ECOLOGY, BEHAVIOR AND BIONOMICS

Nesting Ecology of a Neotropical Solitary Wasp (Hymenoptera: Sphecidae) in Panamá

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Ecologia da Nidificação de uma Vespa Solitária (Hymenoptera: Sphecidae) no Panamá

RESUMO - *Cerceris* é um gênero bastante interessante, cujas espécies estão presentes em todos os continentes, em diferentes condições climáticas, mostrando comportamentos variando do solitário ao social. Com relação ao hábito de nidificação desse gênero, muitos autores descreveram as características do ninho e das presas, provendo material útil para comparações. Além disso, a maioria das espécies estudadas até agora vivem em regiões temperadas e faltam informações sobre as tropicais. O grande número de espécies e sua ampla distribuição sugerem uma adaptabilidade filogenética que merece ser estudada mais profundamente. Neste trabalho foram investigados o ninho e a presa da espécie tropical *Cerceris binodis* Spinola, no Barro Colorado Natural Monument no Panamá. O estudo foi realizado durante três estações secas, marcando e escavando os ninhos, observando o comportamento dos indivíduos e coletando a presa. Os ninhos, reutilizados na mesma estação e nos anos seguintes, são especialmente profundos, como observados somente em outra espécie neotropical, *Cerceris lutzi* Scullen. Esse resultado pode ser correlacionado com a profundidade de solos tropicais e suas características. Apesar de as espécies apresentarem dimorfismo sexual, as celas subterrâneas não mostram diferenças no tamanho ou no seu conteúdo. A presa, identificada dos espécimes carregados pelas fêmeas provedoras, pertencem a Clytrinae (Coleoptera: Chrysomelidae). Os dados quantitativos sobre a presa foram analisados por comparação das estações e locais, evidenciando as diferenças. Infere-se que a adaptabilidade de *Cerceris* às características ecológicas dos trópicos pode explicar essas diferenças assim como as observadas na estrutura do ninho.

PALAVRAS-CHAVE: Adaptabilidade, *Cerceris binodis*, Clytrinae, estrutura do ninho, presa, floresta tropical

ABSTRACT - *Cerceris* is a very interesting genus, providing species living in all continents, in different climatic conditions, showing behaviors ranging from solitary to social. About the nesting habit of this genus, many authors described nest characteristics and prey, providing useful material for comparison. Yet, the majority of species studied so far live in temperate regions and we lack information about the tropical ones. The high number of species and their wide distribution suggest a phylogenetic adaptability that merit to be studied deeper. I investigated nest and prey of a tropical species, *Cerceris binodis* Spinola, on Barro Colorado Nature Monument in Panamá. The study was performed during three dry seasons, marking and excavating nests, observing individual's behavior and collecting prey. The nests, reused over the same season and in following years, are especially deep as observed only in another neotropical species, *Cerceris lutzi* Scullen . This result could be correlated with tropical soils depth and characteristics. In spite of the species being sexually dimorphic, the subterranean cells show no bimodality in size or content. Prey, identified from specimens carried by provisioning females, belongs to Clytrinae (Coleoptera: Chrysomelidae). Quantitative data on prey are analyzed comparing seasons and sites and differences emerged. *Cerceris* adaptability to ecological trait of the tropics is inferred to explain these differences as well as the ones observed in the nest structure.

KEY WORDS: Adaptability, *Cerceris binodis,* Clytrinae, nest structure, prey, tropical wet forest

Cerceris is a large genus, including 850 species (Bohart & Menke 1976). Females usually dig subterranean nests, whose entrances are often aggregated; these nests end with pedotrophic cells where larvae develop. Evans (1971) defined the general nest characteristics in this genus: burrows tend to be vertical or nearly so; cells are constructed at the end of short side-burrows departing from the main tunnel; each side-burrow is closed off with soil when the cell is fully provisioned; cells may be of two sizes in case of sexually dimorphic species.

Some species are solitary, a term that is applied when a female individually digs and provisions her own nest, without the help of other conspecifics. Usually in these cases interactions among females are aggressive. When some kind of cooperation, and consequently tolerance among females, is detected, we are facing a presocial organization in which females are involved at different levels. This is the case of some *Cerceris* living in Australia (McCorquodale 1989).

