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Cover: Emperor Tamarin *Saguinus imperator*: a look into a better world through the mustache lens – mixed media illustration. © Maya Santhanakrishnan.

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ARTICLE

Bio-ecology of the bush cricket *Tarbinskiellus portentosus* (Lichtenstein, 1796) (Insecta: Orthoptera: Gryllidae): a relished edible insect in Nagaland, India

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Abstract: *Tarbinskiellus portentosus* (Lichtenstein, 1796) (bush cricket), also called "viituo" in the Angami dialect, belongs to the order Orthoptera and the family Gryllidae. It is one of the most common edible insects found in Nagaland and is a potential source of animal protein and other nutrients. Despite being highly preferred as food and relished, studying their ecology, biology, and market potential is nonexistent, at least in Nagaland, India. Therefore, the present study was conducted to fill the knowledge gap on the biology and market potential of *T. portentosus.* Insects were collected from the wild and reared as stock at 20–25 °C. The results show that *T. portentosus* undergoes seven nymphal instars to fully develop into an adult with an average growth rate of 9.94 ± 2.43 mg/day. *T. portentosus* is found in the grassland vegetation in burrows up to 800 mm depth. Adult males weigh about 2940 ± 93.0 mg, and females weigh 2980 ± 200 mg. The incubation period of eggs was 33.8 ± 0.96 days and showed a moderate percent of hatching efficiency (45.20 ± 0.28). In laboratory conditions, this cricket completed its life cycle in 341 ± 4.29 days. Collection of adults involves handpicking and pouring water, cleaning involves a gut removal process through head pulling, and preparation for consumption is done by cooking with local spices, fried or roasted. *T. portentosus* are sold in the local market at INR 300/- for 250–300 g. With scanty information on growth and reproduction, the present study serves as a baseline for future studies on the biology of *T. portentosus* that may uplift the local market through mass rearing.

Keywords: Entomophagy, food security, northeastern India, rearing, socio-economy, soil cricket.

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Author contributions: PK—experimentation, field visitation, data collection, manuscript drafting; LJ—experimentation, field visitation, data collection, editing, and statistical analysis; BA—supervision, review, and editing; LK—conceptualisation, supervision, review and editing

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INTRODUCTION

Expansions of food production are the primary source of greenhouse gas emissions, with livestock products being one of the most significant contributors that trouble the ideas of modern sustainable means of livelihood (FAO 2017). Insect productions have little environmental consequences compared to traditional livestock, and due to their physiology, insects have better feed conversion rates and growth efficiency (Oonincx et al. 2015). For instance, *Acheta domesticus* has a feed conversion ratio of 2.1, meaning 2.1 kg of feed is required to produce 1 kg of edible products. In comparison, for other conventional livestock such as cattle, pigs, and poultry, 25, 9.1, and 4.5 kg of feed is required to produce 1 kg of meat (Van Huis 2013). Edible insects provide 5–10% of the animal protein as well as fat and calories, and various vitamins (A, B1, B2, and D) and minerals (iron, calcium) (Gullan & Cranston 2005; McCluney & Date 2008). Therefore, developing a new sustainable source of edible insects has been recommended (Nikkhah et al. 2021). Despite serving as a potential source of nutrients, only a few species of insects are mass-reared. According to the European Food Safety Scientific Committee, only nine different species of insects are being reared and farmed in mass to be used as food (Halloran et al. 2017).

One of the downfalls of unsuccessful mass-rearing of edible insects could be lack of essential information on biology, growth, and reproduction. There are approximately 20,000 insect farms in Thailand that produce 7,500 metric tons per year, and cricket is one of the promising insect that is farmed, produced in mass, and used for domestic consumption as well as for selling in the market (van Huis et al. 2015). South Korea is another leading consumer of edible insects, and their consumption has resulted in increased demand. The edible insect market's value in south Korea increased from 143 million in 2011 to 259 million in 2015 (Shin et al. 2018). Similarly, entomophagy practices are widespread in India (particularly Nagaland, northeastern India) (Kiewhuo et al. 2022). Among the 106 edible insects consumed and relished, bush cricket *Tarbinskiellus portentosus* is one of the most preferred insects in the region (Mozhui et al. 2020). Therefore, studies on its biology and reproduction are essential to enable the efficient usage of this promising insect.

