Arctoa (2019) **28:** *103–115*
doi: 10.15298/arctoa.28.09

ON THE GENUS *HAPLOHYMENIUM* (ANOMODONTACEAE, BRYOPHYTA) IN RUSSIA

РОД *HAPLOHYMENIUM* (ANOMODONTACEAE, BRYOPHYTA) В РОССИИ

IRINA V. CZERNYADJEVA[1] & ELENA A. IGNATOVA[2]
ИРИНА В. ЧЕРНЯДЬЕВА[1], ЕЛЕНА А. ИГНАТОВА[2]

Abstract

The genus *Haplohymenium* is represented in Russia by four species, including *H. sieboldii*, reported for the first time for the country, in southern regions of Primorsky Territory and Transbaikalia. This species is very closely related to *H. triste* and their distinction is discussed in detail. *Haplohymenium longinerve* is usually described as an easily recognizable species due to unipapillose laminal cells, whereas in all other species the cells are multipapillose. However the variation in papillosity is drastic in this species. Different expressions of pseudounipapillose pattern of *Haplohymenium longinerve* are illustrated with different microscopy techniques, revealing a quite delicate structure of large conic papillae, making this character applicable with caution.

Резюме

В настоящее время на территории России известно четыре вида из рода *Haplohymenium*, включая *H. sieboldii*, который приводится для России впервые по образцам из Приморского края и Забайкалья. Этот вид очень близок к *H. triste* по молекулярным маркерам и морфологии; обсуждаются отличия между ними. *Haplohymenium longinerve* обычно описывают как легко отличимый вид, поскольку только у него клетки пластинки листа имеют одну папиллу, тогда как у всех других видов рода клетки мультипапиллозные. Однако выясняется, что характер папиллозности очень сильно варьирует *у H. longinerve*. Клетки этого вида охарактеризованы как псевдоунипапиллозные; различные варианты строения папилл и степени выраженности крупных папилл, которые выглядят как одиночные, показаны с использованием разных методов микроскопирования; крупные конические папиллы *H. longinerve* имеют деликатную структуру, что требует осторожности в применении этого признака как диагностического.

KEYWORDS: mosses, *Haplohymenium*, new record, Russia, papillae.

INTRODUCTION

Haplohymenium is a small moss genus, with a circum-temperate distribution in the Northern Hemisphere. It sometimes is treated within the genus *Anomodon*, as a subgenus or section, especially in regions where just one species of the genus, *H. triste,* occurs (Granzow-de-la-Cedra, 1997, 2014). In East Asia, where all five species of the genus are represented, they form a well delimited group. Molecular phylogenetic analyses support a well delimited position of *Haplohymenium*, as its clade is maximally supported in analyses of different DNA regions (Ignatov *et al*., 2019). Thus, following Ignatov *et al*. (2019), we accept *Haplohymenium* as a separate genus. The important revision of the genus was published by Iwatsuki (1963), and in subsequent publication of Watanabe (1972) and Granzow-de-la-Cedra (1997), who mostly confirmed conclusions achieved by Iwatsuki for species limits and diagnostic characters.

Molecular phylogenetic analysis of *Haplohymenium* in the course of the general study of the Anomodontaceae (Ignatov *et al*., 2019) in general supports the taxonomic conclusions of Iwatsuki (1963), namely the distinction between *H. triste* and *H. pseudotriste* and between *H. longinerve* and *H. flagelliforme*. The distinction between *H. sieboldii* and *H. triste* appeared too small for species resolved in phylogenetic trees, but at least three substitutions differentiate two specimens of *H. sieboldii* from two specimens of *H. triste*, confirming their separation (Ignatov, pers. comm.). Therefore in the present paper, along with the first record of *H. sieboldii* in Russia we provide detailed comparisons of *H. sieboldii* with widespread and very variable *H. triste*.

The second aim of this present paper is the elucidation of variation in papillosity pattern in *H. longinerve*, because some of its morphotypes disagree with the species descriptions commonly presented in literature.

[1] – Komarov' Botanical Institute of Russian Academy of Sciences, Prof. Popov Str., 2, St. Petersburg, 197376 Russia – Россия, 197376, Санкт Петербург, Проф. Попова 2, Ботаническиий институт им. В.Л. Комарова РАН; e-mail: irinamosses@yandex.ru
[2] – Lomonosov Moscow State University, Faculty of Biology, Geobotany Dept., Leninskie Gory Str. 1-12, Moscow 119234 Russia – Россия, 119234, Москва, Ленинские Горы, д. 1 стр. 12, Московский государственный университет им. М.В. Ломоносова, Биологический факультет, кафедра геоботаники. E-mail: arctoa@list.ru

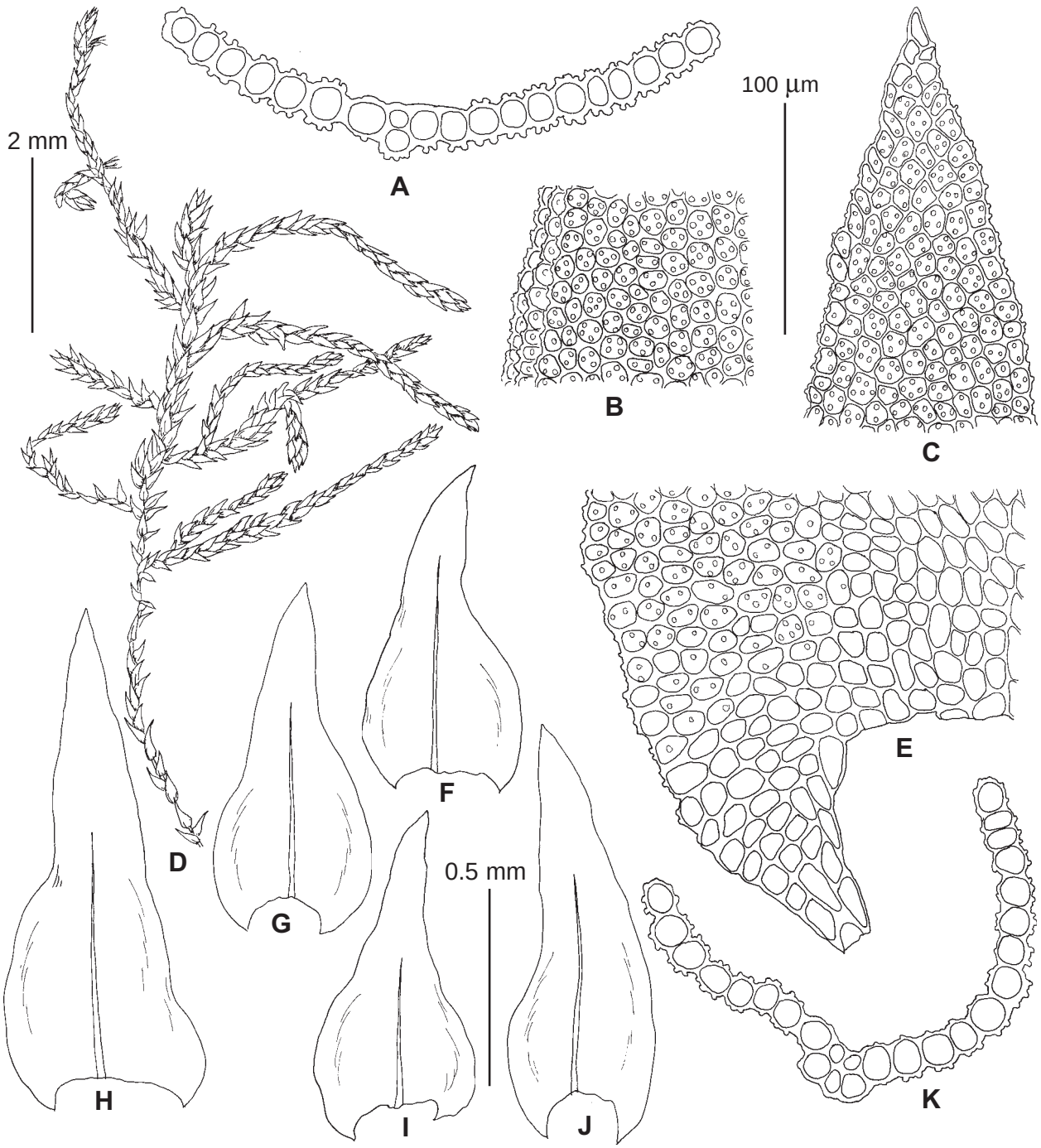

Рис. 1. *Haplohymenium sieboldii* (from: Russia, Primorsky Territory, Elomovskij Creek, *Malashkina & Ivanov Pr-8-25-13*, MHA9035395): A, K: leaf transverse section; B: mid-leaf cells; C: upper leaf cell; D: habit; E: leaf base; F–J: leaves. Scale bars: 2 mm for D; 0,5 mm for F–J; 100 µm: A–C, E, K.

