

Hidden diversity in European bees: *Andrena amieti* sp. n., a new Alpine bee species related to *Andrena bicolor* (Fabricius, 1775) (Hymenoptera, Apoidea, Andrenidae)

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Abstract

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We revise the Alpine bee taxa related to *Andrena bicolor* (Fabricius, 1775), including *A. montana* Warncke, 1973 and *A. allosa* Warncke, 1975, the status of which has remained contentious. Phylogenetic analyses of one mitochondrial gene and one nuclear gene, as well as morphological examination reveal the presence of four Alpine species in this complex, one of which is new to science, *A. amieti* sp. n. This new species is widely distributed in the Alps from southern France throughout Switzerland, northern Italy and southern Germany to Austria; a single record is known from the Apennines. The type locality is located within the Unesco World Heritage site “Swiss Alps Jungfrau-Aletsch”. Two widely divergent mitochondrial lineages are found in sympatry in *A. amieti* sp. n.; the status of these lineages, which together form a paraphyletic unit from which *A. allosa* arose, is briefly discussed. We show that *A. allosa*, *A. amieti* sp. n. and *A. montana* are polylectic but that each species exhibits a distinct spectrum of pollen hosts: the univoltine *A. allosa* shows affinities for pollen of the early-blooming Alpine plant genus *Crocus*. *A. amieti* sp. n. is bivoltine and, as in *A. bicolor*, the summer generation exhibits a distinct preference for Campanulaceae, while the spring generation is widely polylectic. *A. montana* has a single generation in the summer and forages on a diversity of flowers such as Campanulaceae, Cistaceae and Caryophyllaceae. An identification key is presented for central European members of the subgenus *Euandrena* Hedicke, 1932. Lastly, the new Alpine species appears to represent the tip of the iceberg of substantial cryptic diversity in southern European *Andrena* (*Euandrena*): *A. croatica* Friese, 1887 is resurrected from synonymy with *A. bicolor* and treated as a valid species (**stat. rev.**), *A. pileata* Warncke, 1875, described as a subspecies of *A. allosa*, is elevated to species rank (**stat. n.**), and three additional unclear taxa are briefly described.

Introduction

The conservation of species depends on accurate data regarding their distributions and a sound understanding of their ecological requirements. While the distributions and ecology are well-known for a large part of the bee fauna in northern and central Europe (Peeters et al. 2012, Scheuchl and Willner 2016, Else and Edwards 2018, Westrich 2018), some Alpine and numerous southern Euro-

pean bee taxa remain poorly investigated. In part, this situation is due to open taxonomic questions and ambiguous morphology-based identifications, both of which strongly limit studies on species’ distribution and biology.

Within central European bees, one complex of species that remains insufficiently investigated is the group of taxa related to *Andrena bicolor* (Fabricius, 1775), referred to here as the “*bicolor*-group”. *Andrena bicolor* is one of the most widely distributed species of wild bees in

Europe (Gusenleitner and Schwarz 2002, Scheuchl and Willner 2016). In Switzerland it is found in all biogeographic regions in numerous habitats from low elevations to the tree line (Amiet et al. 2010). The taxonomic status of two alpine taxa related to *A. bicolor* remains debated, *A. allosa* Warncke, 1975 and *A. montana* Warncke, 1973. These two taxa were recognized as specifically distinct from *A. bicolor* by Schmid-Egger and Scheuchl (1997), although both Westrich (2006) and Schmid-Egger (2012) indicated that the taxonomy of the *bicolor*-group was not fully elucidated. In agreement with this finding, Amiet et al. (2010) report that the morphological delimitation of *A. allosa* and *A. montana* in the Swiss Alps was difficult because of intermediate forms that bridge the morphological gap among taxa. In particular, Amiet et al. (2010) point to an unclear, Alpine “form” of *A. bicolor* in which the vestiture of both female and male is extensively white instead of yellow-brown and thus transitional between *A. bicolor* and *A. montana*.

In a recent, comprehensive DNA barcoding study of central European bees, Schmidt et al. (2015) presented DNA barcodes for three taxa of the *bicolor*-group and indicated that these taxa were clearly divergent from one another, supporting the hypothesis that there are three well separated species. While this result appears to solve the controversy on the status of these species, sequencing more specimens, especially those intermediate forms mentioned by Amiet et al. (2010), is still critical to our understanding of how to delimitate these species using morphological criteria. Such morphological delimitation is a necessary step for the revision of museum specimens and for obtaining details on species’ life-histories and distributions.

The biology of *A. allosa* and *A. montana* remains virtually unknown. The nominal subspecies of *A. allosa* is so far known from less than 20 individuals from France and Switzerland. Two subspecies have been described from Greece and Turkey, respectively, *A. allosa pileata* Warncke, 1975 and *A. allosa canigica* Warncke, 1975. Gusenleitner and Schwarz (2002: 100) raised doubts that these two infraspecific taxa were conspecific with *A. allosa* and stated that “more taxa are certainly present in the close relatedness of *A. allosa*”. *Andrena montana* is slightly better known and Amiet et al. (2010) indicated that this species flies from late June to mid-August, suggesting a single generation per year. Observations on the floral associations of these two taxa, however, are completely lacking. In contrast, the biology of *A. bicolor* is well-known. This species is bivoltine (e.g., Westrich 2006, 2018), at least at low elevations (Amiet et al. 2010), and broadly polylectic (Stöckhert in Schmiedeknecht 1930, Peeters et al. 2012, Scheuchl and Willner 2016). The cuckoo bee *Nomada fabriciana* (Linnaeus, 1767), similarly bivoltine, is a cleptoparasite of *A. bicolor* (e.g., Westrich 2006).

In the present study, we investigate the systematics of the Alpine taxa of the *bicolor*-group using DNA sequences of a mitochondrial and a nuclear gene. The nuclear gene was used in addition to the mitochondrial

gene given the limitations of relying on a single DNA marker for species delimitation, for example because of incomplete lineage sorting, mitochondrial introgression or deep mitochondrial genetic divergence not accompanied by nuclear differentiation (e.g. Nicholls et al. 2012, Andriollo et al. 2015, Klopstein et al. 2016). Based on combined genetic and morphological results, we demonstrate the presence of four well-separated species in the Alps, one of which is new to science. We also examine the status of some southern European taxa of the *bicolor*-group; this taxonomic treatment does not seek to resolve the taxonomy of this group outside the Alps but simply to ensure that the new species that we describe is not conspecific with a named taxon in southern Europe. Lastly, we revise the available material to determine the distribution of the Alpine taxa and to examine their phenology, study their host plant preferences based on analyses of pollen loads and report on field observations on habitat selection and floral preferences.

Methods

Specimen selection for molecular analyses

The selection of specimens for molecular analyses was performed iteratively. During an initial phase of this project, 14 specimens representing the observed morphological variation in the *bicolor*-group were sequenced, including 11 Alpine specimens as well as three specimens from the Apennines. Based on genetics and subsequent morphological examination of these specimens, we were able to recognize four well-separated species in the Alps. For simplicity, these species are named hereafter in a way that anticipates the species delimitation and the description presented below: *A. allosa*, *A. amieti* sp. n., *A. bicolor* and *A. montana*. Subsequently, 35 additional specimens originating from the Alps were identified using morphology prior to genetic analyses and added to the molecular dataset. Whenever possible, for all Alpine localities where *A. amieti* sp. n. and *A. bicolor* were found in sympatry we sequenced at least one specimen of each species. One specimen of *A. montana* from Greece was sampled, and we included one unclear specimen from the northern Pyrenees that showed morphological affinities with *A. allosa*.

Morphological examinations of further specimens of the *bicolor*-group revealed several additional taxa in southeastern Europe, some of which were very similar to *A. amieti* sp. n. Since at least one species-group name is available for this group of taxa (*A. allosa pileata*), we expanded our sampling to include six specimens of the *bicolor*-group from southeastern Europe. These six specimens include three unclear taxa of the *bicolor*-group, referred to as *Andrena* sp1, sp2 and sp3. Lastly, we sequenced three specimens of *A. rufula* Schmiedeknecht, 1883 to generate reference material for this species since females have so far been misidentified in Switzerland (Artmann-Graf 2017).

Details on locality information and BOLD and GenBank accession numbers, as well as GenSeq categories following Chakrabarty et al. (2013) are given in Table 1. All available sequences for the *bicolor*-group (Schmidt et al. 2015) were downloaded from the BOLD platform (www.boldsystems.org), as well as a selection of European species from the subgenus *Euandrena* Hedicke, 1933 and representatives of the subgenera *Larandrena* LaBerge, 1964, *Andrena* s. str. Fabricius, 1775, *Leucandrena* Hedicke, 1933, *Micrandrena* Ashmead, 1899, *Melandrena* Pérez, 1890 and *Ptilandrena* Robertson, 1902.

Lab protocols

We first amplified the 658-bp “DNA barcode” fragment of the mitochondrial gene cytochrome oxidase I (hereafter COI) using the LepF and LepR primers (Hebert et al. 2004) with the PCR conditions given therein; if no amplification was obtained, we used the alternate forward primer UAE3 (TAT AGC ATT CCC ACG AAT AAA TAA; Lunt et al. 1996) together with LepR to amplify a 409-bp fragment of the same gene applying the same PCR conditions. If the obtained chromatograms were not clean, possibly because of nuclear pseudogenes, contamination or co-amplification of *Wolbachia* sequences, the PCR was repeated with the following specific primers designed for the *bicolor*-group: *bicolor_COX1_F*: RGC AGG AAT RRT TGG WGC AT and *bicolor_COX1_R*: CTC CVC CYA TWG GRT CAA A, with the following PCR conditions: 4' 94°; 35 × 45'' 94°, 45'' 55°, 45'' 72°; 7' 72°. These primers amplified a 563-bp fragment nested within the 658-bp barcoding fragment with a high success rate.

For 22 specimens of the *bicolor*-group as well as for *A. chrysopus* Pérez, 1903, *A. fulvata* Stöckert, 1930 and *A. rufula*, we additionally sequenced an approximately 400-bp fragment of the single-copy nuclear gene LW-rhodopsin (hereafter opsin; Danforth et al. 2004) using the following primers specific to the genus *Andrena*: *Ops-For3_Andrena*: TTC GAC AGA TAC AAC GTR ATY GTM AAR GG and *OpsRev3_Andrena*: GCY ARC TTK GCC TCY GCG CT, with the following PCR conditions: 4' 94°; 35 × 45'' 94°, 45'' 56°, 45'' 72°; 7' 72°.

Lab protocols follow Trunz et al. (2016). DNA was extracted from a single leg using DNA extraction kits (Nucleospin tissue, Macherey-Nagel). PCR-reactions were performed with Hotstart GoTaq polymerase (Promega) with a blank sample as a negative control. PCR products were examined visually using agarose gel electrophoresis and purified enzymatically with a mix of exonuclease and FastAP thermosensitive alkaline phosphatase (Fermentas). Purified PCR products were sequenced bidirectionally with the primers used in the PCR using BigDye Terminator v3.1 technology (Applied Biosystems).

Phylogenetic analyses

Chromatograms were trimmed and assembled in Geneious 6.0.6 (Kearse et al. 2012) and exported consensus were aligned using MAFFT (Katoh and Standley 2013).

The resulting matrices were examined and edited in Mesquite (Maddison and Maddison 2018) and converted to amino acid sequences to verify that no stop codon was present. Maximum likelihood analyses were performed in RAxML 8.2.10 (Stamatakis 2014) on the CIPRES server (Miller et al. 2010), performing 1000 bootstrap replicates and applying a GTR + G model to each partition. COI was partitioned into three partitions corresponding to the three nucleotide positions, while opsin was partitioned into four partitions corresponding to the three nucleotide positions and the intron. Trees were rooted at the base of the subgenus *Euandrena*. Uncorrected p-distances were computed in a test version of Paup 4.0 (Swofford 2002) kindly provided by D. Swofford.

Morphology

Terminology follows Michener (2007); when numbered, metasomal terga and sterna are abbreviated by T and S (e.g., T2 for metasomal tergum 2). When describing vestiture colour in the descriptions and the identification key, “brown” means yellowish-brown, orange-brown or grey-brown and is opposed to “dark brown”, which means dark brown to black. The length of the mouthparts, especially of the maxillary and labial palps, is an important criterion for separating some species of *Euandrena*. The palps are not cylindrical; instead, several segments are rather flattened and triangular. For estimating the length-to-width ratio of the segments, the width was measured on the flattened side, at the widest part, near the apex, perpendicular to the longitudinal axis of the segment. Measurements were made with ImageJ 1.48 (Abràmoff, Magalhães and Ram 2004) on pictures taken with a Keyence digital microscope VHX 1000 with the stacking option turned off. All pictures of specimens presented here were taken with the same digital microscope with the stacking option turned on.

Databasing

The material of the *bicolor*-group deposited in the main entomological collections in Switzerland (Table 2) has been revised. In addition, the material deposited in the Warncke collection (OLML) as well as in some additional collections in Europe (Table 2) has been examined. For *A. amieti* sp. n., every type specimen deposited in a public collection in Switzerland has been provided with a label bearing a unique identifier. Swiss data have been submitted to the Swiss Zoological Records Center (InfoFauna; www.cscf.ch) and data from other countries to GBIF (www.gbif.org) if the specimens are deposited in a public collection in Switzerland. To compare the phenology and altitudinal distribution of the examined species, records from the Alps were filtered to keep only one record per day and locality (regardless of the number of individuals collected). The distribution map was produced with ArcGIS using the “Digital Elevation Model over Europe, EU-DEM” data for relief (available at <https://www.eea.europa.eu/data-and-maps/data/eu-dem>).

Table 1. Locality data (including coordinates), BOLD (for the mitochondrial gene cytochrome oxidase, or COI) or Genbank (LW rhodopsin, or opsin) accession numbers and GenSeq category (after Chakrabarty et al. 2013; 1 = holotype; 2 = paratype; 3 = topotype; 4 = vouchered specimens) for sequences produced in this study.

