

Integrative description of A new *Dactylobiotus* (Eutardigrada: Parachela) from Antarctica that reveals an intraspecific variation in tardigrade egg morphology

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Supplementary Figures and Tables:

Supplementary Figure S1

Study area. a, Antarctica (square: King George Island, arrow: Signy Island). b, King George Island, showing Lake CZO and Lake Jubany of Barton Peninsula and Potter Peninsula respectively.

Supplementary Figure S2

The transverse crests of *Dactylobiotus ovimutans* sp. nov. a, The mouth opening which shows the dorso and the ventromedian transverse crest with pointed tips. b, The mouth opening which shows a pointed tip on the dorsomedian transverse crest only. c, The mouth opening which shows a pointed tip on the dorsomedian transverse crest only. The dorsomedian crest and dorsolateral transverse crest combine. d, The mouth opening which shows a pointed tip on the ventromedian transverse crest only. d: dorsomedian transverse crest, dlc: dorsolateral transverse crest, opa: oval perforated area, v: ventromedian transverse crest, vlc: ventrolateral transverse crest.

Supplementary Figure S3

The cytochrome c oxidase subunit I (COI) alignment of *Dactylobiotus ovimutans* sp. nov. haplotype 1 and 2. A site highlighted in green indicates substitution between haplotype1-*Dactylobiotus* sp. (EF632526) and haplotype 2, yellow indicates substitutions between haplotype 1 and 2.

Supplementary Figure S4

The small ribosome subunit (18S rRNA) alignment of *Dactylobiotus ovimutans* sp. nov. and *D. parthenogeneticus* (HQ604963). Sites highlighted in yellow indicate substitutions.

Supplementary Figure S5

Eggs that DNA extracted in this study. Number indicates the number of processes on the circumference and number in parentheses indicates haplotype 1 or 2.

Supplementary Figure S6

Phase-contrast microscope (PCM) and Scanning Electron Microscope (SEM) images of eggs of *Dactylobiotus* cf. *ovimutans* sp. nov. from Signy Island, Antarctica. a–c, PCM images of eggs with various numbers of processes. d–g, PCM images of processes of eggs. h–i, SEM image of an egg.

Supplementary Table S1

Measurements (in μm) of selected morphological characters of *Dactylobiotus ovimutans* sp. nov. N, the number of specimens; *pt*, the percent ratio of the length of a character to the length of buccal tube; SD, standard deviation; *b*, allometric exponent; *a**, Y-intercept of the regression line of the Thorpe's normalized trait versus buccal tube length; *br*, the percent ratio of the length of the secondary branch to the length of the primary branch of claw.

Supplementary Table S2

Measurements (in μm) of selected morphological characters of eggs of *Dactylobiotus ovimutans* sp. nov. N, the number of specimens; SD, standard deviation.

Supplementary Table S3

Partial gene sequences of cytochrome c oxidase subunit I (COI), small ribosome subunit (18S rRNA), and large ribosome subunit (28S rRNA) of *Dactylobiotus ovimutans* sp. nov.

Supplementary Table S4

Morphometric comparison between *Dactylobiotus ovimutans* sp. nov. and *D. caldarellai*. *pt*, the percent ratio of the length of a character to the length of buccal tube; *br*, the percent ratio of the length of the secondary branch to the length of the primary branch of claw.

Supplementary Table S5

Measurements of *Dactylobiotus* cf. *ovimutans* sp. nov. eggs from Signy Island. All eggs were isolated from the same freshwater sample.

