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Moslem Doostmohammadi, Maryam Malekmohammadi, Morteza Djamali, Hossein Akhani. Is Pteropyrum a pathway to C4 evolution in Polygonaceae? An integrative approach to the taxonomy and anatomy of Pteropyrum (C3), an immediate relative of Calligonum (C4). Botanical Journal of the Linnean Society, 2020, 192 (2), pp.369-400. 10.1093/botlinnean/boz079. hal-02391348

# HAL Id: hal-02391348 https://amu.hal.science/hal-02391348

Submitted on 3 Dec 2019  $\,$ 

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# Is *Pteropyrum* a pathway to $C_4$ evolution in Polygonaceae? An integrative approach to the taxonomy and anatomy of *Pteropyrum* ( $C_3$ ), an immediate relative of *Calligonum* ( $C_4$ )

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Received 14 February 2019; revised 11 September 2019; accepted for publication 24 September 2019

*Pteropyrum* is a small genus of Polygonaceae with four species from the arid regions of Iran and adjacent countries. Pteropyrum spp. are not precisely delimitated and are difficult to identify because of their high plasticity in morphological characters. *Pteropyrum* ( $C_{a}$ ) has a close affinity to *Calligonum* ( $C_{a}$ ) and is therefore a suitable case for  $C_4$  evolutionary studies. We investigated the morphology and micromorphology (including pollen morphology) of Pteropyrum and elucidated the phylogenetic relationships with Atraphaxis and Calligonum using nuclear ITS sequences. Characteristics of the photosynthetic tissues such as volume and number of layers of primary carbon assimilation tissues (PCA) and photosynthetic carbon reduction tissue (PCR) were studied. In addition, the leaf and cotyledon anatomical characters of *Pteropyrum* ( $C_3$ ), *Atraphaxis* ( $C_3$ ) and *Calligonum* ( $C_4$ ), and their  $\delta^{13}$ C values were compared to look for evolutionary changes in assimilating organs. The molecular phylogenetic tree identifies two strongly supported clades in *Pteropyrum* and its close relationship with *Calligonum*, confirming previous studies. Some morphologically similar species belong to different clades, which is probably due to convergent evolution and homoplasy. Leaf anatomical studies show that Atraphaxis has a multilayered mesophyll tissue, whereas *Calligonum* has one-layered mesophyll cells. The volume and layer number of mesophyll tissue cells decreases, whereas water storage tissue area significantly increases from Atraphaxis to Pteropyrum and Calligonum. This phenomenon confirms previous studies in other lineages with C<sub>4</sub> salsoloid anatomy that have evolved through increasing of water storage tissue and succulence of assimilating organs. In the taxonomic part of the paper, a key to identification of accepted taxa of *Pteropyrum*, description of species and distribution maps are presented based on numerous herbarium specimens and our own rich collections from the field. Four new species are described based on a combination of morphology of seedlings and mature plants, pollen morphology and molecular data. A subspecific classification is suggested to show morpho-geographical variation of *Pteropyrum aucheri s.l.* 

KEYWORDS:  $A traphaxis - C_4$  photosynthesis - Caryophyllales - cryptic speciation - desert flora - Irano-Turanian flora - molecular phylogeny - pollen morphology - proto-Kranz anatomy.

# INTRODUCTION

Polygonaceae belong to the non-core Caryophyllales and have a close affinity with Plumbaginaceae. They are well circumscribed with synapomorphies including an ochrea sheath, swollen nodes, five or six petaloid tepals, a single orthotropous ovule and usually trigonal achenes (Cuénoud *et al.*, 2002; Hernández-Ledesma *et al.*, 2015). Recently, Sanchez *et al.* (2011) revised the tribal classification of subfamily Polygonoideae using molecular data and proposed five newly circumscribed tribes of which Calligoneae consist of two poorly studied

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genera, Calligonum L. and Pteropyrum Jaub. & Spach. Close relationships between these two genera have been suggested in previous molecular (Sanchez, Schuster & Kron, 2009; Tavakkoli, Kazempour & Maassoumi, 2010; Schuster et al., 2015) and non-molecular (Haraldson, 1978; Ronse-Decraene & Akeroyd, 1988; Hong, 1995; Hong, Ronse-Decraene & Smets, 1998; Tavakkoli, Kazempour & Maassoumi, 2008) studies. Calligonum is the only  $C_4$  genus in Polygonaceae (Sage, Christin & Edwards, 2011), and species are leafless shrubby plants with assimilating stems with salsoloid anatomy (Muhaidat, Sage & Dengler, 2007). *Calligonum* is a species-rich genus with *c*. 80 species, widely distributed in sand dunes and desert areas of south-western and Central Asia, south-eastern Europe and North Africa. In contrast, Pteropyrum is a small genus with a limited distribution in Iran and adjacent countries (Turkmenistan, Afghanistan, Pakistan, Oman, UAE, and Iraq) (Brandbyge, 1993). Atraphaxis L. is a genus of C<sub>3</sub> shrubs; it was classified in tribe Polygoneae by Sanchez et al. (2011) and occurs widely in Irano-Turanian montane steppes. All these genera appear to have formed an important floristic component of the Irano-Turanian shrub steppes during the Quaternary glacial intervals (Djamali et al., 2008), the long persistence of which in these landscapes may have had a significant role in their evolutionary history.

Pteropyrum spp., with their small leaves (microphylls), are successful xerophytic shrubs usually growing near seasonal water runnels or dry riverbeds or on gypsum hills in desert areas and, rarely, on rocky slopes. Pteropyrum was first described with three species categorized into two sections according to fruit characters: Pteropyrum section Streptocarya Jaub. & Spach (P. aucheri Jaub. & Spach, P. olivieri Jaub. & Spach) and Pteropyrum section Orthocarya Jaub. & Spach (P. scoparium Jaub. & Spach) (Jaubert & Spach, 1846). In most cases, subsequent additional described species (Boissier, 1853; Meisner, 1856; Gilli, 1963) were not significantly different from the original three species; therefore, Rechinger & Schiman-Czeika (1968) accepted the occurrence of only three species in the Flora Iranica area: P. aucheri, P. noeanum Boiss. and P. olivieri. In studies following Flora Iranica, P. naufelum Al-Khayat was introduced from Iraq (Al-Khayat, 1993) and Akhani (2004) reported this species in Iran and synonymized P. noeanum with P. aucheri. Species boundaries in Pteropyrum are not precise, and species growing in different habitats are not easily distinguishable based on their morphology. Leaf size and shape are important diagnostic characters for distinguishing *Pteropyrum* spp., but these traits are often affected by environmental conditions, and there are many apparently intermediate forms that complicate species boundaries.

From an evolutionary point of view, it has been accepted that in some phylogenetic groups, C<sub>3</sub> sister species of C<sub>4</sub> plants exhibit some anatomical traits and subcellular events that are considered to be the initial phase of C, evolution. Anatomical traits such as enlarged bundle sheath (BS) cells and centripetal localization of mitochondria in BS cells form the proto-Kranz anatomy (Muhaidat et al., 2011; Sage, Busch & Johnson, 2013; Voznesenskaya et al., 2013; Sage, Khoshravesh & Sage, 2014). Proto-Kranz anatomy has some adaptive benefits and facilitates the glycine shuttle development towards C<sub>4</sub> evolution. Since *Pteropyrum* is the sister group of Calligonum distributed in hot/arid regions, it is not unexpected that some *Pteropyrum* spp. may show a proto-Kranz condition or even have C<sub>a</sub> photosynthesis. The aims of this paper are: (1) to revise the taxonomy of *Pteropyrum* and clarify the species circumscription using morphological, pollen morphological, anatomical and molecular traits; (2) to reconstruct phylogenetic relationships in *Pteropyrum* spp. and the sister genera using nuclear ITS (internal transcribed spacer) sequence data and testing the accuracy of traditional classifications and (3) to compare the anatomy of Pteropyrum with Atraphaxis  $(C_3)$  and Calligonum  $(C_4)$  to test our hypothesis that Pteropyrum as a sympatric desert plant with Calligonum undergoes anatomical changes towards C4 photosynthesis that finally evolved in Calligonum.

# MATERIAL AND METHODS

### TAXONOMY, MORPHOLOGY AND GROWTH CONDITION

Many natural populations of *Pteropyrum* have been studied in the field in different parts of its range in Iran and Oman by MD and HA. Herbarium specimens including type specimens (or photographs of these) have been studied in herbaria in Iran and Europe including B, G, P, IRAN, TARI, TUH, MIR, W, E and LE (abbreviations after Thiers, 2018+) and the private herbarium of the senior author (Herb. H. Akhani). The holotype specimens of the newly described taxa are preserved in IRAN.

Seeds collected during field studies have been cultivated in the laboratory under the same condition. They were grown in a mix of one part sand, one part clay and one part commercial peat. Seedling morphological traits were studied and measured under an Olympus BZ 21 stereomicroscope and photographed after emergence of the first true leaves using a Sony DSC-HX5Vi digital camera. Studied morphological characters of seedlings include hypocotyl height, cotyledon length, cotyledon width and hypocotyl indumentum.

#### POLLEN MORPHOLOGY

Pollen grains of 23 specimens of Pteropyrum were sampled from dried herbarium specimens and examined using a light microscope (LM) and scanning electron microscope (SEM). Pollen grains were first acetolysed following Erdtman (1960) and Dehghani & Akhani (2009). For LM studies, the pollen grains were mounted in glycerine jelly, and then observed by a Nikon Optiphot-2 light microscope and photographed with a Motic Image Plus 2.0 digital camera. Polar axis, equatorial axis, length of colpi, maximum distance between colpi in mesocolpium, apocolpium diameter and the thickness of exine, sexine and nexine were measured using calibrated Motic Image Plus 2.0 software. In general, the terminology follows Hong (1995) and Mondal (1997), taking into account the standardized pollen and spore terminology proposed by Punt et al. (2007). For SEM studies, acetolysed pollen grains were gold coated and examined using a Zeiss DSM 960 SEM microscope in the Electron Microscopy Laboratory, College of Science, University of Tehran, Iran.

#### ANATOMY OF ASSIMILATING ORGANS

Anatomical studies were conducted on cotyledons, leaf blades and young stem cross sections. Fresh leaf sections from cultivated plants or during field trips were fixed in FAA (one part formaldehyde, one part glacial acetic acid, 18 parts 70% ethanol), then rinsed in distilled water twice and dehydrated in a sequence of ethanol solutions. Acetone was used as the transition solvent to improve the infiltration. Samples were then embedded in pure epoxy resin and incubated for 24-48 h at 60 °C (Davies, 1999). Cross sections were made by Leica Ultracut UCT ultramicrotome (housed in College of Science, University of Tehran), stained with 0.5% (w/v) Toluidine blue in 1% (w/v) Na<sub>o</sub>CO<sub>o</sub> and studied with an Olympus BX51 LM. Cross sections from green stems of *Calligonum* were made by hand using a razor blade. Sections were then stained in aqueous methylene blue/carmine and photographed before dehydration. Dehydrated sections were permanently mounted in Euparal.

To compare the assimilating structures, the volume of primary carbon assimilation tissues (PCA or mesophyll cells), PCR tissues (PCR or BS), their ratios (PCA/PCR), percentages of epidermis and the percentages of aqueous tissues were measured for assimilating stems of seven *Calligonum* spp., mature leaves of six *Calligonum* spp., nine *Pteropyrum* taxa and four *Atraphaxis* spp. and the cotyledons of six *Calligonum* spp. and six *Pteropyrum* taxa. Our sampling strategy for comparative anatomy was based on multiple species of the compared genera rather than multiple sampling of the same species. The cell

volume of each specimen was measured for one section using ImageJ software (Rasband, 1997–2018).

#### DNA SEQUENCING

# Plant material and taxon sampling

All of the *Pteropyrum* samples used in this study were herbarium material mostly deposited in Herb. H. Akhani. The selected specimens cover almost all geographical distribution range and morphological diversity of *Pteropyrum*. Our ITS dataset includes 25 accessions representing all ten taxa of *Pteropyrum*. Four ITS sequences of *Atraphaxis* and *Calligonum* were taken from GenBank. Voucher information and GenBank accession numbers are provided in Appendix 1. In addition to ITS, we also sequenced two plastid markers (*rpl32* and *petD*) for several accessions. Due to failure to complete our data set and low resolution of the obtained trees, however, we prefer not to publish incomplete data.

