Mandibular morphology and dietary preferences in two pygmy mole crickets of the genus *Xya* (Orthoptera: Tridactylidae)

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Abstract: Pygmy mole crickets (Tridactylidae) are an interesting but little-known group of Orthoptera; their food biology and dietary preferences have not been adequately studied. Our study focused on the diet composition and associated functional morphology of the mandibles in two European species: *Xya pfaendleri* Harz, 1970 and *X. variegata* (Latreille, 1809). Based on postmortem gut content analyses, the studied species feed mainly on detritus (decaying organic particles), representing 91% and 97% of the diet in *X. pfaendleri* and *X. variegata*, respectively. The mandibular structures are associated with the processing of such food. The incisor area is equipped with three robust and sharp teeth, and the molar area consists of a small molar ridge forming a triturative area with parallel molar slats. The size of the molar ridge is determined by sex and the side (left/right) of the mandibles: females have larger molar ridges than males and left molar ridges are larger than the right. Food preferences as well as measured parameters of the mandibles were similar in both studied species due to the similar pattern of their biology. The narrow food niche indicates a low diversity of consumed food and uniformity of food intake.

Key words: Detritophagy, detritovory, food biology, mouthparts, exoskeleton

1. Introduction
Although the food strategies of many Orthoptera species are relatively well known, the food strategy in certain species has only been estimated based on the structure of the mouthparts, habitat preferences, or feeding behavior observed in only a few individuals. Species of the suborder Caelifera are mostly herbivorous (Acridomorpha) and only rarely detrito-/bryophagous (Tetrigoiidea). Species of Tridactylidae (pygmy mole crickets) are probably omnivorous, although detailed analyses of their food preferences have not been performed. The consumption of a specific diet is associated with the shape and structure of the mandibles; four types of mandibles (graminivorous, forbivorous, herbivorous, and bryovorous types) have been identified in orthopterans (Isely, 1944; Kauffmann, 1965; Uvarov, 1977; Aguirre et al., 1987; Smith and Capinera, 2005). A correlation between mouthpart structure and the specific diet of orthopterans has been reported for only a few species, i.e. *Locusta migratoria* (L., 1758); *Schistocerca gregaria* (Forskal, 1775); *Melanoplus desultorius* Rehn, 1907; *Trimerotropis pallidipennis* (Burmeister, 1838); *Opeia obscura* (Thomas, 1872); and *Tetrix tenuicornis* (Sahlberg, 1891) (Gangwere, 1960, 1961, 1965; Patterson, 1983, 1984; Kang et al., 1999; Kuřavová et al., 2014).

The food biology and dietary preferences of pygmy mole crickets (Caelifera: Tridactylidae) are poorly understood due to their small body size (up to 5 mm) and hidden way of life. Pygmy mole crickets build horizontal galleries beneath the surface of wet damp substrates, usually on the banks of ponds or rivers, and they are considered to be algae-feeders or omnivorous insects (Blackith, 1987; Deyrup and Eisner, 1996).

Our study focused on determining whether the pygmy mole crickets *Xya pfaendleri* Harz, 1970 and *X. variegata* (Latreille, 1809) are dietary specialists or omnivorous detritus feeders. This study was based on analyses of postmortem gut contents and the mandibular structures associated with food processing. The aims of this study were to describe and compare the structure of the mandibles in the two pygmy mole crickets, evaluate food preferences of pygmy mole crickets, and assess interspecific differences in these highly similar and related species.

2. Materials and methods

2.1. Study organisms
The pygmy mole crickets *Xya pfaendleri* and *Xya variegata* (Orthoptera: Tridactylidae) are small (4–6 mm total body size)
length), shiny, dark or black and cryptic-colored insects. They are characterized by stout and enlarged femurs of the hind legs that are adapted for leaping. They can jump as far as 1 m (Burrows and Picker, 2010; Burrows and Sutton, 2012). Pygmy mole crickets are riparian and thermo-hygrophilous, and they have a Ponto-Mediterranean distribution (Zechner et al., 1999; Berg et al., 2000; Kočárek et al., 2005; Baur et al., 2006; Münsch et al., 2013). They live in shallow burrows, which they build in sandy soil alongside bodies of water, including streams, ponds, or sand quarries with permanent or temporal water (Blackith and Blackith, 1979; Münsch et al., 2013).

