

MORPHOLOGY, ANATOMY, PALYNOLOGY AND CONSERVATION STATUS OF *ACHILLEA KETENOGLUI* H. DUMAN

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Abstract

Achillea ketenoglui H. Duman is an endangered species in Turkey. Its morphological, anatomical and palynological features were studied. Microphotographs of the cross sections of stem and root of this species are provided along with the detailed anatomical features and their morphological description with the help of drawings. Distribution and ecology of *A. ketenoglui* were determined and IUCN risk category was revised. Microphotographs of scanning electron and light microscope was studied, evaluated and discussed for their taxonomic use.

Introduction

Asteraceae (Compositae) is one of the largest families of flowering plants occurring all over the world. According to the angiospermic phylogeny Asteraceae is placed in Asterideae/Asterales. Its members are distributed particularly in semiarid region of the tropics and subtropics with about 1600 - 1700 genera and 24000 species around the world (APG 1998, APG II 2003, APG III 2009, APG IV 2016).

The *Achillea* L. one of the genera of Asteraceae belongs to tribe Anthemideae. It is represented by 115 taxa throughout the world and by 58 taxa in Turkey. Because of its high endemism ratio (53%) *Achillea* L. is considered as an important genus among the Asteraceae genera available in Turkey Arabacı 2012, Aytaç *et al.* 2016). Previously *Achillea* L. was revised by Huber-Morath (1975) in the flora of Turkey.

Thereafter Duman (2000) added new species in second supplement of the Flora of Turkey. The new species were collected and described by Aytaç *et al.* (1990) from marly steppes between Polatlı and Sivrihisar (Duman 2000). Studies were carried out on *Achillea* taxa by both local and foreign workers on different aspects, mostly on chemical components and their importance for anticancer, antimicrobial etc. (Tabanca *et al.* 2016, Haliloğlu *et al.* 2017, Demirci *et al.* 2018, Özek *et al.* 2018). Studies on morphology, anatomy and palynology of *A. ketenoglui* H. Duman are still limited (Arabacı 2006, Akyalçın *et al.* 2009, 2011, 2014, Grytsyk 2016, Dauti *et al.* 2017). In the present study the detailed morphological, anatomical and palynological features of the *Achillea ketenoglui* H. Duman were investigated for the first time.

Materials and Methods

The specimens of *Achillea ketenoglui* were collected from Günyüzü, between Günyüzü-Yazır, (Eskişehir-Turkey) gypsicolous area, N 39° 25' 40.1" - E 31° 49' 29.8", 810 m., 29.06.2018, OUFÉ: 18242 (Fig. 1). In order to carry out a systematic study of the plant material, herbarium samples were prepared and kept as voucher specimens at the Eskişehir Osmangazi University Herbarium (OUFE). For the anatomical studies, fresh plant specimens collected from the natural habitat were fixed in 70% alcohol. Transverse sections of the stem and root were made by hand with the help of a scalpel.

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The thickness of each section was identified by Leica EZ-40 stereomicroscope from the herbarium samples stored in OUFÉ. To identify the range of variation in descriptive morphologic characters, 25 plant specimens from different locations were investigated. A number of relevant literatures were consulted for naming and their comparison made Davis *et al.* (1965, 1988), Esau (1967), Fahn (1967), Yentür (1995), Güner *et al.* (2000).