#### DNA isolation, amplification and sequencing

Total genomic DNA was extracted using the modified CTAB method of Doyle & Doyle (1987). To amplify the ITS region (ITS1 and ITS2 spacers plus the 5.8S gene), the universal primer combination ITS4/ITS5 (White et al., 1990) was used following the protocol of Tavakkoli et al. (2010). Reactions were carried out in the volume of 25 µl, containing 10.5 µl deionized water, 12.5 µl Taq DNA polymerase master mix Red [Ampliqon; Tris-HCl pH 8.5, (NH<sub>4</sub>)<sub>3</sub>SO<sub>4</sub>, 4 mM MgCl<sub>2</sub>, 0.2% Tween 20, 0.4 mM each of dNTP, 0.2 units/µl Ampliqon Taq DNA polymerase, inert red dye and stabilizer], 0.5 µl each primer and 1 µl template DNA. Cycle sequencing reactions (PCR) were performed on a Techne TC-3000 thermocycler (Cole-Parmer, UK). Amplification conditions were an initial denaturation (1 min 30 s at 96 °C), 34 cycles of denaturation (30 s at 95 °C), annealing (1 min at 48 °C), extension (1 min 30 s at 72 °C) and a final extension step (20 min at 72 °C). PCR products were purified using the Avegene PCR cleanup kit following the manufacturer's protocols and sequenced via Macrogen Inc. (Seoul, South Korea). The same primers were used for amplification and sequencing.

# Sequence alignment and phylogenetic reconstruction

PhyDE v.0.9971 (Müller, Müller & Quandt, 2010) was used to edit the electropherograms. Dataset was aligned automatically using Muscle (Edgar, 2004) and edited manually using PhyDE. The ITS matrix was analysed using Bayesian Inference (BI), Maximum Likelihood (ML) and Maximum Parsimony (MP). *Atraphaxis spinosa* Jaub. & Spach and *A. suaedifolia*  L. were used to root trees based on the results of a previous phylogenetic study by Tavakkoli et al. (2010). The best model of molecular evolution was found using jModelTest v.2.1.10 (Darriba et al., 2012). The GTR+G model was found to fit best with the ITS region according to the Akaike information criterion (AIC). MrBayes v.3.2.6 (Ronquist et al., 2012) was used to conduct BI. Two parallel runs of four MCMC chains including three heated and one cold chain was run simultaneously for five million generations for each matrix. The first 25% of generations of each run were discarded and only the trees after the 'burn-in' sampled at a frequency of 1000. When the standard deviation of split frequencies was well below 0.01 the analyses were stopped. RAxML v.8.2.8 (Stamatakis, 2014) was used to conduct ML tree inference and bootstrapping (BS). For this purpose, the model was set to GTRGAMMA and bootstrap analyses were carried out with 1000 replicates. MP analyses were conducted using heuristic searches and tree lengths and homoplasy indices [consistency (CI), retention (RI), rescaled consistency (RC) and homoplasy indices (HI)] were calculated in PAUP v.4.a164 (Swofford, 1991). Bootstrapping (BS) was estimated in PAUP by conducting a heuristic search with 10 000 replicates using TBR branch swapping. Trees were visualized in TreeGraph2 v.2.10.1-641 beta (Stöver & Müller, 2010). All BI, ML and MP analyses were conducted on the Cipres portal (www.phylo.org).

#### CARBON ISOTOPE VALUES ( $\delta^{13}C$ )

The carbon isotope ratio was determined on green stems or leaves of herbarium materials or dried cotyledons of seedlings (Osmond *et al.*, 1975). The samples were first ground to a fine powder and placed in a tin capsule, then combusted in a Heraeus CHN Eurovector elemental analyser (GSF National Research Center for Environment and Health, Munich, Germany for four samples and Iso-Analytical Limited, Cheshire, UK, for other samples). The resulting N<sub>2</sub> and CO<sub>2</sub> were separated by gas chromatography and admitted into the inlet of a Finnigan MAT Delta S (GSF) or Micromass Isoprime Isotope Ratio Mass Spectrometer (IRMS) (Iso-Analytical Limited) for determination of 13C/12C ratios (R).  $\delta^{13}$ C values were calculated using the formula:

 $d = 1000 \times \left( R sample / R standard - 1 \right).$ 

#### RESULTS

#### TAXONOMY AND NOMENCLATURE

An updated revision of *Pteropyrum* is provided in the taxonomic treatment at the end of this paper. Based

on our multidisciplinary approach using morphomolecular data and field observations seven species and ten taxa are recognized in Pteropyrum. Selected figures are provided for the habitat and close up of studied Pteropyrum spp. and two species each of Atraphaxis and Calligonum (Figs 1–17). Widespread species P. aucheri and P. olivieri are merged and the morpho-geographical varieties are classified as subspecies: P. aucheri subsp. aucheri, P. aucheri subsp. olivieri (Jaub. & Spach) Doostmohammadi & Akhani, P. aucheri subsp. ericoides (Boiss.) Doostmohammadi & Akhani and P. aucheri subsp. noeanum (Boiss.) Doostmohammadi & Akhani. Pteropyrum scoparium Jaub. & Spach is restricted to Oman and the UAE and is well-distinguishable by its unique upper part of achenes that are not twisted as in other species. The other five species (P. jakdanense Doostmohammadi, P. macrocarpum Doostmohammadi & Akhani, P. naufelum, P. gypsaceum Akhani & Doostmohammadi and P. zagricum Doostmohammadi & Akhani) are rare plants usually growing on gypsum hills, shale or rocky habitats in a geographical arc from eastern Iraq to south-eastern Iran in Sistan-va Baluchestan Province.

#### MOLECULAR PHYLOGENY

For phylogenetic studies, we generated 21 ITS sequences, and four ITS sequences for *Atraphaxis* and *Calligonum* were taken from GenBank (see Appendix 1 for GenBank accessions). The length of the multiple sequence alignment of the ITS region is 800 characters that is reduced to 693 characters after trimming the ends. The analysed ITS matrix includes 112 potentially parsimony-informative characters. Table 1 shows the sequence statistics of the different matrices.

# ITS phylogenetic trees

The BI, ML and MP analyses of the ITS dataset produced congruent trees without any major difference in topology. Therefore, only the results from the Bayesian analyses are shown here, along with posterior probabilities as well as ML and MP bootstrap values. The phylogenetic tree (Fig. 18) strongly supports the monophyly of *Pteropyrum* (node B) and confirms Calligonum as its sister group (node C). Two strongly supported clades of *Pteropyrum* are detectable: (1) the *P. aucheri* clade [node D; PP = 0.99, BS (ML) = 100, BS (MP) = 100] and (2) the *P. naufelum* clade [node E; PP = 1, BS (ML) = 100, BS (MP) = 100]. The first clade (node D) is an unresolved group comprising several accessions of the highly variable and widely distributed P. aucheri s.l. (i.e. P. aucheri subspp. aucheri, ericoides, olivieri and noeanum) with P. scoparium. In the second clade (node E), P. gypsaceum is sister to the rest of the species. Three accessions of P. naufelum form a subclade



**Figures 1–8.** Natural habitat of several species of *Pteropyrum* and one species of *Calligonum* and *Atraphaxis*: Fig. 1. *P. naufelum* community growing on gypsum hills; Fig. 2. *P. zagricum* on limestone rocks; Fig. 3. *P. jakdanense* on shale hills; Fig. 4. *P. aucheri* subsp. *ericoides* along dry riverbank; Fig. 5. *P. scoparium* on gravelly dry rivulet beds; Fig. 6. *P. macrocarpum* in seasonal water runnel; Fig. 7. *C. amoenum*, sand dunes in Lut Desert; Fig. 8. *A. spinosa* on foothills and mountain slopes. Photographs: H. Akhani: 1, 5, 7, 8; M. Doostmohammadi 2, 3, 4, 6.

[node H; PP = 0.96, BS (ML) = 91, BS (MP) = 63] that is placed along with another group of species including *P. macrocarpum*, *P. jakdanense* and *P. zagricum* [node G; PP = 0.93, BS (ML) = 82, BS (MP) = 63].

# CARBON ISOTOPE COMPOSITION

Carbon isotope compositions of 24 samples of leaves, cotyledons or assimilating stems belonging to four *Atraphaxis* spp., six *Calligonum* spp. and nine taxa of *Pteropyrum* are given in Table 2. All assimilating shoots of *Calligonum* spp. have  $C_4$ -type carbon isotope ratios ranging from -15.13 to -13.96‰ (average of -14.55). Leaves of all studied *Atraphaxis* spp. have

 $\delta^{13}{\rm C}$  ranging from -29.35 to -26.17% (average of -27.65) and leaves of all *Pteropyrum* taxa have  $\delta^{13}{\rm C}$  rations ranging from -29.12 to -24.96% (average of -26.85), indicative of non-C<sub>4</sub> plants (C<sub>3</sub>, proto-Kranz or C<sub>2</sub> species). The values for three cotyledons of *Pteropyrum* are -29.06% (*P. aucheri* subsp. *ericoides*), -30.94% (*P. aucheri* subsp. *olivieri*) and -27.98% (*P. jakdanense*).

# $C_3$ and $C_4$ anatomy of assimilating parts

Selected cross sections of assimilating parts of the studied plants are illustrated in Figs 19–24 and 25–31. The percentages of PCA, PCR, PCA/PCR, epidermal



Figures 9-17. Close up of nine taxa of *Pteropyrum* in their natural habitats; Fig. 9. *P. aucheri* subsp. *ericoides*; Fig. 10. *P. aucheri* subsp. *aucheri*; Fig. 11. *P. aucheri* subsp. *olivieri*; Fig. 12. *P. jakdanense*; Fig. 13. *P. macrocarpum*; Fig. 14. *P. scoparium*; Fig. 15. *P. zagricum*; Fig. 16. *P. naufelum*; Fig. 17. *P. gypsaceum*. Photographs: H. Akhani: 9, 10, 11, 14, 16; M. Doostmohammadi 12, 13, 15, 17.

cells (EP) and water storage tissue (WST) for each plant are given in Supporting Information, Appendix S1). The averages of WST and PCA for leaves or assimilating stems of mature plants are shown in Figs 32 and 33. Based on configuration of photosynthetic cells, percentages of PCA, PCR and WST and the succulence of assimilating organs, four categories are recognized: (1) four *Atraphaxis* spp.; (2) five *Pteropyrum* taxa with flattened leaves; (3) four *Pteropyrum* taxa with terete leaves and (4) six *Calligonum* spp. having only assimilating stems as mature plants.

All four *Atraphaxis* spp. (category 1) investigated here were characterized by flattened ovate to elliptic leaves (Fig. 20), except *A. suaedifolia* having thickened

Table 1. Sequence and tree statistics for the ITS matrix

Complete dataset	
Number of taxa	27
Number of <i>Pteropyrum</i> taxa	23
Aligned length (bp)	800
Length range (bp)	364-703
Mean length (SD)	664.8 (44.4)
GC (%)	61.15
Dataset after trimming	
Aligned length (bp)	693
Length range (bp)	394-620
Mean length (SD)	591.3(67.59)
Transition-transversion ratio (Ti/Tv)	6.020
Divergence (%)	4.65
GC (%)	62.93
Percentage variable characters	18.61
Percentage potentially informative sites	16.01
Tree statistics	
Constant characters	564
Variable uninformative characters	17
Potentially parsimony-informative	112
characters	
Number of shortest trees	1056
Tree length	148
Consistency index (CI)	0.97
Retention index (RI)	0.98
Rescaled consistency index (RC)	0.95
Homoplasy index (HI)	0.03

linear leaves (Fig. 19). The leaves are bifacial with palisade cells on the adaxial and roundish spongy cells on the abaxial side. All species have a typical C<sub>a</sub> structure in leaf sections lacking a Kranz layer and BS. They also lack hypodermis with well-developed mesophyll cells in two to five layers. The number of mesophyll layers on the adaxial side is highest in A. suaedifolia (four or five layers, Fig. 19) and lowest in A. intricata (two or three layers); the other two species have three or four layers. The number of mesophyll layers of the spongy cells ranges from five or six layers in A. spinosa (Fig. 20) to two or three layers in A. intricata; the other two species have three to five layers. WST is not well-developed, consisting of only 2–6% among different species (Fig. 32). The mesophyll volume of mature leaves of Atraphaxis spp. is the highest among three studied genera with an average of 68.1% (Fig. 33; Supporting Information, Appendix S1).