T. portentosus belongs to the order Orthoptera, family Gryllidae, and is commonly found in southern and southeastern Asia, including India, Thailand, and Indonesia. This species spends most of its time under burrows with a single individual per burrow (Tantrawatpan et al. 2011). It feeds on fresh plants and is considered crucial human food as this species has higher economic value in southeastern Asia, especially Thailand (Sverdrup-Jensen 2002). Yhoung-Aree (2010) reported protein and fat content of 12.8/100 g and 5.7/100 g, respectively in *T. portentosus.* Due to its high nutritional value, *T. portentosus* has been consumed in many countries (Buzzetti & Devrisese 2008; Yi et al. 2010). In Thailand, *T. portentosus* is available all year round at US$ 4.8 per kg (Siriamornpun & Thammapat 2008). *T. portentosus* is also an important protein source for fisheries and poultry production (Sverdrup-Jensen 2002; Razak et al. 2012) . A noteworthy study on the life history of *T. portentosus* in the laboratory was conducted at 24.68 ± 1.26 °C by Hanboonsong & Rattanapan et al. (2001). The authors highlighted information on its longevity, number of instars, incubation period, and food habits. A recent studies on genetic variation in mainland Southeast Asia, such as the Lao People's Democratic Republic, Cambodia, and Myanmar, show that three morphotypes of *T. portentosus* are available (Pradit et al. 2022). Although considered highly relished insects with potential economic value, detailed information on their biology under Nagaland's climatic condition is lacking.

In Nagaland, *T. portentosus*, commonly known as 'viituo', is primarily available during the rainy season, with its population peaks during August–October. They are preferred as food by many sections of society due to the nutritional and cultural values associated with them. Although *T. portentosus* is preferred as a food supplement it is not fully explored in Nagaland due to lack of knowledge of its biology. Given the availability of technique to mass-produce, *T. portentosus* has enormous potential to be made available for consumption at a minimal cost of environmental pollution. In its available season (June–August), *T. portentosus* adults are sold at INR 150/- per 300 g in the local market. However, before mass production, life history study is a vital footstep that can facilitate the efficiency of insect utilization. Therefore, the present study assesses the biology and life cycle of *T. portentosus* under laboratory conditions to fill the gap in the existing knowledge on this novel insect**.**

MATERIAL AND METHODS

Study area

The present survey for soil cricket was carried out in one-year-old abandoned Jhum cultivated lands at Lumami and Zaphumi villages, Zunheboto district (26°13´42.82´´N and 94.28´24.70´´E) (Figure 1). Since, the two villages are in close proximity, both areas exhibit primarily grassland type vegetation.

Sample collections

Field visits were carried out to collect *T. portentosus* adults from the above-mentioned sites during the summer season (June–August in each year) consecutively for three years from 2019–2021. Crickets were collected by digging their burrows using a spade/ machete and kept in containers with holes to provide sufficient oxygen till they were brought to the laboratory for rearing. Morphologically, identification was done by Mr. Sawapan Pal, Assistant Zoologist, Orthoptera Section, Zoological Survey of India (ZSI), Kolkata.

Rearing

The natural light and dark cycle during insect rearing ranged from 10–13 h to 12–14 h. Adult crickets were kept in large plastic containers with dimensions of 60 cm (length), 40 cm (breadth), and 45 cm (height) filled with 40 cm of loose soil. The adults of *T. portentosus* were provided with natural food (leaves found in their burrows such as *Brassica oleracea var capitata* (cabbage), *Ageratina adenophora* (Mexican devil) and

