



# Heads or tails: exaggerated morphologies in relation to the use of large bamboo internodes in two lizard beetles, *Doubledaya ruficollis* and *Oxylanguria acutipennis* (Coleoptera: Erotylidae: Languriinae)

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## Abstract

The cavities of bamboos (Poaceae) are used by various animals. Most of the animals access these cavities either by existing cracks or by excavating bamboos with soft walls or small, thin-walled bamboos. Only a few animals excavate into the cavities of large and thick- and hard-walled internodes of mature bamboos. We studied two lizard beetle species (Coleoptera: Erotylidae: Languriinae), *Doubledaya ruficollis* and *Oxylanguria acutipennis*, that excavate into large internode cavities of recently dead mature bamboos and have morphological modifications. We observed that females of *D. ruficollis* used their mandibles to bore oviposition holes on *Schizostachyum* sp. (mean wall thickness = 3.00 mm) and *O. acutipennis* did so on *Dendrocalamus* sp. (3.37 mm) bamboos. Previous studies suggested that the markedly asymmetrical mandibles and needle-like ovipositors of females in the genus *Doubledaya* are adaptive traits for excavating hard-walled bamboos for oviposition. Therefore, we measured their mandibular lengths and ovipositor lengths. *D. ruficollis* females had greater asymmetry in the mandibles and shorter and less-sclerotized ovipositors than females of congeners using small bamboos. In contrast, *O. acutipennis* females had slightly asymmetrical mandibles and elongated, well-sclerotized ovipositors. Oviposition holes of *D. ruficollis* were cone-shaped (evenly tapering), whereas those of *O. acutipennis* were funnel-shaped (tube-like at the internal apex). This suggests that *D. ruficollis* females excavate oviposition holes using the mandibles only, and *O. acutipennis* females use both the mandibles and ovipositors. These differences suggest different oviposition-associated morphological specialization for using large bamboos: the extremely

asymmetrical mandibles in *D. ruficollis* and elongated, needle-like ovipositors in *O. acutipennis*.

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## Introduction

Morphological specialization for boring into hard materials has independently evolved many times in the animal kingdom. Examples of this morphological specialization include the shovel-like front legs of moles and dung beetles for tunneling soil, bills of woodpeckers, mandibles of wood-boring beetles, and needle-like ovipositors of wood wasps for excavating wood. These indicate that the specialization occurred in different parts but sometimes in similar parts (i.e., front legs and mouthparts) among species that are phylogenetically distant from one another.

Plant hollows and cavities are fully- or semi-enclosed spaces located in different parts of the plant body (e.g., stems or leaves). These cavities can be used by a wide variety of animals for shelter

and reproduction (Dajoz 2000; Gibbons and Lindenmayer 2002). We can distinguish two main types of plant cavities. Primary cavities are hollows originally present in the plant which are initially closed to the outside. These types of cavities cannot be used unless an animal (known as initial user) makes holes to access them. The accessibility to these cavities are determined by the physical characteristics (e.g., hardness or thickness) of the cavity wall. An example of primary cavities is the domatia (i.e., structures produced by plants to host symbiotic arthropods) used by some mutualistic ants. Ants excavate holes into the domatia when the tissues around the domatia are young and thin (Fiala and Maschwitz 1992). Secondary cavities, in contrast, are not originally found in the plant but are created by external factors such as fungal infections or animal boring. Secondary hollows are often seen in old tree trunks. This type of cavities is opened to the outside, and so they are easily accessible to animals. In fact, most of the animals that use plant cavities depend on secondary hollows (Dajoz 2000; Gibbons and Lindenmayer 2002).

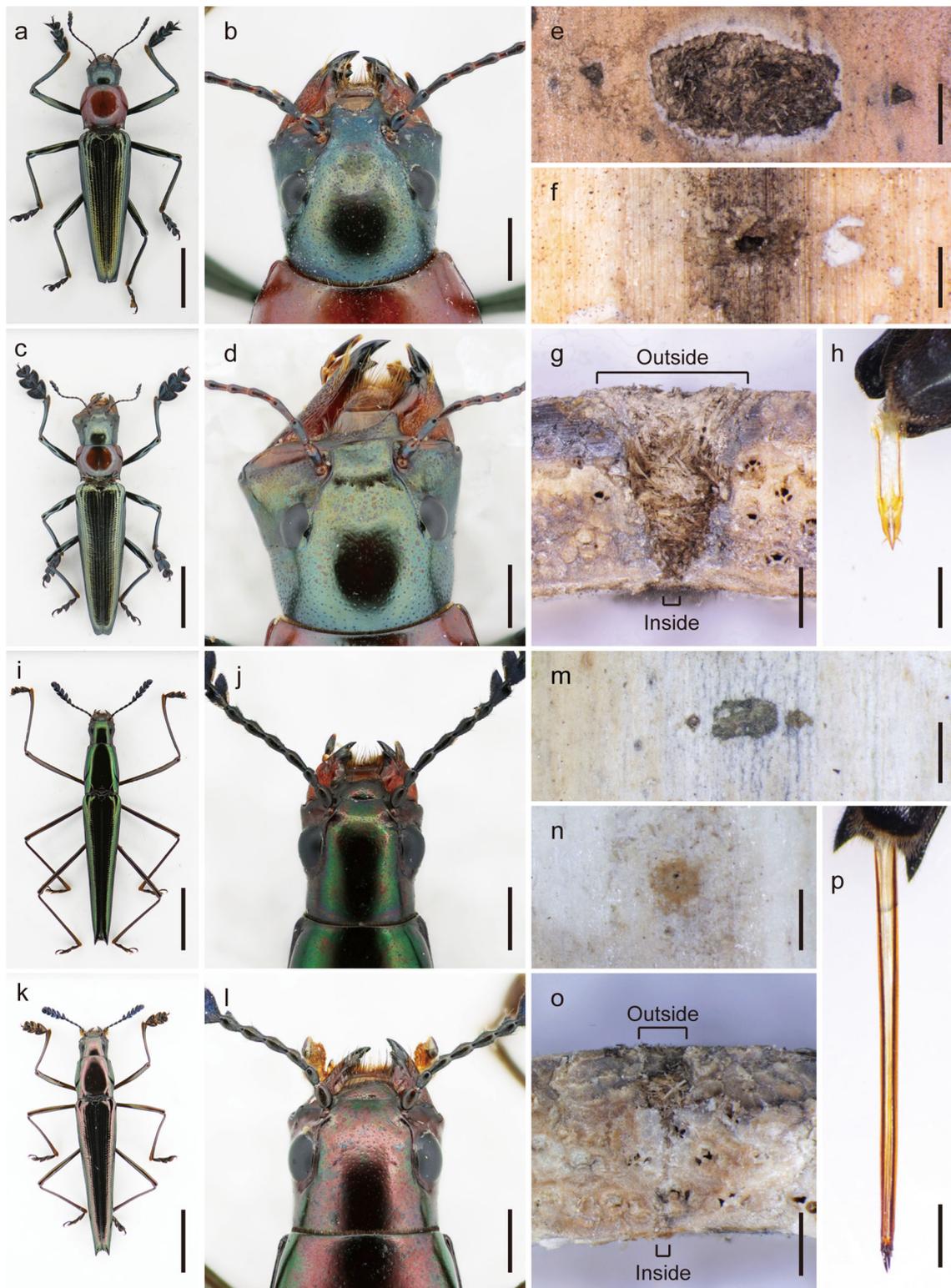
Most of the species of bamboos (Poaceae) have hollow structures in stems (culms). They have natural cavities surrounded by woody tissues consisting of nodes and internode walls. A wide range of vertebrates and invertebrates, including bats, woodpeckers, and insects, are known to use the cavities of bamboos (e.g., Gardner 1945; Medway and Marshall 1970; Round et al. 2012; Kovac and Krocke 2013). Bamboo-cavity users enter into the cavities through cracks or holes made by other animals or by excavating the wall when the bamboos are young and soft. However, there are a few initial users that can bore holes into the cavity of mature bamboo culms with lignified walls. The majority of those who can bore holes in mature and lignified bamboos use small-sized bamboo internodes (i.e., internodes with a small diameter and thin wall), that are likely to be easy to access. Examples of these species include the bamboo carpenter bee *Xylocopa nasalis* Westwood (Apidae) (Hongjamrassilp and Warrit 2014) and the long-horned beetle *Niphona furcata* (Bates) (Cerambycidae) (Kanzaki 1938; Kojima 1955).

