www.czasopisma.pan.pl PAN www.journals.pan.pl

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 55/1: 29–36, 2013
DOI: 10.2478/abcsb-2013-00010

TAXONOMIC UTILITY OF ACHENE MORPHOLOGY AND ANATOMY IN *ANEMONE* L. (RANUNCULACEAE) SPECIES

IRMINA MACIEJEWSKA-RUTKOWSKA[1*] AND WOJCIECH ANTKOWIAK[2]

[1]*Department of Forest Botany, Poznań University of Life Sciences, Wojska Polskiego 71d, 60-625 Poznań, Poland,*
[2]*Department of Botany, Poznań University of Life Sciences, Wojska Polskiego 71c, 60-625 Poznań, Poland*

Received September 9, 2012; revision accepted April 10, 2013

Achene macro- and micromorphology and pericarp anatomy are described in four Polish species of *Anemone* (*A. narcissiflora*, *A. nemorosa*, *A. ranunculoides*, *A. sylvestris*). Biometric analysis showed that achene size varies greatly in all the studied species and is of limited diagnostic value. Three types of sculpture connected with the character of the indumentum were distinguished. The presence or absence of stomata on the achene style and the character of the hair base differentiated *A. nemorosa* and *A. ranunculoides*, which have the same type of pericarp ornamentation. The endocarp (number of layers and outline of its cells) was shown to be useful in the systematics of *Anemone*.

Key words: *Anemone*, achene morphology, pericarp anatomy.

INTRODUCTION

The genus *Anemone* L. *s.str.* (Ranunculaceae) consists of approximately 150 species, distributed mainly in the Northern Hemisphere (Tamura, 1995). The most recent taxonomic revision of this taxon (Ziman et al., 2008) recognized 118 species divided into 15 subgenera. *Anemone* species occur mainly in the northern temperate zones and are considerably less frequent in the cooler mountainous regions of the Southern Hemisphere (Schuettpelz et al., 2002; Zima et al., 2006). About 17 species representing four subgenera grow naturally in Europe (Chater, 1993). Only four *Anemone* taxa are native to the Polish flora: *A. narcissiflora* L. nom. illeg. (subgen. *Omalocarpus*), *A. nemorosa* L., *A. ranunculoides* L. (both belonging to subgen. *Anemonanthea*) and *A. sylvestris* L. (subgen. *Eriocapitella*).

Anemone nemorosa and *A. ranunculoides* are the most common *Anemone* species in Poland, growing in thickets and forests of the *Querco-Fagetea* class. *A. sylvestris* is considerably rarer, with sites scattered over the lowlands. It is a characteristic species of the *Geranio-Anemonetum sylvestris* association, and also grows in thermophilous thickets of *Corylus avellana* and in xerothermic grasslands of the *Festuco-Brometea* class. *A. narcissiflora* is extremely rare and can be found only in mountainous regions of Poland (Sudetes and Carpathians), above the tree line on calcareous and granite substrate. It is a component of rocky grassland belonging to the *Calamagrostion* alliance and the *Sesleretalia variae* order. Both *A. sylvestris* and *A. narcissiflora* are under strict protection in Poland (Gostyńska-Jakuszewska, 1992; Piękoś-Mirkowa and Mirek, 2003; Matuszkiewicz, 2006).

Flowers of the genus *Anemone* are characterized by an apocarpous gynoecium containing anatropous and unitegmic ovules, with the body almost parallel to the funicle and entirely fusing with it (Wang and Ren, 2008). Complete with the ovary tissue, they develop into one-seeded achenes which form heads (fruitlets). Achene length ranges from 1–2 mm to 10 mm. They are most often ovoid, but globose, cylindroid and spindle-like fruits also occur. *Anemone* achenes sometimes are laterally compressed and ribbed or winged. Their bodies are often covered with hairs but sometimes glabrous or nearly so. The style is typically persistent at fruit maturity, and of various length and shape. The pericarp is well-developed and differentiated into a single-layer exocarp, several-layered mesocarp and 1–2-layered endocarp. The seed coat

