



Genetic and multivariate morphometric analyses unveil cryptic diversity in sympatric populations of the Mediterranean wool carder bee, identified as *Anthidium undulatum* Dours, 1873 (Hymenoptera: Megachilidae: Anthidiini)

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Abstract

The distribution of the anthidiine bee identified as *Anthidium undulatum* Dours, 1873 extends from the French Mediterranean coastal region across the Adriatic coast and the eastern Mediterranean to the Caucasus and Iran. Examination of the DNA sequence of the mitochondrial COI gene (“barcoding unit”) of 25 specimens from throughout the distributional range revealed the existence of three distinct clades, which are clearly separated with bootstrap values of 99% and 100% in a Maximum Likelihood Analysis. The genetic distance between these groups ranged from 2.6 to 5.0%, while the intra-group distance did not exceed 0.42%. Two of these clades were found to occur in the same habitat in Lebanon. These three clades also differ in some phenotypic color traits, which, however, are not always fully diagnostic. Despite this, a morphometric analysis of six parameters of the head and wings of the males showed that the three genetic clades also form distinct clusters in a Discriminant Function Analysis. The consistent results of the genetic and morphometric analyses, together with the co-existence of two of the three groups, support the recognition of these groups as distinct species. The three taxa recognized at the species level are: *A. undulatum* Dours, 1873, which has a wide distribution in the Mediterranean and extends eastwards to Iran; *A. wahrmani* Mavromoustakis, 1948 **stat. nov.**, which is found in the southern Levant; and *A. libanicum* Kasperek **sp. nov.**, which is known only from Lebanon, and where it seems to be endemic. The results confirm the high diversity of anthidiine bees in the eastern Mediterranean and indicate that the species richness may be much higher than currently known. The sympatric occurrence of genetically and morphometrically distinct forms within the same habitat is considered strong evidence that these are distinct cryptic species, rather than variations arising from local geographic or environmental adaptations.

Keywords

Cryptic species, endemism, DNA based species delimitation analysis; co-existence, sympatry, genetic barcoding, cyt *b* gene, discriminant function analysis

1. Introduction

The Mediterranean basin is recognized as a global biodiversity hotspot, characterized by a remarkable diversity of plant and animal species (Blondel et al. 2010; Cuttelod et al. 2008; Myers et al. 2000; Thompson 2020). Among its fauna, wild bees (Hymenoptera: Apoidea) are particularly abundant, with the region supporting an exceptionally rich diversity (Ghisbain et al. 2023). The highest concentrations of wild bee species are found at both the western end, in Spain and Morocco, and the eastern end, in Greece and Türkiye (Lhomme et al. 2020; Ascher & Pickering 2024). At the national level, Türkiye stands out, hosting over 1,750 bee species, including approximately 200 endemic species (Lhomme et al. 2020). Resin and wool carder bees of the megachilid tribe Anthidiini follow this general pattern of species richness and endemism, but we are still far from knowing how many species actually exist. The number of valid anthidiine taxa has continuously increased in Europe since 1758 – the beginning of modern taxonomy – and is still on the rise (Warncke 1980; Kasperek 2022). Still, no cryptic species have so far been reported for anthidiine bees. The existence of cryptic species, *i.e.*, species that are morphologically very similar or identical to each other, and are often only identifiable through genetic analysis, are known from some groups of bees such as sweat bees (*Lasioglossum*), mining bees (*Andrena*), and bumblebees (*Bombus*) (Gibbs 2009; Landaverde-González et al. 2017; Mayr et al. 2020; Pauly et al. 2019; Praz et al. 2022; Williams et al. 2012). Unveiling cryptic diversity may help enhance our knowledge on species diversity and species numbers. The number of cryptic bee species was estimated to comprise up to 50% of the known species (Packer & Taylor 1997). This value is similar to some groups of ants (Hymenoptera: Formicidae), where the proportion of cryptic species has been estimated to approximately 50% (Seifert 2009). For insects in general, Xin & Wiens (2023) estimated that each morphology-based insect species contains on average 3.1 cryptic species.

Anthidium undulatum Dours, 1873 is a medium-sized wool carder bee of the tribe Anthidiini, originally described from southern France. It is better known from the eastern Mediterranean, where it has been reported from the northeastern Adriatic coast across Bulgaria, Greece and Türkiye, and extending further to the Caucasus and Iran, as well as to the Levant. In an attempt to establish a library of barcoding sequences of the mitochondrial COI gene of West Palearctic Anthidiini species (M.K.), we found a high genetic diversity in material collected by M.B. in Lebanon and identified as *A. undulatum*. To understand this phenomenon, we produced more COI barcoding sequences from different parts of its distributional area and carried out a morphometric analysis with multivariate statistical methods. The results of this study elucidate the phenotypic and genotypic variability of what is currently regarded as *Anthidium undulatum* and help draw taxonomic conclusions. The results may also serve as a model approach for assessing species diversity in this group of bees.

2. Material and Methods

2.1. Distribution analysis

For the distribution analysis, a comprehensive literature search revealed 52 literature records (listed below) for which geographical coordinates could be determined with sufficient accuracy (locations that could not be identified within a radius of approximately 50 km were excluded from consideration). Data referring only to countries or landscapes without further detail were not taken into account. Additionally, 168 specimens have been examined and the coordinates of the collecting localities determined. Most of these specimens are from M.K.'s collection, but also include, *e.g.*, 18 specimens collected by M.B. in Lebanon, and 31 specimens from the Natural History Museum London (NHMUK), the Zoologisches Museum Hamburg (ZMH), and from the Biodiversity Centre Upper Austria, Linz (OLL).

A comprehensive list of all examined material is provided in Table S1. Additionally, the following distributional records have been obtained through a thorough review of the literature.

AZERBAIJAN: Baku (40.41°N 49.85°E), as *Anthidium littorale* Morawitz, 1873 (Morawitz, 1874; see also Ebmer 2021). — **BULGARIA:** Sandanski (41.56°N 23.28°E); 9–11.vii.1974 (Tkalců 2003). — Sandanski, Lebnica valley (41.52°N 23.24°E); 24.vi.2000, 10.vii.1990 (Tkalců 2003). — Primorsko (42.26°N 27.75°E); vi.1986, 1988 (Tkalců 2003). — **CROATIA:** Ilse of Rab [Arbe] (44.78°N 14.75°E); vi.1914 (Maidl 1922). — Dubrovnik [Gravosa, Lapad] (42.65°N 18.09°E); vi.1914 (Maidl, 1922). — Krk Island (45.08°N 14.59°E); 25.vi.2005 (Józan 2009). — Polača (44.01°N 15.51°E); 21.vi.2007 (Józan 2009). — Hvar Island (43.17°N 16.05°E) (Warncke 1980). — **CYPRUS/NORTHERN CYPRUS:** Limassol (type locality of *A. u. holozonicum*) (34.66°N 33.03°E); Yermasoyia River, Eftagonia, Mesayitonia, Polemedia Hills, Episkopi, Erimi, Ayia Varvara (Stavrovouni), Famagusta, Glypha (near Akanthou), Mt. Troodos (Mavromoustakis 1939; 1949 [“1948”]; 1951; 1952; 1957; Varnava et al. 2020). — **FRANCE:** Montpellier (43.59°N 3.88°E) (Dours 1873). — **GREECE:** Rhodes (36.24°N 27.94°E) and neighbouring mainland (van der Zanden 1980). — Lardos near Lindon (Rhodes) (36.08°N 28.05°E) (Tkalců 2003). — Lefkas (not regarded as reliable, labelled as “holotype”) (Staněk, 1968). — Samos (37.72°N 26.81°E) (ZMB according to drawer picture). — Stavros (Thrace) (40.91°N 24.03°E) (Warncke 1980). — Thessaloniki (40.65°N 22.91°E) leg. et coll. Pelanidou (Tkalců 2003). — Crete (35.24°N 24.80°E) (Tkalců 2003). — Crete: Kavousi (35.12°N 25.85°E); 21.v.1981 (Tkalců 2003). — Crete: Sitia (35.20°N 26.10°E); 20.v.1981, 22.vi.1965, 24.v.1997 (Tkalců 2003). — **HUNGARY:** Listed by Maidl (1922). No further details available. — **IRAN:** Elburz: Damavand, 15 km N Qazvin (36.42°N 49.99°E) (Warncke 1982). — Hamadan: Hamadan (as *A. u. holozonicum*) (34.79°N 48.51°E) (Warncke 1982). — Fars: Zenjun, 40 km W Shiraz (29.53°N 52.29°E); 5.vii.1965 (Warncke 1982). — Fars, Qir, Tange Karzin (28.50°N 53.11°E); 28.vi.2013, and Qir, Shaldan (28.55°N 53.00°E); 5.vii.2013, both records as *A. aff. undulatum* (Falamarzi et al. 2017). — Noorabad, Basharjan (30.10°N 51.52°E); 28.vi.2009 (Zakikhani et al. 2021). — Fars, Kazeroun, Bidzard (29.31°N 51.86°E); 4.viii.2010 (Zakikhani et al. 2021). — Fars, Darab (28.73°N 54.46°E); 9.vii.2011 (Zakikhani et al. 2021). — Isfahan, Chadegan,

Table 1. List of material of *Anthidium libanicum* sp. n., *A. undulatum*, and *A. wahrmani* from which genetic barcodes (sequence of the barcoding unit of the mitochondrial COI gene) were obtained. BIN = Barcode Identification Number; Bp = number of base pairs.

