

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/authorsrights>



Contents lists available at SciVerse ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Research paper

The freshwater fern *Azolla* (Azollaceae) from Eocene Arctic and Nordic Sea sediments: New species and their stratigraphic distributionJohan van der Burgh^{a,*}, Margaret E. Collinson^b, Johanna H.A. van Konijnenburg-van Cittert^{a,c}, Judith Barke^a, Henk Brinkhuis^a^a Marine Palynology Group, Laboratory of Palaeobotany and Palynology, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands^b Department of Earth Sciences, Royal Holloway University of London, Egham, Surrey, TW20 0EX, UK^c Naturalis Biodiversity Center, PO Box 9517, 2300 RA Leiden, The Netherlands

ARTICLE INFO

Article history:

Received 18 July 2012

Received in revised form 21 February 2013

Accepted 22 February 2013

Available online 7 March 2013

Keywords:

Arctic Ocean
Norwegian–Greenland Sea
Eocene
megaspores
microspore massulae
ultrastructure

ABSTRACT

Three new species of the freshwater fern *Azolla* are described from Eocene marine deposits of the Arctic and Nordic seas, bringing the total number of species now documented from these areas to five. *Azolla arctica* Collinson et al., *Azolla jutlandica* Collinson et al., *Azolla nova* sp. nov. and *Azolla nuda* sp. nov. are known from both megaspore apparatuses and microspore massulae, and occur at more than one site. Their microspore massula characters are sufficiently distinctive to allow their recognition in palynological preparations. Presence or absence of hairs on the microspore massula surface and glochidia bases is shown to be a useful species diagnostic character and is worthy of greater attention in *Azolla* taxonomy. The fifth species, *Azolla astroborealis* sp. nov., is only known from a few megaspore apparatuses in the Northstar 1 Well in the western Arctic, but these are strikingly different from those of the other four species e.g., in having nine distinct pseudovacuolate floats.

Samples from cores and most individual sites contain more than one *Azolla* species. This indicates that conditions were particularly favourable for growth of *Azolla* for a prolonged time interval in the latest early and possibly earliest middle Eocene (latest Ypresian and earliest Lutetian) around the western Arctic and the Norwegian–Greenland Sea.

© 2013 Published by Elsevier B.V.

1. Introduction

In the past few years, *Azolla arctica* was described from the Eocene of the central Arctic Lomonosov Ridge, from materials drilled during IODP Leg 302 (Collinson et al., 2009). Further studies, on Eocene North Sea sediments, revealed the presence of an additional species of this genus, *Azolla jutlandica*, which has also recently been described in detail (Collinson et al., 2010, 2013). During studies of material from other Eocene localities in the Arctic and Nordic Seas, three more species were discovered and partly characterized (Barke et al., 2012). In the course of the detailed examination of all the material, it was found that some characters used in descriptive terminology of fossil *Azolla* material were not as typical as they were thought to be; e.g., the presence or absence of recurved hooks on glochidia is a character that can vary within one species depending on the number of massulae studied and the method of observation (LM, SEM or TEM).

The aims of the present paper are (i) to present a full and detailed description of these three new species and compare them with previously described species, (ii) to document the stratigraphic ranges of the

species through the *Azolla* interval and (iii) to document the importance of applying different methods of observation for certain morphological characters.

2. Materials and methods

2.1. Samples

The majority of the material studied comes from multiple sample levels in ODP Leg 151 Site 913B and Leg 104 Site 643A from the Nordic Sea. Additional samples were studied from the Amerada Well Northstar 1 (Alaska, western Arctic Ocean) and Shell Sandpiper Well (western Arctic Ocean) for the systematics, and from Sandpiper, Ellice, Upluk, and Statoil Wells 6608, 6507, 6506, 6406 and 6302 for the stratigraphic interpretation only. For details of sample sites and their geographic distribution see Fig. 1 and Barke et al. (2012).

A GSSP for the base Lutetian (= Ypresian to Lutetian, or early to middle Eocene boundaries) was recently placed within Chron C21r by Molina et al. (2011). The GSSP age, based on Milankovitch cyclicity, was estimated to be approximately 800 kyr younger than the beginning of Chron C21r or c. 47.8 Ma in the Gradstein et al. (2004) timescale. Accordingly the *Azolla* occurrences in Chron C22n are Ypresian and, in consequence of these recent ICS (International Commission on Stratigraphy)

* Corresponding author. Fax: +31 30 2535096.

E-mail address: j.vanderburgh@uu.nl (J. van der Burgh).



Fig. 1. Schematic palaeogeographic reconstruction showing sampling sites and the geographical distribution of the five *Azolla* species in the Arctic Ocean and Nordic Seas based on their microspore massulae and megaspore occurrences. Modified from Barke et al. (2012).

decisions and recommendations, are latest early Eocene, and no longer earliest middle Eocene as previously stated in Barke et al. (2012) and Collinson et al. (2009, 2010).

Following these recent ICS decisions, however, the occurrences in Chron C21r might be either latest Ypresian or earliest Lutetian as we do not know the duration of the section assigned to Chron C21r present in the cores. According to Molina et al. (2011), based on estimated Milankovitch cyclicity, the Ypresian–Lutetian boundary occurs 507 kyr before the beginning of C21n and 819 kyr after the end of C22n, thus in the later part of C21r. According to Gradstein et al. (2004), C22n lasted 828 kyr, thus about the same as that part of C21r that belongs to the Ypresian. The absolute thicknesses in the Norwegian–Greenland Sea

cores (Fig. 2) are similar for the intervals of Chron C22n and C21r from which *Azolla* has been recovered; therefore, if a constant sedimentation rate is assumed, it is most likely that all Norwegian–Greenland Sea *Azolla* occurrences are in effect very latest Ypresian. Further studies should demonstrate whether or not the Ypresian/Lutetian boundary sensu Molina et al., 2011 is straddled by the *Azolla* episode.

2.2. Sample processing and study

Sediment samples were cleaned and oven-dried overnight at 60 °C. WAC wetting agent (former AGEAPON APH12N, Germany) was added and samples were then treated with HCl (30%) and HF (40%) in standard palynological treatment without oxidation. The residues were sieved through 250 µm mesh and the retained fraction was examined under a dissecting microscope. Specimens were picked for SEM and rinsed in distilled water before mounting. Specimens were mounted on a droplet of water onto negative film attached to the stub with Araldite (Ciba Speciality Chemicals Holding Inc. distributed by Bostik Ltd., Leicester, UK) (see Moore et al., 1991), coated with gold in a Bal-Tec (Balzers, Liechtenstein) SGD 050 sputter coater and examined with a JEOL JSM 5300 SEM (Leiden), or coated with platinum using a Cressington 208 h sputter coater (Cressington Scientific Instruments Ltd, Watford, England) and examined under a Philips XL305 FEG SEM (Utrecht). For palynology the <250 µm size fraction from the above treatment was sieved through 15 µm mesh and the retained fraction was mounted on slides using glycerine jelly. Cover slips were sealed with nail varnish. Sampling depths are stated on the slides and these can be interpreted in metres below sea floor (mbsf) in Appendix Table 1 and Fig. 2.

Fifteen specimens of megaspore apparatuses were studied by SEM and then sectioned for TEM (Collinson). Four of these are used in the systematic descriptions. TEM methods are documented in Collinson et al. (2009).

To determine the stratigraphic distribution of *Azolla* species in ODP 151-913B and ODP 104-643A, all the massulae or massula fragments (only those with glochidia tips attached) in two palynological slides were counted. The only exception was in ODP 151-913B 48R 1W 20–22 cm, depth 693.2 mbsf. In those slides they are so abundant that 200 massulae were counted. The total number of massulae was then estimated, based on the ratio between the area in the slide occupied by the counted massulae and the total slide area. The number of massulae actually counted in any one sample ranged from 1 to 381 (Appendix Table 1). As these counts are strongly influenced by concentration of particles on the slides they were converted to percentage values. The relative abundance of each species through ODP 151-913B and ODP 104-643A is plotted in Fig. 2.

3. Descriptive terminology and classification

The species are described following the sequence, style and terminology of Batten and Collinson (2001), to enable comparison with *Azolla arctica* and *Azolla jutlandica* already described from the Arctic and Nordic Seas (Collinson et al., 2009, 2010). The *Azolla* megaspore apparatus is subdivided into a distal megaspore and a proximal float system. The term 'float' is a misnomer; it has been demonstrated conclusively that the floats do not render the megaspores more buoyant (Fowler, 1975; Dunham and Fowler, 1978). *Azolla* microspores are not shed separately, but contained within a perine-derived microspore massula, the outer surface of which is usually ornamented with anchor-like appendages (glochidia) that readily adhere to the hairs (filosum) of the surface of the megaspore apparatus. For further details see Batten and Collinson (2001 and references cited therein). The classification follows Smith et al. (2006). Exoperine surface terminology follows Punt et al. (2007). Glochidium terminology is illustrated in Barke et al. (2012, fig. 3).

4. Systematic palaeobotany

Order Salviniales Britton
Family Azollaceae Wettstein
Genus *Azolla* Lamarck

Species *Azolla nova* Van der Burgh et al. sp. nov.

Plates I, II

Holotype: Plate I, 1; U23536.

Paratypes: Plates I, 2–17, II, 1–13.

Repository: Laboratory of Palaeobotany and Palynology, Utrecht, coll. nr. U23536 (holotype) and U23537, 23538 (paratypes).

Type locality: Sandpiper Well 3570–3600 cm.

Other localities: Upluk (western Arctic) and ODP leg 104, Site 643A, Core 62 sect 1W.

Stratigraphic horizon: latest early Eocene, in lower Chron C22n.

Etyymology: from the new feature of the distinctive microspore massulae in which the microspores are touching and by bulging out influence the massula outline, not known to us in other *Azolla* species.

Specific diagnosis

Megaspore apparatus ovoid pear-shaped to globular (typically <330 µm diameter) with rounded distal pole and obtuse proximal pole. Megaspore inferred to be spherical to sub-spherical when uncompressed, trilete mark on the proximal pole, laesurae extending up to one third of spore radius, lying in a field bordered by a triangular collar. Entire megaspore apparatus covered by a thick mat of intertwined hairs (>0.9 µm wide) arising almost entirely from the proximal region of the megaspore. Remnants of megasporocarp wall present on the proximal pole of the megaspore apparatus.

Megaspore wall with distinct regularly arranged small excrescences. Megaspore surface finely rugulate under LM and SEM; rugulae usually branching and frequently topped by small papillae. Under TEM megaspore wall consisting of a thin exine and a twice as thick two-layered perine; inner surface of the exine forming a more or less continuous layer beneath a more open structure with radially elongated irregular spaces. Endoperine similar to exine with more equidimensional spaces and granular appearance and an undulating surface lacking ornamentation. Excrescences formed by expansion of the endoperine. Exoperine with contorted nodular masses in the inner part and tabular masses on the outerpart giving an undulating exoperine surface. Collar formed from granular endoperine, triangular, linking ends of the laesurae on the proximal pole; modified exoperine on the collar giving rise to hairs (suprafilosum).

Float system a compact dome-shaped structure, occupying the proximal third to one quarter of the megaspore apparatus, slightly overlapping the proximal part of the megaspore; floats not numerous, most likely arranged in one tier, and enmeshed by hairs of the suprafilosum.

Microspore massulae with undulating wavy irregular outline resulting from microspores bulging out from within the massula, microspores filling the massula, touching one another with clearly visible outlines. Massulae containing 1–8 smooth walled triradiate microspores, laesurae extending about half the radius of the spore. Outer surface of microspore massulae with a limited number of strap-shaped aseptate glochidia up to 85 µm long, with very broad basal attachment, narrower lower stalk grading into a wider upper stalk, with a distal dilation and a distinct constriction below an anchor-shaped tip. Flukes long, narrowing gradually, predominantly with recurved hooks. Surface of the microspore massula and glochidia bases covered by long (3–4 µm) hairs.

Description

The megaspore apparatus is variable in shape, most frequently globular or almost so (Plate I, 1, 4, 5, 15), but sometimes ovoid to

pear-shaped (c. 250–350 µm long, c. 230–330 µm wide) (Plate I, 2, 3). The distal pole is rounded and the proximal pole is obtuse. The megaspore apparatus diameter is typically less than 330 µm and the megaspore is usually less than 300 µm (220–320 µm) in diameter. The laesurae are c. 75 µm long. The entire megaspore apparatus is covered by a thick mat of intertwined hairs (0.9–2 µm in diameter) (filosum) (Plate I, 1, 3, 4, 9), sometimes hairs in bundles (Plate I, 9). The hairs arise almost entirely from the proximal region of the megaspore (hence suprafilosum). A few isolated megaspores in palynological slides lack the filosum (Plate I, 8), but in most cases dissection was necessary to reveal the megaspore wall (Plate I, 6, 7) indicating the persistence of the enveloping suprafilosum. Observations of dissected specimens with SEM and thin sections with TEM do not reveal an infrafilosum (Plate I, 10, 11, 14, 16, 17). Remnants of the megasporocarp wall (cap) are one cell layer thick (Plate II, 1) and present on the proximal pole of most specimens (Plate I, 6, 12).

Under SEM and LM the exoperine surface is finely rugulate; the usually branching rugulae are 6–10 µm long and 1.5 µm wide, and are frequently topped by small papillae (c. 2 µm diameter) (Plate I, 10, 11, 13). Typically clearly visible small excrescences are regularly arranged over the entire megaspore surface (Plate I, 6, 7, 10), but in some specimens adjacent papillae fuse into rugulae over the excrescences (Plate I, 11), rendering the excrescences less prominent.

TEM shows that the excrescences are formed by expansion of the endoperine (Plate I, 16). TEM of this species is mainly based on a specimen from the Sandpiper Well identified to *Azolla nova* on the basis of small size and globular shape (Plate I, 15). The inner surface of the thin (1–3 µm thick) exine forms a more or less continuous layer beneath a more open structure with radially elongated irregular spaces (Plate I, 14, 16, 17). The endoperine (c. 2–3 µm thick) is similar to the exine but with more equidimensional spaces, resulting in a granular appearance. The endoperine surface lacks ornamentation (Plate I, 14, 16, 17). The inner part of the c. 3–4 µm thick exoperine consists of contorted nodular masses (Plate I, 14, 16, 17), sometimes elongate parallel to the spore surface (Plate I, 17). The outer part of the exoperine consists of thicker tabular masses (Plate I, 14, 16, 17). The exoperine surface as seen under TEM is undulating. The collar is formed from granular endoperine (Plate II, 1), and is seen on isolated megaspores as a triangular structure, linking the ends of the laesurae on the proximal pole (Plate I, 8). Modified exoperine on the collar gives rise to the hairs of the suprafilosum (Plate II, 1).

The float system is small compared to the megaspore size, and occupies only the proximal third to one quarter of the megaspore apparatus (Plate I, 2, 5). Details of the float system were obscured by compression in both specimens studied by TEM and no pseudovacuolate structure (or central columella) could be discerned. Neither the individual floats nor any float tissue structure can be discerned by LM, SEM or TEM. This may be a result of compaction. Based on the small size and dome shape (Plate I, 5, 6) of the float system and the depression at the proximal end of the megaspore apparatus (Plate I, 2) it is inferred that there is insufficient space for numerous floats. It is most likely that there were three or six floats arranged in one tier.