Mature leaves of nine taxa of *Pteropyrum* and cotyledons of six *Pteropyrum* spp. (categories 2 and 3) were anatomically studied (Figs 21–24, Supporting Information, Appendix S1). Five taxa have flat leaves (*P. aucheri* subsp. *aucheri*, *P. olivieri*, *P. naufelum* and *P. gypsaceum* and *P. zagricum*) and the other four taxa

(P. aucheri subsp. ericoides, P. scoparium, P. jakdanense and P. macrocarpum) have more-or-less succulent leaves that are terete or semi-terete in cross section (Figs 9–17). All species lack a hypodermis layer with a ± equifacial leaves having two or three palisade layers on adaxial and abaxial sides. The palisade cells on the abaxial surface are slightly smaller than the adaxial ones. BS cells are enlarged in all species without chloroplast orientation, but no Kranz layer is developed in the species studied here. In species with cylindrical leaves the vascular bundles scattered around the WST each with a crescent-like layer of BS cells present both in adaxial and abaxial leaf sides (Fig. 21). The vascular bundles of taxa with flat leaves are horizontally scattered with BS cells occurring only on adaxial leaf side (Fig. 22). The PCA volume of both groups is slightly different on average (40.2% in terete leaves vs. 45.6% in flat leaves) and considering the high standard deviation, this feature is not statistically important (Fig. 33). The two groups are well distinguished in the volume of their WST. In flat leaves, WST occupies on average 24.8% of the leaf volume and in the cylindrical leaves it reaches 55.3% in P. scoparium, with an average of 34.7% among all species (Fig. 32; Supporting Information, Appendix S1).

The cotyledons of all studied *Pteropyrum* spp. are flat, even those with cylindrical mature leaves. Similar to mature leaves, the cotyledons of *Pteropyrum* lack hypodermis, but differ markedly in the bifacial configuration of mesophyll cells. The palisade layers consist of three to five layers on the adaxial side and two to five layers of spongy cells in the abaxial side (Figs 23, 24). Another main feature of the cotyledons is a well-developed BS tissue surrounding vascular bundles in both abaxial and adaxial sides (Figs 23, 24).

Anatomy of young stem, mature leaves and cotyledon leaves of seven Calligonum species from three different sections [category 4; Calligonum sections Calligonum, Pterococcus (Pall.) Endl. and Calliphyssa (Fisch. & C.A.Mey.) Endl.] were studied. Leaves and green stems are succulent, terete with a salsoloid Kranz anatomy and a distinct layer of hypodermis in cross section (Figs 25, 26, 29–31). The leaves appear only in young plants or in the early part of the growing season in spring. They soon die and the assimilating green stems take over the CO<sub>2</sub> fixation role. The WST in leaves of Calligonum, with an average of 39%, is higher than that in Atraphaxis and Pteropyrum, and the mesophyll volume, ranging from 22-35% (average of 28%), is the lowest among the three genera (Figs 32, 33, Supporting Information, Appendix S1). Small peripheral vascular bundles are oriented with their xylem side facing towards the BS layer. The anatomy of cotyledons is similar to true leaves, but differs from them in lacking the peripheral vascular bundles (Figs 27, 28).



**Figure 18.** Fifty percent majority-rule phylogenetic tree of *Pteropyrum* inferred from ITS dataset with Bayesian inference. Posterior probabilities obtained from BI (regular) and bootstrap support values for the same nodes found in ML analysis (**boldface**) are shown above branches, and bootstrap support values from MP analysis (*italics*) are indicated below branches.

The anatomy of the stem is markedly different from leaves and cotyledons in the formation of wellstructured supportive tissues of cholenchyma and sclerenchyma cells (Fig. 29–31). The cholenchyma bundles interrupt the hypodermis layer. The sclerenchyma tissue occurs on the outer and, in some cases, alternatively the inner side of central vascular bundles. The water storage cells occupy the largest volume of the stem both between sclerenchyma cells and the whole central parts of the stem. The Kranz ring encircles the complex composed of vascular bundles, WST and the sclerenchyma fibres (Fig. 30).

# SEEDLING MORPHOLOGY

In *Pteropyrum*, the hypocotyl length varies from 1.9-2.6 cm in *P. naufelum* to 5.2-5.6 cm in *P. jakdanense* (Figs 34-41). Apart from these two extremes, the hypocotyl height of most species is *c*. 3-4 cm on average (Table 3). The indumentum of hypocotyl and young stem is different among studied species.

*Pteropyrum aucheri* subsp. *olivieri*, *P. macrocarpum* and *P. zagricum* have glabrous hypocotyls, whereas other taxa are either sparsely papillose or, in the case of *P. jakdanense* (described here from southern Iran), densely papillose (Fig. 34).

#### POLLEN MORPHOLOGY OF PTEROPYRUM

Micrographs (LM and SEM) for ten taxa are shown in Figures 42–91. Measured pollen characters are presented in Table 4. Pollen grains of *Pteropyrum* are prolate-spheroidal to subprolate and trizonocolporate. The pollen size varies between different taxa with many overlapping (Fig. 92). The smallest grains were measured in one population of *P. aucheri* subsp. *aucheri* from Birjand (*Doostmohammadi* 4832) with average polar and equatorial lengths of 23.47 and 20.46  $\mu$ m, respectively. The largest grains were measured in one population of *P. naufelum* collected from northern Khuzestan (*Akhani* 9064-b) with average polar and equatorial lengths of 39.58 and 33.17  $\mu$ m, respectively.

**Table 2.** Carbon isotope values ( $\delta^{13}$ C) of some species of *Atraphaxis* and *Calligonum* and all known *Pteropyrum* species. Voucher specimens of those samples with only collector name and number are listed in the studied specimens of taxonomic treatment.

Species	Voucher	Analysed plant part	δ <sup>13</sup> C (‰)
Atraphaxis seravshanica Pavlov Atraphaxis spinosa L. Atraphaxis spinosa L. Atraphaxis suaedifolia Jaub. & Spach Atraphaxis suaedifolia Jaub. & Spach Atraphaxis tournefortii Jaub. & Spach Average of Atraphaxis Calligonum bungei Boiss. Calligonum caput-medusae Schrenk Calligonum crinitum Boiss.	<ul> <li>Iran: Golestan National Park, Akhani 11039</li> <li>Iran: Golestan National Park, Akhani 9832</li> <li>Iran: Golestan National Park, Akhani 9832</li> <li>Iran: Golestan National Park, Akhani 11702</li> <li>Iran: 38 km NW of Zanjan, Akhani et al. 22515</li> <li>Iran: East Azerbayjan: c. 20 km to Ahar, Wendelbo &amp; Assadi 17119</li> <li>Iraq: Sulaimaniya, Rechinger 10437</li> <li>Iran: Kerman, Doostmohammadi 4658</li> <li>Iran: 22 km N Aranbidgol, Akhani et al. 19769</li> <li>Iran: 11 km NE Aranbidgol, Akhani 21379-b</li> <li>Iran: South Khanagan, Yandan</li> </ul>	Leaf Leaf Leaf Leaf Leaf Leaf Green stem Green stem	$\begin{array}{r} -26.78\\ -29.35\\ -26.80\\ -29.04\\ -26.17\\ -27.80\\ 27.65\\ -14.88\\ -13.96\\ -15.13\\ 14.65\end{array}$
Calligonum persicum Boiss. Calligonum polygonoides L. Average of Calligonum Pteropyrum aucheri subsp. aucheri Pteropyrum aucheri subsp. ericoides Pteropyrum aucheri subsp. ericoides Pteropyrum aucheri subsp. olivieri Pteropyrum gypsaceum Pteropyrum jakdanense Pteropyrum jakdanense Pteropyrum naufelum Pteropyrum naufelum Pteropyrum scoparium Pteropyrum zagricum Average of Pteropyrum (excluding cotyledon values)	Doostmohammadi 4838 Gilan: Near Rudbar, Akhani & Salimain 14345 Azarbaijan, 44 km NW Poldasht, Akhani 18951 Dehghani et al. 4788 Doostmohammadi 4826 Doostmohammadi 4830 Akhani 18571 Doostmohammadi 4673 Doostmohammadi 4861 Doostmohammadi 4861 Doostmohammadi 4861 Doostmohammadi 4729 Doostmohammadi 4729 Doostmohammadi & Noormohammadi 4875 Akhani 24638 Doostmohammadi 4898	Green stem Green stem Leaf Leaf Cotyledon leaf Leaf Leaf Cotyledon leaf Leaf Leaf Leaf Leaf Leaf Leaf Leaf L	$\begin{array}{c} -14.62 \\ -14.07 \\ -14.55 \\ -25.14 \\ -28.40 \\ -29.06 \\ -25.47 \\ -30.94 \\ -25.62 \\ -24.96 \\ -27.98 \\ -27.02 \\ -28.21 \\ -29.12 \\ -27.76 \\ -26.85 \end{array}$

Among the pollen grains of different subspecies of P. aucheri examined here, those of P. aucheri subsp. aucheri had the smallest grains with an average polar length (Pl) of 24.4  $\mu$ m, followed by its close relatives P. aucheri subspp. ericoides (Pl = 30.2 µm), olivieri  $(Pl = 30.8 \ \mu m)$  and *noeanum*  $(Pl = 35.1 \ \mu m)$ . The polar/ equatorial ratio (P/E) of all taxa ranges from 1.1 to 1.3. Other pollen characters such as colpus length, maximum length of mesocolpium and apocolpus diameter show more-or-less similar patterns as pollen size (Table 4). The exine thickness among different species is not significantly different with a range of 1.8-2.4 µm thick. The SEM micrographs of pollen ornamentation provide evidence of two distinct pollen types with different exine sculptures: P. naufelum is characterized by a suprareticulate surface deviating from all other taxa that have a semi-striate tectum

perforatum (Figs 82–91). *Pteropyrum naufelum* also shows other morphological features that make it distinct from other *Pteropyrum* spp., and we provide a more detailed description here.

The exine of *P. naufelum* shows a constant thickness all around the pollen. The ectocolpi are bordered by costae (costa ectocolpi) that thin out towards the colpus ends. Endopori are not always present, but when present are mostly circular to elliptic with the longer axis of the ellipse oriented along the polar axis. Endopori also have costae (costa endopori). Observation under phase contrast at  $1000 \times$  magnification reveals that the columellae mostly occur in groups of five or six. The exine is reticulate with muri formed by fusion of the apices of columellae and organized in a real reticulate and not semi-striate pattern.



**Figures 19–24.** Cross sections of leaves (19–22 and 23–24) and cotyledons (21–22) of *Atraphaxis* and *Pteropyrum*; Fig. 19. A. suaedifolia, Dehghani et al. 4814; Fig. 20. A. spinosa, Akhani 24156; Fig. 21 P. naufelum, seed grown from from Doostmohammadi & Noormohammadi 4876; Fig. 22. P. olivieri, seeds grown from Doostmohammadi 4673; Fig. 23. P. jakdanense, Doostmohammadi 4879; Fig. 24. P. naufelum, Doostmohammadi & Noormohammadi 4876. Arrows represent the enlarged bundle sheath cells.



**Figures 25–31.** Cross-sections of leaves (25–26), cotyledon leaves (27–38) and green stems (29–31) of *Calligonum*. Fig. 25. *C. bungei*, *Doostmohammadi* 4654; Fig. 26. *C. polygonoides*, *Doostmohammadi* 4669; Fig. 27. *C. crinitum*, *Doostmohammadi* 4675; Fig. 28. *C. polygonoides*, *Doostmohammadi* 4669; Fig. 29. *C. denticulatum*, *Doostmohammadi* 4669; Fig. 30. *C. crinitum*, *Doostmohammadi* 4678; Fig. 31. A single peripheral vascular bundle oriented with its xylem side facing towards the Kranz cells. Pa – palisade cells; kr – Kranz cells; xy – xylem; ph – phloem; ws – water storage cells; cb – central bundles; pb – peripheral bundles; hy – hypodermis; ep – epidermis.