2.2. Collection and treatment of samples
Specimens of *Xya pfaendleri* and *X. variegata* were collected from two localities in the southeastern part of the Czech Republic. Specimens of *X. pfaendleri* were collected on 12 August 2013 in a littoral zone (sand substrate) of a small pond (~25 m²) in the area surrounding Lanžhot (48°40′34″N, 16°55′25″E) at approximately 200 m a.s.l. Specimens of *X. variegata* were collected on 4 June 2013 at a sand quarry near Múšlov near Mikulov (48°47′30″N, 16°41′11″E). This locality has an area of 20 m² and an elevation of 250 m a.s.l. The specimens were collected by sweeping and were immediately stored in 70% alcohol for later analysis of the alimentary tract contents and morphology of the mandibles. A total of 24 adult specimens (6 males and 6 females of *X. pfaendleri*, 6 males and 6 females of *X. variegata*) were used for the analysis.

In the laboratory, both mandibles of each specimen were dissected, examined, and documented by scanning electron microscopy (SEM; JEOL JSM-6610LV, Tokyo, Japan) (SEI, 12 kV, WD 41 mm, SS30, 130–1500× magnification). The mandibles were sputter-coated with gold prior to SEM (Automatic Sputter Coater: JEOL JFC-1300, Tokyo, Japan). The parameters of both mandibles (length of the mandible, determined as the distance between the mandibular junction and the peak of the first incisor; surface of the molar ridge; number of slats) were measured using the software SEM JEOL/E0 version 1.0.

The alimentary tract contents of each specimen were permanently mounted in Hoyer’s solution (Anderson, 1954) on a glass microscope slide. The slides were observed using a light microscope (Olympus CX41RF, Hamburg, Germany) at 400× magnification and were then photographed using a camera (Canon EOS 1100D, Tokyo, Japan) mounted on the microscope. The entire alimentary tract preparation from each specimen was photographed. These photographs were evaluated using BaDra 2.0 image analysis software (BaDra, 2011). The software was used to determine the proportions of food components (proportion of detritus + plant cellular matter + other particles = 1):

\[
\text{Proportions of diet} = \sum p_i \quad \% \quad (1)
\]

where \( p_i \) is the proportion in the alimentary tract content, and \( i \) is the type of diet component as either detritus (unidentified decaying organic particles), plant cellular matter (lower plants, algae), or other particles (fragments of arthropod cuticles, mineral particles).

The proportions of the components of the diet were evaluated and were used to calculate the food niche breadth based on the Gini–Simpson index (Gini, 1912, in Di Russo et al., 2014):

\[
\text{Gini–Simpson index} = 1 - \sum p_i^2 \quad (2)
\]

where \( p_i \) is proportion of the \( i \)th component in the alimentary tract content.

2.3. Statistical analysis
The measured parameters of the mandibles (distance between the mandibular junction and the first incisor, surface of the molar ridges, and number of molar slats) are presented as the means ± SD (Table 1). According to the

### Table 1. Parameters measured on the left and right mandibles of *Xya pfaendleri* and *Xya variegata*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Left mandible</th>
<th>Right mandible</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>X. pfaendleri</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>506.9 ± 34.9</td>
<td>8767 ± 703</td>
</tr>
<tr>
<td>Female</td>
<td>676.0 ± 25.9</td>
<td>16204 ± 678</td>
</tr>
<tr>
<td><em>X. variegata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>570.9 ± 0.7</td>
<td>14180 ± 346</td>
</tr>
<tr>
<td>Female</td>
<td>714.7 ± 29.3</td>
<td>21804 ± 1153</td>
</tr>
</tbody>
</table>

A total of 24 specimens of *X. pfaendleri* and *X. variegata* were measured. The values (means ± SD) are in micrometers.
Shapiro–Wilk normality test, all data were not normally distributed. The effects of the side of the mandible, sex, and species on the measured parameters of the mandibles of *X. pfaendleri* and *X. variegata* were evaluated using general linear models (gamma distribution function). Analysis of variance (ANOVA) was used to determine the effects of the left/right mandible, sex, and species on the measured parameters of the mandibles of *X. pfaendleri* and *X. variegata*.

The alimentary data were evaluated using general linear models (Gaussian distribution function). The effects of sex, species, and the interaction of sex versus species on the proportion of food components in the alimentary tracts were tested using multifactorial ANOVA (general linear model with a Gaussian distribution function). The effects of sex, species, and the interaction of sex versus species on the food niche breadth were tested using multifactorial ANOVA (general linear model with a Gaussian distribution function). All analyses were performed using the R Ver. 2 statistical program (R Development Core Team, 2003). The significance level was $\alpha = 0.05$ throughout.

