Structural basis of harmomegathy: evidence from Boraginaceae pollen

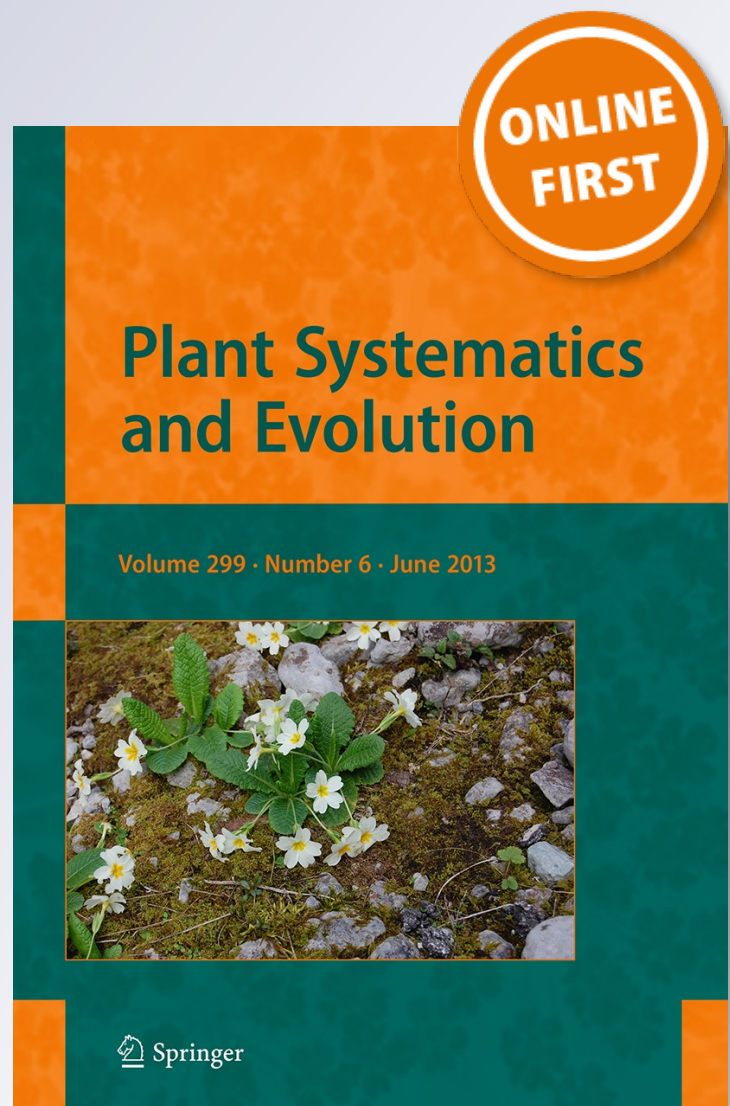## Olga A. Volkova, Elena E. Severova & Svetlana V. Polevova

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Structural basis of harmomegathy: evidence from *Boraginaceae* pollen

**Olga A. Volkova · Elena E. Severova ·
Svetlana V. Polevova**

Abstract Upon release from the anther, pollen grains can be exposed to dry environment and dehydrate. To survive in dry conditions, the pollen wall possesses the ability to fold itself due to water loss-harmomegathic mechanism. Apertures seem to function as the primary elements of harmomegathy as they are more elastic than the remainder of the pollen wall. Contribution of other sporoderm structures, surface features, and pseudocolpi in harmomegathy are usually not considered in palynological studies. The nature of pseudocolpi has not been properly understood until now, partly because of common use of acetolysis method as a standard procedure. Different structures involved in the harmomegathy mechanism were studied in *Cryptantha celosioides*, *Cryptantha coryi*, *Heliotropium europaeum*, *Myosotis palustris*, *Rindera bungei*, and *Rindera tetraspis*. Scanning electron microscopy was used to study harmomegathy in hydrated and dehydrated pollen grains. In addition, transmission electron microscopy was used to elucidate the ultrastructural basis of pseudocolpi and other harmomegathic structures with special attention to intine structure. Our data reveal that additional flexibility of the pollen wall in *Boraginaceae* is provided by pseudocolpi, rugulate surface, tectate–columellate ultrastructure, and a transverse groove. Curious triangular polar poroid areas are described in *M. palustris*.

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Introduction

Harmomegathy is a characteristic infolding of pollen grains to accommodate the decrease in cellular volume due to water loss.

