SPECIES DIVERSITY WITHIN *MACROPODINIUM* (LITOSTOMATEA: TRICHOSTOMATIA): ENDOSYMBIOTIC CILIATES FROM AUSTRALIAN MACROPODID MARSUPIALS

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Ciliates in *Macropodinium* are the most distinctive component of the ciliate fauna of macropodid marsupials. *Macropodinium moiri*, *Ma. setonixum*, *Ma. ennuensis* forma *ennuensis* and *Ma. yalanbense* are redescribed following silver staining to reveal the arrangement of their ciliary bands. *Macropodinium hallae* sp. nov., *Ma. ocallaghani* sp. nov., *Ma. petrogale* sp. nov. and *Ma. titan* sp. nov. and *Ma. ennuensis* f. *dentis* f. nov., are described for the first time. A new key to the species of *Macropodinium* using light microscopic features of silver stained specimens is provided. Species diversity within the genus (13 species) illustrates the morphological variability within the group and its distinctiveness from other genera of endosymbiotic ciliates. ❐ *Ciliophora, Litostomatea, Trichostomatia, Macropodiniidae, parasite specificity, parasite evolution.*

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Most herbivorous mammals are host to a diverse range of ciliated protozoa which inhabit the fermentative digestive organs (Corliss, 1979). Endosymbiotic ciliates have also been detected in macropodid marsupials (kangaroos and their relatives) which are the dominant Australian terrestrial herbivores (Lintern-Moore, 1973; Obendorf, 1984; Dellow et al., 1988; Dehority, 1996; Cameron et al., 2000a; 2000b). Our previous studies have shown that macropodid marsupials are hosts to at least 3 ciliate families: including the Amylovoracidae (Cameron & O'Donoghue, in press); Cycloposthiidae (Cameron et al., 2000); and Macropodiniidae (Cameron et al., 2001). Of these, the monogeneric Macropodiniidae were the most speciose and structurally diverse family with 9 highly host-specific species recovered from 7 host species. The family was originally described by Dehority (1996) on material stained with haemotoxylin, methylene blue and methyl green. This generic diagnosis was amended by Cameron et al. (in press) following studies of material stained with silver proteinate (protargol), which revealed additional structures not described by Dehority (1996). This study re-examines *Macropodinium* spp. described by Dehority (1996) for the new characters described by Cameron et al. (in press) and describes several new *Macropodinium* spp. found in macropodid marsupials.

MATERIAL AND METHODS

Samples of stomach contents were obtained either from road-killed animals or from animals culled from wild populations. The following macropodid species (numbers examined in parentheses) were examined for macropodiniid ciliates: *Macropus eugenii* (14); *M. fuliginosus melanops* (21); *M. fuliginosus fuliginosus* (5); *M. robustus erubescens* (20); *M. robustus robustus* (16); *M. rufogriseus* (32); *Petrogale godmani* (3); and *Setonix brachyurus* (5). Stomach fluid was squeezed from fibrous matter, strained through a triple layer of surgical gauze to remove fine particulate matter and nematodes, and fixed in Bouin's fluid or methyl formol-saline (MFS). Ciliate morphology was determined by microscopic examination of specimens stained with methyl-green, methyl-green pyronin, silver proteinate (protargol), and silver-carbonate (Foissner, 1991). Ciliates were measured using a calibrated eyepiece micrometer and drawn with the aid of a *camera lucida*.

Protargol staining was performed using a combination of the Wicklow & Hill (1992) and Lynn (1992) methods as follows. Cells fixed in Bouin's fluid were washed in distilled water to remove traces of picric acid, dehydrated through a graded series of ethanol solutions (70, 80, 90, 100 and 100% for 10 min each), applied to an albuminised slide and flooded with ethanol.

Evaporation of the ethanol affixes the cells to the albumin. The albumin was fixed by flooding with formol-alcohol (3 parts 10% formaldehyde, 1 part 95% ethanol) followed by immersion in formol-alcohol for 15 min. The specimens were dehydrated in 96% and 100% isopropanol for 2 min each, then immersed in 100% methanol for 3 min. Specimens were coated with 1% parlodion in methanol for 2 sec and air-dried. Slides were coated and air-dried several times, depending on the desired staining effect, rehydrated in a graded series of isopropanol series (70, 50, 30% for 2 min each) and washed two times in distilled water. Cells were oxidised in 0.5% potassium permanganate for 3 min, washed three times in distilled water (for a total of 10 min), bleached in 5% oxalic acid for 5 min and then washed three times in distilled water (for a total of 10 min). Cells were impregnated in 1% protargol at 60°C for 20 min and developed by immersion in 1% hydroquinone dissolved in 2.5% sodium sulphite. Stain development was monitored under a dissecting microscope and halted by immersion in distilled water. The stain was toned by 1-5 min immersion in 2% oxalic acid, then washed in distilled water for 3 min. For some ciliates, additional toning was achieved by a single dip in 2% gold chloride solution followed by washing in distilled water for 3 min. The stain was fixed by immersion in 5% sodium thiosulphate for 5 min followed by a distilled water wash for 3 min. The specimens were dehydrated through a graded series of isopropanol solutions (30, 50, 70, 96, 100 and 100% for 3 min each), cleared by two washes in xylene and mounted in Depex.

The silver carbonate method of Ito & Imai (1998) was modified as follows. Two drops of MFS fixed ciliates, 3-5 drops of pyridine and 5-7 drops of 4% bacteriological proteose peptone were added to 3 mL of distilled water. The resulant suspension was mixed by inversion and left in a dark box for 20 min. Fifteen to 20 drops of ammoniacal silver carbonate solution (made by mixing 5g of silver carbonate in 5mL of distilled water and soluabilising with 25% ammonia; the

TABLE 1. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium moiri* Dehority, 1996 recovered from the quokka, *Setonix brachyurus*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	81.0	12.88	15.9	60.8	100.8	20
Width, W	40.8	4.44	10.9	32.8	48.8	20
Shape index (L/W ratio)	2.0	0.20	9.9	1.4	2.3	20
Macronucleus						
Length	14.4	3.18	22.2	9.6	20.8	20
Width	7.3	0.91	12.4	5.6	9.6	20
Micronucleus						
Length	3.9	0.62	15.9	3.2	4.8	14
Width	2.9	0.60	20.5	2.4	4.0	14
Oral apparatus						
Vestibulum width	15.6	2.48	16.0	11.2	19.2	20
Vestibulum depth	24.5	3.38	13.8	16.8	29.6	20
Cytostome width	3.1	0.51	16.4	2.4	4.0	20
Length of oral cilia	6.2	0.76	12.3	4.8	7.2	7
Somatic ciliature						
Length of somatic cilia	6.1	0.92	15.1	5.6	7.2	3
Miscellaneous						
No Longitudinal grooves, Left side	12.6	0.96	7.6	11	15	19
No Longitudinal grooves, Right side	12.6	0.95	7.5	10	14	20
Width between longitudinal grooves	3.2	4.19	12.9	2.4	4.0	19
Depth DB	6.1	0.83	13.6	4.8	8.0	20
Depth VB	8.6	1.61	18.7	6.4	11.2	20
Cytoproct length	5.2	1.31	25.4	3.2	8.0	20

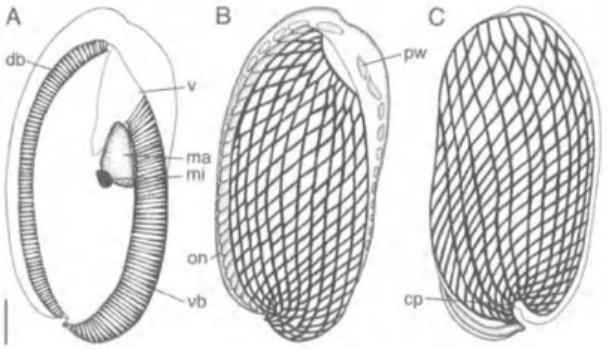

FIG. 1. Morphology of *Macropodinium moiri* Dehority, 1996. A, internal morphology. B, right view, surface features. C, left view, surface features. cp, cytoproct. db, dorsal bars. ma, macronucleus. mi, micronucleus. on, ornamentation. pw, pellicular window. v, vestibulum. vb, ventral bars. Scale bar = 10μm.

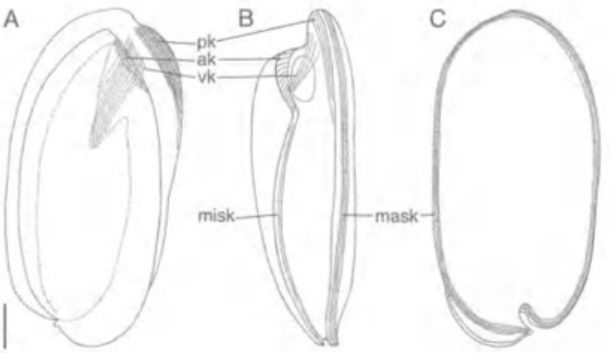

FIG. 2. Infraciliature of *Macropodinium moiri* Dehority, 1996. A, right view, oral ciliary bands. B, ventral view. C, left view, somatic ciliary band. ak, adoral kineties. mask, major somatic kineties. misk, minor somatic kineties. pk, preoral kineties. vk, vestibular kineties. Scale bar = 10μm.

resultant solution was made up to 10mL with distilled water) were then added, mixed and the suspension returned to the dark box for 1 hour. The stain was developed by incubation in a 60°C water bath for several hours until the solution turned dark brown (tea-coloured). Ciliates were removed by pipette and examined immediately as wet preparations.

Samples for scanning electron microscopy were fixed in Bouin's fluid, washed with distilled water and separated from gut debris by centrifugation in a discontinuous Percoll gradient (25%, 50%, 75%, 100%) at 3200 *g* for 10 min. Clean fractions containing ciliates were washed 3 times in Sorensen's phosphate buffer, post-fixed in 4% osmium tetroxide, washed twice in water, dehydrated in a graded ethanol series (30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 100%), and dried in a critical-point drier between Millipore filters. Dried cells were sputter-coated with gold and examined in a scanning electron microscope (JOEL 6300). All measurements are given as a range, followed by the arithmetic mean. Summary statistics of morphometrics were prepared using the Statistix® program. Cell orientation and terminology follows that of Cameron et al., (2001). For each species, representative specimens, stained with protargol and mounted on slides, have been deposited with the Queensland Museum, Brisbane (QM). Abbreviations of cellular characters are: DVG, dorso-ventral groove; DB, dorsal bars; VB, ventral bars.

RESULTS

Macropodiniid ciliates were found in 14 (100%) of *M. eugenii* examined; 6 (29%) of *M. fuliginosus melanops*; 3 (60%) of *M. fuliginosus fuliginosus*; 13 (65%) of *M. robustus erubescens*; 9 (56%) of *M. robustus robustus*; 1 (3%) of *M. rufogriseus*; 3 (100%) of *P. godmani*; and 3 (60%) of *S. brachyurus*. Of the ciliates recovered, 4 conformed to species described by Dehority (1996) and are redescribed here to incorporate the features described by Cameron et al. (2001). Another 5 taxa (4 new species and 1 new forma) are described here for the first time.