Species/BIN	BIN	Sequence No.	Country	Location	No. bp
<i>A. libanicum</i>	BOLD:AEH0696	ABABX058-20	Lebanon	N. Liban, Tannourine	642
		ABABX247-21	Lebanon	N. Liban, Hadath el Jebbe	573
		ABABX250-21	Lebanon	Bekaa, Hadath	593
		ABABX421-22	Lebanon	N. Liban, Tannourine	502
		ABABX422-22	Lebanon	N. Liban, Tannourine	633
		ABABX451-22	Lebanon	N. Liban, Tannourine	648
		ABABX452-22	Lebanon	N. Liban, Tannourine	634
		ABABX453-22	Lebanon	N. Liban, Tannourine	638
		ABABZ092-23	Lebanon	N. Liban, Tannourine	648
		ABABZ093-23	Lebanon	N. Liban, Tannourine	633
<i>A. undulatum</i>	BOLD:AEH3110	ABABX056-20	Croatia	Karlobag Velebit	658
		ABABX101-20	Cyprus	Pomos	642
		ABABX273-21	Iran	Lorestan	563
		ABABX099-20	Türkiye	Muğla	658
		ABABX100-20	Türkiye	Muğla	658
		ABABX129-20	Türkiye	Muğla	658
		ABABX558-22	Türkiye	Antalya	658
<i>A. wahrmani</i>	BOLD:AAO4344	ABABX450-22	Israel/Palestine	Naharya	658
		ABABX538-22	Israel/Palestine	Naharya	658
		ABABX539-22	Israel/Palestine	Naharya	658
		ABABX548-22	Israel/Palestine	Naharya	658
		MGPC093-21	Lebanon	N. Liban, Harissa	658
		ABABX449-22	Lebanon	Mont Liban, Anjar	658

Zayanderud (32.76°N 50.63°E); 8.vii.2012; A. Monfared leg. (Khodarahmi Ghahnavieh et al. 2019; Zakikhani et al. 2021). — Baharestan (51.53°N 32.62°E); 18.v.2012 (Khodarahmi Ghahnavieh et al. 2019). — **ISRAEL/PALESTINE**: Jerusalem (31.76°N 35.21°E), type locality of *A. u. wahrmani*; 1944–1945 (Mavromoustakis 1949). — **JORDAN**: Petra (30.34°N 35.46°E) (Tkalcü 2003). — **LEBANON**: Records by Boustani et al. (2021) here under “material examined”. Additionally, Saidoun El Mrouj (33.57°N 35.44°E); 15.vi.2017 (Boustani et al. 2021). — **MONTENEGRO**: 42.29°N 18.84°E; 18.vii.1967; O. Rebman leg. (OLL). — **PALESTINE**: Ramleh (Ramallah) (31.90°N 35.20°E) (Mavromoustakis 1949). — **SERBIA**: Listed by Mudri-Stojnić et al. (2021). — One specimen in ZMB (drawer picture, not examined). — **SPAIN**: Friese (1898) noted that he examined a specimen from Spain (no further information available). — **TÜRKİYE**: Arkent near Ayvalık (39.43°N 26.81°E) (Tkalcü 2003). — Diyarbakır (37.92°N 40.21°E); 20.vii.1977 (van der Zanden 1980). — Bitlis; 8.vii.1996 (38.40°N 42.10°E) (Tkalcü 2003). — Mersin: Anamur, Abanoz (36.33°N, 32.95°E); 22.vii.2008 (Güler et al. 2014). — Burhaniye (Balıkesir) (39.49°N 27.00°E), Aydın (37.83°N 27.84°E), Ören (Muğla) (37.03°N 27.98°E), and Alanya (Antalya) (36.54°N 31.99°E) (Özbek & van der Zanden 1993). — Elazığ (38.67°N 39.22°E); Ürgüp (Nevşehir) (38°63′N 34.91°E); Gelindere (Mersin) (c. 36.81°N 34.61°E); Sertavul (Mersin) (36.91°N 33.26°E); Beyşehir (37.67°N 31.72°E); Tarsus (36.91°N 34.89°E); Mut (36.79°N 33.99°E); Urfâ (37.16°N 38.79°E); Nizip (Gaziantep) (37.00°N 37.79°E); Halfeti (Urfâ) (37.24°N 37.86°E) (Warncke 1980). — Adana (36.99°N 35.33°E); 22.vi.2001 (Tkalcü 2003).

2.2. Morphometric analysis

For a morphometric analysis, the following measurements were used: (1) shortest distance between com-

pound eye and hind ocellus; (2) distance between hind ocellus and preoccipital ridge; (3) distance between hind ocelli; (4) distance between hind and fore ocelli; (5) diameter of hind ocellus; (6) clypeal length (excluding apical rim); (7) clypeal width at widest point; and (8) length of the marginal cell. The methodological approach for taking the measurements and for evaluating them is described in detail in Kasperek (2018, 2020). Discriminant Function Analyses were carried out with PAST, version 4.15 (Hammer et al. 2001). The morphometric analysis was limited to males due to the insufficient number of females available for statistically meaningful evaluations.

2.3. Genetic analysis

DNA extraction, PCR amplification and sequencing were conducted by the Canadian Centre for DNA Barcoding (CCDB), Guelph, using standardized high-throughput protocols (<http://ccdb.ca/resources>). The barcoding unit of the mitochondrial cytochrome c oxidase subunit I gene (COI) was sequenced, and the results were submitted to Barcode of Life Data System (BOLD), a cloud-based data storage and analysis platform developed by CCDB (www.barcodinglife.com). The list of sequence identifiers is given in Table 1.

The DNA sequences will be made available publicly. Alignments were carried out both with BOLD Aligner and with MUSCLE Aligner. Both methods resulted in the same tree topology. MUSCLE Aligner was used for the further analysis. Model testing in Mega 12 (Kumar et al. 2024) supported the Tamura-3 model as the sub-

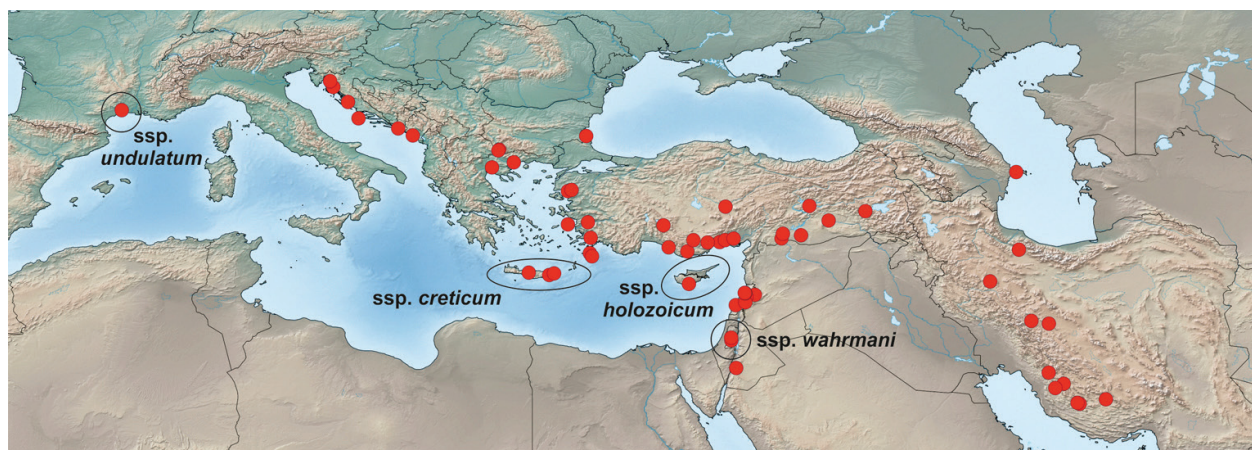


Figure 1. Distribution of *Anthidium undulatum* Dours, 1873, as inferred from literature data. The map also shows the type localities of the historically described subspecies.

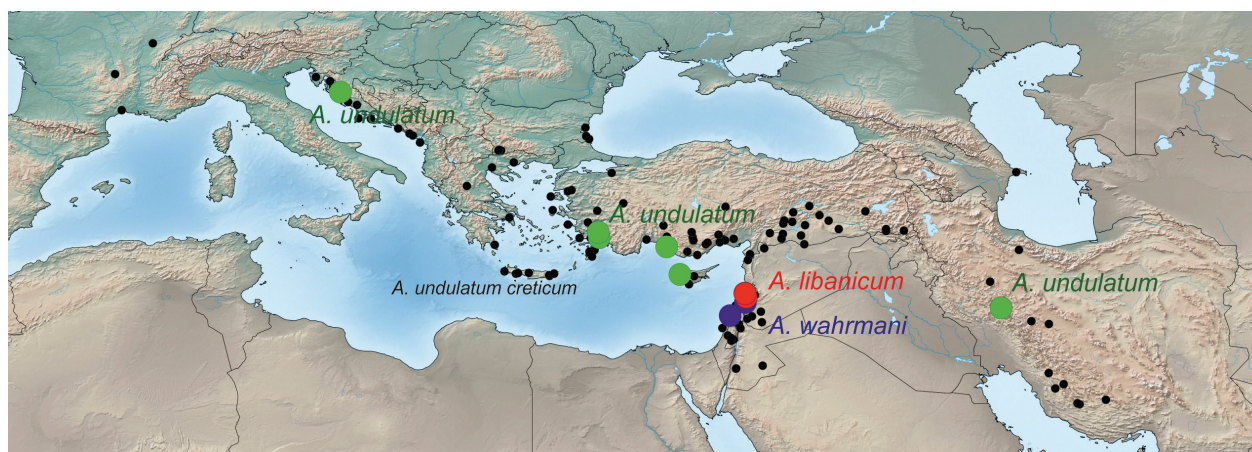


Figure 2. Distribution of the members of the *Anthidium undulatum* species group. Black dots refer to materials examined and literature data. The large dots refer to specimens identified by genetic barcoding (green: *A. undulatum*; red: *A. libanicum* sp. n.; blue: *A. wahrmani* stat. nov.).

stitution model with the lowest BIC score, hence was therefore used for phylogenetic analysis. The data were analyzed in Mega 12 using Maximum Likelihood (ML) phylogenetic framework (Mega 12 software). *Anthidium oblongatum* (Illiger, 1806), specifically a specimen from Türkiye was chosen as outgroup. *Anthidium oblongatum* was selected as it belongs to the same subgenus *Anthidium* (*Proanthidium*) as *A. undulatum*. Haplotype network analysis was carried out with PopART, ver. 1.7 (Bandelt et al. 1999; Clement et al. 2002). Median Joining Network (MJN) was used to depict the phylogenetic relationships.