### 3. Results

#### 3.1. Mandible structures of pygmy mole crickets

The mandibles of pygmy mole crickets of the genus *Xya* have an asymmetrical shape (Figure 1). The left mandibles are longer than the right mandibles (the average difference was approximately 1% in *X. pfaendleri* and 8% in *X. variegata*), although the difference was significant only for *X. variegata* (Table 2). The lengths of the left mandibles are greater than the lengths of the right mandibles, with the exception of males of *X. pfaendleri* and *X. variegata*. Both the right and left mandibles are bigger in females than in males (Table 2; Figure 2). The general shapes of the mandibles are similar in both species: the left mandibles are elongated and narrower and the right mandibles are shorter and wider. Each of the mandibles consists of two functional parts: the incisor area and the molar area (Figure 1). The incisor area is equipped with three robust and sharp teeth. The molar area consists of a small molar ridge, which is strongly concave on both mandibles. The size of the molar ridge is affected by sex and the side (left/
Table 2. ANOVA results for the mandibular parameters in *X. pfaendleri* and *X. variegata* as affected by independent variables, including mandible side, sex, and studied species. LM - length of the mandible, distance between the molar junction and the peak of the first incisor; SMR - surface of the molar ridge; NS - number of slats on the molar ridge.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>LM F-value</th>
<th>LM P-value</th>
<th>SMR F-value</th>
<th>SMR P-value</th>
<th>NS F-value</th>
<th>NS P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left/right side</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>X. pfaendleri</em></td>
<td>2.6</td>
<td>0.11</td>
<td>23.4</td>
<td>&lt;0.001*</td>
<td>0.75</td>
<td>0.39</td>
</tr>
<tr>
<td><em>X. variegata</em></td>
<td>28.1</td>
<td>&lt;0.001*</td>
<td>24.3</td>
<td>&lt;0.001*</td>
<td>2.82</td>
<td>0.10</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>X. pfaendleri</em></td>
<td>73.4</td>
<td>&lt;0.001*</td>
<td>109.6</td>
<td>&lt;0.001*</td>
<td>0.75</td>
<td>0.39</td>
</tr>
<tr>
<td><em>X. variegata</em></td>
<td>191.7</td>
<td>&lt;0.001*</td>
<td>149.3</td>
<td>&lt;0.001*</td>
<td>1.25</td>
<td>0.11</td>
</tr>
<tr>
<td>Between species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left mandible</td>
<td>1.9</td>
<td>0.17</td>
<td>10.3</td>
<td>&lt;0.01*</td>
<td>4.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Right mandible</td>
<td>1.1</td>
<td>0.29</td>
<td>40.7</td>
<td>&lt;0.001*</td>
<td>3.2</td>
<td>0.08</td>
</tr>
</tbody>
</table>

A total of 24 specimens of pygmy mole crickets were analyzed. *P < 0.05.

Figure 2. Comparison of the measured parameters of the mandibles in females (gray boxes) and males (white boxes) of *Xya pfaendleri* and *X. variegata* (Tridactylidae). a, d: length of the mandible (distance between the mandibular junction and the peak of the first incisor); b, e: surface of the molar ridge; c, f: number of molar slats. The box plots show the median (50th percentile) and 25th and 75th quartiles; error bars show the 10th and 90th percentiles. Filled symbols indicate outliers.
right) of the mandibles: females have larger molar ridges than males, and the left molar ridges are larger than the right (Table 2). The molar ridge forms a trituration area with parallel molar slats. The molar slats contain several parallel teeth. These teeth are short and regularly aligned. There was an average of 12 molar slats per molar ridge, and the number of molar slats was not significantly different between the sides of the mandibles or between females and males (Table 2). Differences in measured parameters between the studied species of pygmy mole crickets were significant only with respect to the parameter surface of the molar ridge, with *X. variegata* having a larger molar ridge than *X. pfaendleri* (Table 3; Figure 3).

### 3.2. Dietary preferences

The digestive tracts of the two species of pygmy mole crickets from the genus *Xya* contained mainly detritus, defined as unidentified decomposed organic matter, which accounted for 91% and 97% of the consumed diet in *X. pfaendleri* and *X. variegata*, respectively (Figure 4). Algae formed a minor component and body parts of invertebrates (fragments of cuticles, and setae) were rare, accounting for 9% and 3% of the total diet in the alimentary tract in *X. pfaendleri* and *X. variegata*, respectively. Angiosperms, fungal spores, and mosses were not found. The ratios of detritus/plant cellular matter/other particles were 29:1:3 and 19:1:0 in females and 47:1:1 and 1:0:0 in males of *X.

