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Original article

Nesting biology of *Centris aethyctera* (Centridini, Apidae) in an estuarine environment

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Abstract – Bees from different groups are able to make nests and complete their life cycles in flooded sites. We report the nesting of *Centris aethyctera* in a mangrove area subject to periodic flooding during high tide. Nests in the mangrove are submerged in salty water periodically throughout the year, including in the initial stages of nest construction and provisioning. Furthermore, this is the first report of a bee species using estuarine areas for nesting. We show the life cycle, the characteristics of the substrate, the frequency of flooding, and the rates of brood mortality. Kleptoparasitic bees attack the nests and survive until hatching simultaneously with their host. The highest brood mortality occurred during the period of nest construction and development of the first larval stages. The impermeability and resistance of the cocoon secreted by the prepupa seem to be adequate for resisting submersion. The malleability and temperature of the wet sandy-silty substrate may facilitate the construction of nests and compensate for the disadvantages of its flooding potential.

Bee nests / Flooding / *Centris* / Mangrove / Kleptoparasites

1. INTRODUCTION

The choice of nesting site of solitary bees has a very important impact on their reproductive success. Humidity, texture, internal temperature, stability, and other physical factors can determine the architecture of the nest, the number of cells, and the hatching rate of adults (Danforth et al. 2019; Antoine and Forrest 2021). Biotic factors such as proximity to conspecifics and attacks by parasites (particularly kleptoparasitic bees) can vary depending on the choice of nesting site. It might be thought that water saturated soils that experience periodic or permanent flooding are a poor substrate for nesting by bees because excess water affects the stability of the nests and reduces the availability of oxygen for the development phases of bees, as well as facilitating the growth of pathogenic bacteria and fungi (Antoine and Forrest 2021). In fact, most species of bees that nest in the soil avoid substrates with water content by weight higher than 40% (Cane 1991). In one case in the Andrenidae family, an event of high bee mortality was reported after a flood (Fellendorf et al. 2004), which shows the overwhelmingly negative effect that flooding can have on the life cycle of bees.

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However, in the literature, there are reports of solitary bee populations that nest in periodically flooded areas (Roubik and Michener 1980; Visscher et al. 1994; Cane et al. 1996; Norden et al. 2003) or in substrates with high levels of permanent soil water (Pietsch et al. 2016). Table I summarizes the main characteristics of the environment and the nests in these examples. In the case of bees that nest in periodically flooded areas, the young survive for several months below the water table, even when the water level was high enough to flood aboveground level. None of the populations that nested in flooded soils were species of bees specialized for this type of extreme substrate.

Other characteristics of the substrate that can be critical for the survival of young bees in nests are temperature and salinity. Frankie et al. (1988) reported that temperatures in the range of 40 °C can be lethal for larvae of the first instars in *Centris* species that nest in holes in trees. Cane and Neff (2011) showed that exposure to substrate temperatures 46–50 °C for 27 min can be lethal for broods of *Osmia lignaria* and *Megachile rotunda*, which are two ground-nesting bee species.

Studies on aquatic insects have shown that hyperosmotic conditions in liquid medium can induce physiological problems due to the excessive entry of ions at the intra- and extracellular levels, as well as water stress due to dehydration (Botella-Cruz et al. 2019). High temperatures and salinity are correlated environmental features of coastal and sandy environments. However, the ground-nesting bee species *Nomia melanderi*, a solitary bee managed for alfalfa pollination, prefers to build nests in substrates with high salinity (Cane 2024).

We observed a population of the species *Centris aethyctera* Snelling, building nests in an area that was deforested and transformed to harvest salt 40 years ago within a mangrove swamp in the dry forest of northwestern Costa Rica. This area floods completely on a recurring basis with the highest tides of the year. The flooded nature of this nesting area is very different from the substrates used by this species in other areas of this region; nests of this species have been found in dry environments on clay, rocky or sandy soils, always in open areas within dry forests or grasslands (Vinson and Frankie 1977; 1991). This population permits the study of the adaptability of the nesting strategies of bees in flooded environments. Various aspects of the natural history of this population are described, including nest architecture, development cycle, hatchling survival, presence of kleptoparasites, and nest flooding cycles.

Flood events are expected to become more frequent in all climate regimes (Tabari 2020), and rising sea levels will affect beaches, dunes, or sandy substrates where many bee species are reported to nest. Understanding the capacity of bees to resist flooding is important to predict their resilience in the current climatic scenario.