DNA-based species delineations were tested by the Automatic Barcode Gap Discovery (ABGD), a single locus analysis method (Puillandre et al. 2012). ABGD uses a coalescent model to identify the most likely position of the barcode gap, based on maximal genetic intraspecific distances defined a priori by the user, and uses the DNA barcoding gap to propose species partitions. The default value of 0.001 was used for P_{min} , the minimum value of the genetic distance a priori defined by the user, and the default value of 0.1 for P_{max} , the maximum value. The barcode gap is expected to be found within this range.

Additional to ABGD, the Barcode Index Number (BIN) System, which is embedded into BOLD (<https://bench.boldsystems.org>) was used. The BIN system clusters COI sequence data into Operational Taxonomic Units (OTUs) independent of prior taxonomic assignment. As such, it provides a means of confirming the concordance between barcode sequence clusters and species designations. The BIN algorithm combines the algorithms of five different algorithms (ABGD, CROP, GMYC, jMOTU, RESL) and in most cases correspond to species (Ratnasingham & Hebert 2013). However, as the algorithm is based on machine-learning and therefore not transparently available, it does not represent a taxonomic tool on the level of decision-making.

2.4. Abbreviations

CMK – Collection Max Kasperek, Heidelberg (Germany); **CCDB** – Canadian Centre for DNA Barcoding, Guelph (Canada); **COI** – Mitochondrial cytochrome c oxidase subunit I; **NHMUK** – Natural History Museum London (United Kingdom); **OLL** – Biodiversity Centre

Table 2. Estimates of evolutionary divergence over sequence pairs between the three clades of *Anthidium undulatum* s. l. identified in the Species Identification Tree based on the barcoding unit of the COI gene (Fig. 3), representing three distinct species. The second column shows the within-group distances, the third to fifth column the intergroup distances. Analyses were conducted using the Tamura 3-parameter model. This analysis was conducted using 25 nucleotide sequences. Evolutionary analyses were conducted in MEGA 12.

	Within Group Distance	<i>A. undulatum</i>	<i>A. wahrmani</i>	<i>A. libanicum</i>
<i>A. undulatum</i>	0.0042±0.0015	—	0.009	0.006
<i>A. wahrmani</i>	0.0018±0.0010	0.050	—	0.009
<i>A. libanicum</i>	0.0007±0.0005	0.026	0.049	—

Upper Austria (former Oberösterreichisches Landesmuseum, Biologiezentrum Linz), Linz (Austria); **ZMB** – Museum für Naturkunde – Leibniz-Institut für Evolutions- und Biodiversitätsforschung, Berlin (Germany); **ZMH** – Zoologisches Museum Hamburg (Germany).

Regarding nucleotide bases, A stands for Adenine, C for Cytosine, G for Guanine, and T for Thymine.

3. Results

3.1. Distribution

Anthidium undulatum was first described from France in the 19th century (Dours 1873) but was not reported from there again for almost a century. There are only two recent records from France, one from Issoire (Département Puy-de-Dôme) in 2016, and one from Les Malines (location not identified as there are several locations with the same name) in 1970 (Table S1). This species should be regarded as one of the rarest bee species in this part of Europe. Friese (1898) also mentioned material from Spain, but did not provide details. To the east of France, there is a distributional gap; the next occurrence records are from Croatia. While there is a good number of records available from the northeastern Adriatic coastal areas, inland records from the Balkans are absent (except for one record from Serbia, whose details are not available). The distribution further extends across Bulgaria, Greece and Türkiye, and from there to the Caucasus (Caspian coast in Azerbaijan) and Iran, as well as to the Levantine countries Syria, Lebanon, Israel, Palestine, and Jordan. The distribution includes Crete and Cyprus (Figs 1, 2).

Specimens from Israel/Palestine have been described as a distinct subspecies, *Proanthidium* [= *Anthidium*] *undulatum wahrmani* Mavromoustakis, 1948, specimens from Cyprus as *P. u. holozonicum* Mavromoustakis, 1939, and specimens from Crete as *A. u. creticum* (Tkalčů, 2003) (Mavromoustakis 1939; 1949; Tkalčů 2003). The Cyprus subspecies *holozonicum* has also been reported from Rhodes island (Mavromoustakis 1959), Türkiye (van der Zanden 1980; Özbek & van der Zanden 1993), and Iran (Warncke 1982), the subspecies *wahrmani* also from Iran (Warncke 1982).

3.2. DNA Sequences

A Species Identification Tree (“phylogenetic tree”) inferred from 25 COI barcoding sequences from various parts of the distributional area and constructed by the Maximum-Likelihood (ML) Method with 1000 bootstrap replicates revealed three distinct clades (Fig. 3).

The topology of the tree comprises three main clades, with nodes strongly supported by maximum likelihood (ML) bootstrap values of 99 and 100, respectively. A complementary neighbor joining tree with 1000 bootstrap replicates also supported these three clades, and the nodes were supported by bootstrap values of 100 each.

The three clades were assigned to *Anthidium undulatum* s. str., *A. wahrmani*, and *A. libanicum* **sp. n.** (see taxonomic justification below). The intraspecific genetic distance (within group mean distance) is extremely low in *A. libanicum* (0.0007; N=10), and highest in *A. undulatum* (0.0042; N=7). *Anthidium wahrmani* takes an intermediate position of 0.0018 (N=8) (Table 2).

The average intergroup distance between *A. undulatum* and *A. libanicum* is 2.6%, and between *A. undulatum* and *A. wahrmani* 5.0%. The distance between *A. libanicum* and *A. wahrmani* is 4.9%.

Initial partitioning in the species delimitation analysis ABGD identified 10 OTUs, while the results from recursive partitioning singled out the existence of three OTUs (Fig. 4).

These OTUs are clearly separated by barcoding gaps (Fig. 5). The delimitation results from ABGD are consistent with the genetic distance analysis of the BOLD system. Using the Kimura-2 model provided by the ABGD software as built-in function, this analysis also returned three species, with a mean within-species distance of 0.18% (SE<0.01%), and a minimum between-species distance of 1.96%. Identical results were obtained with the Jukes-Cantor model. The three OTUs identified were attributed by BOLD to three different Barcode Index Numbers (BINs).

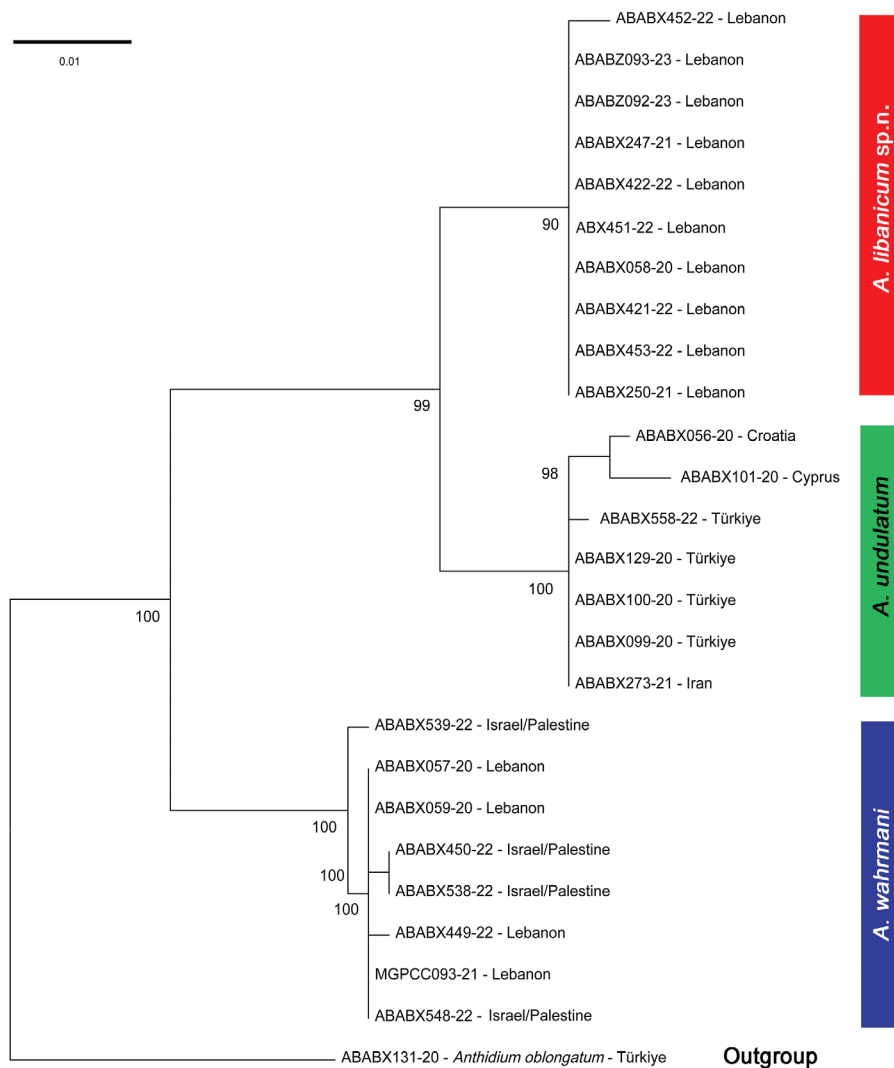
3.3. Haplotype Analysis

The overall number of segregating sites is 32, including 27 parsimony-informative sites. Tajima’s D=1233.2, which is highly significant (p<0.001). The positive and high value of D suggests an excess of intermediate-frequency polymorphisms compared to what is expected

Table 3. Distinct nucleotide positions of the barcoding unit of the COI gene in the three species of *Anthidium undulatum* s. l.

