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New Floral Records in the Region of Southern Jerusalem Hills, Palestine

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Abstract

Palestine has a rich floristic and vegetation diversity due to its geography, topography, as well as the high climatic, lithologic, and edaphic diversity. Our current knowledge of the floral biodiversity within the West Bank is based upon literature data and the observed field records of Al-Sheikh; yet these data do not provide information regarding the location or the distribution range of the plant species. Data were accumulated and updated based upon field observations (Herbarium of Palestine Institute for Biodiversity and Sustainability), published and unpublished available records, and biodiversity databases (BioGIS and GBIF). These studies focus on the southern hills of Jerusalem because it is a newly declared protected area and falls within the Mediterranean key biodiversity areas. Eight significant floral species are notable records and are reported herein: *Crepis reuteriana* Boiss*, Crepis robertioides* Boiss*., Fallopia convolvulus* L*., Fumana scoparia* Pomel*, Glaucium flavum* Crantz*, Sambucus ebulus* L*., Samolus valerandi* L., and *Coronilla cretica* L. *Crepis robertioides* Boiss. is an endangered species according to the IUCN Red list and its population is decreasing globally and locally; *Sambucus ebulus* L. and *Fallopia convolvulus* L. which is an adventive species (Dufour- Dror and Fragman 2019) are recorded in the West Bank for the first time. The remaining species are rare and recorded from new localities. Specimens of the species have been deposited at the Herbarium of Palestine Institute for Biodiversity and Sustainability.

Keywords: Protected area, Flora, Endangered, Adventive species, Palestine.

Introduction

Historic Palestine (Israel and the Occupied Palestinian Territories or West Bank and Gaza Strip) has a unique geography located at the intersection of three continents in addition to its distinctive geology being part of the Great Rift Valley harbouring the lowest point on earth. Palestine is part of the Fertile Crescent, where the domestication of animals and plants happened some twelve millennia ago (Neolithic; Abbo and Gopher 2020). Palestine has five phytogeographical and five ecological zones, which resulted in a quite diverse rich floral and faunal biodiversity. The flora of Historic Palestine is diverse extending over an area of 22,000 km2, comprising 2600 plant species (Thorogood 2019). Studies of floral diversity in the West Bank are accelerating (Qumsiyeh and Al-Sheikh 2023; Al-Sheikh and Qumsiyeh 2021a; Al-Sheikh and Qumsiyeh 2021b; Pahl and Qumsiyeh 2021). The floral diversity in Palestine can be attributed to the climatic history, geography, and geologic changes, for Palestine sits at the southeastern tip of the Mediterranean ecosystem, bordering the vast Saharo-Arabian desert belt to its south and connected via the Rift Valley to the heights of Southeast Asia and the dry tropical ecosystems of East Africa (Soto-Berelov *et al.* 2015). The rate of floral endemism is about 7% (Thorogood, 2019).

The biodiversity is threatened by climate change, habitat destruction, pollution, overexploitation of natural resources, introduction of invasive species which penetrate natural habitats and displace the native floral and faunal species, anthropogenic changes of land confiscation procedures by the Israeli occupation, building illegal settlements, building of bypass roads, and the closure of roads and large areas in the

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West Bank for security reasons. This all leads to habitat degradation and fragmentation as well as the restriction in the range of occurrence of native species resulting in the loss of biodiversity (Tal 2018; ARIJ 2016; Husein and Qumsiyeh 2022; Qumsiyeh and Abu Sarhan 2021; Qumsiyeh and Amr 2016). A new protected area network has been created for the state of Palestine (West Bank and Gaza). One of the areas newly designated is a valley system enclosing a number of towns and villages to the south of Jerusalem called Al-Arqoub (Qumsiyeh *et al.* 2023a). The area includes a UNESCO World Heritage Site (landscape) and is part of the Mediterranean biodiversity hotspot (MOTA 2015). It is also being considered to become the first biosphere reserve in the area. The present paper documents the presence of significant floral records as well as rare species from this area which will be helpful in further conservation efforts.

Methods

Study Area

The study area consists of five studied localities, which are: Cremisan, Al-Makhrour, Battir, Husan, and Wadi Fukin. These areas fall within the Mediterranean key biodiversity areas. The southern Jerusalem Hills region includes several villages (Al-Khader, Battir, Husan, Al-Walaja, and Wadi Fukin) in addition to several wadis (Makhrour, Cremisan, Husan and Fukin) with a rich cultural heritage and rich biodiversity The study area (Figure 1) is found within the Mediterranean biogeographical zone and forms a critical portion of the hydrological system that replenishes the western aquifer. It is an important bird area and an important plant area (Radford *et al.* 2011).

Much of the studied area is located in Area C of the occupied West Bank, which means it is under the Israeli civil and military control

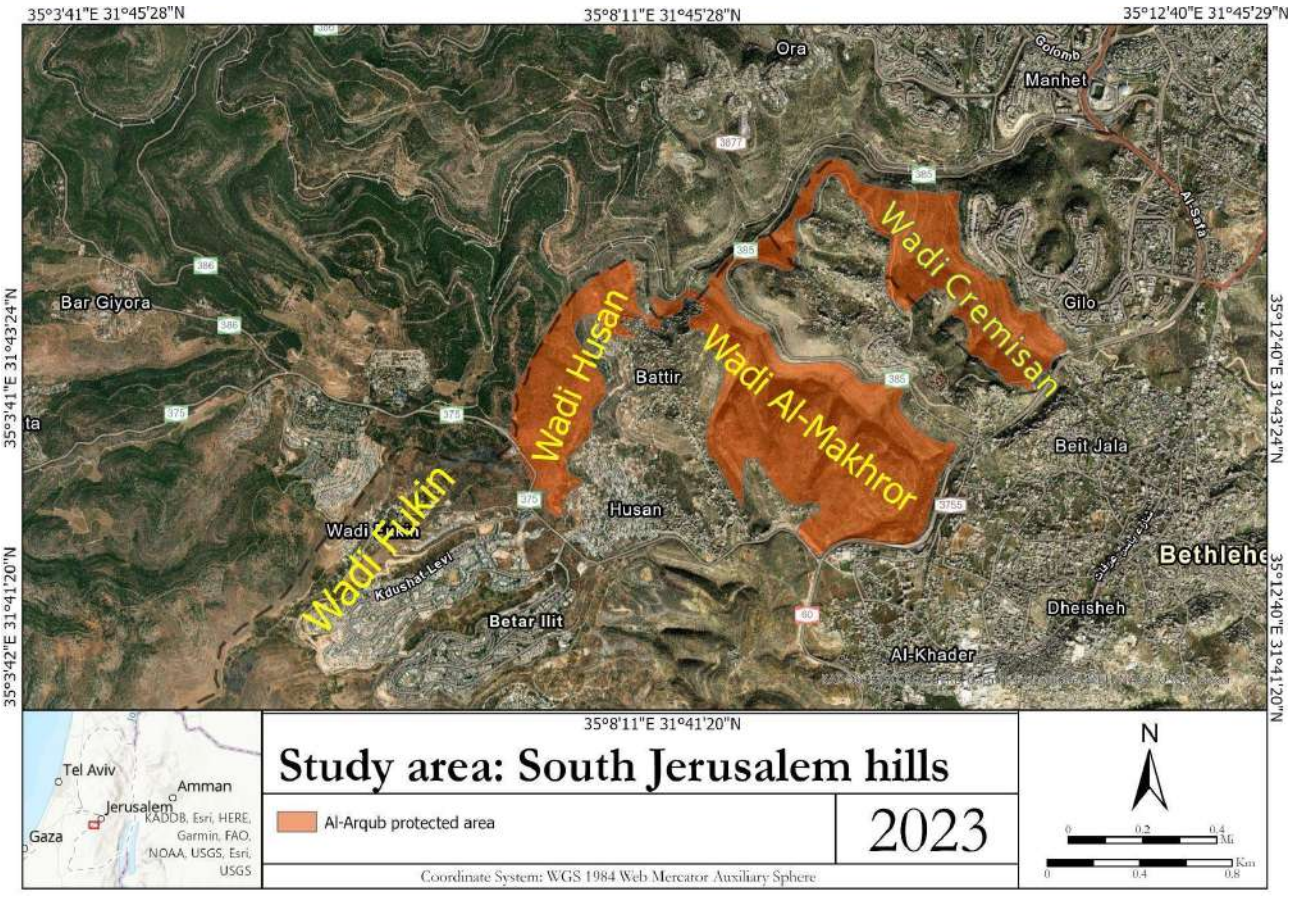

Figure 1: The study area of the southern Hills of Jerusalem (which falls within the newly designated protected area of Al Arqoub); it includes four valleys: A) Cremisan Valley, B) Al- Makhrour Valley, C) Battir/Husan valleys; D) Wadi Fukin Valley; the map was designed specifically for this research paper by our team member Duaa Husein at the Palestine Institute for Biodiversity and Sustainability.

(Qumsiyeh *et al.* 2023a). Part of the area was designated as a threatened UNESCO World Heritage Site (MOTA 2015), and recently much of it is designated as a protected areas re-evaluation (Qumsiyeh *et al.* 2023b).

Data collection: Through the conduction of various field trips, data were collected and the above-mentioned species were documented and their geographical distribution has been updated and recorded in the Al Arqoub region of the West Bank. Such information was either not reported earlier or was dubious and is now confirmed as present and recorded within the region. The floral species' inventory for Wadi Al-Makhrour, Battir, Husan and Wadi Fukin was done based on field trips where floral species were surveyed via meticulous walking in the targeted areas between Spring 2021 and Spring 2022. The classification of plants as far as rarity is based on Al-Sheikh's and Qumsiyeh's list (Al-Sheikh and Qumsiyeh, 2021c). The coordinates of the floral species provided are based on the longitude and latitude of the Global Positioning System (GPS) mapping. Altitudes are given in metres above sea level. Locality data were compared with data from Danin (2015) and GBIF (Horvitz and Danin, 2015). However, the latter includes many amateur reports not confirmed by botanists. Pictures of plants were taken in the field, and some samples were collected and deposited into the herbarium of the Palestine Museum of Natural History and are labelled as PMNH-Hxxxx (which refers to Palestine Museum of Natural History Herbarium). Conservation status was referenced to IUCN (2023) and Sayah *et al.* (2020).

Results

The results of this study confirm that eight taxa in seven families are hereby recorded in the West Bank which were either not reported earlier or were dubious and now confirmed as present in this contested part of Palestine.

***Crepis robertioides*, Boiss, Robertia Hawkweed; Compositae, Figure 2(a)**

Record: Observed specimen, Makhrour, Bethlehem Governorate (31.713778, 35.168855), 798m, 15 June, 2022. Perennial herb which reaches up to 15 cm, with a woody stem. The plant spreads across a width of 20 cm. Leaves; alternate, typical rosette leaf, dissected, less than 10 mm wide, entire with smooth margins, absent stipule. Florets yellow, less than 15 mm in diameter, self-pollinating only. The outer involucral bracts are shorter than its inner bracts. Its inner bracts harden and spread apart after the inner seeds' dispersal. Deciduous. It is considered as an endangered species according to the IUCN Red List of Threatened Species 2020 see table 1 (IUCN 2023; Sayah *et al.* 2020). *Crepis robertioides* Boiss. is found in the extreme north in Majdal Shams, Jabal Alshaikh (Mt. Hermon) as well as the Syrian Golan heights (Danin 2015, https://flora.org.il/en/plants/CREROB/ and https://biogis.huji.ac.il/eng/searchspecies.html). Within the Golan Heights (Syrian Occupied Territory), it was ostensibly recorded by Ofra Friedmann who collected a specimen in Tel-Aviv University Herbarium on 13 July, 2020 (Horvitz 2016). The record provided through this study is far to the south of these localities and is the first record in the West Bank.

***Glaucium flavum*, Crantz, Yellow Horned Poppy; Papaveraceae, Figure 2(b)**

Record: PMNH-H0287, Wadi Fukin (31.7073N, 35.1042E), 645 m, 16 June, 2021 Observed specimen, Wadi Fukin, Bethlehem Governorate, (31.708588N, 35.120448E), 645 m, 20 June, 2022 Observed for another time at the same location on 24 June, 2023 *Glaucium flavum* is a blue-grey, branched biennial to perennial, 30-90 cm. Leaves 15-30 cm with oblong, wavy and pinnately lobed leaves, the upper leaves clasping the stem; stems with a yellowish latex when cut. Flowers bright yellow; petals 30-40 mm long. Fruit narrowly cylindrical, long and narrow, 15-30 cm; curved and hairless but with small whitish tubercles. Mediterranean strand, Coastal sands and shingle, or disturbed habitats inland.It is considered as a very rare Near Threatened species, it could have escaped cultivation, thus it is found in the southern Hills of Jerusalem as its normal habitat is the Mediterranean Sea strands.

Figure 2. a) *Crepis robertioides***,** Boiss., **b)** *Glaucium flavum***,** Cran**tz., c)** *Coronilla cretica***,** L. **d)** *Samolus valerandi* L.

No historical observation has been recorded for it in the West Bank, however it has been observed within the area south of the West Bank (Danin 2015, https://flora.org.il/en/plants/GLAFLA/). This species has been red-listed as a near threatened species (Sapir *et al.* 2003).

Glaucium flavum is documented to be found in Jordan as indicated in the Jordan plant red list by (Taifour, 2017), and in the coastal Mediterranean strands such as Galilee, Acco Plain, Coast of Carmel, Sharon Plain, Philistean Plain. Moreover, there are several observations within the Judean mountains which are considered as the normal habitat of this floral species within the Mediterranean strand (Danin 2015, https://flora.org.il/en/plants/GLAFLA/ and https://biogis.huji.ac.il/eng/searchspecies.html). The record provided through this study extends the distribution of this species within the West Bank.

***Coronilla cretica*, L., Cretan Crownvetch; Fabaceae, Figure 2(c)**

Record: Observed in Makhrour, Bethlehem Governorate (31.714003, 35.168139), 790m, 5 April, 2022.

Coronilla certica is a virtually hairless annual with weak stems 15-60cm, branched form the base. Leaves with 3-6 pairs of oblong-elliptic, blunt leaflets. Flowers in heads of 3-6, borne on straight stalks; corolla 4-7mm,

Table 1. shows the IUCN and regional status of the floral species.

Scientific Name	Family	IUCN Red list Status (IUCN, 2023) Status in Jordan (Taifour, 2017)	Local Status in West Bank (Al-Sheikh and Qumsiyeh, 2021c)
Crepis reuteriana	Compositae (Asteraceae)	- LC in Jordan	Rare
Crepis robertioides	Compositae (Asteraceae)	Endangered (in the Mediterranean region and Globally) Decreasing	Very Rare
Fallopia convolvulus	Polygonaceae	- Endangered in Jordan	Very Rare
Fumana scoparia	Cistaceae	-	Very Rare
Glaucium flavum	Papaveraceae	LC (Europe) LC in Jordan	O= It is found only in 1-3 sites in the West Bank
Sambucus ebulus	Caprifoliaceae	LC (Europe) Critically endangered in Jordan	Very Rare
Samolus valerandi	Primulaceae	LC (in the Mediterranean region, Globally and inEurope) LC in Jordan (Taifour 2017)	Rare
Coronilla cretica	Fabaceae	-	O= It is found only in 1-3 sites in the West Bank

white or pink flowers and straight erect fruits (Zohary and Feinbrun- Dothan, 1966-1986, Thorogood, 2019). It is very rare within the Judean Mountains.

Coronilla cretica has the following geographical distribution where it is common in Hula Plain, Upper Galilee, Mount Carmel, whereas it is very rare within Kinnroth Valley, Coastal Galilee, Acco Plain Nablus (Samaria) and the Judean Mountains (Zohary 1972; Danin 2015 https://flora.org.il/en/plants/SECCRE/). The record provided in this study is the first record in the West Bank as its presence in the West Bank has not been confirmed before as no specific localities were given before.

***Crepis reuteriana*, Boiss., Reuter's Hawk's Beard; Compositae, Figure 3(d)**

Record: PMNH-H0369, Makhrour, Bethlehem Governorate (31.713300, 35.168600), 798 m, 2 April, 2022.

Perennial rhizomatous herb, 30-100 cm. Rhizome woody with numerous fibrous roots. Stems glabrous or crisp- pubescent, erect, long- branched, paniculate- corymbose. Leaves pubescent or hispidulous; radical

Figure 3. a) *Fallopia convolvulus,* L., PMNH-H0715, **b)** *Sambucus ebulus***,** L., PMNH-H0451 **c)** *Fumana scoparia*, Pomel, PMNH-H0368, **d)** *Crepis reuteriana* Boiss, PMNH-H0369.

leaves petiolate, runcinate- pinnately lobed with retrorse oblong- triangular acute lobes; 1-2 lower cauline leaves similar to radical leaves, the others linear- lanceolate, entire. Heads medium- sized. Involucre calculator, 1-1.3 cm, usually glabrous; inner involucral bracts linear-lanceolate, in fruit thickened at base. Florets yellow. Achenes beakless, 3.5-5 mm, greenish or tawny, straight or nearly so, weakly ribbed. Pappus is hardly longer than achene, deciduous (Feinbrun-Dothan, 1978, Thorogood, 2019).

Crepis reuteriana Boiss. is documented to be found in Jordan as indicated in the Jordan plant red list by (Taifour, 2017), the Syrian Golan heights, and in historic Palestine in the Galilee region, including Mount Carmel.

It is reported as being very rare in the south of West Bank (Feinbrun- Dothan 1978, Danin 2015 https://flora.org.il/en/plants/CREREU/ ;https://biogis.huji.ac.il/) this record has confirmed its presence in the West Bank.

***Fallopia convolvulus,* L., Black Bindweed; Polygonaceae, Figure 3(a)**

Record: PMNH-H0715, Husan Ayn Al Hawiyeh, Bethlehem Governorate (31.715556, 35.129167), 690 m, 16 June, 2021.

An annual climber, clockwise- twining, climbing or prostrate vine up to 1 m with angular stems. Leaves; alternate, heart or arrow- shaped, pointed and mealy beneath, 30-70mm long with smooth margins. Flowers have 8 stamens, styles 3, greenish or yellowish- white, borne in loose clusters in the leaf axils. Fruit a triangular nut borne on a short stalk 1-3 mm long. Its normal habitats are ruderal and bare places.

Fallopia convolvulus is new to Jordan as indicated by Al- Eisawi. Also, it is listed as an endangered species in Jordan as indicated by the Jordan plant red list by Taifour; where it is distributed in Wadi Araba and the Jordan Valley see table 1 (Taifour, 2017). Locally, it is found in the north in Majdal Shams, upper Galilee, Mount Carmel; it is reported as being very rare in the mountains near Jerusalem (https://flora.org.il/en/plants/falcon/ and https://biogis.huji.ac.il/eng/searchspecies.html). The record confirmed its presence in the West Bank.

***Fumana scoparia*, Pomel; Cistaceae, Figure 3(c)**

Record: PMNH-H0368, Makhrour, Bethlehem Governorate (31.713300, 35.168600), 798m, 13 April, 2022.

Fumana scoparia differs greatly from the other two local *Fumana* species by the semiterete and unveined leaves, sparsely hairy and deciduous, alternating in a whorl pattern and looking like a rosette from above, and by the thick and smooth green sterile branches, bearing small scars from their fallen leaves. Flowers 25-30 mm in diameter, petals bright yellow (https://flora.org.il/en/plants/fumsco/). A small, finely hairy- stemmed shrub which reaches up to 35 cm similar to *Fumana thymifolia* but more prostrate and with linear leaves 12-18 mm all alternate; stipules absent with many erect flowering stems 40-80 mm curved in bud with 2-3 flowers, inflorescence is very sticky-glandular- hairy.

Fumana scoparia is found in Galilee including Mount Carmel, Shefela, the Judean Mountains to the west of Jerusalem (https://flora.org.il/en/plants/fumsco).

The exact localities of this recorded species were not known before in the West Bank, upon the conduction of this research; it is now confirmed as present in the West Bank within the southern Hills of Jerusalem.

***Sambucus ebulus*, L., Dane Weed; Caprifoliaceae, Figure 3(b)**

Record: PMNH-H0414, Husan, Bethlehem Governorate (31.716717, 35.133280), 622 m, 18 October 2020 PMNH-H0451, Ayn Battir, Bethlehem Governorate (31.727505, 35.138478), 662m, 19 August 2021.

A robust, vigorous, herbaceous perennial to 1.5(2)m with erect, grooved stems; unpleasant-smelling. Leaves pinnately divided with 7-13 narrow leaflets and conspicuous oval stipules at the base. Flowers borne in flat-topped inflorescences with 3 main rays; pinkish-white, with purple anthers. Fruit a black berry. Stream sides and field boundaries (Thorogood 2019).

Sambucus ebulus is documented to be found in Jordan (Ajloun) as indicated in the Jordan plant red list volume II by Taifour. It is considered as a critically endangered species, see table 1 (Taifour 2017). It was recorded as present in Jabal Al Shaikh (Mount Hermon) (https://flora.org.il/en/plants/SAMEBU/ and https://biogis.huji.ac.il/eng/searchspecies.html). The record provided via this study is the first record in the West Bank extending the distribution of this species significantly. It is worth noting that because of its spotty and limited distribution and rarity in Palestine and Jordan, it requires urgent conservation measures.

***Samolus valerandi* L., Seaside Brookweed; Primulaceae, Figure 2(d)**

Record: PMNH-H0306, Husan, Bethlehem Governorate (31.717772, 35.128252), 648 m, 16 June, 2021. Also observed for the second time at same location on18 June, 2022.

A hairless perennial herb with a rosette of leaves 10-15 mm long, and erect flowering stems 50mm- 70cm. Leaves are rather shiny, spoon-shaped and scarcely stalked below; stalkless above. Flowers small and white, cup-shaped to just 3 mm across and with 5 petals. Fruit 3 mm.

Perennial herb, 20-80cm, glabrous, glaucescent. Stems erect, leafy, simple or somewhat branched above. Leaves; obovate to spathulate, obtuse; basal leaves in a rosette, petiolate. Inflorescence a raceme; bracts small, acute; pedicles ascending, in fruit deniculate at the bract- insertion. Calyx campanulate; lobes erect, triangular, acute. Corolla white, 2-3 mm in diameter, somewhat longer than calyx; lobes obovate. Stamens included; staminodes subulate- triquetrous. Capsule 2-3 mm in diameter. Usually self-pollinating. Damp rocks and seeps, often coastal (Feinbrun- Dothan, 1978).

Samolus valerandi was recorded to be found in Jordan as mentioned by the Jordan plant red list volume II (Taifour, 2017) and is found in the coastal regions of Palestine, Upper and Lower Galilee, Judean Mountains, Upper Jordan Valley (Feinbrun-Dothan 1978, https://flora.org.il/en/plants/samval/). The record provided in this study confirmed the presence of this species in the West Bank although it is recorded as a very rare species in the mountains of this region.

In table 1; The status of each of the floral species locally, globally and in Jordan is depicted. *Crepis robertioides* is documented as an endangered species in the Mediterranean region as well as globally; and its population is decreasing (IUCN 2023; Sayah 2020). Furthermore, *Fallopia convolvulus* is considered an adventive species (Dufour-Dror and Fragman- Sapir 2019) and is documented for the first time in the West Bank. It is considered as a new species to Jordan according to Al- Eisawi as well as being an endangered species in Jordan as confirmed by the Jordan plant red list (Taifour 2017). Furthermore, *Sambucus ebulus* is considered as a critically endangered species in Jordan as indicated by the Jordan plant red list vol II (Taifour 2017). Hence, it is quite vital to document their presence in the West Bank of Palestine.

Discussion

Similar studies of the distribution and status of the floral species are highly needed for the betterment of planning and conservation measures and to protect this historic land's fauna and flora which have been neglected in light of the political and social challenges found within Palestine (Al-Sheikh and Qumsiyeh 2021c; EQA 2015; Qumsiyeh *et al.* 2017). Several rare species have been detected in this study within the newly designated protected area of the southern Hills of Jerusalem (Qumseiyeh *et al.* 2023a). *Crepis robertioides* Boiss., which is considered as an endangered species according to the IUCN Red List of Threatened Species 2020 (Sayah *et al*. 2020) has been recorded along with *Fallopia convolvulus* L. and *Sambucus ebulus* L. which are recorded for the first time in the West Bank. This is an indication that the region of the southern Hills of Jerusalem should be subjected to further studies as it has the potential to be an eco-corridor. The area under study is facing numerous challenges, including habitat loss, land fragmentation and the difficult economic and political circumstances (Amr *et al.* 2016; ARIJ 2016; Husein and Qumsiyeh 2022; Qumsiyeh *et al.* 2014). Further study is being undertaken to protect the whole area under a Biosphere Reserve format because it is both a human cultural site as well as an eco- corridor for fauna and flora. This biocultural landscape is even more critical for our area considering the extensive threats that have been documented (Qumsiyeh and Abu Sarhan 2020; Al-Sheikh and Qumsiyeh 2021c; Qumsiyeh *et al.* 2023a).

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Vegetation Cover Assessment at Shaumari Wildlife Reserve Using Satellite Sensors Data

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Abstract

The assessment of rangeland cover and health is fundamental for effective planning and for supporting sustainability efforts in arid lands including Jordan. The objective of this study is to evaluate the vegetation cover density and distribution in the Shaumari Wildlife Reserve over the period between 1991 and 2022 using remote sensing techniques. The Shaumari Wildlife Reserve natural rangelands (total area, 22 km^2) are managed by the Royal Society for The Conservation of Nature. Landsat satellite sensor data from Thematic Mapper (TM, Landsat-TM5), Enhanced Thematic Mapper Plus (ETM+) and Operational Land Imager (OLI) were used to derive the normalized difference vegetation index (NDVI) across growing seasons (March -July) and over the study period (thirty-two years). The results show that bare soil (NDVI: 0.0 – 0.1) and scattered vegetation (NDVI: 0.1-0.2) are the mainland cover classes in the reserve. The NDVI-Landsat showed that 70% (15.3 km^2) to 94% (20.7 km^2) of the reserve is classified as bare soil across the growing seasons (March, July) and over the study period from 1991to 2022. In addition, in July-August (1991-2022), the percentage of reduction in vegetation cover area (compared to March) ranged from 1% (1994) to 20% (2004). In terms of vegetation distribution, most grasses and shrubs were located in the northwestern side of the reserve across years. However, scattered vegetation was noticed in the southern part of the reserve in March 1991, 2004, 2016 and 2022. Interestingly, a significant positive relationship ($R^2 = 0.66$, $P< 0.01$) was found between Landsat-NDVI values and annual precipitation (vegetation cover area = 0.0341 × precipitation + 0.0484). Therefore, the fluctuation in vegetation cover across the years was partially attributed to harsh climatic conditions, especially rainfall. In conclusion, considering the limited vegetation cover density and distribution in the reserve, a potential management and conservation practices should be carried out to sustain and enhance the cover density and distribution for the reserve which is home to the Arabian Oryx in Jordan.

Keywords: NDVI, Landsat, Rangelands, remote sensing, drylands.

Introduction

Evaluating the distribution, quality, and density of rangelands is crucial for a well-informed planning and for the advancement of sustainability initiatives in arid regions (Sawalhah *et al.,* 2021). The appraisal of rangeland health encompasses the examination of soil and site stability, hydrologic functionality, and the overall integrity of the biotic community (Pyke *et al.* 2002; Tahat *et al*., 2020). Covering approximately 80% of Jordan's total land area according to MoEnv (2015), rangelands exhibit features typical of arid and semi-arid climates. In addition, the high grazing pressure decreases primary production, reduces biomass, and perennial grass in these degraded lands. In fact, Jordanian rangelands have been exposed to potential land degradation and biodiversity loss (Al-Karadsheh *et al.,* 2012).

Intense grazing pressure leads to a decline in primary production, a decrease in biomass, and a reduction in the basal cover of perennial grasses (Ash *et al*., 2011). As a result, the

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sustained grazing capacity of the land is diminished over the long term (Ash *et al.,* 2011). Furthermore, low precipitation and high temperatures in these harsh environments play a key role in vegetation distribution (Sawalhah *et al.,* 2021). Therefore, advanced and long-term management systems must be carried out in those dry rangelands to enhance the vegetation cover density, quality, and distribution. Tracking fluctuations in grazing intensity from year to year using conventional methods is a time-consuming and labour-intensive task, often hindered by the absence of suitable equipment (Dara *et al.,* 2020; Tadros *et al.,* 2020). Remote sensing methods offer a promising avenue for identifying rangelands where alterations in surface properties can be detected and linked to land degradation (Sawalhah *et al.*, 2018, 2021). For example, utilizing remotely captured data from the Landsat archives spanning from 1985 to 2017 in conjunction with ground reference information unveiled previously undiscovered areas of intense grazing activity during the Soviet era (Dara *et al*., 2020). Landsat datasets and Google Earth Engine were also employed by Xie *et al.* (2019) who developed a novel approach to mapping changes in vegetation cover density at the pixel level (spatial resolution: 30 m) and identified degraded lands with an overall classification accuracy of 82.6%. In Jordan, satellite images from Landsat effectively evaluated changes in land use and cover with a general precision ranging between 80% to 86% (Tadros *et al.,* 2020). Additionally, this approach demonstrated its capability to suggest the surveillance of invasive plant species such as *Prosopis* spp. within the designated area (Tadros *et al.,* 2020). Overall, Landsat 8 Operational Land Imager (OLI) serves as a practical approach for detecting shifts in vegetation coverage density and measuring degradation within the Middle and Eastern rangelands (Badia) of Jordan (Sawalhah *et al*., 2018). Employing a change detection method with remotely-sensed data allows for the assessment of human impacts on a given area without introducing additional disruptions to ecologically delicate regions (Willis, 2015). In fact, the utilization of a remote sensing change detection strategy can strengthen the preservation of natural resources and ecological surveillance by tracking the diverse and ongoing ecological dynamics of the observed area, whether they are irregular, uniform, or continuous (Sergeant *et al*., 2012; Willis, 2015). Several remotely-sensed indices have been used to detect changes in vegetation cover density (Foran and Pearce, 1990; Othman *et al*., 2014, 2015; Tadros *et al.,* 2020). Perry and Lautenschlager (1984) found that twenty vegetation indices derived from surface reflectance data are functionally equivalent. However, the widely used method for estimating vegetation cover density by assessing chlorophyll content through remotely sensed data is represented by the normalized difference vegetation index (NDVI). This index was used in regional and continental-scale for vegetation monitoring (Foran and Pearce, 1990; Myneni *et al.,* 1997; Wang *et al*., 2004). The Royal Society for the Conservation of Nature (RSCN) established the Shaumari Wildlife Reserve as a wildlife reserve in Jordan in 1975. This reserve serves as a breeding ground and sanctuary for endangered species in Jordan, such as *Oryx leucoryx* in addition to conserving several native wild plant species (Abu Yahya *et al*., 2022; Amr *et al*., 2011). However, long-term changes in vegetation cover area (density and distribution) are not well understood. The missing information is critical for future monitoring and conservation plans. The objective of this study is to evaluate vegetation cover extent and density at the Shaumari Wildlife Reserve over the period from 1991 to 2022 using a remote sensing technique.

