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Nesting behavior of *Osmia tingitana* Benoist (1969) (Hymenoptera: Megachilidae), endemic species of North Africa with first observation of its parasite *Chrysura barbata* Lucas (1849) (Hymenoptera: Chrysididae)

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Abstract
The analysis of six *Osmia tingitana* nests found in snail shells showed that the female selects shells belonging to the *Hygromiidae* family and whose diameter was between 1.8 and 3.5 cm. The nest building materials consist essentially of sand, small pebbles, plant fragments (stems or flower petals) mixed with the pollen grains. The nest was closed with a cap formed of a greenish paste consisting of pine needles chewed and mixed with the bee saliva and pieces of the shell. The number of cells per nest was variable, and range between 4 and 7 cells for multicellular nests.The different development stages were found in the nests analyzed with observation for the first time in one nest of the parasitic species of *osmia,* namely a cuckoo wasp, *Chrysura barbata* of the *Chrysididae* family.

Keywords: *Osmia tingitana*, nesting behavior, parasite, *Chrysura barbata*

1. Introduction
The subgenus *Neosmia* Tkalcu, 1974 is paleartic and distributed across the South-West of Europe and also across the British Islands, the Canary Islands and the Mediterranean zone of Israel, in the Middle East [1, 2] includes 8 species in this subgenus and, according to [3], 4 of them occur in Algérie: *Osmia gracilicronis* Pérez, 1985, *Osmia purpurata* Ducke, 1899, *Osmia rufigastre* Lepletier, 1841 and *Osmia tingitana* Benoist, 1969. Field surveys on the genus *Osmia* Panzer, 1806 carried out by [4] in the East of Algeria show de presence of three of them: *Osmia gracilicronis*, *Osmia purpurata* and *Osmia tingitana.* Species of this subgenus are characterized by a relatively long flight period, from February to May [4]. Bees of the Osmiini tribe are known for their diverse nesting behaviour, having a variety of materials used and ways of building the nest. The nest cells are, depending on the species, made of cemented earth, fragments of masticated leaves or petals and built inside cavities on the ground or rocks, in hollowstems or even in empty snail shells, as is the case of the *Osmia* belonging to the subgenus *Neosmia*. The head morphology is frequently related to the material used for nest construction. Hence, the mason *Osmia* use their facial horns to polish the separation surfaces of the nest [5] cited by [6]. Whereas other *Osmia* have sharp mandibular surfaces, useful for masticating leaves [7] cited by [6].
Two subspecies of the subgenus *Neosmia* are known for nesting inside empty snail shells. The first of them, *Osmia bicolor* Shrank, 1781.uses smashed leaves for making the cells and closes the shells with fibres of pine needles or grass, mingled with saliva secretions [8] cited by [9]. The second one, *Osmia rufigastre*, nests also inside empty snail shells it closes with pieces of broken snail shells embedded in leaves and the female hides the nest under vegetation or stones [9].
The cleptoparasitic bees of the Megachilidae family generally belong to the genus of the same family. Some examples are the genus *Stelis* Panzer, 1806, which parasites the genus *Heriades* Spinola, 1808 and *Hoplitis* Klug, 1807*;* the genus Chelyna, which parasites *Heriades*, *Hoplitis* and *Osmia* and the genus *Dioxys* Lepeletier and Serville 1825, which parasites the genus *Osmia*. According to [10], Parasitic bees are not a solitary bees only though, there are quite a number of other parasitic families of Hymenoptera, for example the genus *Osmia* is parasited by several species of the genus *Chrysis* Linnaeus, 1767 belonging to the Chrysididae family,

such as *Chrysis austriaca* Fabricius, 1804, *Chrysis ignita* Linnaeus, 1758 and *Chrysis trimaculata* Förster, (1853) [11].

Osmia tingitana is an endemics pecies from the North of Africa (Morocco, Algeria, Tunisia, Libya and Egypt [1]. It was first described from Morocco (Tanga) by Benoist in 1969. According to our observations, this red *Osmia*, sized between 10-12 mm, nests in empty snail shells too. In Algeria, the female flies between April and May searching for suitable nesting areas to lay its eggs and visits the regional flora, like Malvaceae (*Malva sylvestris* L) [4]. According to [12], *O. tingitana* is a polylectic bee and the identified pollen carried by it belongs to Fabaceae, Cistaceae, Asteraceae and Brassicaceae families.