	7	34	43	73	82	121	124	163	169	184	190	212	223	293	317	319	325
<i>A. libanicum</i>	A	T	A	A	A	A	C	A	A	G	A	T	A	A	G	A	T
<i>A. undulatum</i>	A	T	A	G	A	T	T	A	A	A	A	T	A	G	G	A	A
<i>A. wahrmani</i>	G	A	G	A	T	A	T	T	T	A	T	A	T	A	T	T	T

	328	335	343	352	359	412	433	451	497	515	547	565	583	619	625	631
<i>A. libanicum</i>	G	G	T	T	A	T	T	T	T	T	A	G	G	A	G	T
<i>A. undulatum</i>	A	G	T	T	C	T	A	A	T	G	A	A	G	A	A	A
<i>A. wahrmani</i>	A	A	A	A	A	A	A	A	C	G	T	A	A	T	T	T

**Figure 3.** Species identification tree as derived from the mitochondrial DNA sequence of the COI barcoding gene of 25 specimens previously assigned to *Anthidium undulatum*. The gene sequences are divided into three groups, which are assigned to *A. undulatum* Dours, 1873; *A. wahrmani* (Mavromoustakis, 1948) **stat. nov.**, and *A. libanicum* Kasperek **sp. nov.** (see text).

under neutral evolution and indicates the existence of distinct groups.

Eight different haplotypes were found in the sample ($N=25$), resulting in an overall haplotype diversity is $H_d=0.797$. *Anthidium undulatum* s. str. and *A. wahrmani* contain three different haplotypes each ($H_d=0.524$ with $N=7$ and $H_d=0.4643$ with $N=8$, respectively), and *A. libanicum* contains two different haplotypes ($H_d=0.200$; $N=10$). All haplotypes are unique to each of these species, i.e., there are no shared haplotypes. These numbers indicate that there is no evidence for gene flow between these species.

The haplotypes of the three clades (species) are characterized by the following unique nucleotide base substitutions. The following list gives those nucleotides which are different from the other two species (bold letters show the actual nucleotides) (see also Table 3).

A. libanicum **sp. n.** (6): COI 184 (A→G), 328 (A→G), 433 (A→T), 451 (A→T), 515 (G→T), 565 (A→G).

A. undulatum s. str. (7): COI 73 (A→G), 293 (A→G), 325 (T→A), 359 (A→C), 625 (G→A, T→A), 631 (T→A).

A. wahrmani (19): COI 7 (A→G), 34 (T→A), 43 (A→G), 82 (A→T), 163 (A→T), 169 (A→T), 190

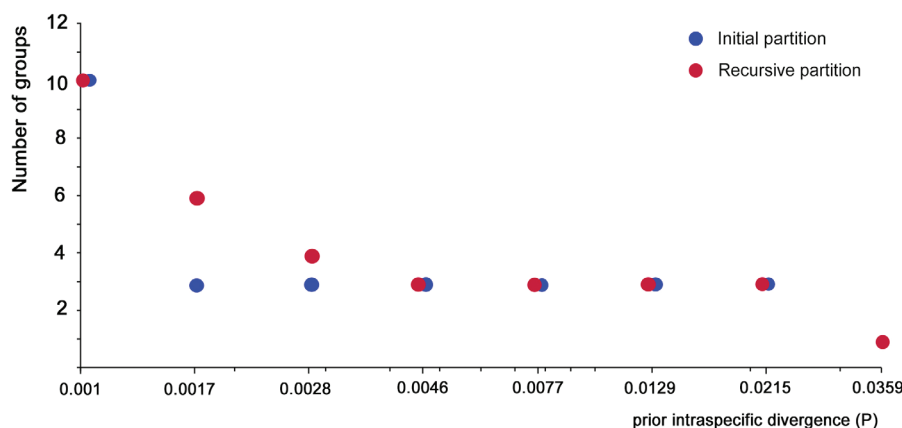


Figure 4. Automatic partition of COI barcode sequences of *Anthidium undulatum* s.l. by the Automatic Barcode Gap Discovery (ABGD) program (Puillandre et al. 2012). The number of OTUs automatically defined by ABGD as a function of the prior intraspecific divergence (P) match the number of groups (putative species) defined by the Species ID Tree and the morphometric analysis.

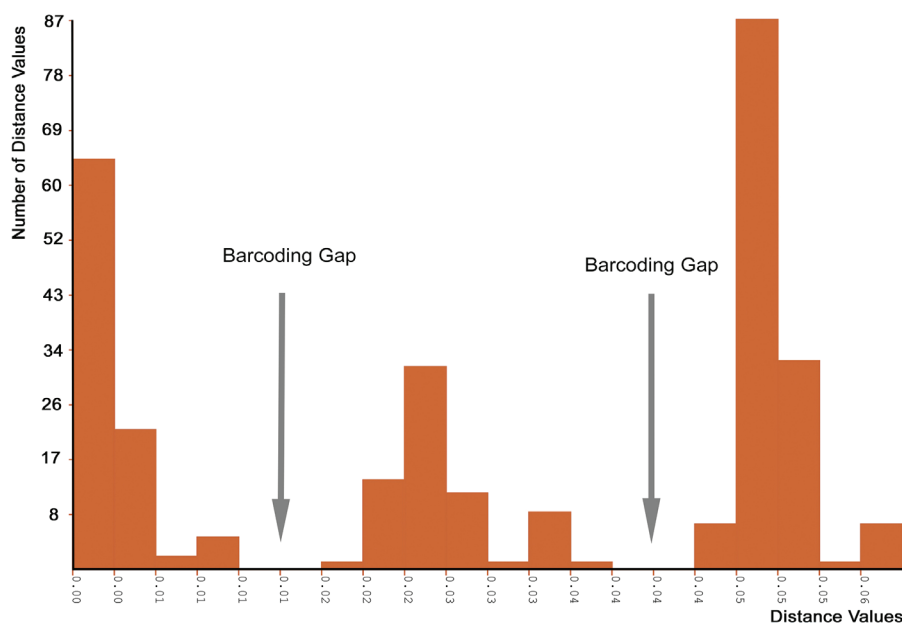


Figure 5. Distribution of pairwise differences of the COI barcode sequences of *Anthidium undulatum* s.l. using the Kimura-2 parameters model. The left peak represents intraspecific distances, while the middle and right peaks correspond to two distinct partitions with varying interspecific distances.

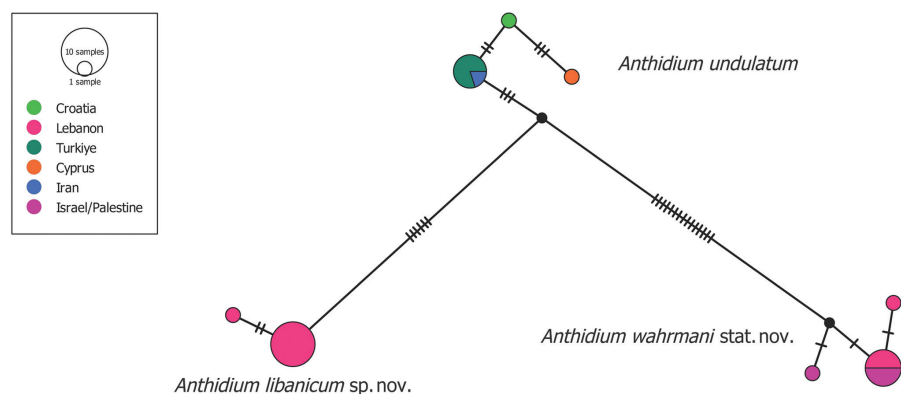


Figure 6. Haplotype network of *Anthidium undulatum* s.l. Minimum spanning network based on the barcoding fragment of the cytochrome oxidase I (COI) gene. Each sequenced haplotype is represented by a circle, the size of which is proportional to its overall frequency in the dataset. Hatch marks on the branches are used to indicate the number of mutations between two different haplotypes.

(A→T), 212 (T→A), 223 (A→T), 317 (G→T), 319 (A→T), 335 (G→A), 343 (T→A), 352 (T→A), 412 (T→A), 497 (T→C), 547 (A→T), 583 (G→A), 619 (A→T).

The Median Joining Network, a graph-based approach used to represent relationships between genetic sequences by connecting them with the least possible genetic distance (or fewest mutations), shows that both *A. libanicum* and *A. wahrmani* are closer to *A. undulatum* than to each other (Fig. 6).

