File SF2.

Identification and description of the identified species according to ecological and taxonomic relationships

1. Identification of associated insect species

All the identification based on morphological traits were confirmed through the comparison of the *COXI* sequences with available databases, with the following exceptions: (i) two specimens of *Trogoderma* (Coleoptera, Dermestidae) for which the species could not be identified based on morphology (ii) a specimen of *Minettia* (Diptera, Lauxaniidae), identified as *M. tabidiventris* according to morphology, but as *M. lupulina* (Fabricius, 1787) according to DNA-barcoding; given the remarkable morphological differences between the two species, we think that the lack of match is possibly due to errors in *M. tabidiventris COXI* sequence reported in NCBI (MZ350575.1); and (iii) two Hymenoptera classified as *Psenulus pallipes* (Panzer,1798) and *Trychosis legator* (Thunberg, 1824) according to DNA-barcoding and as *Psen ater* (Olivier,1792) and *Aritranis director* (Thunberg, 1824), respectively, following morphological traits. Moreover, the morphological identification of four species could not be confirmed, either because the specimen was not sequenced (*Rhodanthidium septemdentatum* (Latreille, 1809), *Attagenus pellio* (Linnaeus, 1758)) or due to unreliable sequencing results (*Euodynerus posticus* (Herrich-Schaeffer, 1841), *Gasteruption jaculator* (Linnaeus, 1758)).
Finally, three ichneumonid species were identified based only on the DNA-barcoding (*Agenioideus cinctellus* Spinola, 1808; *Amblyteles armatorius* (Forster, 1771); *Dichrogaster modesta* (Gravenhorst, 1829).

2. Description of the identified species according to ecological and taxonomic relationships

Out of the 32 species, 14 could not be assigned with regard to their specificity to the host taxa, and among these, eight were considered occasional nest users. Nests of *M. parietina* are indeed very durable and robust (File SF1) and several megachilid species (Tables. 1 and ST1) appear to use them occasionally. Although interspecific nest usurpation among bee species with overlapping flight periods is also possible, we never observed a direct interaction between females of these species and *M. parietina* and, to the best of our knowledge, such observations have never been reported. Therefore, the nesting site included at least seven megachilid species, and this possibly also increases the diversity of parasites and predators. The presence of megachilid species different from *M. parietina* was revealed by the cell opercula, which, although built in mud, differs from those of *M. parietina*. Besides bees, two other species have been reported as nest users. The ichneumonid *Agenioideus cinctellus* Spinola, 1808, has been reported to prey on salticid spiders on vertical surfaces such as old walls and to use empty mud cells as nests. Although in this study we did not consider other arthropods outside insects, several salticid spiders have been observed, and this, together with the presence of available cells, could make *M. parietina* nesting sites very suitable for the reproduction of this ichneumonid species. The Vespinae wasp *Euodynerus posticus* (Herrich-

Schaeffer, 1841) was also considered an occasional nest user since this species, which preys on lepidopteran larvae has been reported to use the nests of mud daubers (*Sceliphron* Klug 1801).
Several specimens of *Minettia tabidiventris* (Papp, 1877), a lauxaniid species whose biology, including the larval diet, is little known, were observed hanging around the cells, in a behaviour recalling that described for the cleptoparasite fly *Cacoxenus indagator* Loew, 1858 (Falk, Lewington, 2018). Taking into account the literature, we have included this species in the "scavengers" category.
The bombyliid species *Spogostylum tripunctatum* (Wiedemann, 1820), already reported as a parasitoid of *M. parietina*, could not be assigned for its specificity to the host taxon, due to the lack of bibliographic information.
The remaining four species, three Ichneumonids and one species of Psenidae, could not be classified with regard to their ecological relationship with *M. parietina* and therefore were considered occasional visitors. Among these, there were several adult females of *Psenulus pallipes* (Panzer,1798) (Psenidae). According to the available literature, this species preys on aphids and nests in stems, which made us consider it as an occasional visitor.

One species the dermestid *Attagenus pellio* (Linnaeus, 1758), identified among specimens that emerged from the collected nests was considered as generalist. This species has previously been reported as commensal in bird nests and also known to damage stored products.

Two species, the crysid *Chrysura radians* (Harris, 1776) and the ichneumonid *Stenerella domator* (Poda, 1761) have been classified as linked to Hymenoptera; however, the relation of this latter with *M. parietina* could be stricter, since the hosts of the subtribe Osprynchotina, to which *S. domator* belongs, are reported to be “wasps whose nests are made of mud or contain mud, and probably sometimes nests of bees” (Townes, 1970).

Fourteen species were considered associated with bees on the base of their obligate or preferred biological relationship.

