

Supplementary Material to:

USING LANDMARK-BASED GEOMETRIC MORPHOMETRICS FOR HOLOTYPE SELECTION IN
CRYPTIC SPECIES: A CASE STUDY OF WESTERN AUSTRALIAN *HALICYCLOPS* (COPEPODA,
CYCLOPOIDA)

BY

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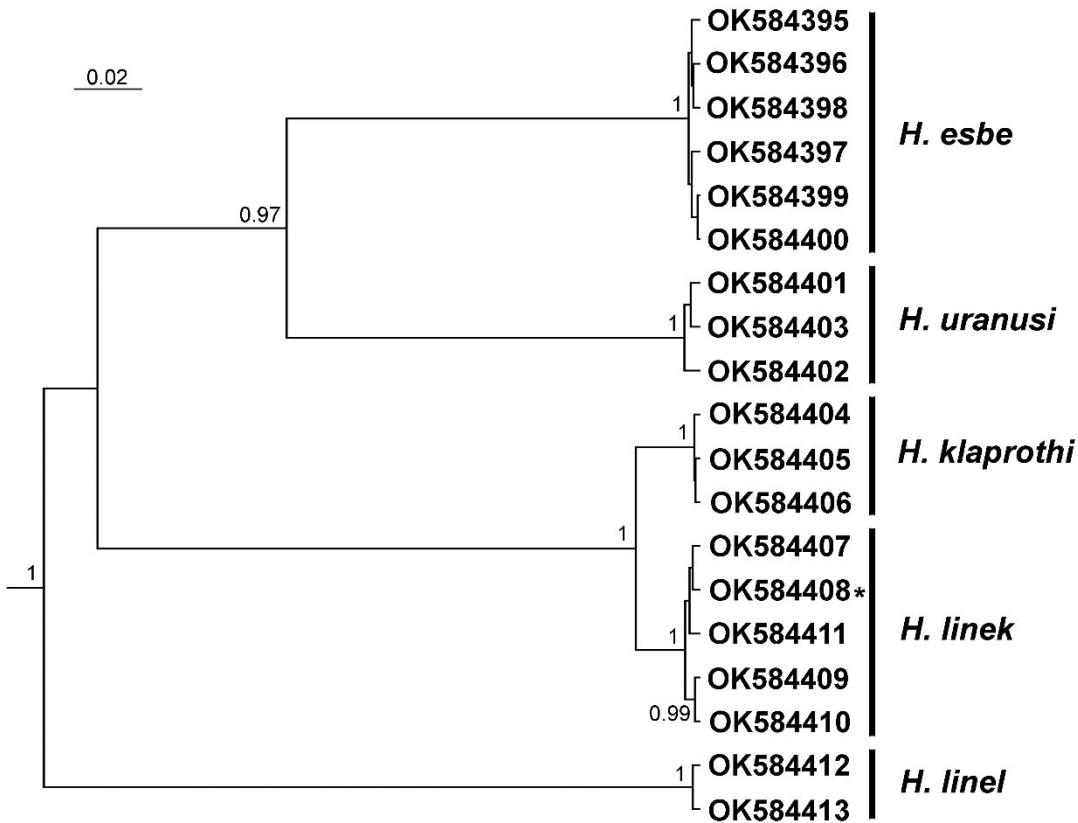


Fig. S1. Bayesian inference (BI) cladogram based on partial 12S sequences from 19 *Halicyclops* specimens (table S-I), showing only BI posterior probabilities larger than 0.9. Five cryptic species indicated on the right are also used for calculating average pairwise distances (table S-IV). Specimen codes are their GenBank accession numbers, and asterisk marks a holotype.

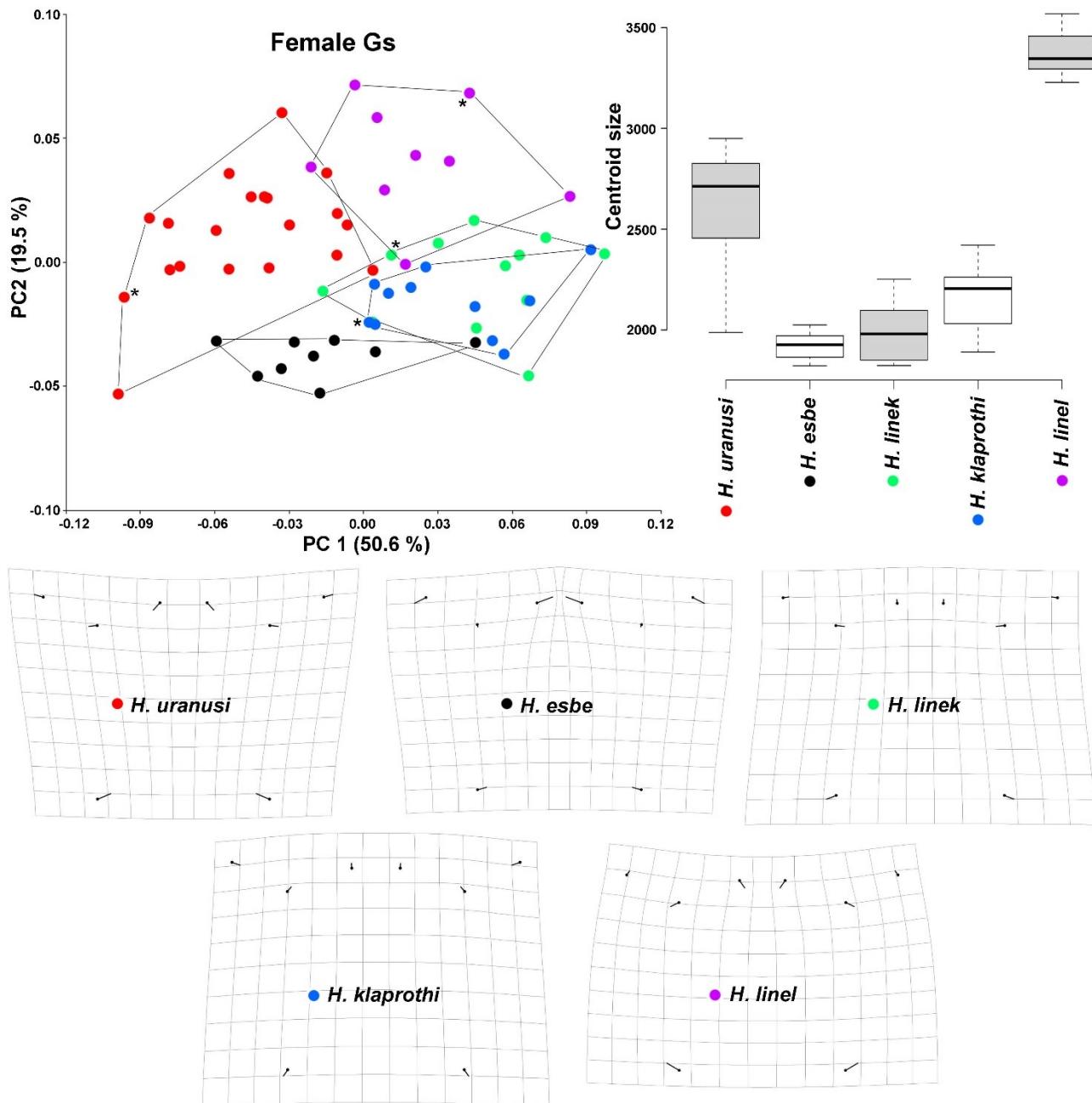


Fig. S2. Graphical visualisation of the geometric morphometric analysis based on eight landmarks (fig. 2) in the female Gs dataset (table S-I). Scatter plot (top left) shows delimitation of species by principal component analysis as convex hulls in morphospace defined by the first two eigenvectors (PCs); relative amount of shape variation explained by PCs is provided in the brackets and holotypes are marked with an asterisk. Box-plot (top right) is based on centroid size; centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend $1.5 \times$ the interquartile range from the 25th and 75th percentiles. Transformation grids (scaled $1.5 \times$) visualize shape changes for each species relative to the mean shape of the whole sample; all scaled to the same centroid size.

