Nest architecture and colony characteristics of three stingless bees in North Vietnam with the first description of the nest of *Lisotrigona carpenteri* Engel (Hymenoptera: Apidae, Meliponini)

Tong X. Chinh¹, Marinus J. Sommeijer², Willem J. Boot³ & Charles D. Michener⁴

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¹ Bee Research and Development Centre, 68 Nguyenhong, Langha, Dongda, Hanoi, Vietnam

² Department of Social Insects, Utrecht University, P.O.Box 80.086, NL- 3508 TB Utrecht, The Netherlands

³ Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands

⁴ Division of Entomology, University of Kansas, 1460 Jayhawk Boulevard (Snow Hall), Lawrence, Kansas 66045-7523, USA.
Reproduction in eusocial bees

Summary. In the tropical primary forest of Cuc Phuong National Conservative Biosphere, North Vietnam and its bufferzones, we studied three sympatric stingless bee species: Lisotrigona carpenteri Engel, Trigona (Tetragonula) laeviceps Smith and Trigona (Lepidotrigona) ventralis Smith. Nest-architecture, adult population, the number of brood cells, the presence of adult reproductives, the proportion of males in brood, the number of queen cells, honey and pollen pots, and other features were recorded from 35 wild nests. Some behavioural observations were made. L. carpenteri and T. laeviceps arrange brood cells in clusters. In contrast, brood cells in T. ventralis are arranged in horizontal combs in a brood compartment that is surrounded by an involucrum of multiple lamellae. L. carpenteri constructed its nests mainly in small crevices of man-made structures while colonies of T. laeviceps and T. ventralis were generally found to nest in live trees. The flexibility for using nest substrates other than living trees appears in these species related to colony size. T. ventralis has large colonies (up to around 10,000 adults), T. laeviceps is intermediate with colonies of up to 1,200 adult bees, and L. carpenteri has by far the smallest colonies of only up to about 400 adult bees. The construction of the external nest entrance was different in three species. L. carpenteri builds a hard brittle cylindrical tube. The nest entrance of T. laeviceps is a hole decorated with a batumen rim. T. ventralis constructs a long medium-soft tube with a funnel-extension at the end. In this paper we present the first description of the nest of the recently described species L. carpenteri.

1. Introduction
Southeast Asia is well-known for its diversity of the social honey bees, the Apini and it is considered to be the origin of the only genus recognized,Apis (Cornuet, 1986). All Apis species are found in Southeast Asia (Otis, 1997), even Apis mellifera that naturally occurs in Africa and Europe (Ruttner, 1988) but has been introduced during the last few hundred years. Despite the sympatric occurrence of all Apis species, the general bee fauna in tropical Southeast Asia is rather poor in species compared with the Neotropics and tropical Africa (Michener, 1979), although this region has a rich angiosperm flora (Withmore, 1984). This may be related to humid weather conditions throughout the year, since bees are most diverse in the warm temperate dry regions (Michener, 1979). It is full of bees, albeit with fewer species.

Both honey bees and stingless bees (Meliponini) are highly social. Like Apini and unlike all other bees, Meliponini live in perennial colonies and have distinct female castes: queen (or gyne if not yet mated) and worker. The distribution of the Meliponini is confined to the tropical and southern subtropical areas throughout the world. Colony size is diverse and stingless
bees live in colonies ranging from a few dozen to 100,000 or more individuals (Michener, 2000). Stingless bees are particularly common in large areas in tropical America and in Southeast Asia. They are considered among the major pollinators in the tropics (Michener, 1974).

All stingless bees build elaborate nests with structures that are often characteristic for the species or for higher taxa (Michener, 1974; Sakagami, 1982). Taxonomically, the stingless bees of Southeast Asia are relatively well known (Schwarz, 1937, 1939; Moure, 1961; Sakagami, 1975, 1978), but bionomical data are still scarce. Even data on nest architecture, information that is basic to any biological and ecological study, are only recorded fragmentarily in a few species as reviewed by Wille and Michener (1973). For neotropical species this problem is partly alleviated by the beautifully illustrated works of Camargo and his associates; for a recent example with references to others, see Camargo and Pedro (2003); for Australian species, see Michener (1961).

Worldwide stingless bees are classified in 23 genera and 18 subgenera, comprising about 374 described species. Of which 43 recognized species belonging to two genera: Lisotrigona and Trigona occur in Asia (Michener, 2000).