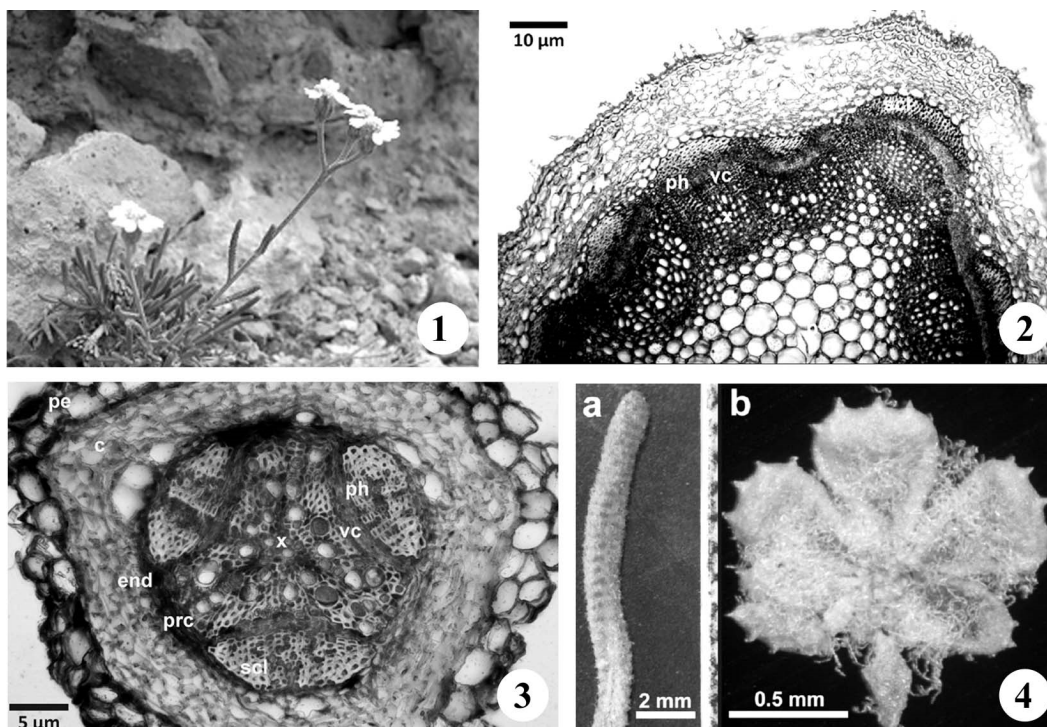
Pollen samples obtained from the herbarium materials of *A. ketenoglui* were stored in OUFÉ. For palynological investigations, pollen grains of 10-15 from each plant specimen collected from different localities were used. Light microscopy and SEM were used for investigation of their morphology. The terminologies used by Faegri and Iversen (1975) were followed to name the exine layers. For the purpose of investigations by light microscope, pollen grains of *A. ketenoglui* were prepared following Wodehouse (1935) and Erdtman (1969) techniques. For identifications and counting of pollen grains simply 10 and 40x objectives were used. Detailed investigations on their identifications were made by 100x plan oil-immersion objective. Thickness of exine and intine of seeds of the taxa was measured following Wodehouse (1935) and Erdtman (1969). Terminologies for pollen morphology were used as par Wodehouse (1935), Kuprianova (1967), Erdtman (1969) and Faegri and Iversen (1975).

Results and Discussion

To identify the anatomical features of *Achillea ketenoglui* the transverse sections of its stem and root were investigated. In stem, the epidermis was covered by simple multicellular hairs. Epidermal cells were surrounded by a thick and slightly undulated cuticle. Cortex was made up of 4-12-layers of parenchymatous cells. One-5-layered collenchymatous tissue located below the epidermis. Cortical cells were found to be ovoid in median layer and squash towards to the phloem. There was a thick sclerenchymatous sheath on the phloem tissue which occupie a wide region. Cambium was not distinguishable and the pith region was composed of large and parenchymatic cells. Starch grains were observed in some cells of the pith (Fig. 2).