Another interesting characteristic of this genus is the specialization. Some species are highly specialized in the capture of a few prey items, often beetles of economic importance (Scullen & Wold 1969). But others are generalists, preying on different species belonging to different genus and even families. *Cerceris* includes species that prey on either adult Hymenoptera or Coleoptera, but no species is known to prey on both. Most *Cerceris*, including all New World species, provision within Coleoptera from at least 13 families (Scullen & Wold 1969). Possible reasons have been inferred to explain specializations, including habitat effects (Linsley & MacSwain 1956), the short flight season of some prey species (Hook & Evans 1991), prey species abundance (Byers 1978), and competition for food sources by wasp species nesting in the same area (Callan 1990). Still there is no evidence of possible links dealing with evolutionary trends.

Cerceris is a very interesting genus, providing species living in all continents (Bohart & Menke 1976, Amarante 2002). Data about it belong mainly to species living in temperate regions and we lack information about the tropical ones. The high number of species and their distribution suggest peculiar adaptations that should be analyzed deeper.

In the Neotropics there are about 250 species (Amarante 2002). *Cerceris binodis* Spinola nests from South America to Mexico (Callan 1990). There are only two brief notes about its prey: from Mexico (Evans 1971) and Trinidad (Callan 1990). No details about the nest structure and nest site selection were provided by these authors. I observed this species in a protected area in Panamá. During a long term study, I recorded nesting behavior and prey, using and comparing different methods of data collection. I will discuss a) the peculiar nest structure and location, and b) prey collection, both related to the ecological conditions of the tropical environment in which this species lives.

Materials and Methods

Barro Colorado Nature Monument (one island and five main peninsulas; 9°09'N, 79°51'W) is situated in the middle of the Gatún Lake in the Panamá Canal. The observed nesting sites were located on the island and on Peninsula Gigante. My study was developed during three following dry seasons: 1996/1997, 1997/1998 and 1998/1999. I made 3-11h per day observations at eight different nesting aggregations during the three periods, totally 110 days and 365 active females observed. Nest entrances were measured and marked. Males and females were individually marked on their thorax with non-toxic dyes. Head width and forewing length of 331 females and 101 males were measured as an estimate of size, using vernier caliper (precision: ± 0.05 mm).

I divided prey in three groups ('prey types'), on the base of their size and the color patterns of their elitra, for an immediate identification in the field. 'Prey type 1' corresponds to large beetles, about half size of the female wasp carrying them, elitra being reddish or brown. 'Prey type 2' corresponds to large beetles, also about half size of the female wasp carrying them, elitra being of a metal green-blue or red. 'Prey type 3' corresponds to small beetles, the wasp often carrying them only by mandibles, elitra brownish with black spots. I adopted three different methods to identify prey, to assess their validity for further field studies. A detailed description of each method follows.

Method 1: prey were collected from provisioning *C. binodis* females returning to the nest. Females were caught while approaching the nest entrance by an insect net or by hand, the prey they were carrying collected and killed, and the female finally released. I first assigned collected prey to a 'prey type'. D. M. Windsor and R. Regalin kindly identified prey specimens to species afterwards. From experts identification, prey species resulted as follows (for complete names see Table 1): the two species of *Megalostomis*, 'prey type 1'; the two species of *Ischiopachys*, 'prey type 2'; *Euriscopa cingulata* (Latreille), 'prey type 3'. The other species of the prey list were recorded only from scattered observations (just one individual preyed) or only from nest excavations, and were not assigned to groups. Eighty-five beetles were randomly collected during the three seasons with this method. This method is the most reliable for what concerns precise identification of species.

Method 2: *C. binodis* females approaching their nest entrance were visually inspected to identify the prey they were carrying. Females were not overdisturbed by netting or handling, but the close proximity of the observer and the size of the species allowed visual identification of the prey characteristics described above. Mis-identifications made using this method were small, given the low number of 'prey types' and their conspicuous differences. This method allowed the identification of other 798 prey.