Supplementary Table S6

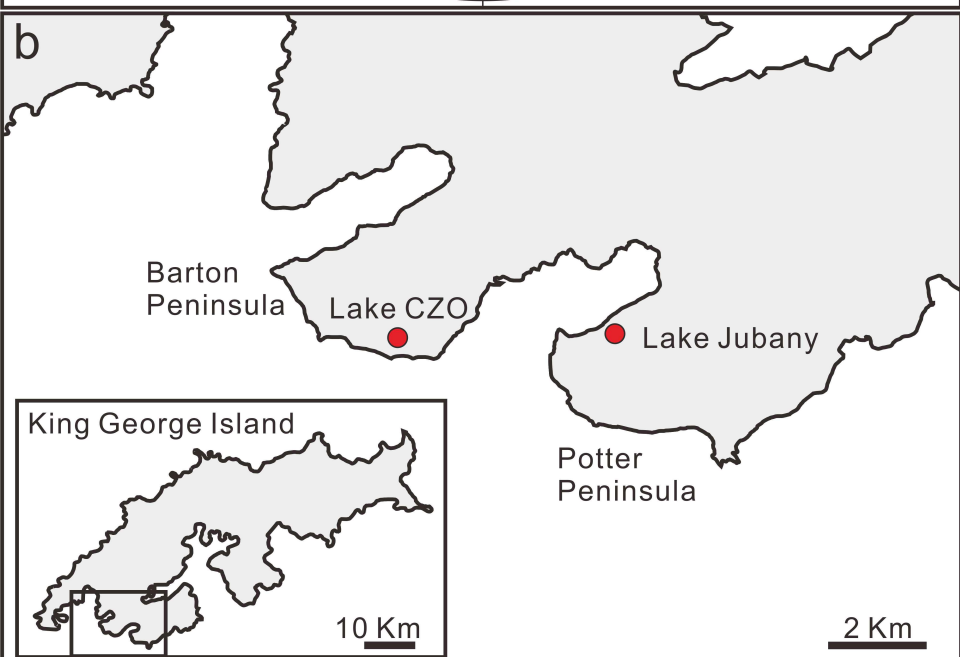
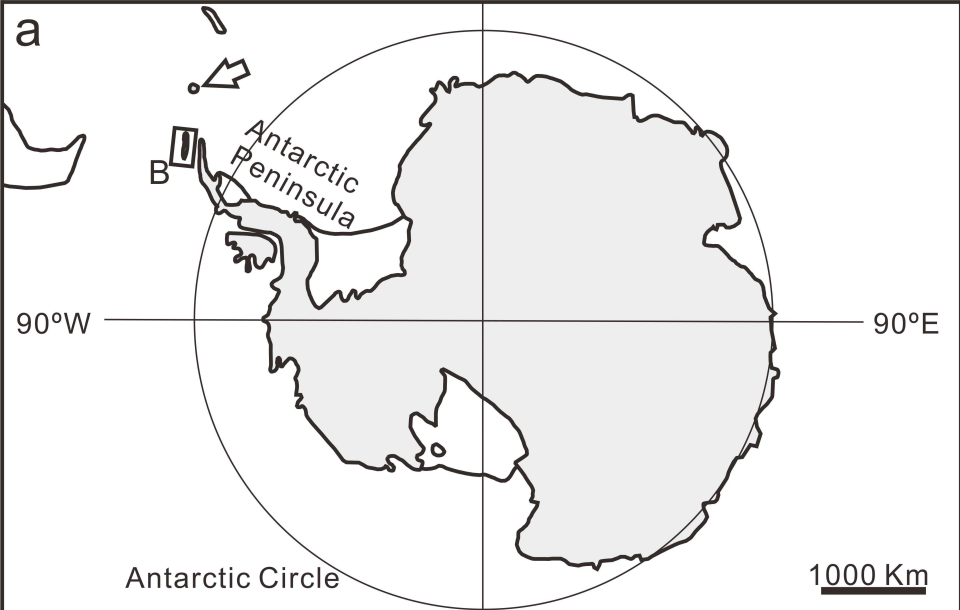
Primers and references for PCR of a cytochrome c oxidase subunit I (COI), a small ribosomal subunit (18S rRNA) and a large ribosomal subunit (28S rRNA) in this study.