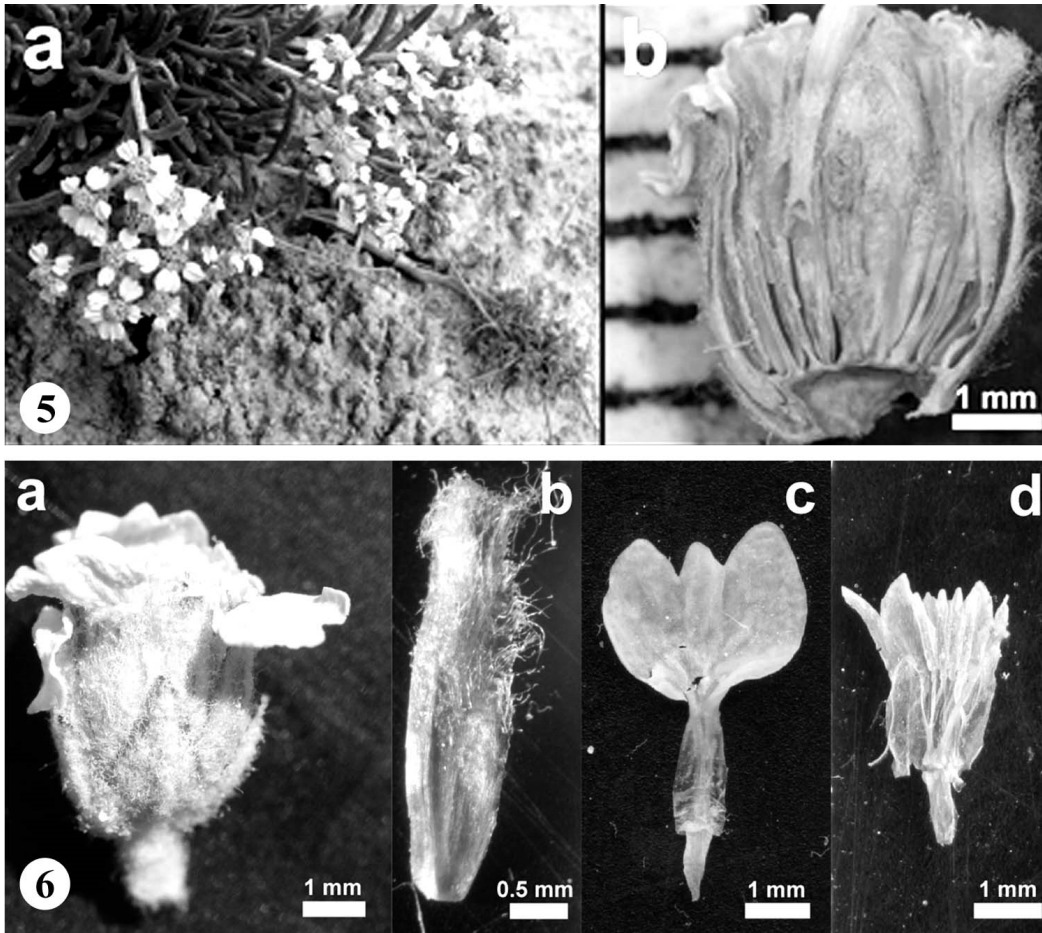
There was a primarily periderm layer on the outer surface of the root. Its cells are crushed, broken up and sometimes worn out parenchymatous cortex is present under the-peridermal layer/s of cells. The breadth of the cells was bigger than the length. These cells have regularly 2-4 layers. Endoderm was located at the end of cortical layer. Phloem occupied a narrow area between scleranchyma bundles and xylem. Cambium cells were distinguishable. In contrast to the older ones, any parenchymatous pith region was not found in young root sections (Fig. 3).