*e-mail: irminamr@up.poznan.pl

PL ISSN 0001-5296

TABLE 1. Collection data for the investigated *Anemone* species

Species	Origin of plant material	No. of achenes
A. narcissifolia	Karkonosze Mts, above Mały Staw (Dolnośląskie Province), M. Nowiński, 1964 (POZNB).	50
	Tatra Field Station and Botanical Garden of W. Szafer Institute of Botany (Małopolskie Province), Polish Academy of Sciences, Cracow, 2009 (POZNB).	39
	Botanical Garden of Maria Curie-Skłodowska University, Lublin (Lubelskie Province), 2009 (POZNB).	50
	Poznań (Wielkopolskie Province), Botanical Garden of Adam Mickiewicz University, 2009 (POZNB).	50
	Rogów (Łódzkie Province), Arboretum of Warsaw University of Life Sciences, 2009 (POZNB).	50
A. nemorosa	Kazimierz Dolny (Lubelskie Province), Botanical Garden of Maria Curie-Skłodowska University, 2009 (POZNB).	50
	Ślęża Mt. (Dolnośląskie Province), Medicinal Plant Garden of Silesian Piasts University of Medicine, Wroclaw, 2009 (POZNB).	45
	Łany (Dolnośląskie Province), Medicinal Plant Garden of Silesian Piasts University of Medicine, Wroclaw, 2009 (POZNB).	50
	Starczanowo near Nekla (Wielkopolskie Province), P. Górski, 2009 (POZNB).	44
	Złotoryja, Olszanica district (Dolnośląskie Province), I. Maciejewska-Rutkowska, 2007 (POZNB).	40
	Goraj (Wielkopolskie Province), I. Maciejewska-Rutkowska, 2008 (POZNB).	50
	Parkowo (Wielkopolskie Province), Maciejewska-Rutkowska, 2008 (POZNB).	50
	Kotlin (Wielkopolskie Province), I Maciejewska-Rutkowska, 2009 (POZNB).	50
	Bukowa Forest (Zachodniopomorskie Province), R. Jagiełło, 2009 (POZNB).	48
A. ranunculoides	Kopaszewo (Wielkopolskie Province), W. Antkowiak, 2009 (POZNB).	50
	Poznań, near Rusałka Lake (Wielkopolskie Province), I. Maciejewska-Rutkowska, 2009 (POZNB).	50
	Zwierzyniec (Lubelskie Province), Botanical Garden of Maria Curie-Skłodowska University, Lublin, 2009 (POZNB).	50
	Kazimierz Dolny (Lubelskie Province), Botanical Garden of Maria Curie-Skłodowska University, Lublin, 2009 (POZNB).	50
	Owińska near Poznań (Wielkopolskie Province), I. Maciejewska-Rutkowska, 2009 (POZNB).	50
	Bukowa Forest (Zachodniopomorskie Province), R. Jagiełło, 2009 (POZNB).	50
	Złotoryja, Olszanica district (Dolnośląskie Province), I. Maciejewska-Rutkowska, 2007 (POZNB).	50
	Goraj (Wielkopolskie Province), I. Maciejewska-Rutkowska, 2008 (POZNB).	50
	Parkowo (Wielkopolskie Province), I. Maciejewska-Rutkowska, 2008 (POZNB).	50
A. sylvestris	Botanical Garden - Center for Biological Diversity Conservation in Powsin (Mazowieckie Province), Polish Academy of Sciences, Warsaw, 2009 (POZNB).	51
	Bolestraszyce Arboretum (Podkarpackie province), 2009 (POZNB).	39
	Opoka Duża (Lubelskie Province), Botanical Garden of Maria Curie-Skłodowska University, Lublin, 2009 (POZNB).	44
	Poznań (Wielkopolskie Province), Botanical Garden of Adam Mickiewicz University, 2009 (POZNB).	21
	Łódź (Łódzkie Province), Botanical Garden, 2009 (POZNB).	45
	Rogów (Łódzkie Province), Arboretum of Warsaw University of Life Sciences, 2009 (POZNB).	25

TABLE 2. Descriptive analysis of quantitative data of achene of *Anemone* species

