

Karyotypic and Morphological Confirmation of Species in *Fredericella australiensis* (Bryozoa: Phylactolaemata)¹

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Abstract. Significant morphological and karyotypic differences between *Fredericella sultana* and *F. australiensis* establish that the latter is a valid species. Chromosome studies show that in *F. australiensis* $2n = 16$, compared with $2n = 14$ in *F. sultana*. Karyotypically, *F. australiensis* appears intermediate between or a common ancestor to *F. sultana* and several *Plumatella* species. Raised together under identical laboratory conditions, zooids of *F. australiensis* produced rounded, thick-rimmed statoblasts in distinct contrast to the elongate, thin statoblasts of *F. sultana*, with a dramatic difference in relative rates of statoblast formation. There also were differences in colony and polypide morphology. Our collecting experience suggests that *F. australiensis* can exploit new or disturbed sites, or is abundant at others on a seasonal basis, but only rarely occurs in association with other phylactolaemate species.

Within the exclusively fresh-water class of Bryozoa (Phylactolaemata), the genus *Fredericella* exhibits two unique but structurally simple characteristics. The expanded circular lophophore contrasts with the inflected horseshoe configuration found in other species, while the statoblasts lack the pneumatic cells common in most phylactolaemates.

The best known species of *Fredericella*, *F. sultana* (Blumenbach, 1779) has a cosmopolitan distribution (Bushnell, 1973) and is generally quite common. Colonies have been found growing at colder temperatures, lower pH, and at greater depths than any other phylactolaemate species. Morphologically, *F. sultana* exhibits considerable plasticity. Variations in zooid and statoblast dimensions, zooecial incrustation, colony growth form, and tentacle number have prompted investigators to split *F. sultana* among numerous new species and subspecies (e.g., Hyatt, 1866; Forel, 1885; Annandale, 1913; Abrikosov, 1927, 1961; Marcus, 1946). Unfortunately, the absence in most cases of preserved specimens precludes the opportunity to examine these types.

Despite the variation in *F. sultana*, however, most workers have considered *F. australiensis* Goddard, 1909 a distinct and valid species. It is characterized by a more robust polypide than *F. sultana* and by the production of numerous, broadly oval statoblasts with distinctively thick shells. Originally reported from water supply reservoirs in New South Wales, Australia (Goddard, 1909),

TABLE I

Measurements from natural populations of *Fredericella australiensis* compared with those from laboratory-reared colonies of *F. australiensis* and *F. sultana*

| Measurement | Goddard (1909) | Marcus (1953) | Rogick (1945) | Bushnell (1971) | Lab-reared <i>F. australiensis</i> | Lab-reared <i>F. sultana</i> |
|---|-------------------|------------------|------------------|--------------------|---------------------------------------|---------------------------------|
| Statoblast dimensions (μm) | | | | | | |
| Length, range | — | 320–420 | 331–461 | —417 | 275–400 | 325–425 |
| Length, mean | — | 360–385 | 382 | 376 | 370 ± 14 | 386 ± 9 |
| Width, range | — | 250–370 | 266–367 | —320 | 200–275 | 200–250 |
| Width, mean | — | 298–325 | 316 | 292 | 243 ± 9 | 224 ± 6 |
| Length:width ratio | 1.3* | 1.19 | 1.21 | 1.29 | 1.52 | 1.72 |
| Number of tentacles | | | | | | |
| Range | 28–30 | 24–27 | 24–28 | — | 22–24 | 21–23 |
| Mean | — | 26 | 26–27 | — | 23 ± 0.4 | 22 ± 0.5 |
| Zooecium diameter (μm) | | | | | | |
| Range | — | 315–500 | 259–576 | 235–510 | 275–375 | 175–275 |
| Mean | — | 450 | 391 | 400 | 334 ± 10 | 230 ± 8 |

* Taken from mean length and width measurements of five figures appearing in Goddard (1909).

this species has since been reported from other parts of the world, particularly in streams and lakes of semiarid or desert regions (Bushnell, 1971, 1973). Specimens collected from Uinta County, Wyoming (Rogick, 1945) and from Lake Titicaca (Marcus, 1953) differ only slightly in tentacle number and statoblast dimensions (Table I). Material collected by Borg (1936) in northern Sweden and from a stream in the central Sahara also may be this species, although descriptions are sketchy. During 1973–80, additional specimens of *F. australiensis* were discovered by the authors in several lake and stream sites in Ohio and the Washington, D.C. area, permitting for the first time close examination of this species from living material. For the defined purposes of this study, all proposed taxonomic variations of *F. australiensis* (see Rogick, 1945) are tentatively considered synonymous.