According to data obtained from morphological studies, detailed description of *A. ketenoglui* is given here. Perennial herb c. 8 - 24 high, not branched, angled, densely lanate-tomentose, bearing many underdeveloped shoots of to 2.7 cm long. Leaves linear-oblong, 10 - 20 (-20.7) × 1 - 2.2 mm, sessile, pinnatisect, divided into dense imbricate segments (0.3 - 0.6 × 0.5 - 1 mm), young segments are not divided, medians are 3 - 5 partitate or 3 - 5 lobed, all lobes are same in shape, orbicular, 0.3 - 0.5 (-0.9) mm long and margine cartilaginous, denticulate, lanate-tomentose (Fig. 4). Capitula 3 - 5 (-8). Corymbs 1 - 5 cm wide: peduncles 0.5 - 1.5 cm long. Involucre broadly ovoid to hemisphaerical. 3 - 5 × 3 - 4 mm, rounded at base (Fig. 5). Phyllaries 4 (-5) serried, with scarios brownish or hyaline margins, tomentose; outhier phyllaries are ovate, lanceolate, 2 × 0.5 - 0.8 mm, acute; inner ones are ovate, 3.3 - 4.2 × 1.2 - 1.7 mm, obtuse. Palea 3 - 3.8 × 0.5 - 1 mm, lanceolate, margin of upper parts and apex hyaline, inner side of palea glabrous, outhier side pilose to villous towards the apex. Ligules 5 - 8, white, 3.8 - 5.5 × 3.5 - 4.5 mm, lamina obrapeziform, 2 - 4 × 2.7 - 4.9 mm, tri-lobed, disc flowers 30 - 40, achenes are oblong to lanceolate, 1.3 - 2.8 × 1.2 - 1.5 mm (Figs 6-7, Table 1).



Figs 1-4: 1. The general appearance of *A. ketenoglui* in nature. 2. Cross section of the stem of *A. ketenoglui* (ep: Epidermis, c: Cortex, scl: Sclerenchyma, ph: Phloem, vc: Vascular cambium, x: Xylem, p: Pith) (10 x 10). 3. Cross section of the root of *A. ketenoglui* (pe: Peridermis, c: Cortex, end: Endodermis, prc: Pericycle, ph: Phloem, x: Xylem, vc: Vascular cambium) (20x10). 4. Leaves of *A. ketenoglui* (a: General view of the leaves; b: Detailed view of a leaf).

To date the descriptive features of the *Achillea ketenoglui* H. Duman are available in only two reports. First one of these reports was published in the second supplement of the Flora of Turkey and in the East Aegean Islands. With the help of detailed description *A. ketenoglui* H. Duman is now introduced to the scientific world through the present study. The detailed description is given in it. Somehow or other, information about most of the important taxonomic characters such as palea and achene are not given here. For example, morphological characteristics of achene in Asteraceae is noted to have especial or high importance for the purpose of classification. Besides, other morphological characters have not also been given in detail in this study too. As such the lapse may create or cause problems revealing the distinction in clear terms between *Achillea ketenoglui* H. Duman and its related taxa. Another important work in which morphological characters of *A. ketenoglui* were discussed is "The revision of *Achillea* L. (Asteraceae) genus grown in Turkey", which was completed by Arabaci in 2006 as a part of his PhD thesis. In this study, some of the missing data) of some taxonomic characters, namely achene and palea are given. The results obtained from the morphological evaluation carried out here are largely in line with the morphological data to those of Arabacı (2006). However, some small deviations have been determined especially in terms of variational values of numerical measurements. In addition, supporting the relevant morphological characters with photographs of living plant specimens eliminates the lack of visual data on these characters.



Figs 5-6: 5. Capitula of *A. ketenoglui* (a: General view of the capitulas at corymb; b: Longitudinal section of a capitula). 6. Phyllaries and flowers of *A. ketenoglui* (a: Phyllaries; b: Palea, c: Ligulate flower, d: Tubulate flower).

A. ketenoglui H. Duman, endemic to Turkey, and distributed in Central Anatolia is located within the borders of the Irano-Turanian phytogeographic region. It is located at the B3 and B4 squares according to Davis' square system. *A. ketenoglui* were found in gypsum and marl areas in the borders of Eskişehir (Bozan, Sivrihisar and Günyüzü), Ankara (Polatlı and Beypazarı) and Kırıkkale (Kesikköprü) provinces.