Until a few years ago, only two species were recognized in the genus Lisotrigona: Lisotrigona cacciae and Lisotrigona scintillans. Both species have been found in the region extending from Sri Lanka and Madhya Pradesh in India to Vietnam, Borneo, and Sumatra (Michener, 2000). Recently, two new species have been described: Lisotrigona carpenteri from northern Vietnam and Lisotrigona furva from Thailand (Engel, 2000). Information on biology, ecology and nests of the genus Lisotrigona has not been described previously.

The genus Trigona encompasses worldwide about 120 species, placed in 10 subgenera, of which Homotrigona, Lepidotrigona and Heterotrigona are endemic subgenera for tropical and subtropical Asia (Michener, 1990, 2000). The subgenus Homotrigona has only one species, Trigona (Homotrigona) fimbriata, and is found from western Malaysia to Vietnam and south to Sumatra and Borneo (Michener, 2000). According to the classification system by Schwarz (1939), Trigona (Lepidotrigona) ventralis is divided into four subspecies: T. v. ventralis, T. v. flavibasis, T. v. doipaensis, and T. v. hoozana. Based on results of a comparison of worker specimens from various localities in Southeast Asia, Sakagami (1975) could not find any superspecific segregation among ventralis, flavibasis and hoozana. He also showed that the body size is smallest in the mountainous areas of central Vietnam, larger in Malaysia and Laos, and largest in northern Thailand.
Reproduction in eusocial bees

The subgenus *Heterotrigona* of the genus *Trigona* consists of about 36 species (Michener, 2000), of which six Australian species have been redescribed (Dollin et al., 1997). According to the classification of Michener, this group includes the subgenus (or genus) *Tetragonula* (Michener, 2000). *Heterotrigona* in this classification is the largest and most widespread subgenus in the Indo-Pacific area and includes the numerous and abundant species of *Tetragonula* (Sakagami and Inoue, 1985). However, in the classification system by Wille (1979), which was later modified by Sakagami and Inoue (1985), *Heterotrigona* and *Tetragonula* are considered separate subgenera of the genus *Trigona*, and we recognize *Tetragonula* as a subgenus in the present work.

The taxonomic characteristics of *Tetragonula* as a separate subgenus have been described by Moure (1961), revised by Sakagami (1978) and further modified by Sakagami & Inoue (1985). *Tetragonula* species are distinguished from *Heterotrigona* species by the conspicuously projecting mesoscutellum of workers and males (not in queens) that is easily recognized by the raised vertex and medially setore propodeum (Sakagami, 1978; Sakagami & Inoue, 1985). In addition, all studied members of *Tetragonula* show the same simple oviposition behaviour (Sakagami & Inoue, 1990). *Tetragonula* is the most successful group of the Indo-pacific stingless bees in terms of the number of species and of their relative abundance in most areas (Dollin et al., 1997; Michener, 2000). In Central Vietnam four of the 13 *Tetragonula* species described have been found: *Trigona laeviceps*, *Trigona gressitti*, *Trigona pagdeni* and *Trigona fuscobalteata* (Sakagami, 1978).

1.1. The objective of this study
Vietnam is situated in the tropical monsoon climatic zone in Southeast Asia. The country stretches from 23°22’ North to 8°30’ South (1650 km) with a coastline of 3260 km (from 21°28’ North to 8°22’ South). Stingless bees are distributed over the country and form an important group of pollinators in agricultural and natural ecosystems. However, hardly any information exists on the biology and ecology of the stingless bees in Vietnam. For this reason we studied the characteristics of three species of stingless bees that are common in the primary forest of Cuc Phuong. In this paper we describe their nest architecture and share our observations on their biology and behaviour.

2. Methods
The study was carried out from March 1999 to December 1999 in the buffer zone around Cuc Phuong National Forest, which is a section of the primary forest that remains on the limestone mountains, located near the Red River Delta. The area is characterized by flora typical for a tropical rain forest. The
forest comprises an area of 22,220 ha (20°14'–20°24' N and 105°29'–105°44' E, 300–400 m above sea level).