3.4. Morphometric Results

In order to find out whether there are morphometric differences between the three groups identified in the genetic analysis, a Discriminant Function Analysis was carried out using eight metric parameters of the head and the wing of males (Fig. 7). The data referring to *A. libanicum* (N=10), *A. wahrmani* (N=8), and *P. undulatum* (N=7) form different, clearly distinct clusters with almost no overlap. In a confusion matrix, 89.5% of all

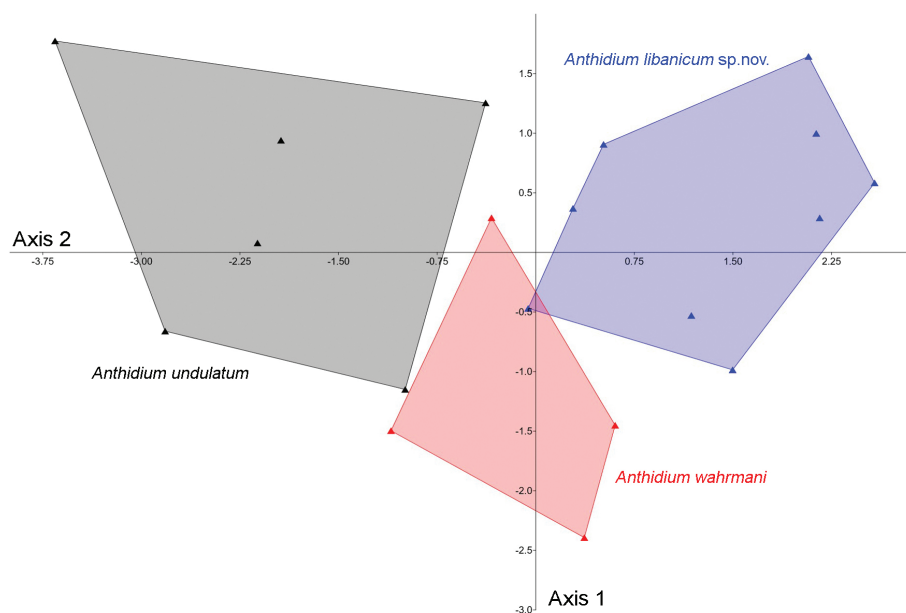


Figure 7. Results of a Discriminant Function Analysis (DFA) of six morphometric parameters of the head and the wing of the males of three species of the *Anthidium undulatum* s. l. (N=19). Only material whose species identity has been previously confirmed by genetic barcoding was used.

specimens could correctly be attributed to the relevant group.

3.5. Co-occurrence

In Lebanon, *A. wahrmani* and *A. libanicum* were found in close proximity to each other in the mountains of the Tan-nourine region in North Lebanon, at elevations between 1,750 and 1,780 meters above sea level. At one specific locality (Harissa, Al-Jawar), they were sampled together; in another case, they were collected from sites just a few kilometers apart. However, *A. wahrmani* appears to be much less abundant, as only two specimens were recorded in this intensively surveyed area. The current data does not suggest a strong habitat preference for either species, as the samples were collected from both semi-arid and humid Mediterranean environments, spanning degraded and well-preserved landscapes (Figs 11–13).

Flower records indicate that both species forage on plants from Fabaceae (mainly *Ononis* species) and Lamiaceae (such as *Stachys* and *Lavandula* species). Records of *Anthidium libanicum* show that the species is active between late June and late August. *Anthidium wahrmani*, on the other hand, was collected only in July, though the limited number of specimens makes it difficult to determine its full activity period.

3.6. Taxonomy

Anthidium undulatum species group

Wool carder bees which have hitherto been identified as *Anthidium undulatum* Dours, 1873 share the following character traits: The female is characterized by a mandible with three strong teeth and approximately 10 minute teeth in between, which together make approximately 13 teeth (drawing in Kasperek 2022: 37). Clypeus

slightly convex, the apical margin smooth and more or less straight or slightly convex. Metasoma with yellow bands on T1–T5, interrupted at most on T1 and T2. T6 steeply (almost vertically) sloping, but with a nearly horizontal apical margin. Males with boomerang-shaped or L-shaped anterolateral yellow band on the scutum, rarely reduced to small yellow remnants; scutellum angulate posterolaterally in dorsal view, with angles formed by a transparent lamella. T6 with an acute lateral projection; depression of T6 punctate. T7 widely rounded in dorsal view, with an almost semicircular median emargination.

Additional to the nominate subspecies described from France, three subspecies of *Anthidium undulatum* Dours, 1873 have been described: *A. u. wahrmani* Mavromoustakis, 1948 from Jerusalem, *A. u. holozonicum* Mavromoustakis, 1939 from Cyprus, and *A. undulatum creticum* Tkalců, 2003 from Crete (Dours 1873; Mavromoustakis 1939; 1949; Tkalců 2003) (Fig. 1). These subspecies were defined on basis of subtle color differences, i.e., differences in the tone and extent of yellow maculations. In an examination of a larger series of specimens, it became evident that the current subspecific classification is insufficient insofar as the character traits are not always diagnostic but are overlapping. Based on the results of the morphometric and genetic examinations, we herein split *A. undulatum* s. l. into three distinct species.

It is suggested to treat the following three sister species as “*Anthidium undulatum* species group” of the subgenus *Anthidium* (*Proanthidium*). They were previously subsumed under the name *A. undulatum* but are formalized here as distinct species.

Anthidium undulatum Dours, 1873

Figures 6–8

Anthidium littorale Morawitz, 1874 (Azerbaijan). – Published in volume 10, 1873 of the *Horae Societatis Entomologicae Rossicae*. Eb-

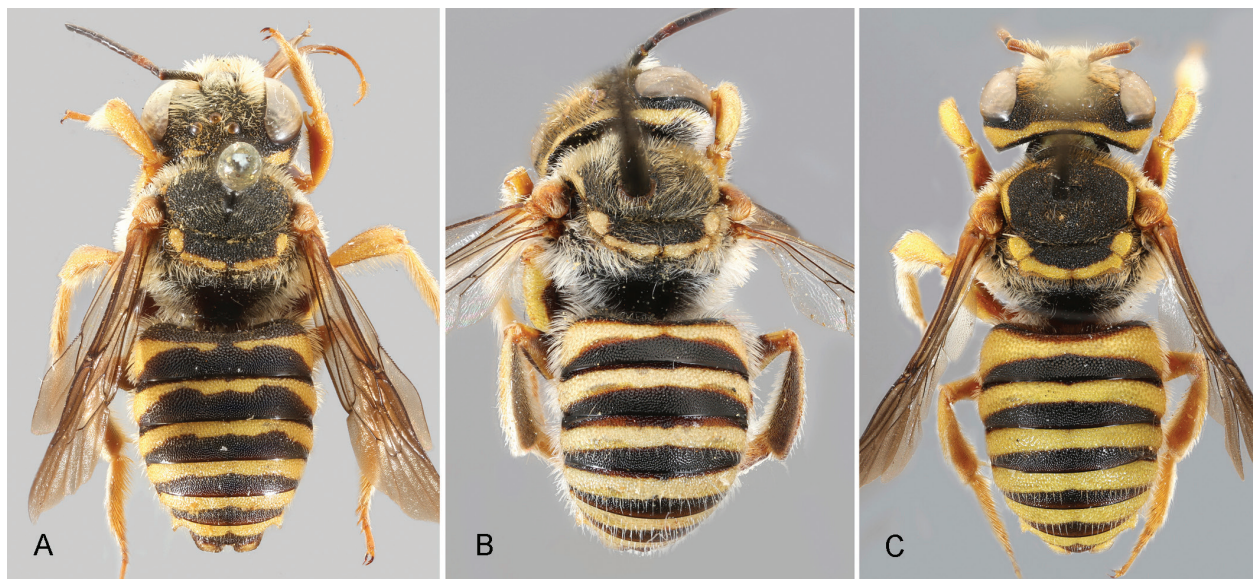


Figure 8. Dorsal habitus in typical males of *Anthidium*: **A** *A. undulatum*, **B** *A. libanicum* sp. n., **C** *A. wahrmani* stat. nov. Note the apically undulated margins of the anterior yellow tergal bands in *A. undulatum* (B), the pale yellow coloration of maculation and the reddish-brown transition zone between the yellow tergal bands and the black ground color in *A. libanicum* sp. n. (B), and the bright yellow tergal bands with straight margins in *A. wahrmani* stat. nov. (C).

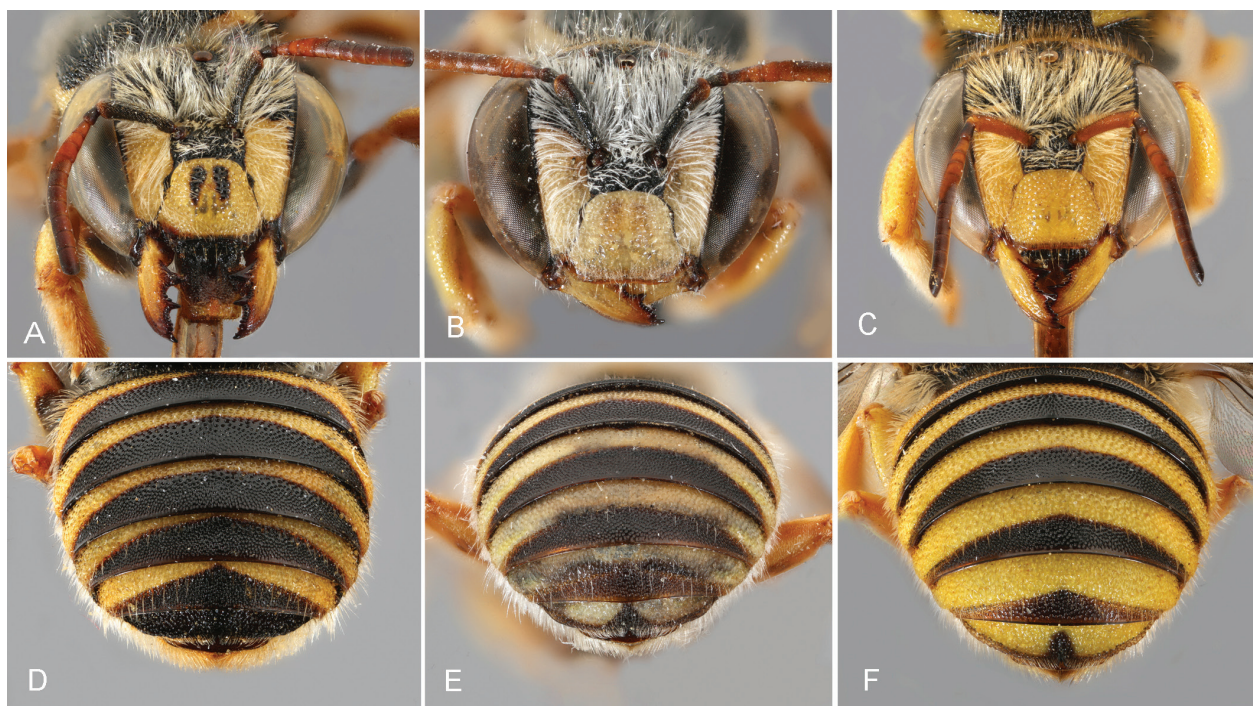


Figure 9. Face and apical terga of typical females of *Anthidium*: **A, D** *A. undulatum*; **B, E** *A. libanicum* sp. n.; **C, F** *A. wahrmani* stat. nov. Note the color of the antennal scape (black in *A. undulatum* and *A. libanicum*), the color pattern of T6 (black in *A. undulatum*, yellow with black apicomedial wedge-shaped incision in *A. libanicum* sp. n. and *A. wahrmani* stat. nov.), and overall tone of yellow (pale yellow in *A. libanicum* sp. n., dark bright yellow in *A. undulatum* and especially in *A. wahrmani* stat. nov.).

mer (2021) found that this volume was distributed in 1874, so that the name *A. undulatum*, published in 1873, has priority and *A. littorale* has to be treated as a nomen oblitum.