SYSTEMATICS

Phylum CILIOPHORA Dolfein, 1901
Class LITOSTOMATEA Small & Lynn, 1981
Subclass TRICHOSTOMATIA Bütschli, 1889
Family MACROPODINIIDAE Dehority, 1996
Macropodinium Dehority, 1996

Macropodinium moiri Dehority, 1996
(Figs 1, 2, 3, 10A; Table 1)

MATERIAL. Voucher specimen QMG463137, from the quokka, *Setonix brachyurus* (Quoy & Gaimard, 1830), Rottnest Is., WA 31°59'S, 115°32'E.

DESCRIPTION. Body oval; 60.8-100.8 (81.0)μm long, 32.8-48.8 (40.8)μm deep, shape index (L/D) 1.4-2.3(2.0); right side abbreviated compared with left side. Single macronucleus round to globo-trianglular; 9.6-20.8(14.4)μm long by 5.6-9.6 (7.3)μm wide; located ventral to the vestibulum. Single micronucleus round to oval; 3.2-4.8 (3.9)μm long by 2.4-4.0 (2.9)μm wide; adjacent to or obscured by the

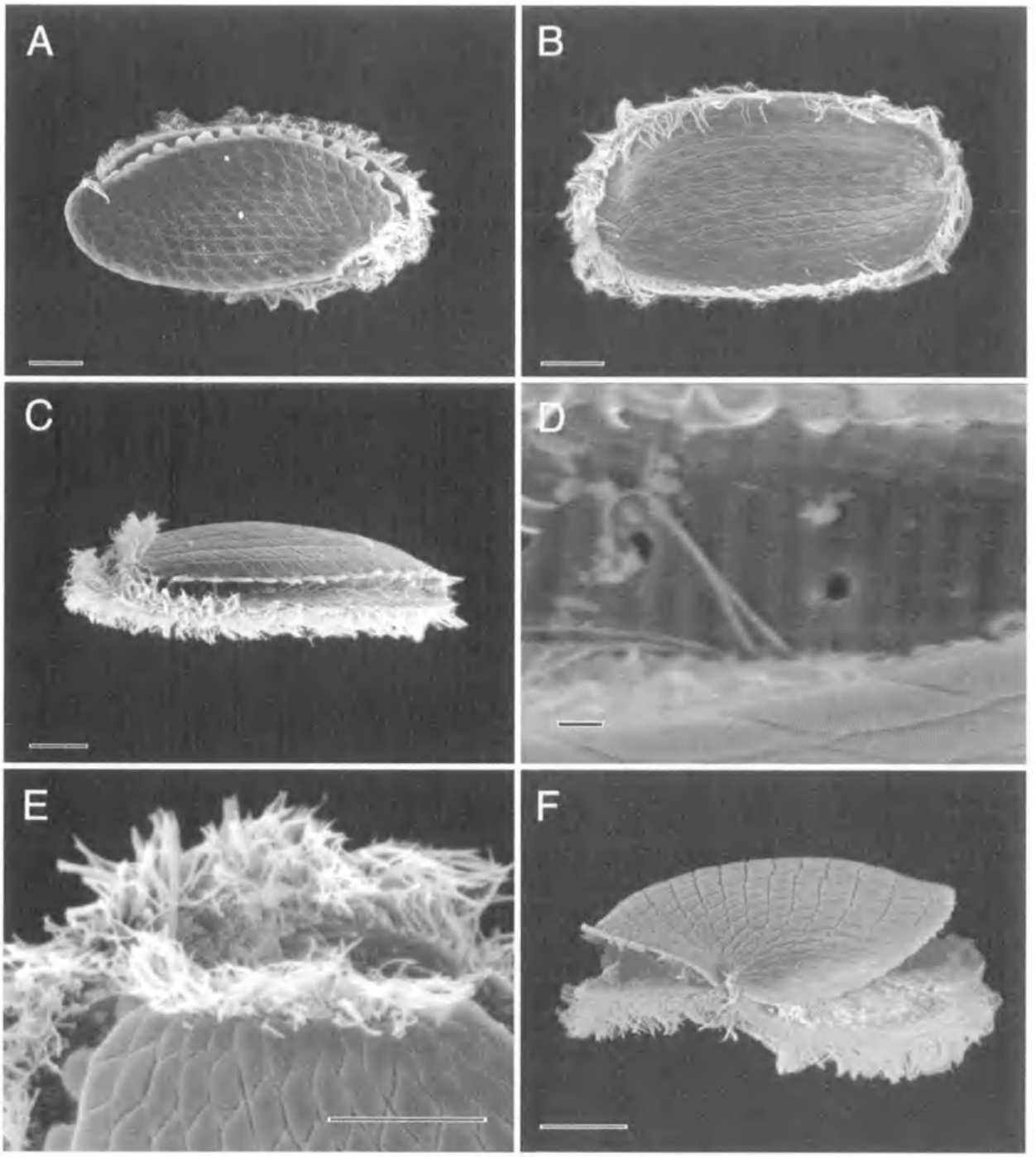

FIG. 3. Scanning electron micrographs of *Macropodinium moiri* Dehority, 1996. A, right view, scale bar = 10μm. B, left view, scale bar = 10μm. C, dorsal view, scale bar = 10μm. D, contractile vacuole pores in ventral DVG, scale bar = 1μm. E, anterior right view, scale bar = 10μm. F, cytoproct, scale bar = 10μm.

macronucleus. Vestibulum conical; 11.2-19.2 (15.6)μm wide by 16.8-29.6 (24.5)μm deep; opening apical, directed anterioventrally; cytostome 2.4-4.0 (3.1)μm wide; cytopharynx composed of short rods directed posteriorly from the cytostome. Somatic cilia 5.6-7.2 (6.1)μm long; adoral cilia 4.8-7.2 (6.2)μm long. Pellicular diamond pattern uniform; left side bears 11-15 (12.6) longitudinal grooves, right side bears 10-14 (12.6) longitudinal grooves; longitudinal

grooves 2.4-4.0 (3.2)µm apart. DVG deep dorsally and ventrally. DB prominent 4.8-8.0 (6.1)µm deep; VB prominent 6.4-11.2 (8.6)µm deep. Flange ornamentations right dorsal. Cytoproct cup-shaped; 3.2-8.0 (5.2) deep; opening posterior.

PREVALENCE. Specimens recovered from 2 of 5 hosts examined.

Macropodinium setonixium Dehority, 1996
(Figs 4, 5, 10B; Table 2)

MATERIAL. Voucher specimen QMG463138, from the quokka, *Setonix brachyurus* (Quoy & Gaimard, 1830), Rottnest Is., WA 31°59'S, 115°32'E.

DIFFERENTIAL DIAGNOSIS. *Ma. setonixium* can be readily distinguished from *Ma. moiri* by its smaller size, anteriorly directed vestibulum and the absence of a preoral window.

DESCRIPTION. Body oval to slightly reniform; 24.8-46.4 (32.1)µm long by 15.2-24.8 (18.8)µm deep, shape index (L/D) 1.3-2.1 (1.7); right side only slightly abbreviated compared with left side. Single macronucleus spherical to ovoid; 4.0-9.6 (6.3)µm long by 3.2-6.4 (5.0)µm wide; located ventral or posterior to the vestibulum. Single micronucleus round to oval; 1.6-2.4 (1.6)µm long by 0.8-1.6 (1.4)µm wide; adjacent to or obscured by the macronucleus. Vestibulum conical; 4.0-6.4 (5.4)µm wide by 6.4-11.2 (8.8)µm deep; opening apical, directed anteriorly; cytostome 0.8-2.4 (1.4)µm wide. Somatic cilia 4.0-8.8 (5.7)µm long; adoral cilia 3.2-9.6 (5.8)µm long. Pellicular diamond pattern uniform; left side bears 7-10 (8.8) longitudinal grooves, right side bears 6-9 (7.1) longitudinal grooves; longitudinal grooves 2.4-4.8 (2.8)µm apart. DVG shallow dorsally and ventrally. DB prominent 2.4-4.8 (3.4)µm deep; VB prominent 2.4-4.0 (3.4)µm deep. No ornamentations. Cytoproct slot-shaped; 1.6-3.2 (2.5) deep; opening posterior.

TABLE 2. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium setonixium* Dehority, 1996 recovered from the quokka, *Setonix brachyurus*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	32.1	4.6	14.4	24.8	46.4	20
Width, W	18.8	2.148	11.4	15.2	24.8	20
Shape index (L/W ratio)	1.7	0.20	11.6	1.3	2.1	20
Macronucleus						
Length	6.3	1.2	19.2	4.0	9.6	20
Width	5.0	0.89	17.9	3.2	6.4	20
Micronucleus						
Length	1.6	0.19	11.8	1.6	2.4	17
Width	1.4	0.38	27.5	0.8	1.6	17
Oral apparatus						
Vestibulum width	5.4	0.68	12.6	4.0	6.4	20
Vestibulum depth	8.8	1.25	14.2	6.4	11.2	20
Cytostome width	1.4	0.50	35.2	0.8	2.4	19
Length of oral cilia	5.8	1.68	29.1	3.2	9.6	19
Somatic ciliature						
Length of somatic cilia	5.7	0.99	17.3	4.0	8.8	18
Miscellaneous						
No Longitudinal grooves, Left side	8.8	0.75	8.6	7	10	17
No Longitudinal grooves, Right side	7.1	0.75	10.6	6	9	17
Width between longitudinal grooves	2.8	0.61	22.0	2.4	4.8	20
Depth DB	3.4	0.62	18.0	2.4	4.8	17
Depth VB	3.4	0.45	13.3	2.4	4.0	17
Cytoproct length	2.5	0.53	21.2	1.6	3.2	19

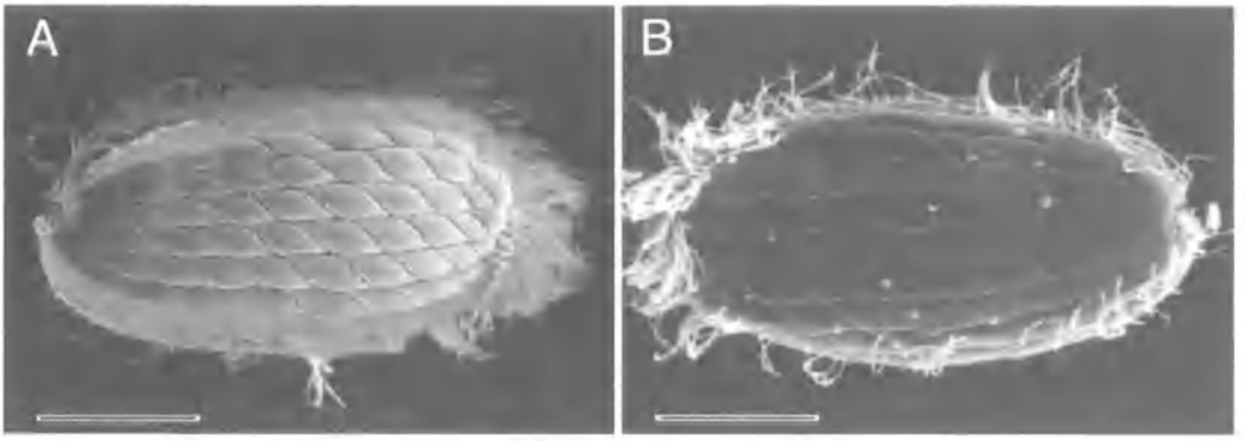

FIG. 4. Scanning electron micrographs of *Macropodinium setonixium* Dehority, 1996. A, right view. B, left view. Scale bars = 10μm.