| Voucher | Name | Sex | Country | Locality | Genseq category | Coord N | Coord E | COI | opsin |
|---------|---------------------------|-----|---------|--|-----------------|---------|---------|-------------|----------|
| 833 | <i>A. chrysopeus</i> | □ | CH | Leuk (S. Gerber) | 4 | 46.303 | 7.678 | NA | MK157241 |
| 483 | <i>A. fulvata</i> | ♀ | CH | Orvin (C. Praz) | 4 | 47.163 | 7.213 | NA | MK157240 |
| 873 | <i>A. rufula</i> | ♀ | FR | St-Guilhem-Le-Désert (34) (J. Litman/C. Praz) | 4 | 43.738 | 3.542 | HYMAA032-18 | MK157242 |
| 874 | <i>A. rufula</i> | ♂ | FR | Blandas (30) (J. Litman/C. Praz) | 4 | 43.912 | 3.513 | HYMAA033-18 | NA |
| 1146 | <i>A. rufula</i> | ♂ | CH | Laupersdorf (SO) (G. Artmann-Graf) | 4 | 47.332 | 7.645 | HYMAA021-18 | NA |
| 558 | <i>A. montana</i> | ♂ | CH | Zermatt, Gornergrat (VS) (J. Litman/C. Praz) | 4 | 45.980 | 7.790 | HYMAA049-18 | NA |
| 559 | <i>A. montana</i> | ♀ | CH | Zermatt, Gornergrat (VS) (J. Litman/C. Praz) | 4 | 45.980 | 7.790 | HYMAA050-18 | MK157255 |
| 800 | <i>A. montana</i> | ♂ | IT | Maiella (Abruzzo) (J. Litman/C. Praz) | 4 | 42.144 | 14.112 | HYMAA053-18 | MK157256 |
| 801 | <i>A. montana</i> | ♀ | CH | Fully, Sorniot (VS) (D. Bénon) | 4 | 46.177 | 7.089 | HYMAA054-18 | MK157257 |
| 962 | <i>A. montana</i> | ♀ | GR | Mt Olympus (Piera) (K. Minachilis) | 4 | 40.105 | 22.390 | HYMAA044-18 | MK157258 |
| 1201 | <i>A. montana</i> | ♀ | CH | Visperterminen (CH) (D. Bénon) | 4 | 46.257 | 7.944 | HYMAA025-18 | NA |
| 1204 | <i>A. montana</i> | ♂ | CH | Glarus Süd, Linthal (GL) (R. Neumeyer) | 4 | 46.841 | 8.932 | HYMAA004-18 | NA |
| 961 | <i>A. sp1</i> | ♀ | GR | Mt Olympus (Piera) (K. Minachilis) | 4 | 40.095 | 22.369 | HYMAA043-18 | MK157259 |
| 1252 | <i>A. sp1</i> | ♀ | GR | Chelmos mountains (Archaea) (A.W Ebmer) | 4 | 38.002 | 22.195 | HYMAA037-18 | NA |
| 1254 | <i>A. sp2</i> | ♀ | GR | Lesbos, Agiassos (North Aegean) (A.W Ebmer) | 4 | 39.054 | 26.401 | HYMAA038-18 | NA |
| 928 | <i>A. sp3</i> | ♀ | GR | Kosmas (Arcadia) (J. Litman/C. Praz) | 4 | 37.107 | 22.728 | HYMAA036-18 | NA |
| 842 | <i>A. allosa</i> | ♀ | FR | Moulinet, Mille Fourches (06) (E. Dufréne) | 4 | 43.992 | 7.435 | HYMAA059-18 | NA |
| 848 | <i>A. allosa</i> | ♀ | CH | Gündlischwand (BE) (F. Amiet) | 4 | 46.649 | 7.928 | HYMAA060-18 | NA |
| 1265 | <i>A. allosa</i> | ♀ | CH | St-Martin, Lovégno (VS) (C. Praz/J. Litman) | 4 | 46.175 | 7.470 | HYMAA020-18 | NA |
| 1271 | <i>A. allosa</i> | ♀ | CH | Leuk (VS) (S. Giriens) | 4 | 46.335 | 7.657 | HYMAA022-18 | MK157244 |
| 1285 | <i>A. allosa</i> | ♀ | FR | Allos, La Foux d'Allos (04) (C. Praz) | 3 | 44.300 | 6.566 | HYMAA039-18 | MK157243 |
| 1293 | <i>A. aff allosa</i> | ♀ | FR | Larrau (64) (D. Genoud) | 4 | 43.035 | -1.023 | HYMAA045-18 | NA |
| 798 | <i>A. amieti</i> group 1 | ♀ | IT | Monte Pollino (Basilicata) (J. Litman/C. Praz) | 2 | 39.904 | 16.181 | HYMAA051-18 | NA |
| 799 | <i>A. amieti</i> group 1 | ♂ | IT | Monte Pollino (Basilicata) (J. Litman/C. Praz) | 2 | 39.904 | 16.181 | HYMAA052-18 | MK157260 |
| 1202 | <i>A. amieti</i> group 1 | ♀ | CH | Kandersteg, Oeschinensee (BE) (C. Praz) | 2 | 46.503 | 7.707 | HYMAA006-18 | NA |
| 802 | <i>A. amieti</i> group 2 | ♀ | CH | Fully, Sorniot (VS) (D. Bénon) | 2 | 46.167 | 7.108 | HYMAA055-18 | MK157261 |
| 804 | <i>A. amieti</i> group 2 | □ | CH | Orsières (VS) (D. Bénon) | 2 | 46.060 | 7.091 | HYMAA057-18 | MK157262 |
| 805 | <i>A. amieti</i> group 2 | ♀ | CH | Fully, Sorniot (VS) (D. Bénon) | 2 | 46.167 | 7.108 | HYMAA058-18 | NA |
| 875 | <i>A. amieti</i> group 2 | ♀ | CH | Bagnes (VS) (D. Bénon) | 2 | 46.008 | 7.346 | HYMAA023-18 | MK157263 |
| 1028 | <i>A. amieti</i> group 2 | ♂ | CH | Domleschg (GR) (H. Martz) | 2 | 46.785 | 9.453 | HYMAA031-18 | NA |
| 1029 | <i>A. amieti</i> group 2 | ♂ | CH | Grindelwald (BE) (M. Haider) | 2 | 46.649 | 8.077 | HYMAA029-18 | NA |
| 1031 | <i>A. amieti</i> group 2 | ♀ | CH | Grindelwald (BE) (M. Haider) | 2 | 46.649 | 8.077 | HYMAA028-18 | NA |
| 1159 | <i>A. amieti</i> group 2 | ♀ | FR | Le Bourg d'Oisan, Lauvitel (38) (M. Aubert) | 2 | 44.954 | 6.060 | HYMAA046-18 | NA |
| 1193 | <i>A. amieti</i> group 2 | ♂ | CH | Ennenda (GL) (A. Müller) | 2 | 47.028 | 9.105 | HYMAA010-18 | NA |
| 1197 | <i>A. amieti</i> group 2 | ♀ | CH | St-Martin, Lovégno (VS) (C. Praz) | 2 | 46.169 | 7.472 | HYMAA007-18 | NA |
| 1203 | <i>A. amieti</i> group 2 | ♀ | CH | Glarus Süd, Linthal (GL) (R. Neumeyer) | 2 | 46.839 | 8.922 | HYMAA005-18 | NA |
| 1207 | <i>A. amieti</i> group 2 | ♀ | CH | Kandersteg, Oeschinensee (BE) (J. Litman) | 2 | 46.501 | 7.712 | HYMAA002-18 | NA |
| 1268 | <i>A. amieti</i> group 2 | ♂ | CH | St-Martin, Lovégno (VS) (C. Praz/J. Litman) | 2 | 46.175 | 7.470 | HYMAA024-18 | MK157264 |
| 1284 | <i>A. amieti</i> group 2 | ♀ | CH | Kandersteg, Oeschinensee (BE) (J. Litman) (HT) | 1 | 46.501 | 7.712 | HYMAA001-18 | NA |
| 1286 | <i>A. amieti</i> group 2 | ♀ | FR | Allos, La Foux d'Allos (04) (C. Praz) | 2 | 44.300 | 6.566 | HYMAA040-18 | MK157265 |
| 556 | <i>A. bicolor</i> clade 1 | ♀ | CH | Emdt, Schalb (VS) (J. Litman/C. Praz) | 4 | 46.221 | 7.816 | HYMAA047-18 | MK157245 |
| 803 | <i>A. bicolor</i> clade 1 | ♀ | CH | Fully, Sorniot (VS) (D. Bénon) | 4 | 46.172 | 7.123 | HYMAA056-18 | MK157246 |
| 871 | <i>A. bicolor</i> clade 1 | ♀ | GR | Kryoneri (East Attica) (J. Litman/C. Praz) | 4 | 36.955 | 22.363 | HYMAA034-18 | NA |
| 900 | <i>A. bicolor</i> clade 1 | ♀ | CH | Leuk (VS) (S. Gerber) | 4 | 46.303 | 7.678 | HYMAA011-18 | NA |
| 903 | <i>A. bicolor</i> clade 1 | ♀ | CH | Vex (VS) (S. Gerber) | 4 | 46.208 | 7.411 | HYMAA013-18 | NA |
| 904 | <i>A. bicolor</i> clade 1 | ♀ | CH | Vex (VS) (S. Gerber) | 4 | 46.208 | 7.405 | HYMAA014-18 | NA |
| 925 | <i>A. bicolor</i> clade 1 | ♀ | CH | Leuk (VS) (S. Gerber) | 4 | 46.303 | 7.678 | HYMAA016-18 | MK157247 |
| 927 | <i>A. bicolor</i> clade 1 | ♀ | CH | Gampel-Bratsch (VS) (C. Praz) | 4 | 46.329 | 7.720 | HYMAA018-18 | MK157248 |
| 1199 | <i>A. bicolor</i> clade 1 | ♀ | CH | St-Martin, Lovégno (VS) (C. Praz) | 4 | 46.169 | 7.472 | HYMAA008-18 | NA |
| 557 | <i>A. bicolor</i> clade 2 | ♀ | CH | St-Niklaus (VS) (J. Litman/C. Praz) | 4 | 46.179 | 7.800 | HYMAA048-18 | MK157249 |
| 876 | <i>A. bicolor</i> clade 2 | ♀ | GR | Kosmas (Arcadia) (J. Litman/C. Praz) | 4 | 37.107 | 22.728 | HYMAA035-18 | NA |
| 902 | <i>A. bicolor</i> clade 2 | ♀ | CH | Naters (VS) (N. Evéquo) | 4 | 46.321 | 7.967 | HYMAA012-18 | MK157250 |
| 924 | <i>A. bicolor</i> clade 2 | ♀ | CH | Yvonand (VD) (M. Khadraoui) | 4 | 46.783 | 6.716 | HYMAA015-18 | NA |
| 926 | <i>A. bicolor</i> clade 2 | ♀ | CH | Nods (BE) (J. Litman/C. Praz) | 4 | 47.135 | 7.063 | HYMAA017-18 | MK157251 |
| 1030 | <i>A. bicolor</i> clade 2 | ♀ | CH | Vals (GR) (A. Müller) | 4 | 46.621 | 9.199 | HYMAA026-18 | NA |
| 1032 | <i>A. bicolor</i> clade 2 | ♀ | CH | Bonaduz (GR) (R. Neumeyer) | 4 | 46.809 | 9.412 | HYMAA019-18 | NA |
| 1033 | <i>A. bicolor</i> clade 2 | ♀ | CH | Domleschg (GR) (H. Martz) | 4 | 46.782 | 9.453 | HYMAA030-18 | NA |
| 1194 | <i>A. bicolor</i> clade 2 | ♂ | CH | Ennenda (GL) (A. Müller) | 4 | 47.026 | 9.104 | HYMAA009-18 | NA |
| 1195 | <i>A. bicolor</i> clade 2 | ♂ | CH | Vitznau (LU) (A. Müller) | 4 | 47.024 | 8.502 | HYMAA027-18 | NA |
| 1205 | <i>A. bicolor</i> clade 2 | ♀ | CH | Kandersteg, Oeschinensee (BE) (J. Litman) | 4 | 46.507 | 7.722 | HYMAA003-18 | NA |
| 1287 | <i>A. bicolor</i> clade 2 | ♂ | FR | Allos, La Foux d'Allos (04) (C. Praz) | 4 | 44.293 | 6.596 | HYMAA041-18 | MK157253 |
| 1288 | <i>A. bicolor</i> clade 2 | ♂ | FR | Allos, La Foux d'Allos (04) (C. Praz) | 4 | 44.300 | 6.566 | HYMAA042-18 | MK157254 |

Table 2. Collections examined with acronyms.

| | |
|-----------|--|
| AEC | Private collection of P. Andreas Ebmer, Puchenau, Austria |
| AMC | Private collection of Andreas Müller, Wädenswil, Switzerland |
| BNM | Bündner Naturmuseum, Chur, Switzerland |
| CPC | Private collection of Christophe Praz, Neuchâtel, Switzerland |
| CSEC | Private collection of Christian Schmid-Egger, Berlin, Germany |
| DGC | Private collection of David Genoud, Arzens, France |
| ESC | Private collection of Erwin Scheuchl, Ergolding, Germany |
| ETHZ | Eidgenössische Technische Hochschule, Zürich, Switzerland |
| FAC | Private collection of Felix Amiet, Solothurn, Switzerland |
| GAC | Private collection of Georg Artmann-Graf, Olten, Switzerland |
| HSC | Private collection of Hans Schwenninger, Stuttgart, Germany |
| JVC | Private collection of Johannes Voith, Augsburg, Germany |
| KHC | Private collection of Karl Hirt, Menziken, Switzerland |
| MAC | Private collection of Matthieu Aubert, Saint-Jean-De-Buèges, France |
| MBC | Private collection of Markus Bur, Rechthalten, Switzerland |
| MHC | Private collection of Mike Herrmann, Konstanz, Germany |
| MHNN | Muséum d'histoire naturelle de la ville de Neuchâtel, Switzerland |
| MNHN | Muséum Nationale d'Histoire Naturelle, Paris, France |
| MZL | Musée cantonal de zoologie, Lausanne, Switzerland |
| NMBE | Naturhistorisches Museum der Burgergemeinde Bern, Switzerland |
| OLML | Oberösterreichisches Landesmuseum, Linz, Austria |
| POL-AEGIS | University of the Aegean Pollinator collection, Mytilene, Lesbos, Greece |
| PRUN | Research collection of Christophe Praz, University of Neuchâtel, Switzerland |
| RNC | Private collection of Rainer Neumeyer, Zürich, Switzerland |
| RNF | Collection of the Natural Reserves of France |
| SRLC | Collection of the Swiss Bee Red List project, Neuchâtel, Switzerland |
| ZMHB | Museum für Naturkunde, Berlin, Germany |

Pollen host preferences

The pollen host preferences of *Andrena allosa*, *A. amieti* sp. n. and *A. montana* were assessed by microscopic analysis of the scopal pollen contents of 24, 50 and 16 female specimens, respectively. These females all originated from the Swiss, French and Italian Alps and were collected from 1921 to 2018. For the bivoltine *A. amieti* sp. n., the results of the pollen analysis were split between females of the first (or spring) generation (n = 22) and females of the second (or summer) generation (n = 28). The methodology for pollen removal, pollen identification and data evaluation follows Müller (2018).

Results

Genetic analyses

Phylogenetic analyses of COI revealed the presence of six taxonomic units (either monophyletic clades or non-monophyletic grades) for Alpine members of the *bicolor*-group (Fig. 1): 1. a clade comprising all specimens of *Andrena montana*. 2. a poorly resolved paraphyletic grade comprising two southern Italian specimens and one Alpine specimen of *A. amieti* sp. n.; this grade is referred to as “*A. amieti* group 1”. 3. a clade comprising most Alpine specimens of *A. amieti* sp. n.; this clade is referred to as “*A. amieti* group 2”. 4. a poorly resolved grade comprising all specimens of *A. allosa*, rendered paraphyletic

by an unclear taxon from Greece, *Andrena* sp1. 5 and 6. two monophyletic clades in *A. bicolor*, referred to as *A. bicolor* clades 1 and 2.

The two clades recovered within *A. bicolor* are those reported in Schmidt et al. (2015); specimens reported therein as *A. montana* in fact belong to *A. amieti* sp. n. group 2; no specimens of *A. montana* were sequenced by Schmidt et al. (2015). For Alpine members of *A. allosa*, *A. amieti* sp. n., *A. bicolor* and *A. montana*, all COI analyses were in agreement with our morphological identifications.

Andrena montana was only distantly related to *A. bicolor*, *A. allosa* and *A. amieti* sp. n. (Fig. 1); genetic distances were on average 6.88% to *A. bicolor* and 9.48% to *A. amieti* sp. n., and maximal genetic distance within *A. montana* was 0.35%. Both groups of *A. amieti* sp. n. were separated by an average distance of 2.41% (range 1.85 - 3.01%), and maximal within-group distances were 0.93 and 0.26% for groups 1 and 2, respectively. These two groups together formed a paraphyletic unit with respect to a clade formed by *A. allosa* and the unclear taxon referred to as *Andrena* sp1 from Greece (bootstrap support 67%; Fig. 1). Maximal genetic distance within Alpine populations of *A. allosa* was 0.48%; the minimal distance between *A. allosa* and the divergent specimen tentatively attributed to *A. allosa* from the Pyrenees (number 1293 on Fig. 1) was 0.73%. Distances between *A. allosa* and *A. amieti* sp. n. ranged from 2.77 to 4.71% (average 3.33%). Maximal distance within *A. bicolor* clade 1 was 0.89%, that within *A. bicolor* clade 2 (with the exception of the divergent



Figure 1. Phylogenetic tree based on maximum likelihood analyses of sequence data of the mitochondrial gene COI; numbers above branches indicate statistical support based on 1000 bootstrap replicates (values below 50 are omitted).

specimen 876 from Greece) 1.44%; the distances between the two clades of *A. bicolor* (except specimen 876) ranged from 3.13 to 4.40% (average 3.68%). Specimen 876 was separated by distances ranging from 1.04 to 1.46% from the rest of clade 2.

Three unclear taxa are additionally revealed in our analyses: *Andrena* sp1 represented by two females (specimens 961 from Mount Olympus, Greece, and 1252 from the Chelmos or Aroania Mountains, Greece) was separated by a minimum distance of 1.82% from *A. allosa* and 4.27% from *A. amieti* sp. n.; *Andrena* sp2, represented by a single female from Lesbos (specimen 1254), was separated by a minimum distance of 5.11% from *A. amieti* sp. n. group 1; and *Andrena* sp3 from the Peloponnese (specimen 928) separated by a distance of 5.71% from *A. bicolor* clade 1. Details on these unclear taxa are provided below.

In the ML analysis of COI, *A. (Ptilandrena) fulvata* and *A. (Ptilandrena) angustior* (Kirby, 1802) were nested within the subgenus *Euandrena*, with a clade composed of *A. ruficrus* Nylander, 1848 and *A. fulvida* Schenck, 1853 sister to a clade composed of these two species of *Ptilandrena* and the rest of *Euandrena* (Fig. 1); *A. amieti*

sp. n., *A. allosa*, *Andrena* sp1 and *Andrena* sp2 formed a well-supported monophyletic group (bootstrap support 96%) that was sister to another well-supported clade (bootstrap support 86%) composed of both clades of *A. bicolor* and *Andrena* sp3 (Fig. 1).

Analyses of opsin (Fig. 2) indicated that all specimens of *A. montana* formed a well-supported clade (bootstrap support 95%) that was sister to another well-supported clade (bootstrap support 86%) composed of both clades of *A. bicolor* and *Andrena* sp3 (Fig. 1). Analyses of opsin (Fig. 2) indicated that all specimens of *A. montana* formed a well-supported clade (bootstrap support 95%) that was sister to another well-supported clade (bootstrap support 86%) composed of both clades of *A. bicolor* and *Andrena* sp3 (Fig. 1). Analyses of opsin (Fig. 2) indicated that all specimens of *A. montana* formed a well-supported clade (bootstrap support 95%) that was sister to another well-supported clade (bootstrap support 86%) composed of both clades of *A. bicolor* and *Andrena* sp3 (Fig. 1). Analyses of opsin (Fig. 2) indicated that all specimens of *A. montana* formed a well-supported clade (bootstrap support 95%) that was sister to another well-supported clade (bootstrap support 86%) composed of both clades of *A. bicolor* and *Andrena* sp3 (Fig. 1).