Upon release from the anther, angiosperm pollen can be exposed to dry environment and dehydrates. The pollen wall consists of two chemically different layers—the exine and the intine. The exine is stiff, chemically resistant, and water impermeable due to its sporopollenin composition and consists of two layers. The ektexine and endexine are slightly different chemically, but it is unknown how this affects the water pathway. The pectocellulosic intine is more permeable to water passing (Heslop-Harrison 1987). The pollen grains should cover the intine to prevent plasmolyze and at the same time to maintain routes of water exchange with environment. The pollen possesses a variety of physiological and structural adaptations to control their water balance. One of them is the ability of pollen wall to infold itself to prevent further desiccation. Wodehouse (1935) invented the term "harmomegathy" for this folding process. The predominant elements of harmomegathy are apertures, where the exine is fragmentary and the intine is usually thickened (Heslop-Harrison 1979b; Blackmore and Barnes 1986). Payne (1972, 1981) has extended Wodehouse's concept of harmomegathy, showing that the entire pollen wall, not just the apertures, functions in volume regulation. Though the exine is extremely rigid, at the same time it is flexible and
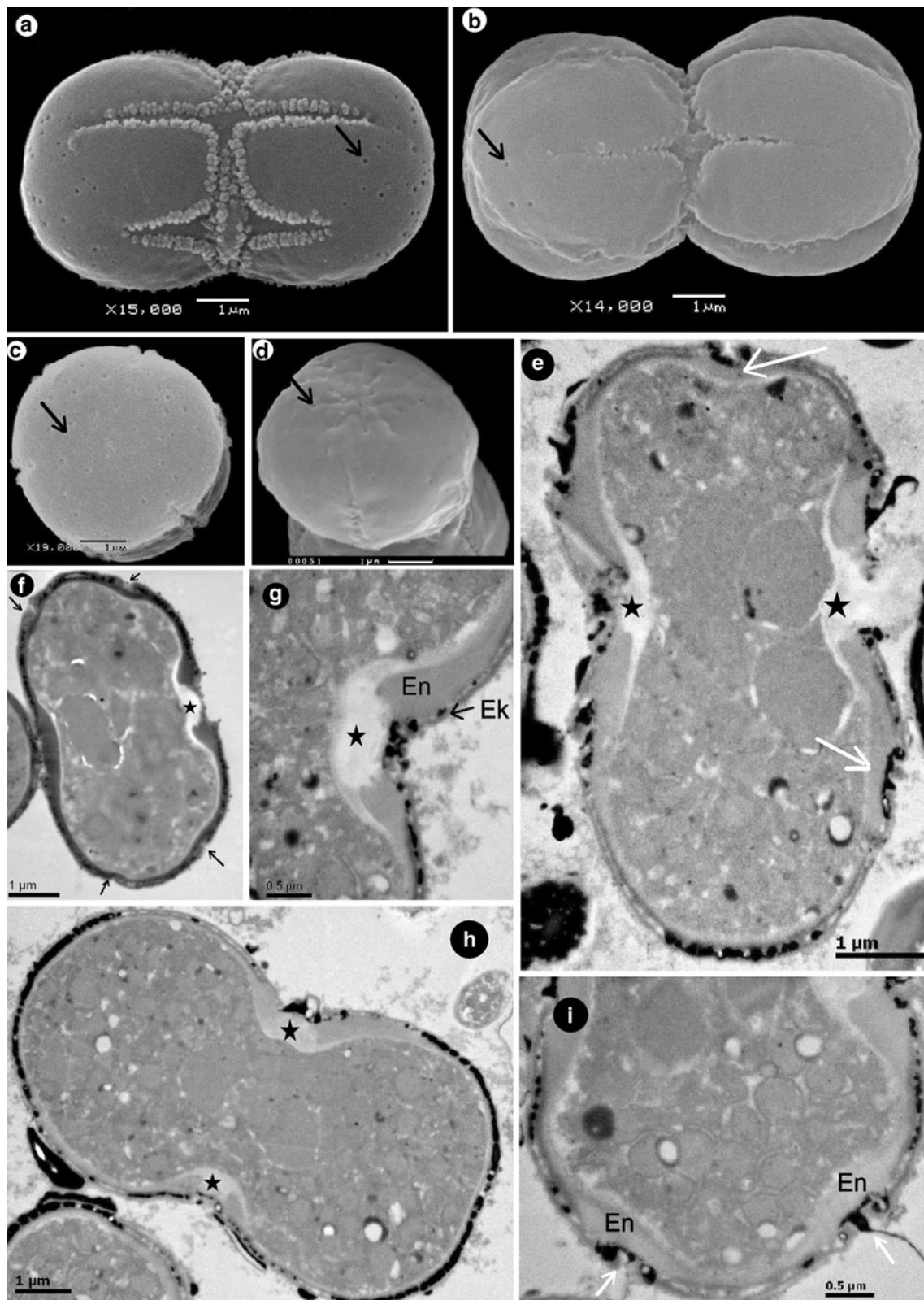
elastic and can contract and stretch (Blackmore and Barnes 1986; Rowley and Skvarla 2000; Halbritter and Hesse 2004; Hesse et al. 2009).

The pollen morphology of *Boraginaceae* is heterogeneous (Erdtman 1966). Previous studies on the family show that pollen grains of two tribes, Cynoglosseae and

◄ **Fig. 1** Pollen grains of **a–e**, **g–i** *Cryptantha coryi* I.M. Johmst. and **f** *C. celosioides* (Eastw.) Payson **a** equatorial view of pollen grain in hydrated state; **b** equatorial view of pollen grain in dehydrated state; **c** polar view of pollen grain in hydrated state. Exine surface is perforated (*black arrows*) at the poles; **d** polar view of pollen grain in dehydrated state, showing folding at the polar area. **e** Section of a pollen grain. The intine is thickened in apertural regions (*filled star*). Single polar poroid area is observed on one of the poles and another poroid area is slightly displaced from the pole (*white arrows*). **f** Section of a pollen grain. The intine is thickened in apertural regions (*filled star*). Four polar poroid areas are observed on the poles (*black arrows*). **g** Pollen wall ultrastructure in apertural region. The intine (*filled star*) and the endexine (*En*) are thickened. The ektexine (*Ek*) is fragmentary. **h** Section of a pollen grain. The intine is thickened in apertural regions (*filled star*). **i** Ultrastructure in two polar poroid areas (*white arrow*). The intine has the same thickness as in the interapertural areas

Eritricheae, are heterocolpate with three apertures and three alternating pseudocolpi (Hargrove and Simpson [2003](#)). The nature of pseudocolpi has not been properly understood until now because of the limitations of employed techniques. With the prevalence of acetolysis as a standard preparation procedure researchers do not pay attention to the intine. Nevertheless, the intine plays an essential role in aperture function, both in germination and harmomegathy (Waha and Hesse [1986](#)). The purpose of this study was to investigate the ultrastructural basis of pseudocolpi and other harmomegathic structures in *Boraginaceae* pollen with special attention to the intine.

Materials and methods

Pollen grains of *Cryptantha* [*C. coryi* I.M. Johmst., *C. celosioides* (Eastw.) Payson.], *Heliotropium* (*H. europaeum* L.), *Myosotis* [*M. palustris* (L.) L.], and *Rindera* (*R. bungei* Gürke, *R. tetraspis* Pall.) were examined using light (LM), scanning (SEM), and transmission (TEM) electron microscopy. Pollen samples were collected from herbarium specimens deposited in the Moscow State University (MW) and Komarov Botanical Institute (LE). Fresh anthers of *Myosotis* were collected in the Botanical Garden of the Komarov Botanical Institute. All studied samples are listed in "[Appendix](#)".

