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X-ray tomographic analysis of the initial structure of the royal chamber and the nest-founding behavior of the drywood termite *Incisitermes minor*

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Abstract

The nesting biology of the drywood termite, *Incisitermes minor*, is poorly understood. To date no published data are available regarding the *in situ* nest-gallery development of *I. minor*. Three naturally infested Sitka spruce (*Picea sitchensis* Bong. Carriere) timbers were analyzed by X-ray computer tomography to observe the structure of the first royal chamber and the termite's nest-founding behavior. One timber was infested by a group of termites which emerged from their natal nest. The other two timbers were infested by dealate reproductives from the nuptial flight. The study revealed that the drywood termite engages in outside foraging activity and has great foraging flexibility. Computer tomographic images also revealed that *I. minor* reproductives showed anatomical selectivity in their nest-founding activity. The structure of the initial royal chambers varied to follow the anatomical texture of the timbers, which resembled either a European pear shape or a cashew-nut shape.

Keywords: Royal chamber, Drywood termite, *Incisitermes minor* , X-ray tomography

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Introduction

The western drywood termite, *Incisitermes minor* (Hagen) (Isoptera: Kalotermitidae) is considered to be the most destructive drywood termite in the western United States (USA) and one of the five most economically important termites in the USA [1]. The colonies live entirely within sound and dry wood [2] and derive their food and water from a single piece of wood [3]. Because of this hidden ecology, *I. minor* can be easily transported around the world within an infested piece of wood as a result of human activities. Originally from the southwestern USA and northern Mexico [2], infestations of this invasive species have been reported in Canada [4], China [5], and Hawaii [6], and more than half of the prefectures in Japan are listed as infested areas [7-10]. In our modern and mobile society, the introduction of such wood-inhabiting termites to new areas is very hard to prevent.

Although it is an economically important pest, there is very little scientific literature on the initial establishment of *I. minor* nest-gallery systems. The most detailed description of *I. minor* biology was provided by Harvey [11], eight decades ago. The cryptic behavior of *I. minor* hinders the study of nest-gallery establishment and of the invasiveness of the colony; thus, early detection of *I. minor* infestation is difficult. Naturally, new colony dissemination is facilitated by swarming activities in the dispersal flight season. Swarming occurs during the summer to the fall on bright, sunny days which attract alates to emerge from the colonies (positive phototaxis) [10-13].

In addition to information on swarming activities, information on the nutritional ecology of *I. minor* and its feeding preferences for various timbers is also very important for investigating initial nest-gallery establishment. *Incisitermes minor* has been reported to attack most common USA timbers, including sycamore, oak, alder, Monterey cypress, redwood, California laurel, buckeye, eucalyptus, willow, peach, pear, almond, cherry, etc. [2]. Rust et al. [14] suggested that Douglas fir was the most highly preferred wood of *I. minor*, while Indrayani et al. [15] found that spruce was the most susceptible wood species after evaluating the termite's feeding responses to 10 commercial timbers from Japan, USA and Malaysia.

It is difficult to use direct dissection to assess drywood termite nest-gallery establishment in situ because it will disturb their habitat and disrupt the continuous development of the nest-gallery. A reliable indirect method involving computer tomography (CT) is a promising approach that allows nest-gallery observation and visualization without damaging the structure. Fuchs et al. [16] showed that the CT scan method was able to provide three-dimensional (3D) image reconstruction of the nest-gallery of the drywood termite *Cryptotermes secundus* and made it possible to display the inner part of the wood. Perna et al. used the technique to reveal the complex systems of connectivity inside the nests of *Cubitermes* sp., leading to a better understanding of the topological efficiency and defense mechanisms of termite nests [17-19].

The purpose of this study was to analyze the initial structure of the royal chamber and the nest-founding activity of *I. minor* in naturally infested timbers using X-ray CT scanning. The study intended to reveal the nest-site selection mechanism in the target timber and the nest-founding and foraging behavior of *I. minor*. As a non-destructive approach, the CT scanning technique could be used to monitor the continuous development of the drywood termite nest-gallery and therefore could be useful for elucidating the hidden biology of this invasive termite.

