Nest structure and notes on the social behavior of *Augochlora amphitrite* (Schrottky) (Hymenoptera, Halictidae)

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Abstract

The nesting biology of *Augochlora* (*Augochlora*) *amphitrite* (Schrottky) in a natural reserve in the Province of Buenos Aires, Argentina, is described. The species nests in decaying wood. Two types of nest architecture were found, which differed according to the substrate where they were built, either soft or hard wood. Nests in soft wood had the cells grouped in clusters surrounded by a cavity, and the clusters were supported by a varying number of pillars. Nests constructed in decomposing portions of cracks in otherwise hard wood had the cells constructed against the walls, without any pillars or surrounding cavity. Cells of both types of nests were oriented in all directions, without any detectable pattern. Measurements and characteristics of the nests are tabulated and compared to those known for other species of *Augochlorina s. str.* Behavioral observations of active nests are indicative of a social division of tasks in *A. amphitrite*. Such observations include nests with several females, some of which were never observed outside the nests, females with different degrees of wear and of ovary development, and at least one female that actively collected pollen which had much worn mandibles and wings, and undeveloped ovaries, all characteristics of the worker caste in social halictids.

Keywords

Augochlorini, *Augochlora*, nesting biology, social behavior, Pampean Region, Argentina
Introduction

The bee tribe Augochlorini has an exclusively New World distribution, with maximal diversity in the Neotropics. This tribe is of particular interest because of the diversity of many of its biological traits, at the genus and species level, as well as within species. The social behavior in this group varies from solitary to primitively eusocial, with various degrees of sociality and transitions, including the origin of solitary behavior from eusocial ancestors (Eickwort 1969, Michener 1990, Danforth and Eickwort 1997, Wcislo and Danforth 1997, Engel 2000, Brady et al. 2006). The structure of the nests also presents ample variation within the tribe (Sakagami and Michener 1962, Eickwort and Sakagami 1979). Although most species nest in the soil, some lineages have shifted to the use of decomposing wood as a nesting substrate. Such behavior has originated repeatedly within the tribe (Engel 2000), and is known in *Augochlora*, *Megalopta*, *Xenochlora*, and some species of *Neocorynura* (Brosi et al. 2006, Wcislo and Gonzalez 2006, Tierney et al. 2008a, b, Tierney et al. 2012).

*Augochlora* is one of the more diverse genera within the tribe, with nearly 120 named species, classified in two subgenera, *Oxystoglossella* and *Augochlora* s. str. (Moure 2007). The genus ranges from southern Canada to northern Patagonia in Argentina, with most species inhabiting tropical areas. In Argentina the number of species strongly diminishes from north to south, being represented in the temperate Pampean region by only five species (Dalmazzo and Roig-Alsina 2011).

The two subgenera of *Augochlora* are considered as behaviorally divergent (Eickwort 1969, Michener 2007, Engel 2000). The subgenus *Oxystoglossella* includes species that nest in the soil and are primitively eusocial, with caste differentiation (Michener and Lange 1958, Eickwort and Eickwort 1972). Species of *Augochlora* s. str. have been considered solitary species that nest in soft wood (Eickwort 1969, Engel 2000). Their mandibles are modified, robust, with a lower preapical expansion and a well developed preapical tooth, suited for the substrate in which they dig. The behavioral characteristics of both subgenera have been inferred from what is known for a rather reduced number of species. In the case of *Augochlora* s. str., nest structure is known for *A. pura* (Say), *A. hallinani* Michener, *A. sidelifoliae* Cockerell, *A. smaragdina* Friese, *A. esox* (Vachal), *A. isthmii* Schwarz, and *A. alexanderi* Engel (Stockhammer 1966, Eickwort and Eickwort 1973, Zillikens et al. 2001, Wcislo et al. 2003), as well as some comments on a nesting site of *A. amphitrite* (Schrottky) (Sakagami and Moure 1967). The concept that the species of *Augochlora* s. str. are solitary has been challenged by Wcislo et al. (2003), who studied two nests of *A. isthmii* with more than one female. Limited data suggested that the nests might have been functioning as colonies, raising the question of whether the social behavior within the genus may be more variable than previously thought.