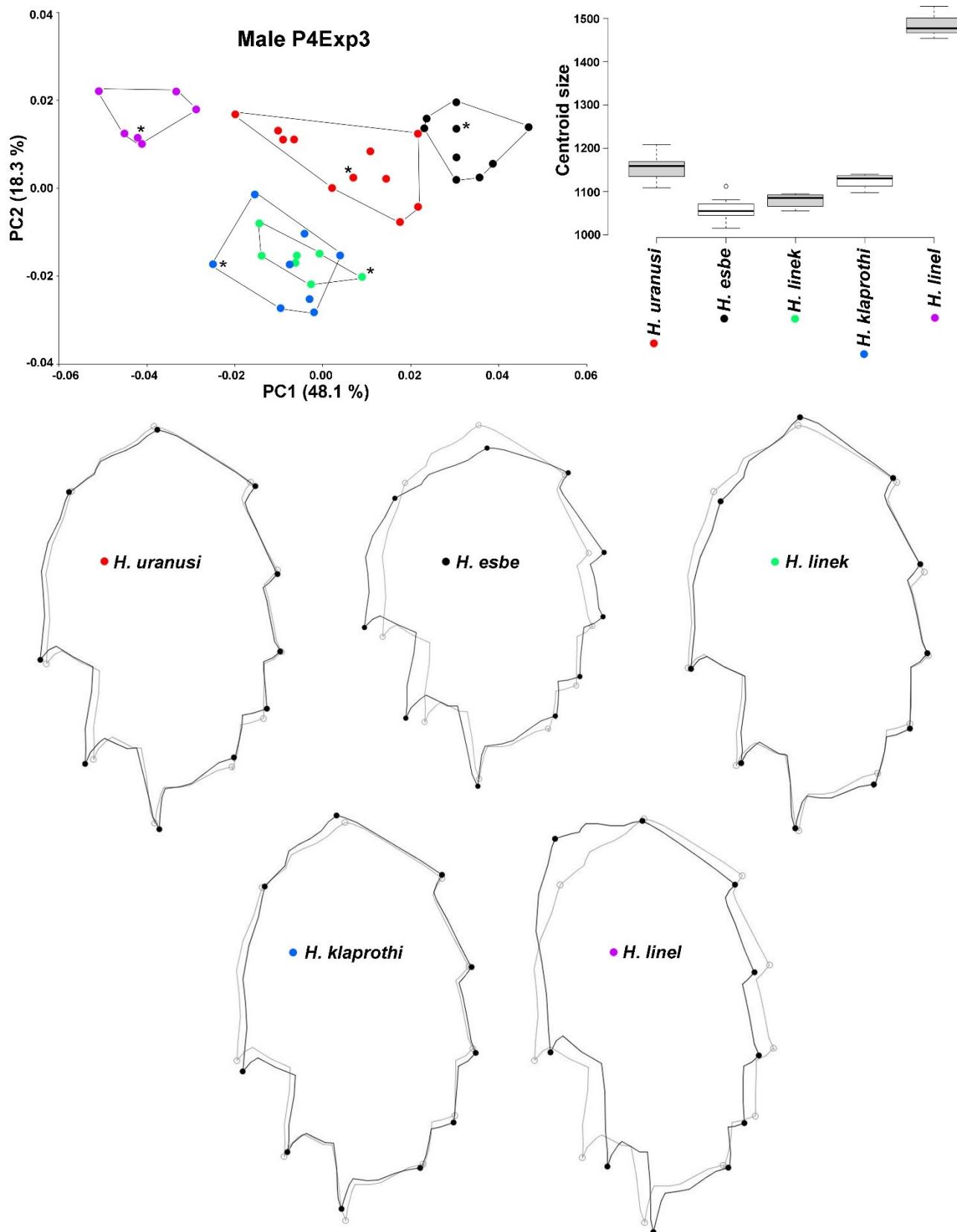


Fig. S3. Graphical visualisation of the geometric morphometric analysis based on 10 landmarks (fig. 2) in the male P4Exp3 dataset (table S-I). Explanations for scatter plot (top left) as in fig. S1, except here allotypes are marked with an asterisk. Explanations for box-plot (top right) as in fig. S1, except here one outlier represented by a dot. Warped outlines (scaled 3×) visualize shape changes for each species (black) relative to the mean shape of the whole sample (grey); all scaled to the same centroid size.