For light microscopy, pollen was acetolysed (Erdtman [1960](#)) and mounted in glycerine gelatin. Ten measurements of pollen grains were made from each specimen in the process of light microscope examinations. For *Cryptantha* pollen measurements are given for *C. celosioides and C.*
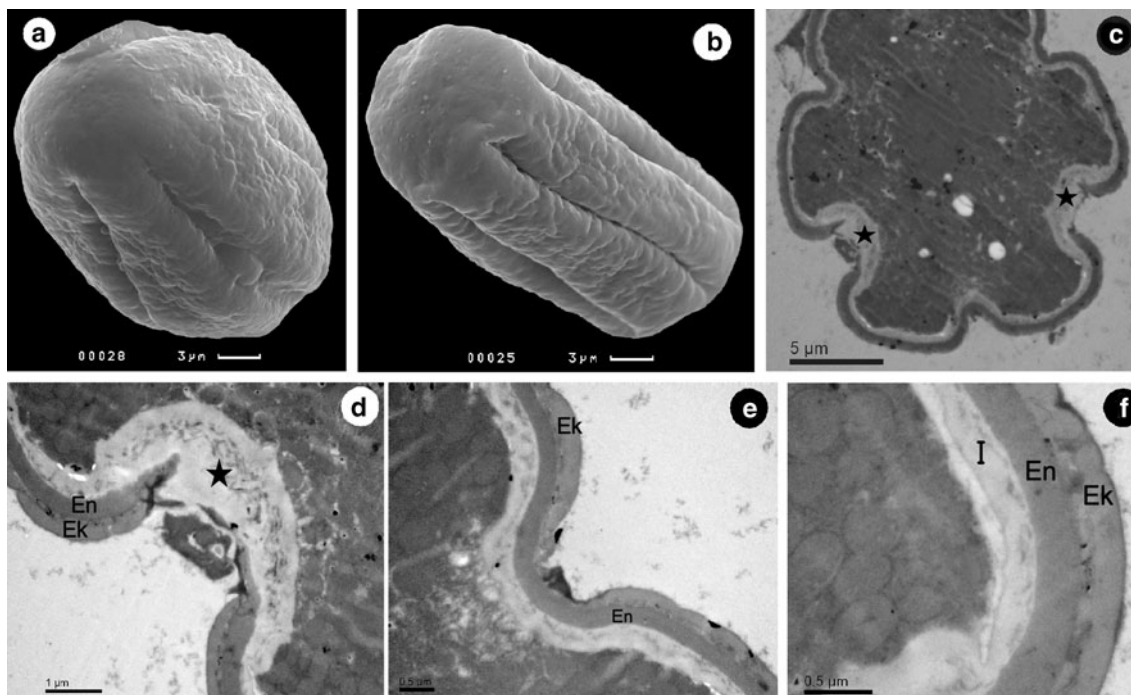
Fig. 2 Pollen grains of *Heliotropium europaeum* L. **a** Oblique polar view of pollen grain in hydrated state. **b** Oblique polar view of pollen grain in dehydrated state. **c** Section of a pollen grain. The intine (*filled star*) is thickened in apertural regions. In pseudoapertural regions, the intine has the same thickness as in the interapertural regions. **d** Pollen wall ultrastructure in apertural regions. The intine beneath the endoaperture is much thicker than in the interapertural region and shows three layers: two thin, homogeneous layers, the outer and the inner, and a thick middle layer, crossed by trabecula. **e** Pollen wall ultrastructure in pseudoapertural region. The intine has the same thickness as in the interapertural region. **f** Pollen wall ultrastructure in interapertural regions consists of intine (*I*) and two exine layers. The exine consists of an endexine (*En*) layer and an ektexine (*Ek*). The ektexine is thick, tectate–columellate, consisting of columellae and tectum. The columellae are very short and thick. The foot layer is absent
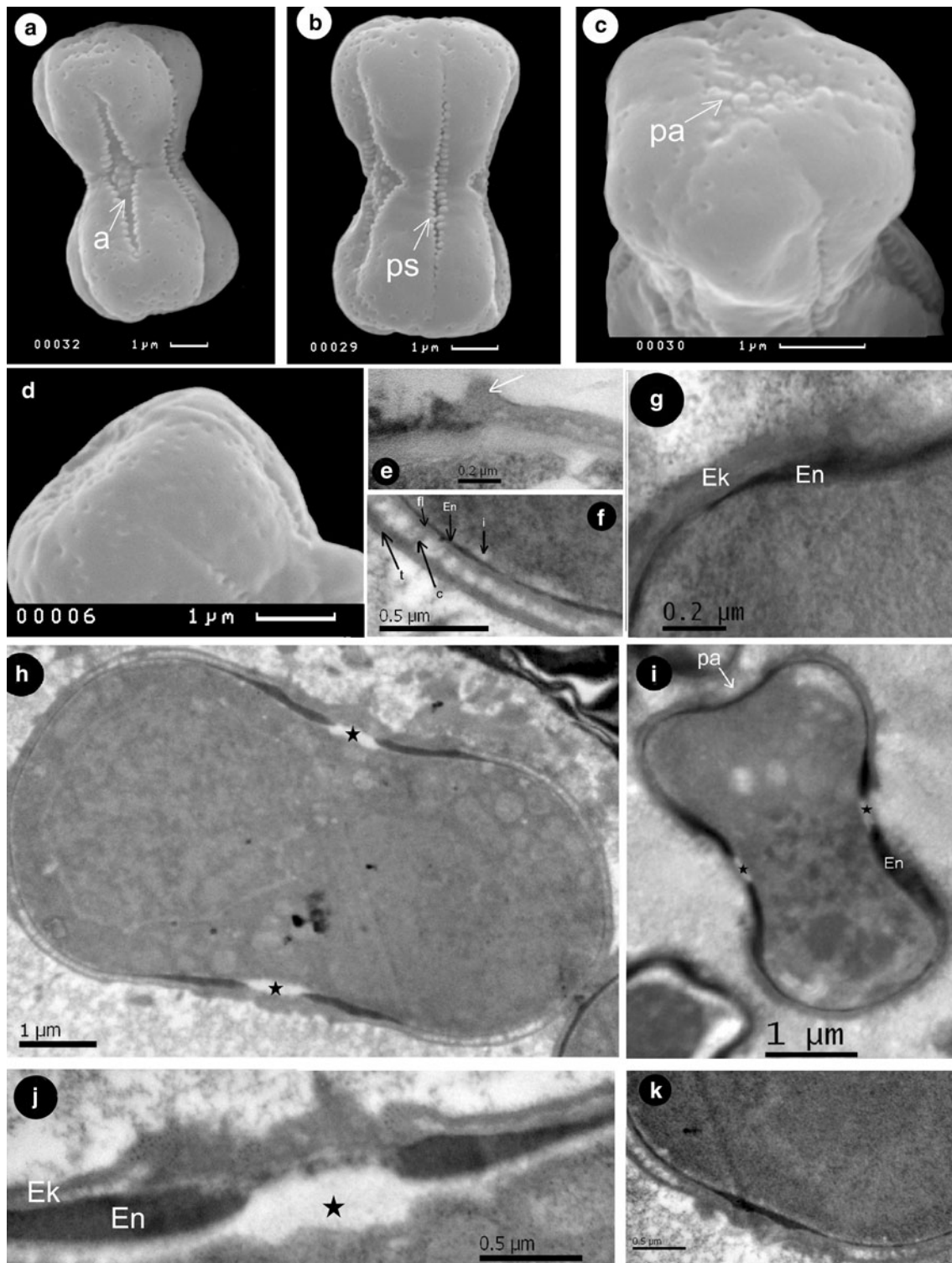
coryi, respectively. Measurements throughout the article are mean values (with ranges in parentheses).

