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A phylogenetic reassessment of Orchidaceae taxonomy in the State of Palestine

Amal P. Handal[1], Huaxi Liu[2], Alfried P. Vogler[2], Johann G. Gedeon[1] and Mazin B. Qumsiyeh[1]*

[1]Palestine Institute for Biodiversity and Sustainability, Bethlehem University, Bethlehem, Palestine; [2]Natural History Museum, London, United Kingdom

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Abstract

Palestine is part of the Eastern Mediterranean biodiversity "hotspot" and possesses exceptionally rich flora owing to its unique geographical position and complex geological history. The occupied Palestinian Territories (West Bank and Gaza) face substantial environmental pressures, yet comprehensive botanical studies remain limited. A total of 144 samples belonging to 14 orchid taxa from 19 localities were gathered across the West Bank. Of these, 44 samples were sequenced using five chloroplast DNA regions: two coding genes (*matK* and *rbcL*) and three intergenic spacers (atpF–atpH IGS, psbK–psbI IGS, and trnH–psbA IGS). The resulting sequences were used to infer phylogenetic relationships among the sampled taxa using three complementary analytical approaches: Maximum Likelihood (ML), Bayesian Inference (BI), and Maximum Parsimony (MP). Notably the results support the recognition of the genera *Anacamptis* and *Serapias* as distinct from the genus *Orchis*. *Ophrys* is recovered as monophyletic, although taxonomic ambiguities remain and hybridization appears to be frequent. The newly described species of *Ophrys palaestinae* (2021) is nested within the clade of *Ophrys adonidis* s.l. Our study further demonstrates that morphological differentiation does not consistently reflect underlying genetic divergence. This study highlights the value of DNA barcoding for resolving systematic challenges in the flora of Palestine, and provides essential baseline data to ecological studies and conservation priorities.

*Corresponding author: info@palestinenature.org

Key words:

DNA barcoding, orchids, phylogeny, atpF–atpH IGS, psbK–psbI IGS, trnH–psbA IGS, matK, rbcL

Introduction

Orchids constitute an integral component of ecosystems (Lussu *et al.,* 2020). Wild orchid populations are undergoing significant declines, and numerous species are currently classified as endangered or threatened, so they serve as effective indicators of biodiversity loss (Newman *et al.,* 2015; Allen *et al.,* 2019; Wraith *et al.*, 2020). Historic Palestine (Israel and the Occupied Palestinian Territories) houses 31 species of orchids, 25 of which have been documented in recent surveys conducted within the Occupied Palestinian Territories (Al Sheikh, 2019; Pahl and Qumsiyeh, 2021). In 2021, three new-to-science orchid species were described in Historic Palestine: *Epipactis odemensis* Kreutz & Shifman, *Ophrys luminosa* Kreutz, Shifman & M. H. Schot, and *Ophrys palaestinae* Kreutz & Shifman (Kreutz *et al.,* 2021). The region forms part of the Mediterranean biodiversity hotspot and possesses a unique geographical position and geological history that have produced a mosaic of habitats spanning five ecogeographical zones, resulting in high biodiversity (Goupil and Qumsiyeh, 2018; Qumsiyeh and Abusarhan, 2020; Gedeon and Khalilieh, 2024). Nonetheless, nearly one-third of the plant species in the Occupied Palestinian Territories are rare or in decline (Al-Sheikh and Qumsiyeh, 2021). Major threats to orchids include overexploitation, habitat modification, land-use change, illegal trade, insecticide application, climate change, and invasive species (Pahl and Qumsiyeh, 2021).

Native orchid species have traditionally been identified solely based on morphological characteristics, with minimal molecular evaluation undertaken to date. Numerous researchers have emphasized the value of integrating molecular approaches with morphological methods for accurate species identification (Savolainen *et al.,* 2005; Vu *et al.,* 2017, 2020; Liu *et al.,* 2022). Therefore, there is a clear and urgent need to apply molecular techniques to the orchids of this region to elucidate their phylogenetic relationships and refine their taxonomic placement. In this context, robust taxonomic resolution is essential for defining conservation priorities and guiding effective habitat protection strategies. Accurate species delimitation and distribution data are critical for monitoring threatened orchid populations and informing evidence-based conservation planning in Palestine.

Materials and methods

Nineteen field trips were conducted between spring 2021 and spring 2022 across the West Bank (see Figure 1 for study area). A total of 144 specimens representing 14 taxa were collected, although some specimens could only be identified to the genus level and were therefore listed as *Ophrys* spp. All specimens were photographed in the field, and GIS coordinates together with ecological data were recorded. Portions of flowers were subsequently collected in 50 mL sterile conical tubes and preserved in a freezer. Species names follow the International Plant Names Index (https://www.ipni.org/). For specimen and locality information, samples designated with Palestine Museum of Natural History (PMNH) numbers correspond to herbarium specimens, whereas those labeled with P numbers represent tissue or DNA samples.

The broader distribution of each species within Palestine was compiled based on field observations, peer-reviewed publications (e.g., Pahl and Qumsiyeh, 2021), and biodiversity recording platforms (https://powo.science.kew.org/, https://biogis.huji.ac.il/, https://www.gbif.org/, https://www.inaturalist.org/).

DNA extraction was performed using the Macherey-Nagel NucleoSpin Plant II kit (Germany). Five chloroplast DNA (cpDNA) regions were selected for amplification in accordance with Kim *et al.* (2014): two coding genes (matK and rbcL), and three intergenic spacers (atpF–atpH IGS, psbK–psbI IGS, and trnH–psbA IGS). Each primer set (Table 1) was amplified and sequenced independently. DNA extraction and amplification were conducted at the PMNH Biodiversity Center. A total of 44 specimens were selected for Sanger sequencing, representing one sample per taxon in addition to samples with taxonomic ambiguity, including presumed varieties, potential subspecies, or individuals displaying morphological anomalies.

All sequence files were initially processed using BioEdit v7.2 (Hall, 2012) to trim low-quality regions. Sequences corresponding to the same fragment were subsequently aligned independently using the ClustalW multiple alignment algorithm (Thompson *et al.,* 1994). In addition to the sequences generated in this study, 47 complete chloroplast genomes of orchid species were downloaded from GenBank (Zhang *et al.,* 2022) and the corresponding regions were extracted for alignment alongside the newly generated sequences.

All alignments were subsequently concatenated into a single matrix for phylogenetic reconstruction using three complementary analytical approaches: Maximum Likelihood (ML), Bayesian Inference (BI), and Maximum Parsimony (MP). The ML approach was selected as the primary phylogenetic framework because it is statistically robust, explicitly models sequence evolution, accommodates partition-specific substitution heterogeneity, and provides reliable branch support estimates, making it particularly suitable for multi-locus datasets. In addition, BI and MP analyses were conducted to further evaluate

Table 1. Primers used for amplification and sequencing of five chloroplast DNA (cpDNA) regions in Orchidaceae.

Region	Primer Name	Sequence (5'—3')	Reference
rbcL gene	Z1F	ATGTCACCACAAACAGAAACTAAAGCAAGT	Soltis *et al.* (1992)
	rbcLaR	GTAAAATCAAGTCCACCRCG	Kress and Erickson (2007)
matK gene	matK_390F	CGATCTATTCATTCAATATTTC	Cuénoud *et al.* (2002)
	matK_1326R	TCTAGCACACGAAAGTCGAAGT	
atpF-atpH IGS	AtpF	ACTCGCACACACTCCCTTTCC	Kim K. J. (Pers. Comm.)
	AtpH	GCTTTTATGGAAGCTTTAACAAT	
psbK-psbI IGS	psbK_F	TTAGCCTTTGTTTGGCAAG	Kim K. J. (Pers. Comm.)
	psbI_R	AGAGTTTGAGAGTAAGCAT	
trnH-psbA IGS	psbAF	GTTATGCATGAACGTAATGCTC	Sang *et al.* (1997)
	trnHR	CGCGCATGGTGGATTCACAAATC	

the stability and congruence of the inferred phylogenetic relationships across different analytical methodologies. Comparison of the resulting topologies enabled assessment of the consistency and robustness of clade recovery and specimen placement throughout the phylogeny. PartitionFinder 2 (Lanfear *et al.,* 2017) was employed to determine the optimal partitioning scheme and evolutionary models (K3Pu+F+R3 for atpF, matK, psbA, and rbcL; GTR+F+I for trnH) prior to ML tree reconstruction with 1,000 bootstrap replicates using IQ-TREE v2 (Minh *et al.,* 2020), based on the selected partitioning strategy. Resulting phylogenies were visualized in FigTree v1.4.4, with *Iris dichotoma* and *Lycoris sanguinea* designated as outgroups.

A subset of 44 specimens was subjected to DNA sequencing of five chloroplast barcode regions and included in all the phylogenetic analysis based on the concatenated data matrix. These specimens represent two orchid subfamilies: Epidendroideae and Orchidoideae. Additionally, published chloroplast genomes for 47 reference species representing all five subfamilies of Orchidaceae were incorporated.