So far, no work has been done on this *Osmia* species nesting behaviour. As it is a North African endemic species, we found very interesting to study its nesting behaviour by determining these parameters: the diameter of the shells selected by the female, the thickness and composition of the closure cap, the different stages of the development and their organization inside the nests. A description of the parasitic species *Chrysura barbata* found in one of the studied nests is also discuss.

2. Material and Methods

2.1 Origin of the studied nests

Six snail shells containing *Osmia tingitana* nests were analyzed. They were collected in the Touchent region, in the Baghai commune (35°29'N;7°12'E; 1016 m), located at the North of the Khenchela wilaya (35°28'N; 7°5'E), in the East of Algeria. The nests (Fig. 1) were collected on the 15/V/2007, hidden under the grass and pine needles. The cap of most of the shells was upside-down, facing the ground. This was an indicative that the female of *O. tingitana* turns the shell after its closure

Fig 1: Nest of *O. tingitana* in a snail shell (original photo).

In the laboratory, nests were deposited in plastic boxes at room temperature of about 28 °C and at 31% of RH. The eggs were laid on the reserve of food (pollen and nectar mixture). They hatched in a dozen days. The larvae develop by feeding on the reserve to which they were hooked during the first ten days. Once the reserve was exhausted, the larvae start weaving a cocoon. Pupation (larvae development into nymphs) occurs in the middle of summer and adult osmia was born in the cocoon in the autumn; it passed through all winter before taking off towards the end of February.

2.2 Description of *C. barbata*, parasite of *O. tingitana*

C. barbata (Lucas, 1849) is a hymenoptera of the Chrysididae family. It belongs to the cuckoo wasps which parasitize the honeybee larvae of the Megachilidae family and the digger wasps of the Sphecidae family [13]. It is spread in North Africa (Morocco, Algeria, Tunisia and Libya). This species is of metallic red color with a green reflection; its body is 9 to 10 mm and it is roughly punctuated with a small head. Between the compound eyes, a depression or facial cavity is found. The antennae of this species are pink. The mesothorax has a median indentation. The scutellum ends with a median and thick tooth. The abdomen has only three visible segments and the other segments are colliding one against the other (Fig.2).

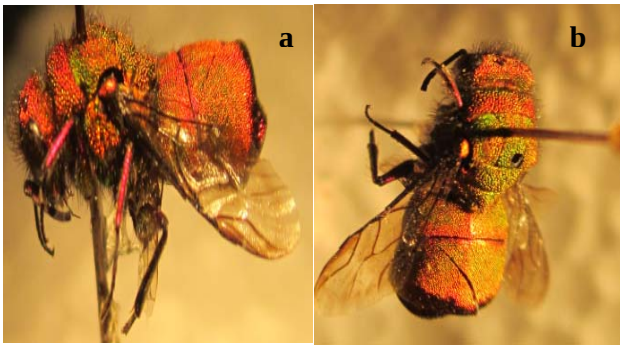

Fig 2 : *C. barbata* female, a: Lateral view, b: Dorsal view (Original Photo).

3. Results

3.1 Determination of shell diameters and their aperture

The diameter of the shells selected by *O. tingitana,* ranges between 1.8 cm and 3.5 cm (Fig.3). The largest nest (n°1) with a diameter of 3.5 cm is a shell with brown stripes. The nest (n°5) is a small white shell without stripes with a diameter of 1.8 cm. With the exception of nest (n°3), the diameter of the shells seemed to be proportional to the aperture diameter.