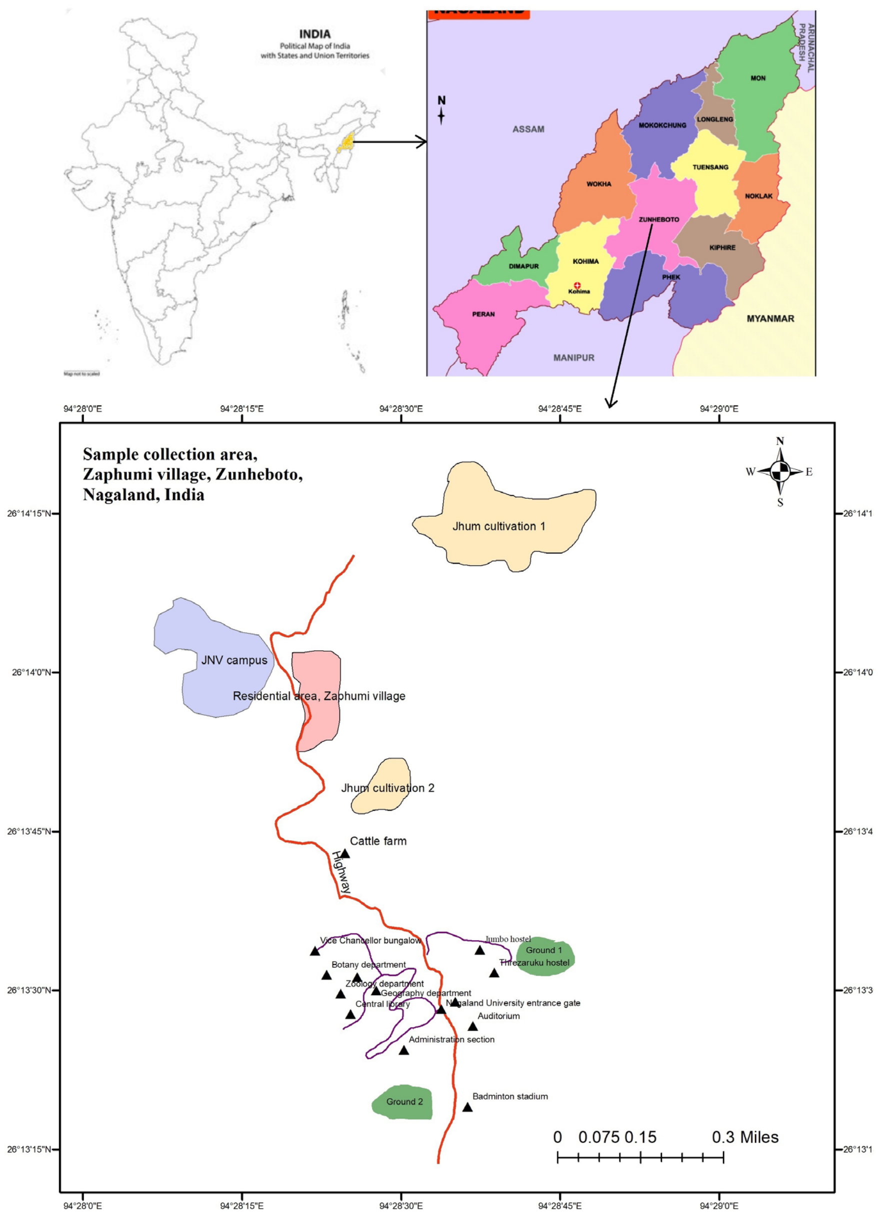

Figure 1. Map showing the sample collection areas at Zaphumi and Lumami village, Zunheboto, Nagaland.

water. The moisture content of the soil was maintained at 35–45 % (Gravimetric method) and the temperature of the rearing room ranged between 20–25 °C. During the year 2019–2021, life cycle studies were repeated thrice (once in each year), and the results were based on a sample size of 75 males and 75 females in each cycle.

For growth rate studies, fresh weight of different nymphal stages (alive) of *T. portentosus* was taken using a portable weighing machine (iScale i-400c) at intervals of seven days (irrespective of nymphal period) until the adult stage, and the growth rate was calculated using the formula.

$$\text{Growth rate} = \frac{\text{Maximum weight–minimum weight}}{\text{Number of days to gain maximum weight}}$$

The final growth rate was reported as mg per day, where, Maximum weight = Final weight of the nymph on the day of measurement | Minimum weight = Weight of the previous measurement (beginning of the 7 day) | Number of days to gain maximum weight = Number of days counted from the day of minimum to maximum weight of the nymph (here we count 7 days and weight was taken in triplicate).

To estimate eggs laid per female, penultimate nymphs were segregated in a separate container at a 2:1 ratio of male to female. This experiment was performed in five containers keeping three individuals (2:1 male:female) in each container; consequently, egg counting per female was carried out using five females. Egg collection was done daily; prior to egg collection, adults were shifted to another container with the help of insect-catching nets. Once all the adults were carefully shifted, eggs were collected manually using a spatula by searching through the soil (Image 1A). A single egg was kept in each of the five containers (100 mm length, 40 mm breadth, 30 mm height) and observed using a Labomed CZM6 microscope. Further, five eggs were taken for morphological observations, such as changes in their color, length (alloet-vernier caliper), weight (iScale i-400c), and incubation period were observed till they hatched. Ten containers were utilized for life cycle studies, each containing soil with an adequate moisture level. Subsequently, a single nymph (first instar) was introduced into each container and observed daily to understand the changes in body coloration, weight, size, and length.