Materials and Methods

Wood specimens and nest-founding activity

The wood specimens were selected based on the feeding responses of *I. minor* to 10 commercial timbers as reported by Indrayani et al. [15]. Sitka spruce (*Picea sitchensis* Bong. Carriere) timbers, with dimensions of 50 (R) × 50 (T) × 1000 mm (L) and made up of a combination of sapwood and heartwood portions, were used in this study. Evaluation of the initial nest-founding activity of *I. minor* in a natural context was carried out at four infested houses in Wakayama Prefecture, Japan. Fifteen pieces of Spruce timbers were laid with gaps of ~10 mm between them in the highly infested attic area at each house. The timbers were laid in random positions, without considering whether tangential sections, radial sections, sapwood parts, and heartwood parts were oriented in any particular direction. The experimental set-up was conducted on August 3, 2012, one month before *I. minor* swarming season in the Wakayama area, which was reported to be in September [10].

Analysis of initial structure of royal chamber

The monitoring was conducted in the following fall season, on November 15, 2012. All of the infested timbers were brought back to the laboratory and prepared for CT scan analysis. Each infested timber was imaged using a large-scale X-ray CT (Y.CT Modular, YXLON International GmBH, Germany) at Kyushu National Museum. The digital data thickness was 1 mm, 1024 × 1024 pixels, with 3.17 two-dimensional (2D) image slices per mm.

CT data were reconstructed into 3D images, 2D section images, and series of virtual cuts using volume graphics software (VGStudio MAX 2.1, Volume Graphics GmbH Germany). The CT images revealed important properties of the chambers: depth, length, diameter and volume. Volume properties were measured by calculating the voxel properties in 3D-image reconstructions. The 3D images of *I. minor* nests were reconstructed from 2D digital image data and displayed as discrete values of a density function F (x, y, z), which represented physical properties. The other important properties were obtained by 2D image analysis.

Results

The monitoring was conducted two months after the *I. minor* swarming season in Wakayama. Three infested spruce timbers, namely the Spruce A, Spruce B, and Spruce C timbers, were analyzed by X-ray CT scan. Two types of infestations were found on those three timbers. Spruce A was infested by a group of termites that emerged from their natal nest. The other two timbers were infested by dealate reproductives from the nuptial flight, with four and one entrance holes on the Spruce B and C timbers, respectively. The details of the infestations of each timber are presented in Table 1, while the nest-founding activities in spruce timbers are displayed in Fig. 1.

The Spruce A timber was infested by a group of termites that emerged from the attic floor and attacked the adjacent bottom surface of the timber. The term pseudergates (false workers) is used to refer to a group of individuals that infested the Spruce A timber, since the profile of the caste pattern of the group members was not fully characterized. This definition of pseudergates follows Roisin's description [20] of pseudergates as totipotent individuals that can give rise to any of the three terminal castes (alate, replacement reproductive, soldier). The group excavated an entrance hole in a fairly wide springwood (Fig. 1a) of a tangential section of the sapwood. The entrance hole was excavated as an ample passageway (~8 mm) and was located adjacent to the emergence hole of the natal colony. Termites surrounded the entrance hole with cement pellets to connect it with the emergence hole of the natal colony. A group of pseudergates was highly engaged in excavation activities when the timber was inspected.

Figure 1b shows the nest-site selection behavior of dealate reproductives. During the monitoring, we observed two pairs of dealate reproductives were assessing a spruce timber apart from the three infested Spruce timbers (Table 1). Several chewed spots, indicated by circles (Fig. 1b), were encountered on the heartwood and sapwood of the radial section. The pairs were assessing the timber in order to establish an entrance hole. One entrance hole on the sapwood, marked by a square (Fig. 1b), was under excavation by a pair of dealate reproductives when the timber was inspected. The entrance hole was not yet sealed as the pair were digging the spot and placing the chewed wood in the surrounding area.

Fig. 1c shows four royal chambers found on the Spruce B timber. The royal chambers were completely excavated, as all the entrance holes were sealed. Alate wings were found in the nearby excavated holes. According to Harvey [11], the alates divest of their wings upon landing from their dispersal by lowering and bracing the wing tips against the timber. Then the dealates crawl to find a mate, and the new pair of reproductives work together to establish the entrance hole [11] in accordance with the case shown in Fig. 1b.

Figure 2 shows 2D and 3D CT images of the newly excavated chamber in the Spruce A timber, while the measurements of relevant properties are shown in Table 2. Termites excavated the big entrance hole (diameter 8.2 mm) on the wider springwood of the tangential section (Fig. 1a) at the edge of the timber (Fig. 2d). The first chamber was transversely excavated 26.2 mm from the surface (Table 2) across several annual growth rings and elongated parallel to the axial system in the sapwood part of the timber (Fig. 2a – c). The chamber (11.9 mm max. diameter and 114.8 mm^3 volume) was spacious enough to accommodate the aggregations of dozens to twenties of termites (Fig. 2b, d). This chamber was evaluated as an extension for infesting a new timber given that the entrance hole was adjacent to the emergence hole of the natal nest.

