

8-9-2016

External morphology of the eggs of *Asplanchnopus multiceps* (Schrank, 1793) (Rotifera): solving the 150-year-old case of mistaken identity

Elizabeth S. Wurdak

College of Saint Benedict/Saint John's University, ewurdak@csbsju.edu

Follow this and additional works at: http://digitalcommons.csbsju.edu/biology_pubs



Part of the [Biology Commons](#), and the [Organismal Biological Physiology Commons](#)

Recommended Citation

Wurdak E. 2016. External morphology of the eggs of *Asplanchnopus multiceps* (Schrank, 1793) (Rotifera): solving the 150-year-old case of mistaken identity. *Hydrobiologia*. doi:10.1007/s10750-016-2831-6

This is a post-print of the publication. The final publication is available at Springer via <http://dx.doi.org/10.1007/s10750-016-2831-6>.

1 **External Morphology of the Eggs of *Asplanchnopus multiceps* (Schrank, 1793; Rotifera):**
2 **Solving the 150 year-old case of mistaken identity.**

3 Elizabeth Wurdak, Biology Department, Saint John's University, Collegeville, MN 56321, USA

4 E-mail: ewurdak@csbsju.edu, Fax: 320-363-3202

5 **Abstract**

6

7 This report calls into question the practice of passing along illustrations and anatomical descriptions
8 from the literature without scrutiny. An error made by Leydig (1854) in characterizing the egg of
9 *Asplanchnopus multiceps* was perpetuated in authoritative publications (Hyman, 1951; Voigt, 1957;
10 Ruttner-Kolisko, 1974; Koste, 1978, 1987) well into the 20th century. Daily tracking of individual
11 mictic and amictic female *A. multiceps* demonstrates that the structure formerly considered to be a
12 diapausing egg is, in fact, subitaneous. It develops without arrest into a male or female rotifer. True
13 resting eggs containing dormant embryos are characterized by a very dark interior. In the majority
14 of these eggs the interior is surrounded by a halo-like zone consisting of a clear space and an external
15 layer. The correct identification of subitaneous and resting eggs in *A. multiceps* confirms the
16 description of Plate (1885) and firmly establishes the mode of reproduction of this species as
17 oviparous. Mictic females lay haploid male eggs first, followed by resting eggs if fertilization has
18 taken place. In the absence of fertilization, mictic females lay significantly more (male) eggs than
19 amictic females lay female eggs; however, their lifespan does not differ markedly.

20

21 **Key words**

22 Oviparous, ovoviparous, subitaneous egg, resting egg, amictic, mictic

23 **Acknowledgements**

24 The author wishes to thank Dr. John J. Gilbert and Dr. William Lamberts for their careful review of the
25 manuscript.

26 **Introduction**

27 *Asplanchnopus multiceps* is a large, predatory rotifer that favors the littoral zones of
28 oligotrophic lakes and rivers. Unlike other members of the Aplanchnidae it is oviparous (Ruttner-
29 Kolisko, 1974; Nandini & Sarma, 2005). Amictic females produce eggs that develop immediately
30 upon laying into diploid females, while mictic females produce haploid ovocytes that develop into
31 males if they are not fertilized or into diploid resting eggs that enclose a dormant embryo if they are
32 fertilized. Resting eggs are capable of withstanding unfavorable environmental conditions and can
33 survive over a prolonged period of time. (Gilbert, 1974; Pourriot & Snell, 1983). They are a source of
34 genetic variability and aid species dispersal (King, 1980). Their external morphology is distinctive
35 enough that, when combined with other features, it can be used to distinguish between closely
36 related species (Gilbert & Wurdak, 1978). Several publications describe shell deposition (Mrázek,
37 1897; Tannreuther, 1920; Bogoslovsky, 1960; Wurdak et al. 1977) and early embryonic development
38 of resting eggs in oviparous (Bogoslovsky, 1929; Nipkow, 1958; de Beauchamp, 1956) and
39 ovoviviparous (Nachtwey, 1925; Lechner, 1966) monogonont rotifer species. However, the internal
40 organization of the resting-egg embryo is not fully understood and significant questions remain
41 regarding the resumption of development prior to hatching (Gilbert, 1988).

42 The current report is part of a broader study on embryonic development in rotifers with
43 particular emphasis on resting eggs. In previous publications the resting egg of *A. multiceps* was
44 presented as a sphere bearing short filaments on its surface (Ruttner-Kolisko, 1974; Koste, 1978).
45 This communication demonstrates that this fuzzy egg is, in fact, subitaneous and presents images
46 obtained through light and scanning electron microscopy of both subitaneous and resting eggs of *A.*
47 *multiceps*. The mischaracterization of the mode of reproduction and of the structure of the resting
48 egg is traced to the earliest reports. The possibility is raised that similar errors may exist in the
49 description of other rotifers leading to problems in identifying closely related and cryptic species.

50 Subitaneous and resting eggs of *A. multiceps* are convenient objects for experimentation due
51 to their large size, abundance, easy collection and handling. The development of both subitaneous
52 and diapausing egg embryos is external in this species, whereas in *Asplanchna* the two types of
53 embryos develop in different environments. Our understanding of resting egg ultrastructure and
54 internal organization in the Rotifera is very limited. Contributions in this area can address larger

55 questions related to the formation and composition of external coverings, yolk and pigment, as well
56 as, the functions of various egg components in protection, dormancy and development.

57 **Materials and Methods**

58 Adult *Asplanchnopus multiceps* females were isolated from Lake Sagatagan in Collegetown,
59 Minnesota during the summer of 2015. They were cultured under continuous light at 21°C on a diet
60 of other rotifers, principally *Philodina*. *Philodina* sp. were collected from local birdbaths and
61 maintained on an algal diet. *A. multiceps* eggs were gently removed from the bottom and sides of the
62 culture vessel using an Irwin loop and transferred to a slide. Egg diameter was measured under a
63 light microscope at 100x magnification. Live eggs and rotifers were photographed using the Leica
64 LasEZ system. Eggs and neonates were placed into individual petri dishes to permit daily
65 observation.