For scanning electron microscopy, pollen samples were soaked in water, dehydrated through ethanol and acetone series, and critical-point-dried using Hitachi HCP-2 critical point dryer. Dry specimens were coated with gold and palladium using Eiko IB-3 ion-coater (Tokyo, Japan) and observed under JSM-6380LA SEM (JEOL, Tokyo, Japan) and CamScan 4 DV (CamScan, UK).

For transmission electron microscopy, fresh material was fixed in 2.5 % glutaraldehyde buffered with phosphate buffer and postfixed with 1 % OsO_4. Following

◄ **Fig. 3** Pollen grains of *Myosotis palustris* (L.) L. **a** Equatorial view of pollen grain in hydrated state, aperture visible (SEM). **b** Equatorial view of pollen grain in hydrated state, "pseudoaperture" visible (*ps*) (SEM). **c** Polar view of pollen grain in hydrated state, polar poroid area are visible (*pa*) (SEM). **d** Polar poroid area at the pole in dehydrated state (SEM); **e** pollen wall ultrastructure near the pseudocolpi. The tectum is raised and arched at the margins (*white arrow*). **f** Pollen wall ultrastructure in interapertural regions consists of an intine (*i*) and two exine layers. The ektexine consists of a foot layer (*fl*), columellae (*c*) and tectum (*t*). The endexine (*En*) is thick and homogeneous. **g** Pollen wall ultrastructure in area of pseudocolpi. It is characterized by thickened endexine (*En*) and thin intine. The ektexine (*Ek*) is fragmentary (TEM). **h** Section of a pollen grain. The intine is thickened in apertural region (*filled star*) (TEM). **i** Section of a pollen grain. The structure of the pollen wall in the polar poroid area (*pa*) resembles the structure of the pseudoapertures (TEM). **j** Pollen wall ultrastructure in colpori region. The intine (*filled star*) beneath the endoaperture is much thicker than in the interapertural region. The exine consists of endexine (*En*) and ektexine (*Ek*). The ektexine is thick, tectate–columellate, consisting of columellae, and tectum (TEM). **k** Pollen wall ultrastructure in polar poroid area. The endexine is thickened (TEM)

dehydration in ethanol and acetone series, the material was embedded in Epon. Leica UC-5 ultramicrotome with diamond knives was used for making ultrathin sections. Sections were stained with uranyl acetate and examined with Jeol 1011 B microscope. The work was performed at User Facilities Center of M. V. Lomonosov Moscow State University under financial support of Ministry of Education and Science of Russian Federation.

For pollen descriptions, we followed the terminology suggested by Hesse et al. (2009). The terms "subsidiary colpi", "simple colpi", and "heteroaperturate" frequently used in publications (Muller 1981; Patel et al. 1984; Booi et al. 2003) were rejected because pseudocolpi do not function as an exit for the pollen tube and, therefore, do not satisfy the term "aperture". The terms "colpori", "pseudocolpi" (=pseudoapertures), and "heterocolpate" were used instead. The term "endoaperture" was used for the ora and the term "ektoaperture" for the colpoid part of the apertures. For polar structures in *Myosotis palustris* the term "polar poroid area" was used.

Results

Cryptantha celosioides (Eastw.) Payson., *Cryptantha coryi* I.M. Johmst (Fig. 1)

Pollen grains are monad, zonocolporate, heterocolpate, isopolar, hourglass-shaped in equatorial view and circular in polar view. $P = (6.73–7.87)\ 7.30–8.92\ (8.53–9.33)$ μm, $E = (3.03–3.96)\ 3.44–4.18\ (3.89–4.91)$ μm at the constricted mid-region; the widest diameter is $(3.87–4.75)\ 4.18–5.16\ (4.90–5.68)$ μm.

The exine surface is psilate in equatorial region and perforated at the poles. Pollen grains are heterocolpate with three colpori and three alternating pseudocolpi (pseudoapertures). The ektoapertures are narrowly rhombic in shape. Endoapertures are equatorial, circular, or circular–elliptical with granular membrane. The exine along the margin of the aperture consists of verrucate deposits, giving the margin a toothed appearance. Three pseudoapertures are parallel to colpori and alternate with them. Pseudoapertures are nearly linear and slightly longer than the apertures. The exine along the edge of the pseudoapertures also consists of verrucate deposits. A narrow, transverse groove appears to extend from aperture to pseudoaperture along the equatorial perimeter (Fig. 1a, b).

