# Determination of Some Structural Features of the Mud Nest Materials of *Sceliphron curvatum* (Smith, 1870), (Hymenoptera: Sphecidae) in Turkey

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# ABSTRACT

The purpose of this paper is to identify the nest materials, some physical characteristics and the elemental composition of the nest of *Sceliphron curvatum* (Smith, 1870). The nest surfaces were observed with a stereomicroscope and scanning electron microscope (SEM). In the inner surface of the *S. curvatum* nest of the fibers in the mud-ball nest varied between 427 nm and 6300 nm, respectively and averaged 1465.730 nm. The thickness of the fibers in the outer and the inner surface section of the mud nest were between 14 nm and 1430 nm, respectively and averaged 629.373 nm. The nitrogen concentration was 3.94%, the percentages of the saliva, soil and the water absorption capacity were calculated to be 4.848%, 95.152%, and 18.51%, respectively. However, the amount of the elements carbon (C), hydrogen (H), sulphur (S) and nitrogen (N) in the nest was found to have a value of 3.38, 1.526, 0.020 and 0.38 weight % respectively. The colors of the individual mud pots are composed of building materials of different origin and with different shades of almost white over light brown to greyish black with long dark and light brown lines. We have found that the ratios and amount of physical characteristics, elemental composition and the fibers in the mud-ball of the nest of *S. curvatum* change with soil structure in which nest is built.

Key words: Sceliphron curvatum, nest, SEM.

Ertürk, Ö., Taş, B., Şahin, H. & Çaldaş, A. (2019). Determination of some structural features of the dud nest materials of *Sceliphron curvatum* (Smith, 1870), (Hymenoptera: Sphecidae) in Turkey. *Journal of the Entomological Research Society*, 21(2), 157-166.

# INTRODUCTION

The guild of mud-daubing spider-hunting sphecid wasps of the subfamily Sceliphrinae is represented with two genera in Europe, Sceliphron (Klug, 1801) currently comprising 10 native and 3 established exotic species (Bitsch, 2010). Sceliphron is a genus of Hymenoptera of the Sphecidae family of wasps, commonly referred to as mud daubers. The European fauna of the genus Sceliphron includes 4 native species, three of them being relatively widespread in southern and to a lesser extent in central and/or eastern Europe: S. destillatorium (Illiger, 1807; Pádr, 1989), S. madraspatanum (Fabricius, 1781), and S. spirifex (L., 1758); the fourth species, S. funestrum (Kohl, 1918), is distributed only in some peripheral Aegean Islands (and also further east, in Asiatic Turkey) (Vecht & Breugel, 1968; Hensen, 1987; Schmid-Egger, 2005). During the second half of the 20th century, two exotic species have become regular and widely represented members of the European fauna, the American S. caementarium (Drury, 1773) and Asian (Bogusch, Liška, Lukáš, & Dudich, 2005). Turkish fauna is characterized by large, but usually not clearly perceived biodiversity. which is in the easiest way expressed by the number of recorded taxa. These include the eventual impoverishment, but also the appearance of migrant species, together with potential invasive ones, which may in one way or another threaten native species Sceliphron curvatum (Smith, 1870), as documented in numerous recent reports and reviews (Pagliano, Scaramozzino, & Strumia, 2000a, b). This species looks very striking, as its body is 30 to 40 mm, (between 15 to 25 mm) long and colored black and yellow. It is known as a rare species nesting around small towns and villages and building nests of mud on rocks houses and but also very often indoor on piles of books, clothes or pieces of furniture (Gepp & Bregant, 1986; Bogusch, Straka, & Srba, 2004). Black and yellow mud daubers primarily prey on relatively small, colorful spiders, such as crab spiders (and related groups), orb weavers and some jumping spiders. Like all Sceliphron species, S. curvatum is not aggressive unless threatened. They usually find them in and around vegetation, S. curvatum appeared in Europe in the 1970s (Vecht, 1984), most likely having been carried over from its natural habitat in Central Asia to Turkey. This is the first Turkish record of S. curvatum, which was made by Gülmez & Can (2015).