66 For scanning electron microscopy, eggs were rinsed 3 times in spring water. Some eggs
67 were treated with 2% bleach to remove adhering bacteria and rinsed again. Eggs were fixed in 2%
68 glutaraldehyde in .05M cacodylate buffer, pH 7.4, overnight at room temperature. The next day they
69 were rinsed in 3 changes of .1M cacodylate buffer and postfixed in 1% osmium tetroxide in .1M
70 cacodylate buffer at 4°C. They were dehydrated in alcohol, critical point dried, sputter coated with
71 gold and observed under a Hitachi S530 scanning electron microscope operated at 5 – 15kV. Images
72 were captured and processed using the QuartzPCI software.

73 **Results**

74 Figures 1 and 2 illustrate a live *A. multiceps* amictic female and male respectively. *A.*
75 *multiceps* males are roughly 2/3 the size of the females (fig. 2). Figure 3 shows a mictic female
76 carrying a developing ovocyte posterior to the stomach. There are no obvious differences between
77 amictic and mictic females in body size or shape. Their size varies with age. Mictic females can be
78 distinguished from amictic females only through observation of the eggs they produce. Male eggs can
79 be recognized by their smaller size. Until they are inseminated, mictic females produce eggs that
80 hatch into males. Once copulation takes place the female produces only resting eggs and will not lay
81 any more male eggs. Consequently, the number of male eggs produced by a given female depends on
82 how soon she is mated. If the male eggs are removed each day prior to hatching the mother will

83 continue to lay haploid male eggs until death. If a male offspring is permitted to hatch in the same
84 culture dish as its female parent he will inseminate her and initiate the development of resting eggs.
85 The number of resting eggs a given female produces depends on diet and on her age at first
86 successful copulation with a male. Typically, a female will lay one or two resting eggs in her lifetime,
87 but five resting eggs were counted in one case. In the confines of the culture dish *A. multiceps*
88 females will cannibalize conspecific males, including their own offspring and those they just mated
89 with.

90 Developing subitaneous ovocytes have medium grey contents, whereas developing resting
91 eggs, along with the adjoining portions of the vitellarium are very dark. At maturity the egg volume
92 is about the same as the volume of the full stomach. Ovocytes remain flexible until late development.
93 One to two hours prior to being laid the shell hardens, the ovocyte becomes visibly spherical and, in
94 the case of subitaneous eggs, covered with a short fuzzy external layer. All eggs are firmly attached
95 to the bottom and sides of the culture dish at the time of laying. Three classes of eggs were observed:

96 1. Large eggs with a fuzzy outer wall having an average diameter of 249 μm (N = 27, SD =
97 10.9), and moderately dense contents (Fig. 4). These eggs are diploid; they develop into
98 amictic or mictic females immediately after deposition.

99 The egg shown in figure 4 is freshly-laid and alive. Beneath the fuzzy shell there is a
100 peripheral zone containing large and small clear vacuoles. These vacuoles disappear when
101 development commences.

102 2. Small fuzzy eggs measuring 192 μm in average diameter (N = 27, SD = 19.5) shown in figure
103 5. These are unfertilized, haploid eggs that develop into males. The egg in figure 5 is in the
104 four-cell stage. There is one large cell and 3 smaller cells. This configuration is very similar
105 to developing embryos in the ovoviviparous *Asplanchna girodi* Guerne, 1888 (Lechner,
106 1966). Figure 6 illustrates the empty shell left behind by a male hatchling. The shell opens
107 into two nearly equal halves. Furrows marking the line of separation between these halves
108 are not externally detectable in intact eggs.

109 3. Large, very dark eggs whose average diameter is 259 μm (N = 28, SD = 9.32). These eggs,
110 illustrated in figure 7, contain diploid dormant embryos. The egg is nearly perfectly

111 spherical. There is a clear zone between the outer shell layer and the very dense interior in
112 the egg illustrated. The outer wall bears very short fibrils. In some instances, however, the
113 clear zone is narrower or missing between the outer wall and the interior and the outer
114 perimeter is smooth. These eggs are presumed to be at an earlier developmental stage.
115 The differences between subitaneous and resting eggs are more evident under the scanning electron
116 microscope. The fuzzy exterior of the wall of the subitaneous egg of the larger variety can be seen in
117 figure 8. Mild bleach treatment fails to remove pieces of debris stuck to the filaments. Figure 9
118 shows the circular adhesive patch that anchors the egg to the substrate. Figure 10 is a high
119 magnification image of the non-adhesive portion of the surface where filaments predominate.
120 Filaments measure around 12 μm in length and splay out into multiple rootlets at the point of
121 attachment. Judging by the variety of their configurations, the filaments are flexible. Between
122 filaments the surface is smooth and clean. Figure 11 is an image of the resting egg taken at the same
123 magnification as the subitaneous egg shown in figure 8. The wall lacks an external layer of filaments
124 altogether and appears somewhat porous. Only a portion of the adhesive patch was preserved. The
125 high magnification view in figure 12 shows both the adhesive patch and the general surface. The
126 adhesive patch is smooth, while the general surface is marked by short microvilli-like projections and
127 minute pores.

128 A comparison of the fecundity and longevity of amictic and mictic females yielded the following
129 data. Amictic females laid an average of 6.85 eggs ($N = 27$, $SD = 3.19$). The number of eggs laid by a
130 single female ranged from 1 to 15. From the egg stage until death their mean lifespan was 8.85 days
131 ($SD = 1.03$). Mictic females laid an average of 11.5 male eggs ($N = 12$, $SD = 4.68$). The number of
132 subitaneous eggs laid by a single female ranged from 5 to 20. Their lifespan averaged 8.67 days ($N =$
133 12 , $SD = 1.37$).