Therefore, we provide a more detailed description of these character states in order to avoid further confusion. As papillae are variously seen depending on method of observation, we tried to show their shape and variation in different states, both dry and wet, and in both front views and cross sections.

MATERIALS AND METHODS

All collections of *Haplohymenium* from Russia kept in LE, MW and MHA were studied, with many duplicates from VLA. Japanese material was used for comparison, as it includes many specimens identified by Iwatsuki, who monographically treated the genus.

Miscoscopic observations were done with the compound light microscope Olympus-C41 with digital camera Infinity 2-2. Scanning electron microscope (SEM) observations were conducted in two ways. First, shoots and separated leaves were simply coated with gold and studied under SEM Cambridge Instruments CamScan S2

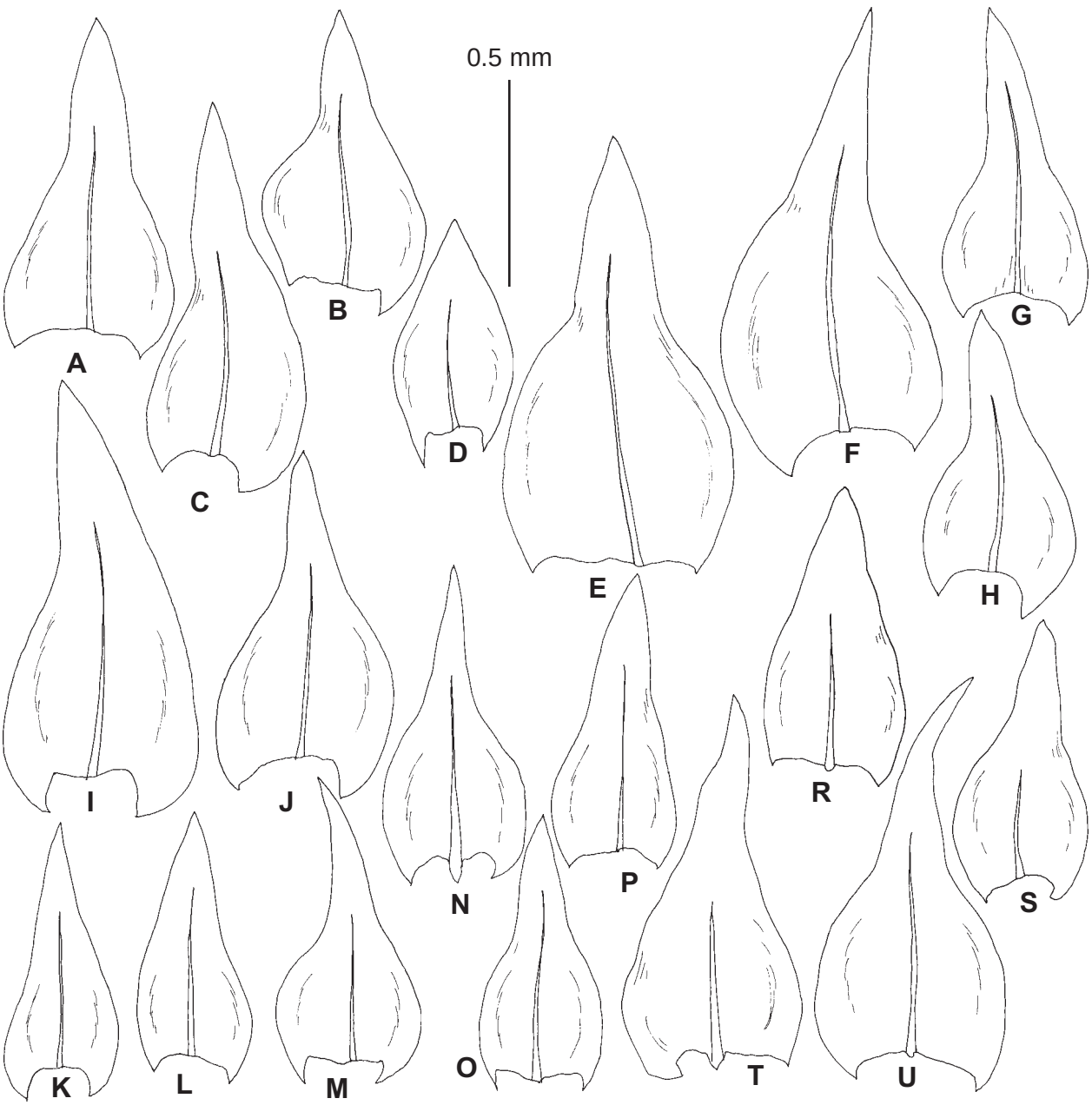

Fig. 2. *Haplohymenium sieboldii* (from: A–D: Japan, Kyushu, X.1946, *Hattori s.n.*, MHA9054150; E–H: Russia, Primorsky Territory, Elomovskij Creek, *Malashkina & Ivanov Pr-8-25-13*, MHA9035395); I–L: Russia, Primorsky Territory, 12 VIII 1929, *Transhel s.n.* (LE); M–P: Russia, Zabaikalsky Territory, 17.VII,2010, *Czernyadjeva 24-10*, LE; R–U: Russia, Primorsky Territory, Ussurijsky Reserve, *Ignatov & Ignatova 06-2326*, MHA9035380). A–B, E–F, I–J, M–N, T–U – stem leaves; C–D, G–H, K–L, O–S – branch leaves. Scale bars: 0.5 mm for all.

(images obtained this way called SEM-simple). Other parts of specimens were also taken from herbarium, wetted in phosphate buffer for 12 hours at room temperature, fixed in 2% glutaraldehyde for 24 hours, washed in water, post-fixed with 2% osmium tetroxide in distilled water for 2 hours, washed in water, dehydrated through an ascending ethanol and then ethanol-acetone series, dried at critical point, covered with gold, and observed under the same CamScan S2 equipment (images obtained after such preparation called SEM-fixed).

Material for Confocal Laser Scanning Microscopy (CLSM) was taken from herbarium specimens and studied with preparation similar to ordinary light microscopy. Shoots without fixation were stained by 0,1mM DAPI and berberin and investigated under Olympus FV-1000, with 405 and 473 nm lasers; series of 5–15 optical sections obtained with 40x objective lens and up to 6x digital zoom were Z-stacked by the microscope software.

Taxonomy

Haplohymenium sieboldii (Dozy & Molk.) Dozy & Molk., Musc. Frond. Ined. Archip. Ind., 4: 127, 40. 1846. — *Leptohymenium sieboldii* Dozy & Molk., Ann. Sci.