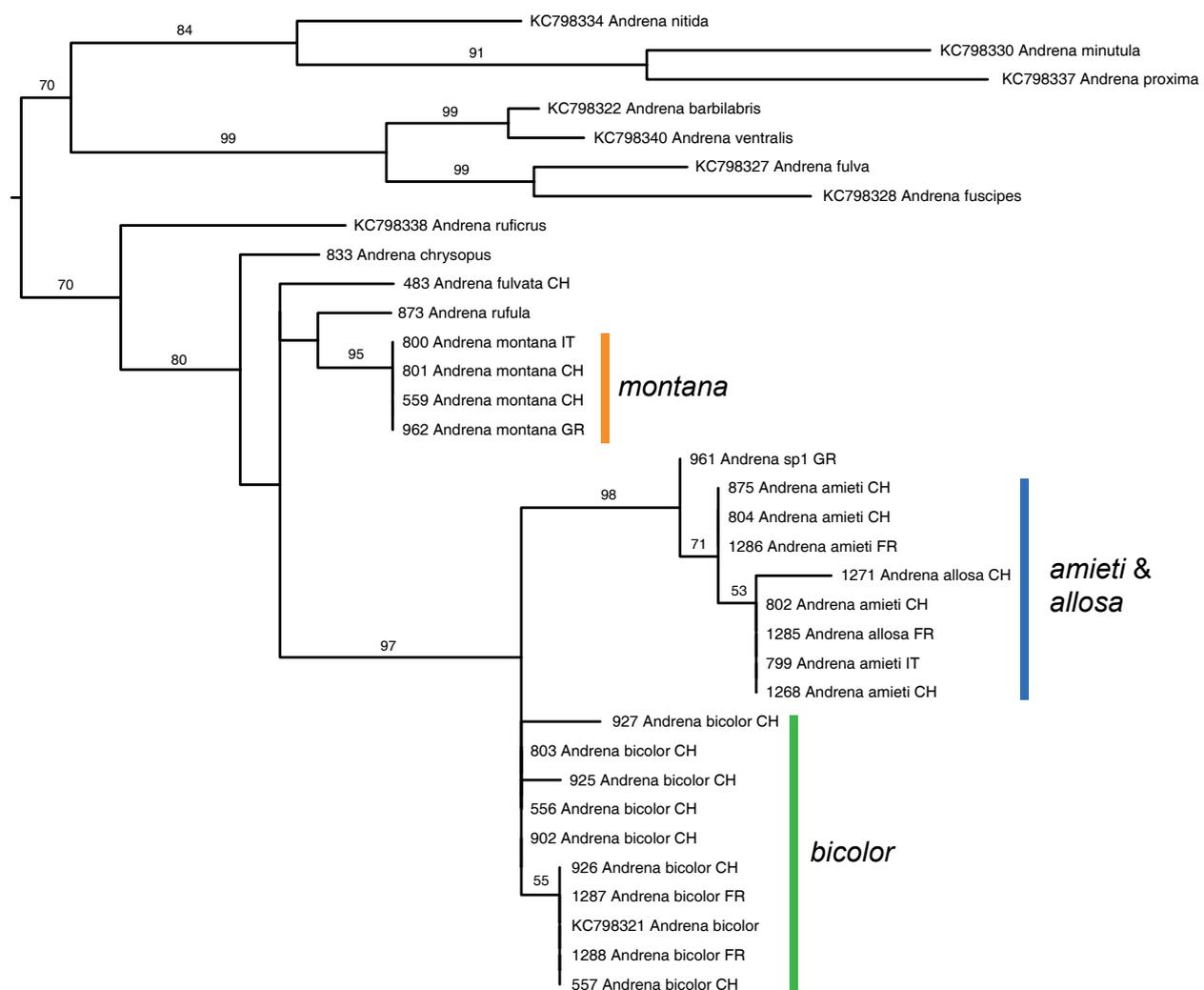


Figure 2. Phylogenetic tree based on maximum likelihood analyses of sequence data of the nuclear gene LW rhodopsin; numbers above branches indicate statistical support based on 1000 bootstrap replicates (values below 50 are omitted).

least *A. ruficrus* (no opsin sequence was available for *A. fulvida*) being sister to a clade including the other members of *Euandrena* and *A. (Ptilandrena) fulvata*.

Species delimitation

Both groups revealed in analyses of COI (Fig. 1) within *Andrena amieti* sp. n. included specimens of the spring and of the summer generations. The lone Alpine specimen of group 1 (specimen 1202) originates from the type locality of *A. amieti* sp. n., thus was found in sympatry with numerous specimens of group 2 and was morphologically not visibly divergent from group 2 specimens. The two southern Italian specimens of *A. amieti* sp. n., both of which were included in group 1, were morphologically slightly distinct from the Alpine specimens (including the lone specimens of group 1 and group 2 specimens). Differences were found in the colour of the vestiture but not in the sculpture. We thus tentatively consider these two groups to represent two mitochondrial lineages within the same biological species. Group 1 lineage appears to be present at low frequency in the Alps since it was represented by only one specimen in the 18 sequenced specimens of *A. amieti* sp. n.

We also consider the Alpine populations of *A. allosa* to constitute a distinct and well-separated species despite lack of monophyly (Fig. 1), lack of divergence with *A. amieti* sp. n. in the nuclear marker (Fig. 2), and the fact that *A. allosa* (and *Andrena* sp1) rendered *A. amieti* sp. n. paraphyletic

in analyses of COI. Alpine populations of *A. allosa* are found in sympatry with *A. amieti* sp. n. but these two taxa are morphologically, phenologically and biologically well-differentiated from one another. In addition, in spite of non-monophyly in COI-based trees, COI sequences of *A. allosa* were unique and diagnostic for this species.

Alpine taxa of the *bicolor*-group

Andrena bicolor is not treated here since this species is widespread and well-known.

Andrena allosa Warncke, 1975

Figs 14, 18, 23, 25, 28, 32, 35, 43, 45, 49, 54.

Andrena allosa Warncke 1975a: 311, ♀, “Allos, Basses-Alpes” [France]. Holotype ♀, OLML, paratypes ♀♀.

Material examined. Type material: holotype female of *A. allosa* (Fig. 18); additional material: 57 females, 2 males from various localities in France and Switzerland (Suppl. material 1).

Distribution. Western Alps from “Alpes Maritimes” in Southeastern France to Western Switzerland (Fig. 3); possibly Northern Spain and Pyrenees (see note below). A mention from northeastern Italy on the map presented

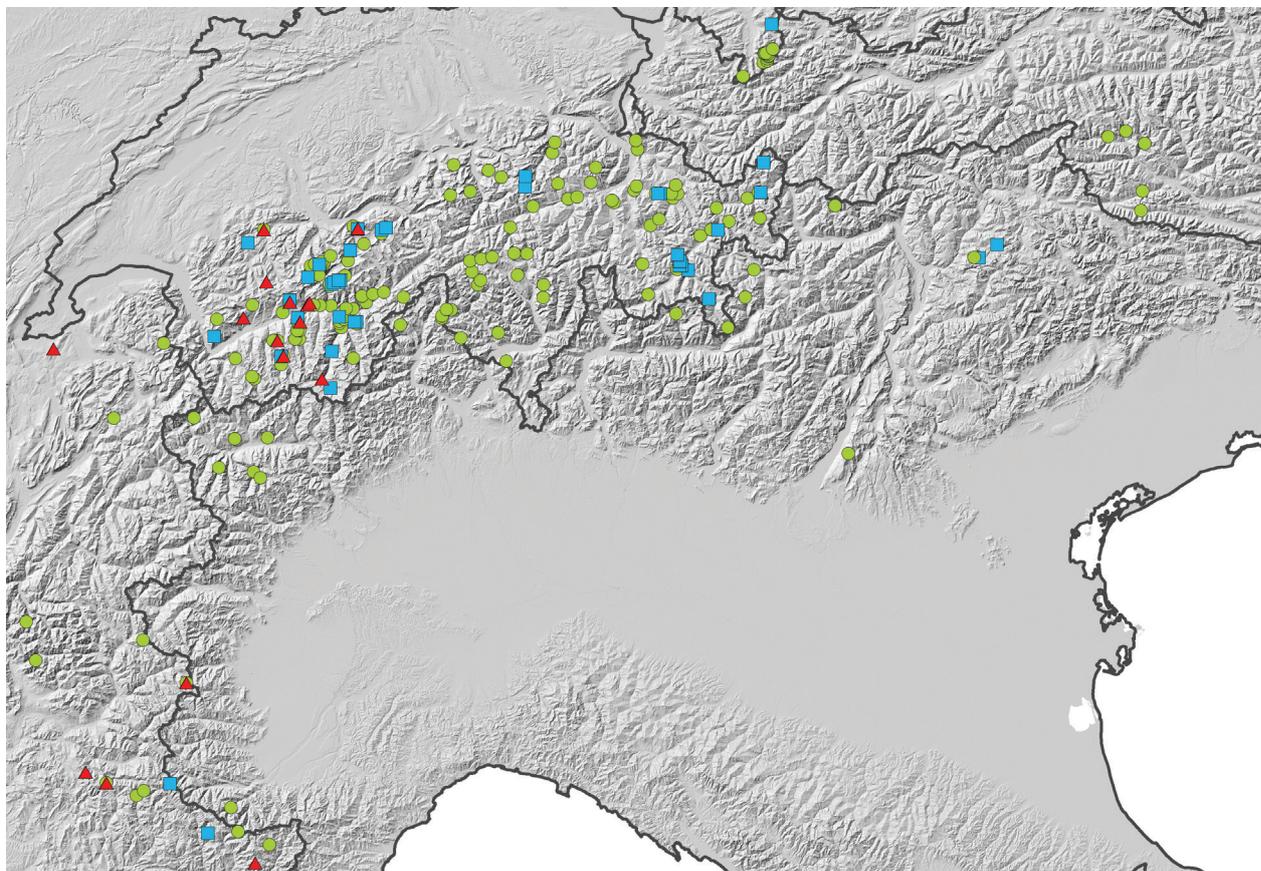


Figure 3. Distribution of *Andrena allosa* (red triangles), *A. amieti* sp. n. (green circles) and *A. montana* (blue squares) in the Alps.

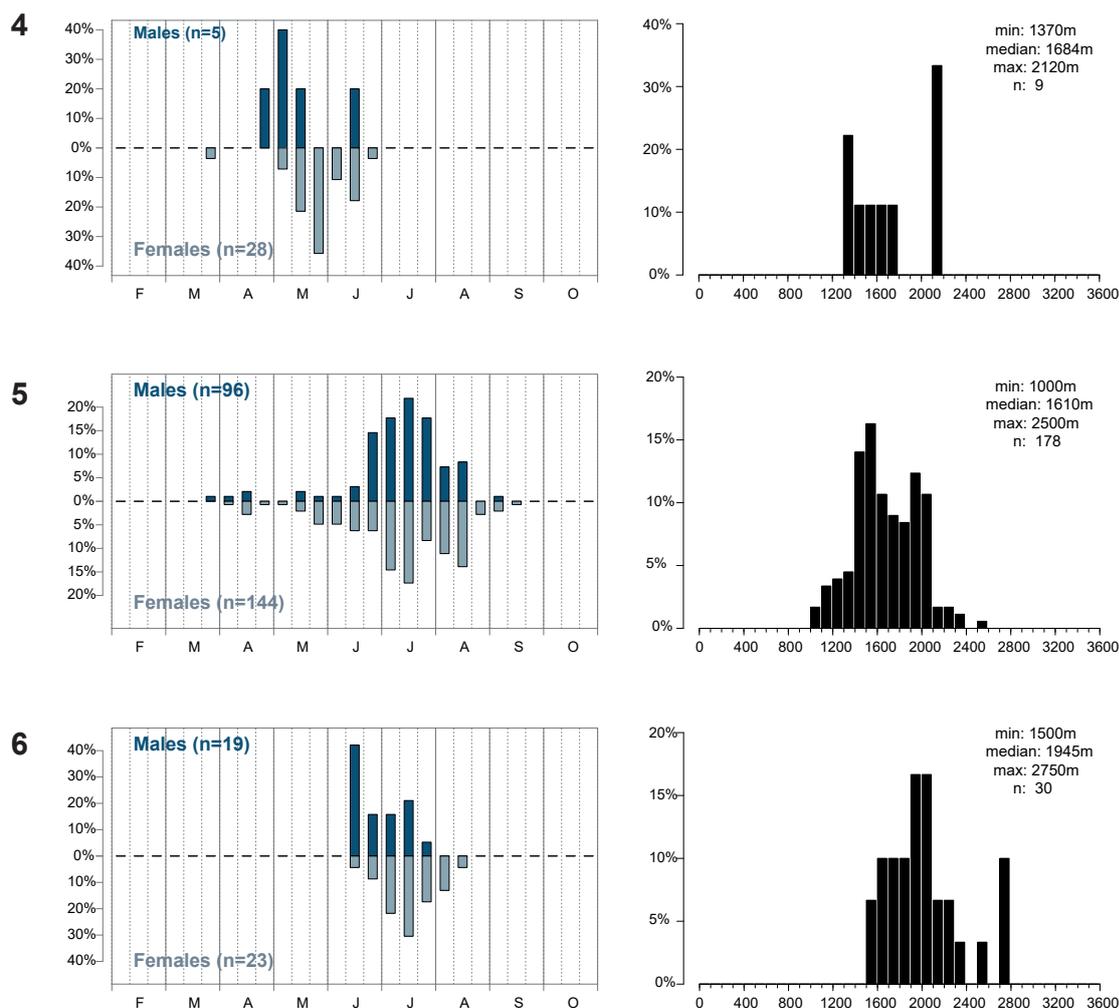
in Gusenleitner and Schwarz (2002: 967) was based on a misidentified specimen of *A. amieti* sp. n. from “Monte Baldo” (OLML).

Notes. We examined one specimen from Spain labelled as follows: “P. Leon Las Senales, 4,875 10.v-12.vi.1967 I & E Yarrow BM 1967-352” (OLML), which agrees with *A. allosa* in the sculpture of the terga and in its vestiture, but which has a shorter malar space, the clypeus that is not flattened apically as well as shorter mouthparts. This specimen appears to be superficially similar to the sequenced female collected at Larrau, in the “Pyrenées Atlantiques” department in France (number 1293; Fig. 1), which was 0.73% divergent from Alpine specimens of *A. allosa*. For now, we consider these two specimens to belong to *A. allosa* in spite of the lack of the most conspicuous diagnostic characters of this species, namely the long malar space and the flattened clypeus.

Phenology. *Andrena allosa* has only one generation per year from the end of March (one isolated record) to the end of June (Fig. 4).

Habitat. We found *Andrena allosa* in various subalpine and alpine grasslands from 1370 m to slightly above the tree line at around 2100 m (Fig. 4), often in close proximity to stands of *Crocus albiflorus* (Fig. 9). Nesting sites are unknown.

Pollen host preferences. *Andrena allosa* collected the pollen on eight plant families (Table 3) but had a pronounced affinity for the pollen of *Crocus* (Iridaceae), which contributed 62.4% to the total pollen grain volume and was recorded in 20 out of 24 scopal loads. This finding is supported by field observations at five different localities in the Swiss and French Alps, where several females of *A. allosa* simultaneously harvested pollen on *Crocus* (Fig. 14). Numerous females were observed to collect the pollen from *Crocus* flowers late in the season, at a time when blooming *Crocus* were restricted to small patches where snow had remained particularly long. Because of the particularly short blooming period of *Crocus*, this observation suggests that *A. allosa* females may locate short-lived stands of *Crocus* throughout their flying season.



Figures 4–6. Phenology (left diagram) and altitudinal distribution (right diagram) of the three Alpine taxa of the *bicolor*-group; only data originating from the Alps were used to produce these graphs. 4, *Andrena allosa*. 5, *A. amieti* sp. n. 6, *A. montana*.