134 **Discussion**

135 Resting eggs of *A. multiceps* have been misrepresented in the literature as spherical objects
136 covered with short filaments (Voigt, 1957; Ruttner-Kolisko, 1974; Koste, 1978). The fuzzy eggs
137 shown in these publications are subitaneous eggs that develop without arrest into females or males.

138 The error is traceable to Leydig (1854) and Weber (1898). Voigt (1957) credits Leydig (1854) for
139 the figure of the, in his opinion, diapausing egg of *A. multiceps* presented in plate 84. The same figure
140 is reproduced in Ruttner-Kolisko (1974) and Koste (1978). Leydig (1854) describes *A. multiceps*
141 under its synonymous name: *Notommata myrmeleo* Ehrenberg, 1834. He assumed that the fuzzy
142 eggs that were laid very rapidly were diapausing. However, both his diagram and description (page
143 24) of the so-called "Wintereier" fit the subitaneous egg perfectly. Leydig did not follow up on the
144 development of these eggs. The only dissenting opinion (Plate, 1885) was disregarded by later
145 authors. Weber (1898) produced a diagram of the adult female *A. multiceps* in which he designated a
146 structure posterior to the vitellarium as an embryo. He noted on page 384 that the description was
147 based on a few poorly preserved specimens. Nonetheless his diagram was passed along with minor
148 modifications by Hyman (1951), Voigt (1957), Ruttner-Kolisko (1974), Koste (1978) and reinforced
149 the misconception that *A. multiceps* is ovoviviparous (Koste, 1987; Shiel & Koste, 1993).
150 Furthermore, investigators may have assumed that *Asplanchnopus* is ovoviviparous like other
151 members of the Asplanchnidae. The fact that three widely separated populations of *A. multiceps* that
152 were recently examined in Berlin (Schröder, T., personal communication, 1993) Mexico (Nandini &
153 Sarma, 2005) and the United States (present communication) are oviparous contradicts the
154 literature.

155 Although this report focuses on a single species, the faulty attribution of reproductive modes
156 and structures may be more widespread due to the practice of passing along diagrams that were
157 created decades or, in some cases over 100 years, previously with minor modifications from one
158 publication to another. Species, like *A. multiceps*, that are seldom encountered are particularly
159 susceptible to misrepresentation. Very dark diapausing eggs stuck to the substratum or vegetation
160 may be missed because they blend in with the sediments. It is important to identify resting eggs
161 correctly because their morphology is one of the criteria utilized in distinguishing closely related
162 species (Gilbert & Wurdak, 1978). Resting egg morphology within a species is not invariable,
163 however. In *A. multiceps* some eggs are dark all the way to the rim, while in others the dark interior
164 is surrounded by a halo consisting of a clear zone followed by a relatively thin outer coat. These
165 configurations represent different stages of development.

166 Securing the eggs to stable supports prevents them from being swept out into the open
167 water and may also facilitate the emergence of the hatchling from the shell. Freshly-laid subitaneous
168 eggs are not buoyant despite the presence of peripheral vacuoles and external filaments. If the egg is
169 detached from the bottom of the Petri dish, lifted and released it will sink back down. The same
170 holds for resting eggs. The fuzzy external coat may hinder the attachment of protozoa and fungi to
171 subitaneous eggs although infected eggs are occasionally encountered. The vacuoles in the
172 peripheral cytoplasm may be associated with the formation of the shell and/or the extrusion of the
173 filaments that cover the surface. Subitaneous and resting eggs coated with short filaments are found
174 in other rotifer genera, eg. *Epiphanes*, *Gastropus*, *Synchaeta* (Ruttner-Kolisko, 1974).

175 Daily tracking of the eggs laid by individual females in isolation failed to demonstrate the
176 existence of amphoteric females in *A. multiceps*. Amphoteric females, capable of both mictic and
177 amictic reproduction, have been identified in other species by Ruttner-Kolisko (1946 and 1977),
178 Sudzuki (1955) and Gilbert (1995).

179 In their population growth study Nandini and Sarma (2005) demonstrated that fecundity
180 and longevity in *A. multiceps* depend on diet. *A. multiceps* reared on *Plationus*, *patulus*, formerly
181 *Brachionus*, *patulus*, had an average lifespan of 6 days and a highest net reproductive rate of 11
182 offspring. It is not clear, however, whether male offspring were included in their calculations.

183 Data collected from individual amictic and mictic females in this investigation vary
184 somewhat from these values; the mean lifespan of both types of females is longer and their fecundity
185 is lower. The discrepancy may be due to the fact that, in the current study, lifespan was measured
186 from the egg stage onward, the temperature was 21°C, versus 22 – 25°C in Nandini and Sarma's
187 (2005), and the diet was not optimized. The data show that the lifespans of amictic and mictic
188 females do not differ significantly ($P = 0.642$), although their fecundity does ($P = 0.001$).

189 *A. multiceps* adult females are hardy; their eggs are large and easily collected with the aid of
190 an Irwin loop. Subitaneous eggs develop on a predictable timetable and may be followed at the light
191 and electron microscope level. The internal structure and development of diapausing eggs in rotifers
192 has not been examined fully. Major questions remain regarding tissue organization in the dormant
193 embryo. Wurdak et al. (1978) agree with Mrázek's (1897) observation that both the ectoderm and

194 inner mass are syncytial. In a fully developed individual, however, some organs, eg. the stomach,
195 consist of separate cells. The process of cell membrane restoration has not been studied. Under
196 appropriate dietary conditions, the percentage of mictic females in *A. multiceps* can reach 80% or
197 more. Consequently, a large number of diapausing eggs is available to investigate the resumption of
198 development. Due to their size and firm consistency these eggs are easily handled. Developmental
199 stages of subitaneous and resting-egg embryos can be compared in the same species as a result.
200 Resting eggs currently in cold storage will be tested to uncover the stimulus required to initiate their
201 further development. Other areas worthy of investigation include the adhesive properties of the eggs
202 and the chemical identity of stored reserves in resting eggs.

