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Jordan Journal of Natural History

Editorial Preface

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At the end of this preface, would like to thank our readers and authors for their continuing interest in JJNH, and each member of our editorial and review boards for their continued hard work, support and dedication, which made it possible to bring another new issue of JJNH to the multidisciplinary international audience. We very much appreciate your support as we strive to make JJNH one of the most leading and authoritative journals in the field of Natural History Sciences.

June, 2026

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The Jordan Journal of Natural History (JJNH) is an open access international scientific journal publishing original research and reviews in nature history in its broadest sense. This is taken to include conservation biology, botany, geology, paleontology, zoology, and ecology, including a broad range of systematics papers encompassing traditional taxonomic revisions and descriptions, cladistics analyses and molecular phylogenetic. The Jordan Journal of Natural History is published by the Nature Conservation Monitoring Centre at the Royal Society for the Conservation of Nature, Jordan.

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Hamidan, NA, Geiger, MF and Freyhof, J. 2014. *Garra jordanica*, a new species from the Dead Sea basin with remarks on the relationship of *G. ghorensis, G. tibanica* and *G. rufa* (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, 25(3): 223-236.

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Determinants of Roost Selection by the Indian Flying Fox (*Pteropus medius*) in Northern Kerala, India: Implications for a Near-Threatened Species

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Abstract

Bat roosting sites are critically important to bats; however, only a few studies are available on the factors influencing the roost selection of the Indian Flying Fox, *Pteropus medius*, in India. Such information is essential for wildlife managers to make decisions on conservation of bat roosts, especially in unprotected areas. Surveying 31 roost sites spread across three districts of Kerala, India, we assessed the determinants of roost selection by *P. medius* between November 2017–March 2018. The abundance of *P. medius* was assessed using the total roost count method. The roost-site attributes *viz.* environmental factors (wind speed, temperature, humidity, elevation, distance nearest to water body), tree characteristics (number of roost trees, nativity of roost trees, tree age, tree height, tree bole height, crown height, crown width, diameter at breast height [DBH], foliage cover, disturbance factors [distance nearest to main roads, human settlements, mobile phone towers]) and other anthropogenic pressure were assessed using standard methods. The abundance of *P. medius varied* significantly in relation to roost sites, roost tree species and their families, and years of roost existence. Among the plant species, *Ficus benghalensis* supported the highest number of bats, followed by *Ficus drupacea*, while *Areca catechu* had the lowest number. Attributes, namely tree height, tree crown length, tree crown width, DBH, canopy cover, distance nearest to human settlement and water body, varied significantly between roost and non-roost trees. The abundance of bats increased with DBH, temperature, tree height, crown width, wind speed, area of roost existence, bole height, canopy cover and less anthropogenic pressure. These factors explained 43% of the variation in bat abundance. Hence, the present study points out that the officials and private stakeholders in Kerala should maintain at least a few mature, native tree species with large canopies for providing suitable roost habitats for the threatened *P. medius.*

Keywords: Abundance, Determinants, Roost sites, Indian Flying Fox, Northern Kerala

Introduction

The Indian Flying Fox (*Pteropus medius*) is currently classified as Near Threatened, based on an estimated population decline of 25–29% over three generations, both in the past and projected into the future (Ahmed *et al.*, 2025). The population decline of *Pteropus medius* is driven by increasing persecution due to orchard raiding, and widespread hunting for bushmeat and traditional medicine. Furthermore, habitat loss and degradation have occurred as roosting sites are increasingly destroyed by urban development and agricultural expansion. Roost-site selection is a vital aspect for the Indian Flying Fox, *Pteropus medius,* that provides shelter against unpredictable weather, facilitates better access to mates, potential cooperation, reduces predation, and increases the foraging efficiency (Sagot, 2016, Dias-Silva *et al.*, 2018, Salvi *et al.*, 2024). Bats are the only flying mammals that use roosts nearly 15 hours/day, and thus diurnal roost selection

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is an important component to understand the habitat requirements for conservation planning (Barclay and Kurta, 2007). Habitat selection by bats for foraging, roosting, swarming, and other uses is typically linked to factors such as microclimatic conditions, vegetation structure, and resource availability (Ruczyński *et al.*, 2010; Ramos Pereira *et al.*, 2010; Barros, 2014; Champness *et al.*, 2023). Globally, large number of studies have been carried out to estimate the effect of habitat loss and fragmentation, toxins, and anthropogenic and poaching pressures on bat species (Willis and Brigham, 2005; O'Keefe *et al,*. 2009; Hahn *et al.*, 2014; Duarte *et al.*, 2015; Kumar and Kanaujia, 2017).

Pteropus medius falls under the family Pteropodidae, and *Pteropus* is the largest bat genus in India (Vendan and Kaleeswaran, 2011). They live in large diurnal roosts, which comprise several hundred individuals, usually in large trees called 'camps' along with suitable topographic features that offer protection against strong wind, assist them in thermoregulation and provide easier flight (Gulraiz *et al*., 2015). However, populations of *P. medius* have been declining across their distribution range, largely due to increasing anthropogenic pressures such as habitat destruction, agricultural expansion, and urban development (Mildenstein *et al*., 2005, Stier and Mildenstein, 2005). Conflict with fruit growers, owing to crop damage caused by bats, has further heightened their vulnerability (Singaravelan and Marimuthu, 2004; Molur *et al.,* 2005). In Kerala, the rapid pace of urbanization, expansion of cash crop plantations, infrastructure development, and other land-use changes continue to exert substantial pressure on forest habitats and biodiversity, including tree-roosting fruit bats like *P. medius*. They are the most misunderstood species, still treated as vermin and listed in Schedule V due to their negative tendencies enroots for fruit farms, but the benefits of its pollination and seed propagation (Singaravelan and Marimuthu, 2004).

There are still relatively few data available on roost sites of *P. medius* in the Indian subcontinent (Chakravarthy and Yeshwanth, 2008; Hahn *et al*., 2014; Gulraiz *et al.*, 2015; Kumar *et al*., 2017; Kumar *et al*., 2022; Kumar and Kanaujia, 2017). Therefore, more studies on *P. medius* are needed with more comprehensive approaches to enable forest managers and officials to make informed decisions concerning the conservation of bats along with their roosts. This is especially important in the Indian state of Kerala, where no ample attempts have been made to date on bat conservation. Existing research on the roost ecology of *P. medius* in the Indian subcontinent remains limited (Chakravarthy and Yeshwanth, 2008; Hahn *et al.*, 2014; Gulraiz *et al*., 2015; Kumar *et al*., 2017; Kumar *et al*., 2022; Kumar and Kanaujia, 2017). Therefore, there is a pressing need for region-specific studies that can inform conservation planning and management. This study aims to address that gap by documenting the ecological and anthropogenic factors influencing roost-site selection by *P. medius* in northern Kerala. By generating baseline data, this research intends to support the conservation strategies for bats and their habitats in the region.

Materials and Methods

Study Area

The study was conducted in the northern part of Kerala, a state located along the southwestern coast of Peninsular India, between 8°18′–12°48′ N latitude and 74°52′–77°22′ E longitude. Covering an area of 38,863 km², Kerala accounts for 1.8% of India's total geographical area. It is bordered by Karnataka to the north and northeast, Tamil Nadu to the east, and the Arabian Sea to the west, with a coastline extending approximately 590 km. The present study focused on three districts in northern Kerala—Kasaragod, Kannur, and Wayanad—collectively referred to as the "crown of Kerala" due to their rich ecological diversity. These districts are bounded by the Western Ghats to the east, Kozhikode and Wayanad to the south, the Lakshadweep Sea to the west and Kasaragod to the north (Figure 1).

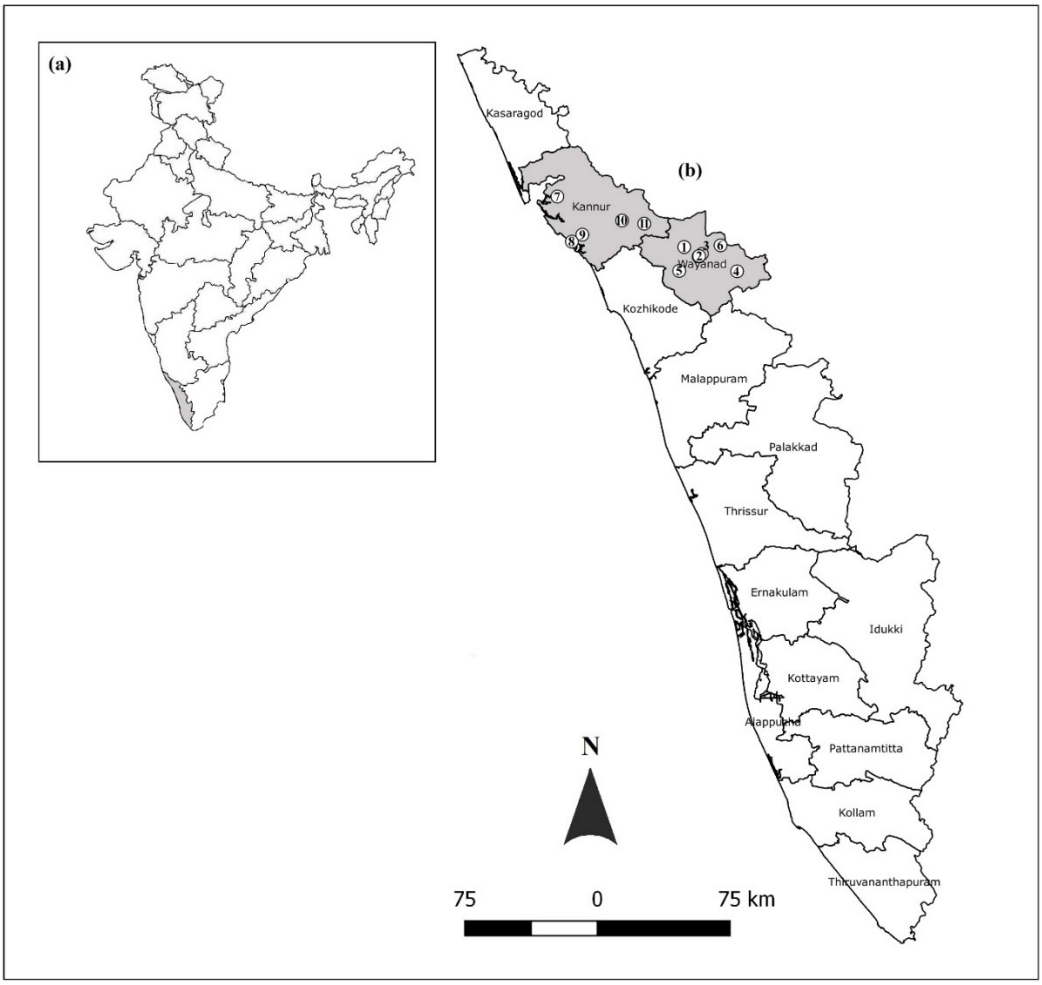

Figure 1. Study area map

Preliminary survey and methods

Field surveys were conducted in selected districts of Kerala to document the distribution, abundance, and roost-site characteristics of *P. medius*. A total of 31 sampling sites were selected based on direct observations, consultations with forest department officials and local residents, and existing literature. Each roost site was monitored over four consecutive days. The abundance of *P. medius* was estimated using the direct roost count method (Kunz *et al.,* 1996; Barlow, 1999), with visual counts conducted twice per day using binoculars (Olympus 10x50) to avoid double counts. Roost coordinates and elevation were recorded using a handheld GPS (Garmin eTrex 10).

Tree characteristics such as height, bole height, and diameter at breast height (DBH) were measured using a Silva Clinometer and Freeman Fiberglass Tape (Gulraiz *et al.,* 2016). Distances from roost trees to nearby roads, human settlements, water bodies, and mobile towers were estimated using a rangefinder (Hawke LRF 600), following standard ecological protocols (Gulraiz *et al.*, 2015; Kumar *et al*., 2017). Crown height was calculated by subtracting bole height from total tree height, while crown width was averaged from measurements in two perpendicular directions (N–S and E–W). Foliage cover was estimated visually based on sunlight penetration (0–100%). Environmental parameters such as temperature and humidity were measured using a digital hygro-thermometer (HTC 288-ATH) and wind speed using a digital anemometer (GM8908). Supplementary weather data were obtained from AccuWeather.com (Manandhar *et al.*, 2018). Roost and non-roost trees at each site were documented, including species name, family, and nativity following (Bentham

and Hooker, 1862). Roost longevity (in years) was estimated through interviews with local residents aged 24–75 years. Anthropogenic disturbances—including tree cutting, poaching, pollution, traffic, fireworks, noise, and infrastructure development—were assessed via semi-structured questionnaires. Each type of disturbance was scored on a scale from 0 (none) to 3 (high) for subsequent analysis.

Statistical Analysis

All data were first tested for normality. Descriptive statistics, including mean and standard error, were calculated. One-way analysis of variance (ANOVA) was performed to assess variations among observations. Where significant differences were detected, Tukey's post hoc test was applied to identify pairwise differences between group means (Zar, 1999). Multiple regression analysis was used to evaluate relationships among variables. A significance level of $p < 0.05$ was adopted for all statistical tests. All analyses were conducted using IBM SPSS Statistics, version 25.0.

Results

Abundance of P. medius in relation to roosts and roost-sites

The abundance of *P. medius* in relation to environmental, tree characteristics, disturbance, and other factors were assessed and summarized in Table 1. The roost-sites with lesser wind speed had a higher number *P. medius*. The roost-sites with the maximum temperature (28–31°C) had the highest number of bats, and the sites with lower relative humidity (<70%) and lower elevation (<500m) had the highest number of bats, indicating the importance of temperature for thermoregulation activity across roost sites. The abundance of *P. medius* was found to be higher in the roost-sites closer to water bodies. The number of bats with reference to roosting trees species varied significantly (One way ANOVA; $F=5.952$; $p<0.05$; df=55). Among the 56 tree species studied, *Ficus drupacea* had the highest number of *P. medius* (300+0 individuals) followed by *Calophyllum inophyllum* (172.5±76.3), while *Areca catechu* (0.3 ± 0.2) had the least (Table 2). The abundance of *P. medius* was relatively higher on the matured trees (37.8 ± 2.8) than that of the younger trees. Similarly, a greater number of *P. medius* roosted on native tree species than the exotic species. The roost-sites on trees that are tall in tree height (>20m) and tree pole height (>15m) had the highest number of *P. medius*. Similarly, trees with higher crown height (>15m), crown width (>10m) and large diameter at breast height (DBH) had the maximum number of *P. medius*, suggesting the significance of microhabitats on the trees for the bats to select their suitable roost-sites (Table 1). The abundance of *P. medius* based on the density of tree branches characterized by an umbrella of dense foliage cover was assessed and the results revealed that the trees with 30–60% foliage cover had the highest number of bats (23.1 ± 3.0) followed by the trees with 60–90% of foliage cover (18.0 ± 1.7). Furthermore, the roost-site located closer to human settlements or towns had a relatively higher number of bats, whereas sites with heavy anthropogenic pressures, including poaching and presence of mobile phone tower stands, had relatively less bats. However, the difference was not statistically significant ($p>0.05$) (Table 1).

Drivers of roost selection of *P. medius*

To understand the preferences for roost-site characteristics of *P. medius*, tree height, bole height, crown height, crown width, DBH, crown closure, distance nearest to town, water bodies, and main roads were assessed from both roosting and non-roosting trees (Table 3). Among the attributes studied, except for roost-site distance nearest to the road, the remaining attributes significantly varied between roosting and non-roosting trees, highlighting the fact that *P. medius* is known to prefer taller trees with maximum tree height, bole height, crown height, crown

Table 1. Roost abundance of *P. medius* in relation roost and roost-site attributes.

Attributes	Category	Abundance Mean ± SE (N)	AANOVA F F value	*p* value
Environmental factor				
Wind speed	1–3 m/hr	22.3 ± 1.9 (538)	16.215	0.000
	4–6 m/hr	9.6 ± 1.4 (202)		
Temperature	22–25 °C	16.8 ± 1.3 (359)	24.288	0.000
	25–28 °C	15.3 ± 2.0 (325)		
	28–31 °C	52.6 ± 11.6 (56)		
Humidity	<70 %	16.9 ± 1.4 (506)	4.036	0.045
	>70 %	23.1 ± 3.3 (234)		
Elevation	<500 m	18.7 ± 1.4 (426)	0.014	0.907
	>500 m	19.1 ± 2.8 (314)		
Distance to nearest water body	<90 m	17.9 ± 2.3 (109)	0.070	0.791
	>90 m	19.0 ± 1.6 (631)		
Tree Character				
Nativity of roost tree	Exotic	17.9 ± 2.5 (231)	0.222	0.638
	Native	19.3 ± 1.7 (509)		
Tree age	Matured	37.8 ± 2.8 (324)	166.702	0.000
	Young	4.1 ± 0.6 (416)		
Tree height	>20 m	25.3 ± 1.9 (531)	55.432	0.000
	<20 m	2.5 ± 0.5 (209)		
Tree bole height	>15 m	33.0 ± 4.2 (129)	21.217	0.000
	<15 m	9.7 ± 2.0 (611)		
Crown height	>15 m	60.2 ± 9.5 (70)	98.738	0.000
	<15 m	14. ± 1.1 (670)		
Crown width	>10 m	40.2 ± 5.6 (141)	56.194	0.000
	<10 m	13.8 ± 1.1 (599)		
Tree diameter at breast height (DBH)	>1 m	30.5 ± 2.3 (421	98.575	0.000
	<1 m	3.5 ± 0.5 (319)		
Foliage cover	>30 %	9.2 ± 2.5 (75)	3.925	0.020
	30–60 %	23.1 ± 3.0 (254)		
	60–90 %	18.0 ± 1.7 (411)		
Disturbance factor				
Distance to nearest town	<500 m	21.2 ± 3.2 (248)	1.351	0.245
	>500 m	17.7 ± 1.4 (492)		
Distance to nearest main road (tar road)	<400 m	18.2 ± 2.4 (103)	0.037	0.848
	>400 m	19.0 ± 1.6 (637)		
Roost site distance to mobile tower	>500	12.7 ± 7.1 (45)	1.000	0.368
	500–1500	21.1 ± 3.2 (234)		
	<1500	18.3 ± 1.4 (461)		
Other anthropogenic pressure around the roost tree	Less	18.7 ± 2.2 (421)	0.011	0.915
	Moderate	19.0 ± 1.7 (319)		
Other factor				
Years of roost existence	10–20	38.1 ± 2.4 (119)	31.101	0.000
	21–40	47.3 ± 7.8 (32)		
	41–60	13.4 ± 1.6 (589)		
Area of roost extant	5–30	17.7 ± 2.5 (218)	0.163	0.687
	30–55	19.2 ± 1.8 (522)		

Table 2. Variation in abundance of *P. medius* in relation tree species.

Tree family	Roosting trees	Mean ± SE	N
Anacardiaceae	*Anacardium occidentale* (E)	42.5 ± 18.5	10
	Holigarna arnottiana (N)	46.8 ± 21.9	8
	Mangifera indica (Nand D)	4.4 ± 1.7	33
	Spondia spinnata (N and D)	20.7 ± 7.4	3
Apocynaceae	*Alstonia scholaris* (N)	145.5 ± 102.9	4
Arecaceae	*Areca catechu* (N)	0.3 ± 0.2	15
	Caryota urens (N)	16.8 ± 3.3	18
Bignoniaceae	*Oroxylum indicum* (N)	38.2 ± 22.5	5
	Spathodea campanulata (E)	1.8 ± 1.1	8
Bombacaceae	*Bombax ceiba* (N)	50.0 ± 0	1
	Butea monosperma (N)	0.5 ± 0.5	2
Calophyllaceae	*Calophyllum inophyllum* (E)	172.5 ± 76.3	4
Casuarinaceae	*Casuarina equisetifolia* (E)	17.6 ± 8.4	7
Combertaceae	*Anogeissus latifolia* (N)	8.0 ± 0	1
	Terminalia bellirica (N and D)	3.8 ± 3.8	5
	Terminalia catappa (E and D)	5.3 ± 5.3	8
	Terminalia elliptica (N)	8.5 ± 6.8	8
Dipterocarpaceae	*Hopea parviflora* (E)	80.0 ± 0	1
	Hopea ponga (N)	23.1 ± 3.8	35
Euphorbiaceae	*Hevea braziliensis* (E)	1.2 ± 0.5	37
	Macaranga peltata (N)	11.5 ± 6.7	27
Fabaceae	*Adenanthera pavonina* (E)	55.0 ± 23.6	4
	Albizia odoratissima (N)	38.0 ± 12.0	2
	Albizia procera (E)	42.7 ± 31.0	3
	Albizia saman (E)	22.3 ± 7.0	25
	Artocarpus fraxinifolius (N and D)	48.0 ± 17.0	2
	Dalbergia latifolia (N)	5.7 ± 4.0	10
	Delonix regia (E)	63.7 ± 48.5	3
	Leucaena leucocephala (E)	8.0 ± 2.8	6
	Pterocarpus marsupium (N)	68.4 ± 22.3	5
	Racosperma auriculiforme (E)	10.5 ± 5.0	10
	Racosperma mangium(E)	18.6 ± 3.1	62
	Tamarindus indica (N and D)	5.7 ± 5.7	3
Loganiaceae	*Strychnos nux-vomica* (N)	11.5 ± 6.0	11
Lythraceae	*Lagerstroemia microcarpa* (N)	3.6 ± 3.6	7
	Lagerstroemia speciosa (N)	26.0 ± 5.5	4
Meliaceae	*Swietenia mahagoni* (E)	10.0 ± 10.0	2
Morceae	*Artocarpus heterophyllus* (N and D)	12.3 ± 2.9	54
	Artocarpus hirsutus (N and D)	37.7 ± 4.8	69
	Ficus drupacea (N and D)	300.0 ± 0	1
	Ficus exasperate (N and D)	3.0 ± 3.0	2
	Ficus hispida (N and D)	16.8 ± 16.8	4
Myrtaceae	*Eucalyptus tereticornis* (E)	24.0 ± 7.5	5
Poaceae	*Bambusa bambos* (N)	16.9 ± 5.8	17

Proteaceae	*Grevillea robusta* (E)	7.8 ± 2.1	16
Rhizophoraceae	*Carallia brachiata* (N)	7.5 ± 7.5	2
Rutaceae	*Melicope lunu-ankenda* (N)	1.8 ± 1.8	9
Sapindaceae	*Dimocarpus longan* (N)	10.8 ± 2.9	5
	Schleichera oleosa (N)	2.7 ± 1.8	7
Sapotaceae	*Chrysophyllum cainito* (N and D)	3.5 ± 3.5	2
Simaroubaceae	*Ailanthus triphysa* (E)	2.8 ± 1.0	6
Sterculiaceae	*Sterculia guttata* (N)	122.0 ± 122.0	2
	Sterculia urens (N)	108.0 ± 0	1
Ulmaceae	*Trema orientalis* (N)	37.3 ± 13.9	12
Verbenaceae	*Gmelina arborea* (N)	121.0 ± 0	1
	Tectona grandis (N)	25.0 ± 4.6	45

width and DBH. Furthermore, a significantly higher number of *P. medius* were found roosting near to water bodies (Table 3, $P<0.05$). Similarly, the bats are avoiding the roost sites with heavy anthropogenic pressures and disturbance. To understand the driving factors of roost-site selection by *P. medius*, the multiple regression tests (stepwise model) were performed (Table 4 and Figures 2-4). The model predicted that the tree diameter at breast height (DBH), temperature, tree height, tree crown width, wind speed and the area coverage of roosts positively influence the roost site selection of bats, whereas variables including anthropogenic pressure, pole height of the trees, and tree crown closure have influenced them negatively (Multiple Regression-Stepwise model; $R^2=0.43$; ANOVA F= 61.509; $p<0.05$; df=18). The data, based on a multiple regression stepwise model, suggests that the tree DBH, temperature, tree height and tree crown width, and wind speed correlated with the roost area coverage, thus suggesting that these were the driving factors which can determine the roost-site selection of *P. medius* in Northern Kerala.