2. MATERIAL AND METHODS

2.1. Study location

The nests of *C. aethyctera* were found in an open area within a mangrove swamp, with small patches of shrubs and herbaceous plants. This area corresponds to the remnants of old salt evaporation ponds used for harvesting sea salt (hereafter saline field) in the town of Cuajiniquil, Guanacaste Province, Costa Rica (Bahía Tomas, 10° 55′ 18″ N, 85° 42′ 58″ W; Figure 1a). In the open area of approximately 31,000 m^2, the original mangrove forest is in the process of being restored through hydrological restoration and reforestation (construction of canals and planting of mangrove seedlings). These actions are reducing progressively the salt concentration of interstitial water and returning the soil structure to their original conditions. The section that corresponds to the old saline field is surrounded by a mangrove of approximately 550,000 m^2, within a landscape composed of fragments of forest and grasslands. The site is very close (~ 200 m) to the boundary of Parque Nacional Guanacaste, a protected area composed of forests with different levels of alteration and extensive areas of tropical dry forest restoration. The average annual temperature in the region is 26.6 to 27.5 °C, and the average annual precipitation is 1390 to 1800 mm,

Table I Reports of nesting bees in hyper humid or flooded environments

Species	Environment	Levels of humidity at bee nesting sites	Special adaptations to excess water [1]	Locality	Reference
Epicharis zonata (Apidae)	Dry forest opening with moist sandy soils	Nests submerged during the peak wet season (2 to 3 months)	Prepupae water -repelling coating, and waterproofing double cell plug	Kourou, French Guiana	Roubik and Michener (1980)
Calliopsis pugionis (Andrenidade)	Alkaline flats in flood plains of a river	Some nests submerged for 3 months	Hydrophobic cell lining	Riverside County, California	Visscher et al. (1994)
Perdita foridensis (Andrenidae)	Seasonal ponds in sandy soils with a high water table	Nests submerged for 6 months	Prepupae water-repelling coating. Oxygen supply by algae	Lake Placid, Florida	Norden et al. (2003)
Augochloropsis caerulans (Halictidae)	Small earth mounds scattered throughout a water-covered swampy area	Permanent waterlogging substrate, nests at least 30 mm above the water table	Cells clusters within cavities that separates brood from substrate	Atlantic rainforest, Rio Grande do Sul, Southern Brazil	Pietsch et al. (2016)
Megachile addenda (Megachilidae)	Dikes and beds in cranberry fields	Annual inundation for 4 months, as part of cranberry management		Burlington county, New Jersey	Cane et al. (1996)

[1] As suggested in the references

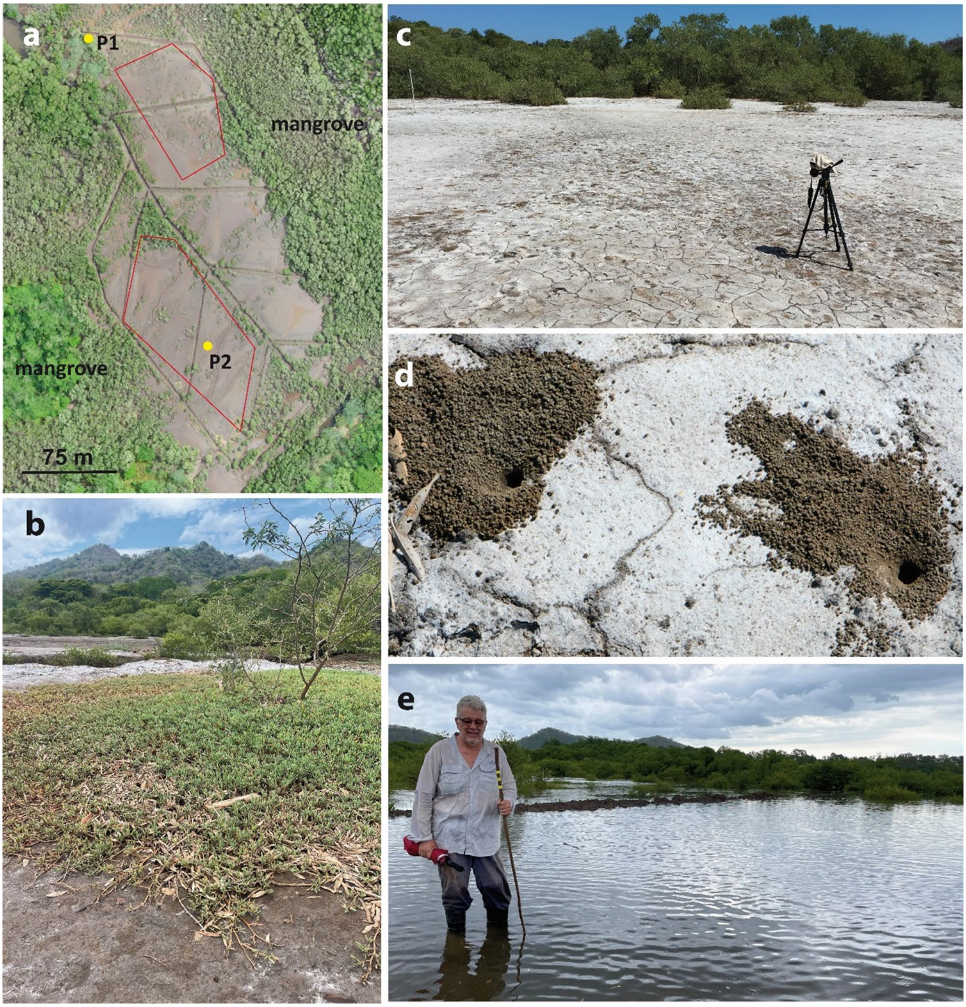


Figure 1. **a** Aerial image of the mangrove swamp and the abandoned salt evaporation ponds used for harvesting sea salt where the nests of *C. aethyctera* were located. Straight lines within the saline field correspond to artificial channels; points P1 and P2 represent sites for observation and excavation of nests, (P1 closer to the sea, P2 a more external site). The red polygons show the areas where nest density was measured. **b** Patch of *Sesuvium portulacastrum* and *Avicennia germinans*, where the excavation activity of females is frequent, as is male patrolling. **c**, **d** Nesting area without vegetation; the white surface salt layer and nests in excavation in an aggregation are apparent. **e** The study area during a high tide that results in a complete flooding event.