The microspore massulae have been encountered attached to the megaspore apparatus (Plate I, 1, 3), as single massulae in palynological slides (Plate II, 2–5), or grouped in clusters (Plate II, 12). The massulae have an undulating wavy irregular outline (Plate II, 2–5) resulting from microspores bulging out from the interior of the massula. Microspores frequently almost completely fill the massula with the spore outlines clearly visible and the spores touching one another (Plate II, 2, 3). The massulae contain 1–8 smooth walled triradiate microspores (20–22 µm diameter) (Plate II, 2–5). The laesurae are c. 5 µm long, extending about half the radius of the spore (Plate II, 9). The outer surfaces of the microspore massulae have a limited number of strap-shaped aseptate glochidia (Plate II, 2–5, 7, 8, 12), sometimes as long as 85 µm (Plate II, 4 right side), with a broad (up to 25 µm wide) basal attachment (Plate II, 3–7); the narrower lower stalk grades

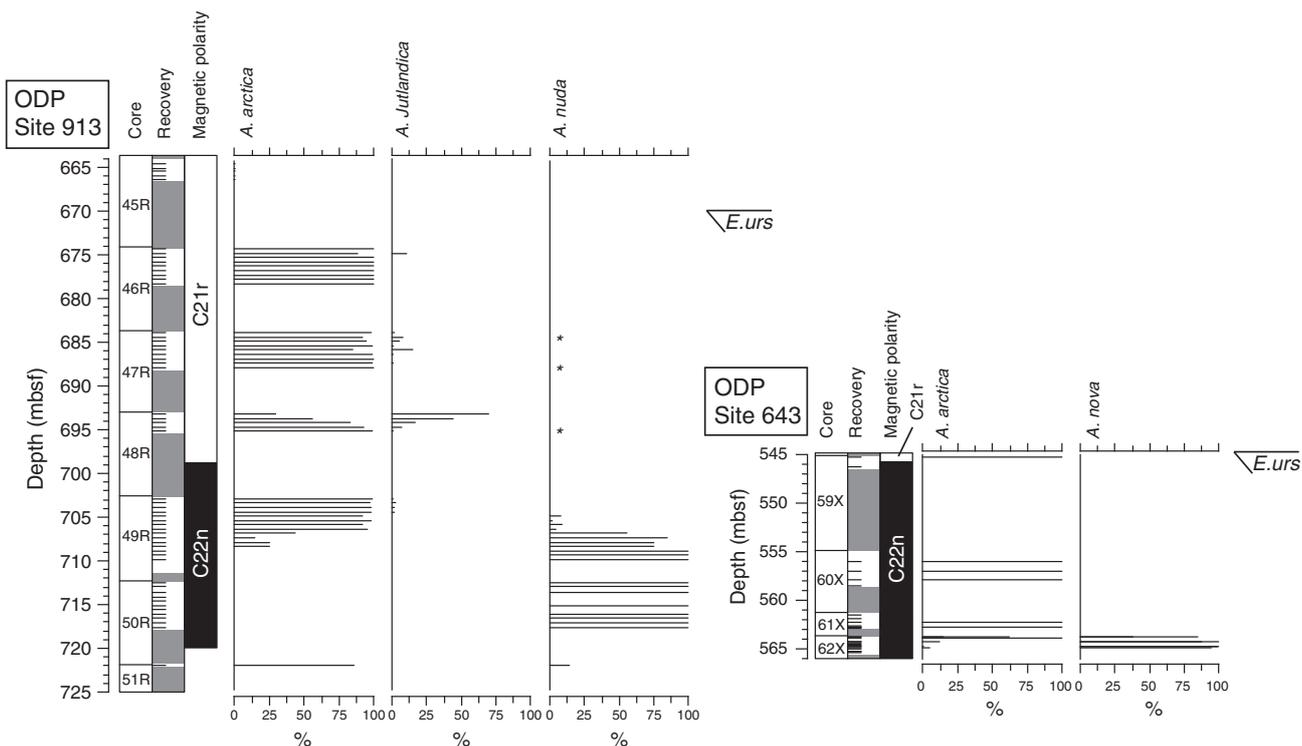


Fig. 2. Stratigraphic distribution of the various *Azolla* species (based on microspore massulae in palynological slides) occurring in holes ODP 913B and ODP 643A. Magnetostratigraphy from Eldrett et al. (2004). Correlation between the cores partly based on Eldrett et al. (2004) and partly on pers. obs. HB. The last occurrence of *Eatonicysta ursulae* is indicated for both holes (Eldrett et al., 2004). In the recovery column, dark grey areas represent 'no recovery'; white areas with lines indicate samples investigated for *Azolla*; white areas without lines indicate sampling gaps. * indicates isolated occurrences of megaspore apparatuses with attached massulae.

Plate I. *Azolla nova* sp. nov. 1–8, 15. Megaspore apparatuses and megaspores showing the various shapes, 9–12 SEM details of megaspore apparatuses, 13 LM details, 14, 16, 17 TEM of megaspore wall. (see on page 5)

1. Holotype, still covered with filiosum and with attached massula (see Plate II, 7). SEM; Sandpiper Well 3570–3600 cm; U23536.
 2. LM of megaspore apparatus with cap, pear-shaped; ODP 104–643 62A 1W 102–105 cm, slide 1; EF: J 34–1.
 3. SEM of megaspore apparatus with filiosum and small cap, ovoid in shape, with attached massula; specimen used for TEM; Sandpiper Well 3570–3600 cm; U23538.
 4. SEM of almost globular megaspore apparatus (debris attached in two places in upper part); ODP 104–643 62A 1W 102–105; U23537.
 5. LM of globular megaspore apparatus with small float zone and cap and small excrescences (arrows) on the megaspore surface; ODP 104–643 62A 1W 102–105 cm, slide 1; EF: T 20–4.
 6. Partly dissected megaspore apparatus showing cap, a little filiosum and megaspore with regular small excrescences; ODP 104–643 62A 1W 102–105; U23537.
 7. Dissected megaspore with regular small excrescences and proximally attached remnants of filiosum; ODP 104–643 62A 1W 102–105; U23537.
 8. LM of megaspore showing triradiate laesurae and triangular collar; ODP 104–643 62A 1W 102–105 cm, slide 1; EF: M 35–3.
 9. Detail of filiosum from Fig. 4.
 10. Megaspore ornamentation with small excrescences and rugulae frequently topped by small papillae, detail of Fig. 7.
 11. Megaspore surface with some fused papillae topping the rugulae; detail of Fig. 6.
 12. Detail of Fig. 6 showing the cap.
 13. LM surface of megaspore showing the rugulate surface; ODP 104–643 62A 1W 102–105 cm, slide 1; EF: H 21–4.
 14. TEM of megaspore wall from Fig. 3 showing exine, endoperine, exoperine and covering of suprafilosul hairs; Sandpiper Well 3570–3600 cm.
 15. SEM of globular megaspore apparatus all of surface obscured; Sandpiper Well 3570–3600 cm; U23538.
 16. TEM of megaspore wall of Fig. 15 showing typical excrescence. e = excrescence.
 17. TEM of megaspore wall of Fig. 15 showing exine, endoperine with radially arranged structure, exoperine with elongated nodular masses and covering of suprafilosul hairs.
- Scale bars: Figs. 1–8, 15: 100 µm, Figs. 9, 10: 20 µm, Figs. 11, 13: 10 µm, Fig. 12: 50 µm, Figs. 14, 16, 17: 2 µm.

Plate II. *Azolla nova* sp. nov. 1. TEM of megaspore; 2–13 microspore massulae in LM and SEM. (see on page 6)

1. TEM of the collar, suprafilosul hairs and cap (beneath gold coating at right of image) of Plate I, 15; Sandpiper Well 3570–3600 cm.
 2. Single massula with undulating, wavy outline, microspores visible; ODP 104–643 62A 1W 112–115 slide 1; EF: J 33–2.
 3. Single massula with undulating, wavy outline, microspores visible; ODP 104–643 62A 1W 102–105 slide 2; EF: L 30–2.
 4. Single massula with undulating, wavy outline showing hairy expanded base of the long glochidia; ODP 104–643 62A 1W 102–105 slide 1; EF: T 36–4.
 5. Single small massula with undulating, wavy outline showing hairy expanded base of the glochidia; ODP 104–643 62A 1W 112–115 slide 1; EF: P 29–1.
 6. Detail of Fig. 5 showing hairs on the base of the glochidia stalk as well as on the expanded basal attachment area.
 7. SEM of massula showing hairs on the massula surface and broad attachment area of glochidia (arrow); from holotype, see Plate I, 1; U23536.
 8. SEM of several massulae and glochidia tips embedded in filiosum; from Plate I, 3.
 9. LM of microspores in a massula, one showing a triradiate mark; ODP 104–643 62A 1 W 102–105 slide 1; EF: P 36–1.
 10. LM of two glochidia anchor tips showing recurved hooks on the flukes; ODP 104–643 62A 1W 112–115 slide 1; EF: P 27–3.
 11. Detail of Plate II, Fig. 8 showing two glochidia anchor tips, one with a twisted recurved hook.
 12. Several massulae grouped in a cluster, surface hairs clearly visible; ODP 104–643 62A 1W 102–105; U23537.
 13. SEM detail showing long hairs on massula surface and glochidia with recurved hooks; ODP 104–643 62A 1W 102–105; U23537.
- Scale bars: Fig. 1: 4 µm, Figs. 2–5, 8, 12: 20 µm, Figs. 6, 7: 10 µm, Figs. 9–11, 13: 5 µm.

into a wider upper stalk (3–4 μm maximum width) (Plate II, 4, 5, 7, 8, 12). The stalk shows a distal dilation and a distinct constriction below an anchor-shaped tip (8–9 μm wide) (Plate II, 10, 11, 12, 13). Total fluke length (including hooked tip) is up to 10 μm long. Flukes narrow gradually and typically have recurved hooks (Plate II, 10, 11, 13). Fluke thickness changes approximately half way down (Plate II, 10, 13), which may be because the anchor tip is solid (borne out by TEM

of glochidia in other species e.g., Collinson et al., 2009 plate III, 7–9), whereas the fluke tip might be hollow. The body of the massula and the bases of the glochidia are covered by hairs, up to 4 μm long (Plate II, 6, 7, 8, 12, 13).

In one sample (Sandpiper), two megaspore apparatuses were found with attached massulae (Plate I, 1, 3). One of these was subsequently used for TEM (see above) and the other was selected as the

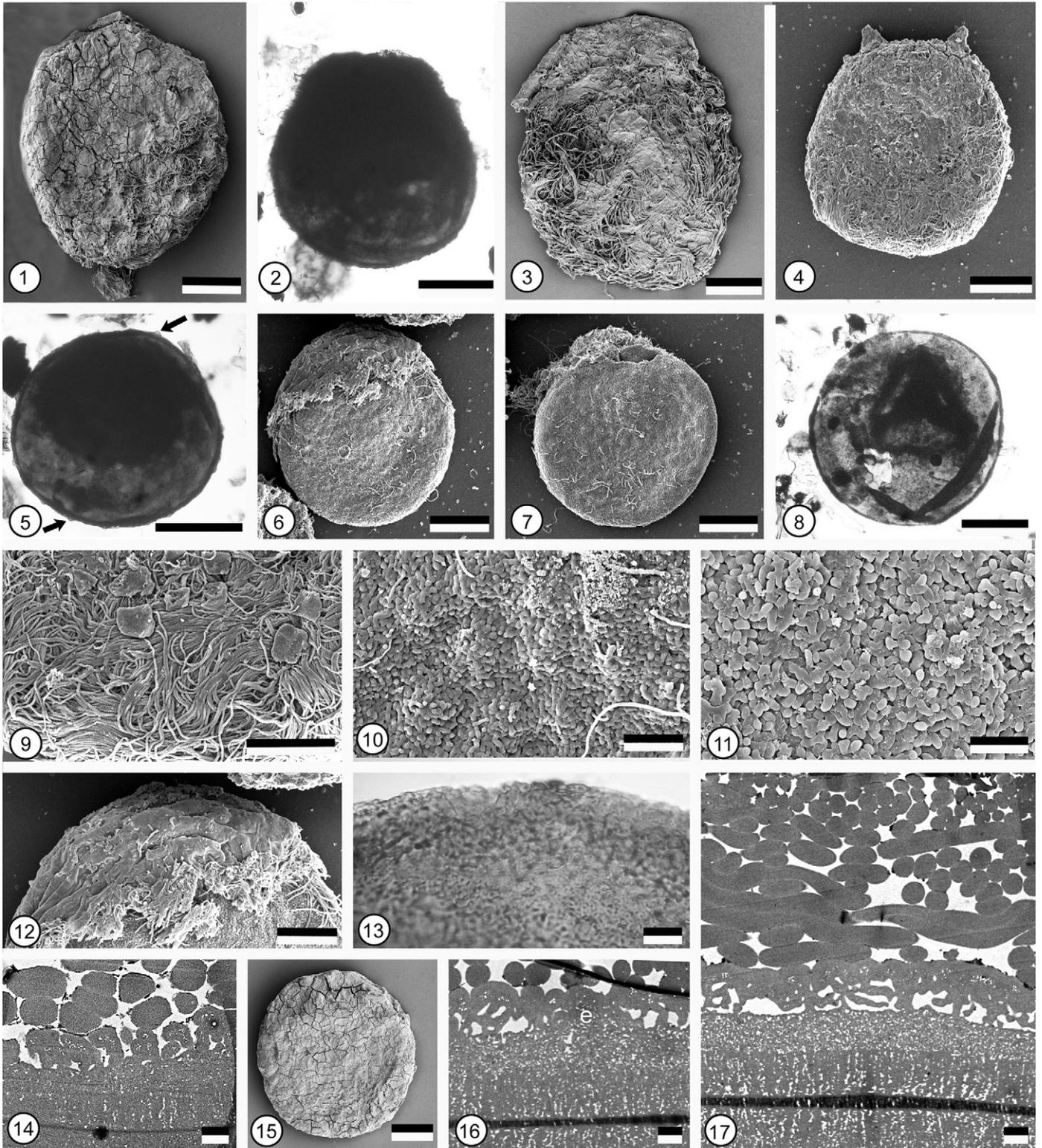


Plate I (caption on page 4).