However, to date, very few initial cavity users of large internodes of mature bamboo culms have been discovered. These species include the bamboo woodpecker (genus *Gecinulus* Blyth), which can use its stout bills to bore a hole in the wooden walls of large internodes of recently dead culms of the large bamboo *Gigantochloa apus* (J.H. Schultes) Kurz ex Munro to make a nest (Round et al. 2012). The long-horned beetle *Abryna regispetri* Paiva has stout mandibles and long legs with enlarged tarsi that are likely to enable the beetle to move on smooth surfaces (Kovac and Yong 1992). Female beetles hold onto the internodes of living and recently felled mature culms of the large bamboo *G. scortechinii* Gamble by extending the legs and excavate holes into the cavity using the mandibles in order to lay eggs (Kovac and Yong 1992). These examples show that the cavities of lignified culms of larger bamboos are relatively inaccessible for most animals and that the few initial users possess specialized traits for the excavation of hard substrates.

Females of lizard beetles (Coleoptera: Erotylidae: Languriinae) in the genus *Doubledaya* White and allied genera exhibit a specialized morphology, such as an asymmetrical head with an enlarged left mandible (Villiers 1945). Additionally, some of the members have elongated legs with enlarged tarsi. There is currently good knowledge on the biology of three species of *Doubledaya* (*D. bucculenta* Lewis, *D. sinuata* Zia, *D. tonkinensis* Zia), all of which are small-bamboo users. Adult females of *D. bucculenta* have a large asymmetrical head with mandibles and make evenly tapered, cone-shaped holes on newly dead culms using the mandibles in order to lay eggs into the cavity (Toki and Togashi 2011, 2013). Females of *D. sinuata* have a less asymmetrical head with mandibles and a sclerotized, needle-like ovipositor (Toki et al. 2014, 2016). The females of this species use their mandibles to make shallow depressions on newly dead culms. Subsequently, they use their ovipositor to penetrate the cavity in order to lay eggs into the cavity, which results in funnel-shaped holes (i.e., cone-shaped at the external apex and tube-like at the internal apex). Females *D. tonkinensis* have a slightly asymmetrical head with mandibles and a needle-like ovipositor (Toki et al. 2016). They make funnel-shaped holes on weakened, young, and soft culms in the same way as *D. sinuata* to lay eggs inside the cavity. Thus, it is suggested that the markedly asymmetrical mandibles and the needle-like ovipositors of *Doubledaya* females play an important role in boring holes into bamboo cavities during oviposition (Toki et al. 2016).

If species in *Doubledaya* and allied genera use large internodes of mature bamboo culms as initial users, it is likely that the females have more asymmetrical heads or more developed needle-like ovipositors than users of small bamboos. Also, the individuals may have long legs for locomotion on the smooth surface of the cylindrical culm. Individuals of *Doubledaya ruficollis* (Kraatz), a species distributed in northern India and northern Vietnam, have long legs and the female's head is extremely asymmetrical compared to the other *Doubledaya* species (Villiers 1945) (Fig. 1a–d). Individuals of *Oxylanguria acutipennis* Crotch, which is related to *Doubledaya* and is distributed in Sumatra, Borneo, and Malacca, also have long legs but the female head is only slightly asymmetrical (Villiers 1945) (Fig. 1i–l). Although their unusual morphologies imply that these beetle species are initial users of large internodes of mature bamboo culms with different morphological specializations, there is no biological information available on them (Crotch 1876; Kraatz 1899).

In the present study, we aimed to determine morphological specialization patterns in relation to the use of large bamboo internodes by studying two lizard beetle species, *D. ruficollis* and *O. acutipennis*, which exhibit remarkably different head morphologies. We observed their oviposition behaviors and determined the degree of asymmetry of the genal width and mandibular length for the adults, and the size of the host bamboos. Finally, we discuss the significance of using the large internode cavity of bamboos.



**Fig. 1** Adults, heads, ovipositors, and completed oviposition marks (COMs) of *Doubledaya ruficollis* and *Oxylanguria acutipennis*. **a–h** *D. ruficollis*. **i–p** *O. acutipennis*. **a, i** Male. **b, j** Male head. **c, k** Female. **d, l** Female head. **e, m** Outside view of a COM. **f, n** Inside view of a

COM. Panel **n** shows an inner opening of a COM from which secretion was removed. **g, o** Cross section of a COM. **h, p** Ovipositor, ventral view. Scale bars = 5 mm (**a, c, i, k**), 1 mm (**b, d–h, j, l–p**)

## Materials and methods

### Insects and bamboos

*Doubledaya ruficollis* and the host bamboos were sampled from clumps of the bamboo *Schizostachyum* Nees sp. in the primary forest of Cuc Phuong National Park, Ninh Binh Province, Vietnam (20° 21' 18" N, 105° 35' 04" E, 377 m altitude), on 5–8 March, 30 May 2015, and 26 May 2016.

The clumps were surrounded by broad-leaved trees (Fig. 2a). The mature culms were large and over 10 m in height.

*Oxylanguria acutipennis* and the host bamboos were sampled from clumps of the bamboo *Dendrocalamus* Nees sp. in the broad-leaved forests of Perak, Malaysia (4° 21' 57"–22' 30" N, 101° 19' 50"–20' 00" E, 600–720 m altitude), on 11 October 2011, 6 March 2012, and 20–23 March 2017 (Fig. 2f, g). The mature culms were large and over 10 m in height.

**Fig. 2** Host bamboos of *D. ruficollis* and *O. acutipennis*. **a–e** *D. ruficollis*. **f–j** *O. acutipennis*. **a** A clump of *Schizostachyum* sp. bamboo. **b** A male adult inhabiting an internode cavity. **c** A female adult inhabiting an internode cavity. **d** An egg in an internode cavity of a weakened culm. **e** A mature larva in an internode cavity of a recently dead culm. **f** A clump of *Dendrocalamus* sp. bamboo. **g** A male adult on a newly dead culm. **h** A female adult making an oviposition hole by her mandibles on a newly dead culm. **i** Inner surface of an oviposition-mark-free internode of a healthy culm. **j** A mature larva in an internode cavity of a recently dead culm. Scale bars = 1 cm (**b–e**, **g–j**)



In addition, we did field observations of the oviposition behavior on the culms of *Dendrocalamus* sp. in Lambir Hills National Park, Sarawak, Malaysia (4° 14' 18" N, 114° 3' 43" E, 50 m altitude), on 26 May 2018.