Method 3: In the laboratory I analyzed the dry content of subterranean pedotrophic cells (each cell content kept separated), after their collection during nest excavation. Each cell contained remains of chitin part of the prey body, as elitra, head and legs, and in some cases entire specimens covered with mold. With the help of a microscope I separated each elitra and assigned it to one of the 'prey types' groups already defined. I analyzed the content of 205 cells. From the elitra and the heads it was possible to estimate the number of prey stored in each cell. The majority of the data refers to old cells, intended as cells where wasp individuals already developed and left the cell, leaving in it dry prey remains and the empty cocoon. In some cells, I found some elitra different in shape, size and color patterns from the collected

Table 1. List of Clytrinae prey of *C. binodis*. The first column represents the number of specimens collected randomly from returning females during the three dry seasons (method 1). One more specimen belongs to Cryptocephalinae and was not identified. Two more species should be added to this list, according to the remains found in the pedotrophic cells, but no key was available to assign them to any subfamily.

n	Clytrinae	Species
19	Megalostomini	*Euryscopa (Coleoneffa) cingulata* (Latreille)
41	Megalostomini	*Megalostomis (Coleobyersa) amazona* Jacoby
15	Megalostomini	*Megalostomis (Coleobyersa) flavipennis* ssp. *dynamica* Monros
1	Ischiopachini	*Ischiopachys bicolor* ssp. *cuprea* (Fabricius)
5	Ischiopachini	*Ischiopachys bicolor* ssp. *violascens* Moldenke
2	Ischiopachini	*Ischiopachys proteus* Lacordaire
1	Babiini	*Urodera* sp.

specimens. They will account for unidentified species in the final prey list and were not considered for comparisons.

I excavated nests at different nesting sites and in different seasons. I assigned at each nest a letter for individual identification (A, B, C, D) and a number related to the year it was excavated (98 = 1998; 99 =1999). The excavation did not reach the end of the main tunnel of all nests. Three nests (Nest A-98, B-98, C-99) were part of aggregations, but somehow isolated from the main group of nests (i.e. cells could be easily assigned to the nest). I excavated six more nests in the middle of a nest aggregation, an area of 2 m x 1.5 m, and data are combined (Nest D-99, corresponds to six different entrances, which cells could not be assigned to a single entrance). I analyzed 87 cells from the isolated nests (Nest A-98, B-98, C-99) and 142 cells from Nest D-99. Nest subterranean structures were illustrated by hand-drawings and using a 3D software, created for the case by D. Romani.

Cells dimensions (n = 55) were measured in the field with the caliper. Cell shape is ovoid: I measured the length between the extreme edges and the width at its half (cm). Then the cell size was estimated as the volume of the cylinder according to the two measures taken in the field.

Statistical analysis were performed using Statistica (StatSoft Inc., Tulsa, Oklahoma, USA) and following Zar (1974).

Voucher specimens of the wasps and the beetles are deposited at the Entomological Collection of the Smithsonian Tropical Research Institute in Panamá. The samples collected from the cells are in the private collection of M. Giovanetti.

Results

Data on characteristics of tumulus, entrance and cells are reported in Table 2. The tumulus is formed by a rim of soil and is typically visible on soil surface. The entrance to the main subterranean tunnel is encountered in the middle

Table 2. Descriptive statistics (mean, standard error, range) of tumulus, entrance and cell of *C. binodis* nests.

	Mean (cm)	SE (cm)	Range (cm)	n
Tumulus	Diameter: 8.8	0.44	3.60 – 13.60	31
	Height: 1.9	0.19	0.40 – 4.10	28
Entrance	Diameter: 0.7	0.02	0.56 – 1.10	31
Cell	Lenght: 2.5	0.06	1.70 – 3.40	56
	Width: 1.4	0.03	0.90 – 1.90	55
	Depth: 79.9	2.63	23 – 153	147

of it, and left open during the female intra-nest activity (excavation of burrows and cells) and hunting. The main tunnel goes down quite perpendicular from the surface level. It is more or less straight, curving when it reaches stones or other obstacles and its depth is estimated at 1-1.50 meters (Fig. 1). Lateral tunnels depart usually very short and lead to the cells, but where filled with earth once the cell is completed. The longest distance measured from the main tunnel to a cell was 21 cm. In only one case (Nest B-98) there was a plug of soil without prey (typical of the genus, where prey should be stored temporally). This nest, containing 20 cells, was also the only case where I reached the very end of the main tunnel. For Nest D-99, actually composed by six different nests, it was not possible to allocate the cells to a specific burrow (Fig. 2) and results were combined.