Results

The collected orchid samples were gathered from 19 localities across the West Bank (Table 2 and Figure 1,2); see Annex 1 for full taxonomic and specimen data. In the resulting phylogenetic trees (Figure 3, Supplementary Figures S1.2, S1.3), our samples form two principal branches that are broadly concordant with the findings of Bernardos *et al.* (2006). Both subfamilies recovered as monophyletic, forming sister clades. The subfamily Epidendroideae contained the Palestinian *Limodorum abortivum* (L.) Sw. (see Figure 2d), a rare species in Historic Palestine. The subfamily Orchidoideae comprised six branches:

1. The *Orchis* genus branch, including *Orchis anatolica* Boiss., an uncommon species in Historic Palestine, and *Orchis galilaea* (Bornm. & M. Schulze) Schlechter, which is uncommon in Historic Palestine and listed as endangered in Jordan according to the Jordan Plant Red List Vol. II (Taifour, 2017). Both species were congruent with their expected monophyletic group.
2. The *Anacamptis* genus branch, including *Anacamptis papilionacea* (L.) R. M. Bateman, Pridgeon & M. W. Chase (endangered in Jordan; Taifour, 2017), *Anacamptis pyramidalis* (L.) L. C. M. Richard (uncommon in Historic Palestine), and *Anacamptis sancta* (L.) R. M. Bateman, Pridgeon & M. W. Chase (rare in Historic Palestine). Each species clustered within its expected monophyletic subgroup.

Table 2. List of sampling localities, geographic coordinates, and corresponding species records. Species numbers correspond to those presented in Annex 1.

Locality	Coordinates	Species
Bethlehem, PMNH	31.7177108N, 35.2052825E	2, 4, 8, 9, 10, 11, 12, 13
Bethlehem, Wadi Al-Makhrour	31.7159647N, 35.1743767E	2, 6, 12, 13, 14
Bethlehem, Wadi Cremisan	31.7267408N, 35.1723939E	5, 6, 12, 13, 14
Hebron, Al-Qarn	31.6192358N, 35.1241150E	2, 6, 12, 13
Jenin, Faqqua’	32.4905342N, 35.3989417E	2, 7, 9
Jenin, Area 70 (near Haddad Village)	32.4405556N, 35.3228222E	4
Jenin, Mount Hureish	32.3373858N, 35.2540247E	10
Jenin, Siris	32.3254197N, 35.2915056E	1, 2, 4
Nablus, New Nablus	32.2143819N, 35.2519650E	6, 10, 12, 13, 14
Nablus, Luban e-Sharkiya	32.0609800N, 35.2448378E	2, 3, 6, 12, 14
Nablus, Sarra	32.2094833N, 35.1897228E	1, 10, 12
Salfit, Wadi Qana	32.1650714N, 35.1294714E	2, 3, 6, 12, 13
Nablus, Yaseed	32.3156814N, 35.3022961E	1, 2, 9, 10, 12
Ramallah, Wadi Al- Haramiyeh	32.006111N, 35.254567E	2, 3, 6, 14
Ramallah, Dayr Ghassana	32.0399000N, 35.0721833E	2, 3, 10, 12, 13
Ramallah, Tal Al-asour	31.9728111N, 35.2764489E	2, 6, 12
Ramallah, Wadi Al-Zarqa	32.0425361N, 35.0749331E	2, 7, 10, 12
Ramallah, Um Safa	32.0152678N, 35.1368856E	2
Hebron, Deir Razeh	31.4688253N, 35.0413964E	2
Hebron, Beit Umar	31.6239369N, 35.1023036E	2

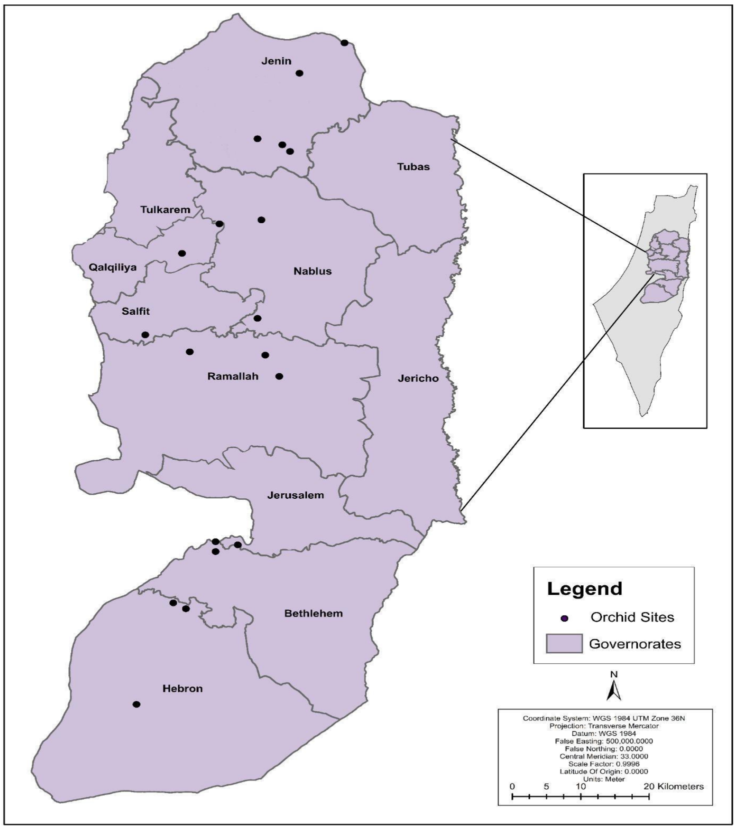

Figure 1. Map of the study area in the West Bank showing the geographic distribution of orchid collection sites.

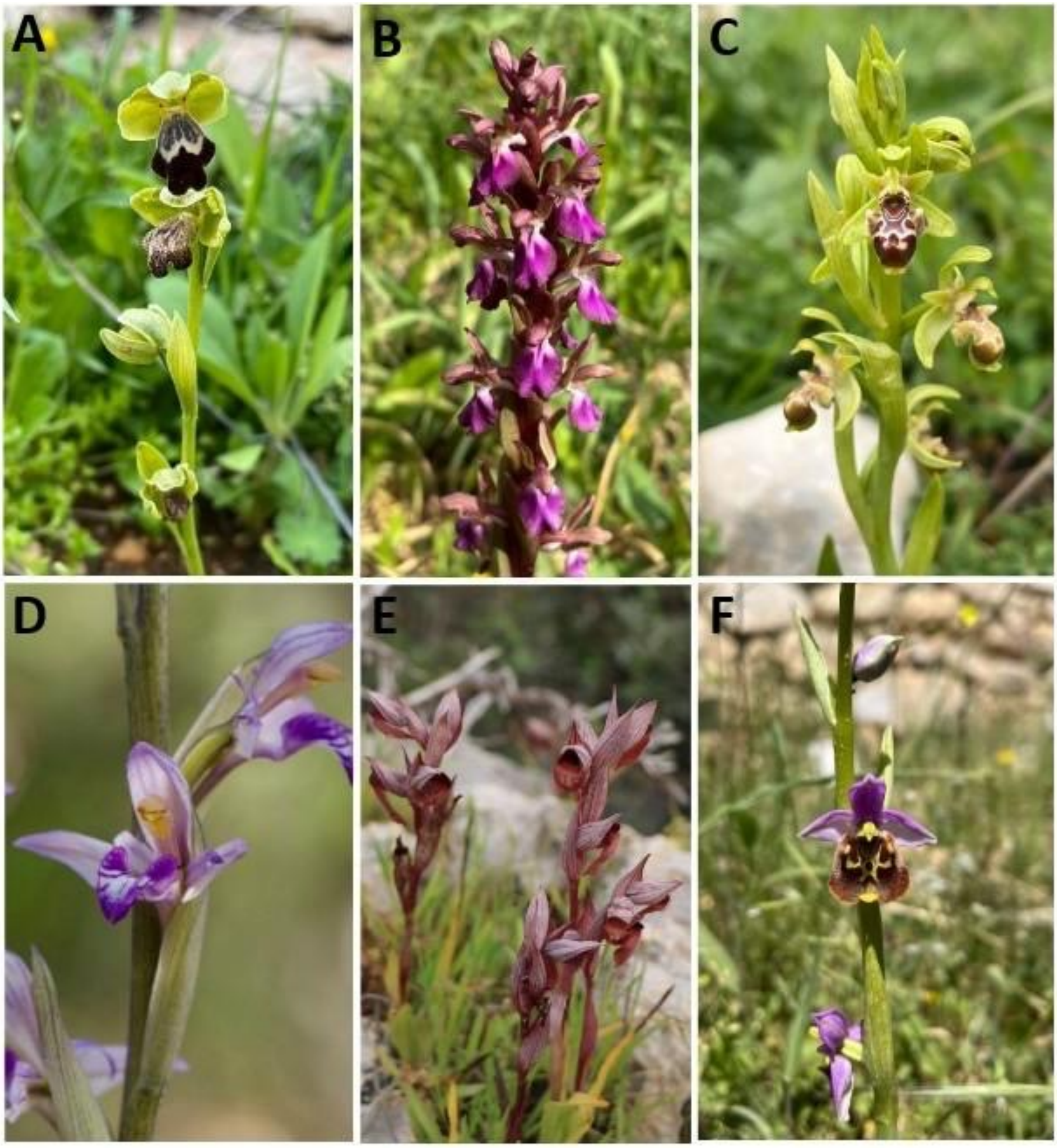

Figure 2. Selected orchid taxa recorded during field surveys in the West Bank, Palestine: (a) *Ophrys omegaifera* H. Fleischm., (b) *Anacamptis collina* (Banks & Sol. ex Russell) R.M. Bateman, Pridgeon & M.W. Chase, (c) *Ophrys umbilicata* Desf., (d) *Limodorum abortivum* (L.) Sw., (e) *Serapias orientalis* (Greuter), (f) *Ophrys holosericea* (Burm.f.) Greuter.