This contribution describes the structure of nests of *A. amphitrite*, and presents information on the nesting biology of the species. The data are compared to those known for other species of the subgenus.
Methods

Study site

The nests were studied in the reserve Refugio Natural Educativo “Ribera Norte” (34°28′10″S, 58°29′40″W), San Isidro, province of Buenos Aires, Argentina. This reserve is on the west margin of the Río de La Plata, and preserves a relict of gallery forest with typical riverine vegetation, including trees such as Ocotea acutifolia (Nees) Mez (Lauraceae), Nectandra falcifolia (Nees) J.A. Castigl. Ex Mart. Crov. & Paccinini (Lauraceae), Pouteria salicifolia (Spreng.) Radlk. (Sapotaceae), Allophylus edulis (A. St.-Hill., A. Juss. & Cambess.) Hieron. ex Niederl. (Sapindaceae), Sebastiania brasiliensis Spreng. (Euphorbiaceae), Sapium haematospermum Müll. Arg. (Euphorbiaceae) and Blepharocalyx tweediei (Hook, et Arn.) O Berg. (Myrtaceae) (Cabrera and Willink 1973).

Field observations

A nesting site of A. amphitrite was discovered in March 2008, near the end of summer. Nests were observed during seven days (35 work hours), from March 12 to April 30, when the nests were excavated. Another nesting site was located the following year in February. It was observed during three days (15 work hours), from February 7 to 14, when the nests were excavated. Although nests were not found in spring, adults flying over flowers (September to November) were collected and kept for dissection.

The activity of the bees was recorded following the methods described by Michener et al. (1955). Nest entrances were marked individually. When possible, females entering and leaving the nests were marked with a two-color code on the mesoscutum using fingernail enamel. One color was used to indicate to which nest a female belonged, and the second one to discriminate between females of the same nest. The length of activity periods, incidence of sunlight, departures and arrivals, and the presence of pollen loads, were recorded.

Nest extraction and description

The methodology described by Sakagami and Michener (1962) was followed. Talcum powder was blown through the nest entrance to assist us to follow the nest as it was excavated with the aid of a knife and a sharp point. A caliper was used for field measurements. In the laboratory, observations and measurements were made with a stereomicroscope with an ocular micrometer. Measurements are given in centimeters, with mean values and standard errors. The contents of each cell were recorded. Voucher specimens are deposited in the collections of the Museo Argentino de Ciencias Naturales, Buenos Aires.
Dissections

The day of nest excavation, arriving bees as well as those found within the nest, were fixed in Kahle's solution. Presence of pollen loads, ovarian development, and presence of fat tissue, were recorded. Length of the body, maximum width of the eye and maximum width of the gena were taken. All measurement are in millimeters.

Three groups of females are recognized according to their ovarian development. The classification of Michener and Wille (1961) is followed, but simplified. Group A: ovaries large, well developed, usually with one or two eggs ready to be laid; posterior portions swollen forcing one or both ovaries to bend (Fig. 1). Group B: ovaries developed, but without eggs ready to be laid, so ovaries not as large as those of group A, and not bent (Fig. 2). Group C: ovaries not developed (Fig. 3).

The degree of wear of mandibles and wings is indicated in a scale from 0 (intact mandibles and wings) to 3 (much worn mandibles and tattered wings).

Results

Nesting site

An aggregation of 18 nests was found in a fallen trunk of *Salix* sp. (Salicaceae) on March 12, 2008. The trunk, 3 m long and 0.8 m in diameter, was in an advanced state of decomposition, with soft wood colonized by fungi and various arthropods. Half of the trunk surface was covered by the plant *Commelina diffusa* Burm. f. (Commelinaceae), but the nests were on the uncovered surface, occupying an area of 0.60 m² on the upper and lateral parts. The nest entrances received sunlight from 11:30 to 15:00, being shaded by surrounding trees the rest of the day.