In total 35 wild nests of three stingless bee species were collected from live tree trunks and walls in different places at the Cuc Phuong National Forest. After opening the cavities, we made estimations of the volume of the nest, population size, size of brood and storage area and the presence of gynes and drones. The volume of the nest cavity was estimated from the length and the average diameter of the cavity in the tree trunk. If cavities were not cylindrical in shape the average height, width and depth were used to estimate the volume. One of the species constructed round combs with an average of 20.5 cells per square cm. The radius of all combs was measured after which the total comb area was calculated. The total number of brood cells per colony was estimated after correction of the comb area for empty cells from which the brood had emerged. The other two species arranged their brood cells in clusters. Here the number of brood cells was estimated by measuring the volume of the brood masses.

Storage pots (for pollen and honey) were normally found clumped in more than one compact cluster, which did in general not allow for a direct count of their numbers. The amount of food reserves was therefore recorded by estimating the total volumes of these cell masses. In some nests, colony stores were assessed by counting and measuring individual pots, however. Here measurements were taken by measuring the height and the width of a number of the smallest and largest pots in each colony and their mean dimensions were used in calculating the volume of food stores by different formulas (either sphere or ellipsoid) depending on the dominant shape (Roubik, 1979). The thickness of storage pot walls was usually less than one mm, and was assumed negligible in the estimation of the storage pot’s volume.

To estimate the numbers of adult bees in each colony as precise as possible, each nest was blocked the entrance tube at night for keeping all the foragers at home. In the next day, the tree log with the nest was cut down and opened in a tent of mosquito netting. Nests constructed in a stone wall were directly opened under a covering net. This facilitates the measurements of the numbers of a swarm of flying bees. Flying bees mostly return to an observation hive to which the nest was transferred at sunset. Estimates of total numbers of adult bees therefore represent the order of magnitude after judging the approximate numbers of bees in flight and on the comb. The number of virgin queens (“gynes”) present in the nest was directly counted, and the number of males was estimated by taking a sample of about 100 adult bees that was preserved in 70% alcohol to be sexed later. The number of queen
cells was directly counted by inspecting the brood combs or the brood cluster. A sample of mature brood of about one hundred cells was taken to determine the percentage of males in the brood. After the colony and nest measurements had been conducted, nests were removed to observation hives and, transported to the Cuc Phuong Commune. They were kept in a dark room of our bee research field station for further behavioural observations. Bee samples were taken and preserved in ethanol 70% and later identified by C.D.M. as belonging to three different species: *Lisotrigona carpenteri*, *Trigona laeviceps* and *Trigona ventralis*.

3. Results

3.1. *Lisotrigona carpenteri* Engel

Workers are pale-coloured, of which body length is 3-3.4 mm. Seventeen nests were opened, of which two were hived for further observations.

In describing *Lisotrigona carpenteri*, Engel (2000) showed (by shading in the illustration) yellow in the lower paraocular areas next to the eye margins. This yellow is absent in the 20 specimens identified by C.D. Michener, in specimens from Cambodia (see below), and in Engel’s paratype in the American Museum of Natural History, New York City. Presumably it was variable in the series studied by Engel. Another character that caused some confusion is the shape of the hind basitarsus, which is not parallel-sided as illustrated by Engel but is widest in its distal half. An additional record for *L. carpenteri*, hitherto known only from Vietnam, is the following: Cambodia, O Rang District, Mandulkari, km 170, 27 November, 2002 (D. Roubik), specimens in the Entomology Division, Natural History Museum, University of Kansas.

3.1.1. Nest architecture and colony characteristics

Unlike the two following species, nests of *L. carpenteri* were found in all kinds of substrates, such as tree trunks, brick walls, rock crevices, and other man-made objects. The inner surface of the nest cavity is usually decorated with a black cerumen (1.5-2 mm). The detailed results concerning the nest architecture and colony characteristics are presented in Table 1. Nest entrances were found at an average height of 90 cm above the ground level. Sometimes two to four nests were found together in crevices of a brick wall. *L. carpenteri* seemed to be the commonest stingless bee species in Cuc Phuong forest. The bees used relatively small cavities for their colonies with volumes ranging from less than 200 cm³ to 1000 cm³. The external entrance tube ranges from 10 to 50 mm long, brownish, precisely cylindrical, thin and brittle. The average outer ø of the tube was 6.5 mm. Sometimes in winter, the entrance tube was temporarily closed at night, possibly helping the very small
colonies to defend the nest or regulate temperature. One colony constructed its nest with two external entrances in a fissure of a main doorframe.