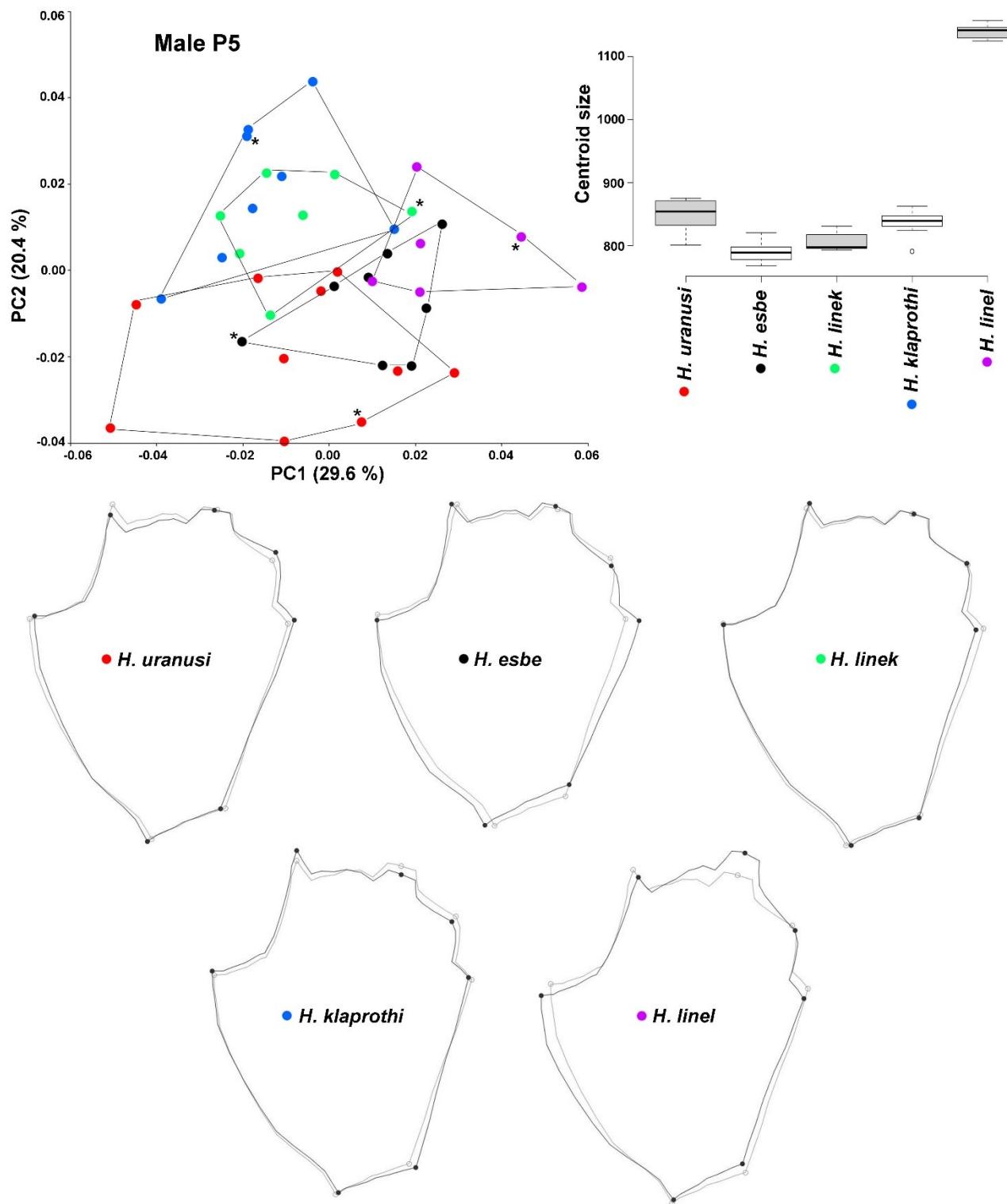


Fig. S4. Graphical visualisation of the geometric morphometric analysis based on seven landmarks (fig. 2) in the male P5 dataset (table S-I). Explanations for scatter plot (top left), box-plot (top right), and warped outlines (scaled 2×) as in fig. S2.

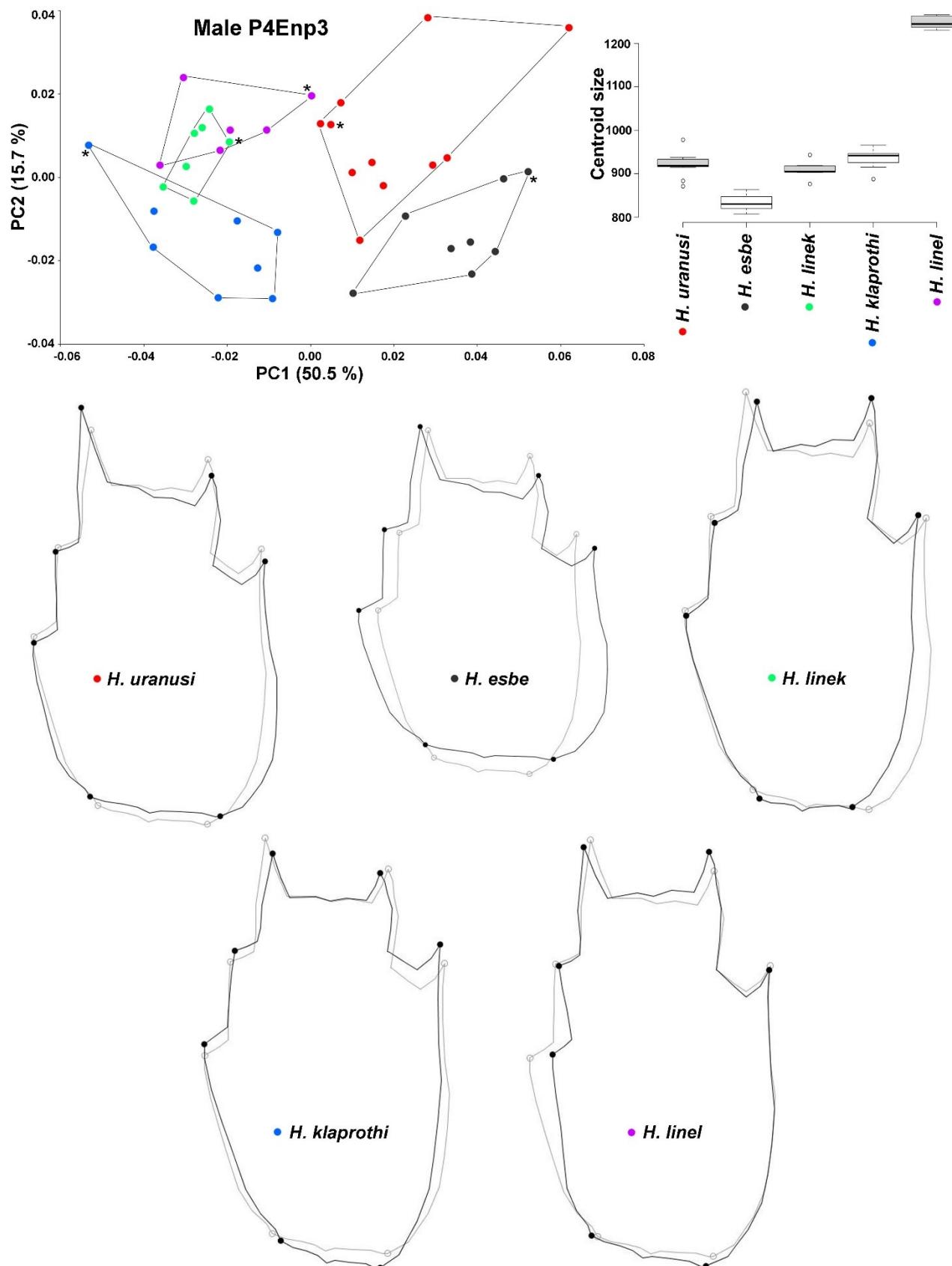


Fig. S5. Graphical visualisation of the geometric morphometric analysis based on seven landmarks (fig. 2) in the male P4Enp3 dataset (table S-I). Explanations for scatter plot (top left), box-plot (top right), and warped outlines (scaled 3×) as in fig. S2.

