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Termites of the Monastery of Saint Catherine (Sinai, Egypt)

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Abstract

The Greek Orthodox Monastery of Saint Catherine is the oldest continuously inhabited Christian Monastery, and is included in a UNESCO World Heritage Site due to its cultural importance. In 2001, termite-related damage was observed in some of the Monastery buildings and in a church in the nearby village of Saint Catherine. The identification of the termite species found in the area of the Monastery was a priority for planning pest control activities. Morphological analysis revealed the presence of two species of higher termite (Termitidae): *Microcerotermes eugnathus* Silvestri and *Amitermes vilis* (Hagen), the latter new to Egypt. Because genetic data on *Microcerotermes* and *Amitermes* species from this area are lacking, we also sequenced mitochondrial DNA genes (COII and 16S) of these species and of two additional species, *Microcerotermes palestinensis* Spaeth and *Amitermes desertorum* (Desneux), occurring in nearby areas. The systematics of *Microcerotermes* species in Africa and the Levant is still confused, some synonymies are suspected, and recent revisions are lacking. As for *M. eugnathus*, no diagnostic differences with the more recently described *M. palestinensis* are known, and we suspect that *M. palestinensis* might be a junior synonym of *M. eugnathus*. In the Monastery, termite-related damage was mainly limited to structural wood and scarce objects of historical value. Termite monitoring and control would be necessary to prevent serious damage to objects of important historical value, especially in consideration of the recent increase in rainfall in the area of the Monastery, which could favour termite abundance and activity.

Key words: *Amitermes vilis*, *Amitermes desertorum*, *Microcerotermes eugnathus*, *Microcerotermes palestinensis*, termite damage.

Introduction

The Greek Orthodox Monastery of Saint Catherine (28°33'22"N 33°58'34"E) was founded in the 6^{th} century at the foot of Mount Sinai, in the South of the Sinai Peninsula (Egypt). It is the oldest Christian monastery still in use for its initial function, and its area is sacred to Christianity, Islam, and Judaism. The area of the Monastery is included in a UNESCO World Heritage Site, due to its cultural and natural importance (UNESCO, 2002).

The Monastery lies at 1580 m a.s.l. in an arid montane valley. The bottom of the valley consists of rocks, gravels and sands, that favour a rapid water drainage. The natural vegetation of the valley is limited to scattered herbaceous or bushy plants, such as *Origanum syriacum* v. *aegyptiacum* (L.) Tackh. and *Phlomis aurea* Decne., which are endemics in southern Sinai (Ayyad *et al.*, 2000) and which grow in dry creek beds and in crevices. In the Monastery, water is regularly available, as it is obtained from wells or, in drought periods, supplied by tankers. Therefore, in the gardens of the Monastery, the vegetation is richer, with native plants such as caper bush, as well as cultivated plants such as cypress, eucalypts, oleander, olive, date palm, apricot, almond, and pomegranate. A large old bramble bush (*Rubus sanctus* Schreb.), which is traditionally believed to be the biblical burning bush, also grows in the Monastery.

In 2001, during extensive maintenance works, the presence of termites was noticed in some of the Monastery buildings. Old termite-related damage was observed in altars, wooden stalls, and other wooden furniture. Termite presence was later noticed also in the church of the Twelve Apostles, near the village of Saint Catherine, on the northern slopes of Mount Raba, about 3 km in a straight line west of the Monastery. During the few years that followed, wooden objects in the church of the Monastery were treated with insecticides, buildings around the church were partially renovated, and several infestation hotspots were eliminated. In 2004, one of the authors (M.M.) was entrusted by the Monastery with the evaluation of the characteristics of the infestation for the planning of pest control activities. In the years 2004-2009, inspections were carried out in the Monastery, in the surrounding area, and in the church of the Twelve Apostles, and termite samples for taxonomic identification were collected.

The morphological analysis of these samples, conducted in this study, shows that two species of higher termite (Termitidae) are present: *Microcerotermes eugnathus* Silvestri and *Amitermes vilis* (Hagen). Because genetic data for *Microcerotermes* and *Amitermes* species from North Africa and the Levant is lacking in the literature, we analyse mitochondrial DNA (partial sequences of the genes COII and 16S) of *M. eugnathus* and *A. vilis*, and of two species occurring in nearby areas: *Microcerotermes palestinensis* Spaeth and *Amitermes desertorum* (Desneux), and we compare them with available sequences from other *Microcerotermes* and *Amitermes* species.