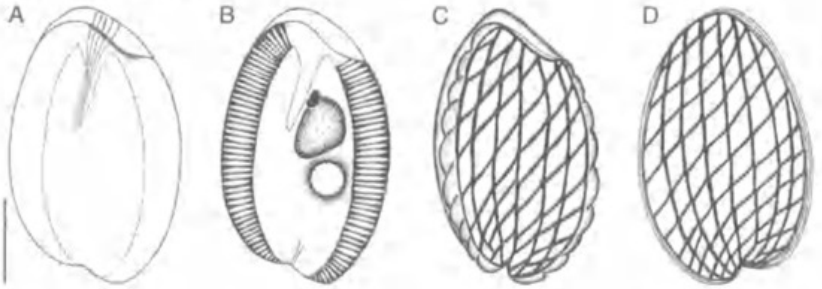

FIG. 5. Morphology and infraciliature of *Macropodinium setonixium* Dehority, 1996. A, infraciliature. B, internal morphology. C, right view, surface features. D, left view, surface features. Scale bar = 10μm.

PREVALENCE. Specimens recovered from 3 of 5 hosts examined.

Macropodinium ennuensis Dehority, 1996
forma **ennuensis**
(Figs 6, 7A,B, 10C; Table 3)

MATERIAL. Voucher specimen QMG463149, from the euro, *Macropus robustus erubescens* Gould, 1841, Pt Augusta, SA, 32°30'S, 137°46'E.

DIFFERENTIAL DIAGNOSIS. *Ma. ennuensis* possesses a VB but lacks a DB, a feature not shared with any other described species.

DESCRIPTION. The species erected as *Ma. ennuensis* by Dehority (1996) is here redescribed as forma *ennuensis*. Body oval to slightly reniform; 37.6-70.4 (56.2)μm long, 20.0-33.6 (26.4)μm deep, shape index (L/D) 1.5-3.0 (2.1); right side abbreviated compared to left side. Single macronucleus, spherical to ovoid; 5.6-15.2 (9.6)μm long by 4.0-8.8 (6.4)μm wide; located ventral to vestibulum. Single micronucleus, spherical to ovoid; 1.6-3.2 (2.4)μm long by 0.8-3.2 (1.8)μm wide; adjacent to the macronucleus. Vestibulum bent conical; 8.8-15.2 (11.8)μm wide by 13.6-27.2 (19.0)μm deep; opening subapically, directed anterioventrally. Somatic ciliation 5.6-10.4 (6.7)μm long; adoral cilia 4.0-10.4 (6.7)μm long. Pellicular diamond pattern uniform; left side bears 8-12 (10.3) longitudinal grooves; right side bears 7-10 (8.0) longitudinal grooves; longitudinal grooves 2.4-4.8 (3.4)μm apart. DVG shallow dorsally and ventrally. DB absent; VB prominent, 3.2-7.2 (5.0)μm deep. Ornamentations absent. Cytoproct slot-shaped; 1.6-3.2 (2.5)μm deep; opening posterior.

REMARKS. This forma was also recorded from the euro, *Macropus robustus erubescens* from Spear Creek Stn., SA, 32°34'S, 137°59'E and from the common wallaroo, *Macropus robustus*

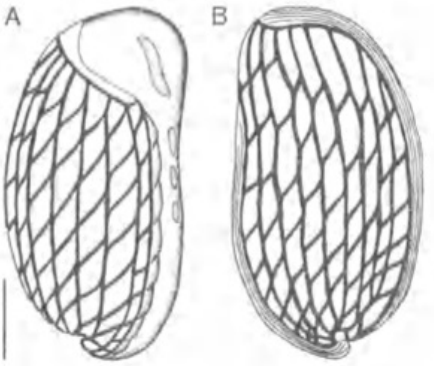

FIG. 6. External morphology of *Macropodinium ennuensis* Dehority, 1996. A, right view. B, left view. Scale bar = 10μm.

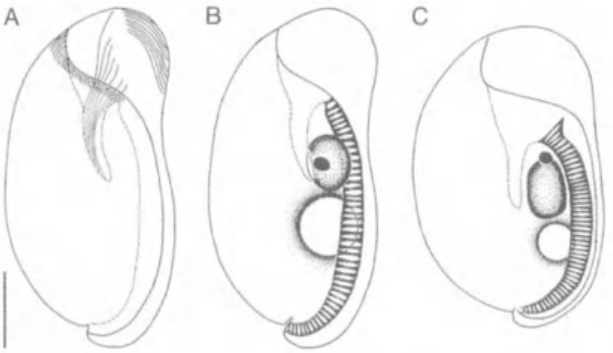

FIG. 7. Infraciliature and internal morphology of *Ma. ennuensis* Dehority, 1996. A, infraciliature. B, internal morphology, *Ma. ennuensis* f. *ennuensis*. C, internal morphology, *Ma. ennuensis* f. *dentis*. Scale bars = 10µm.

robustus Gould, 1841 on Kilclooney Stn., Qld, 18°50'S, 145°48'E and on Lyndhurst Stn., Qld, 19°12'S, 144°20'E.

PREVALENCE. Specimens recovered from 65% (13 of 20) of euros examined and 13% (2 of 16) of common wallaroos examined.

Macropodinium ennuensis Dehority, 1996
forma **dentis** f. nov.
(Figs 7C, 8; Table 4)

ETYMOLOGY. For the single tooth-like spine on the margin of the vestibulum.

MATERIAL. Voucher specimen QMG463150, from the common wallaroo, *Macropus robustus robustus*, Gould, 1841, Mt. Kinoul, Qld, 25°40'S, 149°40'E.

DIFFERENTIAL DIAGNOSIS. *Ma. ennuensis* f. *dentis* f. nov. is almost identical to *Ma. ennuensis* f. *ennuensis*, with the exception of the spine-like projection off the ventral margin of the vestibulum.

DESCRIPTION. Body oval to slightly reniform; 40.0-70.4 (53.3)µm long, 24.0-32.8 (28.8)µm deep, shape index (L/D) 1.6-2.3 (1.9); right side abbreviated compared with left side. Single macronucleus, spherical to ovoid; 8.0-12.8 (10.1)µm long by 5.6-9.6 (6.8)µm wide; located ventral to vestibulum. Single micronucleus, spherical to ovoid; 2.4-3.2 (2.7)µm long by 1.6-3.2 (2.3)µm wide; adjacent to the

TABLE 3. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium ennuensis* Dehority, 1996 f. *ennuensis* f. nov. recovered from the euro, *Macropus robustus erubescens*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	56.2	6.90	12.3	37.6	70.4	80
Width, W	26.4	3.10	11.7	20.0	33.6	80
Shape index (L/W ratio)	2.1	0.23	10.8	1.5	3.0	80
Macronucleus						
Length	9.6	1.90	19.7	5.6	15.2	80
Width	6.4	1.13	17.7	4.0	8.8	80
Micronucleus						
Length	2.4	0.54	22.2	1.6	3.2	34
Width	1.8	0.46	25.2	0.8	3.2	34
Oral apparatus						
Vestibulum width	11.8	1.67	14.2	8.8	15.2	80
Vestibulum depth	19.0	2.60	13.7	13.6	27.2	77
Cytostome width	2.1	0.39	18.6	1.6	2.4	69
Length of oral cilia	6.7	0.86	13.0	5.6	10.4	52
Somatic ciliature						
Length of somatic cilia	6.7	0.98	14.6	4.0	10.4	63
Miscellaneous						
No Longitudinal grooves, Left side	10.3	0.90	8.7	8	12	74
No Longitudinal grooves, Right side	8.0	0.73	9.1	7	10	69
Width between longitudinal grooves	3.4	0.53	15.7	2.4	4.8	79
Depth VB	5.0	0.85	16.8	3.2	7.2	80
Cytoproct length	2.5	0.43	16.8	1.6	3.2	69

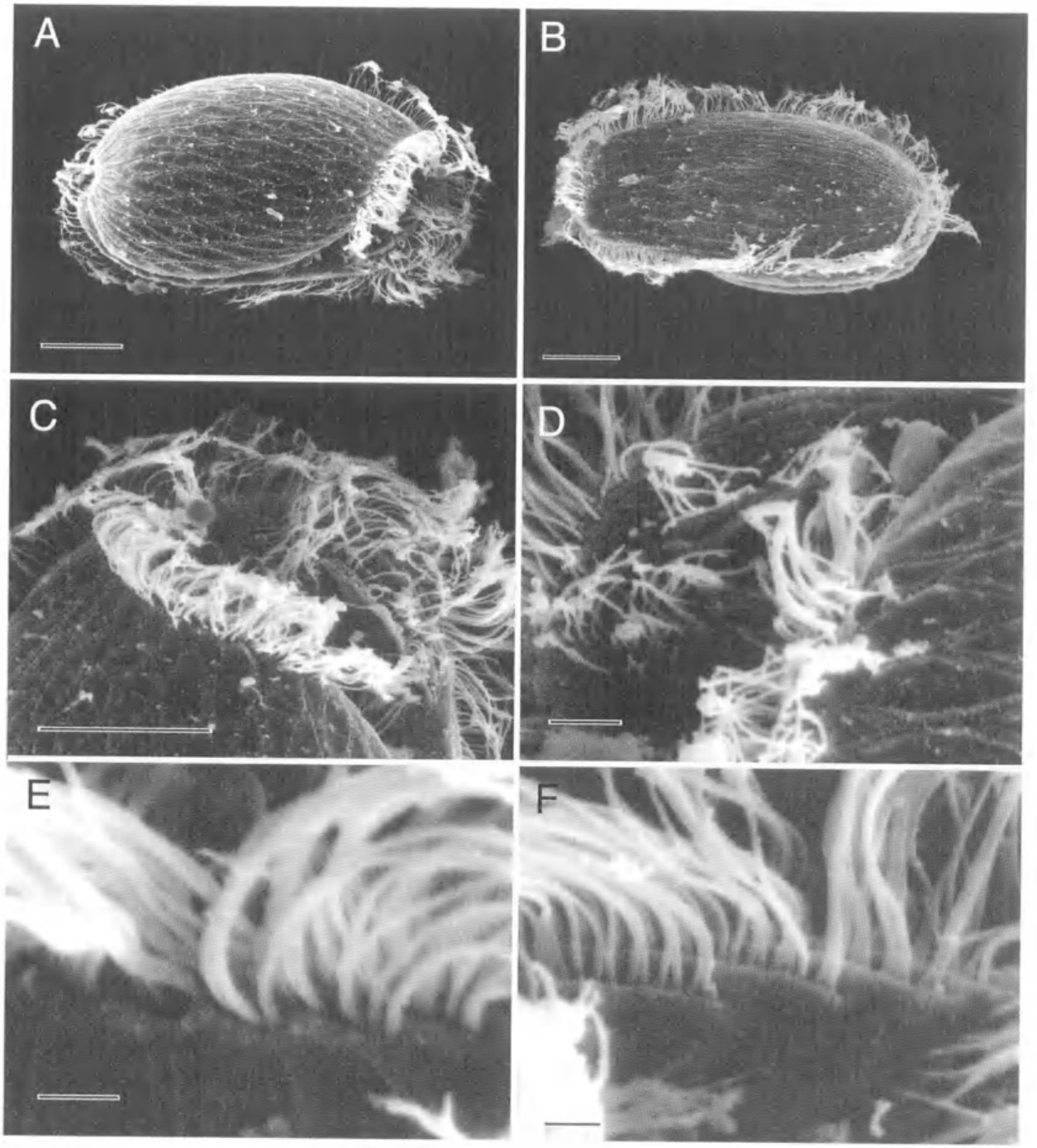

FIG. 8. Scanning electron micrographs of *Macropodinium ennuensis* f. *dentis* f. nov. A, right view, scale bar = 10μm. B, left view, scale bar = 10μm. C, anterior right view, scale bar = 10μm. D, preoral cilia, scale bar = 1μm. E, adoral cilia, scale bar = 1 μm. F, somatic cilia, scale bar = 1μm.