3. *Anacamptis collina* (Banks & Sol. ex Russell) R. M. Bateman, Pridgeon & M. W. Chase, synonym *Orchis collina* Banks & Sol. (see Figure 2b), an uncommon species in Historic Palestine and endangered in Jordan (Taifour, 2017), diverged from the remaining *Anacamptis* species and formed its own distinct clade.
4. *Neotinea tridentata* (Scopoli) R. M. Bateman, Pridgeon & M. W. Chase, synonym *Orchis tridentata* Scop., a common species in Historic Palestine and endangered in Jordan (Taifour, 2017).
5. The *Serapias* genus branch, represented by *Serapias orientalis* (Greuter) synonym *Serapias levantina* H. Baumann & Künkele (see Figure 2e), an uncommon species in Historic Palestine.
6. The *Ophrys* genus branch, comprising

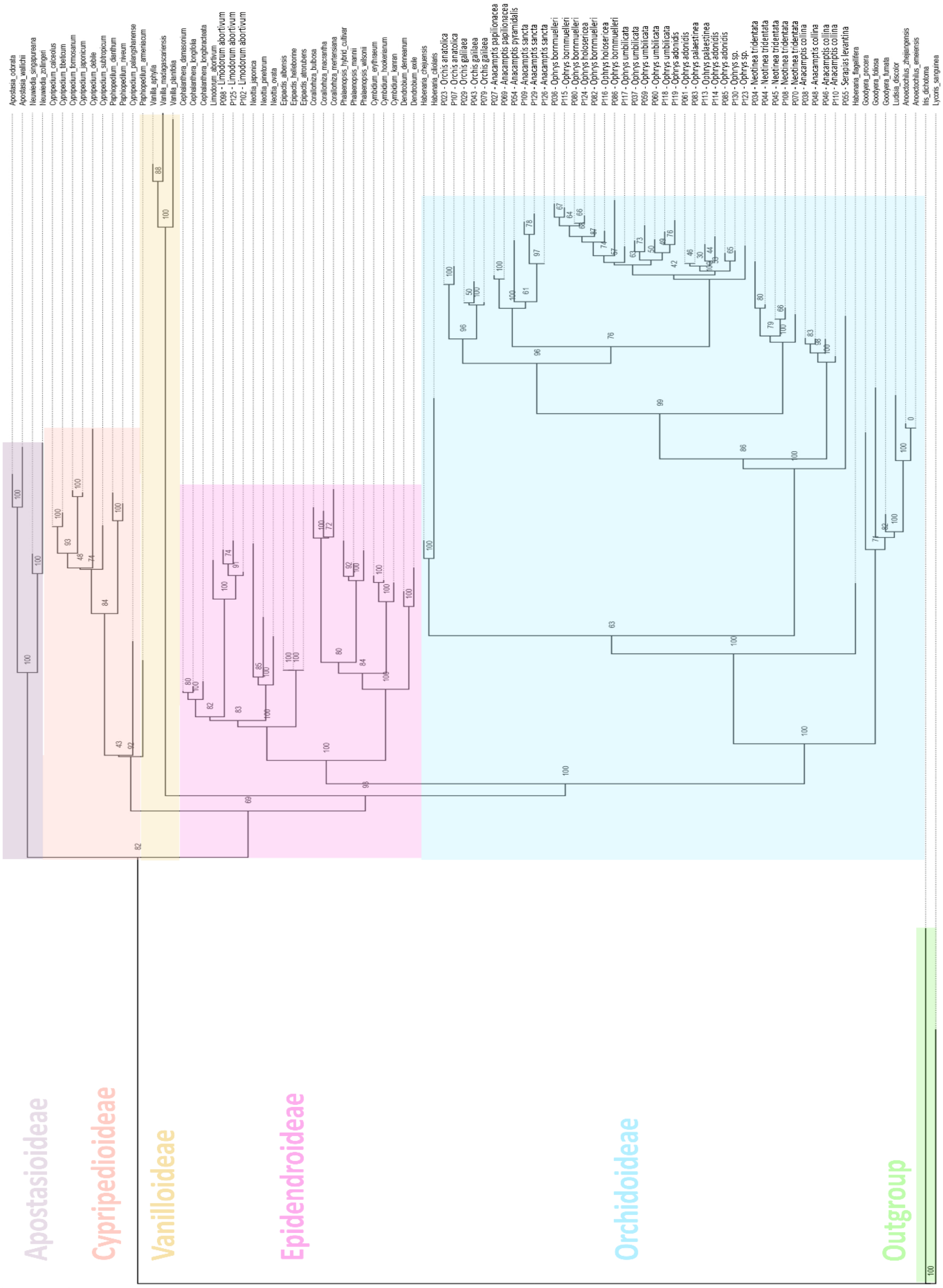

Figure 3. Maximum-likelihood phylogenetic tree of Orchidaceae and Epidendroideae inferred from concatenated sequences of five chloroplast loci. Bootstrap support values are indicated at the nodes to assess the statistical support of the recovered clades. The tree illustrates the phylogenetic relationships among the sampled Palestinian orchid taxa and representative reference sequences across the major Orchidaceae subfamilies, including Epidendroideae and Orchidoideae.

Ophrys spp. (Representative taxa of the genus *Ophrys* are illustrated in Figure 2, including *Ophrys omegaifera* H. Fleischm. (Figure 2a), *Ophrys umbilicata* Desf. (Figure 2c), and *Ophrys holosericea* (Burm.f.) Greuter (Figure 2f), respectively), which formed a monophyletic group. The *Ophrys* genus was recovered as the closest lineage to the *Anacamptis* cluster.

Discussion

The Maximum-Likelihood (ML) phylogenetic tree, produced with the designated outgroups (Figure 3, Supplementary Figure S1.1), recovered the major phylogenetic relationships among the sampled Orchidaceae taxa. To further evaluate the robustness and consistency of the phylogenetic reconstruction, Bayesian Inference (BI) analyses (Supplementary Figure S1.2) and Maximum Parsimony (MP) analyses (Supplementary Figure S1.3) were conducted in parallel with the ML approach. All three analytical methods recovered largely congruent topologies, with consistent placement of all sampled Palestinian specimens (P#) across the principal clades of Orchidaceae. The inferred phylogenetic structure strongly supported the assignment of the investigated taxa within their respective subfamilies and genera. Minor discrepancies among the ML, BI, and MP trees were limited primarily to the phylogenetic placement of Vanilloideae, particularly the genus *Vanilla*, which has been reported in previous phylogenetic studies (Pansarin and Menezes, 2023) as exhibiting unstable placement depending on the analytical method and molecular markers employed. Aside from this variation, the remaining topological relationships were highly consistent across all analyses and agreed with previously published phylogenetic frameworks of Orchidaceae. Additional minor differences were confined to internal branching arrangements within certain clades and were associated mainly with weakly supported nodes; however, these discrepancies did not influence the overall phylogenetic interpretation or taxonomic conclusions of the study. The strong congruence among ML, BI, and MP analyses therefore provides further support for the reliability, stability, and robustness of the inferred evolutionary relationships. The generated inferred trees align with certain previously published phylogenies while providing new insights. The tree structures are congruent with the relationships among the five orchid subfamilies examined. The initial two branches correspond to the Apostasioideae and Cypripedioideae subfamilies, which separate from the remaining four subfamilies. This primary and subsequent split of Cypripedioideae and Vanilloideae is consistent with previous findings (Bernados *et al.,* 2006; Li *et al.,* 2016). Notably, the Epidendroideae subfamily, specifically the Neottieae tribe, exhibits mycoheterotropic characteristics (Zhou and Jin, 2018). Our specimen of *Limordum abortivum* clustered in the Epidendroideae subfamily (Neottieae tribe), is concordant with other published data on this subfamily (Gorniak *et al.,* 2010; Zhou and Jin, 2018). The generated phylogenetic trees (Figure 3, Supplementary Figure S1.2, and Supplementary Figure S1.3) corroborate earlier reports (e.g., Vu *et al.,* 2020) demonstrating a close phylogenetic relationship between *Paphiopedilum* and *Cypripedium* within a monophyletic Cypripedioideae subfamily. Similarly, the trees align with prior literature regarding the relationships among genera within Orchidoideae (*Anacamptis*, *Serapias*, *Orchis*, *Ophrys*, *Neotinea*) (Bateman *et al.,* 2003; Bernardos *et al.,* 2006).

Historically, *Serapias* and *Anacamptis* were occasionally classified under *Orchis* (s.l.); however, cytogenetic evidence supports their recognition as allied yet distinct genera (Tyteca and Klein, 2008). Our data indicate that *Anacamptis* is not strictly monophyletic, with only one branch closely related to the single *Serapias* sequence included. Both genera remain distinct from the monophyletic *Orchis*. Chromosome numbers further support this distinction: *Orchis* s.s. exhibits 2n=42, whereas the *Anacamptis* cluster (including the "Orchis morio" group) and *Serapias* possess 2n=36 (Tyteca and Klein, 2008; DelForge, 2009). Our molecular findings are consistent with these cytogenetic interpretations.

The paraphyly of *Anacamptis*, particularly *A. collina*, merits discussion. Cozzolino et al. (2001) based on ITS sequences, placed *A. collina* within the *Anacamptis* clade of the Eastern Mediterranean. However, other phylogenetic reconstructions demonstrate *A. collina* and *Orchis laxiflora* as distinct, with the latter capable of hybridizing with *Serapias* species (Kretzschmar *et al.*, 2007). Our dataset positions *A. collina* outside the main *Anacamptis* clade. This discrepancy may arise from sequence length: Cozzolino et al. (2001) utilized 226–241 bp fragments, whereas our concatenated sequences encompass up to 4,352 bp. Kreutz *et al.* (2021) classified *A. collina* under the genus *Vermeulenia*, reflecting its phylogenetic divergence from the *Anacamptis* clade. Comparative examination of the sequence data reveals that the *A. collina* clade contains a notably longer psbA gene region than other *Anacamptis* species. This extended psbA sequence supports the observed phylogenetic divergence and underscores the need for additional genomic analyses to fully resolve these taxonomic and evolutionary inconsistencies.