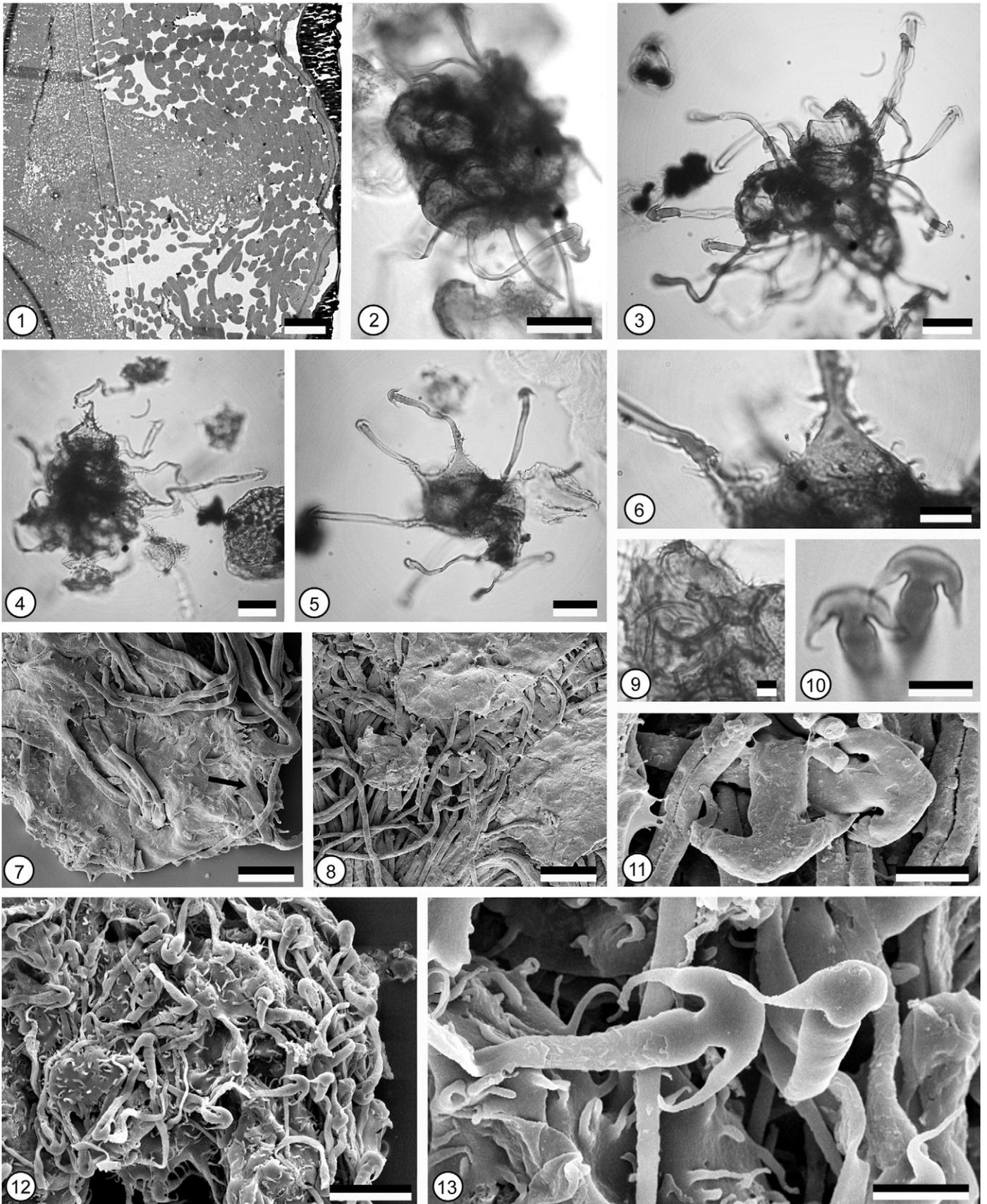


Plate II (caption on page 4).

holotype. The attached massula of the holotype exhibits surface hairs and broad based glochidia attachments typical of the species (Plate II, 7), but unfortunately no glochidia tips are visible, therefore, the characteristic hooks cannot be confirmed.

Specimens studied: Eight megaspore apparatuses by LM, 20 by SEM and two by TEM.

Species *Azolla nuda* Van der Burgh et al. sp. nov.
Plates III, IV

Holotype: Plate III, 1; U23539.

Paratypes: Figured material in Plates III and IV; U23540.

Repository: Laboratory of Palaeobotany and Palynology, Utrecht, coll. nr. U23539 (holotype), U23540 (paratypes).

Type locality: ODP 913B 48R 2W 70–72.

Other localities: ODP Leg 151 Site 913B, cores 47–51 (with main abundance of massulae in cores 49–51) (see Fig. 2), while occasional megaspore apparatuses with attached microspore massulae also occur in cores 47 and 48 (75°29.356'N, 6°56.810'W; Firth, 1996).

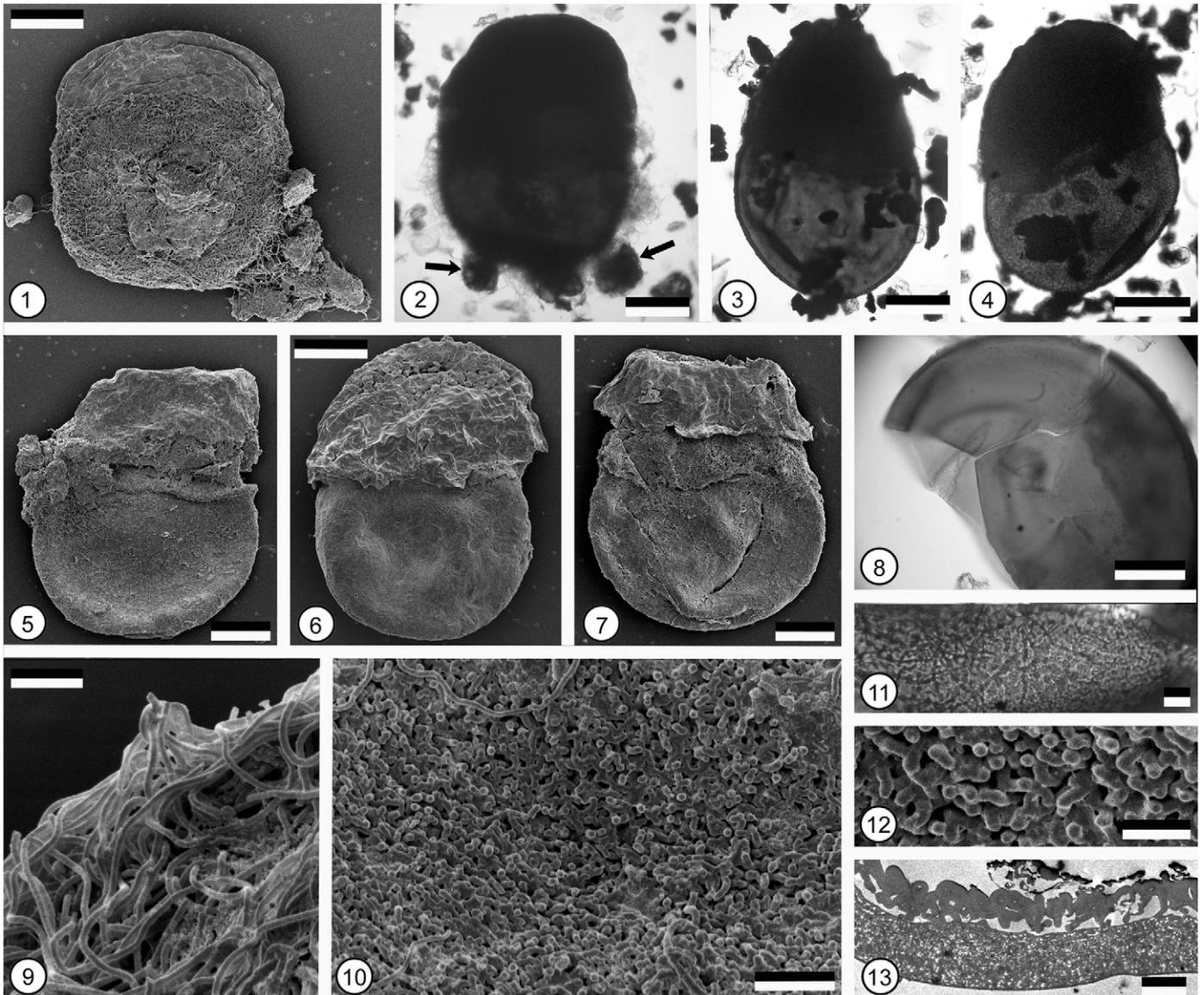


Plate III. *Azolla nuda* sp. nov. 1–8. Megaspore apparatuses and megaspores showing the various shapes (SEM and LM), 9, 10, 12 SEM details of megaspore apparatuses, 11 LM details, 13 TEM of megaspore wall.

1. SEM of holotype, megaspore apparatus, not dissected hence covered with filosum and with attached massulae; ODP 151-913B 48R 2W 70–72; U23539.
2. LM of megaspore apparatus showing several attached massulae (arrows) and filosum cover; ODP 151-913B 49R 4W 120–122, slide 2; EF: R 30-3.
3. Pear-shaped megaspore apparatus, LM; ODP 151-913B 49R 5W 70–73, slide 2; EF: V 21-2; U23540.
4. Ovoid megaspore apparatus. LM; ODP 151-913B 49R 3W 20–22, slide 1; EF: F 29-4.
5. SEM of megaspore apparatus with attached massulae, cap, collar and dissected megaspore; ODP 151-913B 47R 1W 70–72; U23540.
6. SEM of megaspore apparatus with cap covering float system and dissected megaspore; ODP 151-913B 47R 1W 70–72; U23540.
7. SEM of megaspore apparatus with cap partly covering float system and dissected megaspore; specimen used for TEM; ODP 151-913B 47R 1W 70–72; U23540.
8. LM of broken megaspore showing triradiate scar; ODP 151-913B 49R 3W 120–122, slide 2; EF: M 19-4.
9. SEM of filosum; ODP 151-913B 47R 1W 70–72; U23540.
10. SEM of megaspore ornamentation showing narrow rugulae topped by small papillae and baculae; detail of Fig. 5.
11. LM of megaspore ornamentation; ODP 151-913B 49R 4W 20–23, slide 1; EF: M 30-3.
12. Detail of Fig. 10, showing small rugulae and papillae.
13. TEM of megaspore wall; ODP 151-913B 47R 1W 70–72 cm.

Scale bars: Figs. 1–7: 100 μ m, Fig. 8: 50 μ m, Figs. 9, 11, 12: 5 μ m, Fig. 10: 10 μ m, Fig. 13: 4 μ m.

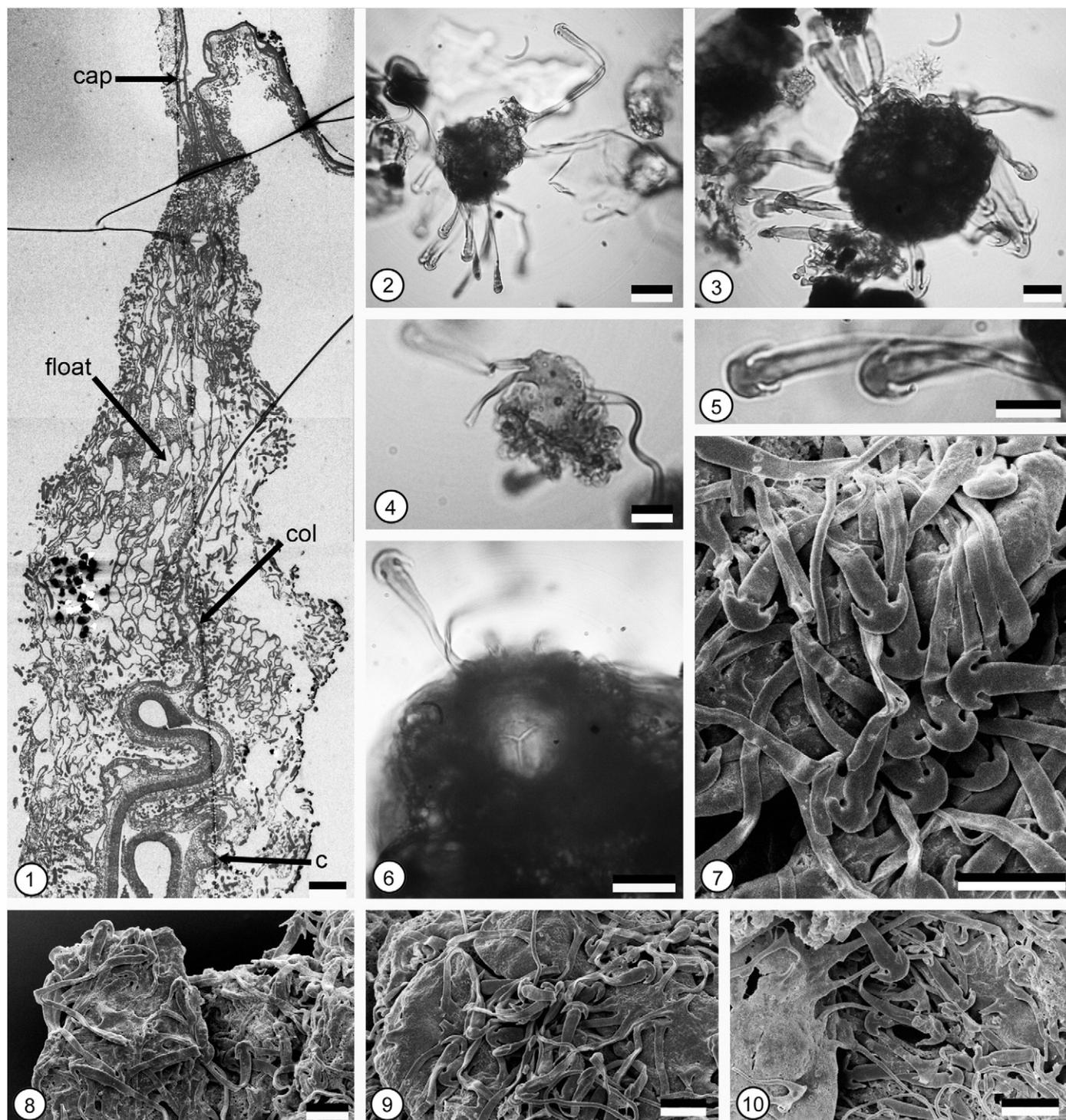


Plate IV. *Azolla nuda* sp. nov. 1. TEM of megaspore; 2–10 microspore massulae in LM and SEM.

1. TEM montage of two images in float zone showing columella (col), part of the cap, floats and filosum; ODP 151-913B 47R 1W 70–72 cm. c = collar; U23540.
 2. Single microspore massula with long glochidia; ODP 151-913B 49R 5W 120–122 slide 1; EF: U 32-4.
 3. Single microspore massula with shorter glochidia; ODP 151-913B 49R 4W 20–23 slide 1; EF: D 19-4.
 4. Part of a microspore massula showing broad attachment area of the glochidia without any hairs; ODP 151-913B 49R 5W 20–23 slide 2; EF: O 22-1.
 5. Two glochidia anchor tips with slightly recurved hooks on flukes; ODP 151-913B 49R 5W 120–122 slide 2; EF: V 24-1.
 6. Part of a microspore massula showing spongy pseudovacuolate structure and a microspore with trilete mark; ODP 151-913B 49R 5W 120–122 slide 1; EF: G 34-2.
 7. SEM of glochidia, anchor tips lacking recurved hooks on flukes; ODP 151-913B 48R 2W 70–72; U23539.
 8. Detail of Plate III, 5, showing attached microspore massula.
 9. Microspore massula with glochidia showing a broad attachment area without hairs; ODP 151-913B 48R 2W 70–72; U23539.
 10. Surface of microspore massula and glochidia lacking hairs; ODP 151-913B 48R 2W 70–72; U23539.
- Scale bars: Figs. 1, 3–10: 10 μ m, Fig. 2: 20 μ m.

Stratigraphic horizon: Latest early Eocene (mainly middle and lower part of Chron C22n and isolated occurrences of megaspore apparatuses with attached massulae in lower part of Chron C21r).

Etymology: from the microspore massula surface which is devoid of hairs.

Specific diagnosis

Megaspore apparatus ovoid to pear-shaped (up to 440 μm long), broader distally. Megaspore inferred to be spherical to sub-spherical when uncompressed, trilete, laesurae extending about half of the radius of the spore. Entire megaspore apparatus covered by a thick mat of intertwined hairs, which arise almost entirely from the proximal region of the megaspore (hence suprafilosum). Collar not visible under transmitted light. Remnants of megasporocarp wall present on the proximal pole of the megaspore apparatus.

Megaspore wall lacking excrescences. Under LM megaspore surface with small-scale ornamentation, combining rugulae and small papillae. Under SEM exoperine surface finely rugulate, rugulae usually branching and consistently topped by small papillae to baculae. Under TEM megaspore-wall consisting of an exine and a two-layered perine; inner surface of the exine forming a more or less continuous layer beneath a more open structure with a small number of radially elongated irregular spaces. Endoperine similar to exine but with more equidimensional spaces, granular appearance and an undulating surface lacking ornamentation. Exoperine with contorted nodular masses in the outer part supported by nodular baculae giving a strongly undulating exoperine surface. Collar formed from endoperine, slightly spongy and pseudovacuate, modified exoperine on the collar gives rise to hairs (suprafilosum). Modified exoperine also forming a columella extending proximally through the float system.

Float system compact dome-shaped, occupying at least the proximal two-fifths of the megaspore apparatus; floats spongy pseudovacuate, probably numerous, arranged in at least two, probably three, tiers. Floats enmeshed by hairs of the suprafilosum.