A. ketenoglui on the gypsum and marl areas of marble bedrocks with high level of CaCO_3 . In natural habitats, it was found in association of other calcicolous taxa, such as, *Alyssum niveum* Dudley, *Asperula nitida* Smith, *Aubrieta canescens* Boissier, *Aethionema subulatum* Boissier, *Astragalus oxytropifolius* Boissier, *Cota tinctoria* J. Gay ex Gussone, *Cephalaria aytachii* Göktürk & Sümbül, *Hedysarum cappadocicum* Boiss., *Scabiosa hololeuca* Bornm., *Achillea gypsicola* Hub.-Mor., *Matthiola anchonifolia* Hub.-Mor., *Linum hirsutum* L. and *Gypsophila perfoliata* L.

According to field surveys made by the authors, natural populations of *A. ketenoglui* H. Duman were also found at different localities from of Eskişehir, Ankara and Kırıkkale provinces.

The area of occupancy (AOO) of the natural populations were changed from 1 to 10 km² and so the size of its population is found to be between 200 to 900 individuals. On the basis of gathered data and on the basis of IUCN Red List Categories (IUCN 2017), *A. ketenoglui* H. Duman was proposed as "Vulnerable [VU: B1ab (i)]" and its no change at the IUCN category is proposed.

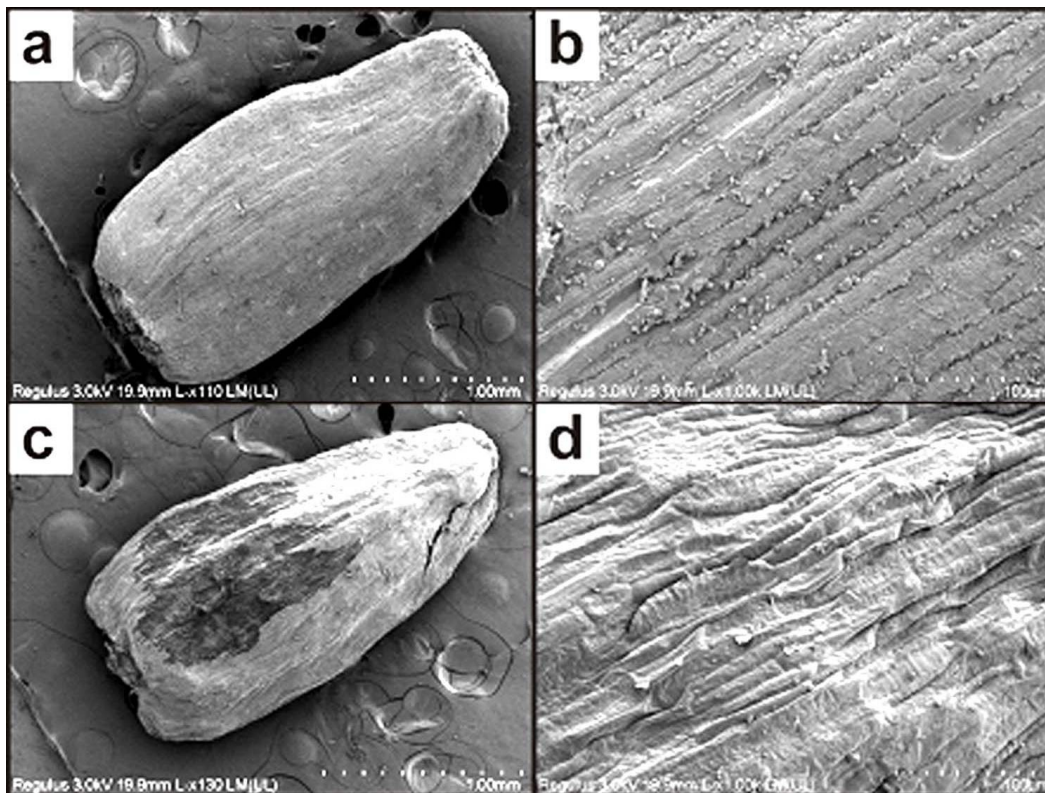


Fig. 7. Achene of *A. ketenoglui* in SEM (a: Ventral view of achene, b: Ventral surface of achene and c: Dorsal view of achene, d: Dorsal surface of achene).