macronucleus. Vestibulum bent conical; 7.2-18.4 (11.1)μm wide by 11.2-22.4 (18.6)μm deep; opening subapically, directed anterioventrally. Somatic ciliation 4.8-8.0 (6.2)μm long; adoral cilia 4.8-8.0 (6.2)μm long. Pellicular diamond pattern uniform; left side bears 9-12 (11.3)longitudinal grooves; right side bears 9-12 (10.2) longitudinal grooves; longitudinal grooves 2.4-4.0 (3.1)μm apart. DVG shallow dorsally and ventrally. DB absent; VB prominent, 3.2-6.4 (4.8)μm deep. Single tooth-like projection within DVG near ventral base of vestibulum. Cytoproct circular; 1.6-3.2 (2.1)μm deep; opening posterior.

TABLE 4. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium ennuensis* Dehority, 1996 f. *dentis* f. nov. recovered from the common wallaroo, *Macropus robustus robustus*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	53.3	5.98	11.2	40.0	70.4	30
Width, W	28.8	2.57	8.93	24.0	32.8	30
Shape index (L/W ratio)	1.9	0.17	9.37	1.6	2.3	30
Macronucleus						
Length	10.1	1.27	12.6	8.0	12.8	30
Width	6.8	0.98	14.5	5.6	9.6	30
Micronucleus						
Length	2.7	0.39	14.6	2.4	3.2	30
Width	2.3	0.35	15.2	1.6	3.2	30
Oral apparatus						
Vestibulum width	11.1	2.25	20.2	7.2	18.4	29
Vestibulum depth	18.6	2.60	14.0	11.2	22.4	29
Cytostome width	2.1	0.40	19.6	1.6	2.4	26
Length of oral cilia	6.2	0.99	16.0	4.8	8.0	19
Somatic ciliature						
Length of somatic cilia	6.2	0.90	14.4	4.8	8.0	21
Miscellaneous						
No Longitudinal grooves, Left side	11.3	0.79	7.0	9	12	30
No Longitudinal grooves, Right side	10.2	0.85	8.3	9	12	30
Width between longitudinal grooves	3.1	0.37	12.0	2.4	4.0	30
Depth VB	4.9	0.66	13.6	3.2	6.4	29
Cytoproct length	2.1	0.47	22.5	1.6	3.2	21

REMARKS. This forma was also recorded from *Macropus robustus robustus* at Kilclooney Stn., Qld, 18°50'S, 145°48'E.

PREVALENCE. Specimens recovered from 3 (19%) of 16 hosts examined.

Macropodinium yalanbense Dehority, 1996 (Figs 9, 10D; Table 5)

MATERIAL. Voucher specimens from the eastern-grey kangaroo, Shaw, 1790: QMG463145, St. George, Qld, 28°01'S, 148°35'E; the western-grey kangaroo, *Macropus fuliginosus melanops* (Desmarest, 1817), QMG463146, Collie, WA, 33°21'S, 116°09'E; the common wallaroo, *Macropus robustus* Gould, 1841 (subspecies unknown), QMG463147, Brisbane, Qld, 27°28'S, 153°01'E; and the red-necked wallaby, *Macropus rufogriseus* (Desmarest, 1817), QMG463148, Brisbane, Qld, 27°28'S, 153°01'E.

DIFFERENTIAL DIAGNOSIS. *Ma. yalanbense* was the first *Macropodinium* species described which lacked both DB and VB; all previously described species possessed either or both features.

DESCRIPTION. Body oval to slightly reniform; 40.8-77.6 (58.4)µm long, 19.2-37.6 (28.2)µm deep, shape index (L/D) 1.5-2.9 (2.1); right side abbreviated compared to left side. Single macronucleus, spherical to ovoid; 6.4-16.8 (10.6)µm long by 4.8-14.4 (7.2)µm wide; located ventral to vestibulum. Single micronucleus, spherical to ovoid; 1.6-5.6 (2.9)µm long by 1.6-4.8 (2.3)µm wide; adjacent to the macronucleus. Vestibulum bent conical; 6.4-20.8 (13.3)µm wide by 11.2-24.8 (17.5)µm deep; opening subapically, directed anterioventrally. Somatic ciliation 4.8-9.6 (6.9)µm long; adoral cilia 4.8-8.8 (6.5)µm long. Pellicular diamond pattern uniform; left side bears 8-12 (10.6) longitudinal grooves; right side bears 8-12 (10.1) longitudinal grooves; longitudinal grooves 2.4-4.0 (3.1)µm apart. DVG shallow dorsally and ventrally. DB and VB absent. Ornamentations absent. Cytoproct slot-shaped; 1.6-4.0 (2.7)µm deep; opening posterior.

REMARKS. This species is also found in the western grey kangaroo, *Macropus fuliginosus*

TABLE 5. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium yalanbense* Dehority, 1996. recovered from the eastern and western grey kangaroos, *Macropus giganteus* and *Macropus fuliginosus*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	58.4	7.85	13.5	40.8	77.6	140
Width, W	28.2	3.35	11.9	19.2	37.6	140
Shape index (L/W ratio)	2.1	0.29	14.1	1.5	2.9	140
Macronucleus						
Length	10.6	1.91	18.0	6.4	16.8	140
Width	7.2	1.29	18.0	4.8	14.4	140
Micronucleus						
Length	2.9	0.63	21.8	1.6	5.6	76
Width	2.3	0.59	26.1	1.6	4.8	76
Oral apparatus						
Vestibulum width	13.3	2.59	19.5	6.4	20.8	140
Vestibulum depth	17.5	2.81	16.0	11.2	24.8	138
Cytostome width	2.7	0.44	16.4	1.6	3.2	138
Length of oral cilia	6.5	0.88	13.6	4.8	8.8	96
Somatic ciliature						
Length of somatic cilia	6.9	0.92	13.3	4.8	9.6	107
Miscellaneous						
No Longitudinal grooves, Left side	10.6	0.65	6.1	8	12	134
No Longitudinal grooves, Right side	10.1	0.93	9.2	8	12	137
Width between longitudinal grooves	3.1	0.37	11.7	2.4	4.0	139
Cytoproct length	2.7	0.46	17.1	1.6	4.0	139

melanops (Desmarest, 1817) from Collie, WA 33°21'S, 116°09'E and Port Augusta, SA, 32°30'S, 137°46'E; the Kangaroo Is. kangaroo, *Macropus fuliginosus fuliginosus* (Desmarest, 1817) from Penneshaw, SA, 35°43'S, 137°56'E; the eastern-grey kangaroo, *Macropus giganteus*, Shaw, 1790 from Bendigo, Vic, 36°45'S, 144°16'E and Wee Jasper, NSW, 35°07'S, 148°40'E.

PREVALENCE. Specimens recovered from 6 (29%) of 21 western-grey kangaroos, 3 (60%) of 5 Kangaroo Is kangaroos, 11 (26%) of 43 eastern-grey kangaroos, 4 (11%) of 36 wallaroos and 1 (3%) of 32 red-necked wallabies examined.

Macropodinium hallae sp. nov.
(Figs 11, 17A; Table 6)

ETYMOLOGY. For our colleague and helminthologist, Kathryn Hall.

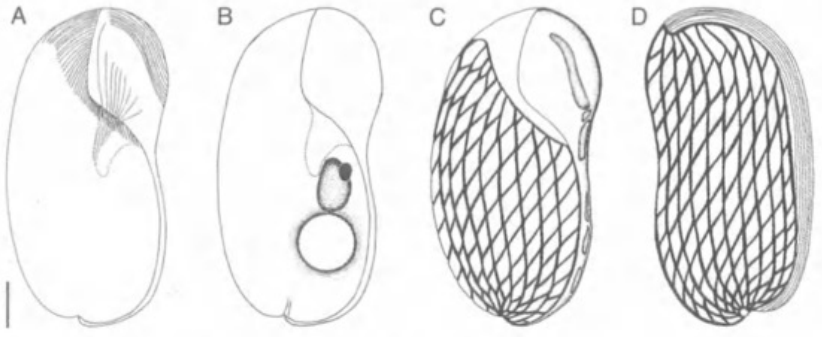

FIG. 9. Morphology and infraciliature of *Macropodinium yalanbense* Dehority, 1996. A, infraciliature. B, internal morphology. C, right view, surface features. D, left view, surface features. Scale bar = 10μm.

MATERIAL. HOLOTYPE QMG463143, from the tammar wallaby, *Macropus eugenii* (Desmarest, 1817), Penneshaw, SA, 35°43'S, 137°56'E.