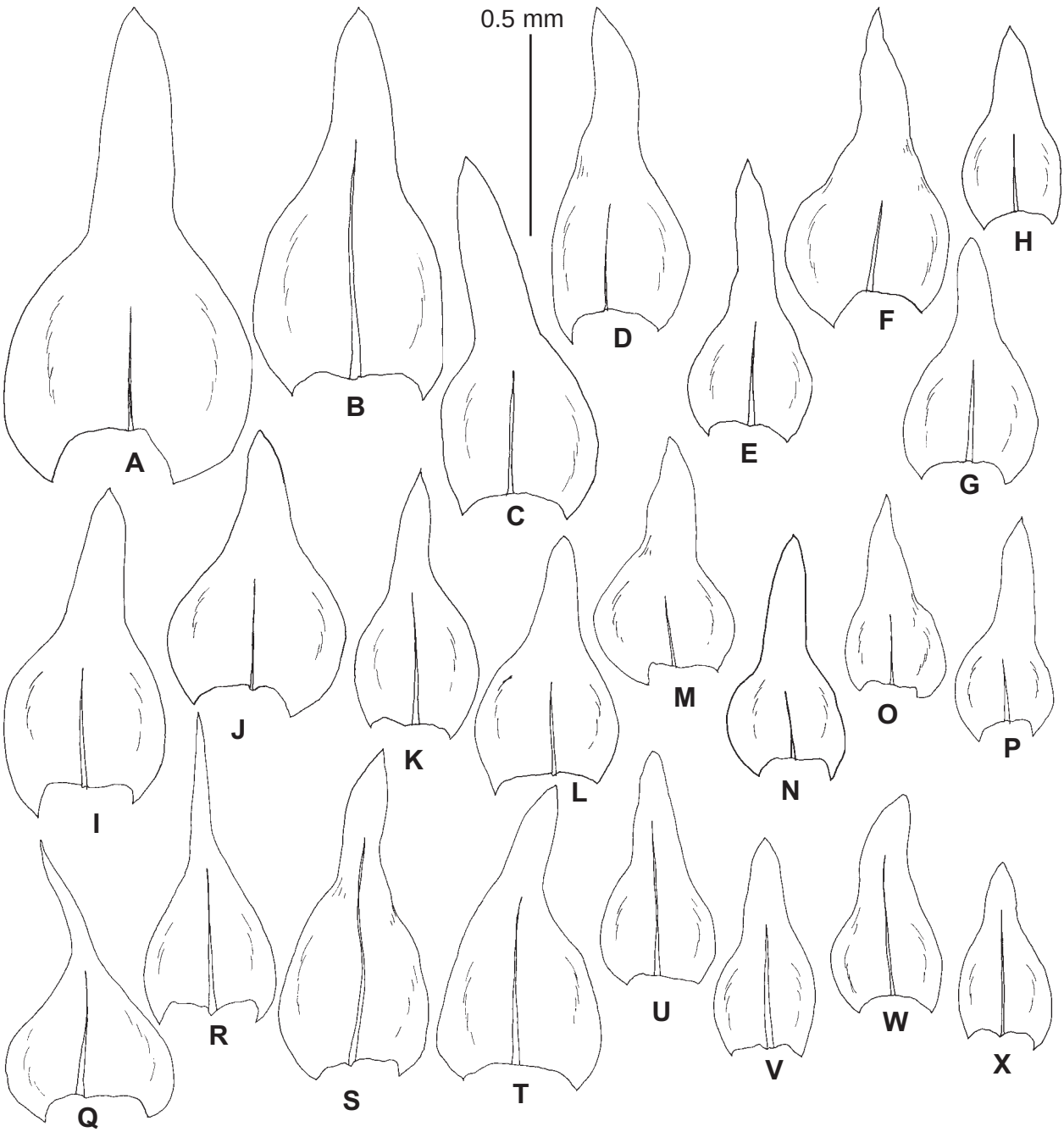

Fig. 3. A–X: *Haplohymenium triste* (from: A–D: Italy, 5.IV.1912, *Dixon s.n.*, MHA9054151; E–H: Russia, Ingushetia, *Ignatov et al. 18-2*, MHA9025858; I–L: Russia, Primorsky Territory, Pidan Mt., *Ignatov & Ignatova 06-2399*, MHA9035384; M–P: Russia, Yakutia, 27.VII.1991, *Ivanova s.n.*, MHA9035415; Q–T: Russia, Zabaikalsky Territory, 12.VIII.2011, *Afonina 3311*, MHA20354010; U–X: *H. pseudotriste* (from: Japan, Kyushu, *Ignatov & Ignatova 98-561*, MHA9054148). A–B, E–F, I–J, M–N, Q–R, U–V – stem leaves; C–D, G–G, K–L, O–P, S–T, W–X – branch leaves. Scale bars: 0.5 mm for all.

Nat., Bot., sér. 3, 2: 310. 1844. — *Anomodon sieboldii* (Dozy & Molk.) Granzow, Contr. Univ. Michigan Herb., 21: 243. 1997. Figs. 1, 2, 5.

Plants small, in loose patches, yellowish- to brownish-green, dull. Stems creeping, 1–3 cm long, irregularly branched, central strand absent. Leaves appressed when dry, erect-spreading when wet; stem leaves 0.5–0.9×0.2–0.4 mm, from ovate base gradually narrowed into lanceolate acumen, acute, occasionally with 1–3 transparent cells at apex, at lower portion of shoot often fragile; margins flat, entire or weakly crenulate; costa reaching 1/2–3/4 the leaf length, smooth or with few papillae on dorsal side; leaf lamina unistratose; upper and median lamina cells 9–13 μm in diameter, round or round-polygonal, not transparent, thin-walled, with several low branched papillae above lumen, basal marginal cells transversely ovate, to 16 μm wide, basal cells near costa

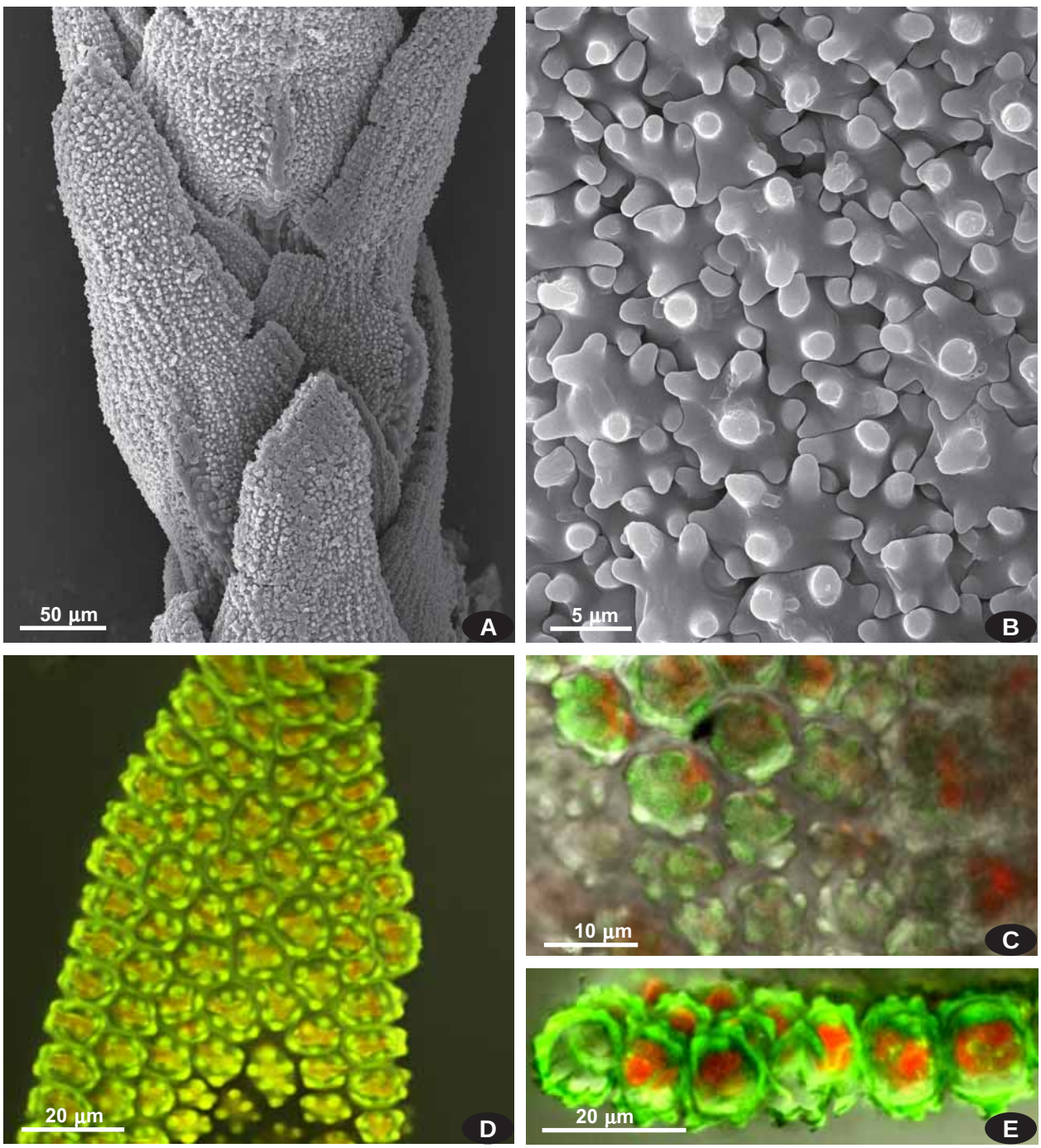

Fig. 4. *Haplohymenium sieboldii* (from: Russia, Primorsky Territory, Komarovka Creek, *Ignatov & Ignatova 06-2326*, MHA9035380). A–B: SEM-simple; C–E: CLSM. A: shoot; B–D: papillae on dorsal leaf surface; E: leaf transverse section.