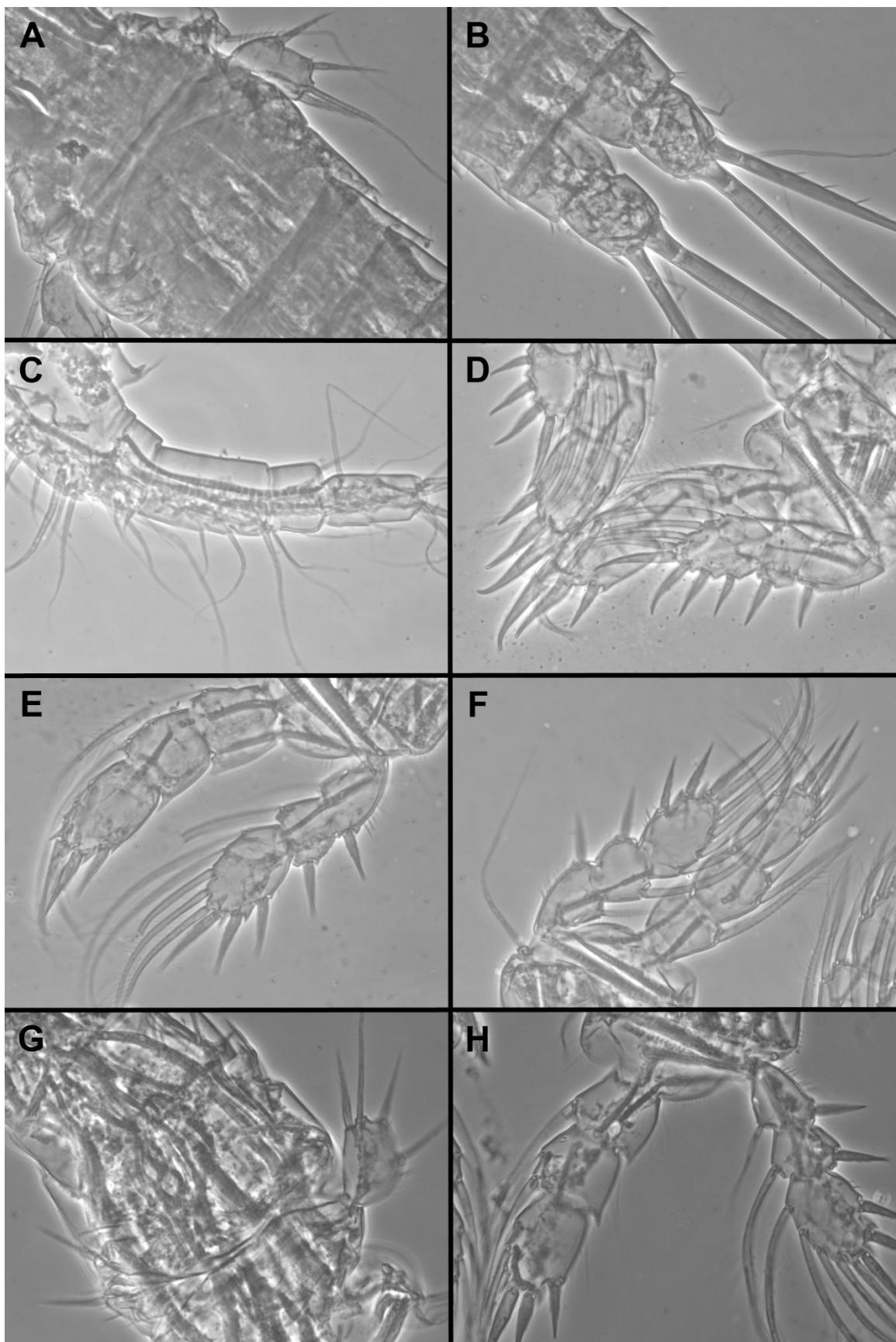


Fig. S6. *Halicyclops eberhardi* De Laurentiis, Pesce & Humphreys, 2001, compound light microscope photographs, all with 40 \times objective; A-F, holotype female; G & H, paratype female: A, genital double-somite and fifth legs; B, last two urosomites and caudal rami; C, antennula; D, second swimming leg; E, third swimming leg; F, fourth swimming leg; G, genital double-somite and fifth legs; H, third swimming leg.

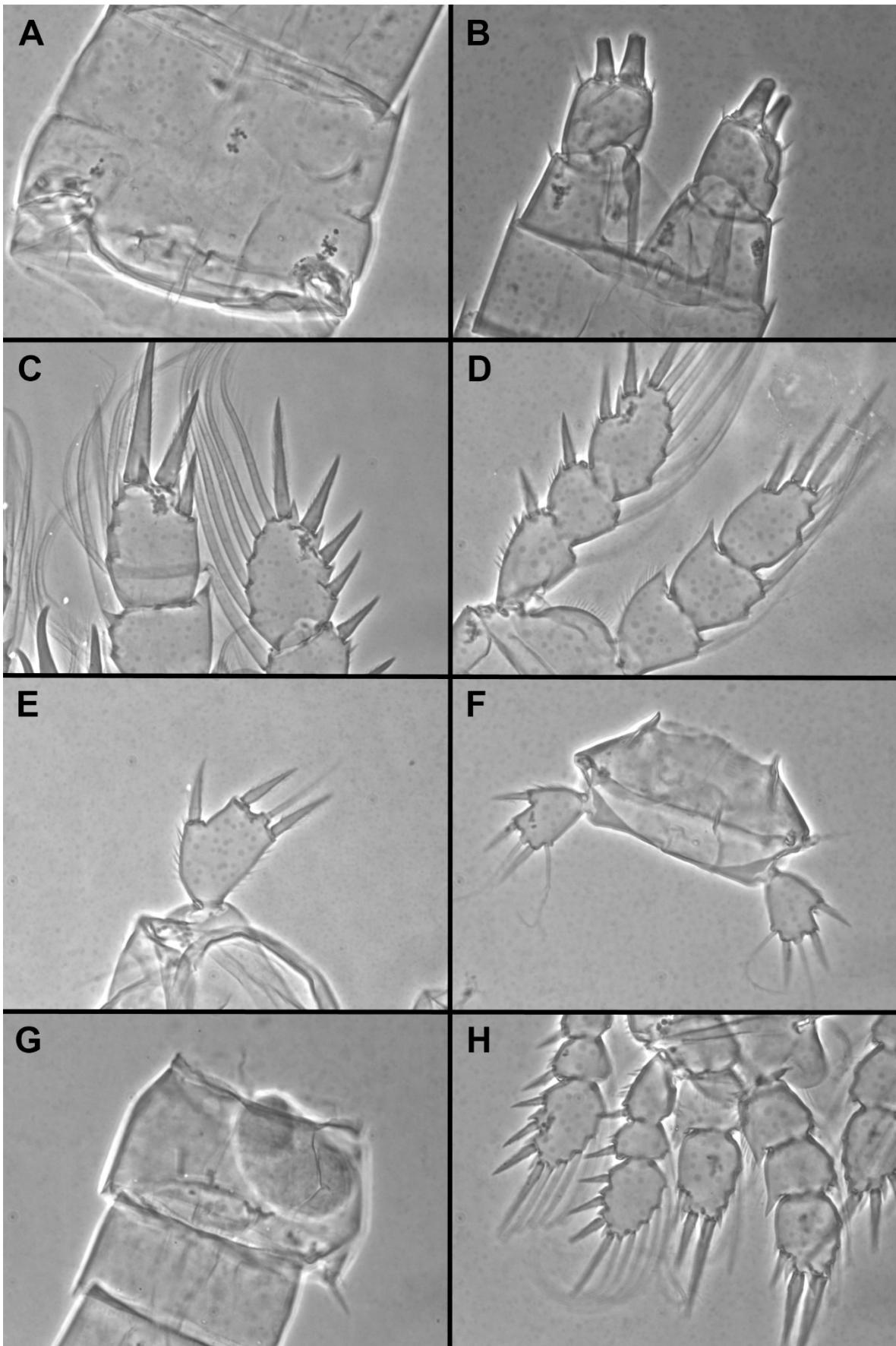


Fig. S7. *Halicyclops uranusi* sp. nov., compound light microscope photographs, all with 40 \times objective; A-F, holotype female; G & H, allotype male: A, genital double-somite; B, last two urosomites and caudal rami; C, third swimming leg; D, fourth swimming leg; E, fifth leg; F, fifth legs; G, genital somite; H, second and third swimming legs.