The aim of this work is to present the current state of knowledge concerning the distribution of *S. curvatum* localities and determination of some properties of nests in Turkey. There are some new and very limited faunistic studies about *S. curvatum* Turkey. *S. curvatum* has been distributed only in the Black Sea region, not the other parts of Turkey so far (Gülmez & Can, 2015). However, new record of *S. curvatum* was made by us from the Ordu district. The mud-ball nesting behavior of the *S. curvatum* is little known. This is the first study about the nesting behavior of *S. curvatum* in Turkey and it will be the base for future studies.

## MATERIALS AND METHODS

#### **Nest collection**

All the mud nest materials of *Sceliphron curvatum* (Smith, 1870) (Hymenoptera: Sphecidae) were collected during May to July 2015 from Perşembe district in Ordu, located in the Eastern Black Sea region of Turkey at an altitude of 27 m. In this area, which is usually warm and rainy, and with forest trees, a lot of flowers prevail. Larvae, pupae, spiders that larvae use as food and eggs were removed from the mud nests. Approximately 20 mud nest of *S. curvatum* were gathered. The mud nests were observed, and the mud nests were removed from curtain. The collected material is deposited in the Microbiology Research Laboratory, in Ordu University, Ordu, Turkey.

#### Observation of fine surface structure

Tittle parts from the mud nest were watched with a Leica Stereo Zoom S8 APO stereomicroscope (Leica Microsystems GmbH-Wetzlar, Germany) and scanning electron microscope (SEM) (Hitachi SU1510, Hitachi High-Technologies Co., Japan). Small piece separated from mud nest materials of *S. curvatum* for electron microscopy shots were secured with double-sided carbon tape and glued on. Fixed samples were coated with 10-30 mA one min gold-palladium (SEM coating system, sputter). Review and SEM shooting in was conducted at 5-15 kV voltage. 5 kV images were placed in this article.

#### Percentage of plant material and oral secretion

The dried clear mud on rocks nest piece was planned carefully. Mud nest piece was engrossed in 0.5 N KOH solutions, and held at 70°C for 3-4 hours. After oral self-secreted materials were dissolved and mud nest piece unconnected, mud nest piece ingredients were filtrated and separated from secretion. The mud nest material was washed with sterile water and dried in 250°C in a hot plate for 10 minutes. It was weighed with filter paper. Then percentage proportion of mud material and oral self-secreted materials were estimated as in the following formula: self-secreted (%) = (k<sub>1</sub>/k<sub>2</sub>) x 100, (where k<sub>1</sub>= dried weight of sample before process, k<sub>2</sub> = dried weight of sample after process) It was slightly modified (Yamane et al, 1998).

## Absorbance

The larvae, pupas and eggs of the mud nest of *S. curvatum* were removed from the mud nest and small pieces were weighed. Each fragment was weighed after immersion in water for 30 s (Curtis, Aponte, & Stamp, 2005). The absorption capacity, given in percentage, was estimated using the following formula: Absorption capacity  $(\%) = [(m_2 - m_1)/m_1] \times 100 m_1 = dried$  weight of sample before the process;  $m_2 = dried$  weight of sample after 30 s.

#### Elemental analysis

Elemental analysis of the mud nest of *S. curvatum* samples were determined for carbon, hydrogen, nitrogen, oxygen and sulphur atoms with an elemental analysis instrument of Elementar-Vario MICRO Cube (VARIO Co.).

# RESULTS

## Observation of surface under light microscope

Investigation of the surface of both inside and outside of mud nest under light microscope on the mud-ball nest of *S. curvatum*. There were grey clear and dark brown and dark yellow lines. While it was especially bright saliva silk inside, soil particles were of different color and size in outside surface because they are small and they are used in small quantities. The saliva shone like varnish in inside surface and the soil particle was short and thick (Fig. 1 A, B, C, Table 3).