### Host-bamboo use by *Doubledaya ruficollis* and *Oxylanguria acutipennis*

The spatial distribution of oviposition marks (OMs) of *D. ruficollis* among host bamboo internodes was determined by taking 34 internodes with OMs from 18 recently dead culms in two *Schizostachyum* sp. bamboo clumps in 2015. These culms were standing or leaning on other trees. In addition, we found a *D. ruficollis* female ovipositing on a *Schizostachyum* sp. culm on 26 May 2016 and recorded its behavior while building a hole to determine whether or not the oviposition behavior is the same as that of congeners.

In the case of *O. acutipennis*, nine internodes with OMs were taken from four recently dead culms of *Dendrocalamus* sp. bamboo in 2017. These culms were standing, leaning on other culms, or lying on the ground. Additionally, we found a female ovipositing on a *Dendrocalamus* sp. culm on 26 May 2018 and recorded its behavior.

Generally, OMs of the genus *Doubledaya* were divided into completed oviposition mark (COM) and incomplete mark (Toki and Togashi 2013; Toki et al. 2016). COM was characterized by a hole penetrating the internode wall. Incomplete mark was characterized by small depressions, the bottoms of which did not reach the internode cavities, indicating they were left behind due to the interruption of ovipositional behavior. To determine whether or not the insects studied are initial users of large bamboos, records were taken on the internode length and the diameter at the middle in each sampled culm. The diameter of the internode at the location of the OMs was also measured. The OMs were counted on all the internodes. The internodes were dissected using pruning clippers and a pruning saw to record the number and developmental stages of the insects present, and to measure the wall thickness of the internode at the middle and at OMs formed. In addition, to make a close observation, COMs were cut transversely at a right angle with internodes and through the bottom of cone-shaped depressions using an ultrasonic cutter USW-334 (Honda Electronics, Aichi, Japan) to determine whether the oviposition hole is cone-shaped (i.e., mandible-associated) or funnel-shaped (i.e., mandible-and-ovipositor-associated). Images of the cross sections of COMs were taken under a stereo microscope SZX10 (Olympus, Tokyo, Japan) with an EOS Kiss X7i digital camera (Canon, Tokyo, Japan). If the hole was funnel-shaped, the depth of the cone-shaped depression of individual COMs made by females with the mandibles was measured using ImageJ 1.47t (Schneider et al. 2012).

### Head morphology

The body and elytral lengths of the collected adults were determined using digital calipers (Mitutoyo, Kanagawa, Japan). The mandibular length and genal width were measured using images taken under a stereomicroscope, SZX10, with an EOS Kiss X7i digital camera following Toki and Togashi (2011). To show the degree of asymmetry of the head traits, we employed the asymmetry index (AI) calculated as  $\{(R - L) \times 100\} / (R + L)$ , where  $R$  was the length of traits on the right side and  $L$  of traits on the left side (e.g., Hosoi et al. 2007).

### Ovipositor morphology

To examine the morphological specialization of the ovipositors for boring holes, we dissected five females of *D. ruficollis* collected in 2015 and 2016 and five females of *O. acutipennis* collected in 2017. The ovipositor length, which was defined as the distance between the proximal margin of the pair of baculi and the apex of the pair of coxites, was measured using digital calipers. The shape and degree of sclerotization of the apex of the pair of coxites were also observed.

### Statistical analysis

We used  $t$  test or Wilcoxon rank sum test to compare the means of any two samples. These tests were used after comparing the sample variances using  $F$  test and testing for normality with the Shapiro-Wilk normality test. Pearson's correlation coefficient and the ordinary least squares (OLS) method were used to determine the relationship between two variables. The paired  $t$  test was used to compare the two means of the diameter and wall thickness of the bamboo internodes.

There are three types of asymmetries distinguished by the mean and variation of the difference between the right-hand and left-hand measurements: fluctuating asymmetry (mean = 0, variation = normal distribution), directional asymmetry (mean  $\neq$  0, variation = normal distribution), and antisymmetry (mean = 0, variation = platykurtic or bimodal) (Palmer 2005). The method of Toki and Togashi (2011) was used to judge fluctuating asymmetry and directional asymmetry of traits as follows. First, the Shapiro-Wilk normality test was applied to determine whether the AI values followed a normal distribution. When the AI values were normally distributed, the  $t$  test was used to determine whether they had a mean of zero for discriminating between fluctuating asymmetry and directional asymmetry. When the test showed under- or overdispersed variance of AI values, the 95% confidence intervals (CIs) given by the percentile bootstrap method (1000 replicates) was used to determine whether or not the AI values had a mean of zero. The trait was judged to be directional asymmetry when the 95% CI did not include zero. Calculations were performed using R 3.4.7 (R Development Core Team 2017).

## Results

### Host-bamboo use by *Doubledaya ruficollis*

Typical OMs of *Doubledaya* species were found on recently dead culms of *Schizostachyum* sp. bamboo in March 2015. There were three depressions in a horizontal line on the culm (Fig. 1e). The depression in the center was large and deep while the remaining two were very small and shallow. There was no difference in the length between the nearest two depressions. Thirty-five OM-present internodes were obtained from 18 recently dead culms in two *Schizostachyum* sp. bamboo clumps. Most traits were not recorded for one of these internodes due to missing parts of the internode during sampling. The culms were standing or leaning on other trees and the surfaces were covered with dark brown mold. In addition, we sampled six OM-present internodes from two weakened culms in another clump of the bamboo on 26 May 2016. The culms were standing with green surfaces, and the leaves and the tips were missing. A female boring a hole for oviposition was observed on one of the culms (see below).

Large diameter internodes had longer lengths (Pearson's  $r = 0.75$ ,  $P < 0.0001$ ,  $n = 39$ ), and a larger wall thickness (Pearson's  $r = 0.86$ ,  $P < 0.0001$ ,  $n = 39$ ), larger outer surface area (Pearson's  $r = 0.95$ ,  $P < 0.0001$ ,  $n = 39$ ), and larger cavity volume (Pearson's  $r = 0.97$ ,  $P < 0.0001$ ,  $n = 39$ ) (Table 1).

Dissection revealed that out of 40 COM-present internodes, 36 internodes harbored three living eggs, 15 living larvae, and 18 living adults of *D. ruficollis* (Table 1; Fig. 2b–e). Each individual was found singly in each internode. The remaining four internodes were empty. When the larvae and adults were present in the internode cavities, no holes were penetrating the division walls separating adjoining cavities. The eggs and the early-instar larvae ( $n = 3$ ) were found in the internodes of weakened culms, and the late-instar larvae ( $n = 12$ ) and the adults were in those of recently dead culms.

The inner surface of the internodes was covered with a large amount of fungal mycelia and white layers when they contained *D. ruficollis* mature larvae or adults. No fungal mycelia or layers were observed on the surface of the internodes containing *D. ruficollis* eggs or early-instar larvae (Fig. 2d, e).