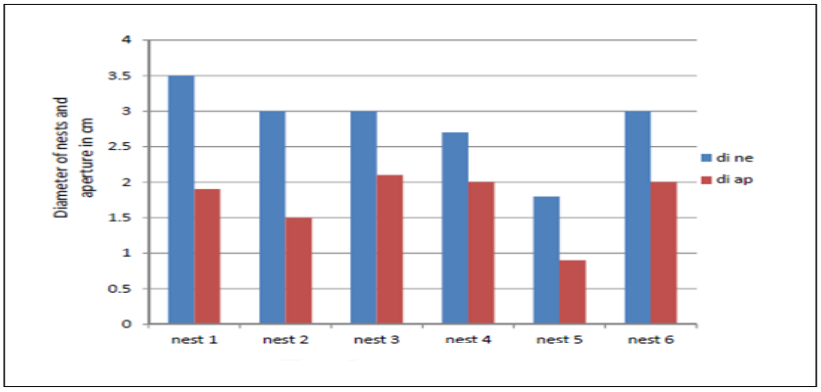

Fig 3: Diameters of the shells and their opening of the nests of *O. tingitana* (di ne: diameter of nest, di ap: diameter of aperture).

3.2 Number of cells per shell

Observations of the inside part of the dissected shells showed that the female builds several cells (between 4 and 7) inside a nest (fig. 4). However, the smallest nest (n° 5) contained only one cell. Inside the nests, the cells took the form of the cocoons they shelter; they are separated by rigid walls or partitions made by the female.

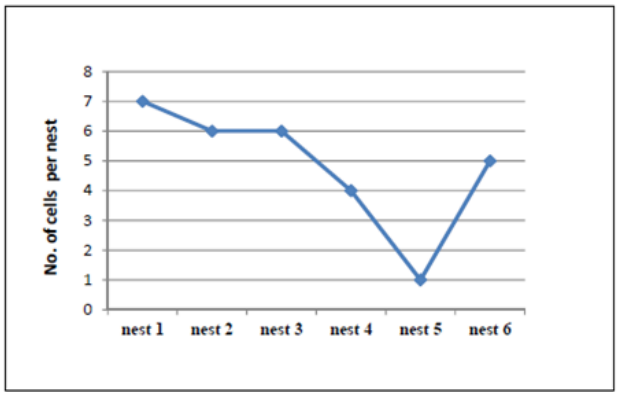

Fig 4: Number of cells per nest in *O. tingitana.*

3.3 Composition and thickness of the closure cap

Composition analysis of the shells 'closure cap showed that the latter was made from pine needles (resulting in a strong pine odor) chewed and mixed with the saliva of the bee and making a greenish paste (vegetable cement) reinforced with parts of the shell (Fig. 5). Hence, the closure cap constituted a solid mass which protected and covered the entire surface of the shell aperture.

The thickness of the shells' closure cap was not homogeneous over the entire surface; there were thicker regions, less thick regions and others thin or narrow. (Fig. 5) showed that the thickest cap is of 0.7 cm (nest n° 3) and the thinnest cap is 0.25 cm (nest n°5). Only nest n° 1 had a homogeneous thickness over its entire surface. Before the shell was closed, the female protected the eggs with different nesting materials such as sand, small pebbles, plant fragments (stems or flower petals) mixed with the pollen grains.