As shown in Fig. 2d, five exploratory tunnels were excavated by the invasive colony. One tunnel gallery (No. 1 in Fig. 2d) was excavated perpendicularly to the axial system to follow the springwood of a particular growth ring of the sapwood. Three tunnel galleries were excavated parallel to the longitudinal axis of the springwood of the sapwood (Nos. 2, 3 and 5 in Fig. 2d). Another tunnel gallery (No. 4 in Fig. 2d) was transversely excavated across the grain from the sapwood to the heartwood (Fig. 2a). The average diameter of the tunnel galleries was so narrow (2.2 mm, Table 2) that only a single termite could pass through it at one time (Fig. 2a – b, d).

Figure 3 presents a CT image reconstruction of four royal chambers found in the Spruce B timber in 3D and 2D CT images of a radial section, cross sections and tangential sections. The measurements of important properties of the chambers are presented in Table 2. The royal chambers were sequentially numbered from the edge of the timber as RC-a; RC-b; RC-c and RC-d (Fig. 3). Four newly sealed entrance holes were found on the sapwood (Fig. 3a – b) with an average diameter of 1.8 mm (Table 2). The excavation followed the springwood grain of an annual growth ring, resulting in a sloping entrance (Fig. 3b). The chambers were excavated on the plane of the springwood (indicated by the darker color in the CT image) (Fig. 3c). The pair excavated a narrow oval-shaped chamber sufficient for them and to store some fecal pellets (Fig. 3c – d).

The chambers were located 6.4 – 11.9 mm beneath the surface to the bottom edge of the chamber (Fig. 3.c, Table 2). The initial structures of the royal chambers were varied to follow the anatomical texture of the particular excavation spot. Two chambers (RC-a and RC-c) resembled the shape of a European pear, supporting the previous findings [11,12], while the other two chambers (RC-b and RC-d) resembled a cashew-nut shape. The cashew-nut-shaped chambers were longer but narrower than those with the European pear shape (Table 2). Fecal pellets were found inside of all the royal chambers (Fig. 3c – d). RC-d was the chamber had the highest volume, 41.0 mm^3, followed by RC-a, RC-c and RC-b with volumes of 33.5 mm^3, 25.6 mm^3, and 17.9 mm^3, respectively.

Figure 4 shows CT images of a royal chamber in the Spruce C timber, and the chamber properties are presented in Table 2. A pairing reproductive excavated a sloping entrance to follow the springwood of the sapwood (Fig. 4a – c) on the bottom side of the timber (radial section). The royal chamber was excavated in an elongated cashew-nut shape (Fig. 4d). The chamber had a 4.6-mm diameter and a 13.0-mm length, and was established 6.7 mm beneath the surface (Table 2). The pair selected a particular spot with a somewhat wider growth ring for establishing the royal chamber (Fig. 4d).

Discussion

Nest-site selection

Two types of infestations found in the three Spruce timbers reflected nest-site selection mechanisms. Even though the three spruce timbers were infested from different planes, all of the entrance holes were excavated on the springwood of the sapwood (Table 1). The selective excavations may be related to the feeding biology of drywood termite, which is suggested to be influenced by both the physical and chemical properties of the wood [21,22].

The drywood termite has the ability to assess physical properties of the wood such as mass, density, and internal damping [23] through the reception of vibratory signals [24]. Indrayani et al. [25] also observed that *I. minor* touches the surface of the timber with its antennae before penetrating the timber by engaging in cutting, pulling and collecting activities. This behavior is apparently suggested as “selection” mechanism, as indicated by the concentration of excavation and foraging activities on the softer part of the timber, i.e. the springwood. Both the foraging activities of individuals in the Spruce A timber in excavating tunnel-galleries and the nest-founding activities of the royal pair in the other two timbers were observed to occur on the springwood, and the termites were seen to avoid the summerwood (Fig. 2 – 4).

The wood selection mechanism is also known to be affected by wood chemistry [26] and the wood’s nutritional value [27]. Wood is primarily composed of three major components: cellulose, hemicelluloses, and lignin, with various other components such as organic extractives and minerals. Extractives are responsible for the color, smell, and durability of the wood [28]. In Sitka Spruce, the heartwood contained about 70% more extractives than the sapwood [29]. Therefore, nest-site selection in the sapwood may be determined by the extractive content.