Observations revealed that all 42 OMs on 40 OM-present internodes were COMs. All the COMs made by *D. ruficollis* had a hole with a tiny depression on each side (Fig. 1e). The external opening was rectangular (mean  $\pm$  SD =  $2.65 \pm 0.34$  mm for length,  $1.59 \pm 0.31$  mm for width,  $n = 10$ ; Fig. 1e), and the internal opening was small oval to indefinite shape ( $n = 10$ ) (Fig. 1f). Of them, 39 OM-present internodes had a single OM in each internode, and one internode had three OMs (mean  $\pm$  SD =  $1.05 \pm 0.32$ ,  $n = 40$ ) (Table 1). The holes were plugged with excised bamboo fibers.

**Table 1** Relationships of *Schizostachyum* sp. internode diameter to the other traits of internodes, number of oviposition marks (OMs), completed oviposition marks (COMs), and individuals of *Doubledaya ruficollis* in 19 recently dead bamboo culms

Internode diameter ( $d$ ) (mm)	$20 \leq d < 30$	$30 \leq d < 40$	$40 \leq d < 50$	$50 \leq d < 60$	$60 \leq d < 70$	Total
No. of culms containing internodes with specified diameter	1	4	8	9	2	19
Internodes with specified diameter						
No. examined	1	6	12	16	4	39
Length (cm)	49.0	$54.6 \pm 4.4$	$70.5 \pm 10.7$	$76.3 \pm 6.3$	$84.2 \pm 6.3$	$71.3 \pm 11.8$
Diameter (mm) <sup>a</sup>	28.1	$35.6 \pm 3.2$	$43.9 \pm 2.6$	$54.0 \pm 3.2$	$63.3 \pm 2.6$	$48.3 \pm 9.4$
Outer surface area (cm <sup>2</sup> )	432.3	$611.3 \pm 86.9$	$974.3 \pm 172.6$	$1293.9 \pm 135.0$	$1676.8 \pm 155.0$	$1107.7 \pm 352.0$
Wall thickness (mm) <sup>a</sup>	2.2	$2.5 \pm 0.2$	$2.7 \pm 0.2$	$3.0 \pm 0.2$	$3.4 \pm 0.1$	$2.9 \pm 0.3$
Cavity volume (cm <sup>3</sup> )	215.4	$406.0 \pm 95.8$	$822.9 \pm 180.1$	$1379.7 \pm 212.3$	$2126.2 \pm 265.9$	$1105.3 \pm 546.5$
No. of internodes with						
0 OM (or COM)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 OM (or COM)	1 (1)	6 (6)	11 (11)	16 (16)	4 (4)	38 (38)
2 OMs (or COMs)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
3 OMs (or COMs)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	1 (1)
Mean number of OMs per internode	1	$1.0 \pm 0.0$	$1.2 \pm 0.6$	$1.0 \pm 0.0$	$1.0 \pm 0.0$	$1.1 \pm 0.3$
Mean number of COMs per internode	1	$1.0 \pm 0.0$	$1.2 \pm 0.6$	$1.0 \pm 0.0$	$1.0 \pm 0.0$	$1.1 \pm 0.3$
No. of living egg present in internodes	0	0	0	0	3	3
No. of living larvae present in internodes	1	1	6	5	1	14
No. of living adult present in internodes	0	4	4	10	0	18

All values are mean  $\pm$  SD

<sup>a</sup>Diameter and wall thickness were measured in the middle part of the internode

The internode diameter at COM was not significantly different from that at the middle part (mean ± SD = 48.10 ± 9.05 mm, *n* = 42 at COM; 48.32 ± 9.36 mm, *n* = 39 at the center) (paired *t* test, *t*<sub>38</sub> = -0.77, *P* = 0.778). This was also the case for internode wall thickness (mean ± SD = 3.00 ± 0.35 mm, *n* = 42 at COM; 2.87 ± 0.33 mm, *n* = 39 at the center) (paired *t* test, *t*<sub>38</sub> = -4.86, *P* = 1.000). Cross sections showed that the *D. ruficollis* holes were cone-shaped (Fig. 1g), indicating that the depth of the hole corresponds to the internode wall thickness at COM. The thickness and diameter of the internode at COM were significantly correlated (Pearson’s *r* = 0.75, *P* < 0.0001, *n* = 42). When the internodes were separated into five diameter classes, the mean numbers of OMs and COMs per internode peaked at internodes of a 40.0 to 50.0 mm diameter class (Table 1).

Two larvae of *D. ruficollis* and an adult long-horned beetle *Abryna* sp., which is an initial-cavity user of mature bamboo culms, were obtained from a recently dead culm of *Schizostachyum* sp. bamboo on 30 May 2015. The larvae of *D. ruficollis* were found in two internode cavities of a recently dead culm, whose surface was dark brown, and the adult of *Abryna* sp. was found on a newly dead culm, whose surface was still green.

A male and two female adults of *D. ruficollis* were found on the surface of weakened mature culms of *Schizostachyum* sp. bamboo on 25 and 26 May 2016. We observed the behavior of excavation of an oviposition hole by a female. A female fixed the tip of one of the two mandibles in one of the two tiny depressions while using the other mandible to cut bamboo tissue in the deep depression. After a while, she alternated the mandibles for cutting fibers on the opposite side of the

deep depression. She went forward and across the deep depression, turned 180°, went forward again and cut the bamboo fibers in the manner described above. Boring behavior was repeated. Although a limited time of the field survey prevented us from observing oviposition itself, the boring behavior of *D. ruficollis* was the same as that of the *Doubledaya* species (Toki et al. 2014, 2016).

### Host-bamboo use by *Oxylanguria acutipennis*

*Doubledaya*-like OMs were found on recently dead culms of *Dendrocalamus* sp. bamboo in March 2017 (Fig. 1m). Nine OM-present internodes were obtained from four recently dead culms of the bamboo. Most traits were not recorded for one of these internodes due to missing parts of the internode during sampling. The culms were standing or leaning on other culms and the color was light brown.

Large-diameter internodes had a longer length (Pearson’s *r* = 0.94, *P* = 0.0004, *n* = 8) and larger outer surface area (Pearson’s *r* = 0.99, *P* < 0.0001, *n* = 8) and cavity volume (Pearson’s *r* = 0.98, *P* < 0.0001, *n* = 8), whereas there was no significant correlation between length and wall thickness (Pearson’s *r* = 0.53, *P* = 0.175, *n* = 8) (Table 2).