Materials and Methods

Site Description

The study was conducted at the Shaumari Wildlife Reserve (Figure 1) between 1991 and 2022. The Royal Society for the Conservation of Nature (RSCN) established

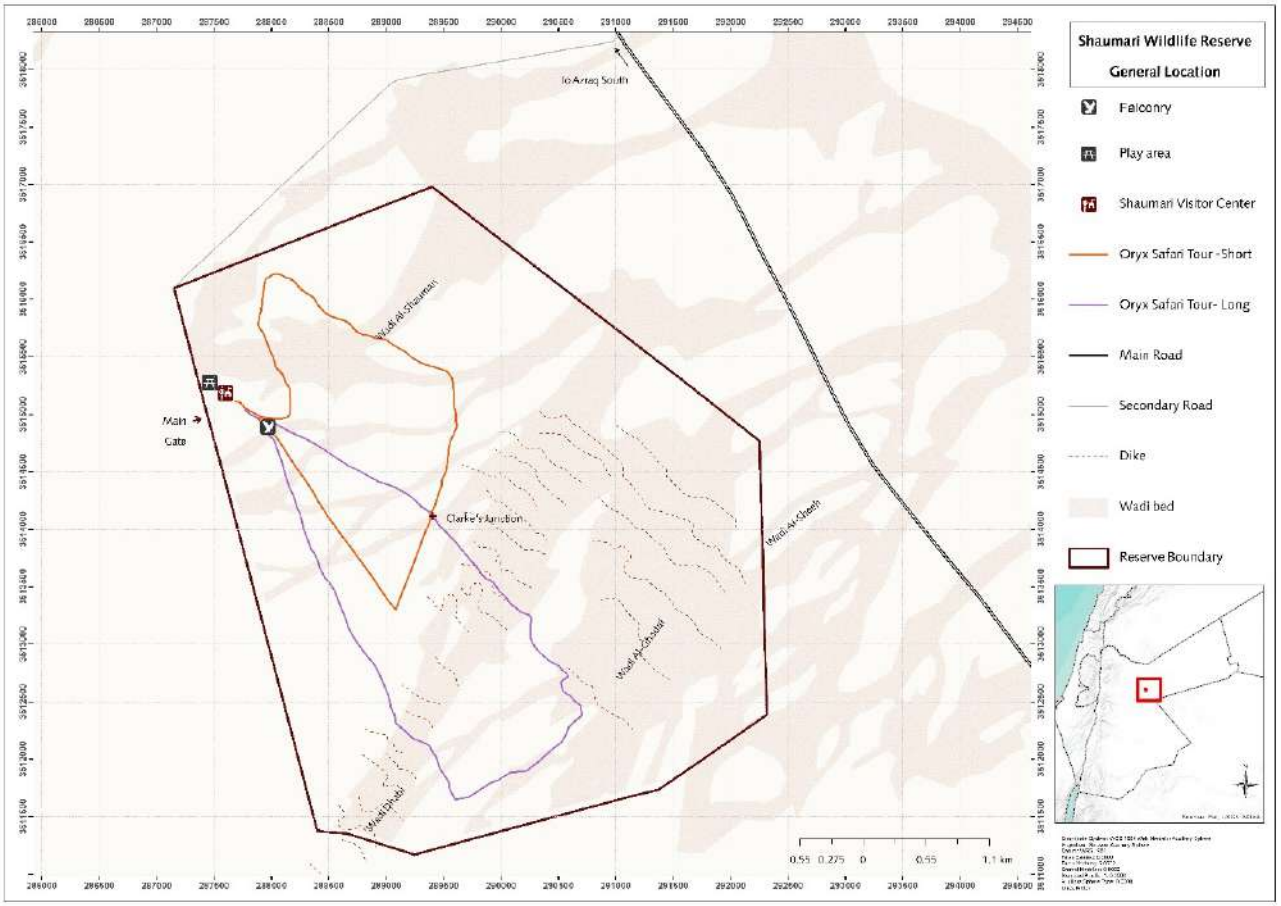

Figure 1. Shaumari Wildlife Reserve, Eastern Badia, Jordan.

the Shaumari Wildlife Reserve as a wildlife reserve in Jordan in 1975. The total area of the reserve is 22 km^2 with 60% of its total area consisting of wadis, while the remaining area is flat land covered in basalt stone (Hamad). The elevation ranges from 510 to 680 m. The reserve is situated in the desert biogeographical zone, which experiences varying temperatures. Summers can be very hot, with temperatures reaching up to 40°C, while cold days in winter can reach -10°C. The total rainfall in the area is very low, averaging less than 50 ml annually. The primary reason for the establishment of this reserve was to serve as a breeding ground and sanctuary for endangered species in Jordan, such as *Oryx leucoryx* (Figure 2), and to conserve wild plant species such as , *Retama raetam, Salsola vermiculata, Artemisia herba-alba, Achillea fragrantissima*, *Astragalus* spp., *Stipa* spp., *Trigonella* spp (Abu Yahya *et al.,* 2022; Amr *et al.*, 2011). Soil is very poor and mostly is hammada, saline, sandy-loam. Land degradation, which can be defined as the extreme reduction of biological diversity, is a significant issue in the area.

Image Acquisition and Processing

Cloud-free Landsat sensor images were downloaded from the earth-explorer portal (http://earthexplorer.usgs.gov/). The Landsat satellite sensor data used were Thematic Mapper (TM, Landsat-TM5), Enhanced Thematic Mapper Plus (ETM+) and Operational Land Imager (OLI). The downloaded images (Landsat-TM5, ETM+, OLI, collection-2 level-2) are classified as a Surface Reflectance Climate Data Records (CDRs) datasets, which are a reliable Landsat source for land cover studies (Qarallah *et al*., 2023). These datasets are atmospherically corrected using the Landsat Ecosystem Disturbance Adaptive Processing System (LEDAPS) program (Qarallah *et al.,* 2021, 2023). Images were geo-rectified using Environment for Visualizing Images (ENVI) 5.0 (Research Systems, Boulder, Colorado, USA).

Figure 2. Arabian Oryx (*Oryx leucoryx*) at the Shaumari Wildlife Reserve, Eastern Badia, Jordan.

Then, NDVI (Eq. 1) was derived and assigned to different classes (Table 1) (Abu Yahya *et al*., 2022; Atun *et al*., 2020).

$$NDVI = \frac{(Near\ infrared\ band - red\ band)}{(Near\ infrared\ band + red\ band)} \quad \text{(Eq. 1)}$$

During the study period, two images per year were selected. The two images (during each year) where when vegetation cover density had the highest (February-March) and lowest (July-August) NDVI values. This step facilitated the comparison process between reserves and determined the maximum and minimum vegetation cover density across the year. In addition, the NDVI classes which represented spares vegetation to dense (0.1-0.6) were summed together and correlated to rainfall data (Figure 3) in order to understand the impact of climatic condition on the reserve vegetation cover density. SigmaPlot software 14.0 was used to graph the data and derive the regression model.

Results

The vegetation cover density and total area for the Shaumari Wildlife Reserve in March (1991 to 2022) are presented in Table 2. Remotely-sensed data revealed that most of the reserve area consisted of bare soil.

The NDVI values in March ranged from < 0.1 (bare soil) to 0.4 (dense vegetation). The NDVI-Landsat showed that the total area, which is classified as bare soil in March, ranged from 15.3 to 20.7 (reserve total area, 22 km^2) over the period from 1991 to 2022 (Table 2). In addition, the maximum vegetation cover density was recorded during the years 1991, 2004 and 2016.

Figure 4 presents the vegetation cover density and distribution in March over the period between 1991 and 2002. Satellite images show that the vegetation density was consistently concentrated in the Northwest side of the reserve over the years (Figure 3). In addition, scattered vegetation was noticed in the southern part of the reserve. In March 1991, 2004, 2016 and 2022.

In July-August 1991-2022, NDVI data which were derived using Landsat-5 (TM), Landsat-7 (ETM+) and Landsat 8 (OLI) showed that the vegetation values in July ranged from < 0.1 (bare soil) to 0.3 (moderate vegetation) (Table 3, Figure 5). In addition, the vegetation coverpercentage in July was lower than that in March (Table 3). The percentage of reduction in the vegetation cover area in July (compared to March) ranged from 1% (1994) to 20% (2004). However, the total reduction in vegetation in

Table 1. Normalized difference vegetation index (NDVI) classes.

Class	Features
Negative values	Deep and shallow water.
0.0 - 0.1	Barren areas of rock and sand.
0.1 - 0.2	Scattered vegetation.
0.2 - 0.3	Moderate shrubs and grasslands.
0.4 - 0.6	Agricultural area, agroforestry, intense vegetation cover density.
0.6 – 0.9	Dense forests.

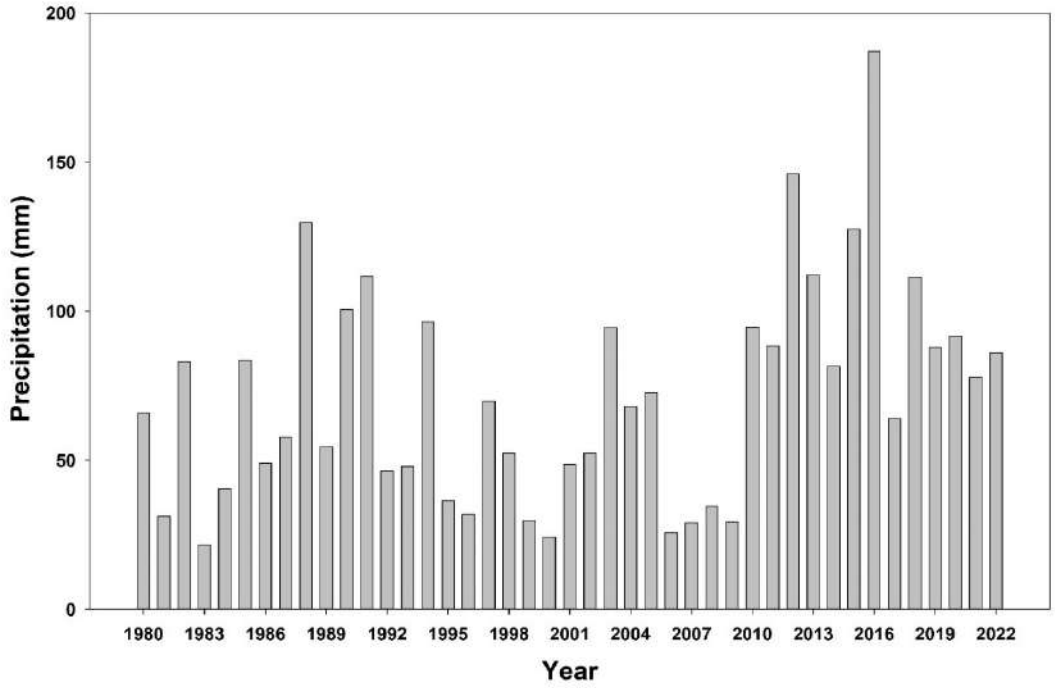

Figure 3. Long-term annual precipitation (1980-2022) for the Shaumari Wildlife Reserve, Eastern Badia, Jordan.

Table 2. Normalized Difference Vegetation Index (NDVI) classes for the Shaumari Wildlife Reserve (total area, 22 km^2) in March 1991-2022. NDVI class's data was derived using Landsat-5 (TM), Landsat-7 (ETM+) and Landsat 8 (OLI) sensors data.

	NDVI classes (area km^2)			
Year	**0.0 - 0.1**	**0.1 - 0.2**	**0.2 - 0.3**	**0.3 - 0.4**
1991	17.4	4.19	0.4	0.006
1994	20.6	1.4	0	0
1999	20.7	1.3	0.003	0
2004	16.7	4.77	0.5	0.027
2006	20.3	1.67	0.03	0
2011	19.6	2.32	0.08	0
2016	15.3	6.63	0.07	0.0008
2021	20.4	1.55	0.05	0
2022	19.7	2.28	0.012	0

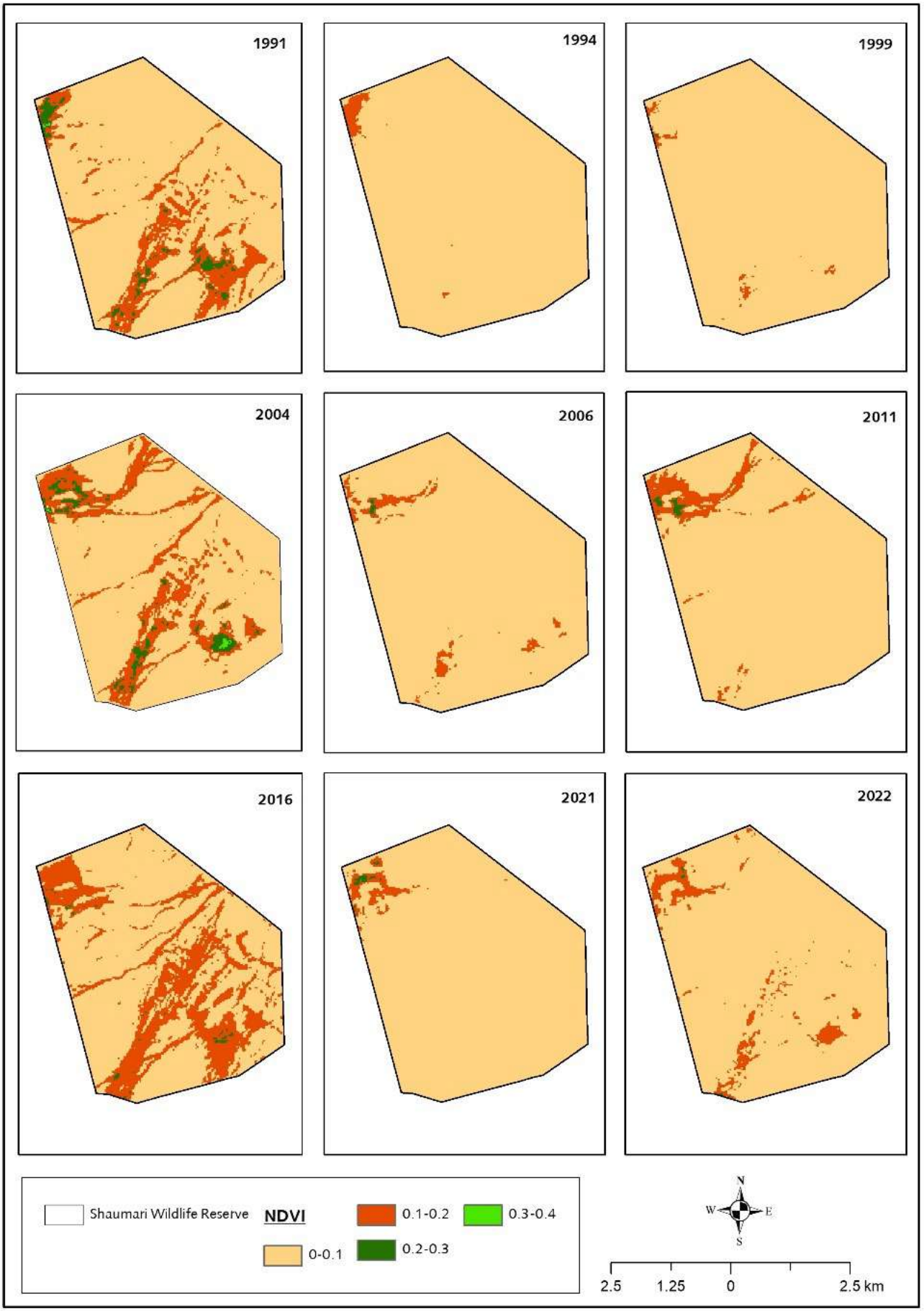

Figure 4. Vegetation cover density and distribution for the Shaumari Wildlife Reserve (total area, 22 km2) in March 1991-2022. NDVI class's data was derived using Landsat-5 (TM), Landsat-7 (ETM+) and Landsat 8 (OLI) sensors data.

July 2022 compared to March 2022 was about 5% (19.7 km^2 vs. 20.7 km^2). The vegetation distribution in July (Figure 5) was similar to that in March (Figure 4). Except in July 1991 and 2016, the vegetation was found in the Northwestern side of the reserve.

The simple linear regression model was used to estimate the relationship between the vegetation cover area, which was derived using normalized difference vegetation index (NDVI), and between precipitation during the study period 1991-2022 revealing a significant (P-value < 0.01) relationship (Figure 6). The coefficient of determination was 66%.

Table 3. Normalized Difference Vegetation Index (NDVI) classes for the Shaumari Wildlife Reserve (total area, 22 km^2) in July-August 1991-2022. NDVI class's data was derived using Landsat-5 (TM), Landsat-7 (ETM+) and Landsat 8 (OLI) sensors data.

	NDVI classes (area km^2)			
Year	**0.0 - 0.1**	**0.1 - 0.2**	**0.2 - 0.3**	**0.3 - 0.4**
1991	19.6	2.40	0	0
1994	20.7	1.30	0	0
1999	20.79	1.21	0	0
2004	20.75	1.25	0	0
2006	20.4	1.60	0	0
2011	20.4	1.60	0	0
2016	17.9	4.10	0	0
2021	20.8	1.19	0.0005	0
2022	20.7	1.29	0.003	0

Discussion

Rangelands, which account for almost a third of the Earth's ice-free land, stand as the most extensive land cover globally (Ellis and Ramankutty, 2008). However, they only contribute to approximately 11% of terrestrial net primary production (Ellis and Ramankutty, 2008). This is because rangeland are found primarily in arid and other low productivity areas with a high percentage of bare earth cover (Ellis and Ramankutty, 2008). In Jordan, rangelands constitute about 80% of Jordan's total land area (MoEnv, 2015) and are characterized by arid and semi-arid climates. While the nomadic pastoral lifestyle of Bedouins depends on animals and includes various practices, rangelands in Jordan face the risk of potential land degradation and a decline in biodiversity (Al-Karadsheh *et al*., 2012; Juneidi and Abu-Zanat, 1993).

Assessing the conditions of remote rangelands in Jordan presents significant logistical obstacles due to the requirement for frequent evaluations of ecological conditions across time and space (Sawalhah *et al*., 2018). Remote sensing was successfully used for land cover/land use assessment (Al-Kofahi *et al*., 2019; Othman *et al*., 2021; Tadros et al., 2020). In this study, NDVI was used to estimate the vegetation cover density in the Shaumari Wildlife Reserve which is managed by RSCN. Landsat-NDVI data for the reserve revealed potential changes in vegetation cover density over the years from 1991 to 2022. In each year, the NDVI values were between 0.1 and 0.4 in March, and ranged from 0.0 to 0.3 in July. Higher vegetation cover density in March (compared to July) can be attributed partially to the amount of soil moisture. During spring period (March-April), grasses were available and covered parts of the reserve. In summer (July-August), those grasses entered the senescence stage and die.

Patterns of change over time demonstrate that both land-use and rainfall variability

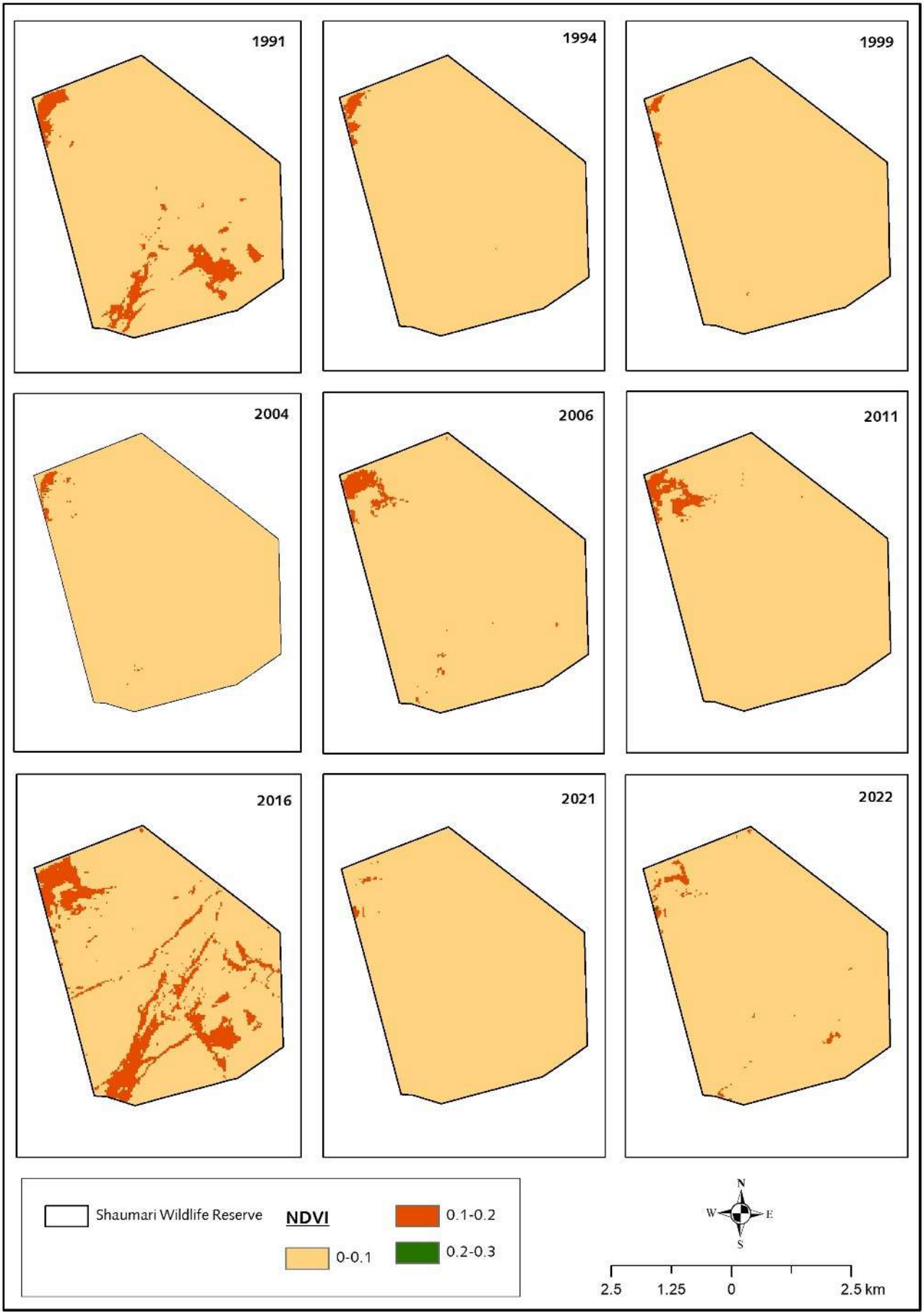

Figure 5. Vegetation cover density and distribution for the Shaumari Wildlife Reserve (total area, 22 km^2) in July 1991-2022. NDVI class's data were derived using Landsat-5 (TM), Landsat-7 (ETM+) and Landsat 8 (OLI) sensors data.

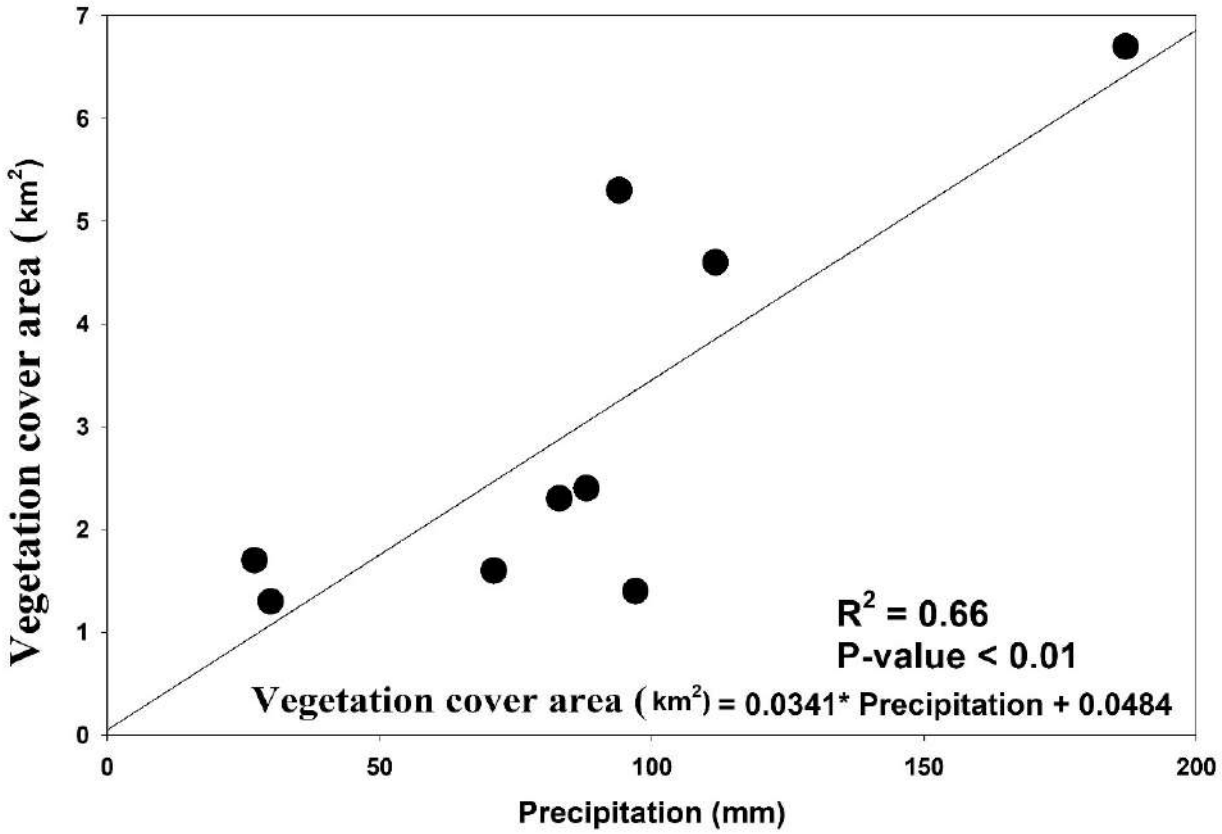

Figure 6. The relationship between the vegetation cover total area (dense + scattered),derived using normalized difference vegetation index (NDVI), and between precipitation during the study period 1991-2022.

influence vegetation cover density (Dube and Pickup, 2001). Given the varying effects of rainfall patterns and land utilization, it is important to consider whether intensified land use might hold comparable or even greater significance than climate change itself (Dube and Pickup, 2001). The application of high grazing intensity has the potential to elevate the vulnerability of both rangeland ecosystems and local populations to drought circumstances (Hein, 2006). Furthermore, during arid years, areas experiencing intensive grazing pressure witness substantial reductions in both above-ground biomass production and rain-use efficiency (Hein, 2006). In this study, the linear regression analysis of remote sensing and rainfall data revealed a moderate association (66%). Therefore, the fluctuation in the density and distribution of vegetation cover across years can be attributed to the harsh climatic conditions, especially rainfall. In Kuwait's rangelands, the plant cover was 83% and 70% less, and herbage production was 76% and 91% less in grazing areas (Zaman, 1997). In the same study, an average seasonal precipitation of 90 mm supported a mean of 223 kg ha^{-1} biomass, whereas an average mean seasonal precipitation of 73 mm maintained a mean phytomass of 102 kg ha^{-1} (Zaman, 1997). The process of vegetation change detection using satellite sensor data can determine pixel variations at different times to identify areas undergoing rapid changes (Willis, 2015). However, for accurate information from moderate resolution satellites (e.g. Landsat) ground surveys are essential for an accurate assessment of the remotely-sensed data (Qarallah et al., 2023; Abu Yahya *et al*., 2022). Sawalhah *et al*. (2018) found that the overall accuracy of remote sensing data derived from Landsat to assess the density and distribution of the rangeland vegetation cover in Jordan in 2016 was 75%. Overall, utilizing open- access (free of charge)

extensive sequences of remotely sensed data, notably Landsat (with over 40 years of free images), in combination with ground-referenced data, is considered as a feasible strategy for quantifying vegetation cover density (Tongway and Hindley, 2004).

Conclusions

Remote sensing techniques hold promises for detecting vegetation cover density and distribution. This advanced sensing approach reduced time, cost, and provided essential historical data records for the released sites (vegetation cover density for the 34 years). Landsat-NDVI images revealed that the northwestern side of the reserve had consistently the highest vegetation cover density over the growing season (March and July) andduringthe study period from 1991to 2022. A moderate and significant relationship was found between vegetation cover density and rainfall during the growing season. However, for precise information, further studies are recommended, especially ground survey assessments.

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The Reproductive Cycle of the Female Cushion Sea Star *Patiriella regularis*

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Abstract

Patiriella regularis or the New Zealand Sea Star spawns small eggs (150 μm) and has indirect development through the bipinnaria and brachiolaria larvae, typical for asteroids. While *P. regularis* has been used in studies related to reproduction and embryo development, the gametogenic cycle in this species has not been studied in terms of histological analysis or division into stages. The current study aims to describe the ovarian cycle and oogenesis of *P. regularis* in terms of organ indices and histological and ultrastructural analysis of the ovary using light and electron microscopy. The reproductive cycle in the female *P. regularis* showed high similarity to that in many other studied asterinid species and was divided into five growth/gametogenic stages: I) The recovery stage, II) The growing stage, III) The maturing stage, IV) The partly- spawned stage, and V) The spent stage. While oocytes at different growing phases were found in the ovary throughout the reproductive cycle, the majority of the oocytes developed synchronously and indicated that *P. regularis* spawns during summer. The outcome of this study provides new insight into the gametogenesis of the *P. regularis* ovary throughout the reproductive cycle, and presents new details pertaining to oogenesis in a major invertebrate phylum that is rarely studied.

Keywords: Reproductive cycle, Oogenesis, Asteroids, Sea star.

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Introduction

The female reproductive cycle in marine invertebrates consists of a series of events that include oogenesis, gonad activation and growth, spawning of gametes, regression of gonadal activity, and a resting period (Giese, 1959). Oogenesis is a complex process that results in the production of a fully mature egg (ovum) from a primordial germ cell. This process involves cell growth, synthesis of organelles, and preparation of a highly specialised cell for fertilisation (Song *et al.*, 2006). In oviparous animals, in which the females deposit eggs that develop and hatch outside the maternal body, the gamete must be prepared for the energy and nutritional requirements of the future embryo (Smiley, 1990; Blackburn, 1999). Oogenesis is the major event during the reproductive cycle. Thus, during oogenesis, nutrients which mainly consist of yolk proteins accumulate in the oocyte, the genes encoding ribosomal ribonucleic acid (rRNA) are amplified, and many types of RNA are synthesised and stored in their inactive form until there is a need to use them by the developing embryos (Carlson, 2009).