Female head width (Mean ± SD: 5.1 ± 0.31 cm, n = 331) was significantly larger (Student $t = 38.15$, df = 426, $P < 0.001$) than that of male (Mean ± SD: 3.7 ± 0.30 cm, n = 101). Female wings (Mean ± SD: 13.5 ± 0.84 cm, n = 329) were significantly longer (Student $t = 27.88$, df = 426, $P < 0.001$) than that of male (Mean ± SD: 10.9 ± 0.83 cm, n = 100). The species is then dimorphic with females larger than males.

The average number of prey per cell, calculated from prey remains, was 5.53 ± 2.25 (Mean ± SD; n = 204). In seven deep cells of Nest D-99, I found cocoons and a developing larva, but I did not succeed in their rearing apart for two males from one-prey cells. *C. binodis* then digs and reuses progressive nests (definition by Iwata 1976): old cells with prey remains are closer to the surface than new cells containing wasp cocoons and larvae of the ongoing season. In 136 out of 205 cells (66.7 %) the remains of a cocoon confirmed the successful development

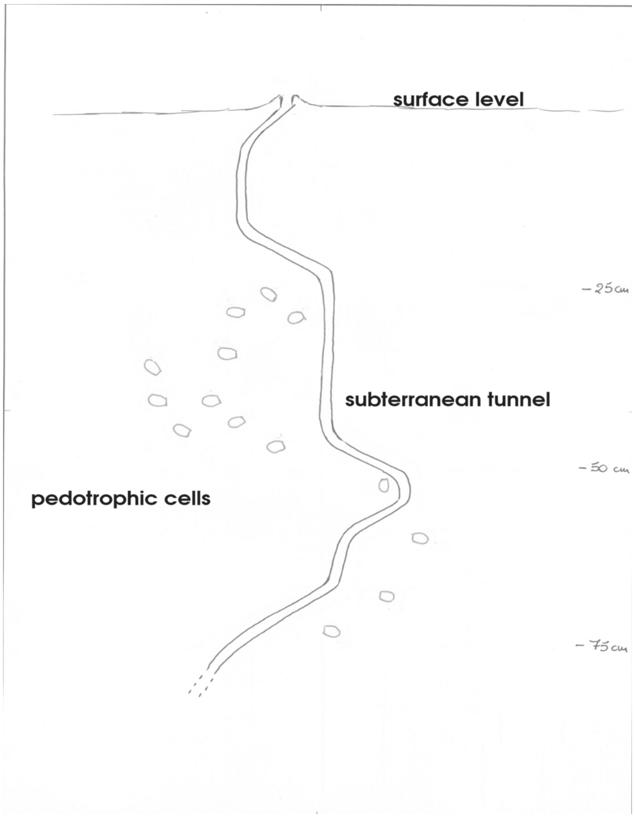

Figure 1. Hand-made illustration of the subterranean structure of a nest of *C. binodis*. The nest was isolated from the main aggregation (40 cm to the closer nest), and its end was not reached due to difficulties in the field. The illustration represents the main tunnel and the associate pedotrophic cells.