Microspore massulae with slightly irregular outline and granular appearance, internally spongy, vacuolated in structure, obscuring the spore outlines. Massulae containing up to 20 smooth-walled trilete microspores, laesurae extending about half the radius of the spore. Outer surface of the microspore massulae with a number of aseptate glochidia up to 75 μm long, with broad basal attachment, a narrow lower stalk grading into a broader upper stalk with a distal dilation and a distinct constriction below an anchor shaped tip. Flukes mostly lacking recurved hooks. Surface of the microspore massulae and glochidia bases lacking hairs.

Description

The megaspore apparatus is typically ovoid (Plate III, 1, 2, 4–7) sometimes pear-shaped (Plate III, 3) broader distally, 310–440 μm long and up to 300 μm wide, always longer than broad. The laesurae of the trilete mark are 50–55 μm long and extend about half of the radius of the megaspore (Plate III, 8). The entire megaspore apparatus is covered by a thick mat of intertwined hairs (c. 0.8–1 μm in diameter) (Plate III, 1, 2, 9). A single isolated megaspore was encountered in the palynological slides (Plate III, 8), but in most cases dissection was necessary to reveal the megaspore wall (Plate III, 5–7), indicating the persistence of the enveloping suprafilosum. The hairs arise almost entirely from the proximal region of the megaspore (hence suprafilosum). Observations of dissected specimens with SEM and thin sections with TEM do not reveal an infrafilosum (Plate III, 10, 12, 13). Remnants of the megasporocarp wall (cap), one cell layer thick (Plate IV, 1), are present on the proximal pole of most specimens (Plate III, 1, 5–7). The collar is not clearly visible in transmitted light.

LM observations (Plate III, 3, 4, 8), specimens dissected to remove filosum studied by SEM (Plate III, 5–7) and intact specimens studied

by TEM (Plate III, 13) show that excrescences are absent on the megaspore wall. The megaspore surface ornamentation is very small, a combination of rugulae and small papillae (Plate III, 5–8, 10–12). In LM a combination of rugulae and small papillae can be seen (Plate III, 11). Under SEM the exoperine surface is finely rugulate; rugulae 4–5 μm long, 1 μm wide, usually branching but the length of the branches is obscured by small papillate to baculate ornamentation (c. 1 μm diameter) that consistently occurs on top of the rugulae (Plate III, 10, 12). TEM observations of this species are based on a single specimen (Plate III, 7) identified to the species (after dissection, hence lacking filosum) on the basis of the megaspore surface ornamentation. TEM pictures (Plate III, 13) show that the inner surface of the 1–3 μm thick exine forms a more or less continuous layer beneath a more open structure with a few radially orientated irregular spaces, otherwise granular. The perine is c. 4 μm thick; the endoperine is very similar to the exine but with more equidimensional spaces giving a granular appearance. The endoperine thickness varies slightly (± 1 –2 μm) around the specimen resulting in an undulating surface, which lacks ornamentation. The exoperine has contorted nodular masses in the outer part supported by nodular baculae resulting in a strongly undulating exoperine surface. The collar is formed from modified endoperine, slightly spongy and pseudovacuate (Plate IV, 1); this modified endoperine also forms a columella extending proximally through the float system (Plate IV, 1). Modified exoperine on the collar gives rise to hairs of the suprafilosum (Plate IV, 1).

The float system is a compact and dome-shaped structure, occupying at least the proximal two-fifths of the megaspore apparatus, extending well over the proximal part of the megaspore (Plate III, 1–7). The floats are spongy pseudovacuate (Plate IV, 1). Although compression partly obscures the details of the float system, at least two (Plate IV, 1) and possibly three tiers are present. The minimum float number is inferred to be nine (an upper tier of three large floats and a lower tier of six smaller floats) which is supported by digital sections in Collinson et al. (2013, fig. 5, C,D). Up to 18 floats is possible in three tiers with three, six and nine floats respectively (from proximal to distal respectively). The fact that individual floats cannot be discerned in SEM or LM (Plate III, 1–7) suggests that this is a multifloated and not a nine-floated species. The floats are enmeshed by hairs of the suprafilosum (Plate IV, 1).

The microspore massulae have been encountered attached to the megaspore apparatus singly or in groups (Plate III, 1, 2, 5) and as single massulae in palynological slides (Plate IV, 2, 3). They have a slightly irregular outline and a granular appearance. Internally they are spongy and vacuolated in structure with the vacuolated tissue obscuring the spores (Plate IV, 2, 3, 6). The massulae contain up to 20 smooth-walled trilete microspores (diameter 20–22 μm) with 5–6 μm long laesurae (Plate IV, 6) extending about half the radius of the spore. The outer surfaces of the microspore massulae have a number of aseptate, broadly attached glochidia, from 30 to 75 μm long (Plate IV, 2–4, 8, 9). The narrow lower stalk gradually broadens (up to 3 μm wide) (Plate IV, 7–10) with a distal dilation and a distinct constriction below an anchor-shaped tip (6–7 μm wide) (Plate IV, 5, 7–10). Total fluke length including tips from 4 to 6 μm ; flukes mostly lacking recurved hooks (Plate IV, 5, 7–10). The surfaces of the microspore massulae and the glochidia bases lack hairs (Plate IV, 4, 6–10).

Specimens studied: Twenty megaspore apparatuses by LM, eight by SEM, one by TEM.

Species *Azolla astroborealis* Van der Burgh et al. sp. nov.
Plates V, VI, Fig. 3, 1, 3

Holotype: Plate V, 1, 5, 8, VI, 1; U 23541.

Paratypes: Plates V, 2, VI, 2, Fig. 3, 1, 3.

Repository: Collection Laboratory of Palaeobotany and Palynology, Utrecht, coll. nr. U 23541.

Type locality: Northstar 1 Well (Amerada) western Arctic sea along the north coast of Alaska, (70°31'42.02"N, 149°5'48.82"W).

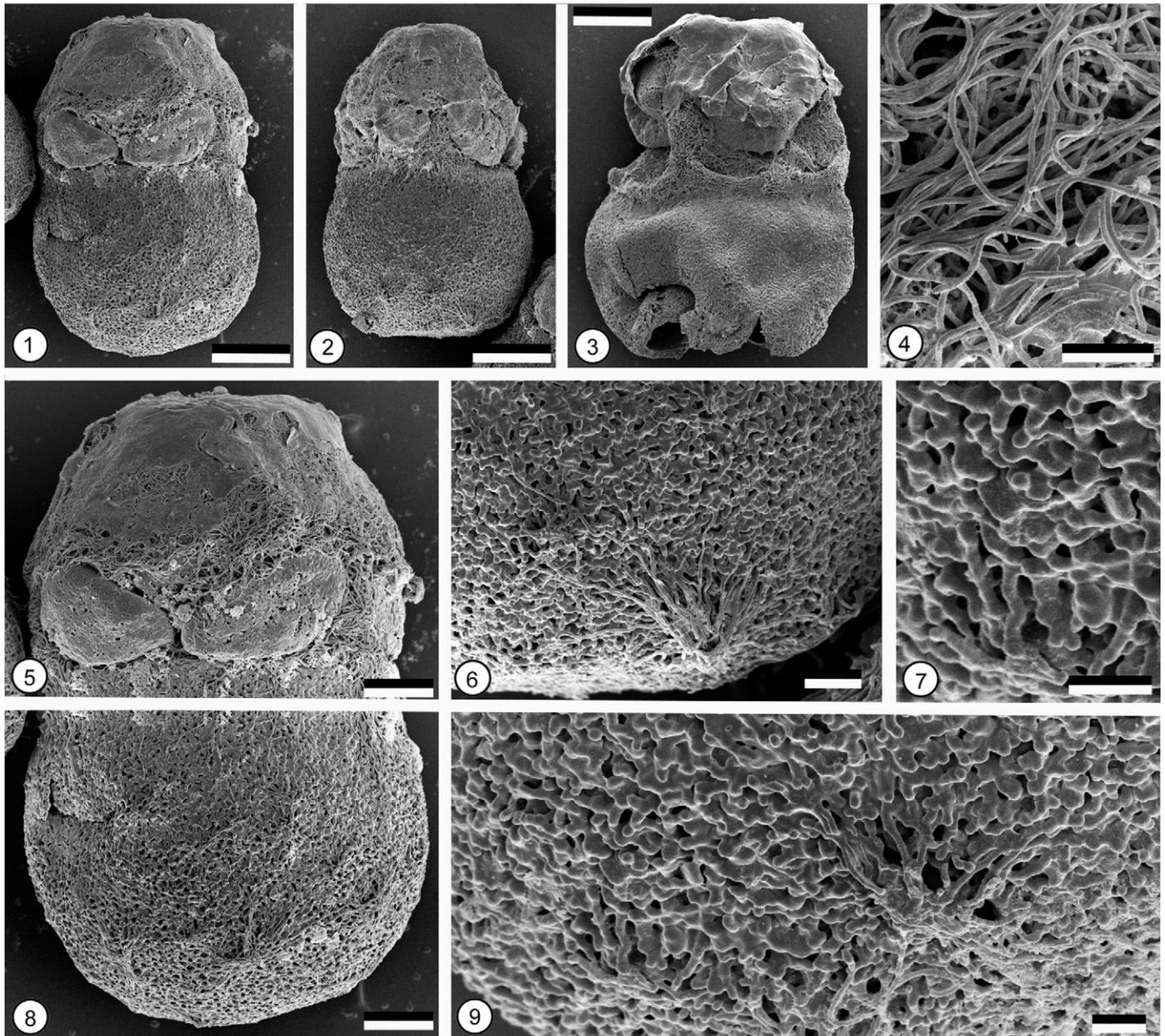


Plate V. *Azolla astroborealis* sp. nov. SEM; all images from Northstar 1 Well (Amerada) western Arctic.

1. SEM of holotype megaspore apparatus with cap, visible lower tier of small floats and megaspore without filousum and showing some excrescences on its distal surface; U23541.
 2. SEM of a similar uncompressed specimen that has been used for TEM; U23541.
 3. Damaged megaspore apparatus revealing part of the floats (under the cap) and the collar; U23541.
 4. Detail of suprafilousum present in the float zone; U23541.
 5. Upper part of holotype showing cap and float zone with filousum between the floats; U23541.
 6. Detail of Plate V, 2 showing excrescence formed from exoperine strands and a rare long infrafilousal hair.
 7. Megaspore wall ornamentation with rugulae frequently topped by small papillae and part of an excrescence (detail of Fig. 9); U23541.
 8. Lower part of holotype megaspore showing distal excrescences and absence of filousum; U23541.
 9. Detail of megaspore wall showing ornamentation and two excrescences; U23541.
- Scale bars: Figs. 1–3: 100 μ m, Figs. 4, 7, 9: 10 μ m, Figs. 5, 8: 50 μ m, Fig. 6: 20 μ m.

Stratigraphic horizon: probably latest early Eocene.

Etymology: after the name of the well site 'Northstar' that yielded this material.

Specific diagnosis

Megaspore apparatus truncated oviform (up to 450 μ m long), wider distally, proximal pole slightly flattened. Filousum lacking over megaspore, apart from rare hairs associated with excrescences. Remnants

of megasporocarp wall, firmly attached over the proximal pole of the megaspore apparatus.

Under SEM megaspore exoperine surface finely reticulate, muri formed by rugulae, rugulae regularly topped by small papillae. Distinct excrescences, formed from exoperine, occurring near the distal pole.

Under TEM megaspore wall consisting of a thin exine and a much thicker two-layered perine. Inner surface of the exine forming a more or less continuous membrane beneath a more open structure, with small irregular radially elongate cavities; endoperine with open spongy

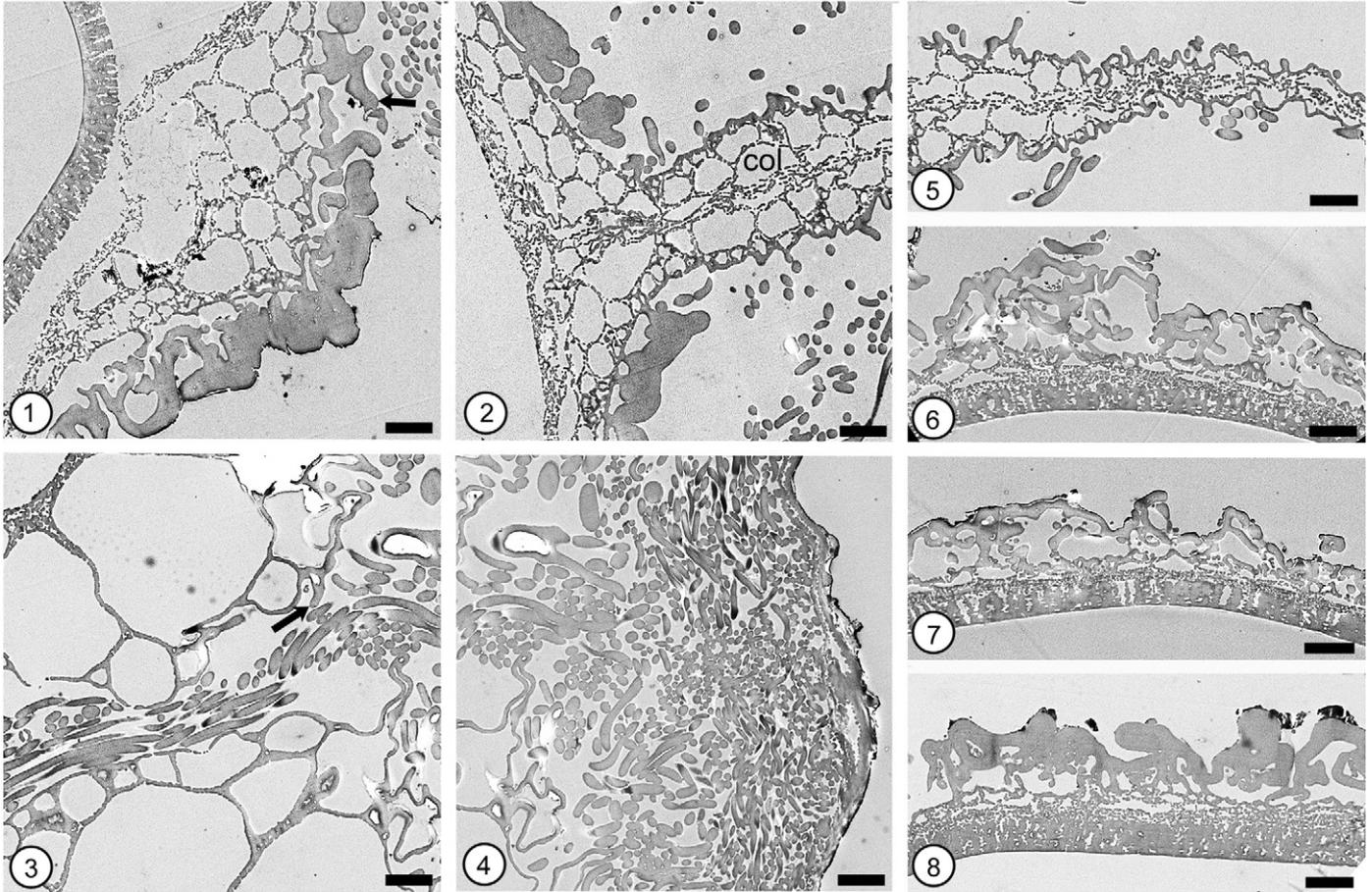


Plate VI. *Azolla astroborealis* sp. nov. TEM of specimen of Plate V, 2; from Northstar 1 Well (Amerada) western Arctic.