Fig. 1. a. *Sceliphron curvatum* mud nest removed from window curtain. Surface of mud nest in stereomicroscope. b. Inward facing of *S. curvatum* mud nest (1×1.25); c. Outward facing of *S. curvatum* mud nest (1×1.25).

## **Observation of surface under SEM**

We first define that *S. curvatum* uses three kinds of nest material in mud ball nest. A SEM observation revealed that low amount of organic matter, tiny vegetable chips, plant hairs and a large amount of soil (ca 1465.730 nm, 14 nm) were used as a major mud- ball nest material in all parts of the surface of both inside and outside of mud nest (Fig. 3 A, B). In addition to the above plant fibers, *S. curvatum* also used moist wet soil particles and clay. On the inside surface of this mud material, there were several plant hairs (6.30-1.19 µm in width) that were regularly oriented parallel to each other. On the outside surface of mud nest, there were only a few very small plant fiber 449 nm in width (Fig. 2 A, B, C, D). From this appearance, such materials, especially the presence of larger plant fibers in the interior keeps the mud-ball nest strong. *S. curvatum*, three parts including fiber, inorganic material soil and the oral secretions were observed both on the surface. The plant fibers were hairs and tiny fiber scrapings. There were inorganic and organic materials, especially soil, oral secretions between the fibers. It was observed that the particles of mud nest material were not water-soluble and not separated easily from each other.

## **EDX Analysis**

S. curvatum: Oxygen (O), aluminum (Al), silicium (Si), potassium (K), iron (Fe), magnesium (Mg), calcium (Ca), sodium (Na), nitrogen (N) and carbon (C) were

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determined in the sludge particle of the mud-ball nest wall with EDX analysis. O, Si, Al, and C were the major elements. Fe and N were higher than the other inorganic elements. Normally, it does not exist in nature. However, in almost all rocks, sand, clay and soil are found in silicates  $(SiO_2)$  or as  $SiO_2$  with other elements such as O and Al, Mg, Ca, Fe, Na, K. The mud of the nest was soil. The other inorganic elements were mixed in the mud. K, Ca, and Na were in very low concentrations. Fe was found as a magnetic mineral (Fig. 3). *Sceliphron curvatum*: O, C, Si and Al were the major elements. Ca, Fe, K and N were in trace amounts. The concentrations of elements are shown in Table 4. EDX spectra are shown in Fig. 4.



Fig. 2. Surface of the mud nest in SEM. a. The inner surface section; b. The outer surface of the section of the mud nest; c. Longitudinal section of mud nest; d. Cross section of the mud nest.

## Percentages of plant material and oral secretion

Oral secretion was mainly used for connecting tiny plant fibers, mud and other nest materials (Fig. 1). It was found by a SEM observation and a stereomicroscope (Leica S8 APO) that very small amounts of oral secretion were used for mud ball nest construction, compared with that used in on the wasps nest. While the coating of oral secretion was seen more on the inside surface of the mud in nest, the coating of oral secretion was seen less on the outside surface of the mud in nest. The percentages

of the soil and saliva in the *S. curvatum* mud-ball nest were calculated as 4.848% and 95.152%, respectively (Fig. 2). A, B, C and D are a SEM micrograph taken at a high magnification on the both surface of the mud-ball nest.



Fig. 3. Surface of the *S. curvatum* mud nest in SEM. a. Longitudinal section view of the fiber samples in the nest from outer surface of mud nest; b. Longitudinal section view of the fiber samples in the mud nest from inner surface of the mud nest.



Fig. 4. EDX spectra of elements embedded in the wall mud nest: S. curvatum.

## Absorption capacity and pH

While the water absorption capacity of the mud nest particles of *S. curvatum* was calculated as 18.51%, pH in water and in 0.5 N KOH were calculated 8.4 and 12.4 respectively. Some measured values of the *S. curvatum* of the mud-ball nest are shown in Table 2.