Three nests were found in railroad sleepers made from *Schinopsis* sp. ("quebracho colorado") (Anacardiaceae) on February 7, 2009. The sleepers (1.0 m long, 0.4 m wide, and 0.15 m thick) lay on the ground, forming the visitors trail in the wettest parts of
the reserve. *Schinopsis* wood is well known for its hardness. The nest entrances were located in knots and cracks, where decomposition had begun to soften the wood. The entrances were on the upper and lateral surfaces, occupying an extension of 0.50 m², and receiving sunlight from 11:00 to 15:00 hours.

**Nest architecture**

Nests on *Salix* and on *Schinopsis* differ considerably in their architecture, mainly in the distribution and arrangement of the cells.

Nest entrances on the trunk of *Salix*, separated by a minimum distance of 10 cm, presented a ring of compacted sawdust 0.75–1.00 cm in diameter ($\bar{x} = 0.85 \pm 0.08$, n = 8) of the same color of the trunk surface. Active nests sometimes presented loose particles beyond the ring, which came from broken nest plugs. The tunnels, all unbranched, penetrated toward the interior of the trunk. They had a length of 7.00–15.00 cm ($\bar{x} = 9.67 \pm 2.56$, n = 8), and a diameter of 0.45–0.50 cm ($\bar{x} = 0.46 \pm 0.03$, n = 8); their smoothed walls were lined with substrate particles. Each tunnel led to a cluster of 2–10 cells ($\bar{x} = 5 \pm 2$, n = 18), irregularly oriented, supported within a cavity by pillars. Two kinds of pillars were observed, those that were remaining parts of the substrate not excavated, and others, more frequent, made of compacted sawdust. The clusters were retrieved intact (Figs 4–5).

Nests on *Schinopsis* had shorter tunnels, 2.00–5.00 cm long ($\bar{x} = 3.10 \pm 1.36$, n = 3). The soft material of the cracks was used for cell construction. The cells were in small groups or isolated, but without any pillars, and lying against the hard wood, with no surrounding cavity, taking advantage of masses of soft substrate within the crack (Fig. 6). Nests had 8–19 cells ($\bar{x} = 13 \pm 5$, n = 3).

Cells of all nests were constructed with compacted particles of ground wood. The external surface was irregular, and the internal surface smooth and shiny, lined with a waxy substance. The cells were ovoid, with the lower surface slightly flattened (Fig. 7); the inner cavity was 0.80–1.45 cm long ($\bar{x} = 1.07 \pm 0.13$, n = 72), 0.30–0.60 cm in diameter ($\bar{x} = 0.46 \pm 0.06$, n = 72), and 0.25–0.45 cm in cell entrance diameter ($\bar{x} = 0.36 \pm 0.04$, n = 72); the cell wall was 0.05–0.30 cm in thickness ($\bar{x} = 0.09 \pm 0.04$, n = 72). The cell plugs were made with the same material as the cell walls, 0.15 cm in thickness, dish-shaped, with the outer surface concave. Table 1 summarizes the architectural characteristics of *A. amphitrite*, comparing them to other species of *Augochlora* s. str. known to date.

**Cell contents**

Nests collected from *Salix* in April had their cells filled with compacted sawdust; they had feces deposited on the posterior portion, oriented toward the bottom of the cell (Fig. 8).