elliptic, transparent, smooth, to 30 µm long; branch leaves ovate-lanceolate, gradually narrowed to acute or subobtuse apex, slightly smaller than stem leaves. Dioicous. Sporophytes unknown from Russian collections. [Perichaetial leaves with oblong base and narrowly lanceolate acumen, to 1.3 mm long. Setae to 5 mm long, yellowish-brown. Capsules erect, symmetric, ovate. Operculum conic. Calyptra with sparse, long hairs, papillose at apex.]

Diagnostic characters of *H. sieboldii* include ovate-lanceolate, gradually tapered, acute leaves, costa longer the half leaf length, and multipapillose lamina cells. This species is closely related to *H. tristie*. The latter species differs from it in leaf shape, from ovate base abruptly narrowed into lanceolate or oblong acumen, often rounded at apex and having small apiculus, more fragile, and with shorter costa. Watanabe (1972) points the presence of stem central strand in *H. triste* and its absence in *H. sieboldii*; however, in specimens of *H. triste* from Asian Russia the stem central strands is very weak or almost absent.

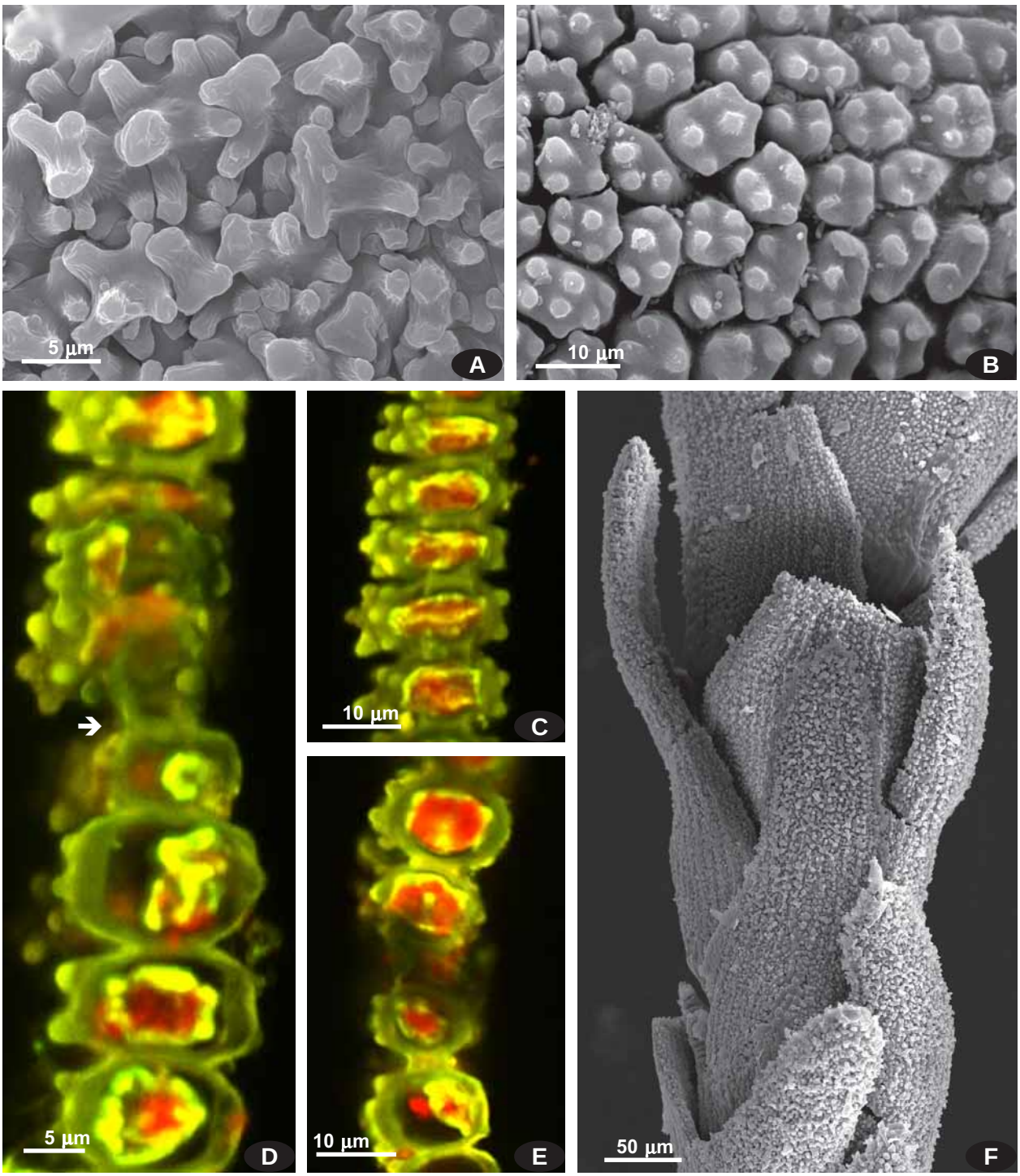

Fig. 5. *Haplohymenium triste* (from: Russia, Ingushetia, *Ignatov et al. 18-2*, MHA). A, F: SEM-simple; B: SEM-fixed: C–E: CLSM. A–B: papillae on dorsal leaf surface; C–E: longitudinal leaf sections from upper part of leaf (C), from lower part of leaf (E) and at the most fragile area of leaf constriction (D), showing narrow joint between leaf parts (arrowed).

Specimens examined: RUSSIA: **Zabaikalsky Territory**: Sokhondinsky State Reserve: vicinity Agutsa River, 49°38'N, 111°27'E, ~1170 m alt., 17.VII.2010, *Czernyadjeva 24-10* (LE), *ibidem*, *Czernyadjeva 28-10* (LE). **Primorsky Territory**: Murav-'ev Amursky Peninsula, 12 VIII 1929, *Transhel s.n.* (LE); Sikhote-Alin, Krinichnaya Mountain, 42°59'41"N, 132°28'58"E, IX.1990, *Czernyadjeva s.n.* (LE). Shkotovsky District, vicinity Novokhotunichi Settlement, 3.VIII.1927, *Transhel s.n.* (LE); vicinity of Vladivostok Sity, 18.VII.1971, *Bondartseva s.n.* (LE).

JAPAN: exsiccata ser. 2 (1948), # 88, Kyushu, X.1946, *Hattori s.n.* (LE); Buttuji, Hiroshima Pref., XII 1963, *Ando s.n.* (LE); Honshu, 6 X 1980, *Mizutani* 6333 (LE).