DIFFERENTIAL DIAGNOSIS. Five other *Macropodinium* species have prominent DB and VB, namely *marai*, *baldense*, *setonixium*, *moiri* and *bicolor*. *Ma. hallae* sp. nov. can be readily distinguished from the former three species on the basis of size

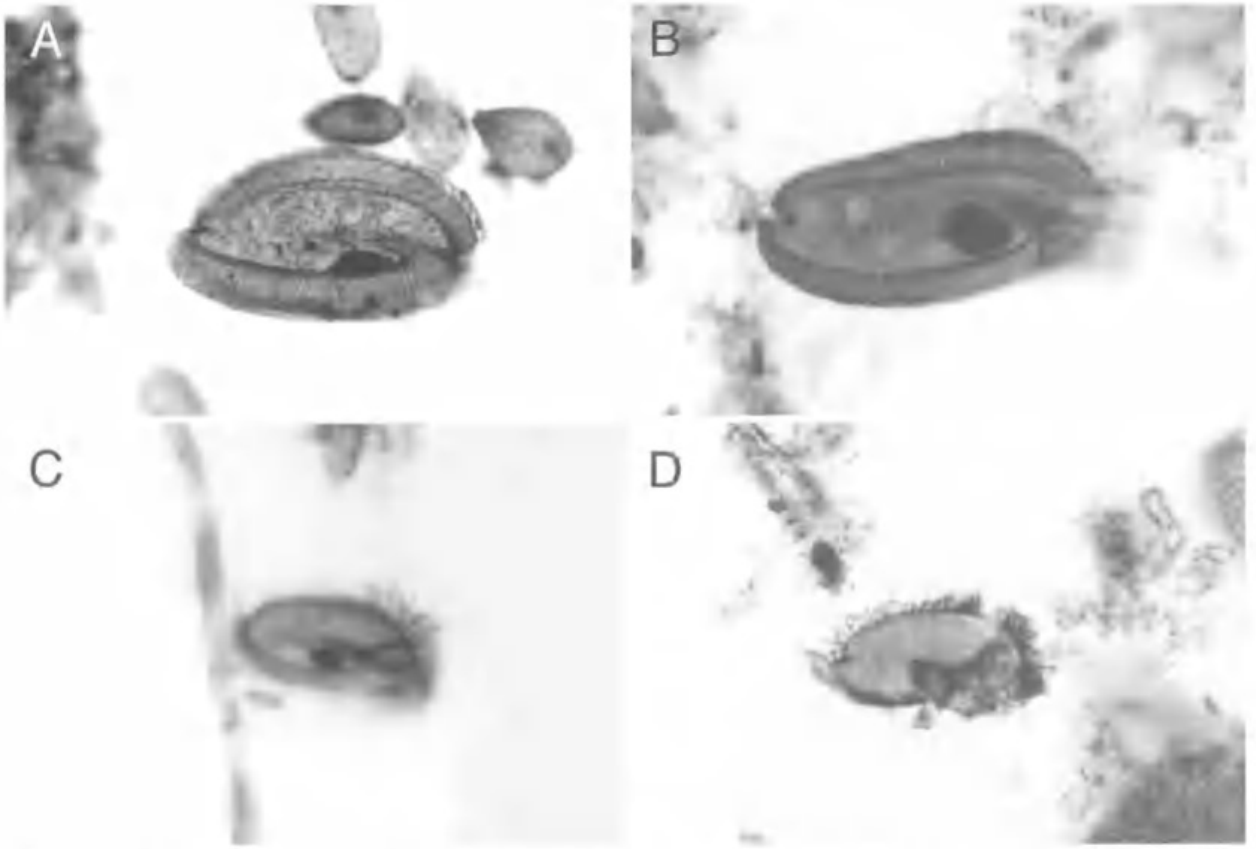

FIG. 10. Light micrographs of *Macropodinium* spp. A, *Ma. moiri*. B, *Ma. setonixium*. C, *Ma. ennuensis*. D, *Ma. yalanbense*. Scale bars = 10μm.

and shape; all are small, oval species whereas *Ma. hallae* sp. nov. is a large, oval to oblong shaped species. *Ma. hallae* sp. nov. can be distinguished from *Ma. moiri* by the ornamentation (strongly crenulate right dorsal margin *vs* weakly flange-like right dorsal margin) and pellicular windows (entirely absent *vs* well-developed in the DVG). *Ma. hallae* sp. nov. can be distinguished from *Ma. bicolor* on the shape (oval *vs* broad with a prominent tail) and ornamentation (crenulate *vs* spine-like).

DESCRIPTION. Body oval to reniform; 53.6-79.2 (63.3)μm long, 25.6-39.2 (33.4)μm deep, shape index (L/D) 1.7-2.1 (1.9); right side slightly abbreviated compared with left side. Single macronucleus, oval to globotriangular; 8.8-14.4 (12.3)μm long by 5.6-12.0 (7.8)μm wide; located ventral to the vestibulum. Single micronucleus, oval to round; 2.4-4.0 (2.7)μm long by 1.6-2.4 (2.2)μm wide; generally adjacent to the macronucleus. Vestibulum funnel-like; 10.4-15.2 (12.4)μm wide by 16.0-23.2 (19.8)μm deep; opening subapically, directed anterioventrally. Cytopharynx composed of short rods issue directly from cytostome. Somatic ciliation 5.6-8.8 (6.9)μm long; adoral cilia 4.8-8.8 (6.2)μm long. Pellicular diamond pattern uniform; left side bears 8-10 (9.2) longitudinal

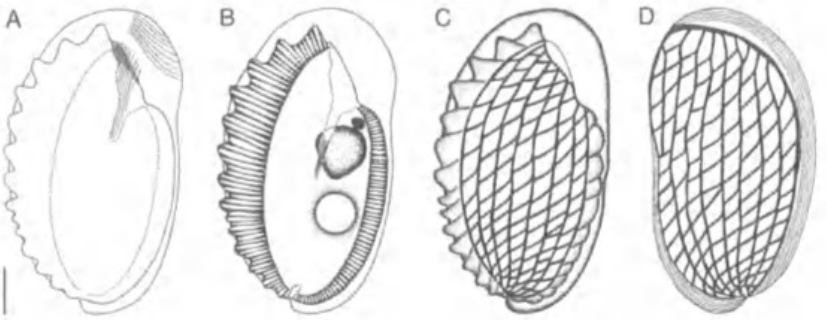

FIG. 11. Morphology and infraciliature of *Macropodinium hallae* sp. nov. A, infraciliature. B, internal morphology. C, right view, surface features. D, left view, surface features. Scale bar = 10μm.

TABLE 6. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium hallae* sp. nov. recovered from the tammar wallaby, *Macropus eugenii*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	63.3	7.65	12.1	53.6	79.2	20
Width, W	33.4	3.98	11.9	25.6	39.2	20
Shape index (L/W ratio)	1.9	0.13	6.6	1.7	2.1	20
Macronucleus						
Length	12.3	1.61	13.1	8.8	14.4	20
Width	7.8	1.58	20.4	5.6	12.0	20
Micronucleus						
Length	2.7	0.63	23.0	2.4	4.0	7
Width	2.2	0.39	18.0	1.6	2.4	7
Oral apparatus						
Vestibulum width	12.4	1.43	11.6	10.4	15.2	20
Vestibulum depth	19.8	1.91	9.7	16.0	23.2	20
Cytostome width	1.6	0.32	19.2	0.8	2.4	20
Length of oral cilia	6.2	1.00	16.3	4.8	8.8	16
Somatic ciliature						
Length of somatic cilia	6.9	0.83	12.0	5.6	8.8	18
Miscellaneous						
No Longitudinal grooves, Left side	9.2	0.66	7.2	8	10	17
No Longitudinal grooves, Right side	7.8	0.58	7.5	7	9	16
Width between longitudinal grooves	3.4	0.44	12.9	2.4	4.0	19
Depth DB	8.6	1.18	13.7	5.6	9.6	10
Depth VB	3.8	1.09	28.9	2.4	6.4	19
Cytoproct length	3.3	0.73	22.2	2.4	4.8	20

grooves; right side bears 7-9 (7.8) longitudinal grooves; longitudinal grooves 2.4-4.0 (3.4)µm apart. DVG deep dorsally and ventrally. DB prominent 5.6-9.6 (8.6)µm deep; VB prominent 2.4-6.4 (3.8)µm deep. Crenulate ornamentations right dorsal, flange ornamentations right ventral. Cytoproct slot-shaped; 2.4-4.8 (3.3)µm deep; opening left.

PREVALENCE. Specimens recovered from 2 (14%) of 14 hosts examined.

Macropodinium ocallaghani sp. nov.
(Figs 12, 13, 17C; Table 7)

ETYMOLOGY. For Michael O'Callaghan who, apart from considerable contributions to wildlife parasitology, also helped to recover this species.

MATERIAL. HOLOTYPE QMG463144, from the tammar wallaby, *Macropus eugenii* (Desmarest, 1817), Penneshaw, SA, 35°43'S, 137°56'E.

DIFFERENTIAL DIAGNOSIS. Aside from *Ma. ocallaghani* sp. nov., only *Ma. yalanbense* lacks both DB and VB. *Ma. ocallaghani* sp. nov. can be distinguished from *Ma. yalanbense* by the structure of the oral aperture which is limited by the dorsal DVG in the former species but not in the latter species.

DESCRIPTION. Body reniform; 38.4-72.8 (56.5)µm long, 20.8-33.6 (26.1)µm deep, shape index (L/D) 1.5-3.0 (2.2); right side abbreviated compared with left side. Single macronucleus, oval to globotriangular; 5.6-16.0 (10.6)µm long by 4.8-9.6 (7.1)µm wide; located ventral to the vestibulum. Single micronucleus, oval to round; 1.6-3.2 (2.2)µm long by 0.8-2.4 (1.7)µm wide; generally adjacent to the macronucleus. Vestibulum bent conical; 10.4-16.0 (12.6)µm wide by 16.0-25.6 (20.2)µm deep; opening subapically, directed anterioventrally. Somatic ciliation 4.0-8.8 (6.7)µm long; adoral cilia 3.2-8.0 (6.7)µm long. Pellicular diamond pattern uniform; left side bears 7-11 (9.6) longitudinal grooves; right side bears 7-12 (8.5) longitudinal grooves; longitudinal grooves 2.4-4.0 (3.0)µm apart. DVG shallow dorsally and ventrally; pellicular windows on DVG anterior and dorsal

TABLE 7. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium ocallanghani* sp. nov. recovered from the tammar wallaby, *Macropus eugenii*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	56.5	6.90	12.2	38.4	72.8	70
Width, W	26.1	2.83	10.9	20.8	33.6	70
Shape index (L/W ratio)	2.2	0.25	11.5	1.5	3.0	70
Macronucleus						
Length	10.6	2.15	20.4	5.6	16.0	70
Width	7.1	1.12	15.8	4.8	9.6	70
Micronucleus						
Length	2.2	0.43	19.5	1.6	3.2	36
Width	1.7	0.36	20.6	0.8	2.4	36
Oral apparatus						
Vestibulum width	12.6	1.46	11.6	10.4	16.0	70
Vestibulum depth	20.2	2.08	10.3	16.0	25.6	70
Cytostome width	1.7	0.31	18.3	0.8	2.4	70
Length of oral cilia	5.7	1.17	20.6	3.2	8.0	60
Somatic ciliature						
Length of somatic cilia	6.7	1.01	15.1	4.0	8.8	66
Miscellaneous						
No Longitudinal grooves, Left side	9.6	0.85	8.8	7	11	58
No Longitudinal grooves, Right side	8.5	1.11	13.0	7	12	58
Width between longitudinal grooves	3.0	0.41	14.0	2.4	4.0	69
Cytoproct length	3.3	1.19	35.9	2.4	8.0	66

left side; DB and VB absent. Cell ornamentations absent. Cytoproct slot-shaped; 2.4-8.0 (3.3)µm deep; opening posterior.