The size of the container for rearing was as per the size of the instar. Water was sprayed regularly to moisten the soil (Image 1B). The first four instars were kept in rounded containers of 110 mm diameter and 120 mm height filled with soil up to 50 mm and covered with net (Image 1C). The last three instars were kept in circular plastic containers of 180 mm in diameter, 200 mm in height, filled with soil up to 100 mm, and covered with nets. Adults were kept in plastic containers 280 mm in length, 200 mm in breadth, and 150 mm in height (Image 1D) filled with loose soil up to 120 mm and provided with sufficient leaves (*Brassica oleracea* var. *capitata*, *A. adenophora*). The excess food in the rearing containers was removed and cleaned by handpicking on a regular basis. Once the nymphs matured into adults, the interactions between males and females were observed in five containers by keeping five males and five females in each container and the experiment was done in five containers simultaneously.

All data were presented as mean ± SD for three years. Analysis of variance (One-way ANOVA) at 95% interval (p <0.05) was done to find the mean significance difference in *T. portentosus* instars. Each test was followed by a multiple comparisons test (Tukey test) to find the mean difference between the variables. Statistical analysis was performed using SPSS-22 software.

Observations of burrows in the wild

The depth of the burrows was estimated by digging from the entrance to the bottom and measuring using a scale. At the same time, the distance between burrows was measured from the entrance of one burrow to the other.

RESULT

Life stages *T. portentosus*

Eggs: Five eggs were observed for morphological study. Freshly laid eggs are oblong in shape, glabrous, and yellowish-white, 3 ± 0.05 mm in length, and 36 ± 0.57 mg in weight. The weight of eggs was recorded on day 2 (36 ± 1.04 mg), day 7 (56 ± 0.21 mg), and day 11 (66 ± 1.23 mg), and it constantly increased till the final weight on day 31 (74.8 ± 1.67 mg). While no difference in egg weight between day 1 (on laying) and day two was observed, a significant increase in weight (p <0.05) was noticed, and eggs became heavier prior to the hatching. There were not many changes in the length of the eggs from the initial to the final stage. The eggs are slightly more pointed towards one end and round towards the other end (Image 2A). The chorion being translucent, the embryo is visible to some extent.

On the 7th day after oviposition, the middle part of the egg becomes bent, forming a convex plane on one side and a concave plane on the other (Image 2B). On

Image 1. A—Collection of the egg using high-intensity light | B—Water sprayed to keep the soil moist for eggs | C—The round container | D—Rectangular box for rearing adult's crickets. © Authors.

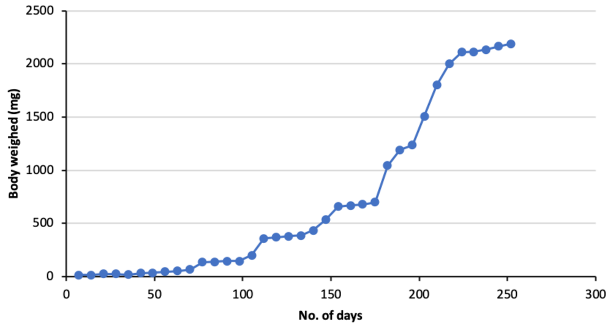

Figure 2. Increase body weight (mg) of *T. portentosus* during different stages of development.

the 13th day, the egg becomes slender and more bent towards the ends (Image 2C). On day 20, the eggs begin to break open, and the embryonic eyes become visible (Image 2D). The eyes, outlines of the wings, and body appendages became distinct on day 28 (Image 2E). The forelegs, hind legs, wings, and cerci became visible as the egg starts to break open widely (Image 2F) and subsequently hatching took place. By the 28th, 29th, and 30th days, the eyes became more prominent, and body appendages were faintly visible until the cracks began to occur till hatching. The final weight and length of egg before hatching was 89.0 ± 0.13 mg and 4.89 ± 0.16 mm. The incubation period of eggs was 33.8 ± 0.96 days. The hatching efficiency was 45.20 ± 5.28 %.

Nymphs

In *T. portentosus,* seven instars were observed*.* The newly hatched nymph was white and possessed a soft body until transitioning into light brown. The main morphological changes between the instars were the development of the wing pad, coloration, body size, body mass, ovipositor development, and an increase in antennal length. A newly hatched instar weighed 34 ± 1.13 mg and had a body length of 4.0 ± 0.00 mm with an 8 mm antenna and 3 mm cerci (Table 1). The coloration of the body was grey-blackish on the abdomen, and its head region was light brown during the first three instars. The first five instars had a round head and abdomen with not-so-visible segments on the body (Image 3A–E). No differences were observed during the first five instars concerning their body color and shapes except for increased body sizes, weight, antennal length, etc. The wing pad developed in the sixth instar (Image 3F) in both male and female nymphs. In females, the ovipositor appeared in the seventh instar (Image 3G) while the body color was darker, and the nymphs became broader and less rounded than the first five instars. With further development, the head, legs, and abdomen became more turgid, and the color became dark brown. After the final molt, the adults weighed 2100 ± 379.86 mg with length 31.7 ± 1.0 mm, antennae 40 mm, and cerci 16 mm.