Regarding *Anacamptis papilionacea*, originally described as *Orchis papilionacea* subsp. *palaestina* by H. Baumann & R. Lorenz (2005), its nomenclature has undergone multiple revisions, including assignments to *Anacamptis* and *Vermeulenia*. Delforge (2015) designated it as *Vermeulenia papilionacea* var. *palaestina*, whereas Shifman (2019) reverted to *Anacamptis papilionacea* subsp. *palaestina*. In our phylogenetic trees, this species clustered with *Anacamptis pyramidalis* and *Anacamptis sancta*, confirming its placement within the genus *Anacamptis* rather than *Vermeulenia*.

During field surveys, an atypical individual of *Orchis galilaea* (specimen P079) was observed exhibiting a distinct deviation from the species' characteristic morphology (Figure 4). Typically, *O. galilaea* displays a "stick man" or "clown"-like labellum structure, with two narrow, elongated lateral lobes and a distinctly forked central lobe bearing a small median tooth (Figure 4A; specimen P043). In contrast, specimen P079 lacked a fully bifurcated central lobe, instead presenting a partially fused, webbed appearance (Figure 4B). This unusual morphology prompted consideration of potential taxonomic significance, including the possibility of a novel taxon or hybrid origin; however, the occurrence of only a single individual made such interpretations tentative. Tissue was collected from the atypical specimen in spring 2021 for molecular analysis. Upon revisiting the site in spring 2022, the same individual was relocated, but it exhibited typical morphology. Subsequent DNA sequencing confirmed no genetic differentiation between the atypical specimen (P079) and a typical individual (P043), indicating that the observed variation likely represents phenotypic plasticity rather than underlying genetic divergence.

The *Ophrys* genus formed a monophyletic clade (Figures 3 and 5, Supplementary Figures S1.1, S1.2, and S1.3) comprising three clusters, with closely affiliated species that may hybridize together, showing in molecular phylogenetic trees as mixed branching. Devey *et al.* explains that this ongoing introgression between species displays the "fuzzy margins" of the species clusters as evidence of ongoing introgression and hybridization, as previously documented (Soliva and Widmer, 2003; Devey *et al.*, 2008; Shifman, 2019). The *Ophrys bornmuelleri-Ophrys holosericea* cluster exhibits transitional forms capable of hybridization (Feinbrun-Dothan, 1986; Kreutz *et al.*, 2021). *Ophrys umbilicata* specimens clustered consistently, except for P117, potentially representing *O. umbilicata* subsp. *carmeli*. The third cluster included taxa belonging to the *Ophrys sphegodes* complex, notably *O. palaestinae* and *O. adonidis*, together with an unidentified taxon. *Ophrys*

Figure 4. Morphological comparison of typical (A; specimen P043) and atypical (B; specimen P079) individuals of *Orchis galilaea*.

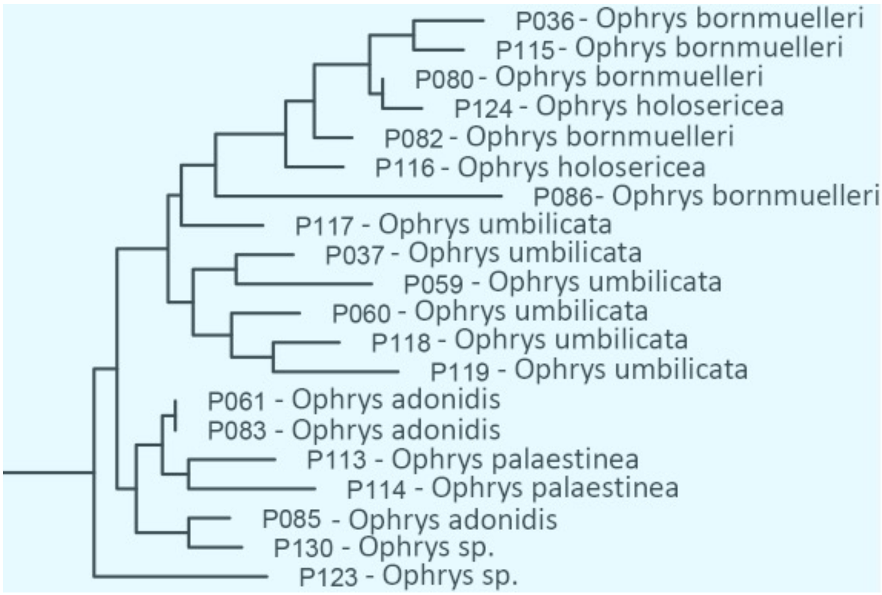

Figure 5. Section of concatenated tree (Figure 3) showing *Ophrys* clade.

Figure 6 *Ophrys palaestinae*: (A) specimen P113 collected for sequencing (photographed 8 April 2022); (B) the same individual at the collection site (photographed 18 April 2023), illustrating the natural (unspread) labellum width, which appears markedly broader when fully spread.

palaestinae, described by Kreutz *et al.* (2021), is morphologically similar to *O. adonidis,* but taller (up to 50 cm vs. 40 cm) with a wider labellum (16–22 mm vs. 9–12 mm) (Figure 6), and a later flowering period (end March–May vs. February–mid-April). Collected specimens (P113, P114) clustered within the *O. adonidis* clade in our phylogenetic analysis (Figure 5).

The current molecular phylogeny clarifies the relationships amid West Bank, Palestine's orchids and thus refines the taxa that conservation must target. In conservation planning, taxa on long, isolated phylogenetic branches such as *Serapias levantina* H.Baumann & Kuenkele warrant special attention (Vitt *et al.*, 2023). By resolving which orchids are evolutionarily distinct or part of young radiation, this study aids in the identification of candidate priority species: since losing an evolutionarily unique orchid (one without close relatives within West Bank, such as *Limodorum abortivum* (L.) Sw.) would erode a disproportionate share of the Epidendroideae subfamily's diversity (Vitt *et al.*, 2023). In practice, the current results can be used to flag such taxa for Red List assessment or *in situ* protection that may deserve focused conservation given its distinct lineage.

The habitats of many of these orchids face increasing anthropogenic threats. Earlier study notes that *all* orchid taxa in this region "suffer from expanding urban areas, increasing land use, overgrazing, fires, changing climatic conditions and decreased precipitation" (Pahl and Qumsiyeh, 2021). Infrastructure urban expansion (roads, settlements) and agriculture have already fragmented or destroyed many orchid habitats (Pahl and Qumsiyeh, 2021). These threats are reflected in Historic Palestine's vascular plant Red List: 28% of native taxa are classified as Critically Endangered, Endangered or Vulnerable (Pahl and Qumsiyeh, 2021). This high proportion of threatened plants underscores the urgency of targeted action. This study recommends explicitly integrating these taxonomic findings into conservation priorities. As Palestine implements its new National Biodiversity Strategy and Action Plan (which emphasizes protection of rare plant species) (Pahl and Qumsiyeh, 2021) alongside the recent assessments of Palestine's protected area network (Qumsiyeh *et al.*, 2023), the need for detailed plant distribution studies to guide conservation is emphasized. Comparative studies of nearby regions reveal unique assemblages, including rare orchids, reinforcing the necessity of local protection (Qumsiyeh and Al-Sheikh, 2023). Improved understanding of phylogenetic relationships provides a robust foundation for conservation planning, thus the clarified species boundaries and phylogenetic distinctiveness from this work should guide those efforts.

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Annex 1: Taxonomic and Specimen Data of Collected Plant Taxa

1. *Anacamptis collina* (Banks & Sol. ex Russell) R. M.Bateman, Pridgeon & M.W.Chase, synonym name: *Orchis collina* Banks & Sol.; Common English Name: Fan-Lipped Orchid

Specimens and localities: PMNH-H0261, Sarra, Nablus Governorate; N: 32.210514, E: 35.189636; Elev: 565m, 22 March 2021; P038, Yaseed, Nablus Governorate; 22 March 2021; P046, Sarra, Nablus Governorate; 22 March 2021; P048, Sarra, Jenin Governorate; P110, Siris, Jenin Governorate; 06 April 2022.

Other localities seen: Jenin Governorate: Faqqua', Siris. Nablus Governorate: Al-Badhan, Osarin, Sebastia, Duma, Aqraba. **Ramallah Governorate:** Al-Mughayer, Turmus Ayya. **Salfit Governorate:** Hares. **Tubas Governorate:** Tamoun.

Characteristics: **Plant:** Grows from bulb. 10–40 cm long. The spur is thick and short and looks like a sac. Cylindrical shapes inflorescence. **Flowers:** 4–30 medium sized flowers. Pale pink to reddish purple in color. **Labellum:** 8–12 mm in length, not split into lobes. Light pink to wine-purple in color. **Flowering in the region:** (January) February to March (April).

Name in Kreutz *et al.* (2021): *Vermeulenia collina* (Banks & Solander ex Russell) P. Delforge

Notes: This species was reclassified as a member of the genus *Anacamptis* instead of *Orchis* based on molecular analysis (Pridgeon et al, 1997), yet Kreutz *et al.* (2021) changed its genus assignation from *Anacamptis* to *Vermeulenia*. This plant is mainly distributed near the Mediterranean and east of it, up to the Caucasus. This is one of the earliest orchid flowers blooming. This flower imitates a nectar-producing flower and tricks bees to pollinate it. Based on molecular analysis, this species is phylogenetically isolated and distant from other *Anacamptis* species.