Despite of apparent difference, the species can be identified for sure only when the collection is large enough. The variation in *Haplohymenium triste* is great and in many collections some less developed shoots might have non-fragile and more gradually tapered leaves. Therefore we presented

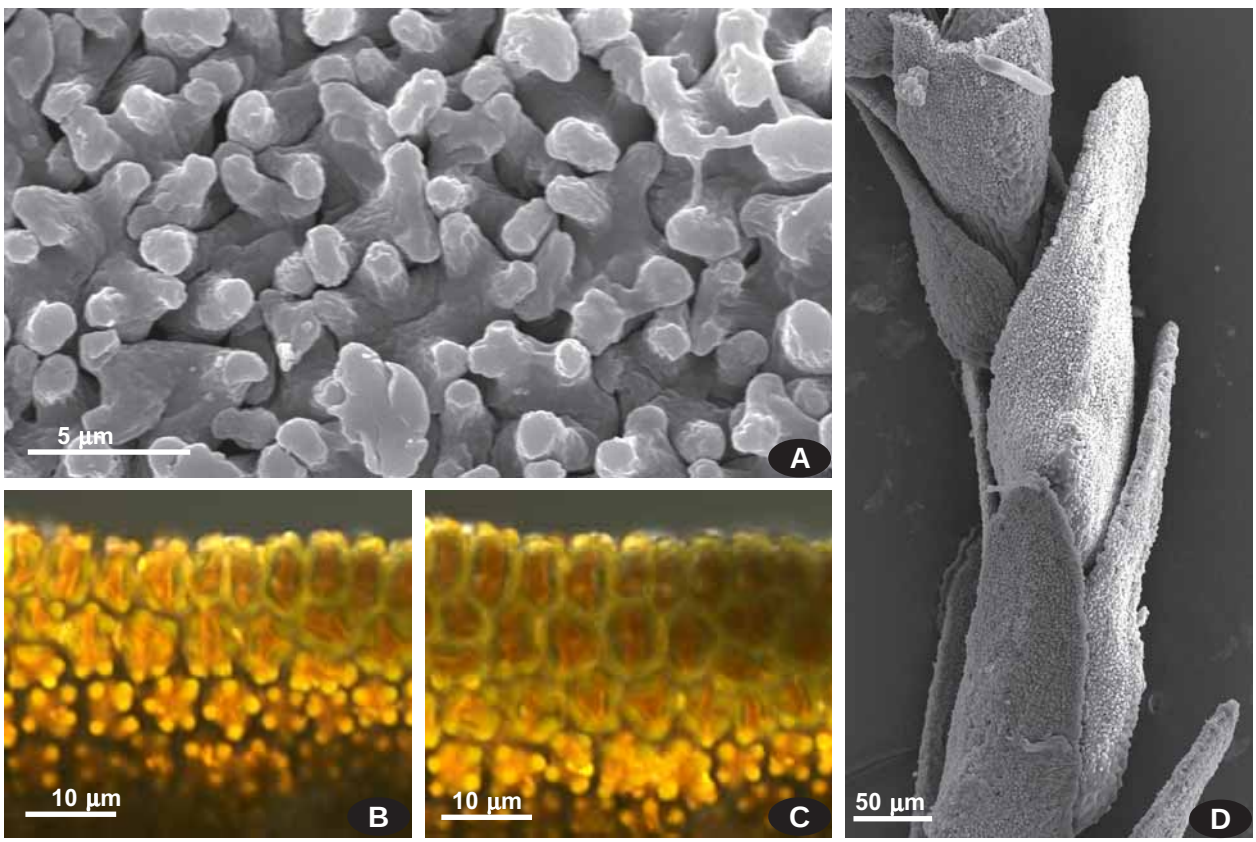

Fig. 6. *Haplohymenium pseudotriste* (from: Japan, Kuishiu, *Ignatov & Ignatova 98-561*, MHA9054148). A, D: SEM-simple; B–C: CLSM: same place at different focus.

in Fig. 2–3 numerous leaves to illustrate the character stressed by Granzow-de-la-Cedra (1997): costa is extended farer than leaf constriction in *H. sieboldii* and only rarely so in *H. triste*. In addition, smaller papillae is a useful character of *H. sieboldii*. Despite of variation of *H. triste,* its papillae are invariably large, especially on dorsal leaf surface.

As we already mentioned, DNA indicated very close relationships of *Haplohymenium triste* and *H. sieboldii,* contrary to *H. pseudotriste*, which is genetically well differentiated (Ignatov *et al*., 2019). The latter species is more southern and so far unknown in Russia, although its findings are possible. This species is the smallest in the genus, with hardly fragile leaves, looking as a strongly minitaturized *Anomodon minor*.

Papillae of *H. pseudotriste* and *H. triste* are rather similar, however their shape depends not only on species, but also of method of preparation: material from the same collection (Fig. 5A, B) looks very different under SEM after different specimen preparation. Cross section illustrates that the connection between neighboring cells in *Haplohymenium* is much less than cell height, thus a considerable portion of the cell is above the level of their joint and can be considered as a large stump-shaped mammilla, further bearing papillae on its upper surface (Figs. 5, 6).

However even more complicated is the case of *H. longinerve*, which may bring light to papillae development and limits of variation in Anomodontaceae as a whole.

* * *

Haplohymenium longinerve is often separated in identification keys at first couplet by the unipapillose laminal cells. The light microscopy usually allows the high papillae to be seen in distal leaf half. They are especially easily observable in upper third of leaf at back of convex part of acumina (Fig. 7A), "in profile". Closer to leaf margin (Fig. 7C) and at leaf middle (Fig. 7D) most cells are already multipapillose. The main problem appears with specimens where most or even all cells are multipapillose (Fig. 7G and 9A,B), which in the case of plants growing in relatively harsh environments, e.g. rock outcrops, but open and sunny, or exposed to winds. Also in plants with well developed high papillae (cf. Fig. 8A) some smaller leaves have only multipapillose cells: pictures in Fig. 7G, H are taken from the same shoot as shown in Figs.7E, F and 8E, F.

No unipapillose cells were seen, in e.g. collections from Kurils Islands by Bakalin, which caused erroneous identification as *H. triste* (Bakalin *et al*., 2009). Its multipapillose cells are shown in Fig. 9A, B. Molecular phylogenetic analysis of Ignatov *et al.* (2019), however, demonstrated that it is identical by all DNA markers with other specimens of *H. longinerve*, including specimen shown in Figs. 7, 8, 9C–F, and 10.

The large papillae of *H. longinerve* are similar to those in *Anomodon abbreviatus* and *A. solovjovii*; such papillosity pattern seemingly needs a special term, so we sug-

gest to call it pseudounipapillose. The high conic papillae dominate in places of maximal development, however small round papillae are also present, albeit not well seen in light microscope.

Close to the leaf base papillae on one cell surface are not so unequal, and therefore cells look multipapillose. In some places within transitional zone the variation in papillae structure characterize the type of their development (Fig. 8C). The stump-like mammillae are well seen here and their surface raises in place, forming various type of projections, where one of those may take over others, or not. Such views suggest that the cell wall in this species is plastic and delicate. The structure of papillae is obviously heterogeneous: autofluorescence at higher magnification shows difference in upper part of these high papillae from its median part (Fig. 8E).

Our attempt to take SEM picture of high papillae of *H. longinerve* (simple mode without special preparation, just coated with gold) brought unexpected results. Stiff plants from the Kuril Islands, where all cells look pluripapillose, resulted in "normal" view (Fig. 9A, B), rather comparable with views of wet plants under CLSM and also comparable with similarly taken pictures for other species (Figs. 3, 4, 5, 11), whereas in more delicate specimens shown in Figs. 7 & 8 the papillae appear terribly damaged by vacuum. Since the preparation was the same and plants were lying side by side during the same session of observation, this difference requires discussion. It seems obvious that cell walls in plants with high conic papillae are extremely delicate and vacuum makes them to collapse, either transforming cell lumina to pit, or leaving at its surface crumplings which we present here with a great hesitation. Few shoots examined this way have no one high papilla in places where they are commonly seen in wet plants in ordinary slide under light microscope and CLSM.

SEM observation of plants fixed in glutaraldehyde and osmium tetroxide, dehydrated in ethanol–acetone series and dried under critical point by the standard protocol also did not succeed to show papillae as large as they are in wet state. Wet papillae are up to 12 μm high (Fig. 8A), while the largest ones in SEM images do not exceed 5 μm (Fig. 10D). The considerable shrinking of large papillae in *H. longinerve* can be seen even in water slide after its complete drying: photos in Fig. 7A and 7B provide such an example.

Assuming papillae pattern as unreliable for identification, we consider the base character to be the long, percurrent to excurrent costa, at least in some leaves. The leaf tip, a continuation of costa is often composed of large smooth cells, occasionally with crown papillae at the tip of apical cell (Fig. 10). This pattern occurs also in *H. flagelliforme.* The latter species differs in invariably multipapillose cells, and long teeth near leaf apex,which often terminate with crown papillae. Molecular phylogenetic analysis (Ignatov *et al*., 2019) placed *H. flagelliforme* in the basal position in the *Haplohymenium* clade.