1. Detail of collar with expanded endoperine and origin of suprafilosum (arrow) from modified exoperine.
 2. Detail of collar showing modification of endoperine and exoperine, both extended to form the columella (col) from which suprafilosum hairs arise.
 3. Detail of float zone with suprafilosum between floats and originating from float surface (arrow).
 4. Suprafilosum below remnant of sporocarp wall (cap) at proximal end of megaspore apparatus (right of image).
 5. Proximal part of columella also showing origin of suprafilosum.
 6. Section of megaspore wall with large alveolae in exoperine bounded by thin elongate strands; exoperine expanded to form an excrescence.
 7. Section of megaspore wall lacking excrescences; with large alveolae in exoperine bounded by thin elongate strands.
 8. Section of megaspore wall in region with more nodular exoperine.
- Scale bars: 1–8: 4 μ m.

porous appearance, granular in the inner part, variable larger spaces in the outer part; endoperine surface slightly undulating, lacking ornamentation; exoperine variable from solid contorted nodular masses to large alveolae bounded by thin elongate strands. Endoperine expanded in thickness and becoming spongy vacuolated in structure near the proximal pole, forming a collar encircling the megaspore, and a narrow columella extending distally through the center of the float system. Modified exoperine of the collar and columella gives rise to hairs (suprafilosum) occurring between the floats and in a thick mat at the proximal pole of the megaspore apparatus beneath the remnant of megasporocarp wall.

Float system dome-shaped, proximal pole slightly flattened, extending over slightly less than one half of the megaspore apparatus. Floats nine, in two tiers, upper tier of three large floats, lower tier of six small floats, one large and two small floats in each third of the float system overlying one contact face of the megaspore.

Microspore massulae not known.

Description

Azolla astroborealis is quite different in appearance compared to the other Arctic and Nordic *Azolla* species (Collinson et al., 2009, 2010; this paper). It is preserved uncompressed so that the megaspore is spherical

and the float tissues show clearly their open spongy, pseudovaculate structure (Fig. 3, 1, 3). This in contrast with *Azolla arctica* from the same sample (Fig. 3, 2), which shows the typical compressed preservation of other Arctic and Nordic species. The megaspore apparatus is truncated oviform (400–450 μ m long, and 280–300 μ m wide), broader distally, proximal pole slightly flattened (Plate V, 1–3). There is no filosum cover over the megaspore (Plate V, 1–3, 6–9). Suprafilosum (Plate V, 4) is represented by hairs (0.8–1.5 μ m wide) between the floats (Fig. 3, 1, 3; Plate V, 5). Remnants of megasporocarp wall (cap), one cell layer thick (Plate VI, 4), are firmly attached over the proximal pole of the megaspore apparatus (Plate V, 1–3, 5) and suprafilosum hairs form a thick mat between the megasporocarp wall remnant and the upper tier of floats (Fig. 3, 1, 3; Plate V, 5; Plate VI, 4).

Under SEM the megaspore wall appears finely reticulate at low magnification (Plate V, 1–3, 8), with lumina 2–4 μ m in maximum dimension. The muri are formed by fine rugulae (2–3.5 μ m wide, up to 20 μ m long) frequently topped by small papillae (2–3.5 μ m diameter) (Plate V, 6, 7, 9). Scattered excrescences of variable height (up to 15–20 μ m) occur near the distal pole and are formed almost entirely from thin strands of exoperine (Plate V, 6–9; Plate VI, 6). Occasional long (up to 100 μ m) hairs (infrafilosum) arise from the exoperine, particularly near to, and on, exoperine excrescences (Plate VI, 6). These

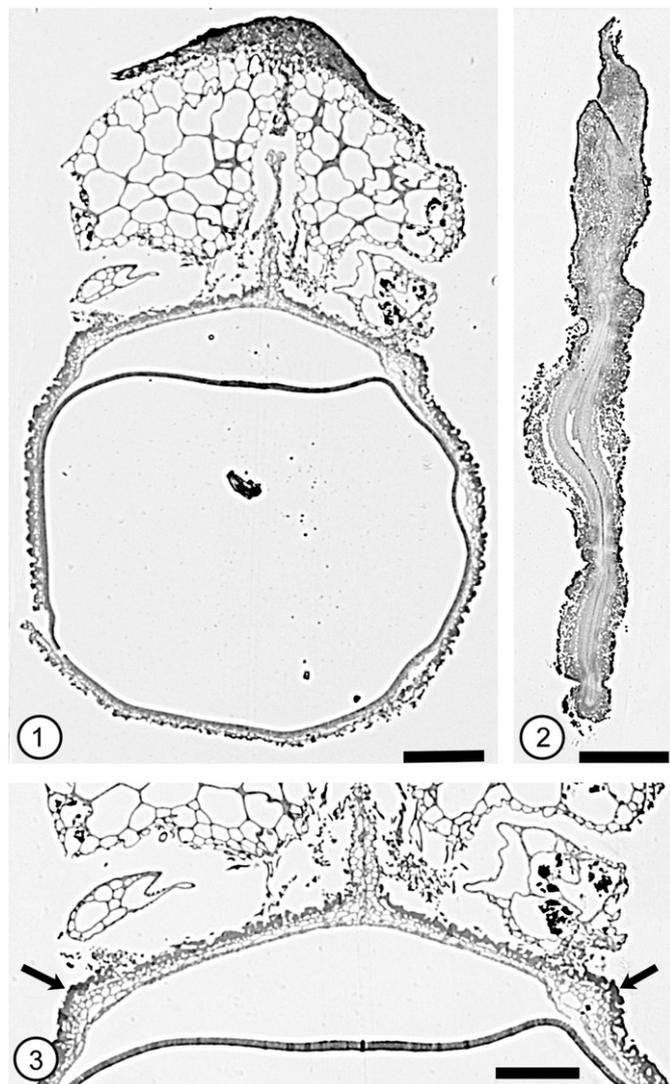


Fig. 3. *Azolla astroborealis* sp. nov. and *A. arctica*; all images from Northstar 1 Well (Amerada) western Arctic.

1. LM of Semi-thin section of uncompressed megaspore apparatus showing distal megaspore and proximal float zone with columella, two tiers of vacuolated floats and proximal cap with underlying mat of suprafilosum; U23541.
2. LM of semi-thin section of heavily compressed megaspore of *A. arctica* Collinson et al., from the same sample as 1.
3. Detail of Fig. 3.1 showing lower part of float zone, columella and collar (arrows). Scale bars: 1–2: 50 μm , 3: 10 μm .

hairs, and shorter strands of exoperine radiating from the tips of the excrescences, result in a volcano-like appearance of the excrescences (Plate V, 6, 9).

TEM analysis of this species is based on a single specimen (Plate V, 2) It shows a wall consisting of a 2–3 μm thick exine and a two-layered perine, c. 4–9 μm thick (Plate VI, 6–8). The inner surface of the exine forms a more or less continuous membrane beneath a more open structure, with small irregular radially elongate cavities (Plate VI, 6–8). The endoperine is c. 2–4 μm thick, extending to at least 11 μm in excrescences. The inner part is granular (c. 200 nm) while the outer endoperine has an open spongy porous appearance with laterally elongated lumina from 1 to 4 μm long. The endoperine surface is slightly undulating and lacks ornamentation (Plate VI, 6–8). The exoperine is variable from solid contorted nodular masses (Plate VI, 8) to large alveolae bounded by thin (<1 μm) elongate strands (Plate VI, 6, 7). The endoperine expands in thickness (to c. 23 μm) and becomes spongy vacuolated in structure

across the proximal pole (Fig. 3, 1, 3), forming a collar (Fig. 3, 3; Plate VI, 1) encircling the megaspore (Plate V, 3) and extended into a narrow columella (Fig. 3, 1, 3; Plate VI, 2, 5) running proximally through the center of the float system. Hairs (suprafilosum) are formed from the modified exoperine of the collar (Fig. 3, 3; VI, 1) and columella (Plate VI, 2, 5). The hairs arise on the proximal flank of the collar and on both sides of the columella. The suprafilosum spreads between the floats (Plate VI, 3) and into a thick mat at the proximal pole of the megaspore apparatus beneath the remnant of megasporocarp wall (Fig. 3, 1; Plate VI, 4).

The float system is dome-shaped and extends over slightly less than one half of the megaspore apparatus (Plate V, 1–3). Nine floats are present in two tiers. The upper tier consists of three large floats and is covered by the cap and underlying filosum. Upper tier float margins are indicated by slight indentations in the proximal part of Plate V, 5, and large upper tier floats are visible in semi-thin sections (Fig. 3, 1). The lower tier of six small floats is exposed in SEM images (Plate V, 1–3, 5) and evident in semi-thin sections (Fig. 3, 1, 3). One large and two small floats occur in each third of the float system (Plate V, 5), overlying one contact face of the megaspore. The float tissue is strongly pseudovaculate (Fig. 3, 1, 3; Plate VI, 3 top left and bottom right), and scattered hairs arise from the float surfaces (Plate VI, 3).

Microspore massulae are not known.

Specimens studied: No megaspore apparatuses were present in LM preparations. Four specimens have been studied by SEM and one of those by TEM.

5. Comparison with other fossil species

In this section comparisons are made with previously described species in the literature including those from the Arctic and Nordic seas. Further discussion of the distinctions between all five Arctic and Nordic species is given in Section 7, and those key differences are summarised in Tables 1 and 2. Blooms of *Azolla* occur at the Bartonian–Priabonian transition (late Eocene) in western Siberia (Iakovleva, 2011; Akhmetiev et al., 2012). However the reproductive material is not yet fully described so comparison with the Arctic and Nordic late early Eocene species is not possible.

Many fossil species are clearly different from *Azolla nova* and *Azolla nuda*, including all species lacking a filosum over the megaspore wall, all species lacking glochidia or lacking anchor-shaped tips on the glochidia, or with septate glochidia. Comparison is made on the basis of float number. *Azolla nova* is interpreted as probably having three or six floats but comparison with nine-floated species is also included here. *Azolla nuda* is interpreted to be multifloated but comparison is also made with nine-floated species as that cannot be totally excluded. In *Azolla astroborealis* massulae are not known but the number of floats is very clear and so comparison is restricted to species with nine floats lacking filosum cover over the megaspore apparatus.

5.1. Fossil species with three floats

Although it is unlikely that any of the three new species has only three floats, there is a chance that the number of floats in *Azolla nova* is only three, and hence a short comparison is also made with three-floated species (see also Collinson et al., 2010).

Azolla intertrappea Sahni and Rao, 1934 from the Eocene of India has massulae and glochidia that are similar to those of *Azolla nova*, but the megaspore is smaller (diameter 215 μm versus c. 300 μm in *A. nova*). Moreover, it does not show excrescences; neither does *Azolla pyrenaica* Florschütz and Menedez Amor, 1960 from the Pliocene and Pleistocene of France.

Azolla indica Trivedi and Verma, 1971, also from the Eocene of India, differs in having septate glochidia and the megaspores lack excrescences unlike *Azolla nova*.

Table 1
Comparison of the main distinguishing characters of the megaspore apparatuses of the five Arctic/Nordic *Azolla* species.

	<i>A. arctica</i>	<i>A. jutlandica</i>	<i>A. nova</i>	<i>A. nuda</i>	<i>A. astroborealis</i>
Megaspore apparatus shape	Oblong	Pear shaped	Pear shaped to globular	Oblong to ovoid to pear shaped	Truncated oviform
Megaspore apparatus mean sizes	350–400 × 250 µm	350–400 × 300 µm	250–350 × 230–330 µm	310–440 × 300 µm	400–450 × 280–300 µm
Megaspore apparatus covered with filosum	Yes	Yes	Yes	Yes	No
Filosum width	0.3–0.6 µm	1.0–1.3 µm	0.9–2.0 µm	0.8–1.0 µm	0.8–1.5 µm
Number of floats	15–18, in 3 tiers	Probably 6, in 1 tier	Most likely 3–6, probably 1 tier	Minimum 9, most likely multi-floated up to 18, at least 2 tiers	9, in 2 tiers
Presence/absence excrescences on megaspore surface	Absent	Present, variable in size and distribution	Present, regularly arranged	Absent	A few near distal pole, variable in size
Excrescences formed by Exoperine surface	–	Endoperine	Endoperine	–	Mainly exoperine
	Rugulate, in several planes	Granulate to baculate to clavate	Rugulate, frequently topped by papillae	Rugulate, consistently topped by small papillae	Reticulate, frequently topped by papillae

Azolla geneseana Hills and Weiner, 1965 has glochidia with usually two flukes, but occasionally three to four, with recurved hooks. The megaspores are larger than in *Azolla nova* (c. 400–500 µm in diameter).

Azolla tomentosa Nikitin, 1948 (see also Dorofeev, 1963) has a cap that covers only the top part of the floats. The filosum appears to be thin and there are no excrescences. The description and illustrations do not enable a more detailed comparison. Bertelsen (1974) gives a better description and figures, describing a reticulate megaspore surface with infrafilosum hairs winding between the cavities of the perine unlike *Azolla nova*.

Azolla tujanensis Dorofeev, 1963 from the Oligocene of Russia and the Miocene of Denmark lacks a diagnosis. The illustrations show medially swollen glochidia and the species differs from *Azolla nova* in this respect, although the recurved flukes are similar to those of *A. nova*. Moreover, the cap only covers the upper part of the float system; the floats can clearly be observed on the megaspores (see also Friis, 1977). The float system of *A. nova* is only small and no individual floats can be observed. No excrescences have been observed in *Azolla tujanensis*.

Azolla primaeva (Penhallow) Arnold, 1955 is discussed in this section on fossil species with three floats, although the number of floats is not conclusively known. Arnold (1955) stated that no floats were visible at all, although the space in the 'swimming apparatus' for floats was quite large. Hall (1969) stated that Arnold's pl. II figs. 1 and 2 demonstrated that there was only a single apical, columellate float. Hills and Weiner (1965) mentioned that no floats were observed on any of the 40 studied megaspores, but that the presence of a distinct triradiate mark on the perispore of a single specimen suggested the presence of three floats (their plate 2, fig. 3). Hills and Gopal (1967) repeated that information but in their discussion it was mentioned clearly that *A. primaeva* had three floats. However, Sweet and Hills (1976, p. 350)

stated that *A. primaeva* possessed nine floats. Although Arnold (1955) mentioned and figured some excrescences on one of his megaspore apparatus specimens (his plate II, fig. 2) none of the other authors has observed any excrescences in this species, and certainly no small, regular excrescences as are present in *Azolla nova*.

5.2. Fossil species with six floats

Azolla nova might have possessed six floats. The only other fossil species with six floats is *Azolla jutlandica* (see Collinson et al., 2010, 2013).

Azolla nova is comparable with *Azolla jutlandica*. The megaspore apparatus is similar in both species though more globular in *A. nova*. Both have wide (c. 1 µm) filosum hairs in bundles, a small float section of probably only one tier and a rugulate to granulate ornamentation with papillae, baculae or clavae on the surface of the megaspore. Also the massulae bear up to 4–5 µm long hairs (Fig. 4 shows the hairs on a massula of *A. jutlandica*, which can be compared with Plate II, 4–6). However, there are differences, especially in the massulae and glochidia. One distinguishing character is that in *A. nova* the glochidia typically have recurved tips (Plate II, 10, 13), while *A. jutlandica* typically lacks recurved tips (Fig. 4). The glochidia in *A. nova* are rather long (45–85 µm), whereas those in *A. jutlandica* are shorter (<55 µm). The widened glochidia base in *A. jutlandica* is up to 5 µm wide, while it is up to 25 µm in *A. nova*. In *A. nova* the spores fill the massula with the spore shape clearly visible and spores touching one another. The spores bulge from the massula causing a wavy deeply indented outline. In the massulae of *A. jutlandica*, the individual spores are hardly visible and the massulae show a granular appearance, because the vacuolated tissue obscures the spores; hence, the massula outline is only slightly irregular (Fig. 4). The distinguishing characters are summarised in general in Tables 1 and 2.