2. *Anacamptis papilionacea subsp. palastina H.*Baumann & R.Lorenz) H.Kretzschmar, Eccarius & H.Dietr.; synonym name: *Orchis papilionacea* L.; Common English Name: Palestinian Orchid, Pink Butterfly Orchid

Specimens and localities: PMNH-H0099, Al-Makhrour, Bethlehem Governorate; N: 31.713311, E: 35.168644, Elev: 980m, 15 March 2018; PMNH-H0427, Battir, Bethlehem Governorate; N: 31.719444, E: 35.140833, Elev: 598m, 15 February 2013; PMNH-H0443, Faqqua, Jenin Governorate; N: 32.487459, E: 35.399795, Elev: 260m, March 2019; PMNH-H0531, Al-Makhrour, Bethlehem Governorate; N: 31.713311, E: 35.168644, Elev: 980m, March 2019; PMNH-H0622, Dair Jreer, Ramallah Governorate; N: 31.964444, E: 35.295278, Elev: 407m, 24 March 2021; PMNH-H0984, Wadi Al Haramiyeh, Ramallah Governorate; N: 32.003757, E: 35.254351, Elev: 137m, 15 March 2022; PMNH-H1186, Battir, Bethlehem Governorate; N: 31.719444, E: 35.140833, Elev: 598m, 9 March 2022; P027, Al Makhrour, Bethlehem Governorate; 18 March 2021; P032, Al-Makhrour, Bethlehem Governorate; 18 March 2021; P069, Tel Asour, Ramallah Governorate; 24 March 2021.

<u>Other localities seen:</u> **Bethlehem Governorate:** Beit Skarya, Artas, Wadi Cremisan, Wadi Fukin, Husan. **Hebron Governorate:** Al-Kanoub, Al-Qarn, Wadi Quff. **Jenin Governorate:** Arraba Valley, Raba. Jericho Governorate: Sartaba, Fasayil. **Jerusalem Governorate:** Beit Hanina, Nabi Samuel, Biddu, Abu Dis. **Nablus Governorate:** Tell, Al-Badhan, Osarin, Sebastia, Aqraba. **Qalqilya Governorate:** Falamiyah, Jit. **Ramallah Governorate:** Deir Abu Mash'al, Deir 'Ammar/Zarqa, Ein Siniya, Jalazone, Khirbet Tana, Kufr Malik, Ein Qinia, Wadi Al-Zarqa, Wadi Ein-Arik, Beitillu, Ein Al Sakiya, Aboud, Deir Al-Sudan, Surda. **Salfit Governorate:** Hares, Um Safa, Deir Ballut. **Tulkarm Governorate:** Beit Lid, Ras Tira. **Tubas Governorate:** Tayasir.

<u>Characteristics:</u> **Plant:** Grows from bulb. 10–30 cm tall. **Flowers:** 10–30 densely arranged flowers, light lilac to purple in color with short lines and dark purple spots. **Labellum:** 8–12 mm in length. **Flowering in the region:** (January) February to April.

<u>Notes:</u> This species was reclassified as a member of the genus *Anacamptis* instead of *Orchis* based on molecular analysis (Pridgeon et al, 1997), yet Kreutz *et al.* (2021) changed its genus from *Anacamptis* to *Vermeulenia*. Common in the eastern Mediterranean region. This flower imitates a nectar-producing flower and tricks bees to pollinate it.

3. *Anacamptis pyramidalis* (L.) L.C.M. Richard; Common English Name: Pyramidal Orchid

<u>Specimens and localities:</u> PMNH-H0449, Rawabi, Ramallah Governorate; N: 32.010031, E: 35.187191, Elev: 209m, 18 April 2013; PMNH-H0464,Wadi Al Haramiyeh, Ramallah Governorate; N: 32.003757, E: 35.254351, Elev: 137m, 4 May 2013; PMNH-H0576, Azoun, Qalqilya Governorate; N: 32.179766, E: 35.067867, Elev: 170m; 8 April 2019; PMNH-H0662, Dayr Ghassana, Ramallah Governorate; N: 32.044167, E: 35.075833, Elev: 407m, 24 March 2021; P054, Dayr Ghassana, Ramallah Governorate; 24 March 2021.

<u>Other localities seen:</u> **Bethlehem Governorate:** Wadi Al-Makhrour. **Hebron Governorate:** Wadi Quff, Wadi Al Sha'er. **Jenin Governorate:** Raba, Faqou'a. **Jerusalem Governorate**: Biddu. **Nablus Governorate:** Osarin, Tal al Khashabe, Duma, Yaseed, Bani Fadil, Aqraba. **Qalqilya Governorate:** Jayyous, Ras Tira. **Ramallah Governorate:** Deir Abu Masha'l, Deir Ammar, Zarqa, Kufr Malik, Ein Qinia, Wadi Al-Zarqa, Wadi Ein-Arik. **Salfit Governorate:** Hares, Wadi Qana, Deir Ballout, Um Safa.

<u>Characteristics:</u> **Plant:** Grows from bulb, 20–50 cm long. It is pyramid-shaped (hence the name). **Flowers:** approximately 10–50 small to medium sized flowers, pale pink to pink or whitish. **Labellum:** 6–10mm long, split into three equal lobes. There are two bulges that are parallel to one another and perpendicular to the labellum. **Flowering in the region:** end of March to end of April.

<u>Notes:</u> Common in the Mediterranean region, preferring more open habitats than *A. papilionacea subsp. palaestina*. The flower produces nectar in its spurs and is pollinated by various species of butterflies and moths.

4. *Anacamptis sancta* (L.) R.M.Bateman, Pridgeon & M.W.Chase; Common English Name: Holy Orchid, Palestine Orchid

<u>Specimens and localities:</u> PMNH-H1432,

Siris, Jenin Governorate; N: 32.316837, E: 35.310123, Elev: 150m, 20 April 2022; P109, Siris, Jenin Governorate; 06 April 2022; P126, Jenin, Jenin Governorate; 20 April 2022; P129 PMNH Botanical Garden, Bethlehem Governorate; 20 April 2022**.**

<u>**Other localities:**</u> **Nablus Governorate:** Tal al Khashabe, Osarin. **Ramallah Governorate:** Um Safa, Wadi Haramiya. **Salfit Governorate:** Wadi Qana.

<u>**Characteristics:**</u> **Plant:** Grows from bulbs, 15–50 cm long. **Flowers:** 10–40 small to medium-sized flowers, odorless, pale to dark pink or brick red (sometimes white). **Labellum:** 10–15mm in length and is triple lobed (with the middle lobe being the longest). Does not have any spots or streaks. Each lobe has three to four sharp teeth. The labellum is pinkish red in color and the edges are greenish. **Flowering in the region:** End of March (April) to late May (June)

<u>**Notes**</u>**:** The holotype of this species was collected from Palestine, hence the name (Holy Orchid from the Holy Land), in 1759. Morphological distinctive for having one of the longest bracts of its genus (Sevgi *et al.*, 2012). This species is pollinated by honey and bumblebees. It was reclassified as a member of the genus *Anacamptis* instead of *Orchis* based on molecular analysis (Pridgeon *et al.*, 1997), yet Kreutz *et al.* (2021) changed its genus assignment to *Anteriorchis*.

5. *Limodorum abortivum* (L.) Sw.; Common English Name:Violet Limodore, Violet Bird's-nest Orchid

<u>**Specimens and localities:**</u> PMNH-H0258, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793 m, 13 April 2021; PMNH-H0283, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793 m, 13 April 2021; PMNH-H1185, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793 m, 20 April 2021; P098, Cremisan Valley, Bethlehem Governorate; 12 April 2021; P102, Cremisan Valley, Bethlehem Governorate; 12 April 2021; P125, Cremisan Valley, Bethlehem Governorate; 20 April 2021.

<u>**Other localities seen:**</u> **Ramallah Governorate:** Um Safa, Wadi Al-Zarqa.

<u>**Characteristics:**</u> **Plant:** Grows from rhizomes, up to 100 cm long, asparagus-like appearance**.** **Flowers:** Large, lilac-purple and the column head is yellow, five to 25 flowers. **Labellum:** 25–30 mm long, it is divided into two by a lace. **Flowering in the region:** (February) March to May (June).

<u>**Notes:**</u> This is a saprophyte orchid with no green leaves; it does not undergo photosynthesis but rather feeds on rotten organic matter. It is parasitic in that it relies on mycorrhizal fungi. It is the only saprophyte orchid in the region. Unlike most orchids that grow from bulbs, this orchid grows from rhizomes. This species produces nectar and is pollinated by various species of bumblebees. It is a rare species in our region. This species was phylogenetically distant from all other samples sequenced from the region. It was clustered with reference *Limodorum abortivum* sequences downloaded from GenBank (Fig. 3, Supplementary figures S1.2, and S1.3).

6. *Orchis tridentata* Scop.; synonym name *Neotinea tridentata*, Common English Name: Three-toothed Orchid

<u>**Specimens and localities:**</u> PMNH-H0097, Al Makhrour, Bethlehem Governorate; N: 31.713311, E: 35.168643815, Elev: 980m, March 2018; PMNH-H0521, Wadi Al Haramiyeh, Ramallah Governorate; N: 31.993611, E: 35.247500, Elev: 598 m, 6 April 2021; PMNH-H0550, Azoun, Qalqilya Governorate; May 2021; PMNH-H0613, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793 m, 9 March 2021; PMNH-H0771, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793 m, 8 March 2021; PMNH-H0897, Wadi Qana, Salfit Governorate; N: 32.165000, E: 35.129444, Elev: 261m, 14 March 2021; PMNH-H1019, New Nablus, Nablus Governorate; N: 32.209167, E: 35.247778, Elev: 827m, 10 April 2022; P034, Al-Makhrour Valley, Bethlehem Governorate; 18 March 2021;

P044, New Nablus, Nablus Governorate; 22 March 2021; P045 , New Nablus, Nablus Governorate; 22 March 2021; P070, Tal Asour, Ramallah Governorate; 24 March 2023; P108, Cremisan Valley, Bethlehem Governorate; 29 March 2021.