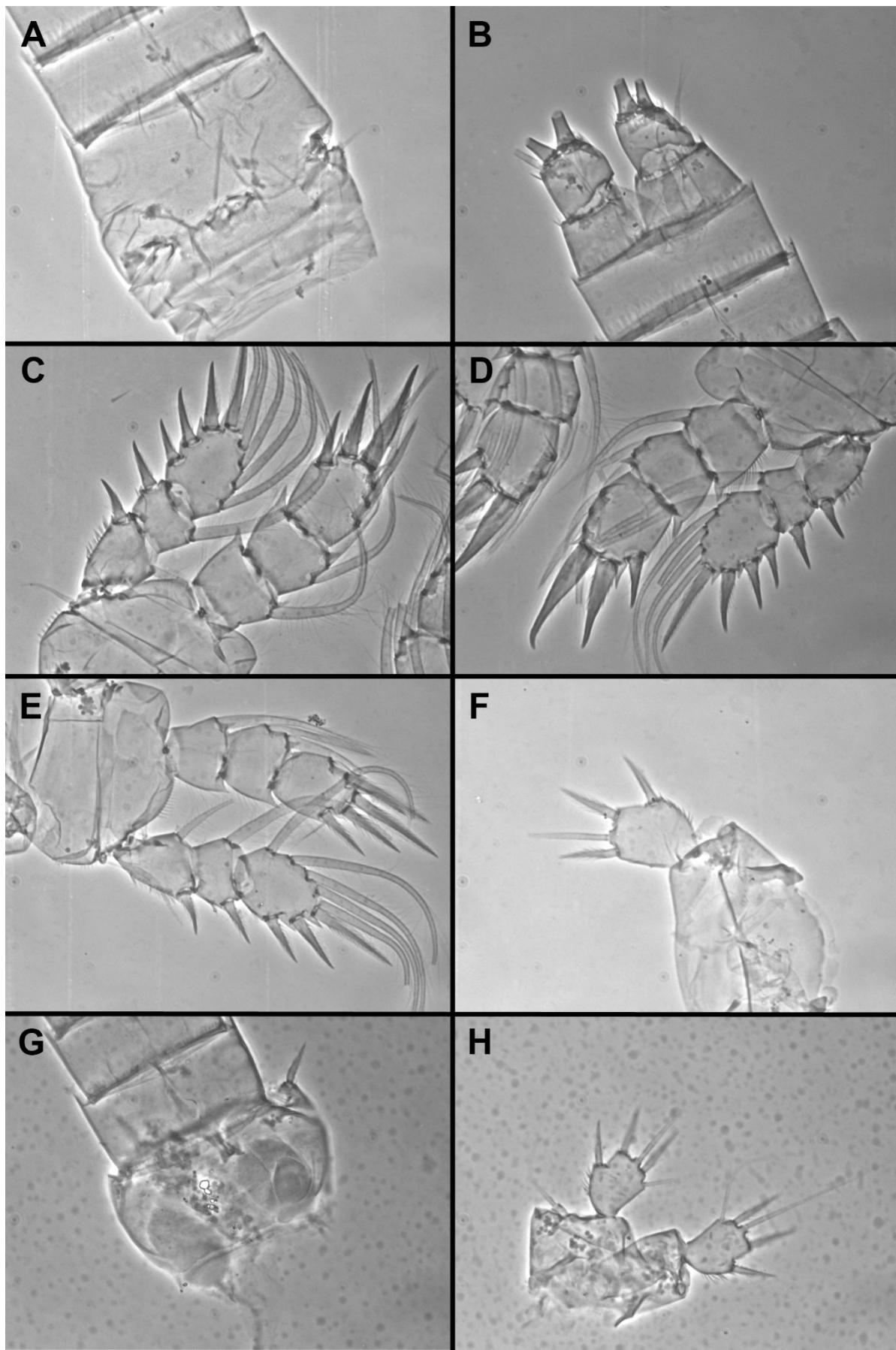


Fig. S8. *Halicyclops esbe* sp. nov., compound light microscope photographs, all with 40 \times objective; A-F, holotype female; G & H, allotype male: A, genital double-somite; B, last two urosomites and caudal rami; C, second swimming leg; D, third swimming leg; E, fourth swimming leg; F, fifth leg; G, genital somite; H, fifth legs.