Nests collected from *Schinopsis* in February were active, and the cell contents consisted of pollen masses with eggs, larvae (in various stages of development), pupae, and
a few cells with feces and filled with sawdust (Table 2). The pupae were all males. The pollen mass, placed near the bottom on the flattened surface of the cell, was slightly wider than long (0.40 × 0.35 cm), 0.43 cm high, and rather spherical, except for the flattened resting surface. The whitish egg was deposited on top of the mass, oriented along the longitudinal axis of the cell (Fig. 7).
Table 1. Architectural characteristics of nests of *Augochlora* s. str. Measurements are given in cm (mean ± SD). Data for species other than *A. amphitrite*, from Wcislo et al. 2003 (*A. isthmii, A. alexanderi*), Zillikens et al. 2001 (*A. esox*), Eickwort and Eickwort 1973 (*A. hallinani, A. sidaefoliae* and *A. smaragdina*), and Stockhammer 1966 (*A. pura*).

<table>
<thead>
<tr>
<th></th>
<th><em>A. amphitrite</em></th>
<th><em>A. isthmii</em></th>
<th><em>A. alexanderi</em></th>
<th><em>A. esox</em></th>
<th><em>A. pura</em></th>
<th><em>A. hallinani</em></th>
<th><em>A. sidaefoliae</em></th>
<th><em>A. smaragdina</em></th>
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<td><strong>Nest entrance</strong></td>
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<td>(diameter)</td>
<td>0.85 ± 0.08</td>
<td>0.34 ± 0.04</td>
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<td><strong>Tunnel</strong></td>
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<tr>
<td>Diameter</td>
<td>0.46 ± 0.03</td>
<td>0.64 ± 0.11</td>
<td>0.52</td>
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<td>0.80</td>
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<td>Length</td>
<td>9.67 ± 2.56</td>
<td>8.60 ± 0.65</td>
<td>5.60</td>
<td>20</td>
<td>2.50</td>
<td>5.00</td>
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<td><strong>Cells</strong></td>
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<td>Arrangement</td>
<td>Clusters supported by pillars. Cells isolated or in groups against substrate without pillars or surrounding cavity</td>
<td>Isolated along the tunnel</td>
<td>Isolated along the tunnel</td>
<td>In groups against substrate without pillars or surrounding cavity</td>
<td>Clusters supported by pillars. Planiform. Along a tunnel. Intermediate forms.</td>
<td>Isolated along the tunnel</td>
<td>Cluster supported by pillars.</td>
<td>In a column along the tunnel</td>
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<tr>
<td>Orientation</td>
<td>Radiated in all directions</td>
<td>Radiated in all directions</td>
<td>Radiated in all directions. Horizontal.</td>
<td>Parallel, sub-horizontal. Radiated in all directions</td>
<td>Horizontal</td>
<td>Horizontal</td>
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<td>Inner length</td>
<td>1.07 ± 0.13</td>
<td>1.39 ± 0.11</td>
<td>0.88</td>
<td>1.2</td>
<td>0.84–1</td>
<td>1.50 (outer dimensions)</td>
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<td>Inner max. diameter</td>
<td>0.46 ± 0.06</td>
<td>0.63 ± 0.06</td>
<td>0.43</td>
<td>0.5</td>
<td>0.4–0.6</td>
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<td>Neck diameter</td>
<td>0.36 ± 0.04</td>
<td>0.48 ± 0.03</td>
<td>0.45</td>
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<td>0.35–0.45</td>
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<td>Wall thickness</td>
<td>0.09 ± 0.04</td>
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<td>0.1–0.4</td>
<td>0.2</td>
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</table>
Behavioral observations

Females observed leaving and entering nests in March-April did not carry pollen loads. Activity began soon after the sunlight hit the trunk; before that, the entrances were covered with closed tumuli. Flights were inconstant, and up to three females were seen leaving and entering the same nest. The females spent 10–15 minutes perching on the surrounding vegetation, where flying males were also observed. Returning females had erratic flights, and inspected cracks and small holes in the trunk.