### Table 3. ANOVA results for the composition of detritus, plant cellular matter, and other particles in *Xya pfaendleri* and *X. variegata* as affected by independent variables, including sex and species.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Detritus F-value</th>
<th>Detritus P-value</th>
<th>Cellular matter F-value</th>
<th>Cellular matter P-value</th>
<th>Other particles F-value</th>
<th>Other particles P-value</th>
<th>Gini–Simpson index F-value</th>
<th>Gini–Simpson index P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>5.52</td>
<td>0.03*</td>
<td>2.74</td>
<td>0.11</td>
<td>2.47</td>
<td>0.13</td>
<td>6.06</td>
<td>0.02*</td>
</tr>
<tr>
<td>Species</td>
<td>2.74</td>
<td>0.11</td>
<td>0.05</td>
<td>0.82</td>
<td>2.47</td>
<td>0.13</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Sex × species</td>
<td>0.73</td>
<td>0.40</td>
<td>2.06</td>
<td>0.16</td>
<td>2.47</td>
<td>0.13</td>
<td>0.40</td>
<td>0.53</td>
</tr>
</tbody>
</table>

A total of 24 specimens of pygmy mole crickets were analyzed. An asterisk indicates significant differences (P < 0.05) between types of diet.

### Figure 3. Differences in the surfaces of the (a) left and the (b) right mandibles in *Xya pfaendleri* and *X. variegata*. The box plots show the median (50th percentile) and 25th and 75th quartiles; error bars show the 10th and 90th percentiles.
The proportion of detritus in the diet differed in males and females, although the proportions of cellular matter and other particles were similar (Table 3; Figure 4). The diet niche breadth according to the Gini–Simpson index was wider in females than in males and was similar between species (Table 3; Figure 5). The maximum average index was 0.1, where values less than 0.5 indicate a low variability of diet and consumption of only a single food resource.

Figure 4. The proportion of detritus in the diet of females (F) and males (M) in (a) *Xya pfaendleri* and (b) *X. variegata* as determined based on the postmortem gut content analyses. The box plots show the median (50th percentile) and 25th and 75th quartiles; error bars show the 10th and 90th percentiles. Open circles indicate outliers.

Figure 5. The food niche breadths in males (M) and females (F) of (a) *Xya pfaendleri* and (b) *X. variegata* (Orthoptera: Tridactylidae). The food niche breadth was cumulatively evaluated based on the Gini-Simpson index. The box plots show the median (50th percentile) and the 25th and 75th quartiles; error bars show the 10th and 90th percentiles.
4. Discussion

We evaluated the dietary preferences and morphological structure of the mandibles of two pygmy mole crickets from the genus *Xys* (Orthoptera: Tridactylidae). The dominant component of the diet was detritus, which we defined as noncellular decomposing organic matter. Differences in the morphological structures of the mandibles of the two species were minimal to nonexistent. The mandibles are asymmetrical in both species; the left and right mandibles differ in size and partly in shape. Uniformity in the diet is associated with the functional morphology of the mandibles as well as similar patterns of the biology and habitat preferences of the studied pygmy mole crickets.

Asymmetry of body structures is a rare characteristic of animals that have evolved independently many times (Palmer, 2009). In Orthoptera, the fixed asymmetry is associated with mouthparts, side of forewing, and shape of genitalia (Huber et al., 2007; Palmer, 2009). Pygmy mole crickets have asymmetrical mandibles, with the left mandible being larger than the right, which is based on the use of the mandibles during food intake. Other studied Caelifera (grasshoppers and groundhoppers) also show asymmetry in mandible size, with the left mandible always overlapping the right (Snodgrass, 1935; Isely, 1944; Uvarov, 1977; Bernays et al., 1991; Chapman, 1995; Köhler et al., 2000 for grasshoppers, and Kuřavová et al., 2014 for groundhoppers). The mandibular asymmetry is important for the correct mutual contact of mandibles during biting to assure that the cusps intermesh with each other (O’Brien, 1984).

Adaptation to food intake has led to behavioral and morphological specialization of mouthparts (Snodgrass, 1935). Grasshoppers (Caelifera: Acridomorpha) are often phytophagous (Fry et al., 1978), and the adaptations of their mandibular structures are associated with different types of food and different species of ecological groups of plants, grasses, forbs, flowers, and seeds (Bernays, 1998; Isely, 1944; Patterson, 1984). Blackith (1987) suggested that many species of the basal orthopteran families, such as the Tridactylidae and Tettigidae, are mainly algae feeders (lower-plant feeders), and the mouthparts are adapted to this food source. We found that the mandible structures of the pygmy mole crickets are reduced in comparison with related groundhoppers of the Tettigidae (Kuřavová et al., 2014). The incisor area of the groundhoppers consists of four incisors, and the molar area bears the central teeth and a molar ridge with parallel molar slats (Kuřavová et al., 2014). Pygmy mole crickets have only three incisors and a molar ridge with only a few molar slats. The basic features (shape of the incisor teeth and teeth on the molar ridge) are similar to those of Tettigidae due to their evolutionary, morphological, and functional association. The incisors of both the left and right mandibles overlap and work as a pair of scissors, and the molar area acts as a grinder to fragment food into small pieces. The mandibular areas of groundhoppers have similar functions (Kuřavová et al., 2014).