Brood cells were positioned in amorphous clusters, of which up to three could occur in a single nest. Brood cells were spherical, pale-coloured, 3.0-3.2 mm long and 2.0-2.2 mm diameter. The numbers of brood cells varied considerably, with an average of 57 cells (SD = 63, range: 0-206, n = 17). Of 17 nests opened, ten nests contained a mated queen, six had no queens and brood and one had only a virgin queen. Queen cells (5.0-5.5 mm long, 3.2-3.4 mm Ø) were randomly found among worker and male brood, and their number ranged from none to fourteen.

Table 1. Characteristics of nest-architecture and colony measurements in 17 wild nests of *L. carpenteri*

<table>
<thead>
<tr>
<th>Measurement criteria</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of external entrance tube (mm)</td>
<td>31.6 ± 11.7</td>
<td>10.0 – 50.0</td>
</tr>
<tr>
<td>Ø of external entrance tube (mm)</td>
<td>6.5 ± 1.5</td>
<td>4.8 – 11.0</td>
</tr>
<tr>
<td>Number of stored honey pots</td>
<td>60.0 ± 42.0</td>
<td>4.0 – 145.0</td>
</tr>
<tr>
<td>Number of stored pollen pots</td>
<td>18.0 ± 16.1</td>
<td>2.0 – 56.0</td>
</tr>
<tr>
<td>Number of brood clusters</td>
<td>1.1 ± 1.1</td>
<td>0.0 – 3.0</td>
</tr>
<tr>
<td>Number of queen cells</td>
<td>4.5 ± 5.4</td>
<td>0.0 – 14.0</td>
</tr>
<tr>
<td>Total number of brood cells</td>
<td>57.0 ± 63.0</td>
<td>0.0 – 206</td>
</tr>
<tr>
<td>Field estimate of total adult bees</td>
<td>144.2 ± 96.0</td>
<td>50.0 – 375.0</td>
</tr>
</tbody>
</table>

Queen cells are also pale, connected with brood cells by from one to four short pillars. All physogastric queens had intact wings, a swollen abdomen and their body length was 4.9-5.2 mm. Of one hundred bees sexed, only two males were found. The external morphology of the males is rather similar to that of the workers and the lacking corbiculae are the convenient feature for discrimination. The population size of this species was small, with an average of 144 (SD = 96, range: 50-375, n = 17). Honey and pollen pots are elliptical or spherical, 7-7.2 mm high and have a remarkably transparent wall (so the colour of honey and pollen in side can be seen). Honey and pollen pots tended to be arranged in separate clusters. The number of honey and pollen pots ranges from 4 to 145 pots, and from two to 56 pots (n = 17) respectively.

3.1.2. Some observations on the behaviour of *L. carpenteri*
For human observers, the guard bees are very timid, withdrawing even when a small stick is inserted in the entrance. Bees do not crawl in the hairs and
clothes, nor do they bite the skin when nests are opened. We observed a special behaviour with regard to waste disposal. Workers carried waste pellets from inside the nest to the outer rim of the entrance tube where they dropped the pellet immediately. As a result, we found a small waste dump was piled right under the entrance tube on the ground.

3.2. *Trigona (Tetragonula) laeviceps* Smith

*Trigona (Tetragonula) laeviceps* is a jet-black species. The worker body length is 4.8-5 mm. Four nests were opened and hived for further observations.

3.2.1. Nest architecture and colony characteristics

Unlike *L. carpenteri*, *T. laeviceps* nested mainly in living tree trunks of different tree species with trunks of about 30-50 cm in diameter. The hollow space they occupied apparently had been formed through a process of decay and rotting. Usually, a nest cavity was cylindrical with a Ø of 8-11 cm, and a length of 40-90 cm. We also found nests of *T. laeviceps* in hollows between rocks. External and internal entrance tubes were not found in nests of *T. laeviceps*. The nest entrance consisted of a hole decorated with a thin rim of black sticky material. In trees, the nest-entrance was found at a level of 2-4 m above ground. The inner walls of the nest were lined with black material of 1-2 mm thickness. The oval brood cells (4.5-5 mm long) were arranged in amorphous clusters. The cells were connected to each other by pillars. New cells were brownish, but appeared yellow after the wax removal exposed the cocoons. Brood clusters were not covered with an involucrum. Several separate clusters of brood cells were found in the nests. There were also several clusters with food pots. Honey and pollen were stored in separate pots of the same size (5-7 mm Ø, 12-15 mm high), but pollen pots were found closer to the brood masses. Several separate masses of plant resin and resinous material with a strong smell were found at the bottom of the nest. Males and workers were reared in identical cells. Queen cells, elliptical, were sporadically constructed at irregular positions in the brood nest. The number of queen cells was found from zero to seven. The volume of the nests was from 2.0 to 8.5 liters. The estimated number of brood cells was from 650 to 3000 cells and the number of adult bees was estimated from 487 to 1,150 bees. The total volume of stored (pollen and honey) food was estimated from 1.0 to 5 liters.