REMARKS. This species also recovered from *Macropus eugenii* from Kangaroo Is., SA, 35°46'S, 137°37'E.

PREVALENCE. Specimens recovered from 12 (86%) of 14 hosts examined.

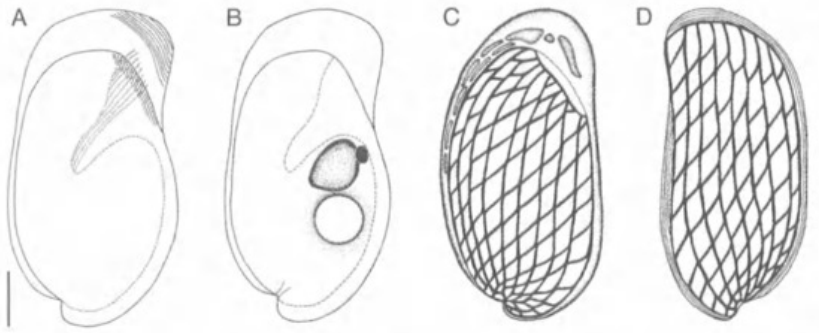

FIG. 12. Morphology and infraciliature of *Macropodinium ocallaghani* sp. nov. A, infraciliature. B, internal morphology. C, right view, surface features. D, left view, surface features. Scale bar = 10µm.

Macropodinium petrogale sp. nov.
(Figs 14, 15, 17C; Table 8)

ETYMOLOGY. For the generic name of its rock-wallaby host.

MATERIAL. HOLOTYPE QMG463140, from Godman's rock-wallaby, *Petrogale godmani* Thomas, 1923, Church Hill, Curraghmore Stn., Qld, 16°27'S, 145°11'E.

DIFFERENTIAL DIAGNOSIS. Aside from *Ma. petrogale* sp. nov., only *Ma. tricresta* and *Ma. spinosus* possess a DB but lack a VB. *Ma. petrogale* sp. nov. can be readily distinguished from both by the absence of ornamentations which are prominent in both *tricresta* and *spinosus*.

DESCRIPTION. Body truncated elliptical, narrowing posteriorly; modest anterio-leftward bend; 36.0-52.0 (44.3)µm long by 19.2-30.4 (24.7)µm deep, shape index

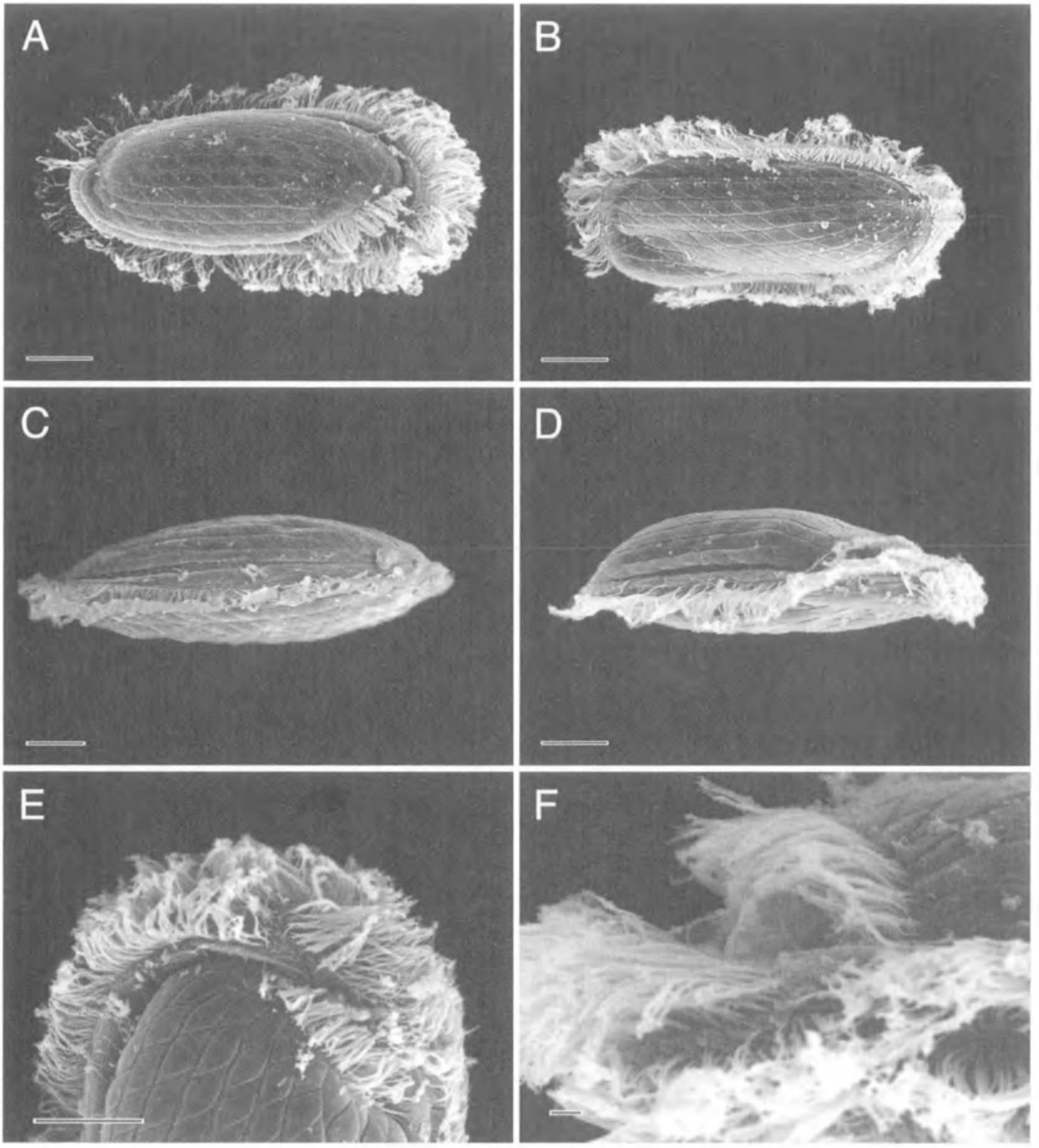

FIG 13. Scanning electron micrographs of *Macropodinium ocallaghani* sp. nov. A, right view, scale bar = 10μm. B, left view, scale bar = 10μm. C, dorsal view, scale bar = 10μm. D, ventral view, scale bar = 1μm. E, anterior right view, scale bar = 1μm. F, anterior dorsal view, scale bar = 1μm.

(L/D) 1.4-2.4 (1.8); right side only slightly abbreviated compared with left side. Single macronucleus spherical to globo-triangular; 6.4-10.4 (8.2)μm long by 4.0-7.2 (5.6)μm wide; located ventral to the vestibulum. Single micronucleus spherical to ovoid; 1.6-2.4 (2.1)μm long by 1.6-2.4 (1.7)μm wide, adjacent to or obscured by the macronucleus. Vestibulum bent conical; 9.6-14.4 (12.2)μm wide by 11.2-21.6 (18.0)μm deep; opening apical, directed anterioventrally; cytostome 0.8-2.4 (1.7)μm wide. Somatic cilia 3.2-7.2 (4.5)μm long; adoral cilia 3.2-7.2 (4.4)μm long. Pellicular diamond pattern uniform; left side bears 9-11 (9.9)

TABLE 8. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium petrogale* sp. nov. recovered from Godman's rock-wallaby, *Petrogale godmani*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	44.3	4.51	10.2	36.0	52.0	30
Width, W	24.7	2.83	11.5	19.2	30.4	30
Shape index (L/W ratio)	1.8	0.20	11.0	1.4	2.4	30
Macronucleus						
Length	8.2	1.18	14.4	6.4	10.4	30
Width	5.6	0.85	15.3	4.0	7.2	30
Micronucleus						
Length	2.1	0.41	12.0	1.6	2.4	14
Width	1.7	0.29	17.0	1.6	2.4	14
Oral apparatus						
Vestibulum width	12.2	1.34	11.0	9.6	14.4	30
Vestibulum depth	18.0	2.37	13.2	11.2	21.6	30
Cytostome width	1.7	0.38	22.9	0.8	2.4	30
Length of oral cilia	4.4	0.21	24.6	3.2	7.2	26
Somatic ciliature						
Length of somatic cilia	4.5	1.09	24.2	3.2	7.2	28
Miscellaneous						
No Longitudinal grooves, Left side	9.9	0.58	5.9	9	11	30
No Longitudinal grooves, Right side	8.0	0.56	6.9	7	9	30
Width between longitudinal grooves	3.2	0.59	18.5	2.4	4.0	12
Depth DB	4.0	0.65	16.1	2.4	4.8	30
Cytoproct length	3.0	0.53	17.5	2.4	4.0	30

longitudinal grooves, right side bears 7-9 (8.0) longitudinal grooves; longitudinal grooves 2.4-4.0 (3.2)µm apart. DVG shallow dorsally and ventrally. DB prominent 2.4-4.8 (4.0)µm deep. VB absent. Ornamentations absent. Cytoproct cup-shaped; 2.4-4.0 (3.0)µm deep; opening posterior.

PREVALENCE. Specimens recovered from 3 (100%) of 3 hosts examined.

Macropodinium titan sp. nov.
(Figs 16, 17D; Table 9)

ETYMOLOGY. For its great size and robust shape.

MATERIAL. HOLOTYPE QMG463151, from Godman's rock-wallaby, *Petrogale godmani* Thomas, 1923, Church Hill, Curraghmore Stn, Qld, 16°27'S, 145°11'E.

DIFFERENTIAL DIAGNOSIS. The only other *Macropodinium* species which possesses a VB but lacks a DB is *Ma. ennuensis* (both forms). *Ma. titan* sp. nov. and *Ma. ennuensis* can be readily distinguished on size (the former is much larger), shape (the former is oval, the latter reniform), cell curvature (the right side envelops the left side in the former species whereas it does not in the latter), host occurrence (rock-wallaby *vs* wallaroo) and absence of the non-patterned strip on the left surface of *Ma. ennuensis*.

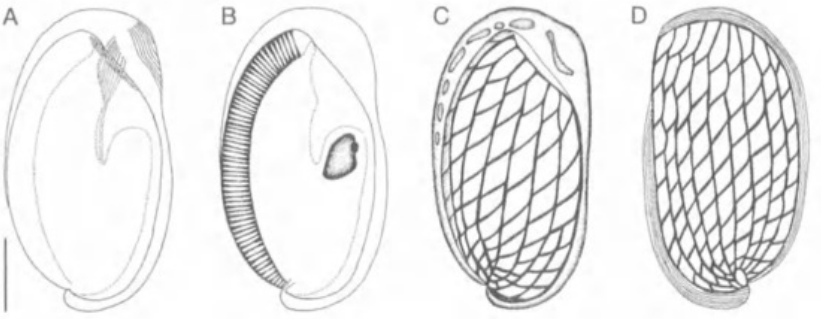

FIG. 14. Morphology and infraciliature of *Macropodinium petrogale* sp. nov. A, infraciliature. B, internal morphology. C, right view, surface features. D, left view, surface features. Scale bar = 10µm.