# DISCUSSION

There are individual pots, which are composed of building materials of different origin and with different shades of almost white over light brown to greyish black. *S. curvatum* preferred to use fine clay, rarely interspersed with recognizable sand and pebble shares for mud-ball nest. *S. curvatum* uses three kinds of nest material,

i.e., small vegetable chips, much of soil (sand, clay) and petty quantity of plant hairs and/or inorganic particles (Egger, 1974; Gepp & Bregant, 1986). According to Wenzel (1991), wasps of genus Polybia utilize a great variety of nest materials. In addition to the above nest materials, some Polybia species use long woody fibers like Polistes (Wenzel, 1991), but in the case of P. paulista, woody fibers were never found in the nests. However the plant fibers of D. sylvestris and D. media nests were long and thin (Bagriacik, 2013 a; b). Matsuura (1991) has reported that the structure of the envelope of a Vespa nest might be ball, bowl- or flask-shaped. The Vespa genus collects rotten wood, the dead parts of live trees and inorganic materials as nest materials (Spradbery, 1973; Matsuura, 1991). Matsuura (1991) has reported that the structure of the envelope of a Vespa nest might be ball, bowl- or flask-shaped. The Vespa collects rotten wood, the dead parts of live trees and inorganic materials as nest materials (Spradbery, 1973; Matsuura, 1991). The nests which were found in this study had no envelopes around the combs. There is only mud-ball cell. Short small plant fiber scrapings were found in the nests. However, contamination with inorganic particles has never been detected during SEM observations of nest material, which consists only of long woody fibers, in two Japanese Polistes genus wasps, P. chinensis (Kudô, Yamane, & Yamamoto, 1998) and P. riparius (Yamane et al, 1998). The fibers of the inside of mud nest were thicker than that of outside nest. We cannot only conclude that P. paulista regularly collects mud and/or inorganic particles in the field, because a possibility that such a material contaminated the plant materials is not excluded. The plant fibers are needed to keep sand and gravel granules together in mud -ball nest. However, contamination with inorganic particles has never been detected during SEM observations of nest material, which consists only of long woody fibres, in two Japanese Polistes wasps, P. chinensis (Kudô et al, 1998). Two subgenera of Polybia, Pedothoeca and Furnariana, rely solely on mud for their nest building (Wenzel, 1991). P. paulista belongs to another subgenus, Myrapetra, but there is still a possibility that this species partially depends on some inorganic materials for its nest building. Similarly Sceliphron curvatum and Sphecidae use soil when they make their nest. The oral secretions were seen as a thin membrane and such as the spider web in the inner region by under light microscope. The oral secretion in the mud nest of S. curvatum in the both regions was richer than that on the inside. The sand and clay in the mud-ball nest were spare and glued irregularly. We found from a SEM observation that S. curvatum uses only a small amount of oral secretion for the construction and maintenance of their nests. However, some researchers said that until this time there was no saliva secretion. Accordingly there are only certain moisture levels of the substrate to form the balls, also their transport and suitable for pottery (Gepp & Bregant, 1986). The nitrogen content of a small amount of mud nest was investigated in as much as nitrogen can serve as an index of the amount of oral secretion in all wild bee nests. The nitrogen content of the nest of *Polybia paulista* was 1.59-2.14%. *P. paulista* uses a small amount of oral secretion for nest construction. The nitrogen content of the Vespa analis nest was 1.1-2.0%, V. simillima 0.9-2.0%, and V. crabro 2.5% (Kudô et al, 2001). In general, the nitrogen content in most Polistes nests varies considerably (Espelie & Himmelsbach, 1990; Singer, Halldorson, Lear,