MATERIALS AND METHODS

Specimens of *Fredericella australiensis* used in laboratory culture studies were collected 16 April 1978 in Montgomery County, Maryland, from a stream flowing from just above Lock 20 of the Chesapeake and Ohio (C. & O.) Canal to the Potomac River at Great Falls. Additional specimens were taken from this site during April–May 1979 and January–April 1980, and from the following areas in and near the C. & O. Canal: overflow from a feeder channel into the Potomac River near Lock 5 (April–May 1978); a stream flowing in the canal bed above Lock 10 (October 1979–January 1980); the canal just above Lock 11 (April 1980); a stream in the canal bed just above Lock 12 (July–August 1980); the bypass channel at Lock 6 (April 1980); and just above

Lock 21 (October 1979). These sites are all in Maryland and, with the exception of Locks 10, 11, and 12, are separated by several km. The specimens of *F. sultana* used for karyotypic comparison were collected above Lock 8 in November 1979; those used in the laboratory culture studies originated from Cowan Lake, a flood-control reservoir 8 km southwest of Wilmington, Clinton County, Ohio.

Freshly-collected material yielded the best chromosome spreads. The following procedure was modified from that of Kligerman & Bloom (1977). Terminal polypides and buds were dissected out, immersed in 0.05% w/v colchicine in tapwater for no more than 55 min, and fixed in 32% acetic acid:32% methanol:36% ethanol for at least 5 min. A single polypide and bud were touched to lens tissue to drain off fixative, then placed in 2–3 drops of 60% acetic acid warmed to 65–70°C on a microscope slide. The tissue was teased apart with forceps under a dissecting microscope; when possible, the intact gut was removed from the slide. The suspension was allowed to stand for 2–5 min with occasional gentle rocking to form a thin film on the slide; a drop of 60% acetic acid added, if necessary, to compensate for evaporation. The slide was warmed (65–70°C) to coalesce the acetic acid into a drop, which in turn was aspirated by microhematocrit pipette. Slides were allowed to dry, immersed in 50% acetic acid for 10 sec, washed in flowing tapwater followed by distilled water, and stained 5–15 min in a 3% dilution of stock Giemsa in pH 7, 0.01 M phosphate buffer. After washing in distilled water, slides were warmed (65–70°C) for at least 10 min, placed in xylene for 10 min, air-dried, and coverslip-mounted using Permount®.

Some slides, before staining, were air-dried for two weeks, then placed in a saline solution (1.75 g NaCl, 0.88 g trisodium citrate in 100 ml distilled water) at 60°C for 60–90 min. They were washed in distilled water, stained for 30–50 min in buffered Giemsa and mounted as above. This is a modification of the technique which Sumner et al. (1971) developed for banding human chromosomes.

Photomicrographs of selected spreads were made using Kodak® High Contrast film at 500× in conjunction with a Leitz 100× oil-immersion achromatic objective.

In laboratory culture studies, living branches of both species were placed in individual 10-cm glass Petri lids submerged in a shallow enamel pan of Cowan Lake water. Within several days, the living zooids had attached sufficiently to the glass substrate so that the lids could be suspended in an inverted position in a specially constructed 30-liter aquarium modified according to Wood (1971). Water in the aquarium, changed weekly, was transported from Huffman Reservoir, a small back-water bay of the Mad River in Dayton, Ohio. Colonies of both species were maintained in the same aquarium at 24°C under a natural lighting regime. Gentle aeration kept the water circulating evenly to all parts of the aquarium. At least twice a week, colonies were examined for growth, zooid longevity, and statoblast production. Various dimensions were measured with an optical micrometer in a dissecting microscope at 30× magnification. Confidence limits of mean data were calculated according to *t* distribution at the 0.95 level.



FIG. 1. Chromosome spread from *Fredericella sultana* ($2n = 14$) prepared using modified alkaline-saline-Giemsa (ASG) technique. FIG. 2. Chromosome spread from *F. australiensis* ($2n = 16$). The two smallest chromosome sets in this species are lacking in *F. sultana*, but are similar to those seen in a number of *Plumatella* species. Same scale as Fig. 1. FIG. 3. Meiotic spread from *F. australiensis*, showing diakinesis; prepared using ASG technique. Same scale as Fig. 1.

Statoblasts were prepared for microscopic examination by placing them in hot saturated KOH solution for approximately 1 min, and then transferring them to distilled water. Photomicrographs were taken with High Speed Ektachrome® film using an Olympus compound microscope with substage illumination.