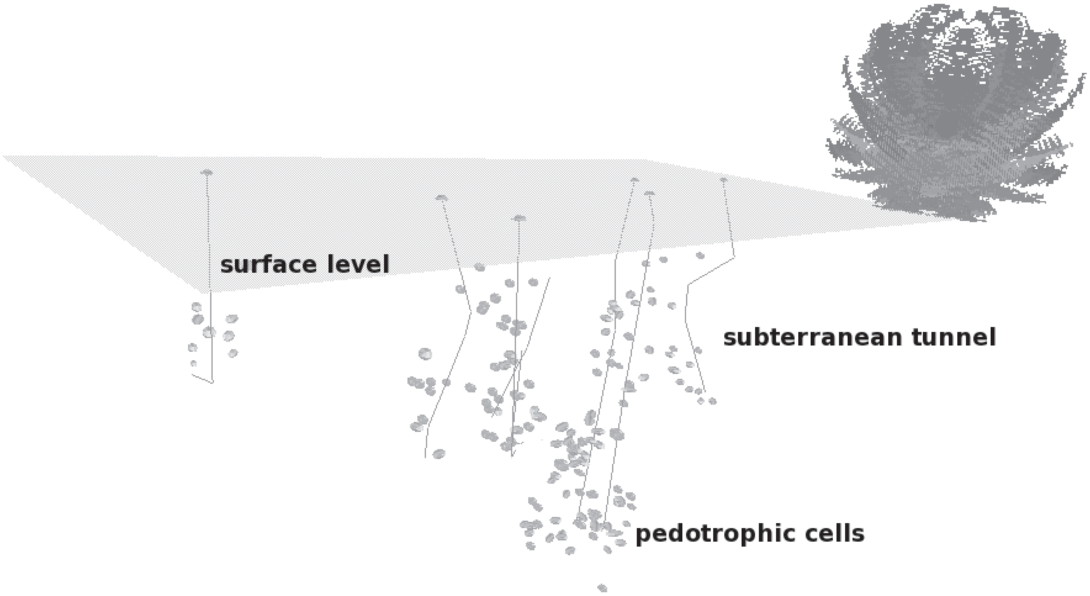

Figure 2. Illustration of the subterranean structure of six nests obtained through a special 3D software created by *D. Romani*. The surface level is represented as a gray rectangle (an hypothetic bush on the right corner), on which truncated cones refer to the tumulus and entrances. Under the surface level, lines report the directions of main tunnels, while gray spots represent the subterranean cells. In this case, cells could not be assigned to a single tunnel and data, in the text, are reported as nest D-99.

Table 3. Percentage of individuals of the three different prey types recorded during three following dry seasons and found in the subterranean cells of four *C. binodis* nests.

		Type 1	Type 2	Type 3
Seasons	1996-97	68.6	11.7	19.7
	1997-98	63.6	0	36.4
	1998-99	48.5	14.1	37.4
Nests	A-98	87.1	4.7	8.2
	B-98	93.1	4.6	2.3
	C-99	5.3	65.3	29.4
	D-99	69.8	1.7	28.5

of a *C. binodis* individual. For the remaining 69 cells where no cocoon was found, 10 cases (5%) of unsuccessful development were certainly due to mold or parasites (during field observations some unidentified Bombyliidae were observed at the nest entrances). I tested with Shapiro-Wilk test for normality the number of prey per cell and the cell volumes: for the number of prey, $W = 0.95$ and $P = 0.00001$ ($n = 204$); for the cell volumes, $W = 0.96$ and $P = 0.05$ ($n = 55$). No clear bimodality is shown by number of prey per cell or cell volumes, in contrast with what would be expected by size dimorphism in the species.

Prey specimens collected (method 1) belonged to eight species of Chrysomelidae, seven of them Clytrinae (Table 1). Only one specimen collected belonged to a different subfamily, Cryptocephalinae. Some elytra (contained in nests A-98 and D-99, six and five respectively; method 3) could not be matched with entire specimens. At least two more unidentified species from nest excavation records should then be added to Table 1.

Prey are carried under the wasp body, their ventral side up, the body embraced by the middle legs of the wasp. The wasp hold a foreleg of the beetle between its mandibles and the clypeus. Among the three seasons, there are not significant differences in number of provisioning females observed and number of prey collected (Chi-square contingency table: $\div^2 = 2.59$, df = 2) or number of days of observation and number of prey collected (Chi-square contingency table: $\div^2 = 4.27$; df = 2). Number of females observed and number of days of observations are then not influencing the number of prey recorded across seasons. There are significant differences among the frequency of prey types across dry seasons (Chi-square contingency table: $\div^2 = 52.03$; df = 4; $P < 0.001$). Prey type 1 frequency declined in following seasons (Table 3), especially in 1998-99, while prey type 3 increased, especially in 1998-99. Interestingly, no individual of prey type 2 was recorded in 1997-98.