The results of the study of LM and SEM revealed that in the echinate tricolporate and exine scabrate of *A. ketenoglui* H. Duman, P/E ratio ranged from 1.07 - 1.12 (Fig. 8, Table 2). Mean values of non-acetolysed pollen grains were 27 µm (P) and 25.30 µm (E) as against 30.20 µm (P) and 26.90 µm in acetolysed pollen grains (E) (Table 2).

The essential criteria used for the determination of the phylogenetic relationship of the characteristics of the aperture and function of this species have been reported by Kuprinova (1967), Cronquist (1968), Walker (1974a-b) and Takhtajan (1980). In the present data analysis of these taxa, authors observed that genetic distinctions encompassed differences in the measurements, raising objections to the possession of a morphological characteristic on to the pollen structure of these species.

Table 1. Comparison of identified morphological characters of *A. ketenoglui* with type description of Güner *et al.* 2000 and Arabacı 2006).

Characters	<i>A.ketenoglui</i> (this study)	<i>A. ketenoglui</i> (Arabacı 2006)	Type description (Güner <i>et al.</i> 2000)
Stem	Perennial herb c. 8-24 high, unbranched, angled, densely lanate-tomentose, bearing many sterile shoots to 2.7 cm long	Perennial herb c. 10-20 high, unbranched, angled, densely lanate-tomentose, bearing many sterile shoots	Perennial herb c.10 cm high, cylindrical, obtuse-angled, densely lanate tomentose, sterile shoots well-developed to 3 cm long
Leaves	Leaves linear-oblong, 10-20 (-20.7) × 1-2.2 mm, sessile, pinnatisect, divided into dense imbricate segments; segments 0.3-0.6 × 0.5-1 mm, young segments not divided, medians are 3-5 partitate or 3-5 lobed, all lobes are same shaped, orbicular, 0.3-0.5(-0.9) mm long and length, margine cartilaginous, denticulate, lanate-tomentose	Homomorphic, linear, 10-20 (-2.30) × 1-2 mm, sessile, sometimes crooked, pinnatisect, divided into dense imbricate segments; segments 0.3-0.5 × 0.7-1 mm, not divided, tri-partitate or tri-lobed, all lobes are same shaped, orbicular, 0.3-0.5 mm long and length, margine cartilaginous, small tooted, lanate-tomentose	Median leaves 10-20 × 1-2 mm, lanate, tomentose, linear oblong, pinnatisect; segments densely imbricate, 3-5 lobed or 3-5 partite, rarely undivided; lobes orbicular, denticulate, 0.5-1 mm long
Capitula	3-5(-8)	3-5(-8)	3-5(-8)
Corymbs	1-5 cm wide	1-5 cm wide	1-5 cm wide
Peduncles	0.5-1.8 cm	0.5-2 cm	0.5-1.5 cm
Involucre	Broadly ovoid to hemisphaerical, 3-5 × 3-4 mm, rounded at base	Broadly ovoid to hemisphaerical, 3-5 × 3-4 mm, rounded at base	Broadly ovoid to hemisphaerical, 3-5 × 3-4 mm, rounded at base
Phyllaries	4(-5) serried, with scarious brownish or hyaline margins, tomentose; outhy phyllaries are lanceolate, 2 × 0.5-0.8 mm, acute; inner ones are ovate, 3.3-4.2 × 1.2-1.7 mm, obtuse	4 serried, with scarious brownish or hyaline margins, tomentose; outhy phyllaries are lanceolate, 2 × 0.5 mm, acute; inner ones are ovate, 3.5-4 × 1.5 mm, obtuse	Ovate-lanceolate, carinate, densely tomentose, obtuse, brownish, with narrow, scarious margins
Palea	Palea 3-3.8 × 0.5-1 mm, lanceolate, margin of upper parts and apex hyaline, inner side of palea glabrous, outhy side pilose to villous towards the apex	Palea lanceolate, 3-3.5 × 0.5-1 mm, acute, apex hyaline, pilose	-
Ligules	5-8, white, 3.8-5.5 × 3.5-4.5 mm, lamina obtrapeziform, 2-4 × 2.7-4.9 mm, tri-lobed	5-7, white, 4-5 × 3.5-4.5 mm, lamina obtrapeziform, 2-4 × 3.5-4.5 mm, tri-lobed	5-7, white, 2-2.5 mm long
Disc flowers	30-40	30-40	30-40
Achene	Obovate to oblong, 1.1-2.3 × 0.7-1.5 mm	Are broadly obovate to oblong, 1-1.5 × 0.6-0.9 mm, lineolate, grey-white colored	-
Flowering	5-6	5	5