The pollen wall in interapertural regions consists of an intine and two exine layers (Fig. 1h). The ektexine is thick, tectate–columellate, consisting of foot layer, columellae, and tectum. The tectum is distinctly perforate at the poles. The endexine is clearly defined, homogeneous, and thicker than the intine.

The apertural and pseudoapertural regions are characterized by a fragmentary ektexine, and along the margins the tectum is raised and arched. These marginal thickenings appear to correspond with the marginal verrucate structures observed with SEM. The endexine thickened close to the colpi and pseudocolpi. In pseudoapertural regions, the intine has the same thickness as in the interapertural areas. The intine beneath the endoapertures is greatly thickened, two-layered (Fig. 1e, f, h). The structure of the pollen wall at the poles is characterized by perforated tectum, slightly thickened endexine, and thin intine (Fig. 1c, d, g). In dehydrated state, small indentations are observed at polar areas (Fig. 1d).

In the equatorial plane of some pollen grains, one or two small polar poroid area are observed on the poles or slightly displaced from the pole (Fig. 1g). The pollen wall in such areas is characterized by the absence or reduction of ectexine, thickened endexine, and thin intine, equal to intine in interapertural regions.

Heliotropium europaeum L. (Fig. 2)

Pollen grains are monad, zonocolporate, heterocolpate, isopolar, prolate in equatorial view, and circular in polar view. $P = 11.29\ (10.4–13.1)$ μm, $E = 7.45\ (7.3–8.43)$ μm.

Exine surface is rugulate, psilate at the poles. Pollen grains are heterocolpate with three colpori and three alternating pseudocolpi (pseudoapertures). The apertures and pseudoapertures are narrow, with smooth margins. Endoapertures are equatorial, circular (Fig. 2a).