Proanthidium undulatum holozonicum Mavromoustakis, 1939 (Cyprus). **Syn. nov.** – Mavromoustakis (1939) was not entirely clear about the deposition of the type specimen, but by analogy with his other material, it can be assumed that he placed it in his own collection. However, it is not listed in the collection inventory (MoA,

1989). The analysis presented here is based on Mavromoustakis' published description.

Anthidium undulatum holozonicum (Mavromoustakis, 1939).

Anthidium undulatum creticum Tkalcū, 2003 (Crete). – Recognised here as valid subspecies. Paratypes examined (CMK). Further examination is required to confirm this taxonomic status.

Barcode Index Number. BOLD:AEH3110.



Figure 10. *Anthidium libanicum* sp. n. Male metasoma, left with black ground color, right a rare color morph with red-brown ground color. The belonging of these two specimens to the same species (*A. libanicum* sp. n.) has been confirmed by genetic barcoding.

Material. Here only material with genetic barcodes: 1♂, Croatia: Karlobag Velebit (44.52°N 15.12°E); 05.vii.2018; H. Wiesbauer leg. (hw029: ABABX056-20; coll. H. Wiesbauer). – 1♀, 1♂ Türkiye: Muğla prov.: Akyaka, Yeşilova (37.05°N 28.42°E), 70 m; 12.vi.2014; M. Kasperek leg. (mk500: ABABX099-20; mk511: ABABX129-20; CMK). – 1♂, Türkiye: Muğla prov.: Kavaklıdere, television tower (37°20'N 28°22'E), 1600 m; 13.vii.2017; M. Kasperek & O. Özgül leg. (mk508: ABABX100-20; CMK). – 1♂, Türkiye: Konaklı 10 km W Alanya (36.58°N 31.89°E), 130 m; 01.viii.2009; C. Schmid-Egger leg. (seg153: ABABX558-22; coll. C. Schmid-Egger). – 1♂, Cyprus: Pomos (35.16°N 32.57°E), 800 m; 14.v.2014; M. Kafka leg. (ms3573: ABABX101-20; CMK). – 1♂, Iran: Lorestan prov.: 10 km SW Dorud (33°27'N 49°05'E), 1520 m; 27.v.2014; J. Halada leg. (ms3567: ABABX273-21; CMK). – For further material, see under “Material” (for literature data, see above; for material examined, see Table S1).

Diagnosis. Antennal scape black in both sexes, contrasting to the reddish-brown inner flagellomeres (as in *A. libanicum*; scape reddish brown in *A. wahrmani*). Typical females with two black, longish maculae on the clypeus next to the middle (rarely absent, and then similar to *A. libanicum* and *A. wahrmani*); apical margin of clypeus black (rarely light brown as in the other two species); yellow preoccipital band narrow, reaching in typical specimens upper third of eye (rarely reaching lower third of eye or reduced to longitudinal spot behind the eye); gena entirely yellow in *A. libanicum* and *A. wahrmani*. Males on average larger than *A. libanicum*, but of similar size as *A. wahrmani* (average radial cell length of barcoded males: 1.83 mm as compared to 1.87 in *A. wahrmani* and 1.60 in *A. libanicum* [an exceptionally large animal not included in the average]). Antennal scape as in female; in both sexes, the yellow anterolateral band on scutum is narrower than in *A. libanicum* and *A. wahrmani*. Yellow tergal bands mostly interrupted on T1 and contiguous on subsequent terga.

Characters such as shape of S6 and the hidden S8, male genitalia, tergal punctuation, etc., did not prove to be diagnostic in distinguishing the other members of the *A. undulatum* group.

Variation. In addition to these “dark” individuals, a light morph was found in Iran. All yellow maculae are broader than in the dark morph, the ground color of the metasoma is dark reddish-brown (not black) and the underside of the scape is yellow (not black). The taxonomic identity of this individual was confirmed by barcoding.

The yellow coloration increases from the north towards the south. While the combination of these character traits characterizes *A. undulatum*, an unambiguous identification is not always possible in southern populations, especially in south-eastern Türkiye and Syria, where the occurrence seems to overlap with *A. wahrmani*, which has a similarly rich yellow color pattern.

Two females from Crete have an almost black clypeus, a black lower margin of the lower paraocular area, the preoccipital band reduced to four small yellow spots, and the anterolateral yellow band and the yellow band on the scutum and the axillar reduced to small remnants.

Taxonomy. Warncke (1980) raised the question of whether the subspecies *holozonicum* described from Cyprus should be recognized as distinct taxonomic entity. Members of that subspecies are, he stated, a little more yellowish than nominate specimens, but the differences are not as strong as between the nominate subspecies and the Levantine subspecies *wahrmani*, and examination of more material would be required to clarify the taxonomic status. Here, our DNA and morphometric data revealed that material from Croatia, Cyprus, Iran, and Türkiye belongs to the same clade and shows very little variation. These populations should be regarded as conspecific with nominate *undulatum*. On the other hand, *wahrmani* with rich dark yellow color should in fact be recognized as a distinct species.

Examination of three male paratypes of *A. undulatum creticum* and two female and one male non-paratypes from Crete (Tkalců 2003, material now in CMK) could not confirm the distinctiveness of the characters described by Tkalců (2003) when compared to nominate *undulatum*. The punctuation on T4, T5 (female) and coloration of hind legs (both sexes) are not diagnostic for this taxon, as shown

in a comparison with various mainland material. However, the black maculation on the clypeus in the female is more extended than in any other female examined from across the distribution area. Also, the yellow preoccipital band, the yellow anterolateral band on the scutum, and the yellow coloration on scutellum and axillae are present only as remnants. A DNA barcode sequence could not be retrieved from the two specimens examined, and material was not sufficient for a morphometric analysis. It is suggested to maintain the subspecies status until further clarification.

The phenotypic character traits of *A. u. holozonicum* Mavromoustakis, 1939 from Cyprus were found to be within the range of the nominate material, and the DNA barcoding sequence concurs with nominate material (Fig. 3). This subspecies is suggested as a junior synonym of the nominate subspecies (*Anthidium undulatum holozonicum* Mavromoustakis, 1939 **syn. nov.**).

Distribution. Widely distributed from southern France across the Adriatic Balkan coast, Greece and Bulgaria, to Türkiye and further to the Caspian coast and Iran. Distribution in south-eastern Türkiye and northern Syria remains ambiguous due to unclear taxonomic status. On Mediterranean islands, the distribution includes Cyprus, while the taxonomic status on Crete needs clarification (distinct subspecies or species?) (Fig. 2).

Anthidium libanicum Kasperek sp. nov.

<https://zoobank.org/FA05A7EB-CC6C-47D4-8A34-A20989B42D78>

Figures 6, 7

Type material. HOLOTYPE: **LEBANON:** male, North Lebanon: Hadath el Jebbe, Road to Wadi al Fouar (34°12'47.9"N 35°55'30.9"E), 1553 m; 22.viii.2018; M. Boustani leg. (CMK: mbou249; ABABX247-21). — PARATYPES: **LEBANON:** 1♂; Beqaa: Hadath (34°00'38.1"N 35°59'01.1"E), 1441 m; 7.vii.2019; M. Boustani leg. (CMK: mbou135; ABABX250-21). — 1♀, North Lebanon: Harissa, Al Jawar (34°11'36.0"N 35°55'25.9"E), 1765 m; 28.vi.2017; M. Boustani leg. (CMK: mbou254; ABABX058-20). — 4♂; North Lebanon: Tannourine, Harissa El Jawar (34°11'36.2"N 35°55'26.1"E), 1736 m; 11.vii.2018; M. Boustani & J. Jabbour (CMK: mbou250-253; ABABX421-22, ABABX453-22, ABABX452-22, ABABX451-22). — 1♂; North Lebanon: Tannourine el Tahta, Mar Boutros Church (34°12'34.8"N 35°54'01.3"E), 1207 m; 27.vi.2019; M. Boustani leg. (OLL: mbou133; ABABZ093-23). — 1♂; North Lebanon: Tannourine El Tahta, Saint Peter Church (34°12'34.70"N 35°54'01.20"E), 1155 m; 27.vi.2019; X. van Achter leg. (coll. M. Boustani: mbou132; ABABX422-22). — 1♂; North Lebanon: Tannourine, Wadi Ain el Raha (34°12'34.5"N 35°54'01.1"E), 1618 m; 29.vi.2017; M. Boustani leg. (CMK: mbou247; ABABZ092-23).