Offspring survival is the main criterion for reproductive success. Therefore, reproduction is often synchronised with environmental conditions that will be most favourable for the success of the offspring (Lawrence, 1987). The synchronisation of gametogenesis and spawning within the populations of marine invertebrates including echinoderms is controlled by various factors.

These factors are generally categorised into two main groups: *i)* exogenous factors and, *ii)* endogenous factors (Giese and Pearse, 1974). The exogenous factors include environmental variables such as temperature, photoperiod, and food availability (Pearse *et al.*, 1986). For example, it was found that shifting the photoperiod regime six months out of phase resulted in shifting the gametogenic cycle by a similar time in the sea stars *Pisaster ochraceus* (Pearse and Eernisse, 1982) and *Asterias vulgaris* (Pearse and Walker, 1986). This shift also resulted in a change of the gonad index and oocyte size in the sea star *Sclerasterias mollis* (Xu and Barker, 1990b).

The reproduction of asteroids has been studied extensively. Like most other marine invertebrates, echinoderms show diversity in reproductive strategies (Carvalho and Ventura, 2002). For example, in the family Asterinidae, *Meridiastra gunnii*, *M. calcar* and *Cryptasterina pentagona* (previously *Patiriella gunnii*, *P. calcar* and *P. pseudoexigua*, respectively) are broadcasters, spawn large eggs, and develop directly through non-feeding brachiolaria larvae (Lawson-Kerr and Anderson, 1978; Grice and Lethbridge, 1988; Byrne, 1991, 1996; Chen and Chen, 1992). In contrast, *Patiriella regularis* spawns small eggs that develop indirectly through feeding bipinnaria and brachiolaria larvae (Crump, 1971; Byrne and Barker, 1991). Unlike the above-mentioned species, *Parvulastra vivipara* and *Parvulastra parvivipara* (previously *Patiriella vivipara* and *P. parvivipara*, respectively) are viviparous, i.e. fertilisation occurs inside the gonad and they brood their embryos in their gonads (Chia, 1976; Chia and Walker, 1991; Byrne, 1996).

P. regularis is one of New Zealand's most common rocky shore sea stars (Morton and Miller, 1973), and embryo development in this species represents a typical asteroid developmental pattern (Byrne and Barker, 1991). *P. regularis* has been used in a number of studies related to reproductive biology (Crump, 1971; Byrne and Barker, 1991; Styan *et al.*, 2005; Prowse and Byrne, 2012; Alqaisi *et al.*, 2016). Crump (1971) studied seasonal changes in gonad and pyloric caeca indices and changes in the oocyte sizes of *P. regularis* in three geographically separated populations and provided useful information regarding the reproductive cycle and spawning time of this species. However, Crump (1971) did not provide a full description of the gametogenic cycle and of cellular changes in gonads during the reproductive cycle. In addition, the reproductive cycle of *P. regularis* remains to be divided into stages that can facilitate future studies on the reproductive biology of this species.

Studies related to reproduction are multidisciplinary; from molecular biology to reproductive ecology. Understanding the cellular processes associated with the reproductive cycle is essential for all studies on reproductive biology. Furthermore, understanding the different reproduction-related processes, such as vitellogenesis, and the factors that regulate reproduction is based on describing the reproductive cycle and how the reproductive status changes in relation to these factors (Chia and Walker, 1991). In general, the reproductive cycle in echinoderms is described by measuring temporal changes in the relative gonad size (i.e. gonad indices) and the histological analysis of the gonads at different reproduction phases (Schoenmakers et al., 1984). The gonad index provides information of relative gonad size and can be helpful to visualise the relationship between the gonad and other body organs. For example, in most studied asteroids, an inverse relationship is found between the pyloric caeca and gonad indices over the course of a reproductive cycle, indicating a transfer of nutrients from the pyloric caeca to the gonads during gametogenesis (Crump, 1971; Byrne, 1992; Chen and Chen, 1992; Carvalho and Ventura, 2002; Georgiades et al., 2006). The histology of gonads is another fundamental approach to describing the reproductive cycle. Staging the reproductive cycle and providing a detailed description of vitellogenin deposition in the ooplasm and of the relationship between the

developing gametes and the surrounding somatic cells in the gonads can be made using histological analysis (Schoenmakers et al., 1981; Beijnink et al., 1984; Schoenmakers et al., 1984; Reimer and Crawford, 1995).

Studies on the oogenesis of invertebrates cover only 1% of the described species in each of major invertebrate's phyla (Eckelbargera and Hodgson, 2021). Thus, studying the reproductive cycle of *P. regularis* in more detail will be useful in future comparative studies on the reproductive biology of other asterinid sea stars and marine invertebrates more broadly. In this respect, the current study aims to describe the female reproductive cycle in a population of the sea star *P. regularis* over one year. In this study, the authors describe the reproductive stages based on: *i)* ovary and pyloric caeca index analysis, and *ii)* a detailed histological and ultrastructural analysis in the ovary throughout the annual reproductive cycle using light and electron microscopy.

Materials and Methods

Animal sampling

Patiriella regularis was collected monthly between March 2010 and February 2011 from a population in the Otago Harbour, New Zealand (45° 48' S; 170° 38' E). Due to difficulties in collecting a large number of samples from one location for all sampling events, samples were collected from one of two locations: Portobello Peninsula or Harrington Point (Figure 1). The samples from the selected locations have similar reproductive potential (Crump, 1971). Around thirty samples were collected each month from a depth of five to eight meters by SCUBA diving. After collection, the samples were returned to the Department of Zoology Aquaria, University of Otago, and were maintained overnight in 30 L glass tanks of sea water from Otago Harbour at ambient temperature and photoperiod during the time of each sampling.

Figure 1. Map of New Zealand (upper left) and Otago Harbour. Labelled red points with numbers represent the sampling locations; 1: Portobello Marine Laboratory wharf; and 2: Harrington Point. Google Map.

Dissection of animals

Only sea stars with a ray (arm) length (measured from the oral opening to the tip of the ray) of more than 30 mm were selected for dissection. Prior to the dissection, the total body wet weight of animals was taken after the animal was gently drained of excess water. Animals were dissected and pieces from gonads were mounted on a slide for a microscopic examination of sex, for this sea star is not sexually dimorphic. Based on the number of females found in each, between eleven and seventeen females were found between the collected sea stars dissected during each monthly sampling. Pyloric caeca and gonads were removed, weighed to the nearest mg, and kept in filtered sea water (FSW) at 4 °C. The ovary index and the pyloric caeca index were calculated for each sample as organ wet weight /total body wet weight * 100 (Broertjes *et al.*, 1984). Ovarian tissues were used for histological examination and staging as shown in the following section.

Histology

Light microscopy analysis

During dissection, fragments of ovary tissues were drained and divided into small pieces (10 – 15 mm each) and were immediately fixed in 1.3 ml of 4 % paraformaldehyde (PFA) in FSW for at least twenty-four hours. the samples were then washed three times with 70 % ethanol for two hours and stored in 70 % ethanol at room temperature until processing. Later, the samples were dehydrated in a graded ethanol series as follows: 80 % for 1 hr, 95% for one hour and 100 % two times for thirty minutes. Subsequently, the dehydrated tissues were embedded in glycol methacrylate resin (Technovit 7100, Heraeus Kulzer GmbH, Germany) as described by the manufacturer and were sectioned at 2 µm thickness using a Reichert-Jung microtome (Cambridge Instruments GmbH, Germany). Two slides were prepared containing serial sections from each sample; one slide was stained with haematoxylin and eosin (H&E) and the other with periodic acid-Schiff (PAS) reagent. The slides were viewed with an Olympus-BX51 microscope and the images were taken with a camera (Olympus-SC100, Japan) connected to a computer and were viewed using Cell Sens (Olympus) software. The images from ovary sections were used for measuring oocyte diameters using the image-analysis software ImageJ (https://imagej.net/ij/) as follows: The area of the biggest thirty oocytes from each sample was measured, and the diameter was calculated by the software as the longest distance between two points along the oocyte area. This method was used to give an estimation of the oocyte diameter since oocytes were not spherical in shape in all stages of the reproductive cycle. To ensure that the oocytes were measured from the centre, only oocytes with visible nucleolus were measured. Ovary sections of five females from each monthly sampling were randomly selected and used for this analysis.

Electron microscopy analysis

Fragments from the ovary were divided into small pieces, approximately 1 mm^3 each and were immediately fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer with 1.68 % NaCl overnight at 4 °C. The samples were sent to the Otago Centre for Electron Microscopy, University of Otago, where they were processed as follows: The fixed samples were washed in 0.1 M cacodylate buffer with 1.68 % NaCl three times, for ten minutes. Subsequently, the samples were post-fixed in 1 % osmium tetroxide in 0.1 M cacodylate buffer with 1.68 % NaCl for one hour, washed in the same buffer three times, ten minutes each, and were stored at 4 °C until further analysis. Prior to subsequent dehydration, the samples were washed in de-ionised water three times for ten minutes, en bloc stained in 1 % uranyl acetate in de-ionised water for one hour and rewashed in de-ionised water two times for five minutes each. The samples were dehydrated in ethanol and were infiltrated with Quetol 651 resin and embedded and polymerised at 60 °C. Finally, the samples were sectioned at 80 nm using a Reichert-Jung Ultracut E ultramicrotome (C. Reichert AG, Vienna, Austria) and were

stained with uranyl acetate and lead citrate for transmission electron microscopy (TEM). The sections were viewed using a Philips CM100 BioTWIN transmission electron microscope (Philips/FEI Corporation, Eindhoven, The Netherland), and images were taken with a MegaView lll digital camera (Olympus Soft Imaging Solutions GmbH, Münster, Germany) connected to a computer.

Data analysis

The statistical analysis of changes in ovary and pyloric caeca indices during the reproductive cycle was done using GraphPad Prism 6 (GraphPad Software, Inc., USA). Graphs were prepared using GraphPad Prism 6 or Office Excel 2010 (Microsoft®). Data were tested for normal distribution using D'Agostino and Pearson omnibus normality tests and were subjected to log-transformation where needed. In doing so, the data still did not achieve normality after log transformation, and therefore, the monthly variations of ovary index or pyloric caeca index were analysed using a nonparametric test, Kruskal-Wallis, followed by Dunn's multiple comparisons test. Regression analysis was used to establish the relationship between ovary index and pyloric caeca index throughout the reproductive cycle. Day length and sea temperature data in Otago Harbour were kindly provided by the Department of Marine Science at the University of Otago, New Zealand.

Results

Organ indices

Monthly variations in ovary index and pyloric caeca index in *P. regularis* varied significantly throughout the reproductive cycle (Kruskal-Wallis test, $P < 0.001$ for ovary and pyloric caeca) (Figure 2). The ovary index peaked in the early austral summer and reached approximately 25 % in December. This was followed by a gradual decrease from January to March during which the ovary index reached its minimum value of about 5 %. The ovary index remained stable during the austral autumn (March to May) with no significant change, then increased

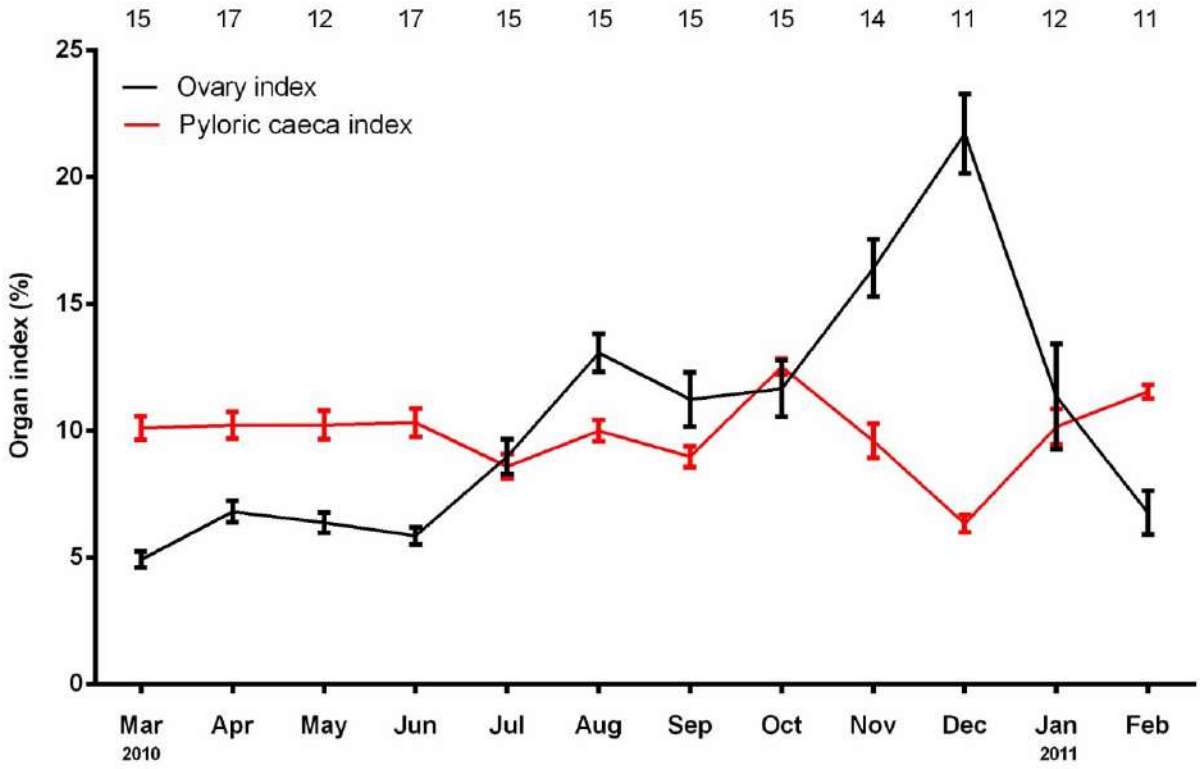

Figure 2. Monthly ovary index and pyloric caeca index (mean ± standard error of the mean) of female Patiriella regularis between March 2010 and February 2011. Numbers across the top indicate sample number for females sampled each month.

gradually through winter, from June to August (Figure 2). Significant differences were found between mean ovary index in August, March, and July (Dunn's multiple comparisons test, α: 0.05). The ovary index increased approximately two-fold during spring and early summer (from 11.5 % in October to 22 % in December).

The pyloric caeca index was stable with no significant change during autumn and winter. It peaked in October during the spring season to 13 % (Figure 2), then decreased and reached its minimum values in December (6 %). In December, the mean pyloric caeca index was significantly different from that in all other months except for July and September (Dunn's multiple comparisons test, α: 0.05). The pyloric caeca index increased during late summer and became stable during autumn. There was no clear inverse relationship between pyloric caeca index and ovary index (r^2= 0.03, $P < 0.02$; Supplementary Figure 1).

Histological analysis and the stages of the reproductive cycle

Based on the histological and ultrastructural analysis of the ovary and ovary index, the reproductive cycle of *P. regularis* was divided into five stages. The following criteria were used to define each stage: the abundance of oocytes at each developmental stage (i.e., young oocytes, growing oocytes and fully-developed oocytes), the thickness of the haemal system (HS), and the presence of HS invaginations in the ovary lumen, the presence/absence of unspawned and degenerating oocytes and the abundance of somatic cells. The stages were named after the previously described other asterinid species by Byrne (1992) as follows: Stage I: The recovery stage; Stage II: The growing stage; Stage III: The maturing stage; Stage IV: The partly-spawned stage; and Stage V: The spent stage. Ovary and oocyte descriptions and terminology for each stage used in the present study were based on Byrne (1992) and Schoenmakers et al. (1981).

Stage I: The recovery stage

Young oocytes were found at this stage lining the ovary wall or attached to HS invaginations into the ovary lumen (Figure 3 (b)). The young oocytes were spherical to oval-shaped, and their diameter reached up to 40 µm (Figure 3 (b)). These oocytes had a large spherical nucleus, their cytoplasm was basophilic and mainly PAS-. Few PAS+, and electron dense granules indicative of yolk deposition were found in the cytoplasm of young oocytes (Figure 3 (d), Figure 3 (e)). Oogonia were found in clusters with young oocytes, forming cell nests (Figure 3 (b)). These cell nests were attached to the ovary wall or HS invaginations. More developed oocytes were pear-shaped, and their size increased to 40 – 100 µm in diameter. These oocytes were attached to the ovary wall and were the most abundant type of primary oocytes in the ovary during this stage (Figure 3 (a)). Pear-shaped oocytes were characterised by PAS+ staining and eosinophilic cytoplasm (Figure 3 (a), Figure 3 (c), Figure 3 (d)); the PAS+ staining reflected yolk deposition in the ooplasm (Schoenmakers et al., 1981; Byrne, 1992). Ultrastructurally, the number of Golgi complexes increased in these growing oocytes and the cytoplasm became more granular and included a large number of electron dense granules (yolk granules). Young oocytes and pear-shaped oocytes were surrounded by somatic cells, mainly follicle cells (Figure 3 (b), Figure 3 (c)).

The lumen of the ovary was devoid of oocytes or contained eosinophilic and PAS+ cell debris resulting from the reabsorption of degenerated unspawned oocytes. Many phagocytes were found dispersed in cell debris and were characterised by a dense basophilic round nucleus (Figure 3 (a)). Many somatic cells were dispersed between young and growing oocytes (Figure 3 (b)). The HS, which appeared to have proliferated from previous stages, was thick and projected into the ovary lumen through invaginations (Figure 3 (b), Figure 3 (c), Figure 3 (d)). The HS and its invaginations included somatic cells that were characterised by densely stained basophilic nuclei (Figure 3 (b)).

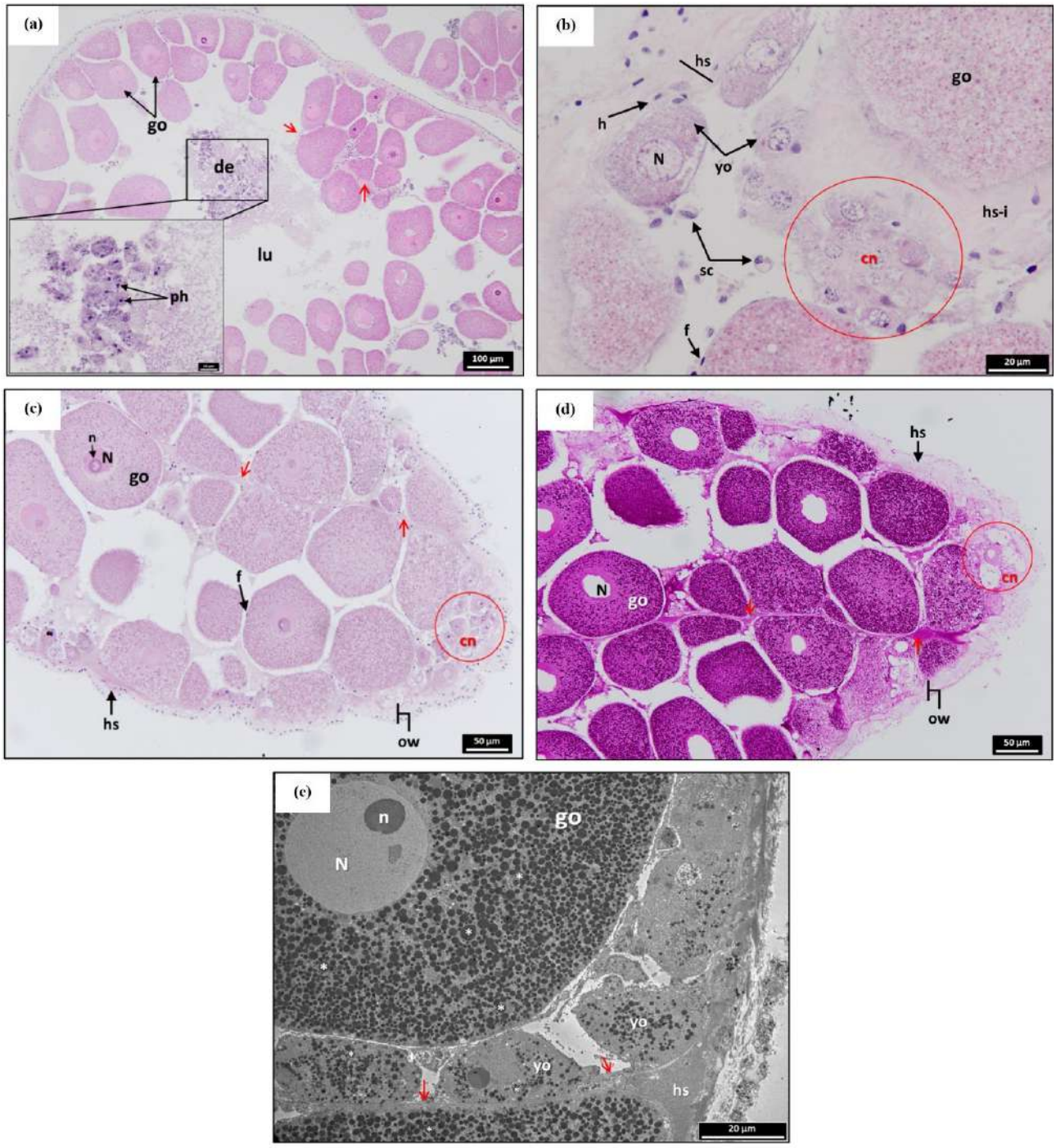

Figure 3. Histology of sea star Patiriella regularis ovaries at recovery stage (Stage I) using light microscopy (a – d) and transmission electron microscopy (e). (a) General section in ovary; growing pear-shaped oocytes (go) are attached to the ovary wall; ovary lumen (lu) contains debris (de); phagocytes (ph) are dispersed in cell debris (enlarged); red arrow heads indicate haemal system invagination into ovarian lumen. (b) Cell nest (cn) containing oogonia and early young oocytes; haemal system (hs) is proliferated and forming invaginations into ovarian lumen (hs-i); somatic cells (sc) scattered between oocytes; young oocytes (yo) and growing oocytes (go) are attached to ovary wall or haemal system invagination; follicle cells (f) surrounding oocytes; h: cell in haemal system; N: nucleus. (c) and (d) are serial sections stained with H&E and PAS respectively; early young oocytes are PAS- and growing oocytes are PAS+; the haemal system (hs) is PAS+; ovary wall (ow). Red arrow heads indicate haemal system invagination in ovary lumen. (e) Section in ovary at late Stage I; young oocytes are attached to the haemal system invagination (red arrow heads), asterisks indicate yolk granules.

Stage II: The growing stage

The ovary at this stage was characterised by increasing numbers of pear-shaped growing oocytes (Figure 4 (a), Figure 4 (c)). Cell nests and young oocyte numbers decreased, and few aggregations were found attached to HS invaginations or the ovary wall (Figure 4 (c), Figure 4 (d)). Somatic cells, including phagocytes, decreased in number at this stage and the HS became attenuated. Toward the end of this stage, the pear-shaped oocytes grew more and became spherical in shape, their size reaching up to 150 µm in diameter. These oocytes detached from the ovary wall and they started to fill the ovarian lumen (Figure 4 (b)). As the oocytes grew and became spherical in shape, the number of basophilic granules increased in their cytoplasm and also, their cytoplasmic PAS+ staining became more intense (Figure 4 (c), Figure (d)). In addition, cortical granules were observed, dispersed throughout the cytoplasm, and they were recognised as a vesicle containing a mix of electron dense and translucent material. The large spherical-in-shape oocytes were still surrounded by follicle cells. Increases in oocyte size at this stage coincided with a gradual increase in the ovary index. Organelle numbers including Golgi complex and mitochondria, increased at this stage (Figure 4 (e)), and electron-dense granules (yolk granules) were abundant in the oocyte cytoplasm (Figure 4 (e)).

Stage III: The maturing stage

During this stage, fully-developed oocytes were the most abundant in the ovary (Figure 5 (a)). These large oocytes (around 200 µm in diameter) were packed in closely together without any spaces between them, and they occupied the entire ovarian lumen (Figure 5 (a)). The fully-developed oocytes were characterised by having a large peripheral nucleus and their cytoplasm was PAS+, but the PAS intensity was less compared to that in growing oocytes (Figure 5 (b), Figure 5 (c), Figure 5 (d)). At the ultrastructural level, the cytoplasm included a large number of electron-dense granules and the cortical granules can be seen arranged under the oocyte cytoplasm (Figure 5 (e), Figure 5 (f)). Many young oocytes and growing pear-shaped oocytes were found in the ovary at this stage (Figure 5 (c), Figure 5 (d)). Few somatic cells, including phagocytes, were seen at this stage and the HS invaginations in the ovary lumen disappeared. At this stage the ovary index reached its maximum values.

Stage IV: The partly-spawned stage

The ovary lumen at this stage became empty as some eggs had been spawned (Figure 6 (a)). Fully-developed oocytes were loosely packed and their number decreased compared to the previous stage (Figure 6 (a), Figure (b)). Pear-shaped growing oocytes and young oocytes lining the ovary wall were abundant (Figure 6). The HS started to proliferate and the ovary wall became thicker than in the previous stage (Figure 6 (c)). Somatic cell numbers increased. Debris resulting from degenerated eggs or oocytes was evident at this stage (Figure 6 (d)).

Stage V: The spent stage

The ovaries at this stage included few unspawned oocytes that were likely to undergo degradation (Figure 7 (a), Figure 7 (b)). Atretic oocytes were characterised by clumping cytoplasm (Figure 7 (c)). Ultrastructurally, the degenerating ooplasm included a vacuole-like structure that seemed to contain cytoplasmic components including yolk granules (Figure 7 (e)). Somatic cells and phagocytes were abundant and found associated with degrading oocytes and cell debris. Dividing oogonia increased and small cell-nests started to form and were attached to the ovary wall or HS invagination (Figure 7 (d)). Toward the end of this stage, the cell-nests became more abundant. The HS proliferated and was visible at this stage (Figure 7 (a), Figure 7 (b)), forming invaginations into the ovary lumen (Figure 7 (d)).

Oocyte size-frequency distributions and gametogenic stages

The results from the oocyte diameter analysis throughout the reproductive cycle revealed a

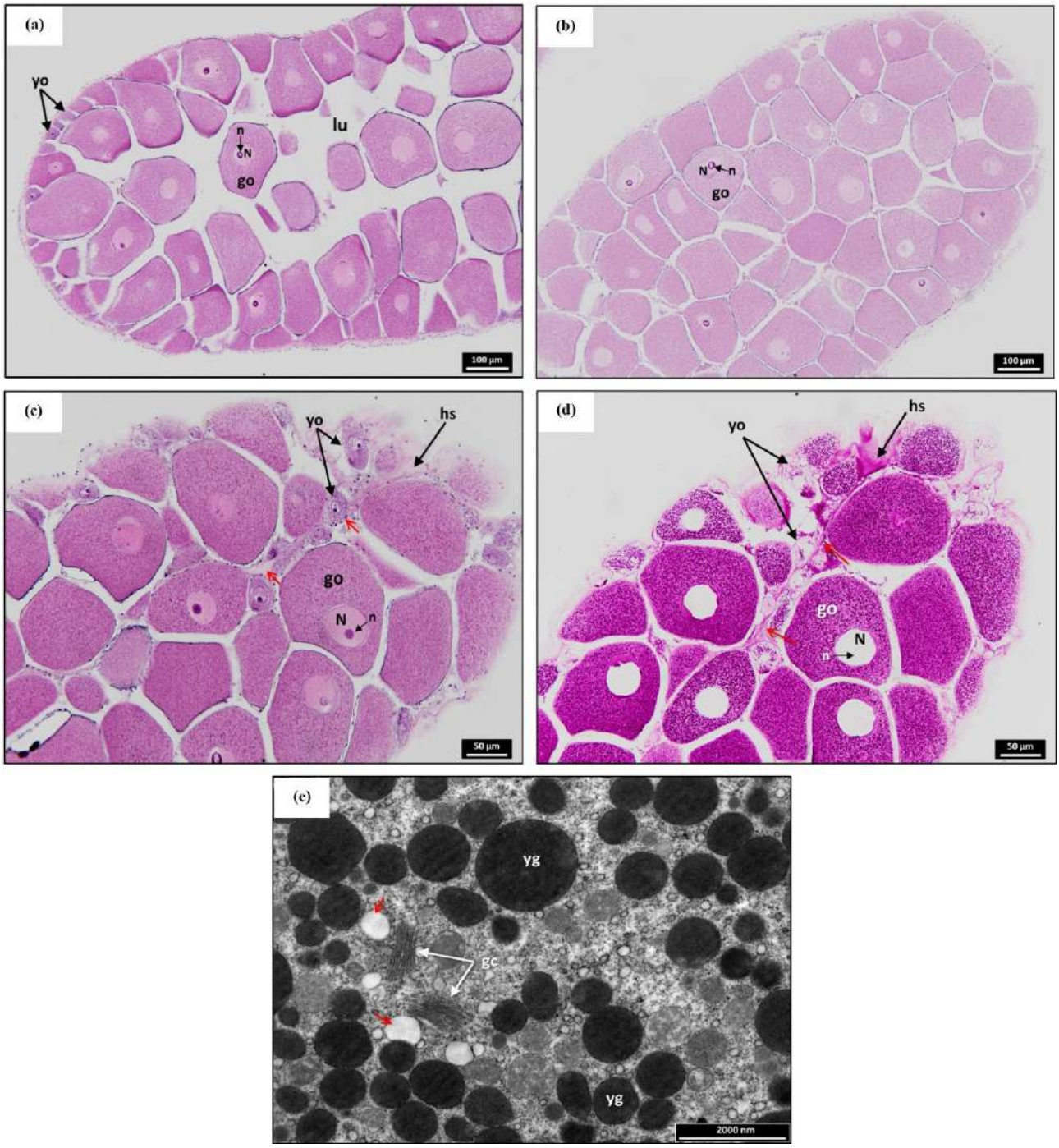

Figure 4. Histology of sea star Patiriella regularis ovaries at growing stage (Stage II) using light microscopy (a – d) and transmission electron microscopy (e). (a) Ovary at early stage II; yo: young oocytes; go: growing oocytes; N: nucleus; n: nucleolus; lu: lumen. (b) Ovary at late stage II; lumen full with oocytes at final growing stage. (c) and (d) are serial sections stained with H&E and PAS respectively; hs: haemal system; red arrow heads indicate haemal system invagination in ovary lumen. (e) Oocyte cytoplasm; gc: Golgi complex; yg: yolk granules, read arrow heads indicate vacuoles.