Table 3. Variation of roost site attributes between roosting and non-roosting trees.

S. No.	Roost site attributes	Roosting trees (n=336)	Non-roosting trees (n=404)	ANOVA	
				***F* value**	***P* value**
1	Tree height (m)	22.6 ± 0.3	14.9 ± 0.3	390.211	0.000
2	Bole height (m)	11.6 ± 0.3	8.1 ± 0.2	119.426	0.000
3	Crown height (m)	10.4 ± 0.2	6.6 ± 0.2	207.524	0.000
4	Crown width (m)	7.7 ± 0.2	6.5 ± 0.1	32.433	0.000
5	DBH (m)	1.3 ± 0.0	0.8 ± 0.0	198.864	0.000
6	Crown closure	52.2 ± 1.2	56.4 ± 1.1	6.773	0.009
7	Distance to nearest town (m)	478.2 ± 24	544.1 ± 21.9	4.104	0.043
8	Distance to nearest water bodies (m)	30.2 ± 2.0	35.6 ± 2.1	3.576	0.05
9	Distance to nearest main road (m)	140.8 ± 8.5	135.2 ± 8.2	0.224	0.636

Table 4. Multiple regressions show the determinants of roost-site selection by P. *medius*.

S. No.	Variables entered	Un-standardized Coefficients		Standardized Coefficients			ANOVA		
		B	Std. Error	Beta	t	Sig.	F	Sig	R^2
	(Constant)	-258.719	24.480		-10.569	0.000			
1	DBH (m)	24.464	2.939	.338	8.324	0.000			
2	Temperature (°C)	7.452	.786	.409	9.480	0.000			
3	Tree height (m)	2.336	.303	.389	7.708	0.000			
4	Crown width (m)	2.351	.516	.177	4.560	0.000	61.509	0.000	0.431
5	Wind speed (m/hr)	5.534	.859	.237	6.440	0.000			
6	Anthropogenic Pressure	-11.899	2.538	-.152	-4.688	0.000			
7	Bole height (m)	-1.145	.384	-.136	-2.981	0.003			
8	Area of roost extent (ha)	0.247	.113	.089	2.177	0.030			
9	Crown closure (%)	-0.118	.054	-.066	-2.175	0.030			

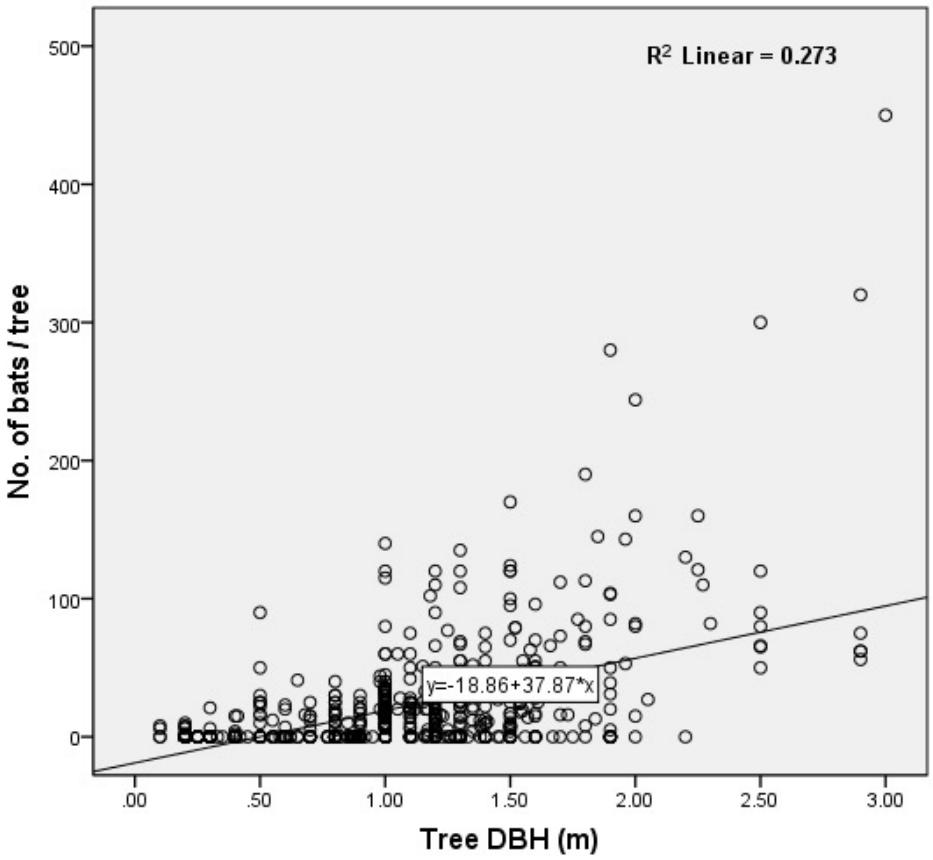

Figure 2. Relationship between diameter of tree at breast height (DBH) and abundance of bat recorded in the study area during November 2017 to January 2018 (Beta = 37.89, $F = 277.7$, $p = 0.000$).

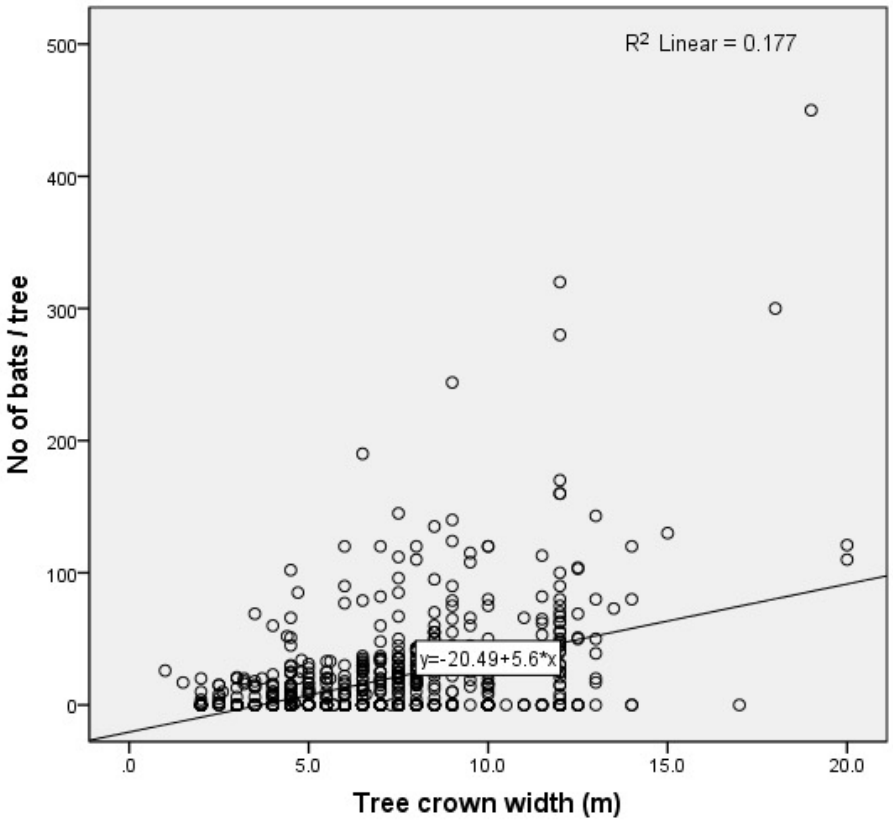

Figure 3. Relationship between tree crown width and abundance of bat recorded in the study area during November 2017 to January 2018 (Beta = 5.559, F = 158.551, p = 0.000

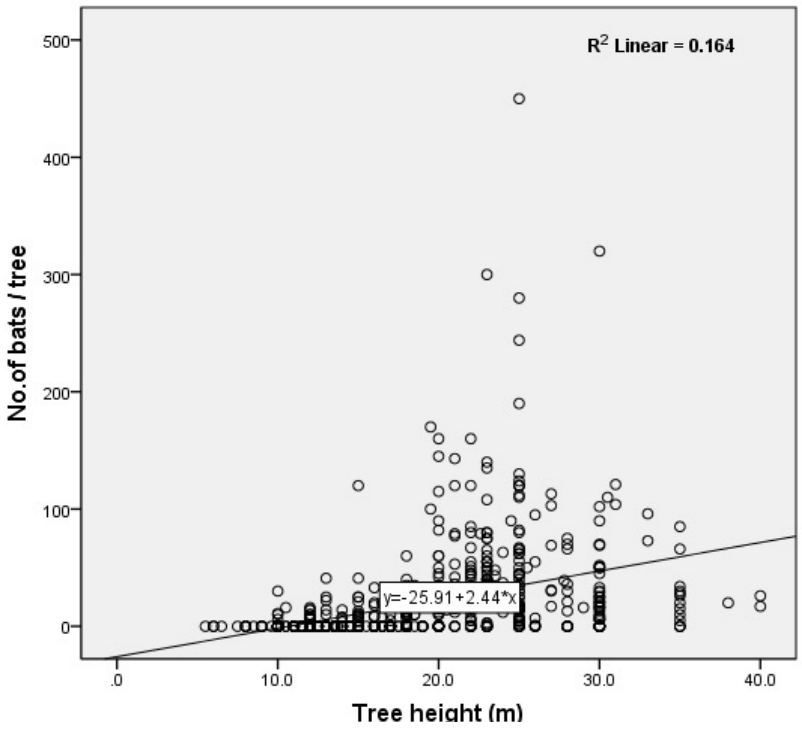

Figure 4. Relationship between tree height and abundance of bat recorded in the study area during November 2017 to January 2018 (Beta = 2.4359, F = 145.151, p = 0.000).

Discussion

Influence of environmental factors on roost selection

P. medius is one of the most widely distributed bat species across the Indian subcontinent (Storz 1998, Kumar and Kanaujia 2015) and their abundance recorded in the present study compares well with studies reported elsewhere (Talmale 2014, Gulraiz *et al*. 2015, Kumar and Kanaujia 2015, 2017, Jeyapraba 2016, Madala *et al.* 2022). In the present study, roost-sites with lesser wind speed, high humidity (>70%), lower elevation (<500m) and high temperature (28–31°C) had the highest number of bats, indicating the importance of temperature and humidity for thermoregulation activity in roost-sites, which agrees with previous studies (Hodgkison *et al*., 2004; Willis and Brigham, 2005; Gulraiz *et al*., 2015; Kumar and Kanaujia, 2017). The majority of bat species that belong to pteropodids roost in habitats with matured trees providing shelter from strong winds, regulating temperature, and providing easy exit for upward flight (Kunz, 1982; Richmond *et al*., 1998; Gulraiz *et al*., 2015; Kumar *et al*., 2017). The ambient temperature and wind speed recorded at the roost site also support the hypothesis that physical factors associated with roosts provide energetic benefits (Willis and Brigham, 2005). In the present study, *P. medius* found roosting near to water bodies, which is in line with *P. alecto* (Palmer and Woinarski, 1999), P. *livingstonii* (Granek, 2002), *Artibeus watsoni* (Cholewa *et al.*, 2001; Chaverri and Kunz, 2006), *Lasiurus borealis* and *Perimyotis subflavus* (O'Keefe *et al*., 2009), *Eptesicus fuscus* (Agosta, 2002), as well as with previous reports on *P. medius* (Gulraiz *et al.*, 2015; Kumar and Kanaujia, 2017). The presence of water bodies closer to roost-sites and on the foraging pathways of bats were reported as one of the essential requirements for bat population, because water bodies are serving as a source of drinking water as well as store house that helps reduce temperature in the roost sites (Welbergen *et al.,* 2008; Russo and Ancillotto, 2015).Availability of such microhabitats is important for nocturnal foraging animals like bats (Kumar and Kanaujia, 2017). Because summer nights are short at the roost-sites located in high latitudes, bats may be confined to roost sites for long periods without access to food or water. Under these circumstances, selecting roosts with specific microclimate conditions plays an important role in maintaining positive energy budgets (Willis and Brigham, 2005; Conenna *et al.,* 2025).

Influence of tree characteristics on roost selection

A total of 659 trees belonging to 56 species in 27 families served as roost trees for *P. medius*. Among the species, the larger and taller hardwood trees species, namely *Ficus drupacea, Calophyllum inophyllum, Alstonia scholaris,* and *Gmelina arborea* had the highest number of bats (Table 1 and 2). Among 56 roosting tree species, 11 species also serve as part of the diet for *P. medius*, which adds value to these roosting trees (Singaravelan and Marimuthu, 2004; Madala *et al.,* 2022). The selection of roost trees by bats recorded in the current study corresponds well with number of other studies, e.g. *Acacia nilotica* (Gulraiz *et al.*, 2016), *Albizzia lebbek* (Baskaran *et al.,* 2016), *Alstonia scholaris* (Ali, 2010), *Azadirachta indica* (Vendan and Kaleeswaran, 2011; Kumar and Elangovan, 2019), *Bassia latifolia* (Senthilkumar & Marimuthu, 2012), *Dalbergia sissoo* (Vyas and Upadhyay, 2014; Kumar and Elangovan, 2019), *Eucalyptus* sp., (Gulraiz *et al.*, 2015; Kumar and Elangovan, 2019), *Ficus racemosa* (Maruthupandian and Marimuthu, 2013; Kumar and Elangovan, 2019), *Ficus benghalensis* (Chakravarthy and Girish, 2003; Goveas *et al.*, 2006; Kumar and Elangovan, 2019), *Holoptelea integrifolia* (Suthari and Raju, 2012), *Mangifera indica* (Singaravelan and Marimuthu, 2004; Kumar and Elangovan, 2019), *Polyalthia longifolia* (Caughlin *et al.,* 2012), *Syzygium cumini* (Tangavelou *et al.*, 2013) and *Terminalia*

arjuna (Dey *et al.*, 2013). Similarly, Javid *et al.* (2017) reported that fruit bats prefer roosting trees that include *Syzygium jambolanum*, *Mangifera indica*, *Manilkara hexandra*, *Ficus glomerata*, *F. elastic*, *F. retusa*, *Bombax ceiba*, and *Dendrocalamus hamiltonii*, and our study coincides well with these findings. Furthermore, Chakravarthy *et al.* (2009) documented that the tree species, namely *Ficus* spp., *Deloni xregia*, *Eucalyptus* spp., *Acacia* spp., *Terminalia* spp., *Casuarina* spp., *Tamarindus indica*, *Mangifera indica*, and *Artocarpus heterophyllus* are the most preferable roosting trees for *P. medius,* which concur well with present observation. The large colony size of *P. medius* was found in large trees such as *F. religiosa, F. glomerata, F. Benghalensis* and *F. virens* (Kumar and Kanaujia, 2015; Madala *et al.*, 2022). The present findings showed that the size of the bat colony was linked to specific tree species, likely due to architectural differences among tree species that allow a larger number of bats to congregate in tree species with numerous branches. Perusal of literature has suggested that these large bats roost in tall trees because they need room to free-fall during take-off (Hahn *et al.*, 2014; Javid *et al*., 2017; Kumar and Elangovan, 2019) and therefore, the larger size of trees is one of the important factors which determines the colony size of *P. medius* both in urban and rural areas.

The abundance of bats is high on the matured and taller native tree species, with higher crown height, crown width, and diameter at breast height (DBH) representing the significance of tree characteristics for tree roosting bats to select their suitable roosts (Perry *et al.*, 2007; Limpert *et al.*, 2007; Madala *et al*., 2022). Several studies have reported that the frugivorous bat species prefers to roost on trees with large diameters (Evelyn and Stiles, 2003; Gulraiz *et al.*, 2015; Kumar and Elangovan, 2019). The majority of the previous studies have found that cavity roosting bats select taller trees and of greater DBH than randomly selected non-roost trees located near roost sites (Kalcounis and Brigham, 1998; Lacki, 2000; Mager and Nelson, 2001). We found that *P. medius* also has similar preference, therefore suggesting that that the larger trees with greater DBH, crown length, and width facilitate bats to gain good protection, presumably from human interference, and enable them to take off and land more easily and provide more space for these large colonies (Hahn *et al.,* 2014; Gulraiz *et al.*, 2015; Kumar and Elangovan, 2019; Madala *et al*., 2022). Furthermore, it could be noted that the larger trees are more stable and long-lasting, whereby providing consistent protection to bats during natural disasters such as high velocity wind, torrential rain, hailstorm, and high intensity sun rays (Hahn *et al.*, 2014). The abundance of *P. medius* was analyzed in correlation to the density of tree branches characterized by an umbrella of dense foliage cover. The results of the present study revealed that the tree species with 30–60% foliage cover had the highest number of bats. Several studies have found a similar behavior of bats selecting their roost sites (Kunz *et al*., 2003; Veilleux *et al*., 2003; Chaverri and Kunz, 2006). Few recent studies have also found that foliage-roosting bats select roosts based on microhabitat characters associated with suitable microclimatic conditions (Willis and Brigham, 2005) or concealment from predators (Perry *et al.*, 2007).

Influence of disturbance and other factors on roost selection

The roost-sites closer to human settlements/ town had a relatively higher number of bats, whereas the sites with heavy anthropogenic pressures, including poaching and logging activities, and the presence of mobile phone tower stands had fewer numbers of bats. Previous studies have shown similar observations, where *P. medius* were found roosting in botanical gardens, cities, and villages closer to human settlements but completely avoided the sites with poaching activities (Krystufek, 2009; Devi and Kumar, 2024). *Pteropus* roost selection is strongly influenced by food availability and food proximity (Palmer and Woinarski, 1999). The reason for *P. medius* roosting preference

in forests near areas of high human density is that homestead gardens provide a diversity of food resources that may not be available in natural forests. It is also observed that the abundance of generalist frugivorous bats was positively associated with the fragmentation of the landscape and it is proposed that their ability to feed on a variety of plant species allowed them to utilize heterogeneous landscapes (Gorresen and Willig, 2004). Subsequently, Pierson and Rainey (1992) found that *P. medius* were documented in forest remnants in populated areas as opposed to only in undisturbed natural forests. Pteropid bats are threatened throughout their range primarily due to hunting and habitat loss (Epstein *et al.*, 2008). Rapid human population growth and deforestation in India will continue to threaten *P. medius* unless comprehensive protection policies and land management practices are established (Streatfield and Karar, 2008). The survival of *P. medius* was severely threatened due to human interference. The present study confirms through inquiries with local people that *P. medius* is being hunted for food and alternative medicine practices in some day roosts. Its meat and oil were believed to cure paralysis, asthma and breathing disorders, and body pains in some villages (Gulraiz *et al*., 2015; Tackett *et al*., 2022), which has led the local people to hunt the bats easily as they live closer to human settlements

On the other hand, bats are recognized as the reservoir hosts for viruses that can cause serious human disease (Calisher *et al.,* 2006; Epstein *et al*., 2008). In Bangladesh, *P. medius* have been implicated as the primary reservoir of Nipah virus (Luby *et al.*, 2009), a disease recently spreading across Kerala and forcing the people to kill the bats or destroy their roosting sites. According to a report by the World Health Organization (WHO), there is strong evidence that the emergence of bat-related viral infections can be attributed to the loss of the animal's natural habitats.

As the *P. medius* habitat is destroyed by human activities, the bats get stressed and hungry, their immune systems get weaker, their viral load goes up and a lot of virus spills out in the urine and saliva. Conservationists fear that the outbreaks could spark a knee-jerk reaction of calls for bat culling. It may seem an easy solution, but studies warn that instead of reducing the outbreak of such diseases, it could cause even more ecological damage.

Despite the ecological, economic, and public health significance of flying foxes, little is known about their habitat requirements. Understanding their habitat selection can provide information for the design of forest management strategies that preserve roosting and foraging landscapes (Mildenstein *et al.*, 2005; Timmiss *et al*., 2021). Our study explored the determinants of roost selection by *P. medius* in northern districts of Kerala, which may be of highly useful in the conservation of roost sites at the local level, as it provides information contributing to local forest management. Despite their beneficial roles, fruit bats have been hunted as a source of protein and for alternative medicinal uses and are persecuted as fruit-eating pests (Tackett *et al*., 2022). The Indian Flying Fox (*Pteropus medius*) is one of the most misunderstood species in India and is listed under Schedule V of the Wildlife (Protection) Act, 1972, categorizing it as vermin, permitting capture or killing. As per the 2024 IUCN Red List assessment, it is classified as Near Threatened (NT) due to a significant population decline (Ahmed *et al*., 2025) and now it is appropriate that the policy makers working in the concerned department has to revisit this issue and to remove these pollinators and seed dispersers from the vermin list under the Wildlife (Protection) Act 1972. Additionally, the land managers and forest officials should come forward to stop the logging activities, particularly cutting down mature tress vicinity to bats' roosting sites and encourage the local people to take part in understanding the habitat requirements of bat species in the entire Kerala. Such efforts may be of useful in conserving this mammalian pollinator in near future and also maintaining their ecosystem.

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Two Flycatchers New to Iraq's Avifauna

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Abstract

Due to contradictory literatures and taxonomic contention, the status of many avifaunal species, especially passerines, in Iraq is still enigmatic. In 2026, the Collared Flycatcher *Ficedula albicollis* and the Indian (Asian) Paradise-flycatcher *Terpsiphone paradisi* were recorded in urban gardens in central and southern Iraq, respectively. These records represent the first confirmed occurrences of these flycatchers in Iraq and presented in this note.

Keywords: Birds of Iraq; Collared Flycatcher *Ficedula albicollis*; First record; Indian Paradise-flycatcher *Terpsiphone paradisi*; vagrant.

Introduction

The avifauna of Iraq, especially the passerine (order: Passeriformes) is unique and diverse, but far from fully explored. The family Muscicapidae (Old-World flycatchers) is represented in Iraq by four species: Spotted Flycatcher *Muscicapa striata*, Semi-collared Flycatcher *Ficedula semitorquata*, Red-breasted Flycatcher *Ficedula parva* (Salim *et al.,* 2012), and European Pied Flycatcher *Ficedula hypoleuca* (Abed *et al.,* 2022). The family Monarchidae (Monarch-flycatchers) has not been confirmed to occur in Iraq by any known species.

Due to taxonomic contention in literatures and "doubtful" misidentification of the seasonal plumage variations of black-and-white *Ficedula* male flycatchers, the occurrence of Collared Flycatcher *Ficedula albicollis* in Iraq was not confirmed (see Salim *et al.,* 2012), yet anticipated to occur on passage. Past ornithological claims of Collared Flycatcher in Iraq are almost referred to races of European Pied (Cumming 1918; Ticehurst *et al.,* 1922; 1926; Johnson 1958; Marchant 1961) and Semi-collared flycatchers (Allouse 1953; 1962; Moor and Boswell 1957). Salim *et al.* (2012) indicated that Iraq's past records were assigned to Semi-collared Flycatcher.

In this note, the occurrence of both Collared Flycatcher and the family Monarchidae (genus: *Terpsiphone*) represented by the Indian Paradise-flycatcher *Terpsiphone paradisi* is confirmed for the first time in Iraq.

Previous Records

The first national record was made at Daik Park (35°33'46.4"N 45°26'49.2"E) in Sulaymaniyah Governorate in northern Iraq (Kurdistan Region) where a first summer male was photographed on 2nd of April 2023 (Figure 1D). The habitat is urban landscape of mixed native and ornamental vegetation of the Zagros Mountains Forest Steppe (PA0446), mainly by scattered Oak tree *Quercus aegilops*, Chinaberry *Melia azedarach*, Plane tree *Platanus orientalis*, and Poplar *Populus nigra* (Figure 1C).