203 **References**

- 204 Bogoslovsky, A. S. 1929. Observations on rotifer ecology. Some data on the ecology of *Conochilus*
205 *volvox* Ehr., *Conochilus unicornis* R., and *Lacinularia socialis* and observations on their resting eggs.
206 Bulletin de la Station Biologique a Bolchevo 3: 7-25. (in Russian).
- 207 Bogoslovsky, A. S., 1960. Observations on the reproduction of *Conochiloides coenobasis* Skorikov and
208 the statement of a physiological category of females new to heterogonous Rotifera. Zoologicheskyy
209 Zhurnal 39: 670-677. (in Russian).
- 210 De Beauchamp, P. 1956. Le développement de *Ploesoma hudsoni* (Imhof) et l'origine des feuilletts
211 chez les rotiferes. Bulletin de la Société zoologique de France 81: 374-383. Gilbert, J. J., 1974.
212 Dormancy in rotifers. Transactions of the American Microscopical Society 93: 490-513.
- 213 Gilbert, J. J., 1983. Rotifera. In K. G. Adiyodi and R. D. Adiyodi (eds.) Reproductive Biology of
214 Invertebrates. Vol. I, part 8. Oogenesis, Oviposition and Oosorption. John Wiley and Sons Ltd.
215 Hoboken, NJ: 181-209.
- 216 Gilbert, J. J., 1988. Rotifera. In K. G. Adiyodi and R. D. Adiyodi (eds.) Reproductive Biology of
217 Invertebrates. Vol. IV, part A. Fertilization, Development, and Parental Care. Oxford and IBH
218 Publishing Company, New Delhi: 179-199.
- 219 Gilbert, J. J., 1995. Structure, development and induction of a new diapause stage in rotifers.
220 Freshwater Biology 34: 263-270.

221 Gilbert, J. J. & Wurdak, E. S., 1978. Species-specific morphology of resting eggs in the rotifer
 222 *Asplanchna*. Transactions of the American Microscopical Society 97:330-339.
 223 Hyman, L. H. 1951. The Invertebrates: Acantocephala, Aschelminthes and Entoprocta. The
 224 pseudocoelomate Bilateria, Volume III. McGraw-Hill Book Company, New York, Toronto, London.
 225 King, C. E., 1980. The genetic structure of zooplankton populations. In: Kerfoot, W. C. (Ed), Evolution
 226 and Ecology of Zooplankton Communities. University press of New England, Hanover, N. H.: 315-328.
 227 Koste, W. 1978. Rotatoria: Die Rädertiere Mitteleuropas begründet von Max Voigt. Monogononta 2.
 228 Auflage neubearbeitet von Walter Koste, Gebrüder Borntraeger, Berlin, Stuttgart: Tafel 163, fig. 2.
 229 Koste, W. 1987. Das Rädertier – Porträt. Ein Rädertier, das Krebse verschlingt: *Asplanchnopus*
 230 *multiceps*. Mikrokosmos 76: 171-175.
 231 Lechner, M., 1966. Untersuchungen zur Embryonalentwicklung des Rädertieres *Asplanchna girodi*
 232 de Guerne. Wilhelm Roux' Archiv Entwicklungsmechanik der Organismen 157: 117-173.
 233 Leydig, F. 1854. Über den Bau und die systematische Stellung der Räderthiere. Zeitschrift für
 234 wissenschaftliche Zoologie 6: 1-120.
 235 Mrázek, A., 1897. Zur Embryonalentwicklung der Gattung *Asplanchna*. Sitzungsberichte der
 236 königlich böhmischen Gesellschaft der Wissenschaften Mathematisch-Naturwissenschaftliche Classe
 237 58: 1-11.
 238 Nachtwey, R., 1925. Untersuchungen über die Keimbahn, Organogenese und Anatomie von
 239 *Asplanchna priodonta* Gosse. Zeitschrift für wissenschaftliche Zoologie 126: 239-492.
 240 Nandini, S. & S. S. S. Sarma, 2005. Life history characteristics of *Asplanchnopus multiceps* (Rotifer) fed
 241 rotifer and cladoceran prey. Hydrobiologia 546: 491-501.
 242 Nipkow, F., 1958. Beobachtungen über die Entwicklung des Dauereies von *Brachionus calyciflorus*
 243 Pallas. Schweizerische Zeitschrift für Hydrologie 20: 186-194.
 244 Plate, L. H., 1885. Beiträge zur Naturgeschichte der Rotatorien. Jenaische Zeitschrift für
 245 Naturwissenschaft 19:1-120.
 246 Pourriot, R. & Snell, T. W., 1983. Resting eggs in rotifers. Hydrobiologia. 104: 213-224.

247 Ruttner-Kolisko, A., 1946. Über das Auftreten unbefruchteter 'Dauereier' bei *Anuraea aculeata*
248 (*Keratella quadrata*). Österreichische Zoologische Zeitschrift 1: 179-191.

249 Ruttner-Kolisko, A., 1974. Plankton Rotifers, Biology and Taxonomy Supplementary Edition of Die
250 Binnengewässer 26, Stuttgart: 1-146.

251 Ruttner-Kolisko, A., 1977. Amphoteric reproduction in a population of *Asplanchna priodonta*. Archiv
252 für Hydrobiologie, Beiheft Ergebnisse der Limnologie 8: 178-181.