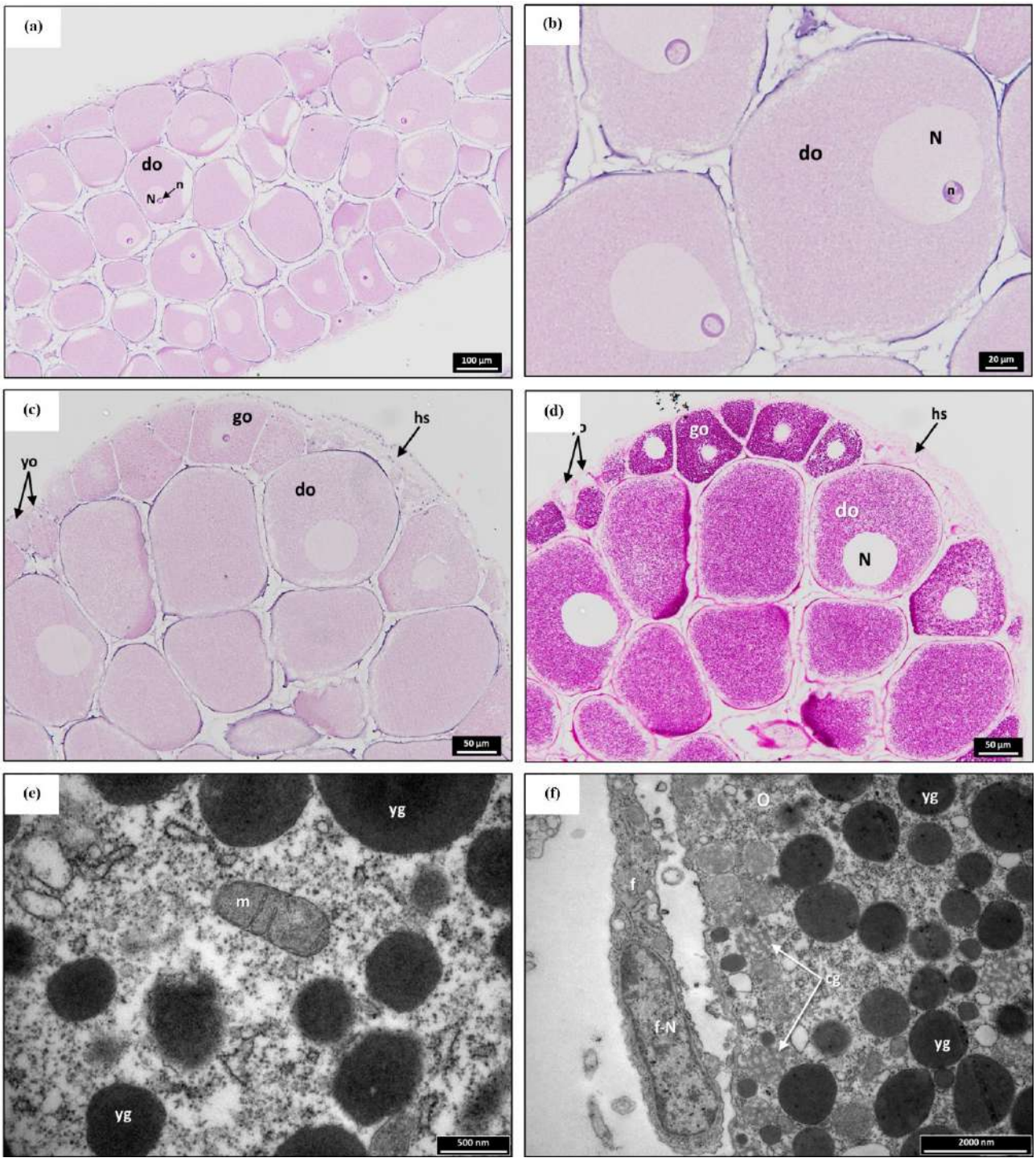

Figure 5. Histology of sea star Patiriella regularis ovaries at mature stage (Stage III) using light microscopy (a – d) and transmission electron microscopy (e and f). (A) Section in ovary showing lumen is full with packed fully-developed oocytes (do); N: nucleus; n: nucleolus. (b) Fully-developed oocytes. (c) and (d) are serial sections stained with H&E and PAS respectively; ovary contains large number of developed oocytes and small number of young oocytes (yo) and growing oocytes (go); hs: haemal system. (e) Ovary cytoplasm; m: mitochondria; yg: yolk granules. (f) Fully-developed oocyte cytoplasm (O) and follicle cells (f) at late Stage III; cortical granules (cg) are abundant at the periphery of oocyte cytoplasm; f-N: follicle cell nucleus.

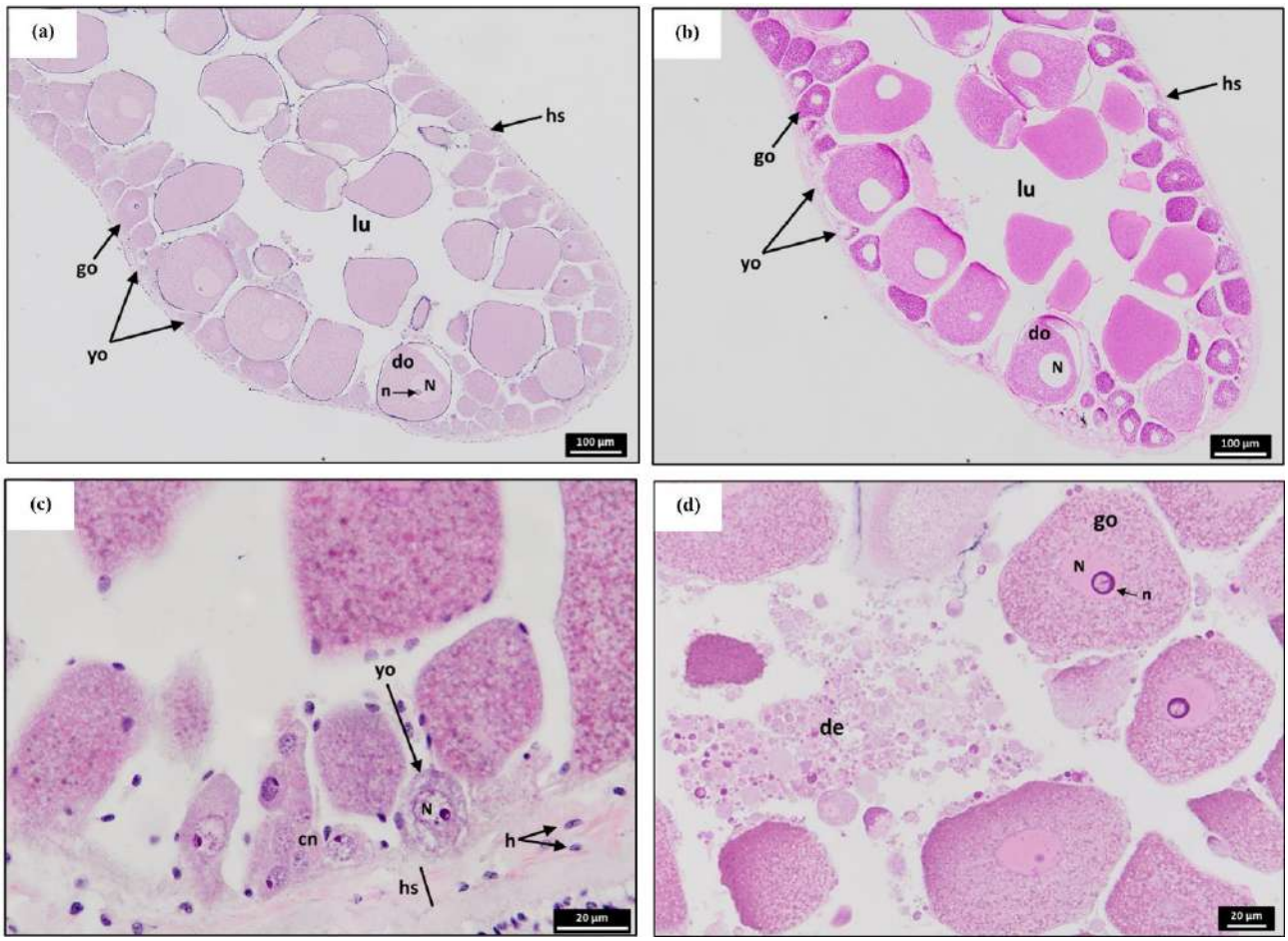

Figure 6. Histology of sea star Patiriella regularis ovaries at partly spawned stage (Stage IV) using light microscopy. (a) and (b) are serial sections stained with H&E and PAS respectively; ovary includes fully-developed oocytes (do) that are not yet spawned; ovary wall is lined with young oocytes (yo) and growing oocytes (go); ovary lumen is clear due to removing fully-developed oocytes during spawning; haemal system (hs) is proliferated; N: nucleus; n: nucleolus. (c) Cell-nest (cn) is abundant with young oocytes at this stage; h: cell in haemal system. (d) Debris (de) in lumen from degenerated oocyte.

seasonal pattern that generally corresponded to the ovary index. The oocyte size-frequency (Figure 8) exhibited a unimodal distribution throughout the annual reproductive cycle, except in the partly spawned stage (Stage IV) due to the abundance of growing oocytes in one cohort and the presence of pre-spawning oocytes on the other. The growing pear-shaped oocytes with diameters between 75 to 150 µm were most abundant between May and July (Figure 8). Most oocyte growth and vitellogenesis occurred during the growing stage in the austral winter between June and September (Figure 9).

From August to December, there was a shift in the most abundant oocytes with diameters between 150 µm to 200 µm (Figure 8). During the same period, there was an increase in ovary index (Figure 2). Females at the mature stage (Stage III) were first observed in late winter, and their number increased toward the summer (Figure 9). During this stage, fully-grown oocytes (175 – 250 µm) were most abundant (Figure 9) which coincided with maximum ovary index values (Figure 2). Spawning did not occur until January in mid-summer when the first partly spawned ovary was observed (Figure 9), and this was followed by a drop in the frequency of large-diameter oocytes (Figure 8). Spawning lasted throughout the summer and early autumn (from January to April). Most females were spent from February to April, by which time most of the ovaries were at the recovery stage of the reproductive cycle.

Young and growing oocytes were found throughout the reproductive cycle which indicated that oogenesis was continuous throughout the year. The majority of oocytes at each stage appeared to develop

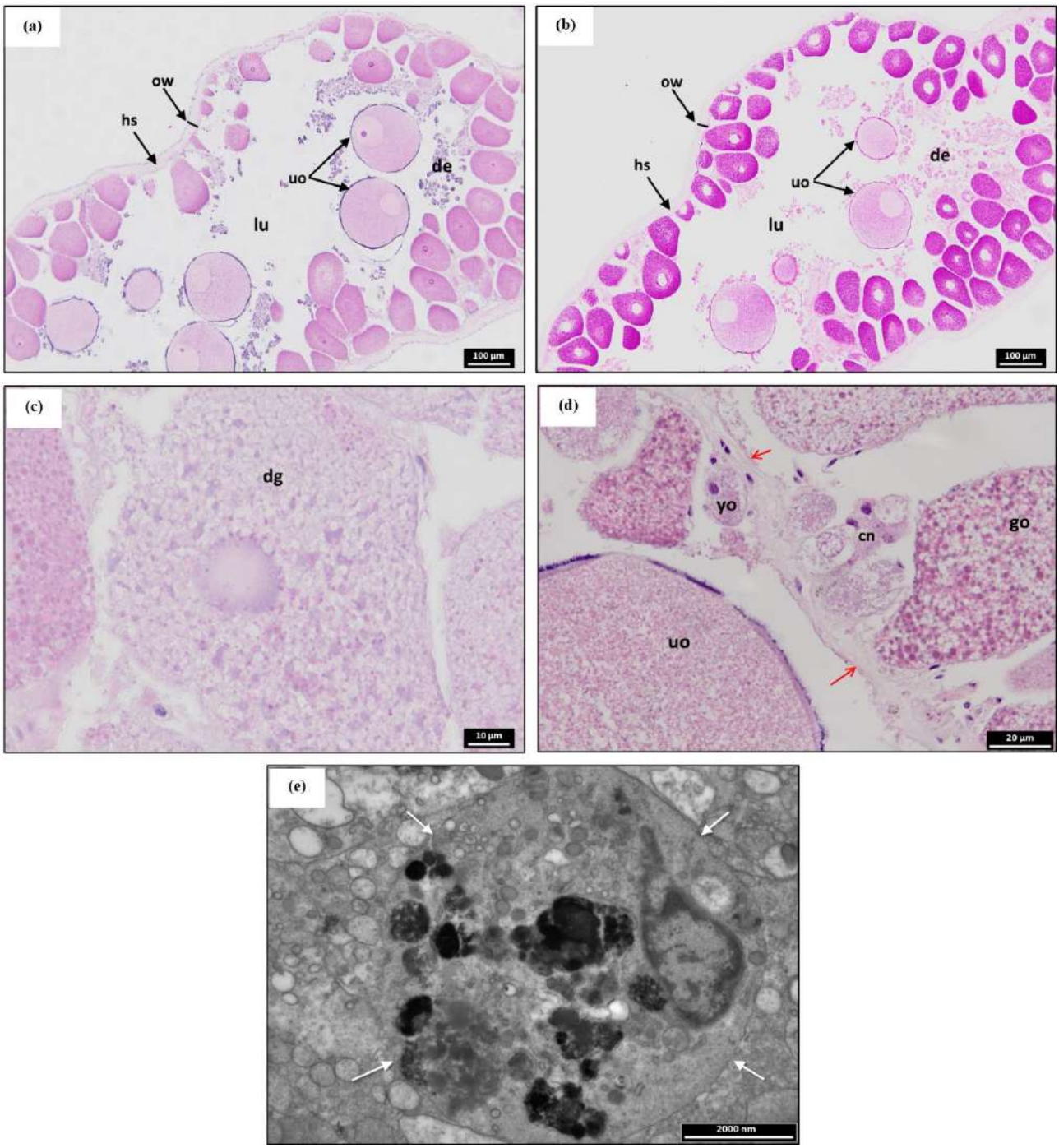

Figure 7. Histology of sea star Patiriella regularis ovaries at spent ovary stage (Stage V) using light microscopy (a – d) and transmission electron microscopy (e). (a) and (b) are serial sections stained with H&E and PAS respectively; ovary lumen (lu) contains few unspawned oocytes (uo) and debris (de) resulted from degenerated oocytes; ow: ovary wall. (c) Degenerating oocytes (dg) with clumping cytoplasm. (d) Cell-nest (cn), young oocytes (yo) and growing oocytes (go) are attached to haemal system invagination (red arrow heads). (e) Cytoplasm of degenerating oocyte; arrow head show the border of vacuole-like structure that contains cytoplasmic components.

synchronously representing one size class. The presence of large oocytes (150 to 200 µm) between May and September resulted from pear-shaped oocytes, diameters being defined as the largest diameter of non-spherical oocytes. Unspawned oocytes (> 200 µm) were present in March and April.

Annual cycle of day length and sea temperature

The annual cycle of day length between March 2010 and February 2011 (Supplementary Figure 2) showed that maximum day length was in late December (approximately 16 hr). This maximum day length just preceded *P.*

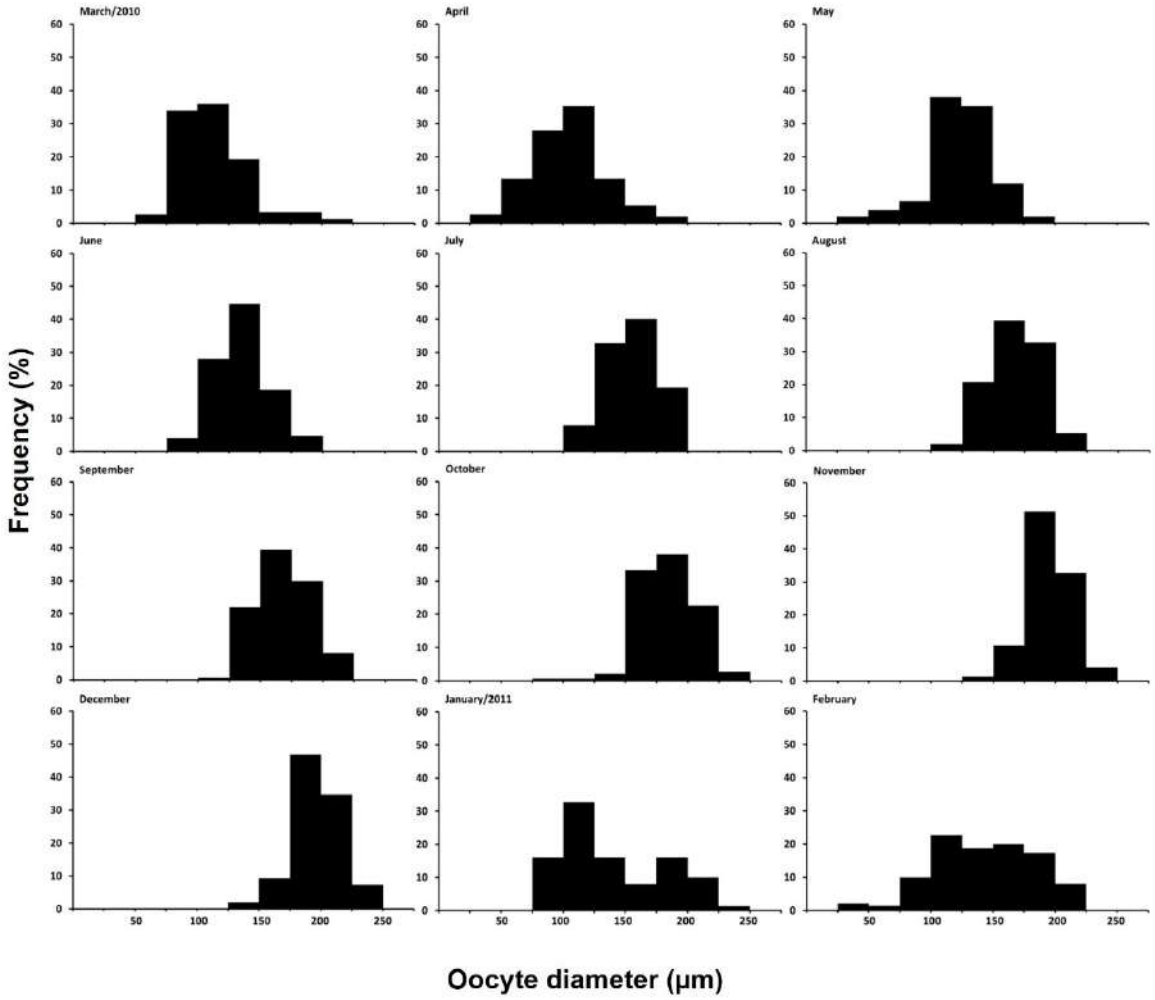

Figure 8. Patiriella regularis. Monthly size-frequency distribution of oocytes from March 2010 to February 2011. n= 150 oocytes per month from across 5 females.

regularis spawning. The shortest day length was on the 21st of June (approximately 9 hr). From August until December, there was an increase in day length which coincided with an increase in ovary index and with the growing stage of the ovary of *P. regularis*. During this period of this study, the maximum sea temperature was in December (average 17.4 °C) when sea stars started spawning. The minimum sea temperature was in July (average 7.9 °C) followed by a gradual increase from August until December.

Discussion

The present study describes the reproductive cycle of the female sea star *P. regularis* based on organ indices and the histological analysis, and the previously described reproductive cycle of other asterinid species (Byrne, 1992). Five stages of the oogenic cycle were defined in *P. regularis*: recovery, growing, maturing, partly-spawned and the spent stage.

A range of reproductive strategies have been reported in the family Asterinidae. For example, *Parvulastra exigua* (previously *Patiriella exigua*) has continuous gametogenesis, whereas *M. gunnii* and *M. calcar* have well-defined seasonal reproductive cycles (Byrne, 1992). *P. regularis*, as in most asteroids, has a distinct annual reproductive cycle (Crump, 1971). This was also evident from the histological analysis of the ovary in the present study. In many temperate asteroids that undergo an annual reproductive cycle, oocyte development occurs mostly during the autumn and winter seasons, such as in *S. mollis* (Barker and Xu, 1991) and *Coscinasterias calamaria* (Crump and Barker, 1985). Similarly, the growing stage of the ovary in *P. regularis* aligned with winter time (Crump, 1971; present study).

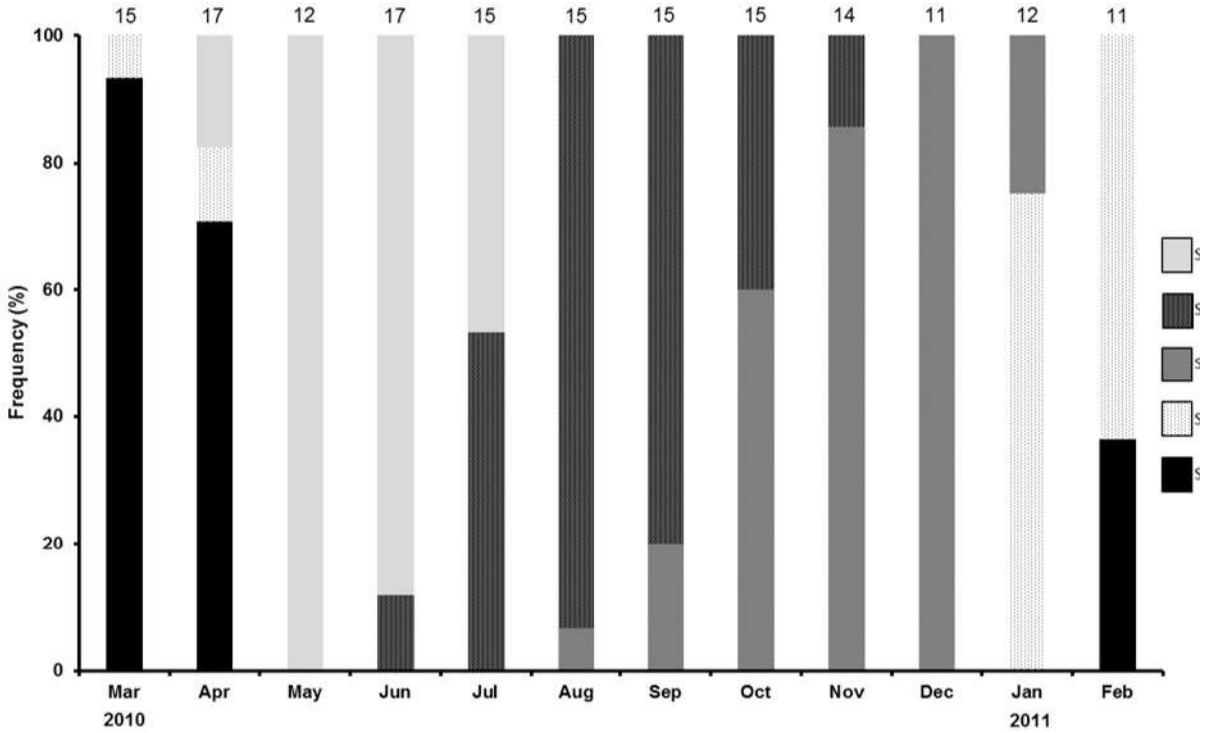

Figure 9. Relative frequencies of ovarian stages in the sea star Patiriella regularis over the annual reproductive cycle. The number of samples for each month is indicated above each histogram. Stage I: recovery stage; Stage II: growing stage; Stage III: mature stage; Stage IV: partly spawned stage; and Stage V: spent stage.

Crump (1971) reported that in *P. regularis*, spawning occurs in mid-summer, between December and February (Southern hemisphere) based on gonadal index. The results from the histological analysis in the present study are in agreement with these previous observations, and showed that spawning occurred in summer, from January to April. However, Crump (1971) noticed a gradual decrease in gonadal index from January to March, which was explained by the presence of ripe and spent animals in the population, and by the assumption that individual sea stars undergo one spawning event. In contrast, the histology of the ovary during the spawning period in the present study revealed the presence of partly-spawned ovaries, including loosely-packed fully-developed oocytes with spaces vacated through spawning. Furthermore, during animal dissection in the present study, shrunken "spent" parts and swollen "mature" parts of ovaries were found in individual sea stars from January to March, with ovary index values being lower than those of the mature ovary and higher than those of the spent ovary. These observations suggest that *P. regularis* is a batch spawner which may explain the gradual decrease in the ovary index during the spawning period. Batch spawning is reported in other asterinids as in *M. calcar* (Byrne, 1992). *P. regularis* spawns over an extended period that lasts for approximately three months (Crump, 1971; present study). Similarly, *M. gunnii* and *M. calcar* spawn for three to four months. Unlike other asterinid species, *C. pentagona* has a restricted spawning season in October (Chen and Chen, 1992).

The reproductive stages described in this study were very similar to those found in other asterinid species studied by Byrne (1992). However, some variation in oocyte size at the onset of vitellogenesis was found between the *P. regularis* and asterinid species studied by Byrne (1992). The histological analysis in the present study revealed that previtellogenic oocytes in *P. regularis* were small, with a diameter of less than 40 µm, and this was evident at higher resolution from electron dense granules in the ooplasm and from PAS+ oocytes. In contrast, the diameter of previtellogenic oocytes in *M. gunnii*, *M. calcar* and *Parvulastra exigua*

ranged from 50 to 100 µm, and oocytes were PAS- and basophilic (Byrne, 1992). The onset of vitellogenesis in these asterinid species occurred when the oocyte diameter was around 100 µm, reflected in the presence of PAS+ oocytes (Byrne, 1992). This comparison shows that vitellogenesis in young oocytes in *P. regularis* started at a smaller size than in *M. gunnii*, *M. calcar* and *Parvulastra exigua*. This may be related to the possibility that in *P. regularis*, oocyte development takes one year (Crump, 1971; Byrne and Barker, 1991; present study), while in *M. gunnii*, *M. calcar* and *Parvulastra exigua*, oocyte development takes two years (Byrne and Barker, 1991; Byrne, 1992).

The ovary wall of *P. regularis* in the present study had a structure similar to previously described asteroids (Walker, 1974; Schoenmakers et al., 1981; Byrne, 1992). In the ovary wall, the haemal system (HS) was observed to have connective tissue characteristics. It consisted of collagen fibers and contained coelomocytes (also called amoeboid cells) (Schoenmakers et al., 1981; Chia and Koss, 1994). In the sea star *Asterias rubens*, the HS proliferates during oocyte growth and vitellogenesis and forms invaginations to which the growing oocytes are attached (Schoenmakers et al., 1981). From late vitellogenesis to spawning, the HS regresses. The same observation was found in the *P. regularis* ovary in the present study, which suggests that HS in asteroids is important for oogenesis, especially during the early stages. Furthermore, vitellogenin was found to be produced in the pyloric caeca and ovaries of *P. regularis* (Alqaisi *et al.*, 2016), and this yolk proteins and other substances are transferred from the pyloric caeca into the ovary through the HS (Beijnink et al., 1984a; Beijnink and Voogt, 1986; Alqaisi *et al.*, 2016). In general, the HS is known to distribute nutrients to different organs in asteroids (Broertjes et al., 1980; Ferguson, 1984; Eckelbargera and Hodgson, 2021).

The asteroid ovaries contain many somatic cells, including follicle cells, phagocytosing cells, and nurse cells, and their abundance depends on the stage of the reproductive cycle (Schoenmakers et al., 1981). In echinoderms, many names have been used to describe somatic cells in the ovary. For example, in the asteroid *Patiria* (*Asterina pectinifera*, the phagocytes were referred to as nurse cells (Aisenshtadt and Vassetzky, 1986), while in echinoids, the somatic cells are known as nutritive phagocytes (Holland and Giese, 1965). Their morphology changes during oogenesis, and functions include the provision of nutrients for germ cells and phagocytosis (Walker et al., 2005; Walker et al., 2007). In the present study, the ovary of *P. regularis* was found to include many somatic cells with varying shapes. Apart from follicle cells that surround oocytes and from the phagocytes that were found scattered within cell debris, it was hard to identify the other somatic cells in the ovary as their function was unclear. Therefore, these cells are referred to as somatic cells in this study. Interestingly, it was noticed in the present study that many cells seemed to originate from the HS and were distributed between the growing oocytes. The fate of those somatic cells and their function are unknown. However, little is known about the somatic cells in the asteroid ovary, and further studies are needed to identify their function. In asteroids, the ultrastructural analysis of oocytes showed an abundance of cortical granules in the mature oocytes that were located close to the plasma membrane (Schoenmakers et al., 1981; Reimer and Crawford, 1995). In addition, yolk granules increased as the oocyte grew, and Golgi complexes increased in abundance during vitellogenesis, indicating a high activity in protein synthesis and processing (Schoenmakers et al., 1981). Similar observations were found in a previous study on *P. regularis* which confirms the abundant expression of vitellogenin genes in ovary and the minor expression of a transferrin-like yolk component termed major yolk protein (MYP) (Alqaisi *et al.*, 2016). The ultrastructural criteria of oocyte atresia in the sea star *P. ochraceus* were studied by Reunov and Crawford (2010). That study showed that oocyte destruction may occur through complex mechanisms that include

elements of necrosis, autophagic cell death, and apoptosis. These atresia patterns were not seen in the present study. This might be because few atretic oocytes were observed.

Oocytes at different stages of development were evident in the *P. regularis* ovary (present study). However, the majority of oocytes grow synchronously at each stage of the ovarian development. The oocyte size-frequency distribution of *P. regularis* during the annual reproductive cycle was studied bimonthly by Crump (1971) and monthly in the present study. The results from both studies are similar and showed general unimodal distribution. Thus, it is concluded that only one oocyte cohort is produced during each reproductive cycle. A similar pattern was found in the sea star *Asterina stellifera* (Carvalho and Ventura, 2002). In comparison, the oocyte size-frequency distributions in *M. gunnii*, *M. calcar*, *Parvulastra exigua* and *C. pentagona* are bimodal which results from the prolonged period of oogenesis that lasts two years (Byrne, 1992; Chen and Chen, 1992).

Pyloric caeca function in digestion and in the storage of nutrients (Lawrence, 1973; Ruppert et al., 2004), in addition to vitellogenin production in *P. regularis* (Alqaisi *et al.*, 2016). The inverse relationship between the gonad index and the pyloric caeca index is commonly observed in asteroids and indicates the transfer of nutrients to the ovary during gametogenesis (Crump, 1971; Barker and Xu, 1991; Byrne, 1992; Chen and Chen, 1992; Carvalho and Ventura, 2002; Georgiades *et al.*, 2006). In the present study, there was no obvious inverse relationship between the pyloric caeca index and the ovary index in *P. regularis* throughout the reproductive cycle. However, the maximum ovary index which was in December coincided with the minimum pyloric caeca index. Crump (1971) reported an approximate inverse relationship between the pyloric caeca index and the ovary index in this same sea star species. Interestingly, while the minimum value for the pyloric caeca index was in December, in Crump (1971) the minimum pyloric caeca value was in March. This difference could reflect differences in food availability and the dependence of ovaries on pyloric caeca food reserves. In this respect, it was suggested that food allocation to different body organs in asteroids is affected by food availability (Harrold and Pearse, 1980), with the ovary being less dependent on stored food reserves in the pyloric caeca when food is readily available (Harrold and Pearse, 1980; Xu and Barker, 1990a).Spawning in asteroids is suggested to be regulated by environmental factors such as photoperiod and temperature (Mercier and Hamel, 2009). In many asteroids, spawning occurs during spring and/or summer when sea temperatures increase seasonally. For example, spawning in *M. gunnii* and *M. calcar* in New South Wales was reported in spring and early summer (August – December) (Byrne, 1992). *P. regularis* in the present study was found to spawn in late December, during the longest period of day length and at maximum sea temperatures. This would be consistent with previous findings in sea stars of photoperiod and sea temperature as cues for maturation and spawning (Pearse and Eernisse, 1982).