Recent Records

1-Collared Flycatcher *Ficedula albicollis*

On 29th of March 2026, an adult male

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was photographed at *ca*. 200m² public garden (32°29'19.78"N 44°26'33.05"E), southwestern edge of Al-Khisrawiyah Park, Hillah City, *ca*. 280m from the eastern bank of Shatt Al-Hillah (Hillah River), Babylon Governorate in central Iraq (Figure 2D). The habitat is urban landscape of mixed mosaic native and ornamental vegetation of the Arabian Desert and East Saharo-Arabian Xeric Shrublands ecoregion (PA1303), dominated majorly by Common Date Palm tree *Phoenix dactylifera* orchards, Common Jujube tree *Ziziphus vulgaris*, and Bitter Orange tree *Citrus* × *aurantium*, Lanceleaf Buttonwood "Damas" *Conocarpus lancifolius* (Introduced), along with riparian bushes and hedges (Figure 2C).

The birds were identified following Svensson *et al.* (2022). Their plumage was unmistakable, instantly recognized by its very distinctive broad white collar complete around the neck (black hindneck in *F. hypoleuca*; white half-collar onto sides of neck in *F. semitorquata*) (Figures 1 and 2 A and B). They had large white forehead patch (small forehead patch narrowing in the middle, sometimes divided into two very small white forehead spots in some *F. hypoleuca* observed in the region; smaller white forehead patch in *F. semitorquata*) (Figure 3 A and B). Extensive white on the wing tertials and large patch on base of the wing primaries (small white streak at base of primaries in *F. semitorquata*; white tips to median wing-coverts which may merge forming large white wing patch in *F. semitorquata*). They had all-black tail (prominent white bases or edges on the outermost tail feathers in *F. hypoleuca*; distinct black-and-white tail pattern with extensive white on the sides outer tail feathers/rectrices in *F. semitorquata*).

The Collared Flycatcher breeds in central and eastern Europe, and winters in central and south-eastern Africa (BirdLife International 2025). In the Arabian Peninsula, it is a common spring and rare autumn passage migrant (OSME 2025).

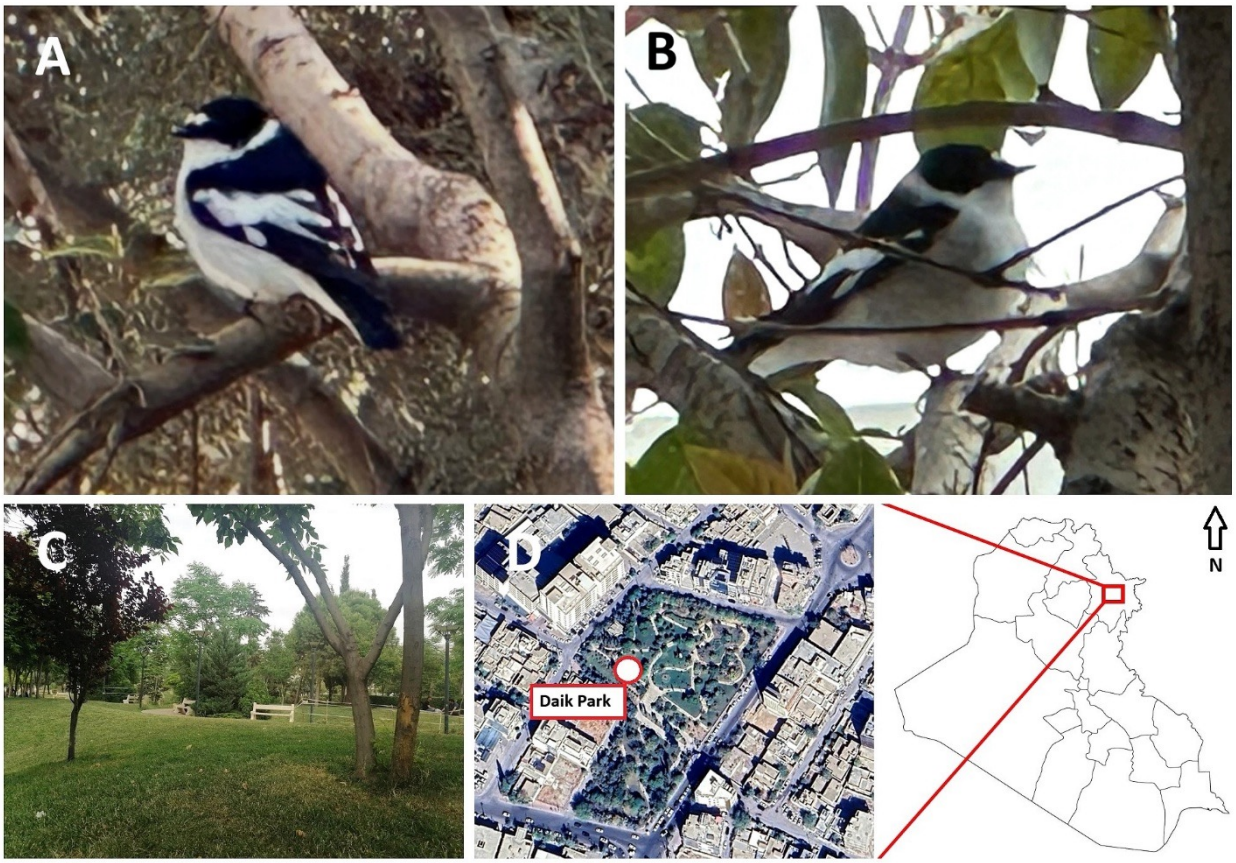

Figure 1: A and B: black-and-white 1st summer male Collared Flycatcher *Ficedula albicollis* in Iraq (Photos ©Korsh Ararat); C: Daik Park (source: https://mindtrip.ai/attraction/sulaymaniyah/daik-park/at-XymFTsKC); D: a Google Map image of the observation site.

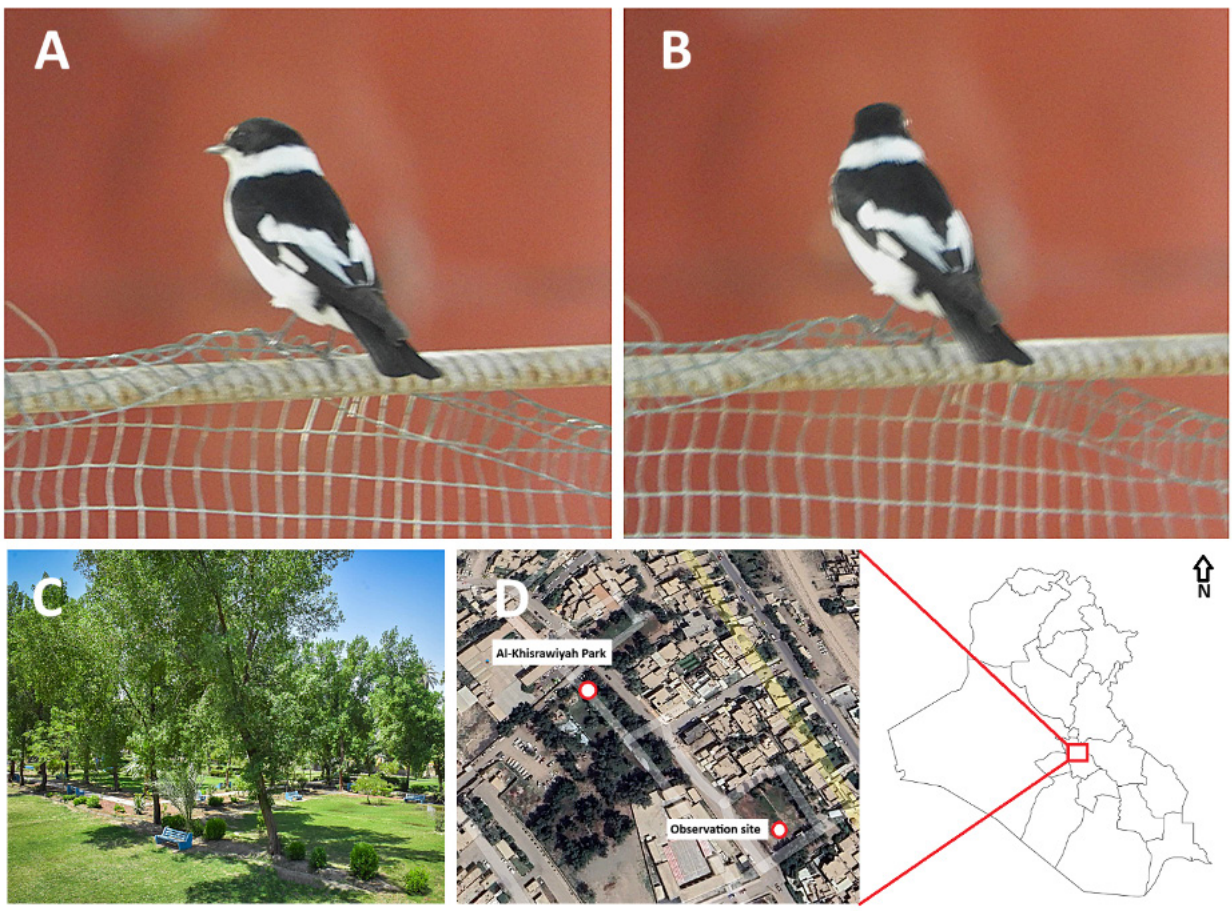

Figure 2. A and B: black-and-white adult male Collared Flycatcher *Ficedula albicollis* in Iraq (Photos © Ameen Ahmed Ali); C: Al-Khisrawiyah Park (source: HillaMunicipal@babel.gov.iq); D: a Google Map image of the observation site.

It is recorded as passage migrant in Jordan (JBW 2025), Syria (Aidek *et al*. 2025), and Gaza Strip-Palestine (Abd Rabou *et al*. 2023). It is recorded as vagrant in Bahrain (BRC 2023); Al Mefah Park, Tanoumah, Saudi Arabia in September 2016 (Harrison and Lamsdell 2016; Alshwamin 2026); Al Abraq in April 2013 and Jahra in May 2018 (KORC 2025; Shobrak and Al-Sirhan 2022), and at Mamzar park in Dubai, United Arab Emirates (UAE) in October 2015 (Campbell *et al.,* 2016). Old ornithological claims from Iran are now assigned to Semi-collared Flycatcher (Scott and Adhami 2006; Khaleghizadeh *et al.,* 2017) similarly to Iraq (Salim *et al.,* 2012).

The species is possibly a passage migrant in Iraq (see Porter and Aspinall 2010); however, additional observations are required to substantiate this claim.

2-Indian Paradise-flycatcher *Terpsiphone paradisi*

On 27th of January 2026, an adult rufous female was captured and photographed by a local person at his house garden (31°50’46.66”N 47° 8’48.10”E), in Shabanah neighborhood, *ca*. 200 m from the western edge of the Tigris River, Amarah City, Maysan Governorate in southern Iraq (Figure 4 D). Later on, the flycatcher was released back into the wild at same capturing site. The habitat is urban landscape at the fringes of the Tigris-Euphrates Alluvial Salt Marsh ecoregion (PA0906), which consists of scattered private and public gardens of native and ornamental plants, majorly Common Date Palm trees, Common Jujube trees, Lanceleaf Buttonwood, along with aquatic riparian bushes and hedges, mainly Common Reed *Phragmites australis* beds alongside Tigris River banks (Figure 4 C).

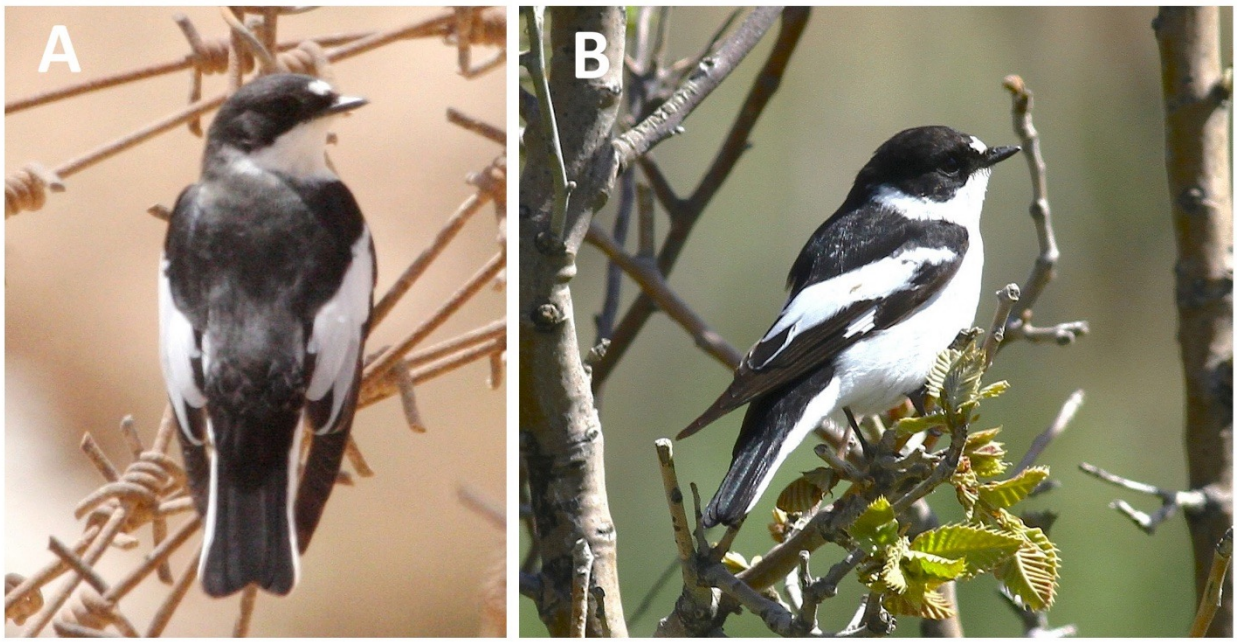

Figure 3. Semi-collared Flycatcher *Ficedula semitorquata* in Iraq. A: Adult male at Jabal Makhool (KBA IQ046) in Salah Ad-Din Governorate (Photo © Omar Al-Sheikhly). B: Adult male at Qara Dagh (KBA IQ039) in Sulaimani Governorate (Photo © Korsh Ararat).

Figure4. A&B: adult female Indian Paradise-flycatcher *Terpsiphone paradisi* in Iraq (Photos © Mahdi Alawi Hanon); C: Shabanah neighborhood (source: https://www.facebook.com/ photo/?fbid=2315487421945604&set=pcb.1457328661468644); D: a Google Map image of the observation site.

The bird was identified according to del Hoyo *et al.,* (2006), and del Hoyo (2020). However, Chhin *et al.* (2012) indicated that the male Indian (Asian) Paradise-flycatchers' plumage changes during their first years. Sub-adult males look very similar to females, but have a black throat and bright blue-ringed eyes (Salomonsen 2008). The female flycatcher had rich rufous plumage with cinnamon-brown upperparts, black-greyish head with short and rounded crest (glossy black head with notably long and pointed crest in males) and pale-whitish throat (full black in males). Tail feathers were taken off accidentally due to capturing (males have long central tail feathers). It had thin and faint pale greyish eye-ring (prominent, well defined vivid blue eye-ring in males – see Robson *et al.,* 2008). The breast was pale-greyish; underparts, belly, and flanks were white (dark-greyish throat and breast, and salty flanks and underparts in female African Paradise Flycatcher, *Terpsiphone viridis* which is confined to southern parts of the Arabian Peninsula – see Porter and Aspinall 2010) (Figure 4 A and B). The female could not be assigned into a subspecies for certainty.

The Indian Paradise-flycatcher is found in west Tien Shan to northern Afghanistan, north and east Pakistan, western and central Nepal, India, central Bangladesh, southwest Myanmar and Sri Lanka (BirdLife International. 2024). The species showed seasonal movements to the west of its range in central and southern Asia, to the south and alongside Arabian Gulf region. It was first recorded for the Arabian Peninsula in UAE as a vagrant at Dalma Island in October 2011, Mushrif Palace Gardens in Abu Dhabi in December 2019–March 2020 (Lloyd *et al.,* 2020), and a record claimed from Ras Al-Khaimah in May 2025 (del Hoyo 2020; Anonymous 2025). In Iran, the species was first recorded near Bandar Abbas in southern Iran in November 2021. Serval additional southern records were made near Gwadar, Pakistan (no details), Mellat Park, Minab, and Hormozgan in February 2023, and Minab in January 2025 (del Hoyo 2020; GBIF 2023; OSME 2025). The species is possibly vagrant or a rare passage migrant in Iraq; though this claim requires further observations.

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A phylogenetic reassessment of Orchidaceae taxonomy in the State of Palestine

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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.

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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

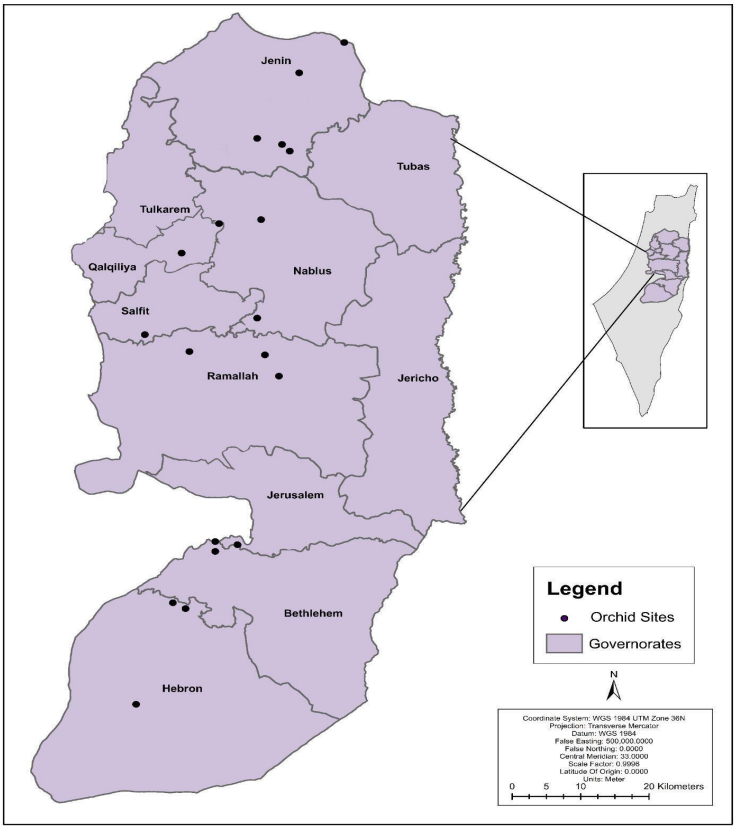

Figure 1. Map of the study area in the West Bank showing the geographic distribution of orchid collection sites.

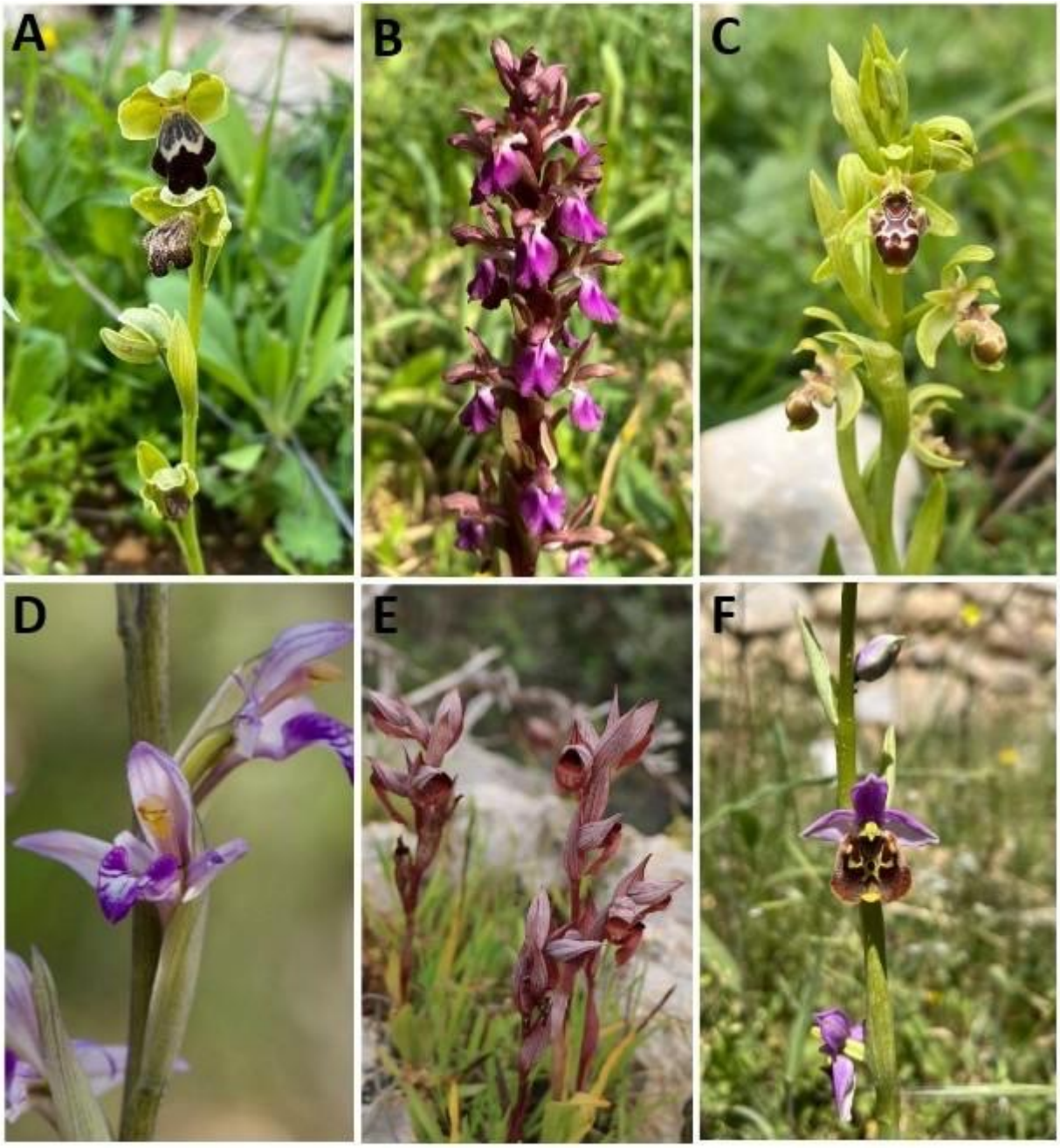

Figure 2. Selected orchid taxa recorded during field surveys in the West Bank, Palestine: (a) *Ophrys omegaifera* H. Fleischm., (b) *Anacamptis collina* (Banks & Sol. ex Russell) R.M. Bateman, Pridgeon & M.W. Chase, (c) *Ophrys umbilicata* Desf., (d) *Limodorum abortivum* (L.) Sw., (e) *Serapias orientalis*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

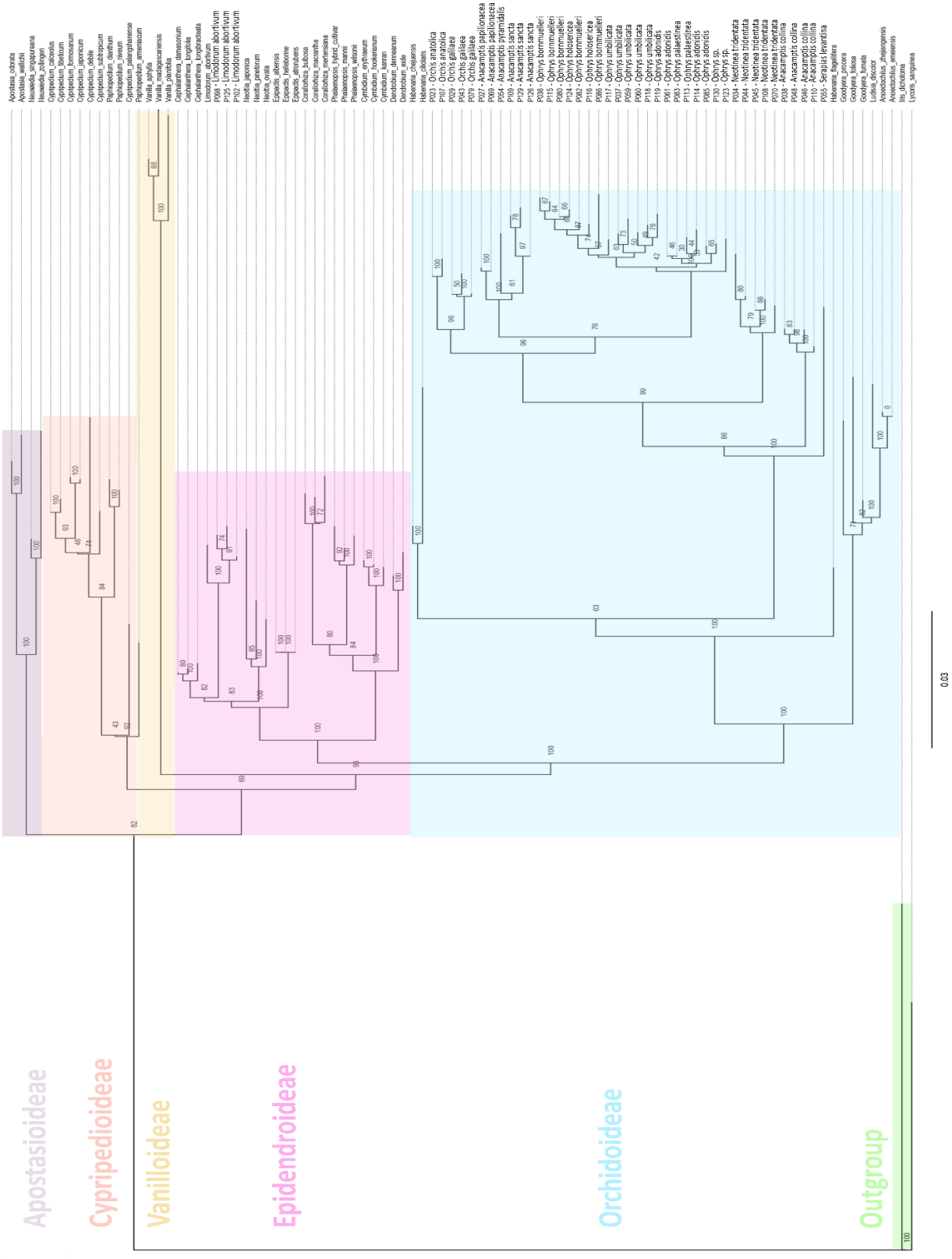

Figure 3. Maximum-likelihood phylogenetic tree of Orchidaceae and Epidendroideae inferred from concatenated sequences of five chloroplast loci. Bootstrap support values are indicated at the nodes to assess the statistical support of the recovered clades. The tree illustrates the phylogenetic relationships 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*.