KEY TO IDENTIFICATION OF *HAPLOHYMNIUM* SPECIES IN RUSSIA

1. Leaf margin below apex with tall, perpendicular to reflexed teeth; very delicate plants hanging on tree trunks and twigs, stems often flagelliform ..... *H. flagelliforme*
— Leaves without long teeth near apex; plants not hanging, stems not or rarely flagelliform 2
2. At least some leaves attenuate, with percurrent or subpercurrent costa vanished in leaf tip; laminal cells in distal part of leaf often with high conical papillae, proximally multipapillose, more rarely multipapillose throughout ..... *H. longinerve*
— Leaves never with long attenuate tips; costa usually up to 0.5(–0.7) the leaf length, laminal cells always multipapillose 3
3. Plants very small; leaves 0.4–0.6 mm long, flat;shoots somewhat complanate when wet .. [*H. pseudotriste*]
— Plants moderately small; leaves (0.4–)0.6–1.0 mm long, somewhat concave; shoots terete 4
4. Leaves from ovate base gradually tapered, acute or acuminate; costa reaches 0.5–0.75 the leaf length, ending above leaf constriction ..... *H. sieboldii*
— Leaves from ovate base abruptly narrowed into lingulate acumen, obtuse or rounded; costa reaches 0.3–0.6(–0.7) leaf length, ending at about the level of leaf constriction ..... *H. triste*

ACKNOWLEDGEMENTS

We cordially thank J. Shevock for improving English of the paper. The work on SEM 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.The study of I.V. Czernyadjeva was carried out within the framework of the institutional research project of the Komarov Botanical Institute of the Russian Academy of Sciences (Flora of lichens and bryophytes of Russia and phytogeographically important regions, № AAAA-A19-119020690077-4) and of E.A. Ignatova in the framework of the MSU research project № 01201157317.

LITERATURE CITED

BAKALIN, V.A., V.YA. CHERDANTSEVA, M.S. IGNATOV, E.A. IGNATOVA & T.I. NYUSHKO. 2009. Bryophyte flora of the South Kuril Islands (East Asia). – *Arctoa* 18: *69–114*.

GRANZOW-DE LA CERDA, I. 1997. Revision and phylogenetic study of *Anomodon* and *Herpetineuron* (Anomodontaceae, Musci). – *Contributions from the University of Michigan Herbarium* **21**: *205–275*.

GRANZOW-DE LA CERDA, I. 2014. Anomodontaceae. – *In: Flora of North America Editorial Committee (eds.), Flora of North America North of Mexico, vol. 28. New York, Oxford University Press: 628–636.*

IGNATOV, M. S., A.V. FEDOROVA & V.E. FEDOSOV. 2019. On the taxonomy of Anomodontaceae and *Heterocladium* (Bryophyta). – *Arctoa* **28**: *75–102*.

IWATSUKI, Z. 1963. A revision of the East Asiatic species of the genus *Anomodon*. – *Journal of the Hattori Botanical Laboratory* **26**: *27–62*.

WATANABE, R. 1972. A revision of the family Thuidiaceae in Japan and adjacent areas. – *Journal of the Hattori Botanical Laboratory* **36**: *171–320.*

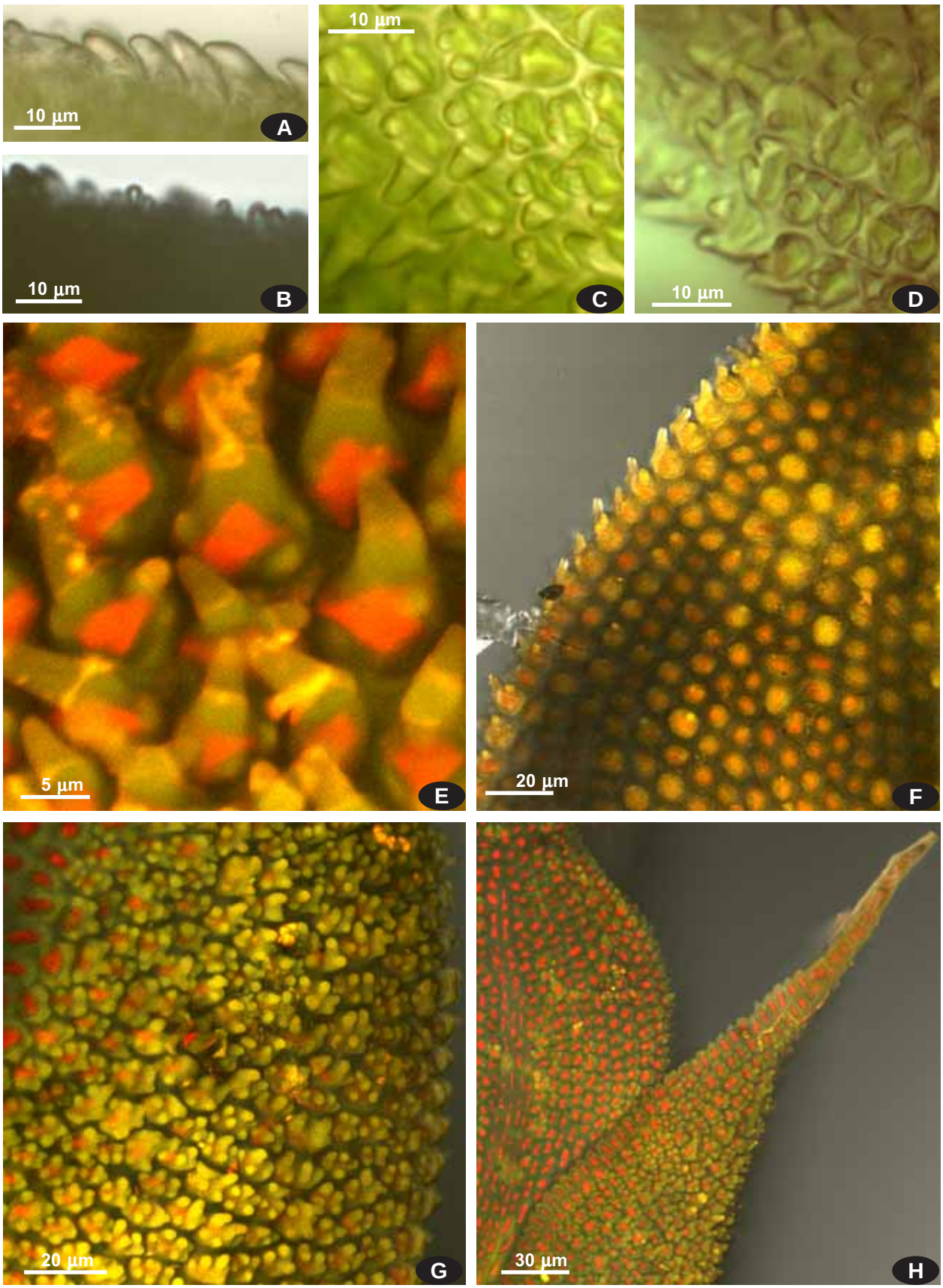

Fig. 7. *Haplohymenium longinerve* (from Russia, Primorsky Territory, Elomovsky Creek, *Ignatov et al. 13-1335*, MHA9035366). A–D: light microscope; E–H: CLSM. A–E, G–H: various views of papillosity pattern on dorsal leaf surfaces; F: ventral surface, papillae seen only along the margin.