Table 2
Comparison between main microspore massula characters of four Arctic/Nordic *Azolla* species (massulae not known for *A. astroborealis*). This table replaces table 2 in Barke et al. (2012). With further detailed study of all specimens throughout their stratigraphic ranges (Fig. 2) it became clear that not all characteristics proposed by Barke et al. (2012) were reliable diagnostic criteria.

	<i>Azolla arctica</i>	<i>Azolla jutlandica</i>	<i>Azolla nova</i>	<i>Azolla nuda</i>
Total length of glochidia	22–30 µm 45–85 µm	15–55 µm	45–85 µm	30–75 µm
Glochidium upper stalk width	Up to 3 µm	Up to 8 µm	3–4 µm	3 µm
Glochidium width of anchor tip	4–5 µm	8–10 µm	8–9 µm	6–7 µm
Total fluke length	Up to 3 µm	Up to 6 µm	Up to 10 µm	Up to 6 µm
Shape of glochidia fluke tips	Not recurved	Straight, typically not recurved	Typically recurved	Straight, typically not recurved
Attachment of glochidia stalks to massulae	Stalk width almost unchanged at attachment area	Stalk width widening	Stalk expanding to wide attachment	Stalk width widening slightly
Hairs on lower glochidia stalks (length)	Absent	Sometimes present (2–5 µm)	Often present (3–4 µm)	Absent
Hairs on massula surface (length)	1–2 µm	2–5 µm	3–4 µm	Absent
Structure massulae	Spongy	Spongy	Microspores filling massula, obscuring minimal spongy tissue	Spongy

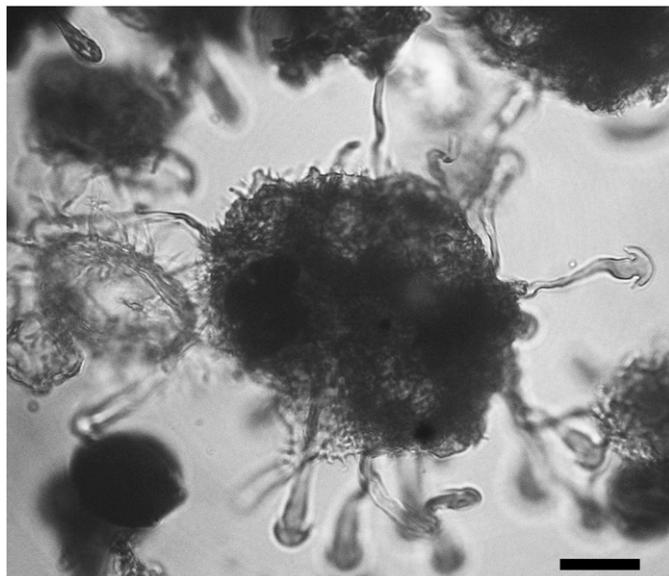


Fig. 4. LM of microspore massula of *Azolla jutlandica* showing hairs on massula surface, glochidia attachment areas and lower glochidia stalk. Statoil 6507/8–4 slide 1; EF: Q 23–4. Scale bar: 20 μ m.

5.3. Fossil species with nine floats

All species with nine floats have an upper tier of three floats and a lower one of six floats, as does *Azolla astroborealis*. It is only necessary to compare this species with other species lacking filosum. There is a slight chance that *Azolla nova* may have had more than six floats and hence we also compare it to some nine-floated species. *Azolla primaeva* has previously been interpreted as possibly having nine floats (see section on three-floated species). However, *A. astroborealis* is completely different from *A. primaeva* in its gross morphology with its megaspore not covered by a filosum.

Azolla tegeliensis Florschütz emend. Bertelsen, 1972 and *Azolla aspera* Dorofeev, 1963 lack glochidia. *Azolla nana* Dorofeev, 1959 differs in its lack of a collar and glochidia. Furthermore, its megaspores are relatively small (only c. 160 μ m in diameter) and have a granulose perispore. These characters also distinguish it from *Azolla astroborealis*.

The Oligocene *Azolla prisca* Reid and Chandler emend. Fowler, 1975 has a cap that covers only the upper tier of floats in the megaspore apparatus, the lower float tier is always visible. The same applies for *Azolla turgaica* Dorofeev, 1959, *Azolla pseudopinnata* Nikitin, 1957, *Azolla sibirica* Dorofeev, 1959, *Azolla parapinnata* Dorofeev, 1963, *Azolla aspera* Dorofeev, 1963 and *Azolla suchorukovii* Dorofeev, 1968a. In this respect, they are quite similar to *Azolla astroborealis*, but differ clearly from *Azolla nova*. *Azolla prisca* is much larger (megaspore apparatus average 455 μ m long) than *A. nova*, but *A. astroborealis* has a more or less similar length; however, *A. prisca* megaspores are 200–250 μ m wide while those of *A. astroborealis* are c. 300 μ m wide. The distal surface of the megaspores in *A. prisca* carries a large number of large excrescences, which are formed from the endoperine (see Fowler, 1975) unlike those in *A. astroborealis*.

The Oligocene *Azolla suchorukovii* Dorofeev, 1968a has a larger megaspore apparatus (up to 650 μ m long) than *Azolla astroborealis* (up to 450 μ m long); it is similar in having some distal excrescences on the megaspore itself, but no further details of the megaspore ornamentation are known, so a detailed comparison cannot be made. The megaspore apparatus of *Azolla sibirica* is the largest so far known (600–900 μ m long) and is thus clearly much larger than any of the species described here (Dorofeev, 1959). That of *Azolla aspera* is just a little shorter (up to 800 μ m long) and the whole of the megaspore wall seems to bear small excrescences (see Dorofeev, 1963, fig. 10)

unlike *A. astroborealis*. The megaspore apparatus of *Azolla parapinnata* Dorofeev, 1963 has more or less the same length as that of *A. astroborealis*, but the megaspore itself is much smaller (only up to 200 μ m wide versus 300 μ m). Moreover, the megaspore wall seems to be covered with small excrescences (see Dorofeev, 1963, fig. 9, 3), but no details of the surface ornamentation are known. *Azolla pseudopinnata* seems to be comparable to *A. parapinnata* (see Dorofeev, 1963), but has much more filosum coming out from the float area and the collar, which covers the upper part of the megaspore, in contrast to *A. astroborealis*.

The Oligocene *Azolla turgaica* Dorofeev, 1959 has a small cap that covers only the upper tier of floats. The float area is smaller than the megaspore, contrary to most of the Russian species (Dorofeev, 1959, 1963). Excrescences might be present on the megaspores but they are not clearly illustrated nor mentioned in the diagnosis, the surface ornamentation is described as reticulate. It differs in this aspect from *Azolla astroborealis*. *Azolla turgaica* has attached massulae that lack glochidia. *Azolla nana* Dorofeev, 1959 is quite similar but has a smaller megaspore apparatus (up to 300 μ m long) and smaller megaspores (maximum 160 μ m wide), and is thus considerably smaller than *A. astroborealis*.

Azolla ventricosa Nikitin, 1965 has been recorded from Oligocene and Miocene floras in Siberia and Europe. Like all Russian species with nine floats, it has a relatively small cap covering only a part of the floats. In *Azolla nova*, the float area is very small, but the float area in *A. ventricosa* occupies at least half of the megaspore apparatus (see also Friis, 1977). The species resembles *Azolla astroborealis* more in this respect and also in having no filosum covering the megaspore, but *A. ventricosa* differs from *A. astroborealis* in lacking excrescences on the megaspore. Moreover, no collar has been described so far for *A. ventricosa* (see Friis, 1977 fig. 10), whereas it is clearly present in *A. astroborealis* (Plate V, 1, 3; Plate VI, 3), where it is formed by the exoperine (a character not described so far from any other *Azolla* species).

The Oligocene–Miocene *Azolla nikitinii* Dorofeev, 1955 has slightly smaller megaspores (diameter c. 200 μ m) than *Azolla nova*, with a distinct collar and excrescences only near the distal pole of the megaspore ('tubercles' in Friis, 1977). The usual sculpture of the megaspore wall consists of verrucae and/or rugulae. No massulae have been described. In all these respects, it resembles *Azolla astroborealis*. However, it differs in the shape of the distal excrescences. In *A. nikitinii* these are 10–20 μ m wide and up to 20 μ m high, consisting of baculate, more or less parallel elements (see fig. 6G in Friis, 1977), while those of *A. astroborealis* are c. 15–20 μ m high and consist of radiating hair-like elements, giving the excrescence the appearance of a volcano (see Plate V, 6, 9). Vanhoorne (1992) records larger measurements for *A. nikitinii* megaspores from the Tongrian of Belgium (megaspore apparatus c. 370 μ m long and megaspore diameter c. 270 μ m). He stated that the base of the distal excrescences have an average diameter of 31 μ m (14.5–47.9 μ m) and a height of 17.5 μ m (10–26 μ m), thus being considerably wider than in *A. astroborealis*. Moreover, the usual sculpture of the megaspore wall is distinctly verrucate to rugulate (Vanhoorne, 1992 pl. 2, 4), and in this respect much coarser than in *A. astroborealis*.

The Miocene *Azolla ucrainica* Dorofeev, 1968b is a maximum of 380 μ m long, usually has only three floats and also has a very large, broad, visible collar encircling the megaspore apparatus and thus differs from *Azolla astroborealis*. Neogene *Azolla sulaensis* Dorofeev, 1968b and *Azolla parvula* Dorofeev, 1968b both have nine floats in two tiers and are similar in size to *A. astroborealis*. However, in both the float system occupies half to two thirds of the megaspore apparatus and the proximal pole is distinctly pointed, not flattened.

Azolla roemoensis Bertelsen, 1974, from the Upper Miocene of Denmark, has larger megaspore apparatuses (445–572 μ m versus 250–350 μ m in *Azolla nova* and 400–450 μ m in *Azolla astroborealis*) and megaspores (292–368 μ m versus c. 300 μ m in *A. nova* and *A. astroborealis*), and a baculate megaspore sculpture (more rugulate with papillae on the rugulae in *A. nova* and *A. astroborealis*). Moreover, clear excrescences have not been described.

Azolla antiqua Dorofeev, 1959 is the most similar in the group of nine-floated *Azolla* species to *Azolla nova* in having excrescences on the megaspore (and thus differing clearly from *Azolla nuda* megaspores). The *A. antiqua* megaspore (apparently meaning megaspore apparatus) is described (in translation) as ellipsoidal or egg-shaped and horizontally cut off or blunt at the top. *Azolla antiqua* has nine floats in two tiers, three upper larger and six lower smaller. The number of lower floats in individual specimens ranges from eight to nine but in a typical assemblage there are six. From the illustrations (Dorofeev, 1959, fig III, 2–5) the float zone is a flattened dome shape. No microspore massulae were observed in *A. antiqua*. *Azolla nova* differs in having a float system in which individual floats are hard to distinguish. *Azolla antiqua* differs from *Azolla astroborealis* in having numerous excrescences on the megaspore surface while *A. astroborealis* only has a few large ones on the distal surface. Moreover, all floats in *A. antiqua* are covered by a cap (Dorofeev, 1959).

5.4. Multi-floated fossil species

Azolla nuda is multi-floated and does not show any large excrescences on its megaspore. Thus we can limit our comparison to a couple of species, excluding e.g., a close comparison with respect to *Azolla teschiana* Florschütz emend. Batten and Collinson, 2001 and *Azolla bulbosa* Snead emend Sweet and Hills, 1976.

Azolla arctica differs clearly in its megaspore ornamentation (see Collinson et al., 2009); a rugulate, undulating, punctate to foveolate exoperine surface (with fusions at several levels) in *A. arctica*, contrary to a rugulate exoperine surface in *Azolla nuda* with narrow rugulae with small (c. 1 µm) papillae. Moreover, *A. nuda* massulae are readily distinguishable from those of *A. arctica* by their completely smooth surface. *A. nuda* glochidia are 30–75 µm long while those of *A. arctica* are in two classes (20–30 µm and 45–85 µm).

Azolla barbata Snead, 1969 has coiled glochidia lacking anchor-shaped tips; in *Azolla extincta* Jain, 1971 the microspore massulae lack glochidia; *Azolla distincta* Snead, 1969 lacks the distal dilation on the glochidia and floats were easily detached from the megaspore; *Azolla boliviensis* Vajda and McLoughlin, 2005 has up to 30 floats in three tiers. The translucent massulae have short (16–30 µm) glochidia with recurved tips (up to 75 µm in *Azolla nuda*). It is similar to *A. nuda* in having a massula surface without any hairs.

Azolla schopfii Dijkstra emend. Batten and Collinson, 2001 has very little suprafilosum, almost entirely restricted to the zone between the floats, and the megaspore surface is ornamented with discrete verrucae to clavae, which are grouped around depressions to form a reticulum. No cap has been recorded. The massula surface appears to be smooth as in *Azolla nuda*; glochidia are rare and short (up to 30 µm), this in contrast to *A. nuda*.

Azolla colwellensis Collinson, 1980 differs from *Azolla nuda* in having 18 (to 24) clearly defined floats and an extensive infrafilosum derived from a columnar to coarsely rugulate exoperine. The massula surface is covered with hairs, the glochidia have recurved hooks and are sometimes septate.

Azolla velus (Dijkstra) Jain and Hall emend. Batten and Collinson, 2001 is superficially similar to *Azolla nuda* as the megaspore apparatus is totally covered by a suprafilosum. However, the exoperine surface is smooth and almost flat with large rounded holes (1–5 µm in diameter) forming a 'reticulum' (see Batten and Collinson, 2001).

Azolla anglica Martin has septate glochidia, which differ from *Azolla nuda* but it is difficult to assess the importance of septae (Collinson, 1980) as both septate and non-septate glochidia are sometimes found on the same massula in modern *Azolla*. *Azolla anglica* glochidia usually have recurved flukes (Collinson et al., 2013) which differs from *A. nuda*. *Azolla anglica* has up to 24 floats in three tiers. However, in strong contrast to *A. nuda*, it has a very limited filiosum such that the exoperine is always revealed, infrafilosum is rare but consistently present (Martin, 1976; Collinson et al., 2013). The exoperine surface is

very different from that of *A. nuda*. *Azolla anglica* has a reticulate foveolate exoperine surface, foveolae have large lumina (up to 3 µm diameter) and are surrounded by broad, rounded muri up to 6 µm wide (Collinson et al., 2013).

Azolla montana Hall and Swanson emend. Jain and Hall has 15–20 small floats (exceptionally, there are ten) in two tiers and descriptions in Hall and Swanson (1968), and in Jain and Hall (1969) indicate that the filiosum is sometimes completely missing. The megaspore surface of *A. montana* is rugulo-reticulate formed of variously fused clavae; the areolae of the reticulum are 1–3 µm wide. This also differs from *Azolla nuda*, where the wall is rugulate with small papillae on the rugulae.

Azolla stanleyi Jain and Hall was described in detail with SEM and TEM observations by Hoffmann and Stockey (1994). The megaspores are very similar to those of *Azolla nuda* having an extensive persistent suprafilosum. They have more than 15 floats roughly organized in three tiers. Hoffmann and Stockey (1994) stated that the megaspore apparatus also has an infrafilosum arising from the exoperine. If correct, *A. stanleyi* would differ from *A. nuda* in this respect, but the SEM illustrations do not demonstrate an extensive infrafilosum, so we do not consider this character appropriate for differentiation. Hoffmann and Stockey (1994) described the exoperine surface as rugulo-reticulate. In our opinion a more appropriate description would be rugulate, and foveolate to fossulate, because the distance between the lumina is much greater than their breadth (rugulae up to 7 µm in width), the holes are 1 µm or more in diameter and some are elongate (e.g., 1 µm × 5 µm; see Collinson et al., 2009). Thus, the exoperine ornamentation is different from that in *A. nuda* with its narrow rugulae covered by tiny papillae.