253 Sudzuki, M., 1955. Studies on the egg-carrying types in Rotifera. I. Genus Pompholyx. Oyo
254 Dobutsugaku Zasshi (Zoological Magazine) Tokyo 64:219-224. (in Japanese).

255 Tannreuther, G. W., 1920. The development of *Asplanchna ebbesbornii* (rotifer). Journal of
256 Morphology 33: 389-422.

257 Voigt, M. 1957. Rotatoria: Die Rädertiere Mitteleuropas. Gebrüder Borntraeger, Berlin-Nikolassee

258 Wurdak, E. S., Gilbert, J. J. & Jagels, R., 1977. Resting egg ultrastructure and formation of the shell in
259 *Asplanchna sieboldi* and *Brachionus calyciflorus*. Archiv für Hydrobiologie Beiheft 8: 298-302.

260 Wurdak, E. S., Gilbert, J. J. & Jagels, R., 1978. Fine structure of the resting eggs of the rotifers
261 *Brachionus calyciflorus* and *Asplanchna sieboldi*. Transactions of the American Microscopical Society
262 97: 49-72.

263 **Figure Captions**

264 **Fig. 1** Amictic female *Asplanchnopus multiceps*
265 She is carrying a developing ovocyte, O, posterior to the stomach, S. The vitellarium, V, is visible
266 beside the stomach.

267 **Fig. 2** Male *A. multiceps*
268 The testis, T, contains typical and atypical spermatozoa.

269 **Fig. 3** Mictic *A. multiceps* female
270 The developing ovocyte, O, is very dark and irregular in shape. The foot, F, on the right is one of the
271 distinguishing features of the genus.

272 **Fig. 4** Freshly-laid subitaneous egg
273 Note the hair-like projections that coat the shell and the vacuoles present in the peripheral
274 cytoplasm. Based on its size this egg will develop into a female.

275 **Fig. 5** Developing male embryo in the 4-cell stage
276 The large cell on the left is the precursor of the gonad and associated structures.

277 **Fig. 6** Empty shell left behind by male hatchling
278 During hatching the shell splits into two symmetrical halves held together at the hinge.

279 **Fig. 7** *A. multiceps* resting egg
280 The egg is perfectly round; the very dark interior is surrounded by a clear zone that is bounded by a
281 thin external coat covered by short fibrils. The peripheral halo-like layer is off center.

282 **Fig. 8** Scanning electron micrograph (SEM) of subitaneous egg
283 The fuzzy external coat is very pronounced.

284 **Fig. 9** Surface detail of subitaneous egg
285 The adhesive patch, P, that secures the egg to the substratum covers a circular area on the underside.

286 **Fig.10** High magnification of subitaneous egg surface
287 Note the filaments that extend from the shell. At their point of attachment to the shell they splay into
288 rootlets. The surface is smooth in the area between the filaments.

289 **Fig.11** SEM of *A. multiceps* resting egg
290 The surface is devoid of the filaments that characterize subitaneous eggs, but the adhesive patch, P, is
291 present here as well.

292 **Fig.12** Detail of resting egg surface
293 The piece of adhesive patch, P, on the right appears smooth while the general surface is marked by
294 minute projections and tiny pores.