with a high degree of interannual variation and a dry season of 6 months between December and May, a period in which most woody plants lose their leaves (Gillespie et al. 2000; Janzen 2000). The peak of flowering of the arboreal flora is observed during the dry season (Frankie et al. 1974). The mangrove species that predominate at the site are *Avicennia germinans* and *Rhizophora mangle*. The bees' nests are in the open sector within the mangrove, with patches of vegetation where *A. germinans* is predominant, along with herbs such as *Sesuvium portulacastrum* (Figure 1b). This sector periodically floods during the highest tides. Floodings usually occur during successive episodes during day and night. In some sectors, the soil is covered by a layer of salt, which has been progressively decreasing in thickness since the beginning of the mangrove recovery process (Figure 1c, d).

In this area, in March 2021, *C. aethyctera* bees were observed flying overhead and building nests (Figure 1d). Two sites were selected in the outermost area of the mangrove swamp, one subject to a less frequent flooding (P2) and another site closer to the main channel of the mangrove, that flooded more often (P1) (Figure 1a).

2.2. Temperature, salinity, and texture of the substrate

As part of a restoration project (Universidad Autónoma de Campeche 2018), data on temperature and salinity were obtained through 4 handmade piezometers located near the excavation sites of bee nests (two at each site). These piezometers were installed at a depth of 0.50 m in the sediment surface (Agraz-Hernández et al. 2011). Additionally, 1-cm-diameter holes were drilled to a depth of 30 cm to allow the interstitial water to flow into the piezometer (Flores-Verdugo et al. 2007). Water samples were collected after the piezometers were drained, and the water was stabilized. Salinity was determined using refractometers (ATAGO, Inc., Bellevue, WA, USA) with a measurement range of 0 to 100 practical salinity units (PSU), and the water temperature was measured using a Multi meter HQ40d device (Hach Company, Loveland, CO, USA). Measurements of these parameters were obtained monthly to assess their variation with the marked seasonality of the climate at the site. Four superficial (0–5 cm depth) and four profound (30 cm depth) soil samples were taken near the bee excavation area using a soil core sampler. The soil samples were processed in the coastal wetland laboratory of the Autonomous University of Campeche using the Bouyoucos hydrometer method, as described by Gee and Bauder (1979).

2.3. Nest flood frequency (flooding records)

In 2021 and 2022, the nesting sites were visited during several episodes of high tides to determine the height required for the flooding of the nesting areas and the submersion time during each tide. During two episodes where the height of the tide exceeded the ground level, the depth of the water surface above the nests was measured to determine the minimum height of the tide that would be sufficient to flood the entrance of the nests.

The tide tables for the period from 2021 to 2022 of the Research Center in Sciences of the Sea and Limnology of the University of Costa Rica (CIMAR-UCR 2020) were used to determine the frequency of the highest tides (flooding episodes) and calculate estimates of the total submersion time of the nests.

2.4. Sampling of adult bees and phenology of adult emergence

Throughout January 2021 to May 2022, the nesting sites were visited every 10 to 15 days to determine the time of reproductive activity of the adult bees and the period when no adults were present. We netted adult *C. aethyctera* and kleptoparasites that were flying and walking around nest entrances. Direct observations and video recordings were made to characterize the digging behavior of the females, the behavior of the males and the activity of the kleptoparasites.

During February–March 2022, two malaise traps, modified to serve as emergence traps, were placed in areas where numerous nest entries had been observed in the previous year. The area covered by each trap was 2 m^2, and the samples were collected in bottles with 70% ethanol. Every fifteen days, the emerged bees were collected, and their species and sex were determined. Taxonomic identification of adult samples were done using the entomological collection of the Zoology Museum of the University of Costa Rica (MZUCR), as well as literature (Snelling 1984). *Mesoplia* species were identified by José Angel Zamarripa Fernández. Voucher specimens were deposited at this museum.

2.5. Nest density

To determine the density and distribution of nests on the site, a survey of the abundance of tunnels under construction and sealed tunnels was carried out in March 2021. For this, 75 circular plots (radius 2.5 m) were set up in different sectors of the mangrove restoration area. The plots were distributed in equal proportions in the different sectors (25 plots in three sectors of 1 ha, at different distances from the coastline: near (site P1), medium, and further away (site P2). In each plot, all tunnels under construction or sealed were counted. The sealed tunnels were easily identified by the mound of soil excavated by the female that contrasted with the white color of the surface covered with salt. This sampling was carried out during the time of maximum activity of the females. Maximum temperatures at all depths were registered at 3 p.m.