Dissection revealed that the nine COM-present internodes harbored eight living larvae and a living pupa of *O. acutipennis* (Fig. 2j). Each individual was found singly in each internode. When the larvae and pupa were present in the internode cavities, no holes were penetrating the division walls separating adjoining cavities. The inner surface of the OM-free internodes was covered with a layer of pith tissue

**Table 2** Relationships of *Dendrocalamus* sp. internode diameter to the other traits of internodes, number of oviposition marks (OMs), completed oviposition marks (COMs), and immatures of *Oxylanguria acutipennis* in three recently dead bamboo culms

Internode diameter ( <i>d</i> ) (mm)	20 ≤ <i>d</i> < 30	30 ≤ <i>d</i> < 40	40 ≤ <i>d</i> < 50	50 ≤ <i>d</i> < 60	Total
No. of culms containing internodes with specified diameter	2	2	1	1	3
Internodes with specified diameter					
No. examined	3	2	2	1	8
Length (cm)	47.7 ± 24.1	90.2 ± 8.1	99.5 ± 0	119.1	80.2 ± 31.3
Diameter (mm) <sup>a</sup>	24.2 ± 4.5	35.5 ± 3.3	45.9 ± 2.7	51.2	35.8 ± 11.3
Outer surface area (cm <sup>2</sup> )	384.6 ± 267.0	1010.4 ± 185.5	1433.4 ± 82.9	1916.5	994.7 ± 601.9
Wall thickness (mm) <sup>a</sup>	2.9 ± 0.2	2.9 ± 0.3	3.1 ± 0.1	3.3	3.0 ± 0.2
Cavity volume (cm <sup>3</sup> )	157.5 ± 158.8	633.4 ± 171.3	1235.1 ± 157.6	1857.3	758.4 ± 641.6
No. of internodes with					
0 OM (or COM)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1 OM (or COM)	3 (3)	2 (2)	2 (2)	1 (1)	8 (8)
Mean number of OMs per internode	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0	1.0 ± 0.0
Mean number of COMs per internode	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0	1.0 ± 0.0
No. of larvae (or living larvae) present in internodes	3 (3)	2 (2)	2 (1)	1 (1)	8 (8)

All values are mean ± SD

<sup>a</sup>Diameter and wall thickness were measured at the middle part of the internode

(Fig. 2i), whereas the pith tissue was lost when *O. acutipennis* mature larvae and a pupa were present (Fig. 2j).

Observations revealed that all OMs on nine OM-present internodes were COMs. All the nine COMs made by *O. acutipennis* had a hole with a tiny depression on each side (Fig. 1m). The external opening was rectangular (mean  $\pm$  SD of  $1.40 \pm 0.58$  mm for length,  $0.67 \pm 0.20$  mm for width,  $n = 9$ ; Fig. 1m) and the internal opening was a small indefinite shape and was covered with secretion ( $n = 9$ ) (Fig. 1n).

The internode diameter at COM was not significantly different from that at the middle part (mean  $\pm$  SD =  $36.04 \pm 11.42$  mm,  $n = 8$  at COM;  $35.83 \pm 11.34$  mm,  $n = 8$  at the center) (paired  $t$  test,  $t_7 = -0.92$ ,  $P = 0.806$ ). This was also the case for internode wall thickness (mean  $\pm$  SD =  $3.37 \pm 0.47$  mm,  $n = 9$  at COM;  $2.99 \pm 0.24$  mm,  $n = 8$  at the center) (paired  $t$  test,  $t_7 = -3.61$ ,  $P = 0.996$ ). Cross sections showed that *O. acutipennis* holes were funnel-shaped (Fig. 1o). The hole was composed of two parts: a cone-shaped depression at the outer part of the internode where the fibers were cut off, and a narrow tube-shaped gap at the inner part where the fibers were not cut (Fig. 1o). The depth of the cone-shaped depression (mean  $\pm$  SD =  $0.99 \pm 0.09$  mm,  $n = 9$ ) was not significantly correlated with diameter or wall thickness of the internode at COM (Pearson's  $r = 0.23$ ,  $P = 0.579$ ,  $n = 8$ , for diameter; Pearson's  $r = -0.04$ ,  $P = 0.912$ ,  $n = 9$ , for wall thickness). When internodes were separated into four diameter classes, the peaks of the mean numbers of OMs and COMs per internode were not clear for the diameter classes (Table 2). Each internode contained a single OM and COM.

Adults of *O. acutipennis* were found on the surface of recently-dead mature culms of *Dendrocalamus* sp. bamboo, whose surface was still green, on 26 May 2018. We observed the behavior of excavation of an oviposition hole by a female. A female fixed the tip of one of the two mandibles in one of the two

tiny depressions while using the other mandible to cut bamboo tissue in the deep depression (Fig. 2h). After a while, she alternated the mandibles for cutting fibers on the opposite side of the deep depression. She went forward and across the deep depression, turned  $180^\circ$ , went forward again, and cut the bamboo fibers in the aforementioned manner. Boring behavior was repeated.

### Head morphology of *Doubledaya ruficollis*

There was no significant difference in body length between the males (mean  $\pm$  SD =  $16.48 \pm 2.93$  mm, range = 11.96 to 21.85 mm,  $n = 19$ ) and females ( $16.83 \pm 1.96$  mm, range = 12.77 to 22.35 mm,  $n = 25$ ) ( $t$  test,  $t_{42} = 0.474$ ,  $P = 0.638$ ) or in elytral length between the sexes ( $10.91 \pm 1.86$  mm, range = 8.35 to 14.08 mm,  $n = 19$  for males;  $11.14 \pm 1.41$  mm, range = 8.73 to 15.33 mm  $n = 25$  for females) ( $t$  test,  $t_{42} = 0.463$ ,  $P = 0.646$ ). A significant positive correlation was found between elytral length and body length in each sex (Pearson's  $r = 0.994$ ,  $P < 0.0001$  for males; Pearson's  $r = 0.990$ ,  $P < 0.0001$  for females). The results indicated that the difference in elytral length directly reflected the difference in adult body size.

The left mandible and gena were longer than the right mandible and gena, respectively, in individual adult females of *D. ruficollis*, indicating a distinctive, directional asymmetry ( $t$  test,  $t_{23} = -101.55$ ,  $P < 0.0001$  for a hypothesis of mean = 0 in mandibular AI values;  $t_{24} = -34.80$ ,  $P < 0.0001$  in genal AI values) (Table 3; Figs. 2d and 3c, d). This was also the case for adult males ( $t$  test,  $t_{18} = -41.77$ ,  $P < 0.0001$  for a hypothesis of mean = 0 in mandibular AI values;  $t_{18} = -4.73$ ,  $P = 0.0002$  for genal AI values) (Table 3; Figs. 2b and 3a, b).

Male and female adults of *D. ruficollis* exhibited a significant, positive correlation between the elytral length and mandibular length (Table 4). They also showed a significant, positive correlation between elytral length and genal width on both sides

**Table 3** Asymmetry index (AI) values of mandibles and genae in relation to body size of *Doubledaya ruficollis* and *Oxylanguria acutipennis*