Feature	Species	mean	min	max	SD	median	CV%
length (mm)	*A. narcissiflora*	7.81	4.99	8.96	0.97	7.85	12.40
	A. nemorosa	3.24	2.12	4.48	0.46	3.20	14.14
	A. ranunculoides	2.62	1.98	3.89	0.30	2.59	11.57
	A. sylvestris	2.57	1.60	3.66	0.46	2.54	18.06
width (mm)	*A. narcissiflora*	6.20	4.01	7.52	0.81	6.25	13.00
	A. nemorosa	2.18	1.32	3.43	0.41	2.13	18.70
	A. ranunculoides	2.07	1.40	2.82	0.22	2.05	10.46
	A. sylvestris	1.59	0.88	2.74	0.38	1.54	23.98
elongation	*A. narcissiflora*	1.26	1.00	1.44	0.14	1.27	11.07
	A. nemorosa	1.50	1.00	2.35	0.32	1.45	21.65
	A. ranunculoides	1.26	1.00	2.08	0.18	1.23	14.54
	A. sylvestris	1.67	1.00	3.31	0.44	1.61	26.33
perimeter (mm)	*A. narcissiflora*	22.64	15.32	25.76	2.57	22.89	11.37
	A. nemorosa	8.64	4.49	11.49	1.02	8.58	11.75
	A. ranunculoides	7.53	6.25	9.67	0.60	7.47	7.92
	A. sylvestris	6.79	4.56	9.42	1.10	6.75	16.22
area (mm^2)	*A. narcissiflora*	34.35	15.83	44.17	7.91	34.55	23.04
	A. nemorosa	4.29	2.29	6.78	0.83	4.32	19.34
	A. ranunculoides	3.73	2.45	5.64	0.57	3.65	15.26
	A. sylvestris	2.42	1.15	4.83	0.86	2.16	35.52
circularity	*A. narcissiflora*	1.21	1.07	1.37	0.08	1.19	6.38
	A. nemorosa	1.41	1.08	2.08	0.15	1.40	10.72
	A. ranunculoides	1.22	1.07	1.61	0.08	1.20	6.47
	A. sylvestris	1.58	1.19	2.54	0.26	1.53	16.17

consists of several layers and is partly compressed (Kuźniewski, 1964; Chaundhry and Trifonowa, 1988; Ziman et al., 2008;).

Fruit morphology has traditionally been accepted as of great value for infrageneric classification of *Anemone*. As many as 25 achene characters are potentially of diagnostic value (Ziman et al., 2008). Chaudhary and Trifonova (1988) demonstrated the usefulness of fruit anatomy in a revision of the systematic position of 11 Nepalese species of this genus.

In this work we examined the achene morphology and anatomy of four *Anemone* species native to the Polish flora, in order to verify the taxonomic utility of fruit characters commonly accepted (e.g., shape, size, pubescence) and other characters (fruit surface sculpture, pericarp structure).

MATERIALS AND METHODS

Samples of fruits were taken from herbarium material, gathered from natural stations in Poland, and obtained from Polish botanical gardens (Tab. 1). All analyses of qualitative and quantitative characters were of ripe and fully developed achenes. The *A. narcissiflora*, *A. nemorosa* and *A. ranunculoides* fruits were not prepared but only cleaned; for *A. sylvestris* fruits the hairs were removed from the achene surface. The number of samples from a given species depended on their availability. Biometry employed DigiShape 1.9.220 (Cortex Nova 2005, Poland). The following achene parameters were measured: length (mm), width (mm), length/width ratio, perimeter (mm), area (mm^2) and circularity [=$perimeter^2/(\pi \times area)$]. Achene length was measured without the achene style. The arithmetical mean, median, standard deviation (SD) and coefficient of variation (CV) were calculated for each of those parameters. Transverse sections were hand-cut in the middle of the fruit. For SEM, dry fruits were mounted on aluminum stubs, sputter-coated with gold and examined with a Hitachi S3000N field emission scanning electron microscope at 5 kV in the Institute of Plant Protection in Poznań (Poland) and with a Zeiss EVO 40 SEM belonging to the Department of Biology of Adam Mickiewicz

University in Poznań. At least five achenes of each species were used for SEM. Terminology follows Bojňanský and Fargašová (2007) and Beentje (2010).

RESULTS

The morphological and anatomical characters of the *Anemone* fruits species showed some similarities and differences between species (Tabs. 2, 3, Figs. 1, 2). The achenes of *A. narcissiflora* were largest, at least twice longer and three times wider than the fruits of the other species. The achenes of *A. nemorosa* were on average 0.6 mm longer, but similar in width to *A. ranunculoides*. Most often the achenes of *A. sylvestris* were almost the same length as those of *A. ranunculoides*, but were distinctly narrower. Biometrical analysis revealed remarkable variability of achene size in all studied species. The CV values were especially high for *A. sylvestris*. The low CV values for elongation and circularity in *A. narcissiflora* and *A. ranunculoides* fruits indicate relative constancy of shape (Tab. 2).