The measured parameters (the distance between the mandible junction and the peak of the incisor, the surface of the molar ridge, and the number of slats) were significantly different between sexes, between sides of the mandibles, and between studied species. Females of Caelifera typically have a larger body size than males (Zechner et al., 1999; Kočárek et al., 2005; Bidau et al., 2013); therefore, the lengths of the mandibles and the molar ridges’ surface are considerably larger in females than in males. The number of molar slats does not differ between sexes (on average 12.8 ± 0.3), and these structures are uniform among individuals of both species and between species. Some tropical species of groundhoppers (Discotettiginae, Cladonotinae, and Scleremeninginae) show different numbers of slats on the molar ridge (from 9 to 19 slats), although differences between sexes have not been reported (Kuřavová et al., unpublished data). In summary, the measured parameters were not significant between the studied pygmy mole crickets; *X. pfaendleri* and *X. variegata* have almost the same features regarding the morphological structures of the mandibles, likely due to similar dietary and behavioral patterns.

The aspects of food strategies, such as food selection, food preferences, feeding patterns, and diet specialization, have been studied by many authors in a variety of grasshoppers (e.g., Williams, 1954; Mulker, 1967; Otte and Joern, 1976; Bernays and Chapman, 1977; Uvarov, 1977; Joern, 1979, 1983, 1985; Bernays and Bright, 1993). The food biology of groundhoppers, as detrito-/bryophagous insects, has been studied only in a few species (for references, see Kuřavová et al., 2014; Kuřavová and Kočárek, 2015). The dietary preferences of pygmy mole crickets (Tridactylidae) have not been studied in detail. Only Deyrup and Eisner (1996) recorded filamentous algae in the gut of the pygmy mole cricket *Neotridactylus archboldi* Deyrup & Eisner, 1996. Blackith (1987) also suggested that pygmy mole crickets are algal feeders. Our analysis of the alimentary tract contents of pygmy mole crickets showed that the species of the genus *Xys* are mainly detritophagous, and cellular matter, such as algae, is consumed only incidentally together with detritus. Detritus is present in large amounts in the pygmy mole cricket habitat and is easily accessible inside their underground corridors. We confirmed that the pygmy mole crickets do not search for specific matter (e.g., lichens, mosses, or plants) as diet but utilize the diet present in their burrows and their surroundings. The determined dietary uniformity could reflect their habitat preferences, especially their subterranean lifestyle. Pygmy mole crickets are forced to stay in subterranean corridors to protect themselves from predators, such as
hymenopteran *Tachytes* spp. (*Hymenoptera*, *Sphecidae*, and *Larrinae*) that dig beneath the surface of the ground to capture pygmy mole crickets (Kurczewski, 1966).

The studied species of pygmy mole crickets had similar diet breadths. The species showed food niche breadth values of up to 0.4, which corresponds with a narrow food spectrum. For comparison, the groundhopper *Tetrix tenuicornis* (*Tetrigidae*) showed a maximum food niche breadth value of 0.6 (Kuřavová and Kočárek, 2015). Males of *X. pfaenderli* and *X. variegata* exhibited a significantly higher uniformity of diet than females, likely because females require a more nutritionally balanced diet for egg production. *X. pfaenderli* and *X. variegata* have almost the same dietary preferences, and the food biology of both is related to their habitat preferences. The studied species have similar demands on habitat quality and occupy the same areas, sometimes in a syntopic manner (Holuša and Kočárek, 1999; Gavlas et al., 2007).

Patterson (1983) compared the structure of grasshopper mandibles with their niche variation. The niche variation hypothesis predicts greater overall mandibular variability in forms having the broadest trophic niches. Comparisons have shown that groundhoppers of the family *Tetrigidae* (Kuřavová et al., 2014; Kuřavová and Kočárek, 2015) and pygmy mole crickets of the family *Tridactylidae* have similar patterns of mandibular variation and dietary preferences. The *Tetrigidae* have more complex mandible structures and more heterogeneous food intake than the *Tridactylidae*. This association of the morphology and dietary preference in each group supports Patterson's niche variation hypothesis.

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