**Figures 32–33.** Proportion of WST (Fig. 32) and PCA (or  $C_3$  mesophyll, Fig. 33) among leaves of *Atraphaxis*, flat leaved species group of *Pteropyrum*, cylindrical leaved species group of *Pteropyrum*, *Calligonum* leaf and *Calligonum* green stems. Vertical lines indicate standard deviations.

# DISCUSSION

# DIVERSITY OF PTEROPYRUM

Pteropyrum is a small exclusively Irano-Turanian genus with several xeromorphic traits, namely sturdy rigid branches and small, more-or-less succulent leaves that often fall in extremely hot and dry seasons (Figs 9–17, 94–101). The species have a limited range in Iran and neighbouring countries including Iraq, Afghanistan, Pakistan, Turkmenistan, the UAE and Oman (Rechinger & Schiman-Czeika, 1968; Nyberg & Miller, 1996; Breckle, Hedge & Rafiqpoor, 2013; Edmondson & Akeroyd, 2016) (Figs 102–107). Occurrence of the genus in Turkey is not confirmed as the plant material cited in *Flora of Turkey* (*Nâbelek* 463, Cullen, 1967) belongs to *Atraphaxis*. So far, only four species have been accepted in the mentioned standard floras (P. aucheri, P. naufelum, P. olivieri and *P. scoparium*). Our multidisciplinary approach revealed presence of additional species particularly in the southern distribution area. These species have been unknown for the botanists due to (1) limited collection and the overlooking of small populations in southern and western Iran and (2) the limitation of classical morphological characters in delineating species diversity in oligospecific genera. In particular, the molecular techniques provide unequivocal insight into species diversity and uncovering cryptic species with scarce morphological characters (Bickford et al., 2006: Shnever & Kotseruba, 2015). Furthermore, relying only on morphological data from herbarium specimens is not informative enough to distinguish species diversity in critical taxa. Therefore, we examined applicability of other characters such as anatomy, micromorphology of epidermis (not presented in this paper), pollen morphology and seedling structure. This approach recently led to the discovery of two additional species of *Bienertia* Bunge with a similar distribution range, which previously was considered as monotypic (Akhani et al., 2005, 2012). We found the importance of habitat, geography and climate as crucial bases for understanding the species area and species delimitation in closely related species groups with minor distinguishing characters in the Irano-Turanian region. Based on combined morpho-molecular data we describe here four new species (P. gypsaceum, P. macrocarpum, P. jakdanense and *P. zagricum*).

The most widespread species in the area is the *P. aucheri* complex, treated here in a broad sense to include *P. olivieri*. We hypothesized that species boundaries in sympatric microspecies are not established and therefore suggested a subspecific classification with four subspecies representing geographical races correlated with some morphological characters (see taxonomic enumeration).

### PHYLOGENY AND CLASSIFICATION

Our sequence analysis of the nuclear (ITS) marker supports the monophyly of *Pteropyrum* as sister to *Calligonum* (Fig. 18), confirming previous studies (Tavakkoli *et al.*, 2010; Sanchez *et al.*, 2009; Schuster *et al.*, 2015). The ITS tree resulted in two highly supported clades in *Pteropyrum*. The *P. aucheri* clade (node D) includes four subspecies of *P. aucheri* recognized in this paper in addition to *P. scoparium*, an endemic species of easternmost parts of the Arabian Peninsula (Fig. 107). Jaubert & Spach (1846) recognized two sections in *Pteropyrum* that are distinguished based on fruit morphology: *P.* section *Orthocarya* characterized by non-rotated upper lobes



Figure 34-41. Seedlings of several species of *Pteropyrum*. Fig. 34. *P. jakdanense*, inset shows the pubescent hypocotyl; Fig. 35. *P. macrocarpum*; Fig. 36. *P. aucheri* subsp. *aucheri*; Fig. 37. *P. gypsaceum*; Fig. 38. *P. aucheri* subsp. *olivieri*; Fig. 39. *P. zagricum*; Fig. 40. *P. aucheri* subsp. *ericoides*; Fig. 41. *P. naufelum*.

Taxa	Hypocotyl length (cm)	Hypocotyl indumentum	
P. aucheri subsp. aucheri	$3.2-3.8(3.6\pm0.1)$	Sparse papillose	
P. aucheri subsp. ericoides	$3.4-3.8(3.6 \pm 0.1)$	Sparse papillose	
P. aucheri subsp. olivieri	$3.2-3.6(3.3 \pm 0.3)$	Glabrous	
P. gypsaceum	$3.5-4.4$ ( $4.2 \pm 0.1$ )	Sparse papillose	
P. jakdanense	$5.2-5.6(5.3 \pm 0.5)$	Dense papillose	
P. macrocarpum	$3.9-4.3$ ( $4.1 \pm 0.2$ )	Glabrous	
P. naufelum	$1.9-2.6(2.3 \pm 0.2)$	Sparse papillose	
P. zagricum	$3.5-4.3(3.6 \pm 0.2)$	Glabrous	

**Table 3.** The length and indumentum of hypocotyls of eight taxa of *Pteropyrum*. The minimum, maximum and average values and their standard deviations  $(\pm)$  are given based on ten measurements

and *P*. section *Streptocarya* with rotated upper fruit lobes. *Pteropyrum scoparium* is the only representative of section *Orthocarya* and the remaining species belong to section *Streptocarya*. This sectional classification based on fruit morphology is not confirmed in our phylogenetic tree (Fig. 18).

There is no obvious morphological trait characterizing the P. aucheri and P. naufelum clades. Ecologically, all members of P. aucheri clade inhabit dry riverbeds, but most of the species of P. naufelum clade inhabit gypsum, rocky or shale habitats with the exception of *P. macrocarpum* that grows on riverbeds, similar to members of the P. aucheri clade. All species of P. naufelum clade have restricted ranges in an arc extending from the Zagros foothills of the Iran-Iraq border to the south-eastern parts of Iran with a similar climate as an ecotone between the Irano-Turanian and tropical climates (Djamali et al., 2011). Occurrence of terete and flat leaf types in both clades supports the convergent evolution of terete leaves from flat leaves pushed by ecological pressures (Nyffeler *et al.*, 2008; Ogburn & Edwards, 2013). Leaf shape diversity in P. aucheri is an example of the role of habitat and climate on leaf morphology: P. aucheri subsp. olivieri with flat leaves inhabits mountain areas with moderate climate and more water supplies; P. aucheri subsp. aucheri with narrower leaves grows in drier habitats partly sympatric with P. aucheri subsp. olivieri (Fig. 102) and P. aucheri subsp. ericoides with almost terete leaves penetrates further into desert areas in central and eastern parts of Iran (Fig. 104).

# $\mathbf{C}_4$ evolution in polygonaceae

Based on leaf anatomy and carbon isotope values, all examined members of *Calligonum* show  $C_4$ photosynthesis, in agreement with previous studies (Winter *et al.*, 1977; Winter, 1981; Muhaidat *et al.*, 2007). The green assimilating shoots including young leaves early in the growing season and green annual stems in *Calligonum* exhibit  $C_4$  photosynthesis. *Calligonum* seeds have a large amount of endosperm, serve as storage organs and the tiny cotyledons have no role in the storage of organic substances. However, as the seeds germinate, cotyledons grow rapidly and, in most cases, they grow to > 5 cm in length. The cotyledons perform  $C_4$  photosynthesis and have an important function in seedling establishment and early carbon gain prior to emergence of true leaves.

Anatomy of cotyledons and mature leaves are not always the same and these two organs may have different anatomical or even different photosynthetic types (Akhani & Ghasemkhani, 2007; Akhani & Khoshravesh, 2013; Pyankov et al., 1999; Pyankov et al., 2000). Anatomy of cotyledons in Calligonum is also different from salsoloid anatomy of leaves and/ or green stems and is similar to salsinoid anatomy. Salsinoid anatomy, which was first introduced by Carolin, Jacobs & Vesk (1975) and then by Jacobs (2001) under the name of Kranz-suaedoid, is different from salsoloid anatomy in lacking the peripheral vascular bundles (Edwards & Voznesenskaya, 2011) and has only previously been detected in Suaeda section Salsina Moq. (Schütze, Freitag & Weising, 2003). Anatomy of cotyledons in *Calligonum* can be regarded as a variant of this type containing a distinct layer of hypodermis.

In most cases, vein density decreases with an increase in leaf succulence, and consequently interveinal distances increases. Vein density decrease leads to a longer transport distance between veins and mesophyll cells that can affect photosynthesis and restrict whole plant growth (Ogburn & Edwards, 2013). Therefore, evolution of succulent plants is restricted, and these plants have to evolve an efficient leaf venation system prior to or while becoming succulent. As a solution it is proposed that, in multiple phylogenetic groups, three-dimensional (3D) venation patterns can maintain the transport pathway in a moderate distance that allows tissues to store a large



**Figures 42–61.** Light microscopy images of pollen grains of five taxa of *Pteropyrum*. Figs 42–45. *P. aucheri* subsp. *aucheri*, *Dehghani et al.* 4788; Figs 46–49. *P. aucheri* subsp. *noeanum*, type specimen of *P. noeanum*, *Noe* 1002; Figs 50–53. *P. aucheri* subsp. *ericoides*, *Doostmohammadi* 4825; Figs 54–57. *P. aucheri* subsp. *olivieri*, *Akhani & Zarrinpour* 14979; Figs 58–61. *P. scoparium*, *Kurschner* 99-33. Scale bar = 10 µm.



**Figures 62–81.** Light microscopy images of pollen grains of five species of *Pteropyrum*. Figs 62–65. *P. naufelum, Akhani* 21580; Figs 66–69. *P. macrocarpum, Doostmohammadi* 4729; Figs 70–73. *P. jakdanense, Doostmohammadi* 4879; Figs 74–77. *P. gypsaceum, Akhani* 16001; Figs 78–81. *P. zagricum, Bagheri et al.* 4780. Scale bar = 10 µm.



**Figures 82–91.** Scanning electron micrographs of pollen grains of ten taxa of *Pteropyrum*. Fig. 82. *P. aucheri* subsp. *aucheri*, *Dehghani et al.* 4788; Fig. 83. *P. naufelum*, *Akhani* 21580; Fig. 84. *P. aucheri* subsp. *noeanum*, type specimen of *P. noeanum*, *Noe* 1002; Fig. 85. *P. macrocarpum*, *Doostmohammadi* 4729; Fig. 86. *P. aucheri* subsp. *olivieri*, *Akhani & Zarrinpour* 14979; Fig. 87. *P. gypsaceum*, *Akhani* 16001; Fig. 88. *P. aucheri* subsp. *ericoides*, *Doostmohammadi* 4825; Fig. 89. *P. jakdanense*, *Doostmohammadi* 4879; Fig. 90. *P. scoparium*, *Kürschner* 99-33; Fig. 91. *P. zagricum*, *Bagheri et al.* 4780. Scale bars = 5 µm (A); 1 µm (B).