The existence of nest-site selection mechanisms was confirmed by the fact that dealate reproductives chewed several areas on the timber before selecting a favorable spot for establishing the royal chamber (Fig. 1b). This result suggests that drywood termites effectively search for nesting sites and start their colonies in favorable environments. This kind of behavior may be highly related to foraging efficiency [30], i.e. it may serve to reduce the energy spent on excavation, and at the same time maximize the termites’ access to an important nutritional diet [27].

Nest-founding and foraging behavior

One-piece nesters spend their entire colony life in a single piece of wood that functions both as a nest and as food [31]. Individuals of one-piece termites with flexible linear caste development [20] have only two options, staying at the natal nest as helpers/workers or developing into winged reproductives that disperse to establish new colonies [32]. The decision as to whether to remain or disperse is influenced by ecological constraints, such as food availability and habitat saturation [33,34]. Korb estimated that the decision to remain in the natal colony had two philopatric benefits: a long-term direct benefit through nest inheritance and an indirect benefit from raising young individuals. However, as the availability of food decreases, both benefits of philopatry decline, thus leading the reproductives to favor choosing dispersal to found new nests [32,33].

An interesting result was observed on the Spruce A timber, as new chamber-gallery establishment was mediated by the foraging activities of individuals that had emerged from their natal nest. The result implied that "one-piece" terminology could not explain this kind of foraging activity outside the natal nest. The designation of drywood termites as "one-piece" termites is only useful as an ecological distinction to distinguish them from "intermediate" (nesting in wood but foraging outside the nest wood) and "separate" (nesting in and foraging through the soil) termites [35]. This finding is in accordance with a previous report [35], suggesting that drywood termites foraged outside the natal nest throughout several adjacent timbers and had extensive gallery systems. The fact that we had collected some pseudergates using a ground sticky trap also supported the outside foraging activity of *I. minor*.

The chamber-gallery excavation in the Spruce A timber showed a different pattern of development compared to the nest-founding activities from nuptial flight in the other two timbers. Entrance holes were excavated in springwood (Fig. 2c), consistent with the wood selection mechanism. The chamber, which was still connected with the natal nest when it was found, functioned as a starting chamber for establishing extended nest-gallery systems on the newly infested timber. Five exploratory tunnel galleries were excavated in five different directions, which may have been part of an "environmental assessment" by the colony before deciding on further nest-gallery excavation. The colony development in the Spruce A timber was in a vulnerable stage, since the presence of a royal pair in the incipient colony was unconfirmed. However, with highly flexible linear caste development, individuals of an *I. minor* colony can develop into replacement reproductives in order to sustain the colony [3,20].

Nest-founding activity by pairing reproductives in the Spruce B and Spruce C timbers indicated that the royal pair carefully "selected" the springwood of the sapwood (Figs. 3 and 4) as the site for excavating the entrance hole (~1.8 mm in diameter) as well as the royal chamber. The pairs excavated the royal chambers beneath the surface, parallel to the annual growth rings, and avoided the summerwood. The royal chambers were pear-shaped, consistent with the previous descriptions given by Harvey [11] and Morimoto[12], and oval cashew-nut shaped with 6 ~ 7 mm elongated narrow diameters, 8~16 mm lengths, and range volumes of 17 ~ 41 mm^3 (Table 2).

Volume properties can be used to estimate in order in which the royal chambers were excavated. The other properties (depth, length, and diameter) are discrete values, as they depend on the anatomical texture of the particular excavated area in the timber. The volume of the chamber represents how much timber was consumed by the royal pair, which is related to how much time has passed since the pair started the excavation. Based on the volume measurements of the royal chambers, RC-e in the Spruce C timber was apparently the first excavated chamber, followed by RC-d, RC-a, RC-c and RC-b in the Spruce B timber.

After the excavation of the royal chamber was completed, the pair sealed the entrance hole using cement feces, and enlarged the diameter and length of the chambers a bit in the plane of the springwood (Fig. 3c and Fig. 4c). Harvey [11] observed that the royal pair had a period of inactivity after spending tremendous energy in swarming, shedding their wings, mating and excavating the chamber. The hibernation period was estimated to last for nine months, and many pairs do not survive through it. However, based on the data from the second CT scan, which was taken six months after the first scan, all of the royal pairs survived. These data indicated that after the royal chambers were established, the pairs continued the nest-gallery excavation for over six months (Himmi, unpublished data). Thus, it is suggested that the nine-month estimate of inactivity was not an entirely accurate description of the hibernation period. Further CT scan analysis of the infested timbers at biannual intervals would help to reveal other processes of nest development and biological information regarding *I. minor*.