In this study we found that the type of pericarp sculpture was the most important qualitative feature differentiating the species' achenes, and that the particular pericarp ornamentation was closely connected with the character of the indumentum. Based on these characters, three fruit types were identified. The first type characterized *A. narcissiflora*, with diverse ornamentation – striate-rugose within the achene body, becoming reticulate toward the margin, and with an entirely glabrous pericarp. The second type was characteristic of *A. sylvestris*, having striate-pustulate pericarp sculpture and completely covered with appressed, woolly hairs. The third type was seen in *A. nemorosa* and *A. ranunculoides*: indistinct, rugulose ornamentation and a downy indumentum. These features made the fruits of the two species very similar, but the achenes of *A. nemorosa* and *A. ranunculoides* differed in the character of the hair base: bulbously swelled in the former, and normal, not thickened, in the latter. Another trait distinguishing the fruits of the two species was the presence (*A. ranunculoides*) or absence (*A. nemorosa*) of stomata on the surface of the beak-like apex (Fig. 1, Tab. 3).

Since the epicarp and mesocarp consisted of flattened and compressed cells, the differences in pericarp anatomy resulted mainly from endocarp structure, which was composed of 3–4 layers in *A. narcissiflora* achenes, 1–2 layers in *A. ranunculoides* achenes, and only one layer in the other two species. An equally significant trait of this part of the pericarp was the outline of its cells, which was connected with cell wall thickness. Those cells were distinctly and evenly thickened in *A. narcissiflora* and *A. ranunculoides*; the cell walls in *A. nemorosa* and *A. sylvestris* achenes were thinner, and much thicker on the inner and lateral sides (Fig. 2, Tab. 3).

DISCUSSION

Our results on the achene morphology of four *Anemone* species are correlated with their taxonomic status. Ehrendorfer et al. (2009) made a similar assertion, but for fruit structure they attached importance mainly to macroscopic traits. Here we verified the taxonomic value of *Anemone* achene micromorphology. In analyzing pericarp ornamentation we distinguished three types of sculpture: two separate ones for *A. narcissiflora* and *A. sylvestris*, and a common one for *A. nemorosa* and *A. ranunculoides*. The latter two species, which are closely related, are very similar in achene surface ornamentation but differ in, for example, the bulbous hair base of *A. nemorosa* and the normal, not thickened hair base in *A. ranunculoides*.

Another specific feature observed only in *A. ranunculoides* is the presence of several stomata in the surface of the achene style. Until now stomata have been noted occasionally on seeds and fruits in a number of unrelated genera (Bergman, 1920; Jernstedt and Clark, 1979; Rugenstein and Lersten 1981). Stomata on achenes are also a characteristic feature of almost all species of *Rosa*, but their taxonomic value within this genus is rather limited (Zieliński et al., 2010).

Macroscopic traits of fruits, mainly their size, shape, indumentum and achene style, have traditionally been used to distinguish *Anemone* species (Saoud et al., 2007; Ziman et al., 2008; 2011). Based on our observations, these characters clearly differentiate the species to subgenus. When closely related species such as *A. ranunculoides* and *A. nemorosa* belong to the same subgenus and even to the same section and series (*Anemonanthea*) the dissimilarities in achene morphology are not so obvious but it is possible to discriminate the species, the most important features for macroscopic determination being the shape of the achene body and achene style. At this point the indumentum is of less diagnostic value.

We noted great remarkable variability of achene size in all the studied species, especially in *A. sylvestris*. Médail et al. (2002) noted large differences in fruit size among *A. palmata* populations in the western Mediterranean region. We infer that the diagnostic value of fruit size is not high.

Authors who have studied the fruit anatomy of different *Anemone* species have emphasized the taxonomic value of pericarp structure (Ziman et al., 2008), but opinions differ. Kuźniewski (1964) stated that an endocarp was not visible in cross-sections of *Anemone* achenes. Chaudhary and

TABLE 3. Comparison of morphological and anatomical characters of achenes of the analyzed *Anemone* species