In conclusion, the present study has examined the female reproductive cycle of *P. regularis* in terms of ovary histology and organ indices and described five reproductive stages. *P. regularis* has an annual reproductive cycle as previously reported. Oogenesis occurs mainly in the autumn and winter seasons. Ready-to-spawn oocytes are found in late spring season, but spawning did not occur until mid-summer which may suggest a link between temperature, photoperiod, and the onset of spawning. The majority of oocytes develop synchronously, and batch spawning was evident in *P. regularis*. The results from the present study provide a histology index for the ovary of *P. regularis* ovary during the reproductive cycle.

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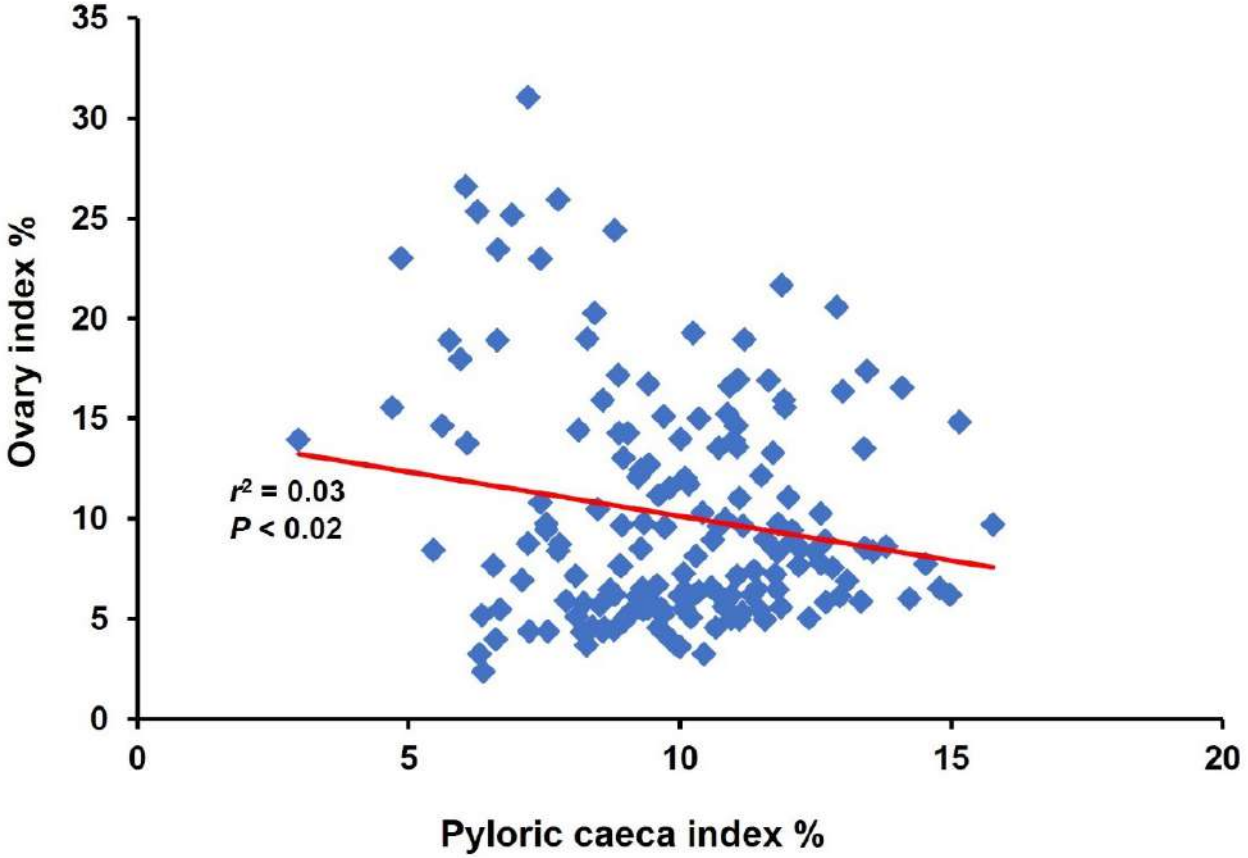
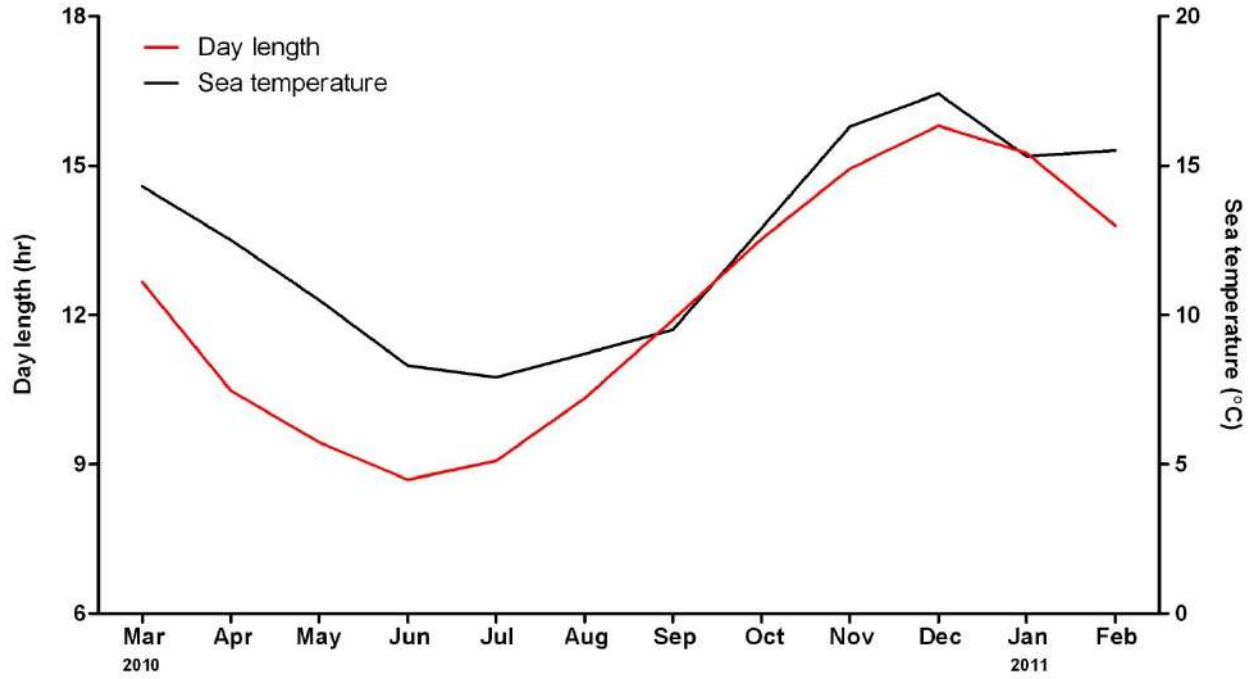
Supplementary Figures



Supplementary Figure 1. Relationship between the pyloric caeca index and ovary index in sea star Patiriella regularis throughout the reproductive cycle. Regression line equation is y = 14.6 – 0.44 X; r2= 0.03; P < 0.02; n= 169.



Supplementary Figure 2. Annual cycle of day length and sea temperature in Otago Harbour, New Zealand (45° 48' S; 170° 38' E) from March 2010 to February 2011.

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Checklist of Trees and Shrubs and their Uses from the Dairy Farm Pasture at the Piedmont of the Barva Volcano, Costa Rica

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Abstract

On the slopes of Barva Volcano in the Central Volcanic Mountain Range of Costa Rica, numerous farms are primarily dedicated to dairy cattle farming. This area belongs to the Lower Montane Tropical Forest life zone, a biome typically found at elevations ranging from approximately 1000 to 2000 meters above sea level. Among the farms located on the slopes of the Barva volcano, where striking remnants of the flora that once populated these areas can still be found, lies La Concordia. This farm measures approximately 400 hectares and varies in altitude between 1800 and 2000 meters above sea level. This farm was selected as study site to conduct a comprehensive survey of all trees and shrubs in the pastures and their uses by the local people. Roads and trails of the farm were walked opportunistically, recording all the trees and shrubs observed in the pastures. Ten one-day surveys were conducted in 1981, and ten more were performed between 1990 and 2000, to include sites not checked during the first ten surveys. After that, then five more were performed between 2001 and 2018, and two in 2023. On at least ten occasions, a local resident took part in these explorations to provide both the local name of the species and their most common uses. At least 141 taxa were identified, but some genera included more than one species totaling about 150 species. 105 taxa were determined at the species level, although nine of those were identified only in terms of genus. All species found belong to ninety-three genera in sixty families. The main uses identified for these species include bearing edible fruits, being valuable for wildlife, charcoal production, providing shade for cattle, being useful as construction materials, and living fences and as a source for firewood, in addition to their ecological benefits. Some species are used for special purposes such as making tool handles, ox yokes, and for medicinal purposes. Trees and shrubs, even as elements outside the forest, continue to be of great importance to the inhabitants of a particular area. It is a priority to care for and sustainably exploit this valuable resource of trees outside forests to continue to benefit from their riches and ecological services.

Key words: Alajuela, *Hamelia patens,* Heredia, La Concordia, vegetation, wildlife

Introduction

On the slopes of Barva Volcano in the Central Volcanic Mountain Range of Costa Rica, numerous farms are primarily dedicated to dairy cattle farming (Boza, 1968; Cascante, 2018). The area, characterized by a cold and rainy climate, is often classified as part of the highlands or cold lands. Authors including Standley (1937) have set the lower limit of these lands at an elevation of 1500 meters. Wercklé (1909) also categorized them as within the cold region or cold lands, although Pittier (1957) argued that the cold lands are those above 2600 meters (which he referred to as alpine). Fournier (1965) recommended discontinuing the use of the term 'alpine' since it is of European origin and is used only due to similarity in climate and vegetation with the Alps. The region above 1500 meters was considered by Wercklé (1909) to have the richest and most interesting flora of all.
This area belongs to the Lower Montane

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Tropical Forest life zone, a biome typically found at elevations ranging from approximately 1000 to 2000 meters above sea level. The climate exhibits moderate temperatures, and there may be a distinct dry season, with variations based on specific geographic locations (Hartshorn, 1983). These forests are renowned for their high biodiversity, hosting a wide variety of plant and animal species. The diverse topography and climate at different elevations contribute to this richness. The vegetation is characterized by a mix of both tropical and temperate species, allowing for a transition from tropical rainforest species to those adapted to cooler conditions. Evergreen broadleaf trees are prevalent, and the forest structure varies from a closed canopy to a more open one, influenced by factors such as elevation and disturbance (Hartshorn, 1983).

Among the farms located on the slopes of the Barva volcano, where striking remnants of the flora that once populated these areas can still be found, lies La Concordia. This farm measures approximately 400 hectares and varies in altitude between 1800 and 2000 meters above sea level. This farm was selected as study site for a comprehensive survey of all trees and shrubs in the pastures. Forest remnants, such as riverine forests, were not surveyed. Trees and shrubs remaining in the pastures may have been common species in the original forests of the area before the region was deforested. Additionally, the uses of these species by the local people were recorded.

Plants are of vital importance for life as they produce the oxygen we breathe and the nutrients we consume (Flagler and Poincelot, 2018). Additionally, they fulfill basic needs such as food, ornamentation, fuel production, insulation, medicine, personal grooming, construction, and dye production, among others (Levy and Aguirre, 1999; Castañeda and Castillo, 2016; Jorquera and Brenes, 2019). The trees in the pastures of La Concordia, whether they are only remnants of the original vegetation or have been planted, continue to provide significant benefits to the people. It is important to understand their uses to help preserve culture and traditions.

Materials and Methods

Study site

The work was carried out at La Concordia, located on the border of the provinces of Alajuela and Heredia, Costa Rica (10.122 N, 84.155 W); its main access is through Cinco Esquinas de Carrizal, province of Alajuela (Figure 1). At an elevation of approximately 1900 m (Cascante, 2018), the farm receives an average annual precipitation of 2906.5 mm, an average relative humidity of 71.7% with minimum and maximum temperatures of 7 °C and 31.5 °C respectively (Cascante, 2018). The farm pasture consists of Kikuyu grass, *Kikuyuocloa clandestine* (Figures 1), which was introduced from Africa to Costa Rica between 1911 and 1922 (Peters, 2008; Cascante, 2018). La Concordia is situated on the piedmont of the Barva Volcano (Figure 2) at the Central Mountain Range of Costa Rica

Trees and shrubs survey

Information was collected over several years by exploring various areas on the farm. Roads and trails of the farm were walked opportunistically, recording all trees and shrubs observed in the pastures. Ten one-day surveys were conducted in 1981, each involving walking from 08:00 to 16:00. Between 1990 and 2000, ten additional sites were surveyed that were not checked during the initial surveys. The farm was visited five more times between 2001 and 2018, and twice in 2023. On at least ten occasions, a local resident took part in these explorations. The elderly participant was able to provide both the local names of the species and their most common use or uses. The observations were recorded along with the species when it was possible to identify the species on-site. For species that could not be identified in the field, a sample was taken for a later identification in the herbarium. However, it was not possible to identify some of the species, nor was it possible to obtain a botanical sample.

Results are presented in separate tables based on the main use of each species. Each species is included only once when possible.

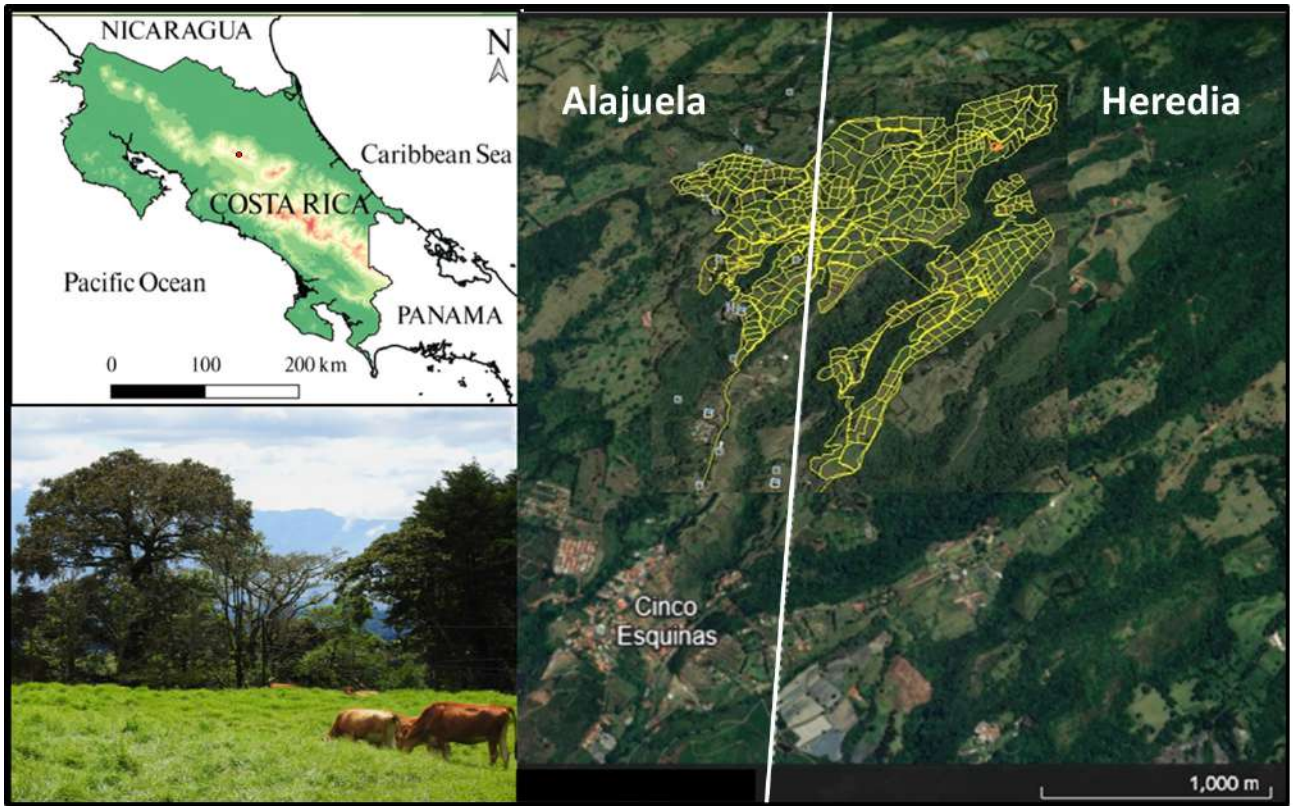

Figure 1. Location of La Concordia dairy farm (red dot) in Costa Rica. The farm pastures (yellow lines) are located at the Barva volcano piedmont on the provinces of Alajuela and Heredia. The pastures are of Kikuyu grass, *Kikuyuocloa clandestina* (lower left).

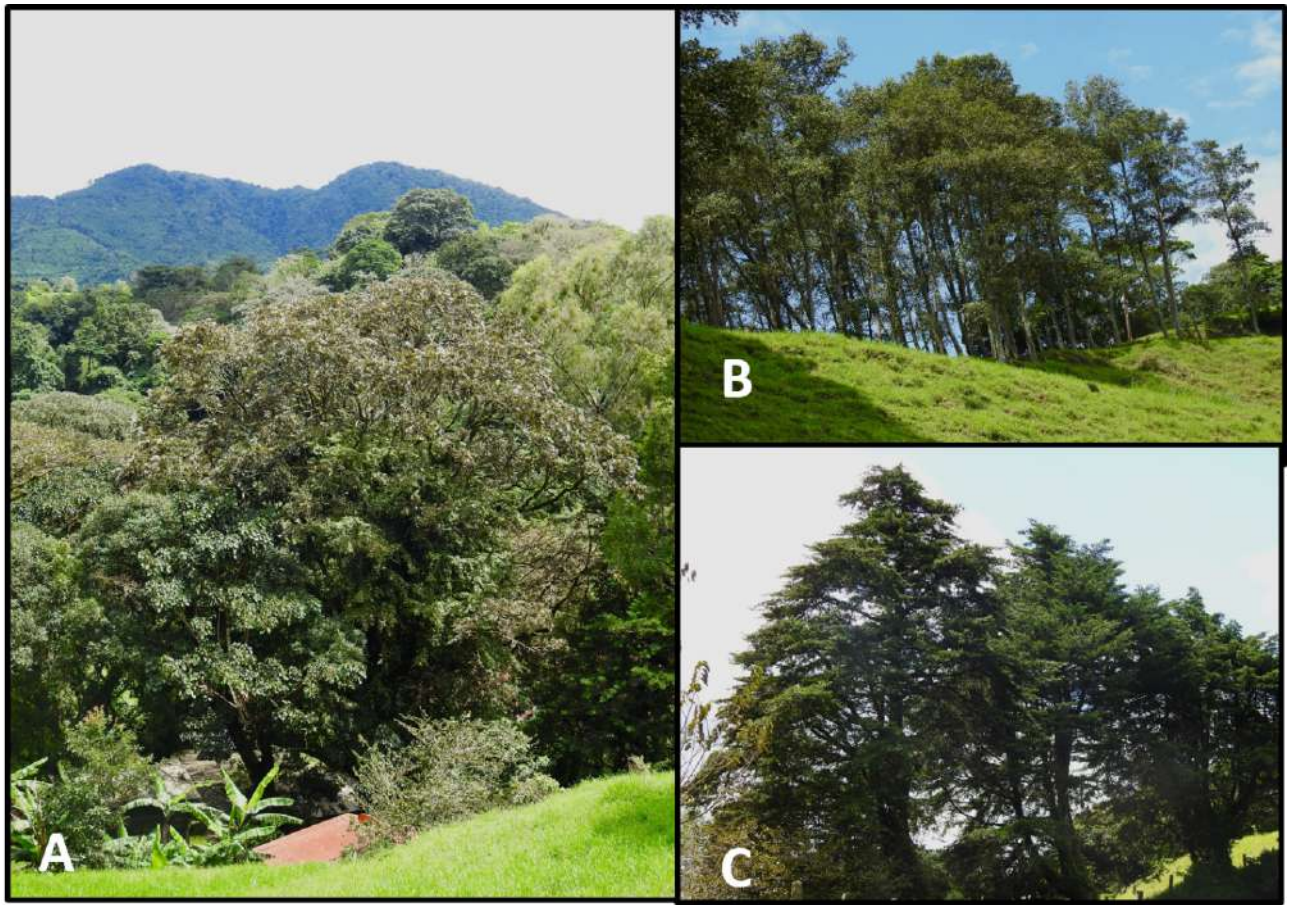

Figure 2. A: View of Barva volcano (background) from one of the westernmost and lowest point of La Concordia dairy farm. B: A stand of jaúl, *Alnus acuminata*, at a nearby farm. C: Ciprés, *Hesperocyparis iusitanica*, at a fence at another dairy farm in the La Concordia area. Costa Rica. Photos by José M. Mora.

However, for some species with multiple uses, they are included in at least two tables. Even if some species have edible fruits, wood, or are valued for firewood or posts, they are listed in the tables only under their main use or their two main uses if included in more than one table.

Results

At least 141 taxa were identified, but some genera included more than one species totaling about 150 species. 105 taxa were determined at the species level, although nine of those were identified only in terms of genus. All species found belong to ninety-three genera in sixty families.

Several of the species found in the pastures of La Concordia are cultivated species. Some of these trees were planted because they bear edible fruits, including peaches (*Prunus persica*), avocado (*Persea americana*), and cherimoya (*Annona cherimola*) (Table 1).

Several of these fruits are also utilized by wildlife, mainly birds (Figure 3). Simultaneously, various other wild species bear fruits highly sought after by wildlife, particularly birds (Table 2).

The production of charcoal has been diminishing in importance in the area and in Costa Rica in general. Although charcoal production was still occurring when this project began, today this practice is almost non-existent in the zone where La Concordia farm is located. Nevertheless, several species, with oaks (genus *Quercus*) being prominent, have been used for charcoal production (Table 3).

Although these species were widely used to produce high-quality charcoal, they still persist in La Concordia's pastures today and are left to provide shade for cattle. There are also other trees with this function that simultaneously serve other additional purposes (Table 4). Some of the species listed in Table 4 are introduced into this region and are mainly used in fence making.

Certainly, one of the most traditional benefits of trees in the region where La Concordia is located is their use in construction. In this regard, there are several tree species on the farm and generally in the area that can be rationally exploited for their timber (Table 5). At the same time, most of these trees have other benefits including their edible fruits, being useful in crafts and making posts in addition to being utilized by wildlife.

Other shrubs and trees in La Concordia and the surrounding areas have been used in particular ways, and that is why they have been classified as special. These uses are important and are still valid, although others

Table 1. Species of shrubs and trees and their local common names at La Concordia, classified as having edible value. Costa Rica.

Scientific name	Family	Local Name
Annona cherimola Mill.	Annonaceae	Anona
Casimiroa tetrameria Millsp.	Rutaceae	Matasano
Satyria spp.	Ericaceae	Muelas
Citrus spp.*	Rutaceae	Several
Eriobotrya japonica (Thunb.) Lindl.*	Rosaceae	Níspero
Panopsis suaveolens (Klotzsch) Pittier	Proteaceae	Papa de Palo
Persea americana Mill.	Lauraceae	Aguacate
Persea schiedeana Nees	Lauraceae	Yas
Prunus persica (L.) Stokes*	Rosaceae	Durazno
Psidium guajava L.	Myrtaceae	Guayaba
Psidium guineense Sw.	Myrtaceae	Güisaro
Saurauia montana Seem	Actinidiaceae	Mocos
Yucca gigantea Lem.	Asparagaceae	Itabo

* Introduced

Figure 3. Fruits of some tree species taken by birds and other wildlife at La Concordia, Costa Rica. A: *Bocconia frutescens*, B: *Oreopanax xalapensis,* C: *Trema micranthum*, D: *Citharexylum donnell-smithii*, E: *Ficus* sp. 1, F: *Ficus* sp. 2 photos by José M. Mora.

are much less common than in the past. However, they are significant because they demonstrate a special characteristic of each species or its use that makes it unique and beneficial for a particular function (Table 6). The targuá, *Croton draco*, (Figure 3) is used in human medicine and to make ox yokes among other uses. The burío, *Heliocarpus appendiculatus*, has a great ecological value, and its bark is also used to wash sugar mills and basketry (Figure 4).

Although few households currently cook using firewood, this has been a very traditional use of several species in the past. However, some of these species continue to be highly important for this purpose (Table 7). A traditional practice that is still in use is to prune the trees in the pastures of the farms to use the branches for firewood. At the same time, most of these trees are useful for making posts, for crafts, and also for their edible fruits.

Some trees and shrubs from La Concordia might be useful for firewood or other purposes, but their primary value is ecological. This is because these trees and shrubs have been left as windbreaks, especially for protection along the edges of streams (Table 8).

Similar to the previous ones (Table 8), several species of trees and shrubs from La Concordia are not highly valued because they do not have a particular use for people. However, many of these species are crucial in the ecological process as they are pioneer species in areas affected by landslides and other natural or anthropogenic events, such as the case of the jaúl, *Alnus acuminata* (Table 9), which is also a species of importance used in silvopastoral practices.

Many other species are used for posts, requiring only that they are hard to ensure

Table 2. Species of shrubs and trees and their local common names at La Concordia, classified as having high value for wildlife. Costa Rica.

Scientific name	Family	Local Name
Ardisia revoluta Kunth	Myrsinaceae	Tucuico
Blakea grandiflora Hemsl*	Melostomaceae	San Miguel
Bocconia frutescens L.	Papaveraceae	Guacamaya
Casearia arguta Kunth.	Salicaceae	Manga larga
Chiococca pachyphylla Wernham	Rubiaceae	Comenegro
Citharexylum caudatum Walp.	Verbenaceae	Dama
Citharexylum donnell-smithii Greenm	Verbenaceae	Flor de dama
Dendrophthora costaricenses Urb.	Viscaceae	Matapalo
Drymis granadensis L.f.	Winteraceae	Chile muelo
Ficus spp.	Moraceae	Higo, higuerón, matapalo, chilamate
Frangula oreodendron (L.O.Williams) A.Pool	Rhamnaceae	Duraznillo
Iochroma arborescens J.M.H.Shaw	Solanaceae	Güitite
Mauria heterophylla Kunth.	Anacardiaceae	Manguillo, cirrí
Miconia oerstediana (O.Berg ex Triana) Michelang.	Melastomataceae	María
Miconia spp.	Melostomataceae	Lengua de vaca
Myrcia splendens DC.	Myrtaceae	Murta
Ocotea spp.	Lauraceae	Aguacatillo
Quercus lancifolia Schltdl. & Cham.	Fagaceae	Roble
Quercus laurina Bonpl.	Fagaceae	Encino
Struthanthus cansjerifolius (Oliv.) Eichler	Loranthaceae	Matapalo
Turpinia sp.	Staphyloceae	No one
Xylosma sp.	Salicaceae	Espino

* Vulnerable (Rodríguez *et al*., 2021)

durability. However, several species root and become trees, forming living fences. Thus, some species like the cedro, *Cedrela odorata*, and poró trees, *Erythrina* spp. (Figure 5), among others (Table 10), are highly valued. Still, other species are important for fences, even as dead posts (Table 10).

Discussion

Forests have provided humanity with multiple goods and ecological services, such as wood, other forest products, and clean air, among other economic and cultural benefits (Jorquera and Brenes, 2019). Forests are essential for climate balance, water reserves, and soil conservation (Ma *et al.,* 2021).

Once the forests are exploited, these lands are converted into farms, some dedicated, as in this case, to dairy production (Boza, 1968). Many trees have disappeared more slowly, but depending on their use, they have been maintained and possibly will be for a long time. Among these, are trees that have been introduced into this area as ornamentals or for their edible fruits, as well as others that grow naturally and are appreciated and maintained for their fruits (Table 1). Some of these species were found in places where houses existed, surely planted by their inhabitants. The loquat or níspero (*Eriobotrya japonica*) and citrus trees (*Citrus* spp.) stand out as a good example, among many others (Table 1). Other trees also bear fruits used by wildlife,

Table 3. Species of shrubs and trees and their local common names at La Concordia, for charcoal production. Costa Rica.

Scientific name	Family	Local Name	Other uses
Chiococca pachyphylla Wernham	Rubiaceae	Comenegro	Posts, fauna
Cleyera theoides (Sw.) Choisy	Pentaphylacaceae	Sierrilla	Fauna Medicine
Mimosa platycarpa Benth.	Mimosaceae	Carboncillo	Firewood
Myrsine coriacea (Sw.)R.Br. ex Roem. &Schult.	Primulaceae	Ratoncillo	Fauna
Oreopanax capitatus (Jacq.) Decne. & Planch.	Araliaceae	Higuerilla	Firewood, posts
Oreopanax xalapensis (Kunth) Decne.&Planch.	Araliaceae	Cacho de venado	Fauna
Quercus lancifolia Schltdl. & Cham	Fagaceae	Roble	Timber, fauna
Quercus laurina Bonpl.	Fagaceae	Encino	Timber, fauna
Roupala montana Aubl.	Proteaceae	Danto	Timber
Sciodaphyllum pittieri (Marchal ex T.Durand & Pittier) Lowry,G.M.Plunkett & M.M.Mora	Araliaceae	Higuerilla	Firewood, posts
Styrax argenteus C. Presl	Styracaceae	Quicirrí	Firewood
Tapirira mexicana Marchand	Anacardiaceae	Dantisco	Firewood
Ulmus mexicana (Liebm.) Planch.	Ulmaceae	Tirrá	Bases and forks

Table 4. Species of shrubs and trees from La Concordia that provide shade for livestock or are used for ornamental purposes, fences, or windbreaks, Costa Rica.