RESULTS

Specimens of *Fredericella sultana* yielded spreads of $2n = 14$ (Fig. 1), with three large metacentric and four smaller submetacentric sets, as previously reported by Backus (1977) and Potter (1979). Colonies of *F. australiensis* consistently gave spreads of $2n = 16$ (Fig. 2). Attention was drawn originally to the material collected 16 April 1978 by this difference in chromosome number; subsequent statoblast development indicated the species was *F. australiensis*. The karyotype consists of three large metacentric, three smaller submetacentric, and two small metacentric sets (Fig. 10). *F. australiensis* collected in November 1979 from above Lock 10 and in January 1980 at Great Falls yielded meiotic spreads (Fig. 3).

Eight colonies of *F. sultana* were reared in the laboratory, forming 218 zooids and 36 statoblasts. The pattern of colony growth and dimensions of body parts were consistent with descriptions by Rogick (1935), Toriumi (1951), Bushnell (1965), Lacourt (1968), and Wood (1973). The narrow tubular branches and widely-spaced zooids grew along the glass surface, sending occasional branches projecting away from the substrate in fragile, antler-like extensions. The external cuticle, mostly free of adhering particles, appeared nearly transparent with a slight amber color. A faintly discernible keel formed along several of the recumbent branches, but none occurred on the free portions of the colony. Uniform sections of the tubular colony branches measured 175–275 μm (average 230 ± 8); septa were infrequent or absent altogether. Lophophores bore 21–23 tentacles (average 22 ± 0.5) arranged in a circle. Mean statoblast dimensions were 366 ± 9 by $224 \pm 6 \mu\text{m}$. Statoblasts exhibited a rough texture on the dorsal valve and a similar but less prominent pattern on the ventral valve (Fig. 9).

The laboratory-grown colonies of *F. australiensis* were similar in appearance to Rogick's (1945) excellent specimens of *F. a. browni* (MDR-471, U.S. National Museum). Eight colonies were reared, with a total of 162 zooids and 106 statoblasts. Zooids were spaced more closely, and the colonies generally were more compact than those of *F. sultana*. All growth occurred along the glass substrate with no substantial free branching. The cuticle was nearly transparent and slightly amber in color. Neither keel nor septa were observed. The tubular branches of the colonies were noticeably wider than those of *F. sultana*, with a mean diameter of $334 \pm 10 \mu\text{m}$ measured in relatively uniform areas. Among full-grown zooids, the polypides appeared consistently shorter and proportionately wider than those of *F. sultana* (Figs. 4, 5) to fully confirm Rogick's (1945) observations of preserved material. Moreover, the epistome was consistently more prominent in *F. australiensis* than *F. sultana*. However, it was impossible to obtain reliable measurements of these parts from