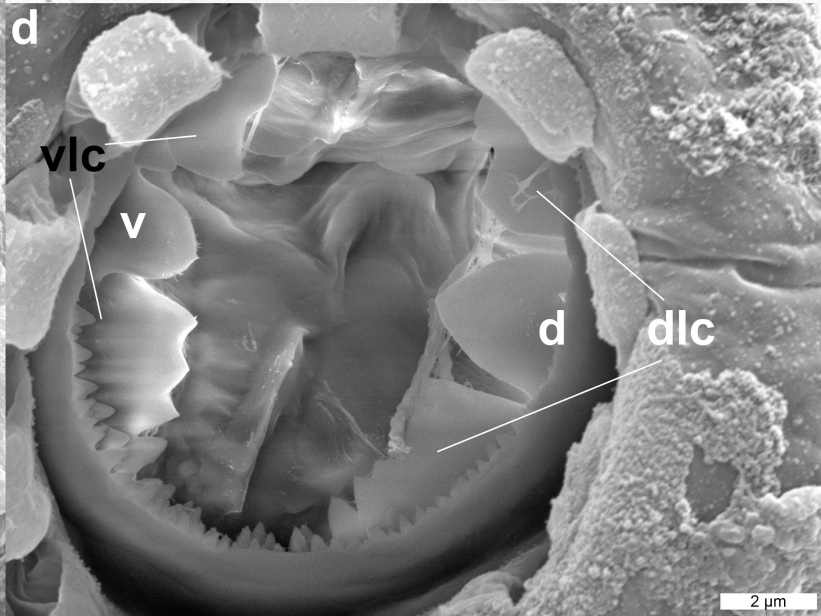
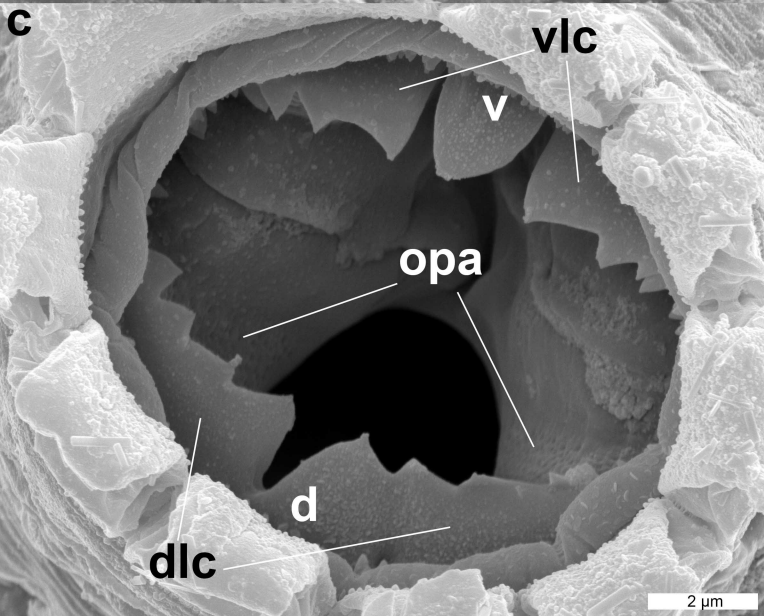
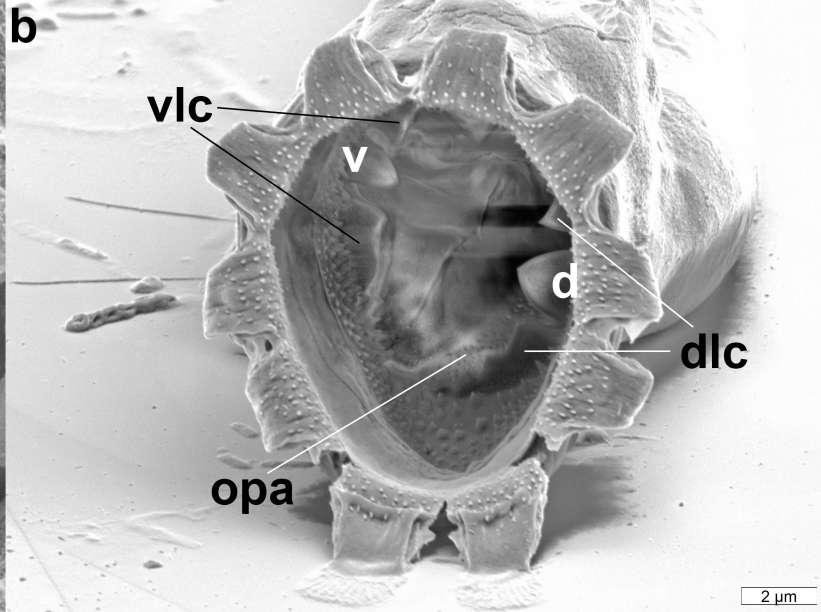
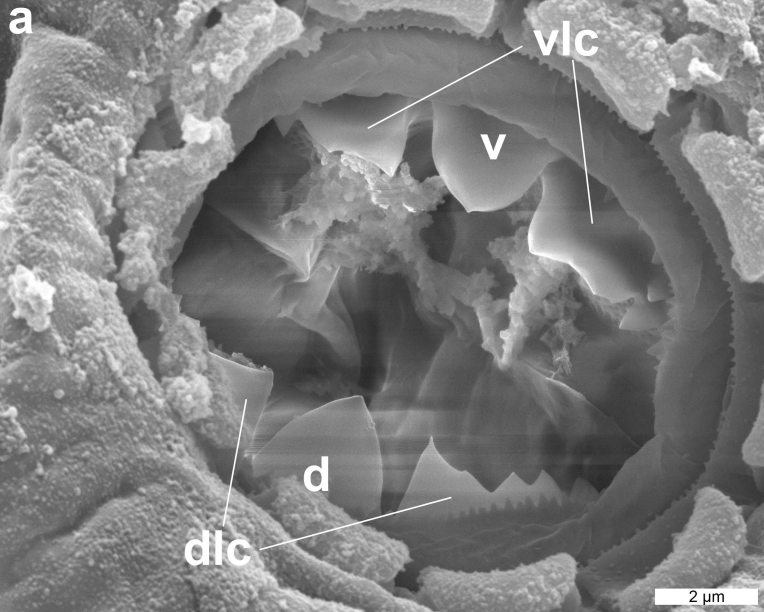
Supplementary Table S7

PCR programmes and references for cytochrome c oxidase subunit I (COI), small ribosomal subunit (18S rRNA) and large ribosomal subunit (28S rRNA) in this study.