Table 3. Pollen host preferences of the three Alpine taxa of the *bicolor*-group. n = total number of pollen loads, N = number of pollen loads from different localities. Countries: CH = Switzerland, F = France, IT = Italy. Plant families: ACE = Aceraceae, AST = Asteraceae, BRA = Brassicaceae, CAM = Campanulaceae, CAR = Caryophyllaceae, CIS = Cistaceae, CLU = Clusiaceae, COL = Colchicaceae, CRA = Crassulaceae, ERI = Ericaceae, FAB = Fabaceae, GER = Geraniaceae, IRI = Iridaceae, LAM = Lamiaceae, LIL = Liliaceae, ORO = Orobanchaceae, PLA = Plantaginaceae, RAN = Ranunculaceae, ROS = Rosaceae, SAL = Salicaceae. Definitions of bee host ranges after Müller and Kuhlmann (2008).

| Bee species | n | N | Origin (and number) of pollen loads | % pollen grain volume (number of loads) | Preferred host | % pollen grain volume of preferred host | % pure loads of preferred host | % loads with preferred host | Host range |
|--|----|----|-------------------------------------|---|----------------|---|--------------------------------|-----------------------------|---|
| <i>Andrena allosa</i> Warncke 1975 | 24 | 9 | CH (20), F (4) | IRI (<i>Crocus</i>) 62.4% (20), AST (Cichorioideae) 7.2% (11), AST (Asteroideae) 2.7% (1), AST (Carduoideae) 1.1% (1), RAN 6.8% (7), BRA 6.2% (4), ROS (cf. <i>Potentilla</i>) 5.0% (5), ROS (<i>Geum</i>) 0.9% (2), CIS (<i>Helianthemum</i>) 4.9% (3), LIL (<i>Gagea</i>) 1.7% (2), FAB 0.1% (1), unknown 1.0% (1) | <i>Crocus</i> | 62.4% | 16.7% | 83.3% | polylectic (8 plant families) with affinity for <i>Crocus</i> (Iridaceae) |
| <i>Andrena amieti</i> sp. n. (first generation) | 22 | 9 | CH (22) | AST (Cichorioideae) 28.9% (14), AST (Asteroideae) 5.3% (2), ROS (cf. <i>Potentilla</i>) 9.2% (6), ROS (<i>Geum</i>) 2.5% (2), ROS (other) 9.4% (4), SAL (<i>Salix</i>) 15.6% (7), BRA 7.5% (4), RAN 6.0% (3), GER (<i>Geranium</i>) 3.1% (1), ORO (cf. <i>Euphrasia</i>) 2.6% (1), IRI (<i>Crocus</i>) 2.6% (1), CAR 2.2% (3), ACE (<i>Acer</i>) 2.1% (1), CAM 1.8% (2), ERI 0.7% (1), unknown 0.5% (1) | - | - | - | - | polylectic (12 plant families) |
| <i>Andrena amieti</i> sp. n. (second generation) | 28 | 26 | CH (28) | CAM 79.9% (24), GER (<i>Geranium</i>) 7.1% (6), CAR 4.0% (2), BRA 3.7% (3), COL (<i>Colchicum</i>) 1.8% (1), CIS (<i>Helianthemum</i>) 1.5% (3), AST (Cichorioideae) 1.4% (6), RAN 0.3% (1), PLA (<i>Plantago</i>) 0.2% (1), unknown 0.1% (1) | Campanulaceae | 79.9% | 39.3% | 85.7% | polylectic (9 plant families) with strong preference for Campanulaceae |
| <i>Andrena montana</i> , Warncke 1973 | 16 | 15 | CH (14), F (1), IT (1) | CAM 27.1% (6), CIS (<i>Helianthemum</i>) 24.3% (6), CAR 22.0% (7), ORO (cf. <i>Euphrasia</i>) 7.8% (2), AST (Cichorioideae) 5.5% (6), AST (Asteroideae) 0.3% (1), RAN 4.0% (3), PLA (<i>Veronica</i>) 2.7% (1), GER (<i>Geranium</i>) 2.1% (2), CRA 1.5% (2), CLU (<i>Hypericum</i>) 1.1% (1), LAM (Nepetoideae) 1.0% (1), ROS 0.6% (2) | - | - | - | - | polylectic (12 plant families) |

Andrena amieti Praz, Müller & Genoud, sp. n.

<http://zoobank.org/6041B44B-3E38-42A4-8C46-7D8886920ECD>

Figs 11, 12, 15–17, 20, 22, 24, 26, 27, 29–31, 33, 37, 39, 40, 42, 44, 46–48, 50, 52, Suppl. material 2: S1.

Type locality. Switzerland, Canton of Bern, Municipality of Kandersteg, Northern shore of Lake Oeschinen [Oeschinensee] 46.502N 7.723E, 1590m. This locality is located within the Unesco World Heritage site “Swiss Alps Jungfrau-Aletsch” (Fig. 7).

Holotype. Female of second generation, pinned (Figs 15–17, 26). Original labels: 1. “CH Oeschinensee, 621750/150000, 1640m, 6.vii.2014, leg. J. Litman 029472”. 2. “ACU 29472, GBIFCH00103472” [unique identifier with a graphical barcode]. 3. “Voucher specimen for DNA extraction, Sample 1284, Christophe Praz, University of Neuchâtel” [yellow label, printed]. 4. Holotype female *Andrena amieti* sp. n. Praz, Müller & Genoud [red label, printed]. Deposited in MHNN.

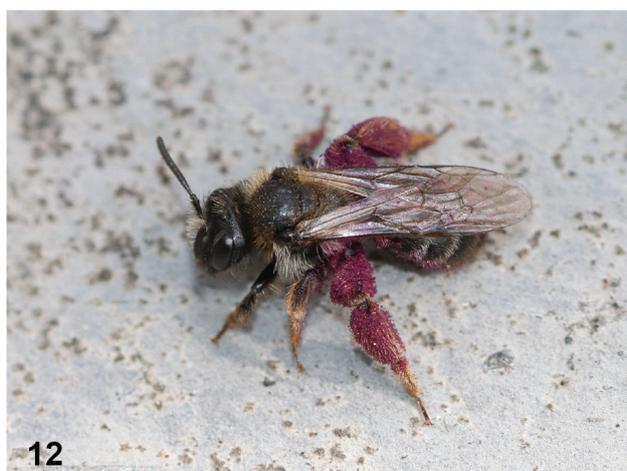
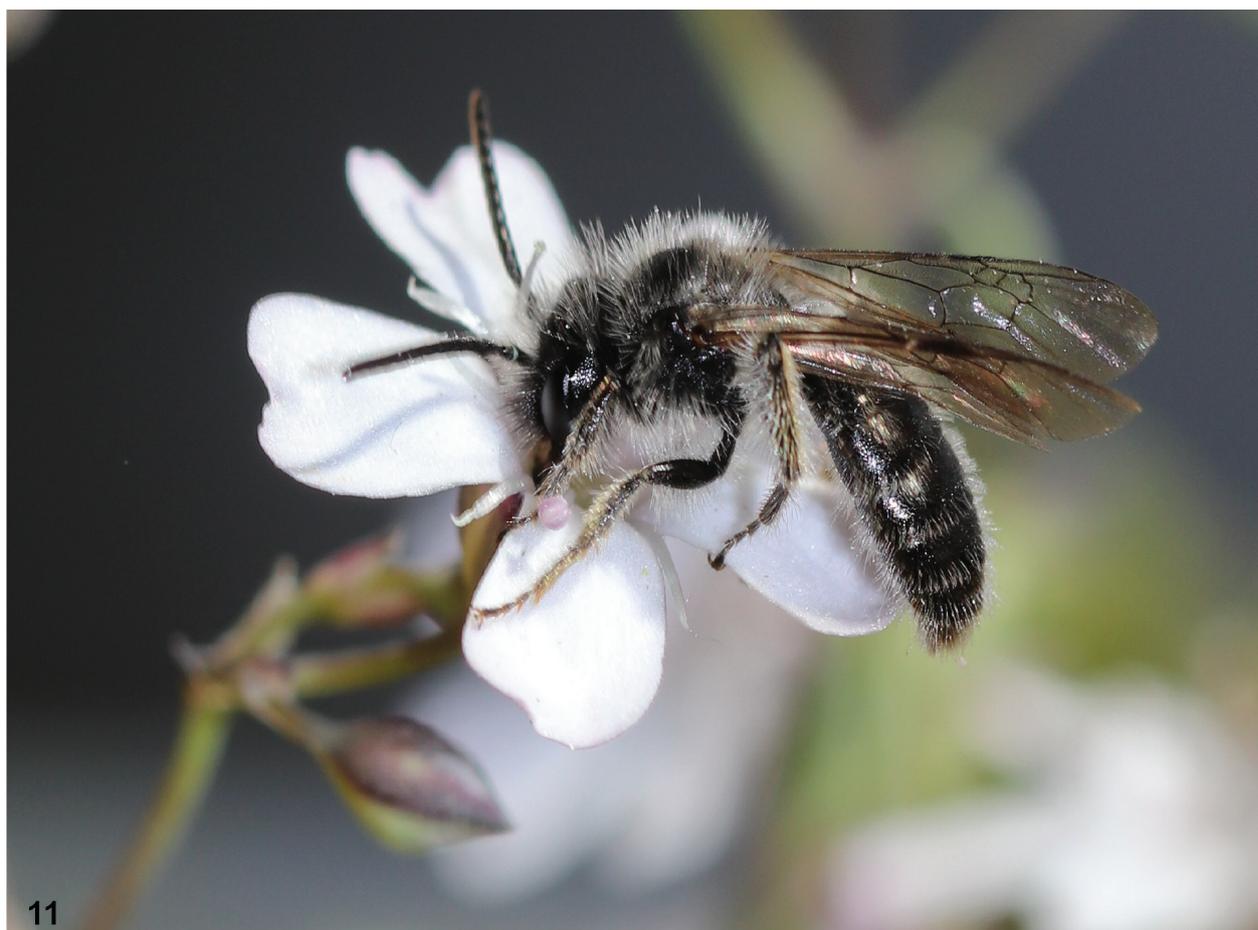
Paratypes. 250 males and 237 females from various localities in France, Switzerland, Italy, Germany and Austria (Suppl. material 1).

Note. The description and diagnosis are based on Alpine populations of this species. Southern Italian specimens (Suppl. material 2: Fig. S1) are slightly divergent in the colour of the vestiture; this variation is presented at the end of the description.

Diagnosis. In the female sex, *Andrena amieti* sp. n. is highly similar to *A. bicolor* and *A. allosa*. All three species can easily be separated from *A. montana* by the dark prepygidial and pygidial fimbria (Fig. 37) (orange-brown in *A. montana*; Fig. 38) and by the presence of at least some dark hairs laterally on the mesosoma (Fig. 15) (hairs on lateral parts of mesosoma entirely brownish-grey in *A. montana*). The female differs from *A. bicolor* in the presence of comparatively long dark hairs on the mesonotum (Fig. 26), usually also on the scutellum; these dark hairs are intermixed with longer, orange brown or grey brown hairs, and many of them are longer than half the length of the light brown hairs. In *A. bicolor*, there are either no dark hairs on the mesonotum and scutellum, or only very short dark hairs on the mesonotum, their length being visibly smaller than half the length of the light brown hairs. In addition, there are in most cases numerous dark hairs on the propodeum in *A. amieti* sp. n. (all hairs usually



Figures 7–10. Habitats of *Andrena amieti* sp. n., *A. allosa* and *A. montana*. **7**, the lake of Oeschinen in the Bernese Alps in Switzerland, UNESCO world heritage site and type locality of *A. amieti* sp. n. (Picture J. Litman). **8**, nesting site of *A. amieti* sp. n. near Disentis in the Grisons, Switzerland (Picture A. Müller). **9**, habitat of *A. allosa* near Chandolin in the Valais, Switzerland, at a time where the females were already actively collecting pollen (Picture S. Giriens, www.swisswildbees.ch). **10**, habitat of *A. montana* near Zermatt in the Valais, Switzerland (Picture S. Giriens, www.swisswildbees.ch).



Figures 11–14. Habitus of *Andrena amieti* sp. n., *A. montana* and *A. allosa*. **11**, *A. amieti* sp. n. male, summer generation, on Caryophyllaceae spec. (Picture S. Giriens, www.swisswildbees.ch). **12**, *A. amieti* sp. n. female, summer generation, with Campanulaceae pollen load (Picture S. Giriens, www.swisswildbees.ch). **13**, *A. montana* female on Caryophyllaceae spec. (Picture D. Bénon, www.swisswildbees.ch). **14**, *A. allosa* female on *Crocus albiflorus* (Picture D. Genoud).

light in *A. bicolor*), the light hairs on T1–T4 are snow white (Fig. 33) (always yellowish-white in fresh specimens of *A. bicolor*; Fig. 34), and some hairs between the antenna are always grey-brown (Fig. 16) (hairs between the antennae commonly entirely dark in *A. bicolor*, although sometimes also grey-brown).

The female of *A. amieti* sp. n. differs from that of *A. allosa* by the shorter clypeus with convex preapical area (Fig. 17) (clypeus produced apically, preapical zone flattened medially in *A. allosa*; Fig. 18) and the short malar space (Fig. 22) (malar space as long as the basal width of antennal segment 3 in *A. allosa*; Fig. 23); in addition,

A. allosa has shagreened, more sparsely punctate tergal discs (especially medially on T2), the margin of T2 hardly impressed medially (Fig. 35), and comparatively longer mouthparts (compare Figs 24, 25).

The male of *A. amieti* sp. n. (Figs 39, 42) is similar to that of *A. bicolor*, *A. allosa* and *A. montana*, and superficially also to that of *A. ruficrus*. Separation from the male of *A. bicolor* may be difficult in worn specimens. In *A. amieti* sp. n., the light vestiture on mesosoma and metasoma is greyish-white without yellowish hue (Figs 39, 42, 50) (hairs on dorsal side of mesosoma and first terga with yellowish hue in fresh specimens of *A. bicolor*; Fig. 51). The vestiture is comparatively long on the terga (Fig. 50), in particular medially on T4, the apical fringe of hairs is longer than the tergal margin (in *A. bicolor* fringe of hairs as long as or shorter than tergal margin; Fig. 51). The disc of T4 has at most a few isolated, dark hairs (disc of T4 with numerous dark hairs in *A. bicolor*). Lastly, the facial vestiture (Figs 39, 42) is on average lighter than in *A. bicolor*, at least some grey hairs are found between and around the antennal sockets (in *A. bicolor* frequently entirely dark).

The male of *A. amieti* sp. n. is highly similar to that of *A. allosa*, especially in the first generation (*A. allosa* has only one generation). Differences are summarized in the key: the maxillary palps are comparatively slightly shorter in *A. amieti* sp. n. (Fig. 44) than in *A. allosa* (Fig. 45), and in *A. amieti* sp. n. the head is comparatively short and broad with little protruding clypeus (Fig. 42) (head comparatively long with protruding clypeus in *A. allosa*; Fig. 43). In addition, the first recurrent vein usually enters the second submarginal cell in or near its middle and the second submarginal cell is subquadrate or longer than broad (Fig. 48) (in *A. allosa*, the first recurrent vein enters the second submarginal cell in its basal half, second submarginal cell broader than long; Fig. 49).

In its vestiture, the male of *A. amieti* sp. n. is similar to that of *A. montana*; the best diagnostic characters are the shape of the labral appendix, which is wider than long in *A. amieti* sp. n. (Fig. 40) and as long as apically wide in *A. montana* (Fig. 41), and the width of the penis valves and of the gonostylus: the gonostylus is more slender and the penis valves broader in *A. montana* (Fig. 61) than in *A. amieti* sp. n. (Fig. 52), although for these genitalic characters direct comparison with reference material is necessary. In addition, the mesonotum is nearly entirely shagreened in males of *A. montana*, while it is at least partly shiny in males of the second generation of *A. amieti* sp. n. (Fig. 47). Lastly, males of *A. amieti* sp. n. are also superficially similar to those of *A. ruficrus*, which usually have the apex of the hind tibia partly orange and the clypeal vestiture entirely show-white; in addition, the penis valves are broader basally in *A. ruficrus* (Fig. 60) than in *A. amieti* sp. n. (Fig. 52).

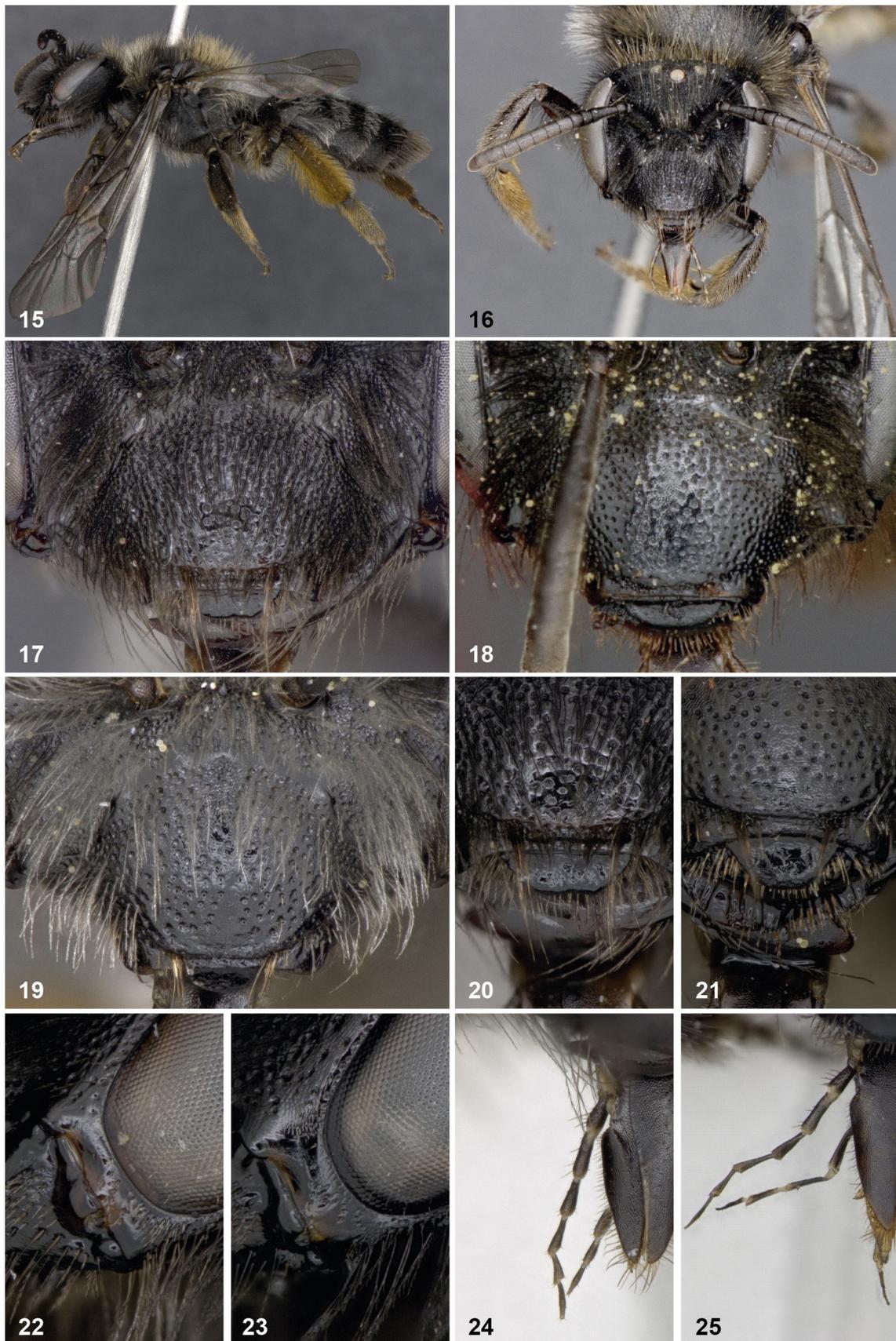
Description. Female. Body size and proportions: Very similar to *A. bicolor*. Body length approximately 9mm,

slightly smaller on average than *A. allosa* (body length approximately 9.5mm-10mm). Head slightly broader than long (Fig. 16), clypeus broader than long. Malar space short (Fig. 22), as in *A. bicolor*, shorter than in *A. allosa* (Fig. 23), length without impressed area at most equal to half the base of third antennal segment. Gena slightly broader than compound eye in lateral view. Interocellar distance approximately 2 times diameter of lateral ocellus. Ocelloccipital distance approximately 0.9 times diameter of lateral ocellus. Third antennal segment longer than fourth and fifth together, the latter two broader than long, segments 6–11 subquadrate, 12 longer than broad (Fig. 16). Labral process trapezoidal, its apical width larger than its length (Fig. 20), comparatively broader than in *A. montana* (Fig. 21). Mouthparts (Fig. 24) as in *A. bicolor*, shorter than in *A. allosa* (Fig. 25), in particular segment 4 of maxillary palpus only three times as long as apically broad (in *A. allosa* at least four times as long as apically broad), and segment 2 of labial palpus hardly longer than broad (in *A. allosa* at least twice as long than broad).