Body weight, total length, antennae, and cerci were significantly different (p <0.05) from instar to instar (Table 1). As shown in Figure 2, the first, second, third, fourth, fifth, sixth, and seventh instars molted on day 42, 77, 119, 147, 182, 210, and 252, respectively. During the rearing period the growth rate ranged 4.2–7 mg/day. Wide variations in growth rate were observed, where the maximum growth rate was observed at 70–80 days (during II instar). With further maturity, the average growth rate declines gradually. The development from the first instar to the final molt required 298 ± 8.24 days. After the final molt, cricket lived for another 43 ± 6.5 days. Therefore, the total number of days required to complete the entire life cycle was found to be 341 ± 4.29 days.

Adults

Adult cricket has a cylindrical body, long slender antennae (34–40 mm), a round head, and two long cerci (15–16 mm) and a long ovipositor (11 mm) for females. The hind leg has an enlarged femur followed by three tarsal segments. The forewings (30 ± 1.4 mm) are smooth in females and rough in males (Image 3H). The hind wings (41.5 ± 2.1 mm) are longer than the fore wings and are lighter in color (Image 4A). The female body measured about 36 ± 1.7 mm in length, and weight about 2980 ± 200 mg, while the male measured about 37.3 ± 0.05 long (Image 4B), weight about 2940 ± 93.0 mg. The morphological study of adults was done based

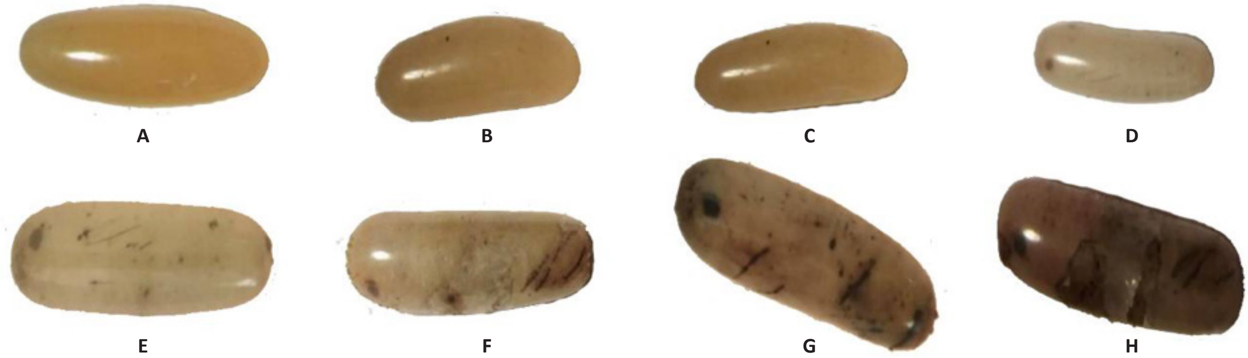

Image 2. A—Day 1 oblong compact yellowish in color | B—Day 7 yellowish-white in color and bent at the center | C —Day 13 more slender and thinner | D—Day 20 eyes become visible, egg white | E—Day 28 egg white and more compact, body appendages visible | F–H—Day 29 to 31 brown dirty look on the inside with stretch marks, cracks appear. © Authors.

Table 1. The instars of *T. portentosus* with details of body weight, body length, antennal length, and cerci length.

Instar	Fresh body weight (mg)	Body length (mm)	Antennal length (mm)	Cerci length (mm)
1st	34 ± 19.13[a]	4.0 ± 0.00[a]	8.0 ± 0.21[a]	3.0 ± 0.76[a]
2nd	137 ± 90.37[b]	13.3 ± 1.7[b]	10 ± 0.44[b]	4.0 ± 0.01[b]
3rd	360 ± 124.34[c]	16.6 ± 2.0[c]	15 ± 0.19[c]	6.0 ± 0.34[c]
4th	561 ± 68.00[d]	21.6 ± 1.5[d]	20 ± 0.32[d]	8.0 ± 0.12[d]
5th	1044 ± 434.72[e]	24.0 ± 1.0[e]	31 ± 1.53[e]	10.0 ± 2.00[e]
6th	1802 ± 296.02[f]	31.0 ± 4.3[f]	37 ± 1.41[f]	12.0 ± 0.45[f]
7th	2100 ± 379.86[g]	31.7 ± 1.0[g]	40.0 ± 1.89[g]	16.0 ± 2.90[g]

Mean with different superscripts indicates the statistically significant difference at $p<0.05$.

on three males and three females.