Supplementary Movie S1

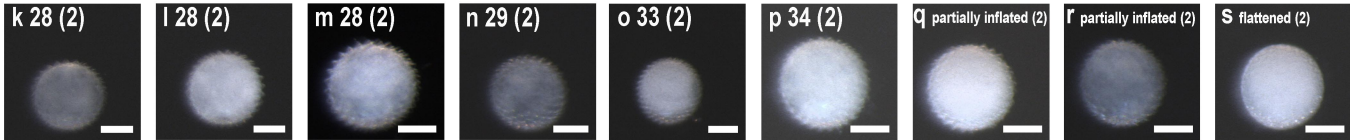
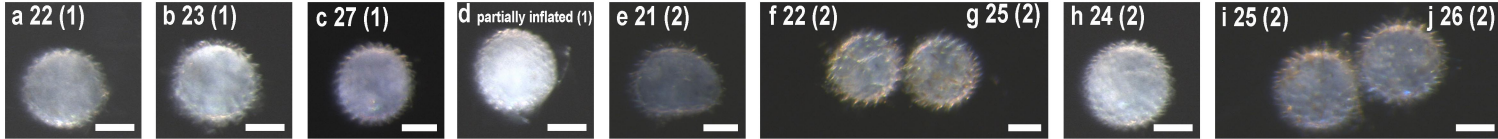
Movie that *Dactylobiotus ovimutans* sp. nov. feeds on a rotifer.

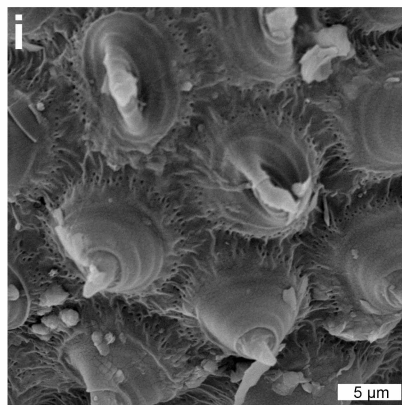
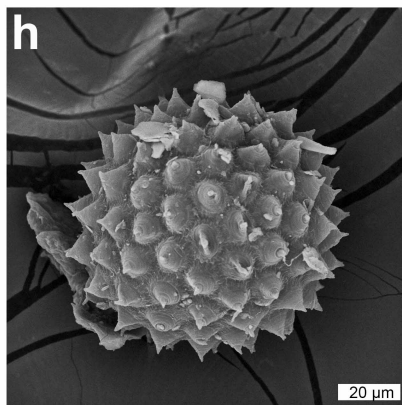
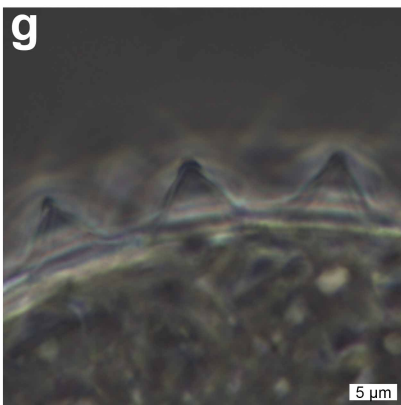
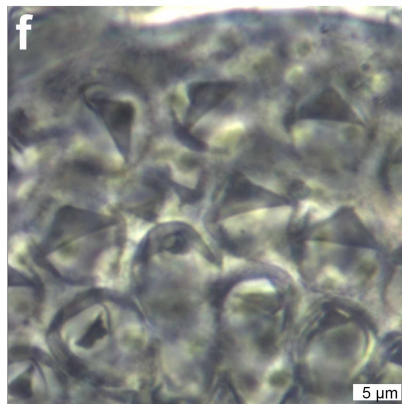
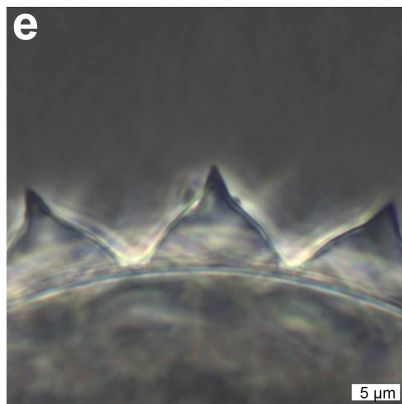
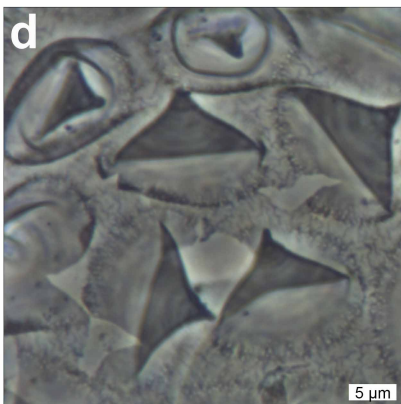
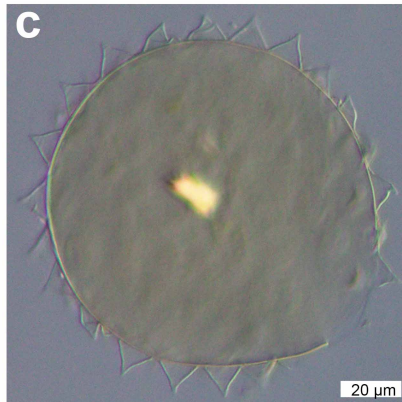
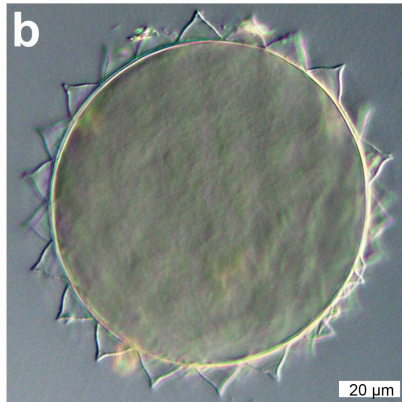
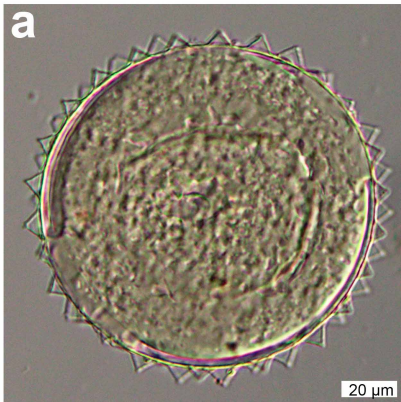