Non-type material (examined). **LEBANON:** 1♀; Beqaa: Jord Aarsal (34°06'27.0"N 36°25'26.4"E), 1975 m; 11.vii.2019; M. Boustani leg. (CMK: mbou137; ABABZ091-23, DNA not retrieved). — 1♂; North Lebanon: Tannourine Cedars (34.20°N 35.92°E); 25.vi.2006; N. Nemer leg. (CMK: mbou248). — 1♂; North Lebanon: Harissa, Al Jawar (34°11'36.0"N 35°55'25.9"E), 1765 m; 28.vi.2017; M. Boustani leg. (coll. M. Boustani: mbou246). Note: Specimens with barcode sequence

have been assigned as type material. Specimens not barcoded are classified as non-type material.

Barcode Index Number. BOLD:AEH0696.

Diagnosis. The species is characterized in both sexes by a combination of several characters. The body size is on average smaller than in *A. undulatum* and *A. wahrmani* (average length of the marginal cell 1.67 [1.53–2.10] mm in 11 males, compared to 2.14 [1.67–2.16] mm in 7 males of *A. wahrmani* and 3.05 [1.46–2.15] mm in 38 males of *A. undulatum*), the color of the body maculations is pale yellow (bright yellow in the other two species), the transition between the black ground color and yellow band on T1 and T2 is rusty (sometimes additionally with some inconspicuous, blurred rusty maculation within the yellow band) (rusty transition zone absent in the other two species), relatively narrow anterolateral yellow band on scutum (broader in the other two species), and a black antennal scape (red-brown in *A. wahrmani*; black or yellow in *A. undulatum*).

Genetically, *A. libanicum* differs from *A. undulatum* and *A. wahrmani* at seven different nucleotide sites in the DNA sequence of the COI gene: 124, 184, 328, 433, 451, 515, 565, and 625 (see Table 3 for details).

Description. *Female.* 8 mm. — *Head:* Black with pale yellow clypeus and lower paraocular area (reaching beyond the antennal socket), preoccipital band extending across vertex and malar area and reaching mandibular area; preoccipital band in contact with the eye only in the lower malar area; clypeus slightly convex, with the highest point in the center; densely punctate laterally, less dense and coarser punctures along the midline; anterior margin almost straight, posterior rim straight and smooth; mandible yellow with dark brown teeth; three large teeth and in between them approximately 10 minute teeth (number slightly variable); antennal scape and pedicel black, flagellum mostly reddish brown (darker on the upper side than on the lower side). — *Mesosoma:* Black; pale yellow anterolateral band on scutum; scutellum as seen from above with margin more or less transverse, angled forward toward axillar margin; posterolateral margin transparent lamellate; scutellum posteriorly with pale yellow band; axilla pale yellow. — *Metasoma:* T1–T5 with narrow, pale yellow transversal band; broader laterally than medially; T6 pale yellow with black outer margin and a black wedge-shaped incision; transition between black ground color and yellow band rusty on T1 and T2, sometimes additionally with some inconspicuous, blurred rusty maculation on the yellow band; femora and tibiae yellow with some black on the inner face; basitarsi covered with dense felt-like pubescence.

Male. 8–9 (10) mm. — *Head:* Yellow maculation similar to female; yellow maculation of paraocular area reaches middle of upper half of eye; preoccipital band variable, sometimes reaching only upper one third or upper half of eye. — *Mesosoma:* As female. — *Metasoma:* Coloration of T1–T5 as in female; apical margin of T6 medially protruding; T6 with lateral hooked extension; outer margin



Figure 11. Habitat of *Anthidium libanicum* sp. n. in Jord Aarsal in the Anti-Lebanon Mountains, Lebanon, at 1.975 m a.s.l.

of T7 as seen from above widely rounded with transparent sides and a deep, almost semi-circular emargination.

Variation. The material from Lebanon includes one male of a light morph. While the general characters agree with those of *A. libanicum*, the ground color of the metasomal sterna and terga is dark reddish brown (not black), the color of the anterior vertical side of T1 light reddish brown (not dark reddish brown). The body size is significantly larger than the other males examined. It has, e.g., a marginal cell length of 2.2 mm, as compared to an average length of 1.60 mm for the remaining specimens (N=8, only barcoded material). The affiliation of this specimen with *A. libanicum* was confirmed by the genetic barcoding sequence.

Habitat and floral relationships. *Anthidium libanicum* was collected visiting flowers of *Ononis natrix*, *O. spinosa* (Fabaceae) and *Stachys distans* (Lamiaceae). The species was collected in both humid Mediterranean and steppic semi-arid habitats (Figs 11, 13). The localities included clearings of oak and cedar woods, scrublands with karstic outcrops and Lamiaceae patches, and degraded roadsides lined with *Ononis* spp. bushes.

Distribution. Endemic to Lebanon. It was found in clearings of oak (1.000–1.400 m a.s.l.) and cedar woods (1.400–1.800 m a.s.l.) on the western slopes of the Mount Lebanon chain, and semi-arid steppes on the eastern slopes of the same mountain chain (1.400 m a.s.l.). It was also found on the peak of the Anti-Lebanon mountain chain near the Syrian border in the Oro-Mediterranean steppe (1.900 m a.s.l.). The lower altitudes were not heavily sampled, and the species could well have a larger distribution than reported (Fig. 2).

***Anthidium wahrmani* Mavromoustakis, 1948 stat. nov.**

Figures 6, 7

Proanthidium undulatum wahrmani Mavromoustakis, 1948. Male, female (Israel/Palestine). – Mavromoustakis (1948), stated that the holotype is deposited in his own collection. However, it has not been listed in the collection inventory (MoA, 1989). The material presented here was attributed to this taxon based on Mavromoustakis' description.

Material examined. ISRAEL/PALESTINE: 2♀, 2♂; R70, 10km NNE Nahariya (33.05N 35.15E), 60 m; 28.iv.2018; M. Halada leg. (CMK: mk1788, ABABX548-22; CMK: mk1789, ABABX450-22; CMK: mk1791, ABABX539-22; CMK: mk1790, ABABX538-22). – 1♀; 15 km E Qiryat Shemona Hermon Foothill (33.25N 36.73E); 16.v.1996; M. Hauser leg. (CMK-MH, mh067). – 1♂; Mt. Carmel, old quarry (32.73N 35.03E); 14.v.2000; M. Török leg. (ZMH: zmh009). – 2♂; Mt. Carmel Wadi Denia (32.75N 35.00E); 04.vii.2000; M. Török leg. (ZMH: zmh010-11). — **JORDAN:** 1♀, 1♂; N. Shuna (32.61N 35.60E); 20.–22.iv.1996; Ma. Halada leg. (CMK: ms0940, 1430). – 1♂; Jordan valley, Mubalath (Tal al Maqlub) (32.40N 35.68E); 27.iv.1996; Ma. Halada leg. (CMK: ms0820). – 1♀; Petra (30.32N 35.44E); 14.v.1995; K. Deneš sen. leg. (OLL: oll0935). — **LEBANON:** 1♂; North Lebanon: Harissa, Al Jawar (34.18N 35.92E), 1765 m; 18.vii.2017; M. Boustani leg. (CMK: MBou245; MGPCC093-21). – 1♂; North Lebanon: Tan-nourine Reserve, Trail 4 (34.20N 35.92E), 1781 m; 27.vii.2019; M. Boustani leg. (CMK: MBou134; ABABX059-20). – 2♀, 1♂; Mount Lebanon: Anjar, Guest House (33.72N 35.93E), 973 m; 23.vii.2019; M. Boustani & X. van Achter leg. (CMK: mbou136, ABABX057-20; CMK: mbou138; ABABX449-22; coll. M. Boustani: mbou139). — **PALESTINE:** 1♂; 10 km E Jerusalem (31.82N 35.33E); 05.v.1996; M. Hauser leg. (CMK: mh068).



Figure 12. Habitat of *Anthidium wahrmani* **stat. nov.** in the Tannourine Nature Reserve, Lebanon, in the Lebanon Mountains at 1.780 m a.s.l.

Barcode Index Number. BOLD:AAO4344.

Diagnosis. Maculation of integument dark yellow (as in *A. undulatum*, but pale yellow in *A. libanicum*). Female: Clypeus yellow without black maculation (as in *A. libanicum*, but present in most *A. undulatum*). Broad yellow preoccipital band, also covering genae entirely; yellow tergal bands broad, uninterrupted; T6 yellow with a black wedge-shaped apical maculation (as in *A. libanicum*; in *A. undulatum* mostly black with a large yellow spot on each side). Male similar in coloration, but preoccipital

band reaching only middle of genae. See also the diagnosis of *A. undulatum*.

Habitat. See under *A. libanicum* and Figs 10, 11.

Distribution. Distribution restricted to the southern Levant with records from Israel/Palestine, Jordan, Lebanon, and southern Syria. The distribution overlaps with *A. libanicum* **sp. n.** in Lebanon. Taxonomic status in south-eastern Türkiye unclear, where it possibly occurs and overlaps with *A. undulatum* (Fig. 2).

Identification Key

Unambiguous identification is only possible through genetic barcoding. The distinguishing base pairs are given under 3.3 and in Table 3. The following key uses morphological traits, which, however, do not always allow for 100% reliable identification. The key is based on the couplets provided by Kasperek (2022).