Ecology of *T. portentosus*

In the wild, we found cricket burrows in grassland-type vegetation with sparse trees. Burrows were located by searching for a heap of loose soil mounted around the entrance (Image 5A). A heap of moist and finely-grained soil at the entrance indicates the presence of cricket inside the burrow. Depending on the instar and texture of the soil, the burrows can be as deep as 50–800 mm. In loose soil areas, adult burrows go as deep as 800 mm, while in rocky soil, if less moisture and roots of trees are present, burrows can be as shallow as 50–200 mm. The burrows do not have any branching at any angle, but towards the bottom, they become less vertical, and that is where crickets store their food brought from outside. The burrows are constructed in such a way that the bottom is not exposed directly to sunlight. At the end of the burrow, crickets were found embedded in soil with its back towards the entrance (Image 5B). Fresh plant leaves such as *Brassica oleracea* (cabbage) and *A. adenophora* (Mexican devil) were found inside the burrow (Image 5C). During the spring season (March–April of each year), in its nymphal stage, cricket burrows were found to be located very close to one another, just 20–50 mm apart. However, in the summer season (August–September, 2019–21), adult burrows were never found close to each other. Single adult per burrow was observed except during mating season, i.e., August–mid-October, when we observed a male and a female together in one burrow. Adults begin to appear on the ground and are attracted towards the light source at night during the month of May, peaking from July–September, with a few still present in October. During mating season, male crickets were observed stridulating near the burrow entrance, (Image 6A); during the evening and night hours, to attract females.

Adults and nymphs mostly stay inside the burrows and are not seen much as they do not venture out during daylight, except for food collection and mating purposes. All stages are nocturnal, and individuals construct their burrows using the mandibles to clear the soil, which