Haplotype 1	5'-ATATTGGAACACTATATTTTATTTTCGGCCTATGGGCTGCTACCGTAGGAACTTCACTAAGATTTATTATTCGATCTGAATTAAGACAACCTGGCTCTATTTTAATA
Haplotype 2	5'-ATATTGGAACACTATATTTTATTTTCGGCCTATGGGCTGCTACCGTAGGAACTTCACTAAGATTTATTATTCGATCTGAATTAAGACAACCTGGCTCTATTTTAATA
Haplotype 1	GACGATCAAATATATAATGTAATTGTAAGTAGACATGCATTTATTATAATTTTCTTTTCGTAATACCTATTCTTATTGGGGGATTGGAACTGACTAGTGCCCTTA
Haplotype 2	GACGATCAAATATATAATGTAATTGTAAGTAGACATGCATTTATTATAATTTTCTTTTCGTAATACCTATTCTTATTGGGGGATTGGAACTGACTAGTGCCCTTA
Haplotype 1	ATAATCGGAGCCCCAGATATAGCTTTCCACGAATAAATAATTTAAGATTCTGACTGCTACCCCCCTCATTCACTAATTACCAGAGGTACAATAAGAGAACAA
Haplotype 2	ATAATCGGAGCCCCAGATATGGCTTTCCACGAATAAATAATTTAAGATTCTGACTGCTACCCCCCTCATTCACTAATTACCAGAGGTACAATAAGAGAACAA
Haplotype 1	GGAGCTGGAACAGGATGAACAGTTTACCCGCCTTTATCCCAATACTTCGCCCATAGTGGACCAAGTGTAGACCTAACTATTTTCTCCCTACATATTGCAGGAGT
Haplotype 2	GGAGCTGGAACAGGATGAACAGTTTACCCGCCTTTATCCCAATACTTCGCCCATAGTGGACCAAGTGTAGACCTAACTATTTTCTCCCTACATATTGCAGGAGT
Haplotype 1	ATCATCCATTCTGGGTGCAATTAATTTTATCTCCACTATTATTAATATACGATCACCAGCAATAAGAATAGAAAATATGCCGCTATTCGTGTGATCTGTACTAATTAC
Haplotype 2	ATCATCCATTCTGGGTGCAATTAATTTTATCTCCACTATTATTAATATACGATCACCAGCAATAAGAATAGAAAATATGCCGCTATTCGTGTGATCTGTACTAATTAC
Haplotype 1	AGCAATCTTACTACTACTTGCCCTACCTGTACTAGCTGGGGGTATTACAATACTACTACTAGATCGTAATTTTAATACTTCATTCTTTGATCCTGCAGGAGGGGG
Haplotype 2	AGCAATCTTACTACTACTTGCCCTACCTGTACTAGCTGGGGGTATTACAATACTACTACTAGATCGTAATTTTAATACTTCATTCTTTGATCCTGCAGGGGGGGGG
Haplotype 1	AGATCCAATTCTATACCAACACTTATTCTGATTTTT-3'
Haplotype 2	AGATCCAATTCTATACCAACACTTATTCTGATTTTT-3'