2.6. Study of the developmental cycle of bees in cells during the year

In the two previously mentioned sites, chosen for their high nesting density, sampling was carried out throughout the year to obtain samples of bee cells and observe the development of the brood. For this, excavations were made in the sediment up to 30 cm deep in randomly selected areas where the presence of nests had been previously observed (evidence of the nest disappears a few weeks after closure). We located cells by screening and manually examining excavated soil. A sample of 20 to 25 sealed cells was obtained in each of the 4 collection timepoints during the dry season (January to April 2021) and 3 collection timepoints during the rainy season (May to December 2021). The larvae, prepupae and pupae were identified with the help of publications that illustrate the external morphology of *Centris* larvae and kleptoparasites (Rozen et al. 2010, 2011). The samples were treated with 10% NaOH to better observe the structures of the head and the segments and thus facilitate their identification.

To estimate the mortality rate of the brood during different phases of its development, the number of empty cells, cells invaded by fungi, cells with rotten content and cells with a brood of kleptoparasites were registered. These censuses were compared between excavations carried out in the months of January, March, and April–May, corresponding to times when different phases of the larval development predominate.

2.7. Nest architecture and cell structure

During the nesting season, excavations were carried out to determine the organization of the nest (length and shape of the main tunnel, presence or absence of lateral branches, arrangement and size of the cells). Liquid plaster and talc were used to guide the excavation or mold the shape of the nest. The cells were collected, and their shape and walls were studied with a stereoscope. The presence of larval provisions or feces in the cells was determined on each collection date.

3. RESULTS

3.1. Nesting cycle

The emergence of adults occurs between December and February (dry season). Based on the adult collections and the emergence traps, we found that the males emerged earlier (from the end of December), while the onset of the emergence of the females occurred approximately 2 weeks later. Males flew over the nesting area, approaching any nearby individuals in flight. Many perched and guarded the entry of nests (exposed by the previous emergence of adult individuals from those nests), and it was common for aggressive behavior between males to occur at these points. Some remained at the entrance of tunnels or entered these tunnels briefly, waiting for the emergence of females. Mating occurred at the exact moment of emerging of the females, immediately after leaving the tunnel, where they were caught by the males that were waiting at the entrance of the tunnel. Both normal males and macrocephalic beta males (Danforth et al. 2019) were collected flying over the nesting sites. Beta males have a greater head width and a more yellowish coloration of the head and thorax than normal males.

Females excavated and provisioned nests during the months of January and February (Figures 2 and 3a). Eggs and the first larval stages were found during 5 to 6 weeks (Figure 3b, c), until the cells mainly contained prepupae at the end of April and the beginning of May (Figures 2 and 3d–f), a unique phase in the nests throughout the rest of the year (rainy season). No early hatchlings were observed during the end of the dry season, nor did they appear in excavations after the beginning of the rainy season, suggesting a univoltine cycle. However, in a collection at the end of April 2023, four dead adult individuals were found inside cells. Few females were observed building nests in this period. This could indicate the presence of a bivoltine cycle in a reduced part of the population or a late emergence of some adults. Despite these observations, the construction of nests was clearly interrupted in the remainder of the year, and the prepupae within the nests excavated in the first quarter remained dormant for the remainder of the year. Pupae were only found at the beginning of the new reproductive season (Figures 2 and 3g).

3.2. Kleptoparasites

Adults of one unidentified species of *Coelioxys* sp. and two species of *Mesoplia* (*M. sapphirina* and *M. rufipes*) were found hovering around the nesting area of *C. aethyctera* during most of the nest excavation phase. These kleptoparasites frequently walked near tunnel entrances, where they were sometimes rejected by nesting females. In many cases, they enter nests not guarded by females. Among cells excavated in April through December, 2–9% contained prepupae of *Mesoplia* and *Coelioxys* (Table II; Figure 3d, h). In these cases, the cocoons were obviously different from those of *Centris* (see the section on nest and cell architecture), as they presented a thick, hard, silky-fibrous texture, of a brownish color, very different from the yellowish laminar appearance of the *Centris* cocoon (Figure 3e, f). We were not able to identify kleptoparasitic brood at the genus level, but we believe most of these brood were *Mesoplia*, because they were more common in the adult population.

3.3. Nesting area, substrate selection, and soil physicochemical variables

Sampling of nesting sites showed that the bees avoid nesting in muddy soils under mangrove trees, preferring sandier substrates. Mean nest density over sandy areas was 0.6 nests/m^2 (open and closed entrances; range 0–1.85). This area can be subdivided into three conditions: open areas without any vegetation cover (with accumulation of salt layers; Figure 1c, d), sandy areas partially covered by patches of the herb *S. portulacastrum* (Figure 1b), and sandy areas with partial cover of small trees of *A. germinans*. We found maximum densities in sectors partially covered by *S. portulacastrum* (0.85–1.85 nests/m^2), while nest density in open areas or areas with partial cover of small trees was minimal (0–0.3 nests/m^2). We also observed dense concentrations of flying males in the herb patches.