Taxon name	Polar length	Equatorial length	P/E	Colpus length	Mesocolpium Maximum distance	Apocolpus diameter	Exine thickness
P. aucheri subsp. aucheri							
Doostmohammadi 4832	$23.47 \pm 1.7$	$20.46 \pm 1.7$	1.14	$18.07 \pm 1.6$	$17.1 \pm 2.5$	$14.55 \pm 3.1$	$1.94 \pm 0.1$
Dehghani et al. 4788	$25.09 \pm 1.2$	$22.25 \pm 1.1$	1.12	$19.74 \pm 1.5$	$16.69 \pm 0.9$	$12.73 \pm 1.6$	$1.83 \pm 0.1$
Dehghani et al. 4782	$25.72 \pm 1.5$	$23.21 \pm 1.4$	1.11	$20.92 \pm 1.5$	$18.99 \pm 2.4$	$16.37 \pm 2.9$	$2.07 \pm 0.1$
Akhani et al. 17728	$23.38 \pm 1.3$	$21.82 \pm 1.1$	1.1	$20.09 \pm 1.3$	$16.86 \pm 1.7$	$14.35 \pm 1.9$	$1.9 \pm 0.1$
Average	24.4	21.9	1.11	19.7	17.4	14.5	1.93
P. aucheri subsp. noeanum							
Noë 1002	$35.5 \pm 2.8$	$28.1 \pm 1.7$	1.26	$29.9 \pm 2.9$	$19.46 \pm 0.1$	$18.06 \pm 0.4$	$2.03 \pm 0.1$
Dinarvand 10142	$34.7 \pm 2.1$	$28.3 \pm 2.3$	1.23	27±1.3	$17.5 \pm 0.1$	$14.9 \pm 3.5$	$2.2 \pm 0.1$
Average	35.1	28.2	1.24	28.4	18.5	16.5	2.1
P. aucheri subsp. ericoides							
Doostmohammadi 4824	$29.5 \pm 1.3$	$24.84 \pm 1.4$	1.2	$22.63 \pm 1.3$	$18.58 \pm 1.2$	$16.48 \pm 1.2$	$2.16 \pm 0.2$
Doostmohammadi 4825	$30.62 \pm 1.5$	$26.29 \pm 1.6$	1.16	$24.71 \pm 1.6$	$20.05 \pm 2.4$	$17.58 \pm 3.5$	$2.02 \pm 0.1$
Doostmohammadi 4826	$30.61 \pm 1.9$	$25.36 \pm 1.5$	1.2	$24.24 \pm 1.9$	$18.82 \pm 1.5$	$15.77 \pm 1.8$	$1.91 \pm 0.2$
Average	30.2	25.5	1.18	23.9	19.2	16.6	2.03
P. aucheri subsp. olivieri							
Doostmohammadi 4852	$28.07 \pm 1.3$	$24.73 \pm 1.1$	1.13	$22.81 \pm 1.3$	$17.57 \pm 1.8$	$16.5 \pm 2$	$1.9 \pm 0.18$
Akhani & Zarrinpor 14797	$28.32 \pm 1.1$	$23.65 \pm 1$	1.2	$23.1 \pm 1.2$	$18.97 \pm 1.3$	$16.25 \pm 2.5$	$1.98 \pm 0.2$
Akhani 1221	$34.81 \pm 2.7$	$26.26 \pm 1.7$	1.32	$28.89 \pm 1.8$	$20.39 \pm 2.6$	$17.95 \pm 2.6$	$2.14 \pm 0.2$
Doostmohammadi 4730	$30.58 \pm 2.1$	$26.95 \pm 2.2$	1.13	$24.57 \pm 1.9$	$21.1 \pm 2.5$	$18.52 \pm 2.8$	$1.89 \pm 0.2$
Doostmohammadi 4673	$31.5 \pm 1.6$	$26 \pm 1.1$	1.21	$25.4 \pm 1.9$	21±2.8	$20.5 \pm 4.3$	$1.9 \pm 0.2$
Akhani et al. 18571	$31.75 \pm 1.9$	$26.09 \pm 1.2$	1.21	$26.17 \pm 1.8$	$20.37 \pm 2.5$	$18.63 \pm 2.8$	$1.98 \pm 0.2$
Average	30.8	25.6	1.2	25.2	19.9	18.1	1.96
P. gypsaceum							
Akhani 16001	$28.77 \pm 1.2$	$25.63 \pm 1.5$	1.12	$23.61 \pm 1.5$	$25.57 \pm 3.9$	$16.95 \pm 1.6$	$1.91 \pm 0.2$
P. jakdanense							
Doostmohammadi 4879	$27.4 \pm 1.8$	$22.7 \pm 1.4$	1.2	$21.3 \pm 1.5$	$21.7 \pm 2.7$	$20.5 \pm 2.4$	$1.8 \pm 0.15$
P. macrocarpum							
Doostmohammadi 4729	$38.7 \pm 2.4$	$33.8 \pm 3.5$	1.14	$31.74 \pm 2.5$	$30.05 \pm 0.5$	$27.6 \pm 1.2$	$2.37 \pm 0.1$
P. naufelum							
Akhani 9064-b	$39.58 \pm 2.4$	$33.17 \pm 1.4$	1.2	$32.46 \pm 2.1$	$25.4 \pm 2.9$	$24.13 \pm 3.4$	$2.3 \pm 0.1$
Akhani 9049	$32.07 \pm 1.8$	$28.39 \pm 1.8$	1.13	$26.48 \pm 1.7$	$23.08 \pm 2.6$	$22.08 \pm 3.4$	$1.8 \pm 0.2$
Akhani 21580	$37.25 \pm 2.3$	$32.94 \pm 2.3$	1.13	$31.27 \pm 2.1$	$23.64 \pm 1.3$	$22.46 \pm 1.7$	$1.94 \pm 0.2$
Average	36.3	31.5	1.15	30.1	24.1	22.9	2.01
P. scoparium							
Kürschner 99-33	$30.1 \pm 2.7$	$25 \pm 1.9$	1.2	$24.4 \pm 3.1$	$23 \pm 3.1$	$21.5 \pm 2.8$	$2.1 \pm 0.2$
P. zagricum	····				··· — •··		
Bagheri et al. 4780	$30 \pm 1.5$	$26.4 \pm 1.4$	1.14	$23.6 \pm 1.5$	$20.9\pm2.4$	$19.7\pm2.8$	$1.81 \pm 0.11$

Table 4. Pollen morphological data for *Pteropyrum* with mean and standard deviations based on 30 pollen grains. All measurements are in  $\mu$ m

amount of water and become succulent (Griffiths, 2013; Ogburn & Edwards, 2013). Ogburn & Edwards (2013) identified two types of 3D venation (types I and II), which differ in the orientation of xylem and phloem strands in abaxial veins. They proposed that 3D type I evolved through the adaxialization procedure. This process is well represented in *Pteropyrum*. Mean WST percentage in *Atraphaxis*, planar leaved *Pteropyrum*, terete leaved *Pteropyrum* and *Calligonum* are 4.9, 24.8, 34.7 and 39.2%, respectively (Fig. 32). *Atraphaxis* 

leaves have little WST, and vascular bundles are normally arranged in the middle of the leaf with their xylem strands facing towards the adaxial surface. Planar leaved *Pteropyrum* spp. are more succulent than *Atraphaxis* and WS cells are placed between the veins and abaxial mesophyll. Therefore, vascular bundles contact the adaxial mesophyll from their xylem side while their phloem side is facing towards the WST. By diminishing the abaxial leaf surface and increase in WST, planar leaves gradually convert to



**Figure 92.** Variation in polar axis length of pollen grains among different populations of *Pteropyrum*. The middle point of each horizontal line indicates the average of 30 counts. Different species are separated by solid lines.

terete leaves with 3D vein systems in which main veins are in the middle of WST and peripheral veins are oriented with their xylem side facing the mesophyll (Fig. 93). Terete leaves in Pteropyrum exhibit a sympegmoid anatomy with two or three layers of mesophyll cells. It has been suggested that salsoloid anatomy has evolved from sympegmoid anatomy in ancestral plants (Voznesenskaya et al., 2013). Kadereit, Askerly & Pirie (2012) showed that in the evolution of succulent  $C_4$  plants (including salsoloid anatomy), succulence and salt tolerance have evolved prior to  $\mathrm{C}_4.$  Hence, succulence is a precondition in the evolution of salsoloid anatomy and on the other hand an efficient vein system (like 3D venation) is required for development of succulence. Occurrence of 3D venation and the consequential

improvement of succulence in salsoloid anatomy have occurred via an adaxialization procedure that is also demonstrated in the leaf anatomy of *Pteropyrum*. It is also proposed here that something opposite to adaxialization (abaxialization) probably occurred during the evolution of tecticornioid anatomy, as the peripheral veins are oriented in the opposite direction to that of salsoloid anatomy.

The polyphyletic origin of  $C_4$  photosynthesis in a few families indicates that there are some preconditioning traits in distinct  $C_3$  plants that increase the potential for  $C_4$  evolution (McKown, Mocalvo & Dengler, 2005; Khoshravesh *et al.*, 2012). Among these preconditions are some anatomical enablers including high vein density and enlarged BS cells (Sage, 2001; Christin *et al.*, 2012; Griffiths *et al.*, 2013). It is largely accepted



**Figure 93.** Evolutionary transition of planar leaves to terete leaves along with conversion of 2D venation type to 3D. A. *Atraphaxis spinosa*; B. *P. aucheri* subsp. *aucheri* and C. *P. jakdanense*. Vascular bundles in planar leaves are oriented with their xylem cells (blue cells) contacting the adaxial leaf mesophyll and phloem cells (red cells) facing towards the abaxial side (Fig. 93B). In terete leaves in both adaxial and abaxial sides, xylem sides of the peripheral vascular bundles are connected to mesophyll cells and their phloem sides are facing towards the central aqueous tissue (Fig. 93C).

that in the evolutionary trajectory of C<sub>4</sub> plants, C<sub>9</sub> Kranz has evolved from C3 ancestors with enlarged BS cells (known as proto-Kranz) (Muhaidat et al., 2011; Sage et al., 2014). In Pteropyrum spp., M tissue area is relatively low (42.9%) compared to Atraphaxis (68.1%) (Fig. 33), and BS cells are enlarged, which may reflect the presence of some anatomical preconditions for  $C_4$  photosynthesis in these species. All the studied species have almost enlarged BS cells; however, PCR percentage is the highest in P. aucheri subsp. aucheri (4.1%) and P. jakdanense (3.8%) among planar leaved and terete leaved species, respectively. These initial steps in the evolution of  $C_4$  are not generally detectable by carbon isotope observations, however, close phylogenetic relationships of Pteropyrum with Calligonum and anatomical evidence, such as enlarged

BS cells, increased WST percentage and presence of 3D venation in some species, implies that some *Pteropyrum* spp. more probably exhibit proto-Kranz or  $C_2$  conditions. However, final confirmation of this hypothesis will require additional studies.

### PALYNOLOGICAL APPLICATION

Pollen morphological characters indicate that *P. nafelum* pollen approaches the *Fagopyrum* esculentum Moench type (Leeuwen, Punt & Hoen, 1988), but with the reticulate structure being more visible and the pollen size variation being more significant in the latter pollen type. In a pollen morphological point of view, the examined pollen of *Pteropyrum* can thus be easily divided into two pollen



**Figures 94–101.** Morphological traits used for species delimitation of *Pteropyrum*. Fruit shapes. Twisted upper wing lobes in *P. aucheri* subsp. *aucheri* (Fig. 94) and *P. aucheri* subsp. *olivieri* (Fig. 95) and straight upper wing lobes in *P. scoparium* (Fig. 96). Three different leaf shapes: spathulate (Fig. 97), linear (Fig. 98) and cylindrical (Fig. 99). Leaf indumentum: glabrous leaf in *P. jakdanense* (Fig. 100, scale bar = 100  $\mu$ m); pubescent leaf in *P. macrocarpum* (Fig. 101, scale bar = 150  $\mu$ m).







106. P. zagricum (dot) and P. macrocarpum (triangle)



Figures 102-107. Distribution maps of *Pteropyrum* species.

types, the P. aucheri and P. naufelum types. The latter type is mainly distinguished by its larger size and suprareticulate ornamentation.

Our palynological results are important in ecological interpretation of fossil pollen data in that they help to distinguish between three pollen types in the Calligonum-Pteropyrum group. This group includes the Calligonum type, indicator of desertification and sand dune expansion, the P. aucheri type, an indicator of desertic areas with welldrained soils and the *P. naufelum* type that can provide two sorts of information, i.e. hot desert conditions with soils containing a high content of gypsum. This information can thus be useful for palaeoecologists working on the Quaternary plant ecology of interior south-west Asia.

103. P. aucheri subsp. olivieri

105. P. naufelum (dot) and P. gypsaceum (triangle)







102. P. aucheri subsp. aucheri (dot), subsp. noeanum (triangle)

### TAXONOMIC REVISION OF PTEROPYRUM

# Morphology and nomenclature

An updated synopsis of the genus including identification key to species, descriptions and distribution maps of all accepted *Pteropyrum* spp. is provided. The importance of habitat and climate is discussed above. Some morphological characters used in delimitation and circumscription of the species are presented in Figures 94–101.