Feature	*A. narcissiflora*	*A. nemorosa*	*A. ranunculoides*	*A. sylvestris*
shape	laterally flattened, inversely urceolate or obovoid, sometimes ellipsoid to almost globose	flattish, ovoid to ellipsoid, slightly biconvex	flattish, ovoid to ellipsoid, often almost globose, biconvex	flattish, oblong ovoid to ellipsoid, often asymmetrical, slightly biconvex
style	beaked, curved or recurved, up to 1.5 mm long; non-persistent	beaked, falcate, exceptionally straight, ±1.0–1.5 mm long; persistent	beaked, double-curved, rarely straight, ±1.0 mm long; persistent	beaked, straight or curved, less than 1.0 mm long; persistent
attachment scar	crater-like, slightly concave; in middle of tapered base, often looks like bottleneck	minute, flat, slightly raised rim in middle of slightly rounded base	minute, slightly raised, conical, in middle of rounded base	minute, poorly marked, on edge of distinctly elongated, slightly asymmetrical base
margin	wide winged, from the outside narrow, blade-like; around the whole achene body	narrow, obtuse, around the whole achene body	narrow, obtuse, around the whole achene body	indistinct, narrow, obtuse, around the whole achene body
indumentum	glabrous	downy, hairs with slightly bulbous base, normal or flagelliform, unicellular, 0.1–0.5 mm long.	downy, hairs normal or flagelliform, unicellular, 0.1–0.5 mm long	appressed-villose, hairs normal or flagelliform, unicellular, 10 mm long.
achene body sculpture	striate-rugose	rugulose	rugulose	striate-pustulate
margin sculpture	outside reticulate, inward gradually becomes striate	rugulose	rugulose	striate-pustulate
style sculpture	striate	striate	striate, some stoma visible	striate
epidermal cell outline	within the zone of achene body quadrangular and somewhat longitudinally elongated; within the zone of margin isodiametric, 5- or 6-gonal	indistinct, variable, often oblong or elliptic and somewhat longitudinally elongated	indistinct, variable, ± isodiametric to longitudinally elongated	oblong or narrowly oblong and longitudinally elongated
anticlinal cell wall	convex, rodlike	convex, of variable thickness, rodlike	convex, rodlike, of variable thickness or concave	mainly convex, rodlike
outer periclinal cell wall	concave, smooth to fine folds	concave; finely coarse	convex to concave; finely coarse	concave to somewhat convex, smooth, with minute bump on one side
pericarp structure	±50–60 µm thick, 6–7 layers	±50 µm thick, usually 4 layers	±50 µm thick, 4–5 layers	±20 µm thick, 4–5 layers
epicarp	1 layer, flattened, tangentially elongated	1 layer, flattened, tangentially elongated	1 layer, flattened, tangentially elongated	1 layer, flattened, tangentially elongated
mesocarp	2–3 layers, cells strongly compressed	2 layers, cells strongly compressed	2 layers, cells strongly compressed	2–3 layers, cells strongly compressed
endocarp	3–4 layers, cells elliptic in outline, with regularly thickened walls	1 layer, cells well preserved, with much thickened inner and lateral walls, forming letter “U”	1–2 layers, cells well preserved, with regularly thickened walls, forming letter “O”	1 layer, cells elliptic or oblong in outline, with much thickened inner and lateral walls