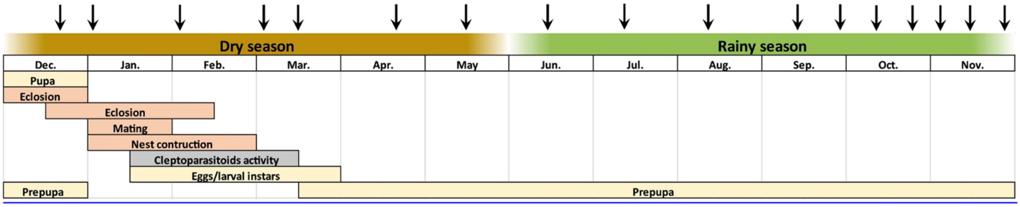
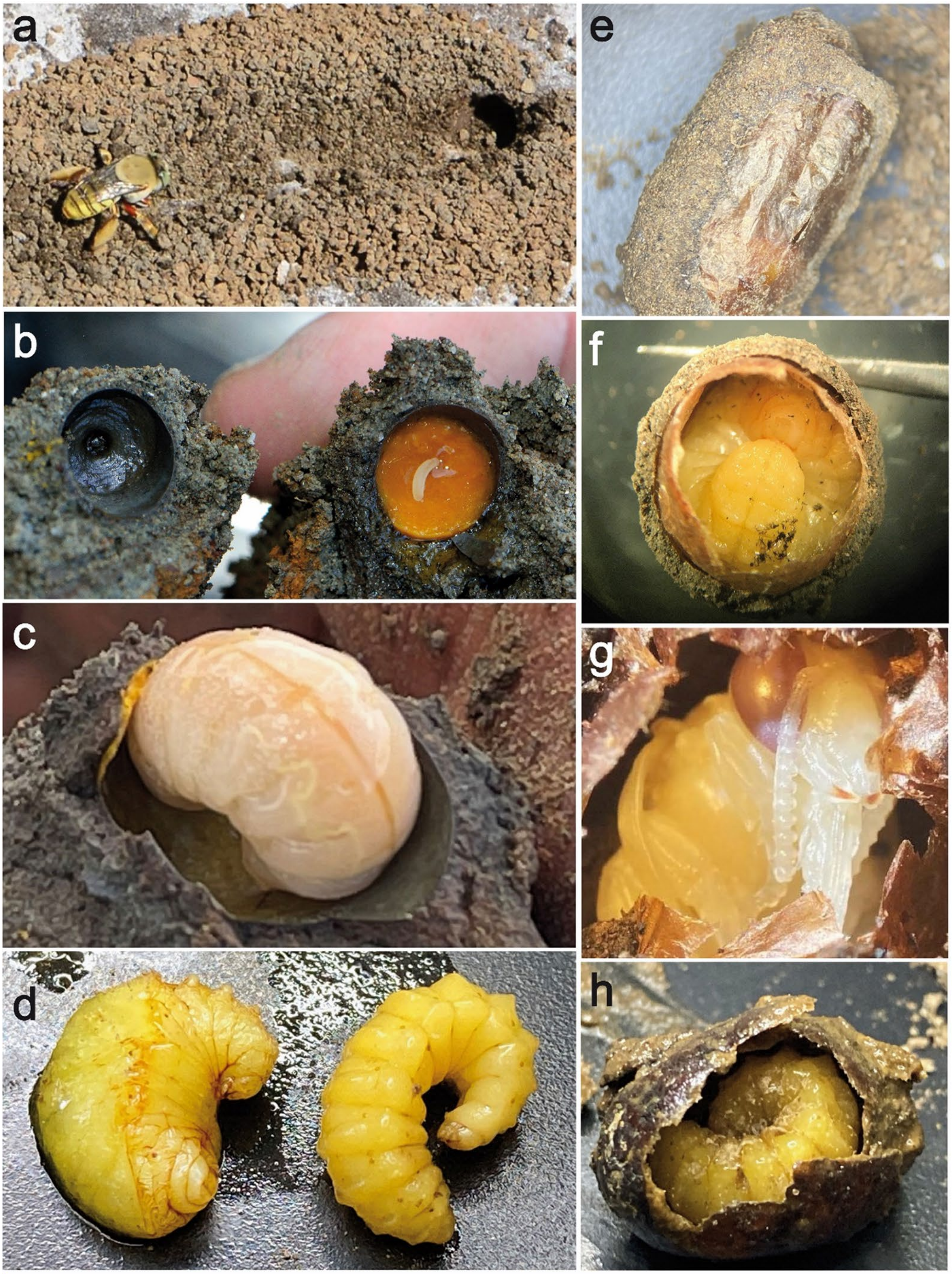


Figure 2. Annual life cycle of the *C. aethyctera* bee and its parasitoids (*Mesoplia* and *Coelioxys*) in an abandoned saline environment within a mangrove swamp in the dry forest of Guanacaste. The start and end dates of each phase are approximations. Arrows indicate flooding episodes (high tide > 2.5 m) during 2022.