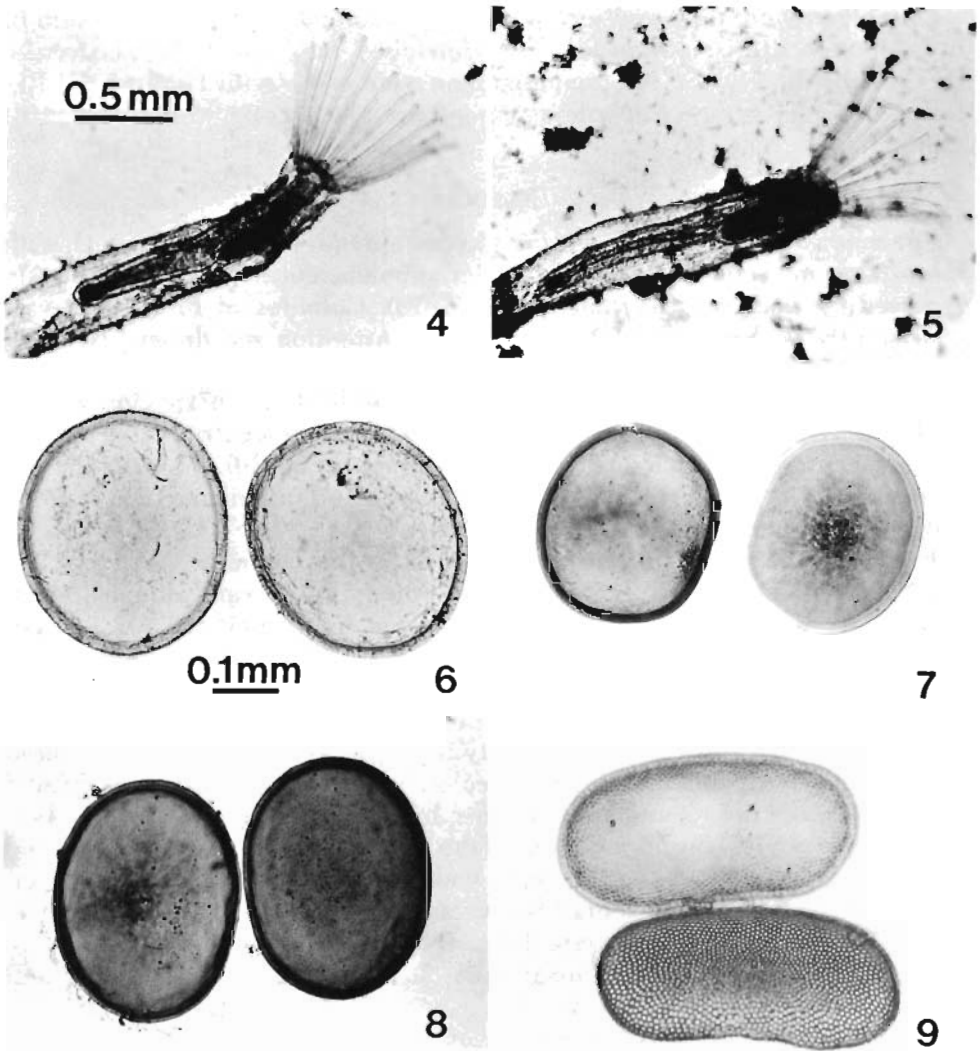


FIG. 4. Typical, fully-grown zooid of *Fredericella australiensis* cultured under laboratory conditions. FIG. 5. Typical, fully-grown zooid of *F. sultana* cultured under laboratory conditions. Same scale as Fig. 4. FIG. 6. Two halves of a single statoblast of *F. australiensis* collected in Uinta County, Wyoming, and described by Rogick (1945). FIG. 7. Two halves of a single statoblast of *F. australiensis* from a stream near Lock 20, Chesapeake and Ohio Canal, Montgomery County, Maryland, collected in April 1978. Same scale as Fig. 6. FIG. 8. Two halves of a single statoblast of *F. australiensis* from laboratory culture. Same scale as Fig. 6. FIG. 9. Two halves of a single statoblast of *F. sultana* from laboratory culture, showing the roughened texture not seen on statoblasts of *F. australiensis*. Same scale as Fig. 6.

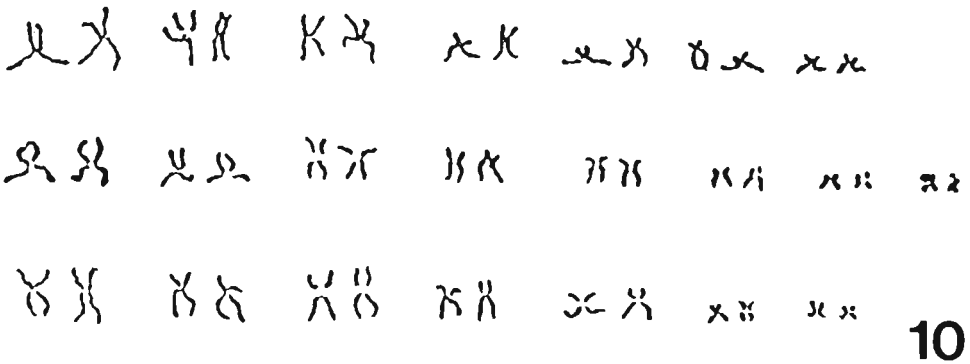


FIG. 10. Comparative karyotypes of *Fredericella sultana* ($2n = 14$, top), *F. australiensis* ($2n = 16$, middle) and a common form of *Plumatella emarginata* ($2n = 14$, bottom). Comparison of gross morphology suggests that the sixth set of *F. sultana* may be equivalent to the two smallest sets of *F. australiensis* which are similar in size and morphology to the two smallest sets of this form of *P. emarginata*. The third set of *F. australiensis* resembles that of *P. emarginata* in that frequently there is a considerable centromere gap, which does not appear in *F. sultana*. However, the metacentric second sets of *F. sultana* and *F. australiensis* are similar, and are in distinct contrast to the submetacentric second set of *P. emarginata*. The sixth set of *F. australiensis* appears similar to the seventh of *F. sultana*; no similar set appears in this form of *P. emarginata*.

living specimens. The lophophores bore 22–24 tentacles (average 23 ± 0.4) in a circular arrangement. Numerous statoblasts, formed end-to-end in continuous series, had average dimensions of 370 ± 14 by $243 \pm 9 \mu\text{m}$. Statoblast valves, isolated and cleaned (Fig. 8), revealed a uniquely thickened rim at the suture area and an absence of the roughened texture so conspicuous in *F. sultana* (Fig. 9).

