addition, combat matches sometimes
resulted in visible tusk breakage ($n = 6$: 2 males and 4 females) due to the strength with which the tusks were used during severe combat (Figure 2A).

During combat, larvae with larger bodies and longer tusks were more dominant and won contests. Additionally, there were positive relationships between the number of cases of aggressive behavior (i.e., tusking) and the size of mandibular tusks in both male and female *Rhoenanthus coreanus* (Yoon and Bae, 1985) larvae ($p < 0.001$; Figure 6). In other words, the longer the mandibular tusks of the individuals, the higher are the cases of tusking. The number of tusking cases was significantly different between males and females (higher in females; $p < 0.001$) (Figure 6).

**Lifting**

In a few extraordinary cases (2.40% of total observed females, and 3.82% of females that displayed tusking behavior; Figure 6, 7), dominant female larvae briefly lifted their opponents upward using only their mandibular tusks, a behavior similar to that observed in combat matches between male horned beetles (Figure 5). In this situation, dominant larvae (lifter larvae) held their bodies on the surface of stones using their fore, middle and hind legs, and then lifted their opponents (lifted larvae) by holding the outer-lateral and basal parts of their opponents’ tusks (Figure 5). During lifting, lifter larvae repeated lifting behavior, and the contest ended with the expelling of the lifted larvae. Lifting was only performed by females bearing large tusks (5.00 ± 0.50 mm) (Figure 6).
Tailing

Tailing was observed when neighboring larvae stole detrital particles from the gills and caudal filaments of other larvae. In some cases, the robbed larvae reacted using the last abdominal segments and caudal filaments, such as a tail, to strike and drive the attacker away (Supplementary video 2). In other cases, the robbed larvae turned around to confront the attacking larvae with their forelegs and tusks. Tailing behavior was observed in both male (13.75% of total observed males) and female (10.42% of total observed females) larvae (Figure 7).

Discussion

In the present study, female larvae, which were larger and more aggressive, appeared to have better survivorship, as indicated by the higher proportion of female individuals at all three study sites (Table 1). This contrasts with previous studies on mature insects, which have generally reported that males are more aggressive (Rillich and Stevenson 2019). Previously, an allometry study on the mandibular tusks of *R. coreanus* found a positive allometry of the tusks only in females, thereby indicating that tusks in females exhibit disproportionately higher rates of growth compared to males (Mayorga and Bae 2021). Thus, this might explain that in this study female individuals with longer mandibular tusks were active competitors, and consequently more aggressive and superior in number than males (Table 1, Figure 6). Therefore, the findings of the present study support those of previous studies in which insect body size and the size of weapon is related to territorial success and fitness (Figure 6) (Zamudio, Huey, and Crill 1995; Peckarsky et al. 2002; Hsu, Earley, and Wolf 2006).

The aggressiveness of larger larvae could be a strategy for avoiding detection by predators by finding the best shelters and meeting their high-energy requirements (Blanckenhorn 2000). However, being large and aggressive can also have negative effects, such as increased mortality rates owing to combat and vertebrate predation (Whitman and Vincent 2008). In *R. coreanus*, this might explain the ‘necessity’ of...
larvae to hide in their shelters. Consequently, the aggressive behavior observed in the larvae of this species (e.g., tusking or even lifting) might be a form of intra-specific competition to defend or find new shelters, as observed in other insects at juvenile stage (Corkum 1978; Grether 2019) (Figure 4).

In addition to their taxonomic study, Gonçalves, Takiya, Salles, Peters, and Nessimian (2017) provided a supplementary video that showed two burrowing mayfly larvae of the genus *Camyplocia* Needham and Murphy, 1924 (Ephemeroidea: Euthyplociidae) from Amazonas State exhibiting aggressive behavior and, in particular, using their tusks and forelegs to fight each other. Although the video was relatively brief, the behavior was similar to that exhibited by *R. coreanus*, and both species bear both long forelegs and elongated mandibular tusks. In the video provided by Gonçalves, Takiya, Salles, Peters, and Nessimian (2017), the dominant larvae can be observed holding and pinching their opponent’s mandibular tusks at the outer-lateral and basal areas, similar to the dominant *R. coreanus* larvae (Figure 5, Supplementary video 1). Moreover, the *Campylocia demoulini* Gonçalves and Salles, 2017 larvae pushed their opponents using their forelegs, similar to the *R. coreanus* larvae.

Aggressive behavior has also been reported in smaller *Potamanthus* sp. larvae (Potamanthidae), which lack exaggerated mandibular tusks and dwell in a micro-habitat similar to that of *R. coreanus* (Mayorga, personal observation). *Potamanthus* sp. did not display tusking behavior, but instead displayed tailing behavior (Mayorga, personal observation).

Mayorga and Bae (2021) in their study on *R. coreanus* reported positive correlations between relative tusk length and relative forefemur and foretibia length in both males and females (Mayorga and Bae 2021). The present study provides evidence of their functionality and how both structures are closely related to the aggressive behavior of this species (Figure 5, 6). Also, a previous study of sheet web spiders observed a positive correlation between forelegs and exaggerated chelicerae, which are used in male-male contest behavior (McCambridge, Painting, Walker, and Holwell 2019).

*Rhoenanthus coreanus*, which belongs to the most basal lineage of the Potamanthidae (Bae and McCafferty 1991), is thought to represent the ancestral morphology of Ephemeroidea, as *Potamanthus* is also a basal lineage of Ephemeroidea (McCafferty 1991; Kluge 2004; Ogden, Breinholt, Bubee, Miller, Sartori, Shiozawa, and Whiting 2019). Therefore, the observations presented here could provide additional insight into the evolution of mandibular tusks in burrowing mayflies, which have previously been explained as burrowing and/or feeding structures (Bae and McCafferty 1993).

**Conclusions**

The current lack of direct observations and documentation regarding the use and function of mandibular tusks in Ephemeroidea pose challenges to the development of a comprehensive comparative phylogeny of Ephemeroidea and the evolution of mandibular tusks. This study provides a new perspective on the use of mandibular tusks as weapons, without an association with direct fitness and sexual
selection, but rather with survivorship and territoriality during the larval stage. In contrast to studies of other animals that bear exaggerated structures, this study shows that the female larvae of the burrowing mayfly *R. coreanus* are most likely to exhibit aggressive behavior, apparently due to their large body size. In conclusion, the present study provides insight into the multifunctionality of mandibular tusks of Ephemeroidea, which serve as burrowing and feeding structures as well as weapons.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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