Scientific name	Family	Local Name
Alnus acuminata Kunth	Betulaceae	Jaúl
Cojoba costaricensis Britton & Rose	Fabaceae	Lorito
Erythrina spp.	Fabaceae	Poró
Hesperocyparis lusitanica (Mill.) Bartel.*	Cupressaceae	Ciprés
Hibiscus rosa-sinensis L.	Malvaceae	Amapolón
Inga spp.	Fabaceae	Guajiniquil
Malvaviscus arboreus Dill. ex Cav.	Malvaceae	Amapola de río
Salix humboldtiana Willd.*	Salicaceae	Sauce
Spathodea campanulata P.Beauv.*	Bignoniaceae	Llama del bosque
Syzygium jambos (L.) Alston*	Myrtaceae	Manzana rosa

* Introduced

Table 5. Species of shrubs and trees in La Concordia that were heavily exploited for construction and still have representatives in the pastures where they have other important uses, Costa Rica.

Scientific name	Family	Local Name
Cedrela odorata L.	Meliaceae	Cedro
Citharexylum caudatum Walp.	Verbenaceae	Flor de dama
Cornus disciflora Moc. & Sessé ex DC.	Cornaceae	Llorón
Guarea guidonia (L.) Sleumer	Meliaceae	Cedro macho
Hesperocyparis lusitanica (Mill.) Bartel.*	Cupressaceae	Ciprés
Lippia myriocephala Schltdl. & Cham.	Verbenaceae	Caragra
Myrcianthes storkii (Standl.) McVaugh	Myrtaceae	Murta
Ocotea sp.	Lauraceae	Quizarrá colpachí
Persea schiedeana Nees	Lauraceae	Yas
Phoebe sp.	Lauraceae	Quizarrá
Phoebe spp.	Lauraceae	Aguacatillos
Quercus lancifolia Schltdl. & Cham	Fagaceae	Roble
Quercus laurina Bonpl.	Fagaceae	Encino
Sloanea ampla I.M.Johnst.	Eleocarpaceae	Peine´mico

* Introduced

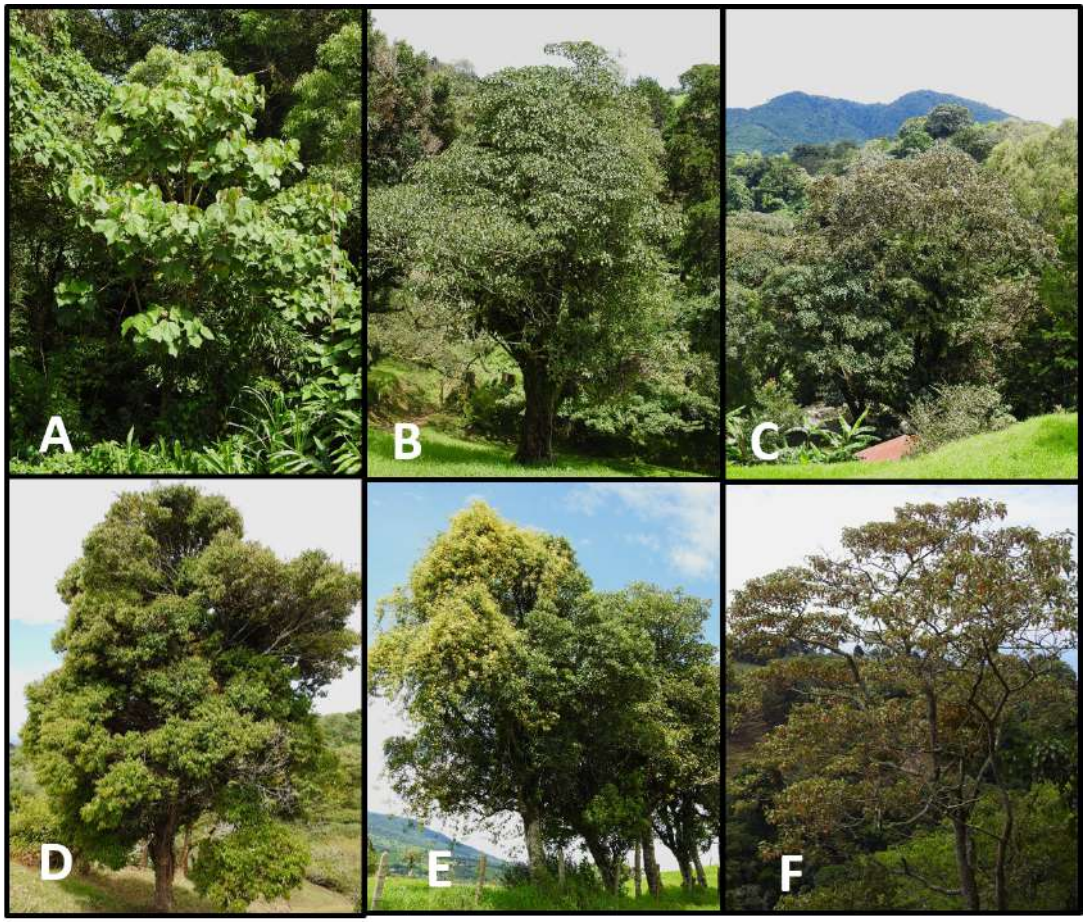

Figure 4. Some selected trees of La Concordia. A: Burío, *Heliocarpus appendiculatus*. B: Higuerilla, *Sciodaphyllum pittieri*. C: Cedro, *Cedrela odorata*. D: Murta, *Myrcianthes storkii.* E: Chile muelo, *Drymis granadensis* (the yellow flowers are from a climbing vine). F: Targuá, *Croton draco*. Photos by José M. Mora.

Table 6. Species of shrubs and trees in the pastures of La Concordia along with their particular use(s), Costa Rica.

Scientific name	Family	Local Name	Uses
Brugmansia arborea (L.) Sweet*	Solanaceae	Floripón	Medicine
Calyptranthes pallens Griseb.	Myrtaceae	Cacique	Handles for hammers, picks, and axes.
Cestrum spp.	Solanaceae	Zorrillo	Medicine
Clusia sp.	Clusiaceae	Copey	Medicine
Croton draco Schltdl.	Euphorbiaceae	Targuá	Ox yokes
Drymis granadensis L.f.	Winteraceae	Chile muelo	Medicine
Ehretia latifolia Loisel	Boraginaceae	Raspaguacal	Washing dishes
Eugenia costaricensis O.Berg	Myrtaceae	Murta	Ox goads, cattle prods
Hedyosmum mexicanum Cordem. ex Baill.	Acanthaceae	Vara de agua	Road protection (corduroy)
Heliocarpus appendiculatus Turcz.	Tiliaceae	Burío	Washing sugar mills, basketry
Ocotea sinuata (Mez) Rohwer	Lauraceae	Quizarrá caca	Yokes
Roupala montana Aubl.	Proteaceae	Danto	Axe handles
Sambucus nigra L.*	Adoxaceae	Sauco	Medicine
Sapium glandulosum (L.) Morong	Euphorbiaceae	Yos	Matches, boxes, rubber
Sciodaphyllum pittieri (Marchal ex T.Durand & Pittier) Lowry,G.M.Plunkett & M.M.Mora	Araliaceae	Higuerilla	Benches
Sloanea ampla I.M.Johnst.	Eleocorpaceae	Peine´mico	Wash tables
Trema micranthum (L.) Blume	Ulmaceae	Capulín	Yokes
Ulmus mexicana (Liebm.) Planch.	Ulmaceae	Tirrá	Bases
Verbesina turbacensis Kunth	Asteraceae	Tora	Bird cages

* Introduced

Table 7. Species of shrubs and trees from La Concordia highly sought after for producing high-quality firewood, Costa Rica.

Scientific name	Family	Local Name	Other key uses
Casearia sylvestris Sw.	Salicaceae	Poipute	Posts
Citrus spp.*	Rutaceae	Limon and others	Edible fruits
Cornus disciflora Moc. & Sessé ex DC.	Cornaceae	Llorón	Timber
Diphysa americana (Mill.) M.Sousa	Fabaceae	Guachepelín	Timber
Eriobotrya japonica (Thunb.) Lindl.*	Rosaceae	Níspero	Edible fruits
Eugenia costaricensis O.Berg	Myrtaceae	Murta	Arches, ox goads, cattle goads
Freziera candicans Tul.	Theaceae	Sierrilla	Fauna
Hesperocyparis lusitanica (Mill.) Bartel.*	Cupressaceae	Ciprés	Timber
Inga spp.	Mimosaceae	Guajiniquil-Guaba-Juaniquil	Edible fruits
Myrcianthes storkii (Standl.) McVaugh	Myrtaceae	Murta	Posts
Psidium guajava L.	Myrtaceae	Guayaba	Edible fruits
Sideroxylon sp.	Sapotaceae	Poipute	Posts

* Introduced

Table 8. Species of shrubs and trees as important components of natural windbreaks and protective forest strips along streams and rivers in La Concordia, Heredia, Costa Rica.

Scientific name	Family	Local Name
Bocconia frutescens L.	Papaveraceae	Guacamaya
Clethra mexicana DC.	Clethraceae	Nance macho
Fuchsia arborescens Sims	Onagraceae	Fucsia
Guatteria sp.	Annonaceae	Anonillo
Heliocarpus appendiculatus Turcz.	Tiliaceae	Burío
Mollinedia viridiflora Tul.	Monimiaceae	Limoncillo
Morus insignis Bureau	Moraceae	Recino
Palicourea sp.	Rubiaceae	Cafecillo
Phenax angustifolius (Kunth) Wedd.	Urticaceae	Yuquilla
Piper spp.	Piperaceae	Anicillo, cordoncillo
Siparuna gesnerioides (Kunth) A.DC.	Siparunaceae	Limoncillo
Urera spp.	Urticaceae	Ortiga

Table 9. Species of shrubs and trees from La Concordia classified as pioneer species, Costa Rica.

Scientific name	Family	Local Name
Alnus acuminata Kunth	Betulaceae	Jaúl
Cestrum spp.	Solanaceae	Zorrillo
Clethra mexicana DC.	Clethraceae	Nance macho
Croton micans Sw.	Euphorbiaceae	Targuacillo
Myrcia splendes DC	Myricaceae	Roblecillo de montaña
Ricinus communis L.*	Euphorbiaceae	Higuerilla
Vernonanthura patens (Kunth) H.Rob.	Compositae	Tuete
Wigandia urens (Ruiz & Pavon) Kunth	Hydrophylaceae	Ortigo

* Introduecd

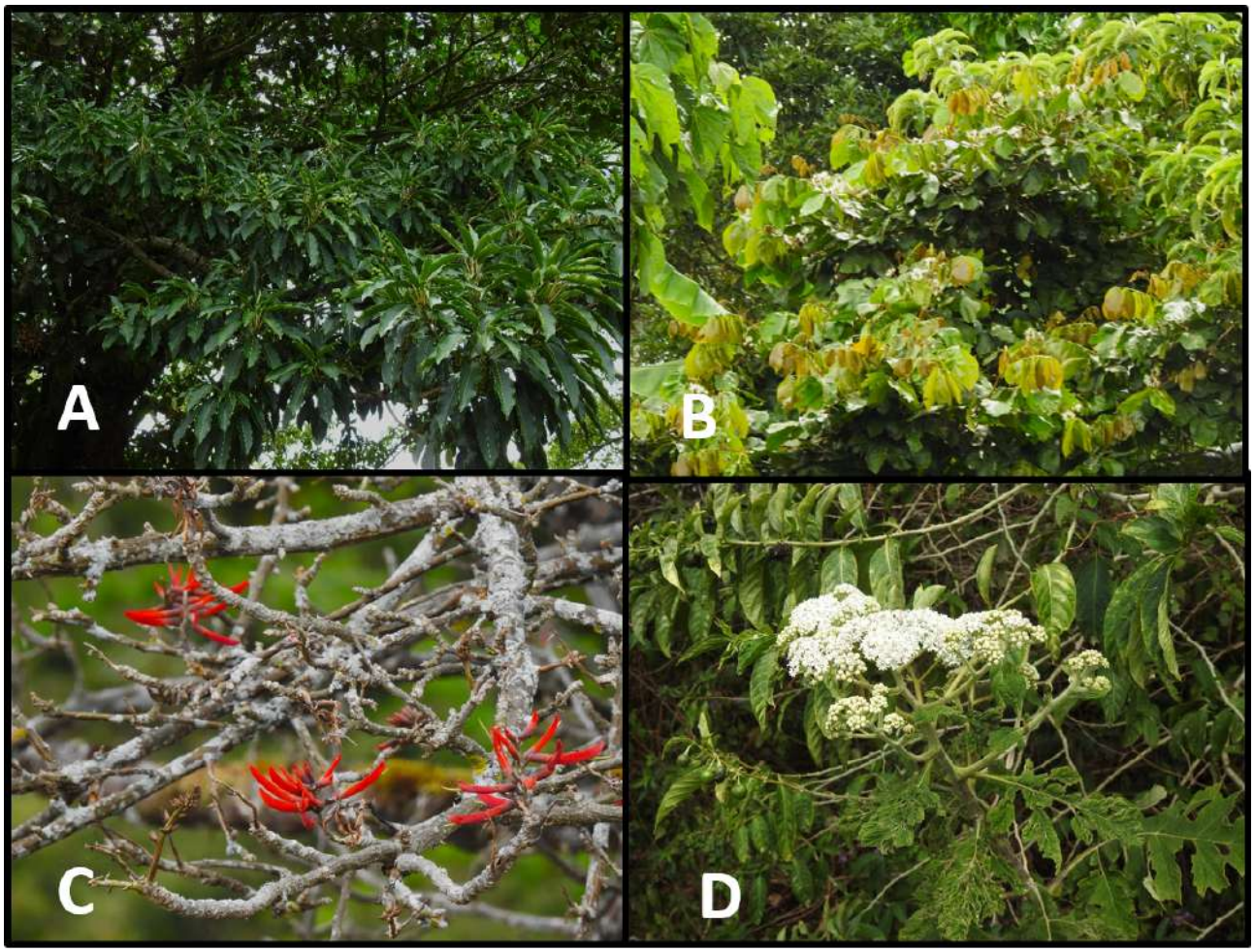

Figure 5. A: Among its other uses, Yos, *Sapium glandulosum*, is sometimes used for making fence posts. B: Guajiniquil, *Inga* sp., has edible fruits and is highly appreciated as a source for firewood. C: Poró, *Erythrina* spp., provides the most favorite posts for living fences, and their knife-like red flowers are eaten by people. D: Tora, *Verbesina turbacensis*, was used to construct bird cages. Photos by José M. Mora.

Table 10. Species of trees from La Concordia used mostly for making posts in living fences (L) and dead fences (D), Costa Rica.

Scientific name	Family	Local Name
Aiouea brenesii (Standl.) R.Rohde	Lauraceae	Ira
Alfaroa costaricensis Standl.	Juglandaceae	Cedro blanco (D)
Billia rosea (Planch. & Linden) C.Ulloa & P.M.Jørg.	Sapindaceae	Cucaracho (D)
Cedrela odorata L.	Meliaceae	Cedro (L)
Miconia oerstediana (O.Berg ex Triana) Michelang.	Melastomataceae	Plomillo (L)
Croton glabellus L.	Euphorbiaceae	Copalchi (L)
Diphysa americana (Mill.) M.Sousa	Fabaceae	Guachepelín (L)
Drymis granadensis L.f.	Winteraceae	Chile-Muelo (L)
Erythrina spp.	Fabaceae	Poró (L)
Ficus spp.	Moraceae	Higuerón (L)
Tecoma stans (L.) Juss. ex Kunth	Bignoniaceae	Vainillo (L)
Terminalia sp.	Combretaceae	Sura
Trichilia havanensis Jacq.	Meliaceae	Uruca (D)
Viburnum costaricanum (Oerst.) Hemsl.	Adoxaceae	Sauco de montaña (D)
Yucca gigantea Lem.	Asparagaceae	Itabo (L)
Zinowiewia costaricensis Turcz.	Celestraceae	Raíz dorada (D) (Zamora, 2021)

such as bats, but they were not included because they were not observed in that regard. However, striking species, including the quetzal (*Pharomachrus mocinno*), primarily feed on aguacatillos (Lauraceae). The duraznillo (*Frangula oreodendron*) attracts the Golden-browed Chlorophonia (*Chlorophonia callophrys*) and the euphonias or agüíos (*Euphonia* spp.), species that were, at least previously, heavily pursued to be kept in cages at homes. However, many small and not so conspicuous species use the flowers of various trees such as *Myrcia splendens*, *Frangula oreodendron*, *Chiococca pachyphylla*, and *Turpinia* sp. (Table 2). The Band-tailed pigeon (*Patagioenas fasciata*) constantly visits the dama (*Citharexylum donnell-smithii*) trees, being one of its main food sources (Alfaro, 2019). In the United States, this pigeon is considered one of the main game birds (Alfaro, 2019), which continues to be hunted in Costa Rica although this is prohibited.

A common use in the area for the güitite (*Iochroma arborescens*) is to have it in house yards as support for orchids; this is one of the most frequent uses cited in the literature for this species (León and Poveda, 2000; Jorquera and Brenes, 2019). However, the güitite has other various uses, including personal hygiene, human food, decoration, human medicine, and animal medicine (Jorquera and Brenes, 2019), and it produces lots of fruits readily taken by birds and other wildlife (Figure 6).

The wildlife value of three Araliaceae species is worth highlighting, even though they were included in Table 3 due to their use in charcoal production. These species, *Oreopanax capitatus*, *Oreopanax*

Figure 6. A female Flame-colored Tanager (*Piranga bidentata*) feeding on güitite fruits (*Iochroma arborescens*) at La Concordia, Costa Rica. Photo by José M. Mora.

xalapensis, and *Sciodaphyllum pittieri*, bear a large number of fruits and are especially appreciated by the Black guan (*Chamaepetes unicolor*) and other bird species.

Charcoal production was once essential here, but it is a practice that has almost disappeared today. Several species had that utility at some point (Table 3), but they were left in the pastures, to provide shade for livestock. Other species also serve this function (Table 4) and can be used as ornamentals or windbreaks. Very few artificial windbreaks (planted exotic species) were observed. However, small forest blocks, used as windbreaks, mainly composed of species of little use to people, were observed. However, these have great ecological importance as they protect the small streams and springs between pastures (Table 8). Specific species play similar roles, such as the jaúl, *Alnus acuminata* (Table 9), which grows in large quantities in areas where landslides have occurred and is also a nitrogen fixer (Guariguata and Ostertag, 2001). In such areas, other species grow, including the ortigo (*Wigandia urens*), nance macho (*Clethra mexicana*), and capulín (*Trema micranthum*).

The jaúl remains very important as it is exploited for its wood and firewood since it is cultivated in pastures for its rapid growth and regeneration.

The purpose or use of wood varies widely depending on the species. For example, the caragra (*Lippia myriocephala*, Table 5) is used to make boards. The Meliaceae species, like the cedro (*Cedrela odorata*), is used for making furniture due to their fine wood; however, as it is now scarce in the area, its primary use is making posts for living fences. Although not a timber species, the coralillo *Hamelia patens* Jacq. (Rubiaceae) was once a very rare shrub species. It is primarily found near some creeks and rivers and, mainly, in open areas with lower lands, that are up to

1700 m in elevation (Estrada and Sánchez, 2012). During the latest observations in 2023, it was clear that this shrub has become widely common and is now found along roadsides, in gardens, and on fences throughout the area, specifically in areas at a bit lower altitude, such as at the village of Cinco Esquinas de Carrizal. The species is appreciated as an ornamental (Figure 7), and its flowers attract a high number of insects and hummingbirds; the fruits are taken by several bird species. It finds application in traditional medicine for various ailments, including athlete's foot, skin issues, insect stings, psychiatric disorders, rheumatism, headaches, asthma, dysentery, menstrual concerns, and ovarian and uterine disorders (Noor *et al.,* 2020).

Although the use of firewood, here as in other parts of the country, has gradually declined, many people still use it, and several species are highly appreciated for this purpose (Table 7). Some of these species, both for timber (Table 5) or firewood (Table 7), were abundant in the past. This is evident along the banks of larger rivers where highly valued species such as the llorón (*Cornus disciflora*) are common and even abundant. Although oaks (*Quercus* spp.) are scattered in the area, in the past, extensive stands of these two species existed (Table 5). *Quercus laurina* is often found regenerating in open sites (Jerome, 2018). Another common species in La Concordia, which was also possibly abundant in the past, is danto (*Roupala montana*).

The manufacturing of handles for hammers, picks, or axes requires hard and manageable woods, such as that of the aforementioned species of the danto *Roupala montana* (Table 6). This in addition to other species are appreciated for this purpose and other uses as well due to their straight and hard branches fit for making ox goads or cattle prods to herd cattle. Some of these species are called "cacique" because, being hardwood, they were used to make the staffs of indigenous chiefs in pre-Columbian times (Fonseca, 1978).

The chile-muelo (*Drymis granadensis*) has a medicinal value, but it also produces a large number of fruits that are highly sought after by birds, especially the Long-tailed Silky-flycatcher (*Ptiliogonys caudatus*). Likewise, zorrillos (*Cestrum* spp.), very abundant in the pastures, have medicinal value (Table 6). For example, their leaves, when rubbed on exposed skin, repel mosquitoes and midges. The copey (*Clusia* spp.) has pleasantly fragrant flowers, which campesinos collect to scent clothes stored in wardrobes or drawers. Several other species are used for medicinal purposes. In a similar study in the Tilarán mountain range in Costa Rica, Jorquera and Brenes (2018) found that 54.38% of species are used for human medicine. Approximately half of the global population relies entirely on plants for medicinal purposes, with many plants serving as the primary source of active ingredients in many traditional medical products (Noor *et al*., 2020). Although they are medicinal, some of these plants are poisonous, for example, the floripón, *Brugmansia arborea*. Other species found in the pastures of La Concordia are poisonous, such as the zorrillos (*Cestrum* spp.) and the higuerilla *Ricinus communis*. However, no health issues were reported for people or livestock.

The Mexican elm (*Ulmus mexicana*) is still abundant because its wood is very hard, and so it was only occasionally cut to be used in the foundation of houses. Another species that has also remained intact for several years is the Water rod (*Hedyosmum mexicanum*), which has been widely used to protect roads by placing logs across (corduroy) to prevent the sinking of roads and the constant passage of cattle from causing too much mud .

Bird capture is almost non-existent in the area today, but those who still engage in it continue to use some species of shrubs to build cages (Table 6). Similarly, another practice that has also lost its importance is the manufacturing of yokes since oxen are almost no longer used as a means of transportation. However, species like capulín (*Trema micranthum*), quizarrá caca (*Ocotea sinuata*), and targuá (*Croton draco*) were important in this regard (Table 6).

In a cattle farm that has been divided into

Figure 7. The coralillo, *Hamelia patens*. Left: a view of the shrub. Right: two close-ups to appreciate the flowers (top) and fruits (bottom). Photos by José M. Mora.

small pastures, posts are a primary need. Many species of trees are used for this purpose, depending mainly on their availability at the time of needing the posts. However, preferred species include porós (*Erythrina* spp.) that sprout again and save money for the landowners (Table 10). Among these species, the uruca (*Trichilia havanensis*) is prominent because it has various uses, especially in other countries. Its branches, once cut, wither very slowly, and for this reason, they have been used to decorate altars, make arches, and ornament religious sites, to which it also gives a pleasant smell (Fonseca, 1978). This species is used as an ornamental in many places, including urban areas.

The discussion would be too extensive if all the different uses of the shrubs and trees included in the results were noted. However, it can be concluded that where there is a tree, there is a source of satisfaction for both humans who feel and appreciate the beauty of nature and for nature itself, which can thus show its wise gifts and generosity. A representative example, that is worth mentioning in the conclusion, is *Diphysa americana*, which is a forage and an ornamental tree used as in making living fences and creating shade in pastures. Also, its dense hard wood is used in house construction, the manufacturing of tool handles, and as firewood (Table 7). Moreover, its bark is used to treat gastrointestinal problems, in addition to being a nitrogen fixer (López de Buen *et al.,* 2019).

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The Diet of Pharoah Eagle Owl *Bubo ascalaphus* (Savigny, 1809) From Al Eraiq Reserve – Qatar

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Abstract

Pellets of the Pharoah eagle owl, *Bubo ascalaphus,* were collected from Al Eraiq Reserve on the southwestern coast of Qatar. The remains of seventy-five prey individuals were retrieved representing five species of mammals, unidentified birds, and scorpions. Small rodents (*Gerbillus cheesmani* and *Jacalus loftusi*) were the most retrieved (36%) while *Meriones crassus* was the least represented (8%). *Lepus capensis* was the most represented in terms of mass intake (79.9%). In addition, birds were considerably high in the owl's diet and constituted 17.33%;scorpions 22.67%. These results reflect the opportunistic diet of the Pharoah eagle owl and its high adaptability to available food resources in ecosystems.

Keywords: *Bubo ascalaphus*, Pharoah eagle owl, Al Eraiq Reserve, Qatar

Introduction

The Pharaoh eagle owl *Bubo ascalaphus* is the largest owl species in the region where it inhabits deserts, arid plains, and rocky mountains. The Pharaoh eagle owl has a wide distribution range extending from Northern Africa to the Middle East (Mohedano *et al*. 2014), and it is a widespread resident in the Arabian Peninsula (Cramp, 1985; Jennings, 2010). However, little is known about its diet in Qatar. Mohedano *et al*. (2014) investigated the diet of *B. ascalaphus* in Al-Wakrah Municipality in southern Qatar. They reported that rodents (*Meriones crassus* and *Jaculus jaculus*) and *Lepus capensis* comprised most of this owl's diet, in addition to birds, reptiles, and arthropods. Evans and Bates (1993) studied the diet composition of the eagle owl in the desert ecosystem in Saudi Arabia. Also, the diet of the eagle owl was examined in Jordan (Amr *et al*., 1997; Rifai *et al*., 2000, Shehab and Ciach, 2008, Obuch, 2018), in Iraq (Al-Sheikhly, 2012; Al-Sheikhly and Al-Azawi, 2019), in UAE (Cunningham and Aspinall, 2001; Llanes *et al.* (2008), Saudi Arabia (Abi-Said *et al* 2020), the Palestinian Territories (Amr *et al.*, 2016), Syria (Shehab, 2004), and Egypt (Goodman, 1990; Sándor and Orbán, 2008; Moldovan and Sándor, 2009).

This study reports on the diet composition of the desert Eagle Owl in Al Eraiq Reserve in the southwestern region of Qatar based on a recent pellet collection.

Methods

A total of thirty-seven intact and three broken pellets were collected from two adjacent rocky hills at Al Eraiq Reserve situated in the southwestern region of Qatar (24° 45' 51.0'' N 50° 54' 19.5'' E altitude 25m a.s.l.). Generally, the area is covered with sand dunes whilesome rocky hills are covered with scattered shrubby vegetation.(Figure 1). The pellets were soaked in hot water until softened; hard material including bones, skulls, mandibles, and scales was removed and placed in a separate petri dish for later identification. Animals were identified based on a reference collection at the Lebanese University Natural History Museum, and Osborn and Helmy (1980), Harrison and Bates (1991), and Amr (2012). The total number of mandibles and maxillas found determined the number of individuals, and the weight of rodents was based on Osborn and Helmy (1980), Harrison and Bates (1991),

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Figure 1. Al Eraiq Reserve on the southwestern coast of Qatar

Scott and Dunstone (2000), and Abu Baker and Amr (2003). The diet composition was expressed by the total number of prey items and their percentage in the diet, frequency of their occurrence, and percentage of mass taken.

Results

The pellets of the Pharoah eagle owl were oval in shape with an average length of 46.51 mm ± 12.39 (range 77.38 – 22.75 mm) and an average width of 24.66 ± 4.77 (range 34.83 – 16.44 mm). A total of seventy-five prey individuals were retrieved from the forty pellets including five mammal species (One Lagomorpha; *Lepus capensis* and four Rodentia: Dipodidae, Cricetidae, and Muridae), unidentified bird species, and scorpions (Table 1).

The pellets contained 1–4 prey items with an average of 1.9 ± 0.87 prey items per pellet. Most pellets (40%) contained one prey item and only 2.5% contained four prey items (Figure 2). Out of the forty pellets, twenty-nine contained rodents, out of which ten contained only remains of rodents, and nineteen contained remains of rodents, hares, birds, and scorpions; seven contained hares, out of which five contained only remains of hares; eleven contained birds out of which three contained only remains of birds; and seventeen contained scorpions. Out of the four rodent species extracted from the pellets, *Gerbillus cheesemani* was the most common (45%), followed by *Jaculus loftusi* (26%), *Meriones crassus* (16%), while *Mus musculus* was the least common rodent (13%). Birds and scorpions constitute a high percentage; 17.33 and 22.67% respectively. In terms of body mass intake, *Lepus capensis* contributed the most (79.9%), followed by *J. loftusi* and *Meriones crassus* (7%), while *M. musculus* had the least contribution of 0.86% (Table 1). Even though *G. cheesmani* was the most common rodent retrieved from the owl's pellet, it only contributed to 4.66% of the body mass taken (Table 1).

Discussion

Bubo ascalaphus exhibits an opportunistic feeding behavior, which plays a vital role in determining its diet. The availability and abundance of prey in its environment influence its dietary habits. Rodents were the

Table 1. Food composition of the Pharoah eagle owl in Al Eraiq Reserve on the southwestern coast of Qatar.