The pollen wall in interapertural regions consists of an intine and two exine layers. The ektexine is thick,
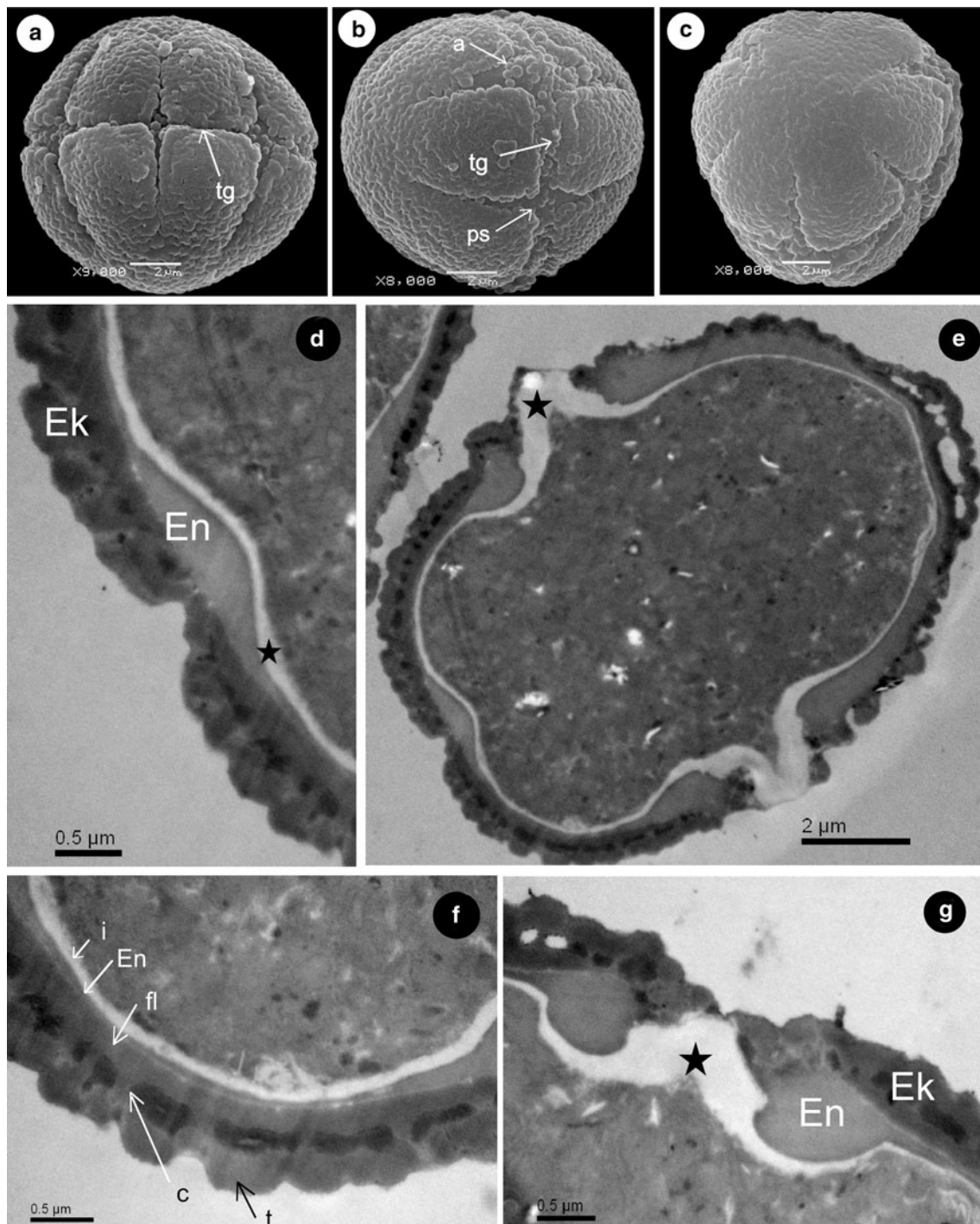
Fig. 4 Pollen grains of *Rindera bungei* Gürke. **a**, **b** Equatorial view of pollen grain in dehydrated state. A narrow, transverse groove (*tg*) extends from aperture (*a*) to pseudoaperture (*ps*) along the equatorial perimeter. **c** Polar view of pollen grain in hydrated state. **d** Pollen wall ultrastructure at the polar area. The tectum is thinned, the intine (*filled star*) has the same thickness as at interapertural regions, and the endexine (*En*) is thickened. Structurally this area looks like polar poroid area. **e** Section of a pollen grain. The endexine is thickened towards endoapertural margins. The intine (*filled star*) is thick in endoapertural region. **f** Pollen wall ultrastructure in interapertural region. It consists of an intine (*i*) and two exine layers. The exine consists of endexine (*En*), foot layer (*fl*), columellae (*c*), and tectum (*t*). **g** Pollen wall ultrastructure in colpori region. The intine beneath the endoaperture (*filled star*) is much thicker than in the interapertural region. The exine consists of an endexine (*En*) and ektexine (*Ek*)
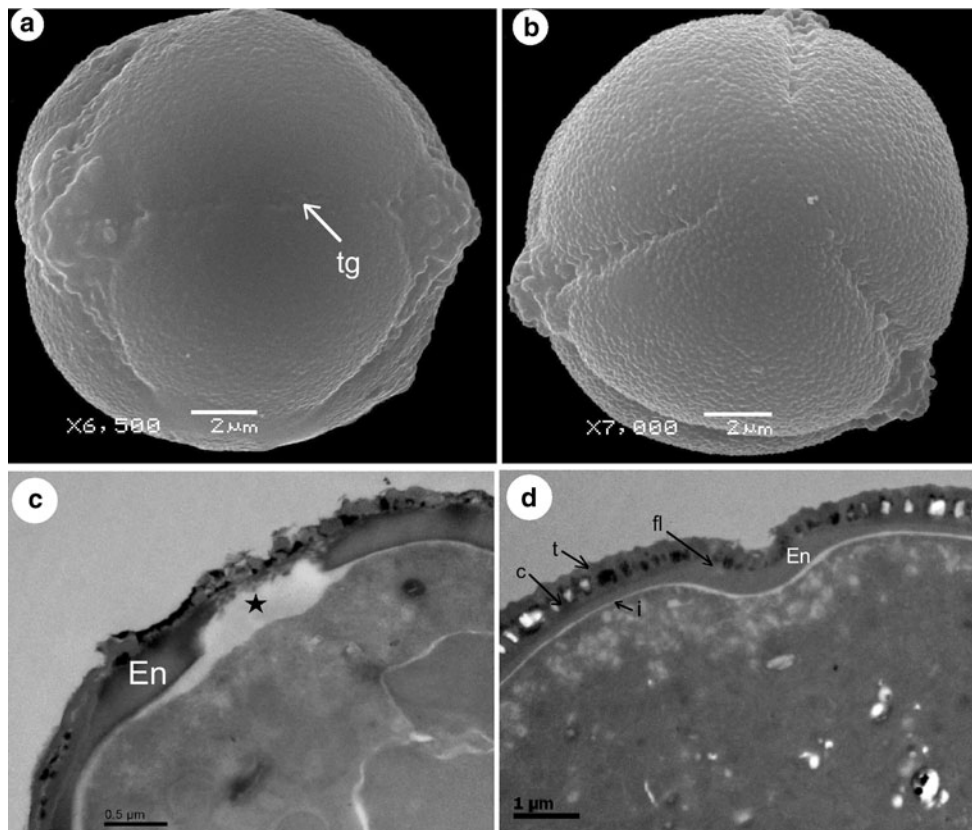
Fig. 5 Pollen grains of *Rindera tetraspis* Pall. **a** Equatorial view of pollen grain in hydrated state. A narrow transverse groove (*tg*) appears to extend along the equatorial perimeter. **b** Polar view of pollen grain in hydrated state. **c** Pollen wall ultrastructure in colpori region. The endexine (*En*) is thickened close to the endoapertures and the intine (*filled star*) is thickened beneath the endoaperture. **d** Pollen wall ultrastructure in interapertural region consists of intine (*i*) and two exine layers. The ektexine consists of a foot layer (*fl*), columellae (*c*), and tectum (*t*). The endexine (*En*) is thick and homogeneous

tectate–columellate, consisting of columellae and tectum. The columellae are very short and thick. The inner part of the exine has high electron density and it is not possible to distinguish the endexine from the foot layer. The intine is thick (Fig. 2f).

The apertural and pseudoapertural regions are characterized by reduced ectexine with thin fragmentary tectum and the lack of columellae. The endexine has the same thickness as in the interapertural regions. The intine beneath the pseudoapertures has the same thickness as in the interapertural regions (Fig. 2e). At the apertural area, the intine consists of three layers: the inner and the outer layers are thin and homogeneous, the middle layer is thick and crossed by trabecula (Fig. 2d). The endexine and the intine beneath the pseudoapertures are similar those to interaperturate regions.

Myosotis palustris (L.) L. (Fig. 3)

Pollen grains are monad, zonocolporate, heterocolpate, isopolar, hourglass-shaped in equatorial view, and circular or triangular in polar view. $P = 6.85$ (6.04–7.43) μm, $E = 3.53$ (2.70–4.32) μm at the constricted mid-region and 4.26 (3.29–5.51) μm at the widest place.

The exine surface is psilate in the equatorial region. Pollen grains are heterocolpate with three colpori and three alternating pseudocolpi (pseudoapertures). Besides six zonal apertures, both poles have additional triangular-shaped poroid areas (Fig. 3c, d). The ektoapertures are rhombic in shape (Fig. 3a). Endoapertures are equatorial, circular or circular–elliptical with granular membrane. The exine along the margin of the apertures consists of verrucate deposits, giving the margin a toothed appearance. Three pseudoapertures are nearly linear, longer than colpori, and alternate with them (Fig. 3b). The exine along the edge of the pseudoapertures also consists of verrucate deposits. The two polar poroid area are triangular with the edges oriented towards the colpori (Fig. 3c). Margins of polar poroid area are ornamented by verrucae, similar to those found in apertures and pseudoapertures. A few median verrucae occur in the center of the polar poroid area.