Data from cell content are analyzed across nests, and consequently sites (Table 3). There are differences in the prey types present in the four nests (Chi-square contingency table: $\div^2 = 510.11$, df = 6; $P < 0.001$). Prey type 1 was the most common in three nests, its percentage ranging from 70 to 93 per cent. In Nest C-99 (observed during the season 1998/1999, excavated in 1999) the percentage of prey type 1 declined abruptly to 5%, while increased the amount of the other two prey types.

Between the data obtained from method 1 (collected) and 2 (from wasps), there is no significant difference among the prey types (Chi-square contingency table: $\div^2 = 4.65$, df = 2). Highly significant differences are found between data from wasps and the ones obtained by nest excavation (Chi-square contingency table: $\div^2 = 21.21$, df = 2, $P < 0.001$).

Discussion

For what concerns main nest characteristics, *C. binodis* resembles what described by Evans (1971). Depths resulted exceptional (more than one meter), similar only to those described for another tropical species, *Cerceris lutzi* Scullen (Evans 1992). These two species differ from temperate ones, whose nest depth usually varies between 10 and 50 cm, as reported in Table 4. In spite of the frequent examination of the entire island, the hilltop was the only place where I found *C. binodis* nests. Soils mantling the island are generally less than 50 cm deep and are rich in clay; soils deeper than 1 m occur only on the flat hilltop, where erosion rates are very low (Dietrich *et al.* 1996). In tropical areas the upper part of the soil is object of great changes across the seasons:

Table 4. Mean nest depth reported for some temperate species. Nests of *C. binodis* resulted exceptionally deep compared to temperate species, reaching more than one meter under the surface.

Temperate species	Nest depth (cm)	Reference
C. acanthophila	32	Hook 1987
C. californica	10	Hook 1987
C. finitima	5	Strandtmann 1945
C. huachuaca	30	Hook 1987
C. morata	27	Alcock 1974
C. rufinoda	6	Strandtmann 1945
C. rufopicta	20	mean obtained from various papers
C. serripes	10	Strandtmann 1945

being moistened by high quantity of water during the rain, forming deep cracks in the dry season. Locating the cells at great depth may be an adaptation to protect larvae from the extreme changes in temperature and humidity of the first 30-50 cm under the surface. Bimodality in cell size and content is commonly found in dimorphic species (Evans 1971, Alcock 1974, Byers 1978). In *C. binodis* bimodality is not evident, but the reasons can not be explained from this work.

All three different methods applied during this study support the prey specialization of *C. binodis* on a few species of Clytrinae (Crysomelidae). 'Prey types' recognized by visual identification (method 2) from flying females hovering on the nests was found to be reliable, considering that there were no significant differences with prey collection (method 1). This assumption is very important considering long-term study on nesting females. The collection of a prey from a returning female is obviously a shocking event for the wasp that could delay or modify its daily activity. While studying host-specific species it may be possible to reduce the displacement of nesting females, obtaining more consistent behavioral data. The previous records of prey of *C. binodis* were made by Evans (1971) and Callan (1990), also observing predation on Crysomelidae. In Panamá, from observation of active females during three subsequent seasons, *Megalostomis* were the most common prey. The other two prey types, smaller in size, may compensate when insufficient quantity of *Megalostomis* are hunted (see correlation in data on season 1998/99 and content of nest C-99), confirming the hypothesis on prey species abundance influence inferred by Byers (1978).

Records of prey species from nest excavation (method 3) is a well established method for ground-nesting wasps, that even resulted in the description of new beetle species (Valentine 1994). In the case of *C. binodis,* this method recorded the presence of some unidentified species that were not present in recent seasonal collections and that was not possible to identify from their elytra. Records from nests, excavated at different sites, showed a significant difference. Not only prey abundance, but also its distribution may explain this result. *Megalostomis* and *Ischiopachys* have been associated with Mimosaceae, and *Euryscopa* with Fabaceae (Jolivet & Hawkeswood 1995). The plant composition of the area, where nests are located, could show a possible association with prey found in subterranean cells. Also myrmecophily, recorded for some species of Clytrinae in the temperate and tropical zones of the world, may play a role. Some *Cerceris* species, nesting close to ant nests, may take advantage of the behavior of myrmecophilous Clytrinae. To investigate the above hypothesis more data are needed, dealing with ecology and hunting behavior.