Materials and methods

Sample collection

Collection sites are shown in figure 1. Termites were looked for in structural wood (beams, window frames, door frames, straw-reinforced concrete in the ceilings), as well as in furniture and wooden objects in churches and other buildings (kitchen, library, cellars). Live plants, logs and stumps were inspected in the garden. Natural areas surrounding the Monastery were also inspected for termite presence. Some of the samples were obtained from monitoring stations that had been in-

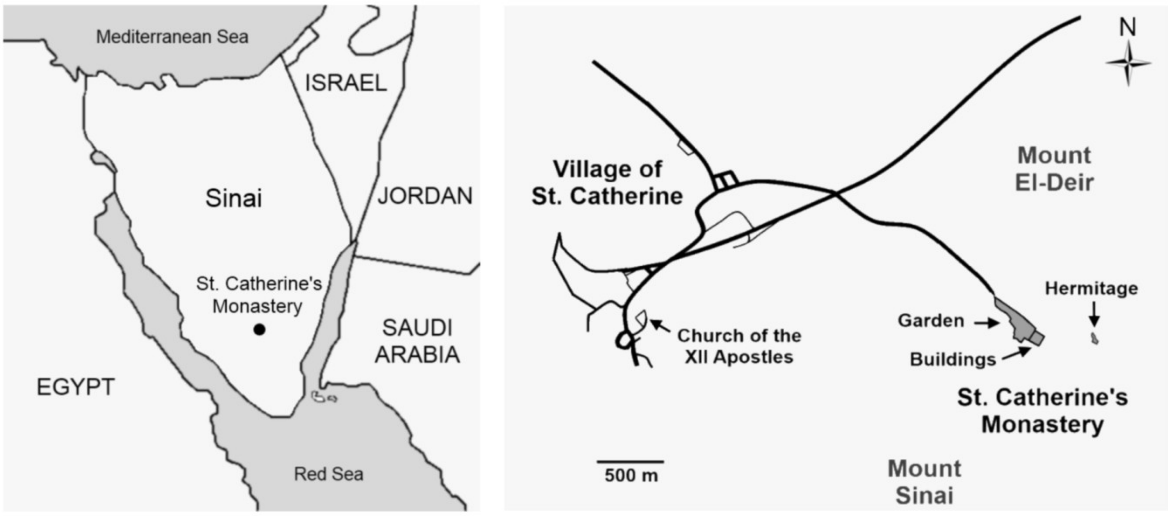

Figure 1. Location of the Monastery in the Sinai Peninsula (left) and topography of the area of the Monastery (right).

stalled in and around the church of the Monastery.

Twenty-eight termite samples were collected, of which 4 were in the Monastery (Church and its surrounding buildings), 8 in the adjoining garden, 2 in the valley upstream of the Monastery, 3 in the hermitage in a side valley upstream of the Monastery, and 11 in the church of the Twelve Apostles. Samples to be used for morphological analysis were preserved in 70% ethanol. Workers from a subset of the samples (1 from the Monastery, 2 from the garden, 1 from the valley, 1 from the hermitage, and 2 from the church of the Twelve Apostles) were preserved in 100% ethanol for genetic analyses.

Morphological analysis

A preliminary morphological observation of soldiers and alates allowed the discrimination of the samples into two genera: *Microcerotermes* and *Amitermes*. Identification at species level was obtained for *Amitermes* samples using the keys for alates and soldiers by Sands (1992). For African and Middle Eastern *Microcerotermes* species, recent taxonomic revisions and identification keys do not exist, so we used descriptions of *Microcerotermes* species available in the literature (Sjöstedt, 1904; Silvestri, 1911; 1920; Weidner, 1955; Spaeth, 1964; Kaschef and El-Sherif, 1972), taking into particular consideration the species known to occur in Egypt, North Africa or the Middle East. The analysis was based mainly on soldiers, because the characters of alates are scarcely useful for the identification of *Microcerotermes* species from North Africa and the Middle East (Spaeth, 1964) and, in the case of the North African species *Microcerotermes palaearcticus* (Sjostedt), no description of the alate exists.