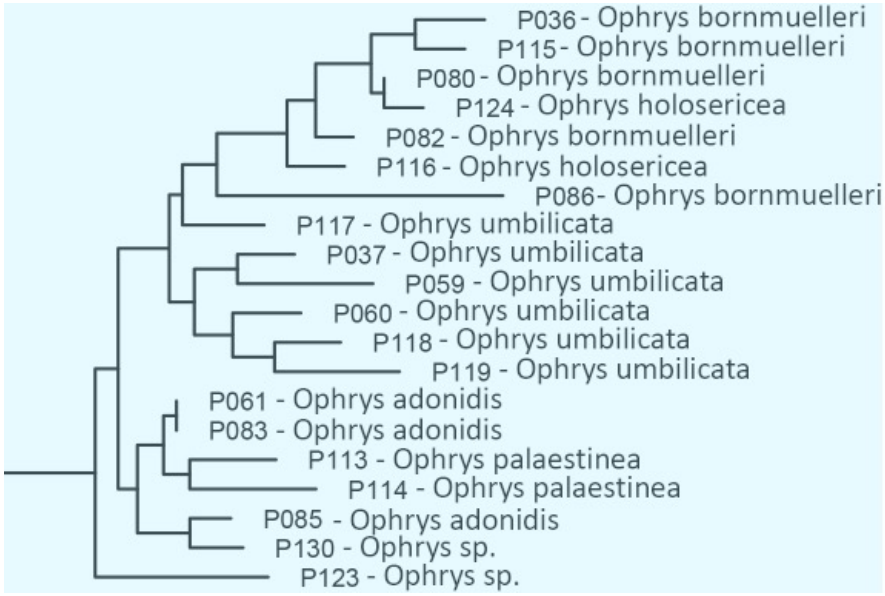

Figure 5. Section of concatenated tree (Figure 3) showing *Ophrys* clade.

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.

Other localities seen: **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.

Characteristics: **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.

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 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

Specimens and localities: 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.

Other localities seen: **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.

Characteristics: **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.

Notes: 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

Specimens and localities: 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.

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

Characteristics: 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)

Notes: 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

Specimens and localities: 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.

Other localities seen: **Ramallah Governorate:** Um Safa, Wadi Al-Zarqa.

Characteristics: 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).

Notes: 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

Specimens and localities: 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).

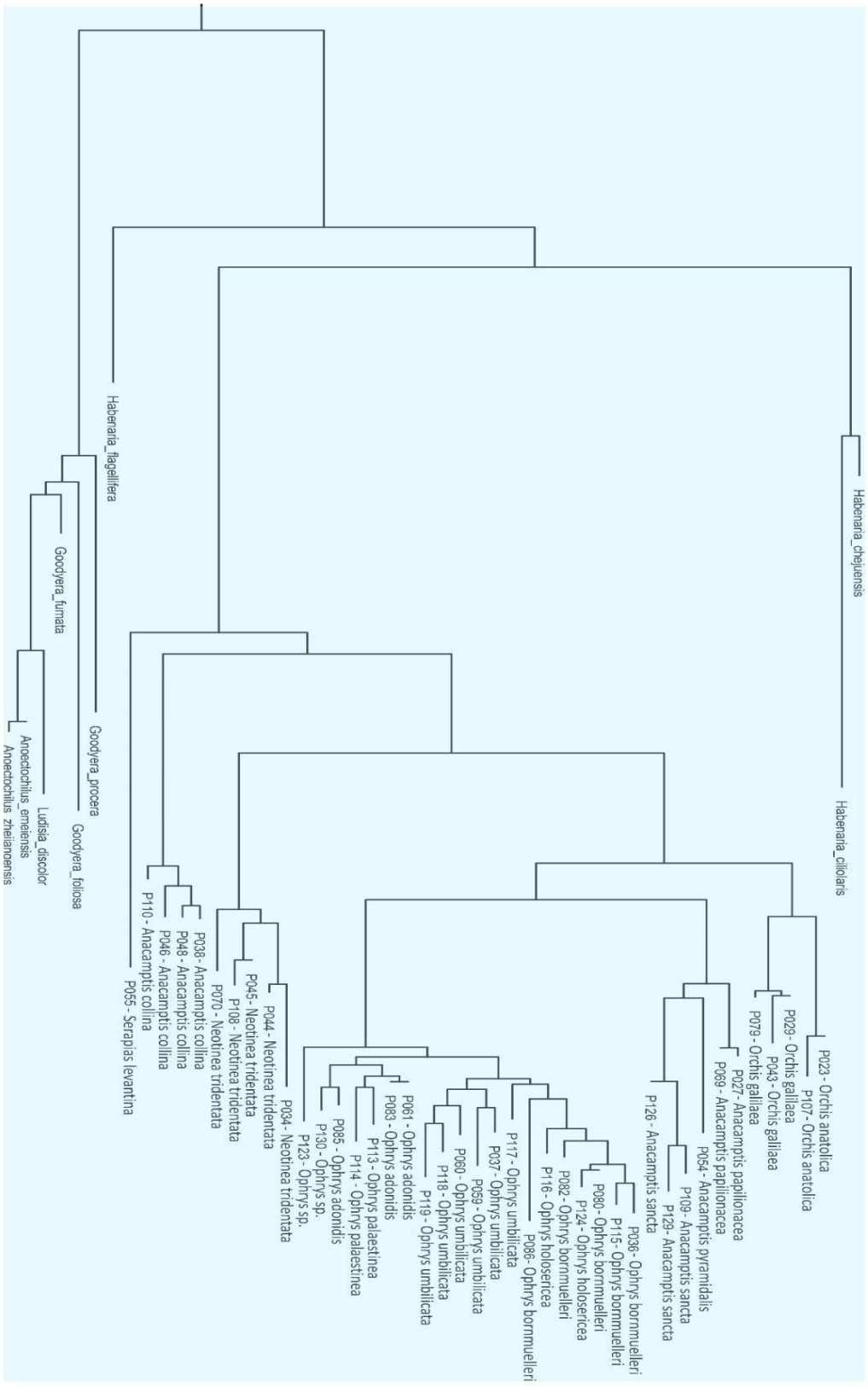
Notes: This species has variable flower colors; it can be purple or white in color. Common in the eastern Mediterranean region. In Arabic, this orchid is referred to as "Qarn al-Ghazzal" which means gazelle horn because its long spur looks like a gazelle's horn. This plant is pollinated by various bees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

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

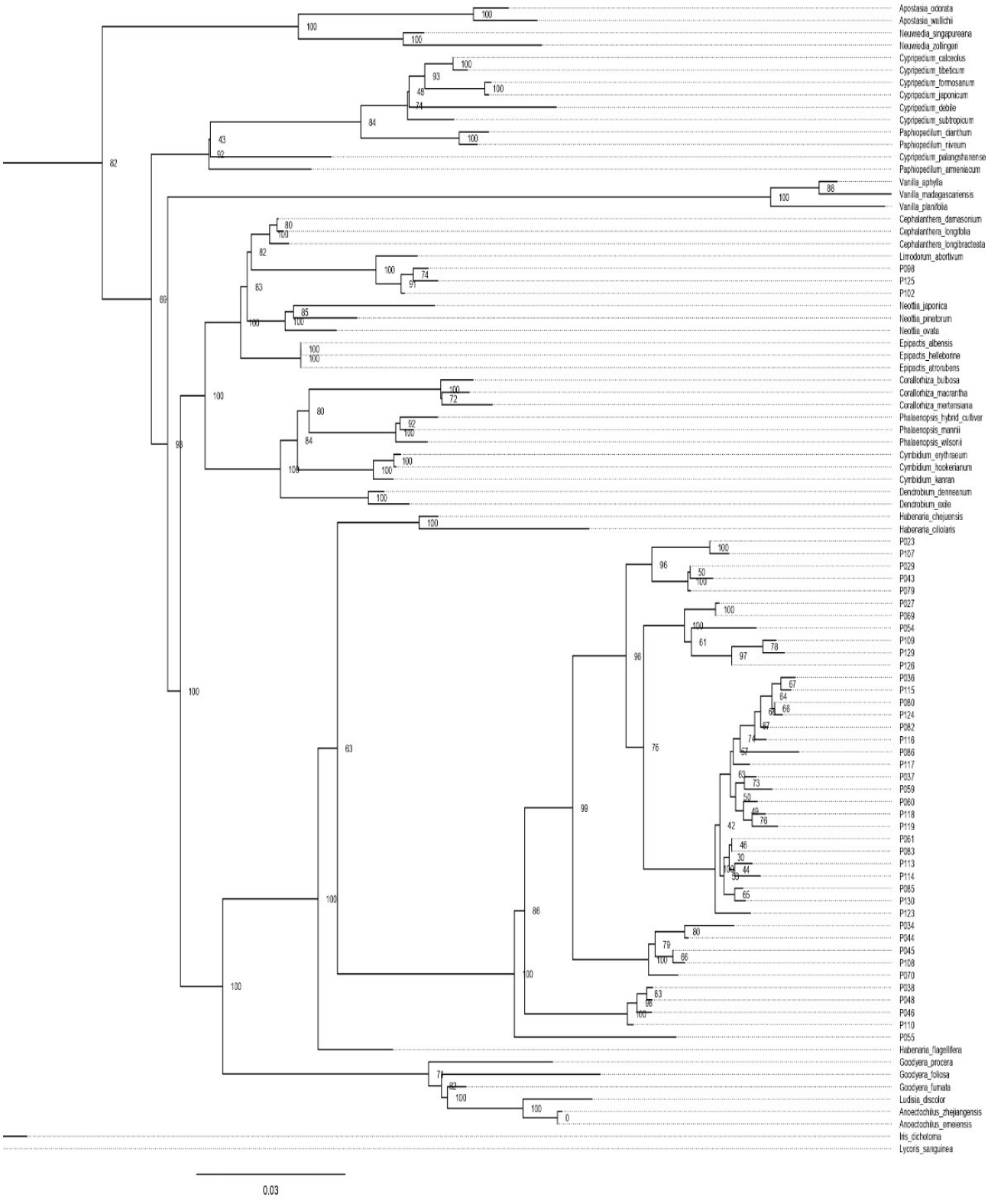
Specimens and localities: PMNH-H0554, Luban e-Sharkiya, Nablus Governorate; N: 31.976667, E: 35.282778, Elev: 930m, 24 March 2021; PMNH-H0596, Wadi Al-Haramiyeh, Ramallah Governorate; N:31.993611, E:35.247500, Elev: 598m, 12 March 2016; PMNH-H0747, Luban e-Sharkiya, Nablus Governorate; N: 31.976667, 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

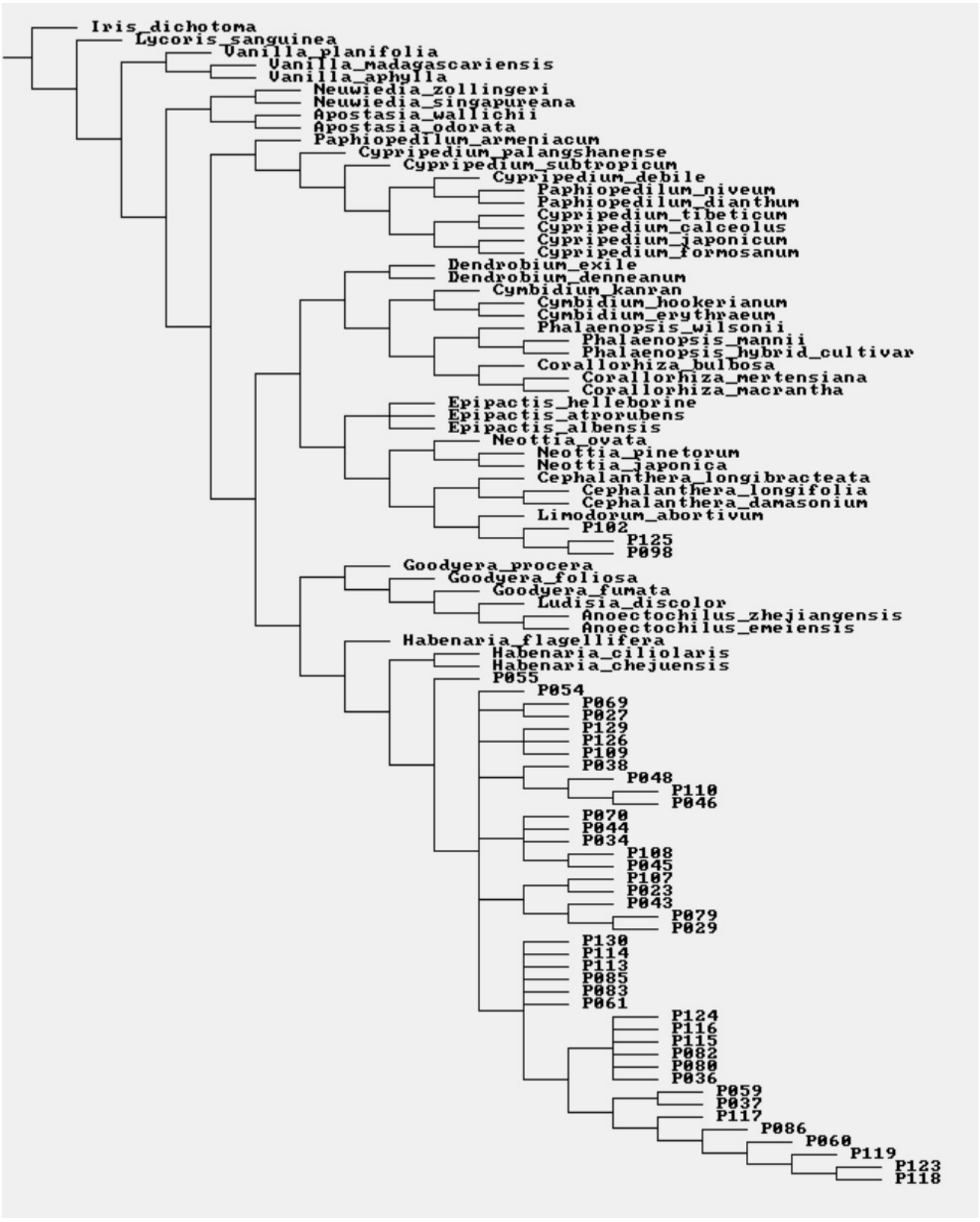
Specimens and localities: PMNH-H0666, Dayr Ghassana, Ramallah Governorate; N: 32.044167, E: 35.075833, Elev: 407m, 24 March 2021; PMNH-H0890, Wadi Qana, Salfit Governorate; N: 32.165000, E: 35.129444, Elev: 261m, 14 March 2021**;** P055, Dayr Ghassana, Ramallah Governorate; 24 March 2021.
Other Localities 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.

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Assessing Water Quality and Macroinvertebrate Assemblage of Ahi River, South-East Nigeria

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Abstract

Natural processes and increasing anthropogenic activities have negatively affected ecosystem health of lotic systems globally. Macroinvertebrate assemblage of a rural river was assessed in relation to anthropogenic stressors and water quality. The study was carried out during the wet season (May-October 2023) in 3 stations with standard protocols. Twelve (12) physicochemical parameters (temperature, flow velocity, transparency, depth, pH, electrical conductivity, total dissolved solids, turbidity, dissolved oxygen, biochemical oxygen demand, phosphate, and nitrate) were evaluated in association with the macroinvertebrates. The parameters were within acceptable limits except for some pH and dissolved oxygen values. The physicochemical parameters were majorly influenced by rainfall rather than anthropogenic activities. Fourteen (14) macroinvertebrate species and 226 individuals were recorded. The assemblage was poor, dominated by tolerant species, with Mollusca (*Bulinus wrighti*) being the most abundant species. The biodiversity indices were low: Shannon-Weiner diversity index (1.114-1.435), Margalef Species Richness Index (1.188-2.217), and Evenness index (0.3705-0.6997) confirmed the status of aquatic biota in the river. It can be concluded that there may be some stressors of possible human or natural sources acting on the environment which were not captured by the physicochemical parameters. This emphasized the need to combine physicochemical assessment with biomonitoring.

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Keywords: biomonitoring, physicochemical parameters, Anthropogenic Activities, rainfall, biodiversity

Introduction

Rivers are naturally occurring freshwater bodies that empty into lakes, the ocean, and other rivers (Erõs and Lowe, 2019). River ecosystems are beneficial to humanity in many ways, including supplying food and water, regulating the climate, and acting as recreation areas. However, human activities like mining, industrial processes, agriculture, the generation of domestic waste, and urbanization contribute significantly to the pollution of aquatic ecosystems. These activities change the physicochemical condition and community structure of aquatic species, including macroinvertebrates (Anyanwu and Umeham, 2020; Odo *et al.,* 2023; Arimoro *et al.,* 2025).

Aquatic macroinvertebrates, which are usually large and visible to ordinary eyes, are important components of the aquatic ecosystem. (Hauer and Resh, 2017). The health of aquatic environments is reflected in the community structure of aquatic macroinvertebrates (Jonah *et al.,* 2020; Qiqi *et al*., 2023). Clean rivers and streams with well-oxygenated water are home to the highest diversity of macroinvertebrates. On the other hand, the diversity and composition of aquatic ecosystems affected by local environmental conditions, including pollution or other manmade influences, is reduced (Morán-Ordóñez *et al*., 2015; Stenert *et al.,* 2019).

Consequently, macroinvertebrates have been extensively used globally for the

determination of aquatic ecosystem health. Macroinvertebrates possess some advantageous characteristics which include universality, difference tolerance capacity, and cheap and easy sampling that have contributed to their use in biomonitoring (Tampo *et al*., 2021). They are also linked to a variety of substrates, including detritus, macrophytes, mineral deposits, and filamentous algae (Anyanwu and Jerry, 2017).

Benthic macroinvertebrates have been used in a number of lotic environment studies for biomonitoring and aquatic ecosystem health assessment due to their potential for long-term anthropogenic impact monitoring programmes (Arimoro *et al.,* 2015; Anyanwu *et al*., 2019; Sumudumali *et al*., 2021; Sabha *et al*., 2022).

Generally, most research on Nigerian lotic systems has been focused on large rivers (Anyanwu and Umeham, 2020; Odo *et al.,* 2023). However, small, rural rivers like Ahi have been found to harbor rich diversity of aquatic biota and/or subjected to intense anthropogenic pressures, but have received much less attention (Anyanwu *et al*., 2021)**.** Ahi River is a rural freshwater body located in Ohiya Community, Umuahia. The river is used for several purposes, particularly in the dry season. Hence, the objective of this study is to evaluate macroinvertebrate community structure in relation to anthropogenic stressors and water quality.

Materials and Methods

Study Area

The study was carried out in Ahi River located in Ohiya Community, Umuahia, Abia State, Nigeria (Figure 1). The area falls within the sub-equatorial zone, characterized by high temperatures (29 - 31°C), high relative humidity (over 70%), and an annual mean rainfall of about 4000 mm. The study area is under the influence of two seasons—the wet season that usually occur between May and October, and the dry season from November to April. A double maximam peak period of rainfall usually occurs between July and September, with a short period of reduced rainfall in August known as the "August break".

Station 1 (N5° 31' 44.91984", E7° 27' 23.61672") was located upstream, and its substrate was a mix of sand, stones, and debris from improperly disposed solid waste. Human visitation and activities were higher due to its location by the road. Observed human activities included laundry and traditional religious activities. Storm water from parts of the community discharged into the river just upstream of Station 1.

Station 2 (N5° 31' 36.68664", E7° 27' 26.61672") was 0.97 km downstream of Station 1, and the sediment was sandy. There was rich riparian vegetation around the station. The entrance of the station had been increasingly used as a solid waste dump, with leachates from the dump being released into the river especially after rains. Human activities here include sand mining, bathing, washing, and food processing.

Station 3 (N5° 31' 53.2452" E7° 27' 13.87944") had a muddy stream bottom and was located in a deep valley, about 1.83 km downstream of Station 2. Riparian vegetation was also sparse. Bathing and washing were the few human activities observed around the station. Additionally, effluents from some cottage industries (oil palm processing mill, cassava processing mill, and pig farm) are discharged into the river around the station.