3.2.2. Some observations on the behaviour of *T. laeviceps*

The temporal pattern of the mass provisioning and oviposition process was facultatively batched (c.f. Sakagami & Inoue, 1990). At some days young
workers (most of them bigger and paler than foragers) hovered above the entrance from 10:00 to 14:00 hrs. to make mass flights. After about half of an hour they returned inside the nest. In general, the defence behaviour of this species is mild, with some withdrawing, and often a mild thrusting with mandibles opened. When the nest was opened, several bees landed on hair and skin of the observer; they mildly bite the skin.

3.3. *Trigona (Lepidotrigona) ventralis flavibasis* Cockerell

*Trigona (Lepidotrigona) ventralis flavibasis* is a bicolour type, with pale white in lateral sides of the abdomen and with pale black in dorsal sides. The worker body length of this species is 5.1-5.2 mm. Fourteen nests were opened and hived for further observations.

3.3.1. Nest architecture and colony characteristics

This species constructed its nests in cavities of trunks of living trees and rarely in branches. Nests were found in different tree species as oak, longan, camellia, and lauraceous trees with a trunk diameter of about 30-60 cm. Usually, a hollow tree trunk provided a cylindrical cavity, of which the average diameter was 10.1 ± 2.0 cm (range: 8.0-13.5, n = 14) and the length of 53.3 ± 21.1 cm (range: 25-90, n = 14). The average volume was 4.8 ± 3.3 liters (range: 1.3-10.7, n = 14).

The wood surrounding the cavity had a thickness of 10-19 cm. Entrance tubes were found at a level of 1-6 m above the ground, and were slightly slanting downward. The tube was projecting 2-15 cm from the outside of the trunk, and it was formed by a very thin wax-like lamella. The average length of the tubes was 7.2 ± 4.0 cm (n = 14). The tube aperture was funnel-shaped, soft, thin-walled (0.3-1 mm) with a smooth surface that unlike the tube of other species like *Trigona terminata* and *T. itama* was hardly sticky. The colour of the entrance tubes was brownish to yellow. The Ø of the opening ranged from 1 to 4.2 cm with an average of 1.9 ± 0.8 cm (n = 14). Fig. 1 presents illustrations of the nest entrance.

The interior of the nests was decorated by a lining layer of resin and cerumen (1-2 mm). As all stingless bee species nests were divided into two major parts: a brood part and an area with storage pots. The brood part (12-40 cm long) was in the middle of the nest, and was nearly completely covered.
Figure 1. Architecture of external entrance tubes and inner nests in three sympatric species: *Lisotrigona carpenteri* (top), *Trigona laeviceps* (middle) and *Trigona ventralis flavibasis* (bottom)
with some soft, thin, brownish-dark sheeted involucrum (2-4 layers). The inner layer (0.2-0.3mm) was thinner than the outer one (0.8-1.1 mm). The involucrum externally connected with the cavity wall by means of short pillars. There was always an internal tunnel (20-30 cm long) directly connecting the external entrance tube to the brood nest chamber.

Brood cells (about 5.5-5.3 mm long, 2.4 mm Ø) were arranged in regular horizontal combs. Brood combs were round in shape or sometimes elliptical with an average Ø of 7 ± 1.9 cm (range: 3.2-9.2, n = 14). Some brownish pillars (1-1.5mm Ø) connected the different brood combs. The number of brood combs ranged from 4 to 32 combs with an average of 15.4 ± 7.3 (n = 14). The total number of brood cells greatly varied in the different nests with an average of 15,118 ± 12,320 cells (range: 659-43,586, n = 14). New cells were brownish, but turned yellow when becoming older. Workers and males were produced in similar cells and both were found in the same combs. Queen cells were elliptical (about 6 mm high, 4 mm Ø). They were mostly positioned at the margin of combs, but in rare cases some of them were found in the middle of the comb.