Wing venation: As in *A. bicolor*; the first recurrent vein enters the second submarginal cell in its middle or nearly so, and second submarginal cell subquadrate or longer than broad (Fig. 27) (in *A. allosa*, the first recurrent vein usually enters the second submarginal cell in its basal half; second submarginal cell broader than long; Fig. 28).

Integument colour: As in *A. bicolor*, integument black or dark brown, including flagellum and tegulae, apical margin of T1–T4 slightly lighter, tarsal segments 2–4 dark orange-brown, tarsal claws weakly ferruginous, hind tibial spurs light brown. Wing venation (including stigma; Fig. 27) predominantly brown as in *A. bicolor*.

Vestiture: Entire body vestiture made of simple to weakly branched hairs, more strongly branched hairs are present between antennal sockets, plumose hairs in propodeal corbicular, floculus, and prepygidial and pygidial fimbria. Hairs on head predominantly dark brown (Fig. 16), always with grey-brown to greyish-white hairs between antennal sockets (sometimes also around antennal sockets) and medially on vertex (in *A. bicolor* hairs on head often entirely dark); extent of grey vestiture on face variable but vestiture on average lighter than in *A. bicolor*. Hairs on mesosoma predominantly grey-brown, including on lateral and ventral sides (Fig. 15); hairs never bright orange-brown as in fresh specimens of *A. bicolor*; mesonotum and usually also scutellum with intermixed long, grey-brown hairs and short, dark brown hairs (Fig. 26) (in *A. bicolor* either no dark hairs, or dark hairs shorter; see above); tegulae covered with short dark hairs (Fig. 26) (in *A. bicolor* light brown hairs); propodeum with intermixed grey and dark brown hairs (in *A. bicolor* usually without dark brown hairs); on lateral side of mesosoma, hairs on average lighter than in *A. bicolor*, sometimes entirely grey-brown (Fig. 15), dark hairs mostly with grey tips except sometimes under tegula, where hairs can be entirely



Figures 15–25. Structure of female of *Andrena amieti* sp. n., *A. allosa* and *A. montana*. **15**, holotype of *A. amieti* sp. n. in lateral view. **16**, holotype of *A. amieti* sp. n. in frontal view. **17**, clypeus of holotype of *A. amieti* sp. n. **18**, clypeus of holotype of *A. allosa*. **19**, clypeus of *A. montana*. **20**, labrum of *A. amieti* sp. n. **21**, labrum of *A. montana*. **22**, malar space of *A. amieti* sp. n. **23**, malar space of *A. allosa*. **24**, section of mouthparts of *A. amieti* sp. n. **25**, section of mouthparts of *A. allosa*.

dark; ventral part of mesosoma with grey-brown hairs becoming lighter apically. Leg vestiture nearly as in *A. bicolor*, flocculus grey-brown to whitish grey, on average lighter than in *A. bicolor*; scopa yellowish brown, hairs on basitarsi 2 and 3 usually yellowish brown (in *A. bicolor* usually dark brown). T1 mostly covered with snow-white hairs (in *A. bicolor* yellowish-white), only a few isolated, dark hairs on anterior part of disc and numerous dark hairs on vertical anterior part of tergum; T2 and T3 covered with long, snow white hairs (Fig. 33) (in *A. bicolor* hairs yellowish-white, Fig. 34 and usually slightly shorter medially) forming very loose apical fringes, not hiding cuticula, discs with short, erect dark hairs, more numerous on T3 than T2; T4 covered with long, dark hairs forming loose apical fringe and with short, erect dark hairs on disc; prepygidial and pygidial fimbriae dark brown (Fig. 37). Sterna covered with dark, erect hairs, apical margin with apical fringes of dark, plumose hairs. In first generation, vestiture on average longer than in second, especially on clypeus, mesosoma and basal terga.

Sculpture: The variation observed in the sculpture of *A. amieti* sp. n. represents the middle range of the much wider sculptural variation observed in *A. bicolor*. **Head:** Facial fovea narrow (Fig. 16), as in *A. bicolor*; clypeus (Fig. 17) densely punctured, more sparsely punctured laterally and basally than apically, there with shiny interspaces that are on average one puncture diameter wide, often with longitudinal irregularities (more so than in *A. bicolor*); apical part of clypeus without flat area as observed in *A. allosa*; frons unpunctured with numerous longitudinal ridges, as in *A. bicolor*. **Mesosoma:** mesonotum (Figs 29, 30) with well-visible punctures, interspaces nearly entirely dull and on average 3–4 puncture diameters in first generation (Fig. 29), shiny or silk-shiny and 2–3 puncture diameters in second generation (Fig. 30). Propodeum (Fig. 31) as in *A. bicolor*, propodeal triangle without punctures, dull, finely sculptured, anteriorly with a few longitudinal wrinkles, wrinkles less visible than in *A. allosa* (Fig. 32). **Metasoma:** terga sparsely punctured (Fig. 33), within variation observed in *A. bicolor* (Fig. 34), on average slightly more shagreened, especially apical tergal margin of T2 (compare specimens of same generation); first generation: disc of T1 very sparsely punctured, that of T2 sparsely punctured with interspaces equal to 4–5 puncture diameters; surface of tergal discs mostly shagreened to silk shiny, apical margins shagreened, mostly unpunctured, that of T2 weakly impressed medially, as in *A. bicolor* but more so than in *A. allosa*; second generation (Fig. 33): punctation on average denser (interspaces equal to 3–4 puncture diameters on disc of T1, and 2–3 puncture diameters on disc of T2; sculpture of surface on average shinier, although usually still weakly shagreened; apical margins as in first generation, usually at least slightly shagreened.

Male: Body size and proportions: Body length approximately 7–8mm, similar to *A. bicolor*; the two males of *A. allosa* examined were 8–8.5mm long. Head slight-

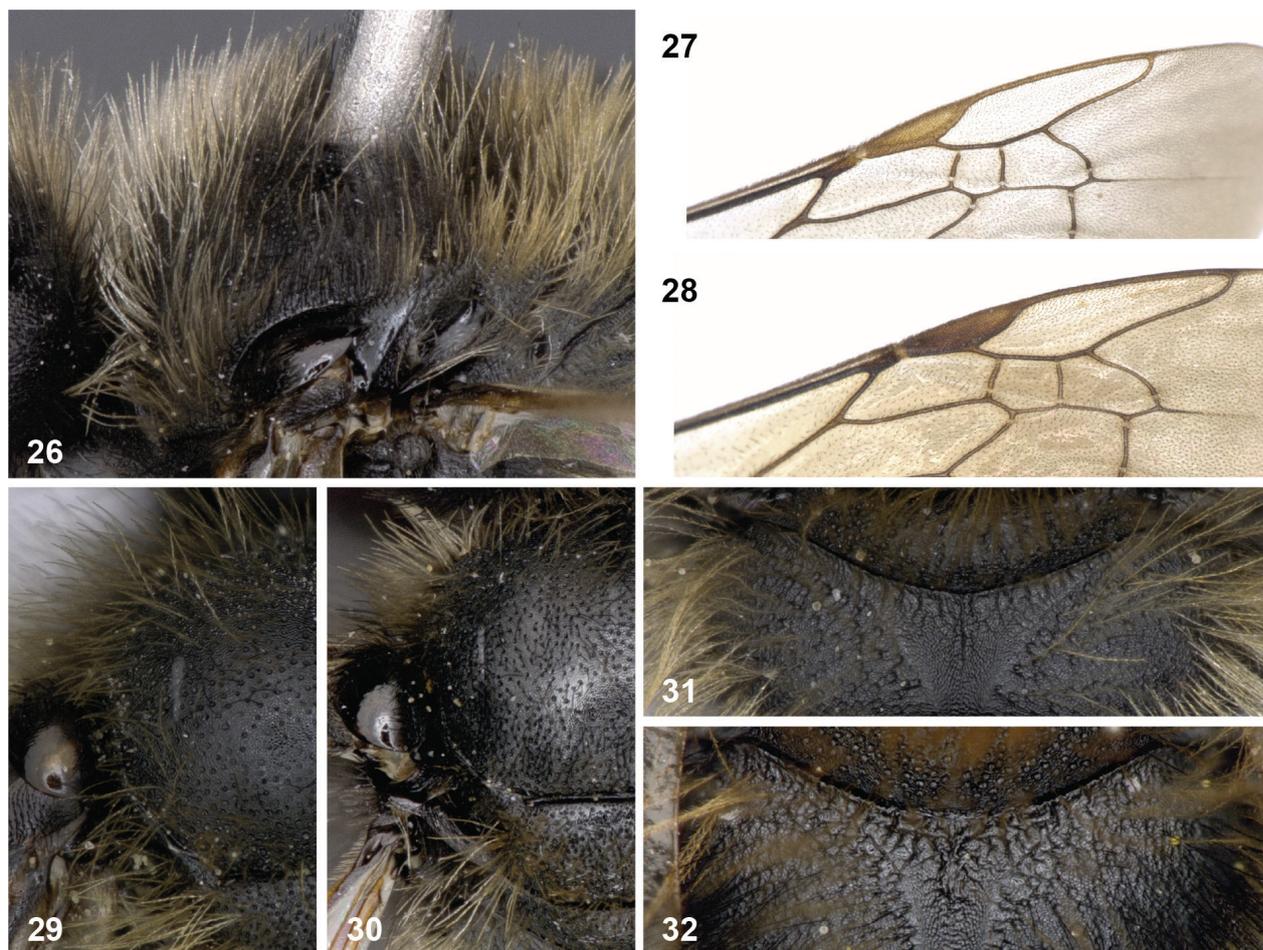
ly broader than long, clypeus little protruding apically (Fig. 42) (in *A. allosa*, clypeus slightly more strongly protruding; Fig. 43); gena slightly broader than compound eye in lateral view; interocellar distance approximately 2.5–3 times diameter of lateral ocellus; ocelloccipital distance approximately equal to diameter of lateral ocellus; length of third antennal segment approximately 1.3 times length of fourth antennal segment (in *A. bicolor* third antennal segment often shorter, 1.1 times length of fourth segment), fourth slightly shorter than broad or subquadrate, segments 5–9 slightly longer than broad, 10–12 subquadrate, 13 longer than broad. Labral process trapezoidal, its apical margin slightly emarginate (Fig. 40) (in *A. montana*, process longer; Fig. 41). Mouthparts (Fig. 44) as in *A. bicolor*, slightly shorter than in *A. allosa* (Fig. 45) although differences not as striking as in female; segment 4 of maxillary palpus only three times as long as apically broad (in *A. allosa* approximately four times as long as apically broad).

Wing venation: As in female, the first recurrent vein usually enters the second submarginal cell in or near its middle; second submarginal cell subquadrate or longer than broad (Fig. 48) (in two examined specimens of *A. allosa*, the first recurrent vein enters the second submarginal cell in its basal half, second submarginal cell broader than long; Fig. 49).

Integument colour: As in female.

Vestiture: Entire body vestiture grey-white without yellowish hue (Figs 39, 42, 50) (light hairs always with yellowish hue in fresh specimens of *A. bicolor*; this yellowish hue is not apparent in worn specimens), except as follows: facial vestiture varying from predominantly dark (as on Fig. 42), with only a few grey hairs between and around antennal sockets, on scape and on vertex medially, to nearly predominantly grey-white, with dark hairs restricted to apical and lateral parts of clypeus (facial vestiture often entirely dark in *A. bicolor*; clypeus entirely covered by snow-white vestiture in *A. ruficrus*, and by light brownish-grey hairs and dark hairs laterally in *A. montana*), area along inner margin of compound eye, and lateral parts of head. Mesonotum with a few isolated dark hairs among longer, grey hairs (Figs 46, 47); lateral side of mesosoma with a few isolated dark hairs below tegula. Propodeum with intermixed dark and light grey hairs. Legs mostly covered with grey-white hairs, hairs on external surface of all tibiae yellowish and on ventral surface of all tarsal segments yellowish-white. Surface of T1 with a few isolated, dark hairs on anterior, vertical part. Vestiture of T2–T4 snow white (Fig. 50) without dark hairs or at most with a few isolated dark hairs basally on disc of T4 (in *A. bicolor*, vestiture of T1–T4 yellowish white in fresh specimens, Fig. 51; disc of T4 predominantly covered with dark hairs, and with numerous dark hairs basally on disc of T3; dark hairs may appear light grey in worn specimens).

Sculpture: As for female, the sculpture of the male of *A. amieti* sp. n. is similar to that of *A. bicolor*,



Figures 26–32. Structure of female of *Andrena amieti* sp. n. and *A. allosa*. **26**, vestiture of dorsal side of mesosoma of holotype of *A. amieti* sp. n. **27**, section of right forewing of *A. amieti* sp. n. **28**, section of right forewing of *A. allosa*. **29**, dorsal view of mesosoma of spring generation of *A. amieti* sp. n. **30**, dorsal view of mesosoma of summer generation of *A. amieti* sp. n. **31**, propodeum of *A. amieti* sp. n. **32**, propodeum of *A. allosa*.

which is particularly variable. *Head*: clypeus densely punctured, interspace shiny or weakly shagreened, mostly narrower than one puncture diameter (Fig. 42); frons nearly unpunctured with numerous longitudinal ridges. *Mesosoma*: mesonotum in first generation (Fig. 46) entirely shagreened with sparse and little visible punctures, interspaces equal to 4–5 puncture diameters (similar to *A. allosa* or to first generation of *A. bicolor*), in second generation (Fig. 47) nearly always with shiny area medially, punctation well-visible, on average slightly sparser than in second generation of *A. bicolor*, interspaces commonly over 3–4 puncture diameters (in *A. bicolor* rarely over 3 puncture diameters); propodeum as in female. *Mesosoma*: terga (Fig. 50) similar to *A. bicolor*, on average slightly more sparsely punctate and more shagreened, but within variation observed in *A. bicolor*; punctures fine (slightly coarser on T1), interspaces often more than 5 times puncture diameters. Tergal margins usually partly shagreened (Fig. 50) (in second generation of *A. bicolor* often entirely shiny). Structure of S8 not visibly different from *A. bicolor*.

Genitalia: As on Fig. 52, very similar to *Andrena bicolor* (Fig. 53), dorsal lobe of gonocoxite weakly developed, gonostylus simple, regularly spatulate, external margin regularly rounded, penis valves narrow, hardly broadened basally.

Geographic variation. In females from Southern Italy (Suppl. material 2: Fig. S1), the vestiture is nearly entirely grey-white, including on all parts of the mesosoma (isolated dark hairs on mesonotum and scutellum excepted), with no brownish hue, in contrast to Alpine specimens where the mesosomal vestiture is predominantly brown. No difference is found in vestiture colour in male specimens.

Etymology. This species is named in honor of Felix Amiet, who has greatly contributed to our understanding of Central European bees, including the four species presented here.

Additional comments. There are numerous species-group names currently treated as junior synonyms

of *A. bicolor* (Gusenleitner and Schwarz 2002). We carefully examined the description of each of these names if their type locality was located within the known range of *A. amieti* sp. n. The description of none of these species-group names points to *A. amieti* sp. n. (see also comments on *A. croatica* Friese, 1887 stat. rev., below). *A. gwynana* var. *testacea* Dalla Torre, 1877, described from Seefeld (Tyrol) and whose type material is presumably lost, originates from a region where *A. amieti* sp. n. might occur. The very brief original description merely mentions the “testaceous” hind tibia, which corresponds neither to *A. bicolor* nor to *A. amieti* sp. n., and which does not point to a differential character between these two species. For these reasons, we keep this taxon as a junior synonym of *A. bicolor*. Lastly, several taxa of *Euandrena* have been described from Ukraine, Central Asia or Russia (Gusenleitner and Schwarz 2002). Given that *A. amieti* sp. n. appears to be restricted to the Alps, the Apennines and the Pyrenees, we consider it unlikely that any of these taxa will turn out to be conspecific with *A. amieti* sp. n.