<i>Dac. ovimutans</i>	5'------TTCTAGAGCTAATACGTGCAATCAGCTTGCTCTCTCGGGAGCAAGCG
<i>Dac. parthenogeneticus</i>	5'-TAAATCAGTTATGGTTCAGTAGATCGTACAGTTTACACGGATAACTGTGGTAATTCTAGAGCTAATACGTGCAATCAGCTTGCTCTCTCGGGAGCAAGCG
<i>Dac. ovimutans</i>	CAGTTATTAGAATAAAAACCAATCCGGCCCTCGGGTCGGTACAATTGGTGACTCTGAATAACCGAAGCGGAGCGCATGGTCTCGTACCGGCGCCAGATCT
<i>Dac. parthenogeneticus</i>	CAGTTATTAGAATAAAAACCAATCCGGCCCTTGGGTTCGGTACAATTGGTGACTCTGAATAACCGAAGCGGAGCGCATGGTCTCGTACCGGCGCCAGATCT
<i>Dac. ovimutans</i>	TTCAAGTGTCTGACTTATCAGCTTGTTGTTAGGTTATGTTCTAACAAGGCTTCAACGGGTAACGGGGTATCAGGGTCCGATACCGGAGAGGGAGCCTGA
<i>Dac. parthenogeneticus</i>	TTCAAGTGTCTGACTTATCAGCTTGTTGTTAGGTTATGTTCTAACAAGGCTTCAACGGGTAACGGGGTATCAGGGTCCGATACCGGAGAGGGAGCCTGA
<i>Dac. ovimutans</i>	GAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAGCTCTAAGC
<i>Dac. parthenogeneticus</i>	GAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAGCTCTAAGC
<i>Dac. ovimutans</i>	TCCTCGTAATCGGAATGGGTACACTTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCG
<i>Dac. parthenogeneticus</i>	TCCTCGTAATCGGAATGGGTACACTTTAAATCCTTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCG
<i>Dac. ovimutans</i>	TATATTAAAGTTGCTGCGGTTAAAAGCTCGTAGTTGGATCTGGGTAGCTGGATGGATGGTGTGCCTTGCGGTATTACTGTCTGTTCGGCACCACAAGCC
<i>Dac. parthenogeneticus</i>	TATATTAAAGTTGCTGCGGTTAAAAGCTCGTAGTTGGATCTGGGTAGCTGGATGGATGGTGTGCCTTGCGGTATTACTGTCTGTTCGGCACCACAAGCC
<i>Dac. ovimutans</i>	GGCCATGTCTTGTATGCCCTTCACTGGGTGTACTTGGCGACCGGAACGTTTACTTTGAAAAATTAGAGTGCTCAAAGCAGGCGTACGGCCTTGCATAAT
<i>Dac. parthenogeneticus</i>	GGCCATGTCTTGTATGCCCTTCACTGGGTGTACTTGGCGACCGGAACGTTTACTTTGAAAAATTAGAGTGCTCAAAGCAGGCGTACGGCCTTGCATAAT
<i>Dac. ovimutans</i>	GGTGCATGGAATAATGGAATAGGATCTCGGATTCTTTTTGTTGGTTTTCGGAACTCGAGGTAATGATTAATAGGAACAGACGGGGGCATTCTGATTGCG-3'
<i>Dac. parthenogeneticus</i>	GGTGCATGGAATAATGGAATAGGCTCTCGGATTCTTTTTGTTGGTTTTCGGAACTCGAGGTAATGATTAATAGGAACAGACGGGGGCATT-----3'