The presence of *S. portulacastrum* reduces maximum surface temperature (43–48 °C) compared to open areas (50 °C). Soil temperatures diminish rapidly with depth level, but this effect is more pronounced in areas with herb patches: in herb patches, soil temperature at 15 cm depth was 31–32 °C, compared to 35–38 °C in open sandy areas. Temperature

◂**Figure 3.** Different stages of development of *C. aethyctera*. **a** Adult female excavating a nest. **b** Newly completed cell showing the semiliquid supply on which an egg rest. On the left, the bright cell lining is the only lining of the cell during the instar larvae before the prepupal phase. **c** Most advanced larval instar that has consumed almost all the maternal supply. **d** Lateral view of the prepupae of *C. aethyctera* (left) and of an unidentified kleptoparasite (right). **e** Lateral view of a cell from which the outer layer of earthy material was removed to show the thin and soft cocoon produced by the prepupa and which isolates it from meconium and the external environment during its entire latency period. **f** Vertical view of a cell in which the cocoon was opened to show the larva in its dormant position. It rests on is back, motionless and arched over the bottom of the cell, with the head at the top of the image. **g** Pupal phase of the host bee, collected during the emergence phase of adults in January. **h** Thick and hard cocoon produced by the prepupa of a kleptoparasite with an individual inside.

at 15 cm depth is relevant because bees used to excavate tunnels and build cells between 5 and 15 cm depths (see below). Therefore, bees show a preference for sandy substrates where vegetation cover reduces surface and underground temperature.

Considering the mean density of nests, we estimated that the entire open area of 31,000 m^2 where the nests are found might contain approximately 9300 nests under excavation or sealed at the time of greatest nest-building activity.

The texture of the substrate used by bees as excavation areas could be classified as silt loam (sand 18%, clay 9.9%, silt 61.4%) in the range of 0 to 15 cm depth and as silty clay loam (sand 28.3%, clay 22.3%, silt 48.3%) at depths of 15 to 30 cm. The density of this substrate increased with depth, from an average of 1.18 g/cm^3 near the surface to an average of 3.49 g/cm^3 at a depth of 30 cm.

The salinity of the interstitial water at ground level was high at both study sites (average 68.8 g/kg), a salinity greater than the average salinity of seawater (35 g/kg), while at a depth of 30 cm, it decreased to 28.9 g/kg at site 1 (closest to the shoreline) and at 7.95 g/kg at site 2 (innermost). These parameters are typical of hyper or mesohaline conditions (Ohrel and Register 2006), where the features resulting from the alteration of the site for salt collection were still present.

Collections carried out in emergence traps placed on areas with nests verified that adult bees emerged from areas with sandier substrate. In one month of collection 22 females and 3 males of *C. aethyctera* emerged, as well as 1 female of *Mesoplia* sp. These emerged from an area of approximately 4 m^2 covered by two emergence traps.

3.4. Nest and cell architecture

The excavation of the nest tunnels to determine their detailed shape and the arrangement of the cells was very difficult in the sandy substrate since the tunnels collapsed easily. We found that the bees excavated a simple tunnel that did not descend vertically but had an oblique inclination at its beginning. The first cells were located 5 cm below ground level, most were between 9 and 10 cm, and the maximum depth of the tunnels was 13 cm. We did not find structures that resembled clusters of cells isolated by empty spaces in the substrate, but we did observe that cells were grouped along the tunnel in certain sectors. The cells were urn-shaped, approximately 19 mm long and 11 mm wide ($n = 22$), with a double cap ending in a small button, typical of the cells of the genus already described (Vinson and Frankie 1977). In February 2022, cells with eggs and cells with larvae in intermediate instars stages (larval instars 2 to 3) were observed on a depression at the bottom of the cell formed by a layer of dry pollen and a nectar film on which the larvae floated (Figure 3b). We could not determine if this liquid contained an oily film originating from floral oils, as described by Vinson and Frankie (1999). The inner walls of the cells with eggs or young larvae looked shiny and polished, typical of the structure described as the "cell lining" in *Centris* nests. The cell lining covered the entire cell until it reached the upper lid (Figure 3b, c). Larval morphology differs between *Centris* and kleptoparasites (Fig. 3d), and in the prepupal and pupal stages, the presence of a cocoon was noted for all the species (Figure 3e, f, h). In *Centris*, the cocoon was soft and thin, although very resistant, and was formed by two waxed paper-like

Table II Number of cells according to their content in *C. aethyctera* nests in excavations carried out during different months in the dry season of 2022 within a mangrove swamp in Cuajiniquil, Guanacaste. In the cells with alive individuals, number of cells containing each developmental phase is indicated. The frequency of each type of cell in its respective monthly census is reported in parentheses

Month	Alive					Dead	Kleptoparasites	Empty	Total cells
	Adult	Pupa	Prepupae	Larvae	Total				
January	3	2	1	0	6 (0.27)	6 (0.27)	2 (0.09)	8 (0.36)	22
March	1	0	14	20	35 (0.81)	2 (0.05)	1 (0.02)	5 (0.12)	43
April–May	0	0	30	0	30 (0.33)	54 (0.60)	6 (0.07)	0	90

sheets of a brown, glossy color, and its outer layer adhered to the sand particles that formed a hard and sandy outer layer (Figure 3e). The dorsal part of the prepupa was in contact with this cocoon and was lying curved on the base of the cell (Figure 3f). At the bottom of the cell, isolated by the cocoon, was the meconium, with abundant digested pollen grains. The cocoon of the kleptoparasites was very hard and thick, more rounded in shape and composed of very fibrous material in some cases (Figure 3h).