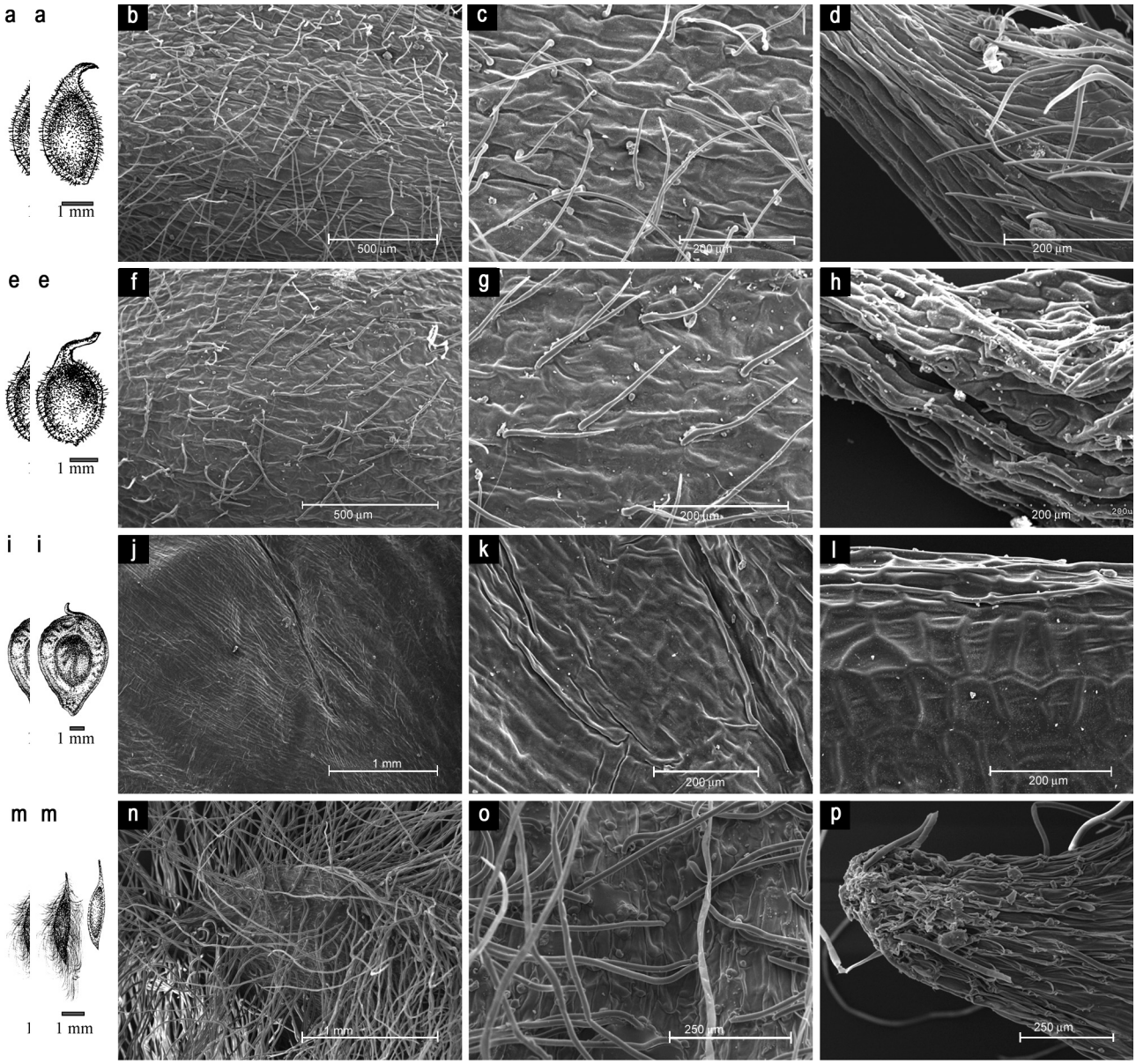

Fig. 1. Morphology of achenes; *A. nemorosa*: (**a**) Outline, (**b**) Rugulose pericarp sculpture, (**c**) Pericarp sculpture – focus on swelled bases of hairs, (**d**) Striate sculpture of style surface; *A. ranunculoides*: (**e**) Outline, (**f**) Rugulose pericarp sculpture, (**g**) Pericarp sculpture – magnification, (**h**) Striate sculpture of style surface, stomata visible; *A. narcissiflora*: (**i**) Outline, (**j**) Striate-rugose pericarp sculpture of body zone, (**k**) Pericarp sculpture of body zone – magnification, (**l**) Reticulate sculpture of wing surface; *A. sylvestris*: (**m**) Outline, (**n**) Striate-pustulate pericarp sculpture of body zone coverd by hairs, (**o**) Pericarp sculpture of body zone – magnification, (**p**) Striate sculpture of style surface.

Trifonova (1988) distinguished epicarp, mesocarp and endocarp, and focused on the number of cell layers in individual pericarp layers and on cell form. We also stress the significance of the endocarp, and specifically its number of layers and the outline of its cells. The differences between authors reflect differences in interpretation of pericarp structure. Most likely the layer described by Kuźniewski (l.c.) as an "external epidermis of integument transformed into sclerenchymatic layer" corresponds with an endocarp. In such a case our results are convergent with those of the cited authors, since both studies show endocarp cells with regularly thickened walls in *A. narcissiflora* and *A. ranunculoides*, and cells with unequally thickened walls in *A. nemorosa* and *A. sylvestris*.