The establishment of a new nest-colony by pairing reproductives has been described as vulnerable solitary founding of claustral independent colony foundation (ICF) [36]. The level of failure is high, as the queen must brood and forage at the same time. Successful colony-founding depends on the royal pair's ability to maintain their energy while producing a workforce and to maintain their foraging efficiency. The colony has to distribute the time and energy spent in excavating the nest-gallery and in foraging in such a way to maintain its fitness [30]. The present study also showed the occurrence of outside foraging activity, indicating that the drywood termite has greater foraging flexibility in response to environmental conditions than is suggested by its classification as a "one-piece" nester. Continuous CT scan analysis to monitor the development of the nest-gallery system would lead to a deeper understanding of the foraging biology of *I. minor*.

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Table

Table 1. Types of new infestation found in spruce timbers

Timber specimen	Details of infestation						
	Number of infestation (Type)	Location of infestation					
		Sapwood	Heartwood	Border	Tangential section	Radial section	Cross section
Spruce A	1 (a group of termites)	○			○		
Spruce B	4 (dealate reproductives)	○				○	
Spruce C	1 (dealate reproductives)	○				○	

Table 2. Measurement of relevant initial nest-gallery properties

Properties	Spruce Timber					
	A	B				C
		RC-a	RC-b	RC-c	RC-d	RC-e
Entrance hole diameter (mm)	8.2	1.9	1.9	1.9	1.9	1.7
Volume* (mm^3)	114.8	33.5	17.9	25.6	41.0	41.4
Depth** (mm)	26.2	7.4	11.9	9.0	6.4	6.7
Length (mm)	49.4	8.7	14.9	12.1	15.6	13.0
Maximum chamber diameter (mm)	11.9	6.4	6.3	7.0	6.3	4.6
Average gallery diameter (mm)	2.3	-	-	-	-	-

*Volume refers to the empty space in the chamber.