Clusters with food storage pots were usually found both at the top and at the bottom of the nest. Storage food pots were spherical or oval; and the walls were soft, thin and dark-brown. Pollen and honey were stored in separate pots of similar shape. However, pollen pots were in general found closer to the brood. The volume of stored food greatly differed from colony to colony, with an average volume of 1.9 ± 1.1 liters (range: 0.4-3.7, n = 14). Honey had a slightly acid taste, was hardly viscous when compared to honey of honeybees, and had a light yellow colour. Soft propolis-masses (10-12 mm high, 5-8 mm Ø), white or yellow in colour, were scatteredly deposited at various places in the nest.

A remarkable observation was their striking variation in size of the colonies. The field estimate of adult bees ranged from 258 to 12,167 with an average of 4.221 ± 3,348 bees. The number of brood cells was about 3-4 times higher and showed the same variation. Male adults were found in seven out of the 14 nests of *T. ventralis*. The analysis of mature brood samples showed that the percentage of male brood to female brood ranged from zero to 41.8 % (10.6 ± 12.9 % on average). The number of queen cells present in a nest ranged from zero to 20 (6.4 ± 6.6 on average), and up to six newly emerged gynes were found together with the mother queen. Detailed observations on production of sexuals in nests of *T. ventralis* have been described in another paper (Chinh & Sommeijer, chapter 5). Further quantitative characteristics of the colonies are listed in Tab. 2.

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3.3.2. Some observations on the behaviour of *T. ventralis*

On summer afternoons, young workers sometimes performed mass flights in front of the entrance. These groups of bees consisted of about 50-120 young workers. Guard bees were normally sitting in the opening. When colonies were disturbed (e.g. by inserting small sticks inside their tubes) the worker bees quickly deposited lots of sticky resin droplets around the inner and outer sides of the opening. We also observed this behaviour when the colony was attacked by ants. When the nests were opened, numerous worker bees landed on the hairs and skin of the observer where they perform disturbing biting.

At two times, once in May and once in August, we observed a group of about 14-25 males to hover over the entrance of a nest containing a mated laying queen and several gynes. This drone congregation was present from 12h to 17h, for a period of 5-6 days.

**Table 2.** Characteristics of nest-architecture and colony measurements in 14 wild nests of *T. ventralis*

<table>
<thead>
<tr>
<th>Measurement criteria</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupied volume of nest site (liter)</td>
<td>4.8 ± 3.3</td>
<td>1.3 –10.7</td>
</tr>
<tr>
<td>Length of external entrance tube (cm)</td>
<td>7.2 ± 4.0</td>
<td>2.0 –15.0</td>
</tr>
<tr>
<td>Ø of external entrance tube (cm)</td>
<td>1.9 ± 0.8</td>
<td>1.0 – 4.2</td>
</tr>
<tr>
<td>Volume of stored food (liter)</td>
<td>1.9 ± 1.1</td>
<td>0.4 – 3.7</td>
</tr>
<tr>
<td>Number of queen-cells</td>
<td>6.4 ± 6.6</td>
<td>0.0 – 20.0</td>
</tr>
<tr>
<td>Percentage of male brood (%)</td>
<td>10.6 ± 12.9</td>
<td>0.0 – 41.8</td>
</tr>
<tr>
<td>Number of brood combs</td>
<td>15.4 ± 7.3</td>
<td>4.0 – 32.0</td>
</tr>
<tr>
<td>Ø of brood comb (cm)</td>
<td>7.0 ± 1.9</td>
<td>3.2 – 9.2</td>
</tr>
<tr>
<td>Total number of brood cells</td>
<td>15,118 ± 12,320</td>
<td>659 – 43,586</td>
</tr>
<tr>
<td>Field estimate of total adult bees</td>
<td>4,221 ± 3,348</td>
<td>258 – 12,167</td>
</tr>
</tbody>
</table>

4. Discussion

*L. carpenteri, T. laeviceps* and *T. ventralis* from northern Vietnam, construct their nests in cavities. This is similar to most stingless bees; only very few species build exposed nests. Of the more than 60 species occurring in Costa Rica, only one (*T. corvina*) is building exposed nests. The three species studied in the Cue Phuong forest were characterised by a different preference for nest substrates. *T. ventralis* has a strong preference for nesting in live tree trunks. *T. laeviceps* uses a somewhat wider variety of cavities, but mainly builds in trees. *L. carpenteri* constructs its nests in a wide variety of substrates and seems to adapt readily to available cavities in man-made structures.