character	N	Range		Average		SD		Holotype		b	a*
		µm	pt	µm	pt	µm	pt	µm	pt		
Body length	58	303.49-759.99	682.44-1123.44	555.33	854.39	127.32	109.43	576.32	868.87	-	-
Buccal-pharyngeal tube											
Buccal tube length	44	37.42-78.02	-	62.84	-	13.05	-	66.33	-	-	-
Stylet support insertion point	44	27.16-57.23	67.47-77.03	45.56	72.65	9.23	2.10	48.93	73.77	0.96	45.63
Buccal tube external width	44	4.15-14.01	11.09-19.31	9.59	14.93	2.98	2.54	12.71	19.16	1.49	9.43
Buccal tube internal width	44	2.83-11.43	7.22-15.38	7.08	10.96	2.48	2.37	9.99	15.06	1.64	6.82
Ventral lamina	41	13.06-42.69	29.52-67.12	31.75	50.30	7.66	6.36	34.08	51.38	1.09	32.60
Placoid lengths											
Macroplacoid 1	43	10.37-39.69	27.24-55.03	26.04	40.33	8.20	6.74	28.55	43.04	1.59	25.74
Macroplacoid 2	43	5.49-21.35	12.94-28.76	13.88	21.44	4.54	3.89	17.40	26.23	1.64	13.65
Macroplacoid row	40	17.26-63.56	42.72-88.58	42.83	66.80	13.98	11.19	48.15	72.59	1.62	42.89
Claw I lengths											
Primary branch	44	13.17-40.37	35.20-62.41	29.23	45.97	7.79	5.73	32.19	48.53	1.27	29.46
Secondary branch	44	4.45-15.79	11.89-24.76	11.44	18.06	2.86	2.01	13.62	20.53	1.19	11.66
Claw II lengths											
Primary branch	41	13.48-38.85	34.41-60.83	28.76	45.48	7.50	4.76	30.81	46.45	1.24	29.27
Secondary branch	41	5.02-15.56	13.42-24.40	11.71	18.54	3.06	1.95	13.33	20.10	1.22	11.90
br	41	30.97-46.18	-	40.91	-	3.45	-	43.27	-	-	-
Claw III lengths											
Primary branch	41	13.01-39.37	33.18-59.21	28.66	45.29	7.77	5.21	31.74	47.85	1.26	28.93
Secondary branch	41	5.70-16.21	14.94-24.08	11.82	18.70	2.91	1.95	13.74	20.71	1.12	11.98
Claw IV lengths											
Primary branch	39	17.12-50.53	40.75-72.87	37.08	58.31	10.90	7.69	46.01	69.37	1.40	37.52
Secondary branch	39	13.25-21.42	17.45-28.87	15.38	24.29	4.35	2.83	18.56	27.98	1.30	15.53
br	39	35.14-51.60	-	41.89	-	3.70	-	40.34	-	-	-

Character	N	Range	Average	SD
Bare diameter (μm)	54	100.72-136.94	114.93	8.97
Full diameter (μm)	54	122.50-158.41	137.52	8.24
Process height (μm)	53	7.03-15.23	11.57	1.87
Process base width (μm)	53	10.54-21.00	15.15	2.22
Process base / height ratio (%)	53	90.79-210.21	133.22	23.19
Number of processes on the egg circumference	53	20-37	26.17	3.97

Supplementary Table S3

CO1 sequence

Haplotype 1:

5’-

ATATTGGAACACTATATTTTATTTTCGGCCTATGGGCTGCTACCGTAGGAACTTCAC
TAAGATTTATTATTCGATCTGAATTAAGACAACCTGGCTCTATTTTAATAGACGATCA
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CTATTCTTATTGGGGGATTTGGAACTGACTAGTGCCCTTAATAATCGGAGCCCCA
GATATAGCTTTCCACGAATAAATAATTTAAGATTCTGACTGCTACCCCCCTCATTC
ATACTAATTACCAGAGGTACAATAAGAGAACAAGGAGCTGGAACAGGATGAACAG
TTTACCCGCCTTTATCCCAATACTTCGCCCATAGTGGACCAAGTGTAGACCTAACTA
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CACTATTATTAATATACGATCACCAGCAATAAGAATAGAAAATATGCCGCTATTCGT
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GGGTATTACAATACTACTACTAGATCGTAATTTTAATACTTCATTCTTTGATCCTGCA
GGAGGGGGAGATCCAATTCTATACCAACACTTATTCTGATTTTT-3’

Haplotype 2:

5’-

ATATTGGAACACTATATTTTATTTTCGGCCTATGGGCTGCTACCGTAGGAACTTCAC
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18S sequence