Among species associated with Anthophila in general, we included one of the two clerid species *Trichodes alvearius* (Fabricus, 1792), a predator of larvae, two commensal *Trogoderma* species (Dermestidae), the bombyliid *Anthrax anthrax* Schrank 1781, and the eulophid species *Melittobia acasta* (Walker, 1839). None of them, apart *M. acasta*, has previously been reported for *M. parietina* nests. *A. anthrax* was very abundant and females were observed several times rubbing their abdomen in the loose soil before releasing eggs while hovering in front of the cells (Video SV1). According to Yeates (Yeates, 1994) eggs stuck on soil particles are more easily directed toward the cell openings. Moreover, because of the dark colour, *A. anthrax* females are mimetic to *M. parietina* females when both species are active and fly in high numbers in front of the nests. Many males and females of both the identified bombyliid species were observed to emerge starting from the beginning of June and to mate close to the nests. After their heads emerge from the cells they remain still for one hour or more and are often preyed on by lizards. This is the only case when predation by vertebrates has been observed on insects present on the nests. The eulophid species *Melittobia acasta* (Walker, 1839) was classified as associated mainly with Anthophila, because it is a well-known parasite or hyperparasite of bees although also reported for wasps (including parasitic wasps), flies (including parasitic flies) Lepidoptera and Coleoptera. Interestingly among the genera including parasites of bees reported as hosts for *M. acasta* (González et al. 2004), three have also been found in the nesting site: namely *Anthrax* Scopoli, 1763; *Monodontomerus* Westwood 1833, and *Chrysis* Linnaeus, 1767. Moreover, several *M. acasta* individuals emerged from the same cell where a male of *M. parietina*, seven *Monodontomerus aeneus* (Fonscolombe, 1832), and one specimen of *Ptinus sp.* were also present, while in another nest, they were found in cells containing only a bee cocoon.

Seven species were classified as associated in general with megachilids: *Ptinus sexpunctatus* Panzer, 1789 (Ptinidae), the clerid *Trichodes apiarius* (Linnaeus, 1758), both predators of larvae, the crysid species *Chrysis marginata aliunda* Linsenmaier, 1959 and *Chrysis sexdentata* Christ, 1791, , *Leucospis dorsigera* Fabricius, 1775 (Leucospidae), *Monodontomerus aeneus* (Fonscolombe, 1832) (Torymidae) and *Gasteruption jaculator* (Linnaeus, 1758) (Gasteruptiidae). Only two of these species had been previously reported for *M. parietina*.
L. dorsigera Fabricius, 1775 whose main hosts are *Osmia* species, has to the best of our knowledge, never been reported for the subgenus *Chalicodoma*; while among the megachilid species present, *M. ericetorum* Lepeletier, 1841 is a known host (Baur, Amiet, 2000). Many males and a lower number of females were seen each year and two specimens emerging from the nests have been observed in the middle of June 2019. Several attempts of egg laying have been observed and one complete deposition was observed in June 2019 (Video SV3); however since the cell on which the deposition occurred had not been marked before, we are not sure if it has been closed by *M. parietina* or by other associated megachilid species
Many specimens of *M. aeneus*, previously reported as *M. nitidus* (Grissell, 2007; Bonelli, Campadelli, 1990), have been observed flying around the nests for almost the whole flying period of *M. parietina* and several individuals have been found in the cells of the collected nests. Given their small size and the limited length of their ovipositor, they are likely to parasitise *M. parietina* cells before they are covered with the outer soil layer (file SF1).
Finally, the Gasteruptiidae *G. jaculator* was considered in this category since it is a parasitoid mainly of Megachilidae (Bogush, 2021).

As expected, the stricter association with the subgenus *Chalicodoma* is found among cleptoparasite bees (cuckoo-bees) and parasitoids. *Stelis nasuta* (Latreille, 1809) and *Coelioxys aurolimbata* Förster, 1853 were among the most common specimens observed . Both species adopt an open-cell parasitic strategy (Danforth et al., 2019) and we observed many females of both species sneaking inside the cells when the host females leave the nests. Every year, *S. nasuta* specimens appear at the end of May, *C. aurolimbata* 7-10 days later (fig.3). Moreover *C. aurolimbata* females start to visit the host cells in the late morning, a couple of hours after *S. nasuta*. With regard to the other solitary bees present, *C. aurolimbata* has also been reported to parasitize *M. ericetorum*, but given the limited number of specimens, *M. parietina* was certainly the main host.
Leucospis gigas Fabricius, 1793 is a parasitoid strictly associated with the subgenus *Chalicodoma*. Contrary to *L. dorsigera*, no males were found for *L. gigas* which reproduces via obligate parthenogenesis (Baur, Amiet, 2000). Several attempts of deposition have been observed towards the end of *M. parietina* flying period when most cells had already been closed; and a complete deposition, lasting 86 minutes, was observed (Video SV2). According to our hypothesis about the nest's external layer as protection against parasitoids, we could observe that *L. gigas* females spend a long time proofing the nest surface with their ovipositor to find a suitable point where to insert their ovipositor.

Very interestingly, despite the very high number of parasites and their proximity to the cells, *M. parietina* females were never observed chasing them away.

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