Samples collection

Water samples and analyses

The water sampling was carried out once every month from May to October 2023. The water samples for physicochemical analysis were collected using a 1 litre water sample and transferred into 1 litre plastic bottles, while samples for biochemical oxygen demand were collected with 250 mL reagent bottles with glass stoppers. All samples collected were transported in an ice-filled chest to the laboratory for analysis. Water temperature, dissolved oxygen, electrical conductivity,

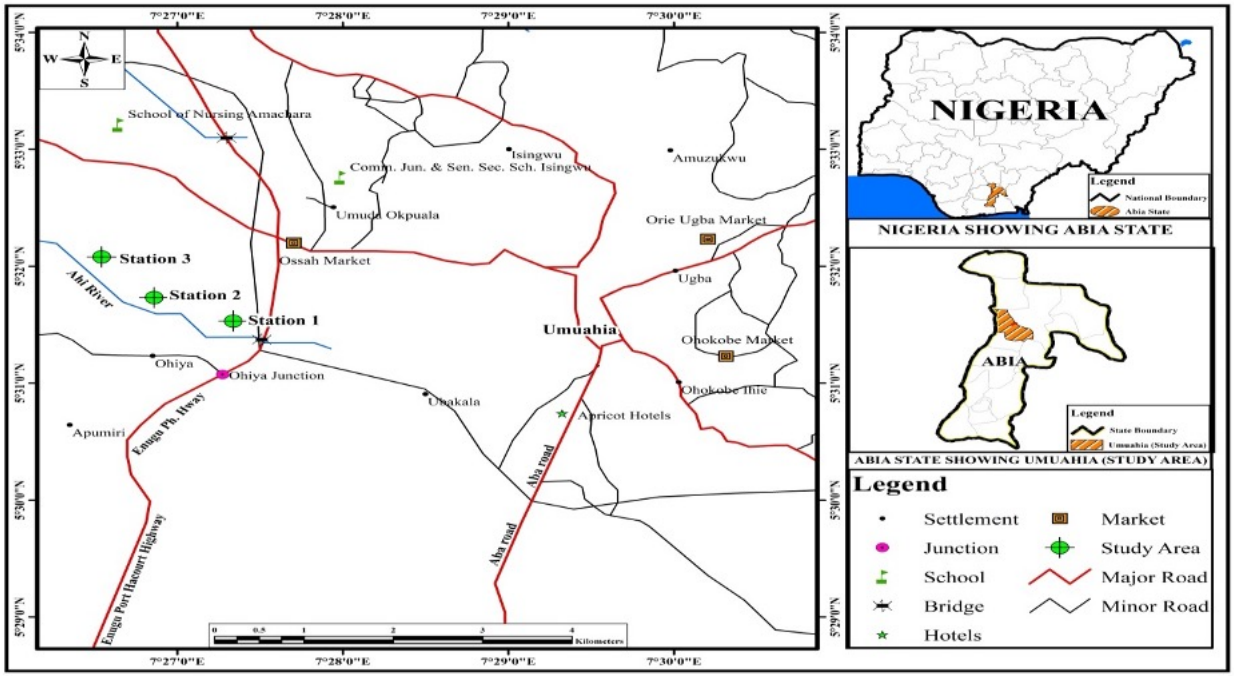

Figure 1: Map of Ohiya Community, Umuahia, Abia State, Nigeria showing the sampling stations in Ahi River

pH, total dissolved solids, flow velocity, transparency, turbidity, and maximum depth were determined *in-situ,* while biochemical oxygen demand, phosphate, nitrate-nitrogen were determined in the laboratory based on AWWA (2017) methods (Table 1).

Macroinvertebrates samples

Macroinvertebrate samples were collected in each of the stations with water samples. The D-net was placed in the downstream and the sediment upstream was disturbed by kicking with the heel for about 5 minutes, and the dislodged organisms were washed into the net. Aquatic macrophytes within the river and around the banks were also disturbed against the current with a sweep net. The samples were preserved with 10% formalin and taken to the laboratory for identification

Table 1: The physical-chemical parameters evaluated and methods of determination

Parameter	Method/Measuring Equipment	Note
Water Temperature	Mercury-in-glass Thermometer	*In-situ*
pH	Jenway 550 Portable pH meter	*In-situ*
Total Dissolved Solids/ Electrical Conductivity	HACH CO. 150 TDS/ Conductivity Meter	*In-situ*
Turbidity	Jenway 6035 Portable Turbidimeter	*In-situ*
Dissolved oxygen	DO meter	*In-situ*
Biochemical Oxygen Demand	Titrimetric Method - Winklers Method (Azide Modification)	*Ex-situ*
Transparency	Secchi Disk	*In-situ*
Flow Velocity	Floatation Method	*In-situ*
Phosphate	Titrimetric Method (Stannous Chloride Method)	*Ex-situ*
Nitrate	UV Spectrophotometric Method	*Ex-situ*
Maximum Depth	Graduated Wooden Pole	*In-situ*

and counting. The identification was carried out using the following taxonomic materials: Willoughby (1976), Merritt and Cummins (1996), and Umar *et al.* (2013).

Statistical Analyses

All data were summarized with Microsoft Excel. One-way Analysis of Variance (ANOVA) was used to determine significant variations (P <0.05) in the spatial mean values of physicochemical parameters, while the Tukey pairwise post-hoc test was used to locate the source of significant difference. Biodiversity indices Shannon-Wiener index (H), Margalef index (d), and evenness index (E) were used to characterize the diversity and determine the ecosystem health of the river.

Results

Spatio-temporal variations of physiochemical parameters

The physicochemical parameters are presented in Table 2. The water temperatures were moderate (25.2–27.0°C). The lowest value was recorded in Station 2 (June 2023) and the highest values in Stations 1 and 2 (May 2023). Station 3 consistently had low values. The flow velocity values were moderate (0.15–0.46 m/s). The lowest and highest values were recorded in August 2023 and October 2023 respectively in Station 1. The water transparency was moderate (14.0-78.5cm). The lowest and highest values were recorded in Station 2 in October 2023 and June 2023.

Table 2. Summary of physiochemical parameters of Ahi River (with range in parenthesis)

Parameters	Station 1 X±SEM	Station 2 X±SEM	Station 3 X±SEM	P- Value	NESREA 2011*
Water Temperature (°C)	26.3±0.17 (25.9-27.0)	26.1±0.25 (25.2–27.0)	25.9±0.07 (25.6–26.0)	F = 0.96 P > 0.05	-
Flow Velocity (m/s)	0.35±0.03[a] (0.27–0.46)	0.31±0.04[a] (0.15–0.41)	0.24±0.01[b] (0.22–0.28)	F = 4.27 P < 0.05	-
Transparency (cm)	49.62±6.44 (17.5–57.5)	49.12±9.96 (14.0–78.5)	45.58±6.34 (15.0–54.0)	F = 0.08 P > 0.05	-
Depth (cm)	63.0±6.62 (54.5-96.0)	83.1±4.67 (65.0-96.0)	61.8±8.65 (51.0–105.0)	F = 3.05 P > 0.05	-
pH	6.85±0.31 (5.7–7.8)	6.85±0.32 (5.7–7.7)	6.81±0.87 (5.6–7.7)	F = 0.00 P > 0.05	6.5-8.5
Elect. Conductivity (µS/cm)	80.33±4.48 (66.0-98.0)	81.66±3.24 (72.0-92.0)	80.0 ± 2.68 (70.0–88.0)	F = 0.06 P > 0.05	-
Total Dissolved Solids (mg/l)	40.17±2.24 (33.0-49.0)	41.00±1.57 (36.0-46.0)	40.17±1.42 (35.0–44.0)	F = 0.07 P > 0.05	-
Turbidity (NTU)	0.88±0.12 (0.3–1.1)	0.80±0.20 (0.2–1.7)	0.65±0.17 (0.1–1.4)	F = 0.47 P > 0.05	5
Dissolved Oxygen (mg/l)	3.93±0.29 (3.2 – 5.2)	3.97±0.30 (3.4–5.2)	4.80±0.38 (3.8–6.4)	F = 0.40 P > 0.05	> 6
Biochemical Oxygen Demand (mg/l)	1.77±0.26 (1.0–2.6)	1.97±0.17 (1.5–2.4)	2.2±0.23 (1.2–2.7)	F = 0.97 P > 0.05	< 3
Phosphate (mg/l)	0.03±0.00 (0.02–0.04)	0.02±0.00 (0.01-0.03)	0.02±0.00 (0.01 – 0.03)	F = 0.62 P > 0.05	3.5
Nitrate (mg/l)	0.05±0.00 (0.03–0.8)	0.05±0.00 (0.03–0.6)	0.04±0.00 (0.02–0.05)	F = 0.72 P > 0.05	9.1

Means with different superscripts in the same row are significantly difference at P < 0.05; SEM - Standard Error of Mean; *Guidelines and Standards for Environmental pollution control in Nigeria, Nigeria Environmental Standard and Regulatory Enforcement Agency (NESREA).

The water depth values were also moderate (51.0–105.0cm). The lowest and highest values were recorded August 2023 and October 2023 respectively in Station 3. The pH was moderate acidic to slight alkaline (5.6–7.8). Most of the values were within the optimal water pH (6.5–8.5) for freshwater organisms set by NESREA (2011). The lowest value was recorded in Station 3 (June 2023), while the highest was in Station 1 (September 2023).

The electrical conductivity values (66.0–98.0 µS/cm) and Total Dissolved Solids (33.0–49.0 mg/l) were also moderate and exhibited similar trend. The lowest value was recorded in Station 1 (August 2023), while the highest was in May 2023.

The turbidity values were low (0.1–1.7 NTU) and well within the acceptable limit (5 NTU) set by NESREA (2011). The lowest value was recorded in Station 3 (June 2023), while the highest recorded was in Station 2 (August 2023).

The Dissolved Oxygen (DO) values (3.2–6.4 mg/l) were generally lower than acceptable limit (6 mg/l) set by NESREA (2011) except one (Station 3, June 2023). The lowest and highest values were in Station 3 in October 2023 and June 2023 respectively.

Biochemical oxygen demand ranged from 1.0 to 2.7 mg/l and within the acceptable limit (3.0 mg/l) set by NESREA (2011). The lowest value was recorded in Station 1 (June 2023) while the highest was in Station 3 (June 2023).

The concentrations of phosphate were 0.01–0.04 mg/l and within the acceptable limit (3.5 mg/) set by NESREA (2011). The highest value was recorded in Station 1 (September 2023) while the lowest values were in Station 3 between June and July 2023 respectively.

On the other hand, nitrate ranged from 0.02 to 0.8 mg/l and within the acceptable limit (9.1 mg/l) set by NESREA (2011). The highest value was recorded in Station 1 (May 2023), while the lowest was in Station 3 (October 2023).

Macroinvertebrates Composition and Diversity

The composition, abundance, and distribution of Macroinvertebrates recorded in Ahi River are presented in Table 3. A total of 226 individuals were recorded from 3 phyla, 11 families and 14 taxa (species). The most abundant phylum was Mollusca with 173 individuals, contributing 76.6% of the total macroinvertebrates recorded. This is followed by Arthropoda with 43 individuals (19.02%), and the least was class Malacostraca with 10 individuals (4.42%). Most of the species recorded were pollution-tolerant species. The most abundant species was *Bulinus wrighti* (129 individuals, 57.1%), while the least was *Macrobrachium macrobrachion* (1, 0.44%). Among the stations, the highest number of individuals was recorded in Station 2 (91) while the least was recorded in Station 1 (67).

Biodiversity Indices

The macroinvertebrates community structure is presented in Table 4. The number of taxa (species) ranged from 6 (Station 1) to 11 (Station 2) and the number of individuals followed the same trend—67 individuals (Station 1) to 91 individuals (Station 2). The biodiversity indices were low. The Shannon-Weiner diversity index was low and ranged between 1.114 and 1.435. The lowest value was recorded in Station 3, while the highest was recorded in Station 1. The Margalef species richness index was also low and ranged between 1.188 and 2.217. The lowest was recorded in Station 1 while the highest was in Station 2. The Evenness index values were also low and ranged between 0.3705 and 0.6997. The lowest value was recorded in Station 2 while the highest was recorded in Station 1.

Discussion

The physicochemical parameters were moderate or within the acceptable limits to support aquatic life, except for pH and dissolved oxygen. The variations in stations

Table 3. Composition, abundance and distribution of Macroinvertebrates

Phylum/Class	Order/Family	Species	Stn 1	Stn 2	Stn 3	Total	R.A. (%)
Mollusca Gastropoda,	Hygrophila Planorbidae	*Bulinus wrighti*	27	57	45	129	57.1
		Bulinus globosus	22	9	2	33	14.6
	Architaenioglossa Viviparidae	*Viviparus fasciatus*	0	0	11	11	4.9
	Subtotal		**49**	**66**	**58**	**173**	**76.6**
Arthropoda Insecta	Ephemeroptera Baetidae	*Baetis* sp. *larvae*	4	0	0	4	1.77
	Caenidae	*Caenis luctuosa*	0	4	0	4	1.77
		Caenis horaria	0	1	0	1	0.44
	Potamanthidae	*Potamanthus luteus*	0	1	0	1	0.44
	Odonata Libellulidae	*Libellula* sp *larvae*	7	1	0	8	3.54
	Coleoptera Haliplidae	*Haliplus immaculicollis*	5	8	0	13	5.75
	Diptera Tabanidae	*Tabanidae larvae*	0	4	0	4	1.77
	Plecoptera Leuctridae	*Leuctra hippopus*	0	3	1	4	1.77
	Plecoptera Perlidae	*Paragnetina fumosa*	0	2	2	4	1.77
	Subtotal		**16**	**24**	**3**	**43**	**19.02**
Malacostraca	Decapoda Palaemonidae	*Macrobrachium rosenbergii*	2	1	6	9	3.98
		Macrobrachium macrobrachion	0	0	1	1	0.44
	Subtotal		**2**	**1**	**7**	**10**	**4.42**
Grand Total			**67**	**91**	**68**	**226**	**100**

Table 4. Macroinvertebrates Community Structure

Index	Station 1	Station 2	Station 3
Taxa	6	11	7
Individuals	67	91	68
Shannon-Weiner Index	1.435	1.405	1.114
Evenness Index	0.6997	0.3705	0.4351
Margalef Species Richness Index	1.189	2.217	1.422

and months were probably influenced by season (rainfall) rather than human activities. There were no significant differences ($p > 0.05$) in all the parameters evaluated across the stations, except flow velocity.

The water temperatures were moderate and consistent with values commonly recorded in tropical waterbodies and/or adapted to by most tropical aquatic organisms (Amusan *et al*., 2024; Arimoro *et al.,* 2025). The lowest value recorded in Station 2 (June 2023) could be a result of the onset of the wet season, while the highest values in Stations 1 and 2 (May 2023) could be due to the effect of the late dry season. The consistently low values recorded in Station 3 were due to the location and time of sampling. Station 3 was located in a deep valley, which could reduce the intensity of the sun reaching the water, and secondly sampling always started from station 3 early in the morning throughout the study. The values were within 21.0–28.0 °C recorded in Ossah

River in the same watershed (Anyanwu *et al.,* 2019). Water temperature is one of the main abiotic factors affecting the structure and functioning of aquatic ecosystems and its alteration can have important effects on biological communities (Bonacina *et al.,* 2023).

The flow velocity values were moderate and lower than 0.21–0.85m/s recorded in Eme River, Umuahia by Anyanwu *et al.* (2022a). The lowest and highest values recorded in August 2023 and October 2023 respectively in Station 1 could be due to rainfall. August is period of reduced rainfall ("August Break") while October is known for intense rainfall. Flow velocity generally increases with rainfall (Koushali *et al*., 2021). Lower flow velocities recorded in Station 3 could be due to its downstream position, while Station 1 was located at a higher gradient. Water flow in a river is usually slower towards its mouth and causes sediment—solid material carried downstream by currents—to settle to the river bottom (Douglas *et al*., 2025), leading to muddy substrate as observed in Station 3. During extreme high flows, river macroinvertebrates may be swept downstream in large numbers (Stamp *et al*., 2020; Asmamaw *et al*., 2021) as reflected in the lower abundance recorded in Station 1.

The water transparency values were also moderate with the lowest and highest values recorded in station 2 in October 2023 and June 2023 attributed to rainfall. The lowest in October was caused by dirt and other debris introduced into the river during the intense rainfall while the highest in June was just after the onset of wet season with cleaner runoff. Other stations also recorded low values in October 2023. Lower transparency is likely to negatively affect primary productivity, resulting in less secondary growth of benthic macroinvertebrates (Shimba *et al*., 2018).

The water depth values were also moderate, with the lowest and highest values recorded in Station 3 (August 2023 and October 2023 respectively), attributable to rainfall. Reduced rainfall was observed in August due to the August break, while October is one of the high periods of rains in the region. Though Station 2 consistently had higher values throughout the study, higher macroinvertebrate abundance are usually associated to riparian areas of rivers due to habitat availability, light penetration, and nutrient cycling and availability of food (Powell *et al*., 2023).

The pH values ranged between moderate acidic and slight alkaline range, with most values within the optimal water pH (6.5–8.5) for freshwater organisms set by NESREA (2011). The lowest and highest values recorded in Station 3 (June 2023) and Station 1 (September 2023) respectively were influenced by rainfall. Acidic values below the acceptable limits were recorded before the onset of the wet season and in early wet season (May and June 2023), and increased with the rains. The 3 stations had lower acidic values in June 2023; this is attributable to a rainfall event recorded on sampling day. Release of organic and inorganic substances into the river due to runoff during rainfall could lower the pH (Ojok *et al*., 2017). On the other hand, the pH generally increased with the rains in all the stations; attributable to dilution. Increased runoff tends to dilute solute concentrations and in turn increases the pH of a river during the wet season (Huang *et al*., 2020). The values were higher than 4.6–6.3 recorded in Ossah River, Umuahia within the same watershed (Anyanwu *et al*., 2019). pH levels can significantly affect macroinvertebrate survival, community structure, and diversity, with extreme highs or lows acting as stressors to organisms and changing their physiology (Dou *et al.,* 2022).

The electrical conductivity and Total Dissolved Solids were also moderate and exhibited a similar trend, suggesting relatively low salt contents in the water. Higher electrical conductivity values (52.9–110.5 µS/cm) and total dissolved solids values (25.7–55.3) were recorded in Ossah River, Umuahia (Anyanwu *et al.,* 2019). The lowest value recorded in Station 1 (August 2023) could be due to dilution, while the highest in May 2023 could be due concentration caused by the late dry season. Low rainfall and flow velocity, high temperatures, and evaporation

that occur in the dry season usually result in the concentration of some parameters (Etesin *et al*., 2013). The distribution of most freshwater invertebrates is influenced by the concentration of Total Dissolved Solids (TDS) and electrical conductivity (Olson and Hawkins, 2017).

The Turbidity values were low and conformed to the acceptable limit. The lowest value recorded in Station 3 (June 2023) could be due to initial dilution caused by the early rains, while the highest recorded in Station 2 (August 2023) could be a result of the August break or allochthonous input as the rain resumed after the break. Highest turbidity values were recorded by Anyanwu *et al* (2022b) in Anya stream in Abia State, Nigeria and Akhere and Kadiri (2023) in Ikoro Stream, Edo State, Nigeria in August. Turbidity has been known to be negatively associated with macroinvertebrate communities (Forio *et al*., 2023) as they can affect transparency and primary productivity (Shimba *et al*., 2018). The Dissolved Oxygen (DO) values did not conform to the acceptable limit, except in Station 3 (June 2023). Higher values were recorded in the 3 stations in June 2023 probably due to the combined effect of more nutrients, good temperature, and transparency that could enhance photosynthesis, as well as the rainfall event on sampling day. The lowest recorded in Station 3 (October 2023) could be due to reduced transparency. Values of the same range (3.2–6.4 mg/l) were recorded in Ossah River, Umuahia (Anyanwu *et al.,* 2019). Ding *et al.* (2016) reported an abnormal macroinvertebrate assemblage dominated by pollution-tolerant groups due to low dissolved oxygen in Ziya River Basin, North China. Tolerant groups were also dominant in this study, especially gastropod molluscs.

Biochemical Oxygen Demand (BOD) values were within the acceptable limit. The lowest recorded in Station 1 (June 2023) could be due to initial dilution from early rains, while the highest value recorded in Station 3 (June 2023) could be due effluent discharge from pig farms and palm oil and cassava processing mills washed into the river by early rains. Studies have shown that effluents contribute to high biochemical oxygen demand in aquatic environment (Cao et al., 2021; Lokman et al., 2021; Izah et al., 2022). The BOD values generally increased with the rains; suggesting that more organic matter was introduced into the river. The values were lower than 2.1–4.2 mg/l recorded in Ossah River, Umuahia (Anyanwu et al., 2019). Extremely high BOD can negatively affect the macroinvertebrate composition by depleting the dissolved oxygen, leading to hypoxia and change in the community composition from sensitive to tolerant groups (Ding et al., 2016; Lee et al., 2025).

The concentrations of the nutrients were low. Phosphate conformed to the acceptable limit. The highest value recorded in Station 1 (September 2023) could be due to allochthonous input, while the lowest values in Station 3 (June and July 2023) could be due to dilution. Phosphate values increased with the rains, suggesting addition through runoff. Nitrate values also conformed to the acceptable limit. The highest value was recorded in Station 1 (May 2023) while the lowest was in Station 3 (October 2023). Nitrate concentration decreased with rains, suggesting dilution (Griffin, 2017). The values of phosphate (0.1–2.7 mg/l) and nitrate (1.1–3.4 mg/l) recorded in Ossah River, Umuahia were higher (Anyanwu *et al.,* 2019). The low nutrient concentrations suggest low input from agricultural activities around the river. Nutrients are essential for maintaining an ecosystem's structure and function (Ouyang *et al.,* 2018).

Macroinvertebrates complement water quality assessment since they serve as crucial bioindicators for aquatic ecosystem health. They have diverse pollution tolerance levels that allow them to reflect the cumulative impact of chemical, physical, and biological disturbances in an ecosystem and integrate multiple stressors into a single biological assessment that cannot be captured by physicochemical measurements (Chen *et al.,* 2019; Arimoro *et al.,* 2025; Mureithi *et al.*, 2025) The number of Macroinvertebrates individuals (226) was higher than the 119

individuals recorded by Anyanwu et al (2019) in Ossah River in the same locality. However, the number of phyla, families and taxa were lower than 5 phyla, 19 families and 20 taxa recorded in Ossah River, Umuahia. Most of the species recorded were pollution tolerant species as observed in Ossah River. The most abundant species was *Bulinus wrighti* (mollusc). The dominance of molluscs in this study is an indication of an unhealthy ecosystem (Nugroho *et al.,* 2025). Chessman (2003) identified gastropod molluscs as a very tolerant group and indictor of disturbed environment. Being the intermediate hosts to several waterborne parasites like *Schistosoma haematobium*, their dominance points to potential public health issues in the area.

Spatially, the highest number of individuals recorded in Station 2 could be due to the rich riparian vegetation despite relatively higher mean depth (Bulto *et al*., 2026). The lowest number of individuals recorded in Station 1 could be due to higher human activities, because the location was the most easily accessible coupled with sparse riparian vegetation, substrate mixed with debris and high flow velocity (Anyanwu *et al*., 2021; 2022b; Asmamaw *et al.,* 2021; Mathers *et al*., 2024). Station 3 was difficult to access with muddy substrate and sparse riparian vegetation. However, it receives effluents from pig farming and palm oil and cassava processing mills, which could negatively affect aquatic organisms (Arimoro *et al*., 2008).

The biodiversity indices were low. All the Shannon-Weiner diversity index and Margalef species richness index values were lower than 3, which is the minimum value for a stable or undisturbed environment as observed in nearby Ossah River (Anyanwu *et al*., 2019). An ecosystem is considered stable and balanced when the values of these indices are higher than 3 (Türkmen and Kazanci, 2010).

The Evenness index values were also low (<0.7) and were well below 1, the maximum for the index. The lowest value recorded in Station 2 was an indication of the dominance of one species (*Bulinus wrighti*). The low diversity as reflected in the Margalef, Shannon-Wiener and Evenness indices was due to the low number of species and individuals recorded in the study, which could be attributed to environmental disturbances caused by human and natural events. However, these impacts were not reflected in the physicochemical parameters evaluated. Macroinvertebrates can integrate changes over time, reflecting the cumulative effects of environmental conditions and human activities in their diversity and community structure because of their sedentary nature (Gouissi *et al*., 2019; Kengne Fotsing *et al*., 2022).

Conclusion

This study showed that all the physicochemical parameters evaluated were moderate or within acceptable limits to support aquatic life, except for some pH and dissolved oxygen values. Rainfall was the major influence on the physicochemical parameters, rather than anthropogenic activities. However, the macroinvertebrate assemblage was poor and dominated by pollution indicators, especially gastropod molluscs, which are indicators of disturbed environment. This was confirmed by the low values recorded in the biodiversity indices. It can be concluded that there may be some stressors of possible human or natural sources acting on the environment which were not captured by the physicochemical parameters. This emphasizes the need to combine physicochemical assessment with biomonitoring.

Conflict of Interest

The authors hereby state that this research work and manuscript production complied with ethical standards, and none of the authors has any potential conflict of interest. We further declare that this research was not funded by any agency.