5’-

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AAGCGGAGCGCATGGTCTCGTACCGGCGCCAGATCTTCAAGTGTCTGACTTATCA
GCTTGTTGTTAGGTTATGTTCTTAACAAGGCTTCAACGGGTAACGGGGTATCAGGG
TCCGATACCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGC

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28S sequence

5'-

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AAACTTTAAATGGGTGAGAAGTCTGGCTTGCTTAAATGCGAAGCTGAAGTCTGGA
CGTTGGATACGAGCGCCTAGTGGGCCACTTTTGGT-3'

character	<i>Dac. ovimutans</i>	<i>Dac. caldarellai</i>
Buccal tube length	54.24 μm	52.20 μm
Body length	437.58	420.00
Ventral lamina (<i>pt</i>)	56.90	43.00
Stylet support insertion point (<i>pt</i>)	69.32	77.82
Primary branch length of claw II (<i>pt</i>)	48.95	34.77
<i>br</i> of claw II	42.34	51.53
Primary branch length of claw IV (<i>pt</i>)	65.69	44.71
<i>br</i> of claw IV	37.19	48.97

Supplementary Table S5

CHARACTER	1	2	3	4	5	6	10	11	12	13	14	15	16	17
Diameter of egg without processes		99.6	90.0		124.0	141.1	128.8	125.7		97.7	93.4	132.8	135.1	134.4
Diameter of egg with processes		123.9	109.2		140.4	154.6	140.0	142.9		122.6	109.2	145.9	151.0	145.1
Process height	9.8	10.6	10.4	9.3	6.1	9.0	7.6	7.8	15.6	14.3	10.5	5.6	9.0	6.0
	8.8	11.7	10.7	10.0	7.6	7.8	7.1	8.4	15.7	14.3	10.4	6.0	10.1	5.5
	6.4	12.8	10.6	8.8	7.0	8.7	7.2	8.7	16.1	15.1	9.5	6.2	8.4	5.9
Process base width	10.9	16.4	14.1	13.6	13.6	14.3	13.0	18.6	10.8	12.9	11.6	12.7	15.6	11.4
	12.0	17.9	13.9	13.9	14.9	13.4	12.2	17.9	8.9	12.6	11.3	12.3	14.2	11.4
	8.3	17.4	15.4	13.8	17.1	14.6	11.6	18.2	10.2	11.6	12.7	13.4	14.1	11.6
Process base/height ratio	111%	155%	135%	146%	224%	160%	171%	239%	69%	90%	110%	227%	174%	191%
	136%	154%	130%	139%	197%	172%	172%	215%	56%	88%	109%	206%	141%	206%
	131%	136%	145%	156%	245%	169%	160%	209%	63%	77%	134%	217%	168%	197%
Distance between processes				1.0	2.0	2.7	5.2	2.2	1.4			2.4	3.5	2.8
				0.8	2.3	3.0	6.7	3.2	1.5			2.7	4.9	2.4
					1.6	3.9	6.6	4.3	2.7			2.8	3.8	1.9
Number of processes on the egg circumference		21	20			24			20					

DNA fragment	Primer name	Primer direction	Primer sequence (5'-3')	Source
18S rRNA	18S-Tard84F	Forward	CCGCGAATGGCTCATTAATCAG	Giribet <i>et al.</i> 1996
	18S-9S	Reverse	GATCCTTCCGCAGGTTCACCTAC	
28S rRNA	28S-1274F	Forward	GACCCGTCTTGAAACACGGA	Markmann 2000
	28S-706R	Reverse	CGCCAGTTCTGCTTACC	
COI	LCO1490	Forward	GGTCAACAAATCATAAAGATATTG	Folmer <i>et al.</i> 1994
	HCO2198	Reverse	TAAACTTCAGGGTCACCAAAAAATCA	

Step	18S rRNA			28S rRNA			COI		
	Temperature [°C]	Time [min:sec]	Cycles	Temperature [°C]	Time [min:sec]	Cycles	Temperature °C	Time [min:sec]	Cycles
Initial denaturation	95	5:00	1	95	5:00	1	94	05:00	1
Denatureation	-	-	-	-	-	-	94	01:00	5
Annealing	-	-	-	-	-	-	45	01:30	5
Elongation	-	-	-	-	-	-	72	01:30	5
Denaturation	94	0:30	30	94	0:30	30	94	01:00	35
Annealing	53	0:30	30	42	0:30	30	50	01:30	35
Elongation	72	0:30	30	72	0:30	30	72	01:00	35
Final elongation	72	10:00	1	72	10:00	1	72	05:00	1
Source	This study			This study			Michalczyk <i>et al.</i> 2012		

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