3.5. Frequency of flooding in the nesting area

Measurements of the water level above the nesting areas, taken during two especially high tides, showed that during flooding, the soil surface was covered by a water column 27 cm deep in open sandy sectors and 15 cm in depth in patches of *S. portulacastrum*. Considering the annual record of tides in the area during 2022, we estimated that the nesting sites studied were partially or totally submerged during 17 cycles per year, corresponding to the periods of the year when the high tide was sufficiently high (>2.5 m) to cover the nesting areas (Figure 1e). During each of these events, depending on the height and duration of the high tide, we estimated that the nests were flooded for 2 to 3 h during each high tide. This flooding occurred once or twice a day and lasted for 3 to 4 continuous days in each of the 16 cycles. These flood cycles were separated by maximum intervals of 1 month and minimum intervals of 15 days. These data allowed us to determine that the nests were submerged for approximately 180 to 300 h per year, depending on the distance to the coastline and the main channels in the mangrove swamp. Four flooding cycles occurred during the nest construction and early larvae life phase (new moons during the first weeks of January–February and the first and middle week of March of 2022; Figure 2). During this period, high tides occurred during day and night times. Therefore, flooding affects the activity of adults (eclosion, mating and nest construction) and early larval instars.

3.6. Breeding mortality rates

Table II shows the percentage of cells with live adults or brood, dead brood (classified as such because damaged brood was observed or they were entire cells with a lid, but empty), cells with kleptoparasite brood, or open empty cells (possible residual hatching cells). Many of the empty cells or cells with dead broods exhibited fungal growth. The data showed a wide variation in the mortality rates of the young throughout the season, since the number of cells with actual presence or possibility of dead brood oscillated between 27% in January, at the end of the prepupa phase and the start of the nesting period, a minimum of 5% in March, in the middle of the egg and first larval instars and the beginning of the prepupal phase, up to a maximum of 60% in April–May, at the end of the larval phase. This pattern suggests higher mortality rate of the larval stages compared to the prepupal-pupal stages.

4. DISCUSSION

4.1. Characteristics of the floodplain in comparison with other cases in the literature

We found that an open area within a mangrove swamp, with a sandier substrate, left over from the operation of a salt plant abandoned for almost 40 years, has become an important nesting site for *C. aethyctera*. In the place, a population of females build several thousand nests at the time of maximum nesting activity. Within this area, bees hatch, mate, and build nests in each annual cycle, choosing sandy areas that are open or partially covered by patches of *S. portulacastrum*. On these herbaceous patches and on the sand, male patrolling and mating take place. We observed that over two years, the site was used repeatedly by females, and there was evidence to suggest that these aggregations of nests have persisted for several years. The exact time that these aggregations began is unknown, and it is not clear if there is a relationship with the timing of the restoration process of the mangrove. Fidelity to the nesting site is one of the factors that explains nest aggregations (Danforth et al. 2019), and it is possible that this population has been maintained by locally hatching females and/or by migrant females.

The nesting area of *C. aethyctera* does not represent a case of bee nests that are permanently submerged during a season of the year, as in other cases reported in the literature (Table I). Instead, temporary submersion events occur throughout the year, including during the time of adult activity and the construction and provision of cells. In most of the cases reported, the time of adult activity, the construction of new nests and the first stages of larval development coincide with a dry season during which the nests are not flooded, since the flooding periods are limited to the rainy season (Table I). In addition, this is the first report of bees using estuarine areas for their nesting activities, and the first case in which flooding of nesting areas of bees with high salinity water has been registered. This increases the level of risk for the brood if water enters the cells. Despite the high mortality of bees in immature stages, the great emergence of adults observed in the dry season of 2022 shows that an important part of the cells managed to complete their development, surviving the recurrent periods of flooding and the high salt concentration.