This study confirms that nesting characteristics of the genus *Cerceris* follow general rules, but that adaptability plays an important role. Tropical species show differences that allow them to nest in the distinct ecological conditions offered by the tropics. Observations and comparison of more species in tropical and temperate areas may confirm a plastic behavior, possibly corresponding to a phylogenetic trait that permitted to this genus to spread in all continents and various climatic conditions.

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Literature Cited

Alcock, J. 1974. The nesting behavior of *Cerceris simplex macrosticta* (Hymenoptera: Sphecidae). J. Nat. Hist. 8: 645-652.

Amarante, F.T. 2002. A synonymic catalog of neotropical Crabronidae and Sphecidae (Hymenoptera: Apoidea). Arq. Zool. São Paulo 37: 1-139.

Bohart, R.M. & A.S. Menke. 1976. Sphecid wasps of the world. A generic revision, Berkeley, Univ. California Press., 695p.

Byers, G.W. 1978. Nests, prey, behavior and development of *Cerceris halone* (Hymenoptera: Sphecidae). J. Kansas. Entomol. Soc. 51: 818-831.

Callan, E. McC. 1990. Nesting behaviour and prey of *Cerceris* wasps in Trinidad (Hymenoptera: Sphecidae). Entomologist 109: 194-199.

Dietrich, W.E., D.M. Windsor & T. Dunne. 1996. Geología, clima e hidrología de la isla de Barro Colorado, p.25-51. In E.G. Leigh, A.S. Rand & D.M. Windsor (eds.), The ecology of a tropical forest, 2nd ed., 503p.

Evans, H.E. 1971. Observations on the nesting behavior of wasps of the tribe Cercerini. J. Kansas Entomol. Soc. 44: 500-523.

Evans, H.E. 1992. The nest and prey of *Cerceris lutzi* Scullen (Hymenoptera: Sphecidae). J. Kansas Entomol. Soc. 65: 91-92.

Hook, A. 1987. Nesting behaviour of Texas *Cerceris* digger wasps with emphasis on nest reutilization and nest sharing (Hymenoptera: Sphecidae). Sociobiology 13: 93-118.

Hook, A.W. & H.E. Evans. 1991. Prey and parasites of *Cerceris fumipennis* (Hymenoptera: Sphecidae) from Central Texas, with description of the larva of *Dasymutilla scaevola* (Hymenoptera: Mutillidae). J. Kansas. Entomol. Soc. 64: 257-264.

Iwata, K. 1976. Comparative Ethology of Hymenoptera. Amerind Publish. Co. PVT. LTD., 535p.

Jolivet, P. & T.J. Hawkeswood. 1995. Host-plants of Chrysomelidae of the world. Backhuys Publ., Leiden, 281p.

Linsley, E.G. & J.W. MacSwain. 1956. Some observations on the nesting habits and prey of *Cerceris californica* Cresson (Hymenoptera, Sphecidae). Ann. Entomol. Soc. Am. 49: 71-84.

McCorquodale, D.B. 1989. Nest sharing, nest switching, longevity and overlap of generations in *Cerceris antipodes* (Hymenoptera: Sphecidae). Insect. Soc. 36: 42-50.

Scullen, H.A. & J.L. Wold. 1969. Biology of wasps of the tribe Cercerini, with a list of the Coleoptera used as prey. Ann. Entomol. Soc. Am. 62: 209-214.

Strandtmann, R.W. 1945. Observations on the nesting habits of some digger wasps (Sphecidae; Hymenoptera). Ann. Entomol. Soc. Am. 38: 305-313.

Valentine, B.D. 1994. Two new species of *Mylscopus* Fairmaire and other Anthribidae (Coleoptera) from nests of *Cerceris* Latreille wasps in Madagascar. Coleopts. Bull. 48: 201-206.

Zar, J.H. 1974. Biostatistical Analysis. Prentic Hall International Edition, Englewood Cliffs, New Jersey, USA, 620p.

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