Fig 1



Fig 2



200 μm

Fig 3



Fig 4



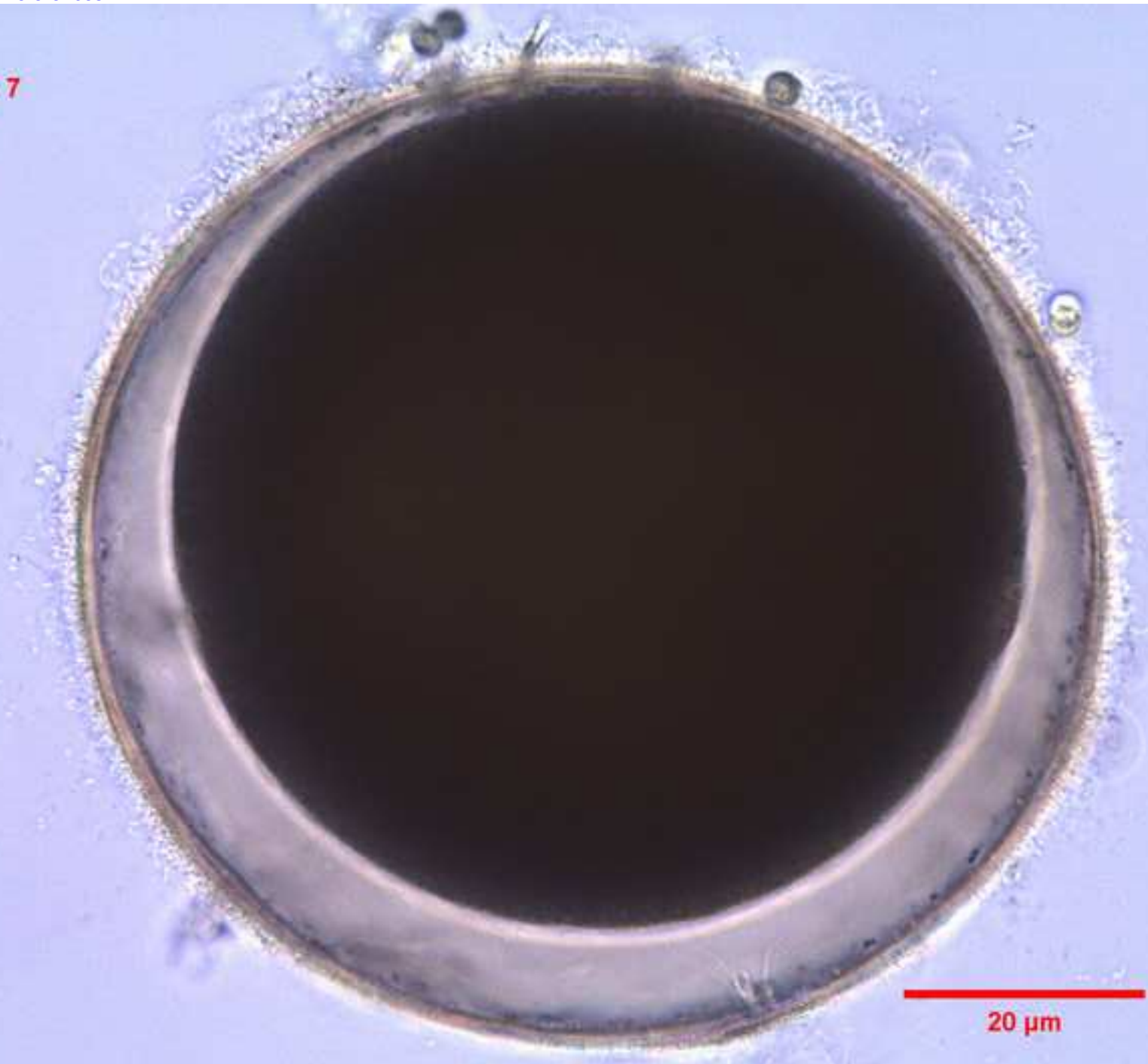
Fig 5



Fig 6