Measurements were taken with an ocular micrometer mounted in a stereomicroscope. All the alates and soldiers found in the samples, from at least two colonies for each species, were measured. For alates, the following measurements were taken: head length (from the distal margin of the clypeus to the posterior end of the head), maximum head width, maximum and minimum eye diameter, distance between the eye and the ocellus, tibia length in the third right leg. For soldiers, the following measurements were taken: head length (from the line joining the two mandible insertions to the posterior end of the head), maximum head width, left mandible length, tibia length in the third right leg. In individuals with complete antennae, the number of antennal articles was also counted.

For scanning electron microscope observations, samples were kept in a 1:1 solution of hexamethyldisilazane/ethanol for 2-3 hours, then immersed in hexamethyldisilazane 100%, air dried, mounted on aluminium supports, and finally metalized with gold in a metalizer BIO-RAD SC 502 (45 seconds at 15 mA). Observations were carried out with a Jeol JSM-5200 microscope. Two alates, 2 soldiers and 2 workers were examined for each genus.

Genetic analysis

In order to compare the samples from Sinai with available DNA sequences of species of the respective genera, the mitochondrial genes COII and 16S were amplified and sequenced in two workers from each sample. Samples of *A. desertorum* from Aswan (Egypt) and *M. palestinensis* from Be'er Sheva (Israel) were also analysed for comparison.

Total DNA was extracted from termite heads with CTAB protocol (Doyle and Doyle, 1987). Two individuals for each sample were analysed. A 702-bp portion of the mitochondrial gene COII and a 516-522-bp portion of the mitochondrial gene 16S were amplified, using the primers TL2-J-3034 (5'-AAT ATG GCA GAT TAG TGC A-3') and TK-N-3785 (5'-GTT TAA GAG ACC AGT ACT TG-3') for COII and LR-J-12887 (5'-CCG GTC TGA ACT CAG ATC ACG T-3' and LR-N-13398 (5'-CGC CTG TTT AAC AAA AAC AT-3') for 16S. PCR was performed in a 50 µl mixture using GoTaq® Flexi DNA Polymerase kit (Promega, USA). Reaction conditions were set as follows: initial denaturation at 95 °C for 5 minutes; 30 cycles composed by denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds, extension at 72 °C for 30 seconds; final extension at 72 °C for 7 minutes. Purification and sequencing were performed by Macrogen Inc. (Amsterdam, Netherlands).

The preliminary analysis and the alignment of DNA sequences were performed with MEGA version 5 (Tamura *et al.*, 2011). For the construction of phylogenetic trees, COII sequences from *Amitermes* and *Microcerotermes* species from different geographic regions, published in previous studies (Ohkuma *et al.*, 2004; Inoue *et al.*, 2005; Bergamaschi *et al.*, 2007; Inward *et al.*, 2007; Ozeki *et al.*, 2007; Monaghan *et al.*, 2009; Hausberger *et al.*, 2011; Zhang and Leadbetter, 2012; Dietrich *et al.*, 2014; Bourguignon *et al.*, 2015; Rahman *et al.*, 2015) were drawn from GenBank and added to the alignments. Sequences from *Neocapritermes talpoides* Krishna et Araujo (Termitidae) and *Reticulitermes urbis* Bagneres, Uva et Clement (Rhinotermitidae) were used as outgroups (A.N.: DQ442201 and AF291736). The 16S gene was not used for phylogenetic analysis because sequences are available only for a few *Amitermes* and *Microcerotermes* species.

Models of nucleotide substitution were tested with JModelTest 2.1.2 (Darriba *et al.*, 2012), with the corrected Akaike Information Criterion. Maximum likelihood trees were obtained with PhyML 3.1 (Guindon and Gascuel, 2003), and bootstrap values were calculated after 200 replicates. Maximum parsimony analyses were performed with PAUP* 4.0 (Swofford, 2003), with bootstrap values calculated after 1000 replicates. Bayesian trees were obtained with MrBayes 3.1.2 (Huelsenbeck *et al.*, 2001; Ronquist and Huelsenbeck, 2003). Convergence was reached after one million generations (average standard deviation of split frequencies <0.01), and 25% of the trees were discarded as burn-in.