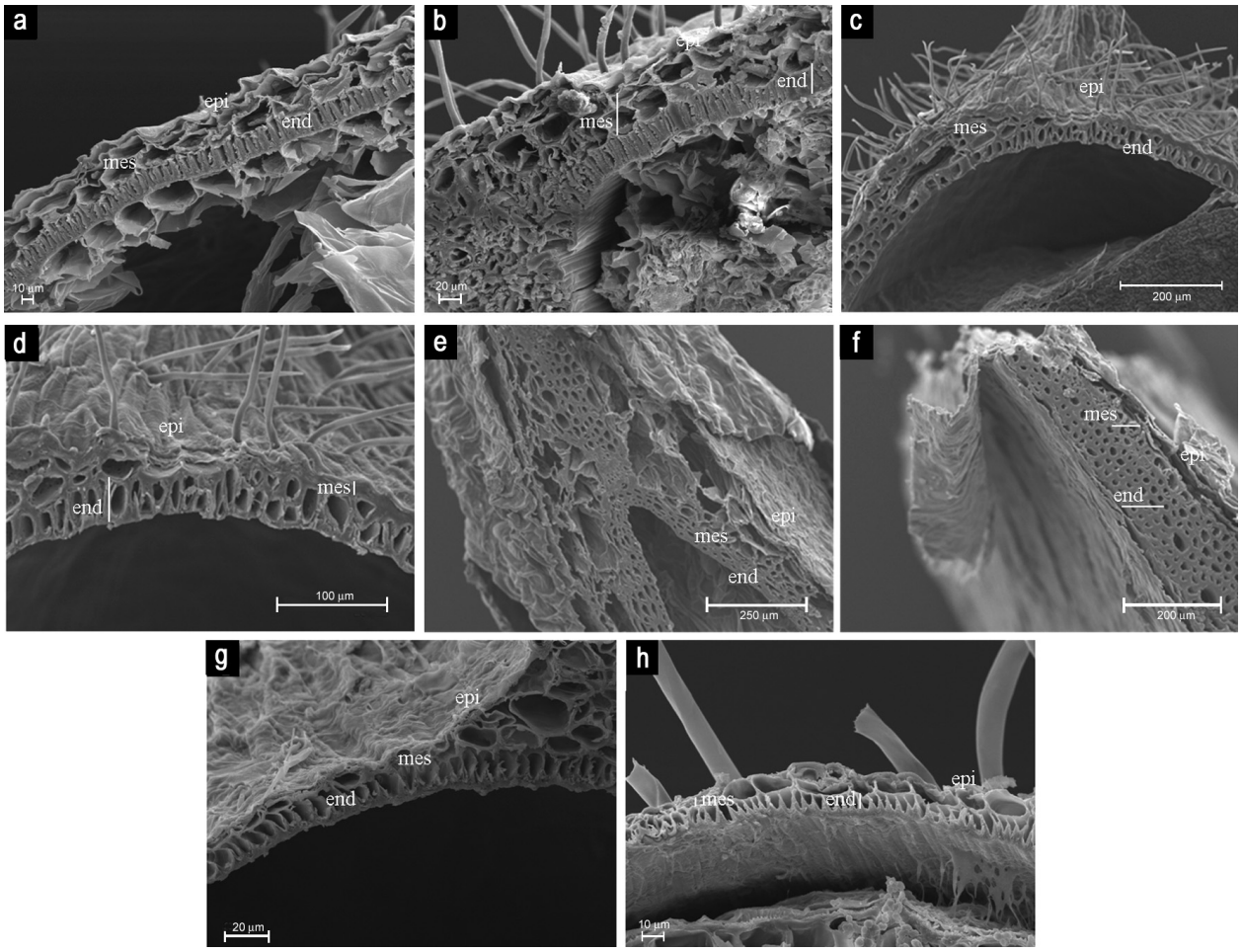

Fig. 2. Achene structure. *A. nemorosa* (**a, b**), *A. ranunculoides* (**c, d**), *A. narcissiflora* (**e, f**), *A. sylvestris* (**g, h**). Pericarp layers: epicarp (epi), mesocarp (mes), endocarp (end).

ACKNOWLEDGEMENTS

We are grateful Tomasz Sudoł for making the drawings of *Anemone* achenes and to Magdalena Gawlak (Institute of Plant Protection in Poznań) and Marcin Kujowa (Faculty of Biology, Adam Mickiewicz University) for making the SEM images.

REFERENCES

BEENTJE H. 2010. *The Kew Plant Glossary. An Illustrated Dictionary of Plant Terms*. Kew Publishing. Royal Botanic Gardens, Kew.

BERGMAN HF. 1920. Stomata in Ericaceous and other unrelated fruits. *Bulletin of the Torrey Botanical Club* 47(5): 213–221.

BOJŇANSKÝ V, and FARGAŠOVÁ A. 2007. *Atlas of Seeds and Fruits of Central and East-European Flora: the Carpathian Mountains Region*. Springer, Berlin.