The megaspore apparatus of *Azolla areolata* Sweet and Hills is larger (mean 479 µm) than that of *Azolla nuda*. *Azolla areolata* usually lacks exoperine excrescences but they do occur rarely (Sweet and Hills, 1976, fig. 62) (they are lacking in *A. nuda*); the exoperine surface of *A. areolata* has large perforations thus differing from that of *A. nuda*. Some glochidia of the microspore massulae in *A. areolata* have recurved hooks (fig. 74 in Sweet and Hills, 1976) just as in *A. nuda*, but the glochidia are much shorter (mean 38 µm) than in *A. nuda* (30–75 µm).

6. Stratigraphic distribution

In the Arctic Ocean Hole IODP 302-4A only *Azolla arctica* is present and it occurs throughout the *Azolla* interval (Brinkhuis et al., 2006; Collinson et al., 2009; Barke et al., 2012), which spans the upper part of Magnetochron C22n to the lower part of Chron C21r, ~ 49.3 to 48.1 Myr ago (Speelman et al., 2009; timescale according to Gradstein et al., 2004).

Within the *Azolla* interval, ODP 151-913B and 104-643A from the Nordic Sea can be correlated using the magnetostratigraphy of Eldrett et al. (2004) with the top of Chron C22n taken as a timeline (Fig. 2). In the subsequent text we refer to the part of the *Azolla* interval in Chron C22n as the lower part of the *Azolla* interval and that in C21r as the upper part of the interval.

In ODP 151-913B in the Norwegian Sea, *Azolla arctica* occurs in both the lower and upper part of the *Azolla* interval (spanning the upper Chron C22n and lower Chron C21r). In the lower part of the *Azolla* interval, *A. arctica* occurs in one sample from Core 55, namely ODP 151-913B 55R CC (core catcher sample), but core was not recovered from most of the interval from core 55 to near the top of 51. The species occurs (Fig. 2) from the uppermost part of Core 51 upwards to the top of Core 46 (with small sampling gaps in Cores 46 and 47, a core recovery gap in Core 48 and a small one in Core 46 and many samples where the species is absent in Core 50). *Azolla jutlandica* co-occurs with *A. arctica* from the top of core 49 across the Chron C22n/C21r boundary upwards through to the top of core 46, however, mainly in low percentages (1–8%), but it reaches 15–44% in three samples and even 70% in one sample.

Based on the LM massula counts in palynology slides *Azolla nuda* occurs only in the lower part of the *Azolla* interval (in Cores 50 and 49 with two outlier occurrences, one in Core 55, core catcher, and another in the top Core 51). In the massula counts *A. nuda* does not co-occur in time with *Azolla jutlandica*. However, in the mesofossil samples there are four specimens where massulae of *A. nuda* are attached to megaspore apparatuses in Core 47R Section 1W Interval 70–72 cm, 684.4 mbsf (1 specimen) and Core 48R Section 2W Interval 70–72 cm, 694.7 mbsf (three specimens) (Plate III, 1,5). One other sample from ODP 151-913B was also examined for mesofossils (Core 47R Section 3W Interval 120–122 cm, 687.9 mbsf) and contains a megaspore apparatus that is poorly preserved but on which the exoperine ornament is most similar to *A. nuda*. *Azolla nuda* massulae were not present in the palynology slides at these three sample levels. These samples are from various positions in Chron C 21r (Fig. 2) and so might perhaps be from the earliest middle Eocene.

In ODP 104-643A in the Norwegian Sea, all the samples studied come from an interval assigned to Magnetochron C22n and therefore represent the lower part of the *Azolla* interval. *Azolla arctica* occurs intermittently throughout the interval studied but there is a large core recovery gap in Core 59. *Azolla nova* occurs only in Core 62 (but very consistently in 7 out of 8 samples studied), in the lower part of the interval studied and hence only in the lower part of the *Azolla* interval. Both *A. nova* and *Azolla nuda*, therefore, occur at different sites in the lower part of the *Azolla* interval.

7. Discussion and wider implications

7.1. Species recognition for Arctic and Nordic *Azolla*

A certain amount of *Azolla* material throughout the whole *Azolla* interval is not assignable to any species. This applies to damaged massulae on which neither intact glochidia nor undamaged surface (essential for identification of presence or absence of hairs and their length) are present. Concerning the megaspore apparatuses, only material that was not covered by persistent sedimentary material could be identified.

The floats in both *Azolla nova* and *Azolla astroborealis* are pseudovacuate. They are compressed in *A. nova* and not compressed in *A. astroborealis*. In *Azolla nuda* they could not be observed properly.

If the megaspore apparatus is covered by a filosum, then the width of the filosum hairs and the shape of the megaspore apparatus are the only available diagnostic characters (Table 1). In all probability most newly found specimens could be identified using a combination of these two characters. In *Azolla nova*, *Azolla jutlandica* and *Azolla nuda* the megaspore exoperine surfaces are rather similar. All combine rugulae and papillae/baculae with only differences in the width of rugulae (twice as broad in *A. nuda* compared with the other two species) and relative abundance of papillae/baculae as possible differentiating characters. In the early stages of our work, when testing for other occurrences of *Azolla arctica*, a number of *Azolla* megaspore apparatuses were dissected to leave just the megaspore prior to the SEM study. In the Statoil 6506 Well these included specimens with a range of baculate to papillate ornaments (Plate VII, 1–3), further indicating the difficulty of species recognition based on exoperine surface alone. A detailed study of a large sample size of dissected megaspores would be necessary to establish if there are morphological breaks in these ornamentation patterns. In the absence of such a study, megaspores with papillate to baculate ornaments cannot be identified to species based on exoperine surface alone. However, if exoperine surface detail is combined with presence and distribution of excrescences, then most specimens can be identified (an exception would be a possible *A. jutlandica* with regularly arranged excrescences).

In palynological preparations the massula characteristics enable species recognition. *Azolla nova* and *Azolla jutlandica* are similar in having long hairs (up to 4 or 5 μm) on the massula and glochidia

bases, but can nevertheless be distinguished as shown in Table 2 using dimensions of glochidia structures and massula structure. *Azolla arctica* has only short hairs (up to 2 μm) and *Azolla nuda* lacks hairs on the massulae. In addition, *A. arctica* is characterised by having thin glochidia with much smaller tips than those of the other species.

7.2. Comments on systematically important characters in *Azolla*

Massula and glochidia base surface hairs

The four Arctic and Nordic Sea species, of which the massulae are known, fall into three clear morphologies with respect to massula surface hairs. *Azolla nova* and *Azolla jutlandica* both have long hairs. *Azolla nuda* lacks hairs and *Azolla arctica* has only short hairs. This character is rarely mentioned in the diagnoses of other species, although it can be seen on some published images (see Section 5). These massula surface hairs and glochidia base hairs therefore represent an undervalued diagnostic criterion, worthy of more consideration in *Azolla* taxonomy. This feature is of additional significance because it can be observed in material in palynological slides and thus will be of value for species recognition in applied stratigraphic studies. In this context, it is important to emphasise that a lot of care is needed to ensure recognition of surface hairs under LM using a $\times 40$ objective, especially if the hairs are short, thin or sparse. It is essential to study both the profile all around the specimen and the surface, and to use focus carefully because of the thickness of the massula (much thicker than a typical palynomorph). With normal to good preservation, and some experience, these characters are easy to observe.

Recurved hooks

Recurved hooks are often used as a diagnostic criterion for *Azolla* species but, having examined a large number of specimens of *Azolla jutlandica* and the new species *Azolla nuda* and *Azolla nova*, it is clear that, at least in these species, the occurrence of recurved tips is variable. Up to 25% of all specimens could be considered atypical for the species i.e., those with glochidia lacking recurved hooks in *A. nova*, or those having recurved hooks in *A. jutlandica* and *A. nuda*. Therefore, not only the presence or absence of recurved tips, but also any variation in this character should be stated along with the number of massulae examined and the method of study (LM, SEM or both).

7.3. The distinctive nature of *Azolla astroborealis*

The combination of 3D preservation, detailed tissue preservation of the pseudovacuate floats, the clear presence of nine floats, the exoperine strands radiating from the tips and down the sides of the excrescences, and excrescences that are formed mainly from exoperine, makes *Azolla astroborealis* totally different from any other species from the Arctic and Nordic Seas. There are only four specimens of this species from a single sample at a single site in the western Arctic. However, apart from the assemblages of *Azolla arctica* and *Azolla jutlandica* (Collinson et al., 2009, 2010, 2013), in which numerous megaspore apparatuses were available for study, we have only been able to study small numbers of megaspore apparatuses (<133 in total, varying from 3 to 19 at each site). Therefore, it cannot be excluded that larger assemblages may yield *Azolla astroborealis* from other sites. However, although the microspore massulae are not known for this species, all recognizable microspore massulae encountered (17 sites, >100 samples studied in palynological slides) can be assigned to one of the other four Arctic and Nordic species. It, therefore, seems likely that *A. astroborealis* had a geographically restricted distribution.

7.4. Stratigraphic distribution

Azolla nova shares a number of characters with *Azolla jutlandica*, and *A. nova* probably occurs earlier in ODP 104-643A than *A. jutlandica* in ODP 151-913B. These two species might be two parts of an evolving lineage, which spread from the area around ODP

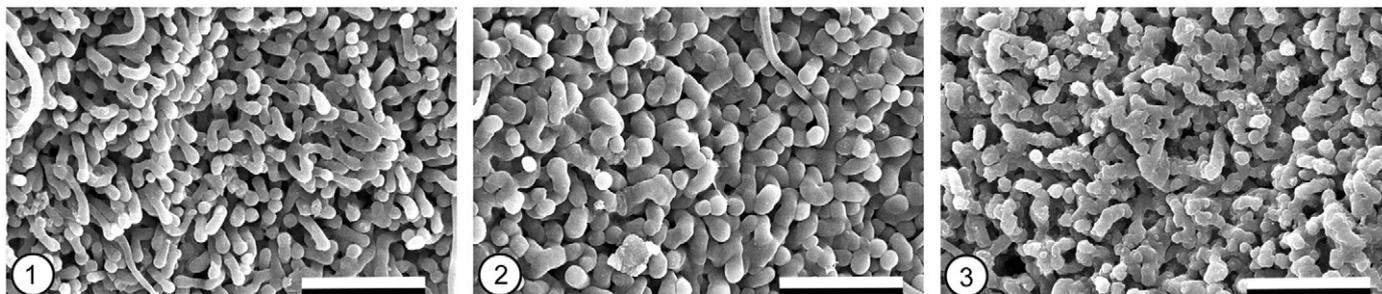


Plate VII. Three exoperine surfaces from different unidentified *Azolla* dissected megaspores showing ornamentation of the megaspore wall varying from baculate to papillate; SEM; Statoil 6506; U23542.

1. Baculate ornamentation.
 2. Mixed baculae and papillae.
 3. Mainly papillate ornamentation.
- Scale bars: 1–3: 10 μ m.

104-643A to that around ODP 151-913B as a result of dispersal, and whose morphology modified as a consequence of vicariance and dispersal of a small gene pool (such as a few fragments of *Azolla* leafy shoots). In the Statoil, Sandpiper, Ellice and Upluk Wells, *Azolla arctica* can be found with either *A. jutlandica* or *A. nova* but never co-occurring with both – supporting the indication from ODP 151-913B and 104-643A that these two species were not contemporaneous. However, the time correlation of ODP 151-913B and 104-643A is uncertain below the single timeline available, so it is not possible to draw a precise conclusion concerning a possible contemporaneous occurrence of these two species.

Azolla species co-occur in the Eocene without interbreeding (at least as far as can be judged from the morphology of the reproductive structures). Modern *Azolla* hybrids have been produced in the laboratory (Zimmerman et al., 1991), showing that the genus can hybridise; however, we are not aware of any papers examining morphological variation in megaspore or microspore massulae across hybrids and their parents, nor of any wild hybrid occurrences. This suggests that *Azolla* does not hybridise in the wild and it is likely that it has not done so at least since the middle Eocene.

More than one species of *Azolla* co-exist and thrive at ODP 151-913B and 104-643A over prolonged intervals. Two species of *Azolla* also co-exist in some samples from the Sandpiper, Ellice, Upluk, and Statoil Wells 6608, 6507, 6506, 6406 and 6302. This suggests that conditions were particularly favourable for extensive growth of *Azolla* across a wide geographic area from the western Arctic to the southern Norwegian–Greenland Sea. In this context it must be noted that in one sample (ODP 151-913B Core 48R Section 1W Interval 20–22 cm) a high concentration of massulae of both *Azolla arctica* and *Azolla jutlandica* is present. About half of this material consists of massula aggregates, representing partial or complete microsporangia. This mixture rules out the possibility of transport-induced sorting as this ought to have resulted in sorting by size. Furthermore, many (unidentifiable) fragments of megaspore apparatuses and some more or less complete megaspore apparatuses of *A. jutlandica* are present. Therefore, we conclude that it is very likely that this sample is a record of local vegetation over the Norwegian Sea, comparable with the occurrence of *Azolla arctica* in the Arctic Ocean.

The western Arctic Well samples (Sandpiper, Northstar and Ellice) and Statoil 6610/2-1S contain only *Azolla nova* with *Azolla arctica*. Extrapolating from the stratigraphic occurrence of *A. nova* in ODP 104-643A indicates that these samples are all from the lower part of the *Azolla* interval.

8. Conclusions

Three new species of *Azolla* are described, bringing the total of known species from the Eocene Nordic and Arctic Seas to five. The

new species are characterized by their megaspore apparatuses as well as by their microspore massulae. The megaspore apparatus of *Azolla astroborealis* is very distinct compared with other species in being uncompressed and having nine distinct pseudovacuate floats, but microspore massulae are unknown. The microspore massulae of the other four species are readily distinguishable in palynological slides and in SEM. Among a number of systematically useful features, massulae are characterized by the presence/absence and length of surface hairs, which have proven to be a very important, but previously overlooked, character. Recurved flukes on glochidia tips have also been used in *Azolla* taxonomy, but this study shows that their presence or absence alone gives insufficient information. In addition data are needed on variation in this character in a large population.

The new species *Azolla nuda* occurs only in ODP 151-913B in the Norwegian–Greenland Sea, *Azolla astroborealis* only in the Northstar 1 Well in the western Arctic while *Azolla nova* occurs in various sites from the western Arctic and the northern Norwegian–Greenland Sea, but is absent from the southern Norwegian–Greenland Sea and the North Sea sites. *Azolla arctica* occurs in both ODP 151-913B and 104-643A, but *Azolla nuda* and *A. nova* are each present at only one of these two sites in spite of their close proximity in the Norwegian–Greenland Sea and their overlapping stratigraphic ranges.

The stratigraphic distribution of *Azolla* species has been investigated in holes IODP 302-4A (central Arctic Ocean) and ODP 151-913B and 104-643A (Norwegian–Greenland Sea). Of the five species, *Azolla arctica* occurs throughout the *Azolla* interval. *Azolla nova* is restricted to the lower part of the *Azolla* event in Chron C22n. *Azolla nuda* is mainly restricted to the lower part, but has a few occurrences (of megaspore apparatuses with attached massulae) in the upper part. *Azolla jutlandica* is absent from the earliest part of the *Azolla* interval in lower Chron C22n, but continues through into the upper part of the *Azolla* interval in Chron C21r. The stratigraphic position of *Azolla astroborealis* within the *Azolla* interval is not exactly known.