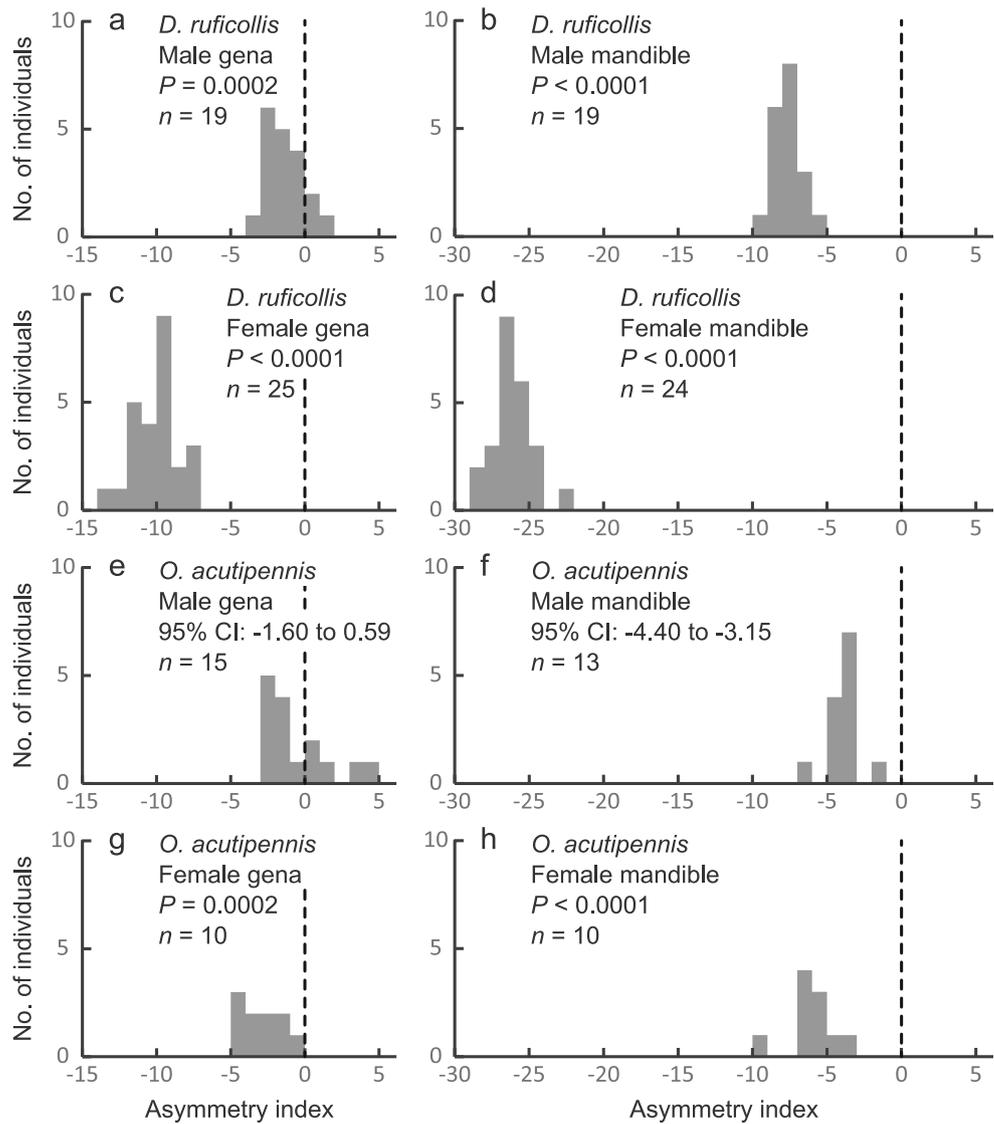
Sex	Trait (no. examined)	AI value	$P$ value of Shapiro-Wilk test	$P$ value of $t$ test (mean = 0) or 95% CI of mean <sup>a</sup>	Slope	$P$ value	$y$ -intercept	$P$ value	$R^2$
<i>D. ruficollis</i>									
Male	M (19)	$-7.64 \pm 0.18$	n.s.	< 0.0001	$0.01 \pm 0.10$	n.s.	$-7.79 \pm 1.15$	< 0.0001	0.001
	G (19)	$-1.35 \pm 0.29$	n.s.	0.0002	$-0.16 \pm 0.16$	n.s.	$0.42 \pm 1.74$	n.s.	0.059
Female	M (24)	$-26.11 \pm 0.26$	n.s.	< 0.0001	$-0.47 \pm 0.22$	0.039	$-20.93 \pm 2.38$	< 0.0001	0.179
	G (25)	$-10.00 \pm 0.29$	n.s.	< 0.0001	$-0.08 \pm 0.21$	n.s.	$-9.15 \pm 2.38$	0.0008	0.006
<i>O. acutipennis</i>									
Male	M (13)	$-3.70 \pm 0.32$	0.032	$-4.40$ to $-3.15$	$0.45 \pm 0.13$	0.004	$-9.38 \pm 1.60$	0.0001	0.540
	G (15)	$-0.60 \pm 0.58$	0.021	$-1.60$ to $0.59$	$-0.20 \pm 0.33$	n.s.	$1.85 \pm 4.17$	n.s.	0.026
Female	M (10)	$-6.01 \pm 0.45$	n.s.	< 0.0001	$-0.42 \pm 0.35$	n.s.	$-0.98 \pm 4.19$	n.s.	0.154
	G (10)	$-2.72 \pm 0.44$	n.s.	0.0002	$0.35 \pm 0.36$	n.s.	$-6.91 \pm 4.25$	n.s.	0.109

AI, asymmetry index, is calculated as  $\{(R - L) \times 100\} / (R + L)$ , where  $R$  and  $L$  are lengths or widths of traits on the right and the left sides, respectively. Slope and  $y$ -intercept were given by linear regression, OLS, of AI value against body size that was expressed by elytral length. All values are the mean  $\pm$  SE

M mandibular length, G genal width, n.s. not significant.

<sup>a</sup>Ninety-five percent CI of mean was obtained by the bootstrap method

**Fig. 3** Frequency distributions of asymmetry index (AI) values of mandibles and genae in *D. ruficollis* and *O. acutipennis* adults. AI value was calculated as  $\{(R - L) \times 100\} / (R + L)$ , where *R* and *L* are length or width of traits on the right and left sides, respectively. The *P* value shows the probability at which data follows the null hypothesis of AI = 0. The 95% confidence interval of mean (95% CI) was obtained by the bootstrap method. **a–d** *D. ruficollis*. **e–h** *O. acutipennis*. **a, e** Male genal width. **b, f** Male mandibular length. **c, g** Female genal width. **d, h** Female mandibular length



(Table 4). There was a significant, negative correlation between elytral length and mandibular AI values in females (Table 3). There was no significant correlation between the elytral length and mandibular AI values in males or between the elytral length and genal AI values in both sexes (Table 3). The means of mandibular and genal AI values were significantly smaller in adult females than males (*t* test,  $t_{41} = -55.63$ ,  $P < 0.0001$  for mandibles;  $t_{42} = -20.93$ ,  $P < 0.0001$  for genae) (Table 3; Fig. 3a–d).

**Head morphology of *Oxylanguria acutipennis***

There was a significant difference in body length between males (mean  $\pm$  SD = 19.11  $\pm$  3.01 mm, range = 11.38 to 21.91 mm,  $n = 14$ ) and females (16.80  $\pm$  2.16 mm, range = 13.45 to 19.54 mm,  $n = 10$ ) (Wilcoxon rank sum test,  $W = 24$ ,  $P = 0.006$ ), but not in elytral length between the sexes (12.40  $\pm$  1.86 mm, range = 8.02 to 14.11 mm,  $n = 15$  for males; 11.90  $\pm$  1.31 mm, range = 9.85 to 13.85 mm  $n = 10$

for females) (Wilcoxon rank sum test,  $W = 47$ ,  $P = 0.129$ ). A significant positive correlation was found between elytral length and body length in each sex (Pearson’s  $r = 0.997$ ,  $P < 0.0001$  for males; Pearson’s  $r = 0.989$ ,  $P < 0.0001$  for females), indicating that the difference in elytral length directly reflected the difference in adult body size for each sex.