Other Localities seen: **Bethlehem Governorate:** Beit Skarya. **Hebron Governorate:** Abu Soda, Ad-Dhahiriya, Al-Qarn. **Jenin Governorate:** Raba, Faqou'a. **Jerusalem Governorate:** Abu Dis, Nabi Samuel. **Nablus Governorate:** Tell, Osarin. **Qalqilya Governorate:** Khallet Afana, Ras Tira. **Ramallah Governorate:** Deir Abu Masha'l, Deir Ammar, Jalazone, Kufer Malik, Um Safa, Ein Qinia, Wadi Ein-Arik, Beitillu. **Salfit Governorate:** Hares. **Tulkarem Governorate:** Beit Lid. **Ramallah Governorate:** Wadi Al-Zarqa.

Characteristics: **Plant:** Grows from bulb, 20–45 cm long. This orchid is referred to as the three-toothed flower because of its three sepals that are sharp at the tip, giving the flower the look of three teeth. Inflorescence is spherical to ovate. **Flowers:** Small to medium sized, pale pink, pink, pale to dark violet, up to 20–50 flowers and are densely packed. **Labellum:** 8–12 mm long and divided into three slightly serrated lobes (the central lobe is larger than the lateral lobes and is divided into two wide lobes). Light purple to deep reddish purple and is dotted with dark purple spots. **Flowering in the region:** (February) March to April (May).

Name in Kreutz *et al.* (2021): *Odontorchis tridentata* (Scopoli) D. Tyteca & E. Klein.

Notes: Very common in Mediterranean habitats, as well as central Europe and the Caucasus. This species is a member of the genus *Neotinea*. The shift from *Orchis* to *Neotinea* is based on modern phylogenetic revisions within Orchidaceae, particularly molecular studies that reassigned several species formerly placed in *Orchis* (Pridgeon *et al.*, 1997), yet Kreutz *et al.* (2021) changed its genus from *Neotinea* to *Odontorchis*.

7. *Ophrys adonidis* A.Camus & Gomb.**, synonym of *Ophrys sphegodes* subsp. *taurica*, Common English Name: Adonis Ophrys**

Specimens and localities: PMNH-H0397, Faqou'a, Jenin Governorate; N: 32.486894, E: 35.394425, Elev: 598m; March 2019; PMNH-H0659, Faqou'a, Jenin Governorate; N: 32.486894, E: 35.394425, Elev: 598m, 29 March 2021; PMNH-H0671, Wadi Al-Zarqa, Ramallah Governorate; N: 32.043333, E:35.091667, Elev: 367m; 24 March 2021; P061, Wadi Al Zarqa, Ramallah Governorate; 24 March 2021; P083, Faqou'a, Jenin Governorate; 29 March 2021; P085, Faqou'a, Jenin Governorate; 29 March 2021.

Other Localities seen: Jerusalem Governorate: Biddu. **Nablus Governorate:** Osarin, Yaseed. **Ramallah Governorate:** Deir Abu Masha'l, Deir Ammar, Ein Qinia, Um Safa, Wadi Al-Zarqa, Wadi Haramiya. **Qalqilya Governorate:** Ras Tira. **Salfit Governorate:** Hares.

Characteristics: **Plant:** Grows from bulb, 30–40 cm in height. **Flowers:** Medium size to large, 3–15 in a plant and distributed along the stem sparsely, dark brown to brown black in color. **Labellum:** 12 to 15 mm long and 9 to 12 mm wide. Labellum is wide at the base and sharp at the tip and has a greenish-brown appendage, divided into three lobes. It has two parallel lengthwise stripes in a shiny blue purple and sometimes make the Greek letter π. **Flowering in the region:** (January) February to mid-April, early.

8. Ophrys sp.

Specimens and localities: P123, PMNH, Bethlehem, 20 April 2022; P130, PMNH, 26 April 2022. Old specimen; did not allow definitive identification of species.

9. *Ophrys bornmuelleri* M. Schulze Common English Name: Bornmueller's Bee Orchid, Bornmueller's Ophrys

Specimens and localities: PMNH-H0262, Yaseed, Nablus Governorate; N: 32.300596, E: 35.277288, Elev: 556m, 22 March 2021; P36 Yaseed, Nablus Governorate; 22 March 2021; P080, Raba, Jenin Governorate; 29 March 2021; P086, Faqou'a, Jenin Governorate; 29

March 2021; P115, PMNH Botanical Garden, Bethlehem Governorate; 11 April 2022.

Other Localities seen: **Jenin Governorate:** Faqqua’. **Nablus Governorate:** Yaseed. **Qalqilya Governorate:** Ras Tira. **Ramallah Governorate:** Al-Mughayer, Deir Abu Masha’l, Deir Ammar, Um Safa, Wadi Al-Zarqa, Wadi Haramiya. **Salfit Governorate:** Wadi Qana. **Tulkarm Governorate:** Beit Lid.

Characteristics: **Plant:** Grows from bulbs, 15–50 cm tall. **Flowers:** Small to medium sized, 3–15 flowers, with no spur. Sepal yellowish green to pale green, rarely pale pink. **Labellum:** 6.5–10 mm in length and 8–12.5 mm in width (when spread). It is almost triangular and not divided into lobes. The labellum is folded down the middle, making the flower look like a tent. The center of the labellum is dark brown, and its edges are light brown with white-silvery hairs. The labellum has a wide brown-light-purple to brown-wine-purple stripe, usually in the shape of an H, but other patterns can be found as well. The tip of the labellum creates a deep notch with a green-yellow appendage that rises (usually split into three flat teeth). **Flowering in the region:** Mid-March to mid-April.

Notes: The original type of this species was first collected in Palestine in 1899. It is distributed in the Eastern Mediterranean region.

10. *Ophrys episcopalis* Poir., synonym name: *Ophrys holoserica* subsp. holosericea (Poir.), Common English Name: Late Bee-Orchid

Specimens and localities: PMNH-H0396, New Nablus, Nablus Governorate; N: 32.209167, E: 35.247778, Elev: 827m, 14 May 2020; P124, Jabal Hreesh, Jenin Governorate; 04 April 2022; P116, PMNH Botanical Garden, Bethlehem Governorate; 11 April 2022.

Characteristics: **Plant:** Grows from bulb, 15–50 (60) cm long. Sparse with flowers, and the sepals are ovate, wide and pinkish green to intense purple in color, with green arteries. **Flowers:** 2–10 flowers. **Labellum:** Convex and almost square and is not divided into lobes. The labellum is 10–15 mm long and 13–20 mm wide (when spread). **Flowering in the region:** (March) April to May.

Notes: This species is found in the Eastern Mediterranean region. It is known to hybridize with *Ophrys bornmuelleri* and *Ophrys apifera*. There is great dispute about what this species that we have in Palestine should be called. Shifman (2019) refers to it as *Ophrys episcopalis* (Poiret) in reference to the same *Ophrys* that is found in Crete. Kreutz (2021) refers to it as *Ophrys libanotica*.

11. *Ophrys palaestinae* Kreutz & Shifman; Common English Name: Palestine Ophrys

Specimens and localities: P113, PMNH Botanical Garden, Bethlehem Governorate; 8 April 2022; P114, PMNH Botanical Garden, Bethlehem Governorate; 11 April 2022.

Characteristics: **Plant:** Grows from bulbs, 30–50 cm long. **Flowers:** Medium size to large, 4–8 flowers, dark brown to brown black in color. **Labellum:** 15–20 mm long and 16–22 mm wide. Labellum is wide at the base and sharp at the tip and has a greenish-brown appendage, divided into three lobes. It has two parallel lengthwise stripes in a shiny blue purple and sometimes make the Greek letter “pi”. **Flowering in this region:** March to April (May), late.

Notes: It is distributed in the Eastern Mediterranean. This is one of the most beautiful *Ophrys* taxa. Compared to the *Ophrys adonidis*, this plant is about 10 cm longer (up to 50 cm long), the flowers are about twice the size, and the flowering period is about three weeks later. *Ophrys adonidis* flowers from (January) February to mid-April (early), whereas *Ophrys palaestinea* flowers from the end of March to April (May), late. In his remarks Kreutz *et al.* (2021) says that *Ophrys adonidis* starts to fade by the end of March, and *Ophrys palaestinea* flowers not before early April.

12. ***Ophrys umbilicata*** **subsp.** ***beerii*** **Shifman, synonym name:** ***Ophrys umbilicata*** **subsp.** ***umbilicata*****; Common English Name: Beeri's Ophrys**

Specimens and localities: PMNH-H0624, Dair Jreer, Ramallah Governorate; N: 31.964444, E: 35.295278, Elev: 407m, 24 March 2021; PMNH-H0946, Sarra, Nablus Governorate; N: 32.210514, E: 35.189636; Elev: 565m, 22 March 2021**;** P037, Yaseed, Nablus Governorate; 22 March 2021; P047, Sarra, Nablus Governorate; 22 March 2021; P060, Al-Zarqa, Ramallah Governorate; 24 March 2021; P117, PMNH Botanical Garden, Bethlehem Governorate; 11 April 2022; P118, PMNH Botanical Garden, Bethlehem Governorate; 11 April 2022.

Other Localities seen: **Hebron Governorate:** Abu Soda. **Jenin Governorate:** Faqou'a. **Jerusalem Governorate:** Abu Dis, Nabi Samuel, Biddu. **Jericho Governorate:** Sartava, Wadi Qelt. Nablus Governorate: Al-Badhan, Osarin. **Ramallah Governorate:** Khirbit Ein Nabi, Kufer Malik, Al-Mughayer, Um Safa, Ein Qinia, Wadi Al-Zarqa, Wadi Ein-Arik, Wadi Haramiya, Wadi Musrara. **Salfit Governorate:** Hares. **Tulkarm Governorate:** Beit Lid, Falamiyeh.