Prey item	Total No.	%	Average body mass (g)	Total mass taken	% mass
L. capensis	7	9.33	1000	7000	79.9
G. cheesmani	17	22.67	24	408	4.66
J. loftusi	10	13.33	67.8	678	7.74
M. crassuss	6	8.00	100	600	6.85
M. musculus	5	6.67	15	75	0.86
Birds	13	17.33			
Scorpion	17	22.67			
Total	75	100	8761		

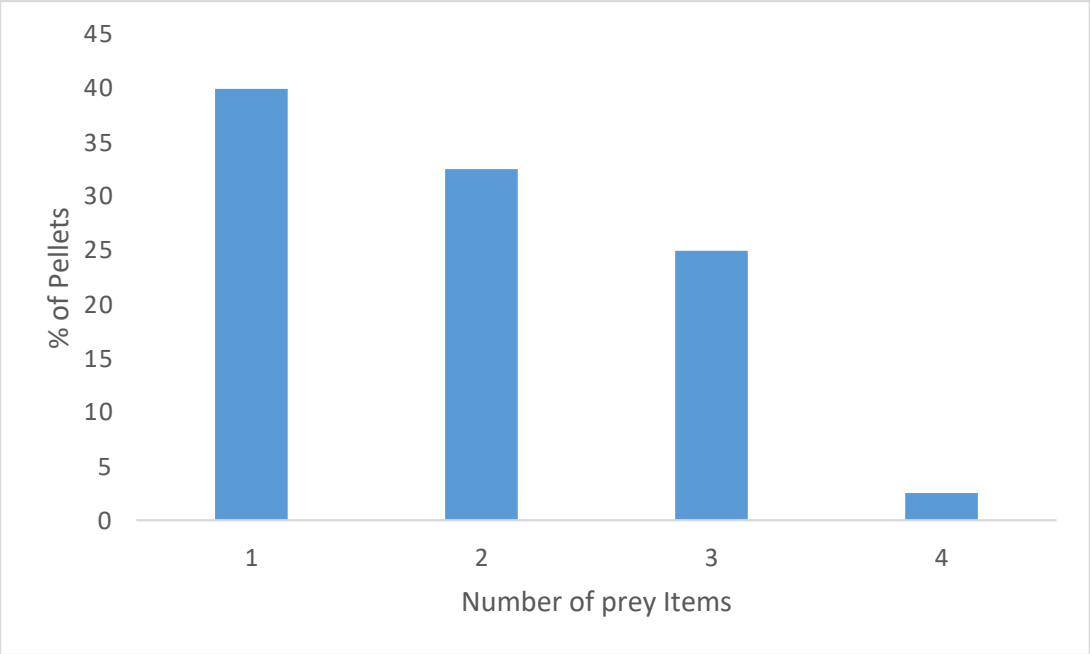

Figure 2. The number of prey items per pellet retrieved from the pellets of the Pharoah eagle owl from Al Eraiq Reserve, southwestern region of Qatar.

main prey item for the Pharoah eagle owl in this study which is consistent with other previous studies on this species' diet (Abi-Said *et al*., 2020; Nouira, 2007, Boukhamza *et al*., 1994, Goodman, 1990, Shehab and Ciach, 2008, Mohedano *et al.,* 2014; Rifai *et al.,* 2000, Sándor and Orbán, 2008; Benamor *et al.*, 2021). Contrary to other studies where large rodents constituted the main prey item for the Pharoah eagle owl (Shehab and Ciach, 2008; Mohedano, 2014; Rifai *et al*., 2000), smaller species of rodents (i.e. *G. Cheesmani* and *J. lofti*) made the highest frequency of the owl's diet in this study. The presence of the high frequency of *G. cheesmani* reflects the owl's nocturnal habit and its opportunistic feeding behavior that was determined by the abundance of its prey within an ecosystem. *Meriones crassus* was the least represented after *Mus musculus* and represented only 6.85% of the body mass consumed, even though many studies reported that *Meriones* sp. was the main prey item of the Pharaoh eagle owl (Boukhamza *et al.,* 1994; Sekour *et al.,* 2010; Abi-Said *et al*., 2020). This low percentage in the diet could be attributed to the low availability of this species in the reserve. Nonetheless, *L. capensis* was represented by 9.33% of the owl's diet but exhibited the highest percentage in terms of body mass intake (79%). This reflects the association of greater biomass to energy needs and energy expenditure. In contrast to Mohendano *et al.* (2014), who reported that Pharaoh eagle owls did not frequently consume birds, in the current study, birds constituted 17.33%

of the owl's diet and were found in 27.5% of the pellets. This reflects the scarcity of resources in the area and the acceptable cost/benefit of prey for the owl to hunt. The importance of scorpions in augmenting the owl diet in this desert ecosystem where the resources are limited was clear in the pellets collected. Arthropods, mainly scorpions, were found in 42.5% of the pellets and constituted 22.67% of the Pharoah owl diet. Owls provide important information on the biodiversity of the ecosystem in which they thrive. Analyzing the remains of prey found in their pellets can provide valuable information on the abundance and presence of small vertebrates and other species upon which they prey on a larger scale (Torre *et al.*, 2004; Heisler *et al.*, 2016). Hence, further studies are needed to thoroughly understand the diet of the Pharoah eagle owl and its seasonality. The Pharaoh eagle owl is considered a bad omen in the Arabic culture and has been frequently persecuted by humans (Al-Sheikhly *et al.*, 2020). Hence, threats impacting the Pharoah eagle owl will help in formulating an awareness program for effective conservation strategies.

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New Records of Ground Beetles (Coleoptera: Carabidae) from Jordan

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Abstract

Three species of ground beetles of the family Carabidae are recorded from Jordan for the first time: *Siagona jenissoni* (Dejean, 1826), *Dixus sphaerocephalus* (Olivier, 1795) and *Laemostenus complanatus* (Dejean, 1828). The occurrence of the following six species in Jordan is confirmed: *Grammognatha euphratica euphratica* (Latreille and Dejean, 1822), *Calosoma (Campalita) algiricum* (Géhin, 1885), *Paussus turcicus* (I. Frivaldszky von Frivald, 1835), *Clivina laevifrons* (Chaudoir, 1842), *Trechus quadristriatus* (Schrank, 1781), and *Sphodrus leucophthalmus* (Linné, 1758). *Taphoxenus* (*Lychnifugus*) *ziegleri* (Casale and Assmann 2017) is collected from a new locality. The collection sites and dates, all available biological or ecological data, and the digital images are given for all the recorded species, including the male genitalia for *Siagona jenissoni* Dejean.

Key words: Carabidae, Jordan, Cicindelinae, Carabinae, Paussinae, Siagoninae, Scaritinae, Trechinae, Harpalinae.

Introduction

The ground and tiger beetles belong to the Family Carabidae (Latreille, 1802), which is a large family of the order Coleoptera comprising more than 40,000 described species worldwide (Kesdek, 2012). They are mostly predators of small insects or other arthropods; some feed on carrion, while other species feed on plant materials. Ground beetles are an essential group for various studies, particularly in ecology (Ghahari *et al.*, 2010) and in biological control in forestry areas. Nasir and Katbeh-Bader (2017) published an annotated checklist of the ground beetles of Jordan, including 136 species, arranged in sixteen subfamilies, twenty-eight tribes, and sixty-nine genera. They also presented literature about the Carabidae from Jordan and the adjacent countries. However, after examining some newly collected specimens of the carabid beetles during 2017-2018, new records were added to the entomofauna of Jordan.

The objectives of this paper are to record ground beetle species from the family Carabidae from Jordan for the first time, in addition to confirming some earlier records, and presenting new collecting localities for some species. Since Lóbl, and Smetana (2003) mentioned in their "Catalogue of Palaearctic Coleoptera" that old records from "Arabia" may pertain to any of the states in the Arabian Peninsula and Jordan. Therefore, the present records confirm the occurrence of species mentioned from Jordan in the catalogue, which may have been recorded from Arabia without a specific locality.

Materials and Methods

The keys of Trautner and Geigenmüller (1987) and the website (http://coleo-net.de/coleo/index.htm) were used to identify the ground beetle specimens. Relevant taxonomic papers dealing with certain groups of the Carabidae of Jordan or adjacent areas were also used (Assmann *et al*., 2012; and Matalin and Chikatunov, 2016; Casale, and Assmann, 2017). The valid names followed by synonyms and world distribution are given for all the recorded species based mainly on the Catalogue of Palearctic Coleoptera (Lóbl and Smetana, 2003). Digital images were taken by Canon EOS 40D equipped with a 100mm Macro lens and ring LED illumination. The digital images of small specimens (less than 5 mm)

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and genitalia preparations were taken using the same camera mounted on a 65mm Macro lens. Many images were taken for each specimen and then stacked using Helicon Focus software and were processed using Adobe Photoshop 2020. Body length was measured from tip of the head to the tip of the abdomen. The male genitalia of *Siagona jenissoni* was dissected, boiled gently for one minute in 10% Koh, and was kept in an incubator at 50°C for one day. Images for paramere slide preparations were taken by a digital camera (CMEX 5.0 M pixel digital USB2 camera Euromex, Arnhem, The Netherlands) attached to the eye tube of a light microscope.

Results and discussion

The following ten species are recorded from Jordan; they are listed according to seven subfamilies of the Carabidae. Each valid species' name is followed by a synonymy list, world distribution, specimens examined, and some remarks.

1 Subfamily Cicindelinae (Latreille, 1802)
***Grammognatha euphratica euphratica* (Latreille and Dejean, 1822) (Figure 1)**
Megacephala euphratica euphratica (Latreille and Dejean, 1822)
Megacephala syriaca (GISTL, 1837)
Megacephala algeriana (Guérin-Méneville, 1846)
Megacephala algirica (Desmarest, 1849)
Megacephala nigra (W. Horn, 1899)
Megacephala nigripennis (Ferrante, 1908)
Megacephala aida (MandL, 1959)
Distribution: The United Arab Emirates, Algeria, Cyprus, Djibouti, Egypt, Greece, Iran, Iraq, Jordan, Kuwait, Libya, Lebanon, Morocco, Oman, Pakistan, Palestine, Saudi Arabia, Spain, Syria, Turkey, Tunisia, West Africa (Senegal), and Yemen.
Specimens examined: Fifa Reserve, 2018, RSCN researcher, no date.
Remarks: This record confirms the occurrence of this species in Jordan. It was recorded as *Megacephala* (*Grammognatha*) *euphratica euphratica* in the Catalogue of Palaearctic Coleoptera (Lóbl and Smetana, 2003), but according to Matalin and Chikatunov (2016), the subgenus *Grammognatha* was elevated to a genus level. Its distribution in Egypt, the Middle East, and Central Asia was studied. New localities were given for *M. e. euphratica* from Turkey, Syria, Saudi Arabia and Buraydah (Franzen, 2001). Franzen and Gigli (2003) confirmed its presence in Cyprus and described its habitat. Aydin (2011) studied some morphological and biological features of this species under laboratory conditions for the specimens collected from Turkey. He found that the duration of the pre-oviposition, oviposition, and post oviposition periods were 15.75, 6.41 and 9.15 days respectively. The shortest duration period for adult males was 14 days, while, it was 23 days for the female.

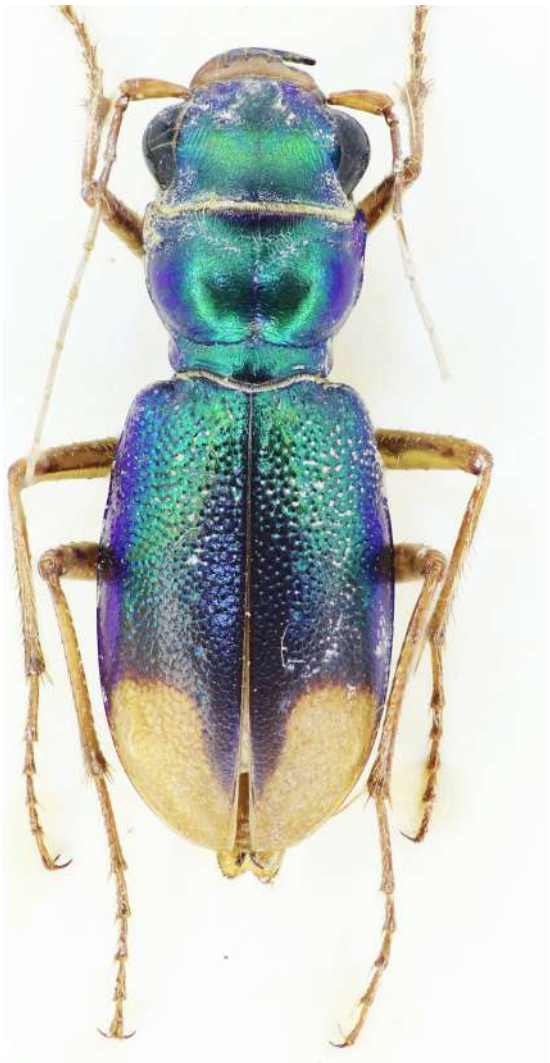

Figure1. *Grammognatha euphratica euphratica* (Latreille and Dejean, 1822)

2 Subfamily Carabinae (Latreille, 1802)
***Calosoma (Campalita) algiricum* (Géhin, 1885) (Figure 2)**
Calosoma petri (Semenov, 1902)
Distribution: Europe: Italy (Sicilia). **North Africa:** Algeria, Libya, Morocco, Tunisia. **Asia:** Iran, Palestine, Jordan, Saudi Arabia, Turkmenistan. Also, it was found in Lebanon (Tripolis) (Deuve 2004), and Syria (Bruschi 2013).
Specimen examined: One specimen. Al Mafraq Gov. III.2019, 32°21'N 36°12'E.
Remarks: This species was recorded previously from Wadi Rum in the south of Jordan by Borzatti von Löwenstern (1987). This is the second record of this species from Jordan since 1987. It is recorded for the first time from the north of Jordan. It prefers desert habitats, especially oases (Breuning 1927; Casale *et al.* 1982).

Figure 2. *Calosoma (Campalita) algiricum* (Géhin, 1885)

3 Subfamily Paussinae (Latreille, 1807)
***Paussus turcicus* (I. Frivaldszky von Frivald, 1835) (Figure 3)**
Paussus foreli (Wasmann, 1922)
Paussus innotatipennis (Pie, 1914)
Paussus mariae (Mulsant, 1855)
Distribution: Armenia, Bulgaria, Georgia, Greece, Iran, Jordan, Kyrgyzstan, Kazakhstan, Lebanon, Macedonia, Russia, Syria, Tadzhikistan, Turkmenistan, Turkey, and Uzbekistan.
Specimens examined: One specimen. Al Jubayhah, Amman. No date.
Remarks: The record confirms the occurrence of this species in Jordan. (Lapeva-Gjonova *et al*. 2011) mentioned that it is called ant nest beetle or the flanged bombardier beetle. Its typical host is the ant *Pheidole pallidula* (Nylander, 1849), but may be found with *Tetramorium semilaeve* (André, 1883) and *Messor barbarus* (Linnaeus, 1767), all of them belong to the subfamily Myrmicinae.

Figure 3. *Paussus turcicus* I. (Frivaldszky von Frivald, 1835)

4 Subfamily Siagoninae (Boftelli, 1813) *Siagona jenissoni* (Dejean, 1826) (Figures 4-9)
Specimens examined: One specimen. Sahab, 8.III.2019. Coll. Ahmad Gasan.
Distribution: **Europe**: Spain (incl. Gibraltar)
North Africa: Morocco (incl. Western Sahara)

Remarks: *Siagona jenissoni* (Dejean, 1826) is a new record to Jordan. It is also a new continental record in Asia. Future work may show its presence in other south European, north African, and Middle Eastern countries. Mediterranean *Siagonia* spp. are adapted to living in ground fissures in clayey soils formed during the dry season. They are nocturnal olfactory hunters, and prey on ants only. Their presence in such a specific habitat may explain why only one specimen was listed in the present collection. The male genitalia structures are displayed in (Figs. 4-9) showing the aedeagus, left and right parameres, and details of their distal part.

5 Subfamily Scaritinae (Bonelli, 1810)
***Clivina laevifrons* (Chaudoir, 1842a: 814) (Figure 10)**
Clivina lernaea (Schaum, 1857)
Clivina subcylindrica (Peyron, 1858)
Distribution: **Europe**: Azerbaijan, Albania, Bułgaria, Georgia, Greece (incl. Crete), Italy (Sicilia), Moldavia, Romania, Slovenia, Turkey, Yugoslavia (Serbia, Montenegro).

Figure 4. *Siagona jenissoni* (Dejean, 1826)

Figure 5. Male Aedeagus and paramesres

Figure 6. Left paramere

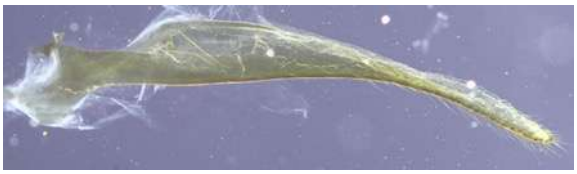

Figure 7. Right paramere

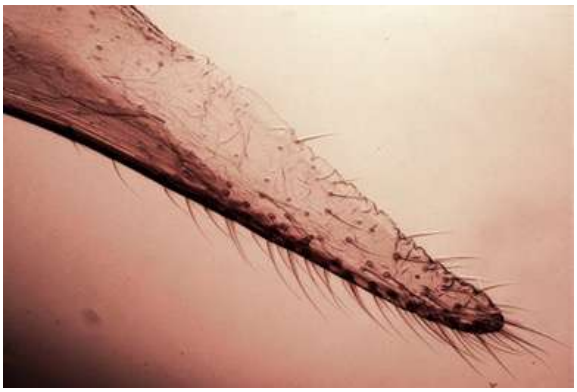

Figure 8. Left paramere distal end enlarged

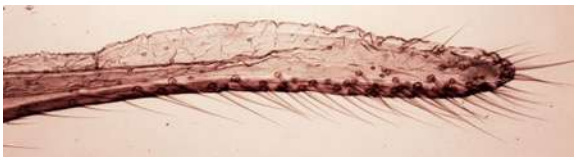

Figure 9. Right paramere distal end enlarged

North Africa: Egypt. **Asia**: Cyprus, Iran, Palestine, Iraq, Jordan, Kazakhstan, Lebanon, Oman, Syria, Turkmenistan, Turkey, Ukrainę, and Yemen. Slavčo and Borislav (2015) recorded the species from Macedonia.
Specimens Examined: One specimen. Al Moujib Reserve. 2.V.2016, Coll. Katbeh and Nasir.
Remarks: This record confirms the presence

Figure 10. *Clivina laevifrons* (Chaudoir, 1842)

Figure 11. *Trechus (Trechus) quadristriatus* (Schrank, 1781)

of *Clivina laevifrons* Chaudoir in Jordan. This is a common species widespread in the Mediterranean area to Middle Asia (Bulirsch and Stachowiak 2017).

6 Subfamily Trechinae (Bonelli, 1810)
***Trechus (Trechus) quadristriatus* (Schrank, 1781) (Figure 11)**

Trechus (Trechus) amaurocephalus (Kolenati, 1845)
Buprestis capitatus (Geoffroy, 1785)
Trechus (Trechus) fascipennis (Stephens, 1828)
Trechus (Trechus) humeralis (Dejean, 1831)
Carabus minutus (Fabricius, 1792)
Trechus (Trechus) piciventris (Graells, 1858)
Trechus (Trechus) politus (Faldermann, 1836)
Trechus (Trechus) syriacus (Putzeys, 1870)
Carabus tempestivus (Panzer, 1799)
Distribution: Afghanistan, Algeria, Albania, Armenia, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Byelorussia, Croatia, Cyprus, Denmark, Egypt, Estonia, Finland, France, Great Britain, Germany, Georgia, Greece, Hungary, Iran, Iraq, Ireland, Palestine, Italy, Jordan, Kyrgyzstan, Kazakhstan, Latvia, Lebanon, Lithuania, Luxembourg, Malta, Macedonia, Moldavia, Morocco, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Syria, Switzerland, Tadzhikistan, Turkmenistan, Turkey, Ukraine, and Uzbekistan.
Specimens examined: one specimen. University of Jordan Farm, Jordan Valley, Light trap 29/4/-3/5 1994.
Remarks: This record confirms the presence of this species in Jordan. Hind wings dimorphic, (in the Middle East predominantly winged specimens). Eurytopic species in meadows (Assmann *et al*., 2012).

7 Subfamily Harpalinae (Bonelli, 1810)
***Dixus sphaerocephalus* (Olivier, 1795) (Figure 12)**
Aristus trogossitoides (L. Dufour, 1820)
Ditomus sphaerocephalus (Olivier, 1795)
Dixus trogossitoides (L. Dufour, 1820)
Scarites sphaerocephalus (Olivier, 1795)
Distribution: **Europę**: Faeroe Islands, Italy (incl. Sardinia, Sicily, San Marino) Portugal, Spain (incl. Gibraltar); **North Africa**: Algeria, Libya, Morocco (incl. Western Sahara), and Tunisia.
Material examined: One specimen. Al Jubayhah, 18.III.2018.
Remarks: *Dixus sphaerocephalus* **(**Olivier, 1795) is a new record to Jordan. It is also a new continental record in Asia. Additional collecting in other countries may reveal its presence in other south European, north African, and Middle Eastern countries.
***Laemostenus* (*Laemostenus) complanatus* (Dejean, 1828) (Figure 13)**
Pristonychus alatus (Wollaston, 1854)
Pristonychus australis (Blackburn, 1888)
Pristonychus australianus (Casale, 1988)
Pristonychus beloni (Raffray, 1870)
Pristonychus chilensis (Gory, 1833)
Pristonychus crassicornis (Fainnaire, 1877)
Pristonychus nanniscus (Péringuey, 1896)
Pristonychus rufitarsis (Curtis, 1839)
Specimens examined: One specimen. Amman, Tabarbour 17.XII.2017.
Distribution: **Europe**: Azores, Croatia, France (incl. Corsica, Monaco), Great Britain (incl. Channel Islands), Greece (incl. Crete), Italy (incl. Sardinia, Sicily, San Marino), Portugal, Spain (incl. Gibraltar), Slovenia, Yugoslavia (Serbia, Montenegro). **North Africa**: Algeria, Canary Islands, Morocco (incl. Western Sahara), Madeira Archipelago, Tunisia. **Asia**: Cyprus, and Lebanon.
Remarks: *Laemostenus* (*Laemostenus) complanatus* (Dejean, 1828) is recorded for the first time from Jordan. It is a cosmopolitan species (Lóbl and Smetana, 2003). Originating from North Africa, it has been dispersed by trade to ports in most continents (Lindroth, 1974).

Figure 12. *Dixus sphaerocephalus* (Olivier, 1795)

Figure 13. *Laemostenus (Laemostenus) complanatus* (Dejean, 1828)

***Sphodrus leucophthalmus* (Linné, 1758) (Figure 14)**

Sphodrus armeniacus (Osculati, 1844)
Sphodrus findus (Chaudoir, 1852)
Carabus obsoletus (P. Rossi, 1790)
Carabus planus (Fabricius, 1792)
Sphodrus siculus (Motschulsky, 1865)
Carabus spiniger (Puykuîl, 1790)

Distribution: **Europe**: Armenia, Austria, Belgium, Bułgaria, Croatia, Czech Republic, Denmark, Finland, Franc, Great Britain, Germany, Georgia, Greece (incl. Crete) Hungary, Italy, The Netherlands Poland Portugal Slovakia Slovenia, Spain (incl. Gibraltar), Russia, Sweden Switzerland, Romania, Ukrainę, Yugoslayia.

North Africa: Algeria, Canary Islands, Egypt, Libya, Morocco, Tunisia. **Asia**: Afghanistan, Cyprus, Himachal, Pradesh, Iraq, Kashmir (India), Saudi Arabia, Syria, Turkey, Uttar Pradesh (India), and Yemen.

Specimens examined: One specimen. Dibeen Reserve.

Remarks: Casale and Assmann (2017) recorded this species from the south of Jordan in Wadi Rum and Dhana Camp syntopically with *Taphoxenus* (*Lychnifugus*) *ziegleri* (Casale and Assmann, 2017). *Sphodrus leucophthalmus* Linné is steppic, eremic and anthropophilic species, rare and disappearing in several European countries. It lives in mammal nests and other damp areas with clay floors. It preys on adults and the larvae of the darkling beetle family Tenebrionidae. *Sphodrus leucophthalmus* is the only species of *Sphorus* present in the southern Levant. Another species of the genus, *S. trochanteribus* (Mateu, 1990), is found in Yemen (Casale and Assmann, 2017).

Figure 14. *Sphodrus leucophthalmus* (Linné, 1758)

***Taphoxenus* (*Lychnifugus*) *ziegleri* (Casale and Assmann, 2017) (Figure 15)**

Distribution: Iraq, Jordan, Syria, and Turkey.

Specimens examined: Two specimens Dibbeen Reserve.

Remarks: The type locality of this species is Madaba, Jordan. Other specimens were collected from Petra, Dhana and Tafila. The record from Dibben Reserve shows the most northern distribution of the species so far. Further investigations are needed to determine the presence of this species

Figure 15. *Taphoxenus (Lychnifugus) ziegleri* (Casale and Assmann, 2017)

in east Mediterranean countries. Casale and Assmann (2017) provided a detailed description of the species, diagnosis, and genitalia drawings.

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A New Record of the Longtail Tadpole Shrimp, *Triops longicaudatus* (LeConte, 1846), from a Temporary Water Body in Azraq, Northeastern Jordan

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Abstract

The longtail tadpole shrimp *Triops longicaudatus* (LeConte, 1846) is recorded from Jordan for the first time. Specimens were collected from Azraq Mudflat, located within the borders of the Azraq Wetland Reserve. This represents the first record of the family Triopsidae and the order Notostraca from the country of Jordan. The current study stresses the need for additional research on the fauna of temporary pond habitats in Jordan as well as the implementation of better conservation management strategies for this important environment.

Key words: Notostraca, first record, distribution, Jordan

Introduction

The family Triopsidae (Crustacea, Branchiopoda) is represented by two extant genera in the order Notostraca: *Triops* (Schrank, 1803) and *Lepidurus* (Leach, 1819). This family is considered as a great example of "living fossils" (Mathers *et al.,* 2013). These freshwater crustaceans mainly occur in temporary water bodies, including freshwater, brackish water, and occasionally saline temporary pond habitats (Su and Mulla, 2001; Damgaard and Olesen, 1998; Brendonck *et al.,* 2008), where they can survive over long dry periods of time by producing and depositing resistant cysts in the sand or sediment that stay viable after extended periods of diapause (Aloufi and Obuid-Allah, 2014).

The *Triops* species are found on all continents except Antarctica and have a dispersed but worldwide distribution (Vanschoenwinkel *et al.,* 2012; Korn *et al.,* 2013).

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Al-Oufi and Obuid-Allah (2014) recorded this species for the first time in the Kingdom of Saudi Arabia, from twelve temporary water bodies in Tabuk and Al-Madinah, which are located to the southeast of Jordan and east of Azraq.

The records made by Al-Oufi and Obaud Allah (2014) indicate that this species probably occurred in Jordan under the same conditions. Freshwater invertebrates are scarcely investigated in Jordan, and there is little information about the Notostraca fauna, despite the fact that these animals received special attention due to their conservation status because they were treated as flagships of temporary water bodies, allowing shrimp tadpoles to eliminate the mosquito larvae that were there as well. Furthermore, in many countries, including the UK, some members of this class are classified as endangered species and are protected by Schedule Five of the Wildlife and Countryside Act 1981.

Materials and Methods

On June 10, 2023, a local guide and the site researcher captured nine specimens in the Azraq mudflat, which is located within the borders of the Azraq Wetland Reserve, after a highly precipitated raining season that enhanced the formulation of the seasonally flooded area in Azraq, Eastern Jordan, known as Qa (Qa=Arabic name of the mud flat). The exact coordinates are N 31.83394°, E 36.81868°, and the altitude is 491m. All the nine specimens captured were collected and preserved in absolute ethanol. The examination and measurements were carried out in the Royal Society for the Conservation of Nature (RSCN)-HQ lab

under a stereomicroscope equipped with a camera and were identified based on morphology according to Longhurst (1955), Alonso (1996), and Obuid-Allah *et al.* (2014). The specimens have been deposited in the Conservation Monitoring Center (CMC) at the RSCN-HQ lab, reference number 0023/ TL.

Results

Systematic Treatment

Class - *Branchiopoda*
Order - *Notostraca*
Family - *Triopsidae*
Species - Longtail Tadpole Shrimp ***Triops longicaudatus* (LeConte, 1846)**(Figure 1)

Description of *Triops longicaudatus* (LeConte, 1846) (Figure 2,Table 1)

Six females and three males were identified among the collected nine shrimp tadpoles. The tadpole shrimps can be recognized by their large, horseshoe-shaped dorsal carapace (Martin and Boyce, 2005, Cáceres and Rogers, 2015), and they also have a pair of dorsal compound eyes. The second maxilla was absent in all the specimens seen (Figure 2). All females were gravid. In general, the female specimens were longer than males, with a 55-mm total body length and a 52-mm total body length for males. The average carapace length in the female specimens was 14.83 mm and was 13.44 mm for the male specimens. The average carapace width in female specimens was 13.83 mm and was 12.74 mm for male specimens. The average body segment in the female specimens was 40.2, while it was 40.7 in the male specimens,. The average furcal rami length in females was 18.83 mm, while it was found to be 22 mm in the male specimens; one specimen of male furcal rami was damaged.

Figure 1. Dorsal and ventral views of a male of *Triops longicaudatus* from Jordan.

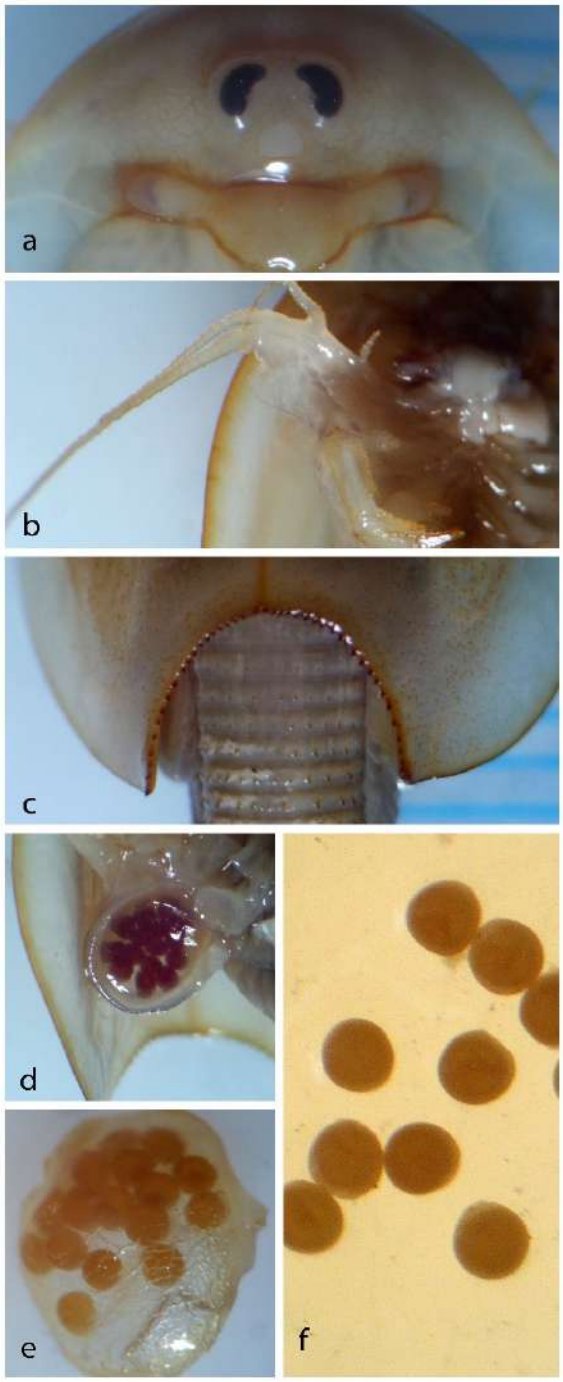

Figure 2. *Triops longicaudatus*, a: enlarged eyes and dorsal organ, b: thoracopod 1, c: enlarged sulcus (dorsal view), d: thoracopod XI (egg pouch), e: egg pouch containing sixteen eggs, f: eggs.