The three nests collected in February were active. Foraging activity began 15–20 minutes after the sunlight hit the entrances (around 11:20). A female pushed the plug of sawdust with its hind legs, scattering the particles 2–3 cm around the tumulus. After that the female remained at the entrance, with only its head visible, for 3–5 minutes before departing. After 7–10 minutes the same female came back to the nest laden with pollen. Usually, as soon as a female left the nest, another one showed its head at the entrance. When disturbed, the female turned around, plugging the hole with its metasomal terga. Activity continued for approximately 4 hours until no more sunlight bathed the nests (around 15:00). Nest 1 had five females, four of which were captured when returning to the nest (two with, and two without pollen loads); the fifth female was never observed outside the nest and was captured when it was extracted. Another nest had two females; only one of them was observed collecting pollen. The third nest had a single female. Recently emerged males were found in two nests, and males were seen flying in the surroundings of the nesting area and on flowers of *Ludwigia* (Onagraceae), 50 cm away from the nests.

Dissections

Inactive nests (end of summer). The six fixed females had slender, undeveloped ovaries (group C, Fig. 3) and mandibles and wings without signs of wear (class 0). All specimens had abundant fat tissue, in the form of small, whitish spheres. None carried pollen loads. Measurements (length of body – maximum width of eye – maximum width of gena): 9.0–0.5–0.4; 10.0–0.5–0.4; 10.0–0.5–0.4; 11.0 –0.6–0.6; 8.0–0.6–0.5; 11.0–0.8–0.8.

<table>
<thead>
<tr>
<th>Table 2. Cell contents of nests of <em>A. amphitrite</em>. Po (pollen), E (egg), Lpo (larva with pollen), pdL (pre-defecating larva), Pu (pupa), F,s (Feces, filled with sawdust), f (female), m (male).</th>
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<tr>
<td><strong>Active nests</strong></td>
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<tr>
<td>Nest 1</td>
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<td>Nest 2</td>
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<td>Nest 3</td>
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<td><strong>Inactive nests</strong></td>
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Active nests (summer)

Nest 1 (5 females). Three females were group A, had unworn mandibles (class 0), and slightly worn wings (class 1); two of them were seen carrying pollen loads to the nest; measurements, 9.0–0.6–0.5; 10.0–0.6–0.8; 9.0–0.6–0.7. One female was group B, with unworn mandibles (class 0), and slightly worn wings (class 1); this female was never observed outside the nest; measurements, 12.0–0.7–0.9. One female was group C, had worn mandibles and wings (class 2); this female carried pollen loads; measurements, 9.5–0.6–0.5. Nest 2 (2 females). Both were group A, with slightly worn mandibles (class 1) and worn wings (class 2); one of them carried pollen loads to the nest. Measurements: 9.5–0.5–0.4; 11.0–0.6–0.7.

Nest 3 (1 female). It was group A, with slightly worn mandibles (class 1) and worn wings (class 2). This female was not observed outside the nest and was captured during excavation. Measurements: 11.0–0.6–0.7.

Spring flying adults. Fourteen females were captured in spring (1, September; 7, October; 6, November). All had developed ovaries (2, group A; 12, group B), and unworn wings and mandibles (class 0). None contained fat tissue. Maximum and minimum values for these females were: length of body, 8.0–12.5, width of eye, 0.5–0.7, and width of gena, 0.5–0.9.

Discussion and conclusions

The nests of *A. amphitrite* presented two types of nest architecture according to the substrate where they were built. Common features to both types were the entrance surrounded by a ring of compacted sawdust, and the unbranched tunnels leading to the cells. Cells of all nests had the same structure, and similar proportions to those of other species of *Augochlora* s. str. (Table 1).

Nests constructed in the thick trunk of *Salix*, with a large mass soft wood, had the cells grouped in clusters surrounded by a gallery of similar diameter to that of the tunnel, and supported by a varying number of pillars. Nests constructed in the decomposing parts of the cracks and knots of the hard wood of *Schinopsis* had the cells toward the end of the tunnel, constructed against the hard walls, without any pillars or surrounding cavity. In both cases the number of cells was variable and the orientation of the cells irregularly radiated.