Characteristics: **Plant:** Grows from bulb, 10–40 cm tall. **Flowers:** Small sized, 4–15 flowers, with no spur. Sepals are predominantly greenish, whitish, or whitish pink to stained pink, with a distinct dark green mid-vein. **Labellum:** 7.5–9.5 mm in length and 8–10.5 mm in width. Labellum is strongly convex, and lateral edges reflexed at an angle of about 90 degrees. **Flowering in the region:** December to April.

Notes: The original type of this species was first collected in Historic Palestine in 2010. This species is endemic to Palestine.

13. ***Orchis anatolica*** **Boiss., Common English Name: Anatolian Orchid**

Specimens and localities: PMNH-H0100, Al-Makhrour, Bethlehem Governorate; N: 31.713311, E: 35.168643815, Elev:980m, 15 March 2018; PMNH-H0339, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793 m, 08 March 2021; PMNH-H0359, Al-Qarn, Hebron Governorate; N: 31.623056, E: 35.129444, 908 m, 31 March 2021; PMNH-H0920, New Nablus, Nablus Governorate; N: 32.209167, E: 35.247778, Elev: 827m, 22 March 2021; PMNH-H1000, New Nablus; Nablus Governorate; N: 32.209167, E: 35.247778, Elev: 827m, 14 March 2022; P023, Al-Makhrour, Bethlehem Governorate; 18 March 2021; P107, PMNH Botanical Garden, Bethlehem Governorate; 22 March 2022.

Other Localities seen: **Bethlehem Governorate:** Nahalin, Beit Skarya, Irtas, Wadi Fukeen, Battir. **Hebron Governorate:** Abu Soda. **Jerusalem Governorate:** Abu Dis, Nabi Samuel, Biddu, East of Al Bireh. **Qalqilya Governorate:** Ras Tira, Jit. **Ramallah Governorate:** Deir Abu Masha'l, Deir Ammar, Um Safa, Ein Qinia, Wadi Al-Zarqa, Wadi Ein-Arik, Wadi Haramiya, Bitlillu, Dayr Ghassana, Beit Rima. **Salfit Governorate:** Hares, Wadi Qana. **Tubas Governorate:** Tarama, Tayasir.

Characteristics: **Plant:** Grows from bulbs, 15–40 cm long. **Flowers:** Medium sized, 6–25 sparse flowers and have a long spur. Wine-purple in color and dotted with brown-purple spots. Sometimes, it can have white flowers without dots. **Labellum:** 8–13 mm long and divided into three lobes. The lateral lobes are rounded, while the central lobe is cuneate, elongated with a depression at the tip. **Flowering in the region:** (February) March to April (May).

Notes: This species has variable flower colors; it can be purple or white in color. Common in the eastern Mediterranean region. In Arabic, this orchid is referred to as "Qarn al-Ghazzal" which means gazelle horn because its long spur looks like a gazelle's horn. This plant is pollinated by various bees because it imitates nectar-producing flowers.

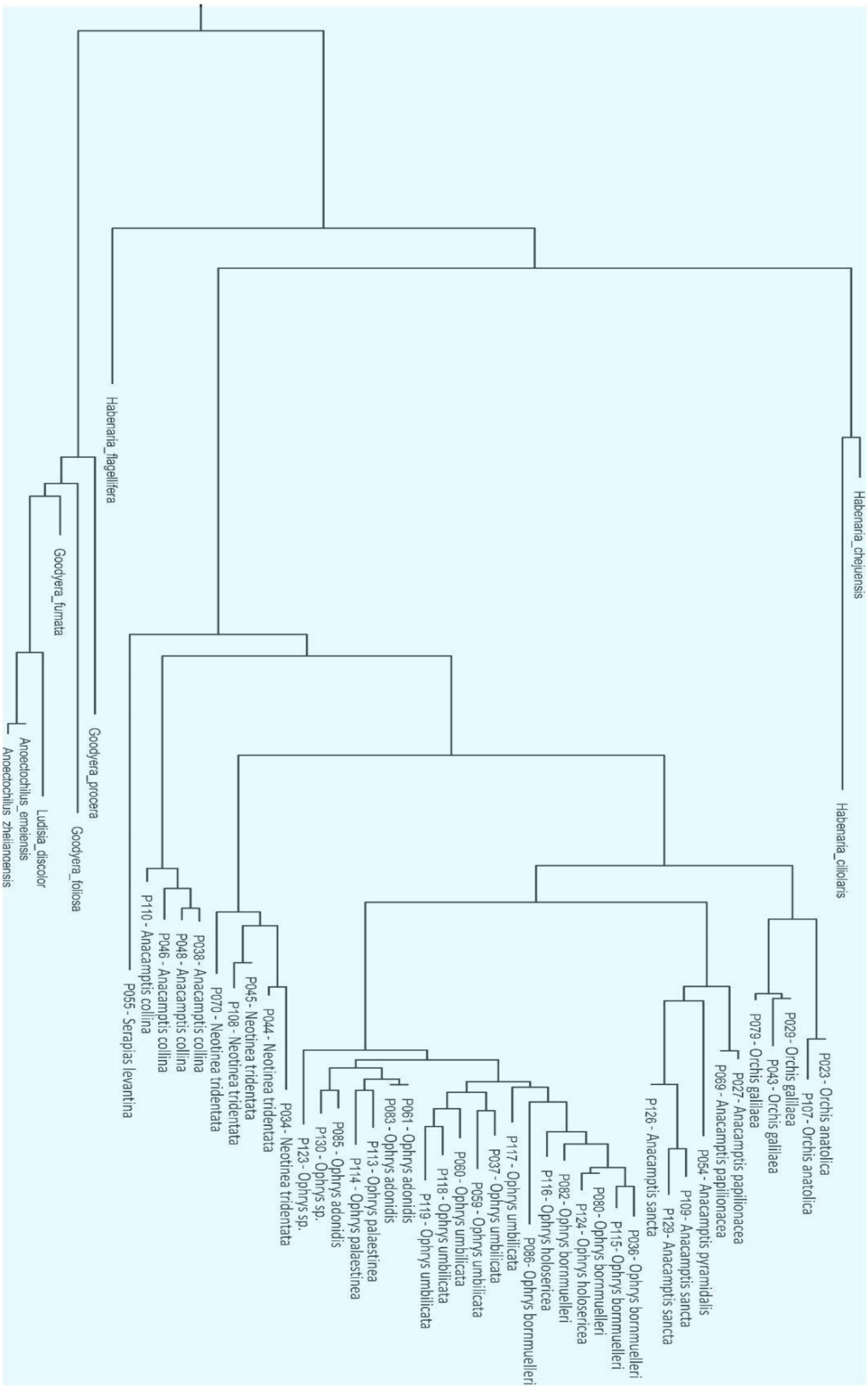
14. ***Orchis galilaea*** **(Bornm. & M. Schulze) Schlechter; Common English Name: Galilee Orchid**

Specimens and localities: PMNH-H0554, Luban e-Sharkiya, Nablus Governorate; N: 31.976667, E: 35.282778, Elev: 930m, 24 March 2021; PMNH-H0596, Wadi Al-Haramiyeh, Ramallah Governorate; N:31.993611, E:35.247500, Elev: 598m, 12 March 2016; PMNH-H0747, Luban e-Sharkiya, Nablus Governorate; N: 31.976667, E: 35.282778, Elev: 930m, 24 March2021;PMNH-H0770,CremisanValley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev:793m, 8 March 2021; PMNH-H0909, Cremisan Valley, Bethlehem Governorate; N: 31.726286, E: 35.172740, Elev: 793m, 8 March 2021; PMNH-H0942, New Nablus, Nablus Governorate; N: 32.217500, E: 35.244167, Elev: 611m, 22 March 2021; P029, Al-Makhrour Valley, Bethlehem Governorate; 18 March 2021; P043, New Nablus, Nablus Governorate; 22 March 2021; P079, Luban e-Sharkiya, Nablus Governorate; 24 March 2021.
Other Localities seen: **Bethlehem Governorate:** Beit Skarya, Wadi Al-Makhrour. **Hebron Governorate:** Wadi Quff. **Jerusalem Governorate:** Biddu. **Nablus Governorate:** Tal al Khashabe. **Ramallah Governorate:** Dayr Ghassana, Beit Rima, Deir Abu Masha'l, Deir Ammar, Jalazone RC, Um Safa, Ein Qinia, Wadi Al-Zarqa, Wadi Ein-Arik. **Tubas Governorate:** Tayasir. **Jenin Governorate:** Faqqua'.
Characteristics: **Plant:** Grows from bulb. 20–70 cm long. **Flowers:** Dense in flowers, about 15–35 medium sized flowers. The flowers have variable colors from white to yellow, to purple. The most common color though is the yellow form. **Labellum:** The labellum looks like a stick man and almost has a "clown" shape. The inflorescence is a cylindrical shape. 7–10 mm long, is divided into three lobes. The lateral lobes are narrow, elongated and slightly bent inwards. The central lobe is forked, with a small "tooth" in the center. **Flowering in the region:** (January) February to April (May).