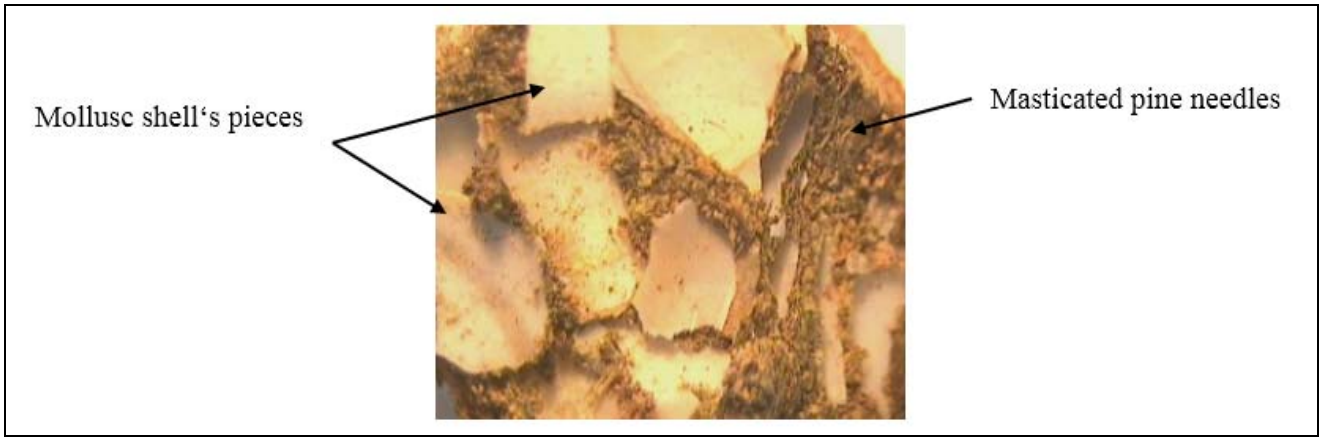

Fig 5: *O. tingitana* nest closure composition.

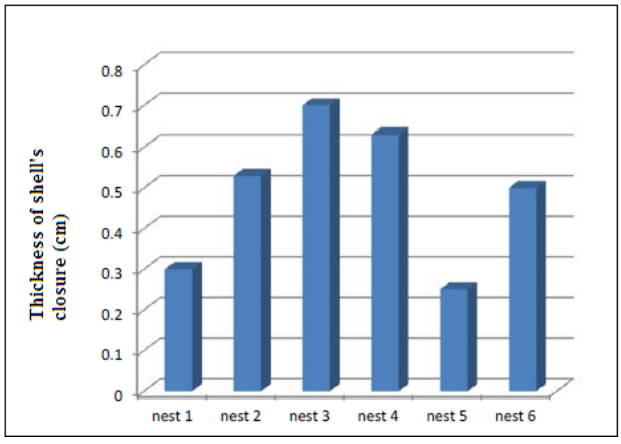

Fig 6: Closure cap's thickness of the shell.

In this study conducted on the six nests, only two emergences were observed as of 25 / II / 2008 at nest (n° 6) with the appearance of two females. The dissection of the harvested nests was carried out 20 days after the emergence of the two females). Two dissections were made to know the position of the cocoons inside the nests and also to know the different nesting materials. The first dissection was performed from the top of the shell towards the base and the second from the opening cap to the top.

Once the closure cap was removed, the nests were struck by different materials (small stones, fragments of stems) mixed with pollen grains.

The analysis of the six nests showed that they contain different development stages (Table 1). Some of them contain only one stage like the case for nest 1 and 5 which contain dead larvae and eggs respectively, nests 2 and 4 contain adults (Fig 8. a). However the other nests (3 and 6) contain nymphs and adults. Males appear to be more abundant than females; on all nests containing adult stages, the proportion of males was 72%.

Table 1: Percentages of the different stages of *O. tingitana* found in the analyzed nests. n = Number of stage recovered

Nests	**nest 1**		**nest 2**		**nest 3**		**nest 4**		**nest 5**		**nest 6**	
Stages	n	%	n	%	n	%	n	%	n	%	n	%
eggs	0	0	0	0	0	0	0	0	23	100	0	0
Larvae	7	100	0	0	0	0	0	0	0	0	0	0
cocoon	0	0	0	0	1	20	0	0	0	0	1	20
♀	0	0	2	33	1	20	0	0	0	0	2	40
♂	0	0	4	67	3	60	4	100	0	0	2	40

Fig 7: Dead larvae of *O. tingitana* (nest 1).

The *O. tingitana* eggs found only at nest 5 are tubular in shape and reddish in color. An anterior side can be distinguished with a posterior side with a black-bordered opening. Eggs vary in size; their length ranges between 3.1 mm and 5 mm and their width varies between 1 and 2 mm (Table 2). Larvae (dead) have also variable dimensions; their length ranges between 0.9 and 1 cm and the width varies between 0.3 and 0.4 cm (Table2, Fig 7).

The cocoons of the different nests are almost of the same size which varies between 1.4 cm and 1.7 cm in length and 0.7 and 0.9 in with (Table 2) (Fig b, c, d, e). The male cocoons are slightly larger than female ones since males are larger than the females. Some cocoons are thinner and of white colorlike nest 6, while the cocoons of other nests are brown in color and have a more rigid wall.