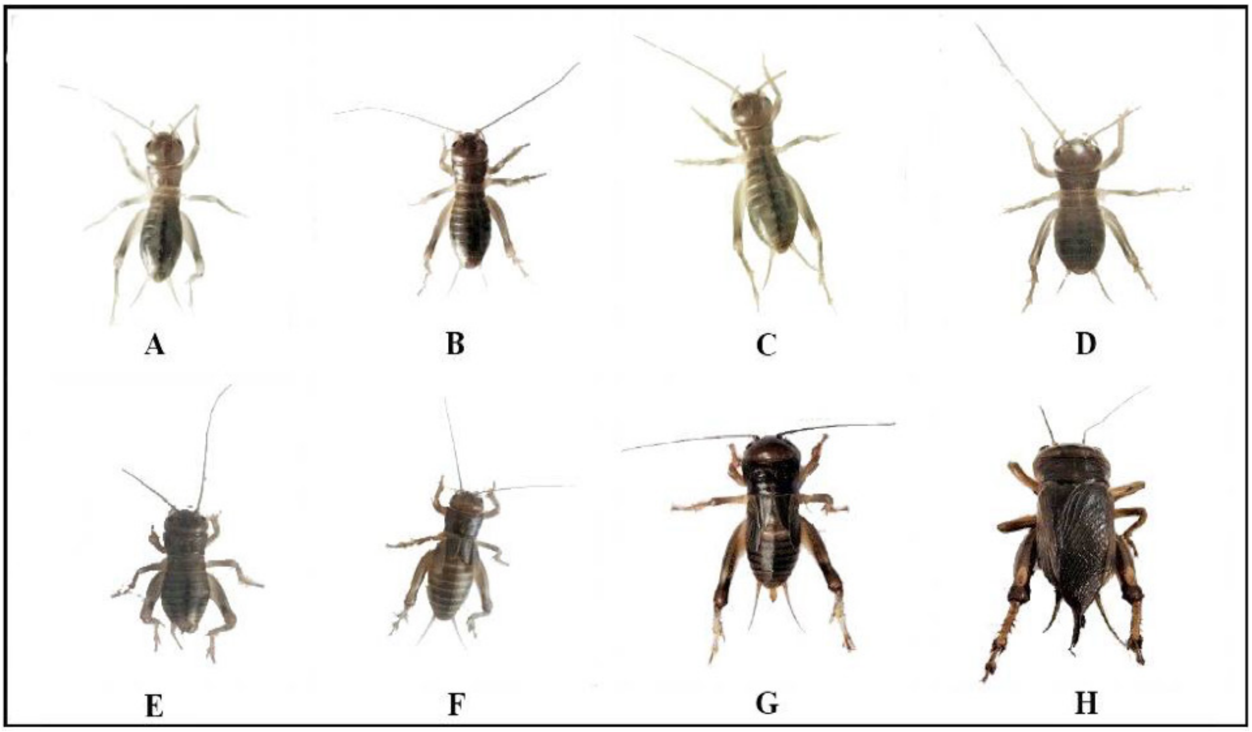

Image 3. A–G—The seven instars | H—an adult *T. portentosus*. © Authors.

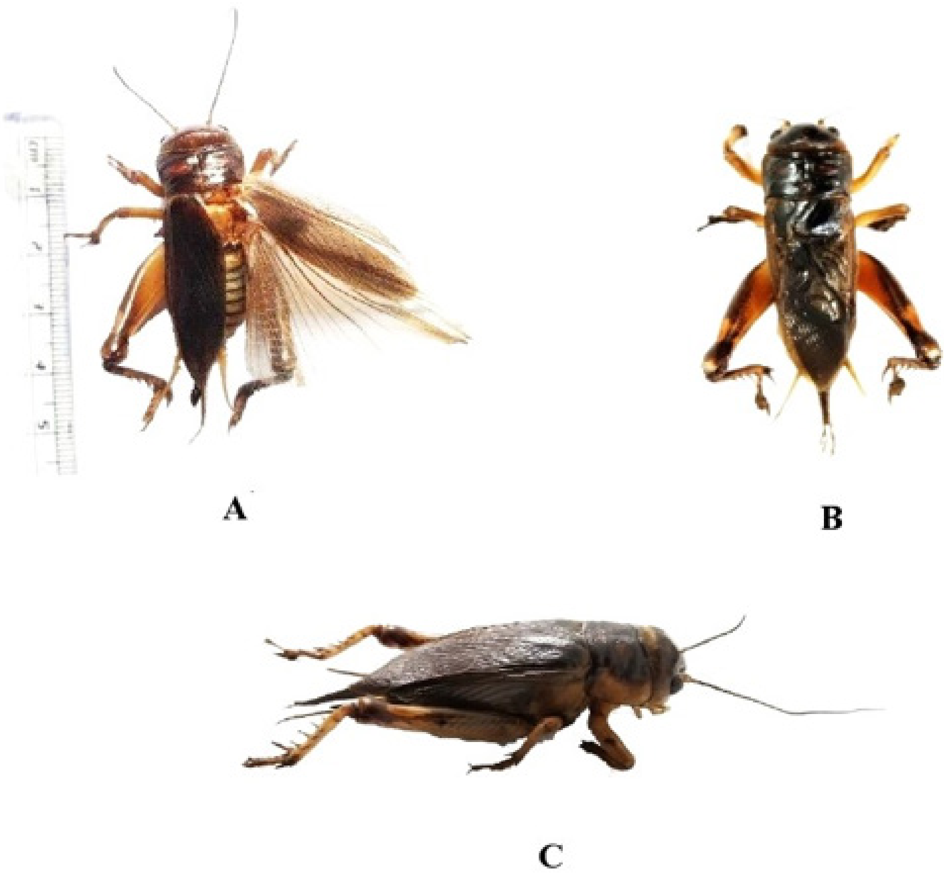

Image 4. A—Adult female | B—Adult male *T. portentosus* | C—Lateral view of *T. portentosus*. © Authors.

Image 5. A—Heap of loose soil mounted around the burrow's entrance | B—*T. portentosus* embedded at the end of the burrow | C—Food stored in burrows. © Authors.

Image 6. A—A male *T. portentosus* | B—Adult *T. portentosus* digging its burrow. © Authors.

is pushed outside the burrows using their hind legs backward (Image 6B). During mid-August, most are in their nymphal stage, while a few are in their adult stage.

Behavioral observation in the laboratory showed that adults of the same gender showed more aggression towards each other when put together for the first time. However, within 5–10 minutes, they start constructing their burrows, which keeps them away from each other. Aggressive behavior such as kicking, chirping, and biting one another was observed mainly in males during mating calls and in ovipositing females. Nymphs showed no sign of aggression towards each other. Eggs are deposited by the female into the soil through ovipositor at the bottom of the burrow at 750.8 ± 60 mm depth.