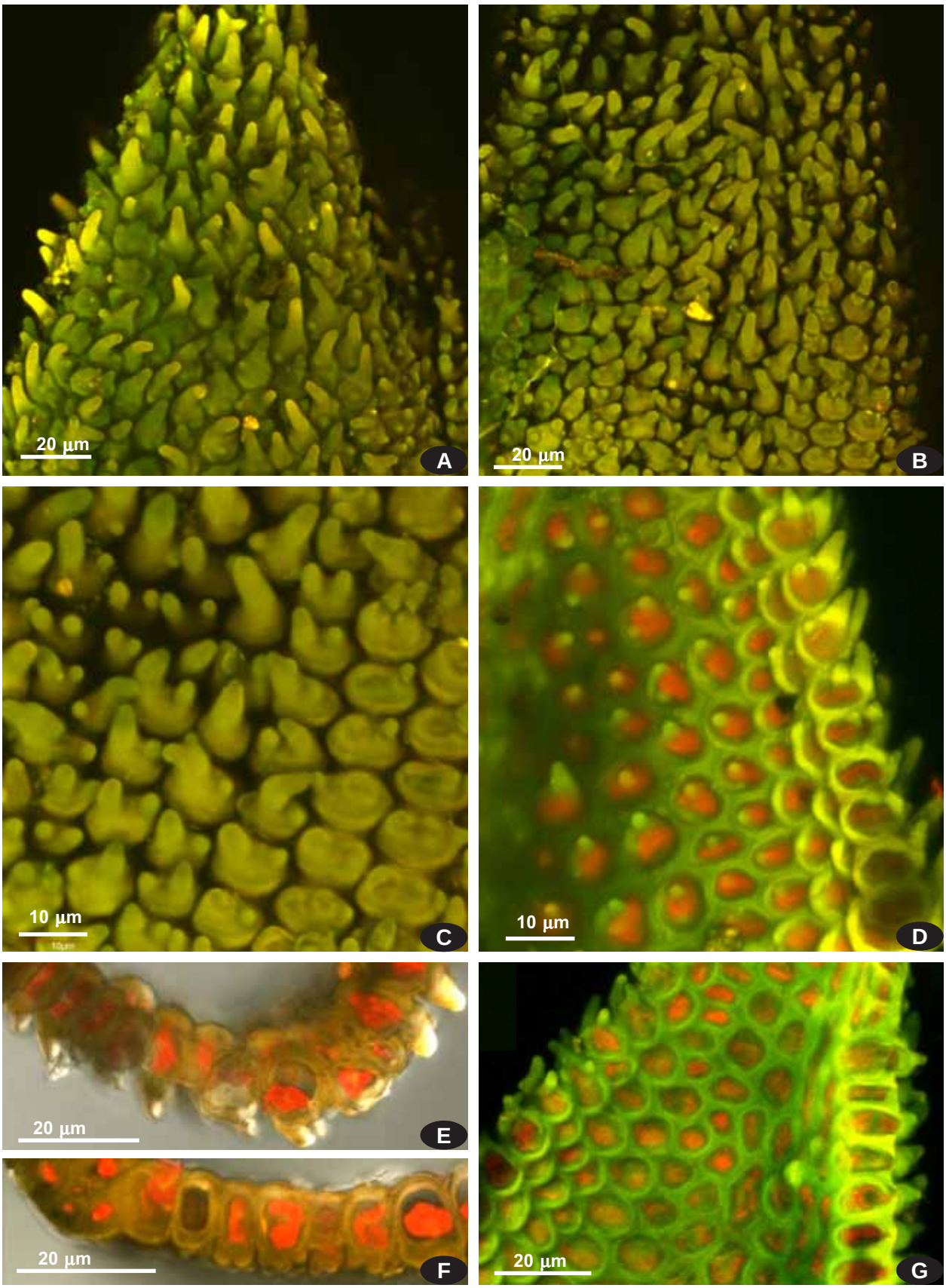

Fig. 8. *Haplohymenium longinerve* (from Russia, Primorsky Territory, Elomovsky Creek, *Ignatov et al. 13-1335*, MHA9035366), CLSM. A–C: dorsal surface, showing subapical (A) to middle (C) parts of leaf; D, G: ventral surface of leaves, with somewhat papillose (D) and smooth (G) cells; E–F: transverse leaf sections showing uni- or multipapillose dorsal surface. Fig. C shows variety of papillae, at various stages of development.

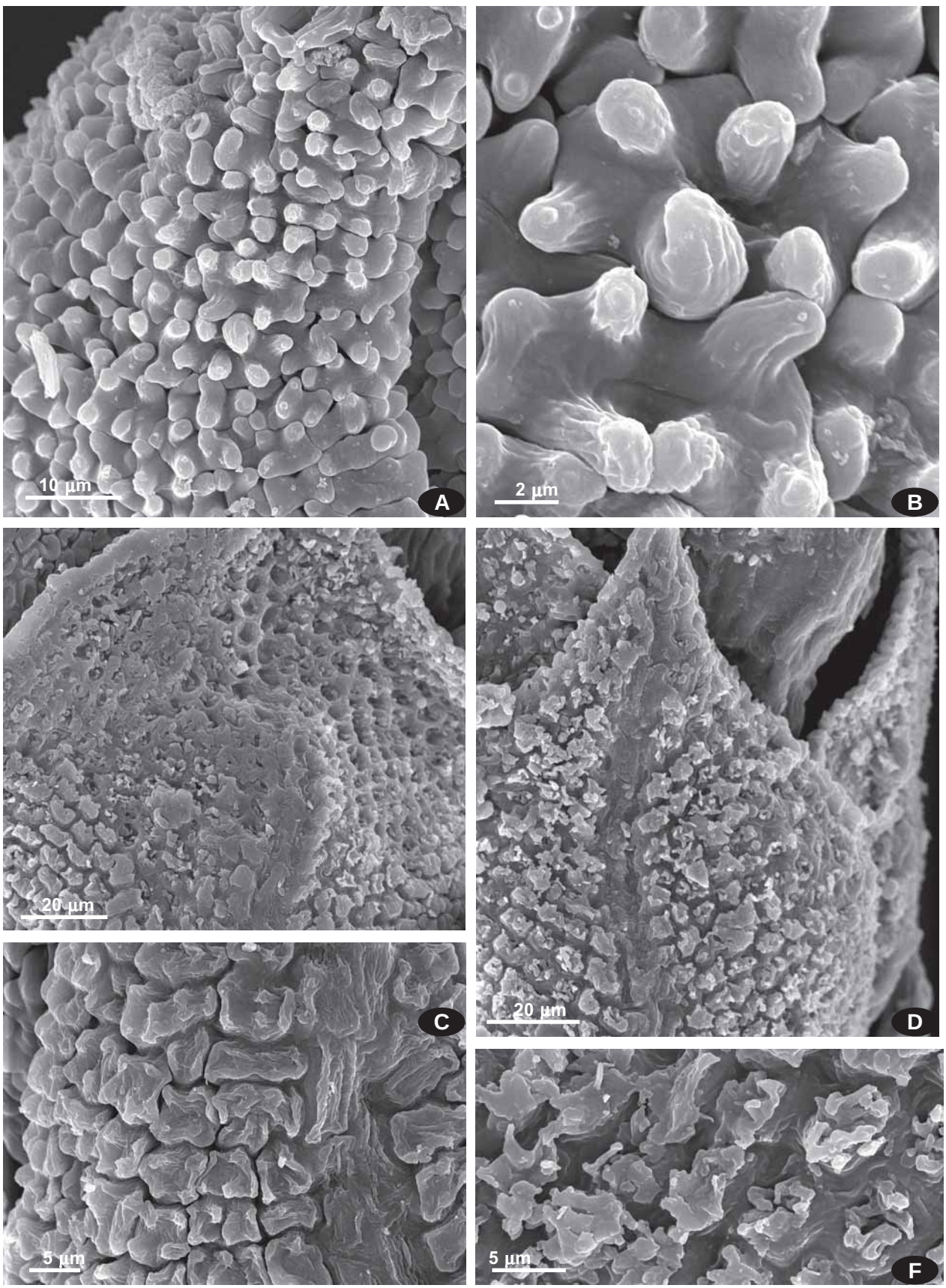

Fig. 9. *Haplohymenium longinerve* (A–B: from Russia, Kuril Islands, *Bakalin-42-18-07,* MHA9035417; C–F: from Russia, Primorsky Territory, Elomovsky Creek, *Ignatov et al. 13-1335*, MHA9035366). SEM-simple. Papillae patterns on dorsal surface in distal part of leaves.