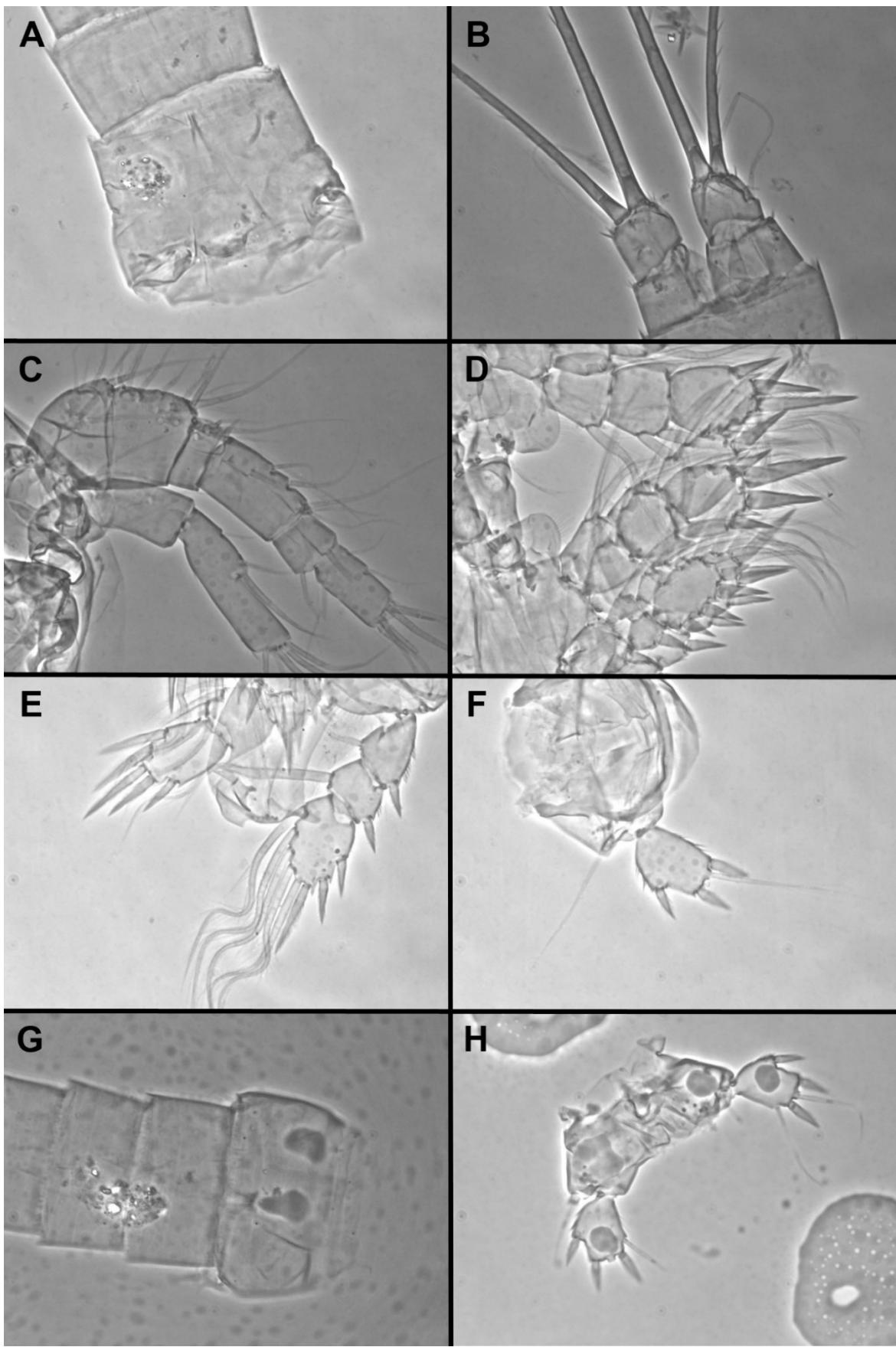


Fig. S9. *Halicyclops linek* sp. nov., compound light microscope photographs, all with 40 \times objective; A-F, holotype female; G & H, allotype male: A, genital double-somite; B, anal somite and caudal rami; C, antennula and antenna; D, second and third swimming legs; E, fourth swimming leg; F, fifth leg; G, genital somite and three posterior urosomites; H, fifth legs.

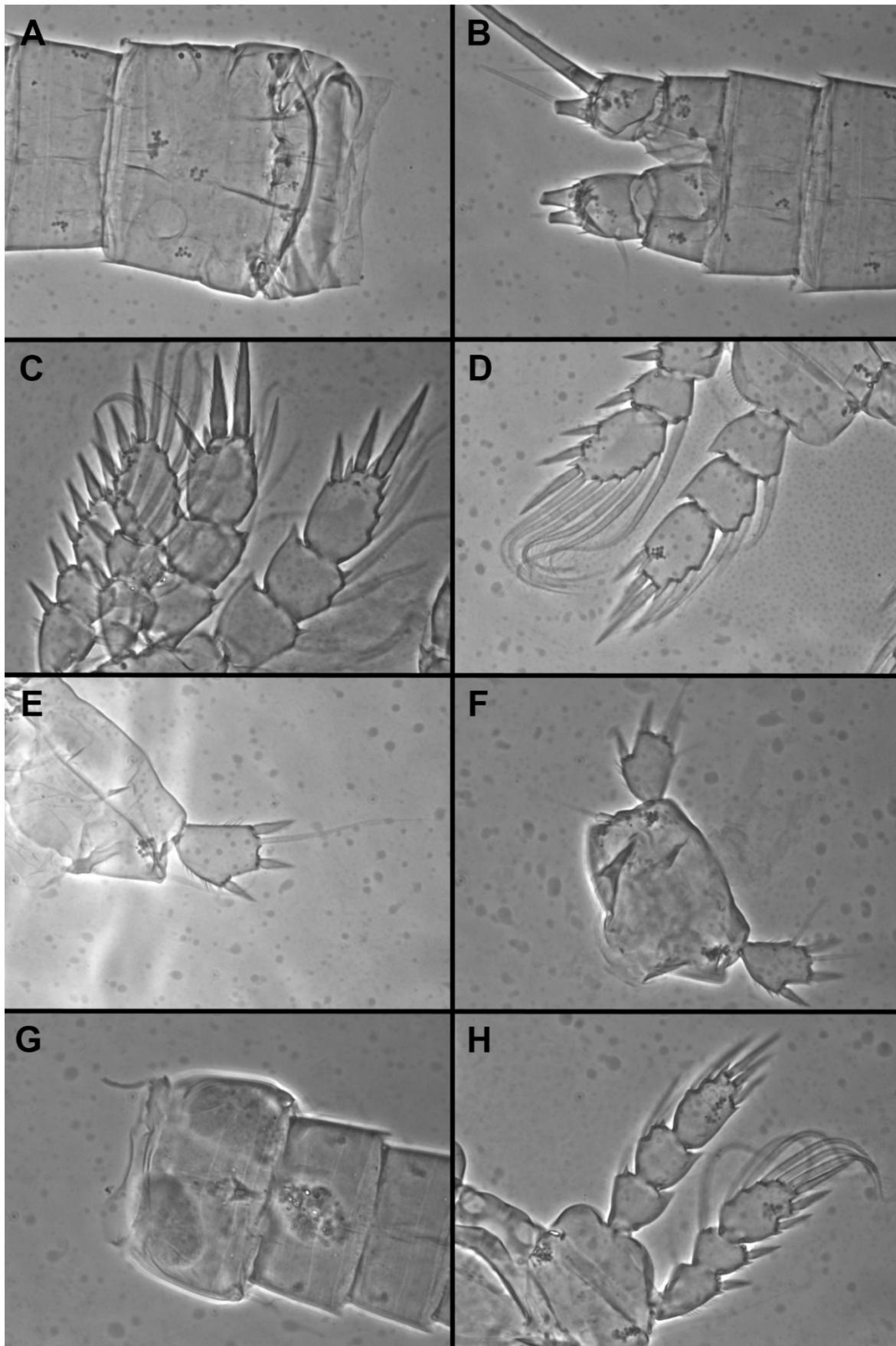


Fig. S10. *Halicyclops klaprothi* sp. nov., compound light microscope photographs, all with 40 \times objective; A-E, holotype female; F-H, allotype male: A, genital double-somite; B, last three urosomites and caudal rami; C, second and third swimming legs; D, fourth swimming leg; E, fifth leg; F, fifth legs; G, genital somite and two posterior urosomites; H, fourth swimming leg.