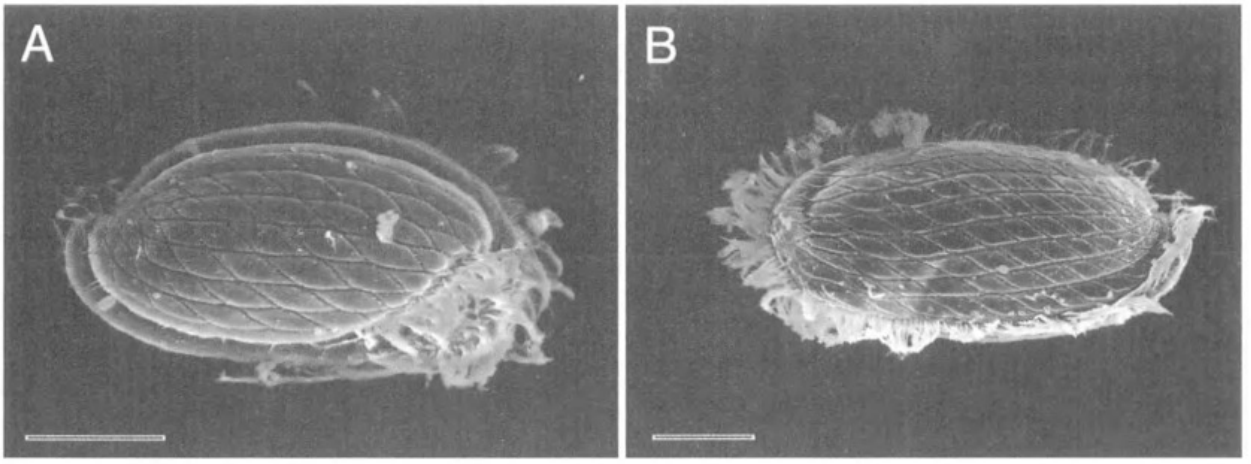

FIG. 15. Scanning electron micrographs of *Macropodinium petrogale* sp. nov. A, right view. B, left view. Scale bars = 10µm.

TABLE 9. Morphometric characterisation of the macropodiniid ciliate, *Macropodinium titan* sp. nov. recovered from Godman's rock-wallaby, *Petrogale godmani*; x: mean; SD: standard deviation; CV: coefficient of variation; min: minimum; max: maximum; n: number of observations.

Character	x	SD	CV	min	max	n
Body dimensions						
Length, L	88.1	10.10	11.5	64.8	104.0	13
Width, W	60.4	3.57	5.9	52.8	64.0	13
Shape index (L/W ratio)	1.5	0.16	10.7	1.2	1.7	13
Macronucleus						
Length	15.7	2.86	18.2	11.2	21.6	13
Width	11.0	1.05	9.6	9.6	12.8	13
Micronucleus						
Length	6.2	1.32	21.5	4.8	8.8	13
Width	5.0	0.87	17.5	4.0	7.2	13
Oral apparatus						
Vestibulum width	22.7	3.26	14.4	15.2	26.4	13
Vestibulum depth	36.5	5.15	14.1	28.8	44.0	13
Cytostome width	2.6	0.38	14.5	2.4	3.2	13
Length of oral cilia	5.5	1.49	27.2	3.2	8.8	13
Somatic ciliature						
Length of somatic cilia	5.4	1.00	18.7	4.0	7.2	10
Miscellaneous						
No Longitudinal grooves, Left side	11.7	0.95	8.1	10	13	13
No Longitudinal grooves, Right side	19.7	1.32	6.7	18	22	13
Width between longitudinal grooves, Left side	3.7	0.46	12.4	3.2	4.0	3
Width between longitudinal grooves, Right side	2.4	0.80	33.3	1.6	3.2	3
Depth VB	13.8	1.57	11.4	11.2	16.0	13
Width non-patterned stripe	9.9	1.29	13.0	8.0	12.8	13
Cytoproct length	5.9	0.70	11.8	4.8	7.2	13

DESCRIPTION. Body ovoid; 64.8-104.0 (88.1)µm long by 52.8-64.0 (60.4)µm deep, shape index (L/D) 1.2-1.7 (1.5); right side not abbreviated compared to left side. Single macronucleus ovoid to pyriform; 11.2-21.6 (15.7)µm long by 9.6-12.8 (11.0)µm wide; located ventral to vestibulum. Single micronucleus spherical to ovoid; 4.8-8.8 (6.2)µm

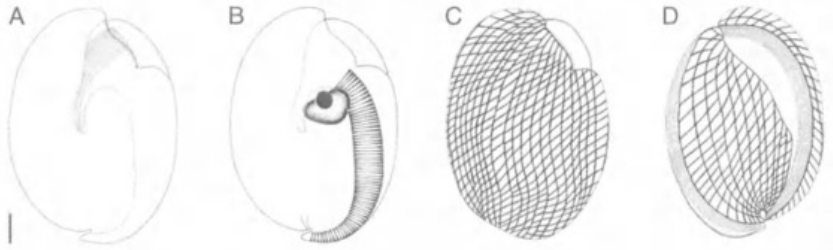

FIG. 16. Morphology and infraciliature of *Macropodinium titan* sp. nov. A, infraciliature. B, internal morphology. C, right view, surface features. D, left view, surface features. Scale bar = 10μm.

longitudinal grooves 3.2-4.0 (3.7)μm apart; right-side longitudinal grooves 1.6-3.2 (2.4)μm apart. Right side envelops dorsal left side; left side convex, curving leftward ventrally. DVG deep ventrally. DB absent. VB prominent 8.0-12.8 (9.9)μm deep. Obvious unpatterned, non-ciliated strip separates left side patterning from dorsal somatic ciliary

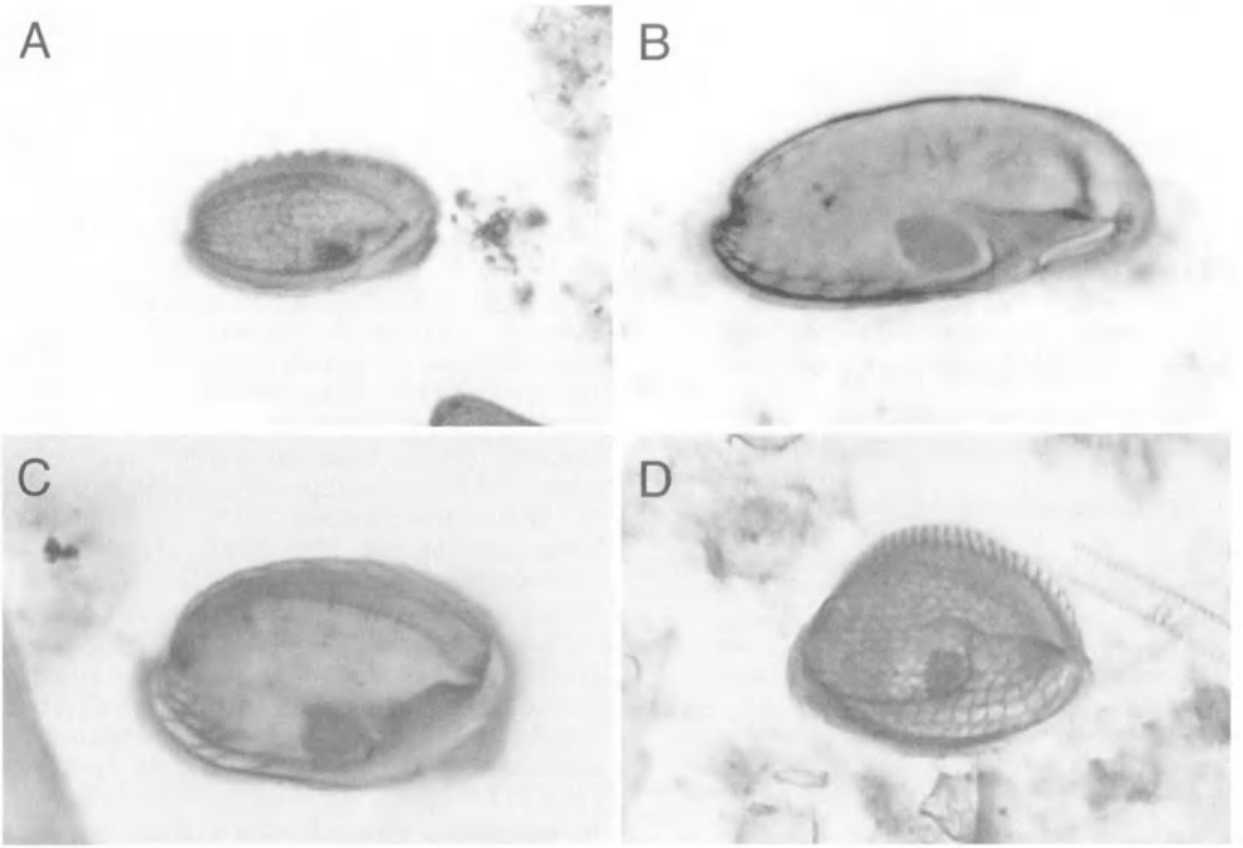

FIG 17. Light micrographs of *Macropodinium* spp. A, *Ma. hallae*. B, *Ma. ocallaghani*. C, *Ma. petrogale*. D, *Ma. titan*. Scale bars = 10μm.

long by 4.0-7.2 (5.0)μm wide; adjacent to or obscured by macronucleus. Vestibulum bent conical; 15.2-26.4 (22.7)μm wide by 28.8-44.0 (36.5)μm deep; opening apical, directed anterioventrally, surrounded by prominent circumoral collar; cytostome 2.4-3.2 (2.6)μm wide. Somatic cilia 4.0-7.2 (5.4)μm long; adoral cilia 3.2-8.8 (5.5)μm long. Pellicular diamond pattern non-uniform; left side bears 10-13 (11.7) longitudinal grooves, right side bears 18-22 (19.7) longitudinal grooves; left-side field. Ornamentations absent. Cytoproct slot-shaped; 4.8-7.2 (5.9)μm deep; opening posteriorly; ventral cytoproct spine present.

PREVALENCE. Specimens recovered from 2 (66%) of 3 hosts examined.

KEY TO THE SPECIES OF *MACROPODINIUM*

1. Prominent ventral and dorsal bars 2
   Prominent ventral bar, dorsal bar absent 6
   Prominent dorsal bar, ventral bar absent 7

TABLE 10. Comparison of morphometrics of *Macropodinium moiri* and *Ma. setonixium*.