Table 2: Dimensions of the egg, larval and cocoon stages of *O. tingitana.*

Stages	Eggs (mm)	Larvae (cm)	Cocoon (cm)
Dimensions	n= 23	n= 7	n= 6
Length	0.4 ± 0,1	0.95 ±0.05	1.55±0.05
Width	0.10± 0.05	0.35± 0.05	0.80± 0.10

(mean ± SE), N = number of observed stages; SE = standard error.

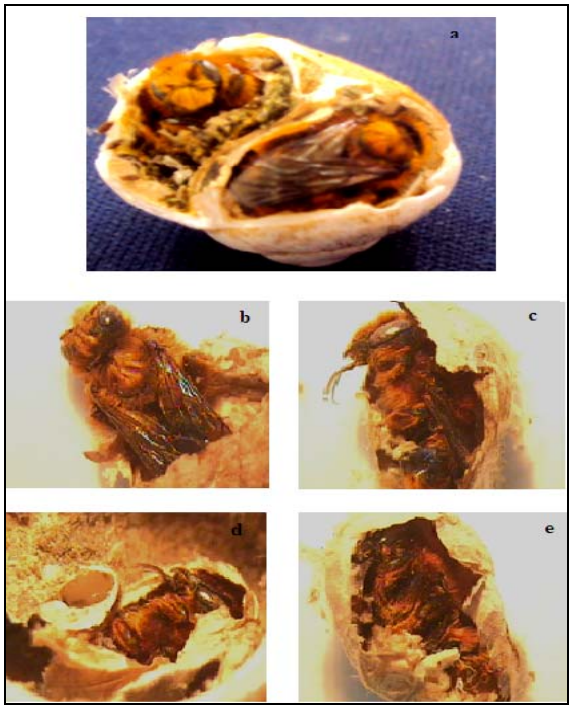

Fig 8: (a) Osmie in their logie (shell of snails)**,** Imago male of *O. tingitana* in her cocoon (**b:** dorsal view, **c**: side view**), (d) :** Female in her cocoon inside the shell, (E): female in her cocoon in lateral view,

3.5 Nest Building Material

The female *O. tingitana* builds her nest with cells of variable number. In each cell, an egg was deposited on a mixture of nectar and pollen. The cells were separated by partitions of vegetable nature (chewed leaves), or by partitions of limestone nature (coming from shells like the nest 4).The outermost cell is closed by a thin wall made of leaves slurry and protected by different nesting materials: soil, clay, mud, small pebbles, parts of leaves, stems or petals of flowers. These materials are mixed with fragments of snail shells. In some nests, nectar and pollen are mixed with these nesting materials. Other nests have particularities like nest 4 which contains a cell divided into two parts, the smallest of which contains provisions, or the presence of an opening in the partitions which separate the cells allowing their communication like in nest n°5.

3.6 Parasitic species of *Osmia tingitana*

Inside nest n°3 and near the top of the shell, two houses were found; one comprising a cocoon containing a female of a parasitic hymenoptera belonging to the cuckoo wasps of the family Chrysididae which is *C. barbata* Lucas (1849) identified for the first time in this study as the parasitic species of *O. tingitana*.

4. Discussion

One of the nesting methods specific to the *Osmia* species is the construction of nests in empty snail shells by females. This mode is observed in three species of the American subgenus *Diceratosmia* Robertson 1904 [6] and several species of the Eurasian subtype *Pyrosmia*. It is also observed at least in seven other species belonging to other genera of *Osmia* [14, 9, 15-18]. This is the case of *O. tingitana*, which belongs to the *Neosmia* genus and most species of which nest in the empty snail shells [12].

The analysis of the six snail shells containing the *Osmia tingitana* nests showed that the female disposes the nests so that the opening of the shell is turned towards the ground and then hides them under the grasses and pine needles. This behavior is also observed in *Osmia bicolor* [18-29] cited by [12]. *Osmia rufigastre* Lepeletier, 1841 behaves differently by transporting closed nests to an appropriate place and burying them in sandy soil 6-8 cm deep. However, *Osmia scutispina* Gribodo, 1894 nests are neither buried nor returned to the soil for protection [30] cited by [12].