In each adult female of *O. acutipennis*, mandibular length and genal width exhibited left-directional asymmetry patterns (*t* test,  $t_9 = -13.48$ ,  $P < 0.0001$  for a hypothesis of mean = 0 in mandibular AI values;  $t_9 = -6.17$ ,  $P = 0.0002$  in genal AI values) (Table 3; Figs. 2l and 3g, h). In each male adult, mandibular length exhibited left-directional asymmetry patterns (95% CI = -4.40 to -3.15) but genal width exhibited no clear asymmetry patterns (95% CI = -1.60 to 0.59) (Table 3; Figs. 2j and 3e, f).

Male and female adults of *O. acutipennis* exhibited significant, positive correlations between the elytral length and mandibular length and between the elytral length and genal width (Table 4). There was a significant, positive correlation between

**Table 4** Relationships of mandibular length and genal width to body size of *Doubledaya ruficollis* and *Oxylanguria acutipennis* adults

Species	Sex	Trait (no. examined)	Length (mm)	Slope	P value	y-intercept	P value	R <sup>2</sup>
<i>D. ruficollis</i>	Male	LM (19)	1.82 ± 0.06	0.15 ± 0.01	<0.0001	0.21 ± 0.07	0.006	0.973
		RM (19)	1.55 ± 0.06	0.13 ± 0.01	<0.0001	0.16 ± 0.07	0.034	0.958
		LG (19)	1.28 ± 0.04	0.10 ± 0.00	<0.0001	0.18 ± 0.04	0.0008	0.975
		RG (19)	1.25 ± 0.04	0.09 ± 0.00	<0.0001	0.22 ± 0.05	0.0002	0.968
	Female	LM (24)	2.93 ± 0.06	0.27 ± 0.01	<0.0001	-0.06 ± 0.16	n.s.	0.940
		RM (24)	1.72 ± 0.04	0.14 ± 0.01	<0.0001	0.16 ± 0.13	n.s.	0.866
		LG (25)	1.89 ± 0.05	0.16 ± 0.01	<0.0001	0.13 ± 0.07	n.s.	0.962
		RG (25)	1.55 ± 0.04	0.13 ± 0.01	<0.0001	0.13 ± 0.08	n.s.	0.934
<i>O. acutipennis</i>	Male	LM (13)	1.44 ± 0.05	0.10 ± 0.01	<0.0001	0.21 ± 0.11	n.s.	0.920
		RM (15)	1.32 ± 0.05	0.10 ± 0.01	<0.0001	0.08 ± 0.11	n.s.	0.911
		LG (15)	1.06 ± 0.04	0.08 ± 0.01	<0.0001	0.10 ± 0.08	n.s.	0.916
		RG (15)	1.05 ± 0.04	0.07 ± 0.01	<0.0001	0.15 ± 0.09	n.s.	0.895
	Female	LM (10)	1.43 ± 0.04	0.09 ± 0.01	<0.0001	0.34 ± 0.10	0.007	0.943
		RM (10)	1.27 ± 0.03	0.07 ± 0.01	0.0009	0.43 ± 0.17	0.033	0.765
		LG (10)	1.11 ± 0.03	0.07 ± 0.01	<0.0001	0.22 ± 0.07	0.016	0.950
		RG (10)	1.05 ± 0.03	0.08 ± 0.01	<0.0001	0.12 ± 0.07	n.s.	0.952

Slope and y-intercept were given by linear regression, OLS, of the traits against body size that was expressed by elytral length. All values are the mean ± SE

LM left mandibular length, RM right mandibular length, LG left genal width, RG right genal width, n.s. not significant

elytral length and mandibular AI values in males (Table 3). There was no significant correlation between the elytral length and mandibular AI values in females or between the elytral length and genal AI values in both sexes (Table 3). The means of mandibular and genal AI values were significantly smaller in adult females than males (Wilcoxon rank-sum test,  $W = 12$ ,  $P = 0.0005$  for mandibles;  $W = 35$ ,  $P = 0.026$  for genae) (Table 3; Fig. 3e–h).

### Ovipositor morphology

Dissection of five *D. ruficollis* females (elytral length: mean ± SD = 11.36 ± 1.54 mm, range = 8.73 to 12.62 mm,  $n = 5$ ) revealed that the ovipositor was short (mean ± SD = 2.01 ± 0.17 mm, range = 1.72 to 2.14 mm,  $n = 5$ ) and less sclerotized (Fig. 1h), suggesting that the ovipositor does not contribute to the path construction to the internode cavity.

In the case of *O. acutipennis*, dissection of five females (elytral length: mean ± SD = 11.70 ± 1.45 mm, range = 10.40 to 13.85 mm,  $n = 5$ ) revealed that the ovipositor was long (mean ± SD = 6.30 ± 0.51 mm, range = 5.69 to 6.92 mm,  $n = 5$ ), strongly sclerotized, and sharpened at the apex (Fig. 1p), suggesting that they could make the ovipositor penetrate the wall of bamboo internodes.

### Discussion

Few animals are known to inhabit the large internode cavities of mature culms of bamboos as initial users, suggesting that these

cavities are an inaccessible niche for most animals. In this study, we observed that both *D. ruficollis* and *O. acutipennis* used the large internodes of mature bamboo culms as oviposition substrate, which constitutes the second record of initial users of the large internodes of woody bamboo culms in insects. *D. ruficollis* females had markedly asymmetrical mandibles and genae and used their mandibles when depositing the eggs in the internode cavities through the bamboo wall. In contrast, *O. acutipennis* females had slightly asymmetrical mandibles and genae and sclerotized long ovipositors, and used their mandibles and ovipositors when ovipositing in the internode cavities through the bamboo wall. These two examples suggest that the specialization of different morphological traits (i.e., mandibles and ovipositor) is related to oviposition strategy for large woody bamboo internodes in languriine beetles.

We found a similar pattern of bamboo use in *D. ruficollis* and *O. acutipennis*. *D. ruficollis* oviposited on a weakened, mature culm using the mandibles. *O. acutipennis* also used the mandibles to oviposit on newly dead, mature culm. The approach of using their mandibles was the same as that described for other *Doubledaya* species (Toki et al. 2014, 2016).

The mean number of COMs per COM-present internode was close to 1 for both *D. ruficollis* and *O. acutipennis*. This is also the case for the grass-feeder *Anadastus pulchelloides* Nakane, *D. bucculenta*, and *D. sinuata* but different from the soft-bamboo user *D. tonkinensis* (Toki and Togashi 2013; Toki et al. 2014). This similarity of oviposition pattern suggests that COMs deter females from ovipositing in internodes that contain COMs in Languriinae.

For both species, an internode cavity was occupied by only a single individual. Individuals occupying an internode cavity did not penetrate the division walls separating adjoining bamboo cavities, which indicate that the beetle larvae did not travel across the internodes. These observations suggest that either only a single egg is deposited in a COM or that there is fatal competition among the multiple larvae developing in the same internode, as it has been suggested in several other languriine species (Genung et al. 1980; Toki and Togashi 2013; Toki et al. 2016). Moreover, our observations suggest that the size of the internode cavity does not determine the coexistence of conspecific individuals in a bamboo internode.