Pteropyrum Jaub. & Spach, Ill. Pl. Orient. 2: 7. 1844. Type: Pteropyrum aucheri Jaub. & Spach.

Shrubby plants; leaves fasciculate; flowers in cluster, pedicels jointed near the base or in the middle, associated with leaf clusters, bisexual, perianth segments five, connate at base, petaloid, white inside, three inner segments slightly larger than two outer segments, inner segments slightly accrescent in fruit, each segment with a greenish central part; stamens eight (three + five), three longer ones opposite to the larger perianth segments, five shorter ones alternate with perianth segments, filament barbellate at base, anthers reddish, versatile; carpels three, style short, with three capitate red stigma; fruit achene, with three usually reddishmembranous wings, wings bilobed, upper parts of fruit in the same direction of lower or twisted at 120°.

# Key to identification of the species

- 1. Leaves flat, oblanceolate to obovate, linear, > 1 mm wide, not terete in cross section ......5

- 4. Gemmae on leafy branches absent, if present short and occurring only in lower leafy branches; hypocotyl 3-4 cm long, glabrous ...... *P. aucheri* subsp. *ericoides*

- 6. Leaves narrowly linear, leaf length more than six times as long as wide ......7
- The polar length of pollen grains 23.3–25.7 μm on average, distribution in central and eastern Iran .....P. aucheri subsp. aucheri
- The polar length of pollen grains 34.7-35.5 μm on average, distribution in south-western parts of Zagros in Iran and adjacent Iraq ..... P. aucheri subsp. noeanum
- Erect shrub, up to 1.5 m height; leaves oblanceolate, acute at apex; habitat on dry water runnels ..... P. aucheri subsp. olivieri

- 9. Leaves 8–12 mm × 3–4 mm, subacute to minutely mucronate, leaves and young branches always densely pubescent-papillose, flowers occurring in winter or early spring, pollen grain ornamentation punctate, polar length 28.77  $\mu$ m on average, on saline-gypsum habitats near the Persian Gulf shores .......*P.gypsaceum*

Pteropyrum aucheri Jaub. & Spach, Ill. Pl. Or. 2: 8 tab. 107 (1844)

*Type:* In collibus aridis Persiae septentrionalis, ad amnem Kizil Ouzein, *Aucher 5269* (Lectotype: P-00734256!, selected here; isotypes: P-00734257, P-00734258, P-00734259. K000830354, K!, LE!).

Description: Shrub, 0.5–1.7 m height, richly branched, rigid, bark of young and mature branches greyish white, older branches darker, young twigs parallel striate; leaves three to seven (to 12) in each cluster, on a short elevated base, the remnants of dead leaves on a very short 0.5–1.0 (2.0) mm gemmae; leaf blades  $5-16 \times 1-6$  mm, glabrous or pubescent, variable in shape, linear-filiform, linear subulate, oblong,

lanceolate, elliptic to obovate, ± succulent, terete to oval in cross section, flat leaves slightly revolute at margin, acute to obtuse at apex, ± sessile or with a short petiole; ochrea membranous, 0.5–0.7 mm long; flowers bisexual, clustered, pedicles capillary, 2.0-5.5 mm long, jointed near the base and rarely up to the middle; perianth segments white at the margins with a green part in the middle, slightly accrescent in fruit, three internal segments  $2.0-2.8 \times 1.5-2.0$  mm, two external segments  $1.7-2.4 \times 1.0-1.4$  mm; stamens eight, the three longer ones opposite to the internal perianth segments 1.4-1.8 mm long, the five shorter ones alternating with tepals 1.0-1.5 mm long, filaments persistent; ovary reddish, styles three, minute, stigma capitate, reddish; achenes coriaceous, red, pale red to pinkish, 6.0-7.5 mm long, 5.0-7.0 mm wide, three-winged, wings two-parted, upper lobes are twisted clockwise or anti-clockwise, upper and lower lobes are overlapped or distant, lower lobes 1.5-2.5 mm wide, upper lobes 1.2-1.5 mm wide.

*Note:* In *Flora Iranica* (Rechinger & Schiman-Czeika, 1968) *P. aucheri*, *P. olivieri* and *P. noeanum* are treated as separate species with the explanation of difficulties to distinguish many specimens due to overlapping characters. *Pteropyrum aucheri* was characterized by its linear and narrower leaves that are often six times longer than wide. In contrast, *P. olivieri* and *P. noeanum* were distinguished by their flat leaves at most six times longer than wide. Checking the type specimen of *P. noeanum* carefully and field studies in western Iran adjacent to Iraq border revealed that these populations have linear to linear-lanceolate leaves matching the type of *P. aucheri*, and therefore it was reduced as synonymy of *P. aucheri* (Akhani, 2004). The only cited specimen of *P. noeanum* (*Rechinger*, *9640*) belongs to *P. naufelum* (see below).

Intensive field studies over the distribution range of P. aucheri and the P. olivieri complex and study of numerous herbarium specimens integrated with our anatomical and micromorphological studies and analysis of nuclear ITS sequences convinced us that (1) distinction between *P. aucheri* and *P. olivieri* is not always possible because of intermediate traits in many cases; (2) a more-or-less geographical correlation is distinguishable with three leaf forms: populations with P. olivieri leaf types (flat-wide) distributed in central and southern parts of Iran (Fig. 103); populations with P. aucheri leaf types (flat-linear) occurring in central and eastern parts of Iran (Fig. 102) and populations with P. ericoides leaf types (cylindrical terete) distributed in eastern and south-eastern parts of Iran (Fig. 104). Leaves of populations in the western parts of Iran and adjacent Iraq are morphologically similar to P. aucheri leaf type but differ markedly in their much larger pollen grains; they are treated here as P. aucheri subsp. noeanum. All these species fall clearly into the P. aucheri clade in ITS tree (Fig. 18). The common character of most ITS sequences is the presence of several polymorphic nucleotide sites that might indicate interpopulation hybrids. This is an indication that over the distribution range of these taxa, some microspecies are interbreeding each other. We can hypothesis that these populations might have undergone several range expansion and range restriction events that have repeatedly mixed different populations during some climatic and geological times. Therefore, we decided to suggest a subspecific categorization of these species into four subspecies. Although this study covers most parts of Iran, but the lack of opportunity to study enough material and field studies in Iraq, Afghanistan and Pakistan requires reassessment of our classification.

Pteropyrum aucheri Jaub. & Spach. subsp. aucheri (Fig. 10)

*Description:* Leaves linear, narrowly oblanceolate, acute,  $7.0-16.0 \times 1.5-2.0$  mm, glabrous; internal perianth segments  $2.5-2.8 \times 1.7-2.0$  mm, external ones  $2.2-2.4 \times 1.3-1.5$  mm; achenes,  $6.0-7.0 \times 5.0-5.5$  mm long, upper and lower lobes are overlapped or distant, lower lobes 1.4-1.7 mm wide, upper lobes 1.0-1.4 mm wide; hypocotyl sparse papillose,  $\pm 3.5$  cm height.

*Habitat:* Gravelly desert steppes along dry riverbeds and water runnels

*Distribution:* Flat plains in southern slopes of Alborz mountain range, central and eastern parts of Iran and adjacent Afghanistan and Pakistan (Fig. 102).

Additional examined material (Supporting Information, Appendix S2)

Pteropyrum aucheri Jaub. & Spach. subsp. noeaum (Boiss.) Doostmohammadi & Akhani comb. & stat. nov.

 $\equiv P.$  noeanum Boiss. ex Meisn. in DC., Prodr. 14: 31 (1856).

*Type:* Ad ripam Dialae et Tigridis, prope Mossul, *Noë 1002* (Lectotype: G-DC!, selected here; isotypes: G-BOIS.! G00330368, LE! B100294865!)

*Description:* Leaves linear, narrowly oblanceolate, acute,  $3.0-15.0 \times 1.2-2.0$  mm, glabrous or pubescent; internal perianth segments  $2.0-2.5 \times 1.2-2.0$  mm, external ones  $1.8-2.2 \times 1.2-1.3$  mm; achenes  $7.0-7.5 \times 7.0-7.5$  mm, lower lobes 1.5-1.8 mm wide, upper lobes 1.2-1.5 mm wide.

Habitat: along dry riverbeds and adjacent low slopes.

*Distribution:* West of Iran close to the Iraqi border and northern and eastern Iraq (Fig. 102).

Additional examined material (Supporting Information, Appendix S2)

*Note: Pteropyrum noeanum* was synonymized with *P. aucheri* by Akhani (2004) and *P. olivieri* by Edmondson & Akeroyd (2016). The pollen morphology of two specimens checked in this study is clearly larger than several examined samples from central Iran (Table 4, Fig. 92). Therefore, we decided to classify the westernmost range of *P. aucheri* complex as a separate subspecies, considering the fact that the leaves of some specimens tend to be closer to *P. aucheri* leaf type and some specimens are more similar to *P. olivieri* leaf type.

Pteropyrum aucheri Jaub. & Spach subsp. olivieri (Jaub. & Spach) Doostmohammadi & Akhani comb. nov. (Fig. 11).

 $\equiv$  *Pteropyrum olivieri* Jaub. & Spach, Ill. Pl. Or. 2: 9 tab. 108 (1844).

*Type:* In 'Persia septentrionali, circa urben Teheran', *Olivier & Bruguiere* (Lectotype: P00734250, selected here; isotype: P00734252).

= P. gracile Boiss., Diagn. Pl. Or. Nov. Ser. 1, 12. 102 (1853). = P. olivieri Jaub. & Spach var. gracile (Boiss.) Boiss. Fl. Or. 4: 1002 (1879).

*Type:* Prope Sonak in monte Elburz, 1843, *Kotschy* 664 (Lectotype: G-BOIS!, selected here; isotypes: G00330367, P00734245-photograph!, LE!).

= *P. griffithii* Meisn. in DC., Prodr. 14: 31 (1856) *Type:* Afghanistan: ln collibus circa Sharkabad, *Griffith* 721 (Lectotypus: K001097690 -photograph!, selected here; isotypes: G-BOIS, P00762383 -photograph!).

Description: Leaves oblanceolate, elliptic, oblong, glabrous or densely pubescent,  $6.0-16.0 \times 2.5-6.0$  mm, acute or rarely obtuse, summer leaves smaller; internal perianth segments  $2.0-2.5 \times 1.5-1.8$  mm, external ones  $1.7-2.0 \times 1.0-1.4$  mm; achenes  $6-7 \times 5-6$  mm, lower lobes 1.5-2.0 mm and upper lobes 1.2-1.5 mm wide, wing margin entire; hypocotyl glabrous,  $\pm 3.5$  cm height.

*Habitat:* Desert steppes, flat plains and foothills on dry water runnels.

*Distribution:* Central and southern Iran, Afghanistan and Pakistan (Fig. 103).

Additional examined material (Supporting Information, Appendix S2)

Pteropyrum aucheri Jaub. & Spach subsp. ericoides (Boiss.) Doostmohammadi & Akhani comb. nov. (Figs 4, 9) *≡ P. ericoides* Boiss., Fl. Or. 4: 1002 (1879). *Type:* Belutschia inferior, *Stocks* 419 (Lectotype: G-BOIS00330366, selected here).

Description: Branches greyish white, milky; leaves fasciculate, linear, subterete, terete, 6–12 mm long, usually < 1 mm wide, glabrous or occasionally sparsely papillose; internal perianth segments  $2.4-2.8 \times 1.5-$ 1.8 mm, external perianth segments  $1.9-2.2 \times 1.0-$ 1.4 mm; achenes  $6.5-7.5 \times 6.0-7.0$  mm, lower lobes 2.0-2.5 mm, upper lobes 1.5-1.8 mm wide, wing margin entire, denticulate or sinuate;. hypocotyl glabrous,  $\pm 3.5$  cm height; cotyledons straight.