DISCUSSION

Karyotypic comparison between *Fredericella sultana* and *F. australiensis* (Fig. 10) suggests that one of the smaller submetacentric sets (probably the sixth) of *F. sultana* is equivalent to the two smallest sets in *F. australiensis*. However, more substantial rearrangements may have occurred than are evident from chromosome morphology.

The two smallest chromosome sets of *F. australiensis* are similar in size and morphology to the two smallest sets of the $2n = 14$ *Plumatella emarginata* (Fig. 10). The third set of *F. australiensis* also resembles that of *P. emarginata* in that frequently there is a considerable centromere gap (Fig. 10), which does not appear in *F. sultana*. However, the metacentric second sets of *F. sultana* and *F. australiensis* are morphologically similar, and are in distinct contrast to the submetacentric second set of *P. emarginata*. The sixth set of *F. australiensis* and seventh of *F. sultana* also appear to be identical; no similar set appears in the $2n = 14$ *P. emarginata*.

Hyalinella punctata and most *P. repens* have $2n = 14$ karyotypes extremely similar or identical to that of $2n = 14$ *P. emarginata* (Backus, 1977, 1979).

originate from bodies of slow-moving water, which may reach a peak in plankton productivity during late fall and early spring when they are not shaded by trees. These seasons also may be periods of relatively high dissolved oxygen content. At two of the lotic sites where *F. australiensis* is usually abundant in early spring, luxurious colonies of *Plumatella emarginata* are subsequently common.

Like *F. sultana*, *F. australiensis* is morphologically variable. At Great Falls in cold, rapidly-flowing water, smaller colonies of *F. australiensis* tend to be closely adherent to the substrate, with no free branching. As growth continues, these colonies may remain surprisingly compact, developing a columnar mass of branches growing directly away from the substrate. The same area also has yielded dense masses of colonies 20–30 cm in diameter intertwined with submerged tree roots. Chromosome preparations from younger polypides of massive colonies showed considerable meiotic activity (Fig. 3), while older zoecia contained many statoblasts, arranged end-to-end. A large number of planarians, insect larvae, protozoans, and nematodes usually are associated with the dense growth.

By contrast, colonies of *F. australiensis* collected just above Lock 11 in early April 1980 from cold, slow-moving water were considerably less compact and only loosely attached to the substrate, being distinguishable from *F. sultana* by statoblast morphology and slightly greater zoecial diameter. Statoblasts occurred singly. Colonies of *F. australiensis* collected from warm, rapidly-flowing water above Lock 12 during July–August 1980 were similar, but were remarkable in being mixed with considerable *H. punctata*, some *P. emarginata*, and specimens of the fresh-water endoproct, *Urnatella gracilis*. This site had been dry during May 1980 while repairs were being made between Locks 10 and 11.

It is not certain whether the variations we have seen are genetic or environmental; however, the laboratory-cultured *F. australiensis* which was primarily adherent to the substrate, and which produced many statoblasts, was from Great Falls.

With so little data yet available, our knowledge of the ecology of *F. australiensis* remains rudimentary. The species has been reported previously from relatively arid regions of the world, including southeastern Australia, Lake Titicaca, the central Mexican plateau, and an alkaline pond in southwestern Wyoming, although Lacourt (1968) reported it in Belgium. The colonies with which we have worked were collected from sites in southwestern Ohio and near Washington D.C., areas which experience over 90 cm of precipitation annually. The unifying feature of these sites may be simply the frequency of rapid, short-term fluctuations in environmental conditions. Sudden changes in water level, salinity, or temperature may create a habitat where favorable conditions are of short duration but quite suitable for the rapid growth of this species. It also is possible that *F. australiensis* has some of the attributes of a fugitive species (Hutchinson, 1951), being capable of rapidly colonizing new or disturbed sites, or of seasonally exploiting existing sites, but not normally competing or associating with other bryozoans. We

frequently (but not invariably) have found that when *F. australiensis* is flourishing, other phylactolaemates are either absent, or are present as young or senescent colonies.

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