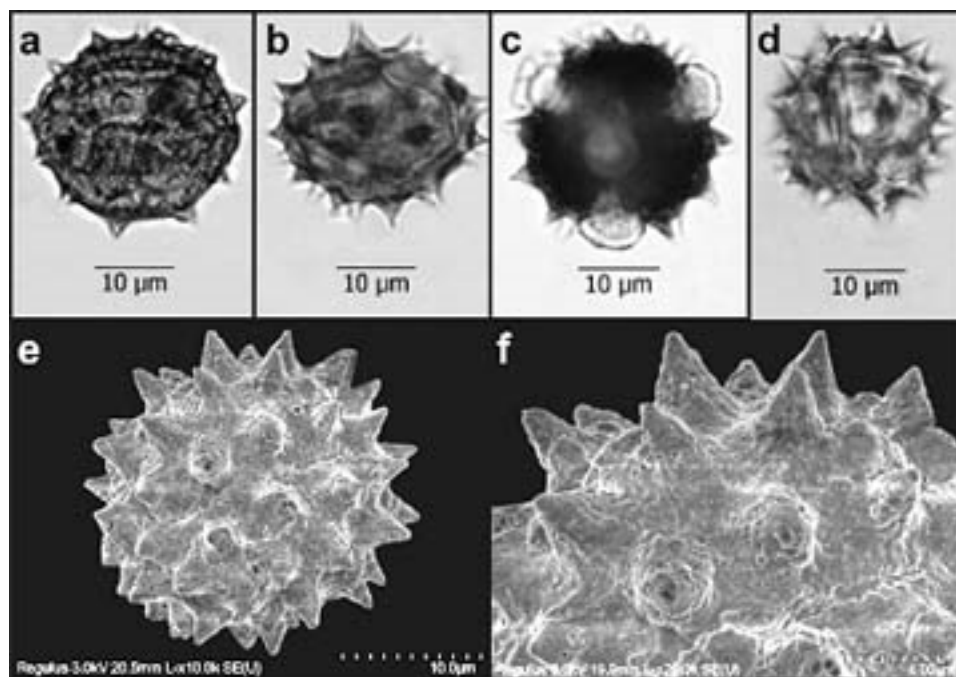


Fig. 8. Pollen grains of *A. ketenoglui* (a: Equatorial view of non-acetolysed pollen grain; b: Equatorial view of acetolysed pollen grain, c: Polar view of non-acetolysed pollen grain, d: Polar view of acetolysed pollen grain e: General view of pollen grain in SEM and f: Exine ornamentation of pollen grain in SEM).

Table 2. Pollen morphometrical parameters of *A. ketenoglui* (μm); (P: Polar axis; E: Equatorial axis; M: Mean; S: Standard deviations).

	P		E		P/E		Aperture number	Ornamentation	Pollen shape	Exine		Intine	
	M	S	M	S	M	S				M	S	M	S
Non-acetolysed (this study)	27	± 0.4	25.3	± 0.4	1.07	± 0.24	3	Echinate	Prolate-spheroidal	4.2	± 0.2	1.1	± 0.4
Acetolysed (this study)	30.2	± 0.3	26.9	± 0.3	1.12	± 0.17	3	Echinate	Prolate-spheroidal	4.6	± 0.4	-	-

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