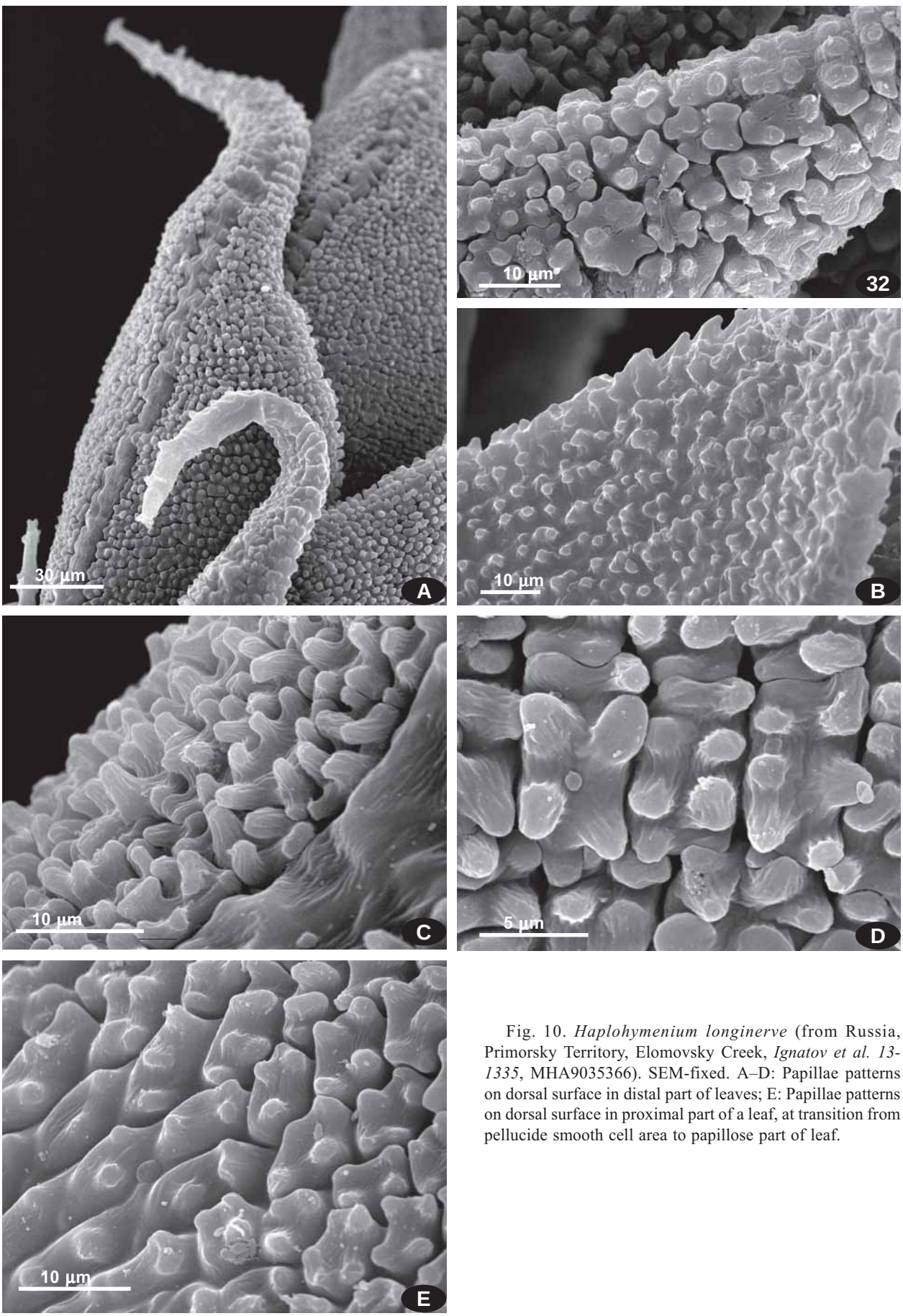

Fig. 10. *Haplohymenium longinerve* (from Russia, Primorsky Territory, Elomovsky Creek, *Ignatov et al. 13-1335*, MHA9035366). SEM-fixed. A–D: Papillae patterns on dorsal surface in distal part of leaves; E: Papillae patterns on dorsal surface in proximal part of a leaf, at transition from pellucide smooth cell area to papillose part of leaf.

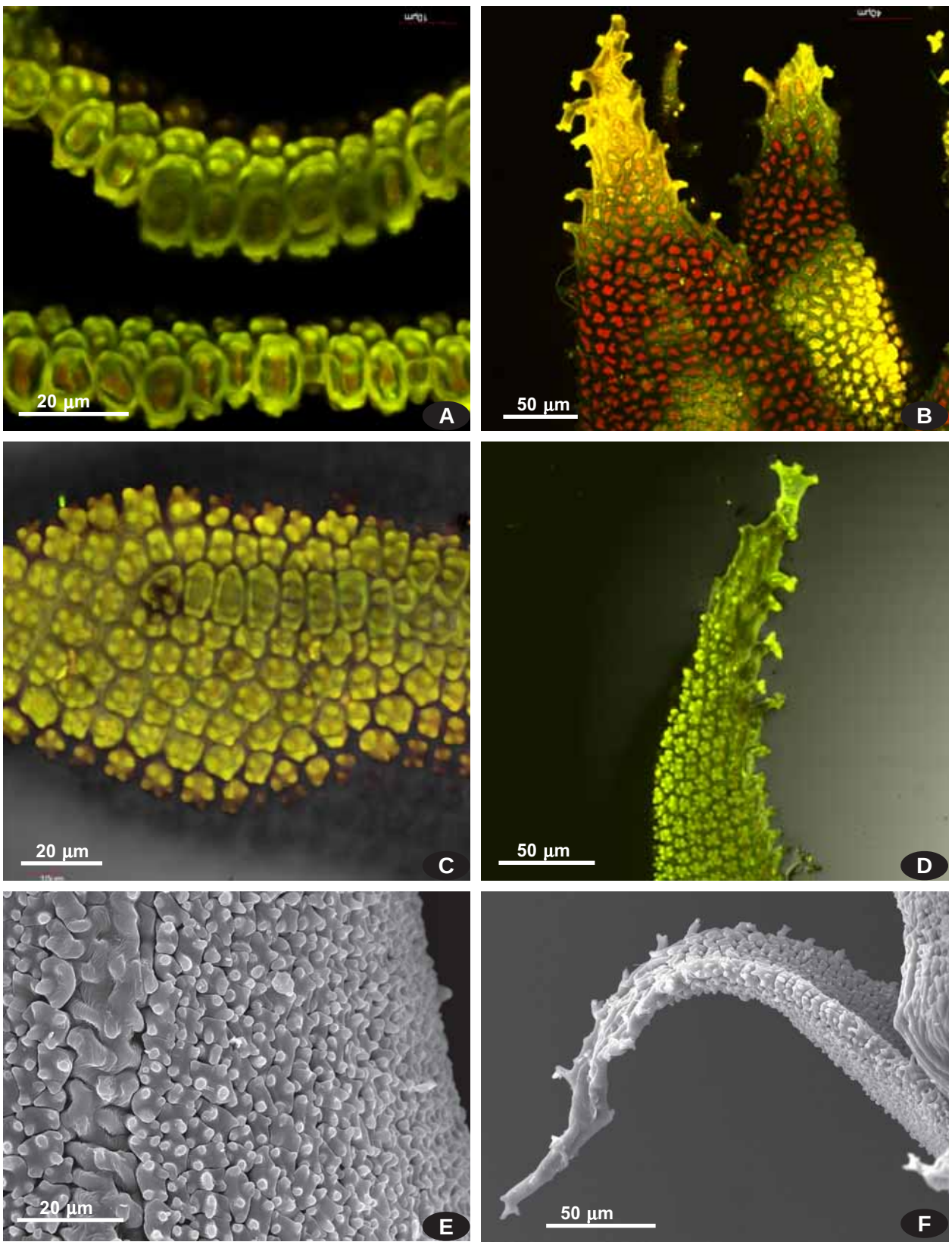

Fig. 11. *Haplohymenium flagelliforme* (from: Russia, Primorsky Territory, Ignatov #08-20, MHA). A–D: CLSM, E–F: SEM-simple. A: transverse section of two leaves (ventral side up), showing papillae on both surfaces; B, D, F: leaf apices, showing high papillae / projections on leaf margin, and crown papillae on their tips; C, E: papillae on dorsal leaf surface.