Discussion

According to Longhurst's (1955) review of Notostraca, there is considerable difficulty in the species identification because most species exhibit significant morphological diversity. Three features were deemed significant by Longhurst (1955) in terms of taxonomy: the telson's armature, the second maxilla's presence or absence, and the positioning of the dorsal organ and eyes within this group. The species was identified as *Triops longicaudatus* (LeConte, 1846) based on the aforementioned characteristics and comparison with earlier research on the same species conducted by: Aloufi and Obuid-Allah, (2014) (Table 2). The researchers found that some of these characteristics, such as the carapace and telson width, fall within the range reported by Aloufi and Obuid-Allah's (2014). The ranges found in this study, however, are primarily smaller than those found in Aloufi and Obuid-Allah's (2014). Habitat, sex, or the total number of specimens analyzed might all be factors in these variations.

The current research showed some significant differences between *T. longicaudatus* females and males. The number of body lengths and the number of posterior segments not covered by the carapace were among the discrepancies. This finding is consistent with Longhurst's (1955) study, which confirms that some characteristics are connected to the sex of the specimen (sexual dimorphism).

This is the first record of *T. longicaudatus* from Jordan. Figure 3 shows the collecting site in mudflat within the Azraq Wetland Reserve. Figure 4 shows the global distribution of *T. longicaudatus* including this new record from Jordan. This species is currently known from the Kingdom of Saudi Arabia, Jordan, Hawaii, New Caledonia, Japan, the West Indies, the Galapagos Islands, and Central and South America. Members of Notostraca are widely distributed due to their passive distribution. The dried viable eggs must be blown around by wind and transported by birds because the eggs, when laid, are extremely sticky and remain so for several days while the shell hardens. Thus, they could presumably adhere to large animals. Further studies should address the life history of *T. longicaudatus* , including feeding habits and reproduction in Jordan.

Table 1. Morphometric (in mm) and meristic characters of shrimp tadpole.

The character / Spec. no	Total Body length (^{tot} L)	Carap ace length (Cl)	Carapace width (CW)	Length of Telson (T)	Telson width (W)	Furcal rami length	Number of posterior segments not covered by the carapace	Number of body segments
Female 1	50	16	15	2.2	3.3	18	26	42
Female 2	45	15	15	1	2	15	25	41
Female 3	41	15	15	2	2	14	25	37
Female 4	55	15	13	3	3.8	24	28	43
Female 5	45	13	11	2.1	2	20	27	40
Female 6	49	15	14	1.6	2.6	22	25	38
Male 7	52	14	12	2.6	2.3	25	23	44
Male 8	33.5	12.7	13.8	1	2	19	31	36
Male 9	31.2	13.7	12.5	1	2.5	?	28	42

Table 2: Some morphometrics of *Triops longicaudatus* compared with those confirmed by the study of Aloufi and Obuid-Allah (2014).

The character	Sex	Present study (in mm)	Aloufi and Obuid- Allah, (2014) (in mm)
Body length	Female	41- 55	19- 65
	Male	31.5- 52	20- 55
Carapace length	Female	13-16	6- 20
	Male	12.7- 14	6-18
Carapace width	Female	11-15	5- 15
	Male	12-13.8	6- 17
Number of body segments	Female	37-43	35-39
	Male	36- 42	36-39
Length of Telson	Female	1.2.2	1-2
	Male	1-2.6	1-3
Telson width	Female	2-3.8	1-3
	Male	1-2.5	1-2.5

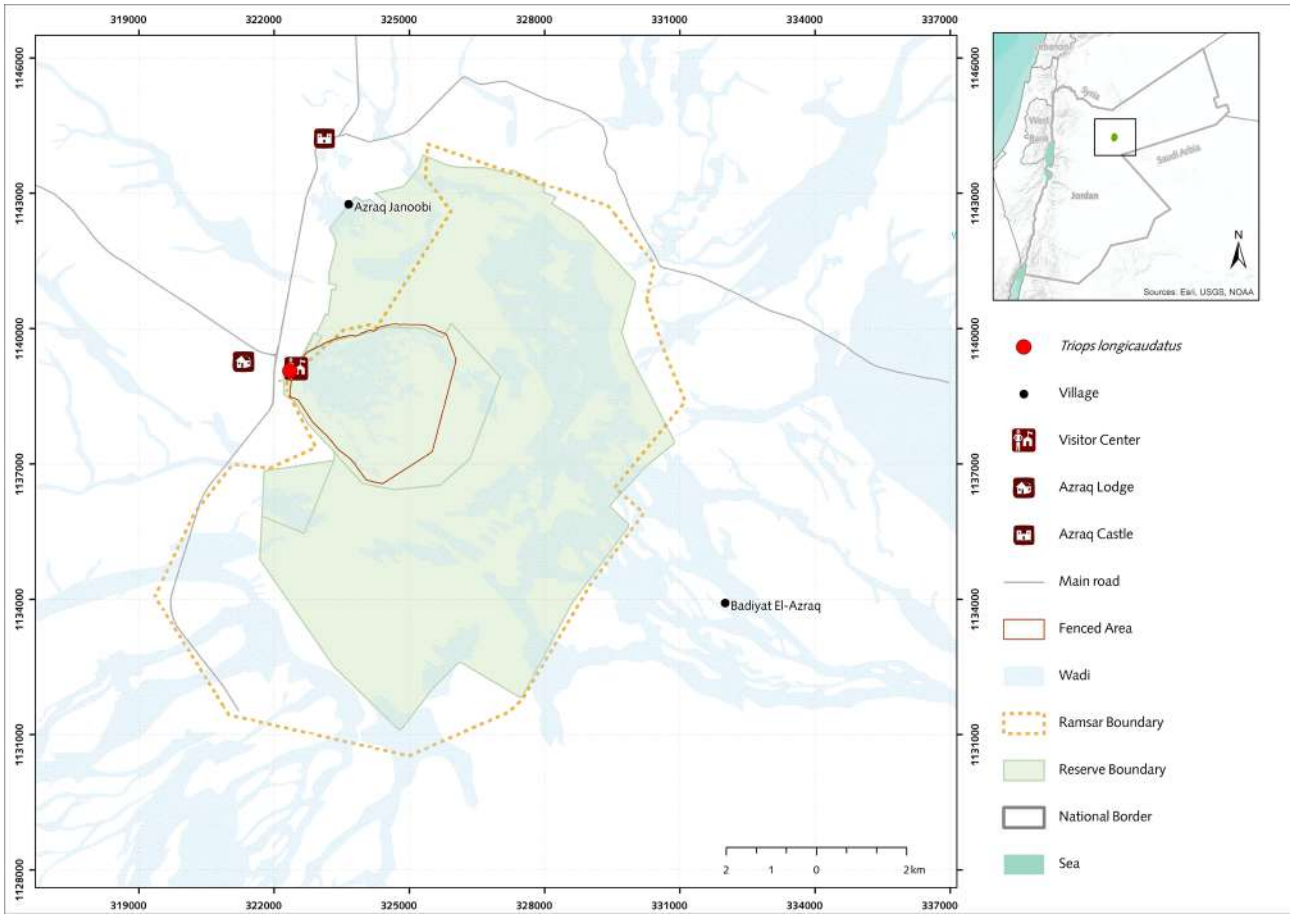

Figure 3. Collection site of *Triops longicaudatus* from Azraq, Eastern Jordan.

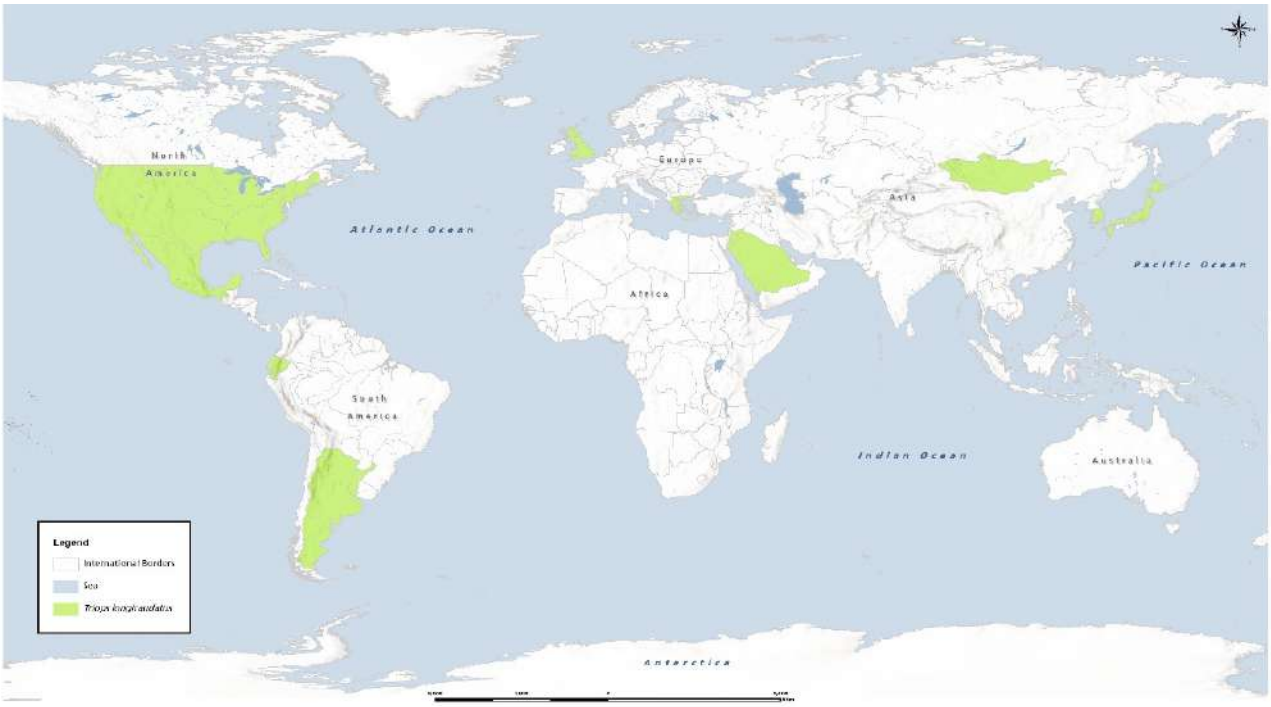

Figure 4. The global distribution of *Triops longicaudatus*

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Short Communication

A Teratological Record of *Spilostethus pandurus* (Hemiptera, Heteroptera, Lygaeidae) from the Occupied Palestinian Territories, West Bank

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Abstract

A teratological case of the Seed Bug (*Spilostethus pandurus*) is recorded from the Mar Saba area in the Bethlehem District, Palestine. This anomaly appears on the pronotum, scutellum, corium, and the membrane part of the specimen, which seems to occur less often than antennal anomalies in true bugs (Heteroptera).

Keywords

Malformation, anomaly, true bug, West Bank, Palestine.

Introduction

The Milkweed Bug (*Spilostethus pandurus*) is widely distributed in tropical and subtropical areas (Awad, *et al*., 2013). In some countries, it is considered as a serious pest on the seeds ofsome plants with highly economic important such as in Egypt where at times it causes serious damage (Meguid, *et al*., 2013; Kugelberg, 1973). It infests many crops including sunflower seeds, watermelon seeds, squash seeds, cantaloupe seeds, pea nuts, cotton, sorghum, sesame, lobia, tomato, eggplant, sugarcane, okra, pecans, whole kidney seeds, wheat and cabbage (Thangavelu, 1979). Teratology can be exhibited as a simple change in structure (morphology) (Faúndez and Rider, 2017; Faúndez and Rocca, 2016). In other cases, it can be more complex especially when there is an extra part in the insect's body such as having an extra segment of the antennas (Burke, *et al*., 2018) or compound eyes (Clark and Neto, 2010). Morphological anomalies appear occasionally in insects especially the True Bugs (Heteroptera) group, and most records focused on the antenna and other structures including claws (Asiain and Márquez 2009; Carvajal, *et al*., 2019; Taszakowski and Kaszyca-Taszakowska, 2020).

The Heterpotera fauna were collected from Mar Saba area (31°42'16.6"N 35°19'51.5"E), the Bethlehem District (West Bank) on the 20th of Feb. 2022 by a member of the Palestine Museum of Natural History (PMNH) and Biodiversity Center research team. A population of the species *Spilostethus pandurus* was collected during the survey. One specimen of the Seed Bug *Spilostethus pandurus* was found to have morphological malformations (Figure 1). This appeared on the pronotum as wrinkled aberrance from the middle and affected mainly the posterior margin which was indented anteriorly. The scutellum showed shrinkage from one side, and the left clavus showed huge shrinkage (see Figure 1: both normal specimen A and the malformation specimen B).

Solbreck and Anderson (1989) described short-winged individuals of Spilostethus pandurus from laboratory cultures on Cyprus. The wings were about the length of the scutellum, but the flight muscles were fully developed. Crossing data showed that shortwing-edness is determined by a recessive gene. The findings could be explained as early steps in the evolution of wing reduction.

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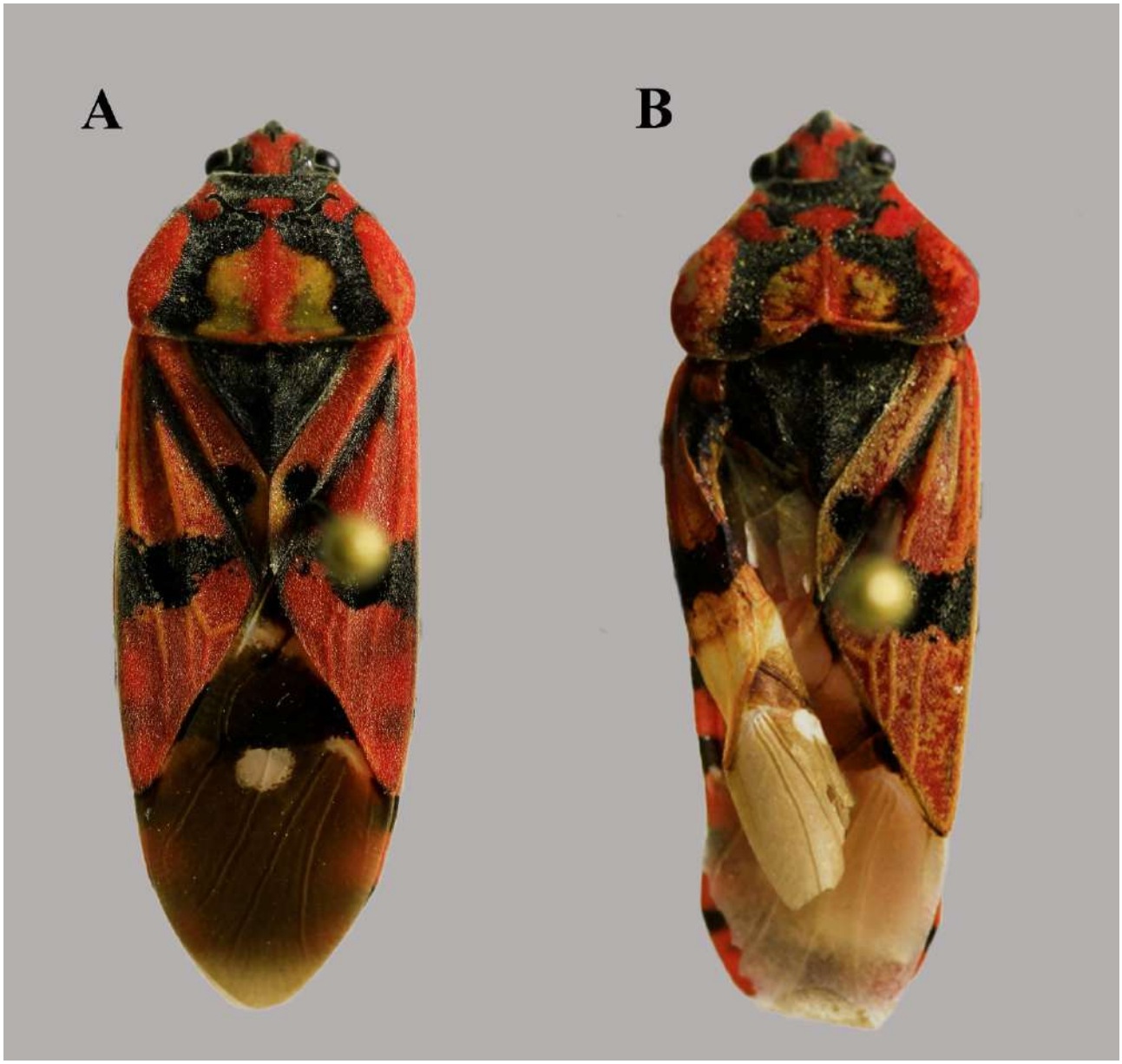

Figure 1: Dorsal view of Spilostethus pandurus; A: Normal specimen, B: Malformed specimen.

In general, anomalies of the pronotum, scutellum, and clavus seem to occur less often than antennal anomalies in true bugs (Heteroptera). A record from Palestine for the same case of malformation was made for The Southern Green Stink Bug (*Nezara viridula*) (Carvajal, *et al*., 2019; Steinhaus and Zeikus, 1968; Taszakowski and KaszycaTaszakowska, 2020; Handal, 2021). Some recent studies suggest that global warming will be a force actor for abnormalities in insects and could show malformation in some of them. This effect should be studied and observed more extensively to understand the effect of climate change on insect fauna and its morphological changes (Polidori, *et al*., 2020; McCauley, *et al*., 2018; Nijhout and Emlen, 1998). Our suggestion is that the cause of an aberrant specimen may incur some physical damage to the nymph before the final molt or accident during the molding process.

Bernal and Romo (2018) described four teratological cases in Lygaeoidea (including *Spilostethus pandurus*. They concluded that the simple unilateral oligomery was more likely due to the loss of one of four antennal segments in an early immature stage.

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Short Communication

The Occurrence of the Reticulated Python *Malayopython reticulatus* (Schneider, 1801) (Serpentes: Pythonidae) as evidence of Alien Species' introduction into Iraq

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Abstract

Wildlife researchers should be cautious about the risks associated with the spread of non-native alien species. Ancestral stories on the occurrence of a giant mythical serpent were circulated among local communities in Iraq; however, their validity was rarely tested by researchers. In fact, most of these allegations were often regarded as unsubstantiated superstitions and remained unverified. In a rare case, the researchers report the occurrence of the Reticulated Python *Malayopython reticulatus* (Schneider, 1801) as an emergent alien species in the Iraqi environment possibly introduced from wildlife trade; its possible introduction pathways, movement, and fate are also discussed.

Key words: Exotic species; herpetofauna of Iraq; human-wildlife interaction; Invasive species; wildlife trafficking.

Introduction

The Reticulated Python *Malayopython reticulatus* (Schneider, 1801) is one of the world's largest snakes inhabiting river ecosystems as well as rainforests, woodlands and the adjacent grassland areas (Stuart *et al*. 2018). Its zoogeographical range is restricted to Southeast Asia, and it is widely spread throughout Nicobar Islands Bangladesh (Chittagong), Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia and Singapore, eastern Indonesia and the Indo-Australian Archipelago and the Philippines (Auliya *et al*. 2002; Reptile Database 2023). However, the Reticulated Python is one of the most economically important reptile species for wildlife trade (Groombridge and Luxmoore 1991; Kasterine *et al*. 2012; Natusch *et al*. 2016; Murray-Dickson *et al*. 2017). It is also part of the trade in non-native reptile pets worldwide (Luiselli *et al*. 2012) and in the Middle East in particular (El-Sayed 2018; Farashi and Alizadeh-Noughani 2021). The species is listed in Appendix II of the Convention on International Trade in Endangered Species of Fauna and Flora (CITES) (CSW 2015). However, due to its wide distribution, adaptability, and abundance even in some areas where it faces heavy exploitation, the Reticulated Python is listed as Least Concern by the International Union for Conservation of Nature (IUCN) Red List (Stuart *et al*. 2018).

Observations and records

In Iraq, unverified stories of the "giant snake" have appeared since the early 1980s, where scattered locals' testimonies described a gigantic snake locally named "*Afa'ah*" coiling on the branches of big trees in the northern mountains and were much feared

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by locals. Similar observations were also claimed by villagers in eastern and southern Iraq. However, all of these testimonies were regarded as "mythical superstition" by researchers, for they were not supported by credible perceptible evidence, and were, therefore, overlooked. For decades, the story of this terrifying snake remained shrouded in mystery.

In recent years (≥ 2003), new observations on the Reticulated Python were frequently reported by locals in central and southern Iraq (Figure 1); however, the researchers only report confirmed incidents that were verified by their investigations (Table 1).

The first sighting was made by local villagers in a water canal branched from the Diyala River (one of Tigris River tributaries) in Khirnabat Village, ca. 5 km to the north of Baqubah, Diyala Province over the period from the 26th of July to the 1st of August 2018. The locals have repeatedly contacted the Iraqi Green Climate Organization (IGCO) reporting a fierce looking "giant snake" inhabiting the densely vegetated canal and aroused fear among the people. A joint team

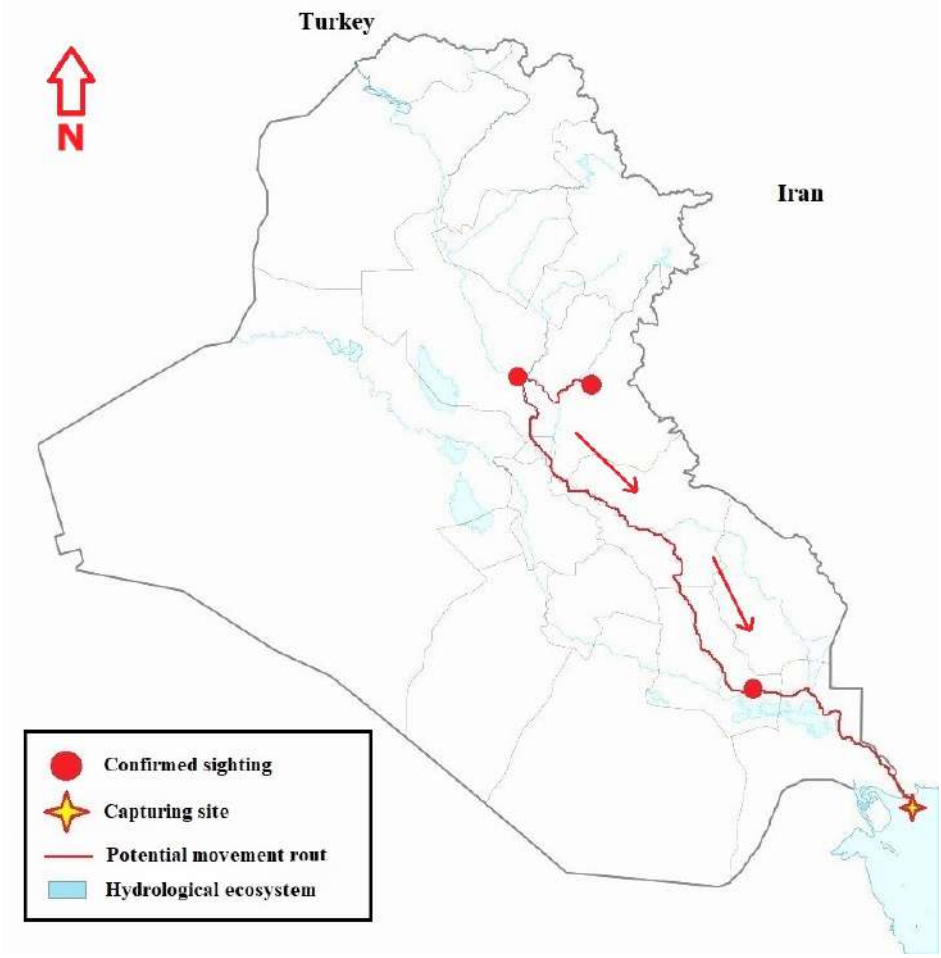

Figure 1: Reticulated Python *Malayopython reticulatus* confirmed records with possible pathway in Iraq.

Table 1: Reticulated Python *Malayopython reticulatus* observations and records in Iraq.

No.	Site Name	Coordinates	Date	
			1st observation	2nd observation
1	Khirnabat Village	33°45'N 44°36'E	26-7-2018	1/2-8-2018
2	Ad'jeel area	33°49'N 44°17'E	5-9-2018	Repeated observations on 21-7-2021
3	Central Marshes	31°2'N 47°5'E	20-9-2018	23-9-2018
4	Basra Oil Harbor	29°40'54.11"N 48°48'57.96"E	10-2018	

from the Iraqi Ministry of Environment (IMoEn) and IGCO (see acknowledgments) was formed to investigate the locals' claims. The researchers' interviews with local communities show that the snake was seen multiple times within an area estimated by ca.2 km^2 over a period of one week by three villagers (requested to be anonymous). The villagers' testimonies indicated that they have confidently eye-witnessed an "anaconda-like giant snake", referring to the massive body size of the animal, with a big head, and an olive-grey skin color with yellow spots alongside. The first testimony came from a driver who took a short rest on the western bank of the canal where he thought he had seen a big truck tire then realized it was a coiling hissing "big snake" among the riparian thickets. The other two testimonies revealed that a 3–5m long "giant snake" was seen moving from the canal banks towards the nearby wheat fields at mid-day. Electronic photographs of both Anaconda *Eunectes* and Python spp. were shown to the villagers to be able to recognize which snake species they had encountered. According to the morphological features described by the villagers; they were clearly referring to the photos of a Python. Despite the wide use of mobile phone devices supported by digital cameras among local communities, however, none of these observations was documented, which raised suspicions. Observations unsubstantiated by visual evidence (e.g., photos/videos) could be attributed to the fact that the eyewitnesses were terrified from the snake which they had never encountered in the Iraqi environment and were forced to run away. The researchers' investigation lasted two days (1st –2nd of August 2018) and no tangible evidence (tracks, fecal samples, shed skin, etc.) confirming the presence of this "giant snake" was obtained and mission was aborded.

The second sighting was in the Ad'jeel (Dejail) area, ca. 30 km to the northwest of the Khirnabat village in Salahadin Province, central Iraq. A "giant snake" moving on the eastern bank of a shallow pool branched from the Ishaqi River was seen by a local fisherman on the 5th of September 2018; this incident was ignored by local authorities as no persuasible evidence was provided. In July 2021, the appearance of a "giant snake" in Ad'jeel was reported again and several local testimonies were widely spread on local social media. The locals' claims were reliably considered by the Iraqi authorities who launched campaigns to find and kill the snake (Anonymous 2021). Two years later, the illusional psychological impact of a "giant snake" swimming in the river was still expressed and feared by the Ad'jeel's locals. However, no credible results were obtained and the fate of that "giant snake" in this area remained enigmatic.

In September 2018, the third sighting was made in the Central Marshes in southern Iraq. The Central Marshes are a part of the Mesopotamian wetlands, Iraq's Protected Area, and are considered a site of international importance (Al-Sheikhly and Al-Azawi 2019). In addition to prey abundance, the landscape consists of open shallow ponds bordered by dense reed beds and marshland vegetations which are somewhat compatible to the Reticulated Python's ecological requirements (see Auliya *et al*. 2002, Stuart *et al*. 2018). The "giant snake" was frequently seen by local fishermen and villagers of the Marsh Arabs (native inhabitants of the marshes) within an area of ca. 4.5–5 km^2, extending from Al-Manthar (30°59'6.81"N 47° 2'55.97"E) and Abu-Subat Village (30°59'55.93"N 47° 1'17.20"E). The locals' observations confirmed that the snake was observed sneaking among reed beds and entering the densely vegetated watercourses. Moreover, the Marsh Arabs expressed worries and fears saying that the snake could prey on their livestock; therefore, they organized a retaliation campaign to kill it. Despite intensive in situ surveys conducted for three continuous days (20–23 September; see Laith 2018), no plausible evidence was obtained, and the presence of the "giant snake" in the Central Marshes remained a puzzling question.

In October 2018, the fate of the "giant snake" in Iraq was finally determined. As expected,

based on photographic documentation, an adult Reticulated Python (2.5–3 m long) was reported swimming in the marine waters in Basra Oil Harbor (Al-Baker Harbor) in the coastal line of the Arabian Gulf in extreme southern Iraq. The snake was captured and killed by the Iraqi marine troops and disposed in the marine waters, but no specimen was obtained (Figure 2A and B).

In addition to receiving visual evidence made by the marine troops (see acknowledgments), the interviews indicated that the snake was drifted by sea tide from Khawr Abdualla opening (29°51'32.91"N 48°27'43.22"E) through Khawr al'Amaya, along the western bank of the Iraqi Faw Peninsula (29°53'N 48°33'E) towards the harbor. Based on the spatial and temporal pattern of previous incidents mentioned above, this pathway supported the researchers' assumption that this snake was originated somewhere inside Iraq and introduced/escaped into inland waters. It is most probable that the python swam from central Iraq through the watercourse system crossing the Central Marshes to the Tigris-Euphrates confluence, through Shatt Al-Arab towards the Arbian Gulf in southern Iraq (Figure 1). Nevertheless, due to the cryptic behavior of the snake, this interpretation is hypothetical and requires further investigation.

Stuart *et al*. (2018) indicated that the Reticulated Python is an excellent swimmer and tolerant of highly modified habitat; it has been reported far out at sea and has consequently colonized many small islands within its range. Regardless, there was no concrete evidence which supports the idea that this snake has escaped/been introduced from pet trade to the environments of the

Figure 2: A & B-The Iraqi marines with the killed Reticulated Python *Malayopython reticulatus* in Basra Oil Harbor, Arabian Gulf coast, Basra Province. Photos © Yasir Wathiq

nearby countries (e.g., Kuwait; Iran) and/or leaked into the Iraqi marine waters from cruising ships.

The trade in non-native reptile species as pets is an increased business worldwide with a potential impact on the Middle East (Farashi and Alizadeh-Noughani 2021). These animals are caught and smuggled out of their natural habitats or raised in captivity (Reed 2005). However, non-native reptiles might escape or be introduced into natural ecosystems where they establish populations in the wild, which in turn could lead to becoming invasive (Stringham and Lockwood 2018; Bartoszek *et al*. 2021). Non-native reptiles produce adverse impacts on ecosystems through predation, herbivory, competition, and genetic hybridization (Kraus 2015). The impact of invasive alien species in Iraq is not fully known (Nature Iraq 2017). Moreover, types and numbers of non-native species imported for wild trade in Iraq is far to be determined (Al-Sheikhly *in press*). The occurrence of the Reticulated Python in Iraq is probably attributed to the pet trade where many non-native reptilian species are deliberately introduced or accidentally escaped/leaked from captivity into the environment as a result of irresponsible management (see Hulme 2015, Willson *et al*. 2011; Guzy *et al*. 2023). According to the biodiversity national target no.19 in Iraq's NBSAP (2015): "*Invasion pathways of the 30 most dangerous/problematic (known or assumed) alien species of the list*" should be achieved; therefore, monitoring the distribution pathways of these alien species, including this current case, is urgently required.

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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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