**Depth is measured from the particular surface where the entrance hole was excavated

Figure

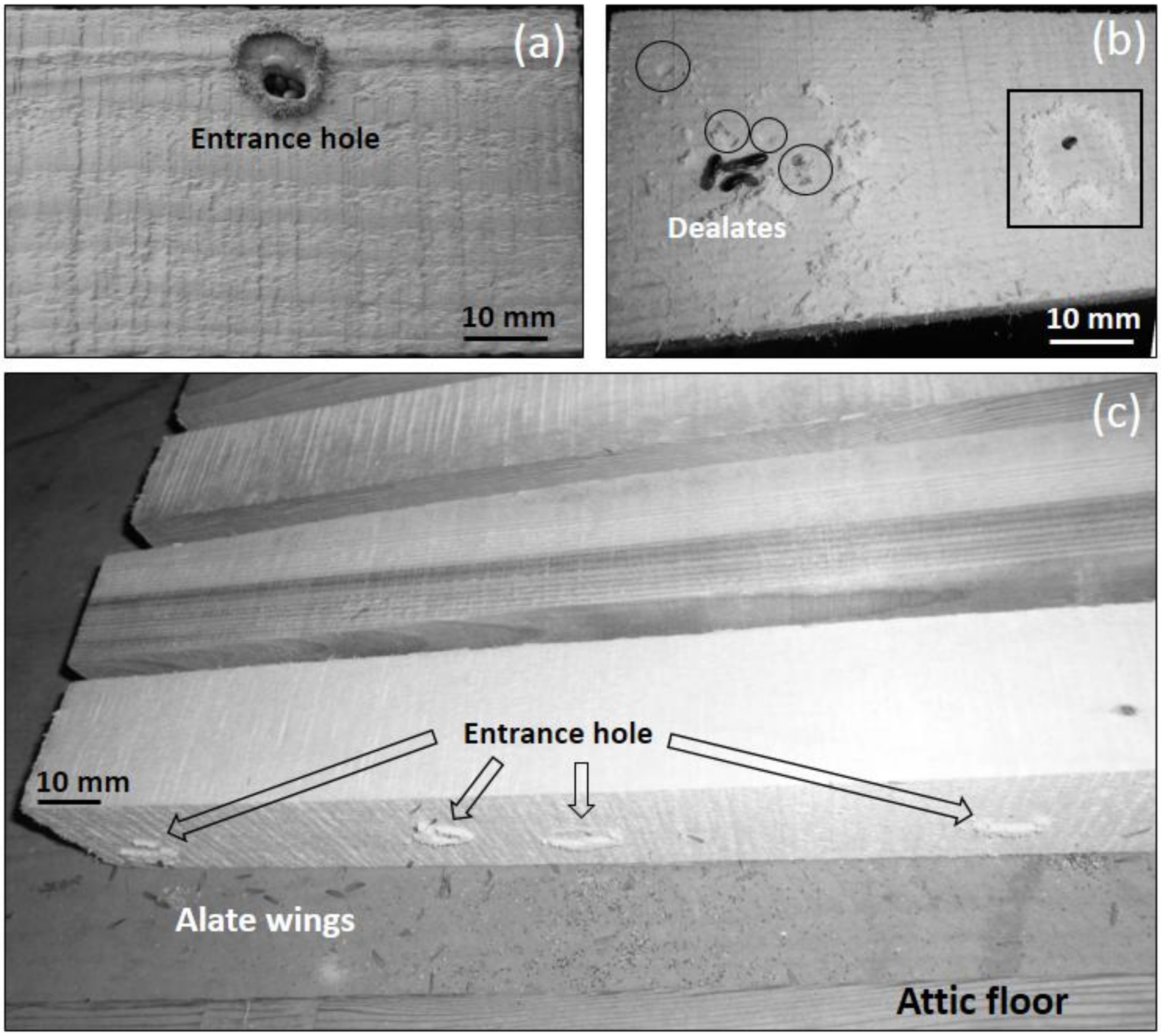

Fig. 1. Nest-founding activity of *I. minor*. (a) New infestation hole created due to colony expansion found on the Spruce A timber. (b) Nest-site selection behavior: dealate reproductives assessed the timber to establish the royal chamber. Chewed spots are marked with circles, while an under-excavated entrance hole is marked with a square. (c) Nest-founding activity from the nuptial flight in the Spruce B timber: four sealed royal chambers. Alate wings were found on the attic floor near the excavated holes.