*Note:* Boissier (1879) described *P. ericoides* based on Stocks' collection from Baluchistan (*Stocks 419*, G-BOIS00330366) mentioning that the sterile collection of Haussknecht (Persiae australis monte Sawers in glareosis fl. Chyrsan. JE00021268, JE00021269) from south-western Iran is also the same. The leaves of Haussknecht's collection are not completely cylindrical and its distribution in the Khersan Valley in western Iran is far from the range of *P. aucheri* subsp. *ericoides* (Fig. 104). We studied pollen morphology of another specimen from the Khersan Valley (10142) that confirms this population belongs to *P. aucheri* subsp. *noeanum*.

Pteropyrum aucheri subsp. ericoides is the most xerophytic taxon of the *P. aucheri* complex distributed in desertic parts of central and eastern Iran and adjacent parts of Afghanistan, Pakistan and Turkmenistan (Fig. 104). It is characterized by usually linear and terete leaves. However, in many cases as in the type specimen the leaves are linear with revolute margins that cause difficulties in distinguishing it from *P. aucheri* subsp. *aucheri*. The species protologue of Boissier (1879) described the species with densely pubescent leaves. This character is not constant in this subspecies.

*Habitat:* Widespread in desert areas of Iran and surrounding area in Afghanistan and Pakistan, commonly grow on temporary water runnels, flood contexts and sometimes on sand hills.

*Distribution:* Central and eastern parts of Iran, Afghanistan, Pakistan and Turkmenistan (Fig. 104).

Additional examined material (Supporting Information, Appendix S2)

*Pteropyrum naufelum* Al-Khayat, Nordic. J. Bot. 13 (1): 33 (1993) (Figs 1, 16).

*Type::* Iraq: Hashima, on the Iraqi-Persian border, near Badra, Muthanna Province, dry stony hill, 2 Dec.

1962; A. D. Q. Agnew, W. El-Hashimi & S. Haddad (Holotype: BUH; isotype: BAG, K).

Description: Small shrubs, up to 50 cm height, stems blackish grey; leaves leathery, obovate, obtuse, rounded at apex, mostly densely papillose, sometimes glabrous,  $8-18 \times 3-8$  mm, two to seven in each cluster; ochrea short, 1.0-1.5 mm long, hyaline; flowers axillary, pedicles capillary, minutely papillose, 4-8 mm long, jointed above the base; perianth segments  $2.2-2.8 \times 1.2-1.6$  mm; achene coriaceous, red becoming brown when ripe,  $6-7 \times 6-7$  mm, three-winged, wings two-parted, upper lobes twisted clockwise or anti-clockwise, lower lobes 1.2-2.0 mm, upper lobes 1.0-1.4 mm wide; hypocotyl sparsely papillose, relatively short,  $\pm 2.5$  cm height.

Habitat: gypsum hills (see Akhani, 2004).

*Distribution:* Western and south-western Iran in Khuzestan, Ilam and Lorestan Provinces and adjacent gypsum hills in Iraq (Fig. 105).

Additional examined material (Supporting Information, Appendix S2)

*Note: Pteropyrum naufelum* is a typical gypsophyte frequently growing on low gypsum hills along the Iran-Iraq border; its distribution extends further eastwards in Lorestan and southwards in Khuzestan Provinces in Iran. On gypsum hills between Mehran and Dehloran it forms a community with many gypsophilous plants, including Euphorbia acanthodes Akhani, Gypsophila linearifolia (Fisch. & C.A.Mey.) Boiss., Diplotaxis harra (Forssk.) Boiss., Albraunia fugax (Boiss. & Noë) Speta, Crepis aspera L., Onobrychis gypsicola Rech.f., Scabiosa leucactis Patzak, Cleome glaucescens DC., Convolvulus gonocladus Boiss. Astrgalus akhanii Podlech and Rumex ephedroides Bornm. (Akhani, 2004). The field observations in this area suggest possible hybridization of P. naufelum growing on the hills and P. aucheri subsp. noeanum growing along the rivers.

Pteropyrum scoparium Jaub. & Spach, Ill. Pl. Orient 2. 10 tab: 109 (Figs 5, 14)

*Type:* In Arabiae regno Mascate, *Aucher 5270* (Lectotype: P04927957!, left specimen, selected here; isotypes: P04927956, K000830353!, LE!).

*Description:* Shrub, up to 170 cm height, bark of mature branches milky-white, smooth, much branched, young twigs striate; leaves linear, succulent, glabrous,  $3.0-12.0 \times 0.5-1.0$  mm, terete, acutish at apex, three to ten in each cluster; ochrea short (0.8–1.2 cm), membranous; flowers bisexual; pedicles capillary, 3–5 mm long, jointed above the base; perianth segments five, petaloid, white to cream, oblong-obovate  $1.5-2.5 \times 1.0-1.5$  mm,

in fruiting time slightly accrescent; stigma capitate; achenes coriaceous,  $6.0-7.5 \times 6.0-7.5$  mm, threewinged, wings two-parted, upper lobes not twisted, lower lobes 2.5-3.0 mm, upper lobes 1.5-2.0 mm wide; seeds rounded-cordate in outline, not sinuate at the base of rostrum.

*Habitat:* Gravelly wadis, dry water runnels and dry riverbeds in desert areas, *Acacia* Mill. shrubland.

Distribution: Northern Oman and UAE (Fig. 107).

Additional examined material (Supporting Information, Appendix S2)

Pteropyrum jakdanense Doostmohammadi sp. nov. (Figs 3, 12).

*Holotype:* Iran: Hormozgan: Bashagard, 5 km after Jakdan towards Sardasht, 26°24'33"N, 57°47'52"E, 833 m, 16.6.2013, *Doostmohammadi 4860* (Holotype: IRAN; isotype: Hb. Akhani)

Diagnosis: P. jakdanense is superficially similar to P. aucheri subsp. ericoides. It differs in the presence of a well-developed leaf base branchlet up to 5 mm long (absent or short in P. aucheri), 5.2–5.6 mm long hairy hypocotyls (3.2–3.8 mm long and glabrous or sparsely pubescent in P. aucheri) and the habitat on shale. Based on the ITS tree (Fig. 18), P. jakdanense shows affinity with species of the P. naufelum clade. From P. naufelum, P. gypsaceum and P. zagricum it differs in its linear terete leaves (not flat) and from P. macrocarpum in its smaller fruits 7.0–7.5 × 6.0–7.0 mm (9.0–13.0 × 8.0–10.5 mm in P. macrocarpum).

Description: Robust and rigid shrub, up to 150 cm height, branches erect, bark blackish grey, young branches whitish striate; leaves fasciculate, clustered on well-developed leaf base branchlets 2–5 mm long, linear-filiform, 4.0–10.0 × 0.4–0.7 mm, three to 12 leaves in each cluster, glabrous, terete in cross section, acute; ochrea 0.8–1.5 mm long, membranous; flowers bisexual, pedicles capillary, 3.0–5.5 mm long, jointed above the base; perianth segments 2.2–2.6 × 1.3– 2.2 mm; stigmas capitate; achenes coriaceous, 7.0– 7.5 × 6.0–7.0 mm, three-winged, wings two-parted, upper lobes are twisted clockwise or anti-clockwise, lower lobes 1.5–2.5 mm wide, upper lobes 1.0–1.5 mm wide; cotyledons straight.

*Habitat:* Lowland undulating hills with shale substrate.

*Distribution:* Endemic to southern Iran, Hormozgan Province, Bashagard Area (Fig. 107).

Additional examined material (Supporting Information, Appendix S2)

*Etymology:* The specific epithet '*jakdanense*' refers to the geographical distribution of this species near Jakdan.

*Pteropyrum zagricum* Doostmohammadi & Akhani sp. nov. (Figs 2, 15).

= *P. aucheri* Jaub. & Spach subsp. *caespitosum* Ghahreman & Mozaffarian in Fl. Iran [Ghahreman] 21: number 2593. 2001; *nom. inval*.

*Type:* Fars: c. 17 km N of Mamasani (Noorabad) on the road towards Yasuj, 30°22'05"N, 51°28'31"E, 1410m, 23.11.2012, *F. Bagheri, T. Chatrenoor, M. Dehghgani & M. Doostmohammadi 4780* (Holotype: IRAN; isotype: Hb. Akhani).

Diagnosis: Pteropyrum zagricum is unique in its chasmophytic habitat on rocky crevices of oak (Quercus brantii Lindl.) forests; the other species growing on dry riverbeds or gypsum or shale habitats. It differs from *P. aucheri s.l.* by its larger achenes ( $8.5-10.0 \times 7.5-$ 9.0 mm vs.  $6.0-7.5 \times 5.0-7.0$  mm). In the ITS tree *P. zagricum* belongs to the *P. naufelum* clade (Fig. 18). From *P. macrocarpum* and *P. jakdanense* it differs in its flat or semi-terete leaves (not cylindrical and terete) and from *P. naufelum* and *P. gypsaceum* in its unique habitat and different achene sizes ( $8.5-10.0 \times 7.5-$ 9.0 mm vs.  $6.5-7.5 \times 6.5-7.0$  mm) and light brown bark (not blackish, greyish or whitish as in other species).

Description: Prostrate-ascending shrubs, up to 50 cm height, spreading on rocks forming large mats to 2 m diameter, bark light brown; leaves fasciculate, in clusters of three to nine, dark green, variable, linear, linear-lanceolate to oblanceolate,  $2.0-12.0 \times 1.5-$ 4.0 mm, pubescent, margin revolute, ±semi-terete in cross section, acute at apex; ochrea 1.0-1.5 mm long, membranous; flowers clustered, pedicle 4–8 mm long, jointed above the base; perianth segments  $1.5-2.3 \times 1.0-1.7$  mm, achenes coriaceous, pink and becoming red when ripe,  $8.5-10.0 \times 7.5-9.0$  mm, threewinged, wings two-parted, upper lobes are twisted clockwise or anti-clockwise, lower lobes 2–3 mm and upper lobes 1.5-2.0 mm wide; hypocotyl glabrous, ± 3.5 cm height; cotyledons curved and convex.

*Habitat:* Steep limestone rocks in *Quercus brantii* steppe forest.

Distribution: Western Iran (Fig. 106).

Additional examined material (Supporting Information, Appendix S2) *Etymology:* The epithet '*zagricum*' refers to the type locality of the species in Zagros Mountains Range, western Iran.

*Note: Pteropyrum zagricum* is a unique species in its habitat on limestone cliffs and spreading habit. It shows leaf variability similar to *P. aucheri* in which linear to linear-lanceolate leaves occur on the same plant. The leaves of studied specimens are pubescent, despite glabrous cultivated seedlings based on one population. Whether the seeds of a plant with pubescent leaves produce glabrous plants or the indumentum develops latter in mature plants should be checked.

The illegitimate taxon *P. aucheri* Jaub. & Spach subsp. *caespitosum* Ghahreman & Mozaffarian (Ghahreman 2001) was published with photographs and descriptions in Persian, French and English, but without designation of type or mention of other herbarium vouchers. The locality was given between Andimeskh (Iran: Khuzestan Prov.) and Malavi (Iran: Lorestan Prov.). Herbarium specimens cited in *Flore de L'Iran* are preserved in TUH. Our efforts to see a specimen of the photographed plant in TUH was not successful. The attempt by one of the authors (H.A.) to collect this plant between Andimeshk and Malavi was not successful.

*Pteropyrum macrocarpum* Doostmohammadi & Akhani sp. nov. (Figs 6, 13).

*Type:* Iran: Kerman Province: 42 km on the road from Kahnuj towards Rudan, 27°33'44"N, 57°35'59"E, 479 m, 16.3.2013, *Doostmohammadi 4722* (Holotype: IRAN; isotype: Hb. Akhani).

Diagnosis: P. macrocarpum is superficially similar to P. aucheri subsp. ericoides but differs clearly in much larger achenes  $(9.0-12.0 \times 8.0-10.5 \text{ mm vs}, 7.0 7.5 \times 6.5-7.0 \text{ mm})$  and densely pubescent leaves (not glabrous or sparse pubescent) and presence of short leaf base branchlet to 4 mm. In the ITS tree (Fig. 18) it belongs to the P. naufelum clade. This species differs from P. naufelum, P. gypsaceum and P. zagricum in its cylindrical and terete leaves (not flat) and from P. jakdanense with larger achenes (9.0-12.0 × 8.0-10.5 mm vs. 7.0-7.5 × 6.0-7.0 mm) and its larger pollen grains (Fig. 94).