Results and discussion

The morphological analysis of the samples collected in the area surrounding the Monastery of Saint Catherine revealed the presence of two termite species: *M. eugnathus* in the Monastery, the adjoining garden, and the hermitage; *A. vilis* in the valley upstream of the Monastery and in the church of the Twelve Apostles (village of Saint Catherine). The two genera can be easily distinguished based on the shape of the head, and particularly of the mandibles of soldiers: the mandibles of *A. vilis* are shorter than those of *M. eugnathus* and have an evident tooth on the inner margin, while the mandibles of *M. eugnathus* have a minutely serrate inner margin (figure 2). The mandibles of *Amitermes* spp.

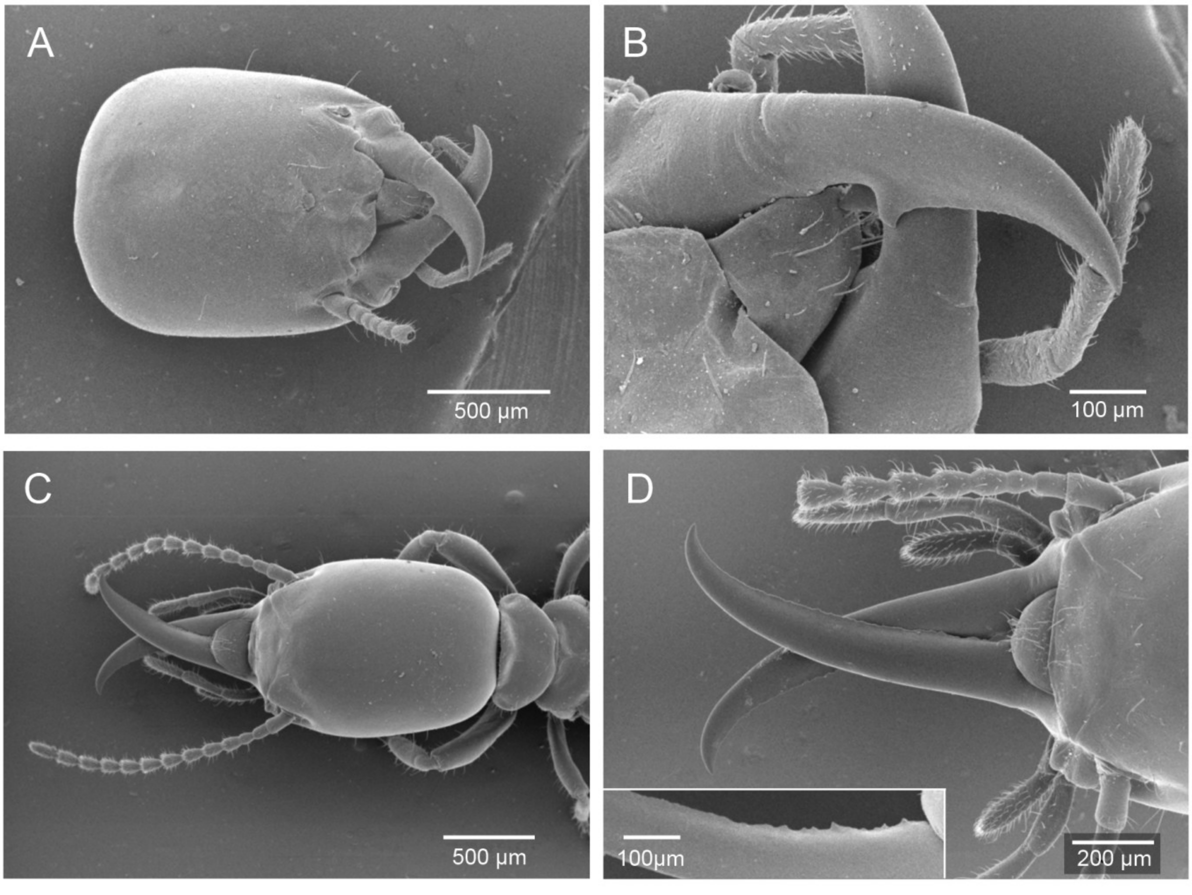

Figure 2. Soldier of *A. vilis*: A) head, B) detail of the left mandible, showing the marginal tooth. Soldier of *M. eugnathus*: C) head, D) mandibles, and (in the inset) the minutely serrate inner margin of the basal portion of the left mandible.