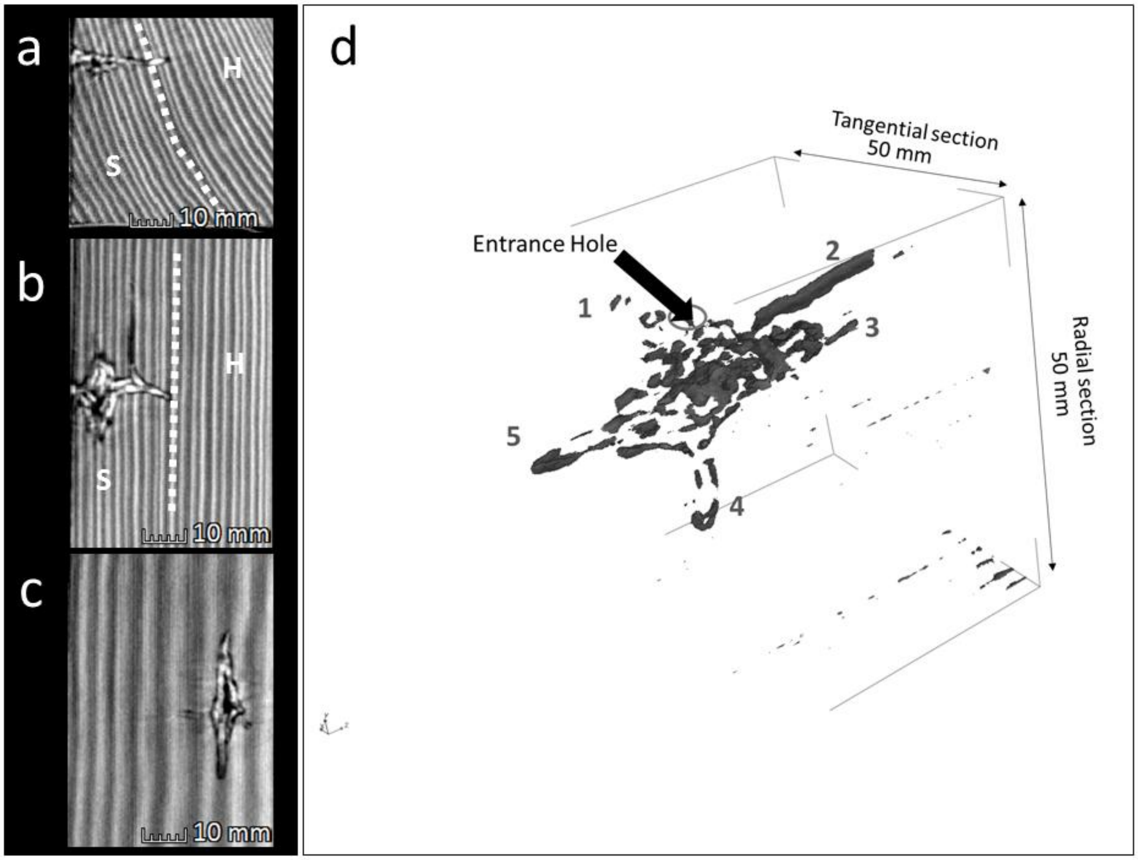

Fig. 2. CT images of the newly excavated chamber in the Spruce A timber. (a) A tunnel gallery was transversely excavated to several layers of annual growth rings. (b) A 2D image of the chamber as viewed along from the radial section, showing an aggregation of termites inside the chamber. (c) A 2D image of the chamber as viewed along the tangential section (sapwood). (d) A 3D image of the chamber: exploratory tunnels in five directions are indicated by number (1 – 5). The dark-grey color on the 3D image indicates the empty space inside the chamber, and termite presence is indicated by the uncolored area inside the chamber. S: sapwood; H: heartwood. Springwood and summerwood are indicated by the darker and lighter colors in 2D CT images, respectively.