Comparison with nests and other populations of *C. aethyctera.*

Despite the difficulties of obtaining a complete view of the architecture of the nests, our observations show that the entrance, the shape of the tunnels, and the arrangement, shape and size of the cells of this population were very similar to those already described for *C. aethyctera* at dry nesting sites in the same geographic region (Vinson and Frankie 1977, 1991). An important difference from the observations in dry sites was the minimum depth at which the females began to build the cells. Whereas studies of *C. aethyctera* in dry soils reported nest cells at depths of 2–5 cm (Vinson and Frankie 1991), those at our study site occurred at depths of 5–13 cm. This small difference can be very important in terms of the probability of flooding and the effect of high surface temperatures. On the other hand, Vinson and Frankie (1991) observed that the maximum depth of the tunnels in nests of *C. aethyctera* in sandy soils within grasslands was only 5 cm. They proposed that short tunnels in sandy soils may be the product of the lack of stability of the sandy walls, which prevents the construction of deeper tunnels, typical of nests on rocky sites or clay soils. In the present study, bees excavate profound tunnels (15 cm) in a substrate with a high content of sand. The tunnels reached depths like those observed in nests on rocky sites or clay soils. It is possible that the high humidity of the soil in our study area permitted deeper tunnels than those possible in drier areas, or the extreme surface temperature (~ 50 °C) required a greater depth to facilitate adequate larval development.

The characteristics of the cell lining and the larval cocoon are important for evaluating the survival of the bee brood in flooded conditions. Our observations of the appearance of the cells did not differ from the characteristics reported for these structures in excavations at dry sites (Vinson and Frankie 1977), either in their general appearance or in their dimensions. We can assume that both the cell lining and the cocoon function as preadaptations to flood conditions since they would repel water enough to allow the survival of the young in short periods of flooding. However, a minority (< 5%) of the cells with dead pupae obtained from excavations had water inside them, indicating that this barrier was not totally effective. These accidents may be the cause of the high mortality (60%) in the cells observed in excavations at the end of the nesting period, which would especially affect the egg stage or first larval stages. The mortality rate decreased to moderate levels in nests excavated at the beginning of the nesting period, which would correspond to the death of young in the prepupal stage. These data reveal that a cocoon with a glossy and laminar appearance is possibly more effective than a cell lining in the isolation from harsh external condition and excessive humidity. Minckley and Danforth (2019) reported that the average brood mortality in solitary bees that nest on the ground was 21.5% (range 0 to 87%), and the main cause of death was attack by kleptoparasites, although no data were presented by phase of larval development. Thus, the overall brood mortality rate in the study population at the end of the nest activity phase was within the upper limit observed in solitary bees, although the mortality resulting from kleptoparasites was relatively low (2–9%).

We propose that the area formed in the open region with sandy-silty substratum within the mangrove swamp is favorable for the construction of nests, overcoming the problems of flooding and high salinity. The sandy substrate at this site, moistened by the proximity of the water table and high tide events, appeared ideal for the construction and filling of tunnels and cells. Humidity reduces the temperature a few centimeters deep. Humidity at bee nests tunnels can also provide water vapor that moistens the hygroscopic pollen masses, a source of water and nutrients for larval development (Cane and Love 2021).

The survival of the kleptoparasite reared in the site conditions was noteworthy. The breeding and survival of these species was verified in the prepupal stage until the hatching period at the beginning of the dry season. This proves that the environment of the tunnels and the cells of the host bee, as well as the structures of the cocoon made by these kleptoparasites, efficiently withstood the flood cycles of the mangrove swamp. In addition, it confirms the synchrony of the life cycles of the host and its kleptoparasites. We did not have enough data to compare the mortality rate of kleptoparasite rearing between this site and other drier sites or to determine if the level of kleptoparasitism in this population is lower than in other populations of *C. aethyctera*. In *C. flavofasciata*, a species that builds nests in beach sands, Vinson et al. (1987) and Rozen et al. (2011) found rates of kleptoparasitism by *Mesoplia* of 30 to 59% of cells.

The survival of *Centris* bee nesting aggregations to periodical flooding is important from the perspective of rising sea levels or increasing hurricane episodes, both as a consequence of climatic change. Cane (1997) reports the survival of a melittid bee population in coastal dunes after the impact of temporal flooding caused by a category 3 hurricane. Bee species adapted to nesting in coastal areas can be more resistant to these climatic phenomena than previously thought.

5. CONCLUSIONS

The floodplain offers advantages and disadvantages for the nesting and maintenance of populations of *C. aethyctera* and its kleptoparasites. Although the cell layers protects the brood within the cells and allow larval development in a univoltine cycle, this protection does not prevent mortality, especially in the earliest stages. On the other hand, the unique characteristics of the sandy-silty substrate at this site appear to facilitate excavation activity and may reduce mortality from kleptoparasites. This balance seems to operate in the other cases of nesting of bees in flooded conditions, where species of bees of very diverse taxonomic groups (Apidae, Andrenidae, and Halictidae; Table I) seem to have colonized a habitat for which they had preadaptations in terms of the characteristics of the materials that protect the cells. More studies can reveal if estuarine habitats are an important nesting substrate for more bee species, justifying their preservation as part of bee conservation strategies.

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AUTHOR CONTRIBUTION

JAL and MFO conceived this research, designed the field study, and collected and analyzed field data; MMC found the bee nesting area and contributed to field work; CMAH contributed to field work and analysis of soil samples. The first draft of the manuscript was written by JAL, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

CODE AVAILABILITY

Not applicable.

DECLARATIONS

Ethics approval This study received the corresponding collection and research licenses from national official agencies. They reported that no ethical approval was required.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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