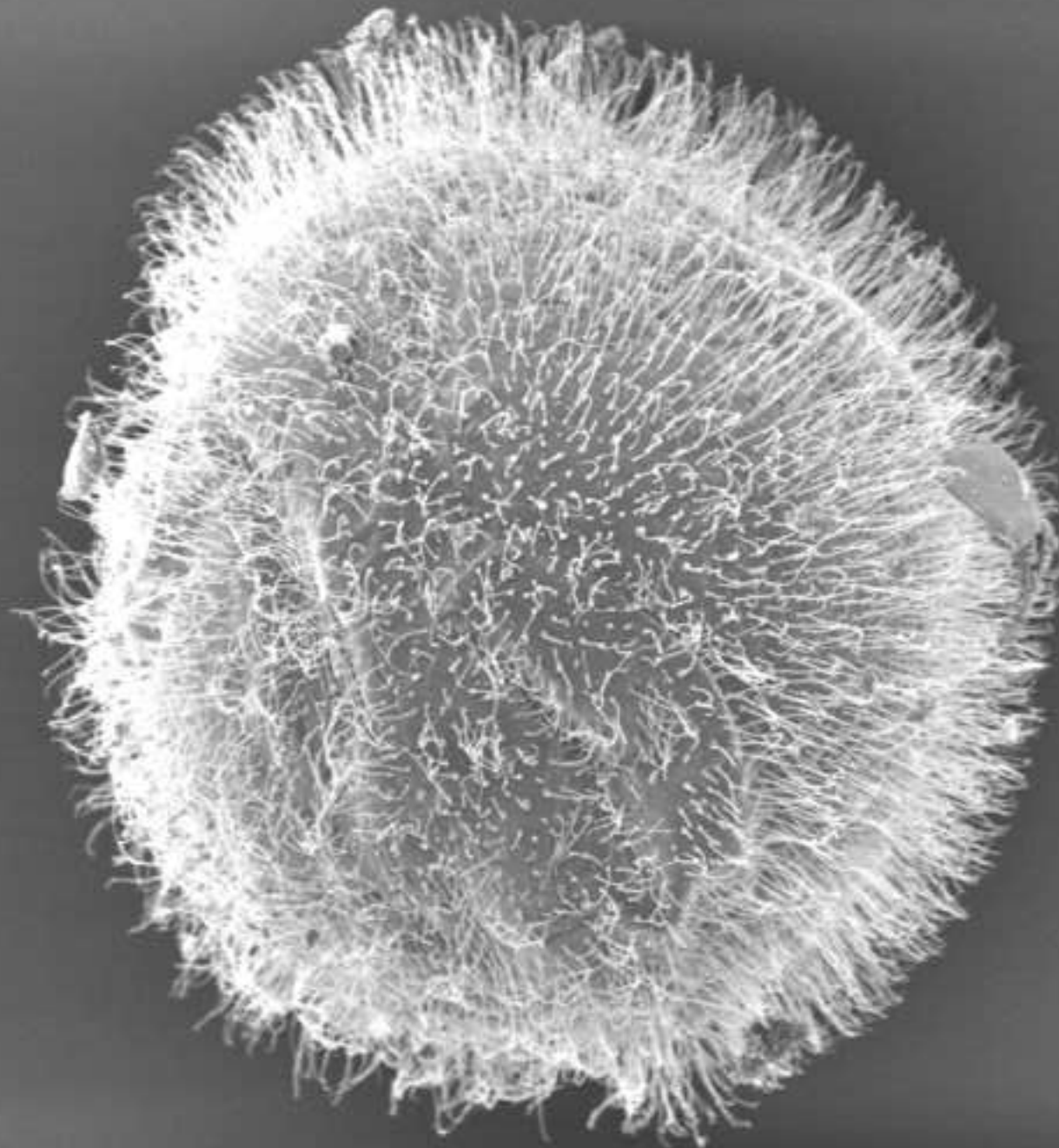


Fig. 7



20 μ m

Fig 8



50 μm

Fig 9

P

20 μ m

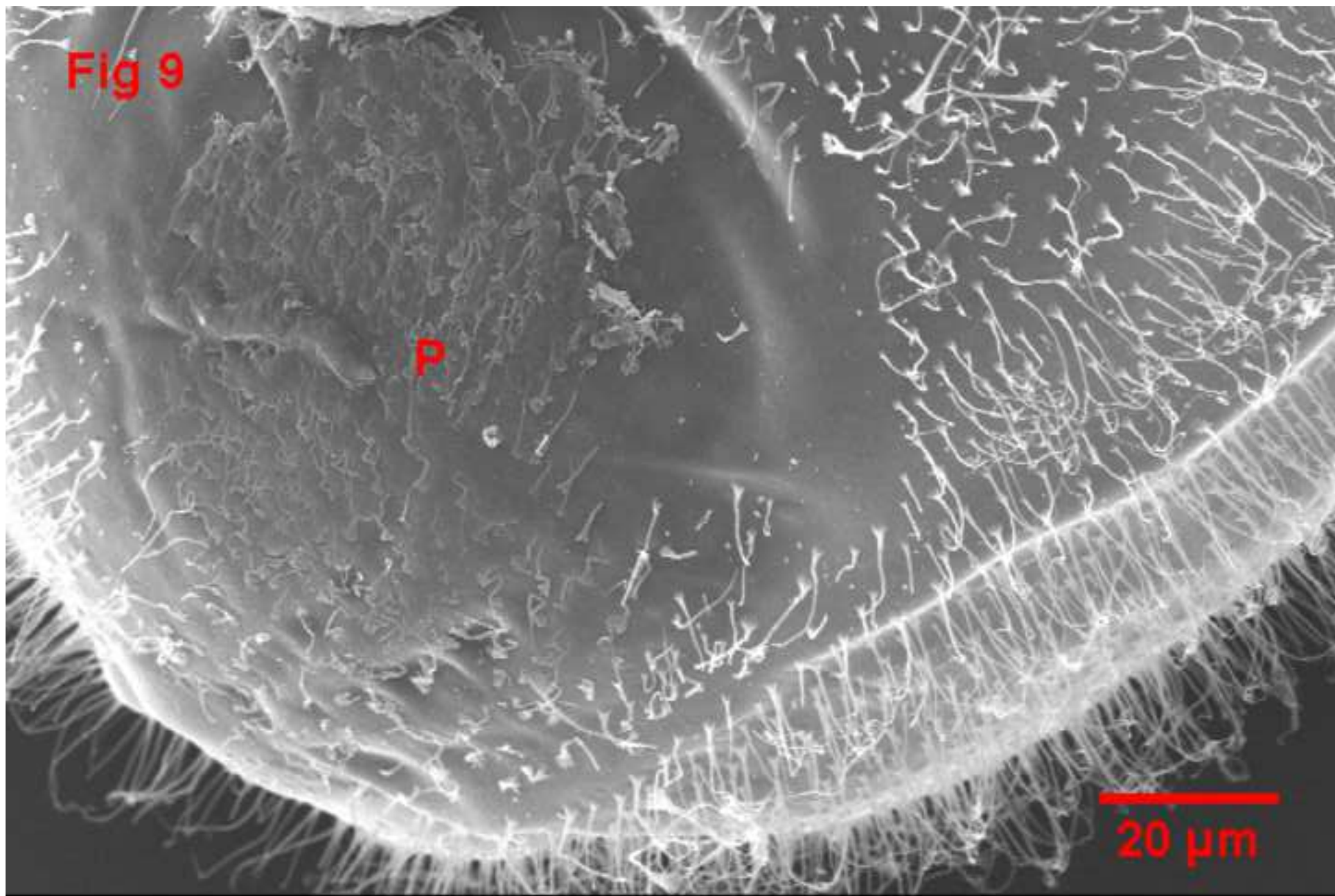