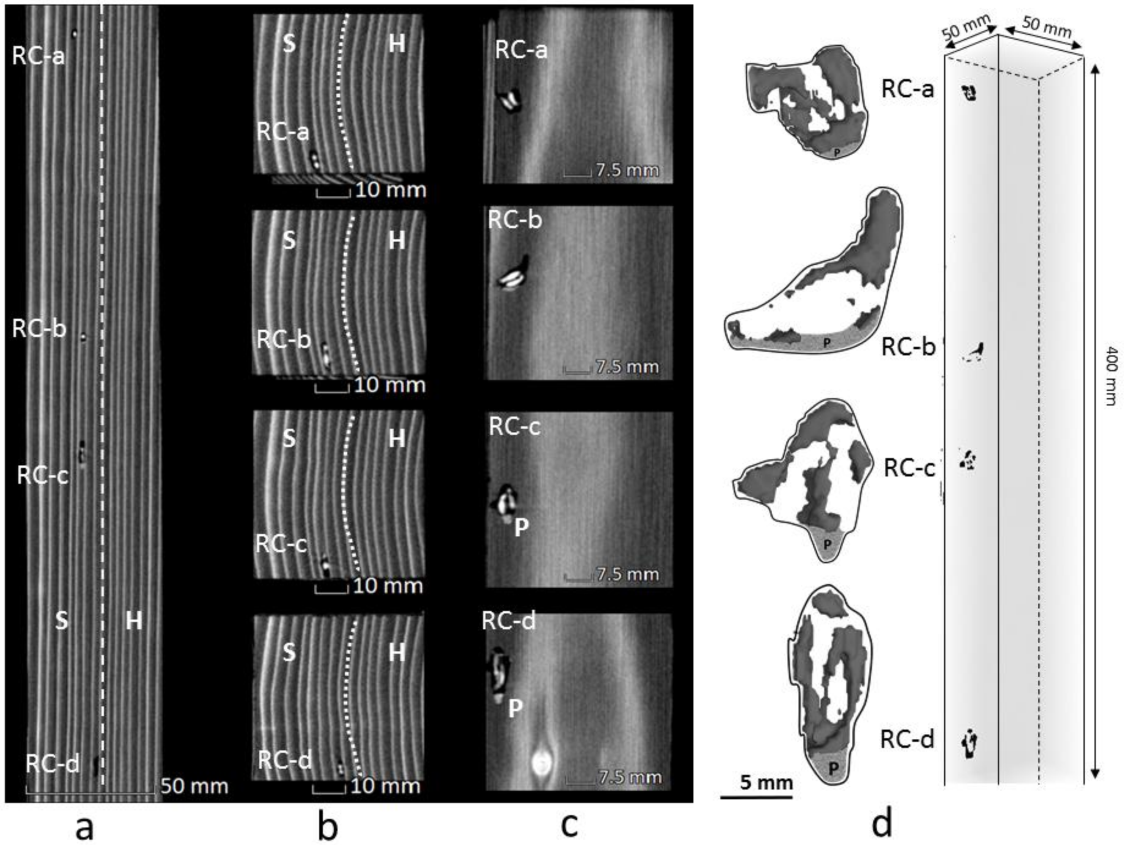

Fig. 3. CT images of four royal chambers in Spruce B timber. (a) A 2D CT image of a radial section, showing the sites of the entrance holes on the timber. (b) 2D CT images of cross sections, indicating that the royal chambers were excavated following the grain of the annual growth ring. (c) 2D CT images of a tangential section showing the presence of royal pairs and the shape and depth of the chambers. (d) 3D CT images of four royal chambers; the dark-grey color represents the empty space inside the chamber; the presence of royal pairs is indicated by the uncolored area inside the chamber. RC: royal chamber; S: sapwood; H: heartwood, P: fecal pellets. Springwood and summerwood are indicated by the darker and lighter colors in the 2D CT images, respectively.

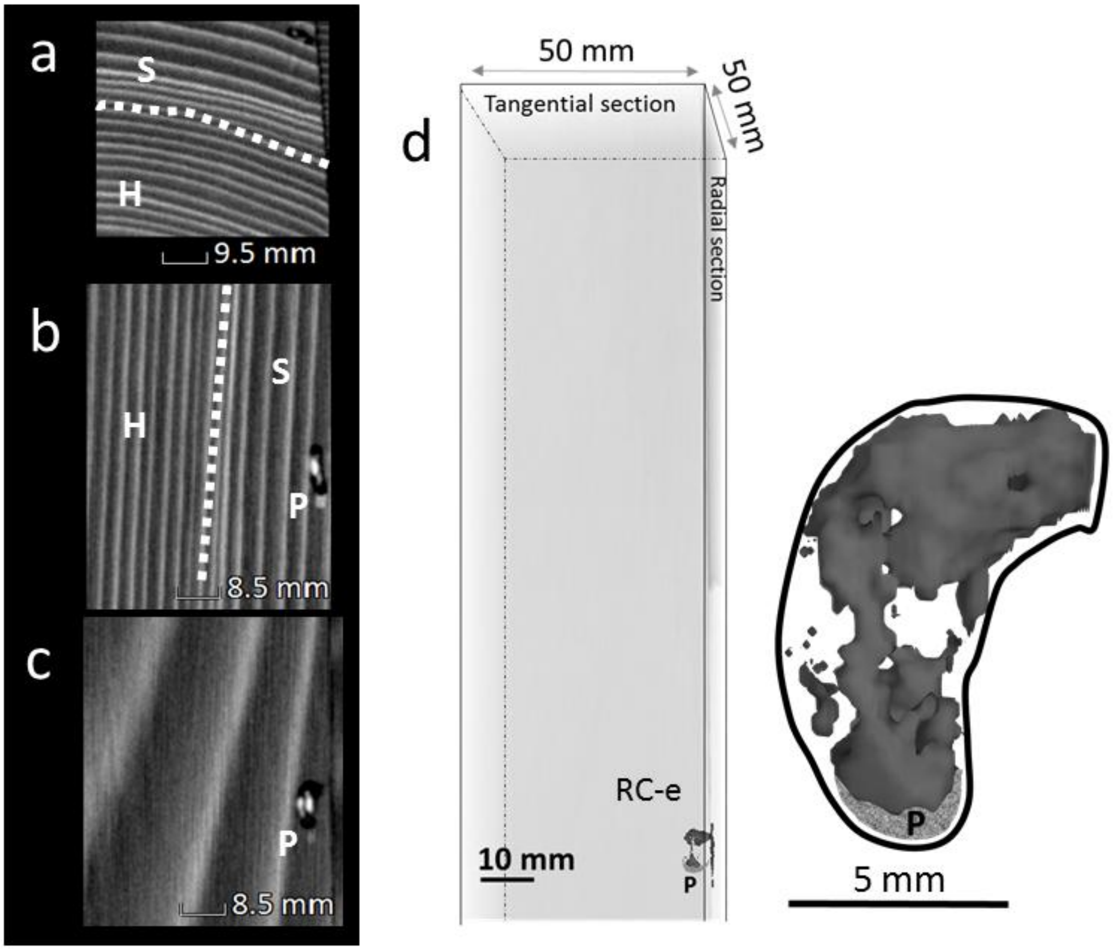

Fig. 4. CT images of a royal chamber in Spruce C timber: (a) A 2D image of a cross section; (b) A 2D image of a radial section; (c) A 2D image of a tangential section; (d) A 3D image of the royal chamber. RC: royal chamber; S: sapwood; H: heartwood, P: fecal pellets. Springwood and summerwood are indicated by darker and lighter colors in the 2D CT images, respectively.