Table 1. Measurements (mm) and number of antennal articles of soldiers (n = 6) and alates (n = 2) of *A. vilis.*

	Soldiers		Alates	
	Range	Mean ± S.D.	Range	Mean ± S.D.
Head length	1.13 - 1.31	1.22 ± 0.10	0.94 - 0.95	0.94 ± 0.01
Head width	1.01 - 1.04	1.02 ± 0.01	1.01 - 1.03	1.02 ± 0.02
Mandible length	0.69 - 0.74	0.72 ± 0.02		
Eye: maximum diameter			0.24 - 0.28	0.26 ± 0.03
Eye: minimum diameter			0.22 - 0.27	0.24 ± 0.03
Distance eye-ocellus			0.04 - 0.06	0.05 ± 0.02
Tibia length	0.93 - 1.01	0.97 ± 0.04	0.98 - 1.01	1.00 ± 0.02
N. of antennal articles			14	

were traditionally classified as biting mandibles, while those of *Microcerocerotermes* spp. as slashing mandibles (Prestwich, 1984), but recent studies show that may be both included in the biting type (Scholtz *et al.*, 2008).

M. eugnathus had already been reported in Egypt (Kashef and El-Sherif, 1972; Moein and Farrag, 1998) but not in Sinai, while *A. vilis* is new to Egypt.

Amitermes vilis

Until the present study, *A. vilis* was known to occur in the Middle East, with Israel at the western limit of its distribution range (Krishna *et al.*, 2013). The populations of *A. vilis* from Israel were formerly known as *Amitermes wahrmani* Spaeth, which is now a junior synonym (Sands, 1992).

Two species in the genus *Amitermes* were known from Egypt: *Amitermes desertorum* (=*Amitermes santschi*) Desneux and *Amitermes messinae* (=*Amitermes harlei*) (Fuller). All the castes of *A. vilis* can be distinguished from *A. messinae* because they have no prominent spine-like setae on the fore coxae. The soldier of *A. vilis* can be distinguished from those of *A. messinae* and *A. desertorum* based on the point of the marginal tooth: erect from the inner curve of the mandible blade in *A. vilis*, in line with the inner curve of the mandible blade in *A. messinae* and *A. desertorum* (Sands, 1992).

Morphological data on *A. vilis* samples from Sinai are reported in table 1.

COII and 16S sequences of *A. vilis* and *A. desertorum* obtained in this study are deposited in GenBank under accession numbers KU523912-KU523914 and KU523917-KU523919.

In *A. vilis* samples, two COII haplotypes and two 16S haplotypes were identified. Samples from the church of the Twelve Apostles and from the valley upstream of the Monastery have different haplotypes for both genes. In the phylogenetic reconstruction based on COII alignment (figure 3) species from different biogeographic realms belong to separate clades. Phylogenetic relationships among clades from different realms are not resolved. The species most related to *A. vilis* is *A. desertorum* from Egypt.