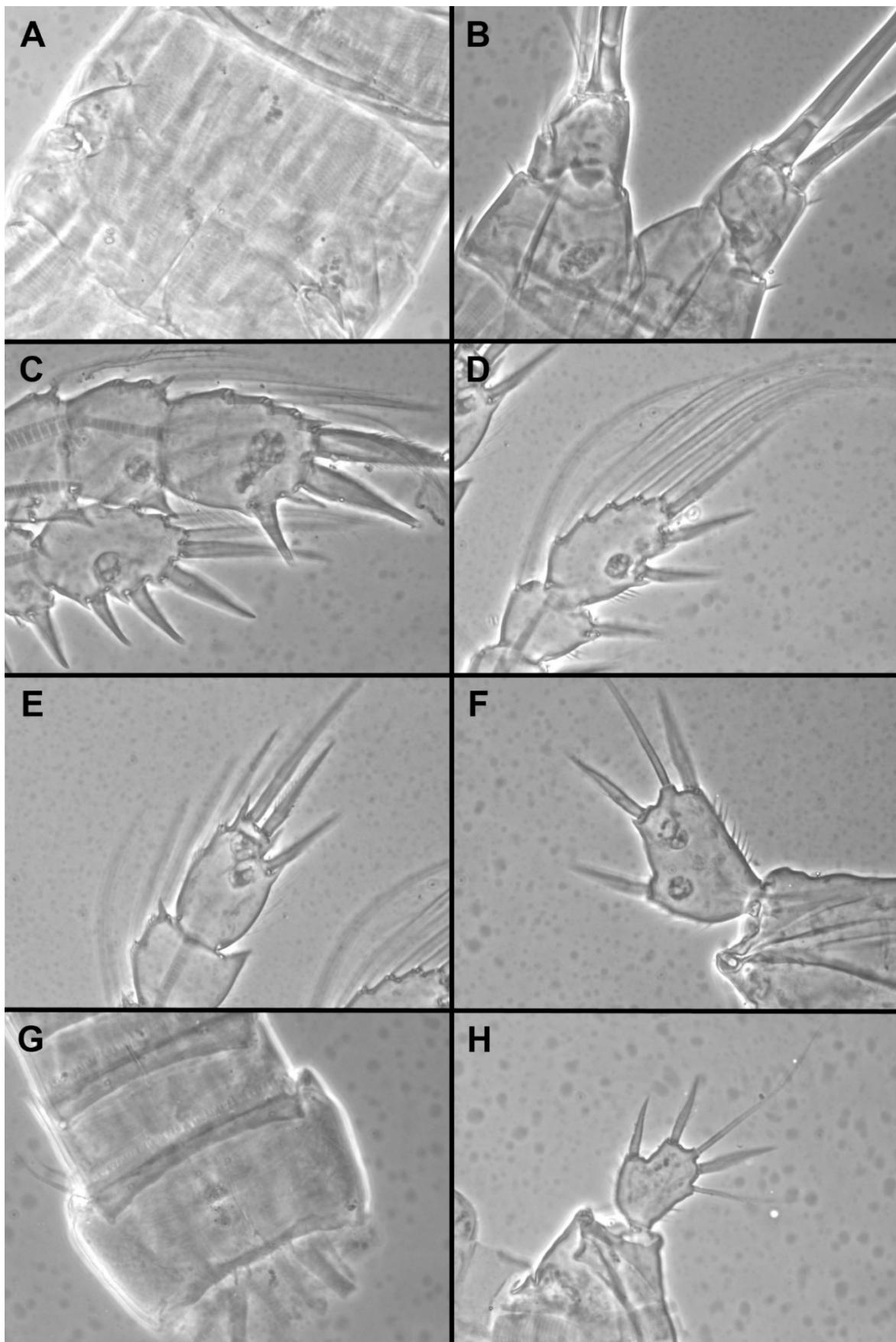


Fig. S11. *Halicyclops linel* sp. nov., compound light microscope photographs, all with 40 \times objective; A-F, holotype female; G & H, allotype male: A, genital double-somite; B, anal somite and caudal rami; C, third swimming leg; D, exopod of fourth swimming leg; E, endopod of fourth swimming leg; F, fifth leg; G, genital somite and one posterior urosomite; H, fifth leg.

TABLE S-I

List of examined specimens belonging to five cryptic species of *Halicyclops* from Yeelirrie and *H. eberhardi*. For abbreviations see text

TKN	Species	Sex	Type	WAM	Date	Line	Bore	Sample	Coordinates	LBGM datasets	COI	12S
1	<i>esbe</i>	F	Paratype	76340	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301712	no
2	<i>esbe</i>	F	Paratype	76341	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301713	no
3	<i>esbe</i>	F	Paratype	76342	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301714	OK584395
4	<i>esbe</i>	F	Paratype	76343	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301715	OK584396
5	<i>esbe</i>	F	Paratype	76344	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3	MW301716	OK584397
6	<i>esbe</i>	F	Paratype	76345	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301717	no
7	<i>esbe</i>	F	Paratype	76346	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301718	no
8	<i>esbe</i>	F	Holotype	76347	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301719	no
9	<i>esbe</i>	F	Paratype	76348	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301720	no
10	<i>esbe</i>	F	Paratype	76349	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301721	no
11	<i>esbe</i>	F	Paratype	76350	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301722	OK584398
12	<i>esbe</i>	F	Paratype	76351	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3	MW301723	no
13	<i>esbe</i>	F	Paratype	76352	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301724	no
14	<i>esbe</i>	F	Paratype	76353	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301725	no
15	<i>esbe</i>	F	Paratype	76354	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301726	no
16	<i>esbe</i>	M	Paratype	76355	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301727	no
17	<i>esbe</i>	M	Paratype	76356	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301728	OK584399
18	<i>esbe</i>	M	Paratype	76357	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301729	no
20	<i>esbe</i>	M	Paratype	76358	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	MW301730	OK584400
21	<i>esbe</i>	M	Paratype	76359	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Exp3	MW301731	no
22	<i>esbe</i>	M	Paratype	76360	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P4Emp3, P4Exp3	MW301732	no
23	<i>esbe</i>	M	Paratype	76361	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	no	no
24	<i>esbe</i>	M	Allotype	76362	16/3/10	SB	SB14	LN8516	27.344283 120.307708	Gs, P5, P4Emp3, P4Exp3	no	no
25	<i>esbe</i>	M	Paratype	76363	16/3/10	SB	SB14	LN8516	27.344283 120.307708	P5, P4Emp3, P4Exp3	MW301733	no
26	<i>linex</i>	F	Paratype	76281	2/03/10	K	YHHC048KA	LN8463	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301734	no
27	<i>linex</i>	F	Paratype	76282	2/03/10	K	YHHC048KA	LN8463	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301735	no
28	<i>linex</i>	F	Paratype	76283	2/03/10	K	YHHC048KA	LN8463	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301736	no
29	<i>linex</i>	F	Paratype	76284	2/03/10	K	YHHC048KA	LN8463	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301737	no
31	<i>linex</i>	M	Paratype	76285	2/03/10	K	YHHC048KA	LN8463	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301738	no
32	<i>linex</i>	M	Paratype	76286	2/03/10	K	YHHC048KA	LN8463	27.247640 120.054900	Gs	MW301739	no
33	<i>linex</i>	F	Paratype	76287	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301740	no
34	<i>linex</i>	F	Paratype	76288	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301741	no
35	<i>linex</i>	F	Paratype	76289	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301742	no
36	<i>linex</i>	F	Paratype	76290	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301743	no
37	<i>linex</i>	F	Paratype	76291	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301744	OK584411
38	<i>linex</i>	F	Holotype	76292	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301745	OK584408
39	<i>linex</i>	F	Paratype	76293	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	P5, P4Emp3, P4Exp3	MW301746	OK584407
40	<i>linex</i>	F	Paratype	76294	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301747	no
41	<i>linex</i>	F	Paratype	76295	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301793	no
42	<i>linex</i>	F	Paratype	76296	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	P5	MW301748	OK584409
43	<i>linex</i>	M	Paratype	76297	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301749	OK584410
44	<i>linex</i>	M	Paratype	76298	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	P5, P4Emp3, P4Exp3	MW301750	no
45	<i>linex</i>	M	Paratype	76299	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301751	no
46	<i>linex</i>	M	Paratype	76300	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301752	no
47	<i>linex</i>	M	Paratype	76301	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	MW301705	no
48	<i>linex</i>	M	Allotype	76302	2/03/10	K	YHHC048KA	LN100467	27.247640 120.054900	Gs, P5, P4Emp3, P4Exp3	*	no
51	<i>linel</i>	F	Paratype	76392	18/3/10	L	L-UNK-1	LN7137	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	MW301803	no
52	<i>linel</i>	F	Paratype	76393	18/3/10	L	L-UNK-1	LN7137	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	MW301802	no
53	<i>linel</i>	F	Paratype	76394	18/3/10	L	L-UNK-1	LN7137	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	MW301801	no
54	<i>linel</i>	M	Paratype	76395	18/3/10	L	L-UNK-1	LN7137	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	MW301805	no
55	<i>linel</i>	M	Paratype	76396	18/3/10	L	L-UNK-1	LN7137	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	MW301804	no
56	<i>linel</i>	F	Paratype	76364	16/3/10	L	L-UNK-1	LN8854	27.329832 120.150590	P5, P4Emp3, P4Exp3	MW301800	no
57	<i>linel</i>	F	Paratype	76365	16/3/10	L	L-UNK-1	LN8854	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	MW301799	no
58	<i>linel</i>	M	Paratype	76366	16/3/10	L	L-UNK-1	LN8854	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	no	no
59	<i>linel</i>	M	Paratype	76367	16/3/10	L	L-UNK-1	LN8854	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	no	OK584412
61	<i>linel</i>	M	Paratype	76368	16/3/10	L	L-UNK-1	LN8854	27.329832 120.150590	Gs, P5, P4Emp3, P4Exp3	no	OK584413
62	<i>klaprothi</i>	F	Paratype	76303	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301752	no
63	<i>uranusi</i>	F	Paratype	76304	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301753	no
66	<i>uranusi</i>	F	Paratype	76305	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301754	no
69	<i>uranusi</i>	F	Paratype	76306	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301755	OK584401
70	<i>klaprothi</i>	F	no	no	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	no	MW301756	no
71	<i>uranusi</i>	M	Paratype	76307	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301757	OK584403
74	<i>uranusi</i>	F	Paratype	76308	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301758	OK584402
75	<i>uranusi</i>	F	Paratype	76309	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301759	no
77	<i>uranusi</i>	F	Paratype	76310	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301760	no
78	<i>klaprothi</i>	F	Paratype	76311	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301711	no
79	<i>uranusi</i>	F	Paratype	76312	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301761	no
80	<i>uranusi</i>	F	Paratype	76313	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301762	no
81	<i>klaprothi</i>	F	Paratype	76314	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301763	OK584404
82	<i>uranusi</i>	F	Paratype	76315	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301764	no
83	<i>uranusi</i>	F	Holotype	76316	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301765	no
84	<i>uranusi</i>	F	Paratype	76317	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301766	no
85	<i>uranusi</i>	F	Paratype	76318	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301767	no
86	<i>uranusi</i>	F	Paratype	76319	2/1/10	1.5	YYAC33	LN8303	27.169565 119.871815	Gs, P5, P4Emp3, P4Exp3	MW301768	no
87	<i>klaprothi</i>	F	Paratype	76320	2/1/10	1.5	YYAC					