Distribution. Alps from Southern France to East Tyrol (Austria), including most of the Swiss Alps and the Allgäu Alps in Southern Germany, as well as the Italian Alps (Fig. 3); Monte Pollino in Southern Italy; one isolated record from the Pyrenees.

Phenology. According to our data and field observations, *Andrena amieti* sp. n. has two generations per year (Fig. 5). The earliest males of the first generation were collected at the end of March, and some worn females collected during the first half of June probably belonged to the first generation (thus some females collected in June and seemingly belonging to the second “peak” on Fig. 5 were in fact most likely of the first generation). The second generation is active from mid-June to the first half of September. While the first generation was underrepresented in the examined material, intensive surveys in three Swiss localities where the species was found to be abundant consistently revealed the presence of two generations, with several females observed from May to early June, then numerous fresh males and fresh females observed from the end of June to mid-August.

Habitat. *Andrena amieti* sp. n. has been found from an altitude of approximately 1000m up to slightly above the tree line at around 2300m in the Valais; one isolated record is from an elevation of 2500m (Fig. 5). Foraging females were observed in various habitats, often in clear forests but also in meadows, subalpine grasslands and scree slopes. Patrolling males, probably indicating the location of nesting aggregations, were regularly found on disturbed terrains with little vegetation, such as dry river beds, scree slopes or avalanche corridors (Fig. 8). In these presumed nesting sites, the soil was not particularly sandy, but rather consisting of gravel mixed with sand or clay.

Pollen host preferences. *Andrena amieti* sp. n. collected the pollen from flowers belonging to 15 plant families (Table 3). Females of the spring generation exploited a wide spectrum of pollen hosts, among which Asteraceae, Rosaceae and *Salix* (Salicaceae) predominated; pollen of these three plant taxa represented 70.9% of the total pollen grain volume. In striking contrast, females of the summer generation exhibited a strong preference for the pollen of Campanulaceae, which contributed 79.9% to the total pollen grain volume and was recorded in 24 out of 28 scopal loads (Fig. 12). Field observations revealed that flowers of both *Campanula* and *Phyteuma* serve as pollen hosts among the Campanulaceae.

Andrena montana Warncke 1973

Figs 13, 19, 21, 36, 38, 41, 61.

Andrena montana Warncke 1973: 33, ♀♂, “Bozen/Italien [Bolzano, Italy]” Holotype ♀, OLML, paratypes ♀♀♂♂.

Material examined. Holotype female (OLML); additional material: 31 males, 29 females originating from France, Switzerland, Germany, Italy, Greece and Macedonia (Suppl. material 1).

Distribution. Alps from “Alpes Maritimes” in Southeastern France to Switzerland, Northern Italy and southern Germany (Fig. 3); Apennines; Balkans (Greece, Macedonia). The species is expected to also occur in the western Austrian alps. One mention from Albania (Warncke 1973) in fact refers to *A. ruficrus*.

Phenology. *Andrena montana* has only one generation per year from mid-June to August (Fig. 6).

Habitat. *Andrena montana* has been found from 1500 m to 2750m (Fig. 6) and is more often observed above the tree line than any other Alpine species of the *bicolor*-group. Pollen-collecting females were observed in flower-rich alpine grasslands (Fig. 10). A few males were observed to patrol on areas with bare soil sparsely covered by vegetation; the soil was not particularly sandy but rather made of gravel mixed with sand and clay.

Pollen host preferences. *Andrena montana* collected the pollen on 12 plant families (Table 3). Flowers of the Campanulaceae, *Helianthemum* (Cistaceae) and Caryophyllaceae were the most important pollen hosts (Fig. 13); their pollen contributed 73.4% to the total pollen volume, whereas the pollen of all other taxa was represented by less than 10% each.

Identification key to Central European species of *Andrena* subgenus *Euandrena*

Unique characters in bold; non-unique characters in regular font; characters given in order of importance. This

key will work in most of Continental Europe except in the Pyrenees, in Spain and in the Balkans.

Females

***Euandrena*:** body-length 9–11 mm. Fovea comparatively narrow, its broadest width less than 1.5 times broadest width of scape (slightly broader in *A. fulvida*). Third antennal segment longer than segments 4 and 5 together. Metasoma-

mal integument predominantly dark-brown. Mesonotal vestiture consisting of long, simple or shortly branched hairs. Flocculus neither particularly long nor bent apically. Propodeal triangle at most weakly wrinkled, coarsely wrinkled anteriorly in *A. fulvida* and in *A. allosa* (Fig. 32); propodeum laterally with little-developed corbicula. Hind femur without row of thorn-like projections. Dorsolateral angle of pronotum without elevated transverse carina.

- 1 **Head clearly longer than broad. T2-T4 with dense apical fringes of snow-white hairs, fringes continuous on T3 and T4 and hiding cuticula.** Head vestiture entirely light grey. Hind basitarsus ferruginous. Surface of metasomal terga smooth, not shagreened. Prepygidial and pygidial fimbria orange to brown-orange. *Andrena chrysopus*
- Head at most as long as broad. If present on T2-T4, apical fringes of hairs not hiding cuticula or interrupted medially. Combination of other characters different. 2
- 2 **Hind basitarsus and hind tibia ferruginous.** Terga regularly finely shagreened, silk-shiny, with comparatively fine, little visible punctures. Fovea comparatively short and broad, little narrowed inferiorly, 1.5 times broader than broadest width of flagellum. Facial vestiture brown medially, dark brown laterally. *Andrena ruficrus*
- Hind basitarsus and tibia dark brown. Terga shagreened or shiny with often distinct punctures. Fovea usually longer and often narrower (not clearly so in *A. fulvida*). Vestiture variable. 3
- 3 **Clypeus shiny, with particularly coarse punctures and slightly elevated, impunctate longitudinal line** (Fig. S2). Entire body vestiture brown, except dark on T6, sterna, tibia and tarsi, as well as a few isolated dark hairs on face along compound eye. Mesonotum and terga shagreened, the latter with isolated, little visible punctures. *Andrena symphyti*
- Clypeus shiny or shagreened, without particularly coarse punctures, with or without impunctate longitudinal line, but longitudinal line never elevated. Mesosomal vestiture variable. Sculpture of mesonotum and terga variable. 4
- 4 **Facial vestiture nearly entirely brown, with at most a few isolated dark hairs along compound eye and vestiture on mesonotum brown intermixed with numerous, slightly shorter dark hairs. Anterior area of propodeum comparatively coarsely wrinkled** (as on Fig. 32). Surface of T1 shiny or only weakly shagreened, **medially between disc and depression with a few longitudinal irregularities [as in the distantly related *A. (Melandrena) vaga*]**. Vestiture on lateral side of mesosoma entirely brown, without black hairs. T2 and T3 fringed laterally with weak, brownish-white apical fasciae. Mesonotum and scutellum mat, the former comparatively densely punctate. Terga regularly, finely punctate, shiny or only slightly shagreened. *Andrena fulvida*
- Vestiture of head and mesonotum different, never with the combination of predominantly brown facial hairs and intermixed brown and dark hairs on mesonotum. Propodeum more finely wrinkled (except in *A. allosa*). Surface of T1 shagreened or shiny, without longitudinal irregularities. Vestiture and sculpture variable. 5
- 5 **Integument of apical margin of T1-T4 ivory-colored** (Figs S3, S4), basal part of tergal margins weakly translucent. Entire body vestiture brown, except facial vestiture, which is predominantly dark with more or less brown hairs medially, a few isolated, dark hairs on mesonotum and some dark hairs on T5 and T6. 6
- **Integument of apical margin of T1-T4 not ivory-colored**, at most narrowly light brown apically; basal part of tergal margins not translucent; vestiture variable. 7
- 6 **Tergal discs on average more coarsely and densely punctate, interspaces on disc of T2 equal to 1.5-2 puncture diameters** (Fig. S3). Surface of tergal discs strongly convex, apical margin strongly impressed. Fovea comparatively long, starting below middle of antennal sockets. *Andrena granulosa*
- Tergal discs on average more finely and sparsely punctate, interspaces on disc of T2 equal to 3-4 puncture diameters or more (Fig. S4). Surface of tergal discs nearly flat, apical margins less strongly impressed. Fovea comparatively short, starting well-above middle of antennal sockets. *Andrena vulpecula*
- 7 **Vestiture of mesosoma entirely brown-orange** (quickly fading to grey brown), **including on lateral and ventral sides**, mesonotum without intermixed long or short dark hairs. **Facial vestiture predominantly brown orange**, only a few dark hairs along compound eyes and sometimes on clypeus (Fig. S5). Scopa orange. Prepygidial and pygidial fimbria dark brown. Facial fovea comparatively little narrowed ventrally (Fig. S5). Inferior part of clypeus shiny, sparsely and coarsely punctate (Fig. S5). Mesonotum strongly shagreened, with shallow punctures. Tergal discs superficially shagreened, silk-shiny, sparsely punctate, interspaces 4-5 puncture diameters medially on disc of T3. Apical margins of T2 and T3 slightly depressed, shagreened, with few, shallow punctures, often light-brown apically. [Females of *A. rufula* are often confused with those of *A. (Ptilandrena) fulvata*, which can be recognized by the less shiny clypeus with a weak, longitudinal impression medially (sometimes not clearly visible!), by the strongly shagreened and very sparsely punctate terga, and by the medially not depressed apical margins of T2 and T3]. *Andrena rufula*
- Lateral and ventral parts of mesosoma either with at least some dark hairs, or entirely grey-brown, but then mesonotum with some intermixed dark hairs; other characters variables. 8

- 8 **Prepygidial and pygidial fimbria yellowish-orange** (Fig. 38), at most with a few brown hairs next to pygidial plate. **Hairs on face nearly entirely grey-white**, only a few dark hairs laterally and on vertex (Fig. 19). **Hairs on lateral and ventral parts of mesosoma light grey**, at most with isolated dark hairs. **Labral appendix long, approximately as long as wide apically** (Fig. 21). **Hairs on T1-T4 entirely grey-white, without erect, dark hairs** (Fig. 36). Fovea comparatively broad, broadest width wider than broadest width of flagellum and little narrowed ventrally. Tergal discs entirely finely shagreened (Fig. 36), silk-shiny, regularly sparsely punctate, punctures fine, interspaces 3-4 puncture diameters. Margins of T2-T4 hardly depressed, shagreened..... *Andrena montana*
- Prepygidial and pygidial fimbria dark brown (Fig. 37), never orange (but whitish brown in old specimens). Hairs on face predominantly dark, light brown or grey only between and around antennae. Lateral and ventral sides of mesosoma with extended areas covered with dark hairs. Labral appendix wider than long (Fig. 20). Sculpture variable..... 9
- 9 **Smallest length of malar space** (without impressed margin close to mandible) **comparatively long, approximately as long as basal width of antennal segment 3** (Fig. 23). **Clypeus laterally convex, medially nearly flat**, densely and coarsely punctate, interspaces conspicuously shiny at least apically (Fig. 18). **Terga entirely shagreened, disc of T2-T4 very sparsely punctate, nearly impunctate medially** (Fig. 35); **T2 and T3 with apical margin weakly impressed medially** (Fig. 35) [sculpture nearly as in *A. (Ptilandrena) fulvata*]. **Anterior part of propodeum with comparatively strong wrinkles** (Fig. 32). First recurrent vein enters second submarginal cell in basal half, second submarginal cell usually longer than broad (Fig. 28). Vestiture dark on head (except for some brown hairs on vertex), lateral and ventral sides of mesosoma, and T4-T6. Mesonotum with brown hairs intermixed with some dark hairs that are 2/3 as long as the brown hairs..... *Andrena allosa*
- Malar space shorter (Fig. 22) (in some rare females with particularly long head, malar space nearly as long as in *A. allosa*). Clypeus regularly convex medially (Fig. 17), shiny or shagreened. Sculpture of terga variable, but disc of T2-T4 comparatively more densely punctate (Figs 33, 34); apical margin of T2 and T3 visibly impressed medially (Figs 33, 34). Anterior part of propodeum at most with only fine wrinkles (Fig. 31). First recurrent vein usually enters second submarginal cell in its middle, second submarginal cell usually quadrate (Fig. 27). Mesonotal vestiture variable..... 10
- 10 **Brown vestiture of mesonotum and usually also of scutellum intermixed with numerous, comparatively long dark brown hairs; dark hairs on average at least half as long as light hairs** (Fig. 26). **Light hairs on T1-T4 snow white without yellowish hue** (Fig. 33). Hairs on mesonotum greyish brown (Figs 15, 26), never bright orange brown. Hairs laterally and ventrally on mesosoma dark grey to dark brown (Figs 15, 26), but dark hairs often with greyish tips. Propodeum with numerous dark hairs. Hairs on face predominantly dark but always grey between or around antennal sockets (Fig. 16). Sculpture variable, mesonotum sparsely punctate, medially with interspaces up to 3 puncture diameters, punctures well visible (Figs 29, 30), interspaces often silk-shiny medially, never entirely smooth with no sculpture. Tergal discs shagreened and comparatively sparsely punctate (Fig. 33)..... *Andrena amieti* sp. n.
- **Mesonotum either without intermixed dark brown hairs, or with very short dark hairs, then dark hairs on average less than half the length of light hairs** (very rarely longer). **Scutellum without intermixed dark hairs. Light hairs on T1-T4 yellowish-brown** (Fig. 34) **in fresh specimens** (but grey-white in worn specimens). Hairs on mesonotum orange-brown in fresh specimens. Hairs laterally and ventrally on mesosoma mostly dark, dark hairs without grey tips, except in second generation, especially in the southern and western Alps; propodeum usually without dark hairs. Hairs on face usually entirely dark, although in the southern and western Alps often with numerous grey-brown hairs in second generation. Sculpture of mesonotum and of terga variable and encompassing variation seen in previous species..... **A. bicolor**

Males

Euandrena: The delineation of the subgenus *Euandrena* is challenging in males and it is thus advised to use available keys to the genus *Andrena* (e.g., Schmid-Egger and Scheuchl 1997; Amiet et al. 2010) in addition to this diagnosis. Body-length 7–11mm. Integument of clypeus and of terga dark brown. Propodeal triangle at most weakly wrinkled (coarsely wrinkled anteriorly in *A. fulvida*). Third antennal segment usually clearly

longer than fourth (except in *A. rufula*). Labral appendix not projecting anteriorly. Apical margin of clypeus regularly rounded, not curved anteriorly. Male genitalia usually simple (Figs 52–61), dorsal gonocoxite lobes short and rounded, penis valves usually narrow basally, their maximal width subequal to apical width of gonostylus (except in *A. fulvida*, Fig. 57 and *A. chrysopus*, Fig. 55), gonostylus not enlarged apically (except in *A. fulvida*, Fig. 57).

- 1 **Antennal segment 3 as long as segments 4 and 5 together, segment 4 clearly wider than long, segment 5 wider than long to approximately as long as wide.** Entire vestiture brown or grey, especially facial vestiture nearly entirely grey, at most with a few isolated dark hairs laterally..... 2
- Antennal segment 3 shorter than segments 4 and 5 together, segment 4 as long as wide or longer, segment 5 longer than wide. Vestiture variable..... 3
- 2 Terga shiny and densely punctate, interspaces approximately 1-2 puncture diameters on terga 2-4. Hind basitarsus and usually also hind tibia ferruginous. **Penis valves basally broadened with lateral, angular extension** (Fig. 55) *Andrena chrysopus*