CHATER AO. 1993. *Anemone* L. In: Tutin TG [ed.] *Flora Europaea*. Vol. 1 *Psilotaceae to Platanaceae*, 262–264. 2nd edition, Cambridge University Press.

CHAUDHARY RP, and TRIFONOVA VI. 1988. Morphology of fruit and comparative anatomy of pericarp and seed coat in the Nepal species of the genus *Anemone* (Ranunculaceae). *Botanicheskii Zhurnal* 73(6): 803–817.

EHRENDORFER F, ZIMAN SN, KÖNIG CH, KEENER CS, DUTTON BE, TSARENKO ON, BULAKH EV, BOSCAIU M, MÉDAIL F, and KÄSTNER A. 2009. Taxonomic revision, phylogenetics and transcontinental distribution of *Anemone* section *Anemone* (Ranunculaceae). *Botanical Journal of the Linnean Society* 160: 312–354.

GOSTYŃSKA-JAKUSZEWSKA M. 1992. *Anemone* L., Zawilec. In: Jasiewicz A [ed.]. *Flora Polski. Rośliny Naczyniowe*. Vol. 4, 46–50. 2nd edition. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

JERNSTEDT JA, and CLARK C. 1979. Stomata on the fruits and seeds of *Eschscholzia* (Papaveraceae). *American Journal of Botany* 66(5): 586–590.

Kuźniewski E. 1964. Studia systematyczne rzędu Ranales Engler. Część I. Morfologia i anatomia owoców niektórych gatunków *Anemone* L. *OTPN Zeszyty Przyrodnicze* 4: 135–148.

Matuszkiewicz W. 2006. *Przewodnik do Oznaczania Zbiorowisk Roślinnych Polski.* Wydawnictwo Naukowe PWN, Warszawa.

Médail F, Ziman S, Boscaiu M, Riera J, Lambrou M, Vela E, Dutton B, and Ehrendorfer F. 2002. Comparative analysis of biological and ecological differentiation of *Anemone palmata* L. (Ranunculaceae) in the western Mediterranean (France and Spain): an assessment of rarity and population persistence *Botanical Journal of the Linnean Society* 140: 95–114.

Piękoś-Mirkowa H, and Mirek Z. 2003. *Flora Polski. Atlas Roślin Chronionych*. Multico Oficyna Wydawnicza, Warszawa.

Rugenstein SR, and Lersten NR. 1981. Stomata on seeds and fruits of *Bauhinia* (Leguminosae: Caesalpiniodeae). *American Journal of Botany* 68(6): 873–876.

Saoud SN, Knio K, and Jury S. 2007. Phenetic analysis of *Anemone coronaria* (Ranunculaceae) and related species. *Botanical Journal of the Linnean Society* 153: 417–438.

Schuettpelz EJ, Hoot SB, Samuel R, and Ehrendorfer F. 2002. Multiple origins of Southern Hemisphere *Anemone* (Ranunculaceae) based on plastid and nuclear sequences. *Plant Systematics and Evolution* 231: 143–151.

Tamura M. 1995. Angiospermae: Ordnung Ranunculales. Fam. Ranunculaceae In: Hiepko P [ed.], *Die Natürlichen Pflanzenfamilien*. Vol. 17a(4), 324–349. Duncker & Humblot, Berlin.

Wang ZF, and Ren Y. 2008. Ovule morphogenesis in Ranunculaceae and its systematic significance. *Annals of Botany* 101: 447–462.

Zieliński J, Tomaszewski D, Guzicka M, and Maciejewska-Rutkowska I. 2010. Stomata on the pericarp of species of the genus *Rosa* L. (Rosaceae). *Plant Systematics and Evolution* 284: 49–55.

Ziman S, Bulakh E, and Tsarenko O. 2011. *Anemone* L. (Ranunculaceae): comparative morphology and taxonomy of the species from the Balkan flora. *Botanica Serbica* 35(2): 87–97.

Ziman SN, Bulakh EV, Kadota Y, and Keener CS. 2008. Modern view on the taxonomy of the genus *Anemone* L. sensu stricto (Ranunculaceae). *The Journal of Japanese Botany* 83: 127–155.

Ziman SN, Keener CS, Kadota Y, Bulakh EV, and Tsarenko ON. 2006. A revision of *Anemone* L. (Ranunculaceae) from the Southern Hemisphere. *The Journal of Japanese Botany* 81(4): 193–224.