One approach that females can follow to excavate the bamboo wall involves fixing a tip of one mandible on the surface of the bamboo and using the other mandible to cut into the wall. In this case, the distance between the mandibular tips (DMT) at the maximum opening of the mandibles (i.e., the outer edges of the mandibles are parallel to the body axis) equals the maximum wall thickness through which she can reach a cavity by the mandibles. DMT is calculated as  $\sqrt{(LM-RM)^2 + (LG+RG)^2}$ , where LM is left mandibular length, RM is right mandibular length, LG is left genal width, and RG is right genal width. We found that the mean DMT of female *D. ruficollis* (3.65 mm) is longer than the mean wall thickness of the host internodes at COM (3.00 mm), whereas the mean ovipositor length (2.01 mm) was shorter than the wall thickness. Besides, the oviposition hole made by *D. ruficollis* was cone-shaped (Fig. 1g). The relative sizes of DMT, COM, and ovipositor as well as their shape suggest that the females of this species excavate holes by penetrating the internode wall of the host bamboo by the mandibles, then insert a short, less-sclerotized ovipositor into the internode cavity through the hole. Thus, the ovipositor does not contribute to the construction of a hole into the internode cavity. The same oviposition method has been described for *D. bucculenta* (Toki et al. 2012, 2013).

In contrast, the ovipositor seems to play an important role in the construction of holes to reach the bamboo internode cavity in females of *O. acutipennis*. The mean DMT (2.17 mm) in *O. acutipennis* females was shorter than the mean wall thickness of the internodes at COM (3.37 mm). This indicates that the females of the species cannot penetrate the wall into the bamboo cavity using only the mandibles. Interestingly, however, the mean ovipositor length (6.30 mm) was much longer than the wall thickness. We observed that the oviposition hole of *O. acutipennis* was funnel-shaped (Fig. 1o) and females excavated only 29% (0.99 mm/3.37 mm) of the internode wall thickness using the mandibles. In other words, more than 70% of the wall thickness was penetrated by the well-developed ovipositor. Funnel-shaped oviposition holes are also made by the hard-bamboo user,

*D. sinuata*, and the soft-bamboo user, *D. tonkinensis*, both of which have long, needle-like ovipositors (Toki et al. 2016).

The two studied large-bamboo users also showed contrasting morphological patterns. A more markedly asymmetrical head with mandibles was observed in *D. ruficollis* and a less asymmetrical head with mandibles but long and strongly sclerotized, needle-like ovipositor was observed in *O. acutipennis*. The level of dependence on the mandibles to construct a path varies among hard-bamboo users. Species such as *D. ruficollis* and *D. bucculenta* have a 100% dependency, whereas *D. sinuata* depends on its mandibles only 57% (1.37 mm/2.40 mm), and *O. acutipennis* only 29% (Toki et al. 2016). AI values of the mandibular length and genal width of female beetles also differ among species. *D. ruficollis* shows the smallest, i.e., the most asymmetrical, AI values (mean  $\pm$  SE of AI values =  $-26.11 \pm 0.26$  for mandibles,  $-10.00 \pm 0.29$  for genae), followed by *D. bucculenta* ( $-25.53 \pm 0.17$  for mandibles,  $-7.77 \pm 0.29$  for genae), *D. sinuata* ( $-14.46 \pm 0.22$  for mandibles,  $-7.31 \pm 0.60$  for genae), and *O. acutipennis* ( $-6.01 \pm 0.45$  for mandibles,  $-2.72 \pm 0.44$  for genae) (Toki and Togashi 2011; Toki et al. 2016). The contrasting morphological patterns of the two studied large-bamboo users suggest different evolutionary trajectories in the utilization of large bamboo internodes between them, but phylogenetic studies will be required to test this hypothesis.

The mean depth of cone-shaped depressions in the oviposition holes of *O. acutipennis* was slightly shallower than that reported for the soft-bamboo user *D. tonkinensis*. The females of *D. tonkinensis* excavate 23% (1.03 mm/4.53 mm) of the internode wall thickness using the mandibles (Toki et al. 2016). However, AI values of the mandibular length and genal width of *O. acutipennis* females were more markedly asymmetrical than those previously observed in *D. tonkinensis* females (mean  $\pm$  SE of AI values =  $-4.41 \pm 0.20$  for mandibles,  $-1.58 \pm 0.31$  for genae) (Toki et al. 2016). The ovipositor of *O. acutipennis* (mean  $\pm$  SD =  $6.30 \pm 0.51$  mm) was much longer than that reported for *D. tonkinensis* ( $3.44 \pm 0.24$  mm) (Toki et al. 2016). The higher asymmetry of the head with mandibles and the longer sclerotized ovipositor observed in *O. acutipennis* may be related to the hardness of the internode wall as well as the wall thickness.

*D. ruficollis* and *O. acutipennis* showed similar bamboo-using patterns but strikingly different morphologies associated with the excavation of holes into the bamboo wall. Different morphological specialization may not necessarily indicate the use of different niches in bamboo-using languriines, in the same way that similar morphological specialization does not necessarily indicate the use of similar niches. For example, *D. tonkinensis* and *O. acutipennis* show similar morphologies (i.e., slightly-asymmetrical mandibles and needle-like ovipositor) but a different bamboo-using pattern (*D. tonkinensis* uses soft-walled bamboos, and *O. acutipennis* uses hard-walled bamboos) (Toki et al. 2016).

The body sizes of *D. ruficollis*, one of the largest species of *Doubledaya* (Villiers 1945), and *O. acutipennis* are not different from that of *D. bucculenta*. However, the host bamboo internodes occupied by a single individual are much larger in *D. ruficollis* and *O. acutipennis* than in *D. bucculenta* (Toki and Togashi 2011). The absence of correlation between the body size and the host bamboo size may be due to a larval dietary difference among them. It is known that *D. bucculenta* farms a yeast symbiont for food, resulting in a large body size to host bamboo size (Toki et al. 2012, 2013). Further study of larval diets of *D. ruficollis* and *O. acutipennis* is required to test this hypothesis.

Although the two langurine beetles examined in this study oviposit in the cavity of large-sized bamboos, the host bamboo walls are relatively thin compared with those being penetrated by the other known initial cavity users of large-sized bamboos, such as the woodpecker (10 mm) and the long-horned beetle (4–11 mm) (Kovac and Yong 1992; Round et al. 2012). This preference for thin-walled bamboo species may be due to the fact that langurine beetles are smaller in size than woodpeckers and, unlike the long-horned beetle, they do not depend on woody tissue for nutrition. Langurine beetles depend on pith tissue or fungi grown on the pith. It is also possible that langurine beetles are physically unable to access the cavity of the internodes with too thick walls for oviposition.

The present study suggests that morphological specialization of oviposition-related body parts (either mandibles or ovipositor) enable the langurine beetles to access the large internode cavities of mature culms of bamboos. These environments are competitor-free, as very few species are known to be able to access them. After the individuals of these species reach adulthood, they make exit holes and leave the natal bamboo internodes, leaving the holes open through which secondary users can access the cavities. As such, the large-bamboo-using lizard beetles *D. ruficollis* and *O. acutipennis* may play an important role in the bamboo ecosystem, especially given that they show an allopatric, wide distribution in tropical Asia.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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