TABLE S-II

List of internal primers developed from an initial alignment of 526 bp (obtained with universal COI primers), with their start and stop positions in that alignment

Primer code	Primer structure	Start	Stop
Frag1.F1	5'GCTTTTATYATAATTTTTTACAG	17	41
Frag1.R1	5'ARWGAAAAAAATAGCAAAATC	282	263
Frag1.F2	5'CTTTTATYATAATTTTTTACAG	18	41
Frag2.R3	5'AYCACARAATAAHGGAGTW	392	372
Frag2.R1	5'CAACARAATAAHGGAGTWCG	390	371

TABLE S-III

Average pairwise K2P distances within (diagonal) and between five new *Halicyclops* species from Yeelirrie, based on 115 partial mtCOI sequences (see fig. 3; table S-I)

Species	1	2	3	4	5
1. <i>H. klaprothi</i>	0.0102				
2. <i>H. uranusi</i>	0.2117	0.0179			
3. <i>H. esbe</i>	0.2199	0.1777	0.0015		
4. <i>H. linek</i>	0.0730	0.2045	0.2160	0.0056	
5. <i>H. linel</i>	0.2423	0.2504	0.1956	0.2772	0.0053

TABLE S-IV

Average pairwise K2P distances within (diagonal) and between five new *Halicyclops* species from Yeelirrie, based on 19 partial 12S sequences (see fig. S1; table S-I)

Species	1	2	3	4	5
1. <i>H. klaprothi</i>	0.0000				
2. <i>H. uranusi</i>	0.2164	0.0047			
3. <i>H. esbe</i>	0.2643	0.1916	0.0009		
4. <i>H. linek</i>	0.0335	0.2234	0.2594	0.0024	
5. <i>H. linel</i>	0.2567	0.2179	0.3069	0.2414	0.0024

TABLE S-V

Cross-validation scores (as percentages) from discriminant function analysis (DFA) among six examined *Halicyclops* species for all morphological structures separated for females (F) and males (M). Asterisks mark values that do not show statistically significant Procrustes distances after 10,000 runs in permutation tests ($P \geq 0.05$); abbreviations for morphological structures (datasets) as in fig. 2; *Halicyclops eberhardi* is known only after females and for this species data were also not scored for Gs, so some comparisons are not applicable (n/a)

Species pairs	F Gs	M Gs	F P5	M P5	F P4Exp3	M P4Exp3	F P4Emp3	M P4Emp3
<i>uranusi-esbe</i>	100-100	89-100	86-71	90-100	80-85	73-89	95-80	82-75
<i>uranusi-linek</i>	95-83	78-86	68-64	70-86	95-100	82-57	95-100	100-100
<i>uranusi-klaprothi</i>	95-100	78-88	73-58*	60-63	90-91	91-75	100-100	91-88
<i>uranusi-linel</i>	95-78	78-83	95-100	90-100	95-100	91-83	95-90	91-83
<i>uranusi-eberhardi</i>	n/a	n/a	100-0	n/a	100-100	n/a	95-33	n/a
<i>esbe-linek</i>	100-92	88-86	79-93	75-86	100-100	100-100	100-100	100-100
<i>esbe-klaprothi</i>	100-100	100-100	93-67	88-88	100-100	100-88	100-100	88-88
<i>esbe-linel</i>	100-100	88-100	93-100	75-100	100-100	100-100	100-100	75-83
<i>esbe-eberhardi</i>	n/a	n/a	93-50	n/a	92-100	n/a	87-67	n/a
<i>linek-klaprothi</i>	67-82*	57-63*	86-58	57-75*	100-91	71-63	85-75	57-63
<i>linek-linel</i>	92-78	86-100	79-70	100-67	85-100	100-100	85-80	71-33
<i>linek-eberhardi</i>	n/a	n/a	100-50	n/a	85-33	n/a	92-67	n/a
<i>klaprothi-linel</i>	100-67	100-83	92-90	88-100	82-70	100-100	83-80	63-33
<i>klaprothi-eberhardi</i>	n/a	n/a	100-50	n/a	100-67	n/a	83-100	n/a
<i>linel-eberhardi</i>	n/a	n/a	70-50	n/a	100-100	n/a	80-33	n/a