The pollen wall in interapertural regions consists of an intine and two exine layers. The ektexine is thick,

tectate–columellate, consisting of a foot layer, columellae, and tectum. The tectum is distinctly perforate at the poles. The endexine is thick, homogeneous, and thicker than the intine.

The apertural and pseudoapertural regions are characterized by fragmentary ektexine. At the margins of them, the tectum is raised and arched (Fig. 3e). The endexine thickened close to the colpi and pseudocolpi. In pseudoapertural regions, the intine has the same thickness as at interapertural ones. The intine beneath the endoapertures is markedly thickened.

The structure of pollen wall of the polar poroid area resembles the structure of the pseudoapertures. It is characterized by fragmentary ectexine, thickened endexine, and thin intine. These similarities allow us to consider them as additional pseudoapertures.

Rindera bungei Gürke (Fig. 4)

Pollen grains are monad, zonocolporate, heterocolpate, isopolar, prolate in equatorial view, and hexagonal in polar view. $P = 15.09$ (14.2–16.2) µm, $E = 11.88$ (10.5–13.6) µm.

The exine surface is rugulate. Pollen grains are heterocolpate with three colpori and three alternating pseudocolpi (pseudoapertures). The pseudoapertures are very narrow with smooth margins, shorter than the apertures. The apertures are spindle-shaped. Endoapertures are equatorial, elongated with granular membrane. A narrow, transverse groove extends from aperture to pseudoaperture along the equatorial perimeter (Fig. 4a, b).

The pollen wall in interapertural regions consists of an intine and two exine layers. The ektexine is thick, tectate–columellate, consisting of a foot layer, columellae, and tectum. The endexine is thick, homogeneous, and thicker than the intine (Fig. 4f).

The apertural and pseudoapertural regions are characterized by a fragmentary ektexine. The endexine is thickened close to the colpi and pseudocolpi. In pseudoapertural regions, the intine has the same thickness as in interapertural ones. The intine beneath the endoaperture is greatly thickened (Fig. 4g).

In the equatorial plane of some pollen grains, polar poroid area are observed (Fig. 4d, e). The pollen wall in polar poroid area has fragmentary ectexine, thickened endexine, and thin intine, equal to interapertural regions.

Rindera tetraspis Pall. (Fig. 5)

Pollen grains are monad, zonocolporate, 3-colporate, isopolar, prolate in equatorial view, and circular in polar view. $P = 14.15$ (13.5–14.9) µm, $E = 12.9$ (12.4–13.7) µm.

The exine surface is verrucate. The apertures are narrow, with smooth margins. Endoapertures are equatorial, circular with granular membrane. A narrow transverse groove extends along the equatorial perimeter (Fig. 5a, b).

The pollen wall in interapertural region consists of an intine and two exine layers. The ektexine is thick, tectate–columellate, consisting of an foot layer, columellae, and tectum. The endexine is homogeneous and thicker than the intine (Fig. 5d).

The apertures are characterized by a fragmentary ektexine. The endexine is thickened close to the colpi. The intine beneath endoapertures is greatly thickened (Fig. 5d).

Discussion

The pollen grains reveal a great diversity in apertures and surface sculpture. Such diversity is influenced by various factors (Thanikaimoni 1986). The primary one is the function of pollen grains in plant reproduction. Pollen germination requires hydration with liquids from the stigma that need to enter the pollen grain quickly and easily. This problem can be solved by large apertures. On the other hand, large apertures are the places of intensive transpiration and the pollen grains should reduce their size to decrease the water loss. It is especially important for wind-pollinated plants (Punt 1986). The exine structure and surface should be integrated with aperture into a unified structure which allows to minimize the chance of exine rupture and to provide efficient germination and metabolic processes. The attempts to reconcile different functions with conflicting demands lead to great morphological diversity in pollen structure, but all attempts are successful only to some degree; none can be perfect (Heslop-Harrison 1979a; Muller 1979; Punt 1986).

One of the major trends of structural evolution is specialization towards controlled harmomegathy (Muller 1979). One of the most widespread harmomegathic systems involves compound apertures wherever the endoapertural areas are more elastic than the remainder of the colpi (Blackmore and Barnes 1986). This type of harmomegathic mechanism is observed in *R. tetraspis*. When pollen dehydrates, the granules of sporopollenin in apertural areas become compacted, and the margins of the apertures are drawn inward to protect the intine. To provide resistance to compression thickening of the endexine occurs in aperture (Payne 1981). During rehydration aperture membrane is primarily exposed to stretching. In this type of harmomegathy colpori guided longitudinally folding are the main elements. When the apertural membrane folds inward, the interapertural region should stretch gradually and thus the entire pollen wall may be involved in stretching and contraction. Participation of non-aperturate

Table 1 Structures involved in harmomegathy

	Discontinuous surface	Compound apertures	Transverse groove	Pseudoapertures	Tectate–columellate ultrastructure	Polar poroid areas	Perforated tectum on the poles	Verrucate deposits at the margins
Cryptantha celosioides (Eastw.) Payson., *C. coryi* I.M. Johmst.		±	±	±	±		±	±
Heliotropium europaeum L.	±	±		±	±			
Myosotis palustris (L.) L.		±		±	±	±		±
Rindera bungei Gürke	±	±	±	±	±			
Rindera tetraspis Pall.	±	±	±	±	±			

areas in harmomegathy is usually not considered in palynological studies, but there is some evidence pointing to their important role in that process (Payne 1972; Blackmore and Barnes 1986). Payne, Blackmore, and Barnes showed that discontinuous exine, tectate–columellate ultrastructure, and any thinned regions provide additional elasticity of the pollen wall. Flexibility of the sporopollenin walls is also evident from photographs of stressed pollen, published by Katifori et al. (2010). In *R. tetraspis*, additional flexibility of the wall is provided by rugulate surface, tectate–columellate ultrastructure, and a transverse groove. Payne (1981) noted that tectate–columellate ultrastructure of the wall provides great resistance to compressive forces. Transverse groove is a latitudinally oriented harmomegathic system. Such type of harmomegathy can be achieved by different ways. Muller (1979) described this type in *Rhizophora racemosa* where colpi are reduced and the polar area is more flexible in comparison with the remainder of the wall. In some species of *Tournefortia* a latitudinally oriented system is more expressed. It consists of a rigid, smooth, and thick polar cap that is separated from more flexible verrucate equatorial area (Nowicke and Skvarla 1974). This type of harmomegathy is observed in taxonomically distant groups that can be considered as convergent development of highly specialized pollen structure. Possibly it also can indicate the influence of similar environmental factors influenced the harmomegathic efficiency.