- Terga dull with little visible and sparse punctation. Hind basitarsus dark. Penis valves basally without lateral extension (Fig. 56), although broader than in *A. bicolor*. *Andrena symphyti*
- 3 **Penis valves basally broad, broader than gonostylus apically** (Fig. 57). **Gonostylus apically broad, spatulate, inner angle nearly quadrate** (Fig. 57). Propodeum anteriorly coarsely wrinkled. Entire body vestiture brown, except a few isolated dark hairs laterally on face. *Andrena fulvida*
- Penis valves basally narrow, subequal to apical width of gonostylus (Figs 52-54, 58-61). Gonostylus apically not broadened (Figs 52-54, 58-61). Propodeum comparatively finely wrinkled anteriorly. Vestiture variable. 4
- 4 **Apical margin of terga 1-5 ivory coloured** (Figs S6, S7). **Terga impressed basally and apically, thus tergal discs comparatively strongly convex**. Penis valves basally slightly broader than in *A. bicolor*, approximately as broad as apical width of gonostylus (Fig. 58). 5
- **Apical margin of terga 1-5 not ivory-coloured**, at most T3-5 with a narrow, yellowish brown margin. Terga little impressed basally, disc comparatively flat. Width of penis valves variable 6
- 5 Tergal discs coarsely and densely punctate, on T2 interspaces equal to 2 puncture diameters (Fig. S6). Surface of tergal discs strongly convex, apical margin strongly impressed, separated from disc by an abrupt step even medially (Fig. S6). *Andrena granulosa*
- Tergal discs finely and sparsely punctate, on T2 interspaces equal to 3-4 puncture diameters (Fig. S7). Surface of tergal discs not strongly convex (similar to *A. bicolor*), apical margin weakly impressed, separated from disc medially by a gradual step (Fig. S7). *Andrena vulpecula*
- 6 **Apex of gonostylus triangular, external angle acute** (Fig. 59). Third antennal segment subequal to or only slightly longer than fourth. Vestiture entirely brown, except a few dark hairs laterally on face. Penis valves slightly broadened basally (Fig. 59). Mesonotum shagreened with little visible punctures. *Andrena rufula*
- Apex of gonostylus rounded, without acute angle (Figs 52-54, 60, 61). Third antennal segment visibly longer than fourth. Vestiture, basal width of penis valves and sculpture of mesonotum and terga variable. 7
- 7 **Hind tibia usually more or less ferruginous near apex, hind basitarsus sometimes ferruginous** (hind leg rarely entirely dark). **Clypeal vestiture predominantly snow white and propodeum laterally with numerous dark hairs**. Clypeus densely and finely punctate, interspaces very narrow, mat. **Sterna with dense apical fringes of snow-white hairs**. Vestiture laterally on mesosoma and on terga predominantly snow white, on mesonotum grey-brown. Terga silk-shiny, punctation very fine, little visible. Penis valves slightly broadened basally (Fig. 60). *Andrena ruficrus*
- Hind tibia and basitarsus dark. Clypeal vestiture dark or light, but *if* light grey-brown (fading to grey-white), *then* propodeum without dark hairs. Clypeus more coarsely punctate, interspaces wider and at least partly shiny (Figs 40-42). Vestiture and basal width of penis valves variable. 8
- 8 **Facial hairs predominantly grey-white, especially on clypeus, including hairs along apical clypeal margin; dark hairs restricted to area along compound eyes and on frons. Labral appendix longer than apically wide** (Fig. 41). Mesonotum with grey-brown hairs with a yellowish hue in fresh specimens and with isolated, comparatively long intermixed dark hairs. Propodeum and lateral parts of mesosoma without dark hairs. **Penis valves basally slightly enlarged** (difference with the following species only visible in direct comparison!), **its maximal width approximately equal to the width of the gonostylus; apex of gonostylus comparatively narrow, parallel-sided, exterior margin straight or very slightly concave** (Fig. 61). Discs of T2-T5 finely shagreened, silk-shiny, never entirely smooth, finely and regularly punctate, punctures comparatively small. *Andrena montana*
- Facial hairs usually predominantly dark, grey hairs, if present, restricted to area around antennal sockets or to base of clypeus (Figs. 39, 42), hairs along apical clypeal margin predominantly dark. Labral appendix wider than long (Fig. 40). Vestiture on mesosoma variable, at least some dark hairs present laterally. Penis valves narrow basally, not broadened and clearly narrower than apical width of gonostylus; gonostylus apex regularly rounded, external margin convex (Figs 52-54). Sculpture of tergal discs variable, often with smooth and shiny area, or punctation coarser. 9
- 9 **Light hairs on dorsal parts of mesosoma, terga** (Fig. 51) **and sterna yellowish white in fresh specimens** (although snow white in worn specimens). T2-T4 with comparatively short apical fringes of hairs, long only laterally, fringes medially not longer than apical tergal margin. **Erect hairs on disc of T4 usually predominantly dark** (lighter in worn specimens). Facial vestiture often entirely dark (but frequently light medially in second generation, especially in southern and western parts of the Alps). *Andrena bicolor*
- Light hairs on dorsal parts of mesosoma, terga and sterna snow white, **without yellowish hue even in very fresh specimens** (Figs 39, 50). T2-T4 with comparatively long, snow white apical fringes of hairs, fringes medially clearly longer than apical tergal margin (Fig. 50). **Disc of T4 at most with a few isolated, erect dark hairs**. Face always with some grey-white hairs medially, at least between and around antennal sockets (Figs 39, 42). 10
- 10 Maxillary palps comparatively longer, segments 4 and 5 at least four times as long as maximal apical width (Fig. 45). Body length 8.5mm. First recurrent vein entering second submarginal cell comparatively closer to first submarginal crossvein (Fig. 49), second submarginal cell broader than long. Head comparatively long with protruding clypeus (Fig. 43). Mesonotum matt, without extended silk-shiny area medially (as on Fig. 46). *Andrena allosa*
- Maxillary palps comparatively shorter, segments 4 and 5 at most three times as long as maximal apical width (Fig. 44). Slightly smaller, body length on average 7-8mm. First recurrent vein entering second submarginal cell comparatively

closer to middle of cell, second submarginal cell often subquadrate (Fig. 48). Head comparatively short with little protruding clypeus (Fig. 42). Sculpture of mesonotum variable, in first generation matt (Fig. 46), in second generation with extended silk-shiny area medially (Fig. 47)..... *Andrena amieti* sp. n.

(Only two males of *A. allosa* were examined and the characters mentioned to distinguish *A. allosa* from *A. amieti* sp. n. are tentative.)

Additional taxonomic notes on selected *Andrena* (*Euandrena*) species

Andrena croatica Friese, 1887, stat. rev.

Suppl. material 3: Figs S8, S9

Andrena croatica Friese 1887: 85, ♀, ♂, “Prope Orechovicam non procul ab urbe St. Veithii, mense Martio” [Near Orehovica (nowadays a district of Rijeka), not far from the city of St. Veith (Rijeka, Croatia) month of March]. Syntypes ♀♀♂♂ (NMBE, OLML, ZMHB).

Notes. We examined two possible syntype females of *Andrena croatica*, which perfectly agree with the original description. No specimen was available for genetic study. These two females are markedly divergent from *A. bicolor*, and thus *A. croatica*, which is currently considered as a synonym of *A. bicolor* (Gusenleitner and Schwarz 2002), probably represents a valid species (stat. rev.). Striking divergent features in the female sex include the white scopa, the fine, regular punctuation of the terga (Suppl. material 3: Fig. S9), the mesonotal vestiture comprising intermixed grey-brown hairs and numerous, comparatively long, dark hairs, and the numerous dark hairs on the propodeum. This species also has long mouthparts approaching the condition observed in *A. allosa* (Suppl. material 3: Fig. S8). The male is unknown to us.

Examined material. 2 possible syntype females from Fiume [Croatia, Rijeka] and an additional female from Croatia (Suppl. material 1).

Andrena pileata Warncke, 1975, stat. n.

Suppl. material 3: Figs S10–S12

Andrena allosa pileata Warncke 1975b: 85, ♀ nec ♂, “Chelmos, Peloponnes” [Chelmos mountains, Greece]. Holotype ♀, OLML.

Notes. Warncke (1975b) described *Andrena allosa pileata* from the Chelmos mountains in Greece. The type series of *A. allosa pileata* is mixed and includes two distinct species. The first species, represented by the holotype, is briefly redescribed here. No specimen was available for genetic study. This first species appears clearly distinct from *A. allosa* and *A. amieti* sp. n., and we treat it as a valid species, *A. pileata* Warncke stat. n.

Description. Female: Body length 9mm. Clypeus shorter than in *A. allosa*, as in *A. bicolor*, without flattened apical area, regularly convex, densely punctate, interspaces weakly shagreened even apically (Suppl. mate-

rial 3: Fig. S10). Malar space as in *A. bicolor*, shorter than in *A. allosa*. Fovea as in *A. bicolor* or *A. allosa*, markedly narrower inferiorly than superiorly. Mesonotum nearly entirely matt, silk-shiny only medially, comparatively densely punctate, except medially (there interspaces up to 3–4 puncture diameters). Terga entirely shagreened, finely, regularly punctate even medially on discs, interspaces 3 puncture diameters (Suppl. material 3: Fig. S12). Apical tergal margins weakly impressed apically, more so than in *A. allosa*. The sculpture of the terga is similar to that of *A. ruficrus* and unlike *A. amieti* sp. n. or *A. allosa*. Vestiture predominantly brown (both specimens are not fresh), medially on face grey-brown, laterally dark; vestiture on mesonotum orange-brown, made of comparatively short, strongly plumose hairs, with only few, very short dark hairs (Suppl. material 3: Fig. S11). Hairs on lateral and ventral sides of mesosoma grey-brown. Scopa brown-orange. Hairs on metasoma comparatively short, metasomal terga without loose apical fringes of white hairs, at most with isolated, short hairs (Suppl. material 3: Fig. S12).

Male: unknown; the male paratype is likely not conspecific and probably belong to *Andrena* sp1.

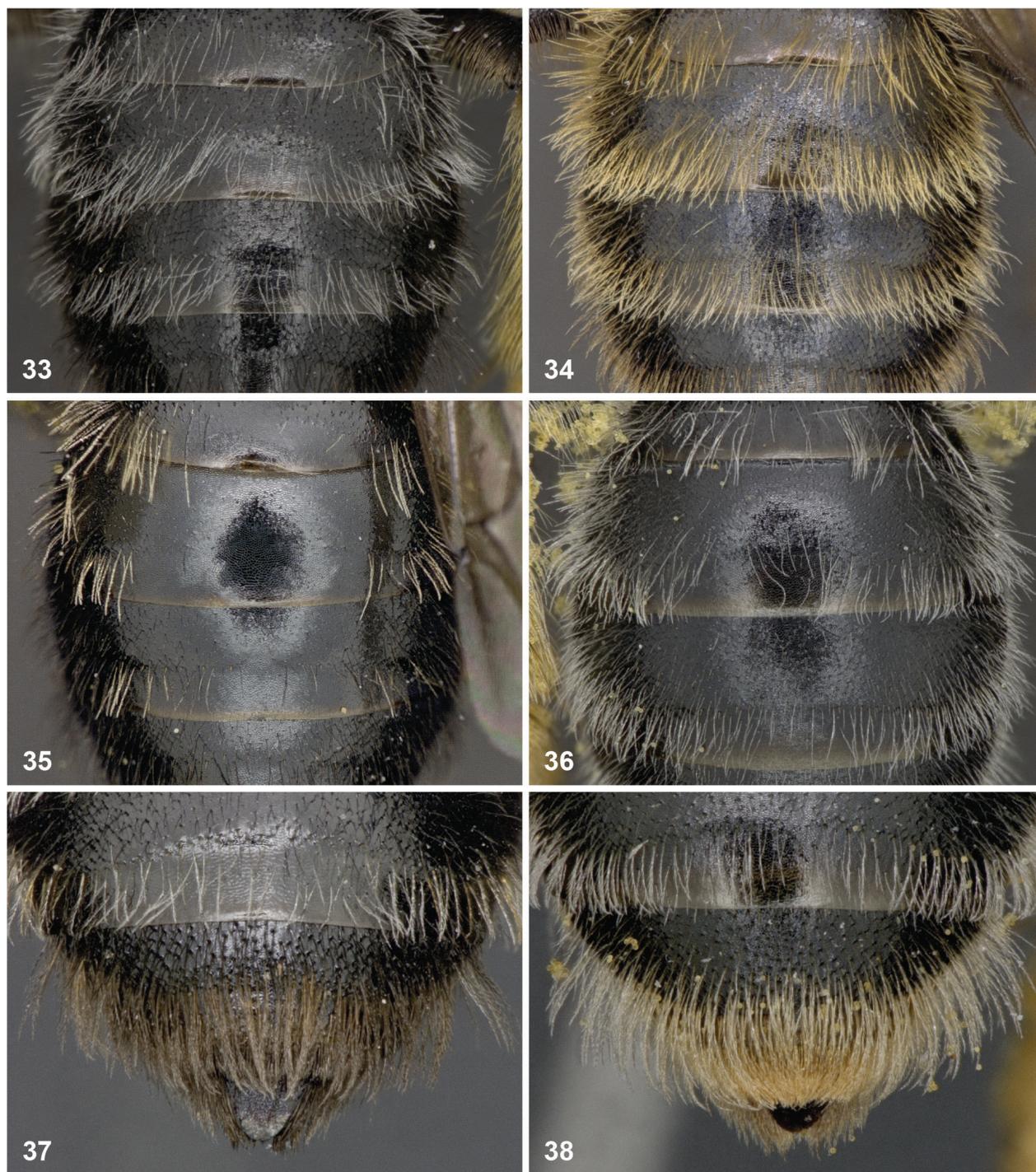
Examined material. Holotype female of *A. pileata*; one additional female from the Chelmos mountains, Greece (Suppl. material 1).

Andrena sp1

Suppl. material 3: Figs S13–S15

Andrena allosa pileata Warncke, 1975b, *partim*

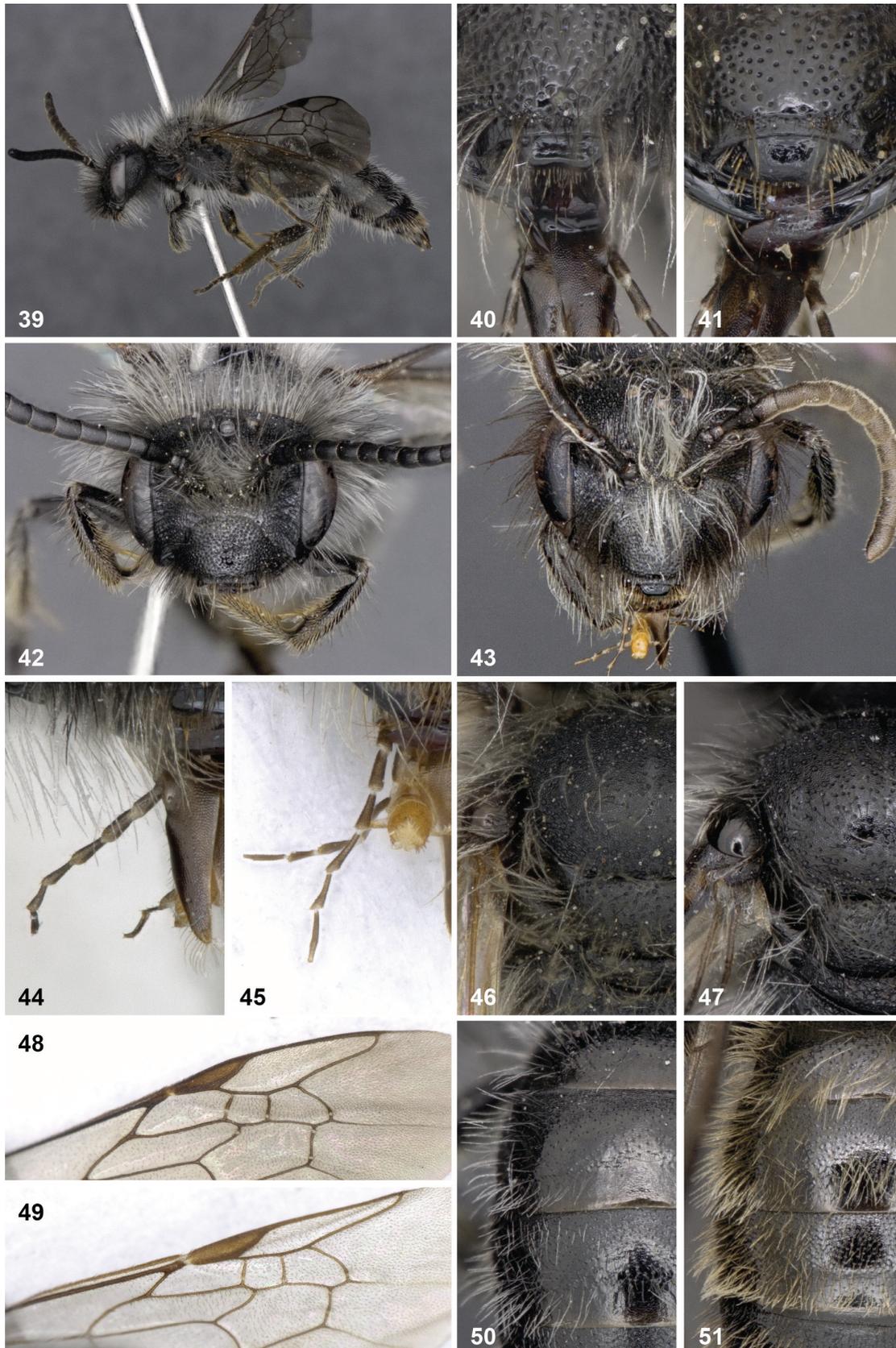
Notes. The second species included in the type series of *Andrena allosa pileata* is referred to as *Andrena* sp1. This species shares similarities with both *A. amieti* sp. n. and *A. allosa*. It is superficially similar to specimens of *A. amieti* sp. n. from Southern Italy (Suppl. material 2: Fig. S1), which have entirely grey vestiture even in the female sex. Two specimens were available for genetic study (specimens 961 and 1252; Figs 1, 2). These two specimens were closely related to *A. allosa* and separated from that species by a genetic distance of 1.82%. We do not consider this species to be conspecific with *A. amieti* sp. n. for three reasons: first, genetic distances to *A. amieti* sp. n. (4.27%) were higher than to *A. allosa*; second, based on the available material, this species appears to have only one generation per year unlike *A. amieti* sp. n.; third, there are slight, but consistent morphological differences compared to *A. amieti* sp. n. *Andrena* sp1 is characterized as follows:



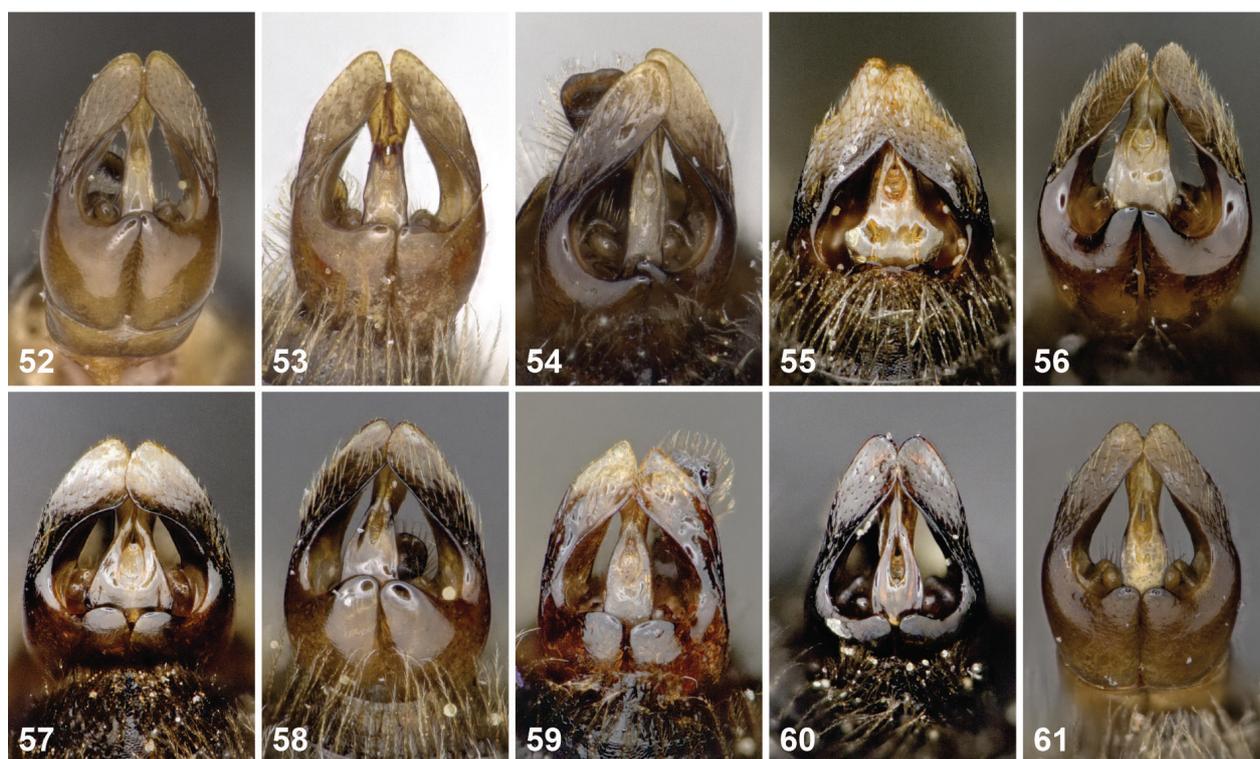
Figures 33–38. Structure of female of *Andrena amieti* sp. n., *A. bicolor*, *A. allosa* and *A. montana*. **33**, T1–T4 of *A. amieti* sp. n. **34**, T1–T4 of *A. bicolor*. **35**, T1–T4 of *A. allosa*. **36**, T1–T4 of *A. montana*. **37**, prepygidial and pygidial fimbria of *A. amieti* sp. n. **38**, prepygidial and pygidial fimbria of *A. montana*.

Description. Female: Body size, 7–8mm. Clypeus shorter than in *A. allosa*, as in *A. bicolor*, without flattened apical area, in most specimens, apical area with irregular, longitudinal concavity (not clearly visible on Suppl. material 3: Fig. S15). Clypeus less densely punctate than in *A. pileata* stat. n., interspaces shinier, very weakly shagreened or completely shiny medially (Suppl. material 3: Fig. S15). Malar space as in *A. bicolor*, shorter than in

A. allosa. Fovea as in *A. bicolor* or *A. allosa*, markedly narrower inferiorly than superiorly. Mesonotum nearly entirely matt, finely sculptured, silk-shiny medially, comparatively sparsely punctate, interspaces medially up to 5 puncture diameters. Terga weakly shagreened, entirely silk-shiny, shinier than in *A. allosa*, irregularly, very sparsely punctate to nearly impunctate, apical margin weakly impressed apically (Suppl. material 3: Fig. S14)



Figures 39–51. Structure of male of *Andrena amieti* sp. n., *A. montana*, *A. allosa* and *A. bicolor*. 39, male of *A. amieti* sp. n. in lateral view. 40, labrum of *A. amieti* sp. n. 41, labrum of *A. montana*. 42, male of *A. amieti* sp. n. in frontal view. 43, male of *A. allosa* in frontal view. 44, section of mouthparts of *A. amieti* sp. n. 45, section of mouthparts of *A. allosa*. 46, dorsal view of mesosoma of spring generation of *A. amieti* sp. n. 47, dorsal view of mesosoma of summer generation of *A. amieti* sp. n. 48, section of right forewing of *A. amieti* sp. n. 49, section of right forewing of *A. allosa*. 50, T1–T4 of *A. amieti* sp. n. 51, T1–T4 of *A. bicolor*.



Figures 52–61. Male genitalia of species of *Euandrena*. 52, *Andrena amieti* sp. n. 53, *A. bicolor*. 54, *A. allosa*. 55, *A. chrysopus*. 56, *A. symphyti*. 57, *A. fulvida*. 58, *A. vulpecula*. 59, *A. rufula*. 60, *A. ruficrus*. 61, *A. montana*.

although more so than in *A. allosa*. Vestiture predominantly white-grey, without yellowish or orange hue (Suppl. material 3: Figs S13, S14). Face darker than in *A. pileata* stat. n., grey only around antennal sockets. Vestiture on mesonotum predominantly grey, made of comparatively long, weakly plumose grey hairs and numerous, comparatively long dark hairs (Suppl. material 3: Fig. S13) (as in *A. amieti* sp. n. but unlike *A. pileata* stat. n.). Hairs on lateral side of mesosoma dark, on ventral side grey. Scopa white. Hairs on metasoma comparatively long, nearly entirely whitish grey, except dark on disc of T4, and on T5 and T6. The most distinctive features are the white scopa, the grey mesonotal vestiture with long, dark hairs, and the nearly impunctate terga.

Male: No difference was found between the three males examined and the first generation of *A. amieti* sp. n.

Examined material. 3 males and 10 females from the Chelmos Mountains, the Mount Olympus and the Mount Tymfristos, Greece (Suppl. material 1).

Andrena sp2

Suppl. material 4: Figs S16, S17

Notes. One female specimen from Lesbos included in our genetic study (specimen 1254) is morphologically distinct from both *A. pileata* stat. n. and *Andrena* sp1 and is referred to as *Andrena* sp2. In the female sex, it is similar to *Andrena* sp1, except that the clypeus lacks the longi-

tudinal impression (Suppl. material 4: Fig. S17) observed in *Andrena* sp1, the mesonotum is more densely punctate, the terga are more shiny (disc of T1 and T2 nearly completely shiny; Suppl. material 4: Fig. S16) and more clearly punctate. The mesonotum has grey-brown vestiture, the hairs are weakly plumose, there are a few long dark hairs on the mesonotum and numerous dark hairs on the propodeum. The scopa is yellowish white, in contrast to *Andrena* sp1. Male unknown.

Examined material. 2 females from two localities on the Island of Lesbos, Greece (Suppl. material 1).

Andrena sp3

Suppl. material 4: Figs S18, S19

Notes. Specimen 928 in our genetic dataset, a female collected in Greece, appears to be morphologically distinct from *A. bicolor* and from any taxa mentioned above. In addition, we have examined a series of females from the Island of Lesbos, as well as one female from Northern Italy, all of which putatively belong to *Andrena* sp3. This species is presumably widely distributed in southeastern Europe. In the female sex, *Andrena* sp3 is similar to some forms of *A. bicolor*. The vestiture is nearly entirely brown-orange, including on all sides of the mesosoma, with only a few dark hairs laterally on face (Suppl. material 4: Fig. S19), on the disc of T4, as well as numerous dark hairs on T5 and T6. The mesonotum

is conspicuously shiny with coarse and dense punctation (Suppl. material 4: Fig. S18). In its vestiture this species is highly similar to *A. rufula*, from which it can easily be separated by the shiny sculpture of the mesonotum. Male unknown to us.

Examined material. 5 females from the Island of Lesbos, Greece; 1 female from the Peloponnese, Greece; one female from northwestern Italy (Suppl. material 1).

Discussion

Species delimitation in the *bicolor*-group

The taxonomy of the *bicolor*-group in the Alps has long remained controversial. In light of our results, there appears to be little doubt that four well-separated biological species co-occur in the Alps, for the following reasons. First, DNA barcodes are diagnostic for each of these species in spite of the observed paraphyly in *Andrena amieti* sp. n. in mitochondrial trees (Fig. 1); these distinct barcodes were concordant with morphology in all cases, suggesting no mitochondrial introgression. Second, all four species occur in sympatry and maintain distinct morphology. *Andrena montana* differs from the other three species in numerous sculptural characters in addition to conspicuous characters in vestiture colour; similarly, this species was only distantly related to the other three species (Figs 1, 2). *Andrena allosa* differs from *A. bicolor* and *A. amieti* sp. n. in subtle but constant sculptural characters in the female, while *A. amieti* sp. n. and *A. bicolor* can be distinguished by constant differences in vestiture colour. Third, *A. allosa* and *A. montana* have a single generation per year while *A. bicolor* and *A. amieti* sp. n. have two generations per year. Fourth, *A. allosa* differs from *A. bicolor* and *A. amieti* sp. n. in its pollen host choice: the second generation of the latter two species have a pronounced affinity for Campanulaceae, a pollen type that is completely absent from the pollen diet of *A. allosa*.

These clear differences in morphology and life-history traits are in contradiction with the results of our analyses of the nuclear gene, where one specimen of *A. allosa* (1285; Fig. 2) was not separated from some specimens of *A. amieti* sp. n. *A. amieti* sp. n. and *A. allosa* have probably diverged recently, and the observed pattern in the nuclear gene sequences is likely due to either lack of variation in the selected marker, incomplete lineage sorting or rare instances of horizontal gene flow, all of which are expected to occur in complexes of closely related species.

While our study solves a long controversy in the systematics of central European bees, it also raises additional questions that will require future work. In both *A. bicolor* and *A. amieti* sp. n., two distinct clusters of sequences were found in phylogenetic analyses of COI. In both cases these two clusters were found in sympatry

and they conflict with current species concepts. The same two clades occurring in sympatry within *A. bicolor* were previously reported (Schmidt et al. 2015). Based on available sequences on BOLD these two clades appear to be widespread in Europe. In contrast to the situation in *A. bicolor* where both clades are sister groups, the two clusters found in *A. amieti* sp. n. form a paraphyletic unit with respect to a distinct biological species occurring in sympatry, *A. allosa*.

Two hypotheses can be formulated to explain these discrepancies between mitochondrial gene trees and species trees (Mutanen et al. 2016). First, these clusters may point to additional cryptic diversity. For example, species paraphyly in the pierid butterfly *Leptidea reali* Reissinger, 1989 has recently been demonstrated to be due to the presence of an additional cryptic species, *L. juvernica* Williams, 1946 (Dincă et al. 2011). Second, these clusters may merely represent deep intraspecific divergences within one biological species, without reproductive isolation between mitochondrial lineages. Such a situation has been suggested for two divergent clades within the Common Redstart *Phoenicurus phoenicurus* (Linnaeus, 1758) (Hogner et al. 2012) and for the paper wasp *Polistes dominula* (Christ, 1791) (Neumeyer et al. 2014, Schmid-Egger et al. 2017). To discriminate between these two alternative hypotheses for the *bicolor*-group, future research is needed, ideally using genomic-scale, nuclear markers.

Our study reveals that there are probably five additional species in the *bicolor*-group in southeastern Europe: *A. croatica* stat. rev., *A. pileata* stat. n., *Andrena* sp1, *Andrena* sp2 and *Andrena* sp3. How these species relate to *A. allosa*, *A. amieti* sp. n., or to additional species from Eastern Europe (*A. asperula* Osytshnjuk, 1977 from Ukraine) or from the East Palearctic (e.g., *A. capillosella* Osytshnjuk, 1986, *A. khosrovi* Osytshnjuk, 1993), remains unclear and requires future investigation. We strongly recommend the use of genetic analyses to clarify the taxonomy of these challenging taxa.

Delimitation of *Euandrena* and *Ptilandrena*

Our study suggests that *Andrena fulvata* and the closely related *A. angustior*, currently placed in the subgenus *Ptilandrena*, may in fact belong to the subgenus *Euandrena*. These two species differ from other species of *Euandrena* in the long mandibles of the male and the presence of an elevated carina on the pronotum in both sexes. They differ from other members of *Ptilandrena* such as *A. vetula* Lepeletier, 1841, in the narrow facial fovea of the female, a character shared with species of *Euandrena*. Future studies should examine the phylogenetic placement of other members of the morphologically heterogeneous subgenus *Ptilandrena*.

Pollen host preferences

The high proportion of *Crocus* pollen in the larval diet of *Andrena allosa* was unexpected. *Crocus albiflorus*, which is the only native *Crocus* species in the Central

Alps, starts to bloom immediately after the snowmelt (Fig. 9). The early flight period of *A. allosa* in combination with its restricted host plant choice might explain why this bee species has so rarely been found in the past. We hypothesize that *A. allosa* will turn out to be more common than hitherto assumed when flowering *Crocus* stands will be systematically checked for the presence of this species.

Surprisingly, the spring and summer generations of the bivoltine *A. amieti* sp. n. distinctly differ in their pollen host selection in that the females of the latter exhibit a strong preference for the pollen of Campanulaceae. This preference is also suggested by our observations that males of the summer generation patrolled *Campanula* flowers in their search for females and that summer generation males and females visited *Geranium sylvaticum* (Geraniaceae). The latter observation is in agreement with the finding that *Geranium* was the second most important pollen host of the summer generation (Tab. 3). *Geranium* flowers often serve as a source of nectar or a “rendezvous” point for oligolectic bees specialized on *Campanula*, such as *Andrena curvungula* Thomson, 1870, *A. pandellei* Pérez, 1895 or *Chelostoma rapunculi* (Lepeletier, 1841), which might possibly be due to similar colour or olfactory cues exerted by the flowers of the two unrelated taxa. Attraction to similar cues might also explain why *Geranium* is regularly visited by *A. amieti* sp. n. Interestingly, a preference for Campanulaceae was also reported for the summer generation of the bivoltine *A. bicolor*, which is closely related to *A. amieti* sp. n. (Stöckhert in Schmiedeknecht 1930, Müller et al. 1997, Peeters et al. 2012, Scheuchl and Willner 2016). Differing pollen host choice by spring and summer generations of the same species as in *A. amieti* sp. n. and *A. bicolor* suggest that the two generations have evolved their own phenotypes as a result of different selection regimes in spring and summer (Simpson et al. 2011). In a recent study, however, Milet-Pinheiro et al. (2016) did not detect seasonal polyphenism in *A. bicolor* and found instead that females of the first and the second generation have the same innate flower search image in spite of the different spectrum of pollen hosts they exploit.

Conclusion

Genetic analyses, in combination with morphological analysis, were decisive in solving an important controversy in the taxonomy of European wild bees. However, our study also raised new questions, in particular the intriguing case of parapatry observed in mitochondrial gene trees in *Andrena amieti* sp. n. This new species appears to be the tip of the iceberg of cryptic diversity in southern Europe. Time and funds should be dedicated to taxonomic research on wild bees in species-rich Mediterranean ecosystems.

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Supplementary material 1

Database for all specimens examined in this study (Occurrences)

Authors: Christophe Praz, Andreas Müller, David Genoud

Data type: specimens data

Explanation note: Complete database including all examined specimens of *Andrena allosa*, *A. amieti* sp. n. (including holotype and paratypes), *A. croatica* stat. rev., *A. montana*, *A. pileata* stat. n., and the three unclear taxa referred to as *Andrena* sp1, sp2 and sp3.

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Link: <https://doi.org/10.3897/alpento.3.29675.suppl1>

Supplementary material 2

Supplementary figures S1-S7

Authors: Christophe Praz, Andreas Müller, David Genoud

Data type: multimedia

Explanation note: Fig. S1, female paratype of *Andrena amieti* sp. n. from Southern Italy, lateral view. Fig. S2, clypeus of *A. symphyti* female. Fig. S3, T1–T3 of *A. granulosa* female. Fig. S4, T1–T4 of *A. vulpecula* female. Fig. S5, head of *A. rufula* female in frontal view. Fig. S6, T1–T3 of *A. granulosa* male. Fig. S7, T1–T3 of *A. vulpecula* male.

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Link: <https://doi.org/10.3897/alpento.3.29675.suppl2>

Supplementary material 3

Supplementary figures S8-S15

Authors: Christophe Praz, Andreas Müller, David Genoud

Data type: multimedia

Explanation note: Fig. S8, mouthparts of *Andrena croatica* stat. rev. female. Fig. S9, T1–T3 of *A. croatica* stat. rev. female. Fig. S10, head in frontal view of *A. pileata* stat. n. female. Fig. S11, vestiture on mesonotum and scutellum of *A. pileata* stat. n. female. Fig. S12, T1–T3 of *A. pileata* stat. n. female. Fig. S13, vestiture on mesonotum and scutellum of *Andrena* sp1 female. Fig. S14, T1–T4 of *Andrena* sp1 female. Fig. S15, head in frontal view of *Andrena* sp1 female.

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Link: <https://doi.org/10.3897/alpento.3.29675.suppl3>

Supplementary material 4

Supplementary figures S16-S19

Authors: Christophe Praz, Andreas Müller, David Genoud

Data type: multimedia

Explanation note: Fig. S16, T1–T4 of *Andrena* sp2 female. Fig. S17, head in frontal view of *Andrena* sp2 female. Fig. S18, mesosoma in dorsal view of *Andrena* sp3 female. Fig. S19, head in frontal view of *Andrena* sp3 female.

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