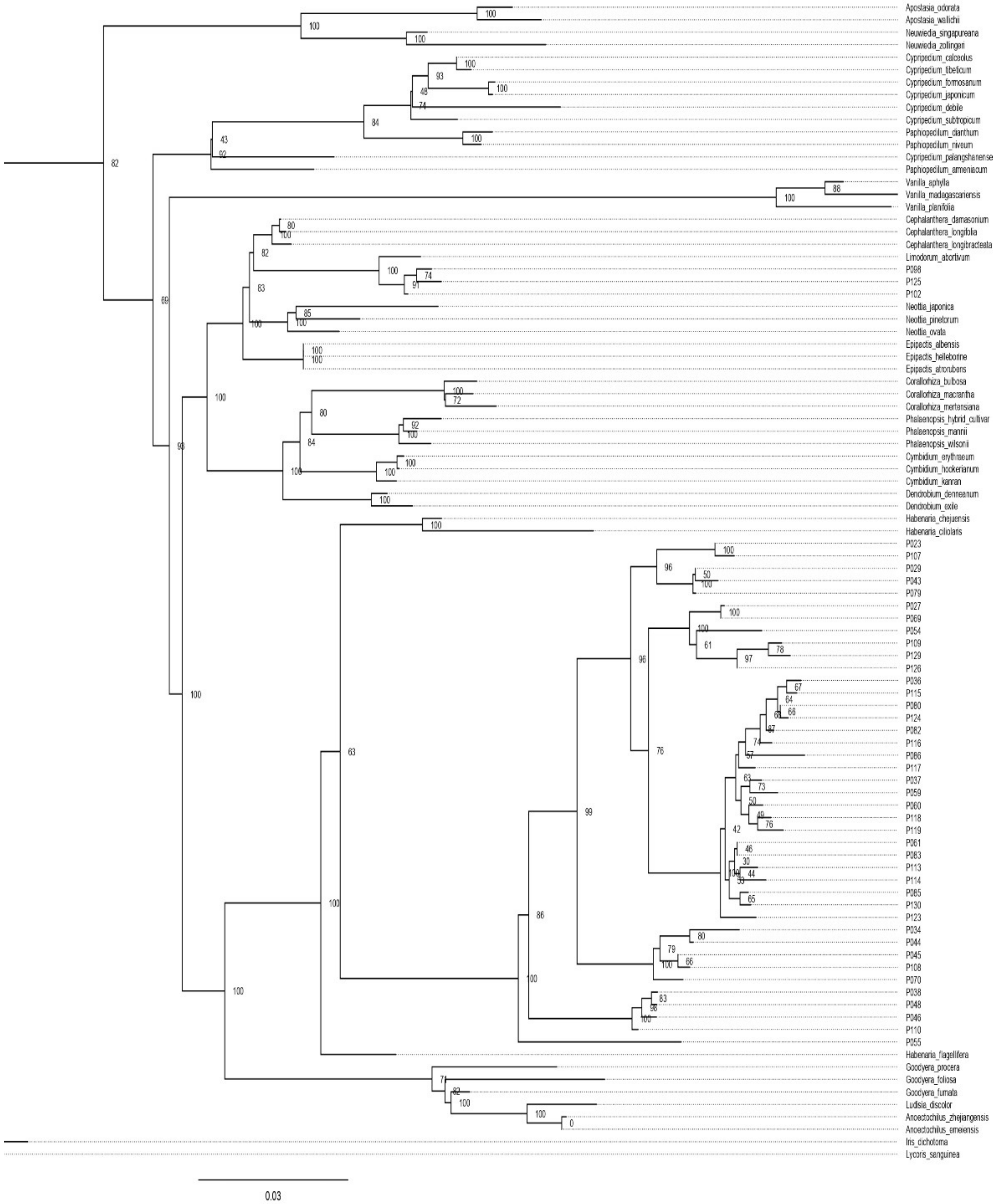
Notes: The original type of this species was first collected in Palestine in 1898. Common in the eastern Mediterranean region. The flower has a scent that is like the pheromone secreted by the female *Lassiglossum marginatum* bee, so it attracts male bees of that species to pollinate it.

15. ***Serapias levantina*** **Baumann & Künkele; Common English Name: Levantine tongue-orchid**

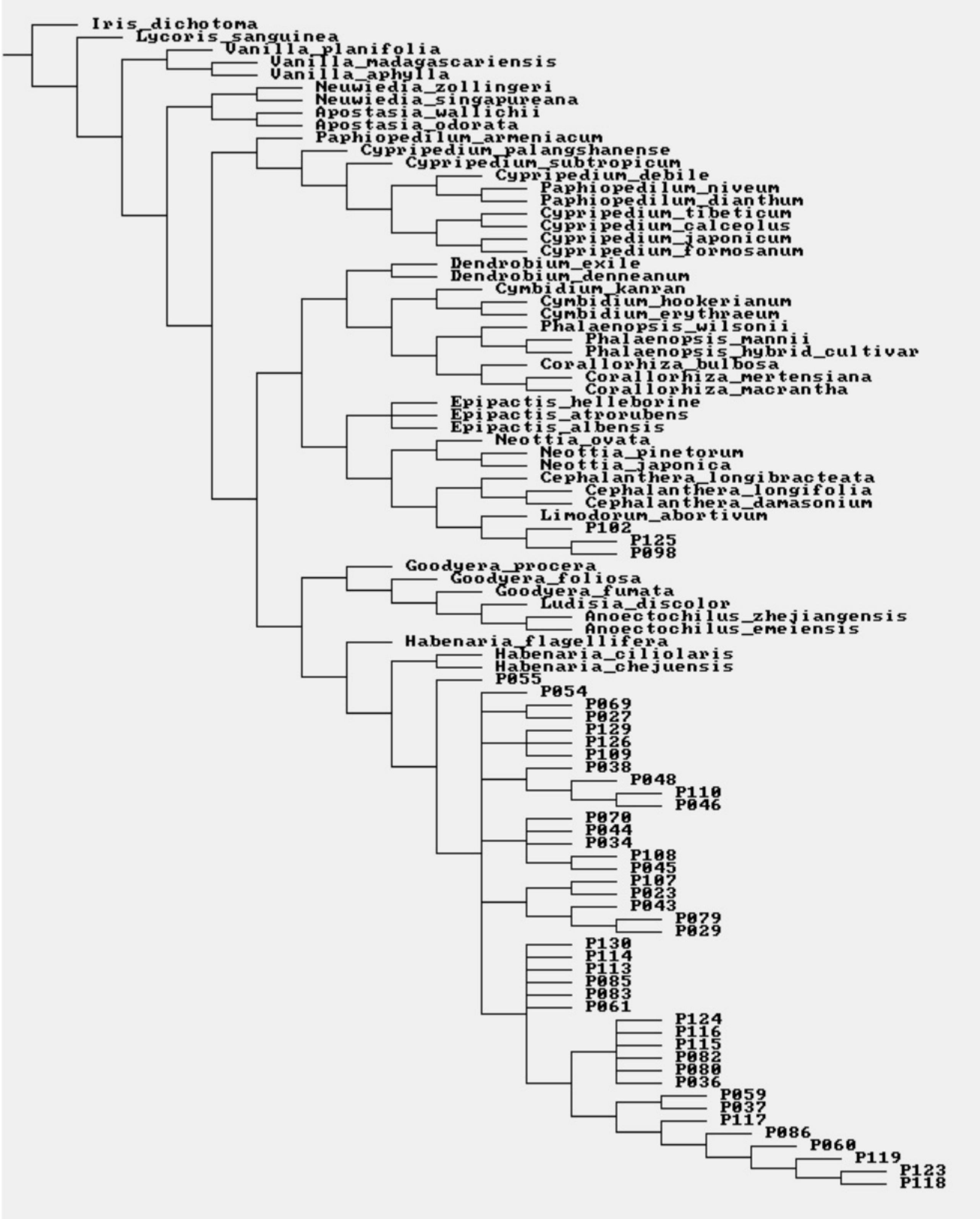
Specimens and localities: PMNH-H0666, Dayr Ghassana, Ramallah Governorate; N: 32.044167, E: 35.075833, Elev: 407m, 24 March 2021; PMNH-H0890, Wadi Qana, Salfit Governorate; N: 32.165000, E: 35.129444, Elev: 261m, 14 March 2021**;** P055, Dayr Ghassana, Ramallah Governorate; 24 March 2021.
Other Localities seen: **Ramallah Governorate:** Deir Abu Masha'l, Deir Ammar, Al-Mughayer, Um Safa, Wadi Al-Zarqa, Wadi Haramiya. **Salfit Governorate:** Wadi Qana.
Characteristics: **Plant:** Grows from bulb. 20–60 cm long. **Flowers:** 3–10 large flowers. Bordeaux color, with reddish arteries. Flowers do not have a spur. **Labellum:** 25–45 mm long. **Flowering in the region:** (January) February to April (May).
Notes: Common in the eastern Mediterranean region. This plant imitates holes in the ground that house bugs and male bees, and therefore it attracts these insects to pollinate it.



Supplementary Figure S1.1. Maximum-likelihood phylogenetic tree of Orchidodeae inferred from concatenated DNA sequences. To enhance clarity and resolution, a cropped version focusing specifically on the Orchidodeae clade is provided, allowing improved visualization and interpretation of the relationships among the study taxa within this lineage.



Supplementary Figure S1.2. Bayesian inference phylogenetic tree of the studied Orchidaceae taxa inferred from concatenated DNA sequence data. Posterior probability support values and bootstrap values are indicated at the nodes to assess branch support and phylogenetic confidence. The Bayesian topology was largely congruent with the maximum-likelihood analysis, recovering consistent placement of all sampled Palestinian specimens (P#) within their respective major clades and subfamilies.



Supplementary Figure S1.3. Maximum Parsimony (MP) phylogenetic tree of Orchidaceae inferred from concatenated sequences of five chloroplast loci. The MP analysis recovered a topology largely congruent with the Maximum-Likelihood and Bayesian Inference analyses, with consistent placement of the sampled Palestinian orchid specimens (P#) across the major Orchidaceae clades.