In some pollen grains, folding has been displaced from the apertures to interapertural regions. In this case folding upon dehydration occurs in specific areas within interapertural region with reduced thickness of the wall. When these areas are distinctly delimited, they are called "pseudoapertures" because they never serve as sites of germination but look like real apertures. Such pseudoapertures are additional elements of harmomegathy in *C. coryi*, *C. celosioides*, *R. bungei,* and *H. europaeum*. These species demonstrate various combinations of harmomegathic structures. In *H. europaeum*, longitudinally oriented

harmomegathic system is formed by meridional pseudoapertures and rugulate surface, whereas the latitudinally oriented system consists of a rugulate exine surface in interapertural areas and psilate at the poles.

Rindera and *Cryptantha* combine both longitudinally and latitudinally oriented harmomegathy systems. Pollen grains of *C. coryi* and *C. celosioides* have pseudoapertures, and transverse groove, but psilate surface. It makes the wall more rigid, and decreases folding ability. To provide greater flexibility of this rigid wall, pollen grains possess perforated tectum on the poles (Fig. 1a–c). These perforations look like breaks in the tectum and in dehydrated state are often depressed (Fig. 1d). Sometimes the endexine is thickened beneath the pole areas. These features resemble polar poroid area but are not always clearly visible. Varied exine thickness at the polar areas was observed in other *Boraginaceae* species. In *Elizaldia calycina* the variation in the polar exine thickness is due to the different height of the columellae (Bigazzi and Selvi 1998). Contraction of the polar areas may be achieved by forming syncolpate pollen grains in some species of *Onosma* (Retief and Van Wyk 1997). Syncolpy allows only limited infolding, with stresses more equally distributed over the polar hemispheres (Muller 1979). The tendency for syncolpy is common in *Boraginaceae* and closely related families Lennoaceae, Hoplestigmataceae, and especially Hydrophyllaceae (Walker and Doyle 1975). Harmomegathic system in *M. palustris* pollen grains involves pseudoapertures, perforated tectum, and two well-defined triangular polar poroid areas, characterized by thickened endexine and thin intine. These polar poroid areas are folded inward in dehydrated state and structurally resemble meridional pseudoapertures (Fig. 3c, d, i). Hargrove and Simpson (2003) also suggested that polar poroid areas may be structural remnants of sporogenesis. Structures similar to the polar poroid area of *M. palustris* were described by Gentrya and Carr (1976-cited by Hargrove and Simpson 2003) as "irregular spaced shallow depressions at the poles" in *Hackelia* species (close relatives of *Cryptantha*)

and are probably homologous. Perhaps, these cases of polar exine variation may reflect the hypothetical way of polar poroid area formation.

The verrucate deposits at the margins of the apertures in *M. palustris* serve as struts (comparable with the costae of colpus margins). They provide additional resistance to compressive forces and help to prevent aperture rupture.

Structures involved in harmomegathy in all investigated species are summarized in Table 1.

Thus, in heterocolpate pollen two main functions of apertures—harmomegathy and germination—are performed by different parts of the wall that allow to control the processes of hydration and dehydration. Colpori are associated with the intine thickening due to the functions of the intine in germination. Absence of endoaperture in the pseudoaperture leads to the loss of germination function but provides additional flexibility. Such transference of functions can be assumed as one of the methods of evolution (Corner 1958). Muller (1979) considered heterocolpate pollen as an example of both more efficient harmomegathic system and successful germination, providing fast and efficient stigmatic contact. Blackmore and Barnes (1986) suggested that apertures combining both harmomegathy and germination are primitive, whereas the displacement of functions to different structures may be considered as derived.

Appendix

Cryptantha celosioides (Eastw.) Payson (MHA) (1) Fremont Co. T 40 N R106W S14, SE EDGE of Trail Lake at inlet of torrey creek, CA 8.2 air Mi SSE of Dubois, CA 6.9 MI S ON trail lake road. Edge of meadow North of Greek. 27.07.1985. Elev. 7,400 ft. June Haines 5169 with georgia haines. Rocky mountain herbarium (RM). (2) Two miles NE of Omak, Okanogan Co. Rare on open rocky Sagebrush slopes at edge of valley; flowers white. No 16141. Col. J. A. Calder, J. A. Parmelee, R. L. Taylor. 8.05.1956. *Cryptantha coryi* I.M. Johnst. (MHA) Val Verde Co.: About ten miles east of Langtry Corolla white, turning orange with age. D. S. Correl and Helen B. Correl. No 30818. 3.04.1965. *Heliotropium europaeum* L. (MW) Tauricheskaya province, Berdyansk county, Girsovka village, Plowing-salt marshes. 18.07.1998. D. Duz. *Myosotis palustris* (L.) L. Botanical Garden of the Komarov Botanical Institute. 07.2010. *Rindera bungei* Gürke (MHA) USSR. Turkmenistan. Ashhabatskaya area. Central Kopetdag, Gopal-Dag, on the rocky substrate summit. 2,886 m above sea level. Coll. and Def. V. V. Nikitin. 06.25.1958. *Rindera tetraspis* Pall. (MHA) Uzbekistan, western spur of Zeravshanskogo ridge, env. per. Tyahta-karacha. Altitude of about 1,700 m, rocky slope. 27.04.1958. Col. A. K. Skvortsov, Def. N. Y. Stepanova. 10.06.2010.

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