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A Review of the Terrestrial Mammalian Fauna of Egypt: New Records, Distribution Records, and Extinctions

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Abstract

The present review deals with the changes that occurred in the list of the terrestrial mammals of Egypt during the last 50 years. A total of 14 newly-recorded species and subspecies, belonging to four orders: Eulipotyphla, Rodentia, Carnivora, and Hyracoidea—were documented, the presence of 3 of which is questionable. New collection localities of 19 species and subspecies, belonging to the 4 orders Eulipotyphla, Rodentia, Carnivora, and Artiodactyla were documented, and the local extinction or extinction of five species of order Artiodactyla was declared. The total number of the terrestrial mammals of Egypt has thus increased from 108 to 115 species and subspecies over the last 50 years, i.e., an increase of about 6.5% of the original number.

Keywords: Egypt; terrestrial mammals; new records; new distribution records; local extinctions.

Introduction

The first published works on the mammals of Egypt date back to the 18th century. Osborn and Helmy (1980) cited the works of several early naturalists such as Alpini (1735), Forskal (1775), and Bruce (1790).

The monumental work: "Description de L'Égypte", published in a series of volumes over a period of about 13 years (1809–1822) (published online by the Bibliotheca Alexandrina starting from 2007) addressed, among other aspects of Egypt, the natural history of the country. The "Histoire Naturelle" volumes contained highly artistic plates of Egyptian bats, lagomorphs, rodents, and artiodactyls collected by the French naturalists who accompanied Napoleons's military campaign on Egypt (1798-1801).

Several works on the taxonomy, description, biology, and geographical distribution of Egyptian mammals have been published since the beginning of the 20th century. The earliest and most comprehensive work was the Anderson's "Zoology of Egypt: Mammalia" published in 1902. He described the taxonomy and geographical distribution of Egyptian bats as well as land and marine mammals. Many years later, Flower (1932) updated the list of the mammalian species of the country including the domesticated ones. The work of El-Nigoumy (1952), in Arabic, presented brief notes on the description and geographical distribution of the mammalian species known from Egypt and introduced them with scientific and Arabic vernacular names.

The second most comprehensive work on the Egyptian mammals was that of Osborn and Helmy (1980), who gave detailed cranial, dental, and morphological descriptions, taxonomic revisions with lists of synonyms, detailed distribution maps, and notes on the biology and ecology of the Egyptian modern land mammals. Qumsiyeh (1985), five years later, reviewed the systematics, zoogeography, and ecology of the Egyptian bats.

Wassif and Soliman (1993) wrote on the habitat diversity and Egyptian mammals, and Wassif (1995) presented, in Arabic, an illustrated guide to the mammals of protected areas of Egypt. Few years later, Hoath (2003) presented an illustrated field guide to the Egyptian mammals, including bats, land and marine mammals. He described the known forms of Egyptian mammals and discussed

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the probability of the presence of several new mammalian species.

Scattered information on the taxonomy, ecology, and biology of Egyptian mammals are also found in other works on the mammals of neighboring countries such as Libya (Ranck, 1968; Hufnagl, 1972), Arabia (Harrison and Bates, 1991), and the Holy Land (Qumsiyeh, 1996). A lot of information included in the above works is based on the publications of many Egyptian and visiting naturalists. The list of these publications is too long to be reviewed herein.

The distribution of animals is a dynamic process. Since 1980, the year of Osborn and Helmy's published work, new species records and collection localities were documented and the local extinction of other species was declared. The present work briefly reviews the changes that occurred in the list of Egyptian land mammals over nearly the past 50 years. Taxonomic, biological, ecological, and behavioral aspects that correlate with the below-mentioned mammalian species are beyond the scope of the present work.

Materials and Methods

The study areas

Geography: A brief description of the geography, topography, and biogeography of Egypt is given below. For more details, refer to Osborn and Helmy (1980). Egypt is divided, on geographical and topographical bases, into the following five provinces: Sinai Peninsula, Eastern Desert, Nile Delta and Valley, Western Mediterranean Coastal Desert, and Western Desert (Figure 1).

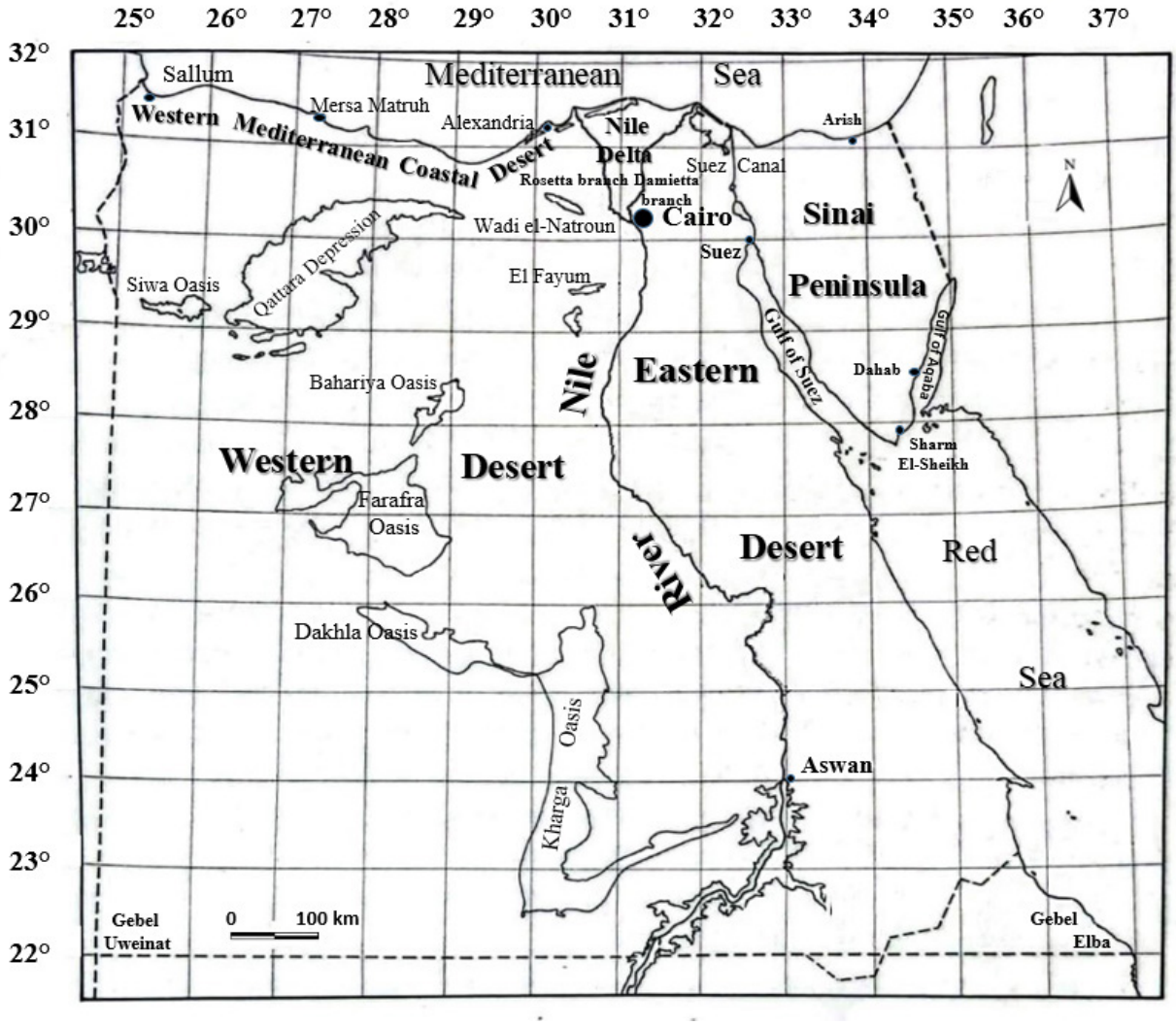

Figure 1. Geographic and topographic provinces of Egypt, showing the country's division into five major regions: the Sinai Peninsula, Eastern Desert, Nile Delta and Valley, Western Mediterranean Coastal Desert, and Western Desert.

Sinai is considered as a part of Asia and serves as a link for the exchange of Asian and African fauna.

At the extreme southeastern corner of the Eastern Desert, the outstanding Gabal Elba is located. The Nile Delta and Valley divides the mainland of Egypt into two different parts: the Eastern and Western Deserts. It serves as a link between south and north and a barrier between east and west.

The Western Mediterranean Coastal Desert is a distinct northern belt of the Western Desert. It acts as a corridor for the spread of terrestrial Libyan fauna to Egypt (Ayyad and Ghabbour, 1986). The Western Desert is characterized by depressions and oases. The highest point of the Western Desert is Gabal Uweinat at the southwestern corner of Egypt.

Biogeography: The Egyptian land is part of the Sahara Desert of north Africa. Several opinions had been proposed for assigning the Sahra to a given biogeographical region. It was considered by some authors to be a transitional area between the Mediterranean and Ethiopoan regions (Ayyad and Ghabbour, 1986). Some other naturalists considered the Sahara as part of a much broader area that extends from the Sahara to Sind in northwestern India; namely, the Saharo-Sindian region (Zohary, 1954; Ranck, 1968). They based their opinion on the close taxonomic similarities of the plant and animal species of this vast area. The mammals of the Sahara and Arabia thus became known as the Saharo-Sindian mammals (Atallah, 1977). Osborn and Helmy (1980), from a zoogeographical point of view, suggested the name "Saharo-Sindian Sub-region of the Palearctic". It is a meeting place of species from the Ethiopian, Palaearctic, and, to a much lesser extent, the Oriental or Indomalayan Biogeograpical Regions (Ghabbour, 1997).

Baseline list of terrestrial mammals of Egypt

A review of the recent literature dealing with the terrestrial mammals of Egypt was carried out using online search engines. Reference was also made to many publications related to the taxonomy, ecology, and geographical distribution of Egyptian mammals that the author collected during the last 50 years. The results of field surveys and ecological studies that were conducted by the author and his colleagues, and by other Egyptian colleagues, on the Egyptian mammals were also considered. These field surveys covered many parts of Egypt such as Sinai Peninsula, the Western Desert, Gabal Elba region in the Eastern Desert, and villages of the Nile Delta region (Soliman, 2006; Soliman and Muhallal, 2006, 2014, 2016). During field trips, verbal information provided by locals was taken into consideration. As a review article, the different opinions regarding the proper identification of the species and subspecies treated in the present work were thoroughly discussed. Reference was made to the latest version of the IUCN Red List of threatened species for two reasons; firstly, to check the present status of the Egyptian mammals, and secondly, to check the updated scientific names of the mammalian species of the present work.

The baseline list of mammals for this review is based upon the detailed work of Osborn and Helmy (1980) on the contemporary land mammals of Egypt. They reviewed all the former publications on the taxonomy and distribution of the terrestrial mammals of the country and added more collection localities of them. Reference was also made to two other important works on Egyptian mammals: the "Zoology of Egypt: Mammalia" (Anderson, 1902) and the updated list of Egyptian mammals published by Flower (1932).

Results and Discussion

I- New records:

1. Order: Eulipotyphla
Family: Erinaceidae
North African hedgehog, *Atelerix algirus* (Lereboullet, 1842)

The presence of this species in Egypt is based on seven specimens collected from near el-Negila village west of Mersa Matruh during

the period extending from 2001 to 2011 (Saleh and Basuony, 2014b). The geographic range of the species extends from western Egypt through North Africa to Morocco. The nearest locality from which the species was recorded was Derna, Libya (Hufnagl, 1972), about 500 km to the west. According to the same author, the species geographical range had also extended to Spain.

2. Order: Rodentia
Family: Muridae
Subfamily: Gerbillinae
Allenby's lesser gerbil, *Gerbillus andersoni allenbyi* (Thomas, 1918)

It was recorded for the first time in Egypt by Soliman and Muhallal (2006). They based their record on 14 specimens (7 males and 7 females) collected from al-Dhahir and Wadi el-Agra, and two specimens collected from al-Matly Island of al-Zaranik protected area, all located in northeastern Sinai Peninsula. Harrison and Bates (1991) considered *allenbyi* as a subspecies of *G. andersoni* and briefly compared between it and *bonhotei***,** which is the subspecies known from Sinai and northern Jordan. The conspecific status of *allenbyi* and *andersoni* was confirmed by Qumsiyeh (1996).

Family: Dipodidae
Long-legged lesser Egyptian jerboa, *Jaculus Jaculus longipodus* Soliman and Mohallal, 2006

A sample of 88 individuals of this subspecies was collected from three localities in northeastern Sinai: namely, al-Dhahir, al-Gora, and Wadi el-Agra. (Soliman and Mohallal, 2006). The subspecies differs from the other four subspecies known from Egypt, especially *J. j. schlueteri* of Sinai and the Holy Land, in a number of morphological and cranial characters and measurements. A prominent difference was that the average length of the hind foot was longer than that of *J. j. schlueteri*, characterized by having the longest hind feet of all Egyptian subspecies of *J. jaculus*.

Family: Hystricidae
Indian crested porcupine, *Hystrix indica* Kerr, 1792

The first record of a porcupine in Sinai was that of Wassif (1953), who collected a porcupine's quill, presumably of *Hystrix cristata*, from Ain Gudeirat, 90 km southeast of el-Arish, northeastern Sinai. Osborn and Helmy (1980) thought that the Sinai quill might belong to *H. indica*, which is present in the Arabia and the Levant, not to *cristata*. They based their opinion on the probable limited distribution of the latter species in Egypt, which is confined to the cliffs north of el-Sallum of the Western Desert where it is possibly extinct. In 1990, two specimens of porcupines were collected by Bedouins from Nekhel and Bir el-Hasana, 70 and 135 km south of el-Arish, respectively (Wassif, 1995). Saleh and Basuony (1998) collected three specimens of porcupines from Gabal el-Maghara in northern Sinai. They compared the external and cranial characters of these specimens with the corresponding ones of *H. indica*, and *H. cristata* and indicated that their external characters were clearly closer to those of *indica* and that the rest of characters overlapped with those of the other two species. Due to the small number of Sinai specimens (only 3 specimens), they could not reach at a definite conclusion regarding the specific identification of the collected specimens and recommended that further examination of more specimens must be carried out. Hoath (2003) thought that the widely separated geographical ranges of the two species of porcupines under consideration—one close to the western and the other close to the eastern frontiers of the country—would exclude any confusion about their identification. He also pointed out the two individual porcupines of el-Arish Zoo that were captured in Wadi el-Arish and were tentatively identified as *H. indica*. He considered, however, that the presence of the Indian crested porcupine in the country had not been proven yet.

3. Order: Carnivora
Family: Canidae
Golden jackal, *Canis aureus* Linnaeus, 1758

Golden jackals were recorded from Egypt for the first time by Saleh and Basuony (2014a). They identified two subspecies: the Syrian golden jackal, *C. a. syriacus* Hemprich and Ehrenberg, 1833 of Sinai Peninsula and the Qattara desert jackal, *C. a. qattarensis* Saleh and Basuony, 2014 of the northern region of the Western Desert. According to Saleh (2024), the previous records of the golden jackal in Egypt belong, in fact, to the Egyptian/African golden wolf, *Canis lupaster* Hemprich and Ehrenberg, 1833 that was erroneously identified as a subspecies of the golden jackal under the name *C. a. lupaster*. Flower (1932), however, wrote on the wolf-like jackal or the so called "Egyptian Wolf", *Canis lupaster* and described it as having an intermediate size between the real wolves of northern countries and the jackals of other parts of Africa and Asia. Wolves were widely distributed in Egypt except Sinai (El-Nigoumy, 1952; Osborn and Helmy, 1980). The record of the true golden jackal in northeastern Sinai was based on an adult female from el-Magdaba, north Sinai, and a male from near Quosiyma, eastern Sinai (Saleh and Basuony, 2014a). The animals were repeatedly recorded afterwards in considerable numbers in different parts of southern Sinai, such as Ras Mohammed National Park and the neighboring Sharm el-Sheikh area, the coastal plain of Nabq Nature Protectorate, and Saint Catherine Nature Protectorate (Saleh, 2024)). These records were based on photographs captured in 2023 and early 2024 and sight records of the species obtained by rangers of the protected areas as well as on dead golden jackals including museum specimens. The number of observed jackals ranged between one or two individuals, to up to seven animals. The record of *C. a. qattarensis* of the northern region of the Western Desert of Egypt was based on nine specimens collected from Qattara Depression, el-Dabaa, Sitra and Siwa Oases, and el-Fayum (Saleh and Basuony, 2014a).

Grey wolf, *Canis lupus* Linnaeus, 1758

The presence of the Arabian wolf in Sinai was questionable since it depended on sight records or verbal information by local Bedouins. The real presence of this wolf in Sinai was documented by Saleh and Basuony (2014a). They collected 5 specimens from the Saint Catherine area and west of Dahab in south Sinai, and Quosiyma, east Sinai. They also depended on sight records and photographs captured by rangers of Saint Catherine Natural Protectorate and Ras Mohammed National Park, and tracks observed in Abu Gallum, Ras el-Naqab, Quosiyma, and Wadi el-Arish. Based on these observations, they concluded that the Arabian wolf is widely distributed throughout Sinai, from sea level to the high elevations of south Sinai mountains. They referred the Arabian wolf of Sinai to the subspecies *C. l. arabs* Pocock, 1934.

Blanford's fox, *Vulpes cana* (Blanford, 1877)

Many authors have recorded the presence of this fox species in Egypt. According to (Hoath, 2003), the first specimen of the Blanford's fox from Egypt was collected in 1977 from Sinai. A skull was recovered from Gabal Sirbal by Mendelssohn et al. (1987) and a specimen of this fox was collected in 1988 at Wadi Quoseib, about 60km south of Suez along the coast of the Gulf of Suez (Peters and Rödel, 1994), which was a confirmation that Blanford's fox has extended its distribution to the African Continent. Hoath (2003) indicated that very few specimens were known from southeastern Sinai since the early 1980s and wondered whether this fox species was very rare or just extremely elusive. Sight records of the Blanford's fox were later reported by Saleh and Basuony (1998) who observed a very small fox with large ears and huge bushy tail for several minutes in a rocky wadi north of Ras Mohammed, southern Sinai. They also mentioned the repeated observation of this fox by the members of the Multinational Peace Keeping Forces on the summit of

Gabal el-Rashrash, southeastern Sinai, who described it as a small agile fox with a large bushy tail. The foot tracks of a probable Blanford's fox in Abu Gallum, south Sinai, were also observed by the same authors.

Family: Mustelidae
The common badger, *Meles meles* (Linnaeus, 1758)

It was recorded for the first time in Egypt from northeastern Sinai Peninsula by Soliman (2006). A skeleton of the animal together with a bunch of its hair were provided to the author by a local Bedouin in "Sayalat Al-Bawati" area, about 30 km to the south of the Mediterranean seashore and about 5 km to the west of the eastern frontiers of Egypt, in winter 2004. The animal is locally known as "Al-Ke'eby". It seemed that it was killed by locals since the left frontal of the skull was severely damaged. Several years later, Saleh and Basuony (2014b) included the common badger among the mammalian records from the country. They based their record on a live specimen captured by a Bedouin in 2006 near Ein el-Godierat in northeastern Sinai. They added that another specimen had been captured by Bedouins in the same area and sold to an animal collector in Cairo.

The honey badger, *Mellivora capensis* (Schreber, 1776)

The honey badger is a close relative of the common badger. It was believed to be found along the eastern frontiers of Egypt (Hoath, 2003). The first record of the animal was reported by Saleh and Basuony (2014b). It was based on a captive male captured by Bedouins just south of Ein el-Godierat, northeastern Sinai. They indicated that the species was very rare in the area since locals neither had seen it before nor known any local name of it. A skull of an adult animal was found in March, 2023 in a sandy shrubland area, about 5 km from Zaranik Protected Area in northeast Sinai (Rabia, 2025).

The marbled polecat, *Vormela peregusna* (Güldenstädt, 1770)

It was recorded for the first time in Egypt by Saleh and Basuony (1998) from Bir el-Abd and Gabal el-Maghara (fragmented skull) in northern Sinai Peninsula. They also observed the tracks of the animal in sandy habitats throughout this region and indicated that the species was common and well known to local Bedouins throughout northern Sinai. The tracks of the animal were also observed throughout Wadi el-Agra in northeastern Sinai by Soliman and Mohallal (2016) who collected a specimen from the same Wadi. Bedouins of Wadi el-Agra know the species by the local name "Do'wai". Two other dead specimens from unknown locality in Sinai were also provided by an animal collector to the authors.

Family: Herpestidae
White-tailed mongoose, *Ichneumia albicauda* (G. Cuvier, 1829)

The species has a wide distribution that extends through sub-Saharan Africa and southern Arabia. The possible presence of this species in Egypt was suggested by Taylor (1972). The inclusion of this species in the list of Egyptian mammals was considered by Hoath (2003) to be without a basis and did not depend on a collection locality. He added, however, that if the species was present in Egypt, it would most likely occur in the Gabal Elba region, southeast of the Eastern Desert. Egypt was included again among the African and Asian countries covered by the geographical range of this species (Do Linh San, 2015). The map provided by the author includes the southeasternmost part of the country as part of the species geographical range.

Family: Hyaenidae
Spotted hyena, *Crocuta crocuta* (Erxleben, 1777)

The first record of the spotted hyena in Egypt was published by Mahdy *et al.* (2024). They

based their record on an adult male killed by local people on March 24th, 2024 in Wadi Yahmib, Elba Protected Area, southeast Egypt. Nagy *et al.* (2025), however, published a record of a spotted hyena and said that it was the first record of the species in modern Egypt since its extinction from the country over 5,000 years ago. They based their record on an individual hyena killed on February 24th, 2024 by local people, also in Wadi Yahmib. They added that the record was 500 km north of the known range of the spotted hyena and discussed the possibility of its dispersal from neighboring Sudan along a corridor created by increased rainfall and grazing practices. It is strongly believed, based on the dates and locality of observation, and a photograph of the killed hyena, that the above two records are based on the same individual hyena.

4. Order: Hyracoidea
Family: Procaviidae
Bush hyrax or Yellow-spotted hyrax, *Heterohyrax brucei* (Gray, 1868)

The presence of the yellow-spotted hyrax in Egypt is questionable. Its presence in the Red Sea hills and Sinai was mentioned by several authors (e.g., Barry and Shoshani, 2000; Hoath, 2003) without giving specific localities for sight records or collected specimens. The records of this species along the Red Sea coast of Egypt, however, were thought to be erroneous and relate to *Procavia* (Hoffman *et al*., 2008). They have concluded, based on a review of the literature and classification of this species, that there was no evidence to support the presence of *H. brucei* in Egypt, or anywhere north of northern Eritrea and the Sudan. The same authors added that collecting further materials in the form of specimens, photographs, and tissue materials for genetic analysis was required before taking a final decision on the northern range of this species.

II- New distribution records:

1. Order: Eulipotyphla
Family: Erinaceidae
Desert hedgehog, *Paraechinus aethiopicus deserti* (Loche, 1858)

The desert hedgehog, identified by Osborn and Helmy (1980) as *P. d. deserti*, was previously recorded from the northwestern margin of the Nile Delta and the Western Mediterranean Coastal Desert of Egypt. Saleh and Basuony (2014b) collected an adult male and reported on several sight records of this hedgehog from an olive grove in Siwa Oasis. According to these authors, the new records were several hundred kilometers far from the nearest known points of the distribution of this species.

Sinai desert hedgehog, *Paraechinus aethiopicus pectoralis* (Heuglin, 1861)

This subspecies, identified by Osborn and Helmy (1980) as a *P. d. dorsalis*, was recorded from different localities in southern Sinai. The subspecies proved to be more widespread in Sinai since it was recorded by Saleh and Basuony (1998) from a wider area that included northern, central, and southern parts of the Peninsula.

Family: Soricidae
Dwarf shrew, *Crocidura nana* Dobson, 1890

The geographical range of the species included the southern part of Nile Delta and possibly Nile Valley (Osborn and Helmy, 1980). An adult female was collected later from a densely vegetated, sandy island located about 5 km from the eastern shore of Lake Bardawil of northern Sinai (Saleh and Basuony, 2014b).