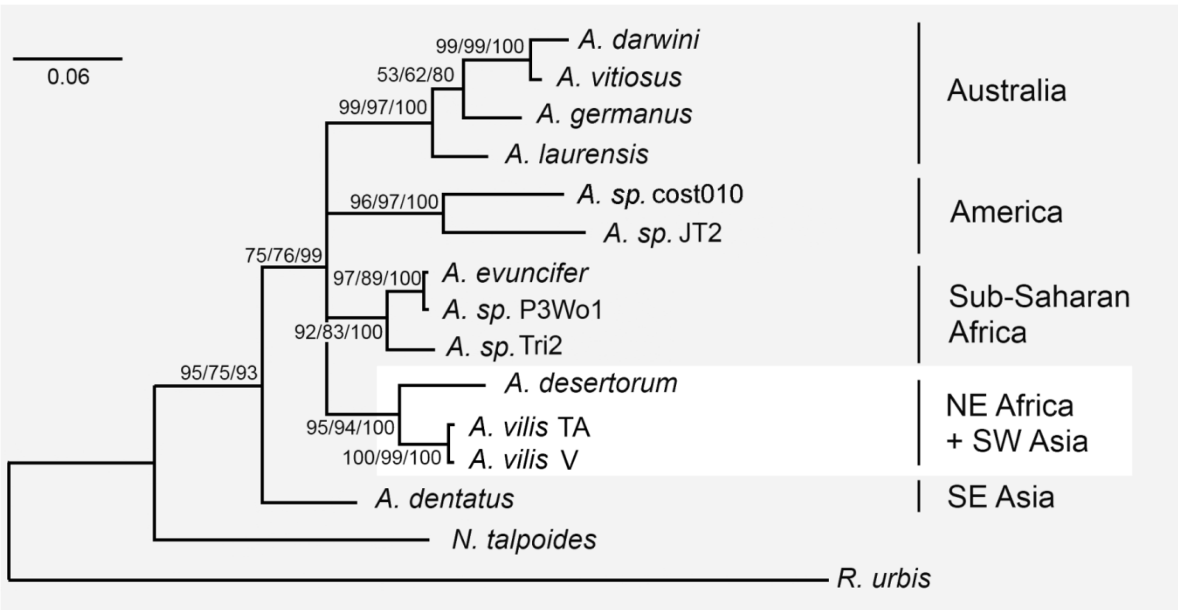

Figure 3. Phylogenetic tree of *Amitermes* spp., based on COII alignment. Support values higher than 50 are shown at the nodes (MP/ML/BI). Nodes supported by a single method are collapsed. TA = church of the Twelve Apostles; V = valley upstream of the Monastery.

Table 2. Measurements (mm) and number of antennal articles of soldiers (n = 6) and alates (n = 6) of *M. eugnathus.*

	Soldiers		Alates	
	Range	Mean ± S.D.	Range	Mean ± S.D.
Head length	1.19 - 1.33	1.26 ± 0.07	0.88 - 1.05	0.95 ± 0.07
Head width	0.95 - 1.02	0.96 ± 0.03	0.89 - 0.98	0.96 ± 0.04
Mandible length	1.05 - 1.23	1.13 ± 0.07		
Eye: maximum diameter			0.21 - 0.25	0.23 ± 0.02
Eye: minimum diameter			0.17 - 0.24	0.19 ± 0.03
Distance eye-ocellus			0.03 - 0.10	0.07 ± 0.03
Tibia length	0.75 - 0.82	0.78 ± 0.03	0.93 - 1.07	1.01 ± 0.06
N. of antennal articles	13	13.00 ± 0.00	13 - 14	13.60 ± 0.55

Microcerotermes eugnathus

The alates and soldiers of *Microcerotermes* from Sinai correspond morphologically with the descriptions of both *M. eugnathus* (Silvestri, 1911; Kashef and El-Sherif, 1972) and *M. palestinensis* (Spaeth, 1964). No diagnostic differences between the two species are known, as the description of *M. palestinensis* (Spaeth, 1964), based on samples from Israel, inexplicably makes no reference to *M. eugnathus.* In this study, we choose to indicate *Microcerotermes* samples from the Monastery as *M. eugnathus*, while keeping the binomial *M. palestinensis* for the sample from Israel.

M. eugnathus is distributed in Tunisia and Egypt (Silvestri, 1911; Kashef and El-Sherif, 1972; Moein and Farrag, 1998). In Egypt, this species was reported to occur in the northwestern coast (Moein and Farrag, 1998) and, further south, in the oasis of Dakhla in the Western Desert (Kashef and El-Sherif, 1972). Until the present study, *M. eugnathus* was not known for the Sinai Peninsula.

Morphological data on *M. eugnathus* samples from Sinai are reported in table 2.