The flexibility for using other nest substrates appears in these species.
related to colony size. *T. ventralis* has large colonies (up to around 10,000 adults), *T. laeviceps* is intermediate with colonies of up to 1200 adult bees, and *L. carpenteri* has by far the smallest colonies of only up to about 400 adult bees. The fact that colonies of *L. carpenteri* are much smaller than those of the other two species, may allow for the use of all kinds of small cavities. The frequent occurrence of nests of *L. carpenteri* in diverse substrates may result from a focus on colonizing new holes, whereas the other two species described here focus more on colony defence and growth of the colony itself. This is in agreement with the common occurrence of *L. carpenteri* in this area.

Of *Tetragona angustula* in Costa Rica (C.A.) and of *Trigona nigra* in Trinidad and Tobago (W.I.), two species that also use a wide variety of nest substrates, is known that both species swarm more frequently than other stingless bees, for example *Melipona beecheii* (Sommeijer, personal observation). It is obvious that the very common neotropical *T. angustula*, because of a great adaptableness to all sorts of cavities, of which many are man-made, is a frequently swarming species. This species is considered as a very “weedy bee” (D.W. Roubik pers. com.) We have as yet no information about the swarming frequency of *L. carpenteri* from Vietnam, but the occurrence of aggregations of this species may be a further indication for frequent colony division. The observation that a number of the colonies in the aggregation in the brick wall had very weak nests, may also be an indication for the colonising tendency of this species.

Possibly, the aggregation of nests of *L. carpenteri* may not have a function in colony defence, as has been suggested for the aggregations of *T. cupira* nests (c.f. Seeley et al., 1981). The defensive behaviour of *L. carpenteri* was, at least during interference by human observers, very weak. Of *T. nigra*, which is very common in Trinidad and Tobago, also many nearby nests can be observed, when suitable nest cavities are available. For example, it is common to find tens of nests of this species in one abandoned building that provides ample nesting sites in hollow walls etc. Such occurrence of nest aggregations may result also from the typical colony multiplication of these bees: daughter colonies are established within the flight range of the mother colony from which food and nest materials are transferred to the new nest. The characteristic lacking of a distinct nest exit tunnel in *L. carpenteri* also resembles the situation in the neotropical *T. nigra*.

The queenless colonies in the aggregation of *L. carpenteri* could have been the result of recent colony foundation. Virgin queens of recently established colonies could have died because of various reasons, for example they could have been lost in a nuptial flight by predation.

The architecture of the comb builder *T. ventralis* demonstrates some
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differences with that of the related *T. terminata*: *T. ventralis* covers the brood nest with a multi-layer involucrum, while *T. terminata* does not covers the brood nest with involucrum, but with a pillar systems (Sakagami et al., 1983). The involucrum is a general feature for all horizontal comb building species (Michener, 2000). In our study area in northern Vietnam, nests of *T. ventralis flavibasis* contain two to four layers of involucrum, whereas five to ten layers have been recorded in *T. v. hoozana*, from in Taiwan (Sakagami & Yamane, 1984). A higher number of involucrum layers may be related to harsh weather conditions and temperature may be the main factor for this. We observed in *T. ventralis* colonies that the number of involucrum layers was increased in winter and reduced in summer, which suggests that bees adjust the involucrum for temperature regulation. Unlike in *Apis* species, where the temperature of brood combs can be kept constant by clustering or fanning behaviour and by water vaporization (Seeley, 1985), thermo-regulation in stingless bees is limited (Michener, 1974; Wille, 1983; Sakagami et al., 1983). The lack of temperature homeostasis may be a factor that restricts the geographic distribution of stingless bees outside the tropics and that influences nest sites for some species (e.g. in large trunks providing thick wood protection).

*Lisotrigona carpenteri* was only recently described by Engel (2000) from Nghe An and Ha Tinh province, northern Vietnam. In this study we report for the first time about the nest architecture of this species. Our study shows that many interesting biological data on social bees still have to be revealed, not only of rare species, but also of general ones that are expected to play important roles in the ecosystem.