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& Andrusiak), however the variation is not much elevated in the nests of *P. paulista* and Vespa species. Major dissimilarity in nitrogen content between distinct parts of the mud-ball nest in most polistine wasps can be seen because of differences in the frequency of licking different areas with oral secretion. In other words, these wasps would smear different nest parts with different amounts of the secretion. The nitrogen concentration in the V. orientalis nest was 18.75%, V. crabro nest 27.93% (Bagriacik, 2011). We found that the nitrogen concentration in the S. curvatum mud nest was 4.848%. We found this nitrogen concentration is less than that of the other species. The amount of oral secretion was equal to the amount of plant fibers, but the amount of soil was higher than the amount of oral secretion and plant fibers in the S. curvatum mud nest. The amount of saliva was less than the amount of fiber and soil in the mud nest, the shape of the mud nest depends on the sticky materials in the soil. There were sand, red soil and garden soil as nest materials in the S. curvatum mud nest from Turkey. The soil particles were composed mainly of O, Fe, Ti, Si, C, and Al with traces of Ca, K, Na and Mg according to the EDX spectra (Ishay, Riabinin, Kozhevnikov, van der Want, & Stokroos, 2003; Ishay et al, 2008). In this study, the concentration of O, Si, C and Fe in the mud nests of S. curvatum species was higher than the other elements (Table 4). The soil in the nest of S. curvatum was sand and fine gravel. The sand was collected from the garden by the female S. curvatum. There was a small amount of C, H, N, O and S atoms elemental in the mud-ball nest piece of S. curvatum (Table 1). Mud-ball nest strength is highly dependent on its moisture content. There is a relationship between the water absorbency and low moisture content of the mud nest. The processing of sludge dough affects the sticking ability of the fibers and sand, the absorbency of nest mud and its durability (Basil-Edwardes, 1921; Vecht, 1961; 1984; Biermann, 1993). The saliva protects the nest from rain and other weather conditions (Kudô et al, 2001). In particular anthropogenic substances are preferred as underground for the planting of clay pots: porozell, bakelite, paper (books, wallpaper), rare bricks and artificial marble. Somehow, S. curvatum has managed in selection processes to ensure the required rain protection through the selection of the storage substrates. The reason for the preference of anthropogenic substrates becomes evident by a test: If the pots are sufficiently wetted with water or immersed completely in water, they break down into pulpy constituents within a few seconds. The soil nests of S. curvatum exposed to rain and droplet, were under threat from open skies (Vecht, 1984). In this study, the mud-ball nest wall of S. curvatum was very fragile. When it was touched, the mud nest was broken. This is likely because the moisture content of the mud-ball nest we analyzed was very low, 18.51% and flexible plant fibers were not preferred by female S. curvatum. The saliva was not effective on the water permeability of mud-ball nest because the mud nest has not included dry dense fibers and soil which is hydrophilic.

Area					Weight %				
Elements	N	С	н	S	N	С	н	S	
Nest sample	186.5	787	2158.5	55	0.38	3.38	1.526	0.020	

Table 1. Element analysis of the mud nest of S. curvatum samples.

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		S. curvatum				
	10	Outer surface of nest	Inner surface of nest			
Thickness (nm)	Min. Max. Average	14 nm 1430 nm 629.373 nm	427 nm 6300 nm 1465.730 nm			

Table 2. The thickness of the fibers of the mud nest (nanometer).

# Table 3. Values of parameters of nests of *S. curvatum* mud nest within the area at the final stage of their development.

Some parameters for mud nest	Dimensions of small mud nest cells	Dimensions of medium mud nest cells	Dimensions of large mud nest cells		
N	10	10	10		
Nest weight ±SE (mg)	866.6±0.060	935.3±0.059	1100.20±0.032		
Nest internal volume±SE(µI)	850.35±0.076	890.44±0.063	1020.12±0.042		
Nest diameter±SE (cm)	0.634±0.054	0.765±0.032	0.825±0.065		
Nest height±SE (cm)	1.910±0.041	2.104±0.092	2.458±0.012		

Table 4. Elements and their concentration % in a fragment according to EDX analysis.

Element	0	AI	Si	к	Fe	Mg	Ca	Na	N	С	Total
Weight %	53.08	9.80	16.04	1.77	3.99	0.75	0.47	0.37	3.94	9.79	100.00

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