Females

- 65 Large species (13–15 mm); mostly 9–10 teeth; teeth with the exception of the innermost and the outermost tooth almost equal in size; apical margin of clypeus crenulate to dentate ***Anthidium undulatifforme***
- Smaller species (≤10 mm); teeth alternate between large and small; apical margin of clypeus smooth **65a**
- 65a Combination of the following characters: two black, longish maculae on the clypeus next to the middle; apical margin of clypeus black (rarely light brown as in the other two species); yellow preoccipital band narrow, reaching in typical specimens upper third of eye (rarely reaching lower third of eye or reduced to longitudinal spot behind the eye) ***A. undulatum***
- Black maculation on clypeus usually absent; apical margin of clypeus light brown; yellow preoccipital band broad, reaching gena; gena entirely yellow **65b**
- 65b Maculation of integument dark yellow; scape reddish brown; abrupt transition between tergal bands and ground color on T1 and T2 (rusty transition zone absent) ***A. wahrmani* stat. nov.**
- Maculation of integument pale yellow; scape black, contrasting to the reddish-brown inner flagellomeres; rusty transition between black ground color and yellow band on T1 and T2 ***A. libanicum* sp. nov.**



Figure 13. Mountainous habitat in Harissa al Jawar, North Lebanon, in which *Anthidium wahrmani* and *A. libanicum* **sp. n.** were found to occur sympatrically.

Males

- 73 Scutellum nearly rectangular with posterolateral corners rounded (corners often transparent and lamellate); median tooth at apical margin of T6 absent; T2–T6 with uninterrupted yellow bands **73a**
- Scutellum with posterolateral tooth; T6 with median tooth at apical margin; T2–T3 with lateral yellow bands.....
..... *Anthidium oblongatum*
- 73a Combination of the following characters: antennal scape black; gena black or with some yellow in the upper part; yellow anterolateral band on scutum broad *A. undulatum*
- Antennal scape; gena entirely yellow; yellow anterolateral band on scutum narrow **73b**
- 73b Antennal scape reddish brown; yellow margin of scutellum broad; on average larger species *A. wahrmani*
- Antennal scape black, contrasting to the reddish-brown inner flagellomeres; yellow margin of scutellum narrow; on average smaller species..... *A. libanicum*

4. Discussion

In a sample of wool carder bees identified as *Anthidium undulatum*, we could distinguish three distinct groups, and we conducted a thorough investigation to determine whether these groups represent distinct taxonomic units. Key results of our morphological, morphometric, and genetic analyses that led to the suggestion of these groups as distinct species include: The three groups form clearly distinct clusters in an analysis of the DNA barcoding sequence of the COI gene and do not share haplotypes. The genetic barcode gap of the mitochondrial COI gene is much higher between the members of the three groups than the intra-group variation. The three groups are distinguished by a combination of several morphometric traits and form in a multivariate analysis three distinct clusters with little overlap. The three groups also exhibit differences in color and color patterns, while these differences are

not fully diagnostic in the central and southern Levant. The restricted-range populations assigned to *A. libanicum* **sp. n.** and *A. wahrmani* **stat. nov.** occur sympatrically without evidence for interbreeding.

The applicability of COI barcode sequence for species identification and taxonomic decisions largely depends on the barcode gap, which refers to the phenomenon where the genetic variation within a species (intraspecific variation) is significantly smaller than the genetic variation between different species (interspecific variation) (Meyer & Paulay 2005; Hebert et al. 2003). The gap itself is the difference between the highest level of intraspecific variation and the lowest level of interspecific variation. A large barcode gap indicates that species can be reliably distinguished based on their DNA barcodes, as there is little overlap between the genetic variation within species

and between species. However, it needs to be considered that a sufficient number of samples is needed to assess full range of genetic variation, and that genetic variation may change across the distribution area, resulting in a higher variation in samples from different and distinct populations than samples from a single local population. In our samples, the barcode gap between *A. undulatum* and *A. libanicum* was 1.96%, and 4.43% between *A. undulatum* and *A. wahrmani*. It was 4.54% between the sympatrically occurring *A. libanicum* and *A. wahrmani*. These values were many times higher than the intraspecific variation (Table 2).

Since *A. libanicum* and *A. wahrmani* currently occur in the same area, geographic distance can be ruled out as the primary factor driving the genetic differences between these species. However, it is possible that these species originated from two populations that were historically separated in distribution for an extended period. After the post-glacial period, these populations may have come back into contact. For *A. undulatum*, the closest sampling localities are 280 km apart, while the farthest are over 2000 km apart, and we did not observe any correlation between geographic distance and genetic distance. Therefore, we concluded that genetic differences between the three groups are not a result of a rapid adaptation to environmental conditions in different geographic areas.

Studies on the size of the barcode gap rarely take geographic variation into account. For example, Gaytan et al. (2020) found in moths feeding upon *Quercus* sp. in Europe that pairwise intraspecific genetic divergence increased along with spatial distance and thus resulted in a geographical bias of the barcode gap. Ashfaq et al. (2013) compared barcoding sequences of butterflies from Pakistan with those from other parts of the world and noted that the relationship between geographical distance and the level of intraspecific divergence was in general not strong. They concluded that the increase is mostly too small to impede the identification of species. In a study on 40 species of butterflies (Nymphalidae) from India, Gaikwad et al. (2012) found very little intraspecific genetic variation also when they compared their results with conspecifics from different, often distant geographic regions – except for a few cases which may represent cryptic diversity. Lukhtanov et al. (2009) found in a study on 353 Central Asian butterflies that although expanded geographical coverage substantially increased intraspecific variation and reduced the barcoding gap between species, this did not decrease species identification.

Genetic distance between species may vary greatly and is also known to depend on the kind of organism. While the genetic distance between bee species is mostly below 2.5%, distances up to 12% have been reported in bees, particularly in Halictidae (Gibbs et al. 2018; Schmidt et al. 2015; Villalta et al. 2021). In Anthidiini, the lowest reported genetic interspecific distance is 2.4% between *Anthidiellum africanum* Kasperek, 2022, and *A. breviusculum* (Perez, 1890) (Kasperek et al. 2022). Litman et al. (2021) also reported very low genetic distances between some members of the *Pseudoanthidium scapulare* complex, which are likely even lower than this value, al-

though they did not provide specific figures. In *Rhodanthidium caturigense* (Giraud 1863), a genetic distance of 2.7% was found still to be regarded as intraspecific variation (Kasperek 2021). The interspecific genetic differences of 2.6% to 5.0% among *A. undulatum* s.l. (Table 2) are within these ranges. The co-existing species pair *A. libanicum* / *A. wahrmani* exhibit a genetic distance of 4.9% and represent a good benchmark for genetic distances in this group of anthidiine species.

We also evaluated whether the morphometric differences observed among the three groups could be attributed to geographic variation. In anthidiine bees, body size has been found to correlate with geographic latitude in species such as *Rhodanthidium caturigense* and *Eoanthidium insulare* (Kasperek 2021; Kasperek et al. 2023). Additionally, the combination of various morphometric parameters reflecting both size and shape has been instrumental in identifying previously unknown taxa (e.g., Kasperek 2018, 2020). In the case of *A. libanicum* and *A. wahrmani*, the morphometric differences cannot be considered influenced by geographic variation, as these species coexist in the same, localized area. In *A. undulatum*, this geographic range could account for the larger convex hull (smallest convex polygon) observed for *A. undulatum* compared to *A. libanicum* and *A. wahrmani* in the Discriminant Analysis (Fig. 5). Despite this, *A. undulatum* still forms a distinct cluster, separate from *A. libanicum* and *A. wahrmani*.

A general trend was observed in *A. undulatum*, insofar as specimens exhibit increasingly rich yellow coloration towards the south. Populations in southern regions particularly in the xeric areas of southeastern Türkiye, often display coloration resembling that of *A. wahrmani*. This similarity can make phenotypic distinction between the two species challenging. Although both species appear to be present in overlapping distribution areas in southeastern Türkiye, further confirmation, e.g., through genetic barcoding is still needed. Similar trends in the geographic variation of coloration have been documented in various other anthidiine species (Kasperek 2021; Kasperek et al. 2023; Warncke 1980).

In *A. undulatum* and *A. libanicum*, one male of each was found with abnormal coloration: these two males were markedly lighter than the other specimens, with all yellow maculae broader than in the dark individuals (Fig. 8). The underside of the scape was yellow (not black) in *A. undulatum*, and in particular, the ground color of the metasoma was dark reddish-brown (not black). The species identity of these individuals was confirmed by genetic barcoding. These individuals are regarded as color morphs, i.e., a distinct intraspecific color variation, most likely arising from genetic differences and possibly also influenced by environmental factors. A very similar situation has been described for *A. dalmaticum* Mocsáry, 1884, where such light color morphs have previously even been described as distinct taxa (Kasperek 2024).

In this study, *A. libanicum* and *A. wahrmani* were shown to be closely related species with weak phenotypic but clear genotypic differences, coexisting in sympatry in the central Levant. Therefore, the possibility of sympatric

speciation (where new species arise without geographical isolation) may be considered. De Vries (1901–1902) was possibly the first to claim that new species can arise through mutation within the ancestral range, while Mayr (1963) emphasized that the sympatric occurrence of sibling species is not necessarily the result of sympatric speciation but may instead be due to a secondary contact zone. There is a rich body of literature discussing the pros and cons of these different approaches (Bolnick & Fitzpatrick 2007). The Levant is known as a land bridge between the Palearctic and Africa, where climatic oscillations have caused species' range sizes to expand and contract during various glacial periods (e.g., Por 1987; Thompson 2020). Therefore, it seems plausible that the speciation of *A. libanicum* and *A. wahrmani* occurred in distinct locations, but their distributional areas shifted and overlapped subsequently during one of the postglacial periods, rather than through sympatric speciation in the same area.

The Levant is the region where the three species converge. However, little is known about their local distribution patterns, the degree of overlap or mutually exclusive distributions, and their specific habitats or ecological preferences. In particular, Syria and southern to south-eastern Türkiye present promising areas for more detailed studies of these aspects.

Overall, the case of *A. undulatum* is considered a rare example in which cryptic diversity could be shown for sympatric populations and hereby confirm the level of diversity at the species level.

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Supplementary Material 1

Table S1

Authors: Kasperek M, Boustani M (2025)

Data type: .xlsx

Explanation notes: List of material of *Anthidium undulatum* s. l. used for this study.

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