DISCUSSION

Due to bigger body size than most crickets, it is one of the Nagaland's most preferred edible insects (Mozhui et al. 2020). This is considered as one of the giant and prominent edible cricket found in Asia, with a body length and weight of 37.30 ± 5.0 mm and 2980 ± 200 mg. In Lao PDR, Hanboonsong & Durst (2014) reported that adult body length of *T. portentosus* is 50 mm, the slight variations in body size could be due to differences in climatic conditions, food availability, and nutritional content of their food.

Hanboonsong & Rattanapan (2001) reported that a single female laid about 123.00 ± 46.44 eggs, and the incubation period of eggs was 56.10 ± 15.03 days, with 40.70 ± 4.74 % of hatching, while the whole growth period including seven instars of nymph and adult was 173.70 ± 19.86 days. In the present study, the incubation period of eggs was 33.8 ± 0.96 days and showed a moderate percentage of hatching efficiency (45.20 ± 0.28%). Lesser incubation period and more hatching efficiency than that found in our population could be attributed to difference in climatic conditions. Based on five observed females, the present study records that females individually lay 98 ± 11.4 eggs throughout its reproductive life cycle. *T. portentosus* is found in abandoned jhum cultivated areas and is available for consumption from May till September. The crickets live in burrows, where they store food and lay eggs. With the growth and development of the nymph, its burrow goes as deep as 50–800 mm, and only a single generation of *T. portentosus* is produced per year. The use of mandibles and forelegs during the construction of burrows recorded in this study agrees with the study on *Anurogryllus muticus* (Lee & Loher 1996), which uses its forelegs to push out the soil substrates when digging burrows. *T. portentosus* lives inside its burrows and comes out primarily for food collection and mating. Usually, collected food is taken back to the bottom of the tunnel for feeding. During mating season, adult males come out of burrows in search of a mate and call for partner by stridulating at the edge of their burrows. After attracting a female, mating takes place, and the mated female remains within the burrow to lay eggs at the bottom. The present study observed that mated females lay eggs at the bottom of the tunnel, and similar behavior is also found in *A. muticus* (Lee & Loher 1996).

The burrow serves as a congenial environment for *T. portentosus*, allowing mating and providing protection against predators, rain, sun, and wind and acts as a convenient environment for reproduction. In addition, a sealed burrow offers protection from predators, further enhanced by increased aggression of the females during oviposition and brood care (West & Alexander 1963; Alexander & Otte 1967). Females of *Brachytrupes achatinus* Stoll (Brachytrupinae), the 'big-brown cricket' of India, deposit their eggs in shallow burrows at the end of one of their galleries, and the young nymphs leave the parental burrow a few days after hatching (Ghosh 1912). In the present study also, it has been observed that in natural condition, *T. portentosus* nymphs leave the original burrow to construct independent burrows of their own.

After the final molt, *T. portentosus* lives for another 43 ± 6.5 days; therefore, the total number of days required to complete the entire life cycle was 341 ± 4.29 days. Hanboonsong & Rattanapan (2001) also reported that *T. portentosus* completes its life cycle in 333.30 ± 20.06 days and has 6–7 instars at 24.68 ± 1.26 °C. In the present study, seven (7) nymphal instars were observed. The first molting took the most extended period, which could be attributed to the colder climate and lesser humidity (October, November, December, and January, 2019–22). The subsequent instars required lesser days for molting, which could be due to warmer season.

Egg morphology shows that on the seventh day after oviposition, it bent towards the middle, and by the 12th day, eyes were visible, and the body appendages were visible on day 28. In *Acheta domesticus*, also called house cricket, after four days of oviposition, as the eggs undergo developmental changes it become slightly curved for a few days. By the 12th day, eyes become visible; by the 16th day, just before hatching, inner body appendages are visible (Douan et al. 2021). Present study also shows similar morphological changes in the egg, but the number of days differed. The variation between species explains most of this difference, although experimental conditions could also affect the developmental time and growth of the egg (McCluney & Date 2008; Doherty et al. 2018).

CONCLUSIONS

T. portentosus is a promising edible insect that shows positive prospects for future food development. Compared to most crickets, it has a larger body mass, making it more preferred as food, especially in Asian countries. For better access to any food product, continuous market supply is an important key that should depend on a mass production unit. With proper knowledge of its biology and domestication, *T. portentosus* can be a potential insect as food in Nagaland, India. However, further extensive study of its biology, along with technology for mass rearing, can boost the economy and provide a livelihood for the weaker sections of society.

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