Cluster nests are known for *A. pura* and *A. sidaefoliae*, while studied nests of *A. isthmii, A. alexanderi, A. hallinani* and *A. smaragdina* had tunnel nests with sessile cells distributed along the tunnel, and *A. essox* had nests with grouped cells, but not forming clusters (Table 1) (Stockhammer 1966, Eickwort and Eickwort 1973, Zillikens et al. 2001, Wcislo et al. 2003). A nest with a cluster of cells supported by pillars within a cavity is the predominant and probably plesiomorphic type of nest within the tribe Augochlorini (Eickwort and Sakagami 1979, Danforth and Eickwort 1997, Engel 2000), and it is the plesiomorphic condition for the monophyletic *Augochlora*...
genus-group (Engel 2000, Coelho 2004), indicating that departures from the cluster type of nest are derived conditions within Augochlora. Although the nests of few species of Augochlora s. str. have been studied, their structure is highly variable. The two types of nests found in the present study indicate that this variation can be intraspecific, coincidently with the variation found by Stockhammer (1966) for A. pura, which had tunnel, planiform, and cluster nests, with some intermediate forms. This variation observed within Augochlora s. str., greater than in other genera of Augochlorini, would result from the irregularity of the nesting substrate. Species that nest in the soil, may be less constrained by the substrate, and can fully express their behavioral capabilities. Species of Augochlora nest on the substrate offered by diverse plants, usually trees, but also bromeliads (Zillikens et al. 2001), which offer a heterogeneous supply regarding the size, shape and degree of decomposition of the nesting sites. Probably all species of Augochlora s. str. can construct well defined clusters when an unconstrained substrate is available, as is the case in A. pura and A. amphitrite.

The daily activity pattern of the females was limited by the forest environment where the bees were studied. Females left the nests to collect pollen during a period of 3.5–4 hours, while the sunlight hit the nesting site.

The annual cycle in the study area was typical of the cycle of most halictids in temperate regions, although the winters in the study area are mild and the temperatures in July are rarely freezing. Activity begins in spring (September-October), when posthibernating females begin to visit flowers. Females captured at this time showed well developed ovaries and would be the foundresses of the first nests. The activity continues until mid March, when the nests become inactive and females of the last generation are looking for hibernacula. Females captured at this time had undeveloped ovaries and abundant fat tissue.

Nests studied in summer (February) contained larvae in various stages of development, male adults, male pupae, and adult females, which correspond at least to the first brood of the foundress (Table 2). Although possible, we are not certain whether more generations are bred between spring and mid-summer. A further brood is produced by the end of summer, so at least two broods are produced during the activity cycle. Although most females collected in February had developed ovaries, one female, from nest 1, had the ovaries undeveloped and actively collected pollen. It also had worn mandibles and wings, all characteristics of the worker caste in social halictids (Michener et al. 1955). Also, the presence within the excavated nests of females that were never observed outside the nest, is indicative of a social division of tasks.

Values taken from the fixed females show size variation among females with enlarged ovaries. The female that was never observed outside the nest in multi-female nest 1 was distinctly larger than the others in the same nest. It also had an allometrically enlarged head, with a broad gena. Females of A. amphitrite have distinct cephalic polymorphism (Dalmazzo and Roig-Alsina 2011), which can be indicated by the maximum width of the eye - maximum width of the gena coefficient. A few other females of nests 1–3 had moderately enlarged heads.
Although the number of studied nests is very low, the information recovered is suggestive of social behavior in *A. amphitrite*. Wcislo et al. (2003) reached similar conclusions for *A. isthmii*, pointing out that social behavior within *Augochlora s.str.* is more variable than previously thought, since members of the subgenus had been considered as solitary and derived from an eusocial ancestor (Eickwort 1969, Michener 1990, Danforth and Eickwort 1997, Wcislo and Danforth 1997, Engel 2000, Brady et al. 2006). Further studies are needed, both in the field and laboratory, to understand the degree of sociality in *Augochlora*, and whether its occurrence is widespread in the subgenus.

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