The choice of shells used for the construction of nests does not seem to be random; all the shells selected by the female belong to the Hygromidae family, often found in dry environments. They are white, varying in diameter (between 1.8 and 3.5 cm), globular and often spiral with regular spots. The number of cells per nest seems to depend on the size of the shells; the nests containing several cells (between 2 and 7) are constructed in larger shells; the shells of small diameters (nest n°5) are composed of only two cells. [31] also revealed that the species *Osmia aurulenta*, Panzer 1799 makes nests with multiple cells, while females of *Osmia bicolor* and *Osmia rufohurta* Latreille, 1811 make unicellular nests in small shells. However, according to [12] (pers. obs) *Osmia cinnabarina* Pérez 1895 builds nests of cells in three shells of 15 mm diameter only, and for the species *O. scutispina* Gribodo, 1894 the shells are found to contain 06 Cells. The walls that separate the cells from the nests are made of leaves slurry. This is the case for *Osmia bicolor* [32, 22, 23, 26, 27, 30, 15, 18] cited by [12].

Analysis of the shells closure cap showed that the female *O.*

tingitana closes her nest with a paste made from pine needles masticated and mixed with the bee saliva; this cap is cemented with pieces of snail shells. This nest closure mode is observed in all *Neosmia* species. In *O.rufigastre*, the female builds a wall made up of fragments of snail shells cemented with leaf pulp according to [30].

The same way of nest closing is observed in the species: *Osmia gracilicornis* and *O. cinnabarina* according to [12] (pers obser), and *Osmia bicolor* according to [27, 28 18]; cited by [12]. In this study, the closure cap consists of several layers of leaves and its thickness varies between 0.3 and 0.7 cm. After the closure cap there are various nesting materials such as sand, small stones, stems fragments and petals. According to [12] (Pers obs) *O. cinnabarina* Pérez, 1895 protects cells from its nest by a thin wall (leaves pulp) followed by dense particles (pebbles, fragments of snail shells, pieces of petals and stems). The same components of nesting materials are observed in other species of the *Neosmia*.

In this study, partition walls of the nest cells of *O. tingitana* are made of leaf slurry and other partitions are of limestone origin as the case of nest 04. According to [19] cited by [12], *Osmia bicolor* creates partitions between two cells from chewed leaves of different plants (*Potentilla sp*, *Fragaria sp*, *Glaucium sp* and *Ononis sp*. Etc).

The analyzed nest cells contain different stages of development; the number of stages found in the nests coincides with the number of cells constructed by the females with the exception of nest 3 which contains 06 cells and in which there are 5 stages (1 nymph and 4 adults), and nest 5 which comprises 23 eggs whereas it is a nest of two cells. This high number of eggs deposited by the female one cell can be explained by the unavailability of empty shells which probably forces the female to lay the eggs in small shells.

The nests of many bees can be parasitized by other insects including bees known as "cuckoo bees" or even wasps (cuckoo wasps) such as those of the family Chrysididae and whose hosts belong to two Families of Hymenoptera: Megachilidae and Sphecidae. Among the Megachilidae, the genus *Osmia*, *Heriades* and *Megachile* are among the genera parasitized by Chrysididae [13]. In this case, it was possible to observe and identify for the first time the parasite of this *osmia*; a cuckoo wasp of the Chrysididae family, namely *C. barbata* with the presence of only one individual (one Female) in one of the nests. According to [29], multicellular nests, often not hidden, are strongly parasitized by Chrysididae, while unicellular or nests hidden by females are less exposed to cuckoo wasps. The vast majority of the Chrysides show a preference for hot and sunny places but the first condition of their presence is that of their hosts; a vast majority of which are themselves thermophilous insects.

5. Conclusion

The present study on the species *O.tingitana* gave an idea of the mode of nesting in the empty shells of snails as well as the identification of their parasite *C. barbata*, this species produces multicellular, nests and lays several eggs in Each shell. It would be interesting to study the nesting behavior of other species of the genus *Osmia* but also of the genera *Megachile* Latreille, 1802 or *Anthidium* Fabricius, 1804 several species of which are endemic to Algeria or the Maghreb region.

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