COII and 16S sequences of *M. eugnathus* and *M. palestinensis* obtained in this study are deposited in GenBank under accession numbers KU523915-KU523916 and KU523920-KU523921. In *M. eugnathus*, one COII haplotype and one 16S haplotype were identified. In the phylogenetic reconstruction based on COII alignment (figure 4) species from different biogeographic realms belong to separate clades. Phylogenetic relationships among clades from different realms are not resolved. The species most related to *M. eugnathus* is *M. palestinensis* from Israel, with a p-distance of 0.021. Compared with the lowest genetic distance between the other *Microcerotermes* species for which COII sequences are available (0.029, between *Microcerotermes crassus* Snyder and *Microcerotermes paracelebensis* Ahmad), the distance between *M. eugnathus* and *M. palestinensis* is lower, and suggestive of geographical variation.

The systematics of *Microcerotermes* species from Sub-Saharan Africa is still confused and the identification of samples at the species level is difficult (Kifukieto *et al.*, 2014). We found this is also true for

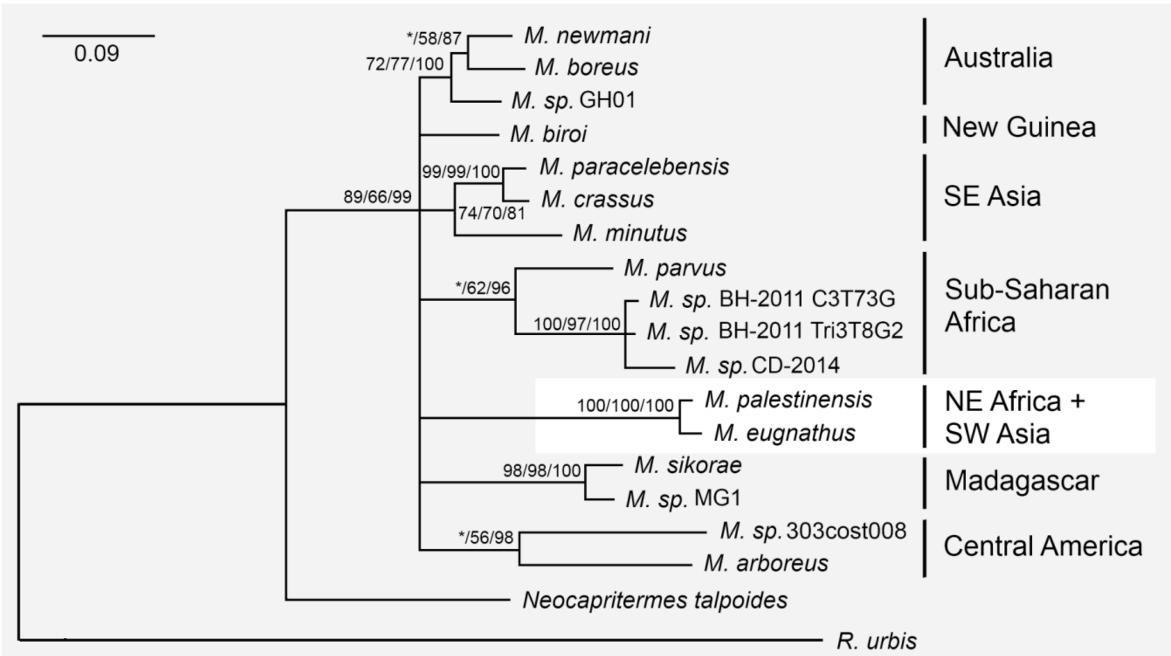

Figure 4. Phylogenetic tree of *Microcerotermes* spp., based on COII alignment. Support values higher than 50 are shown at the nodes (MP/ML/BI). Nodes supported by a single method are collapsed.

Microcerotermes species of North Africa and the western Middle East. Five *Microcerotermes* species are found in this area, which for some of them is a part of a wider range of distribution: *M. palaearcticus* in Morocco and Algeria, *M. eugnathus* in Tunisia and Egypt, *M. palestinensis* in Israel, *Microcerotermes diversus* Silvestri in Iraq, Iran, and the Arabian Peninsula, and *Microcerotermes gabrielis* Weidner in Iraq, Iran, and Afghanistan (reviewed in: Harris, 1970; Krishna *et al.*, 2013).