	Ma. moiri Dehority (1996)	*Ma. moiri* Present study	*Ma. setonixium* Dehority (1996)	*Ma. setonixium* Present study
Length				
Range	65-127	60.8-100.8	24-50	24.8-46.4
X	85.2	81.0	38.0	32.1
SD	13.1	12.88	5.9	4.6
Width				
Range	41-73	32.8-48.8	18-40	15.2-24.8
X	54.8	40.8	27.9	18.8
SD	7.0	4.44	4.6	2.148
Shape index				
Range	1.2-2.0	1.4-2.3	1.0-1.6	1.3-2.1
X	1.56	2.0	1.37	1.7
SD	0.18	0.2	0.12	0.2

Both dorsal and ventral bars absent 9

2. Prominent marginal ornamentations on at least one part of the cell 3

No ornamentations, at best slight marginal flange . . . 5

3. Pellicle plates in DVG left dorsal and right ventral . . . . . *maira*

Pellicle plates not wrapped into DVG 4

4. Cell oval with prominent tail; left cell half ornamented . . . . . *bicolor*

Cell reniform without tail; right cell half ornamented . . . . . *hallae*

Cell oval without prominent tail; right cell half ornamented . . . . . *baldense*

5. Cell oval, small (<50μm long), no preoral kineties . . . . . *setonixium*

Cells elongate, large (> 60μm long), preoral kineties . . . . . *moiri*

6. Cell small & reniform, prominent pellicular windows. . . . . *ennuensis*

Cell large, oval and twisted left, lacks pellicular windows *titan*

7. Prominent cell ornamentations 8

No cell ornamentations, small oval ciliate . . . . . *petrogale*

8. Cell wedge-shaped; ventral spines, dorsal flange . . . . . *spinosus*

Cell broadly oval; ventral spines, dorsal crenulations; strong left bend; extracalary dorsal DVG. . . . . *tricresta*

9. Prominent dorsal pellicular windows, limited mouth . . . . . *ocallaghani*

Prominent ventral pellicular windows, entire mouth . . . . . *yalanbense*

DISCUSSION

Macropodinium comprises the most diverse and distinctive component of the ciliate fauna of macropodid marsupials. Collectively (Cameron et al. 2001; present study) we have described 12 species within the genus, including 4 redescriptions of species originally erected by Dehority (1996). Redescriptions were deemed necessary on the basis of staining techniques. Dehority (1996) used methylene blue, methyl green and haematoxylin which failed to resolve the fine differences between the three components of the oral ciliature of *Macropodinium* spp., namely the adoral, vestibular and preoral kinety bands. We used protargol and silver carbonate staining which produced finer resolution of the somatic kineties, dorsal and ventral bars and the pellicular windows. Comparisons of the morphometric descriptors published here and in Dehority (1996) (Tables 10, 11) show that they are broadly in agreement, although with a slight tendency towards the specimens recorded here being smaller. We are therefore confident that we have accurately redescribed 4 of the species presented in Dehority (1996) to include the additional features of the genus first reported in Cameron et al. (2001), namely cell orientation, cell ornamentations and oral ciliation.

TABLE 11. Comparison of morphometrics of *Ma. ennuensis*[1] and *Ma. yalanbense*. [1]*Ma. ennuensis* f. *ennuensis* as this was the form described by Dehority (1996).

	Ma. ennuensis Dehority (1996)	*Ma. ennuensis* Present study1	*Ma. yalanbense* Dehority (1996)	*Ma. yalanbense* Present study
Length				
Range	43-66	37.6-70.4	47-75	40.8-77.6
X	55.3	56.2	62.6	58.4
SD	4.6	6.9	6.9	7.85
Width				
Range	23-33	20.0-33.6	29-41	19.2-37.6
X	28.3	26.4	34.2	28.2
SD	2.6	3.10	2.7	3.35
Shape index				
Range	1.6-2.4	1.5-3.0	1.5-2.2	1.5-2.9
X	1.96	2.1	1.83	2.1
SD	0.16	0.23	0.14	0.29

Intraspecific variation was examined in detail in *Ma. yalanbense* and *Ma. ennuensis* by examination of the morphometric differences between isolates from different host species and subspecies. *Ma. yalanbense* is the only *Macropodinium* species which has been recorded in more than one host species. It has been recovered from native populations of the eastern

TABLE 12. Comparative morphometrics of *Macropodinium yalanbense*, Dehority 1996, isolates from *Macropus giganteus*, *M. fuliginosus melanops* and *M. fuliginosus fuliginosus*; [1]expressed as a range with the mean in parentheses.

Character	*M. giganteus*	*M. f. fuliginosus*	*M. f. melanops*
Body dimensions			
Length, L	40.8-77.6 (61.7)	45.6-63.2 (54.5)	43.2-69.6 (53.5)
Width, W	21.6-37.6 (28.0)	24.8-36.0 (29.3)	19.2-34.4 (27.5)
Shape index (L/W ratio)	1.7-2.9 (2.2)	1.7-2.1 (1.9)	1.5-2.7 (2.0)
Macronucleus			
Length	6.4-16.8 (11.1)	7.2-11.2 (9.3)	7.2-16.8 (10.5)
Width	4.8-10.4 (7.2)	4.8-10.4 (6.8)	4.8-14.4 (7.4)
Micronucleus			
Length	2.4-5.6 (3.0)	1.6-3.2 (2.5)	2.4-4.0 (2.6)
Width	1.6-4.8 (2.4)	1.6-2.4 (1.7)	1.6-3.2 (2.1)
Oral apparatus			
Vestibulum width	8.8-20.8 (14.3)	8.0-18.4 (12.6)	6.4-15.2 (11.3)
Vestibulum depth	11.2-24.8 (18.5)	13.6-22.4 (17.3)	11.2-19.2 (15.3)
Cytostome width	1.6-3.2 (2.7)	1.6-3.2 (2.6)	1.6-3.2 (2.6)
Oral ciliary length	4.8-8.8 (6.5)	4.8-8.8 (6.5)	4.8-7.2 (6.3)
Somatic ciliature			
Somatic ciliary length	4.8-9.6 (7.0)	5.6-8.8 (6.4)	6.4-8.8 (6.9)
Miscellaneous			
No Longitudinal grooves, Left side	8-12 (10.5)	10-12 (10.8)	10-12 (10.8)
No Longitudinal grooves, Right side	8-12 (10.1)	8-12 (9.7)	9-12 (10.6)
Width between longitudinal grooves	2.4-4.0 (3.2)	2.4-4.0 (3.2)	2.4-3.2 (2.9)
Cytoproct length	1.6-3.2 (2.6)	1.6-4.0 (2.7)	2.4-3.2 (2.7)

and western grey kangaroo (*M. giganteus* and *M. fuliginosus*) and captive populations of wallaroos (*M. robustus*) and red-necked wallabies (*M. rufogriseus*). The two native hosts are bigemminate species which diverged during the last ice age due to separation of a formerly widespread species to the eastern and southwestern fringes of the continent (Flannery, 1989). At the end of the ice age, the retreat of the central desert established favourable habitats across southern Australia and the two species now overlap in regions around the South Australian/New South Wales border (Strahan, 1996). No significant differences were found between isolates from the two subspecies of western grey kangaroos (*M. f. fuliginosus* and *M. f. melanops*) or between the two grey kangaroo species (Table 12). These hosts also share the amylovoracid ciliates *Amylovorax dehorityi* and *Bitricha obolata* (Cameron et al., 2000a). Finally, there have been no obvious local acquisitions of ciliates from sympatric macropodids. It is probable that the ciliate fauna found predates the split between the host species. As the habitat and diet of the host probably did not change through the period of isolation (Flannery, 1989), it is possible that there was no selective pressure for differentiation in the ciliate species. Any speciation in the ciliates would therefore be the result of genetic drift rather than directional selection. There are three possible explanations for the observed pattern. First, the ciliates failed to diverge sufficiently due to random chance alone for speciation to result. Secondly, they may have speciated cryptically. Thirdly, one of the lineages of ciliates may have successfully displaced its bigemminate pair in the other host species once the kangaroos were again sympatric. Assessments of genetic variability in the ciliates across a wide geographic range, including the sympatric zone, would greatly contribute to our understanding of the parasite species flow within grey kangaroos.

In contrast, *Ma. ennuensis* which is associated with the wallaroo, *M. robustus*, appears to have differentiated more despite less host divergence. Consistent differences were found between ciliates from the two subspecies of host. *Ma. ennuensis* f. *dentis* from the eastern subspecies (*M. r. robustus*) has an oral spur and 2

more longitudinal grooves on the right cell side than *Ma. ennuensis* f. *ennuensis* from the western subspecies (*M. r. erubescens*). The divergence between the two forms is modest in comparison to the difference between other *Macropodinium* species, and for this reason they have not been erected as separate species. The biogeographic history and distribution of *M. robustus* is not as well understood as *M. giganteus*/*M. fuliginosus* but it is also believed to have been marginalised by ice age expansions of the central deserts of Australia. *M. robustus* is more xerically adapted than either *M. giganteus* or *M. fuliginosus* and thus probably experienced less habitat contraction due to desert expansion (Strahan, 1995). This would explain the failure of the host to speciate but not the divergences within the ciliates. Assessments of the ciliate fauna of the northern wallaroo subspecies, *M. robustus woodwardi*, and the other wallaroo species, *M. antilopinus* and *M. bernadus*, and the genetic structure of their faunas would help explain the evolution of ciliate diversity within the wallaroos.

While most species of *Macropodinium* utilise only a single species of host, several host species harbour more than one species of *Macropodinium*. The quokka (*Setonix brachyurus*) had *Ma. moiri*, *Ma. setonixium* and *Ma. baldense* (Dehority, 1996), the black-striped wallaby (*M. dorsalis*) had *Ma. tricresta* and *Ma. spinosus* (Cameron et al., 2001), the tammar wallaby (*M. eugenii*) had *Ma. hallae* and *Ma. ocallaghani* (present paper) and Godman's rock-wallaby (*Petrogale godmani*) had *Ma. petrogale* and *Ma. titan* (present paper). These 4 hosts have little in common in terms of phylogenetic history or biogeographic distribution. All 4 are considered to be browsers. There are, however, species of browsing macropodids which are host to only a single *Macropodinium* species (*Thylogale billardierii* and *Wallabia bicolor*) (Cameron et al., 2001) or none at all (*M. agilis*, *M. parryi*, *M. rufogriseus*, *Petrogale assimilis*, *P. herberti* and *P. sharmani*). Therefore, simple dietary descriptors alone cannot explain this pattern.

The presence of multiple congeners in a single host species (and indeed single host animals) is common within the trichostome ciliates, and it appears that *Macropodinium* is no different. The reasons for this pattern of co-occurrence are unknown. Within the ophryoscolecids, which are associated with ruminants, multiple species of *Entodinium* commonly occur in domesticated livestock. It has been proposed that this is due to species which have host switched from non-domesticated ruminants such as deer or antelope accumulating over time and being spread globally by livestock trading (Williams & Coleman, 1991). Most macropodid species are sympatric with at most 2 other species, so opportunities for such profligate host switching are therefore less prevalent.

Macropodinium is the most diverse genus of ciliates in macropodid marsupials, in terms of both species diversity and structural complexity. The patterns of their host occurrence may, in some cases, be related to either biogeographic history or dietary preferences of their hosts. However, these conclusions are hampered by a lack of knowledge of phylogenetic relationships between both ciliates and their hosts. Such knowledge will greatly contribute to understanding how the Australian fauna of trichostome ciliates has evolved.

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