Fig 10

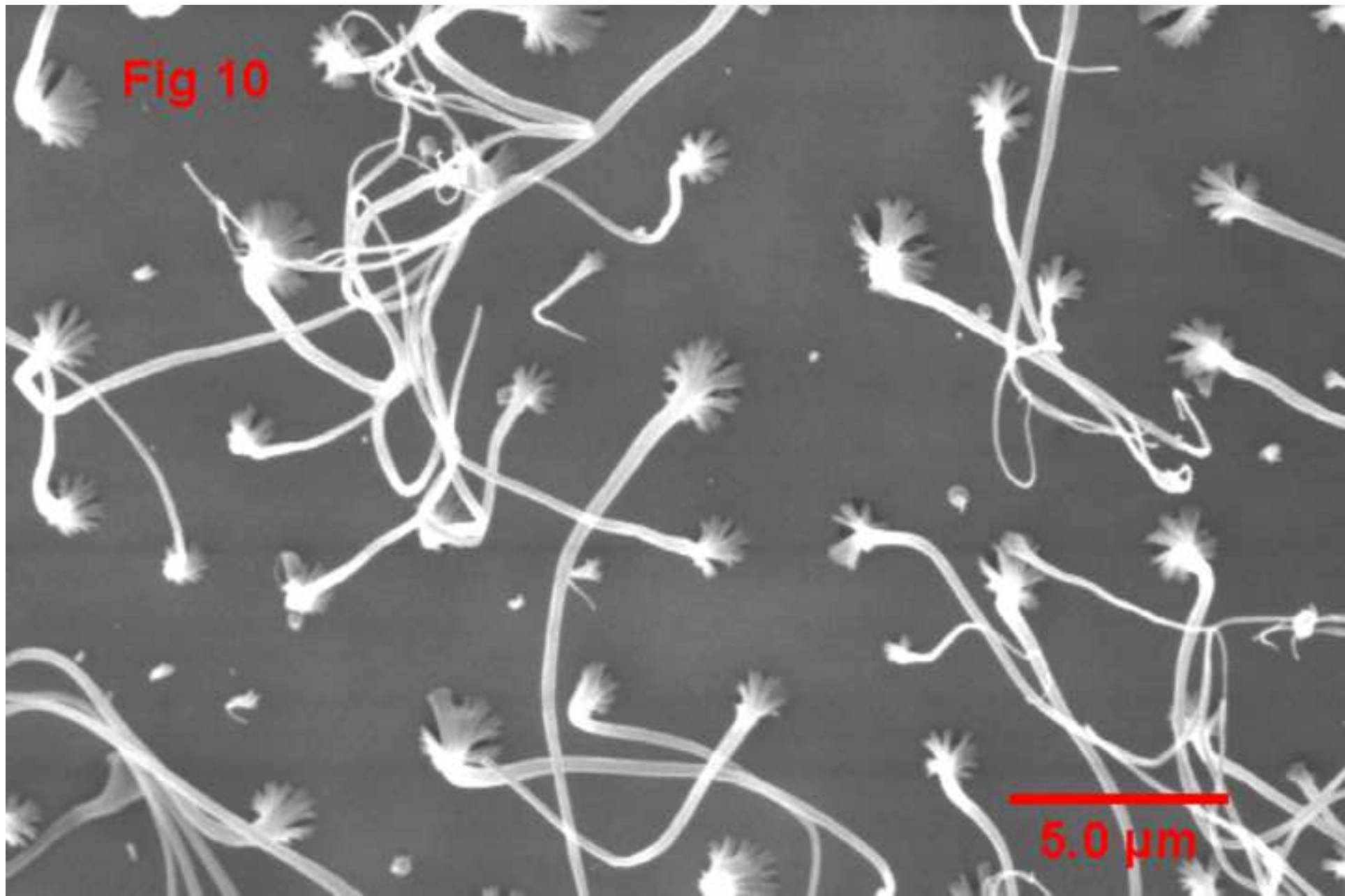
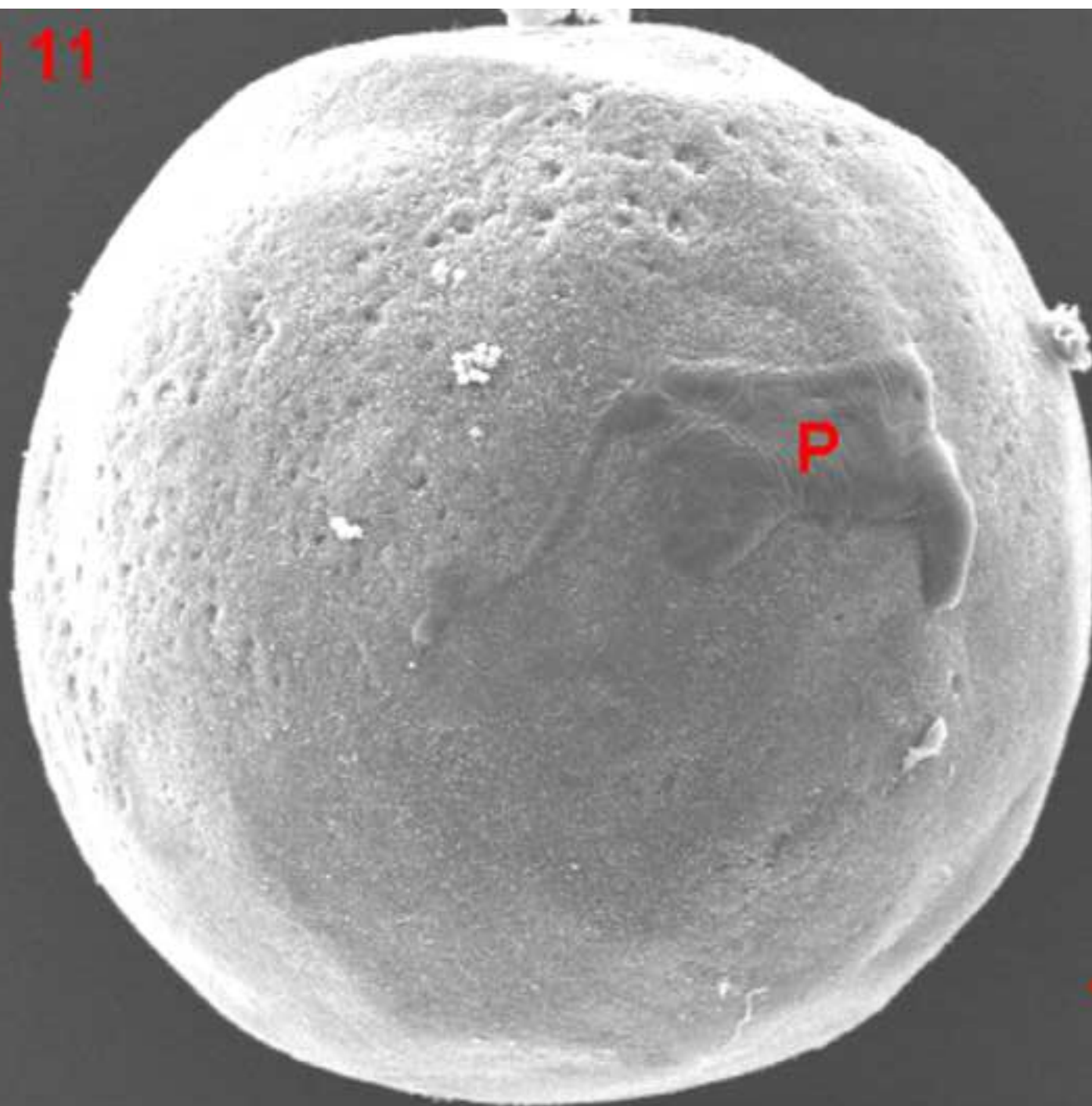


Fig 11



50 μ m

Fig 12