Guldenstadt's shrew, *Crocidura gueldenstaedtii* (Pallas, 1811)

The presence of this species in Egypt was based on a single female specimen recorded from South Sinai at an elevation of 1,500 m inside a small monastery west of St. Catherine (Osborn and Helmy, 1980, Hoath, 2003). Two more specimens were later collected from Wadi el-Arish in northeastern Sinai (Saleh and Basuony, 1998).

House shrew, *Suncus murinus* Linnaeus, 1766

The house shrew was known in Egypt only from a single specimen collected from a house in Suez on the Red Sea (Osborn and Helmy, 1980). A female specimen was later collected from a house in Motobus, Kafr El-Sheikh Governorate, Nile Delta (Saleh and Basouni, 2014b).

2. Order: Rodentia
Family: Muridae
Subfamily: Gerbillinae
Lesser Egyptian gerbil, *Gerbillus gerbillus* (Olivier, 1801)

It is a widely distributed desert rodent in Egypt. Different subspecies of the lesser gerbil are found in Sinai Peninsula, northern and southern parts of the eastern Desert, Nile Delta and Valley, and Western Desert (Osborn and Helmy, 1980). 13 specimens of this gerbil were recently collected from Baltim, Kafr el-Skeikh Governorate, at a Mediterranean coastal area of the Nile Delta (Younis, 2023). He did not refer the collected specimens to any known subspecies from Egypt, but he noticed that they were larger than them all.

Pleasant gerbil, *Gerbillus amoenus* (de Winton, 1902)

It is grouped either under the genus *Gerbillus* or *Dipodillus*. It is a widely distributed dipodil in Egypt since it is known from different localities in the Western Desert of Egypt including, Wadi el-Natroun, el-Fayum and Dakhla Oasis, and from southern Eastern Desert including Wadi Allaqi (Osborn and Helmy, 1980). Four individuals were collected from different localities of Gabal Elba region in the far southeast of Egypt, just to the north of the Egyptian-Sudanese frontiers (Mohallal, 2007). These localities represent the southeasternmost collection localities of this species in Egypt. It was suggested that the charming dipodil could exploit the southern part of the Eastern Desert of Egypt by trans-Nubian Nile crossing (Ayyad and Ghabbour, 1986). Specimens of this species were also collected for the first time from four localities in Siwa Oasis; namely, al-Gabal al-Akhdar, al-Senoseya, Gabal Zomat, and al-Ghweisat (Soliman and Mohallal, 2014). The disjunct distribution of the species suggests that it is more widespread than the records indicate (Hoath, 2003).

Pigmy gerbil, *Gerbillus henleyi* (de Winton, 1903)

It is grouped either under the genus *Gerbillus* or *Dipodillus*. Specimens of this species (2 males and one female) were collected from three localities in northeastern Sinai; namely, Wadi Umm Shehan (gravely habitat), Bir Lehfen (moderately vegetated habitat), and Wadi el-Agra (soft sand and moderate vegetation) (Soliman and Mohallal, 2016). The three areas represent new collection localities of this rodent species from Sinai, since it was collected before only from the southwest of the Peninsula (Osborn and Helmy, 1980).

Libyan jird, *Meriones libycus* Lichtenstein, 1823

The distribution of the Libyan jird covers the northern part of the Western Desert (Osborn and Helmy, 1980). It was recently recorded by Mohalla and Ahmed (2018) from Kharga and Dakhla Oaes. No details about the number of collected specimens or the names of collection localities were given.

Shaw's jird, *Meriones shawi* (Duvernoy, 1842)

The species is known to occur in various localities of the Western Mediterranean Coastal Desert and el-Maghra (Osborn and Helmy, 1980). According to Hoath (2003), the southernmost collection locality of this species was the northeastern corner of Qattara Depression. It was recorded for the first time by Soliman and Mohallal (2014) from an area close to Siwa Oasis, far south to the Mediterranean coastline. They

collected a single individual of this jird from Umm Hwemel, about 300 km south of the Mediterranean Coastal Desert, in a sparsely vegetated locality characterized by the presence of isolated scattered trees of genus *Vachellia* (= *Acacia*). The specimen may belong to a relict population of this species.

Fat tailed gerbil, *Pachyuromys duprasi* Lataste, 1880

The distribution of the species covered various localities in the northern part of the Western Desert of Egypt including Wadi el-Natroun, and Abu Rawash in Giza Governorate (Osborn and Helmy, 1980). A male gerbil was collected from a barren isolated stand of 14 trees of *Vachellia raddiana* east of Qattara Depression, about 280 km southwest of Cairo and 350 km south of the Mediterranean coastline (Saleh and Basuony, 2014b). They trapped and released 5 more specimens (2 males and 3 females) in the same locality during the period extending from 1995–2006, and suggested that these individuals belong to a relict isolated population of this gerbil.

Subfamily: Murinae
Brown rat, *Rattus norvegicus* (Berkenhout, 1769)

The distribution of this commensal rat in Egypt covers the coastal towns and Nile Delta and Valley (Osborn and Helmy, 1980). Hoath (2003) added Aswan, el-Fayum, Suez Canal area, with one isolated record from Gabal Elba area. It was recently recorded from Kharga and Dakhla Oases by Mohallal and Ahmed (2018). The authors did not give details about the numbers of collected specimens or the names of collection localities.

House mouse, *Mus musculus* Linnaeus, 1758

This commensal mouse is widely distributed in Egypt. It was recorded from different localities in Sinai Peninsula, Suez Canal area, Red Sea coast, Nile Delta and Valley, Western Mediterranean Coastal Desert, and oases of the Western Desert (Osborn and Helmy, 1980). According to Hoath (2003), it is absent from the Eastern Desert except the settlements along the Red Sea coast south to Mersa Alam. Four males were later collected from two localities in Gabal Elba region in far southeastern Egypt; namely, Abu Ramad and Sarara Eshabae just north of the Egyptian-Sudanese frontiers (Mohallal, 2007). Commensal rodents likely expand their range following the spread of new human societies.

Family: Dipodidae
Four-toed jerboa, *Allactage tetradactyla* (Lichtenstein, 1823)

The former geographic range of this species in Egypt included the northern part of the Western Mediterranean Coastal Desert. It was reported to be locally extinct or threatened with extinction since early 1960s (Hoogstraal, 1963). The threats facing this rodent species were attributed to anthropogenic activities such as habitat destruction through land reclamation (Hoogstraal, 1963), summer resort development along the Mediterranean coast (Hoath, 2003), and the extensive collection of this rodent for commercial purposes in response to the great demand for it as a house pet (Saleh and Basuony, 2014b). The last authors added that the animal became a locally extinct species in Egypt except for a very small area near el-Negila, west of Mersa Matruh, where they observed some individual jerboas and captured and released some others.

3. Order: Carnivora
Family: Canidae
Red fox, *Vulpes vulpes* (Linnaeus, 1758)

The red fox is widely distributed in Egypt (Osborn and Helmy, 1980) and continuously expands its range of distribution in the country as it follows the spread of human activities (Hoath, 2003). It was recorded for the first time from Siwa Oasis by Soliman and Mohallal (2014), who collected three

individuals (2 males and one female) from Ghet-Akhlel area. The tracks of this fox were also observed by the authors in different localities of the Oasis. According to Osborn and Helmy (1980), the fox was recorded from various localities in north Sinai coastal areas and the northern coast of the Gulf of Suez. A live specimen was later collected from the Wadi el-Agra area in northeastern Sinai by Soliman and Mohallal (2016). The fox has also become very common throughout southern Sinai from sea level up to 1800 m altitude (Saleh and Basuony, 1998). According to Hoath (2003), the red fox has extended its distribution range as far south as Ras Mohammed.

Fennec fox, *Vulpes zerda* (Zimmermann, 1780)

The distribution of the species covers Sinai Peninsula and the Western Desert south of the Mediterranean Coastal Desert, where it was recorded from various localities including el-Maghara, Wadi el-Natroun, el-Fayum, Wadi el-Rayan, Farafra, Dakhla and Kharga Oases (Osborn and Helmy, 1980). The presence of this fox in Siwa oasis was inferred from the observation of its foot tracks, a burrow opening in Ain al-Safa, probably belonging to it, as well as verbal information of local people (Soliman and Mohallal, 2014). The species was also very common in sandy areas of northern Sinai (Saleh and Basuony, 1998).

Family: Felidae
Sand cat, *Felis margarita* Loche, 1858

The distribution of this elusive little cat in Egypt is not well known. Flower (1932) wrote about a very long-haired wild cat of unknown locality which he did not examine but he thought it was a sand cat. He also reported on two living cats that he saw in the Zoological Garden at Zagazig, east of Nile Delta, in the years 1912–1914, which were collected from sandy tracts of unknown localities. The external features of these two cats conformed with hose of the sand cat. This cat species was not, however, recorded among the cats of Egypt by El-Nigoumy (1952). The presence of the sand cat was confirmed in southeastern part of Egypt by the collection of only one specimen by Ibrahim Helmy in 1975 (Osborn and Helmy, 1980). Several specimens of the sand cat were later collected. The following records of sand cats were reported by Hoath (2003): records from the Qift-Quseir road in the Eastern Desert and Ras Abu Fatma in the far southeast of Egypt in 1980s; an individual cat trapped in North Sinai and sent to al-Arish Zoo in 1995; several specimens obtained from northeastern Sinai; 7 live cats of unknown locality possessed by an animal dealer in 1996 and at least 4 stuffed specimens of unknown locality displayed in Kerdasa, an important center of animal trade, since 1990. Five specimens of this cat species were collected by Saleh and Basuouny (1998) from northern Sinai Peninsula: a male and a female from Bir el-Abd and a male and two females from Gabal el-Maghara. They also observed the tracks of this cat throughout the sandy desert of northern Sinai. The above records indicate that the sand cat is widespread in Egypt.

4. Order: Artiodactyla
Family: Bovidae
Dorcas gazelle, *Gazella dorcas* (Linnaeus, 1758)

Different subspecies of the dorcas gazelle are present in the Sinai Peninsula, Eastern and Western Deserts (Osborn and Helmy, 1980). They did not examine any specimens from Sinai Peninsula. Anderson (1902) recorded this gazelle from Ayun Musa in western Sinai. According to El-Nigoumy (1952), the dorcas gazelle was observed in el-Fayum desert, the oases of the Western Desert and the Nile Valley. This gazelle became greatly reduced over much of its former range in both the Western and Easter Deserts, and Sinai (Hoath, 2003). Due to the illegal hunting of this species, he did not publish the details of the sight records of this gazelle outside protected areas of Egypt. Individuals and small herds of this gazelle species were sighted in Sahl El-Qa, Nabq Protected Area

to the west of Dahab, southeast Sinai by Saleh and Basuouny (1998), and the presence of this gazelle in St. Katherine Protected Area, south Sina, was reported by Hoth (2003).

Mountain gazelle, *Gazella gazella* (Pallas, 1766)

The presence of the Arabian gazelle in Egypt is based on sight records in Wadi el-Arish in north Sinai (Flower, 1932). According to El-Nigoumy (1952), Arabian gazelles also inhabited the valleys of Sinai Peninsula between Suez and el-Tor in the south. It was not collected from Sinai Peninsula by Osborn and Helmy (1980), who only examined four specimens from Saudi Arabia. Few individuals of Arabian gazelle together with the foot tracks and fresh fecal pellets were observed in sandy areas close to Wadi el-Agra in northeastern Sinai (Soliman and Mohallal, 2016). A local Bedouin provided the authors with a part of male gazelle head covered with skin, with only one ear pinna and one horn, and a leg shaft with hoofs.

III- Locally extinct species:

The following five mammalian species of order Artiodactyla were already locally extinct in Egypt in 1980, the time of publishing the work of Osborn and Helmy on the contemporary land mammals of Egypt. They, however, included them in their book.

Order: Artiodactyla
Family: Suidae
The wild boar, *Sus scrofa* Linnaeus, 1758

The wild boar is locally extinct in Egypt (Keuling and Leus, 2019). They added that over-hunting and changes in land use had resulted in the extermination of this species from many parts of its geographical range, including North Africa. The distribution of this species in the prehistoric and historic times of Egypt included the Nile Delta, Wadi el-Natroun, el-Faiyum, and probably most of the Nile Valley (Osborn and Helmy, 1980). They were observed, perhaps for the last time in Egypt in Wadi el-Natroun in 1895 and in the neighborhood of Damietta in 1897 (Anderson, 1902). Before that time, wild boars occurred in many parts of the country during the 19th century (Flower, 1932).

Family: Hippopotamidae
The hippopotamus, *Hippopotamus amphibious* Linnaeus, 1758

The hippopotamus is locally extinct in Egypt (Lewison and Pluháček, 2017). They added that until about the year 1700, the animal sporadically occurred in Egypt in two areas: the Nile Delta to the north, and upper Nile to the south. It was, however, not present in Rosetta branch (Osborn and Helmy, 1980). The animal was not present in Egypt at the time of Anderson (1902), who concluded that it had disappeared after the spread of firearms. According to Flower (1932), the animal became extinct in Egypt within the 19th century. Based on information provided by early writers, he reported that the last hippopotamus in the Nile Delta had been killed near Damietta in the year 1815, and that the last sight record of the animal in the upper Nile was documented 37 km north of Aswan in about the year 1816.

Family: Bovidae
The scimitar horned oryx, *Oryx dammah* (Cretzschmar, 1827)

The scimitar horned oryx is extinct from Egypt (IUCN SSC Antelope Specialist Group, 2023). The date of the disappearance of the species from the country is not clear cut. Flower (1932) considered this animal species to be extinct in Egypt and added that the last individual had been killed in about the year 1850. About 125 years later, however, an individual oryx was observed by Ibrahim Helmy in 1975, about 160 km south of Mersa Matruh, Western Desert (Osborn and Helmy, 1980). It was perhaps the last sight record of the oryx in Egypt. The distribution of the animal in the early years of the 19th century included the west side of Giza Province and round el-Fayum (Flower, 1932). He added, based on collected reports, that a small herd of oryx was observed in the desert near Wadi el-Natroun about the year 1831.
The addax, *Addax nasomaculatus* (Blainville,

1816)

The addax is locally extinct in Egypt (IUCN SSC Antelope Specialist Group, 2016). The previous distribution of the addax in the country probably included most of the Western Desert (Osborn and Helmy, 1980). The geographical range of the animal progressively shifted southwards until it disappeared from Egypt (Malinus, 2000). There is, however, no agreement about the date of the disappearance of addax from the country. It was thought that the last individual addax in Egypt was shot in about the year 1900 at a district located more than 60 km to the west of Alexandria (Flower, 1932). Malinus (2000), however, reported that a stray living addax was killed in a car collision southwest of the Kharga depression in 1931, and El-Nigoumy (1952) mentioned that a live addax was collected from the desert areas to the south of Gebel Uweinat of southwest Egypt and brought to the Giza Zoo. According to Malinus (2000), only few animals still visited the extreme southwest of the country in search of food plants during the period extending from the beginning of the 20th century and the end of the 1960s. Hoath (2003) wrote that it was unlikely that this endangered species still existed in the country. If so, he added, it would only be present in very small numbers in the remote parts of the Egyptian deserts. On a worldwide basis, the addax is listed today as a critically endangered species and almost all of its remaining individuals live in Niger (IUCN SSC Antelope Specialist Group, 2016).

The bubal hartebeest, *Alcelaphus buselaphus* (Pallas, 1766)

Bubal hartebeest is extinct worldwide (IUCN SSC Antelope Specialist Group, 2017). The previous distribution of the animal in Egypt included Oases and oasis-like depressions of the Western Desert and the Western Mediterranean Coastal Desert (Osborn and Helmy, 1980). Based on verbal information, the animal was present west of el-Fayum in the Western Desert of Egypt until 1893 (Anderson, 1902). There were no records of wild individuals of the species from the country thereafter (Flower, 1932).

Conclusion

The distribution of animals on a national, regional, or a worldwide scale is a dynamic process; new species cross the borders from neighboring areas, others become locally extinct or exceedingly rare, while new collection localities are discovered. The reasons for this dynamic nature of animal distribution are many and are related to such factors as anthropogenic activities (habitat destruction, fragmentation, or degradation) and global climatic changes. The regular survey of the fauna of different parts of the world is badly needed to update the lists of animal groups inhabiting different parts. Updated lists are needed by conservation authorities to set priorities and propose conservation action plans for saving threatened or near-threatened species from going extinct.

According to the baseline list of Osborn and Helmy (1980), the total number of the terrestrial mammals of Egypt was 108 species and subspecies. During the last 50 years, a total of 14 species and subspecies were added to this list, the presence of 3 of which is questionable. They were, however, discussed in the present work, but not added to the list of the present mammals of Egypt, to draw the attention of Egyptian naturalists to the probable presence of these species. A taxonomic revision of genus *Canis* in Egypt (Saleh and Basuony, 2014a) increased the number of canid species and subspecies to 5 (3 of which are included in the newly recorded forms in the country) instead of one subspecies in the baseline list. Five extinct species were, on the other hand, removed from the baseline list. The total number of the extant mammals of Egypt has thus raised to 115 species and subspecies (Appendix 1). In other words, during the last 50 years, the list of Egyptian mammals increased by 7 species and subspecies that represent about 6.5% of the original number of species and subspecies (108) of the country.

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Appendix 1: Updated List of Egyptian Mammals

1. Order: Eulipotyphla

Family 1: Erinaceidae

Hemiechinus auritus aegyptius (Fischer, 1829)
H. a. libycus (Ehrenberg, 1833)
Paraechinus aethiopicus deserti (Loche, 1858)
P. a. aethiopicus (Ehrenberg, 1833)
P. a. pectoralis (Heuglin, 1861)
Atelerix algirus (Lereboullet, 1842)

Family 2: Soricidae

Crocidura flavescens deltae Heim de Balsac and Barloy, 1966
C. floweri Dollman, 1916
C. nana Dobson, 1890
C. gueldenstaedtii (Pallas, 1811)
C. suaveolens matruhensis Setzer, 1960
Suncus murinis sacer (Ehrenberg, 1833)
S. etruscus Savi, 1822

2. Order: Lagomorpha

Family: Leporidae

Lepus capensis sinaiticus (Ehrenberg, 1833)
L. c. aegyptius (Dresmarest, 1822)
L. c. isabellinus (Cretzschmar, 1826)
L. c. rothschildi (De Winton, 1902)

3. Order: Rodentia

Family 1: Muridae

Subfamily 1: Gerbillinae

Gerbillus pyramidum pyramidum I. Geoffroy St. Hilaire, 1825
G. p.gedeedus Osborn and Helmy, 1980
G. p. elbaensis Setzer, 1958
G. floweri (Thomas, 1919)
G. perpallidus Setzer, 1958
G. a. andersoni De Winton, 1902
G. a. inflatus (Ranck, 1968)
G. a. bonhotei (Thomas, 1919)
G. a. allenbyi (Thomas, 1918)
G. gerbillus asyutensis Setzer, 1960
G. g. sudanensis Setzer, 1956
G. g. gerbillus (Olivier, 1801)
G. amoenus (de Winton, 1902)
G. h. henleyi (de Winton, 1903)
G. h. mariae (Bonhote, 1909)
Dipodillus campestris wassifi (Setzer, 1958)
D. c. haymani (Setzer, 1958)
D. c. patrizii (de Beaux, 1932)
D. c. venustus (Sundevall, 1843)
D. d. dasyurus (Wagner, 1842)
D. mackilligini Thomas, 1901
D. simoni kaiseri (Setzer, 1958)
Sekeetamys c. calurus (Thomas, 1892)
S. c. makrami (Setzer, 1961)

Meriones c. crassus Sundevall, 1842
M. c. perpallidus Setzer, 1961
M. c. pallidus Bonhote, 1912
M. sacramenti Thomas, 1922
M. libycus Lichtenstein, 1823
M. shawi (Duvernoy, 1842)
M. t. tristrami Thomas, 1892
Pachyuromys duprasi natronensis De Winton, 1903
Psammomys o. obesus Cretzschmar, 1828
P. o. nicoli Thomas, 1908
P. o. terraesanctae Thomas, 1902

Subfamily 2: Murinae

Arvicanthis n. niloticus (Desmarest, 1822)
Rattus rattus (Linnaeus, 1758)
R. norvegicus (Berkenhout, 1769)
Mus musculus praetextus (Brants, 1827)
Acomys r. russatus (Wagner, 1840)
A. r. aegyptiacus Bonhote, 1912
A. c. cahirinus (Desmarest, 1819)
A. c. viator (Thomas, 1902)
A. c. hunter (De Winton, 1901)
A. c. dimidiatus (Cretzschmar, 1826)
A. c. megalodus (Setzer, 1959)
A. c. helmyi Osborn, 1980
Nesokia indica suilla (Thomas, 1907)

Family 2: Spalacidae

Nannospalax ehrenbergi (Nehring, 1898)

Family 3: Muscardinidae

Eliomys quercinus melanurus (Wagner, 1840)
E. q. cyrenaicus (Festa, 1921)

Family 4: Dipodidae

Allactaga tetradactyla (Lichtenstein, 1823)
Jaculus o. orientalis Erexleben, 1777
J. j. schlueteri (Nehring, 1901)
J. j. flvillus Setzer, 1955
J. j. butleri Thomas 1922
J. j. longipodus Soliman and Mohallal, 2006
J. j. jaculus (Linnaeus, 1758)

Family 5: Hystricidae
Hystrix cristata Linnaeus, 1758

4. Order: Carnivora

Family 1: Canidae

Canis aureus syriacus Hemprich and Ehrenberg, 1833
C. a. qattarensis Saleh and Basuony, 2014
C. lupus arabs Pocock, 1934
C. lupaster lupaster (Hemprich and Ehrenberg, 1833)
C. l. doederleini Hilzheimer, 1906
Vulpes v. aegyptiaca (Sonnini, 1816)
V. v. arabica Thomas, 1902
V. r. rueppelli (Schinz, 1825)
V. zerda (Zimmermann, 1780)
Vulpes cana (Blanford, 1877)

Family 2: Mustelidae

Poecilictis l. libyca (Hemprich and Ehrenberg, 1833)
Ictonyx striatus erythreae De Winton, 1898
Vormela peregusna (Güldenstädt, 1770)
Mustela nivalis numidica (Hemprich and Ehrenberg, 1833)
Meles meles (Linnaeus, 1758)
Mellivora capensis (Schreber, 1776)

Family 3: Viverridae
Genetta genetta senegalensis (Fischer, 1829)

Family 4: Herpestidae

Herpestes i. ichneumon (Linnaeus, 1758)

Family 5: Hyaenidae

Hyaena hyaena dubbah (Meyer, 1793)
Crocuta crocuta (Erxleben, 1777)
Proteles cristatus pallidior Cabrera, 1910

Family 6: Felidae

Felis chaus nilotica De Winton, 1898
F. sylvestris libyca (Froster, 1780)
F. s. tristrami (Pocock, 1944)
F. m. margarita Loche, 1858
Caracal caracal schmitzi (Matschie, 1912)
Panthera pardus jarvisi Pocock, 1932
P. p. pardus (Linnaeus, 11758)

Acinonyx jubatus (Schreber, 1776)

5. Order: Hyracoidea

Family: Procaviidae

Procavia cpensis syriaca (Schreber, 1784)
P. c. ruficeps (Hemprich and Ehrenberg, 1832)

6. Order: Perissodactyla

Family: Equidae

Equus asinus africanus (Fitzinger, 1857)
7. Order: Artiodactyla

Family: Bovidae

Gazella l. leptoceros (F. Cuvier, 1842)
G. dorcas littoralis (Blaine, 1913)
G. d. dorcas (Linnaeus, 1758)
G. d. saudiya (Carruthers and Schwarz, 1935)
G. gazella arabica (Lichtenstein, 1827)
Capra ibex nubiana (F. Cuvier, 1825)
Ammotragus lervia ornatus (I. Geoffroy St. Hilaire, 1827)

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First Record of the Invasive Australian red gum lerp psyllid, *Glycaspis brimblecombei* Moore, 1964 (Hemiptera: Psylloidea: Aphalaridae), in Jordan

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Abstract

The Australian red gum lerp psyllid, *Glycaspis brimblecombei* Moore, 1964, and its parasitoid *Psyllaephagus bliteus* Riek, 1962 (Hymenoptera: Encyrtidae) are recorded for the first time in Jordan. Other insect species associated with the psyllid are also recorded. Digital images of the pest, its natural enemies, and other associated insects are provided. The distribution of this introduced pest in Jordan is still unknown and further research is needed to evaluate its damage and to construct an integrated management program.