Azolla jutlandica co-occurs with *Azolla arctica* through most of ODP 151-913B spanning the Chron C21r to C22n boundary. *Azolla nuda* co-occurs with *A. arctica* but not with *A. jutlandica* in this core. *Azolla nova* and *A. arctica* co-occur through a short time interval in ODP 104-643A within Chron C22n. Individual samples from other sites also contain more than one species. These co-occurrences suggest very favourable growth conditions for *Azolla* for a long time interval around the Arctic and Norwegian–Greenland seas. High numbers of aggregated microspore massulae indicate in situ growth of *Azolla* on the ocean surface during at least one time interval in the Norwegian–Greenland Sea at the site of hole 151-913B.

Acknowledgements

We would like to thank J. van Tongeren in Utrecht and B.J. van Heuven in Leiden for technical support with the SEM work; and T. Brain in Kings College London for his extensive support with both TEM and SEM work during this study. We thank L. Bik and N. Welters for their support. We are indebted to A.F. Lotter and P. K. Bijl (LPP Foundation) for their support and discussion during this project,

and the latter especially for the drafting of Fig. 2. We thank Jerry Hooker for very helpful discussion of the Ypresian/Lutetian GSSP. The samples and accompanying data for *Azolla arctica* (Collinson et al., 2009), were provided by the Integrated Ocean Drilling Program (IODP). We thank Statoil and the Canadian Geological Survey for providing samples for this study, especially Jonathan Bujak and Martin Pearce. We thank the Darwin Centre and Statoil for their financial support.

Appendix A

Table 1

Counts of massulae in holes ODP 913B (left) and ODP 643A (right) (in percentages). In cases of very low amounts of material present the actual counts are added within brackets.

Sample	Species			Sample	Species	
	<i>A.arctica</i>	<i>A.jutl.</i>	<i>A.nuda</i>		<i>A. arctica</i>	<i>A. nova</i>
45R 1W 20–22	–	–	–			
45R 1W 70–72	–	–	–			
45R 2W 20–23	–	–	–			
45R 2W 70–72	–	–	–			
45R 2W 120–122	–	–	–			
46R 1W 20–23	100	–	–			
46R 1W 70–72	89	11	–			
46R 1W 120–122	100	–	–			
46R 2W 20–23	100	–	–			
46R 2W 70–72	100(2)	–	–			
46R 2W 120–122	100(1)	–	–			
46R 3W 20–24	100(7)	–	–			
46R 3W 70–72	100	–	–			
46R 3W 120–122	100	–	–			
47R 1W 20–23	98	2	–			
47R 1W 70–72	92	8	–			
47R 1W 120–122	95	5	–			
47R 2W 20–23	99	1	–			
47R 2W 70–72	85	15	–			
47R 2W 120–122	99	1	–			
47R 3W 20–24	100	–	–			
47R 3W 70–72	99	1	–			
47R 3W 120–122	100	–	–			
48R 1W 20–22	30	70	–			
48R 1W 70–72	56	44	–			
48R 2W 20–23	83	17	–			
48R 2W 70–72	93	7	–			
48R 2W 120–122	99	1	–			
49R 1W 20–22	99	1	–	59 1W 6–8	100(3)	–
49R 1W 70–72	97	3	–	59 CCW	–	–
49R 1W 120–122	98	2	–	60 1W 110–112	100(2)	–
49R 2W 20–23	98	2	–	60 2W 56–58	100	–
49R 2W 70–73	92	–	8	60 3W 3–5	100	–
49R 2W 120–122	98	–	2	60 CCW 22–24	–	–
49R 3W 20–22	92	–	8	61 1W 32–35	–	–
49R 3W 70–72	96	–	4	61 1W 70–75	–	–
49R 3W 120–122	44	–	56	61 1W 113–116	100	–
49R 4W 20–23	15	–	85	61 CCW 13–16	–	–
49R 4W 70–72	25(1)	–	75(3)	61 CCW 23–25	–	–
49R 4W 120–122	25	–	75	61 CCW 33–36	100	–
49R 5W 20–23	–	–	100	61 CCW 43–46	–	–
49R 5W 70–73	–	–	100	62 1W 2–5	62	38
49R 5W 120–123	–	–	100	62 1W 12–15	15	85
50R 1W 20–25	–	–	100(5)	62 1W 22–25	100(1)	–
50R 1W 66–68	–	–	100	62 1W 52–55	–	100(1)
50R 2W 20–23	–	–	100	62 1W 62–65	12	88
50R 2W 70–72	–	–	–	62 1W 72–75	–	–
50R 2W 120–122	–	–	–	62 1W 82–85	–	–
50R 3W 20–23	–	–	100(5)	62 1W 92–95	–	–
50R 3W 70–72	–	–	–	62 1W 102–105	1	99
50R 3W 120–122	–	–	100	62 1W 112–115	–	100
50R 4W 20–23	–	–	100	62 1W 123–125	5	95
50R 4W 70–72	–	–	100	62 1W 131–134	–	–
50R 4W 120–123	–	–	100	62 CCW 10–12	–	–
51R 1W 0–5	86	–	14	62 CCW 20–22	–	–
55R CCW 0–5	98	–	2	62 CCW 30–32	–	–

References

- Akhmetiev, M.A., Zaporozhets, N.I., Benyamovskiy, V.N., Aleksandova, G.N., Iakovleva, A.I., Oreshkina, T.V., 2012. The Paleogene history of the Western Siberian seaway – a connection of the Peri-Tethys to the Arctic Ocean. *Austrian Journal of Earth Sciences* 105, 50–67.
- Arnold, C.A., 1955. A Tertiary *Azolla* from British Columbia. *Contributions from the Museum of Paleontology, University of Michigan*, XII, 4 37–45.
- Barke, J., Van der Burgh, J., Van Konijnenburg-van Cittert, J.H.A., Collinson, M.E., Pearce, M.A., Bujak, J., Heilmann-Clausen, C., Speelman, E.N., Van Kempen, M.M.L., Reichart, G.-J., Lotter, A.F., Brinkhuis, H., 2012. Coeval Eocene blooms of the freshwater fern *Azolla* in and around Arctic and Nordic seas. *Palaeogeography, Palaeoclimatology, Palaeoecology* 337–338, 108–119.
- Batten, D.J., Collinson, M.E., 2001. Revision of *Minerisporites*, *Azolla* and associated plant microfossils from deposits of the Upper Palaeocene and Palaeocene/Eocene transition of the Netherlands, Belgium and the USA. *Review of Palaeobotany and Palynology* 115, 1–32.
- Bertelsen, F., 1972. *Azolla* species from the Pleistocene of the central North Sea area. *Grana* 12, 131–145.
- Bertelsen, F., 1974. Late Tertiary *Azolla* species from Rømø, SW Denmark. *Danmark Geologiske Undersøgelser, Årbog* 1973, 15–25.
- Brinkhuis, H., Schouten, S., Collinson, M.E., Sluijs, A., Sinninghe Damste, J.S., Dickens, G.R., Huber, M., Cronin, T.M., Onodera, J., Takahashi, K., Bujak, J.P., Stein, R., Van der Burgh, J., Eldrett, J.S., Harding, I.C., Lotter, A.F., Sangiorgi, F., Van Konijnenburg-van Cittert, J.H.A., De Leeuw, J.W., Matthiessen, J., Backman, J., Moran, K., the Expedition 302 Scientists, 2006. Episodic fresh surface waters in the Eocene Arctic Ocean. *Nature* 441, 606–609.
- Collinson, M.E., 1980. A new multiple-floated *Azolla* from the Eocene of Britain with a brief review of the genus. *Palaeontology* 23, 213–229.
- Collinson, M.E., Barke, J., Van der Burgh, J., Van Konijnenburg-van Cittert, J.H.A., 2009. A new species of the freshwater fern *Azolla* (Azollaceae) from the Eocene Arctic Ocean. *Review of Palaeobotany and Palynology* 155, 1–14.
- Collinson, M.E., Barke, J., Van der Burgh, J., Van Konijnenburg-van Cittert, J.H.A., Heilmann-Clausen, C., Howard, L.E., Brinkhuis, H., 2010. Did a single species of Eocene *Azolla* spread from the Arctic Basin to the southern North Sea? *Review of Palaeobotany and Palynology* 159, 152–165.
- Collinson, M.E., Smith, S.Y., Van Konijnenburg-van Cittert, J.H.A., Batten, D.J., Van der Burgh, J., Barke, J., Marone, F., 2013. New observations and synthesis of Paleogene heterosporous water ferns. *International Journal of Plant Sciences* 174 (3), 350–363.
- Dorofeev, P.I., 1955. Sarmatian plants from the river Tiligul and S. Bug. *Trudy Botanicheskogo Instituta Akademii Nauk SSSR Seriya 1* 11, 144–160 (in Russian).
- Dorofeev, P.I., 1959. New species of *Azolla* Lam in Tertiary flora of USSR. *Botanicheskij Zhurnal* 44, 1756–1763.
- Dorofeev, P.I., 1963. The Tertiary flora of Western Siberia. *Izd. Akad. Nauk. SSSR. Moscow and Leningrad*. 345 pp. (in Russian)
- Dorofeev, P.I., 1968a. Oligocene flora of Transuralia. *Paleontological Journal* 2, 248–255.
- Dorofeev, P.I., 1968b. On megaspores of *Salvinia*, *Azolla* and *Pilularia* from Neogene deposits of the Ukraine. *Ukrainskij Botanicheskij Zhurnal* 25, 63–72. [In Russian].
- Dunham, D.G., Fowler, K., 1978. Megaspore germination, embryo development and maintenance of the symbiotic association in *Azolla filiculoides* Lam. *Botanical Journal of the Linnean Society* 95, 43–53.
- Eldrett, J.S., Harding, I.C., Firth, J.V., Roberts, A.P., 2004. Magnetostratigraphic calibration of Eocene–Oligocene dinoflagellate cyst biostratigraphy from the Norwegian–Greenland Sea. *Marine Geology* 204, 91–127.
- Firth, J.V., 1996. Upper middle Eocene to Oligocene dinoflagellate biostratigraphy and assemblage variations in Hole 913B, Greenland Sea. In: Myhre, A.M., Thiede, J., Firth, J.V., Johnson, G.L., Ruddiman, W.F. (Eds.), *Proc. ODP, Sci. Results*, 151. Ocean Drilling Program, College Station, TX, pp. 203–242.
- Florschütz, F., Menedez Amor, J., 1960. Une *Azolle* fossile dans les Pyrénées Orientales. *Pollen et Spores* 2, 285–292.
- Fowler, K., 1975. Megaspores and massulae of *Azolla prisca* from the Oligocene of the Isle of Wight. *Palaeontology* 18, 483–507.
- Friis, E.M., 1977. EM studies on Salviniaceae megaspores from the Middle Miocene Fæsterholt flora, Denmark. *Grana* 16, 113–128.
- Gradstein, F.M., Luterbacher, H.P., Ali, J.R., Brinkhuis, H., Gradstein, F.M., Hooker, J.J., Monechi, S., Ogg, J.G., Powell, J., Röhl, U., Sanfilippo, A., Schmitz, B., 2004. The Paleogene period. In: Gradstein, F.M., Ogg, J.G., Smith, A.G. (Eds.), *A Geologic Time Scale*. Cambridge University Press, Cambridge, UK, p. 396.
- Hall, J.W., 1969. A reappraisal of the megaspores of two Eocene species of *Azolla*. *Journal of Paleontology* 43, 528–531.
- Hall, J.W., Swanson, N.P., 1968. Studies in fossil *Azolla*: *Azolla montana*, a Cretaceous megaspore with many small floats. *American Journal of Botany* 55 (9), 1055–1061.
- Hills, L.V., Gopal, B., 1967. *Azolla primaeva* and its phylogenetic significance. *Canadian Journal of Botany* 45, 1179–1191.
- Hills, L.V., Weiner, N., 1965. *Azolla geneeana*, n. sp., and revision of *Azolla primaeva*. *Micropaleontology* 11, 255–261.
- Hoffmann, G.L., Stockey, R.A., 1994. Sporophytes, megaspores and massulae of *Azolla stanleyi* from the Paleocene Joffrey Bridge locality, Alberta. *Canadian Journal of Botany* 72, 301–308.
- Iakovleva, A.I., 2011. Palynological reconstruction of the Eocene marine palaeoenvironments in south of Western Siberia. *Acta Palaeobotanica* 51, 229–248.
- Jain, R.W., 1971. Pretertiary records of Salviniaceae. *American Journal of Botany* 58, 487–496.
- Martin, A., 1976. Some structures in *Azolla* megaspores, and an anomalous form. *Review of Palaeobotany and Palynology* 21, 141–169.
- Molina, E., et al., 2011. The global stratotype section and point (GSSP) for the base of the Lutetian Stage at the Gorrondatxe section, Spain. *Episodes* 34, 86–108.
- Moore, P.P., Webb, J.A., Collinson, M.E., 1991. *Pollen Analysis*, second ed. Blackwell Scientific Publications, Oxford, UK 216 pp.
- Nikitin, P.A., 1948. Pliocene flora from the River Ob' in Tomsk Region. *Doklady Akademii Nauk SSSR* 61, 1103–1106. (in Russian).
- Nikitin, P.A., 1957. Pliocene and Quaternary floras from the Voronezh district. *Akademi Nauk. SSSR, Leningrad*. 200 pp. (in Russian).
- Nikitin, P.A., 1965. An Aquitanian seed floras from Lagernogo Sad (Tomsk). *Tomsk University, Tomsk*, 119 pp. (in Russian).
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S., Le Thomas, A., 2007. Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* 143, 1–81.
- Sahni, B., Rao, H.S., 1934. The silicified flora of the Deccan Intertrappean Series, part IV. *Azolla intertrappea* sp. nov. *Proceedings of the 21st Indian Science Congress Bombay*, pp. 318–319.
- Smith, A.R., Pryer, K.M., Schuettelpelz, E., Korall, P., Schneider, H., Wolf, P.G., 2006. A classification for extant ferns. *Taxon* 55, 705–731.
- Snead, R.G., 1969. Microfloral diagnosis of the Cretaceous–Tertiary boundary, Central Alberta. *Research Council of Alberta Bulletin* 25, 1–148.
- Speelman, E.N., Van Kempen, M.L., Barke, J., Brinkhuis, H., Reichart, G.J., Smolders, A.J.P., Roelofs, J.G.M., Sangiorgi, F., De Leeuw, J., Lotter, A.F., Sinninghe Damste, J.S., 2009. The Eocene Arctic *Azolla* bloom: environmental conditions, productivity and carbon drawdown. *Geobiology*. <http://dx.doi.org/10.1111/j.1472-4669.2009.0195.x>.
- Sweet, A.R., Hills, L.V., 1976. Early Tertiary species of *Azolla* subg. *Azolla* sect. *Kremastospora* from western and Arctic Canada. *Canadian Journal of Botany* 54, 334–351.
- Trivedi, B.S., Verma, C.L., 1971. Contributions to the knowledge of *Azolla indica* sp. nov. from the Deccan Intertrappean Series M.P. India. *Palaeontographica B* 136, 71–82.
- Vajda, V., McLoughlin, S., 2005. A new Maastrichtian–Paleocene *Azolla* species from Bolivia, with a comparison of the global record of coeval *Azolla* microfossils. *Alcheringa* 29, 305–329.
- Vanhoorne, R., 1992. *Azolla* and *Salvinia* species (Azollaceae and Salviniaceae, Pteridophyta), from the Cenozoic of Belgium. *Bulletin de l'Institut Royal des Sciences Naturelles de Belgique, Science de la Terre* 62, 229–255.
- Zimmerman, W.J., Watanabe, I., Ventura, T., Payawal, P., Lumpkin, T.A., 1991. Aspects of the genetic and botanical status of Neotropical *Azolla* species. *New Phytologist* 119, 561–566.