In the soldier of *M. eugnathus* and *M. palaearcticus*, the third antennal article is the shortest (Sjöstedt, 1904; Silvestri, 1911; Kashef and El-Sherif, 1972), while in the soldier of *M. gabrielis* and *M. diversus* the fourth antennal article is the shortest (Silvestri, 1920; Weidner, 1955). In the description of *M. palestinensis* (Spaeth, 1964), no information about antennal articles is given. In soldiers of *M. palestinensis* in our sample from Israel, the third antennal article is the shortest. In comparison with soldiers of *M. palaearcticus*, *M. eugnathus* and *M. palestinensis*, those of *M. gabrielis* and *M. diversus* also have more robust serrations in the inner margin of mandibles (Spaeth, 1964), and the mandibles of the soldier of *M. gabrielis* are straighter than those of the other species considered here (Weidner, 1955). Therefore, the shortest antennal article and the shape of the mandibles allow us to discriminate between two groups, one including *M. palaearcticus*, *M. eugnathus*, and *M. palestinensis*, and the other, *M. gabrielis* and *M. diversus*.

The distinction among *M. palaearcticus*, *M. eugnathus*, and *M. palestinensis* is not sufficiently clear. *M. palaearcticus* was described based on a sample containing workers and a single soldier (Sjöstedt, 1904). The characteristics of this soldier are compatible with those of *M. eugnathus* and *M. palestinensis*, except for the length of its mandibles (0.8 mm), which is shorter than those of *M. eugnathus* (0.85-1.20 mm) and of *M. palestinensis* (0.93-1.12 mm), leading to a lower ratio between mandible length and head length: 0.63 in *M. palaearcticus*, 0.84-0.92 in *M. eugnathus*, 0.89 in *M. palestinensis* (ranges of variation and ratios calculated based on data and images in Sjöstedt, 1904, Silvestri, 1911; Weidner, 1955; Spaeth, 1964; Kashef and El-Sherif, 1972). However, measurements can be quite variable even among soldiers of the same colony, and in some *Microcerotermes* species, such as *M. diversus*, soldiers of two different size classes have been found (Weidner, 1955), so measurements on a single soldier might not always be sufficient to define or identify a species.

Some synonymies have been suspected between species of the area considered in this study. According to an unpublished note by Emerson, *M. eugnathus* should be considered as a synonym of *M. palaearcticus* (Krishna *et al.*, 2013). *M. palestinensis* has even been suspected by some authors (S. Bacchus, W. A. Sands, R. M. C. Williams) to be a synonym of *M. diversus* (reviewed in Kugler, 1988). We suspect that *M. palestinensis* could be a junior synonym of *M. eugnathus*, because no diagnostic differences between the two species are known.

A systematic revision of the genus *Microcerotermes* in Africa and the Middle East would be needed, considering both morphological and genetic characters, and possibly based on several samples, in order to take into account intra-specific variability.

Concluding remarks

A. vilis and *M. eugnathus* are adapted to semi-arid or arid regions (Harris, 1970), where they can be found where some water is available, such as in the bottom of valleys, in oases, vegetated patches, or irrigated areas. The area where the Monastery lies has an arid climate, with flash floods occurring once or twice annually (Youssef *et al.*, 2011). In the Monastery, termite survival and recruitment from the surrounding territory are favoured by the availability of soil rich in organic matter and water. In fact, the garden is irrigated, and drains from the kitchens discharge water into the nearby ground. In the period 2007-2009, the area where the Monastery lies became progressively more arid, making the environment less suitable for termites. In the Monastery, water use decreased, as the main well went completely dry and water had to be supplied to the Monastery by tankers.

Termite monitoring activities were carried out regularly from 2004 to 2009. Until 2009, termites had caused minor damage to objects of important historical value in the Monastery. Heavier damage was limited to wooden objects of lesser value and to structural wood, which was largely replaced with concrete. From 2010 onwards, international security issues have prevented the scheduled continuation of the activities. In recent years, floods have occurred almost annually and due to the higher availability of water, it is likely that termite abundance and activity has increased, making the need of termite monitoring and control more urgent.

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