Keywords: *Eucalyptus camaldulensis*; *Psyllaephagus bliteus*; biological control; natural enemies; pest management.

Introduction

Psylloidea (Hemiptera: Sternorrhyncha) (jumping plant-lice or psyllids) constitute one of the superfamilies of Sternorrhyncha with more than 4,000 described and probably just as many undescribed species (Burckhardt *et al.*, 2012). There were 33 previously recorded species of Psylloidea from Jordan. Their distributions within and outside Jordan were given as well as their host plants and zoogeographic affinities (Al Khawaldeh *et al.,* 1997). Recently, the bay sucker, *Lauritrioza alacris* (Flor) (Hemiptera: Triozidae) was recorded for the first time in Jordan (Bader *et al.,* 2021).

Glycaspis brimblecombei is a native psyllid in Australia which feeds on *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) and other *Eucalyptus* species (Moore, 1970). It was first recorded outside Australia in California in 1998 on *E. camaldulensis* (Brennan *et al.,* 1999). Later, it was reported from the Iberian Peninsula in 2007 (Hurtado Hernández and Reina Belinchón, 2008; Valente and Hodkinson, 2009), Italy (Laudonia and Garonna, 2010; Garonna *et al.,* 2011), France (Cocquempot *et al.,* 2012), Montenegro (Malumphy *et al.,* 2013), Greece (Bella and Rapisarda, 2013), Turkey (Karaca *et al.,* 2015) and Cyprus (Karaca *et al.,* 2017), Croatia (Pintar *et al.,* 2020). In Africa, *G. brimblecombei* was reported from Algeria (Reguia and Peris-Felipo, 2013), Mauritius (Sookar, Seewooruthun & Ramkhelawon, 2003), Morocco (Maatouf and Lumaret, 2012), Tunisia (Ben Attia and Rapisard, 2014) and South Africa (Bush et al. 2016), and Zambia (Chungu *et al.,* 2016).

The objective of this paper is to officially record *G. brimblecombei* from Jordan as well as its associated natural enemies.

Materials and Methods

Infested leaves of *E. camaldulensis* were collected in plastic bags and then kept in the refrigerator for several hours. Adult insects were picked up with a fine brush immersed in 70% alcohol. Adults were identified morphologically according to the description and illustrations of Moore (1964), descriptions and key provided in Hollis (2004) and keys developed by Halbert *et al.* (2001) and Laudonia and Garonna (2010). Male genitalia were also examined to confirm the identification. Voucher specimens of the psyllids and all other insects associated with it were collected and deposited at the University of Jordan Museum. Digital images of adult flies were taken using a 65 mm macro lens mounted on a Canon (Tokyo,

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Japan) 5D Marl IV camera with an LED ring light. Images were stacked using Helicon Focus software and then processed using Adobe Photoshop 2020.

Results and Discussion

Conical, white, scale-like coverings (Figure 1) were first observed on the leaves of *E. camaldulensis* in Day Abi Said (North Jordan) in 2021. The insect causing these white lerps was identified as *G. brimblecombei* Moore, 1964. After our initial observation, the insect was seen in other parts of Jordan in Ajloun, Jerash, and Amman. Almost all trees were infested with different infestation densities. The origin of the infestation is expected to be a natural extension of the geographical distribution from neighboring countries.

The eggs are laid in clusters on leaf surfaces (Figure 2). The first instar nymphs build their lerps as soon as they start feeding. The lerp is attached to the leaf surface. The following instars (Figure 3) increase the diameter and height of the lerp by adding new sugary secretions under its outer edge. The secretions appear as an opaque thick liquid which solidifies soon after contact with the air (Hollis, 2004). After the nymphs become adults, they leave the lerp and fly to feed on new *E. camaldulensis* trees (Laudonia and Garonna, 2010). Adults (Figure 4) of *G. brimblecombei* are 2–4 mm long, light green in color (Figure 5), with dark eyes (Karaca *et al.,* 2017).

The parasitoid *Psyllaephagus bliteus* Riek, 1962 (Hymenoptera: Encyrtidae) was collected from the infested leaves (Figure 6). This parasitoid was recovered from *G. brimblecombei* in Greece (Bella and Rapisarda 2013), Italy (Caleca *et al.,* 2011), Spain (Pérez-Otero *et al.,* 2011), Turkey (Karaca *et al.,* 2015), and Algeria and Morocco (Reguia and Peris-Felipo 2013). It is assumed that it was introduced by coincidence associated with its host, *G. brimblecombei*.

Several insect species were seen associated with the psyllid: the Cardinal Ladybird (Figure 7), *Novius cardinalis* (Mulsant, 1850) (Coleoptera: Coccinellidae), ants (Hymenoptera: Formicidae), the oriental wasp, *Vespa orientalis* (Hymenoptera: Vespidae), honeybees, *Apis mellifera* (Hymenoptera: Apidae), and other wild bees, *Anthocoris* sp. (Hemiptera: Anthocoridae) were observed and collected. Before an integrated pest management program is suggested, some necessary data should be collected about the distribution of this pest in Jordan, assessment of its damage to established trees and new seedings, presence of other host species, its number of generations a year, and the presence of native natural enemies and their role in reducing its populations.

Figure 1. *Glycaspis brimblecombei* lerps on *Eucalyptus camaldulensis.*

Figure 2. Egg cluster of *Glycaspis brimblecombei* on *Eucalyptus camaldulensis* leaf. Enlarged eggs.

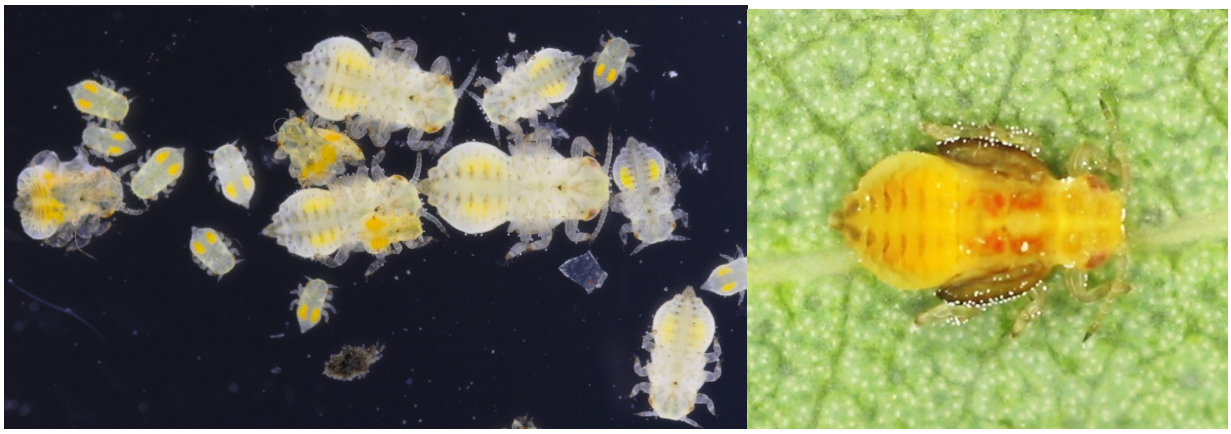

Figure 3. Different nymphal instars of *Glycaspis brimblecombei* extracted from under the lerps and a late instar nymph.

Figure 4. *Glycaspis brimblecombe*i adult male.

Figure 5: *Glycaspis brimblecombei* adult female.

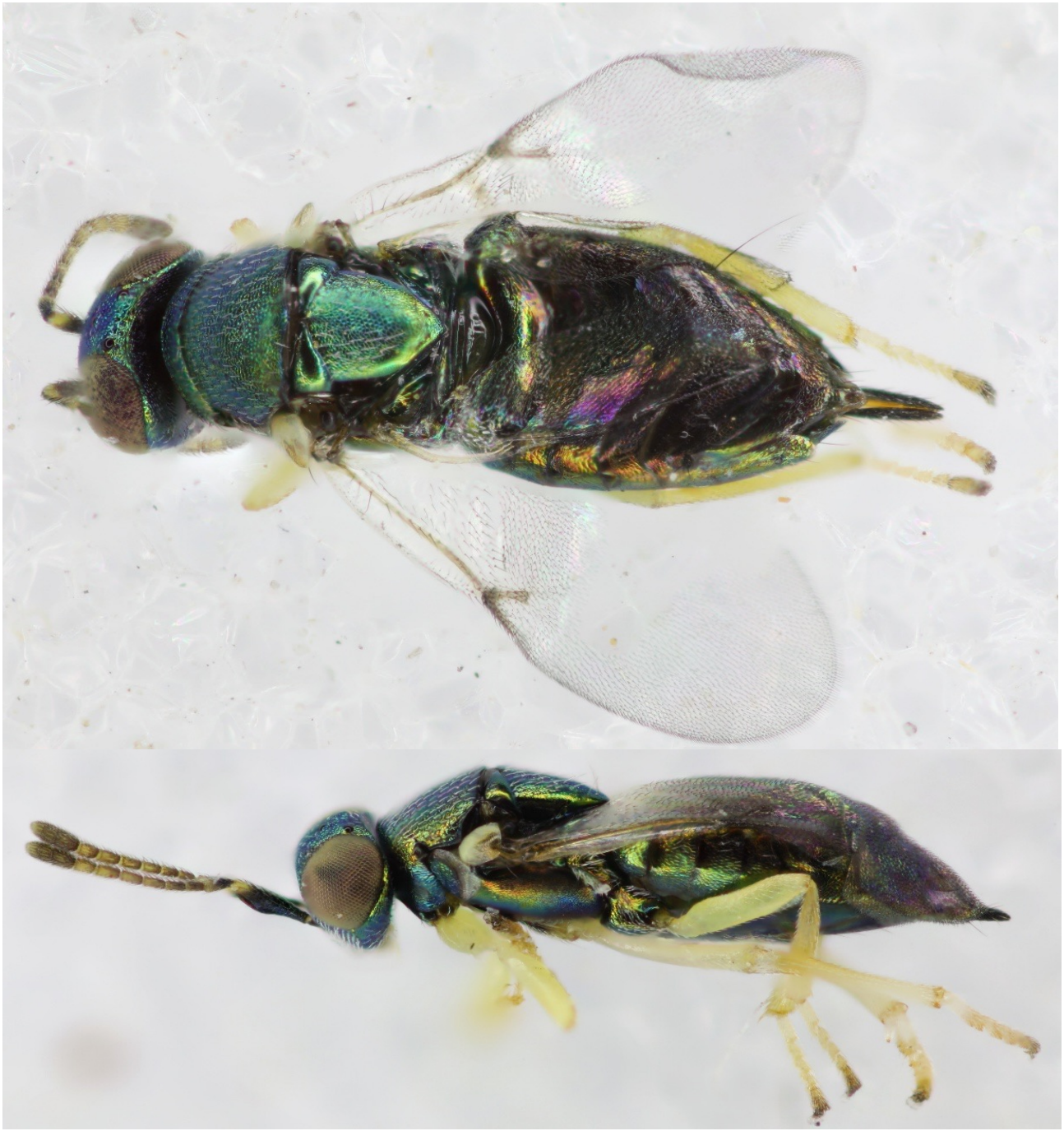

Figure 6. The parasitoid *Psyllaephagus bliteus* Riek, 1962 (Hymenoptera: Encyrtidae) (Dorsal and lateral views)

Figure 7. The Cardinal Ladybird, *Novius cardinalis* (Mulsant, 1850).

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Short Communication

Documented Opportunistic Predation by the Squacco Heron *Ardeola ralloides* on the Ocellated Skink *Chalcides ocellatus* in an Artificial Wetland at Ayla Oasis, Aqaba, Southern Jordan

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Jordan lies along one of the principal migratory flyways linking Eurasia and Africa, and supports a wide range of waterbirds that use its wetlands as stopover, wintering, or occasional breeding sites (Porter and Aspinall 2010; BirdLife International 2023). Within this context, Aqaba represents a particularly important location as the country's only coastal outlet on the Gulf of Aqaba. Its position at the southern extremity of Jordan, combined with the presence of both natural and artificial wetlands, provides suitable habitats for numerous waterbird species throughout the year.

Among these, herons are regularly observed in the Aqaba region, where shallow water bodies offer favourable foraging conditions. Species such as the Squacco Heron *Ardeola ralloides* are attracted by the availability of small aquatic and semi-aquatic prey, as well as the relatively stable environmental conditions compared to inland wetlands (Cramp and Simmons 1977; Hancock and Kushlan 1984). The development of artificial habitats has further enhanced these opportunities.

The Ayla Oasis Project, in particular, has created a network of artificial wetlands consist of sea water lagoons and fresh water bodies among the Golf course that maintain permanent water and support diverse prey communities. These conditions have made the site a consistent foraging area for herons and other waterbirds. Despite the well-documented presence and feeding activity of herons in such habitats, certain aspects of their diet, particularly involving non-aquatic prey, remain infrequently reported (Kushlan *et al.,* 2020; del Hoyo *et al.,* 2024).

The observation was made on 14 April 2026 at approximately 11:00 h during a birdwatching visit by the author at Ayla Oasis, Aqaba, southern Jordan. An individual Squacco Heron *Ardeola ralloides* was initially observed foraging along the shallow edge of the fresh water wetland. During the observation, the bird was noted handling and consuming an unusual prey item. The event was documented photographically using a 600 mm Sigma lens. Subsequent examination of the images allowed the prey to be identified as an Ocellated Skink *Chalcides ocellatus* (Figure 1).

This behaviour can be interpreted as opportunistic prey switching by the Squacco Heron *Ardeola ralloides* under local prey limitations. The predation on the Ocellated Skink *Chalcides ocellatus* represents a deviation from the typical diet of *A. ralloides*, which is generally dominated by aquatic and semi-aquatic prey such as insects, fish and amphibians (Hancock and Kushlan 1984; Kushlan 1978). Studies on heron feeding ecology indicate that these species rely on visually detected prey and employ a range of foraging techniques shaped by local environmental conditions and prey availability (Kasprzykowski and Goławski 2024).

At Ayla Oasis, the absence of freshwater amphibians such as frogs and toads remove one of the key vertebrate prey groups

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Figure 1: The Squacco Heron is eating the Ocellated Skink *Calcides ocellatus*.

commonly exploited by *A. ralloides* in freshwater wetlands. Although small fish occur in shallow areas, their relatively low abundance may constrain prey availability. Under such conditions, herons are known to broaden their prey spectrum and take alternative prey encountered within their foraging range. This pattern is consistent with general ardeid feeding ecology, where diet composition reflects local prey availability rather than strict specialization (Kushlan 1978; *Fasola et al.,* 1993). Similar responses have been documented in arid-zone wetlands, where variation in prey availability influences foraging tactics and prey selection in herons (Kasprzykowski and Goławski 2024).

Comparable evidence from studies of heron assemblages indicates that smaller species such as *A. ralloides* typically exploit smaller prey items, mainly invertebrates, while larger prey are taken less frequently (Kasprzykowski and Goławski 2024). Occasional inclusion of reptiles, such as small lizards, has been reported within the family Ardeidae, but these represent a minor component of the diet relative to aquatic prey (Hancock and Kushlan 1984). Such records support the interpretation that predation on *Chalcides ocellatus* by *Ardeola ralloides* falls within the broader, but infrequently documented, feeding plasticity of the group.

The present observation is therefore best interpreted as a locally driven trophic response, where *A. ralloides* exploits an available terrestrial prey item, *C. ocellatus*, at the interface between aquatic and terrestrial habitats. Artificial wetlands such as those at

Ayla Oasis may enhance such interactions by concentrating both foraging individuals of *A. ralloides* and small reptiles within restricted habitat margins. Similar patterns have been described in modified or resource-variable wetlands, where prey composition influences foraging behaviour and trophic interactions among herons (Fasola et al. 1993; Kasprzykowski and Goławski 2024).

The present observation highlights the importance of continued, site-specific documentation of feeding ecology in waterbirds, particularly in artificial wetlands such as the Ayla Oasis Project. Such systems may facilitate interactions between aquatic and terrestrial prey within confined habitat margins. Targeted studies on the diet composition of *Ardeola ralloides* in the Aqaba region, based on systematic field observations and, where feasible, direct assessment of prey remains, would help to quantify the contribution of non-aquatic prey such as reptiles under local conditions. More broadly, further research is needed to evaluate how artificial wetlands in arid environments influence trophic interactions and foraging behaviour of waterbirds. These findings also emphasise the ecological significance of the Ayla wetlands as functional habitats supporting waterbird communities along the southern Jordanian flyway.

Acknowledgements

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Short Communication

First Documented Breeding Evidence of Lichtenstein's Sandgrouse *Pterocles lichtensteinii* in Jordan

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Abstract

Lichtenstein's Sandgrouse *Pterocles lichtensteinii* has long been considered a resident species in southern Jordan, although breeding has not previously been documented. On 24 July 2025, at 18:20 h, a family group consisting of two adults and two chicks was observed at Aqaba Bird Observatory (ABO), southern Jordan (Figure 1). The chicks remained in close association with the adults, were observed walking throughout the observation period, and were still too small to be capable of sustained flight, indicating that they were still dependent on parental care. A total of 17 adult Lichtenstein's Sandgrouse were recorded at the observatory on the same day. The record was subsequently reviewed by the Jordan Bird Records Committee (JBRC), which accepted it as the strongest evidence to date and a strong indicator of breeding by the species in Jordan. This observation contributes important information on the breeding status of Lichtenstein's Sandgrouse, and further highlights the conservation importance of Aqaba Bird Observatory for resident desert birds.

Keywords:

Lichtenstein's Sandgrouse, *Pterocles lichtensteinii*, breeding evidence, Aqaba Bird Observatory, Jordan, Jordan Bird Records Committee, resident birds.

Observation

Lichtenstein's Sandgrouse *Pterocles lichtensteinii* is a desert-adapted species inhabiting arid and semi-arid landscapes across parts of the Middle East and northeastern Africa (Porter & Aspinall, 2010; BirdLife International, 2024). In Jordan, it has been recorded mainly in the southern desert region; however, evidence of breeding has remained undocumented (RSCN, 2025).

On 24 July 2025, at approximately 18:20h, the authors observed a family group of Lichtenstein's Sandgrouse at Aqaba Bird Observatory, southern Jordan. The group comprised two adults accompanied by two chicks. The birds were observed walking together within the observatory, with the chicks remaining in close proximity to the adults throughout the observation. The chicks were noticeably small and had not yet developed the ability to fly, indicating that they were still dependent on parental care and were unlikely to have dispersed any significant distance from their hatching site. These observations strongly suggest that breeding had occurred locally.

During the same survey, a total of 17 adult Lichtenstein's Sandgrouse were recorded within the observatory. The occurrence of a family group together with this relatively high number of adults suggests that the site provides suitable habitat during the breeding season and may support a local breeding population.

The observation was submitted to the Jordan Bird Records Committee (JBRC), which reviewed the documentation and accepted

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Figure 1. A family group of two adults and two chicks observed at Aqaba Bird Observatory.

the record as a strong indicator of breeding by Lichtenstein's Sandgrouse in Jordan (JBRC, 2026). To our knowledge, this represents the strongest breeding evidence available for the species in the country.

Significance of the record

The observation provides important new information on the breeding status of Lichtenstein's Sandgrouse in Jordan and substantially improves current knowledge of the species' distribution and ecology in the country. The presence of two dependent, flightless chicks accompanying two adults constitutes compelling evidence of local reproduction, and supports the conclusion that breeding occurs in the Aqaba region.

The record also emphasizes the ecological significance of Aqaba Bird Observatory, where artificial wetlands are embedded within a surrounding arid landscape, creating a diverse habitat that supports both migratory and resident bird species. Continued monitoring during the breeding season is recommended to determine the size and status of the local population, identify breeding territories, and improve understanding of the breeding ecology of Lichtenstein's Sandgrouse in Jordan.

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Short Communication

Return of Verreaux's Eagle *Aquila verreauxii* to Southern Jordan after 15 Years

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Abstract

A Verreaux's Eagle (*Aquila verreauxii*) was observed and photographed in Wadi Alyoutom, Aqaba, southern Jordan, on 6 October 2025. This represents the first confirmed national record of the species in 15 years. The sighting was made during ecological monitoring conducted under the Environmental and Social Impact Assessment (ESIA) for the Aqaba–Amman Water Desalination and Conveyance Project. This record highlights the value of systematic ecological monitoring for documenting regionally rare or uncommon species in southern Jordan.

Keywords

Middle East, Raptor, Aqaba, rare bird record, biodiversity monitoring.

Introduction

The Verreaux's Eagle (*Aquila verreauxii*) is a large raptor widely distributed across sub-Saharan Africa and parts of the Arabian Peninsula. It is typically associated with rugged, mountainous terrain, and preys mainly on rock hyraxes (*Procavia capensis*) (Ferguson-Lees & Christie, 2001). Although listed as Least Concern by the IUCN (BirdLife International, 2024), the species is rarely reported in the Middle East, where its distribution is limited and records are sporadic (Ferguson-Lees & Christie, 2001).
In Jordan, confirmed sightings of Verreaux's Eagle have been exceedingly rare, with the last verified record in the 29th April 2010 (Al Awaji *et al.,* 2010). The present report documents the return of the species to southern Jordan after a 15-year absence, based on field observations conducted as part of an environmental impact assessment project.

Observation

On 6th October 2025, an adult Verreaux's Eagle was observed in Wadi Alyoutom (29°38′22.61″ N, 35°10′25.49″ E), approximately 35 km north of Aqaba, southern Jordan. The bird was detected at a distance approximetly less than 1 km soaring along the slopes of a rugged mountain wadi at 08:00 h under clear weather conditions. ocasionally, the bird perched on some rockes (Figure 1).
The observation was made by the authors during bird survey conducted for the Environmental and Social Impact Assessment (ESIA) of the Aqaba–Amman Water Desalination and Conveyance Project. The bird was identified based on the species' large size, characteristic all-black plumage, white sides of the mantle forming a large U-shaped marking on the upperparts in flight, white rump and upper tail-coverts, slightly wedge-shaped tail and distinctive large white patch formed by the primaries' bases underneath. Photographs taken during the observation clealy confirmed all these features (Figure 1). No additional individuals were observed, and the bird was not seen during subsequent visits to the area.

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Figure 1. Verreaux's Eagle photographed in the field

Discussion

This record represents the first confirmed sighting of *Aquila verreauxii* in Jordan since 2010, when the species was last recorded in the Dana area (Al Awaji *et al.,* 2010), approximately 200 km to the north-northeast of Aqaba. The reappearance of the species after 15 years is noteworthy and could represent occasional dispersal from populations in the Hijaz Mountains of north-western Saudi Arabia, although the origin of the observed individual remains unknown.

The record highlights the ecological significance of southern Jordan's arid and mountainous environments, which

may provide habitat for regionally rare or uncommon species. It also demonstrates the importance of systematic biodiversity monitoring associated with major infrastructure projects, which can yield valuable data for conservation planning. Continued environmental monitoring and habitat protection are important for safeguarding these ecosystems, particularly given the expansion of development activities in southern Jordan.

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The Royal Society for the Conservation of Nature

Is a national organization devoted to the conservation of Jordan's wildlife. It was founded in 1966 under the patronage of His Majesty the late King Hussein and has been given responsibility by the government to establish and manage protected areas and enforce environmental laws. As such, it is one of the few non-governmental organizations in the Middle East to be granted such a public service mandate.

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