*Description:* Small shrubs, with rigid erect branches, up to 100 cm height, barks grey, young twigs parallel striate; leaves fasciculate, on well-developed leaf base branchlets up to 4 mm long, three to 12 in each cluster, linear,  $3.0-11.0 \times 0.8-1.0$  mm, densely pubescent, terete in cross section, acute at apex; ochrea 0.5-1.5 mm long,

membranous; flowers bisexual, pedicles 4–6 mm long, papillose, jointed above the base; perianth segments  $2.8-3.0 \times 1.8-2.2$  mm; achenes coriaceous,  $9.0-13.0 \times 8.0-10.5$  mm, three-winged, wings two-parted, lower lobes almost completely overlap the upper ones and reach the fruit apex, upper lobes are twisted clockwise or anti-clockwise, lower lobes 3–4 mm, upper lobes 2.0–3.5 mm wide; hypocotyl glabrous, ± 4 cm height.

Habitat: along water runnels and gravelly alluvial plains associated with Ziziphus spina-christii (L.) Desf. and Calligonum denticulatum Bunge ex Boiss., C. bungei Boiss. and C. comosum L'Hér.

*Distribution:* South-eastern Iran; Kerman, Hormozgan and Sistano va Baluchestan Provinces (Fig. 106).

Additional examined material (Supporting Information, Appendix S2)

*Etymology:* The epithet name of *'macrocarpum'* refers to large fruits of this remarkable species.

*Pteropyrum gypsaceum* Akhani & Doostmohammadi sp. nov. (Fig. 17).

*Type:* Hormozgan: 56 km E of Bandare-Lengeh towards Bandare-Khamir, 26°47'17"N, 55°19'42"E, gypsum hills near the coasts, 5 m, 20.12.2001, *H. Akhani 16001* (Holotype: IRAN; isotype: Hb. Akhani).

Diagnosis: Pteropyrum gypsaceum is superficially similar to P. aucheri subsp. olivieri but differs in the presence of a well-developed leaf base branchlet up to 5 mm (absent or short in *P. aucheri s.l.*), and its smaller leaves  $(8.0-12.0 \times 3.0-4.0 \text{ mm vs.} 6.0-16.0 \times 2.5-$ 6.0 mm). Based on the ITS tree it has a close affinity with P. naufelum but shows some differences in leaf sizes  $(8-12 \times 3-4 \text{ mm vs. } 8-18 \times 3-8 \text{ mm})$  and leaf apex (mucronate vs. not mucronate) and also micromorphological characters such as the polar length of pollen grain (average of 28.77 µm vs. 36.30 µm in P. naufelum; Fig. 94) and ornamentation (punctate, not obscurely reticulate as in *P. naufelum*). From P. macrocarpum and P. jakdanense it differs in its flat leaves (not linear terete) and from P. zagricum in achene size  $(7.0-7.5 \times 6.5-7.0 \text{ mm vs. } 8.5-10.0 \times 7.5-9.0 \text{ mm})$ .

*Description:* Dwarfshrub, up to 40 cm height, spreadingascendant, branches light grey; leaves fasciculate, clusters on a small branchlet up to 5 mm long, two to seven in each cluster, elliptic,  $8-12 \times 3-4$  mm wide, mucronate at apex, densely pubescent-papillose; ochrea 0.5-1.0 mm long, membranous; flowers bisexual, pedicles capillary, 4.5-5.5 mm long, jointed above the base; perianth segments  $1.5-2.5 \times 1.0-1.5$  mm; achenes coriaceous, 6.5-7.5 mm long, 6-7 mm wide, three-winged; wings two-parted, upper lobes twisted clockwise or anti-clockwise, lower lobes 1.5-2.5 mm, upper lobes 1-2 mm wide; hypocotyl sparsely papillose,  $\pm 4$  cm height; cotyledons straight.

*Habitat:* A halo-gypsophytic shrub confined to gypsum-saline hills near the Persian Gulf coasts.

*Distribution:* Southern Iran; Hormozgan Province along the Persian Gulf coasts (Fig. 105).

Additional examined material (Supporting Information, Appendix S2)

*Etymology:* The epithet '*gypsaceum*' refers to the habitat of the species mostly on gypsum soils.

Doubtful taxa and specimens from Afghanistan and Pakistan

Pteropyrum olivieri Jaub. & Spach var. scabrida Gilli, Feddes Repert. 68: 242 (1963)

*Type:* Bei Kabul, Tangi Gharu, Felsen und Schutthalden oberhalb des Wasserfalles Maipar, 1540 m, 11. V. 1951, fol. 2215, *A. Gilli 2215* (W).

We have only seen a photograph of this taxon available at http://herbarium.univie.ac.at/database/detail. php?ID=1160180. It superficially resembles *P. naufelum* and other related species of the *P. naufelum* clade described from western and southern Iran. We hypothesized that these specimens and many other immature plants seen in different herbaria from eastern Afghanistan and adjacent Pakistan (particularly from Quetta) represent a distinct species. To clarify its identity, recent collections will need to be sequenced. Here, we list a number of specimens that should be further reconsidered. Examined specimens studied based on virtual herbarium images are indicated by an asterisks (\*).

Afghanistan: 5 km W of Sarobi, gravelled plains and slopes, 34°36'35"N, 69°42'32"E, 1200 m, 7.11.1967, Toncev 4066 (W\*); Sarobi, 25 km SW at road to Lataband pass, 34°28'58"N, 69°37'35"E, 1300 m, 16.6.1967, on vertical rocks of conglomerates, Freitag 6174 (W\*); Kabul: c. 5 km W Sarobi, 1200 m, 24.10.1970, Podlech 20018 (W\*); Hills 15 km SW Kandahar, desertic to semi-desertic hill sides, 31°33'44"N, 65°31'04"E, 1100 m, 22.6.1967, Freitag 6340 (W\*); Kabul: In the mid of Tange-Gharu, 2 km to Mahipar, 1250 m, 22.5.1970, Anders 3718 (W\*); Between Sarobi and Jalalabad, 900 m, 26.3.1967, Breckle 6580 (W\*); Nangarha: between and Jalalabad, 820 m, 8.10.1969, Sharifi 449 (W\*).

Pakistan: Quetta: Murgha Kibzai to Fort Sandeman, 12–25 km from Murgha Kibazai, c. 1600 m, 19.5.1965, J. Lamond 1434 (E); Quetta: Mekhtar to Murgha Kibazi, stony and sandy plain, c. 1500 m, 18.5.1965, J. Lamond 1418 (E00191345); Quetta, Murdar Range, 14.9.1942, J. Sinclair 2465 (E); Ornach cross, c. 30 miles from Wadh on road to Karachi, 30.4.85, Abdolghafoor & Rizwan Yousuf 983 (B).

Furthermore, delimitation of specimens of *P. aucheri s.l.* collected from Baluchistan and Sind provinces in western Pakistan are also controversial. They appear to have an intermediate leaf shape between *P. aucheri* subsp. *aucheri* and *P. aucheri* subsp. *olivieri* for which their identification requires additional studies:

Pakistan: Baluchistan, 47 km from Awaran on way to Jhal Ihao and Las-Bella, sedimentary rocks, erect shrub, c. 40 cm tall, flower white; fruit winged, common, 23.3.1990, A. Ghafoor & S. M. Goodman 4862 (E); Karachi: Khadeji, c. 46 km ENE of Karachi, rocks at side of river, 29.4.1965, J. Lamond 759 (E); Baluchistan: Kalat; way to Bela, variations in colour, 1100–1250 m, 2.4.1965, J. Lamond 216 (E); Baluchistan: Kalat; Jhal Jhao to Awaran, roadside, plant of mountain areas of loose shale, 10.4.1965, J. Lamond 331, 323, 339 (E); Kachal, c. 5 miles from Naigaj to Karach Mountain, 8.11.1982, K. A. Malik, M. Qaiser, S. Omar & G. Khan. 2138 (B); 4 miles from Hubchauki on way to Bande-Murad, 24.3.1973, Sultan Alabedin et al. 9809 (B).

# **ACKNOWLEDGEMENTS**

This study is the result of a long-term research started in 1990 by H. Akhani. M. Doostmohammadi continued the research as his Master's thesis. The field and herbarium visits have been supported through different projects and organizations. The expeditions supported by the Department of Environment (Natural History Museum of Iran) and Research Council University of Tehran. The herbarium visits to B, E, G, LE, P and W were supported by German Academic Exchange Service (DAAD), Sibbald Trust (Edinburgh Botanical Garden), Alexander von Humboldt Foundation and University of Tehran. Some of the sequences granted by the Botanical Garden and Botanical Museum, Berlin, Dahlem with great help of Prof. Thomas Borsch. The stable isotope analyses performed at the ISO Analytical where funded by LIA HAOMA 'Human adaptation to environmental constraints on the Iranian plateau since the late glacial' project of the CNRS (Centre National de REcherche Scientifique) in France. We appreciate the Central Laboratory of College of Science for SEM and ultramicrotome facilities. The improving suggestions by Alexander Suchorukov (Moscow) and an anonymous reviewer are much appreciated.

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# SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Appendix S1.** Measurements of stem, leaf and cotyledon leaf anatomical characters for *Calligonum*, *Pteropyrum* and *Atraphaxis*. PCA – primary carbon assimilation, M –mesophyll, PCR – photosynthetic carbon reduction, BS – bundle sheath, EP – epidermis, MCL – mesophyll cell layers, HY – hypodermis, AB – abaxial, AD – adaxial. **Appendix S2.** List of additional examined specimens: Examined specimens studied based on virtual herbarium images are indicated by an asterisks (\*).

# APPENDIX 1. GENBANK ACCESSION NUMBER OF ITS SEQUENCES STUDIED IN THIS PAPER. DETAILS OF HERBARIUM VOUCHERS OF ALL NEW SEQUENCES ARE AVAILABLE IN THE TAXONOMIC ENUMERATION.

Taxon	Voucher collector(s) and herbarium number	GenBank accession numbers
Pteropyrum aucheri subsp. aucheri	M. Doostmohammadi 4832	MN437492
Pteropyrum aucheri subsp. aucheri	H. Akhani 24127	MN437491
Pteropyrum aucheri subsp. noeanum	H. Akhani 8397	MN437490
Pteropyrum aucheri subsp. ericoides	H. Akhani et al. 23487	MN437495
Pteropyrum aucheri subsp. ericoides	M. Doostmohammadi 4679	MN437493
Pteropyrum aucheri subsp. ericoides	M. Doostmohammadi 4826	MN437494
Pteropyrum aucheri subsp. olivieri	H. Akhani 24224	MN437502
Pteropyrum aucheri subsp. olivieri	H. Doostmohammadi 4852	MN437503
Pteropyrum aucheri subsp. olivieri	H. Akhani 7941	MN437504
Pteropyrum aucheri subsp. olivieri	M. Doostmohammadi 4673	MN437505
Pteropyrum aucheri subsp. olivieri	M. Doostmohammadi 4730	MN437506
Pteropyrum aucheri subsp. olivieri	V. Mozaffarian 49456	MN437507
Pteropyrum gypsaceum	H. Akhani 16001	MN437509
Pteropyrum jakdanense	M. Doostmohammadi 4861	MN437498
Pteropyrum macrocarpum	M. Doostmohammadi 4722	MN437496
Pteropyrum macrocarpum	M. Doostmohammadi 4729	MN437497
Pteropyrum naufelum	M. Doostmohammadi & A. Noormohammadi 4875	MN437500
Pteropyrum naufelum	H. Akhani 21580	MN437499
Pteropyrum naufelum	H. Akhani 9064-b	MN437501
Pteropyrum scoparium	Kürschner 99-33	MN437508
Pteropyrum zagricum	F. Bagheri et al. 4780	MN437510
Calligonum polygonoides	Tavakoli et al. 2010	AB542779
Calligonum persicum	Tavakoli et al. 2010	AB542777
Atraphaxis spinosa	Tavakoli et al. 2010	AB542772
Atraphaxis suaedifolia	V. Mozaffarian 87202	AB542773